ELSEVIER

Journal of Chromatography A, 947 (2002) 119–127

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of endosulfan isomers and endosulfan sulfate in air and tomato leaves by gas chromatography with electron-capture detection and confirmation by gas chromatography–mass spectrometry ☆

J. Castro[a], R.A. Pérez[b], E. Miguel[a], C. Sánchez-Brunete[a], J.L. Tadeo[a,*]

[a]*Departamento de Uso Sostenible del Medio Natural (INIA), Carretera de La Coruña, Km 7, 28040 Madrid, Spain*
[b]*Departamento de Protección Vegetal (INIA), Carretera de La Coruña, Km 7, 28040 Madrid, Spain*

Received 12 June 2001; received in revised form 7 December 2001; accepted 7 December 2001

Abstract

Rapid analytical methods for the determination of endosulfan isomers and endosulfan-sulfate in air and plant samples were developed. The insecticides were trapped from air using a column containing Florisil and extracted with a low volume of ethyl acetate, assisted by sonication. Pesticide residues were determined by gas chromatography with electron-capture detection using a nonpolar capillary column. Residue identities were confirmed by gas chromatography coupled with mass spectrometry. Recoveries of these compounds from air samples were always higher than 78% with an RSD lower than 11% and the detection limits obtained were at least 0.3 ng/l air. Leaf samples were homogenised with ethyl acetate and extracts cleaned-up on an aluminium oxide column. Pesticides were eluted with a hexane–ethyl acetate (80:20, v/v) mixture. Recoveries obtained from plant samples were higher than 78% with an RSD lower than 14% and detection limits in leaves were 0.02 μg/g for each pesticide. These methods were applied to study the volatilisation of endosulfan from tomato leaves under laboratory conditions. A volatilisation rate near 1% of the initial amount of endosulfan per hour was obtained during the first 24 h at room temperature.

Keywords: Air analysis; Plant materials; Vegetables; Endosulfan; Pesticides

1. Introduction

Volatilisation is a significant pathway for the dissipation of pesticides, mainly in the first hours after their application. This process is crucial for global distribution of pesticides in the atmosphere and it is also very important for potential human exposure to these chemicals. Different sampling methods have been followed in the determination of pesticides in air involving the use of several solvents [1] or various adsorbents, such as Tenax, chromatographic packings, resins, and polyurethane foam (PUF) [2–6]. Glass fibre filters [7], activated carbon [8], and Florisil [9,10] have also been used. However, in these methods, pesticide extraction from adsorbents is generally time-consuming and a large amount of glassware and organic solvents are often

☆Presented at the 30th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques/1st Meeting of the Spanish Society of Chromatography and Related Techniques, Valencia, 18–20 April, 2001.

*Corresponding author. Tel.: +34-913-476-856; fax: +34-913-572-293.

E-mail address: tadeo@inia.es (J.L. Tadeo).

0021-9673/02/$ – see front matter
PII: S0021-9673(01)01598-9

required (e.g. in Soxhlet extraction). Some other alternatives, such as supercritical fluid extraction (SFE) [9] or sonication [5,11] have recently been used in the extraction of different pesticides from adsorbents, but SFE requires special and expensive equipment and sonication is often applied using a large volume of solvent.

Endosulfan, Fig. 1, is a broad-spectrum insecticide used for pest control on fruits and vegetables. Analysis of this compound in air is generally based on retention on an adsorbent and subsequent extraction using various organic solvents [11]. Determination of endosulfan residues in plants is usually carried out by homogenisation with organic solvents followed by clean-up of extracts using several analytical procedures [12–14]. Analysis of residues is generally performed by gas chromatography with electron-capture detection (GC–ECD) [15–17], although gas chromatography coupled with mass spectrometry has also been used [12].

The aim of this work was to develop rapid analytical methods, requiring low volumes of organic solvents, for determination of endosulfan isomers together with endosulfan-sulfate, the main endosulfan metabolite, in air and plant samples. These methods were applied to study volatilisation of endosulfan from tomato leaves treated with a commercial formulation of this pesticide.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Insecticide standards of endosulfan-I, endosulfan-II, and endosulfan-sulfate were obtained from Reidel-de Häen (Steinheim, Germany). Ethyl acetate and *n*-hexane for pesticide residue analysis were from Scharlau (Madrid, Spain). Thimul [endosulfan-I+endosulfan-II, 70:30, 35% (w/v) suspensible liquid], obtained from Rhône Poulenc Química (Madrid, Spain), was used as a commercial formulation. Florisil 60–100 mesh, activated by heating at 180 °C overnight before use, was purchased from Aldrich (Steinheim, Germany). Anhydrous sodium sulfate and aluminium oxide (0.063–0.200 mm) 90 standardized were from Merck (Darmstadt, Germany).

