

KKU Res. J. 2012; 17(1):14-24 http://resjournal.kku.ac.th

### Optimization of Headspace Solid-Phase Microextraction Technique for the Determination of Trace Organochlorine Pesticides in Surface Sediments

Amornrat Wongklom\* and Janpen Intaraprasert

Department of Chemistry, Faculty of Science, Ubon Ratchathani University \*Correspondent author: amornrat\_dekarnkon@hotmail.com

> Received November 14,2011 Accepted january 26,2012

### Abstract

Headspace solid-phase microextraction (HS-SPME) with gas chromatography – electron capture detection (GC-ECD) for the determination of trace organochlorine pesticides(OPCs) in sediment was developed. The optimum condition of GC-ECD was as follows. The injector and ECD temperatures were 270°C and 280°C, respectively. A 15-m DB-1 capillary column was held at 100 °C for 2 min, increased to 180 °C at a rate of 10 °C/ min, held for 4 min, and finally ramped to 280°C at a rate of 10 °C/ min, held for 6 min. The nitrogen gas was used as a carrier gas and making up gas at a flow rate of 1.5 mL/min and 28 mL/min, respectively. The HS-SPME technique was used for extraction of the OCPs from sediments samples. The suspension of 0.5-g dried sediment in 1- mL of water was extracted using 100- $\mu$ m PDMS fiber with extraction temperature of 70°C for 60 min with stirring. The addition of other hydrophilic solvents and salt had different effects on the extraction of OCPs . Higher responses of OCPs were obtained when water was added to the sediment. The linear regression was greater than 0.995 (0.004 - 0.4 ng/g, dry weight of sediment). The relative standard deviation was found to be lower than 10% (n=5). Limit of detection was lower than 0.01 ng/g, dry weight. The mean recoveries were between 80 and 99% (2 level of concentration; 0.01 ng/g and 0.1 ng/g). The optimized HS-SPME procedure has been shown to be reliable for fast, accurate and precise monitoring of OCPs in extracted sediment samples. This method has been expanded for application to a wide range of sediment and soil matrices.

Keywords: headspace solid-phase microextraction, organochlorine pesticides, sediment

#### 1. Introduction

The contamination of persistent organic pollutants (POPs) in the environments has become a global concern due to their carcinogenic and multagenic properties. Organochlorine pesticides(OCPs) have been extensively used in past decades against vegetal pests and vectorborne deseases, and have highly contaminated the soil and sediment environments. Several OCPs including HCHs, aldirn/dieldrin, endosulfan and DDTs have been promulgated as POPs or endocrine disrupting chemicals (1). Although they have been prohibited in developed countries since 1970s due to their toxicity and tendency to accumulate in living organisms, they have been recently detected in soils (2). Organochlorines characteristically have very low solubilities in water, fat soluble, and resistant to metabolism. The combination of their persistence in the environment, toxicity and ability to bioaccumulate has caused them to be labiled as environmental hazards (3).

The primary step in soil and sediment analysis involves the separation of OCPs from the matrix. Several methods have been developed to accomplish this often difficult task, including soxhlet extraction (4), ultrasonic solvent extraction (5,6), accelerate solvent extraction (7,8) and microwave extraction (9). The most popular technique usually comprised of extraction, clean-up and analysis, which require a long time, quantities of expensive, toxic solvent that can be harmful to the environment (3). The procedure itself is time-consuming, tedius, and requires pre-concentration of the analytes and remove them from interference components in the matrix prior to chromatographic analysis (10). In comparison with headspace (HS)-SPME, the fiber is exposed in the vapor phase above a liquid or solid sample. In direct immersion (DI)-SPME, the fiber is directly immersed in liquid samples. HS-SPME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in gaseous phase than in the aqueous solution. HS-SPME is especially suitable when used in the analysis of volatile and semivolatile organic compound because these compounds can easily diffuse into the sample headspace from other sample matrices.

