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Gas chromatographic determination of organochlorine and pyrethroid pesticides of horticultural concern

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Abstract

An optimized gas chromatographic method is described for the determination of thirteen organochlorine and pyrethroid pesticides currently applied to vegetable and fruit crops. The selected pesticides are extracted with ethyl acetate–sodium sulphate and an aliquot is evaporated to dryness and reconstituted in 10 ml of light petroleum. Sample clean-up is accomplished by aspirating 2 ml of the light petroleum extract through a silica gel solid-phase disposable cartridge. Following aspiration, the sample is eluted with 2 ml of diethyl ether–light petroleum (50:50) and is ready for GC with electron-capture detection. The method provides an excellent clean-up for all matrices studied. The recoveries varied from 73 to 106%, except for captan (51%), with relative standard deviations from 3.5 to 20.4% in all instances. Detection limits of less than 0.01 mg/kg were obtained. The sample throughput is ca. 51 per 24 h. These results were confirmed by GC–MS. Results are also presented for extracts of four different food product types fortified with the target pesticides. Data for real residues of these pesticides found in vegetables during 1 year routinely applying the multi-residue method are also presented.

1. Introduction

Monitoring of pesticide residues in agricultural products has become a priority field in pesticide research and analysis. The main objectives of such residue monitoring are to enforce tolerance levels in food, to acquire incidence/level data on designated commodity pesticides and to carry out total diet studies. This concern is reflected in the publication by the EEC jointly with the government of each country of the maximum recommended limits (MRLs) for pesticide residues in a variety of agricultural foods [1]. Hence the development of improved multi-residue methods (MRMs) to cover the agricultural chemicals of current interest in which the repetition of time-consuming operations is minimized is of great interest in monitoring food supplies.

Several multi-residue procedures have been proposed in recent years for the determination of organochlorine compounds (OCs) and organophosphorus compounds (OPs) in crops by using

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GC to separate the individual residues followed by detection with selective and sensitive methods such as electron-capture detection (ECD), nitrogen–phosphorus detection and flame photometric detection [2–8]. Among these, few are adequate for screening both classes of pesticides (OPs and OCs) in horticultural samples with a single sample preparation [6,7].

Chlorinated hydrocarbon pesticides are of special concern because of their persistence in the environment and in animal tissues [9] and their significance to human health [10], and so are usually an important objective in food analysis. Acetonitrile extraction (Mills method) [11] is usually preferred in the determination of these compounds in horticultural samples when no clean-up methods are applied as vegetable extracts in acetonitrile are cleaner than those obtained with other solvents of similar polarity. Owing to its high separation power and sensitivity, GC–ECD is the favoured technique for the determination of OC pesticides [12]. However, carefully control of degradation due to thermal or column reaction processes is necessary with this technique, especially with phthalimide fungicides such as captan and folpet, which are notoriously prone to adsorption and degradation processes [13,14].

Nowadays, the use of ethyl acetate for multi-residue solvent extraction is replacing with good results other MRM procedures [15–17] owing to its simplicity, speed and the solvent, resulting in cheaper analyses. Thus, the National Food Administration of Sweden adopted in 1989 an ethyl acetate multi-residue method as a single sample preparation for OCs and OPs [18]. Unfortunately, this extraction also removes an abundance of co-extractives in most agricultural commodities. In such a situation an efficient clean-up is required before determination, especially in complex matrices such as pepper. Solid-phase extraction (SPE) provides a useful alternative [19–23] to the traditional liquid–liquid extraction-based methods [24] or gel permeation chromatographic (GPC) clean-up methods [7,25].

In a previous paper [26], we described an ethyl acetate method (MRM) for the separation of a selected group of OP pesticides, but its direct application to OC determination suffered from serious interference problems, as mentioned above.

In this study, this rapid and accurate multi-residue protocol was developed by incorporating a clean-up step for the determination of thirteen organochlorine and pyrethroid pesticides (for structures see Fig. 1) currently applied to horticultural crops grown in greenhouses in south-eastern Spain. The work described proceeded in

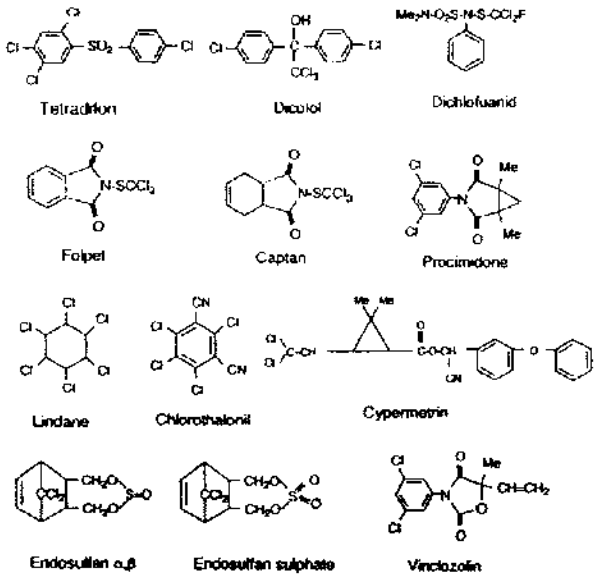


Fig. 1. Structures of the compounds studied.