2.1.2. Pesticide solutions

Five stock solutions of the selected compounds were prepared containing 0.1, 0.2, 0.5, 1 and 2 μg/ml of each insecticide and were used to fortify Florisil columns and plant samples in the recovery study. A solution containing 200 μg pesticide/ml was prepared from a commercial formulation of endosulfan for the volatilisation assays.

2.1.3. Columns

Polypropylene columns (12 ml) and polyethylene frits purchased from Supelco (Bellefonte, PA, USA) were used for the retention of pesticides from air samples. Glass columns (20 cm×10 mm I.D.) used in the clean-up of plant extracts were purchased from Pobel (Madrid, Spain).

2.2. Apparatus

2.2.1. GC–ECD

A Hewlett-Packard 5890 Series II gas chromatograph (Waldbronn, Germany) equipped with an electron-capture detector and automatic split-splitless injector was used for the analysis of insecticides. A nonpolar fused-silica capillary column, HP-1 (30

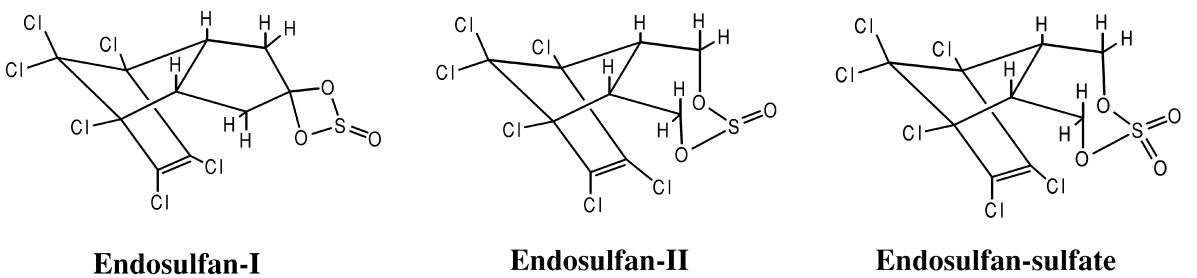

Fig. 1. Structures of endosulfan and endosulfan-sulfate.

m×0.25 mm I.D.) and 0.25 μm film thickness supplied by Agilent (Madrid, Spain), was employed, with helium as carrier gas at 1 ml/min. The column temperature was maintained at 130 °C for 1 min, then programmed at 15 °C/min to 220 °C, held 0.5 min and programmed at 8 °C/min to 280 °C, held 5 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 gas chromatograph equipped with a HP5973 mass-selective detection system and an automatic split-splitless injector was used for the confirmation of the studied pesticides. A nonpolar fused-silica capillary column, HP-1 (30 m×0.25 mm I.D.) and 0.25 μm film thickness supplied by Agilent, was employed, with helium as carrier gas at 1 ml/min. The column temperature was maintained at 120 °C for 1 min, then programmed at 25 °C/min to 230 °C, held 0.5 min and programmed at 15 °C/min to 280 °C, held 1 min. The injector port was maintained at 270 °C and a 2 μl volume was injected in the splitless mode.

Mass spectrometric parameters: electron impact ionisation mode with 70 eV energy, mass range, m/z 50–450; ion source temperature 230 °C, MS Quad temperature 150 °C, scan rate, 3.62 s per scan, electron multiplier voltage 1000; solvent delay, 5 min.

2.2.3. Laboratory equipment

An ultrasonic water bath (Raypa, 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 in the pesticide extraction from the Florisil column.

A Q-Max air sampling pump and a Q-Max twin port sampler purchased from Supelco (Bellefonte, PA, USA) were used in the air analyses. A homogeniser DI 148 from Schott Iberica (Madrid, Spain) was employed to homogenise the vegetable samples.

2.3. Plant samples

Tomato plants were planted into individual pots and maintained in an incubation chamber under controlled conditions (24 °C and 70% air humidity) for 2 months before use in these assays.

2.4. Procedure

2.4.1. Analysis of air samples

Analysis of endosulfan in air was carried out by using a double neck round-bottom flask with a column placed in a cap with a perforated silicone septum. Florisil (2 g) was sandwiched between two frits at the base of the column and a pump was connected at the head providing a flow-rate of 0.5 l/min. Different sampling periods from 1 to 24 h were employed at room temperature (22 °C). Pesticide extraction from the column was carried out, assisted by sonication, using low volumes of ethyl acetate (2×3 ml) and pesticide residues were determined by GC–ECD under the conditions described above.