In this study eight OCPs, heptachlor, aldrin, endosulfan I, DDE, endrin, endosulfan II, 2,4-DDT and 4,4-DDT were selected as important insecticides that have been concerned and prohibited due to their persistent nature and chronic adverse effect on wildlife and humans. The aim of this work was to develop an efficient multi-residue method on the basis of HS-SPME and gas chromatography with electron capture detection (ECD). For pre-concentration and chromatographic analysis of the selected pesticides. To demonstrate the ability of this method to extract the selected semi-volatile organochlorine pesticides in surface sediment.

#### 2. Materials and Methods

#### 2.1 Reagents and standards

The tested pesticides; heptachlor, aldrin, endosulfan I, DDE, endrin, endosulfan II, 2,4-DDT and 4,4-DDT were purchased from AccuStandard, Inc., New Haven,USA. Stock standard solution of 2,000  $\mu$ g/mL of each compound was prepared in hexane. Working standard solutions were prepared by diluting the stock solution with hexane. The stock and working standard solutions were stored at 4°C. The sediment samples were prepared by spiking with an appropriate amount of the working standard (to be final concentration of 0.04 ng/g). Organic solvent including hexane, methanol and acetonitrile were purchased from LabScan Asia. Sodium chloride was from Carlo Erba (Milan, Italy).

#### 2.2 SPME fibers

SPME holder and fiber assemblies for manual sampling were provided from Supelco (Bellefonte, PA, USA). The fiber coatings assayed were 100- and 7-µm poly (dimethylsiloxane) (PDMS) fiber, 75-µm carboxen (CAR)-PDMS, 85-µm polyacrylate (PA) and 65-µm PDMS-divinylbenzene (DVB). All fibers were conditioned in the hot injector part of the gas chromatograph, according to instructions provided by the manufacture. Then the fibers was repeatedly injected into the GC injector until interfering peak disappeared. During this fiber desorption process the GC column temperature was maintained at 250°C.

### 2.3 Headspace solid-phase microextraction (HS-SPME) procedure

Approximately 0.5-g of dried sediment sample was placed in 15-mL amber vial capped with PTFE-coated septa. Sediment samples were prepared by spiking appropriate amount of the OCPs standard solution (2 ng/mL) to sediments to get the final concentration of 0.04 ng/g-dry weight. The samples were left to evaporate the solvent and added 1-mL of deionized water into the amber vial. Magnetic stirring with a 8-mm long teflon coated stir bar was used to agitate the slurry. Sample vials were equilibrated by water bath at 70°C for 5-min. After that the needle of the SPME device pierced the septum of the vial and the SPME fiber was exposed at 70°C for 60-min to the headspace of the vial and stirred by a small PTFE-coated bar. Finally the fiber was retracted into the needle, pulled out from the vial and inserted into the hot injector port of the GC systems at 270°C for 5-min. Reinserting the SPME fiber after the run was performed to ensure that there was no contaminants on the fiber.



**Figure 1.** Headspace solid-phase microextraction of OCPs from sediment

#### 2.4 Instrumental analysis

Chromatographic analysis was carried out using a Varian 3600 CX gas chromatograph equipped with electron capture detection (ECD). A DB-1 capillary column for OCPs analysis (F&W Scientific, Folsom, CA) of 15mx0.25mm I.D. coated with 0.25  $\mu$ m film thickness was used. The oven temperature was held at 100°C for 2 min, increased to 180 °C at a rate of 10 °C/min, held for 4 min, and finally ramped to 280 °C

at a rate of 10 °C/min, held for 6 min. The GC injector was operated in a splitless mode. Injector and ECD temperature were set at the highest temperature allowable for each SPME fiber and 280 °C, respectively. Nitrogen was used as the carrier gas at 1.5 mL/min and used as make-up gas at 28 mL/min.

#### 3. Results and Discussion

An effective HS-SPME procedure for the determination of OCPs concentrations in sediments, SPME fiber type, extraction temperature and time, amount of sediment, solvent type, liquid volume and the addition of hydrophilic solvent and salt were optimized.

