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Selective pressurized liquid extraction of polychlorinated biphenyls from fat-containing food and feed samples Influence of cell dimensions, solvent type, temperature and flush volume

Sune Sporring, Erland Björklund*

Department of Analytical Chemistry, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

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Abstract

Sulphuric acid impregnated silica was used for the lipid free extraction of polychlorinated biphenyls from fat containing food and feed matrices using pressurized liquid extraction on a Dionex ASE300, with 34 mL cells. Data were compared to a previous publication where extractions had been performed on a Dionex ASE200, with 33 mL cells. Four different fat/fat retainer ratios (FFRs) were tested (0.100, 0.075, 0.050 and 0.025) at 50 and 100 °C using *n*-pentane, *n*-hexane or *n*-heptane as extraction solvent. The best results were obtained with a FFR of 0.025 when applying a temperature of 100 °C. Both *n*-pentane and *n*-heptane were capable of replacing *n*-hexane as extraction solvent. A flush volume of 60% was sufficient as suggested in US Environmental Protection Agency Method 3545. The applicability of the method was demonstrated for naturally contaminated fish meal as well as various spiked and certified materials.

Keywords: Pressurized liquid extraction; Extraction methods; Food analysis; Polychlorinated biphenyls

1. Introduction

As a consequence of the Belgian dioxin crisis [1] the European Union has initiated a large research project to develop fast and cheap analytical methodologies for the determination of polychlorinated biphenyls (PCBs) and dioxins in food and feed matrices [2]. A substantial part of this project is aiming at improved extraction and clean-up methodologies or ultimately a combined approach for a maximized sample throughput. It is well known that extraction of minute levels of contaminants in the presence of sample components such as lipids causes injection problems in gas chromatography, if these are not removed prior to injection. A number of well-established methods for removing these interfering components are available such as gel permeation chromatography or column chromatography on Florisil. The main disadvantages with external clean-up procedures are labour intensiveness and consumption of large volumes of organic solvent waste. Additionally, the classical way of extracting organic contaminants rely on time, and solvent consuming extraction techniques and are therefore gradually being replaced with modern extraction technique such as pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) [3,4].

Until now only a few publications have been published dealing with combined extraction and clean-up procedures utilizing selective PLE with on-line clean-up [5–10]. This combined extraction/clean-up strategy has drastically decreased the time spent on sample handling. Some investigations have been performed for relatively polar compounds such as musk components in fish [6] and corticosteroids in bovine liver [9]. Musk components were selectively extracted from lipids with a mixture of ethyl acetate–hexane (1:5) utilising alumina in the extraction cell to hinder the co-extraction of lipids [6]. In the case of corticosteroids, the fat was removed by selectively extracting it with pure hexane, leaving the analytes of interest behind. These could then be extracted in a second step using ethyl acetate–hexane

* Corresponding author. Tel.: +46-46-2229468; fax: +46-46-2224544.
E-mail address: erland.bjorklund@analykem.lu.se (E. Björklund).

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(1:1) as extraction solvent [9]. The first on-line clean-up attempt ever in PLE was performed for the extraction of PCBs from fish, utilizing acidic alumina in the extraction cell [5]. Thereby the obtained extracts could be analysed directly by GC–electron-capture detection (ECD). In a later work by Björklund et al. several fat retainers (basic alumina, neutral alumina, acidic alumina, Florisil and sulphuric acid impregnated silica) were tested on their fat retaining capability for the extraction of PCBs using *n*-hexane as extraction solvent at 100 °C, and it was found that sulphuric acid impregnated silica was the better choice due to the cleanness of the extracts [7]. Sulphuric acid impregnated silica was then later investigated in more detail changing the static extraction time and the number of cycles [8]. In this paper it was found that a static extraction step of 5 min in combination with two cycles was the best choice for a number of matrices using a fat/fat retainer ratio (FFR) of 0.025. Until now all publications dealing with selective PLE have been utilising the commercial instrumentation ASE200 from Dionex. The largest extraction cell in this system is 33 mL, and this was also the cell size used in the experiments investigating sulphuric acid impregnated silica as fat retainer [7,8]. In this paper the fat retaining capability of sulphuric acid impregnated silica is investigated in the larger system from Dionex, called ASE300. In this system the smallest extraction cell has an extraction cell volume of 34 mL, which is very close to the volume in the 33 mL cells (ASE200). However, the dimensions differ drastically since the cross sectional area in the 33 mL cell is 46% of that in the 34 mL cells. The main objectives of this paper are to investigate possible changes in fat retaining capability with different cell dimensions as well as to study effects of extraction solvent. Most applications dealing with extraction of PCBs from environmental matrices make use of *n*-hexane [including US Environmental Protection Agency (EPA) Method 3545], but since this is a more toxic solvent than other linear alkanes [11], it should be replaced with something less harmful. Additionally the effect of temperature on fat retaining capability of sulphuric acid impregnated silica is scarcely investigated and will be given increased attention here. Another important aspect is to make the packing procedure of the sample cell as simple as possible, and therefore a simplified approach is evaluated here (in comparison to previously published procedures [7,8]) in order to make selective PLE as straight forward as possible.

