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ACCELERATED SOLVENT EXTRACTION METHOD FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN SOIL

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This paper describes a fast and simple method for the determination of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in soil samples using accelerated solvent extraction (ASE). Spiked soil samples were extracted by ASE using a mixture of hexane/acetone (3/1, *v/v*). The extracts were cleaned on silica impregnated with concentrated sulfuric acid and the final analysis was performed with GC-ECD and GC-MS. The accuracy of the method was assessed through analysis of a certified reference material (CRM-481) contaminated with PCBs. The method limits of quantification ranged between 0.1 - 1.8 ng/g soil and were dependent on the levels of analytes in the procedural blanks. The average recoveries of OCPs and PCBs in spiked soil (range 66 - 149%) were considered satisfactory. The procedure was applied to 18 soil samples from Moldavia province in which DDT and analogues were the major contaminants.

INTRODUCTION

The need to reduce the amounts of hazardous organic solvents used in analytical extraction has contributed in the last years to the development of new extraction techniques.[1] One of such techniques is the accelerated solvent extraction (ASE) which is based on the use of small volumes of solvents at elevated temperatures and pressures to obtain in short time a complete extraction of analytes from solid and semi-solid samples.[2,3] ASE has some advantages over other extraction techniques such as shorter extraction time and lower consumption of solvent than Soxhlet and ultrasonic extraction. In the last years, ASE has been applied to the extraction of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzodioxins and furans (PCDD/PCDFs) and polybrominated biphenyl ethers (PBDEs) from different matrices, such as solid wastes,[4] soil,[5-7] fish,[8] mosses and pine needles,[9] feedingstuffs and food matrices[10] and sediments.[11] ASE is accepted by the United States Environmental Protection Agency (US EPA) as Method 3545A for the extraction of the organic compounds covered by the Resource Conservation and Recovery Act.[12] The investigation of the environmental occurrence, biochemical/toxicological effects and human exposure of persistent organochlorine pollutants (POPs), such as PCBs and OCPs, is a major issue of research. Soils are an important reservoir for POPs and agricultural soil is likely the largest sink, but also a major source of emission of OCPs.

The aims of the study were: 1) to develop and validate an analytical method based on ASE for the determination of PCBs and OCPs from soil; 2) to assess the contamination with PCBs and OCPs in soil samples from eastern part of Romania (Moldavia province).

EXPERIMENTAL

Materials

The OCPs under investigation were α-, β-, γ-HCH, DDT and analogues (op-DDE, pp-DDE, op-DDD, op-DDT, pp-DDD, pp-DDT) and hexachlorobenzene (HCB). The following PCB congeners (IUPAC numbers) were targeted: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194 and 199. Internal standards (IS)

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were PCB 46, PCB 143 and ε-HCH, while 1,2,3,4-tetrachloronaphtalene (TCN) was used as a recovery standard. All individual PCB and OCP standards were purchased from Dr. Ehrenstorfer Laboratories (Germany). Acetone, hexane, dichloromethane, and iso-octane were of pesticide grade (Merck, Germany). Analytical grade concentrated sulfuric acid (95-97%) was purchased from Merck. Anhydrous sodium sulfate (Na_2SO_4) and silica gel (70-230 mesh) (Merck) were washed with hexane and used after heating overnight at 120°C. The acidified silica gel (44% H_2SO_4, *w/w*) was prepared as previously described.[13] Empty polyethylene cartridges (25 ml) were purchased from Alltech (Belgium).

Samples

Surface soil samples (max. 5 cm deep) were collected in October 2005 from rural and industrial area from the eastern part of Romania (Moldavia province). For rural zones, samples were collected from area belonging to Strunga, Basta, Raducaneni, Dragesti, Rafaila, Gadinti, Breazu, Deleni, Botosani and Dorohoi. For industrial areas, samples were collected from Roman, Vaslui, Barlad and Galati. Samples were homogenized, sieved through a steel mesh (500 µm grid size), dried at room temperature and stored in air-tight polyethylene containers at room temperature until analysis.

Extraction and clean-up. Extractions were performed using an ASE system (Dionex, USA). The used extraction parameters were based on instrumental settings previously suggested[6] and are listed in Table 1. To eliminate interfering peaks and avoid cross-contamination, the extraction cells were pre-extracted with hexane-acetone (3:1, *v/v*) at 100°C and 2000 psi during 5 min. One gram of dried soil was introduced in the cell and spiked with 15 ng of each IS. The extract was concentrated in the extractor vials to 2 mL under a nitrogen stream. The clean-up procedure was described by Covaci et al. (2002) and consists in the purification of the extract on 8 g acidified silica and elution with 15 mL hexane and 10 mL dichloromethane. The purified extract was further concentrated under a nitrogen stream until dryness, resolubilized in 100 µl iso-octane and transferred to a vial for GC analysis.

