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Short communication

Multiresidue determination of pesticides in apples and pears by gas chromatography–mass spectrometry

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Abstract

This paper describes a rapid, specific and sensitive multiresidue method for the routine analysis of several classes of pesticides used for the treatment of apples and pears, involving a rapid extraction procedure at pH 4.5 with a mixture of acetone–dichloromethane–hexane (50:20:30, v/v/v) and gas chromatography coupled to mass-selective detection, in order to achieve quantitative analysis down to their respective maximum residue limit. Extraction recoveries were between 55 and 98%. Limits of detection and limits of quantitation ranged respectively, from 0.01 to 0.05 mg/kg and from 0.02 to 0.1 mg/kg. Intra-assay relative standard deviation was less than 19% for all compounds. An excellent linearity was observed from these LOQs up to 500 mg/kg. Intermediate (inter-assay) precision and accuracy were satisfactory. The method has been applied to many fruit samples intended for commercialisation. © 1998 Elsevier Science B.V.

Keywords: Apples; Pears; Fruits; Food analysis; Pesticides

1. Introduction

There is a need to develop multiresidue methods for pesticides in food, for the protection of environment and for the evaluation of food quality. Pesticides are usually minimal in fruits and have to be below the maximum residue limits (MRLs) [1–4].

Since our laboratory became involved in the control of commercial apples and pears to determine the compliance with MRLs, we had to develop an efficient method for the determination of pesticides. The molecules investigated were selected among those most frequently used by fruits growers, the volatility, good thermal stability and low polarity of which rendered them suitable for gas chromatographic analysis: endosulfan (α and β), lindane, methyl-parathion, phosalone, propargite, captan, bifenthrin, deltamethrin and tolylfluanid.

Most of the multiresidue procedures proposed for the determination of volatile pesticides in fruits used gas chromatography (GC), either with electron-capture (ECD) [5–8], nitrogen–phosphorus (NPD) [9,10] or flame ionization (FID) [11,12] detection. GC coupled to mass spectrometry (GC–MS or GC–MS–MS) was used when a highly selective detection was required [13–17].

Numerous published methods used an extraction step followed by a clean-up procedure prior to chromatographic analysis, using conventional liquid–liquid partitioning [18,19], chromatography on

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Florisil or on alumina [20–22], gel permeation chromatography [6,7,16,23], solid-phase extraction (SPE) [7,12,24] or supercritical fluid extraction (SFE) [25,26]. All these methods using clean-up procedure are time-consuming, which is a determining factor in routine analysis, together with the cost of the extraction procedure.

The detection and quantification limits of most recently published techniques were usually below tolerance levels (MRLs) [4], which range from 0.1 to 3 mg/kg for these pesticides in apples and pears [1–3].

This paper presents a rapid, specific and sensitive method for the simultaneous quantitation of nine pesticides from several classes, in apples and pears down to or below their respective MRL.

2. Experimental

2.1. Reagents and materials

Endosulfan, lindane, methyl-parathion, phosalone, propargite, captan, bifenthrin, deltamethrin and tolylfluanid were purchased from Cluzeau Info Labo (Libourne, France). A stock standard solution for each pesticide was prepared at 1 g/l in methanol. Dichloromethane (DCM), acetone, hexane, acetonitrile (of Pestinorm grade), acetic acid and sodium acetate were purchased from Prolabo (Fontenay-sous-bois, France). All were of chromatographic purity. The working solutions were prepared by appropriate dilution of a mixture of stock solutions in acetone–dichloromethane–hexane (50:20:30, v/v/v) at the following concentrations: 1, 10 and 50 mg/l. The internal standard solution of heptachlor was prepared at 50 mg/l in the same solvent mixture. All standards and stock solutions were stored at +4°C The stock solutions stability was verified over a three-month period by comparing two different stock solutions after extraction of six spiked samples at two concentration levels (0.5 and 1 mg/kg).

2.2. Samples

Samples of apples and pears were collected from various fruit growers in Haute-Vienne (France). Pesticide-free fruits, controlled by ECOCERT (control office, certified by the European Union) were provided by La Vie Claire (Limoges, France) and used as blank matrix to prepare matrix matched standards for calibration.

