ELSEVIER

Journal of Chromatography A, 712 (1995) 355–364

JOURNAL OF CHROMATOGRAPHY A

Steam distillation–solvent extraction, a selective sample enrichment technique for the gas chromatographic–electron-capture detection of organochlorine compounds in milk powder and other milk products☆

Gerda Filek, Marcello Bergamini, Wolfgang Lindner*

Institut für Pharmazeutische Chemie, Karl-Franzens-Universität Graz, A-8010 Graz, Austria

First received 21 February 1995; revised manuscript received 2 May 1995; accepted 8 May 1995

Abstract

The application of a simultaneous water steam distillation–organic solvent extraction (SDE) method for the GC–electron-capture detection of hexachlorobenzene, α-hexachlorocyclohexane (α-HCH), γ-HCH, *cis*-heptachlorepoxide, 2,4′-DDE, 4,4′-DDE, dieldrin, 2,4′-DDT and 4,4′DDT in milk powder and other milk products is described. The SDE apparatus was a newly designed modification of the apparatus of Likens and Nickerson and Godefroot and co-workers and light petroleum was used as the extraction solvent. The method extracted the organochlorine pesticides selectively and with high recovery from interfering matrix compounds. Extraction and clean-up were performed in one step, followed by a simple concentration step. Quantification was performed using pentachlorobenzene and Mirex as internal standards. The limit of determination was between 0.5 and 2 ng/g, depending on the analyte.

1. Introduction

Several organochlorine pesticides (OCPs) have been widely used in Europe and are still used by some countries for agricultural and sanitary purposes. Because of their stability towards biodegradation and their lipophilic character, they can accumulate in fatty tissues of human, animal or plant origin. In this context a number of studies have been carried out on the OCP content of human milk [1–6]. In three studies, carried out in France [1], Yugoslavia [4] and Norway [3], hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, DDE, DDT and polychlorinated biphenyls (PCBs) were detected in human milk samples. The DDE levels in French human milk were above the EEC Directive level of 10 ppb in milk products. Jensen [5] calculated the maximum tolerable concentrations for these compounds in human milk from acceptable daily intakes of these compounds. The PCB levels of breast milk samples in some Yugoslavian areas exceed the proposed tolerable values [4].

With respect to the trace determination of organochlorine compounds in milk and particu-

☆ Dedicated to Professor Karl Winsauer on the occasion of his 70th birthday.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00544-7

larly in milk powder, these matrices are regarded as difficult to handle. The main problem is the separation of OCPs, highly fat-soluble compounds, from milk fat. The membrane of the milk fat globules has to be disrupted in order to obtain sufficient recoveries of the OCPs. Milk powder, however, contains denatured milk proteins and clusters thereof, which have to be broken up because they enclose or entrap the OCPs.

Conventional methods for the determination of OCPs in milk or milk powder involve the extraction of OCPs from matrix components by liquid–liquid partitioning with organic solvents [7] or solid-phase extraction [8], mostly followed by the isolation of the OCPs from interfering co-eluted lipophilic compounds by column chromatographic separation. Clean-up by means of alumina [9], Florisil [7,8], C_{18}-modified silica gel cartridges [10], gel permeation [11], HPLC [12] and in some cases with the use of sulphuric acid [13,14] or potassium hydroxide [15] as efficient chemical emulsifiers has been described. A straightforward on-line extraction and clean-up procedure for OCPs using "normal" solid-phase extraction was described by Steinwandter [16] and a "reversed" solid-phase extraction method by Mañes et al. [17].

In this paper, and as an alternative to conventional methods, the application of simultaneous steam distillation–solvent extraction (SDE) as a sample preparation technique for the enrichment and determination of OCPs in a variety of sample matrices including milk and milk products is described. The SDE technique was employed by Likens and Nickerson [18] in 1964 for the determination of essential oil contents in flowers. In the meantime, the original apparatus [18] has been modified by incorporating a vacuum jacket in the arm which conducts the solvent vapour to the extractor body [19] and by introducing more efficient cooling surfaces [20]. Godefroot and co-workers [21,22] modified the apparatus by scaling down the original device. In this work, the application of a newly designed SDE apparatus [23] for the GC-ECD determination of OCPs in milk and milk powder is described. The apparatus is constructed in such a way that permits more complete mixing of steam vapour, e.g., of water and of an organic solvent such as light petroleum, and condensing the gas phase on a greater surface, which allows the use of tap water as coolant (10–12°C). Moreover, it permits the use of a larger amount of sample, thus enhancing the overall sensitivity of the method.

