การหาสภาวะเหมาะสมและทดสอบการใช้ได้ของวิธีการสกัดระดับไมโครด้วยเฟสของแข็งบริเวณเฮดสเปซเพื่อวิเคราะห์ปริมาณสารฆ่าแมลงกลุ่มออร์กาโนคลอรีนในตะกอนดินโดยวิธีแก๊สโครมาโทกราฟี

Optimization and Validation of a Headspace Solid-Phase Microextraction for the Determination of Organochlorine Pesticides in Sediment by Gas Chromatography

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บทคัดย่อ

งานวิจัยนี้ได้ศึกษาสภาวะเหมาะสมและทดสอบการใช้ได้ของวิธีการสกัดระดับไมโครด้วยเฟสของแข็งบริเวณเฮดสเปซเพื่อวิเคราะห์ปริมาณสารฆ่าแมลงกลุ่มออร์กาโนคลอรีน (เอ็นดริน เอ็นโดซัลแฟน I เอ็นโดซัลแฟน II เฮปตาคลอร์ 4,4-ดีดีอี 4,4-ดีดีที) ในตัวอย่างตะกอนดินโดยวิธีแก๊สโครมาโทกราฟี ศึกษาปัจจัยที่มีผลต่อประสิทธิภาพการสกัด ได้แก่ ชนิดของไฟเบอร์ อุณหภูมิและเวลาการสกัด และผลการเติมตัวทำละลายมีขั้ว พบว่า สภาวะเหมาะสมของการสกัด ได้แก่ สกัดด้วยไฟเบอร์ชนิด PDMS 100 ไมโครเมตร ควบคุมอุณหภูมิ 70 องศาเซลเซียส เป็นเวลา 60 นาที ผลการเติมตัวทำละลายมีขั้วต่างชนิดกันจะมีประสิทธิภาพการสกัดแตกต่างกัน โดยจะให้สัญญาณการวิเคราะห์ของสารเป้าหมายเกือบทุกชนิดสูงขึ้น ในขณะที่สัญญาณการวิเคราะห์ของสารเฮปตาคลอร์จะลดลงเล็กน้อย วิธีวิเคราะห์นี้เป็นวิธีที่มีประสิทธิภาพ ค่าความเป็นเส้นตรงจะมีพฤติกรรมเป็นเส้นตรงที่ดีในช่วง 0.004-0.4 นาโนกรัม/กรัมของน้ำหนักแห้งโดยให้ค่าความสัมพันธ์เชิงเส้นมีค่าอยู่ระหว่าง 0.9952 ถึง 0.9992 ขีดจำกัดต่ำสุดของการวัดประเมินจากความเข้มข้นของสารที่ให้สัญญาณเป็น 3 เท่าของสัญญาณรบกวนซึ่งมีค่าระหว่าง 0.002 ถึง 0.004 นาโนกรัม/กรัม ความแม่นยำของการทวนซ้ำมีค่าเป็นที่น่าพอใจคือ 2.37-7.98% (ทำการวิเคราะห์ 5 ซ้ำ) ค่าเปอร์เซ็นต์การได้กลับคืนมีค่าอยู่ระหว่าง 83.66 ถึง 99.02% วิธีนี้เป็นวิธีที่น่าเชื่อถือในด้านความรวดเร็วในการวิเคราะห์ ผลการวิเคราะห์มีความแม่นและความเที่ยง ซึ่งแสดงให้เห็นว่าวิธีการสกัดระดับไมโครด้วยเฟสของแข็งบริเวณเฮดสเปซสามารถนำมาใช้เพื่อวิเคราะห์ปริมาณสารฆ่าแมลงกลุ่มออร์กาโนคลอรีนในตัวอย่างดินและตะกอนดินได้อย่างมีประสิทธิภาพ

คำสำคัญ : สารฆ่าแมลงกลุ่มออร์กาโนคลอรีน ตะกอนดิน วิธีการสกัดระดับไมโครด้วยเฟสของแข็งบริเวณเฮดสเปซ

