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Determination of organochlorine compounds in fatty matrices Application of rapid off-line normal-phase liquid chromatographic clean-up

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Abstract

A clean-up method for organochlorine compounds in fatty samples based on normal-phase liquid chromatography (NPLC) is described. To this end, an existing clean-up procedure which uses column switching for the separation of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) from the fat matrix was simplified to a single silica LC column procedure. The use of an LC column packed with 3 μm silica enables complete fat/OCP separation in a total fraction volume of 12 ml, and results in a fully automated clean-up procedure that takes only 32 min per sample. The method showed average recoveries of 80–110% in the concentration range of 1–510 μg/kg, with relative standard deviations of less than 10%. Limits of determination were in the range of 0.5–50 μg/kg.

The simplified approach has shown its potential for a variety of samples, such as milk, pork fat, animal feed and cod liver oil, showing its general applicability to fatty samples.

Keywords: Milk; Food analysis; Sample preparation; Pesticides; Polychlorinated biphenyls; Organochlorine compounds

1. Introduction

The accumulation of organochlorine compounds in the fatty part of the food chain is still a matter of major concern. Therefore, the development and/or improvement of analytical methods for the efficient determination of these compounds in fatty samples is needed for monitoring purposes. Since 1968, Dutch trend studies on organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human tissue, human milk and human blood showed decreasing levels of OCPs, except for *p,p'*-DDE, to around 10–30 μg/kg of fat, while levels of the predominant PCB congeners numbered 118, 138, 153 and 180 remained at constant levels of approx. 0.1 μg/kg of fat [1,2].

The analysis of organochlorine compounds in fatty samples usually comprises three principal steps; extraction, clean-up and gas chromatographic (GC) analysis. The clean-up step usually serves two purposes; elimination of the fat matrix, which is incompatible with GC analysis, and removal of compounds that may interfere in the final GC analysis. In most of the analytical procedures described to date, the clean-up step is the most laborious one. Therefore, a variety of different clean-up methods have been studied in the literature.

An overview of sample pretreatment techniques used in the analysis of organic micropollutants in fatty samples is given by Liem et al. [3]. The

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provisional standard issued by the European Committee for Standardisation [4] as well as the standard for pesticide analysis provided by the International Dairy Federation (IDF) [5] describe a wide variety of methods applying adsorption chromatography on silica, florisil and alumina as well as sweep-co-distillation and gel permeation chromatography (GPC). The absence of a single reference method for the clean-up of fatty samples indicates that there is still a need for novel approaches. Next to the established methodology cited in the normative literature [4,5], several approaches have been described recently such as the removal of the fatty matrix by means of solid-phase extraction over C_{18} [6], supercritical fluid extraction (SFE) in the presence of a silica sorbent [7] and SFE coupled to supercritical fluid chromatography–GC–electron-capture detection (ECD) [8].

The most widely used clean-up technique for pesticides in fatty foodstuffs nowadays is GPC. Important advantages of this technique are that it can easily be automated and has a large scope for pesticide residue analysis, since separation occurs on the basis of molecular size. Hence, relatively large molecules such as chlorophylls and triglycerides are separated from the lower-molecular-mass pesticides. An important drawback of GPC is that the triglycerides, which are usually present at the mg level, elute before the pesticides, which are present at the μg to ng level. Some tailing of the large triglyceride peak is inevitable and makes on-line combinations of GPC and capillary GC difficult to realize [9,10]. To overcome this problem, some authors suggest an additional off-line clean-up step over silica [11,12]. However this leads to a much more laborious clean-up procedure with extra evaporation steps prior to the second clean-up and the actual GC analysis. For this reason one-step clean-up procedures are preferable since they allow a high sample throughput with a minimal amount of manual evaporation procedures.

