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Solid-phase extraction on C_{18} in the trace determination of selected polychlorinated biphenyls in milk

Y. Picó*, M.J. Redondo, G. Font, J. Mañes

Laboratori de Toxicologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andres Estelles s/n, 46100 Burjassot, València, Spain

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Abstract

The utility of solid-phase extraction with octadecylsilica for determining fifteen polychlorinated biphenyl (PCB) congeners from milk samples was examined. Recoveries higher than 80% and relative standard deviations better than 10% were obtained for PCBs from different kinds of milk (whole, skimmed, 2%, powdered, breast and evaporated). A comparison with other procedures was made. The described method provides better detection limits than those attainable with the liquid–liquid extractions currently used as standard methods, when capillary gas–liquid chromatography is used for the final determination. A study of the separation was also performed using six different fused-silica capillary columns and an electron-capture detector.

1. Introduction

Different solid-phase extraction (SPE) procedures for the determination of polychlorinated biphenyls (PCBs) based on adsorption chromatography using Florisil [1], anhydrous sodium sulphate [2], silica gel [3] or lipophilic gels [4] have been used. The advantage of the reversed-phase mode is that the amount of solid phase and organic solvent used and the analysis time are reduced. Octadecylsilica has been used to isolate PCBs in different matrices, such as water [5], fat [6] and blood and serum [7]. The procedure could be applied to milk samples, but the results obtained in previous studies demonstrate that the method must be optimized to obtain satisfactory recoveries of PCBs from whole milk [8] and that the fat globules in milk must be disrupted [9].

The main objective of this study was therefore the application of the SPE procedure to the determination of PCBs in milk, previously proposed for the determination organochlorine pesticides [9], and to verify its applicability to different kinds of milk.

2. Experimental

2.1. Chemicals

Fifteen biphenyls, 2-PCB (Ballschmiter No. 1), 2,2'-PCB (No. 4), 2,4-PCB (No. 7), 4,4'-PCB (No. 15), 2,4,4'-PCB (No. 28), 2,4,5-PCB (No. 29), 2,2',5,5'-PCB (No. 52), 3,3',4,4'-PCB (No. 77), 2,2',4,5,5'-PCB (No. 101), 2,3',4,4',5,5'-PCB (No. 118), 2,2',3,4,4',5,5'-

* Corresponding author.

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PCB (No. 138), 2,2',4,4',5,5'-PCB (No. 153), 2,2',3,3',4,4',5-PCB (No. 170), 2,2',3,4,4',5,5'-PCB (No. 180) and decachlorobiphenyl (No. 209) were chosen as model compounds. Analytical standards were purchased from Riedel-de Haën with a purity of 99%.

A certified standard of powdered milk (CRM 450) was supplied by the EEC Community Bureau of Reference (BCR).

n-Hexane and methanol were of pesticide grade. Solvents were shown to be free of interfering residues by GC with electron-capture detection following 200-fold concentration.

Potassium hydroxide solution (2 *M* in ethanol) was purchased from Merck (Darmstadt, Germany). To prepare chromic acid solution, 5 g of chromium(VI) oxide (Merck) were dissolved in 3 ml of water, and 60 ml of glacial acetic acid (Merck) were added.

Preparative octadecylsilica (55–105 μm) was obtained from Water–Millipore (Bedford, MA, USA).

2.2. Capillary gas chromatographic analysis

The gas chromatograph used for PCB isomers was a Konik 3000 equipped with a ^{63}Ni electron-capture detector and the following fused-silica columns: two Supelco BP-5 columns (25 × 0.22 mm I.D. and 50 m × 0.22 mm I.D., respectively); a Supelco BP-10 column (50 × 0.22 mm I.D.); a J & W Scientific DB-17 column (30 × 0.22 mm I.D.); a Scharlau OV-1701 column (25 × 0.25 mm I.D.); and a Delta Scientific CP-cyclodextrin-B-2,3,6-M-19 column (30 m × 0.25 mm I.D.). All of them had a film thickness of 0.25 μm.

The temperature programme for the columns with lengths between 25 and 30 m was 0.8 min at 50°C then increased at 30°C min^{-1} to 140°C, which was held for 2 min, then at 5°C min^{-1} to 280°C, the final temperature being held for 10 min. The temperature programme for the columns with a 50-m length was 0.8 min at 50°C then increased at 30°C min^{-1} to 200°C, which was held for 2 min, then at 2°C min^{-1} to 260°C. The final temperature was held for 8 min.

