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SELECTIVE, ON-COLUMN EXTRACTION OF ORGANOCHLORINE PESTICIDE RESIDUES FROM MILK

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SUMMARY

A rapid procedure has been developed that allows a single-step, selective extraction of organochlorine pesticide (OCP) residues from milk on solid-matrix disposable columns by means of acetonitrile–saturated light petroleum. Recovery experiments were carried out on homogenized milk (3.6% fat content) spiked with an ethanolic solution of nine OCPs, *viz.*, HCB, α-HCH, β-HCH, γ-HCH, heptachlor epoxide, dieldrin, endrin, *p,p'*-DDE and *p,p'*-DDT, at levels ranging from 0.002 mg/kg for α-HCH to 0.008 mg/kg for *p,p'*-DDT. Average recoveries of four replicates were 77% for HCB and almost quantitative (94–113%) for the other pesticides, with relative standard deviations from 2.9 to 7.3%. Coextracted fatty material amounted to about 5 mg/ml of milk before the clean-up. The described procedure also showed a satisfactory performance with milk powder. The extraction procedure requires about 60 min. The main advantages are that 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.

INTRODUCTION

The determination of organochlorine pesticide (OCP) residues in milk has always presented problems because the most common approach has involved total extraction of fat together with other lipophilic compounds including OCP residues. This approach has been based on the assumption that complete extraction of OCP residues from fatty foodstuffs is not possible unless all of the fat is extracted[1,2]. As a result, complex and lengthy procedures are in use for the extraction of milk fat and lipophilic pesticides. For instance, one of the most commonly used procedures[3] employs triple extraction of fluid milk with light petroleum–diethyl ether (1:1) with addition of sodium or potassium oxalate and ethanol to break up the emulsions, disrupt the fat globule membrane and improve the recovery of both fat and OCP residues. Combined extraction solvents are washed with salt solution, dried by passage through anhydrous sodium sulphate and concentrated to dryness to obtain milk fat. Another popular extraction system employs triple extraction of milk with *n*-hexane–acetone (1:1)[4]. Both procedures have some drawbacks, *e.g.*, the amounts of solvents

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and glassware used, the number of manual operations involved, which strongly affect the throughput of residue laboratories, centrifugation after each extraction and troublesome emulsions, which sometimes are not easily controlled by centrifugation and addition of ethanol, especially with whole milk.

Further, OCP residues need to be separated from the relatively large amount of milk fat so obtained by means of liquid–liquid partition[5,6], size-exclusion chromatography[7–10] or sweep co-distillation[11–15]. Adsorption column chromatography on Florisil[16–18], alumina[2,19] or silica gel[20] has been used as a final clean-up step before determination by gas chromatography (GC) with electron-capture detection (ECD)[21–24]. When a sufficient sensitivity is available in the final GC–ECD determination, it is also possible to clean up only a portion of the extracted fat by means of adsorption chromatography alone using minicolumns of Florisil[25,26], alumina[2,27] or silica gel[28].

In contrast to such conventional extraction procedures, selective extraction of OCP residues from milk has been reported by Suzuki *et al.*[26]. The addition of small amounts of acetonitrile and ethanol to the milk, before extraction with *n*-hexane, allows almost complete recoveries of OCP residues with minimum extraction of fatty substances. This method offers a substantial advantage over classical procedures in that the small weight of fatty extracts so obtained represents a significant fraction of the original sample, but requires only a minicolumn of adsorption material to remove it before GC–ECD. In our hands, the major drawback of this selective extraction procedure is the formation of stable emulsions in the second and third extraction stages, which are not controlled by centrifugation. Particularly in the extraction of whole-milk powder, which is reconstituted with water and extracted like fluid milk, the formation of a semi-solid gel in the organic phase almost completely prevents the recovery of the extraction solvents.

In order to minimize this effect we have developed a single-step, selective method for the extraction of OCP residues from milk and milk powder which is carried out on disposable, ready-to-use, solid-matrix cartridges where the efficiency of extraction is improved and emulsions do not occur.

EXPERIMENTAL

Reagents

Analytical-reagents grade light petroleum ether (b.p. 40–60°C), isooctane, acetonitrile and ethanol were redistilled in glass.

Ready-to-use Chem Elut CE 1010 cartridges were obtained from Analytichem International (Harbor City, CA, U.S.A.).

Organochlorine pesticide reference standards were from the collection in this laboratory.

Apparatus

The GC analyses were carried out on a DANI 6800 gas chromatograph equipped with an electron-capture detector. A glass column (1.8 m × 4 mm I.D.) was packed with OV-17–QF-1 (1.5% + 1.95%) on Chromosorb W HP (100–120 mesh). The temperatures were as follows: oven, 210; inlet block, 230; outlet block, 250; and detector, 250°C. The carrier gas was nitrogen at a flow-rate of 55 ml/min.

