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On-line clean-up of pressurized liquid extracts for the determination of polychlorinated biphenyls in feedingstuffs and food matrices using gas chromatography–mass spectrometry

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Abstract

This paper describes a fast and simple pressurized liquid extraction method for the determination of polychlorinated biphenyls (PCBs) in feedingstuffs and food matrices. The method is based on a simultaneous extraction/clean-up step requiring a minimum of sample handling. The final analysis was performed with gas chromatography–mass spectrometry. Seven PCBs (28, 52, 101, 118, 138, 153 and 180) were analyzed, all of which are indicator congeners that, according to European legislation should be included in the analytical monitoring program. The extracted matrices were spiked feed for poultry and two certified reference materials naturally contaminated with PCBs (cod-liver oil and milk powder), which showed excellent conformity with certified data.

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

1. Introduction

Today there is a major concern regarding the quality of food and feedingstuffs because of the feed poisoning episode that occurred in Belgium in May 1999 [1]. Various feedingstuff samples contained high levels of dioxins as well as polychlorinated biphenyls (PCBs), and analysis of food samples such as pork and chicken meat showed a large contamination with PCBs, exceeding the tolerance level set by the European Commission (200 ng, sum of seven indicator PCBs/g fat, including PCBs 28, 52, 101, 118, 138, 153 and 180) by a factor of 250 [2]. In order to handle such crises, rapid and reliable methods must be available to instantaneously give relevant concentration levels for political action. Some of the most frequently used methods are given in the CEN guidelines (European Committee for Standardization) presenting various strategies for extraction, clean-up and quantitative analysis of PCBs [3–6]. Most of these methodologies are however rather tedious and there is a great need for novel and faster technologies. Recently Von Holst et al. [7] presented a simplified method employing a cold column extraction procedure followed by a sulfuric acid treatment as clean-up step, decreasing the

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overall time spent on each sample. The upper *n*-hexane phase was injected directly into a gas chromatography–mass spectrometry (GC–MS) system. This method was used to analyze a large number of pork samples belonging to a shipment of meat sent from Belgium to Russia [8]. Even though this method reduces the workload compared to normally used extraction and clean-up procedures, avoiding the initial cold column extraction step could save additional time. In the last few years a number of new sample preparation techniques have entered the market such as supercritical fluid extraction (SFE) [9], pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) [10], and microwave-assisted extraction (MAE) [11,12] These techniques have several advantages compared to the conventional approaches such as reduced solvent consumption, decreased extraction time and possibilities of automation The latest contribution to this increasing number of extraction techniques is PLE, which appeared for the first time in the mid-1990s [13,14] presenting extraction data for PCBs, polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides and herbicides from environmental matrices. The technique is relatively matrix independent and the main reason for an improved extraction speed is the possibility of using elevated temperatures and pressures, similarly to other modern extraction techniques (SFE and MAE). Until now, PCBs have been determined in a variety of environmental matrices including soil [15], sediment [16,17], sewage sludge [17,18], urban dust [19] and suspended particle material [20], showing good recoveries compared to Soxhlet data and certified values. PLE has also been used with some success for the extraction of PCBs in whole ground carp [16], mussel tissue [16] and oyster tissue [18] demonstrating that this technique is also promising in the food application area. Unfortunately, the lipids present in food samples co-elute with the PCBs and must be removed prior to injection into the chromatographic system. For mussel and fish tissues, size-exclusion chromatography has been utilized on PLE extracts (using as much as 70 ml of methylene chloride) prior to injection into a gas chromatograph with electron-capture detection [16]. It would of course be desirable to obtain a PLE extract ready for analysis without any time consuming clean-up steps and additional solvent consumption. Simultaneous extraction and clean-up of various contaminants in biological matrices were developed for the determination of PAHs in crab tissues [21] employing SFE and PCBs in human blood using solid-phase extraction [22]. Moreover, selective PLE of PCBs from fish tissue at a concentration level of about 200 ng/g fat for each congener has been reported by Ezzel et al. [23] using alumina as fat retainer.