The injector and detector temperatures were 285 and 300°C, respectively. Helium was used as the carrier gas at a flow-rate of 1 ml min^{-1}. A sample volume of 3 μl was injected in the splitless mode and the splitter was opened after 0.7 min.

2.3. Procedure

Octadecylsilica (1 g) was transferred to a 100 mm × 9 mm I.D. glass column fitted with a coarse frit (No. 3) and covered with a silanized glass-wool plug. The microcolumn was treated with 10 ml of methanol and 10 ml of distilled water.

In an erlenmeyer flask, 5 ml of milk, 5 ml of water and 10 ml of methanol were mixed by sonication and passed through the microcolumn. A vacuum was applied to obtain a flow-rate about 1–20 ml min^{-1}. The microcolumn was washed twice with 10 ml of distilled water and the washings were discarded. The C_{18}-bonded silica was then dried by passing room air (previously filtered) through the column using a vacuum. The adsorbed residues were then eluted with 10 ml of *n*-hexane. The extract was concentrated at 45°C to 0.5 ml and 3 μl were injected into the gas chromatograph.

For milk powder, 1 g of the powder milk was reconstituted with distilled water (1:9), and for evaporated milk, 2 ml of milk were mixed with 6 ml of distilled water.

Recovery experiments were made preparing contaminated milk at different concentrations, 1 l of milk was transferred into a glass bottle and fortified with a known amount of the analytes by adding 20 μl of the stock solution (prepared in hexane) directly into the milk, the mixture was shaken vigorously for 2 h in an automatic vibrator and left to stand overnight at 4°C. The samples were equilibrated to room temperature before proceeding with the above procedure.

The quantification of the PCBs was made on the basis of individual congeners and not as Aroclors [10].

Alkali and oxidative treatment procedures have fully described elsewhere [11,12], and can be summarized as follows: extract-containing PCBs were mixed with 2 *M* ethanolic potassium

hydroxide or chromium(VI) oxide in glacial acetic acid at 75–80°C, shaken for a few minutes, washed to eliminate the excess of the reagents and then the organic layers were recovered and re-analysed by GC.

3. Results and discussion

3.1. Chromatographic separation

The selection of PCB congeners to evaluate the analytical method was based on their common presence in milk (Nos. 28, 52, 101, 118, 138, 153 and 180), on their proved toxicity (Nos. 77 and 170) and the fact that they cover all the possible range of the retention times in GC analysis (Nos. 1, 4, 7, 15, 29 and 209) to enclose the retention time interval where the PCBs can appear.

The fifteen PCBs studied are well separated with the six columns, except the pair PCB 15–29, which cannot be separated with the OV-1701 and cyclodextrin columns, which are the most polar columns used. Additional problems arise when a PCB fraction is studied in milk samples because among the 209 possible PCB congeners, around 150 have been found in environmental samples. Although the use of a single capillary column of BP-5, DB-17 or cyclodextrin phase allowed the separation and determination of the PCB congeners studied, a combination of two of them was selected to avoid quantification errors caused by other PCBs congeners not included in this study.

The use of capillary columns is essential for congener-specific analysis, but there is no single column available that can separate all 209 PCB congeners. The literature data reported that using the most common stationary phases, which usually contain 95% dimethyl–5% phenyl polysiloxane (BP-5, DB-5, SE-54), there are co-elution problems with PCBs 110–77, 126–129, 101–84–90, 123–149, 153–105–132, 123–149 and 171–156–202 [10,13]. This problem has also been noted for PCBs 31–28 using a more polar stationary phase containing 50% phenylmethyl siloxane (DB-17, BP-10) [13]. However, the PCBs can be determined unambiguously in terms of the individual congeners using different polarity capillary columns in combination with electron-capture detection (ECD).

3.2. Solid-phase extraction on C_{18}

The different variables of the solid-phase extraction procedure were checked to establish which values provide the best recoveries. The optimum conditions for the solid-phase extraction on C_{18} in the trace determination of polychlorinated biphenyls in milk are the same as were established in a previous study for the organochlorine pesticides in the same matrix [9]. Organochlorine compounds are usually present in milk samples and can constitute interfering compounds.

Whole milk with a fat content of 3.4% spiked with fifteen PCBs was analysed according to the present procedure.

Data referring to the recovery and the repeatability of the method are given in Table 1. For the fifteen PCB congeners, the recovery was better than 83% and the relative standard deviation was below 11%. The detection limits (signal-to-noise ratio = 3) are also given in Table 1.

