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Journal of Chromatography A, 782 (1997) 123–132

JOURNAL OF CHROMATOGRAPHY A

Supercritical fluid extraction of pesticide residues in fortified apple matrices[1]

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Received 10 June 1996; received in revised form 12 May 1997; accepted 10 June 1997

Abstract

In the present work, by means of the supercritical fluid extraction (SFE) technique, we developed a method for pesticide residue extraction which proved fast and effective in extracting the largest possible number of plant protection products in a short time and with acceptable recoveries and reproducibility. We observed the recoveries in fortified apple matrices while the instrument conditions varied, and obtained eventually parameters which allowed us to extract – by one and the same method – 92 pesticides, with good levels of recovery and reproducibility. Then, the pesticides were assessed by means of multiresidue methods, gas chromatography and high-performance liquid chromatography. © 1997 Elsevier Science B.V.

Keywords: Apple; Fruits; Food analysis; Extraction methods; Pesticides

1. Introduction

The possibility to analyse and ascertain foreign substances in food – their presence being due to chemical and technological treatments or processes or through environmental pollution – has become an important issue nowadays.

The determination of pesticide residues on food products has thus become a more and more essential requirement for consumers [1], producers and the authorities that are responsible for quality controls. As a consequence, the need has arisen to set up increasingly fast and simple analysis methods which can provide the largest possible range of results. The present work aims at contributing in this direction.

In a previous work [2] we compared a system we called traditional [3–10] and one based on the supercritical fluid extraction (SFE) [11–13] technique. In that work we tried to determine if the SFE technique could be used for samples which would be multiresidue-analysed, and we had stressed the advantages of the SFE technique in comparison to the one previously used in our laboratory.

On that occasion we managed to extract and purify at the same time 44 active components with recoveries and reproducibility at an extent that enabled us to apply this technique to routine analysis.

Encouraged by those results, we tried to develop the method even further, trying to increase the number of active components extracted including also those, as omethoate, vamidothion and acephate, which prove difficult to extract and analyse even by traditional methods.

Therefore, we decided to study the recoveries of a mixture of pesticide standards and apple samples (used as a matrix) fortified with pesticides according

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[1]Presented at the 1st European Workshop on Pesticide Residues, Alkmaar, 10–12 June 1996.

0021-9673/97/$17.00
PII S0021-9673(97)00671-7

to the variation of the SFE operating conditions; we aimed at a method that would be suitable for the application on plant products, fast and capable of extracting the largest possible number of plant protection products in a short time and with acceptable recoveries and reproducibility.

We obtained satisfactory results, as we were able to extract by means of one method 92 different pesticides which were then assessed and analysed by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Reagents and standards

Cylinder of carbon dioxide with dip tube, purity 99.998% (Rivoira, Torino, Italy); cylinder of technical carbon dioxide with dip tube (Rivoira); pesticide standard solutions in cyclohexane at 100 mg/l (Labor Dr. Ehrenstoffen, Augsburg, Germany); multistandard solution of organic chlorine derivative pesticides in cyclohexane (at 0.02 mg/l) by dilution from single standards; multistandard solution of common chlorinated pesticides in cyclohexane (at 0.02 mg/l) by dilution from single standards; two multistandard solutions in cyclohexane of organophosphorous and organonitrogenous pesticides (at 0.5 mg/l) by dilution from single standards; multistandard solution of pyrethroid pesticides in cyclohexane (at 0.5 mg/l) by dilution from single standards; multistandard solution in acetonitrile for HPLC analysis (at 0.5 mg/l) by dilution from single standards; solvents with residue pesticides analysis grade or better; anhydrous sulphate sodium calcined at 600°C for 1 h; Celite BDH 545 (BDH, Poole, UK) [14]; pesticide-free apples from controlled cultures.

2.2. Sample preparation

A 1 ml volume of multistandard solution placed on filter paper and then, after vaporization of the solvent, into the extraction cell.

Fortified matrix: as the extraction cell had a volume of 7 ml, we amalgamated 2 g pesticide-free apple+0.8 g Celite+2.5 g anhydrous calcined Na_2SO_4+1 ml multistandard solution.

