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Selective extraction of pyrethroid pesticide residues from milk by solid-matrix dispersion

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Abstract

A rapid procedure has been developed that allows a single-step, selective extraction and clean-up of pyrethroid (PYR) pesticide residues from milk dispersed on solid-matrix diatomaceous material filled into disposable cartridges and eluted by means of light petroleum saturated with acetonitrile and ethanol. The extract was cleaned up by high-performance size-exclusion chromatography. Determinations were carried out by gas chromatography with electron-capture detection. Recovery experiments were carried out on homogenized commercial milk (3.6% fat content) that was spiked with solutions of 14 PYR pesticides, viz., tefluthrin, tetramethrin, cyphenothrin, cyfluthrin, flucythrinate, fluvalinate, deltamethrin, bioallethrin, fenpropathrin, λ-cyhalothrin, permethrin, α-cypermethrin, esfenvalerate and tralomethrin, at levels ranging from 0.04 to 0.41 mg/kg for the different PYR pesticides. Average recoveries were in the range 60–119% for the different PYR pesticides, with relative standard deviations from ca. 2.5 to 14.4%. Coextracted fatty material amounted to an average of ca. 5 mg/ml of milk. The sole extraction step requires about 30 min. The main advantages of the procedure are that extraction of PYR pesticides (with a minimum carry over of fat) is performed in a single step, emulsions do not occur, several samples can be run in parallel by a single operator, reusable glassware is not needed and simple operations are required.

Keywords: Milk; Extraction methods; Food analysis; Pyrethroids; Pesticides

1. Introduction

Synthetic pyrethroid (PYR) insecticides are widely used because they are effective against many insect pests and show favourable selective toxicity towards insects and low toxicity to mammals and birds. Their main uses include field-treatment of crops and protection of stored products and hygienic treatments in houses and stable premises and on animals to control ecto- and endo-parasites [1]. Thus, despite the fact that modern pyrethroids can undergo a relatively rapid biotransformation and excretion in mammals compared to well-known persistent organochlorinated compounds, there are a number of ways in which they can reach milk. Among them, possible sources of contamination of milk are (i) foodstuffs containing high levels of PYR pesticide residues from post-harvest treatment; (ii) foodstuffs manufactured from plant material that has been treated during the growing season with insecticides; (iii) use of insecticides directly on the animal against disease vectors and (iv) use of insecticides against insects in stables and in milk processing factories.

Although ingested PYR pesticides are not always excreted as such in milk, nevertheless, some of the

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previously discussed routes can lead to the contamination of milk by parent PYR pesticides.

Indeed, maximum residue limits for PYR pesticides have been set by several organizations, such as FAO-Codex Alimentarius [2] and the European Union [3] (see Table 1), thus requiring adequate methodology for enforcement.

Several methods have been described for extraction and determination of PYR pesticide residues in vegetables [4–7], but only one paper [8] deals specifically with the determination of one pyrethroid (deltamethrin) in milk and butter.

The scheme of this method follows the conventional approach in use for the analysis of traces of lipophilic compounds, such as PYR pesticides, in fatty foods, i.e., extraction with acetone and partition into *n*-hexane to recover fat and pesticide residues, followed by separatory funnel partition between immiscible solvents to remove the major part of fat, and a final clean-up step by size-exclusion chromatography (SEC) before the determination by GC–electron-capture detection (ECD). Some drawbacks are common to most methods [9–12] based on this approach. They include (a) several separate steps for separate functions, such as extraction, separatory-funnel partition and/or SEC and clean-up of the extract before the final determination by GC–ECD; (b) time-consuming operations, such as concentration, transfer and solvent exchange; (c) the use of quite large volumes of solvents and glassware that has to be recovered and reconditioned.

Table 1
Maximum residue limit (MRL) in milk for some of the considered pyrethroids according to European Union legislation and FAO — Codex Alimentarius

Compound	MRL (mg/kg)	
	EU	FAO
Tefluthrin		
Tetramethrin		
Cyphenothrin		
Cyfluthrin	0.02[a]	0.01[a]
Flucythrinate		
Fluvalinate		
Deltamethrin		0.02
Fenpropathrin		
λ-Cyhalothrin	0.05	
Permethrin	0.05	0.1
α-Cypermethrin	0.02	0.05
Esfenvalerate	0.05	
Tralomethrin		

[a]At or about the limit of determination.

To overcome most of the drawbacks of conventional methods for the determination of pesticide residues in milk, we have discussed and reported, in two previous papers [13,14], the performances of a selective extraction of organochlorinated (OC) and organophosphate (OP) pesticide residues from milk. Briefly, the milk sample, mixed with acetonitrile and ethanol, is absorbed into a macroporous diatomaceous material filled in a disposable cartridge. OC and OP pesticide residues are recovered by eluting the cartridge with light petroleum saturated with acetonitrile and ethanol, with a minimum carry over of fatty material.

