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Strategic and Advancing Regulatory Science Approach in Pharmaceutical Development of Complex Generic Drug Products

Tese no âmbito do Doutoramento em Ciências Farmacêuticas, especialidade de Tecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Paulo Magalhães Simões e Professor Doutor João José Martins Simões de Sousa, e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

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Faculdade de Farmácia da Universidade de Coimbra

STRATEGIC AND ADVANCING REGULATORY SCIENCE APPROACH IN PHARMACEUTICAL DEVELOPMENT OF COMPLEX GENERIC DRUG PRODUCTS

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Dissertação de Doutoramento na área científica de Ciências Farmacêuticas, especialidade de Tecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Paulo Magalhães Simões e Professor Doutor João José Martins Simões de Sousa, e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

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Doctoral thesis in Pharmaceutical Sciences, specialization in Pharmaceutical Technology, with coordination and supervision of Sérgio Paulo Magalhães Simões (PharmD, Ph.D., Associate Professor with habilitation in the Faculty of Pharmacy of the University of Coimbra) and João José Martins Simões de Sousa (PharmD, Ph.D., Associate Professor with habilitation in the Faculty of Pharmacy of the University of Coimbra), presented to the Faculty of Pharmacy of the University of Coimbra.

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A todos vós o meu profundo agradecimento.

If I have seen further than others, it is by standing upon the shoulders of giants.

Isaac Newton

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List of Abbreviations

AA	Amino Acid
AAS	Atomic Absorption Spectroscopy
ACN	Acetonitrile Nonconforming Copolymer
AFFF	Asymmetric Field-Flow Fractionation
AFM	Atomic Force Microscopy
AIDS	Acquired Immunodeficiency Syndrome
ALL	Acute Lymphoblastic Leukemia
AMD	Age-Related Macular Degeneration
AML	Acute Myeloid Leukemia
AML-MRC	Acute Myeloid Leukemia With Myelodysplasia-Related Changes
ANDA	Abbreviated New Drug Application
ANOVA	Analysis of Variance
APC	Antigen-Presenting Cell
API	Active Pharmaceutical Ingredient
ASA	Acetylsalicylic Acid
ASO	Antisense Oligonucleotide
ASTM	American Society for Testing and Materials
AT	Austria
ATM-FTIR	Attenuated Total Reflection-Fourier Transform Infrared
AUC	Area Under the Curve
BBB	Blood-Brain Barrier
BCPI	Biologics Price Competition and Innovation Act
BCS	Biopharmaceutics Classification System
BDNF	Brain-Derived Neurotrophic Factor
BE	Belgium
BEq	Bioequivalence
BEWGG	Bioequivalence Working Group for Generics
BG	Bulgaria
BLA	Biologics License Application
BPCIA	Biologics Price Competition and Innovation Act
BPF	Bilayer Phospholipid Fragments
BPH	Benign Prostatic Hyperplasia
BsUFA	Biosimilar User Fee Act
BW	Body Weight
BWG	Biosimilars Working Group
CBA	Cell-Based Assay
CBB	Coomassie Brilliant Blue
CD	Circular Dichroism
CDER	Center for Drug Evaluation and Research
CEX	Cation Exchange Chromatography
CFR	Code of Federal Regulations
cGMPs	Current Good Manufacturing Practices
CHMP	Committee For Medicinal Products For Human Use
cIEF	Capillary Isoelectric Focusing Electrophoresis
CKD	Chronic Kidney Disease
CLSM	Confocal Laser Scanning Microscopy

CMA	Critical Material Attribute
Cmax	Maximum (or peak) serum concentration
CMC	Chemistry, Manufacturing, and Controls
CMDh	Coordination Group for Mutual Recognition and Decentralized Procedures - Human
CMS	Concerned Member State
CNPq	National Council for Scientific and Technological Development
CNS	Central Nervous System
CP	Centralized Procedure
CPI	Critical Path Initiative
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
CRCG	Center for Research on Complex Generics
CTD	Common Technical Document
CTWG	Cell Therapy Working Group
CY	Cyprus
CZ	Czech Republic
Da	Dalton
DCAP	Drug Competition Action Plan
DCP	Decentralized Procedure
DE	Germany
DK	Denmark
DLS	Dynamic Light Scattering
DMTs	Disease-Modifying Treatments
DNA	Deoxyribonucleic Acid
DoE	Design of Experiments
DP	Drug Product
DS	Drug Substance
DSC	Differential Scanning Calorimetry
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DSPE	1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine
DVT	Deep Vein Thrombosis
EAE	Experimental Autoimmune Encephalomyelitis
EDQM	European Directorate for the Quality of Medicines and HealthCare
EDTA	Ethylenediaminetetraacetic Acid
EDX	Energy-Dispersive X-Ray Spectroscopy
EE	Estonia
%EE	Encapsulation Efficiency
EEA	European Economic Area
EFTA	European Free Trade Association States
EL	Greece
ELISA	Enzyme-Linked Immunosorbent Assay
ELS	Electrophoretic Light Scattering
ELSD	Evaporative Light Scattering Detector
EMA	European Medicines Agency
EMR	Electron Magnetic Resonance Spectroscopy
EPAR	European Public Assessment Report
EPO	Erythropoietin

EPR	Enhanced Permeability and Retention Effect
EPRS	Electron Paramagnetic Resonance Spectroscopy
ES	Spain
ESEM	Environmental Scanning Electron Microscopy
ESMO	European Society for Medical Oncology
ESRD	End-Stage Renal Disease
EU	European Union
EXAFS	Extended X-Ray Absorption Fine Structure
FAPESP	São Paulo Research Foundation
FDA	U.S. Food And Drug Administration
FDC	Fixed-Dose Combination
FFDCA	Federal Food, Drug, and Cosmetic Act
FFF	Field-Flow Fractionation
FI	Finland
FID	Flame-Ionization Detection
FMEA	Failure Mode Effect Analysis
FMECA	Failure Mode, Effects, and Criticality Analysis
FOGA	Follow-On Glatiramer Acetate
FR	France
FTA	Fault Tree Analysis
FTIR	Fourier Transform Infrared Spectroscopy
GA	Glatiramer Acetate
GALA	Glatiramer Acetate Low-Frequency Administration
GAO	United States Government Accountability Office
GATE	Glatiramer Acetate Clinical Trial to Assess Equivalence with Copaxone®
GC	Gas Chromatography
GC-FID	Gas Chromatography with Flame Ionization Detection
GCRSR	Global Coalition for Regulatory Science Research
GDUFA	Generic Drug User Fee Act
GEICO	Spanish Ovarian Cancer Research Group
GI	Gastrointestinal
GPC	Gel Permeation Chromatography
GRAS	Generally Recognized As Safe
GSRS	Global Summit on Regulatory Science
GTR	Generic Glatiramer Acetate
GTWG	Gene Therapy Working Group
HACCP	Hazard Analysis and Critical Control Points
hATTR	Hereditary Transthyretin-Mediated Amyloidosis
HAZOP	Hazard Operability Analysis
HCl	Hydrochloride
HILIC	Hydrophilic Interaction Liquid Chromatography
HIV	Human Immunodeficiency Viruses
HLB	Hydrophilic-Lipophilic Balance
HMA	Heads of Medicines Agency
HMWID	High Molecular Weight Iron Dextran
HPH	High-Pressure Homogenization
HPLC	High Performance Liquid Chromatography
HR	Croatia

HSPC	Fully Hydrogenated Soy Phosphatidylcholine
HU	Hungary
IBD	Inflammatory Bowel Disease
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDMPWG	Identification of Medical Products Working Group
IE	Ireland
IFN	Interferon
IMMS	Ion Mobility Mass Spectrometry
IND	Investigational New Drug Application
INN	International Nonproprietary Name
IP	Intellectual Property
IPE	Individual Protection Equipment
IPRP	International Pharmaceutical Regulators Programme
IRIS	Regulatory & Scientific Information Management Platform
IS	Iceland
ISO	International Organization for Standardization
IT	Italy
ITZ	Itraconazole
IV	Intravenous
IVIVC	In Vitro-In Vivo Correlation
IVRT	In Vitro Release Test
IWG	Implementation Working Group
IWGG	Information Sharing for Generics Working Group
kDa	Kilodaltons
LAI	Long-Acting Injection
LAL	Limulus Amebocyte Lysate
LC-MS	Liquid Chromatography Coupled With Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem-Mass Spectrometry
LD	Laser Diffractometry
LDA	Laser Doppler Anemometry
LDH	Lactate Dehydrogenase
LEP-ETU	Liposome Entrapped Paclitaxel Easy to Use formulation
LMWH	Low-Molecular-Weight Heparin
LMWID	Low Molecular Weight Iron Dextran
LT	Lithuania
LTLD	Lyso-Thermosensitive Liposomal Doxorubicin
LU	Luxembourg
LV	Latvia
MAbs	Monoclonal Antibodies
MAH	Marketing-Authorization Holder
MALLS	Multi-Angle Laser Light Scattering
MAs	Material Attributes
MBP	Myelin Basic Protein
MHLW	Ministry of Health, Labour and Welfare
MLV	Multilamellar Vesicles
MMS	Mean Maximal Score Ratio

MPEG	Poly(ethylene glycol) methyl ether
MPS	Mononuclear Phagocyte System
MRI	Mutual Recognition Information Product Index
MRP	Mutual Recognition Procedure
MS	Mass Spectrometry
MSB	Magnetic Susceptibility Balance
MSCH	Mouse Spinal Cord Homogenate
MT	Malta
MTD	Maximum Tolerated Dose
MVDA	Multivariate Data Analysis
MW	Molecular Weight
MWCO	Molecular Weight Cut-Off
MWD	Molecular Weight Distribution
NaCl	Sodium Chloride
NBCD	Non-Biological Complex Drug
NBED	Nanobeam Electron Diffraction
NCAs	N-Carboxyanhydrides
NCE	New Chemical Entities
NCI	National Cancer Institute
NDA	New Drug Application
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIR	Near-Infrared Spectroscopy
NL	Netherlands
NLC	Nanostructured Lipid Carrier
nm	Nanometer
NMR	Nuclear Magnetic Resonance Spectroscopy
NO	Norway
NOR	Normal Operating Range
NP	National Procedure
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
NSOM	Near-field Scanning Optical Microscopy
NTA	Nanoparticle Tracking Analysis
NTBI	Non-Transferrin-Bound Iron
NWG	Nanomedicines Working Group
O/W	Oil-In-Water
OBP	Office of Biotechnology Products
OEL	Occupational Exposure Limit
OGD	Office of Generic Drugs
OND	Office of New Drugs
ONDQA	Office of New Drug Quality Assessment
OPQ	Office of Pharmaceutical Quality
ORP	Oxidation-Reduction Potential
PAbs	Polyclonal Antibodies
PALM	Post-Approval Lifecycle Management Plan
PAR	Proven Acceptable Range
PAT	Process Analytical Technology
PBS	Phosphate-Buffered Saline

PC	Phosphatidylcholine
PCS	Photon Correlation Spectroscopy
PD	Pharmacodynamics
PDA	Parenteral Drug Association
PDI	Polydispersity Index
PE	Pharmaceutical Equivalence
PEG	Polyethylene Glycol
Ph Eur.	European Pharmacopoeia
PHA	Preliminary Hazard Analysis
PHS Act	Public Health Service Act
PIL	Patient Information Leaflets
PK	Pharmacokinetics
PL	Poland
PLD	Pegylated Liposomal Doxorubicin
PLGA	Poly(DL-Lactic-Co-Glycolic Acid)
PLM	Polarized Light Microscopy
PM	Polymeric Micelle
PPMS	Primary Progressive Multiple Sclerosis
PPs	Process Parameters
PPV	Packed Particle Volume
PRMS	Progressive Relapsing Multiple Sclerosis
PSA	Parallel Scientific Advice
PSC	Polyglucose Sorbitol Carboxymethylether
PSD	Particle Size Distribution
PSG	Product-Specific Guidance
PSUR	Periodic Safety Update Report
PT	Portugal
PVWG	Pharmacovigilance Working Group
QbD	Quality by Design
QbR	Question-based Review
QC	Quality Control
Q-IWG	Quality-Implementation Working Group (Q-IWG)
QTPP	Quality Target Product Profile
QWGG	Quality for Generics Working Group
R&D	Research and Development
RA	Risk Assessment
REM	Risk Estimation Matrix
RES	Reticuloendothelial System
RI	Refractive Index
RLD	Reference Listed Drug
RMP	Risk Management Plan
RMS	Reference Member State
RNA	Ribonucleic Acid
RO	Romania
ROS	Reactive Oxygen Species
RPLC	Reverse Phase Liquid Chromatography
RPM	Revolutions Per Minute
RRMS	Relapsing-Remitting Forms Of Multiple Sclerosis

RTRT	Real Time Release Testing
SAED	Selected Area Electron Diffraction
SAXS	Small Angle X-ray Scattering
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SE	Sweden
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Scattering
SI	Slovenia
sIL-1Ra	Interleukin-1 Receptor Antagonist
siRNA	Small Interfering Ribonucleic Acid
SK	Slovakia
SLN	Solid Lipid Nanoparticle
SLS	Static Light Scattering
SmPC	Summary Of Product Characteristics
SOS	Double-Distilled Water Containing Sodium Oleate
SPMS	Secondary Progressive Multiple Sclerosis
SPOS	Single Particle Optical Sensing
SQUID	Superconducting Quantum Interference Device
SrLC	Drug Safety-related Labeling Changes
STEMI	ST-Segment Elevation Myocardial Infarction
STM	Scanning Tunneling Microscopy
SUV	Small Unilamellar Vesicles
SV-AUC	Sedimentation Velocity Analytical Ultracentrifugation
t-AML	Therapy-Related Acute Myeloid Leukemia
TBI	Transferrin-Bound Iron
TDI	Total Dose Infusion
TE	Therapeutic Equivalence
TEM	Transmission Electron Microscopy
TERS	Tip-Enhanced Raman Spectroscopy
TFF	Tangential Flow Filtration
Tg	Glass Transition Temperature
TGA	Thermal Gravimetric Analysis
Th1	T Helper Type 1 Cell
Th2	T Helper Type 2 Cell
TI	Total Iron
TLC	Thin Layer Chromatography
TMP	Transmembrane Pressure
TTR	Transthyretin
UC	Ulcerative Colitis
UK	United Kingdom
ULV	Unilamellar Vesicle
UPLC	Ultra Performance Liquid Chromatography
URT	Ultrasonic Resonator Technology
US	United States
USD	United States Dollar
USP	United States Pharmacopeia
UV	Ultraviolet

UV/VIS	Ultraviolet–Visible
VSM	Vibrating Sample Magnetometer
W/O	Water-In-Oil
WAXD	Wide-Angle X-Ray Scattering
WFI	Water for Injection
WHO	World Health Organization
XANES	X-Ray Absorption Near-Edge Structure
XRD	X-Ray Diffractometry

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List of Publications

Scientific Articles published from the work presented in this thesis:

Review Article: 'A Quality by Design (QbD) Approach in Pharmaceutical Development of Lipid-based Nanosystems: A Systematic Review'. Journal of Drug Delivery Science and Technology. April 2022. Volume 70, 103207. DOI: https://doi.org/10.1016/j.jddst.2022.103207.

Review Article: 'Quality by Design (QbD) Approach in Marketing Authorization Procedures of Non-Biological Complex Drugs: A Critical Evaluation'. European Journal of Pharmaceutics and Biopharmaceutics: Under Review.

Review Article: 'Regulatory Science Approach in Pharmaceutical Development of Follow-On Versions of Non-Biological Complex Drug Products'. Journal of Pharmaceutical Sciences: Under Review.

Poster/Oral communication:

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Presentations:

Presentation of Seminar I included in the plan of doctoral studies: entitled 'QbD Approach to Advance Lipid Based Nanoparticles Product and Process Understanding' and presented at the Faculty of Pharmacy of the University of Coimbra.
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Presentation of Seminar II included in the plan of doctoral studies: entitled 'Regulatory Science Approach in Pharmaceutical Development of Non-Biological Complex Drug Products' and presented at the Faculty of Pharmacy of the University of Coimbra.
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Abstract

The major scientific and technological advances in the field of Nanotechnology in the last decades have contributed to the emergence of more increasingly complex drug products. Currently, a great number of nanotechnology-based products correspond to the class of Non-Biological Complex Drugs (NBCDs). Alongside the fast-growing market of NBCDs, nowadays also exist a rising interest in the development of their complex generic drug products, due to the many advantages they bring to cost-saving for patients and health care services, as well as, the competitive benefits and attractive business models to generic developers.

The NBCDs have been defined in the scientific literature as complex drug products, where the active substance, does not have a homo-molecular structure, corresponds to different closely related structures that cannot be isolated and fully quantitated, characterized, and described by available physicochemical analytical means. The NBCDs include a wide group of medicinal products such as micelles, nanoemulsions, iron-carbohydrate complexes, polymers, liposomes, transferosomes, dendrimers, nanoparticles, glatiramoids, or other products intimately related to nanoparticular structure and properties, upon which the composition, quality, and in vivo performance are highly dependent on the manufacturing process.

The heterogeneity, diversity, and unique characteristics of NBCDs provide serious challenges to the pharmaceutical development and regulatory approval of complex generic drug products. In line with the principle 'The Product is the Process', any change in the manufacturing process or formulation, even though it is small, can bring variations in quality, efficacy, and safety properties of the final drug product. This can be a problem, especially to ensure both reproducibility and batch-to-batch consistency of reference drug products and their complex generics. For some classes of NBCDs, the structure-function relation or mechanism of action has not yet been fully described, as well as, the completeness characterization of their physicochemical and structural properties. The lack of critical quality attributes (CQA) assessment, makes it impossible to demonstrate therapeutic equivalence and consequently hamper the approval and market access of complex generics. On the other hand, the regulatory basis for NBCDs is much more unclear and extensively unavailable compared to the biological complex drugs, not existing a distinct and dedicated regulatory pathway for the approval of their generic versions. Therefore, the lack of an adequate and effective approach for each type of NBCDs leads to a wide diversity of regulatory landscapes throughout Europe and the United States.

The main goal of this thesis is to deeply investigate, discuss and provide insight into the pharmaceutical development of complex generic drug products, identifying points of consensus and outstanding potential challenges for current regulatory frameworks. The key issue to be discussed in this context will be what is the best possible way to place the generic versions of NBCDs in the current regulatory system, seeking to balance the burden of scientific proof with an efficient

marketing authorization procedure. Thus, a reflection will be developed about the importance of new developments and strategies in regulatory science and science-based multi-stakeholder interactions to stimulate the rethinking of regulatory pathways and identify the needs for global harmonization of evaluation procedures of safety, quality, and therapeutic performance of complex generic drug products. A higher level of knowledge and understanding regarding these strategies will have a definite contribution to overcoming more easily the countless scientific and regulatory challenges associated with the development of these very complex pharmaceutical products, reducing the risk of development failure and increasing the probability of generic complex drug products to reaching the market in the near future, with a significant clinical and economic impact on the health care systems worldwide. Lastly, this thesis will attempt to provide a substantial contribution to tackling the complexity of generic drug development, underlining the need for regulatory science to keep pace with innovation in the NBCDs field.

Keywords

Non-Biological Complex Drugs; Complex Generic Drug Products; Therapeutic Equivalence; Regulatory Science; Global Regulatory Harmonization.

Resumo

Os grandes avanços científicos e tecnológicos no campo da Nanotecnologia nas últimas décadas têm contribuído para o surgimento de medicamentos cada vez mais complexos. Atualmente, um número crescente de produtos resultantes da Nanotecnologia corresponde à classe de Medicamentos Complexos Não Biológicos (NBCDs). Paralelamente à rápida expansão do mercado de NBCDs, existe igualmente um interesse crescente no desenvolvimento de suas versões genéricas, devido às inúmeras vantagens que oferecem na redução de custos para pacientes e serviços de saúde, bem como os benefícios competitivos e modelos de negócios atrativos para os fabricantes de medicamentos genéricos.

Os NBCDs foram definidos na literatura científica como medicamentos complexos, em que a substância ativa, não sendo uma estrutura homomolecular, corresponde a diferentes estruturas intimamente relacionadas que não podem ser isoladas e totalmente quantificadas, caracterizadas e descritas através das metodologias analíticas físico-químicas disponíveis. Os NBCDs incluem uma ampla gama de produtos, como micelas, nanoemulsões, complexos de ferro, polímeros, lipossomas, transferossomas, dendrímeros, nanopartículas, complexos de acetato de glatirâmero, ou outros produtos intimamente relacionados à estrutura e propriedades nanoparticulares, nas quais a sua composição, qualidade e desempenho in vivo são altamente dependentes do processo de produção.

A heterogeneidade, diversidade, e características únicas dos NBCDs providenciam importantes desafios para o desenvolvimento farmacêutico e aprovação regulamentar de medicamentos genéricos complexos. Segundo o princípio 'O Produto é o Processo', qualquer alteração no processo de produção ou formulação, mesmo que pequena, pode originar alterações nas propriedades de qualidade, eficácia ou segurança do produto final. Isto pode constituir um problema, especialmente para assegurar a reprodutibilidade e a consistência de lote para lote de medicamentos complexos, bem como dos respetivos genéricos complexos. Para algumas classes de NBCDs, a relação estrutura-função ou mecanismo de ação ainda não foram totalmente descritos, bem como a caracterização completa das propriedades físico-químicas e estruturais. A falta de avaliação e descrição dos atributos críticos de qualidade (CQAs), impossibilita a demonstração da equivalência terapêutica e, consequentemente, dificulta a aprovação e o acesso ao mercado de genéricos complexos. Por outro lado, a base regulamentar para os NBCDs é pouco clara e extensivamente indisponível comparativamente com os medicamentos complexos biológicos, não existindo um caminho regulatório distinto e dedicado para a aprovação de suas versões genéricas. Portanto, a falta de uma abordagem adequada e eficaz para cada tipo de NBCDs origina uma ampla diversidade regulamentar em toda a Europa e nos Estados Unidos.

Esta tese tem como principal objetivo investigar, discutir e analisar extensivamente o desenvolvimento farmacêutico de medicamentos genéricos complexos, identificando pontos de consenso e desafios potenciais das estruturas regulatórias atuais. A questão-chave a ser discutida

neste contexto será qual a melhor forma de inserir as versões genéricas dos NBCDs no sistema regulamentar disponível, procurando um equilíbrio entre a exigência da comprovação científica e um procedimento de autorização de introdução no mercado eficiente. Assim, será desenvolvida uma reflexão sobre a importância de novos desenvolvimentos e estratégias de ciência regulamentar e interações multissetoriais baseadas na ciência para estimular o repensar das abordagens regulamentares e identificar as necessidades de harmonização global dos procedimentos de avaliação da segurança, qualidade e desempenho terapêutico de medicamentos genéricos complexos. Um maior nível de conhecimento e compreensão sobre essas estratégias terá uma contribuição decisiva para superar mais facilmente os inúmeros desafios científicos e regulatórios associados ao desenvolvimento de produtos farmacêuticos complexos, reduzindo o risco de falha no desenvolvimento e aumentando a probabilidade de mais produtos chegarem ao mercado num futuro próximo, com significativo impacto clínico e económico nos sistemas de saúde em todo o mundo. Concluindo, esta tese tentará fornecer uma contribuição significativa para enfrentar a complexidade do desenvolvimento de medicamentos genéricos, destacando a necessidade da ciência regulamentar acompanhar a inovação na área dos NBCDs.

Palavras-chave Medicamentos Complexos Não-Biológicos; Medicamentos Genéricos Complexos; Equivalência Terapêutica; Ciência Regulamentar; Harmonização Regulamentar Global.

Outline of the Thesis

The current dissertation is divided into the following chapters:

Chapter I: *General Introduction: Review Analysis of Non-Biological Complex Drug Products.*

The first chapter briefly introduces the concept of Non-Biological Complex Drug Products (NBCDs), highlighting the main points for the reader's contextualization. It discusses the current technology and scientific trends in the pharmaceutical industry fostering the advancement of innovative medicines, such as Nanotechnology-based products. Several basic insights are introduced, from the definition of complex drug products, a general description of the characteristics of Small Molecule Drugs compared to complex drug products, just as the categorization of different NBCD-families that have been dealt with in the succeeding chapters. Moreover, this chapter was also presented a systematic analysis surrounding the NBCDs approved by the U.S. Food and Drug Administration and European Medicines Agency, as well as the NBCDs that being tested at the clinical level. All information contained in this chapter served as the basis for the remaining chapters.

Chapter II: *Generic Complex Drug Products: Challenges in Pharmaceutical Development and Marketing Approval.*

The diverse nature and variety of complex drug products, such as the Non-Biological Complex Drug Products (NBCDs), provide significant issues for the pharmaceutical development and marketing approval of the reference products and their generic versions (also referred to as follow-on products). Chapter II contributes to the discussion and knowledge about the current scientific and regulatory challenges related to the development of complex generic drug products. Thus, the emerging trends and specific challenges related to their complexity, interchangeability, therapeutic equivalence, heterogeneity in the regulatory approaches adopted by each regulatory authority, complex manufacturing process, sterilizing filtration, scalability issues, and the translation towards clinical applications were critically discussed. Another aim of this chapter includes a brief discussion of the reflection papers and guidance documents published by the regulatory authorities, which may be related or applied to the pharmaceutical development of NBCDs and their follow-on versions. Knowing and understanding the principles and recommendations included in the guidance documents constitute a powerful lever for the beginning of pharmaceutical development of each type of NBCDs, establishing the science-based regulatory approaches, and making the review of regulatory submissions more effective.

Chapter III: *The Regulatory Landscape of Non-Biological Complex Drug Products from the EMA and US-FDA Perspective.*

One of the main challenges identified in Chapter II corresponds to the difficulty to place the Non-Biological Complex Drugs (NBCDs) in an efficient regulatory system with adequate scientific substantiation that would allow the approval of high-quality, safe, and efficacy drug products. Thus, Chapter III deeply investigates insight into the pharmaceutical legislation and regulatory landscape of NBCDs and follow-on versions currently adopted by the regulatory authorities. It provides several examples of regulatory uncertainty and disparities in the existing legislative framework, specifically in assessing the pharmaceutical development and therapeutic equivalence of NBCDs products.

Chapter IV: *Pharmaceutical Quality by Design (QbD): A Strategic Approach to Risk Management and Regulatory Compliance.*

I. *Quality by Design (QbD) Approach in Marketing Authorization Procedures of Non-Biological Complex Drugs: A Critical Evaluation.*

The main purpose of Chapter IV(I) is to introduce the quality-by-design (QbD) principles and provide an overview of the QbD implementation in the development and approval procedures of Non-biological Complex Drugs (NBCDs) in the Europe and United States, through the analysis of the available data from their regulatory dossiers. Additionally, it aims to understand and discuss in what way the QbD approach is established and operated by the Pharmaceutical Industry for complex drug products, as well as, highlight the gaps and challenges related to the implementation of this approach. The advantages and disadvantages of the QbD approach concerning the regulatory flexibility, product quality assessment, or the possible reasons for market withdrawal are also be addressed.

II. *A Quality by Design (QbD) Approach in Pharmaceutical Development of Non-Biological Complex Drug Products: A Systematic Review.*

Chapter IV(II) aims to map and provide a basic understanding of the current state of implementation of the QbD approach in the pharmaceutical development of Complex Drug Products, through the analysis of the existing literature (survey methodology: 118 scientific articles) and databases regarding Complex Drug Products already approved by the regulatory authorities. This systematic analysis discloses the most common material attributes, process parameters, quality attributes, and other variables that are critical for the quality, efficacy, and safety of Complex Drug

Products. On the other hand, include the analysis of current trends of risk assessment tools, design of experiments (DoE) methodologies, and characterization techniques applied to the development of these products. This higher level of knowledge will have a definite contribution to overcoming the gaps related to the QbD implementation identified in the previous section (Chapter IV(I)).

Chapter V: *Case Study I: Generic Development of Iron-Carbohydrate Complexes: Regulatory and Scientific Considerations.*

Chapter V describes the regulatory challenges involved in the marketing authorization of the iron-carbohydrate complexes and the absence of harmonization in the assessment of therapeutic equivalence. In general, the main issues addressed in this chapter are related to: the definition of iron-carbohydrate complexes; an overview of physicochemical and clinical characteristics of IV iron-carbohydrate complexes; the implications of their complexity in bioequivalence evaluation; the regulatory landscape of iron-carbohydrate complexes approved in Europe and the United States; comparative evaluation of the FDA and EMA requirements for the demonstration of therapeutic equivalence; physicochemical, non-clinical, and clinical characterization; and corresponding analytical techniques.

Chapter VI: *Case Study II: Generic Development of Glatiramer Acetate Complex Products: Regulatory and Scientific Considerations.*

Chapter VI provides an overview of the regulatory landscape of glatiramer acetate complex products approved in Europe (EU) and the United States (US), and outlines the regulatory challenges to establishing therapeutic equivalence of their follow-on versions. Also, it aims to highlight issues related to their classification, complexity, pharmaceutical quality, clinical efficacy, safety, and tolerability profiles, which may be helpful for the re-examination and optimization of the approval pathways for these complex generic drug products. On the other hand, it also addresses the comparative evaluation of FDA and EMA requirements for the demonstration of therapeutic equivalence, as well as, the appropriate analytical techniques to perform the physicochemical, non-clinical, and clinical characterization of glatiramer acetate complex products. Ultimately, discuss possible future directions of harmonization to the approval pathways for the assessment of therapeutic equivalence between the reference products and their follow-on versions.

Chapter VII: *Case Study III: Generic Development of Complex Injectable Liposomal Formulation: From the Bench to Approved Drug Products.*

Chapter VII describes the development and optimization of complex generic injectable liposomal formulation through the application of Quality by Design (QbD) principles. Slight variations in physicochemical properties resulting from the manufacturing process of liposomal formulations can impact the particle size distribution, encapsulation efficiency, stability under physiological conditions, and drug release at the target tissue, among many other critical attributes. This chapter has focused particularly on the control and optimization of the particle size distribution since the earliest stages of the manufacturing process of doxorubicin hydrochloride liposomal drug products, such as the preparation of Multilamellar Vesicles (MLVs) by Ethanol Injection Method and the liposomes size reduction and formation of Unilamellar Vesicles (ULVs) by the High-Pressure Extrusion Method. Some of the main findings of the scientific work performed are reported in this chapter.

The galenical development work undertaken includes the following objectives: patent landscape and literature review of liposome injection for intravenous infusion; development of an adequate formulation (qualitative and quantitative) based on the reference product characteristics; development of an adequate manufacturing process; development following the QbD principles with the definition of Quality Target Product Profile (QTPP); identification of Critical Quality Attributes (CQAs); use of risk assessment tools to identify potential Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs); investigation of the effect and relationship of material attributes and process parameters on the CQAs through Design of Experiments (DoE). This chapter also considered issues related to the future direction of the experimental work, such as the definition of the design space, as well as, the performance of pre-stability studies with the most promising prototype.

Chapter VIII: *Strengthening Regulatory Science Research in Pharmaceutical Development of Non-Biological Complex Drug Products.*

Chapter VIII intends to identify the needs and priorities for global harmonization of evaluation procedures between regulatory authorities in different places worldwide, as well as, demonstrate the importance of regulatory science research and science-based multi-stakeholder interactions to stimulate the rethinking of regulatory pathways. This chapter also focuses on the role and importance of scientific advice among regulatory authorities and developers for the submission and approval procedures of complex drug products. The latest Parallel Scientific Advice (PSA) program plays a significant contribution to increasing the clarity of regulatory approaches for complex

generics, improving patient access to essential and more affordable drug products, and therefore promoting the sustainability of the healthcare system.

Chapter IX: *Concluding Remarks and Future Perspectives.*

Chapter IX summarizes the major findings of this thesis and discusses the future perspectives of the pharmaceutical development of NBCDs and their follow-on versions. Some observations related to the regulatory science framework for NBCDs are expressed in this chapter.

Chapter I. General Introduction: Review Analysis of Non-Biological Complex Drug Products

Abstract

Over the last few decades, the advanced scientific insights into the Nanotechnology field led to the development of innovative therapeutics becoming increasingly complex.

An important class of these complex drug products corresponds to Non-Biological Complex Drugs (NBCDs), that as its name suggests, don't fall in the category of biological complex drug products as they are not derived from living materials. These products can be defined as a synthetic medicinal products with an active substance that is not homo-molecular but contains different (closely related and often nanoparticulate) structures that cannot be fully quantitated, characterized and/or described by physicochemical analytical means. The NBCDs are dependent upon a well-controlled robust manufacturing process, so that slight variations in this process can substantially change the quality, safety, and efficacy profile of the final drug product. Some examples of NBCDs include micelles, nanoemulsions, iron-carbohydrate complexes, polymers, liposomes, transferosomes, dendrimers, nanoparticles, glatiramoids, among others.

The present chapter is designed to introduce the concept of Non-Biological Complex Drugs (NBCDs), highlighting the main points for the reader's contextualization. Moreover, this chapter was also presented a systematic analysis surrounding the NBCDs approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), as well as the NBCDs that being tested at the clinical level, which will serve as a basis for further discussions that have been dealt in the succeeding chapters.

Keywords

Pharmaceutical Development; Pharmaceutical Technology; Nanotechnology; Innovative Medicines; Nanotechnology-based Products; Nanomedicines; Nanomaterials; Differentiated Technologies; Drug Repurposing; Complex Drug Products; Non-Biological Complex Drugs; U.S. Food and Drug Administration; European Medicines Agency; Clinical Trials.

1. Introduction

Over the last decades, there has been impressive progress in the transition of pharmaceutical development from conventional to novel advanced therapeutics through the use of Nanotechnology.

Nanotechnology is an emerging, dynamic and innovative technology that plays a key role in the design, development, and manufacture of the new generation of life-saving pharmaceutical products. This technology comprises a general term that covers a wide range of drug products, including biologics, medical devices, and pharmaceuticals, that have very different characteristics and functionalities depending on a design principle, formulation, manufacturing process, structure, function, shape, charge, or other physical or chemical properties [1,2]. The development of sophisticated therapeutics in the medical field offers relevant improvements and significant opportunities to attend to the unmet therapeutic needs of patients and the healthcare system. Thus, this technology presents numerous applications in the diagnosis, treatment, and prevention of different diseases [3–5].

Nanotechnology-based products (also known as nanomedicines) can comprise complex multifunctional conjugates with specific molecules dissolved, encapsulated, or adsorbed to their surface (e.g. coatings, drug delivery/targeting molecules, therapeutic agents, prodrugs, contrast agents, imaging agents, or tracking moieties) [2,6–9]. In accordance with the U.S. Food and Drug Administration (FDA) Guidance for Industry 'Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology' and 'Drug Products, Including Biological Products, that Contain Nanomaterials', the particle size may range from 1 to 100 nanometer (nm) that exhibits distinctive chemical or physical properties, with a significant impact in the bioavailability and targeting to different sites within the body [1,10]. Additionally, the National Nanotechnology Initiative Program defines Nanotechnology as 'the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications' [11]. However, it should also be recognized that vesicles with upper dimensions are also sometimes considered (e.g. nanoscale range up to 1000 nm) [2,6,8,9].

The majority of conventional drug products provide an immediate and high release after drug administration, which can result in an increased dosage frequency [12]. Moreover, it has been widely known that conventional drugs present considerable drawbacks in the treatment of some diseases and should be urgently replaced by other technologies. Thus, several drug products containing nanomaterials are already in the phase of clinical development or have been approved in several therapeutic areas worldwide [12]. These systems have been extensively investigated to improve some issues relating to their quality, safety, efficacy, and cost-effectiveness, through the handling of the biopharmaceutical and pharmacokinetic properties. Therewith, Nanotechnology has become a rapidly growing field with potential advantages attributed to its physicochemical properties, such as the improvement of site-specific delivery, drug targeting, controlled release,

bioavailability, potency, and the effectivity of drug products [1,3–5]. The nanotechnology-based products enable the drug release and distribution to be controlled according to the site of action, increasing the therapeutical effectiveness and minimizing potential adverse effects. This is partly due to the possibility of functionalization of the nanosystem surface through receptor-specific ligands (e.g. peptides, antibodies, and small molecules), the modulation of particle size distribution and surface charge, or their large surface/volume ratio that promote the simultaneous interaction with a great variety of molecules [13]. Thus, the development of drug products at the nano-scale offers new opportunities for interaction with biological systems due to their distinct physicochemical characteristics.

On the other hand, the need to overcome the limitations of susceptible drugs, namely low solubility, permeability, and drug stability, poor pharmacokinetics, non-specific distribution, or potential toxicological effects in the case of cytotoxic drugs, was also the main driving force behind this development [3–5,12]. Through the application of nanotechnology-based products, the cytotoxic drugs or other with high toxicity can be effortlessly encapsulated and distributed in a specific tissue, without compromising the surrounding healthy tissues and causing undesirable or harmful effects. This plays an important role since it allows a more targeted, precisely, effective, and personalized therapeutic, with high toxicity drugs that would not be used otherwise [12,14].

The emergence of more sophisticated and breakthrough technologies, like Nanotechnology, has also facilitated the development and implementation of drug repurposing strategies. These strategies comprise the use of existing medicines (including approved, discontinued, or experimental drugs) for establishing a new technological platform, different formulations, or finding new therapeutic uses, providing value-added solutions in drug development (Figure 1) [15]. Contrary to the traditional drug discovery process, repurposing already known drugs present multiple advantages in the treatment of both common or rare, neglected, and particularly difficult to treat diseases, such as: reducing development timelines and costs, maximizing the therapeutic value of an existing drug, and mitigating the risk of failure in the development and approval procedures. It is an alternative and highly efficient approach, often requiring a lower investment by developers/pharmaceutical industry [15].

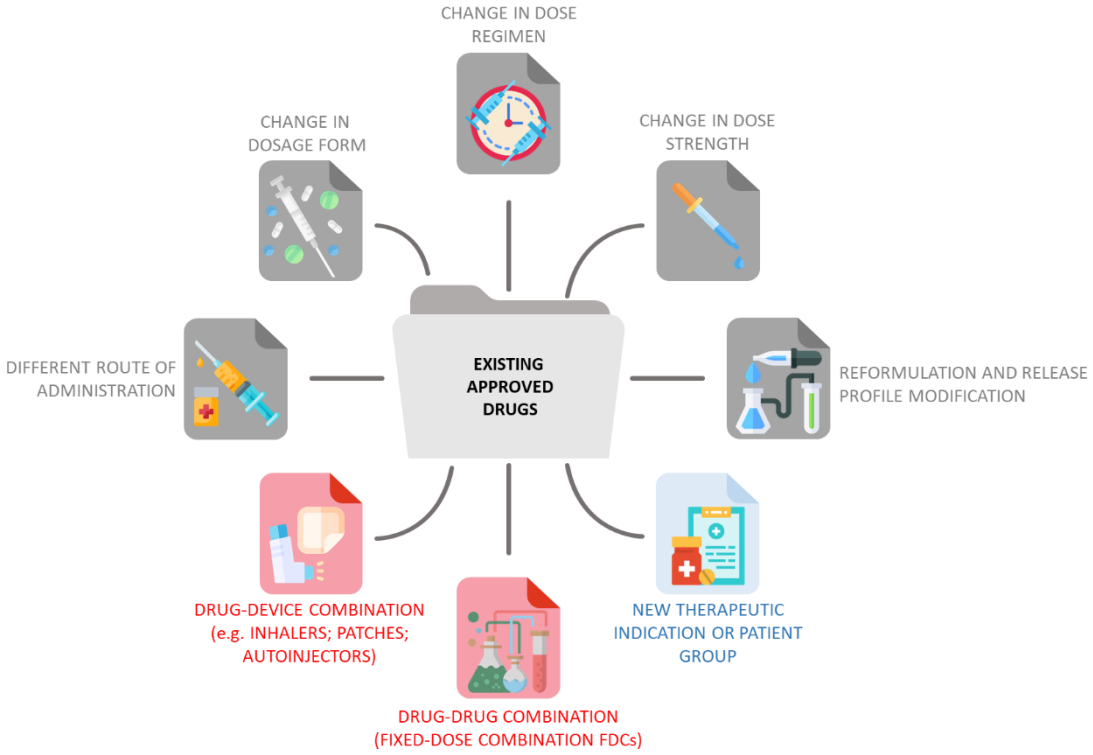

Figure 1. Drug repurposing strategies and types of value-add medicines. Each strategy is represented by a color: Drug Repositioning (blue): development of a drug product with a new therapeutic indication or patient group of an already known drug; Drug Reformulation (grey): development of different formulations (different routes of administration, dosage form, dose regimen, dose strength, drug release) for the same pharmaceutical drug; and Complex Combinations (red): development of novel drug-drug or drug-device combination products of existing drugs.

1.1. Non-Biological Complex Drugs (NBCDs)

Novel advanced therapeutics are becoming ever more complex as a result of the increasing use of Nanotechnology. A growing number of nanotechnology-based products correspond to the class of Complex Drug Products. The FDA in the 'Generic Drug User Fee Act (GDUFA) II Commitment Letter' describes 'complex products' more broadly, such as:

'1. Products with complex active ingredients (e.g., peptides, polymeric compounds, complex mixtures of [active pharmaceutical ingredients], naturally sourced ingredients); complex formulations (e.g., liposomes, colloids); complex routes of delivery (e.g., locally acting drugs such as dermatological products and complex ophthalmological products and otic dosage forms that are formulated as suspensions, emulsions, or gels); or complex dosage forms (e.g., transdermals, metered-dose inhalers, extended-release injectables);

2. Complex drug-device combination products (e.g., auto-injectors, metered dose inhalers);

3. Other products where complexity or uncertainty concerning the approval pathway or possible alternative approach would benefit from early scientific engagement' [16].

For the purpose of this thesis, the distinct category of Complex Drug Products could be organized into two broad categories: Biological Complex Drugs (Biologics or Biologicals) and Non-Biological Complex Drugs (NBCDs) [17–20]. The general characteristics of these classes are briefly described in the following table (Table 1) [21–25].

Table 1. Overview of the general characteristics of Small Molecule Drugs compared to Complex Drug Products (including Biological Complex Drugs and Non-Biological Complex Drugs) (adapted from [21–25]).

	CONVENTIONAL DRUGS	**COMPLEX DRUG PRODUCTS**	
Category	**Small Molecule Drugs**	**Biological Complex Drugs**	**Non-Biological Complex Drugs**
	e.g. Aspirin, acetylsalicylic acid (ASA)	e.g. Monoclonal antibody (Trastuzumab)	e.g. Polymeric nanoparticle
Size	Small (single molecule)	Large (mixture of related molecules)	Large (complex macromolecules or complex mixtures)
Molecular weight	Low molecular weight (<500 Da)	High molecular weight (5 – 900 kDa)	Variable
Structure	Relatively simple. Well-defined physicochemical properties. Easy to purify. Independent of manufacturing process.	Complex. Heterogeneous mixture. Difficult purification process. Defined by the exact manufacturing process.	
Modifications	Well-defined	Several options	
Production	Chemical synthesis	Living cell culture or organisms (e.g. bacteria, yeast)	Synthetic technologies (including Nanotechnology)
Manufacture	Completely characterized. Not affected by minor changes in the process.	Susceptible to subtle changes in the manufacturing process	
Stability	Generally stable. Predictable.	Generally unstable. Sensitive to external conditions. Aggregation.	
Immunogenicity/ Toxicity	Mostly non-immunogenic. Generally specific toxicity. Antigenicity is not often. Higher toxicity than complex drug products.	Mostly immunogenic. Antigenic.	Immunogenicity varies
Pharmaceutical identity	Fully characterizable	Not fully characterized	
Pharmacokinetics/ Route of Administration	Many routes of administration. The oral route is very usual. Rapidly diffuse across membranes.	Mostly parenterally. Reach circulation through the lymphatic system.	Variable
Pharmacodynamics	By binding to receptors (e.g. enzymes)	Alleviation of deficiencies. Alter physiological effects (e.g. enhance cellular immune responses).	Variable

		Specifically inhibit targets' activity (e.g. monoclonal antibodies).	
Clinical Requirements	Bioequivalence (pharmacokinetic) studies	Extensive clinical studies	Variable
Regulatory Framework	Automatic substitution is allowed. National and multinational approval procedures. Pharmacokinetic similarity (bioequivalence) implies therapeutic similarity.	Demonstrate biosimilarity and interchangeability. Automatic interchangeability is not allowed.	Automatic interchangeability is not allowed. Impossible to ensure identical copy versions.

The Biological Complex Drugs are defined by regulatory authorities as a medicine that contains active substances isolated from a variety of biological sources (human, animal, or microorganism), and are usually produced by advanced technologies [24,26–29]. The main components of this class are sugars, proteins, nucleic acids or complex combinations of them, or also living components like cells and tissues [24,26–29]. Thus, the Biological Complex Drugs include a wide range of products with high complexity, such as proteins, monoclonal antibodies, vaccines, blood and blood components, allergenic, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins [24,26–29].

Although there is a classification for Complex Drug Products based on several factors as described above, and specifically for the Biological Complex Drugs, regulatory authorities like the European Medicines Agency (EMA) and FDA, do not provide any official definition for NBCDs [16,18,19,22,30]. However, the term NBCDs has been extensively discussed in the scientific community. Crommelin *et al* defined NBCDs as:

'*A medicinal product, not being a biological medicine, where the active substance is not a homo-molecular structure, but consists of different (closely related and often nanoparticulate) structures that can't be isolated and fully quantitated, characterized, and/or described by physicochemical analytical means. It is also unknown which structural elements might impact the therapeutic performance. The composition, quality, and in vivo performance of NBCD are highly dependent on the manufacturing processes of both the active ingredient as well as the formulation'* [28]. Such a class of products contains mostly nanoparticulate structures with highly complex, multi-component, and multi-functional materials, giving rise to high variability in terms of size, shape, composition, and structure. This multitude of closely related structures with particular physicochemical properties can be substantially altered in the manufacturing process, with the consequent change in the quality, safety, and clinical performance of the product [17,22,24,31,32]. Thus, it is also possible to infer that the complexity of NBCDs is close to the biological products, and that subtle changes in nanoparticulate structure can impart drastic variations in its function [33].

In addition, the categorization of different types of NBCDs by regulatory authorities, scientific experts, and health care professionals is still far away from the expected. In the literature, the NBCDs include a wide group of medicinal products such as micelles, nanoemulsions, iron-carbohydrate complexes, liposomes, transferosomes, dendrimers, nanoparticles, glatiramoids, nanocrystals, or other products intimately related to nanoparticular structure and properties (Figure 2) [16,17,22,24,27,30]. A proper distinction between each class of NBCDs is critical to the implementation of an adequate and effective regulatory approach. However, while there are several guidelines and regulatory approaches successfully defined and established for Small Molecule Drugs and Biological Complex Drugs, the regulatory basis for NBCDs is much more unclear and extensively unavailable [25,34,35].

The first chapter of this thesis aims to map and provide an overview and basic understanding of the concept of Non-Biological Complex Drugs (NBCDs). The methodology applied relied on the thorough analysis of the existing literature and databases regarding NBCDs already approved by the FDA, EMA, and under clinical trials, in order to try to understand trends and future prospects of this type of complex drug product. Several key insights are introduced, such as: the analysis of the general characteristics and definition of complex drug products; definition of NBCDs; types of NBCDs; NBCDs approved in the United States; NBCDs approved in Europe; just as the NBCDs in Clinical Trials. The diversity of NBCDs described provides the background for the next chapters of this thesis.

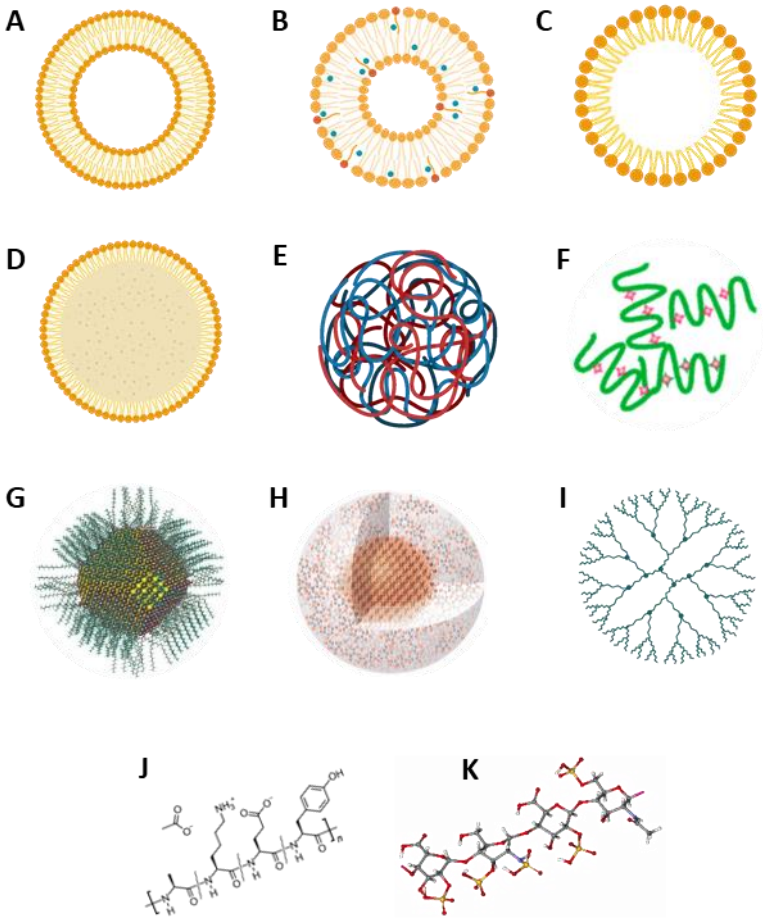

Figure 2. Illustration of the several types of Non-Biological Complex Drugs (NBCDs): [A - Liposome; B – Transferosome; C – Micelle; D – Nanoemulsion; E – Nanoparticle; F – Polymer-drug Conjugate; G – Nanocrystal; H – Iron-carbohydrate Complex; I – Dendrimer; J – Glatiramer Acetate Complex; K – Low Molecular Weight Heparin (LMWH)].

2. Methodology

This chapter provides an overview of NBCDs already approved by the U.S. Food and Drug Administration (FDA) (Section 3), the European Medicines Agency (EMA) (Section 4), and the NBCDs that are being tested at the clinical level (Section 5). To carry out this analysis, a general list of NBCDs already approved by the FDA, EMA, or in clinical trials was primarily outlined as a scientific basis for the Table 48, Table 49, and Table 50 respectively (see Appendix I. Supplementary Data).

The information included in Table 48 (see Appendix I. Supplementary Data) regarding the NBCDs already approved by the FDA, was collected from different databases. A first search was conducted using the platform Cortellis™ and query terms: '(Complex Drugs)', '(Liposome)', '(Transferosome)', '(Nanoparticle)', '(Nanoemulsion)', '(Micelle)', '(Polymer-drug Conjugate)', '(Dendrimer)', '(Glatiramoid)', '(Iron-carbohydrate Complex)', '(Nanocrystal)' and '(Low Molecular Weight Heparin (LMWH))'. Another search was conducted using the 'Drugs@FDA: FDA Approved Drug Products' database in the section 'New drug application (NDA)', with further analysis of the Drug Approval Package and FDA Application Review Files of each drug product, such as the Chemistry Review(s) [36–131]. Additionally, was analyzed recent scientific literature [5,132–139], but also a list published by the United States Government Accountability Office (GAO) for drugs that are simultaneously classified by the FDA as complex and non-biologic drug products [27]. Subsequently, Table 48 (see Appendix I. Supplementary Data) provides detailed information about the brand name (reference product), follow-on product, type of NBCDs, drug name, therapeutic indication, route of administration, dosage form, approval date, sponsor (company), regulatory pathway, application type, application number, submission classification, categories of drugs considered to be complex by the FDA, availability of information contained in each dossier (e.g. Chemistry Review), and the classification of non-QbD (Quality by Design) and QbD-developed products approved by the FDA.

To identify all medicinal products approved by the EMA, comprehensive bibliographic research was carried out through the analysis of the European Public Assessment Reports (EPARs) Database published on the EMA website; the book 'Non-biological complex drug: The science and regulatory landscape' [132]; and scientific literature [5,30,133,134,136,138,140–144]. Other search platforms applied are the Cortellis™, Heads of Medicines Agency (HMA) Mutual Recognition Information (MRI) product index, Summaries of Product Characteristics (SmPCs), Periodic Safety Update Reports (PSURs), Patient Information Leaflets (PIL), or EMA Human Medicines Highlights. Thus, Table 49 summarizes the essential aspects to be considered for the NBCDs already approved by the EMA, such as the brand name (reference product), follow-on product, type of NBCDs, drug name, therapeutic indication, route of administration, dosage form, authorization date, marketing authorization holder (MAH), authorization procedure, Reference Member State

(RMS) (if applicable), Concerned Member State (CMS) (if applicable), application procedure, and the classification of non-QbD and QbD-developed products.

The NBCDs highlighted in bold correspond to Reference products and the NBCDs underlined in gray are their follow-on versions (Table 48 and Table 49). It is important to emphasize that part of the information contained in Table 48 and Table 49 will be used only for the analysis performed in subsequent chapters (e.g. follow-on versions, regulatory landscape, or the implementation of the QbD approach).

By following the same principle in the previous segments, the clinical trials listed on the website ClinicalTrials.gov related to NBCDs were analyzed to identify trends and prospects for these types of complex drug products. A search was conducted using the query terms '(Liposomal Formulation)', '(Liposome)', '(Ethosomes)', '(Niosome)', '(Aspasome)', '(Transferosome)', '(Nanoemulsion)', '(Solid Lipid Nanoparticle)', '(Nanostructured Lipid Carrier)', '(Nanoparticle)', '(Lipid-Based Self-Nanoemulsifying)', '(Iron-Carbohydrate Complex)', '(Polymeric Micelle)', '(Polymer-Drug Conjugate)', '(Nanocrystal)', '(Dendrimers)', '(Low Molecular Weight Heparin (LMWH))', '(Glatiramer Acetate Complex)', and '(Glatiramoids)'. From the 124 studies initially retrieved, 82 were selected for further evaluation (Table 50). The 43 clinical trials excluded were not related to NBCDs. Table 50 represents various products undergoing clinical trial investigation and their classification in terms of the drug name, type of NBCDs, therapeutic regimen and indication, route of administration, authors' affiliation, study start date, and current development phase and status.

The critical discussion of the analysis of Table 48, Table 49, and Table 50 is present in the following sections.

3. An overview of Non-Biological Complex Drugs (NBCDs) approved in the US

3.1. Analysis by Type of Drug

The discovery of new chemical entities (NCE) with a potential therapeutic action is a time-consuming, expensive and challenging process [133,145]. Furthermore, a wide number of NCE do not achieve the clinical development phases before market authorization, due to the several issues related to their low solubility, permeability, and bioavailability, or lack of efficacy and safety [133,145].

According to the Biopharmaceutics Classification System (BCS), the active pharmaceutical ingredients (API) are classified into four categories depending on their solubility and permeability properties: class I presents higher solubility and permeability; class II has lower solubility and higher permeability; class III exhibits higher solubility and less permeability; and finally, the class IV that representing lower solubility and permeability [145–147]. Thus, the application of Nanotechnology is considered a prominent and challenging approach specifically designed for the encapsulation of a number of therapeutics agents (BCS class II and IV compounds) into the NBCDs [145,146]. Some examples of drug substances BCS class II are shown in Figure 3 as: nabilone (n=1, 2%), propofol (n=1, 2%), daunorubicin (n=1, 2%), fenofibrate (n=2, 4%) and paliperidone palmitate (n=2, 4%). On the other hand, some drug substances classified as BCS class IV corresponds to: amphotericin B with maximum number of applications (n=3, 6%), followed by cyclosporine (n=2, 4%), docetaxel (n=1, 2%), aprepitant (n=1, 2%) and paclitaxel (n=1, 2%) (Figure 3).

In addition, most anti-neoplastic agents are highly cytotoxic and present solubility problems, requiring drug delivery systems, such as NBCDs, for efficient drug targeting to a specific site [141]. The doxorubicin (n=1, 2%), daunorubicin (n=1, 2%), and irinotecan (n=1, 2%) are just a few examples of cytotoxic drugs successfully encapsulated in liposomes approved for clinical use (Figure 3). The combination of different cytotoxic agents could result in a significant increase in the therapeutic efficacy of NBCD products. An example of this is the Vyxeos® (liposomal encapsulation of daunorubicin and cytarabine), which is capable to release synergistic ratios of these two drugs, demonstrating superior antileukemia activity for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC) [122,148]. This liposomal formulation revealed a significant posological improvement over the conventional treatment, since the liposomes provide the release of different active substances, with a longer duration of action in the body, enhanced therapeutic efficacy, and improved survival rates [122,148].

Another relevant example of the clinical application of NBCDs corresponds to Onpattro® (patisiran lipid complex injection), a lipid nanoparticle-based short interfering ribonucleic acid (siRNA) therapeutics for the treatment of polyneuropathy caused by hereditary transthyretin-

mediated amyloidosis (hATTR) (U.S. FDA Approval, 2018). The gene-delivery systems used in gene therapy transport nucleic acids (e.g. DNA or RNA) to target tissues, modifying the genetic information or the expression of specific proteins at the cellular level, allowing to provide new cell functions, replace missed functionalities in a disease, or even suppress the expression of certain genes [13]. This product opens up the way for the clinical development of other nucleic acid drug technologies based on gene-delivery strategies, with applicability in the imaging and diagnostic techniques (e.g. identification of the disease stage) or in the treatment of different pathologies (e.g. cancer, autoimmune, neurodegenerative, cardiovascular diseases, and so on) [13,149]. The same applies to the encapsulation of active molecules of biotechnological origin (e.g. peptides, proteins, antisense oligonucleotides, plasmids) that are subject to physicochemical and enzymatic degradation after administration, have difficulty in crossing the biological barriers, and whose function and efficacy depends on the capability to reach a precise cellular compartment [13,150].

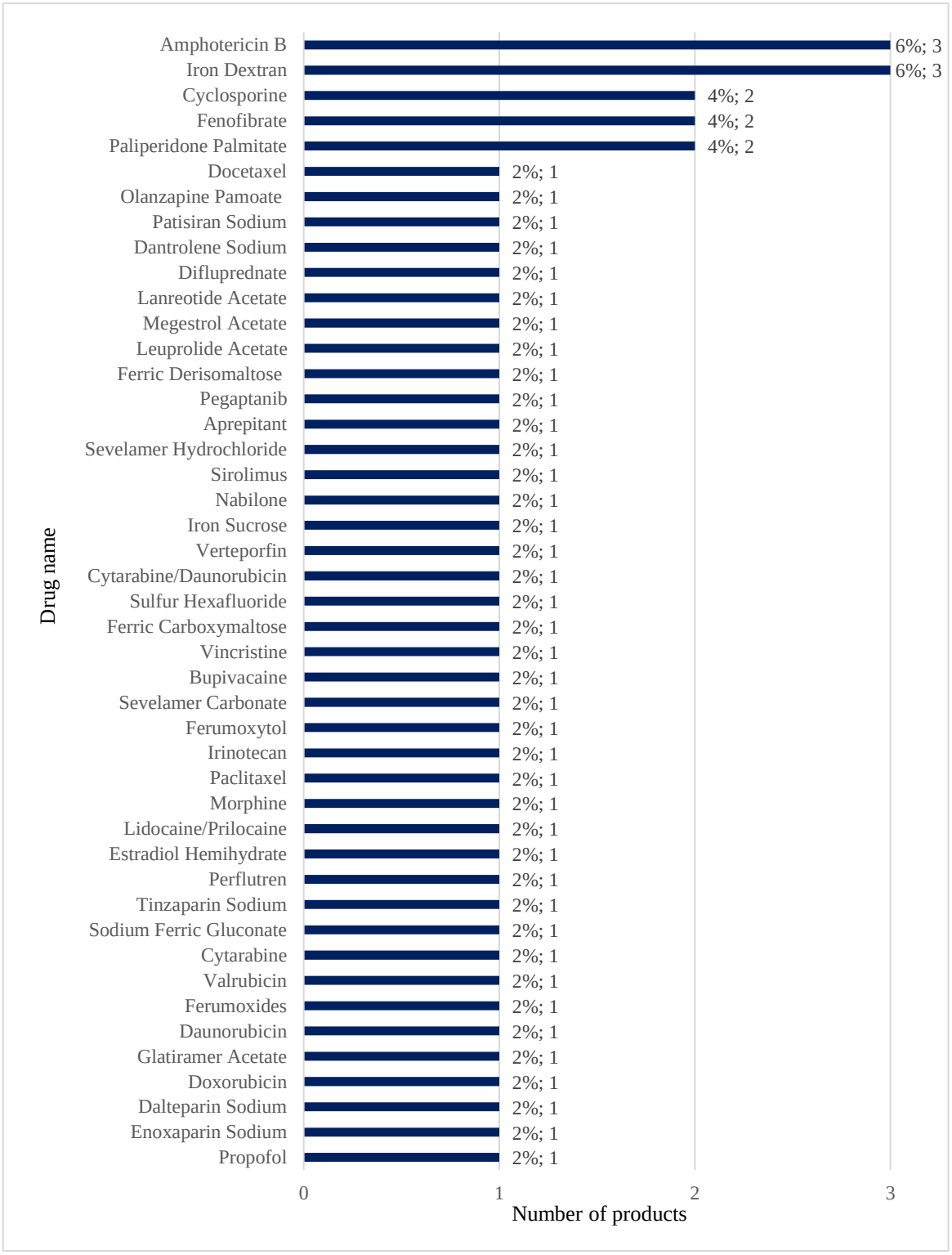

Figure 3. Type of drugs identified for Non-Biological Complex Drugs (NBCDs) approved by the FDA.

3.2. Analysis by Type of NBCDs

The most common NBCD products that were approved by the FDA are liposomes (n=13, 25%), followed by nanocrystals (n=10, 19%), and iron-carbohydrate complexes (n=8, 15%) (Figure 4). Only seven products are related with nanoparticles (n=7, 13%), six with emulsions (n=6, 11%) and three with low-molecular-weight heparins (LMWHs) (n=3, 6%) and microspheres (n=3, 6%) (Figure 4). One product corresponds to polymeric micelles (n=1, 2%), and another product is a glatiramer acetate complex (n=1, 2%) (Figure 4).

Liposomes have been described by A.D. Bangham in the early 1960s, comprising one of the first complex drugs to be developed [151]. Thus, the widespread use of the liposomes is not surprising, considering that liposomal formulations were one of the first nanotechnology-based products to be developed, with indubitable advantages over other systems in terms of preparation, scalability, stability, and biocompatibility [8,152]. They correspond to vesicular structures of concentric lipid bilayers with a hollow core resulting from the organization of amphipathic lipids when in contact with an aqueous medium [5,151,153]. This versatile structure allows the encapsulation of hydrophobic drugs within the lipid bilayer, hydrophilic substances within the aqueous core, and amphiphilic molecules in the lipid-aqueous interface [151,154,155]. The main components of the phospholipid bilayer are phospholipids and cholesterol, which play an important role in the modification of lipid membrane properties (e.g. surface charge, membrane permeability, or lipid stability in the bilayer), and hence in the performance, safety, and stability profile of liposomal drug products [5,13,153]. Due to the variety of available phospholipids and the sound knowledge regarding the development of liposomes, they can be formulated with different compositions, sizes, morphology, and surface charge, which in turn allows the change of the route of administration, change the drug release profile, facilitate drug penetration through biological barriers, improves organ distribution, targeted delivery, bioavailability, stability, increase maximum tolerated doses, and reduce systemic toxicity [152,153,156,157]. For example, the phospholipids used in the production of liposomes will determine their degrees of stiffness and permeability depending on whether incorporate phospholipids with saturated or unsaturated hydrocarbon chains in the formulation, just as their specific net surface charge according to the use of anionic or cationic phospholipids [13].

In addition, the stealth effect triggered by PEG (polyethylene glycol) moieties on the liposome surface avoids the recognition by the mononuclear phagocytic system, and hence allows an extended circulation time and increase in the bioavailability and drug accumulation. PEG plays an important role in the ‘steric stabilization’ effect which consists in the formation of a protective hydrophilic layer on the surface of liposomes, avoiding the interaction with each other (aggregation) and with components in blood circulation, such as the capture of macrophages [140,158]. As shown

in Table 48, the liposomal formulation has been approved for a variety of therapeutic indications including cancer, infectious diseases, and pain management.

However, the need to improve the performance in other application areas and routes of administration led to the development of other types of NBCDs [159,160].

Nanocrystals correspond to another type of NBCDs which are defined as a nanoparticulate system of pure API [5,132]. These systems have been developed to overcome the lack of solubility associated with drugs of class II of the BCS system and improve oral absorption and bioavailability [25,132,137]. Several nanocrystal formulations already have obtained marketing authorization by the FDA, predominantly for oral administration, such as: Cesamet® (Nabilone), Rapamune® (Sirolimus), Emend® (Aprepitant), Tricor® (Fenofibrate), and Triglide® (Fenofibrate) [25,132]. The complexity of nanocrystals is associated with the manufacturing process, the ratio of amorphous to crystalline drug form, stability, and particle size distribution, which should be strictly controlled [5,25,132,133].

Iron-carbohydrate complex products are colloidal dispersions composed of polynuclear iron(III)-hydroxide cores stabilized by carbohydrate ligands [34,134,137,161–163]. They are nanometer-range particles with 5 to 100 nm, commonly administered by the intravenous (IV) route [34,134,137,161]. These classes of NBCD products are the most widely used in the clinical treatment of diseases associated with iron deficiency and anemia, such as chronic kidney disease (CKD) [34,134,137,162,164]. The iron-carbohydrate complex products approved by the FDA include: InFed® (Iron dextran), Dexferrum® (Iron dextran), Ferrlecit® (Sodium ferric gluconate), Venofer® (Iron sucrose), Feraheme® (Ferumoxytol), Injectafer® (Ferric carboxymaltose) and Monoferric® (Ferric derisomaltose) [36,62,63,79,111,119,123]. The stability of each formulation and the release of iron depend on the iron core size, surface properties according to the carbohydrate coating material, and hydrodynamic size of the final nanoparticle [34,134,137,161]. The iron-carbohydrate complex products will be discussed in more detail in Chapter V.

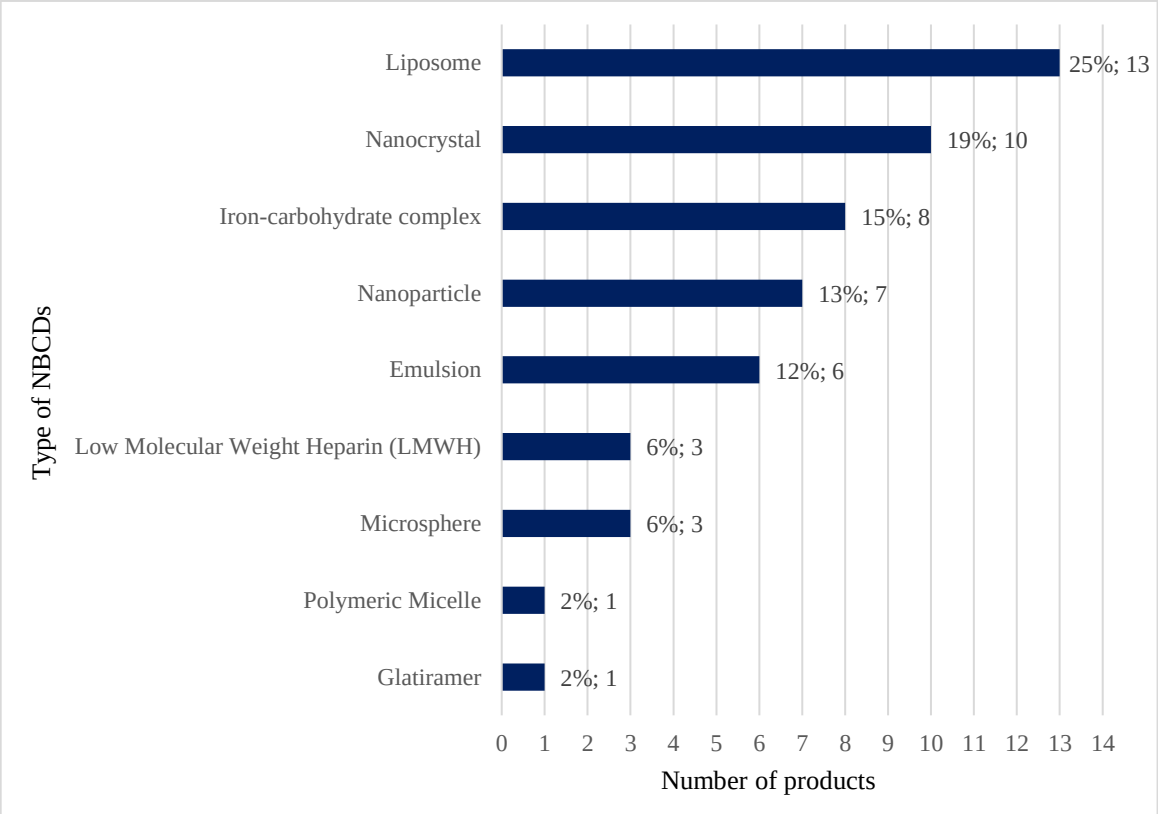

Figure 4. Type of Non-Biological Complex Drugs (NBCDs) approved by the FDA.

The NBCDs could be formulated through different types of dosage forms, mainly in drug delivery technologies for complex injectable products (Figure 5). The high demand for these dosage forms is due in particular to the wide benefits and applications in the reformulation of existing pharmaceutical products with limited clinical utility, greater control over the pharmacokinetic and pharmacodynamic properties of the active ingredient, design of sustained-release dosage forms over extended periods (e.g. long-acting injectables), reduction of dosing frequency and toxicity, increase solubility, enhancement of bioavailability, targeted delivery, and the improving therapeutic effectiveness [165–169].

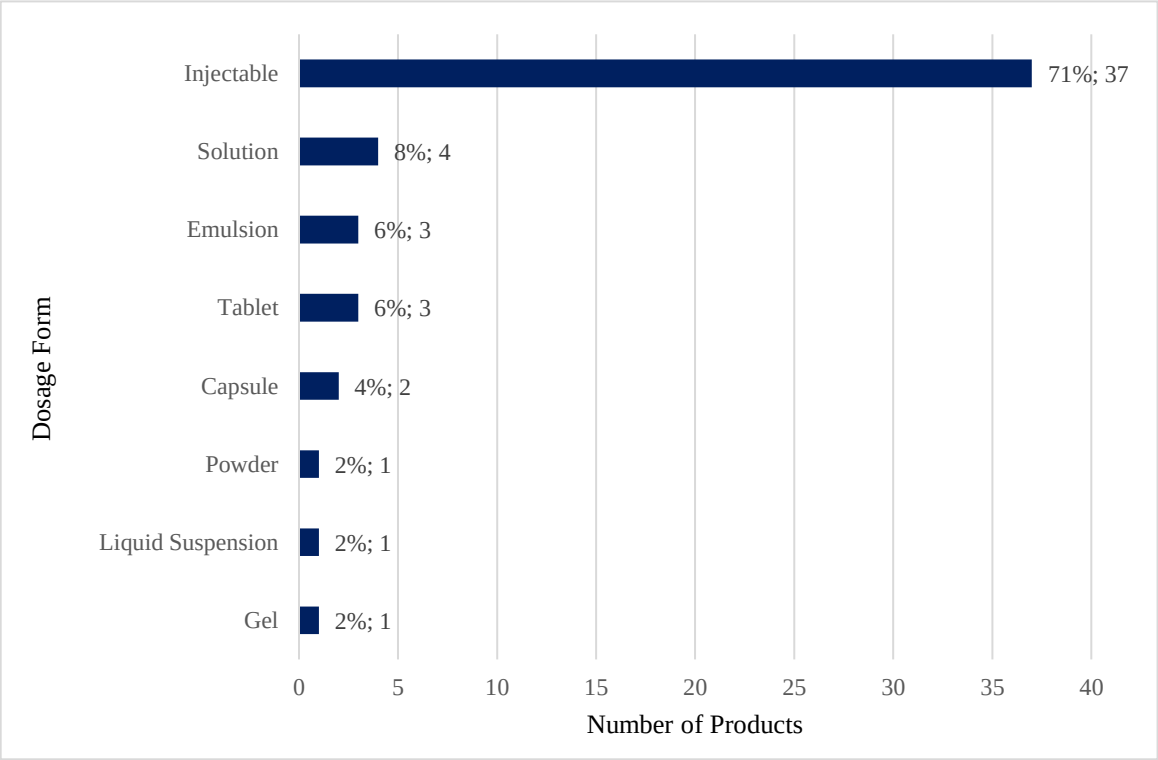

Figure 5. Dosage form identified for Non-Biological Complex Drugs (NBCDs) approved by the FDA.

3.3. Analysis by Route of Administration

NBCDs have been developed for different routes of administration, such as: intravenous (n=25, 48%), oral (n=9, 17%), subcutaneous (n=6, 12%), intramuscular (n=3, 6%), intrathecal (n=2, 4%), ophthalmic (n=2, 4%), transdermal (n=1, 2%), intravitreal (n=1, 2%), soft tissue injection (n=1, 2%), periodontal (n=1, 2%), and intravesical (n=1, 2%) (Figure 6). The therapeutic success of NBCDs mainly depends on their capability to overcome several biological barriers in the human body, and strike targeted organs according to the route of administration selected.

Most of the NBCDs already on the market are administered through an intravenous route (Table 48). The intravenous administration is mostly used, due to it allows a more rapid onset of action with full bioavailability into the systemic circulation, just as the capability to achieve site-specific delivery [137,140,166]. Furthermore, this route overcomes the problem of first-pass metabolism and the degradation by proteolytic enzymes and is preferred for drugs that cannot be administered orally or through other means in specific situations (e.g. palliative care or esophageal dysphagia) [137,140,170].

As it is possible to see in Figure 6, oral administration is the second most common route used for NBCDs. From the total of NBCDs administered orally, nanocrystals were the majority type identified, followed by polymeric nanoparticles and emulsions [37,52,81,85,91,95,110,113]. This route is convenient and preferably employed to allow the faster dissolution of the drug, especially

for drugs that are easily absorbed in the gastrointestinal tract [132]. However, despite the oral administration is commonly used, there are some key challenges stemming from its use. The low solubility, stability, and bioavailability of some drugs prevent the effective use of this route. Additionally, the large numbers of degrading enzymes, mucus layers that cover epithelial surfaces, and the pH across the gastrointestinal tract promote the inactivation or difficulty in the absorption of some drugs [12].

The bioavailability of topical formulations administered through the skin can be extremely low. The effectiveness of drug transport through the skin depends on the capability to overcome its natural barrier on the level of the stratum corneum, increase drug skin permeation, enhance the time and concentration in the stratum corneum and epidermis, or ability to disrupting the integrity of the superficial layers [8]. Thus, the development of innovative NBCDs offers tremendous opportunities to enhance drug administration through the skin. The use of a subcutaneous route for the administration of NBCDs was one of the main paths identified. The advantages associated with this route are the capacity to prevent the first-pass metabolism, the drug concentration fluctuations, and allowing a controlled drug release (e.g. drug delivery depot). On the other hand, it also allows a minimization of the side effects and improves the safety and therapeutic response of the drug product [171,172].

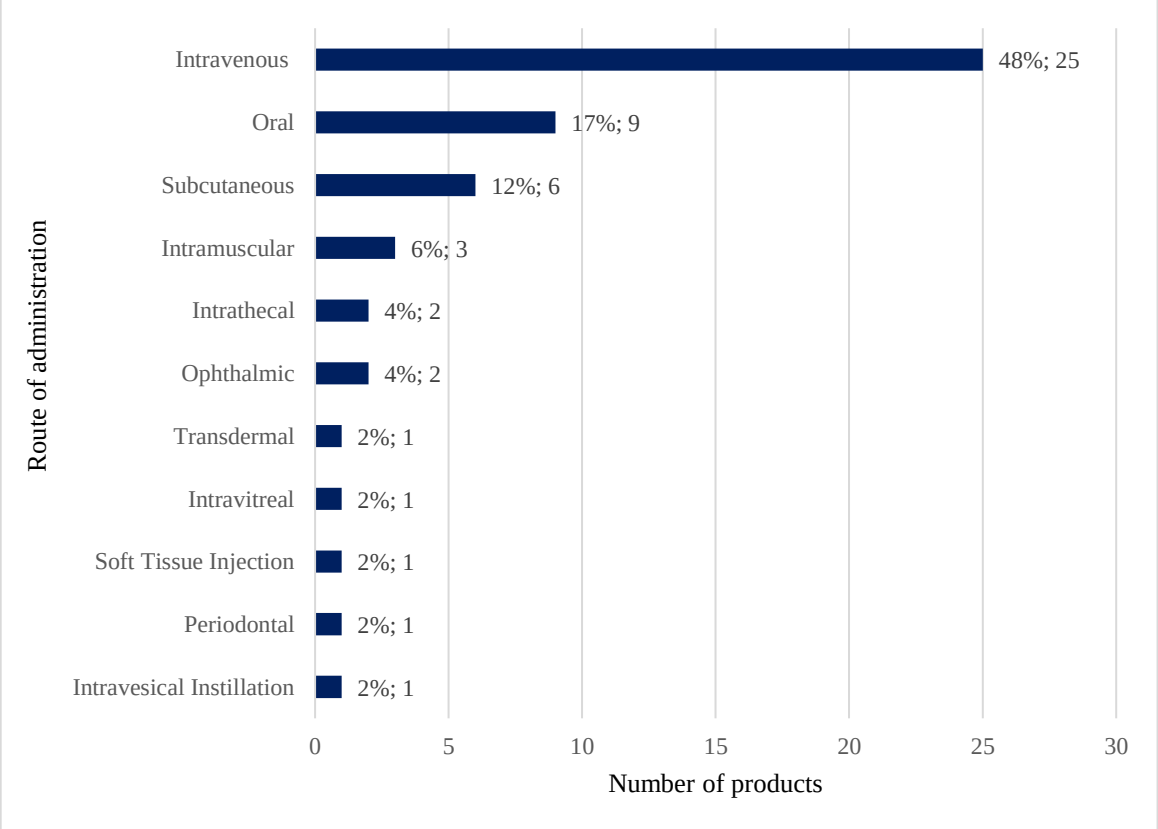

Figure 6. Route of administration identified for Non-Biological Complex Drugs (NBCDs) approved by the FDA.

3.4. Analysis by Therapeutic Indication

According to the World Health Organization (WHO), cancer is a leading cause of death worldwide, being responsible for approximately 10 million deaths in 2020 [173]. Therefore, increasing access to essential and effective cancer medicines is urgently needed to control the high mortality rate associated with this disease.

The mechanism of action of most antineoplastic drugs depends on the interruption of proliferation and division of cells to avoid uncontrolled growth of tumor tissue and the spreading metastasis. However, the healthy cells are also subject to a constant proliferation process, wherefore the chemotherapeutic agents do not discriminate between a healthy and a cancer cell [13]. Thus, the antineoplastic drug encapsulation in NBCDs allows overcome some of the drawbacks of conventional chemotherapy, such as the inability to provide a suitable therapeutic drug concentration to the target tissues, low solubility, low specificity, just as the high potential to cause severe adverse effects during treatment due to the inherent toxicity that could affect both normal or cancer cells [3,136,140,141,152].

Most NBCDs approved by the FDA, currently available in the U.S. market, were developed for cancer therapy (n=11, 21%) (Figure 7). This is expected due to the potential ability of NBCDs to deliver therapeutic and diagnostic agents based on the type and location of the tumor. These systems are mainly biocompatible, biodegradable, and present the ability to protect the content encapsulated and minimize systemic toxicity and side effects [3,136,140,141]. They can promote the increase of drug efficacy due to the rise in the time of systemic circulation and concentration gradient to the tumor microenvironment [3,136,140,141,152]. Thus, the encapsulation of antineoplastic drugs in NBCDs leads to an improvement in its efficacy and the quality of life for cancer patients.

The unique pathophysiology of tumor tissues is characterized by stimulation of angiogenesis that plays a critical role in guaranteeing the blood supply for the tumor growth, resulting in a defective architecture formed by a highly porous system. Moreover, the uncontrolled growth of tumor cells could also be promoted by membrane receptors overexpressing in those cells [13]. Over the years, different strategies have been developed to target the tumor, such as: active targeting driven by receptors overexpressed by tumor cells or tumor vasculature, passive targeting based on the Enhanced Permeability and Retention (EPR) effect, stimuli-responsive tumor targeting, among others.

The EPR effect corresponds to passive diffusion and accumulation in specific regions of the tumor due to the leaky configuration of the tumor compartments in combination with insufficient lymphatic drainage [3,12,13,136,140]. Thus, the nanotechnology-based products enter to tumoral microenvironment through the pores by passive diffusion where are remain concentrated for a longer period of time. Doxil® (doxorubicin HCl liposome injection) for intravenous infusion is a pegylated liposomal formulation making use of the passive EPR targeting and particle-size control

mechanisms to overcome the recognition by the reticuloendothelial system and extend circulation time [49]. Other examples of FDA-approved liposomal formulations characterized by their effective accumulation in tumor tissue based on the EPR effect include the DaunoXome® (daunorubicin citrate liposome injection), Marquibo® (vincristine sulfate liposome injection), and Onivyde® (irinotecan liposomal injection) [61,109,118].

On the other hand, Abraxane® (paclitaxel albumin-bound particles for injectable suspension) for intravenous use is a good example of the active-targeting nanomedicine, which results in an increased accumulation of ligand-coated nanoparticles at the target site or an enhanced cellular uptake of nanoparticles by expressing the target receptor to the tumor markers [108].

The stimuli-responsive tumor-targeting corresponds to an innovative strategy that allows the target release of drug contents through the exposition to external stimuli (e.g. temperature, light, or ultrasound). Lyso-thermosensitive Liposomal Doxorubicin (LTLD, ThermoDox®) (NCT02536183) broadly studied in clinical trials is a striking example of stimuli-responsive tumor-targeting, throughout raising temperature via the application of radiofrequency [174].

Besides cancer therapy, NBCDs are applied in many other therapeutic indications, including: iron deficiency (n=8, 15%), infectious diseases (n=3, 6%) and deep vein thrombosis (DVT) (n=3, 6%) (Figure 7). Other common indications are related with pain management (n=3, 6%), schizophrenia (n=3, 6%), dyslipidemia (n=2, 4%), organ transplantation (n=2, 4%), anesthesia (n=2, 4%), nausea and vomiting (n=2, 4%), age-related macular degeneration (n=2, 4%), and kidney diseases (n=2, 4%) (Figure 7).

Iron deficiency is the most common nutritional deficiency worldwide, which can be progressively evolved into anemia in patients with chronic diseases [136,141,162,163]. Iron-carbohydrate complex products have been frequently used to treat these deficiencies when the oral administration might not be appropriate due to the extended periods of treatment or lack of the effectiveness of the same [136,141,162,163]. Thus, the IV iron-carbohydrate complex products enable the management of large concentrations of iron quickly and safely [136,141,162,163]. The iron uptake and bioavailability are critical for essential mechanisms of maintenance and proper functioning of tissue and body organs [136,141,162,163]. The IV iron-carbohydrate complex products will be discussed in greater detail below (Chapter V).

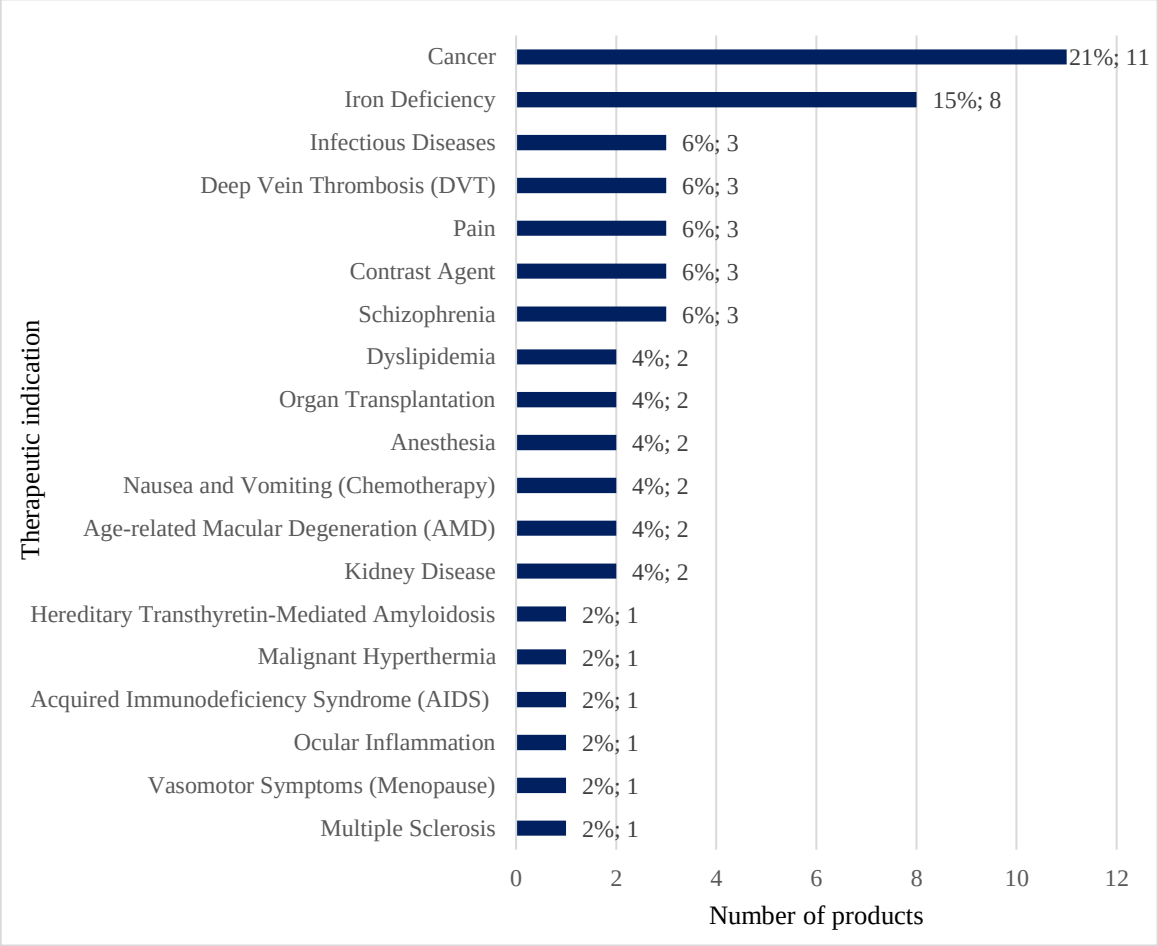

Figure 7. Therapeutic indication identified for Non-Biological Complex Drugs (NBCDs) approved by the FDA.

3.5. Analysis by Sponsor (Company)

Pacira Pharmaceuticals appear at the top of the list of companies with NBCD products approved by the FDA (n=3, 6%) (Figure 8). However, two of those products were withdrawn from the market, such as the Depocyt® and Depodur®. The reasons for their discontinuation are discussed below in Chapter VI.I. (Section 4.5). Subsequently, the American Regent Inc (n=3, 6%), Sanofi Aventis US (n=3, 6%) and Janssen Cilag Ltd (n=2, 4%) are listed (Figure 8). It is important to highlight the fact that the majority of products are developed by capital-intensive industries, such as multinational groups. Multinational pharmaceutical companies can dominate NBCDs markets due to the know-how and expertise in a specific research area, greater investment capacity to support innovative research projects, acquire production facilities, request a full range of outsourced services of the contract research organizations (CROs) and contract manufacturing organizations (CMOs), just as in building robust approval procedures and business strategies to the manufacturing and marketing of complex drug products.

On the other hand, the rising of R&D studies, information sharing, and significant investments by the pharmaceutical industry, just as the technological and scientific developments in the field of nanotechnology contribute to the growth of the NBCDs market. In 2020, the global nanomedicine market size reached USD 171,7 million and is projected to reach USD 350.8 billion by 2030 [12]. In the next years, it is expected that NBCDs keep up with the healthy market growth of Nanotechnology-based products.

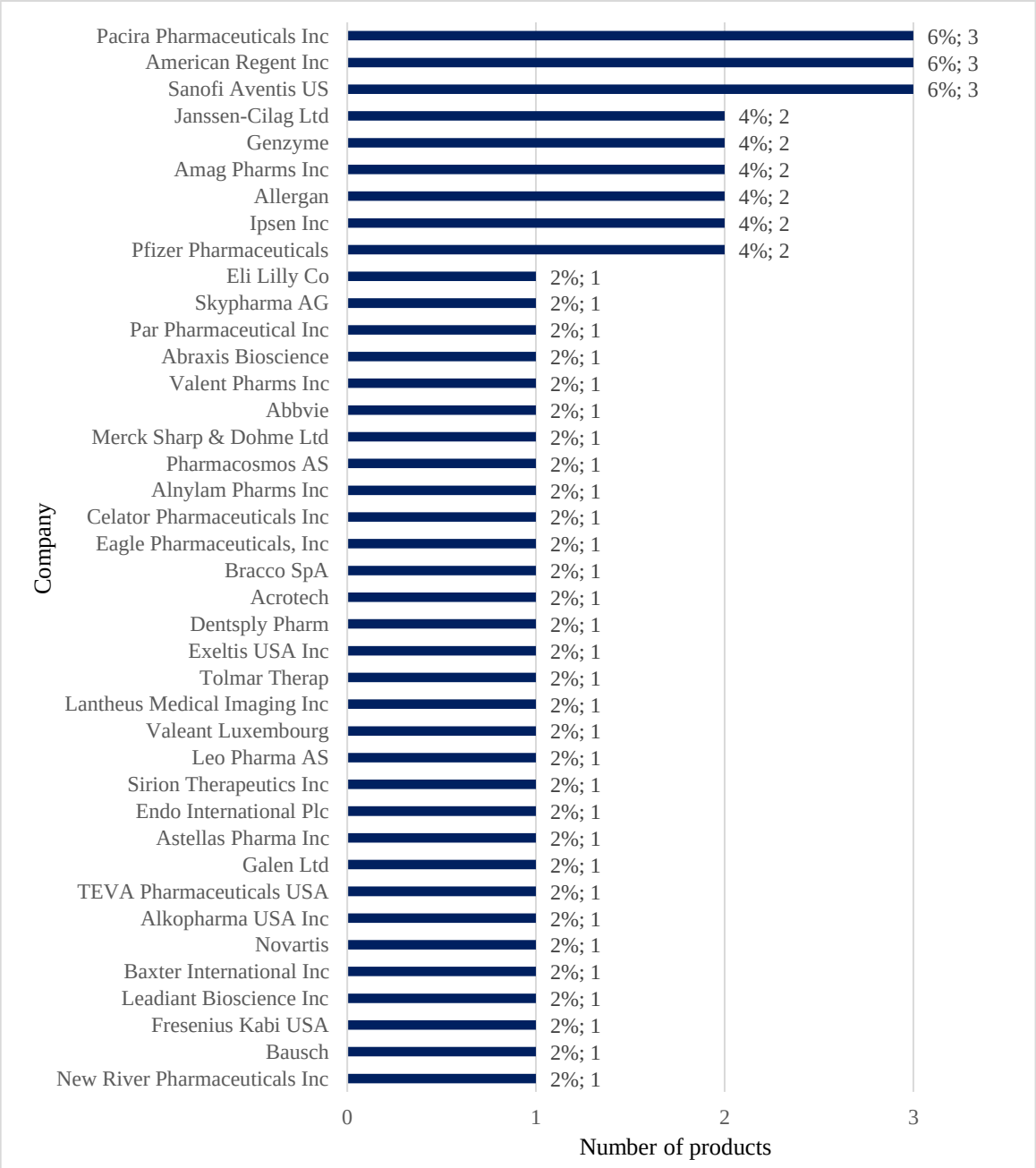

Figure 8. Type of companies holding Non-Biological Complex Drugs (NBCDs) approved by FDA.

3.6. Analysis by Approval Year

Figure 9 indicates the number of products approved by the FDA from 1974 up until 2020. As shown in Figure 9, the maximum number of NBCD products was approved in the year 1996, following the year 2000, 2003, and 2009.

It is very clear that the number of approved products each year is quite limited. Contrary to what one might expect for this emerging area, it is not possible to draw a clear growing trend of the number of NBCD products approved by the FDA. Some of the obstacles that have contributed to the low number of these medicinal products on the market are the complexity of formulation development and optimization, as well as the manufacturing process validation that makes it very difficult to comply with reproducibility requirements and quality standards [20,24,25,35,141]. The challenges related with the pharmaceutical development of NBCDs will be discussed in more detail in the next chapter.

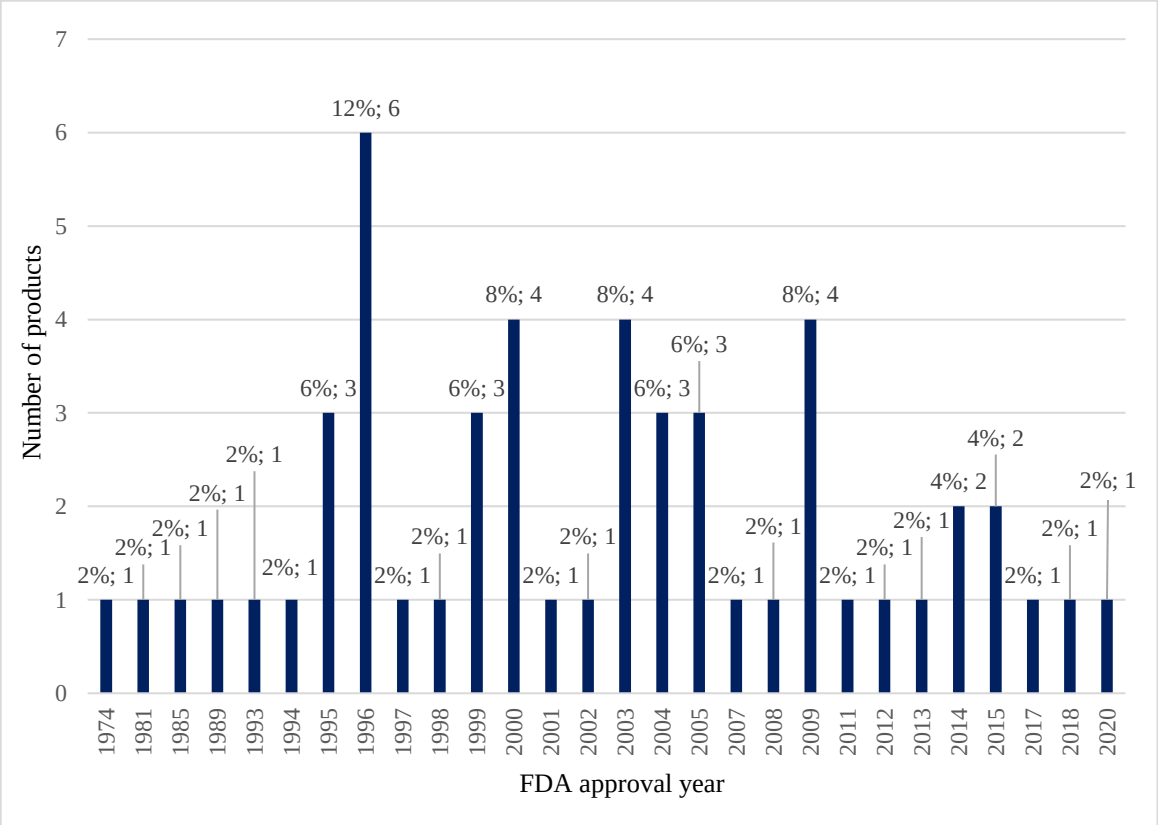

Figure 9. FDA approval year of Non-Biological Complex Drugs (NBCDs).

4. An overview of Non-Biological Complex Drugs (NBCDs) approved in the EU

4.1. Analysis by Type of Drug

As shown in the figure below (Figure 10), the type of drugs with maximum number of applications occurs for ferric compounds, such as iron dextran (n=4, 8%), iron sucrose (n=3, 6%), ferric derisomaltose (n=2, 4%), ferumoxytol (n=1, 2%), ferumoxides (n=1, 2%), ferric carboxymaltose (n=1, 2%), and sodium ferric gluconate (n=1, 2%).

It must be highlighted that the majority of APIs are the same as those that were identified in the FDA analysis. The only different API found was the mifamurtide (Mepact®). Mepact® is a liposomal suspension of mifamurtide, an immunomodulator indicated for the treatment of high-grade resectable non-metastatic osteosarcoma [175].

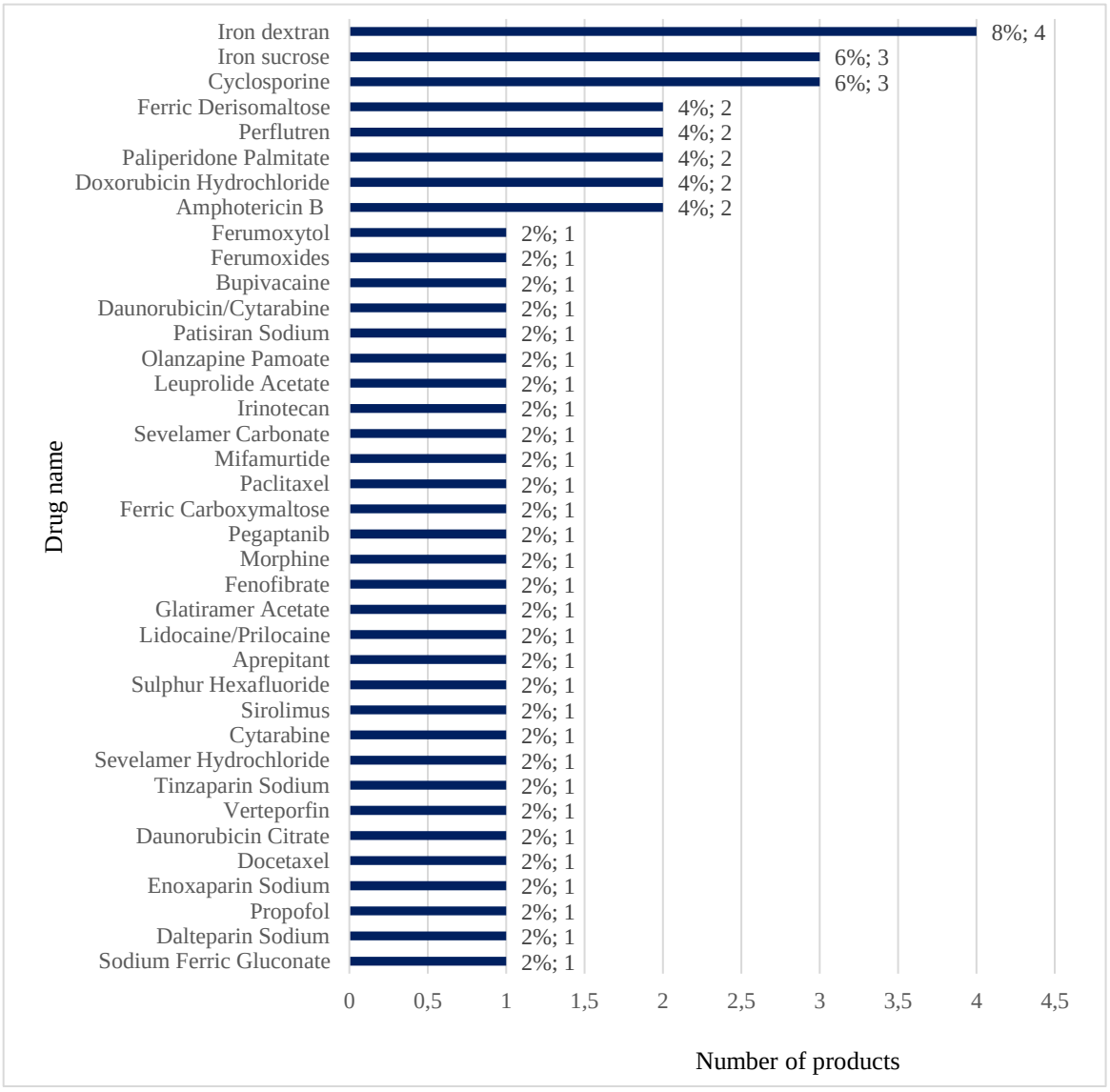

Figure 10. Type of drugs identified for Non-Biological Complex Drugs (NBCDs) approved by the EMA.

4.2. Analysis by Type of NBCDs

In Figure 11 it is possible to observe that the most common NBCD products that were approved by the EMA are liposomes (n=12, 24%) and iron-carbohydrate complex (n=12, 24%), followed by nanoparticles (n=7, 14%), and nanocrystals (n=6, 12%). Only five products are related to emulsions (n=5, 10%) and three with Low-Molecular-Weight Heparins (LMWH) (n=3, 6%). The classes with the lower number of approved products are lipid microspheres (n=2, 4%), gas dispersions (n=1, 2%), glatiramer acetate complexes (n=1, 2%), or polymeric micelles (n=1, 2%) (Figure 11). On the other hand, it is possible to verify that the injectables are the pharmaceutical dosage forms predominantly applied (Figure 12). The search results obtained for the type and dosage forms of products approved in the EU market are in line with the conclusions of section 3.2.

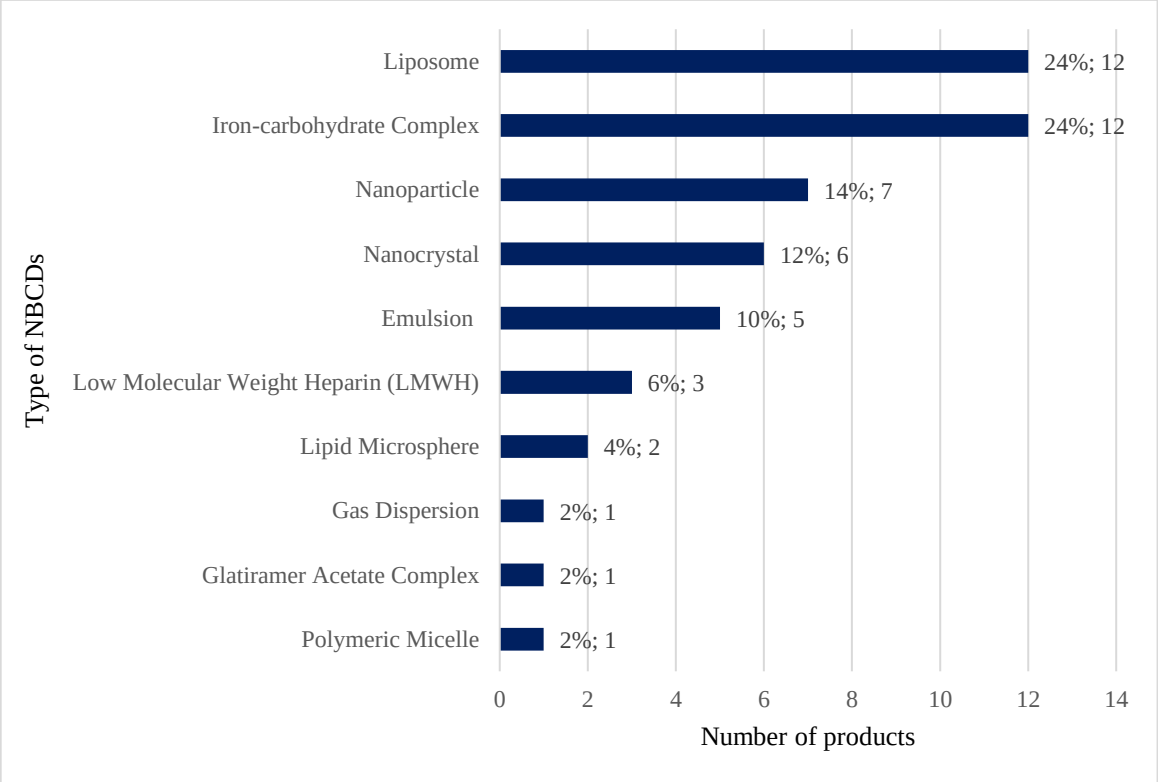

Figure 11. Type of Non-Biological Complex Drugs (NBCDs) approved by the EMA.

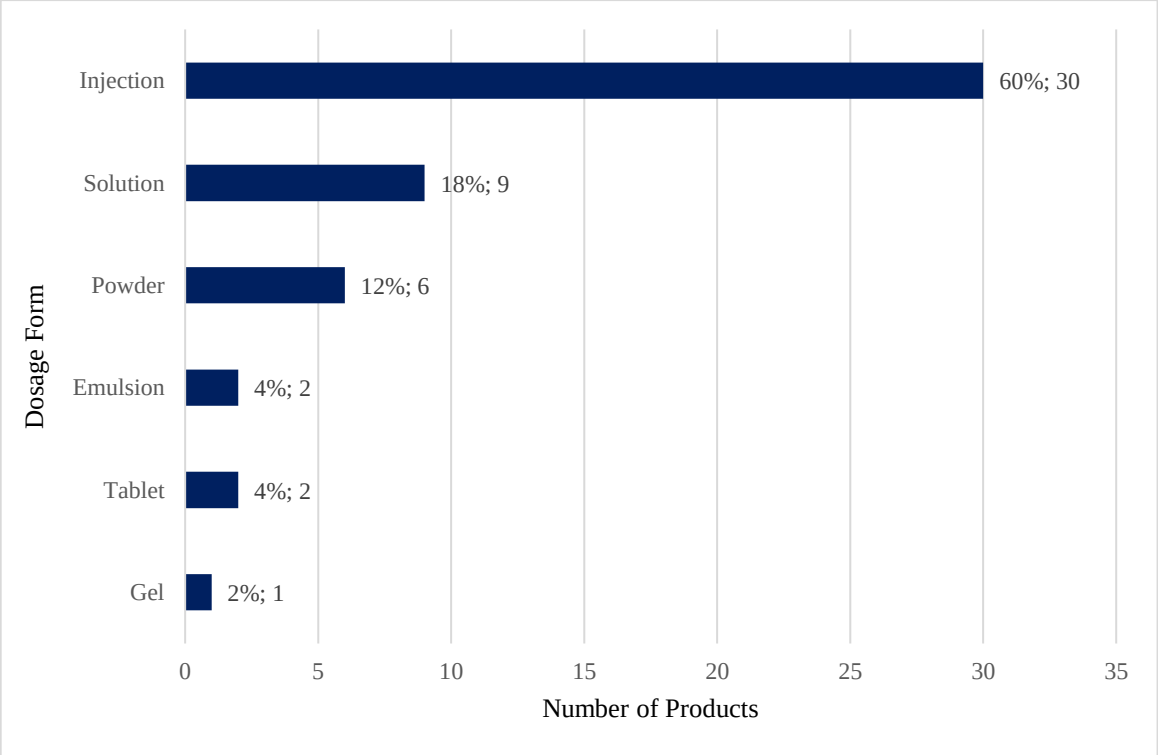

Figure 12. Dosage form identified for Non-Biological Complex Drugs (NBCDs) approved by the EMA.

4.3. Analysis by Route of Administration

Figure 13 presents the number of products approved by the EMA as a function of the route of administration. The distribution was as follows: intravenous (n=29, 58%), oral (n=6, 12%), subcutaneous (n=5, 10%), intramuscular (n=3, 6%), intrathecal (n=2, 4%), ophthalmic (n=2, 4%), infiltration (perineural use) (n=1, 2%), intravitreal (n=1, 2%) and periodontal (n=1, 2%). This distribution follows an identical line to the routes of administration of NBCDs approved by the FDA. No NBCD product approved by the EMA has been identified for transdermal or intravesical instillation, in contrast to what was previously specified for FDA approval.

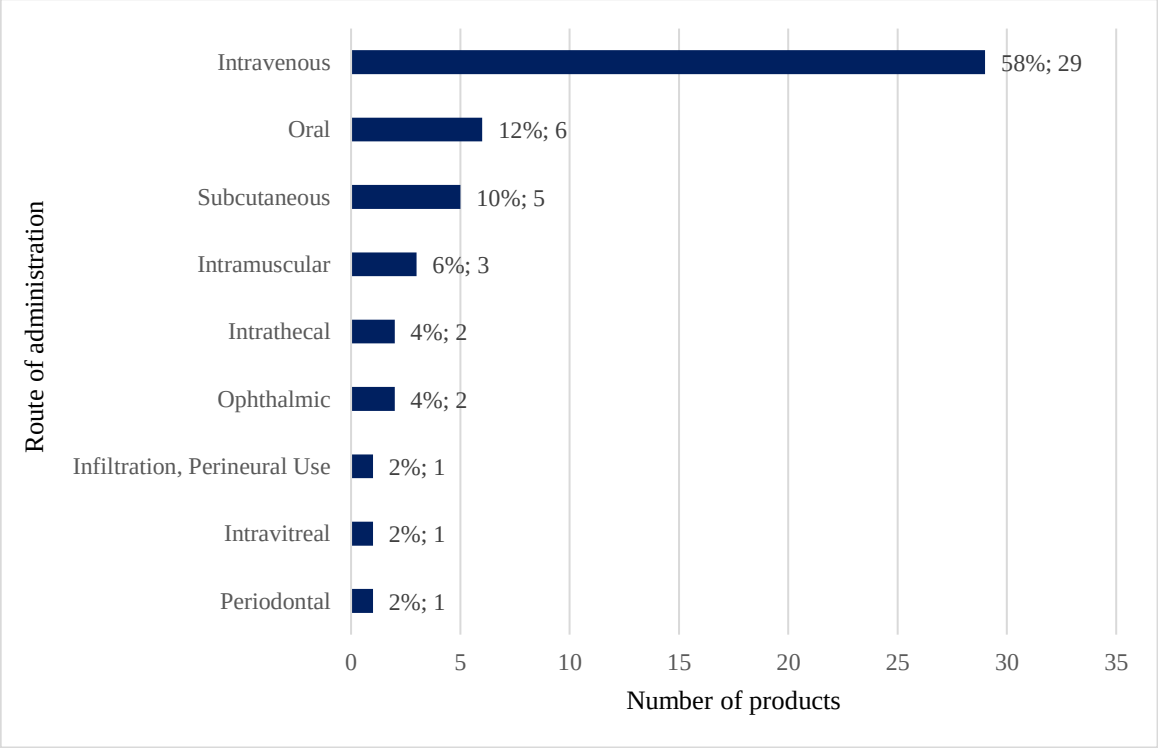

Figure 13. Route of administration identified for Non-Biological Complex Drugs (NBCDs) approved by the EMA.

4.4. Analysis by Therapeutic Indication

Interestingly, the number of NBCDs approved by the EMA for iron deficiency are at the top of the list (n=12, 24%), contrasting with section 3.4 where the cancer appears in first place. Other therapeutic indications include: cancer (n=10, 20%), deep vein thrombosis (DVT) (n=3, 6%), schizophrenia (n=3, 6%), macular degeneration (n=2, 4%), infectious diseases (n=2, 4%), anesthesia (n=2, 4%), kidney disease (n=2, 4%), ophthalmic diseases (n=2, 4%), pain management (n=2, 4%), organ transplantation (n=2, 4%), hereditary transthyretin-mediated amyloidosis (n=1, 2%), dyslipidemia (n=1, 2%), multiple sclerosis (n=1, 2%), nausea and vomiting (n=1, 2%) (Figure 14).

Four of the NBCD products approved by EMA are used for diagnostic, such as Endorem® (dextran-coated ferumoxide nanoparticles), Optison® (perflutren lipid microspheres), SonoVue® (sulphur hexafluoride gas dispersion), and Luminity® (perflutren lipid microspheres). SonoVue® is a dispersion of sulphur hexafluoride for ultrasound imaging [176]. Similarly, Endorem corresponds to a superparamagnetic contrast agent of dextran-coated iron oxide nanoparticles, used for magnetic resonance imaging [177]. Optison® and Luminity® are contrast agents that contain microspheres (tiny bubbles) of perflutren gas as the active substance. They are for diagnostic use only, which helps to make internal body structures visible during imaging tests. Specifically, are

used in patients with suspected or confirmed cardiovascular disease, when the image obtained without a contrast agent has not been conclusive (optimal) [178,179]. It is possible to infer that NBCDs are also applied to medical diagnosis with an increased capability of detection and detailed examination of tissues (e.g. cellular, subcellular, and molecular levels) [12].

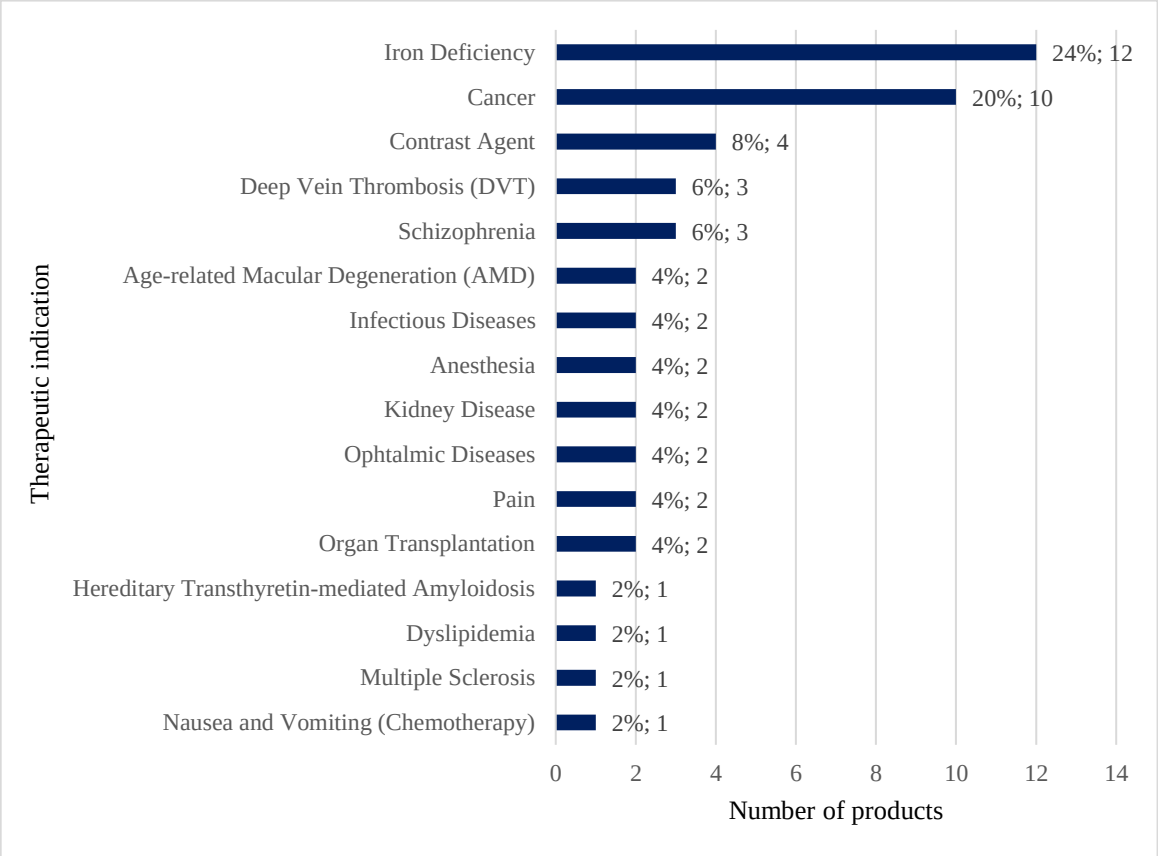

Figure 14. Therapeutic indication identified for Non-Biological Complex Drugs (NBCDs) approved by the EMA.

4.5. Analysis by Marketing-Authorisation Holder (MAH)

At the top of the list are the Pharmacosmos A/S (n=4, 8%), Teva Pharmaceutical Industries Ltd (n=3, 6%), Janssen-Cilag Ltd (n=3, 6%), Sanofi-Aventis (n=3, 6%), Takeda Pharma A/S (n=3, 6%), and then appears the Genzyme (n=2, 4%), Novartis Europharm Ltd (n=2, 4%), Pfizer Pharmaceuticals (n=2, 4%), and Pacira Ltd (n=2, 4%) (Figure 15). The majority of the companies belonging to multinational groups have also been identified in section 3.5 (analysis of NBCD products approved by the FDA).

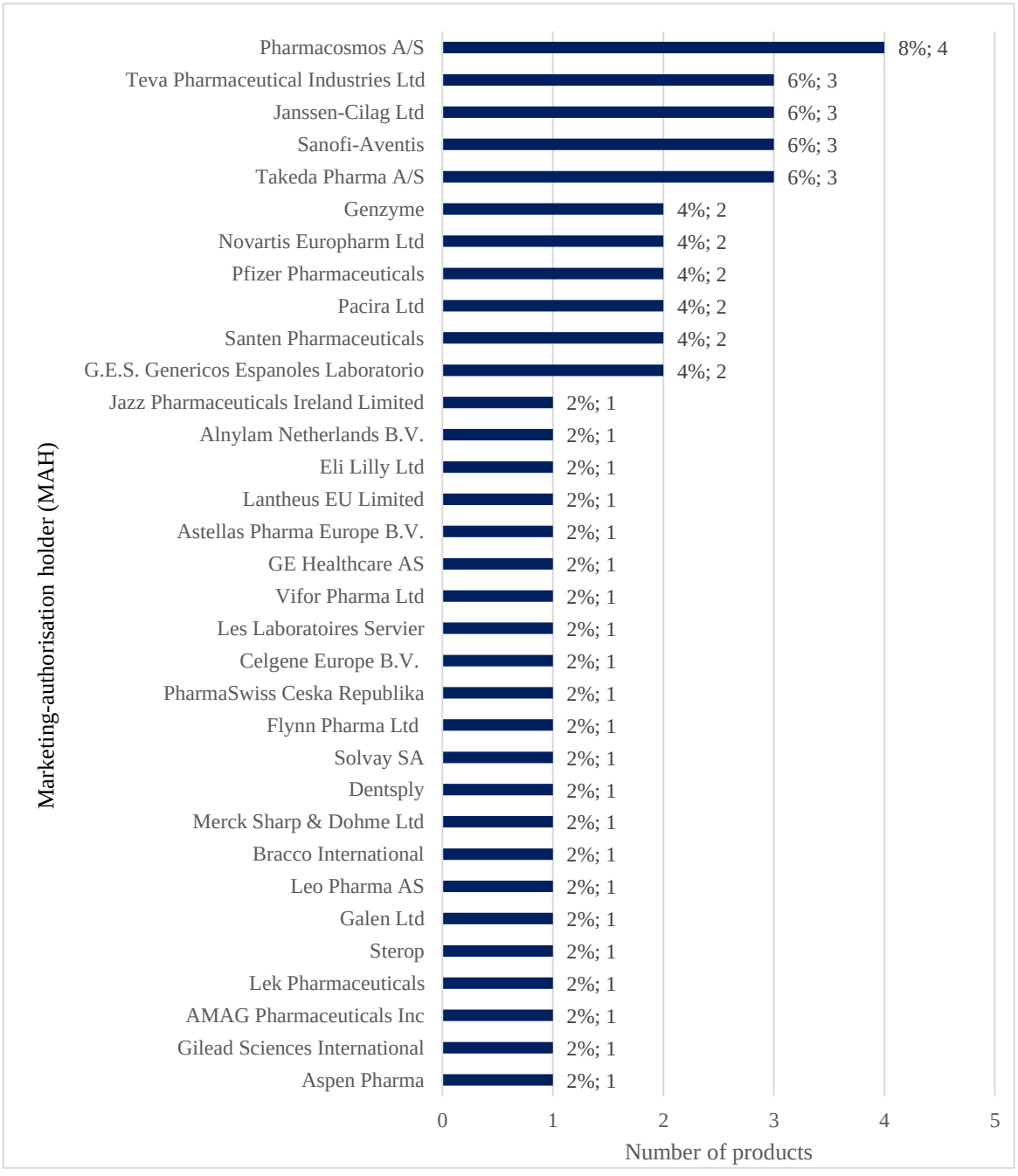

Figure 15. Type of marketing-authorization holders (MAH) of Non-biological Complex Drugs (NBCDs) approved by the EMA.

4.6. Analysis by Approval Year

Figure 16 highlights the trend of the number of NBCD products approved between 1963 and 2020. The few number of NBCD products approved each year followed the same remarks identified for approvals by the FDA (section 3.6).

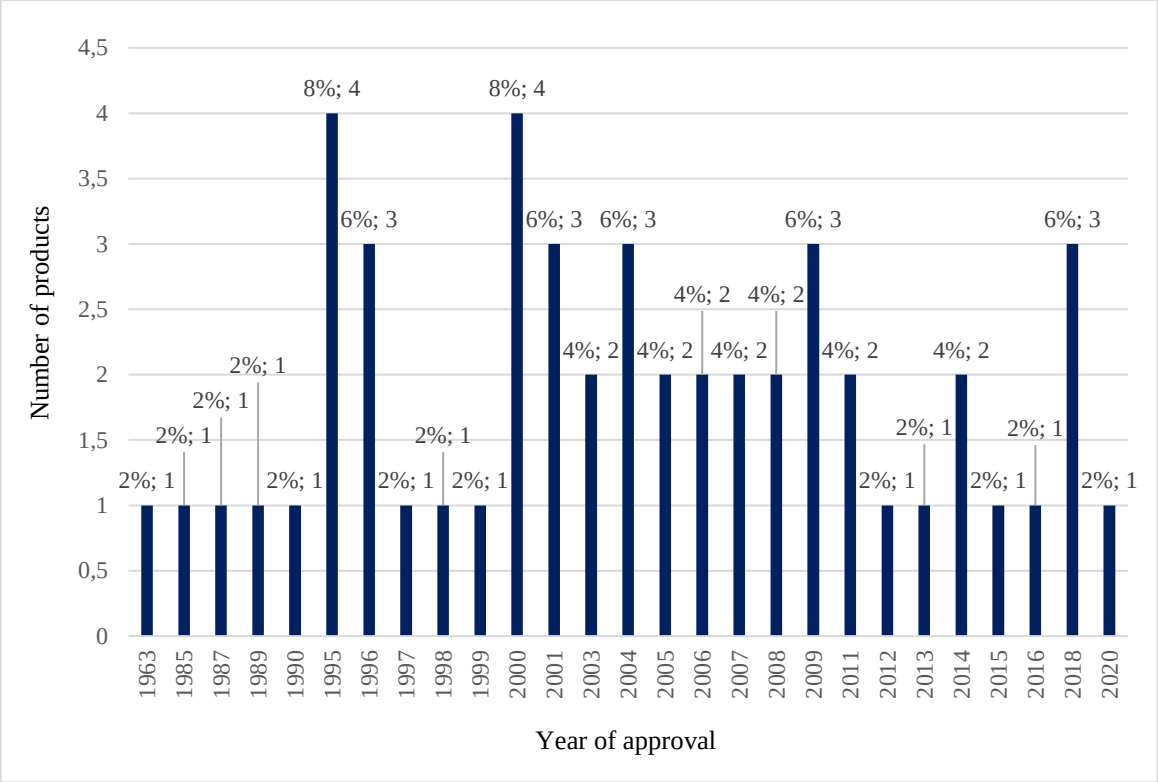

Figure 16. EMA approval year of Non-Biological Complex Drugs (NBCDs).

5. An overview of Non-Biological Complex Drugs (NBCDs) in Clinical Trials

5.1. Analysis by Type of Drug

As previously stated, a large number of APIs can be used for the development of NBCDs. The main APIs identified under clinical evaluation are: paclitaxel (n=14, 17%), bupivacaine (n=13, 16%), doxorubicin (n=10, 12%) amphotericin B (n=6, 7%), docetaxel (n=4, 5%) and irinotecan (n=4, 5%) (Figure 17).

Paclitaxel is a highly effective anti-neoplastic agent, currently used for treating a broad range of cancers [180]. This agent is classified as BCS class IV and presents high toxicity such as neutropenia and peripheral neuropathy [133,180]. Two different formulations, Genexol-PM (Paclitaxel loaded polymeric micelles) and LEP-ETU (Liposome Entrapped Paclitaxel Easy to Use formulation), have been highlighted in the clinical trials identified for the paclitaxel (Table 50). Genexol-PM is a formulation of paclitaxel encapsulated in copolymeric micelles, developed for various types of cancer, such as metastatic breast cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, or colon cancer [141,180–186]. This formulation is currently undergoing several clinical trials, especially due to its high efficiency and reduced toxicity [141,180–186]. Similarly, the clinical trials with LEP-ETU have described several advantages of paclitaxel encapsulation, such as: enhancing the anti-tumor properties of paclitaxel and increasing the dose administered, greater therapeutic effectiveness, and reduced infusion time and side effects [187,188].

All the clinical studies of bupivacaine are related to liposomal bupivacaine extended-release injectable suspension (Table 50). The currently approved liposomal bupivacaine (Exparel®, Pacira Pharmaceuticals Inc) consists of a non-opioid, single-dose, long-acting local analgesic used for postsurgical pain management [116,189,190]. In general, clinical studies have been designed to evaluate the effectiveness, safety, and health economic benefits of bupivacaine formulations [191–203].

A significant number of clinical studies used doxorubicin hydrochloride as the API. The most common reasons given for the high number of the clinical trials with liposomal doxorubicin are: the liposomal formulation demonstrated a greater efficacy compared with free doxorubicin; presented a passive targeting property and tumor accumulation by the EPR effect; and exhibited a lower toxicity profile, with better cardiac tolerance and less myelosuppression, alopecia, nausea, and vomiting [19,135,136,140,204]. Furthermore, additional factors are the high commercial value achieved over the years and the existence of a lot of knowledge and documentation disclosed for being the first liposomal formulation approved by the FDA [19,135,136,140,204].

The product-specific guidelines published by regulatory authorities for the assessment of bioequivalence of several NBCDs are also an important reason to propel the high number of clinical studies [205–208].

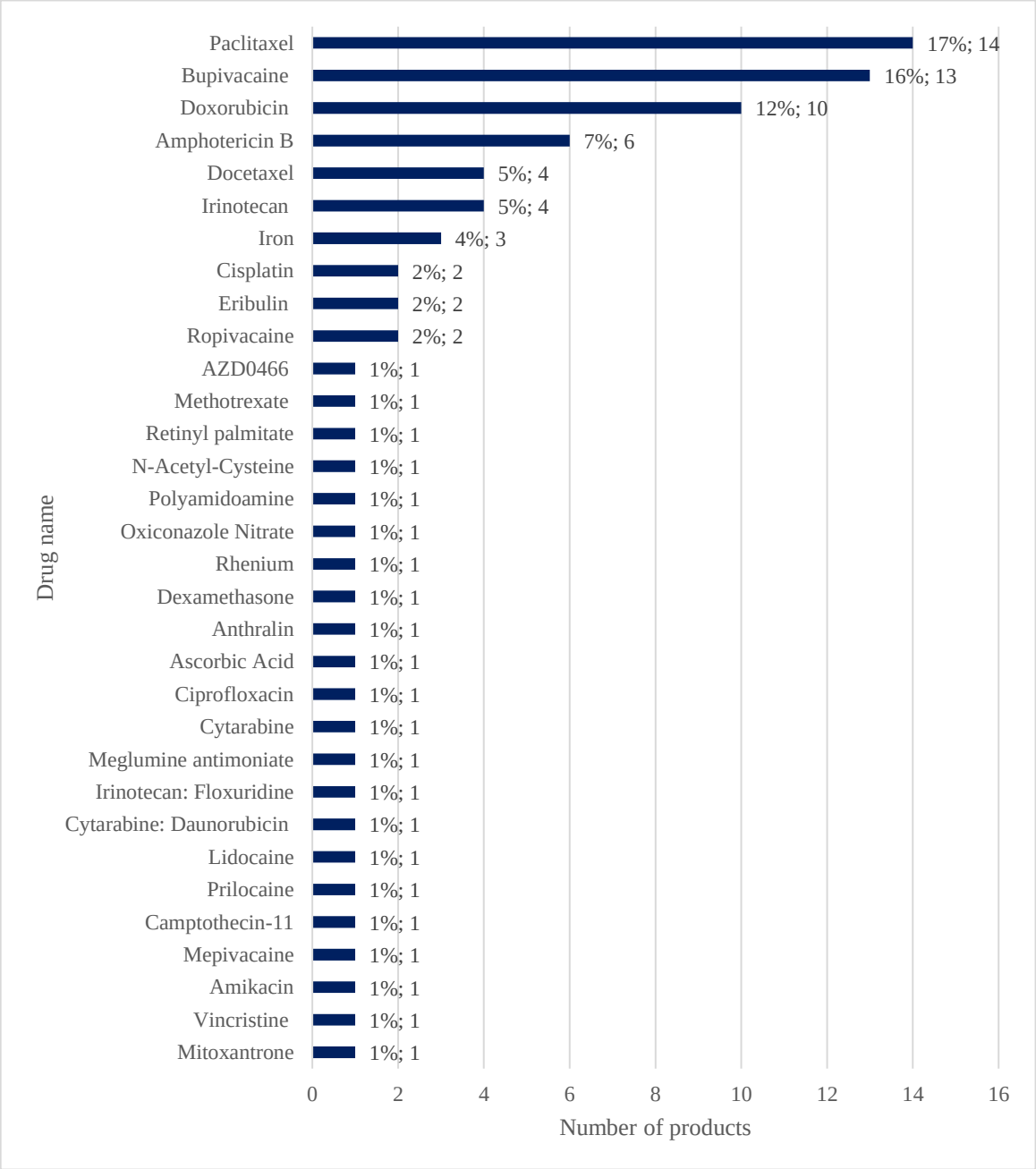

Figure 17. Clinical study classified by type of drug.

