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Methods for the determination of organochlorine pesticide residues in human serum

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Abstract

The effectiveness of solid-phase extraction with Florisil for the determination of 12 organochlorine pesticide residues from human serum was examined. Recoveries greater than 84% and coefficients of variation better than 19% were obtained. Others methods, such as column partition and matrix solid-phase dispersion, were compared. The better method provides quantification limits ranging from 1.08 μg/l for γ-HCH and 37.5 μg/l for *p,p′*-DDT when capillary gas–liquid chromatography with electron-capture detection is used for the final determination.

Keywords: Organochlorine pesticides

1. Introduction

The investigation of organochlorine (OC) residues in human serum requires a study of validation methods which present adequate accuracy and precision.

Most papers reporting the determination of OC residues in human serum are based on the method of Dale et al. [1]. However, some researchers have used laborious methodologies which employ pre-treatment with a specific solvent, solvent extraction and different types of column adsorption chromatography [2], where columns for clean-up were sometimes used [3]. Although some of the available methodologies are more rapid, they require treatment with sulphuric acid [4], but this degrades some compounds, such as heptachlor epoxide, dieldrin and endosulfan isomers [5,6], or Florisil preparation [7,8]. With other methods [9] the clean-up step is eliminated, raising problems for electron-capture detection (ECD).

The purpose of this investigation was to find a rapid method that permits quantitative and adequate recovery of 12 pesticide residues in human serum: hexachlorocyclohexane (HCH) isomers (α, β, γ), aldrin and dieldrin, heptachlor epoxide (HE), hexachlorobenzene (HCB), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (*p,p′*-DDT), 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane (*o,p′*-DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p′*-DDE) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane (*p,p′*-DDD), and endosulfan sulphate.

Twelve procedures were used based on different extraction solvents and clean-up by solid-phase

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extraction (SPE), column partition, adsorption chromatography, or matrix solid-phase dispersion (MSPD), not using the special C_{18} adsorbent [10–12], but the same adsorbents used for column partition.

To determine the difference between the extraction solvents, *n*-hexane and *n*-hexane–acetone (9+1) were used.

To avoid the extraction phase, column partition and MSPD procedures with 1 g SPE Florisil, 1 g SPE C_{18} and 2 g 2% deactivated Florisil were used.

2. Experimental

2.1. Reagents

Anhydrous sodium sulphate [granulated for residue analysis (Merck)]; *n*-hexane, acetonitrile, acetone, dichloromethane, and iso-octane (Carlo Erba, Milan, Italy) were of pesticide residue grade; water purified via Milli Q (Millipore, Bedford, MA, USA); pesticide standards (Dr. Ehrenstorfer, Augsburg, Germany); elution solvents: *n*-hexane, dichloromethane–*n*-hexane (1+5), and dichloromethane–*n*-hexane (5+5), prepared daily. Florisil (60–100 mesh, Fluka Chemika, USA) was heated to 300°C in a furnace for 3 h, cooled in a desiccator prior to appropriate deactivation with water (2%) and used within the next 48 h.

A standard pesticide mixture consisting of a stock standard solution (approximately 500 mg/ml) of each pesticide was prepared separately in *n*-hexane. A standard working solution (100 mg/ml) was also prepared. Two fortification solutions were also prepared in *n*-hexane. For the first fortification solution (A) concentrations ranged between 0.1 and 1.25 ng/μl, and for the second (B) between 5.0 and 62.5 ng/μl.