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Abstract

A headspace solid-phase microextraction (HS-SPME) method for the determination of organochlorine pesticides (OCPs ; endrin, endosulfan I, endosulfan II, heptachlor, 4,4-DDE, 4,4-DDT) in sediment samples by gas chromatography has been optimized and validated. The fiber selection, extraction temperature and time, and the addition of hydrophilic solvent parameters on influence the extraction efficiency were studied. The study conditions were: a 100-μm polydimethylsiloxane(PDMS) fiber, 70°C of controlled extraction temperature, 60 min of extraction time; the addition of hydrophilic solvents have different effects on the extraction efficiency. The higher responses of the analytes were obtained when hydrophilic solvents were added to the sediment while the response of heptachlor slightly decreased. This method showed a good performance. The linearity showed a good linear behavior in the range of 0.004-0.4 ng/g-dry weight, with correlation coefficients ranging between 0.9952 to 0.9992. The limit of detection (LOD) was estimated as the analyte concentration that produced a peak signal of three times the background noise. The detection limit ranged from 0.002 to 0.004 ng/g. The repeatability (2.37-7.98%) was shown to be satisfactory (n=5). The recovery ranged from 83.66 to 99.02%. The optimized procedure has been shown to be fast, accurate and precise. The results indicate that HS-SPME-GC-ECD can be considered for the determination of OCPs in soil and sediment environments.

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

Introduction

Organochlorine pesticides (OCPs) were extensively used as pest control chemicals to increase agricultural production. In Thailand, persistent insecticides like HCHs, DDTs, aldrin and dieldrin were used in large quantities for agriculture and public health (Boonyatumanond *et al*., 2002). They are discharged into riverine and estuarine environments through different input pathways, such as discharge of domestic sewage and industrial wastewater, runoff from nonpoint sources, and atmospheric deposition (Doong *et al*., 2002). In 1983 the usage of some imported pesticides such as DDT were banned. Endrin, aldrin, dieldrin and heptachlor were banned in 1988. Although OCPs were banned, based on their mutagenic, carcinogenic and endocrine disrupting properties. They still have been used in agriculture and domestic activity. Therefore, they still have been contaminated and detected in environmental samples such as soil, sediment and water from main rivers and agricultural areas due to their persistence and lipophilic properties (Siriwong *et al*., 2009; Lesueur *et al*., 2008; Chang *et al*., 2006). Sediment is one of the principle reservoirs of environmental pesticides. Residue can be released to the groundwater and living organisms. Thus, it is important to monitor and analyze OCPs residues in sediments that serve as the primary sink for a majority of pesticides used in agriculture.

Ideally, a sample preparation and analysis of OCPs in soil and sediment should be rapid, simple, cheap, and environmental friendly and provide clean extracts (Lesueur *et al*., 2008). Many traditional methods have been proposed for the separation and analysis of OCPs in sediment samples such as soxhlet extraction, ultrasonic solvent extraction, accelerate solvent extraction, microwave extraction and the others. These analytical procedures comprised of three steps: extraction, clean-up and analysis. The drawbacks include time and solvent consumption. However, these procedures are usually expensive and both labor-and time-consuming because typical environmental samples cannot be directly analyzed by the chromatographic techniques (Keithmaleesatti *et al*., 2009; Siriwong *et al*., 2009; Doong *et al*., 2002).