2.3. Supercritical fluid extraction

The samples, placed in an extraction cell, were extracted by means of a Hewlett-Packard Model HP 7690 T (Hewlett-Packard, Palo Alto, CA, USA) supercritical fluid extractor, operated through the Hewlett-Packard Model HP G 1225 C (Hewlett-Packard) software, under the conditions reported in Section 4.8.

2.4. Analysis

Analyses were performed using the following equipment: gas chromatograph Varian 3400 with a thermoionic specific detection (TSD) system and autosampler 8100 (Varian, Palo Alto, CA, USA), Hewlett-Packard fused-silica capillary column HP-50+ cross linked 50% phenyl-methyl silicone, 15 m×0.53 mm (Hewlett-Packard), injector temperature 250°C, detector temperature 260°C, carrier He at 5 p.s.i. (1 p.s.i=6894.76 Pa), column oven: 60–150°C at a rate of 50°C/min, at 150°C for 5 min, followed by a ramp of 10°C/min to 186°C, at 186°C for 2 min, followed by a ramp of 5°C/min to 250°C and finally at 250°C for 20 min and finally at 250°C for 5 min, work station Varian mod. Star Integration; gas chromatograph Carlo Erba Model HRGC 5300 with an electron-capture detection (ECD) system and autosampler A200S (Carlo Erba, Milan, Italy), Hewlett-Packard fused-silica capillary column HP-1 cross linked methyl silicone, 30 m×0.32 mm (Hewlett-Packard), injector temperature: 250°C, detector temperature: 270°C, carrier: H_2 at 50 kPa, column oven: 60–120°C at a rate of 30°C/min, at 120°C for 1 min, followed by a ramp of 5°C/min to 250°C and finally at 250°C for 20 min, work station Hewlett-Packard mod. HP 3365 series II Chem Station, HPLC chromatograph Varian 9010 with autosampler 9095 and diode array detector Varian 9065 Policrom, chromatographic Merck LiChrospher 100 column, RP 18, endcapped (5 μm) 250×4 mm (Merck, Darmstadt, Germany), mobile phase: A=acetonitrile, B=water, gradient programme: time 0 min, A 30%–B 70%; 2 min, A 30%–B 70%; 15 min, A 60%–B 40%; 21 min, A 60%–B 40%; 26 min, A 70%–B 30%; 33 min, A 100%; 37 min, A 100%; flow-rate: 1.0 ml/min; λ: 205, 224, 244 nm; work station Varian mod. Star Integration.

3. Procedure

Before proceeding with our work – aimed at developing a pesticide residue extraction method which could apply to the largest possible amount of active components – we thought it necessary to set up the parameters representing the variables in the SFE [15].

Because of the number of variables to be considered, we thought it best to leave aside the parameters that had been already optimized in our previous work, i.e.,: (i) nozzle temperature; (ii) trap temperature and (iii) trap type.

As recovery tests were planned, we had to decide first whether to use pure standards or a fortified matrix. Thus we performed some recovery tests using pure standard solutions compared to pesticide-free apple matrices, to which we added the same standard amount. From the results given by those tests, we decided to carry out the subsequent tests using fortified apple matrices. Results are reported in Section 4.9.

Once the type of sample was set, we focused on the supercritical fluid density, i.e., its polarity, trying to improve the extraction through this parameter. The supercritical CO_2 flow was also investigated.

Besides, the extraction temperature was also observed, recovery varying according to this parameter.

Particular attention was also paid to the parameters extraction time and method (static and dynamic), observing the pesticide recovery variation according to the variation of these parameters. Results are given in Section 4.5, like those relating to the other factors.

A further step was connected with finding the most suitable solvent for washing the absorbtion trap. As a matter of fact, as we wanted to recover the numerous substances extracted and deposited by CO_2, it was vital that we employed a solvent having remarkable characteristics both as extractor and solvent for all the pesticides, given the limited washing volume and the wide variety of chemical characteristics of the different active components. Therefore we performed a series of trials with different solvents and mixtures of solvents, in different proportions.

Finally, we worked on the number of extraction steps, checking if the result for some pesticides could be improved.