#### 3.1 Selection of SPME fiber coating

Five commercially available SPME fibers (100-,7-µm poly(dimethylsiloxane) (PDMS) fiber, 75-µm carboxen (CAR)-PDMS, 85-µm polyacrylate (PA), 65-µm PDMS-divinylbenzene (DVB) fiber were compared for efficiently determining the OCPs. The extraction temperature and time were 70°C and 30 min, respectively. Fig 2 illustrates the effect of type of SPME fibers on the extraction OCPs. The OCPs with different chemical characteristics showed different extraction behaviors (11). Almost SPME fiber can extract all OCPs except CAR-PDMS. A low adsorption efficiency of the CAR-PDMS fiber was observed for the extraction of 2,4-DDT and 4,4-DDT (11,12,13). Application of the 75-µm carboxen (CAR)-PDMS fiber showed a decrease in signal response of 2,4-DDT and 4,4-DDT because it has a more polar coating and also has a low affinity to the OCPs. When PDMS fiber were used, all OCPs could be effectively extracted with the equilibration time of 30 min. The extraction efficiencies decreased with the

decreasing coating thickness (7-µm PDMS fiber) and 100-um PDMS fiber extracted the highest amounts of OCPs and their metabolites. The OCPs are typically considered as hydrophobic organic compounds. The log values of octanol-water partition coefficients (K\_) range from 3.7 to 7.4 and the water solubilities were from 5 µg/L (4,4-DDT) to 7.3 µg/L (gamma-BHC) (11). Therefore, these analytes would be expected to partition readily into a more non-polar fiber coating rather than a polar fiber such PA, CAR-PDMS, PDMS-DVB. Due to the high capacity of the PDMS-DVB coating to extract volatile compound, some additional peak appeared on the chromatogram of OCPs when applied to sediment extraction. The 100-µm PDMS fiber was selected for the further experiments based on the criteria of the OCPs amount extracted onto the fiber and the reproducibility. SPME fibers  $(K_{snme})$  between stationary phase and analytes, subsequently shifting the sorption equilibria. Moreover, elevated temperatures can decrease the partition coefficient of OCPs in soil particles (11). Extraction temperature should be optimized since it controlled the diffusion rate of the OCPs into the fiber coating. Fig 3 illustrated the effect of extraction temperature of OCPs adsorbed by a 100-µm PDMS fiber with an extraction time of 30 min at temperature ranging from 40 to 95°C. An increase in extraction efficiency of OCPs was observed when temperature increased, the increase of extraction temperature decrease the partition coefficient between OCPs and sediment particle and increasing the desorption rates of OCPs from the surface of sediment particle to solution. The elevated temperature increase the diffusion of the OCPs from the



**Figure 2.** Effect of five SPME fiber, extraction temperature 70oC, extraction time 30 min, addition of water 5 mL

#### 3.2 Effect of extraction temperature

Sample temperature has an influence on the extraction efficiency. Higher temperatures can decrease the diffuse coefficient of analytes in water and shorten the extraction time (9,11). Also, elevated temperatures decrease the distribution coefficient of solution to gaseous phase. However, a decrease in sensitivity was observed for heptachlor and aldrin when the extraction temperature exceed 70°C. These compounds have relatively high vapor pressures;  $3x10^{-4} - 6.3x10^{-6}$ mmHg, 1 mmHg = 133.3 Pa (11). Since the adsorption of the target analytes by the SPME fiber is an exother-



Figure 3. Effect of extraction temperature, using 100-  $\mu$ m PDMS fiber, extraction time 30 min, addition of water 5 mL

mic process. The SPME distribution coefficients ( $K_{spme}$ ) between coating materials and analytes also decrease at the elevated temperature (14) and extraction disfavored at high temperature. Thus, the optimum extraction efficiency was achieved at 70°C and selected for the further experiments.

