

*Research article*

## **Development of sample preparation method for organochlorine pesticides analysis in soil samples**

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**Abstract:** OCPs are the persistent organic pollutants which are highly toxic to the environment and can accumulate in humans by foods such as rice, vegetable, and fruits. The aim of this research was to develop a rapid and easy sample preparation method for determination 4 OCPs, aldrin, dieldrin,  $\beta$ -endosulfane, and p-p' DDT in soil samples by gas chromatography with electron capture detection. The analysis was performed by using HP-5 capillary column with the isothermal condition at 250 °C and carrier gas flow rate of 1 mL/min. The results show that the 4 OCPs and internal standard, pentachloronitrobenzene, were analyzed within 6 min. The optimized condition of the sample preparation method was using ultrasonic assisted solvent extraction with 20 mL of hexane and ethyl acetate (9:1 v/v) as an extractant for 15x2 min. The results obtained from the proposed sample preparation method have demonstrated that the limit of detection was 0.628–3.68  $\mu\text{g kg}^{-1}$ . Percent recoveries were in the range of 81.42 to 110.7% and RSD were 1.68–9.43%. Correlation coefficients of calibration graphs were more than 0.99. The developed sample preparation method exhibited simplicity rapid and low chemical reagent consumption with satisfactory results.

**Keywords:** none clean-up method; ultrasonic extraction; Thai soil sample; electron capture detector

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**Abbreviations:** OCPs: Organochlorine pesticides; p-p'-DDT: 1,1,1-Trichloro-2,2-bis (4-chlorophenyl) ethane; HP-5: 5% Diphenyl / 95% Dimethylpolysiloxan; RSD: Relative standard deviations

## 1. Introduction

Organochlorine pesticides (OCPs) were widely used after World War II due to cheap, high stability, and bioaccumulation property. However, these persistent chemicals can be transferred and accumulate in the environment [1]. They are the large group of persistent organic pollutants (POPs) with high lipophilicity, bioaccumulation activities, long half-life in the food chain [2]. OCPs have been banned for agricultural and other uses in Europe, North America South America and many countries of Asia including Thailand because of their carcinogenic, mutagenic, and toxicity properties. However, OCPs still can be found in environmental samples such as water, agriculture products, animal, and soils caused by their long half-life and non-polar properties [1]. It is widely known that OCPs still contaminated in soils because they do not decompose due to their physical properties and it is widely recognized that most non-polar organic compounds, such as the OCPs retained in soils, are bound to solid organic matter with covalent bonds, and hydrophobic interactions [3]. Mostly, the interaction between soil matrices and OCPs is stronger than in several agriculture products [4].

Therefore, the development of sample preparation method for analysing and monitoring OCPs in soil samples is still important. Many studies have shown that the extraction and clean-up of OCPs from soils are problematic steps in sample preparation methods and OCPs analysis [5]. Numerous papers have been published about OCPs extraction from soils, namely applying the soxhlet extraction [6] solid phase microextraction [7] and tradition shake flask extraction [8] followed in some cases by a clean-up step with solid-phase extraction. Tor and co-worker present the ultrasonic technique combined the solvent extraction of OCPs from environment matrices and clean-up by solid phase extraction method [9] but this methodology still require expensive equipment, high amount of toxicity solvent, complexation procedure, and long-time extraction. Thus the aim of this work was to develop a cheap, safety, simple, and rapid sample preparation method with satisfying precision and accuracy for determination of OCPs in soils.

## 2. Material and methods

The four standard compounds of OCPs namely, aldrin, dieldrin,  $\beta$ -endosulfane, and p-p'-DDT were purchased from Chem service (USA) and pentachloronitrobenzene as an internal standard (IS) were purchased from Sigma aldrich (USA). The stock solutions of these compounds were prepared by dissolving in ethyl acetate (RCI Labscan, Thailand) and kept storing in the dark place at 4 °C. The extraction solvents namely, hexane, ethyl acetate, dichloromethane were purchased from Acros Organics (USA) and sodium chloride was purchased from Merck (Germany). Two types of clean-up materials including Alumina and C-18 cartridges were purchased from Merck (Germany) and Chromabound (USA), respectively. The deionized water was obtained by using a Milli-Q water purification system.

### 2.1. Reference soil samples

The reference soil samples were collected from the Department of Agriculture, Thailand. The soils were collected in plastic bags (1 kg) from 10 to 20 cm in the ground. The reference sample was heated for the elimination of OCPs in soils by drying oven at 100 °C for 24 h and ground sample to powder. Soil samples were spiked with a mixture of the standard solution prior to analysis by GC-ECD under each optimization parameter.

