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Determination of pyrethroid pesticide residues in fatty materials by solid-matrix dispersion partition, followed by mini-column size-exclusion chromatography

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Abstract

The method studied uses a combination of a solid-matrix dispersion partition (SMDP) followed by high-performance size-exclusion chromatography on a minicolumn (HPmSEC) of 7.8 mm I.D. for the separation of pyrethroid (PYR) residues from fatty material. The solid-matrix dispersion extraction is carried out by absorbing a fat solution onto an Extrelut-3 cartridge (filled with a macroporous diatomaceous material) and extracting the PYR residues with acetonitrile. Up to 1 g of fatty material can be extracted with 15 ml acetonitrile. The small amount (mean±S.D.=12.4±5.9 mg) of fatty material which is eluted into the acetonitrile is further removed by HPmSEC. PYR pesticide residues are collected in a 2-ml fraction between 7 and 9 ml, the column being washed up to 24 ml. The two techniques used in series allow a better removal of fat, a greater input of sample and a lower consumption of solvent compared to the sole SEC on macrocolumns, and a lower limit of determination compared to the sole SEC on minicolumns. Recoveries of 9 PYR out of the 14 investigated residues from soya oil were in the range 66–83% at spiking levels ranging 0.49–2.57 mg/kg, while for 6 PYR residues tested at spiking levels in the range 0.13–0.53 mg/kg the recoveries were in the range 80–111%. Recovery of fluvalinate and permethrin could not be calculated due to interferences from soya oil, while λ-cyhalothrin, esfenvalerate and tralomethrin gave low recovery. The final extract contains small amount (mean±S.D.=2.4±0.9 mg) of lipid residue and is not completely free from interferences.

Keywords: Solid-matrix dispersion partition; Soya oil; Sample handling; Pyrethroids; Pesticides

1. Introduction

Synthetic pyrethroid (PYR) pesticides are widely used both in agriculture on a variety of fruits and vegetables, to protect stored commodities, and for the control of household, industrial and veterinary pests. PYR pesticides are lipophilic compounds and their extraction from fatty matrices, such as food of animal origin, oils, cereals and oilseeds, is accompanied by the simultaneous extraction of consider-

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able amounts of fatty material. Also the use of supercritical fluid extraction (SFE) and pressurized liquid extraction [PLE, Dionex trade name Accelerated Solvent Extraction (ASE)] cannot overcome this condition [1].

So, like for other lipophilic pesticide residues such as organochlorine (OCs) and organophosphate (OPs) pesticides in fatty extracts, the crucial step in the analytical procedure for their determination is the separation of the pesticide residues from the bulk of the lipidic material. To this task, two main approaches have been used, namely size-exclusion chromatography (SEC) and liquid–liquid partition (LLP).

SEC in recent years has become increasingly indicated for separation of the general group of pesticides residues from the crude fatty extracts [2–14], but some of the applications mainly consider the determination of OCs and OPs [4,6,9]. Hong et al. [11] reported the analyses of 25 pesticides in soybeans and rice, but no pyrethroids were considered. Other papers [2–4,6–10] deal with only a few pyrethroids, mainly fenvalerate and permethrin [3], resmethrin [4], permethrin, deltamethrin and cypermethrin [6], deltamethrin [7], *cis*-permethrin, cypermethrin and deltamethrin [8], deltamethrin [10]. SEC is normally carried out on Bio-Bead SX-3, in columns of 25 mm I.D. (macrocolumns), which are eluted at 5 ml/min, and a single analysis run requires large volumes of eluents in the order of 250 ml [2–4,6–10,12–14], not considering the time and solvents spent in the equilibration step. This fact as well as the need to maintain electrical equipment in good performance are significant drawbacks of the SEC techniques.

Furthermore, some of the above quoted references claim an efficiency for the removal rate of fat of 100% of butter fat in the separation from deltamethrin [7], 95–100% of wheat and rice fat in the separation from permethrin, cypermethrin, deltamethrin [6]. While other workers do not report quantitative information on separation of fat from pesticide residues or state that there is an excellent separation [2] or just present data in graphical form [5,11]. In any case, it is clear that the separation depends largely on the type of fat and pesticides. In the case of PYR residues, the fraction to be collected elutes earlier than other pesticides, such as the OC fraction, and almost together with the OP fraction. So, it appears that there is a significant overlap of the PYR fraction with the fat fraction and that there is need for further cleanup [6]. Indeed, also a 95% removal rate can leave some 25 mg of fatty residue when 0.5 g of fat is injected into the SEC macrocolumn. This amount of residue is not compatible with modern gas chromatographic injectors, capillary columns and detectors.

Adsorption chromatography on silica [4,9,12,13] alumina [3,6,10] Florisil [2,7,8] have been used before the determination by gas chromatography (GC) with electron- capture detection (ECD). Also SEC columns of 10 mm I.D. (minicolumns) have been indicated as useful for the said separation and for saving time and solvents [11,12]. However they can accept lower loads and this can impair the limit of determination. Also in this case, an insufficient separation (75%) [11] has been reported and the need for further clean-up was just overcome by the use of a selective determination, such as gas chromatography–mass spectrometry (GC–MS).

The second most important approach is liquid–liquid partition between immiscible solvents such as the classical *n*-hexane–acetonitrile partition followed by Florisil adsorption cleanup [1,15]. Determination without cleanup after partition was also considered feasible by using GC with ion trap mass spectrometry (GC–IT-MS) [16]. But, also here, there are no indications of the amount of fat remaining in the extract. Even if the GC–IT-MS can selectively detect the compound of interest, according to our experience with liquid–liquid partition, the amount of fatty material remaining can not be afforded by the GC system for more than a few injections.

In our laboratory, SEC techniques with a high-performance macrocolumn (see Section 2) have been tested and are in use for the determination of OC pesticide residues in fatty materials. When trying to extend the application of these techniques to the determination of PYR pesticide in oils, we found the techniques unsuitable due to the poor efficiency (70–75%) in fat removal. This finding agree with the indication of Krahn et al. [5] who, while proposing the column we tried, specify in a footnote that the use of two preparatory size-exclusion columns in series allows a greater margin for error in cutting fractions and results in a greater separation of

analytes from interferences. With this dual in-series column system, the flow was increased to 7 ml/min. These quite extreme conditions, the poor efficiency in the separation of PYR and the cost of such columns are indications that this techniques is not a routinely suitable tool at least for PYR pesticide residues determination.

In previous papers [17,18], we have demonstrated that the classical *n*-hexane–acetonitrile separatory-funnel partition can be carried out on solid-matrix cartridges with good efficiency in fat removal and recovery of OC and OP residues. Taking into considerations the drawbacks of SEC techniques and our positive experience with solid-matrix partition, we studied the possibility of extending the applicability of these partition techniques to the PYR pesticide determinations.

2. Experimental

2.1. n-Hexane–acetonitrile solid-matrix partition into acetonitrile with back-extraction into light petroleum

Reagents and materials used: analytical-reagent grade solvents: light petroleum (b.p. 40–60°C), *n*-hexane, isooctane and acetonitrile, redistilled from an all-glass apparatus; distilled water, extracted with dichloromethane (4 l with 2×100 ml CH_2Cl_2); sodium sulfate and sodium chloride, heated at 500°C for at least 6 h; Extrelut-3 cartridge (Merck, Darmstadt, Germany, cat. No. 15372); Extrelut-1 cartridge (Merck, cat. No. 15371); C_{18} Isolute, 40–60 mesh, International Sorbent Technology Part No. 9221-1000, obtained through StepBio, Bologna, Italy. Prepare cleaned C_{18} by washing 22 g of material, held in a 200×20 mm I.D. tube, with, in the order, 100 ml each of *n*-hexane, dichloromethane and methanol. Remove any remaining solvents by keeping the material in an Erlenmeyer flask in a desiccator under water-jet pump vacuum. Store the material in a brown glass bottle with a PTFE-lined rubber septum and screw cap.

