ELSEVIER

Journal of Chromatography A, 754 (1996) 497–506

JOURNAL OF CHROMATOGRAPHY A

Selective, solid-matrix dispersion extraction of organophosphate pesticide residues from milk

Alfonso Di Muccio[a,*], Patrizia Pelosi[a], Ivano Camoni[a], Danilo Attard Barbini[a], Roberto Dommarco[a], Tiziana Generali[a], Antonella Ausili[b]

[a]*ISS-Istituto Superiore di Sanità (National Institute of Health), Viale Regina Elena, 299, 00161 Rome, Italy*
[b]*ICRAM-Istituto Centrale per la Ricerca Applicata al Mare, Via Lorenzo Respighi, 5, 400197 Rome, Italy*

Abstract

A rapid procedure has been developed that allows a single-step, selective extraction and cleanup of organophosphate (OP) pesticide residues from milk dispersed on solid-matrix diatomaceous material filled into disposable cartridges by means of light petroleum saturated with acetonitrile and ethanol. Recovery experiments were carried out on homogenized commercial milk (3.6% fat content) spiked with ethanolic solutions of 24 OP pesticides, viz., ethoprophos, diazinon, dimethoate, chlorpyrifos-methyl, parathion-methyl, chlorpyrifos-ethyl, malathion, isofenphos, quinalphos, ethion, pyrazophos, azinphos-ethyl, heptenophos, omethoate, fonofos, pirimiphos-methyl, fenitrothion, parathion, chlorfenvinphos, phenthoate, methidathion, triazophos, phosalone, azinphos-methyl, at levels ranging for the different OP pesticides from 0.02 mg/kg to 1.11 mg/kg. Average recoveries of four replicates were in the range 72-109% for the different OP pesticides, with relative standard deviations (R.S.D.) from ca. 1 to 19%, while dimethoate and omethoate were not recovered. Coextracted fatty material amounted to an average of about 4.0 mg/ml of milk. The extraction procedure requires about 30 min. The main advantages are that extraction and cleanup are carried out 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; Food analysis; Extraction methods; Solid-matrix dispersion; Pesticides; Organophosphorus compounds

1. Introduction

Being at one of the highest levels of the trophic chain and due to its lipophilic nature, milk has been usually studied as an indicator of the bioconcentration process of environmentally persistent organic micropollutants, such as organochlorinated (OC) pesticides [1]. Although organophosphate (OP) pesticides are relatively less stable and persistent, there is 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 OP pesticide residues from post-harvest treatment or contamination, for instance by drift during commercial aerial application; (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; (iv) use of insecticides in stables (treatment against flies); (v) hygienic treatments against insects in milk processing factories.

The contamination of milk from source (i) and (ii) with OP pesticides depends on the stability of the compound, its mode of application, the duration of intake or exposure, and its metabolic fate in the animal. Contamination from source (iii) is more

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00535-3

important, especially in tropical countries where the use of insecticides (cattle dipping) is necessary to protect the health and productivity of the animals. Spraying of stables frequently leads to contamination of milking equipment, and treatment of factory premises against cockroaches and other insects may introduce significant quantities into the milk products. Although ingested OP pesticides are not always excreted as such in milk, nevertheless some of the previously discussed routes can lead to the contamination of milk by parent OP pesticides. Indeed, maximum residue limits for parent OP pesticides have been set by several organizations such as FAO-Codex Alimentarius [2] and European Union [3], thus requiring adequate methodology for enforcement.

As the occurrence of OC pesticide residues in milk has been extensively studied, a vast choice of methodologies is available for the determination of OC pesticide residues which are in principle applicable to OP pesticide residues determination.

Milk falls into the category of fatty foods and, in general, the analysis of OC as well as OP pesticide residues follows the conventional approach of the multi-matrix, multiresidue methods in which the total residues are extracted together with the total fatty material [4–9].

For milk, the bulk of fat together with lipophilic pesticide residues can be obtained by extraction with light petroleum–diethyl ether (1:1, v/v) with addition of potassium oxalate and ethanol [10], with *n*-hexane–acetone (1:1, v/v) [11], or with acetone extraction followed by partition into dichloromethane [8].

Raw extract solution is usually dried by passage through anhydrous sodium sulfate before concentration to obtain milk fat. Such procedures have some drawbacks, e.g., the amounts of solvents and glassware used, the number of manual operations involved, centrifugation after extraction and occurrence of troublesome emulsions, which sometimes are not easily controlled.

Further, pesticide residues need to be separated from the relatively large amount of milk fat so obtained before the sample extract can be amenable to the usual analytical techniques chosen for the determination, e.g. gas chromatography (GC). The extent of removal of fat and other unwanted coextracted material is governed by the characteristics of the analytical system (type of injector, columnn, detector).