2.4.2. Plant analysis

Each sample consisted of a whole leaf (0.4 g) cut from the tomato plants. Samples were placed in glass tubes, homogenised twice with 4 ml of ethyl acetate. An additional solvent (2 ml) was utilised each time for washing. The homogenised samples were centrifuged for 10 min at 4600 rev./min, the extract was transferred to another tube and concentrated to 1 ml. Clean-up was accomplished by passing the extract through a column containing a small amount of glass wool at the base and 3.5 g of aluminium oxide with a thin layer of anhydrous sodium sulfate lying on top. A hexane–ethyl acetate (80:20, v/v) mixture (10 ml) was used to elute the pesticides from the column. Finally, the extracts were concentrated to an appropriate volume (2–10 ml) and analysed by GC–ECD.

2.4.3. Storage

The stability of the studied compounds once they were trapped in the adsorbent was evaluated using the following procedure. The pump, at a flow-rate of 1 l/min, was connected to one cap of the double neck round-bottom flask and in the other cap, a Q-Max twin port sampler with a Florisil column attached to each channel was fitted and adjusted to allow a flow-rate of 0.5 l/min through each channel. A pair of fortified columns were taken after 1 h sampling. One column was extracted and analysed in the same day and the other was stored at 4 °C in the

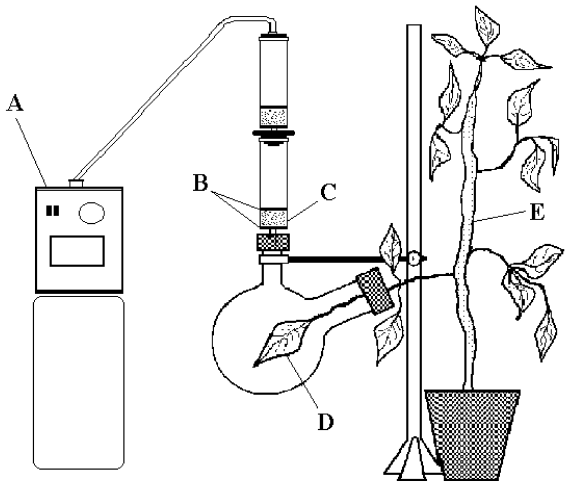

Fig. 2. Assembly used for the volatilisation study: (A) air sampling pump, (B) frits, (C) Florisil, (D) treated leaf and (E) tomato plant.

dark for 1 day and then analysed. Another set of columns were tested under the conditions described above, but the stored columns remained in the dark for 1 week at 4 °C.

2.4.4. Volatilisation studies

These methods of air and plant analysis were applied to study the volatilisation of endosulfan from tomato leaves. One leaf was introduced through an open cap with a perforated silicone septum in a double neck round-bottom flask, as shown in Fig. 2, and treated with the pesticide. Air was sampled during the first 24 h after treatment. A second Florisil column was connected in series to test breakthrough during sampling. Endosulfan levels in air and leaf samples were determined under the conditions described above.

3. Results and discussion

3.1. Recoveries

To evaluate recovery from the adsorbent, 2 g of Florisil placed in a polypropylene column with a frit at the base were fortified with 0.1, 0.5, or 1 μg of the pesticides studied, extracted with ethyl acetate and analysed by GC–ECD following the procedure described above. The results obtained are shown in Table 1. Recoveries of endosulfan-I, endosulfan-II, and endosulfan-sulfate were always higher than 85% with a relative standard deviation (RSD) lower than 11% for the range of concentrations studied.

To assess the retention efficiency of the columns used in these experiments, 2 g of Florisil were fortified with the same amounts of pesticides used above and a pump was attached at the end of the column delivering a flow-rate of 0.5 l/min. Two sampling periods were studied, 1 and 24 h. The Florisil columns were then extracted and pesticides were analysed by GC–ECD (Table 1). Retention efficiencies for the compounds studied after 1 h sampling ranged from 80 to 95% with RSDs between 3 and 9% and from 78 to 90% with RSDs between 3 and 11% after 24 h sampling. Initially, a hexane–ethyl acetate (80:20, v/v) mixture was employed for the extraction of pesticides from Florisil columns but retention efficiencies lower than 70% were obtained in some cases, mainly after 24 h of air sampling. Therefore, ethyl acetate was selected as extraction solvent and high recoveries were obtained in all cases.