#### 3.3 Effect of extraction time

The extraction time required to reach the equilibrium at  $70^{\circ}$ C between the 100-µm PDMS fiber and the sediment sample was studied. Fig 4 illustrated

the effect of equilibration time on the extraction of the OCPs target analytes, in the range of 15 to 120 min. The difference in response depended on the volatilities, distribution coefficients and structure of the OCPs (1). The short equilibrium time for heptachlor, aldrin (60 min) may be due to the high vapor pressure at 70°C. The long equilibrium time for endrin, endosulfan I, DDE, endrin, endosulfan II, 2,4-DDT and 4,4-DDT (120 min) are due to the low water solubilities and high  $K_{ow}$  values of these compounds. The higher response was



**Figure 4.** Effect of extraction time, using 100-µm PDMS fiber, extraction temperature 70oC, addition of water 5 mL

observed when using long equilibrium time for all OCPs (120 min). However, the extraction time of 120 min is too long for HS-SPME procedures to extract OCPs and no significant difference in sensitivity between the extraction time of the OCPs for 60 and 120 min were observed. Also, the response of some OCPs including heptachlor and aldrin decreased after 60 min. Thus, the extraction time of 60 min was selected to perform the sediment sample analysis.

#### 3.4 Effect of sample weight

The partition of semi-volatile organic compounds between the sediment and the headspace is generally low, and the total sample amounts would influence the release rate of OCPs from the sediment matrix. Thus, amounts of sediment samples were varied from 0.5 to 2.0 g that were added into the amber vial containing 5-mL of deionized water. Fig 5 illustrated the effect of sediment weight on the extraction efficiency of OCPs by the HS-SPME at 70°C. The response of OCPs increased upon decreasing the amount of sediment sample, 0.5 g of sediment showed the highest response. The high loading of sediment hampered the release of OCPs from sediment to water, subsequently the response of OCPs in headspace decreased. Because of the turbulence level in the sediment was low and the viscosity was high, the time required for equilibration and extraction of OCPs from solid to gaseous phase was long when high amount of sediment was used. The results showed that small amounts of sediment sample were needed and sufficient for HS-SPME analysis. Thus, sample amount of 0.5 g was selected for the further experiments.

## 3.5 Effect of the addition of the different solvent to the sediment matrix

The addition of solvent can enhance the release of target analytes from the matrix. Therefore, the effect of solvent was investigated. The experiments were done at 70°C after addition 5 mL of water and different type of solvents such as methanol, methanol:water (1:1), acetone, acetone:water (1:1) to 0.5 g of spiked sediment sample. Sediment samples were extracted for 60 min using a 100-µm PDMS fiber. Fig 6 shows the response for all OCPs after add-ing of different solvents. The highest response was



**Figure 5.** Effect of sample weight, using 100-µm PDMS fiber, extraction temperature 70oC and extraction time 60 min, addition of water 5 mL

obtained when 5 mL of water was added to the spiked sediment while the response of OCPs with the presence of methanol:water(1:1), acetone:water(1:1), methanol and acetone were not observed. The enhanced sensitivity obtained when adding water to sediment could be due to the displacement of analytes from the active sites in the sediments and to the low solubility of the target analyte in water (2). Therefore, the addition of water was selected to the further experiments. dilute the concentration of the OCPs and increase the diffusion barrier of the OCPs from aqueous phase to gaseous phase (11). Therefore, amount of 1-mL water was selected for the further experiments.

# 3.7 Effect of the addition of hydrophilic solvent and salt

The effect of the addition of hydrophilic solvent (methanol and acetonitrile) and salt to the samples was studied. Fig 8 shows the higher response



Figure 6. Effect of the addition of the different solvent to the sediment matrix(0.5 g), using 100-μm PDMS fiber, extraction temperature 70°C and extraction time 60 min

#### 3.6 Effect of liquid volume

The partitioning of semi-volatiles organic compounds between the soil and the headspace is generally low, and the addition of water can enhance the release of volatile organic compounds(VOCs) from the soil matrix (11). To increase the release of OCPs from sediment, different volumes of water ranging from 1 to 10 mL were added into 0.5 g of sediment. Fig 7 illustrates the effect of water/sediment ratio on the extraction efficiency of OCPs using the HS-SPME procedure at 70°C for 60 min. The response of OCPs increased with the decreasing water/sediment ratio. A highest response was obtained when 1 mL of water was added to 0.5 g sediment, the addition of higher amount of water would observed in heptachlor, aldrin, endosulfan I, 4,4-DDE and endosulfan II when adding the hydrophilic solvent (1% v/v) but the response of endrin, 2,4-DDT and 4,4-DDT slightly decreased. The poor response was observed when adding NaCl solution(1% w/v). This study showed that no increase in the response was observed after the addition of salt. Since the OCPs analytes are very low polarity (15). In contrast with other reports, the addition of salt to aqueous sample is frequently used to enhance the sensitivity of HS analysis of polar compounds; for non-polar compound this effect is expected to be insignificant (14,15). The final proposed method for the simultaneous extraction of the OCPs was: HS-SPME of 0.5 g sediment sample (0% hydrophilic solvent and



