Rusong Zhao · Xia Wang · Shan Fu · Jinpeng Yuan ·
Ting Jiang · Xiaobai Xu

A novel headspace solid-phase microextraction method for the exact determination of organochlorine pesticides in environmental soil samples

Received: 22 November 2005 / Revised: 26 January 2006 / Accepted: 27 January 2006 / Published online: 21 March 2006
© Springer-Verlag 2006

Abstract A novel method of determining organochlorine pesticides (OCPs) is described. It is based on solid-phase microextraction (SPME) and gas chromatography–electron capture detection. During the development of the method, soil samples were prepared, spiked with standard solution, and then aged for some time. Extraction conditions such as the extraction time, the NaCl content, the volume of water, the extraction temperature and the desorption time were investigated and optimized. The limits of detection obtained using the method ranged from 0.10 to 0.51 ng g⁻¹, and relative standard deviations were lower than 10% for most organochlorine pesticides. Real soil samples were successfully analyzed using the proposed method. The results from the method developed here were in good agreement with those obtained using ultrasonic extraction. The result demonstrates that aging soils spiked with standard solution is an important method development step, because the soil samples obtained using this approach are more like real soils than those obtained when aging is not used.

Keywords Organochlorine pesticides · Solid-phase microextraction · Environmental soil samples · Gas chromatography–electron capture detector

Introduction

Organochlorine pesticides are one of the most persistent organic pollutants (POPs) present in the environment. These compounds exhibit high toxicity and bioaccumulation and have been shown to be carcinogenic in animals and humans. They have been included in list of priority pollutants compiled by the U.S. Environmental Protection Agency (EPA) and also appear in the list of priority pollutants in Chinese waters [1]. Therefore, it is imperative to develop rapid, simple and accurate methods for determining them in different matrices.

Sample preparation is a critical step in the analytical process. Conventional soxhlet extraction [2] and ultrasonic extraction [3] have been widely used for the extraction of organochlorine pesticides from soils. It is well known, however, that these methods are time-consuming, tedious, often require large amounts of organic solvent, and can be relatively expensive. Although ultrasonic extraction can save a lot of time and can be automated, the residue and the extract need to be separated by centrifugation.

Solid-phase microextraction, introduced by Pawliszyn and his coworkers, is a novel and solvent-free sample pretreatment technique [4]. The method has been successfully used to analyze volatile and semivolatile environment pollutants [4–6]. Aguilar et al. optimized the conditions needed for direct SPME of organochlorine pesticides in water, while Goncalves and Alpendurada compared the extraction efficiencies of different coatings used in the analysis of pesticide multiresidues in water samples [7, 8]. Tomkins and Barnard determined 19 organochlorine pesticides in groundwater using solid-phase microextraction based on a PDMS-coated (30 µm-thick) fiber coupled to dual-column gas chromatography with satisfactory results [9]. Researchers found that the recoveries of organic pollutants were obviously affected by the complex sample matrix [10]. However, the application of headspace solid-phase microextraction avoids such matrix effects [11, 12]. At the same time, it can prolong the life of an SPME coating. Doong and Liao have successfully analyzed 18 organochlorine pesticides and their metabolites in soil

R. Zhao (✉) · X. Wang · J. Yuan · T. Jiang
Key Laboratory for Applied Technology of Sophisticated Analytical Instruments of Shandong Province,
Analysis and Test Center, Shandong Academy of Sciences,
Jinan, Shandong 250014, P. R. China
e-mail: zrswangx@yahoo.com.cn

R. Zhao · S. Fu · X. Xu
State Key Laboratory of Environmental Chemistry
and Ecotoxicology,
Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences,
Beijing 100085, P. R. China

using the headspace mode [13]. However, the analytical results were biased toward some compounds. The main reason for this is that soils spiked with standard solutions during method development are not close enough to real soil sample. Furthermore, the desorption temperature and ionic strengths were not considered.