## 2.2. Sample preparation procedure

### 2.2.1. Optimization of type of extraction solvent and extraction time

Soil powder (10.00 grams) that spiked with 10.00  $\mu\text{g kg}^{-1}$  of 4 OCPs were placed into 100 mL of the beaker. Then, 20 mL of two different types of extraction solvents (mixture solvent between hexane:ethyl acetate 9:1 v/v and dichloromethane) were added and the sample was extracted by ultrasonic technique for five different time periods (5, 10, 15, 20, and 25 min). The sample matrix was then filtrated with Whatman no.42 and extraction again. After the twice extraction, the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of each extraction solvents and then immediately injected into the GC-ECD to prevent the analytes from losing in the injection step.

### 2.2.2. Optimization of clean up methods.

The spiked soil powder was placed into 100 mL of the beakers. Then, 20 mL of mixed solvents were added and the soil sample was extracted by ultrasonic technique for 15 min. The supernatant was then filtrated and extracted again. After the twice extraction, The clean-up was carried out for three different methods (Alumina, C-18, and without clean-up). The extracts were clean-up by using Alumina and C-18 solid phase extraction with hexane:ethyl acetate 9:1 v/v as an eluent solution. All of the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of mixed solvent and then immediately injected into the GC-ECD

## 2.3. Real sample analysis

Soil samples such as S101, S102, S103, and S104 were obtained from several regions of Department of Agriculture, Thailand. Soils were ground to powder and added 20 mL of mixture extraction solvent and extracted by ultrasonic technique. The sample matrix was then filtrated with Whatman no.42 and extraction again. After the twice extraction, the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of mixed solvent between hexane and ethyl acetate (9:1 v/v) and then immediately injected into the GC-ECD.

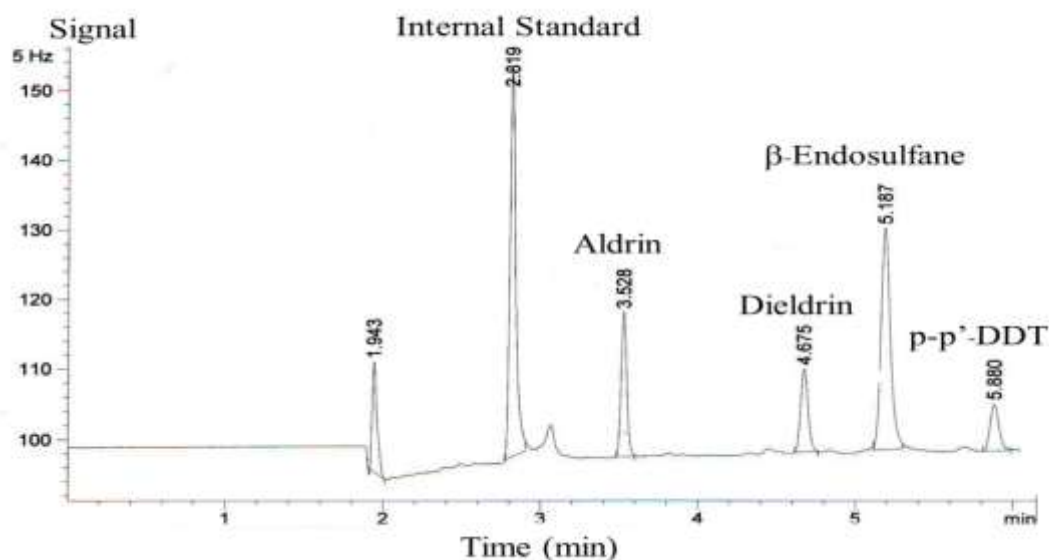
**Table 1.** The Gas chromatography (GC-ECD) conditions.

Parameters	Optimized conditions
Column temperature	250 °C
Detector temperature	320 °C
Injection system	split mode: split ratio 64.4:1
Injection temperature	260 °C
Injection volume	1.0 $\mu\text{L}$
Carrier gas	Helium
make up gas	Nitrogen
Rate of carrier gas	1 mL/min

## 2.4. GC-ECD analysis of soil samples

The extracts of OCPs in soil samples were analyzed by gas chromatography with electron capture

detector (GC-ECD) (Hewlett-Packard). The capillary column was HP-5 (5%-phenyl 95%-dimethylpolysiloxane 30.0 m x 320  $\mu\text{m}$  i.d., 0.25  $\mu\text{m}$  film thickness). Helium was used as carrier gas at a flow rate of 1  $\text{mL min}^{-1}$ . The isothermal program was set at 250  $^{\circ}\text{C}$  (6 min). Other operating conditions and the retention time of 4 OCPs are shown in Table 1 and the chromatograms of 4 OCPs and IS in reference soil samples are shown in Figure 1.