Since 1968, our Institute has been involved in the determination of OCPs in human tissues. The clean-up procedure initially used, consisted of an off-line procedure over alumina during which triglycerides were removed from the analytes, followed by separation of the PCBs and OCPs over silica [13]. The drawback is the tedious adjustment of the alumina by means of water addition. Adjustment of the sorbent requires off-line recovery experiments which may take a considerable amount of time. Normal-phase column liquid chromatography (NPLC) is a good alternative for the above procedure, because it takes less time for optimisation of the method, since the process can be monitored on-line with an UV detector. In an earlier study, Hogendoorn et al. [14] reported a method for the clean-up of OCPs and PCBs in fatty samples that was based on a coupled-column LC system. The reason for using coupled column chromatography was that the inherent separation power of such a system allowed separation between the OCPs and the PCBs. The firm establishment of capillary GC as well the reduction of the levels of the OCPs in real samples diminishes the requirement for separation between the OCPs and the PCBs. Therefore, in this study, the potential of single-column LC is explored. A wide range of fatty sample types is investigated including pork fat, human milk, cod liver oil and animal feed.

2. Experimental

2.1. Materials

Chemicals: All OCPs were obtained from Promochem (purity of >99%, Dr. S. Ehrenstorfer, Wesel, Germany). All stock solutions and further dilutions were prepared in *n*-hexane (Promochem). *n*-Hexane from Baker (Deventer, Netherlands) and dichloromethane from Promochem were used as LC eluents without any further pretreatment.

LC equipment: The set-up of the LC system used for all sample types is depicted in Fig. 1. The LC system used in the clean-up procedure consisted of a Gilson 231 autosampler from Gilson (Villiers le Bel, France) equipped with an injection loop of 1 ml. A Spectroflow 400 pump (Ramsey, NJ, USA) was used for the delivery of *n*-hexane, at a flow-rate of 1.0 ml/min, and a Model 9208 pump from Kipp Analytica (Delft, Netherlands) was used for the delivery of dichloromethane, which was used to backflush the LC column at a flow-rate of 2.0 ml/min. Furthermore a high-pressure six-port valve Type 7000 and a low-pressure three-way valve Type 5300 from Rheodyne (Cotati, CA, USA), a Model 441 UV detector from Waters (Milford, MA, USA)

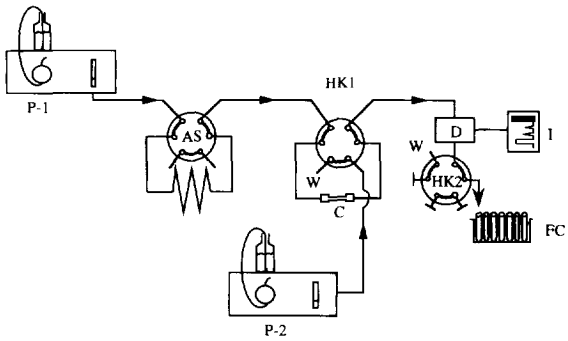

Fig. 1. Schematic representation of the equipment used in the NPLC clean-up of fatty samples. P-1, LC-pump (*n*-hexane); P-2, LC-pump (dichloromethane); AS, autosampler; HK1-2, high-pressure six-way valves; FC, fraction collector; C, LC column; D, UV detector; I, integrator; W, waste.

equipped with a zinc lamp and a 214-nm filter, a Model 7000 fraction collector from LKB (Bromma, Sweden) and a laboratory-made programmable logic controller for the time-based switching of the two LC valves were used.

Fat clean-up was performed on either a 60×4.6 mm I.D. column that was laboratory-packed with 10 μm LiChrosorb from Merck (Darmstadt, Germany) or on a 50×4.6 mm I.D. column packed with 3 μm Chromspher from Chrompack (code no. 28002, Bergen op Zoom, Netherlands). After LC clean-up, the fat was removed from the LC column by a backflush step with 10 ml of dichloromethane at a flow-rate of 2 ml/min. Prior to the next injection, the LC column was reconditioned over 15 min with *n*-hexane at a flow-rate of 1.0 ml/min.

GC equipment: GC analysis was performed on a Varian 3500 GC–ECD system (Walnut Creek, CA, USA) equipped with a type 8100 autosampler. By means of splitless injection, aliquots (2 μl) were analysed. GC separation was performed on a 25 m×0.32 mm capillary column coated with SE-54 (d_f 0.5 μm) (Macherey-Nagel, Düren, Germany) or on a 30 m×0.32 mm I.D. column coated with HP-1 (d_f 0.17 μm) (Hewlett-Packard, Avondale, PA, USA) with a typical oven temperature programme as follows; 80°C over 2 min, at 30°C/min to 150°C, at 2°C/min to 220°C, at 15°C/min to 260°C with a final hold for 15 min.