Tomato leaves were fortified at 0.1, 1, and 3 μg pesticide/g of leaf; the recoveries obtained are shown in Table 2. Values were in the range 79–93% with RSDs between 5 and 9% for endosulfan-I, from

Table 1
Recoveries and retention efficiencies obtained from Florisil with ethyl acetate[a]

Pesticide amount (μg)	%Recovery (mean±RSD)			%Retention efficiencies (mean±RSD)[b]					
	Endosulfan-I	Endosulfan-II	Endosulfan-sulfate	1 h sampling			24 h sampling		
				Endosulfan-I	Endosulfan-II	Endosulfan-sulfate	Endosulfan-I	Endosulfan-II	Endosulfan-sulfate
0.1	100.8±3.9	101.9±6.0	95.2±6.8	94.7±7.7	90.7±8.1	89.9±8.9	83.7±6.6	81.3±4.9	82.9±4.8
0.5	90.7±8.4	89.9±8.7	85.1±10.2	86.3±3.6	86.4±3.8	86.2±3.9	78.4±8.0	80.7±2.8	81.0±3.4
1.0	89.1±6.7	89.8±2.9	88.5±3.3	80.2±3.5	88.7±2.8	88.1±2.9	85.5±7.5	89.4±10.7	87.6±10.7

[a] Results are the mean of four replicates±relative standard deviation.
[b] Results obtained after 1 or 24 h sampling with a pump at a flow-rate of 0.5 l/min.

Table 2
Pesticide recoveries obtained from tomato leaves

Concentration (μg/g)	%Recovery (mean±RSD)[a]		
	Endosulfan-I	Endosulfan-II	Endosulfan-sulfate
0.1	79.1±8.3	84.4±13.4	81.6±12.6
1.0	88.0±6.3	88.9±4.1	90.7±8.9
3.0	92.8±5.5	91.8±5.4	91.0±6.0

[a] Results are the mean of four replicates±relative standard deviation.