As PYR pesticides have a mobility on siliceous materials of the same order as that of most OP pesticides, we have investigated the possibility of a direct, selective extraction of PYR pesticide residues from milk and we report the results in this paper.

2. Experimental

2.1. Reagents and materials

Analytical-reagent-grade chemicals were used. Light petroleum (40–60°C), acetonitrile, ethanol, dichloromethane and toluene were redistilled from an all-glass apparatus.

Ready-to-use Chem Elut 1005 (AI-12198006) cartridges were obtained from Varian (Leini, Italy). Florisil, PR (60–100 mesh; Supelco cod. 2-0280) was from Supelchem (Milan, Italy). Rotary evaporator was from Buechi, Switzerland. The homogenizer was an Ultra-Turrax T25, IKA, with a S 25-8G dispersing tool. A 500 μl microsyringe, Hamilton model 750 SNR 80865, was also used. The micropipette used was a Transferpettor with fixed volume 50 and 100 μl capillaries from Brand, Germany (Nos. 7018 60 and 7018 73, respectively) obtained in Italy from Carlo Erba (Milan, Italy). The column used for adsorption chromatography was made of glass (300×10 mm I.D.) and had a PTFE stopcock.

Pyrethroid reference standards were kindly sup-

Table 2
Concentration (μg/ml) of standard mixtures for group A consisting of seven PYR pesticides used for either GC quantitation (in toluene) or for spiking milk (mg/kg) samples (in acetonitrile), and the corresponding spiking levels obtained by adding 4 ml of each solution+1 ml of acetonitrile (instead of 5 ml of acetonitrile) to 10 ml of milk

Pesticides Pyr A	Concentration for				
	GC determination (μg/ml)	Spiking			
		Pyr A-1 (μg/ml)	Level 1 (mg/kg)	Pyr A-2 (μg/ml)	Level 2 (mg/kg)
(1) Tefluthrin	0.12	0.12	0.05	0.25	0.10
(2) Tetramethrin	0.51	0.51	0.21	1.03	0.41
(3) Cyphenothrin	0.25	0.25	0.10	0.51	0.20
(4) Cyfluthrin	0.20	0.20	0.08	0.39	0.16
(5) Flucythrinate	0.25	0.25	0.10	0.50	0.20
(6) Fluvalinate	0.30	0.30	0.12	0.61	0.24
(7) Deltamethrin	0.15	0.15	0.06	0.30	0.12
I.S.=PCB 153	0.05				

plied by the main manufacturer of each pesticide (1). Single-compound stock solutions were prepared at about 1 mg/ml in toluene and working standard mixtures containing internal standard were prepared in toluene for GC–ECD quantitation, while standard solutions without internal standard were prepared in acetonitrile to produce the two spiking levels (see Table 2 for Pyr A and Table 3 for Pyr B). Internal standard solution: PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), 1.0 μg/ml in toluene, to be added to the sample extract before GC–ECD.

2.2. Apparatus

High-performance size-exclusion chromatography (HPSEC) was carried out with an apparatus composed of (1) a LKB 2150 HPLC pump; (2) a Rheodyne mod. 7125 injector equipped with a 500-μl loop; (3) an Envirosep ABC pre-column (50×7.8 mm I.D.), Phenomenex part 03B-3035 KO and an Envirosep ABC column (cross-linked styrene–divinylbenzene), 300×7.8 mm I.D., Phenomenex part 00H-3035 KO, both obtained in Italy through Lab

Table 3
Concentration (μg/ml) of standard mixtures for group B consisting of seven PYR pesticides used for either GC quantitation (in toluene) or for spiking milk (mg/kg) samples (in acetonitrile), and the corresponding spiking levels obtained by adding 4 ml of each solution+1 ml of acetonitrile (instead of 5 ml of acetonitrile) to 10 ml of milk

Pesticides Pyr B	Concentration for				
	GC determination (μg/ml)	Spiking			
		Pyr B-1 (μg/ml)	Level 1 (mg/kg)	Pyr B-2 (μg/ml)	Level 2 (mg/kg)
(1) Bioallethrin	0.10	0.10	0.04	0.20	0.08
(2) Fenproppathrin	0.10	0.10	0.04	0.20	0.08
(3) λ-Cyhalothrin	0.11	0.11	0.04	0.21	0.08
(4) Permethrin	0.20	0.20	0.08	0.40	0.16
(5) α-Cypermethrin	0.25	0.25	0.10	0.51	0.20
(6) Esfenvalerate	0.10	0.10	0.04	0.20	0.08
(7) Tralomethrin	0.16	0.16	0.06	0.31	0.21
I.S.=PCB 153	0.05				

Service Analitica (Bologna, Italy); (4) a fraction collector (LKB 2212 Helirac); fractions were collected in glass tubes on a time basis mode. The mobile phase was toluene with a flow-rate of 1 ml/min.