In procedures where a 10-μl volume of the final organic extract was to be injected, a Mega 5160 GC–ECD system (Fisons, Milan, Italy) with an AS-550 on-column autosampler with a large-volume option was used. A volume of 10 μl was introduced within 8 s into a 3 m×0.53 mm I.D. phesil deactivated retention gap (code number 723560.25) from Macherey-Nagel, which was connected to a 30 m×0.32 mm I.D. GC separation column coated with SE-54 (d_f 1.0 μm) from Macherey-Nagel or to a CP-Sil 19 column (d_f 0.2 μm) from Chrompack. A typical oven temperature programme was 75°C over 3 min, at 20°C/min to 200°C, at 3°C/min to 240°C, at 10°C/min to 270°C, with a final hold for 15 min.

2.2. Procedures

Pork fat: Raw porkfat, cut into small cubes, was transferred into a glass funnel and placed on top of a conical flask. The fat was melted in a heating cabinet at a temperature of 65°C over 6 h. The rendered fat was dissolved in *n*-hexane at a concentration of 45 mg of pork fat/ml. An aliquot containing 1.0 ml of this solution was injected on the LC clean-up system, which was equipped with a laboratory-packed 60×4.6 mm I.D. 10 μm LiChrosorb silica column. The pesticide fraction was eluted in a total fraction volume of 43 ml, after which evaporation in a Kuderna-Danish apparatus was performed to a final sample volume of 1 ml. In order to analyse this final extract by GC–ECD, a 2-μl volume was injected by means of splitless injection.

Human milk: Extraction of the human milk samples was performed according to a modified AOAC procedure [15]. A 1-g amount of sodium oxalate was added per 100 g of sample before extraction. Equal volumes of methanol (Merck), diethyl ether (Merck) and light petroleum (J.T. Baker) were added to the milk sample and extraction was performed manually for 1 min. After extraction, the organic layer was dried over sodium sulfate and evaporated in a Kuderna-Danish apparatus to a few milliliters and then to dryness with nitrogen. Finally, the extract containing the triglycerides, OCPs and PCBs was placed for 30 min in an oven held at 120°C to remove residual traces of solvent. After preparing a solution containing 45 mg of fat/ml, a 0.6-ml volume of this solution was injected in the LC clean-up system. Fat separation was performed on the 50×4.6 mm I.D. Chromspher 3 μm silica

column; the total elution volume for the OCP fraction was 12 ml. After evaporation of this fraction to 1.0 ml with nitrogen, a 10-μl volume was introduced by means of on-column injection in order to determine the OCPs with GC–ECD.

Cod liver oil: 900 mg of fat were dissolved in 20 ml of *n*-hexane to obtain a final fat concentration of 45 mg/ml. The clean-up procedure was identical to that used with the human milk.

After evaporation to 200 μl, GC–ECD analysis was performed by means of the split/splitless injection of 2 μl of sample.

Animal feed: The extraction procedure consisted of the addition of 40 ml of *n*-hexane to 10 g of animal feed. After 1 min of manual shaking, static extraction was performed overnight. Fat determination of the final extract was performed by evaporation to dryness of 2.0 ml of the hexane extract. Taking into account the amount of fat that was originally present in the animal feed sample, an extra amount of soya oil was added to the hexane extract in order to prepare a final fat solution of 45 mg/ml of *n*-hexane. Fat clean-up and GC–ECD analyses were performed as described for human milk.

3. Results and discussion

LC clean-up. During method development for the LC clean-up of fatty samples, several aspects had to be taken into account, such as the maximum solubility of the triglycerides in the mobile phase, the required sensitivity for GC–ECD analysis and the separation capacity of the clean-up column, defined as the amount of fat that can be injected without coelution of the analytes. During the extraction of e.g. milk, polar compounds will remain in the aqueous layer, while the triglycerides will be transferred to the organic layer, together with the more apolar compounds such as the OCPs and PCBs. After extraction, the obtained fat residue containing the compounds of interest must be dissolved in an appropriate organic solvent. In the case of NPLC, *n*-hexane is suitable because of its low solvent strength in NPLC. The solubility of triglycerides in non-polar solvents is limited and from earlier experience we know that precipitation of fat occurs at levels above 45 mg of fat/ml of *n*-hexane. Therefore, the maximum concentration of fat in the extract was fixed at this level.