**Figure 1.** The chromatogram of 4 OCPs and internal standard

### 3. Results and discussion

#### 3.1. Optimization of sample preparation method

The effects of some sample preparation parameters were studied including extraction solvent, extraction time, and type of clean-up methods, affecting to the extraction efficiency of the sample preparation method.

##### 3.1.1 Optimization of type of extraction solvent

The characteristic requirements of the extraction solvent in sample preparation are composed of suitable volatility, low viscosity, and polarity nearly to OCPs. The extraction solvents which have been used for the ultrasonic assisted solvent extraction that gave good results in earlier work, namely, mixture solvent between hexane and ethyl acetate (9:1 v/v) and dichloromethane were investigated in detail as shown in Figure 2A. It was indicated that the highest signal of these OCPs was obtained by using mixture solvent between hexane and ethyl acetate (9:1 v/v) as the extraction solvent. In fact, the trend of extraction efficiency between mixture solvent and dichloromethane were closely but p-p'-DDT was not found when used dichloromethane as extraction solvent. Thus, mixture solvent between hexane and ethyl acetate (9:1 v/v) was choosing as the extraction solvent in this study.

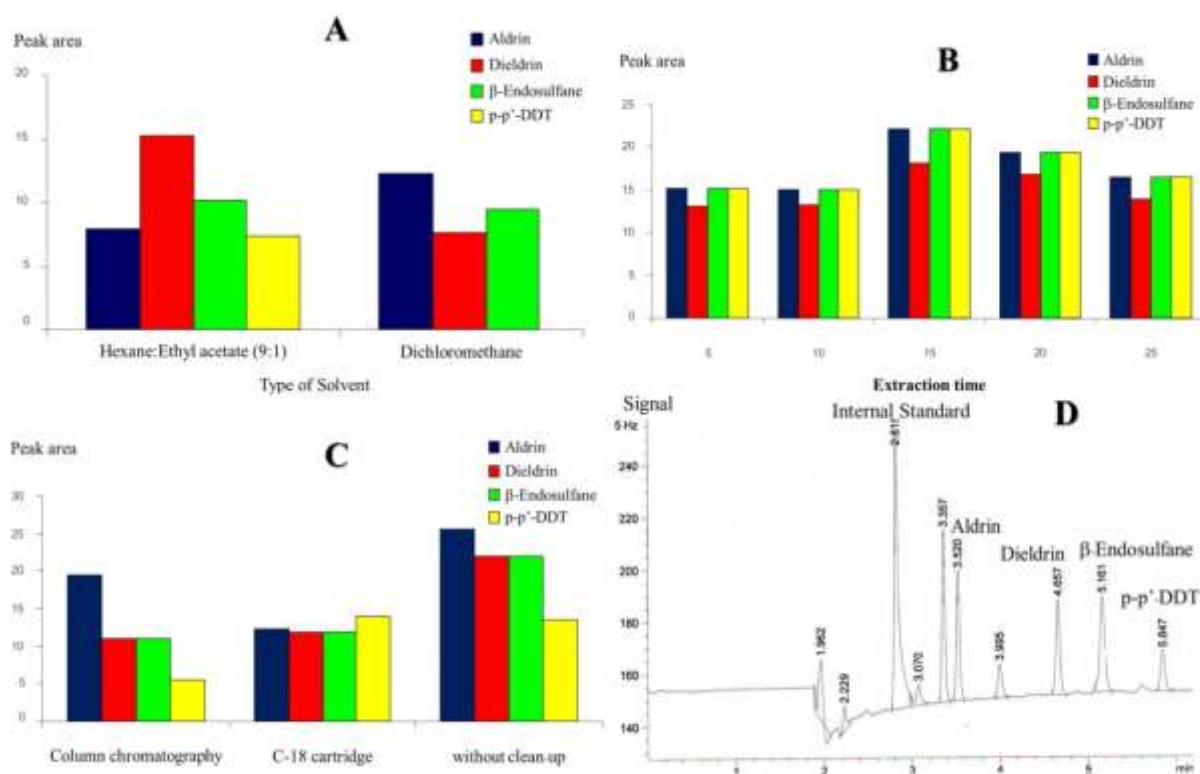
##### 3.1.2. Optimization of extraction time

Extraction time is the time to extract 4 OCPs from the soil samples by extraction solvent. The

effect of the extraction time on the signal of OCPs in soils was performed from 5 to 25 min shows that in Figure 2B. It was found that the signal trend of 4 OCPs increased with an increase of the extraction time up to 15 min after that they slightly decrease. It might relate to the back extraction of the analytes caused by a long time of the extraction. So, the extraction time of 15 min was an appropriate case in this study.

