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Clean up Methods for Soil Samples Before Injecting in GC/ECD.

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ABSTRACT

Although many organochlorine pesticides (OCPs) have been forbidden or reduced to apply, large quantities of residual fractions still remain in the natural environment due to their persistence, and cause adverse effects on organisms and human beings. Sediment and biota and groundwater are the main sinks of OCPs based on actual measurements and simulation results using the multimedia fate model.[1] Therefore, precise analysis of OCPs in these substrates may offer accurate information on the distribution of persistent organic pollutants and provide background data for further ecological risk assessment. In the course of quantitative determinations, extraction and cleanup pretreatment of raw samples are the fundamental procedures, since the purity of sample input for gas chromatography (GC), commonly employed as the analytical technique of OCPs, would directly affect the accuracy and sensitivity. Many impurities (e.g., triglyceride and humic substances) having similar solubility in the extracting solvents may coexist in the extract of OCPs. The presence of impurities can decrease the analytical performance of instrument via retaining in the injection port and/or in the chromatographic separation column. The main objective of this study was to optimize the sample cleanup conditions for determiningOCPs in natural sediment and fishmuscle after extraction by PLE, in which the deactivated ratio of florisil and the eluting properties of OCPs concerned were investigated. Moreover, the effects of two assistant procedures, as the preliminary purification step, are compared between sulphonation and liquid–liquid distribution.

Keywords: Sample purification; soil; Organochlorine pesticides; sulfur removal

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INTRODUCTION

The methods of sample purification for determinations of organochlorinepesticides (OCPs) in agricultural soils were investigated in this study. The analysis of organochlorine pesticides (OCPs) residues in trace levels usually requires a simple, fast, and sensitive method of analysis. The most crucial point in pesticides residue analysis is the sophisticated sample preparation and method validation procedure. A modification on the analytical procedure was performed as the slurry analytical multi-method was not suitable for our soil sample matrix. The efficiency of the extraction procedure of OCPs in soil was evaluated according to the following:

- Including high number of pesticides as much as possible in a single procedure (multiresidual assay).
- Extraction Recoveries as close as possible to 100%,
- Sufficient removal of potential interferences from the sample to increase the selectivity and avoid undesirable matrix effects.
- Decrease concentration of the analytes and hence increase the sensitivity of the assay method.
- Acceptable precision and accuracy.
- Low costs.
- High speed.
- Easy.
- Safe (low amount of solvents, less harmful solvents).

Soil sample was spiked with different known concentration levels of the target pesticides. The sample was extracted using conventional liquid solid extraction techniques and concentration of the pesticides was determined using GC-ECD. The recovery rate, limit of detection, limit of quantitation and other validation parameters of the analytical method will be determined. Based on the validation parameters the extraction procedure will be optimized.

MATERIAL AND METHODS

Sample preparation

Slurry extraction

The wet homogenized soil samples containing 50 g of soil material were placed separately into 500 mL Erlenmeyer flask. Extraction was carried out with 2:1 acetone/water mixture (100mL: 50mL); soil weight was calculated for each sample according to calculated amount of water (moisture content). The mixture was shaken overnight using a horizontal shaker at shaking velocity of 220 cycle min^{-1}.

After adding 15 g of NaCl and 100 mL cyclohexane, the mixture was shaken additionally for 1 h for completing the liquid/liquid partitioning. The organic layer was decanted into 250 mL Erlenmeyer flask and dried over 15 g anhydrous sodium sulfate. Then, 100 mL of the extract were evaporated using rotary evaporator and dissolved in 5 mL of (1:1, V: V) n-Hexane and ethyl acetate mixture.

Clean up

During the extraction step many interfering (mainly organic) components are co-extracted from soil samples together with target analytes. The aim of the clean-up stage is to remove these substances that can interfere with the identification and the quantitation of analytes. Clean up procedure was developed to enhance the quality of the chromatographic analysis, because most of the co-extractants and the instrumental interfering materials are removed. A large number of sorbents are used to isolate organic compounds from the extracted solutions, including alumina, ion-exchange resins and silica gel. For example, silica and Florisil sorbents were not suitable for extract clean-up prior to the final determination of analytes ranging widely in polarity as in our case.

In original method, the extracts were purified by column chromatography using silica gel as adsorbent. The column was eluted with 65 mL n-hexane, and then with 50 mL ethyl acetate, but the obtained recovery rate was poor (≤70%). Therefore, alumina was used to clean up the sample; the column was eluted with co-solvent of n-hexane and ethyl acetate. The best recovery rates were obtained with (1:1) co-solvent of n-hexane and ethyl acetate (v:v).

Table 1: The recovery rates of OCPs in fortified soil samples.

