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Journal of Chromatography A, 1007 (2003) 137–143
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of endosulfan isomers and endosulfan sulfate in tomato juice by matrix solid-phase dispersion and gas chromatography☆

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Received 7 March 2003; received in revised form 21 April 2003; accepted 22 May 2003

Abstract

A rapid method based on matrix solid-phase dispersion was developed for the determination of endosulfan isomers and endosulfan sulfate in commercial tomato juice. After the optimisation of different parameters such as the type of adsorbent, the extraction solvent, and the extraction assistance by sonication, the recoveries obtained ranged from 81 to 100% with relative standard deviations equal to or lower than 10%. The analysis of samples was accomplished using gas chromatography with electron-capture detection and the identity of endosulfan residues was confirmed by gas chromatography–mass spectrometry with selected ion monitoring. The detection limit for these compounds, calculated as three times the background noise, was 1 μg/kg. The proposed method was applied to the analysis of these compounds in commercial juice samples and levels of endosulfan between 1 and 5 μg/kg were detected in some samples.

Keywords: Fruit juices; Food analysis; Matrix solid-phase dispersion; Extraction methods; Endosulfan; Pesticides

1. Introduction

Insecticides are often used in horticultural crops to control pests that may produce important yield reductions. Endosulfan is a broad-spectrum insecticide frequently applied, as a mixture of isomers, for pest control on fruits and vegetables. Fig. 1 shows the chemical structures of these insecticides. These compounds may persist over the season and, therefore, their residues may be found in fruits after harvest. Fruit juices constitute an important share of the commercially processed fruit products and are a staple component of the diet of wide number of countries. Therefore, the analysis of insecticide residues in fruit juices is necessary in order to know their concentration in those matrices.

The conventional methods used for the determination of chlorinated pesticide residues in fruits and vegetables are usually based on liquid–liquid extraction [1–6] followed by a cleanup step [1,7,8]. During the past few years, many new extraction

☆Presented at the 2nd Meeting of the Spanish Society of Chromatography and Related Techniques, Barcelona, 26–29 November 2002.

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00954-3

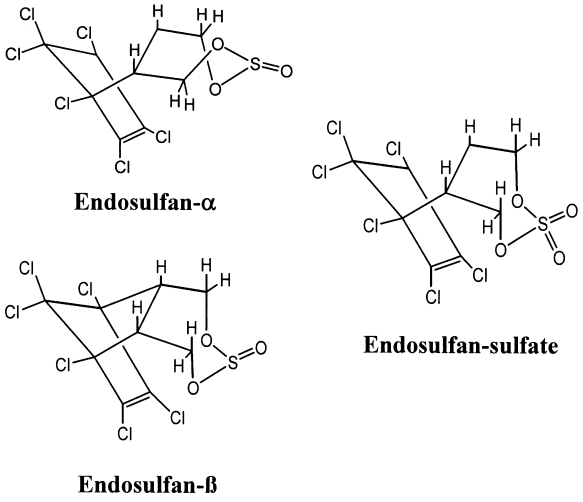

Fig. 1. Chemical structures of endosulfan and endosulfan sulfate.

techniques have appeared, such as supercritical fluid extraction (SFE) [9,10], solid-phase microextraction (SPME) [11], and solid-phase extraction (SPE) [12]. Matrix solid-phase dispersion (MSPD), based on the dispersion of the sample on an adsorbent generally Florisil or C_{18}, is a technique that allows the extraction and cleanup in one single step [13,14]. Recently, this technique has been applied, using diatomaceous earth, to determine some pesticides in fruit juices [15].

The analysis of endosulfan residues is generally performed by gas chromatography with electron-capture detection (GC–ECD), although gas chromatography coupled with mass spectrometry (MS) [13,16] and GC with tandem mass spectrometry (MS–MS) [17,18] have also been used.

The aim of this work was to develop an MSPD method for the determination of endosulfan isomers together with endosulfan sulfate in commercial tomato juices. Residues were determined by GC–ECD and their identity was confirmed by GC–MS with selected ion monitoring (SIM).