The amount of sample that can be injected determines the sensitivity of the overall method. In the GC–ECD determination of OCPs, a convenient amount to analyse is about 10 pg injected per OCP. The equivalent of at least 0.2 mg of fat must be injected into the GC in order to reach quantification levels in the order of 10 μg/kg, which is sufficient for most applications.

The main purpose of the clean-up procedure described here is the separation of the fatty matrix from the OCPs, rather than fractionation of the analytes. Hence, complete separation of the fat matrix from the last-eluting OCP, dieldrin, is of major importance. As shown in experiments performed by Hogendoorn et al. [13], the maximum amount of fat that can be injected on a 60×4.6 mm I.D. column packed with 10 μm silica is approx. 45 mg. Increasing the injection volume from 1.0 to 1.2 ml, which corresponds to a fat amount of 54 mg, indeed resulted in an unacceptable loss of separation between dieldrin and triglycerides. In order to determine the fraction volume to be collected after LC clean-up of pork fat, human milk, cod liver oil and animal feed samples, the final fat solution was spiked with dieldrin at a level of approx. 0.1 μg/ml. This solution was used to check the repeatability of the LC procedure by monitoring the UV chromatogram. After selection of the suitable LC conditions, such as injection volume and run time, fat clean-up was performed automatically. Table 1 shows a typical time programme of the clean-up procedure.

Determination of OCPs in pork fat: LC clean-up of the pork fat was performed on 10 μm LiChrosorb silica; the clean-up of 45 mg of matrix resulted in a total OCP fraction of 43 ml. Prior to GC analysis, off-line evaporation in a Kuderna-Danish apparatus was utilized to concentrate the final LC fraction to 1 ml. The OCP recoveries were between 80 and 98%, with a maximal relative standard deviation of 7%; limits of determination (LODs) were 10–50 μg/kg (Table 2).

Cod liver oil: A major drawback of the method described above is the evaporation step which requires considerable manual operation. Decreasing the run-time of the NPLC procedure has two advantages. Obviously, the sample-throughput increases, while

Table 1
Typical time schedule used for the automated sample clean-up (abbreviations according to Fig. 1)

Step	Time (min)	Activated device	Event
1	0	AS	autosampler injects 600 μl and activates the programmable logic controller, integrator and fraction collector
2	0.5	HK-2	start of fraction collection
3	12.0	HK-1, P-2	silica column backflushed with dichloromethane
4	13.0	HK-2	transfer tubing HK-1 to FC is cleaned from step 3
5	17.0	HK-1, P-2	end of backflush and start of column conditioning with *n*-hexane
6	32.0	None	end of column conditioning with *n*-hexane prior to next injection.

the analytes are found in a smaller fraction of solvent, which may make an evaporation step superfluous. In this approach, the retention of analytes is to some extent inversely proportional to the amount of fat injected. However, speeding up the run-time this way also decreases the separation between triglycerides and dieldrin. The column used in the pork fat application given above was a laboratory-packed column with 10 μm LiChrosorb. Smaller particles are known to give faster separations [16]. Therefore, a 50×4.6 mm I.D. column packed with 3 μm silica was tested. A typical chromatogram obtained during optimisation of the LC clean-up on this column is given in Fig. 2. The effect on separation is shown by an injection of increasing amounts of fat. Fig. 2A shows an injection of a 27-mg amount of fat (injection volume, 600 μl), dieldrin is well separated from the first eluting triglycerides at 19 min. Fig. 2B shows an injection of a 32-mg amount of fat (injection volume, 700 μl). Obviously, a small increment in the amount of triglycerides is sufficient to diminish the separation between dieldrin and triglycerides completely.