Compound	Recovery (%) (mean ± SD), RSD					
	Procedure M1 (n=5)		Procedure M2 (n=4)		Procedure M3 (n=7)	
HCB	57 ± 3.0	5.4	58 ± 3.0	5.2	84 ±3.0	3.5
HCH	58 ± 6.6	11.3	53 ± 6.4	12.2	85 ± 1.5	1.8
Aldrin	62 ± 3.2	5.2	64 ± 5.0	7.9	91 ± 3.0	3.2
Procymidone	68 ± 4.1	5.9	55 ± 13	23.6	97 ± 3.5	3.6
Hexy thiazox	43 ± 6.0	14	26 ± 9.5	36.7	70 ± 7.0	10
O,P-DDE	45 ± 2.5	5.6	44 ± 4.6	10.5	76 ± 1.2	1.6
Endosulfan	46 ± 0.7	1.5	47 ± 4.6	9.7	86 ± 1.3	1.5
4,4`-DDE	71 ± 3.1	4.3	65 ± 5.5	8.5	96 ± 3.6	3.8
Myclobutanil	_______	___	67 ± 4.7	7.3	86 ± 2.5	4.2
O,P-DDT	53 ± 3.8	7.2	47 ± 4.1	8.6	80 ± 4.4	5.4
β-Endosulfan	41 ± 1.7	4.2	42 ± 7.2	17	83 ± 2.4	3
O,P-DDD	71 ± 26	37	54 ± 10.2	18.9	100 ± 5.7	5.7
Endosulfan sulfate	64 ± 7.2	11.3	68 ± 9.0	13.2	95 ± 5.1	5.3
Phosalone	50 ± 3	6	46 ± 12.5	27.2	105 ± 10.4	9.9
Fenarimol	13 ± 5	38	7 ± 4.2	59.8	129 ± 32.5	25
Cypermethrin 1	_______	___	26 ± 0.7	2.7	78 ± 15.3	19.6
Cypermethrin 2	_______	___	64 ± 18.6	29	120 ± 25.9	21.6
Cypermethrin 3	_______	___	30 ± 5.7	18.8	89 ± 29.3	3.3
Cypermethrin 4	_______	___	214 ± 0	0	82 ± 12	14.7

Procedure M1: the concentrated extracts were washed with 5 mL of 1:1 cyclohexane and ethylacetate, silica gel was used for clean up, elution was achieved using 65 mL n-hexane followed by 50 mL ethyl acetate.

Procedure M2: the concentrated extracts were washed with 5 mL of 1:1 n-hexane and ethylacetate, florisil was used for clean up, 50 mL of co-solvent of n-hexane and ethyl acetate (3:1) was used for elution.

Procedure M3: the concentrated extracts were washed with 5 mL of 1:1 n-hexane and ethylacetate, alumina for clean up was used, 50 mL of co-solvent of n-hexane and ethyl acetate (1:1) was used for elution.

Chromatographic detection problem

For many reasons, sulfur offers a number of challenges for GC analysis. The chemical reactivity of many sulfur-containing compounds can make both analysis of the compound at high temperature of concern, but also relating the compound in a sample to the level that is measured at the detector somewhat problematic.

Effect of sulfur on GC/ECD

In GC/ECD analysis, Sulfur contamination causes a rise in the baseline of a chromatogram and may interfere with the analyses of the later eluting organochlorine compounds. Detection of the organochlorine compounds was not possible in the presence of the elemental sulfur. Sulfur is removed using tetrabutylammoniumsulfite (TBA) method [7]. The elemental sulfur is converted to the thiosulfate ion, which is water-soluble, according to the following reaction:

$$(\mathrm{TBA}^{+})_2\ \mathrm{SO}_3 + \mathrm{S(s)} \rightarrow 2\mathrm{TBA} + \mathrm{S_2O_3}$$

Effect of sulfur on detection of electron capture detector (ECD) and sample matrix before and after its removal is shown in Figures 1 and 2.

