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Application of single-drop microextraction and comparison with solid-phase microextraction and solid-phase extraction for the determination of α- and β-endosulfan in water samples by gas chromatography–electron-capture detection

M.C. López-Blanco, S. Blanco-Cid, B. Cancho-Grande, J. Simal-Gándara*

Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E-32004 Ourense, Spain

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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 the measurement of pesticides residues 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 necessary. The application of a new extraction technique: single-drop microextraction (SDME), followed by gas chromatography with electron-capture detection, was assessed for determining α-endosulfan and β-endosulfan in water samples. Experimental parameters which control the performance of SDME, such as selection of microextraction solvent and internal standard, optimization of organic drop volume, effects of sample stirring, temperature and salt addition, and sorption time profiles were studied. Once SDME was optimized, analytical parameters such as linearity, precision, detection and quantitation limits, plus matrix effects were evaluated. The SDME method was compared with solid-phase microextraction and solid-phase extraction with the aim of selecting the most appropriate method for a certain application.
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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.

Monitoring pesticide residues in waters is important for human health protection and environmental control. The European Union has set a maximum admissible concentration of 1.0 μg/l for each pesticide in surface water and 0.1 μg/l in drinking water [1].

Prior to chromatographic separation, pesticides are extracted and preconcentrated from the aqueous

*Corresponding author. Tel.: +34-988-387-000; fax: +34-988-387-001.
E-mail address: jsimal@uvigo.es (J. Simal-Gándara).

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medium and interfering compounds in the matrix are removed at the same time. Liquid–liquid extraction (LLE) is the classical approach for pesticide extraction from waters [2–4] but this technique is time consuming and requires large volumes of expensive and toxic solvents. A substantial improvement for aqueous sample preparation techniques resulted from the development of solid-phase extraction (SPE), using bonded silica sorbents. SPE offers the advantages of a shorter analysis time, lower cost, and the consumption of very low volumes of organic solvents [5–7]. Extraction methods are continually revised and improved with new technologies in order to reduce the laboratory staff resources, especially time required for sample extraction and preparation. Solid-phase microextraction (SPME), the extraction technique developed by Pawliszyn and co-workers [8–11], has become popular for the analysis of organic compounds because it combines sampling and pre-concentration in one step. SPME has been applied extensively to determine pesticide residues in water samples [12–22].

Single-drop microextraction (SDME) has been recently developed as an alternative extraction technique [23–27]. SDME provides analyte extraction in a single drop of organic solvent; therefore small volumes of organic solvent (from 0.5 to 2.5 μl) are used. When extraction is finished, the single drop of organic solvent is injected into the GC port for analysis. SDME avoids the problems of solvent evaporation as seen in LLE and SPE as well as fiber degradation of SPME; it is also fast, inexpensive and uses simple equipment. SDME has been successfully applied for the determination of alcohols [28], nitroaromatic explosives [29], chlorobenzenes [30], drugs [31,32] and also for the screening of pesticides [33,34] and volatile organic compounds (VOCs) in water [35].

The main objective of this paper is to study the applicability of SDME followed by GC with electron-capture detection (ECD) to determine endosulfan in water samples. Experimental parameters affecting the extraction of the studied pesticides, such as selection of organic solvent, organic drop volume, sample stirring, salt addition, temperature and sampling time, were assessed and optimized. Quality parameters and matrix effects were evaluated by analyzing spiked water samples. The optimized SDME–GC–ECD procedure was compared with SPE–GC–ECD and SPME–GC–ECD [36] for various applications.

2. Experimental

2.1. Chemicals, solvents and disposables

α-Endosulfan (97%) and β-endosulfan (98.5%) were obtained from Dr Ehrenstorfer Lab. (Augsburg, Germany). Lindane, used as internal standard, was obtained from Aldrich (Milwaukee, WI, USA). Other reagents used were methanol *purge and trap grade* from Aldrich; hexane *for trace analysis* and isooctane *for trace analysis* from Merck (Seelze, Germany); and water *for chromatography* from Merck).

For SDME analysis, water samples were placed in 2-ml glass vials from Supelco (Bellefonte, PA, USA), equipped with stir bars (10×6 mm I.D.) from Afora (Barcelona, Spain), and sealed with PTFE-faced silicone septa. Water samples were stirred with a magnetic stirrer from Bunsen (Barcelona, Spain). SDME was performed with a 10 μl-microsyringe (model 701 and needle point 1) from Hamilton (Reno, NV, USA).