Independently from the analytes searched for, the major part of coextracted fatty material is generally removed by liquid–liquid partitioning [5,12–17], size-exclusion chromatography [18–20] or sweep co-distillation [21,22].

The determination of OC pesticides by GC with electron-capture detection (ECD) requires a further cleanup, usually an adsorption chromatographic separation that, owing to the small difference in polarities, combines the most commonly sought OC pesticides in a single fraction [23]. On the contrary, for the OP pesticide residues determination, taking advantage of the selectivity of GC with flame photometric detection (FPD), less stringent cleanup is sufficient and no adsorption chromatographic cleanup is used unless one wants to obtain groups of pesticides in simplified fractions separated according to polarity.

To overcome the drawbacks of conventional methods, procedures have been reported in which there is no intermediate separation of total fat. The method of Toyoda et al. [24] reports extraction of OP pesticide residues with three portions of acetonitrile, removal of fat by zinc acetate addition and separation-funnel partition of OP pesticides from aqueous phase into dichloromethane. While there is no intermediate separation of fat and no cleanup, drawbacks of this methods are the quite large volume of solvents, the use of glassware (particularly separation funnels), reagents (sodium chloride, zinc acetate, sodium sulfate) and the number of operations (decantation, filtration, anhydrification).

Baynes and Bowen [25] reported the use of SPE C_{18} cartridges to isolate parathion-methyl and paraoxon-methyl from water-diluted milk, which were analyzed with no further cleanup. However, the applicability of the method has been demonstrated only with these two compounds and no data have been reported on the amount of coextracted matrix material appearing in the final extract. Furthermore, the eluate consisted of a two-phase aqueous system, and the aqueous phase has to be removed and discarded to let the organic phase containing the residues be concentrated and used for GC determination.

In a previous paper [26] we have shown that in contrast to the above mentioned conventional extraction procedures, selective extraction of OC pesticide residues, i.e., extraction of pesticide residues with a minimum of fat from milk is possible by distributing a mixture of milk, acetonitrile and ethanol over a macroporous diatomaceous material and eluting the columnn with light petroleum saturated with acetonitrile and ethanol.

In this paper we report the performance of this selective extraction procedure as applied to the determination of a number of OP pesticide residues in milk.

2. Experimental

2.1. Reagents and materials

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

Ready-to-use Chem Elut 1005 (AI-12198006) and Chem Elut 1010 (AI-12198007) cartridges were obtained from Varian, Leini, Italy. Rotary evaporator. Homogenizer, Ultra-Turax T25, IKA, with S 25-8G dispersing tool.

2.1.1. Reference standards

OP reference standards were from the collection in this laboratory and were kindly supplied by the main manufacturer of each pesticide. Primary, single-compound solutions were prepared at ca. 1 mg/ml in benzene. By sequential dilution, two working mixtures (Mix A and Mix B) for GC–FPD quantitation and four mixtures, for both group A and group B, to produce four spiking levels were prepared (see Table 1 for Mix A and Table 2 for Mix B, respectively). Internal standard solution: triphenylphosphate, 1.8 mg/ml in acetone.

2.2. Apparatus

The GC analyses were carried out on a Perkin-Elmer Model 8500 gas chromatograph equipped with a flame photometric detector operated in the P mode (interference light filter to pass the band around 596 nm). A wide-bore capillary, fused-silica columnn, SPB-608, 15 m×0.53 mm I.D.×0.83 μm film thickness was used. The temperature program of the columnn oven was as follows: 80°C (2 min.), 10°C/min to 150°C, then 5°C/min to 260°C and finally 260°C (20 min). The carrier gas was helium, supplied through a flow controller at 9 ml/min (set at room temperature). The injector, flash, glass lined, was set at 240°C. Nitrogen as auxiliary gas, hydro-

Table 1
Composition of standard mixture A consisting of 12 OP pesticides used for either GC quantitation or for spiking milk samples

No.	Pesticide	Retention time (min)	Concentration (μg/ml) for				
			GC determ.	Spiking levels			
				1	2	3	4
1	Ethoprophos	13.23	0.40	0.20	0.40	1.00	2.00
2	Diazinon	15.96	0.42	0.21	0.42	1.04	2.09
3	Dimethoate	17.00	0.81	0.41	0.81	2.03	4.07
4	Chlorpyrifos-ethyl	18.30	0.80	0.40	0.80	2.00	4.00
5	Parathion-methyl	18.73	0.80	0.40	0.80	2.00	4.00
6	Chlorpyrifos-ethyl	19.62	0.80	0.40	0.80	2.00	4.00
7	Malathion	19.94	1.50	0.75	1.50	3.75	7.50
8	Isofenphos	21.19	0.78	0.39	0.78	1.94	3.89
9	Quinalphos	21.92	0.79	0.39	0.79	1.97	3.95
10	Ethion	25.15	0.50	0.25	0.50	1.25	2.50
11	Pyrazophos	30.62	0.80	0.40	0.80	2.00	4.00
12	Azinphos-ethyl	32.14	1.00	0.50	1.00	2.50	5.00

Retention times were obtained with an SPB-608 column.