The column packed with 3 μm particles proved to be very suitable since an injection of 600 μl of the fat solution, corresponding to 27 mg of fat sample, resulted in an improvement in the total fraction volume of 12 ml, which is a decrease in fraction volume of a factor of almost four, when compared with the 10 μm column. This combination of a 3-μm LC column and 27 mg of fat was applied for the clean-up of the cod-liver samples. Overall OCP recoveries, which are in a range of 83–100% with relative standard deviations not greater than 12%, are shown in Table 3. Fig. 3A shows a GC–ECD chromatogram of a cod liver oil sample analyzed after NPLC clean-up; the peaks marked in the chromatogram are in the concentration range of 50 μg/kg for dieldrin (No. 4) to over 600 μg/kg for *p,p'*-DDE (No. 5). The majority of the interfering peaks that are eluted at retention times between 17–27 min are probably caused by toxaphenes present in the cod liver oil. Since toxaphene compounds show a behavior similar to OCPs in the extraction and clean-up procedures, they coelute in the GC chromatogram [17].

Animal feed: In our laboratory, background levels

Table 2
Recoveries and LODs of OCPs in the analysis of pork fat

Compound	Spiking level (μg/kg fat)	Recovery (%)±R.S.D.[a]	LOD[b] (μg/kg fat)
HCB	260	95±6	20
α-HCH	230	96±5	10
γ-HCH	250	98±3	10
β-HCH	510	96±3	40
β-Heptachlorepoxide	190	98±4	30
Dieldrin	200	93±7	30
p,p'-DDE	260	94±4	30
Endrin	310	80±2	40
p,p'-TDE	450	93±5	50
p,p'-DDT	390	94±5	50

[a] $n=4$
[b] $S/N=3$

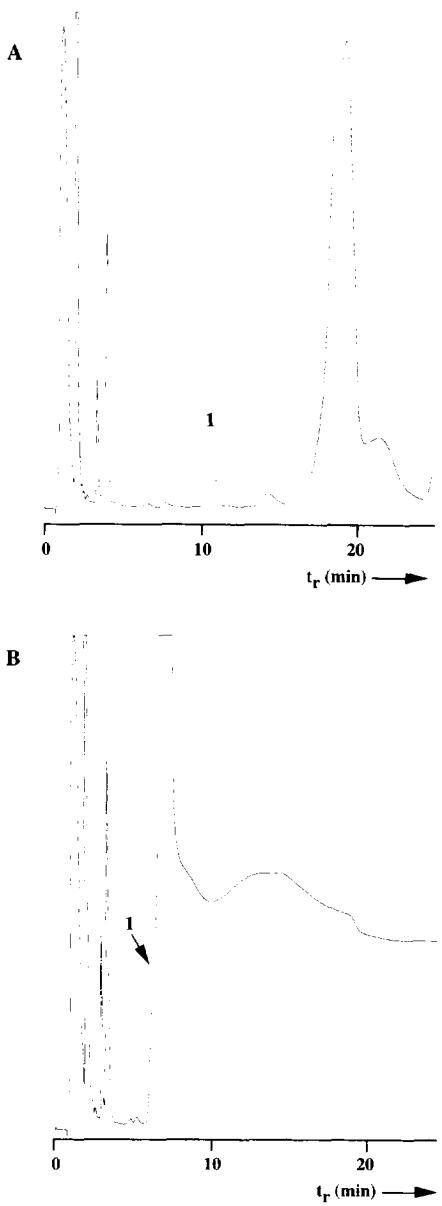

Fig. 2. LC–UV chromatograms in the NPLC clean-up of 27 mg of fat injected (A) and 32 mg fat injected (B) spiked with dieldrin (1) at a level of 0.1 μg/ml.