Table 1

Accelerated solvent extraction parameters

Parameters	Values
Temperature	100°C
Pressure	2000 p.s.i.
Static time	5 min
Heat time	5 min
Solvent	n-hexane/acetone (3/1, *v/v*)
Cycle	3
Flush volume	60%
Purge time	100s
Cell volume	5 mL

Instrumentation

Instrumental conditions have been previously described[13,14] and are briefly presented below. An Agilent Technologies (USA) 6890 GC-µECD was equipped with a 25m x 0.25mm x 0.25µm HT-8 capillary column (SGE, Belgium). One µl was injected in pulsed splitless mode (pulse pressure = 40 psi, pulse time = 1.5 min) with the split outlet opened after 1.5 min. Injector and detector temperatures were set at 300 and 330°C, respectively. Helium was used as carrier gas at constant flow (1.0 mL/min), while argon/methane (95/5, *v/v*) was used as make-up gas (25 mL/min). The temperature program of the oven was set to 90°C for 1.5 min, then with 30°C/min to 180°C, then with 5°C/min to 300°C, kept for 15 min.

Samples with high concentrations of POPs were confirmed by GC/MS (Agilent Technologies) operated in electron impact ionization mode and equipped with a 30m × 0.25mm × 0.25µm DB-1 capillary column (J&W Scientific, USA). The ion source, quadrupole and interface temperatures were 230, 150 and 300°C, respectively. Helium was used as carrier gas at constant flow of 1.0 mL/min. One µl of the extract was injected in cold splitless mode (injector temperature at 100°C, then heated with 700°C/min to 300°C, pulse time 1.5 min, splitless time 1.50 min, pulse pressure 25 psi). The temperature program of the oven was set to 90°C for 1.5 min, then with 15°C/min to 180°C, kept for 1 min, then with 5°C/min to 280°C, and further by 40°C/min to 300°C, kept for 16 min. Dwell times were set to 25 ms. Specific ions for the investigated POPs were monitored for the entire run.

Recovery experiments

To evaluate the absolute recoveries of the method, soil samples was spiked in triplicate before extraction at 100 ng/g soil for each OCP and 2.5, 5 or 10 ng/g soil for various PCB congeners. Non-spiked soil samples were also processed in duplicate and the obtained values were subtracted from the values found in the spiked soil samples. In order to estimate the accuracy of the method, a reference material CRM 481 (PCBs in industrial soil, BCR, Belgium) was diluted 1000 times with Na_2SO_4 and analyzed in triplicate.

RESULTS AND DISCUSSION

Method validation

A major advantage of the ASE system is that extraction time and solvent consumption are greatly reduced, while the whole extraction process is fully automated. Tab. 1 shows the optimized parameters for the extraction of spiked soil samples and certified reference materials samples. A temperature of 100°C, previously[6] shown to give the best results, was selected for the extraction, while the hexane/acetone (3/1, *v/v*) mixture was

shown to be the most efficient solvent mixture for extraction of OCPs and PCBs from soil samples.[13] However, this solvent mixture was not yet evaluated for extraction of OCPs and PCBs by ASE.

Multi-level curves were created for the quantification and good linearity (r^2>0.999) was achieved for the tested intervals that included the whole concentration range found in the samples. The identification of analytes was based on their relative retention time to the internal standard used for quantification. Recoveries of internal standards (calculated based on TCN) ranged in samples between 64 and 99%. Analyte recoveries were calculated using the spiked soil samples and ranged from 65 to 157% (Tab. 2). In general, higher recoveries were obtained for PCBs than for OCPs.

Table 2

Limits of quantification (ng/g soil), percentage recoveries (%) and relative standard deviation (RSD) of target analytes

Compounds	LOQ	Recovery (%)	RSD (%)
CB28	0.5	79	3
CB52	1.8	149*	23
CB101	0.3	157*	24
CB153	0.1	103	11
CB105	0.1	137*	14
CB138	0.1	102	11
CB156	0.1	85	10
CB180	0.1	85	13
CB194	0.1	84	15
HCB	0.1	71	5
α-HCH	0.2	65	11
β-HCH	0.4	66	9
γ-HCH	0.1	70	11
δ-HCH	0.9	84	14
op-DDE	0.4	66	9
pp-DDE	0.2	86	9
op-DDD	0.2	90	10
op-DDT	0.2	72	8
pp-DDD	0.2	78	9
pp-DDT	0.2	84	13

