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Optimization of solid-phase extraction and solid-phase microextraction for the determination of α- and β-endosulfan in water by gas chromatography–electron-capture detection

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Abstract

Water contamination due to the wide variety of pesticides used in agriculture practices is a global environmental pollution problem. The 98/83/European Directive requires to measure residues of pesticides at a target concentration of 1.0 μg/l in surface water and 0.1 μg/l in drinking water. In order to reach the level of detection required, efficient extraction techniques are required. Although solid-phase extraction (SPE) is the most common technique for isolation and concentration of pesticides from water, solid-phase microextraction (SPME) is being increasingly applied for this purpose. In this study, a direct-SPME procedure has been developed for the determination of α-endosulfan and β-endosulfan in waters; experimental parameters such as selection of SPME coating, effect of temperature, effect of salt addition, optimization of the sample volume, adsorption and desorption profiles and desorption temperature were studied and optimized. Analytical parameters such as linearity, precision, detection and quantitation limits, and matrix effects for SPE and SPME methods were evaluated for comparison purposes with the aim of selecting the most appropriate for a certain application. Both extraction techniques, SPE and SPME, were followed by gas chromatography with electron-capture detector.

Keywords: Extraction methods; Water analysis; Endosulfan; Pesticides

1. Introduction

Endosulfan (mixture of α- and β-isomers) is an insecticide and acaricide which acts as a poison to a wide variety of insects and mites on contact. Although it may also be used as a wood preservative, it is used primarily on a wide variety of food crops, including tea, coffee, fruits and vegetables, as well as on cereals such as rice, maize, sorghum or other grains.

Endosulfan is moderately persistent in soils and transport of this pesticide into waters is most likely to occur if endosulfan is adsorbed to soil particles in surface runoff [1,2]. It is not likely to be very mobile or to pose a threat to groundwater. It has, however, been detected in well and surface waters near areas of application [3] at very low concentrations, but also in drinking waters due to the fact that some of these waters are used for drinking.

Monitoring the trace levels of pesticides in waters is important for human health protection and environmental control. The European Union has set a

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maximum admissible concentration of 1.0 μg/l for each pesticide in surface water and 0.1 μg/l in drinking water [4].

The main objective of this study was to develop an analytical method based in SPE or SPME and followed by GC–electron-capture detection (ECD) to determine endosulfan in waters. A new SPME coating never used for endosulfan microextraction, divinylbenzene–Carboxen–polydimethylsiloxane (DVB–CAR–PDMS), was evaluated. Method parameters such as linearity, precision, and limits of detection and quantification were determined and compared in order to select the most suitable extraction technique depending on the application.

2. Experimental

2.1. Chemicals, disposables and materials

α-Endosulfan (97%) and β-endosulfan (98.5%) were purchased from Dr. Ehrenstorfer Lab. (Augsburg, Germany). Lindane from Aldrich (Milwaukee, WI, USA) was used as internal standard (I.S.). Other reagents used were methanol, purge and trap grade, from Aldrich (Milwaukee, WI, USA), hexane, for organic trace analysis, from Fluka (Buchs, Switzerland); ethyl acetate, suprasolv grade, from Merck (Darmstadt, Germany) and sodium chloride ACS-ISO, for analysis, from Panreac (Barcelona, Spain). Ultrapure water was from a Milli-Ro Waters purification system (Milford, MA, USA).

Waters 360 mg Sep-Pak C_{18} Plus cartridges were used as solid-phase extraction (SPE) minicolumns for purification and concentration. A Visiprep SPE vacuum manifold (Supelco, Bellefonte, PA, USA) is used to simultaneously process up to 24 SPE tubes. The visidry drying attachment (Supelco) is used to dry up to 24 SPE tubes at one time, and can be used with any inert gas supply. It is also useful for evaporating and concentrating recovered samples. Nitrogen C_{50} of analytical quality was supplied by Carburos Metálicos (Spain). SPE extracts were placed in 2-ml vials (Supelco). Homogenization of SPE extracts was achieved by vortex agitation (Heidolph Reax Top, Germany).