The GC analyses were carried out on a Hewlett-Packard 5890 series II plus gas chromatograph equipped with twin split–splitless injectors, twin columns and twin detectors (ECD). Injections were simultaneously carried out on both injectors with two autosamplers (HP 7673A). The columns used were: (1) a wide-bore, fused-silica column, DB-1, J&B (P/N 125-1012), 15 m×0.53 mm I.D., 1.5 μm film thickness; (2) a wide-bore, fused-silica column, DB 1701, J&B (P/N 125-0712), 15 m×0.53 mm I.D., 1.0 μm film thickness (used as the confirmatory column). Both were used with the protection of a retention gap, Hewlett-Packard (part 19095-10050), 0.9 m×0.53 mm I.D., thin film coated fused-silica. The temperature program of the column oven was as follows: 60°C, held for 2 min; increased at a rate of 10°C/min to 160°C, then at 3°C/min to 250°C, where it was held for 20 min (overall run time 62 min). The carrier gas, helium, was supplied in constant flow mode at 5 ml/min (set at an oven temperature of 60°C). Injectors, with bottom-tapered glass liners, were used in splitless mode with a purge-off time of 1 min, at the operating temperature of 240°C. Nitrogen (the auxiliary gas) was delivered to each ECD system at a flow-rate of 50 ml/min. The temperature of the detectors was set at 300°C.

2.3. Procedure

2.3.1. Extraction

In an erlenmeyer flask mix 10 ml of milk, 5 ml of acetonitrile and 1 ml of ethanol. Homogenize with an Ultra-Turrax for 3 min at 9500 rpm. Pipette 4 ml of this mixture into a Chem Elut CE 1005 solid-matrix, ready-to-use cartridge, allow it to drain and wait 10 min to obtain an even distribution. Attach a hypodermic needle (30×0.60 mm) to the column outlet as a flow regulator. Add to the column 5 ml of the upper phase (UP) obtained by equilibrating light petroleum–acetonitrile–ethanol (100:25:5, v/v/v). Wait 10 min, then elute with a further 4×5 ml of UP. Collect the eluates in a 50-ml erlenmeyer flask from the first addition of eluting mixture and concentrate to a small volume by rotary evaporation (40°C; 400 mbar), then to dryness by manually rotating the flask.

2.3.2. Size-exclusion chromatography clean-up

Dissolve the residue with 2 ml of dichloromethane, washing the side walls of the flask. Keep the flask in an inclined position to let the solution collect in a spot at the bottom of the flask on one side. Let the solution evaporate spontaneously. Dissolve the residue again with 450 μl of toluene, washing the area around the spot. Keep the flask in the inclined position to let the solution collect at the same spot. Carefully aspirate all the solution with a 500-μl microsyringe washed with toluene and containing a 50-μl toluene plug. Inject into the HPSEC apparatus and collect the fraction (2 ml) from 7.5 to 9.5 min, continue the elution to wash the HPSEC column up to 24 min. Add 50 μl of internal standard solution to the collected fraction and analyze by injecting 1 μl into the GC–ECD apparatus. Quantitation was carried out by peak area comparison and internal standard techniques with a single level calibration. Add the internal standard solution to the final extract of samples until the component–internal standard solution ratio is about the same in the sample as in the standard mixture used for calibration. In this case, problems arising from a possible lack of linearity of the ECD can be circumvented.

2.4. Recovery experiment

For recovery experiments, 4 ml of standard solution in acetonitrile, containing either Pyr A or Pyr B, plus 1 ml of acetonitrile (instead of the 5 ml of acetonitrile used in the sample preparation) are added to 10 ml of milk (Tables 2 and 3). After the homogenization step, keep the milk sample at room temperature for 3–4 h before proceeding with the above procedure, to mimic samples with incurred residues. Internal standard solution (50 and 100 μl) was added to the final extract for recovery experiments at levels 1 and 2, respectively, to keep the component–internal standard ratio at about the same value as that of the standard mixture used for calibration.