Apparatus and glassware used: Rotary evaporator; Balance at ±0.01 g; Erlenmeyer flasks, pear shaped: 100 ml with ground glass neck; erlenmeyer flasks: 100 ml with PTFE-lined rubber septum and screw cap; Glass tube, without stopcock, 150×15 mm I.D. with a restriction 50×5 mm I.D.

2.1.1. Procedure

Dissolve 2 g of the fatty material with *n*-hexane making up the volume to 5 ml. Record the mass of the fatty material as P_1 and the mass of fatty material+solvent as P_{tot}. Weigh an Extrelut-3 cartridge. Transfer into the cartridge 2.5 ml of the fat solution. Calculate by difference the exact amount of solution transferred and record it as P_2. Calculate and record the fat portion transferred to the Extrelut-3 cartridge by P_1P_2/P_{tot}.

Let the solution drain into the cartridge and wait 10 min to obtain an even distribution into the filling material. Remove *n*-hexane by passing a stream of Nitrogen through the cartridge at 0.5 l/min for 30 min, from bottom to top. Empty an Extrelut-1 cartridge so as to leave only 1 cm height of the Extrelut material. Add into the Extrelut-1 cartridge 0.72 g of a 1:1 (w/w) mixture of Extrelut+C_{18} material. Position the Extrelut-1 tube under the Extrelut-3 cartridge. Prepare a 100 ml screw-cap erlenmeyer flask containing 100 ml distilled water and 5 g sodium chloride. Add 10 ml light petroleum and shake the flask until the sodium chloride is dissolved. Let the phases separate. Pipet and discard the light petroleum. Add 10 ml light petroleum into the flask and position it under the system of the combined cartridges. Elute the system of the combined cartridges with 3×5 ml portions of acetonitrile collecting the eluates into the 100 ml erlenmeyer flask. Cap the flask, shake, let the phases separate. Transfer the light petroleum phase into a column of 10 g anhydrous sodium sulfate (ca. 4 cm height in the glass tube 150×15 mm I.D.) collecting the solution into a 100 ml pear shaped erlenmeyer flask. Repeat the extraction with 2×10 ml portions of light petroleum passing each portion through the Na_2SO_4 column. Wash the Na_2SO_4 column with 2×5 ml light petroleum and collect washings in the same flask.

Concentrate the combined solvent to a small volume (ca. 1 ml) and then to dryness by manually rotating the flask by rotary evaporator (bath temperature, 40°C; reduced pressure).

2.2. Size-exclusion chromatography with 7.8 mm I.D. column (mini-SEC)

Reagents and materials used: dichloromethane (DCM), HPLC-grade or analytical-reagent grade redistilled from an all glass apparatus and filtered through Anodisc 47, 0.20 μm membrane filter; membrane filter, Anodisc 47, 0.2 μm, 47 mm diameter, Merck cat. 11460 obtained through Bracco, Milan, Italy; syringe filter, Anodisc 10, 0.2 μm, Merck cat. 1.11318 obtained through Bracco.

Apparatus and glassware used:

(i) HPLC apparatus composed of a LKB 2150 pump, a Rheodyne model 7125 injector equipped with a 1-ml loop and a Envirosep ABC (cross-linked styrene–divinyl benzene) column, 300×7.8 mm I.D., Phenomenex part No. 00H-3035 KO, with a Envirosep ABC pre-column, 50×7.8 mm I.D., Phenomenex part No. 03B-3035 KO [both obtained through Lab Service Analitica, Anzola Emilia (BO) Italy]. Conditions: column eluted with dichloromethane at 1 ml/min.

(ii) LKB 2212 Helirac Fraction collector.

(iii) A 1-ml microsyringe Hamilton model 1001 TLLSL, P/N 203240/00, obtained through Carlo Erba, Milan, code 0862.63240 with a 22 gauge needle, Hamilton type KF722, obtained through Carlo Erba, code 0862.91122.

(iv) Rotary evaporator.

2.2.1. Procedure

Dissolve the residue resulting from the partition step 2.1 with 0.6 ml of dichloromethane, washing the side walls of the flask. Keep the flask in upright position to let the solution collect at the bottom. Carefully aspirate all the solution with the microsyringe previously washed with dichloromethane and containing a 200 μl of dichloromethane plug. After the sample solution, aspirate another 300 μl of dichloromethane. Attach to the Luer tip of the microsyringe an Anodisc 10 syringe filter and discharge ca. 100 μl to prime the Anodisc filter. Then, inject into the HPLC apparatus and collect the fraction from 7 to 9 min. Wash the column up to 24 min and waste this fraction.

Concentrate the solution to a small volume (ca. 0.5 ml) by rotary evaporator (bath temperature, 40°C; reduced pressure). Manually rotate the flask until complete removal of dichloromethane from the extract. Add 1 ml of polychlorinated biphenyl (PCB) 153 at 0.05 μg/ml concentration in toluene, as internal standard (I.S.).

2.3. Preparation of spiked soya oil

The low level spiked samples were prepared by mixing ca. 10 g of oil and 100 μl of either PYR-A stock solution, or PYR-B stock solution, and diluting to 25 ml with *n*-hexane. The high level spiked samples were prepared similarly by mixing ca. 10 g of oil and 500 μl of either PYR-A stock solution or PYR-B stock solution, and diluting to 25 ml with *n*-hexane. The resulting spiking levels for the different pyrethroids are shown in Table 1.

2.4. Techniques used in method development

2.4.1. Size-exclusion chromatography with 21.2 mm I.D. column (macro-SEC)

Reagents and materials used: DCM HPLC-grade or analytical-reagent-grade redistilled from an all glass apparatus and filtered through 0.20 μm membrane filter; 5-ml glass syringe, Hamilton mod. 1005 TLL-SL, P/N 20326 0/00 obtained through Carlo Erba, code 0862.63260 with a 22 gauge needle, obtained through Carlo Erba type KF722 code 0862.91122; membrane filter, Anodisc 47, 0.2 μm, 47 mm diameter, Merck, code 11460, obtained through Bracco; syringe Filters: Anotop 10, 0.2 μm, 10 mm diameter, Merck, code 11318 obtained through Bracco.

Apparatus and glassware used: HPLC apparatus composed of a Shimadzu model 10 LC-10 AD pump, a Rheodyne model 7125 injector equipped with a 5-ml loop and a Phenogel 10 μm, 100 Å column, 300×21.2 mm I.D., Phenomenex part No. 00H-0642-PO with a Phenogel-10 μm precolumn, 50×7.8 mm I.D., Phenomenex part No. 003B-2090-KO (both obtained through Lab Service Analitica); conditions: column eluted with dichloromethane at 5 ml/min; LKB 2212 Helirac fraction collector; rotary evaporator.

2.4.1.1. Procedure

Dissolve the oil with dichloromethane to obtain a solution of ca. 0.1 g/ml. Inject 5 ml into the HPLC apparatus and collect the fraction from 12 to 16 min

Table 1
Concentrations of pyrethroids in mixtures A and B used in different steps and corresponding spiking levels

Mixture	Compounds	Concentration (μg/ml)							
		Stock solution PYR-A (Toluene)	PYR-A-GC (Toluene)	PYR-A-mSEC (CH_2Cl_2)	PYR-A-MSEC (CH_2Cl_2)	PYR-A-2 for spiking (*n*-hexane)		PYR-A-1 for spiking (*n*-hexane)	
						conc.	level 2 (mg/kg)	conc.	level 1 (mg/kg)
A	1-Tefluthrin	12.25	0.12	0.25	0.12	0.25	0.61	0.05	0.13
	2-Tetramethrin	51.40	0.51	1.03	0.51	1.03	2.57	0.21	0.53
	3-Cyphenothrin	25.30	0.25	0.51	0.25	0.51	1.27	0.10	0.26
	4-Cyfluthrin	19.50	0.20	0.39	0.20	0.39	0.98	0.08	0.20
	5-Flucythrinate	25.18	0.25	0.50	0.25	0.50	1.26	0.10	0.26
	6-Fluvalinate	30.41	0.30	0.61	0.30	0.61	1.52	0.12	0.31
	7-Deltamethrin	15.15	0.15	0.30	0.15	0.30	0.76	0.06	0.16
	PCB 153 (Internal St.)	5.00		0.05					
		Stock solution PYR-B (Toluene)	PYR-B-GC (Toluene)	PYR-B-mSEC (CH_2Cl_2)	PYR-B-MSEC (CH_2Cl_2)	PYR-B-2 for spiking (*n*-hexane)			
						conc.	level 2 (mg/kg)		
B	1-Bioallethrin	10.06	0.10	0.20	0.10	0.20	0.49		
	2-Fenpropathrin	10.00	0.10	0.20	0.10	0.20	0.49		
	3-λ-Cyhalothrin	10.56	0.11	0.21	0.11	0.21	0.51		
	4-Permethrin	20.00	0.20	0.40	0.20	0.40	0.97		
	5-Cypermethrin-*cis*	25.33	0.25	0.51	0.25	0.51	1.23		
	6-Esfenvalerate	10.10	0.10	0.20	0.10	0.20	0.49		
	7-Tralomethrin	15.62	0.16	0.31	0.16	0.31	0.76		
	PCB 153 (Internal St.)	5.00	0.05						

