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Automated Sample Clean-up and Fraction of Organochlorine Pesticides in Sediments Using the SPE–GPC–VAP System

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Abstract This paper describes an automated sample clean-up and fraction method using the SPE–GPC–VAP system for the determination of organochlorine pesticides (OCPs) in sediments. The method is based on a simultaneous extraction/clean-up step to reduce analysis time and solvent consumption. The parameters of a solid-phase extraction procedure, a gel permeation chromatography procedure and an AccuVap procedure were optimized to obtain maximum recovery of the target analytes with minimum presence of matrix-interfering compounds. The detection limits ranged from 0.03 to 0.13 ng g^{-1} and the recovery ranged from 50.05% to 104.39%. The relative standard deviation was lower than 19.34% when OCPs concentrations in sediments ranged from 1.25 to 125 ng g^{-1}.

Keywords Automated sample clean-up · SPE–GPC–VAP system · ASE · Organochlorine pesticides · Sediment

Organochlorine pesticides (OCPs) are chemical substances that persist in the environment, bio-accumulate through the food web and pose adverse effects to human health and the environment (Zhou et al. 2007; Carvalho et al. 2008). OCPs are usually determined by GC–ECD, GC–MS or HPLC (Zhou et al. 2007; Carvalho et al. 2008). However, a number of sample preparation steps are required to determine OCPs in sediment matrix, because of the strong interactions between the OCPs and different constituents of the sediments, particularly the organic matter (Carvalho et al. 2008). At present, solid-phase extraction (SPE) methods are routinely used to purify pesticides in sediment because of the wide availability of selective sorbent material. SPE methods have been extensively reported in the literature (Schade and Heinzow 1998; Schinas et al. 2000). The lipid (fat) matrix or sulfur, which adversely impacts the detection of the target OCPs, is often difficult to be removed from the sediments. The application of concentrated sulfuric acid for sample clean-up has been reported (Kalra et al. 1994; Barkatina et al. 1998), but it is not suitable for some less stable pesticides such as DDT, which may decompose (Burke et al. 2003; Hoff and Zoonen 1999). Gel-permeation chromatography (GPC) is an attractive clean-up alternative for sample purification, in which organic solvents and a hydrophobic gel are employed to separate compounds based on their sizes. Therefore, the GPC could separate the sulfur, chlorophyll or fat matrix due to the difference of molecular size between triglycerides and pesticides (Hoff and Zoonen 1999). The combination of SPE–GPC may be much more effective than either of them applied separately.

The traditional analysis systems for OCPs consist of soxhlet extraction, florisil purification (Tao et al. 2007), rotary evaporation and analysis by gas chromatography. They are laborious, time-consuming and require high volumes of solvents as well as skills of operators. In addition, poor inter- and intra-laboratory reproducibility may occur.

This study aimed at developing a fully automated, simple and robust method for the analysis of OCPs in sediments. The sample processing method is controlled by

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PrepLinc software which facilitates the automation of the experimental set-up. Furthermore, since this method is capable of running the instrument unattended, it will be less labor intensive. This method was first verified with a standard sample and then applied to determination of OCPs in several surface sediments in Bohai Bay, China.

Materials and Methods

A standard solution containing eight OCPs (α-, β-, γ- and δ-hexachloro-cyclohexane, p, p′-DDT, o, p′-DDT, p, p′-DDD, p, p′-DDE) in toluene/hexane (1:1 v/v) for quantification was obtained from the National Research Center for Certified Reference Materials of China (Beijing, China). It was also employed as the matrix spike for precision and recovery studies. Decachlorobiphenyl ($^{13}C_{12}$PCB209) was obtained from ChemService (West Chester, PA, USA) and 2,4,5,6-tetrachloro-m-xylene (TCMX) was purchased from Supelco (Bellefonte, PA, USA).