2. Experimental

2.1. Materials

2.1.1. Chemicals

Endosulfan-α, endosulfan-β, and endosulfan sulfate standards were purchased from Riedel-de Häen (Steinheim, Germany). Ethyl acetate, acetone and *n*-hexane (pesticide grade) were obtained from Scharlau (Madrid, Spain). Florisil 60–100 mesh, heated at 200 °C overnight before use, was obtained from Fluka (Buchs, Switzerland). Anhydrous sodium sulfate and aluminium oxide 90 standardised were from Merck (Darmstadt, Germany).

2.1.2. Pesticide solutions

Three stock solutions containing 0.4, 0.2, and 0.1 μg/ml of each insecticide in acetone were prepared and used to fortify the tomato juice samples. Standard solutions containing 0.1, 0.05, 0.02 and 0.01 μg/ml of each insecticide in ethyl acetate were used as chromatographic standards.

2.1.3. Columns

Glass columns (10 cm×20 mm I.D.) were purchased from Pobel (Madrid, Spain) and Whatman No. 1 filter paper circles of 2 cm diameter placed at the bottom end, were from Whatman (Maidstone, UK).

2.2. Apparatus

2.2.1. GC–ECD

A Hewlett-Packard 5890 Series II gas chromatograph (Waldbronn, Germany) equipped with a Model HP 6890 automatic split–splitless injector and an electron-capture detector was used. A fused-silica capillary column, HP-1, with crosslinked dimethylpolysiloxane as the nonpolar stationary phase (30 m×0.25 mm I.D., 0.25 μm film thickness) supplied by Agilent (Madrid, Spain) was employed, with helium as the carrier gas at a flow-rate of 1 ml/min. The column temperature was maintained at 90 °C for 1 min, then programmed at 5 °C/min to 220 °C, held for 10 min and programmed at 10 °C/min to 260 °C, held for 2 min. The injector port was maintained at 270 °C and the detector temperature was 300 °C. A 2-μl volume was injected in the splitless mode.

2.2.2. GC–MS

A Hewlett-Packard 6890 equipped with an automatic split–splitless injector Model HP 7683 and a 5973 series mass-selective detector was used for the confirmation of the pesticides studied. A fused-silica

capillary column (ZB-5MS), 5% phenyl polysiloxane as nonpolar stationary phase (30 m×0.25 mm I.D.) and 0.25 μm film thickness, supplied by Phenomenex, was employed, with helium as carrier gas at 1 ml/min. The column temperature was maintained at 80 °C for 1 min, then programmed at 8 °C/min to 220 °C, held for 10 min and programmed at 10 °C/min to 260 °C, held for 2 min. The injector port was maintained at 270 °C and a 2-μl volume was injected in pulsed splitless mode (pulsed pressure 45 p.s.i. for 1.5 min; 1 p.s.i.=6894.76 Pa).

Mass spectrometric parameters: electron impact ionisation mode with an ionising energy of 70 eV, ion source temperature 230 °C, MS Quad temperature 150 °C, electron multiplier voltage 1000; solvent delay, 5 min.

Analysis was performed in the SIM mode using two acquisition windows as follows: (1) from 0 to 29.01 min, *m/z* 195, 241, 339; (2) from 29.01 to 37.50 min, *m/z* 229, 272, 387.

2.2.3. Laboratory equipment

An ultrasonic water bath (Raypa, Barcelona, Spain) was used in the extraction procedure. The generator of this apparatus has an output of 150 W and a frequency of 33 kHz. A 12-port vacuum manifold from Supelco was employed.

2.3. Juice samples

Various commercial brands of tomato juice were purchased from supermarkets in Madrid.

2.4. Procedure

Glass columns, with Whatman No. 1 filters placed at the bottom end, were filled with 2.5 g of Florisil. A 2-ml volume of tomato juice was transferred to the glass column, fortified when required with 0.5 ml of the pesticide mixture in acetone. A 0.5-ml volume of acetone was added instead to unfortified samples. Acetone was used to allow a better sample distribution throughout the column and an additional amount of Florisil (1 g) was added to enhance the dispersion of the tomato juice sample in the matrix. The columns were placed in a tube rack and closed with one-way stopcocks. Juice samples were extracted twice with ethyl acetate (5 ml) for 15 min in an ultrasonic bath at room temperature. Water level was adjusted to solvent level inside the columns. After sonication, columns were placed in a vacuum manifold and the extraction solvent was filtered. The extracts were collected in 10-ml graduated glass tubes and concentrated, with a gentle stream of air, near 1 ml. The concentrated extracts were diluted to 10 ml with *n*-hexane for the highest fortification level and to 5 or 2 ml for the other two levels. Extracts from commercial juice samples were diluted to 2 ml. The use of *n*-hexane together with the addition of anhydrous sodium sulfate (ca. 0.2 g) facilitated the drying of extracts and improved the chromatograms obtained.