Table 2
Composition of standard mixture B consisting of 12 OP pesticides used for either GC quantitation or for spiking milk samples

No.	Pesticide	Retention time (min)	Concentration (μg/ml) for				
			GC determ.	Spiking levels			
				1	2	3	4
1	Heptenophos	12.96	0.40	0.20	0.40	1.00	2.00
2	Omethoate	14.90	4.04	2.02	4.04	10.10	20.20
3	Fonofos	16.20	0.60	0.30	0.60	1.50	3.00
4	Pirimiphos-methyl	19.26	1.00	0.50	1.00	2.50	5.00
5	Fenitrothion	19.74	1.00	0.50	1.00	2.50	5.00
6	Parathion	19.89	1.00	0.50	1.00	2.50	5.00
7	Chlorfenvinphos	21.63	2.06	1.03	2.06	5.15	10.31
8	Phenthoate	22.39	1.00	0.50	1.00	2.50	5.00
9	Methidathion	23.39	1.00	0.50	1.00	2.50	5.00
10	Triazophos	27.27	2.23	1.11	2.23	5.57	11.14
11	Phosalone	29.72	1.01	0.50	1.01	2.52	5.05
12	Azinphos-methyl	31.42	2.00	1.00	2.00	5.00	10.00

Retention times were obtained with an SPB-608 column.

gen and air flow-rates to the detector were set according to the manufacturers directions. The temperature of detector base was set at 300°C.

The GC analyses for fenitrothion and parathion were carried out on a Perkin-Elmer model 8700 gas chromatograph equipped with a flame photometric detector operated in the P mode. A wide-bore capillary, fused-silica column, SPB-1, 15 m×0.53 μm I.D.×0.53 μm film thickness was used. The temperature program of the columnn oven was as follows: 80°C (2 min.), 10°C/min to 150°C, then 3°C/min to 260°C, and finally 260°C (10 min). The carrier gas was helium, supplied through a flow controller at 9.8 ml/min (set at room temperature). The injector, flash, glass lined, was set at 240°C. Nitrogen as auxiliary gas, hydrogen and air flow-rates to the detector were set according to the manufacturer's directions. The temperature of the detector base was set at 300°C.

2.3. Procedure

In an Erlenmeyer flask, 10 ml of milk, 5 ml of acetonitrile and 1 ml of ethanol were mixed and homogenized with an Ultra-Turrax for 3 min at 9500 rpm. A 4-ml aliquot of this mixture was pipetted onto a Chem Elut CE 1005 solid-matrix, ready-to-use cartridge, which was allowed to drain with a further 10 min allowed to obtain an even distribution. A hypodermic needle, 0.60×30 mm, was attached to the columnn outlet as a flow regulator. A 5-ml volume of the upper phase (UP) obtained by equilibrating light petroleum–acetonitrile–ethanol (100:25:5)was added to the column. After 10 min, the column was eluted with a further 4×5 ml of UP. The eluates from the first addition of the eluting mixture were concentrated to a small volume by rotary evaporation (40°C; reduced pressure), then to dryness by manually rotating the flask. The residue was dissolved in 1 ml of internal standard solution and analyzed by injecting 1 ml into the GC–FPD apparatus.

For recovery experiments, 1 ml of an ethanolic solution of either Mix A or Mix B was added instead of 1 ml of ethanol. After the homogenization step, the milk sample was kept at room temperature for 3–4 h before continuing with the procedure described above.

If necessary, 8 ml of mixture of milk–acetonitrile–ethanol can be added onto a Chem Elut 1010 cartridge, which is then eluted with 5×10 ml portions of UP eluting mixture.

Quantitation was carried out by peak area comparison and internal standard techniques.

3. Results and discussion

Chem Elut 1005 and 1010 cartridges are ready-to-use, disposable cartridges filled with a flux-calcined macroporous diatomaceous material with a nominal volume of 5 ml and 10 ml, respectively. The described procedure consists of on-columnn partitioning on the macroporous diatomaceous material. The milk sample is dispersed over a large surface area. A relatively large volume of solvent is passed over a thin film of sample, and as a result, OP pesticides along with a small amount of fatty material are extracted efficiently.