In this paper a PLE methodology with on-line lipid removal (sulfuric acid treatment) is outlined with a final detection using GC–MS in the single ion monitoring (SIM) mode. This approach combines a fast extraction technique with a highly selective final detection system, drastically increasing the sample throughput. The method proposed in this paper is capable of screening a high number of samples and selecting for positive samples that could be extracted again for subsequent fat determination if this is required. Applying this method is therefore an accurate and cost-effective program for the high throughput detection of PCBs in food and feed samples.

2. Experimental

2.1. Samples

Commercially available feed for poultry ''Becco Giallo'' (Raggio di Sole Mangimi, Italy) was used as feedingstuff matrix. The main ingredients of the feedingstuff were maize, soybean, wheat and maize gluten, with a total fat content of 3% according to the producer of the feed. The fat content of the feed was increased to 10% by adding pure pork fat (lard). The lard was bought in a local Italian supermarket. The feeding stuff was spiked with 25 to 100 μl of a PCB spiking solution depending on the final matrix amount subjected to analysis. This resulted in a total of 20 ng/g fat of each congener (see Section 2.2). This level is clearly above the decision limits which previously have been shown to be 3 and 7 ng/g fat for food and feeding stuffs, respectively, using a similar approach [7]. However, the sum of the seven indicator PCBs was 140 ng/g fat which is well below the tolerance level of 200 ng/g fat.

The certified reference materials (CRMs), cod-

liver oil CRM 349 [24] and milk powder CRM 450 [25] were provided by the Institute for Reference Materials and Measurements of the European Commissions Joint Research Centre (IRMM, Geel, Belgium). These certified reference materials represents both high PCB concentration levels in the range of 70–1000 ng/g cod-liver oil for individual congeners (CRM 349) and low PCB concentration levels in the range of 1–19 ng/g milkpowder for individual PCB congeners (CRM 450).

2.2. Chemicals

Sodium sulfate (AnalaR) was obtained from BDH (Poole, UK). Sulfuric acid (95–98%), sea sand (analytical-reagent grade), *n*-hexane (SupraSolv, organic trace analysis), were all purchased from Merck (Darmstadt, Germany). The sodium sulfate and the sea sand were heated to 500°C for 6 h before usage.

Silica gel 60 was obtained from Fluka Chemie AG (Buchs, Switzerland), SFE Wet support was delivered from Isco Inc. (Lincoln, NE, USA), and cellulose filters for extracting cell caps came from Dionex Corp. (Sunnyvale, CA, USA). Impregnated silica gel was prepared by heating 600 g of silica gel 60 over night at 200°C and adding to the cold material 400 g of sulfuric acid. Finally this mixture was mixed in a head over heel mixer for 4 h. A PCB spiking solution was prepared by dissolving PCBs 28, 52, 101, 118, 153, 138 and 180 in *n*-hexane. The concentration of each congener was 200 ng/ml. This solution was added to all poultry feed samples in the PLE experiments.

An internal standard solution with a concentration of 1000 ng/ml was prepared by dissolving PCB 209 in *n*-hexane. This standard was added directly to the PLE extracts in all experiments. All PCB congeners were from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Quantitation was based on a three-point calibration curve in the concentration interval 2.5–10 ng/ml with PCB 209 added at 50 ng/ml.

2.3. Equipment

2.3.1. Gas chromatographic analysis

The analyses of the PCB congeners were carried out on a HP GC 6890 equipped with a HP MSD 5973 and a HP 5 MS capillary column (30 m×0.25 mm I.D., film thickness 0.25 μm). All quantifications were based on the added internal standard (PCB 209). The mass spectrometer was operated in the SIM mode and the following masses were measured for each chlorination level of the analyzed PCBs: molecule mass (M) and M+2 for PCBs 28 and 52; M+2 and M+4 for PCBs 101, 118, 138, 153, 180 and 209.

2.3.2. Standard parameters for PLE

The extractions were performed on an ASE 200 system (Dionex). The parameters used during the extraction procedure are listed in Table 1.