2. Experimental

2.1. Capillary gas chromatography

A Hewlett-Packard HP 5890 A gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. Sample volumes of 1 and 2 μl were injected using an HP 7673 A autosampler equipped with a 10-μl Hamilton syringe into a capillary inlet with a glass liner in the splitless mode. The column was a fused-silica capillary column (30 m × 0.25 mm I.D.), coated with 0.25-μm cross-linked 65% dimethyl–35% diphenylpolysiloxane (RTX-35; Restec). The injector was heated at 290°C and the detector at 325°C. As carrier and make-up gas, nitrogen of 5.5 grade at 18 p.s.i. (125 kPa) column head pressure (corresponding to a flow-rate of 55 ml/min at split vent) was used. The temperature programme was as follows: initial temperature, 100°C for 1 min, then increased at 12°C/min to 220°C, followed by (A) 1.5°C/min to 240°C and (B) 6°C/min to 290°C, the final temperature of 290°C being held for 5 min.

GC control and data processing were performed with an HP 5895 A ChemStation.

2.2. Chemicals and sample materials

Standards

All pesticide standards (HCB, α-HCH, γ-HCH, *cis*-heptachlorepoxide, 2,4′-DDE, 4,4′-DDE, dieldrin, endrin, 2,4′-DDT, 4,4′-DDT) were obtained from Dr. Ehrenstorfer (Augsburg, Germany), each in the form of a stock solution in isooctane or cyclohexane (10 ng/μl). For the spiking procedure two different mixtures and

dilutions of these pesticide stock solutions were prepared, one with a concentration of 1 ng per 10 μl and the other 1 ng/μl of each pesticide. As internal standards pentachlorobenzene (PCBz) and Mirex in the form of a stock solution (10 ng/μl) in cyclohexane were used. To obtain a concentration of 1 ng/μl for the spiking procedure a dilution of this stock solution was prepared.

Reagents

Light petroleum (b.p. 40–60°C) for residue analysis was obtained from Merck (Darmstadt, Germany) and ethanol for UV spectroscopy from Fluka (Buchs, Switzerland). As antifoam agent Simethicon (polydimethylsiloxane) from Aldrich was used; a stock solution containing 800 mg of Simethicon in 10 ml of light petroleum was prepared.

Milk powder

Milk powder was purchased in a supermarket and characterized as containing 1% (w/w) fat, 3.3% (w/w) protein and 4.7% (w/w) lactose.

Two certified milk powder reference samples (natural milk powder CRM 187 and spiked milk powder CRM 188) were supplied by the EEC Community Bureau of Reference (BCR) (Brussels, Belgium).

2.3. Steam distillation–solvent extraction apparatus

The SDE apparatus used in this work (Fig. 1) was designed as a modification of the original apparatus of Likens and Nickerson [18] and Godefroot and co-workers [21,22]. The extraction head chamber was constructed in such a way as to provide more complete mixing of solvent and steam vapours, and the condensing surface area was increased to about 500 cm^2, which allows the use of tap water (10–12°C) as coolant. More details with respect to the design and function of the SDE apparatus can be found in a paper by Seidel and Lindner [23].