2.2. Stock standard solutions

Stock standard solutions (ca. 1000 mg/l) were prepared in methanol, separately, by accurately weighing approximately 0.01 g of analyte into 10-ml volumetric flasks and diluting to volume. Intermediary mix standard solutions were prepared by diluting the stock standard solutions in methanol. Stock and intermediary standard solutions of the internal standard, lindane, were prepared in the same way. All solutions were stored at 4 °C in the dark.

Water samples were prepared by spiking with different volumes of intermediary standard solutions and were used for the evaluation of the method performance.

2.3. SDME procedure

A water sample (1.8 ml) was placed into a 2-ml

glass vial equipped with PTFE-coated magnetic stir bar and screw capped with a PTFE-faced silicone septum. Isooctane, containing a fixed concentration of lindane (25 μg/l) as internal standard, was drawn into a microsyringe. The needle of the microsyringe was inserted through the septum and directly immersed into the water sample. The microsyringe plunger was depressed to expose the isooctane drop (1.5 μl) to the sample. SDME was performed for 20 min with magnetic stirring (800 rev./min) at room temperature (22 °C). After microextraction, the organic drop was drawn back into the syringe and the needle removed from the vial and transferred immediately into the GC injection port for analysis.

2.4. Analytical instrumentation and operating conditions

A Fisons (Rodano, Italy) 8000 series gas chromatograph equipped with an ECD system was used. Chromatographic separations were performed using a Supelco MDN-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: initially 80 °C, immediately ramped at 15 °C/min to 250 °C, then ramped at 5 °C/min to 300 °C and finally held for 10 min at 300 °C. A split/splitless injector was used in the splitless mode (1 min) for SDME analyses. Helium (125 kPa) and nitrogen (150 kPa) were used as carrier and make-up gases, respectively. The injector temperature was 250 °C and the detector temperature was 300 °C.

2.5. Water samples

Tap water samples were collected from the local water supply (Ourense, Spain). Surface water samples were collected from A Limia basin (Ourense, Spain). Both tap and surface water samples were collected in glass bottles and stored at 4 °C before use. Surface and tap water samples were free of the selected pesticides as found by previous analysis. Spiked surface and tap water samples were analyzed after 24 h in order to allow the equilibration of α-endosulfan and β-endosulfan.

3. Results and discussion

3.1. SDME optimization

To develop a SDME method for determining α- and β-endosulfan in water samples, several parameters controlling optimum performance, such as the selection of organic solvent, optimization of internal standard, optimization of organic drop volume, effect of sample stirring, effect of salt addition and effect of temperature and sorption time profiles were assessed.

3.1.1. Selection of organic solvent

The choice of an appropriate water-immiscible solvent is essential for establishing the SDME method, which depends on the solubility of target compounds in such a extraction solvent. Two water-immiscible solvents (hexane and isooctane) were tested to select the best one for the extraction of α- and β-endosulfan in water samples with this technique.

Solvent selection was performed by extraction of a fortified ultrapure water sample (1.8 ml at level of 2 μg/l with each fungicide and internal standard) with a single solvent drop (1.5 μl). Spiked water samples were extracted at room temperature for 15 min without stirring. After extraction, the plunger was withdrawn and the microdrop was retracted into the microsyringe which was transferred to the split/splitless injector of the GC–ECD instrument. Three replicate analysis were performed for each solvent.

Average peak areas obtained for each pesticide with the two solvents are shown in Fig. 1. Non-polar hexane is recommended by de Jager and Andrews [34] but it had a lower extraction capability than isooctane. Non-polar isooctane extracted both pesticides better, with an acceptable reproducibility, since it is less soluble in water and higher boiling point than hexane; it was chosen as the extraction solvent in further experiments. A value of drop volume 97% was estimated to be withdrawn into the microsyringe after the microextraction.

3.1.2. Optimization of internal standard

Lindane was used as internal standard to correct for variation in injection volumes. It can be added directly to each water sample or it can be added to

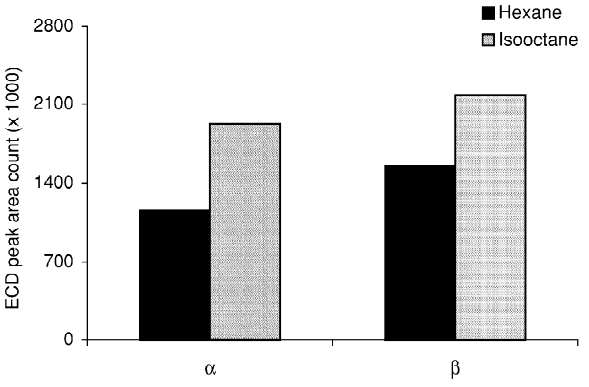

Fig. 1. Efficiency of different organic solvents evaluated for extraction for α-endosulfan, β-endosulfan by SDME. Aqueous samples (1.8 ml) containing both pesticides (2 μg/l of each compound) were analyzed in triplicate. Isooctane was used for the remaining experiments.