The major objective of this study was to enhance the SPME-based method used in previous reports by aging the soil samples spiked with standard solution and by considering all of the analytical conditions used in the technique.

Experimental

Instruments

A Varian (Palo Alto, CA, USA) 3700 gas chromatograph equipped with ^{63}Ni electron capture detection system was used for all analyses. The gas chromatograph was fitted with a HP-17 capillary column (40 m $\times$ 0.25 mm I.D.). The gas chromatography conditions were as follows: injector temperature 250 °C; detector temperature 320 °C; initial oven temperature 80 °C (held for 10 min), increased to 250 °C at a rate of 4 °C min $^{-1}$, and then maintained at 250 °C for 3 min. The total time required for one GC run was 55.5 min. The inlet was operated in splitless mode. High-purity nitrogen was used as carrier gas. A WDL-95 workstation (Dalian Inst. Chem. Phys, CAS, Dalian, China) was utilized to control the system and to acquire the analytical data.

Materials and reagents

The eight pesticides and their metabolites (α -HCH, β -HCH, γ -HCH, δ -HCH, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, used were purchased from ChemService Inc. (West Chester, PA, USA). All solvents, including acetone, petroleum ether and isooctane, were of analytical grade and were redistilled in an all-glass system to remove impurities prior to use. An ultrasound extraction device was used (from the Institute of Acoustics, Chinese Academy of Sciences, Beijing, China). The 2,4,5,6-tetrachloro-*m*-xylene internal standard was purchased from Supelco (Bellefonte, PA, USA).

The soil used to develop the HS-SPME method was a sandy-loam deep soil collected from Qinghe, Beijing. After removing twigs and extraneous material it was air-dried at room temperature and sieved to a particle size of 300 μm . This soil did not contain pesticides and their metabolites. About 200 g of soil was weighed into a jar and spiked with 100 mL of hexane containing the target pesticides and their metabolites. The slurry was allowed to stand, loosely covered to protect from dust, and was stirred occasionally until the hexane had completely evaporated after $\sim$ 10 h. The final soil concentration, assuming that no loss occurred during preparation, was 2.0 ng g $^{-1}$ of each compound. The soil was stored at room temperature. The first analysis was performed one month later. The soils prepared in this

manner more closely resembled real soil samples than those obtained using the more common technique of spiking one spot in the soil matrix just before analysis, because the target analytes spend more time in intimate contact with the soil particles, thus maximizing the analyte–matrix interactions.

In the calibration studies, different levels of organochlorine pesticides were added to aliquots of 0.5 g of clean soil in a 15-mL vial to give a final soil concentration of 0.2–5.0 ng g $^{-1}$ of each organochlorine pesticide. The vials were tumbled and allowed to equilibrate at room temperature for one month before sampling and analysis.

HS-SPME procedure

A stir bar, 5.0 mL water and 0.5 g soil were introduced into a 15 mL vial. Then the vial was sealed with an aluminum cap that had a septum on it with Teflon lining inside and rubber outside. Sampling is performed by immersing the SPME fiber directly into the headspace above the sample for 60 min at the extraction temperature of 60 °C. Then the fiber was removed from the sample solution and immediately inserted into the GC injector, and at the same time the temperature program for the GC column was initiated. The desorption time was 7.5 minutes and the desorption temperature was 250 °C.

Results and discussion

Organochlorine pesticides are well-known as being hydrophobic compounds. Their octanol–water distribution constants K_{ow} are $7.8 \times 10^3 \sim 8.1 \times 10^6$, and their solubilities range from 5.5 $\mu\text{g L}^{-1}$ (for *p,p'*-DDT) to 31.4 mg L $^{-1}$ (for γ -HCH) (not including *o,p'*-DDT) [14]. They are nonpolar or weakly polar compounds. According to the extraction principle “like dissolves like”, they should easily enter nonpolar coatings. Considering all of the factors mentioned above, a PDMS (100 μm -thick) coating was chosen for use in the subsequent experiments.