Table 1
Maximum residue limits (MRLs) in mg/kg established in two European countries and USA and EEC regulations

No.	Compound	Sweden	Spain	USA	EEC
1	Lindane	1.0	1.0	1.0	1.0
2	Chlorothalonil	1.0	0.01	–	–
3	Vinclozolin	2.0	2.0		
4	Dichlofuanid	5.0	5.0		5.0
5	Captan[a]	0.1	0.1	25.0	0.1
6	Folpet[a]	0.1	0.1		
7	Procimidone	0.1	2.0		–
8–10	Endosulfan[b]	0.5	1.0	2.0	1.0
11	Dicofol	3.0	0.5		0.5
12	Tetradifon	2.0	1.0	1.0 –	
13	Cypermetrin	2.0	1.0		

[a] As sum of captan and folpet.
[b] As sum of endosulfan I, II and III.

the following stages: (a) optimization of the GC conditions taking into account the factors mentioned below which gave the best chromatographic resolution and the shortest time of analysis; (b) confirmation by GC–MS; (c) clean-up study on silica gel cartridges; (e) determining the linearity, reproducibility and recoveries of the method; (g) evaluating the performance of the method on different samples fortified with selected target compounds; and (f) application of the proposed method to crops possibly containing residues of OCs and pyrethroid pesticides in routine practice for nearly 1 year. A list of the compounds included in the present study together with maximum limits (MRLs) for peppers established according to different regulations are shown in Table 1.

2. Experimental

2.1. Chemicals

Pesticide-grade ethyl acetate, light petroleum and anhydrous sodium sulfate (12–60 mesh) were obtained from Merck (Darmstadt, Germany). Solid-phase extraction disposable columns (6 ml) containing 500 mg of silica gel were obtained from Varian (Harbor City, CA, USA). The pesticide standards (Pestanal quality) listed in Table 1 were obtained from Riedel-de Haën (Seelze, Germany). Stock standard solutions were prepared by dissolving 10.0–15.0 mg of each purity-certified pesticide in 100 ml of light petroleum to give 100.0–150.0 mg/l stock standard solutions. A working standard solution was prepared by transferring 1 ml of each stock standard solution into a 100-ml volumetric flask and diluting to volume with light petroleum–diethyl ether (1:1), giving a 1.0–1.5 mg/l working standard solution.

2.2. Chromatographic analysis

GC–ECD

A Perkin-Elmer (Beaconsfield, UK) model 8600 gas chromatograph equipped with a ^{63}Ni electron-capture detector was used for GC analysis. An HP1 fused-silica capillary column (30 m × 0.53 mm I.D.) coated with methylsilicone (2.65 μm) (Hewlett-Packard, Palo Alto, CA, USA) was used. Helium was the carrier gas at a flow-rate of 8 ml/min. The temperatures of the injector and detector were maintained at 240 and 300°C, respectively. The conditions used for gas chromatography were optimized. The injection volume was 1 μl.

GC–MS

A Hewlett-Packard Model 5995 system with a Model 59970 data system was used for GC–MS in the electron impact (EI) mode. The same

fused-silica column as described above was used. The sample was introduced directly into the ion source. The carrier gas was helium. The other chromatographic conditions were identical with those described for GC–ECD. EI mass spectra were obtained at 70 eV.

2.3. GC optimization

The optimization of the temperature programming cycle was carried out by a computer-assisted method [27] taking into account the following parameters.

Response function

The selection of a chromatographic response function (*CRF*) based on the criteria given by Schoenmakers [28] is defined as

$$CRF = n + \sum R_{i,j} + 1/2(T_T - T_L) \quad (1)$$

where T_L is the retention time of the last peak and T_T the target retention time for the last peak; the term $T_T - T_L$ in the function is only included if T_L exceeds T_T; n is the number of peaks detected; $R_{i,j}$ is the resolution between adjacent peaks i and j ($R_{i,j}$ is limited to a maximum value of 1.5 to avoid $\Sigma R_{i,j}$ being determined largely by the largest values of $R_{i,j}$).

Only the four least well resolved pairs of peaks were considered in the present calculations of n and $R_{i,j}$. The target retention time was fixed at $y_1 = 25$ min as the maximum acceptable retention time of the last peak. Hence a maximum value of $CRF = 14$ can be expected.

