ORIGINAL RESEARCH

Enantioselective HPLC-UV method for determination of eslicarbazepine acetate (BIA 2-093) and its metabolites in human plasma

Gilberto Alves,[1] Isabel Figueiredo,[1] Margarida Castel-Branco,[1] Ana Loureiro,[2] Ana Fortuna,[1] Amílcar Falcão[1]* and Margarida Caramona[1]

[1]Laboratory of Pharmacology, Faculty of Pharmacy, Coimbra University, 3000-141 Coimbra, Portugal
[2]Department of Research and Development, BIAL, 4745-457 S. Mamede do Coronado, Portugal

ABSTRACT: Eslicarbazepine acetate (BIA 2-093) is a novel central nervous system drug undergoing clinical phase III trials for epilepsy and phase II trials for bipolar disorder. A simple and reliable chiral reversed-phase HPLC-UV method was developed and validated for the simultaneous determination of eslicarbazepine acetate, oxcarbazepine, *S*-licarbazepine and *R*-licarbazepine in human plasma. The analytes and internal standard were extracted from plasma by a solid-phase extraction using Waters Oasis® HLB cartridges. Chromatographic separation was achieved by isocratic elution with water–methanol (88:12, v/v), at a flow rate of 0.7 mL/min, on a LichroCART 250-4 ChiraDex (*β*-cyclodextrin, 5 µm) column at 30°C. All compounds were detected at 225 nm. Calibration curves were linear over the range 0.4–8 µg/mL for eslicarbazepine acetate and oxcarbazepine, and 0.4–80 µg/mL for each licarbazepine enantiomer. The overall intra- and interday precision and accuracy did not exceed 15%. Mean relative recoveries varied from 94.00 to 102.23% and the limit of quantification of the assay was 0.4 µg/mL for all compounds. This method seems to be a useful tool for clinical research and therapeutic drug monitoring of eslicarbazepine acetate and its metabolites *S*-licarbazepine, *R*-licarbazepine and oxcarbazepine. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: eslicarbazepine acetate (BIA 2-093); oxcarbazepine; human plasma; bioanalytical method validation; enantioselective HPLC-UV method

INTRODUCTION

Until the last decade of the twentieth century, epileptic seizures were still managed by antiepileptic drugs (AEDs) such as carbamazepine (CBZ), phenytoin, valproic acid, ethosuximide, phenobarbital and some benzodiazepines (Levert *et al.*, 2002). However, since the beginning of the 1990s, an explosion in knowledge about the neurobiology of epileptogenesis has taken place and several new AEDs have been introduced in clinical practice, such as vigabatrin, zonisamide, lamotrigine, gabapentin, felbamate, topiramate, tiagabine, oxcarbazepine (OXC) and levetiracetam (Bazil and Pedley, 1998; Ducan, 2002). Despite the currently available therapeutic arsenal of old and new AEDs, about 30% of epileptic patients are still not seizure-free, and

therefore, there is a substantial need to develop more effective drugs simultaneously with less potential for drug interactions and side effects (Deckers *et al.*, 2003; Bialer, 2006).

Eslicarbazepine acetate (ESL) [*S*-(−)-10-acetoxy-10,11-dihydro-5H-dibenz/b,f/azepine-5-carboxamide], previously known as BIA 2-093, is a novel chiral drug presently completing phase III clinical trials, as add-on therapy in refractory partial epilepsy, and undergoing phase II clinical trials, as monotherapy in partial epilepsy and in bipolar disorder (Almeida and Soares-da-Silva, 2007). ESL is chemically related to CBZ and OXC (Benes *et al.*, 1999). CBZ is a very effective first-generation AED that is used as a major first-line drug for partial and generalized tonic–clonic seizures (Arroyo and Kramer, 2001; Coppola, 2004). In humans, the major metabolic pathway of CBZ is oxidation to carbamazepine-10,11-epoxide, which seems to be responsible for several unwanted adverse effects and CBZ-related drug interactions resulting from the induction of hepatic microsomal cytochrome P450 enzymes (Myllynen *et al.*, 1998; Arroyo and Kramer, 2001; Coppola, 2004). OXC is a second-generation CBZ, presenting a similar spectrum of anticonvulsant activity but showing a more favourable tolerability profile (Reinikainen *et al.*, 1987;