2.3. Gas chromatography–mass spectrometry

A Shimadzu GC 17A gas chromatograph, equipped with a split/splitless injector operated in the splitless mode, with a AOC SPL 1400 automatic sampler and coupled to a Shimadzu QP-5000 mass spectrometer (Touzart et Matignon, France) was used. The analytical column used was a 30 m×0.25 mm I.D., 0.25 μm film thickness, PTE5 (Supelco, St. Quentin-Fallavier, France), coated with a 5% biphenyl–95% dimethylsiloxane stationary phase. The chromatograph was programmed from an initial temperature of 60°C, increased at 10°C/min to 250°C, and held at 250°C for 6 min. The temperatures of the injector and of the transfer line were 250°C and 280°C, respectively. Helium was used as the carrier gas (flow-rate: 2.1 ml/min). The mass spectrometer was operated in the electron impact (70 eV), selected ion monitoring (SIM) mode. For each analyte, the most abundant and characteristic mass fragment was chosen for quantitation and two others for confirmation (Table 1). These mass-to-charge ratios were carefully selected to avoid all those belonging to other pesticide residues of the same class. Pesticides analytes were subsequently identified by their relative retention time and by the ratios of their respective confirmation ions to their quantitation ion.

2.4. Extraction procedure

After homogenisation of 1 kg of fruits, 10 g portions were sampled, to which were sequentially added 100 μl of internal standard (I.S.) solution (50 mg/l) and 10 ml of 3 *M* sodium acetate solution (pH 4.5). The mixture was extracted with 25 ml of an acetone–dichloromethane–hexane (50:20:30, v/v/v) mixture, by shaking for 15 min and centrifuging at 3000 rpm (1600 *g*) for 5 min. The organic phase was concentrated to 1 ml by evaporation at 50°C under a gentle stream of nitrogen. Then 1 μl of this solution was injected into the GC–MS system.

Table 1
Quantitation and confirmation ions selected for the GC–MS determination of nine pesticides in fruits

Pesticides	Relative retention time	Quantitation ions (m/z)	First confirmation ions		Second confirmation ions	
			m/z	Relative intensity (%)	m/z	Relative intensity (%)
Captan	0.710	79	80	60	151	44
Lindane	0.914	183	219	80	181	102
Parathion-methyl	0.996	263	125	140	109	222
Heptachlor (I.S.)	1.000	272	237	75	337	40
Tolylfluanid	1.100	137	238	32	181	25
Endosulfan	1.138	195	241	86	265	40
Propargite	1.275	135	173	39	201	10
Bifenthrine	1.317	181	165	31	166	33
Phosalone	1.353	182	121	77	367	15
Deltamethrin	1.617	253	181	142	172	38

2.5. Validation

All validation procedures were performed using pesticide-free fruits. Calibration standards were prepared by adding standard solutions to 10 g of pesticide-free fruit samples to obtain concentrations ranging from 0.05 to 5 mg/kg [27]. Recovery was determined in triplicate at three concentration levels (0.1, 0.5 and 2 mg/kg) by comparing the analyte/I.S. peak area ratios obtained with those of unextracted solutions.

The intra-assay precision was assessed at 0.1, 0.5 and 2 mg/kg by extraction and analysis on the same day of six fortified fruit samples for each level. For