(60–80 ml). Wash the SEC system up to 28 min and discard. When multiple samples have to be run in sequence, each successive sample can be injected at time 18 min from the previous injection. That is, the wash time (18–28 min) of the previous sample is also the dump time for the following sample (0–12 min).

Concentrate the solution to a small volume (ca. 0.5 ml) by rotary evaporator (bath temperature, 40°C; reduced pressure). Manually rotate the flask until complete removal of dichloromethane from the extract.

Note: the collection window should be defined by running spiked oil samples and checked from time to time.

2.4.2. Cleanup with Si–CN cartridges

Activate a Si–CN cartridge (500 mg, 3 ml, Analytichem International, code 6133C3) with 2×2 ml of *n*-hexane, apply the sample residue dissolved in 1 ml *n*-hexane, elute the cartridge with either of the two following scheme.

Scheme A:
Fraction 1 1 ml *n*-hexane
Fraction 2 2×1 ml *n*-hexane–DCM (75:25)
Fraction 3 2×1 ml *n*-hexane–DCM (50:50)
Fraction 4 2×1 ml *n*-hexane–DCM (25:75)
Fraction 5 2×1 ml DCM
Fraction 6 2×1 ml DCM–acetone (90:10)

Scheme B:
Fraction 1 1 ml *n*-hexane
Fraction 2 2×1 ml *n*-hexane–acetone (97:3)
Fraction 3 2×1 ml *n*-hexane–acetone (95:5)
Fraction 4 2×1 ml *n*-hexane–acetone (90:10)
Fraction 5 2×1 ml *n*-hexane–acetone (85:15)
Fraction 6 2×1 ml *n*-hexane–acetone (80:20)

Carefully concentrate the fractions to dryness. Redissolve in 1 ml of the I.S. solution for the GC–ECD analysis.

2.4.3. Cleanup with alumina [6]

Pour 2 g of alumina (Merck 90, neutral, code

1077, without activation), in a glass column (300× 10 mm I.D. with a PTFE stopcock) and cover with a 1-cm layer of Na_2SO_4. Redissolve the residue in 1 ml of dichloromethane–hexane (70:30, v/v) and transfer to the column.

Elute the column with 3×5 ml of dichloromethane–hexane (70:30, v/v), using small portions of the solvent mixture to wash the flask and transfer to the column. Collect all the eluates from the first application of the sample.

Carefully concentrate the eluates to dryness. Redissolve in 1 ml of the I.S. solution for the GC–ECD analysis.

2.4.4. Cleanup with 5% deactivated alumina [3]

Activate 10 g of alumina (Merck 90, neutral, code 1077) to 205°C for 2 h. Cool in a desiccator. Mix 9.5 g of activated alumina and 0.5 g of distilled water. Shake occasionally for 3 h. Store in a desiccator and let stand overnight. Pour 2 g of alumina 5% deactivated in a glass column (300×10 mm I.D. with a PTFE stopcock) and cover with 1-cm layer of Na_2SO_4. Redissolve the residue in 1 ml of dichloromethane–*n*-hexane (15:85) and transfer onto the column.

Elute the column with 3×5ml of the solvent mixture using small portions to wash the flask and transfer onto the column. Collect the eluates from the first application of the sample. Carefully concentrate the eluates to dryness. Redissolve in 1 ml of the I.S. solution for the GC–ECD analysis.

2.4.5. Cleanup with 10% deactivated Florisil [19]

Activate 10 g Florisil at 130°C overnight. Mix 9 g of activated Florisil and 1 g of distilled water. Shake for 3 h occasionally. Store in a desiccator and let stand overnight.

Pour 1 g of 10% deactivated Florisil in a glass column (300×10 mm I.D. with a PTFE stopcock) and cover with a 1 cm layer of anhydrous sodium sulfate.

Redissolve the residue in 1 ml *n*-hexane and transfer to the column. Wash the flask with 3×0.5 ml of *n*-hexane. Elute the column with 5 ml dichloromethane–*n*-hexane (10:90, v/v) and collect. Change the receiver and elute with 10 ml dichloromethane–*n*-hexane (20:80, v/v). Concentrate separately the two fractions to dryness and redissolve in 1 ml of the I.S. solution for the GC–ECD analysis.

2.5. Gas chromatographic analysis

The analyses were carried out on a HP 5890 Series II Plus gas chromatograph equipped with two split–splitless injectors and two electron-capture detectors. The main column was a capillary column (15 m × 0.53 mm I.D., 1.5 μm) DB-1 (crosslinked Methyl Silicone Gum, JW code 1251012), used with a retention gap, 0.9 m × 0.53 mm I.D., thin-film coated fused-silica (HP code 19095-10050). The second column (used for confirmation) was a fused-silica capillary column (15 m × 0.53 mm I.D., 1.00 μm) DB-1701 (14% CNPrPhMe Siloxane, JW code 1250712), used with a retention gap, 0.9 m × 0.53 mm I.D., thin film coated fused-silica (HP code 19095-10050). The column oven temperature programme was as follows: 60°C (2 min), 10°C/min to 160°C, 3°C/min to 250°C, finally at 250°C (50 min), overall runtime was 92 min. The detector temperature was set at 300°C. The carrier gas was helium at a flow-rate of 5.05 ml/min (39.8 cm/s average linear velocity at 60°C, supplied in constant flow mode) for both columns. Column head pressure 2.1 p.s.i. (1 p.s.i.=6894.76 Pa) at 60°C. Injectors, with dual-tapered deactivated glass liners (HP, code 5181-3315) were used in splitless mode with a purge-off time of 60 s, at the operating temperature of 240°C. Split vent flow and septum purge flow were 15 ml/min and 3 ml/min, respectively. Nitrogen (the au×iliary gas) was supplied to each ECD system at a flow-rate of 57 ml/min. Quantitation was carried out through peak area comparison by the internal standard technique and a single-level calibration.