All solvents were of pesticide residue grade and were purchased from J. T. Baker (Philipsburg, NJ, USA). LC-18 (6 mL), ENVI-18 (6 mL), LC-Si (1 g, 6 mL), and LC-Florisil (1 g, 6 mL) were supplied by Supelco (Bellefonte, PA, USA). Preplinc instrumentation was obtained from J2 Scientific Corporation (Columbia, MO, USA) and equipped with AccuVap, SPE, GPC and a UV detector. Accelerated solvent extraction (ASE) and diatomaceous earth were purchased from Dionex (Sunnyvale, CA, USA). Florisil (60–100 mesh) was purchased from Supelco (Bellefonte, PA, USA) and activated in air at 130°C for 16 h. Anhydrous sodium sulfate (Beijing Chemical Factory, Beijing, China) was heated at 600°C for 6 h.

Sediment samples were collected using a stainless steel grab sampler from the surface layer (0–10 cm) at four estuaries (Dagu River, Ziya River, Nanpai River, and Dashentang River) and Gaoshaling intertidal zone of the Bohai Bay, China. All samples were wrapped in aluminum foil and sealed in plastic bags to minimize the possibility of contamination. During a 2-day sampling trip, the samples were stored in a cool box. Upon arrival at laboratory, the samples were immediately transferred to a freezer where they were stored until treatment. In the laboratory, sediment samples were thawed and freeze-dried, pulverized and sieved through a 100-meshed stainless steel mesh prior to analysis.

The sediment sample was extracted by ASE system (ASE300, Dionex, Sunnyvale, CA, USA). About 2.000 g of freeze-dried and homogenized sediment was spiked with 5 ng of $^{13}C_{12}$PCB 209 and 10 ng of TCMX as surrogates, homogenized with diatomaceous earth (2:1), equilibrated for 24 h, and then extracted with solvent (hexane/acetone (1:1 v/v)) at 100°C under a pressure of 1,500 psi. The extraction was carried out in two cycles with 5 min static extraction. The extracts were concentrated to 1–2 mL by rotary evaporation and then further concentrated to 0.5 mL under a gentle nitrogen flow prior to instrument analysis. The samples for the matrix spike recovery experiment was additionally spiked with 1 mL of standard material (using acetone as the solvent) at OCPs concentrations of 2.50, 25.00 and 250.00 μg L^{-1}, respectively.

The automation of purification system consists of three parts: SPE, GPC and AccuVap (Preplinc AccuVap, J2 Scientific's automatic sample concentrator), and the extracts could pass through the three parts sequentially. Key instrumental conditions of clean-up can be separately programmed. The effluent from the GPC was operated in-line with the AccuVap and was automatically concentrated during the elution process.

A Supelclean LC-Florisil (6 mL, 1 g) cartridges (Bellefonte, PA, USA) was chosen as a clean-up column, which was placed in a nine port Visiprep manifold and conditioned with 15 mL of cyclohexane. The extract was loaded onto the cartridge by an auto-sampler. The extracts were thoroughly transferred to the cartridge, the cartridge was then eluted with 8 mL co-solvent (cyclohexane: ethyl acetate (1:1 v/v)) at a flow rate of 1.1 mL min^{-1}.

The effluent after purification was subjected to GPC. The operating conditions for the AccuPrep GPC were: sample loop volume was 5 mL, column: glass 30 mm × 400 mm, packed with Bio-Beads SX-3; the mobile phase was ethyl acetate/cyclohexane (1:1 v/v) at a flow rate of 4.7 mL min^{-1}. The fractions eluting from 0 to 8.0 min and from 18.0 to 30.0 min were discarded, while the fraction eluting from 8.0 to 17.0 min containing the analytes of interest was collected and automatically concentrated in the VAP to a final volume of 2 mL.

The AccuVap methods are defined as either an Inline or EVS method. In the Inline mode, the sample is added to the AccuVap from another process such as GPC or SPE. In the EVS mode, the sample is transferred directly from a vial or container to the auto-sampler. The inline mode was chosen since it was desirable to automatically concentrate the GPC effluent.