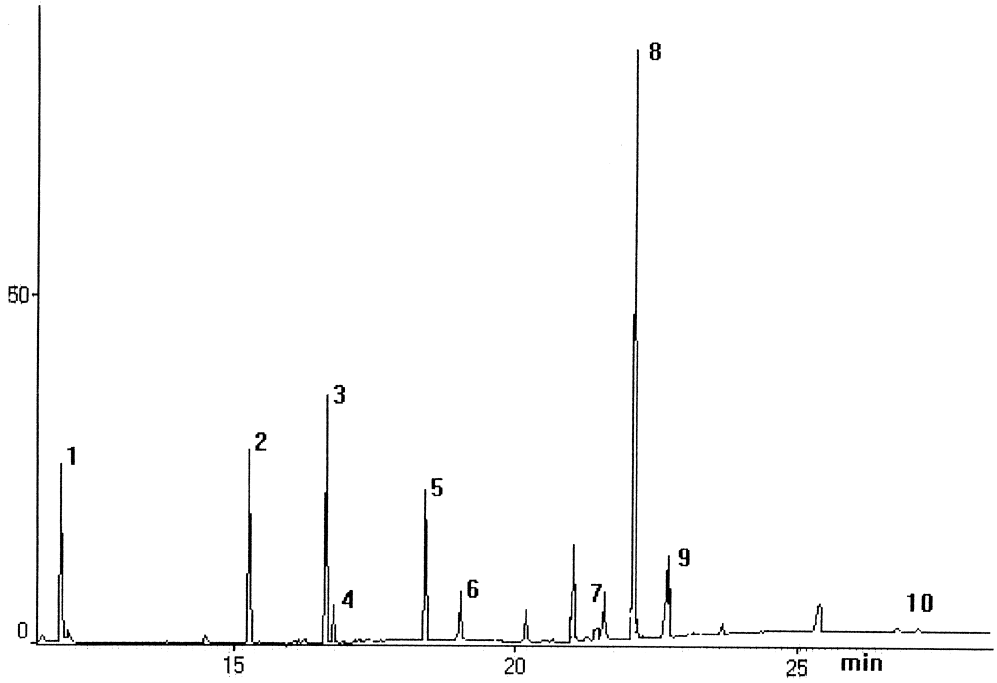

Fig. 1. Selected ion chromatogram of an apple sample spiked with 1 ppm of each pesticide mixture. Peaks: 1=captan, 2=lindane, 3=methyl-parathion, 4=I.S., heptachlor, 5=tolylfluanid, 6=α-endosulfan, 7=propargite, 8=bifenthrin, 9=phosalone, 10=deltamethrin.

the intermediate (''inter-assay'') precision a set of calibrating samples (0.05, 0.1, 0.5, 1, 2 and 5 mg/kg) was analysed each day for five days. The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average of the baseline noise defined from three unfortified samples. Limits of quantitation (LOQs) were determined as the lowest amount of a given pesticide giving a response that can be quantified with an accuracy and an inter-assay relative standard deviation (R.S.D.) lower than 20%. Calibration graphs of the pesticide-to-internal standard peak-area ratios of the quantitation ions versus theoretical pesticide concentration were constructed, using a least-square linear regression analysis, in order to verify the linearity.

3. Results and discussion

Figs. 1 and 2 show chromatograms obtained respectively, from a 1 mg/kg spiked apple sample and from a real sample. The background obtained is very low and thus the extracts did not require further clean-up operation. Analysis of blank samples revealed no trace of the pesticides studied.

Average recoveries were in the range of 55 to 98% (see Table 2) with R.S.D.s less than 19%, for the three levels of concentration. The crucial problem was to define optimal extraction conditions, in order to obtain satisfying recoveries for each of the nine compounds, belonging to six different classes of pesticides (phthalimide, pyrethroid, sulfamid, organochlorine, organophosphorus and organosulfur class). Even in the case of the lowest recoveries (ca. 55%), the overall repeatability and sensitivity of the method were good enough to ensure a reliable determination at levels lower than the respective MRL.

This multiclass/multiresidue extraction method, using only a mixture of acetone–dichloromethane–hexane suitable for both non-polar and slightly polar pesticides, is a combination of acetone–water extraction and hexane–dichloromethane partitioning [4,18,28]. In this mixture, the proportion of acetone is determinant to ensure the best penetration of the