On the other hand, we did not consider the possibility of using a modifier, e.g., MeOH, since the aim of this work was to find a method capable of extracting and analyzing the highest possible number of pesticides in one process [16–19].

4. Results and discussion

4.1. Choice of sample type

The first decision to take was that about whether using pure standards fortified matrices. Thus we performed some recovery tests using pure standard solutions and a pesticide-free matrix (apple, in our case) to which we added the same pesticide amount. If we had obtained consistent recovery data, we could have carried out the following tests in the easiest way, i.e., recovery tests from standard solutions. We noticed that the recoveries in the two cases differed from each other and that a better recovery could be obtained from the standards added to the matrix: thus this technique was used in the subsequent tests. We did not investigate the reasons why that happened; we will look for an explanation in a further work.

Table 1 shows some recovery tests performed with both methods: for convenience we do not show all the cases, just some representative examples.

Table 1
Recoveries from pure standard and from standard+matrix

Pesticide	Recovery (%) pure std.	R.S.D. (%) ($n=3$)	Recovery (%) std. in matrix	R.S.D. (%) ($n=3$)	Detection
α-BHC	68	8.4	70	7.7	ECD
β-BHC	61	17.5	83	15.3	ECD
Dieldrin	56	18.1	74	13.2	ECD
Isofenphos	94	14.1	95	10.5	TSD

As observed from the examples considered, the recovery of some active components (α-BHC and isofenphos) showed no difference between pure standards and fortified matrices, whereas for the others (β-BHC and dieldrin) the extent of recovery differed in the two cases: therefore we decided to use the fortified matrix sample for the subsequent tests.

4.2. Supercritical fluid density

Since the first results proved contradictory – because some active components were recovered with good yields and reproducibility, while others showed poor and scarcely reproducible recoveries – we thought of adjusting the supercritical fluid density, i.e., its polarity, by trying tests at different densities. In this way we achieved some progress, as in the example shown in Table 2, which registers the recoveries for some of the pesticides which were problematic at first [20–22].

4.3. Supercritical carbon dioxide flow

Some tests were carried out varying the supercritical CO_2 flow. Only a slight increase was obtained by intensifying the supercritical CO_2 flow, which dissuaded us from insisting on searching optimal extraction conditions through this parameter [23].

4.4. Extraction temperature

As regards the extraction cell temperature, the trials showed a slight improvement of recoveries when the temperature increased to 45°C; no noticeable change being observed beyond that temperature, we set 45°C as extraction working temperature, without insisting too much on this parameter.

4.5. Extraction time and method

Particular attention was paid to the parameters extraction time and method (static and dynamic), by observing the plant protection products recovery according to the variation of parameters [24,25].

We expected that at first – when increasing the extraction time – the recovery would improve up to a maximum and then it would keep constant even for a longer extraction time. The actual results did not always correspond to our expectation.

As regards the static extraction time, we noticed that while a short static extraction gave a better yield, a longer extraction time scarcely affected the recovery percentages of the tested substances. Table 3 provides examples of that. Every static extraction was followed by a 10 min dynamic extraction.

Unlike static extraction, dynamic extraction brought about different situations after an initial increase of recovery percentages according to the extension of extraction time. In fact some pesticides behaved as expected, i.e., once they reached a maximum recovery after a certain extraction time, they did not show any remarkable variation at longer extraction times, while others – after reaching their maximum recovery – decreased their recovery as the extraction time lengthened. Table 4 shows examples of that.

Therefore we took 10 min as the most suitable dynamic extraction time.

An explanation of that behaviour could lie in the fact that the whole extraction probably takes place in a limited time and that keeping extracting CO_2

Table 2
Recoveries at different supercritical CO_2 densities

Pesticide	Density			
	0.6 g/l		0.8 g/l	
	Recovery (%)	R.S.D. (%) (n=3)	Recovery (%)	R.S.D. (%) (n=3)
Methamidophos	0	–	12	44.6
Terbuconazole	24	27.8	39	25.3
Triadimenol	10	32.3	60	22.7

Detection: TSD.