To test the performance of the method, commercial pasteurized homogenized whole milk (3.6% fat content) samples were spiked with 24 typical OP pesticides. The compounds studied were not separated in a single run under either of the GC conditions adopted. Hence, the recovery experiments were carried out with two mixtures (Mix A and Mix B) of OP compounds that could be separated in a single run with SPB-608 columnn, which gave complete resolution of compounds included in either Mix A or Mix B except for parathion and fenitrothion. For the complete separation and quantitation of these two compounds, the SPB-1 columnn was used. Wide-bore capillary columns were used because they are well-recognized as giving the best compromise among factors such as resolution, sample capacity, ease of installation and handling.

Recovery experiments were carried out at least in quadruplicate at four spiking levels (scaled in the order 1:2:5:10) ranging for the different compounds from 0.02 to 1.11 mg/kg. The elution of OP compounds was carried out with a total of 25 ml (5×5 ml portions) after stepwise elution of the cartridges with 6×5 ml portions showed that no elution of the studied compounds occurred in fraction number 6. Analyses for recovery experiments were carried out 3–4 h after spiking in order to allow at least a partial entrainment of the residues in the milk matrix and to mimic samples with incurred residues. The results obtained are presented in Table 3 and Table 4 for Mix A and Mix B, respectively. Recoveries were satisfactory at all spiking levels ranging from 72–109% for all compounds, except omethoate and dimethoate, with standard deviation (S.D.) in the range 0.8–18.6%. Neither average recoveries nor standard deviations are correlated with the spiking level in the range studied. In some experiments, especially for azinphos-methyl and azinphos-ethyl, the S.D. is somewhat high. No clear explanation for this can be proposed, except perhaps that variability in packing density of the Chem Elut cartridges could have caused 'channelling' effects and variable recoveries. Omethoate and dimethoate are not recovered, which is predictable given the analytical conditions, such as adsorption properties of the solid matrix and low polarity of the eluting mixture, that do not favour the elution of polar, water-soluble compounds such as omethoate and dimethoate which, containing a carbamoyl group, are quite different from the other investigated compounds. To explore the extraction performance of the procedure from samples with incurred residues, two spiked milk samples were analyzed at $t=0$ days (i.e. as usual after 3–4 h from spiking) and at $t=14$ days. During this period of time the samples were kept at 4°C and rehomogenized before analysis. Results have been reported in Table 5 and do not show any significant differences. Longer periods were not explored on the basis of the possibility that the microbiological activity could degrade some of the OP compounds. In this experiment, fenitrothion, chlorpyrifos-ethyl and malathion were not quantified because spiking was carried out with a mixture used in preliminary experiments which contained the three compounds together. But, fenitrothion overlaps with chlorpyrifos-ethyl and is not fully resolved from malathion on SPB-608 columnn. Indeed, afterwards fenitrothion was included in Mix A, leaving chlorpyrifos-ethyl and malathion well resolved in Mix B.

The method detection limit (MDL) of the different compounds is reported in Tables 3 and 4 and was estimated on the basis of results for the lowest spiking level according to the formula reported by Baynes and Bowen [25]: $\mathrm{MDL}=t_{(0.99)}\times\mathrm{S.D.}$ where $t_{(0.99)}$ is the Student's 1-tailed t value at the 99% confidence level and with $(n-1)$ degrees of freedom, and S.D. is the standard deviation of replicate analyses.

The present procedure can be considered selective because the OP pesticides, the analytes of interest in this work, are satisfactorily recovered with a minimum carry over of fatty substances. Indeed, the amount of fatty material in the eluate was determined

Table 3
Recovery values of 12 OP compounds from commercial pasteurized whole milk spiked at different levels with Mix A

No.	Pesticide	MDL	Spiking level 1 (mg/kg)	Recovery % $n=5$		Spiking level 2 (mg/kg)	Recovery% $n=4$		Spiking level 3 (mg/kg)	Recovery % $n=4$		Spiking level 4 (mg/kg)	Recovery % $n=4$	
				mean	S.D.		mean	S.D.		mean	S.D.		mean	S.D.
1	Ethoprophos	0.007	0.02	89	9	0.04	90	17	0.10	87	6	0.20	90	4
2	Diazinon	0.003	0.02	88	4	0.04	94	13	0.10	75	4	0.21	86	4
3	Dimethoate	n.r.	0.04	n.r.	n.r.	0.08	n.r.	n.r.	0.20	n.r.	n.r.	0.41	n.r	n.r
4	Chlorpyrifos-methyl	0.003	0.04	88	2	0.08	91	12	0.20	88	2	0.40	93	2
5	Parathion-methyl	0.010	0.04	101	7	0.08	90	7	0.20	100	2	0.40	98	7
6	Chlorpyrifos-ethyl	0.007	0.04	89	5	0.08	93	14	0.20	88	1	0.40	89	5
7	Malathion	0.011	0.08	91	4	0.15	88	5	0.38	94	1	0.75	95	6
8	Isofenphos	0.009	0.04	95	6	0.08	91	14	0.19	91	4	0.39	96	6
9	Quinalphos	0.006	0.04	98	4	0.08	90	8	0.20	91	4	0.39	99	4
10	Ethion	0.005	0.03	90	6	0.05	87	4	0.13	88	2	0.25	91	5
11	Pyrazophos	0.007	0.04	96	5	0.08	92	5	0.20	96	4	0.40	101	3
12	Azinphos-ethyl	0.010	0.05	95	6	0.10	97	19	0.25	97	14	0.50	101	5