2.5. Adsorption chromatography on Florisil (used only in preliminary experiments)

Insert a small plug of cotton wool at the end of the void glass column and pour in 2.5 g of Florisil powder. Add 1 cm of anhydrous sodium sulphate. Wash the column with 3×5 ml of *n*-hexane and drain the solvent until the meniscus reaches the top of the sodium sulphate and then discard the wash. Quantitatively transfer the residue obtained at the end of the extraction step onto the column with 1 ml of *n*-hexane followed by 3×1 ml of the *n*-hexane used to wash the flask. Start collecting fractions. Elute the column according to the following scheme and collect five fractions: 1st fraction, 25 ml of *n*-hexane–benzene (80:20, v/v); 2nd fraction, 30 ml of *n*-hexane–benzene–ethyl acetate (180:19:1, v/v/v); 3rd fraction, 30 ml of *n*-hexane–benzene–ethyl acetate (176:19:5, v/v/v); 4th fraction, 20 ml of *n*-hexane–benzene–ethyl acetate (171:19:10, v/v/v); 5th fraction, 60 ml of *n*-hexane–benzene–ethyl acetate (171:19:10, v/v/v).

3. Results and discussion

Chem Elut 1005 cartridges are ready-to-use, disposable cartridges filled with a flux-calcined macroporous diatomaceous material with a nominal volume of 5. Following our previous findings with OC [13] and OP [14] pesticide residues, the extraction step of the described procedure consists of an on-column partitioning, carried out after dispersing the milk sample over the large surface area of the diatomaceous material. A relatively large volume of solvent is passed over a thin film of sample and, as a result, PYR pesticide residues are extracted efficiently along with a small amount of fatty material. The eluting solvent is essentially *n*-hexane saturated with acetonitrile and ethanol (the UP obtained by equilibrating *n*-hexane, acetonitrile and ethanol).

To test the suitability of the on-column extraction of PYR pesticide residues and of the eluting solvent, the extraction was first tested without the milk matrix, so that PYR pesticide could be analyzed by GC–ECD without any clean-up step, and it was found to be suitable. As found in previous experiments [13,14], there is a minimum carry over of fatty material from milk, of the order of ca. 5 mg/ml of milk (i.e. an average of ca. 13 mg per sample). So, a clean-up step had to be inserted before GC determination to remove the remaining fatty material.

Two alternative clean-up steps have been tried: adsorption chromatography on Florisil and HPSEC. Adsorption chromatography on Florisil follows the scheme adopted to clean up extracts containing OC pesticide residues [13], but a sequence of increasingly polar eluent mixtures was used to elute PYR. The elution behaviour of the considered compounds from the Florisil column was tested with 1 ml of either Pyr A-1 or Pyr B-1 standard solutions (same concentration as in Tables 2 and 3, but prepared in *n*-hexane) containing ca. 13 mg of milk fat residue from the Chem Elut 1005 extraction step. Fractions (10 ml) of each eluent mixture were collected. The presence of fatty material and PYR pesticides in each fraction was monitored by weighing the residues after evaporation and by GC–ECD, respectively. The elution behaviour of both fatty material and PYR pesticides is shown in Table 4. It appears that a small amount of fatty material is eluted in the third fraction where most of the PYR pesticides are recovered. Although the combined fractions of interest could be analyzed by GC–ECD without any appreciable disturbance of the baseline, we considered the small amount of fat remaining to be undesirable in GC analysis.

Therefore, we looked for clean-up steps that could remove the fatty material from the solution more efficiently, prior to GC–ECD determination. The best technique was that of size-exclusion chromatography, which was used in the mini preparative range in order to reduce solvent consumption. The elution profile of fatty material and PYR pesticides from the SEC column was studied by using artificial solutions containing ca. 13 mg of milk fat residue from Chem Elut 1005, dissolved in either Pyr A or Pyr B standard solutions.

Since there is a transfer involving solvent exchange between the extraction step and the HPSEC clean-up step, it is desirable that the volume of the new solvent (the mobile phase) is as large as possible, to wash completely the flask containing the raw extract. The maximum useful volume is mandated by the maximum volume accepted by the SEC column. For the size of the column used (7.8 mm

Table 4
Fractional elution of the two groups of pyrethroids (Pyr A and Pyr B) from 2.5 g of activated Florisil in the presence of ca. 13 mg of fat residue from commercial pasteurized whole milk samples

Pesticides	Recovery (%)				
	n-Hexane–benzene (80:20, v/v) 25 ml	*n*-Hexane–benzene–ethyl acetate (180:19:1, v/v/v) 30 ml	*n*-Hexane–benzene–ethyl acetate (176:19:5, v/v/v) 30 ml	*n*-Hexane–benzene–ethyl acetate (171:19:10, v/v/v)	
				20 ml	60 ml
Pyr A					
(1) Tefluthrin		88			
(2) Tetramethrin (a+b)					61
(3) Cyphenothrin			85		
(4a) Cyfluthrin			93		
(4b) Cyfluthrin			93		
(4c) Cyfluthrin			86		
(5a) Flucythrinate			92		
(5b) Flucythrinate			89		
(6a) Fluvalinate			86		
(6b) Fluvalinate			85		
(7) Deltamethrin			92		
Pyr B					
(1) Bioallethrin				97	
(2) Fenpropathrin			87		
(3) λ-Cyhalothrin			86		
(4) Permethrin		97			
(5a) α-Cypermethrin			86		
(5b) α-Cypermethrin			88		
(6) Esfenvalerate			93		
(7) Tralomethrin			89		
mg of fat		0.1	6.6	1.8	0.5