Table 3
Recoveries at different static extraction times

Pesticide	Static extraction time (min)						Detection
	0		1		10		
	Recovery (%)	R.S.D. (%) (n=3)	Recovery (%)	R.S.D. (%) (n=3)	Recovery (%)	R.S.D. (%) (n=3)	
Acephate	7	48.7	22	31.1	19	29.5	TSD
Azinphos ethyl	50	28.1	58	25.3	62	27.9	TSD
Azinphos methyl	65	39.1	74	31.4	71	30.3	TSD
Formothion	83	12.6	84	8.5	91	9.6	TSD
Heptenophos	74	19.4	88	13.7	89	12.0	TSD
Nuarimol	54	16.8	77	12.6	73	11.1	ECD
Phosalone	50	27.7	56	24.3	58	26.8	TSD
Pirazophos	44	31.5	60	34.1	55	36.0	TSD
Vinclozolin	55	19.9	67	14.7	62	21.3	ECD

through the trap may tend to make the previously deposited substances soluble again resulting in part of them being carried along by entrainment. This might happen in particular for more volatile pesticides.

4.6. Trap washing solvent

The choice of an absorption trap washing solvent was a particularly important one, since it is necessary to recover small volumes of pesticides having sometimes very different chemical characteristics [26].

In order to solve this problem we performed a series of trials with different solvents and mixtures of solvents. In particular, we noticed that the mixture hexane–acetone provided the best results as it combines the action of a non-polar solvent (hexane) with that of a polar solvent (acetone). After focusing on this mixture, we searched for the best ratio between the two solvents by performing an extraction series with mixtures at different ratios. We obtained the best results from a mixture hexane–acetone (1:1), which was then adopted. Table 5 shows the results of some of the tests.

4.7. Number of extraction steps

At this stage the results obtained by employing the above conditions were good for most of the plant protection products considered, with average recoveries over 80% and a good reproducibility. Nevertheless, some pesticides from the groups of phosphorothioates and phosphoraminodithioates – that show extraction and analysis difficulties also

Table 4
Recoveries at different dynamic extraction times

Pesticide	Dynamic extraction time			
	10		20	
	Recovery (%)	R.S.D. (%) (n=3)	Recovery (%)	R.S.D. (%) (n=3)
Heptenophos	88	13.7	83	10.9
Triadimenol	82	27.5	82	23.3
Buprofezin	75	19.9	72	26.4
Azinphos methyl	74	31.4	65	40.1
Azinphos ethyl	58	25.3	47	39.9
Pyrazophos	60	34.1	47	40.0
Procymidone	85	10.4	73	26.2

Detection: TSD.

Table 5
Recoveries for different hexane–acetone mixtures

Pesticide	Acetone in hexane (%)						Detection
	10		20		50		
	Recovery (%)	R.S.D. (%) (n=2)	Recovery (%)	R.S.D. (%) (n=2)	Recovery (%)	R.S.D. (%) (n=2)	
Heptenephos	77	18.5	84	14.1	97	17.0	TSD
Parathion ethyl	73	25.8	70	27.4	85	15.0	TSD
Procymidone	70	16.5	82	19.3	101	12.2	ECD
Quinalphos	74	31.0	81	27.3	92	20.6	TSD

with traditional extraction methods – showed a very low recovery and a scarce reproducibility. In particular, that happened for pesticides such as acephate, omethoate and vamidothion, highly polar compounds showing an average recovery lower than 20%.

We could have improved the extraction yield by adding some MeOH, which would have aided the polar compounds recovery. But we chose not to use that method, trying instead to work on the number of steps, since the aim of our work was to develop one extraction method which could be applied to a large number of pesticides without much modification, and which could therefore be applied to routine analyses. We worked on the number of extraction steps. In fact, by using two steps, i.e., two subsequent extractions on the same sample, we obtained recovery and reproducibility data which were comparable to those relating to the other active components. The two steps were like each other, except for the volume of trap washing solvent, which was 1.0 ml in the first extraction and 0.5 ml in the second one. The washing liquids were collected in the same vial, in order to obtain a single analysis sample.

It is useful to note that the two-step procedure is worth performing only if those pesticides which we called "difficult", the highly polar ones have to be assessed; in the other cases it is better to use the one-step method, both because of the quicker extraction, which implies sparing time and improving effectiveness, and because the two-step method produces a higher outcoming washing solvent volume, implying a lower concentration of analytes in the solution which in the end makes them less analytically perceptible [27].