5.2. Analysis by Type of NBCDs

In Figure 18 it is possible to observe the most common NBCDs that were evaluated in clinical trials. From the 82 clinical trials pool (Table 50), 58 were related with liposomes (n=58, 71%), 13 were related with polymeric micelles (n=13, 16%), 4 were related with dendrimers (n=4, 5%), 2 were related with ethosomes (n=2, 2%) and iron-carbohydrate complexes (n=2, 2%), and only one clinical trial aimed to study a lipid nanoemulsion (Effect of Methotrexate Carried by a Lipid Nanoemulsion on Left Ventricular Remodeling After STEMI (ST-segment elevation myocardial infarction)) (n=1, 1%) [209]. No study was found on ClinicalTrials.gov for glatiramoids and transferosomes. The high number associated with liposomes is very much in line with the advantages observed for the NBCDs approved by the FDA (Table 48) and EMA (Table 49).

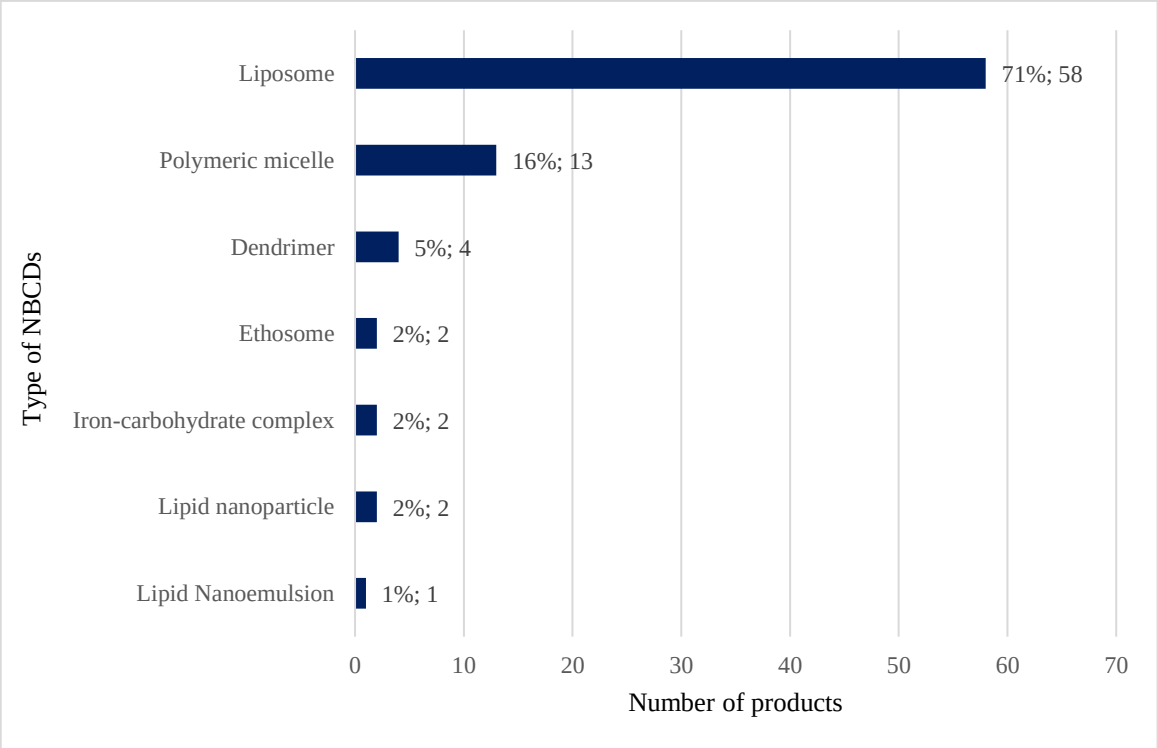

Figure 18. Clinical study classified by type of NBCDs.

5.3. Analysis by Route of Administration

Regarding the route of administration, the distribution was as follows: intravenous (n=45, 55%), intra-articular (n=13, 16%), oral (n=9, 11%), topical (n=6, 7%), periodontal (n=4, 5%), inhalation (n=2, 2%), intra tumoral (n=1, 1%), intrathecal injection (n=1, 1%) and intravesical administration (n=1, 1%) (Table 50). Most of the NBCDs already on the market are also administered intravenously (Table 48 and Table 49). Likewise, the intravenous administration is the main route of products under clinical development (Figure 19).

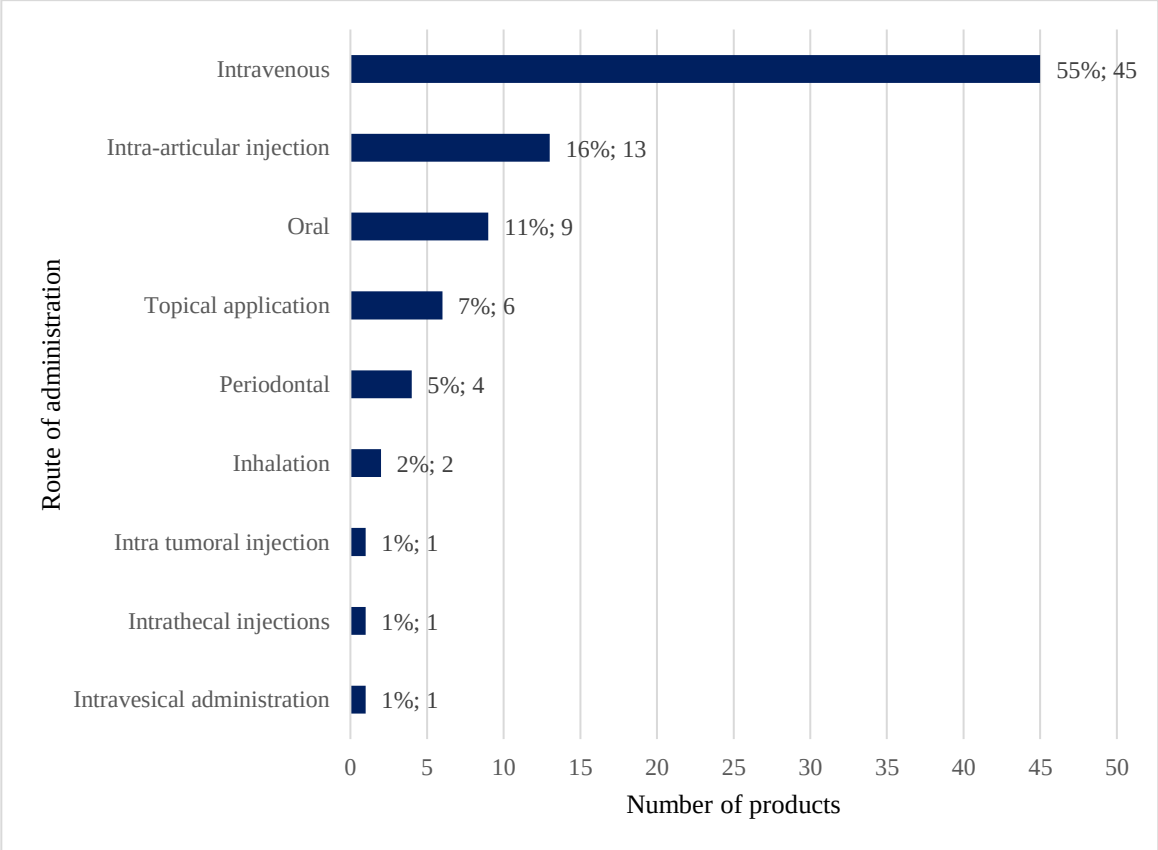

Figure 19. Clinical study classified by route of administration.

5.4. Analysis by Therapeutic Indication

Cancer is the leading therapeutic indication with a total of 46 clinical studies (n=46, 56%) (Figure 20), which follows the same trend found in the analysis of the products approved by the FDA (Table 48). Pain is the second therapeutic indication with 18 clinical studies (n=18, 22%), followed by infection diseases with 7 clinical studies (n=7, 9%) (Figure 20).

The clinical studies for cancer therapy had several objectives, such as the optimization of dose with an increase in the effectiveness of treatment, reduction in toxicity and side effects, and to promote the synergistic activity using a combination of drugs or other therapeutic modalities such as radiotherapy or hyperthermia [210–213].

Another area of application of NBCDs is pain. The entrapment of local anesthetics or nonsteroidal anti-inflammatory drugs (NSAIDs) provides a sustained and controlled release of the drugs with several advantages: prolonged duration of action, decreased plasma concentrations and reduced toxicity, increased pain control with subsequent reduction of consumption of pain medications, and their adverse effects [106–109]. This has a considerable impact on the effectiveness of therapy and the reduction of treatment costs and hospitalizations. Currently, the most common liposomal formulations with local anesthetics in clinical trial development are amides like bupivacaine, ropivacaine, mepivacaine, prilocaine, or lidocaine [106–109]. For example, a study developed by Burnett and colleagues demonstrated that the use of bupivacaine extended-release liposomal injection allows a multimodal pain control regimen with a reduction of 30% opioid consumption in the first 72h post-operative period, in comparison to a gel formulation containing a free drug. Other studies provide strong evidence for the benefits of this formulation in significantly and effectively reduction in pain and the need for opioid use [214–217].

Moreover, liposomes are the main type of NBCDs widely used as efficient delivery systems for drugs or antigens to obtain desired immune responses against a variety of infectious diseases. The biodegradability, biocompatibility, lack of immunogenicity, versatility in composition, size, structure, electrical charge, or method of production, and adequate safety profile for human use are some of the benefits encountered for these formulations [218–224]. In addition, they have high efficiency of antigen encapsulation, protect from premature proteolytic degradation, reducing the required dose, which consequently reduces the systemic adverse reactions [218–224]. They function as effective antigen-delivery systems with the ability to ‘passively’ accumulate and depot formation at sites of increased vasculature permeability with an efficient presentation of antigens by APCs (antigen-presenting cells), i.e., potentiate the immunomodulatory functions [218–224].

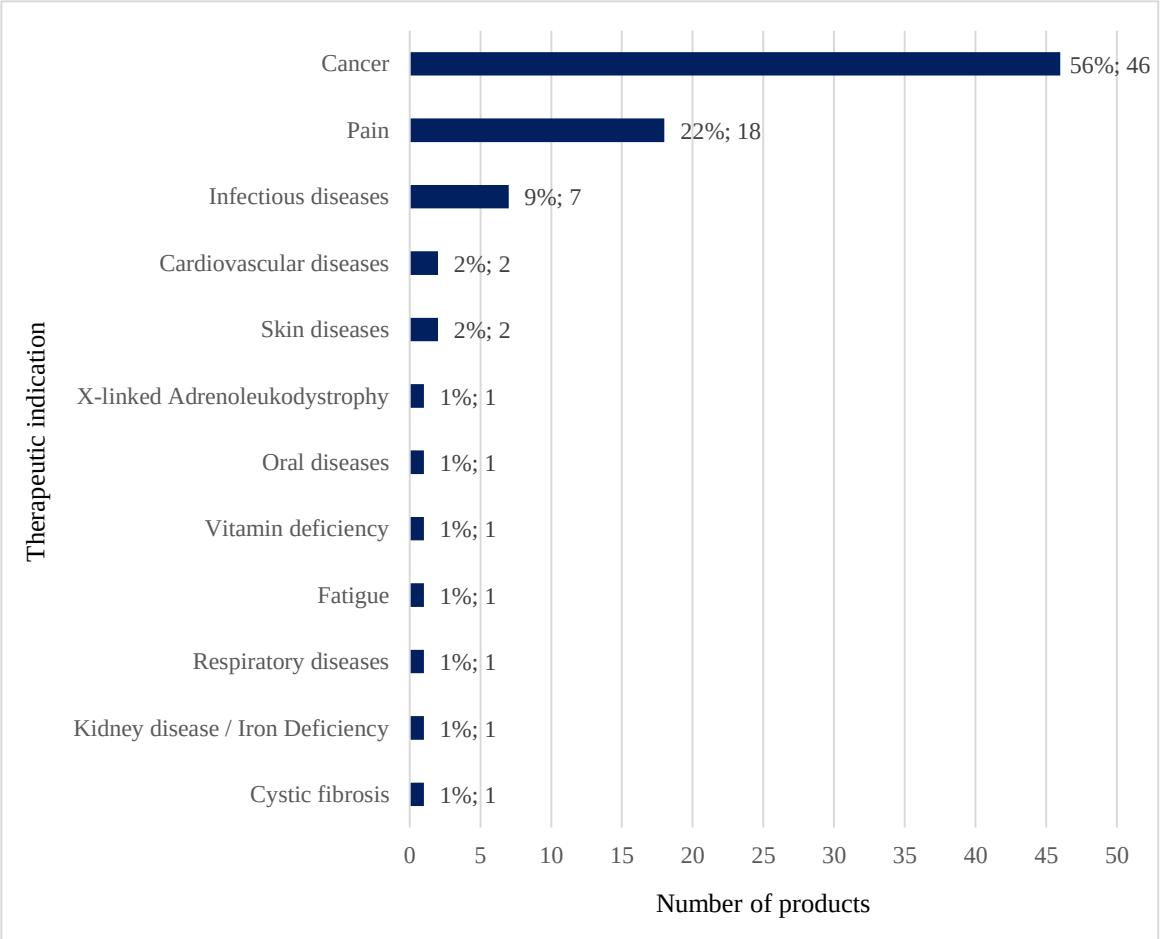

Figure 20. Clinical study classified by therapeutic indication.

5.5. Analysis by Type of Therapeutic Regimen

Most of the clinical trials (n=61, 74%) correspond to single-agent therapies and 21 clinical trials involve the study of multi-agent therapies (n=21, 26%) (Figure 21).

Cancer is the main therapeutic indication identified in clinical trials with a multi-agent therapy regimen (Table 50). This therapy is much more effective than single-agent therapy, due to allowing an increase in drug accumulation in tumors and a decrease in dose intensity and dose-limiting toxicity [225]. Moreover, the drugs widely used for cancer therapy in combination with other drugs are doxorubicin, irinotecan, and paclitaxel (Table 50).

The common objectives listed for clinical trials with doxorubicin (NCT00001059, NCT00944801, and NCT00407888) are: to determine the delivered dose intensity of the regimen and whether the drug combination enhances the disease-free survival; and to evaluate the dose limiting toxicity, but also the incidence and severity of adverse events [225–227].

The clinical studies with irinotecan (NCT02640365, NCT02697058, and NCT03337087) present similar objectives such as: extending plasma circulation and increasing accumulation in the

tumor through the EPR effect; comparing the efficacy of single-agent with multi-agent therapy; and assessing the safety, tolerability and pharmacokinetic profiles of distinct therapies [228–230].

Paclitaxel presents the greatest number of clinical trials with the same concept of combining free drugs and nano-drug delivery systems, as NBCDs [182–184,186,231–233].

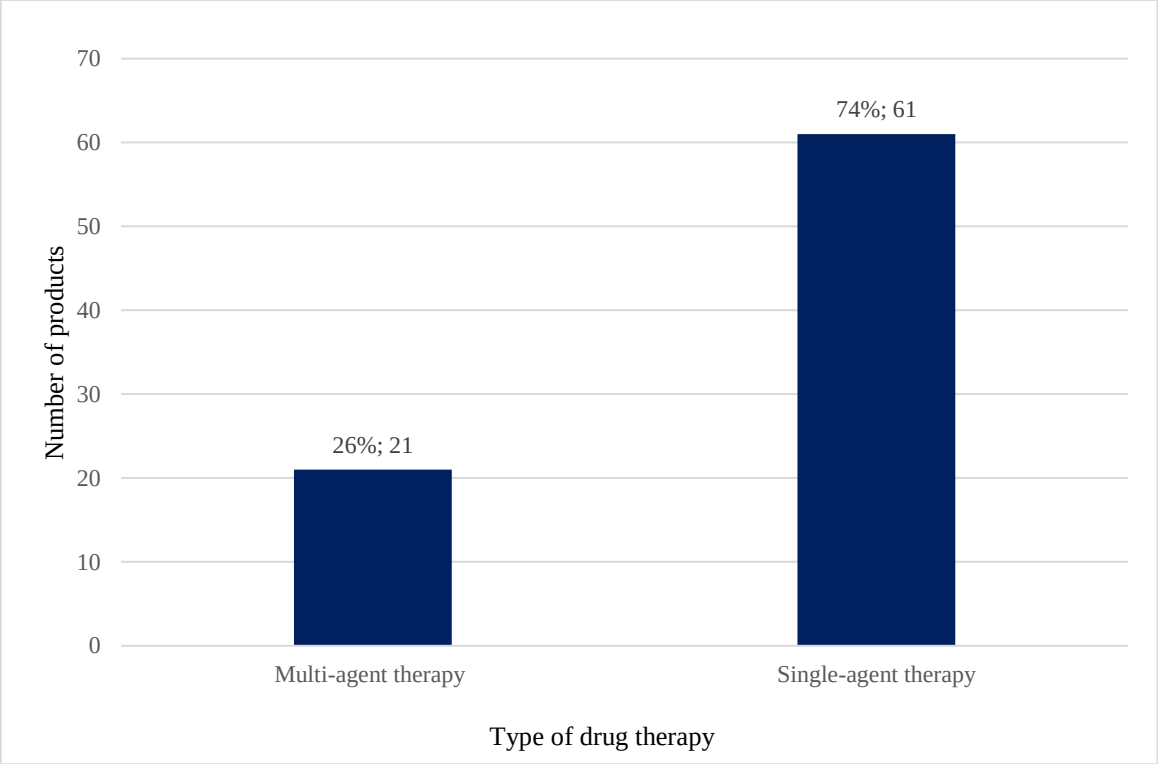

Figure 21. Clinical study classified by therapeutic regimen.

5.6. Analysis by Authors' Affiliation

Now analyzing the type of sponsor, nearly half of the clinical trials (n=34, 41%) were sponsored by companies and the remaining ones were sponsored by non-profit organizations (Universities, research centers, hospitals, etc.) or by strategic partnerships (Figure 22). Examples of these partnerships between two interesting parts are academia with research centers or institutes (n=18, 22%), academia with industry (n=6, 7%), and research centers or institutes with industry (n=5, 6%) (Figure 22).

The companies with a higher number of clinical trials sponsored are as follows: Pacira Pharmaceuticals (n=6), Matinas Biopharma Nanotechnologies (n=5), and Insys therapeutics (n=4) (Table 50). As can be seen in Table 48, Pacira Pharmaceuticals had already 3 products approved by the FDA, such as Depocyt®, Depodur®, and Exparel®.

Out of a total of 17 phase 4 clinical studies, 12 are related to bupivacaine extended-release liposomal injection (Exparel®). What is surprising is that half of Phase 4 clinical trials of bupivacaine extended-release liposomal injection were not sponsored by Pacira (Table 50).

Insys therapeutics and Matinas Biopharma Nanotechnologies currently don´t have any NBCD product on the market. It is also interesting to note that 7 clinical trials were sponsored by Brazillian Universities (University of Campinas, University of Brasilia and University of São Paulo) (Table 50).

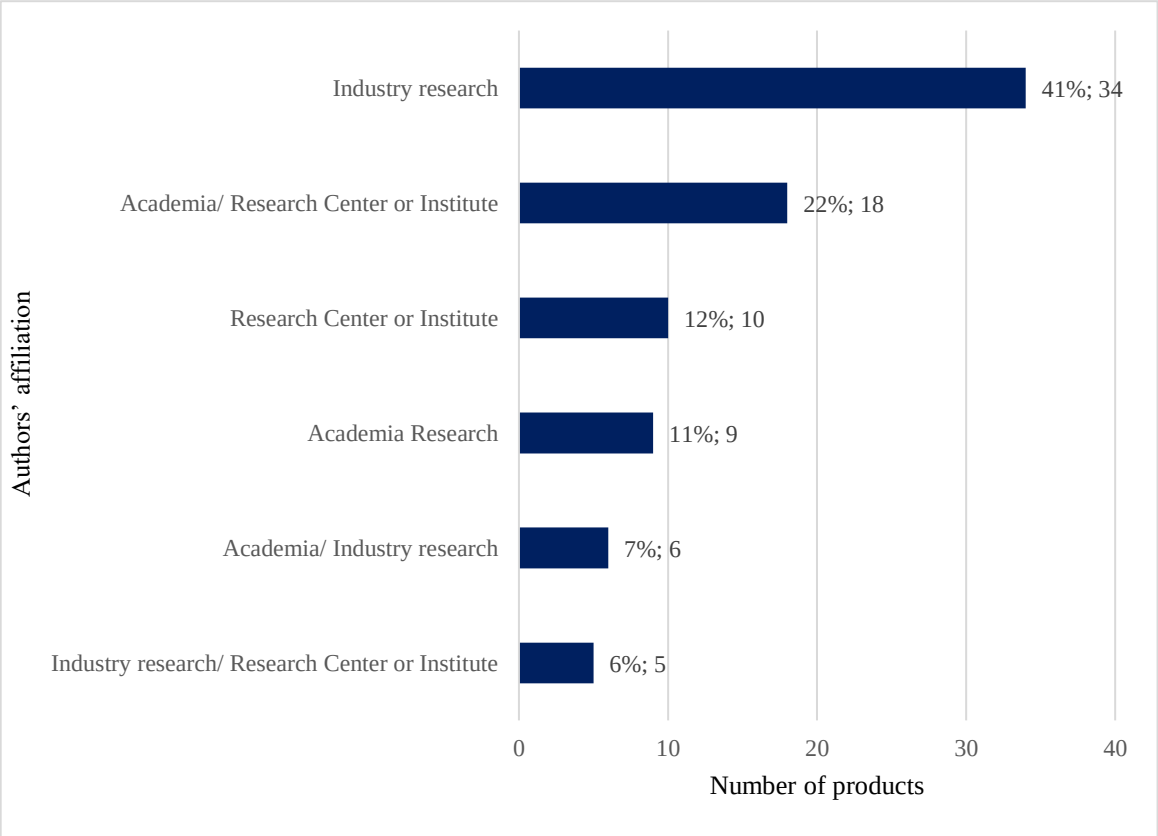

Figure 22. Clinical study classified by the Authors' affiliation.

5.7. Analysis by Status and Phase of Clinical Trials

Clinical studies are classified into four temporal phases according to their objectives: Phase I (Human Pharmacology), Phase II (Therapeutic Exploratory), Phase III (Therapeutic Confirmatory), and Phase IV (Therapeutic Use) [234]. The initial and exploratory trials intend to assess dose-tolerability, side effects, drug metabolism, drug interactions, drug activity, and pharmacokinetic and pharmacodynamic data [234]. Confirmatory studies are larger and comparative trials that establish the efficacy and safety profile, dose-response, and benefit/risk relationship to support licensing [234].

From the 82 studies found, 28 clinical studies are phase 1 (n=28, 34%), 21 are phase 2 clinical studies (n=21, 26%), four are phase 3 studies (n=4, 5%) and 17 correspond to phase 4 studies (n=17, 21%) (Figure 23). Eight clinical trials correspond to phase I/phase II clinical studies (n=8, 10%), and another one was classified as a phase II / phase III clinical trial (n=1, 1%) (Figure 23). Three of the all clinical trials identified have not presented any indication of the study phase (n=3, 4%) (Figure 23). From the information available, it is possible to conclude that most of NBCDs in clinical trials are still in the early stages of development (Phase I or II). This may be due to the fact that just a limited number of NBCDs advance to the next stages of clinical development and reach the market [235]. There are many problems or challenges associated with the failures in clinical trials such as lack of efficacy and safety, problems with resources, time, and funding, and also issues related to patient recruitment and retention [236].

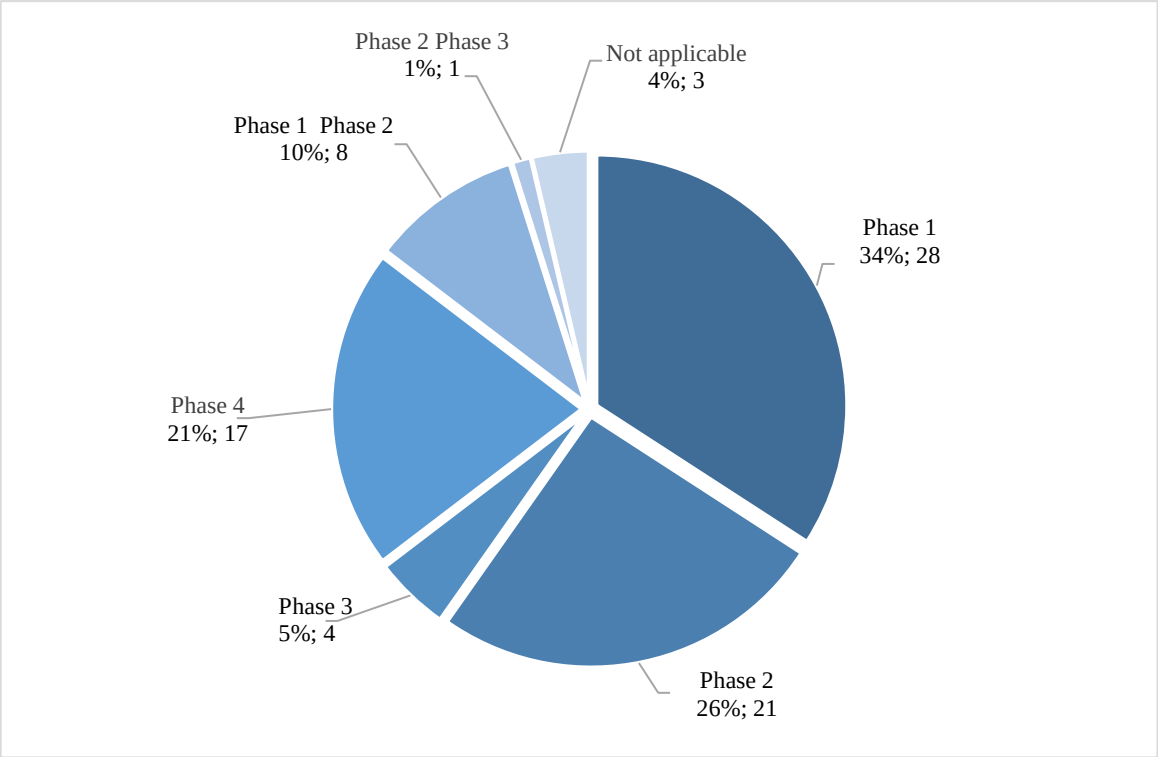

Figure 23. Phase of Clinical Trials with Non-Biological Complex Drugs (NBCDs).

Moreover, in relation to the status of clinical trials, 46 were completed (n=46, 56%), 19 were recruiting (n=19, 23%), six were terminated (n=6, 7%), three were withdrawn (n=3, 4%), not yet recruiting (n=3, 4%) and unknown (n=3, 4%), and only two were active but not recruiting (Figure 24). The greatest number of ongoing clinical studies expands the possibilities of more NBCDs becoming available in the pharmaceutical market with novel and distinct clinical applications.

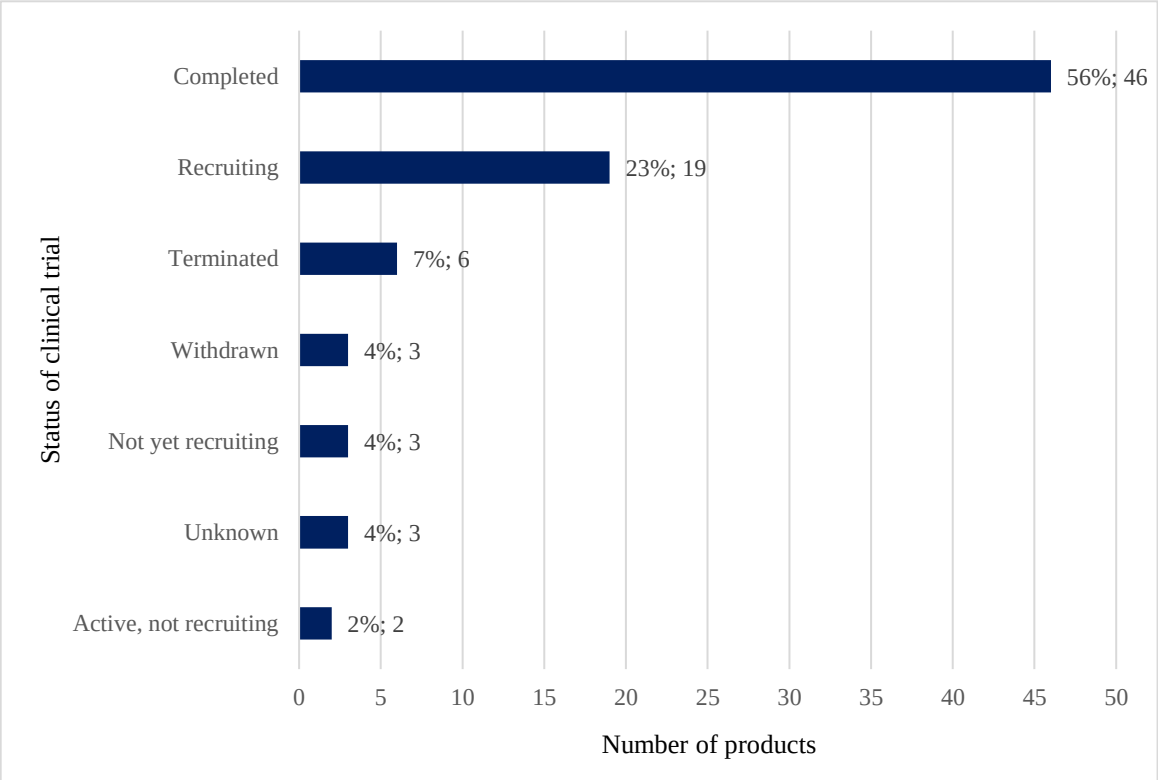

Figure 24. Status of Clinical Trials with Non-Biological Complex Drugs (NBCDs).

6. Concluding Remarks

Over the past years, there is an increasing interest in the development of nanotechnology-based products. A big part of that interest is owed to the high value of the market due to the great advantages of nanomedicines when compared with conventional medicines. Indeed, the conventional therapies could have limitations on the clinical practice, especially because of the severe toxicity for healthy cells and tissues, just as the limited capacity to release active substances to the target tissues in a concentration that allows the desired therapeutic effects.

An increasing number of nanotechnology-based products correspond to the class of Complex Drug Products, particularly Non-Biological Complex Drugs. NBCDs are defined as medicinal products containing mostly nanoparticulate and non-homo-molecular structures, which cannot be isolated and fully quantitated, characterized, or described by available physicochemical analytical means. Their composition, structure, quality, and in vivo performance are highly dependent on the manufacturing process of the active ingredient, just as the formulation (in most cases). The category of NBCDs doesn't fall under the definition of biological complex drug products since they correspond to synthetically derived complex drug products.

The NBCDs comprise a wide range of drug products that present different features and functionalities depending on a design principle, formulation, manufacturing process, composition, and structure. Such class comprises micelles, nanoemulsions, iron-carbohydrate complexes, liposomes, transferosomes, dendrimers, nanoparticles, glatiramoids, nanocrystals, or other products intimately related to nanoparticular structure and properties. The NBCDs are developed for numerous therapeutic indications, such as cancer, iron deficiency, pain management, cardiovascular, infectious, or skin diseases. This diversity shows the importance and impact of NBCDs to address unmet medical needs.

The complexity related to the NBCDs can be derived from the Active Pharmaceutical Ingredient (API), product manufacturing process, route of administration, dosage form, or even the delivery device selected. Most of the NBCDs already on the market or in clinical studies are administered through an intravenous route and are often formulated through the use of the injectable dosage form. These products are often designated as complex injectables belonging to the class of differentiated technologies. In line with this, liposomes are the most common NBCD products that were approved by the regulatory authorities or are under evaluation in clinical trials with expanded medical applications, particularly in the vehiculation of anticancer drugs. The most important reasons behind the success of liposomal formulations are the multiple advantages in terms of preparation, scalability, stability, versatility, and biocompatibility.

In this chapter, it was also possible to identify that the number NBCDs approved on the market is not very extensive at present, probably due to the several scientific and regulatory challenges in

their development and approval procedures. These hurdles will be discussed in the subsequent chapters.

Chapter II. Generic Complex Drug Products: Challenges in Pharmaceutical Development and Marketing Approval

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Abstract

Scientific and technological breakthroughs have been a driving force throughout the development and approval of Complex Drug Products, such as Non-Biological Complex Drugs (NBCDs). Therefore, the complexity and diversity of drug products are increasing at an accelerating pace and so are the questions around their quality, handling, and affordability.

The fast-growing of NBCDs and the advent of their follow-on versions (generic complex drug products) have brought with it diverse challenges for regulatory systems worldwide, which strongly limit the development, evaluation, and marketing approval of high-quality, safe, and effective drug products.

Chapter II critically discusses the emerging trends and specific challenges related to their complexity, such as the lack of standardization of nomenclature, the complicated therapeutic equivalency assessment and interchangeability, the heterogeneity and divergence in the regulatory approaches, the absence of suitable analytical characterization techniques, and so on. Furthermore, this chapter highlights the main needs in terms of regulation, legislation, alignment, and harmonization in developing an improved regulatory strategy adapted to the complexity of NBCDs.

Keywords

Complex Drug Products; Non-Biological Complex Drugs; Complex Generic Drugs; Follow-on Versions; Generic Development; Therapeutic Equivalence; Pharmaceutical Equivalence; Bioequivalence; Interchangeability; Regulatory Approach; Regulatory Compliance; Regulatory Density; Regulatory Science Research.

1. Introduction

The scientific and technological advancements in Nanotechnology and Biotechnology have led to numerous cases of success in the pharmaceutical market with the emergence of novel complex drug products, such as the Non-Biological Complex Drugs (NBCDs). Notwithstanding this success and the increased number of market approvals in the last decades, the pharmaceutical development of complex drug products has been marked by countless challenges in discovery, product development, manufacturing process, clinical evaluation, regulatory approval, and lifecycle management. These challenges become even more prevalent in the development of its copy versions, also referred to as 'follow-on products' or 'complex generics', with unproductive efforts and high failure rates from the earliest steps of the manufacturing process to the clinical development.

As described in the Generic Drug User Fee Amendments (GDUFA) II Commitment Letter of the U.S. Food and Drug Administration (FDA), complex generics correspond to generic versions of drug products that generally present a complex active ingredient(s) (e.g., peptides, polymeric compounds, complex mixtures of APIs, naturally sourced ingredients), a complex formulation (e.g., liposomes, colloids), a complex route of delivery (e.g., locally acting drugs such as dermatological products and complex ophthalmological products and otic dosage forms that are formulated as suspensions, emulsions or gels), or a complex dosage form (e.g., transdermals, metered-dose inhalers, extended-release injectables). This definition also covers complex drug-device combination products (e.g., auto-injectors, metered-dose inhalers), and other products where complexity or uncertainty concerning the approval pathway or possible alternative approaches would benefit from early scientific engagement [16].

On the other hand, complex generics are considered 'hybrid medicines' by the European Medicines Agency (EMA), which defines them as 'medicines whose authorization depends partly on the results of tests on the reference medicine and partly on new data from clinical trials'. In order to meet this definition, the manufacturer develops a generic medicine that is based on a reference medicine but has a different strength, a different route of administration, or a slightly different indication from the reference medicine [16].

The complex generics assume an increasingly prominent and differentiating position in the pharmaceutical market, assisting in the response to address unmet medical needs, providing enhanced patient access, just as providing cost savings to the healthcare systems. In addition to cheaper products than their reference products (innovators), the complex generics add financial value and significant opportunities for business and economic growth for pharmaceutical companies.

For that purpose, the complex generics need to surpass the strong intellectual property barriers of innovator drug products, the difficulty in the therapeutic equivalence assessment, establishment

of in vitro and in vivo assessment, complicated physicochemical, functional, and structural characterization, safety evaluation, demonstrate structural or device sameness, laborious and relatively complex manufacturing process, batch-to-batch reliability and reproducibility, biocompatibility, biodistribution and toxicity issues, final sterilization and scale-up problems, long-term stability issues, lack of precise in-process control methods, regulatory uncertainty in the approval process, among others [2,12,19,22,34,132,237–239]. The main underlying challenges of the pharmaceutical development of complex generics are summarized in Figure 25. Different sources of complexity can be distinguished in the process of development of NBCDs, such as the complexity derived from materials, formulation, manufacturing process, therapeutic equivalence assessment, and regulatory approval procedures.

Contrarily to the development of standard generic products, the complex generics require a higher level of expertise, more planning and intensive development process, as well as, a deep understanding of the regulatory environment and quality assessment. As referred to in the meeting 'FDA kicks off GDUFA III reauthorization process', the complex generic drug products are harder to 'genericize' and often have less market competition [240]. The absence of a consistent, well-defined, and science-based regulatory pathway in response to particular, and unique properties of complex generics of NBCDs promotes high regulatory uncertainty for drug developers, creates vulnerabilities for potential drug shortages, and therefore compromises the patient access to safe, affordable, quality, and effective drug products [167].

This chapter aims to discuss and deepens the several challenges involved in the pharmaceutical development and marketing authorization procedures of NBCDs and their follow-on versions. Accordingly, the chapter also deals with the complexity and heterogeneity of NBCDs and their implications on the therapeutic equivalence assessment of complex generics, just as the absence of harmonization between regulatory authorities in different places worldwide. Another aim includes a brief discussion of the reflection papers and (draft) guidance documents published by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), which may be related or applied to the pharmaceutical development of NBCDs and their follow-on versions. The whole discussion around the NBCDs is crucial for creating clear and suitable regulatory approaches for the development of complex generic drug products.

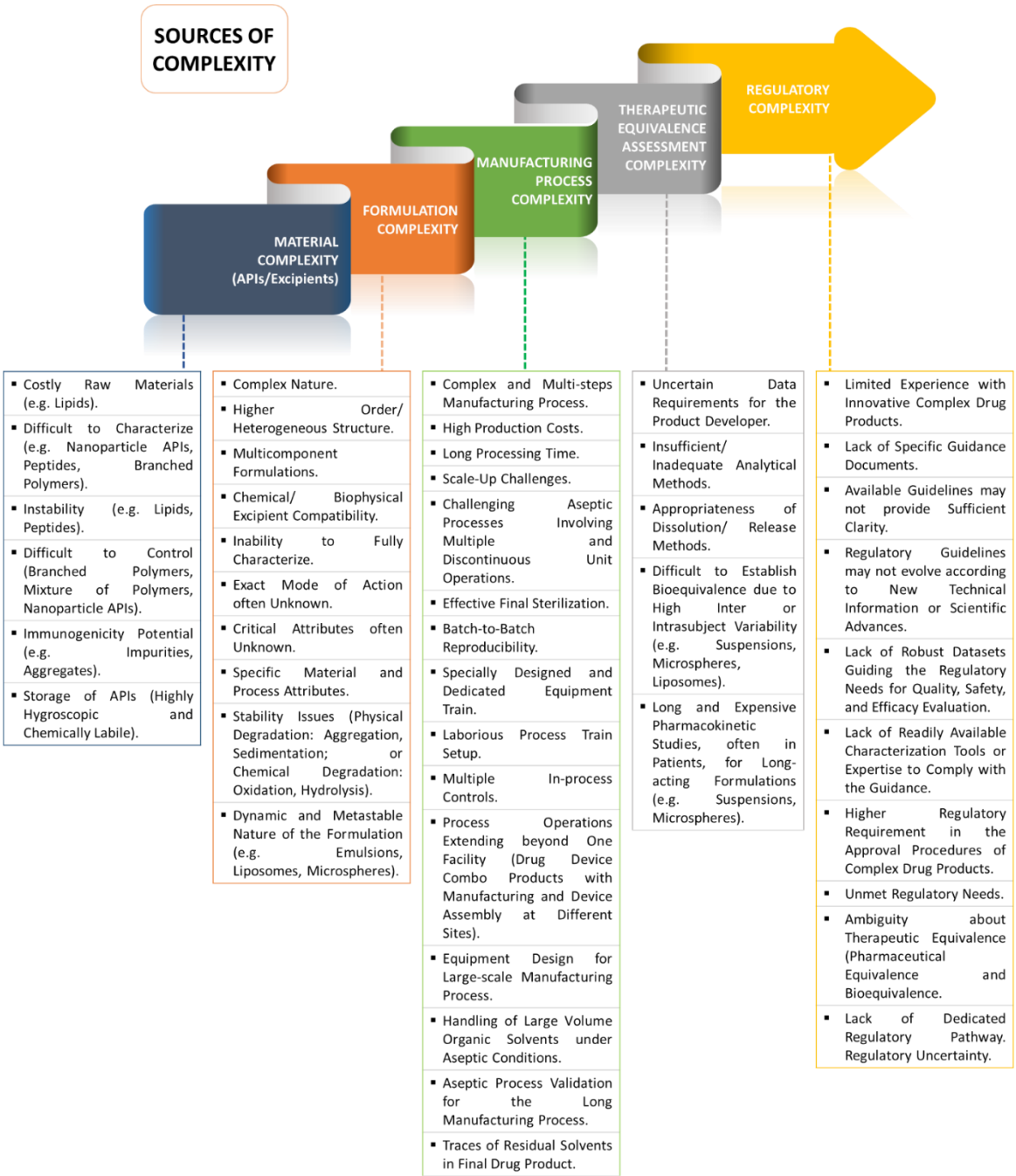

Figure 25. Sources of Complexity related to the Pharmaceutical Development of Complex Generic Drug Products.

2. Regulatory Challenges in Pharmaceutical Development of NBCDs and Follow-On Versions

2.1. Positioning NBCDs Families on the Nanomedicine Field: Need to Harmonize the Terminology

As already stated in the previous chapter, certain classes of nanotechnology-based products correspond to the NBCDs. With the advance of new and innovative technologies, has arisen a whole set of different and non-harmonized terms, definitions, and concepts worldwide. Until recently there were no established and standardized regulatory definitions for 'nanotechnology', 'nanotechnology-based products', 'nanomaterial', 'nanoscale', 'nanomedicines', 'nano drugs', or other related terms [1,241].

Likewise, the regulatory agencies do not provide any official definition for NBCDs, just as does not consider NBCDs as a distinct category of complex drug products [132,138]. The NBCD products display several particular characteristics that can be easily distinguished from Small Molecule Drugs and don't fall under the definition of biologicals either since they aren't derived from living material [132,138]. Therewith, the definition of NBCDs is solely described in the scientific literature [17,22,24,31]. This makes challenging the categorization of different types of NBCDs, as well as, the definition of the respective regulatory approaches. The lack of harmonized terminology can give rise to the multiple interpretations of guidance documents, reflection papers, and regulatory requirements, the application of a distinct regulatory approach, or the non-compliance with quality, efficacy, and safety properties [142]. Despite their diversity, each subclass should be uniformly and precisely defined, to avoid the inherent ambiguity that exists regarding their classification [4,25,242]. Consequently, there must be a constant struggle to obtain the exact meaning of the terms used for complex drug products and proposals for standardization, with a clear distinction between the terminology of biological complex drug products and NBCDs in different jurisdictions.

Standardized terminology is a primordial requisite for the success of global harmonization of regulatory requirements and procedures, and consequently to the approval and market access of nanomedicines. The article 'Different Pharmaceutical Products Need Similar Terminology' published by Crommelin *et al* reflects this need and offers a proposal for some designations globally accepted. This article aimed to describe the meaning of relevant terms in several jurisdictions (EMA, FDA, WHO) that need to be harmonized, and hence, reach a global consensus regarding the terminology among multiple stakeholders [28]. Also, the article doesn't only refer to the concept of NBCDs, but other terms and definitions related to the therapeutic equivalence assessment [28]. As mentioned by *Crommelin et al.*, 'the pharmaceutical 'rules of engagement' are more and more

becoming global. The use of common and accepted terminology is the first requirement for the global harmonization of regulatory rules and actions. It is critically important for authorities, health care professionals, scientific experts, and patients to have one unified terminology to guarantee consistent quality and use of generic versions of complex innovator products' [28].

2.2. Hard-to-Access the Demonstration of Therapeutic Equivalence

The 'Drug Price Competition and Patent Term Restoration Act of 1984', also referred to as the Hatch-Waxman Act, led to the promotion and development of a regulatory pathway for the approval of generic drug products by the FDA [28,243,244]. According to FDA, a generic drug is 'a medication created to be the same as an existing approved brand-name drug in dosage form, safety, strength, route of administration, quality, and performance characteristics' [245]. In the same way, the EMA defines that 'a generic medicine contains the same active substance(s) as the reference medicine, and it is used at the same dose(s) to treat the same disease(s)' [16]. The marketing authorization of Small Molecule Drugs is obtained through the generic drug pathway and requires a demonstration of Therapeutic Equivalence (TE) to the Reference product (innovator), i.e., based on proof of the Pharmaceutical Equivalence (PE) and Bioequivalence (BEq) (Figure 26). Therefore, the Reference product and follow-on versions are PE and BEq, and consequently therapeutically equivalents and interchangeable, with the possibility of being automatically replaced among themselves [2,18,25,132,164].

Pharmaceutical equivalence (PE) requires that the follow-on versions present the same active ingredient(s), dosage form, route of administration, strength, concentration as the reference product, and that it meets the compendial or other applicable standards of quality, purity, and identity [20,136,137,164]. On the other hand, the main aim of the bioequivalence is to demonstrate that there is no significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (21 CFR 320.1(e)) [246].

The regulatory procedures for developing and approval of the generic versions of Small Molecule Drugs with a well-described molecular structure are correctly defined and established in many places across the world. Thus, is important to emphasize that the generic drug pathway is considered a solid and well-established regulatory framework generally applied to Small Molecule Drugs when the molecular structure is known and might be fully reproduced and characterized, and when the physical-chemical characteristics predict the biological effects, and the pharmacokinetic data can be used as a substitute for clinical efficacy (Figure 26) [22,31,132].

However, the NBCDs are fairly complex, and unlike follow-on versions of Small Molecule Drugs, a proper demonstration of therapeutic equivalence is considerably more challenging or even

impossible [14,17,19,25]. Whereas for the small-molecule drugs can be applied the term equal or identical according to the generic drug pathway, it is more appropriate to use the term ‘similar’ or ‘quasi-similar products’ for follow-on versions of the complex drug products (Figure 26) [22].

Differences in the complex heterogenous structures, physicochemical characteristics, form, size, manufacturing process, clinical efficacy, and safety profile, unknown mode of action, uncertain regulatory data requirements, or lack of proper analytical methods, constitute important hurdles that limit or delay the development and market access of complex drugs [19,27,34,132,247]. Consequently, the NBCD products are recognized for presenting a special position in the regulatory landscape (Figure 26). It is well established that the conventional approach (generic drug pathway) may not be appropriate for NBCDs, due to the potential safety and efficacy problems in clinical practice [14,25]. If it is not possible to obtain a complete pharmaceutical characterization and the comparability of bioequivalence, the generic paradigm must not be applied, it is necessary to determine the extent of the similarity [2]. The lack of comparative safety and efficacy clinical data makes the demonstration of equivalence unsuitable and increases the regulatory uncertainty relating to the overall risk-benefit and patient safety assessment [239]. The clinical problems closely linked with the interchangeability decisions are also related to the lack of the appropriate level of clinical evidence, non-recognition of the inherent complexity of NBCDs, the diversity of regulatory approaches that can be applied, as well as, the only use of the common INN-based approach (International Nonproprietary Name) that hinder the perception of the relationship between an adverse effect and the responsible drug product (reference product or follow-on version) [136,239]. Contrary to the biological drug products, the NBCDs do not fall under the European centralized pharmacovigilance procedures, once they are not required to be identifiable by brand name and batch number in reports of adverse reactions. This is quite problematic, as it makes it more difficult to identify possible differences in the safety and efficacy profiles of NBCD products from different manufacturers [136,239,248]. Other important questions focus on the possibility to consider the biosimilar approach, based on the ‘totality of evidence’ (including physicochemical, non-clinical, and clinical studies), as a guiding principle for the development and approval of NBCD follow-on versions, often involving even more complex structures than biosimilars (Figure 26) [2,31,132]. Evaluation of the present state of the legislation for the NBCDs and their follow-on versions, just as the disparities in existing regulatory pathways and lack of harmonization between the EMA and FDA will be discussed in more detail in the next chapter.

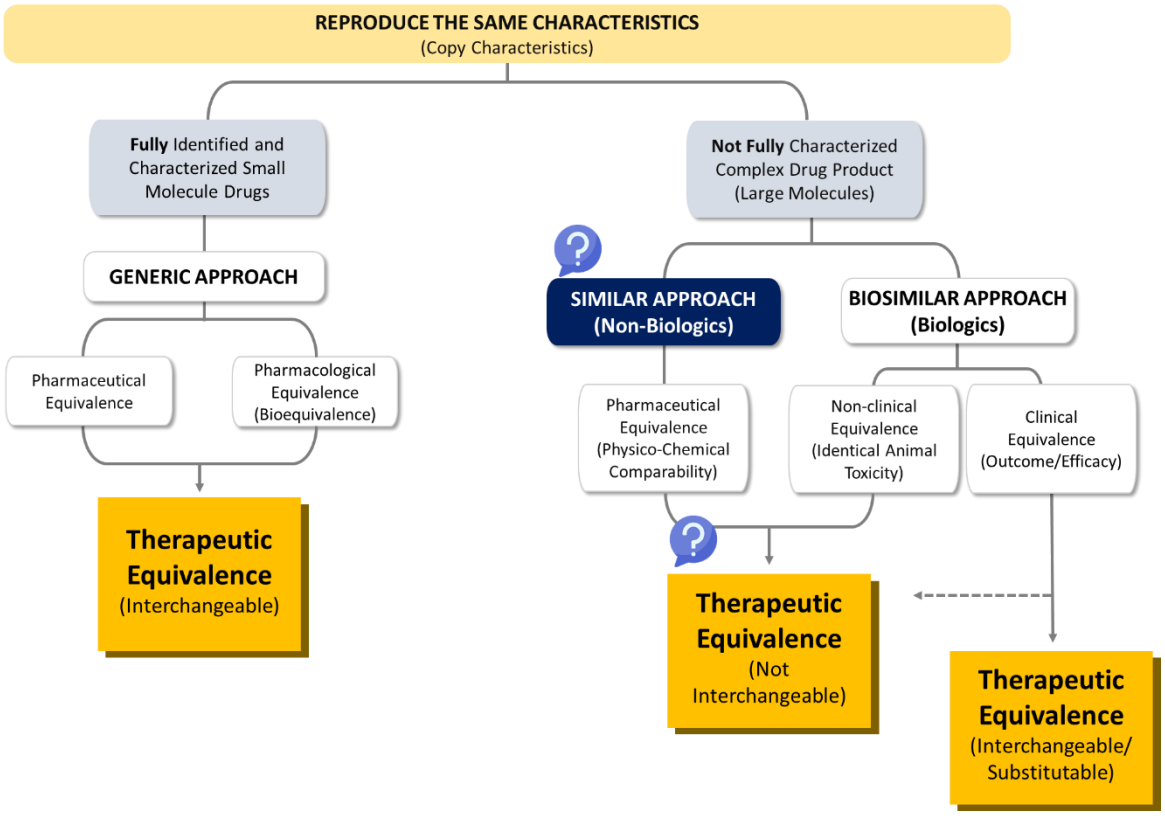

Figure 26. Therapeutic equivalence assessment for small and complex drug products (adapted from [31,132]).

The complexity associated with each type of drug product is responsible for the definition of the degree of risk and uncertainty related to the different stages of the pharmaceutical development and approval procedures of follow-on versions. The higher the risk and uncertainty level, the greater will be the regulatory density linked to that product, i.e., the relative quantity of standards, regulatory requirements, measures, resources, clinical, epidemiological, and statistical tools, or information gathering systems allocated to each regulatory procedure, including in the post-marketing phase [237].

Until now, the regulatory thinking and consequent application of a specific regulatory pathway were made on the basis of the identification of the product category to develop (e.g. generic pathway for Small Molecule Drugs). However, the NBCDs comprise a range of diverse medicinal products with different complexities, tackling varying degrees of risk and uncertainty, wherefore the strict categorical pathways may not be necessarily sufficient to address the complexity and large variability between drug products that belong to the same regulatory category [237].

For a better understanding of the hard-to-access demonstration of Therapeutic Equivalence, Figure 27 presents the positioning of the drug products in accordance with their degree of risk and

uncertainty to demonstrate the pharmaceutical equivalence (PE) and bioequivalence (BEq) between the reference product and their follow-on version.

Through Figure 27 it is possible to verify that for the small molecule drug products (shown in green) the assessment of the PE and BEq is reasonably straightforward, due to the possibility to be exactly reproduced and fully characterized. On the other hand, for biological complex drug products (shown in yellow) the demonstration of the PE and BEq is slightly more difficult than for Small Molecule Drugs. However, despite the biological medicinal products corresponding to a category with a great product diversity (e.g. small-sized recombinant peptides, large complex recombinant monoclonal antibodies, or recombinant coagulation factors), this demonstration is relatively more simple than other complex drug products with high molecular weight, such as the NBCDs. This is due to the definition, classification, and regulatory basis of biological complex drug products being well established compared with the NBCDs (shown in blue). Thus, Figure 27 shows that the demonstration of PE and BEq of most of the NBCDs are challenging, especially due to their structural complexity, sensitivity to changes in the physicochemical and functional properties, sophisticated manufacturing methods, difficulty in fully characterizing, lack of proper analytical methods, unknown mode of action, or uncertain regulatory data requirements [34,237,247]. Consequently, NBCDs show the highest degree of regulatory density (Figure 27).

It must be highlighted that some complex drug products, such as the albumin-bound nanoparticles for injectable suspension or the low-molecular-weight heparins (LMWHs), are classified in different ways in distinct jurisdictions. These complex drug products might not entirely fall under the definition of NBCDs but share many of their features [132]. Accordingly, the albumin-bound nanoparticles and LMWHs are considered biological medicinal products by the EMA and complex drug products by the FDA [31,34,138]. For this reason, these products present the color blue with a yellow outline (Figure 27). This constituted a huge challenge, as it leads to the application of different regulatory requirements and approval pathways in different parts of the world and, as a consequence, a high regulatory heterogeneity.

Therewith, the high level of regulatory density of NBCDs has led to growing concerns about the adequacy of current available regulatory approaches to face the development challenges of these highly complex drug products, which may request the creation of a more flexible, personalized, and effective regulatory approach.

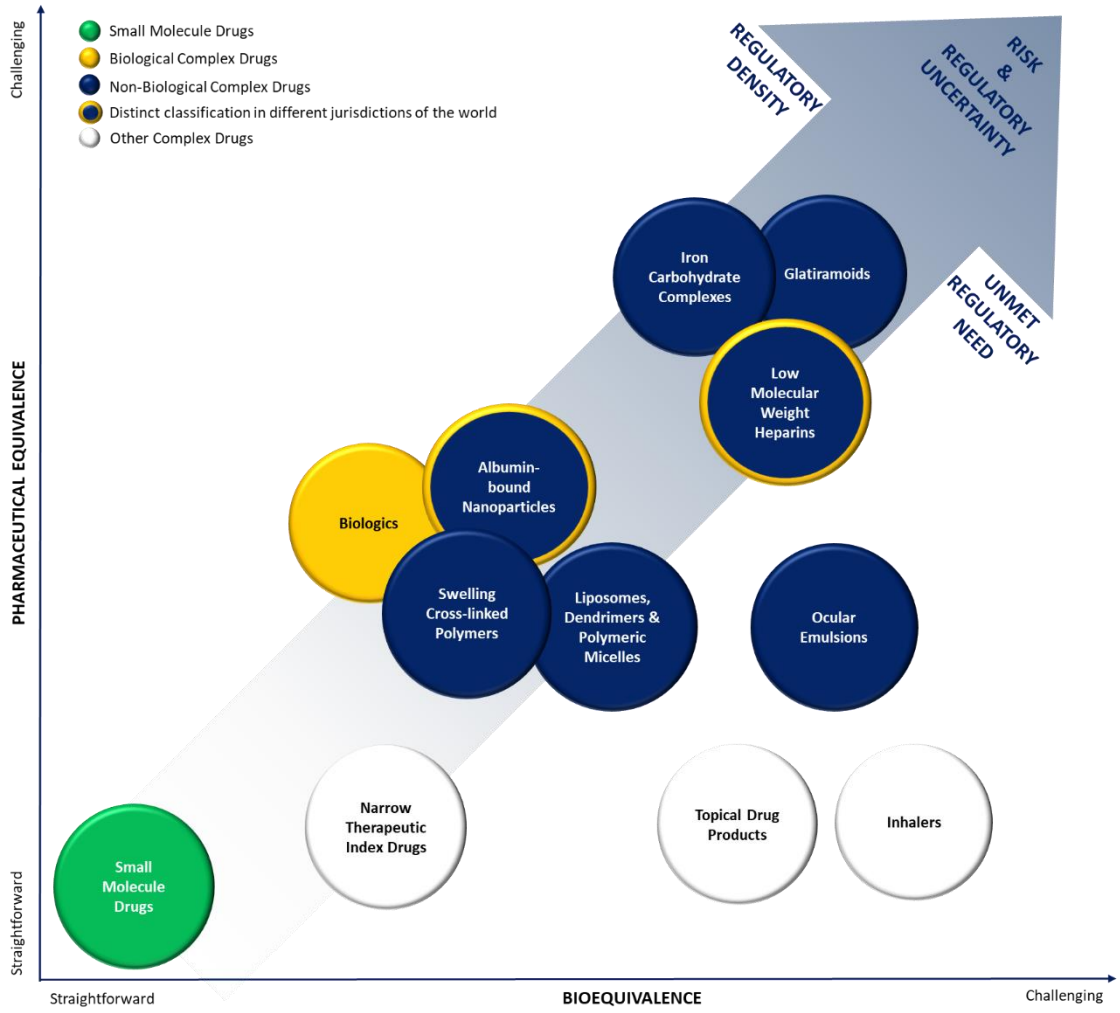

Figure 27. The positioning of the products in accordance with the challenge to demonstrate the pharmaceutical equivalence (PE) and bioequivalence (BEq) between the reference product and their follow-on version (adapted from [34,237]).

2.3. Regulatory Guidance Issuance

The growing awareness of the different stakeholders about the need to develop and harmonize the regulatory requirements for NBCDs and follow-on versions should be accompanied by an increase in the guidance documents or reflection papers published worldwide [17,143]. The publication of guidelines/reflection papers plays a central role to improve the understanding of pharmaceutical development for each type of NBCDs, establish the science-based regulatory approaches, clarify regulatory expectations early in product development, assisting applicants to develop more complete submissions, making the review process of regulatory submissions more efficient and effective, help to reduce the number of review cycles required to obtain market approval, and consequently increase patient access by potentially faster approval procedure [14,16,22,249]. Knowing and understanding the principles and recommendations included in the guidance documents constitute a powerful lever for the beginning of pharmaceutical development for any drug product.

However, a major challenge confronting the development and approval of NBCDs is the lack of specific guidance documents and regulatory approaches successfully defined and established for each type of NBCDs. Part of the difficulty to create and establish suitable regulatory guidance documents arises from the lack of scientific expertise and limited knowledge about the NBCDs, wherefore the existing procedures may not be sufficient to afford the patient safety and regulate the use of complex products in a clinical setting [250]. Thus, these regulatory authorities have not published any guidelines with the term NBCDs but developed a set of (draft) guidance documents and reflection papers that can be applied to certain families of NBCDs and their follow-on versions [251,252]. Table 2 and

Table 3 include a brief summary of the reflection papers and (draft) guidance documents published by the FDA and EMA respectively, which may be related or applied to the pharmaceutical development of complex drug products and their follow-on versions. These tables also comprise the classification of the published documents according to the year of publication and the main focus of them.

A common example of overarching guidance applied to the pharmaceutical development of liposomal formulations and their follow-on versions is the ‘FDA Guidance for Industry: Liposome Drug Products - Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation’ [253,254]. The discussion in this guidance focus on the liposome drug products and the kind of information needed for submission of a new drug application (NDA) or abbreviated new drug application (ANDA) [253]. Topics covered in the guidance included: (A) chemistry, manufacturing, and controls (CMC); (B) human pharmacokinetics and bioavailability or, in the case of an ANDA, bioequivalence; and (C) labeling in NDAs and ANDAs [253,254].

On the other hand, the FDA published a number of new and revised product-specific guidances (PSGs) documents to support the development and approval of safe and effective complex generic drug products, such as: Draft Guidance on sirolimus (2005), nabilone (2008), sevelamer hydrochloride (2008), fenofibrate (2008), doxorubicin hydrochloride (liposomal injection) (2010), morphine sulfate (2010), enoxaparin sodium (2011), sevelamer carbonate (2011), paliperidone palmitate (2011), dalteparin sodium (2012), paclitaxel (2012), ferumoxytol (2012), iron sucrose (2012), budesonide (2012), sodium ferric gluconate complex (2013), cyclosporine (2013), daunorubicin citrate (liposomal injection) (2014), lidocaine-prilocaine (2014), verteporfin (liposomal injection) (2014), amphotericin B (liposomal injection) (2014), propofol (2016), ferric carboxymaltose (2016), iron dextran (2016), glatiramer acetate injection (2016), aprepitant (2017), perflutren (2018), estradiol hemihydrate (2018), bupivacaine (liposomal injection) (2018), and sulfur hexafluoride lipid-type A microsphere (2018) [18,22,138,140,205–208,251,255–279]. It should be highlighted that the FDA publishes PSGs to assist the generic pharmaceutical industry/developers with identifying the most appropriate methodology and evidence needed to support a specific complex generic drug's development and approval [280]. More specifically, the information contained in the PSGs for complex generic drug development address the current thinking on in vivo bioequivalence (BEq) approaches that are more challenging to conduct for this class of drug products, covering such recommended number of studies, type of study, study design, study population, parameters to measure, fasting conditions, analyte(s) to measure, appropriate biological matrix, among others [280]. In this way, in assessing the planned revised PSGs for complex generic drug products, it is possible to verify that the revisions are focused mainly on the BEq issues, such as 'harmonize the language for BEq recommendations across similar PSGs in alignment with the general guidances' [280]. However, these product-specific guidance documents do not make any reference to the regulatory approval pathway that should be applied for each situation.

In the same way as the FDA, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) presents specific reflection papers to communicate its current state of regulatory thinking regarding the iron-based nano-colloidal products (2015), intravenous liposomal products (2013), intravenous medicinal products containing active substances solubilized in micellar systems (2012), coated nanomedicine products (2013), and block copolymer micelle medicinal products (2013) [161,162,252,281–285].

Table 2. Guidance Documents published by the FDA related to Pharmaceutical Development of Complex Drug Products.

US Guidance documents (FDA)	Type of Guidance	Year	Reference
Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice	Pharmaceutical development	2004	[286]
Draft Guidance on Sirolimus	Product-specific guidance document	2005	[255]
Draft Guidance on Nabilone	Product-specific guidance document	2008	[256]
Draft Guidance on Sevelamer Hydrochloride	Product-specific guidance document	2008	[257]
Draft Guidance on Fenofibrate	Product-specific guidance document	2008	[258]
Draft Guidance on Doxorubicin Hydrochloride (liposomal injection)	Product-specific guidance document	2010	[206]
Draft Guidance on Morphine Sulfate	Product-specific guidance document	2010	[259]
Draft Guidance on Enoxaparin Sodium	Product-specific guidance document	2011	[260]
Draft Guidance on Sevelamer Carbonate	Product-specific guidance document	2011	[261]
Draft Guidance on Paliperidone Palmitate	Product-specific guidance document	2011	[262]
Draft Guidance on Dalteparin Sodium	Product-specific guidance document	2012	[263]
Draft Guidance on Paclitaxel	Product-specific guidance document	2012	[205]
Draft Guidance on Ferumoxytol	Product-specific guidance document	2012	[264]
Draft Guidance on Iron Sucrose	Product-specific guidance document	2012	[265]
Draft Guidance on Budesonide	Product-specific guidance document	2012	[266]
Guidance for Industry: Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA	Therapeutic Equivalence	2013	[287]
Draft Guidance on Sodium Ferric Gluconate Complex	Product-specific guidance document	2013	[267]
Draft Guidance on Cyclosporine	Product-specific guidance document	2013	[268]
Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology	Nanotechnology	2014	[1]
Draft Guidance on Daunorubicin Citrate (liposomal injection)	Product-specific guidance document	2014	[269]
Draft Guidance on Lidocaine - Prilocaine	Product-specific guidance document	2014	[270]
Draft Guidance on Verteporfin (liposomal injection)	Product-specific guidance document	2014	[271]
Draft Guidance on Amphotericin B (liposomal injection)	Product-specific guidance document	2014	[208]
Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product	Biosimilarity	2015	[288]
Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product	Biosimilarity	2015	[289]
Draft Guidance on Propofol	Product-specific guidance document	2016	[272]
Draft Guidance on Ferric Carboxymaltose	Product-specific guidance document	2016	[273]

Draft Guidance on Iron Dextran	Product-specific guidance document	2016	[274]
Guidance for Industry: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product	Biosimilarity	2016	[290]
Draft Guidance on Glatiramer Acetate Injection	Product-specific guidance document	2016	[275]
Guidance for Industry: Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA	Generics	2017	[16]
Guidance for Industry: ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin	Generics	2017	[291]
Draft Guidance for Industry: Comparative Analyses and Related Comparative Use Human Factors Studies for a Drug-Device Combination Product Submitted in an ANDA	Generics	2017	[292]
Guidance for Industry: Drug Products, Including Biological Products, that Contain Nanomaterials	Pharmaceutical Quality	2017	[10]
Draft Guidance on Aprepitant	Product-specific guidance document	2017	[276]
Guidance for Industry: Statistical Approaches to Evaluate Analytical Similarity	Biosimilars	2017	[293]
Guidance for Industry: Assessing Adhesion with Transdermal Delivery Systems and Topical Patches for ANDAs	Generics	2018	[294]
Guidance for Industry: Assessing the Irritation and Sensitization Potential of Transdermal and Topical Delivery Systems for ANDAs	Generics	2018	[295]
Guidance for Industry: Liposome Drug Products - Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation	Pharmaceutical Quality	2018	[253]
Draft Guidance on Perflutren	Product-specific guidance document	2018	[277]
Draft Guidance on Estradiol Hemihydrate	Product-specific guidance document	2018	[278]
Draft Guidance on Bupivacaine (liposomal injection)	Product-specific guidance document	2018	[207]
Draft Guidance on Sulfur Hexafluoride Lipid-type A Microsphere	Product-specific guidance document	2018	[279]
Guidance for Industry: Questions and Answers on Biosimilar Development and the BPCI Act Guidance for Industry	Biosimilars	2018	[296]
Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products	Biosimilars	2018	[297]
Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs - General Considerations	Clinical development	2019	[246]
Guidance for Industry: Considerations in Demonstrating Interchangeability With a Reference Product	Biosimilars	2019	[298]
Guidance for Industry: Determining Whether to Submit an ANDA or a 505(b)(2) Application	Generics	2019	[299]
Guidance for Industry: Competitive Generic Therapies	Generics	2020	[300]

Table 3. Guidance Documents published by the EMA related to Pharmaceutical Development of Complex Drug Products.

EU Guidance documents (EMA)	Type of Guidance	Year	Reference
Specifications and control tests on the finished product	Pharmaceutical Quality	1991	[301]
Note for Guidance on Pharmacokinetics: Repeated dose tissue distribution studies (ICH Topic S3B)	Clinical development	1995	[302]
Note for Guidance on general considerations for clinical trials (ICH Topic E8)	Clinical development	1998	[303]

Note for Guidance on duration of chronic toxicity testing in animals (rodent and non-rodent toxicity testing)	Clinical development	1999	[304]
Note for Guidance ICH Q6A specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances	Pharmaceutical Quality	2000	[305]
Note for Guidance on safety pharmacology studies for human pharmaceuticals	Clinical development	2001	[306]
Annex II to note for Guidance on Process validation: non-standard processes	Pharmaceutical Quality	2004	[307]
Note for guidance on Biotechnological/Biological Products subject to changes in their manufacturing process (ICH Q5E)	Pharmaceutical development	2005	[308]
Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product	Pharmaceutical development	2007	[309]
Reflection paper on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products.	Clinical development	2007	[310]
Comparability of biotechnology-derived medicinal products after a change in the manufacturing process; non-clinical and clinical issues	Clinical development	2007	[311]
Guideline on clinical evaluation of diagnostic agents.	Clinical development	2009	[312]
Guideline on the investigation of bioequivalence.	Therapeutic Equivalence	2010	[313]
Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications	Product-specific guidance document	2011	[281]
ICH guideline Q11 on development and manufacture of drug substances (chemical entities and Biotechnological/Biological entities)	Pharmaceutical Quality	2011	[314]
Reflection paper on the pharmaceutical development of intravenous medicinal products containing active substances solubilised in micellar systems	Product-specific guidance document	2012	[282]
Reflection paper on considerations given to designation of a single stereo isomeric form (enantiomer), a complex, a derivative, or a different salt or ester as new active substance in relation to the relevant reference active substance.	Pharmaceutical development	2012	[315]
Reflection paper on surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products	Product-specific guidance document	2013	[283]
Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product	Product-specific guidance document	2013	[284]
Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products	Product-specific guidance document	2013	[285]
Concept paper on the need for a reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development	Biosimilars	2013	[316]
Guideline on similar Biological Medicinal Products containing biotechnology-derived proteins as active substance: quality issues	Biosimilars	2014	[317]
Guideline on similar Biological Medicinal Products	Biosimilars	2014	[318]
Guideline on similar Biological Medicinal Products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues	Biosimilars	2014	[319]
Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product	Product-specific guidance document	2015	[161]

Guideline on non-clinical and clinical development of similar Biological Medicinal Products containing low-molecular-weight-heparins	Product-specific guidance document	2016	[320]
Guideline on manufacture of the finished dosage form	Pharmaceutical development	2017	[321]
ICH guideline Q8 (R2) on pharmaceutical development	Pharmaceutical development	2017	[322]
Guideline on the requirements for the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials	Pharmaceutical Quality	2017	[323]
Guideline on clinical development of fixed combination medicinal products	Clinical development	2017	[324]
Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development	Pharmaceutical Quality	2017	[325]
Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance	Product-specific guidance document	2018	[158]
European Medicines Agency procedural advice for users of the Centralised Procedure for similar Biological Medicinal Products applications	Biosimilars	2019	[326]
European Medicines Agency procedural advice for users of the Centralised Procedure for generic/hybrid applications	Generics	2019	[327]

Despite the attempt to increase the dissemination of regulatory guidelines, the FDA issued the relevant product-specific guidance documents much later than the approval of generic versions. Table 4 provides some examples of the chronological gap that exists between the generic application submission and approval of NBCDs products and the corresponding product-specific FDA guidance issuance. For example, the submission of the first approved generic application for glatiramer acetate injection occurred in December 2007, the first generic approval arose in April 2015, whereas the first product-specific guidance was only published in April 2016 [27,275]. Other examples correspond to the Sodium ferric gluconate complex (first generic approval in March 2011) with the product-specific guidance published in June 2013, Propofol (first generic approval in January 1999) with the product-specific guidance issued in June 2016, or the Enoxaparin sodium injection (first generic approval in July 2010) with the product-specific guidance launched in October 2011 [27,260,267,272]. The same thing happens in the Europa, where the follow-on versions were previously approved before the introduction of current regulatory guidance documents [239].

It is possible to infer that there is a mismatch between the pace at which complex drug products are developed and the rate at which a set of policies, procedures, and regulatory guidelines are produced to guide their development and approval criteria. This is a source of concern, as the non-issuance of the draft guidance before a generic sponsor submitted the first generic application for a given product, can hinder the fully informed decisions by the sponsor, and delay market access and the availability of more affordable versions of NBCDs.

Such a chronological gap may be due to the quick rise in the rate of complex products introduced onto the market, without a weight of evidence and data support to keep track of these scientific advancements. The 'FDA Should Make Public Its Plans to Issue and Revise Guidance on Nonbiological Complex Drugs' report (U.S. GAO, 2017) also discusses the problematic issue stemming from a lack of advance communication on product-specific guidance issuance and subsequent revisions from the competent regulatory authority. This can create setbacks for generic drug developers, such as a great deal of time, effort, and other resources for them to update the content of their regulatory dossiers in response to unexpected changes in guidance, therefore delaying the entry of some generics to the market [27]. However, it should be taking into account the period of time that the regulatory agency needs to gain extensive knowledge and expertise on the innovative character of the complex drug products, and subsequently, produce, review and release a draft document for public comments, which afterward will constitute the official version of the final guidance.

Table 4. Examples of the chronological gap between the Generic Application Submission and Approval of NBCDs products and their Product-Specific FDA Guidance Issuance (adapted from [27]).

Drug Name	Type of NBCDs	Submission of First Approved Generic Application	First Generic Approval	First Publicly Available Product-Specific Guidance Issued
Propofol	Emulsion	March 1997	January 1999	June 2016
Enoxaparin Sodium Injection	Low Molecular Weight Heparin (LMWH)	August 2005	July 2010	October 2011
Sodium Ferric Gluconate Complex in Sucrose	Iron-Carbohydrate Complex	March 2006	March 2011	June 2013
Glatiramer Acetate Injection	Glatiramoid	December 2007	April 2015	April 2016
Doxorubicin Hydrochloride	Liposome	June 2011	February 2013	February 2010

2.4. Complex Drugs, but not Biological

The biological complex drugs and NBCDs constitute the distinct category of complex drug products. In line with discussed previously (Chapter I), NBCDs share a number of specific characteristics with Biological Complex Drugs, such as: the complex and heterogeneous structure that cannot be fully quantitated, characterized, or described by physicochemical analytical methods, and the reproducible quality attributes and therapeutic performance based on the tightly controlled manufacturing process [17,20,30]. Contrarily to the NBCDs, the Biological Complex Drug Products presented for many years a well-established and harmonized regulatory approach for the Reference products or Biosimilars, which resulted in successful applications for marketing

authorization. In other words, the regulatory approach of Biologics and Biosimilars is far ahead of developing a specific approach for approval of NBCDs and their follow-on versions.

The innovator Biological Complex Drugs are licensed by FDA under the Biologics License Application (BLA) pathway, and the follow-on versions are approved by the biosimilar or interchangeable licensure pathway under the Public Health Service Act [18,25,328]. On the other hand, the EMA uses the biosimilar pathway for evaluating the applications to market Biosimilar medicines, through a step-wise process dependent on each product [17,328]. Although NBCDs are complex molecules and share some characteristics with biological complex drug products, the application of the biosimilar approach is not allowed [19,25]. However, given the complexity of these products, it is logical to analyze and understand the lessons learned with the biosimilar pathway and the published guidelines as instructive models for the regulation of NBCD follow-on products, especially regarding the inclusion of non-clinical and clinical studies to demonstrate the therapeutic equivalence [17,18,25,132,136,142,329,330]. Thus, the above tables (Table 2 and

Table 3) also include some examples of guidance documents applied to Biological Medicinal Products and Biosimilars to serve this purpose [14,18,330].

In the general Biosimilar guidelines published by the EMA, the regulatory pathway consists of a stepwise approach, starting with a comprehensive physicochemical and biological characterization. Subsequently, are performed nonclinical and clinical studies according to the level of evidence and robustness obtained in the previous characterization (physicochemical, biological, and non-clinical in vitro data) [14,317,318]. The goal of clinical data is to exclude any significant differences between the biosimilar and the reference product and to confirm comparable clinical performance between them [318]. If it cannot be shown the biosimilarity, should be selected as a stand-alone development to support a full Marketing Authorization Application [318]. Similarly, the FDA guidance documents provide a 'totality-of-the-evidence' and stepwise approach to demonstrating biosimilarity, including comparative structural analyses, functional assays, animal testing, toxicity, human pharmacokinetics (PK) and pharmacodynamics (PD) studies, clinical immunogenicity assessments, and clinical safety and effectiveness studies [14,288,290]. The FDA Guidance for Industry 'Drug Products, Including Biological Products, that Contain Nanomaterials' (2017) or 'Scientific Considerations in Demonstrating Biosimilarity to a Reference Product' (2015) also proposes a risk-based approach and defines that the 'development of drug products entails a continual reduction of residual uncertainty throughout a product's lifecycle' [10,288].

Accordingly, the principles of regulatory approaches to establish the similarity in quality, safety, and efficacy, published in EMA and FDA guidelines/reflection papers, comprise a full quality analysis with physicochemical characteristics, pre-clinical and clinical data [14,17,31]. Both regulatory authorities include the 'similarity' paradigm rather than the demonstration of therapeutic equivalence by the equality (e.g. for small molecules). This corresponds to an evolutionary, stepwise, and science-based approach, centered on 'totality of evidence', that examines the drug

product on a case-by-case basis [22]. There is, however, a need for caution, because the high degree of scrutiny of this approach can greatly increase the development cost of follow-on versions, rendering the undertaking unworkable.

From the total list of guidance documents published by the FDA (Table 2), the draft guidance for the industry entitled 'Statistical Approaches to Evaluate Analytical Similarity' (2017), has been withdrawn [293]. The guidance aimed to provide advice for sponsors developing biosimilars regarding the evaluation of analytical similarity between a proposed biosimilar product and the reference product [293]. The reasons for withdrawal of the guidance were a 'range of issues that could impact the cost and efficiency of biosimilar development, including the number of Reference product lots the draft guidance would recommend biosimilar developers sample in their evaluation of high similarity and the statistical methods for this evaluation' [293].

Another important aspect of the 'Guideline on similar biological medicinal products' (EMA, 2014) is the possibility to compare the Biosimilar with a non-EEA authorized comparator, to facilitate the development and avoid unnecessary repetition of clinical trials. In this case, it is necessary to demonstrate that the comparator authorized outside the EEA is representative of the Reference product authorized in the EEA [143,318]. In the FY 2021 Generic Drug Science and Research Initiatives Public Workshop, Raja Velagapudi, Executive Director of Clinical Development at Sandoz Pharmaceuticals (US), also defended this approach, claiming that the FDA should approve the ANDA based on its bioequivalence to a non-US reference listed drug (RLD), easing regulatory burdens in the BEq tests for certain generics, such as the long-term injectables, inhalation products, or other drugs products with a 'high clinical burden' (e.g. oncology and antipsychotic drugs). Velagapudi also proposed that the drug would have to be assessed according to similar pharmacokinetic and bioequivalence studies as the US RLD, and suggested that the FDA work in conjunction with the International Council of Harmonization (ICH) develop a 'common understanding for mutual utilization of bioequivalence data as much as possible for products that require high clinical burden to register' [331].

2.5. The Product is the Process

The class of NBCDs encompasses different products with a wide variation in terms of architecture, multiple components, particular arrangements, and distinctive critical parameters [12]. Thus, issues concerned with the physicochemical properties and comprehensive characterization of their structure and therapeutic performance are also of increasing importance. For some NBCDs, the structure-function relation or mechanism of action is not completely known (e.g. glatiramer acetate complex products) and it is not possible to specify the critical attributes for the demonstration of similarity [136,164]. Thereby, the characterization of innovator NBCDs or their follow-on versions, and the knowledge of how formulation variables and manufacturing process

parameters impact the final product's critical quality attributes (CQAs) and in vivo performance, constitutes an important analytical challenge [19,20,24,25,136]. The challenges are evident either in the diverse list of critical attributes that should be evaluated for each product (e.g. particle size, polydispersity index, content uniformity, surface morphology, surface chemistry, zeta potential, encapsulation efficiency, drug release kinetics, physical stability, impurity profile, among others), just as the capability to properly characterize them [1,10,253].

On the other hand, the limited assessment of critical quality attributes (CQA) and the lack of scientific knowledge about them hamper the development and implementation of suitable regulatory strategies [20,24,25,35,141]. Thus, the demonstration of pharmaceutical equivalence is more difficult for some complex drug products due to the lack of design studies with sufficient statistical power to detect in vivo slight differences, but clinically significant between the reference product and their follow-on version [12,138,164,239].

The development of advanced and sophisticated analytical methods, novel statistical methods, or predictive approaches, as well as, the implementation of a suitable control strategy, will be of extreme importance to overcome the problems listed above and further the scientific understanding of the product complexity, the successful physicochemical characterization of the components, the impact of each critical process parameters towards the definition of critical quality attributes, the interaction of critical components and performance relationships, the exact mechanism of action, and the immunological, pharmacological and toxicological profiles (Figure 28) [12,332–334].

As referred to in the definition of NBCDs by *Crommelin et al.,* 'the composition, quality, and in vivo performance of NBCDs are highly dependent on the manufacturing processes of both the active ingredient as well as the formulation' [132]. The complexity of NBCDs requires a well-controlled manufacturing process, since any slight variation in the manufacturing process or formulation may lead to changes in the inherent properties of the final drug product, and hence in their safety and efficacy profile (often referred to as 'The product is the process') [14,30,31,136,138,239,333,335–338]. This corresponds to a huge challenge for maintaining batch-to-batch consistency and reproducibility in the manufacturing of these products as well as of their follow-on versions since might be impossible to formulate exact, comparable, or even interchangeable products that translate into similar disposition in vivo [17,30,134]. Hence, there is required the high control of the manufacturing processes and stringent protocols applied on small-scale, and subsequently on the large scale, to ensure a strong and consistent production process, which defines the quality, efficacy, stability, and safety of NBCD products and their follow-on versions (Figure 28) [250]. Better knowledge and understanding of critical components during the early stages of pharmaceutical development is reflected in the higher likelihood of success in achieving an effective reproducible manufacturing process and the corresponding therapeutic purpose.

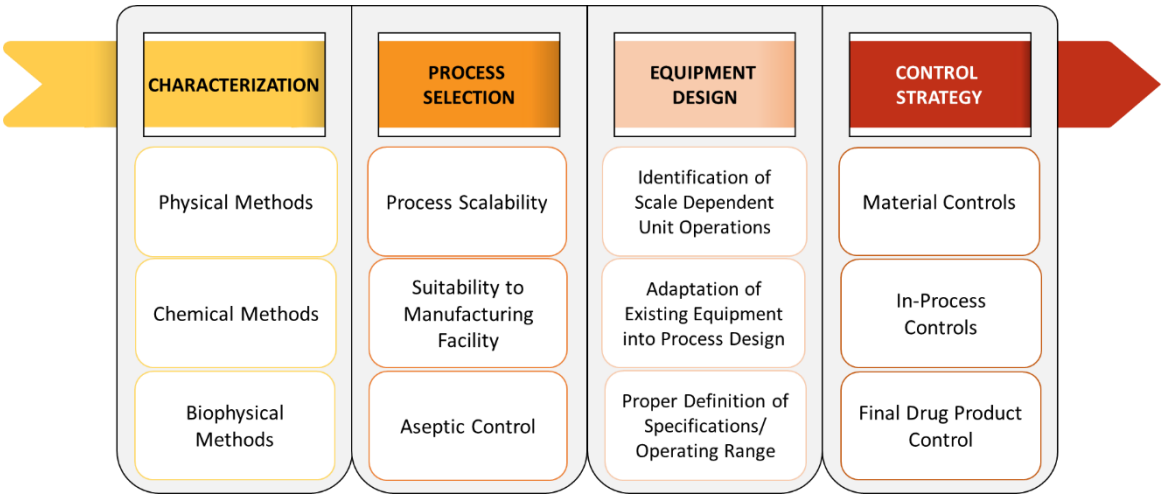

Figure 28. Critical Factors in Robust Design and Control of Complex Drug Products (adapted from [840]).

2.6. Complex Injectables

A large proportion of NBCDs already on the market corresponds to complex injectables. The nanoparticulate structures with highly complex, multi-component, and multi-functional materials of these types of products result in numerous barriers in their manufacturing process, demonstration of therapeutic equivalence, sterilization, likewise the scaling-up process from laboratory scale to industrial scale [12]. Therefore, it is critical in injectable formulations (e.g. solutions, suspensions, or other dispersed systems) to consider the desired formulation requirements (e.g. dispersibility, stability, particulate matter, injection volume, viscosity, compatibility), therapeutic criteria (e.g. indication, required administration route, intended patient population, drug release profile, pharmacokinetic/pharmacodynamic PK/PD profile), as well as, their physiological constraints (e.g. site of administration, ease of withdrawal, injection volume, and speed, accuracy of dose, frequency, blood flow, evenness of flow, local site reactions, tissue damage, injection site pain) (Figure 29) [249,339,340].

One of the biggest challenges to face in the development of complex injectables is the sterilization process, a mandatory requirement for formulations administered through the parenteral, ophthalmic, inhaled, or otic route [12,341]. Examples of such complex drug products include sterile injectables, reconstituted lyophilized powders for injection, ophthalmic suspensions, aqueous-based aerosols for inhalation, among others. This challenge is closely connected to the unique properties and sensitive nature of complex injectables, like the size and composition of the particles in the formulation. This limitation is also related to the susceptibility to chemical and physical degradation, heat-sensitive solutions in thermolabile products (e.g. liposomes), or the high viscosity and low surface tension of these formulations [341–343]. For that, it is fundamental the

selection of appropriate sterilization methods, throughout studies that ensure the process is convenient for a given component and does not cause degradation or failure of them. The method selected should be accompanied by written procedures and a definition of specifications for acceptance or rejection of contaminated components.

On the one hand, no is possible to use certain conventional techniques of terminal sterilization due to the risk of degradation or loss of performance of the formulation, such as the ultraviolet and gamma irradiations, dry heat, saturated steam, ethylene oxide, or dense gas technique [341,343]. On the other hand, filtration and aseptic manufacturing can only be applicable for a limited range of particle-size distribution, resulting in a real risk of to damage the structures and loss of a large quantity of active or inactive ingredients in the filtration process (e.g. loss of lipids in liposomal formulation subject to the tangential flow filtration technique) [341,343]. For instance, the most common standard sterilizing grade filters present a pore size of only 0.22 μm [286,341–343].

Further challenges include the complex manufacturing process in a sterile environment with multiple stages, the need to have dedicated cleanroom facilities, investment into specialized equipment, sterile areas, and the use of specific individual protection equipment (IPE), designed to minimize contamination risk from personnel, materials, and equipment [286,341,344]. Many of the drug products encapsulated in complex injectables correspond to cytotoxic drugs (e.g. doxorubicin, daunorubicin, irinotecan, vincristine), which can also involve hazards to human health and the environmental safety, and require double the care in their handling [12] (Chapter I).

The formulation of complex injectables may request the use of excipients and solvents with particular features (e.g. ionic strength, pH, osmolarity, viscosity, surfactants) which are not compatible with the nature of the materials that constitute the filters or sterilizing grade membranes (e.g. surface chemistry, hydrophilicity, composition, surface tension, pore size, structure). The incompatibility between the formulation and filter materials can result in kinds of complications, such as the blockage and high pressure during filtration, compromising product integrity and structure, and leading to serious failures in the filter integrity tests required for drug products release [345]. Thus, after the filtration process is needed to completely characterize the formulation to ensure that integrity and structure remain unchanged. Other parameters of the sterile filtration are also critical factors in achieving the success of the process, such as the temperature, time, flow dynamic, pressure, and flow rate [342]. The ‘Sterile Filter Master Plan’ of the Parenteral Drug Association (PDA) includes a raft of elements of sterile filtration validation that must be followed: ‘*1. Integrity Testing: Prove the filter’s bacterial retention capabilities with a non-destructive test; 2. Fit for Use: Prove the filter meets all requirements within the product & process conditions; 3. Stability: Prove the filter does not adversely affect the process stream; 4. Sterilization: Prove the sterilization method is effective and does not compromise the filter; 5. Binding: Prove the filter does not remove stream components; 6. Compatibility: Prove the stream does not adversely impact the filter; 7. Extractables/Leachables: Identify, quantify, and assess the impact of compounds that*

migrate from filter to process stream; 8. Retention: Prove the filter removes bacteria from the stream per ASTM 838-05' [346].

The success of sterile operations is no trifling matter since relies on mitigating contamination from different sources, such as the personnel, drug product components and container systems, cleanroom facilities, just as the equipment and processes, whether on a clinical or commercial scale. Moreover, the type of sterilization method, conditions, and facilities must be selected to the detriment of the formulation properties to develop. The high costs associated with the establishment of the desired facility, dedicated instrumentations, manufacturing equipment, training of personnel, sterilization, or the scale-up of complex injectables shall also be taken into account [12].

In keeping with the above, the presence of particulate matter in complex injectables, for instance intravenously administered, is considered critical due to the possibility to jeopardize patient safety through life-threatening health hazards. More recently, the FDA announced the availability of a guidance for the industry entitled 'Inspection of Injectable Products for Visible Particulates' (FDA, 2021) [347]. This guidance document discusses the development and implementation of a holistic, risk-based approach to visible particulate control that incorporates product development, manufacturing controls, visual inspection techniques, particulate identification, investigation, and corrective actions designed to assess, correct, and prevents the risk of visible particulate contamination [347]. However, different stakeholders encouraged the FDA to align the content of this guidance (e.g. classification categories for visible particles) with the US Pharmacopoeia's (USP) Chapter <1790> Visual Inspection of Injections or make mention to the EU Annex 1 on good manufacturing practices (GMPs) for 'Manufacture of sterile medicinal products' [348–350].

In the demonstration of therapeutic equivalence, the parenteral drug product should contain the same inactive ingredients (Q1) and in the same concentration (Q2) as the reference listed drug (RLD), although there may be some differences in preservative, buffer, or antioxidant (21 CFR 314.94 (a)(9)(iii) – *Inactive ingredient changes permitted in drug products intended for parenteral use*) [351]. However, it is necessary that the applicant identifies and characterizes the differences and provides information demonstrating that the differences do not affect the safety or efficacy of the proposed drug product [351]. That increases the regulatory burden around the development of a generic drug product of a complex injectable. On the other hand, the non-identification and understanding of the critical process parameters, critical material attributes, and critical quality attributes of the drug product in the early steps of development, just as the lack of adequate characterization techniques and analytical criteria, result in more difficult access to the large-scale manufacturing method and specification that guaranteeing the reproducibility of the complex product [12].

Additionally, the bioequivalence demonstration of generic injectable drug products presents other considerable challenges, such as the absence of a standard in vitro release method, the

restricted number of patients for in vivo pharmacokinetic studies, or non-viability linked to the intensive pharmacokinetic sampling in certain physiological sites [339,352]. As described in the publication 'Approved Drug Products With Therapeutic Equivalence Evaluations' (Orange Book), 'injectable suspensions are subject to bioequivalence problems because differences in particle size, the polymorphic structure of the suspended active ingredient, or the suspension formulation can significantly affect the rate of release and absorption' [353]. In the present year (June 2022), FDA and the Center for Research on Complex Generics (CRCG) are dedicated to advancing programs, such as the 'Public Workshop: In Vitro Release Test & In Vitro-In Vivo Correlation of Complex Generic Ophthalmic, Injectable, Implantable, and Inserted Products', to stimulate scientific dialogue, disseminate current insights, and generate new knowledge about complex generics [354]. This program has the main aim of discussing the scientific principles and practical considerations that inform current FDA thinking for in vitro release test (IVRT) and in vitro-in vivo correlation (IVIVC) studies to support the development and approval of complex generic ophthalmic, injectable, implantable, and inserted drug products [354].

The concept of 'complex products' defined by the FDA in the 'Generic Drug User Fee Act (GDUFA) II Commitment Letter' also includes the complex drug-device combination products (e.g., pre-filled syringe, pre-filled auto-injector) [16]. Other relevant matters are raised for the generic drug-device combination product development, such as the regulatory environment, product design, integrity of the product, device impacts on drug delivery, or the evaluation of the differences in the user interface for the device constituent between complex generic and their reference product [249,355]. Some of GDUFA Science and Research Priority Initiatives include the evaluation of the impact of differences in the user interface between complex generic drug-device combination products and their reference listed drugs (RLD) on therapeutic equivalence, just as the development of criteria for device performance comparisons that would support bioequivalence (BEq) demonstration by in vitro methods and may eliminate the need for in vivo comparative clinical endpoint BEq studies [355].

Given the importance of these issues, it is highly recommended the analysis of Guidance for Industry 'Container Closure Systems for Packaging Human Drugs and Biologics' (FDA, 1999), 'Technical Considerations for Pen, Jet, and Related Injectors Intended for Use with Drugs and Biological Products' (FDA, 2013), 'Regulatory Science Report: Long-Acting Injectables and Implants' (FDA, 2018), 'Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms' (EMA, 2014), and the 'Guideline on quality documentation for medicinal products when used with a medical device' (EMA, 2021) [356–360].

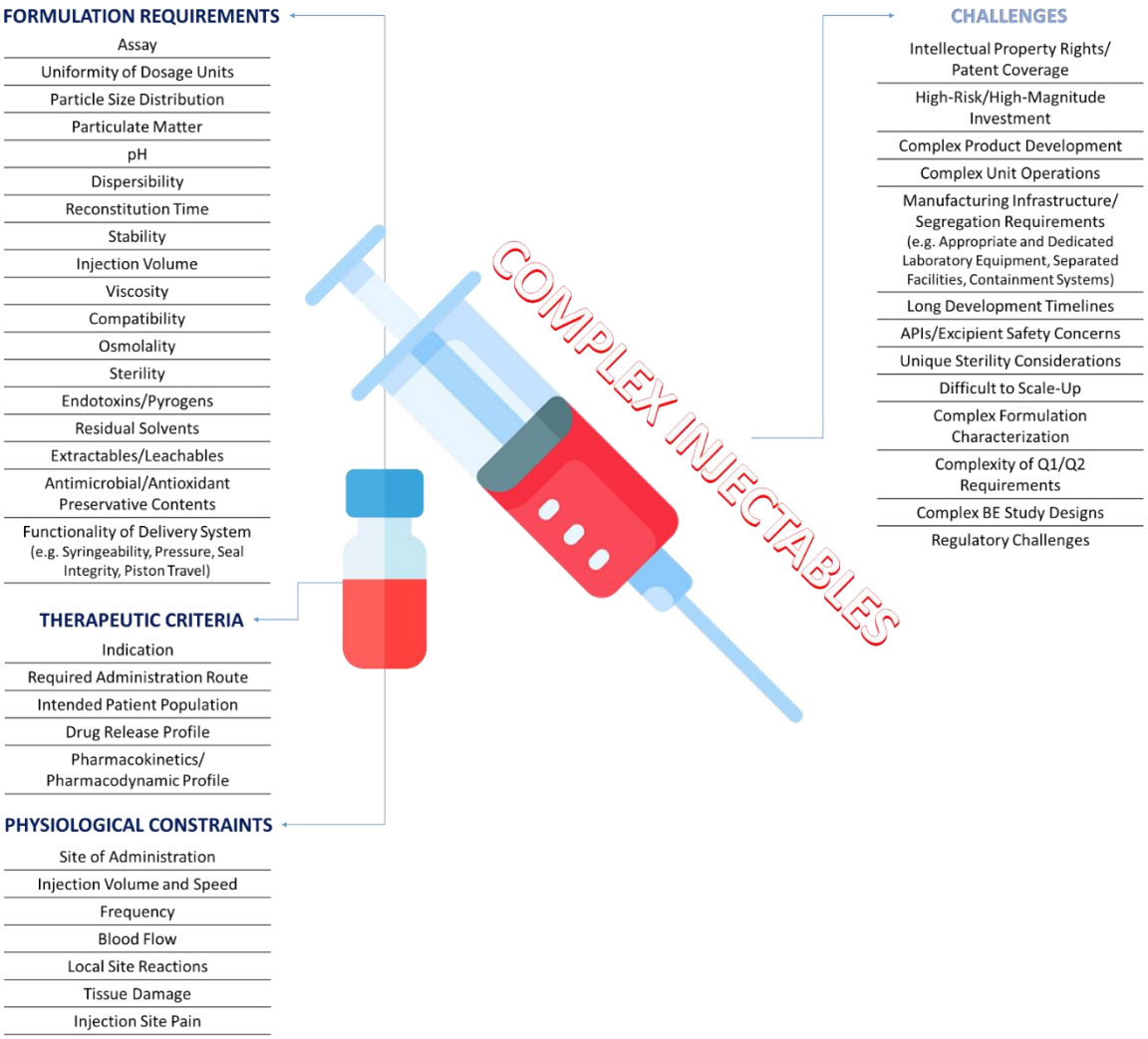

Figure 29. Challenges and Formulation Aspects of Complex Injectables.

3. Concluding Remarks

During the past few decades, complex drug products, including Biological and Non-Biological Complex Drugs (NBCDs), have been approved worldwide, marking an unprecedented step forward in several therapeutic areas. The greater pressure to reduce prices or the loss of exclusivity conferred by a patent expiration is also responsible for promoting the pharmaceutical development of copy versions, also known as the 'follow-on versions', 'generic versions', or 'similar versions'.

As technology advancements enable new opportunities for the complex drug segment, regulatory systems faced countless challenges and multiple concerns over the quality, efficacy, and safety profile of complex generic drug products. Some of those hurdles result from the lack of clear definition and classification of NBCD products by the regulatory authorities. A proper distinction between each class of NBCDs and the harmonization across different jurisdictions is critical to the implementation of an adequate and effective regulatory approach globally accepted. On the other hand, it was also possible to identify classes of NBCDs (such as LMWHs and albumin-bound particles) which are differently classified by each regulatory authority, and consequently approved under different regulatory pathways.

Furthermore, it is particularly challenging to evaluate the therapeutic equivalence of NBCD products and ensure their quality, safety, and clinical effectiveness. These hurdles are directly related to their complexity and heterogeneity, as well as, the variability in their manufacturing process. Issues related to the physicochemical properties and characterization of their structure and therapeutic performance are also of increasing importance. On the other hand, it could be inferred that the complexity associated with the NBCDs, the limited assessment of critical quality attributes (CQA), and the lack of scientific knowledge about them hamper the development and implementation of suitable regulatory strategies.

While there are several guidelines and regulatory approaches successfully defined and established for Small molecule drugs and Biological complex drugs, the regulatory basis for NBCDs is much more unclear and extensively unavailable. This chronological gap and regulatory uncertainty related to the guidance documentation specified for each class of NBCDs may be due to the 'baby steps' taken by the regulatory authorities to create regulatory procedures tailored to their high complexity and diversity. There is a general agreement among the scientific community, industry, and regulatory authorities that the authorization pathways used to accomplish the marketing authorization of NBCDs are frequently inadequate. Therefore, there is a need to re-evaluate existing regulatory frameworks and implement an adapted regulatory approach and specific data requirements for the assessment of therapeutic equivalence to each sub-class of NBCDs. Due to these challenges related to complex drug products, more importance has been given to regulatory decision-making based on a case-by-case analysis, just as the definition of an adequate

and tailored level of regulatory density according to the complexity of the drug product. All of these topics will be deeply addressed and discussed in the following chapters of this thesis.

Chapter III. The Regulatory Landscape of Non-Biological Complex Drug Products from the EMA and US-FDA Perspective

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Abstract

The heterogeneity, diversity, and unique characteristics of Non-Biological Complex Drugs (NBCDs) provide challenges to the pharmaceutical development and regulatory approval of complex generic drug products.

The definition of NBCDs is still not officially recognized by the regulatory authorities, and there is no dedicated regulatory pathway addressing the particular features of NBCDs and their follow-on versions. The lack of clear and consistent regulatory guidance documents in this field, as well as, the inconsistency across different regulatory agencies, impact negatively on the acceptance and huge potential of these drug products. This regulatory uncertainty coming from the use of different regulatory approaches worldwide, just as within the same class of products, may affect the patient's access to valuable high-quality NBCD follow-on versions.

A central issue relates to the demonstration of the similarity between a reference-listed drug product and its follow-on version. How is the therapeutic equivalence demonstrated? Which scientific approaches should be applied? What regulatory requirements are necessary to demonstrate therapeutic equivalence? Which product characteristics are critical to obtaining the intended therapeutic performance and safety? And what are the appropriate analytical methods? How similar is similar?

This chapter has attempted to provide a critical overview of the regulatory landscape of NBCDs and follow-on versions currently adopted by the FDA and EMA, and compare the specific pathways and scientific considerations used by each regulatory authority. The dissemination of knowledge and discussion in this field constitutes an important contribution to increasing the clarity of the legislation, policies, and regulatory approaches for complex generics, filling regulatory and scientific gaps in the therapeutic equivalence establishment. It also reinforces the need to develop a specific regulatory pathway compliant with the complexity of NBCDs and their follow-on versions or, alternatively, makes better use of available regulatory pathways.

Keywords

Non-Biological Complex Drugs; Complex Generics; Follow-on Versions; Therapeutic Equivalence; Pharmaceutical Equivalence; Bioequivalence; Regulatory Evaluation; EU Legislation; U.S. Legislation; Regulatory Landscape; Regulatory Frameworks; Drug Approval Procedures; Marketing Authorization; Regulatory Science Research; Regulatory Burden; Global Regulatory Harmonization; European Medicines Agency; U.S. Food and Drug Administration.

1. Introduction

The scientific and regulatory framework for developing generic versions of their brand-name counterparts have been established by each regulatory authority in every world location. It is a well-known fact that individual regulatory agencies have their regulatory procedures to ensure that the drug products approved for marketing achieve the desired quality and are safe and effective for the people in their countries. Therefore, the regulatory burden for the approval process of a generic drug depends on the classification and complexity of the drug product, the location of submission, the type of marketing authorization application, and the completeness of the drug approval procedure [33]. This process comprises a rigorous regulatory review to ensure that the proposed generic drug product meets the same high standards as reference products (brand-name drugs/innovators), such as the same active/key ingredient, strength, dosage form, route of administration, safety, effectiveness, stability, and quality [16,245,361].

However, the substantial advances in the Nanotechnology field and the appearance of a multitude of complex drug products meant that the global regulatory systems be confronted by unprecedented challenges in the pharmaceutical equivalence (PE) and/or bioequivalence (BEq) assessment to generic versions. The complexity of development and the lack of well-defined regulatory approaches to address the unique characteristics of complex generics led to major regulatory uncertainty and problems concerning the guarantee of product quality and safety [14,17,19,25,27,34,132].

Currently, discussions regarding the assessment of therapeutic equivalence of complex generics and the adoption of appropriate regulatory procedures are still underway. There is a need to define which studies and requirements to be required to demonstrate therapeutic equivalence, as well as, which type of regulatory approval pathway best matches each type of complex generics. Thus, the steady increase in the complexity level of generic drug products should go hand in hand with efforts to improve the scientific and technical capability of product characterization and data analysis.

This chapter describes and compares the specific regulatory pathways, policies, and scientific considerations used by each regulatory authority, given the respective quality, safety, and efficacy requirements of Non-Biological Complex Drugs (NBCDs). Also provides several examples of challenges and regulatory disparities involved in applications for marketing authorization of NBCDs and their follow-on versions. Other key aims are related to the discussion of the absence of harmonization between regulatory authorities in different places worldwide, just as understanding trends and future perspectives surrounding the therapeutic equivalence and clinical use of complex drug products within available regulatory pathways.

2. Regulatory Approval Procedures of the EMA and FDA

The EMA is a regulatory agency responsible for the scientific evaluation and supervision of applications for marketing authorization of medicines in the European Union (EU), whereas the FDA is responsible for ensuring the safety, efficacy, quality, and security of human and veterinary drugs, biological products, and medical devices in the United States [18,362,363].

In the EU, there are two main pathways for marketing authorization depending on the nature of the drug product: Centralized Procedure (CP) and National Authorization Procedures (NP). The National Authorization Procedures include the Mutual Recognition Procedure (MRP), Decentralized Procedure (DCP), and National Procedure (NP) [34,364].

The Centralized Procedure allows that the medicines can be authorized in all EU Member States, as well as European Free Trade Association (EFTA) states (Iceland, Norway, and Lichtenstein), through a single marketing-authorization application procedure [364]. Upon submission of a valid marketing-authorization application, the EMA's Committee for Medicinal Products for Human Use (CHMP) provides a scientific evaluation of the application and gives an opinion on whether the medicine should be authorized. Subsequently, the European Commission is responsible for granting the marketing authorization after receipt of the CHMP opinion [364].

The marketing authorization in each EU Member State, outside the extent of Centralized Procedures, can be made from the following National Authorization Procedures: the National Procedure, whereby each member states approve the medicines for use in their territory; the Mutual Recognition Procedure (MRP), in which an already existing marketing authorization is extended for the other Member States; the Decentralized Procedure (DCP), which simultaneously applies for marketing authorizations in more than one Member State, for a medicine that has not yet been authorized in the EU [30,364].

The Directive 2001/83/EC presents the legal basis for the submission of medicinal products for human use and addresses the applications regulated under Article 8 and Article 10 [365]. This directive sets the specific data requirements for each application and differentiates among the full dossiers regulated under Article 8(3) Stand-alone Application, Article 10(a) Bibliographic/Well-Established Use Application, Article 10(b) Fixed-combination Application, and Article 10(c) Informed Consent Application, but also the 'abridged applications' regulated under Article 10(1) Generics Application, Article 10(3) Hybrid Application, and Article 10(4) Biosimilar Application [30,34,143,365,366]. Table 5 illustrates the schematic representation of the types of approval applications used in the EU.

The generic application under Article 10(1) only can be applied for a medicinal product that has: the same qualitative and quantitative composition in active substance(s) as the reference product, the same pharmaceutical form as the reference medicinal product, and when the bioequivalence with the reference medicinal product can be demonstrated by appropriate

bioavailability studies, without requiring results of pre-clinical tests and clinical trials [327]. Conversely, the hybrid applications under Article 10(3) differ from generic applications in that the results of appropriate pre-clinical tests and clinical trials will be required in particular circumstances, such as: where the medicinal product does not fall within the definition of a generic medicinal product; where the bioequivalence cannot be demonstrated through bioavailability studies; or in case of changes in the active substance(s), therapeutic indications, strength, pharmaceutical form, or route of administration of generic drug product in comparison with the reference medicinal product [327,367].

Table 5. Schematic representation of the types of approval applications in the EU (adapted from [19,25,34,136]).

European Medicines Agency [EMA]						
Full Dossier				**Abridged Applications**		
Stand Alone Application	Bibliographic/ Well-Established Use Application	Fixed Combination Application	Informed Consent Application	Generics Application	Hybrid Application	Biosimilar Application
Article 8(3)	Article 10(a)	Article 10(b)	Article 10(c)	Article 10(1)	Article 10(3)	Article 10(4)
Innovators	Api presents well-established medicinal use	Two or more APIs (registered in EU) used in combination in the same Medicinal Product	Holder of Authorized Medicinal Products allows data to be used for subsequent applications	Generics	Products that do not meet the strict definition of a ‘Generic’	Biosimilars

In the US, the NBCDs are considered synthetically derived complex drug products, but not Biological Complex Drugs, being automatically regulated under the Federal Food, Drug, and Cosmetic Act (FFDCA) [34,164,368]. Under that Act, the new drug application (NDA) regulatory pathway is subdivided into 505(b)(1) route for innovator drug products and 505(b)(2) route for products closely related to innovators. On the other hand, the abbreviated new drug application (ANDA) regulatory pathway corresponds to the 505(j) route used for generics/follow-on versions [17,22,34,137,368].

As per the FDA Guidance for Industry ‘Determining Whether to Submit an ANDA or a 505(b)(2) Application’ the applicant may submit an ANDA (505(j)) to the Office of Generic Drugs (OGD) if the product is intended to have the same active ingredient(s), conditions of use, route of administration, dosage form, strength, labeling (with certain permissible differences), and is bioequivalent to the RLD [299]. On the other hand, the applicant should contact the Office of New

Drugs (OND) if intend to apply a 505(b)(2) pathway, where the product has a different active ingredient, conditions of use, route of administration, dosage form, strength, or labeling than a listed drug [299]. The question remains as to what is the most appropriate regulatory pathway (e.g. 505(j) or 505(b)(2) route) for the approval of follow-on versions of NBCDs. The Biological License Application (BLA) with section 351(a) for regulatory approval of biological drug products, just as the 351(k) route for biosimilars, cannot be applied since both are outside the scope of the NBCDs [18,25,328]. The schematic representation of the types of approval applications used by the FDA is illustrated in Table 6.

Table 6. Schematic representation of the types of approval applications in the US (adapted from [19,25,34,136]).

U.S. Food and Drug Administration [FDA]				
Food, Drug and Cosmetics Act			**Public Health Service Act**	
New Drug Application [NDA]		Abbreviated New Drug Application [ANDA]	Biologic License Application [BLA]	Biologic Price Competition and Innovation Act [BCPI]
Section 505(b)(1)	Section 505(b)(2)	Section 505(j)	Section 351(a)	Section 351(k)
Innovator Drug Product	Products closely related to Innovators	Generics	Biological Drug Product	Biosimilar

3. Regulatory Landscape of Non-Biological Complex Drugs (NBCDs)

3.1. Methodology

This section examines the regulatory landscape of NBCDs and their follow-on versions approved by the FDA and EMA until the end of 2020. To carry out this analysis was used the general list of NBCDs already approved by the FDA (Table 48) and EMA (Table 49) (see Appendix I. Supplementary Data). The methodology employed to reach Table 48 and Table 49 is described in section 2 of Chapter I.

3.2. Analysis by Type of NBCDs and Follow-On Versions

Over the past few years, there has been a slight increase in the development of NBCDs, but also their follow-on versions. A big part of that interest is owed to the high value of the market due to the great advantages of these systems when compared with conventional medicines, the loss of exclusivity conferred by a patent expiration, the greater pressure to reduce prices, and the increased knowledge and documentation disclosed about them [19,30,34,162].

From the total list of 52 NBCD Reference products approved by the FDA, 27% of them present follow-on versions (n=14, 27%), such as: Copaxone®, Diprivan®, Doxil®, Emend®, Ferrlecit®, Lovenox®, Megace ES®, Neoral®, Rapamune®, Renagel®, Renvela®, Taxotere®, Tricor® and Valstar® (Table 48). In the US, have been identified 54 NBCD follow-on products FDA approved (Table 48). Likewise, from the total list of 50 NBCD Reference products approved by the EMA, 12% of them present follow-on versions (n=6, 12%), such as: Abraxane®, Copaxone®, Diprivan®, Lovenox®, Renvela®, and Venofer® (Table 49). In total, have been identified 89 NBCD follow-on products approved in the EU (Table 49).

Despite the growing interest in this field, it is possible to verify that the number of reference products with therapeutic equivalents is still considerably reduced. The small number of NBCDs with follow-on versions is exclusively due to the complexity that can be found in various stages of product development. The main difficulties attributed to the development of complex generics are related to the technical and manufacturing complexity, demonstration of the bioequivalence, regulatory requirements required, and the patents/intellectual property associated with the reference products [139,140]. These challenges are often wholly incompatible with the generic pharmaceutical business due to the long, expensive, and uphill development pathways [139].

There are several categories of NBCDs (e.g. liposomes, iron-carbohydrate complexes, or glatiramoids) where the establishment of therapeutic equivalence and marketing approval is extremely difficult, with highlighted regulatory gaps. These categories are discussed more fully throughout this thesis.

3.2.1. Liposome

As mentioned in Chapter I, the widespread use of liposomal formulations is not surprising, considering the multiple advantages of liposomes over other systems in terms of preparation, scalability, biocompatibility, targeted delivery, bioavailability, and low systemic toxicity [8,152,153,156,157].

However, it is also important to highlight that, up to date the analysis (end of 2020), it had not been possible to approve any liposomal follow-on versions in the EU. For example, the applications for marketing authorization of the follow-on version of liposomal doxorubicin hydrochloride that was withdrawn correspond to the following products: Doxorubicin SUN® (2011), Doxolipad® (2019), and Doxorubicin Hydrochloride Tillomed® (2020) [369–371]. In accordance with the CHMP assessment report for Doxorubicin SUN®, the nonclinical and clinical results did not provide enough evidence to show that Doxorubicin SUN® was similar to the reference medicine, as well as the company did not provide enough data to support their submission [370]. In the case of Doxolipad®, developed as a 'hybrid medicine', the bioequivalence results demonstrated that this follow-on version is comparable to Caelyx® in terms of 'liposome-encapsulated doxorubicin', but did not show the same amount of 'free doxorubicin'. Thus, the application of Doxolipad® has been refused due to insufficient evidence to show the bioequivalence to Caelyx®, and the impossibility to establish that the benefits outweigh its risks [369]. Finally, for the doxorubicin hydrochloride Tillomed®, the company withdrew the application in March 2020 because of the identified deficiencies in Good Clinical Practice at the test site [371]. On the other hand, it is possible to observe that only two liposomal formulations approved by FDA (Doxil® and Valstar®) provide follow-on versions.

It is also important to highlight that recently the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion about the granting of a marketing authorization for the medicinal product Zolsketil (pegylated liposomal doxorubicin, 2 mg/ml concentrate for solution for infusion), intended for the treatment of metastatic breast cancer, advanced ovarian cancer, progressive multiple myeloma, and AIDS-related Kaposi's sarcoma. This product is a hybrid medicine of Adriamycin® (non-liposomal doxorubicin hydrochloride injection), containing the same active substance as Adriamycin®, but occurs in a pegylated liposomal formulation. Zolsketil has demonstrated a satisfactory quality and its bioequivalence to Caelyx®, which was chosen as the comparator. Since 25 March 2022, Zolsketil is pending EC decision and detailed recommendations for the use of this product will be described in the summary of product characteristics (SmPC), which will be published in the European public assessment report (EPAR) and made available in all official European Union languages after the marketing authorization has been granted by the European Commission [372].

The limited number of submissions for liposomal follow-on versions is related to remarkable challenges that occur during the development and manufacturing of liposomal drug products. Some reasons include the complex nature of liposomes that hamper the comparison to the RLD, deficiencies from the quality evaluation, and the protection granted by a patent that remains active for the majority of products. Additionally, many of them are parenterally administered which requires compliance to additional and strict specifications, as mentioned above in Chapter II (Section 2.6) [10,286,340]. On the other hand, it is also difficult to prove the pharmaceutical equivalency due to the complexity of in vitro/in vivo studies and pharmacokinetic evaluation for the lack of proper analytical methods [139,140,373,374].

Any change to the policies by the regulatory authorities over time has a significant impact on the market trends, R&D investments accomplished, and subsequently the approval of generic drug products. This is particularly noticeable within the framework of regulatory policies defined for liposomal formulations. For example, the version of Guidance for Industry 'Liposome Drug Products - Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation' published in 2018 presents updated contents of science-based regulatory procedures for liposomal formulations compared to the older version of liposome guidance released in 2002 [253,375]. The first version of Draft Guidance for Industry on Liposome Drug Products (2002) provided recommendations to applicants on the CMC, human pharmacokinetics and bioavailability, and labeling documentation for liposome drug products submitted in NDAs, but did not cover the recommendations on clinical efficacy and safety studies, nonclinical pharmacology and toxicology studies, bioequivalence studies or those to document sameness, liposomal formulations of vaccine adjuvants or biologics, or drug-lipid complexes [375]. The most recent version of this guidance addresses instructions for both new drug applications (NDAs) and abbreviated new drug applications (ANDAs) regulatory submissions and though does not provide recommendations specific to liposomal formulations to be marketed under biologics license applications (BLAs), many scientific principles described therein may also apply to these products. This guidance does not yet provide recommendations on clinical efficacy and safety studies, nonclinical pharmacology/toxicology studies, or drug-lipid complexes [253]. On the other hand, EMA has not yet published a general and specific guideline covering the approval procedures for innovators and follow-on versions of liposomal formulations, but only a reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA, 2013) [284]. This reflection paper provides some insight on the quality, non-clinical and clinical data to support a marketing authorization of intravenous liposomal products, including some information concerning the toxicological studies and safety issues [284].

Another substantial challenge widely recognized in the establishment of therapeutic equivalence of liposomal drug products is diverging regulatory and authorization requirements provided by each competent authority, as can be observed in the specific case of doxorubicin

hydrochloride liposome injection. In February 2010, FDA published the first product-specific guidance to abbreviated new drug applications for pegylated liposomal doxorubicin formulation, entitled 'Draft Guidance on Doxorubicin Hydrochloride', which subsequently has undergone four major revisions (revision of November 2013, December 2014, April 2017, September 2018) [206]. In February 2013, EMA published the 'Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product', and a couple of years later issued identical guidance named 'Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance' (2018) [158,284]. From the comparison of regulatory requirements of both regulatory authorities, it was possible to verify that the first versions of guidances contained slightly different recommendations, particularly in non-clinical and clinical studies for the demonstration of safety and efficacy for follow-on versions (Table 7) [137,206,284]. In general, the FDA did not require non-clinical and clinical studies, whereas the EMA reflection papers stated that the in vivo studies could not be only replaced by physicochemical characterization and pharmacokinetic BEq studies. Thus, it was clear that the two regulatory authorities were not completely aligned due to the highlighted differences within their regulatory guidance, especially the different regulatory grades for the demonstration of bioequivalence (Table 7) [137,206,284].

Nowadays the last version of the FDA guideline (2018) includes regulatory requirements for the demonstration of bioequivalence based on physicochemical characterization, in vitro and in vivo studies [206]. The development of a follow-on version of the liposomal reference drug product requires that the test product meets the following criteria: qualitatively (Q1) and quantitatively (Q2) the same as the Reference Listed Drug (RLD); manufactured by an active liposome loading process with an ammonium sulfate gradient; at least one batch of the test product should be produced by the commercial scale process and be used in the in vivo bioequivalence study; and shares equivalent liposome characteristics at the CMC level including liposome composition, state of encapsulated drug, the internal environment of liposome, liposome size distribution, number of lamellar, grafted PEG at the liposome surface, electrical surface potential or charge, and in vitro leakage rates comparable to the reference standard [206]. At clinical level should include the following tests: a single-dose, two-way cross over study in ovarian cancer patients whose disease has recurred or progressed after platinum-based chemotherapy; a dose of 50 mg/m2; bioequivalence based on 90% CI; and a pivotal bioequivalence study conducted using test product produced by the proposed commercial-scale manufacturing process [206]. The large number of patients required for this last study can be a problem, deriving from the low free doxorubicin plasma level and large patient-to-patient variability [2].

Despite the significant improvements with the guidance updates, there are still some challenges related to the development of follow-on versions of liposomal formulations, such as the establishment of the correlations between in vitro release and in vivo pharmacokinetic data (in vivo

performance) [345,376]. This poor in vitro/in vivo correlation arise mainly from the physiological conditions at the systemic circulation and the tumor sites, the presence of macrophages, and the necessity to cross multiple biological membranes and compartments [376]. Thus, the conventional method of measuring the total drug concentration in blood circulation/plasma may not be the most appropriate to reflect the bioavailability of the drug and therapeutic performance in the intended target organ (e.g. cancer tissues) [253,345].

The reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (EMA, 2013), pointing out the importance of using validated analytical methods to quantify encapsulated and unencapsulated drugs in blood/plasma and unencapsulated drugs in tissues, just as the care that should be taken in sample processing procedures during the method development to prevent the destruction of liposomes and the erroneous results [284].

Furthermore, the FDA Guidance for Industry 'Drug Products, Including Biological Products, that Contain Nanomaterials' stated that a fully validated dissolution/in vitro release method 'should be able to discriminate batches that are not bioequivalent to the pivotal clinical batch, which will have demonstrated efficacy and safety'. Also, the details of the proposed dissolution/in vitro release test and the developmental parameters (equipment/apparatus, media, agitation/rotation speed, pH, sink conditions, surfactant type and concentrations) must be included in the submission. On the other hand, the drug release profiles should e complete (reach a plateau), without significant increase over three consecutive time points, and guarantee at least 85 percent release of the labeled amount of active ingredient(s). If this does not happen, the application submitted should provide additional data to explain the reasons for incomplete release, which can be considered very challenging. An applicant also could develop their own in vitro release/dissolution method for a specific product, on the premise that will be subject to a regulatory evaluation regarding feasibility, scientific rationale, and method validation to ensure that such a method is reproducible, reliable, and sensitive to variations in the product's formulation and manufacturing processes [10].

Table 7. Comparison of therapeutic equivalence recommendations of FDA and EMA on generic liposomal injection (adapted from [137,206,284]).

Requirements	US FDA Guidance (released in 2010)	EU (EMA) Guidance (released in 2013)
Physicochemical characterization		
Lipid and non-lipid excipients	Yes	Yes
Histidine, sucrose, internal and total sulfate concentrations	Yes	Yes
Active substance /lipids ratios	Yes	Yes
Liposome morphology, number of lamellae	Yes	Yes
Mean size and size distribution	Yes	Yes
Fraction of free and encapsulated drug	Yes	Yes
Osmolarity	Yes	Yes

Stability of active substance, lipids and functional excipients (e.g., lyso-PC)	Yes	Yes
Stability studies under proposed in-use conditions	Yes	Yes
In vitro drug release rate in relevant media and under stress conditions (to simulate release in storage, blood circulation and target site of action)	Yes	Yes
Validated process for reconstitution and/or pharmacy preparation	No	Yes
Liposomal formulation integrity in plasma	Yes	Yes
Lipid bilayer phase transition temperature	Yes	Yes
Liposome surface charge	Yes	Yes
Physical state of the active substance inside the liposome	Yes	Yes
Internal environment (volume, pH, ion concentration)	Yes	Yes
Distribution of active substance within liposome (e.g., surface, bilayer, interior, etc.)	No	Yes
PEG layer thickness	Yes	No
PEG-lipid linkage chemistry, molecular weight and size distribution, disposition of PEG, stability of PEGylation (if applicable)	Yes	Yes
Manufacturing controls To identify impact of process changes on product quality (quality by design)	Yes	Yes
Non-clinical study		
In vivo pharmacokinetics, distribution and elimination	No	Yes
Drug concentration in tissues relevant to toxicity and/or efficacy	No	Yes
In vivo pharmacodynamic study	No	Yes
In vitro efficacy study on target cells	No	Yes
Toxicity studies using unloaded liposomes	No	No
In vivo studies		
Equivalence in total exposure (non-encapsulated and encapsulated)	Yes	Yes
Equivalence in exposure of non-encapsulated drug	Yes	Yes
Equivalence in exposure of encapsulated drug	Yes	Yes
Excretion of active substance in urine	No	Yes
Pharmacokinetics of at least one metabolite	No	Yes
Clinical efficacy studies	No	No

3.3. Analysis by Submission Classification

Figure 30 presents the submission classification of the New Drug Application (NDA) used for NBCDs approved by the FDA. From the total list of New Drug Applications (NDAs) (n=52) approved by FDA, 37% of them were approved under the type 1 of submission as ‘New Molecular Entity’ (n=19, 37%), 6% through the type 2 as ‘New Active Ingredient’ (n=3, 6%), 33% through the type 3 as ‘New Dosage Form’ (n=17, 33%), 4% thought the type 4 as ‘New Combination’ (n=2, 4%), and 19% thought the type 5 as ‘New Formulation or New Manufacturer’ (n=10, 19%) (Figure 30). The NBCD Reference products submitted as ‘New Active Ingredient’ are Abelcet®, Ambisome®, and Feraheme® [48,72,111]. On the other hand, the NBCDs submitted as ‘New Combination’ correspond to Oraqix® (emulsion with a combination of lidocaine and prilocaine)

and Vyxeos® (daunorubicin and cytarabine liposomal formulation) [89,122]. The Dexferrum® is the only NBCD Reference product where the type of submission is not identified [62].

It is important to highlight the fact that there is significant progress in the pharmaceutical development of innovative formulation platforms. The discovery and development of Small Molecule Drugs are no longer the main focus of the pharmaceutical industry. As can be seen in Figure 30, several NDA submissions of complex drug products are related to 'New dosage forms', 'New combinations', and 'New formulation or New manufacturer', i.e., novel technological platforms that often incorporate one or more existing approved drugs. Therefore, there is a paradigm shift centered on the development of new classes of drugs, such as complex drug products, with significant advantages compared to conventional forms. As previously mentioned, these products have been extensively investigated due they enable overcoming the limitations of some drugs, namely low solubility, low permeability, stability, or toxicological effects [3–5]. On the other hand, they present attractive properties and numerous advantages related to site-specific delivery, drug targeting, bioavailability, potency, and the effectivity of drugs [3–5].

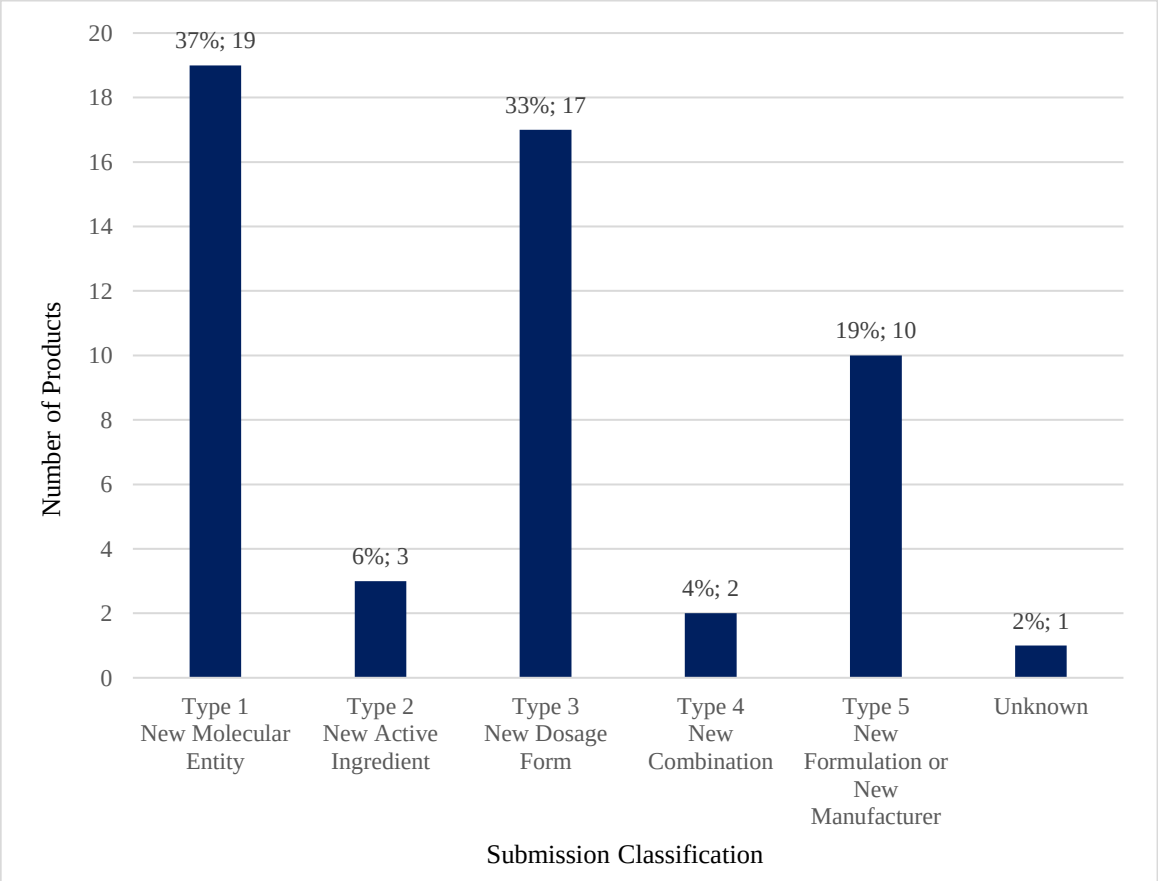

Figure 30. Submission Classification of NBCD Reference products approved by FDA.