### 3.1.3. Optimization of clean-up methods

Various clean-up methods have been used by different research groups for extract OCPs from soil samples. In this work, ultrasonic assisted solvent extraction with column chromatography, C-18 cartridge, and the extraction without clean-up method was studied. Figure 2C indicates that the ultrasonic assisted solvent extraction without the clean-up method provided a slight increase in the extraction efficiencies of 4 OCPs as compared with the other two clean-up procedures for soil samples. It might have been the OCPs extracts were loss in the clean-up methods and affecting to the signal of 4 OCPs. From the signal trends with all of three methods, without the clean-up method is the best choice for the sample preparation of 4 OCPs compounds from soil samples.



**Figure 2.** The effect of A) extraction solvent B) Extraction time C) clean-up methods on 4 OCPs analysis and D) the chromatogram of 4 OCPs in real soil samples.

### 3.2. Features of the analytical method

To validate the optimum conditions of propose sample preparation method, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (%RSD) and accuracy (% recovery) were investigated. The results of the validated methods for sample preparation and GC-ECD are shown in

Table 2. Linearity for the five-point calibration curves were satisfied with linear coefficient values greater than 0.99 at the concentration range of 5–200  $\mu\text{g kg}^{-1}$  for 4 OCPs. The obtained LOD values were in the range from 0.628–3.68  $\mu\text{g kg}^{-1}$  while LOQ were in the range between 2.093–12.27  $\mu\text{g kg}^{-1}$ . Both LOD and LOQ demonstrate that the proposed sample preparation method is sensitive enough to determine these OCPs in soil samples. The method precision was expressed as %RSD in the range of 1.68–9.43 %. The recovery (%) studied was determined by spiking of the mixed solution of OCPs standard into the soil sample which was prepared to be a blank without any residual OCPs. Then the spiked sample was analysed under the optimum conditions of the proposed sample preparation-GC-ECD method. The analytical recoveries of these OCPs represent a satisfactory value in the range from 81.42–110.7%. It is shown that the developed method gives an acceptable precision and accuracy.

### 3.3. Analysis of OCPs in soil samples

The developed sample preparation method was then applied for the determination of 4 OCPs in soil samples. The chromatogram of 4 OCPs are shown in Figure 2D and Table 3, respectively. It was found that there were high contents of Dieldrin in S101–S103 and  $\beta$ -Endosulfane in S101 and S104. Additionally, Aldrin and p-p'-DDT was not detectable in all samples. From the results, it is indicated that the developed sample preparation method with GC-ECD is a rapid, simple and suitable method for the determination of these OCPs from the soil samples.

**Table 2.** Linear range of calibration curve, LOD, LOQ, %RSD and % recovery of the proposed method.

OCPs	Linear range ( $\mu\text{g kg}^{-1}$ )	$R^2$	LOD ( $\mu\text{g kg}^{-1}$ )	LOQ ( $\mu\text{g kg}^{-1}$ )	%RSD (n=6)	%Recovery (n=3)
Aldrin	5–150	0.991	1.186	3.954	1.68	110.7 $\pm$ 11.1
Dieldrin	5–150	0.997	0.628	2.093	9.08	105.2 $\pm$ 6.57
$\beta$ -Endosulfane	5–150	0.991	2.259	7.530	9.43	106.4 $\pm$ 11.6
p-p'-DDT	10–200	0.998	3.681	12.268	4.46	81.42 $\pm$ 10.77

**Table 3.** The contents of some OCPs found in soil samples determined by the proposed method and GC-ECD (mean  $\pm$ SD, n=5, ND = not detected).

Sample code	OCPs ( $\mu\text{g kg}^{-1}$ )			
	Aldrin	Dieldrin	$\beta$ -Endosulfane	p-p'-DDT
S101	ND	17.09 $\pm$ 0.69	15.96 $\pm$ 0.52	ND
S102	ND	280.50 $\pm$ 6.8	ND	ND
S103	ND	5.68 $\pm$ 0.78	ND	ND
S104	ND	ND	265.25 $\pm$ 7.92	ND

## 4. Conclusion

In this study, an optimized ultrasonic extraction without the clean-up procedure followed by analysis with GC-ECD has been indicated a good alternative method for the analysis of toxicity organochlorine pesticides in soil. This method exhibited none complexation procedure and lower chemical reagent consumption than tradition extraction method with satisfactory precision and

accuracy results. Moreover, the ultrasonic extraction without clean-up method have shown the rapid sample preparation method because the OCPs extracted from soil samples by ultrasonic extraction and filtrated were clean enough for injecting to GC-ECD analysis.

### Conflict of interest

All authors declare no conflicts of interest in this paper.

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