The parameters of zone settings for sample introduction, endpoint and endpoint zone settings were optimized for this application but may be changed as required by the operator. The unique chamber is equipped with level sensors which monitor three zones for liquid levels and apply heating rates and vacuum appropriate for each zone in order to facilitate efficient and controlled removal of solvent. Zone 1 is below the lower level sensor, zone 2 is between the lower and upper level sensor and zone 3 is above the upper level sensor. If the solvent level is in zone 3 at the start of endpoint, zone 2 values will be used until the level reaches zone 1. There are two sample endpoints which may be used: the operator may choose whether it is

desired to remove all the solvent (dryness endpoint) or whether the sample should be reduced to a preset volume level (level sense). In this study, the solvent is reduced to a preset volume.

Finally, the extracts from the GPC–SPE–VAP system were further concentrated to 0.5 mL under a nitrogen stream for GC–MS analysis.

The traditional purification method: the OCPs in the sediments were first extracted and concentrated with use of hexane, then purified by using an adsorption chromatography column (10 mm internal diameter, and 30 cm long) which was packed with 4 g of activated Florisil and 2 g of anhydrous sodium sulfate and conditioned with 30 mL of hexane/diethyl ether (4:1, v/v) prior to loading of the extracts. The fraction containing OCPs was first eluted with 60 mL of hexane/diethyl ether (4:1, v/v) (Fu et al. 2009), then concentrated and further cleaned up by the GPC column. The effluents were concentrated to 0.5 mL prior to GC–MS analysis.

OCPs were analyzed by an Agilent 7890 gas chromatograph coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, Inc. CA, USA) using an electron impact ionization source (EI) in the selected ion monitoring (SIM) mode. A 1 μL aliquot of the sample was injected in splitless mode (splitless time of 1 min) onto a DB-5 MS fused silica capillary column (30 m × 250 μm i.d., film thickness 0.25 μm) with helium as carrier gas, at a constant flow rate of 0.8 mL min^{-1}. The injector and the interface temperature were 225 and 290°C, respectively. The oven temperature program was as follows: started at 70°C, held for 1 min, increased to 280°C at 4°C min^{-1} and held for 5 min. Quantification was performed by an external standard method.

The average recovery was determined in triplicate by spiking blanks with known concentrations of the standards. The values ranged between 70% and 95% (RSD < 15%) for OCPs. The ongoing recoveries of TCMX and $^{13}C_{12}$-PCB209 ranged from 70% to 105% (RSD < 20%). The amounts OCPs in the blanks were below detection limits. The detection limits were defined as three times the signal-to-noise ratio, and ranged from 0.03 to 0.13 ng g^{-1} for OCPs. Instrumental quality control was done by regular injection of solvent blanks and standard solutions. The concentrations of OCPs were measured using an external quantification standard consisting of known amounts of the target compounds, and the results were corrected by the recovery factor.

The OCPs concentration in the standard reference material® 1941b (organics in marine sediment, National Institute of Standards and Technology, Gaithersburg, MD, USA) was analyzed. Comparison between our experimental values and the reference values showed that our results were reliable, as indicated by the RSD < 20%.

Results and Discussion

For optimization of SPE, 25 μL aliquot of 1 μg mL^{-1} OCPs standard solution was added to the 0.5 mL of solvent (cyclohexane) to give 50 ng mL^{-1} of OCPs. After rinsing the sample vial twice using a small amount of cyclohexane, the sample was combined and transferred to the cartridge, which had been conditioned with 15 mL of cyclohexane. The effluents was collected at a flow rate of 1.1 mL min^{-1} and concentrated to 0.5 mL under a gentle nitrogen stream for GC–MS analysis. The recovery was calculated for HCHs and DDTs.

Four kinds of SPE cartridges: LC-18, ENVI-18, LC-Si, and LC-Florisil were compared for their recoveries of the spiked analytes. LC-Florisil cartridge showed obviously higher recovery (87.23%–117.95%) than did the LC-18, LC-Si, and ENVI-18 cartridges.