2.2. Materials and apparatus

A gas–liquid chromatograph Carlo Erba Mega HRGC 5300 equipped with a ^{63}Ni electron-capture detector was used. Two fused-silica capillary columns, 30 m×0.25 mm I.D., 0.25 μm, with chemically bonded phases DB-5 and DB-17 (J&W Scientific) were used. The first column was used for quantification and the second as confirmation column. For both columns, a 1-μl sample was injected in the splitless mode and the splitter was opened after 60 s. Chromatographic conditions were 280°C for the detector, 220°C for the injector, and 150°C held for 1 min and programmed at 10°C/min to 210°C, held for 1 min and programmed at 3°C/min to 230°C, held for 5 min and finally programmed at 3°C/min to 250°C, held for 3 min for the first column and 10 min for the second column. Gases used were: carrier gas helium N60 carrier at 2 ml/min, split valve 100 ml/min, purge valve 2 ml/min, make-up gas, nitrogen at 120 kPa. A Spectra-Physics 4270 integrator was used for quantification; a centrifuge Model Meditronic (Selecta) was used; the vacuum system for SPE and vacuum pump B-160 were from Vacobox Büchi (Switzerland); Si–C_{18} glass cartridges (6 ml) and Florisil glass cartridges (6 ml) were from J.T. Baker (Deventer, Holland); glass columns, 8×150 mm filled with 2 g 2% deactivated Florisil. A rotary vacuum evaporator (Heidolph VV 2001) was also used.

2.3. Sample preparation

Blood was collected in glass centrifuge tubes (80 ml) and placed in an oven at 37°C for 10 min to clot. Serum was removed with a pipet and placed in 15 ml centrifuge Pyrex tubes.

2.4. Recovery

For recovery, 1 ml of fortification solution A was added to 1 ml of human serum in methods 1, 2, 3, and 4, 20 μl of fortification solution B was used in methods 5–10, and 150 μl of fortification solution B was added in methods 11 and 12, and allowed to stand for 15 min before extraction, for five replications.

2.5. Extraction and clean-up methods

One milliliter of serum sample, placed in 15 ml centrifuge Pyrex tubes, was used for all extraction methods.

2.5.1. Methods 1 and 2

Extraction solvents were *n*-hexane for method 1 and *n*-hexane–acetone (90+10) for method 2.

In these methods, following the addition of 2×5 ml of the respective organic solvents, the sample was shaken for 1 min on a Vortex mixer. The sample was centrifuged at 1520*g* for 5 min. The *n*-hexane (method 1) or *n*-hexane–acetone extracts (method 2) were decanted into 15 ml graduated centrifuge tubes, three drops of iso-octane were added and the solutions concentrated to 2 ml.

Sodium sulphate (1 cm) was added to a Florisil SPE cartridge and the column washed with 10 ml *n*-hexane without drying. The concentrated hexane (method 1) and hexane–acetone (method 2) extracts were transferred to the columns and left to flow under the influence of gravity. Two different eluents were used: E_1 6 ml *n*-hexane and E_2 6 ml *n*-hexane–Cl_2CH_2 (5+1). The eluates were collected in graduated centrifuge tubes, added of three drops of iso-octane, and concentrated to 1 ml for quantification by gas chromatography–electron-capture detection (GC-ECD).

2.5.2. Other methods

For methods 3, 5, 7, 9, and 11, the sample was only homogenized for 1 min on a Vortex mixer before clean-up.

For methods 3 and 5, the homogenized samples were placed directly on top of the SPE Florisil cartridge. The eluents described for methods 1 and 2 were used. The eluates were collected in graduated centrifuge tubes, three drops of iso-octane were added, and the solution concentrated to 1 ml for quantification by GC-ECD.

For method 7, SPE C_{18} cartridges were conditioned and then the homogenized sample was transferred to the column. The vacuum was adjusted to elute the extract at ≤5 ml/min. Pesticide residues were eluted in the same way as described for the other methods.

For methods 9 and 11, adsorption chromatography columns (8×150 mm) filled with glass wool washed with acetone, 2 g 2% deactivated Florisil and 1 cm sodium sulphate were used. The concentrated hexane extracts were transferred to the columns and left to flow under the influence of gravity. The eluents used in method 1, but in greater quantity (10 ml), were used in both methods. Another eluent was also used: E_3 10 ml *n*-hexane–Cl_2CH_2 (5+5).