3. Results and discussion

In preliminary work we have tried to separate PYR pesticide fraction from soya oil used as model fat using HP-macro-SEC. Half gram of soya oil was injected as 5 ml×0.1 g/ml in CH_2Cl_2 (the SEC eluent). The elution profile of PYR pesticides was assessed by injecting 5.0 ml of the mixtures of standards PYR-A-MSEC and PYR-B-MSEC (for concentration see Table 1) and analysing by GC–

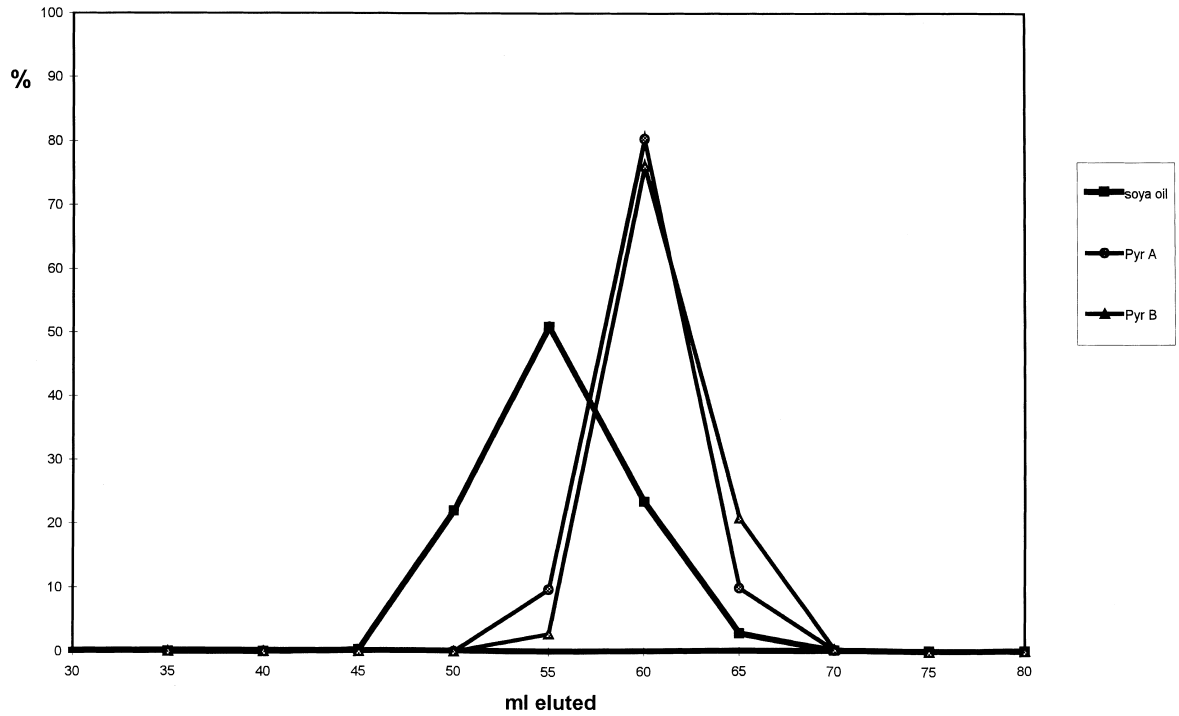

Fig. 1. Elution profiles of soya oil mass, and PYR-A-MSEC and PYR-B-MSEC solutions from macro-SEC column. Volumes injected=5 ml; total oil mass injected=0.5 g.

ECD the 5-ml fractions collected over 28 min (140 ml). The elution profile of oil was assessed by injecting 'blank' soya oil and weighing the residue in the fractions similarly collected. The elution profiles are shown in graphical form in Fig. 1 and in tabular form in Table 2. It appears a significant overlap of the fractions. The efficiency in removing the fat can be estimated in the order of 70–75% when the PYR fraction is collected from 50 to 65 ml. That means wasting 5–10% of PYR compounds in the oil

Table 2
Elution pattern of PYR-A-MSEC and PYR-B-MSEC mixtures, and soya oil from Macro-SEC column

Fractions		Soya oil residue (mg)	% of soya oil	% of PYR A	% of PYR B
number	ml				
1	0–30	0.0	0.0	0.0	0.0
2	30–35	0.1	0.0	0.0	0.0
3	35–40	1.5	0.3	0.0	0.0
4	40–45	125.5	22.0	0.0	0.0
5	45–50	290.3	50.9	9.7	2.8
6	**50–55**	**133.4**	**23.4**	**80.3**	**76.1**
7	**55–60**	**16.1**	**2.8**	**9.9**	**20.9**
8	**60–65**	**0.7**	**0.1**	**0.2**	**0.3**
9	65–70	0.7	0.1	0.0	0.0
10	70–75	0.4	0.1	0.0	0.0
11	75–80	0.3	0.1	0.0	0.0
12	80–140	1.2	0.2	0.0	0.0

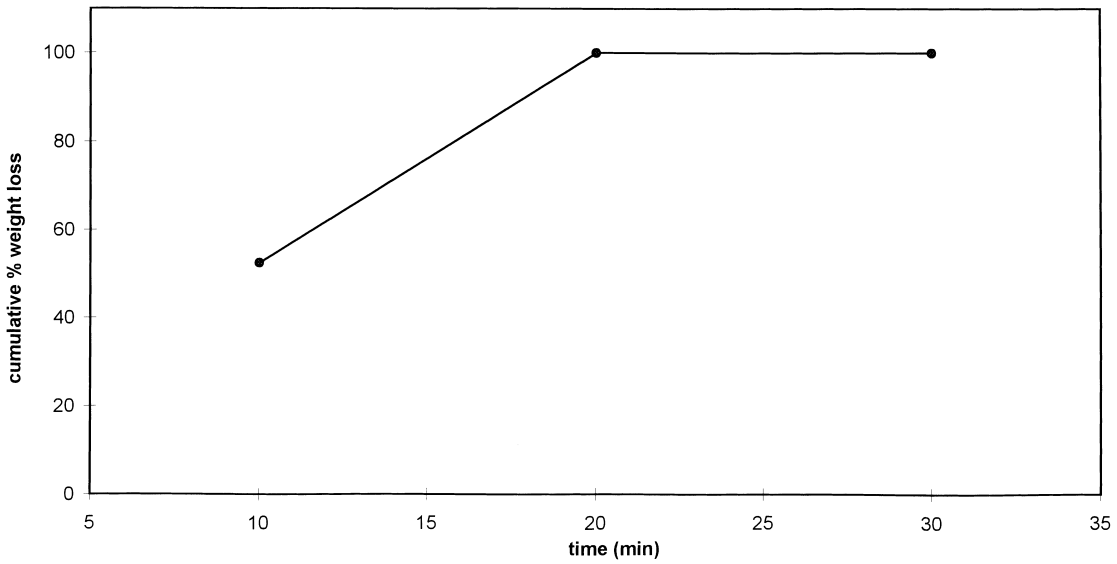

Fig. 2. Cumulative % (w/w) loss of an Extrelut-3 cartridge loaded with ca. 1 g soya oil in 2.5 ml *n*-hexane under a nitrogen flow of 0.5 l/min.

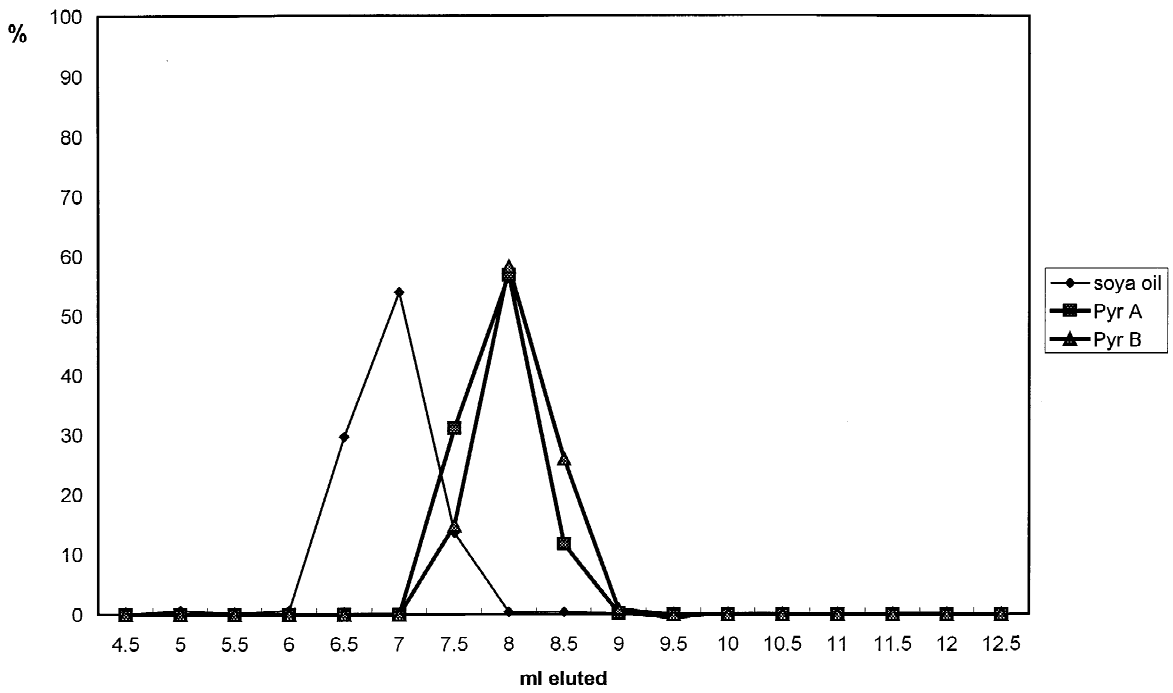

Fig. 3. Elution profiles of soya oil mass, and PYR-A-mSEC and PYR-B-mSEC solutions from mini-SEC column. Volumes injected=500 μl; total oil mass injected=50 mg.

fraction. It means also that some 150 mg of oil are collected in the PYR fraction, thus making it difficult to use any kind of further cleanup. It is noteworthy that the same column, used for separation of fat from OC pesticides, shows satisfactory separation, because the OC fraction is collected from 60 to 80 ml [20], while the PYR fraction elutes early (from 50 to 65 ml).