Six SPME fibers were considered in this study: 100 μm polydimethylsiloxane (100-PDMS), 85 μm polyacrylate (PA), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 65 μm Carbowax–divinylbenzene (CW–DVB), 65 μm Carboxen–polydimethylsiloxane (CAR–PDMS) and 50/30 μm Stable Flex divinylbenzene–Carboxen–polydimethylsiloxane (DVB–CAR–PDMS). The commercially available SPME device and fibers were purchased from Supelco. Fibers were initially conditioned according to the manufacturer's instructions in order to remove contaminants and to stabilize the polymeric phase. For the SPME extraction, water samples were placed in 40-ml EPA vials (Wheaton, USA) equipped with stir bars and sealed with PTFE-faced silicone septum, and stirred with a magnetic stirrer (Raypa, Spain).

2.2. Standard solutions

Stock standard solutions (1000 mg/l) of α- and β-endosulfan were prepared in methanol, separately, by weighing approximately 0.01 g of analyte into a 10-ml volumetric flask and diluting to volume. An intermediary standard solution (200 mg/l) was prepared by dilution in methanol of the both stock standard solutions. Stock and intermediary standard solutions of the internal standard, lindane, were prepared in the same way. All solutions were stored at 0–4 °C in the dark. Ultrapure water solutions were prepared by spiking with different volumes of the intermediary standard solutions and used for calibration.

2.3. Extraction procedure

2.3.1. Solid-phase extraction

The 360 mg C_{18} Sep-Pak cartridge was previously conditioned with 5 ml of methanol followed by 10 ml of ultrapure water without allowing the cartridge to dry out. To each aqueous sample (100 ml), lindane was added as an internal standard (1 μl of the intermediary solution of 100 mg/l). The aqueous sample was passed through the cartridge at a rate of 4 ml/min. The cartridge was dried by blowing nitrogen for 15 min. Adsorbed pesticides were eluted by 5 ml of hexane; hexane was then evaporated to dryness under a gentle stream of nitrogen and the residue redissolved, finally, with 1

ml of ethyl acetate. Homogenization of the final extract was achieved with vortex agitation.

2.3.2. Direct solid-phase microextraction (direct-SPME)

For direct-SPME, water samples (40 ml) were placed into 40-ml EPA glass vials equipped with PTFE-coated magnetic bars and capped with a PTFE-faced silicone septum. To each sample, lindane was added as an internal standard (1 μl of the intermediary solution of 200 mg/l). The holder needle was inserted through the septum and the fiber DVB–CAR–PDMS was directly immersed in the sample solution during 30 min under magnetic stirring at room temperature (22 °C). Magnetic stirring facilitates mass transport of the analyte between the water sample and the fiber, reducing the equilibration times. After extraction, the fiber was withdrawn into the holder needle, removed from the vial and immediately introduced into the GC injector port for 2 min at 270 °C for thermal desorption.

2.4. Analytical instrumentation and operating conditions

A Fisons (Rodano, Italy) GC 8000 series gas chromatograph equipped with an ECD system was used. Chromatographic separations were performed using a Supelco MD*N*-5S (30 m×0.25 mm I.D.) fused-silica capillary column with 5% diphenyl–95% dimethylsiloxane liquid phase (0.25 μm film thickness). The oven temperature was programmed as follows: 80 °C ramped at 15 °C/min to 250 °C, ramped at 5 °C/min to 300 °C and held for 10 min. A split/splitless injector was used in the splitless mode (1 min) for the SPE extract analyses, and in the split mode (1/100) for the SPME desorption. Helium with a column head pressure of 125 kPa and nitrogen (150 kPa) were used as a carrier and make-up gases, respectively. Injector temperature was 270 °C. Detector temperature was 300 °C.

3. Results and discussion

3.1. SPE method characterization

The SPE procedure of α- and β-endosulfan from waters is based on the literature consulted [5–10]; it is a simple, automatable and quantitative SPE procedure for routine analysis. A chromatogram of a SPME standard solution is shown in Fig. 1 (top).