2. Materials and methods

2.1. Samples

Lard fat consisting of triglycerides was supplied by Swedish Meat Research Institute, Kävlinge, Sweden. Cod-liver oil CRM 349 and pork fat IRMM 446 served as fatty food matrices, and were provided by the IRMM of the European Commission, Joint Research Centre (Geel, Belgium). Naturally contaminated fish meal (11.3% fat) came from State Official Laboratory (ROLT, Tervuren, Belgium) and was used for method development as the PCBs had been naturally incorporated into the matrix. Commercially available feed for poultry “Becco Giallo” (Raggio di Sole Mangimi, Italy) and a vegetable feedstuff prepared at the JRC of the European Commission (Ispra, Italy) were used as feeding stuff matrices. Feed for poultry contained mainly maize, soybean, wheat and maize gluten, with a fat content of 4.0%, while the vegetable feedstuff contained some 13 ingredients e.g. wheat, citrus pulp, molasses, minerals etc., with a fat content of 4.0%.

2.2. Chemicals

Acetone D (for analysis of dioxins), *n*-pentane, *n*-hexane, *n*-heptane (Pestanal grade) were obtained from Riedel-de Haën, Germany, who also supplied the sulphuric acid (analytical reagent grade, 95–97%). Sodium sulphate (purriss, analytical-reagent grade >99%) and silica gel 60 were purchased from Fluka, Buchs, Switzerland, Germany and baked at 400 °C for 10 h prior to use. Impregnated silica was prepared by heating 600 g of silica gel 60 for 10 h to 400 °C and adding to the cold material 400 g of sulphuric acid. Glass microfibre filters GF/A for covering cell caps came from Whatman, Maidstone, UK.

2.3. PCB-standard solutions

PCB standard solutions were prepared from a certified reference material, NIST 2262 (Gaithersburg, MD, USA) containing a total of 28 PCBs. Seven different calibration solutions were prepared in the interval 1–40 ng/mL in *n*-heptane. PCB 35 and PCB 169 (Larodan, Malmö, Sweden) were used as internal standards (IS). PCB 35 (time reference) and PCB 169 (quantification) were added (50 μL) to each sample prior to evaporation and analysis. The concentration of this IS solution was 540 and 400 ng/mL in *n*-heptane for the two PCBs, respectively.

2.4. Equipment

2.4.1. Standard parameters for the PLE extraction

All extractions were performed on an ASE300 System (Dionex, Sunnyvale, CA, USA). Sodium sulphate was used to fill up the dead volume. The samples were mixed with sodium sulphate. Grinding was performed with mortar and pestle. The packing of the extraction cell can be seen in Fig. 1. When compared to the previously published procedure [7], this is a simplified packing procedure since only sodium sulphate is used to fill up the dead volume of the extraction cell. Another advantage is the low cost of purchase for all components in this packing procedure. Sodium sulphate, sulphuric acid and silica are all cheap components. Additionally both sodium sulphate and silica can easily be cleaned by heating to high temperatures. The final extract was always in the range of 30–40 mL when using a thimble

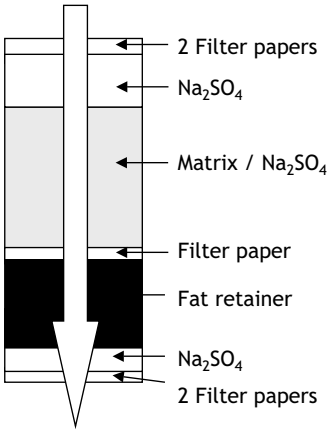

Fig. 1. Packing of the extraction cell.