Thus, we have studied the applicability of the *n*-hexane–acetonitrile partition in the form previously reported by some of us [17,18]. The partition step uses Extrelut-3 cartridges (filled with a macroporous diatomaceous material) as a support for the *n*-hexane solution of the fatty material. The partitioning solvent (acetonitrile) is simply passed through the cartridges. A commercial plastic C_{18} cartridge was added downstream to increase the retention of oil. With this combination [18] of Extrelut-3+C_{18} cartridge, the oil carried in the acetonitrile eluate amounted to (average±S.D) 39±26 mg (n=14) when ca. 1.0 g of oil is partitioned on the cartridge. Despite this satisfactory performance, sometimes it was observed that minute droplets of *n*-hexane oil solution are displaced by the acetonitrile. This results in higher amounts of oil carried in the eluate. So, wishing to reduce this amount of oil, in this work we tried to remove the *n*-hexane from the cartridge by passing, from bottom to top, a stream of nitrogen at 0.5 l/min. The cumulative percent weight loss is shown in Fig. 2. It can be seen that almost all *n*-hexane is removed already after 20 min. The amounts of oil released by the sole Extrelut-3 from 1.0 g oil after 20 min and 30 min of nitrogen flow were (average±S.D) 54.1±8.6 mg (n=6) and 40.3±2.3 mg (n=7), respectively. So, we adopted 30 min as standard condition. Removal of *n*-hexane is an improvement compared to our previous method [18] because under these conditions, the sole Extrelut-3 partition has quite the same efficiency in removing fat as the previous combination of Extrelut-3+C_{18} cartridge with a lower S.D.

Wishing to further reduce the amount of oil released into the acetonitrile and to avoid the interferences from the plastic material of C_{18} cartridges, we decided to use a specially washed C_{18} material mixed 1:1, (w/w), with Extrelut material (as a flow aid) contained in a small glass tube, downstreem to the Extrelut-3 cartridge. Indeed, we obtained a further reduction of the oil carried into the acetonitrile eluate. The mean and standard deviation were 12.4±5.9 mg (n=25). Thus the average efficiency of the new arrangement in removing the fat can be estimated to be ca. 98.8%. This is an improvement compared to our previous application [18].

Table 3
Elution pattern of PYR-A-mSEC and PYR-B-mSEC mixtures, and soya oil from mini-SEC column

Fractions		Soya oil residue (mg)	% of soya oil	% of PYR A	% of PYR B
number	ml				
1	0–4.5	0.0	0.0	0.0	0.0
2	4.5–5.0	0.3	0.6	0.0	0.0
3	5.0–5.5	0.1	0.2	0.0	0.0
4	5.5–6.0	0.3	0.6	0.0	0.0
5	6.0–6.5	13.9	29.6	0.0	0.0
6	6.5–7.0	25.3	53.9	0.0	0.0
7	**7.0–7.5**	**6.4**	**13.6**	**31.1**	**14.9**
8	**7.5–8.0**	**0.2**	**0.4**	**56.9**	**58.3**
9	**8.0–8.5**	**0.2**	**0.4**	**11.8**	**26.0**
10	**8.5–9.0**	**0.1**	**0.2**	**0.2**	**0.8**
11	9.0–9.5	−0.3	−0.6	0.0	0.0
12	9.5–10.0	0.1	0.2	0.0	0.0
13	10.0–10.5	0.1	0.2	0.0	0.0
14	10.5–11.0	0.0	0.0	0.0	0.0
15	11.0–11.5	0.1	0.2	0.0	0.0
16	11.5–12.0	0.1	0.2	0.0	0.0
17	12.0–12.5	0.0	0.0	0.0	0.0

Table 4
Mean recovery values of seven PYR compounds from commercial soya oil spiked at two levels with PYR-A-1 and PYR-A-2 mixtures, respectively

Pesticides PYR-A	Retention time (min)	Spiking level 1 (mg/kg)	Recovery (%) (n=7)		Spiking level 2 (mg/kg)	Recovery (%) (n=4) Mean	S.D.
			Mean	S.D.			
1-Tefluthrin	17.553	0.13	111.3	37.1	0.61	65.7	7.3
2-Tetramethrin	31.994	0.53	102.2	22.6	2.57	82.5	6.1
3-Cyphenothrin	36.943	0.26	102.5	18.1	1.27	80.6	7.3
4-Cyfluthrin	39.631	0.20	89.9	21.2	0.98	73.6	4.2
5-Flucythrinate	40.855	0.26	85.8	17.2	1.26	76.6	5.9
6-Fluvalinate	44.305	0.31	[a]	[a]	1.52	154.0	7.4
7-Deltamethrin	45.443	0.16	80.2	20.9	0.76	70.5	7.3

Retention times are those obtained with the DB-1 column.
[a] Not quantified because of a high interference.