Headspace solid-phase microextraction (HS-SPME) is a technique that allows simultaneous extraction and preconcentration of analytes from sample matrix (Gonzalez *et al*., 2007). Several advantages of SPME include low price, being solvent-free, using the whole sample for analysis, and being a convenient and simple analytical method. The fiber was not directly contact the matrix sample can be pointed out when this technique is compared to the conventional method (Kataoka *et al*., 2000). The SPME device consists of the syringe assembly and fiber assembly. A fused-silica fiber has been coated with a liquid polymeric phase, allowing adsorption of the analyte according to their affinity toward the fiber coating. The analytes are thermally desorbed from the fiber in the hot injector of a gas chromatograph and are subsequently analyzed (Magdic *et al*., 1996). HS-SPME has been widely accepted for the determination of volatile and semivolatile organic compounds in different samples. Many authors have proposed HS-SPME methods for the determination of chlorobenzene in soil (Santos *et al*., 1997), trihalomethane and BTEX in drinking water (Ezquerro *et al*., 2004), volatile and semivolatile pollutants in soil (Llompart *et al*., 1999), organophosphorus insecticide in natural water and environmental samples (Lambropoulou *et al*., 2001; Magdic *et al*.,1996), pyrethroids, organochlorine pesticide and other main plant protection agents in agricultural soil and sediment (Chang *et al*., 2006; Alvarez *et al*., 2008; Doong *et al*., 2001). However, there is no official guideline or regulations for OCPs in river sediment in Thailand, and the information on pollution potential of OCPs in river sediment is still limited. The new method in routine laboratory needs to be optimized and validated before being used for official purposes.

The aim of this study was to optimize and validate an extraction efficiency of HS-SPME for six selected OCPs

from sediment samples. Six OCPs including endrin, endosulfan I, endosulfan II, heptachlor, 4,4-DDE and 4,4-DDT pesticides, were selected as the model compounds. The HS-SPME method was optimized with variables involving SPME fiber selection, extraction temperature and time and the addition of hydrophilic solvent. The OCPs were identified and quantified by GC-ECD.

Materials and Methods

Materials

All OCPs analytical standards (endrin, endosulfan I, endosulfan II, heptachlor, 4,4-DDE, 4,4-DDT) were purchased from AccuStandard, Inc., New Haven, USA. An individual stock standard solution of 2000 mg/l was prepared in hexane by exact weighing of high-purity substance. A mixture was daily prepared in hexane containing 1.0 mg/l each individual pesticide. A working solution containing all studied OCPs at a concentration of 20 µg/l in hexane was prepared daily. Five kinds of SPME fibers, 7-µm and 100-µm poly (dimethylsiloxane) (PDMS) fiber, 75-µm carboxen (CAR) - PDMS, 85-µm polyacrylate (PA) and 65-µm PDMS-divinylbenzene (DVB) fiber, solid-phase microextraction system and 15-ml amber vials capped with PTFE-coated septa were purchased from Supelco Co., Inc. (Supelco, Bellefonte, PA, USA). All chemicals and solvents were analytical reagent grade; purchased from Sigma Chemical Co. Deionized water was obtained from a Milli-Q water purification system.

HS-SPME procedures

All fibers were conditioned in the injection port of the gas chromatograph for 0.5-2 hr at 250-300°C according to the instruction of supplier before use. A manual solid-phase microextraction system was used for extracting OCPs. The HS-SPME was performed by placing approximately 0.5-g of seived sediment sample and 1-ml of deionized water into 15-ml amber vials capped with PTFE-coated septa. A 8-mm long teflon coated stir bar was used to agitate the slurry. The samples were equilibrated into water bath controlled temperature at 70°C for 5 min before extracting the OCPs. The 100-µm PDMS fiber was immersed into the headspace above the sample. The OCPs adsorbed on the liquid polymeric stationary phase of the fibers at the extraction temperature of 70°C for 60 min. The sample agitation was used at 500 rpm. After extraction, the fiber was thermally desorbed for 5 min into the glass liner of the GC injector at 270°C and analysis by GC-ECD

Gas chromatographic analysis and Validation

The chromatographic analyses were carried out by Varian Star 3600 CX gas chromatograph (GC) equipped with an electron capture detector (ECD). High-purity nitrogen gas at a constant flow rate of 1.5 ml/min and 28 ml/min was used as the carrier gas and make-up gases, respectively. A 15-m DB-1 capillary column (0.25 mm inner diameter, 0.25 µm film thickness (F&W Scientific, Folsom,CA) was used for separating OCPs. The column oven temperature program 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 at 270°C. The temperature of ECD was 280°C.