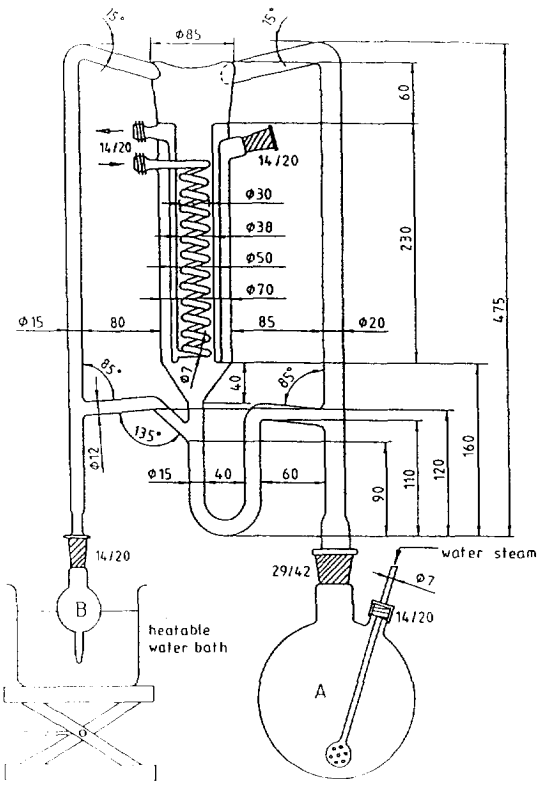

Fig. 1. Scale drawing (dimensions in millimetres) of the continuous steam distillation–solvent extraction (SDE) apparatus. Vessel A, sample in water, total volume 700 ml; vessel B, organic solvent, e.g., light petroleum, total volume 30 ml.

The small exit opposite the condenser inlet serves as a pressure-regulating vent and should have a minimum aperture of 7 mm. Water steam was generated from tap water by a Büchi (Flawil, Switzerland) DG 1500 steam generator and the steam flow was controlled by hydrostatic pressure regulation. The SDE apparatus was constructed for the use of organic solvents with density lower than that of water (in contrast to the apparatus developed by Godefroot and co-workers). To avoid contamination of the apparatus with interfering organochlorine compounds stemming from external components, all tubing was made of PTFE or glass and for the sealing of glass connections PTFE sleeves were used.

2.4. Steam distillation solvent extraction procedure

A 5-g amount of milk powder was weighed into the water steam extraction chamber (round-bottomed flask A), wetted with 80 ml of 2 M sulfuric acid, spiked with internal standard solution (PCBz and Mirex in cyclohexane) and ultrasonicated for 1 min. Subsequently, 1 ml of UV-grade ethanol and 0.5 ml of Simethicon solution (corresponding to 40 mg) were added before the water steam flow was switched on and the SDE process was carried out. After filling the U-shaped separation chamber with tap water and the small conical-tapered vessel (B, 50 ml) with the extraction solvent (e.g., light petroleum, b.p. 40–60°C), the apparatus was first filled with organic vapour by heating vessel B in a water-bath at 70°C. Subsequently, the water steam, generated and flow controlled by a separate steam generator, was blown through the milk sample to extract the volatile OCPs. After an efficient gas-phase nebulization-type extraction by spiral (helical) vapour flows in the extraction head chamber (Fig. 1), the vapours were condensed by a cooling device and the light petroleum flowed back to flask B and the water back to flask A. After about 1.5 h, the sample flask A (1000 ml) was filled with about 800 ml of condensed water and at this volume the steam distillation was stopped. In this way all the lipophilic, volatile and water steam-distillable compounds were extracted and collected in the final, ca. 20-ml, light petroleum extract (vessel B), which was concentrated to 1 ml by means of a Kuderna–Danish concentrator [23]. This sample solution was then ready for GC analysis without any further purification steps.

2.5. Determination of OCPs

The determination of the OCPs was performed via calibration graphs obtained with spiked milk powder samples [or alternatively with spiked whole milk samples (see below)], whereby the area of each identified peak was referred to the peak area of the internal standard (PCBz and Mirex). "Blank" milk powder (5 g) was spiked with 25, 50, 100, and 250 μl of the 1 ng per 10 μl pesticide mixture, corresponding to concentrations of 0.5, 1, 2 and 5 ng/g. To obtain concentrations of 10, 20 and 50 ng/g, 5 g of "Blank" milk powder were spiked with 50, 100 and 250 μl of the 1 ng/μl pesticide mixture. Each of these calibration samples was spiked with 3 ng/g of PCBz and 5 ng/g of Mirex. Because of the injection volume of 2 μl, in the low ng/g range (0.5–10 ng/g) the detector response was not linear, and did not become so until the higher range (20–50 ng/g). For this reason, an injection volume of only 1 μl for the 20 and 50 ng/g calibration samples was chosen.

In addition, a calibration graph for whole milk (cow milk, 3.6% fat content) was obtained. A 5-g amount of whole milk was spiked with the OCP standard solution in the same way as for the milk powder calibration samples. The concentration range was between 0.5 and 50 ng/g, and 3 ng/g of PCBz and 5 ng/g of Mirex were added as internal standards.