Table 2 demonstrates the performance of the SPE when skimmed, 2%, powdered, breast and evaporated samples were spiked with a mixture of PCBs. The recoveries were similar for the different kinds of milk, except for decachlorobiphenyl, the recovery of which varied between 89 and 43% depending on the kind of milk. This may be due to the binding of the high-chlorine-content compounds to proteins [4].

Recovery data for whole milk spiked with fifteen PCBs and analysed according to Suzuki et al. [14] and the sulphuric acid–hexane extraction procedure [15] are similar. Although the recoveries obtained by the three procedures are satisfactory, the detection limits obtained by the solid-phase extraction procedure are better.

The extraction efficiency can be checked by analysing field-treated samples and comparing the data with those obtained by specific methods of known effectiveness. The results obtained

Table 1
Results obtained using SPE on C_{18}

PCB	Detection limit ($\mu g l^{-1}$)[a]	Concentration ($\mu g l^{-1}$)	Recovery (%)[b]
1	12.4	500	92 ± 6
		50	91 ± 7
4	9.9	250	94 ± 6
		25	96 ± 10
7	1.3	50	93 ± 5
		5	96 ± 9
15	9.9	250	92 ± 10
		25	97 ± 13
29	0.8	30	91 ± 9
		3	87 ± 8
28	5.0	200	99 ± 11
		20	102 ± 15
52	0.7	50	97 ± 8
		5	101 ± 13
101	0.4	20	96 ± 9
		2	93 ± 9
77	0.6	30	97 ± 8
		3	104 ± 10
118	0.2	8	98 ± 7
		0.8	91 ± 11
153	0.2	9	83 ± 10
		0.9	79 ± 14
138	0.2	8	99 ± 8
		0.8	102 ± 10
180	0.1	8	85 ± 9
		0.8	92 ± 12
170	0.1	10	88 ± 9
		1	80 ± 9
209	0.1	6	83 ± 10
		0.6	75 ± 16

[a] Signal-to-noise ratio = 3.
[b] Mean ± R.S.D. ($n = 5$).

with the certified standard are given in Table 3 and corroborate the previous results.

The identification and determination of PCBs in milk samples are sometimes difficult because there are a large number of chlorinated compounds with similar chemical and physical properties, including pesticides (e.g., DDT, hexachlorobenzene, dieldrin, chlordane), polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzo furans (PCDFs) [16].

However, the interferences produced by PCDDs and PCDFs are highly hypothetical

Table 2
Results obtained using for different kinds of milk SPE on C_{18}

PCB	Recovery (%)[a]			Detection limit ($\mu g l^{-1}$)	Powdered milk		Evaporated milk	
	2% milk	Skimmed milk	Breast milk		Recovery (%)[a]	Detection limit ($\mu g kg^{-1}$)	Recovery (%)[a]	Detection limit ($\mu g l^{-1}$)
1	90 ± 8	88 ± 10	91 ± 10	13.0–15.0	96 ± 7	120	94 ± 7	42.0
4	89 ± 9	98 ± 7	83 ± 9	9.0–10.0	94 ± 9	99	96 ± 9	33.0
7	87 ± 6	87 ± 8	94 ± 8	1.0–2.0	91 ± 7	20	88 ± 9	4.4
15	89 ± 5	93 ± 9	82 ± 9	8.0–10.0	94 ± 8	99	95 ± 6	34.0
29	93 ± 6	98 ± 8	104 ± 8	0.6–0.8	95 ± 10	7	94 ± 9	2.8
28	94 ± 9	90 ± 8	86 ± 10	5.0–7.0	94 ± 8	50	93 ± 8	17.0
52	90 ± 7	92 ± 8	96 ± 11	0.7–1.0	96 ± 6	7	94 ± 9	2.4
101	89 ± 5	94 ± 7	91 ± 9	0.4–0.6	94 ± 7	4	90 ± 10	1.5
77	92 ± 8	96 ± 10	100 ± 8	0.5–0.7	91 ± 7	6	88 ± 8	2.3
118	86 ± 9	90 ± 8	106 ± 9	0.1–0.4	84 ± 4	2	82 ± 6	1.0
153	82 ± 6	95 ± 8	95 ± 5	0.1–0.2	92 ± 9	2	90 ± 8	1.0
138	83 ± 10	89 ± 10	74 ± 10	0.2–0.4	89 ± 9	2	84 ± 7	1.0
180	87 ± 5	80 ± 8	67 ± 9	0.1–0.6	77 ± 9	1	76 ± 10	0.5
170	85 ± 8	79 ± 9	84 ± 10	0.1–0.3	77 ± 5	1	77 ± 9	0.5
209	70 ± 9	89 ± 10	54 ± 11	0.1–0.3	54 ± 11	1	43 ± 12	0.5

Extraction recoveries and R.S.D.s (at the higher concentrations indicated in Table 1). Detection limits obtained depending on the sample.
[a] Mean ± R.S.D. ($n = 5$).