The polarity of the mobile phase directly affects recovery efficiencies. If the polarity is too strong, impurities will be eluted along with the desired analytes; whereas, if the polarity is too weak, some components cannot be eluted completely. In this study, the solvents of pure cyclohexane and a mixture of ethyl acetate/cyclohexane (5:95, 10:90, 50:50 v/v) were compared. The results demonstrated that cyclohexane alone as mobile phase on the LC Florisil matrix could not totally elute the target compounds, while the recovery was lower than 71.34% when 5:90 (v/v) ethyl acetate/cyclohexane was used as the mobile phase. In contrast, sufficient recoveries were obtained using 10:90(v/v) and 50:50 (v/v) ethyl acetate/cyclohexane for elution, ranging in 84.06%–118.66%, and 87.23% –117.95%, respectively. In order to facilitate the purification by GPC, 50:50 (v/v) ethyl acetate/cyclohexane was selected for elution. Subsequent experiments indicated that an elution volume of 8 mL was sufficient to totally elute the desired analytes.

To evaluate the aforementioned methods reproducibility, a spiked solution (OCPs level at 12.5 ng g^{-1}) was analyzed in triplicate under abovementioned experimental conditions. The recovery ranged from 81.14% to 118.84% and the RSD was between 1.69% and 8.72%.

The application of GPC as a technique for sample matrix clean-up and separation of pesticide residues will be used more frequently (Liu et al. 2005), as GPC with improved automation is becoming more commercially available.

In order to verify the effectiveness of GPC as a purification technique in this application, a preferred GPC configuration was selected, in which the UV detector trace was used to determine the components elution window and elution volume (time). A 100 ng mL^{-1} standard solution was prepared in 1:1 ethyl acetate/cyclohexane (v/v) which flowed through the column at a rate of 4.7 mL min^{-1}. The

OCPs components in the standard solution were eluted between 10.5 and 13.5 min.

According to the UV-absorbance trace, a wider collection time, i.e. 8.0–17.0 min was used to collect the effluents, which was tended to obtain good recovery and allow for variation of sample concentrations in sediment samples. As presented in Table 1, the calculated recovery of all components were between 85.55% and 115.27%. Therefore, an optimum elution profile for GPC analysis was comprised of: 0–8.0 min of washing, 8.0–17.0 min collects to AVP and 18.0–30.0 min of cleaning (to waste).

In the AccuVap concentration process, if the temperature was too high and/or the degree of vacuum was too low, sample decomposition and/or loss of analytes due to evaporation was likely to occur. The optimum heating rates and vacuum levels to accommodate minor solvent concentration fluctuations and to assure that the solvent was slightly boiling were determined. In the endpoint determination, the level sensor mode was selected in a final volume of 2 mL (ranging from 1 to 5 mL). The extracts were transferred to the K-D tube, and concentrated under small stream of nitrogen at room temperature for GC/MS analysis.

The performance of the whole SPE–GPC–VAP system was evaluated for spiked blank and spiked sediment matrix, in which the spike gave 1.25, 12.5 and 125 ng g^{-1} of OCPs, in order to obtain 5, 50, 500 ng g^{-1} OCPs in sediment respectively. As shown in Fig. 1, the recoveries of spiked blank varied from 81.73 ± 2.86% to 104.30 ± 13.77% while from 50.05 ± 10.00% to 104.39 ± 6.07% for the spiked sediment matrix over the entire concentration range. RSD of OCPs ranged from 1.35% to 19.34%. The recoveries of surrogates for $^{13}C_{12}$PCB209 and TCMX lied in 68.21%–95.91% and 70%–90%, respectively.

As shown in the Fig. 1, higher levels of HCHs may have a reduced recovery, while the recovery for the DDTs ranged from 70.01 ± 6.56% to 104.39 ± 6.07%. Recovery experiments conducted using the traditional purification technologies gave recoveries of 56.4%–117.1% at a spike level of 12.5 ng g^{-1}.