n.r.=not recovered.
MDL=Method Detection Limit=$t_{(0.99)}$×S.D. (mg/kg) of fat for lowest spiking level.
$t_{(0.99)}$=Student's 1-tailed t value at the 99% confidence level for $(n-1)=4$ degrees of freedom is 3.747.

Table 4
Recovery values of 12 OP compounds from commercial pasteurized whole milk spiked at different levels with Mix B

No.	Pesticide	MDL	Spiking level 1 (mg/kg)	Recovery % $n=4$		Spiking level 2 (mg/kg)	Recovery % $n=4$		Spiking level 3 (mg/kg)	Recovery % $n=4$		Spiking level 4 (mg/kg)	Recovery % $n=4$	
				mean	S.D.		mean	S.D.		mean	S.D.		mean	S.D.
1	Heptenophos	0.012	0.02	89	13	0.04	88	8	0.10	82	4	0.20	82	9
2	Omethoate	n.r.	0.20	n.r.	n.r.	0.40	n.r.	n.r.	1.01	n.r.	n.r.	2.02	n.r.	n.r
3	Fonofos	0.014	0.03	89	10	0.06	86	2	0.15	80	2	0.30	78	2
4	Pirimiphos-methyl	0.018	0.05	92	8	0.10	91	2	0.25	95	1	0.50	90	2
5	Fenitrothion	0.015	0.05	83	7	0.10	85	5	0.25	93	3	0.50	92	2
6	Parathion	0.016	0.05	84	7	0.10	91	6	0.25	93	1	0.50	96	2
7	Chlorfenvinphos	0.020	0.10	109	4	0.21	97	1	0.52	101	2	1.03	98	5
8	Phenthoate	0.007	0.05	72	3	0.10	79	8	0.25	89	3	0.50	93	2
9	Methidathion	0.010	0.05	94	5	0.10	95	4	0.25	101	1	0.50	96	9
10	Triazophos	0.026	0.11	95	5	0.22	101	3	0.56	102	2	1.11	98	7
11	Phosalone	0.012	0.05	97	5	0.10	96	4	0.25	97	10	0.51	96	4
12	Azinphos-methyl	0.066	0.10	85	15	0.20	90	4	0.50	94	6	1.00	95	15

n.r.=not recovered.
MDL=Method Detection Limit=$t_{(0.99)}$×S.D. (mg/kg) of fat for lowest spiking level.
$t_{(0.99)}$=Student's 1-tailed t value at the 99% confidence level for $(n-1)=3$ degrees of freedom is 4.541.

Table 5
Recovery values of 24 OP compounds from spiked commercial pasteurized whole milk analyzed at different times after spiking

No.	Pesticide	Standard conc. (μg/ml)	Spiking level (mg/kg)	$t=0$ days Recovery % $n=2$		$t=14$ days Recovery % $n=2$	
				mean	S.D.	mean	S.D.
1	Heptenophos	0.20	0.04	91.5	1.6	97.5	4.4
2	Omethoate	2.02	0.40	n.r.	n.r.	n.r.	n.r.
3	Fonofos	0.40	0.08	90.0	6.7	94.2	0.6
4	Pirimiphos-methyl	0.80	0.16	89.7	8.6	90.2	3.5
5	Fenitrothion	0.80	0.16	n.q.	n.q.	n.q.	n.q.
6	Parathion	0.80	0.16	92.7	5.0	98.8	10.0
7	Chlorfenvinphos	2.03	0.41	108.2	2.8	105.8	5.3
8	Phenthoate	0.80	0.16	84.5	1.4	86.1	23.7
9	Methidathion	0.80	0.16	110.0	10.4	99.2	7.0
10	Triazophos	1.67	0.33	81.6	1.0	95.5	9.4
11	Phosalone	0.80	0.16	102.4	7.9	91.9	6.4
12	Azinphos-methyl	1.00	0.20	109.8	5.7	105.0	8.7
13	Ethoprophos	0.32	0.06	82.4	6.6	98.3	10.8
14	Diazinon	0.42	0.08	84.4	3.9	84.0	17.5
15	Dimethoate	0.81	0.16	n.r.	n.r.	n.r.	n.r.
16	Chlorpyrifos-methyl	0.80	0.16	85.2	5.2	83.0	3.2
17	Parathion-methyl	0.80	0.16	90.6	3.8	103.5	8.3
18	Chlorpyrifos-ethyl	0.80	0.16	n.q.	n.q.	n.q.	n.q.
19	Malathion	1.50	0.30	n.q.	n.q.	n.q.	n.q.
20	Isofenphos	0.80	0.16	83.9	2.4	87.1	4.7
21	Quinalphos	0.79	0.16	85.9	5.1	90.8	3.6
22	Ethion	0.60	0.12	78.6	4.8	79.9	5.0
23	Pyrazophos	0.80	0.16	79.3	2.3	94.3	3.8
24	Azinphos-ethyl	1.00	0.20	89.2	11.8	102.9	2.6