3. Results and discussion

3.1. Optimisation of the MSPD method

The proposed method was used to determine endosulfan and endosulfan sulfate in tomato juice. First of all, the influence of the adsorbent in the recoveries was evaluated. Florisil and aluminium oxide were assayed using ethyl acetate–hexane (70:30, v/v) as extraction solvent and without assisted sonication at 0.1 μg/g fortification level. Although similar results were obtained for both adsorbents, around 65% of average recovery, Florisil was chosen because cleaner extracts were obtained. Different amounts of Florisil were tried to optimise the adsorbent–sample ratio but similar results were obtained when 3 or 4 g of Florisil were used.

Different extraction solvents were assayed to improve the extraction procedure. Ethyl acetate and mixtures of ethyl acetate and *n*-hexane were tried out. Best recoveries were obtained when ethyl acetate was used as extraction solvent.

The effect of sonication in the extraction procedure was studied at 0.1 μg/g with two different extraction solvents. Table 1 shows that recoveries improved when extraction was assisted with sonication, as recoveries without sonication were always lower than those obtained with sonication. The average recoveries of ethyl acetate extraction with sonication were equal or higher than 80% for all the pesticides.

To enhance sample dispersion and facilitate extraction, an additional amount of Florisil (1 g) was added to tomato juice samples fortified at 0.1 μg/g.

Table 1
Influence of extraction solvent, assisted or not by sonication, on the pesticide recovery (0.1 μg/g)[a]

Pesticide	Recovery (%) (mean±RSD)			
	Ethyl acetate–hexane (70:30, v/v)		Ethyl acetate	
	Without sonication	With sonication	Without sonication	With sonication
Endosulfan-α	67.6±9.8	70.1±8.2	74.4±3.8	86.9±4.2
Endosulfan-β	63.7±7.6	68.6±7.5	70.4±4.1	80.0±3.9
Endosulfan sulfate	67.1±4.1	73.8±9.4	77.0±8.8	94.9±5.9

[a] Results are the mean of four replicates ±RSD.

The recoveries obtained were higher than 86% for all the pesticides. Mean relative standard deviations (RSDs) for all the pesticides studied were lower than 6% at this fortification level.

3.2. Recoveries

Tomato juice samples were fortified at 0.1, 0.05 and 0.025 μg endosulfan/g juice, and the recoveries obtained following the proposed procedure are shown in Table 2. Endosulfan recoveries ranged from 81 to 100% with RSDs equal or lower than 10%. Fig. 2 shows representative GC–ECD chromatograms of a standard solution of 0.020 μg/ml and a tomato juice sample fortified at 0.05 μg/g. Some matrix peaks that appeared in the tomato juice

Table 2
Insecticide recoveries obtained from tomato juice

Concentration (μg/g)	Recovery (%) (mean±RSD)[a]		
	Endosulfan-α	Endosulfan-β	Endosulfan sulfate
0.1	91.0±2.3	86.7±2.0	100.6±6.1
0.05	81.3±3.8	81.3±4.2	87.6±4.5
0.025	90.3±10.00	81.0±6.9	86.7±8.6

[a] Results are the mean of four replicates ±RSD.