Initial calibration of the procedure was performed by regressing α- and β-endosulfan peak areas, separately, relative to that of lindane versus the analyte concentration using standard fortified aqueous samples after applying the SPE method to all standards. Analysis of a blank ultrapure water did not give any response at the retention time of the α- and β-endosulfan. The 10-point calibration line was found to have good linearity as can be seen in Table 1.

The recovery±repeatability of α- and β-endosulfan from water was measured at levels of 0.1 and 1.0 μg/l by the analysis of three samples (100 ml) of ultrapure water fortified with a pesticide methanolic solution (internal standard concentration was 5 μg/l). These samples were quantified with α- and β-endosulfan standard solutions injected directly into the GC column to estimate absolute recoveries±RSD for α- and β-endosulfan. Relative recoveries±RSD were estimated by using the initial calibration procedure in the paragraph described above (see Table 1).

Limits of detection (LOD) and quantitation (LOQ) were evaluated on the basis of the noise obtained with the analysis of unfortified water samples ($n=5$) LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively [11] (Table 1).

Duplicate samples of ultrapure waters and surface fortified at levels of 0.5 and 5.0 μg/l of endosulfans were analyzed (Table 2). Standard deviations and mean values obtained were compared using, respectively, the Fischer *F*-test (95% probability) and the Student two-tailed *t*-test (95% probability) [12]. No significant differences between both matrices were obtained. This method can then be applied to surface samples.

3.2. SPME method characterization

3.2.1. Optimization

Since α- and β-endosulfan are polar analytes with high affinity toward aqueous matrices, immersion or