Selection variables

The next step is to define the variable space or search region. Relying on literature data [29] and previous experience, we selected the following temperature programming cycle:

A final temperature of 260°C was chosen to avoid column bleeding. An initial temperature of 150°C (1-min hold) and a rate 30°C/min were chosen for the initial conditions, ramp 1 and time 1. Thus a three-variable space (temperature 2, time hold 2 and ramp 2) with ranges 180–230°C, 0–6 min and 5–20°C/min, respectively, was used in the optimization procedure.

Experimental design

A very general second-order linear model with interaction Eq. 2 was fitted to the experimental data:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 \quad (2)$$

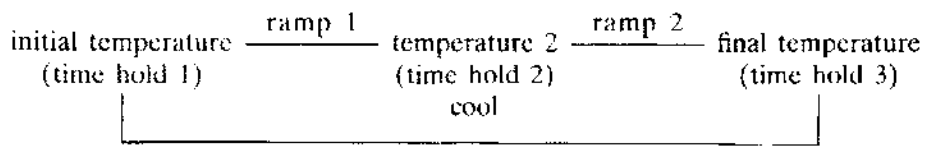
where X are the independent variables (X_1 = temperature 2, X_2 = time hold 2 and X_3 = ramp 3), y is the dependent variable (measured response *CRF*, from the experimental runs) and b_0–b_9 are the coefficients to be evaluated.

The experimental data were obtained by carrying out, in randomized order, twenty experimental runs according to the rotate central composite design proposed by Box and Hunter [30]. Each response value was calculated from two replicate injections for each set of parameters. The XYZ computer program used [27] computes the coefficients of the selected model.

The optimum conditions selected were an increase from 150°C (1-min hold) at 30°C/min to 215°C (6-min hold) and then at 15°C/min to 260°C.

2.4. SPE clean-up

Solid-phase disposable extraction cartridges with silica as packing material were used. The breakthrough volumes were established in the range 2.5–3.5 ml for the pesticides studied in



light petroleum. Therefore, 2-ml volume of light petroleum extract can be safely used for vegetable sample extracts. The elution behaviour of the OCs and pyrethroid compounds on the silica solid-phase cartridges was studied by application of 2 ml of a pepper extract containing a standard solution of pesticides to the silica minicolumn and subsequent elution with 2-ml volumes of elution solvent. Light petroleum with diethyl ether as modifier was used in the desorption process in order to avoid dilution and to limit the final volume to 2 ml. If pure light petroleum ether was used an elution volume of at least 5 ml was necessary to recover all thirteen compounds. Fractions of 2 ml of diethyl ether in light petroleum in different ratios were collected separately in each clean-up experiment and analysed off-line by GC.

2.5. Sample preparation

Different fruit and vegetable samples were collected at greenhouses in the vicinity of Almería, Spain (where all of the OCs compounds mentioned in Table 1 are currently used) and were extracted in our laboratory according to the following procedure.

Weigh 50 g of chopped sample into a high-speed blender and add 40 g of anhydrous sodium sulfate. Mix thoroughly, add 100 ml of ethyl acetate and blend the mixture for 5 min. Filter the supernatant liquid with suction through a filter-paper and a layer of 20 g of anhydrous sodium sulfate. Rinse the filter with 50 ml of ethyl acetate and evaporate the combined extracts on a vacuum rotary evaporator using a 40–60°C water-bath. Dissolve the residue in 10 ml of light petroleum and pass through to a silica gel (500 mg) disposable SPE cartridge previously conditionated with 5 ml of light petroleum and 2 ml of the vegetable extract in light petroleum. Elute the SPE minicolumn with of 2 ml of diethyl ether–light petroleum (50:50) at a flow-rate of 1–2 ml/min and adjust the volume to 2 ml with a diethyl ether–light petroleum (50:50). Inject 1 μl of this eluate into the GC–ECD system.

2.6. Recovery studies

Fresh green pepper samples, which had not been treated with the pesticides studied, were fortified with 0.20–0.30 mg/kg of each pesticide as follows. A 10-ml volume of the working standard solution described above was added to 50 g of chopped sample in a high-speed blender jar. After evaporation of the light petroleum with an air stream, the sample was homogenized for 2 min. After 1 h, the sample was again homogenized for 1 min and immediately analysed by application of the previously described method. The recovery assays were replicated ten times. For three other food products types the recovery studies were carried out identically but in duplicate.

3. Results and discussion

3.1. GC optimization

After the twenty experiments mentioned above, the values of the different coefficients were as follows: $b_0 = 11.56$, $b_1 = -0.46$, $b_2 = 0.79$, $b_3 = -0.49$, $b_4 = -0.24$, $b_5 = -0.23$, $b_6 = 0.24$, $b_7 = 0.27$, $b_8 = 0.31$ and $b_9 = 0.22$. These parameters were calculated by a least-squares method and all the coefficients were representative ($t_{Student} \geq 1.8$). An ANOVA test was applied to validate the model. The resulting F values calculated from the experimental data were higher than the F values tabulated for three and six degrees of freedom at a level of significance $\alpha = 0.05$. A correlation coefficient $R^2 = 0.91$ between the theoretical and experimental models shows that a high degree of explanation of the variability of the experimental data is achieved by the model. To obtain a graphical representation of the response surface of the variables to be optimized it was necessary to fix one variable and to represent the response of the other two. Fig. 2 shows the representation of the isoresponse as a function of the temperature 2 and ramp 2 at a constant time hold (time hold 2) of 6 min.