n.r.=not recovered.
n.q.=not quantified.

by weighing the residue after evaporation of the solvent and was found between 6.4 and 18.8 mg per 2.5 ml of milk loaded onto the Chem Elut 1005 cartridge, the average and standard deviation being 9.8±2.6 mg ($n=39$), corresponding to ca. 4.0 mg/ml of milk. This amount compares favourably with the nominal total fat amount (ca. 90 mg) that would have been extracted with conventional total fat and residues extraction procedures. The concentration of fatty material in the final solution ready for GC–FPD determination was ca. 10 mg/ml, that is some 10 μg per 1 μl injection. This amount of coextractives is compatible with the GC system used, i.e. injector with glass liner and a short length of retention gap. Impairment of the performance of the GC columnn was not observed during all this work that required some 300 injections of non-spiked and spiked samples, 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–FPD chromatograms obtained with the SPB-608 column are shown in Figs. 1 and 2. In the chromatograms of non-spiked milk samples (Fig. 1a) no interfering peaks appear. Peaks A, B and C are compounds from the cartridge. Separate analyses of the diatomaceous material and the plastic void tube showed that peaks A and B come from the diatomaceous material, while peak C comes from the tube material. Peak B was identified by GC–MS as tri-*n*-butyl phosphate by searching the Wiley 138 Library of electron impact mass spectra. Peak A and peak C have not been conclusively identified, but may be supposed to be plasticizers.

Chromatograms of spiked milk (both Figs. 1 and 2) were indistinguishable from those obtained with the standard solution of pure pesticides.