3. Results and discussion

3.1. Determination of OCPs

The determination of the OCPs was performed via internal standard calibration, whereby each peak area value was referred to that of the internal standard (PCBz and Mirex). For the low-boiling compounds HCB, α-HCH, γ-HCH and *cis*-heptachlorepoxide, PCBz was chosen as the internal standard, whereas the high-boiling compounds 2,4′-DDE, 4,4′-DDE, dieldrin, 2,4′-DDT and 4,4′-DDT were calibrated with Mirex; for calibration data, see Table 1. The recoveries of the analytes and analyte groups are comparable to those of the internal standard (see also Table 3). In order to increase the determination sensitivity in the low ng/g range (0.5–10 ng/g), an injection volume of 2 μl was chosen, otherwise 1 μl was injected.

3.2. Performance and ruggedness of the method

A 5-g amount of milk powder was spiked with 10 ng/g of nine OCPs and distilled with the SDE apparatus. In the first attempts the samples were

Table 1
Calibration data for the determination of OCPs in milk powder and whole milk

Compound	Milk powder (0.5–50 ng/g)			Whole milk (0.5–50 ng/g)		
	Correlation coefficient	Slope	Intercept	Correlation coefficient	Slope	Intercept
HCB	0.999	0.388	0.213	0.999	0.383	0.223
α-HCH	0.999	0.331	−0.335	0.999	0.343	−0.789
γ-HCH	0.998	0.226	−0.039	0.998	0.228	−0.134
HEPO[a]	0.999	0.236	−0.153	0.999	0.241	−0.364
2,4'-DDE	0.999	0.191	0.077	0.999	0.190	0.103
4,4'-DDE	0.996	0.249	−0.302	0.999	0.233	−0.168
Dieldrin	0.998	0.226	0.032	0.998	0.227	−0.027
2,4'-DDT	0.999	0.184	0.197	0.998	0.182	0.267
4,4'-DDT	0.998	0.077	0.096	0.999	0.073	0.223

[a] *cis*-Heptachlorepoxide.

distilled without the addition of 2 M sulfuric acid; later they were distilled with the addition of 2 M sulfuric acid (see also Experimental). The recoveries from spiked milk powder obtained without sulfuric acid treatment were compared with those obtained with sulfuric acid treatment (Table 2). Without sulfuric acid treatment the recoveries were relatively low, but with the addition of sulfuric acid the recovery increased significantly and the results were satisfactory. The limit of determination was determined for the nine OCPs and the data are included in Table 2. The recoveries from 5 g of spiked whole milk (3.6% fat) obtained with sulfuric acid treatment were also determined and compared with the results for spiked milk powder (1% fat). The results are given in Table 3. For high-boiling OCPs and Mirex the recovery was lower.

The day-to-day reproducibility for an 8-day period was determined by analysing milk powder samples that had been spiked with OCPs corresponding to a concentration of 10 ng/g each. The reproducibility is expressed as the relative standard deviation of these eight values and the data are given in Table 2.

The method was cross-validated with certified milk powder reference samples (natural milk powder CRM 187 and spiked milk powder CRM 188) from the EEC BCR [24–27]. The results obtained with these reference samples are given in Table 4. The techniques for the determination of OCPs in the certified milk powder samples

Table 2
Day-to-day reproducibility, recoveries and limits of determination of the SDE technique for milk powder samples

Compound	R.S.D. (%) ($n = 8$)	Recovery without acid (%)	Recovery with acid (%)	Limit of determination (ng/g)
HCB	8	87	97	0.5
α-HCH	8	85	79	1
γ-HCH	11	61	96	1
HEPO[a]	7	n.d.[b]	93	1
2,4'-DDE	7	73	94	1
4,4'-DDE	9	71	83	1
Dieldrin	9	82	121	1
2,4'-DDT	10	57	87	2
4,4'-DDT	13	42	78	2

[a] *cis*-Heptachlorepoxide.
[b] Not determined.