of OCP residues are monitored in animal feed for rabbits, rats and guinea pigs. The purpose of the analysis is to guarantee the absence of OCPs above a certain level. In order to ensure a safe margin, fortification for recovery experiments is performed at a level approx. 2–10 times lower than the maximum OCP levels tolerated in these samples. The composition of animal feeds can vary from feed with an almost entirely vegetable origin, which contain a minimal amount of fat, to feed that contains additional products of animal origin. The latter results in a rather fatty final extract after static extraction with *n*-hexane, while the first results in an essentially fat-free extract. The extraction procedure results in an extract of 250 mg of animal feed/ml of *n*-hexane. However, the amount of fat injected on the LC column has a strong influence on the separation, hence on the total volume of the OCP fraction that has to be collected. Low fraction volumes have the advantage of less dilution of the sample during the LC step. Therefore, in these cases readjustment of the fat concentration to 45 mg of fat/ml is performed. A vegetable oil is preferable since it usually does not contain the organochlorine compounds of interest in this study. This is done by the addition of soya oil to the original extract to obtain a final fat concentration of 45 mg/ml of *n*-hexane. Table 4 shows the mean recovery results and relative standard deviations as well as the corresponding fortification levels of samples spiked with OCPs at the levels indicated. Fig. 3B shows the GC–ECD chromatogram recorded for a rabbit feed sample spiked with OCPs at a level of 1–6 μg/kg. The limits of determination in these sample types are around 0.1 μg/kg.

3.1. Human milk

OCP analysis. Also in this application, an LC column packed with 3 μm rather than 10 μm LiChrosorb was used and the sample size was reduced to 27 mg as mentioned under cod liver oil. Since no blank human milk is available to check the performance of the extraction and the clean-up method, recovery and repeatability experiments for the determination of OCPs were obtained from spiked cows' milk (Table 5). Method performance was monitored by analysing six double-blind samples, prepared from pooled human milk. These samples were analyzed randomly from among 90 human milk samples during a period of twelve months (Table 6). The results of these control samples show good long-term repeatability for the whole procedure, since the level of the average plus

Table 3
Recoveries and LODs in the determination of OCPs in cod liver oil

Compound	Spiking level (μg/kg oil)	Recovery (%)±R.S.D.[a] (μg/kg oil)	LOD[b]
HCB	14	83± 8	2
α-HCH	53	98± 5	3
γ-HCH	24	90±10	8
β-HCH	80	100± 9	3
α-Chlordane	44	95± 7	6
γ-Chlordane	44	89± 5	6
Oxy-chlordane	68	84± 8	6
Trans-nonachlor	53	88± 8	6
Dieldrin	51	92± 7	6
o,p'-DDE	69	92± 5	9
p,p'-DDE	93	92±12	7
o,p'-TDE	175	93± 9	12
p,p'-TDE	79	99± 7	10
o,p'-DDT	86	90± 7	10
p,p'-DDT	180	98± 7	10

[a] $n=6$
[b] $S/N=3$

twice the standard deviation was never exceeded. Fig. 3C shows a GC–ECD chromatogram of a pooled control sample after NPLC clean-up.

PCB analysis: The application of NPLC clean-up for the determination of indicator PCBs in fatty matrices was performed analogously to the clean-up procedure used for the OCPs, and the same final hexane extract was used as in the OCP clean-up procedure.

In order to determine the elution window of PCBs in human milk, the *n*-hexane extract containing 45 mg of fat/ml was spiked with PCBs 77 and 180, the first and the last eluting PCB of all congeners, respectively. Injection of the spiked fatty extract resulted in a fraction volume of approx. 2 ml only. After clean-up of the samples, the extracts were evaporated and GC analysis was performed without further sample pretreatment for the determination of fourteen PCBs with PCB numbers 28, 52, 60, 74, 101, 105, 114, 118, 138, 153, 156, 157, 167 and 180. Fig. 3D shows a typical GC–ECD chromatogram of a human milk sample after NPLC clean-up. Analogously to the OCP analysis, the method performance of the total procedure was monitored by means of the six double-blind quality control samples. For all PCBs analysed in the range 1–122 ng/g of fat, an overall relative standard deviation of 3–12% was found ($n=6$), except for PCB 101 (24%). The detection limits ($S/N=3$) were 0.5–1.5 ng of PCB/g of fat. When both GC–ECD chromatograms for the human milk samples C (OCPs) and D (PCBs) in Fig. 3 are compared, an ECD baseline with many more interfering peaks is seen in Fig. 3C than in Fig. 3D. The difference is even larger when the absolute amounts of sample injected are taken into account, being a 0.27-mg fat extract for the OCPs and a 1.8 mg fat extract for the PCBs. Absence of interfering peaks is due to the fact that the PCB clean-up is far more selective because only 2 ml is taken from the LC eluent, while for the OCPs a 12-ml fraction is collected.