The linearity of the method was tested by extracting six spiked sediment samples range from 0.004 to 0.4 ng/g. The accuracy was estimated by means of recovery experiments, analyzing sediment samples (n=5) spiked at 0.01 and 0.1 ng/g–dw. The limit of detection (LOD) was estimated as the analyte concentration that produced a peak signal of three times the background noise from the chromatogram at the lowest fortification level tested.

Results and Discussion

Optimization of HS-SPME

An effective HS-SPME procedure for the determination of 6-OCPs concentrations in sediments, fiber selection, extraction temperature and time, the addition of hydrophillic solvents were optimized.

The experiments for the selection of the fiber were carried out in the GC-ECD. A sediment sample of 0.5 g into 5 ml of deionized water and 30 min of extraction time with constant agitation at 70°C were used. Desorption of the fiber was carried out at 270°C for 5 min. Five different fibers, 7-μm and 100-μm poly (dimethylsiloxane) (PDMS) fiber, 75-μm carboxen (CAR) - PDMS, 85-μm polyacrylate (PA) and 65-μm PDMS-divinylbenzene (DVB) fiber were evaluated for the extraction of studied OCPs. The sensitivity of each fiber is different depending on the molecular mass and polarity of the analytes to be extracted (Lambropoulou *et al*., 2006; Alvarez *et al*., 2008). Results are shown in Figure 1. All fibers showed a response to all OCPs. A poor adsorption efficiency of the CAR-PDMS fiber was observed for the extraction of 4,4-DDT due to it has low vapor pressure. The 100-μm PDMS fiber obtained high efficiency for all OCPs corresponding to the authors report that the 100-μm PDMS and 65-μm PDMS-DVB fiber showed good extraction efficiency for OCPs (Doong *et al*., 2001; Gonzalez *et al*.,2007). Therefore, the 100-μm PDMS fiber was selected for further optimization experiments.