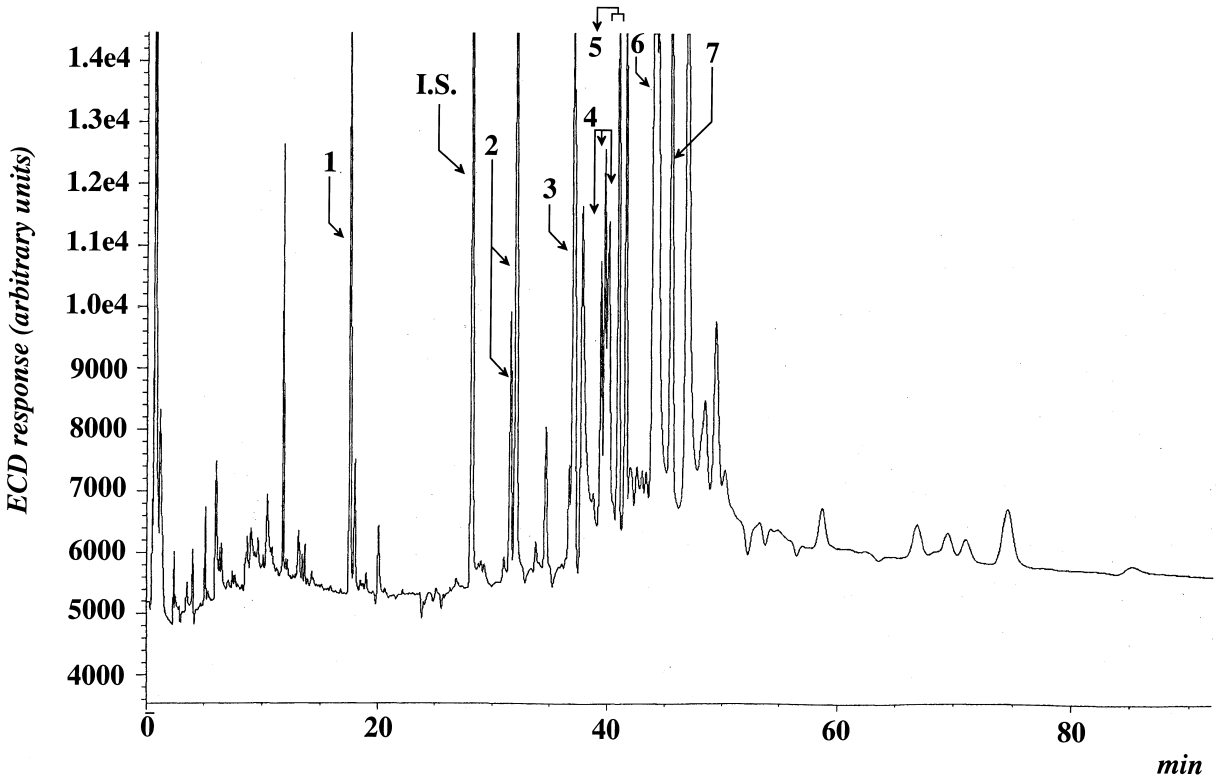

Fig. 4. Chromatogram of soya oil spiked at the higher level with PYR-A-2 (for spiking levels, see Table 1). Matrix concentration 1 g/5 ml, 1 μl injected. I.S.= PCB 153, 0.05 ng; 1=tefluthrin; 2=tetramethrin; 3=cyphenothrin; 4=cyfluthrin; 5=flucythrinate; 6=fluvalinate; 7=deltamethrin.

Table 5
Mean recovery values of seven PYR compounds from commercial soya oil spiked at one level with PYR-B-2 mixture

Pesticides PYR-B	Retention time (min)	Spiking level 2 (mg/kg)	Recovery (%) (n=5)	
			Mean	S.D.
1-Bioallethrin	23.157	0.49	80.3	11.6
2-Fenpropathrin	32.732	0.49	78.2	11.7
3-Λ-Cyhalothrin	35.657	0.51	33.0	4.5
4-Permethrin	37.898	0.97	155.2	60.6
5-Cypermethrin	40.715	1.23	72.6	11.2
6-Esfenvalerate	43.508	0.49	28.2	4.7
7-Tralomethrin	45.450	0.76	29.5	3.0

Retention times are those obtained with the DB-1 column.

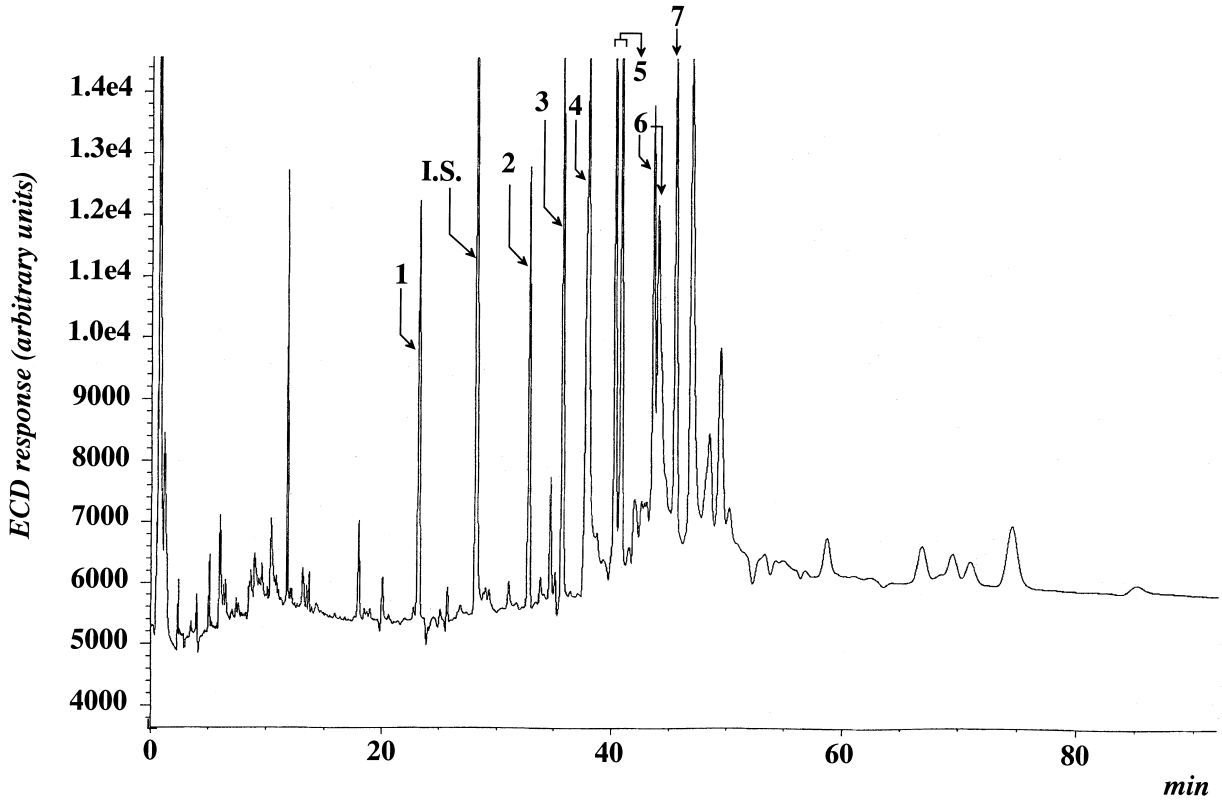

Fig. 5. Chromatogram of soya oil spiked at the higher level with PYR-B-2 (for spiking levels, see Table 1). Matrix concentration 1 g/5 ml, 1 μl injected. I.S.=PCB 153, 0.05 ng; 1=bioallethrin; 2=fenpropathrin; 3=λ-cyhalothrin; 4=permethrin; 5=cypermethrin; 6=esfenvalerate; 7=tralomethrin.

At this point we tried to remove the amount of oil remaining after SMDP+C_{18} by using the mini-SEC we have already reported [21]. The residue after the mini-SEC was an average of 2.4±0.9 mg (n=21). The efficiency of removal of fat by the sole mini-SEC can be estimated to be 80%. The overall removal of fat mass by the combination SMDP+C_{18} followed by mini SEC can be estimated to be an average of 99.8%. This efficiency is better than any reported cleanup for pyrethroid determination in fatty material.

The elution profile on mini-SEC of fat mass was assessed by injecting 500 μl of 0.1 g/ml solution of oil in DCM i.e., 50 mg of oil, an amount slightly in excess of that appears in the acetonitrile eluate. Similarly, the elution profile of PYR pesticides was assessed by injecting 500 μl of standard mixtures PYR-A-mSEC and PYR-B-mSEC (see Table 1), and analyzing by GC–ECD the 0.5 ml fractions collected up to 24 ml. Elution profiles are shown in graphical form in Fig. 3 and in tabular form in Table 3.

The recovery experiments were carried out at two levels with PYR-A-1 and PYR-A-2 mixtures and only at the higher level with PYR-B-2 mixture (for concentration see Table 1). The results are reported in Tables 4 and 5 for PYR-A and PYR-B mixtures, respectively. As can be seen from Table 4, the recovery values with PYR-A-2 (the higher level), ranged from 66 to 83% with S.D. in the range 4–7%. Fluvalinate gave 154% recovery because of an interference on one of the two peaks. As can be seen from Table 5 the recovery values with PYR-B-2 (the higher spiking level) ranged from 73 to 80% for three compounds with S.D. of about 11%. Permethrin showed a high recovery (155%) because of an interference while λ-cyhalothrin, esfenvalerate and tralomethrin gave unexpectedly lower recoveries. Both for PRY-A-2 and for PYR-B-2, recovery values did not improve significantly when the quantitation was carried out by using ‘matrix-matched calibrant solution‘, i.e. a calibrant solution containing the same amount of lipid extract as that resulting in the