* - interferences

In order to estimate the accuracy and precision of the analytical method (Tab. 3), the reference material CRM 481, an industrial soil contaminated with PCBs, was extracted in triplicate and analyzed by GC-ECD and GC/MS. The accuracy (calculated as the deviation from the certified values) was within 10-15% for the investigated PCBs congeners, while the precision (calculated as the RSD of the triplicate measurements) was always >90% (RSD<10%) (Tab. 3). Method limits of quantification (LOQs) for individual PCBs and OCPs ranged between 0.1 and 0.5 ng/g, with exception of CB 52 and δ-HCH (1.8 and 0.9 ng/g, respectively). LOQs were dependent on the analyte value in the procedural blanks and were established at 3xSD of the value in the procedural blanks, resulting in a certainty of more than 95% for results given for the samples.[14] For calculation of concentrations in the samples, the value of each compound in the procedural blank was subtracted from the corresponding value in the sample and the resulting value was compared to the LOQ calculated for each compound.

Table 3

Concentrations of PCBs (ng/g soil) and RSD (%) in the reference material CRM 481.

Compounds	Certified values	Measured values on GC-MS	RSD (%)	Measured values on GC-ECD	RSD (%)	Coelutions
CB 101	37	29	1.7	29	3.0	-
CB 118	9.4	12	3.8	10.6	4.5	-
CB 128	9.1	8.9	0.3	49	4.3	-CB128/CB174

Tabel 3 (continues)

Tabel 3 (continued)

CB 149	97	84	0.6	85	2.7	-
CB 153	137	132	1.4	123	4.1	-
CB 156	7.0	8.3	1.0	17	7.4	CB156/CB172
CB 170	52	55	1.4	57	7.2	-
CB 180	124	125	1.3	136	8.2	-

Application to real sample

The ASE method has then been used for the determination of OCPs and PCBs in soil samples collected form eastern part of Romania (Tab. 4). In all samples from the agricultural and forest zones, PCB congeners were found at very low levels (< 6 ng/g soil for sum PCBs), suggesting that atmospheric deposition is the predominant pollution source with PCBs. In these samples, tri- and tetra-CBs congeners were below LOQ. The penta-CB congeners were predominant (46-57%), followed by hexa-CBs (37-50%), hepta-CBs (10-31%) and octa-CBs (9-15%). Soil samples collected from the industrial sites showed much higher concentrations of PCBs, ranging between 9 - 332 ng/g soil for the sum PCBs (mean value 91 ng/g soil). One sample (industrial soil) from Galati exceeded the Roumanian norms of 250 ng/g soil for sum PCBs.[15] A more heterogeneous PCB distribution (high standard deviation) was also observed in these samples (Tab. 4) due to very different contamination degrees of the investigated sites. Furthermore, an increase in the proportion of heavier congeners (hepta- and octa-CBs) was observed, suggesting local sources. Concentrations of HCB were very low (up to 0.1 ng/g soil) in all samples in accordance to previously reported concentrations of HCB in Roumanian agricultural soils.[16]

Similarly, HCHs had a relatively homogeneous distribution throughout the 18 investigated sites and their concentrations were low (range 0.7 - 12 ng/g soil) (Tab. 4). In forest soils, a higher contribution of the most volatile HCH isomer (α-HCH) was observed emphasizing the predominant contamination through atmospheric deposition in these locations. In agricultural and industrial soils, the contribution of γ-HCH was higher suggesting a shift in the use of HCH formulations (pure lindane (γ-HCH) vs. technical HCH (α-HCH major isomer)).

Compared to HCHs, higher concentrations of DDTs were found in samples collected from the agricultural and industrial sites. However, only two samples exceeded the Roumanian norms of 500 ng/g soil for sum DDTs.[15] A large variation in the concentrations (high standard deviation) was observed between the sites and high concentrations were measured in each of the investigated types of soil. Unexpectedly, a soil samples from the forested zone contained also high concentrations of DDTs, while similar samples contained much lower concentrations of DDTs, in accordance with the presumed contamination through atmospheric deposition in these locations. In all samples, pp-DDT was the major contributor to the sum DDTs (Tab. 4), proving that the soil texture and other local conditions delayed the chemical or biochemical biodegradation of pp-DDT.