I.D.), it can be estimated to be approximately a few hundred microlitres. So, the influence of injection volume on the elution profile was studied by injecting 200, 300, 400 and 450 μl of the said artificial solutions, in which the amounts of both fat residue and PYR pesticide injected were kept almost constant. This was achieved by dissolving ca. 13 mg of fat residue in a series of either Pyr A or Pyr B standard solutions of different concentrations that were inversely proportional to the volume injected so that for each volume to be tested, the concentration of each pesticide multiplied by the volume injected is almost the same across all levels. It was observed that in the range of volumes studied, the separation of fatty material from the PYR pesticide fraction is almost equivalent and that volume injected has no effect. Thus, a volume of 450 μl of toluene was used to transfer the sample to the SEC column. Before transferring the sample with toluene, a low boiling solvent (dichloromethane) is used to collect the sample extract in a small corner of the container, so that a small volume of toluene (the mobile phase) is sufficient to quantitatively transfer the sample. The elution profile obtained by injecting 450 μl of Pyr A or Pyr B together with ca. 13 mg of fat residue is shown in Fig. 1. The compounds of interest are eluted in a small-volume fraction (2 ml) from 7.5 to 9.5 min.

To test the performance of the method, commercial pasteurized homogenized whole milk (3.6% fat content) samples were spiked with typical PYR pesticides. The compounds studied were not separated in a single run with either of the GC columns used. Tralomethrin and deltamethrin appear at the same retention time, due to the fact that tralomethrin is converted to deltamethrin at high temperature in

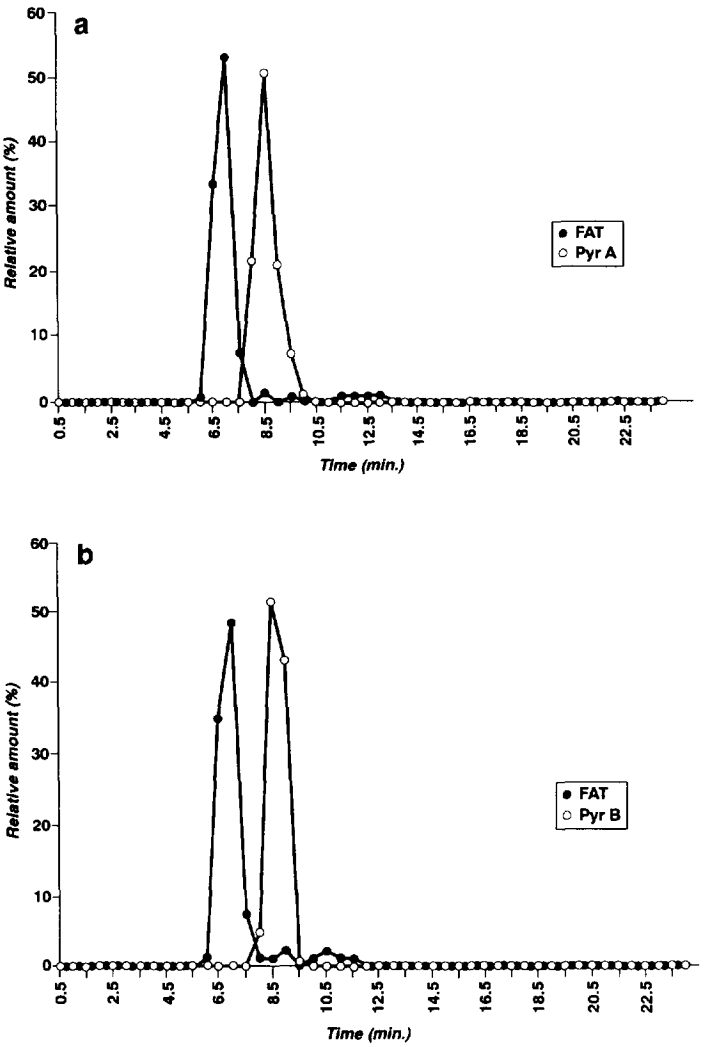

Fig. 1. Elution profile from the SEC column obtained by injecting 450 μl of Pyr A (a) and Pyr B (b) together with ca. 13 mg of milk fat residue from the extraction step.

the injection port, as we proved by GC–MS examination. Hence, the recovery experiments were carried out with two mixtures (Pyr A and Pyr B) comprising those pyrethroid compounds that could be separated in a single run. The recovery of PYR compounds from milk absorbed into the Chem Elut cartridge is carried out in a total volume of 25 ml (5×5 ml portions), as stepwise elution of the cartridges with 6×5 ml volumes showed that no elution of the studied compounds occurred in the sixth fraction.