4. Conclusions and future research

Sample clean-up with a NPLC column has been shown to be an efficient and robust way to separate triglycerides from organochlorine compounds for their analysis in a wide range of fatty samples. Complete fat–OCP separation is obtained in a fraction volume of 12 ml, which results in an automated clean-up procedure of only 32 min per sample. Depending on the determination level required, aliquots of 1 to 100 μl can be introduced into capillary GC contemporary injection techniques after

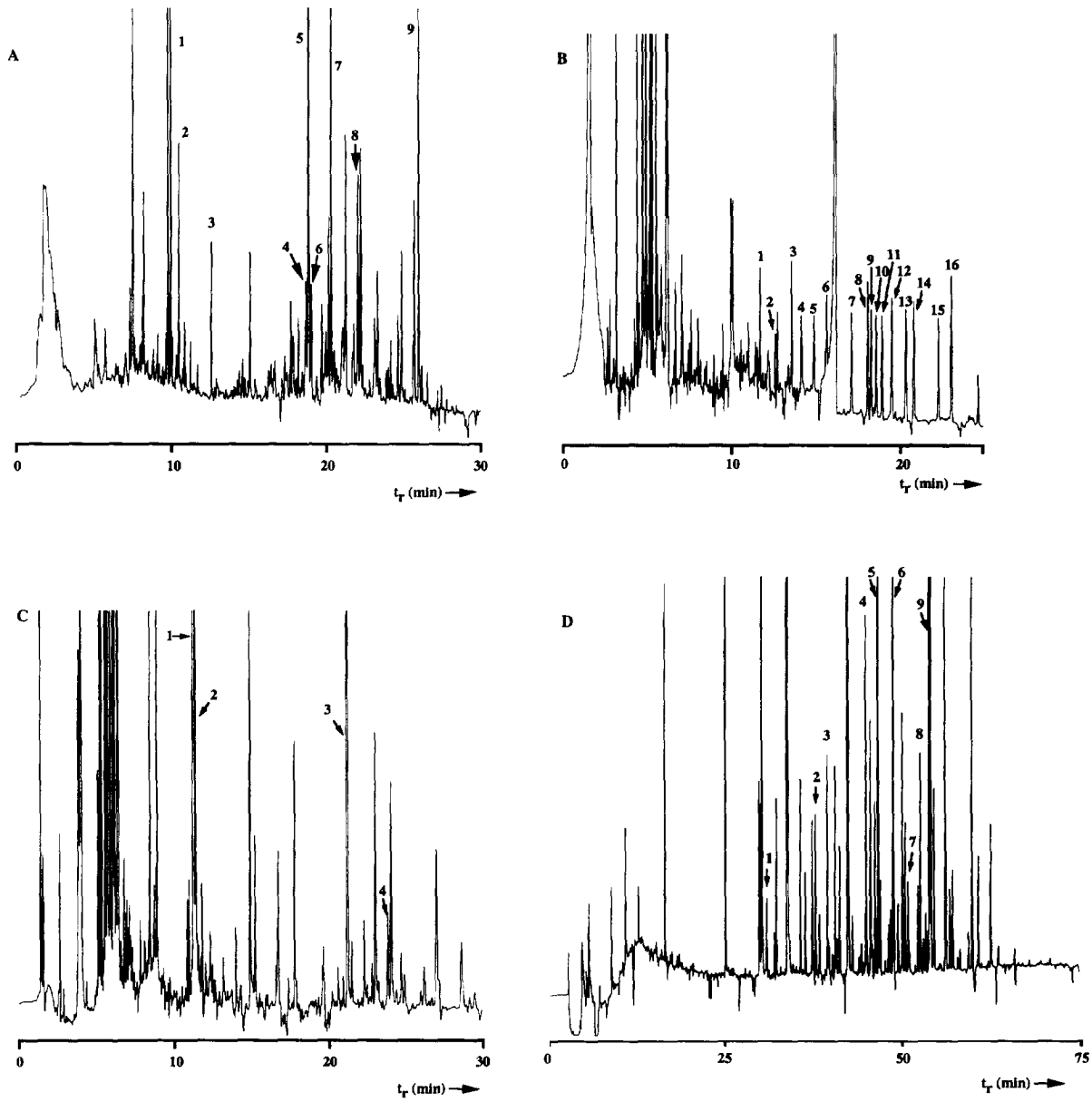

Fig. 3. (A) A GC–ECD chromatogram obtained after LC clean-up of cod liver oil (attenuation ×64). Peak identification: α-HCH (1), HCB (2), PCB 29 (3), dieldrin (4), *p,p'*-DDE (5), *o,p'*-TDE (6), *p,p'*-TDE (7), *p,p'*-DDT (8) and PCB 198 (9). (B) A GC–ECD chromatogram obtained after LC clean-up of rabbit feed (attenuation ×32). Peak identification: HCB (1), α-HCH (2), γ-HCH (3), heptachlor (4), aldrin (5), β-HCH (6), β-hepo (7), α-endosulfan (8), τ-chlordane (9), α-chlordane (10), *p,p'*-DDE (11), dieldrin (12), endrin (13), *o,p'*-DDT (14), *p,p'*-TDE (15) and *p,p'*-DDT (16). (C) A GC–ECD chromatogram of OCPs in human milk after NPLC clean-up (attenuation ×32). Peak identification: HCB (1), β-HCH (2), *p,p'*-DDE (3) and *p,p'*-DDT (4). For the concentration levels see Table 4. (D) A GC–ECD chromatogram of PCBs in human milk after NPLC clean-up. Peak identification: PCB 28 (1), PCB 74 (2), PCB 118 (3), PCB 153 (4), PCB 138 (5), PCB 167 (6) and PCB 180 (7).

Table 4