Table 3
Recoveries for milk powder samples (1% fat content) compared with recoveries for whole milk samples (3.6% fat content)

Compound	Recovery for milk powder (%)	Recovery for whole milk (%)
PCBz	90	88
HCB[a]	97	97
α-HCH[a]	79	88
γ-HCH[a]	96	91
HEPO[a,b]	93	85
2,4'-DDE	94	67
4,4'-DDE	83	65
Dieldrin	121	57
2,4'-DDT	87	47
4,4'-DDT	78	46
Mirex	75	51

[a] To calibrate these OCPs PCBz was used as internal standard; for the others Mirex was used.
[b] *cis*-Heptachlorepoxide.

can be summarized as follows: the pesticides were extracted commonly with milk fat and other interfering compounds from reconstituted milk with various solvents. For the clean-up of the sample extracts, aluminium oxide (ten laboratories), Florisil (seven laboratories) and silica gel (one laboratory) were used, and gel permeation chromatography was also employed by four laboratories [24].

The problem with the BCR reference samples was that they were 2–3 years old. In order to obtain sufficient recoveries, we had to break up the sample matrix by a combined sulfuric acid treatment, ultrasonication and heat. Nevertheless, the result for 4,4'-DDE in CRM 187 (natural milk powder) was not in agreement with the certified levels (see Table 4). The samples were analysed in triplicate, but for 4,4'-DDE the analysis value remained low. We assume that the milk powder sample and its composition may have changed as an effect of aging.

3.3. Extraction

The SDE technique has proved to be a powerful method to extract OCPs from complex matrices such as low-fat milk powder. The advantage of steam distillation is that the OCPs, as volatile compounds, can be separated selectively from interfering matrix compounds. However, milk powder, rich in proteins, lecithins, carbohydrates, etc., sometimes several years old, is a very difficult to handle matrix, because the OCPs can become entrapped by denatured milk proteins. Matrices rich in fat components need emulsifiers to make possible a "homogeneous" aqueous matrix; in order to optimize this process, we added lecithin as emulsifier to extraction flask A, but the recoveries of the OCPs were not significantly improved. We assume that the lipo-

Table 4
Cross-validation of the SDE technique with BCR certified milk powder reference samples (natural milk powder CRM 187 and spiked milk powder CRM 188)

Compound	Concentration (μg/kg)[a]			
	CRM 187, certified value	CRM 187, SDE method	CRM 188, certified value	CRM 188, SDE method
HCB	1.5 ± 0.20	1.1	37.4 ± 2.7	47.2
α-HCH	1.8 ± 0.14	1.3	20.0 ± 0.9	16.5
γ-HCH	5.7 ± 0.70	6.3	45.4 ± 2.9	47.0
HEPO[b]	n.d.[c]	n.d.	32.0 ± 1.8	36.0
4,4'-DDE	6.6 ± 0.60	2.5	51.3 ± 3.5	48.3
Dieldrin	n.d.	n.d.	36.1 ± 2.4	35.8
4,4'-DDT	n.d.	n.d.	69.0 ± 4.6	62.6

[a] Contents in dry mass.
[b] *cis*-Heptachlorepoxide.
[c] n.d. = Not determined.

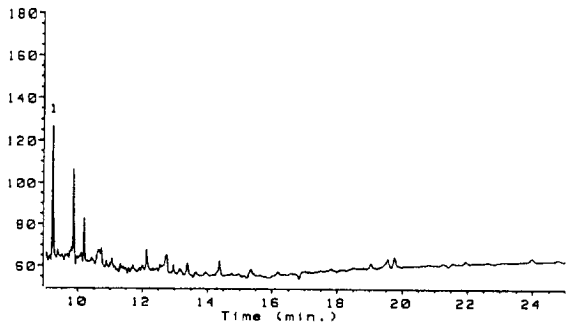

Fig. 2. GC–ECD of milk powder "blank". The milk powder was distilled with the SDE apparatus after it had been spiked with pentachlorobenzene (1) and wetted with 80 ml of 2M sulfuric acid (see also Experimental).

philic OCPs become "attached" to the lipophilic parts of the lecithins, which act like surfactants forming, e.g., micelles. In order to obtain sufficient recoveries, milk proteins have to be broken up, which can be stimulated by ultrasonication, sulfuric acid treatment and heat: the SDE technique allows the simultaneous treatment of the sample in the above-described way.