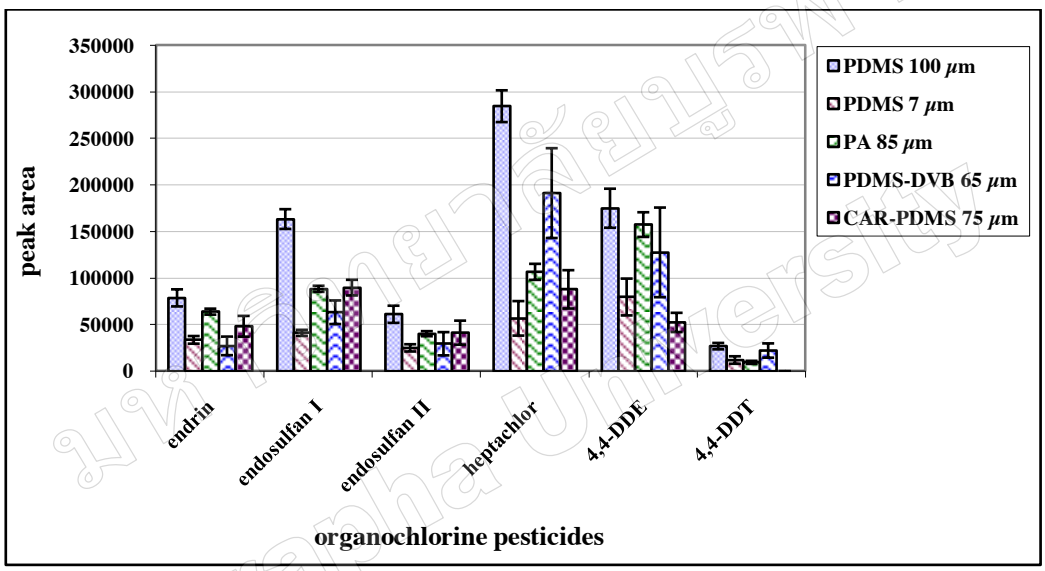

Figure 1 Effect of the different coating fiber on the HS-SPME efficiency

The change in extraction temperature can influence the extraction efficiency of OCPs. Figure 2 illustrates the signals of OCPs adsorbed by a 100-μm PDMS fiber with an extraction time of 30 min with temperature range of 40-95°C. Generally, the increase in temperature increased the extraction efficiency of OCPs, due to the decrease in the partition coefficient (Kp) between OCPs and sediment particles. The elevated temperature significantly enhanced the mass transfer and diffusion rates of the OCPs from solid phase to aqueous solution, and then to gaseous phase (Chang *et al*., 2006; Kataoka *et al*., 2000). Therefore, an increase in temperature produced an improvement in the extraction efficiency for most of the OCPs. On the other hand, the extraction efficiency of a few OCPs (heptachlor) slightly decreased when the temperature was higher than 70°C. The decrease of OCPs response may be due to it has high vapor pressure. The adsorption of analyte by the fiber is an exothermic process, a high temperature could decrease the SPME distribution coefficients of analyte (Chang *et al*., 2006; Doong *et al*., 2001). Thus, 70°C was selected as the optimum extraction temperature for further experiments.

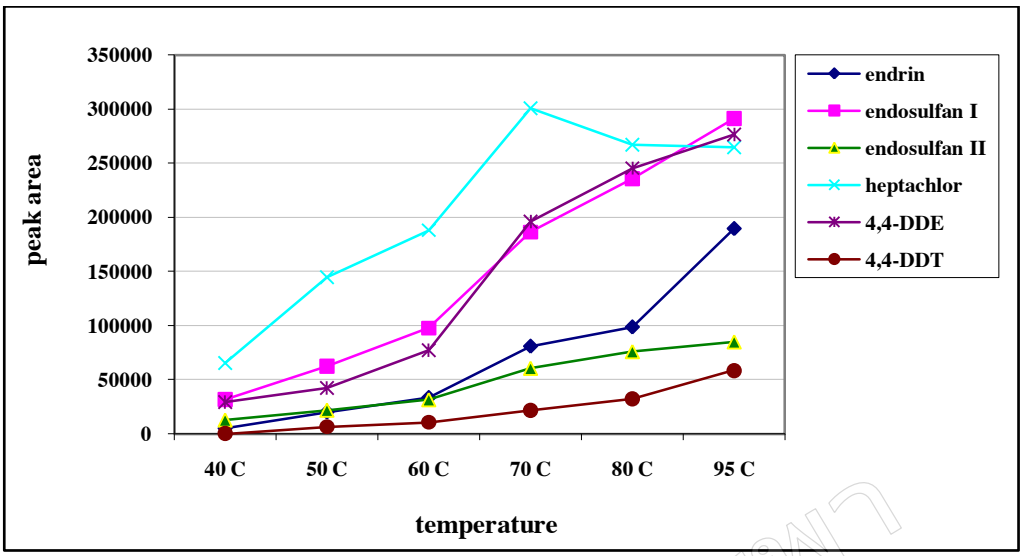

Figure 2 Effect of the extraction temperature on the HS-SPME efficiency, using a 100-µm PDMS fiber and extraction time was 30 min

The HS-SPME technique is an equilibrium process of the analyte between the vapor phase and fiber coating, the extraction time required to reach the equilibrium between the fiber stationary phase and the sediment sample was determined. The analytes with high molecular mass are expected to need longer equilibrium times, due to their lower diffusion coefficient (Lambropoulou *et al*., 2001; Santos *et al*., 1997). It is important to determine the time required reaching the equilibrium. The extraction time was determined in the ranged of 15 to 120 min, at 70°C. Figure 3 shows the difference in response depended on the volatilities, distribution coefficients and structure of the OCPs. The short equilibrium time for heptachlor (60 min), the responses slightly decreased after 60 min, may be due to the high vapor pressure at 70°C. The long equilibrium time for endrin, endosulfan I, endosulfan II, 4,4-DDE and 4,4-DDT (120 min) are due to the low vapor pressure of these compounds. The higher response was observed when using long equilibrium time (120 min) because the partition coefficient between the coating and headspace increased for all OCPs. However, the extraction time of 120 min was so long time for HS-SPME procedures to extracting the analytes. An extraction time of 60 min was selected to perform the sample analysis.