Recovery levels with corresponding spike levels in the determination of OCPs in rabbit feed

Compound	Spiking level (μg/kg)	Recovery (%)±R.S.D.[a] (μg/kg)	LOD[b]
HCB	1	111±11	0.1
α-HCH	2	62±10	0.1
γ-HCH	2	77±30	0.1
Heptachlor	1	76±15	0.1
Aldrin	2	75± 9	0.1
β-HCH	3	89±10	0.2
β-Heptachlorepoxide	2	84±11	0.1
α-Endosulfan	3	84±11	0.2
γ-Chlordane	3	89± 6	0.2
α-Chlordane	3	78± 7	0.2
p,p'-DDE	3	87± 3	0.2
Dieldrin	3	101±24	0.2
Endrin	4	71± 9	0.2
o,p'-DDT	6	77± 5	0.3
p,p'-TDE	5	76± 4	0.3
p,p'-DDT	6	82± 7	0.3

[a] $n=4$
[b] $S/N=3$

Table 5
Recoveries of OCPs in the analysis of milk

Compounds	Spiking level (μg/kg fat)	Recovery (%)±R.S.D.[a]	LOD[b] (μg/kg fat)
HCB	60	101±5	10
α-HCH	30	107±6	10
β-HCH	90	96±6	10
γ-HCH	50	107±5	10
β-Heptachlorepoxide	80	94±3	20
p,p'-DDE	100	101±5	30
Dieldrin	110	107±7	30
p,p'-TDE	170	96±6	30
o,p'-DDT	180	102±5	30
p,p'-DDT	190	98±4	30

[a] $n=4$
[b] $S/N=10$

Table 6
Results of the double-blind pooled quality control samples in the analysis of OCPs in human milk

Compound	Levels (mg/kg fat)	R.S.D.(%)[a]
HCB	0.06	14
β-HCH	0.07	11
p,p'-DDE	0.87	10
p,p'-DDT	0.05	17

[a] $n=6$

which the obtained fractions can then be analysed by GC–ECD directly.

Future research will focus on on-line coupling of the LC clean-up to GC for this type of application. To that end, LC columns with smaller internal diameters have to be tested in order to study the possibility of obtaining the total OCP fraction in a volume of approx. 200 μl. The application of the method to non-fatty samples is currently under investigation.

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