Journal of Chromatography A, 655 (1993) 293–300
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 110

Gas chromatographic analysis of organophosphorus pesticides of horticultural concern☆

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(First received December 30th, 1992; revised manuscript received March 17th, 1993)

ABSTRACT

An optimized GC method for the determination of 25 organophosphorus pesticides (OPs) currently applied to horticultural crops was developed. Four additional compounds were evaluated for their suitability as internal standards. The linearity, reproducibility and recoveries of the method are discussed. The reliability of the method for routine analysis of fruit and vegetable samples is demonstrated. Registration of the analytical results for a control sample in quality control charts demonstrated the performance of this method. The limits of detection are in the range 5–20 μg/kg. The sample throughput is *ca.* 68 per 24 h. Results are also presented for extracts of four real samples fortified with the target OPs. The method was evaluated and the results were confirmed by GC–MS.

INTRODUCTION

During the last decade the number of pesticides, including isomers and breakdown products, applied to fruits and vegetables and for which analytical methods are available has increased to about 180 [1]. Many official and commercial laboratories regularly examine these food products to determine compliance with maximum residue limits (MRLs) [2]. Such analyses also yield information on the effect of pesticide degradation processes on the horticultural crop and on the average amount of pesticide ingested by the consumer.

The importance of revising and improving multi-residue analytical methods periodically is readily apparent. The demand for efficient monitoring programmes requires that a set of multi-residue methods and measurement regimes be designed by taking into account the need for greater efficiency and lower costs and the requirements of consumer safety. Design considerations must also include recent technological advances such as macro open-tubular columns and new stationary phases [3–5].

Gas chromatography (GC) with phosphorus flame photometric detection (FPD-P) has frequently been the instrumental technique of choice for the analysis of volatile organophosphorus pesticides for reasons of selectivity, sensitivity and reproducibility. However, optimum GC temperatures and confirmation methods are necessary [6,7]. Interferences in analytical determinations may occur when FPD-P is used in the phosphorus mode and sulphur is present [8], when some thermally labile OPs, such as tri-

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☆ Presented at the *21st Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Granada, October 21–23, 1992.*

0021-9673/93/$06.00

chlorfon [9–11], are present or when bleeding of the liquid phase occurs [12]. On the other hand, experience gained during interlaboratory evaluations [13–15] of methodologies for this class of compounds indicates the need for analysts to be familiar with the OPs currently used on specific crops in each region.

In this study, a rapid, sensitive and accurate chromatographic procedure for the determination of 25 OPs currently applied to horticultural crops grown in greenhouses in southeastern Spain was developed. The work described proceeded in 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 for analysis; (b) confirmation by GC–MS; (c) study and selection of internal standards; (d) study of the behaviour of working and internal standards; (e) determination of the linearity, reproducibility and recoveries of the method; and (f) evaluation of the performance of the method on control samples and on different real samples fortified with selected target compounds. A list of the compounds included in this study together with total turnover values from three European countries and determinations of many of these compounds in residue amounts ≥20% of MRLs from one European country are given in Table I.

EXPERIMENTAL

Chemicals

Pesticide-grade ethyl acetate, acetone, light petroleum, anhydrous sodium sulphate (12–60 mesh) and triphenyl phosphate were obtained from Merck (Darmstadt, Germany). The pesticide standards (Pestanal quality) listed in Table I 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 or acetone (methamidophos) to give 100.0–150.0 mg/l solutions. A working standard mixed solution of 1.0–1.5 mg/l was prepared by transferring 1 ml of each stock standard solution into a 100-ml volumetric flask and diluting to volume with light petroleum.

Chromatographic analysis

GC–FPD. A Perkin-Elmer (Beaconsfield, UK) Model 8600 gas chromatograph equipped with a flame photometric detector was used with an SPB 1701 fused-silica (0.5 μm) capillary column (30 m × 0.53 mm I.D.) coated with cyanopropylphenylmethylsiloxane (Supelco, Bellefonte, PA, USA). The carrier gas was helium at a flow-rate of 10 ml/min. The temperatures of the injector and detector were maintained at 220 and 300°C, respectively. The conditions used for GC were optimized. The injection volume was 3 μl.

GC–MS. A Hewlett-Packard (Palo Alto, CA, USA) Model 5995 instrument with a Model 59970 data system were 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–FPD analysis. EI spectra were obtained at 70 eV.