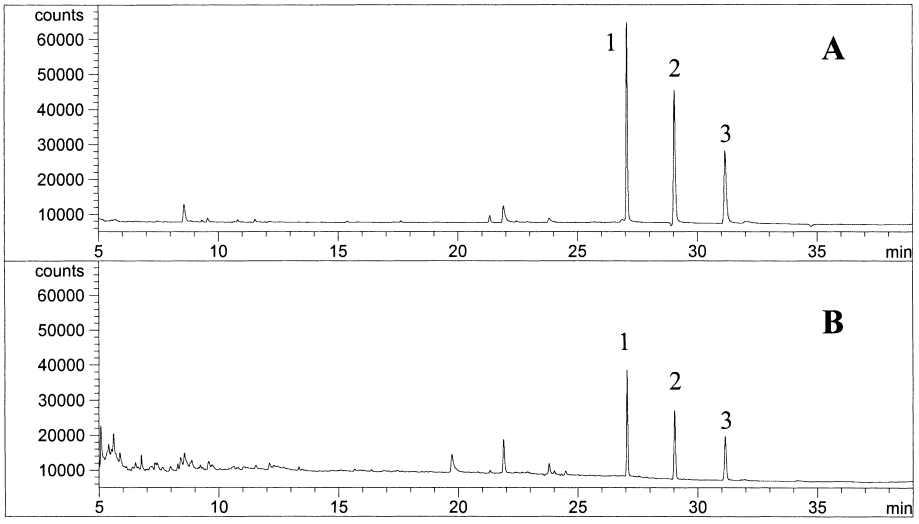

Fig. 2. GC–ECD chromatograms of a standard mixture solution at 0.02 μg/ml (A) and a fortified juice sample at 0.05 μg/g (B), where: (1) endosulfan-α, (2) endosulfan-β, (3) endosulfan sulfate.

Table 3
Limit of detection (LOD), limit of quantification (LOQ) and linearity

Pesticide	GC–ECD		Calibration data		LOD (μg/kg)	LOQ (μg/kg)
	t_R (min)	RSD (%)	Equation	Correlation coefficient		
Endosulfan-α	27.12	0.01	$y=1.4\cdot10^7x+2.1\cdot10^4$	0.999	1.0	3.0
Endosulfan-β	29.11	0.02	$y=1.3\cdot10^7x+4.1\cdot10^4$	0.998	1.0	3.0
Endosulfan sulfate	31.23	0.01	$y=1.1\cdot10^7x+8.5\cdot10^3$	0.999	1.0	3.0

sample chromatogram do not interfere with the studied pesticides and, therefore, a further cleanup step was not necessary.

3.3. Detection limits, quantification limits and linearity

The limits of detection (LODs) of the proposed method were determined by considering a value ≥3 times the background noise obtained for blank samples whereas the limits of quantification (LOQs) were determined considering a value 10 times the background noise. The detection limit for endosulfan-α, endosulfan-β, and endosulfan sulfate was considered 1 μg/kg and the quantification limit was 3 μg/kg for all the compounds.

The detector response was linear in the range of concentrations studied. The linearity of the method was assayed by analysing standard solutions in the range from 0.01 to 0.10 μg/ml. Correlation coefficients (r) for all the pesticides were ≥0.998. Table 3 summarises the retention times, calibration data, LODs and LOQs of the studied pesticides.

3.4. GC–MS confirmation

The identity of endosulfan residues was confirmed by GC–MS in the SIM mode. Previously, a total ion chromatogram of a standard solution of each compound was obtained to determine its main ions and retention time. Table 4 shows the retention time and the main ions with the relative abundance of each compound.

3.5. Real samples

After developing this MSPD method, seven commercial brands of tomato juice were analysed. The results obtained showed that some commercial samples contained endosulfan residues. One sample contained 1 μg/kg of endosulfan-α and endosulfan-β and another one contained 4 μg/kg of endosulfan-α, 5 μg/kg of endosulfan-β and 3 μg/kg of endosulfan sulfate. Residue levels for the rest of the tomato juice samples were below the detection level. The identity of endosulfan residues in the samples was confirmed by GC–MS-SIM. Fig. 3 shows the GC–ECD and GC–MS chromatograms of the juice sample with the highest levels of endosulfan.

4. Conclusions

The proposed MSPD method allows the determination of endosulfan isomers and endosulfan sulfate in tomato juice samples at low levels. This method is rapid due to the simultaneous extraction and cleanup of the sample, and requires a low consumption of organic solvents. The proposed method can be used as a routine technique in the laboratory for monitoring programmes to determine low residue levels of those pesticides in commercial samples of tomato juice.

Table 4
Main ions and their relative abundance for the studied insecticides

Pesticide	t_R (min)	m/z (% relative abundance)
Endosulfan-α	23.36	195(76), 241*(100), 339(37)
Endosulfan-β	26.48	195*(100), 241(80), 339(36)
Endosulfan sulfate	29.46	229(75), 272*(100), 387(62)

*Quantitation ion.