3.4. Analysis by Categories of Complex Drug Products

The FDA in the ‘Generic Drug User Fee Act (GDUFA) II Commitment Letter’ recognizes six complex drug product categories based on the sources of complexity: Complex Active Ingredients, Complex Formulations, Complex Dosage Forms, Complex Routes of Delivery, Complex Drug–Device Combinations, and other products where complexity or uncertainty concerning the approval pathway or possible alternative approach would benefit from early scientific engagement [16]. The outline of different categories of complexity for several complex drug products provides a strong basis to understand the most appropriate regulatory application procedure/regulatory density which needs to be implemented for each type of drug product.

Table 48 present the classification of NBCDs according to these six categories. Thus, 40% of NBCDs are classified as ‘Complex Active Ingredients’ (n=21, 40%) and 44% as ‘Complex Formulations’ (n=23, 44%) (Figure 31). Only 10% are identified as ‘Complex Dosage Forms’ (n=5, 10%), and 6% as ‘Complex Routes Of Delivery’ (n=3, 6%) (Figure 31). Neither NBCDs has been identified as ‘Complex Drug–Device Combinations’ or ‘other products where complexity or uncertainty concerning the approval pathway or possible alternative approach would benefit from early scientific engagement’ (Figure 31). In Figure 31 it is possible to observe that the ‘Complex Formulations’ and ‘Complex Active Ingredient’ are the most common types of NBCD products, which is in accordance with the current trends in pharmaceutical development.

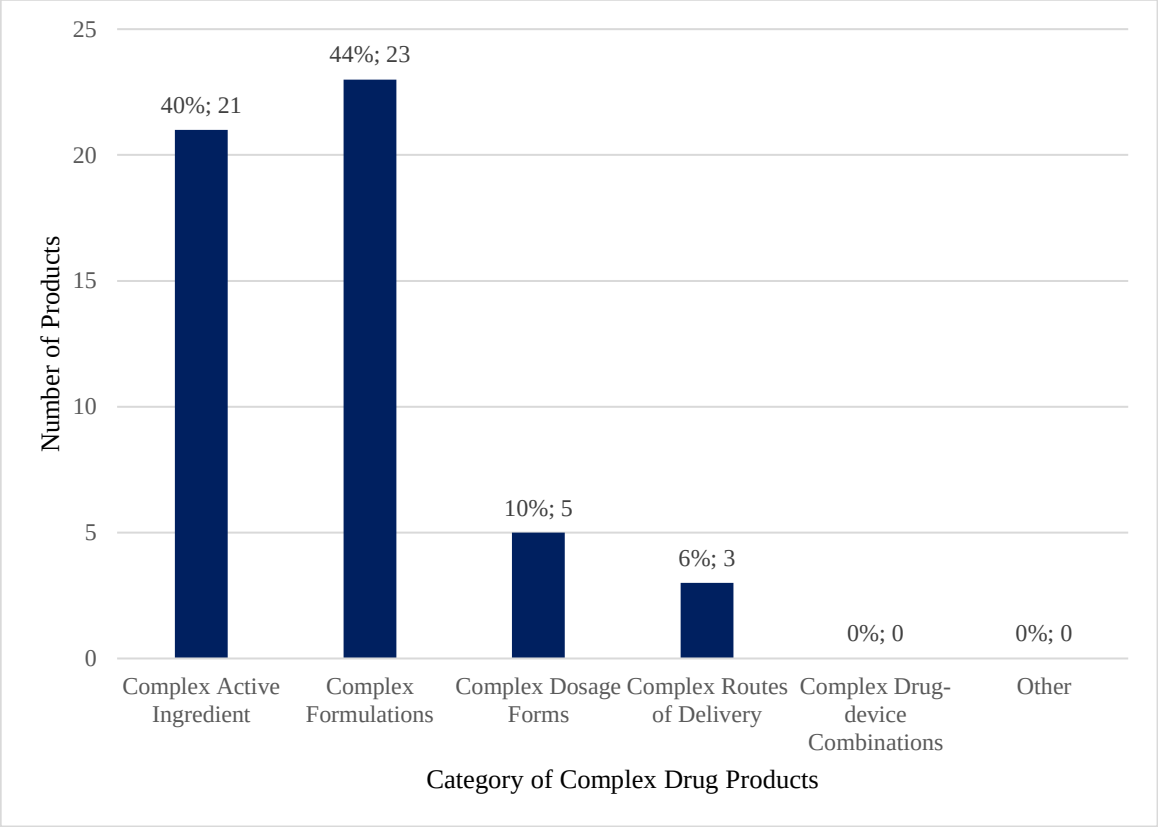

Figure 31. Classification of Non-biologic Complex Drugs (NBCDs) according to the categories of drugs considered to be complex by the FDA.

3.5. Analysis by Marketing Authorization Procedures in the EU

As previously stated, the NBCDs and follow-on versions have not even been recognized as a distinct category by regulatory authorities, with no existing specific and dedicated pathways for their marketing authorization [18,19,22,30]. However, the follow-on versions need to be regulated by the EU legislation available (Section 2).

According to several scientific publications, the NBCD follow-on products can fall within the Generics Application under Article 10(1) or the Hybrid Application under Article 10(3), depending on the necessary data requirements to support the bioequivalence studies [19,34,136,143]. In accordance with Falk Ehmann and Ruben Pita, the Generics Application under Article 10(1) can be used '*if the follow-on medicinal product has the same qualitative and quantitative composition in active substance(s) and the same pharmaceutical form as the reference medicinal product, and whose bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies*'. Alternatively, the Hybrid Application under Article 10(3) might be used '*where the bioequivalence cannot be demonstrated through bioavailability studies or in case of changes in the active substance(s), therapeutic indications, strength, pharmaceutical form or route*

of administration, vis-à-vis the reference medicinal product, the results of the appropriate preclinical tests or clinical trials shall be provided' [143]. As indicated earlier, there are differing opinions about the most suitable EU legislation for the evaluation and regulation of NBCDs and follow-on products. Some authors argue that the Generics Application is not appropriate, whereas others claim that the Hybrid Application is a suitable and flexible regulatory approach for the approval of follow-on versions of NBCDs [136].

In the analysis conducted in this article, it is possible to verify that the NBCD follow-on products were approved through four different application procedures under European legislation (Figure 32): 55% through the Generic Application under Article 10(1) (n=49, 55%), 36% through the Hybrid Application under Article 10(3) (n=32, 36%), 6% through the Biosimilar Application under Article 10(4) (n=5, 6%), and 3% through the Informed Consent Application under Article 10(c) (n=3, 3%).

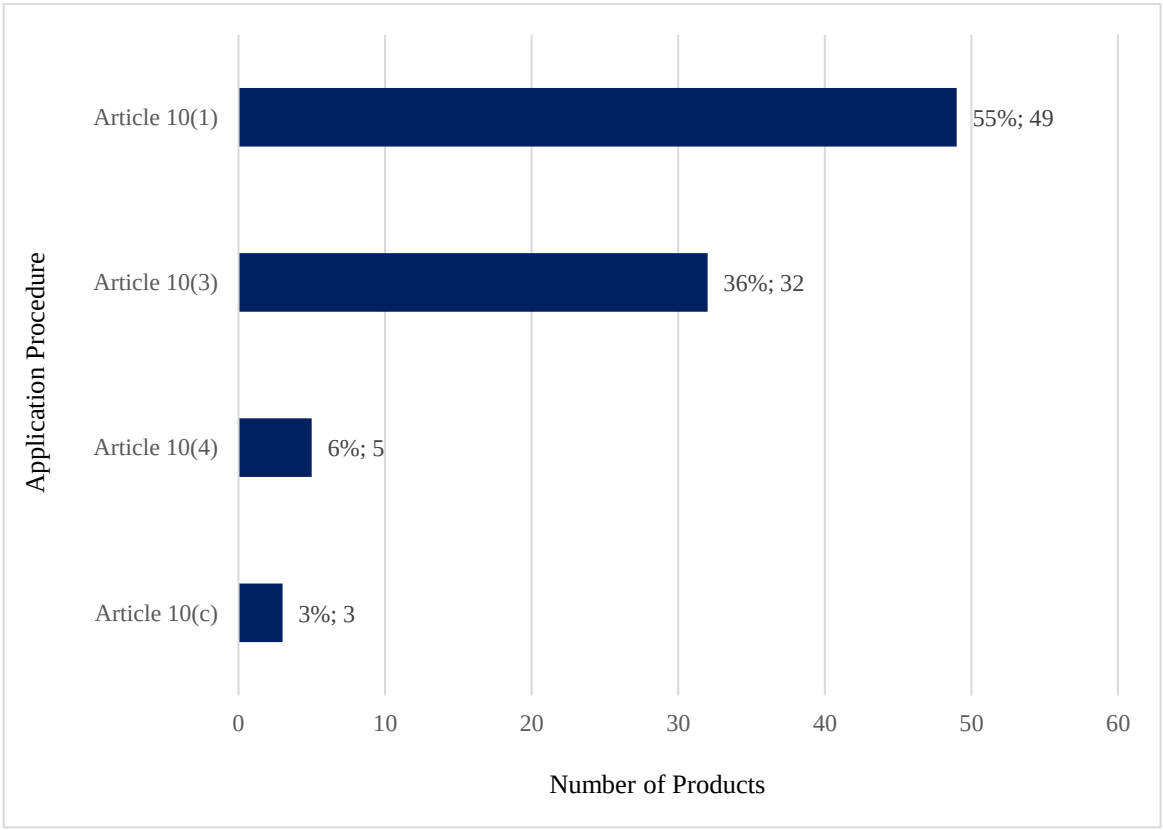

Figure 32. Application Procedures of NBCD follow-on products approved by the EMA.

In Figure 32 it is possible to observe that the follow-on versions were approved predominantly through the Generic Application, despite an increase in the use of the Hybrid Application Procedure for recent approvals of follow-on versions of Renvela® (Sevelamer carbonate) and Copaxone® (Glatiramer acetate). The upward trend in the use of Hybrid Application Procedure can be broadly linked to the uncertainty of the performance of certain classes of NBCDs, where is required complementary clinical data for marketing approval [239].

The results also indicate a tendency to use the same application procedure inside the same class of products (Table 49). The Hybrid Application Procedure under Article 10(3) was predominantly used for the follow-on versions of Renvela® (Sevelamer carbonate) and Copaxone® (Glatiramer acetate) (Table 49). On the other hand, the Generic Application Procedure under Article 10(1) was mostly applied for the approval of follow-on versions of Venofer® (Iron sucrose complex) and Diprivan® (Propofol) (Table 49).

Regarding the marketing authorization procedures used for approval of NBCD Reference products, 50% of them were approved via the Centralized Procedure (n=25, 50%), 20% through the National Procedure (n=10, 20%), 12% through the Mutual Recognition Procedure (n=6, 12%), and 4% were approved via the Decentralized Procedure (n=2, 4%) (Figure 33). Some Reference products present more than one approval procedure, such as: MRP/NP (n=5, 10%), and MRP/DCP/NP (n=2, 4%) (Figure 33).

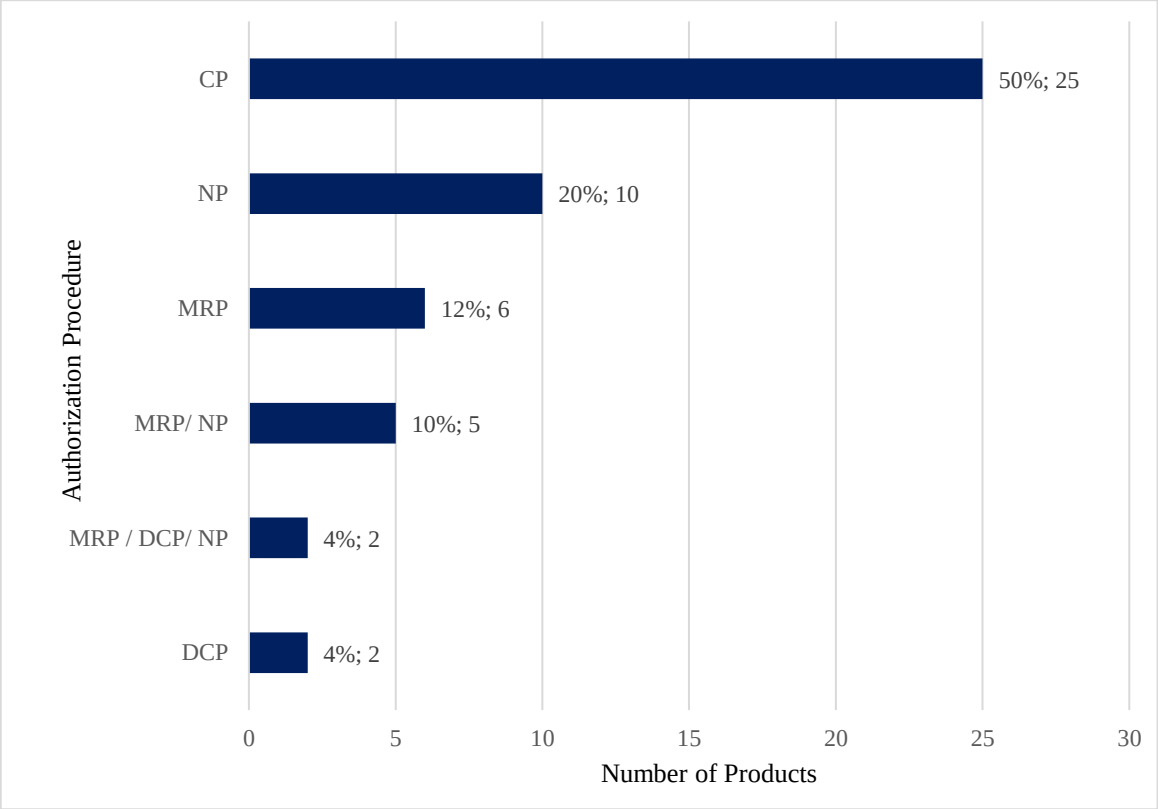

Figure 33. Marketing Authorization Procedures of NBCD Reference Products approved by the EMA.

On the other hand, Figure 34 highlights the trend of the marketing authorization procedures used for approval of NBCD follow-on versions. Approximately half of all follow-on products were approved via the Decentralized Procedure (n=47, 53%), and 30% through the National Procedure (n=27, 30%). Only 9% were approved via the Mutual Recognition Procedure (n=8, 9%), 4% through the Centralized Procedure (n=4, 4%), and 3% through both MRP/NP Procedures (n=3, 3%) (Figure 34).

While the biotechnology-derived medicinal products, like Biosimilars, have to follow a Centralized Procedure (CP), the follow-on versions of NBCDs might receive the marketing authorization through Non-Centralized Procedures (Figure 34) [22,30,362]. This can lead to impressive heterogeneity in regulatory approaches used for each product, and consequently result in high variability of regulatory requirements demanded and uncertainties related to their safety and efficacy evaluations [30,132]. Regarding the authorization procedures, there is broad consensus that the follow-on versions of NBCDs must follow the Centralized Procedures, and not the National Authorization Procedures. This opinion, shared by many experts as the NBCD Working Group of Lygature, emerges from the negative results of clinical studies of follow-on versions of nanomedicines authorized following National Procedures that 'clearly showed differences in clinical performance between the innovator and follow-on products' [18,362].

An interesting example that might illustrate the discussion around the Marketing Authorization Procedures is the case of the approval of a generic version of innovator Forteo® (US) /Forsteo® (EU) (teriparatide [rDNA origin] injection), a biological drug product marketed by Eli Lilly and Company (Lilly) for the treatment of osteoporosis [377,378]. Although this product presents biosimilar versions approved in the market (Movymia® and Terrosa®), Teva Pharmaceutical Industries, Ltd. developed a follow-on version of Forteo® by chemical synthesis, via the solid-phase peptide synthesis method. Thus, the generic version of TEVA was developed by a distinct manufacturing process, and falls under a different regulatory framework with different requirements for documentation, prescribing, and dispensing than biosimilar products that use a centralized registration procedure, despite referencing the same drug product (RLD) [237]. Teva may have been the 'first applicant' to submit an abbreviated new drug submission (ANDA) pathway for a generic equivalent of a biotech medicine. In Europe, the product was registered using a decentralized procedure under Article 10(3) (hybrid application), but without impositions to register the product through a centralized procedure [237,379]. This constitutes an unprecedented and intriguing situation, sometimes referred to as a 'regulatory tangle', since that for a common biological innovator (Forsteo®), exists two biosimilars registered through centralized procedures, and one follow-on version registered as a generic drug product through a decentralized hybrid application [237,379]. Other questions have arisen regarding the appropriateness of the hybrid application (Article 10(3)) for this follow-on version. The application of the hybrid pathway was justified by the fact that the Teva product cannot be strictly defined as a generic medicine, because

of qualitative and quantitative differences between the Teva product and Forsteo®, despite using Forsteo® as the Reference product in the BEq registrative study [379]. It is important to underscore that the reference product Forteo/Forsteo® not be included in the list of products of the bibliographic corpus since it corresponds to a biotechnology-derived product (biological drug product), which is not within the scope of the NBCDs. Its generic version may be considered as NBCDs.

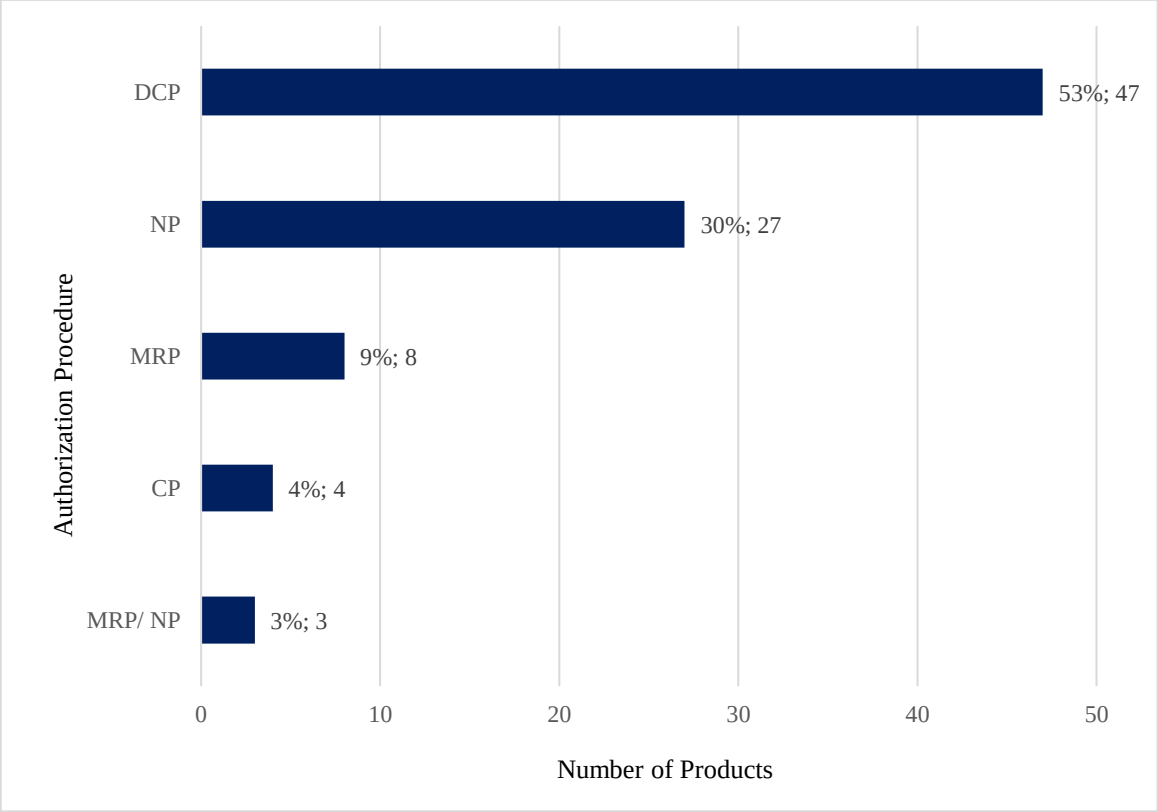

Figure 34. Marketing Authorization Procedures of NBCD Follow-on Products approved by the EMA.

In the EU, the authorization procedures selected for the marketing authorization of NBCDs and their follow-on versions depend on the year of submission (Table 49). Interestingly, the Decentralized Procedure is much more widely used in recent authorization applications than in older applications (Table 49). This is due to the fact that the Coordination Group for Mutual Recognition and Decentralized Procedures - Human (CMDh) has been only established in 2005 [30,380]. This can be verified for follow-on products of Diprivan®, which at first used the Mutual Recognition Procedure and National Procedure, and more recently used the Decentralized Procedure (Table 49).

On the other hand, several differences were found in the same class of products. For example, the LMWHs are considered biological medicinal products by EMA and complex drug products by FDA, consequently approved under different regulatory pathways by distinct regulatory authorities

[31,34,138]. According to the EMA classification, the LMWHs must be approved through a biosimilar approach under a Centralized Procedure [138,326]. However, in Table 49 is possible to observe that two follow-on versions of Lovenox® were approved under the Centralized Procedure, but the other three were approved via the Decentralized Procedure. The use of distinct authorization procedures was also verified for follow-on versions of Diprivan®, Renvela®, and Venofer® (Table 49).

With regard to Application Procedures, the follow-on products of LMWHs are evaluated under Article 10(4) for Biosimilars in the EU (EMA), and require the use of the Generic Application through the ANDA pathway in the US (FDA) [25,30,34,132,138]. As it is possible to see in Table 49, all follow-on products with enoxaparin sodium (n=5, 6%) were approved via the Biosimilar Application Procedure under Article 10(4). Contrarily to the EMA, in Table 48 is possible to observe that the follow-on products with enoxaparin sodium were approved via the Abbreviated New Drug Application (ANDA) under the 505(j) route [44,45].

This class of complex drug products is a clear example of the striking differences between the regulatory approaches of the EMA and FDA [25,30,34,132,138]. In particular, it is quite significant the contrast between the reflection and guidance documents published by each regulatory authority [31,34,138]. In accordance with the 'Draft Guidance on Enoxaparin Sodium' (FDA, 2011), in vivo BEq study requirements may be exempted if the follow-on product meets the five requirements for demonstrating active ingredient sameness (equivalence of physicochemical properties; equivalence of heparin source material and mode of depolymerization; equivalence in disaccharide building blocks, fragment mapping, and sequence of oligosaccharide species; equivalence in biological and biochemical assays; equivalence of in vivo pharmacodynamic (PD) profile), and is qualitatively (Q1) and quantitatively (Q2) the same as the Reference Listed Drug (RLD) [260].

Currently, there is a trend towards regulatory alignment between the EMA and FDA, which is reflected in a convergence of regulatory requirements [34,138]. For example, whereas the first version of 'Guideline on the non-clinical and clinical development of similar biological medicinal products containing low molecular-weight-heparin' (EMEA/CHMP/BMWP/118264/2007) required a comparative clinical trial by default, in the last revision (EMA, 2016) the clinical efficacy and safety studies are not considered necessary and the demonstration of biosimilarity is based on physicochemical and functional results and comparable pharmacodynamic profiles [320]. The last revision (EMA, 2016) of this guideline could have been responsible for the changes in the authorization procedures preferably used. As can be seen in Table 49 three products are approved under a Decentralized Procedure after 2016, instead of a Centralized Procedure [30,320].

3.6. Analysis by Marketing Authorization Procedures in the US

Figure 35 presents the regulatory pathways identified for NBCD Reference products and follow-on versions approved by FDA. There are approximately 48% of New Drug Applications (NDA) (n=55, 48%), corresponding to the NBCD Reference products, and 52% of Abbreviated New Drug Application (ANDA) (n=51, 52%), corresponding to the follow-on products (Figure 35).

The vast majority of NBCD follow-on products are approved through an Abbreviated New Drug Application (ANDA) under the 505(j) route (Figure 35). Despite the 505(j) route being proper for generic products of small molecules, this pathway can even result in problems in the PE/BE assessment of complex generics. The lack of comparative safety and efficacy clinical data makes the demonstration of equivalence inappropriate and increases the regulatory uncertainty relating to the overall benefit-risk assessment and patient safety [239]. Consequently, it is necessary to act with utmost caution regarding the selection of the ANDA application. A thorough physicochemical comparability study between the reference product and their generic version should be included to achieve pharmaceutical equivalence with the similarity in structural arrangement and formulation. Depending on the cases, the equivalence demonstration can involve clinical studies [32,299].

An interesting finding of the analysis is that only three follow-on products of RLD Taxotere® were approved through the New Drug Application (NDA) under the 505(b)(2) route [65,67,381] (Figure 36). Even though not as commonly applied, the 505(b)(2) route can be used for follow-on versions with significant differences compared to the RLD, such as a new indication, dosage form, strength, formulation, or route of administration [299,368]. Both applications of Taxotere® (application No. 201195, 203551, and 205934) correspond to submission of type 5 (New Formulation or New Manufacturer) since there are additional or different inactive ingredients in the formulation [65,67].

The 505(b)(2) route is economically unviable for the development of complex generics, and hence much less used than the 505(j) route [139]. This is due to the need to present full reports of investigations of the safety and effectiveness of complex generics versus reference products, adequately supported by additional clinical and/or nonclinical studies [139,299]. According to the differences between the RLD and follow-on products, the 505(b)(2) application shall include sufficient and appropriate data to support those variations [299].

However, the 505(b)(2) application presents a significant degree of flexibility compared with the 505(j) route, since there is the possibility to select the type of studies, data, and information to be included in the submission. The 505(j) route is significantly more limited concerning the additional physicochemical characterization and in vivo bioequivalence studies [32,299].

Therefore, it is possible to infer that the 505(b)(2) application can also be an attractive and scientifically robust alternative to the development and approval of follow-on versions of NBCDs.

This is particularly advantageous when the clinical studies requested do not comply with the assumptions of the 505(j) route [32,299].

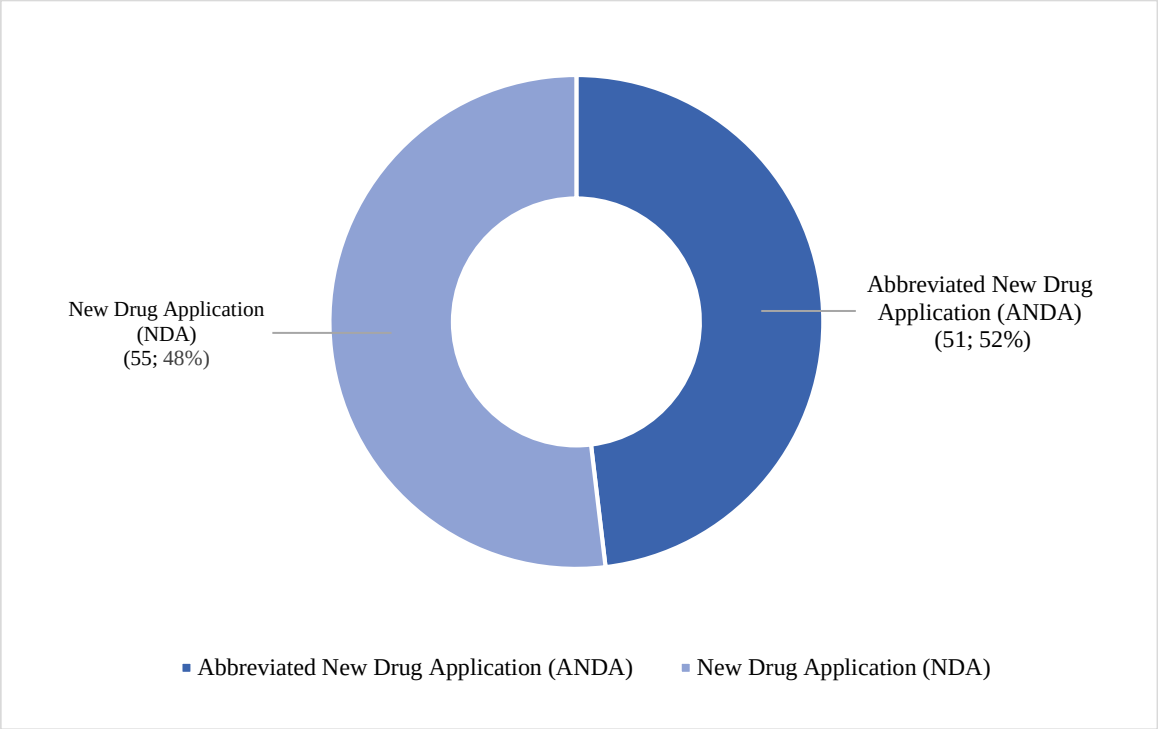

Figure 35. Regulatory Pathways of NBCDs and Follow-on Products approved by the FDA.

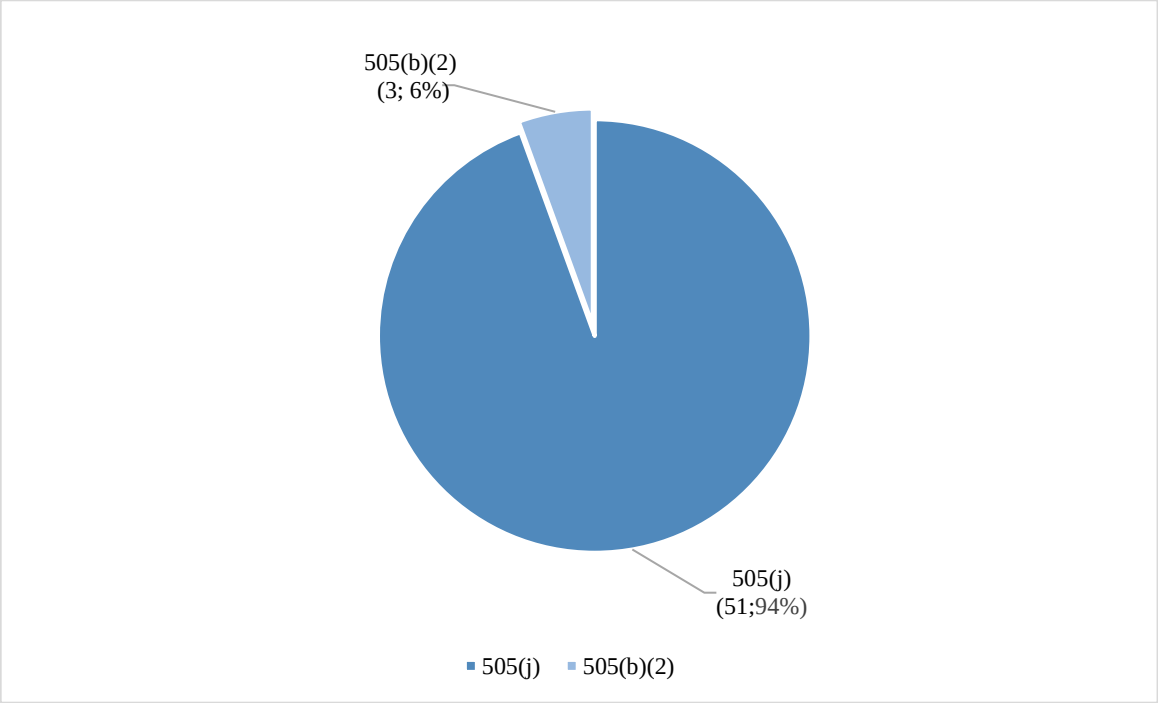

Figure 36. Type of Application for NBCD Follow-on Products approved by the FDA.

From the total of 52 NBCD products submitted under the New Drug Application (NDA), 46% of them used the 505(b)(1) route (n=24, 46%) and 29% used the 505(b)(2) (n=15, 29%) (Figure 37). It should be noted that for 25% of the total of NDAs, it was not possible to understand the route of submission (Figure 37). In this case, it was unable to determine the route of submission due to the lack of complete and publicly available information on the FDA website for older products, mainly approved before the year 2000. In addition, this lack of information can also difficult to understand approval pathways implemented and limit the pharmaceutical development of and launching new products in the market rapidly and efficiently.

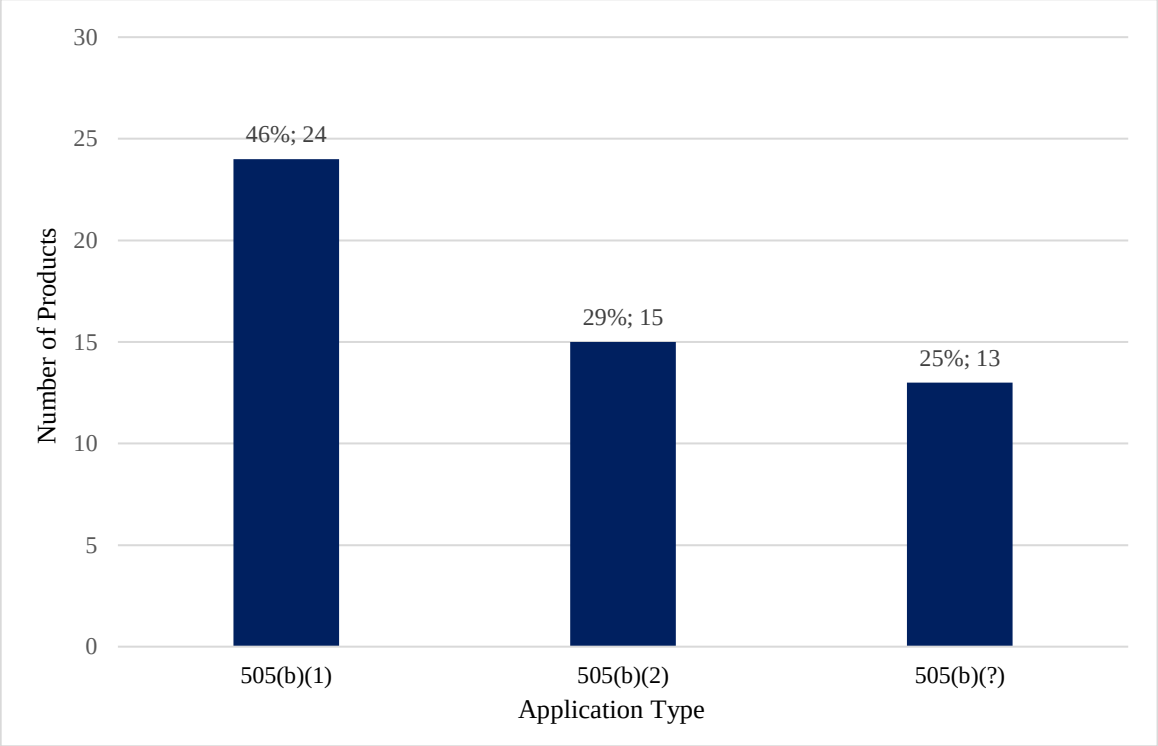

Figure 37. Type of Application for NBCDs Reference Products approved by the FDA.

4. Concluding Remarks

The diverse and complex nature of Non-Biological Complex Drugs (NBCDs) led to global challenges in the establishment of regulatory approaches and guidance documents for approving complex generics. Accordingly, there are divergent opinions about the regulatory approach, submission strategies, and outcomes of the approval of follow-on versions of NBCDs, depending on the countries and competent authorities involved, as well as the type of NBCDs.

The lack of a distinct regulatory approach for each category of NBCDs and their follow-on versions led to a wide diverse regulatory landscape throughout the EU and the US. Similarly, also some striking differences in the approval pathway within the same type of NBCDs have been uncovered. For example, the analysis of the regulatory approach implemented in the EU shows that the Non-Centralized Procedures are most widely used for follow-on versions than the Centralized Procedures (Table 49). On the other hand, it could be verified that the follow-on versions were approved predominantly through the Generic Application under Article 10(1), despite there being an increase of the Hybrid Application under Article 10(3) for recent approvals of NBCD follow-on versions (Table 49). In the specific case of follow-on versions of iron-carbohydrate, sevelamer carbonate, and glatiramer acetate, the regulatory pathway mainly applied was been the hybrid application procedure via Article 10(3).

In the U.S., the majority of NBCD follow-on products are approved through an Abbreviated New Drug Application (ANDA) under the 505(j) route, including the complex generics of iron-carbohydrate, sevelamer carbonate, and glatiramer acetate (Table 48). It means, the FDA used a more simplistic regulatory approach (generic pathway) for these 'Complex Active Ingredients', which is not analogous to the hybrid pathway (505(b)(2) application) used by EMA. It is important to infer that the conventional approach (generic drug pathway) used in FDA approvals may not be the most appropriate to establish the therapeutic equivalence of these follow-on versions, due to the difficulty, or even impossibility, in obtaining the complete characterization of the active pharmaceutical ingredient (API). Therefore, it would be recommendable the use of 505(b)(2) application with the provision of additional/comparative clinical studies for safety and efficacy assessment of the complex follow-on versions.

The NBCDs constitute a diverse group with varying degrees of complexity, which therefore hinder the existence of a universal regulatory approach that applies to all classes of products. It should be noted that in the specific case of the NBCDs, 'there is no 'one-size-fits-all approach' when looking at the relevance of pre-clinical data and the availability of clinical data for marketing approval', as referred by Gaspar et al [239]. Accordingly, it is critical to perform a case-by-case analysis depending on the type of complex product to be developed, and the scrutiny of the available product-specific guidelines.

For complex products on which it is possible to achieve the complete characterization of the API or the pharmaceutical equivalence through an additional physicochemical characterization and/or in vivo BE studies, the traditional generic approach under 505(j) route (ANDA) or the Article 10(1) should be selected for the applications submitted in the United States (FDA) or Europe (EMA), respectively (Figure 38). On the other hand, the hybrid applications covered by section 505(b)(2) (U.S., FDA) and Article 10(3) (EU, EMA), can be considered an appropriate pathway when it is impossible to establish the therapeutic equivalence through a complete characterization of the drug product (Figure 38). This means that we could potentially follow an identical evaluation path to the totality of evidence pathway that is already established for approval of biosimilars of complex biologics, in particular with the inclusion of analytical comparability and functional comparability study followed by non-clinical and clinical studies to examine similarity. In duly justified specific cases of NBCDs there is the possibility that some of the so-called complex generics may not be covered by the genuine definition of generics, wherefore need to be looked at as new complex drug products.

In the light of the above, is extremely important to develop consistent and well-established strategies of regulatory science for NBCDs and their follow-on versions, to form a similar and harmonized basis of approval, ensure the highest level of safety and effectiveness of products, promote the clarity and consensus among the regulatory authorities, decrease the assessment time and the costs of development, and promote the access to more affordable drugs. For example, the possibility to perform a Pre-Investigational New Drug (IND) meeting with the FDA can be very helpful in planning a drug development program, especially if sponsors' concerns are not fully answered by guidance and other information provided by the regulatory agency. This early interaction with the regulatory authority provides sponsors information that will assist to submit complete investigational new drug applications, preventing clinical hold issues from arising [382]

On the other hand, there is an urgent need to fill the gap in publishing product-specific guidance documents (PSGs) for each category of NBCDs, including detailed information on the approval pathways and regulatory requirements that should be followed. For example, the definition and uniformization of qualitative and quantitative (Q1/Q2) sameness requirements for inactive ingredients are of utmost importance for certain types of complex generics (e.g. liposomal formulations). It is also relevant to underline the need to make a public statement on the planned significant revisions to existing PSGs for complex drugs.

Substantially increased capability to understand and define relevant critical quality attributes (CQAs) will be needed to achieve greater robustness in therapeutic equivalence assessments. This can be particularly useful for drug products containing complex APIs or when the exact mechanism of action is not yet known (e.g. glatiramer acetate complex products). The next chapters will attempt to provide an overview of critical characteristics that have an impact on the quality, safety, and

clinical profile of every single product, just as the corresponding characterization techniques (Chapters 5, 6, and 7).

Additionally, regulators must participate actively in the appropriateness of available regulatory approval procedures for the therapeutic equivalence assessment of complex generics, defining the degree of regulatory exigency - highest requirements - in accordance with the type and complexity of each drug product. In this regard, it would be essentially the publication of a comprehensive and up-to-date list of complex drug products and follow-on versions approved in the U.S. and European markets, specifically highlighting complex drug product categories, to facilitate the early detection of the level of regulatory exigency required. In conclusion, the sharing and alignment of state-of-the-art knowledge and regulatory harmonization involving science-based multi-stakeholders brings a clear added value to advancing regulatory science and overcoming the several challenges related to NBCDs.

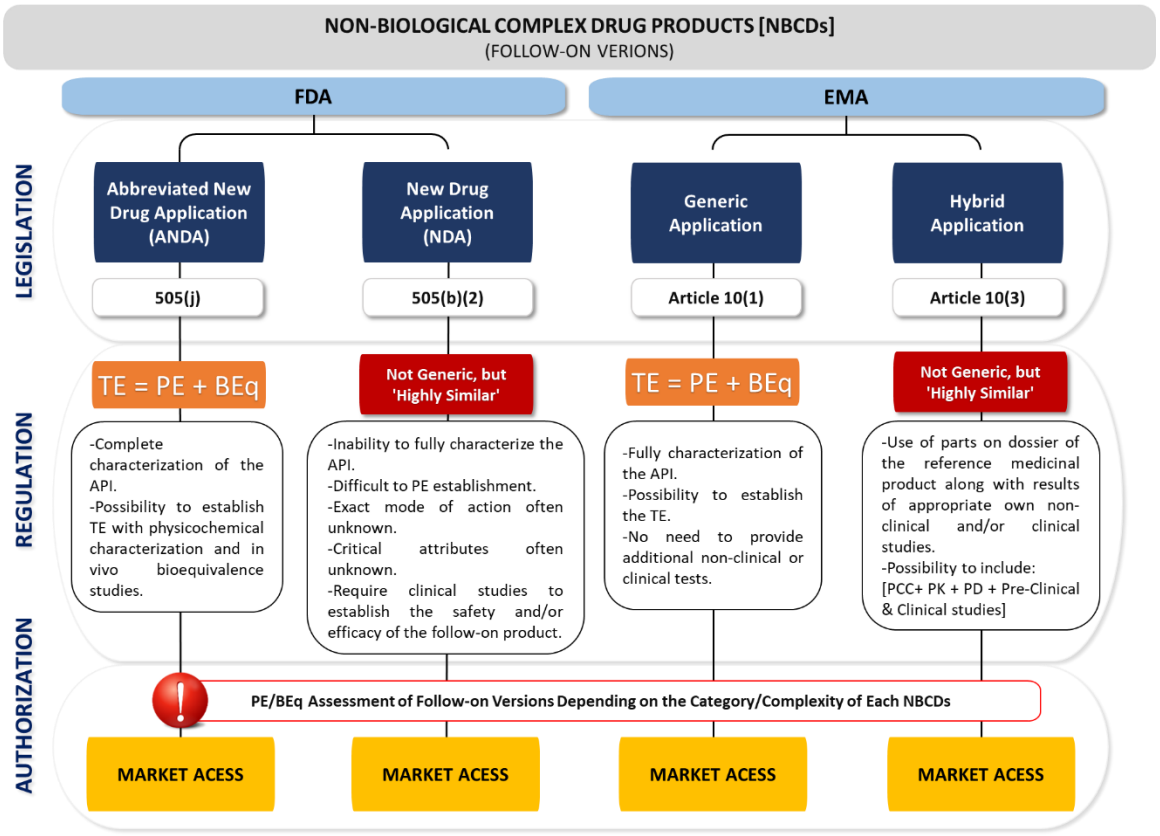

Figure 38. Existing Regulatory Science Framework for the Approval of Follow-On Versions of Non-Biological Complex Drugs (NBCDs) in the United States (FDA) and Europe (EMA).

Chapter IV. Pharmaceutical Quality by Design (QbD): A Strategic Approach to Risk Management and Regulatory Compliance

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IV.I. Quality by Design (QbD) Approach in Marketing Authorization Procedures of Non-Biological Complex Drugs: A Critical Evaluation

Abstract

The emergence of innovator-driven complex drug products, such as the Non-Biological Complex Drugs (NBCDs), has provided disruptive advances in the Nanotechnology and Biotechnology fields. However, the design and development of NBCDs can be especially difficult with some unresolved regulatory challenges related to the pharmaceutical quality assessment.

The application of a more holistic, systematic, integrated science and risk-based approach, such as Quality by Design (QbD), is essential to address key scientific and regulatory challenges in the research and development of the NBCDs. The deeper product and process understanding derived from the implementation of the QbD approach ensures consistent, reliable, and high-quality pharmaceutical products, and promotes innovation and continuous improvement in the entire product lifecycle.

The prime aim of this chapter is to provide deeper insight and understanding about the current state of implementation of the QbD approach in the pharmaceutical development and approval of NBCDs in Europe and the United States, through the analysis of the available data from their regulatory dossiers. Additionally, it aims to understand and discuss in what way the QbD approach is established and operated by the Pharmaceutical Industry for complex drug products, as well as, highlight the gaps and challenges related to the implementation of this approach. The advantages of the QbD approach concerning the increase of knowledge, regulatory flexibility, and faster development based on processing big data, just as the reduction of the risk of failure in regulatory procedures or market withdrawal of NBCDs are also be addressed.

Keywords

Non-Biological Complex Drugs; Pharmaceutical Development; Pharmaceutical Quality by Design; Risk Assessment; Design of Experiments; Design Space; Critical Control Strategy; Product Life Cycle Management; Regulatory Science Research; Marketing Authorization; Drug Approval; Marketing Withdrawal; European Medicines Agency; U.S. Food and Drug Administration.

1. Introduction

The development of advanced and innovative drug delivery systems, such as Non-Biological Complex Drugs (NBCDs), has become highly prevalent in recent years. Its popularity has been related to the countless advantages compared to conventional medicines, particularly in quality, safety, and efficacy improvement. The NBCDs have attractive biological properties and numerous benefits such as their ability to deliver both hydrophilic and lipophilic drugs, to increase drug solubility and permeation through biological membranes, to respond to specific microenvironments, but also due to their biocompatibility and biodegradability properties [6,135,383–386]. Besides their capacity to direct the delivery of drugs to specific targets in the human body, these nanosystems can shelter their content, increasing the drug half-life in circulation and their efficacy, and preventing the uptake by immune cells thus minimizing systemic toxicity. Another important characteristic of NBCDs is the possibility of fine-tuning multiple properties such as lipid composition, ratios, and surface chemistry, including their size, charge, and surface functionality [6,8,9,387].

Despite these advantages, concerns regarding the control of quality, efficacy, and safety of these nanosystems have increased along with the widespread of this technology.

As mentioned in previous chapters, the NBCDs present heterogeneous structures that cannot be fully quantitated, characterized, or described by physicochemical analytical methods [17,20,30]. The composition is complex and the manufacturing process is challenging, costly, and hard to upscale, hindering the compliance with quality standards of Good Manufacturing Practice guidelines [8,388]. Thus, their complexity constitutes a significant challenge in the proper demonstration of therapeutic equivalence of complex generics with their brand-name counterparts [14,17,19,25]. For some classes of NBCDs, the structure-function relation or mechanism of action has not yet been fully described, as well as, the completeness characterization of their physicochemical and structural properties [136,164].

Thereby, the knowledge of how formulation variables and manufacturing process parameters impact the final product's critical quality attributes (CQAs) and *in vivo* performance is still limited [25,389]. As mentioned in 'Progress report on the 3rd International Symposium on Scientific and Regulatory Advances in Biological and Non-Biological Complex Drugs: A to Z in Bioequivalence', the '*identification and a thorough (physicochemical) characterization of CQAs is an important step towards the development of a follow-on or similar complex drug product*' [390]. The lack of critical quality attributes (CQA) assessment, makes it impossible to demonstrate therapeutic equivalence and consequently hamper the approval and market access of complex generics [25,389].

Apart from the complex and heterogeneous structure, the quality and therapeutic performance of NBCDs be based on a tightly controlled manufacturing process [17,20,30]. In line with the principle 'The Product is the Process', any change in the manufacturing process or formulation,

even though it be small, can bring variations in quality, efficacy, and safety properties of the final product [14,30,31,136,138,239,333,335–338]. This can be a problem, especially to ensure both reproducibility and batch-to-batch consistency of complex drugs and their complex generics [17,30,134]. On the other hand, the technology transfer from lab-scale to large-scale constitutes an important challenge [391].

The pharmaceutical quality assessment of the finished drug product constitutes one of the main focuses of pharmaceutical R&D, providing the basis for the patients' confidence in the safety and effectiveness of medicines. Thus, the product quality monitoring should be performed throughout the product lifecycle, as well as, in the post-approval to ensure the product quality consistency, therapeutic efficacy, and patient safety.

However, despite the regulatory oversight of drug product quality through the review of drug applications and inspection of compliance with current good manufacturing practices (cGMPs), there are still some complex hurdles to the pharmaceutical quality such as: inherent defects in product and process design, failures in the implementation of manufacturing process scale-up and routine production, outdated equipment, product recalls, lack of effective quality management systems, or regulatory review and inspection practices that analyze all products equally without taking into account the specific risks to the consumer or individual product failure modes [392].

To address the gaps identified above, the Food and Drug Administration (FDA) published a significant initiative designated as the '*Pharmaceutical cGMP Initiative for the 21st Century – a Risk-Based Approach*' (2002), to promote and modernize the regulation of pharmaceutical manufacturing and product quality [393]. As mentioned by Janet Woodcock (FDA CDER, 2004), this initiative aims to promote 'a maximally efficient, agile, flexible manufacturing sector that reliably produces high-quality drug products without extensive regulatory oversight' [392].

On the other hand, the Center for Drug Evaluation and Research (CDER) established the Office of Pharmaceutical Quality (OPQ) (2015), to guarantee the availability of high-quality drug products across all sites of manufacture and human drug product areas, such as the new drugs, generics, biologics, biosimilars, over-the-counter drugs, and compounded drug products [392]. OPQ is responsible for bringing a comprehensive approach to quality oversight to strengthen and ensure a consistent pharmaceutical quality over the drug product lifecycle, through the integration of review, inspection, surveillance, policy, and research. This '*One Quality Voice*' strategy promotes transparency and communication between the agency and pharmaceutical industry, streamlines regulatory processes, advances quality standards, and initiates surveillance function of quality within CDER [392].

The EMA (European Medicines Agency) 'Regulatory Science to 2025 Strategic reflection' and the 'Framework for FDA's regulatory science initiative' define several strategies to overcome the scientific and regulatory challenges related to the pharmaceutical quality of Complex Drug Products [389,394]. These include the need for the development and standardization of

sophisticated analytical techniques, understanding the product complexity and the correlation between critical quality attributes and in-vivo behavior, monitoring, and controlling the continuous manufacturing process, and developing new statistical tools to detect changes in process or product quality [389,394].

Moreover, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and regulatory agencies bring the Quality by Design (QbD) concepts originally proposed by Joseph M. Juran (1970), through the launch of pharmaceutical guidance documents ICH Q8 (Pharmaceutical Development), Q9 (Quality Risk Management), Q10 (Pharmaceutical Quality System), Q11 (Development and Manufacture of Drug Substances), and Q12 (Pharmaceutical Product Lifecycle Management) [7,314,395–398].

The application of a more holistic, systematic, and risk-based approach, such as Quality by Design (QbD), is essential to overcome the technical and quality challenges through a deeper product and process understanding, ensure high-quality pharmaceutical products, and promote the grant of marketing authorization [395–397]. This approach is distinct from the conventional pharmaceutical development of 'Quality by Testing', since that enables building quality and safety from the earliest steps of the design and manufacturing process [7,399].

The European Medicines Agency (EMA) and the United States Food and Drug Administration (US FDA) launched a joint pilot program for the parallel assessment of applications containing Quality by Design (QbD) elements (March 2011) [400]. The main purpose of this program was to promote and facilitate the consistent implementation of QbD concepts and relevant regulatory requirements introduced through the International Council for Harmonization (ICH) Q8, Q9, and Q10 documents, but also to harmonize regulatory decisions to the greatest extent possible between both regions [400].

On the other hand, the release of the final FDA guidance on liposome drug products comprising QbD principles according to ICH Q8(R2) Pharmaceutical Development, including screening of critical variables (CQAs) and establishment of a design space, illustrates the effort of the regulatory authorities to provide recommendations focused on the unique technical aspects of such dosage forms [253]. Other recent initiatives related to the complex drug products include the 'Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA Guidance for Industry' (FDA, 2020), and the Pilot Program 'EMA-FDA Parallel Scientific Advice for Hybrid/Complex Generic Products' (FDA/EMA, 2021) [16,367]. Thus, the development and implementation of regulatory initiatives and appropriate guidance documents demonstrate the acceptability by the regulatory authorities of the application of the QbD approach for pharmaceutical product design [7,314,392,393,395–398,401,402].

The timeline of Pharmaceutical Quality by Design implementation, initiatives, guidance documents, or other key developments related to the pharmaceutical quality is presented in Figure 39 [7,16,253,299,314,367,395–398,400,403–409].

Despite the Quality by Design (QbD) approach is not a new concept, its implementation in the pharmaceutical development of complex drug products has not been widely established. Thus, the main purpose of this chapter is to understand and discuss in what way the QbD approach is established and operated by the Pharmaceutical Industry for complex drug products, as well as, highlight the gaps and challenges related to the implementation of this approach. The advantages and disadvantages of the QbD approach concerning the regulatory flexibility or product quality assessment have also been addressed.

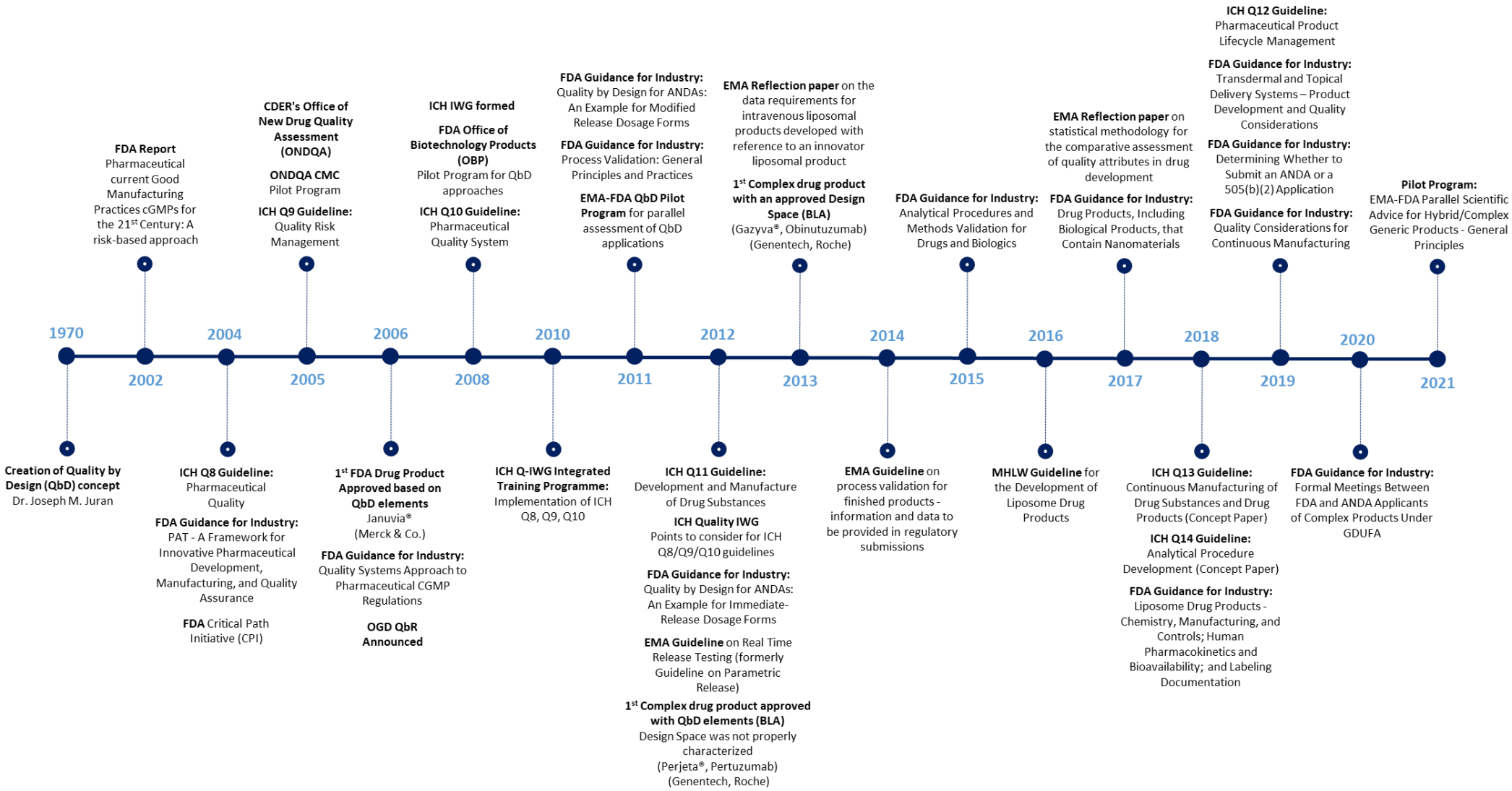

Figure 39. Timeline of QbD Implementation and Regulatory Initiatives related to the Pharmaceutical Quality [7,16,253,299,314,367,395–398,400,403–409].

2. Pharmaceutical Quality by Design (QbD) Concepts

QbD is defined in ICH Q8(R2) as a *'systematic approach to product development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management'* [395]. The pharmaceutical QbD approach seeks to ensure the desired quality of the pharmaceutical products as early as possible, through the early evaluation of the product and process specifications, just as the appropriate manufacturing controls [395,397,410].

One of the aims of this approach is to guarantee the identification, explanation, and management of variability sources that affect a process through the application of statistical, analytical, and risk-management methodology in the design, development, and manufacturing of drug products. Therefore, this systematic approach goes further to identify and select the limiting factors of each step in the development process, providing the necessary means to perceive how each step of the process affects the final product quality attributes.

As referred by EMA, the quality by design enables the finished medicine to consistently meet its predefined characteristics from the start - so that it is *'right first time'* [411]. Therefore, the product quality should be built from the beginning based on knowledge and a thorough understanding of its characteristics just as their manufacturing process.

An increased focus on the QbD concept is also due to the reduction of regulatory oversights. The incorporation of QbD principles in regulatory submissions guarantees less hassle during the review, fewer manufacturing problems, lesser time-consuming approvals, a reduced number of post-market manufacturing supplements, and the capability to implement different technologies that enhance manufacturing without regulatory scrutiny [8,412].

Thus, the application of a systematic approach will shorten the development times and costs, reduce the likelihood of manufacturing failures, enhance the formulation design and performance, and the success rate in regulatory approvals will be higher, providing opportunities for continuous improvement [395,410,413,414].

The QbD implementation encompasses the definition of the Quality Target Product Profile (QTPP), identification of Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs), and Critical Process Parameters (CPPs). This quality-improving scientific approach also includes the quality risk management principles to establish an appropriate control strategy, through a proposal for a Design Space(s) and/or Real-Time Release Testing (RTRT). The product lifecycle management, including continual improvement, is also part of a QbD framework [395–397]. The definition of QbD elements according to the ICH guidance documents and regulatory authorities is summarized in Table 8.

The QTPP is an overview of the quality characteristics of the drug product that should be reached to provide the desired quality considering the target product's safety and efficacy. Their

identification depends on prior scientific knowledge and may include the dosage form, dosage strength, drug product quality criteria (e.g. assay, dissolution, impurities profiles), and so on [152,395,415,416].

A CQA, as defined in ICH Q8(R2), is a '*physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality'* [395]. The quality attributes of the drug product include normally physical attributes, residual solvents, drug assay, drug content uniformity, identity, and impurities. Their criticality is assessed through risk assessment tools considering the severity of harm to the patient [152,395,415,416].

The identification of potential CMAs and CPPs is performed using different risk assessment tools such as the Cause and Effect Diagram, Risk Estimation Matrix (REM), and Failure Mode Effects Analysis (FMEA). CMAs are characteristics of input materials (drug substance and excipients) and CPPs are process parameters that can have an impact on the CQAs of the drug product [152,395,415,416].

The Design of Experiments (DoE) methodology is used to investigate the effect and relationship of process parameters and material attributes on the CQAs. This will allow establishing the design space, which is the region where the product quality is ensured, and to define a control strategy [152,395,415,416].

The main steps for the implementation of the Pharmaceutical QbD approach are described in Figure 40 [391,395–397,399,403,417–419].

Table 8. Pharmaceutical Quality by Design (QbD): Definition of QbD elements ([395–397]).

QbD Element	Definition
Continuous Process Verification	An alternative approach to process validation in which manufacturing process performance is continuously monitored and evaluated.
Control Strategy	A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.
Critical Material Attribute (CMA)*	A physical, chemical, biological, or microbiological property or characteristic of an input material (e.g., drug substance, excipients, primary packaging materials) that should be within an appropriate limit, range, or distribution to ensure the desired quality of that drug substance, excipient, or in-process material. The control of input material attributes is based on an understanding of their impact on processability or product quality.
Critical Process Parameter (CPP)	A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.
Critical Quality Attribute (CQA)	A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality (e.g. physical attributes, residual solvents, drug assay, drug content uniformity, identity, and impurities).

Design Space	The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.
Design of Experiments (DoE) (also referred as 'Formal Experimental Design')	A structured, organized method for determining the effect and relationship between factors affecting a process and the output of that process, i.e., process parameters and material attributes on the CQAs.
Lifecycle Management	Lifecycle is defined as all phases in the life of a product from the initial development through marketing until the product's discontinuation. The lifecycle management includes preventive actions and continual improvement, a recurring activity to increase the ability to fulfill requirements (ISO 9000:2005).
Manufacturing Process based on QbD	Adjustable within design space. Lifecycle approach to validation and, ideally, continuous process verification. Focus on control strategy and robustness. Use of statistical process control methods.
Normal Operating Range (NOR)*	The region around the target operating conditions that contain common operational variability (variability that can't always be controlled). NOR is not an established ICH term.
Pharmaceutical development based on QbD	Systematic, relating mechanistic understanding of material attributes and process parameters to drug product CQAs. Multivariate experiments to understand product and process. Possibility of establishing of design space. Possibility of implementing the PAT tools.
Process Analytical Technology (PAT)	A system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.
Process Control	The process control strategies can include: PAT tools utilized with appropriate feedforward and feedback controls; or process operations tracked and trended to support continual improvement efforts post-approval.
Process robustness	Ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality.
Product Specification	Part of the overall quality control strategy; Based on desired product performance with relevant supportive data.
Proven Acceptable Range (PAR)	A characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria.
Quality	The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as identity, strength, and purity (from ICH Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances).
Quality by Design (QbD)	A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.
Quality Risk Management	A systematic process for the assessment, control, communication, and review of risks to the quality of the drug (medicinal) product across the product lifecycle.
Quality Target Product Profile (QTPP)	A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product (e.g. dosage form, dosage strength, drug product quality criteria (e.g. assay, dissolution, impurities profiles)). Their identification depends on prior scientific knowledge.
Real-Time Release Testing (RTRT)	The ability to evaluate and ensure the quality of in-process and/or final product based on process data, which typically include a valid combination of measured material attributes and process controls.

*Definition not included on ICH Guidelines

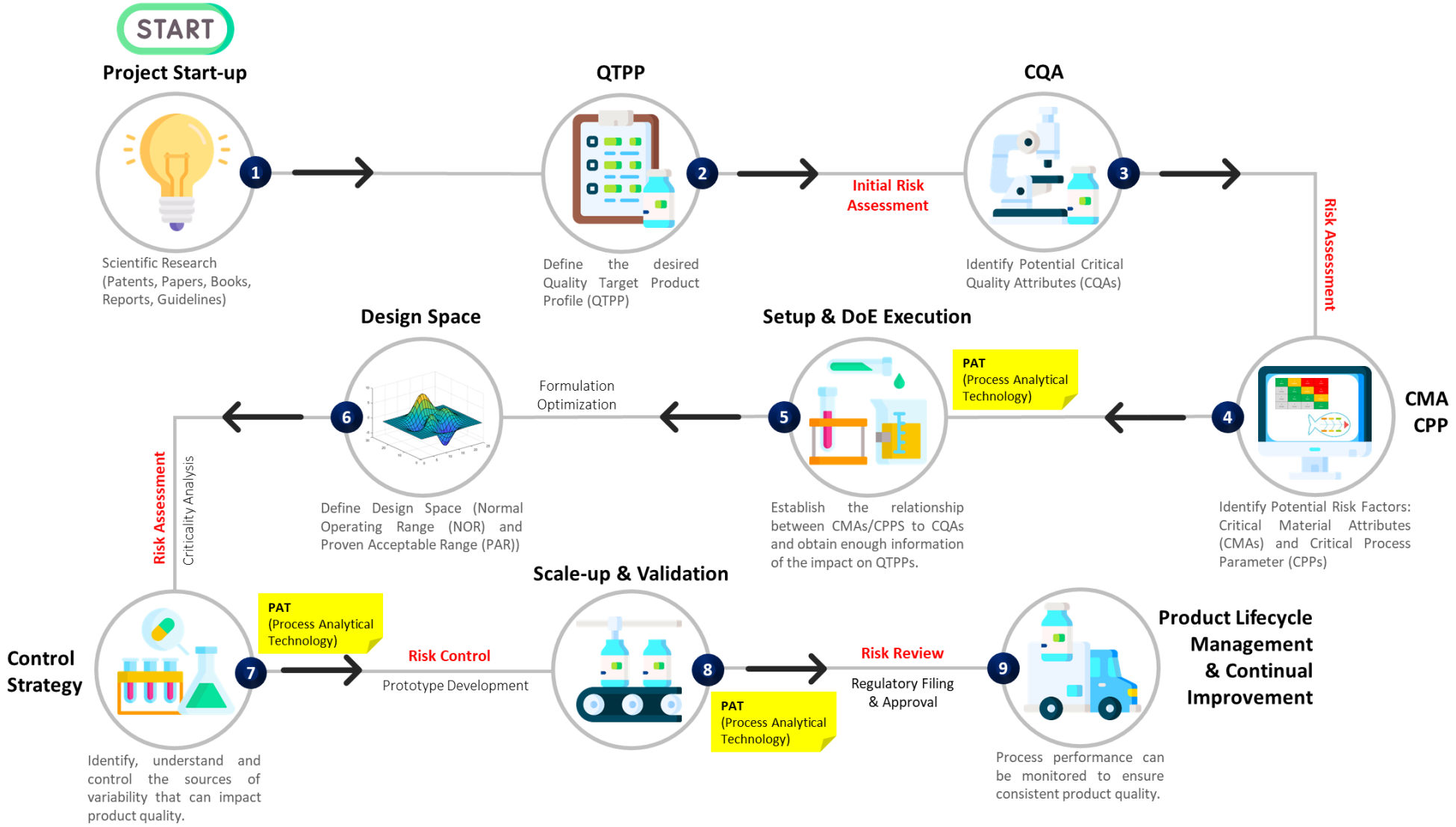

Figure 40. Roadmap for Pharmaceutical QbD Implementation (adapted from [391,395–397,399,403,417–419]).

Several scientific publications and regulatory guidance documents allowed the dissemination of the QbD principles as a valuable approach in the development and approval of drug products, including generics and new pharmaceutical products. The extended use of the QbD approach presents several advantages for the pharmaceutical industry, but also the regulators. The application of a systematic approach will shorten the development times and costs, reduce the likelihood of manufacturing failures, enhance the formulation design and performance, and the success rate in regulatory approvals will be higher, providing opportunities for continuous improvement [395,410,413,414]. Figure 41 provides a brief description of the inherent and significant value of the Pharmaceutical QbD approach concerning the impact on product quality and the regulatory framework [8,395,401–403,410,413,414,417,418,420].

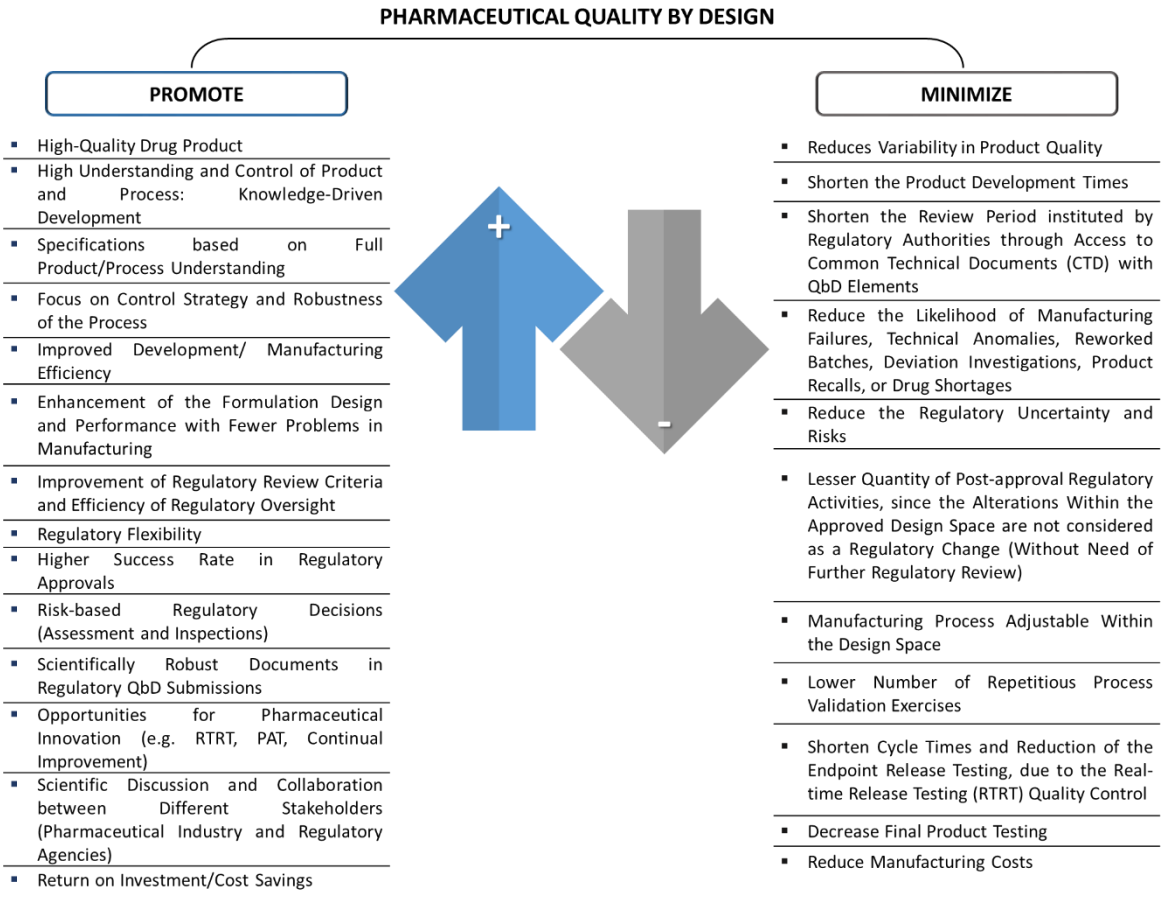

Figure 41. Scheme of the Advantages of Quality by Design Approach Implementation in Pharmaceutical Development of Complex Drug Products [395,401–403,410,413,414,417,418,420].

Despite the obvious benefits related to the QbD approach, for applicants as well as regulatory bodies, the pharmaceutical industry still considers some hurdles of the QbD implementation in the pharmaceutical development of complex drug products. These obstacles are frequently linked to the perception that QbD is an expensive approach, the lack of understanding of the QbD concepts and their advantages, or the idea that is needed an initial investment in instrumentation and training of a qualified workforce in QbD, statistical software, or multivariate modeling. Other reasons for uncertainty in QbD implementation are related to the higher complexity of the product, the limited number of complex drug products whose development is based on the QbD approach, the need for more advanced methods, or high process robustness. From a regulatory point of view, the main barriers to the QbD implementation are the regulatory guidance documents do not adequately deal with the product complexity, unpublished information or data due to the confidentiality issues, the need to determine what relevant data is required in submissions, lack of international harmonization of regulatory requirements, need a 'regulatory agreement' or post-market management plan, or the need to assure collaboration and coordination between inspectors, compliance and review [401,403,417,418,420,421]. The next sections will outline the truthful rate of implementation of the QbD approach in the pharmaceutical development of complex drug products.

3. Methodology

The main goal of this chapter is to evaluate the implementation of the QbD approach in the pharmaceutical development and marketing applications of approved NBCDs by the FDA and EMA. Therefore, this chapter is organized as follows. Section 1 (Introduction) contextualizes the research scope of this chapter relating to the Pharmaceutical Quality System in the development and approval of complex drug products. Section 2, it is presented the pharmaceutical Quality by Design (QbD) concepts and the roadmap for QbD implementation. This section has also addressed the advantages and limitations related to the QbD implementation in the pharmaceutical development of complex drug products. Section 3 described the survey methodology used in this chapter. Hereinafter, Section 4 provides an analysis of the marketing authorization applications of NBCDs already approved by the FDA and EMA, in order to gather valuable information about the Non-QbD or QbD-based developments, identification of the QbD elements, screening of critical quality attributes (CQAs), and factors that can impact the CQAs. Also considered was the establishment of design space, the analysis of risk assessment tools, strategies of lifecycle management, or the discussion of the reasons for market withdrawal (if applicable). Lastly, Section 5 summarizes the major findings of this chapter and discusses the future perspectives of QbD implementation in the pharmaceutical development of NBCDs.

For this purpose, the exhaustive list of NBCDs already approved by the FDA and EMA outlined in Chapter I was used as a scientific basis for this analysis and discussion. Each approved NBCD was assessed relating to the QbD principles and elements incorporated into their pharmaceutical development and consequently described in the submission documents. Some query terms were surveyed in each regulatory document, such as: ‘Quality by Design’, ‘QbD’, ‘Quality Target Product Profile’, ‘QTPP’, ‘Critical quality attributes’, CQAs’, ‘Critical process parameters’, ‘CPPs’, ‘Critical material attributes’, ‘CMAs’, ‘risk assessment’, ‘design space’, or other QbD elements defined above (Figure 42). In this analysis, have not been considered QbD applications, the product submissions that do not include QbD elements or explicitly specified by the regulatory authority that it was not a QbD application. Thus, Table 48 and Table 49 also provide an overview of non-QbD and QbD-developed products approved by the FDA and EMA, respectively (see Appendix I. Supplementary Data).

For the FDA-approved products, the analysis is performed from the ‘Drugs@FDA: FDA Approved Drug Products’ database in the section ‘New drug application (NDA)’, with further analysis of Drug Approval Package and FDA Application Review Files, such as the Chemistry Review(s) or Product Quality Review(s) (Table 48). On the other hand, the databases used in the EMA approvals include non-confidential information from European public assessment reports (EPARs) for medicinal products authorized via the centralized procedure (coordinated by EMA). Particularly examines the relevant information on the pharmaceutical development of the

products discussed under the Quality section of these reports. For products authorized via the Mutual Recognition Procedure (MRP) or National Procedure (NP), it was necessary to analyze and assessed other sources of information such as the Heads of Medicines Agency (HMA) Mutual Recognition Information (MRI) product index, Summaries of Product Characteristics (SmPCs), Periodic Safety Update Reports (PSURs), Patient Information Leaflets (PIL), or EMA Human Medicines Highlights (Table 49).

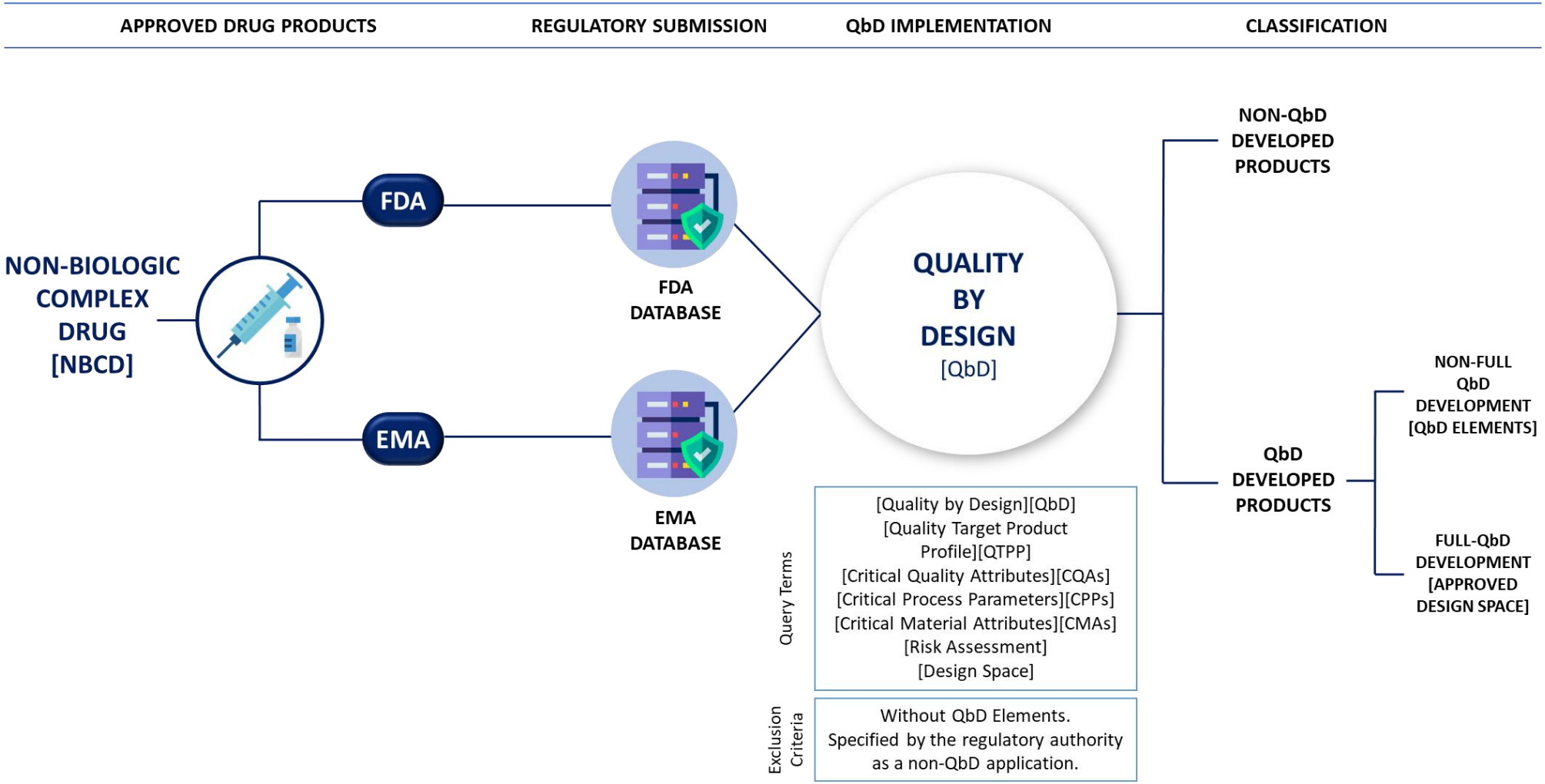

Figure 42. The descriptive diagram of the research methodology used in this study.

4. Results

4.1. Quality by Design (QbD) Approach in U.S. and EU Submissions

The starting point of the QbD implementation in pharmaceutical development occurred with the FDA approval of the non-complex drug product Januvia® (sitagliptin phosphate, Merck & Co.) in 2006, indicated as an adjunct to diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. The CMC portion of this NDA (Application No.: 021995) was submitted under the ONDQA Pilot Program to explore science- and risk-based approaches to assuring product quality through several QbD elements provided in the product design and process understanding [405,422].

Subsequently, FDA approved the first biological complex drug product with QbD elements in 2012, called Perjeta® (pertuzumab, Genentech, Inc.). This corresponds to a Biologics License Application (BLA), where the Design Space was not properly characterized [408]. As referred to in the Assessment Report of Perjeta® (EMA, 2012), 'the principles used to define the proposed Design Space were endorsed. However, there were several issues that, taken together, led to a Major Objection on Day 120 and Day 180 of the procedure that precluded the approval of the Design Space. As a consequence, the claimed Design Space was withdrawn' [406]. Thus, FDA rejected the attempt to file Perjeta® (Pertuzumab) as a full-QbD submission, and the design space was not approved.

In the following year (2013), FDA approved the Gazyva® (obinutuzumab, Genentech, Inc.), the first biological complex drug product with an approved design space and a post-approval lifecycle management (PALM) plan [407]. It means, that Gazyva® corresponds to the first complex drug product with a full-QbD submission with an approved design space [407].

In the particular case of the NBCDs approved by the FDA (Table 48), it was not possible to perform the analysis of the QbD approach in eight drug products, due to the lack of available chemistry reviews, such as: InFed® (1974), Proferdex® (1981), Abelcet® (1995), Neoral® (1995), Amphotec® (1996), DaunoXome®(1996), Dexferrum® (1996), and Feridex® (1996) [36,46,48,52,61–63,124]. Thus, these products can be classified as Non-QbD Developed Products, since the implementation based on the QbD approach has to be after the date of Januvia® approval (2006).

On the other hand, it is important to emphasize that from 2011, some examples of NBCDs (e.g. Exparel®, Marqibo®, or Injectafer®) mention the concepts of 'Quality by design (QbD)' or 'Process Analytical Technology (PAT)' in their chemistry reviews, although not still applied for the drug substance (DS) or final drug product (DP) (Table 9) [116,118,119].

Table 9. Examples of FDA-approved Non-biologic Complex Drug Products where the 'Quality by Design' and 'Process Analytical Technology' are cited in Chemistry Review.

Questions / FDA approved NBCDs	Exparel® (2011)	Marqibo® (2012)	Injectafer® (2013)
1. Does the section contain a description of the DS (drug substance) manufacturing process?	Yes	Yes	Yes
2. Does the section contain identification and controls of critical steps and intermediates of the DS?	Yes	Yes	Yes
3. Does the section contain information regarding the characterization of the DS?	Yes	Yes	Yes
4. Does the section contain controls for the DS?	Yes	Yes	Yes
5. Has stability data and analysis been provided for the drug substance?	Yes	Yes	Yes
6. Does the application contain **Quality by design (QbD)** information regarding DS?	**No**	**No**	**No**
7. Does the application contain **Process Analytical Technology (PAT)** information regarding the DS?	**No**	**No**	**No**
8. Is there a description of manufacturing process and methods for DP (drug product) production through finishing, including formulation, filling, labeling and packaging?	Yes	Yes	Yes
9. Does the section contain identification and controls of critical steps and intermediates of the DP, including analytical procedures and method validation reports for assay and related substances if applicable?	Yes	Yes	Yes
10. Is there a batch production record and a proposed master batch record?	Yes	Yes	Yes
11. Has an investigational formulations section been provided? Is there adequate linkage between the investigational product and the proposed marketed product?	Yes	Yes	Yes
12. Have any biowaivers been requested?	No	No	No
13. Does the section contain a description of to-be-marketed container/closure system and presentations)?	Yes	Yes	Yes
14. Does the section contain controls of the final drug product?	Yes	Yes	Yes
15. Has stability data and analysis been provided to support the requested expiration date?	Yes	Yes	Yes
16. Does the application contain **Quality by design (QbD)** information regarding the DP?	**No**	**No**	**No**
17. Does the application contain **Process Analytical Technology (PAT)** information regarding the DP?	**No**	**No**	**No**
References	[116]	[118]	[119]

Of the total of NBCDs already approved by the FDA (n=52), 90% of the products not have been developed based on the QbD approach (n=47) (Figure 43). Invega Trinza® (paliperidone palmitate extended-release injectable suspension), Monoferric® (ferric derisomaltose injection), Onpattro® (patisiran lipid complex injection), Onyvide® (irinotecan liposomal injection), and Vyxeos® (daunorubicin and cytarabine liposomal injection) are the only products where it is possible to find some information regarding CQAs of the drug product, although not in much detail because only a small part of the drug product information is available on chemistry review (Table 48). Thus, it may be inferred that only 10% (n=5) of the total of NBCDs are approved by FDA under the QbD approach (Figure 43). However, it should be considered the proviso that these products are not fully developed with the QbD approach since did use only a few QbD

elements during their development (non-full QbD developed products). Therefore, no products were identified as full QbD-development with approved design space.

Regarding the total of NBCDs already approved by the EMA (n=50), only six of them applied QbD elements in their pharmaceutical development: Emend® (nanocrystals dispersion of aprepitant), Exparel® (bupivacaine liposomal injectable suspension), Onpattro® (patisiran lipid complex injection), Onivyde® (irinotecan liposomal injection), Vyxeos® (daunorubicin and cytarabine liposomal injection), and Zypadhera® (olanzapine long-acting injection) (Table 49). It should be noted that the Emend®, despite being approved in 2003, only presents the QbD elements in an extension of the marketing authorization consisting of the addition of a new pharmaceutical form (2015) [423]. It means, that the implementation of the QbD approach was later than the initial marketing application. Thus, can be defined that 88% (n=44) of all EMA-approved NBCDs not have been developed based on the QbD approach, while only 12% (n=6) are considered QbD-developed products (Figure 43).

Table 10. The Number of Approved Non-Biological Complex Drugs (NBCDs) According to the Type of Pharmaceutical Development.

Regulatory Authority (Marketing Approval)	**Total of Approved NBCDs**	**Total of Non-QbD Developed Products**		**Total of Full QbD-Developed Products** (Approvals with QbD approach)		**Total of Non-Full QbD Developed Products** (Only on the basis of some QbD Elements)		**Total of Products with Approved Design Space**	
	n	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
FDA	52	47	90%	0	0%	5	10%	0	0%
EMA	50	44	88%	0	0%	6	12%	0	0%

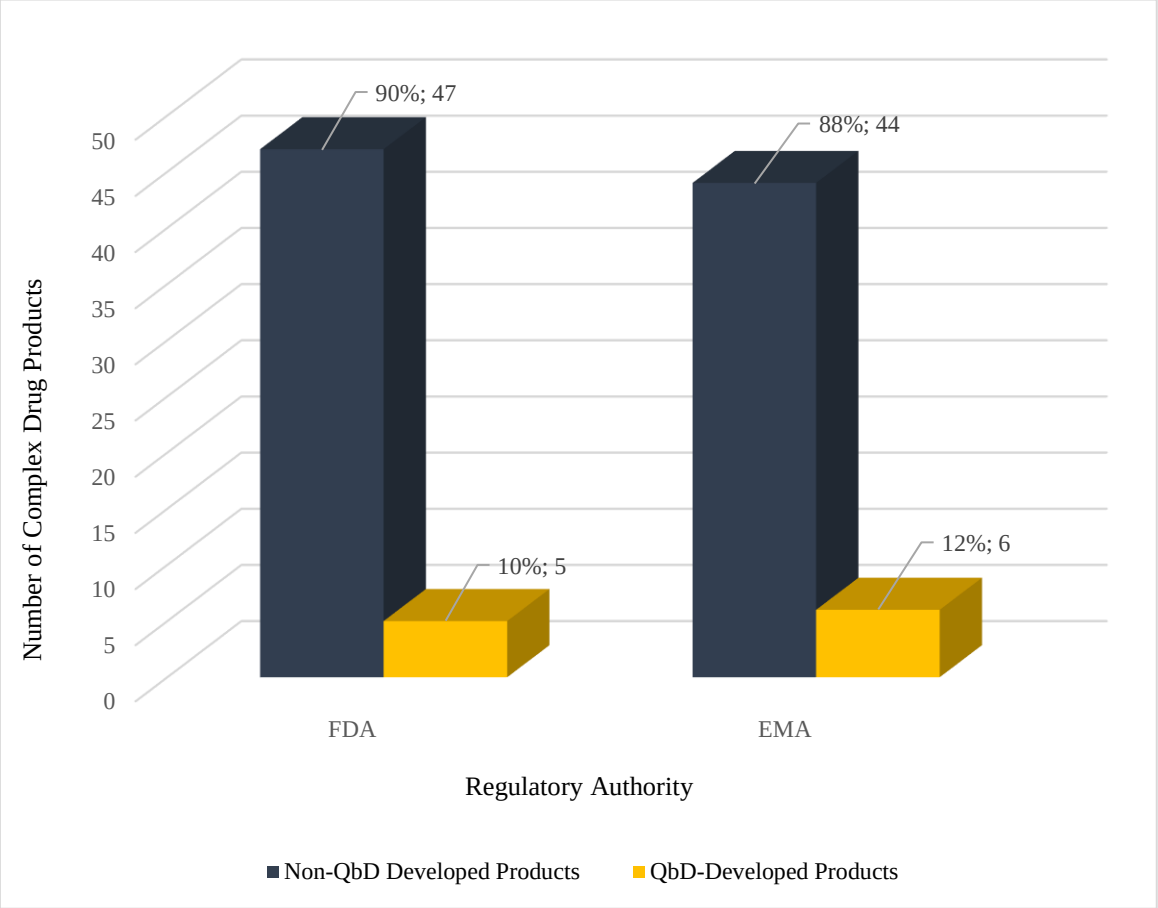

Figure 43. Classification of Non-Biological Complex Drugs (NBCDs) Approved by the FDA and EMA based on QbD or Non-QbD Development.

Given this data, it is possible to infer that exist a relatively small number of NBCDs were approved by the FDA or the EMA with the application of the QbD approach in their development and marketing approval (Figure 43). Despite the significant progress reached in recent years related to the QbD acceptance by the pharmaceutical industry, the much-reduced number of products and QbD elements described suggests that there is still a major gap in the widespread implementation of the QbD approach in the pharmaceutical development of NBCDs. Examples of lacking detail in this reviews derived from the confidentiality issues on the reference products include the absence of identification and description of specific CQAs for each drug product, specific CMAs and CPPs that impact on the CQAs, the rationale to be considered critical parameters, manufacturing process steps and in-process controls, type of characterization techniques applied and methodology of analysis, risk assessment tools, the process of obtaining the design space, lifecycle management strategies, among others.

Regarding the number of FDA approved products based on the QbD approach by the approval year, it has been possible to verify that the QbD implementation occurs in recent years: 2015

(Invega Trinza® and Onivyde®), 2017 (Vyxeos®), 2018 (Onpattro®), and 2020 (Monoferric®) [109,121–123,149]. For the NBCDs approved by the EMA, it has also been possible to infer a tendency towards the QbD implementation in more recent years, as verified in FDA analysis. Examples include the years 2015 (extension of the marketing authorization of Emend®), 2016 (Onivyde®), 2018 (Onpattro® and Vyxeos®), and 2020 (Exparel®) [423–427]**.** This can be explained by the fact that exists a greater number of regulatory initiatives and guidance documents related to the QbD approach and pharmaceutical development of complex drug products over the last few years (Figure 39). Additionally, exist wider dissemination of knowledge and benefits of the QbD approach within the Pharmaceutical Industry.

Moreover, it is also possible to verify that the QbD was applied for distinct types of NBCDs (e.g. liposomes, nanocrystals, lipid nanoparticles, and iron carbohydrate complex), underlining the versatility of this approach.

4.2. Quality by Design (QbD) Elements

Although most proprietary information is hidden in the regulatory dossiers of NBCD products approved by the FDA and EMA, it is important to highlight some relevant information about the QbD elements scrutinized (Figure 44).

The only QbD elements identified for the five products approved by the FDA are the CQAs, risk assessment tools, and lifecycle management strategies (control strategy) (Figure 44). Also been identified within the QbD analysis, factors that can impact the CQAs. However, do not necessarily specify the exact CMAs and CPPs of each drug product, but only a general description of these factors (e.g. formulation, container closure, raw materials, process parameters, scale/equipment, and site) (Table 48). On the other hand, the regulatory documentation of the EMA-approved products presents a greater level of detail involved in the description of the QbD elements, mentioning the QTPPs, CQAs, Materials/Process Parameters, Risk Assessment, Design of Experiments, or Control Strategy (Figure 44).

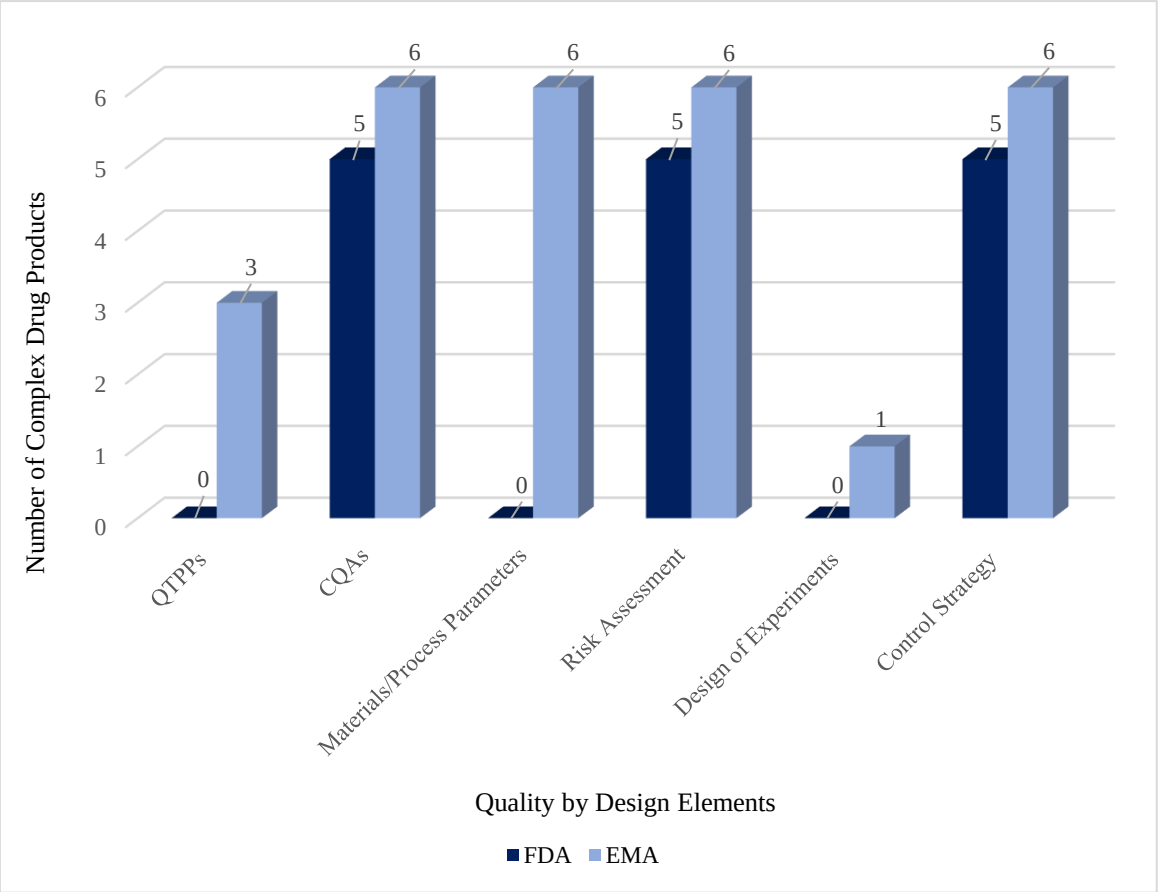

Figure 44. Quality by Design Elements Identified for Non-Biological Complex Drugs (NBCDs) Approved by the FDA and EMA.

Interestingly, the CQAs identified are similar to all FDA approved products, while corresponding to different types of NBCDs with different active substances, therapeutic indications, and routes of administration (e.g. liposomes, nanocrystals, lipid nanoparticles, and iron carbohydrate complex) (Table 11) [109,121–123,149]. The main CQA listed are appearance, sterility, endotoxins/pyrogens, drug substance assay, in vitro release, physical stability, leachable/extractables, dose and content uniformity, osmolality, pH, particle size distribution, and particulate matter (Table 11) [109,121–123,149]. It is possible to identify this similarity between the CQAs of different NBCDs since they correspond to general CQAs that should be identified and studied in all drug products due to the impact on their quality, safety, and efficacy (not specific of each type of NBCDs). This corresponds to one of the greatest shortcomings found in the Chemistry Review(s) of these products. It would be important to include a more complete list of specific CQAs of each type of NBCDs, including the justification for their classification as critical. The same should be applied in the case of CMAs and CPPs.

For example, in the case of liposomes, already exist particular guidance documents where are described recommendations for unique attributes and specific information that sponsors might best consider for new liposome drug applications (NDAs), but also generic drug products through the abbreviated new drug applications (ANDAs). Examples of such guidance documents include: 'FDA Guidance for Industry: Drug Products, Including Biological Products, that Contain Nanomaterials', 'EMA: Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product', 'FDA Guidance for Industry: Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation', or 'MHLW Guideline for the Development of Liposome Drug Products' [10,253,284,428].

The FDA guidance for industry entitled 'Drug Products, Including Biological Products, that Contain Nanomaterials' (2017), gives guidance on the pharmaceutical development of human drug products containing nanomaterials in the finished dosage form (e.g. nanocrystals, liposomes, polymeric nanoparticles, gold nanoparticles, or surface-modified nanoparticles). Despite this guidance providing the general principles and specific considerations for quality assessment for several nanomaterials (e.g. nanomaterial's critical quality attributes (CQAs)), fails to provide specific information on each type of drug product [10]. It was very interesting to understand that the following FDA specific guidance for liposomes ('FDA Guidance for Industry: Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation'), does not mention anywhere the term 'nanomaterial', which demonstrates the need to harmonize the concepts related to complex drug products [253].

Particularly, the following critical quality attributes should be considered to ensure the quality, safety, and efficacy of the liposome drug product [8,10,253,284,428,429]: components of the liposome; quantities of the active substance and each lipid; lipidic components (description, source and characterization, manufacture, assay, impurity profile, isomers, and stability characteristics); quality, purity, and stability characteristics of other critical excipients; molar ratio or percentage by weight of the lipid (including functional lipid) to the active substance; stability of the active substance, lipids, and functional excipients in the finished product, including quantification of critical degradation products (e.g. lyso phosphatidylcholine, oxidated/hydrolytic moieties); particle size distribution and polydispersity index; morphology and/or structure of the liposome including, if applicable, lamellarity determination; surface characteristics of the liposomes, as applicable, e.g., pegylation; surface charge (zeta potential); drug product viscosity; drug concentration, encapsulation efficiency, and loading capacity; liposome phase transition temperature; thermodynamic properties of the liposome membrane; drug release kinetics; stability; appearance (color/turbidity/caking); osmolality; pH; impurities; physical state of the encapsulated active substance; and so on.

Indeed, even though there is a greater discussion about the pharmaceutical quality of liposomes and the QbD implementation in their development, this has not occurred at the same scale as other NBCDs. As mentioned previously, the wide application of the 'FDA Guidance for Industry: Drug Products, Including Biological Products, that Contain Nanomaterials' for all types of nanosystems may be too generalist, with the risk of not discriminating the specific CQAs, CMAs, CPPs, characterization techniques, or other important issues of each drug product [10]. Furthermore, the existence of guidance documents with the QbD approach for the development of other NBCDs remains scarce.

In the particular case of the iron-carbohydrate complexes (e.g. InFed®, Proferdex®, Dexferrum®, Venofer®) contain specific critical attributes for both iron core and carbohydrate shell. The CQAs of the iron core include the identification of their chemical structure, the elemental ratio of iron and carbon, iron content (total iron, ionic iron, colloidal iron), iron core size and morphology, and iron core environment. For the carbohydrate shell, is important to identify carbohydrate matrix (structure and composition), characterization of polysaccharides, carbohydrate-iron core interactions, and surface charge. Furthermore, other attributes of iron colloid particles that should be included in the characterization are the labile iron determination under physiologically relevant conditions, average molecular weight, and molecular weight distribution. These attributes are still poorly discussed in pharmacopoeial monographs and product-specific guidance documents, and the reliable and validated analytical techniques for their characterization have been undocumented [265,267,273,274,430–432].

The same specificity is applied to complex glatiramer mixtures (e.g. Copaxone®), with the following CQAs: amino acid content and sequence, optical purity, molecular charge, charge distribution, proteolytic digestion profile, molecular weight distribution and profile, hydrophobicity correlation, molecular size distribution, higher-order structures, polydispersity, biological activity, gene expression assay, cytotoxicity, and immuno-recognition [275].

Table 11. Non-biological Complex Drug Products Approved by the FDA based on the Quality by Design (QbD) Approach.

NBCDs (FDA Approved)	Pharmaceutical Application	QbD Approach Implementation	QbD Elements	CQAs	Factors that can Impact the CQAS (General Description)	Risk Assessment Tools	Design Space	Lifecycle Management	References
Invega Trinza®	Use of a water-insoluble palmitate ester analogue of paliperidone: allows an extended drug release.	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs Risk assessment tools	Sterility Endotoxins Assay - drug substance Physical stability Dose uniformity Content uniformity Osmolality pH Particle size distribution Particulate matter Leachables, Extractables Re-dispersability Appearance In vitro release	Formulation Container closure Raw materials Process parameters Scale/equipments Site	Risk Estimation Matrix (REM)	Not listed	Identified	[121]
Onivyde®	Longer half-life of drug release. Increased length of tumor exposure to both irinotecan and its active form (SN-38).	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs Risk assessment tools	Sterility Endotoxin, pyrogen Assay (API), stability Uniformity of dose (fill volume/deliverable volume) Osmolality pH (Low) Particle size distribution (suspension) Particulate matter	Formulation Container closure Raw materials Process parameters Scale/equipments Site	Failure mode, effects, and criticality analysis (FMECA)	Not listed	Identified	[109]

				(non aggregate for solution only)					
				Leachable/Extractables					
				Appearance (color/turbidity)					
Vyxeos®	Unique combination of two established therapies at a synergistic ratio. Increased activity: greater cell uptake. Prolonged delivery: longer half-life (greater drug exposure within the plasma and bone marrow).	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs Risk assessment tools	Assay (API), stability	Formulation Container closure Raw materials Process parameters Scale/equipments Site	Risk Estimation Matrix (REM)	Not listed	Identified	[122]
				Sterility					
				Endotoxin, pyrogen					
				Physical stability (solid state)					
				Assay (preservative)					
				Assay (anti-oxidant)					
				Uniformity of dose (fill volume/deliverable volume)					
				Osmolality					
				pH (High)					
				pH (Low)					
				Particulate matter					
				Leachable/Extractables					
				Re-dispersability, reconstitution time					
				Moisture content					
				Appearence (caking)					
				Appearance (color/turbidity)					
				Microbial limits					
				In vitro drug release					
				Liposome particle size distribution;					
Onpattro®	RNAi therapeutic.	Identified*	CQAs	Appearance	Formulation Container closure Raw materials	Risk Estimation Matrix (REM)	Not listed	Identified	[149]
				Assay (active), stability					
				Lipid component assay					

	Silence messenger RNA. Inhibit the synthesis of TTR protein (transthyretin). Prevent the deposition of the TTR amyloid in tissues.	(Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	Risk assessment tools	Lipid entrapment efficiency (bound vs. free drug) In vitro release Particle size distribution Sterility Endotoxin, pyrogen Fill volume/delivered volume Osmolality pH (high) pH (low) Particulate matter Leachable/Extractables	Process parameters Scale Equipment Site				
Monoferric®	Controlled release of iron in the body (through carbohydrate matrix structure). High dosing flexibility.	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs Risk assessment tools	Assay (at release), stability Osmolality Uniformity of dose (fill volume, deliverable volume) Sterility Endotoxin pH Particle matter (non aggregate for solution only) Leachable, extractables	Formulation; Container closure; Raw materials; Process paramters; Scale, equipments; Site;	Risk Estimation Matrix (REM)	Not listed	Identified	[123]

*QbD principles and elements have been implemented into the product development and consequently described in the submission documents.

Similar to the FDA products, the NBCDs approved by the EMA are not classified as fully developed with the QbD approach, presenting only a few QbD elements during their development (e.g. CQAs and Risk assessment tools) (Table 12).

However, an analysis of each assessment report showed that the information submitted for the individual products is most detailed, as compared with the FDA data. On this basis, the assessment reports described specific CQAs of the drug products, as well as, the corresponding characterization techniques [423,424,433].

For example, in the assessment report of Onivyde®, the finished product release specifications include appropriate tests for this kind of dosage form, such as: appearance (Ph. Eur.), irinotecan identity (HPLC, UV), DSPC identity (HPLC-ELSD), cholesterol identity (HPLC-ELSD), MPEG2000-DSPE identity (HPLC-ELSD), irinotecan concentration (assay) (HPLC-UV), percent encapsulated drug (HPLC-UV), irinotecan impurities (HPLC-UV), lipid impurity (HPLC-ELSD), residual solvents (GC), residual trimethylamine (GC-FID), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), drug to phospholipid ratio (calculation), DSPC to cholesterol ratio (calculation), extractable volume (Ph. Eur.), container closure integrity (in-house), in vitro drug release (HPLC-UV), osmolality (Ph. Eur.), particle size (Ph. Eur.), particle size distribution (Ph. Eur.), particulate matter in injections (Ph. Eur.), pH (Ph. Eur), and zeta potential (in-house) [424].

Perhaps, the higher degree of details in the assessment report of Onivyde® is due to the publication of the ‘EMA: Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product’ in 2013, well before the publication of ‘FDA Guidance for Industry: Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation’ (2018) [253,284,424]. Accordingly, the earlier the specific guidance documents are published for a type of NBCDs, the greater the tendency to comply with these recommendations.

Furthermore, it has been possible to verify in this analysis that the other NBCDs classified as Non-QbD Developed Products (e.g. Abraxane®, Mepact®, Rienso®, Renvela®, Xeplion®, Ikervis®), present important quality aspects of scientific discussion in their assessment reports [434–439]. For example, identifies the finished product release specifications, critical process parameters and in-process controls, appropriate characterization tests, among others. This occurred even without any mention of the ‘Quality by design’, ‘QbD elements’, or ‘CQAs’.

Table 12. Non-biological Complex Drug Products Approved by the EMA based on the Quality by Design (QbD) Approach.

NBCDs (EMA Approved)	QbD Approach Implementation	QbD Elements	CQAs	CMAs	CPPs	Risk Assessment Tools	Design Space	Lifecycle Management	References
Emend®	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs CPPs Risk assessment tools Lifecycle Management	Identity Appearance Content Uniformity (API) Assay (API) Degradants/Impurities Aprepitant PSD Sachet Hold-Up and Dosing	Not listed	Mentioned (Proven acceptable ranges (PARs))	Failure Mode Effect Analysis (FMEA)	Not listed	Mentioned	[423]
Zypadhera®	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	CQAs CMAs CPPs Risk assessment tools Lifecycle Management	Active Substance Identification (solubility, pKa, melting point) Crystal Form Assay Related Substances In vitro Dissolution Residual Solvents Sulphated Ash Appearance, Colour, Clarity Water Content Particle Size Distribution Specific Surface Area Bacterial Endotoxins Microbial Quality Sterility Particulate Matter Purity/Impurity Profile Stability	Mentioned	Mentioned (Proven acceptable ranges (PARs))	Failure Mode Effect Analysis (FMEA)	Not listed	Mentioned	[433]

			Uniformity of Dosage Units						
			Viscosity						
			Injectability (Force)						
Onivyde®	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	QTPPs CQAs CMAs CPPs Risk assessment tools Lifecycle Management	Visual Appearance Irinotecan Identity Lipid Identity Cholesterol Identity Irinotecan Concentration Percent Encapsulated Drug Irinotecan Impurities Lipid Impurity Residual Solvents Bacterial Endotoxins Bioburden and Sterility Drug to Phospholipid Ratio DSPC to Cholesterol Ratio Extractable Volume in Container *In Vitro* Release Osmolality Particle Size Particle Size Distribution Particulate Matter in Injections, pH Zeta Potential	Mentioned	Mentioned	Failure Mode Effect Analysis (FMEA)	Not listed	Mentioned	[424]
Onpattro®	Identified* (Not classified as fully developed with QbD	CQAs CMAs CPPs	Dosage Form Visual Appearance Identification by Molecular Mass	Mentioned	Mentioned (Proven acceptable	Mentioned	Mentioned (multifactorial design of	Mentioned	[425]

	approach - use of one or more of the QbD elements during the development)	Risk assessment tools DoE Lifecycle Management	Identification by Single Strand Retention Time Purity and Impurities Assay Lipid Identity Lipid Content Duplex (siRNA) Encapsulation pH Osmolality Particle Size Elemental Impurities Residual Ethanol Residual EDTA Particulate Matter Bacterial Endotoxins Sterility Content Uniformity Volume In Container Container Closure Integrity siRNA In Vitro Release		ranges (PARs))		experiment (DoE))		
Vyxeos®	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	QTPPs CQAs CPPs Risk assessment tools Lifecycle Management	Appearance of Lyophilized Cake and Post-Reconstitution Suspension Reconstitution Time Water Content of the Lyophilized Cake pH of the Reconstituted Suspension Particle Size Osmolality	Not listed	Mentioned	Mentioned	Not listed	Mentioned	[426]

			Particulate Matter						
			Identification of Cytarabine and Daunorubicin						
			Cytarabine and Daunorubicin Assay						
			Cytarabine and Daunorubicin % Encapsulation						
			Cytarabine and Daunorubicin Impurities						
			Cytarabine and Daunorubicin Content Uniformity						
			DSPC Assay						
			DSPG-Na Assay						
			Cholesterol Assay						
			Lipid Impurities						
			Copper Assay						
			Residual Solvents						
			Endotoxin						
			Sterility						
			In-Vitro Release						
Exparel®	Identified* (Not classified as fully developed with QbD approach - use of one or more of the QbD elements during the development)	QTPPs CQAs CMAs CPPs Risk assessment tools Lifecycle Management	Appearance	Mentioned	Mentioned	Mentioned	Not listed	Mentioned	[427]
			Identity						
			Total Bupivacaine						
			Free Bupivacaine						
			Packed Particle Volume (PPV)						
			Bupivacaine Degradation Products						
			Cholesterol						
			Particle Size Distribution						
			In Vitro Release						

pH
Residual Solvent (GC)
Lipid Degradation
Particulate Contamination: Subvisible Particles
Osmolality
Uniformity of Dosage Units
Extractable Volume
Bacterial Endotoxins
Sterility

*QbD principles and elements have been implemented into the product development and consequently described in the submission documents.

4.3. Quality Risk Management

The risk assessment tools form part of the quality risk management (ICH Q9), a process that supports science-based and practical decisions when integrated into quality systems, through the current knowledge about assessing the probability, severity, and sometimes detectability of the risk [396]. According to the ICH Q9, effective quality risk management 'can facilitate better and more informed decisions, can provide regulators with greater assurance of a company's ability to deal with potential risks, and might affect the extent and level of direct regulatory oversight. In addition, quality risk management can facilitate better use of resources by all parties' [396]. There are different options of tools available on ICH Q9, which should be appropriately selected in accordance with the assessment purpose. Examples of these risk management tools are the basic risk management facilitation methods (e.g. Ishikawa diagram), Failure Mode Effects Analysis (FMEA), Failure Mode, Effects and Criticality Analysis (FMECA), Fault Tree Analysis (FTA), Hazard Analysis and Critical Control Points (HACCP), Hazard Operability Analysis (HAZOP), Preliminary Hazard Analysis (PHA), Risk ranking and filtering, or other supporting statistical tools [396].

The risk assessment tool most commonly used in NBCDs FDA approved is the Risk Estimation Matrix (REM) (Table 11). The Failure Mode Effect and Criticality Analysis (FMECA) has only been applied for Onivyde® [109,121–123,149]. The greater use of REM can be related to the simplicity of this tool to graphically compile a Risk Estimation Matrix assigning low, medium, and high potential risks to the factors based on their potential impact on the process and product performance. This risk assessment tool allows the simple establishment of the relationship between the specific material attributes and process parameters to the potential CQAs of the drug product [8,403]. On the other hand, the FMECA includes an investigation of the variables according to the degree of severity of the consequences, their respective probabilities of occurrence, detectability, and criticality, through a quantitatively ranks of the variables with a relative risk 'score' for each failure mode [396,403].

For some NBCDs approved by EMA, the risk analysis was performed using the Failure Mode Effect Analysis (FMEA) method. Particularly, the FMEA was identified in the assessment report of Emend®, Zypadhera®, and Onivyde® (Table 12). The FMEA is a powerful tool identical to the FMECA analysis that ranks the variables based on probability, severity, and detectability. This tool provides an appraisal of potential failure modes for processes, factors causing these failures, and the likely effects of these failures on outcomes and product performance. Additionally, FMEA may be employed to prioritize risks and monitor the effectiveness of risk control activities [396]. For the remaining NBCDs (Onpattro®, Vyxeos®, and Exparel®), the risk assessment was conducted even though is not specify the risk management tool applied (Table 12).

4.4. Design Space: Highly Desirable, but Not Mandatory

The risk assessment tools can lead to an understanding of the relationship and effects of the process inputs (material attributes and process parameters) on the product critical quality attributes (CQAs), and thereby allow the identification and selection of the variables and their ranges for inclusion in the design space within which consistent quality can be achieved. Thus, the operation within the design space (multidimensional parametric space) will result in a product meeting the defined quality. This QbD element is submitted by the applicant and consequently subject to regulatory assessment and approval.

From the NBCDs approved by the FDA based on the Quality by Design (QbD) approach (Table 11), it was possible to understand that none of the five products provided a design space, nor an in-depth description of CMAs and CPPs [109,121–123,149]. Similarly, the design space was also rarely applied for NBCDs approved by the EMA (Table 12). Onpattro® is a unique example of NBCDs where a multifactorial design of experiments (DoE) is mentioned in their public assessment report [425]. Contrarily to the biological complex drug products, more specifically to the Gazyva® that present a full-QbD submission with approved design space, the submission and approval of this QbD element in the development of NBCDs have not been widely discussed.

This may be owing to design space being an optional element (not mandatory) of the QbD approach, subject to regulatory assessment and approval [395,403,418]. Moreover, design space is not extensively applied in the Pharmaceutical Industry due to the difficulty to be accepted by regulatory authorities, high demand for extensively detailed data, the inherent uncertainty in design space, or concerns related to the return on investment [418,440]. For example, a description and rationale for the inclusion of the process inputs (material attributes and process parameters) in the design space, and their effect on product quality, shall be supplied. It should also be described the rationale as to why some parameters were excluded, or the process parameters and material attributes that were not varied through development (can include parameters that were held constant).

As stated by Tone Agasoester (Norwegian Medicines Agency) and Graham Cook (Pfizer), in the presentation entitled ‘Development and Verification of Design Space’, the ‘*industry experience to date suggests that design spaces for more complex products (e.g., biopharmaceuticals) may be harder to get approved’* [441].

The applicant can choose between the independent design spaces for one or more unit operations (simpler to develop) or establish a single design space that comprises multiple operations of the entire process that provide more operational flexibility. Therefore, the applicant needs to define the type of operational flexibility desired, in accordance with the production scale. The applicant should justify the importance of an independent design space developed at a small

or pilot scale to the proposed production scale manufacturing process and discuss the potential risks in the scale-up operation. For the multiple operational scales, the design space shall be defined in terms of significant scale-independent parameters.

As mentioned in the ICH-Endorsed Guide for ICH Q8/Q9/Q10 Implementation, the design spaces are typically developed at a small scale, and 'while the entire design space does not have to be re-established (e.g., DoE) at commercial scale, design spaces should be initially verified as suitable prior to commercial manufacturing' [404].

Despite being an optional feature, the achievement of a design space specific to a product and process is desirable due to the unquestionable usefulness and operating flexibility that allows the post-approval change process, without the need for additional regulatory scrutiny. As disclosed in the ICH Q8, 'working within the design space is not considered as a change' [395,403,442]. Thus, design space is a key element of the QbD approach defined by the multidimensional combination and interaction of CMAs and CPPs, which had proven to assure consistently the desired quality requirements of the final drug product [395].

4.5. Market Withdrawal

One of this chapter's objectives was to understand the number of NBCDs discontinued, the reasons for this market withdrawal, and if the withdrawal is related or not to the QbD-Developed Products. Accordingly, it was tried to gather information regarding the reasons for their discontinuation to understand if they were related to the manufacturing process, market strategies, and product quality, efficacy, or safety issues that could have been avoided or solved with a more holistic development as the QbD approach advocated in this chapter.

The reasons for withdrawal can be classified mainly into commercial issues, manufacturing compliance issues, quality issues, safety issues, efficacy issues, clinical issues, or other issues related to the insufficient data in documents submitted in the marketing application. The quality issues include any deviation from product specifications, such as stability, dissolution, assay, impurity, particle matter, pH, endotoxin, uniformity of dose, among others. Other questions related to the control of manufacturing processes and compliance with GMP comprise the inappropriate sites for the manufacturing process, improper procedures, or contaminations. On the other hand, the commercial issues can comprehend the relatively high production costs, lack of demand, low usage of the products and sales, commercial partners' termination of the licensing, distribution, and marketing agreement, or the competitive prices of alternative products that limit the market penetration. Furthermore, other issues directly responsible for market recalls are the safety issues by the adverse events, the lack of efficacy of the product, the need for additional clinical data, insufficient evidence to show the bioequivalence, or the impossibility to establish that the benefits of the drug product outweigh its risks [440,443–445].

Under this analysis, there are about nine FDA-approved drug products with a marketing withdrawal and seven products approved by the EMA (Table 13). In both regulatory classes, all products present a Non-QbD Development. The reasons for the marketing withdrawal of NBCDs approved by the FDA and EMA have been illustrated in Figure 45 and discussed in greater detail in Table 14 (FDA-approved products) and Table 15 (EMA-approved products).

Table 13. Number of Withdrawn Non-QbD Developed Products of the United States (FDA) and European (EMA) Market.

Reason	FDA		EMA	
	n	%	n	%
Commercial Issues	4	44%	3	43%
Clinical Issues	1	11%	0	0%
Manufacturing Compliance Issues	4	44%	0	0%
Quality Issues	2	22%	0	0%
Safety Issues	4	44%	4	57%
Not found	0	0%	2	29%
Total	9	100%	7	100%

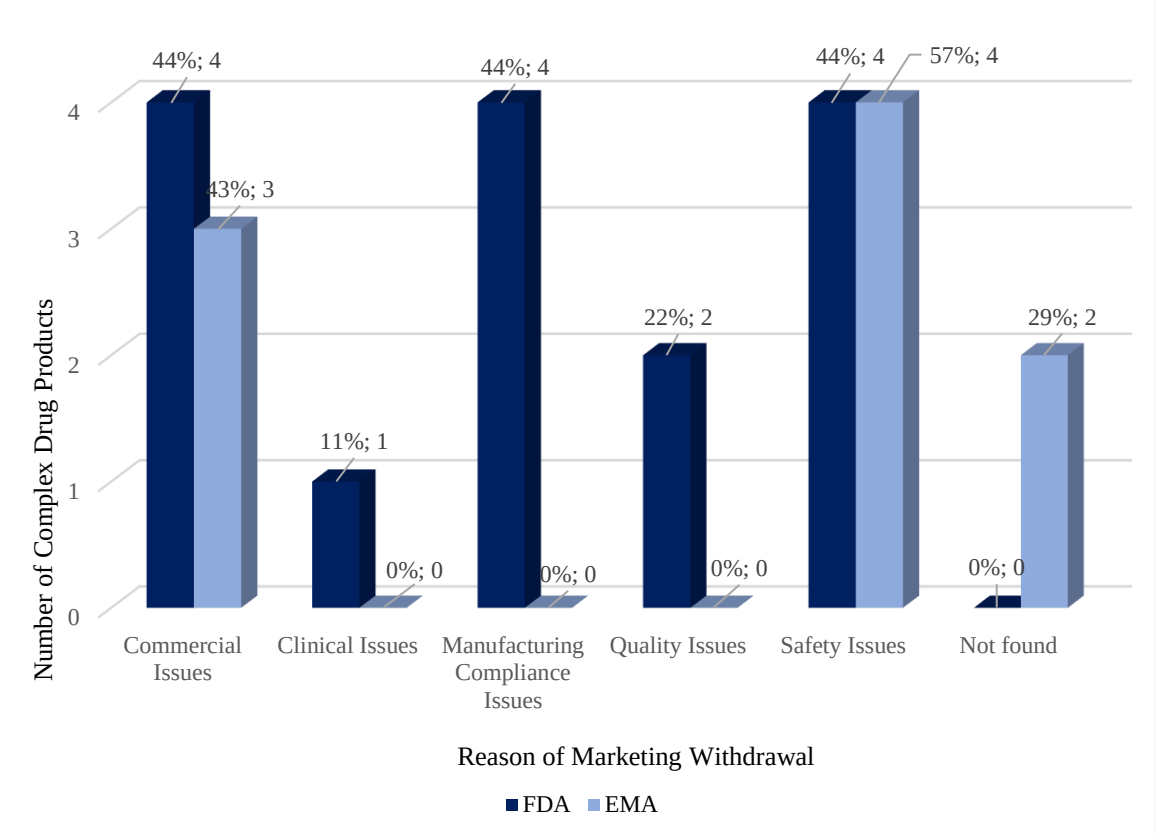

Figure 45. Reasons for Marketing Withdrawal of Non-biological Complex Drugs (NBCDs) Approved by the FDA and EMA based on Non-QbD Development.

The NBCDs approved by the FDA that were discontinued, as well as, the possible reasons for their market withdrawal are described in Table 14. From the total list of 52 NBCD products approved by the FDA, 9 of them were discontinued (n=9, 17%): Amphotec®, DaunoXome®, Depocyt®, Depodur®, Dexferrum®, Estrasorb®, Feridex®, Innohep®, and Proferdex® [46,61–63,75,78,88,94,124]. For these products, it was possible to identify several reasons for market withdrawal (commercial, clinical, quality, safety, and manufacturing compliance issues), just as more than one issue for each drug product.

The reasons founded for Proferdex® withdrawal encompass the manufacturing compliance issues at the firm's facility, but also the safety issues related to the anaphylactic-type reactions and fatalities following the iron dextran injection [124,446,447]. In the same way, American Regent discontinued marketing Dexferrum® injection in 2014, on account of the serious adverse reactions that followed the parenteral administration of iron dextran injection, such as anaphylactic-type reactions and fatalities [62,448–450]. The discontinuation of Feridex® occurred in 2008, due to undesirable side effects like hypotension, lumbar pain, and leg cramps [451,452]. Another reason found for the Feridex® withdrawal has been the lack of clinical users [63,453,454]. Regarding Amphotec®, the major reasons behind their discontinuation are the prevalence and severity of adverse side effects associated with infusion, lower acceptance by the medical community, and the competitive prices of alternative products that limit the market penetration [46,455]. In March 2016, Galen U.S. Inc informed that not be able to supply DaunoXome® due to manufacturing and commercial issues. This unfortunate situation occurred mainly due to the lack of their manufacturer's ability to produce the DaunoXome®. The plan to qualify a new manufacturer for DaunoXome® and guarantee the manufacturing technology transfer requirements required a substantial investment beyond the financial capabilities of Galen U.S. Inc [61,456,457]. Likewise, in Pacira's annual report for the fiscal year of 2012, it is reported that EKR Therapeutics, Inc. (the commercial partner for Depodur®) decided to exit the DepoDur® market. Following this decision, Pacira considered its inability to re-sublicense the product due to minimal supply revenue for the product both in the U.S. and in Europe as well as DepoDur's complex manufacturing process. As a consequence, NDA for DepoDur® was also withdrawn from the FDA by commercial issues, and the company stated that they don´t expect future DepoDur sales [94,453,458]. Regarding Depocyt®, Pacira announced the discontinuation of Depocyt, due to critical and persistent technical issues related to the product's manufacturing process [75,458,459]. LEO pharmaceutical company discontinued marketing all Innohep® presentations from the U.S. in 2011. The withdrawal from the U.S. market was mainly due to the limited usage of the products in the U.S. market (low sales), as well as, the theoretical risk of the presence of particulate matter in the released vials (contamination issue) verified during an FDA inspection at the production facilities [78,460,461]. This suspension is not related to any side effects. Lastly, Novavax Inc. has decided to withdraw its application to sell Estrasorb® after

receiving a request for more information from the FDA. The issues raised are not related to the efficacy and safety of the drug product, but rather due to manufacturing compliance issues and chemistry requests with an impact on the product's stability [88,462].

From the survey results of Table 14, it is important to highlight that all NBCDs discontinued are non-QbD developed products. Thus, the absence of market withdrawals for QbD-developed products can emphasize the advantages of a systematic QbD approach to achieve high-quality products, through a deep understanding of the product and process, just as the importance to reduce the variability and the risk of failure in marketing authorization procedures. Therefore, it is possible to infer that QbD plays a crucial role to improved manufacturing efficiency and minimizing the possibility of market withdrawal due to quality, efficacy, or safety issues. The advantages of the QbD approach are summarized in Figure 41 (Section 2) of this chapter.

Table 14. Reasons for Marketing Withdrawal in Non-biological Complex Drug Products Approved by the FDA.

Brand Name (Reference Product)	Type of NBCDs	Marketing Status	Non-QbD or QbD-Developed Products	Reason to Withdrawal	Remarks	References
Proferdex®	Iron-Carbohydrate Complex	Discontinued	Non-QbD Developed Products	Manufacturing Compliance Issues	Manufacturing compliance problems at the firm's facility.	[124,446,447]
				Safety Issues	Increased frequency of adverse reactions, such as anaphylactic-type reactions and fatalities.	
Amphotec®	Liposome	Discontinued	Non-QbD Developed Products	Commercial Issues	Pricing of alternative/ competitive products limit Amphotec's market penetration. Acceptability of Amphotec as a safe and effective therapy for invasive aspergillosis.	[46,455]
				Safety Issues	Prevalence and severity of adverse side effects associated with Amphotec, such as infusion-related side effects, including high levels of chills and fever.	
DaunoXome®	Liposome	Discontinued	Non-QbD Developed Products	Manufacturing Compliance Issues	The existing DaunoXome® manufacturer notified Galen that their ability to manufacture the product was coming to an end.	[61,456,457]
				Commercial Issues		

					The financial investment necessary to meet the manufacturing technology transfer requirements is simply beyond Galen's financial capabilities.	
Dexferrum®	Iron-Carbohydrate Complex	Discontinued	Non-QbD Developed Products	Safety Issues	Anaphylactic-type reactions, including fatalities.	[62,450]
Feridex®	Superparamagnetic Iron Oxide Nanoparticle	Discontinued	Non-QbD Developed Products	Clinical Issues	Lack of clinical users.	[63,451–454]
				Safety Issues	Undesirable side effects (e.g. hypotension, lumbar pain, and leg cramps).	
Depocyt®	Liposome	Discontinued	Non-QbD Developed Products	Manufacturing Compliance Issues	Critical and major deficiencies to comply with the Principles and Guidelines of Good Manufacturing Practices related to Depocyt® manufacturing facility - which have persisted despite corrective efforts. The remediation of these deficiencies result in additional and significant costs or delays in the production and sale of DepoCyt®, and consequent adverse effect on business, financial position, and results of operations.	[75,458,459]
Innohep®	Low Molecular Weight Heparin (LMWH)	Discontinued	Non-QbD Developed Products	Commercial Issues	Lack of demand (low usage of the products/low sales)	[78,460]
				Quality Issues	Contamination issue. Risk of presence of particulate matter in the released vials.	
Estrasorb®	Emulsion (with Micellar Nanoparticles)	Discontinued	Non-QbD Developed Products	Manufacturing Compliance Issues	Lack of information concerning the Chemistry, Manufacturing, and Controls (CMC) section of the filing.	[88,462]
				Quality Issues	Chemistry issues related to product's stability.	
DepoDur®	Liposome	Discontinued	Non-QbD Developed Products	Commercial Issues	EKR Therapeutics, Inc. (the commercial partner for Depodur) notices the termination of the licensing, distribution, and marketing agreement relating to DepoDur®.	[94,453,458]

From the total list of 50 NBCDs approved by the EMA, seven of them were market withdrawn: Endorem®, DaunoXome®, Depocyte®, DepoDur®, Macugen®, Ferrisat®, and Rienso® (Table 15). The possible reasons for their withdrawal are present in Table 15.

Endorem® (Guerbet, France) was withdrawn from the market due to not being economically viable (commercial issues), although is effective and safe [463,464]. In the same way, the marketing authorization holder of Depocyte® (Pacira Ltd) notified the European Commission of its decision to permanently discontinue the marketing of the product for commercial reasons [465]. Also, the company decided to withdraw the application for Depocyte® due to the CHMP's opinion that the data provided would not allow a conclusion to be drawn on a positive benefit-risk balance (safety issues) [466,467]. On the other hand, the Macugen® has been withdrawn at the request of the marketing authorization holder (PharmaSwiss Ceska Republika) on account of the Committee for Medicinal Products for Human Use CHMP's preliminary assessment, who stated that the data provided does not allow to conclude a positive benefit-risk balance [468–470]**.** The withdrawal of the Ferrisat® derives from the increased frequency of adverse reactions, such as hypersensitivity reactions [471]. Finally, the marketing authorization holder of Rienso® (Takeda Ltd) notified the European Commission of its decision to permanently discontinue the marketing of the product for commercial reasons [472]. In addition, appeared post-marketing reports of serious or fatal hypersensitivity (allergic) reactions were observed in regular ongoing safety monitoring. According to CHMP, the benefits of Rienso in the treatment of iron deficiency anemia in the extended population do not outweigh its risks [473]. Lastly, it is important to highlight that has not been found the possible reasons for the discontinuation of the DaunoXome® and DepoDur® in Europe. Assessing the marketing status of some products approved by EMA might be more difficult since the status is only described on the EMA website for medicinal products authorized via the centralized procedure, which was not the case for DaunoXome® (MRP/NP) and DepoDur® (NP).