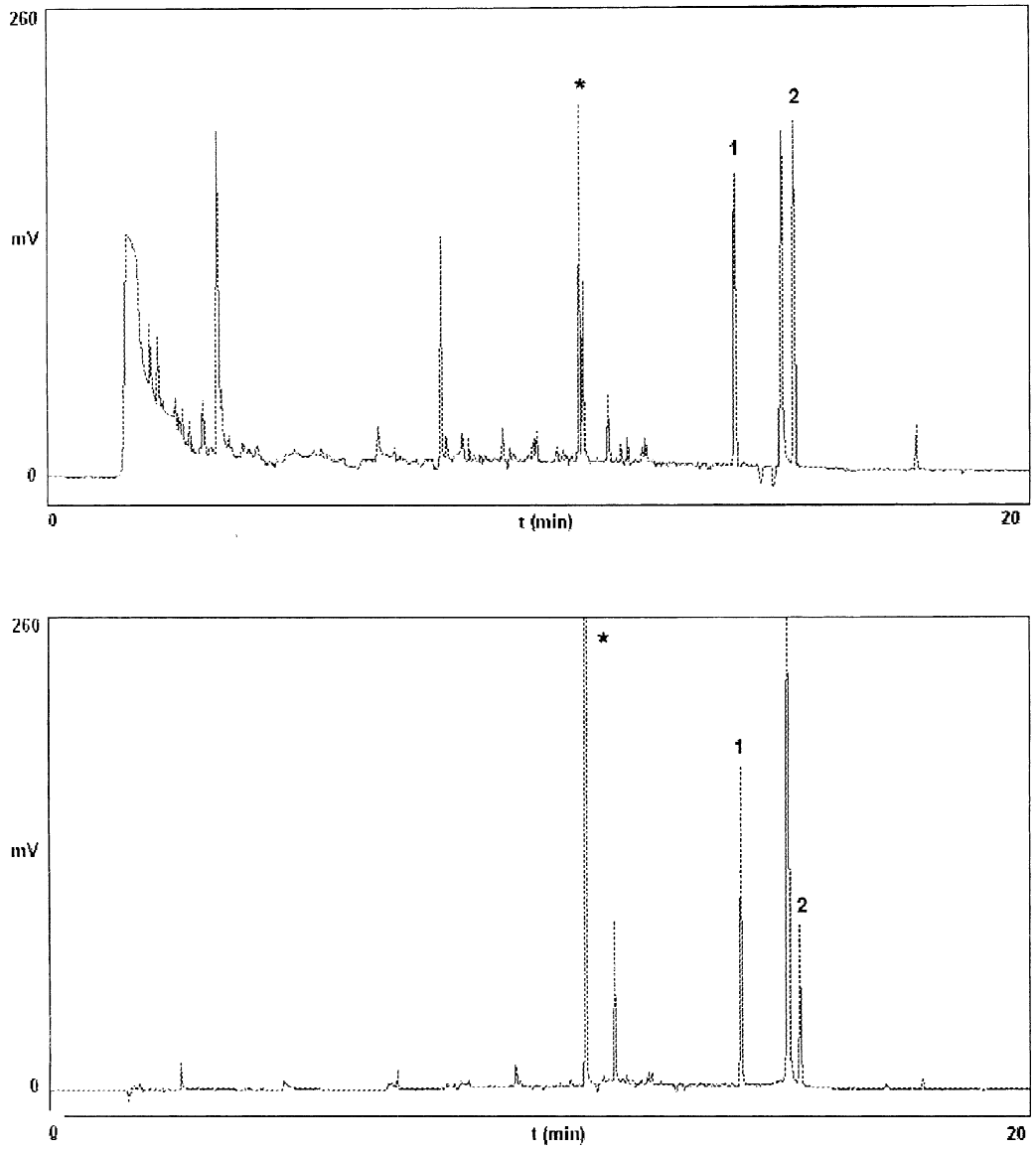

Fig. 1. SPE–GC–ECD (top) and SPME–GC–ECD (bottom) chromatograms registered for α-endosulfan, β-endosulfan and lindane as internal standard (*) of water spiked at 5 μg/l at the optimized and validated conditions. Peaks: (*) internal standard, lindane; (1) α-endosulfan; and (2) β-endosulfan. Chromatographic conditions as described in Section 2.4.

direct-SPME sampling was selected as extraction mode rather than headspace-SPME. In order to develop a direct-SPME procedure for the analysis of endosulfans in waters, several parameters related to the extraction and desorption processes were evaluated.

3.2.1.1. Selection of SPME coating

Six SPME fiber coatings were evaluated to select the most appropriate. Fortified aqueous samples (40 ml spiked at 5 μg/l with each pesticide and internal standard) were analyzed by triplicate with each fiber. The extraction time was 30 min at room temperature

Table 1
Repeatability, reproducibility, linear dynamic ranges, determination coefficients (r^2) and limit of detection (LOD) and quantification (LOQ) of the SPE and the SPME techniques followed by GC–ECD for determining endosulfan in waters

Extraction techniques	Absolute[a] recovery			Relative[a] recovery			Linearity[b] range (μg/l)	r^2	LOD[c] (μg/l)	LOQ[c] (μg/l)
	μg/l	%	RSD (%)	μg/l	%	RSD (%)				
α-Endosulfan										
SPE	0.1	115	6.4	0.1	120	0.3	0.05–1.0	0.999	0.02	0.04
	1.0	115	3.4	1.0	100	3.4				
SPME	0.1	<0.1	12.4	0.1	97.8	12.4	0.1–4.5	0.994	0.06	0.13
	5.0	<0.1	2.1	5.0	100	2.8				
β-Endosulfan										
SPE	0.1	108	8.1	0.1	100	8.1	0.05–1.0	0.995	0.02	0.03
	1.0	108	8.1	1.0	100	8.1				
SPME	0.1	<0.1	19.2	0.1	105	18.6	0.1–5.0	0.996	0.05	0.10
	5.0	<0.1	3.0	5.0	100	4.4				

[a] (n=3) mean of determinations.
[b] (n=10) mean of determinations.
[c] (n=5) determinations.