The effect of the addition of hydrophilic solvents and salt on the HS-SPME efficiency was studied. Figure 4 shows the responses of adding water as well as the responses obtained after adding 10 µl of hydrophilic solvents into 0.5 g of the sample matrix. The addition of hydrophilic solvents (methanol, acetonitrile and diethyl ether) the response increased, such as endosulfan I, endosulfan II, heptachlor and 4,4-DDE. The hydrophilic solvents are the displacement of the analytes from the active sites in the soil. The active sites are usually polar functional groups that have more affinity for polar compounds than for less polar ones. The polar compounds added to the soil will substitute, at least in part, the OCPs on these active sites and in consequence, They will be released into the headspace (Llompart *et al*., 1999; Ezquerro *et al*., 2004). However, these added solvents would also decrease the response of certain OCPs, like endrin and 4,4-DDT. The effect of the addition of salt to the sediment samples was also studied. A 1% (w/v) of NaCl was added to each vial of the samples and HS-SPME was performed. No increase in the response was observed after addition of salt corresponding to the authors reported that the aliquot of salt (NaCl ,KCl) was added to the soil samples. No significant enhancement in sensitivity of VOCs

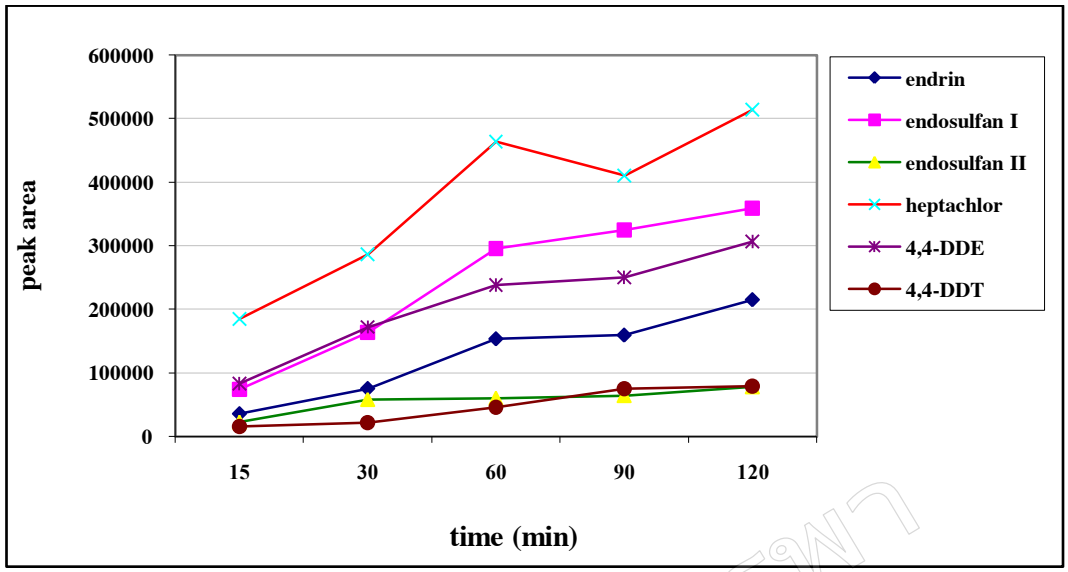

Figure 3 Effect of the extraction time on the HS-SPME efficiency, using a 100-μm PDMS fiber and extraction temperature was 70°C