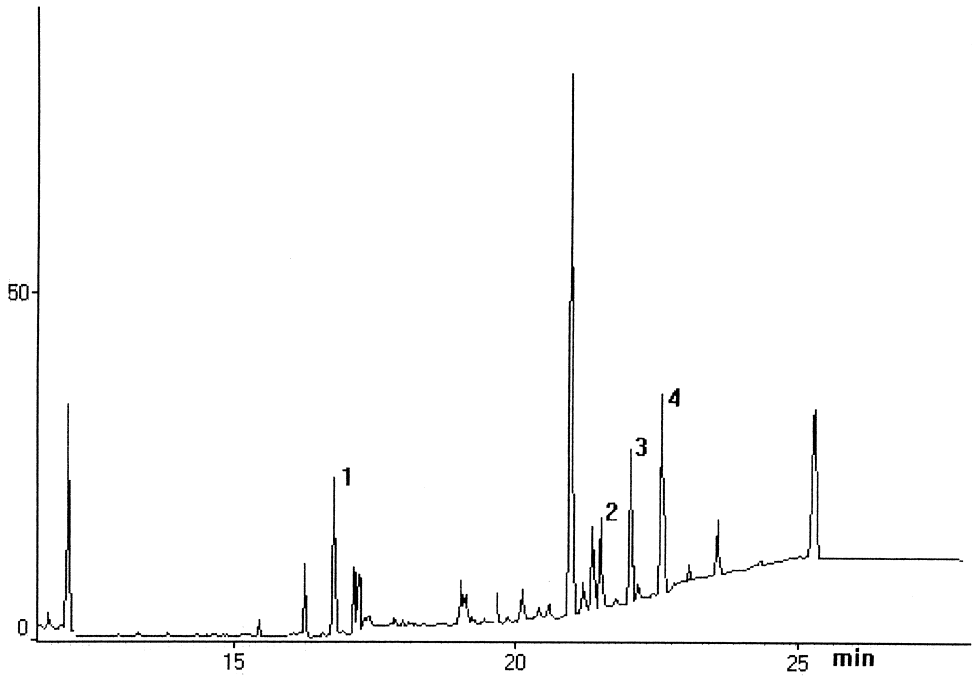

Fig. 2. Example of a selected ion chromatogram of a positive real apple sample. Peaks: 1=I.S., hepachlor, 2=propargite, 3=bifenthrin, 4=phosalone.

Table 2
Results of the validation procedure of the GC–MS analysis of nine pesticides in apples and pears

Concentration (mg/kg)	Recovery (%)		Repeatability (n=5)		Reproducibility (n=5)	
	Apples Average (R.S.D., %)	Pears Average (R.S.D., %)	Apples Average (R.S.D., %)	Pears Average (R.S.D., %)	Apples Accuracy (R.S.D., %)	Pears Accuracy (R.S.D., %)
Captan						
0.05					112 (14.2)	
0.10	89.17 (13.3)	89.20 (1.7)	0.12 (12.7)	0.14 (6.8)	110 (16.6)	59 (19.3)
0.50	76.97 (11.9)	87.97 (1.3)	0.58 (4.6)	0.65 (9.2)	106 (8.1)	80 (18.8)
1.00					105 (5.4)	92 (17.9)
2.00	79.35 (8.6)	79.35 (9.4)	2.23 (2.5)	3.13 (5.5)	90.5 (3.3)	94 (8.1)
5.00					101 (0.2)	101.4 (1.9)
r					0.999 (0.067)	0.998 (0.243)
Parathion-methyl						
0.01					66 (17.3)	
0.02					65 (8.5)	70 (10.0)
0.05					66 (8.0)	76 (16.2)
0.10	74.65 (12.7)	84.36 (3.8)	0.14 (6.7)	0.09 (5.5)	78 (9.9)	87 (16.0)
0.50	85.63 (11.8)	66.01 (11.6)	0.37 (3.5)	0.50 (6.0)	80 (5.5)	85 (8.7)
1.00					87 (1.7)	97 (5.9)
2.00	71.41 (8.0)	65.44 (9.2)	1.78 (2.0)	2.19 (5.2)	85 (2.6)	97.5 (4.7)
5.00					102.4 (0.4)	103 (0.7)
r					0.997 (0.080)	0.999 (0.069)
Tolylfluanid						
0.01					70 (18.1)	
0.02					95 (17.7)	160 (13.3)
0.05					98 (10.0)	100.2 (12.9)
0.10	73.17 (11.4)	79.01 (2.6)	0.11 (7.7)	0.12 (1.2)	120 (10.3)	130 (14.6)
0.50	82.34 (11.9)	58.05 (8.1)	0.55 (5.4)	0.49 (17.7)	106 (8.2)	128 (10.3)
1.00					130 (3.3)	128 (3.2)
2.00	64.29 (8.0)	66.17 (2.5)	2.12 (4.4)	1.64 (5.1)	104 (9.1)	113.5 (7.5)
5.00					130 (1.5)	82 (1.6)
r					0.999 (0.099)	0.995 (0.554)
Endosulfan						
0.02					110 (11.1)	
0.05					102 (15.5)	112 (10.4)
0.10	75.19 (14.4)	81.16 (4.4)	0.11 (8.0)	0.14 (9.0)	113 (10.4)	120 (12.1)
0.50	88.64 (12.1)	65.61 (9.4)	0.48 (1.3)	0.64 (6.3)	102.4 (6.4)	108 (8.3)
1.00					104 (1.6)	113 (5.0)
2.00	69.52 (7.3)	67.71 (9.1)	2.09 (2.1)	2.40 (5.4)	98 (3.4)	105.5 (5.1)
5.00					100 (0.6)	98.6 (1.0)
r					0.999 (0.023)	0.998 (0.168)
Propargite						
0.02						140 (14.0)
0.05					128 (12.3)	70 (13.8)
0.10	63.73 (15.5)	81.55 (1.3)	0.16 (9.9)	0.10 (5.9)	190 (17.1)	140 (10.9)
0.50	78.88 (11.3)	64.53 (11.9)	0.44 (2.7)	0.56 (6.4)	138 (17.5)	104 (8.3)
1.00					131 (4.3)	128 (5.2)
2.00	63.02 (7.0)	69.23 (9.5)	2.31 (13.7)	2.25 (7.2)	112.5 (11.4)	116 (8.3)
5.00					196 (2.7)	95.8 (1.9)