These extraction conditions were based on instrumental settings suggested by the manufacturer of the instrument [26]. In some experiments a few of these parameters were changed, as will be seen from the discussion below. The investigated parameters were the extraction temperature, the static time and

Table 1
Standard extraction parameters used in all experiments unless otherwise stated

Temperature[a]	100°C
Static time[a]	5 min
Cycle[a]	2
Solvent	*n*-Hexane
Pressure	10.34 MPa
Heat time	5 min (pre-set value depending on temperature)
Flush volume	60% (pre-set value)
Purge time	90 s (pre-set value)
Cell volume	33 ml
Dead volume material	Sand–sodium sulfate (1:1, w/w)+1 ml SFE Wet support
Sample support	Sand–sodium sulfate (1:1, w/w)

[a] Indicates that the influence of changes on this parameter was tested in some experiments. These changes are presented in Tables 2 and 3.

the number of cycles, otherwise the parameters in Table 1 apply. The reason for not investigating the influence of the pressure is that this parameter has been demonstrated not to have any significant effect on the extraction process [16,27]. The main purpose of applying an increased pressure is to keep the liquid from boiling. The solvent volume ending up in the glass vial was always in the range of 30–40 ml, using a cell size of 33 ml.

Volumes of 100–250 μl of the internal standard, depending of the final volume of the extract subjected to analysis, were added directly to the PLE extracts, resulting in a final concentration of 50 ng/ml of PCB 209.

The PLE extracts were reduced prior to analysis. In the case of poultry feed and cod liver oil the final volume was 5 ml, while for milkpowder it was 2 ml. The heat time, the flush volume and the purge time were preset by the instrument and were not investigated further. In all experiments the dead volume material was sand–sodium sulfate (1:1, w/w) and about 1 ml of SFE wet support was added on top of this. The SFE wet support was introduced for practical reasons, in order not to have grains of sands all the way to the top of the cell when closing it. In all experiments the extraction cell was completely filled according to recommendations from the instrument producer. The matrix was always mixed with a sample support consisting of sand–sodium sulfate (1:1, w/w). The grinding was performed with a mortar and pestle, where the presence of sand supported the mechanical breaking and grinding of the matrix. The extraction cell was packed according to various schemes depending on the parameters to be investigated. These packing procedures are presented in Fig. 1.

In experiments where fat was co-eluted with the extracted PCBs, the fat was removed in an external clean-up procedure by adding concentrated sulfuric acid to the extracts and shaking the samples with a Vortex (FORLAB MT 135) followed by centrifugation and injection of the organic solvent supernatant.

2.4. Fat determination

The fat content was determined gravimetrically using an analytical balance Mettler PM 400 (Mettler Strumenti, Milan, Italy). An aliquot of the extract was transferred to a flask, the solvent was evaporated and the glass and residue were weighted until a constant mass was achieved.

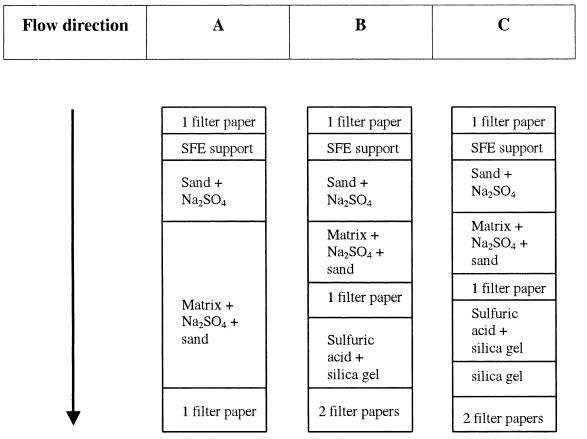

Fig. 1. Packing procedures of the cells in the various method development experiments.

3. Results and discussion

3.1. Extraction of feed for poultry spiked with PCBs

3.1.1. Fat remover and temperature

In some initial experiments the extraction recoveries of fat and PCBs were investigated using *n*-hexane as solvent. This solvent was chosen since it was suggested by the producers of the PLE instrument [26]. Additionally, *n*-hexane will not dissolve any water that might be present in the matrix, which is advantageous for the sulfuric acid clean-up, where water will deactivate the sulfuric acid impregnated silica. In these initial experiments the packing procedure of the extraction cell was according to cells A and B in Fig. 1. Experiments with and without fat remover present in the extraction cell were performed and the effect of a temperature below 100°C was also investigated. The PCB results from these experiments are presented in Table 2.