Based on this analysis, the main reasons found for market withdrawal were the commercial and safety issues, such as the lack of the assessment of a positive benefit-risk balance or adverse reactions. No rationale is related to the quality issues, which might be prevented by a systematic QbD approach. Similar to the analysis of the FDA, all NBCDs withdrawn in the EU are non-QbD-developed products, which could be in line with the capability of the QbD approach to provide high-quality products. However, the majority of withdrawal NBCDs are older products and the use of the QbD strategy was not so much 'en vogue' at the time of their development and marketing approval. Moreover, the number of QbD-developed products is rather limited, which leads to the necessity to take these conclusions with caution. In this specific case, it would be interesting to check whether this trend continues with more complex drug products approved in the future by the QbD approach.

Table 15. Reasons for Marketing Withdrawal in Non-biological Complex Drug Products Approved by the EMA.

Brand Name (Reference Product)	Type of NBCDs	Marketing Status	Non-QbD or QbD-Developed Products	Reason to Withdrawal	Remarks	References
Endorem®	Nanoparticle	Withdrawn	Non-QbD Developed Products	Commercial Issues	Endorem® (Guerbet, France) was withdrawn from the market due to not being economically viable, although is effective and safe.	[463,464]
DaunoXome®	Liposome	Withdrawn	Non-QbD Developed Products	Not found	Not found	NA
Depocyte®	Liposome	Withdrawn	Non-QbD Developed Products	Commercial Issues	The marketing authorization holder of Depocyte® (Pacira Ltd) notified the European Commission of its decision to permanently discontinue the marketing of the product for commercial reasons.	[465]
				Safety Issues (Lack of a positive benefit-risk balance)	The company decided to withdraw the application for Depocyte® due to the CHMP's opinion that the data provided would not allow a conclusion to be drawn on a positive benefit-risk balance.	[466,467]
DepoDur®	Liposome	Withdrawn	Non-QbD Developed Products	Not found	Not found	NA
Macugen®	Polymeric nanoparticle	Withdrawn	Non-QbD Developed Products	Safety Issues (Lack of a positive benefit-risk balance)	Pfizer Limited withdraws its application for an extension of the indication for Macugen® based on the CHMP's view that the data provided so far does not allow the Committee to conclude on a	[468–470]

					positive benefit-risk balance in the applied for indication.	
Ferrisat®	Iron-carbohydrate complex	Withdrawn	Non-QbD Developed Products	Safety Issues	Increased frequency of adverse reactions, such as hypersensitivity reactions.	[471]
Rienso®	Iron-carbohydrate complex	Withdrawn	Non-QbD Developed Products	Commercial Issues	The marketing authorization holder of Rienso® (Takeda Ltd) notified the European Commission of its decision to permanently discontinue the marketing of the product for commercial reasons.	[472]
				Safety Issues (Lack of a positive benefit-risk balance)	Post-marketing reports of serious or fatal hypersensitivity (allergic) reactions observed in regular ongoing safety monitoring. According to CHMP, the benefits of Rienso in the treatment of iron-deficiency anemia in the extended population do not outweigh its risks.	[473]

5. Concluding Remarks

Over the last few decades, great emphasis has been placed on the control of the pharmaceutical quality of complex drug products and their generic versions. The complexity of these drug products could be translated into several challenges in identification, establishment, and guarantee of quality. One of the key questions remains how to comply with reproducibility requirements and quality standards for complex drugs, to ensure the availability of high-quality, safe, and effective pharmaceutical products.

Therefore, the pharmaceutical industry has been adopting increasingly more holistic, systematic, and risk-based approaches, as in the case of the Quality by Design (QbD). The Quality by Design (QbD) concepts, originally proposed by Joseph M. Juran (1970), were well established in guidance documents ICH Q8 to ICH Q12. The QbD approach implementation provides an in-depth and advanced understanding of the product and process, and the establishment of an appropriate control strategy, based on science-driven and quality risk management. Thereby, this approach enables obtaining the desired quality of the drug products (QTPP), through the prior identification, analysis, and control of all attributes (CMAs and CPPs) that could have an impact on the product quality (CQAs). Implementing QbD in development offers greater robustness in product and process, fewer variability, lower number of off-specification outputs, reduce supplement submission burden and regulatory oversights, and a consequent reduction in the likelihood of product recalls, or drug shortages. Also, this robustness is reflected in the greater ease of technology transfer from lab-scale to large-scale. The absence of a QbD-developed products market withdrawn highlights the benefits of systematic QbD development in obtaining high-quality drug products (Section 4.5).

Despite the clear advantages of QbD and the wide dissemination of the QbD concepts in pharmaceutical development, the industry still demonstrates some resistance to the acceptability of the QbD approach implementation in their marketing-authorization applications. As discussed in this chapter, a relatively low number of complex products approved by the FDA or EMA implement the QbD approach or describe QbD elements in their regulatory dossier submitted in marketing authorization applications (Section 4.1). The NBCDs already approved by the FDA where it is possible to find some information related to the QbD are Invega Trinza®, Onyvide®, Vyxeos®, Onpattro®, and Monoferric®. Similarly, the Emend®, Zypadhera®, Onivyde®, Onpattro®, Vyxeos®, and Exparel® are examples of EMA-approved NBCDs that applied QbD elements in their pharmaceutical development. Thus, it is possible to deduce that the marketing application with QbD implementation is still far from becoming a true standard approach for the pharmaceutical development of NBCDs in the EU and US. Indeed, these could not be expected since the QbD approach has already been included in ICH guidance documents (Q8/Q9/Q10, 2010), approximately 11 years ago.

The widespread implementation of the QbD approach still is challenging, due to several hurdles for both the pharmaceutical industry and regulatory agencies. The QbD implementation requires extensive resources for experiments, data collection, and documentation, just as the proper use of QbD tools as the design of experiments, quality management system, statistical process control methods, and multivariate modeling. The higher level of details associated with the documentation and the hard classification of parameters criticality are other issues of the QbD approach. The lack of clear and sufficient data in certain submissions, or the high complexity of statistical results and multivariate analysis, can constitute huge challenges for the regulators. Also, there can be some skepticism as to the regulatory benefits of QbD, regulatory flexibility, acceptance and treatment of QbD by regulators, availability of equipment and qualified workforce, the limited number of complex drug products whose development is based on the QbD approach, and the return on investment. Another key issue has been the need for global regulatory alignment of assessment methods by the regulators, as well as, the identified differences in terminology used in dossier submissions, showing that ICH terminology was not always adopted.

On the other hand, it could be verified during this investigation that the manufacturers included only a few QbD elements during the pharmaceutical development of complex products, not following all requirements recommended in the guidance documents. Both in the United States and in Europe, the commonly QbD elements identified for the NBCDs are the CQAs and risk assessment tools. Particularly in the FDA-approved products, it is possible to identify general CQAs in the Chemistry Review(s), i.e. not specific to each type of NBCDs. One of the strategies should be to include a more complete list of specific CQAs, CMAs, and CPPs of each type of NBCDs, including the justification for their classification as critical, just as the corresponding characterization techniques available for the critical attributes. Currently, there is an increasing number of scientific articles and guidance documents that leverage the development of liposomal formulations based on the QbD approach, detailing specific requirements for this type of NBCDs (e.g. unique CQAs). However, this has not occurred at the same scale as other NBCDs.

Furthermore, only one NBCD (Onpattro®, EMA) referenced a multifactorial design of experiments (DoE) on its regulatory dossier (Section 4.4). Although the establishment of design space is a non-mandatory element, this provides unquestionable advantages in improved quality and regulatory flexibility. The reasons why the design space may not be attractive enough for the manufacturers are the need for substantial resources and detailed data to answer the questions of regulators during the review process, an active dialogue between the stakeholders, non-viability in a short development period, or the ability to claim the DS at a commercial scale.

Looking ahead will be needed more extensive efforts and resources from regulatory agencies in the development of internationally harmonized guidelines for each type of complex drug product, including specific and clear quality standards/requirements. Similarly, the development of innovative methods of manufacturing, new control strategies, advanced analytical techniques,

and statistical methods must keep the pace of technological innovations and the complexity of drug delivery systems. It is also very important the application of the orthogonal and multiple complementary techniques to gain a more complete characterization picture of the complex drug products, just as the proper validation and justification of the instrumentation and methodology selected for characterization of them.

Despite still a long way to go in the wide QbD implementation within regulatory procedures, the close cooperation and scientific advice between the science-based multi-stakeholders, and interdisciplinary research, can facilitate innovation in an impact area on the development of high-quality complex drug products.

Chapter IV. Pharmaceutical Quality by Design (QbD): A Strategic Approach to Risk Management and Regulatory Compliance

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IV.II. A Quality by Design (QbD) Approach in Pharmaceutical Development of Non-Biological Complex Drug Products: A Systematic Review

Chapter IV (II) has resulted in a review article entitled [A Quality by Design (QbD) Approach in Pharmaceutical Development of Lipid-based Nanosystems: A Systematic Review], published in the Journal of Drug Delivery Science and Technology.

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Abstract

Over the last few decades, there has been impressive progress in developing novel drug delivery systems and targeted therapies through the use of Nanotechnology. In particular, complex drug products attracted attention due to their interesting biological properties and potential to improve the safety, stability, and delivery efficiency of susceptible drugs.

Despite the technological breakthroughs and multiple advantages related to drug-delivery systems, concerns regarding quality, efficacy, and safety have increased along with the widespread of these drug products. The complex and heterogeneous structure that cannot be fully quantitated, characterized, or described by physicochemical analytical methods, as well as, the nonstandard manufacturing process, makes it very hard to comply with reproducibility requirements and quality standards in their pharmaceutical development.

Therefore, the application of a more holistic and systematic approach, such as Quality by Design (QbD), may be an effective way of surpassing technical and quality challenges. The present chapter aims to map and provide a basic understanding of the current state of implementation of the QbD approach in the pharmaceutical development of Non-Biological Complex Drugs, particularly in the subcategory of the lipid-based nanosystems. The survey methodology applied relied on the thorough analysis of the existing literature and databases regarding lipid-based nanosystems already approved by the regulatory authorities. This analysis discloses the most common material attributes, process parameters, quality attributes, and other variables that are critical for the quality, efficacy, and safety of lipid-based nanosystems. It also includes a brief survey of current trends of risk assessment tools, design of experiments (DoE) methodologies, and characterization techniques applied to the development of these products. This higher level of knowledge will have a definite contribution to facilitating pharmaceutical development and the increase of the number of lipid-based nanosystems reaching the market in the future.

Keywords

Non-Biological Complex Drugs; Lipid-based Nanosystems; Pharmaceutical Development; Drug Development; Quality by Design; Critical Quality Attributes; Critical Process Parameters; Critical Material Attributes; Risk Assessment; Design of Experiments; Design Space; Critical Control Strategy.

1. Introduction

Despite the disruptive advances and extensive popularity of Non-Biological Complex Drugs, there are still unique regulatory challenges in the pharmaceutical development and marketing approval of the reference products and their follow-on versions (also referred to as the complex generic drug products). Significant issues linked to the control of the pharmaceutical quality of the complex drug products, and consequent efficacy and safety profile, have extended over the widespread of these drug delivery systems. The main challenges in this respect are: their complexity and heterogeneous structure that may not be isolated and fully quantitated, characterized, or described by physicochemical analytical means; the composition, quality, and in vivo performance are highly dependent on the manufacturing process; and the structure-function relation or mechanism of action has not yet been fully described for some products. In addition, further challenges deserve attention, including: the absence of complete characterization of their physicochemical and structural properties; the non-understanding of how CMAs and CPPs impact the final product's critical quality attributes (CQAs) and therapeutic performance; difficulty to ensure both reproducibility and batch-to-batch consistency; the pitfalls in the technology transfer from lab-scale to the large-scale; and consequently the impossibility to demonstrate therapeutic equivalence of complex generics with their reference product [14,17,19,20,25,30,31,134,136,138,164,239,333,335–338,389,391]. These obstacles have hampered the market approval of reference products and their follow-on versions since it makes it more difficult to comply with reproducibility requirements and quality standards that ensure every dose is safe and effective, free of contamination and defects.

The application of a more holistic and systematic approach, such as Quality by Design (QbD), through the application of pharmaceutical guidance documents ICH Q8 (*Pharmaceutical Development*), Q9 (*Quality Risk Management*), Q10 (*Pharmaceutical Quality System*), Q11 (*Development and Manufacture of Drug Substances*), and Q12 (*Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management*), is mandatory to surpass the technical and quality challenges through a deeper product and process understanding that will lead to more robust and consistent complex drug products [314,395–398].

As indicated in Chapter IV (I), there is a major gap in the widespread implementation of the QbD approach in the pharmaceutical development of NBCDs. On the other hand, specific needs have been acknowledged in this area, such as the suitable identification of specific CQAs, CMAs, and CPPs of each type of NBCDs, including the justification for their classification as critical, just as the corresponding characterization techniques available for the critical attributes. In addition, the information and contents present in the regulatory guidance documents are scarce to the NBCDs and are often neither tailored to the complexity of these drug products.

This chapter aims to map and provide a basic understanding related to general issues of QbD application namely the material attributes, process parameters, quality attributes, and other variables that are critical for the quality, efficacy, and safety of NBCDs. The bibliographical review and analysis also provide an evaluation of risk assessment tools, experimental design methods, and characterization techniques applied for the pharmaceutical development of these products. The higher level of knowledge will have a definite contribution to facilitating pharmaceutical development and the increase the number of complex drug products reaching the market in the future.

2. Methodology

The survey methodology used in the systematic analysis of the application of QbD in the pharmaceutical development of NBCDs is depicted in Figure 46. A search was conducted on PubMed® using the query terms [Quality by design], [Liposome], [Nanoemulsion], [Nanoparticle], [Polymeric Nanoparticle], [Lipid Nanoparticle], [Solid Lipid Nanoparticle], [Nanoestructured Lipid Carrier], [Niosome], [Ethosome], [Aspasome], [Transferosome], [Nanocapsule], [Micelle], [Polymeric Micelle], [Iron-Carbohydrate Complex], [Dendrimer], [Glatiramer Acetate Complex], or [Glatiramoid]. A total of 118 results (the bibliographic corpus of this chapter) were selected for further evaluation after careful analysis to exclude scientific publications prior to 2000, clinical trials, food industry, or absence of QbD implementation [154,155,157,415,474–587].

The bibliographic corpus of this chapter comprises different classes of NBCDs that include, but are not limited to, liposomes, niosomes, ethosomes, aspasomes, transferosomes, nanoemulsions, nanoparticles, micelles, and nanocapsules. However, it was not possible to find scientific articles related to the application of QbD in the pharmaceutical development of iron-carbohydrate complexes, dendrimers, or glatiramoids. Thus, the main focus of this chapter will be centralized in lipid-based nanosystems.

The results were organized in order to gather all the knowledge generated through QbD application in the development of lipid-based nanosystems, as well as to understand R&D trends, such as the type of lipid-based nanosystems more often investigated, authors' affiliations, therapeutic indication, CQAs, CMAs, CPPs, risk assessment tools, characterization techniques and type of DoE study applied (See Table 51: Appendix II Supplementary Data) [154,155,157,415,474–587].

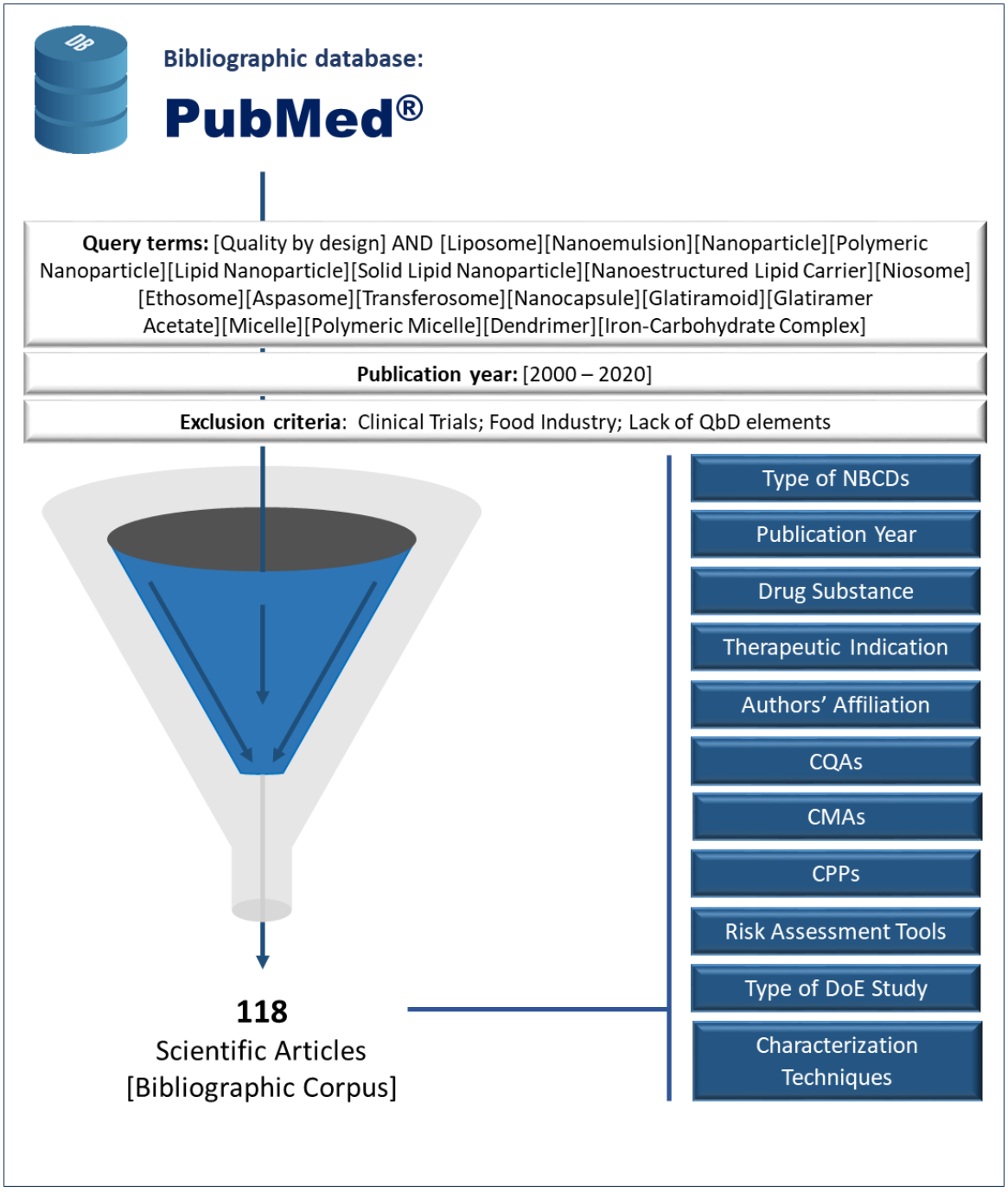

Figure 46. Methodology used for bibliographic corpus selection.

3. Systematic Analysis of the QbD Implementation in Pharmaceutical Development of Lipid-Based Nanosystems

3.1. Authors' Affiliation

From the total number of scientific articles in the bibliographic corpus (n=118), the authors' affiliation was identified and categorized into six main sections, such as: 'academia research', 'industry research', 'research center or institute', as well as, the collaborations 'academia/research center or institute', 'academia research/FDA', 'academia/industry research' (Figure 47).

The examined QbD studies applied to lipid-based nanosystems come mainly from academia (n=73, 62%) and collaborations between academia and the research centers or institutes (n=22, 19%) (Figure 47). Ten publications include authors from a regulatory authority (FDA), through collaboration with academia (n=10, 8%). Under this analysis, the FDA presents an increased involvement in QbD implementation compared to other regulatory authorities, since the FDA was the only agency identified. This is in line with their well-known efforts to provide product-specific guidances for Complex Drug Products that facilitate the submissions of dossiers that focus the product quality, as well as, a thorough understanding of the manufacturing process. Likewise, the Drug Competition Action Plan, Regulatory Science Research Program of GDUFA, and Complex Generic Drug Product Development Workshop are important examples of initiatives undertaken by the FDA to help address the scientific challenges related to Complex Drug Products [16,27,280,588,589]. For example, the FDA-supported Regulatory Science Research Projects related to its equivalence of Complex Products priority area includes 'evaluation of dissolution methods for complex parenteral liposomal formulations', 'development of a liposome doxorubicin product drug release assay', 'computational drug delivery; leveraging predictive models to develop bioequivalent generic long-acting injections', 'novel method to evaluate the bioequivalence of nanomedicines', 'critical process parameters for the preparation of amphotericin B liposomes', among others [27].

On the other hand, only eight articles correspond to research collaborations between academia and the pharmaceutical industry (n=8, 7%). One paper corresponds to a partnership with Atral Pharmaceutical S.A that studied the application of the QbD approach on starch-based nanocapsules for topical drug delivery [585]. Another research paper involving AbbVie Deutschland GmbH & Co includes the results of the characterization and optimization of the encapsulation process of itraconazole (ITZ) into the PEGylated liposomes [495]. Another example is the research paper published by Novartis, entitled 'Confocal Raman microscopy to probe content uniformity of a lipid-based powder for inhalation: A Quality by Design approach' [497].

Concerns related to confidentiality and loss of commercial value can be the root cause of the low number of scientific publications involving the pharmaceutical industry. The content of scientific articles that arise from this collaboration has serious shortcomings in the QbD application, due in part to the dearth of information and details related to the product and process development. Other issues related to the low number of scientific articles from the pharmaceutical industry, may be due to the small number of approved lipid-based nanosystems with the full application of the QbD approach described in their dossiers. There is also a higher uncertainty surrounding the regulatory approval of lipid-based nanosystems developed based on the QbD approach, and the inconsistency of processing by regulatory authorities.

Furthermore, it is important to highlight the existence of scientific articles resulting from different collaborations, as an effort of some stakeholders to promote the communication, cooperation, and application of translational research, to achieve the full potential of the scientific findings related to the QbD implementation.

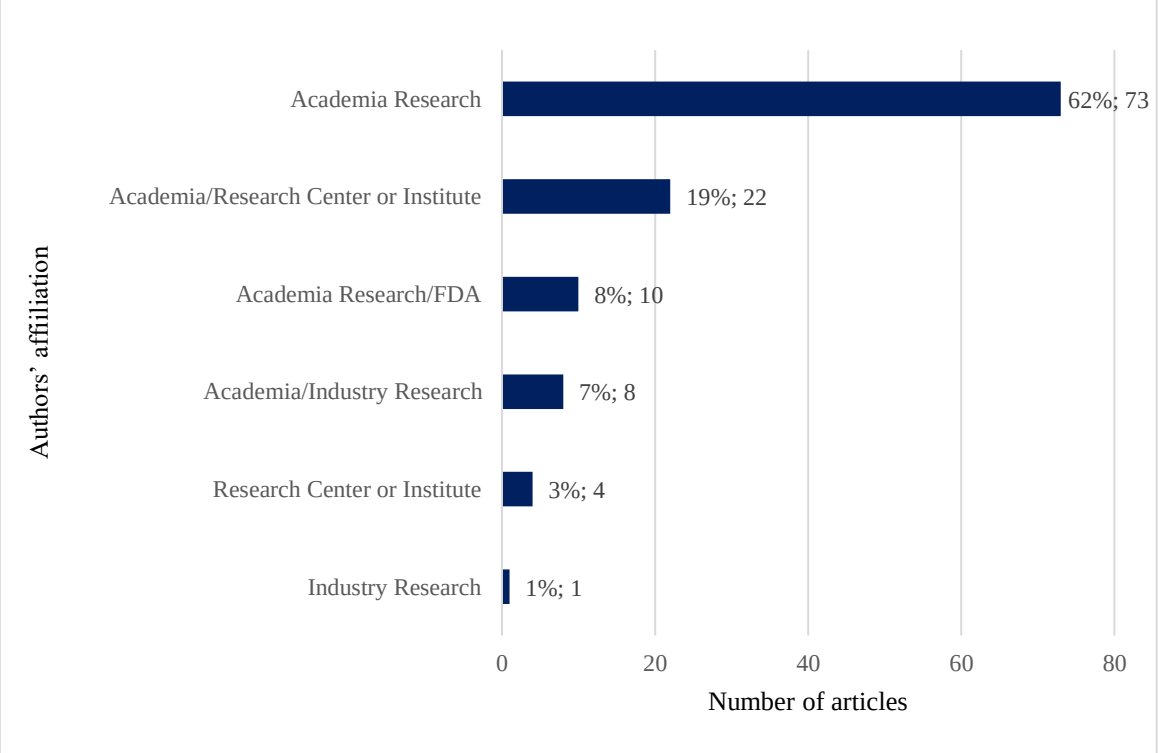

Figure 47. Authors' affiliation distribution of the bibliographic corpus.

3.2. Publication Year

The current section highlights the trend of the number of scientific articles related to lipid-based nanosystems published during the latest 15 years (Figure 48). The number of scientific articles applying the QbD principles to the development of lipid-based nanosystems is higher over the last five years compared to the previous years (Figure 48). The year 2012 appears to be a turning point in the timeline of QbD implementation in lipid-based nanosystems. It is noted that the search was conducted until June 2020, wherefore is too early to have a conclusion on the publication number for subsequent years.

There are several factors related to the increasing interest in the development of lipid-based nanosystems, such as the high value of the market, the great advantages of targeted systems, the loss of exclusivity conferred by a patent expiration, and the increased knowledge and documentation disclosed about them [19,30,34,162].

The starting point of the QbD implementation in pharmaceutical development occurred with the FDA approval of the non-complex drug product Januvia® (sitagliptin phosphate, Merck & Co.) in 2006 [405,422].

In 2008 the ICH Q8 revision clarified the QbD concepts present in the first version of the guideline [395], in 2010 FDA issued draft guidance on doxorubicin hydrochloride liposomes stating that the applicants should follow a QbD approach for the development [206] and, in 2011 [590] and 2012 [591] FDA published examples of reports using QbD for generics development. Even in the year 2011, the EMA and FDA launched a joint pilot program for the parallel assessment of applications containing Quality by Design (QbD) elements [400].

Subsequently, FDA approved the first biological complex drug product with QbD elements in 2012, called Perjeta® (pertuzumab, Genentech, Inc.). This corresponds to a Biologics License Application (BLA), where the Design Space was not properly characterized [408]. In the following year (2013), FDA approved the Gazyva® (obinutuzumab, Genentech, Inc.), the first biological complex drug product with an approved design space and a post-approval lifecycle management (PALM) plan [407]. It means, Gazyva® corresponds to the first complex drug product with a full-QbD submission with an approved design space [407].

Furthermore, EMA published a ‘Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product’ (2013) [284].

The main published documents in the year 2017 are the EMA ‘Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development’ [325], just as the FDA Guidance for Industry ‘Drug Products, including biological products, that contain nanomaterials’ [10]. On the other hand, the release of the final FDA guidance on liposome drug products (2018) comprising QbD principles according to ICH Q8(R2) Pharmaceutical Development, including screening of critical variables (CQAs) and establishment of a design

space, illustrates the effort of the regulatory authorities to provide recommendations focused on the unique technical aspects of such dosage forms [253].

As an application of QbD principles can be rather complex, the examples provided by the regulatory authorities could have stimulated the work in this field, which in turn may have resulted in an increased number of publications in the last years.

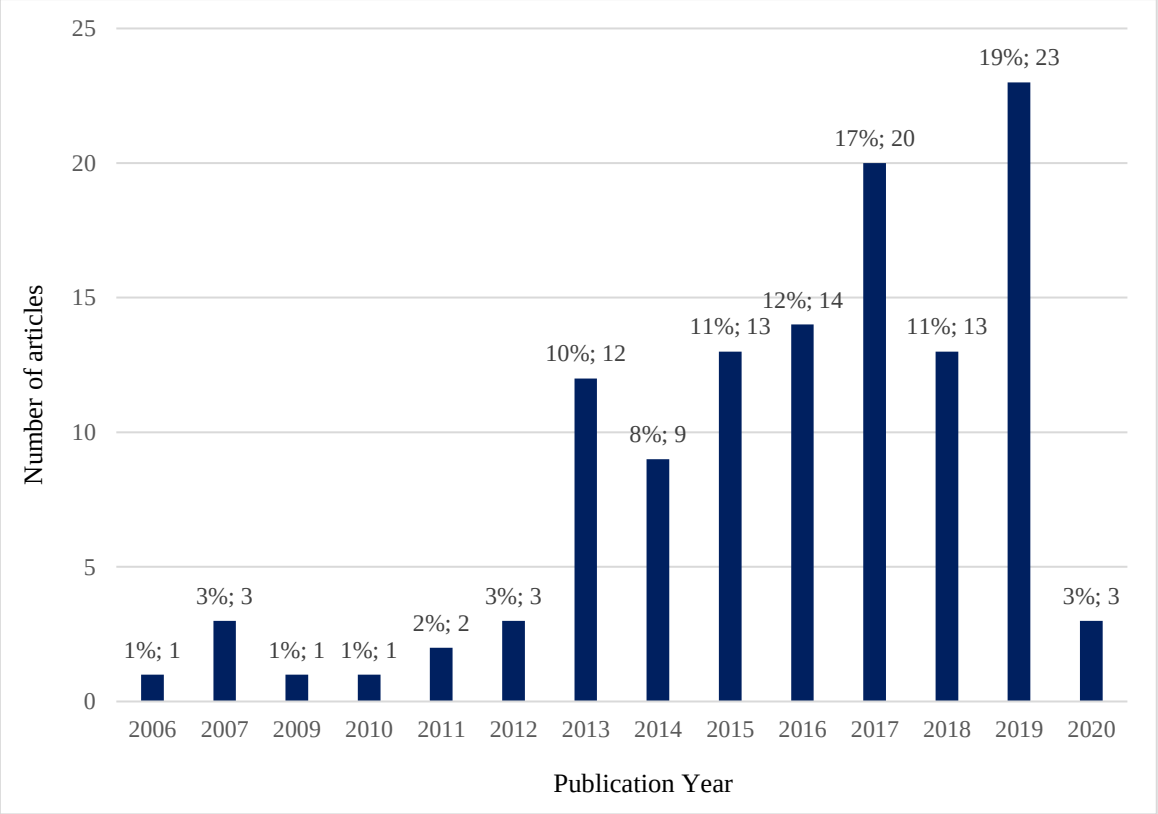

Figure 48. Distribution of the bibliographic corpus according to the year of publication.

3.3. Type of Lipid-Based Nanosystems

Liposomes were the most common type of lipid-based nanosystems identified in bibliographic corpus (n=29, 25%), followed by polymeric nanoparticle (n=28, 24%), nanoemulsions (n=19, 16%), nanostructured lipid carriers (NLC) (n=12, 10%) and solid lipid nanoparticles (SLN) (n=11, 9%) (Figure 49). The widespread use of liposomes is in line with the results and advantages described in the first chapter of the thesis (Chapter I, Section 3.2.).

Nevertheless, the need to overcome specific problems related to the physical and chemical instability of vesicular systems, and to improve the performance in other applications areas, led to the development of other types of nanosystems [159,160]. In this analysis, it can be noted in this regard that the nanoparticles as a whole (polymeric nanoparticles, SLN, and NLC), overcome

the number of liposomal formulations (Figure 49). Polymeric nanoparticles are a polymer-based controlled release technology, composed of a core of biodegradable polymers [525,532,533,535,539]. The poly(DL-lactic-co-glycolic acid) (PLGA) is an example of polymers most commonly used, due to their nontoxicity, good colloidal stability, biocompatibility, biodegradability, and GRAS certification (Generally recognized as safe) [525,532,533,535,539]. The polymeric nanoparticles present numerous applications of systemic delivery in different therapeutic areas including oncology, neurological disorders, cardiovascular disease, gene therapy, diabetes, infectious diseases, among others [518,523,525,532,533,535,539,542,543]. The higher shelf-life stability during the storage, the structural integrity, the sustained release of the therapeutic agents, the improved specific biodistribution, and easy administration through the intravenous route are the major advantages of polymeric nanoparticles compared with other delivery systems [518,523,525,532,533,535,539,542,543].

Solid lipid nanoparticles (SLN) have a solid lipid core matrix stabilized by surfactants [82,120–122]. During the process of production, the solid lipids crystallize and generate an unstable polymorphic form, which consequently suffers a high organization and leads to the expulsion of drugs, and a reduction in encapsulation efficiency (%EE) [82,120–122]. Due to these limitations, the second generation of lipid nanoparticles arose, the Nanostructured lipid carriers (NLC) [82,120–122]. The NLCs are constituted by solid lipids and liquid lipids, thus creating an amorphous structure, preventing the drug expulsion and increasing the %EE [82,120–122]. Solid lipid nanoparticles (SLN) and Nanostructured lipid carriers (NLC) combine the advantages of other lipid-based nanosystems, featuring high versatility in distinct routes of administration, such as oral, dermal, pulmonary, parenteral, and brain targeting [570,592–594]. The main advantages identified are the improvement of the control over the release due to the incorporation of drug substances in the lipid matrix, as well as, higher long-term stability, higher capacity of drug loading, large-scale and cost-effective production [570,592–594].

On the other side, nanoemulsions are thermodynamically stable isotropic systems with small size droplets (20–200 nm), that allow a large surface area to provide better absorption and stability to sedimentation, flocculation, and coalescence [511,594–598]. Furthermore, it also exhibits enhanced solubility for hydrophilic and lipophilic substances, high percentages of drug loading, and the ability to use excipients that play an important role in interaction with specific tissues [511,594–598].

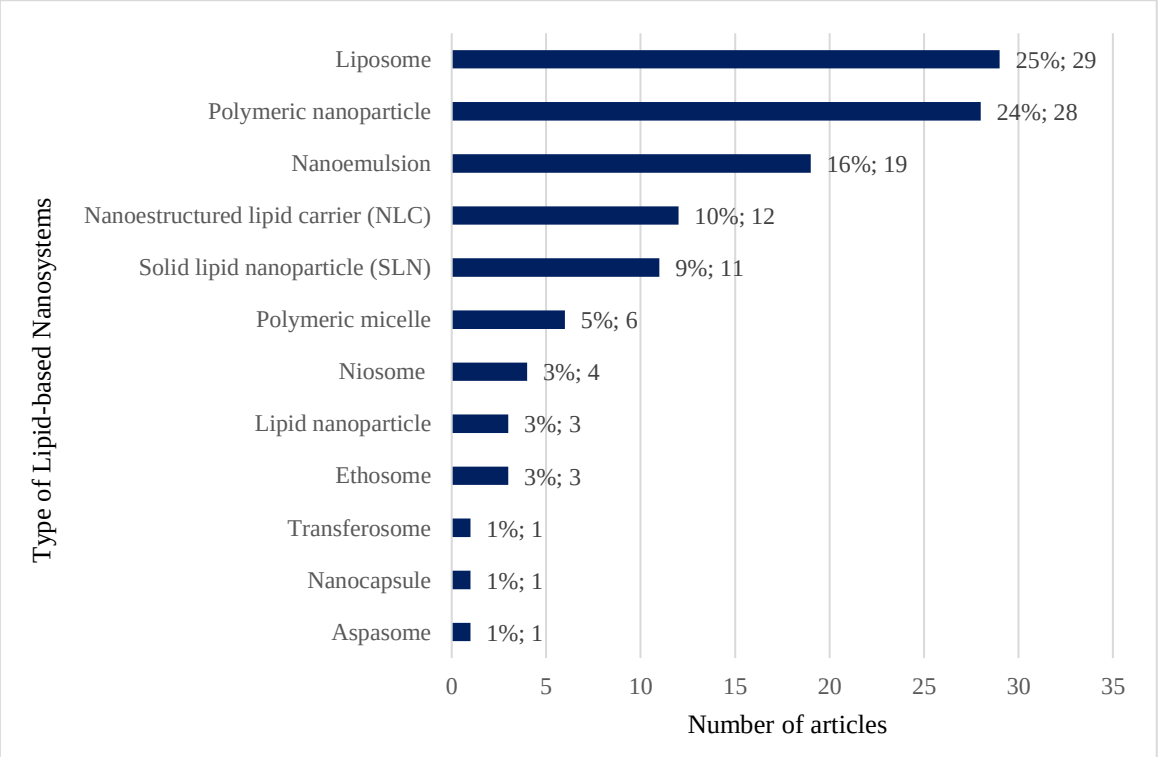

Figure 49. Type of Lipid-based Nanosystems identified in the bibliographic corpus.

3.4. Therapeutic Indication

From the analysis of the bibliographic corpus, it was possible to observe that the majority of the lipid-based nanosystems were developed for cancer therapy (n=26, 22%), followed by neurological disorders (n=15, 13%), infectious diseases (n=10, 8%), HIV/AIDS (n=9, 8%), hypertension (n=7, 6%), lipid disorders (n=6, 5%), among others (Figure 50).

Most lipid-based nanosystems have been widely applied to the treatment of cancer, as it is in accordance with the results described in Chapter I (Section 3.4.). The drug encapsulation in a nanosystem allows for overcoming some of the drawbacks of chemotherapy, such as low specificity, high toxicity, and high potential to cause adverse effects. That is because lipid-based nanosystems are biocompatible, biodegradable, nonimmunogenic, and present the ability to protect the content encapsulated, promote the accumulation in tumor tissues, minimize systemic toxicity and side effects of oncolytic therapeutics, promote the increase of drug efficacy due to the rise in the time of systemic circulation, and promote the synergistic activity using a combination of drugs. Additionally, the tumor microenvironment allows an enhanced permeability and retention effect (EPR effect) in specific regions of tissues, and tumor cells

overexpress genetic markers which constitute important therapeutic targets [3,136,140,141,152,535].

On the other hand, certain types of lipid-based nanosystems (liposomes, nanoemulsions, or nanoparticles) consist of promising strategies for overcoming the problems of crossing the blood-brain barrier (BBB), a major challenge to treatment of most neurological disorders [476,488,491,507,511,532,536,540,554,557,559,560,569,571].

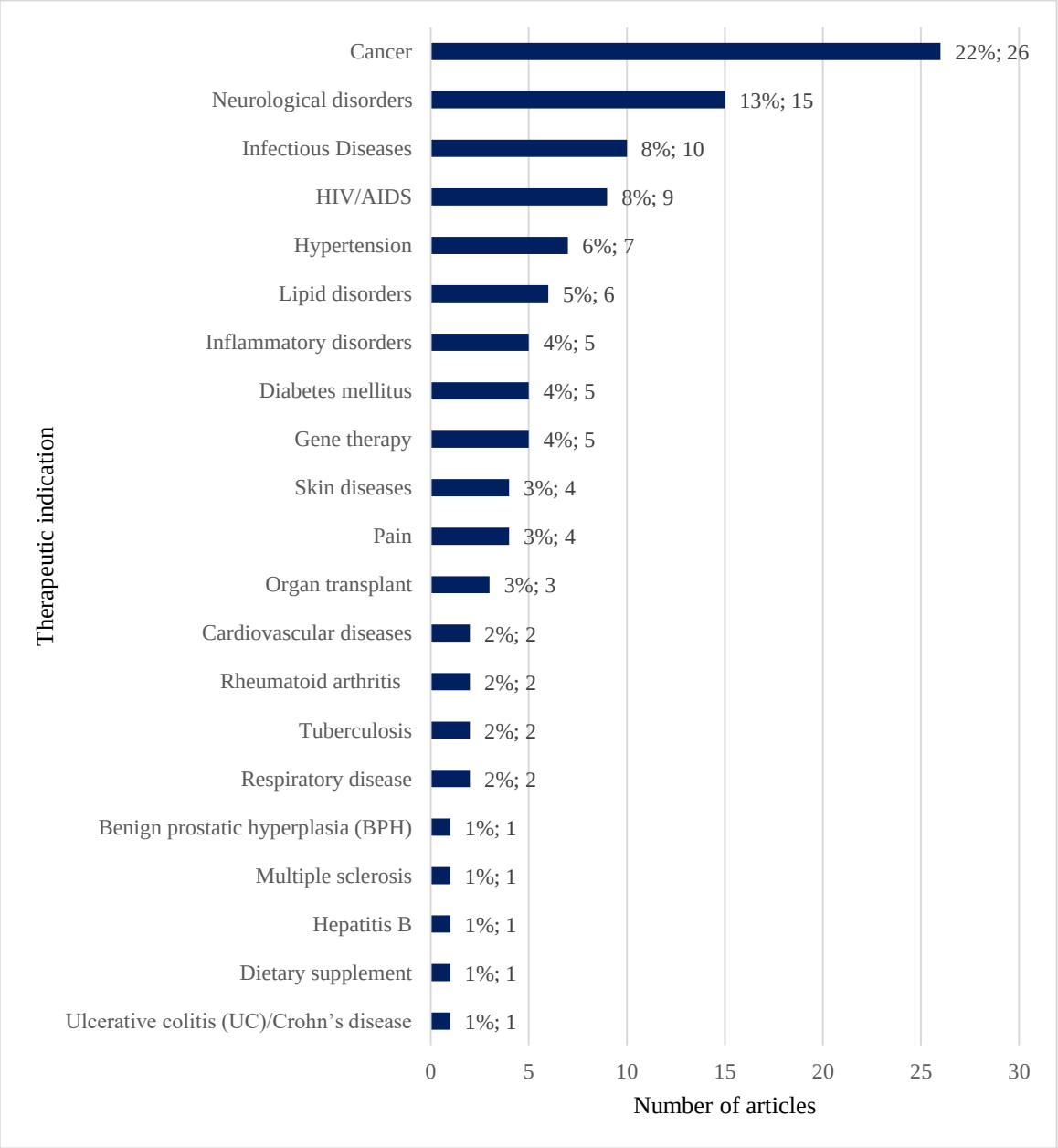

Figure 50. Therapeutic indication of lipid-based nanosystems identified in the bibliographic corpus.

3.5. Critical Quality Attributes (CQAs) of Lipid-Based Nanosystems

Over the analysis of the bibliographic corpus, it was possible to identify a diversified list of CQAs (Figure 51). The CQAs most frequently referred are: particle size (n= 117, 99%), polydispersity index (PDI) (n=87, 74%), encapsulation efficiency (n=86, 73%), zeta potential (n=85, 72%), drug release (n=67, 57%) and morphology (n=56, 47%).

Our results showed that most of the CQAs identified in the bibliographic corpus are specific for lipid-based nanosystems. Surprisingly, some CQAs that must be identified and studied in all medicinal products because they are key for product safety and efficacy were lacking in the papers analyzed (e.g. assay, uniformity of dose, impurities, etc).

Due to this gap, a more complete list of potential CQAs for lipid-based nanosystems is proposed by the authors in Table 16, along with the justification for their classification as critical. The table is divided into two sections. In the first section, there are the CQAs that although are not specific to lipid-based nanosystems must be studied because they are critical to ensuring the safety and efficacy of the medicinal product. In the second section of Table 16, the specific CQAs are described. Some of the CQAs listed in Table 16 are also referred to in numerous guidelines such as: 'ICH Q6' [305]; 'Drug Products, Including Biological Products, that Contain Nanomaterials' [10], 'Development of Liposome Drug Products' [428]; 'Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product' [284] and 'Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation' [253].

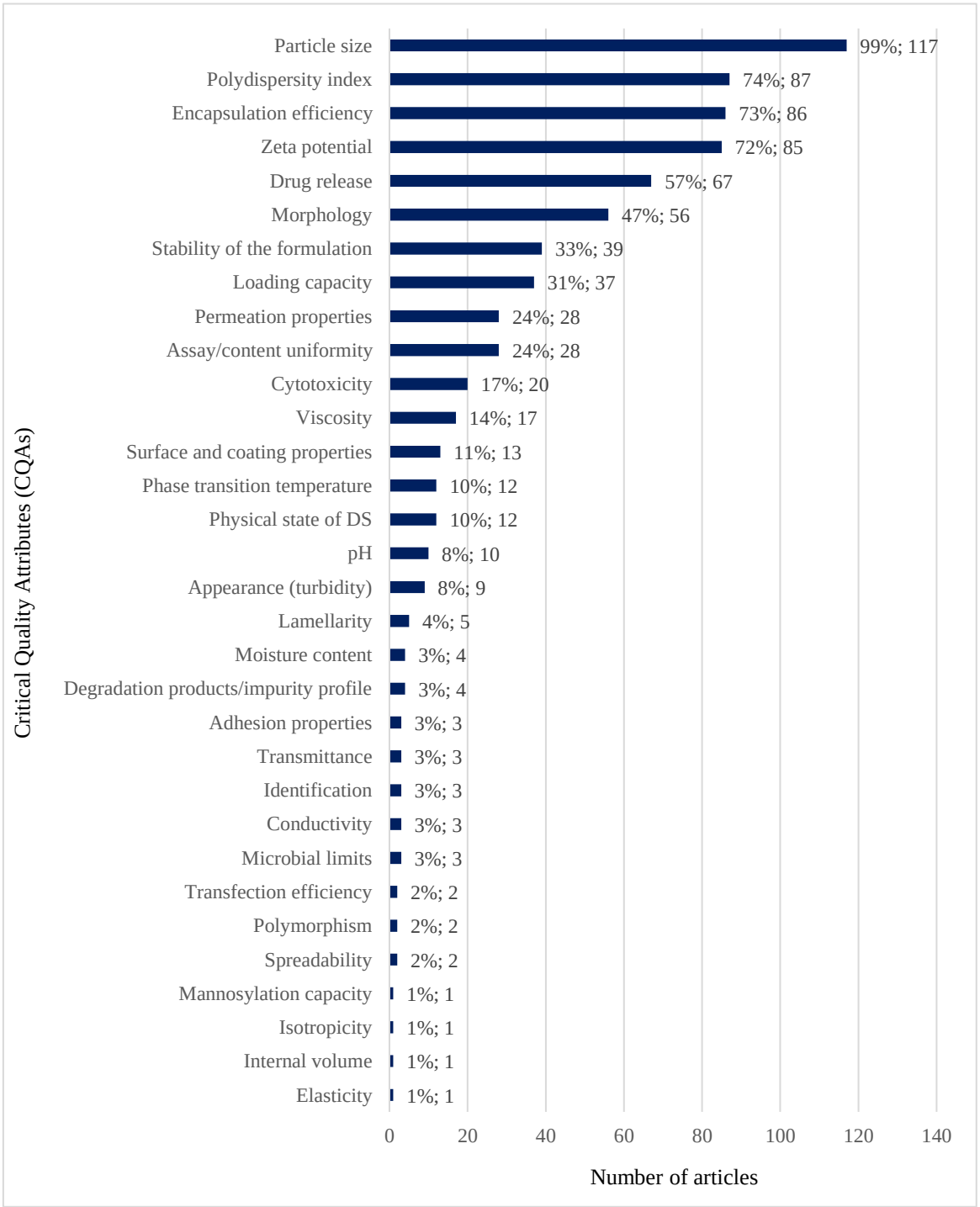

Figure 51. Critical Quality Attributes (CQAs) identified in the bibliographic corpus.

Table 16. List of CQAs proposed for Lipid-based Nanosystems.

General CQAs	Justification	Reference
Assay/ Content Uniformity	The DS (drug substance) assay and content uniformity have an impact on the drug concentration in plasma, so they are critical for the safety and efficacy of any drug product. Thus, the assay and content uniformity should be evaluated throughout product and process development and should conform to USP <905> Uniformity of Dosage Units.	[157,474,476,478,481,482,486,493,497,500–502,507–509,512,514,523,534,535,548,554,562,563,567,569,571,587,599]
Cytotoxicity	The cytotoxicity has a great impact on the safety profile of the drug product and may be influenced by the particle size, shape, composition, surface charge, and hydrophobicity of these types of dosage forms. For example, positively charged nanoparticles tend to present a higher cytotoxicity, since they may promote cellular membrane disruption and death. Conversely, distinct components used in formulation may be more or less toxic. Cytotoxicity studies are performed to verify the absence of toxic effects, indicating a safe and biocompatible profile.	[474,480,486,499,502,510,519,533–535,546,550–552,560,563,565–567,569]
Degradation products/ Impurity profile	Degradation products can compromise the safety profile of the drug product and must be controlled based on compendial/ICH requirements or a reference listed drug (RLD) characterization, to limit patient exposure. The target for any unknown impurity is set according to the ICH identification threshold for each drug product. Therefore, degradation products should be assessed during product and process development conforms according to ICH Q3B(R2) requirements.	[21,47,51,156–159,162]
Drug release	Drug release can impact on bioavailability and clinical performance of the drug product, e.g. the drug pharmacokinetics and pharmacodynamics, and therefore the therapeutic efficacy and the safety profile.	[23–29,36–38,40,43,49–52,55,57–60,62–65,67,70,73,74,80,82,84–87,90,92,97–99,101–103,105–115,117–121,126,129,131,135–137,156–159]
Identification	The identification of formulation components including the drug substance, lipid components, and functional excipients, should be assessed during product and process development, due to can largely affect the quality, efficacy, and safety profile of the drug product.	[10,497,535]
Leachable/ extractables	The leachables and extractables from components of the primary packaging (e.g. plastic and rubber) can compromise the safety profile of drug product, due to the generation of impurities. Thus, they should be evaluated throughout the product and process development, according to USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems and USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery systems.	[600,604]
Particulate matter (not aggregate for solution only)	The presence of particulate matter in formulations intravenously administered is considered critical due to the potentially life-threatening health hazards. They can cause irritation, phlebitis, anaphylactic shock, embolism, and even death. This CQA should be evaluated throughout the product and process development, by USP <788> Particulate matter in injections and USP <790>Visible particulates in injections.	[428,605]

General CQAs	Justification	Reference
Physical state of the DS	The specific physical state of the drug substance within the drug product may impact both, the stability of the encapsulated drug and the apparent drug release rate, which therefore have an impact on drug pharmacokinetics (PK). In turn, the drug PK greatly determines the therapeutic efficacy and the safety profile of the drug product. For example, a DS in a precipitated state is related to limited solubility, which leads to a slower drug release, compared with an amorphous form that results in a faster drug release from the formulation.	[10,157,463,497,501,511,523,525,543, 548,560,561,566]
Polymorphism	As referred for the CQA 'Physical state of DS', the solubility of a drug (for example in the lipid matrix) becomes a very important controlling factor for the drug release rate from the drug product. Different polymorphs can exhibit different solubility and, therefore, different bioavailability, which have an impact on the efficacy and safety of the drug product. Thus, the polymorphism is an attribute that should be evaluated throughout product and process development.	[10,284,463,497]
Residual solvents	Residual solvents can impact the drug product safety profile when used in the manufacturing process because most of the time they cannot be completely removed from the drug product. Thus, this CQA should be evaluated and quantified during product and process development, by USP <467> Residual solvents and ICH Q3C(R6) requirements in accordance with each type of solvent used.	[606,607]
Stability of the formulation	The physicochemical stability of drug products is required in order to maintain therapeutic potential and ensure the quality of the medicinal product during the entire shelf-life. Stability studies should include tests to assess the microbiological, physical, and chemical stability of the formulation. Some products are susceptible to fusion, aggregation, or leakage of the contained drug substance. The stability of the drug substance, lipid components, and functional excipients should be appropriately evaluated in accordance with the concepts included in guideline ICH Q1A(R2), ICH Q5C, and USP <1049> Quality of Biotechnological Products.	[12,23–25,29,37,41,43,44,46,48,50,51,57–59,61,64,73–75,80,84,89,92,93,96,100,103,105–108,113,116,117,120,121,132,156–159,167,168]
Sterility and bacterial endotoxins	Non-compliance with microbial limits has the potential to harm the patients particularly when the medicinal product is intended to be administered intravenously. The sterility/pyrogen content and bacterial endotoxins may be influenced by process parameters and formulation variables, which can impact patient safety. Therefore, these attributes should be investigated during product and process development and conform to USP <71> Sterility tests and USP <85> Bacterial endotoxins.	[10,603,610,611]
Uniformity of dose (Fill volume)	An accurate fill volume is crucial to ensure the required dosage, which is mandatory to ensure the efficacy and safety of the drug product. Fill volume per vial should be investigated during product and process development, by USP <697> Container content for injections and USP <905> Uniformity of dosage units.	[206,599,612]
Specific CQAs of Lipid-based Nanosystems	**Justification**	**Reference**

General CQAs	Justification	Reference
Adhesion properties	The adhesion properties are related to the driving force for drug permeation that can have a significant impact on transport through the skin. Therefore, this quality attribute should be investigated during product and process development, for the impact that it has on the effectiveness of complex drugs administered on the skin, specifically NLC, niosomes, or transferosomes.	[481,569,579]
Appearance (color/turbidity/caking)	The changes in the appearance of formulations can indicate physical instability that can be due to degradation, phase separation, caking, or aggregation. These phenomenons can compromise the quality, efficacy, and safety of drug products.	[482,484,501,502,512,515,535,563]
Assay of lipid components	The lipid content is a CQA which can affect the particle size, polydispersity index, %EE, loading capacity, and zeta potential, and hence may influence the therapeutic efficacy and the safety profile of the drug product.	[284,428,603]
Conductivity	The conductivity measured in an emulsion is related to its nature and stability. For example, water in oil emulsions (w/o) present lower conductivity compared with o/w emulsions. This parameter is often used as a confirmation of the type of emulsion obtained.	[491,507,511]
Degradation products related to lipid components	Lipids with unsaturated acyl chains are subject to oxidative degradation, while both saturated and unsaturated lipids are subject to hydrolysis to form lysolipids and free fatty acids. This degradation process can change the phase transition temperature and, consequently the stability of drug products. Therefore, degradation products should be assessed during product and process development according to ICH Q3B(R2) requirements.	[21,47,51,156–159,162]
Elasticity	The elasticity related to some drug delivery systems (e.g. ethosomes) is a physicochemical property that assumes great importance for skin permeation, because it allows their passage through the small skin pores. Therefore, this CQA should be investigated during the R&D of this type of formulation intended to be administered through the skin.	[583]
Encapsulation efficiency (%EE)	Encapsulation efficiency is one of the most important CQA. The drug encapsulation efficiency (the amount of drug contained inside of drug system, compared with the total amount of drug) has a large impact on drug release from the drug product, and hence, in their effectiveness and safety profile. In addition to the importance of drug delivery in the target tissue, the %EE is also very important for quality control purposes to demonstrate that the drug concentration encapsulated is consistent between lots.	[12,22–40,42–46,48,64,67–69,71–74,76,78–83,85–92,94–99,101–115,117–122,124,127,129–134,136,137,157,159]
Internal volume	The internal volume is directly related to the particle size, polydispersity index, loading capacity, and %EE. For example, an increase in the total number of liposomes formed leads to an increase in total internal volume and consequently a higher %EE.	[516]
Isotropicity	The isotropicity corresponds to an optical property used to characterize the isotropic nature of the systems, particularly in the formation of emulsions. This parameter is determined through a refractive index (RI) measurement.	[514]
Leakage	Any leakage during storage can have a significant impact on drug biodistribution, in vivo clearance from plasma, and the effectiveness of the drug product. The leakage might be due to hydrolyzation and degradation of the lipids that can change	[10,34,428]

General CQAs	Justification	Reference
	the original function of lipid bilayer structure or lead to stability problems like vesicle aggregation, disintegration, or fusion that compromises stability.	
Lipid bilayer phase transition temperature	At temperatures close to the phase transition temperature of the lipids there is an enhanced lipid mobility that results in increased lipid bilayer permeability and hence rapid drug diffusion and leakage. Thus, this CQA affects the drug release and biodistribution that may influence the efficacy and safety of the drug product. In addition, it can also accelerate collision and coalescence rates of the nanosystems that have an impact on the stability of formulations.	[428,475,483,484,524,525,532,539,543,548,554,566,583]
Loading capacity	The drug loading capacity is the amount of drug-loaded per weight of the lipid used, thus it is strongly related to the encapsulation efficiency of the drug. The loading capacity depends on several factors like the method of drug loading (e.g. active, passive), the extent of solubility of the drug in the lipid matrix, drug physical state, and polymorphism. Therefore, it is important to ensure that the drug loading is within a predefined range to ensure the desired drug release, bioavailability, efficacy, and safety of the drug product.	[428,475,477,478,480,483,487,489,495,498,518,519,521,523,524,526,529,533,535,536,539,541,546,552,553,557–562,567,569,570,572,584,586]
Mannosylation capacity	The mannosylation is a strategy to increase the immunogenicity of liposomes and consists of the conjugation of mannopyranoside moieties with the vesicular surface. This CQA significantly affects the target specificity and uptake that, consequently, has an impact on the effectiveness of the drug product. This attribute was found critical on liposomes used for brain-targeting with a region-specific distribution	[488]
Morphology	Morphology and lamellarity reflect the ability of the drug products to contain and to retain the drug substance, and in that sense may affect the drug loading capacity and the rate of drug release. These CQAs should be investigated throughout product and process development, due to their impact on biodistribution, efficacy, and safety of the drug product.	[10,154,155,157,428,475,477,481,482,488,493,497,499,500,502,506–508,510,511,514,518,523–525,528,530,532–534,536,539,540,543,546,548,550,552–554,557–563,565–567,570,571,579,581–583,585–587]
Lamellarity		
Osmolality	In the guideline ‘Development of Liposome Drug Products’ it is described that, for injectable products, the reconstituted drug solution should preferably be isotonic (295 mOsm/kg) to prevent rupture or contraction of the lipid-based nanosystem structure. Thus, the nanosystem integrity is influenced by the osmolality. Additionally, osmolality values different from plasma osmolality may cause tissue irritation and damage to blood cells. This CQA should be evaluated during product and process development, by USP <785> Osmolality and osmolarity.	[428,613]
Particle size	The particle size is the most extensively studied CQA (Figure 51), because it has a significant impact on stability, encapsulation efficiency, drug release profile, biodistribution, cellular uptake, bioavailability and, as a consequence, the	

General CQAs	Justification	Reference
	efficacy and safety profile of the drug product. The control of particle size in the range between 10 and 100 nm is important to avoid the elimination by kidneys or liver, and to promote the efficient enhanced permeability and retention (EPR) effect in the tissues. The particle size also affects the drug release, i.e., smaller sizes are normally associated with faster drug release rates. On the other hand, small sizes are crucial for sterile filtration necessary to ensure the sterility of the final product, and to prevent embolism or thrombosis issues.	[154,155,157,284,415,428,474–577,579–587,603]
Permeation properties	The permeation properties through biomembranes are indicative of the availability of the drugs in the systemic circulation, i.e., it is a critical quality attribute to ensure a drug concentration in the blood within the therapeutic window.	[481,482,499,501,502,505–509,512,524,549,551,557,560–562,565,569,570,576,579,580,582–584,587]
pH	This quality attribute has an impact on active drug loading driven by a pH-gradient as it generates a driving force for the accumulation of drugs in the interior of the vesicles. In turn, it affects the drug release, permeation, and stability of the drug product. Also, the pH of the final product is critical for the safety profile of formulations intravenously administered, that must be biocompatible. pH values different from plasma pH may cause irritation, vasculitis, thrombosis, and emboli.	[428,476,501,504,507,511,535,554,565,582,587,603]
Polydispersity index	Polydispersity is a physical parameter related to the particle size distribution, which, in turn, influences the pharmacokinetic profile and the product performance (safety and efficacy). Also, the tendency of complex drugs to accumulate in the target tissue depends on this quality attribute. The polydispersity index can also affect the bulk properties, processability, stability, and appearance of the final product. Thus, it is very important to obtain drug products with low values for the polydispersity index indicative of a monodisperse population.	[157,476–478,481,482,485,486,488,489,491–497,499,500,503,504,507,511,513–515,518,519,521–534,537–543,545–552,554–567,569–576,581–583,585–587]
Residual moisture content	The residual moisture content is a CQA that can affect the stability of the freeze-dried products, due to the impact on the transition temperature of the system and molecular mobility. It has been verified that the increased moisture content leads to a decrease in the transition temperature and increased molecular mobility that results in the fusion of vesicles. A low residual moisture content is a requirement for the storage stability and therapeutic potential of the products.	[483,484,494,521]
Spreadability	The spreadability is a quality attribute specific to topical formulations, such as ethosomes. This parameter depends on the viscosity of the formulation and has an impact on drug availability at the site of action, and hence in the efficacy of the drug product.	[582,587]
Surface and coating properties	The surface modification process can have a substantial impact on the tissue and intracellular distribution which in turn affects both the efficacy and safety of the drug product. For example, the stealth effect triggered by PEG moieties on the surface of the nanosystems avoid the recognition by macrophages, and hence increases the bioavailability and therapeutic potential of the encapsulated drug, due to an extended circulation time. Other polymers are used to enhance mucosal immune response or targeting the drug at specific tissue through changes in pH.	[157,476,477,479,492,497,524,535,550,561,570,579,586]

General CQAs	Justification	Reference
Transfection efficiency	The transfection efficiency, specifically for genetic material, is a quality attribute that promotes the pass across different biological barriers, favoring an efficient drug release. This QA is also related to a significant reduction of safety hazards concerned with material transfected.	[533,550]
Transmittance	The percent transmittance is a measure of the optical clarity of a nanoemulsion and is commonly used to characterize the isotropic nature and physical stability of the drug product. The percent transmittance is a function of the droplet size, thus it may indicate changes in size distribution. The lower the droplet size, the higher the transmittance of nanoemulsion, and the clarity of the formulation.	[507,514,576]
Viscosity	Viscosity is an important rheological CQA that should be evaluated throughout the product and process development, because it may impact the efficacy, safety, and physical stability of different drug products. For transdermal delivery of therapeutic agents, the viscosity may influence the drug diffusion rate at the microstructural level and, consequently the effectiveness of the treatment regarding the delivered dose. Conversely, the low viscosity of formulations is more suitable when parenteral administration is intended.	[481,498,501–503,507,508,511,514,549,561,562,565,570,582,586,587]
Zeta potential	Zeta potential is the electric charge on the particle surface and is an important quality attribute for the evaluation of the physical stability of colloidal systems, since that reflects the electrostatic repulsive force between particles, and may influence their efficient interactions with cells and tissues, in vivo clearance, tissue distribution, and intracellular uptake. The particle aggregation, sedimentation, or flocculation is prevented when the formulation exhibits a zeta potential higher than +30 mV or lower than − 30 mV, being considered stable colloidal dispersions. The positive charge of the formulations promote the interaction with the negatively charged lipid membranes, facilitating drug delivery for specific tissues, increasing the cellular uptake, and enhancing the therapeutic effectiveness. However, the cationic particles are associated with reduced colloidal stability, non-specific tissue internalization, shorter blood circulation time, cytotoxicity, cell membrane disruption, and consequent cell death. On the other hand, it was also demonstrated that negatively charged liposomes used for transdermal drug delivery may also exhibit high drug permeation.	[10,154,155,157,284,415,428,475–481,484,487–489,492,493,496,498,499,502,504,507,508,510,511,513–515,518,519,521–534,536,537,539,540,542–544,546–560,562–568,570,574–576,579,581–587,603]

3.6. Critical Material Attributes (CMAs) of Lipid-Based Nanosystems

A significant number of the identified CMAs in the bibliographic corpus are related to the lipid concentration (n=63, 53%), type of lipid (n=23, 19%), lipid: lipid molar ratio (n=25, 21%), drug: lipid molar ratio (n=13, 11%), solid lipid concentration (n=8, 7%), liquid lipid concentration (n=7, 6%), type of solid lipid (n=5, 4%), type of liquid lipid (n=3, 3%), phase transition temperature of lipids (n=7, 6%), among others (Figure 52). That is not surprising because the pharmacological and toxicological properties, as well as the quality of several drug products (e.g. liposomes, nanoemulsion, lipid nanoparticles, micelles), can vary significantly with changes in the lipid composition and quality of the lipid components [155,415,492,496,555,558]. For that reason, the companies that develop these types of products must provide information concerning the chemistry, manufacturing, and control of the lipid components at the same level of detail expected for a drug substance as defined in the relevant guidelines [10,284,305,428].

Other identified CMAs include surfactant concentration (n=58, 49%), drug concentration (n=46, 39%), aqueous: organic phase volume (n=23, 19%), polymer concentration (n=27, 23%), type of surfactant (n=25, 21%), and co-surfactant concentration (n=13, 11%).

The CMAs identified for lipid-based nanosystems studied in the bibliographic corpus are listed in Table 17, just as the justification for their classification as critical.

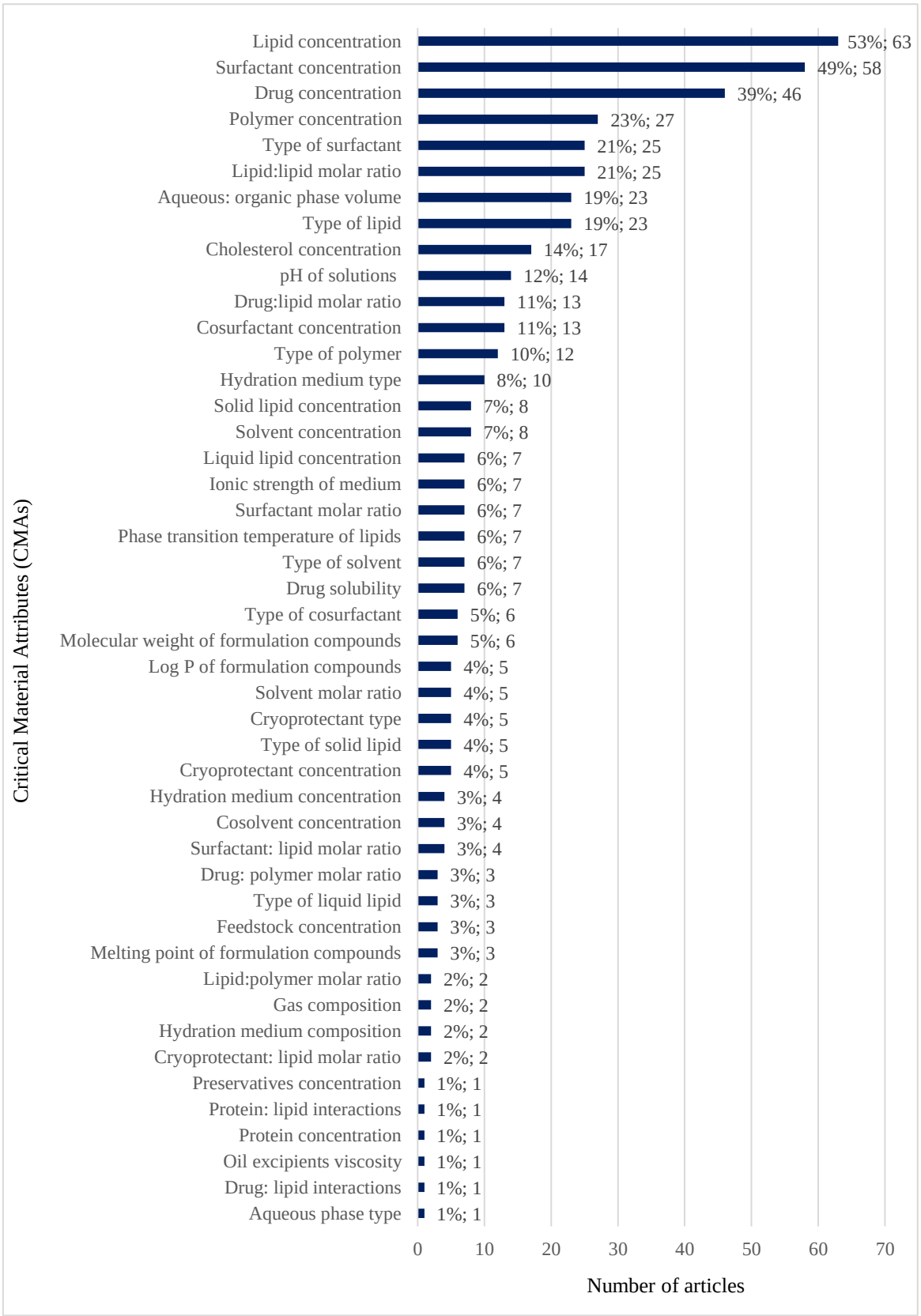

Figure 52. Critical Material Attributes (CMAs) identified in the bibliographic corpus.

Table 17. Specific CMAs identified for Lipid-based Nanosystems.

Specific CMAs of Lipid-based Nanosystems	Justification	Reference
Aqueous: organic phase volume	Maintaining the optimum aqueous: organic phase ratio is critical to obtain the desired CQAs, as the particle size and %EE. An increase in the aqueous: organic phase ratio results in a decrease in the particle size by modifying the internal volume, which in turn affects the %EE due to the lesser drug entrapment.	[155,477,487,492,522,529,531,536,539,540,544–546,548,552,562,567,568,570,574,578,582,583]
Aqueous phase type	The type of aqueous phase has an impact on the physicochemical properties and stability of drug products. According to *Dordevic et al*, the type of aqueous phase revealed a positive influence on the particle size distribution of nanoemulsions, e.g., the aqueous phase containing PBS (phosphate-buffered saline) leads to a smaller droplet size, while the larger droplet sizes were found to correlate with aqueous phase with SOS (double-distilled water containing sodium oleate). On the other hand, the nanoemulsions prepared with an aqueous phase (SOS) are more robust and stable, compared to the aqueous phase (PBS).	[511]
Cholesterol concentration	The incorporation of cholesterol in the formulation plays a strategic role in the rigidity, membrane elasticity, mechanical strength, and stabilization of the lipid bilayers. This is important for formulations with phospholipids (e.g. liposomes) that have a phase transition temperature close to the physiological temperature (37°C), which can lead to early drug release. Cholesterol decreases the fluidity and permeability of the bilayers and increases the packing density of phospholipids as a function of the proportion included, which increases drug retention and %EE. On the other hand, it also allows an extended circulation time by an increase in the drug product stability in biological fluids.	[13,155,157,415,476,478,479,482,483,485,486,489,491,492,496,579–581]
Cryoprotectant type/ concentration Cryoprotectant to lipid molar ratio	The type and concentration of cryoprotectants have a great influence on particle size, polydispersity index, stability, and drug leakage of products that are lyophilized (e.g. liposomes). A suitable concentration of cryoprotectant maintains the membrane integrity and protects against aggregation and fusion.	[483,484,489,521,522,529,568]
Drug concentration	The drug concentration affects the particle size, drug loading capacity, and %EE of drug products. The value of %EE increases with higher drug concentration but only up to a certain limit, due to the drug-lipid interaction. Likewise, the particle size increases with higher drug concentration, as well as, the drug loading capacity.	[155,391,415,475,477,479,480,482,483,486,487,489–492,495,501,502,518,522,523,526–529,531,533,541,543–549,553–557,559,561,562,565,567,568,571,574,586]
Drug to lipid molar ratio/ interactions	The drug to lipid molar ratio has an impact on the particle size, polydispersity index, drug loading capacity, %EE, and drug release, which in turn influences the pharmacokinetic and pharmacodynamic properties, bioavailability, and the efficacy of the drug product. If there is no drug-lipid interaction, the %EE only depends on the internal: external volume ratio, regardless	[391,478,481,482,485,489,493,519,521,533,548,554,564,586]

Specific CMAs of Lipid-based Nanosystems	Justification	Reference
	of drug concentration. Conversely, if there are drug-lipid interactions, a portion of free drug concentration in the medium will be linked to the surface of the nanosystem and leading to an increase in %EE.	
Drug to polymer molar ratio	According to *Sawant et al*, the drug: polymer molar ratio impacts the size of the polymeric nanoparticles and %EE. The increase of particle size is related to the increase of the viscosity of the organic phase due to the increase of polymer concentration. This observation is well correlated to the literature *Shirmard et al.* On the other hand, the study of *Saadat et al* (synthesis and optimization of a novel polymeric micelles), showed that increasing the drug to polymer ratio led to a decrease in %EE due to the limited capacity of the micelle cargo for drug loading.	[536,572,577]
Drug polymorphism	Different polymorphic forms of drug substances have a different impact on the physicochemical properties of the drug product such as drug release, drug solubility, chemical, and physical stability of the drug product.	[10,284]
Drug solubility	Drug solubility inside the vesicles may have an important impact on particle size and drug release from the drug product. Precipitated forms of DS have a limited solubility, which leads to a slower drug release when compared with amorphous forms or drugs dissolved that lead to a faster drug release profile. On the other hand, the hydrophilicity or lipophilicity properties of drugs have a significant influence on the drug release rate, due to their different diffusion capacity across the biological membranes.	[476,482,501,527,554,562,568]
Feedstock concentration	The feedstock concentration used during specific manufacturing process (e.g. spray-drying, solvent displacement process) influences the particle size, internal volume, and %EE. An increase in the feedstock concentration correlated to the higher particle size. According *Ingvarsson et al*, the '*positive effect of the feedstock concentration is a result of the increased solid content in each droplet generated during atomization, eventually resulting in an increased particle size'*.	[494,521,545]
Gas composition	The gas composition corresponds to a critical air parameter that needs to be controlled in the spray drying process.	[494,521]
Ionic strength of medium	The ionic strength of the medium used in specific manufacturing process, like ethanol injection or thin lipid film, has an impact on the zeta potential and %EE. A change in the ionic strength of the medium may lead to a shift in zeta potential which affects the drug encapsulation. For example, the orientation of polar groups of zwitterionic-type phospholipids depend on the ionic strength. The negative surface charge occurs in low-ionic strength conditions, whereas the positive surface charge arises in high-ionic strength conditions.	[155,486,489,495,496,504,578]
Lipid concentration	The lipid concentration impacts the particle size, polydispersity index, zeta potential, and %EE. Higher lipid concentration increased the number of vesicles formed, the internal volume for drug encapsulation, which consequently leads to an increase in the particle size and EE%. However, above a certain lipid concentration, a plateau is reached due to a significant increase in sample viscosity. On the other hand, the concentration of charged lipids has an impact on the zeta potential and stability of the drug product. Positively charged lipids play an important role in zeta potential and the formulation stability because the electrostatic repulsive forces prevent particle aggregation, lipid fusion, and drug leakage.	[154,155,157,415,475–480,482,483,486,487,489,491,492,495,496,499–502,505–510,512–517,519,521,524–526,533,539,549,551,552,554–559,561,566–568,576,580,582,584–587]

Specific CMAs of Lipid-based Nanosystems	Justification	Reference
Lipid origin	For naturally-sourced lipid mixtures, it is necessary to provide the lipid composition as a range of percentages for each stated lipid present in the mixture and its fatty acid composition. It is important to ensure the removal and inactivation of infectious agents, viruses, or animal proteins that may affect the quality and safety. For synthetic or semi-synthetic lipids, it is necessary to specify the percentage of each lipid and fatty acid, positional specificity of acyl side chains, and degree of fatty acid unsaturation. Thus, the lipid origin can affect the quality, safety, and performance of the drug product.	[603]
Lipid to lipid molar ratio	The lipid to lipid molar ratio can have an impact on particle size, drug release, and, mainly, in %EE. The increase in the amount of lipids leads to the formation of numerous vesicles with higher internal volume for drug encapsulation that consequently increases the % EE. For example, an increase in cholesterol may be associated with an increase in the particle size, rigidity of membrane, drug retention, and stability of the formulation. This is designated by 'pocket theory' on which the cholesterol promotes the drug encapsulation in pockets formed in the membrane bilayer.	[154,157,476,477,479–481,486,490,492,493,498,525,532,546,553,561,562,566–568,570,571,583,586]
Lipid to polymer molar ratio	Lipid to polymer molar ratio has a significant impact on particle size, polydispersity index, zeta potential, and stability. This effect depends on the type of polymer and lipids selected for the formulation composition, and the respective ratio between them. Particularly, this CMA applies to the polymeric nanoparticles.	[525,532]
Log P of formulation components	The partition coefficient (log P) of drug substance and excipients (emulsifying agent, preservatives, antioxidants) has a large influence on the stability of the emulsion, permeation properties, and drug release.	[476,482,501,518,568]
Hydration medium concentration/ composition/ type	The selection of the type of medium, composition, and concentration used in specific manufacturing process, like thin lipid film, is crucial for each type of formulation due to the impact in osmolality, stability, morphology, pH, drug loading, and size distribution.	[155,415,476,478,479,483,486,489,495,496,498,582,586]
Melting point of the formulation components	The melting point of each component of the formulation (e.g. drug substance, lipids, emulsifying agent) can play an important role in viscosity, stability, and *in vitro* drug release. The melting point of each component will influence the phase transition temperature of the formulation.	[482,501,568]
Molecular weight of formulation compounds	According to *Simões et al*, the molecular weight of drug substances is recognized a CMA due to their role on the percutaneous permeation of topical formulations. The molecular weight of other formulation compounds (e.g. molecular weight of polymers) is also considered a CMA.	[482,492,501,544,568,575]
Oil excipients viscosity	The viscosity of oil excipients used in formulation can have an impact on particle size, viscosity, stability, drug release, permeation properties, and content uniformity of the final product.	[501]
pH of solutions used in manufacturing process	The pH of the different solutions used in different steps of the manufacturing process can have a definitive impact on certain CQAs. For pH-gradient loaded nanosystems, the pH and composition of the aqueous phase inside and outside of the system are critical for the drug loading, which has a direct impact on drug release, in pharmacokinetics and pharmacodynamics, and therefore in the efficacy and safety profile of the drug product.	[155,475,478–480,486,487,489,492,495,496,504,568,578,603]

Specific CMAs of Lipid-based Nanosystems	Justification	Reference
Phase transition temperature of lipids	Some formulations have a characteristic phase transition temperature, the temperature at which the lipids that are in a gel state transition into a liquid-crystalline state. At higher temperatures, the lipid molecular mobility increases due to accelerated collision and coalescence rates. This can affect drug product stability and lead to drug diffusion and consequent drug leakage. This temperature is a decisive factor for the selection of the lipid composition of the drug products.	[25,26,30,36,39,44,46]
Polymer concentration	The polymer concentration, solubility, and molecular weight have an important impact on particle size, zeta potential, %EE and drug release. The coating with polymers corresponds to one more layer in the formulation, which is correlated with an increase in the particle size. It can also influence drug release and biodistribution. According to *Cunha et al*, the increase in PEGylating agent concentration in NLC formulations decreased zeta potential and increased %EE.	[391,483,491,492,518,520,522,523,525,527–532,534,537,540–545,553,569,573–575,585]
Preservatives concentration	The preservative content and antimicrobial effectiveness are critical material attributes typically included in the drug product specification. The concentration used has to be justified in terms of efficacy and safety and should be a minimum acceptable limit that gives the required level of efficacy the drug product shelf-life.	[501]
Protein concentration Protein: lipid interactions	The protein-lipid interaction is related to a 'pocket theory' of the lipid bilayer, which consists of pockets generated in between the cholesterol molecules that favor the interactions with proteins. The size of pockets depends on the cholesterol content (lower cholesterol content leads to larger pockets), therefore it requires an optimization of the size of the pocket according to the size of proteins. This is particularly important for the ability of liposomes to effectively deliver protein/enzyme in targeted cancer therapy. One example of this was been described by *Xu et al* for liposomal protein therapeutics using superoxide dismutase protein.	[496]
Solvent/ cosolvent concentration Solvent molar ratio	The solvent and cosolvent concentration has a great influence on the particle size, polydispersity index, zeta potential, %EE, and loading capacity. For example, the study of *Dawoud et al*, demonstrated that the EE% of insulin-loaded liposomes increased as the organic solvent volumetric ratio (chloroform: methanol) decreased. Another study by *Patel et al*, defines that the solvent composition affect the proliposome quality, since the factors like volume of chloroform or volume of methanol, were associated with low and medium risk for the defined CQAs (vesicle size, %EE, drug release).	[479,482,487,491,494,495,505,506,508,509,523,530,570,572,576,582,584]
Surfactant/ cosurfactant concentration Surfactant molar ratio	The surfactant/ cosurfactant concentration can have a significant impact on the particle size, polydispersity index, %EE, and stability. A higher surfactant/cosurfactant concentration leads to a decrease in particle size and polydispersity index, which might be due to a decrease in the interfacial tension between the lipid and aqueous phase, facilitating the particle partition and preventing particle agglomeration. Also, the increase in surfactant/cosurfactant concentration is related to an increment in encapsulation efficiency and drug release. This can occur due to the decrease in the particle size that results in a significant increase in the surface area.	[157,485,489,491,499–501,503,505–512,515–518,523,524,527–532,534,536,539,541,543,544,546,547,549,551–555,557–559,561–564,566–568,570–572,576,580,581,585]

Specific CMAs of Lipid-based Nanosystems	Justification	Reference
	On the other hand, it also was verified a high stability of formulations with surfactants and aggregates in formulations without stabilizers.	
Surfactant: lipid molar ratio	The surfactant: lipid molar ratio significantly influences the particle size, polydispersity index, zeta potential, lamellarity, morphology, and stability. This parameter is related to the surfactant critical micellar concentration and the distribution coefficients on the lipid bilayer or aqueous phase.	[487,498,556,587]
Type of lipid	The type of lipid selected for the formulation has an important impact on particle size and %EE, due to the differences in lipid chain length that may influence lipid bilayer properties such as bilayer thickness, elasticity, fluidity, and permeability. Thus, the different types of lipids may have a different impact on particle size. Longer lipid chain length is normally related to a higher lipid phase transition temperature that has an impact on stability and drug release. On the other hand, unsaturated lipids are susceptible to degradation reactions such as oxidation or hydrolysis and can promote the formation of pockets in the lipid bilayer that allow the encapsulation of lipophilic drugs. In the specific case of SLN or NLC, the type and concentration of liquid lipid and solid lipid has a huge impact on particle size, %EE, loading capacity, and drug release. The liquid lipids present a better capacity of drug solubility than solid lipids, which leads to a decrease in drug retention capacity. On the other hand, the incorporation of solid lipids produces a less-ordered matrix with a disorganized crystalline structure and imperfections that provide an enhanced drug loading capacity and %EE. As a result of that, solid lipid matrices offer the possibility of an extended and controlled drug release due to the limited diffusion. These types of complex drug products are appropriate for transdermal administration due to the small size and easy penetration into the skin.	[25,26,30,33,36,40,42,44–46,50,54,55,62,69,71,97,104–106,118,132,135]
Type of polymer	The type of polymer used for coating and surface modification can have a great impact on particle size, biodistribution, and drug release. The physical and chemical properties of polymers must be evaluated, such as solubility, molecular weight, and density.	[518,520,521,527,529,530,534,540,544,575,577,585]
Type of solvent	The type of solvent selected for each formulation can influence their critical attributes. According to *Sousa et al*, the type of solvent used in the film hydration method is depicted as a factor potentially affecting the attributes of the polymeric micelles. Another study by *Pallagi et al*, identified the type of the organic solvent used in liposome preparation by film hydration method, as a CMA in the risk assessment analysis.	[476,479,529,544,545,574,578]
Type of surfactant/cosurfactant	The type of surfactant selected has a significant effect on particle size, %EE, and stability of drug products. This occurs due to the difference in the hydrophilic-lipophilic balance (HLB) of each surfactant. For example, the increase in the HLB value results in lower surface interfacial tension, which leads to the formation of more stable nanoemulsions. Hence, the selection of suitable surfactant and cosurfactant is important to generate a uniform size distribution, avoid drug precipitation, and delay the vesicle flocculation or coalescence.	[500,501,503–505,512,518,521,527,529,530,534,544,546,554,555,561–563,568,570,579,581,585,587]

3.7. Critical Process Parameters (CPPs) of Lipid-Based Nanosystems

The Critical Process Parameters identified in the bibliographic corpus are detailed in Figure 53. The most common CPPs are: temperature of the different steps of the manufacturing process (n=50, 42%); stirring speed (n=36, 31%), time (n=19, 16%) and type (n=10, 8%); sonication time (n=27, 23%), amplitude (n=10, 8%), speed (n=8, 7%) and type (n=2, 2%); number of cycles (n=19, 16%); and pressure (n=24, 20%). In Figure 53 it is possible to find the CPPs that were identified in the bibliographic corpus, just as the rationale for their classification as critical.

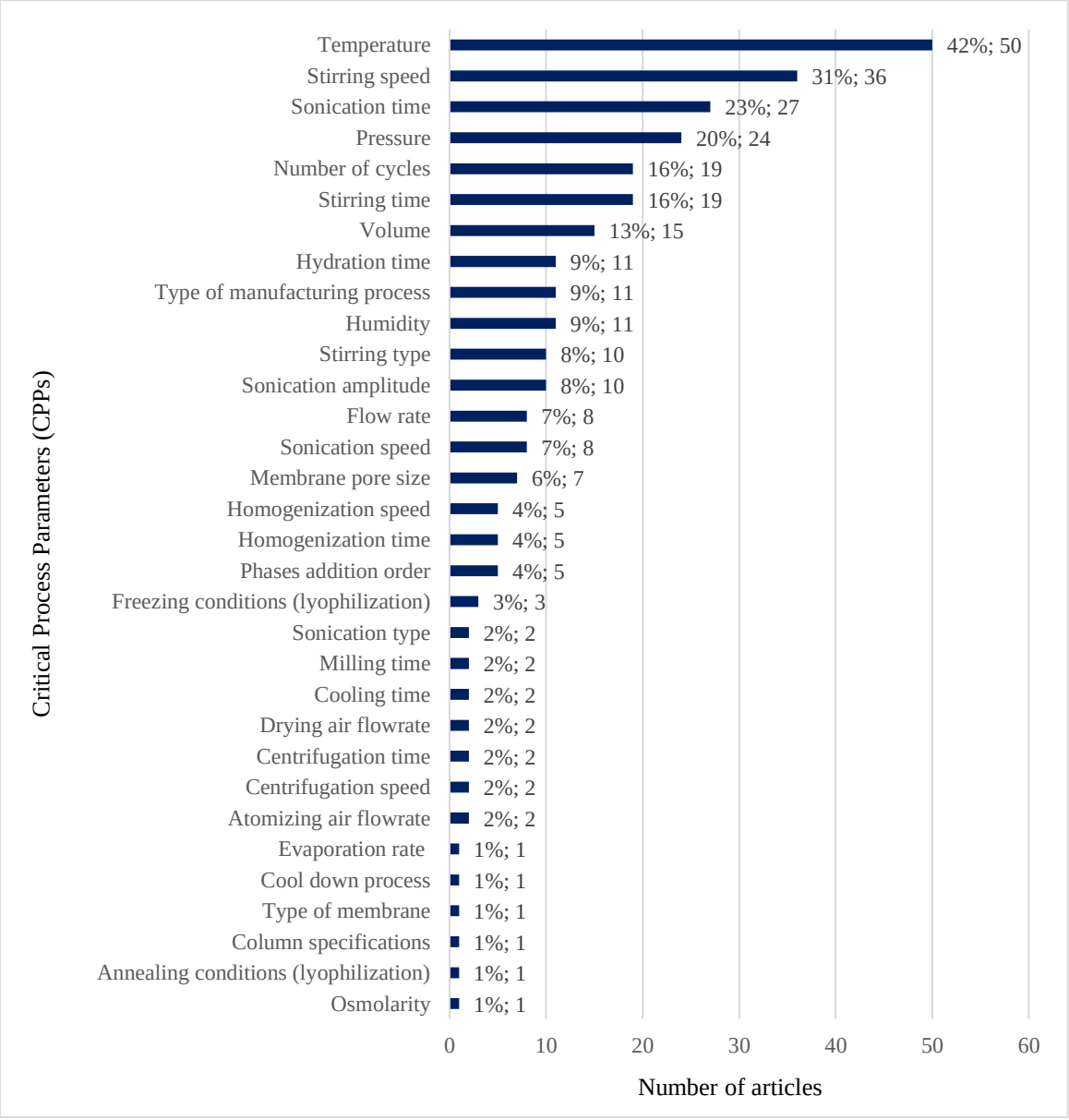

Figure 53. Critical Process Parameters (CPPs) identified in the bibliographic corpus.

Table 18. Specific CPPs identified for Lipid-based Nanosystems.

Specific CPPs of Lipid-based Nanosystems	Justification	References
Annealing conditions (lyophilization)	The annealing conditions (temperature and time) during lyophilization influence the thermal behavior of the formulation, due to maximize crystallization of the bulking agents.	[484]
Atomizing air flowrate during spray drying	The atomizing air flowrate has a great influence on the particle size. The high energy applied to atomize leads to an increase of dispersion for drying, which hence results in a decrease in particle size.	[494,521]
Centrifugation time/ speed	The scientific articles of *Vardhan et al* and *Pallagi et al* (development of polymeric nanoparticles), include in the risk analysis the centrifugation time and speed as a CPP, with potential impact on particle size, polydispersity index, zeta potential, %EE, and drug loading.	[529,530]
Column specifications (type, dimension)	The column specifications were identified as CPP in the application of QbD-based development and validation of an HPLC (High-Performance Liquid Chromatography) method for drug quantification (analytical development).	[578]
Cool down process/ Cooling time	The cooling process, time, and temperature mainly affect the particle size, polydispersity index, stability, %EE, and loading capacity. Three scientific articles related to the development of NLCs (*Kang et al*, *Cavalcanti et al*, and *Beg et al*), include the cooling conditions as CPPs in risk assessment analysis, with impact on particle size, polydispersity index, encapsulation efficiency, and loading capacity.	[561,567,568]
Drying air flowrate during spray drying	The drying air flowrate used during the spray drying technique has an impact on particle size and polydispersity index of products. It has been demonstrated that an increase in the drying air flowrate may decrease particle size by improving the efficiency of particle separation inside the container.	[494,521]
Evaporation rate	The time, temperature, and flowrate of solvent evaporation can impact on particle size, polydispersity index, stability, morphology, and residual solvents/degradation products. According to a research article by *Yadav et al*, the evaporation rate is a critical process parameter in the modified solvent diffusion-evaporation method employed in the preparation of phospholipid nanoparticles.	[548]
Flowrate	The feed flowrate has an important influence on morphology, particle size, polydispersity index, drug loading, residual solvents, and degradation products. For example, the feed flowrate used during spray-drying influences the particle size and polydispersity index, due to an increase of the kinetic energy and dispersion. On the other hand, the feed flowrate used during tangential flow filtration (TFF) has a great influence on the residual solvents, assays, molar ratios, degradation products, and particle size. Thus, depending on the process used, it is very important the control this CPP.	[53,71,77,86,88,95,123,128]
Freezing conditions (lyophilization)	The time of freezing and drying cycles has an impact on particle size, polydispersity index, lamellarity, physical stability (aggregation and collapse, drug leakage), and chemical stability (lipid degradation and hydrolysis) of freeze-dried materials.	[483,484,529]

	After lyophilization, an increase in particle size can suggest a possible aggregation of particles, and the decrease in zeta potential can indicate a weaker electrostatic repulsion, with consequent fusion of particles.	
Homogenization time/ speed	Homogenization time and speed are PP that can affect the particle size, polydispersity index, %EE, zeta potential, and physical stability (coalescence or phase separation). For example, in a scientific article of *Beg et al* (development of NLCs) the homogenization time and speed are present in FMEA, with impact in CQAs as particle size and %EE. On the other hand, the scientific article of *Yerlikaya et al*, demonstrates that the increase in homogenization rates, decreases the particle size of paclitaxel nanoparticles, mainly due to the higher efficiency of shearing rates in the rupture of larger into smaller droplets.	[530,534,544,568,574]
Humidity	Some environmental variables can have a great impact during the product development phase. For example, it is required a humidity-controlled working room to control the moisture content of the spray-dried products, which influences the product stability due to the impact in the glass transition temperature (Tg) of the system, and the molecular mobility in the final product. This is discussed by *Ingvarsson et al*, where it has been described that the plasticizer effect of water can reduce Tg, increase molecular mobility of the system, and lead to the fusion of spray-dried liposomes.	[494,501,512,518,521,527,546,563,567,578,585]
Hydration time	The time of hydration in the thin lipid film technique has a significant impact on particle size, polydispersity index, %EE, and stability because it is during film hydration that the formation of the phospholipid bilayer takes place. Dependent on the formulation, a suitable hydration time can lead to an increase and optimization of %EE and optimal stability (no aggregation).	[155,415,476,478,479,481,486,489,491,495,496]
Membrane pore size Type of membrane	The particle size, polydispersity index, and lamellarity can be controlled by the pore size of the membrane used in different operating units of the manufacturing process (e.g. extrusion, tangential flow filtration, sterile filtration). For example, in the extrusion process, the use of a smaller pore size leads to a decrease in particle size, polydispersity index, as well as, lamellarity. Also, these attributes can be controlled by the membrane stacking during extrusion. The type of membrane depends essentially on the process employed and can also impact the morphology, lamellarity, and particle size. For example, in sterile filtration techniques or tangential flow filtration, it is important to verify the compatibility between membranes and each type of formulation, to prevent problems like leakage or clogging. Thus, the pore size, type, and number of membranes need to be optimized in accordance with the drug product desired.	[155,476,483,486,489,496,503]
Milling time	The milling time mainly affects the mean particle size and zeta potential. Usually, a high milling time is associated with a decrease in mean particle size and an increase in zeta potential. It was described by *Patel et al* that the zeta potential increase due to the greater mobility of the particles and adsorption of steric and electrostatic stabilizers.	[527,543]
Number of cycles	The number of cycles used for extrusion or during high-pressure homogenization affects the morphology, lamellarity, particle size, polydispersity index, degradation products, and stability. On the other hand, the increase in the number of cycles at high temperatures during freeze-thaw (lyophilization process) can lead to drug leakage and, consequently, to a lower %EE. Thus, it is very important the control this CPP to get the desired product characteristics.	[155,415,476,478,483,486,489,491,492,496,501,503,504,507,518,542,557,563,565]

Osmolarity	The osmolarity is an environmental process parameter that must be monitored during liposome production, to prevent rupture or contraction of the liposome structure and loss of formulation stability.	[492]
Phases addition order	The order of addition of the different components of the formulation has a significant impact on stability, content uniformity, and %EE. This is particularly important in the internal and external phase addition order in water-in-oil emulsions (w/o) and oil-in-water emulsions (o/w).	[482,501,555,563,568]
Pressure	Pressure is necessary in different steps of the manufacturing process of these products (e.g. extrusion, high-pressure homogenization, lyophilization, tangential flow filtration). The pressure used may have a significant impact on morphology, lamellarity, particle size, %EE, and physical stability (drug leakage and aggregation). Higher pressures narrow down the particle size/lamellarity and lower polydispersity due to the shear forces and the applied cavitation and disruption in vesicles. The pressure used during lyophilization is important to ensure an effective stabilization of freeze-dried materials. On the other hand, the pressure of the backpressure valve in tangential flow filtration has a great influence on the drug assay, molar ratios, degradation products, and particle size distribution.	[155,415,476,478,482,483,486,489,492,496,501,503,504,507,512,518,538,544,557,563,565,568,574,578]
Sonication amplitude	Particle size, PDI, and entrapment efficiency parameters are significantly affected by the amplitude of sonication. Higher sonication amplitudes lead to higher dispersions in the formulation due to increased kinetic energy during sonication, which induces a decrease in particle size. On the other hand, the %EE decreases with the increase in sonication amplitude, due to probable leakage of the drug from the lipid bilayers because of repetitive sonication cycles.	[481,488,489,534,542,546,547,551,562,567]
Sonication speed	The sonication speed has a large impact on particle size, polydispersity index, internal volume, %EE, and loading capacity. For example, a higher sonication speed can be used to reduce the particle size.	[155,492,496,516,529,531,534,582]
Sonication time	The sonication time has a significant impact on particle size and %EE. The sonication process leads to a decrease in particle size and an increase in the total internal volume, which hence influences the %EE.	[155,415,478,479,481,487–490,492,496,521,522,524,529–531,534,546,551,553,562–564,566,567,582]
Sonication type	The sonication type has a great influence on particle size, polydispersity index, and %EE. For example, it was demonstrated that the sonotrode (probe sonicator) leads to a smaller particle size in a short period of time, compared to the water bath sonication.	[495,582]
Stirring speed	Stirring is necessary in different steps of the manufacturing process of these products (e.g. ethanol injection, homogenization, emulsification). The stirring speed has an important influence on morphology, particle size, polydispersity index, drug loading, and %EE. Lower stirring speeds tend to lead to larger particle sizes. Conversely, higher stirring speeds are selected when the aim is to obtain more homogeneous formulations with smaller particle size and polydispersity index, preventing agglomeration and sedimentation. It also promotes an increase in drug encapsulation due to an increase in the surface area of vesicles.	[477,481–483,486,487,492,496,500,501,505,506,509,512,527,530,536,541,545–548,550,551,554–556,561–563,567,568,570,574,582,585]

Stirring time	The particle size, polydispersity index, %EE, and stability of formulations change dramatically with different stirring times. Particle size and polydispersity index decrease with the greatest stirring time, through the kinetic energy applied. However, the longer stirring times can lead to instability of structures, disruption, aggregation, and formation of larger particles. The %EE can also decrease if the stirring times are too long, due to the disintegration and escape of drug encapsulated. The research paper of *Khurana et al*, suggested that stirring speed and stirring time were associated with medium risk, affecting the CQAs of self-nanoemulsifying lipidic nanomicellar formulations.	[477,487,492,500,501,505,506,509,512,530,536,551,554,556,563,570,574,582,585]
Stirring type	The stirring type has an important influence on particle size, polydispersity index, %EE, and stability of drug products. This effect depends on the type of manufacturing process selected for the development of a specific drug product. In the scientific article of *Panigrahi et al*., the stirring type is present as a CPP in the design matrix for factor screening as per Taguchi design with high level and low levels of various CMAs and CPPs.	[500,501,505,506,509,512,563,568,582,585]
Temperature	The control of temperature during the manufacturing process plays an important role in the pharmaceutical development of drug products. The temperature selected in each step of the manufacturing process depends, among other things, on the operation unit in question (e.g. ethanol injection, hydration step on the thin lipid film technique, extrusion, high-pressure homogenization (HPH), emulsification, lyophilization, spray drying, etc) and the intended result, because it can greatly affect some CQAs such as morphology, particle size, polydispersity index, drug loading, degradation products and stability. For example in the case of liposomes, very often it is used a process temperature above phase transition temperature during drug loading, to increase the fluidity of the membrane and to increase %EE. On the other hand, the temperature of the HPH method had the largest influence on particle size and polydispersity index of nanoemulsions. The hot HPH allows a smaller particle size and polydispersity index compared to cold HPH, due to the higher energy input during homogenization, the decrease in viscosity as well as in the interfacial tension. Also, freezing and drying temperatures can have an impact on the physical stability (aggregation, collapse, drug leakage) and chemical stability (lipid degradation and hydrolysis) of freeze-dried materials. This temperature also affects the ice nucleation rate, crystal growth, and morphology of the final dry product.	[155,476,478–483,486–490,492,494–496,500,501,505,506,509,511,512,516,518,521,527,529,536,538,544,546,550,554–557,561–563,567,568,570,573,574,577,578,582,585]
Type of manufacturing process	The type of manufacturing process has a pronounced effect on the bulk characteristics of each type of complex drug products, in particular on the particle size distribution, morphology, lamellarity, stability, %EE, loading capacity, among others. Thus, it is crucial the selection of the most appropriate manufacturing process for each formulation in particular. For example, the film hydration technique mainly leads to the formation of multilamellar structure and particle size in the micrometer range, while the ethanol injection allows to obtain particles with a size in the nanometer range. Size-reduction techniques such as sonication, freeze-thaw cycling, or extrusion have a significant impact on particle size distribution, internal volume, and %EE. In all of them, it is necessary to optimize process conditions (number of cycles, temperature, pressure, speed), to get the desired particle size distribution.	[486,489,492,493,511,512,518,521,551,568,582]

Volume	Film rehydration fluid volume in the thin lipid film technique has a significant impact on particle size, polydispersity index, and %EE. Usually, the increasing hydration volume resulted in larger vesicle formation, variations in internal volume, polydispersity index, and %EE.	[155,478,481,482,484,489,491,492,495,496,527,546,567,574,578]

3.8. Risk Assessment Analysis

According to the ICH Q9, the risk assessment is defined as *'a systematic process of organizing information to support a risk decision to be made within a risk management process'* [396]. Thus, the risk assessment analysis is a valuable science-based process used in quality risk management that allows identifying and organizing the critical material attributes and process parameters that potentially have an impact on the CQAs of the product [395,396].

The purpose of this section is to provide a general overview of risk assessment tools used in quality risk management that were identified in the bibliographic corpus, such as: Ishikawa diagram (n=39, 33%), Risk Estimation Matrix (REM) (n=13, 11%), Failure Mode and Effect Analysis (FMEA) (n=2, 2%), Failure Mode and Effect Critically Analysis (FMECA) (n=5, 4%), and other supporting statistical tools (n=104, 88%) (Figure 54).

The Ishikawa diagram, also known as Fishbone or Cause and Effect diagram, is a basic risk assessment facilitation method able to identify the potential risk factors and corresponding causes assessed during formulation development. It is established the relation between the different process/formulation variables and the characteristics affected by them, which allows for identifying and list of the potential CQAs of the product [395,505,506,512,546,578,585]. This diagram can be elaborated for each potential CQA individually, or for all potential product CQAs at the same time [396,614].

The application of other risk assessment tools with the ability to provide qualitative and quantitative information regarding risk levels is essential to complement the Ishikawa analysis and prioritize risks. Thus, in some cases, this tool has been combined with REM, which is used to rank the parameters that may affect quality attributes and thus should be studied during product development [482,505,506,512,551]. The REM is a simple tool that allows to evaluate, prioritize, select and construct the risk estimation matrix for identifying the potential risk(s) or failure mode(s) of process and product performance, considering the probability of the risk and the severity of the associated impact [396,482,505,506,512,551,614]. On the other hand, the FMECA quantitatively ranks the variables according to their probability of occurrence, severity, detectability, and criticality [396,614].

The number of articles using risk assessment tools like Ishikawa diagram (n=39, 33%), REM (n=13, 11%), FMEA (n=2, 2%), FMECA (n=5, 4%) is quite small, compared to the total number of articles of the bibliographic corpus (Figure 54). Despite that, we cannot entirely exclude the possibility that risk assessment tools were used by the authors but the results were not published. The value of risk assessment is stressed out in ICH Q8(R2) [395] and in several review articles referring to QbD [155,156,415,476,482,492,494–496,501,505,506,508,509,512,546,549–565,567,570,571,579,581,582,585,586]. Nevertheless, lack of knowledge regarding risk assessment tools and their importance may also explain these findings.

In this literature analysis, it was also verified that the Ishikawa diagram had been described in 39 articles: in three of them it was used alone and in 36 it was used in combination with other tools. The combination of risk assessment tools significantly improves the amount and quality of information obtained from that risk assessment study and reduces the time and costs necessary for pharmaceutical development [482,505,506,512,551].

In addition, supporting statistical tools were identified in 104 articles (n=104, 88%), such as Pareto charts, histograms, statistical models, or control charts. These support tools are important to facilitate quality risk management, thus allowing an efficient data assessment and definition of their significance [396]. Subsequently, the critical variables identified during risk assessment should be investigated and optimized with experimental designs (DoE), as further described in the below section [395].

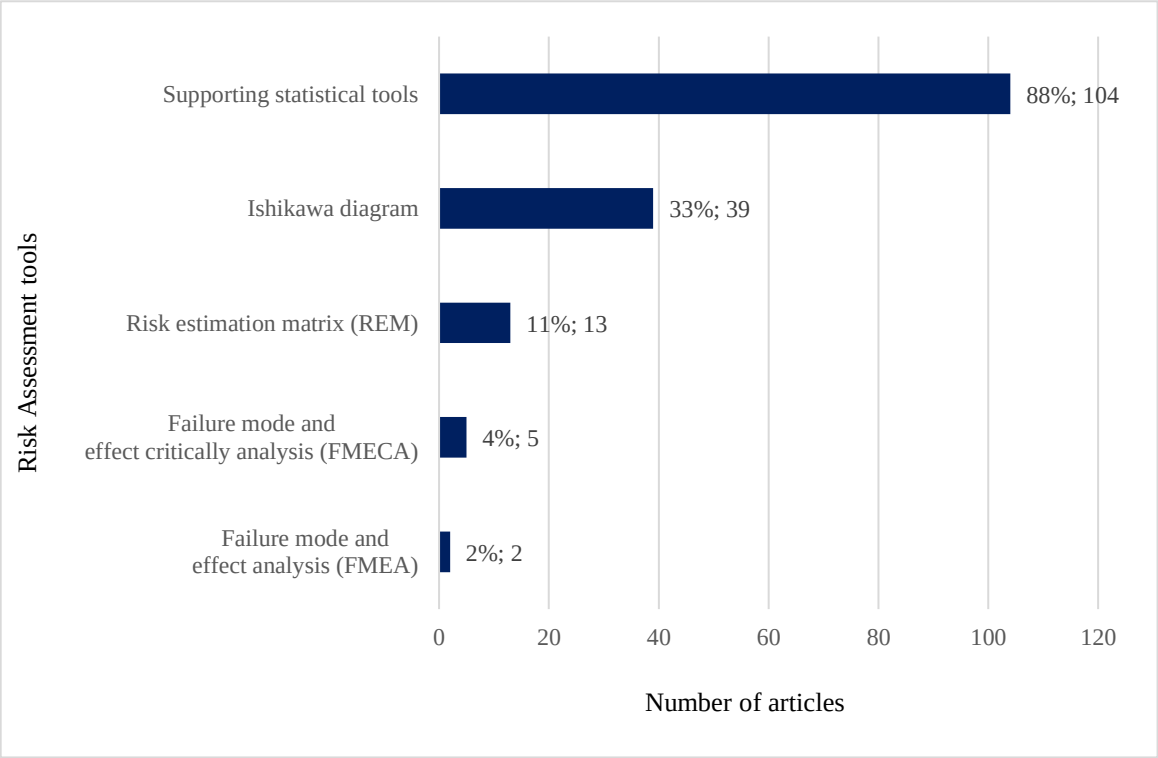

Figure 54. Risk assessment tools identified in the bibliographic corpus.

3.9. Design of Experiments (DoE)

One of the most critical issues in the application of DoE in the QbD approach is the choice of a suitable experimental design [403,615]. This choice depends on the objective of the study, the available resources, prior knowledge, investigator's experience, and sensitivity, problem complexity, number of factors, levels, and quantitative or qualitative nature of the factors being studied [616,617]. Three main DoE applications usually occur sequentially: **screening** to explore a large number of independent variables and identify which ones can be more influential and their appropriate ranges to reduce the number of variables being studied in the following experiments; **optimization** with the goal of finding optimum operating conditions and predict the response values taking into consideration the main independent variables identified in the screening phase; and **robustness** testing to determine if the product or process is robust to small variations in the independent variable levels [403,617].

Eight types of DoE studies were identified in this literature review (Figure 55). Full factorial design (n=30, 25%), Fractional factorial design (n=16, 14%), Placket-Burman design (n=13, 11%), and Taguchi design (n=7, 6%) are examples of screening designs, where a large number of independent variables were evaluated to identify the most critical MAs or PPs to be further studied during the next steps of development (Figure 55). Box-Behnken design (n=33, 28%), Central composite design (n=29, 25%), D-optimal mixture design (n=10, 8%) and I-optimal mixture design (n=3, 3%) are optimization DoEs, i.e., they are used to determine the optimal conditions of the critical factors and to define the design space (Figure 55).

It is worth noting that some authors followed the approach of first using a screening design and then an optimization design. The Full factorial design followed by central composite design (n=6), Plackett-Burman design followed by Box-Behnken design (n=3), and Taguchi design followed by central composite design (n=3) were the most commonly used combinations (Table 51).

The full factorial design allows the efficient use of the data which provides a clear evaluation of the independent variables and interaction effects. On the other hand, the number of experimental runs increases exponentially with the increase in the number of independent variables and their number of levels [494,525,542,546,567,617].

Fractional factorial designs are very often used for estimating the main and the interaction effects, performing only a particular subgroup of the full factorial experiments, when the number of parameters increases [616–618].

In addition, the Plackett-Burman design is widely used for estimating the main factors that cause product variability. This design allows the screening of a large number of factors with a relatively few number of experiments. The disadvantage of the Plackett-Burman design is that the interactions between variables are commonly mistaken with main effects [616,618,619].

On the other hand, the Taguchi design, often called orthogonal arrays allows determining the interaction and influence between the control variables and noise variables (factors that can only be controlled in the laboratory experiments) [616,618].

The Box-Behnken design is useful for product optimization since it uses a smaller number of experimental runs, i.e., which limits and simplifies the execution of the experiments as the number of independent variables that need to be studied increases [494,525,542,546,567,617].

Moreover, the Central composite designs are used for building response surfaces and are preferably selected due to an increase in the robustness of the model and the possibility to include several points (center and corner points) [616–618].

Optimal designs, such as D-optimal design and I-optimal design, are on the type of response-surface-oriented method, enabling to test of different datasets of samples until it is possible to define the design space where the products meet the target product profile. The main disadvantage of these designs is a long time needed for them to be completed [616].

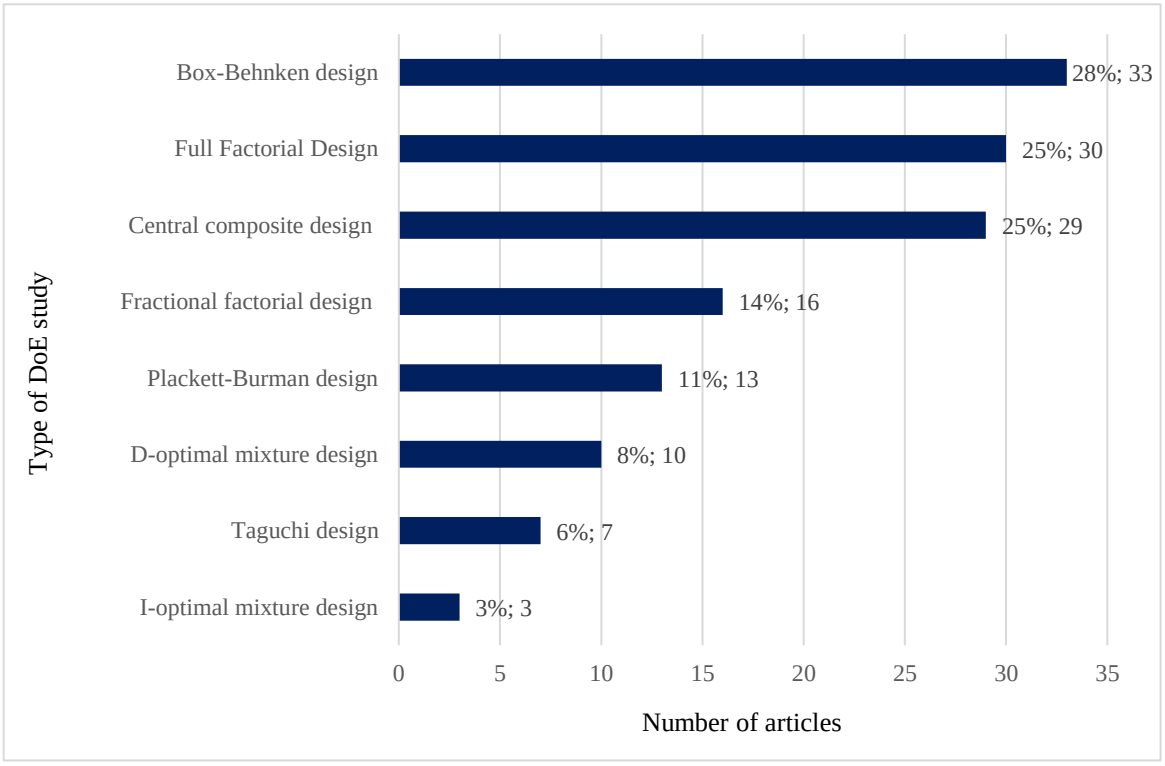

Figure 55. Experimental Design Methods identified in the bibliographic corpus.

3.10. Characterization Techniques

The analytical techniques play a crucial role in the comparability exercise between the reference products and their generics, through a comprehensive side-by-side analysis with the identification and justification of similarities in critical quality attributes, just as the potential differences. The development and validation of advanced and sophisticated analytical techniques, statistical methods, or predictive approaches is fundamental to guarantee suitable characterization of them [332–334]. On the other hand, should be implemented orthogonal and complementary analytical techniques to improve the consistency and accuracy of the results obtained [161].

The characterization techniques described in the bibliographic corpus are detailed in Figure 56. Dynamic Light Scattering (DLS) (n=103, 87%), High-Performance Liquid Chromatography (HPLC) (n=74, 63%), Transmission Electron Microscopy (TEM) (n= 58, 49%) and UV/Vis Spectrophotometry (n= 55, 47%) are the most used characterization techniques (Figure 56).

DLS, also known as photon correlation spectroscopy or quasi-elastic light scattering, is applied to the measurement of particle size, particle size distribution (polydispersity), and zeta potential (surface charge), which are three of the most important CQAs as discussed above. DLS is listed in the USP chapter <729> [340] as one of the non-imaging techniques available for the determination of particle size and, there are specific publications with guidance documents regarding the method development and acceptance criteria [620,621]. The principle of DLS is based on the particle diffusion coefficient determined by the relation between light intensity scattered as a function of time at a fixed angle, which is related to particle diameter [622–627]. This non-invasive technique enables the evaluation of size ranges from nanometers to micrometers and is considered a fast and reliable measurement for routine analysis [622–627]. On the other hand, because the DLS technique is used for providing the hydrodynamic diameter, it has some limitations in differentiating aggregates from false results due to ligands on the particle surface and does not provide the particle concentration [622–627].

The morphology of several lipid-based nanosystems is also considered a CQA [628–631]. Therefore, imaging techniques (e.g. transmission electron microscopy (TEM), scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), optical microscopy, atomic force microscopy (AFM)) with the capacity to provide structural information of nanoscaled systems are commonly used [628–631].

Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM), are powerful complementary techniques to non-imaging techniques for comparison and confirmation of the values measured [628–631]. In the articles analyzed, the TEM was used for assessing the morphology and lamellarity and allowed to corroborate DLS size and distribution results [154,475,481,497,499,500,502,506,507,509,510,512,514,523–525,532,533,539,543,546–548,550,551,554,556,559,561–563,565–567,570,579,581,582,585,586]. In images obtained by

TEM, the particle size determined was a little smaller than the particle size obtained through DLS. This difference is related to the fact that DLS measures the hydrodynamic diameter of particles [532,567]. The inherent differences across a range of analytical methods (e.g. DLS and TEM can give rise to significant variations in measurement endpoints of critical quality attributes. Therefore, the basis of each analytical technique should be described and justified in accordance with potential differences. On the other hand, SEM technique is performed when the aim is to investigate the morphology, topography, physical state and surface characteristics of the product [156,157,476,482,488,493,494,497,502,513,514,524,543,552,553,557,558,562,566,569–571,579]. The major limitations of TEM and SEM are the changes in shape or morphology that can be induced by the sample preparation. To overcome this limitation, cryo-transmission electron microscopy (cryo-TEM) was developed. Cryo-TEM enables a higher differentiation and particle resolution [628–631].

HPLC is an efficient, rapid, accurate and reproducible quantitative method used for the separation and quantification of the different formulations components (e.g. lipids and drug substances) with high resolution as well as for the determination of the encapsulation efficiency, drug loading capacity, or drug release profile [155,156,474,475,480–482,488,489,493,495–497,501,505–510,513–515,523–526,532,533,543,547–549,551,552,554–556,558–561,563,565,569–571,578,579,583,586].

Differential scanning calorimetry (DSC) and other spectroscopic techniques, like X-ray diffraction (XRD), are used to characterize the solid-state form of the materials (namely the drug substance), which can influence the stability, solubility, and drug release [632–634].

The different analytical techniques used for the characterization of the main CQAs are outlined in Table 19. The authors included not only the ones identified in the bibliographic corpus but also other techniques that we believe are also relevant for this purpose (Table 19).

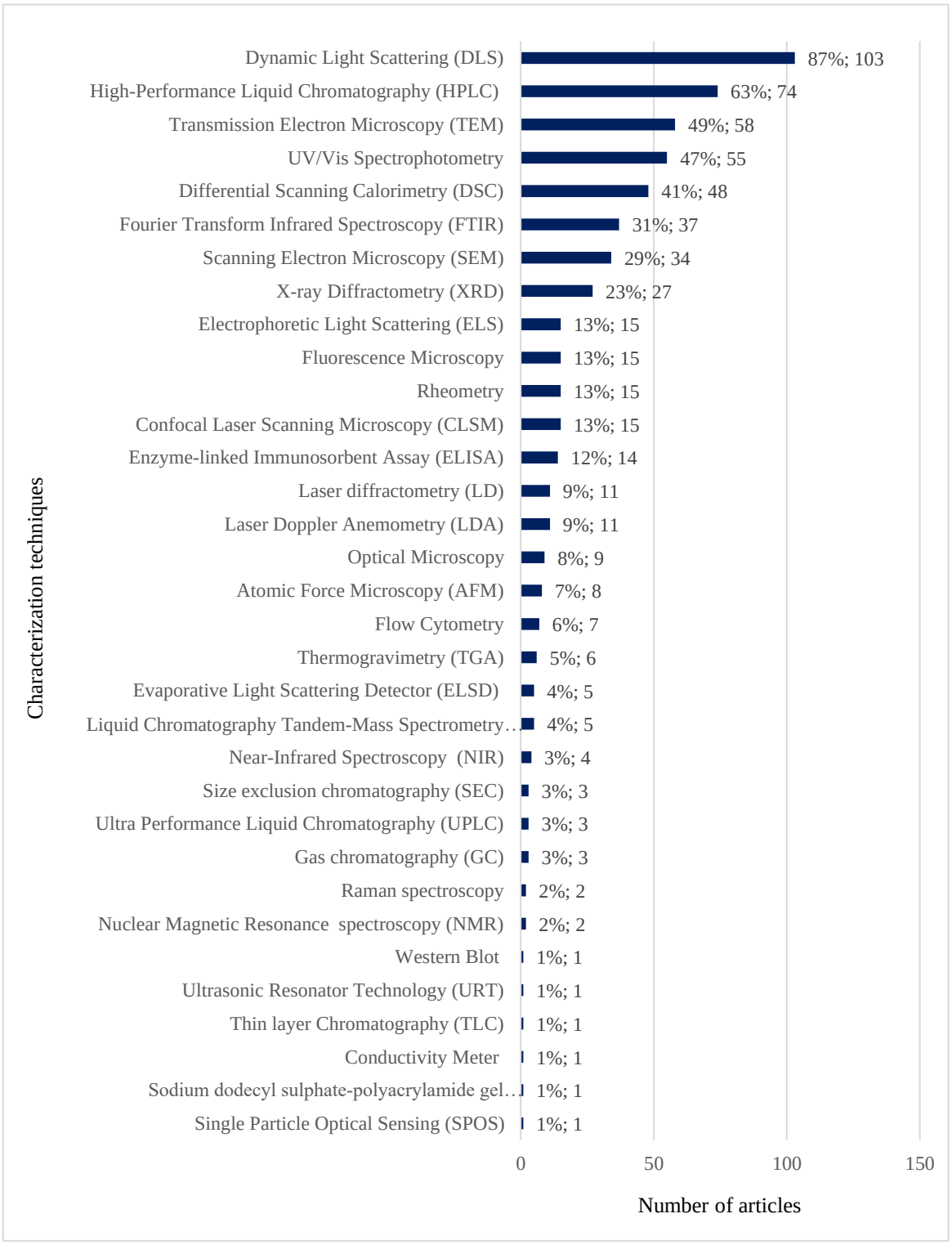

Figure 56. Characterization techniques identified in the bibliographic corpus.

Table 19. Resume table of CQAs and respective characterization techniques identified for Lipid-based Nanosystems [154,155,157,415,474–587].

CQAs	Analytical| Instrumental Techniques
Assay/Content Uniformity	Evaporative Light Scattering Detector (ELSD)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	UV/Vis Spectrophotometry
Cytotoxicity	Enzyme-Linked Immunosorbent Assay (ELISA)
Degradation Products/Impurity Profile	Evaporative Light Scattering Detector (ELSD)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	Raman Spectroscopy
Drug Release	Enzyme- or Protein-based Assays
	Evaporative Light Scattering Detector (ELSD)
	Fluorescence Spectrometry
	Gel Electrophoresis
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	UV/Vis Spectrophotometry
Elasticity	Size Exclusion Chromatography (SEC)
Encapsulation Efficiency (%EE)	Evaporative Light Scattering Detector (ELSD)
	Enzyme- or Protein-based Assays
	Fluorescence Spectrometry
	Gas Chromatography (GC)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	UV/Vis Spectrophotometry
Identification	Evaporative Light Scattering Detector (ELSD)
	Fourier Transform Infrared Spectroscopy (FTIR)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	Nuclear Magnetic Resonance Spectroscopy (NMR)
	Raman Spectroscopy
	Thin Layer Chromatography (TLC)
	UV/Vis Spectrophotometry
Internal Volume	Conductivity Meter
	Confocal Laser Scanning Microscopy (CLSM)
	Flow Cytometry
	Fluorescence Microscopy
	Freeze Fracture Microscopy
	Transmission Electron Microscopy (TEM)
Lamellarity	Atomic Force Microscopy (AFM)
	Cryo-Transmission Electron Microscopy (cryo-TEM)

	Confocal Laser Scanning Microscopy (CLSM)
	Freeze Fracture Microscopy
	Nuclear Magnetic Resonance Spectroscopy (NMR)
	Transmission Electron Microscopy (TEM)
	X-ray Diffractometry (XRD) Wide-Angle/Small-Angle X-ray Scattering (WAXD)/(SAXS)
Leachables/Extractables	Evaporative Light Scattering Detector (ELSD)
	Fourier Transform Infrared Spectroscopy (FTIR)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	Raman Spectroscopy
Leakage	Evaporative Light Scattering Detector (ELSD)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
Loading Capacity	Evaporative Light Scattering Detector (ELSD)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
Morphology	Atomic Force Microscopy (AFM)
	Cryo-Transmission Electron Microscopy (cryo-TEM)
	Confocal Laser Scanning Microscopy (CLSM)
	Extended X-ray Absorption Fine Structure (EXAFS)
	Fluorescence Microscopy
	Freeze Fracture Microscopy
	Optical Microscopy
	Polarized Light Microscopy (PLM)
	Scanning Electron Microscopy (SEM)
	Size exclusion chromatography (SEC)
	Transmission Electron Microscopy (TEM)
	X-ray diffractometry (XRD) Wide-Angle/Small-Angle X-ray Scattering (WAXD)/(SAXS)
Particle Size	Atomic Force Microscopy (AFM)
	Confocal Laser Scanning Microscopy (CLSM)
	Cryo-Transmission Electron Microscopy (cryo-TEM)
	Dynamic Light Scattering (DLS) (also known as Photon Correlation Spectroscopy (PCS))
	Electrophoretic Light Scattering (ELS)
	Field-Flow Fractionation (FFF)
	Flow Cytometry
	Fluorescence Microscopy
	Freeze Fracture Microscopy
	Laser Diffraction Spectroscopy
	Nanoparticle Tracking Analysis (NTA)
	Optical Microscopy
	Polarized Light Microscopy (PLM)
	Scanning Electron Microscopy (SEM)
	Single Particle Optical Sensing (SPOS)
	Size Exclusion Chromatography (SEC)
	Transmission Electron Microscopy (TEM)
Particulate Matter	Optical Microscopy
Physical State of DS	Differential Scanning Calorimetry (DSC)
	Nuclear Magnetic Resonance Spectroscopy (NMR)

	X-ray Diffractometry (XRD) Wide-Angle/Small-Angle X-ray Scattering (WAXD)/(SAXS)
Polydispersity Index	Atomic Force Microscopy (AFM)
	Dynamic Light Scattering (DLS) (also known as Photon Correlation Spectroscopy (PCS))
	Electrophoretic Light Scattering (ELS)
	Field-Flow Fractionation (FFF)
	Laser Diffraction Spectroscopy
Polymorphism (Phase behavior)	Differential Scanning Calorimetry (DSC)
	Fluorescence Probe Polarization
	Fourier Transform Infrared Spectroscopy (FTIR)
	Nuclear Magnetic Resonance Spectroscopy (NMR)
	Thermogravimetry (TGA)
	Scanning Electron Microscopy (SEM)
	Transmission Electron Microscopy (TEM)
	X-ray Diffractometry (XRD) Wide-Angle/Small-Angle X-ray Scattering (WAXD)/(SAXS)
Residual Moisture Content	Thermogravimetry (TGA)
Residual Solvents	Evaporative Light Scattering Detector (ELSD)
	Gas Chromatography (GC)
	High-Performance Liquid Chromatography (HPLC) Ultra Performance Liquid Chromatography (UPLC)
	Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)
	Near-Infrared Spectroscopy (NIR)
	Raman Spectroscopy
Stability (Agglomeration; Aggregation)	Atomic Force Microscopy (AFM)
	Confocal Laser Scanning Microscopy (CLSM)
	Extended X-ray Absorption Fine Structure (EXAFS)
	Fluorescence Microscopy
	Freeze Fracture Microscopy
	Optical Microscopy
	Polarized Light Microscopy (PLM)
	Scanning Electron Microscopy (SEM)
	Size Exclusion Chromatography (SEC)
	Thermogravimetry (TGA)
	Transmission Electron Microscopy (TEM)
	Ultrasonic Resonator Technology (URT)
Surface and Coating Properties	Atomic Force Microscopy (AFM)
	Confocal Laser Scanning Microscopy (CLSM)
	Scanning Electron Microscopy (SEM)
	Transmission Electron Microscopy (TEM)
	Western Blot
Viscosity	Rheometry
Zeta Potential	Conductivity Meter
	Dynamic Light Scattering (DLS) (also known as Photon Correlation Spectroscopy (PCS))
	Electrophoretic Light Scattering (ELS)
	Laser Doppler Anemometry (LDA)
	Scanning Electron Microscopy (SEM)

4. Concluding Remarks

The application of nanotechnology in the field of medicine has shown gradual progress, due to the huge potential to improve drug delivery, efficiency, and stability, with applicability in detection, prevention, or treatment. In particular, lipid-based nanosystems are being defined as a promising strategy for improving the specificity and efficiency of therapeutics, and reducing the toxicity and hence side effects of conventional medicines, such as chemotherapy.

Despite technological advances related to lipid-based nanosystems, there are growing concerns about their reproducibility requirements, safety, and quality standards. This is related to the inherent complexity of the formulations, costly and hard manufacturing processes, and limited knowledge of how formulation variables and manufacturing process parameters impact the final product's critical quality attributes (CQAs) and in vivo performance.

Given the challenges related to lipid-based nanosystems, it is essential to provide an individual, specific and deeper knowledge about the product and process, to achieve the intended product quality, based on sound science and quality risk management.

This chapter gives an overview of the implementation of QbD in the pharmaceutical development of lipid-based nanosystems. The application of the QbD approach corresponds to an adaptive, integrative, and flexible strategy for the development and optimization of lipid-based nanosystems. The continuing emphasis on product and process understanding and process control is reflected in the success of technology transfer up-scaled from lab-scale to large-scale, with significantly reduces the amount of time, cost, and effort associated with the pharmaceutical development of the lipid-based nanosystems, just as the lowest number of failures in their regulatory approval.

In this chapter, it was possible to verify that the liposomes are the most common type of lipid-based nanosystem identified. Further, it was possible to establish that cancer, neurological disorders, as well as, infection disease are the emerging areas of application of lipid-based nanosystems. On the other hand, it was also possible to identify common critical attributes selected in the bibliographic corpus, as well as, a diversity of statistical tools and design models applied. The main CQAs identified (dependent variables), are the particle size, polydispersity index, encapsulation efficiency, zeta potential, and drug release. Also, it was possible to understand the most common CMAs and CPPs, which will depend on the type of product to be developed and the manufacturing process selected. Therewith, the CMAs identified (independent variables) are mainly related to the formulation composition, such as the type and concentration of lipids, phase transition temperature of lipids, type and concentration of (co-)surfactant, drug concentration, among others. The key CPPs (independent variables) comprises the temperature of the different steps of the manufacturing process, stirring speed, time and type, sonication time, amplitude, speed and type, number of cycles, or pressure.