and extraction time 60 min, addition of water into sediment sample 0.5g



Figure 8. Effect of the addition of hydrophilic solvent and salt, using 100-μm PDMS fiber, extraction temperature 70°C and extraction time 60 min, addition of water into sediment sample 0.5g

NaCl) with addition of 1 mL of water, using  $100-\mu m$ PDMS fiber at  $70^{\circ}C$  for 60 min with stirring.

#### **3.8 Analytical Performance**

The repeatability of HS-SPME procedure was obtained by analyzing five replicate spiked sediment samples consecutively at two concentration levels (0.01 ng/g and 0.1 ng/g). The %RSD obtained was between 1.41 and 7.98%, and the precision of this method was good. The limit of detection (LOD) was evaluated by comparing the signal to noise ratio (S/N) of the lowest concentration to a S/N = 3. The results showed that the method allowed detection of OCPs in sediment samples at concentration lower than 0.01 ng/g.

Series of six concentration levels were obtained by spiking sediment sample with all the organochlorine pesticides in a concentration ranging from 0.004 to 0.4

KKU Res. J. 2012; 17(1)

ng/g, dry weight. Each solution was analyzed in five replicates. The linearity of the method has been investigated over the range of 0.004 - 0.4 ng/g and the eight OCPs had correlation coefficients of the calibration graphs greater than 0.995. The mean recoveries obtained for the eight organochlorine pesticides spiked in sediment samples. The recovery was determined as the peak area of real sample spiked with analyte at two concentration levels. The recoveries of all analyte ranged between 80 and 99%. This demonstrates that HS-SPME with polydimethylsiloxane is a simple, fast, solvent free, inexpensive, precise and accurate technique for quantitative determination of the OCPs in river sediment samples when compared to the conventional method. The conventional method such as soxhlet extraction, ultrasonic solvent extraction and accelerate solvent extraction that analytical procedures usually comprised of extraction, clean-up and analysis with drawbacks including time, solvent consumption, expensive and both labor-and time-consuming because typical sediment samples cannot be directly analyzed by the chromatographic techniques (3,14). With the HS-SPME technique, the limitations of the conventional technique can be removed. Optimization of the HS-SPME parameter affecting the method sensitivity should be developed in order to enable increase in the amount of most OCPs and to improve the limit of detection. The combination of HS-SPME with GC-ECD can be achieved very low limits of detection (0.002-0.004 ng/g-dry weight), as the total amount of extracted analytes is used for the determination. Thus the HS-SPME-GC-ECD can be considered as an alternative for the determination of the OCPs in sediment samples and can be verified without difficulty.

#### 3.9 Application to real sediment samples

The sediment samples from Moon river were determined the OCPs using the developed HS-SPME technique. These samples were collected from six stations, during November 2010 to September 2011. The contamination of heptachlor, aldrin, endosulfan I, endosulfan II, 4,4-DDE, endrin, 2,4-DDT and 4,4-DDT were in the range of 0.0022 to 0.5212 ng/g, 0.0060 to 0.0097 ng/g, 0.0025 to 0.0079 ng/g, 0.0063 to 0.0090 ng/g, 0.0027 to 0.0214 ng/g, 0.0040 to 0.0054 ng/g, 0.0045 to 0.0079 ng/g, 0.0040 to 0.0066 ng/g, respectively. The quantities of the OCPs found in sediment samples were not over the soil and sediment quality standard by the Pollution Control Department.