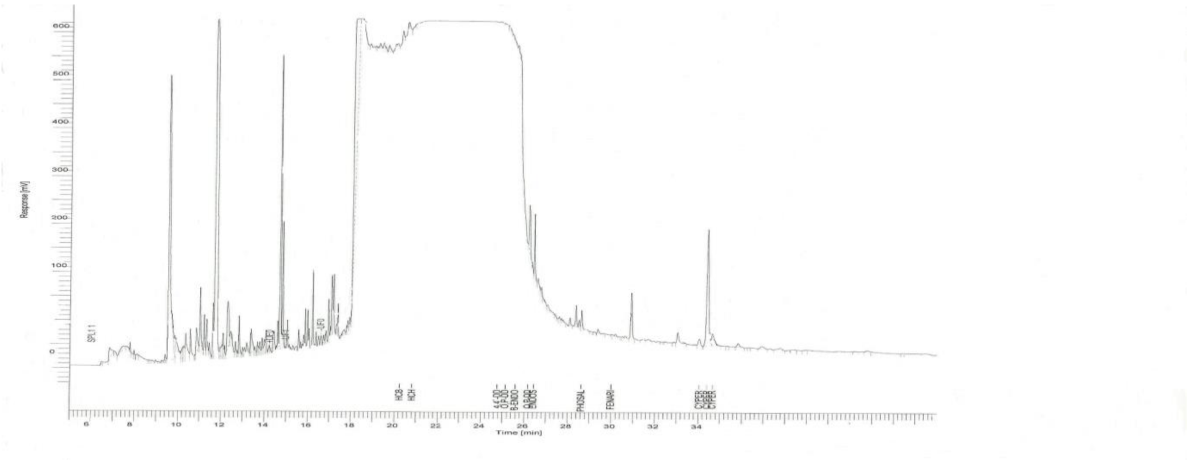

Figure 1: GC/ECD chromatogram in presence of sulfur.

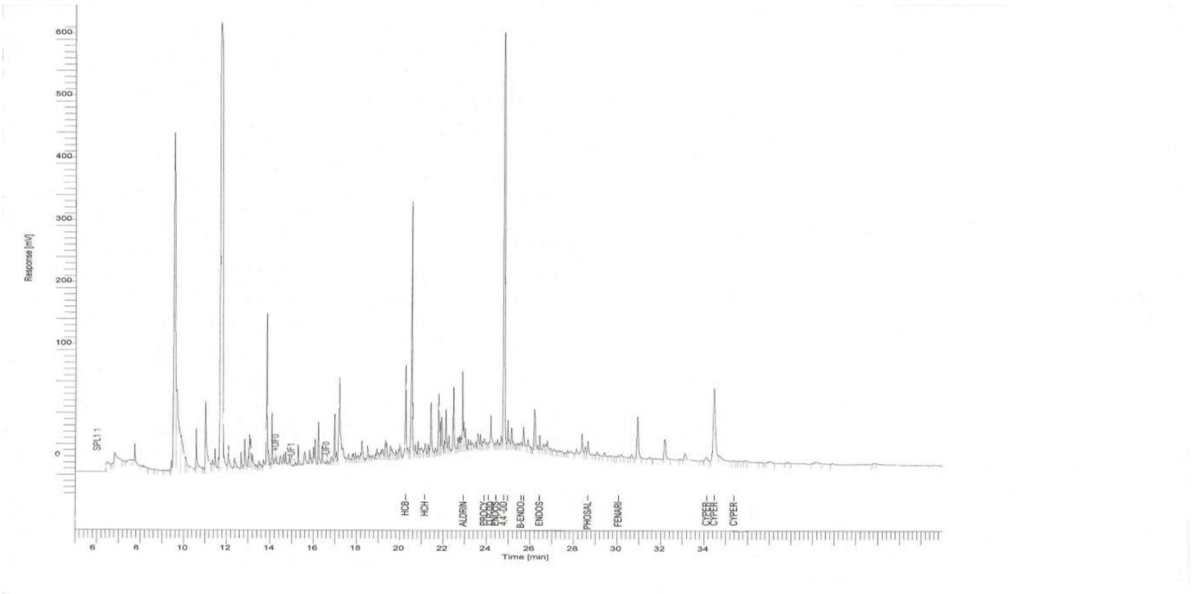

Figure 2: GC/ECD chromatogram after sulfur removal.

Sulfur removal

The elemental sulfur was removed before analysis using tetrabutylammoniumsulfite (TBAS) method [7]which converts solid sulfur from the organic phase into aqueous phase as soluble thiosulfate. Prepare 0.1 M TBAS reagent by dissolved 0.68 g TBAS and 5.0 g Na2SO3 in 20 mL deionized water. Then mix 1 mL of 0.1 M TBAS reagent with 1.0 mL isopropanol and few crystals of sodium sulfite, then the sample is transferred quantitatively to a test tube, the mixture was shaked for 1 min. The phase separation was carried out by adding 2.5 mL deionized water and then shaking for 1 min; few crystals of sodium sulfite were added until the appearance of a white precipitate. Finally, the clear upper n-hexane layer was collected and transferred into a GC vial.

CONCLUSION

A multi-residue analytical procedure was modified for analysis of 17 different organochlorine compounds in using a gas chromatography equipped with electron capture detector (GC-ECD).

The results of this study have shown that the alumina clean-up method is more fitting for Moroccan soils regarding the highest quantity of sulfur that contains.

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