For methods 4 and 6, 1 ml of serum was placed onto 1 g of Florisil, from the SPE cartridges, contained in a glass mortar. The serum was then gently blended into the Florisil with a glass pestle. The resultant homogeneous adsorbent/serum was transferred into the glass column that contained the adsorbent. A frit was placed on top of the mixture and the column was not overcompressed with a glass rod.

For method 8, the clean-up scheme used in methods 4 and 6 was followed, but 1 g of C_{18}, derived from the SPE cartridges, was used. For methods 10 and 12, the same scheme with 2 g 2% deactivated Florisil was used.

3. Results and discussion

Serum extracts containing the organochlorine pesticide residues were analysed on a high-resolution GC system with DB-5 and DB-17 columns. The first column separated the 12 compounds, but in the second *p,p′*-DDD and *o,p′*-DDT are coeluted.

In order to study the difference between the extraction solvents, *n*-hexane and *n*-hexane–acetone (9+1), we used methods 1 and 2, respectively, because pesticides with different polarities are involved. For both methods, clean-up with 1 g SPE Florisil was performed.

The recovery mean, standard deviation, and coefficients of variation for the different methods are presented in Tables 1 and 2. The data show good recoveries for all compounds when method 1 and, especially, method 2 were used, except for endosulfan sulphate (Table 1 Fig. 1). Comparison of the results shows that the best precision values were obtained with method 2. In this method the accuracy and precision values obtained were acceptable for all compounds, except for endosulfan sulphate where the recovery values were low. The same was observed when SPE clean-up with Florisil cartridges was used for medicinal plants [13] and for linden infusion [14]. This phenomenon may be due to the high polarity of that compound, and perhaps a more polar eluent will be required.

The limits of quantification for serum with method

Table 1
Recovery [mean±RSD (CV) (%) (n=5)] of OCs from fortified blood samples using different methods

Pesticide	Fortification level (μg/ml)	Method					
		1	2	6	8	9	10
α-HCH	0.1	85±10 (11)	87±7 (8)	76	106	150±94 (78)	45±30 (66)
HCB	0.25	78±9 (12)	84±6 (8)	nd	34	38±(10 (25)	2±1 (27)
β-HCH	0.1	80±28 (35)	86±10 (12)	75	358	197±116 (137)	8±10 (20)
γ-HCH	0.15	87±20 (23)	94±9 (9)	47	70	38±12 (15)	3±2 (60)
Aldrin	0.5	78±13 (17)	91±11 (12)	3	67	152±149 (98)	13±10 (78)
Heptachlor epoxide	0.5	90±15 (17)	95±18 (19)	nd	nd	nd	nd
p,p'DDE	1.25	83±17 (20)	86±7 (8)	41	96	54±29 (54)	26±4 (16)
Dieldrin	0.25	99±24 (24)	99±10 (10)	35	59	12±8 (13)	16±8(12)
p,p'DDD	1.25	90±22 (24)	91±7 (7)	107	135	61±43 (71)	4±2 (38)
o,p'DDT	1.25	84±21 (25)	86±8 (9)	77	169	207±247 (120)	22±16 (71)
Endosulfan sulphate	0.25	36±9 (24)	40±(12)	nd	186	57±72 (126)	nd
p,p'DDT	1.25	77±24 (31)	94±10 (10)	109	253	40±43 (106)	20±14 (72)

2 ranged from 1.08 μg/l for γ-HCH and 37.5 μg/l for p,p'-DDT.

To avoid the extraction phase, column partition procedures with 1 g SPE Florisil, 1 g SPE C_{18} and 2 g 2% deactivated Florisil were used after mixing the serum on a Vortex mixer for methods 3, 5, 7, 9, and 11. For methods 3, 5 and 7, SPE columns remained clogged. To avoid column clogging, vacuum was applied in method 9, but very inconsistent results were obtained for all compounds (Table 1). This phenomenon may be due to the formation of an emulsion, but some researchers have reported good recoveries when similar procedures were attempted using C_{18} SPE for other biological fluids such as milk [15]. Very low recovery values were obtained with method 11, ranging from 29% for p,p'-DDE and 57% for α-HCH (Table 2).