WILEY InterScience®
DISCOVER SOMETHING GREAT

Schachter, 1999). Chemically, OXC differs from CBZ by a keto-group in the tenth position. Therefore, in humans, the achiral prodrug OXC is rapidly reduced in liver by cytosolic arylketone reductases to the pharmacologically active metabolite licarbazepine or 10-hydroxy-carbazepine (May *et al.*, 2003; Flesch, 2004). Nevertheless, OXC first-pass reduction to licarbazepine is stereoselective, appearing in plasma as *S*-licarbazepine (*S*-LC) and *R*-licarbazepine (*R*-LC) in the enantiomeric ratio of approximately 4:1 (Flesch *et al.*, 1992; Volosov *et al.*, 1999). In fact, it is well known that enantiomers of chiral drugs may have different pharmacodynamic, pharmacokinetic and toxicological effects in biological systems (Misl'anová and Hutta, 2003). Accordingly, the enantiomeric dilution of the active metabolite of OXC is a negative aspect and the development of a single enantiomer (eutomer) or the manipulation of the enantiomeric ratio could be performed in order to optimize the therapeutic index. Thus, ESL, a third-generation CBZ or second-generation OXC, was specifically designed to circumvent its further biotransformation to toxic metabolites, such as epoxides, and to avoid enantiomeric impurity and unnecessary production of enantiomers or diastereoisomers of its metabolites, without losing anticonvulsant potency (Benes *et al.*, 1999; Hainzl *et al.*, 2001). As a result, ESL exhibits a 'cleaner' metabolism and higher enantiomeric purity than OXC (Hainzl *et al.*, 2001). Previous reports indicated that ESL is extensively metabolized to *S*-LC (95–98%) and to a minor extent to *R*-LC and OXC (Almeida and Soares-da-Silva 2004; Almeida *et al.*, 2005).

Over the years, several chromatographic methods for OXC and racemic licarbazepine determination have been developed (Noirfalise and Collinge, 1983; Menge *et al.*, 1987; Elyas *et al.*, 1990; Rouan *et al.*, 1994; Pienimaki *et al.*, 1995; Souppart *et al.*, 2001; Levert *et al.*, 2002; Mandrioli *et al.*, 2003; Franceschi and Furlanut, 2005; Lanckmans *et al.*, 2006). However, only three chiral HPLC assays have been validated to determine *S*- and *R*-LC enantiomers, in which a very expensive column and mobile phase were employed (Flesch *et al.*, 1992; Pichini *et al.*, 1995; Volosov *et al.*, 2000). ESL and its metabolites *S*-LC, *R*-LC and OXC have also been previously evaluated by a chiral HPLC-MS method, but it was not completely described (Hainzl *et al.*, 2001; Almeida *et al.*, 2005).

As ESL is a new drug in final phase of clinical trials, in our opinion it would be essential to develop and validate a simple chiral HPLC-UV method for simultaneous and stereoselective determination of ESL and its main metabolites in human plasma. Moreover, considering that epilepsy is a neurological disease with high prevalence and high incidence in patients younger than 1 year and older than 75 years (Arroyo and Kramer, 2001; Jarrar and Buchhalter, 2003), the availability of an easy-to-use HPLC-UV method is also imperative to

achieve individualized dosage regimens and to improve the overall clinical outcomes by monitoring plasma drug concentrations in these special populations (Perucca, 2000; Johannessen *et al.*, 2003; Neels *et al.*, 2004).

The purpose of this paper is to describe an accurate, sensitive and practical enantioselective HPLC-UV assay to quantify ESL and its metabolites in human plasma, within a wide concentration range, which could be applied not only to clinical research but also to therapeutic drug monitoring.

EXPERIMENTAL

Chemicals and reagents. Standards of ESL (BIA 2-093, lot number 0000012976, 100% pure by HPLC), *S*-LC (BIA 2-194, lot number PC020131B, 99.79% pure by HPLC), *R*-LC (BIA 2-195, lot number PC040414, 100% pure by HPLC), OXC (lot number 97.12.17, >98% pure by HPLC) and BIA 2-265 (used as internal standard, lot number PC050704, 97.4% pure by HPLC) were kindly supplied by BIAL (Porto, Portugal; Fig. 1). Methanol (HPLC grade, SDS), water milli-Q (HPLC grade, >15 MΩ, home-made), acetonitrile, ethyl acetate, sodium dihydrogen phosphate dihydrate, di-sodium hydrogen phosphate dehydrate and hydrochloric acid fuming 37% were purchased from Merck KGaA (Darmstadt, Germany). Drug-free human plasma from healthy donors was kindly provided by the Portuguese Blood Institute after the written consent of each subject.

Chromatographic apparatus and conditions

The HPLC system used for analysis was composed of a BAS-480 liquid chromatograph equipped with a PM-80 pump, a Rheodyne manual injector with a 20 μL loop, a BAS UV-116 UV–vis detector, a BAS LC-22C temperature controller, a BAS DA-5 chromatography control and a data system interface (all from Bioanalytical Systems, West Lafayette, IN, USA). Data collection and integration were achieved by means of BAS Chromgraph Control and Chromgraph Report software version 2.30.

The chromatographic separation of all four drugs and internal standard was carried out at 30°C by isocratic elution with water–methanol (88:12, v/v), at a flow rate of 0.7 mL/min, on a LiChroCART 250-4 ChiraDex (β-cyclodextrin, 5 μm) column protected by a LiChroCART 4-4 ChiraDex (β-cyclodextrin, 5 μm) guard column purchased from Merck KGaA (Darmstadt, Germany). The mobile phase was filtered through a 0.45 μm filter and degassed ultrasonically for 15 min before use. A sample volume of 20 μL was injected and the analytes were detected at 225 nm.