Recovery experiments were carried out at least in quadruplicate at two spiking levels (scaled in the order 1:2) ranging from 0.04 to 0.41 mg/kg for the different compounds (see Tables 2 and 3). Analyses for the recovery experiments were carried out 3–4 h after spiking, in order to allow at least a partial entrapment of the residues in the milk matrix and to mimic samples with incurred residues. The results obtained are presented in Tables 5 and 6 for Pyr A and Pyr B standard solutions, respectively. Recoveries were in the range of 60–119%, with relative standard deviations (R.S.D.s) in the range of 2.5–14.4% and with no apparent differences between the spiking levels studied.

Table 5
Recovery values of seven PYR compounds from commercial pasteurized whole milk spiked at two levels with Pyr A-1 and Pyr A-2, respectively

Pesticides Pyr A	Retention time (min)	Spiking level 1 (mg/kg)	Recovery (%) (n=6)		Spiking level 2 (mg/kg)	Recovery (%) (n=4)	
			Mean	R.S.D.		Mean	R.S.D.
(1) Tefluthrin	19.958	0.05	64	13.4	0.10	73	5.2
(2a) Tetramethrin	34.588	0.21	113	4.2	0.41	114	7.7
(2b) Tetramethrin	35.034		119	10.6		111	8.4
(3) Cyphenothrin	39.945	0.10	88	5.4	0.20	93	2.5
(4a) Cyfluthrin	42.338	0.08	83	8.4	0.16	93	5.5
(4b) Cyfluthrin	42.648		75	5.5		93	4.3
(4c) Cyfluthrin	43.041		60	7.7		73	5.0
(5a) Flucythrinate	44.054	0.10	100	9.1	0.20	110	2.6
(5b) Flucythrinate	44.831		102	9.3		115	3.2
(6a) Fluvalinate	48.321	0.12	80	14.4	0.24	90	5.0
(6b) Fluvalinate	48.670		68	12.8		76	6.1
(7) Deltamethrin	50.310	0.06	95	8.6	0.12	98	5.7

Retention times were obtained by injecting the samples onto a DB-1 column.

This extraction procedure can be considered to be selective because the PYR pesticides, the analytes of interest in this work, are satisfactorily recovered with a minimum carry over of fatty material. Indeed, the amount of fatty material in the eluate (determined by weighing the residue after evaporation of the solvent) was found to be between 8.5 and 17.8 mg per 2.5 ml of milk loaded onto the Chem Elut 1005 cartridge, the average and standard deviations being 13.3±2.4 mg (n=48), corresponding to ca. 5 mg/ml of milk. This amount is in agreement with our previous work and compares favourably with the nominal total fat amount (ca. 90 mg) that would have been extracted using conventional total fat and residue extraction procedures. The final extract after HPSEC is almost free from fatty material and is compatible with the GC system used, i.e., an injector with a glass liner and a short retention gap. Impairment of the performance of the GC column did not occur during this work, in which some 300 non-spiked and spiked samples were injected, with the only normal maintenance operations being the removal of the rubber septum and the cleaning of the glass liner every 50–70 injections. Typical GC–ECD chromatograms obtained with the DB-1 column are shown in Figs. 2–7, for the blank method, "blank"

Table 6
Recovery values of seven PYR compounds from commercial pasteurized whole milk spiked at two levels with Pyr B-1 and Pyr B-2, respectively

Pesticides Pyr B	Retention time (min)	Spiking level 1 (mg/kg)	Recovery (%) (n=6)		Spiking level 2 (mg/kg)	Recovery (%) (n=4)	
			Mean	R.S.D.		Mean	R.S.D.
(1) Bioallethrin	26.047	0.04	93	5.7	0.08	102	4.5
(2) Fenpropathrin	35.796	0.04	87	5.1	0.08	91	4.3
(3) λ-Cyhalothrin	38.652	0.04	78	5.3	0.08	65	9.0
(4) Permethrin	40.945	0.08	76	10.7	0.16	75	4.8
(5a) α-Cypermethrin	43.278	0.10	77	8.7	0.20	66	6.5
(5b) α-Cypermethrin	43.896		79	5.3		74	7.0
(6) Esfenvalerate	47.647	0.04	87	6.4	0.08	74	7.5
(7) Tralomethrin	50.341	0.06	94	14.0	0.12	80	4.6

Retention times were obtained by injecting the samples onto a DB-1 column.