Table 2
Estimated concentrations and standard deviations of α- and β-endosulfan in spiked HPLC water and surface spiked water (italics) determined by SPME and SPE methods

Compound	SPE–GC–ECD				SPME–GC–ECD			
	0.5 μg/l		5 μg/l		0.5 μg/l		5 μg/l	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
α-Endosulfan	0.49	0.02	4.93	0.29	0.50	0.04	5.00	0.19
	0.49	*0.04*	*4.86*	*0.60*	*0.50*	*<0.01*	*5.02*	*0.41*
β-Endosulfan	0.51	0.03	5.12	0.14	0.50	0.03	4.99	0.15
	0.51	*0.04*	*5.01*	*0.78*	*0.50*	*<0.01*	*4.99*	*0.08*

(n=3) determinations.

for all fibers. The samples were magnetically stirred during the extraction process. The desorption time was 2 min (split mode 1/100) at 250 °C for all fibers. Areas obtained for each pesticide and for internal standard with the different fibers are shown in Fig. 2. DVB–CAR–PDMS, the SPME coating not evaluated in the literature as far as we know [13–24], resulted to be the most effective due to the presence of two adsorbents, DVB and CAR, and was selected for further experiments.

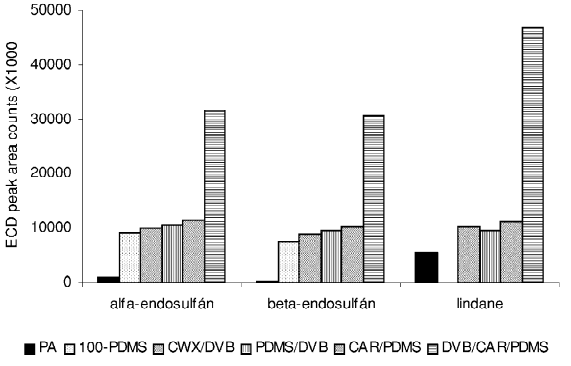

Fig. 2. Extraction efficiencies of SPME fiber coatings evaluated for sampling α-endosulfan, β-endosulfan and lindane by direct-SPME. Aqueous samples (40 ml) containing both pesticides (5 μg/l of each compound) were analysed.

3.2.1.2. Effect of salt addition

Fortified aqueous samples (40 ml spiked at 5 μg/l with each pesticide and internal standard) were unsalted and salted with NaCl (1–3 g) and analyzed

twice as above. The addition of salt is specially helpful when analysis of polar analytes in water is performed. Experimental ECD areas obtained for salty and non-salty solutions were similar. Further experiments were performed without addition of salt.

3.2.1.3. Effect of temperature

The effect of temperature was evaluated when fortified aqueous samples were extracted at 22, 45 and 75 °C by holding the sample in a water bath controlled at a constant temperature; desorption process was carried out as before. It was observed that ECD areas increased but very slightly. In order to simplify the SPME procedure, further experiments were performed at room temperature.

3.2.1.4. Sorption and desorption time profiles

Duplicate fortified aqueous samples (40 ml spiked at 5 μg/l for each pesticide and lindane) were analyzed considering different extraction times (5, 10, 15, 20, 30 and 45 min) at room temperature; desorption was carried out as before. The extraction time profiles of this fiber were obtained by plotting the ECD response versus the extraction times evaluated (Fig. 3) for each pesticide. The extraction time profile shows that α- and β-endosulfan reached the maximum extraction yield in 30 min. Further experiments were performed selecting this time.

3.2.1.5. Desorption temperature and desorption time profiles

Temperature of GC injector and desorption time were tested in order to guarantee the complete desorption of fungicides and to avoid carryover. For the DVB–CAR–PDMS fiber, temperatures ranging between 230 and 270 °C were tested. High desorption temperatures can enhance the process but they can also degrade analytes. The best temperature for desorption was 270 °C. Desorption profiles of α- and β-endosulfan were obtained by plotting the detector response versus different desorption times (1, 2, 3, 5 and 7 min). Desorption profiles showed that a 2-min period was sufficient to desorb both pesticides in the GC injector port (Fig. 4). When chromatographic analysis was completed, the fiber was immediately thermally desorbed again at these conditions to determine carryover; no pesticide peaks were registered. The chromatogram obtained once SPME was optimized is shown in Fig. 1 (bottom).