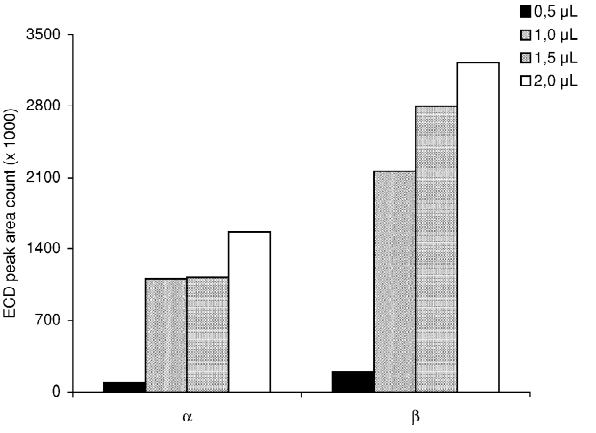

Fig. 2. Efficiency of different isooctane drop volumes evaluated for extraction of α-endosulfan, β-endosulfan by SDME. Aqueous samples (1.8 ml) containing both pesticides (2 μg/l of each compound) were analyzed in triplicate.

the organic extractant. In order to evaluate whether lindane should be added to the water samples or to the extracting solvent, fortified water samples (at a level of 2 μg/l for each pesticide) were analyzed in triplicate with lindane (4 μg/l) added to water samples and added to isooctane (25 μg/l). No significant differences in α- and β-endosulfan extraction were obtained when lindane was present in the organic or aqueous phases. Further experiments were performed considering the organic extractant containing a fixed concentration of lindane to simplify the SDME procedure.

3.1.3. Optimization of organic drop volume

To increase the sensitivity of the SDME procedure, the organic drop volume was optimized. For this purpose, experiments were performed by increasing the drop volume from 0.5 to 2.0 μl for extracting fortified water samples (at a level of 2 μg/l for each pesticide), in triplicate, under the experimental conditions described above. As can be shown in Fig. 2, peak areas of pesticides increased with drop volume. However, using high drop volumes of organic solvent can result in the loss of the organic drop. To avoid these losses, drop volumes of 1.5 μl were considered in further experiments.

3.1.4. Effect of sample stirring

Sample stirring increases extraction efficiencies and reduces extraction time, as the equilibrium betweeen the aqueous and the organic phases is established more rapidly. To evaluate the effect of sample stirring, spiked water samples (at a level of 2 μg/l for each pesticide) were extracted, in triplicate, with an isooctane drop (1.5 μl) for 15 min at room temperature and at different stirring rates (0, 400 and 800 rev./min). Higher stirring rates were not evaluated because they damaged the drop. Experimental results (Fig. 3) showed that peak areas of

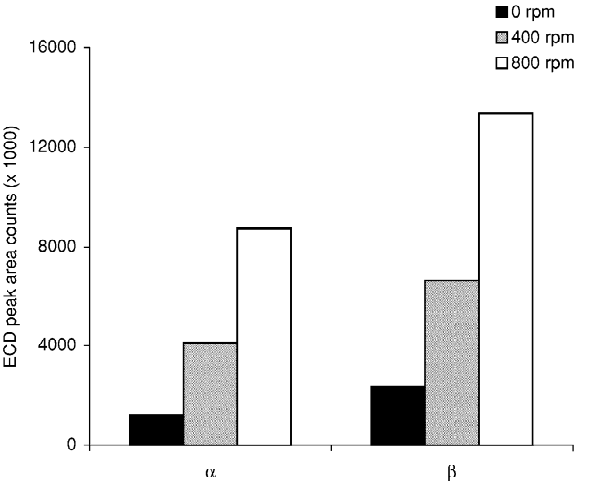

Fig. 3. Efficiency of different stirring rates evaluated for extraction of α-endosulfan, β-endosulfan by SDME. Aqueous samples (1.8 ml) containing both pesticides (2 μg/l of each compound) were analyzed in triplicate.

pesticides increased with stirring rate. Further experiments were performed with a stirring rate of 800 rev./min.