Table 6
Recovery of organic chlorine derivative pesticides from spiked apple matrix

Pesticide	Detection	Recovery (%)	R.S.D. (%) (n=6)	Detection limit (μg/l)
Bromopropylate	ECD	101	4.1	0.36
Captafol	ECD	105	4.1	33.1
Captan	ECD	107	3.3	0.37
Chlorthalonil	ECD	131	10.3	0.05
Chlozolinate	ECD	97	9.0	0.09
Dichlofluanid	ECD	80	4.2	0.17
Difenconazole	TSD	88	10.1	19.3
Endosulfan sulph.	ECD	98	4.9	0.11
fenarimol	ECD	113	2.7	0.52
Folpet	ECD	110	2.6	0.61
Hesaconazole	TSD	92	10.9	51.2
Iprodione	ECD	104	3.3	0.55
Nuarimol	ECD	109	7.8	0.24
Penconazole	TSD	86	15.9	31.5
Procymidone	ECD	116	4.4	0.53
Tetradifon	ECD	93	7.5	0.34
Vinclozolin	ECD	95	10.6	0.12

4.8. Extraction parameters

After considering and assessing the different parameters involved in this extraction technique, we adopted the following procedure:

	Step 1		Step 1
Fluid type		CO_2	
Fluid density (g/l)		0.8	
Pressure (bar)		189	
Liquid flow-rate (ml/min)		2.5	
Extraction cell temperature (°C)		45	
Static extraction time (min)		1	
Dynamic extraction time (min)		10	
Trap type		ODS (octyl decylsilane)	
Nozzle temperature (°C)		60	
Trap temperature (°C)		45	
Extraction solvent in the trap		Hexane–acetone (1:1)	
Extraction solvent volume (ml)	1.0		0.5

4.9. Results

Ninety-two active components were extracted and subsequently assessed under the above conditions.

For easier reading Tables 6–11 report the pesticide groups, including the recoveries obtained, the amount of repetitions, the relative standard deviation (R.S.D.), the analytical technique used in the assessment, as well as the recovery data for the pesticides we called "difficult".

Table 7
Recoveries of common chlorinated pesticides from spiked apple matrix

Pesticide	Recovery (%)	R.S.D. (%) ($n=9$)	Detection limit (μg/l)
α-BHC	69	6.0	0.29
Aldrin	61	10.9	0.32
β-BHC	71	13.1	1.51
Dieldrin	61	17.6	0.29
Endrin	71	7.1	0.41
Heptacloro	56	14.9	0.39
Heptacloro epox.	64	7.2	0.26
Lindane[1]	68	6.0	0.26
o,p'-DDD	76	22.5	0.57
o,p'-DDE	71	8.1	0.35
o,p'-DDT	70	7.4	0.52
p,p'-DDD	74	10.8	0.63
p,p'-DDE	52	18.3	0.40
p,p'-DDT	72	16.5	1.22

Detection: ECD.
[1] $n=10$.

Table 8
Recoveries of organophosphorous and organonitrogenous pesticides from spiked apple matrix

Pesticide	Recovery (%)	R.S.D. (%) ($n=13$)	Detection limit (μg/l)
Aacephate	85	13.0	12.2
Azinphos ethyl	95	17.5	5.5
Azinphos methyl	104	24.4	8.2
Bitertanol	85	12.8	29.3
Buprofezin	90	13.5	15.6
Chlorfenvinphos	103	16.7	11.2
Chlormephos	80	11.3	7.8
Chlorpyr. ethyl	72	14.9	6.3
Chlorpyr. methyl	78	13.4	5.1
Cymoxanil	81	12.8	33.1
Cyproconazole	69	27.3	57.8
Demeton	121	14.7	2.3
Diazinon	82	12.1	4.1
Dimethoate	95	13.3	5.4
Fenitrothion	87	6.1	6.8
Formothion	88	7.6	5.6
Heptenophos	99	10.3	7.6
Isofenphos	103	12.9	8.9
Methamidophos	73	17.7	4.3
Methidathion	97	7.8	10.4
Methomyl	89	10.4	9.8
Myclobutanil	89	20.8	21.9
Ometoathe	98	11.2	12.6
Paraoxon	102	8.3	4.7
Parathion ethyl	80	12.3	6.3
Parathion methyl	90	11.9	4.6
Phosalone	85	17.7	6.7
Phosfamydon	107	9.9	9.5
Pirimicarb	92	9.1	11.1
Propiconazole	88	8.0	31.3
Propoxur	128	15.7	23.2
Pyrazophos	92	15.6	5.0
Pyridafenthion	103	9.1	7.4
Quinalphos	90	18.3	9.3
Tebuconazole	94	21.3	32.8
Triadimefon[a]	76	10.6	40.9
Triadimenol	94	19.4	56.4
Triclorfon	100	15.8	15.0
Vamidothion	117	17.9	18.3