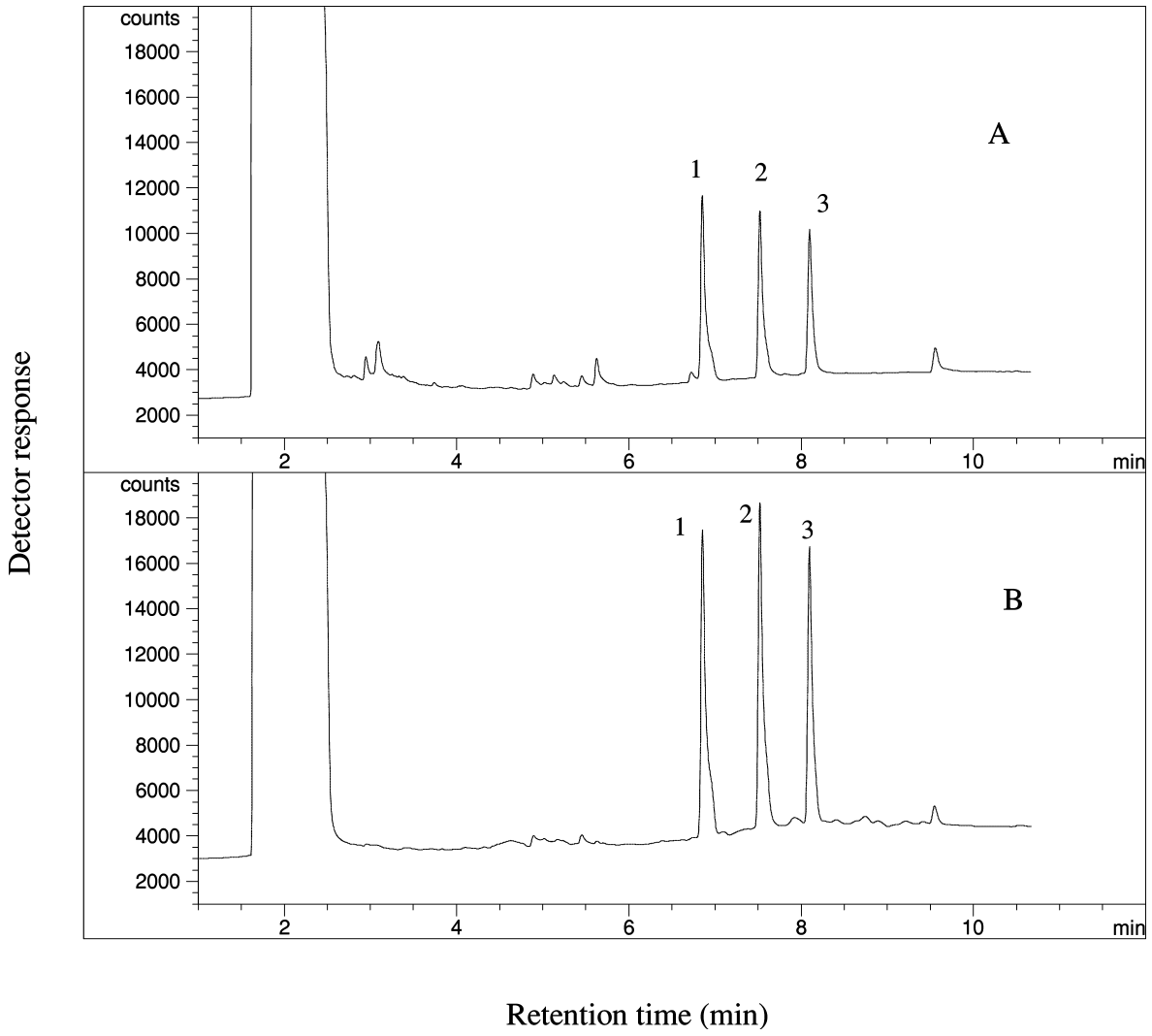

Fig. 3. Chromatogram of pesticides recovered from Florisil fortified at 0.1 μg after 24 h sampling (A) and of a plant sample fortified at 0.6 μg/g (B), where: (1) endosulfan-I, (2) endosulfan-II and (3) endosulfan-sulfate.

84 to 92% with RSDs between 4 and 13% for endosulfan-II, and from 82 to 91% with RSDs in the range 6–13% for endosulfan-sulfate. Representative chromatograms of pesticides recovered from air and plant samples are depicted in Fig. 3.

3.2. Detection limits and linearity

The detection limits (LODs) obtained in the GC–ECD analysis were 9 ng/ml for endosulfan-I, endosulfan-II, and endosulfan-sulfate considering a signal-to-noise ratio higher than 3. This LOD value represents 0.3 ng of each compound per litre of air for the lowest volume of air sampled, 30 l (1 h of sampling at a flow-rate of 0.5 l/min). When higher volumes of air are sampled, LODs can be improved, e.g. a LOD of 0.01 ng/l air can be obtained for 24 h sampling at a flow-rate of 0.5 l/min. Fig. 4 shows representative chromatograms at the limit of detection together with a standard solution of the compounds studied and a blank air sample.

With regard to plant analysis, the detection limits for the compounds studied were 0.02 μg pesticide/g of leaf for the GC–ECD analysis. Fig. 5 depicts chromatograms corresponding to an unfortified vegetal sample and to the limit of detection.

The detector response was linear over the range of concentrations assayed. The linearity of the method