Optimization

Response function. The selection of an objective function (*OF*) based on a penalty function (*p*-) was realized by applying criteria given by Deming [16]. This function is defined as

$$OF = n + \sum R_{i,j} + p$$

where

$$p = 0 \quad \text{for } y < y_t$$

$$p = b(y - y_t) \quad \text{for } y_t < y < y_t + 5$$

$$p = -\infty \quad \text{for } y > y_t + 5$$

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 $R_{i,j}$), y is the retention time of the last peak considered and y_t is the target retention time.

Only the four worst resolved pairs of peaks were considered in the present calculations. The target retention time was fixed at $y_t = 20$ min as

TABLE I

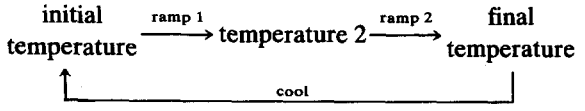
ORGANOPHOSPHORUS COMPOUNDS STUDIED, TOTAL TURNOVER OF SELECTED PESTICIDES IN THREE EUROPEAN COUNTRIES IN 1989 AND RESIDUES OF ORGANOPHOSPHATES FOUND AT ≥20% OF THE MRL IN FRUITS AND VEGETABLES IN 1991

No.	Compound	Total turnover (tonnes)[a]			Found at ≥20% of MRLs[b]
		Greece	Sweden	Denmark	
1	Dichlorvos	25.6	0.1	4.9	×
2	Methamidophos	317			×
3	Mevinphos		0.4		
4	Acephate	21	0.9	1.3	×
5	Naled				
6	Omethoate	29			×
7	Etrimphos		0.3		
8	Monocrotophos	118			×
9	Dimethoate	106	9.5	88.8	
10	Chlorpyriphos methyl				×
11	Pirimiphos methyl	20			×
12	Chlorpyriphos				×
13	Parathion methyl	25			×
14	Malathion	42.5	3.2	11.0	×
15	Fenitrothion	5.3	0.5	4.4	×
16	Quinalphos	4.3			
17	Chlorfenvinphos		0.7	1.3	×
18	Mecarbam	42			×
19	Metidathion	230			
20	Fenamiphos	42			
21	Carbophenothion				×
22	Triazophos	27.7			×
23	Pyridafenthion	1.2			
24	Phosalone	30.9			
25	Pyrazophos	7.1			

[a] Ref. 28.
[b] In fruits imported from Spain to Sweden; × = compound found. Ref. 1.

the maximum acceptable retention time of the last peak.

Selection variables. The next step was to define the variable space or search region. Relying on literature data [17] and previous experience, we selected the following temperature programming cycle:



Five preliminary runs (Table II) were made in order to establish the variables and ranges to be optimized and the survey limits of each of them.

A final temperature of 260°C was chosen to avoid column bleeding. A rate of 30°C/min was chosen for ramp 1. Thus a three-variable space (temperature 1, temperature 2 and ramp 2) with ranges 90–110°C, 180–240°C and 2–10°C/min, respectively, was used in the optimization procedure.

OPSP optimization. Over this domain an automated sequential method, optimization by search point (OPSP) as described previously [18], was used to determine the optimum GC conditions to accomplish the separation of the OPs [highest criterion value (*OF*)] within the desired time limits. A series of chromatograms were generated for which the experimental conditions were

TABLE II

PRELIMINARY RUNS

$OF_{max} = 14$.

Temperature 1 (°C)	Ramp 1 (°C/min)	Temperature 2 (°C)	Ramp 2 (°C/min)	Temperature 3 (°C)	*OF* value
100	10	210	2	260	0
100	30	210	2	260	10.02
100	10	210	10	260	8.76
100	30	210	10	260	9.72
120	20	230	5	260	9.43

set as indicated by the search algorithm. The optimum conditions were known after 23 experimental chromatographic runs had been performed. These optimum conditions temperature programme was an initial temperature of 110°C (held for 1 min), increased to 226°C at 30°C/min and then to 260°C at 4°C/min.