From the first experiments without fat remover it is clear that *n*-hexane is capable of extracting the spiked PCBs from the sample matrix. However, the fat recovery with packing procedure A (no fat

Table 2
Recovery of spiked PCBs in feed for poultry at two temperatures, with and without sulfuric acid–silica gel as fat remover present in the extraction cell

PCB congener	100°C, no fat remover[a]		100°C fat remover[b]		70°C, fat remover[b]	
	PCB recovery (%)	RSD (%) (n=4)	PCB recovery (%)	RSD (%) (n=4)	PCB recovery (%)	RSD (%) (n=4)
28	97	6.0	99	2.3	103	3.2
52	96	5.2	94	1.9	103	8.0
101	102	3.0	104	6.1	108	4.0
118	99	8.7	104	10.0	106	4.0
153	104	4.7	102	8.4	110	5.8
138	101	4.9	100	4.5	112	4.4
180	104	3.1	99	9.7	108	4.5
Average	100		100		107	

The total sample mass was ca 10 g (9 g matrix spiked to a level of 1 g fat). The matrix was mixed with 5 g sample support. The cell was packed according to procedure A or B in Fig. 1. The coextracted fat was removed by external sulfuric acid clean-up according to the procedure described in the Experimental section. All measurements were made in quadruplicate.

[a] Cell packed according to procedure A in Fig. 1.

[b] Cell packed according to procedure B in Fig. 1.

remover) was 103% (RSD=1.5%) and consequently a fat remover was introduced to decrease the amount of co-extracted fat.

In the first attempts to remove the fat, 5 g sulfuric acid–silica gel (40:60, w/w) was added according to packing procedure B (Fig. 1). Additionally, two filter papers were placed at the bottom of the cell since the usage of only one filter paper showed that this was somewhat darkened by sulfuric acid.

The data clearly show that at both temperatures 70°C and 100°C, *n*-hexane is capable of transporting all PCBs through the sulfuric acid–silica gel mixture since quantitative PCB recoveries were obtained. Applying a fat/fat remover ratio (FFR ratio) of 0.2 (1 g fat to 5 g sulfuric acid impregnated silica gel) the fat recoveries for 70°C and 100°C were 48% (RSD=3.6%) and 45% (RSD=12%), respectively. Even though no difference in extraction behavior for PCBs or fat were observed comparing the two temperatures, the higher temperature was still chosen for the further trial since this is recommended by the instrument producer [26].

3.1.2. Static time, cycles and fat/remover ratio

Some additional trials were performed using *n*-hexane at 100°C, but changing the static extraction time and the number of cycles as presented in Table 3. In these experiments, pure silica gel (1 g) was placed on top of the two bottom filter papers of the extraction cell, just below the sulfuric acid–silica gel mixture (see Fig. 1, packing procedure C). This was done in order to further prevent the sulfuric acid from being transported out of the cell. By doing this neither of the two bottom filter papers darkened in reaction to sulfuric acid, demonstrating that this additional layer of silica gel efficiently stopped the sulfuric acid from leaving the extraction cell.

The PCBs were quantitatively extracted when the number of cycles were decreased from 2 to 1 (keeping the extraction time at 5 min) and likewise the fat recovery was relatively constant (48%, RSD= 5.5%) corresponding to 0.48 g of extracted fat. When the extraction time was decreased to 1 min with one cycle the PCB recoveries were however not quantitative. Therefore a combination of 1 min with two cycles was tested and this once again re-established the PCB quantitative extraction performance with a fat recovery of 45% (RSD=2.7%) since 0.45 g of fat was coextracted. The fact that the fat recovery was very constant (ca. 45–50%) in all experiments where 1 g of fat was applied together with 5 g of fat remover demonstrated that the static time and the number of cycles, only had a small influence on the fat extraction performance. Instead the most im-

Table 3
Recovery of spiked PCBs in feed for poultry at various conditions using sulfuric acid–silica gel as fat remover