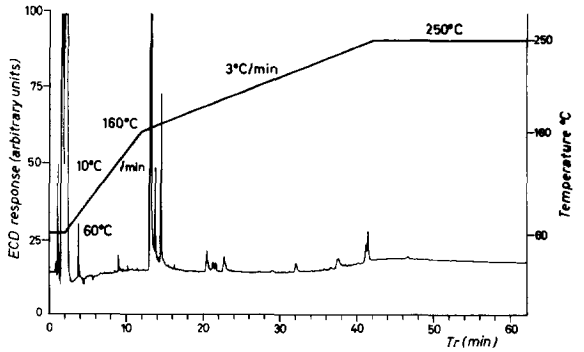

Fig. 2. GC–ECD chromatogram of the blank method on a DB-1 column (for conditions see Section 2).

milk, standard mixtures of the pyrethroids of groups A and B, and milk spiked at level 1 with Pyr A-1 and Pyr B-1 standard mixtures.

Indeed, in a manner that is different from the quoted methods, the described procedure performs both the extraction and a low-activity clean-up in a single step, giving a raw extract containing only a small amount of fatty material that can be easily cleaned up by using miniaturized steps. For the SEC clean-up step, only 24 ml of toluene per sample are needed. In terms of handling operations, also, the described procedure compares favourably with the conventional schemes in which the same functions are carried out through separate, time-consuming and labour- and glassware-intensive operations. It is noteworthy also that in sophisticated automated procedures, for instance the normal-phase HPLC clean-up of milk extract for OC determination [15], the preparation of fatty extract is a conventional

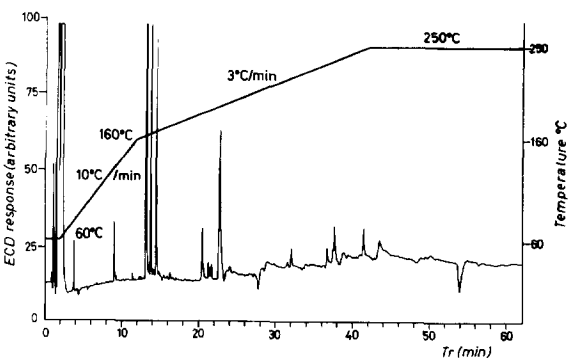

Fig. 3. GC–ECD chromatogram of "blank milk" on a DB-1 column (for conditions see Section 2).

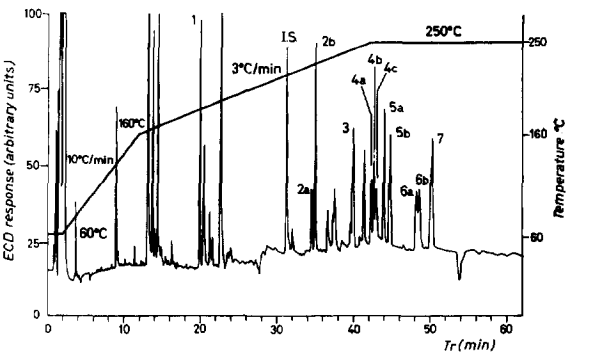

Fig. 4. GC–ECD chromatogram of whole milk spiked at level 1 with Pyr A-1: 1, 0.125 ng of tefluthrin (t_R=19.96 min); I.S., PCB 153, 0.05 ng (t_R=31.28); 2a, 0.525 ng of tetramethrin (t_R=34.59); 2b, 0.525 ng of tetramethrin (t_R=35.03); 3, 0.25 ng of cyphenothrin (t_R=39.95); 4a, 0.20 ng of cyfluthrin (t_R=42.34); 4b, 0.20 ng of cyfluthrin (t_R=42.65); 4c, 0.20 ng of cyfluthrin (t_R=43.04); 5a, 0.25 ng of flucythrinate (t_R=44.05); 5b, 0.25 ng of flucythrinate (t_R=44.83); 6a, 0.30 ng of fluvalinate (t_R=48.32); 6b, 0.30 ng fluvalinate (t_R=48.67); 7, 0.15 ng of deltamethrin (t_R=50.31).

off-line step that represents the bottle-neck of the entire analytical method. In contrast, the described procedure includes a straight extraction step that allows a high sample throughput, requiring only a few, simple handling operations.