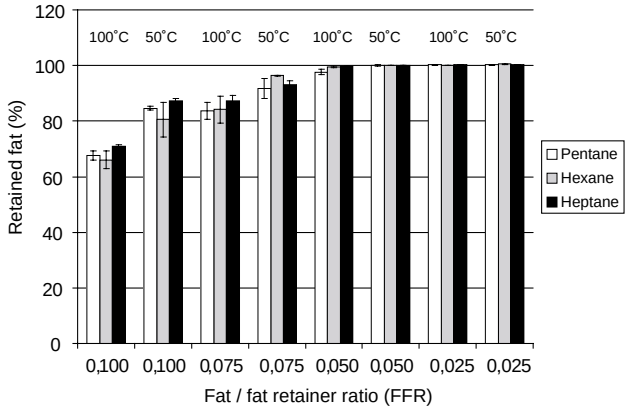

Fig. 2. Amount of fat retained for sulphuric acid impregnated silica using different FFR ratios at two temperatures (50 and 100 °C) with three different solvent types (*n*-pentane, *n*-hexane and *n*-heptane). All extractions were performed with 0.50 g lard fat and increasing amounts of fat retainer. Each data point is an average of three measurements, error bars represents S.E.M.

size of 34 mL. Directly after the extraction step, 50 μL of the IS solution was added to the extracts and concentrated to about 1 mL using a rotary evaporator.

2.4.2. Sample clean-up

The samples from the non-selective extraction (using EPA Method 3545) were evaporated to 1 mL using a rotary evaporator. Samples were then passed through columns packed with silica gel impregnated with sulfuric acid (40% (w/w), H_2SO_4). The packed columns were pre-conditioned with 50 mL *n*-hexane prior to adding the sample extracts. Elution was done with 50 mL of *n*-hexane. After clean-up the samples were once again reduced to 1 mL and transferred to GC vials for analysis.

2.4.3. Fat determination

The fat content was determined gravimetrically using an analytical balance Sartorius MC1 RC 210D (Sartorius, Göttingen, Germany). By weighing the residue in the glass after evaporation of the solvent, the fat content of the sample could be determined.

2.4.4. Gas chromatographic analysis

All PCB analyses throughout the experiment were done by dual column GC–μECD using an Agilent 6890N GC system with a 7683 auto injector and auto sampler (Agilent, Palo Alto, CA, USA). All other GC parameters were as previously described for a dual-column system using a DB17 in parallel to a combined HP5–HT5 [12].

3. Results and discussion

The starting point for the investigation was to study the fat retaining capability of sulphuric acid impregnated silica under different extraction conditions using pure lard fat (triglycerides) as fat matrix. In all cases 0.50 g of lard fat was used in four different combinations; 0.50 g fat combined with 5.0, 6.7, 10.0 or finally 20.0 g retainer. These combinations resulted in FFR ratios of 0.100, 0.075, 0.050 and 0.025. All combinations were tested at two temperatures (50 and 100 °C) with three different solvent types (*n*-pentane, *n*-hexane and *n*-heptane). Each experiment was performed in triplicate. The results are presented in Fig. 2.

When applying a FFR of 0.025 the amount of co-extracted fat never exceeded 0.5 mg for any solvent at any temperature. This was also true when applying a FFR of 0.050 at 50 °C. However, the most interesting observation to be made was that a FFR of 0.050 also gave rather low levels of co-eluting fat at 100 °C. For *n*-hexane and *n*-heptane the amount of co-eluted fat was 2.2 and 1.2 mg, respectively, while *n*-pentane seemed less efficient with a co-elution of 12.5 mg fat. These findings differed somewhat from previous experiments where *n*-hexane was applied in 33 mL cells in the ASE200 system [7]. In that case only 95% of the triglycerides were retained meaning that ca. 25 mg of the total amount of 500 mg fat was co-eluted. Even as little as 10 mg of co-eluted fat has been shown to cause suppressed recoveries of PCBs when raw PLE extracts were injected directly into the chromatographic system [8]. These results indicated that the 34 mL cells, with a larger cross-sectional area, might be somewhat more efficient in removing fat, but without doubt they were not less efficient. Consequently, also for the larger cell size of 34 mL, a FFR ratio of 0.025 can be used (and possibly also a FFR ratio of 0.050). Secondly, it can be concluded that all three solvent types behave basically the same, meaning that *n*-hexane can be replaced with one of the other less harmful solvents. Finally, the fat retaining capability increases when the temperature goes down. Similar findings have been presented in another application where PCBs were extracted from dried spoonbill eggs with a lipid content of 42% [10]. In that case fat retention was performed with Florisil using 15% dichloromethane in *n*-pentane as extraction solvent. The changes in fat retaining capability, when lowering the temperature from 125 to 60 °C, went