Milk and milk powder are matrices which cause numerous negative peaks in the GC–ECD trace, which interfere seriously with the peaks of some OCP analytes (see Fig. 3A). The sulfuric acid treatment, however, was very helpful in suppressing the negative peaks in the chromatograms to a large extent (see Figs. 2 and 3B). As shown earlier and instead of cleaning up the sample extract on an Extrelut column charged with concentrated sulfuric acid [13,14,28], we wetted the milk powder sample with 80 ml of 2 M sulfuric acid prior to the steam distillation procedure. With concentrated sulfuric acid the fat and the triglycerides are destroyed by this harsh chemical treatment, and the addition of only dilute sulfuric acid helps mainly to break up denatured milk proteins. In this way we achieved sufficient recoveries for all OCPs except endrin; the gas chromatograms had good baselines (see Fig. 3B). Endrin is unstable to acid treatment; according to published data [13,29] it is converted into the ketone. Dieldrin, DDE and DDT, however, showed good recoveries (between 80 and 95%; see Table 2), indicating that these compounds are not degraded by means of this relatively mild sulfuric acid treatment. It should be mentioned that the 2 M sulfuric acid is diluted to 0.2 M during the steam distillation process and buffered by sample matrix components.

With the object of achieving optimum extraction of the OCPs in a suitable time, it was necessary to add a co-distillation solvent to the extraction flask A. Polar solvents such as ethanol, 2-propanol and acetonitrile were tested. The addition of 10–15% of ethanol (referred to the volume in the extraction flask A prior to the distillation; see Experimental) under the SDE

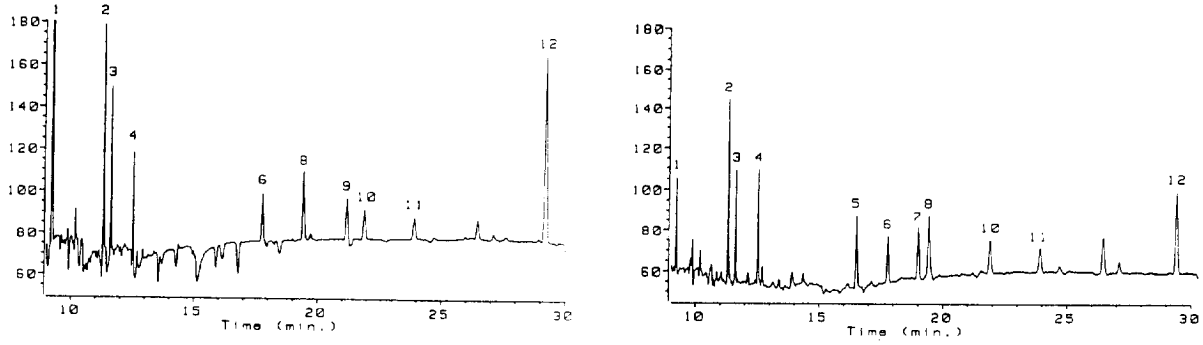

Fig. 3. GC–ECD of spiked milk powder (5 ng/g of each OCP) after sample pretreatment with the SDE technique. The distillation process was carried out (A) without and (B) with the addition of 2 M sulfuric acid to the sample. Pentachlorobenzene and Mirex were added as internal standards. Peaks: 1 = pentachlorobenzene; 2 = hexachlorobenzene; 3 = α-HCH; 4 = γ-HCH; 5 = *cis*-heptachlorepoxide; 6 = 2,4′-DDE; 7 = 4,4′-DDE; 8 = dieldrin; 9 = endrin; 10 = 2,4′-DDT; 11 = 4,4′-DDT; 12 = Mirex. For GC conditions, see Experimental.

starting conditions was sufficient, although it should be noted that a steady dilution of the ethanol content in vessel A occurred.

The milk powder samples showed severe foam formation during the distillation process, so we tested various substances to reduce the foaming. Celite, Kieselguhr (diatomaceous earth), NaCl and Simethicon (a polysiloxane) were examined for their antifoaming properties. With the addition of 25% (w/w) of Celite to the milk powder the foam was suppressed well, but the recoveries for the OCPs were very low. The addition of 20, 30 or 50 g of NaCl to 10 g of milk powder did not suppress the foam formation. However, the addition of 40 mg of Simethicon to 5 g of milk powder reduced the foaming without influencing the recoveries of the OCPs.