Unlike the classical schemes, with our procedure,

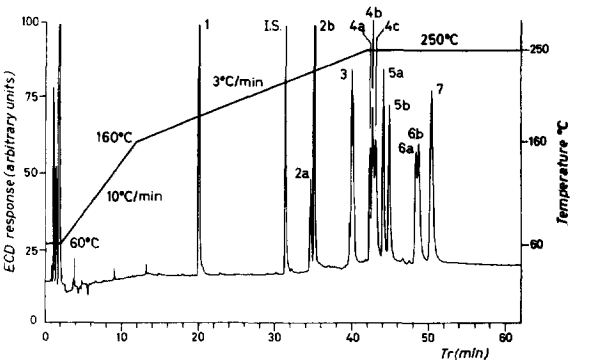

Fig. 5. GC–ECD chromatogram of a standard mixture for pyrethroids of group A: 1, 0.12 ng of tefluthrin (t_R=19.96 min); I.S., PCB 153, 0.05 ng (t_R=31.28); 2a, 0.51 ng of tetramethrin (t_R=34.59); 2b, 0.51 ng of tetramethrin (t_R=35.03); 3, 0.25 ng of cyphenothrin (t_R=39.95); 4a, 0.20 ng of cyfluthrin (t_R=42.34); 4b, 0.20 ng of cyfluthrin (t_R=42.65); 4c, 0.20 ng of cyfluthrin (t_R=43.04); 5a, 0.25 ng of flucythrinate (t_R=44.05); 5b, 0.25 ng of flucythrinate (t_R=44.83); 6a, 0.30 ng of fluvalinate (t_R=48.32); 6b, 0.30 ng of fluvalinate (t_R=48.67); 7, 0.15 ng of deltamethrin (t_R=50.31).

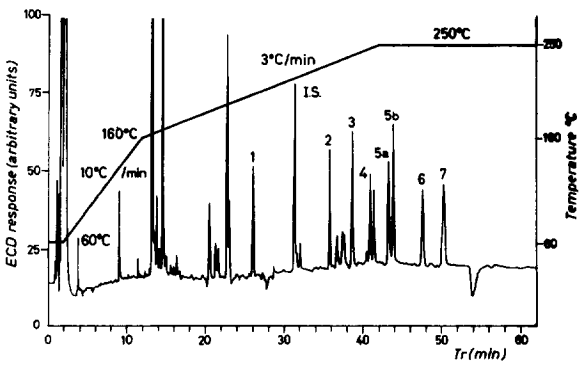

Fig. 6. GC–ECD chromatogram of whole milk spiked at level 1 with Pyr B-1: 1, 0.10 ng of bioallethrin (t_R=26.05 min); I.S., PCB 153 0.05 ng (t_R=31.28); 2, 0.10 ng of fenpropathrin (t_R=35.80); 3, 0.10 ng of λ-cyhalothrin (t_R=38.65); 4, 0.20 ng of permethrin (t_R=40.95); 5a, 0.25 ng of α-cypermethrin (t_R=43.28); 5b, 0.25 ng of α-cypermethrin (t_R=43.90); 6, 0.10 ng of esfenvalerate (t_R=47.65); 7, 0.15 ng of tralomethrin (t_R=50.34).

the extraction is rapid (ca. 30 min), emulsions do not occur, mainly disposable items are used, and small volumes of solvents and very few items of glassware are needed.

Compared to our procedure, the method specifically designed for the determination of deltamethrin [8] in milk samples has significant drawbacks, which are similar to those of the conventional procedure for fatty foods.

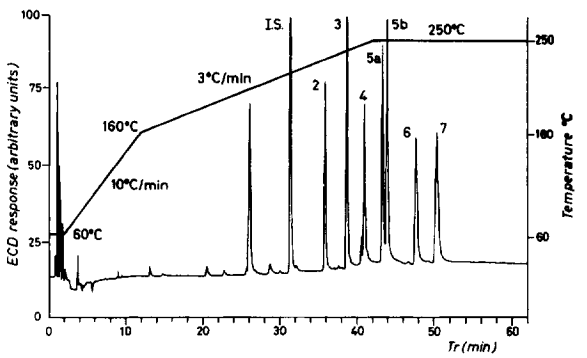

Fig. 7. GC–ECD chromatogram of a standard mixture for pyrethroids of group B: 1, 0.10 ng of bioallethrin (t_R=26.05 min); I.S., PCB 153 0.05 ng (t_R=31.28); 2, 0.10 ng of fenpropathrin (t_R=35.80); 3, 0.11 ng of λ-cyhalothrin (t_R=38.65); 4, 0.20 ng of permethrin (t_R=40.95); 5a, 0.25 ng of α-cypermethrin (t_R=43.28); 5b, 0.25 ng of α-cypermethrin (t_R=43.90); 6, 0.10 ng of esfenvalerate (t_R=47.65); 7, 0.16 ng of tralomethrin (t_R=50.34).

In conclusion, the main features of the described procedure are that, in a single step and with a minimum of glassware, solvents and reagents, a rapid selective extraction of PYR pesticide residues from milk can be carried out and that the clean-up is easily performed by HPSEC with a reduced consumption of solvent.

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