However, it was established that exist inadequacies in the implementation of QbD, particularly the incomplete identification of CMAs, CPPs, or CQAs for each formulation individually, lack of prior risk analysis, implementation of adequate risk assessment tools and control strategies throughout the whole process. For example, some general CQAs that must be identified and studied in the development of all medicinal products due to their impact on final product safety and efficacy was lacking in the most of articles in the bibliographic corpus (e.g. identification, assay, content uniformity, or degradations products/impurity profile). This can occur due to the limited number of products approved by regulatory agencies with known strategies of implementation of QbD, the information was not publicly available for confidentiality issues, the specific guidance documents do not adequately address the product complexity, or the need for investment in instrumentation and training a qualified workforce in QbD, DoE software, multivariate modeling, or control strategy development.

Moreover, despite the abundant quantity of information provided by the QbD approach was considered a significant advantage to facilitate the risk evaluation and reduce the likelihood of failure, in practice, the management and control of them can be fairly challenging. The overarching aim is to find as soon as possible the optimal operating conditions to obtain the most promising lipid-based nanosystem according to the established target product profile, without the need to test all experimental settings. Accordingly, it is fundamental the implementation of adequate control and risk assessment strategies, select the most appropriate mathematical models in the design of experiments (DoE), continuously process monitoring, in-process or real-time release testing through the Process Analytical Technology (PAT) in lieu of end-product testing, implementation of efficient statistical tools such as the Multivariate data analysis (MVDA), or other predictive models. These concepts can be closely followed through the analysis of ICH guidelines on pharmaceutical development (Q8), quality risk management (Q9), and pharmaceutical quality system (Q10) [395–397].

Therefore, the framework for future applications should also include the efforts to follow the recommendations present in specific guidelines and the implementation of the structured methodology of QbD to ensure a great enhancement in the design of these products, reducing the product and process variability, matching the target product profile previously defined, and overcome the hurdles related to the demonstration of therapeutic equivalence of complex generics.

Furthermore, the close cooperation between the science-based multi-stakeholders, such as the regulatory institutions, national agencies, pharmaceutical industry, academia, and medical community, is the key to progress in the field of lipid-based nanosystems, through the scientific discussions, meetings, interdisciplinary research, and publication. Also, the cooperation and scientific advice provided by the regulatory authorities to manufacturers can ease the pharmaceutical development and marketing approval of lipid-based nanosystems in the pre-registration phase.

Other strategies should include the development of internationally harmonized guidelines on scientific and technical standards for lipid-based nanosystems, increasing the efficiency of regulatory oversight, and implementing common standards and requirements to increase the consistency in the quality of complex generics. In parallel, it is essential to develop new and advanced analytical techniques and statistical methods for assessing the critical parameters and minimizing the risk of development failure in clinical development.

This will further step up innovation in an area that holds a severe impact to revolutionize advances in bio- and nanotechnologies with high-quality lipid-based nanosystems coming on the market.

Chapter V. Generic Development of Iron-Carbohydrate Complexes: Regulatory and Scientific Considerations

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Abstract

Iron-carbohydrate complexes are nano colloidal intravenous (IV) suspensions used in the treatment of diseases or conditions associated with iron deficiency and anemia. Despite the numerous advantages of these complex drug products, different adverse event profiles and safety concerns have been associated with IV-iron formulations. This can mainly be attributed to the high difficulty of providing a complete physicochemical characterization of iron colloids, the performance of adequate non-clinical and clinical studies, the establishment of the pharmaceutical equivalence, as well as, the substitutability and interchangeability issues arising from the standard generic approach.

One major objective of Chapter V is to provide comprehensive knowledge and full characterization of different physicochemical properties and biological responses, i.e., how quality attributes relate to in vivo performance. Another priority target is to provide substantial evidence that facilitates the proper demonstration of therapeutic equivalence/similarity between products, the re-examination of the approval pathways, and the definition of an adequate regulatory approach to ensure improved quality, efficacy, and safety of the IV iron-carbohydrate complex products and its follow-on products.

The growing scientific evidence base related to the IV iron-carbohydrate complexes has the potential to contribute to the adequate product quality assessment, facilitate generic complex development, and accelerate the introduction of newly developed products on the pharmaceutical market.

Keywords

Non-Biological Complex Drugs; Iron-Carbohydrate Complexes; Nanomedicines; Colloidal Nanoparticles; Intravenous Suspension; Parenteral Formulations; Iron Deficiency; Iron Supplementation; Follow-on Products; Therapeutic Equivalence; Physicochemical Characterization; Clinical Evaluation; Regulatory Science Research; Regulatory Evaluation; Regulatory Landscape; Regulatory Guidance.

1. Introduction

As mentioned in previous chapters, have emerged several discussions on the challenges related to the therapeutic equivalence and regulatory frameworks of NBCDs and their follow-on versions. The main focus of this chapter is the iron-carbohydrate complexes, precisely, the regulatory challenges involved in the marketing authorization of complex generic products and the absence of harmonization in the assessment of therapeutic equivalence.

Iron-carbohydrate complexes are colloidal intravenous (IV) dispersions with a complex structure that consists of polynuclear iron(III)-hydroxide cores stabilized by carbohydrate ligands [34,134,137,161–163]. They are usually considered to be nanometer-range particles with 5 to 100 nm, commonly administered by the intravenous route [34,134,137,161]. Relating to the therapeutic indication, these classes of NBCD products are the most widely used in the clinical treatment of diseases or conditions associated with iron deficiency and anemia, such as chronic kidney disease (CKD), end-stage renal disease (ESRD), inflammatory bowel disease (IBD), pregnancy, post-partum period, chronic heart failure, cancer, and post-bariatric surgery [34,134,137,162,164,238,635,636].

According to the World Health Organization (WHO), iron deficiency is the most common and widespread nutritional disorder worldwide, which can be progressively evolved into anemia [136,141,162,163,637]. Surprisingly, the WHO numbers suggest that there are over 30% of the world's population (approximately 2 billion people) with anemia, many due to iron deficiency [637]. The iron uptake and bioavailability are critical for essential mechanisms of maintenance and proper functioning of tissue and body organs, such as metabolic processes, synthesis of hemoglobin for oxygen transport, redox reactions in cellular respiration, and cellular proliferation [136,141,162,163,635,638,639]. Thus, iron deficiency can lead to harmful and severe health consequences like impaired physical and cognitive performance, increased risk of morbidity in children, reduced work productivity, reduced quality of life, and poor pregnancy outcomes [639,640]. It is important to highlight that the IV administration of iron-carbohydrate complexes has been frequently used to treat these deficiencies when the oral administration might not be appropriate due to the limited oral bioavailability, extended periods of treatment required to achieve iron stores, poor tolerability due to adverse drug reactions or lack of the effectiveness of the same [136,141,162,163,238]. Therefore, the IV iron-carbohydrate complexes are widely recommended in the treatment of iron deficiency in clinical practice to offer benefits over oral iron administration, such as the capacity to manage large concentrations of iron quickly (rapid repletion of iron stores), effectively (significant increase of hemoglobin levels), and safely (low rate of treatment-related adverse events) [136,141,162,163,329,638,641].

However, the several IV iron-carbohydrate complexes present significant differences in terms of molecular weight distribution, type, size and chemical nature of carbohydrate shell coating

material, size of the iron core, surface properties, hydrodynamic particle diameter, crystalline structure, conformation, osmolality, and labile iron content [17,132,137,141,238,329,635,638–640,642–645]. Consequently, the physicochemical differences between the IV iron-carbohydrate complexes lead to significant changes in the complex stability, iron release, absorption, bioavailability, pharmacokinetics and pharmacologic properties, immunogenicity, maximum tolerated dose and safety profile, and efficacy of the product [17,141,238,635,643,645]. As detailed in the 'Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product' (EMA, 2015), 'the release of iron appears to be influenced by the size and surface properties of the colloidal iron complex and the matrix' [161]. The following table shows an analysis of the physicochemical and clinical characteristics of the approved IV iron-carbohydrate complexes (Table 20).

Table 20. Overview of physicochemical and clinical characteristics of IV Iron-carbohydrate complexes (Adapted from [132,134,137,329,639,642,644–654]).

Type of iron-carbohydrate complex	Sodium iron gluconate complex	Iron sucrose complex	Iron dextran complex (HMWID and LMWID)	Iron carboxymaltose complex	Iron isomaltoside 1000 complex	Ferumoxytol complex
Core structure/Crystallite structure	Akaganeite/Ferrihydrite/ Lepidocrocite	Akaganeite/Ferrihydrite/ Goethite/Lepidocrocite	Akaganeite	Akaganeite	Akaganeite	Magnetite/Maghemite
Carbohydrate shell	Gluconate (mono-saccharide)	Sucrose (di-saccharide)	Dextran (branched poly-saccharide)	Carboxymaltose (branched poly-saccharide)	Isomaltoside (linear oligo-saccharide)	Polyglucose sorbitol carboxymethylether (branched poly-saccharide)
Complex type	Type III Labile and weak	Type II Semi-robust and moderately strong	Type I Robust and strong	Type I Robust and strong	Type I Robust and strong	Type I Robust and strong
Molecular weight (kDa)	289-440	30-60	165 (LMWID) 265 (HMWID)	150	150	750
Relative stability	Low	Medium	High	High	High	High
Reactivity with transferrin	High	Medium	Low	Low	Low	Low
Relative labile iron release	High	High	Medium	Low	Low	Low
Relative osmolalities	Hypertonic	Hypertonic	Isotonic	Isotonic	N/A	Isotonic
Maximal single dose (mg)	125 – 187.5	200-300	20 mg/kg BW	20 mg/kg BW (max. 1000)	20 mg/kg BW	510
Minimum administration time	10-60 min	10-30 min	60 min	15 min	15 min	15 min
Iron content (mg/ml)	12.5	20	50	50	100	30
Vial volume (ml)	5	5	2 and 10	2 and 10	1, 5 and 10	17
Labile iron (% of dose)	3.3	3.4	1.9	0.6	1	0.99
Direct iron donation to transferrin (% injected dose)	5-6	4-5	1-2	1-2	<1	<1
Test dose required	No	Yes (EU)/No (US)	Yes	No	No	No
Total dose infusion (TDI)	No	No	Yes	Yes	Yes	No
Premedication	No	No	No (LMWID)	No	No	No

			TDI only (HMWID)			
Administration (IV push) rates	12.5 mg/min	20mg/min	50mg (1ml/min)	Bolus push	50mg/min	30mg/s
Initial distribution volume, L	6	3.4	3.5	3.5	3.4	3.16
Plasma half-life (h)	1.42	5.3	5-30 (LMWID) 60 (HMWID)	7.4 - 9.4	20.8 - 22.5	14.7
Examples (Brand Name)	Ferrlecit®	Venofer®; Feriv®; Fer Panpharma®	Cosmofer®; Infed® Imferon®; Dexferrum®; DexIron®; Ferrum lek ®; Fercayl®	Injectafer®; Ferinject®	Monofer®; Monoferric®; Diafer®	Rienso®; FeraHeme®

Abbreviations: BW, body weight; DLS, dynamic light scattering; HMWID, high-molecular-weight iron dextran complex; IV, intravenous; LMWID, low-molecular-weight iron dextran complex.

As demonstrated in Table 20, a wide variety of mono- or polymeric carbohydrates can be used for this shell, such as sucrose, carboxymaltose, dextran, gluconate, isomaltoside, and polyglucose sorbitol carboxymethylether (PSC) [22,141,162,635,643,655,656]. Thus, the physicochemical and clinical characteristics of IV Iron-carbohydrate complexes specifically depend on the interaction between the iron (III)-hydroxyde core and the surrounding carbohydrates [657]. The type of carbohydrate shell influences the conformation and stability of Fe(III)-oxyhydroxide complex core, protects the iron core from hydrolysis, precipitation, and polymerization, prevents particle aggregation, and keeps the particles in colloidal suspension. Furthermore, the amenability of the carbohydrates to intracellular degradation, influences and controls the iron release profile [17,22,132,161,635,642,643,656]. As stated in the 'Assessment report for: Iron-containing intravenous (IV) medicinal products' (EMA, 2013), the potential impact on the stability of the drug product is extremely important because the weakly bound iron can readily dissociate and catalyze the generation of reactive oxygen species (ROS), induce oxidative stress, inflammation, tissue damage and, consequently lead to serious adverse events [31,238,635,639,657,658]. As a result, several carbohydrate shells play a crucial role in the quality and safety/tolerance of the drug product, but also in the efficacy, pharmacokinetics, and immunogenicity profile [17,22,132,635,642,643,656].

Therefore, the comprehensive knowledge and fully characterization of different physicochemical properties and biological responses, i.e., how quality attributes relate to in vivo performance, is one of the priority objectives of this chapter, to ensure improved quality, efficacy, and safety of each IV iron-carbohydrate complex product and their follow-on versions. Other aims include providing substantial evidence that facilitates the proper demonstration of therapeutic equivalence/similarity between products, and the re-examination of the approval pathways for these complex generic products.

2. Complexity of IV Iron-Carbohydrate Complexes and Implications for Therapeutic Equivalence Evaluation

The IV iron-carbohydrate complexes are defined by the complex, extensive, robust, multi step, and proprietary manufacturing process [17,22,30,31,132,636,643,645,655,659,660]. Thus, the structure, physicochemical and pharmacological properties, and clinical performance are strongly dependent on their specific process of production [17,22,30,31,132,636,643,645,655,659,660]. Consequently, any changes during the manufacturing process with slight structural modifications may affect the stability, physicochemical and biological properties, and therefore, the quality, safety and efficacy of them [17,22,30,31,132,636,643,645,655,659,660].

There is a broad range of parameters that can be changed during the manufacturing process such as starting materials, pH, temperature, reaction time, and other conditions that lead to the production of different types of iron-carbohydrate complexes [31,134]. This corresponds to a huge challenge for maintaining batch-to-batch consistency and reproducibility in the manufacturing of follow-on versions of iron-carbohydrate complexes [17,30,134]. Therefore, it could lead to a follow-on version with distinct physicochemical properties and biopharmaceutical profile compared with the reference product [17,31,162,164].

As stated in the 'Assessment report for: Iron-containing intravenous (IV) medicinal products' (EMA, 2013), any variation in the iron carbohydrate complex structure could affect the stability of the iron formulation, and hence promote the formation of reactive oxygen species (ROS), oxidative stress and tissue damage [17,20,31,132,162,658]. These differences might not be detectable by the available methods and consequently may result in inconsistency and higher inter-batch variability related to lack of robustness and control of their manufacturing process, the absence of a clear definition of critical quality attributes, and, accordingly, the different clinical outcomes observed in patients when using the originator product or one of its generic product [17,31,162]. A scientific article by *Francesco et al*, demonstrated a considerable dissimilarity in potential critical quality parameters between the iron sucrose originator product and iron sucrose complex generics, with statistically significant differences in size, size distribution, morphology, stability, and the fraction of labile iron [660,661]. The inconsistency and higher inter-batch variability were related to a lack of robustness and control of their manufacturing process, the absence of a clear definition of critical quality attributes, and, accordingly, the different clinical outcomes observed in patients when using the originator product or one of its generic product [660,661]. Thus, not only do different carbohydrate shells have an impact on their characteristics, but every change in the manufacturing process could lead to follow-on versions with distinct properties compared with the reference product, with drastic implications for the establishment of therapeutic equivalence and product safety [17,31,162,164].

On the other hand, the IV iron-carbohydrate complexes are characterized by a complex uptake mechanism. They are described as prodrugs, due to the dissociation from the carbohydrate shell and release of iron from the iron(III)-hydroxide core. Firstly, when administered intravenously, the iron-carbohydrate complexes are recognized and phagocytosed by cells of the reticuloendothelial system (RES) (macrophages), where the iron is released through the cleavage of the carbohydrate shell from the core. Subsequently, the iron is transiently stored bound to ferritin or taken up extracellularly by transferrin (transferrin-bound iron (TBI)) followed by transport and deposition in the sites where they are needed (e.g. bone marrow for hemoglobin synthesis) [22,31,132,137,161,635,638–640,642,645,652,656,662]. The type of carbohydrate shell influences the uptake mechanism and the clearance rate of the iron-carbohydrate complexes from the plasma [663]. For example, the majority of the iron-carbohydrate complexes, such as

the iron sucrose, ferric gluconate, or iron carboxymaltose complex, present a partial enzymatic degradation of shell in the plasma and an uptake by endocytosis in macrophages [663]. Nevertheless, the iron dextran complex does not present dissociation of the carbohydrate shell in plasma and can be uptake by a receptor-mediated mechanism [663]. The general schematic sequence that illustrates the uptake mechanism of iron–carbohydrate complexes is described in Figure 57.

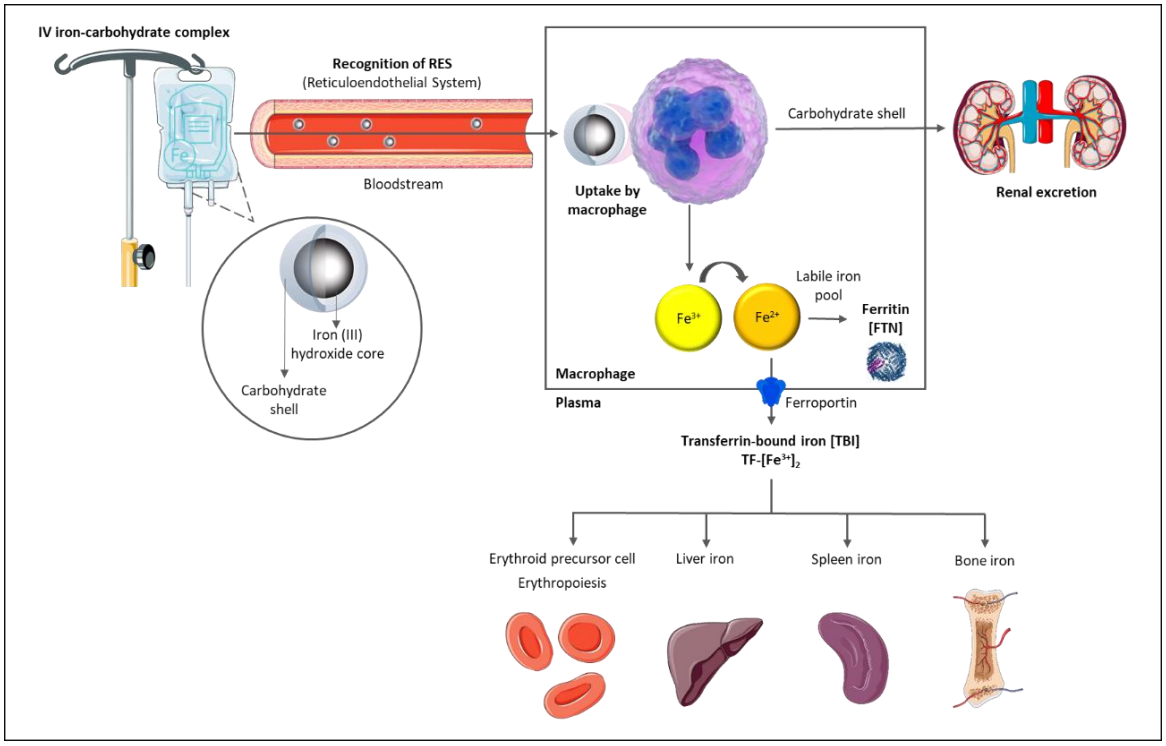

Figure 57. General schematic sequence illustrating the uptake mechanism of iron–carbohydrate complexes (based on [132,137,645,663]).

When the serum transferrin is already saturated with iron, the labile iron weakly binds to serum components, forming a significant amount of non-transferrin-bound iron (NTBI). The NTBI can lead to different side effects under the treatment of iron such as oxidative stress by catalyzing lipid peroxidation, reactive oxygen, and nitrogen species formation, inflammation, apoptosis, endothelial damage, hemodynamic alterations, myocardial infarction, heart failure, Alzheimer's disease, Friedreich's ataxia, Parkinson's disease, among others. The incidence of adverse effects arises mostly with changing product availability and disposition, when high doses are administered, or in less stable complexes that contain substantial amounts of weakly bound iron which lead to a rapid saturation of transferrin [31,640,642,645,652,659].

At this level, the account needs to be taken, of the interaction between the iron(III)-hydroxide core and the type of surrounding carbohydrates, which has an important role to stabilize the iron

core and delay the release of labile iron potentially harmful [31,238,635,639]. Thus, the incidence of adverse effects has been shown to correlate with different thermodynamic stabilities of IV iron-carbohydrate complexes, and consequently with the transferrin saturation and the NTBI formation. The structures with smaller thermodynamic stability (e.g. iron sucrose, Venofer®) are quickly dissolved and present significant quantities of weakly bound iron, which results in a greater number of NTBI species and enhanced toxicity [31,134,238,635,639,644]. Conversely, the complexes with high thermodynamic stability (e.g. ferumoxytol, FeraHeme®) are characterized by a slow degradation, low bioavailability of the labile iron, and minimized adverse effects. This is important to allow a rapid and high-dose infusion of iron replacement, with diminished risk of infusion reactions and improved tolerability [31,134,238,635,639,644].

Another example corresponds to the significant differences verified in iron dextran complexes with distinct molecular weight. The iron dextran complexes with high molecular weight (HMWID), e.g. Dexferrum®, are associated with severe and immune life-threatening anaphylactic reactions and clinical hypersensitivity. On the other hand, the iron dextran formulations with low molecular weight (LMWID), e.g. Cosmofer®, present a better profile of efficacy and tolerability, exhibiting fewer incidence of adverse effects than high molecular weight iron dextran [141,329,635,643,652].

Also has been extensively documented in the literature a lack of therapeutic and safety equivalence, and problems of automatic interchangeability in follow-on versions of iron sucrose complexes (Venofer®) [132,134,238,641,651]. Unfortunately, significant differences were observed in some parameters of follow-on versions, such as pH, titratable alkalinity, turbidity point, or iron release rate, without fulfilling the USP criteria nor exhibiting physicochemical similarity to Venofer® [134,137,656]. It is also important to highlight that the majority of generic iron complexes are approved within particular countries in the European Union, without fulfilling the pharmaceutical comparability requirements as described in the EMA guidance documents [137]. Another issue raised was the capacity of analytical techniques to detect the relevant differences in the critical quality attributes of follow-on versions with an impact on clinical outcomes [660].

Clinical and non-clinical studies demonstrated significant differences in efficacy and safety profiles between the iron sucrose complex formulations and their follow-on versions [17,20,25,31,34,132,162,164,238,659,662]. Surely, this issue is of the greatest importance because the substitution of Venofer® with a follow-on version induced oxidative stress, cytokine activation, apoptosis, endothelial dysfunction, life-threatening hypersensitivity reactions, inflammation, hypotension, phlebitis, and thereby hospitalization in subjects who previously tolerated the reference product [20,30,134,164,238,656,658,662]. Also was verified reduced efficacy in controlled trials in anemic patients, with decreased hemoglobin levels and reduced iron indices despite higher doses of the follow-on version [164].

All issues previously referred to surrounding the efficacy and safety of iron-carbohydrate complexes have led to public concerns and have been cited in scientific discussions, publications, citizen petitions, and reflection papers about the arisen regulatory challenges of demonstrating the substitutability and interchangeability between a follow-on version and its reference product [17,20,25,31,34,132,162,164]. In the same way, the adverse reactions by immunologic responses to the several iron-carbohydrate complexes led to drug safety-related labeling change data. For example, in 2009 the American Regent and FDA notified healthcare professionals of the Dexferrum® labeling change warning of possible anaphylactic-type reactions, including fatalities, have followed the parenteral administration of iron dextran injection [638]. It is also possible to identify the Drug Safety-related Labeling Changes (SrLC), approved by FDA Center for Drug Evaluation and Research (CDER), for Infed® (Iron Dextran) and Venofer® (Iron Sucrose) [664].

3. The Regulatory Landscape of Iron-Carbohydrate Complexes Approved in the US

This section examines the regulatory landscape of iron-carbohydrate complexes and their follow-on versions approved by the FDA, intending to understand the disparities in the regulatory pathways and the possible impact on the safety and efficacy of these products (Table 21) [36–123]. The information included in Table 21 was collected from the information of the regulatory landscape initially drawn up for Chapter I (Table 48) (see Appendix I. Supplementary Data). Thus, the following Table 21 provides detailed information about the approval year, brand and drug name, therapeutic indication, company, route of administration, marketing status, application number, regulatory pathway, application type, submission classification, and categories of complex products. The iron-carbohydrate complexes highlighted in bold correspond to reference products and the iron-carbohydrate complexes underlined with gray are their follow-on versions. The analysis of Table 21 is described in the following sections.

Table 21. Regulatory landscape of iron-carbohydrate complexes and their follow-on versions approved by the FDA.

Approval Date	Brand name (Reference product)	Follow-on product	Drug name	Therapeutic inification	Company	Route of administration	Marketing status	Regulatory Pathway	Application type	Application Number	Submission classification	Categories of drugs considered to be complex by the FDA	References
1974	**InFed®**	Not applicable	Iron dextran	Iron deficiency	Allergan	Intravenous	Prescription	New Drug Application (NDA)	505(b)(?)	017441	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	[36]
1981	**Proferdex®**	Not applicable	Iron dextran	Iron deficiency	New River Pharmaceuticals Inc	Intravenous	Discontinued	New Drug Application (NDA)	505(b)(?)	017807	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	[124]
1996	**Dexferrum®**	Not applicable	Iron dextran	Iron deficiency	American Regent Inc	Intravenous	Discontinued	New Drug Application (NDA)	505(b)(2)	040024	Unknown	Complex active ingredient	[62]
1996	**Feridex®**	Not applicable	Ferumoxides	Contrast agent	AMAG Pharmaceuticals Inc	Intravenous	Discontinued	New Drug Application (NDA)	505(b)(1)	020416	Type 1 - New Molecular Entity	Complex active ingredient	[63]
1999	**Ferrlecit®**	Not applicable	Sodium ferric gluconate complex	Iron deficiency	Sanofi Aventis US	Intravenous	Prescription	New Drug Application (NDA)	505(b)(1)	020955	Type 1 - New Molecular Entity	Complex active ingredient	[76]
2011	Ferrlecit®	Sodium iron Gluconate Complex in Sucrose Injection	Sodium ferric gluconate complex	Iron deficiency	West-Ward Pharmaceuticals	Intravenous	Prescription	Abbreviated New Drug Application (ANDA)	505(j)	078215	Not applicable	Complex active ingredient	[77]
2000	**Venofer®**	Not applicable	Iron sucrose	Iron deficiency	American Regent Inc	Intravenous	Prescription	New Drug Application (NDA)	505(b)(1)	021135	Type 3 - New Dosage Form	Complex active ingredient	[79]

2009	**Feraheme®**	Not applicable	Ferumoxytol	Iron deficiency	Amag Pharms Inc	Intravenous	Prescription	New Drug Application (NDA)	505(b)(1)	022180	Type 2 - New Active Ingredient	Complex active ingredient	[111]
2013	**Injectafer®**	Not applicable	Iron carboxymaltose	Iron deficiency	American Regent Inc	Intravenous	Prescription	New Drug Application (NDA)	505(b)(1)	203565	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	[119]
2020	**Monoferric®**	Not applicable	Iron derisomaltose	Iron deficiency	Pharmacosmos AS	Intravenous	Prescription	New Drug Application (NDA)	505(b)(1)	208171	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	[123]

In the US, the iron-carbohydrate complex products approved by the FDA include: Dexferrum® (Iron dextran) under section 505(b)(2); Feraheme® (Ferumoxytol), Venofer® (Iron sucrose), Ferrlecit® (Sodium ferric gluconate complex), Monoferric® (Ferric derisomaltose), and Injectafer® (Ferric carboxymaltose) under section 505(b)(1); InFed® (Iron dextran) and Proferdex® (Iron dextran) where the type of application is not known (Table 21) [36,62,76,79,111,119,123,124]. Thus, from the total of reference products submitted under the New Drug Application (NDA), 67% of them used the 505(b)(1) route (n=6, 67%) and 11% used the 505(b)(2) (n=1, 11%) (Figure 58). It should be noted that for 22% of the total of NDAs, it was not possible to understand the route of submission (Figure 58). The information on the FDA website is not always complete and it is common to have incomplete information for older products, as in the case of the InFed® (1974) and Proferdex® (1981).

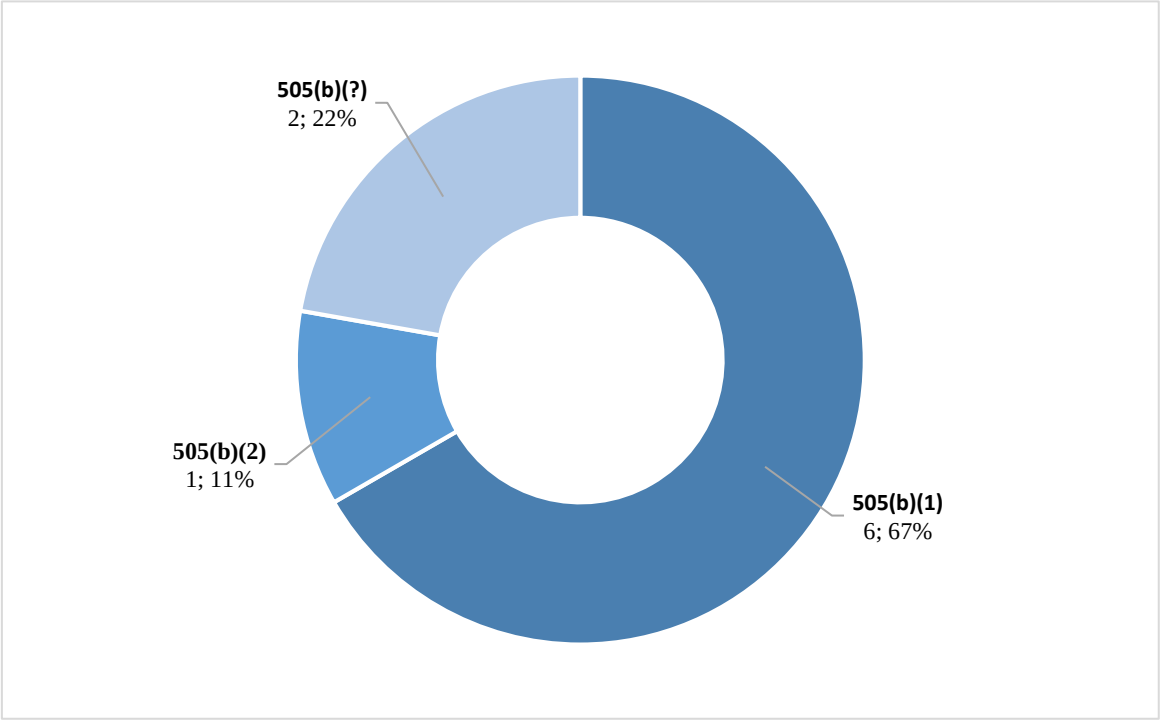

Figure 58. Type of New Drug Application (NDA) for iron-carbohydrate complexes approved by the FDA.

The FDA in the 'Generic Drug User Fee Act (GDUFA) II Commitment Letter' recognizes six complex categories based on the sources of complexity, such as complex active ingredients, complex formulations, complex routes of delivery, complex dosage forms, complex drug-device combinations, or 'other products where complexity or uncertainty concerning the approval pathway or possible alternative approach would benefit from early scientific engagement' [27,138]. Therewith, the iron-carbohydrate complexes identified were classified according to these categories as complex active ingredients (Table 21).

Regarding the submission classification of New Drug Application (NDA) approved by the FDA, 22% of them were approved under the type 1 of submission as 'New Molecular Entity' (n=2, 22%), 11% through the type 2 as 'New Active Ingredient' (n=1, 11%), 11% through the type 3 as 'New Dosage Form' (n=1, 11%), and 44% thought the type 5 as 'New Formulation or New Manufacturer' (n=4, 44%) (Figure 59). None of them has approved thought the type 4 as 'New Combination'.

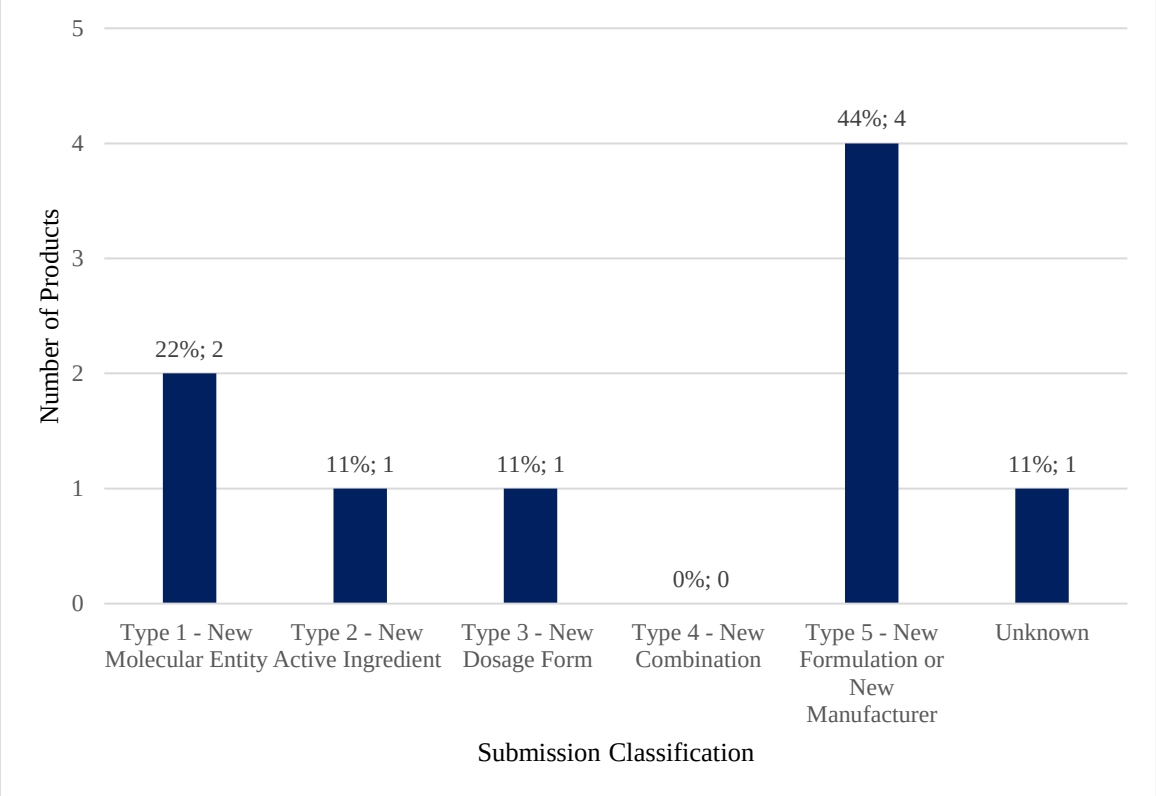

Figure 59. Submission Classification of New Drug Application (NDA) of iron-carbohydrate complex reference products approved by FDA.

The pharmaceutical generic development plays a fundamental role in cost reductions and increasing accessibility to medicinal products with adequate quality, safety, and efficacy [638]. To date, only one follow-on version among all intravenous iron–carbohydrate complexes was approved by the FDA [77]. This follow-on product corresponds to the therapeutic equivalent of Ferrlecit®, the generic sodium ferric gluconate complex in sucrose, submitted pursuant to abbreviated new drug application (ANDA) under section 505(j) in March 2011 [77,638,661].

While in certain countries no appropriate regulatory pathway for follow-on versions of iron-carbohydrate complexes has been defined, the FDA follows the abbreviated new drug application (ANDA) regulatory pathway under the 505(j) route [17,20,164]. Nevertheless, there is a need to understand if the ANDA regulatory pathway is more suitable for the approval of follow-on versions of iron-carbohydrate complexes [17,20,164]. According to the commentary 'Reflections on FDA Draft Guidance for Products Containing Nanomaterials: Is the Abbreviated New Drug Application (ANDA) a Suitable Pathway for Nanomedicines?', the 505(j) ANDA pathway is not adequate to approve safe and effective complex generic versions of all drug products containing nanomaterials, specifically drug products for systemic action and that are administered intravenously, which generally present a higher risk [661]. The former FDA Commissioner Scott Gottlieb, in Public Meeting on Generic Drug Competition, acknowledges that '*the traditional requirements used to demonstrate sameness may not be appropriate when it comes to complex drugs that can't be easily measured in the blood, or where the drug's therapeutic effect is delivered locally to a particular organ, rather than systemically, through the blood*' [665].

There are some notable examples of petitions filed to the FDA by manufacturers of iron-carbohydrate complexes, such as the petition of Luitpold Pharmaceuticals 'requesting that the FDA withhold approval of any Abbreviated New Drug Application (ANDA) or any 505(b)(2) application for any generic version or other pharmaceutical alternatives of Venofer® (iron sucrose injection, USP), unless and until any such applicant satisfies all of the conditions outlined in Luitpold's Petition to ensure appropriate safety and efficacy of any such generic version or pharmaceutical alternative of Venofer®' [642,666].

In short, these petitions arise from limitations already described, such as: the physicochemical techniques being insufficient to fully characterize the iron-carbohydrate complexes; lack of clinical data which supports the justification that certain physicochemical attributes recommended by the FDA have an impact on clinical efficacy and safety; the plasma pharmacokinetic results do not ensure equivalent tissue distribution; and the cases of iron complexes approved under much less rigorous standards showed the nonequivalent preclinical or clinical performance to reference products [642,666].

4. The Regulatory Landscape of Iron-Carbohydrate Complexes Approved in EU

By following the same principle in the previous segment, this section includes the regulatory landscape of iron-carbohydrate complex drugs and follow-on products approved by the EMA. To carry out this analysis, a list of iron-carbohydrate complexes already approved by the EMA (Table 22) was taken from Table 49 (see Appendix I. Supplementary Data). Thus, Table 22 gives a summary information about the approval year, brand and drug name, therapeutic indication, marketing-authorization holder (MAH), route of administration, marketing status, authorization procedure, Reference Member State (RMS), Concerned Member State (CMS), and application procedure for iron-carbohydrate complexes and their follow-on versions. The iron-carbohydrate complexes highlighted in bold correspond to reference products and the iron-carbohydrate complexes underlined with gray are their follow-on versions.

Table 22. Regulatory landscape of iron-carbohydrate complexes and their follow-on versions approved by the EMA [5,30,133,134,136,138,140–144].

Approval date	Brand name (Reference product)	Follow-on product	Drug name	Therapeutic indication	Marketing authorisation holder (MAH)	Route of administration	Marketing status	Authorization procedure	Reference Member State (RMS) (if applicable)	Concerned Member State (CMS) (if applicable)	Application procedure
1963	**Ferrlecit®**	Not applicable	Sodium iron gluconate	Iron deficiency	Sanofi-Aventis	Intravenous	Authorized	NP	Not applicable	NP: CZ, HU, DE, IT	Not applicable
1994	**Endorem®**	Not applicable	Ferumoxides	Contrast agent	AMAG Pharmaceuticals Inc	Intravenous	Withdrawn	MRP	FR	MRP: EL, IT, LU, NL, PT, SE, UK	Not applicable
1995	**Ferrum lek®**	Not applicable	Iron dextran	Iron deficiency	Lek Pharmaceuticals	Intravenous	Authorized	NP	Not applicable	NP: EE, PL, LV, LT, SI	Not applicable
1995	**Fercayl®**	Not applicable	Iron dextran	Iron deficiency	Sterop	Intravenous	Authorized	NP	Not applicable	NP: BE	Not applicable
1997	**Venofer®**	Not applicable	Iron sucrose complex	Iron deficiency	Vifor Pharma Ltd	Intravenous	Authorized	MRP/ NP	UK	MRP: AT, BE, DK, EL, ES, FI, IE, IT, LU, SE NP: CZ, EE, FR, HR, HU, IS, NL, NO, PT, SI, SK, LT	Not applicable
2005	Venofer®	Ferrovin	Iron sucrose complex	Iron deficiency	Refarm	Intravenous	Authorized	NP	Not applicable	EL, MT	Article 10(1)
2007	Venofer®	Óxido Férrico Sacarosado Generis	Iron sucrose complex	Iron deficiency	Generis	Intravenous	Authorized	NP	Not applicable	PT	Article 10(1)
2008	Venofer®	Alvofer	Iron sucrose complex	Iron deficiency	Cooper Pharmaceuticals	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)

2008	Venofer®	Dextrifer-S	Iron sucrose complex	Iron deficiency	Intermed	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2008	Venofer®	Ferrinemia	Iron sucrose complex	Iron deficiency	Help Pharmaceuticals	Intravenous	Authorized	NP	Not applicable	EL, MT	Article 10(1)
2008	Venofer®	Hemafer-S	Iron sucrose complex	Iron deficiency	Uni-Pharma	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2008	Venofer®	Intrafer	Iron sucrose complex	Iron deficiency	Vianex	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2008	Venofer®	Ironcrose	Iron sucrose complex	Iron deficiency	Target Pharma	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2008	Venofer®	Fer Mylan	Iron sucrose complex	Iron deficiency	Mylan	Intravenous	Authorized	NP	Not applicable	FR	Article 10(1)
2008	Venofer®	Fer Sandoz	Iron sucrose complex	Iron deficiency	Sandoz	Intravenous	Authorized	NP	Not applicable	FR	Article 10(1)
2008	Venofer®	Faremio	Iron sucrose complex	Iron deficiency	Demo	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2008	Venofer®	Óxido Férrico Sacarosado Accord	Iron sucrose complex	Iron deficiency	Accord Helathcare	Intravenous	Authorized	NP	Not applicable	PT	Article 10(1)
2009	Venofer®	Venotrix	Iron sucrose complex	Iron deficiency	Alternova	Intravenous	Authorized	NP	Not applicable	FI	Article 10(1)
2009	Venofer®	Nefro-Fer	Iron sucrose complex	Iron deficiency	Medice Arzneimittel Pütter	Intravenous	Authorized	DCP	DE	DE, AT, LU	Article 10(1)

2009	Venofer®	IJzerhydroxide sacharose complex	Iron sucrose complex	Iron deficiency	Teva	Intravenous	Authorized	NP	Not applicable	NL	Article 10(1)
2010	Venofer®	Veniron	Iron sucrose complex	Iron deficiency	Viofar	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2010	Venofer®	Fer Arrow	Iron sucrose complex	Iron deficiency	Arrow Generiques	Intravenous	Authorized	NP	Not applicable	FR	Article 10(1)
2011	Venofer®	Nephroferol	Iron sucrose complex	Iron deficiency	Verisfield	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2012	Venofer®	Ferracin	Iron sucrose complex	Iron deficiency	Acino	Intravenous	Authorized	NP	Not applicable	NL	Article 10(1)
2012	Venofer®	Järnsackaros Rechon	Iron sucrose complex	Iron deficiency	Rechon Life Science	Intravenous	Authorized	NP	Not applicable	SE	Article 10(1)
2012	Venofer®	Reoxyl	Iron sucrose complex	Iron deficiency	Medicus	Intravenous	Authorized	NP	Not applicable	EL	Article 10(1)
2018	Venofer®	Sucrofer	Iron sucrose complex	Iron deficiency	Claris Lifesciences	Intravenous	Authorized	DCP	UK	DE, FR	Article 10(3)
1999	**Cosmofer®**	Not applicable	Iron dextran	Iron deficiency	Pharmacosmos A/S	Intravenous	Authorized	MRP/ NP	DK	MRP: EE, DE, IE, LV, LT, LU, NL, NO, SE, UK, DK, ES NP: FR, FI, PL	Not applicable
2005	**Feriv®**	Not applicable	Iron sucrose	Iron deficiency	G.E.S. Genericos Espanoles Laboratorio	Intravenous	Authorized	NP	Not applicable	NP: ES	Not applicable
2007	**Ferinject®**	Not applicable	Iron carboxymaltose	Iron deficiency	G.E.S. Genericos Espanoles Laboratorio	Intravenous	Authorized	MRP/ DCP/ NP	UK	DCP: AT, CZ, DK, EE, FI, DE, EL, IE, LV, LT, LU, NL, PL, PT, SK, ES, SE, UK;	Not applicable

										MRP: BE, BG, CY, FR, HU, IS, IT, MT, NO, RO, SI NP: HR	
2007	**Ferrisat®**	Not applicable	Iron dextran	Iron deficiency	Pharmacosmos A/S	Intravenous	Withdrawn	MRP	DK	FR	Not applicable
2009	**Monofer®**	Not applicable	Ferric derisomaltose	Iron deficiency	Takeda Pharma A/S	Intravenous	Authorized	DCP	SE	DCP: AT, BE, BG, CY, DK, EE, FI, DE, EL, HU, IS, IE, LV, LT, LU, NL, NO, PL, PT, RO, ES, UK	Not applicable
2012	**Rienso®**	Not applicable	Ferumoxytol	Iron deficiency	Takeda Pharma A/S	Intravenous	Withdrawn	CP	Not applicable	Not applicable	Article 8(3)
2013	**Diafer®**	Not applicable	Ferric derisomaltose	Iron deficiency	Pharmacosmos A/S	Intravenous	Authorized	DCP	SE	AT, BE, DK, FI, IE, NL, NO, PL, RO, UK	Not applicable
2014	**Fer Panpharma®**	Not applicable	Iron sucrose	Iron deficiency	Pharmacosmos A/S	Intravenous	Authorized	NP	Not applicable	NP: FR	Not applicable

Abbreviations: AT, Austria; BE, Belgium; BG, Bulgaria; CMS, Concerned Member State; CP, Centralized Procedure; CY, Cyprus; CZ, Czech Republic; DCP, Decentralised Procedure; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, Ireland; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MRP, Mutual Recognition Procedure; MT, Malta; NL, Netherlands; NO, Norway; NP, National Procedure; PL, Poland; PT, Portugal; -RMS, Reference Member State; RO, Romania; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

The iron-carbohydrate complex products approved by the EMA include: Ferrlecit® (sodium iron gluconate complex), Ferrum lek® (iron dextran complex), Fercayl® (iron dextran complex), Feriv® (iron sucrose complex), and Fer Panpharma® (iron sucrose complex) under the National Procedure; Endorem® (Ferumoxides) and Ferrisat® (iron dextran complex) under the Mutual Recognition Procedure; Rienso® (Ferumoxides) under the Centralized Procedure; Monofer® (iron isomaltoside 1000 complex) and Diafer® (Ferric derisomaltose) through the Decentralized Procedure; Venofer® (iron sucrose complex) and Cosmofer® (iron dextran complex) under the MRP/NP; and Ferinject® (iron carboxymaltose complex) through the MRP/NP/DCP (Table 22).

Therefore, regarding the Marketing Authorisation procedures used for approval of iron-carbohydrate complex reference products, 39% of them were approved via the National Procedure (n=5, 39%), and only two were approved via the Mutual Recognition Procedure (n=2, 15%), and through the Decentralised Procedure (n=2, 15%). On the other hand, one of them was approved via the Centralized Procedure (n=1, 8%) (Figure 60). Some reference products present more than one approval procedure, such as: MRP/NP (n=2, 15%), and MRP/DCP/NP (n=1, 8%) (Figure 60).

Under this analysis, it is important to emphasize that the iron-carbohydrate complexes were approved through distinct and not comparable pathways. This could lead to impressive heterogeneity in regulatory approaches used for each product, and consequently result in high variability of regulatory requirements demanded and uncertainties related to their safety and efficacy evaluations [30,132].

According to *Crommelin et al*, 'considering the complex and also nanoparticular nature with the pharmaceutical, pharmacological and toxicological intricacies inherent to this family of NBCD products (…) should follow the centralized procedure and undergo a drug development program according to the ICH Common Technical Document', that it does not in line with the results found in this analysis.

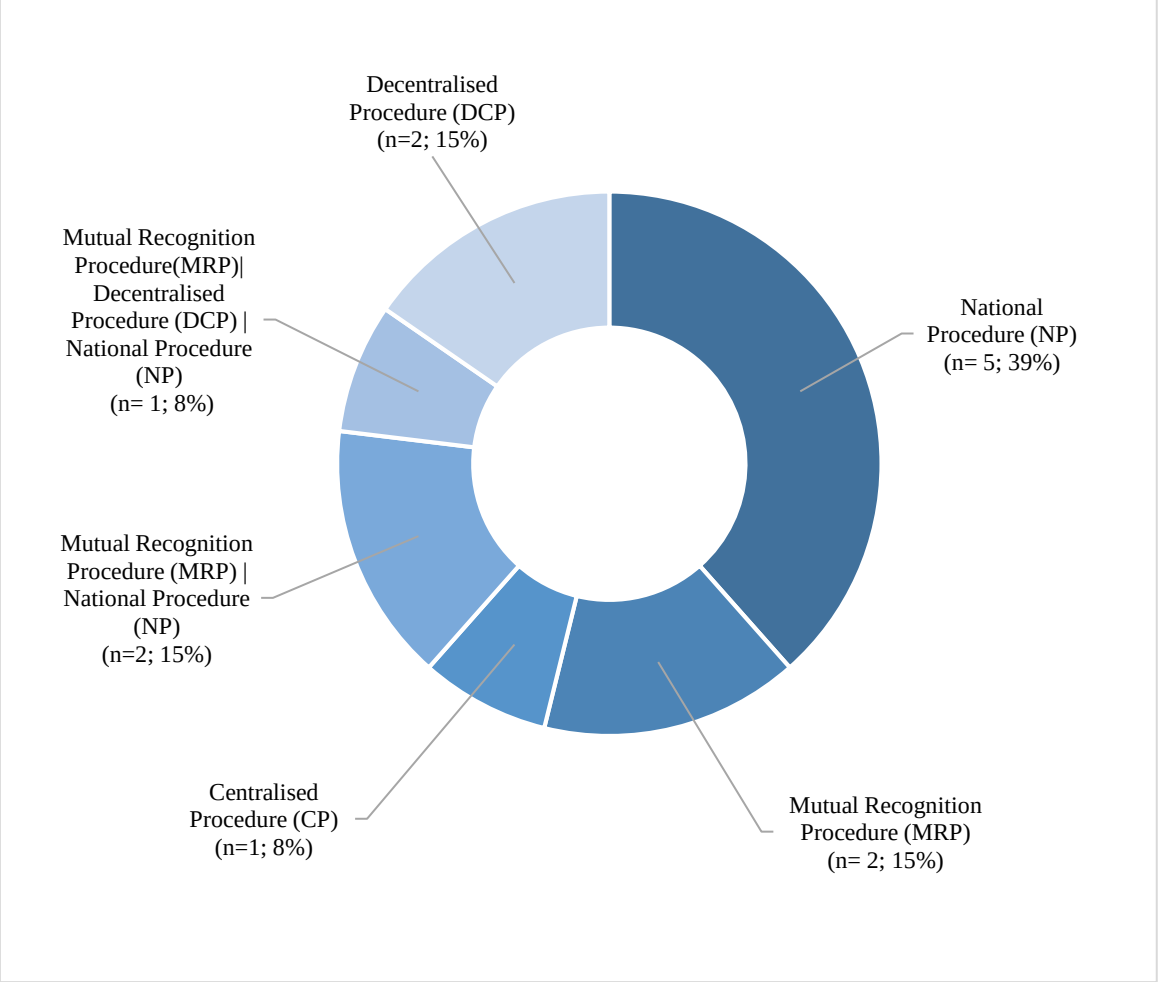

Figure 60. Marketing Authorisation Procedures of iron-carbohydrate complex reference products approved by the EMA.

From the total list of 13 iron-carbohydrate complex reference products approved by the EMA, only one of them presents follow-on versions (Venofer®). It is interesting to notice that the several follow-on versions of the Venofer® (iron sucrose complex) are approved by the EMA, mostly through the National Procedure under the Generic Application (Article 10(1)), without considering the consequences of their nanoparticulate structure and complexity (Table 22). Only two follow-on versions of the Venofer® are approved under the Decentralized Procedure (DCP), one of them by the Hybrid Application under Article 10(3) (Table 22).

While the biotechnology-derived medicinal products, like biosimilars, have to follow a Centralized Procedure (CP), the follow-on versions of iron-carbohydrate complexes might receive the marketing authorization through non-centralized procedures [22,30,362]. Regarding the authorization procedures, there is broad consensus that the follow-on versions of NBCDs must compulsorily follow the centralized procedure, and not the National Authorization Procedures. This opinion, shared by many experts, emerges from the negative results of clinical studies of follow-on versions authorized following national procedures that ‘clearly showed differences in

clinical performance between the innovator and follow-on products', i.e., an inappropriate assumption of interchangeability between the reference products and their follow-on versions [18,362,655]. It is important to emphasize that the FDA has not approved a generic version of iron sucrose injection, and defined the evaluation of the equivalence of iron-carbohydrate complexes as a key regulatory scientific priority [27,667].

In Table 22 it is possible to observe that the most follow-on versions were approved predominantly through the generic application under Article 10(1), and only one product was approved by hybrid application procedure under Article 10(3). Therefore, despite the results indicating a tendency to use the same application procedure inside of the same class of iron-carbohydrate complexes, a paradigm shift is expected in the use of the hybrid application procedure for recent approvals of follow-on versions (e.g. Sucrofer®, 2018).

Regarding the marketing status, there are three products discontinued (Endorem®, Rienso®, and Ferrisat®). Endorem® (Guerbet, France) was withdrawn from the market due to not being economically viable (commercial issues), although is effective and safe [463,464]. The withdrawal of the Ferrisat® derives from the increased frequency of adverse reactions, such as hypersensitivity reactions [471]. The withdrawal of the application for a change to the marketing authorization for Rienso® (ferumoxytol) resulted from the need for additional clinical data to support wider use of the medicine, as well as, the commercial reasons [472,473]. In addition, appeared post-marketing reports of serious or fatal hypersensitivity (allergic) reactions were observed in regular ongoing safety monitoring. According to CHMP, the benefits of Rienso® in the treatment of iron deficiency anemia in the extended population do not outweigh its risks [473].

5. Iron-Carbohydrate Complexes: Therapeutic Equivalence Recommendations

5.1. Comparative Evaluation of FDA and EMA Requirements for the Demonstration of Therapeutic Equivalence

The major challenges of the regulatory evaluation related to the similarity and therapeutic equivalence between a follow-on version and its reference product have been acknowledged by the regulatory agencies through the scientific discussions, publications, guidance documents, and reflection papers [17,20,25,31,34,132,162,164,329]. They perceived the complexity of IV iron formulations and the impossibility to use the well-established generic approval paradigm of small molecules for the approval of the iron follow-on versions [645,657]. Both regulatory agencies also agree that the straightforward analysis of drug kinetics, the non-clinical biodisposition, and the evaluations of in vitro pharmaceutical quality do not guarantee the interchangeability and full efficacy and safety of these follow-on versions, i.e., are not sufficient to prove the similarity between the originator product and their follow-on version [17,132,161,162,642]. An example

widely mentioned in the literature is the non-clinical assessments in plasma or tissues, which do not necessarily predict a direct extrapolation of tissue biodistribution in humans, as the physiological or pathophysiological iron homeostasis differs substantially [132,642].

Table 23 summarizes the FDA and EMA data requirements for generic iron-carbohydrate complexes developed as a treatment for iron deficiency with reference to an innovator product, including the comparison of guidance documents and reflection papers to support their regulatory approval [132,161,162,264,265,267,273,274,281,640].

The 'Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product' (2015) published by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA), describes the current state of regulatory thinking regarding the iron-based nano-colloidal products and takes a 'weight of evidence approach' requiring data from quality, non-clinical and human pharmacokinetic studies for the evaluation of therapeutic equivalence to the reference product [161]. The non-clinical comparability studies shall be performed to assess the iron distribution in at least three biological compartments: plasma, reticuloendothelial system (RES), and pharmacological/toxicological target tissues [18,136,161,162,668]. Thus, depending on each case and its complexity, the non-clinical data including comparative tissue distribution, toxicology, and pharmacodynamic studies are applied before performing BEq studies in human subjects [137]. Nevertheless, it is widely recognized the lack of experience in the statistical analysis of the equivalence results of non-clinical studies, as well as, the need for validation studies of different methods with the definition of sensitivity, variability, and detection limits [132,137].

Similarly, the FDA published product-specific draft guidance documents containing recommendations on the assessment of bioequivalence for the generic version of Iron Sucrose (2012), Ferumoxytol (2012), Sodium Ferric Gluconate (2013), Iron Dextran (2016), and Ferric Carboxymaltose (2016) [264,265,267,273,274]. The comparison of these draft guidances demonstrates that differ in some requirements, such as the in vivo evaluation. While the iron–dextran guidance employs patients with iron-deficiency anemia, the sodium ferric gluconate complex, and iron–sucrose include healthy volunteers [18].

Moreover, the regulatory agencies present different opinions concerning the requirements to demonstrate the bioequivalence of intravenous generic iron-carbohydrate complexes [642]. Contrary to the EMA, the FDA does not include non-clinical studies in the demonstration of bioequivalence. On the other hand, it includes clinical studies in healthy subjects or patients with iron deficiency anemia, determination of Q1/Q2 sameness with the equivalence of stoichiometric ratios of formulation components, and sameness in physicochemical properties (such as particle morphology, core size determination, ferric oxyhydroxide crystalline structure, iron core environment, the composition of carbohydrate shell, carbohydrate-iron core interactions, surface

charge, molecular weight distribution, the labile iron determination under physiologically relevant conditions, among others) [265,267,274,646]. The FDA recommends carrying out a 'single-dose, randomized, parallel pharmacokinetic (PK) bioequivalence study in healthy subjects or patients with iron deficiency anemia, with measurement of plasma total iron (TI) and transferrin-bound iron (TBI)'. When demonstrating the bioequivalence of follow-on product with the originator product, 'the 90% confidence intervals of the generic drug for the maximum value of the difference in concentration and area-under-the-curve between TI and TBI over all time points measured should be within an 80.00% to 125.00% range of innovator product's values' [264,265,267,273,274,638]. It is recommended that the demonstration of the physicochemical properties sameness be carried out under the least three lots of the originator product and the follow-on version [264,265,267,273,274,638].

Both regulatory agencies agree that the stability of formulations depends on the quality attributes and physicochemical properties, which may affect the safety and efficacy of iron-carbohydrate complexes [161,264,265,267,273,274,281,645]. These attributes are carefully detailed in the next section (section 5.2), and appropriately justified concerning the potential impact on the product safety and efficacy [161].

It must be highlighted that Table 23 also includes some examples of guidance documents applied to biological medicinal products and biosimilars, as these complex drugs present a harmonized regulatory approach well-established for some years [14,18,330]. Since NBCDs share many characteristics with biological medicinal products, the experience and guidance documents of biologicals can be used to define and improve the regulatory systems of NBCDs and their follow-on versions [14,18,330].

Table 23. Comparative Evaluation of FDA and EMA requirements for the demonstration of Therapeutic Equivalence for the Follow-On Versions of Iron-Carbohydrate Complexes [132,161,162,264,265,267,273,274,281,430–432,640].

Evaluation of Therapeutic Equivalence for the Follow-On Versions of Iron-Carbohydrate Complexes		
Regulatory Authority	**US (FDA)**	**EU (EMA)**
Type of Regulatory Pathway	Abbreviated New Drug Application (ANDA)	National Procedure (NP) or Decentralized Procedure (DCP)
	Generics Application - 505(j)	Generics Application - Article 10(1) or Hybrid Application - Article 10(3)
Equivalence Requirements (Recommended studies)	**<u>In vitro studies</u>**	**<u>Quality characterization</u>**
	Q1 sameness (Qualitative - same active/inactive ingredient(s)) and Q2 sameness (Quantitative - same concentrations). (e.g. stoichiometric ratios of iron, carbohydrate, and other relevant components).	Q1 (Qualitative) and Q2 (Quantitative) sameness.
	Physicochemical properties sameness on ≥3 batches of the ANDA versus RLD (Reference Listed Drug) (e.g. iron core size, iron oxide crystalline structure, iron environment, composition of carbohydrate shell, overall particle morphology, comparable labile iron under physiologically relevant conditions).	Physicochemical properties sameness (critical quality attributes: e.g. structure and composition of carbohydrate matrix, spectroscopic properties, size of the iron core, labile iron released, polymorphic form of the iron comprising the core, impurities, morphology, ratio of bound carbohydrate to iron, particle size, size distribution, charge, surface properties, degradation path, stability on storage, in-use stability).
	<u>In vivo BEq studies</u>	**<u>Non-clinical studies</u>**
	Single-dose, randomized, parallel pharmacokinetic (PK) bioequivalence study in healthy subjects or patients with iron deficiency anemia, with measurement of plasma total iron (TI) and transferrin-bound iron (TBI).	Bio-distribution studies - Plasma (or serum) and red blood cells. - RES: macrophages (e.g. in spleen, liver (Kupffer cells)). - Target tissues: pharmacological target tissues (e.g. bone marrow) and toxicological target tissues (e.g. kidney, liver (hepatocytes), lungs, heart).
	The 90% confidence intervals of the generic drug for the maximum value of the difference in concentration and area-under-the-curve between TI and TBI over all time points measured should be within an 80.00% to 125.00% range of innovator product's values.	**<u>Clinical studies</u>**
		Pharmacokinetics studies Bioequivalence based on: maximum value of the difference in concentration between total iron and transferrin-bound iron over all time points measured; and difference in AUC (area under the curve) between total iron and transferrin-bound iron.

		The 90% confidence interval of the baseline-corrected values should be in the 80-125% range.
		Efficacy and Safety studies Clinical trial: least 3 months in duration and performed in a group of patients with a similar etiology for their anemia (e.g. chronic renal failure); endpoints - ferritin, transferrin saturation, hemoglobin, total iron dose administered overstudy, total EPO (erythropoietin) dose administered over study. Safety endpoints - short-term adverse safety profile (anaphylactoid reaction rate, Non-transferrin bound iron (NTBI), overall adverse event rates, markers of oxidative stress, and free radical activity.
		Pharmacovigilance / Risk Management Plan Adverse safety profile in the post-marketing period.
Guidance documents	Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice: Guidance for Industry (2004)	Specifications and control tests on the finished product (1991)
	Draft guidance on Iron Sucrose: Product-specific guidance document (2012)	Note for Guidance on Pharmacokinetics: Repeated dose tissue distribution studies (ICH Topic S3B) (1995)
	Draft Guidance on Ferumoxytol: Product-specific guidance document (2012)	Note for Guidance on general considerations for clinical trials (ICH Topic E8) (1998)
	Draft Guidance on Sodium Ferric Gluconate Complex: Product-specific guidance document (2013)	Note for Guidance on duration of chronic toxicity testing in animals (rodent and non-redent toxicity testing) (1999)
	Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA: Guidance for Industry (2013)	Note for Guidance ICH Q6A specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances (2000)
	Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology: Guidance for Industry (2014)	Note for Guidance on safety pharmacology studies for human pharmaceuticals (2001)
	Scientific Considerations in Demonstrating Biosimilarity to a Reference Product: Guidance for Industry (2015)	Note for guidance on biotechnological/biological products subject to changes in their manufacturing process (ICH Q5E) (2005)
	Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product Guidance for Industry (2015)	Reflection paper on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products (2007)
	Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product Guidance for Industry (2016)	Comparability of biotechnology-derived medicinal products after a change in the manufacturing process - non-clinical and clinical issues (2007)
	Draft Guidance on Ferric Carboxymaltose: Product-specific guidance document	Guideline on the investigation of bioequivalence (2010)

	(2016)	
	Draft Guidance on Iron Dextran: Product-specific guidance document (2016)	ICH guideline Q11 on development and manufacture of drug substances (chemical entities and biotechnological/biological entities) (2011)
	Drug Products, Including Biological Products, that Contain Nanomaterials: Guidance for Industry (2017)	Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications: Product-specific guidance document (2011)
	Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA Guidance for Industry (2017)	Concept paper on the need for a reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (2013)
	Draft Guidance for Industry: Statistical Approaches to Evaluate Analytical Similarity (2017)	Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (2014)
	Questions and Answers on Biosimilar Development and the BPCI Act Guidance for Industry: Guidance for Industry (2018)	Guideline on similar biological medicinal products (2014)
	Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products: Guidance for Industry (2018)	Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (2014)
	Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs - General Considerations: Guidance for Industry (2019)	Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product: Product-specific guidance document (2015)
	Considerations in Demonstrating Interchangeability With a Reference Product: Guidance for Industry (2019)	Guideline on manufacture of the finished dosage form (2017)
	Determining Whether to Submit an ANDA or a 505(b)(2) Application: Guidance for Industry (2019)	ICH guideline Q8 (R2) on pharmaceutical development (2017)
	Competitive Generic Therapies: Guidance for Industry (2020)	Guideline on the requirements for the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (2017)
		Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (2017)
		European Medicines Agency procedural advice for users of the centralised procedure for similar biological medicinal products applications (2019)
		European Medicines Agency procedural advice for users of the centralised procedure for generic/hybrid applications (2019)
Other documents	Iron Sucrose Injection - USP Monograph	Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines
	Iron Dextran Injection - USP Monograph	
	Ferumoxides Injection - USP Monograph	

5.2. Pharmaceutical Quality: Physicochemical Characterization of Iron-Carbohydrate Complexes

The proper physicochemical characterization and the comparability of the follow-on version to the reference product, constitute part of the process of demonstrating therapeutic equivalence and are an important means to ensure consistent quality of the iron-carbohydrate complexes [161]. This can be only achieved by the combination of a well-defined and controlled manufacturing process and comprehensive characterization of the complex product [161]. According to the FDA, it is essential the implementation of the risk-based approach and defines that the 'development of drug products entails a continual reduction of residual uncertainty throughout a product's lifecycle' [10,661].

Table 24 describes the critical quality attributes, that potentially impact the quality, safety, and efficacy of iron-carbohydrate complexes, based on respective pharmacopeia monographs, specifications listed in product-specific guidance documents, and scientific literature. This table are differentiated into two main categories: general and specific critical quality attributes (including the iron core properties, and carbohydrate shell properties) [18,123,132,137,161,264,265,267,273,281,635,639,642,643,669].

As acknowledged in the draft guidance 'Drug Products, Including Biological Products, that Contain Nanomaterials', 'the active ingredients of some nanomaterials are generally heterogeneous mixtures which may require considerable characterization to demonstrate drug substance sameness. Some critical excipients for the formation of nanomaterials are also complex' [10]. However, the challenges related to the identification of clinically meaningful quality attributes are much deeper, since the analytical methods are not advanced enough to evaluate and ensure an adequate characterization of them [10,661]. On the other hand, the results of characterization may differ depending on the method selected, and must therefore be employed orthogonal and complementary analytical techniques, to ensure the accuracy and consistency of the data [161]. To overcome these problems, it is necessary effectively to implement an extensive comparability exercise with comprehensive side-by-side analysis between the follow-on versions and reference products, through the determination and justification of similarities in quality attributes, but also potential differences [284]. It is also fundamental to develop additional, reliable, and robust analytical techniques [132].

The analytical techniques available for the characterization of iron-carbohydrate complexes are described in Section 6 of this chapter.

Table 24. List of Critical Quality Attributes (CQAs) proposed for Pharmaceutical Development of Iron-carbohydrate complexes [18,123,132,137,161,264,265,267,273,281,430–432,635,639,642,643,669].

	Critical Attributes	Justification	Analytical methods
General Critical Attributes (Formulation/Whole Particle)	Absorption from injection site	The absorption from the injection site is an attribute identified as a specific test of the USP monograph of Iron Dextran Injection, and is related to the control of heavy tissue deposit of unabsorbed iron compounds.	USP monograph of Iron Dextran
	Assay (iron)	The assay of iron has an impact on the concentration in plasma, so they are critical for the iron release rate, pharmacokinetics, toxicological profile, safety, and efficacy of the iron-carbohydrate complexes.	Atomic absorption spectroscopy (AAS)
	Assay (carbohydrate)	The carbohydrate shell influences the conformation and stability of the iron complex core, controls the iron release profile, and has a significant impact on the quality, safety, and efficacy of the product. For example, complexes with smaller carbohydrate shell coating material are more labile and present higher rates of iron release compared to complexes with larger carbohydrate shell.	High-performance liquid chromatography (HPLC)
	Bacterial endotoxins	Non-compliance with microbial limits has the potential to harm the patients particularly when the medicinal product is intended to be administered intravenously. The pyrogen content and bacterial endotoxins may be influenced by process parameters, formulation variables, container closure, scale/equipments, and site, which can impact patient safety. Therefore, these attributes should be investigated during product and process development and conform to limit bacterial endotoxins as defined in Bacterial Endotoxins Test ⟨85⟩ or Pyrogen Test ⟨151⟩. The bacterial endotoxin is an attribute present in the USP monograph of Iron Sucrose Injection, Ferumoxides Injection, and Iron Dextran Injection.	Bacterial endotoxin test (LAL test) Chromogenic technique Gel-clot technique Turbidimetric technique
	Cytotoxicity	The cytotoxicity has a great impact on the safety profile of the drug product and may be influenced by the particle size, shape, composition, and surface charge of these types of dosage forms.	USP monograph Iron Dextran Injection Method of Acute Toxicity
	Degradation products/impurity profile	Degradation products can compromise the safety profile of the drug product and must be controlled based on compendial/ICH requirements or a reference listed drug (RLD) characterization, to limit patient exposure. The target for any unknown impurity is set according to the ICH identification threshold for each drug product. Therefore, degradation products should be assessed during product and process development conforms according to ICH Q3B(R2) requirements.	Potentiometric titration Cerimetric titration

		The Content of Chloride, Nonvolatile Residueis, and Limit of Iron [Fe(II)] are included in the impurities tests present in the USP monograph of iron sucrose injection and Iron Dextran Injection.	
	Equivalence in stoichiometric ratios of iron, free and other relevant components	Equivalence in stoichiometric ratios of iron, free and other relevant components is a Q1/Q2 sameness requirement, i.e., the test product used the same inactive ingredient(s) as the reference product (Q1. Qualitative sameness), and the concentrations of the inactive ingredient(s) used in the test product are within ±5% of those used in the reference product (Q2, Quantitative sameness). This CQA can impact assay, stability, iron release rate, pharmacokinetics, impurity profile, safety, and efficacy of the iron-carbohydrate complexes.	Atomic absorption spectroscopy (AAS) Energy-dispersive X-ray spectroscopy (EDX) High-performance liquid chromatography (HPLC) Inductively coupled plasma mass spectrometry (ICP-MS)
	Identification	The identification of formulation components (chemical composition) including the iron, carbohydrates, and functional excipients, should be assessed during product and process development, due to can largely affect the quality, efficacy, and safety profile of the drug products. Therewith, becomes crucial the identification and control of key intermediates in the manufacturing process.	Differential scanning calorimetry (DSC) Extended X-ray absorption fine structure (EXAFS) Fourier transform infrared spectroscopy (FTIR) Mössbauer spectroscopy Polarography Raman spectroscopy Thermal gravimetric analysis (TGA) X-ray absorption near-edge structure (XANES) X-ray diffraction (XRD)
	Labile iron	The iron release is influenced by the particle size, surface properties, and carbohydrate matrix of iron-carbohydrate complexes. Conversely to dialyzable low molecule weight iron or free iron, the labile iron released from the product when administered, bind to the iron carbohydrate matrix and are not dialyzable. In the same way, the release in blood circulation leads to the formation of NTBI (Nontransferrin-bound iron), which potentiates oxidative stress, inflammation, cellular damage, and other toxic effects. Thus, the labile iron can impact bioavailability, pharmacological, and toxicological target tissue distribution, and therefore in safety profile, therapeutic efficacy, and clinical performance of the product. The determination of labile iron should be performed under physiologically relevant conditions.	Catalytic bleomycin assay of spiked human serum samples Ultra-filtration In vitro dialysis system Iron (III) reduction by acid degradation and UV spectrophotometric measurement In vitro labile iron donation studies

	Leachable/extractables	The leachables and extractables from components of the primary packaging (e.g. plastic and rubber) can compromise the safety profile of drug product, due to the generation of impurities. Thus, they should be evaluated throughout the product and process development, according to USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems, USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery systems, USP <381> Elastomeric Closure for Injections, USP <660> / EP 3.2.1 Glass Containers for Pharmaceutical Use, and <87> Biological Reactivity Tests.	USP <1663> USP <1664>
	Low molecular weight iron species	The Low molecular weight iron (or free iron) is related to the formation of NTBI (Nontransferrin-bound iron), which are impurities with potential oxidative toxicity and consequent serious adverse reactions.	In vitro dialysis system Ultra-filtration
	Molecular weight distribution	The determination of average molecular weight and molecular weight distribution are essential as indicators of the quality and stability of iron formulations because they allow the detection of variations in product quality, potential degradations, or aggregation of colloidal iron nanoparticles. These attributes are identified in the USP monograph of iron sucrose injection.	Analytical ultracentrifugation Asymmetric field-flow fractionation (AFFF) Gel permeation chromatography (GPC) Mass spectroscopy (MS) Size exclusion chromatography (SEC) Static light scattering (SLS)
	Morphology	The particle morphology (e.g., shape and particle agglomeration profile) should be investigated throughout the product and process development, due to their impact on stability, iron release rate, biodistribution, efficacy, and safety profile. As the polydispersity index, the microscopic evaluation of the surface and morphology of particles can be indicative of a monodisperse or polydisperse population.	Atomic force microscopy (AFM) Transmission electron microscopy (TEM)
	Osmolarity/osmolality	Osmolality values different from plasma osmolality may cause tissue irritation and damage to blood cells. This CQA should be evaluated during product and process development, in accordance with USP <785> Osmolality and osmolarity. The factors that can impact this CQA are the formulation, raw materials, process parameters, scale/equipments, or site.	Freezing point depression
	Particulate matter	The presence of particulate matter in formulations intravenously administered is considered critical because they are potentially life-threatening health hazards due to the fact that they can cause irritation, phlebitis, anaphylactic shock, embolism, and even death. This CQA should be evaluated throughout the product and process development, in accordance with USP <788> Particulate matter in injections and USP <790> Visible particulates in injections.	Light obscuration particle count test Microscopic particle count test

	Particle size distribution and uniformity	The particle size distribution is one of the most extensively studied CQA, because it has a significant impact on stability, opsonization, MPS uptake, iron release profile, PK, target tissue distribution and, as a consequence, in the efficacy and safety profile of the iron-carbohydrate complexes.	Atomic force microscopy (AFM) Dynamic light scattering (DLS) Near-field scanning optical microscopy (NSOM) Scanning Electron Microscopy (SEM) Scanning tunneling microscopy (STM) Sedimentation velocity analytical ultracentrifugation (SV-AUC) Small angle x-ray scattering (SAXS) Surface-enhanced Raman scattering (SERS) Transmission electron microscopy (TEM) X-ray diffraction (XRD)
	pH	The pH of the final product is critical for the safety profile of formulations intravenously administered, which must be biocompatible. pH values different from plasma pH may cause irritation, vasculitis, thrombosis, and emboli. This CQA can be impacted by formulation variables, container closure, raw materials, process parameters, scale/equipments, and site. This attribute is present in a specific test of the USP monograph of Iron Dextran Injection, Ferumoxides Injection, and Iron Sucrose Injection, with different intervals of pH value.	USP <791> pH
	Physical appearance	The changes in appearance of formulations can indicate physical instability that can be due to degradation, phase separation, aggregation, fusion, and structure modifications. These phenomenons can compromise the quality, efficacy, and safety of drug products.	Particulate Matter in Injections ⟨788⟩ (Light obscuration particle count test Microscopic particle count test) Visual inspection Visible Particulates in Injections ⟨790⟩
	Polydispersity index (PDI)	Polydispersity is a physical parameter related to the particle size distribution, which, in turn, influences the pharmacokinetic profile and the product performance (safety and efficacy). Also, the tendency of iron-carbohydrate complexes to accumulate in the target tissue depends on this quality attribute. The polydispersity index can also affect the bulk properties,	Dynamic light scattering (DLS)

		processability, stability, and appearance of the final product. Thus, it is very important to obtain products with low values for the polydispersity index indicative of a monodisperse population.	
	Residual solvents	Residual solvents can impact the drug product safety profile when used in the manufacturing process because most of the time they cannot be completely removed from the drug product. Thus, this CQA should be evaluated and quantified during product and process development, according to USP <467> Residual solvents and ICH Q3C(R6) requirements, and for each type of solvent used.	Evaporative Light Scattering Detector (ELSD) Gas chromatography (GC) High-Performance Liquid Chromatography (HPLC)/ Ultra Performance Liquid Chromatography (UPLC) Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS) Near-Infrared Spectroscopy (NIR) Raman Spectroscopy
	Specific gravity	Relative density, or specific gravity, is a USP requirement present in the USP monograph of Iron Sucrose Injection and Ferumoxides Injection. This requirement is defined as the ratio of the weight of a substance to a weight of an equal volume of standard (water). The density and suspension proprieties of the nanoparticles in the formulation should be considered due to an impact in physical stability, potential for agglomeration, and the performance characteristics of the drug product.	USP <841> Specific gravity Oscillating transducer density meter Pycnometry
	Stability of the formulation	The physicochemical stability of the iron-carbohydrate complexes includes the stability on storage of the product, in order to maintain therapeutic potential and ensure the quality of the medicinal product during the entire shelf-life, as well as, the in-use stability (including after re-constitution with recommended diluents for administration) with consideration to instructions for administration in the SmPC, e.g. concentration. Thus, the stability of the iron-carbohydrate complexes is critical das it relates to the fraction of labile iron released at the time of administration and the short term stability in plasma, as labile iron has well known direct toxic effects and may influence pharmacokinetics and body distribution. Stability studies should include tests to assess the microbiological, physical, and chemical stability of the formulation. Nanoparticulate products are susceptible to fusion, aggregation, leakage, or structure and surface chemistry modifications. The stability of the formulation	Atomic force microscopy (AFM) Dynamic light scattering (DLS) Energy-dispersive X-ray spectroscopy (EDX) Small angle x-ray scattering (SAXS) Transmission electron microscopy (TEM) X-ray diffraction (XRD)

		should be appropriately evaluated in accordance with the concepts included in guideline ICH Q1A(R2), ICH Q5C, and USP <1049> Quality of Biotechnological Products.	
	Sterility	Non-compliance with microbial limits has the potential to harm the patients particularly when the medicinal product is intended to be administered intravenously. The sterility may be influenced by process parameters, formulation variables, container closure, scale/equipments and site, which can impact patient safety. Therefore, these attributes should be investigated during product and process development and conform to methods described in Sterility Tests ⟨71⟩ or by an approved alternative method.	USP <71> Sterility test
	Surface and coating properties	Upon injection into the bloodstream, the size and surface properties of the iron-carbohydrate complexes could change significantly depending on the stability of the complex and the different environments between the storage conditions and body. The surface properties of iron nanoparticles should be assessed, due to the strong impact on the cellular uptake, biodistribution, and hence the product's safety and efficacy profile.	Atomic force microscopy (AFM) Attenuated total reflection-Fourier transform infrared (ATM-FTIR) Confocal Laser Scanning Microscopy (CLSM) Scanning Electron Microscopy (SEM) Transmission Electron Microscopy (TEM)
	Titratable alkalinity	Alkaline measurements are of great importance in formulation intravenous administrated, due to the impact in tolerability and safety profile. The alkalinity is an attribute present in a specific test of the USP monograph of Iron Sucrose Injection.	Titration method with hydrochloric acid
	Turbidity point	The turbidity point of the iron-carbohydrate complexes can indicate the stability of the formulation in the physiological environment, which is related to the safety profile of him. This attribute is a specific test present in the USP monograph of iron sucrose injection.	Electrometric determination
	Uniformity of dose (fill volume/deliverable volume)	An accurate fill volume is crucial to ensure the required dosage, which is mandatory to ensure the efficacy and safety of the drug product. Fill volume per vial should be investigated during product and process development, in accordance with USP <697> Container content for injections and USP <905> Uniformity of dosage units.	Atomic absorption spectroscopy (AAS)
	Viscosity	Viscosity is an important rheological QA that should be evaluated throughout the product and process development, because it may impact the efficacy, safety, and physical stability of the drug products.	Rheometry
	Zeta Potential/surface charge	Zeta potential is the electric charge on the particle surface and is an important quality attribute for the evaluation of the physical stability of colloidal systems, since that reflects the electrostatic repulsive force between particles, and may influence their efficient interactions with cells and tissues, in vivo clearance, tissue distribution, and intracellular uptake. The	Conductivity Meter Dynamic Light Scattering (DLS) Electrophoretic Light Scattering (ELS)

		particle aggregation, sedimentation, or flocculation is prevented when the formulation exhibits a zeta potential higher than +30 mV or lower than − 30 mV, being considered stable colloidal dispersions.	Laser Doppler Anemometry (LDA)
Specific Critical Attributes (Iron core)	Chemical structure of iron core	The polymorphic form of the iron comprising the core, i.e. the crystallinity of iron oxide or oxyhydroxide core, is a critical quality attribute that should be characterized, due to the impact on the in vivo stability and iron release from the core.	Extended X-ray absorption fine structure (EXAFS) Mössbauer spectroscopy Raman spectroscopy Transmission electron microscopy/nano beam electron diffraction (TEM/NBED) Transmission electron microscopy/selected area electron diffraction (TEM/SAED) X-ray absorption near-edge structure (XANES) X-ray diffraction (XRD)
	Elemental ratio of iron and carbon	The elemental iron to carbohydrate molar ratio has an impact on the iron content, structure, and composition of iron complexes, which in turn influences the pharmacokinetic and pharmacodynamic properties, bioavailability, toxicological profile, and efficacy of the drug product.	Elemental analysis Atomic absorption spectroscopy (AAS) Energy-dispersive X-ray spectroscopy (EDX)
	Iron content (total iron, ionic iron, colloidal iron)	The content uniformity of iron has an impact on the concentration in plasma, so they are critical for the iron release rate, pharmacokinetics, toxicological profile, safety, and efficacy of the iron-carbohydrate complexes.	Atomic absorption spectroscopy (AAS)
	Iron core size and morphology	The iron core size and morphology have a direct influence on the iron release rate, in vivo stability, macrophage uptake, PK, and tissue distribution.	Atomic force microscopy (AFM) Mössbauer spectroscopy Transmission electron microscopy (TEM) X-ray diffraction (XRD)
	Iron core environment	The iron core environment could affect the in vivo stability, iron release, and toxicologic profile of iron-carbohydrate complexes. Thus, must be identified the valence state of iron, the spin state of ferric, type of coordination state of iron atoms, details of ligand binding, or presence of iron-oxygen bonds.	Electron paramagnetic resonance spectroscopy (EPRS) Mössbauer spectroscopy Raman spectroscopy UV/Vis spectroscopy

	Magnetic properties of the core	For the iron-carbohydrate complexes with magnetic properties, is essential to perform a comparative magnetic characterization (field frequency, temperature-dependent magnetization, dynamic magnetic susceptibility), to measure overall sameness in the iron core, sense subtle differences in iron core structure and environment or detect impurities. This attribute is present in the USP monograph of Ferumoxides Injection.	C-13 nuclear magnetic resonance (13C NMR) Electron magnetic resonance spectroscopy (EMR) Electron paramagnetic resonance spectroscopy (EPRS) Magnetic susceptibility balance (MSB) Mössbauer spectroscopy Neutron scattering Superconducting quantum interference device (SQUID) Vibrating sample magnetometer (VSM)
	Reduction potential (Fe3+ to Fe2+) and Fe(II) content	Reduction potential (Fe3+ to Fe2+) is related to in vivo stability and toxicological/safety profile of the product. The determination of the ratio of divalent and trivalent iron or divalent iron content is controlled as an impurity in intravenous iron-carbohydrate complexes.	Potentiometric titration Cerimetric titration Polarography
Specific Critical Attributes (Carbohydrate shell)	Carbohydrate-iron core interactions	Significant differences in tissue distribution and toxicological profiles have also been described for different types of carbohydrates. Therefore, the carbohydrate shell properties (type and composition), are an important CQA that should be evaluated throughout the product and process development. Where applied, the high processing temperatures or heat sterilization of the finished product can lead to changes in the composition of the carbohydrate matrix.	Changes in particle size under dilution Polarography
	Characterization of polysaccharides	The control of monograph specifications and suitable characterization of the quality standards and purity of the carbohydrate starting materials is fundamental for the manufacture of the active substance and finished product (description, source and characterization, manufacture, assay, impurity profile, and stability characteristics). The carbohydrate starting material may be subject to modifications, as the activation to enable binding. In the same way, the carbohydrate matrix composition can be changed due to the high processing temperatures or heat sterilization of the finished product (if applicable). It is also required additional characterization and comparability studies when are used several suppliers.	Copper assay Nuclear magnetic resonance (NMR) Size exclusion chromatography (SEC)
	Identification of carbohydrate matrix	The quality standard for carbohydrates used in the manufacture of the active substance and the finished product must be assessed (description, source and characterization, manufacture, assay, impurity profile, and stability characteristics), due to the influence on the	Changes in particle size under dilution

		pharmacokinetics and tissue distribution, formation of coating specific degradation products and potential to cause adverse reactions (anaphylactic/anaphylactoid reactions).	Differential scanning calorimetry (DSC) Fourier transform infrared spectroscopy (FTIR) Polarography Thermal gravimetric analysis (TGA)
	Structure and composition of carbohydrate matrix	The properties of the carbohydrate shell coating materials and carbohydrate shell-iron core interactions affect the opsonization, MPS uptake, PK, biodistribution, iron release rate, and in vivo stability, and efficacy of iron-carbohydrate complexes. In this way, is necessary to implement procedures for control and characterization of the physicochemical properties of the carbohydrate matrix, due to the potential for anaphylactic/anaphylactoid reactions, the influence on the pharmacokinetics and body distribution, and the formation of coating specific degradation products.	Differential scanning calorimetry (DSC) Fourier transform infrared spectroscopy (FTIR) Thermal gravimetric analysis (TGA)
	Zeta Potential/surface charge	The type of carbohydrate selected has a significant impact on surface charge or zeta potential of nanoparticles, with the consequent impact in the ways and different velocities of opsonization, uptake in the reticuloendothelial system (RES), PK, and tissue distribution. On the other hand, the internalization of iron varies according to the surface properties of the carbohydrate shell, which results in significant variability for different types of iron-carbohydrate complexes.	Conductivity Meter Dynamic Light Scattering (DLS) Electrophoretic Light Scattering (ELS) Laser Doppler Anemometry (LDA)

5.3. Non-Clinical Characterization of Iron-Carbohydrate Complexes

The pharmaceutical quality characterization itself does not provide an adequate guarantee as to the full comparability between the iron reference product and its follow-on version [132,161]. Consequently, the regulatory evaluation should consist of a 'stepwise and weight of evidence approach', including the non-clinical and clinical studies [132,161].

The 'Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product' (EMA, 2015), provided useful support to establishing pharmaceutical comparability between the reference products and follow-on versions, through the description of a series of non-clinical and clinical tests [161]. Likewise, as referred to in the 'Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications' the comparative data from non-clinical studies on the time-dependent iron content in the major target organs may be used to support the claim of the essential similarity of generic and reference nanoparticle iron medicinal products [281].

The non-clinical studies are important to provide the first evidence about the biodistribution of colloidal IV iron carbohydrate complexes, as well as, broadening the knowledge of the degradation profile and uptake mechanism by macrophages [132,161,635].

As described in Table 23 (Section 5.1), the biodistribution studies should be based on the distribution, accumulation, and retention of intravenous iron-based nanoparticles in at least three relevant compartments, such as: plasma (or serum) and red blood cells; macrophages of the reticular endothelial system (RES) (e.g. in the spleen, liver (Kupffer cells)); and target tissues/organs (pharmacological target tissues e.g. bone marrow, and toxicological target tissues e.g. kidney, liver (hepatocytes), lungs, or heart) [161].

The design of the non-clinical studies of evaluation of distribution in rodents shall include the establishment of effective dosage regimens, sampling time points, as well as, the definition of target organs and tissues selected [132,161].

The development of other methods to measure the distribution, the uptake of the nanoparticles, and their degradation or solubilization products are acceptable if shown to be appropriate (accuracy/sensitivity of the method) [132,161].

The prior knowledge of the biodistribution of the iron reference product plays an important role in the design of non-clinical studies, such as the target organs and tissues selected for the measurement of analytes and the distribution pattern of the reference product [132,161].

One of the main research shortcomings to demonstrate the similarity of iron-carbohydrate complexes is the insufficient regulatory experience with comparative non-clinical bio-distribution studies, which bridge the physicochemical characterization with the clinical outcomes [161,635]. This limitation is because the physiological mechanisms and the critical attributes relevant to the uptake of the iron-carbohydrate complexes are still not fully identified [132]. Identifying critical

quality attributes (Section 5.2) aims to tackle part of these identified weaknesses of the regulatory framework of iron-carbohydrate complexes. Another issue that was identified by non-clinical studies is the demonstration that similar physicochemical properties do not necessarily ensure similar biodistribution. Thus, the clinical studies are critical to the extrapolation of efficacy and safety results in the human body [132].

5.4. Clinical Characterization of Iron-Carbohydrate Complexes

As mentioned above, the clinical studies need to be performed to demonstrate sufficient evidence of similarity and detect the clinically significant differences that may not be properly identified in quality and non-clinical studies [132,161,162]. These include the evaluation of the pharmacokinetic profile, the efficacy and safety studies, and the post-marketing pharmacovigilance control through the Risk Management Plan (RMP) [161].

The pharmacokinetics studies should include the comparability between the follow-on versions and their reference products [132,161]. It is also recommended a single-dose parallel or crossover design, the inclusion of primary variables, such as the AUC and Cmax of total and transferrin-bound iron, and the baseline correction to decrease interindividual variability [132,161].

On the other hand, the clinical assessment must be accompanied by scientific advice in drawing up the study design [132,161]. As recommended in the 'Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed concerning an innovator medicinal product', the clinical trial shall be at least 3 months; performed in patients with a similar aetiology for anemia; and should include several endpoints as ferritin, transferrin saturation, hemoglobin, total iron dose administered over study, and total EPO dose administered over study [161].

Regarding the evaluation of the safety, it is important to take into consideration the monitoring of adverse events and markers, such as the anaphylactoid reaction rate, non-transferrin bound iron (NTBI), overall adverse event rates, and markers of oxidative stress and free radical activity [161]. Many authors have reported the clinical data that needs to be considered for the demonstration of therapeutic equivalence and/or interchangeability of iron-carbohydrate products [162,645,646,667].

However, the quality, non-clinical, and clinical studies in pre-authorization procedures may not provide sufficient data to establish the full therapeutic equivalence of follow-on versions [34,132,161,162]. For example, the pharmacokinetic studies that are underway in a short period, might not reflect the real incidence of adverse reactions in the post-marketing authorization [161]. Therefore, it is essential the implementation of an appropriate post-marketing surveillance program and the respective Risk Management Plan (RMP) [161]. Thus, the international

harmonization of the protocols of pharmacovigilance, as well as, of observational or epidemiological studies might be of even greater importance to identify and monitor the potential differences in quality, safety, and efficacy of each follow-on version post-authorization [34,132,161,162].

In March 2011, FDA approved the first follow-on version of Sodium Ferric Gluconate Complex of the reference product Ferrlecit®, through the demonstration of equivalence in formulation composition (qualitatively and quantitatively the same), product physicochemical characteristics, as well as equivalence in vivo pharmacokinetics [76,77]. Subsequently, the FDA issued a solicitation in 2014 to evaluate the therapeutic equivalence through the in vivo studies to compare plasma total iron (TI), transferrin bound iron (TBI), non-transferrin bound iron (NTBI) levels, and oxidative stress after i.v. administration of RLD and generic sodium ferric gluconate injections in healthy subjects. This constitutes a strategy of post-market surveillance on approved generic iron complex products, intending to promote a better knowledge and discussion of potential concerns regarding the quality of follow-on versions and support the Agency's review standards [670].

6. Analytical Techniques for Physicochemical Characterization of Iron-Carbohydrate Complexes

As mentioned in Section 5.2, a well-defined manufacturing process with satisfactory process controls and extensive product characterization, are critical to assure consistent quality of iron-carbohydrate complexes, as well as, to determine the therapeutic equivalence between the follow-on version and their reference product [161,642]. Therewith, it is crucial the definition and development of specific techniques for the characterization of iron-carbohydrate complexes, according to the critical quality attributes aforementioned [161,642].

Although these attributes are defined with a suitable control strategy, based on relevant pharmacopoeial monographs and product-specific draft guidance, the corresponding techniques are not properly documented [161,642]. Consequently, the comparability of two iron-carbohydrate complexes is hampered by the lack of availability of reliable and validated analytical techniques for some types of critical attributes [132,161,642]. As specified in the scientific article 'Physicochemical Characterization of Iron Carbohydrate Colloid Drug Products', 'to ensure accurate and reproducible characterization of iron colloids, the characterization methods for quantitative analysis of stoichiometric ratio, particle size, molecular weight, ferrous content, low molecular weight iron, labile iron, and other properties need to be appropriately validated for measurement range, robustness, repeatability, precision, and accuracy' [642]. This information is also available on FDA product-specific guidance documents, where the regulatory authority

acknowledges that shall be demonstrated the suitability (accuracy and precision) of the different analytical methods [132,264,265,267,273,274].

On the other hand, due to the high complexity associated with the iron formulations, the use of a single analytical technique might be insufficient to demonstrate the similarity of one critical attribute between two products, with considerable accuracy [161,642]. As indicated earlier, the results depend on the method selected and should be taken into account the use of orthogonal and complementary analytical techniques to guarantee their consistency [161,642].

For example, the morphological characterization of size and polymorphic form of the iron core is challenging, as it depends directly on the degree of crystallinity of the compounds. The use of X-ray Diffraction (XRD) has demonstrated conflicting, unclear, and inaccurate results for the inconsistent crystallite structures of Ferrlecit® and Venofer®, to present a mixture of other species besides the akaganeite structure. The poor crystallinity of these complexes, the free carbohydrates interferences, and the variability in experimental conditions, led to the poor quality of XRD results. Thus, the use of a single detection technique was not sufficiently sensitive or reproducible to detect the subtle differences of two iron-carbohydrate complexes, which implies the use of additional techniques [132,642].

Furthermore, have also been identified difficulties, disagreements, and variability in the characterization of size distribution through different techniques, such as DLS, TEM, Cryo-TEM, or AFM. The results associated with the particle size measurement strongly depend on several factors, such as the sample preparation, the number of measurements, particle concentration, dispersion medium composition and viscosity, refractive index, set temperature, cuvette size and type. It has been documented important differences in iron core size measurements by different techniques. For example, the iron core size of the iron dextran complex was measured to be 1.9 ± 0.3 nm by Cryo-TEM, and 5.6 ± 1.2 nm by TEM [137]. Furthermore, Di Francesco *et al* refer that the value of iron sucrose complex diameter is approximately 10 nm by DLS technique and 4 to 80 nm by AFM technique [660]. On the other hand, the scientific article of Yong Wua *et al* set out several examples of iron-carbohydrate complexes with distinct results of core size and particle size determined by the same technique [639].

The considerable variations in the data obtained may result from different factors such as the sample preparation (e.g. dilution or dialysis) and creation of artifacts in iron core structure, the presence of free sucrose or other excipients, instrument variability, the lowest detection limit of the equipment, or data analysis [642,660].

A further important example is the kinetic studies of Fe(III) reduction by acid degradation, which are performed in vitro under experimental conditions that do not predict the physiological environment within the human body, it is impossible to draw any relevant conclusions with significantly different conditions [132].

It becomes crucial the head-to-head comparative analysis and extensive investigations, as effective strategies to eliminate the variability associated with the analytical characterization [161,642]. Such a strategy should include a sufficient number of batches of iron formulations to provide a robust and statistically meaningful comparative analysis [161,642]. The comparative analysis also should be considered when there is a change in the manufacturing process, manufacturing site, scale-up, among others [161,642]. The results of each study are dependent and sensitive to the sample preparation, experimental conditions, and data analysis, therefore the methods developed should be guaranteed the integrity/stability of the iron-carbohydrate complexes, and must be conducted under the same experimental conditions [161,642].

Table 25 provides an overview of the available characterization techniques commonly recommended and reported in the literature, to demonstrate the therapeutic equivalence of iron-carbohydrate complexes. The description and discussion related to the characterization of iron-carbohydrate complexes are aimed to fill the knowledge gap on analytical techniques more appropriate to support the development and drug product quality.

Table 25. Analytical techniques for the characterization of the iron-carbohydrate complexes [142,161,238,430–432,635,639,642,644,655,660,667,671,672].

Technique	Analytical purpose Main role	Adavantages	Disadvantages
Analytical ultracentrifugation **Sedimentation velocity analytical ultracentrifugation (SV-AUC)**	Molecular weight distribution. Particle size distribution and uniformity.	Absolute method. Dispersive method - mixtures are fractionated during analysis. Gives access to geometric (size, shape, structure) and thermodynamic properties (equilibrium constants, free energies, enthalpies, entropies). High statistical reliability for complex mixtures fractionated - detection of all particles sedimented. Sedimentation and spectroscopic properties - allows resolution on Ångström scale. Versatile tool - multiple, synchronous optical systems. Wide range of molecular sizes/densities.	Require highly trained personnel for instrumentation operation and data analysis. The precision and accuracy are not well established for low levels of aggregates.
Asymmetric field-flow fractionation (AFFF)	Molecular weight distribution. Particle size distribution.	Allows to provide information about the size distribution of complex samples (high polydispersity). The particles are separated by the mobility induced by a laminar flow field and consequent interaction with a second perpendicularly field force.	Agglomerates and aggregates of particles are not determined. Complex algorithm to conclude about the size distribution. Complicated and expensive analysis. Limited to semipermeable membranes and size range of 1 µm. The ionic strength of the components influences the retention of analytes.
Atomic absorption spectroscopy (AAS)	Equivalence in stoichiometric ratios of iron, free and other relevant components. Iron content (total iron, ionic iron, colloidal iron). Uniformity of dose (fill volume/deliverable volume).	Easy data collection - simple to use. High analytical selectivity. High analytical sensitivity. Rapid measurement. Small amount of sample is required.	Expensive (high cost related to the type of atomizer and individual source lamps required for each component). Low sample yield. Requires a high skill level of analytical operator. The sample shall be in a solution or volatile form.
Atomic force microscopy (AFM)	Iron core size and morphology. Particle size and size distribution, shape heterogeneity,	Allows obtaining high-resolution optical images of distinct characteristics (shape, size distribution, surface properties).	Difficult to standardize and validate. Possibility of artifacts concerning the iron core size or structure modifications during sample preparation

	agglomeration, and aggregation status. Surface and coating properties. Uniformity and stability of the formulation.	Direct measurement in dry sample or aqueous suspension; not required to operate in a vacuum system. Non-invasive. Possibility to perform nanometer-scale measurements - mapping a three-dimensional sample surface resolution (both vertical height and lateral diameter of iron complexes). The surface topography is detected by a scanning probe, through the forces measured from the interaction between both surfaces.	(washing, drying steps, support surfaces of equipments, the shape of the probe, and scan mode). Possibility of interference from carbohydrates at different concentrations. The morphology and measurement of lateral dimensions are dramatically influenced by the sample concentration. Time-consuming.
Conductivity Meter	Surface charge. Zeta Potential.	Easy data collection. Fast responding. Low maintenance cost. Provides information about zeta potential, surface charge, and electrophoretic mobility. Reliable.	Limited sensitivity. Requires electrical conductivity of formulation.
Colorimetric assays (Bleomycin assay Chromazurol B assay Copper assay Ferrozine assay *MAK025 assay*)	Characterization of polysaccharides. Labile iron.	Bleomycin assay specifically detects redox-active iron. Ferrozine assay determines the low molecular weight iron, labile iron, and transferrin bound iron in serum - can detect the iron in both ferrous or ferric state	Bleomycin assay cannot distinguish free iron and labile iron - the amount of free iron is included in the measured labile iron. High cross-lab variability in labile iron measurement. The acidic conditions (low pH) lead to the degradation of carbohydrates into reducing sugars (e.g. hydrolysis of sucrose into glucose and fructose), resulting in overestimation of labile iron. The binding affinity of selected chelators and their concentration influences the values of labile iron assays.
Confocal Laser Scanning Microscopy (CLSM)	Surface and coating properties.	Allows obtaining superior and high-resolution optical images of distinct characteristics (shape, size, and internal structure of iron nanoparticles). Direct and non-invasive technique.	Expensive. Requires the sample preparation. Unable to produce high definition images of small nanoparticles.
Differential scanning calorimetry (DSC)	Identification of formulation components (chemical composition) including the iron, carbohydrates, and functional excipients.	Allows the characterization of the carbohydrate shell. Provides detailed information about thermal behavior and stability of iron complex structures. Provides information about the water loss as well as the melting of the complex.	It applies only to formulations containing components with the phase transition temperature in the operating temperature range of the equipment. Relative low accuracy and precision.

		Rapid measurement. Small amount of sample is required.	
Dynamic light scattering (DLS)	Hydrodynamic diameter of iron nanoparticles in suspension. Particle size distribution - Polydispersity index (PDI). Stability and uniformity of the formulation. Zeta potential and surface charge.	Moderate expenses on equipment. Rapid, precise, accurate, versatile, and straightforward method, with good reproducibility for routine analyses. Reliability of the results. Simple preparation of the sample. Small volume of sample required.	Can provide misleading and non-reliable results, due to polydisperse samples, dust, or aggregates. Limited information about morphology/shape. Low resolution for polydisperse samples. Only applicable to suspensions. Overestimation of the particle size in the presence of larger structures or aggregates in the sample bulk. Size determination restrictions. The dilution of samples impacts in quality of results. The particle size determinations are affected by the experimental conditions, such as the existence of free sucrose and/or gluconic acid in the sample. The zeta potential of iron complexes is sensitive to sample preparation and measurement conditions (e.g. pH, buffer, concentration). Unable to determine the size of the iron core. Unable to sufficiently distinguish subtle differences between distinct iron-carbohydrate complexes.
Electron paramagnetic resonance spectroscopy (EPRS) **Electron magnetic resonance spectroscopy (EMR)**	Iron core environment. Magnetic properties of the iron core.	Non-destructive technique. Provide useful information related to the iron core environment and magnetic properties of iron structure. Rapid data collection. Small sample size is required. Spectra and g-factor values of iron species are dependent on different temperatures.	Complexity of spectra obtained. Sensitive towards (para)magnetic species.
Electrophoretic Light Scattering (ELS)	Zeta potential and surface charge.	Provides information about electrophoretic mobility of particles in dispersion, which is converted to zeta potential.	Can provide non-reliable results in low concentrations of smaller particles.
Energy-dispersive X-ray spectroscopy (EDX)	Elemental ratio of iron and carbon. Equivalence in stoichiometric ratios of iron, free and other relevant components. Stability of the formulation.	Allows performing elemental analysis to determine the amount (wt%) of the carbon (C), hydrogen (H), nitrogen (N), and iron in the complexes. Easy data collection, processing, and interpretation. Good spatial resolution. Possibility of use as an attachment to the SEM.	Expensive (high cost of instrumentation). High volume of sample. Limited sensitivity at low concentrations. Provides strictly atomic information.

		Rapid measurement (analysis performed in minutes).	
Evaporative Light Scattering Detector (ELSD)	Residual solvents	High reproducibility. Robust.	Destructive. Interference. Low sensitivity. Requirement for highly volatile mobile phases.
Extended X-ray absorption fine structure (EXAFS) **X-ray absorption near-edge structure (XANES)**	Identification of chemical structure of iron core.	Provides information on the types of ligands, distances, oxidation state, covalency and coordination number of the atoms surrounding the iron, indicative of structure and chemical stability of the complex.	Provides only radial structure information (no angular resolution) - it is not possible to directly infer about geometry.
Fourier transform infrared spectroscopy (FTIR) **Attenuated total reflection-Fourier transform infrared (ATM-FTIR)**	Identification of structure, chemical composition and conformation. Surface and coating properties.	Accurate and reproducible method. Evaluation of the iron core crystallinity and the characteristic functional groups of iron oxides, specifically the presence of Fe (II) and Fe (III) within the core structure, and characterization of precursors from different synthesis methods. Provides information about the composition of the carbohydrate shell. Rapid measurement.	Complex sample preparation - need to remove free carbohydrates before FTIR analysis due to the signals interference in the spectra (e.g. free sucrose or gluconic acid). Complicated interpretation of spectra derived from complex samples. Difficulty in analyzing aqueous samples.
Freezing point depression	Osmolarity/ Osmolality.	Inexpensive. Rapid measurement. Simple to use. Small sample size is required.	Not suitable for formulation with high molality. Samples should be low viscosity.
Gas chromatography (GC)	Residual solvents.	High resolution. High sensitivity. Quantitatively analysis. Reliable method. Short analysis time.	Limited to thermolabile samples. Require other complementary techniques for confirmation of peak identity. Requires volatile samples.
Gel permeation chromatography (GPC)	Molecular weight distribution.	Can provide narrow bands. Good sensitivity. No sample loss. Short analysis time. Small amount of mobile phase is required. The flow rate can be set. Well defined separation.	Cannot be used for high molar mass or insoluble polymers. Limited number of peaks that can be resolved. Need to select an appropriate molecular weight calibration standards and calibration curve plotting method(chromatographic conditions, columns, and calibration standards).

			Require pre-filtrations of sample - performed before using the instrument.
Gel-clot technique	Bacterial endotoxins.	Easy to perform. High sensitivity. Inexpensive. Low equipment costs. Low variability. Qualitative test – quick and simple. Reference method of USP.	Compliance issues. Fixed incubation time. Limited 'limit of detection'. No automation. Possibility of interferences. Product compatibility. Quantitation is difficult. Subjective results - Margin of errors.
High-performance liquid chromatography (HPLC) **Ultra Performance Liquid Chromatography (UPLC)**	Assay (carbohydrate). Equivalence in stoichiometric ratios of iron, free and other relevant components. Residual solvents.	High analytical selectivity. High analytical sensitivity. Provides information about the identification and quantification of different components in the formulation. Robust and reliable.	Expensive (high cost of instrumentation). Requires a high skill level of analytical operator. Requires sample processing. Time-consuming.
In vitro dialysis system	Labile iron. Low molecular weight iron species.	Easy to perform. Possibility to perform the method in a large volume of samples.	The low molecular weight iron quantification depends on the components in dialysis buffer, pH of the buffer, volume ratio of iron colloid suspension and dialysis buffer, and the dialysis membrane cutoff.
Laser Doppler Anemometry (LDA)	Zeta potential and surface charge.	High-frequency response. Provides information on surface charge of iron nanoparticles.	Expensive. Possibility of measurement errors when the velocities of beam scattering particles and flow velocity of fluid are not the same. Requires the insertion of tracer particles for operating of the method.
Light obscuration particle count test	Particulate matter. Physical appearance.	Automatic determination of the size of particles and the number of particles according to size. Easy to use. High resolution. Reference method of USP (USP <788>).	Need to check the particle-free environment - preparatory steps of cleaning. Need to perform the test under conditions limiting particulate matter, (preferably in a laminar flow cabinet). Not suitable for preparations with reduced clarity or increased viscosity. Sample preparation (dilution) is required.
Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)	Residual solvents.	Combines the capacity of physical separation of HPLC and the capacity of mass analysis by mass spectrometry (MS).	Expensive (higher operational cost). Unable to provide direct structural information.

Magnetic susceptibility balance (MSB)	Magnetic properties of the iron core.	Allows the measurements at a wide range of diamagnetic and paramagnetic materials. Ease to use. Sensitivity and accuracy. Small sample size is required.	Complex and laborious process. Complexity. Expensive instrumentation. Sensitive to electromagnetic fields. Time-consuming (in some equipments).
Mass spectroscopy (MS) **Inductively coupled plasma mass spectrometry (ICP-MS)**	Equivalence in stoichiometric ratios of iron, free and other relevant components. Molecular weight distribution.	High accuracy and precision. High sensitivity to detection. Small amount of sample is required.	Expensive equipment. Limited complete databases for the determination of certain molecular species.
Method of Acute Toxicity	Cytotoxicity.	Reference method of USP (Iron Dextran Injection monograph). Suitable to predict toxicity level.	Expensive – high labor costs. Sacrifice of animals. Time-consuming.
Microscopic particle count test	Particulate matter. Physical appearance.	Easy data collection, processing, and interpretation. Possibility to use manual, semi-automated, or fully-automated microscope systems. Reference method of USP (USP <788>).	Need to check the particle-free environment - preparatory steps of cleaning. Need to perform the test under conditions limiting particulate matter, (preferably in a laminar flow cabinet). Requires an adequate illumination and well-aligned optics. Requires operator training. Time-consuming.
Mössbauer spectroscopy	Identification of morphology and chemical structure of the iron core. Iron core environment. Magnetic properties of the iron core.	Allows the evaluation of iron core crystallinity and the presence of Fe (II) and Fe (III) within the core structure. High resolution. Low maintenance. Provides important information about the qualitative (structural) and quantitative changes in iron complexes, as well as, the distribution and electronic structure of iron.	Difficult analysis. Large amounts of sample are needed for analysis. Long data acquisition time.
Near-field scanning optical microscopy (NSOM)	Uniformity and stability concerning to charge on surface, shapes and particle size distribution.	High optical resolution. Instant measurement of fluorescence and spectroscopy. No special sample preparation needed.	High scanning time. Implies a detailed examination of sample area.
Near-Infrared Spectroscopy (NIR)	Residual solvents.	Non-invasive, non-destructive and fast method that allows the identification of compounds.	Expensive (high cost of instrumentation).

			The control of temperature, qualitative calibration model and data evaluation algorithms are required. Time-consuming.
Neutron scattering	Magnetic properties of the core.	A wide range of wavelengths can be achieved. Neutrons interact through nuclear interactions – high penetration for most elements making neutron scattering a bulk probe. Scattering nuclei are point particles. The detection signal-to-noise ratio is high.	A large amount of sample is required. High maintenance cost. Neutron sources are characterized by low fluxes - limited use in processes that depend on rapid time. Neutron sources are very expensive.
Nuclear magnetic resonance (NMR)	Characterization of polysaccharides. Magnetic properties of the core.	Easy sample preparation. Noninvasive and constructive procedure.	Low sensitivity. Need to use the same iron concentration and temperature in comparative NMR measurements, since they impact the magnetism of iron colloid samples. Requires a high amount of sample for detection. The characterization of iron complex-bound carbohydrates with 1H NMR and 13C NMR is hampered by the interference of the iron core - is needed the isolation of the carbohydrates bonded to iron cores and consequent analysis of shell. Time-consuming.
Oscillating transducer density meter	Specific gravity.	Low complexity. Reliable density determination. Short measuring time. Small sample size is required. The extraction of an exact volume is not required. The temperature measurement in situ.	Low sensitivity. Mechanical properties are influenced by pressure and temperature
Polarography	Identification of the iron core structure, carbohydrate matrix, and carbohydrate-iron core interactions. Reduction potential (Fe3+ to Fe2+) and Fe(II) content.	Electrochemical method. Provides information about the iron core structure, carbohydrate interactions, assay of iron, iron core stability, and analysis of reducible or oxidizable substances in a solution. Provides specific and characteristic polarogram shapes and reduction potentials for each type of iron colloid formulation.	Low reproducibility and high variability for accurate measurement of Fe(II) in the iron sample. Low sensitivity in the quantitation of low molecular weight iron species. The Fe(II) concentration is usually highly overestimated with pulse polarography measurement. The polarogram obtained depends on the method selected, the type of indicator electrode used, the potential ramp applied, and the pH of the solution.

		The absence of low molecular weight iron complex can be demonstrated through the absence of additional peaks in the polarogram.	
Potentiometric titrationCerimetric titration	Reduction potential (Fe3+ to Fe2+) and Fe(II) content.	Absence of side reactions. Auxiliary products are not formed. Large ORP (Oxidation-Reduction Potential) value. Simplicity of fixing the equivalence point. Stability of the standard solution of the titrant. Titration is possible at the presence of chloride ions.	Possibility of complexing or sedimentation reactions. Possibility of photochemical reduction in the presence of hydrochloric medium solutions. Require the use of indicators.
Pycnometry	Specific gravity.	Easy data collection - simple to use. Low equipment costs. Rapid measurement. Reference method of USP (USP <841>) Small size equipment.	Possibility of misinterpretation or reading errors of results. Requires a large sample volume. Requires a large solvent volume (cleaning). Time-consuming cleaning procedures.
Raman spectroscopy **Surface-enhanced Raman scattering (SERS)** **Tip-enhanced Raman spectroscopy (TERS)**	Identification of conformational variations in the chemical structure of iron core. Iron environment. Particle size distribution and hydrodynamic size. Residual solvents.	Can be used to investigate and characterize the structural stability and chemical structure of iron oxyhydroxide core, and detection/quantification of impurities and degradation products. Enables to obtain a characteristic Raman spectrum for each crystalline form of iron oxide and iron oxyhydroxide. Enhanced spatial resolution. Non-destructive and rapid technique. Sample preparation is not required. The deconvolution analysis of Raman spectra can provide useful information about the specific Raman bands of akaganéite, magnetite, and maghemite.	Enormously minute cross-section. Expensive. Fluorescence interference. Need to exclude the vibration signals from the carbohydrate shell through deconvolution analysis or dialysis analysis. The intense Raman vibration signals from the carbohydrate shell can hide the Raman vibrations related to the iron core. Weak single restricted spatial resolution.
Rheometry	Viscosity.	Allows obtaining information about the rheological properties of materials. Easy to use. Inexpensive.	Low accuracy. Possibility of deformation under stress conditions.
Scanning Electron Microscopy (SEM) **Environmental Scanning Electron Microscopy (ESEM)**	Crystal structure. Particle size and size distribution, shape heterogeneity,	Can be used to visualize small vesicles under a very large depth of field and high resolution. Easy to interpret results. Provides information about morphology, size, shape, and surface characteristics.	Cannot provide detailed information on the internal structure. Complex sample preparation. Expensive. Need of dry samples.

	agglomeration, and aggregation status.	Versatile technique.	Operation in the high-vacuum system. Requirement of solid and conductive materials. Risk of shape modification due to sample preparation required (e.g. staining processes or high vacuum system). Time-consuming.
Scanning tunneling microscopy (STM)	Particle size distribution and uniformity.	Can operate on several surfaces and temperatures. Give a 3D profile of the surface. High spatial resolution. Highly versatile.	Complex to operate effectively. Conductive surfaces are required. Demand of very clean surface and excellent vibration control. Expensive. Sensitive to external vibrations and contaminations.
Size exclusion chromatography (SEC)	Characterization of polysaccharides. Molecular weight distribution.	High reproducibility and reliability. Provides information about the stability and aggregation of nanoparticles. Separation of nanoparticles depending on its shape, size, rigidity and composition.	Low recovery. Possibility of damage of the sample.
Small-angle x-ray scattering (SAXS)	Particle size distribution, shape, and structure of iron nanoparticles. Uniformity and stability of the formulation.	Accurate. Not require a crystalline sample. Rapid data collection. Simple sample preparation. Small amount of sample is required.	Complex data analysis. Expensive and complex instrumentation. Low information content in data analysis - possible misinterpretation. Low resolution.
Static light scattering (SLS)	Molecular weight distribution.	Absolute method. Presents advantages in comparison with analytical ultracentrifugation or size exclusion chromatography to provide independent access to the basic property of mass. Provide enhanced evaluations about molecular masses, particle shape, and interactions, enabling a complete characterization of complex systems.	High demanding of experimental setup. High risk of misleading artifacts. Limited to monomodal systems. Strong restrictions on experiment and detection - physical limitation of light scattering intensity variation with particle size.
Superconducting quantum interference device (SQUID)	Magnetic properties of the core.	Provide electromagnetic measurements at much higher levels than conventional techniques. Very sensitive detector of changes in magnetic flux (magnetization measurements) and other electrical measurements.	Noise limitations of the device.

Thermal gravimetric analysis (TGA)	Identification of structure and chemical composition of the carbohydrate matrix.	Provides detailed information about the thermal behavior of the system. Provides information about the decomposition pattern of the carbohydrate shell, specifically water loss and degradation of the carbohydrate shell.	Difficult interpretation of results for complex samples.
Transmission electron microscopy (TEM) **Transmission electron microscopy/nanobeam electron diffraction (TEM/NBED)** **Transmission electron microscopy/selected area electron diffraction (TEM/SAED)**	Crystal structure. Iron core size and morphology. Particle size and size distribution, shape heterogeneity, agglomeration, and aggregation status. Surface and coating properties. Uniformity and stability of the formulation.	Allows visualization of detailed characteristics, such as particle size distribution, shape, and internal structure. Comparatively with conventional TEM, cryo-TEM maintains the native state and morphology of iron nanoparticles and prevent particle aggregation. Also, provide a higher resolution image and a more accurate measurement of iron core size compared with TEM. High spatial resolution (higher than SEM). Wide range of operational magnifications for direct visualization of iron nanoparticles.	Complex sample preparation. Expensive. Inability to distinguish the agglomeration of individual iron cores (a specific case of Injectafer®). Need to apply a proper staining to detect carbohydrate shell by TEM (due to the lower electron density of the carbohydrate). Operation in the high-vacuum system. Possibility of artifacts, damage, or structure modifications during routine sample preparation procedures (chemical fixation, rinsing, and dehydration). Time-consuming.
Ultrafiltration	Labile iron. Low molecular weight iron species.	Easy to perform. Low driving force.	Expensive. Laborious and time-consuming. Low selectivity. Membrane fouling. The low molecular weight iron quantification depends on the pH of the buffer, membrane cutoff, and centrifugation speed.
UV/Vis spectroscopy	Iron core environment.	Provides information regarding the several characteristic absorption bands, used to estimate the valence state of iron, the spin state of ferric, and the coordination state between iron atoms and ligands. The demonstration of the presence of octahedrally coordinated high spin Fe(III) ions corresponds to the standard measure of degradation kinetics of intravenous iron formulations.	Can only be applied for compounds that do absorb light at UV/Vis wavelength region Destructive. Limitations in detectable concentrations. Need to remove free carbohydrates before the UV-Vis analysis of iron colloid samples. Nonselective. Possibility of interference of free carbohydrates - UV absorption of free carbohydrates (e.g. sucrose and

			gluconic acid) hidden the UV-Vis absorption bands of iron core.
Vibrating sample magnetometer (VSM)	Magnetic properties of the iron core.	Allows the measurements at a range of angles. High precision and accuracy. Non-destructive technique. Provides information about the magnetic field/magnetic properties of the iron core.	Limited field Not suitable for the determination of the magnetization loop or the hysteresis curve. Possibility of self demagnetizing effects of the sample.
X-ray diffraction (XRD)	Evaluation of the presence of Fe (II) and Fe (III) within the core structure. Identification of chemical structure, morphology, crystallinity, and size of the iron core. Particle size distribution, shape, and structure of crystalline materials. Uniformity and stability of the formulation.	Easy data collection, processing, and interpretation. High sensitivity to long-range crystalline order - enable the distinction between several crystallite structures of iron oxide or iron oxyhydroxide. High spatial resolution. Non-destructive method. Provides information about the iron oxyhydroxide crystallite dimensions, through the XRD diffraction pattern with multiple diffraction peaks corresponding to different planes. Provides useful information about chemical structure, crystallinity (angle position, width, and intensity), atomic structure level, phase transitions, and polymorphism of the iron core. Reliability.	Can also occur peak overlay, which could hinder the interpretation of results obtained. Complex sample preparation is related to the small nanocrystals of the iron oxide or hydroxide in the 'core' and the low levels of crystallinity due to the interaction with the carbohydrate shell of the iron complex. Homogeneous and single-phase materials are preferred for identification. Possible influence of the sample preparation on the quality and reliability of results (the overlying crystalline sucrose signal hidden the iron oxide/hydroxide signals).

7. Concluding Remarks

The IV iron-carbohydrate complexes represent a group of NBCDs successfully used to treat iron deficiency, particularly in patients with chronic kidney disease (CKD). There are several types of parenteral iron-carbohydrate complexes available in the European or United States market, such as iron dextran, iron sucrose, sodium ferric gluconate, ferumoxytol, iron carboxymaltose, and iron derisomaltose. They feature a polydisperse and nanoparticular structure, with an iron (III)-oxyhydroxide core stabilized by a carbohydrate complex, which is highly dependent on a well-controlled manufacturing process. The specific disposition of the nanoparticular structure defines the pharmacodynamics of the drug product and, accordingly, its efficacy and safety profile. As a result of the advantages related to the iron-carbohydrate complexes, there has been growing interest in the development and introduction of follow-on versions on the market.

The purpose of this chapter was to discuss issues related to the current regulatory landscape involved in the assessment of therapeutic equivalence and marketing authorization of complex generic products, more precisely, the follow-on versions of colloidal IV iron-carbohydrate complexes. Therefore, the systematic analysis of the regulatory landscape was enabled to draw some conclusions, such as: the iron-carbohydrate complexes were approved through a distinct and not comparable pathway, which lead to impressive heterogeneity in regulatory approaches used for each product, and uncertainties related to their safety and efficacy evaluations; the number of reference products with therapeutic equivalents is considerably reduced due to the technical and manufacturing complexity, problems related to their efficacy and safety, regulatory challenges of demonstrating the therapeutic equivalence, the patents/intellectual property associated with the reference products, and the strict specifications for the parenteral formulations; and then withdrawn from the market of some products is mainly due to the safety issues, such as serious adverse reactions.

Clinical and non-clinical studies demonstrated significant differences in efficacy and tolerability between the follow-on versions and iron sucrose reference product when the generic approach was applied. The problems of substitutability and interchangeability arising from the standard generic approach, lead to increased awareness of the need to establish appropriate data requirements and an adapted regulatory approach for approval of follow-on versions of iron-carbohydrate complexes. Other issues raised included the difficulty of fully characterizing and defining iron-carbohydrate complexes, the impact of the manufacturing process, uncertainties about the clinically meaningful quality attributes with impact on in vivo performance, lack of advanced analytical techniques, large variability and poor accuracy of currently available testing methods, conflicting results of multiple techniques applied, and absence of guidance documents with validated methods. Therefore, it is necessary effectively to develop additional, reliable, and robust analytical techniques, and implement an extensive comparability exercise with comprehensive side-

by-side analysis between the follow-on versions and reference product, to ensure the accuracy and consistency of the data. Thus, the critical physicochemical properties which may affect the quality, safety, and efficacy of iron-carbohydrate complexes were made available in this chapter, in particular their specific critical quality attributes (including the iron core and carbohydrate shell properties).

As was to be expected, both regulatory agencies should include similar requirements for the demonstration of therapeutic equivalence of iron-carbohydrate complexes. However, the FDA did not include non-clinical studies in the demonstration of bioequivalence, contrary to the EMA that comprised the quality characterization, non-clinical and clinical studies, and pharmacovigilance system. It may be inferred the heterogeneity of the regulatory requirements has a major impact on the degree of rigidity in the approval approaches in the different Member States. It is perceptible that the straightforward analysis of drug kinetics, comparative physicochemical characterization, and qualitative and quantitative (Q1/Q2) sameness, are insufficient to guarantee the interchangeability and full efficacy and safety of these follow-on versions. Also, there are no clinical data that support that the physicochemical attributes have an impact on clinical efficacy and safety, and the equivalent plasma pharmacokinetic data do not necessarily predict tissue distribution in humans, due to the physiological iron homeostasis differs substantially in humans. As a result, supplementary non-clinical and head-to-head clinical studies are required to support the approval of generic iron-carbohydrate complexes. Thus, it is also expected that the FDA revise the guidance documents to include appropriate requirements for the approval of follow-on versions of iron-carbohydrate complexes, for example, the incorporation of clinical studies.

The regulatory framework should be composed of quality, non-clinical, and clinical studies to document the therapeutic equivalence of the follow-on versions, as well as, a post-marketing surveillance program and the respective Risk Management Plan (RMP), to reflect the real incidence of adverse reactions in the post-marketing authorization. It is also fundamental the multidisciplinary research, consensus discussions, and in-depth dialogue between the different stakeholders to establish a global, harmonized, and stepwise similarity approach that considers the complexity, serious inequalities, and problems related to the approval of follow-on versions of iron-carbohydrate complexes. Until such a regulatory approach is developed and implemented, there has to be a doubled effort by the healthcare professionals concerning the potential differences in the safety and efficacy profiles of iron-carbohydrate complexes. Consequently, the harmonization of the protocols of the post-marketing pharmacovigilance system might be of even greater importance and highly desirable, when the pharmaceutical equivalence cannot be established completely. Finally, some documents also provide support recommendations to ensure the early detection and effective management of allergic reactions that may occur, such as the ‘New recommendations to manage risk of allergic reactions with intravenous iron-containing medicines’ of the European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP).

Chapter VI. Generic Development of Glatiramer Acetate Complex Products: Regulatory and Scientific Considerations

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Abstract

Glatiramer acetate consists of the complex heterogeneous mixture of peptide copolymers indicated for the treatment of patients with relapsing-remitting forms of multiple sclerosis (RRMS).

The development and approval of follow-on versions of glatiramer acetate products are critical to improving the access of patients to this type of complex product. However, the establishment of therapeutic equivalence and marketing approval of follow-on versions presents a wide range of scientific and regulatory challenges. One of the main challenges is related to the complex nature of glatiramoids with an incalculable number of structurally closely related active peptide moieties that cannot be isolated, quantified, or identified by the available characterization techniques. The active epitopes in the glatiramer structure are not yet known, and the exact immunomodulatory mechanism of action has still not been fully understood. On the other hand, the identity, quality, and in vivo performance are inexorably dependent on the manufacturing process, since any slight variation may lead to changes in polypeptide sequences, and hence in the safety and efficacy profile of the complex drug product. Other key challenges include the absence of a globally regulatory approach to the approval of generic glatiramoid products, just as the lack of consensus regarding regulatory requirements and criteria needed to establish therapeutic equivalence.

Chapter VI provides an overview of the regulatory landscape of glatiramer acetate complex products approved in Europe (EU) and the United States (US) and outlines the regulatory challenges of establishing therapeutic equivalence of their follow-on versions. Also, it aims to highlight issues related to their classification, complexity, pharmaceutical quality, clinical efficacy, safety, and tolerability profiles, which may be helpful for the re-examination and optimization of the approval pathways for these complex generic drug products. On the other hand, it also addresses the comparative evaluation of FDA and EMA requirements for the demonstration of therapeutic equivalence, as well as, the appropriate analytical techniques to perform the physicochemical, non-clinical, and clinical characterization of glatiramer acetate complex products. Ultimately, discuss possible future directions of harmonization to the approval pathways for the assessment of therapeutic equivalence between the reference products and their follow-on versions.

Keywords

Non-Biological Complex Drugs; Glatiramer Acetate Complex; Glatiramoids; Copaxone®; Colloidal Suspension; Copolymeric Mixture; Multiple Sclerosis; Follow-on Products; Therapeutic Equivalence; Physicochemical Characterization; Clinical Evaluation; Regulatory Science Research; Regulatory Evaluation; Regulatory Landscape; Regulatory Guidance.

1. Introduction

The recent scientific advances in the Biotechnology and Nanotechnology field allowed the development and marketing approval of distinct Complex Drug Products. The main focus of this chapter is the glatiramer acetate complex products (or glatiramoids), an NBCDs that correspond to complex active pharmaceutical ingredients (API), according to the FDA 'Generic Drug User Fee Act (GDUFA) II Commitment Letter' [16].

Glatiramer acetate (GA) consists of the complex heterogeneous mixture of peptide copolymers containing four specific amino acids, L-glutamic acid, L-alanine, L-tyrosine, and L-lysine, with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively [275,333,673–675]. The average molecular weight of glatiramer acetate is 5000 to 9000 Da (Daltons), while the polypeptides in the glatiramer acetate mixture present a molecular weight distribution range of approximately 2500 to 20.000 Da [132,138,673,676–678]. This product is obtained from the ring-opening polymerization reaction of the corresponding activated amino acids NCAs (N-carboxyanhydrides), followed by side-chain depolymerization of the intermediate copolymers [679,680]. The fundamental reaction scheme for the synthesis of glatiramer acetate has been published in the US (United States) Patent previously listed in FDA's Approved Drug Products With Therapeutic Equivalence Evaluations (the Orange Book) [353,681,682].