If the fat content of the milk product sample is not higher than 5%, a selective and adequate isolation of the OCPs from such samples can be achieved by the SDE method. The recovery, however, is dependent on the fat content, but also on the volatility of the analyte. If the fat content is about 1%, the recoveries of all the OCP analytes examined (low and high boiling) ranged between 78 and 95%. For whole milk (3.6% fat), the recoveries of PCBz, HCB, α-HCH, γ-HCH and *cis*-heptachlorepoxide were hardly influenced, whereas the recoveries of 2,4'DDE, 4,4'-DDE, dieldrin, 2,4'DDT, 4,4'-DDT and Mirex decreased in the specified sequence (see also Table 3). When analysing milk samples with a 3.6% fat content, the calibration should be carried out with whole milk. With a higher fat content of the samples the recoveries of certain OCPs drop significantly, but even if the fat content exceeds 10% this method is applicable, provided that one calibrates with spiked samples of the same fat content. If the distillation process could be extended for a longer period of time, a further increase in the recovery would result. In the present case the water steam is not generated by a separate steam generator, but by heating the sample flask A with an external heat source. The water steam formation in flask A could be extended for a long period of time (a condensation volume of 800 ml was not final), giving much higher recoveries.

The sensitivity of the overall method could easily be further increased by, e.g., concentrating the organic sample solution to 100 μl instead of 1 ml (see Experimental). Many volatile and ECD-sensitive compounds such as chlorophenols, PCBs and dioxanes could possibly interfere in the course of the GC determination of OCPs. On the other hand, the SDE method could also be extended to preconcentrate a wide variety of analytes from a complex matrix followed by additional chromatographic work-up and analysis techniques. Ramos et al. [30] described the application of the SDE technique to the determination of PCBs, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in water samples.

3.4. OCP content found in various milk samples

Various whole milk and breast milk samples were analysed for their OCP content with the SDE method described above. The fat content of some of these samples and the OCP values determined are summarized in Table 5.

4. Conclusion

The simultaneous water steam distillation–organic solvent extraction (SDE) method represents a selective sample enrichment technique for the determination of organochlorine pesticides in milk, milk powder and other milk products. This technique is reasonably fast; the distillation process for the extraction and clean-up method takes 1–1.5 h per sample and the following concentration of the organic extract takes 15–30 min. Owing to the unique SDE set-up, several selective sample pretreatment steps, e.g. ultrasonication, chemical treatment and heating, can be integrated. The resulting sample extracts are very suitable for direct GC analysis, resulting in long term stability of the GC columns and the injector liners. Another noticeable advantage is that one needs only small amounts of organic solvents for extraction. However, compounds that are unstable to heat, acid

Table 5
OCP content of various milk samples

Sample	Fat content	Concentration (ng/g)					
	(%)	HCB (d.l.[a] 0.5)	α-HCH (d.l. 1)	γ-HCH (d.l. 1)	4,4′-DDE (d.l. 1)	Dieldrin (d.l. 1)	4,4′-DDT (d.l. 2)
Milk	3.6	2	<d.l.	2	<d.l.	<d.l.	<d.l.
Breast milk sample 1	4	5	<d.l.	5	8	<d.l.	3
Breast milk sample 2	4	10	<d.l.	5	4	<d.l.	2

[a] d.l. = Determination limit (ng/g).

and oxidation (e.g., endrin) cannot be determined with the SDE method.

The separation and enrichment principles of this method are based on simultaneous steam distillation–solvent extraction, which are controlled by liquid–liquid extraction equilibria influenced by the type and volume of extraction media (equal to the amount of steam generated) and to a certain extent the matrix component and composition. Thus, the extraction recoveries of the highly lipophilic OCPs are influenced by the fat content of the matrix and by the volatility of the analyte. A high fat content ($>5\%$) and low volatility lead to a decrease in recovery, although the value is reproducible owing to the equilibria to be controlled. The decisive step for obtaining reliable results is calibration with an internal standard of a similar volatility to the analyte and calibration with a matrix of similar fat content.

In the analysis of complex matrices other than milk powder, the addition of dilute sulfuric acid prior to the distillation process might not be required.