(Cont.)

Table 2 (*Continued*)

Concentration (mg/kg)	Recovery (%)		Repeatability (n=5)		Reproducibility (n=5)	
	Apples Average (R.S.D., %)	Pears Average (R.S.D., %)	Apples Average (R.S.D., %)	Pears Average (R.S.D., %)	Apples Accuracy (R.S.D., %)	Pears Accuracy (R.S.D., %)
r					0.998 (0.140)	0.988 (0.206)
Bifenthrine						
0.01						70 (11.2)
0.02					85 (5.1)	180 (11.2)
0.05					102 (14.9)	112 (9.9)
0.10	61.74 (19.2)	61.99 (7.5)	0.11 (4.6)	0.12 (18.2)	81 (10.4)	73 (13.9)
0.50	82.49 (11.0)	56.22 (9,8)	0.39 (10.8)	0.85 (14.5)	113.2 (18.5)	104 (12.7)
1.00					121 (7.5)	119 (4.5)
2.00	60.86 (6.7)	61.67 (7.4)	1.86 (9.2)	2.14 (5.2)	121 (3.2)	107 (6.9)
5.00					95.4 (1.9)	97.8 (1.4)
r					0.999 (0.049)	0.988 (0.881)
Phosalone						
0.01					70 (14.3)	
0.02					130 (10.7)	130 (20.1)
0.05					98.2 (11.2)	160 (13.2)
0.10	64.65 (11.0)	93.53 (8.2)	0.13 (8.3)	0.15 (3.8)	94 (10.3)	71 (16.2)
0.50	79.43 (11.3)	68.05 (9,6)	0.42 (2.3)	0.54 (1.7)	97.2 (6.2)	78 (7.9)
1.00					99.9 (3.1)	123 (5.7)
2.00	63.84 (6.5)	66.04 (8.8)	1.82 (3.1)	2.51 (4.9)	99 (7.0)	105.5 (5.3)
5.00					100.2 (1.1)	101.4 (0.9)
r					0.999 (0.013)	0.998 (0.128)
Deltamethrin						
0.10	71.82 (11.2)	98.44 (4.1)	0.12 (12.9)	0.06 (20.0)	80 (7.3)	152 (9.4)
0.50	75.63 (7.3)	59.08 (7.6)	0.43 (5.5)	0.56 (16.6)	82.8 (6.5)	70 (13.4)
1.00					85.6 (5.6)	92 (13.6)
2.00	57.28 (6.9)	55.00 (1.2)	1.58 (2.2)	2.13 (5.0)	97 (8.8)	102 (6.1)
5.00					100.4 (2.0)	102 (1.6)
r					0.999 (0.057)	0.998 (0.090)