#### 4. Conclusion

Headspace solid-phase microextraction technique is a fast, inexpensive and solvent free technique that has been proved accurate in the analysis of organochlorine pesticides in sediment and soil. HS-SPME-GC-ECD procedure has been optimized using a 100-µm PDMS fiber for extraction of the OCPs in real samples. HS-SPME extraction was finally carried out using the extraction time of 60 min at the extraction temperature 70°C, maintaining a stirring of the slurry, then desorption in the GC injector was kept at 270°C for 5 min. The optimized HS-SPME procedure has been shown to be reliable for fast, accurate and precise monitoring of OCPs in the river sediment samples.

#### 5. Acknowledgement

We are grateful to the Chemistry program, Faculty of Science, Ubon Ratchathani Rajabhat University and Department of Chemistry, Faculty of Science, Ubon Ratchathani University for financial support and providing us with the facilities for OCP analyses.

#### 6. References

- Chang S, Doong R. Concentration and fate of persistent organochlorine pesticides in estuarine sediments using headspace solid-phase microextraction. Chemosphere. 2006;62: 1869-1878.
- (2) Alvarez MF, Lloblmpart M, Lamas JP, Lores M, Jares CG, Cela R, et al. Simultaneous determination of traces of pyrethroids, organochlorines and other main plant protection agents in agricultural soils by headspace solidphase microextraction-gas chromatography. Journal of Chromatography A. 2008;1188: 154-163.
- (3) Magdic S, Pawliszyn J. Analysis of organochlorine pesticides using solid –phase Microextraction. Journal of Chromatography A. 1996;723: 111-122.
- (4) Fatoki OS, Awofolu RO. Methods for selective determination of persistent organochlorine pesticide residues in water and sediments by capillary gas chromatography and electron-capture detection. Journal of Chromatography A. 2003;983: 225-236.
- (5) Castro J, Brunete CS, Tadeo JL. Multiresidue analysis of insecticides in soil by gas chromatography with electron-capture detection and confirmation by gas chromatography-mass spectrometry. Journal of Chromatography A. 2001;918: 371-380.
- (6) Vagi MC, Petsas AS, Kostopoulou MN, Karananoli MK, Lekkas TD. Determination of organolchlorine pesticides in marine sediments samples using ultrasonic solvent extraction followed by GC/ECD. Desalination. 2007;210: 146-156.

- (7 Yehouenou E, Pazou A, Boko M, Cornelis AMG, Ahissou H, Laleye P, et al. Organochlorine and organophosphorus pesticide residues in the Oueme River catchment in the Republic of Benin. Environment International. 2006;32: 616-623.
- (8) Keithmaleestti S, Varanusupakul P, Siriwong W, Thirakhupt K, Robson M, Kitana N. Contamination of Organchlorine Pesticides in Nest soil, Egg, and Blood of the Snail-eating Turtle (Malayemys macrocephala) from the chao Phraya River Basin, Thailand. World Academy of Science, Engineering and Technology. 2009;52: 444-449. Thai.
- (9) Lambropoulou DA, Alanis TA. Optimization of headspace solid phase microextraction conditions for the determination of organophosphorus insecticides in natural waters. Journal of Chromatography A. 2001;922: 243-255.
- (10) Wurl O, Obbard JP. Organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in Singapore's coastal marine sediments. Chemosphere. 2005;58: 925-933.
- (11) Doong R, Liao PL. Determination of organochlorine pesticides and their metabolites in soil samples Using headspace solid-phase microextraction. Journal of Chromatography A. 2001;918: 177-188.
- (12) Wongklom A, Intaraprasert J. Determination of organochlorine pesticides residues in sediment from Shi river and Moon river by gas chromatography. Journal of Srinakharinwirot University. 2011; 3 suppl 1: 20-26. Thai.

- (13) Wongklom A, Intaraprasert J. Contamination of organochlorine pesticides in sediment from Shi and Moon river, Thailand. Loas Journal on Applied Science. 2011;2(1): 822-827.
- (14) Santos FJ, Sarrion MN, Galceran MT. Analysis of chlorobenzenes in soils by headspace solid-phase microextraction and gas chromatography-ion trap mass spectrometry. Journal of Chromatography A. 1997;771: 181-189.
- (15) Llompart M, Li K, Fingas M. Headspace solid-phase microextraction (HS-SPME) for the determination of volatile pollutants in soils. Talanta. 1999;48: 451-459.