3.1.5. Effect of salt addition

The effect of increasing the ionic strength of the water sample was evaluated, in triplicate, by adding NaCl amounts from 0 to 400 mg into spiked water samples (at a level of 2 μg/l for each pesticide). SDME experimental conditions were the same as those described above. Experimental results showed that pesticide peak areas decreased when NaCl amounts increased, as can be seen in Fig. 4. This behaviour was also observed by Psillakis and Kalogerakis who determined nitroaromatic explosives in water samples with this technique [29]; they explained that NaCl dissolved in water might have changed the physical properties of the Nerst diffusion film and reduced the rate of diffusion of the target analytes into the drop [37]. To increase extraction efficiency, no salt addition was performed in further experiments.

3.1.6. Effect of temperature

The effect of temperature was studied by exposing an isooctane drop for 5 min in fortified water samples (at a level of 2 μg/l for each pesticide), in triplicate, at 22 and 45 °C. Experimental results showed that, by increasing the temperature, extraction efficiency was increased. However, high temperatures can cause solvent drop damage and decrease the reproducibility of SDME procedure. To simplify the method, further experiments were performed at room temperature.

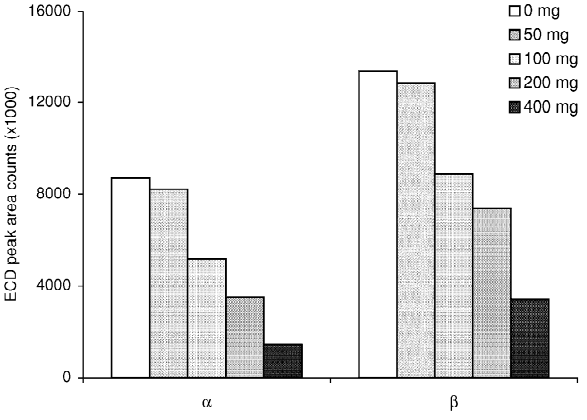

Fig. 4. Efficiencies of different NaCl amounts for extraction of α-endosulfan, β-endosulfan by SDME. Aqueous samples (1.8 ml) containing both fungicides (2 μg/l of each compound) were analyzed in triplicate.

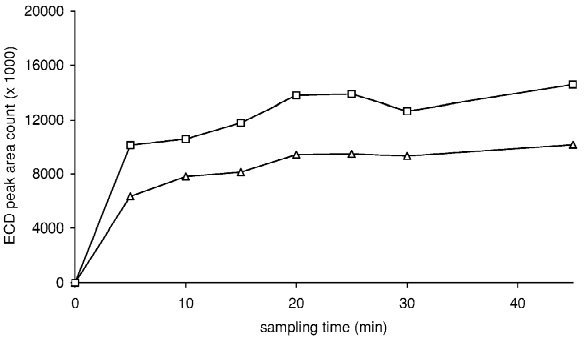

Fig. 5. Sorption time profiles for α-endosulfan (Δ), β-endosulfan (□) by SDME. Aqueous samples (1.8 ml) containing both pesticides (2 μg/l of each compound) were analyzed in duplicate.

3.1.7. Sorption time profiles

It is necessary to select an exposure time that guarantees the equilibrium between aqueous and organic phases has been achieved and the amount of pesticides extracted is maximum. The sorption time profile for each pesticide was obtained by plotting the ECD response vs. the extraction time evaluated (from 0 to 45 min) for each pesticide to obtain the partition equilibrium curve (Fig. 5). Fortified water samples (at level of 2.0 μg/l) were analyzed, by duplicate, under the experimental conditions described in the SDME procedure. Sorption time profiles indicated that the equilibium between both phases was reached after 20 min, as can be seen in Fig. 5.

3.2. Evaluation of method performance

Quality parameters of the SDME–GC–ECD method, such as linearity, repeatability, reproducibility and limits of detection and quantitation, were calculated under the optimized conditions described in the SDME procedure.

The linearity of the method was calculated by analyzing fortified water samples (from 0.05 to 2.0 μg/l). The eight-point calibration curves were found

Table 1
Quality parameters for SDME, SPME and SPE techniques, followed by GC–ECD, for determining α- and β-endosulfan from water samples

	α-Endosulfan			β-Endosulfan		
	SDME	SPME[a]	SPE[a]	SDME	SPME[a]	SPE[a]
Recovery[b] (%)						
0.1 μg/l	3.8	<0.1	115	9.2	<0.1	108
Repeatability[b] RSD (%)						
0.1 μg/l	5.5	12.4	6.4	4.9	19.2	8.1
1.0 μg/l	1.7	2.1	3.4	4.9	3.0	8.1
Reproducibility[c] RSD (%)						
0.1 μg/l	10.3	14.5	6.5	17.7	13.6	14.8
1.0 μg/l	10.2	6.0	13.3	11.1	5.6	11.8
Linearity[d]						
r^2	0.999	0.994	0.999	0.998	0.996	0.995
Linear range (μg/l)	0.1–0.9	0.1–4.5	0.05–1.0	0.1–0.9	0.1–5.0	0.05–1.0
LOD[b] (μg/l)	0.01	0.06	0.02	0.01	0.05	0.02
LOQ[b] (μg/l)	0.02	0.13	0.04	0.03	0.10	0.03
Matrix effects	No	No	No	No	No	No