Stock solutions, calibration standards and quality control samples

The stock solutions of ESL (2 mg/mL), *S*-LC (10 mg/mL), *R*-LC (10 mg/mL), OXC (2 mg/mL) and internal standard (1 mg/mL) were prepared by dissolving appropriate amounts of each compound in acetonitrile. These solutions were
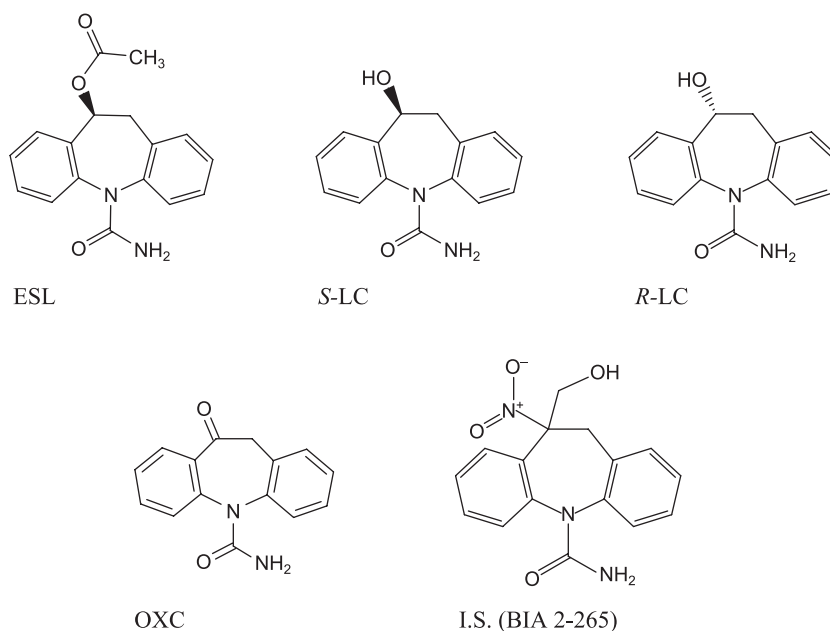
Figure 1. Chemical structures of eslicarbazepine acetate (ESL), *S*-licarbazepine (*S*-LC), *R*-licarbazepine (*R*-LC), oxcarbazepine (OXC) and BIA 2-265 used as internal standard (IS).

adequately diluted with acetonitrile in order to give *S*-LC and *R*-LC 2 mg/mL solutions and ESL, *S*-LC, *R*-LC and OXC 200 μg/mL solutions. The stock and working solutions were used appropriately to afford five combined spiking solutions of all four drugs with final concentrations of 10, 50, 500, 1000 and 2000 μg/mL for *S*-LC and *R*-LC and 10, 20, 50, 100 and 200 μg/mL for ESL and OXC. An internal standard working solution of 200 μg/mL was prepared by diluting appropriately the respective stock solution. All standard solutions were stored at approximately 4°C and protected from light for one month, except the internal standard working solution, which was prepared daily.

Calibration standards at 0.4, 0.8, 2, 4 and 8 μg/mL for ESL and OXC and at 0.4, 2, 20, 40 and 80 μg/mL for *S*-LC and *R*-LC were prepared by adding known amounts of the appropriate combined spiking solutions to blank human plasma. Quality control (QC) samples were prepared independently in the same matrix (blank human plasma).

Extraction procedure

Aliquots of human plasma (250 μL) were added to 750 μL of 0.1 M sodium phosphate buffer (pH 5) spiked with 10 μL of the internal standard working solution. The samples were vortex-mixed and loaded into Oasis® HLB (30 mg, 1 mL) cartridges (Waters, Milford, MA, USA), which were previously conditioned with 1 mL of methanol, 1 mL of acetonitrile and 1 mL of water–acetonitrile (95:5, v/v). After sample elution, the loaded cartridges were submitted to −30 kPa and washed twice with 1 mL of water and twice with 1 mL of water–acetonitrile (95:5, v/v). After drying the sorbent under airflow for 5 min, the analytes were eluted with 1 mL of ethyl acetate under gentle vacuum and then the cartridges were dried for 30 s at −30 kPa. The eluates were evaporated to

dryness under a nitrogen stream at 45°C and the residues reconstituted in 100 μL of HPLC mobile phase, vortexed for approximately 30 s and placed in an ultrasonic bath at room temperature for approximately 1 min. Following this, the reconstituted extracts were transferred to 0.22 μm Spin-X centrifugal filters, centrifuged at 13,400 rpm for 2 min and 20 μL of the final filtered samples were injected onto the HPLC system.

Method validation

The method was validated according to the general recommendations published in the last few years concerning to bioanalytical method validation and acceptance criteria for validation parameters: selectivity, linearity, sensitivity, precision, accuracy, sample dilution, recovery and stability (Shah *et al.*, 2000; Peters and Maurer, 2002).

Method selectivity. The extracts of blank human plasma samples from six different subjects were tested for endogenous interferences. Furthermore, solutions of several commonly co-prescribed AEDs such as phenobarbital, phenytoin, carbamazepine, valproic acid, felbamate, gabapentin, vigabatrin, lamotrigine, topiramate and levetiracetam, and solutions of non-AED cotherapies including antidepressants (dothiepin, mirtazapine, sertraline, trazodone, escitalopram and paroxetine), anxiolytics or hypnotics (diazepam, cloxazolam, lorazepam and ethyl loflazepate) and antipsychotics (risperidone, olanzapine, chlorpromazine and zuclopenthixol) were also injected to check for drugs which could potentially interfere with eslicarbazepine acetate and its metabolites.