Table 3
Concentrations of PCBs in a certified standard (CRM 450 natural milk powder).

PCB	Concentration ($\mu g kg^{-1}$)[a]			
	Certified value[b]	C_{18}	Suzuki et al. [14]	H_2SO_4–*n*-hexane [15]
1				
4				
7				
15				
29				
28				
52	1.16 ± 0.17			
101				
77				
118	3.3 ± 0.4	3.2 ± 0.5		
153	19.0 ± 0.7	18.5 ± 1.6	18.1 ± 2.0	19.7 ± 2.3
138		14.8 ± 2.01	14.0 ± 2.5	15.03 ± 1.3
180	11.0 ± 0.7	10.1 ± 1.25	9.2 ± 1.5	8.3 ± 2.2
170	4.8 ± 0.7	4.0 ± 0.6		5.8 ± 1.76
209				

[a] Mean ± S.D. ($n = 5$).
[b] The standard also certified the value for PCB 156, which was not included in this study.

when ECD is used because the level of these substances found in milk are about 50 times lower than those of PCBs [4] and strong alkali degrades PCDDs and PCDFs [16].

Concentrated sulphuric acid destroys certain pesticides, e.g., dieldrin [11,12,15], and chromic acid eliminates most of the organochlorine pesticides [11,12]. No drastic difference in the PCB composition was observed in the milk samples after the treatments.

In this study, the treatments were applied only to real samples and not spiked samples because the latter were checked before spiking in order to obtain extracts where probably no organochlorine contamination occurred.

3.3. Application

Samples of whole, 2%, skimmed, evaporated, powdered and human milk (two samples of each)