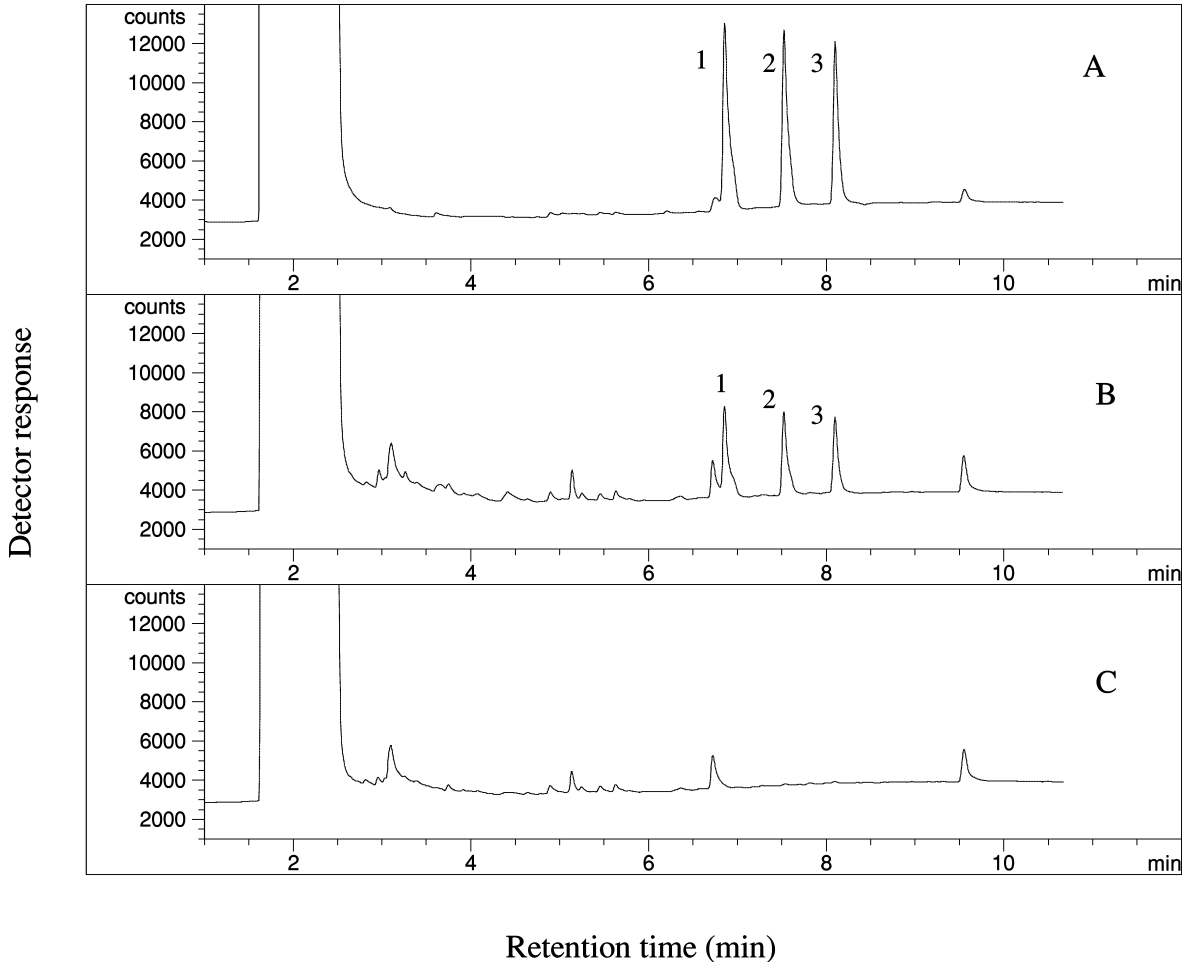

Fig. 4. Chromatogram of a mixture of the compounds studied at 0.01 μg/ml (A) of a sample containing 0.3 ng of each pesticide per litre of air sampled (B) and of a blank air sample (C), where: (1) endosulfan-I, (2) endosulfan-II and (3) endosulfan-sulfate.