from about 60 to 90%. The FFR ratio used was 0.14 (0.84 g fat/6 g Florisil), however, it should be noted that Florisil has a much lower density, and therefore only half the amount of Florisil is normally used for this type of fat retainer when compared to for example alumina or sulphuric acid impregnated silica [7]. On the other hand Florisil is more efficient in removing fat on a weight basis, and therefore a FFR ratio of 0.14 for Florisil equals a FFR ratio of about 0.070 for alumina and sulphuric acid impregnated silica [7,8]. Comparing the above increase of fat retaining capability from 60% (125 °C) to 90% (60 °C) with corresponding values of sulphuric acid impregnated silica at 100 and 50 °C, with a FFR ratio of 0.075 (Fig. 2), gives at hand that the change in fat retaining capability for pure *n*-pentane is only about 8%. This demonstrates that sulphuric acid impregnated silica is much less sensitive to changes in temperature, even though part of this of course could be due to that the experiments were not performed under exactly identical conditions. However, it should be pointed out that in order to get a completely fat free extract in the application with spoon bill eggs and Florisil, the temperature had to be lowered to 30 °C, which is rather unsatisfactory in terms of recovery of the PCBs. Additionally dichloromethane had to be utilised, which should be avoided since it today is considered an environmental contaminant in itself. Based on the data presented in Fig. 2 and the above discussion, sulphuric acid impregnated silica is still considered the best choice for on-line fat removal when extracting acid resistant analytes, keeping the temperature reasonably high.

In order to verify that the two alternative solvents *n*-pentane and *n*-heptane were capable of replacing *n*-hexane they were also tested on a naturally contaminated fish meal sample to determine whether they behaved differently when extracting a real world matrix. This fish meal has been extensively investigated and data are available from a European Intercomparison study [13] as well as from previously published in-house determinations [7]. It was also available in relatively large quantities. Prior to testing the performance of *n*-pentane and *n*-heptane, the fish meal was extracted with non-selective PLE according to EPA Method 3545 using *n*-hexane–acetone (1:1 (v/v)) as extraction solvent, followed by external clean-up on sulphuric acid impregnated silica. These data are presented and compared to previously published values in Table 1.

From the table it is clear that the values obtained with non-selective PLE combined with dual-column GC–ECD compares reasonably well with both Intercomparison values as well as values obtained with different extraction techniques in a different laboratory using GC–MS as the final analytical method. However, it is inevitable that the obtained values will differ from previous data, but it is also clear that no general trend in terms of over or underestimation of the investigated PCBs occurs. In the following the data obtained for the fish meal with EPA Method 3545 combined with dual-column GC–ECD will be used as a measure of 100% recovery except for PCB 101 as indicated in Table 1. Additionally PCB 28 is excluded from the recovery studies below since it is at the limit of detection of the system used in this study.

The PCB recoveries obtained with on-line clean-up of fishmeal with a FFR ratio of 0.050 are presented in Table 2. The reason for applying a FFR ratio of 0.050 is that the data presented in Fig. 2 indicated that this might be possible, still achieving reasonably fat free extracts.

Quantitative recoveries for all combinations of solvents and temperatures (column 1–4 in Table 2) were obtained, even though a small decrease in recovery was observed for some PCBs. From previous investigations it is known that this can be caused by co-extraction of fat [8]. However, an alternative explanation might be that a too small flush volume (FV) was used since it has been shown that a FV value of 150% in some cases increases the extraction efficiency [10]. The results in column 1–4 in Table 2 were all done with a FV of 60%, which is a standard setting according to US EPA Method 3545. Therefore, some additional experiments were performed at 100 °C with *n*-heptane, varying the

Table 1
Determination of PCBs using PLE according to US EPA Method 3545 with *n*-hexane/acetone (1:1 (v/v)) as extraction solvent, followed by external clean-up on sulphuric acid impregnated silica