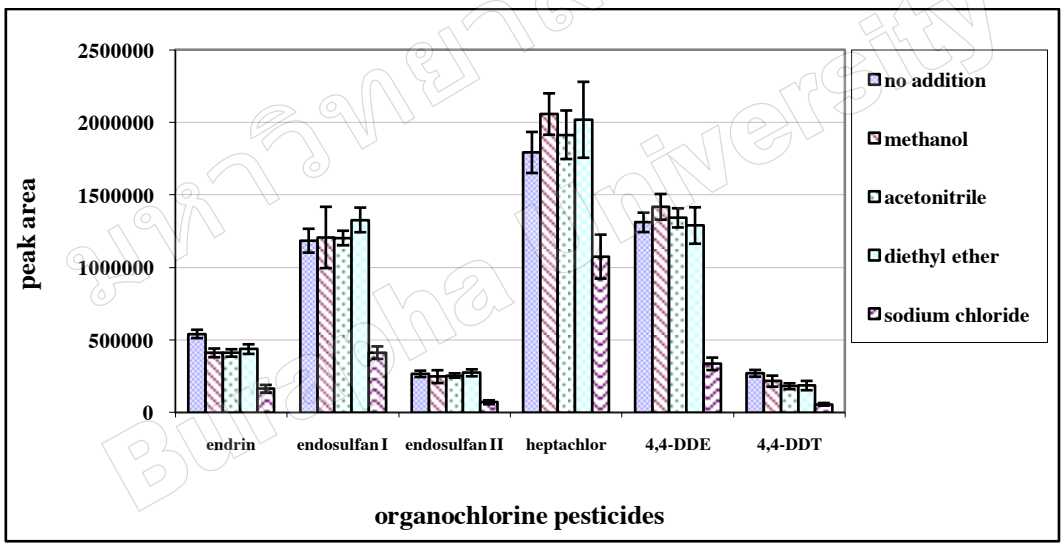

Figure 4 Effect of addition of the different hydrophilic solvent and salt on the HS-SPME efficiency, using a 100-μm PDMS fiber, extraction temperature was 70°C for 60 min

should be expected after the addition of salt (Llompart *et al*., 1999; Alvarez *et al*., 2008). Because the addition of hydrophilic solvents and salt would also decrease the response of endrin and 4,4-DDT. While the ECD response of them was lower than the other OCP. DDT was mainly usage in agriculture. Therefore, addition of 1-ml of water into the 0.5-g sediment without the addition of hydrophilic solvent and salt was selected for the experiment.

The desorption temperature in the GC injector must be enough high to desorb all OCPs but it is necessary to take into account the stability and lifetime of the fiber, and the decomposition of some OCPs when the desorption temperature is too high (Gonzalez *et al*., 2007). For this reason, a relatively low desorption temperature was selected (270°C). To ensure the complete desorption of the analytes and avoid the carryover, a fiber exposure

time in the injection port of 5 min was chosen.

The intensity of stirring is one important parameter that affects the time profile. For headspace, stirring should be vigorous and has to be maintained constant in all experiments (Lambropoulou *et al*., 2001). The actual stirring required depends on the dimensions of the vial (15 ml) and the magnetic stirring bar (8 mm). Stirring the sample has an important repercussion on the kinetics, speeds up the equilibrium process and the response obtained with the sample extraction time is higher with stirring than with out it (Gonzalez *et al*., 2007). In this work, sediment sample were stirred during the extraction time with a 8-mm long teflon coated stir bar at 500 rpm.The response with stirring was about 2-3 times higher than without stirring for all OCPs.

The validation of HS-SPME-GC-ECD

The analytical performance of HS-SPME-GC-ECD; the linearity, accuracy, repeatability and limits of detection (LODs) of the OCPs were determined. The results were showed in Table 1. The limit of detection (LOD) ranged from 0.002 (endosulfan I, heptachlor, 4,4-DDE) to 0.004 ng/g (endrin, endosulfan II, 4,4-DDT) for sediment analysis.