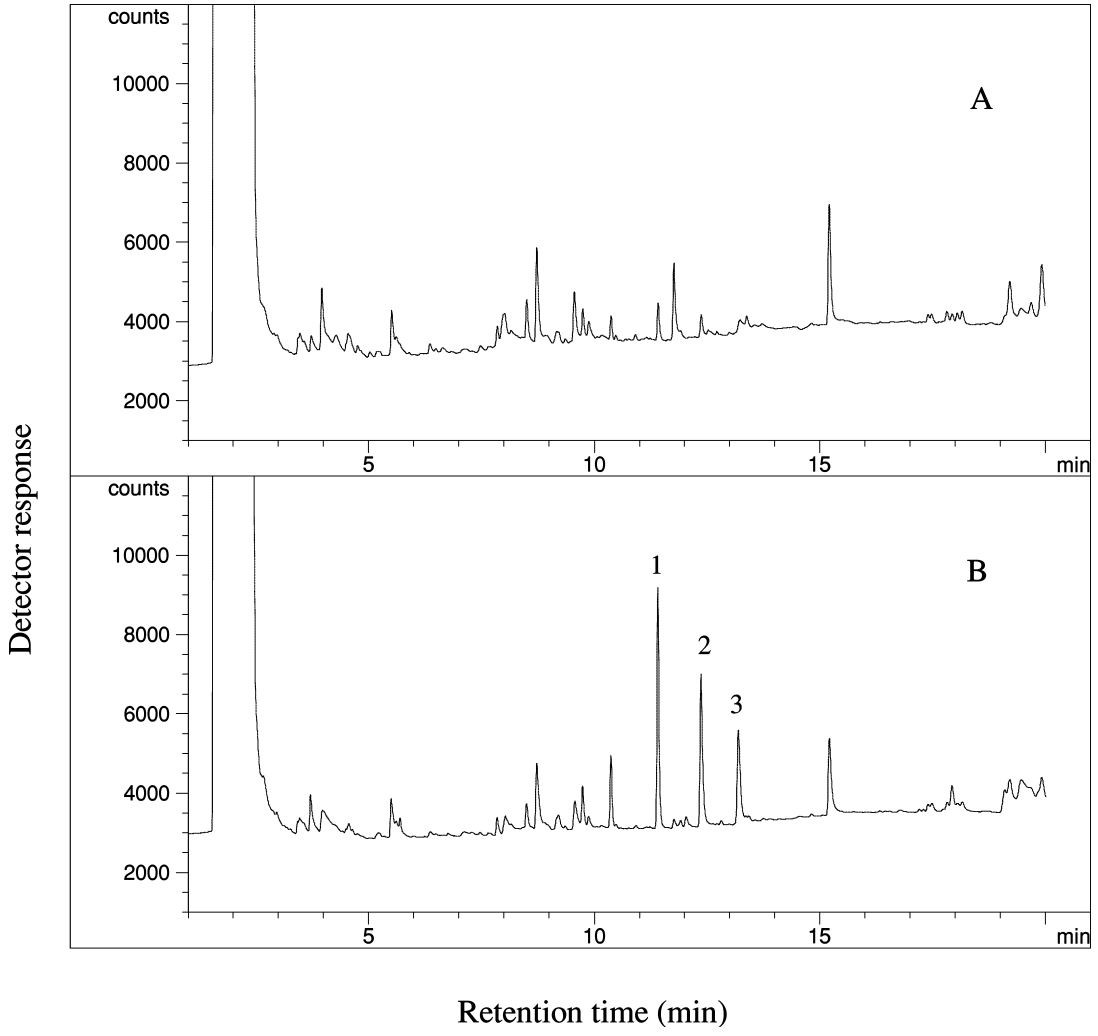

Fig. 5. Chromatogram of an unfortified vegetal sample (A) and of a fortified plant sample at 0.02 μg/g (B), where: (1) endosulfan-I, (2) endosulfan-II and (3) endosulfan-sulfate.

was tested by analysing solutions over the range 0.01–0.2 μg/ml. Correlation coefficients (linear regression analysis) for the pesticides varied from 0.992 to 0.997. Table 3 shows the calibration data and the detection limits obtained for the compounds studied.

3.3. GC–MS confirmation

Confirmation of the identity of the pesticides studied was performed by GC–MS. Pesticides were quantitated by selecting the base peak of their mass spectra, after the acquisition of the total ion chromatogram of the sample. The retention time, the main ions obtained for each pesticide and their relative abundances in GC–MS analysis are summarised in Table 4. These data are in agreement with those published by other authors [18–20]. The absence of coextracted interferences at the insecticide retention times was confirmed by analysing blank extracts. The insecticides studied can be confirmed at the detection limits indicated above (0.3 ng/l in air and 0.02 μg/g in plant samples) by using their respective main ions.

Table 3
Limits of detection (LOD) and linearity

Pesticides	t_R (min),	Calibration data		LOD[a]	
	GC–ECD	Equation	Correlation coefficient	Air (ng/l)[b]	Plant (μg/g)
Endosulfan-I	6.85	$y = 3.3 \cdot 10^6 x + 11655$	0.997	0.3	0.02
Endosulfan-II	7.52	$y = 2.6 \cdot 10^6 x + 12840$	0.995	0.3	0.02
Endosulfan-sulfate	8.09	$y = 1.7 \cdot 10^6 x + 10763$	0.992	0.3	0.02

[a] LOD for the analysis by GC–ECD.
[b] The LOD for air corresponds to the lowest volume of air sampled, 30 l.

Table 4
Main ions and their relative abundance in the mass spectra of pesticides

Compound number	Pesticide	t_R (min)	m/z (%)
1	Endosulfan-I	6.94	195 (100), 241 (70), 339 (40)
2	Endosulfan-II	7.56	195 (100), 241 (70), 339 (40)
3	Endosulfan-sulfate	7.99	272 (100), 229 (80), 387 (80)

3.4. Storage

The stability of pesticides sorbed on Florisil was determined. The adsorbent was fortified with 0.1 μg of each compound and air was pumped through the columns at a flow-rate of 0.5 l/min for 1 h. Half of the columns were extracted and analysed in the same day (day 0); the remainder of the columns were extracted and analysed after storage at 4 °C in the dark, for 1 day, in a capped plastic flask. The stability of the compounds studied after 1 week of storage was also tested. Good results were obtained after the storage of columns (Table 5). The recovery expressed as percentage of recovery at day 0 varied from 97 to 100% after 1 day of storage and from 89 to 95% after 1 week. These values are similar to the retention efficiencies obtained for the compounds studied with the proposed method. Therefore, samples can be stored up to 1 week at 4 °C before being analysed.