Sample preparation

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

The sample was macerated in a food chopper, 50 g of chopped sample were weighed into a high-speed blender and 40 g of anhydrous sodium sulphate were added. After thorough mixing, 100 ml of ethyl acetate were added and the mixture was blended for 5 min. The supernatant liquid was filtered with suction through a filter-paper and a layer of 20 g of anhydrous sodium sulphate. The extraction and filtration were repeated once. The filter was rinsed with 50 ml of ethyl acetate and the combined extracts were evaporated on a vacuum rotary evaporator using a 40–60°C water-bath. The volume of the residue solution was reduced to exactly 10 ml [19].

Recovery assays

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 mixed solution described above was added to 50 g of chopped sample in a high-speed blender jar. After evaporation of the light petroleum with a stream of air, the sample was homogenized for 2 min. After 30 min, the sample was again homogenized for 1 min and immediately analysed by application of the previously described method. The recovery assays were replicated three times.

RESULTS AND DISCUSSION

GC separation

The optimum temperature programme (see Experimental) resulted in the retention behaviour indicated in Table III. An example of a gas chromatogram of the mixed OP compounds is shown in Fig. 1A. Two critical pairs of compounds, chlorpyriphos–methylparathion and quinalfos–chlorfenvinos, remained partially overlapping, but the second pair could be well resolved using the detector in the sulphur mode (Fig. 1B). The total run time was 16 min plus 5 min for equilibration at the initial temperature. Hence the analysis of *ca.* 68 samples in 24 h is feasible.

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 IV. These data are in good agreement with the

TABLE III

RELATIVE RETENTION TIMES (RRT) AND THEIR RELATIVE STANDARD DEVIATIONS (R.S.D.s) AND AVERAGE RECOVERIES AND THEIR R.S.D.s OF TEST COMPOUNDS IN GREEN PEPPER SAMPLES USING GC–FPD-P

Fortification level 0.20–0.30 mg/kg ($n = 10$). Chromatographic conditions and sample preparation are described in the text.

No.	Compound	RRT[a]	R.S.D. (%)	Average recovery (%)	R.S.D. (%)
1	Dichlorvos	0.540	0.17	83.0	6.9
2	Methamidophos	0.602	0.17	68.4	33.9
3	Mevinphos	0.665	0.24	79.2	8.2
4	Acephate	0.737	0.26	69.1	30.8
5	Naled	0.793	0.28	70.2	8.4
6	Omethoate	0.835	0.27	65.1	21.2
7	Etrimphos	0.868	0.30	108.1	6.6
8	Monocrotophos	0.904	0.29	69.3	23.8
9	Dimethoate	0.925	0.30	78.8	12.7
10	Chlorpyriphos methyl	0.934	0.31	94.3	6.3
11	Pirimiphos methyl	0.965	0.31	96.2	6.8
12	Chlorpyriphos	1.000	0.32	98.2	4.9
13	Parathion methyl	1.006	0.39	96.9	7.9
14	Malathion	1.029	0.23	105.2	3.4
15	Fenitrothion	1.043	0.32	94.4	4.2
16	Quinalphos	1.120	0.35	93.7	10.6
17	Chlorfenvinphos	1.125	0.33	90.5	15.6
18	Mecarbam	1.152	0.34	95.7	7.5
19	Metidathion	1.217	0.34	94.5	7.9
20	Fenamiphos	1.268	0.37	99.9	11.6
21	Carbophenothion	1.425	0.36	96.0	3.7
22	Triazophos	1.496	0.35	100.2	8.2
23	Pyridafenthion	1.756	0.32	102.1	5.9
24	Phosalone	1.928	0.32	99.3	6.5
25	Pyrazophos	2.001	0.35	96.5	3.7

[a] Absolute retention time of chlorpyriphos = 7.698 min.

different diagnostic ions reported for these organophosphorus pesticides [20–26].

Selection of internal standard

Four compounds were considered for use as internal standards. Three of them, chlormephos, fenthion and demeton, are OP compounds but are not used on horticultural crops in this area, and triphenyl phosphate has been proposed in EPA Method 507 and National Pesticide Survey Method 1 [27]. Selection was based on both retention time and detector response. Chlormephos met all the criteria for an internal standard (retention time 5.05 min; detector response 2.5 times more than that for chlorpyrifos; recovery = 88%) and is therefore, recommended as the internal standard. Of the other three compounds investigated two (fenthion and demeton) co-eluted with the target compounds. Triphenyl-phosphate eluted at the end of the desired retention time range.