The chemical structure of amino acid polymer chains of glatiramer acetate complexes corresponds to [678]:

$$(Glu, Ala, Lys, Tyr)_x.xCH_3COOH$$

$$(C_5H_9NO_4.C_3H_7NO_2.C_6H_{14}N_2O_2.C_9H_{11}NO_3)_x.xC_2H_4O_2$$

CAS – 147245-92-9

The Copaxone® (Teva Pharmaceutical Industry), whose active substance is the glatiramer acetate, corresponds to an NBCD approved in 1996 by the US as a first-line immunomodulatory therapy indicated for the treatment of patients with relapsing-remitting multiple sclerosis (RRMS) [679,683–686].

Multiple sclerosis is considered to be a neurodegenerative, chronic autoimmune inflammatory disorder of the central nervous system (CNS), progressively debilitating and characterized by multifocal inflammation, demyelination, cell apoptosis, oligodendrocyte, and neuroaxonal loss, impaired nerve conduction, progressive brain, and spinal cord atrophy [337,679,683,686–692]. The immune-mediated process which is responsible for this disease consists of an erroneous response of the immune system directed against myelin in the CNS [683,686,689]. The disease onset can be manifested mainly in young people aged between 20 and 50 years, affecting approximately 2.8 million people worldwide, with nearly 1 million in the US [679,683,693]. Despite the unpredictable course, this disease can be categorized as relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS), and

progressive relapsing multiple sclerosis (PRMS) [686,692]. There are several disease-modifying treatments (DMTs) approved for reducing the risk of relapses and disease progression, such as the interferon-beta-1a IFN-β1a (Avonex, Rebif), interferon-beta-1b IFN-β1b (Betaseron), glatiramer acetate (Copaxone®), dimethylfumarate, fingolimod, teriflunomide, natalizumab (Tysabri), daclizumab, alemtuzumab, and ocrelizumab [683,688].

The Summary of Product Characteristics (SmPC) of Copaxone® state that this product is not indicated in primary or secondary progressive multiple sclerosis [694]. Copaxone® is available in dosages of 20mg/mL and 40 mg/ml of sterile and nonpyrogenic solution for injection in a pre-filled syringe [678]. The glatiramer acetate mimics the myelin basic protein (MBP) considered to be one of the main autoantigens in multiple sclerosis, with higher immunomodulatory and neuroprotection activity [684,686,687,695].

The exact mechanism of action of glatiramer acetate in multiple sclerosis is not fully elucidated, but it is believed that relates to the immunomodulation of both the adaptive and innate immune systems, i.e. as an antigen-based therapeutic vaccine [132,164,338,685,696–699]. As described in the Highlights of Prescribing Information (Label) of Copaxone®, 'this hypothesis is supported by findings of studies that have been carried out to explore the pathogenesis of experimental autoimmune encephalomyelitis, a condition induced in animals through immunization against central nervous system derived material containing myelin and often used as an experimental animal model of MS. Studies in animals and in vitro systems suggest that upon its administration, glatiramer acetate-specific suppressor T-cells are induced and activated in the periphery' [678].

According to current scientific literature, this mechanism of action encompasses anti-inflammatory and neuroprotective activities. The anti-inflammatory properties include the high-affinity binding to antigen-presenting cells (APCs) and alteration of APC function, induction of a shift from a T-helper 1 (Th1) cytokine profile to a T-helper 2 (Th2) anti-inflammatory profile, and modulation of the functional properties of regulatory B cells [132,338,683,687,696,697,700–702]. On the other hand, the neuroprotective effects comprise the promotion of neurotrophic factors mediated by brain-derived neurotrophic factor (BDNF) production, the axonal protection, the decrease of glutamate-mediated neurotoxicity and demyelination, as wells as, the promotion of remyelination [132,683,696,697,702].

This chapter will provide an overview of the regulatory landscape of glatiramer acetate complex products approved in Europe (EU) and the US, and outlines the regulatory challenges of establishing therapeutic equivalence of their follow-on versions. Thus, this chapter is divided into the following sections. Section 1 provides the background concepts related to the glatiramer acetate complexes and contextualizes the intended aims of this chapter. Section 2 aims to highlight issues related to their classification, complexity, pharmaceutical quality, clinical efficacy, safety, and tolerability profiles, which may be helpful for the re-examination and optimization of the approval pathways and reduction of regulatory uncertainty for these complex generic drug products. Section

3 comprehends a regulatory landscape of glatiramer acetate complex products approved in the Europe and United States. On the other hand, Section 4 also addresses the comparative evaluation of FDA and EMA requirements for the demonstration of therapeutic equivalence of glatiramer acetate complex products, as well as, the appropriate analytical techniques to perform their physico-chemical, non-clinical, and clinical evaluation. Ultimately, Section 5 discusses possible future directions of harmonization to the approval pathways for the assessment of therapeutic equivalence between the reference products and their follow-on versions and summarizes the essential conclusions obtained with the chapter.

2. Complexity of Glatiramer Acetate Complex Products and Implications for Therapeutic Equivalence Evaluation

2.1. Classification of Glatiramer Acetate Complex Drug Products: A Faint Line between the Non-Biological and Biological Drug Products

The ‘FYs 2013-2017 Regulatory Science Report: Complex Mixtures and Peptides’ highlights that the complex drug substances market in the United States, such as the peptide drug market, grew from less than $11 billion in 2012 to more than $18 billion in 2016 [703]. This growth can be also accompanied by an increased interest in the pharmaceutical development of their follow-on versions.

The provision of follow-on versions of glatiramer acetate products is critical to improving the access of patients to this type of complex product. However, the establishment of therapeutic equivalence and marketing approval of follow-on versions presents a wide range of scientific challenges [138,334,335,679,697]. These challenges arise particularly from the classification of glatiramer acetate products (Copaxone®) as complex active pharmaceutical ingredients (API), according to the classes described in the FDA ‘Generic Drug User Fee Act (GDUFA) II Commitment Letter’ [16].

On the other hand, there are important discussions about the regulatory uncertainty related to the definition and class of products in which the glatiramer acetate is inserted, such as the NBCDs or biological drug products. The glatiramer acetate products can appear incorrectly classified as a biologic complex product in articles or conference presentations [704]. Although being synthesized by chemical processes and not derived from living sources, glatiramer acetate resembles biologics due to its immunogenicity, molecular complexity, higher-order structures, and manufacturing process dependence [164,333]. This product features a protein-like structure, with polypeptides composed of amino acids linked by peptide bonds, that does not present a specific and defined sequence, wherefore cannot be classified as a protein, neither a biological product. It corresponds

to a heterogeneous mixture of copolymers, chemically synthesized, much more complex than a polypeptide or protein derived from a biotechnological process [31,337,696,705].

In March 2010, one provision of the BPCIA (Biologics Price Competition and Innovation Act) expanded the scope of the biological products to include in their definition the proteins ‘except any chemically synthesized polypeptide’ [706]. However, ten years later, the Further Consolidated Appropriations Act (2020) amended the definition of a biological product, removing the specific exclusion of ‘chemically synthesized polypeptides’, i.e., the biologic products will now include all proteins, even the chemically synthesized polypeptides [706].

On the other hand, the FDA published a list of approved New Drug Applications (NDAs) that were converted to biologics license applications (BLAs) under section 351 of the Public Health Service Act (PHS Act), due to the Biologics Price Competition and Innovation Act of 2009 and the removal of the exception ‘any chemically synthesized polypeptide’ from the category of ‘protein’ in the statutory definition of the biological product. The FDA did not include the glatiramer acetate on the list, highlighting what has previously been said about the Copaxone® ‘is not a protein because it does not have a defined and specific sequence’ [707,708].

However, Teva Pharmaceuticals argues that the Copaxone® is ‘noticeably absent from this list’, as well as, ‘the chemically synthesized polypeptides that the FDA carefully carved out of biological products now fall within the scope of the term ‘protein’, and must be regulated as biological products’, requesting on the regulatory agency its to be introduced in them. Furthermore, Teva referred to two examples of drugs similar to Copaxone® (Vitrase® (hyaluronidase for injection) and Creon® (pancrelipase)) included in the FDA’s transition list, and emphasizes that ‘glatiramer acetate has an amino acid sequence that is at least as ‘specific’ and ‘defined’ as either of these products, and therefore should similarly meet the ‘specific, defined sequence’ portion of the FDA’s protein definition. Another issue that was raised by TEVA is that even if the Copaxone® does not constitute part of the definition of a biological product, ‘it fits squarely into the catchall category of an analogous product’ both structurally and functionally (modulates the immune response, potential immunogenicity, absorption through the lymphatic system, high molecular weight structure, among others) [706]. These requests can be considered a way of restricting the competition and effects of incoming other generic versions of Copaxone® in the pharmaceutical market, since the regulatory approval by biosimilar pathway can be much more demanding, compared with a generic approach [706,709,710].

The European Directorate For The Quality Of Medicines & Healthcare (EDQM) classified the glatiramer acetate complex product as synthetic peptides, but not Biotherapeutics or Biological Complex Products [711]. In 2016, a follow-on glatiramer acetate product gained European approval, with the tradename Synthon’s EU FOGA. This name varies according to the Member States where the product was approved (e.g. Copemyl (SE, Sweden), Clift (DE, Germany), Remurel (SK, Slovakia)). The marketing authorization of this follow-on product was granted under Article

10(3) of Directive 2001/83/EC as a hybrid product. Despite not being classified as a biological complex drug product, Synthon's EU FOGA follows an identical strategy to the requirements of biosimilars, including additional quality, nonclinical and clinical data to demonstrate the product equivalence [337,712].

For the issues enumerated above, it was possible to verify that the glatiramer acetate products stayed in a regulatory limbo relating to the uncertainties of their classification and approval requirements in the US and EU.

2.2. Batch-to-Batch Variability

The intrinsic complexity of this product is mainly due to the potentially incalculable number of non-identified peptide moieties in random order in the heterogeneous mixture, where the glatiramer acetate sequences can reach up to 1029 possible variants [337,338,679]. Thus, the amino acid chains in glatiramer acetate products may vary in length and molecular weight, rendering it impossible to guarantee the complete replication of the final amino acid sequences in each chain [27,338]. Consequently, there is a low probability of the amino acid sequences remaining identical throughout the entire chain in different batches, since the conserved sequences are restricted to small sections within the copolymer chain [682]. Therefore, the composition, physicochemical properties of the starting materials, fundamental reaction chemistry used in the manufacturing process, or variations of other characteristics are essential to establish the sameness criteria for the generic glatiramer acetate complexes [682].

However, the specific amino acid sequences or structures ('immunological epitopes') responsible for the efficacy and safety of the product are impossible to isolate, quantify, sequence, and fully characterize, through the current available discriminatory analytical technology, which hinders the proper definition of critical quality attributes and the complete physico-chemical characterization [27,335,337,674,687,697]. This is an important issue, because the changes in the physicochemical profile of glatiramer acetate (e.g. aggregation behavior, secondary and tertiary structure, molecular mass distribution, disperse electric charge distribution), result in distinct immunological and toxicological responses [333]. These challenges, together with the potential immunogenicity of glatiramer acetate and the exact mechanism of action has still not been fully understood, made it is widely recognized that the demonstration of equivalence of follow-on products with glatiramer acetate is rather difficult or even impossible, existing a considerable discussion surrounds the most appropriate regulatory requirements for follow-on versions of glatiramer acetate [34,132,138,368].

The development of advanced analytical methods and novel statistical methods will be of extreme importance to overcome the problems listed above and furthering the scientific understanding of the product complexity, the impact of each process parameter, the definition of

critical quality attributes, the exact mechanism of action, and the immunological, pharmacological and toxicological profiles [332–334].

2.3 Manufacturing Process Complexity

As referred to in the definition of NBCDs by Crommelin et al, 'the composition, quality, and in vivo performance of NBCDs are highly dependent on the manufacturing processes of both the active ingredient as well as the formulation' [17,22,24,31,661]. The complexity of glatiramer acetate requires a well-controlled manufacturing process, since any slight variation to the process may lead to changes in polypeptide sequences, and hence in the safety and efficacy profile of the product (often referred to as 'the process is the product') [31,138,333,335–338].

Of all NBCDs, the glatiramer acetate present the greatest degree of difficulty in the replication of a reference product, due to it does not have a fixed single sequence or combination of sequences, and the impossibility of generating an exact sequence data. Thus, there is no chance of obtaining batches exactly alike, due to the inherent variability of products with active structures that cannot be guaranteed identical, even where the product and process are tightly controlled [31,333,336,713].

For example, Teva produced a new glatiramer acetate product (denoted TV-5010 or protiramer), a result of the minor changes introduced in the manufacturing process of Copaxone®. Despite presenting similarity of amino acid ratio and physical properties to Copaxone®, TV-5010 showed a higher molecular mass distribution (molecular weight (MW) at 13,500–18,500 Da) and significant differences in vivo safety profile with toxic effects in long-term and repeat-dose studies (e.g. fibrosis in rats and eosinophilia in monkeys). Other studies with TV-5010 have demonstrated differences in immunogenicity profile and increased potency, as a result of structural differences and the impact on the immune response [31,138,333,335].

Other comparative gene expression studies between the reference drug product Copaxone® and the follow-on versions showed significant challenges related to the manufacturing process of high complexity mixture, and differences in their physicochemical and biological characteristics with impact on the safety and efficacy profile of them [132,335,337,674].

2.4 Regulatory Uncertainty

The glatiramer acetate products are injectable colloidal solutions, particularly subject to much higher critical requirements related to injectable formulations, such as: dispersibility, stability, injection volume, viscosity, compatibility, drug release profile, pharmacokinetic/pharmacodynamic (PK/PD) profile, site of administration, local site reactions, tissue damage, injection site pain, and injection volume, speed, or frequency [339,340].

Due to their composition and administration route, the glatiramer acetate products present considerable challenges in the establishment of bioequivalence [132,353]. One of the main problems linked to the glatiramer acetate products corresponds to the inadequacy of conventional PK studies to demonstrate glatiramer bioequivalence, due to the immediately hydrolyzed of a substantial fraction of glatiramer acetate complex at the site of the injection and uptake by local APCs, leading to an unmeasurable systemic PK profile [31,132,164,332,336,338,674,679]. In accordance with the Approved Drug Label of Copaxone®, the results of PK studies performed in humans (healthy volunteers) and animals confirm that 'a substantial fraction of the therapeutic dose delivered to patients subcutaneously is hydrolyzed locally' and 'some fraction of the injected material, either intact or partially hydrolyzed, is presumed to enter the lymphatic circulation, enabling it to reach regional lymph nodes, and some may enter the systemic circulation intact' [678]. Furthermore, these products do not present identified or validated PD markers for demonstration of clinical efficacy [332,338,687]. Thus, there is substantial uncertainty in establishing equivalence, in both quality and clinical characterization. The lack of PK/PD biomarkers established increases substantially the complexity to develop the follow-on versions of glatiramer acetate complexes and does not guarantee the safety and efficacy profile without conducting appropriate clinical studies [336,674].

In 2015, the United States Pharmacopeia (USP) established a Glatiramer Expert Panel that includes specialists from academia, regulatory authorities, and manufacturers to discuss issues related to the definition of critical quality attributes for glatiramer acetate products, development of glatiramer specifications, development of USP Glatiramer Acetate and Glatiramer Injection monographs, reviewing submitted methods (bioassays/ lot release methods that should be included in the monograph to demonstrate the efficacy and safety), characterization methods and batch data [335,677,714]. In 2020, the USP proposed a new monograph <1503>, entitled 'Quality Attributes Of Synthetic Peptide Drug Substances'. This monograph aims to discuss the potential critical quality attributes and associated test methods that should be included in specifications for peptide drug substances, particularly on peptide-related impurities, their identification, and the development of methods for their quantification [715]. On the other hand, the European Directorate for the Quality Of Medicines & Healthcare (EDQM) also features new Synthetic peptides Ph. Eur monographs in preparation, such as Glatiramer (3057) and Glatiramer injection (3104) (finished product monograph) [677,711]. The comparative evaluation of FDA and EMA requirements for the demonstration of the pharmaceutical equivalence and bioequivalence according to the available product-specific guidance documents of glatiramer acetate complex products is discussed below in section 4.

3. The Regulatory Landscape of Glatiramer Acetate Complex Drug Products approved in the EU and US

The regulatory landscape of glatiramer acetate complexes and their follow-on versions approved by the EMA and FDA are discussed in the following section. To carry out this analysis a list of glatiramer acetate complex products already approved by the EMA and FDA (Table 26) was selected through the general tables of the regulatory landscape of NBCDs and their follow-on versions approved by the FDA (Table 48) and EMA (Table 49) (see Appendix I. Supplementary Data). Thus, Table 26 divided into different columns, provides detailed information about the application number, approval date, and regulatory approach for glatiramer acetate complexes and their follow-on versions, by the respective regulatory authority. The glatiramer acetate complexes highlighted in bold correspond to reference products and the products underlined with gray are their follow-on versions. The analysis of Table 26 is described below.

Table 26. Regulatory landscape of glatiramoids and their follow-on versions approved by the EMA and FDA [27,30,60,132,138,336,338,677,678,682,684,687,689,691,692,694,696,697,702,712,716–719].

Regulatory authority	Reference product	Follow-on product	Authorisation number	Authorization date	Authorization procedure	Reference Member State (RMS) (if applicable)	Concerned Member State (CMS) (if applicable)	Application procedure
EMA	**Copaxone® 20mg/ml**	Not applicable	DE/H/5283/002 UK/H/0453/002	2004	MRP/DCP	DE	AT, BE, CY, CZ, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK	Not applicable
	Copaxone® 40mg/ml	Not applicable	DE/H/5283/004/DC	2014	DCP	DE	AT, BE, CY, CZ, DK, EE, EL, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI, SK, UK	Not applicable
	Copaxone® 20mg/ml	Brabio (20mg/ml) Glatiramer acetate Actavis (20mg/ml) Remurel (20mg/ml) Glatimyl (20mg/ml) Copemyl (20mg/ml) Meglarat (20mg/ml)	NL/H/3211/001	2016	DCP	NL	BG, CY, CZ, DK, EE, EL, FI, HR, HU, IE, IS, LT, LV, MT, NO, PL, RO, SE, SI, SK, UK	Article 10(3)
	Copaxone® 20mg/ml	Sclerthon (20mg/ml) Perscleran (20mg/ml) Glatoxone (20mg/ml) Galtipex (20mg/ml)	NL/H/3212/001	2016	DCP	NL	AT, LU, MT	Article 10(3)
	Copaxone® 20mg/ml	Glatiramer acetate Mylan (20mg/ml) Clift (20mg/ml) Glatiramyl (20mg/ml) Copemyl (20mg/ml)	NL/H/3213/001	2016	DCP	NL	BE, DE, ES, FR, IT, PT	Article 10(3)

		Glatsyn (20mg/ml)						
	Copaxone® 40mg/ml	Sclerthon (40mg/ml) Perscleran (40mg/ml) Galtipex (40mg/ml)	NL/H/3779/001	2017	DCP	NL	AT, MT	Article 10(3)
	Copaxone® 40mg/ml	Glatiramer acetate Alvogen (40mg/ml) Remurel (40mg/ml)	NL/H/3778/001	2017	DCP	NL	BG, CZ, EE, HR, HU, IS, LT, LV, PL, RO, SI, SK	Article 10(3)
	Copaxone® 40mg/ml	Glatiramer acetate Mylan (40mg/ml) Clift (40mg/ml) Copemyl (40mg/ml) Brabio (40mg/ml) Glatimyl (40mg/ml) Glatiramyl (40mg/ml)	NL/H/3777/001	2017	DCP	NL	BE, CY, DE, DK, EL, ES, FI, FR, IE, IT, NO, PT, SE, UK	Article 10(3)
	Copaxone® 40mg/ml	Marcyto (40mg/ml)	NL/H/3776/001	2017	DCP	NL	LU	Article 10(3)
	Copaxone® 20mg/ml	Glatiramer acetate Teva (20mg/ml) Glataxon (20mg/ml) Copaxobene (20mg/ml) Glatiraxone (20mg/ml)	DE/H/5449/001	2018	DCP	DE	AT, BE, HR, LU, PL, PT, SK	Article 10(c)
	Copaxone® 40mg/ml	Glatiramer acetate Teva (40mg/ml) Glataxon (40mg/ml) Copaxobene (40mg/ml) Glatiraxone (40mg/ml)	DE/H/5449/002	2018	DCP	DE	AT, BE, FI, HR, LU, PL, PT, SK	Article 10(c)

Regulatory authority	Reference product	Follow-on product	Application Number	Approval Date	Regulatory Pathway	Application procedure	Company
FDA	**Copaxone® 20mg/ml**	Not applicable	N020622	1996	New Drug Application (NDA)	505(b)(**?**)	TEVA Pharmaceuticals USA
	Copaxone® 40mg/ml	Not applicable	N020622	2014	New Drug Application (NDA)	505(b)(**?**)	TEVA Pharmaceuticals USA
	Copaxone® 20mg/ml	Glatopa (Glatiramer Acetate Injection, 20 mg/mL)	A090218	2015	Abbreviated New Drug Application (ANDA)	505(j)	Sandoz Inc
	Copaxone® 20mg/ml	Glatiramer Acetate Injection Mylan (20 mg/mL)	A091646	2017	Abbreviated New Drug Application (ANDA)	505(j)	Mylan
	Copaxone® 40mg/ml	Glatiramer Acetate Injection Mylan (40 mg/mL)	A206936	2017	Abbreviated New Drug Application (ANDA)	505(j)	Mylan
	Copaxone® 40mg/ml	Glatopa (Glatiramer Acetate Injection, 40 mg/mL)	A206921	2018	Abbreviated New Drug Application (ANDA)	505(j)	Sandoz Inc

Abbreviations: ANDA, Abbreviated New Drug Application; AT, Austria; BE, Belgium; BG, Bulgaria; CMS, Concerned Member State; CY, Cyprus; CZ, Czech Republic; DCP, Decentralised Procedure; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; EMA, European Medicines Agency; ES, Spain; FDA, U.S. Food and Drug Administration; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, Ireland; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MRP, Mutual Recognition Procedure; MT, Malta; NDA, New Drug Application; NL, Netherlands; NO, Norway; PL, Poland; PT, Portugal; RMS, Reference Member State; RO, Romania; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

The reference product Copaxone® (glatiramer acetate injection, Teva Pharmaceuticals USA), was approved by the FDA in 1996, as a once-daily 20 mg/mL injection for subcutaneous use (not administered intravenously). Afterward, a new dosing strength of Copaxone® 40 mg/mL three-times-weekly (and at least 48 hours apart) was approved in 2014 as a 'New Dosing Regimen' [58,336,337,678,684]. It is to be noted that the Copaxone® 20 mg/mL and Copaxone® 40 mg/mL are not interchangeable [678].

Regarding the submission classification, Copaxone® was approved under Type 1 (New Molecular Entity) and as an Orphan Product [716]. For this NDA (Application Number N020622), it was not possible to understand the route of submission (505(b)(1) or 505(b)(2)) [58]. This may be owing to the information is not been accessible to the public for some older products which makes it more difficult the understanding of regulatory approaches implemented, as mentioned above for certain iron-carbohydrate complexes.

In the US, the reference product Copaxone® is the most often prescribed treatment for relapsing forms of MS, becoming an important and particularly lucrative area with widespread use. Thus, there is a rapidly increasing interest in the development of follow-on versions of Copaxone®, in order to improve the access of patients to complex products with affordable prices [696,697]. So far there are four follow-on versions of glatiramer acetate products approved by the FDA, through an Abbreviated New Drug Application (ANDA) pathway, according to section 505(j) route (Generic approach) of the Federal Food, Drug, and Cosmetic Act (Table 26) [60,682,692,696,697]. The approval of follow-on versions was based on the establishment of the active ingredient sameness through the physicochemical characterization and biological data, excluding the clinical trials [138,164,337,700]. Due to this incomplete approach, the complexity of products, and the impossibility to be fully quantitated, characterized, or described by physicochemical analytical tools, certain questions have been raised concerning the appropriateness of the generics pathways in assessing public health safety and clinical efficacy [132,164].

Moreover, Copaxone® (20 mg/ml and 40 mg/ml) and their corresponding complex generic drug products (marketed as Glatopa® and Glatiramer acetate Mylan) are classified as to be therapeutically equivalent and fully substitutable (AP code for injectable aqueous solution in the Orange Book - Approved Drug Products with Therapeutic Equivalence Evaluations) [138,353,684,689,692].

The submission of the first approved generic application occurred in December 2007, while their first generic approval only happens in April 2015 [27]. This time difference of over 7 years, resulted largely from the complexity and challenges related to the product development and demonstration of therapeutic equivalence, as well as, the years of scientific and regulatory discussions, litigations, and citizen petitions submitted by Teva Pharmaceuticals to the regulatory authority (FDA) [720].

The approval of Copaxone® 20mg/ml (2004) and Copaxone® 40mg/ml (2014) occurred later in Europe, compared with the United States [689]. Regarding the glatiramer acetate copies, EMA approved 9 follow-on versions through the Decentralized Authorization Procedures: 7 follow-on versions by the Hybrid Application under Article 10(3) and 2 follow-on versions by the Informed Consent Application under Article 10(c), with different brand names depending on the Reference Member State (RMS) and Concerned Member State (CMS) (Table 26) [138,677,689,717,719].

The first generic version of glatiramer acetate was approved in June 2016 (NL/H/3211/001/DC) [336,717]. The Coordination Group for Mutual Recognition and Decentralised Procedures - Human (CMDh) advised the use of the application procedure under Article 10(3), referring that the PK study would be insufficient ('simple pharmacokinetic studies would not be appropriate for bridging the current product to the innovator product Copaxone®'), along with the importance of considering the detailed comparative characterization study with Copaxone® or other additional data required to demonstrate the similarity [721].

In contrast with the FDA classification as a generic product, the EMA considers the glatiramer acetate follow-on versions as a hybrid product, using a different approach based on detailed comparative physicochemical characterization, just as non-clinical and clinical studies [30,138,336,337,712,719]. Indeed, contrary to the generic approach of the FDA, the EMA recognizes the complexity of glatiramer acetate products and the several challenges involved in the demonstration of therapeutic equivalence with the reference products, including additional information as, for example, the clinical experience obtained with the GATE trial (Glatiramer Acetate Clinical Trial to Assess Equivalence with Copaxone®: large-scale, randomized, double-blind, placebo-controlled, multicenter phase 3) [30,138,336,692,700,712,719].

On the other hand, there are two applications, Glatiramer acetate Teva 20mg/ml (DE/H/5449/001) and Glatiramer acetate Teva 40mg/ml (DE/H/5449/002), which used the Article 10(c) (informed consent application), instead of the Article 10(3) (hybrid application) (Table 26). The informed consent application can be used by innovator companies for ensuring the market leader when there is a patent expiration, and so delay the impact of entry of generic drugs into the marketplace. This strategy is referred to as 'branded generics' [30].

As a result of this analysis, it is possible to identify that the glatiramer acetate products are a particularly important example of the scientific and regulatory divergences between US and EU regulatory agencies. It is important to emphasize that the regulatory agencies used distinct and not comparable pathways, demonstrating the need to create similar and harmonized procedures [30,138,689]. There is a general acknowledgment that the correct way to evaluate and guarantee the same quality, safety, and efficacy of the follow-on version of glatiramer acetate is to perform comparative and long-term clinical trials with a high number of patients and appropriate clinical endpoints, as well as through the characterization and documentation of the immunogenicity of the product [17,132,138].

4. Glatiramer Acetate Complex Drug Products: Therapeutic Equivalence Recommendations

4.1. Comparative Evaluation of FDA and EMA requirements for the demonstration of Therapeutic Equivalence

In line with the regulatory approach variability verified in the previous section, is important the comprehensive review and comparability of the similarities and differences of the specific regulatory requirements for evaluation and approval of follow-on versions of glatiramer acetate products in the United States and Europe.

Table 27 summarizes the FDA and EMA data requirements for the demonstration of therapeutic equivalence of follow-on versions of glatiramer acetate complex products, including the description of regulatory pathways and the guidance documents to support their regulatory approval.

In the US, the follow-on versions of Copaxone® are considered to be generic products and, consequently, follow the 505(j) approach, which requires the demonstration of therapeutic equivalence (both pharmaceutical equivalence and bioequivalence) [275,689]. The FDA published a Product-Specific Guidance (PSG) document containing recommendations on the assessment of API sameness, such as Glatiramer Acetate Injection (2016) [275]. This PSG form part of the efforts of agency research (GDUFA), to establish an efficient and consistent regulatory standard and enable the proper approval of generic products with complex drug substances.

According to the FDA's Draft Guidance on Glatiramer Acetate Injection (2016), the equivalence between follow-on versions and Copaxone® can be established across four major criteria by orthogonal analytical measurements: Equivalence of fundamental reaction scheme; Equivalence of physicochemical properties including compositions; Equivalence of structural signatures for polymerization and depolymerization; and Equivalence of biological assay results [275]. In this guidance, the FDA argues that 'since the Reference Listed Drug (RLD) product is a parenteral solution, if the proposed generic (Test) product meets the following criteria for demonstrating API sameness and is qualitatively (Q1) and quantitatively (Q2) the same in terms of active and inactive ingredients as the RLD product, the generic sponsor may request to waive the requirement of in vivo bioequivalence (BEq) study based on 21 CFR 320.22(b)(1)' [275]. It is also advisable to perform side-by-side comparative studies using the Test API and the API obtained from the RLD product, as well as, the characterization of at least three batches of the Test API and three batches of API from the RLD, to assess API sameness and robustness in the manufacturing process [275]. A particularly important aspect described in this guidance is that the biological assay, as a confirmatory test of equivalence, is performed in experimental

autoimmune encephalomyelitis (EAE) models, and the tests in humans are non-requested (i.e. without clinical studies) [275].

When the generic approach is applied, preclinical or clinical data are usually not required to define the safety and efficacy of the follow-on versions, which leads some authors to argue that the generic application is not the most appropriate [697,702]. Besides, this application may not be applicable for glatiramer acetate products due to their complexity and the impossibility to be fully quantitated, characterized, or described by physicochemical analytical tools [19,132]. On the other hand, the PK studies cannot be applied to glatiramer bioequivalence due to the rapid hydrolysis of the polypeptides in a glatiramer mixture at the site of injection [132,164]. Furthermore, these products do not present validated pharmacodynamic (PD) markers for pre-clinical and clinical studies [332,338,687]. As the glatiramer acetate complexes are NBCDs and not biological product, the application of the biosimilar approach is also not allowed [702].

In contrast to the US, the glatiramer acetate follow-on versions were approved by the EMA mainly through a hybrid application under Article 10(3), which requires a comparative characterization study with Copaxone®, including non-clinical and clinical results for assessing their efficacy, safety, and tolerability profile. Also in the EU (EMA), glatiramer acetate complexes are not considered a biological product, and therefore the centralized procedures are not mandatory and the biosimilar approach is not applicable [31,702]. Interestingly, the applicant of Sclerthon (follow-on version of Copaxone® approved by EMA, 2017) followed a similar approach to the biosimilar applications, providing an adequate bridging strategy with an extensive set of quality, non-clinical and clinical data [34,138,718].

On the other hand, the Public Assessment Report of Glatiramer acetate Mylan 40 mg/ml (solution for injection, pre-filled syringe) include the data submitted by marketing authorization holder (MAH) to demonstrate therapeutic equivalence between Glatiramer acetate Mylan 40 mg/ml and Copaxone® 40 mg/ml, such as: analytical and in vivo and in vitro biological studies comparing Copaxone® 20 mg/ml, Copaxone® 40 mg/ml, Glatiramer acetate Mylan 20 mg/ml and Glatiramer acetate Mylan 40 mg/ml; preclinical toxicological studies; the GATE (Glatiramer Acetate Clinical Trial to Assess Equivalence with Copaxone®) study comparing Copaxone® 20 mg/ml to Glatiramer acetate Mylan 20 mg/ml strength; the GALA (Glatiramer Acetate Low-Frequency Administration) clinical study comparing Copaxone® 40 mg/ml to placebo; and published data on clinical trials (four published clinical studies, used in the application for the 40 mg/ml strength of the innovator Copaxone®, including a report for a meta-analysis with weekly doses of 120 mg, 140 mg and 280 mg Copaxone®) [719].

Therefore, it is possible to understand that both regulatory authorities used distinct and not comparable pathways to the evaluation and approval of glatiramer acetate follow-on versions, which leaves some questions unanswered about the most appropriate regulatory requirements to establish the therapeutic equivalence of these complex generic drug products. Due to the quality,

efficacy, and safety issues, there is a pressing need to reach a consensus of regulatory agencies related to the regulatory requirements and a globally defined pathway for these follow-on versions.

The therapeutic equivalence between products cannot simply be established based ‘on bulk physicochemical characteristics’, since the minor modifications in the primary structure could lead to different antigenic epitopes, distinct immunogenicity profile, anti-glatiramoid antibodies, neutralization of drug efficacy, interference in the recognition of foreign antigens, hypersensitivity reactions, drug-related eosinophilia, progression of neurologic disability, additional autoimmune disorders, demyelination, general immune suppression, or death [132]. Thus, the inclusion of characterization of immunogenicity is of utmost importance in the regulatory approval process, due to the inherent immunogenic activity of glatiramer acetate complexes and their impact on both safety and efficacy [132]. On the other hand, is of equally high important the performance of comparative clinical trials in patients with MS, using an active comparator, encompasses a sufficient number of patients, performing long-term monitoring (at least 2 years), and a proper evaluation of clinical endpoints (i.e., relapse rate) [132].

Table 27. Comparative Evaluation of FDA and EMA requirements for the demonstration of Therapeutic Equivalence for the Follow-On Versions of Glatiramer Acetate Complex Products [1,10,16,246,275,286,288–290,293,296–300,308,311,313,314,317–319,321–323,325–327,712,719,722,723].

Evaluation of Therapeutic Equivalence for the Follow-On Versions of Glatiramer Acetate Complexes		
Regulatory Authority	**US (FDA)**	**EU (EMA)**
Type of Regulatory Pathway	Abbreviated New Drug Application (ANDA)	Decentralized Procedure (DCP)
	Generics Application - 505(j)	Hybrid Application - Article 10(3)
Equivalence Requirements (Recommended studies)	**Quality characterization**	**Quality characterization**
	• **Equivalence of fundamental reaction scheme** Using the same (or equivalent): (1) NCA-amino acids and polymerization initiator to yield the intermediate copolymer; and (2) chemical reagent(s) for acid-catalyzed cleavage conditions. The elements of a fundamental reaction scheme to manufacture glatiramer acetate can be determined and confirmed using publicly available information on the synthesis process in conjunction with diagnostic analysis of the RLD by orthogonal analytical measurements. • **Equivalence of physicochemical properties including composition** Side-by-side comparative physicochemical characterizations of Test API and the API from the RLD: (1) Amino acid content and optical purity of the four amino acids; (2) Molecular weight distribution, including the molar mass moments (Mn, Mw and Mz) and polydispersity; (3) Spectroscopic fingerprints, including but not limited to, Fourier Transformation Infrared spectroscopy (FTIR), nuclear magnetic resonance spectra (1H and 13C NMR) and circular dichroism (CD). • **Equivalence of structural signatures for polymerization and depolymerization** -Structural signatures for polymerization initiation (the distribution of the four amino acid-initiator adducts, the initiator content in the copolymer). -Structural signatures for propagational shift during polymerization (identify relevant amino acid sequence properties and corresponding analytical procedures, which can quantitatively measure the propagational shift in the generic and RLD products, and demonstrate that the propagational	• Fixed the drug substance manufacturing conditions rigorously to ensure compositional reproducibility. • Extensive physicochemical and biological characterization program comparing the active substance present in follow-on version and Copaxone®, using a panel of chemical and biological assays. • Quality control of drug substances. • Stability of drug substance. • Control of excipients. • Quality control of drug product - finished product specifications (appearance, color, clarity, pH, particle contamination, extractable volume, assay, identification, molecular weight distribution, impurities, potency, immunoassay, sterility, and bacterial endotoxins). • Stability of drug product.

	shift resulting from its process is the same (or equivalent) as the propagational shift present in the RLD). -Structural signatures for cleavage reactions in partial depolymerization (characterize any preference at the site of cleavage and average number of cleavages for an intermediate copolymer chain).	
	Non-clinical studies	**Non-clinical studies**
	• **Equivalence of biological assay results: experimental autoimmune encephalomyelitis (EAE) assays** (1) Prophylactic dosing in the active C57BL/6 mouse model induced by immunization with myelin oligodendrocyte glycoprotein peptide 35-55 in adjuvant (2) Therapeutic dosing in the passive SJL mouse model induced by adoptive transfer of encephalitogenic T cells activated in vitro with proteolipid lipoprotein peptide	• Experimental autoimmune encephalitis (EAE) mouse model • Cell-based potency assay in THP-1 cells • Comparative toxicity studies
	Clinical studies	**Clinical studies**
	Not required	• GATE clinical study: Multicentre, randomized, double-blind, placebo-controlled, parallel-group, 9-month equivalence trial comparing the efficacy, safety, and tolerability of follow-on version to Copaxone® (Teva Pharmaceuticals Ltd) in subjects with RRMS.
Guidance documents	Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice: Guidance for Industry (2004)	Note for guidance on biotechnological/biological products subject to changes in their manufacturing process (ICH Q5E) (2005)
	Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology: Guidance for Industry (2014)	Guideline on clinical investigation of medicinal products for the treatment of Multiple Sclerosis (EMEA/CHMP/EWP/561/98) (2005)
	Scientific Considerations in Demonstrating Biosimilarity to a Reference Product: Guidance for Industry (2015)	Comparability of biotechnology-derived medicinal products after a change in the manufacturing process - non-clinical and clinical issues (2007)
	Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product: Guidance for Industry (2015)	Guideline on the investigation of bioequivalence (2010)
	Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product: Guidance for Industry (2016)	ICH guideline Q11 on development and manufacture of drug substances (chemical entities and biotechnological/biological entities) (2011)
	Guidance on Glatiramer Acetate Injection (2016)	Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (2014)

	Drug Products, Including Biological Products, that Contain Nanomaterials: Guidance for Industry (2017)	Guideline on similar biological medicinal products (2014)
	Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA: Guidance for Industry (2017)	Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (2014)
	Statistical Approaches to Evaluate Analytical Similarity: Guidance for Industry (2017)	Guideline on manufacture of the finished dosage form (2017)
	Questions and Answers on Biosimilar Development and the BPCI Act: Guidance for Industry (2018)	ICH guideline Q8 (R2) on pharmaceutical development (2017)
	Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products: Guidance for Industry (2018)	Guideline on the requirements for the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (2017)
	Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs - General Considerations: Guidance for Industry (2019)	Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (2017)
	Considerations in Demonstrating Interchangeability With a Reference Product: Guidance for Industry (2019)	European Medicines Agency procedural advice for users of the centralised procedure for similar biological medicinal products applications (2019)
	Determining Whether to Submit an ANDA or a 505(b)(2) Application: Guidance for Industry (2019)	European Medicines Agency procedural advice for users of the centralised procedure for generic/hybrid applications (2019)
	Competitive Generic Therapies: Guidance for Industry (2020)	

4.2. Pharmaceutical Quality: Physicochemical Characterization of Glatiramer Acetate Complex Products

The glatiramer acetate complexes comprise a heterogeneous mixture of an incalculable number of peptide moieties in random order which cannot be isolated, quantified, or identified [132,337,338,677,679]. As mentioned above, the intrinsic heterogeneity and complexity of glatiramer acetate products become the process of establishment of therapeutic equivalence more difficult and particularly demanding. Therefore, there are considerable regulatory challenges still, particularly related to the establishment of quality standards.

The framework for the assessment of quality standards of glatiramer acetate complexes and the impact on their safety and efficacy profile should include a stepwise risk analysis approach with the continual decrease of residual uncertainty through a product's lifecycle [10,724]. This can comprise the deep knowledge of their complexity, product composition, manufacturing process, and clinical use; evaluation of the critical process parameters; assessment of the physicochemical and biological attributes; the accomplishment of additional in vitro and in vivo studies; definition of test methods, in-process controls, and acceptable specifications [724].

According to the scientific discussion of the Public Assessment Report Glatiramer acetate Mylan 40 mg/ml (NL/H/3777/001/DC, 2018), 'there are inherent limitations for drawing a conclusion on similarity/comparability of highly heterogeneous mixtures such as glatiramer', such as the comparative tests patterns only provide 'fingerprints' instead of an absolute result and the impossibility of accessing the individual related impurities for compounds with numerous possible combinations [719].

One of the major challenges related to the glatiramer acetate complexes is the availability of competent analytical techniques with the capacity to assess the critical quality attributes (CQAs) with impact on clinical performance, identify differences and sources of variability between the RLD and follow-on products, or establish batch-to-batch consistency in compliance with current regulatory requirements and studies recommended. Despite the impossibility of completely characterizing the glatiramer acetate complex mixtures, there are cutting-edge and advanced analytical techniques with the capability to evaluate and differentiate distinct products through physicochemical and biological properties [132,677,680].

The following table summarizes the critical quality attributes and available characterization techniques related to the pharmaceutical development of glatiramer acetate complexes, to support and facilitate the process of the demonstration of therapeutic equivalence (Table 28Table 28).

The quality, efficacy, and safety of glatiramer acetate products are dependent on some general CQAs that must be identified and studied in all medicinal products (identification, assay, impurities, degradation products), CQAs related to injectable/colloidal suspensions (particle size distribution, sterility, and bacterial endotoxins, stability, particulate matter, agglomeration), as

well as, specific CQAs related to complex glatiramer mixtures (amino acid content, molecular weight distribution, molecular charge, higher-order structures, polydispersity, conformation, biological activity, gene expression assay, cytotoxicity, immuno-recognition) (Table 28) [27,132,275,333,336–338,340,349,599,600,604–612,676,677,679,680,684,689,697,703,712,719,724–729].

The combination of several techniques increases the discriminatory power among samples and the ability to identify and understand slight differences in peptide sequences, molecular weight distribution, amino acid compositions, among others [334,337,677,689,703,730]. Thus, the orthogonal characterization analytical techniques play a crucial role to determine the main features of glatiramer acetate complexes. Examples of orthogonal high resolution and multi-dimensional analytical methods correspond to asymmetric field flow fractionation coupled with multi-angle laser light scattering (AFFF-MALLS), Reverse phase liquid chromatography 2-dimensional multi-angle laser light scattering (RPLC-2D-MALLS), or Liquid Chromatography Coupled with Mass Spectrometry (LC-MS) (Table 28).

The discriminatory power of comparative studies involved negative controls, which correspond to polymers with similar composition but using intentional variations in the manufacturing process, resulting in product changes of the primary structure/structural signatures [275,336,719].

It is important to highlight that the adequate and reliable manner of characterizing the glatiramer acetate complexes is in the unchanged form since the chemical or enzymatic cleavage of the polypeptides leads to the loss of the original complex structures of the parent sequences [132,689]. For example, the identification of amino acid sequences on polypeptidic chains through proteolyzed materials is not suitable and sensitive enough for the characterization of glatiramer acetate complexes and identification of small compositional differences, due to the irreversibility and less structural complexity of the mixture obtained [132,689].

The book entitled ‘Non-Biological Complex Drugs: The Science and the Regulatory Landscape’ described several examples of the loss of discriminatory power of analytical methods when applying the fragmentation of glatiramer acetate structure, also referred to as peptide mapping [132]. The observed differences between the intact glatiramer acetate complexes ‘was masked when the mixtures were fragmented and analyzed using a conventional nonspecific method’. This enabled them to establish that ‘the more exhaustive the extent of cleavage, the weaker the correlation between the digested fragments and the parent molecules’ [132].

Table 28. Pharmaceutical Quality System: Critical Quality Attributes and Characterization Techniques related to the Pharmaceutical Development of Glatiramer Acetate Complex Products.

	Critical Quality Attribute	Analytical technique	Justification	Support documentation (method identified in Guidance or USP Monograph)	References
Physicochemical characterization	Appearance (Color/Turbidity/Caking)	USP Product Quality Test	The changes in the appearance of formulations can indicate physical instability that can be due to degradation, phase separation, caking, or aggregation. These phenomenons can compromise the quality, efficacy, and safety of drug products.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests USP <381> Elastomeric Components Used in Injectable Pharmaceutical Packaging/Delivery Systems USP <1790> Visual Inspection of Injections	[340,349,719, 725]
	Container Closure Systems	USP Product Quality Test	The identity and description of materials of construction of container closure system, just as their compatibility with glatiramer acetate formulation is required due to the physical and chemical interaction between the packaging system and the preparation, with potential changes in their strength, quality, or purity.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests USP <381> Elastomeric Components Used in Injectable Pharmaceutical Packaging/Delivery Systems USP <659> Packaging and Storage Requirements USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery systems	[340,604,724, 725,727,729]
	Degradation Products/ Impurity Profile	USP/ICH Product Quality Test	The peptide-related impurities may result either from peptide synthesis or from its degradation. The impurities/degradation products can compromise the safety profile of the drug product (risk of immunogenicity) and must be controlled based on compendial/ICH requirements or an RLD characterization, to limit patient exposure. The target for any unknown impurity is set according to the ICH identification threshold for each drug product.	ICH Q3B(R2) USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests	[132,340,600, 719,724]
	Identification	USP Product Quality Test	The identification and description of formulation components including the active and inactive ingredients, as well as, their amounts and function, should be assessed during product and process	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests	[340,719,724]

			development due to can largely affect the quality, efficacy, and safety profile of the peptide drug products.		
	Leachable/ Extractables	USP Product Quality Test	The leachables and extractables from components of the primary packaging can compromise the safety profile of drug products, due to the generation of impurities.	USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery systems	[604,719,724, 727]
	Particulate Matter	USP Product Quality Test	According to the United States Pharmacopoeia (USP), the particulate matter in injections and parenteral infusions consists of 'extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions'. As stated in USP ⟨1⟩ Injections and Implanted Drug Products, solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of USP ⟨788⟩ Particulate Matter in Injections. The presence of particulate matter in formulations is considered critical due to the potential health hazards, such as the local adverse reactions.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests USP <788> Particulate matter in injections USP <790>Visible particulates in injections	[340,605,719, 726]
	pH	USP Product Quality Test	The pH of the final product is critical for the safety profile of formulations injected subcutaneously, which must be biocompatible. It is recommended that the pH is closer to the physiological one to minimize the occurrence of pain, irritation, and tissue damage arising from injection.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests	[340,719]
	Residual Solvents	USP/ICH Product Quality Test	Residual solvents can impact the drug product safety profile when used in the manufacturing process because most of the time they cannot be completely removed from the drug product.	USP <467> Residual solvents ICH Q3C(R6)	[606,607,724]
	Stability	USP/ICH Product Quality Test	The physicochemical stability of drug products is required to maintain therapeutic potential and ensure the quality of the medicinal product during the entire shelf-life. The stability protocols and results support the proposed expiration date and storage conditions of glatiramer acetate formulations.	ICH Q1A(R2) ICH Q5C USP <1049> Quality of Biotechnological Products	[608,609,719, 724,728]

			Stability studies should include tests to assess the microbiological, physical, and chemical stability of the formulation.		
	Sterility And Bacterial Endotoxins	USP Product Quality Test	Non-compliance with microbial limits has the potential to harm the patients particularly when the medicinal product is intended to be injected subcutaneously. The sterility/pyrogen content and bacterial endotoxins may be influenced by process parameters and formulation variables, which can impact patient safety.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests USP <71> Sterility tests USP <85> Bacterial endotoxins	[340,610,611, 719]
	Uniformity of Dose (Fill Volume)/ Content Uniformity	USP Product Quality Test	An accurate fill volume is crucial to ensure the required dosage, which is mandatory to ensure the efficacy and safety of the drug product.	USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests USP <[599]> Uniformity of dosage units USP <697> Container content for injections	[340,599,612, 724]
	Primary Structure of Polypeptides (Peptide Sequencing)	Edman Degradation	The primary structure of the polypeptides chain, i.e., the amino acid sequence, might impact on the peptide immunogenicity, which in turn, lead to potential clinical consequences such as: production of antibodies against the glatiramer product, loss of therapeutic efficacy, neutralization of the human peptide counterpart, or immunological adverse effects as allergy and anaphylaxis. There are numerous analytical techniques used to the evaluation of the primary structure of a peptide, performed alone or in conjunction with other methods. For example, the Edman degradation consists of a sequencing method used for the analysis of the primary structure of a polypeptide chain (amino acid sequence), through the N-terminal residue identification.	Not Found	[677,684,697, 724]
	Primary Structure of Polypeptides (Amino Acid Content and Optical Purity)	Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR)	Nuclear magnetic resonance (NMR) is applied to identify and compare the amino acid composition of the intact molecular peptide structure (amino acid content), i.e., the relative ratios and positions of four amino acids that comprised the glatiramer acetate complexes. The NMR allows the determination of Diethylamine-N	Draft Guidance on Glatiramer Acetate Injection (FDA, 2016)	[275,336,676, 677,689,703, 724]

			terminal AA (amino acid) of digested compounds, although do not provide an evaluation of the sequences of all individual peptides in glatiramer acetate mixtures. This technique may also be used for the characterization of higher-order structure between the follow-on version and its reference product (Copaxone®).		
	Primary Structure of Polypeptides (Amino Acid Sequence, Charge Distribution)	Capillary Isoelectric Focusing Electrophoresis (cIEF)	Capillary isoelectric focusing (cIEF) electrophoresis is used to evaluate the heterogeneity of the polypeptide sequences of different glatiramer products, through the analysis of polypeptide primary structures, charge distribution, and sequence composition. This method is based on dissimilarities in isoelectric points obtained for distinct glatiramer batches, i.e., differences in the number and distribution of detected peaks across the entire cIEF pattern. The charge distribution of the polypeptide chains composed with charged residues (Lys and Glu), constitute a specific critical attribute of these type of products since that reflects the primary structure through arrangement of the charges. The main advantages associated with the IEF are related to the excellent batch-to-batch consistency; high-resolution separation technique; and sensitive discriminatory analytical technique with the capability to discriminate among glatiramer acetate products manufactured by different processes.	Not Found	[132,336,689]
	Peptide Mapping/Proteolytic Digestion Profile	Liquid Chromatography Coupled with Mass Spectrometry (LC–MS)	The liquid chromatography-mass spectrometry (LC-MS) analyses allow the identification of distinguishable and repeatable peptide sequences, commonly designated ‘comparable digestion fingerprints’. To obtain the proteolytic digestion profile, can be used the enzyme trypsin, which cleaves the glatiramer sequences in the carboxyl side of the amino acid lysine. The peptide mapping can be achieved by analysis of a comparable number of peaks corresponding to each	Not Found	[336,676,677, 724]

			glatiramer digested (comparable retention times and intensities). The main disadvantage associated with this analytical technique is the digestion and separation of complex copolymers by enzymatic degradation, resulting in the loss of the complex structures of the original sequences and low sensitivity in the screening of small compositional differences.		
	Identification and Separation of Polar Compounds	Hydrophilic Interaction Liquid Chromatography (HILIC)	The hydrophilic interaction liquid chromatography (HILIC) is used specifically for the separation of polar compounds that are poorly retained and resolved by other separation analytical techniques. The peptide sequences comprised Glu and Asp amino acids are examples of these polar compounds.	Not Found	[676]
	Higher Order Structure (Secondary Structure)	Circular Dichroism (CD)	The secondary structure corresponds to random coils and limited degree structures (e.g. α-helices and β-sheets) with high conformational flexibility, which impacts the biological activity of the glatiramer acetate product. As in the primary structure of polypeptides, there is a wide range of analytical techniques for the secondary peptide structure characterization. Circular Dichroism (CD) is a spectroscopic technique used to determine the extent of the secondary structure of the polypeptide chains, through the three-dimensional folding of its amino acid chains (related to α-helix, β-sheet, and/or random coil conformational structures). The CD spectra is obtained from a difference in absorption of right- and left-circularly polarized light by optically active peptide structures, in the wavelengths between 190 and 300 nm. A disadvantage described for the CD technique is the low sensitivity to determine the structural differences	Draft Guidance on Glatiramer Acetate Injection (FDA, 2016)	[27,275,336,677,697]

			based on the comparison of CD spectra among the reference product and follow-on versions.		
	Higher Order Structure (Secondary Structure)	X-Ray Crystallography	X-ray crystallography is a technique capable of the characterization of the entire molecular structure of the polypeptide chain, i.e., the relative positions of all amino acids.	Not Found	[724]
	Higher Order Structure (Secondary Structure)	Fourier-Transform Infrared Spectroscopy (FTIR)	Fourier-transform infrared spectroscopy (FTIR) is a technique used to characterization of sequences of amino acids in glatiramer acetate complexes. Also, is described their capability to estimate the b-sheet formation and further discrimination among parallel and antiparallel forms, as well as, the aggregates.	Draft Guidance on Glatiramer Acetate Injection (FDA, 2016)	[275,679,724]
	Higher Order Structure (Secondary Structure)	Raman Spectroscopy	The Raman spectroscopy enables the analysis of peptide conformation and local interactions related to the Cys or Tyr groups.	Not Found	[724]
	Intrinsic Fluorescence of the Polypeptides	Intrinsic Fluorescence Spectroscopy	The aromatic residue tyrosine that composed the polypeptide chains of glatiramer acetate complexes is responsible for the intrinsic fluorescence of the polypeptide structures. Thus, Fluorescence spectroscopy provides intrinsic fluorescence emission spectra to the analysis of structural differences of the polypeptides in glatiramer acetate complexes. This analysis allows to compare if the intrinsic fluorescence emission spectra for a follow-on version is similar to the reference product (Copaxone®), as well as if the variability in fluorescence intensity of this product is within the range of variability for Copaxone®.	Not Found	[336]
	Identification and Quantification of Characteristic Polypeptides	Ultraviolet Spectroscopy (UV)	The Ultraviolet spectroscopy (UV) allows the identification and quantification of characteristic polypeptides in glatiramer, through the absorbance maxima measurements at three characteristic transition wavelengths, such as the peptide bond around 200 nm,	Not Found	[336]

			the α-helix around 220 nm, and the tyrosine moieties around 280 nm. With this analysis is possible to conclude if the polypeptides in follow-on versions contain the same conformational regions as reference product (Copaxone®).		
	Molecular Charge	Coomassie CBBG-250	Coomassie Brilliant Blue (CBB) Dye-250 (CBBG-250) is a dye with affinity to several proteins and peptide structures, promoting distinct color changes in solution as a result of this interaction. The Coomassie Brilliant Blue (CBB) binding is applied for glatiramer acetate complexes to evaluate the specific interaction between the CBBG-250 dye and glatiramer peptides, which are indicative of characteristic molecular charge distributions in polypeptide sequences. UV-Vis absorbance spectra obtained is dependent on specific chemical properties and higher-order structure of each glatiramer acetate complex. Thus, the comparable UV-Vis absorbance spectra indicating similarities in the binding behavior of CBB, and consequently, comparable polypeptide higher-order structures between the follow-on version and reference product. This analysis should be performed according to the reference product release specifications (Copaxone®) and relative to a CBBG-250 dye control solution.	Not Found	[336,712]
	Molecular Weight Distribution (including Molecular Mass Moments and Polydisperisty)	Size Exclusion Chromatography (SEC)	The Size-Exclusion Chromatography with Multi-Angle Laser Light Scattering Detection (SEC-MALLS) allows the analysis of Molecular Weight Distribution (MWD) of the polypeptides in glatiramer acetate mixtures and higher molecular weight precursors, in accordance with their size and relative abundance.	Not Found	[132,275,336, 337,677,689, 712,719]

			MWD constitutes a critical quality attribute that should be assessed in basic bulk physicochemical characterization. The retention time of each compound in the SEC column is dependent on hydrodynamic size, instead of the primary structure of the constituents. The SEC technique constitutes a nonspecific analytical method, which presents the disadvantage of not be able to discriminate among structurally related constituents, and consequently just exhibit small differences in molecular weight distributions of glatiramer acetate complexes.		
	Molecular Weight Profile	Asymmetric Field Flow Fractionation (AFFF)	The Asymmetric Field-Flow Fractionation coupled to a Multi-Angle Laser Light Scattering (AFFF-MALLS) is an analytical technique used to measure critical quality parameters of glatiramer acetate mixture, such as the average molecular weight, polydispersity, mass, and the root mean square radius. AFFF-MALLS uses distinct glow streams to separate sample components according to the speed at which they elute, and consequently, giving rise to the elution profiles with particular retention times. Thus, it is possible the distinction between a follow-on version with a different molecular weight range than a reference product (Copaxone®). A positive aspect of AFFF-MALLS is the capability to perform the characterization of original peptide structures in the glatiramer acetate mixture, without the need for glatiramer compounds are digested by chemical or enzymatic cleavage. This technique can also be applied to the aggregate profile characterization.	Not Found	[676,677,724]
	Molecular Size Distribution	Viscotek	Viscotek TDAmax gel permeation chromatography allows the comprehensive conformational characterization of constituents in glatiramer mixture, such as the molecular weight and distribution,	Not Found	[336–338,676,677,712]

			molecular size, intrinsic viscosity, hydrodynamic radius, and polydispersity. This multidetector size-exclusion chromatography analysis system is considered as a high-resolution approach for polymers and macromolecules, due to their triple detector array of refractive index, viscometer, and light scattering detector.		
	Molecular Weight – Hydrophobicity Correlation	Two Dimensional Multi-Angle Laser Light Scattering (2D RPLC MALLS)	Reverse phase liquid chromatography 2-dimensional multi-angle laser light scattering detector (RPLC 2D-MALLS) is used to obtain comparative molecular mass elution profiles as a function of hydrophobicity for distinct glatiramer acetate mixtures. Thus, this technique allows to infer that the products with the same characteristic multi-component peak, present similar hydrophobic interaction properties and a comparable composition of amino acid sequences.	Not Found	[336–338,677,712]
	Average Particle Size	Dynamic Light Scattering (DLS)	The particle size distributions are one of the critical quality attributes most extensively studied due to the impact on stability, biodistribution, bioavailability, and as a consequence, in the efficacy and safety profile of the glatiramer acetate complexes. The Dynamic light scattering (DLS) analysis is used to assess particle size distribution, by measuring the variations of the scattering of laser light by particles in suspension subject to Brownian motion. Thus, their hydrodynamic diameters and size distribution can be inferred from the evaluation of the particles diffusion speed through the fluctuations in scattering signal intensity. It is noted that the DLS microscopic technique does not have the capacity to identify and quantify monomeric peptide form or aggregated material, since the results should be interpreted with caution and using other techniques as the analytical ultracentrifugation.	Not Found	[132,677,689]

	Charge Distribution	Cation Exchange Chromatography (CEX)	The surface charge distribution of the polypeptides is an important critical quality attribute that impacts the binding properties of antigens to the antigen-presenting cells and T cells (immunological counterpoints), presenting a key role in the molecular recognition and interactions that determine the therapeutic efficacy. Variations in surface charge distribution parameters of distinct glatiramer acetate complexes are indicative of differences in the polypeptide primary structure. The Cation Exchange Chromatography (CEX) technique is considered a gold-standard for charge-sensitive antibody analysis, based on a non-destructive separation of polypeptide mixtures in accordance with the intensity of the average overall charge. Thus, the separation of polypeptide subpopulations occurs due to the affinity of components to the negatively charged stationary phase of the column, and gives rise to distinct peaks on CEX chromatograms.	Not Found	[132,333,337, 338,680,712]
	Aggregate Profile	Analytical Ultracentrifugation	The presence of aggregates could lead to serious safety problems, as the immunogenicity risks, due to the increase of immune responses with production of antibodies by the reinforced activation of T helper cells. The aggregates can arise during the manufacturing process (variations of temperature, light, stirring, pH adjustments) or formed during the storage (denaturation effects due to thermal, pH, dielectric constant and ionic strength changes, or peptide sequence variations by oxidation or deamidation). Consequently, some process parameters should be highly controlled (e.g. temperature, pH, light, or mechanical stress). The evaluation of aggregate profiles can be achieved by different analytical techniques, such as analytical ultracentrifugation, asymmetric field flow fractionation (AFFF), atomic force microscopy (AFM), or ion mobility mass spectrometry (IMMS).	Not Found	[724]

	Aggregate Morphology and Charge	Atomic Force Microscopy (AFM)	The nature of peptide aggregate population in the colloidal suspensions and the morphology of these aggregates, constitute a critical quality attribute with an impact on the efficacy and safety of glatiramer acetate complexes. The Atomic Force Microscopy (AFM) is a sensitive and standard microscopic technique that provides valuable information regarding the sample topography, such as aggregation morphology. This technique allows the identification of variations in aggregate appearance of follow-on versions, such as the large globular particles and heterogeneous structures, compared to consistent structures with linear shapes of the reference product (Copaxone®).	Not Found	[132,337,338, 677,689,712]
	Amino Acid Sequence, Size, Charge and Shape	Ion Mobility Mass Spectrometry (IMMS)	The Ion mobility mass spectrometry (IMMS) is a two-dimensional analytical technique applied to the structural analysis of heterogeneous mixtures, aggregates, and charge hydrophobicity, according to the separation of ionized molecules based on the molecular size, shape, and mass-to-charge ratio (m/z). This technique is recommended by the FDA and can detect the differences in polypeptide composition between the follow-on versions and reference product, as well as, differentiate among closely related moieties as the isomeric peptides. The analysis is performed with non-digested peptides with a high sensitivity level in the 2-dimensional separation of ionized molecules.	Not Found	[132,338,677, 689,712]

4.3. Non-Clinical and Clinical Evaluation of Glatiramer Acetate Complex Drug Products

As described throughout this chapter, there is a huge difficulty in the demonstration of therapeutic equivalence of follow-on versions of glatiramer acetate complexes.

According to the Public Assessment Report Glatiramer acetate Mylan 40 mg/ml (NL/H/3777/001/DC, 2018), 'glatiramer parent compound molecules cannot be quantified in body fluids or tissues' and 'given the nature of the product, accurate detection methods to monitor exposure to glatiramer in the systemic circulation (or in other readily available biological matrices) are not available' [719]. Also, the inadequacy of conventional PK studies to demonstrate bioequivalence, and the lack of identified or validated PD biomarkers for demonstration of clinical efficacy, hindered the establishment of a proper PK/PD profile [31,132,164,332,336,338,662,674,679,687].

However, it is impossible to predict the efficacy and safety profile of glatiramer acetate complexes in humans only through the physicochemical characterization or shorter-term toxicity studies [31]. Thus, the performance of non-clinical and clinical studies constitutes an absolute priority to obtain a complete analysis of the immunogenicity, gene expression, potency, toxicity profile, among others [677]. For example, as Larisa Wu stated in the book chapter entitled 'Regulatory Considerations for Peptide Therapeutics', '*an in vitro bioassay may be included as part of the characterization of higher-order structure and biological activity of complex peptides as it provides essential information on the peptide structure–activity relationship*' [724].

The following table provides a summary of the main attributes and analytical techniques described in scientific literature, that can be implemented in nonclinical (in vitro and in vivo studies) and clinical evaluation of glatiramer acetate complexes (Table 29). The non-clinical assessment includes several studies such as: immuno-recognition, gene expression modulation, potency, biological activity, cytotoxicity, nonclinical toxicity and safety analysis [275,336,337,677,678,696,702,712,719]. Furthermore, the biological characterization is corroborated by additional clinical data resulting from the GATE study [132,164,336,677,683,685,688–690,692,701,702,719,731].

Table 29. Nonclinical and Clinical evaluation in the Pharmaceutical Development of Glatiramer Acetate Complex Products.

	Specific attribute	Analytical technique/study	Description	References
Nonclinical Evaluation	Immuno-recognition (GA specific monoclonal antibodies)	Western Blot	The Western blot technique detects the relative binding of glatiramer to polyclonal antibodies (pAb) - anti-GTR (generic glatiramer acetate) and anti- Copaxone® (reference product). If it is identified a similar variety of epitopes, i.e. similar affinities to the same antibodies, it can be inferred that both products present similar recognition moieties and specificities.	[336]
	Immuno-recognition (GA specific monoclonal antibodies)	Enzyme-Linked Immunosorbent Assay (ELISA)	The Enzyme-linked immunosorbent assay (ELISA) technique constitutes an anti-Glatiramer acetate antibody biorecognition assay. This technique was employed for the specific bio-recognition of Glatiramer Acetate (GA) using distinct antibodies: - one assay using two anti-GA monoclonal antibodies (MAbs); - second assay using rabbit IgG polyclonal antibodies (PAbs). These antibodies' bio-recognition assays based on the ELISA technique allows an assessment of relative binding of the GA-specific monoclonal and polyclonal antibodies to follow-on version (GTR), compared to the reference product (Copaxone®). Therewith it is possible to infer if follow-on versions contain the same epitopic polypeptide sequences as Copaxone®.	[336,337,712]
	Potency	Cell-Based Assay (CBA)	The soluble Interleukin-1 Receptor antagonist (sIL-1Ra) plays an important role as a mediator in the pathogenesis of MS. The Potency ex vivo by Cell-Based Assay (CBA) is used to quantify the in vitro biological activity of follow-on versions (GTR) or reference product (Copaxone), through measurement of glatiramer-induced secretion of soluble Interleukin-1 Receptor antagonist (sIL-1Ra), by GA-primed human monocytic THP-1 cell line following response to recall antigen (GA). The results of the cell-based assay allows to infer if both products induce a similar anti-inflammatory sIL-1Ra response, through the production of soluble Interleukin (sIL-1Ra) by THP-1 cell line in comparable proportion. Thus, the potency assay demonstrates the comparable bioactivity at a functional level as relative to the reference product (relative potency).	[336,337,712,719]
	Gene expression	Micro Array Study	The microarray technology constitutes another test to evaluate the equivalence of biological responses through gene expression profiling and pathway modulation. This biological test system detects the genome-wide perturbations through the modulating gene expression in the human monocytic THP-1 cell line.	[336,696,712,719]
	Gene expression	Acetonitrile Nonconforming Copolymer (ACN) Study	The acetonitrile nonconforming copolymer (ACN) constitutes a polymer chains compositionally similar to glatiramer acetate complexes (same molecular weight distribution and amino acid composition), but structurally distinct. Different manufacturing conditions lead to a structurally nonequivalent mixture,	[696]

			which may be differentiated from reference product (Copaxone®) and other glatiramer acetate complexes in biological assays. This molecule is mainly used to establish the sensitivity, robustness, and reproducibility of biological test system, and hence increase their discriminatory capability to determine differences in biological activity of glatiramer acetate complexes. The experimental system applied in the ACN study includes a well-established whole-genome microarray technology platform, with a population of the Th2-polarized T cells.	
	Biological activity	Experimental Autoimmune Encephalomyelitis (EAE) Blocking Test	Experimental Autoimmune Encephalomyelitis (EAE) Blocking Test allows to infer about the biological activity of glatiramer acetate complexes, through the capability to block the induction of EAE in mice. For the EAE induction is commonly used the encephalitic antigen designated as mouse spinal cord homogenate (MSCH). The EAE blocking capability is defined as the reduction of the disease appearance (% Activity) and disease severity (mean maximal score ratio (MMS ratio)). This test has been recommended by the FDA as a confirmatory assay for testing the clinical performance of glatiramer acetate complexes. As mentioned in the Draft Guidance of Glatiramer Acetate (2016), 'a biological assay can serve as a confirmatory test of equivalence and provide complementary confirmation of API sameness. FDA recommends generic sponsors to conduct at least the following two EAE assays: 1) prophylactic dosing in the active C57BL/6 mouse model induced by immunization with myelin oligodendrocyte glycoprotein peptide 35-55 in adjuvant, and 2) Therapeutic dosing in the passive SJL mouse model induced by adoptive transfer of encephalitogenic T cells activated in vitro with proteolipid lipoprotein peptide 139 – 151'. The disadvantage related to the EAE model is the considerable inter-and intra-assay variability, which hinder to draw valuable conclusions on the comparability of two glatiramer acetate complexes. Also, has been identified the absence of predictive potential for the occurrence of infections, and the lack of genetic variability of animal tests due to inbreeding, which do not reflect the patient population.	[275,336,337,677,702,712,719]
	Cytotoxicity	In Vitro Cytotoxicity Assay (Human B Cell Lines)	The cytotoxicity evaluation is achieved based on the induction of in vitro cytotoxicity using an established human B cell lines. The cell-based in vitro assay allows the determination of the dose-dependent cytotoxic effect of tested product lots in serial concentrations. This assay is performed through the measurement of a marker of cytotoxicity effect, the lactate dehydrogenase (LDH), a cytosolic enzyme released in the cell lysis.	[337,712]
	Nonclinical toxicity and safety	Long-term toxicity study in rats	The long-term toxicity and safety comparative studies can be assessed in a 28-day and 90-day repeat dose study in rats, in which the follow-on versions and reference product (Copaxone®) are administered subcutaneously by daily injection to four different injection sites in a rotating schedule (one site each day).	[336,678,702,719]

			This study enables the analysis of differences in frequency and severity of analogous local reactions and organ effects between the glatiramer acetate complexes, such as: local effects at the injection sites (dark red foci and dark red discoloration of the subcutis/muscle); perilobular fibrosis of liver; increase in relative liver weight; tubular basophilia, hyaline cast(s) and glomerulopathy in the kidney; systemic perivascular (lympho) plasmacytic infiltrates in kidneys, liver, parotid glands and injection sites; changes in biochemical and hematological parameters; among others.	
Clinical Evaluation		GATE study	The GATE clinical study (ClinicalTrials.gov NCT01489254) is a multi-center, randomized, double-blind, placebo-controlled, parallel-group, 9-month, equivalence trial comparing the efficacy and safety and tolerability of follow-on version to Copaxone® (Teva) in subjects with RRMS, followed by an open-label 15-month follow-on treatment part evaluating the long-term follow-on version treatment effects. The primary endpoint used in this study corresponds to the total number of gadolinium-enhancing lesions (during months 7, 8, and 9), and the additional endpoints comprised other magnetic resonance imaging parameters, annualized relapse rate, and Expanded Disability Status Scale score. On the other hand, the assessment of safety and tolerability profile included the monitorization of adverse events (MS relapse, bronchitis, anaphylactoid reaction, angioedema), injection site reactions, and laboratory test results. The results of the GATE study have been published by Cohen et al, in the research paper entitled 'Equivalence of Generic Glatiramer Acetate in Multiple Sclerosis: A Randomized Clinical Trial', which concluded that the follow-on version and brand drug had equivalent efficacy, safety, and tolerability [732]. Based on the results obtained, Synthon BV achieved the marketing authorization for follow-on version of Copaxone® (GTR 20 mg/mL pre-filled syringe) in 28 member-states of the European Union, as well as, in Iceland, Liechtenstein, and Norway.	[132,164,336,677,683,685,688–690,692,701,702,719,731,732]

5. Concluding Remarks

Glatiramer acetate consists of the complex heterogeneous mixture of peptide copolymers containing four specific amino acids (L-glutamic acid, L-lysine, L-alanine, and L-tyrosine) in a defined ratio. Copaxone® (Teva Pharmaceutical Industry) is the first glatiramer acetate complex product approved for the treatment of patients with relapsing-remitting forms of multiple sclerosis (RRMS).

Despite the development and approval of several options for the treatment of MS, the continual rising costs of therapeutics is a matter of serious concern due to the risk of restricting market access to the patients. Therefore, efforts should focus on the introduction of follow-on versions of glatiramer acetate complexes into the MS treatment landscape. Approval of the follow-on versions plays a crucial role to drive a significant reduction of medication costs, promote price competition, expansion of the market, and increasing the affordability of a higher number of MS therapies. The effect of the development and approval of follow-on versions of glatiramer acetate complexes is dependent on their price, the extent of use, and the capability to ensure comparable quality, efficacy, and safety to the RLD.

In the scientific literature is strikingly evident several challenges related to the pharmaceutical development of follow-on versions of glatiramer acetate complexes and the hurdles in the demonstration of therapeutic equivalence of this type of highly complex drug product. The main challenges correspond as follows:

- Nano-sized complexes of synthetic polypeptides (high complexity);
- Heterogeneous mixture in colloidal suspension for subcutaneous injection;
- Regulatory uncertainty related to the definition and class of products in which the glatiramer acetate is inserted;
- Inherent batch-to-batch heterogeneity: amino acid sequences are not completely conserved;
- Polypeptide sequences not fully identified, isolated or quantified by current available discriminatory analytical technology;
- The precise mechanisms of the immunomodulatory activity responsible for its therapeutic efficacy remain poorly understood;
- Quality, efficacy, and safety profiles are highly dependent on complex and multistep manufacturing processes;
- Minor changes to the manufacturing process give rise to distinct and unique compositions of glatiramer acetate mixtures;
- Minor changes to the manufacturing process lead to distinct biological activities, such as alterations in clinical efficacy and safety properties;

- The proper definition of critical quality attributes and the complete physico-chemical characterization is not yet well established;
- Lack of proposed regulatory approaches or dedicated pathways for follow-on versions of glatiramer acetate complexes;
- Regulatory approach variability - use of distinct approval pathways between US and EU regulatory agencies;
- Divergences in regulatory requirements between US and EU regulatory agencies;
- Delay or lack of relevant product-specific guidance documents published;
- PK and PD profiles are not well established.
- Inadequacy of conventional PK studies to demonstrate bioequivalence - immediately hydrolyzed of a substantial fraction of glatiramer acetate complex at the site of the injection and uptake by local APCs;
- Lack of identified or validated PD markers for demonstration of clinical efficacy;
- Immunomodulatory activity with potential implications in mechanisms of immunopathology, immunotoxicity, induction of autoimmune disorders, among others;
- There are no reliable analytical methods for the establishment of therapeutic equivalence;
- Low-resolution methods show similarities between the follow-on versions and RLD - has not the statistical power to detect differences between glatiramer mixtures - whereas more sensitive higher-resolution techniques and biological studies show striking differences.

According to the analysis of the regulatory landscape, there are significant discrepancies between both regulatory agencies. The follow-on versions of glatiramer acetate products were approved by the FDA, through an ANDA pathway, according to section 505(j) route (Generic approach), without requiring additional clinical studies. On the other hand, EMA approved the majority of follow-on versions through the decentralized authorization procedure under Article 10(3) of Directive 2001/83/EC (hybrid application), through the detailed comparative characterization and clinical studies (e.g. Phase III study). Thus, certain questions have been raised concerning the appropriateness of the generic pathway in assessing public health safety and clinical efficacy, since is considered an incomplete approach that does not recognize the real complexity of the glatiramer acetate products. Moreover, there is a risk of efficacy and safety profiles being inadequately analyzed, due to the important differences that cannot be identified when only using the physicochemical and biological evaluation.

The understanding and control of product and manufacturing process, just as the identification and characterization of their critical quality parameters and biological effects, are key elements to guarantee its quality, efficacy, and safety. The best strategy to demonstrate the active ingredient sameness includes comparative physicochemical, nonclinical (in vitro and in vivo studies), and clinical studies, using cutting-edge and orthogonal analytical techniques, with differentiation of

mixtures in the unchanged form, without chemical or enzymatic cleavage of the polypeptides. The use of negative control polymers with different physicochemical properties boosts the discriminatory power of analytical techniques to characterize the microheterogeneity of glatiramer mixtures.

The wide set of orthogonal analytical techniques allows the characterization of glatiramer acetate complexes with some precision, but not be able to ensure an unambiguous, exhaustive, and complete characterization. Therewith, the development of additional reliable and robust analytical techniques to increase the accuracy and consistency of the data is considered a key priority.

Another complementary strategy includes the analysis of the regulatory approach used for marketing authorization of biosimilar drugs, which can steer the development of similar requirements for the glatiramer acetate complexes. For example, in the EU the interchangeability between the biosimilars and the reference biological product is not mandatory, and the product is considered a new entity analyzed with high scrutiny, while in the US the clinical studies are included in the establishment of therapeutic equivalence.

Lastly, the future prospects for the development of follow-on versions of glatiramer acetate complexes should be included: the clear definition of dedicated regulatory pathways, specific guideline documents, and approval requirements; the implementation of a post-marketing surveillance program; and the promotion of multidisciplinary research, consensus discussions, and in-depth dialogue between the different stakeholders.

Thus, an alignment and harmonization of regulatory requirements and approval pathways between the US and EU should be a priority target to be achieved in the coming years. Additionally, the recent advances in the preparation of glatiramer acetate guidance documents, drafted both the EDQM [new synthetic peptides Ph. Eur monographs in preparation, such as Glatiramer (3057) and Glatiramer injection (3104) (finished product monograph)], and also by the USP [Glatiramer Acetate and Glatiramer Injection monograph], show the current effort to overcome the challenges in the development and approval of this type of complex drug products.

Chapter VII. Generic Development of Complex Injectable Liposomal Formulation: From the Bench to Approved Drug Products

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Abstract

Despite increasing interest in the development of generic liposomal drug products, its complexity raises significant issues related to pharmaceutical quality assessment. Any change in the chemical, physical or microbiological properties of the liposomal drug product arising from the manufacturing process might impact and modify the quality target product profile. Therefore, one of the main scientific and technical challenges in the development and approval of generic liposomal formulations is linked to the difficulty of complying with reproducibility requirements and quality standards. The greater investment into advanced analytical techniques, novel control strategies, or the application of statistical and modeling tools are indispensable for a better understanding of the product and process.

In this chapter, the Quality by Design (QbD) approach was used to optimize and define the optimal conditions in the preparation of doxorubicin hydrochloride liposomal formulation, particularly in the obtaining of the target criteria for mean particle size and polydispersity index of Unilamellar Vesicles (ULVs). Size and size distribution is one of the most important physicochemical properties of the liposomal formulation, due to the significant impact on drug loading, encapsulation efficiency, drug release profile, stability, biodistribution, cellular uptake, and bioavailability of the final drug product. Thus, the formulation optimization from the earliest stages brings a very significant added value to the manufacturing process of doxorubicin hydrochloride liposomal drug products, ensuring the consistency between batches of generic products and the reference listed drug. Furthermore, the development of novel technological platforms through this systematic and risk-based approach presents unprecedented advantages, such as lower development time and costs, increased formulation design and performance, less scope for manufacturing failures, a higher success rate at the regulatory level, and accordingly increased affordability to the essential medicines.

Keywords

Non-Biological Complex Drugs; Liposome; Drug Delivery System; Doxil®; Doxorubicin Hydrochloride; Complex Generics; Formulation Development; Formulation Optimization; Experimental Trials; Quality by Design; Quality Target Product Profile; Critical Quality Attributes; Critical Material Attributes; Critical Process Parameters; Risk Assessment; Liposome Characterization.

1. Introduction

Doxil® (doxorubicin HCl liposome injection) was the first nano-drug delivery system based on PEGylated liposome technology, to obtain regulatory approval by the Food and Drug Administration (FDA) (US, 1995) [733–736]. In the European Union, the doxorubicin HCl liposome injection is namely Caelyx® and marketed by Janssen Pharmaceuticals NV (EU, 1996) [737]. This drug product consists of a sterile, translucent, red liposomal dispersion, containing a liposomal form of doxorubicin hydrochloride 2 mg/mL, intended for intravenous administration [733]. The liposomal suspension contains small unilamellar vesicles (SUVs) with an average size range of 85 - 100 nm [733].

The active ingredient doxorubicin hydrochloride is a cytotoxic anthracycline topoisomerase II inhibitor isolated from the bacterium *Streptomyces peucetius* var. *caesius* [369,738–741]. Despite being a potent chemotherapeutic agent, its use is rather limited in the clinical environment, due to its inherent cardiotoxicity. This is one of the major drawbacks of conventional or non-liposomal anthracyclines. The positively charged doxorubicin has the capacity to interact strongly with the cardiolipin (anionic diphosphatidylglycerol) of the mitochondria in the cardiac muscle, which consequently leads to lipid peroxidation within cardiac tissue [734,739]. Thus, the encapsulation of doxorubicin HCl (remote loading) in long-circulating STEALTH® liposomes is one of the main development strategies to overcome the systemic toxicity related to the administration of the free drug [738,739]. Greater than 90% of the drug is encapsulated in the STEALTH® liposomes, i.e., the encapsulation efficiency of the drug is more than 90% [6,10].

The STEALTH® liposomes of Doxil® are formulated with surface-bound methoxypolyethylene glycol (MPEG), a process often referred to as pegylation, to protect liposomes from detection by the mononuclear phagocyte system (MPS). Thus, STEALTH® technology is a liposomal coating that evades detection and destruction by the immune system, reduces clearance by the mononuclear phagocyte system, and thereby increases blood circulation time. The encapsulation in STEALTH® liposomes enables the protection of the active drug (doxorubicin HCl) to enhance the chance of reaching the tumor, where the medication is released [369,733,736,738]. It is hypothesized that because of their small size (approximately 85 to 100 nm) and persistence in the circulation, the pegylated Doxil® liposomes can penetrate the altered and often compromised vasculature of tumors. Once the STEALTH® liposomes distribute to the tissue compartment (passively targeted to tumors), the encapsulated doxorubicin HCl becomes available, due to the enhanced permeability and retention (EPR) effect [733,734,738]. The targeted therapy through the EPR effect comprises a fundamental pathophysiological phenomenon of targeting delivery and progressively retention of pharmacological compounds (anti-cancer drugs) into the vascularized area of solid tumor tissue [742]. Other factors that contributed to the good therapeutic performance of doxorubicin liposomal formulations are the lipid composition and liquid-ordered

phase of the membrane bilayer composed of the cholesterol, MPEG-2000-DSPE, and mainly fully hydrogenated soy phosphatidylcholine (HSPC) with a high phase transition temperature (53 ºC), that helps maintain a stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, excellent drug retention during storage and in vivo administration, as well as, a zero-order slow drug release at the tumor tissue [2]. A schematic representation of the STEALTH® liposomal doxorubicin is presented in Figure 61 [733].

Therewith, Doxil® provides a significant therapeutic benefit over the conventional treatment in therapeutic indications such as the treatment of ovarian cancer (after the failure of platinum-based chemotherapy), acquired immune deficiency syndrome (AIDS)-related to Kaposi's Sarcoma (after the failure of prior combination chemotherapy or intolerance to such therapy), metastatic breast cancer (compared with free doxorubicin), and relapsed or refractory multiple myeloma (in combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy) [733].

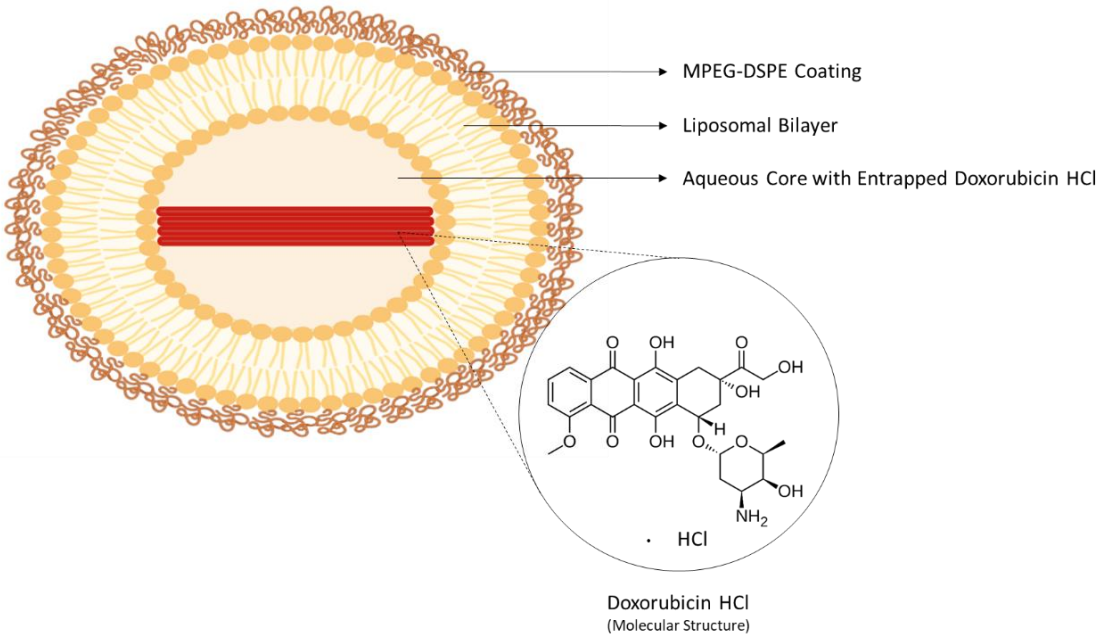


Figure 61. Schematic depiction of a STEALTH® liposomal doxorubicin (based on [733]).

Although the patent expiry has occurred in 2010, the number of approved generic complex drug products of liposomal doxorubicin is still very low at present. In 2012, the FDA allowed the temporary importation supply of Sun Pharma Global FZE's doxorubicin hydrochloride liposome injection (Lipodox®) to totally alleviate the critical shortage of doxorubicin hydrochloride

liposome injection (Doxil®) in the US, and gradually address the patient needs. During this period, the agency has exercised enforcement discretion for the importation of Lipodox®, manufactured in a facility that has been inspected by the FDA and found to comply with good manufacturing practices. At that moment, as the product has not been approved by FDA, it could not be considered a 'generic' of Doxil® [743]. Accordingly, despite the phenomenal sales success of Doxil® around the world, more than 10 years after the last Doxil®-related lost patent protection, there are only two FDA-approved generic versions available in the US market (ANDA 203263, 2013, Sun Pharma Global FZE; ANDA 208657, 2017, Dr. Reddy's Laboratories Inc) [50,51]. On the other hand, there is no approved generic drug product available on the EU market.

The expiry of patent rights with the consequent development of generic liposomal formulations plays a very important role in lowering the cost of prescription medication for both patients and payers, improving patient access to essential and more affordable anti-cancer drug products. In addition, the liposomal formulations are recognized as value-added medicines, in a high potential return market that has attracted significantly the attention of manufacturers over the past few years. For example, the global liposomal doxorubicin market size is experiencing considerable growth due to the increasing awareness regarding the benefit of liposomal doxorubicin formulation over the traditional doxorubicin, with a revenue forecast of USD 1.39 billion in 2025 [744].

However, there are still unlimited scientific, technological, and regulatory challenges in obtaining marketing approval for the generic versions of the existing reference listed drug product Doxil®, which makes it almost inconceivable and inefficient the development of these formulations. On the other hand, there are also commercial issues that hinder liposomal production and constitute a barrier to their translation from bench to bedsides, such as the extremely high costs of the manufacturing procedures and raw materials.

Some of the main liposome-specific quality issues that delayed generic liposomal drug development are related to the complexity of drug products, parenteral administration route, difficulty in physicochemical and structural characterization, or strict quality controls. Further concerns include the multi-step and relatively complex manufacturing process, the necessity of specialized equipment for size reduction or filtration, limited batch size, batch-to-batch reliability and reproducibility, scale-up problems, long-term stability issues, or effective sterilization of the final liposome drug product in specific injectable production sites.

On the other hand, there is also high regulatory demand for the qualitatively (Q1) and quantitatively (Q2) requirements and bioequivalence studies (prior in vivo efficacy and toxicology studies) to the demonstration of therapeutic equivalence of generic liposomal formulations with the reference product. The complex nature of liposomal formulations, just as the difficulties of exact reproducibility and characterization of the vital physical and chemical parameters, do give rise to serious challenges in the adequate definition of regulatory requirements and tests for the development and approval of generic versions. In addition, there are also different regulatory grades

for the demonstration of bioequivalence between the two regulatory authorities, as stated in Chapter III (Section 3.2.1). Additionally, there is a great discrepancy between the FDA and EMA related to the year of issue of the first publicly available product-specific guidance for generic development of doxorubicin liposome injection. The chronological gap occurs between 2010, with the issue of 'Draft Guidance on Doxorubicin Hydrochloride' by the FDA [206], and the year 2018, with the publication of the 'Pegylated liposomal doxorubicin hydrochloride product-specific bioequivalence guidance' by the EMA [158].

Therewith, it is very important to promote advancement and innovation in liposome manufacturing processes, making the drug-delivery systems more reliable and attractive, particularly to the generic product manufacturers. The application of a systematic approach based on sound science and quality risk management, such as the Quality by Design (QbD), introduces a different quality concept of 'Quality by Testing', since that enables 'to build quality into the product instead of testing it' [399]. The QbD principles focus on the quality target profile of the final drug product, improving knowledge and control of the product and process, and the underlying sources of variability [395]. In this chapter, an optimization process supported by a Quality by Design (QbD) approach was applied to the development of generic doxorubicin hydrochloride liposomal formulation.

2. Quality by Design in Pharmaceutical Development of Complex Generic Injectable Liposome Drug Products: Experimental Section

The objective of the galenical development work was to establish a suitable formulation and manufacturing process to obtain a doxorubicin hydrochloride liposomal injection for intravenous infusion, with the same drug product composition, equivalent liposome characteristics and bioequivalent to Reference Listed Product (RLD) marketed in the United States under the name Doxil®, from Janssen Products, LP. Hence, the pharmaceutical development focused on obtaining a product, which was both qualitatively and quantitatively similar to Doxil®, and devising suitable manufacturing controls to reliably and consistently manufacture a product of the same quality and performance profile as the RLD.

The development and optimization of the generic dosage form of the liposomal injection of doxorubicin hydrochloride should include the following steps:

- Patent landscape and literature review of liposome injection for intravenous infusion.
- Production of suitable liposomes:
    - Analysis and characterization of the reference product (Doxil®).
    - Development of an adequate formulation (qualitative and quantitative) based on the reference product characteristics.
    - Development of an adequate manufacturing process.

- Development following the QbD principles:
    - Definition of Quality Target Product Profile (QTPP), based on analysis and characterization of the RLD (Doxil®).
    - Identification of Critical Quality Attributes (CQAs) for the finished product.
    - Use of risk assessment tools to identify potential risks for each unit operation, and to identify potential Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs).
    - Investigation of the effect and relationship of material attributes and process parameters on the CQAs through Design of Experiments (DoE).
    - Identification of CMAs and CPPs and definition of the design space.
    - Performance of pre-stability studies with the most promising prototype.
    - Development of a robust process based on risk assessment (RA).
    - Establishment of control strategies.
    - Applicability of the several physicochemical characterization procedures to guarantee consistency between batches of generic doxorubicin hydrochloride liposome injection and their reference listed drug.

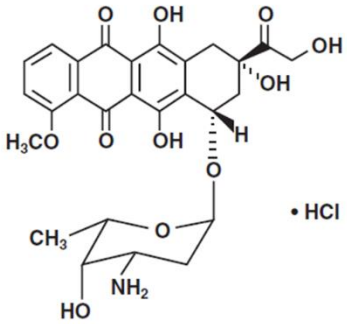
It should be highlighted that this chapter focuses above all on the control and optimization of the first steps in the manufacturing process, particularly the optimization of the preparation step of Unilamellar Vesicles (ULVs) by the high-pressure extrusion method.

2.1. Drug Substance Information

The active ingredient of DOXIL® is doxorubicin hydrochloride, an anthracycline molecule with cytotoxic/antineoplastic activity. Doxorubicin Hydrochloride is the hydrochloride salt of doxorubicin. Doxorubicin, isolated from the bacterium *Streptomyces peucetius* var. *caesius*, is also defined as the hydroxylated congener of daunorubicin [369,738–741].

The therapeutic activity of the doxorubicin arises from more than one mechanism of action. Firstly, its capacity to bind and intercalate between base pairs in the DNA helix, thereby preventing DNA replication and ultimately inhibiting nucleic acid synthesis. Cell structure studies have demonstrated rapid cell penetration and perinuclear chromatin binding, rapid inhibition of mitotic activity and nucleic acid synthesis, and induction of mutagenesis and chromosomal aberrations. Furthermore, this substance inhibits topoisomerase II, resulting in an increased and stabilized cleavable enzyme-DNA linked complex during DNA replication, which in turn prevents the linkage of the nucleotide strand after double-strand breakage. On the other hand, the generation of oxygen free radicals results in cytotoxicity secondary to lipid peroxidation of cell membrane lipids (dose-dependent cardiotoxicity) [733,738–740]. The drug substance (DS) characteristics of the Reference Listed Product (RLD) Doxil® are described in Table 30 [740,741].

Table 30. Drug substance (DS) (doxorubicin hydrochloride) characteristics of the Reference Listed Product (RLD) Doxil® (information retrieved from PUBCHEM Open Chemistry Database [740]; USP-NF Doxorubicin Hydrochloride [741]; and Highlights Of Prescribing Information of Doxil® [733]).

Chemical Name	(8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride
Molecular Formula	$C_{27}H_{29}NO_{11}HCl$
Molecular structure	
MW (g/mol)	579.99
Cas No.	25316-40-9
Pharmacological Class	Antineoplastic agent of the topoisomerase II inhibitor class.
Physical Properties	
Appearance	Orange - red crystalline powder
Solubility	Soluble in water, normal saline, aqueous alcohols, acetonitrile, and tetrahydrofuran; moderately soluble in anhydrous methanol; practically insoluble in non-polar organic solvents (acetone benzene, chloroform, ethyl ether and petroleum ether).
Melting Point	Doxorubicin hydrochloride melts with decomposition at about 204 – 205ºC (399 to 401ºF)
Dissociation Constant (pKa)	pKa1 = 5.9; pKa2 = 8.2; pKa3 = 10.2; pKa4 = 13.2
Hygroscopicity	Hygroscopic
pH	A 5 mg/mL aqueous solution of Doxorubicin hydrochloride has a pH of between 4.0 and 5.5
Polymorphism	There is no polymorphism in doxorubicin hydrochloride.
Storage	Protection from light and moisture. Preservation in airtight containers, and store at controlled room temperature except where it is labeled as amorphous, in which case it should be stored in the freezer.
Safety	Based on animal data and the mechanism of action, doxorubicin HCl is to be considered a genotoxic/ mutagenic, carcinogenic and reproductive toxicant/teratogenic molecule. Occupational Exposure Limit (OEL): 0,47 µg/m^3. Due to its mechanism of action and pharmacology properties, this API should be handled in full contention, particularly in the dedicated containment area.

2.2. Patent Landscape

An initial and continuous overview of the existing patents was conducted during the entire project to avoid any IP (intellectual property) infringements. A thorough literature review was performed to understand the state of the art regarding doxorubicin hydrochloride liposomal formulation composition, manufacturing processes, characterization, and control strategies.

It is important to highlight that the most relevant families of patents in the development of the reference product Doxil® are related to the transmembrane-driven remote loading of amphipathic weak bases such as doxorubicin, as well as, the contribution of the lipopolymer PEG-DSPE as a lipid component of liposome membrane for prolongation liposome circulation time and RES avoidance [734].

In March 2010, occurred the expiry of the patent covering the remote loading, which means that the period of patent protection of Doxil in the USA was approximately 14 years.

Table 31 depicts a selection of patents related to the pharmaceutical development of doxorubicin hydrochloride liposomal formulation.

Table 31. Examples of patents related to the pharmaceutical development of doxorubicin hydrochloride liposomal formulation.

Patent Number (ID)	Patent Title	Publication Date	Patent Holder (Inventor)	Outcomes	References
US4529561A	Method for producing liposomes in selected size range	1985	C. Anthony Hunt; Demetrios P. Papahadjopoulous	Liposomes of uniform size are produced by forming liposomes in relatively random sizes, and extruding the liposomes under pressure through a uniform-pore-size membrane to force at least some of the liposomes into smaller sizes. Extrusion may be repeated to increase uniformity of the liposomes. The liposomes may contain an encapsulated drug.	[745]
US4837028A	Liposomes with Enhanced Circulation Time	1989	Theresa M. Allen	A composition of liposomes which contain an entrapped pharmaceutical agent and are characterized by: (a) liposome sizes predominantly between about 0.08 and 0.5 microns: (b) at least about 50 mole percent of a membrane-rigidifying component, such as sphingomyelin or neutral phospholipids with predominantly saturated acyl chains: and (c) between about 5-15 mole percent ganglioside GM1. The liposomes show a blood/RES tissue distribution ratio, two hours after intravenous administration, which is substantially greater than the sum of the distribution ratios observed with similarly constructed liposome compositions containing the membrane-rigidifying agent alone and gangliosides alone. Also disclosed are methods for enhancing the blood/RES ratio of intravenously administered liposomes, and for assessing the effect of selected liposome components on in vivo uptake of liposomes by cells of the reticuloendothelial system (RES).	[746]
US5013556A	Liposomes with Enhanced Circulation Time	1991	Martin C. Woodle; Francis J. Martin; Annie Yau-Young; Carl T. Redemann	The present invention relates to liposome therapeutic compositions, and, more particularly, to liposome compositions which have enhanced circulation time when administered intravenously.	[747]
US5192549A	Method of Amphiphatic Drug Loading in Liposomes by pH Gradient	1993	Yechezkel Barenolz; Gilad Haran	An improved simple, efficient, safe, economical, and fast transmembrane loading procedure for efficient active loading of weak amphiphatic drugs into liposomes using the transmenbrane gradient. The resulting liposomes loaded with the amphiphatic drug are stable and safe. A storageable form of loadable liposomes has extended period of stability. The reversed procedure is applicable for sustained release of liposome encapsulated drugs from ammonium liposomes.	[748]
EP0361894B1	Loading and Controlled Release of Amphiphatic Molecules to and from Liposomes.	1994	Yechezkel Barenholz; Gilad Haran	The present invention relates to a transmembrane loading procedure for loading of amphiphatic drugs and chemicals into liposomes using the transmembrane gradient. The procedure is equally applicable for sustained release of liposome encapsulated drugs.	[749]

EP1089713B1	Temperature-sensitive Liposomal Formulation	1998	David Needham	The present invention relates to thermosensitive liposomes, and more specifically to liposomes comprising phospholipids and a surface active agent, wherein the liposomes release their contents at mild hyperthermic temperatures.	[750]
US2005/0129752A1	Use and Manufacturing Process for Liposomal Doxorubicin Pharmaceutical Composition	2005	Te-Jung Chen; Sze-Yuan Yang; Chia-Ning Liu; Chun-Ying Huang; Jung-Chin Lin	The present invention provides a method of treating mammals having pancreatic cancer by administering a liposomal doxorubicin pharmaceutical composition, and a process of manufacturing the composition.	[751]
WO/2005/046643	Method for Drug Loading in Liposomes	2005	Alberto A. Gabizon; Yechezkel Barenolz;	A liposome composition having a protonatable therapeutic agent entrapped in the form of a salt with a glucuronate anion is disclosed. Methods for preparing the composition using an ammonium ion transmembrane gradient having glucuronate as the counterion are also disclosed. In one embodiment where the protonatable agent is doxorubicin, the method of the invention has comparable loading efficiency, faster release rate, without compromising the therapeutic efficacy compared to loading with an ammonium ion gradient having sulfate as the counterion.	[752]
WO2010/092590A2	Process for the Preparation of Doxorubicin Liposomes	2010	Subhas Balaram Bhowmick; Alok B. Namdeo; Jayaganesh Natarajan; Pankaj Jain	The present invention discloses a novel process of preparation of doxorubicin liposomal suspension having entrapment efficiency greater than or equal to 95 %.	[753]
US20100209348A1	Methods for Determining Liposome Bioequivalence	2010	Francis J. Martin	This invention provides methods for determining liposome bioeduivalence between a generic drug product and a reference brand-name product. Specific methods for determining bioequivalence between doxorubicin hydrochloric acid (HCl) liposome injection product (Doxil (R) and a generic pegylated liposome doxorubicin product are disclosed herein.	[753]
US20120288558A1	Method for Administration of Pegylated Liposomal Doxorubicin	2012	Alberto A. Gabizon	An embodiment of the present invention comprises a method of treating malignancies in a subject in need of treatment comprising administering to the subject a high loading dose of a pegylated liposomal doxorubicin (PLD) in an initial cycle, followed by a reduced dose in a second cycle, wherein the second cycle reduced dose is in the range of 20% to 50%, preferably 50%, of the initial loading dose, and thereafter one or more maintenance doses in further cycles. The interval between dose cycles is in the range of about three-to-four weeks, preferably about four weeks. The initial loading dose is in the range of between the maximum tolerated dose (MTD) and the recommended dose, preferably the MTD (for instance, in the range of about 70 mg/m2 to 50 mg/m2, preferably 60 mg/m2). The one or more maintenance doses are in the range of about 40 mg/m2 to 50 mg/m2, preferably 45 mg/m2).	[754]

EP3753549A1	Liposomal Doxorubicin Formulation, Method for Producing a Liposomal Doxorubicin Formulation and Use of a Liposomal Doxorubicin Formulation as a Medicament	2020	Stéfan Jonathan Halbherr; Pascal Halbherr; Christoph Mathieu; Patrick Buschor	The present invention relates to a liposomal doxorubicin formulation, a method for producing a liposomal doxorubicin formulation and a liposomal doxorubicin formulation for use as a medicament, in particular for use in the treatment of cancer, uterine leiomyosarcoma and adnexal skin cancer.	[755]

2.3. Quality Target Product Profile and Critical Quality Attributes

Principles of QbD had been applied since the project's early stage, with the definition of Quality Target Product Profile (QTPP).

According to the ICH Q8 (R2), the Quality Target Product Profile (QTPP) is defined as a 'prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product' [395]. The quality target product profile is the basis of design for the development of the intended drug product, constituting the starting point for the implementation of the QbD approach.

Generally, the predefinition of the desired final quality profile (QTPP) includes the outline of the drug product quality criteria (e.g., sterility, purity, stability, and drug release), route of administration, dosage strength(s), dosage form, delivery systems, container closure system, attributes affecting pharmacokinetic profile, bioavailability, among others.

In order to set out appropriately the QTPP of the generic doxorubicin hydrochloride (HCl) liposomal injection, was necessary to take into account the main purposes of the project, previous knowledge of the dosage form, internal knowledge of liposome formulations, preliminary formulation studies, characterization of RLD, as well as, the review of the available scientific literature [206,369,733].

A list of QTPP elements for generic liposomal injection of doxorubicin hydrochloride, target product profile, and respective justifications are described in Table 32. This table encompasses standard quality and regulatory compliance requirements for the parenteral dosage form, as well as standard bioequivalence requirements for generic products.

Table 32. Quality Target Product Profile (QTPP) for generic liposomal injection of doxorubicin hydrochloride [206,369,733].

QTPP Elements	Target Product Profile	Rationale
Dosage Form	Liposomal dispersion for injection.	Pharmaceutical equivalence requirement: same dosage form.
Dosage Design	The suspension must be diluted prior to administration (doses up to 90 mg in 250 mL of 5% Dextrose Injection). Treatment regimen: -Ovarian Cancer- 50 mg/m^2 IV infusion over 60 minutes every 28 days. -AIDS-Related Kaposi's Sarcoma- 20 mg/m^2 IV infusion over 60 minutes every 21 days. -Multiple Myeloma- 30 mg/m^2 IV infusion over 60 minutes on day 4 of each 21-day cycle for 8 cycles or until disease progression (day 1 of each cycle initiates with bortezomib 1.3 mg/m^2 IV bolus).	Pharmaceutical equivalence requirement: same dosage design.

<table>
<tr><td>Route of Administration</td><td>Intravenous.</td><td>Pharmaceutical equivalence requirement: same route of administration.</td></tr>
<tr><td>Administration</td><td>Intravenous infusion over 60 min. Single use.
Intravenous infusion at an initial rate of 1 mg/min. If no infusion-related adverse reactions occur, increase the infusion rate to complete administration over one hour. Single use. Administration as bolus injection or undiluted solution is not possible.</td><td>Same as listed on the RLD label.</td></tr>
<tr><td>Alternative Methods of Administration</td><td>None.</td><td>None listed in the RLD label.</td></tr>
<tr><td>Dosage Strength</td><td>2.0 mg/mL (doxorubicin hydrochloride (HCl)) (20 mg/10 mL or 50 mg/25 mL).</td><td>Pharmaceutical equivalence requirement: same dosage strength.</td></tr>
<tr><td>Pharmacokinetics</td><td>PK Parameters of Total Doxorubicin from Doxil® in Patients With AIDS-Related Kaposi’s Sarcoma: Doxil® displayed linear PK over the range of 10 to 20 mg/m^2. Relative to Doxil® doses at or below 20 mg/m^2, the PK of total doxorubicin following a 50 mg/m^2 Doxil® dose are nonlinear. At this dose, the elimination half-life of Doxil® is longer and the clearance lower compared to a 20 mg/m^2 dose.
<table>
<tr><th rowspan="2">Parameter (units)</th><th colspan="2">Dose</th></tr>
<tr><th>10 mg/m^2</th><th>20 mg/m^2</th></tr>
<tr><td>Peak Plasma Concentration (μg/mL)</td><td>4.12 ± 0.215</td><td>8.34 ± 0.49</td></tr>
<tr><td>Plasma Clearance (L/h/m^2)</td><td>0.056 ± 0.01</td><td>0.041 ± 0.004</td></tr>
<tr><td>Steady State Volume of Distribution (L/m^2)</td><td>2.83 ± 0.145</td><td>2.72 ± 0.120</td></tr>
<tr><td>AUC (μg/mL•h)</td><td>277 ± 32.9</td><td>590 ± 58.7</td></tr>
<tr><td>First Phase (λ_1) Half-Life (h)</td><td>4.7 ± 1.1</td><td>5.2 ± 1.4</td></tr>
<tr><td>Second Phase (λ_1) Half-Life (h)</td><td>52.3 ± 5.6</td><td>55.0 ± 4.8</td></tr>
</table></td><td>Bioequivalence requirement based on (90% CI): AUC and C_{max} for free doxorubicin and liposome encapsulated doxorubicin.</td></tr>
<tr><td>Stability</td><td>Shelf life of unopened vial: 20 months at 2ºC-8°C (36ºF-46°F). Protection from light and freezing.
After dilution: up to 24 hours at 2ºC-8°C (36ºF-46°F); from a microbiological point of view, the product should be used immediately.</td><td>Equivalent to or better than RLD shelf life.</td></tr>
<tr><td>Drug Product Quality Attributes</td><td>Physical attributes:
- Appearance
- Mean particle size
- Size distribution
- Morphology/ Lamellarity
- Surface charge
- State of encapsulated drug (must contain an equivalent doxorubicin sulfate precipitate inside the liposome)
- Internal environment of liposome (volume, pH, sulfate and ammonium ion concentration)
- Grafted PEG at the liposome surface
Liposome composition (lipid content, free and encapsulated drug, internal and total sulfate and ammonium concentration, histidine concentration, and sucrose concentration should be measured)
DS and lipids identification
DS and lipids assay</td><td>Pharmaceutical equivalence requirement for physicochemical characterization: must meet the same compendia or other quality standards (equivalent liposome characteristics). General compendia requirements to ensure patient safety for the chosen dosage form.</td></tr>
</table>

	Liposome encapsulated and free DS	
	DS and lipids degradation products	
	Drug to lipid molar ratio	
	Histidine and sucrose assay	
	Polymorphism	
	Uniformity of dosage units (Fill volume *per* vial)	
	Residual solvents	
	In vitro release of DS from the liposome drug product	
	In vitro drug leakage	
	Osmolality	
	pH	
	Viscosity	
	Phase transition temperature	
	Sterility	
	Pyrogen test	
	Bacterial endotoxins	
Fill Volume *per* vial/ deliverable volume	A volume that enables the extraction of the labeled volume that is to be withdrawn: 10 mL (vial size 10 mL) and 25 mL (vial size 30 mL).	Meet the compendial recommendation (USP <1151>) Same as listed on the RLD label.
Drug Product Composition	Qualitatively and quantitatively the same as the RLD or reference standard: Doxorubicin HCl 2.00 mg/mL; HSPC 9.58 mg/mL; Cholesterol 3.19 mg/mL; MPEG-2000-DSPE 3.19 mg/mL; Ammonium Sulfate 2.0 mg/mL; Sucrose 94.00 mg/mL; Histidine 1.55 mg/mL; Hydrochloric acid or Sodium Hydroxide q.b.p. pH 6.5.	Pharmaceutical equivalence requirement: same drug product composition (drug substance and lipid components), except differences in buffers, preservatives and antioxidants provided that the applicant identifies and characterizes these differences and demonstrates that the differences do not impact the safety/efficacy profile of the drug product. FDA has no recommendations for these type of studies.
Drug Loading Process	Active loading with ammonium sulfate gradient (high encapsulation efficiency, >90%).	Pharmaceutical equivalence requirement: same active loading process and same encapsulating agent.
Container Closure System	Container closure system qualified as suitable for this drug product. Type I glass vial with a siliconized grey bromobutyl stopper and an aluminium seal. RLD packaging material needs to be characterized.	Same as listed on the RLD label. Needed to achieve the target shelf-life and to ensure vial integrity during shipment. Absence of incompatibility and interaction with product formulation.

The next step in the QbD-based development is the identification of the potential critical quality attributes (CQAs).

Under the ICH Q8 (R2) Guideline, the CQAs are defined as ‘a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality’ [395]. Thus, the procedure requires the identification of potential critical quality attributes (CQAs) of the drug product, just as the study and control of the material attributes and process parameters that due to the impact on CQAs should be within a proper range, to guarantee compliance with the desired product profile.

An example of potential CQAs for the liposomal formulation of doxorubicin hydrochloride is provided in Table 33 [206,369,733]. The critical quality attributes (CQAs) being investigated throughout product and process development were identified from the QTPP based on the severity of harm to a patient due to failure to meet the quality target.

Table 33. Critical Quality Attributes (CQAs) for generic liposomal injection of doxorubicin hydrochloride [206,369,733].

Quality attributes		Target	Justification
Physical Attributes	Appearance	Translucent, red liposomal dispersion.	Equivalence requirement for physicochemical characterization.
	Mean particle size	Similar to RLD (85-100 nm).	Liposome mean particle size and size distribuition are critical to ensure passive targeting and their study on at least three batches of both test and RLD is a regulatory requirement to demonstrate bioequivalence. Process and formulation variables may affect the liposomes size. Thus, mean particle size and size distribution (polydispersity index and D_{10}, D_{50}, D_{90}) will be evaluated throughout product and process development.
	Size distribution	Similar to RLD (D_{10}, D_{50}, D_{90} and polydispersity index).	
	Morphology / Lamellarity	Similar to RLD (small unilamellar lipid bilayer vesicles).	Liposome morphology and lamellarity should be determined as drug loading, drug retention, and rate of drug release from the liposomes are likely influenced by the degree of lamellarity. These CQAs may be affected by process variables. Therefore, lamellarity and morphology will be investigated throughout product and process development.
	Surface charge	Similar to RLD (zeta potential).	Surface charge on liposomes can affect the clearance, tissue distribution, and cellular uptake. The surface charge may be influenced by formulation and process parameters, so the zeta potential will be investigated throughout formulation and process development.
	State of encapsulated drug	Similar to RLD (the same form of doxorubicin sulfate precipitate).	The form of the doxorubicin sulfate precipitate inside the liposome could influence its release from the liposomes. Formulation and manufacturing parameters may affect this CQA and, therefore, it will be evaluated during product and process development.
	Internal environment	Similar to RLD (the same internal volume, pH, sulfate and ammonium ion concentration).	The internal environment of the liposome, including its volume, pH, sulfate and ammonium concentration, maintains the precipitated doxorubicin and, therefore, may affect the drug release and leakage. Formulation and manufacturing parameters may influence the liposomal internal environment, so this CQA will be assessed throughout product and process development.
	Grafted PEG at the liposome surface	Similar to RLD.	The surface-bound methoxypolyethylene glycol (MPEG) polymer coating is critical for efficicay, since protects liposomes from clearance by the mononuclear phagocyte system (MPS) and increases blood circulation time. Formulation and manufacturing parameters may influence this CQA. Thus, PEG layer thickness will be determinated during product and process development.
Drug Identification		Positive for doxorubicin HCl.	Identification is critical for safety and efficacy. Sponsors should obtain lipids from the same category of synthesis route (natural or synthetic) as found in the RLD or reference standard. Formulation and manufacturing process are unlikely to impact drug and lipid identity and this CQA can be effectively controlled by the quality management system and will be monitored at drug product release.
Lipid Identification		Positive for HSPC, Cholesterol and MPEG-2000-DSPE.	
Drug Assay		100% (90.0% - 110.0%) of the label claim.	Assay variability is critical for safety and efficacy. Formulation and manufacturing process may affect the assay of the drug substance. Thus, assay will be evaluated throughout product and process development.

Lipids Assay	Molar ratio doxorubicin HCl: total lipids similar to RLD (0.16:1). Molar ratio lipid to lipid similar to RLD (HPSC:Cholesterol:MPEG-2000-DSPE = 1:0.67:0.09).	Assay variability will impact on efficacy and safety. The lipid content demonstrates consistency with the intended formulation and influence the drug loading, leakage, and release from the liposome. Formulation and process variables may affect lipid content so this CQA will be evaluated throughout product and process development.
Percentage and state of encapsulated drug	Same as RLD. Greater than 90% of the drug is encapsulated in the form of a doxorubicin sulfate precipitate.	Variability in encapsulated and free drug levels will impact on safety and efficacy due to changes in the biodistribution. Formulation and manufacturing parameters may affect this CQA. Thus, it will be studied during product and process development.
Drug degradation products	Meet ICH Q3B(R2) requirements.	Degradation products can impact safety and must be controlled based on compendial/ICH requirements or RLD characterization to limit patient exposure. The target for any unknown impurity is set according to the ICH identification threshold for this drug product. The limit for total impurities is based on RLD analysis. Formulation and process variables can impact degradation products. Therefore, degradation products will be assessed during product and process development.
Lipids degradation products	Meet ICH Q3B(R2) requirements.	Degradation products can impact safety and must be controlled based on compendial/ICH requirements or RLD characterization to limit patient exposure. Lipids with unsaturated fatty acids are subject to oxidative degradation, while both saturated and unsaturated lipids are subject to hydrolysis to form lysolipids and free fatty acids. Thus, degradation products will be assessed during product and process development.
Drug to Lipid Molar ratio	Molar ratio doxorubicin HCl: total lipids similar to RLD (0.16:1).	Drug to lipid molar ratio can affect drug leakage and drug delivery to the target cells and, consequently, can impact safety and efficacy. This CQA may be influenced by formulation and process variables. Thus, it will be assessed during development.
Histidine and sucrose assay	Similar to RLD (Histidine: 1.55 mg/mL; Sucrose: 94.00 mg/mL).	Variability in histidine and sucrose assay may impact the safety/efficacy profile of the drug product. Formulation and manufacturing process may affect the this CQA. Thus, histidine and sucrose assay will be assessed throughout product and process development.
Polymorphism	Similar to RLD.	Different polymorphs may result in solubility and bioavailability changes. Process variables may affect this CQA. Thus, polymorphism will be evaluated throughout product and process development.
Container content (Fill volume per vial)	Meet the compendial recommendation (USP <697>). 10 mL (vial size 10 mL). 25 mL (vial size 30 mL).	Inadequate fill volume in the vial may lead to insufficient withdrawal and administration of the labeled volume. Process parameters and the container closure affect this CQA. Fill volume per vial will be investigated during product and process development, through the determination of uniformity of dosage.
Osmolality	Conforms to USP <785>. Isotonic (295 mOsm/kg).	Osmolality values different from plasma osmolarity may cause tissue irritation and damage to blood cells. The integrity of the liposomes is also influenced by osmolality that can cause liposome disruption and premature leakage of the drug substance. The osmolality may be affected by process and formulation variables, so

		this CQA will evaluated during product and process development.
pH	Similar to RLD (pH 6.5 as listed on the RLD label).	pH values different from physiological pH may cause irritation and lead to liposome disruption causing premature leakage of the drug substance. Both formulation and process variables affect the pH. Thus, pH will be assessed during product and process development.
In vitro drug release	Similar to RLD. % drug release acceptance criteria at specific conditions and timepoints (0.5h, 1.5h, 3h, 6h) to be agreed with FDA (info not disclosed on RLD documents).	Failure to meet the in vitro release specification can impact safety and efficacy. Both formulation stability and process variables affect the in vitro release. This CQA will be investigated throughout formulation and process development.
In vitro drug leakage	Similar to RLD.	In vitro drug leakage characterizes the physical state of the lipid bilayer and encapsulated doxorubicin. This test should support a lack of uncontrolled leakage under a range of physiological conditions and equivalent drug delivery to the tumor cells. Formulation and manufacturing variables may affect this CQA, so it will be investigated throughout formulation and process development.
Viscosity	Similar to RLD.	Viscosity can impact efficacy and safety. Formulation and process variables impact on viscosity. Thus, this CQA will be evaluated throughout the development.
Lipid bilayer phase transition	Similar to RLD (equivalent phase transition profiles of raw lipid excipients and liposomes).	Equivalence in lipid bilayer phase transitions will contribute to demonstrating equivalence in bilayer fluidity and uniformity. This affects drug release and the biodistribution. Formulation and manufacturing parameters may impact phase transition temperature. Thus, phase transition temperature will be assessed during product and process development. By using the same qualitatively and quantitatively lipid composition as the RLD or RS and the same manufacturing process, the phase transition profiles of the raw lipid excipients and liposomes should be comparable to those of the RLD or reference standard.
Residual solvents	Meet ICH Q3C(R6) requirements.	Residual solvents can impact patient safety. Ethanol, a class 3 solvent, is used in the manufacturing of the drug product (ethanol injection technique) and is removed by Tangential Flow Filtration (TFF). Considering that solvents may not be completely removed during manufacturing, ethanol will be quantified during product and process development.
Sterility	Meet <71> USP requirements.	Non-compliance with established microbial limits, the presence of any pyrogens or bacterial endotoxins will impact patient safety. Avoidance and/or removal of pyrogenic material and bacterial endotoxins should be addressed by establishing appropriate controls during manufacturing process. These CQAs will be investigated during product and process development.
Bacterial endotoxins	Meet <85> USP requirements.	
Pyrogen test	Meet <151> USP requirements.	
Particulate matter in injections	Meet <788> USP and <790> USP requirements.	All products intended for parenteral administration must be visually inspected for the presence of particulate matter (i.e. extraneous mobile undissolved particles present in solution), which may represent a potentially life-threatening condition. Both formulation and process

		variables may influence this CQA, so it will be evaluated throughout product and process development.
Leachable/ Extractables	Conforms to USP <1663> and <1664> requirements.	Lipid emulsions have higher potential to extract from container-closure systems (plastic and rubber components). This may influence patient safety, so leachable/ extractables will be evaluated throughout product and process development.

2.4. Risk Assessment

The risk assessment is defined in the ICH Q9 as a systematic process of organizing information to support a risk decision to be made within a risk management process [396]. Thus, this valuable science-based process used in quality risk management constitutes an important approach to identifying and prioritizing critical material attributes and process parameters that potentially affect the final quality of the drug product (critical quality attributes, CQAs).

The identification and ranking of potential Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) may be achieved through different tools, based on prior knowledge and initial experimental data, including the Cause and Effect Diagram (also referred to as the Ishikawa diagram, or Fishbone diagram), Risk Estimation Matrix (REM), Failure Mode Effects Analysis (FMEA), or other multivariate data analysis tools.

In this work, the preliminary approach applied to the initial risk assessment of a liposomal formulation was carried out using an Ishikawa diagram, which was subsequently complemented by a Risk Estimation Matrix (REM).

The Cause and Effect Diagrams (also called an Ishikawa diagram or fishbone diagram) is considered a basic and simple risk management facilitation method commonly used to structure risk management by organizing data and facilitating decision-making. This diagram allows the recognition of potential risk factors which can have an impact on the desired quality attributes, enabling to found a list of the potential CQAs of the drug product. An example of the Ishikawa diagram, with the establishment of the set of potential cause-effect relationships of a liposomal formulation, is depicted in Figure 62.

Afterward, a risk estimation matrix (REM) was applied to rank the process parameters that may influence the CQAs of the drug product, in accordance with its potential criticality in terms of risk. This qualitative and quantitative information regarding risk level, and the consequent prioritization of risks, is crucial to complement the cause-effect analysis.

The following tables (Table 34, Table 35, Table 36) give an example of the Risk Estimation Matrix (REM) applied to the manufacturing process of ULVs, specifically the MLVs preparation and extrusion. Based on the severity and the probability of occurrence of the impact on the CQAs, the level of risk for each process parameter was ranked as low, medium, and high. Subsequently,

the experimental design was selected taking into account the data and risk assessment analysis derived from the cause-effect diagram and a Risk Estimation Matrix.

In the QbD-based development, the ultimate objective is to generate a predictive model through DoE, tested for accuracy and robustness, that allows the definition of the design space of the final product.

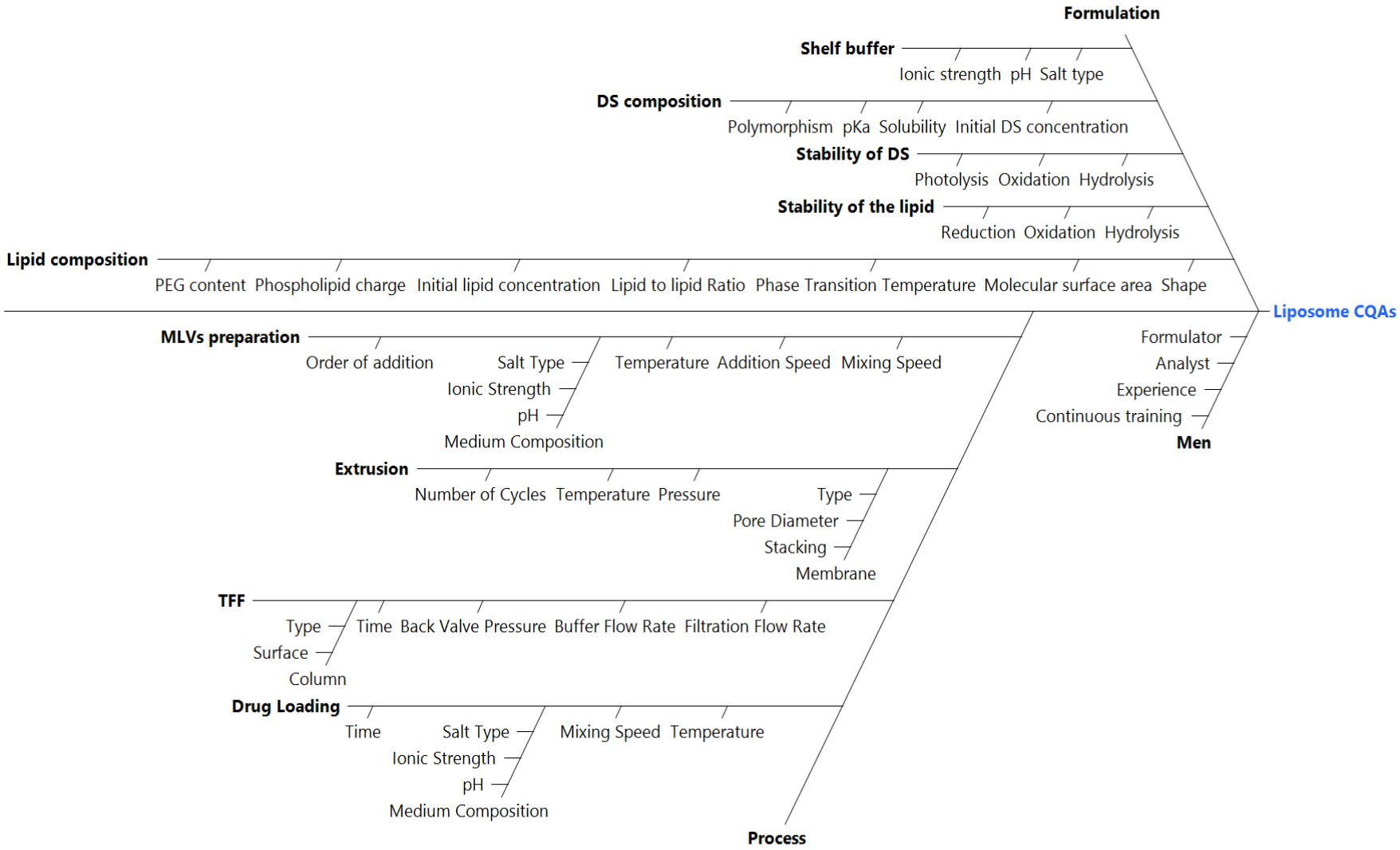


Figure 62. Ishikawa diagram listing the factors that may have impact on liposomes Critical Quality Attributes (CQAs).

Table 34. Risk Estimation Matrix for initial risk assessment of generic doxorubicin hydrochloride liposomal formulation - Part I.

	Formulation									
	Shelf buffer			DS						
Selected CQAs	**Ionic strength**	**pH**	**Salt type**	**pKa**	**Solubility**	**Initial concentration**	**Photolysis**	**Oxidation**	**Hydrolysis**	**Thermal degradation**
Mean particle size	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Size distribution	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Morphology/lamellarity	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Surface charge	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
State of encapsulated drug	Low	Low	Low	Low	High	Medium	Medium	Medium	Medium	Medium
Internal environment	Low	Low	Low	Low	Low	Medium	Low	Low	Low	Low
Grafted PEG at the liposome surface	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Assay DS	Low	Low	Low	Low	Low	High	High	Medium	Medium	Medium
Assay Lipids	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Percentage of encapsulated drug	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
DS degradation products	Low	Low	Low	Low	Low	Low	High	Medium	Medium	Medium
Lipids degradation products	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Drug to Lipid Molar ratio	Low	Low	Low	Low	Low	High	Low	Low	Low	Low
Histidine and Sucrose assay	High	Low	High	Low	Low	Low	Low	Low	Low	Low
Polymorphism	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Container Content	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Osmolality	High	Low	High	Low	Low	Low	Low	Low	Low	Low
pH	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
In vitro drug release	Low	Low	Low	Low	Low	High	Low	Low	Low	Low
In vitro drug leakage	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Viscosity	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Lipid Bilayer Phase Transition	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Residual solvents	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

Table 35. Risk Estimation Matrix for initial risk assessment of generic doxorubicin hydrochloride liposomal formulation - Part II.

	Formulation							
	Lipid							
Selected CQAs	**Initial concentration**	**Lipid to lipid ratio**	**Phase transition temperature**	**Molecular surface area**	**Shape**	**Oxidation**	**Hydrolysis**	**Thermal degradation**
Mean particle size	High	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Size distribution	High	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Morphology/lamellarity	Medium	Medium	Low	Medium	Medium	Low	Low	Low
Surface charge	Medium	Medium	Low	Low	Low	Low	Low	Low
State of encapsulated drug	Low	Low	Low	Medium	Medium	Medium	Medium	Medium
Internal environment	High	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Grafted PEG at the liposome surface	High	High	Low	High	Medium	Medium	Medium	Medium
Assay DS	Low	Low	Low	Low	Low	Low	Low	Low
Assay Lipids	High	Low	Low	Low	Low	Medium	Medium	Medium
Percentage of encapsulated drug	Medium	High	High	Medium	Medium	Medium	Medium	Medium
DS degradation products	Low	Low	Low	Low	Low	Low	Low	Low
Lipids degradation products	Low	Low	Low	Low	Low	Medium	Medium	Medium
Drug to Lipid Molar ratio	High	Medium	Medium	Low	Low	Low	Low	Low
Histidine and Sucrose assay	Low	Low	Low	Low	Low	Low	Low	Low
Polymorphism	Low	Low	Low	Low	Low	Low	Low	Low
Container Content	Low	Low	Low	Low	Low	Low	Low	Low
Osmolality	Low	Low	Low	Low	Low	Low	Low	Low
pH	Low	Low	Low	Low	Low	Low	Low	Low
In vitro drug release	Low	Medium	Medium	Low	Low	Low	Low	Low
In vitro drug leakage	Low	Medium	Medium	Low	Low	Low	Low	Low
Viscosity	Low	Low	Low	Low	Low	Low	Low	Low
Lipid Bilayer Phase Transition	High	Medium	High	Low	Low	Medium	Medium	Medium
Residual solvents	Low	Low	Low	Low	Low	Low	Low	Low

Table 36. Risk Estimation Matrix (REM) for initial risk assessment of generic doxorubicin hydrochloride liposomal formulation (Part III): specific steps of MLVs preparation and extrusion. The critical process parameters were qualitatively classified as high, medium, or low-risk(s) level, according to the severity and the probability of occurrence of the impact on the critical quality attributes selected.