3.5. Volatilisation study

Tomato leaves were fortified with a commercial endosulfan formulation at 12 μg/leaf and pesticide volatilisation was studied under the conditions described above. Under those conditions no breakthrough was observed during sampling. A volatilisation rate near 1% of the initial amount of endosulfan per hour was obtained during the first 24 h at room temperature (22 °C). The mass balance of this assay showed that more than 80% of the initial endosulfan amount applied was recovered. The amount remaining in the leaf was around 70%. Our results indicate that endosulfan-I is the isomer contributing around 90% to the total volatilised endosulfan, which is in

Table 5
Stability of the selected pesticide at 4 °C, in the dark, after 1 day or 1 week of storage

Compound	% Recovery (mean±RSD)[a]			
	1 day of storage		1 week of storage	
	Analysed day 0	Analysed after 1day	Analysed day 0	Analysed after 1 week
Endosulfan-I	88.4±3.8	88.9±5.3	91.1±6.0	80.8±6.7
Endosulfan-II	88.2±2.2	85.8±4.5	87.3±4.8	79.6±6.6
Endosulfan-sulfate	87.0±3.9	84.6±4.3	85.9±4.7	81.3±6.9

[a] Results are the mean of four replicates±relative standard deviation.

agreement with previously published findings [17]. Therefore, the proportion of endosulfan-II remaining in the plant increases with time and this compound becomes the dominant isomer sometime after endosulfan treatment.

4. Conclusions

The results of this study point out that the proposed methods of analysis of air and leaves samples provide a rapid and suitable procedure for the determination of endosulfan-I, endosulfan-II, and endosulfan-sulfate in these matrices at low levels. The methods are simple, requiring a low volume of organic solvents and can be employed in the determination of the volatilisation rates of the compounds studied under laboratory conditions. The pesticide stability obtained in our study allows the samples to be stored at 4 °C for 1 week prior to analysis.

References

[1] R. Grover, Weed Sci. 23 (1975) 529.
[2] J.W. Russell, Environ. Sci. Technol. 9 (1975) 1175.
[3] T.C. Thomas, J.W. Jackson, Y.A. Nishioka, Am. Ind. Hyg. Assoc. 41 (1980) 599.
[4] M.T. Tabernero, J. Álvarez-Benedí, J. Atienza, A. Herguedas, Pest. Manag. Sci. 56 (2000) 175.
[5] F.J.E. González, M.L.C. Cano, J.L.M. Vidal, M.M. Galera, J. AOAC Int. 80 (1997) 1091.
[6] M. Clément, S. Arzel, B. Le Bot, R. Seux, M. Millet, Chemosphere 40 (2000) 49.
[7] A. Gudéhn, B. Kolmodin-Hedman, J. Chromatogr. A 387 (1987) 420.
[8] K. Kawata, H. Mukai, A. Yasuhara, J. Chromatogr. A 710 (1995) 243.
[9] K. Swami, A. Marjit, S. Narang, R.S. Narang, J. AOAC Int. 80 (1997) 74.
[10] L. Martínez, C. Sánchez-Brunete, J.L. Tadeo, Quím. Anal. 13 (1994) 206.
[11] J.L.M. Vidal, F.J.E. González, C.R. Glass, M.M. Galera, M.L.C. Cano, J. Chromatogr. A 765 (1997) 99.
[12] G.F. Antonious, M.E. Byers, J.C. Snyder, Pestic. Sci. 54 (1998) 61.
[13] J.E. Beard, G.W. Ware, J. Agric. Food Chem. 17 (1969) 216.
[14] I. Mukherjee, M. Gopal, Pestic. Sci. 40 (1994) 103.
[15] M. Gopal, I. Mukherjee, Pestic. Sci. 37 (1993) 67.
[16] A. Aguilera-del Real, A. Valverde-García, A.R. Fernandez-Alba, F. Camacho-Ferre, Pestic. Sci. 51 (1997) 194.
[17] N.C. Singh, T.P. Dasgupta, E.V. Roberts, A. Mansingh, J. Agric. Food Chem. 39 (1991) 575.
[18] W. Liao, T. Joe, W.G. Cusick, J. Assoc. Off. Anal. Chem. 74 (1991) 554.
[19] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821.
[20] A. Gelsomio, B. Petrovicová, S. Tiburtini, E. Magnani, M. Felice, J. Chromatogr. A 782 (1997) 105.