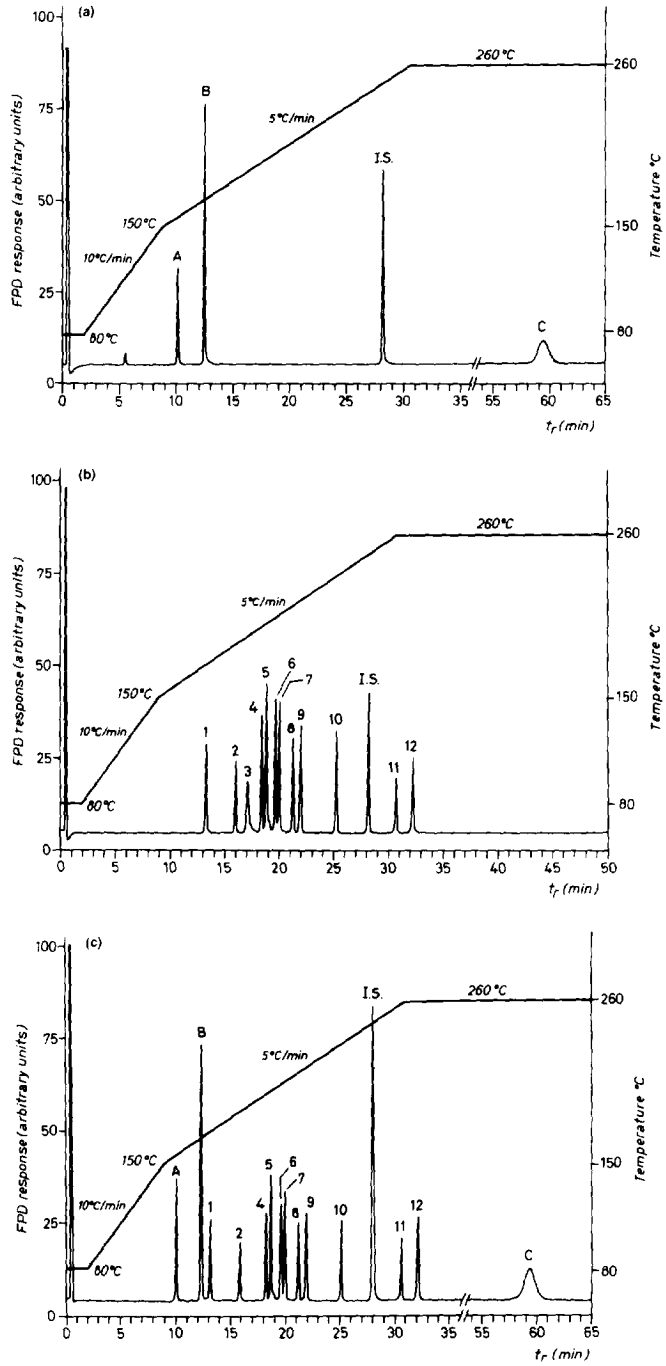

Fig. 1. GC–FPD chromatograms on SPB-608 column by injecting 1 μl of: (a) extract (see procedure) of non-spiked milk sample. (b) standard Mix A, for numbering and concentrations see Table 1; I.S.=triphenylphosphate 1.44 ng. (c) extract (see procedure) of a whole milk sample spiked with standard Mix A at spiking level 3 (see Table 3); Peaks: A=unknown peak; B=tri-*n*-butylphosphate; peaks 1 through 12 see Table 3; I.S.=triphenylphosphate 1.8 ng; C = unknown. t_R (min): A=10.1; B=12.4; I.S.=28.1; C=59.4.

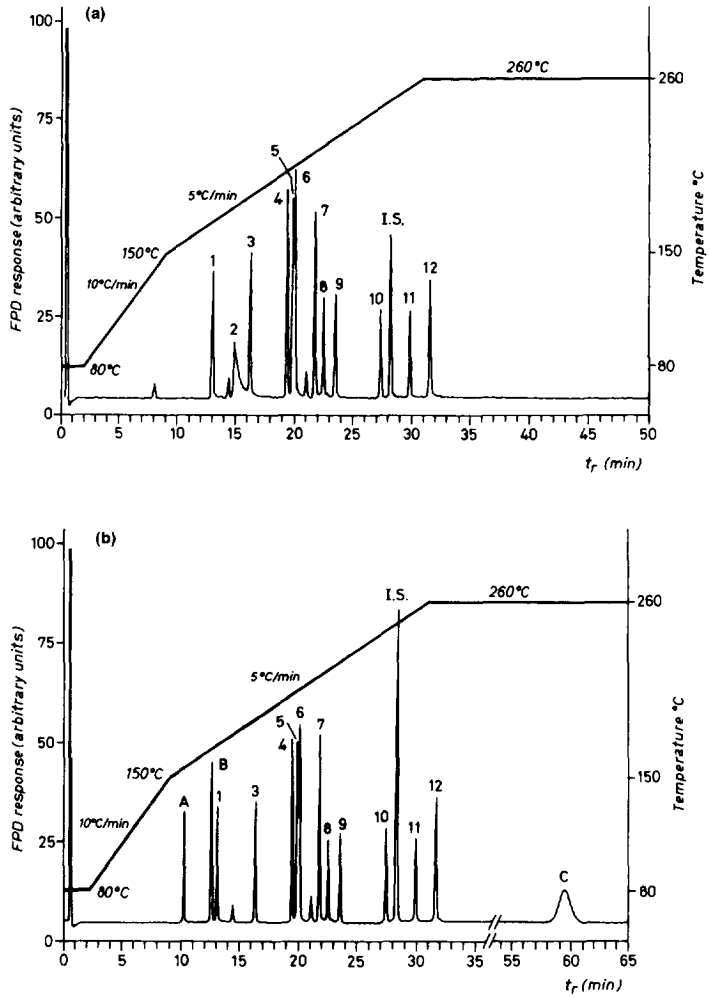

Fig. 2. GC–FPD chromatograms on SPB-608 column by injecting 1 μl of: (a) standard Mix B, for numbering and concentrations see Table 2; I.S.=triphenylphosphate 1.44 ng. (b) extract (see procedure) of a whole milk sample spiked with Standard Mix B at spiking level 3 (see Table 4); A=unknown peak; B=tri-*n*-butylphosphate; peaks 1 through 12 see Table 4; I.S.=triphenylphosphate 1.8 ng; C=unknown. t_R (min): A=10.1; B=12.4; I.S.=28.1; C=59.4.

Indeed, differently from the quoted methods, the described procedure performs in a single step both the extraction and a low-activity cleanup giving a solution amenable, without further cleanup, to the characteristics of the GC–FPD determinative system, namely, injector and column type, and selectivity of the detector. 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 and the preparation of fatty extracts of, for instance, milk is an off-line step that represents the bottle-neck of the entire analytical method. On the contrary, the described procedure is a one-step extraction and cleanup procedure that allows a high sample throughput with a few and simple handling operations and has potential for automation.