Procedure

In an erlenmeyer flask mix 10 ml of milk, 5 ml of acetonitrile and 1 ml of ethanol. Pipette 8 ml of this mixture on to a Chem Elut CE 1010 solid-matrix, ready-to-use column, allow it to drain and wait 10 min to obtain an even distribution. Attach a hypodermic needle to the column outlet as a flow regulator. Add to the column 10 ml of the upper phase (UP) obtained by equilibrating light petroleum–acetonitrile–ethanol (100:25:5). Wait 10 min, then elute with further 40 ml of UP. Collect the eluate and concentrate it to dryness. Dissolve the residue in 1–2 ml of light petroleum and clean up the extract by Florisil minicolumn adsorption chromatography[26]. Analyse the final sample by GC–ECD.

For milk powder, reconstitute 1 g of the powder with pesticide-grade water (1:9), then proceed as for fluid milk.

For recovery experiments, add 1 ml of an ethanolic solution containing OCPs instead of 1 ml of ethanol. Allow the mixture to stand overnight at 4°C. Equilibrate the sample to room temperature before proceeding with the above procedure.

RESULTS AND DISCUSSION

Chem Elut CE 1010 columns are ready-to-use, disposable cartridges filled with a macroporous diatomaceous earth with a nominal volume of 10 ml. In this procedure, the cartridges were used as a solid support to carry out the selective extraction of OCP residues from milk. As observed previously[1], OCP residues are more readily (but not completely) extracted than milk lipids with hydrocarbon solvents. However, the addition of a small amount of acetonitrile to the milk significantly improved the pesticide recoveries without increasing the fatty extracts[26]. Following these indications, a selective extraction of OCP residues from milk supported on a solid matrix was accomplished by eluting the column with acetonitrile-saturated light petroleum. The selective character of the extraction may be attributed to the phase-transfer properties of acetonitrile[29].

To test the performance of the method, milk and a sample of milk powder were analysed. Commercial pasteurized homogenized milk (3.6% fat content) was spiked (see *Procedure*) with nine typical OCPs at levels ranging from 2 to 8 μg/kg of milk and analysed according to the above procedure; the weight of the crude extract was calculated before the clean-up. The results obtained with fluid milk are presented in Table I. Typical chromatograms of spiked milk, unspiked milk and "blank" analyses are shown in Fig. 1. They show that all nine OCPs were satisfactorily recovered from milk with a carryover of fatty substances of only 5 mg/ml. This amount of fatty substances is of the same order as that reported by Suzuki *et al.*[26], thus allowing the use of a Florisil minicolumn for the clean-up. The AOAC[3] and *n*-hexane–acetone[4] extraction procedures would have extracted the nominal fat content and would have required either partition followed by adsorption chromatography or the sole adsorption chromatography of a fraction of the total fatty extract.

The milk powder sample (*ca.* 24% fat content) was prepared from milk to which a solution of nine OCPs in milk fat had been added before the spraying process. The sample was kindly provided by the EEC Community Bureau of Reference (BRC) and analysed under blind conditions according to the present procedure and, for comparison, also according to the AOAC[3] and *n*-hexane–acetone[4] extraction

TABLE I

RECOVERY OF NINE ORGANOCHLORINE PESTICIDES FROM FORTIFIED WHOLE MILK (3.6% FAT CONTENT)

Pesticide	*Spike level (μg/kg)*	*Mean recovery (%) (n = 4)*	*Relative standard deviation (%)*
HCB	4	77	7.3
α-HCH	2	94	6.7
γ-HCH	4	105	4.9
β-HCH	8	113	4.5
Heptachlor epoxide	4	99	6.4
p,p'-DDE	4	99	3.3
Dieldrin	4	106	2.9
Endrin	6	103	2.9
p,p'-DDT	8	98	4.1
Fatty extract (mg/ml milk)*		5.0	4.0

* Weight of extract prior to clean-up.