Evaluation of working and internal standards

An important criterion by which to assess the quality of the analytical results is the accuracy of determination. For this purpose we evaluated the stability, detector response and random deviations of the working and internal standard by quality control charts (QCC). The QCC for two representative OPs, methamidofos (high polarity) and chlorpyrifos (low polarity), and the internal standard chlormephos showed no results

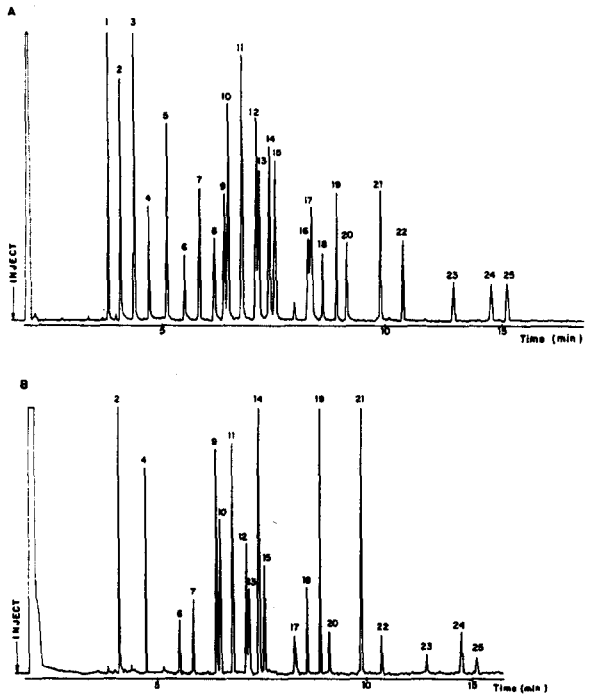


Fig. 1. Gas chromatogram of the working standard solution containing the 25 organophosphorus pesticides (concentrations 1.0–1.5 mg/l). (A) FPD in phosphorus mode; (B) FPD in sulphur mode. Numbers above the peaks correspond to compound numbers in Table I.

outside the limiting lines (three times the standard deviation) during at least 15 days if the standard solutions (see Experimental) were kept at 4°C for all compounds except methamidofos, for which it is recommended that a daily check is made.

Linearity, recoveries and repeatability

The linear dynamic range of the detector response was checked for all the target compounds in the working standard solution and appeared to be 0.15–50 ng absolutely amount injected (on-column) and the correlation coefficients were higher than 0.991 in all instances. This is also the practical working range. Detection limits based on a signal-to-noise ratio of 3:1 were on average 50 pg absolute amount injected.

The retention times and recoveries of the different OPs were tested by fortifying ten fresh pepper samples, using the procedure described above (Table III). The repeatability of retention time was satisfactory in all instances. The recoveries were in the range 68–108% with a relative standard deviation (R.S.D.) of less than 30% in all instances except methamidophos (33.90%) and acephate (30.83%), which is considered acceptable.

Control sample

As a quality assurance measure, a control sample was repeatedly analysed over a 2-month period. Green pepper was chosen as the relevant matrix, and it was shown that residues of target compounds and internal standard were stable for a long time if the sample is kept in a freezer at −18°C. The analytical data for the fortified control sample were registered on a control chart according to Shewhart. The results for chlorpyriphos are shown in Fig. 2 and confirm the overall reliability of optimized method in routine analysis.

Method performance with cucumber, beans and grapes

The proposed screening method was assessed for the analysis of cucumber, beans and grapes, collected from 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, using the procedure described above, with the 25 target compounds in the range 0.20–0.30 mg/kg and analyses were carried out in duplicate (in this test green pepper samples were also included). All compounds were identified correctly

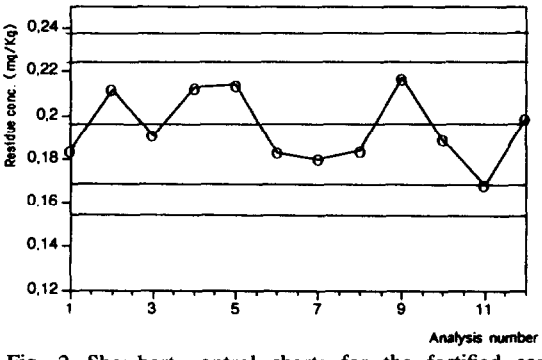


Fig. 2. Shewhart control charts for the fortified control sample for chlorpyrifos. The analytical results were registered over a 2-month period.