Due to the lack of success with methods 3 and 7, MSPD procedures were used. The serum was blended with 1 g Florisil (from the SPE column) in a glass mortar and transferred to the SPE column (methods 4 and 6). Similar procedures were used with SPE–C_{18} for method 8 and with 2 g 2% deactivated Florisil for methods 10 and 12.

Recoveries were not good for HCB, γ-HCH, aldrin, HE, p,p'-DDE and dieldrin with method 6, and for HCB, β-HCH, HE, dieldrin, p,p'-DDD, o,p'-DDT, p,p'-DDT and endosulphan sulphate with method 8 (Table 1).

The best results obtained for method 12 in contrast with method 10 may be due to overcompressing in method 10, giving, in this case, very low recoveries for all compounds (Tables 1 and 2). For method 12, recovery values are low for dieldrin and endosulphan sulphate, and the precision is not good for most compounds (Table 2).

Table 2
Recovery [mean±RSD (CV) (%) (n=5)] of OCs from fortified blood samples using methods 11 and 12

Pesticide	Fortification level (μg/ml)	Method 11	Method 12
α-HCH	0.75	57±3 (5)	76±16 (21)
HCB	1.875	36±6 (18)	79±18 (23)
β-HCH	0.75	50±14 (28)	80±54 (68)
γ-HCH	0.75	54±6 (11)	116±45 (39)
Aldrin	3.75	30±17 (58)	89±28 (32)
p,p'DDE	9.375	29±6 (19)	79±7 (9)
Dieldrin	1.875	31±5 (18)	54±22 (40)
p,p'DDD	9.375	31±4 (14)	81±11 (14)
o,p'DDT	9.375	34±3 (9)	85±17 (19)
Endosulfan sulphate	1.875	35±6 (16)	10±3 (35)
p,p'DDT	9.375	31±4 (14)	87±22 (26)