Table 4

Means, standard deviation and concentration range (ng/g soil dry weight) of PCB homologue groups and individual OCPs in agricultural, forest and industrial soil samples from Eastern Roumania

	Agricultural soil (n=8)		**Forest soil (n=5)**		**Industrial soil (n=5)**	
	Mean (SD)	Range	Mean (SD)	%	Mean (SD)	%
sum tri-CBs	nd		nd		1.3 (0.9)	
sum tetra-CBs	nd		nd		nd	
sum penta-CBs	1.0 (1.2)		1.0 (0.8)		10 (11)	
sum hexa-CBs	1.1 (0.4)		0.7 (0.1)		35 (54)	
sum hepta-CBs	0.7 (0.8)		0.2 (0.1)		35 (54)	
sum octa-CBs	0.3 (0.2)		0.2 (0.1)		11 (17)	
sum PCBs	2.3 (2.0)	nd -5.5	1.8 (1.2)	0.2 -3.5	91 (136)	9.0 - 332
HCB	0.3 (0.1)	nd - 0.5	0.2	nd - 0.2	0.4 (0.2)	0.2 - 0.6
α-HCH	1.8 (0.9)		4.6 (3.1		2.1 (1.4)	
γ-HCH	2.2 (2.5)		2.2 (1.2)		2.5 (1.1	

Table 4 (continues)

Table 4 (continued)

β-HCH	2.8 (2.9)		1.3 (1.2)		1.3 (0.7)	
δ-HCH	nd		nd		1.1	
sum HCHs	5.1 (4.7)	0.7 - 12.2	5.5 (3.6)	1.5 - 9.9	5.1 (1.3)	3.7 - 6.5
op-DDE	3.1 (4.2)		3.0		0.7 (0.3)	
pp-DDE	61 (116)		28 (52)		26 (35)	
op-DDD	3.0 (4.0)		2.2 (3.5)		5.2 (3.1)	
op-DDT	12 (18)		25		16 (17)	
pp-DDD	12 (18)		8 (15)		32 (29)	
pp-DDT	44 (56)		17 (30)		153 (177)	
sum DDTs	127 (202)	5.5 - 599	59 (111)	0.9 - 258	232 (259)	30 - 665
pp-DDT/sumDDTs	0.5 (0.2)		0.5 (0.2)		0.4 (0.2)	

nd – not detected

CONCLUSIONS

ASE appears to be a promising technique that requires a small amount of solvent, a short extraction time, and that can result in a lower exposure to the solvents. ASE has been shown to be efficient at extracting OCPs and PCBs from soil samples.

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REFERENCES

1. L. Ken, M. Landriault, M. Fingas and M. Llompart, *J. Hazard. Mat*., **2003**, *102*, 93-104.
2. B. E. Richter, J. L. Ezzell, D. Felix, K. A. Roberts and D. W. Later, *Am. Lab*., **1995**, 27, 24-28.
3. B. E. Richter, *J. Chromatogr. A*, **2000**, *847*, 217-224.
4. P. Popp, P. Keil, M. Moder, A. Paschke and U. Thuss, *J. Chromatogr. A* , **1997**, *774*, 203-211.
5. A. Hubert, K-D. Wenzel, M. Manz, L. Weissflog, W. Engewald and G. Schüürmann, *Anal. Chem.,* **2000**, *72*, 1294-1300.
6. E. Concha-Graña, M. I. Turnes-Carou, S. Muniategui-Lorezo, P. Lopez-Mahia, E. Fernández- Fernández and D. Prada-Rodriguez, *J. Chromatogr. A*, **2004**, *1047*, 147-155.
7. S. Sporring, S. Bøwadt, B. Svensmark and E. Bjorklund, *J. Chromatogr. A*, **2005**, *1090*, 1–9.
8. M. Weichbrodt, W. Vetter and B. Lukas, *AOAC International*, **2000**, *83*, 1334-1343.
9. K-D. Wenzel, A. Hubert, M. Manz, L. Weissflog, W. Engewald and G. Schüürmann, *Anal. Chem*., **1998**, *70*, 4827-4835.
10. A. Muller, E. Bjorklund and C. Van Host, *J. Chromatogr. A*, **2001**, *925*, 197-205.
11. A. De La Cal, E. Eljarrat and D. Barceló, *J. Chromatogr. A*, **2003**, *1021*, 165-173.
12. US EPA-SW-846, Update III; Test Methods for Evaluating Solid wastes, Method 3545; Fed. Reg. Vol. 62, 114: 32451 US Washington DC, 13 June 1995.
13. A. Covaci, P. Manirakiza and P. Schepens. *Bull. Contam. Environ. Toxicol.,* **2002***, 68*, 97-103.
14. A. Covaci, A. Gheorghe, S. Voorspoels, J. Maervoet, E. Steen Redeker, R. Blust and P. Schepens. *Environ. Intern.,* **2005***, 31*, 367-375.
15. Monitorul Oficial al României, partea I, Nr. 303 bis 6, Nov. 1997.
16. A. Covaci, C. Hura and P. Schepens. *Sci. Total Environ.,* **2001,** *280*, 143-152.