Optimizing the conditions used

It is known that SPME is an equilibrium process [15]. The principle behind SPME is the partitioning of analytes between the sample matrix and the extraction medium [4, 16]. The effect of extraction time on the adsorption was investigated by plotting the profile of the GC peak area versus the extraction time. All of the experiments were carried out under the same conditions. The conditions were as follows: the extraction temperature was 60 °C, 5 mL of water was added, and the adsorption time was 7.5 min. The results are shown in Fig. 1. From Fig. 1, it is clear that the responses to the organochlorine pesticides greatly increase with increasing extraction time, but only gradually increase when the extraction time increases beyond 40 min. For most of the organochlorine pesticides, equilibrium was

Fig. 1 Effect of extraction time on extraction efficiency

reached at 60 min. At the same time, the analytical time used for a GC run is 55.5 min. Considering the above results, a time of used 60 min for one SPME operation was chosen for the following experiments.

It has been shown that the addition of water to soil samples improves the release and transport of volatile and semivolatile compounds from soil samples to the SPME fiber [11, 17]. Initial experiments showed that, without the addition of water, the release of organochlorine pesticides to the headspace is very low. The effect of the volume of water added to the sample was investigated. The samples were spiked with different amounts of water (from 2.5 to 10 mL). The results are shown in Fig. 2. From Fig. 2, it is clear that the absorptions of *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT were maximized when the amount of water was 5 mL. The molecular structures, molecular weights and polarities of the pesticides should play important roles in this respect. Llompert et al. [17] and Maria et al. [18] also found that, in order to release volatile compounds, it was necessary to add an appropriate amount of water to the soil. However, experimental results show that the release of hexachlorocyclohexane isomers decreased with increasing

Fig. 2 Effect of water volume on extraction efficiency

Fig. 3 Effect of amount of NaCl added on the extraction efficiency

Fig. 4 Effect of extraction temperature on extraction efficiency

water volume. Considering the results above, the amount of water was fixed at 5 mL in the following experiments.

The effect of the ionic strength on the SPME was assessed by adding NaCl, and Fig. 3 shows the effect of the amount of NaCl on the extraction efficiency. When the solution is agitated, the salt has the opposite effect on the four hexachlorocyclohexanes to its effect on the other four organochlorine pesticides. From Fig. 3, it can be concluded that the peak areas of the four hexachlorocyclohexanes increase with the amount of NaCl, while the peak areas of the others decrease. Generally, adding a certain amount of salt

Fig. 5 Effect of absorption time on the release of organochlorine pesticides from the coating

(NaCl, Na₂SO₄) decreases the solubilities of the analytes in the aqueous sample and enhances their partitioning into the SPME fiber [19]. However, some studies report that NaCl does not work or has an adverse effect. For example, Pawliszyn and his coworker found that the addition of NaCl has no effect on the adsorption of some nitrogen-containing herbicides [20].

The extraction temperature has a double impact on the HS-SPME: at higher temperatures, the diffusion coefficients in both the water and the headspace are higher and so the extraction time may decrease; however, the partition coefficients between the SPME fiber and the gaseous phase drop. The impact of temperature depends on the magnitudes of these two effects. It is therefore necessary to find a suitable extraction temperature experimentally. Different temperatures, from 40 to 70 °C, were investigated under the following conditions: no NaCl addition, 400 rpm stirring rate, 5 mL water added, extraction time 60 min and desorption time 7.5 min. The results are shown in Fig. 4. From Fig. 4, it is clear that the peak areas of organochlorine pesticides and their metabolites increase as the temperature rises from 40 to 60 °C. For the four hexachlorocyclohexane isomers, greatest peak areas were reached at 60 °C. The peak areas for the other four compounds kept increasing as the temperature rose above 60 °C. A possible reason for this is that the molecular weights of the four hexachlorocyclohexanes are lower than the other four compounds, which could lead to different volatilities under the same

conditions. Finally, an extraction temperature of 60 °C was selected.