Calibration curve. The linearity of the analytical method was assessed by using calibration standards at five different

concentrations within the range of 0.4–8 µg/mL for ESL and OXC and 0.4–80 µg/mL for *R*-LC and *S*-LC. Calibration curves were constructed by plotting drug–internal standard peak height ratio as a function of the respective concentrations in the calibration samples. The data were subjected to a weighted linear regression analysis using $1/x^2$ as weighting factor, which was chosen taking the plots and the sums of absolute percentage relative error into account (Almeida *et al.*, 2002). The sensitivity was evaluated by determining the limit of quantification (LOQ), which is defined as the lowest concentration of the calibration curve that can be measured with acceptable inter- and intraday precision and accuracy, assessed respectively by the coefficient of variation (CV) and the deviation from nominal value (bias) within 20%. The limit of detection (LOD), defined as the lowest concentration that can be distinguished from the noise level, was determined for ESL and its metabolites by analysing plasma samples with known concentrations, after successive dilutions, and was established by visual evaluation of the minimum level at which the analytes can be reliably detected.

Precision and accuracy. Intra- and interday precision and accuracy were assessed by using QC samples in replicate at three different concentration levels representative of all calibration ranges (*n* = 5). The concentrations tested were 0.4, 4 and 8 µg/mL for ESL and OXC and 0.4, 40 and 80 µg/mL for each licarbazepine enantiomer. The acceptance criterion, for precision, was a CV within 15% (or 20% in the LOQ) and, for accuracy, a bias within 15% (or 20% in the LOQ).

Sample dilution. Dilution effect (1:10) was also investigated with QC samples at 40 µg/mL for ESL and OXC and at 400 µg/mL for *R*-LC and *S*-LC to ensure that plasma samples exceeding the highest concentration of the calibration range could be diluted with blank human plasma and accurately quantified. The precision and accuracy of the diluted QC samples were determined in both intra- and interday assays (*n* = 5).

Recovery. The relative recovery from human plasma was calculated at 0.4, 4 and 8 µg/mL for ESL and OXC and at 0.4, 40 and 80 µg/mL for licarbazepine enantiomers (*n* = 5). The absolute recovery of the internal standard was also evaluated at the concentration used in sample analysis. The relative recoveries of the analytes were calculated by comparing the analyte–internal standard peak height ratios for extracted samples with the corresponding ratios obtained with non-extracted standards that represent 100% of recovery. The absolute recovery of the internal standard was also determined by calculating the peak height ratio of the internal standard in extracted samples and non-extracted standards.

Stability. Human plasma stability of ESL and its metabolites was assessed, at low and high concentration levels, by 24 h at 4°C and 30 days at –30°C to simulate sample handling and storage time in the freezer before analysis (*n* = 5). Stability was assessed by comparing the data of QC samples analyzed before (reference samples) and after being exposed to the conditions for stability assessment (stability samples). The stability sample/reference sample ratios of 85–115% were accepted as stability criterion.

RESULTS

Chromatographic separation and selectivity

The separation of *R*-LC, *S*-LC, OXC, ESL and internal standard in spiked human plasma was achieved successfully using the chromatographic conditions and the solid-phase extraction (SPE) procedure previously described. Under these analytical conditions the last-eluting analyte was ESL, with a retention time of approximately 24 min, and the order of elution was internal standard, *R*-LC, *S*-LC, OXC and ESL, respectively.

Representative chromatograms of the extracts of blank and spiked human plasma samples are shown in Fig. 2. The lack of response in blank human plasma samples at internal standard and analytes retention times was demonstrated at 225 nm. Likewise, none of the possibly co-prescribed drugs or commonly prescribed AED cotherapies tested interfered with the internal standard or analytes.

Calibration curve

The calibration curves obtained in human plasma from five calibration standards were linear for ESL and OXC in the range of 0.4–8 µg/mL and for licarbazepine enantiomers in the range of 0.4–80 µg/mL. Respectively, the regression equations of calibration curves were $y = 0.0547x + 0.0005$ ($r^2 = 0.993$) for ESL, $y = 0.1609x - 0.0061$ ($r^2 = 0.998$) for OXC, $y = 0.1320x - 0.0005$ ($r^2 = 0.998$) for *R*-LC and $y = 0.1086x - 0.0009$ ($r^2 = 0.998$) for *S*-LC. These results demonstrated a good linearity ($r^2 > 0.99$) between peak height ratios and concentrations. The LOQ of the assay was set at 0.4 µg/mL for all analytes with acceptable precision and accuracy (Table 1), and the LOD was 0.1 µg/mL for ESL and 0.04 µg/mL for OXC, *R*-LC and *S*-LC.