Unlike the classical schemes, with the described procedure the complete preparation of the sample extract is rapid (ca. 30 min), emulsions do not occur, mainly disposable items are used, and small volumes

of solvents and a very few items of glassware are needed. Compared with instrumental cleanup techniques (size-exclusion chromatography, sweep co-distillation) the described procedure is very simple, rapid, low-cost and does not require the preparation or maintenance of costly apparatus or skilled operators.

In conclusion, the main feature of the described procedure is that, in a single step and with a minimum of glassware, solvents and reagents, a rapid extraction and cleanup can be carried out for the OP pesticide residue determination in milk by GC–FPD.

Acknowledgments

This paper has been funded by Prevention and Control of Disease Factors, Sub-project Quality of the Environment and Health, Research line Toxicological Risk from Pesticides: Development and Integration of Methodologies (Contract C.N.R./FAT.MA. 95.00938 PF41)

References

[1] A. Di Muccio, I. Camoni, R. Dommarco, A. Santilio, A. Ausili, M. Rizzica, B. Gigli and C. Calzolari, Ann. Ist. Super. Sanità, 26 (1990) 155.
[2] Joint FAO/WHO Food Standards Programme-Codex Alimentarius Commission-Codex Alimentarius-Volume II-Pesticide Residues in Food. Food and Agriculture Organization and World Health Organization, Rome, 1993.
[3] European Union Council Directive n 93/57/CEE of June 29, 1993, Off. J. Eur. Communities N. L211/1 of 23.8.1993.
[4] K. Helrich (Editor), Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, Section 970.52A.
[5] H.-P. Thier and H. Zeumer, DFG-Manual of Pesticide Residue Analysis, Vol. I, 1987, VCH. Weinheim, Method S 10, p. 309.
[6] Ministry of Welfare, Health and Cultural Affairs, Rijswijk, The Netherlands, Analytical Methods for Residues of Pesticides in Foodstuffs, 5th ed., 1988, Multi-Residue Method 1, Submethod 5.
[7] M. Beroza and M. Bowmann, J. Agric. Food Chem., 3 (1968) 399.
[8] V. Leoni, A.M. Caricchia and S. Chiavarini, J. Assoc. Off. Anal. Chem. Int., 75 (1992) 511.
[9] R. Frank, H.E. Braun, G.H. Sirons, J. Rasper and G.G. Ward, J. Food Protect., 48 (1985) 499.
[10] K. Helrich (Editor), Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, Section 970.52L(c).
[11] N.F. Wood, Analyst (London), 94 (1969) 399.
[12] K. Helrich (Editor), Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, Section 970.52N.
[13] A. Di Muccio, A.M. Cicero, I. Camoni, D. Pontecorvo and R. Dommarco, J. Assoc. Off. Anal. Chem., 70 (1987) 106.
[14] A. Di Muccio, A. Ausili, L. Vergori, I. Camoni, R. Dommarco, L. Gambetti, A. Santilio and F. Vergori, Analyst, 115 (1990) 1167.
[15] A.M. Gillespie, S.L. Daly, D.M. Gilvydis, F. Schneider and S.M. Walters, J. Assoc. Off. Anal. Chem. Int., 78 (1995) 431.
[16] H.-P. Thier and H. Zeumer, DFG-Manual of Pesticide Residue Analysis, Vol. I, 1987, VCH, Weinheim, Method S 9 p. 297.
[17] W.E. Dale and J.W. Miles, J. Assoc. Off. Anal. Chem., 59 (1976) 165.
[18] K. Helrich (Editor), Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, Section 984.21.
[19] J.J. Blaha and P.J. Jackson, J. Assoc. Off. Anal. Chem., 68 (1985) 1095.
[20] D.M. Holstege, D.L. Scharberg, E.R. Richardson and G. Moeller, J. Assoc. Off. Anal. Chem., 74 (1991) 394.
[21] K. Helrich (Editor), Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 15th ed., 1990, Section 968.24.
[22] H.-P. Thier and H. Zeumer, DFG-Manual of Pesticide Residue Analysis, Vol. I, 1987, VCH, Weinheim, Clean-up Method 2, p. 57.
[23] T. Suzuki, K. Ishikawa, N. Sato and K. Sakai, J. Assoc. Off. Anal. Chem., 62 (1979) 681.
[24] M. Toyoda, K. Adachi, T. Ida, K. Noda and N. Minagawa, J. Assoc. Off. Anal. Chem., 73 (1990) 770.
[25] R.E. Baynes and J.M. Bowen, J. Assoc. Off. Anal. Chem. Int., 78 (1995) 812.
[26] A. Di Muccio, M. Rizzica, A. Ausili, I. Camoni, R. Dommarco and F. Vergori, J. Chromatogr., 456 (1988) 143.