	Critical Process Parameters (CPP)												
	MLVs preparation							**Extrusion**					
Selected Critical Quality Attributes (CQAs)	Order of addition	Medium salt type	Medium pH	Medium ionic strength	Temperature	Addition speed	Mixing speed	Extrusion Time (Number of Cycles)	Temperature	Pressure	Membrane type	Membrane pore diameter	Membrane stacking
Mean particle size	Medium	High	Medium	High	High	High	High	High	High	Medium	Low	High	High
Size distribution	Medium	High	Medium	High	High	High	High	High	High	Medium	Low	High	High
Morphology/lamellarity	Medium	Low	Low	Low	High	Medium	Medium	High	High	Medium	Low	High	High
Surface charge	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
State of encapsulated drug	Low	High	High	High	Low	Low	Low	Low	Low	Low	Low	Low	Low
Internal environment	Medium	High	High	High	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Grafted PEG at the liposome surface	Medium	Low	Low	Low	Low	Medium	Medium	Low	Low	Low	Low	Low	Low
Assay DS	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Assay Lipids	Low	Low	Low	Low	Low	Low	Low	High	High	Low	Low	Low	Low
Percentage of encapsulated drug	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
DS degradation products	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Lipids degradation products	Low	Low	Low	Low	Low	Low	Low	High	High	Low	Low	Low	Low
Drug to Lipid Molar ratio	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Histidine and Sucrose assay	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Polymorphism	Low	Low	Low	Low	Low	Medium	Medium	Low	Medium	Medium	Low	Low	Low
Container Content	Low	Low	Low	Low	Low	Medium	Medium	Medium	Medium	Medium	Low	Low	Low

Osmolality	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
pH	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
In vitro drug release	Medium	High	Medium	High	High	High	High	High	High	Low	Low	Low	Low
In vitro drug leakage	Medium	High	Medium	High	High	High	High	High	High	Low	Low	Low	Low
Viscosity	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Lipid Bilayer Phase Transition	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Residual solvents	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

3. Formulation Development Steps of a Complex Generic Injectable Liposomal Drug Product

3.1. Materials

The doxorubicin hydrochloride liposomal formulation is composed of three vesicle forming lipids (excipients), such as: cholesterol (3.19 mg/mL), fully hydrogenated soy phosphatidylcholine (HSPC) (9.58 mg/mL), and N-(carbonyl-methoxypolyethylene glycol 2000)-1.2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-2000-DSPE) (3.19 mg/mL). The HSPC and MPEG-2000-DSPE were obtained from Lipoid GmbH (Ludwigshafen, Germany), whereas the cholesterol HP was achieved from Dishman Carbogen Amcis Limited (Singapore).

Each mL also contains ammonium sulfate (intraliposomal entrapping agent) (2.0 mg/mL), histidine as a buffer (1.55 mg/mL), and sucrose to maintain isotonicity (osmolality control) (94.0 mg/mL). The pure and pharma-grade L-Histidine base (Ph. Eur., USP) was acquired from PanReac AppliChem ITW Reagents GmbH (Germany). The ammonium sulfate (EMSURE® ACS, ISO, Reag. Ph. Eur.), as well as the sucrose (Reag. Ph. Eur.), were purchased from Merck KGaA (Darmstadt, Germany).

The active ingredient doxorubicin hydrochloride (2.0 mg/mL) was obtained from DZD (Heze) Pharmaceutical Co., Ltd. (Heze, Shandong). The Reference Listed Drug (RLD) used for comparative analysis with our prototype is Caelyx®, acquired from Janssen–Cilag International NV (Belgium). Each single-dose vial contains 20mg or 50 mg doxorubicin hydrochloride at a concentration of 2 mg/mL (equivalent to 1.87 mg/mL of doxorubicin), with a pH of 6.5 [733]. The quantitative and qualitative composition of the finished product is summarized in Table 37 [733].

All of the other reagents used were of analytical grade or better, such as the absolute ethanol, or water for injection (WFI) used at various stages of the manufacturing process.

Table 37. Quantitative and qualitative formulation of finished product Doxil® as described in the label [733].

Component	Quality Reference	Function	Quantity (mg/mL)
Doxorubicin Hydrochloride	USP	Active Ingredient	2.00
HSPC	Company/In-house Specification	Excipient, Vesicle Forming Lipid, Liposome Ingredient	9.58
Cholesterol	NF	Excipient, Vesicle Forming Lipid, Liposome Ingredient	3.19
MPEG-2000-DSPE	Company/In-house Specification	Excipient, Vesicle Forming Lipid, Liposome Ingredient	3.19
Ammonium Sulfate	USP/NF	Excipient, Intraliposomal Entrapping Agent, Ionic gradient	2.0
Sucrose	USP/NF	Excipient, Isotonicity Reagent, Osmolality control	94.00[1]
Histidine	USP/NF	Excipient, Buffer	1.55[1]

Hydrochloric Acid	USP/NF	Excipient, pH Adjustment	-
Sodium Hydroxide	USP/NF	Excipient, pH Adjustment	-
Water for injections	USP/NF	Excipient, Solvent	-

HSPC: Fully hydrogenated soy phosphatidylcholine; **MPEG-2000-DSPE**: N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.
[1]Information available from CAELYX® Pegylated Liposomal Doxorubicin Hydrochloride for Injection (Janssen Inc., 2018) [756].

3.2. Methods

The manufacturing process of doxorubicin hydrochloride liposomal formulation comprises five major steps, wherein the first is the preparation of multilamellar vesicles (MLVs) containing ammonium sulfate, through the ethanol injection method. The second step consists of the liposome size reduction and formation of unilamellar vesicles (ULVs). Then, to generate an ammonium sulfate concentration gradient, the ammonium sulfate that is outside the liposomes is exchanged for the loading buffer and simultaneously the ethanol is also removed. After this step, the liposomes are loaded by an active loading process with the ammonium sulfate concentration gradient that promotes the diffusion of doxorubicin into the liposomes. Finally, the unencapsulated drug substance (DS) is removed and, if the loading and shelf buffer are different, the loading buffer is exchanged for the shelf buffer.

A schematic representation of the experimental design applied in the development of generic doxorubicin hydrochloride liposomal prototype is detailed in Figure 63.

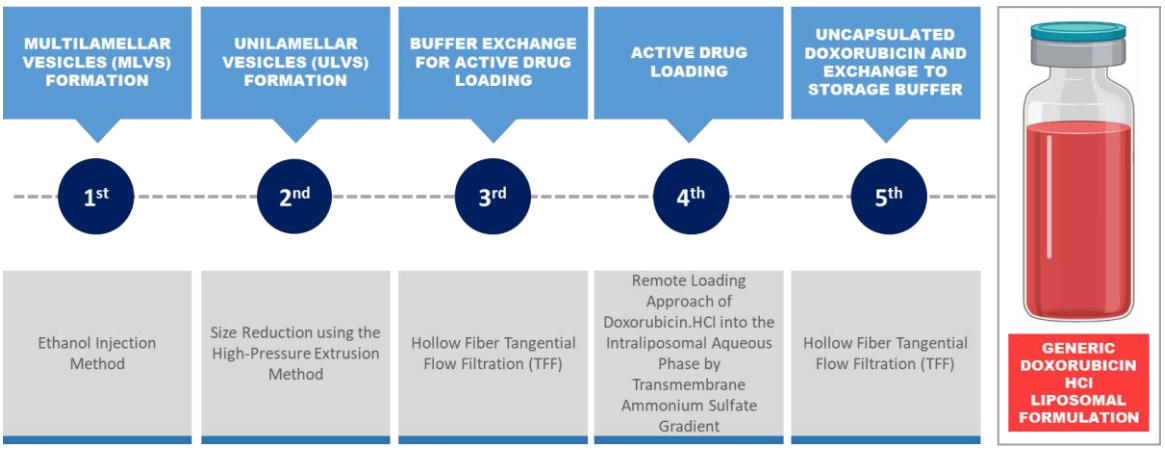


Figure 63. Schematic representation of the manufacturing process applied for the development of generic doxorubicin hydrochloride liposomal formulation.

1st Step: Preparation of Multilamellar Vesicles (MLVs): Ethanol Injection Method

The first step of the manufacturing process of liposomes is the preparation of Multilamellar Vesicles (MLVs). The MLVs were obtained by the ethanol injection method, where the ethanolic solution of lipids was rapidly injected through a thin needle, drop-by-drop, into a certain volume of aqueous phase (ammonium sulfate solution) under continuous vortexing. Both solutions have to be kept above the 53°C phase transition temperature of HSPC (the major component of the lipid membrane), at which membrane fluidity is superior favoring membrane formation [734,735]. The MLVs are formed through the unfavorable interaction of lipids with the aqueous medium resulting in an arrangement in the form of bilayer phospholipid fragments (BPF), that are organized in a phospholipid bilayer in such a way as to limit their exposure to the aqueous environment [757].

This method is one of the preferred reported techniques applied to the manufacturing of stable liposomal formulations since it corresponds to a straightforward, simple, rapid, safe, and reproducible approach. By using ethanol rather than other organic solvents (e.g. chloroform), this technique was considered the safest. Moreover, the ethanol injection does not depend on the sonication step as occurs in the thin-film hydration method, wherefore is not subject to the degradation and toxicity arising from this process [757]. Despite being extensively used, this technique requires the application of several additional steps, such as extrusion, buffer exchange by tangential flow filtration, or drug loading.

The step of MLVs formation is dependent on several parameters (highlighted in yellow) that should be evaluated and controlled throughout the product and process development, such as the lipid concentration, lipid composition, medium composition (salt type, strength, pH), temperature (ºC), mixing/stirring speed (rpm), type of addition (manual or peristaltic pump), order of addition, and addition flow rate (ml/min) (Figure 64).

The suspension contains multilamellar vesicles (MLVs) of a large size, which subsequently need to be down-sized by the extrusion method for the formation of Unilamellar Vesicles (ULVs).

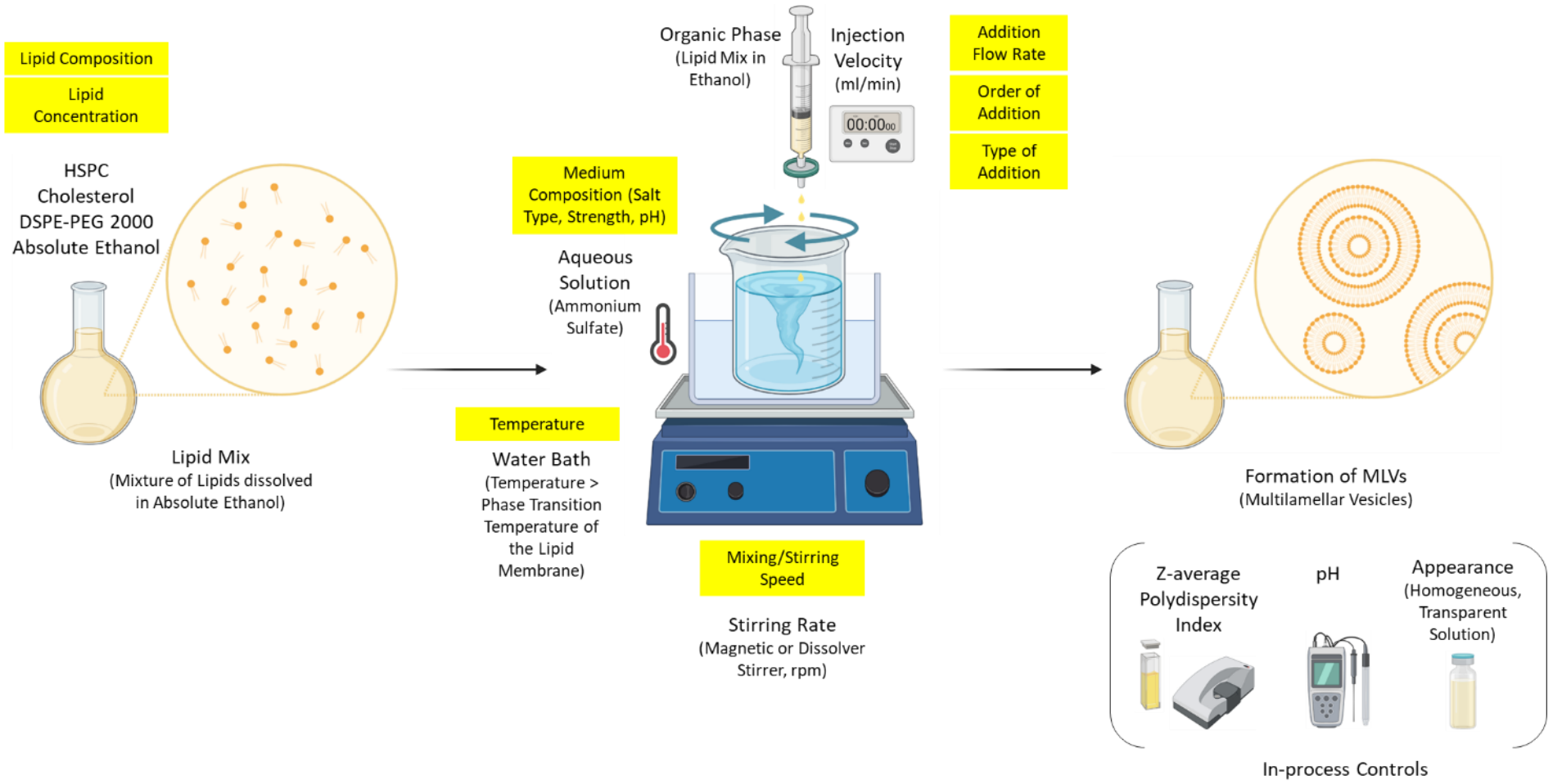


Figure 64. Schematic Representation of Preparation Step of Multilamellar Vesicles (MLVs) by the Ethanol Injection Procedure (the critical processing parameters that can have an impact on the CQAs of the drug product are highlighted in yellow).

2nd Step: Size Reduction and ULVs Formation

The liquid suspension (containing the MLVs) is processed through an extruder for MLVs size reduction and obtaining the Unilamellar Vesicles (ULVs) (Figure 65).

The equipment used in the extrusion process is an Avestin® Emulsiflex C3, a high-pressure homogenizer coupled to an extruder. The process of extrusion is performed through stacked polycarbonate membranes of selected pore size, by applying high pressure in a closed system. The liquid suspension containing MLVs passes through the extrusion device in a specified number of cycles required to obtain ULVs with certain characteristics of mean particle size and polydispersity index (Figure 66) [758]. Before the load of the liposomes onto the extruder, the membranes need to be hydrated with the ammonium sulfate solution. The closed extruder device with all fittings and secure seals, and the regulated pressure system allow the extrudate begins to continually circulate within the system. The temperature during the extrusion step should be kept above the phase transition temperature of HSPC (53ºC), as mentioned in the first step of MLVs formation [734,735]. To obtain a higher temperature than the phase transition temperature in the entire extruder unit, it is necessary to couple a circulating bath at the sample cylinder, as depicted in Figure 66. The formulation of extruded ULVs should be collected in a clean sterile vial after processing finishes.

The extrusion time (defined as the time over which batch volume passes through the extrusion membranes), temperature, pressure, membrane type, membrane pore diameter, and membrane stacking must be controlled to obtain the target mean particle size and the target polydispersity index for the ULVs after the size reduction step, and consequently obtain a final formulation of liposomes with mean particle size and size distribution compliant with the QTPP (85 – 100 nm and < 0.05, respectively) (Figure 66).

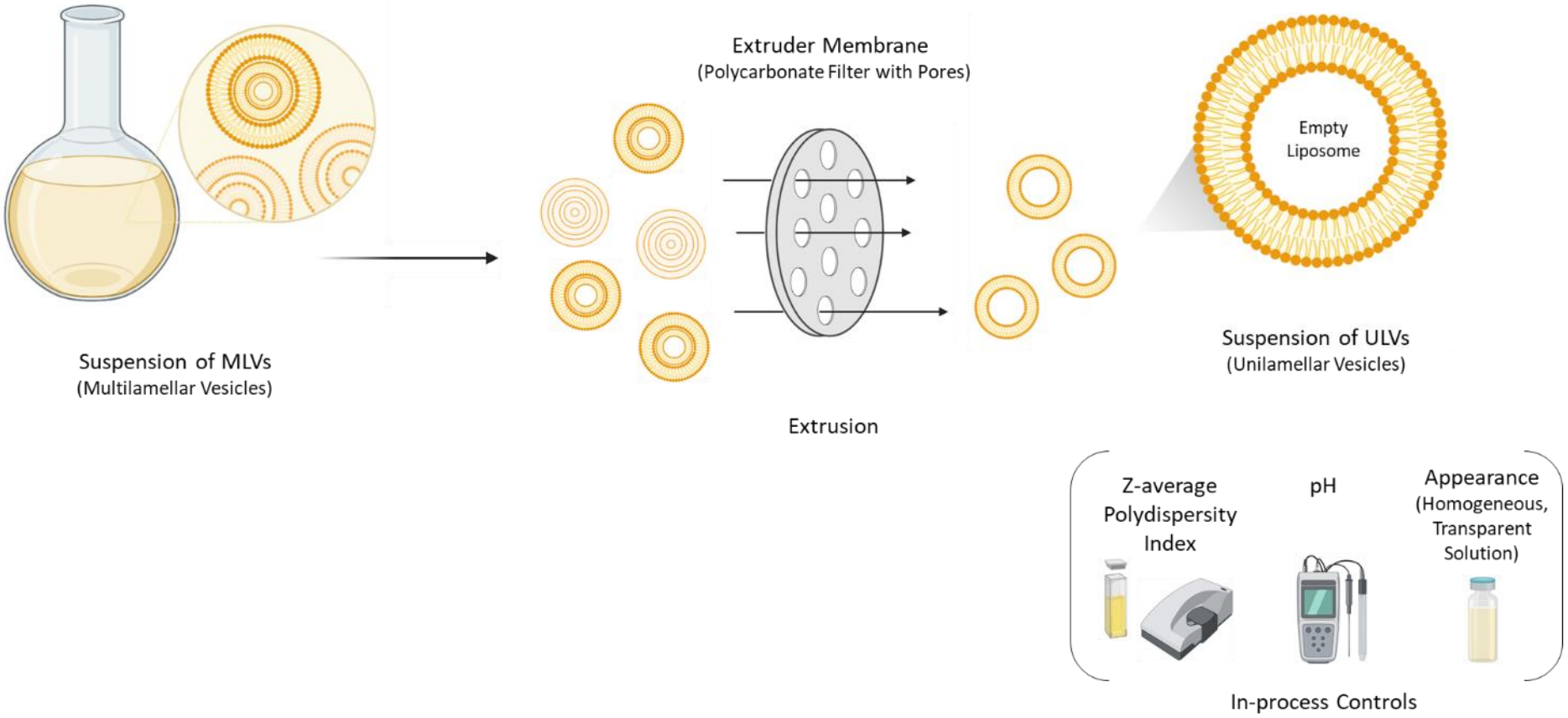


Figure 65. Schematic Representation of Preparation Step of Unilamellar Vesicles (ULVs) by the High-Pressure Extrusion Method.

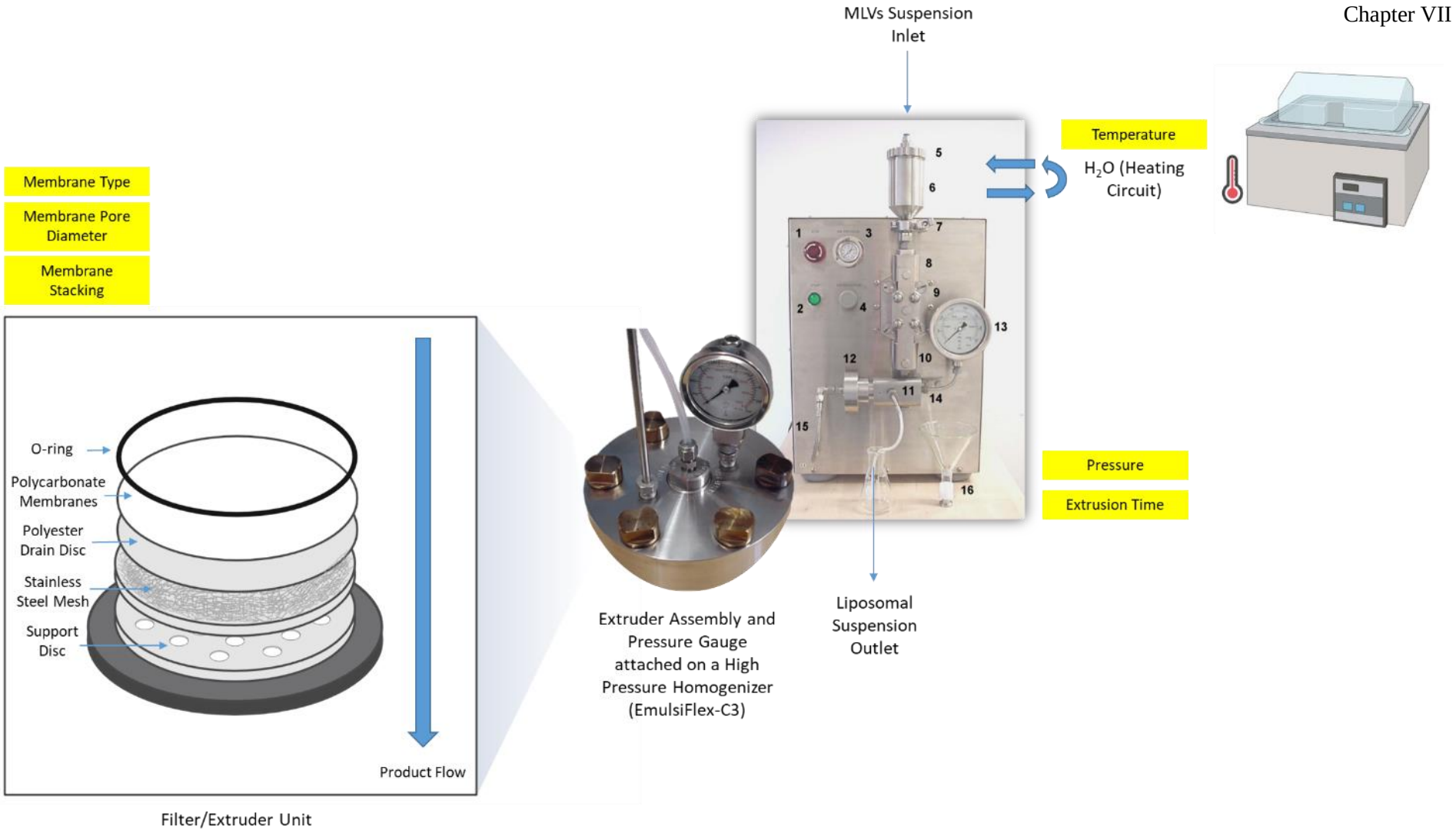


Figure 66. Schematic Representation of the Extruder Unit attached on a High Pressure Homogenizer (Avestin® Emulsiflex C3) on the Laboratory Scale. The critical processing parameters that can have an impact on the CQAs of the drug product are highlighted in yellow. The standard Avestin® Emulsiflex C3 equipment include: 1 - red stop button; 2 - green start button; 3 - air/gas pressure gauge; 4 - air/gas pressure regulator; 5 - sample cylinder cap; 6 - stainless steel sample cylinder; 7 - inlet sanitary fitting; 8 - inlet check valve; 9 - pump body; 10 - outlet check valve; 11 - homogenizing valve; 12 - pneumatic control cylinder; 13 - homogenizing pressure gauge; 14 - gauge nipple; 15 - pneumatic control air supply hose; 16 - glass sample cylinder.

3rd Step: Buffer Exchange for Active Drug Loading

To perform the remote loading of doxorubicin into the preformed liposomes, it is necessary to remove the ethanol excess and more importantly, replace the ammonium sulfate in the exterior of the liposomes to further reinforce the ammonium and pH gradient, in and out of the liposomes. This goal was achieved by using the diafiltration technique, commonly referred to as the tangential flow filtration (TFF) method (Figure 67).

Hollow fiber tangential flow filtration (TFF) modules provide high-performance separation in various upstream and downstream bioprocessing unit operations, including diafiltration and concentration. These modules feature low binding modified polyethersulfone membranes (m-PES) that deliver consistently high process flux and product yields. They are commonly used for the 'washing' or removing a permeable molecule (impurities, salts, solvents, small proteins, etc) from a solution, as well as, to exchange buffers, narrow particle size distributions, modify salt concentrations, or maximize protein recovery in a clarification process. The success of a diafiltration (Buffer Exchange) - the process of using an ultrafiltration membrane to rapidly and gently replace one buffer with another - is largely determined by the selection of an appropriate membrane, and taking into account the molecular weight cut-off (MWCO) of them, composition, surface area, effective length, feed flow rate/shear rate, transmembrane pressure (TMP), among others. For example, the size of the pore membrane must be large enough to allow the permeable species to pass through and small enough to retain the larger species [759].

Figure 67 shows a typical diafiltration system (TFF) using a hollow fiber membrane accomplished by pumping the process solution into the inner diameter of a tubular fiber. In this system, the buffer is continuously added back into the process reservoir through vacuum flow rate based upon permeate flow (continuous diafiltration), i.e. the diafiltration buffer is added to the process vessel at the same rate permeate is being extracted. The outcome is a decrease in the concentration of the permeable species while the retained species remains in the solution which is gently circulating through the tangential flow system [759]. Thus, the primary applications of TFF are:

- **Concentration**: involves the removal of liquid from the liposomal formulation, while retaining the liposome particles. The concentration of the retained particles increases in direct proportion to the decrease in the sample volume.

$$Concentration\ Factor = \frac{(initial\ feed\ weight + feed\ hold - up)}{(initial\ feed\ weight + feed\ hold - up) - (final\ feed\ weight + feed\ hold - up)}$$

- **Diafiltration**: involves the washing of smaller molecules through the TFF membrane, leaving the target liposome particles in the retentate without changing the sample

concentration. This step is often used to remove unencapsulated material, salts, ethanol, small solvents, and additives or to exchange buffers.

$$Diafiltration\ volume = Number\ of\ diafiltration\ volumes\ x\ (initial\ feed\ weight + feed\ hold - up)$$

In the recirculation loop of the TFF system is of utmost importance to careful measurements of the pressure and permeate flow rate to control the driving force through the membrane, just as the process optimization and accuracy in the scale-up. Therewith, two important process variables which need to be controlled in TFF are:

- **Transmembrane pressure (TMP)**: the force that drives solution through the membrane, carrying the permeable molecules.

$$TMP = \frac{(P_{feed} + P_{retentate})}{2} - P_{permeate}$$

- **Shear rate**: the velocity of the solution flow through the feed channel and across the membrane.

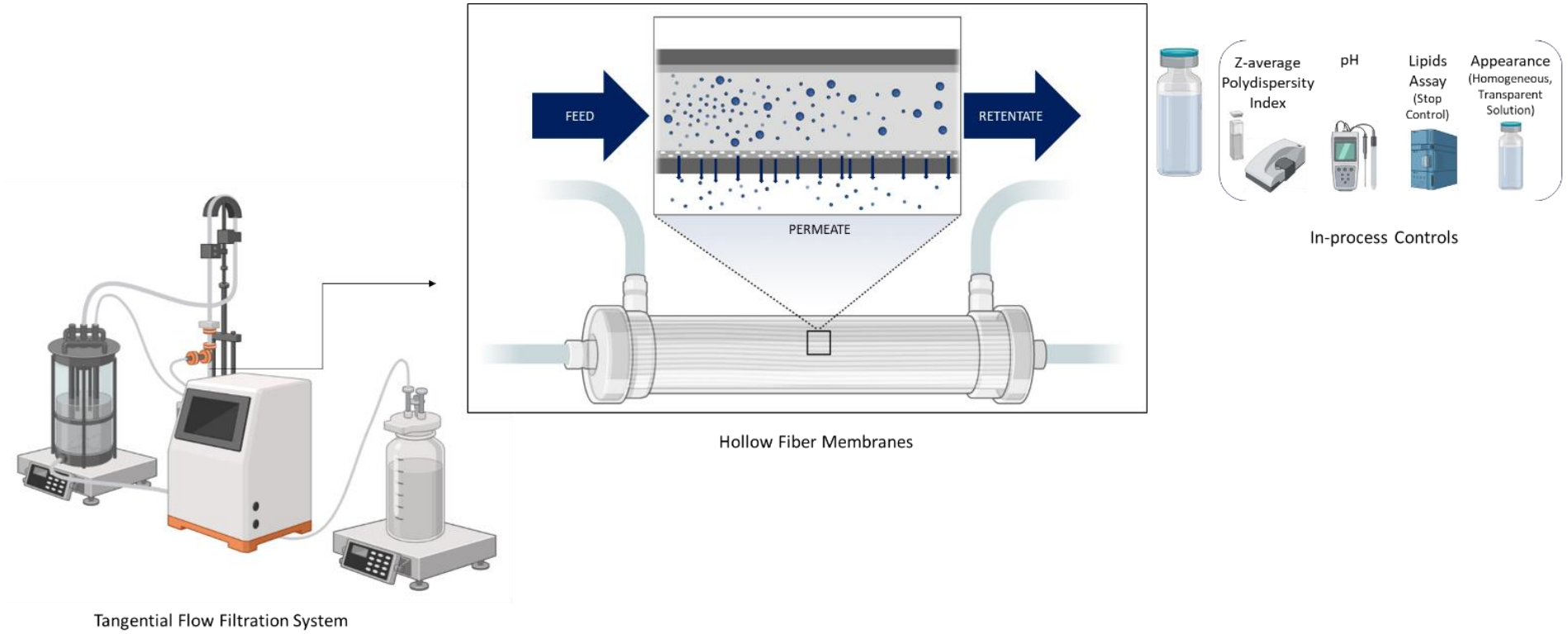


Figure 67. Schematic Representation of Buffer Exchange Step by using the Tangential Flow Filtration (TFF) Method. The Tangential Flow Filtration (TFF) system includes a pump, pressure measurement device, flow measurement device, process reservoir, buffer reservoir, and hollow fiber filter module. The pump circulates the process solution from the process reservoir through the filter and back to the process vessel at a controlled flow/shear rate [759].

4th Step: Active Drug Loading

The remote loading of drug substance (doxorubicin) into preformed liposomes, also referred to as the active loading process, is typically driven by a transmembrane pH gradient, in this particular case, an ammonium sulfate gradient (Figure 68). In the particular case of doxorubicin liposomal formulation, the transmembrane gradient operates as a driving force for the remote loading of the amphipathic weak base drug [735].

The drug internalization into liposomes through a remote loading mechanism by the generation of a pH gradient occurs as follows: the higher concentration of ammonium in the aqueous phase within the liposomes causes the diffusion of the neutral ammonia molecules, i.e. a continuous efflux of ammonia gas generated by the pH-dependent dissociation of the intraliposomal NH_4^+ to neutral ammonia plus a proton. Thus, for every ammonia molecule that leaves the intraliposomal medium, one proton is left inside of the vesicle. This mechanism creates a transmembrane pH gradient ($pH_{liposome} \ll pH_{medium}$), making the intraliposomal aqueous phase then more acidic. Subsequently, the unionized doxorubicin that disseminates to the internal aqueous medium throughout this gradient becomes protonated and gives rise to a form of intraliposome-insoluble doxorubicin sulfate (dox-sulfate) salt. Moreover, the own protonated base buildup inside the liposome leads to elevation of the internal pH, which increases the level of NH_3 with consequent pH reduction, thus further promoting the drug encapsulation. After the remote loading inside of the liposomes of nearly the entire amount of doxorubicin, the dox-sulfate-insoluble salt arranges into nanorod crystals [735,736,760–762]. Thus, the success of the development of liposomal doxorubicin is derived from the huge difference in the permeability coefficients between the neutral ammonia and the sulfate anion, the efficient precipitation (gelation) of anthracycline salt (doxorubicin sulfate) in the intraliposome aqueous phase, just as the low octanol/intraliposome aqueous phase partition coefficient (Figure 68) [760].

The transmembrane ammonium sulfate gradient besides allowing a highly efficient and stable remote loading of doxorubicin required for clinical use (~50 mg/m2), also plays a major role in the retention of doxorubicin in the form of a crystalline-like precipitate during long-term storage and in the blood circulation after the intravenous administration. This gradient also imparts an additional function to the ammonium-induced drug release at the tumor tissue, through the ammonia continuously produced by the exclusive tumor metabolic pathways (e.g. glutaminolysis) [735,736,760,761].

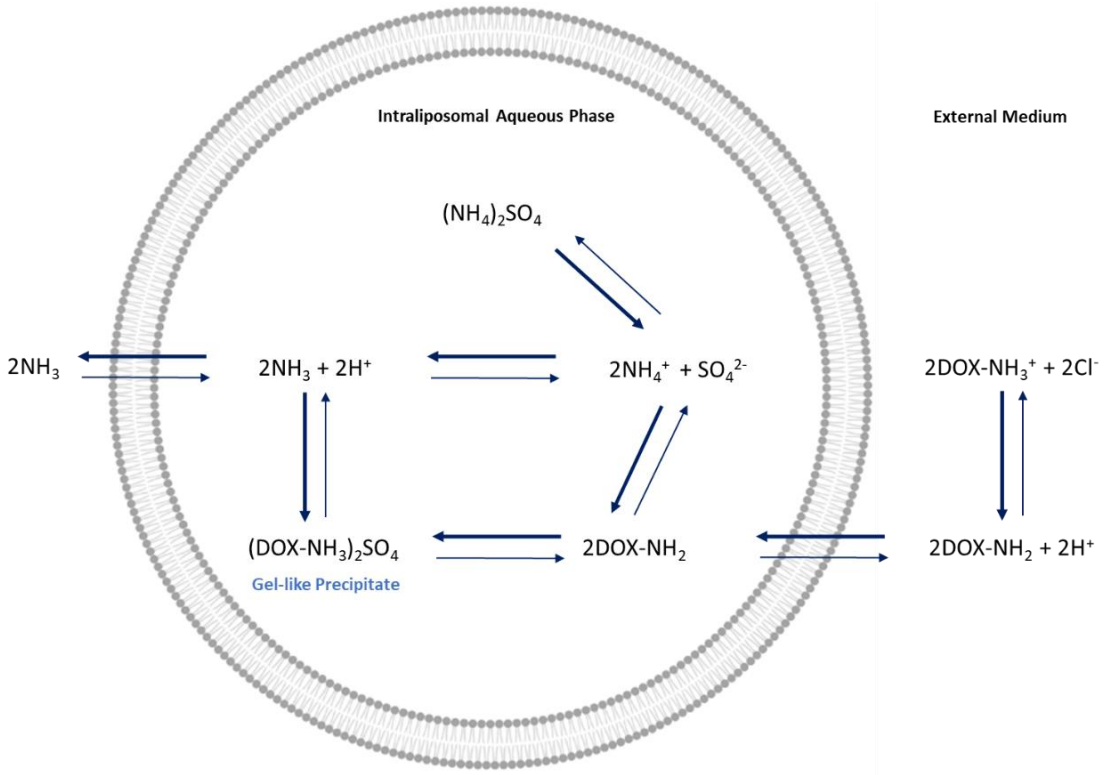


Figure 68. Mechanism of Remote Loading Approach of Doxorubicin into the Intraliposomal Aqueous Phase by Transmembrane Ammonium Sulfate Gradient (based on [735,736,760–762]).

The remote loading of doxorubicin into preformed liposomes is performed above the 53°C phase transition temperature of HSPC, enabling doxorubicin to cross the liposomal bilayer. Initial mixing of a solution of doxorubicin (in 'Loading buffer') and the liposomes (also in 'Loading buffer') is performed at room temperature with a short gentle mix. The mixture is then transferred to a water bath (temperature above 53ºC). The time of incubation of 30 min at 60ºC it has been suggested in the scientific article 'Cardinal Role of Intraliposome Doxorubicin-Sulfate Nanorod Crystal in Doxil Properties and Performance' [735]. To stop the remote loading, and further stabilize the bilayer membrane to avoid any doxorubicin loss from the inner liposome core, the mixture is transferred to an ice-cold water bath at 0-2ºC (Figure 69). Finally, the mixture is then allowed to reach room temperature before performing the final TFF for free drug removal and buffer exchange to the storage buffer.

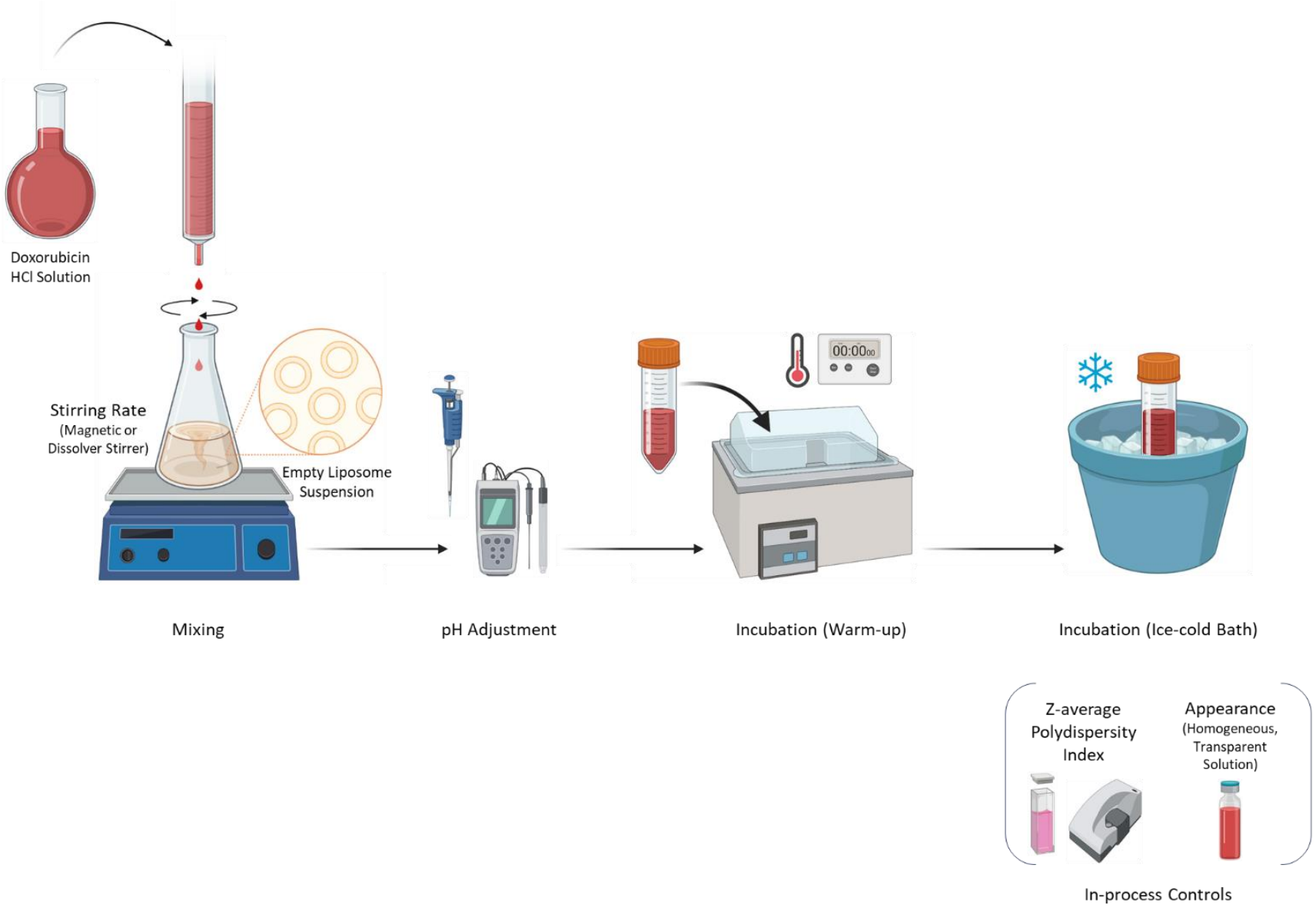


Figure 69. Schematic Representation of Remote Loading of Drug Substance into Preformed Liposomes.

5th Step: Removal of Unencapsulated DS and Buffer Exchange to Shelf Buffer

The process of removal of the unencapsulated drug substance and buffer exchange to shelf buffer (also known as 'storage buffer') is carried out using mPES membrane hollow fibers columns, likewise in the first TFF. To perform the TFF, columns are first equilibrated with Shelf buffer, and the drug-loaded liposomes are diluted with the same Shelf buffer and then concentrated. During this process, the free drug is removed while exchanging the external buffer for a 'Storage buffer' (Figure 70). Due to the acidic pH of doxorubicin hydrochloride in aqueous solution, the inclusion of pH adjustment buffering agent L-histidine in the finished product formulation excipients and consequent buffer exchange to shelf buffer represents fundamental steps to bring the finished product to physiological pH range, feasible for intravenous administration [369].

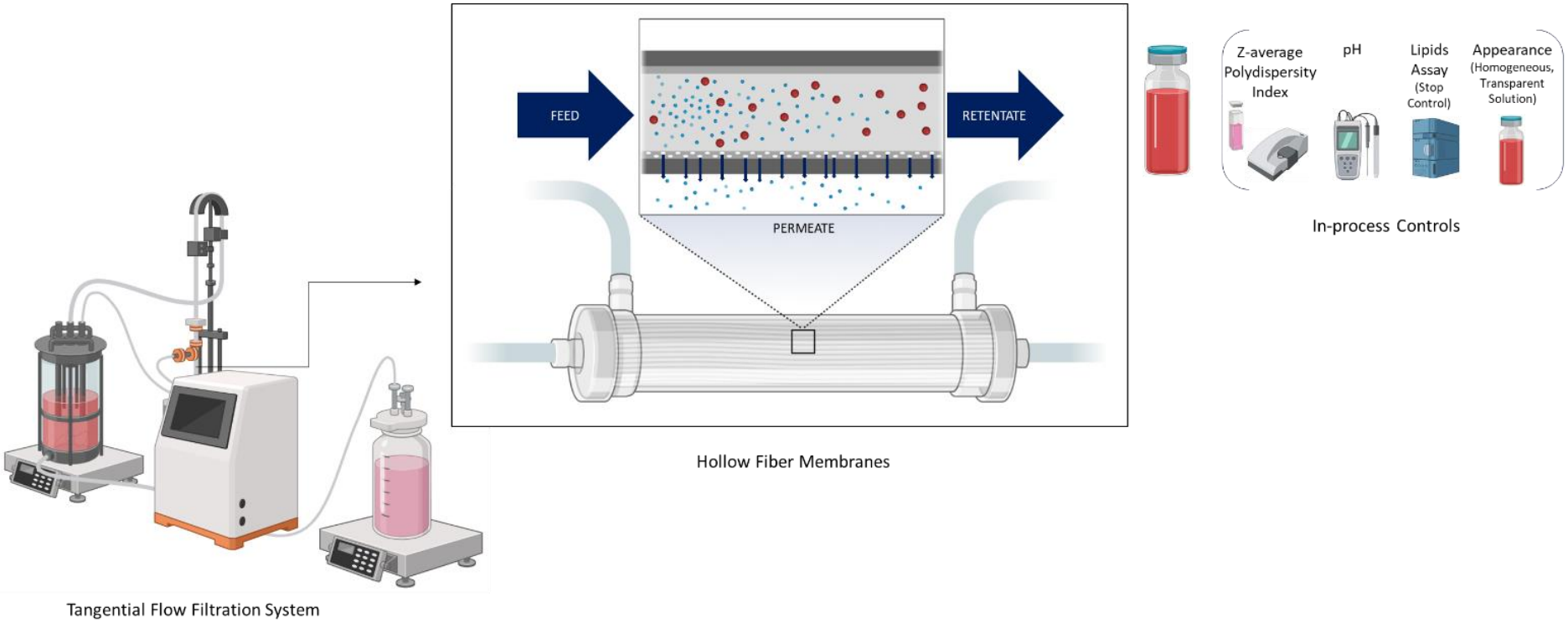


Figure 70. Schematic Representation of the Unencapsulated Drug Substance Removal and Buffer Exchange by using the Tangential Flow Filtration (TFF) Method.

3.3. Liposomal Formulation Characterization (In-Process Controls)

3.3.1. Appearance

The general appearance of the ULVs suspension was checked by visual inspection and includes morphological characteristics like color and opacity. The liposomal dispersion should be homogeneous, translucent, and free of bubbles and particulate matter.

3.3.2. Dynamic Light Scattering (DLS) Characterization

Particle size and size distribution (PDI) were determined by the dynamic light scattering (DLS) technique, acquired with a Zetasizer Nano ZS of MalvernPanalytical® Mastersizer.

Firstly, the NaCl solution of 0.9% (w/v) was filtered with a Nylon VWR filter of 0.2 µm and a syringe. The cuvette was rinsed three times with ultrapure water, and subsequently three times with the previously filtered NaCl solution of 0.9%. Processed samples (10 µL) were diluted into the cuvette in 3 mL of the filtered NaCl solution of 0.9%. Lastly, the cuvette (with the cap) is gently inverted to homogenize the dispersion, ensuring that the presence of bubbles is null, cleaning the walls of the cuvette before starting the analysis. The measurements were performed at a temperature of 25ºC, in replicated (n=3), and results were described through the mean and standard deviation.

3.3.3. Cryogenic Transmission Electron Microscopy (Cryo-TEM) Characterization

The morphology and structure of the liposomal formulations can be determined by Cryo-Transmission Electron Microscopy (Cryo-TEM). The resultant micrographs can be used to give information on the state of the encapsulated drug, liposomal lamellarity, sphericity, size, diameter, volume, and wall thickness. It is necessary to take into account the fact that the particle size determined by Cryo-TEM is a little smaller than the particle size obtained through DLS, due to the fact that DLS measures the hydrodynamic diameter of particles.

The Cryo-TEM analysis was required by the Intertek Pharmaceutical Services (Manchester, United Kingdom).

A drop of each sample was placed on a Lacey carbon film on a 300 mesh Copper TEM grid and blotted for 1.2 secs to produce a thin film across the grid. Each prepared grid was plunged into liquid ethane using the Leica GP plunge freezer and stored under liquid nitrogen.

Prior to the examination, the frozen grid/sample was loaded into a Gatan 626 cryo holder for transfer into the FEI Tecnai 200kV TEM for examination. The plunge frozen specimen is loaded on the Cryo stage, evacuated, and maintained throughout the period of experimentation at a

temperature below -160°C using liquid nitrogen. Before, and during the analysis, the TEM's anti-contamination device was liquid nitrogen cooled to limit any column vacuum deterioration. Preceding the introduction of the specimen into the column, maintained at ultra-high vacuum, the field emission filament was initiated and aligned at 200kV accelerating voltage. The cooled specimen/specimen holder was removed from its covered evacuated storage system and inserted into the TEM set for Cryo examination.

The analysis was performed under extremely low electron illumination (Low Dose Imaging), in order to avoid structure degradation.

The FEI Tecnai Cryo-TEM system used within this study is equipped with software-controlled beam blanking and was therefore used throughout the image capture process. The images were captured using an FEI BM Eagle digital camera.

3.4. Scientific Problem Statement

In order to assess the feasibility of developing generic doxorubicin hydrochloride liposomal formulation through the manufacturing process described above, some preliminary studies were performed based on the compilation of scientific literature and the patent landscape [206,253,369,383,733–735,748,749,753,763]. To characterize and, hence, assess the feasibility of prototype production, the following critical parameters were evaluated: mean particle size, size distribution, morphology, lamellarity, liposomal internal environment, doxorubicin HCl assay, total lipid assay, doxorubicin HCl: total lipid molar ratio, and the loading efficiency.

The preliminary tests showed significant differences between the RLD and prototype obtained. For example, the Cryo-TEM analysis (Figure 71) evidences morphologic differences between the RLD and prototype since the RLD presents an ellipsoid morphology (oblate), unlike the prototype liposomes, which have a deformed ellipsoid morphology (prolate), as also observed by the sphericity values on Table 38. In spite of this, regarding lamellarity, the membrane of the prototype liposomes is unilamellar, as verified for the RLD liposomes. Individual strands can clearly be identified within the internal crystalline fiber structures, suggesting that the structures have formed correctly due to the intra-liposomal drug precipitation, although over-extended resulting in the ellipsoid liposomes.

It is noteworthy that the modifications in the morphology and, thus, in the size of the liposomes, occur after the ULVs formation as a result of the drug loading step. Therefore, this increase may be a possible consequence of the expansion of doxorubicin fiber bundles precipitated inside liposomes that impacts the liposomes' morphology/size. Therewith, the superior size/internal volume of ULVs in the manufacturing process of the prototype translates into a rise in the doxorubicin encapsulated,

with the expansion of crystalline strands and consequently, the greatest deformation of liposomes, as suggested in Figure 71.

In the initial experiments, are there any other issues that should be taken into account about the steps of ethanol injection and extrusion:

- Rupture of O-ring due to the high pressures and loss of sample during the extrusion. The pressure progressively increased until the O-ring of the extruder was broken, leading to the extravasation of the sample through the extruder. The collapse of the O-ring occurs when the pressure increases at a certain level (e.g. above 200 bar), either because of the large dimension of MLVs (before extrusion: 293.5 ± 2.82 nm) or also due to the use of a superior number of membranes in stacking (e.g. more than five membranes) in combination with an excessively low pore diameter.
- Incorrect definition of the target mean particle size and the target polydispersity index for the liposomes after the size reduction (extrusion) step. It is necessary to reduce the target mean particle size and the target polydispersity index to accomplish the specifications of the final product, taking into account the amendments following the drug loading process (in this case, the increase of mean particle size ~5-10% after the doxorubicin fiber bundles formation).
- The need to control the time and temperature of incubation, as well as, the drug substance solution volume that is used in the drug loading step to avoid a superior drug substance assay than the target value and the formation of over-extended ellipsoid liposomes.
- The need to control the pH adjustment during the production process (e.g. pH adjustment before the first TFF) in a way that does not compromise the transmembrane pH gradient required to promote the diffusion of doxorubicin into the liposomes.

Table 38. Summary of the images analysis measurements for the prototype and the Reference Listed Drug (RLD).

Trial ID	Diameter (nm)		Sphericity	Wall Thickness (nm)	Crystal Strands Width (nm)	Internal Structure
	Mean Major Axis	Mean Minor Axis				
RLD	86.14 (238) [109.35/51.31]	71.83 (238) [108.28/48.23]	0.68 (194) [1.0/0.33]	Unilamellar 6.89 (269) [10.16/4.29]	17.07 (208) [30.11/6.01]	Internal Crystalline Strands
Prototype	128.87 (102) [192.54/57.49]	74.59 (102) [129.95/41.75]	0.36 (135) [1.0/0.06]	Unilamellar 5.73 (136) [9.53/3.26]	22.06 (110) [30.32/12.09]	Internal Crystalline Strands

Note: Red bracketed numbers = counts, and Blue numbers = Maximum/Minimum values

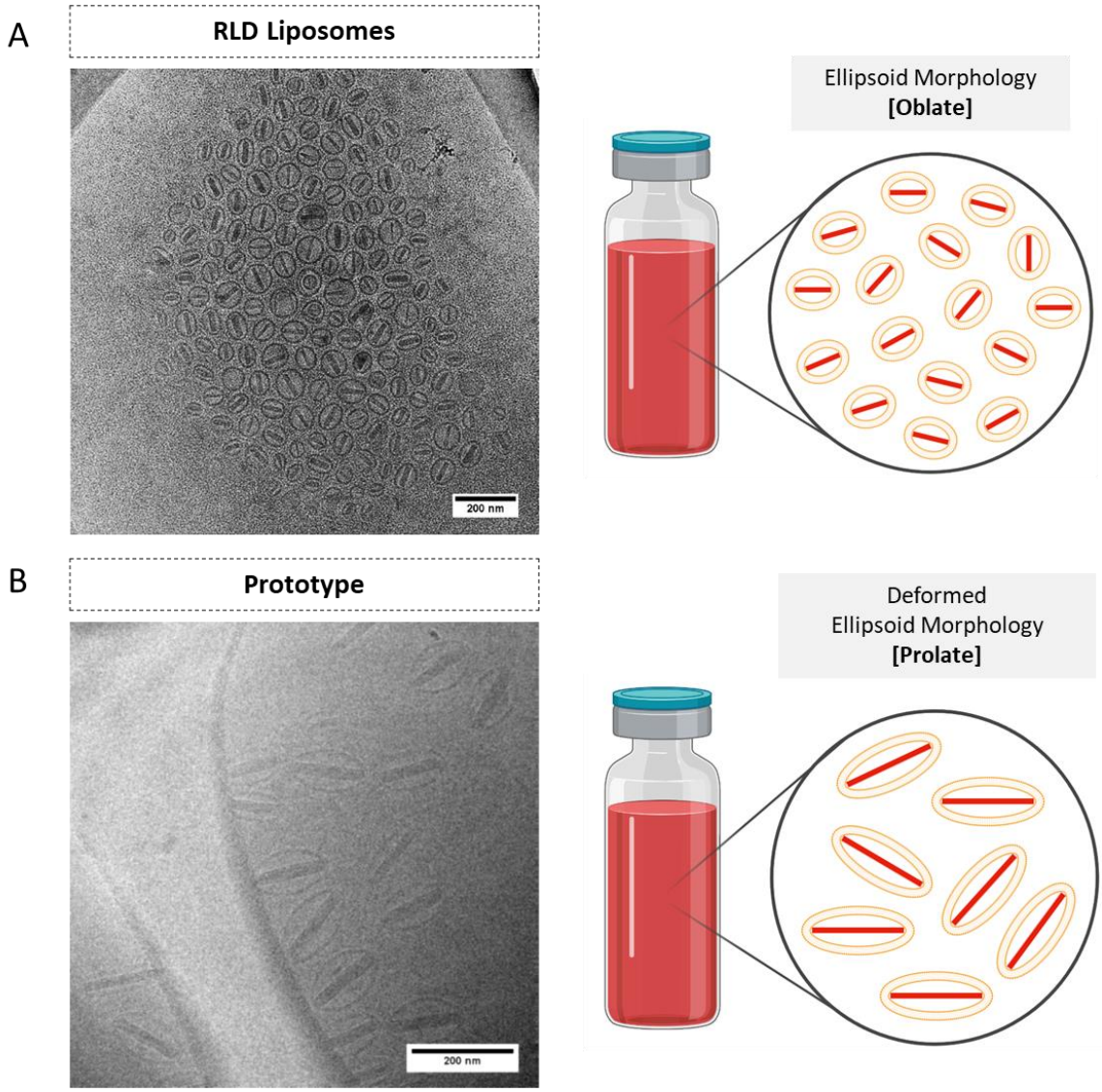


Figure 71. Schematic representation and Cryogenic Transmission Electron (Cryo-TEM) analysis of the: A) RLD liposomes; and B) prototype liposomes; at the micro bar scale of 200 nm (Diluted 1:9 Shelf Buffer). Shelf Buffer: Sucrose 9.4% [m/v] and Histidine 10mM, pH 6.5.

The results of prototype manufacturing indicate that the ethanol injection and extrusion conditions need to be optimized since the conditions tested in this experimental trial were not suitable to accomplish the target mean particle size and the target polydispersity index.

Therefore, the target range of mean particle size (nm) and polydispersity index of ULVs should be redefined for lower values at the extrusion stage (in-house defined as PS < 75 nm, and PdI < 0.05), in a manner that takes into account the change of morphology/size as a result of remote loading step, and consequently, achieve the specifications of the final product.

Thus, the objective of the experimental work focused on the optimization of the processing parameters on the ULVs formation, more specifically in the initial steps of the size reduction. The ULVs preparation procedure was divided into two steps to clarify the design of the experiment: the first step corresponds to the Multilamellar Vesicles (MLVs) preparation by the Ethanol Injection Procedure; followed by the Unilamellar Vesicles (ULVs) formation by the High-Pressure Extrusion Method. The optimization of the first steps plays a crucial role to develop a generic dosage form of the doxorubicin hydrochloride liposomal injection for intravenous infusion, with the same drug product composition, equivalent liposome characteristics, and bioequivalent to their Reference Listed Product (RLD) (Doxil®/Caelyx®) (i.e. obtain a product qualitatively and quantitatively similar to RLD, with the same quality and performance profile).

In accordance with the results previously described, a trial was implemented to obtain a decrease in mean particle size (nm) and polydispersity index of MLVs, to facilitate the extrusion process and avoid the rupture of the O-ring due to the high pressures and loss of sample. The decrease in mean particle size (nm) of MLVs is crucial to obtaining ULVs (size reduction step) with values inside the target range 75 nm ± 5% nm and polydispersity index < 0.05.

In order to reach this aim, specific production settings (stirring speed [800-1000rpm], temperature [60-65ºC], and addition flowrate [1-5 ml/min]) were selected to understand which is the most suitable for the preparation of MLVs with desired characteristics (Table 39). On the other hand, has been used a cowles disperser much more efficient in the dispersion of drops of lipid mix (ethanolic solution) in the aqueous phase compared with the 4-bladed propeller stirrer. It should be ensuring that the cavitation is minimal in the several stirring speed applied. Moreover, has also been used a peristaltic pump to control the injection velocity of the lipid mix drops (constant velocity) to avoid the variability in flowrate during ethanol injection performed manually. The control of temperature in the glass beaker performed through an external temperature test probe (before and after the ethanol injection) has also been considered.

Table 39. Results of Manufacturing Process of Multilamellar Vesicles (MLVs) by Ethanol Injection Method

ID	Stirring speed (rpm)	Temperature (ºC)	Addition flowrate (ml/min)	pH	Temperature (pH)	Mean particle size (nm)	Polydispersity index
MLVs	800 - 1000	60 - 65	1 - 5	6.126	21.3	196.2	0.442

4. Optimization of Extrusion Step and Unilamellar Vesicles (ULVs) Formation

4.1. Experimental design

Based on the initial risk assessment and the preliminary feasibility studies, an I-Optimal design with four factors and two responses, was performed for the optimization of the extrusion step and ULVs formation.

I-Optimal design belongs to the group of custom designs, created using search routines that depend on a mathematical optimality criterion. This design is more appropriate for prediction since that minimizes the average variance of prediction inside the region of the factors (design space). In comparison with D-optimal designs, the I-optimality criterion is more suitable if the primary experimental goal is not to estimate coefficients, but rather to predict a response, determine optimum operating conditions, or determine regions in the design space where the response falls within an acceptable range [764].

Thus, an I-Optimal design approach resulting in a total of 12 experimental runs (including 2 center points), was designed to examine the influence and interactions of the independent variables (factors) on the product quality attributes (dependent variables or responses).

The construct model effects (factors) include the X1: temperature (ºC) (Continuous), X2: membrane pore diameter (Discrete Numeric), X3: membrane stacking (Discrete Numeric), and X4: extrusion time (min) (Continuous). Two responses (dependent variables) were evaluated, particularly the particle size (nm) of ULVs after extrusion (Match target) (Y1), and the polydispersity index (Minimize) (Y2).

The inclusion of the two center points is proposed to increase the number of data points in a DoE (replicates), thus allowing the increased power of the model. By adding just a few center points to the design is possible to estimate pure error for the lack of fit test, i.e., the probability of detecting significant parameters, and estimate the variability. It can also be helpful to check the reproducibility of the model operated on different days, ensuring that the analysis is carefully designed and statistically significant.

The matrix of the experimental design includes 12 ULVs formulations and the results obtained are presented in the next section (Section 3.4.3). Table 40 describes the analytical settings of the design of experiments for extrusion step optimization.

Table 40. Experimental design matrix for extrusion step optimization.

Experiment Number	Temperature (ºC)	Membrane pore diameter (nm)	Membrane stacking (number)	Extrusion time (min)
1	65	50	4	8
2	60	50	5	15
3	65	50	4	8
4	70	80	5	15
5	70	50	3	15
6	60	50	5	1
7	70	80	4	15
8	70	80	5	1
9	60	80	3	1
10	60	80	4	1
11	60	80	3	15
12	70	50	3	1

Subsequently, the prediction profiler was applied to identify optimal settings based on custom design results and to construct a desirability function based on the response Limits information.

Lastly, from the prediction profile obtained for maximum desirability of the target level of responses, were carried out validation tests for the extrusion settings. The range of the critical process parameters to achieve the target criteria for mean particle size and polydispersity index were obtained through the use of the contour plots function.

4.2. Statistical analysis

The design and layout of the set of experiments and consequent mathematical modeling and statistical analysis were carried out in the JMP® screening platform, from SAS Institute, Inc. (North Carolina, USA).

4.3. Results and Discussion

Regarding the extrusion step optimization, the results obtained are presented in Table 41. The analysis of the results obtained was performed through the application of advanced statistical tools, such as the data visualization (from Graph Builder), prediction profiler, and contour profiler.

Table 41. Results of Design of experiments for extrusion step optimization.

Experiment Number	Temperature (ºC)	Membrane pore diameter (nm)	Membrane stacking (number)	Extrusion time (min)	Mean particle size (nm)	Polydispersity index	Pressure (bar)	pH	Temperature (ºC) (pH measurement)
1	65	50	4	8	66.25	0.037	80	6.072	21.9
2	60	50	5	15	60.76	0.069	150	6.049	22.0
3	65	50	4	8	58.96	0.059	100	6.024	21.8
4	70	80	5	15	80.75	0.042	15	5.839	21.8
5	70	50	3	15	63.59	0.029	50	5.771	21.7
6	60	50	5	1	71.71	0.064	110	6.063	21.7
7	70	80	4	15	77.09	0.05	30	5.868	21.7
8	70	80	5	1	89.36	0.109	30	5.883	21.8
9	60	80	3	1	96.09	0.073	10	6.076	21.7
10	60	80	4	1	89.66	0.039	35	6.008	21.5
11	60	80	3	15	80.56	0.071	30	6.002	21.6
12	70	50	3	1	73.66	0.05	50	5.957	21.4

The graph visualization is obtained through the JMP Graph Builder platform. This option allows a rapid and interactive visualization to explore and describe the data collected.

In Figure 72 it is possible to verify that the membrane pore diameter is a factor that significantly impacts the mean particle size. As expected, the tendency is the decrease of the membrane pore diameter lead to the decrease of the particle size, i.e. the particle size is lower when working with 50 nm of membrane pore diameter than using the pore diameter of 80 nm.

On the other hand, the stacking of the four membranes seems to result in the lowest values of particle size. However, it is necessary to treat the graphics and draw conclusions with caution, due to the points which were scattered outside the linear regression and confidence intervals, as occurs for the temperature and extrusion time parameters.

Likewise, it is possible to infer that the membrane pore diameter has an impact on the polydispersity index (Figure 73). The PDI is lower when working with 50 nm of membrane pore diameter, compared with the PDI values obtained with a pore size of 80 nm. Nevertheless, besides the tendency analysis previously discussed, it was not possible to infer any trend for the impact of membrane stacking, extrusion time, and temperature on the polydispersity index.

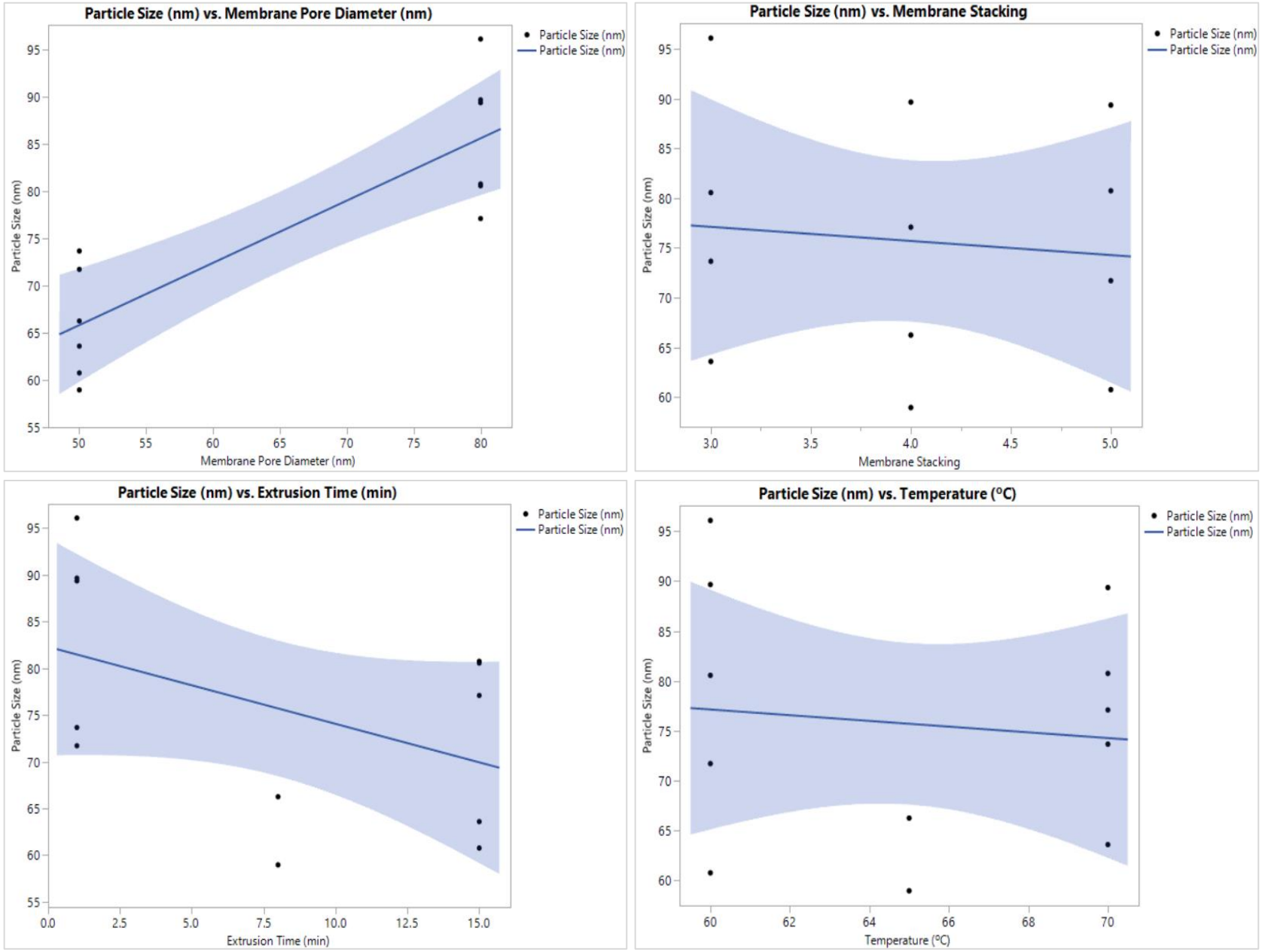


Figure 72. Data visualization of the impact of membrane pore diameter, membrane stacking, extrusion time, and temperature on the mean particle size of ULVs.

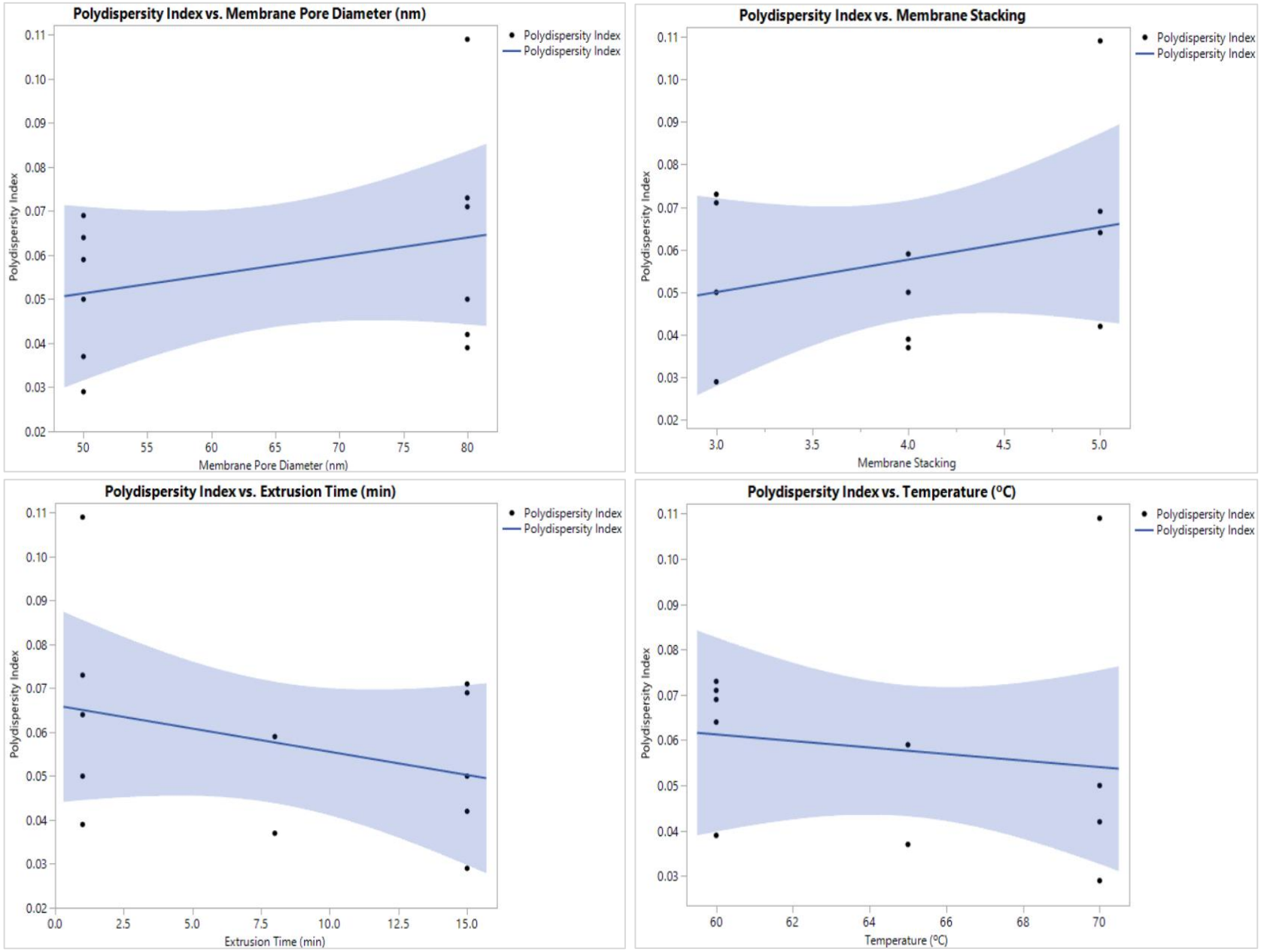


Figure 73. Data visualization of the impact of membrane pore diameter, membrane stacking, extrusion time, and temperature on the polydispersity index of ULVs.

The data relative to the extrusion step was used for data modeling and construction of predictive regression models for mean particle size and size distribution. The factors (membrane pore diameter; membrane stacking; temperature; extrusion time) and the responses (particle size; PdI) were estimated using the JMP software by following the path: Fit Model > Standard Least Squares > Fit Separately > Run. The model parameters were estimated using ordinary Standard Least Squares, where the CPPs analyzed included the membrane pore diameter, membrane stacking, temperature, and extrusion time. All models were thoroughly assessed from the standpoint of their fitting quality and statistical significance by means of the coefficient of determination (R^2) and several statistical hypothesis tests, such as ANOVA and individual tests to the significance of the regression parameters (p-value). Table 42 includes a Summary of Fit for each response.

Table 42. Summary of Fit for each response of the extrusion step.

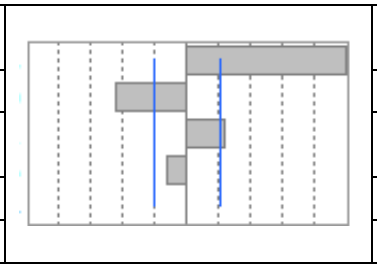
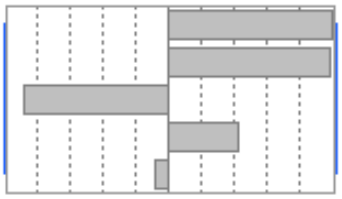
Responses	Summary of Fit		Analysis of Variance
	RSquare	RSquare Adj	Prob>F
Mean particle size	0.958922	0.933248	<0.001
Size distribution (Polydispersity Index)	0.552346	0.272562	0.1872

Table 43 presents the CPPs coefficient estimates in the models developed for CQAs: mean particle size and size distribution. A negative estimate means that the higher the parameter value the lower is the value of the CQA, and a positive estimate indicate that the higher the parameter value the higher is the value of the CQA.

Only certain CPPs were found to have a significant impact on the mean particle size (p-value < 0.05), such as the membrane pore diameter and extrusion time. For example, the membrane pore diameter has a positive effect on the mean particle size, since an increase in the pore diameter results in a higher mean particle size. On the other hand, the extrusion time has a negative impact on this CQA, once the higher the value of this CPP, the lower is the mean particle size. For the membrane stacking and temperature was not possible to estimate the positive or negative effect on mean particle size, due to these parameters individually showing no significant effect as denoted by the p-value higher than 0.05.

Similarly, it was not possible to estimate the positive or negative effect of several factors on particle size distribution (polydispersity index).

Table 43. Sorted effect estimates for Critical Process Parameters (CPPs) used in the model.

Sorted Parameter Estimates					
Term	**Estimate**	**Std Error**	**t Ratio**		**Prob>|t|**
	CQA: Mean particle size				
Membrane pore diameter (nm)	10.120514	0.904945	11.18		<0.001
Extrusion Time (min)	-5.41194	1.104543	4.90		0.0012
Membrane stacking* Membrane stacking	4.8265141	1.772342	2.72		0.0261
Membrane stacking	-1.695467	1.245254	-1.36		0.2104
Temperature (ºC)	0.0457362	1.031395	0.04		0.9657
	CQA: Size distribution				
Membrane stacking* Membrane stacking	0.0164031	0.01056	1.55		0.1590
Membrane pore diameter (nm)	0.0083131	0.005392	1.54		0.1617
Extrusion Time (min)	-0.009096	0.006581	-1.38		0.2043
Membrane stacking	0.0049108	0.00742	0.66		0.5267
Temperature (ºC)	-0.000787	0.006145	-0.13		0.9012

Furthermore, considering the desirability limits indicated in Table 44 below, a prediction profiler has been created and is represented in Figure 74.

The Prediction Profiler uses the Response Limits information to construct and define a Desirability function, which in turn is used in the Prediction Profiler to find optimal factor settings. This approach is a simplified form to predict the optimal factor settings within the experimental space (even if have not been performed), which gives rise to optimal formulation composition based on the target response.

Table 44. Desirability limits for the responses of the extrusion step, used on the design of experiments.

Response	Desirability
Mean particle size	High: 70 Middle: 65 Low: 55 Match target
Polydispersity index	High: 0.05 Middle: 0.035 Low: 0.02 Minimize

Figure 74 displays the local dependencies of the different models regarding all the CPPs. The prediction profiler (Figure 74) shows, in a graphical way, how the parameters studied influence the CQAS. The lines' slopes indicate if the influence is positive or negative and higher slopes indicate the higher impact on certain CQA. The dashed vertical lines with the red color represent the current value of the factor, whereas the value of the horizontal line corresponds to the predicted response based on the current values of the factors. The black line on the predicted plot corresponds to the prediction trace for each process parameter, while the bottom row displays desirability traces.

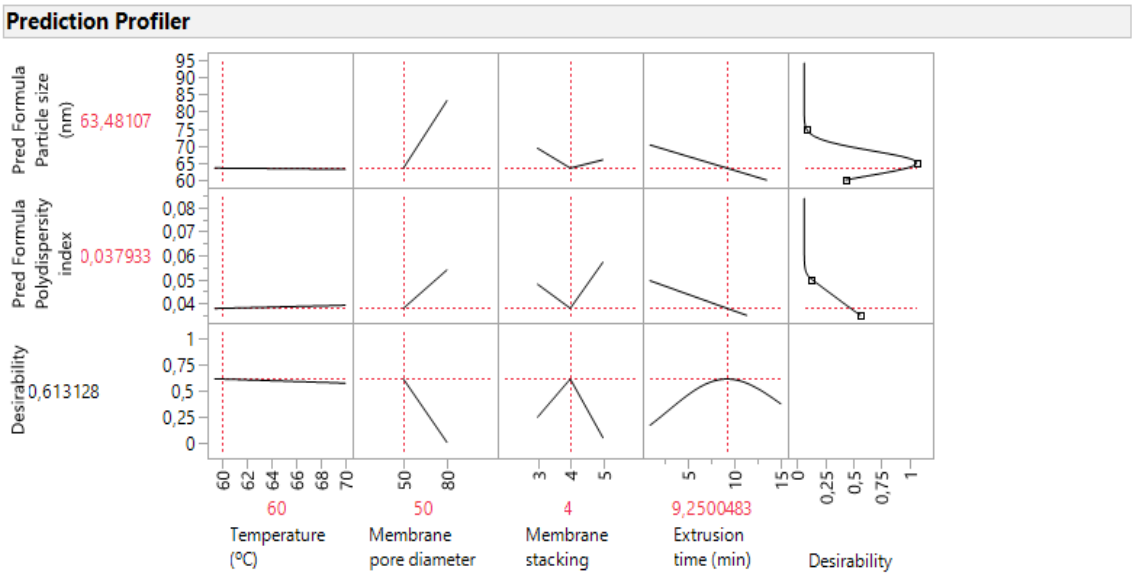


Figure 74. Prediction profiler obtained in the design of experiments of the extrusion step.

As stated in the previous analysis, some process parameters selected for extrusion were found to have a significant impact on the mean particle size (p-value < 0.05). Extrusion time has a negative impact on this CQA (the higher the value of this CPP, the lower is the mean particle size), while membrane pore diameter has a positive effect (an increase in the pore diameter results in a higher mean particle size). It was not possible to conclude about the influence of membrane stacking on size distribution, because this parameter did not show a linear correlation.

In relation to the desirability function, when using four membranes, with a membrane pore diameter of 50 nm, during 9.25 minutes at 60°C the predicted mean particle size is approximately 63.48 nm and the size distribution (polydispersity index) is 0.038.

After analysis of the results in the JMP software, the validation tests for the extrusion settings were performed (Table 45), to obtain the target mean particle size and the target polydispersity index for the liposomes after the size reduction step. The settings selected from a desirability

analysis (prediction profiler) and two points of design space (contour profiler), were included in validation tests (Figure 75).

Table 45. Validation of results of design of experiments (DoE extrusion) for the size reduction and Unilamellar Vescicles (ULVs) formation step.

Source/ Analysis	Experiment Number	Temperature (ºC)	Membrane pore diameter (nm)	Membrane stacking (number)	Extrusion time (min)	Mean particle size (nm)	Polydispersity index	Pressure (bar)	pH	Temperature (ºC) (pH measurement)
Desirability	13	60	50	4	9.17	61.87	0.041	110	5.987	21.1
Contour Profiler	14	65	50	4	4.22	61.62	0.041	110	5.692	21.3
	15	70	50	4	9.93	63.89	0.028	60	4.877	22.3

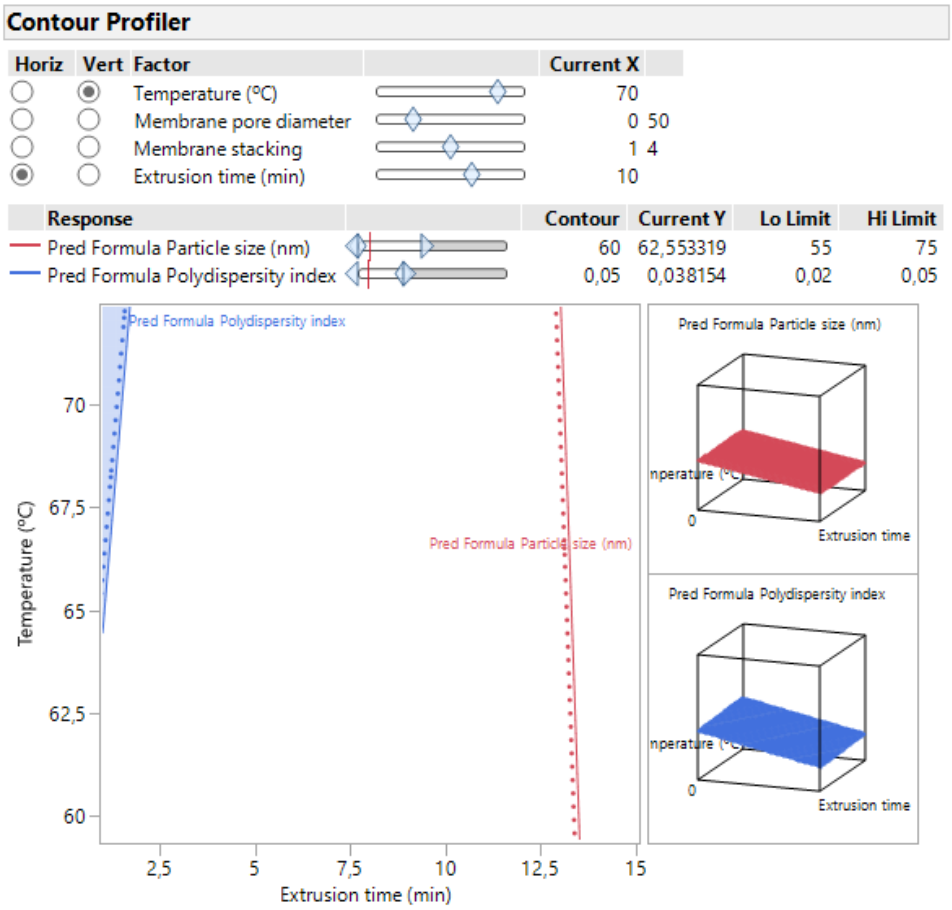


Figure 75. Coutour profiler obtained in the design of experiments of the extrusion step.

Regarding the DoE of extrusion, this set of experiments demonstrated that it is possible to optimize the manufacturing process of ULVs and obtain the mean particle size and polydispersity index within the target established (in-house defined as PS < 75 nm, and PdI < 0.05).

The optimal conditions found for the manufacturing process of ULVs encompass the stacking of four membranes in the extruder, with a pore diameter of 50 nm, during an extrusion time of 10 minutes. Concerning the extruder temperature is advisable for an evaluation of pH observed after the extrusion step when the temperature is 70ºC. While not expected lipid degradation due to their phase transition temperature, it is important to apply an analytic method for the determination of the impurity profile of lipids.

The optimized formulation of ULVs was used to give continuity to the manufacturing process of generic doxorubicin hydrochloride liposomal drug product, following the process of buffer exchange for active drug loading using the tangential flow filtration (TFF) method, active drug loading of drug substance into preformed liposomes by transmembrane ammonium sulfate gradient and, finally, the removal of unencapsulated drug substance and buffer exchange to storage buffer using the TFF method.

The examination of the prototype and RLD through the Cryo-TEM analysis illustrates the similarity between the samples with the majority of the liposomes being consistent in appearance (Figure 76). They presented predominantly a spherical shape with the internal structure having a crystalline strand formation. Moreover, the sphericity, mean diameter, and wall thickness of the liposomes were comparable between the two samples (Table 46).

Table 46. Average measurements from the prototype and the Reference Listed Drug (RLD).

Trial ID	Diameter (nm) [count] (max/min)	Sphericity [count]	Wall Thickness (nm) [count] (max/min)	Lamellarity	Internal Structure
RLD	67.62 (107) [98.39/33.12]	0.91 (107)	6.89 (111) [9.73/4.56]	Unilamellar	Internal Crystalline Strands
Prototype	62.0 (116) [111.06/37.54]	0.91 (116)	6.18 (113) [8.46/4.19]	Unilamellar	Internal Crystalline Strands

Note: Red bracketed numbers = counts, and Blue numbers = Maximum/Minimum values

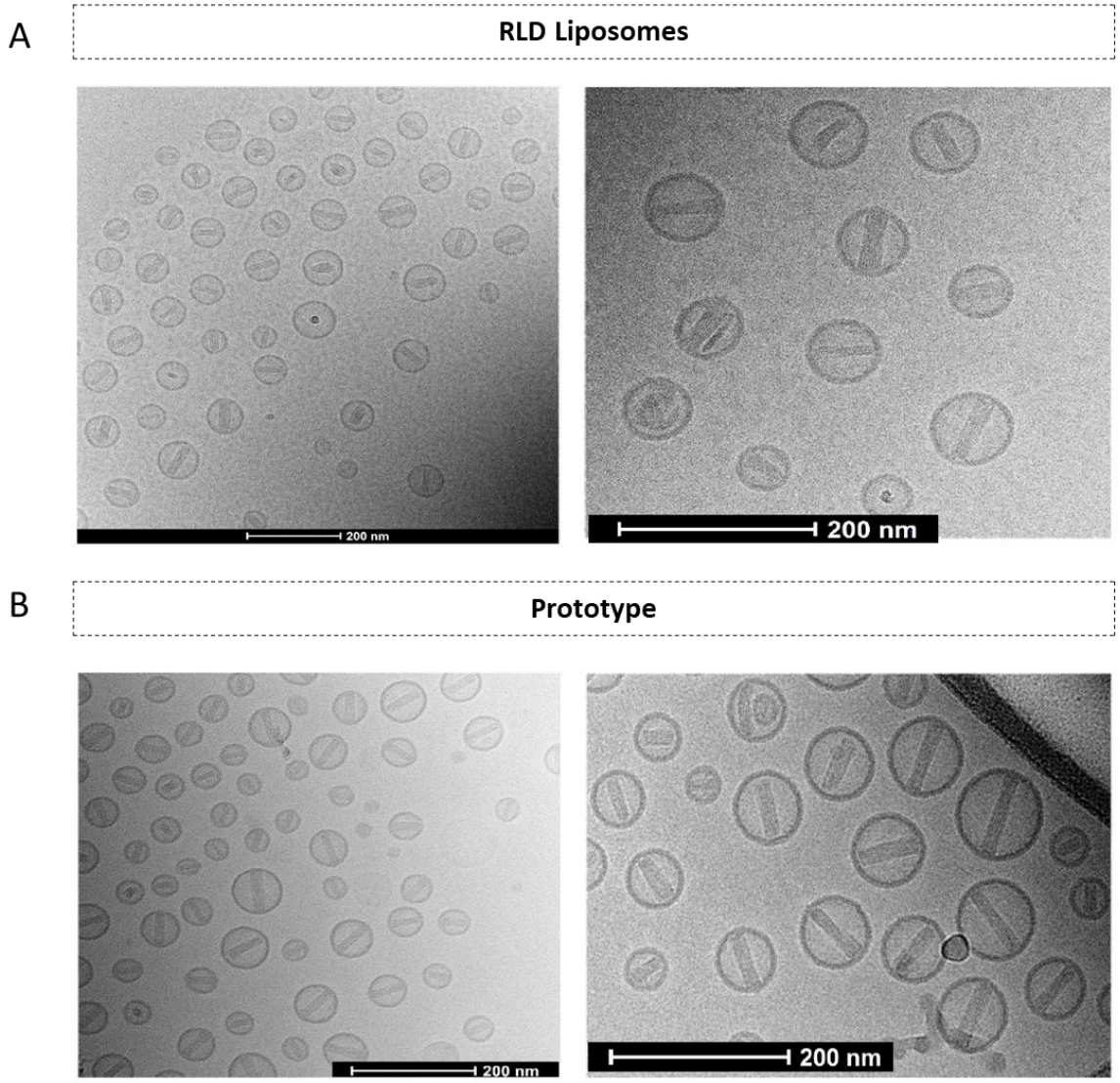


Figure 76. Schematic representation and Cryogenic Transmission Electron (Cryo-TEM) analysis of the: A) RLD liposomes; and B) prototype liposomes; at the micro bar scale of 200 nm (Diluted 1:4 WFI, Water for Injection).

5. Next Steps of Formulation Development

Following the extrusion optimization test should be performed optimizations tests of the Tangential Flow Filtration (TFF) step using the KrosFlo® KR2i Tangential Flow Filtration (TFF) System (Figure 77).

One important factor that affects the optimization of a TFF process is the tendency to be formed a concentration gradient of material (gel layer), on the surface of the filter membrane. This fact reduces the flux and blocks the permeate flow. Therewith, the aim of this test is the improvement of the TFF efficiency, through the definition of the optimal conditions of shear rate and transmembrane pressure (TMP), to give the highest flux rate without forming a gel layer, while maintaining the integrity of the product. Thus, the optimal condition corresponds to the moment immediately before the gel layer formation (passage of liposomes between the membrane pore favoring the membrane clogging) and is the result of the balance between TMP and shear rate. The optimal condition should allow obtaining the permeate flux as higher as possible without compromising the integrity of the liposome structure. These TFF optimization tests consist of consecutively increasing the TMP for a given shear rate, to determine the optimal TMP for that shear rate, which means, the maximum TMP value before the permeate flux is constant (before the gel layer formation). Moreover, this test is used to define the optimal TMP at a single shear rate (Table 47) between the range of 4500 s-1 and 6000 s-1 (the maximum recommended for low-fouling samples). Before testing a new shear rate (with a different sub-batch), the cleaning procedure of the equipment should be performed and the Normalized Water Permeability (NWP) then be determined to evaluate the efficacy of the cleaning procedure, to ensure that the filter was not clogged when a new condition was tested. Considering this information, in each TMP condition, for each shear rate, the particle size and polydispersity index should be evaluated.

Since the generic doxorubicin hydrochloride liposomes size is typically about 85 -100 nm, the use of a filter with 500 kDa of MWCO (pore size of 20 – 22 nm) is near the lower limit of the recommended range for the ratio liposomes size: pore size of 3-6, thus increasing the probability of liposomes permeation through the filter pores. Therewith, to prevent permeation through the pores and gel layer formation, a hollow fiber filter with a smaller MWCO (300 kDa) is required. The choice for the hollow fiber filter comes from the analysis of the ‘Hollow fiber selection guide for nanoparticle retention’ and the general rule that ‘the molecular weight cut-off (MWCO) of a membrane should be a third to a sixth the molecular weight of the molecule to be retained’ [765,766]. On the other hand, it is important to emphasize that the volume used for TFF optimization tests should be enough to allow stabilization of flux just long enough and avoid bubbles in the feed container.

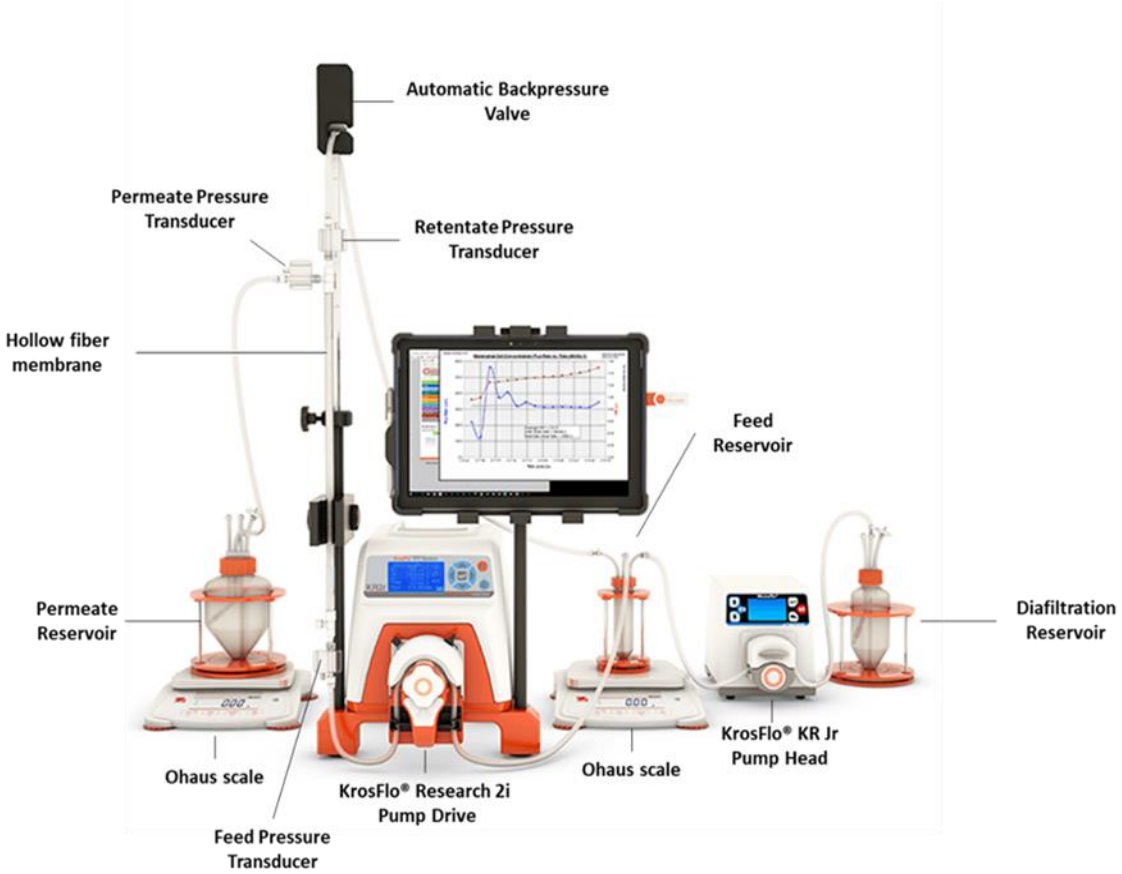


Figure 77. Schematic Representation of KrosFlo® KR2i Tangential Flow Filtration (TFF) System used In-House.

Table 47. Shear rate and transmembrane pressure conditions to be tested for each sub-batch during the TFF optimization tests.

Sub-Batches	Shear Rate (s^{-1})	TMP (bar)
#1	6000	Beginning with the least possible value and slowly increase the TMP until the permeate flux does not increase with the TMP increase
#2	5500	
#3	5000	
#4	4500	

After the corresponding TFF conditions have been selected, the pre-stability studies will be carried out using the selected prototype during a period of 3 months stored in several types of primary packaging material in two different conditions: accelerated conditions (25 °C±2 ºC/ 60 % RH±5% RH) and long term storage conditions (5ºC±3ºC) as described in the ICH guideline Q1A (R2) [609]. The main aim is to investigate the material attributes and process parameters that influence the stability of the formulation, to select the best primary packaging material and the storage conditions. It is also necessary to evaluate the chemical stability of each lipid component and encapsulated drug in the liposomal formulation in such a way as to determine storage conditions and retest periods (period during which the substance is expected to meet the defined specifications). The steps outlined above should be supported by the development of appropriate analytical methods to quantify the free DS, lipids purity, and in vitro drug release.

6. Concluding Remarks

The market of doxorubicin hydrochloride liposomal injections is mainly driven by oncologic needs across the globe. Despite the countless advances in this field, and the liposomal formulations are being considered highly effective drug delivery systems, some scientific and regulatory challenges are still unresolved.

One of the main limitations in the development of liposomal drug products is related to quality assurance. The identification, control, and thorough physicochemical characterization of the critical quality attributes (CQAs) particular to liposome drug products (e.g. morphology, particle size, and size distribution) is an important step toward ensuring their quality, efficacy, and safety. This becomes even more relevant in the evaluation and demonstration of the therapeutic equivalence between a reference-listed drug product and its generic version.

In this study, the manufacturing process of ULVs was investigated and optimized using the Quality by Design (QbD) approach. The I-optimal design was the most appropriate strategy in the field of the QbD approach, taking into consideration the correlations observed among the critical process parameters of the extrusion step on critical quality attributes of ULVs. The temperature of extrusion, membrane pore diameter, membrane stacking, and extrusion time was identified as critical parameters affecting CQAs, such as the mean particle size and polydispersity index. The predictive model that we generated through the I-optimal experimental design was used to find the optimal conditions for the step of ULVs formation at the laboratory scale. Thus, the results of this study demonstrated that ULVs can be successfully designed in compliance with the target values desired for the mean particle size and polydispersity index after this specific process step (in-house defined as PS < 75 nm and PDI < 0.05).

Although this study concentrates solely on the extrusion step of the manufacturing process of generic doxorubicin hydrochloride liposomal drug products, this optimization plays a key role to the achieve the Quality Target Product Profile (QTPP) of the final drug product. As described in problem elicitation, if has not reached a target value of particle size of ULVs, the drug loading and consequent precipitation of doxorubicin inside them, can lead to excessive deformation of liposomes beyond what is desired (prolate morphology instead of oblate).

Therefore, the QbD implementation in the optimization of the multi-steps of the liposomal formulation manufacturing process is a fundamental strategy to foster in-depth knowledge of the product and process, and consequently, achieve the intended robustness and quality target product profile. Through this systematic and risk-based approach is also more easily reached the qualitatively (Q1) and quantitatively (Q2) sameness between the generic drug products and the reference listed drug product, which will translate into the greatest success in the scientific and regulatory field.

Chapter VIII. Strengthening Regulatory Science Research in Pharmaceutical Development of Non-Biological Complex Drug Products

Abstract

Complex generic drug products are more difficult to develop due to the nature of their formulation or delivery system, requiring a higher level of expertise compared to the development of simple generic drug products using traditional equivalence approaches. Some of the key challenges faced by the regulatory system in bringing complex generics to the pharmaceutical market are related to the lack of specific regulatory guidance documents for each NBCD-families or the increased scrutiny and exigence from regulatory agencies for quality systems and data integrity. Thus, in parallel with the progress achieved by this innovative and promising class of complex drug products, there remains the need to proactively develop and implement effective strategies to obtain regulatory approval.

Chapter VIII intends to identify the needs and priorities for global harmonization of evaluation procedures between regulatory authorities in different places worldwide, as well as, demonstrate the importance of regulatory science research and science-based multi-stakeholder interactions to stimulate the rethinking of regulatory pathways. Another aim includes a brief discussion of the reflection papers and guidance documents published by the regulatory authorities, which may be related or applied to the pharmaceutical development of NBCDs and their follow-on versions. Knowing and understanding the principles and recommendations included in the guidance documents constitute a powerful lever for the beginning of pharmaceutical development for each type of NBCDs, establishing the science-based regulatory approaches, and making the review of regulatory submissions and approval procedures more effective.

Keywords

Non-Biological Complex Drugs; Complex Generics; Follow-on Versions; Therapeutic Equivalence; Regulatory Pathways; Regulatory Science Research; Regulatory Evaluation; Global Regulatory Harmonization; Standardization Programs; Scientific Advice; Scientific Stakeholder Exchange; European Medicines Agency; U.S. Food and Drug Administration; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

1. Introduction

The pharmaceutical development of Non-biological Complex Drugs (NBCDs) offers innovative therapeutic and diagnostic opportunities to address unmet medical needs so far, in emerging areas such as gene therapy, cell therapy, nanotechnology, or personalized medicines. However, as described in the previous chapters, the increased complexity and specific particularities of such complex drug products lead to several scientific and regulatory challenges.

The proper definition and classification rules for NBCDs are not well established in the literature or by regulatory authorities. Consequently, the selection of the regulatory approach and the definition of required regulatory requirements for each type of complex drug product can be undoubtedly a serious challenge. This is becoming increasingly evident in the lack of a specific regulatory framework for the demonstration of the therapeutic equivalence of follow-on versions of NBCDs, such as the appropriate standardized methods to assess their bioequivalence.

On the other hand, the limited guidance documents in current regulatory practice is one of the gravest challenges facing manufacturers in the pharmaceutical industry. Therefore, the absence of guidance documents and proper definition of regulatory requirements leads to high regulatory uncertainty and handicaps to prove compliance of the quality, efficacy, and safety data. This hinders the pharmaceutical development and marketing approval of NBCDs and their follow-on versions (Figure 78).

To provide the required requirements on the quality, safety, and efficacy of the complex drug products and thus break this cycle of challenges (Figure 78), translational and regulatory science strategies have to be implemented. Thus, the purpose of this chapter is to stimulate discussions related to regulatory challenges of the pharmaceutical development of NBCDs, just as the strategies required to meet them. Moreover, it intends to demonstrate the importance of regulatory science and science-based multi-stakeholder interactions to stimulate the rethinking of regulatory pathways, which must be flexible and adaptive to increasingly complex drug products. Lastly, also allows the identification of needs for global harmonization of evaluation procedures between the several jurisdictions, as well as, raising regulatory awareness on quality, efficacy, and safety concerns.

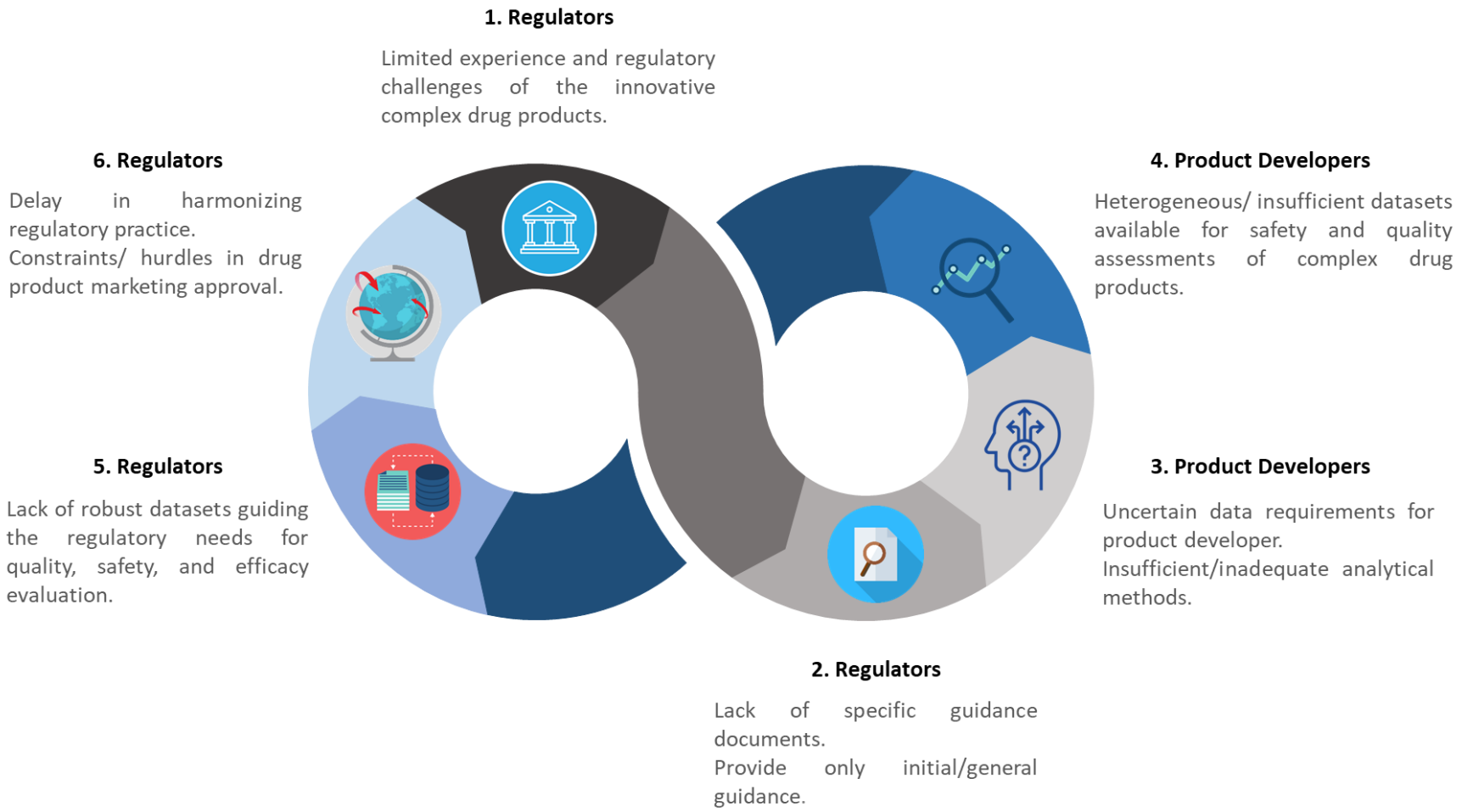


Figure 78. Continuous loop diagram displaying the interdependence between the issuance of regulatory guidance documents and the generation of quality, efficacy, and safety datasets, and subsequent marketing approval of complex drug products.

2. The Importance of Innovative Regulatory Science Approach

The science and technology breakthroughs and the development of new formulations, such as NBCDs and their follow-on versions, have a significant impact on regulatory science and consequently on evaluation and approval processes.

In the 'Regulatory Science to 2025' strategy published by the European Medicines Agency (EMA), the regulatory science is described as 'the range of scientific disciplines that are applied to the quality, safety and efficacy assessment of medicinal products and that inform regulatory decision-making throughout the lifecycle of a medicine. It encompasses basic and applied biomedical and social sciences, and contributes to the development of regulatory standards and tools' [389]. Likewise, the Food and Drug Administration (FDA) has defined regulatory science as the 'science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products' [767,768].

The former Commissioner of the U.S. FDA, Margaret Hamburg, in Workshop Summary 'Building a National Framework for the Establishment of Regulatory Science for Drug Development', states that '*regulatory science not only takes place in laboratories, but it also may involve clinical, epidemiological, and statistical tools and information-gathering systems*' [769]. Thus, regulatory science is an interdisciplinary research area that comprises a wide variety of scientific disciplines, laws, procedures, guidelines and product regulations, which should be fully integrated throughout the complete product lifecycle [389,394,414,768,769]. This requires a qualified and well-trained workforce, with strong knowledge and expertise in the technological and scientific fields. As referred to in 'Advancing Regulatory Science at FDA Report' (2021), regulatory science research arises a variety of outcomes, including the development of assays, animal models, data analysis tools, and reference material or standards applied for developing FDA-regulated products [770].

It is also defined as a critical bridge to speed the translation of pharmaceutical development from the bench to the launch of the products on the market, due to the increase of regulatory capacity, support the transparent and science-based decision-making, definition of new regulatory policy orientations, consumer advisories, labeling, or industry warnings [389,414,768–770]. Moreover, it provides an improvement in the efficiency of regulatory evaluation systems and allows the development of new regulatory approaches or modernizing existing ones [768,769]. Lastly, this approach ensures drug safety and the absence of damage to patients, promoting patient safety and public health, through the scientific, non-biased, and objective requirements [770].

3. Advancing Regulatory Science Strategies

With science and technology development and progress, regulatory authorities should be positioned itself to keep the pace of innovation and remain at the forefront of regulatory science research [770]. The application of regulatory science research encompasses a scientific, regulatory, and operational strategy, through an advanced collaborative approach involving different stakeholders.

Thus, the collaboration and communication involving science-based multi-stakeholders is an important driving force to advance regulatory science. Examples of advantageous collaborations developed in order to improve the efficiency of regulatory submissions and to develop product-specific guidelines are: [Ad-Hoc Nanomedicines Expert Group] established by EMA (2009); [Non-Biological Complex Drugs Working Group] hosted at the Dutch Top Institute Pharma (2009) and currently supported by Vifor Pharma International Inc., Teva Pharmaceutical Industries Ltd. and Sanofi-Aventis Group; [FDA-NIH leadership council] (2010); [Working Party on Non-biological Complexes] established by EDQM (European Directorate for the Quality of Medicines and HealthCare) (2011); [Office of Generic Drugs - Generic Drug User Fee Amendments (GDUFA) regulatory science research program] (2012), and International Pharmaceutical Regulators Programme (IPRP) (2018) that comprises 8 Working Groups in Bioequivalence for Generics (BEWGG), Biosimilars (BWG), Cell Therapy (CTWG), Gene Therapy (GTWG), Identification of Medical Products (IDMPWG), Information Sharing for Generics (IWGG), Nanomedicines (NWG), Pharmacovigilance (PVWG), and Quality for Generics (QWGG) [2,14,19,34,249,414,768].

Concerning the continuous efforts of regulatory authorities, the FDA published in the year 2011, a strategic plan that consists of eight priority objectives of regulatory science such as: ‘modernize toxicology to enhance product safety; stimulate innovation in clinical evaluations and personalized medicine to improve product development and patient outcomes; support new approaches to improve product manufacturing and quality; ensure FDA readiness to evaluate innovative emerging technologies; harness diverse data through information sciences to improve health outcomes; implement a new prevention-focused food safety system to protect public health; facilitate the development of medical countermeasures to protect against threats to the US and global health and security; and strengthen social and behavioral science to help consumers and professionals make informed decisions about regulated products’ [414,768].

Subsequently, a ninth priority area was added in 2013, entitled ‘Strengthening the Global Product Safety Net’. This topic shall be to support efforts to build regulatory capacity through training, tools to strengthen surveillance systems in developing countries, and harnessing informatics to ensure the safety of FDA-regulated products [771]. Also in this very year, the Global Coalition for Regulatory Science Research (GCRSR) (2013) was established under the leadership

of the US-FDA, including specified aims as facilitating education, scientific training, and scientific exchanges in the field of regulatory science and its impact on public health [772]. This international coalition is liable for the institution of 'Global Summit on Regulatory Science (GSRS)' conferences, where regulators and researchers can discuss the innovative technologies and partnerships to enhance the translation of basic science into regulatory applications within the global context, as well as, address the challenges and needs in the interest of advancing regulatory science [772].

In 2017, the U.S. Government Accountability Office (GAO) published a report referred to as 'FDA Should Make Public Its Plans to Issue and Revise Guidance on Non-biological Complex Drugs', which touches on many issues related to the regulatory development in the area of NBCDs [27]. This report identifies, examines, and discusses the scientific and regulatory challenges related to the review of follow-on versions of NBCDs, the existing regulatory pathways and product-specific guidance available for such complex products, the need for rethinking the requirements and pathways within its regulatory framework, and adjusting the delineations between product classifications [27,33].

Still in the year 2017, FDA announced the Drug Competition Action Plan (DCAP) intending to encourage robust and timely market competition for generic drugs and help bring greater efficiency and transparency to the generic drug review process, removing barriers to generic drug development and their entry into the pharmaceutical market, just as promote greater access to the medicines with affordable prices [773].

Another significant development is the establishment of the Center for Research on Complex Generics (CRCG) in 2020 to enhance research collaborations between the FDA, the generics industry, and stakeholders, and increase access to safe and effective generic products, through collaborative research, training, and exchange of resources [167].

That same year, FDA created an Agency-wide committee to develop and communicate efficiently its regulatory science strategies, just as keep track of the rapid pace of scientific advancement, evolving priorities, frequent updates and revisions, and research activities. The FDA committee published the 'Advancing Regulatory Science at FDA: Focus Areas of Regulatory Science' report (2021), which outlines and communicates areas that the FDA has identified as needing continued targeted investment in regulatory science research to facilitate the development of innovative products, provide data and methods to inform regulatory decision-making and improve guidance to sponsors [770].

On the other hand, on 31 March 2020, EMA published the 'Regulatory Science to 2025' strategy presenting five similar goals for regulatory science: 'catalyzing the integration of science and technology in medicines development; driving collaborative evidence generation improving the scientific quality of evaluations; advancing patient-centered access to medicines in partnership with healthcare systems; addressing emerging health threats and availability/therapeutic challenges;

enabling and leveraging research and innovation in regulatory science' [389]. Therefore, it is possible to note the growing evolution and interest in regulatory science as a valuable field in the area of pharmaceutical development, and specifically in the development and approval of complex drug products.

More recently, the FDA Office of Generic Drugs (OGD) provided an overview of the current science and research priorities for the fiscal year (FY) 2022 to spur the development of complex generic drugs, addressing complex ingredients, formulations, or dosage forms, complex delivery routes, drug-device combination products, as well as, tools and methods used to determine their BE and therapeutic equivalence [774].

On the other hand, there are already been disclosed the performance goals and program enhancements for the Generic Drug User Fee Amendments (GDUFA) reauthorization for fiscal years (FYs) 2023-2027, hereinafter referred to as GDUFA III [775].

4. Scientific Advice for Complex Generic Drug Products

The several challenges related to the development and approval of complex drug products require a higher level of regulatory support, through programs designed for scientific advice. Scientific advice is crucial in submitting applications for innovative therapies, such as the case of NBCDs and their follow-on versions, for which scientific guidance has not been developed yet or is still limited. The growing complexity of NBCDs is also expressed in the higher probability of disparities between the scientific advice provided [248]. In addition, the creation of a harmonized and centralized system of scientific advice is even more necessary at the European level, due to the national scientific advice provided for many different Member States [248].

The scientific advice provides developers with detailed information on the most appropriate way to generate robust evidence that demonstrates that a drug product is effective, safe, and of high quality. The regulatory advice may be provided through several types of complementary communication pathways available to drug developers, such as scientific guidelines, face-to-face discussion meetings, pre-ANDA meetings, and controlled correspondences [16,776,777]. For example, the pharmacokinetics studies developed by applicants for liposomal formulations are significantly dependent on factors such as the dosing regimen in the intended patient population and the proposed therapeutic indication for the specific drug. Thus, FDA recommends product-specific advice in the conduct and design of pharmacokinetic studies, data requirements, or post-approval changes. Therefore, for both liposomal formulations and other complex drug products, scientific advice is crucial for a case-by-case analysis [253].

It is important to emphasize that the scientific advice does not correspond to a pre-assessment of the benefits and risks of medicine, nor guarantee that a medicine will receive marketing authorization and enter the pharmaceutical market [776,777].

As drug development proceeds towards a globalized and harmonized approach, various stakeholders increasingly seek opportunities to proactively engage early in product development and promote regulatory success in your marketing approval procedures.

In 2020, the EMA launched the Regulatory & Scientific Information Management Platform (IRIS) to request scientific advice for handling product-related scientific and regulatory procedures. The usefulness of this platform resides in a greater capacity of applicants and the regulatory agency to submit requests, communicate, share information and deliver documents concerning each scientific advice procedure [778].

Another recent example addressing this point is the Parallel Scientific Advice (PSA) program (2021) shared by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA), which provides a mechanism for experts to concurrently engage in scientific discourse with sponsors on key issues during the development phase of new medicinal products (e.g. complex drugs, biologicals, vaccines, advanced therapies, among others) [367]. The program is mainly intended to provide parallel scientific advice to applicants of marketing authorization applications for EMA's hybrid products or abbreviated new drug applications (ANDAs) for complex generic drug products, hereafter referred to as 'complex products' (FDA). Although complex drug products (FDA) and hybrid products (EMA) have different regulatory definitions, this program will be available to those products where EMA and FDA's definitions overlap [367].

Contrary to the standard and unilateral scientific advice provided by each regulatory agency, in the new PSA method, the agencies conduct a preparatory bilateral meeting (EMA-FDA), followed by a trilateral meeting with the applicant (Sponsor-FDA-EMA) (Figure 79).

The main objectives of the PSA program are to provide an interaction mechanism between the two agencies and applicants from the beginning of the lifecycle of a hybrid/complex generic drug product, jointly exchange with applicants the agencies' views on scientific questions during the development phase, increase dialogue and the deeper understanding of the basis of regulatory decisions, optimize the application and decision-making processes, opportunity to simultaneously solicit and receive 'official' feedback from regulatory agencies, understanding of the reasons for potentially remaining divergences of them, avoid unnecessary replication of studies (e.g. clinical and pre-clinical data) or unnecessary testing methodologies, and accordingly reducing the approval time. Therefore, the close collaboration between both regulatory authorities represents an excellent and much-needed opportunity to streamline regulatory decisions in the development of complex drug products and promote regulatory success in the product approval procedures [367].

A.

B.

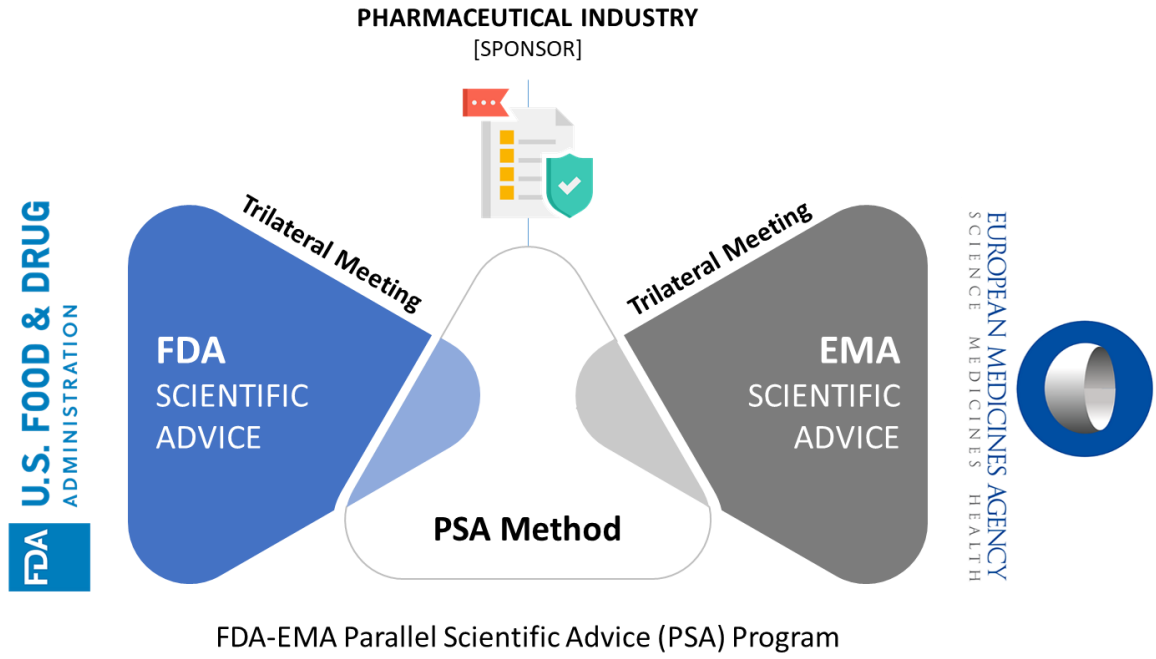


Figure 79. Schematic representation of the Standard Method for Scientific Advice (A) and the Parallel Scientific Advice (PSA) method (B) of the regulatory authorities for the pharmaceutical development of complex generic drugs [367].

5. Regulatory Science Strategies trying to Keep Up with the Breakthrough in NBCDs Field: Further Opportunities for Global Regulatory Harmonization

Recent scientific and technological advances in the field of complex drug products have driven forward some challenges, as outlined throughout this thesis. The regulatory authorities need to be abreast of these advances, strengthening an adaptive and innovative regulatory system with efficient and complementary strategies to ensure the quality, safety, and efficacy of complex drug products.

One of the primordial regulatory strategies to being implemented is related to the comparative characterization of critical quality attributes specified for each formulation, and the definition of the impact of these attributes on the biodistribution, efficacy, and safety of the product. It is necessary effectively to implement an extensive comparability exercise with comprehensive side-by-side analysis between the follow-on versions and reference products, through the determination and justification of similarities in quality attributes, but also potential differences. In this regard, it is also fundamental to develop and validate additional, reliable, and robust analytical techniques, advanced enough to evaluate and ensure an adequate characterization of them. The results of characterization may differ depending on the method selected, and must therefore be employed orthogonal and complementary analytical techniques, to ensure the accuracy and consistency of the data [161]. On the other hand, improving and implementing pre-clinical and clinical studies also plays an important role in the process of the suitable assessment of therapeutic equivalence of NBCDs.

Another strategy corresponds to continuous improvement, reinforcement, and clarification of regulatory procedures and guidance documents by the regulatory authorities [239].

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has a preponderant role in the alignment of scientific principles, practices, and procedures between different states, just as in the development and implementation of internationally recognized technical guidance documents and regulatory approaches for the development of complex generic drugs [588,779–781]. Thus, the ICH is the bridge between the regulatory authorities and the pharmaceutical industry and constitutes an important instrument in which the main goal is to ‘achieve greater harmonization worldwide to ensure that safe, effective and high-quality medicines are developed, and registered and maintained in the most resource-efficient manner whilst meeting high standards’ [780]. However, as previously established, the regulatory frameworks for NBCDs and follow-on products are not harmonized across jurisdictions. There are several examples of NBCDs where it is desirable the development of ICH Harmonized Tripartite Guidelines, for example, follow-on versions of liposomal doxorubicin formulations (Doxil®) or glatiramer acetate (Copaxone®), that have been evaluated through different approaches by both agencies. The ICH Reflection Paper ‘Further Opportunities for Harmonization of Standards

for Generic Drugs' (2018) outlines a strategic approach for developing and enhancing ICH guidelines to support the harmonization of scientific and technical standards for demonstrating the equivalence of complex dosage forms and products [781].

Likewise, the statement of the former FDA Commissioner Scott Gottlieb (2019) maintains that the '*complex medicines are becoming increasingly important to the economic stability of the generic drug industry' and 'the ultimate goal of the global harmonization of scientific and technical requirements would be the attainment of a single global generic drug development program that can support simultaneous regulatory filings across multiple markets*', which allows a global approval for high-quality generic drugs [247,588].

The other essential point in this analysis concerns the diversity of designations (trade names) used for the same product due to the marketing authorization by Decentralized Procedures in the several EU Member States, such as the case of the follow-on products of Copaxone (Table 49). On the other hand, different follow-on products from some Marketing authorization holders can be manufactured by the same manufacturer, as can be seen for follow-on products of Renvela® (manufacturer Synthon). This variability may significantly delay the rapid distinction between products in post-marketing surveillance when there are efficacy or safety problems in the clinical practice [30]. According to *Klein et al.*, it would be important to apply to NBCDs the legislative framework of biologics for brand name and batch number traceability (Directive, 2010) [30].

Moreover, in the methodology used to carry out this analysis, it is possible to see that the information about the EU legislation and marketing approval is segmented into several databases, which can increase the difficulty to understand how many follow-on versions are approved for one reference product, just as the regulatory requirements followed for drawing up the dossier for submission to the competent authority. The creation of a single database by the EMA would be an improvement to simplify the availability and speediness of access to information.

On the other hand, it is mandatory for close cooperation and communication involving science-based multi-stakeholders to solve the challenges in the field of complex generic drug products, such as the regulatory institutions, national agencies, research scientists, manufacturing engineers, and medical community [17,141,164]. The key to progress is based on scientific discussions, professional meetings, interdisciplinary research, publications of findings, and knowledge exchange at the international level, constituting an important driving force to advance regulatory science [17,20,25,141,164]. This will provide a common understanding and consensus among different authorities, achieve shared comprehension of the new analytical technologies, an enhancement of regulatory sciences, and develop meaningful guidance documents in the light of NBCDs and follow-on products [17,20,25,141,164,239]. Furthermore, this cooperation allows it possible to make informed decision-making based on sound scientific knowledge, when it is intended for the interchangeability of NBCD products in clinical practice [239]. The early dialogue

and scientific advice between manufacturers and regulatory authorities in the pre-registration phase, can also facilitate the pharmaceutical development of NBCDs, and reduce time-to-market.

Another priority issue corresponds to the need to ensure the safety and well-being of patients that cannot be compromised with the automatic substitution or interchange of NBCD follow-on products [136,164]. Therewith, the systems for post-marketing surveillance (risk management programs) and monitoring of new formulations should be implemented to ensure a suitable clinical practice and the protection of patients [136,164].

In short, the main strategies of regulatory harmonization of technical and scientific standards for complex generic drug products and their potential benefits are described in the figure below (Figure 80) [19,588,779,781].

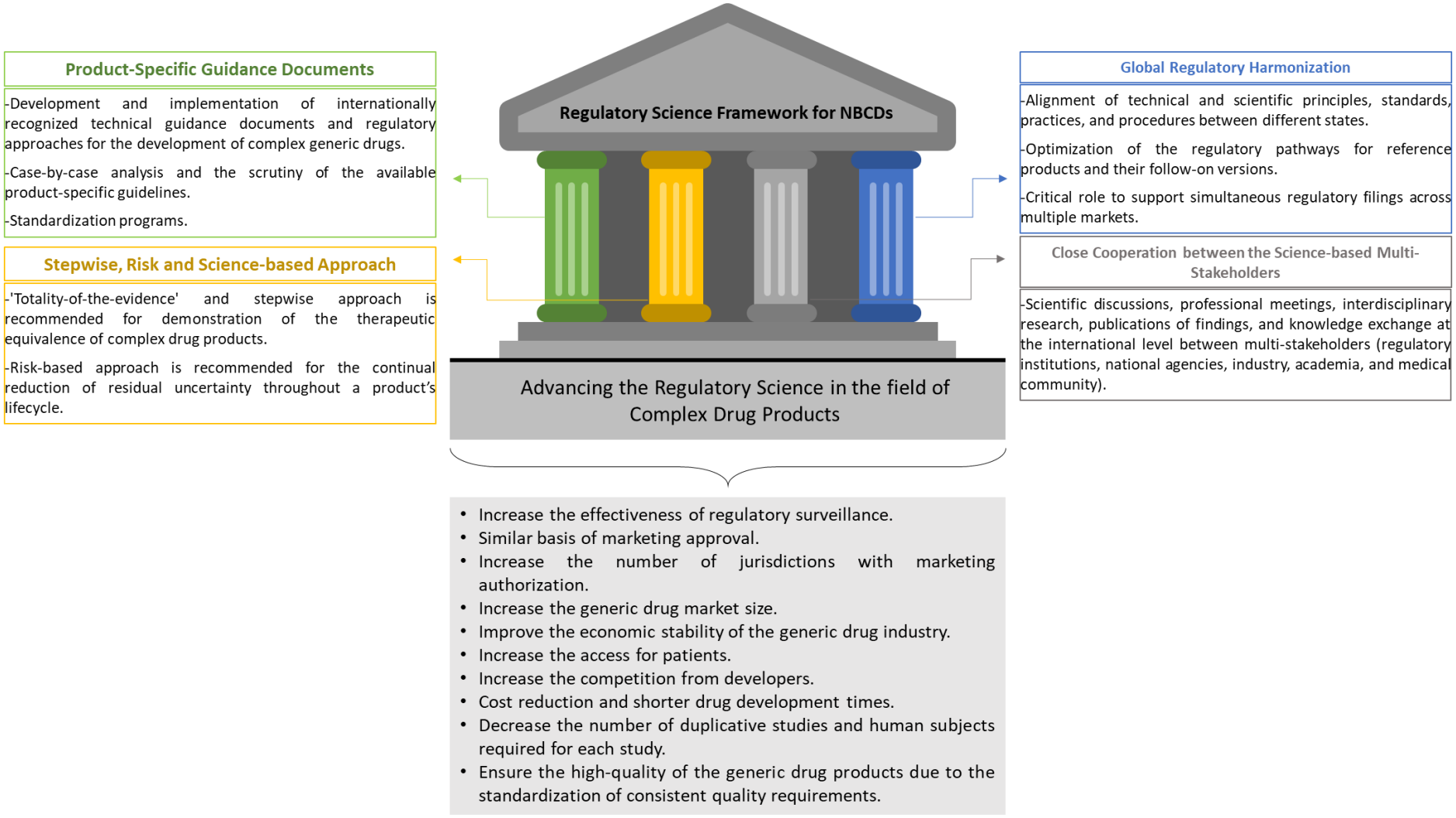


Figure 80. Regulatory Science Strategies in the field of Non-Biological Complex Drug Products.

6. Concluding Remarks

The rapid innovation speed with drug products increasingly complex demands that the regulatory authorities remain abreast of the emerging science and technologies. Despite the NBCDs market's boosting, there are several challenges behind the development of this class of complex drug products. Some of the significant obstacles inherent to the development and approval of NBCDs are related to the lack of specific guidance documents, absence of a definition of proper approval procedures, different regulatory pathways and requirements between jurisdictions, as well as, the major pitfalls in the demonstration of therapeutic equivalence of follow-on versions.

Thus, regulators must participate actively in continuous innovation and improved regulatory science strategies in accordance with the type of complex drug product. These strategies are essential to increase clarity from the regulatory agencies for the development of complex drug products and their follow-on versions, just as the enhancement of a well-established basis of regulatory approval.

In line with this, the sharing of knowledge and regulatory harmonization involving science-based multi-stakeholders brings a clear added value to advancing regulatory science and overcoming the several challenges related to NBCDs. Thus, a cross-disciplinary and continuous learning approach must be integrated into the whole product's lifecycle in order to define the regulatory policies, appropriate level of regulatory density, and acceptable thresholds of risk and uncertainty for a particular category of NBCDs. This approach shall also include post-market monitoring procedures through the use of effective strategies to analyze and evaluate the treatment results at the individual patient level and support regulatory decision-making, such as the real-time monitoring, data-driven medical research, establishment of global data repositories, validated prediction models, digital technologies, or artificial intelligence systems.

The application of a stepwise and risk-based approach centered on the 'totality-of-the-evidence' must also be taken into account, to ensure the continual reduction of residual uncertainty throughout a product's lifecycle and allows the approval of complex drug products with high quality, safety, and effectiveness. This approach should also have an adaptive and flexible character with the capability to continually updated in response to the changes in the regulatory systems, periodic evaluations of existing or new regulations and guidelines, or coming from the new scientific knowledge and technologies in a continuous learning environment. This adaptative capability might be particularly useful for the improvement of regulatory response capacity and crisis management in specific circumstances, such as the increased complexity of drug products, or even the unexpected appearance of serious public health threats (e.g. pandemics).