Precision and accuracy

Precision and accuracy data for intra and interday QC plasma samples are presented in Table 1. The overall intra- and interday CV value was ≤9.25%. Intra- and interday bias varied between –0.56 and 4.98%. These data indicated that the developed HPLC method is reproducible and accurate, given that neither CV nor bias exceeded 15% (or 20% in the LOQ), in agreement with literature recommendations. The precision and accuracy of the diluted plasma samples varied from 0.89 to 2.63% and from –1.96 to 0.38%, respectively. These results revealed that a 10-fold dilution with blank human plasma can be rightly applied if the concentration of a trial sample exceeds the highest concentration of the calibration curve.
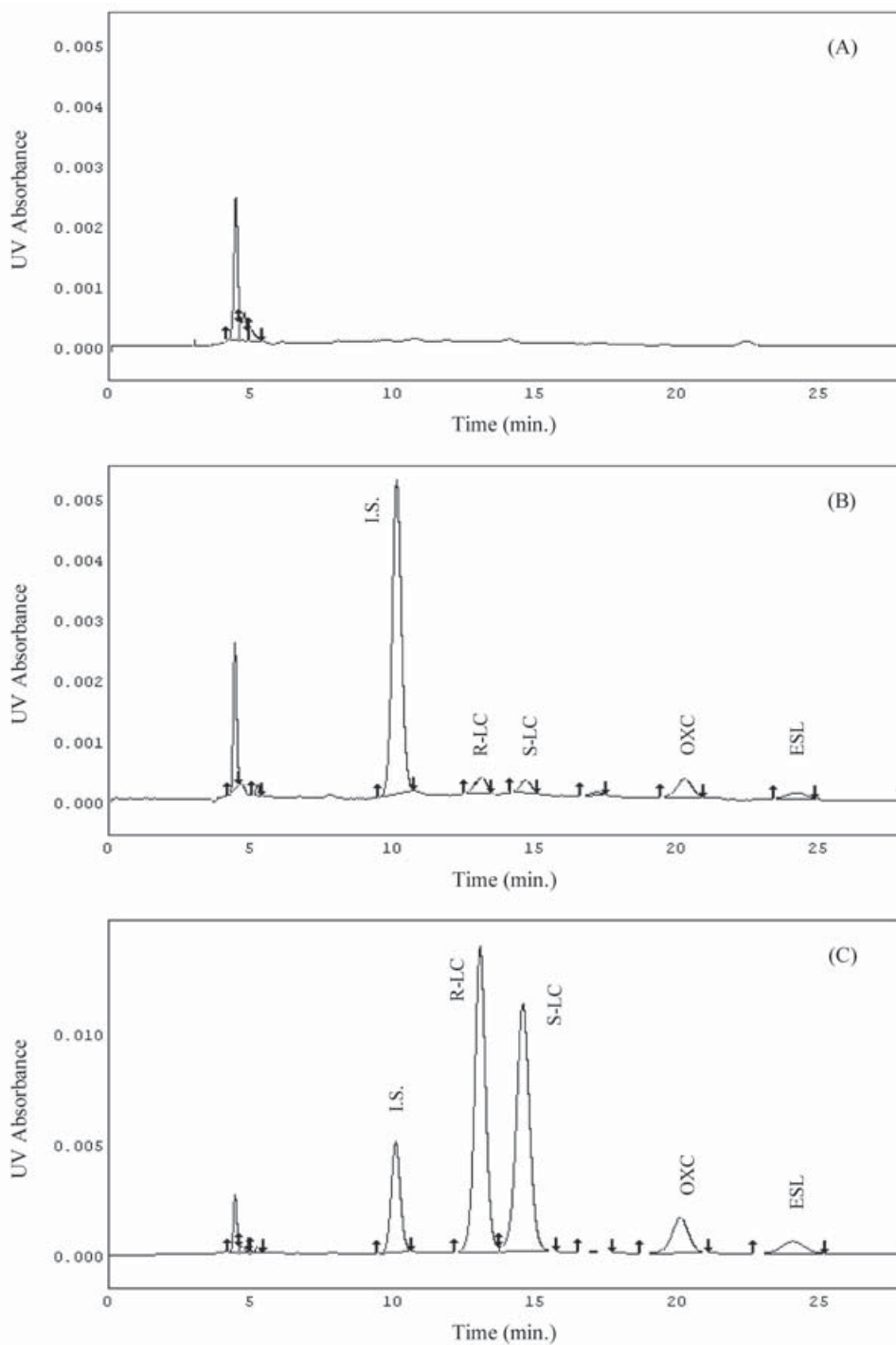
Figure 2. Typical chromatograms of extracted human plasma: (A) blank human plasma; (B) human plasma spiked with internal standard (IS) (concentration = 8 μg/mL), *R*-licarbazepine (*R*-LC), *S*-licarbazepine (*S*-LC), oxcarbazepine (OXC) and eslicarbazepine acetate (ESL) (concentration = 0.4 μg/mL); (C) human plasma spiked with IS (concentration = 8 μg/mL), *R*-LC and *S*-LC (concentration = 20 μg/mL), OXC and ESL (concentration = 2 μg/mL); (D) human plasma spiked with IS (concentration = 8 μg/mL), *R*-LC and *S*-LC (concentration = 80 μg/mL), OXC and ESL (concentration = 8 μg/mL).
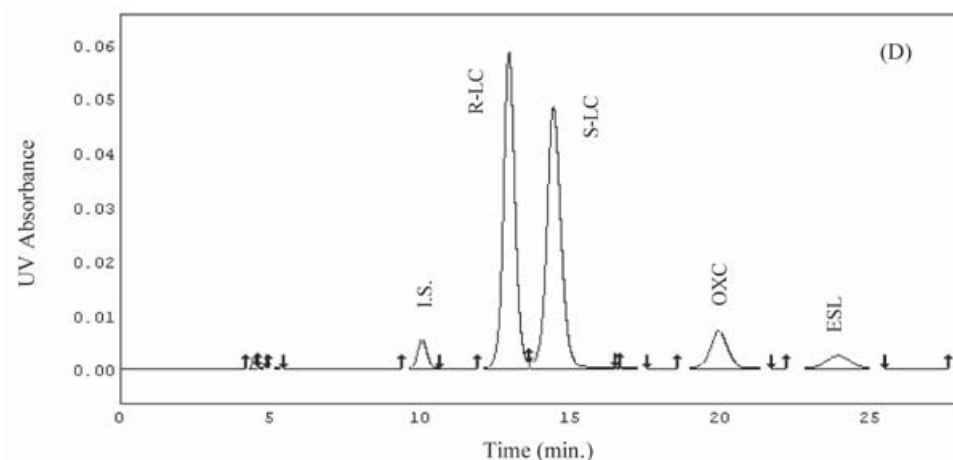
Figure 2. (*Continued*)