PCB	Intercomparison [13]		Soxhlet [7]		Cold column [7]		PLE EPA method 3545	
	Concentration (ng/g)	R.S.D. (%) (n = 20–23)	Concentration (ng/g)	R.S.D. (%) (n =3)	Concentration (ng/g)	R.S.D. (%) (n = 8)	Concentration (ng/g)	R.S.D. (%) (n = 3)
28	0.56	36	0.52	3.6	0.48	4.9	0.39	14
52	1.31	17	0.96	3.2	1.05	5.4	1.14	10
101	2.76	14	2.30	5.8	2.31	4.5	3.30[a]	10
118	4.07	15	4.12	1.1	4.06	5.6	4.51	5
138	9.55	10	10.11	2.8	9.67	2.4	8.56	10
153	13.46	13	12.50	6.0	12.52	2.6	9.06	11
180	4.32	13	5.34	2.1	5.61	1.9	4.80	2

These data are presented and compared to previously published data from a European Intercomparison study [13] as well as previously published determinations [7].

[a] Overestimated value due to unknown interfering peak. The value from the European Intercomparison study is used as a measure of 100% for this congener.

Table 2
PCB recoveries from fishmeal extracted with *n*-pentane and *n*-heptane at 50 and 100 °C, excluding PCB 28 from the average calculations

PCB	*n*-Pentane 50 °C, FV 60%		*n*-Pentane 100 °C, FV 60%		*n*-Heptane 50 °C, FV 60%		*n*-Heptane 100 °C, FV 60%		*n*-Heptane 100 °C, FV 50%		*n*-Heptane 100 °C, FV 100%		*n*-Heptane 100 °C, FV 150%	
	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)
28	70	4.7	85	20	65	12	82	2.2	47	3.5	44	34	45	2.3
52	113	3.3	98	10	109	6.6	109	15	63	16	72	4.7	66	7.9
101	83	5.3	78	8.7	77	3.1	69	5.0	51	7.5	49	5.2	47	5.8
118	91	3.5	89	12	91	3.7	90	2.0	62	5.3	57	6.8	58	3.2
138	91	5.6	93	11	92	1.1	87	2.9	63	6.1	64	2.7	65	3.5
153	101	3.0	104	7.1	104	1.6	100	5.3	80	11	84	8.2	87	1.3
180	88	1.3	85	8.9	85	1.1	86	3.6	66	10	71	3.1	73	0.1
Average	94		91		93		90		64		66		66	
Fat (mg)	1.0	8	0.9	9	0.9	4	2.0	19	8.1	36	15.4	14	13.3	34

Extraction conditions were 5 min with two cycles using 5.0 g fishmeal (corresponding to 560 mg fat) combined with 11.3 g sulphuric acid impregnated silica giving a FFR ratio of 0.050. The flush volume (FV) was varied between 50–150%, where 60% FV is a standard setting according to EPA Method 3545. All recoveries are based on the PCB concentrations obtained with EPA Method 3545 seen in Table 1.

Table 3
PCB recoveries from fishmeal extracted with *n*-heptane at 100 °C, excluding PCB 28 from the average calculations

PCB	*n*-Heptane 100 °C, FV 50%, Step 1		*n*-Heptane 100 °C, FV 100%, Step 1		*n*-Heptane 100 °C, FV 150%, Step 1		*n*-Heptane 100 °C, FV 50%, Step 2		*n*-Heptane 100 °C, FV 100%, Step 2		*n*-Heptane 100 °C, FV 150%, Step 2	
	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)	Recovery (%)	R.S.D. (%) ($n = 3$)
28	94	23	69	30	85	3.5	17	19	9	87	13	15
52	112	15	94	24	94	5.2	4.1	23	3.4	87	6.3	31
101	74	3.3	72	6.2	74	11	1.3	40	1.2	11	1.4	32
118	93	3.0	91	2.4	93	3.9	1.3	41	1.9	32	1.1	25
138	95	4.5	94	2.7	91	3.0	0.9	36	0.5	90	0.8	10
153	92	2.3	92	4.9	89	4.2	0.5	30	0.4	17	0.4	18
180	93	1.7	98	3.8	91	2.0	1.0	15	1.2	24	1.3	14
Average	93		90		89		1.5		1.4		1.9	
Fat (mg)	0.3	14	0.3	15	0.4	7	0.1	74	0.2	17	0.1	57

Extraction conditions were 5 min with two cycles using 2.5 g fishmeal (corresponding to 280 mg fat) combined with 11.3 g sulphuric acid impregnated silica giving a FFR ratio of 0.025. The flush volume (FV) was varied between 50–150%, where 60% FV is a standard setting according to EPA Method 3545. All recoveries are based on the PCB concentrations obtained with EPA Method 3545 seen in Table 1.