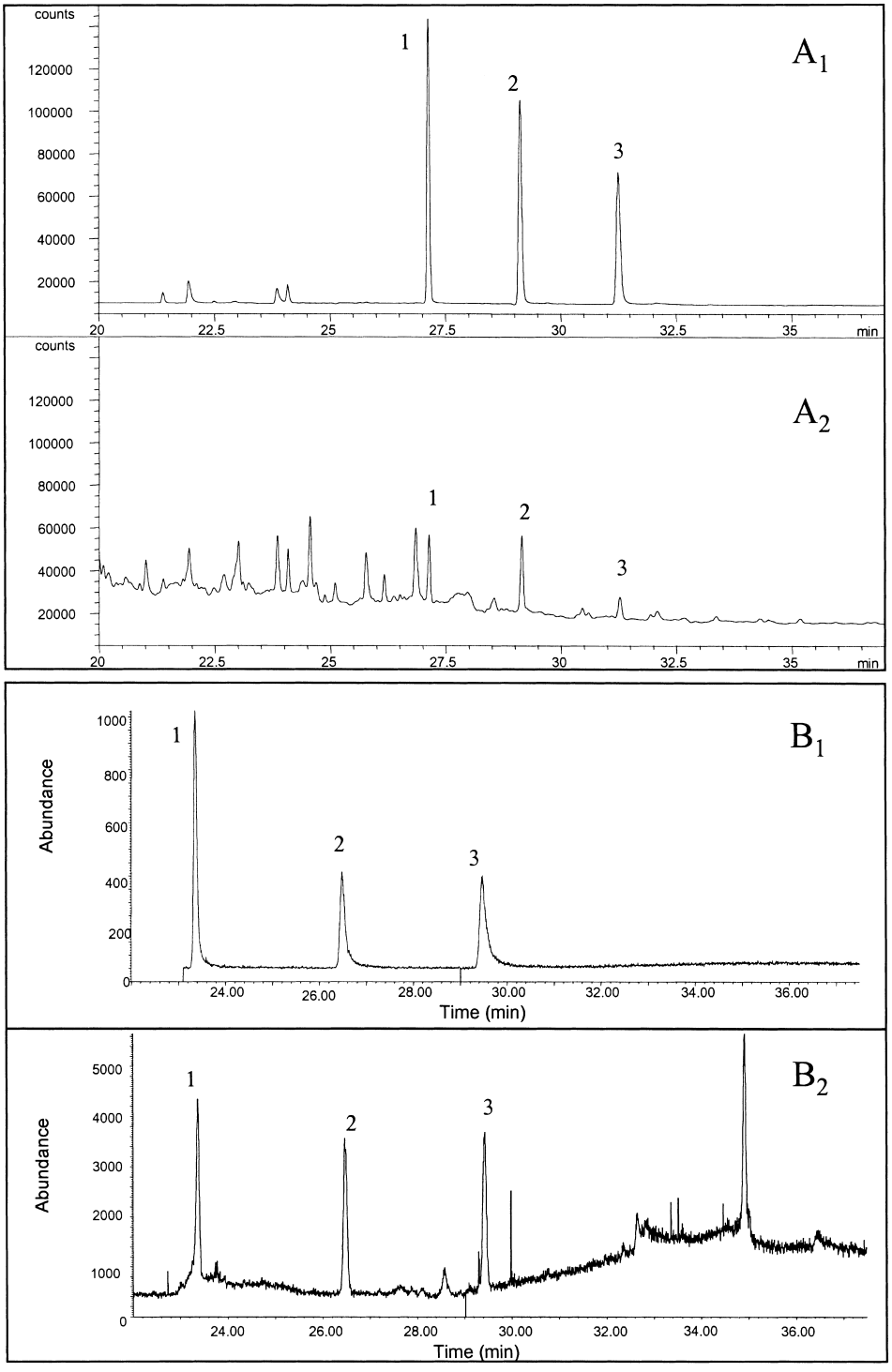

Fig. 3. GC–ECD chromatograms of a standard mixture solution at 0.025 μg/ml (A_1) and of a commercial juice sample (A_2) and GC–MS-SIM chromatograms of a standard mixture solution at 0.01 μg/ml (B_1) and of a commercial juice sample (B_2), where: (1) endosulfan-α, (2) endosulfan-β, (3) endosulfan sulfate.

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