Table 1. Intra- and interday precision (CV, %) and accuracy (bias, %) for the simultaneous determination of eslicarbazepine acetate (ESL), oxcarbazepine (OXC), S-licarbazepine (*S*-LC) and *R*-licarbazepine (*R*-LC) in human plasma (*n* = 5)

	Assay							
	Intraday				Interday			
$C_{Nominal}$[a]	CV %	Bias %	CV %	Bias %	CV %	Bias %	CV %	Bias %
Plasma	ESL		OXC		ESL		OXC	
0.4	5.52	1.65	6.22	2.53	9.25	1.23	2.63	1.70
4	1.24	0.39	1.65	0.36	2.86	−0.56	1.20	−0.54
8	1.91	3.42	2.16	4.05	0.98	1.97	0.91	3.60
	S-LC		*R*-LC		*S*-LC		*R*-LC	
0.4	4.10	1.18	3.21	1.02	6.26	0.11	4.22	0.49
40	1.92	0.76	1.85	0.50	2.57	−0.38	2.60	−0.09
80	1.99	4.98	1.98	4.22	0.97	3.29	1.00	3.05

[a] Nominal concentration (μg/mL); CV, coefficient of variation.

Recovery

The relative drug recoveries from human plasma are presented in Table 2. The mean relative recoveries, taking ESL and its metabolites into account, ranged from 92.36 to 102.85%. The absolute recovery of the internal standard was 84.99%, with a CV of 2.78%. All average recoveries calculated are high and reproducible, either for analytes or internal standard.

Table 2. Relative recovery (%) of eslicarbazepine acetate (ESL), oxcarbazepine (OXC), *S*-licarbazepine (*S*-LC) and *R*-licarbazepine (*R*-LC) in human plasma

		Relative recovery			
$C_{Nominal}$[a]	*n*	Mean (%)	CV (%)	Mean (%)	CV (%)
Plasma		ESL		OXC	
0.4	5	90.10	8.06	85.78	3.27
4	5	100.00	1.87	94.36	1.71
8	5	101.16	1.98	96.93	2.26
	15	97.09	6.67	92.36	5.71
		S-LC		*R*-LC	
0.4	5	97.36	5.38	102.09	4.71
40	5	104.09	1.38	104.99	1.24
80	5	101.57	1.33	101.49	1.30
	15	101.01	4.22	102.85	3.25

[a] Nominal concentration (μg/mL); *n*, number of samples; CV, coefficient of variation.

Stability

From the stability data obtained at 0.8 and 4 µg/mL for ESL and OXC and at 2 and 40 µg/mL for *S*-LC and *R*-LC no significant loss was observed when spiked plasma samples were stored for 24 h at 4°C or for 1 month at −30°C. Thus, ESL, OXC and both licarbazepine enantiomers were revealed to be stable in the assayed conditions.

DISCUSSION

The present paper describes the first reversed-phase HPLC-UV-SPE chiral assay fully validated to quantify ESL and its main metabolites *S*-LC, *R*-LC and OXC in human plasma. This HPLC method appears to be accurate, precise and sensitive in assessing ESL and its metabolites in human plasma, but its major advantages involve the very simple chromatographic conditions. Unlike the methods previously published by Flesch *et al.* (1992), Pichini *et al.* (1995) and Volosov *et al.* (2000) concerning to independent quantification of licarbazepine enantiomers, this enantioselective assay was carried out on reversed-phase using a safe and economical mobile phase essentially composed of water. In the preceding methods, the analysis took place on normal-phase chromatographic columns using more expensive, pollutant and toxic mobile phases, such as *n*-hexane:2-propanol, *n*-hexane:ethanol or *n*-hexane:ethanol:2-propanol. Besides the specific chromatographic conditions, the SPE procedure used here in sample pretreatment offers many other benefits over the traditional liquid–liquid extraction methods, such as faster sample preparation, lower cost, greater recoveries, less sample handling, improved safety and easy automation (Soriano *et al.*, 2001; Camel, 2003). Even though ESL and its metabolites could be analyzed using MS detection systems (Hainzl *et al.*, 2001; Almeida *et al.*, 2005), most hospitals and laboratories are not equipped with such high-cost instruments (Velpandian *et al.*, 2004; Janchawee *et al.*, 2007). Therefore, the availability of the current method will be important and it may be easily adopted to perform clinical studies. Among the several strategies to separate enantiomers, the enantiomeric resolution of *S*-LC and *R*-LC was achieved by the easiest of them, direct HPLC using a chiral stationary phase composed of *β*-cyclodextrins bounded covalently to silica gel.