References

[1] F. Bordet, J. Mallet, L. Maurice, S. Borrel and A. Venant, Bull. Environ. Contam. Toxicol., 50 (1993) 425–432.

[2] Y.K. Matuo, J.N.C. Lopes, I.C. Casanova, T. Matuo and J.L.C. Lopes, Arch. Environ. Contam. Toxicol., 22 (1992) 167–175.

[3] J.U. Skaare and A. Polder, Arch. Environ. Contam. Toxicol., 19 (1990) 640–645.

[4] B. Krauthacker, Bull. Environ. Contam. Toxicol., 46 (1991) 797–802.

[5] A.A. Jensen, Residue Riv., 89 (1983) 1–128.

[6] M.A. Alawi, N. Ammari and Y.Al-Shuraiki, Arch. Environ. Contam. Toxicol., 23 (1992) 235–239.

[7] T. Suzuki, K. Ishikawa, N. Sato and K.I. Sakai, J. Assoc. Off. Anal. Chem., 62 (1979) 681.

[8] A. Di Muccio, M. Rizzica, A. Ausili, I. Camoni, R. Dommarco and F. Vergori, J. Chromatogr., 456 (1988) 143–148.

[9] A.M. Gillespie and S.M. Walters, J. Assoc. Off. Anal. Chem., 67 (1984) 290.

[10] T. Prapamontol and D. Stevenson, J. Chromatogr., 552 (1991) 249–257.

[11] D.P. Goodspeed and L.I. Chesnut, J. Assoc. Off. Anal. Chem., 74 (1991) 388.

[12] E.A. Hogendoorn, G.R. van der Hoff and P. van Zoonen, J. High Resolut. Chromatogr., 12 (1989) 784.

[13] A. Di Muccio, A. Santilio, R. Dommarco, M. Rizzica, L. Gambetti, A. Ausili and F. Vergori, J. Chromatogr., 513 (1990) 333–337.

[14] A. Di Muccio, A. Ausili, R. Dommarco, D. Attard Barbini, A. Santilio, F. Vergori, G. De Merulis and L. Sernicola, J. Chromatogr., 552 (1991) 241–247.

[15] R. Frank, H.E. Braun, G.H. Sirons, J. Rasper and G.G. Ward, J. Food Protect., 48 (1985) 499.

[16] H. Steinwandter, Fresenius' J. Anal. Chem., 312 (1982) 342–345.

[17] J. Mañes, G. Font and Y. Picò, J. Chromatogr., 642 (1993) 195–204.

[18] S.T. Likens and G.B. Nickerson, Am. Soc. Brew. Chem. Proc., (1964) 5.

[19] H. Maarse and R.E. Kepner, J. Agric. Food Chem., 18 (1970) 1095.

[20] R.A. Flath and R.R. Forrey, J. Agric. Food Chem., 25 (1977) 103.

[21] M. Godefroot, P. Sandra and M. Verzele, J. Chromatogr., 203 (1981) 325–335.

[22] M. Godefroot, M. Stechele, P. Sandra and M. Verzele, J. High Resolut. Chromatogr. Commun., 5 (1982) 75–79.

[23] V. Seidel and W. Lindner, Anal. Chem., 65 (1993) 3677–3683.

[24] B. Griepink, E.J. Mulders, C.G. van der Paauw and J.K. Quirijns, BCR Information Reference Materials, Report EUR 12319 EN, 1989.

[25] C.G. van der Paauw, E.J. Mulders, J.K. Quirijns and B. Griepink, Fresenius' Z. Anal. Chem., 322 (1988) 698–700.
[26] J.K. Quirijns, C.G. van der Paauw, E.J. Mulders and B. Griepink, J. Assoc. Off. Anal. Chem., 73 (1990) 773–776.
[27] B. Griepink, J.K. Quirijns, C.G. van der Paauw and E.J. Mulders, J. Assoc. Off. Anal. Chem., 73 (1990) 777–782.
[28] V. Seidel, I. Tschernuter-Meixner and W. Lindner, J. Chromatogr., 642 (1993) 253–262.
[29] A.S.Y. Chau, Bull. Environ. Contam. Toxicol., 8 (1972) 169.
[30] L. Ramos, G.P. Blanch, L. Hernández and M.J. González, J. Chromatogr. A, 690 (1995) 243–249.