The linearity of the method was tested by extracting the OCPs in the gas phase. The concentration applied was in the range of 0.004 to 0.4 ng/g–dry weight of sediment at six concentration levels (0.004, 0.008, 0.02, 0.08, 0.2 and 0.4 ng/g). The results have shown the all studied pesticides presented a good linear behavior in the whole range studied, the linearity range between 0.004 and 0.4 ng/g. The correlation coefficients (r^2) of endrin, endosulfan I, endosulfan II, heptachlor, 4,4-DDE and 4,4-DDT were 0.9985, 0.9973, 0.9952, 0.9988, 0.9986 and 0.9992, respectively.

Repeatability was determined by performing five consecutive extractions with concentration of 0.01 ng/g. The repeatability was calculated as within-day RSD of peak areas using 5 replicates analyzed in the same day and by the same analyst. Results for repeatability showed the good precision of the method with values as relative standard deviation (%RSD) between 2.37 (4,4-DDT) and 7.98% (endosulfan I).

The accuracy was investigated using sediment matrix at two concentration levels of 0.01 and 0.1 ng/g–dry weights. Analytical recoveries were assessed by comparing the chromatogram of calibration standards in samples with spiked sediment samples. The results obtained (n=5) are presented in Table 1. For most of the compounds in the matrix at that concentration level, the mean recovery values (%) were between 83.66 and 91.16% for 0.01 ng/g-dw, and 88.94 and 99.02% for 0.1 ng/g-dw. These results indicate that HS-SPME show a good performance for extraction the OCPs in sediment when compared

Table 1 Analytical performance for the determination of 6-OCPs in sediment samples by HS-SPME-GC-ECD

OCPs	Linear range (ng/g)	Linearity (6 level)	repeatability (%RSD, n=5)	Mean Recovery (n=5)		LOD (ng/g)
				Spiked 0.01 ng/g	Spiked 0.10 ng/g	
Endrin	0.004 – 0.400	0.9985	7.76	83.66	88.94	0.004
Endosulfan I	0.004 – 0.400	0.9973	7.98	89.00	94.12	0.002
Endosulfan II	0.004 – 0.400	0.9952	3.83	88.78	89.66	0.004
heptachlor	0.004 – 0.400	0.9988	4.71	84.16	93.86	0.002
4,4-DDE	0.004 – 0.400	0.9986	3.95	84.18	92.14	0.002
4,4-DDT	0.004 – 0.400	0.9992	2.37	91.16	99.02	0.004

with using soxhlet extraction. Doong *et al*. (2002) have determined the concentration of OCPs in sediments by using a soxhlet extraction. The results found that the MDLs of OCPs ranged from 0.05 to 0.35 ng/g-dry weight and the recoveries of OCPs in the sediments were in the range of 70-124%. The detection limits reported by US Environmental Protection Agency (EPA) Method 8081 were between 1.1 and 5.7 ng/g-dry weight (Doong *et al*., 2001).

Application to real sediment samples

The sediment samples from Moon river were determined the studied OCPs by HS-SPME-GC-ECD. The sediment samples were collected from six sampling points, during April to September 2011. The contamination of heptachlor, endosulfan I, endosulfan II, 4,4-DDE, endrin and 4,4-DDT were ranged from 0022 to 0.5212 ng/g, 0.0025 to 0.0079 ng/g, 0.0063 to 0.0090 ng/g, 0.0027 to 0.0214 ng/g, n.d. to 0.0054 ng/g, and n.d. to 0.0066 ng/g, respectively. The quantities of the OCPs found in sediment samples were not over the soil quality standard by the Pollution Control Department.

Conclusion

The HS-SPME-GC-ECD method for the analysis of OCPs for sediment samples was developed. The method is sensitive, simple and useful in routine laboratories. The 100-μm PDMS fiber has demonstrated a good suitability for multiresidue analysis of studied pesticides. The HS-SPME procedure was finally carried out within 60 min at 70°C, maintaining a stirring of the slurry. The desorption temperature was 270°C for 5 min. The results obtained in the validation of HS-SPME-GC-ECD for the determination of 6-OCPs showed that this method is appropriate for routine analysis of OCPs in sediment, showing adequate sensitivity (LOD ranged from 0.002-0.004 ng/g), good linearity and accuracy in sediment samples.

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