Thus, the present method seems to be a useful tool to individually assess the pharmacologically active metabolites licarbazepine enantiomers and their pro-drugs ESL and OXC. The wide working range tested, as well as all the other advantages previously referred to, will allow its application not only in the support of clinical trials but also in the routine therapeutic drug monitoring assays or in bioequivalence studies that involve the new antiepileptic drug ESL and the already marketed OXC.

REFERENCES

Almeida AM, Castel-Branco MM and Falcão AC. Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. *Journal of Chromatography B* 2002; **774**: 215–222.

Almeida L and Soares-da-Silva P. Safety, Tolerability, and pharmacokinetic profile of BIA 2-093, a novel putative antiepileptic, in a rising multiple-dose study in young healthy humans. *Journal of Clinical Pharmacology* 2004; **44**: 906–918.

Almeida L and Soares-da-Silva P. Eslicarbazepine acetate (BIA 2-093). *Neurotherapeutics* 2007; **4**: 88–96.

Almeida L, Falcão A, Maia J, Mazur D, Gellert M and Soares-da-Silva P. Single-dose and steady-state pharmacokinetics of eslicarbazepine acetate (BIA 2-093) in healthy elderly and young subjects. *Journal of Clinical Pharmacology* 2005; **45**: 1062–1066.

Arroyo S and Kramer G. Treating Epilepsy in the Elderly: Safety Considerations. *Drug Safety* 2001; **24**: 991–1015.

Bazil CW and Pedley TA. Advances in medical treatment of epilepsy. *Annual Review of Medicine* 1998; **49**: 135–162.

Benes J, Parada A, Figueiredo AA, Alves PC, Freitas AP, Learmonth DA, Cunha RA, Garrett J and Soares-da-Silva P. Anticonvulsant and sodium channel-blocking properties of novel 10,11-dihydro-5h-dibenz[b,f]azepine-5-carboxamide derivatives. *Journal of Medicinal Chemistry* 1999; **42**: 2582–2587.

Bialer M. New antiepileptic drugs that are second generation to existing antiepileptic drugs. *Expert Opinion on Investigational Drugs* 2006; **15**: 637–647.

Camel V. Solid phase extraction of trace elements. *Spectrochimica Acta Part B* 2003; **58**: 1177–1233.

Coppola G. Treatment of partial seizures in childhood: an overview. *CNS Drugs* 2004; **18**: 133–156.

Deckers CLP, Genton P, Sills GJ and Schmidt D. Current limitations of antiepileptic drug therapy: a conference review. *Epilepsy Research* 2003; **53**: 1–17.

Duncan JS. The promise of new antiepileptic drugs. *British Journal of Clinical Pharmacology* 2002; **53**: 123–131.

Elyas AA, Goldberg VD and Patsalos PN. Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbazepine. *Journal of Chromatography* 1990; **528**: 473–479.

Flesch G. Overview of the clinical pharmacokinetics of oxcarbazepine. *Clinical Drug Investigation* 2004; **24**: 185–203.

Flesch G, Francotte E, Hell F and Degen PH. Determination of the *R*-(−) and *S*-(+) enantiomers of the monohydroxylated metabolite of oxcarbazepine in human plasma by enantioselective high-performance liquid chromatography. *Journal of Chromatography* 1992; **581**: 147–151.

Franceschi L and Furlanut M. A simple method to monitor plasma concentrations of oxcarbazepine, carbamazepine, their main metabolites and lamotrigine in epileptic patients. *Pharmacological Research* 2005; **51**: 297–302.

Hainzl D, Parada A and Soares-da-Silva P. Metabolism of two new antiepileptic drugs and their principal metabolites *S*(+)- and *R*(−)-10,11-dihydro-10-hydroxy carbamazepine. *Epilepsy Research* 2001; **44**: 197–206.

Janchawee B, Keawpradub N, Chittrakarn S, Prasettho S, Wararatananurak P and Sawangjareon K. A high-performance liquid chromatographic method for determination of mitragynine in serum and its application to a pharmacokinetic study in rats. *Biomedical Chromatography* 2007; **21**: 176–183.

Jarrar RG and Buchhalter JR. Therapeutics in pediatric epilepsy, part 1: the new antiepileptic drugs and the ketogenic diet. *Mayo Clinic Proceedings* 2003; **78**: 359–370.

Johannessen SI, Battino D, Berry DJ, Bialer M, Kramer G, Tomson T and Patsalos PN. Therapeutic Drug Monitoring of the Newer Antiepileptic Drugs. *Therapeutic Drug Monitoring* 2003; **25**: 347–363.