3.2.2. Validation

The linearity of the method was evaluated by regressing α- and β-endosulfan peak areas, separately, relative to that of the lindane versus the analyte concentration using standard fortified aqueous samples after applying the optimized SPME procedure to all standards. The 10-point calibration line was found to have good linearity (see Table 1).

The recovery±repeatability of SPME–GC–ECD

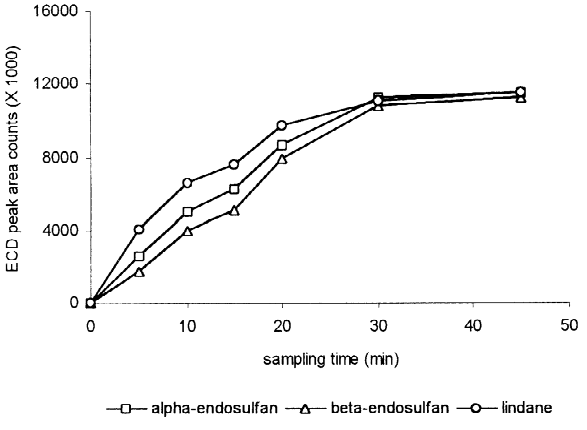

Fig. 3. Sorption time profiles for α-endosulfan (□), β-endosulfan (△) and lindane (○) by direct-SPME using DVB–CAR–PDMS fiber. Aqueous samples (40 ml) containing both pesticides (5 μg/l of each compound) were analysed.

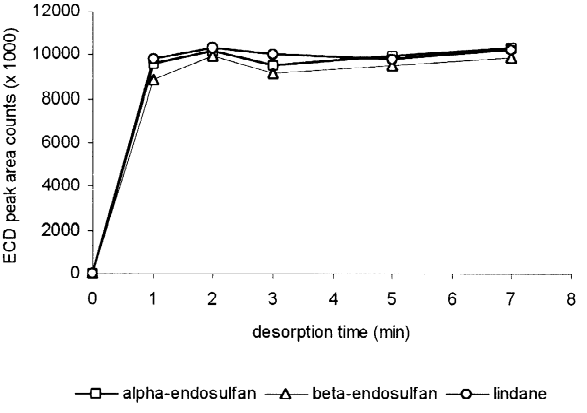

Fig. 4. Desorption time profiles for α-endosulfan (□), β-endosulfan (△) and lindane (○) by direct-SPME using DVB–CAR–PDMS fiber. Aqueous samples (40 ml) containing both pesticides (5 μg/l of each compound) were analysed.

for α- and β-endosulfan from water was measured at levels of 0.1 and 1.0 μg/l by the analysis of three samples (100 ml) of ultrapure water fortified with a pesticide methanolic solution (internal standard concentration was 5 μg/l). These samples were quantified with α- and β-endosulfan standard solutions injected directly into the GC column to estimate absolute recoveries±RSD for α- and β-endosulfan. Relative recoveries±RSD were estimated by using the initial calibration procedure in the paragraph described above (see Table 1).

Limits of detection and quantitation were evaluated on the basis of the noise obtained with the analysis of unfortified aqueous samples, and then tested experimentally.

Duplicate samples of spiked ultrapure and surface waters were analyzed and results obtained are in Table 2. Standard deviations and mean values obtained were compared using, respectively, the Fischer *F*-test (95% probability) and the Student two-tailed *t*-test (95% probability) [12]. No significant differences between both matrices were obtained. This method can then be applied to surface water samples.

4. Conclusions

The present study has shown that the SPME–GC–ECD method is suitable for monitoring both endosulfan isomers in water samples. The DVB–CAR–PDMS fiber, which use for endosulfan determination is not found in the literature, is now proposed for extracting them. Repeatability, detection and quantitation limits are in the same order than the SPE, allowing to detect contaminant levels below the legal maxima in drinking and surface waters required by European Union; no matrix effects interfere in the quantitation process. Although SPME avoids the use of organic solvents, SPE can be automatable and calibration can be performed using aqueous standards injected directly into the column due to the absolute recovery for both isomers of 100%.

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