Chapter IX. Concluding Remarks and Future Perspectives

As Nanotechnology advancements enable the emergence of new and innovative complex drug products, there was a clear acknowledgment of the value of the pharmaceutical development of their follow-on versions. The placing on the market of complex generic drug products plays important role in the increasing drug product competition, reduction of the pressure on health care costs, and ensuring the availability of more affordability options for patients. However, the emergence of increasingly complex products through Nanotechnology has provided additional layers of constraints in developing their follow-on versions. Therefore, the development and approval of complex generics raise considerable scientific, technological, and regulatory challenges, changing the investment needs and strategic priorities of both pharmaceutical companies/research centers and regulatory agencies. It is imperative to solve the regulatory gaps related to their development and approval, and to guarantee an appropriate balance between the innovation, the degree of the regulatory exigency of each drug product, and the adequacy of the regulatory structures of competent agencies in response to these advancements. Moreover, the pharmaceutical companies must adapt and restructure according to the complex nature of drug products and their complicated development process, positioning advantageously compete in the emerging pharmaceutical market.

This doctoral thesis provides a comprehensive regulatory landscape of NBCDs and follow-on versions approved in the European and United States markets, just as the future perspectives of the regulatory science efforts in the assessment and marketing approval procedures. Even though some scientific discussions, publications, and international meetings surrounding the NBCDs are underway, there is a long way to go and outstanding issues in the assessment of the therapeutic equivalence and the creation of protocols for the characterization, evaluation, and process controls. Given the regulatory challenges and the absence of specific pathways for the approval of follow-on versions of NBCDs, such products need to sustain in the regulatory procedures currently available. As NBCDs present a significant diversity resulting from different technologies and multiple clinical indications, it is not possible to design and implement a universal regulatory pathway. Thus, the selection of the regulatory approach must be made based on the degree of complexity for each product individually, as well as, whether it is possible or not to establish the therapeutic equivalence through a complete characterization of the drug product using additional physicochemical analysis and/or in vivo BE studies.

Surely, the choice of hybrid procedures by the FDA (505(b)(2)) or EMA (Article 10(3)) may be the best option to consider for the regulatory submissions for approving follow-on versions of NBCDs. The use of this procedure can bring added advantages to both patients and developers, since it ensures the establishing safety and efficacy of drug products through the use of additional data from more extensive and rigorous clinical studies before market approval, especially when is impossible to establish pharmaceutical equivalence or the complete characterization of the API. The hybrid pathway is the closest to the totality of evidence approach for biosimilars and

represents the ideal strategy to avoid the pitfalls of unforeseen severe adverse reactions or the lack of desired efficacy that cannot be predicted when regulatory decisions are based exclusively on preclinical data or too simplistic physicochemical characterization, as in the case of using the conventional generic pathway (FDA: 505(j) or EMA: Article 10(1)). Despite this procedure offering the developers the possibility to add a higher level of protection/intellectual property and differentiating their products in the competitive pharma market, hybrid pathways also may be an increased risk of investment due to the need to often perform additional tests and the impossibility to make full use of same data as the conventional generic procedures.

Therewith, efforts should focus on a case-by-case analysis of the interchangeability and substitutability among products based on an adequate level of clinical evidence, putting aside the unjustified generalizations behind incomparable data. In summary, the main steps that the manufacturer should have to take into consideration in the assessment of therapeutic equivalence for follow-on versions of NBCDs are as follows:

- In-depth understanding of specific sources of complexity that makes a generic product complex.
- It is recommended to establish early advice with the regulatory agencies (well before the pre-registration phase) for a better understanding, clarification, and more precise definition of the required data or procedures (regulatory density) through a face-to-face meeting, controlled correspondence, scientific advice programs, etc.
- To make better use of systematic approaches, such as the QbD principles, to obtain extensive knowledge of formulation (critical material attributes (CMAs)) and manufacturing process (critical process parameter (CPPs)) at a small scale before making great investments in upscaling operations.
- Comparative and thorough quality characterization of the previously established critical quality attributes (CQAs) to achieve the desired quality, safety, and efficacy of the follow-on version.
- Execution of in-depth physicochemical and structural characterization studies using orthogonal and complementary analytical techniques, to increase the robustness of assessments for follow-on versions.
- Particular care should be taken over the selection of nomenclature of ‘generic’, ‘follow-on version’, or ‘quasi-similar products’, just as their impact on substitution/interchangeability practices, traceability in case of a product specific safety issue, and post-marketing surveillance strategies.
- Evaluate the potential impact of product variations concerning biodistribution, efficacy, and safety profile using modeling and simulation approaches.

- Anticipate the consequences of quality-related differences through confirming pre-clinical in vitro studies, and subsequently, by comparative clinical studies (in vivo efficacy and safety studies).
- The existence of greater support and a certain amount of regulatory flexibility by the competent authorities in the approaches for follow-on versions of NBCDs.
- Implementation of adequate risk management strategies for both known and unknown risks (e.g. post-marketing surveillance program).
- Quick regulatory actions to guarantee patient safety and security when substandard follow-on versions are detected.
- Publishing scientific and clinical findings in the public domain, and knowledge transfer and education in clinical practice, to strengthen further progress in the NBCDs field.
- A comprehensive and publicly available list of all complex products and their follow-on versions, just as a more investment in the publication of product-specific guidances.
- Recognition of commonalities between the NBCDs and biological complex drug products, and the necessity of inspiring from the concepts of ‘totality of evidence’ and ‘stepwise approach’ of the biosimilar approach which has proved successful in the past years.

The implementation of regulatory science strategies, scientific discussions, and multidisciplinary research between different stakeholders will enable to overcome traps in the transition to complex generics, while helping to ensure the long-term supply of more efficacious, safer, and higher quality drug products, with consequent public health protection. The culture of patient safety throughout the entire health system should constitute the uppermost priority in research-based pharmaceutical development. Of the countless regulatory strategies listed in this thesis, the Parallel Scientific Advice (PSA) program (2021) shared by the EMA and FDA comprises the latest example of an open industry-regulator communication channel to assist the scientific and regulatory decision-making during the development stages of new complex drug products, representing significant progress in global regulatory harmonization of the marketing approval procedures. Accordingly, the planning strategies and relevant initiatives described throughout the present thesis are headed in the right direction to improve and streamline the regulatory procedures for obtaining market authorization for the follow-on versions of NBCDs, much-needed to improve the sustainability of health care provision.

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Appendix I. Supplementary Data

Table 48. Regulatory landscape of Non-biological complex drugs (NBCDs) and their follow-on versions approved by the FDA.

Brand name (Reference product)	Follow-on product	Type of NBCDs	Drug name	Therapeutic indication	Route of administration	Dosage Form	Approval Date	Sponsor (Company)	Regulatory Pathway	Application type	Application Number	Submission classification	Categories of drugs considered to be complex by the FDA	Marketing Status	Review (e.g. Chemistry Review)	QbD Approach Implementation	References
InFed®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Injectable	1974	Allergan	New Drug Application (NDA)	505(b)(**?**)	017441	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	Prescription	Not available	Non-QbD Developed Products	[36]
Proferdex®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Injectable	1981	New River Pharmaceuticals Inc	New Drug Application (NDA)	505(b)(**?**)	017807	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	Discontinued	Not available	Non-QbD Developed Products	[124]
Cesamet®	Not applicable	Nanocrystal	Nabilone	Nausea and vomiting (chemotherapy)	Oral	Capsule	1985	Bausch	New Drug Application (NDA)	505(b)(**?**)	018677	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[37]
Diprivan®	Not applicable	Emulsion	Propofol	Anesthesia	Intravenous	Injectable Emulsion	1989	Fresenius kabi USA	New Drug Application (NDA)	505(b)(?)	019627	Type 1 - New Molecular Entity	Complex formulation	Prescription	Available	Non-QbD Developed Products	[38]
Diprivan®	Propofol Injectable Emulsion 1% (10mg/ml)	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	1999	Sagent Pharms Inc	Abbreviated New Drug Application (ANDA)	505(j)	075102	Not applicable	Complex formulation	Prescription	Available	Not applicable	[39]
Diprivan®	Propofol Injectable	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	2005	West-Ward	Abbreviated New Drug Application (ANDA)	505(j)	074848	Not applicable	Complex formulation	Prescription	Not avai	Not applicable	[129]

	Emulsion 1% (10mg/ml)							Pharms Int							labl e		
Diprivan ®	Propofol Injectable Emulsion 1% (10mg/ml)	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	2006	Hospira	Abbreviated New Drug Application (ANDA)	505(j)	077908	Not applicable	Complex formulation	Prescription	Available	Not applicable	[40]
Diprivan ®	Propofol Injectable Emulsion 1% (10mg/ml)	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	2015	Watson Labs Inc	Abbreviated New Drug Application (ANDA)	505(j)	205307	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[41]
Diprivan ®	Propofol Injectable Emulsion 1% (10mg/ml)	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	2018	Dr Reddys	Abbreviated New Drug Application (ANDA)	505(j)	205067	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[42]
Diprivan ®	Propofol Injectable Emulsion 1% (10mg/ml)	Emulsion	Propofol	Anesthesia	Intravenous	Injectable	2020	Innopharma	Abbreviated New Drug Application (ANDA)	505(j)	205576	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[130]
Lovenox ®	Not applicable	Low molecular weight heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Injectable	1993	Sanofi Aventis US	New Drug Application (NDA)	505(b)(1)	020164	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[43]
Lovenox ®	Enoxaparin sodium injection	Low molecular weight heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Injectable	2011	Sandoz Inc	Abbreviated New Drug Application (ANDA)	505(j)	078660	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[44]
Lovenox ®	Enoxaparin sodium injection	Low molecular weight heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Injectable	2019	Amphastar Pharms Inc	Abbreviated New Drug Application (ANDA)	505(j)	208600	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[45]

Fragmin®	Not applicable	Low molecular weight heparin (LMWH)	Dalteparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Injectable	1994	Pfizer Pharmaceuticals	New Drug Application (NDA)	505(b)(**?**)	020287	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[47]
Abelcet®	Not applicable	Liposome	Amphotericin B	Infectious Diseases	Intravenous	Injectable	1995	Leadiant Bioscience Inc	New Drug Application (NDA)	505(b)(**?**)	050724	Type 2 - New Active Ingredient	Complex formulation	Prescription	Not available	Non-QbD Developed Products	[48]
Doxil®	Not applicable	Liposome	Doxorubicin	Cancer	Intravenous	Injectable	1995	Baxter International Inc	New Drug Application (NDA)	505(b)(1)	050718	Type 3 - New Dosage Form	Complex formulation	Prescription	Available	Non-QbD Developed Products	[49]
Doxil®	Doxorubicin Hydrochloride Liposome Injection (2 mg/mL)	Liposome	Doxorubicin	Cancer	Intravenous	Injectable	2013	Sun Pharm	Abbreviated New Drug Application (ANDA)	505(j)	203263	Not applicable	Complex formulation	Prescription	Available	Not applicable	[50]
Doxil®	Doxourbicin Hydrochloride Liposome Injection (2 mg/ml)	Liposome	Doxorubicin	Cancer	Intravenous	Injectable	2017	Dr Reddys Labs Ltd	Abbreviated New Drug Application (ANDA)	505(j)	208657	Not applicable	Complex formulation	Prescription	Available	Not applicable	[51]
Doxil®	Doxourbicin Hydrochloride Liposome Injection (2 mg/ml)	Liposome	Doxorubicin	Cancer	Intravenous	Injectable	2020	Zydus	Abbreviated New Drug Application (ANDA)	505(j)	212299	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[131]
Neoral®	Not applicable	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	1995	Novartis	New Drug Application (NDA)	505(b)(2)	050715	Type 3 - New Dosage Form	Complex formulation	Prescription	Not available	Non-QbD Developed Products	[52]
Neoral®	Cyclosporine Soft Gelatin Capsules (modified)	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	2000	Mayne Pharma	Abbreviated New Drug Application (ANDA)	505(j)	065044	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[53]

Neoral®	Cyclosporine Soft Gelatin Capsules (modified)	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	2000	Sandoz	Abbreviated New Drug Application (ANDA)	505(j)	065017	Not applicable	Complex formulation	Prescription	Available	Not applicable	[54]
Neoral®	Gengraf (cyclosporine soft gelatin capsules modified)	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	2000	Abbvie	Abbreviated New Drug Application (ANDA)	505(j)	065003	Not applicable	Complex formulation	Prescription	Available	Not applicable	[55]
Neoral®	Cyclosporine Soft Gelatin Capsules (modified)	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	2005	Ivax Sub Teva Pharms	Abbreviated New Drug Application (ANDA)	505(j)	065110	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[56]
Neoral®	Cyclosporine Soft Gelatin Capsules (modified)	Emulsion	Cyclosporine	Organ transplantation	Oral	Solution	2019	Apotex	Abbreviated New Drug Application (ANDA)	505(j)	210721	Not applicable	Complex formulation	Prescription	Not available	Not applicable	[57]
Amphotec®	Not applicable	Liposome	Amphotericin B	Infectious Diseases	Intravenous	Injectable	1996	Alkopharma USA Inc	New Drug Application (NDA)	505(b)(**?**)	050729	Type 3 - New Dosage Form	Complex formulation	Discontinued	Not available	Non-QbD Developed Products	[46]
Copaxone®	Not applicable	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Injectable	1996	TEVA Pharmaceuticals USA	New Drug Application (NDA)	505(b)(**2**)	020622	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[58]
Copaxone®	Glatopa (Glatiramer Acetate Injection, 20 mg/mL)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Injectable	2015	Sandoz Inc	Abbreviated New Drug Application (ANDA)	505(j)	090218	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[59]
Copaxone®	Glatiramer Acetate Injection Mylan (20 mg/mL)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Injectable	2017	Mylan	Abbreviated New Drug Application (ANDA)	505(j)	091646	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[60]
Copaxone®	Glatiramer Acetate Injection Mylan (40 mg/mL)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Injectable	2017	Mylan	Abbreviated New Drug Application (ANDA)	505(j)	206936	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[127]

Copaxone®	Glatopa (Glatiramer Acetate Injection, 40 mg/mL)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Injectable	2018	Sandoz Inc	Abbreviated New Drug Application (ANDA)	505(j)	206921	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[128]
DaunoXome®	Not applicable	Liposome	Daunorubicin citrate	Cancer	Intravenous	Injectable	1996	Galen Ltd	New Drug Application (NDA)	505(b)(**?**)	050704	Type 3 - New Dosage Form	Complex formulation	Discontinued	Not available	Non-QbD Developed Products	[61]
Dexferrum®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Injectable	1996	American Regent Inc	New Drug Application (NDA)	505(b)(2)	040024	Unknown	Complex active ingredient	Discontinued	Not available	Non-QbD Developed Products	[62]
Feridex®	Not applicable	Nanoparticle	Superparamagnetic iron oxide nanoparticle	Contrast agent	Intravenous	Injectable	1996	Amag Pharms Inc	New Drug Application (NDA)	505(b)(1)	020416	Type 1 - New Molecular Entity	Complex active ingredient	Discontinued	Not available	Non-QbD Developed Products	[63]
Taxotere®	Not applicable	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	1996	Sanofi Aventis US	New Drug Application (NDA)	505(b)(1)	020449	Type 1 - New Molecular Entity	Complex formulations	Prescription	Available	Non-QbD Developed Products	[64]
Taxotere®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2011	Accord Healthcare Inc	New Drug Application (NDA)	505(b)(2)	201195	Type 5 - New Formulation or New Manufacture	Complex formulations	Prescription	Available	Not applicable	[381]
Taxotere®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2013	Actavis LLC	New Drug Application (NDA)	505(b)(2)	203551	Type 5 - New Formulation or New Manufacturer	Complex formulations	Prescription	Available	Not applicable	[65]
Taxotere®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2014	Dr Reddys Labs Ltd	Abbreviated New Drug Application (ANDA)	505(j)	204193	Not applicable	Complex formulations	Prescription	Not available	Not applicable	[66]
Taxotere®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2015	Teikoku Pharma	New Drug Application (NDA)	505(b)(2)	205934	Type 5 - New Formulation or New Manufacturer	Complex formulations	Prescription	Available	Not applicable	[67]

Taxotere ®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2017	DFB Oncology Ltd	Abbreviated New Drug Application (ANDA)	505(j)	206177	Not applicable	Complex formulations	Prescription	Not availlable	Not applicable	[68]
Taxotere ®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2017	Jiangsu Hengrui Med	Abbreviated New Drug Application (ANDA)	505(j)	207252	Not applicable	Complex formulations	Prescription	Not availlable	Not applicable	[69]
Taxotere ®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2018	Amneal	Abbreviated New Drug Application (ANDA)	505(j)	209640	Not applicable	Complex formulations	Prescription	Not availlable	Not applicable	[70]
Taxotere ®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2019	Shilpa Medicare Ltd	Abbreviated New Drug Application (ANDA)	505(j)	210327	Not applicable	Complex formulations	Prescription	Not availlable	Not applicable	[71]
Taxotere ®	Docetaxel Injection Concentrate	Polymeric micelle	Docetaxel	Cancer	Intravenous	Injectable	2021	Hikma	Abbreviated New Drug Application (ANDA)	505(j)	204490	Not applicable	Complex formulations	Prescription	Not availlable	Not applicable	[782]
AmBisome®	Not applicable	Liposome	Amphotericin B	Infectious Diseases	Intravenous	Injectable	1997	Astellas Pharma Inc	New Drug Application (NDA)	505(b)(**?**)	050740	Type 2 - New Active Ingredient	Complex formulation	Prescription	Availble	Non-QbD Developed Products	[72]
Valstar ®	Not applicable	Liposome	Valrubicin	Cancer	Intravesical instillation	Sterile Solution	1998	Endo International Plc	New Drug Application (NDA)	505(b)(**?**)	020892	Type 1 - New Molecular Entity	Complex formulation	Prescription	Availble	Non-QbD Developed Products	[73]
Valstar®	Valrubicin Sterile Solution for Intravesical Instillation	Liposome	Valrubicin	Cancer	Intravesical instillation	Sterile Solution	2019	Custopharm Inc	Abbreviated New Drug Application (ANDA)	505(j)	206430	Not applicable	Complex formulation	Prescription	Not availlable	Not applicable	[74]
Depocyt ®	Not applicable	Liposome	Cytarabine	Cancer	Intrathecal	Injectable	1999	Pacira Pharmaceuticals Inc	New Drug Application (NDA)	505(b)(**?**)	021041	Type 3 - New Dosage Form	Complex formulation	Discontinued	Availble	Non-QbD Developed Products	[75]
Ferrlecit®	Not applicable	Iron-carbohydra	Sodium ferric gluconat	Iron deficiency	Intravenous	Injectable	1999	Sanofi Aventis US	New Drug Application (NDA)	505(b)(1)	020955	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Availble	Non-QbD Develo	[76]

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Ferrlecit®	Sodium Ferric Gluconate Complex in Sucrose Injection	Iron-carbohydrate complex	Sodium ferric gluconate complex	Iron deficiency	Intravenous	Injectable	2011	West-Ward Pharms Int	Abbreviated New Drug Application (ANDA)	505(j)	078215	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[77]
Rapamune®	Not applicable	Nanocrystal	Sirolimus	Organ transplantation	Oral	Solution	1999	PF Prism C.V. (Pfizer Pharmaceuticals)	New Drug Application (NDA)	505(b)(**?**)	021083	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[81]
Rapamune®	Sirolimus Oral Solution (1 mg/mL)	Nanocrystal	Sirolimus	Organ transplantation	Oral	Solution	2019	Amneal	Abbreviated New Drug Application (ANDA)	505(j)	211212	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[82]
Rapamune®	Sirolimus Oral Solution (1 mg/mL)	Nanocrystal	Sirolimus	Organ transplantation	Oral	Solution	2019	Apotex	Abbreviated New Drug Application (ANDA)	505(j)	211406	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[83]
Rapamune®	Sirolimus Oral Solution (1 mg/mL)	Nanocrystal	Sirolimus	Organ transplantation	Oral	Solution	2019	Novitium Pharma	Abbreviated New Drug Application (ANDA)	505(j)	211040	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[84]
Innohep®	Not applicable	Low molecular weight heparin (LMWH)	Tinzaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2000	Leo Pharma AS	New Drug Application (NDA)	505(b)(1)	020484	Type 1 - New Molecular Entity	Complex active ingredient	Discontinued	Available	Non-QbD Developed Products	[78]
Venofer®	Not applicable	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Injectable	2000	American Regent Inc	New Drug Application (NDA)	505(b)(1)	021135	Type 3 - New Dosage Form	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[79]
Visudyne®	Not applicable	Liposome	Verteporfin	Age-related macular	Intravenous	Sterile, Lyo	2000	Valeant	New Drug Application (NDA)	505(b)(1)	021119	Type 1 - New Molecular Entity	Complex formulation	Prescription	Available	Non-QbD Develo	[80]

				degeneration (AMD)		philized Powder for Injection		Luxembourg							ped Products	
Renagel ®	Not applicable	Polymeric nanoparticle	Sevelamer hydrochloride	Hyperphosphatemia (End-Stage Renal Disease (ESRD))	Oral	Tablet	2000	Genzyme	New Drug Application (NDA)	505(b)(1)	021179	Type 3 - New Dosage Form	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[85]
Renagel ®	Sevelamer Hydrochloride Tablets	Polymeric nanoparticle	Sevelamer hydrochloride	Hyperphosphatemia (End-Stage Renal Disease (ESRD))	Oral	Tablet	2019	Glenmark Pharms Ltd	Abbreviated New Drug Application (ANDA)	505(j)	204724	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[86]
Definity ®	Not applicable	Lipid microsphere	Perflutren	Contrast agent	Intravenous	Sterile Injectable Suspension	2001	Lantheus Medical Imaging Inc	New Drug Application (NDA)	505(b)(2)	021064	Type 1 - New Molecular Entity	Complex formulation	Prescription	Available	Non-QbD Developed Products	[87]
Eligard ®	Not applicable	Polymeric nanoparticle	Leuprolide Acetate	Cancer	Subcutaneous	Powder for Injectable Suspension	2002	Tolmar Therap	New Drug Application (NDA)	505(b)(**?**)	021343	Type 3 - New Dosage Form	Complex dosage forms	Prescription	Available	Non-QbD Developed Products	[783]
Estrasorb®	Not applicable	Emulsion (with micellar nano	Estradiol hemihydrate	Moderate to severe vasomotor sympto	Transderma l	Emulsion	2003	Exeltis USA Inc	New Drug Application (NDA)	505(b)(1)	021371	Type 3 - New Dosage Form	Complex formulation	Discontinued	Available	Non-QbD Developed Products	[88]

		particles)		ms associated with menopause												
Oraqix®	Not applicable	Emulsion	Lidocaine/ Prilocaine	Anesthesia	Periodontal	Gel	2003	Dentsply Pharm	New Drug Application (NDA)	505(b)(1)	021451	Type 4 - New Combination	Complex route of delivery	Prescription	Available	Non-QbD Developed Products	[89]
Restasis®	Not applicable	Emulsion	Cyclosporine	Ocular inflammation	Ophthalmic	Emulsion	2003	Allergan	New Drug Application (NDA)	505(b)(1)	021023	Type 3 - New Dosage Form	Complex route of delivery	Prescription	Available	Non-QbD Developed Products	[90]
Emend®	Not applicable	Nanocrystal	Aprepitant	Nausea and vomiting (chemotherapy)	Oral	Capsule	2003	Merck Sharp & Dohme Ltd	New Drug Application (NDA)	505(b)(1)	021549	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[91]
Emend®	Aprepitant Capsules	Nanocrystal	Aprepitant	Nausea and vomiting (chemotherapy)	Oral	Capsule	2012	Sandoz	Abbreviated New Drug Application (ANDA)	505(j)	090999	Not applicable	Complex active ingredient	Prescription	Available	Not applicable	[92]
Emend®	Aprepitant Capsules	Nanocrystal	Aprepitant	Nausea and vomiting (chemotherapy)	Oral	Capsule	2017	Glenmark Pharms SA	Abbreviated New Drug Application (ANDA)	505(j)	207777	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[93]
Emend®	Aprepitant Capsules	Nanocrystal	Aprepitant	Nausea and vomiting (chemotherapy)	Oral	Capsule	2020	Torrent	Abbreviated New Drug Application (ANDA)	505(j)	211835	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[126]
DepoDur®	Not applicable	Liposome	Morphine	Pain management	Intrathecal	Extended-Release	2004	Pacira Pharmaceuticals Inc	New Drug Application (NDA)	505(b)(2)	021671	Type 3 - New Dosage Form	Complex formulation	Discontinued	Available	Non-QbD Developed	[94]

					Liposome Injection											Products	
Tricor®	Not applicable	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2004	Abbvie	New Drug Application (NDA)	505(b)(2)	021656	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[95]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2011	Lupin Ltd	Abbreviated New Drug Application (ANDA)	505(j)	090856	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[96]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2012	Mylan Pharms Inc	Abbreviated New Drug Application (ANDA)	505(j)	202856	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[97]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2012	Valeant Pharms North	Abbreviated New Drug Application (ANDA)	505(j)	090715	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[98]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2016	Aurobindo Pharma Ltd	Abbreviated New Drug Application (ANDA)	505(j)	205118	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[99]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2016	Cipla	Abbreviated New Drug Application (ANDA)	505(j)	208709	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[100]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2016	Hetero Labs Ltd III	Abbreviated New Drug Application (ANDA)	505(j)	204598	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[101]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2017	Sun Pharm	Abbreviated New Drug Application (ANDA)	505(j)	200884	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[102]
Tricor®	Fenofibrate tablet	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2018	Amneal	Abbreviated New Drug Application (ANDA)	505(j)	209951	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[103]

Tricor®	Fenofibrate tablet	Nano crystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2018	Prinston Inc	Abbreviated New Drug Application (ANDA)	505(j)	211080	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[104]
Tricor®	Fenofibrate tablet	Nano crystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2019	Alembic Pharms Ltd	Abbreviated New Drug Application (ANDA)	505(j)	210476	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[105]
Tricor®	Fenofibrate tablet	Nano crystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2020	Gravitipharms	Abbreviated New Drug Application (ANDA)	505(j)	211122	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[106]
Tricor®	Fenofibrate tablet	Nano crystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2021	Austarpharma	Abbreviated New Drug Application (ANDA)	505(j)	208476	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[784]
Macugen®	Not applicable	Polymeric nanoparticle	Pegaptanib	Age-related macular degeneration (AMD)	Intravitreal	Injectable	2004	Valeant Pharms Inc	New Drug Application (NDA)	505(b)(1)	021756	Type 1 - New Molecular Entity	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[107]
Abraxane®	Not applicable	Polymeric nanoparticle	Paclitaxel	Cancer	Intravenous	Lyophilized Powder for Injection	2005	Abraxis Bioscience	New Drug Application (NDA)	505(b)(2)	021660	Type 5 - New Formulation or New Manufacturer	Complex formulation	Prescription	Available	Non-QbD Developed Products	[108]
Megace ES®	Not applicable	Nano crystal	Megestrol Acetate	Acquired Immunodeficiency Syndrome (AIDS)	Oral	Liquid Suspension	2005	Par Pharmaceutical Inc	New Drug Application (NDA)	505(b)(2)	021778	Type 5 - New Formulation or New Manufacturer	Complex formulation	Prescription	Available	Non-QbD Developed Products	[785]
Megace ES®	Megestrol Acetate	Nano crystal	Megestrol Acetate	Acquired Immunodeficiency Syndrome (AIDS)	Oral	Liquid Suspension	2014	Twi Pharms	Abbreviated New Drug Application (ANDA)	505(j)	203139	Not applicable	Complex formulation	Prescription	Not available	Not applicable	

Megace ES®	Megestrol Acetate	Nano crystal	Megestrol Acetate	Acquired Immunodeficiency Syndrome (AIDS)	Oral	Liquid Suspension	2017	Breckenridge	Abbreviated New Drug Application (ANDA)	505(j)	204688	Not applicable	Complex formulation	Prescription	Not available	Not applicable	
Triglide®	Not applicable	Nano crystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2005	Skyph arma AG	New Drug Application (NDA)	505(b)(2)	021350	Type 3 - New Dosage Form	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[110]
Somatuline Depot®	Not applicable	Polymeric Microsphere	Lanreotide Acetate	Cancer	Subcutaneous	Injectable	2007	Beafour Ipsen	New Drug Application (NDA)	505(b)(1)	022074	Type 1 - New Molecular Entity	Complex dosage forms	Prescription	Available	Non-QbD Developed Products	[786]
Durezol®	Not applicable	Emulsion	Difluprednate	Pain management/ Inflammation	Ophthalmic	Emulsion	2008	Sirion Therapeutics Inc	New Drug Application (NDA)	505(b)(1)	022212	Type 1 - New Molecular Entity	Complex routes of delivery	Prescription	Available	Non-QbD Developed Products	[787]
Ferahe me®	Not applicable	Iron-carbohydrate complex	Ferumoxytol	Iron deficiency	Intravenous	Sterile Solution	2009	Amag Pharms Inc	New Drug Application (NDA)	505(b)(1)	022180	Type 2 - New Active Ingredient	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[111]
Invega Sustenna®	Not applicable	Nano crystal	Paliperidone palmitate	Schizophrenia	Intramuscular	Extended release injectable suspension	2009	Janssen-Cilag Ltd	New Drug Application (NDA)	505(b)(1)	022264	Type 3 - New Dosage Form	Complex dosage form	Prescription	Available	Non-QbD Developed Products	[112]
Renvela®	Not applicable	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for Susp	2009	Genzyme	New Drug Application (NDA)	505(b)(1)	022318	Type 3 - New Dosage Form	Complex active ingredient	Prescription	Available	Non-QbD Developed	[113]

						ension										Products	
Renvela ®	Sevelamer Carbonate for Oral Suspension	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for Suspension	2017	Aurobindo Pharma Ltd	Abbreviated New Drug Application (ANDA)	505(j)	207624	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[114]
Renvela ®	Sevelamer Carbonate for Oral Suspension	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for Suspension	2018	Dr. Reddy's Laboratories Ltd	Abbreviated New Drug Application (ANDA)	505(j)	210464	Not applicable	Complex active ingredient	Prescription	Not available	Not applicable	[115]
Zyprexa ®	Not applicable	Nanocrystal	Olanzapine Pamoate	Schizophrenia	Intramuscular	Injectable Suspension	2009	Eli Lilly Co	New Drug Application (NDA)	505(b)(1)	022173	Type 3 - New Dosage Form	Complex dosage forms	Prescription	Available	Non-QbD Developed Products	[788]
Exparel ®	Not applicable	Liposome	Bupivacaine	Pain management	Soft Tissue Injection (intra wound)	Injectable	2011	Pacira Pharmaceuticals Inc	New Drug Application (NDA)	505(b)(2)	022496	Type 3 - New Dosage Form	Complex formulation	Prescription	Available	Non-QbD Developed Products	[116]
Marqibo®	Not applicable	Liposome	Vincristine sulfate	Philadelphia chromosome negative acute lymphoblastic leukemia (ALL) in second relapse	Intravenous	Injectable	2012	Acrotech	New Drug Application (NDA)	505(b)(2)	202497	Type 5 - New Formulation or New Manufacturer	Complex formulation	Prescription	Available	Non-QbD Developed Products	[118]
Injectafer®	Not applicable	Iron-carbohydrate complex	Ferric carboxymaltose	Iron deficiency	Intravenous	Injectable	2013	American Regent Inc	New Drug Application (NDA)	505(b)(1)	203565	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	Prescription	Available	Non-QbD Developed Products	[119]

Lumason®	Not applicable	Lipid microsphere	Sulfur hexafluoride lipid-type A microspheres	Contrast agent	Intravenous	Lyophilized Powder for Injection	2014	Bracco SpA	New Drug Application (NDA)	505(b)(1)	203684	Type 1 - New Molecular Entity	Complex formulation	Prescription	Available	Non-QbD Developed Products	[120]
Ryanodex®	Not applicable	Nanocrystal	Dantrolene Sodium	Malignant Hyperthermia	Intravenous	Lyophilized Powder for Injection	2014	Eagle Pharmaceuticals, Inc	New Drug Application (NDA	505(b)(2)	205579	Type 3 - New Dosage Form	Complex formulation	Prescription	Available	Non-QbD Developed Products	[789]
Invega Trinza®	Not applicable	Nanocrystal	Paliperidone palmitate	Schizophrenia	Intramuscular	Suspension, Extended Release	2015	Janssen-Cilag Ltd	New Drug Application (NDA)	505(b)(2)	207946	Type 5 - New Formulation or New Manufacturer	Complex dosage form	Prescription	Available	QbD Developed Products	[121]
Onivyde®	Not applicable	Liposome	Irinotecan hydrochloride	Cancer	Intravenous	Injectable	2015	Ipsen Inc	New Drug Application (NDA)	505(b)(2)	207793	Type 5 - New Formulation or New Manufacturer	Complex formulation	Prescription	Available	QbD Developed Products	[109]
Vyxeos®	Not applicable	Liposome	Cytarabine/ daunorubicin	Acute myeloid leukemia (AML)	Intravenous	Lyophilized Powder for Injection	2017	Celator Pharmaceuticals Inc	New Drug Application (NDA)	505(b)(2)	209401	Type 4 - New Combination	Complex formulation	Prescription	Available	QbD Developed Products	[122]
Onpattro®	Not applicable	Lipid nanoparticle	Patisiran Sodium	Hereditary transthyretin-mediated amyloidosis	Intravenous	Injectable	2018	Alnylam Pharms Inc	New Drug Application (NDA)	505(b)(1)	210922	Type 1 - New Molecular Entity	Complex formulation	Prescription	Available	QbD Developed Products	[149]

Monoferric®	Not applicable	Iron-carbohydrate complex	Ferric derisomaltose	Iron deficiency	Intravenous	Injectable	2020	Pharmacosmos AS	New Drug Application (NDA)	505(b)(1)	208171	Type 5 - New Formulation or New Manufacturer	Complex active ingredient	Prescription	Available	QbD Developed Products	[123]

Table 49. Regulatory landscape of Non-biological complex drugs (NBCDs) and their follow-on versions approved by the EMA [5,30,133,134,136,138,140–144].

Brand name (Reference product)	Follow-on product	Type of NBCDs	Drug name	Therapeutic indication	Route of administration	Dosage Form	Authorization date	Marketing authorization holder (MAH)	Authorization procedure	Reference Member State (RMS) (if applicable)	Concerned Member State (CMS) (if applicable)	Application procedure	Marketing Status	QbD Approach Implementation
Ferrlecit®	Not applicable	Iron-carbohydrate complex	Sodium ferric gluconate complex	Iron deficiency	Intravenous	Solution for Injection	1963	Sanofi-Aventis	NP	Not applicable	NP: CZ, HU, DE, IT	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Fragmin®	Not applicable	Low Molecular Weight Heparin (LMWH)	Dalteparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	1985	Pfizer Pharmaceuticals	NP	Not applicable	NP: AT, BE, BG, HR, CZ, DK, EE, FI, FR, DE, EL, HU, IS, IT, LV, LT, LU, NL, NO, PL, PT, RO, SK, SI, ES, SE, UK	Not specified	Authorized	Non-QbD Developed Products
Diprivan®	Not applicable	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	1987	Aspen Pharma	NP	Not applicable	NP: IE, MT, BE, UK, LU, PT, LV, ES, IT, FR, NO, SE, CY, EL, DK, NL	Not specified	Authorized	Non-QbD Developed Products
Diprivan®	Propofol Lipuro 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	1999	B. Braun	MRP/ NP	DE	MRP: IE, LV, PT, ES, UK, PL, SK, CZ, DK, IT, AT, EE, FI, DE, HU, LT, LU, NL, NO, SI, SE, EL; NP: BE, HR, RO	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol IBI 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	1999	Genthon	MRP	UK	IT, ES	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol IBI 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injectio	2000	Genthon	MRP	UK	IT, ES	Article 10(1)	Not applicable	Not applicable

						n/infusion								
Diprivan®	Propofol Genthon 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2000	Genthon	NP	Not applicable	NL	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Genthon 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2000	Genthon	NP	Not applicable	NL	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2001	Fresenius Kabi	MRP/ NP	DE	MRP: BE, DK, DE, EL, FI, IE, PT, ES, UK; NP: RO, LV, LT, EE	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Lipuro 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2001	B. Braun	MRP/ NP	DE	MRP: IE, LV, PT, ES, UK, PL, SK, CZ, DK, IT, AT, EE, FI, DE, HU, LT, LU, NL, NO, SI, SE, EL; NP: BE, HR, RO	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Mylan 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2003	Mylan	NP	Not applicable	FR	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol MCT/LCT Fresenius 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2005	Fresenius Kabi	MRP	DE	AT, BE, CY, CZ, DK, EE, DE, EL, FI, HU, IS, IE, IT, LV, LT, LU, NL, NO, PL, PT, SK, SI, ES, SE, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol MCT/LCT Fresenius 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injectio	2005	Fresenius Kabi	MRP	DE	AT, BE, CY, CZ, DK, EE, DE, EL, FI, HU, IS, IE,	Article 10(1)	Not applicable	Not applicable

						n/infusion					IT, LV, LT, LU, NL, NO, PL, PT, SK, SI, ES, SE, UK			
Diprivan®	Propofol Claris 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2006	Claris Lifesciences	MRP	NL	AT, BE, DK, EE, IT, LT, LU, LV, NO, PL, PT, SI, FI, SE, SK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Claris 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2006	Claris Lifesciences	MRP	NL	AT, BE, DK, EE, IT, LT, LU, LV, NO, PL, PT, SI	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Panpharma 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2008	Claris Lifesciences	NP	Not applicable	FR	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Lipuro 5 mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2008	B. Braun	DCP	DE	AT, DK, SI, ES, FI, FR, HU, IE, IT, LU, NO, PL, PT, UK, CZ, SE	Article 10(3)	Not applicable	Not applicable
Diprivan®	Propofol Primex 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2009	Primex Pharmaceuticals	MRP	FI	CY, MT, ES, FI, NO, SE	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Primex 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2009	Primex Pharmaceuticals	MRP	FI	CY, MT, FI, NO, SE	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Norameda 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2011	UAB Norameda	DCP	DE	AT, BE, CZ, DK, EE, FI, FR, HU, IE, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK	Article 10(1)	Not applicable	Not applicable

Diprivan®	Propofol Norameda 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2011	UAB Norameda	DCP	DE	AT, BE, CZ, DK, EE, FI, FR, HU, IE, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol BioQ Pharma 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2012	BioQ Pharma	DCP	NL	DE, ES, FR, IT, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol BioQ Pharma 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2012	BioQ Pharma	DCP	NL	ES, FR, IT, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Sandoz 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2012	Sandoz	DCP	NL	DE, ES, FR, IT, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Sandoz 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2012	Sandoz	DCP	NL	DE, ES, FR, IT, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol MCT/LCT Fresenius pre-filled syringe 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2013	Fresenius Kabi	DCP	DE	AT, BE, CY, CZ, DK, EE, ES, FI, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, SE, SI, SK, UK	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol MCT/LCT Fresenius pre-filled syringe 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2013	Fresenius Kabi	DCP	DE	AT, BE, CY, CZ, DK, EE, ES, FI, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, SE, SI, SK, UK	Article 10(1)	Not applicable	Not applicable

Diprivan®	Ripol 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2013	Corden Pharma	DCP	IT	IT	Article 10(1)	Not applicable	Not applicable
Diprivan®	Ripol 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2013	Corden Pharma	DCP	IT	IT	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Demo 10mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2017	Demo	DCP	PT	CY, EL	Article 10(1)	Not applicable	Not applicable
Diprivan®	Propofol Demo 20mg/ml	Emulsion	Propofol	Anesthesia	Intravenous	Emulsion for injection/infusion	2017	Demo	DCP	PT	CY, EL	Article 10(1)	Not applicable	Not applicable
Lovenox®	Not applicable	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	1989	Sanofi-Aventis	MRP	AT	MRP: BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, HR, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, RO, SE, SI,SK, UK	Article 30 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Lovenox®	Inhixa	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2016	Techdow Europe	CP	Not applicable	Not applicable	Article 10(4)	Not applicable	Not applicable
Lovenox®	Thorinane	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2016	Techdow Pharma	CP	Not applicable	Not applicable	Article 10(4)	Not applicable	Not applicable
Lovenox®	Enoxaparin Becat	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2017	Laboratorios Farmacéuticos Rovi	DCP	DE	AT, BE, BG, CZ, DK, EE, ES, FI, FR, HR, HU, IE, IT, LU, LV,	Article 10(4)	Not applicable	Not applicable

										NL, NO, PL, PT, RO, SE, SI, SK, UK			
Lovenox®	Crusia	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2017	Laboratorios Farmacéuticos Rovi	DCP	DE	AT, BE, BG, CZ, DK, EE, ES, FI, FR, HR, HU, IE, IT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK, UK	Article 10(4)	Not applicable	Not applicable
Lovenox®	Ghemaxan	Low Molecular Weight Heparin (LMWH)	Enoxaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for Injection	2018	Chemi	DCP	UK	BE, DE, DK, EL, ES, FI, IT, NL, NO	Article 10(4)	Not applicable	Not applicable
Ambisome®	Not applicable	Liposome	Amphotericin B	Infectious Diseases	Intravenous	Suspension for Injection	1990	Gilead Sciences International	NP	Not applicable	NP: AT, BE, DE, DK, ES, FI, FR, HU, IE, IS, NL, NO, PT, SE, SI, UK	Not specified	Authorized	Non-QbD Developed Products
Taxotere®	Not applicable	Polymeric micelle	Docetaxel	Cancer	Intravenous	Concentrate and solvent for solution for infusion	1995	Sanofi aventis	CP	Not applicable	Not applicable	Article 2 of Directive 93/41/EEC	Authorized	Non-QbD Developed Products
Endorem®	Not applicable	Nanoparticle	Dextran-coated ferumoxide	Contrast agent	Intravenous	Suspension for Infusion	1995	AMAG Pharmaceuticals Inc	MRP	FR	MRP: EL, IT, LU, NL, PT, SE, UK	Article 31 of Directive 2001/83/EC	Withdrawn	Non-QbD Developed Products
Ferrum lek®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Solution for Injection	1995	Lek Pharmaceuticals	NP	Not applicable	NP: EE, PL, LV, LT, SI	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Fercayl®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Solution for Injection	1995	Sterop	NP	Not applicable	NP: BE	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products

Caelyx®	Not applicable	Liposome	Doxorubicin hydrochloride	Cancer	Intravenous	Concentrate for solution for infusion	1996	Janssen-Cilag Ltd	CP	Not applicable	Not applicable	Article 2 of Directive 93/41/EEC	Authorized	Non-QbD Developed Products
DaunoXome®	Not applicable	Liposome	Daunorubicin citrate	Cancer	Intravenous	Concentrate for solution for infusion	1996	Galen Ltd	MRP/ NP	DE	MRP: AT, DK, EL, IE, IT, NL, PT NP: FI, FR, NO, UK	Not specified	Withdrawn	Non-QbD Developed Products
Abelcet®	Not applicable	Liposome	Amphotericin B	Infectious Diseases	Intravenous	Suspension for Injection	1996	Teva Pharmaceutical Industries Ltd	MRP	IT	UK	Not specified	Authorized	Non-QbD Developed Products
Venofer®	Not applicable	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	1997	Vifor Pharma Ltd	MRP/ NP	UK	MRP: AT, BE, DK, EL, ES, FI, IE, IT, LU, SE NP: CZ, EE, FR, HR, HU, IS, NL, NO, PT, SI, SK, LT	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Venofer®	Ferrovin	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2005	Refarm	NP	Not applicable	EL, MT	Article 10(1)	Not applicable	Not applicable
Venofer®	Óxido Férrico Sacarosado Generis	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2007	Generis	NP	Not applicable	PT	Article 10(1)	Not applicable	Not applicable
Venofer®	Alvofer	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Cooper Pharmaceuticals	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Dextrifer-S	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Intermed	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable

Venofer®	Ferrinemia	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Help Pharmaceuticals	NP	Not applicable	EL, MT	Article 10(1)	Not applicable	Not applicable
Venofer®	Hemafer-S	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Uni-Pharma	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Intrafer	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Vianex	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Ironcrose	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Target Pharma	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Fer Mylan	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Mylan	NP	Not applicable	FR	Article 10(1)	Not applicable	Not applicable
Venofer®	Fer Sandoz	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Sandoz	NP	Not applicable	FR	Article 10(1)	Not applicable	Not applicable
Venofer®	Faremio	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Demo	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Óxido Férrico Sacarosado Accord	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2008	Accord Helathcare	NP	Not applicable	PT	Article 10(1)	Not applicable	Not applicable
Venofer®	Venotrix	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2009	Alternova	NP	Not applicable	FI	Article 10(1)	Not applicable	Not applicable
Venofer®	Nefro-Fer	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2009	Medice Arzneimittel Pütter	DCP	DE	DE, AT, LU	Article 10(1)	Not applicable	Not applicable
Venofer®	IJzerhydroxide sacharose complex	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2009	Teva	NP	Not applicable	NL	Article 10(1)	Not applicable	Not applicable

Venofer®	Veniron	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2010	Viofar	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Fer Arrow	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2010	Arrow Generiques	NP	Not applicable	FR	Article 10(1)	Not applicable	Not applicable
Venofer®	Nephroferol	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2011	Verisfield	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Ferracin	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2012	Acino	NP	Not applicable	NL	Article 10(1)	Not applicable	Not applicable
Venofer®	Järnsackaros Rechon	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2012	Rechon Life Science	NP	Not applicable	SE	Article 10(1)	Not applicable	Not applicable
Venofer®	Reoxyl	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2012	Medicus	NP	Not applicable	EL	Article 10(1)	Not applicable	Not applicable
Venofer®	Sucrofer	Iron-carbohydrate complex	Iron sucrose complex	Iron deficiency	Intravenous	Solution for Injection	2018	Claris Lifesciences	DCP	UK	DE, FR	Article 10(3)	Not applicable	Not applicable
Optison®	Not applicable	Lipid microsphere	Perflutren	Contrast agent	Intravenous	Dispersion for Injection	1998	GE Healthcare AS	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Cosmofer®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Solution for Injection	1999	Pharmacosmos A/S	MRP/ NP	DK	MRP: EE, DE, IE, LV, LT, LU, NL, NO, SE, UK, DK, ES NP: FR, FI, PL	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Myocet®	Not applicable	Liposome	Doxorubicin hydrochloride	Cancer	Intravenous	Dispersion for infusion	2000	Teva Pharmaceutical Industries Ltd	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products

Visudyne®	Not applicable	Liposome	Verteporfin	Macular degeneration	Intravenous	Powder for solution for infusion	2000	Novartis Europharm Ltd	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Innohep®	Not applicable	Low Molecular Weight Heparin (LMWH)	Tinzaparin sodium	Deep vein thrombosis (DVT)	Subcutaneous	Solution for injection	2000	Leo Pharma AS	MRP/ NP	DK	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Renagel®	Not applicable	Polymeric nanoparticle	Sevelamer hydrochloride	Hyperphosphatemia (End-Stage Renal Disease (ESRD))	Oral	Film-coated tablet	2000	Genzyme	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Depocyte®	Not applicable	Liposome	Cytarabine	Cancer	Intrathecal	Suspension for Injection	2001	Pacira Ltd	CP	Not applicable	Not applicable	Not specified	Withdrawn	Non-QbD Developed Products
Rapamune®	Not applicable	Nanocrystal	Sirolimus	Organ Transplantation	Oral	Solution	2001	Pfizer Pharmaceuticals	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
SonoVue®	Not applicable	Gas dispersion	Sulphur hexafluoride	Contrast agent	Intravenous	Powder and solvent for dispersion for injection	2001	Bracco International BV	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Emend®	Not applicable	Nanocrystal	Aprepitant	Nausea and vomiting (chemotherapy)	Oral	Powder for Suspension	2003	Merck Sharp & Dohme Ltd	CP	Not applicable	Not applicable	Not specified	Authorized	QbD Developed Products
Oraqix®	Not applicable	Emulsion	Lidocaine/ prilocaine	Anesthesia	Periodontal	Gel	2003	Dentsply	MRP	SE	MRP: AT, DK, FI, DE, IS, NL, NO, SE, BE, LU, IE, UK, FR, PT, ES, IT	Not specified	Authorized	Non-QbD Developed Products

Copaxone ®	Not applicable	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2004	Teva Pharmaceutical Industries Ltd	MRP / DCP/ NP	UK	MRP/DCP: AT, BE, CY, CZ, DE, DK, EE, EL, ES, FI, HU, IE, IS, IT, LT, LU, LV, MT, NL, NO, PL, PT, SE, SK NP: FR, HR	Not specified	Authorized	Non-QbD Developed Products
Copaxone®	Brabio (20mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2016	Synthon	DCP	NL	BG, CY, CZ, DK, EE, EL, FI, HR, HU, IE, IS, LT, LV, MT, NO, PL, RO, SE, SI, SK, UK	Article 10(3)	Not applicable	Not applicable
Copaxone®	Sclerthon (20mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2016	Synthon	DCP	NL	AT, LU, MT	Article 10(3)	Not applicable	Not applicable
Copaxone®	Glatiramer acetate Mylan (20mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2016	Mylan	DCP	NL	BE, DE, ES, FR, IT, PT	Article 10(3)	Not applicable	Not applicable
Copaxone®	Glatiramer acetate Alvogen (40mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2017	Alvogen	DCP	NL	BG, CZ, EE, HR, HU, IS, LT, LV, PL, RO, SI, SK	Article 10(3)	Not applicable	Not applicable
Copaxone®	Glatiramer acetate Mylan (40mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2017	Mylan	DCP	NL	BE, CY, DE, DK, EL, ES, FI, FR, IE, IT, NO, PT, SE, UK	Article 10(3)	Not applicable	Not applicable
Copaxone®	Marcyto (40mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2017	Synthon	DCP	NL	LU	Article 10(3)	Not applicable	Not applicable
Copaxone®	Sclerthon (40mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2017	Synthon	DCP	NL	AT, MT	Article 10(3)	Not applicable	Not applicable

Copaxone®	Glatiramer acetate Teva (20mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2018	Teva Pharmaceutical Industries Ltd	DCP	DE	AT, BE, HR, LU, PL, PT, SK	Article 10(c)	Not applicable	Not applicable
Copaxone®	Glatiramer acetate Teva (40mg/ml)	Glatiramer	Glatiramer acetate	Multiple sclerosis	Subcutaneous	Solution for Injection	2018	Teva Pharmaceutical Industries Ltd	DCP	DE	AT, BE, FI, HR, LU, PL, PT, SK	Article 10(c)	Not applicable	Not applicable
Eligard®	Not applicable	Polymeric nanoparticle	Leuprolide Acetate	Cancer	Subcutaneous	Powder and Solvent for Solution for Injection	2004	Astellas Pharma Europe B.V.	MRP	DE	AT, BE, BG, CY, CZ, DK, EE, ES, FI, FR, HU, IE, IS, IT, LT, LU, LV, NL, NO, PL, PT, RO, SE, SI, SK	Not specified	Authorized	Non-QbD Developed Products
DepoDur®	Not applicable	Liposome	Morphine	Pain management	Intrathecal	Sustained-release injectable formulation	2004	Flynn Pharma Ltd	NP	Not applicable	NP: UK	Not specified	Withdrawn	Non-QbD Developed Products
Tricor®	Not applicable	Nanocrystal	Fenofibrate	Dyslipidemia	Oral	Tablet	2005	Solvay SA	MRP/ NP	DE	MRP: AT, BE, CZ, FI, FR, EL, ES, HU, IE, IT, LU, PL, SK NP: HR	Not specified	Authorized	Non-QbD Developed Products
Feriv®	Not applicable	Iron-carbohydrate complex	Iron sucrose	Iron deficiency	Intravenous	Solution for Injection	2005	G.E.S. Genericos Espanoles Laboratorio	NP	Not applicable	NP: ES	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Macugen®	Not applicable	Polymeric nanoparticle	Pegaptanib	Age-related macular degeneration (AMD)	Intravitreal	Solution for Injection	2006	PharmaSwiss Ceska Republika	CP	Not applicable	Not applicable	Article 8(3)	Withdrawn	Non-QbD Developed Products
Luminity®	Not applicable	Lipid microsphere	Perflutren	Contrast agent	Intravenous	Dispersion for injection/infusion	2006	Lantheus EU Limited	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products

Ferinject®	Not applicable	Iron-carbohydrate complex	Ferric carboxymaltose	Iron deficiency	Intravenous	Solution for Injection	2007	G.E.S. Genericos Espanoles Laboratorio	MRP/ DCP/ NP	UK	DCP: AT, CZ, DK, EE, FI, DE, EL, IE, LV, LT, LU, NL, PL, PT, SK, ES, SE, UK; MRP: BE, BG, CY, FR, HU, IS, IT, MT, NO, RO, SI NP: HR	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Ferrisat®	Not applicable	Iron-carbohydrate complex	Iron dextran	Iron deficiency	Intravenous	Solution for Injection	2007	Pharmacosmos A/S	MRP	DK	FR	Not specified	Withdrawn	Non-QbD Developed Products
Abraxane®	Not applicable	Polymeric nanoparticle	Paclitaxel	Cancer	Intravenous	Powder for suspension for infusion	2008	Celgene Europe B.V.	CP	Not applicable	Not applicable	Article 8(3)	Authorized	Non-QbD Developed Products
Abraxane®	Pazenir	Polymeric nanoparticle	Paclitaxel	Cancer	Intravenous	Powder for suspension for infusion	2019	Ratiopharm GmbH	CP	Not applicable	Not applicable	Article 10(1)	Not applicable	Not applicable
Zypadhera®	Not applicable	Nanocrystal	Olanzapine pamoate	Schizophrenia	Intramuscular	Powder and solvent for prolonged release suspension for injection	2008	Eli Lilly Ltd	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	QbD Developed Products
Mepact®	Not applicable	Liposome	Mifamurtide	Cancer	Intravenous	Powder for	2009	Takeda Pharma A/S	CP	Not applicable	Not applicable	Article 8(3)	Authorized	Non-QbD Developed Products

						concentrate for dispersion for infusion								
Monofer®	Not applicable	Iron-carbohydrate complex	Ferric derisomaltose	Iron deficiency	Intravenous	Solution for Injection	2009	Takeda Pharma A/S	DCP	SE	DCP: AT, BE, BG, CY, DK, EE, FI, DE, EL, HU, IS, IE, LV, LT, LU, NL, NO, PL, PT, RO, ES, UK	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Renvela®	Not applicable	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2009	Genzyme	CP	Not applicable	Not applicable	Article 8(3)	Authorized	Non-QbD Developed Products
Renvela®	Sevelamer carbonate Heaton 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Heaton	DCP	CZ	BG, CZ, RO, SK	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Synthon 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Synthon	DCP	DK	DK, BG, CZ, EE, FI, EL, HU, IS, LV, LT, PL, RO, SK, SI	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Housthon 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Amneal Pharma Europe	DCP	DK	DK, ES, UK	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Aurobindo 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Aurobindo Pharma	DCP	DK	BE, DK, IT, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevemed 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Medice Arzneimittel Pütter	DCP	DK	DK, DE, LU, NL, AT, PL	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Sandoz 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Sandoz	DCP	DK	DK, AT, BE, HR, CY, BG, CZ, FI, FR, IE, LV, LT, LU, NL,	Article 10(3)	Not applicable	Not applicable

										NO, PL, RO, SK, SI, SE				
Renvela®	Sevelamer carbonate Teva 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Teva	DCP	DK	DK, AT, BG, DE, EL, ES, FR, IT, LU, NL, SE, SL, UK	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Genthon 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Genthon	DCP	DK	DK, EL, BE, ES, FR, IE, IT, NL, PT, UK	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Stada 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Stada Arzneimittel	DCP	DK	DK, DE, IT, NL, AT, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Mylan 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Mylan	DCP	DK	CZ, DK, FR, DE, EL, IE, IT, NL, NO, PT, ES, SE, SK, UK	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Sandoz 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Sandoz	DCP	DK	DK, EL, DE, IT	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate AL 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2014	Aliud Pharma	DCP	DK	DK, DE	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Sandoz 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2015	Sandoz	DCP	DK	DK, BE, HR, FR, DE, IT, LU, NL, ES, SE	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Zentiva 800 mg, 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2015	Genzyme	CP	Not applicable	Not applicable	Article 10(c)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Ratiopharm 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2015	Ratiopharm	DCP	DK	DK, NL, PT, HR	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Genthon 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2016	Genthon	DCP	DK	DK, IT, UK	Article 10(3)	Not applicable	Not applicable

Renvela®	Sevelamer carbonate Stada 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2016	Stada Arzneimittel	DCP	DK	DK, DE, IT, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Fosquel 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2016	Avansor Pharma	DCP	DK	DK, LU, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Aurobindo 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Aurobindo Pharma	DCP	DK	DK, IT, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevemed 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Medice Arzneimittel Pütter	DCP	DK	AT, DE, PL	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Mylan 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Mylan	DCP	DK	DK, FR, ES	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Aurobindo 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Aurobindo Pharma	NP	Not applicable	NL	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Arrow 800 mg	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Arrow Generiques	NP	Not applicable	FR	Article 10(3)	Not applicable	Not applicable
Renvela®	Sevelamer carbonate Arrow 2.4 g	Polymeric nanoparticle	Sevelamer carbonate	Chronic kidney disease	Oral	Powder for oral suspension	2017	Arrow Generiques	NP	Not applicable	FR	Article 10(3)	Not applicable	Not applicable
Xeplion®	Not applicable	Nanocrystal	Paliperidone palmitate Long-acting depot im injection (LAI)	Schizophrenia	Intramuscular	Prolonged release suspension for injection	2011	Janssen-Cilag Ltd	CP	Not applicable	Not applicable	Article 8(3)	Authorized	Non-QbD Developed Products
Neoral®	Not applicable	Emulsion	Cyclosporine	Organ Transplantation	Oral	Solution (microemulsified	2011	Novartis Europharm Ltd	NP	Not applicable	NP: FR, HR, IE, NL, UK	Article 30	Authorized	Non-QbD Developed Products

						formulation)							
Rienso®	Not applicable	Iron-carbohydrate complex	Ferumoxytol	Iron deficiency	Intravenous	Solution for Injection	2012	Takeda Pharma A/S	CP	Not applicable	Not applicable	Article 8(3)	Withdrawn	Non-QbD Developed Products
Diafer®	Not applicable	Iron-carbohydrate complex	Ferric derisomaltose	Iron deficiency	Intravenous	Solution for Injection	2013	Pharmacosmos A/S	DCP	SE	AT, BE, DK, FI, IE, NL, NO, PL, RO, UK	Not specified	Authorized	Non-QbD Developed Products
Fer Panpharma®	Not applicable	Iron-carbohydrate complex	Iron sucrose	Iron deficiency	Intravenous	Solution for Injection	2014	Pharmacosmos A/S	NP	Not applicable	NP: FR	Article 31 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Trevicta®	Not applicable	Nanocrystal	Paliperidone palmitate	Schizophrenia	Intramuscular	Prolonged-release suspension for injection	2014	Janssen-Cilag Ltd	CP	Not applicable	Not applicable	Not specified	Authorized	Non-QbD Developed Products
Ikervis®	Not applicable	Emulsion	Cyclosporine	Ophtalmic diseases	Ocular	Eye drops, Emulsion	2015	Santen Pharmaceuticals	CP	Not applicable	Not applicable	Article 8(3)	Authorized	Non-QbD Developed Products
Onivyde®	Not applicable	Liposome	Irinotecan hydrochloride	Cancer	Intravenous	Concentrate for dispersion for infusion	2016	Les Laboratoires Servier	CP	Not applicable	Not applicable	Article 8(3)	Authorized	QbD Developed Products
Onpattro®	Not applicable	Lipid nanoparticle	Patisiran Sodium	Hereditary transthyretin-mediated amyloidosis	Intravenous	Concentrate for solution for infusion	2018	Alnylam Netherlands B.V.	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	QbD Developed Products
Vyxeos®	Not applicable	Liposome	Daunorubicin/ Cytarabine	Acute myeloid leukemia (AML)	Intravenous	Powder for concentrate for	2018	Jazz Pharmaceuticals Ireland Limited	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	QbD Developed Products

						infusion								
Verkazia®	Not applicable	Emulsion	Cyclosporin	Ocular inflammation	Ophthalmic	Eye drops, Emulsion	2018	Santen Oy	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	Non-QbD Developed Products
Exparel®	Not applicable	Liposome	Bupivacaine	Pain Management	Infiltration, Perineural use	Prolonged-release dispersion for injection	2020	Pacira Ireland Limited	CP	Not applicable	Not applicable	Article 8.3 of Directive 2001/83/EC	Authorized	QbD Developed Products

Abbreviations: AT, Austria; BE, Belgium; BG, Bulgaria; CMS, Concerned Member State; CP, Centralized Procedure; CY, Cyprus; CZ, Czech Republic; DCP, Decentralized Procedure; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, Ireland; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MRP, Mutual Recognition Procedure; MT, Malta; NL, Netherlands; NO, Norway; NP, National Procedure; PL, Poland; PT, Portugal; RMS, Reference Member State; RO, Romania; SE, Sweden; SI, Slovenia; SK, Slovakia; UK, United Kingdom.

Table 50. Resume table of the Clinical Trials of Non-biological complex drugs (NBCDs).

Clinical trials	Drug name	NBCDs type	Therapeutic regimen	Route of administration	Therapeutic Indications	Sponsor	Collaborations	Study start date	Status of Clinical Trial	Phase of Clinical Trial	References
NCT00001059	Doxorubicin	Liposome	Multi-agent therapies	Intravenous	Cancer	National Institute of Allergy and Infectious Diseases (NIAID)	Sequus Pharmaceuticals | Amgen	Not provided	Completed	Phase 2	[225]
NCT00024492	Mitoxantrone	Liposome	Single-agent therapy	Intravenous	Cancer	INSYS Therapeutics Inc	Not provided	2001	Completed	Phase 1	[790]
NCT00170573	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	North Eastern Germany Society of Gynaecologic Oncology	Not provided	2001	Completed	Phase 2	[791]
NCT00046540	Irinotecan	Liposome	Single-agent therapy	Intravenous	Cancer	INSYS Therapeutics Inc	Not provided	2002	Completed	Phase 1	[792]
NCT00944801	Doxorubicin	Liposome	Multi-agent therapies	Intravenous	Cancer	University of Regensburg	Essex Pharma (Schering-Plough) Germany	2002	Completed	Phase 1 Phase 2	[226]
NCT00080418	Paclitaxel	Liposome	Single-agent therapy	Intravenous	Cancer	INSYS Therapeutics Inc	Not provided	2003	Completed	Phase 1	[187]
NCT00100139	Paclitaxel	Liposome	Single-agent therapy	Intravenous	Cancer	INSYS Therapeutics Inc	Not provided	2004	Completed	Phase 1	[188]
NCT00111904	Paclitaxel	Polymeric micelle	Single-agent therapy	Intravenous	Cancer	Theradex	National Cancer Institute (NCI)	2005	Completed	Phase 2	[793]
NCT00361842	Irinotecan: Floxuridine	Liposome	Single-agent therapy	Intravenous	Cancer	Jazz Pharmaceuticals	Not provided	2006	Completed	Phase 2	[794]
NCT00407888	Doxorubicin	Liposome	Multi-agent therapies	Intravenous	Cancer	University of Washington	National Cancer Institute (NCI)	2006	Completed	Phase 2	[227]
NCT01054547	Ropivacaine	Liposome	Single-agent therapy	Topical application	Pain	University of Campinas, Brazil	São Paulo Research Foundation (FAPESP)	2006	Completed	Phase 1	[795]
NCT00583349	Paclitaxel	Lipid nanoparticle	Single-agent therapy	Intravesical administration	Cancer	Columbia University	Celgene Corporation	2007	Unknown	Phase 1 Phase 2	[796]
NCT00506142	Vincristine	Liposome	Single-agent therapy	Intravenous	Cancer	Spectrum Pharmaceuticals, Inc	Not provided	2007	Completed	Phase 2	[797]
NCT00777296	Amikacin	Liposome	Single-agent therapy	Inhalation	Cystic fibrosis	Insmed Incorporated	Not provided	2007	Completed	Phase 1 Phase 2	[798]
NCT01032798	Mepivacaine	Liposome	Single-agent therapy	Periodontal	Pain	University of Campinas, Brazil	São Paulo Research	2007	Completed	Phase 1	[799]

							Foundation (FAPESP)				
NCT01307969	Ropivacaine	Liposome	Single-agent therapy	Periodontal	Pain	University of Campinas, Brazil	São Paulo Research Foundation (FAPESP)	2007	Completed	Phase 1	[800]
NCT01426126	Paclitaxel	Polymeric micelle	Single-agent therapy	Intravenous	Cancer	Asan Medical Center	Samsung Medical Center| Kangdong Sacred Heart Hospital	2007	Completed	Phase 2	[181]
NCT00606515	Paclitaxel	Liposome	Single-agent therapy	Intravenous	Cancer	Shandong Luye Pharmaceutical Co., Ltd.	Nanjing Sike Pharmaceutical Co., Ltd.	2008	Completed	Phase 4	[801]
NCT00734682	Camptothecin-11	Liposome	Single-agent therapy	Intravenous	Cancer	University of California, San Francisco	Not provided	2008	Completed	Phase 1	[802]
NCT01073371	Prilocaine	Liposome	Single-agent therapy	Periodontal	Pain	University of Campinas, Brazil	São Paulo Research Foundation (FAPESP) | National Council for Scientific and Technological Development (CNPq) (Brazil)	2008	Completed	Phase 1	[803]
NCT01425840	Lidocaine	Liposome	Single-agent therapy	Topical application	Pain	University of Campinas, Brazil	São Paulo Research Foundation (FAPESP)	2008	Completed	Phase 1	[804]
NCT00886717	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Asan Medical Center	National Cancer Institute (NCI)	2008	Unknown	Phase 1 Phase 2	[231]
NCT01023347	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Samyang Biopharmaceuticals Corporation	Not provided	2008	Completed	Phase 2	[182]
NCT00882973	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Samyang Biopharmaceuticals Corporation	Not provided	2008	Completed	Phase 1	[183]
NCT00875693	Cytarabine: Daunorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	Weill Medical College of Cornell University	Jazz Pharmaceuticals	2009	Completed	Phase 1	[805]
NCT01041235	Docetaxel	Liposome	Single-agent therapy	Intravenous	Cancer	Azaya Therapeutics, Inc.	Not provided	2009	Completed	Phase 1	[806]

NCT00912639	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Korean Breast Cancer Study Group	Not provided	2009	Unknown	Phase 4	[232]
NCT01050777	Meglumine antimoniate	Liposome	Multi-agent therapies	Topical application	Infectious diseases	Tehran University of Medical Sciences	Mashhad University of Medical Sciences |Center for Research and Training in Skin Diseases and Leprosy	2011	Completed	Phase 1	[807]
NCT01310738	Amphotericin B	Liposome	Multi-agent therapies	Intravenous	Infectious diseases	University of Brasilia	Ministry of Health, Brazil | Drugs for Neglected Diseases | National Council for Scientific and Technological Development (CNPq) (Brazil)	2011	Terminated	Phase 4	[808]
NCT01507246	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2011	Completed	Phase 4	[191]
NCT01864161	Iron	Liposome	Single-agent therapy	Oral	Kidney disease / Iron Deficiency	Federico II University	Not provided	2011	Completed	Phase 4	[809]
NCT02058290	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2011	Terminated	Phase 4	[192]
NCT01770795	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Gachon University Gil Medical Center	Not provided	2011	Completed	Phase 2	[184]
NCT01507220	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2012	Terminated	Phase 4	[193]
NCT01507233	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2012	Terminated	Phase 4	[194]
NCT01509638	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2012	Completed	Phase 4	[195]
NCT01509807	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Pacira Pharmaceuticals, Inc	Registrat-Mapi	2012	Completed	Phase 4	[196]

NCT01593488	Cytarabine	Liposome	Single-agent therapy	Intrathecal injections	Cancer	National Cancer Institute, Naples	Santobono-Pausilpon Hospital |Azienda Ospedaliera Universitaria di Bologna Policlinico S.Orsola Malpighi |University of Bologna	2012	Recruiting	Phase 2	[810]
NCT01945710	Eribulin	Liposome	Single-agent therapy	Intravenous	Cancer	Eisai Ltd	Not provided	2012	Completed	Phase 1	[811]
NCT01853176	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Emory University	Not provided	2013	Terminated	Phase 4	[197]
NCT01861496	Cisplatin	Liposome	Single-agent therapy	Intravenous	Cancer	Oncology Venture	Not provided	2013	Recruiting	Phase 1 Phase 2	[812]
NCT01515007	Ciprofloxacin	Liposome	Single-agent therapy	Inhalation	Respiratory disorder	Aradigm Corporation	Grifols Therapeutics LLC	2014	Completed	Phase 3	[813]
NCT01977352	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	St. Luke's-Roosevelt Hospital Center	Not provided	2014	Completed	Phase 4	[198]
NCT02237690	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co.,Ltd.	Not provided	2014	Completed	Phase 1	[814]
NCT02260544	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	Dr. Reddy's Laboratories Limited	Not provided	2014	Completed	Phase 1	[815]
NCT02064829	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Sorrento Therapeutics, Inc.	Not provided	2014	Completed	Not Applicable	[233]
NCT01912261	Iron	Iron-carbohydrate complex	Single-agent therapy	Oral	Fatigue	Nova Scotia Health Authority	Capital Health, Canada | Dalhousie University	2014	Terminated	Phase 3	[816]
NCT02188784	Iron	Iron-carbohydrate complex	Single-agent therapy	Oral	Cardiovascular diseases	Adrian Hernandez	National Heart, Lung, and Blood Institute (NHLBI)	2014	Completed	Phase 3	[817]
NCT02428751	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	Wenqi Jiang, Sun Yat-sen University	Not provided	2015	Recruiting	Phase 3	[818]

NCT02606773	Ascorbic Acid	Liposome	Single-agent therapy	Oral	Vitamin deficiency	Semmelweis University	Novonex Pharma Kft	2015	Completed	Phase 1	[819]
NCT02640365	Irinotecan	Liposome	Multi-agent therapies	Intravenous	Cancer	GERCOR - Multidisciplinary Oncology Cooperative Group	Merrimack Pharmaceuticals	2015	Completed	Phase 1	[228]
NCT02639858	Docetaxel	Polymeric micelle	Single-agent therapy	Intravenous	Cancer	Samyang Biopharmaceuticals Corporation	Not provided	2015	Recruiting	Phase 2	[820]
NCT02536183	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	AeRang Kim, Children's Research Institute	Not provided	2016	Recruiting	Phase 1	[174]
NCT02629419	Amphotericin B	Liposome	Single-agent therapy	Oral	Infectious diseases	Matinas BioPharma Nanotechnologies, Inc.	Not provided	2016	Active, not recruiting	Phase 2	[821]
NCT02697058	Irinotecan	Liposome	Multi-agent therapies	Intravenous	Cancer	Baxalta now part of Shire	Not provided	2016	Completed	Phase 2	[229]
NCT02947178	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Walter Reed National Military Medical Center	Not provided	2016	Completed	Phase 4	[199]
NCT02971007	Amphotericin B	Liposome	Single-agent therapy	Oral	Infectious diseases	Matinas BioPharma Nanotechnologies, Inc.	Not provided	2016	Completed	Phase 2	[822]
NCT03008512	Paclitaxel	Polymeric micelle	Single-agent therapy	Intravenous	Cancer	Gachon University Gil Medical Center	Young Saing Kim, Gachon University Gil Medical Center	2016	Recruiting	Phase 2	[185]
NCT02817113	Cisplatin	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Orient Europharma Co., Ltd.	NanoCarrier Co., Ltd.	2016	Recruiting	Phase 1	[823]
NCT02739529	Paclitaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Korean Breast Cancer Study Group	Not provided	2016	Recruiting	Phase 1	[186]
NCT03348462	Anthralin	Ethosome	Single-agent therapy	Topical application	Skin diseases	Assiut University	Not provided	2017	Active, not recruiting	Phase 4	[824]
NCT03033316	Dexamethasone	Liposome	Single-agent therapy	Intravenous	Cancer	Enceladus Pharmaceuticals BV	University Hospital, Aachen |Accelovance	2017	Not yet recruiting	Phase 1 Phase 2	[825]
NCT03076372	Docetaxel	Liposome	Single-agent therapy	Intravenous	Cancer	Merrimack Pharmaceuticals	Not provided	2017	Recruiting	Phase 1	[826]
NCT03161132	Doxorubicin	Liposome	Multi-agent therapies	Intravenous	Cancer	Spanish Ovarian Cancer Research Group (GEICO)	AstraZeneca	2017	Recruiting	Phase 2	[827]

NCT03207672	Eribulin	Liposome	Single-agent therapy	Intravenous	Cancer	Eisai Ltd	Not provided	2017	Recruiting	Phase 1	[828]
NCT03250507	Bupivacaine	Liposome	Multi-agent therapies	Intra-articular injection	Pain	Henry Ford Health System	Not provided	2017	Completed	Phase 4	[200]
NCT03255343	Rhenium	Dendrimer	Single-agent therapy	Intra tumoral injection	Cancer	French Association for the Advancement Medical Research	Shanghai Tongji Hospital | Tongji University School of Medicine	2017	Recruiting	Not applicable	[829]
NCT03823040	Oxiconazole Nitrate	Lipid nanoparticle	Single-agent therapy	Topical application	Infectious diseases	Minia University	Not provided	2018	Completed	Phase 1	[830]
NCT03167957	Amphotericin B	Liposome	Single-agent therapy	Oral	Infectious diseases	Matinas BioPharma Nanotechnologies, Inc.	Not provided	2018	Withdrawn	Phase 2	[831]
NCT03196921	Amphotericin B	Liposome	Single-agent therapy	Oral	Infectious diseases	Matinas BioPharma Nanotechnologies, Inc.	University of Minnesota - Clinical and Translational Science Institute	2018	Withdrawn	Phase 1 Phase 2	[832]
NCT03318757	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	Boston University	Not provided	2018	Not yet recruiting	Phase 4	[201]
NCT03337087	Irinotecan	Liposome	Multi-agent therapies	Intravenous	Cancer	Academic and Community Cancer Research United	National Cancer Institute (NCI)	2018	Recruiting	Phase 1 Phase 2	[230]
NCT03387917	Doxorubicin	Liposome	Single-agent therapy	Intravenous	Cancer	Swiss Group for Clinical Cancer Research	Not provided	2018	Recruiting	Phase 1	[833]
NCT03393117	Bupivacaine	Liposome	Multi-agent therapies	Intra-articular injection	Pain	Case Comprehensive Cancer Center	Not provided	2018	Recruiting	Phase 2	[202]
NCT03574376	Bupivacaine	Liposome	Single-agent therapy	Intra-articular injection	Pain	OSF Healthcare System	University of Illinois College of Medicine at Peoria	2018	Recruiting	Phase 4	[203]
NCT03516903	Methotrexate	Lipid Nanoemulsion	Multi-agent therapies	Intravenous	Cardiovascular diseases	University of São Paulo, Brazil	São Paulo Research Foundation (FAPESP)	2018	Recruiting	Phase 2 Phase 3	[209]
NCT03585673	Docetaxel	Polymeric micelle	Multi-agent therapies	Intravenous	Cancer	Sung Yong Oh	Dong-A University Hospital	2018	Recruiting	Phase 2	[834]

NCT04262076	Polyamidoamine	Dendrimer	Single-agent therapy	Periodontal	Oral diseases	Al-Azhar University	Not provided	2018	Completed	Not applicable	[835]
NCT03500627	N-Acetyl-Cysteine	Dendrimer	Single-agent therapy	Intravenous	X-linked Adrenoleukodystrophy	Orpheris, Inc.	Not provided	2018	Completed	Phase 1	[836]
NCT04080869	Retinyl palmitate	Ethosome	Single-agent therapy	Topical application	Skin diseases	Assiut University	Not provided	2019	Not yet recruiting	Phase 2	[837]
NCT03187691	Amphotericin B	Liposome	Single-agent therapy	Oral	Cancer	Matinas BioPharma Nanotechnologies, Inc.	University of Cologne |The Clinical Trials Centre Cologne	2019	Withdrawn	Phase 2	[838]
NCT04214093	AZD0466	Dendrimer	Single-agent therapy	Intravenous	Cancer	AstraZeneca	Not provided	2019	Recruiting	Phase 1	[839]

Appendix II: Supplementary Data

Table 51. Summary table of the articles included in the bibliographic corpus, organized by type of lipid-based nanosystems and year of publication.

Type of Lipid-based Nanosystems	Publication Year	Drug Substance	Therapeutic Indication	Authors' affiliations	CQAs	CMAs	CPPs	Risk assessment tools	Characterization Techniques	Type of DoE study	Reference
Liposome	2019	Amphotericin B	Infectious Diseases	Academia Research/FDA	Assay/content uniformity Cytotoxicity Degradation products/ Impurity profile Particle Size Polydispersity Index	N/S	N/S	N/S	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) UV/Vis Spectrophotometry	N/S	[474]
Liposome	2019	CAF01	Infectious Diseases	Academia Research	Encapsulation Efficiency Loading capacity Morphology Particle Size Phase transition temperature Polydispersity Index Zeta potential	Drug concentration Lipid concentration pH of solutions	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Evaporative light scattering detector (ELSD) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM)	Full Factorial Design Central composite design	[475]
Liposome	2019	Lamotrigine	Neurological disorders	Academia Research	Assay/content uniformity Drug release Encapsulation Efficiency Particle Size pH Polydispersity Index Stability	Cholesterol concentration Drug solubility Lipid concentration Hydration medium composition Lipid: lipid molar ratio	Hydration Time Membrane pore size Number of cycles Pressure Temperature	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Dynamic light scattering (DLS) Laser diffractometry (LD) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry	Fractional factorial design	[476]

					Surface and coating properties Zeta potential	Log P of formulation compounds Type of solvent					
Liposome	2019	Eugenol and Dacarbazine	Cancer	Academia/Research Center or Institute	Drug release Encapsulation efficiency Loading capacity Morphology Particle size Polydispersity index Stability Surface and coating properties Zeta potential	Aqueous organic phase volume Drug concentration Lipid concentration Lipid: lipid molar ratio	Stirring speed Stirring time	Supporting statistical tools	Dynamic Light Scattering (DLS) Flow Cytometry Scanning Electron Microscopy (SEM) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Central composite design	[477]
Liposome	2019	Erlotinib	Cancer	Academia Research	Assay/ content uniformity Drug release Encapsulation efficiency Loading capacity Microbial limits Morpghology Particle size Polydispersity index Stability Zeta potential	Cholesterol concentration Drug: lipid molar ratio Hydration medium type Lipid concentration pH of solutions Phase transition temperature of lipids Type of lipid	Hydration time Number of cycles Sonication time Temperature Volume Pressure	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Transmission Electron Microscopy (TEM) X-ray Diffractometry (XRD)	Box–Behnken design	[478]
Liposome	2019	Insulin	Diabetes mellitus	Academia Research	Drug release Encapsulation efficiency Particle size Surface and coating properties Zeta potential	Cholesterol concentration Drug concentration Hydration medium type Ion strength of medium Lipid concentration	Hydration time Sonication time Temperature	Ishikawa diagram Supporting statistical tools	Dynamic Light Scattering (DLS) Optical Microscopy Rheometry Scanning Electron Microscopy (SEM) UV/Vis Spectrophotometry	Box–Behnken Design Fractional Factorial Design	[479]

						Lipid: lipid molar ratio pH of solutions Phase transition temperature of lipids Solvent molar ratio Type of lipid Type of solvent					
Liposome	2017	Doxorubicin Curcumin	Cancer	Academia Research/ Research Center or Institute	Cytotoxicity Drug release Encapsulation Efficiency Loading Capacity Particle Size Zeta potential	Drug concentration Lipid concentration Lipid: lipid molar ratio pH of solutions	Temperature	Supporting statistical tools	Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) Electrophoretic light scattering (ELS) Fluorescence microscopy High-performance liquid chromatography (HPLC) Laser Doppler anemometry (LDA)	Full Factorial Design	[480]
Liposome	2017	Terbinafine	Infectious Diseases	Research Center or Institute	Adhesion properties Assay/content uniformity Drug release Encapsulation Efficiency Morphology Particle Size Permeation Properties Polydispersity Index Viscosity Zeta potential	Drug: lipid molar ratio Lipid: lipid molar ratio	Hydration Time Sonication amplitude Sonication time Stirring speed Temperature Volume	Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Box-Behnken design	[481]

Liposome	2017	Lopinavir	HIV AIDS	Academia Research/Research Center or Institute	Appearance (Turbidity) Assay/content uniformity Drug release Encapsulation Efficiency Morphology Particle Size Permeation Properties Polydispersity Index Polymorphism Stability	Cholesterol concentration Drug concentration Drug: lipid molar ratio Drug solubility Lipid concentration Log P of formulation compounds Melting point of formulation compounds Molecular weight of the formulation compounds Solid lipid concentration Solvent molar ratio Type of solid lipid	Phases addition order Pressure Stirring speed Temperature Volume	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Optical microscopy Scanning electron microscopy (SEM) X-ray diffractometry (XRD)	Central composite design	[482]
Liposome	2017	Simvastatin	Lipid disorders	Academia Research	Encapsulation Efficiency Loading capacity Moisture content Particle Size Phase transition temperature	Cholesterol concentration Cryoprotectant: lipid molar ratio Drug concentration Hydration medium type Lipid concentration Phase transition temperature of lipids Polymer concentration Type of lipid	Freezing conditions (lyophilization) Membrane pore size Number of cycles Pressure Stirring speed Temperature	Ishikawa diagram Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Thermogravimetry (TGA) UV/Vis Spectrophotometry	N/S	[483]

Liposome	2017	Pravastatin	Lipid disorders	Academia Research	Appearance (turbidity) Encapsulation efficiency Moisture content Particle size Phase transition temperature Zeta potential	Cryoprotectant type Cryoprotectant concentration Cryoprotectant: lipid molar ratio	Annealing conditions (lyophilization) Freezing conditions (lyophilization) Volume	Ishikawa diagram Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) Laser Doppler Anemometry (LDA) Thermogravimetry (TGA) UV/Vis Spectrophotometry	D-optimal mixture design	[484]
Liposome	2017	Busulfan	Cancer	Academia Research	Encapsulation efficiency Particle size Polydispersity index	Cholesterol concentration Drug: lipid molar ratio Surfactant concentration	N/S	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Gas chromatography (GC) High-Performance Liquid Chromatography (HPLC) Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS) Transmission Electron Microscopy (TEM) X-ray Diffractometry (XRD)	Full factorial design	[485]
Liposome	2016	Prednisolone	N/S	Academia Research/ Research Center or Institute	Assay/content uniformity Cytotoxicity Encapsulation Efficiency Particle Size Polydispersity Index	Cholesterol concentration Drug concentration Hydration medium composition Hydration medium type Ionic strength of medium Lipid concentration	Hydration time Membrane pore size Number of cycles Pressure Stirring speed Temperature Type of manufacturing process	Ishikawa diagram Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) Fourier transform infrared spectroscopy (FTIR) High-performance liquid	D-optimal mixture design	[486]

						Lipid: lipid molar ratio pH of solutions Type of lipid			chromatography (HPLC) Near-infrared spectroscopy (NIR) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry		
Liposome	2016	Gedunin	Cancer	Academia Research	Encapsulation efficiency Loading capacity Particle size Zeta potential	Aqueous organic phase volume Drug concentration Lipid concentration pH of solutions Solvent molar ratio Surfactant: lipid molar ratio	Stirring speed Stirring time Sonication time Temperature	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Placket - Burman design	[487]
Liposome	2015	Sertraline hydrochloride	Neurological disorders	Academia Research	Encapsulation Efficiency Mannosylation capacity Morphology Particle Size Polydispersity Index Zeta potential	N/S	Sonication amplitude Sonication time Temperature	Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM)	Box-Behnken design	[488]
Liposome	2015	Glimepiride	Diabetes mellitus	Academia Research	Drug Release Encapsulation Efficiency Loading Capacity Particle Size Polydispersity Index Zeta potential	Cholesterol concentration Cryoprotectant type Cryoprotectant concentration Drug concentration Drug: lipid molar ratio	Hydration Time Membrane pore size Number of cycles Pressure Sonication amplitude Sonication time Temperature Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Near-infrared spectroscopy (NIR)	Plackett-Burman design	[489]

						Hydration medium concentration Hydration medium type Ionic strength of medium Lipid concentration pH of solutions Phase transition temperature of lipids Surfactant concentration Type of lipid	Type of manufacturing process				
Liposome	2014	Beta-lactoglobulin	N/S	Academia Research/Research Center or Institute	Drug release Encapsulation Efficiency Particle Size Stability	Drug concentration Lipid: lipid molar ratio	Sonication time Temperature	Supporting statistical tools	Laser diffractometry (LD) UV/Vis Spectrophotometry	Central composite design	[490]
Liposome	2014	Citalopram hydrobromide	Neurological disorders	Academia Research	Conductivity Drug release Encapsulation efficiency Particle size Polydispersity index	Cholesterol concentration Drug concentration Lipid concentration Polymer concentration Solvent concentration Surfactant concentration	Hydration time Number of cycles Volume	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Scanning Electron Microscopy (SEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Fractional factorial design	[491]

Liposome	2013	Tramadol	Pain	Research Center or Institute	Encapsulation Efficiency Particle Size Polydispersity Index Surface and coating properties Zeta potential	Aqueous organic phase volume Cholesterol concentration Drug concentration Lipid concentration Lipid: lipid molar ratio pH of solutions Phase transition temperature of lipids Polymer concentration Molecular weight of the formulation compounds	Number of cycles Osmolarity Pressure Sonication speed Sonication time Stirring speed Stirring time Temperature Type of manufacturing process Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) UV/Vis Spectrophotometry	Plackett-Burman design	[492]
Liposome	2013	Rifapentine	Tuberculosis	Academia Research	Assay/content uniformity Drug Release Encapsulation Efficiency Morphology Particle Size Physical state of DS Polydispersity Index Zeta potential	Drug: lipid molar ratio Lipid: lipid molar ratio Type of lipid	Type of manufacturing process	Ishikawa diagram Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)	Full Factorial Design	[493]

Liposome	2013	CAF01	Tuberculosis	Academia Research	Moisture content Particle Size Polydispersity Index Stability	Feedstock concentration Gas composition Solvent concentration	Atomizing air flowrate Drying air flowrate Humidity Temperature	Ishikawa diagram Supporting statistical tools	Differential scanning calorimetry (DSC) Scanning electron microscopy (SEM) Thermogravimetry (TGA)	Central composite design	[494]
Liposome	2013	Itraconazole	Infectious Diseases	Academia/ Industry Research	Encapsulation Efficiency Loading Capacity Particle Size Polydispersity Index	Drug concentration Lipid concentration Type of lipid Hydration medium type Ionic strength of medium pH of solutions Solvent concentration	Hydration Time Sonication type Temperature Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC)	Full Factorial Design Fractional factorial design Central composite design	[495]
Liposome	2013	Budesonide	Ulcerative colitis (UC) Crohn's disease	Academia Research	Assay/content uniformity Drug Release Encapsulation Efficiency Morphology Particle Size Physical state of DS Polydispersity Index Stability Surface and coating properties Zeta potential	Cholesterol concentration Lipid concentration Lipid: lipid molar ratio Surfactant concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry	Box-Behnken design	[157]
Liposome	2012	Superoxide dismutase	Rheumatoid arthritis Cancer Respiratory Disease	Academia Research/FDA	Encapsulation Efficiency Particle Size Polydispersity Index Stability Zeta potential	Cholesterol concentration Hydration medium type Ionic strength of medium Lipid concentration	Hydration time Membrane pore size Number of cycles Pressure Sonication speed	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC)	D-optimal mixture design	[496]

						pH of solutions Phase transition temperature of lipids Protein concentration Protein: lipid interactions Type of lipid	Sonication time Stirring speed Temperature Volume				
Liposome	2012	pDNA	Gene Therapy	Academia Research	Encapsulation Efficiency Morphology Particle Size Zeta potential	Lipid concentration Lipid: lipid molar ratio Type of lipid	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Box-Behnken design	[154]
Liposome	2011	Tenofovir	HIV AIDS	Academia Research/FDA	Encapsulation Efficiency Lamellarity Particle Size Stability Zeta potential	Aqueous organic phase volume Cholesterol concentration Drug concentration Drug: lipid interactions Hydration medium type Ionic strength of medium Lipid concentration pH of solutions Phase transition temperature of lipids Type of lipid	Hydration Time Membrane pore size Number of cycles Pressure Sonication speed Sonication time Temperature Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC)	N/S	[155]
Liposome	2011	Tenofovir	HIV AIDS	Academia Research/FDA	Encapsulation Efficiency Particle Size Stability Zeta potential	Cholesterol concentration Drug concentration	Hydration Time Number of cycles Pressure	Supporting statistical tools	N/S	Central composite design Plackett-Burman design	[415]

						Hydration medium concentration Lipid concentration	Sonication time				
Liposome	2009	API unspecified	N/S	Academia/Industry Research	Assay/content uniformity Degradation products/ Impurity profile Identification Lamellarity Morphology Particle Size Physical state of DS Polydispersity Index Polymorphism Surface and coating properties	N/S	N/S	Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Laser diffractometry (LD) Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	N/S	[497]
Liposome	2006	Coenzyme Q10	Dietary supplement	Academia Research	Encapsulation Efficiency Loading capacity Particle Size Stability Viscosity Zeta potential	Hydration medium concentration Lipid: lipid molar ratio Surfactant: lipid molar ratio	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Fluorescence microscopy UV/Vis Spectrophotometry	Fractional factorial design	[498]
Nanoemulsion	2019	Paclitaxel	Cancer	Academia Research	Cytotoxicity Drug release Morphology Particle Size Permeation Properties Polydispersity Index Zeta potential	Lipid concentration Surfactant concentration Surfactant molar ratio	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	I-optimal mixture design D-optimal mixture design	[499]

Nanoemulsion	2018	Bosentan	Hypertension	Academia Research	Assay/content uniformity Drug release Morphology Particle Size Polydispersity Index Stability	Cosurfactant concentration Lipid concentration Surfactant concentration Type of cosurfactant Type of lipid Type of surfactant	Stirring speed Stirring time Stirring type Temperature	Supporting statistical tools	Laser diffractometry (LD) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Taguchi design Central composite design	[500]
Nanoemulsion	2018	API unspecified	N/S	Academia Research/Research Center or Institute	Appearance (Turbidity) Assay/content uniformity Degradation products/ Impurity profile Drug release Identification Microbial limits Particle Size Permeation Properties pH Physical state of DS Stability Viscosity	Drug concentration Drug solubility Lipid concentration Log P of formulation compounds Melting point of formulation compounds Molecular weight of formulation compounds Oil excipients viscosity Preservatives concentration Surfactant concentration Type of surfactant	Humidity Number of cycles Phases addition order Pressure Stirring speed Stirring time Stirring type Temperature	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	High-performance liquid chromatography (HPLC)	Full Factorial Design Fractional factorial design Plackett-Burman design Central composite design Box-Behnken design	[501]
Nanoemulsion	2018	Raloxifene	Cancer	Academia Research/Research Center or Institute	Appearance (Turbidity) Assay/content uniformity Cytotoxicity Drug release Morphology Particle Size Permeation Properties Viscosity	Drug concentration Lipid concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) Fourier transform infrared spectroscopy (FTIR) Optical microscopy	D-optimal mixture design	[502]

					Zeta potential				Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)		
Nanoemulsion	2017	API unspecified	N/S	Academia Research	Particle Size Polydispersity Index Viscosity	Surfactant concentration Type of surfactant	Flowrate Membrane pore size Number of cycles Pressure Type of membrane	N/S	Laser diffractometry (LD) Rheometry	D-optimal mixture design	[503]
Nanoemulsion	2017	API unspecified	N/S	Academia Research	Particle Size pH Polydispersity Index Zeta potential	Ionic strength of medium pH of solutions Type of cosurfactant Type of lipid Type of surfactant	Number of cycles Pressure	N/S	Dynamic light scattering (DLS)	Fractional factorial design	[504]
Nanoemulsion	2017	Rosuvastatin	Lipid disorders	Academia Research	Drug release Particle Size Permeation Properties	Cosolvent concentration Lipid concentration Surfactant concentration Type of lipid Type of surfactant	Stirring speed Stirring time Stirring type Temperature	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) UV/Vis Spectrophotometry	D-optimal mixture design Fractional factorial design	[505]

Nanoemulsion	2017	Docetaxel	Cancer	Academia Research/ Research Center or Institute	Drug release Morphology Particle Size Permeation Properties	Cosolvent concentration Lipid concentration Surfactant concentration	Stirring speed Stirring time Stirring type Temperature	Failure mode and effect analysis (FMEA) Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Dynamic light scattering (DLS) Flow cytometry Fluorescence microscopy High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) Ultra Performance Liquid Chromatography (UPLC)	Plackett-Burman design Fractional Factorial Design D-optimal mixture design I-optimal mixture design	[506]
Nanoemulsion	2016	Selegiline	Neurological disorders	Academia Research	Assay/content uniformity Conductivity Drug release Morphology Particle Size Permeation Properties pH Polydispersity Index Stability Transmittance Viscosity Zeta potential	Lipid concentration Surfactant concentration Surfactant molar ratio	Number of cycles Pressure	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Rheometry Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Central composite design Box-Behnken design	[507]
Nanoemulsion	2016	Artemether	Infectious Diseases	Academia Research	Assay/content uniformity Drug release Morphology Particle Size Permeation Properties Stability Viscosity Zeta potential	Cosolvent concentration Lipid concentration Surfactant concentration Surfactant molar ratio	N/S	Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Rheometry Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Box-Behnken design	[508]

Nanoemulsion	2016	Lopinavir	HIV AIDS	Academia Research	Assay/content uniformity Drug release Particle Size Permeation Properties Stability	Cosolvent concentration Lipid concentration Surfactant concentration	Stirring speed Stirring time Stirring type Temperature	Failure mode and effect critically analysis (FMECA) Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Fractional factorial design	[509]
Nanoemulsion	2015	Olmesartan Medoxomil	Hypertension	Academia Research/ Research Center or Institute	Cytotoxicity Drug release Morphology Particle Size Zeta potential	Cosurfactant concentration Lipid concentration Surfactant concentration	N/S	Risk estimation matrix (REM) Supporting statistical tools	Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	D-optimal mixture design Taguchi design	[510]
Nanoemulsion	2015	Risperidone	Neurological disorders	Academia/Industry Research	Conductivity Morphology Particle Size pH Physical state of DS Polydispersity Index Stability Viscosity Zeta potential	Aqueous phase type Cosurfactant concentration Type of cosurfactant	Temperature Type of manufacturing process	Supporting statistical tools	Atomic force microscopy (AFM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Laser diffractometry (LD) Liquid chromatography tandem-mass spectrometry (LC–MS/MS) Rheometry	Full Factorial Design	[511]

Nanoemulsion	2014	Lovastatin	Lipid disorders	Academia Research	Appearance (Turbidity) Assay/content uniformity Drug release Particle Size Permeation Properties	Cosurfactant concentration Lipid concentration Surfactant concentration Type of cosurfactant Type of lipid Type of surfactant	Humidity Pressure Stirring speed Stirring time Stirring type Temperature Type of manufacturing process	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Taguchi design Central composite design	[512]
Nanoemulsion	2014	Cilostazol	Cardiovascular diseases	Academia Research	Drug release Particle Size Polydispersity Index Zeta potential	Cosurfactant concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM)	Full Factorial Design	[513]
Nanoemulsion	2013	Irbesartan	Hypertension	Academia Research	Assay/content uniformity Drug release Encapsulation Efficiency Isotropicity Morphology Particle Size Polydispersity Index Stability Transmittance Viscosity Zeta potential	Lipid concentration Surfactant molar ratio	N/S	N/S	Conductivity meter Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography(HPLC) Optical microscopy Rheometry Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)	Full Factorial Design	[514]

Nanoemulsion	2007	Cyclosporine A	Organ transplant	Academia Research/FDA	Drug release Particle Size Polydispersity Index Appearance (Turbidity) Zeta potential	Cosurfactant concentration Lipid concentration Surfactant concentration	N/S	N/S	Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Near-infrared spectroscopy (NIR)	N/S	[515]
Nanoemulsion	2007	Cyclosporine A	Organ transplant n	Academia Research/FDA	Internal volume Particle Size	Cosurfactant concentration Lipid concentration Surfactant concentration	Sonication speed Temperature	N/S	Dynamic light scattering (DLS) Ultrasonic resonator technology (URT)	Box-Behnken design	[516]
Nanoemulsion	2007	Cyclosporine A	Organ transplant	Academia Research/FDA	Drug release Encapsulation Efficiency Particle Size Appearance (Turbidity)	Cosurfactant concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Potenciometry Tensiometry Turbidimetry	Box-Behnken design	[517]
Polymeric nanoparticle	2020	Doxorubicin Paclitaxel Etoposide	Cancer	Academia Research	Encapsulation efficiency Loading capacity Morphology Particle size Polydispersity index Zeta potential	Drug concentration Log P of formulation compounds Polymer concentration Surfactant concentration Type of polymer Type of surfactant	Number of cycles Pressure Temperature Humidity Type of manufacturing process	Ishikawa diagram	Dynamic Light Scattering (DLS)	N/S	[518]
Polymeric nanoparticle	2019	Antisense oligonucleotides (ASOs)	Gene Therapy	Academia Research	Cytotoxicity Encapsulation efficiency Loading capacity Particle size	Drug: lipid molar ratio Lipid concentration Type of lipid	N/S	Supporting statistical tools	Dynamic Light Scattering (DLS) Fluorescence Microscopy High-Performance Liquid	Fractional factorial design	[519]

					Polydispersity index Zeta potential				Chromatography (HPLC) Laser Doppler Anemometry (LDA) Raman spectroscopy		
Polymeric nanoparticle	2019	Sorafenib	Cancer	Academia/Industry Research	Drug release Particle size	Polymer concentration Type of polymer	N/S	Supporting statistical tools	Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) UV/Vis Spectrophotometry	Box–Behnken design	[520]
Polymeric nanoparticle	2019	Small interfering RNA (siRNA)	Gene Therapy	Academia Research/Research Center or Institute	Encapsulation efficiency Loading capacity Moisture content Particle size Polydispersity index Zeta potential	Cryoprotectant type Drug: lipid molar ratio Feedstock concentration Gas composition Lipid concentration Type of lipid Type of polymer Type of surfactant	Atomizing air flowrate Drying air flowrate Feed flowrate Humidity Sonication time Temperature Type of manufacturing process	Ishikawa diagram Supporting statistical tools	Dynamic Light Scattering (DLS) Laser Doppler Anemometry (LDA) Scanning Electron Microscopy (SEM) Thermogravimetry (TGA) UV/Vis Spectrophotometry	Central composite design	[521]
Polymeric nanoparticle	2019	Liraglutide	Diabetes mellitus	Academia Research	Encapsulation efficiency Particle size Polydispersity index Zeta potential	Aqueous organic phase volume Cryoprotectant concentration Cryoprotectant type Drug concentration Polymer concentration	Sonication time	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Liquid Chromatography Tandem-Mass Spectrometry (LC–MS/MS)	Plackett-Burman design	[522]

									Scanning Electron Microscopy (SEM) X-ray Diffractometry (XRD)		
Polymeric nanoparticle	2019	Paclitaxel	Cancer	Academia Research/Research Center or Institute	Assay/content uniformity Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Physical state of DS Polydispersity Index Stability Zeta potential	Polymer concentration Solvent concentration Surfactant concentration	N/S	Supporting statistical tools	Atomic force microscopy (AFM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	Box-Behnken design	[523]
Polymeric nanoparticle	2019	Risperidone	Neurological disorders	Academia Research	Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Permeation Properties Phase transition temperature Polydispersity Index Stability Surface and coating properties Zeta potential	Lipid concentration Surfactant concentration	Sonication time	N/S	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)	Box-Behnken design	[524]

Polymeric nanoparticle	2018	Antigen CTH522	Infectious Diseases	Academia Research	Morphology Particle Size Phase transition temperature Physical state of DS Polydispersity Index Stability Zeta potential	Lipid concentration Lipid: lipid molar ratio Lipid: polymer molar ratio Polymer concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) Evaporative light scattering detector (ELSD) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Laser Doppler anemometry (LDA) Transmission electron microscopy (TEM)	Central composite design Full Factorial Design	[525]
Polymeric nanoparticle	2018	Budesonide	Respiratory Disease	Academia Research	Encapsulation Efficiency Loading Capacity Particle Size Polydispersity Index Zeta potential	Drug concentration Lipid concentration	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Evaporative light scattering detector (ELSD) High-performance liquid chromatography (HPLC) Laser Doppler anemometry (LDA) UV/Vis Spectrophotometry	Central composite design	[526]
Polymeric nanoparticle	2018	Nifedipine	Hypertension	Academia/Industry Research	Particle size Polydispersity index Zeta potential	Drug concentration Drug solubility Polymer concentration Surfactant concentration Type of polymer	Feed flowrate Humidity Milling time Stirring speed Temperature Volume	Ishikawa diagram Supporting statistical tools	Differential Scanning Calorimetry (DSC) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Optical Microscopy	Box-Behnken design	[527]

						Type of surfactant			Rheometry X-ray Diffractometry (XRD)		
Polymeric nanoparticle	2018	Recombinant hepatitis B surface antigen (HBsAg)	Hepatitis B	Academia Research	Encapsulation efficiency Morphology Particle size Polydispersity index Zeta potential	Drug concentration Polymer concentration Surfactant concentration	N/S	N/S	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Enzyme-linked Immunosorbent Assay (ELISA) Fluorescence Microscopy Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) Transmission Electron Microscopy (TEM)	Central composite design	[528]
Polymeric nanoparticle	2018	Liraglutide	Diabetes mellitus	Academia Research	Encapsulation efficiency Loading capacity Particle size Polydispersity index Zeta potential	Aqueous organic phase volume Cryoprotectant concentration Cryoprotectant type Drug concentration Polymer concentration Surfactant concentration Type of polymer Type of solvent Type of surfactant	Sonication time Sonication speed Centrifugation time Centrifugation speed Temperature Freezing conditions (lyophilization)	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	N/S	Full factorial design	[529]

Polymeric nanoparticle	2017	Docetaxel	Cancer	Academia Research	Drug release Encapsulation efficiency Morphology Particle size Polydispersity index Stability Zeta potential	Polymer concentration Solvent molar ratio Surfactant concentration Type of polymer Type of surfactant	Centrifugation speed Centrifugation time Homogenization speed Homogenization time Sonication time Stirring speed Stirring time	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Atomic Force Microscopy (AFM) Confocal Laser Scanning Microscopy (CLSM) Dynamic Light Scattering (DLS) Electrophoretic Light Scattering (ELS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Scanning Electron Microscopy (SEM) Transmission Electron Microscopy (TEM) X-ray Diffractometry (XRD)	Box–Behnken design Placket - Burman Design	[530]
Polymeric nanoparticle	2017	Doxorubicin	Cancer	Academia Research/ Research center or Institute	Encapsulation efficiency Particle size Polydispersity index Zeta potential	Aqueous organic phase volume Drug concentration Polymer concentration Surfactant concentration	Sonication speed Sonication time	Ishikawa diagram Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fluorescence Microscopy Fourier Transform Infrared Spectroscopy (FTIR) Transmission Electron Microscopy (TEM)	Box–Behnken design	[531]
Polymeric nanoparticle	2017	Rutin	Neurological disorders	Academia Research	Drug release Encapsulation Efficiency Morphology Particle Size Phase transition temperature Polydispersity Index Zeta potential	Lipid: polymer molar ratio Polymer concentration Surfactant concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Laser Doppler anemometry (LDA) Liquid chromatography tandem-mass	Full Factorial Design	[532]

									spectrometry (LC–MS/MS) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry		
Polymeric nanoparticle	2017	RNA	Gene Therapy	Academia Research	Cytotoxicity Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index Transfection efficiency Zeta potential	Lipid concentration Drug: lipid molar ratio	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Evaporative light scattering detector (ELSD) Flow cytometry Fluorescence microscopy High-performance liquid chromatography (HPLC) Thin layer chromatography (TLC) Transmission electron microscopy (TEM)	Full Factorial Design	[533]
Polymeric nanoparticle	2016	Gefitinib	Cancer	Academia Research	Assay/content uniformity Cytotoxicity Drug release Morphology Particle Size Polydispersity index Stability of the formulation Zeta potential	Cosurfactant concentration Polymer concentration Type of cosurfactant Type of polymer Type of surfactant Surfactant concentration	Homogenization speed Homogenization time Sonication amplitude Sonication speed Sonication time	Ishikawa diagram Supporting statistical tools	Atomic Force Microscopy (AFM) Dynamic light scattering (DLS) Enzyme-linked Immunosorbent Assay (ELISA) High-performance liquid chromatography (HPLC)	Full factorial design	[534]
Polymeric nanoparticle	2016	API unspecified	N/S	Industry Research	Appearance (Turbidity) Assay/content uniformity Cytotoxicity Degradation products/impurity profile	N/S	N/S	Failure mode and effect critically analysis (FMECA) Supporting	Dynamic light scattering (DLS) Enzyme-linked Immunosorbent Assay (ELISA) Gas chromatography (GC)	N/S	[535]

					Drug release Encapsulation efficiency Identification Loading capacity Microbial limits Particle size pH Surface and coating properties			statistical tools	Nuclear Magnetic Resonance spectroscopy (NMR) Single Particle Optical Sensing (SPOS) Size exclusion chromatography (SEC) Ultra Performance Liquid Chromatography (UPLC)		
Polymeric nanoparticle	2016	Aripiprazole	Neurological disorders	Academia Research/ Research Center or Institute	Drug release Encapsulation efficiency Loading capacity Morphology Particle size Zeta potential	Aqueous organic phase volume Drug: polymer molar ratio Surfactant concentration	Flowrate Stirring speed Stirring time Temperature	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Optical Microscopy Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Box-Behnken design	[536]
Polymeric nanoparticle	2016	Indomethacin	Inflammatory disorders Pain	Academia Research/ Research Center or Institute	Drug release Encapsulation efficiency Particle size Polydispersity index Zeta potential	Polymer concentration Surfactant molar ratio	N/S	Supporting statistical tools	Dynamic Light Scattering (DLS) Enzyme-linked Immunosorbent Assay (ELISA) Laser Doppler Anemometry (LDA) Nuclear Magnetic Resonance spectroscopy (NMR) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Full factorial design	[537]

Polymeric nanoparticle	2016	Ciprofloxacin	Infectious Diseases	Academia/ Industry Research	Particle size Polydispersity index Encapsulation efficiency	N/S	Flowrate Pressure Temperature	Supporting statistical tools	Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) Scanning Electron Microscopy (SEM)	Box-Behnken design	[538]
Polymeric nanoparticle	2015	CAF01	Infectious Diseases	Academia Research/ Research Center or Institute	Encapsulation Efficiency Loading Capacity Morphology Particle Size Phase transition temperature Polydispersity Index Stability Zeta potential	Aqueous organic phase volume Lipid concentration Lipid: lipid molar ratio Surfactant concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Enzyme-linked immunosorbent assay (ELISA) Flow cytometry Fourier transform infrared spectroscopy (FTIR) Transmission electron microscopy (TEM)	Full Factorial Design Central composite design	[539]
Polymeric nanoparticle	2015	Zolmitriptan	Neurological disorders	Academia Research	Drug release Encapsulation efficiency Morphology Particle size Polydispersity index Zeta potential	Aqueous organic phase volume Polymer concentration Type of polymer	N/S	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Thermogravimetry (TGA) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Full factorial design	[540]

Polymeric nanoparticle	2015	Fingolimod	Multiple sclerosis	Academia/ Research Center or Institute	Encapsulation efficiency Loading capacity Particle size Polydispersity index	Drug concentration Polymer concentration Surfactant concentration	Stirring speed	Supporting statistical tools	Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Scanning Electron Microscopy (SEM)	Box–Behnken design	[541]
Polymeric nanoparticle	2014	Heparzine	Cancer	Academia Research	Drug release Encapsulation Efficiency Particle Size Polydispersity Index Stability Zeta potential	Polymer concentration	Number of cycles Sonication amplitude	Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Evaporative light scattering detector (ELSD) Liquid chromatography tandem-mass spectrometry (LC–MS/MS) Size exclusion chromatography (SEC)	Full Factorial Design	[542]
Polymeric nanoparticle	2014	Efavirenz	HIV AIDS	Academia Research/ Research Center or Institute	Morphology Particle Size Phase transition temperature Physical state of DS Polydispersity Index Stability Zeta potential	Drug concentration Polymer concentration Surfactant concentration	Milling time	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Box-Behnken design	[543]

Polymeric nanoparticle	2013	Paclitaxel	Cancer	Academia Research/FDA	Encapsulation efficiency Particle size Zeta potential	Aqueous organic phase volume Drug concentration Molecular weight of formulation compounds Polymer concentration Surfactant concentration Type of polymer Type of solvent Type of surfactant	Homogenization speed Homogenization time Pressure Temperature	Ishikawa diagram Supporting statistical tools	Atomic Force Microscopy (AFM) Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Enzyme-linked Immunosorbent Assay (ELISA) Fourier Transform Infrared Spectroscopy (FTIR) Gas chromatography (GC) Scanning Electron Microscopy (SEM) Thermogravimetry (TGA) Ultra Performance Liquid Chromatography (UPLC) X-ray Diffractometry (XRD)	Box–Behnken design Plackett-Burman Design	[544]
Polymeric nanoparticle	2013	Dutasteride	Benign prostatic hyperplasia (BPH)	Academia Research	Encapsulation efficiency Particle size Polydispersity index	Aqueous organic phase volume Drug concentration Feedstock concentration Polymer concentration Type of solvent	Stirring speed Flowrate	N/S	Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC)	Plackett - Burman design Central composite design	[545]
Lipid nanoparticle	2017	Lamivudine	HIV AIDS	Academia Research	Cytotoxicity Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index	Aqueous organic phase volume Drug concentration Lipid: lipid molar ratio Liquid lipid concentration	Humidity Sonication amplitude Sonication time Stirring speed Temperature Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Full Factorial Design Central composite design	[546]

					Stability Zeta potential	Solid lipid concentration Surfactant concentration Surfactant molar ratio Type of surfactant					
Lipid nanoparticle	2016	5-Fluorouracil	Cancer	Academia Research	Drug release Encapsulation Efficiency Particle Size Polydispersity Index Zeta potential	Drug concentration Liquid lipid concentration Type of lipid Surfactant concentration	Sonication amplitude Stirring speed	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	N/S	[547]
Lipid nanoparticle	2015	Docetaxel	Cancer	Research Center or Institute	Assay/content uniformity Drug Release Encapsulation Efficiency Morphology Particle Size Phase transition temperature Physical state of DS Polydispersity Index Zeta potential	Aqueous organic phase volume Drug concentration Drug: lipid molar ratio	Evaporation rate Stirring speed	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Fluorescence microscopy High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)	Central composite design	[548]
Solid lipid nanoparticle	2019	Ibuprofen	Inflammatory disorders	Academia Research	Drug release Encapsulation Efficiency Particle Size Permeation Properties	Drug concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) High-performance liquid	Box-Behnken design	[549]

					Polydispersity Index Viscosity Zeta potential				chromatography (HPLC) Rheometry		
Solid lipid nanoparticle	2019	siRNA	Gene Therapy	Academia Research	Cytotoxicity Morphology Particle Size Polydispersity Index Stability Surface and coating properties Transfection efficiency Zeta potential	N/S	Stirring speed Temperature	Failure mode and effect critically analysis (FMECA) Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Laser diffractometry (LD) Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	Fractional factorial design	[550]
Solid lipid nanoparticle	2017	Rosuvastatin	Lipid disorders	Academia Research/ Research Center or Institute	Cytotoxicity Drug release Encapsulation Efficiency Particle Size Permeation Properties Polydispersity Index Zeta potential	Lipid concentration Surfactant concentration	Sonication amplitude Sonication time Stirring speed Stirring time Type of manufacturing process	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Dynamic light scattering (DLS) Enzyme-linked immunosorbent assay (ELISA) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM)	I-optimal design Taguchi design	[551]
Solid lipid nanoparticle	2016	Diallyl disulfide	Cancer	Academia Research	Cytotoxicity Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index	Aqueous organic phase volume Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Confocal laser scanning microscopy (CLSM) Flow cytometry Fluorescence microscopy	Box-Behnken design	[552]

					Zeta potential				Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography(HPLC) Scanning electron microscopy (SEM) Western blot		
Solid lipid nanoparticle	2016	Isradipine	Hypertension	Academia Research	Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Stability Zeta potential	Drug concentration Lipid: lipid molar ratio Polymer concentration Surfactant concentration	Sonication time	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry	Taguchi design	[553]
Solid lipid nanoparticle	2015	Rivastigmine	Neurological disorders	Academia Research/ Research Center or Institute	Assay/content uniformity Encapsulation Efficiency Morphology Particle Size pH Phase transition temperature Polydispersity Index Zeta potential	Drug concentration Drug solubility Drug: lipid molar ratio Lipid concentration Surfactant concentration Type of lipid Type of surfactant	Stirring speed Stirring time Temperature	Ishikawa diagram Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)	Full Factorial Design	[554]
Solid lipid nanoparticle	2015	Fenofibrate	Lipid disorders	Academia Research	Drug Release Encapsulation Efficiency Particle Size Polydispersity Index Stability Zeta potential	Drug concentration Lipid concentration Surfactant concentration Type of lipid Type of surfactant	Phases addition order Stirring speed Temperature	Supporting statistical tools	Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) UV/Vis Spectrophotometry	Plackett-Burman design	[555]

Solid lipid nanoparticle	2013	Paclitaxel	Cancer	Academia Research	Drug release Encapsulation Efficiency Particle Size Polydispersity Index Stability Zeta potential	Drug concentration Lipid concentration Surfactant: lipid molar ratio Type of lipid	Stirring speed Stirring time Temperature	Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Optical microscopy Transmission electron microscopy (TEM)	Full Factorial Design	[556]
Solid lipid nanoparticle	2013	Ropinirole	Neurological disorders	Academia Research	Drug Release Encapsulation Efficiency Loading Capacity Morphology Particle Size Permeation Properties Polydispersity Index Stability Zeta potential	Cosurfactant concentration Drug concentration Lipid concentration Surfactant concentration	Number of cycles Pressure Temperature	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry	Central composite design Full Factorial Design	[557]
Solid lipid nanoparticle	2012	Nimodipine	Hypertension	Academia Research	Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index Stability Zeta potential	Cosurfactant concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM) UV/Vis Spectrophotometry	Full Factorial Design	[558]

Solid lipid nanoparticle	2010	Risperidone	Neurological disorders	Academia Research/FDA	Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index Zeta potential	Drug concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Near-infrared spectroscopy (NIR) Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	Box-Behnken design Fractional factorial design Central composite design	[559]
Nanoestructured lipid carrier	2019	Donepezil	Neurological disorders	Academia Research	Cytotoxicity Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Permeation Properties Physical state of DS Polydispersity Index Zeta potential	N/S	N/S	Supporting statistical tools	Atomic force microscopy (AFM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) X-ray diffractometry (XRD)	N/S	[560]
Nanoestructured lipid carrier	2019	Tripterine	Skin diseases	Academia Research	Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Permeation Properties Physical state of DS Polydispersity Index	Drug concentration Lipid concentration Lipid: lipid molar ratio Surfactant concentration Type of liquid lipid Type of solid lipid Type of surfactant	Cooling time Stirring speed Temperature	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Rheometry Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	Box-Behnken design	[561]

					Surface and coating properties Viscosity						
Nanoestructured lipid carrier	2019	Voriconazole	Infectious Diseases	Academia Research	Assay/content uniformity Drug release Encapsulation Efficiency Loading capacity Morphology Particle Size Permeation Properties Polydispersity Index Viscosity Zeta potential	Aqueous organic phase volume Cosurfactant concentration Drug concentration Drug solubility Lipid: lipid molar ratio Liquid lipid concentration Solid lipid concentration Surfactant concentration Type of cosurfactant Type of surfactant	Sonication amplitude Sonication time Stirring speed Temperature	Ishikawa diagram	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Fourier transform infrared spectroscopy (FTIR) Rheometry Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry X-ray diffractometry (XRD)	Central composite design Fractional factorial design Box-Behnken design	[562]
Nanoestructured lipid carrier	2019	Lopinavir	HIV AIDS	Academia Research	Appearance (Turbidity) Assay/content uniformity Cytotoxicity Drug release Encapsulation Efficiency Morphology Particle Size Polydispersity Index Stability Zeta potential	Liquid lipid concentration Solid lipid concentration Surfactant concentration Type of liquid lipid Type of solid lipid Type of surfactant	Humidity Number of cycles Phases addition order Pressure Sonication time Stirring speed Stirring time Stirring type Temperature	Failure mode and effect critically analysis (FMECA) Ishikawa diagram Supporting statistical tools	High-performance liquid chromatography (HPLC) Laser diffractometry (LD) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Box-Behnken design Plackett-Burman design	[563]

Nanoestructured lipid carrier	2019	Clobetasol	Skin diseases	Academia Research	Drug release Encapsulation Efficiency Particle Size Polydispersity Index Zeta potential	Drug: lipid molar ratio Surfactant concentration	Sonication time	Supporting statistical tools	Enzyme-linked immunosorbent assay (ELISA) Laser diffractometry (LD) UV/Vis Spectrophotometry	N/S	[564]
Nanoestructured lipid carrier	2019	5-Fluorouracil	Skin diseases	Academia Research	Cytotoxicity Drug release Encapsulation Efficiency Morphology Particle Size Permeation Properties pH Polydispersity Index Viscosity Zeta potential	Drug concentration Lipid concentration Liquid lipid concentration Solid lipid concentration Type of solid lipid	Number of cycles Pressure	Supporting statistical tools	Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography (HPLC) Rheometry Transmission electron microscopy (TEM) X-ray diffractometry (XRD)	N/S	[565]
Nanoestructured lipid carrier	2018	API unspecified	N/S	Academia Research	Cytotoxicity Morphology Particle Size Phase transition temperature Physical state of DS Polydispersity Index Stability Zeta potential	Lipid concentration Lipid: lipid molar ratio Surfactant concentration	Sonication time	Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Fluorescence microscopy Fourier transform infrared spectroscopy (FTIR) Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)	Plackett-Burman design	[566]

Nanoestructured lipid carrier	2018	Zidovudine	HIV AIDS	Academia Research	Assay/content uniformity Cytotoxicity Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Polydispersity Index Stability Zeta potential	Aqueous organic phase volume Drug concentration Lipid concentration Lipid: lipid molar ratio Surfactant concentration Surfactant molar ratio	Cool down process Humidity Sonication amplitude Sonication time Stirring speed Temperature Volume	Ishikawa diagram Supporting statistical tools	Dynamic light scattering (DLS) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Full Factorial Design Central composite design	[567]
Nanoestructured lipid carrier	2017	Olmesartan Medoxomil	Hypertension	Academia Research/ Research Center or Institute	Drug release Encapsulation efficiency Particle size Zeta potential	Aqueous organic phase volume Cryoprotectant concentration Drug concentration Drug solubility Lipid concentration Lipid: lipid molar ratio Log P of formulation compounds Melting point of formulation compounds Molecular weight of the formulation compounds pH of solutions Solid lipid concentration Surfactant concentration Type of lipid	Cooling time Homogenization speed Homogenization time Phases addition order Pressure Stirring speed Stirring type Temperature Type of manufacturing process	Failure mode and effect analysis (FMEA) Ishikawa diagram Supporting statistical tools	Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Central composite design	[568]

						Type of surfactant					
Nanoestructured lipid carrier	2017	Olanzapine Simvastatin	Neurological disorders	Academia Research	Adhesion properties Assay/content uniformity Cytotoxicity Drug release Encapsulation Efficiency Loading Capacity Particle Size Permeation Properties Polydispersity Index	N/S	N/S	N/S	Confocal laser scanning microscopy (CLSM) Dynamic light scattering (DLS) Fluorescence microscopy Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM)	Full Factorial Design	[569]
Nanoestructured lipid carrier	2017	Aceclofenac	Inflammatory disorders	Academia Research	Drug release Encapsulation Efficiency Loading Capacity Morphology Particle Size Permeation Properties Polydispersity Index Stability Surface and coating properties Viscosity Zeta potential	Aqueous organic phase volume Lipid: lipid molar ratio Liquid lipid concentration Solid lipid concentration Solvent molar ratio Surfactant concentration Type of liquid lipid Type of solid lipid Type of surfactant	Stirring speed Stirring time Temperature	Ishikawa diagram Risk estimation matrix (REM) Supporting statistical tools	Confocal laser scanning microscopy (CLSM) High-performance liquid chromatography (HPLC) Laser Doppler anemometry (LDA) Rheometry Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)	Box-Behnken design Taguchi design	[570]

Nanoestructured lipid carrier	2013	Iloperidone	Neurological disorders	Academia Research	Assay/content uniformity Drug Release Encapsulation Efficiency Morphology Particle Size Polydispersity Index Stability	Drug concentration Lipid: lipid molar ratio Liquid lipid concentration Solid lipid concentration Surfactant concentration	N/S	Supporting statistical tools	High-performance liquid chromatography(HPLC) Laser diffractometry (LD) Scanning electron microscopy (SEM)	Box-Behnken design	[571]
Polymeric micelle	2020	Lapatinib	Cancer	Academia Research/ Research Center or Institute	Encapsulation efficiency Loading capacity Particle size Polydispersity index	Cosurfactant concentration Drug: polymer molar ratio Solvent concentration	N/S	Supporting statistical tools	Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) Optical Microscopy Scanning Electron Microscopy (SEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Box-Behnken design	[572]
Polymeric micelle	2020	Curcumin	N/S	Academia Research/FDA	Loading capacity Particle size Polydispersity index	Polymer concentration	Flowrate Temperature	N/S	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) Optical Microscopy Raman Spectroscopy Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry X-ray Diffractometry (XRD)	Full factorial design	[573]
Polymeric micelle	2019	Salinomycin	Cancer	Academia Research	Encapsulation efficiency Particle size Polydispersity index Zeta potential	Aqueous organic phase volume Drug concentration	Homogenization time Homogenization speed Pressure Stirring speed	Ishikawa diagram	Confocal Laser Scanning Microscopy (CLSM) Dynamic Light Scattering (DLS) Flow Cytometry	Central composite design Fractional	[574]

						Polymer concentration Type of solvent	Stirring time Temperature Volume		High-Performance Liquid Chromatography (HPLC) UV/Vis Spectrophotometry	factorial design	
Polymeric micelle	2018	API unspecified	Cancer	Academia Research	Particle size Polydispersity index Zeta potential	Polymer concentration Molecular weight of the formulation compounds Type of polymer	N/S	Supporting statistical tools	Atomic Force Microscopy (AFM) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) Laser Doppler Anemometry (LDA)	Central composite design	[575]
Polymeric micelle	2017	Quercetin and salicyli acid	Cancer	Academia/ Research Center or Institute	Drug release Particle size Permeation properties Polydispersity index Transmittance Zeta potential	Lipid concentration Solvent concentration Surfactant concentration	N/S	Supporting statistical tools	Confocal Laser Scanning Microscopy (CLSM) Differential Scanning Calorimetry (DSC) Flow Cytometry Fourier Transform Infrared Spectroscopy (FTIR) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Box–Behnken design	[576]
Polymeric micelle	2014	Paclitaxel	Cancer	Academia Research	Encapsulation efficiency Particle size	Drug: polymer molar ratio Type of polymer	Temperature	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) High-Performance Liquid Chromatography (HPLC) Transmission Electron Microscopy (TEM)	D-optimal mixture design	[577]

Niosome	2015	Ketoprofen	Pain	Academia/Industry Research	N/S	Aqueous organic phase volume Ionic strength of medium pH of solutions Type of solvent	Column specifications (type, dimension) Flowrate Humidity Pressure Temperature Volume	Ishikawa diagram Supporting statistical tools	High-performance liquid chromatography (HPLC)	Central composite design Taguchi design	[578]
Niosome	2014	Tenofovir	HIV AIDS	Academia Research	Adhesion properties Drug release Encapsulation Efficiency Morphology Particle Size Permeation properties Surface and coating properties Zeta potential	Cholesterol concentration Type of surfactant	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Fluorescence microscopy High-performance liquid chromatography (HPLC) Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)	Full Factorial Design	[579]
Niosome	2014	Pioglitazone	Diabetes mellitus	Academia Research	Encapsulation efficiency Particle size Permeation properties	Cholesterol concentration Lipid concentration Surfactant concentration	N/S	Supporting statistical tools	Confocal Laser Scanning Microscopy (CLSM) Dynamic Light Scattering (DLS) High-Performance Liquid Chromatography (HPLC) Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Box–Behnken design	[580]
Niosome	2013	Rutin	Cardiovascular diseases	Research Center or Institute	Drug release Encapsulation Efficiency Morphology Particle Size Polydispersity Index Zeta potential	Cholesterol concentration Surfactant concentration Type of surfactant	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	N/S	[581]

Ethosome	2019	Resveratrol	Inflammatory disorders	Academia Research	Encapsulation Efficiency Lamellarity Morphology Particle Size Permeation Properties pH Polydispersity Index Spreadability Stability Viscosity Zeta potential	Aqueous organic phase volume Hydration medium type Lipid concentration Hydration medium concentration Type of lipid Solvent concentration	Sonication speed Sonication time Sonication type Stirring speed Stirring time Stirring type Temperature Type of manufacturing process	Failure mode and effect critically analysis (FMECA) Ishikawa diagram Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Dynamic light scattering (DLS) Rheometry Size exclusion chromatography (SEC) Transmission electron microscopy (TEM) UV/Vis Spectrophotometry	Full Factorial Design	[582]
Ethosome	2015	Diclofenac	Inflammatory disorders Pain	Academia Research	Elasticity Encapsulation Efficiency Lamellarity Morphology Particle Size Permeation Properties Phase transition temperature Polydispersity Index Zeta potential	Aqueous organic phase volume Lipid: lipid molar ratio	N/S	Supporting statistical tools	Confocal laser scanning microscopy (CLSM) Differential scanning calorimetry (DSC) Dynamic light scattering (DLS) High-performance liquid chromatography(HPLC)	Full Factorial Design	[583]
Ethosome	2015	Methoxsalen	Skin diseases	Academia Research	Encapsulation efficiency Loading capacity Particle size Permeation properties	Lipid concentration Solvent concentration	N/S	Supporting statistical tools	Dynamic Light Scattering (DLS) Fluorescence Microscopy High-Performance Liquid Chromatography (HPLC) Rheometry Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Central composite design	[584]

Nanocapsule	2016	API unspecified	N/S	Academia/Industry Research	Drug release Morphology Particle Size Polydispersity Index Zeta potential	Lipid concentration Polymer concentration Surfactant concentration Type of lipid Type of polymer Type of surfactant	Humidity Stirring speed Stirring time Stirring type Temperature	Ishikawa diagram Supporting statistical tools	Atomic force microscopy (AFM) Confocal laser scanning microscopy (CLSM) Differential scanning calorimetry (DSC) Electrophoretic light scattering (ELS) Fluorescence microscopy Fourier transform infrared spectroscopy (FTIR) Laser diffractometry (LD) Laser Doppler anemometry (LDA) Transmission electron microscopy (TEM)	Central composite design	[585]
Aspasomes	2018	Methotrexate	Rheumatoid arthritis	Academia Research	Drug release Encapsulation Efficiency Lamellarity Loading Capacity Morphology Particle Size Polydispersity Index Surface and coating properties Viscosity Zeta potential	Drug concentration Drug: lipid molar ratio Hydration medium type Lipid concentration Lipid: lipid molar ratio	N/S	Supporting statistical tools	Dynamic light scattering (DLS) Electrophoretic light scattering (ELS) Enzyme-linked immunosorbent assay (ELISA) Fluorescence microscopy Fourier transform infrared spectroscopy (FTIR) High-performance liquid chromatography (HPLC) Transmission electron microscopy (TEM)	Full Factorial Design	[586]

Transferosome	2018	Miconazole Nitrate	Infectious Diseases	Academia Research	Assay/content uniformity Drug release Encapsulation efficiency Morphology Particle size Permeation properties pH Polydispersity index Spreadability Viscosity Zeta potential	Lipid concentration Surfactant: lipid molar ratio Type of surfactant	N/S	Supporting statistical tools	Differential Scanning Calorimetry (DSC) Dynamic Light Scattering (DLS) Fourier Transform Infrared Spectroscopy (FTIR) Rheometry Transmission Electron Microscopy (TEM) UV/Vis Spectrophotometry	Fractional factorial design	[587]

N/S: Not Specified