Lanckmans K, Clinckers R, Eeckhaut AV, Sarre S, Smolders I and Michotte Y. Use of microbore LC-MS/MS for the quantification of oxcarbazepine and its active metabolite in rat brain microdialysis samples. *Journal of Chromatography B* 2006; **831**: 205–212.

Levert H, Odou P and Robert H. Simultaneous determination of four antiepileptic drugs in serum by high-performance liquid chromatography. *Biomedical Chromatography* 2002; **16**: 19–24.

Mandrioli R, Ghedini N, Albani F, Kenndler E and Raggi MA. Liquid chromatographic determination of oxcarbazepine and its metabolites in plasma of epileptic patients after solid-phase extraction. *Journal of Chromatography B* 2003; **783**: 253–263.

May TW, Korn-Merker E and Rambeck B. Clinical pharmacokinetics of oxcarbazepine. *Clinical Pharmacokinetics* 2003; **42**: 1023–1042.

Menge GP, Dubois JP and Bauer G. Simultaneous determination of carbamazepine, oxcarbazepine and their main metabolites in plasma by liquid chromatography. *Journal of Chromatography* 1987; **414**: 477–483.

Misl'anová C and Hutta M. Role of biological matrices during the analysis of chiral drugs by liquid chromatography. *Journal of Chromatography B* 2003; **797**: 91–109.

Myllynen P, Pienimaki P, Raunio H and Vahakangas K. Microsomal metabolism of carbamazepine and oxcarbazepine in liver and placenta. *Human & Experimental Toxicology* 1998; **17**: 668–676.

Neels HM, Sierens AC, Naelaerts K, Scharpé SL, Hatfield GM and Lambert WE. Therapeutic drug monitoring of old and newer anti-epileptic drugs. *Clinical Chemistry and Laboratory Medicine* 2004; **42**: 1228–1255.

Noirfalise A and Collinge A. Quantitative determination of oxcarbazepine. *Journal of Chromatography* 1983; **274**: 417–420.

Perucca E. Is there a role for therapeutic drug monitoring of new anticonvulsants? *Clinical Pharmacokinetics* 2000; **38**: 191–204.

Peters FT and Maurer HH. Bioanalytical method validation and its implications for forensic and clinical toxicology—a review. *Accreditation and Quality Assurance* 2002; **7**: 441–449.

Pichini S, Altieri I, Passa AR, Zuccaro P and Pacifi R. Stereoselective bioanalysis of oxcarbazepine and the enantiomers of its metabolites by high-performance liquid chromatography. *Journal of Liquid Chromatography* 1995; **18**: 1533–1541.

Pienimaki P, Fuchs S, Isojarvi J and Vahakangas K. Improved detection and determination of carbamazepine and oxcarbazepine and their metabolites by high-performance liquid chromatography. *Journal of Chromatography B* 1995; **673**: 97–105.

Reinikainen KJ, Keranen T, Halonen T, Komulainen H and Riekkinen PJ. Comparison of oxcarbazepine and carbamazepine: a double-blind study. *Epilepsy Reseach* 1987; **1**: 284–289.

Rouan MC, Decherf M, Le Clanche V, Lecaillon JB and Godbillon J. Automated microanalysis of oxcarbazepine and its monohydroxy and transdiol metabolites in plasma by liquid chromatography. *Journal of Chromatography B* 1994; **658**: 167–172.

Schachter SC. Oxcarbazepine: current status and clinical applications. *Expert Opinion on Investigational Drugs* 1999; **8**: 1103–1112.

Shah VP, Midha KK, Findlay JWA, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT and Yacobi A. Bioanalytical method validation—a revisit with a decade of progress. *Pharmaceutical Research* 2000; **17**: 1551–1557.

Soriano T, Jurado C, Menéndez M and Repetto M. Improved solid-phase extraction method for systematic toxicological analysis in biological fluids. *Journal of Analytical Toxicology* 2001; **25**: 137–143.

Souppart C, Decherf M, Humbert H and Maurer G. Development of a high throughput 96-well plate sample preparation method for the determination of trileptal (oxcarbazepine) and its metabolites in human plasma. *Journal of Chromatography B* 2001; **762**: 9–15.

Velpandian T, Mathur R, Agarwal NK, Arora B, Kumar L and Gupta SK. Development and validation of a simple liquid chromatographic method with ultraviolet detection for the determination of imatinib in biological samples. *Journal of Chromatography B* 2004; **804**: 431–434.

Volosov A, Xiaodong S, Perucca E, Yagen B, Sintov A and Bialer M. Enantioselective pharmacokinetics of 10-hydroxycarbazepine after oral administration of oxcarbazepine to healthy Chinese subjects. *Clinical Pharmacology & Therapeutics* 1999; **66**: 547–553.

Volosov A, Bialer M, Xiaodong S, Perucca E, Sintov A and Yagen B. Simultaneous stereoselective high-performance liquid chromatographic determination of 10-hydroxycarbazepine and its metabolite carbamazepine-10,11-*trans*-dihydrodiol in human urine. *Journal of Chromatography B* 2000; **738**: 419–425.