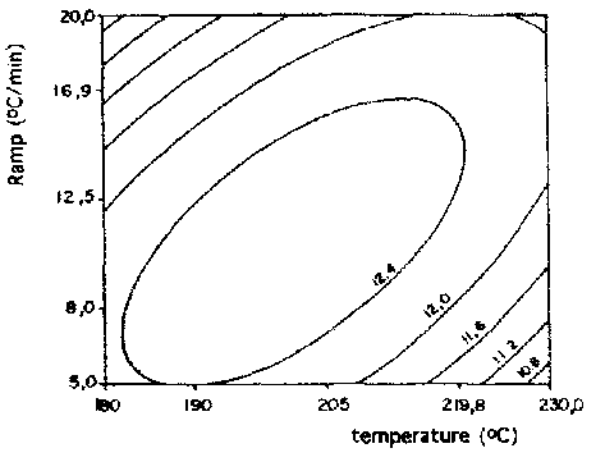


Fig. 2. Isoresponse ($CRF = 10.8$, 11.2, 11.6, 12.0, 12.4) contour plot obtained from Eq. 2 at a constant time hold (time hold 2) of 6 mins. See text for more details.

The optimum conditions selected were an increase from 150°C (1-min hold) at 30°C/min to 215°C (6-min hold) and then at 15°C min to 260°C.

3.2. GC analysis

The optimum temperature programme resulted in the retention behaviour shown in Table 2. An example of a gas chromatogram of the mixture of OCs and pyrethroid compounds is shown in Fig. 3. Two problems were noticed: (i) a minor degradation of captan and folpet confirmed by GC–MS (see below); these degradation products of captan and folpet appeared at 7.7 min, which is a common problem in the GC determination of these phthalimide pesticides, which are prone to adsorption and degradation in the column [13,14]; (ii) the overlapping of the four N° 13) cypermetrin isomers (*cis*-A, *trans*-C, *cis* B and *trans* D) (peaks 13) typical in the determination of this pyrethroid [31,32]. Therefore, the determination of this pesticide was carried out as the sum of the four peaks. The total run time is 23 min plus an extra 5 min for equilibration at the initial temperature conditions. Hence the analysis of 51 samples within 24 h is feasible.

The linear dynamic range of the detector response was checked for all the target compounds in the working standard solution and appeared to be from 0.05 to 5 ng, except for captan, folpet and procymidone (0.2–5 ng), absolute injected amount and the correlation coefficients were higher than 0.999 in all instances except for captan (0.991) and folpet

Table 2
Retention times (t_R) and relative standard deviations (R.S.D.s) of retention times and average recoveries and R.S.D.s for test compounds in green pepper samples using GC–ECD.

No.	Compound	t_R(min)	R.S.D. (%)	Average recovery (%)	R.S.D. (%)
1	Lindane (γ-HCH)	6.351	0.57	97	4.9
2	Chlorothalonil	6.823	0.62	95	9.5
3	Vinclozolin	7.996	0.61	100	4.4
4	Dichlofuanid	9.242	0.62	105	3.5
5	Captan	11.233	0.49	51	20.4
6	Folpet	11.564	0.41	106	16.0
7	Procimidone	11.841	0.39	100	12.5
8	α-Endosulfan	12.748	0.31	92	5.2
9	β-Endosulfan	13.845	0.23	89	6.3
10	Endosulfan sulphate	14.695	0.25	79	10.6
11	Dicofol	10.015	0.61	73	15.8
12	Tetradifon	16.823	0.31	105	14.5
13	Cypermetrin[a]	22.099	0.33	98	16.2

Fortification level 0.20–0.30 mg/kg ($n = 10$). Chromatographic conditions and sample preparation are described in text.
[a] Only the first peak is considered.