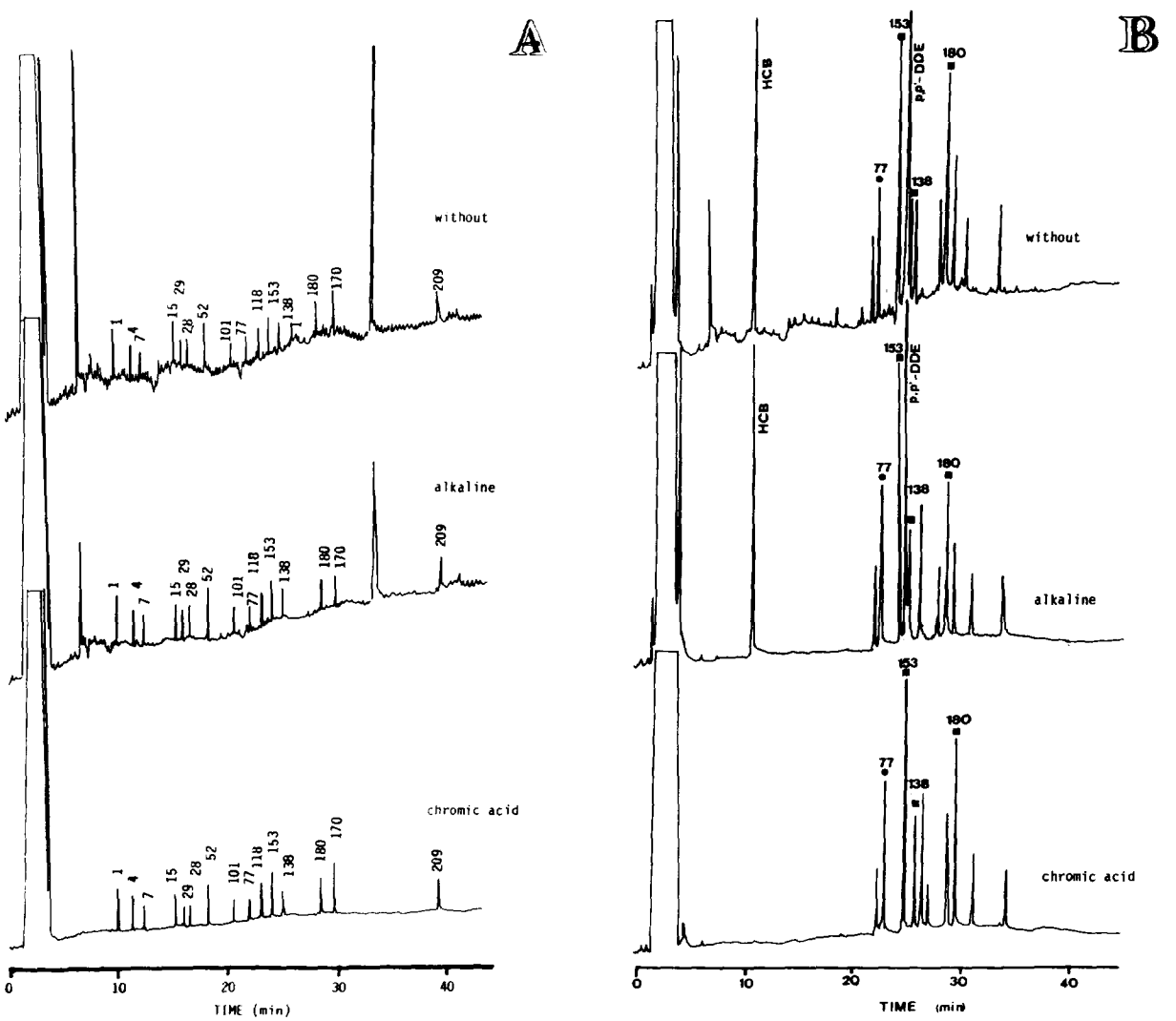

Fig. 1. Chromatograms obtained using a BP-5 column (25 m × 0.25 mm I.D.) for extracts processed according to the proposed procedure without treatment, after ethanolic KOH treatment and after chromic acid treatment of (A) spiked human milk at detection limit levels and (B) human milk sample No. 1 (the levels found are given in Table 4). ● = possible PCB congener not confirmed with other columns; ■ = possible PCB congener confirmed with other columns.

Table 4
Content of PCB congeners and organochlorine pesticides found in human milk samples using two extraction methods

Sample No.	Compound	Concentration ($\mu g l^{-1}$)	
		C_{18}	H_2SO_4–*n*-hexane [15]
1	HCB	5	5
	p,p'-DDE	12	11
	PCB 138	2	3
	PCB 153	8	8
	PCB 180	6	7
2	HCB	10	8
	p,p'-DDE	20	15
	PCB 138	0.3	0.3
	PCB 153	10	9.8
	PCB 180	4	4.5
	PCB 170	0.8	0.7

were analysed by the proposed method and by the sulphuric acid–hexane procedure. Table 4 shows the PCB isomer contents of positive samples determined by two extraction techniques. Only in human milk could some PCBs be detected, always together with HCB and p,p'-DDE, the concentrations of which are also given in Table 4. The results demonstrate that the PCB concentrations are in most instances lower than those of organochlorine pesticides, as has been noted by other workers [4]. In the other kinds of milk analysed, some organochlorine pesticides were identified but PCBs were not detected.

The alkali treatment to eliminate organochlorine compounds and the chromic acid treatment to destroy the organochlorine pesticides was applied to all the real samples.

Fig. 1 shows chromatograms of spiked (at the detection limit level) and real human milk samples. In the real sample, the importance of co-elution of the different PCB congeners can be observed.

Using the BP-5 column, PCB 77 was found at twice the concentration of PCB 138. However, the latter is one of the main environmental PCB compounds whereas the former is one of the coplanar congeners typically found at far lower levels [7,10,12]; as an example, Norén et al. [4] reported the PCB 138-to-PCB 77 concentration ratio in human milk to be approximately 5000:1.

Using the DB-17 or OV-1701 column, no peak appears at the retention time of PCB 77. The erroneous identification with BP-5 could be caused by PCB 110, which co-elutes with PCB 77 in a BP-5 column [10,13] and is typically present in environmental samples.

4. Conclusions

Solid-phase extraction is advantageous compared with liquid–liquid partitioning as no emulsions are formed and the passage of the sample through the column bed replaces repeated extractions and centrifugations. The use of capillary columns of different polarity and an electron-capture detector allows the accurate assessment of the milk levels of the different PCB congeners.

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