Because of the high temperature of the injector, the analytes on the fiber are easily desorbed and transferred to the injector. However, the analysis will be affected if the analytes are not completely desorbed from the fiber. Different adsorption times (from 2.5 to 10 min) were investigated under the same conditions: extraction temperature 60 °C, stirring rate 400 rpm, volume of water 5 mL, extraction time 60 min, and no NaCl addition. From Fig. 5, it can be seen that the adsorption time does not have an obvious effect on the absorption of four hexachlorocyclohexanes, while the absorptions of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD increase with the increasing adsorption time and reach their maximal values when the adsorption time is 7.5 min. Therefore, in the following experiments, an adsorption time of 7.5 min was used.

Linear range, limits of detection and repeatability

Calibration curves were obtained under the optimized conditions, and the results from these curves are shown in Table 1. Linearity was observed over the range 0.2–5.0 ng g⁻¹ for most organochlorine pesticides and their metabolites. Coefficients of correlation (*R*²) ranged from 0.9577 to 0.997. The LODs, based on a signal-to-noise ratio (*S/N*) of 3, ranged from 0.10 to 0.51 ng g⁻¹, which are much lower than those obtained using traditional Soxhlet extraction [13]. The reproducibilities of the peak responses were investigated using six replicate experiments under the optimized conditions. At the level of 2.0 ng g⁻¹, the relative standard deviations (RSDs) of the pesticides were lower than 10% except for *p,p'*-DDD (11.4%) and β-HCH (11.4%).

Environmental soil sample analysis

This HS-SPME method was then successfully applied to the analysis of environmental soil samples. The samples were collected from the Sichuan province. In the soil samples, eight organochlorine pesticides and their metabolites were detected, and they were confirmed by comparing with the results from a standard chromatogram (Fig. 6). The results showed that the concentrations of organochlorine pesticides in the soil samples were much

Table 1 Linear ranges, correlation coefficients (*R*²), precisions and limits of detection (LODs) obtained using HS-SPME–GC–ECD analysis

Compounds	Linear range (ng g ⁻¹)	Equation of line fit to data	<i>R</i> ²	LOD (ng g ⁻¹)	RSD (%) (<i>n</i> =6)
α-HCH	0.2–2.0	<i>Y</i> =502640 <i>X</i> +56090	0.994	0.11	9.28
γ-HCH	0.2–3.0	<i>Y</i> =314100 <i>X</i> +60540	0.9947	0.10	6.82
β-HCH	0.2–5.0	<i>Y</i> =33600 <i>X</i> +48420	0.991	0.51	11.4
δ-HCH	0.2–5.0	<i>Y</i> =106230 <i>X</i> +990	0.9971	0.41	8.64
<i>p,p'</i> -DDE	0.5–4.0	<i>Y</i> =265340 <i>X</i> +944940	0.997	0.12	5.17
<i>o,p'</i> -DDT	1.0–4.0	<i>Y</i> =287840 <i>X</i> +503300	0.9801	0.15	8.81
<i>p,p'</i> -DDD	0.5–4.0	<i>Y</i> =333300 <i>X</i> +358800	0.9577	0.13	11.4
<i>p,p'</i> -DDT	0.2–5.0	<i>Y</i> =135680 <i>X</i> –22680	0.9956	0.18	8.85

Fig. 6 a, b Chromatograms of soils analyzed by HS-SPME-GC-ECD **a** Real soil sample. **b** Standard soil sample. 1 α -HCH; 2 γ -HCH; 3 β -HCH; 4 δ -HCH; 5 p,p' -DDE; 6 o,p -DDT; 7 p,p' -DDD; 8 p,p' -DDT

lower than the First Standard for the Chinese Soil Environment (GB15618–1995). In order to validate the HS-SPME method, the results from one sample were compared with those obtained using an ultrasonic extraction method (Table 2). From Table 1, it is clear that the results from the HS-SPME method agree well with those from the ultrasonic extraction, which are better results than those published in a previous report [13]. The main reason for this is that aging the soils spiked resulted in samples that were closer to real soils than those that had not been aged. The recoveries of organochlorine pesticides present at the 2.0 ng g⁻¹ level in real soil samples ranged from 76.3 to 121.4%.