R.S.D.: relative standard deviation, *r*: average of coefficients of variation.

fruit sample. Moreover, other works have reported that the mixture of acetone and dichloromethane (widely used in liquid–liquid extraction procedures [15,16], despite its toxicity [6]) is sufficiently polar to extract a wide range of pesticides [29,30], whereas hexane lowers the extraction of polar coextractives. We have also tested polar solvents (methanol, acetonitrile) which revealed inefficient for pesticide and coextractive partitioning. In fact, the mixture acetone–dichloromethane–hexane yielded the maximal recoveries together with a low background for the nine compounds. The pH influence was also tested: high pH values (using phosphate buffer at pH 9.5) induced a 30% drop in recovery for captan, tolylfluanid and propargite; the optimum value was found to be pH 4.5.

Despite the absence of clean-up procedure (based on Florisil, alumina or silica gel) and of derivatization before GC–MS analysis, the present technique allows a good selectivity for the nine pesticides, owing to the mass detector. This extraction and liquid–liquid partitioning procedure is easier, faster than SPE or SFE, does not sacrifice the sensitivity of the method, which is comparable to those published

for multiresidue methods in apples and pears [13,15,18,19] and produces relatively clean extracts. We observed no significant differences in recoveries between apple and pear matrices. According to recent results with other fruits, this extraction is applicable to a great number of fruit matrices.

The results of the validation procedure are summarised in Table 2. LODs varied from 0.01 (lindane, parathion-methyl, tolylfluanid, bifenthrine) to 0.1 mg/kg (deltamethrin). They were lower than those obtained by previously published GC–MS methods (0.025–0.1 mg/kg) [13,25] and comparable to those obtained by GC–NPD or GC–ECD [6,7,15,30]. However, these last methods required additional clean-up steps (on Florisil, silica gel or C_{18} SPE columns) which were time-consuming. LOQs varied from 0.02 to 0.1 mg/kg, the highest values being obtained for deltamethrin, captan, endosulfan and propargite. All were lower than the MRLs (Table 3).

The present method is repeatable for all the compounds, as shown by the intra-assay precision (generally R.S.D.$<$15%). The intermediate precision for the nine analytes was excellent above their respective LOQ, with R.S.D.s lower than 15%. The calibration curve of each analyte was linear from its respective LOQ up to 5 mg/kg, with a correlation coefficient (r) between 0.995 and 0.999.

The routine efficiency of this method was verified on hundreds of apple and pear samples intended for commercialisation. Our results showed that these pesticides were absent or found at very low levels in fruits, confirming previously published results [4,31].

4. Conclusion

The present method, developed for the simultaneous determination of nine pesticides in apples and pears involves a rapid and non-selective extraction procedure and a specific GC–MS determination with satisfactory recoveries and LOQs among the lowest published to date for a multiresidue method using a single mass analysing instrument. Routine use demonstrated that this method is suitable for the analysis of residual amounts of pesticides in fruit products, down to or below MRLs.

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Table 3
Maximum residue limits (MRLs) authorized in France, limits of detection and limits of quantitation of the nine pesticides assayed

	MRLs (mg/kg)	Limit of quantification (mg/kg)		Limit of detection (mg/kg)	
Pesticides		Apples	Pears	Apples	Pears
Captan	3.00	0.10	0.10	0.05	0.05
Lindane	1.00	0.02	0.02	0.01	0.01
Parathion-methyl	0.20	0.02	0.02	0.01	0.01
Tolylfluanid	2.00	0.02	0.02	0.01	0.01
Endosulfan	1.00	0.05	0.05	0.02	0.02
Propargite	2.00	0.10	0.10	0.05	0.05
Bifenthrine	0.10	0.02	0.02	0.01	0.01
Phosalone	2.00	0.05	0.05	0.02	0.02
Deltamethrin	0.20	0.10	0.10	0.05	0.05

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