[a] Data taken from our previous work published in Ref. [36].
[b] $n=5$ determinations.
[c] $n=6$ determinations.
[d] $n=8$ determinations.

to have good linearity. Linear ranges and r^2 values are presented in Table 1. The repeatibility and reproducibility of the method were calculated for two different concentrations separately (0.1 and 1.0 μg/l) by analyzing five replicate water samples ($n=5$) and a total of three replicate water samples per day for 2 days in different weeks ($n=6$), respectively. Relative standard deviations (RSDs) for repeatability and reproducibility are given in Table 1. Limits of detection (LODs) and quantitation (LOQs) were evaluated following the recommendations of the American Chemical Society [38]. Limits are given in Table 1 and are lower than the maxima admissible concentrations established by the European Directive [1].

3.3. Matrix effects assessment

Common components of natural water samples, including humic and fulvic acids, inorganic salts and others, could reduce the applicability of the method in the analysis of water samples by decreasing the quantitative recovery or by interfering in the determination. To evaluate the application of the SDME procedure as a pesticide screening method, spiked ultrapure, surface and tap waters were analyzed.

Triplicate samples of ultrapure and natural water samples, spiked with the studied pesticides at a 0.1 and 1 μg/l, were analyzed. The results obtained are reported in Table 2. The standard deviations and

Table 2
Mean concentrations and standard deviations of α- and β-endosulfan measured in spiked ultrapure, tap and surface waters at 0.1 and 1.0 μg/l of each pesticide determined by SDME followed by GC–ECD

	Water sample concentration (μg/l)±SD					
	Ultrapure water		Tap water		Surface water	
	0.1 μg/l	1.0 μg/l	0.1 μg/l	1.0 μg/l	0.1 μg/l	1.0 μg/l
α-Endosulfan	0.10±0.01	1.00±0.02	0.10±<0.01	0.95±0.02	0.09±<0.01	0.90±<0.01
β-Endosulfan	0.10±0.01	1.00±<0.01	0.09±<0.01	0.98±<0.01	0.10±<0.01	0.99±0.02

$n=3$ determinations.

mean values obtained were compared using the Student two-tailed *t*-test (95% probability) [39]. No significant difference was obtained for values within the statistical allowances. It was concluded that matrix effects do not interfere in the quantitation process and SDME–GC–ECD may be used as an alternative method for screening organochlorine pesticides in water samples.

3.4. Comparison of SDME performance vs. SPME and SPE

The optimized SDME–GC–ECD procedure was compared with SPME–GC–ECD and SPE–GC–ECD (Table 1). All experimental data on SPME and SPE methods were taken from Ref. [36].

Water extraction and analysis of α- and β-endosulfan is possible with all three methods. In terms of analysis time, SDME and SPME are equilibrium techniques which allow the determination of the target compounds in 20 and 15 min, respectively. However, they are not exhaustive extraction techniques (recoveries <10 and 0.1%, respectively). SPE is an exhaustive extraction technique (recovery= 100%) but needs additional analysis time and higher volumes of organic solvent.

Regarding quality parameters for the three techniques, there are some differences, as can be seen in Table 1. The repeatability and reproducibility, expressed as RSD, were quite similar for both compounds, independently of the extraction technique considered. The determination coefficient (r^2) for the three techniques are in the same order (>0.995); linear ranges obtained for SDME and SPE were similar and SPME presented a higher concentration in the upper linear range. The three preconcentration methods yielded limits of detection below those established by the European Directive [1] but SDME is more sensitive. Matrix effects do not affect the correct quantitation of the SDME, SPME and SPE methods.

4. Conclusions

In the present study an alternative method, SDME–GC–ECD, for the determination of α- and β-endosulfan in water samples was evaluated. Adequate repeatability, reproducibility, linearity, limits of detection and the absence of matrix effects indicated that SDME–GC–ECD can be used for screening target compounds from ultrapure, tap and surface water samples. The use of organic solvents for SDME is negligible compared to SPE. The cost of SDME is negligible compared to the cost of commercial SPME fibers and SPE cartridges. In conclusion, SDME–GC–ECD may allow fast and inexpensive screening methods to be developed for other groups of environmental pesticides.

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