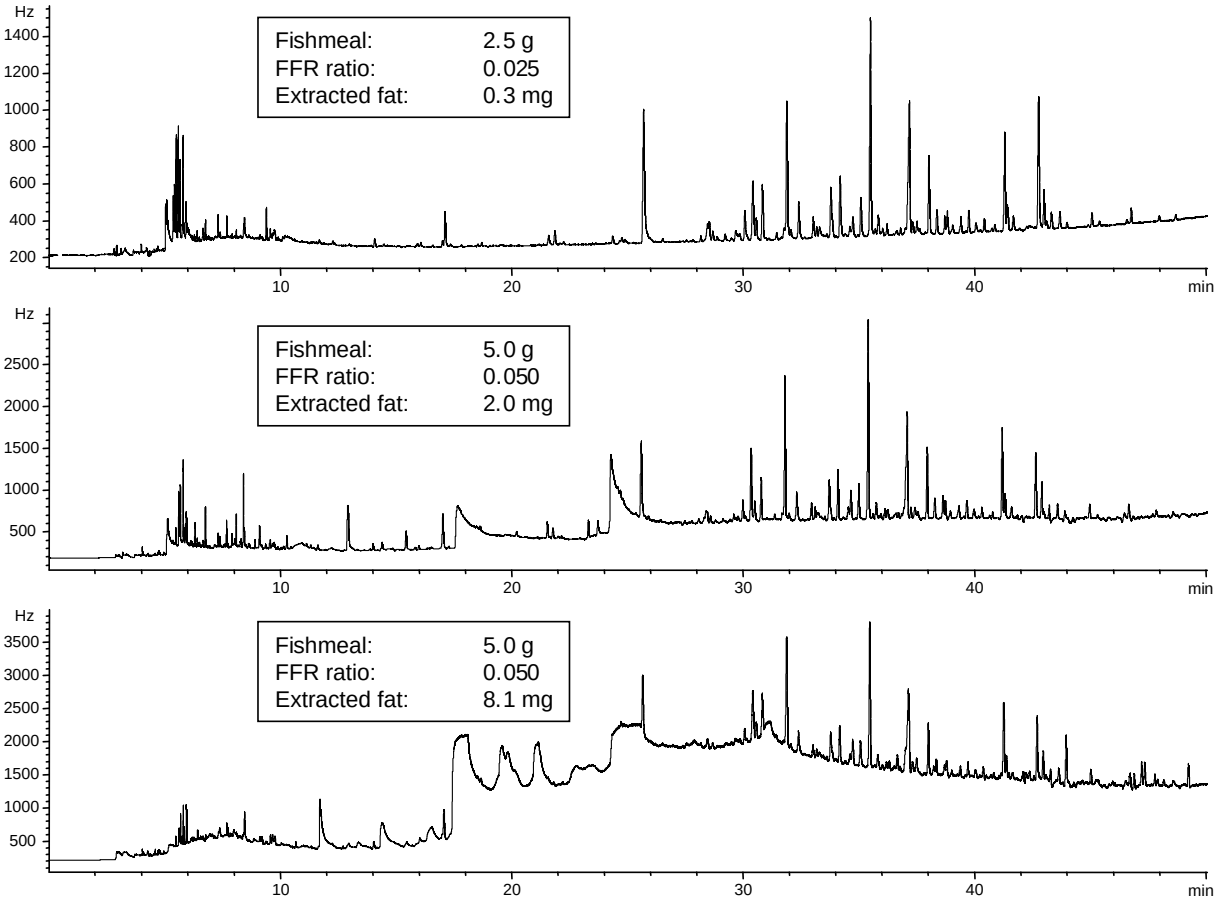

Fig. 3. Influence of the amount co-extracted fat on the chromatographic behaviour of PCBs.