Conclusions

Headspace solid-phase microextraction coupled to capillary column gas chromatography–electron capture detection was successfully used to determine organochlorine pesticides in environmental soil samples. During the process of method development, soils were prepared and spiked with standard solution and then aged for some time. Extraction conditions such as the extraction time, the content of NaCl, the volume of water, the extraction temperature and the desorption time were investigated and optimized. Real soil samples were successfully analyzed

Table 2 Comparison of results from SPME and ultrasonic extraction

Compounds	Mean±relative deviation (ng g ⁻¹) (n=3)	
	Ultrasonic extraction	SPME
α -HCH	7.17±0.69	5.12±0.60
γ -HCH	3.02±0.28	3.41±0.21
β -HCH	0.48±0.11	0.67±0.15
δ -HCH	1.69±0.19	1.97±0.17
p,p' -DDE	1.18±0.19	1.41±0.21
o,p -DDT	3.81±0.41	3.15±0.34
p,p' -DDD	0.84±0.07	0.71±0.19
p,p' -DDT	3.81±0.50	3.52±0.32

using the proposed method. The results from the method developed here showed good agreement with those from ultrasonic extraction. The result demonstrates that aging the soils spiked with standard solution during method development is important because it makes them closer to real soils than soil samples that are not aged.

Acknowledgements This work was jointly supported by the Ministry of Science and Technology of the People's Republic of China (2003CB415005) and the Shandong Academy of Sciences.

References

1. Zhou WM, Fu DQ, Sun ZG (1991) *Res Chinese Environ Sci* 4:9–12
2. Bidleman TF, Leone AD (2004) *Environ Pollut* 128:49–57
3. Zhao RS, Chu SG, Zhao RB, Xu XB, Liu XF (2005) *Anal Bioanal Chem* 381:1248–1252
4. Arthur CL, Pawliszyn J (1990) *Anal Chem* 62:2145–2148
5. Boyd-Boland AA, Magdic S, Pawliszyn J (1996) *Analyst* 121:929–938
6. Llompert M, Li K, Fingas M (1998) *Anal Chem* 70:2510–2515
7. Goncalves C, Alpendurad MF (2002) *J Chromatogr A* 963:19–26
8. Goncalves C, Alpendurad MF (2002) *J Chromatogr A* 968:177–190
9. Tomkins BA, Barnard JJ (2002) *Chromatogr A* 964:21–33
10. Buchholz KD, Pawliszyn J (1993) *Environ Sci Technol* 27:2844–2848
11. Zhang Z, Pawliszyn J (1993) *J High Res Chromatogr* 16:689–692
12. Guan F, Watanabe K, Ishii A, Seno H, Kumazawa T, Hattori H, Suzuki O (1998) *J Chromatogr B* 714:205–213
13. Doong RA, Liao PL (2001) *J Chromatogr A* 918:177–188
14. Jin XC, Cheng ZH, Xu NY, Li HS (1990) *Pollution chemistry of organic compounds: chemistry of toxic organic pollutants*. Tsinghua University Press, Beijing
15. Wu GH, Huang MY (1992) *J Forensic Sci* 37:1213
16. Zhang Z, Yang MJ, Pawliszyn J (1994) *Anal Chem* 66:844A
17. Llompert M, Li K, Fingas M (1999) *Talanta* 48:451–459
18. Maria L, Belen B, Rafael C (2000) *J Microcolumn Sep* 12:25–32
19. Berrada H, Font G, Moltoa JC (2000) *J Chromatogr A* 890:303–312
20. Boyd-Boland AA, Pawliszyn J (1995) *J Chromatogr A* 704:163–172