Table 1 Recovery of OCPs for GPC purification

Pesticide	Spiked amount(ng/mL)	Recovery (%)	RS%(n = 3)
α-HCH	0.025	89.02	2.81
β-HCH	0.025	99.79	4.33
γ-HCH	0.025	93.42	3.07
δ-HCH	0.025	102.1	6.77
p, p′-DDE	0.025	122.1	5.17
p, p′-DDD	0.025	112.9	9.04
o, p′-DDT	0.025	105.3	5.91
p, p′-DDT	0.025	111.7	7.22

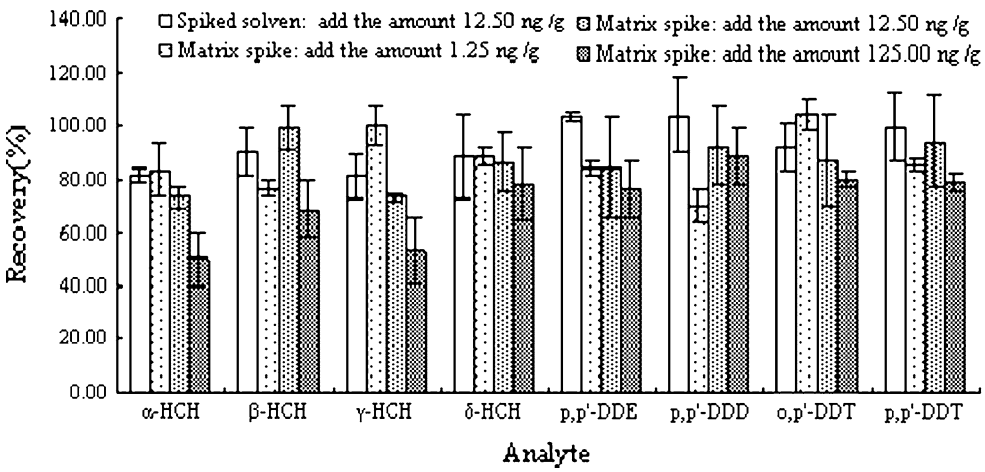

Fig. 1 The recovery and RSD of this method in different OCPs level in sediments

Table 2 The levels of OCPs in sediments from Bohai Bay (ng/g dry weight)

	α-HCH	β-HCH	γ-HCH	δ-HCH	p, p′-DDE	p, p′-DDD	o, p′-DDT	p, p′-DDT	OCPs
NE	2.10	0.57	0.55	0.85	1.02	3.55	2.12	4.55	15.29
ZE	1.46	2.09	0.35	0.98	4.82	0.37	0.44	0.59	11.10
DGE	16.95	6.23	8.62	1.38	0.56	0.70	1.05	0.87	36.36
DSE	<0.13	0.83	0.30	<0.48	0.09	0.08	<0.16	0.18	1.48
GIZ	3.10	2.19	2.32	1.63	0.20	0.53	0.29	0.92	11.19

A series of standard solutions were prepared (2.5, 5, 10, 50, 100, 500, and 1,000 ng mL^{-1}) and analyzed by GC/MS as outlined in section 2.5. The correlation coefficients (r) of all the calibration curves for the OCPs were all $\geq$0.998. The detection limits ranged between 0.03 and 0.13 ng g^{-1} for this method.

To evaluate the performance of the developed method, the surface sediment and the standard reference material® 1941b (organics in marine sediment, National Institute of Standards and Technology, Gaithersburg, MD, USA) was analyzed for selected OCPs. The surface sediments were from four estuaries (Dagu River, Ziya River, Nanpai River, and Dashentang River) and Gaoshaling intertidal zone of the Bohai Bay. The levels of eight kinds of OCPs in sediments decreased in the order of Dagu estuary (36.36 ng g^{-1} dry weight) > Nanpaihe estuary (15.29 ng g^{-1} dry weight) > Ziyahe estuary (10.10 ng g^{-1} dry weight), Dashentang estuary (11.19 ng g^{-1} dry weight) > Gashaling intertidal zone (1.48 ng g^{-1} dry weight), the levels of individual OCPs were provided in Table 2. Moreover, reliable results were obtained for the standard reference material® 1941b, in good agreement with the reference values (RSD < 20% on results).

The method presented in this study is a fully automated purification procedure for OCPs in sediment samples, and it adequately fulfils the basic requirements for multi-residue pesticide detection. Recoveries ranging between 50.05% and 104.39% with RSD less than 19.34% were obtained for eight OCPs.

The automated method was advantageous over the traditional purification methods, in that the former is completely automated, labor-efficient and simple to operate, and satisfies all the requirements for high sample purification efficiency and performance. However, it may be less cost-effective than traditional methods.

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