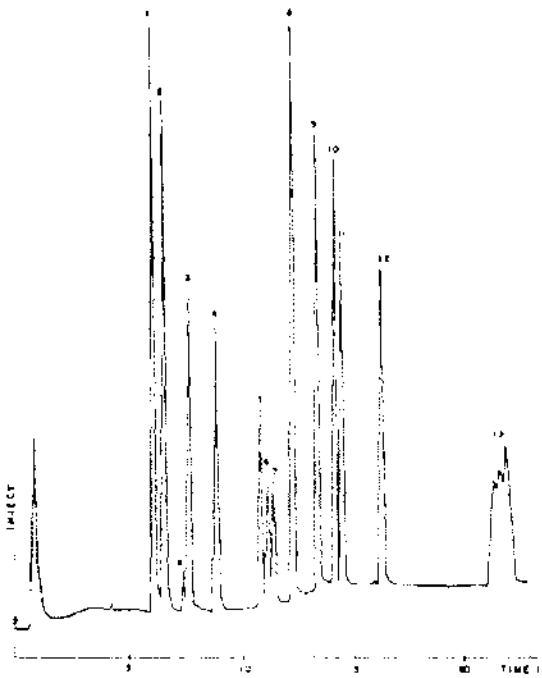


Fig. 3. GC–ECD of the pesticide mixture containing 1.0–1.5 mg/l (injection volume 1 μl). Numbers above the peaks correspond to compounds numbers in Table 1.

(0.989), probably owing to the decomposition processes mentioned above. This is also the practical working range. These results were calculated using peak-height measurements, except for cypermetrin (peak-area measurements). Detection limits based on a signal-to-noise ratio of 3 are on average 10 pg, except for captan, folpet and procymidone (40 pg), absolute injected amount, equivalent to 0.002 and 0.008 mg/kg, respectively.

3.3. GC–MS confirmation

Solutions containing all the target compounds were analysed by GC–MS in the EI mode with a scan range from m/z 30 to 600 under full-scan conditions. The main fragments obtained and their relative abundances are shown in Table 3. These data are in good agreement with the different diagnostic ions reported for these organochlorine and pyrethroid pesticides [20–26].

In order to detect the possible decomposition processes than can occur in the GC analysis, a solution containing 50 mg/l of captan and folpet was analysed separately under identical conditions but varying the injector temperature (150, 200 and 250°C) with intense cleanness of the injector liner. In all instances temperature-dependent decomposition products for captan and folpet were detected at 7.7 min (Fig. 4). Based on the sensitivity requirements for captan and folpet, the injector temperature selected was 240°C.

The EI mass spectra of captan and folpet degradation products are shown in Fig. 5 as unknowns 1 and 2. The mass spectrum of the unknown 1 contained fragments ions at m/z 79

Table 3
Main ions and relative abundances in EI mass spectra of the organophosphorus pesticides studied

No.	Compound	M_r	Main ions [m/z (relative abundance, %)]
1	Lindane	288	77 (25), 111 (66), 147 (29), 183 (100), 219 (66), 254 (19)
2	Chlorothalonil	264	98 (19), 109 (30), 124 (24), 133 (22), 133 (18), 266 (100)
3	Vinclozolin	285	97 (31), 124 (95), 178 (86), 198 (80), 212 (100), 285 (61)
4	Dichlofluanid	332	77 (18), 92 (20), 123 (100), 167 (32), 224 (26)
5	Captan	299	79 (100), 117 (12), 149 (28), 264 (8)
6	Folpet	295	76 (68), 104 (100), 130 (80), 260 (94), 295 (20)
7	Procimidone	283	67 (53), 96 (100), 186 (8), 255 (9), 283 (19)
8	α-Endosulfan	404	109 (69), 121 (68), 160 (79), 195 (99), 237 (100), 265 (52), 339 (21)
9	β-Endosulfan	404	109 (32), 120 (50), 160 (91), 195 (100), 237 (96), 267 (47), 339 (30)
10	Endosulfan sulphate	420	109 (18), 121 (26), 170 (34), 237 (58), 272 (100), 387 (24)
11	Diocfol	368	75 (41), 111 (42), 139 (100), 250 (16)
12	Tetradifon	354	75 (78), 111 (100), 159 (89), 229 (43), 356 (32)
13	Cypermetrin	415	77 (31), 91 (39), 127 (32), 163 (100), 181 (89), 209 (29), 415 (3)