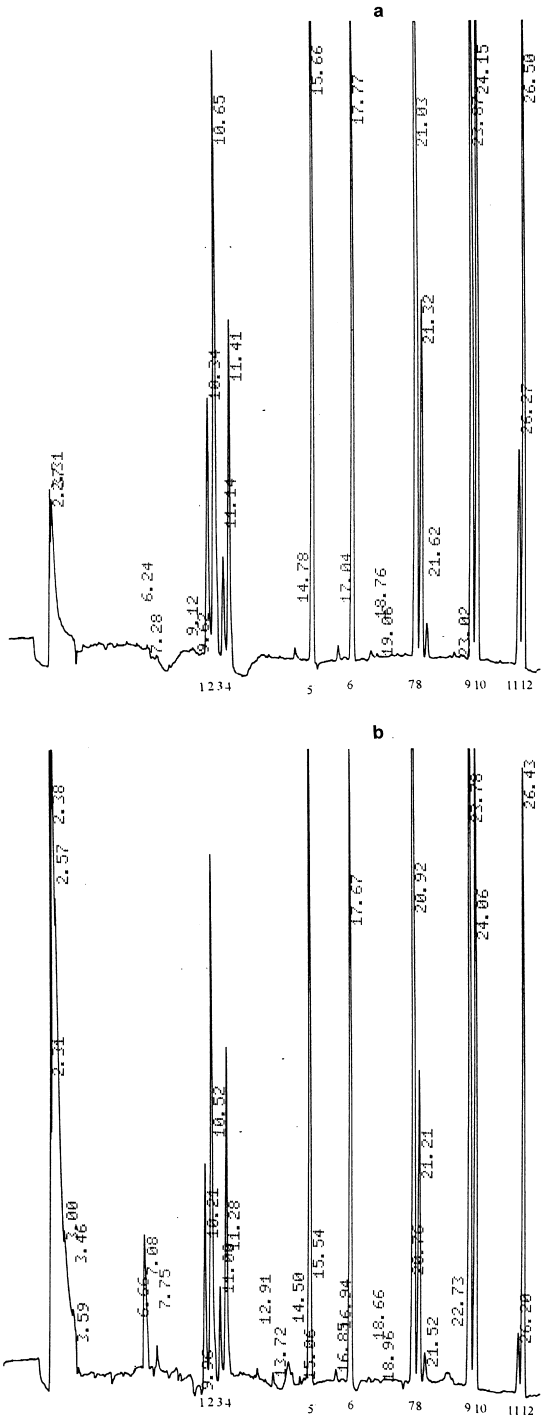

Fig. 1. Representative gas chromatograms (DB-5 column) of method 2: 1 μl fortification solution A (a) and 1 μl spiked serum (b). Peaks: 1=α-HCH; 2=HCB; 3=β-HCH; 4=γ-HCH; 5=aldrin; 6=heptachlor epoxide; 7=*p,p′*DDE; 8=dieldrin; 9=*p,p′*DDD; 10=*o,p′*DDT; 11=endosulfan sulphate; 12=*p,p′*DDT.

Eluate with emulsion and total or partial clogging was observed for methods where column partition (3, 5, 7, and 9) and homogeneous adsorbent/serum (4, 6, 8, and 10) were used.

4. Conclusions

Methods 1 and 2 and the GC procedure described are suitable for multi-residue screening of serum for 11 organochlorine residues, but the results obtained with method 2 show a higher measure of precision than method 1. That method is rapid, simple, and more precise and accurate than the others methods.

Column partition and MSPD procedures were not suitable for the determination of organochlorine residues in serum, presenting bad results for accuracy and/or precision.

The quantity of solvents is small: in extraction procedures only 10 ml *n*-hexane (method 1) or 8 ml *n*-hexane and 2 ml acetone (method 2) are required; SPE Florisil clean-up requires 11 ml *n*-hexane and 1 ml dichloromethane per sample.

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References

[1] W.E. Dale, J.W. Miles, T.B. Gaines, J. Assoc. Off. Anal. Chem. 53 (1970) 1287.
[2] V.W. Burse, S.L. Head, M.P. Korver, P.C. McClure, J.F. Donahue, L.L. Needham, J. Anal. Toxicol. 14 (1990) 137.
[3] M.B. Krawinkel, G. Plehn, H. Kruse, A.M. Kasi, Bull. Environ. Contam. Toxicol. 43 (1989) 821.
[4] B. Krauthacker, Bull. Environ. Contam. Toxicol. 46 (1991) 797.
[5] D.E. Wells, Pure Appl. Chem. 60 (1988) 1437.
[6] J. Mañes, G. Font, Y. Picó, J. Chromatogr. 642 (1993) 195.
[7] J. Gómez-Catalán, J. To-Figueras, J. Planas, M. Rodamilans, J. Corbella, Hum. Toxicol. 6 (1987) 397.
[8] J. Mes, Bull. Environ. Contam. Toxicol. 48 (1992) 815.
[9] US EPA Manual of Analytical Methods for the Pesticides in Human and Environmental Samples, Health Effects Research Laboratory, Research Triangle Park, NC, 1980.

[10] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Assoc. Off. Anal. Chem. 72 (1989) 813.
[11] H.M. Lott, S.A. Barker, Assoc. Off. Anal. Chem. Int. 76 (1993) 663.
[12] S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr. 475 (1989) 353.
[13] C.M. Lino, M.I.N. Silveira, J. Chromatogr. A 769 (1997) 275.
[14] C.M. Lino, M.I.N. Silveira, J. Agric. Food Chem. 45 (1997) 2718.
[15] R. Baracorolo, E. Tealdo, C. Tutta, J. High-Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 539.