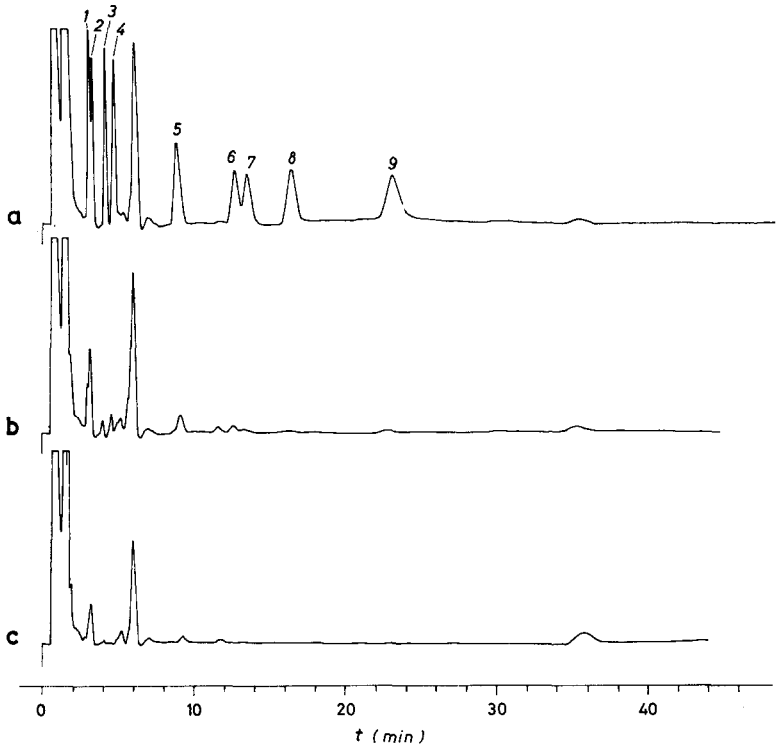

Fig. 1. Gas chromatograms of (a) spiked milk, (b) unspiked milk and (c) "blank", analysed according to the described procedure. Peaks: (1) HCB, 4 ppb; (2) α-HCH, 2 ppb; (3) γ-HCH, 4 ppb; (4) β-HCH, 8 ppb; (5) heptachlor epoxide, 4 ppb; (6) *p,p'*-DDE, 4 ppb; (7) dieldrin, 4 ppb; (8) endrin, 6 ppb; (9) *p,p'*-DDT, 8 ppb.

procedures. In all instances the fatty extracts were cleaned up by Florisil minicolumn chromatography[26] using a ratio of not more than 80 mg of fatty extract per 2.5 g of Florisil.

The results obtained with the milk powder sample are presented in Table II. The results obtained with this procedure appear in general to be slightly higher than the corresponding values obtained with the *n*-hexane–acetone procedure. In contrast, the AOAC procedure gave slightly higher results, at least for certain pesticides, notably γ-HCH, *p,p'*-DDE and *p,p'*-DDT. Although the results are in general satisfactory, the discrepancies may be an indication that, at least for a difficult sample such as milk powder which has a high fat and protein content, the pesticide–matrix interaction has not been completely broken. Hence some further refinements of the extraction conditions may be necessary in the present procedure.

However, it is noteworthy that the extraction of the milk powder according to this procedure did not pose particular problems, whereas it was not possible according to the procedure reported by Suzuki *et al.*[26] because of the formation of a semi-solid gel in the organic phase. Troublesome emulsions with this sample also occurred when the AOAC and *n*-hexane–acetone procedures were used. The results obtained with the present procedure are slightly more scattered (as judged from the relative standard deviation) than those given by the other two methods. Possible explanations for this may include variability of the packing characteristics of the Chem Elut material with possible "channelling" effects and variability of the blank analyses which were subtracted from the sample values. Indeed, blank analyses of the Chem Elut columns showed variable amounts of a peak with the retention time of α-HCH, of another

TABLE II

CONCENTRATION OF NINE ORGANOCHLORINE PESTICIDES IN A MILK POWDER SAMPLE

Results in μg/kg expressed on a dry-mass basis.

Pesticide	*Extraction procedure*					
	This work		*AOAC*[3]		*n-Hexane–acetone*[4]	
	Mean value (n = 4)	*Relative standard deviation (%)*	*Mean value (n = 5)*	*Relative standard deviation (%)*	*Mean value (n = 4)*	*Relative standard deviation (%)*
HCB	41.1	11.8	42.1	10.7	37.0	8.8
α-HCH	23.1	26.7	28.0	7.2	20.0	7.6
γ-HCH	45.5	21.1	52.9	4.4	42.6	13.8
β-HCH	11.8	14.7	13.9	1.4	10.4	6.4
Heptachlor epoxide	39.2	13.1	38.4	3.5	32.3	11.3
p,p'-DDE	49.1	6.6	62.9	10.8	46.7	9.7
Dieldrin	46.4	2.9	45.4	4.3	35.2	12.1
Endrin	6.8	10.9	10.2	2.5	5.1	10.5
p,p'-DDT	64.4	12.7	72.8	10.5	58.1	2.0
Fatty extract on dry-mass (%)*	5.9	42.3	24.5	2.9	22.4	3.7

* Weight of extract prior to clean-up.

major peak that does not interfere with the OCPs investigated and some minor (mostly early eluting) peaks.

In conclusion, we can say that, although the purity of the Chem Elut columns is not at present completely satisfactory as a pesticide-grade product, the results presented indicate that a simple, straightforward extraction of OCP residues from fluid milk and milk powder can be carried out on a solid matrix with satisfactory recoveries. As the extraction procedure requires only disposable items and almost unattended operations, it offers a means of improving the throughput of the residue laboratory with significant savings of reagents, glassware and time.

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