TABLE IV

MAIN IONS AND RELATIVE ABUNDANCES IN EI MASS SPECTRA OF THE ORGANOPHOSPHORUS PESTICIDES

No.	Compound	Molecular mass	Main ions, m/z (relative abundance, %)					
			I	II	III	IV	V	VI
1	Dichlorvos	220	79 (20)		109 (100)		185 (18)	220 (4)
2	Methamidophos	141	79 (14)	94 (100)				141 (39)
3	Mevinphos	224			109 (19)	127 (100)		224 (4)
4	Acephate	183		94 (68)		125 (14)	136 (100)	183 (4)
5	Naled	380			109 (100)			301 (5)
6	Omethoate	213	79 (48)		110 (100)	125 (17)		213 (4)
7	Etrimphos	292	79 (71)		109 (43)	125 (100)		292 (36)
8	Monocrotophos	223			109 (13)	127 (100)		223 (4)
9	Dimethoate	367				125 (90)	182 (100)	367 (9)
10	Chlorpyriphos methyl	321	79 (35)		109 (25)	125 (100)		286 (60)
11	Pirimiphos methyl	305	79 (40)		109 (44)	125 (100)		305 (57)
12	Chlorpyriphos	349		97 (47)			197 (100)	314 (47)
13	Parathion methyl	263	79 (30)		109 (100)	125 (83)		263 (35)
14	Malathion	330		93 (87)		125 (100)	173 (75)	330 (3)
15	Fenitrothion	277	79 (26)		109 (79)	125 (100)		277 (45)
16	Quinalphos	298		97 (39)			146 (100)	298 (17)
17	Chlorfenvinphos	358	81 (100)		109 (62)		267 (45)	323 (44)
18	Mecarbam	329		97 (82)		125 (49)	131 (100)	329 (12)
19	Metidathion	302		93 (21)		125 (18)	145 (100)	302 (3)
20	Fenamiphos	303			109 (14)		154 (100)	303 (69)
21	Carbophenothion	342		97 (66)		125 (49)	157 (100)	342 (15)
22	Triazophos	313		97 (55)			161 (100)	313 (7)
23	Pyridafenthion	340		97 (100)			188 (52)	340 (44)
24	Phosalone	367				121 (56)	182 (100)	367 (9)
25	Pyrazophos	373		97 (30)			221 (100)	373 (11)

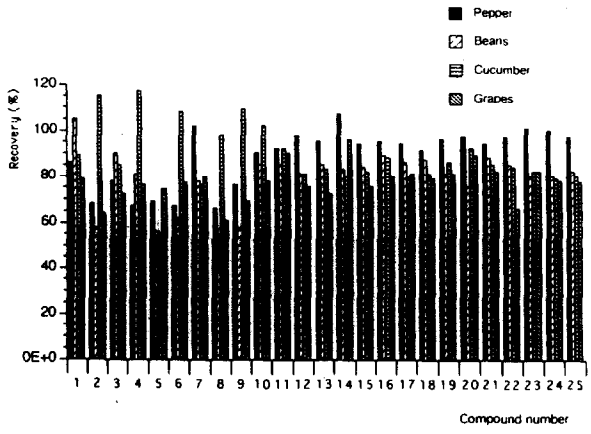


Fig. 3. Average recoveries (%) from duplicate determinations of the 25 OP compounds as a function of the matrix. Fortification level 0.20–0.30 mg/kg.

and the average recovery data were, as expected, dependent on the matrix, but nevertheless acceptable (Fig. 3). These values were in the range 65–111% in all instances, except for methamidophos, acephate, naled, omethoate and dimethoate, with values in the range 58–119%.

CONCLUSIONS

An optimized chromatographic method covering 25 organophosphorus pesticides in fruit and vegetable samples has been developed. The sample throughput was increased considerably (*ca.* 68 per 24 h) with the optimized method. The minimum detectable amount is about 50 pg (signal-to-noise ratio = 3) for each pesticide,

which means that residue levels in real crop samples down to 10 μg/kg can be detected.

ACKNOWLEDGEMENTS

The authors are grateful to J. Moliz Medina for performing the analyses of the OP compounds by GC–MS and to J. Colominas for his collaboration in this work.

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