Detection: TSD.
[a] Detection: diode array detector.

The above reported results were obtained by GC and HPLC techniques. The extracts obtained by SFE provided clear chromatograms, easy to evaluate and integrate. Some of the chromatograms obtained are also shown.

Given the high amount of organophosphorous and

Table 9
Recoveries of pyrethroid pesticides from spiked apple matrix

Pesticide	Recovery (%)	R.S.D. (%) (n=5)	Detection limit (μg/l)
Cyfluthrin	88	7.1	10.1
Cypermethrin	84	11.9	15.6
Deltamethrin	88	7.7	3.5
Fenpropathrin	91	9.3	2.9
Fenvalerat	86	19.8	8.2
Flucythrinate	90	6.5	9.6
Fluvalinate	90	7.8	11.3
Permethrin	90	7.5	10.1

Detection: ECD.

Table 10
Recoveries of other pesticides from spiked apple matrix

Pesticide	Recovery (%)	R.S.D. (%) (n=6)	Detection limit (μg/l)
Benzoximate	69	16.5	34.1
Carbaryl	89	5.3	17.2
Clofentezine	73	6.8	34.1
Diflubenzuron	88	12.8	48.6
Fenoxycarb	86	21.2	49.0
Flusilazole	91	6.2	75.6
Hexaflumuron	76	14.7	37.7
Hexytiazox	60	19.7	48.8
Propargite	66	14.9	98.8
Tebufenozide	76	19.6	55.7
Teflubenzuron	62	6.8	63.5
Triflumuron	73	8.7	68.8

Detection: diode array detector.

organonitrogenous derivatives, we thought it best for a clearer GC reading and interpretation to split the group into two subgroups, in order to avoid any possible interpretation problems.

Fig. 1 shows a chromatogram relating to the recovery of the first organophosphorous and organonitrogenous pesticide subgroup, obtained

Table 11
Recoveries of difficult pesticides from spiked apple matrix

Pesticide	Recovery (%)	R.S.D. (%) (n=13)	Detection limit (μg/l)
Acephate	85	13.0	12.2
Omethoate	98	11.2	12.6
Vamidothion	117	17.9	18.3

Detection: TSD.

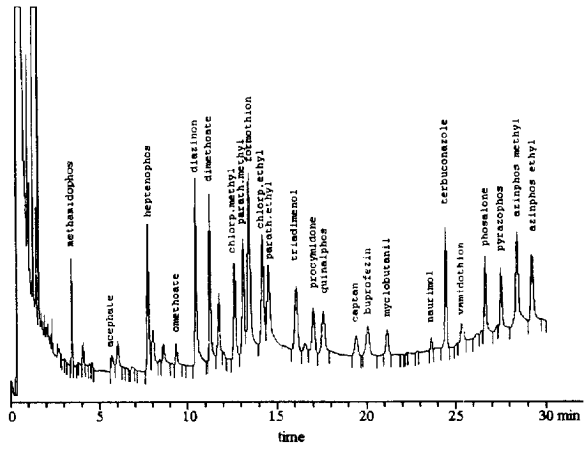

Fig. 1. Chromatogram of organophosphorous and organonitrogenous pesticides (first step).

through one step, while Fig. 2 shows the chromatogram relating to the second step recovery. As can be observed, the second-step chromatogram includes acephate, omethoate and vamidothion, which were hardly detected in the one-step recovery. It should also be considered here that the method we set up automatically combines the two recoveries, so producing one chromatogram; the two recoveries were observed separately only because of investigation reasons, in order to obtain the variation of recoveries through the steps.