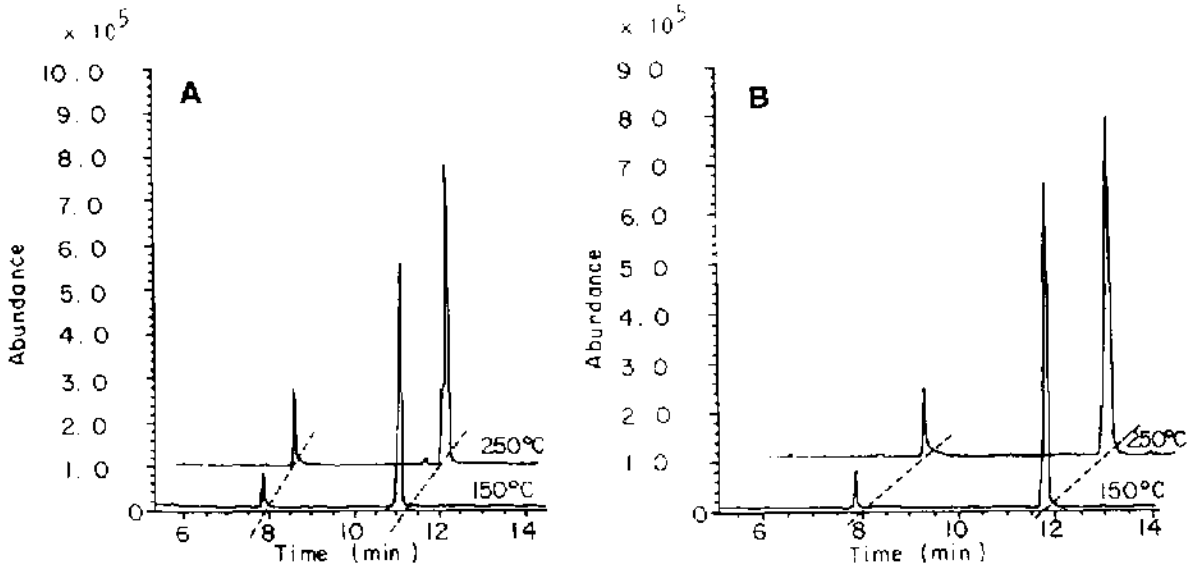


Fig. 4. GC–MS of (A) captan and (B) folpet (50 mg/l) at different injector temperatures: 150°C; 250°C.

(100%), *m/z* 123 (9%) and *m/z* 151 (50%), and it can be assigned to the degradation of captan in tetrahydrophthalimide according to the tentative fragmentation pattern indicated in Fig. 5. The mass spectrum of unknown 2 contained fragments ions at *m/z* 76 (90%), *m/z* 104 (75%) and *m/z* 147 (100%) and it can be assigned to the degradation of folpet in phthalimide according to the tentative fragmentation pattern indicated in Fig. 5. Hence we can expect a peak at 7.7 min from co-eluting degradation products of captan and folpet under these chromatographic conditions.

3.4. SPE clean-up

Fractions of 2 ml of diethyl ether in light petroleum in different ratios were collected separately in each clean-up experiment and analysed off-line by GC. The elution pattern at different diethyl ether percentages (10, 20 30 and 50%) is presented in Fig. 6.

It is apparent that all the target compounds except captan and folpet elute in the first 2 ml. If complete elution of captan and folpet in the two first 2 ml is required, the volume fraction of diethyl ether in the eluent has to be increased to 70%. Increasing the volume fraction of the diethyl ether, however, also increases the concentration of interfering compounds in the eluate, thus making the clean-up less effective. The reproducibility of the elution pattern was optimum at a flow-rate of 1–2 ml/min.

It can be seen that the silica column clean-up (Fig. 7) removes the interferents efficiently from the matrix.

3.5. Recoveries and repeatability

The retention times and recoveries of the different OCs and pyrethroid compounds were tested by fortifying ten fresh pepper samples, using the procedure described above (Table 2). The repeatability of retention time was satisfactory in all instances. The recoveries of the different OCs and pyrethroids appeared to be in the range 73–106%, except for captan (51%), with a R.S.D.s less than 17% in all the instances except for captan (20.4%), which is acceptable.