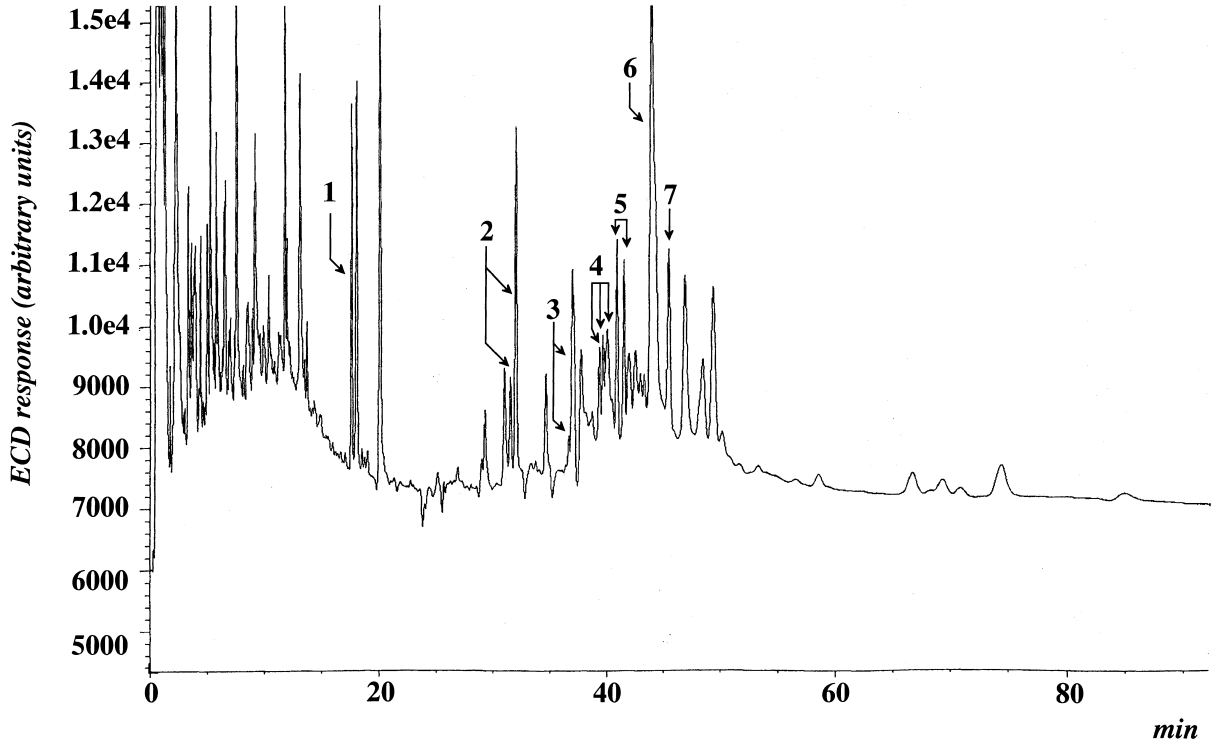

Fig. 6. Chromatogram of soya oil spiked at the lower level with PYR-A-1 (for spiking levels, see Table 1). Matrix concentration 1 g/ml, 1 μl injected. 1=tefluthrin; 2=tetramethrin; 3=cyphenothrin; 4=cyfluthrin; 5=flucythrinate; 6=fluvalinate; 7=deltamethrin.

spiked samples. Mean recovery values at the lower level with PYR-A-1 were satisfactory except for fluvalinate, but high variation was observed as demonstrated by the high S.D. (see Table 4). Chromatograms obtained from the higher spiking levels were reasonably clean and allow a fair quantitation (see chromatograms in Figs. 4 and 5), while the chromatogram obtained from the lower spiking level (see Fig. 6) shows the presence of significant peaks from the soya oil (see also the chromatogram of blank oil in Fig. 7) that prevent the quantitation of fluvalinate and permethrin, and disturb some others or the allocation of a reliable baseline. Also at the higher level the interferences prevent the quantitation of fluvalinate and permethrin. Typical chromatograms of standard compounds are reported in Figs. 8 and 9. Tralomethrin has the same retention time as deltamethrin, because it is converted into deltamethrin in the injection port, as previously reported by us [21]. It is noteworthy that some peaks from soya oil elute up to 85 min. Thus, the run time had to be set at 92 min to avoid peaks appearing in the next run.

A rough estimation of the limit of determination can be drawn from PYR-A-1 gas chromatogram (Fig. 6). It can be seen that at least one fifth of the height or area of these peaks can be measured that corresponds to about 0.05 mg/kg of oil. This value is in line with the lowest maximum residue limits (MRLs) set for a range of crops by the Italian and European legislation [22].

Indeed, even if a few milligrams of lipid material remain in the final extract, several interferences from soya oil should be further removed. The major interferences were identified as tocopherols and sterols by GC–mass spectrometry and library search against the Wiley Library of Mass Spectra.

Adsorption chromatography on alumina (either as received or 5% water deactivated) or Florisil (10% deactivated) were tried on the extract after mini-SEC in our effort to remove or reduce these interferences.

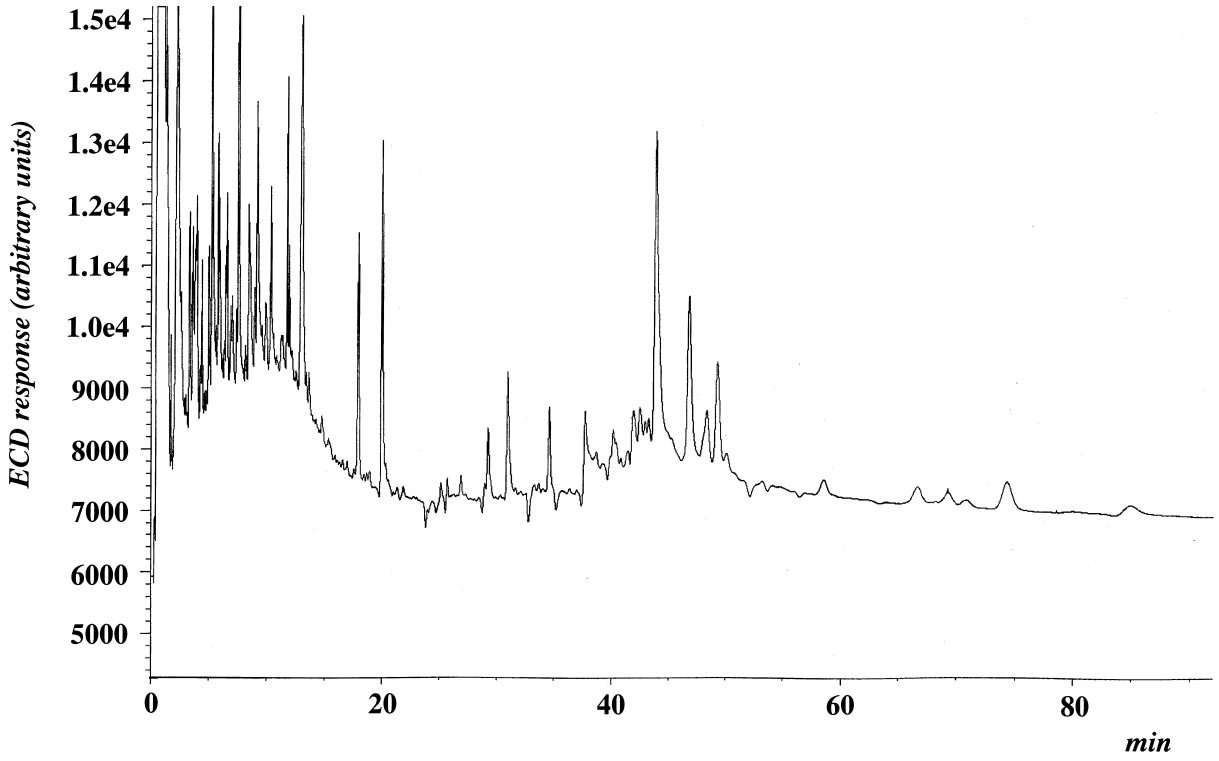

Fig. 7. Chromatogram of blank soya oil. Matrix concentration 1 g/ml, 1 μl injected.