Figs. 3 and 4, respectively show the chromato-

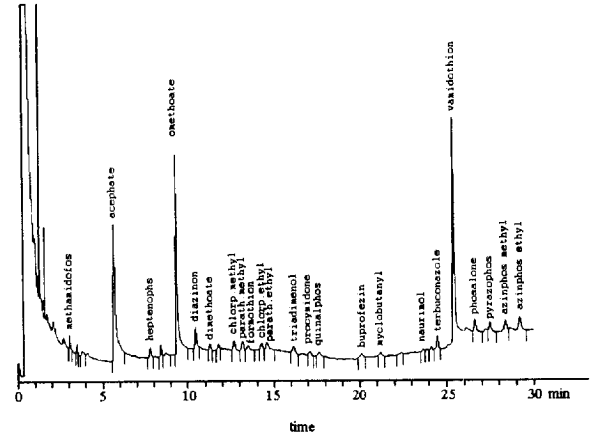

Fig. 2. Chromatogram of organophosphorous and organonitrogenous pesticides (second step).

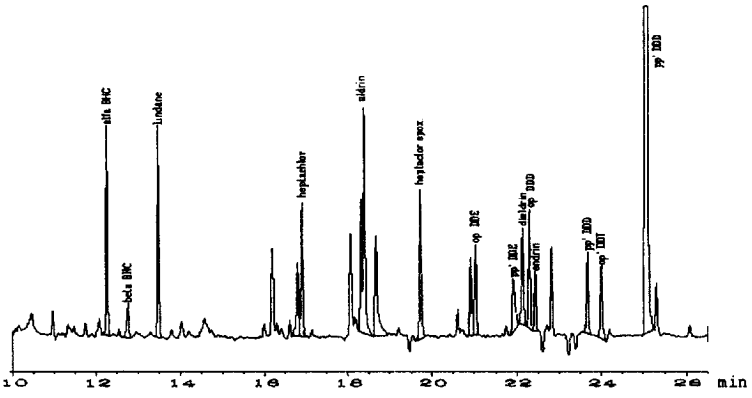

Fig. 3. Chromatogram of common chlorinated pesticides.

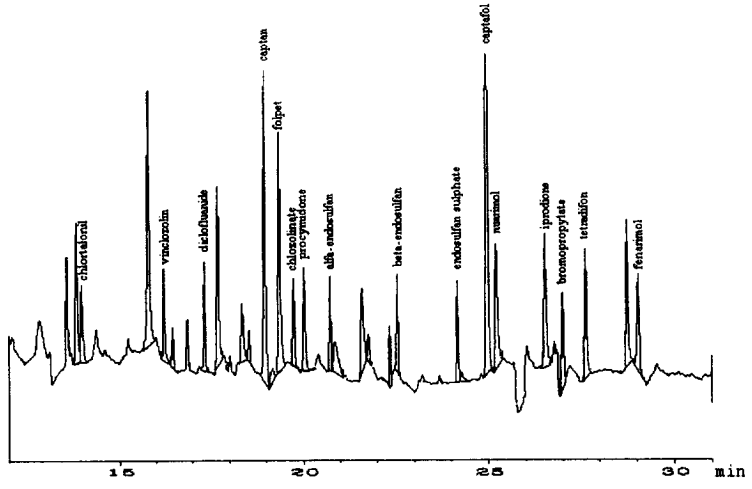

Fig. 4. Chromatogram of organic chlorine derivative pesticides.

grams relating to the common chlorinated pesticides and to the organic chlorine derivative pesticides.

5. Conclusions

The above results lead to the conclusion that the SFE technique, if properly used, may be a valuable support for those who are concerned with or work in the field of pesticide residues, as it allows the extraction of a large number of active components (92 in the present work) in a short time, with a good recovery and reproducibility and avoiding massive employment of expensive and dangerous organic solvents.

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