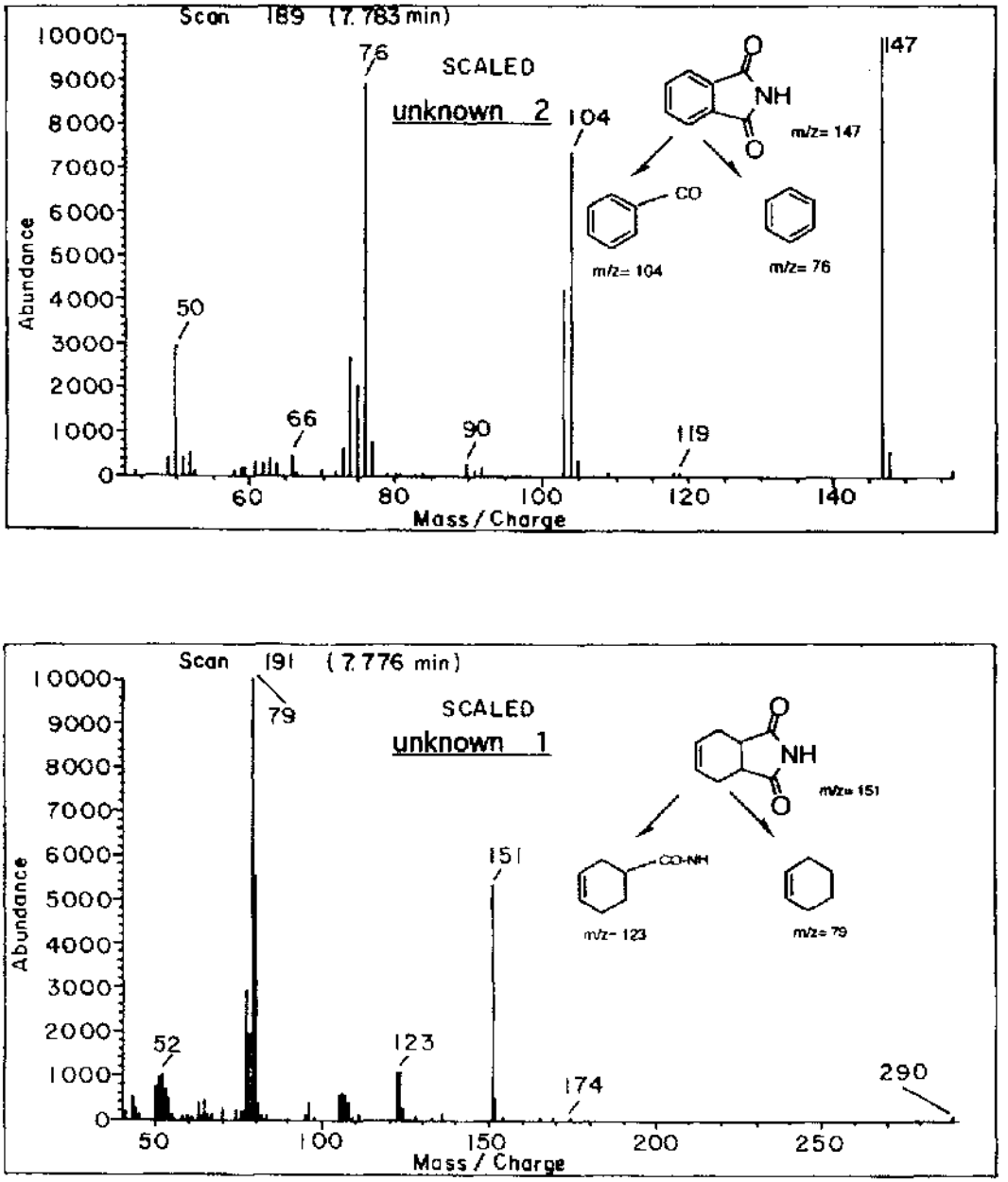


Fig. 5. Mass spectra of captan (unknown 1) and folpet (unknown 2) degradation products.

3.6. *Method performance with cucumber, beans and melons*

The proposed screening method was assessed for the analysis of cucumber, beans and melon, collected at a greenhouse in Almería (Spain), in order to observe the effect of the matrix on the recoveries, separation and interfering peaks. The homogenized samples were fortified with the thirteen target compounds in the range 0.20–

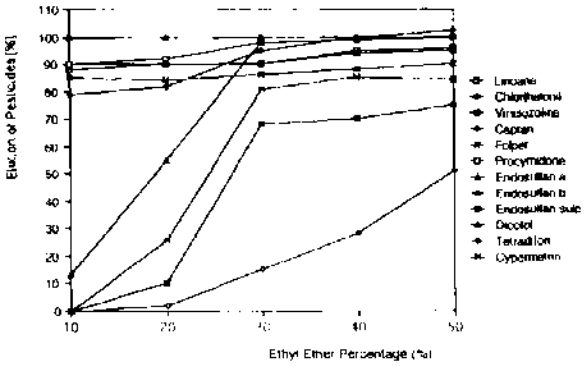


Fig. 6. Elution pattern of target compounds from silica cartridges at different diethyl ether percentages.

0.30 mg/kg. The analyses were carried out in duplicate (in this test, pepper samples were also included). All compounds were identified correctly and the average recoveries were dependent, as expected, on the matrix, but nevertheless were acceptable (Fig. 8). These values are in the range 87–114% in all instances except for captan, endosulfan sulphate and dicofol (44–80%).

3.7. Routine crop analysis

In our laboratory, the proposed GC method for the determination of residues of OCs and pyrethroid pesticides in fruits and vegetables has now been in use interruptedly for 3 years. During this period, the method has evolved from the AOIAC-recommended method to the ultimate SPE clean-up–ethyl acetate method. In this latter form, the method has demonstrated, during more than 1 year of routine application, very high efficiency, sensitivity, selectivity and precision. In Table 4, the residue data for a 1-year period (January 1993–January 1994) are summarized. Positive residues could be detected in nearly 40% of all samples analysed. Endosulfan occurred most frequently and procimidone, vic-