flush volume from 50–150% (Table 2). In these experiments major fat breakthrough (10–15 mg) occurred independent of flushvolume, and as a consequence all PCB recoveries were suppressed. From these findings it was clear that a FFR ratio of 0.050 was too close to the limit of what could be used, even though the results in Fig. 2 showed a potential for using a FFR ratio of 0.050. However, for *n*-pentane in Fig. 2 the breakthrough was 12.5 mg, which is in the same range as those observed for *n*-heptane in Table 2 meaning that the low co-elution of fat in Fig. 2 for *n*-heptane is coincidental. Since the extracts in column 1–4 had fewer breakthroughs than those in column 5–7, the exact FFR ratios were calculated for all columns. For column 1–4 they were always between 0.0499 and 0.0501, while for column 5–7 they varied between 0.0463 and 0.0499. Even though the ratios in column 5–7 were in better favour of generating fat free extracts, this was not the case. It could therefore be concluded that small differences in FFR ratios were not causing these problems, but simply being close to a FFR ratio of 0.050, from time to time caused incomplete burning of fat. Since the extracts in column 5–7 contained larger quantities of fat, the chromatograms were also more severely contaminated (Fig. 3, discussed below). A more detailed analysis of the amount of co-extracted fat in the various experiments in Fig. 2, Tables 2 and 3 using FFR ratios of 0.050 and 0.025 at 100 °C for different solvents revealed that when using a FFR ratio of 0.050, the amount of co-extracted fat can be anything from less than 1 mg up to almost 20 mg. However, with a FFR ratio of 0.025 the amount co-eluted fat never exceeds 0.5 mg.

New experiments with a FFR ratio of 0.025 were therefore performed at 100 °C with *n*-heptane, varying the flush volume between 50–150% (Table 3.).

No fat was co-extracted when using a FFR ratio of 0.025 (columns 1–3, Table 3), and the chromatograms showed very nice base-lines as seen in Fig. 3. From this figure the effects of co-extracted fat are also clearly indicated such as the problem of performing a good quantitative chromatography when unwanted matrix components are present in the extracts. Since the PCB recoveries in columns 1–3 in Table 3 were between 90 and 93%, the extraction cells were extracted one more time to verify that the extraction process was quantitative. This was the case since the recoveries in this second step were only 1–2%. Additionally no difference between FV values could be observed, and therefore the standard setting of 60% suggested in EPA Method 3545 was still considered a good choice keeping the total amount of solvent used low. Since good recoveries in combination with fat free extracts were obtained, the final method of *n*-heptane at 100 °C for 5 min in two cycles with a FV of

Table 4
PCB recoveries for spiked and certified reference materials extracted with *n*-heptane at 100 °C, excluding PCB 28 from the average calculations

PCB	Vegetable feedstuff			Feed for poultry			Mackerel oil (BCR 350)			Pork fat (IRMM 446)		
	Spiked (ng/g)	Recovery (%)	R.S.D. (%)	Spiked (ng/g)	Recovery (%)	R.S.D. (%)	Certified (ng/g)	Recovery (%)	R.S.D. (%)	Certified (ng/g)	Recovery (%)	R.S.D. (%)
28	9.7	103	1.2	9.7	94	2.4	22.5	73	7.8	29.6	91	11.7
52	9.9	110	4.0	9.9	91	2.2	62.0	100	5.5	25.5	89	6.2
101	9.8	105	7.2	9.8	84	4.7	165.0	93	4.0	30.0	81	10.2
118	9.8	98	4.8	9.8	88	2.8	143.0	89	4.7	30.2	88	8.6
138	9.6	113	1.8	9.6	87	1.6	317.0	55	1.0	30.8	46	2.2
153	9.6	109	1.6	9.6	86	0.2	–	–	–	32.0	69	7.9
180	9.6	131	2.2	9.6	92	5.1	73.0	77	8.6	29.8	73	7.3
Average	–	110	–	–	89	–	–	81	–	–	77	–

Extraction conditions were 5 min with two cycles and a FV of 60%. The spiked matrices were 5 g vegetable feedstuff and 5 g feed for poultry (corresponding to 200 mg fat) combined with 8.0 g sulphuric acid impregnated silica giving a FFR ratio of 0.025. Certified reference materials were 250 mg mackerel oil and 250 mg pork fat combined with 10.0 g sulphuric acid impregnated silica giving a FFR ratio of 0.025. All recoveries are based on spiked concentrations or those reported for certified reference materials.

60% and a FFR ratio of 0.025 was tested on several certified food and feed matrices. The results from these extractions are seen in Table 4.

The results demonstrate that the method is capable of generating quantitative, fat free extracts ready for analysis with a minimum of time spent on sample handling for various fatty food and feed matrices.

Acknowledgements

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