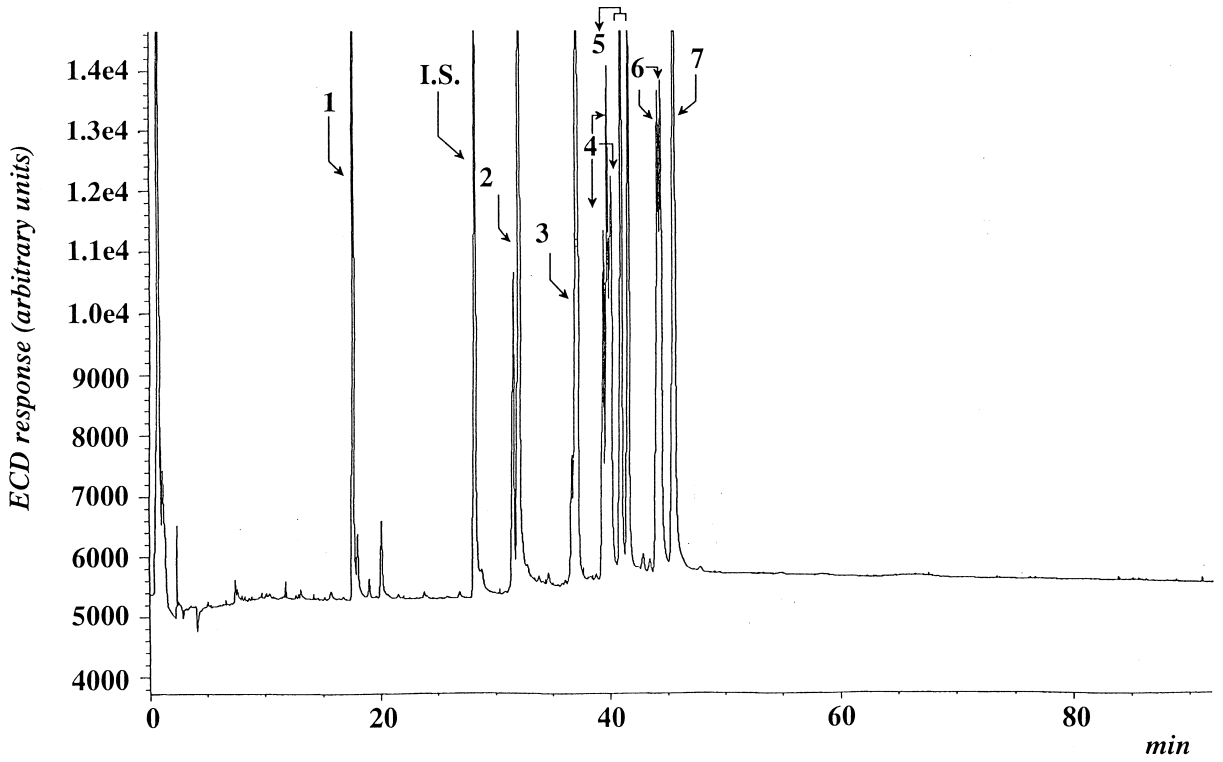

Fig. 8. Chromatogram of the standard mixture PYR-A-GC; (for concentrations, see Table 1). 1 μl injected. I.S.=PCB 153, 0.05 ng; 1=tefluthrin; 2=tetramethrin; 3=cyphenothrin; 4=cyfluthrin; 5=flucythrinate; 6=fluvalinate; 7=deltamethrin.

With the non-deactivated alumina all the pyrethroids in PYR-A were not eluted under the conditions used. With the 5% water deactivated alumina, tetramethrin was retained, while fluvalinate and the interference that prevented its quantitation were almost completely retained. Even if with alumina the fat residue was almost completely reduced, the problems of baseline allocation and incomplete removal of interferences still remains. With 10% water deactivated Florisil, tefluthrin was eluted in the first fraction and tethrametrin was retained, while the remaining compounds in PYR-A were eluted in the second fraction. SPE with Si–CN in normal-phase operation was tried on extracts obtained both after SMDP+C_{18} (without mini-GPC) and after SMDP+C_{18} followed by mini-SEC. Although it was possible to calculate the recovery values for PYR that were eluted, most of lipid extract was not separated by the PYR fraction.

With all the additional cleanups tried there was only a slight improvement of baseline.

Other possibilities of improving the cleanup should consider the use of water–acetonitrile mixtures in the partition step and the different eluent in the SEC.

4. Conclusion

The solid-matrix dispersion partition with in-line mixed Extrelut+C_{18} cartridge, followed by mini-SEC proved an efficient (99.8%) tool to get rid of the fatty matrix in the analysis of pyrethroids. The quantitation was acceptable for 9 out of 14 PYR tested, but several interferences still remain that prevent quantitation of, for instance, fluvalinate and permethrin. Low recoveries were misured with λ-cyhalothrin, esfenvalerate and tralomethrin. So con-

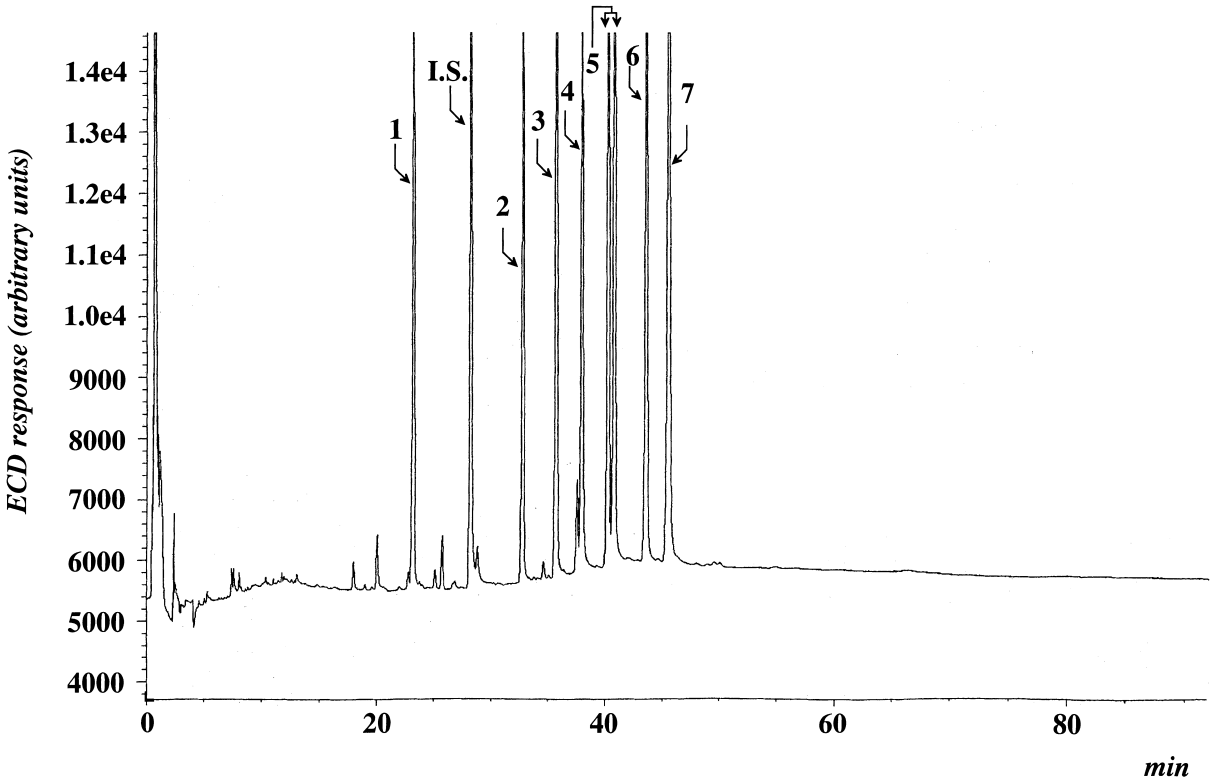

Fig. 9. Chromatogram of the standard mixture PYR-B-GC; (for concentrations, see Table 1). 1 μl injected. I.S.=PCB 153, 0.05 ng; 1=bioallethrin; 2=fenpropathrin; 3=λ-cyhalothrin; 4=permethrin; 5=cypermethrin; 6=esfenvalerate; 7=tralomethrin.

firmation and/or quantitation by GC–MS is recommended.

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