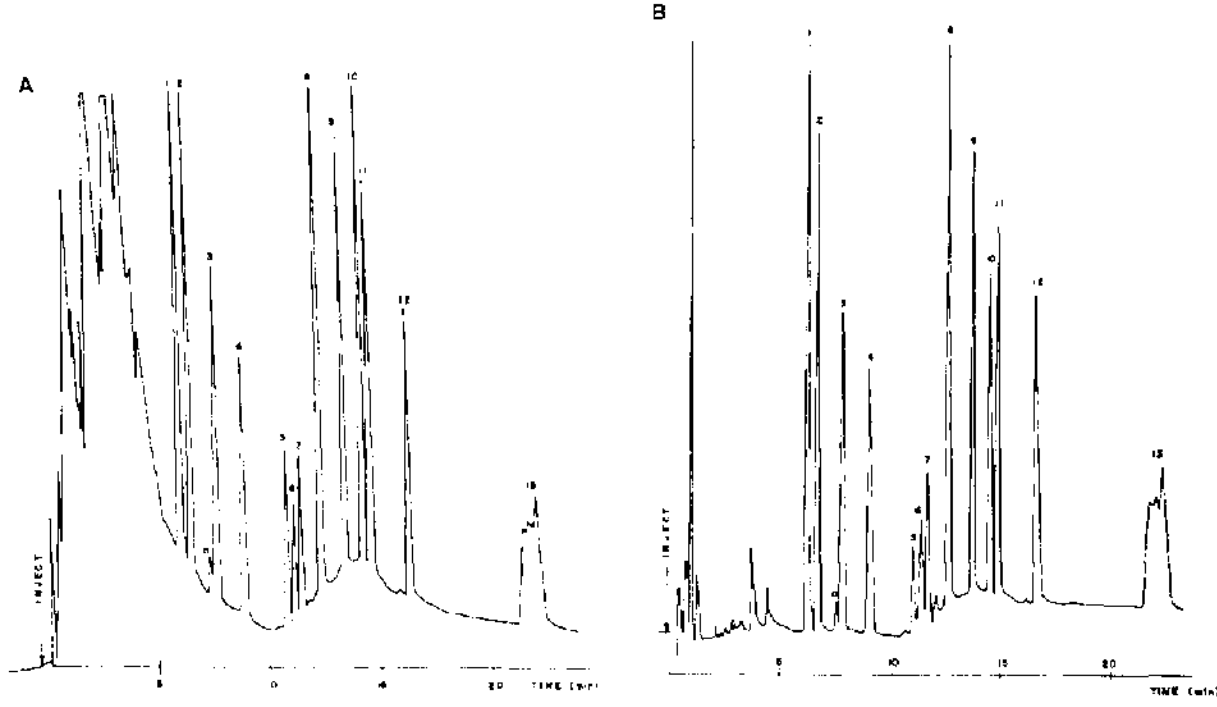


Fig. 7. GC–ECD of fortified green peppers (0.2–0.30 mg/kg) obtained by operating (A) without clean-up and (B) with clean-up using silica cartridges.

Table 4
Residues of organochlorine and pyrethoid pesticides found in vegetables in 1993–94

Compound	Commodity[a]										No. of positive residues
	Peppers		Cucumbers		Tomatoes		Egg-Plants		Beans		
	A	B	A	B	A	B	A	B	A	B	
Lindane	5										5
Chlorothalonil	2	2	1	1							3
Vinclozolin	16										16
Dichlofuanid	2		1								3
Captan											
Folpet											
Procimidone	32	1	9		7		2		2		52
Endosulfan	193	10	27		3				4		227
Dicofol											
Tetradifon											
Cypermetrin	1				2	1					3
Total analysed	546		141		41		26		20		309

[a] (A) Positive residues; (B) exceeding of residue tolerances (Swedish maximum residue limits).

lozolin, lindane, cypermetrin and chlortalonil were detected occasionally. The Swedish maximum residue limits (MRLs) for these compounds were exceeded ca. 2% of the number of all samples analysed or 5% of the number of all samples that were found to contain residues of OCs and pyrethroids.

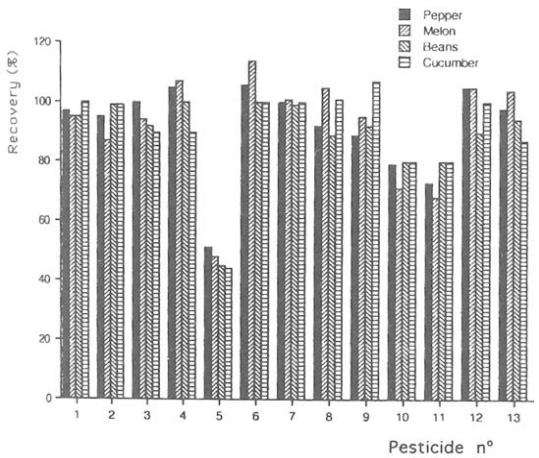


Fig. 8. Average recoveries from duplicate determinations of the target compounds as a function of the matrix. Fortification level, 0.2–0.3 mg/kg.

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