PCB congener	5 min, 1 cycle, 10 g matrix, 1 g fat, 5 g fat remover, external clean-up		1 min, 1 cycle, 10 g matrix, 1 g fat, 5 g fat remover, external clean-up		1 min, 2 cycles, 10 g matrix, 1 g fat, 5 g fat remover, external clean-up		1 min, 2 cycles, 5 g matrix, 0.5 g fat, 10 g fat remover				1 min, 2 cycles, 2.5 g matrix, 0.25 g fat, 10 g fat remover			
							External clean-up		Injection of raw extract		External clean-up		Injection of raw extract	
	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)
28	97	3.0	84	23.0	101	3.7	97	1.2	90	2.2	88	11.9	85	12.8
52	101	2.9	88	26.0	102	8.2	95	2.8	93	4.1	94	8.5	89	12.2
101	105	2.1	91	26.2	112	4.1	93	3.4	84	5.5	101	9.9	95	11.7
118	100	6.2	86	28.0	103	4.1	95	3.4	79	14.5	106	10.1	101	14.7
153	106	4.6	89	28.2	113	6.5	100	1.4	80	13.3	104	11.9	100	13.2
138	103	5.9	89	28.5	112	6.3	98	2.8	81	11.5	113	12.2	105	19.0
180	103	5.7	132[a]	43.5	110	4.7	95	2.0	80	10.0	109	15.7	109	16.6
Average	102		88[b]		108		96		84		102		98	
Extracted fat	0.48 g		0.49 g		0.45 g		–		0.01 g		–		<0.001 g	

The temperature was kept at 100°C. The total sample mass was 10, 5 or 2.5 g (9, 4.5 or 2 g matrix spiked to a total level of 1, 0.5 or 0.25 g fat). The matrix was mixed with 5 g sample support. The cell was packed according to procedure C in Fig. 1. The coextracted fat was removed by external clean-up according to the procedure described in the Experimental section, or in some cases the raw PLE extracts were directly injected into the GC–MS system. All measurements were made in triplicate.

[a] Unknown interfering peak.

[b] Excluding PCB 180.

portant factor is the ratio between the fat and the fat remover (sulfuric acid–silica gel). In order to accomplish a fat free extract the amount of fat was decreased to 0.5 g using only a total of 5 g matrix. Additionally the amount of fat remover was increased to 10 g giving a FFR ratio of 0.05. This resulted in a fat recovery of 2% (0.01 g of coextracted fat) where the PCB congeners were still quantitatively extracted. In order to test the effects of fat present in the extract, the same untreated raw PLE extracts containing 2% fat were also directly injected into the GC–MS system. This suppressed the PCB recoveries giving an average recovery of the seven indicator PCBs of 84% as compared to 96% when performing external sulfuric acid clean-up (Table 3). Consequently the FFR ratio was further decreased by extracting 2.5 g matrix containing 0.25 g fat leading to a FFR ratio of 0.025. After external sulfuric acid clean-up, the recoveries were quantitative with an average of 102% of the seven indicator PCBs (Table 3). Likewise, when injecting untreated raw PLE extracts the individual PCB recoveries were nearly identical to those using external clean-up with an average quantitative recovery of 98%. In this extract less than 1% of the fat was coextracted and not quantifiable (<0.001 g of fat), demonstrating that on-line clean-up is possible when applying a fat/fat remover ratio of 0.025. When performing external sulfuric acid treatment of this PLE extract no reaction was observed, verifying that all fat present in the sample had been destroyed during the PLE session.

3.2. Extraction of certified reference materials

The applicability of the on-line clean-up concept was tested by extracting two different types of CRMs representing both a high and a low level PCB concentration. These materials also represents naturally contaminated matrices which might behave differently from spiked matrices.

3.2.1. Cod-liver oil, CRM 349

The highly contaminated cod-liver oil (CRM 349) was first extracted for 1 min in two cycles using 500 mg of oil with 10 g of sulfuric acid impregnated silica gel giving a fat/fat remover ratio of 0.05 (Table 4).

Table 4
Recoveries of extracted PCBs calculated versus certified values for cod-liver oil CRM 349

PCB congener	Certified values (ng/g)	1 min, 2 cycles, 500 mg cod-liver oil, 10 g fat remover				1 min, 2 cycles, 250 mg cod-liver oil, 10 g fat remover				5 min, 2 cycles, 250 mg cod-liver oil, 10 g fat remover			
		External clean-up		Injection of raw extract		External clean-up		Injection of raw extract		External clean-up		Injection of raw extract	
		Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)
28	68	83	4.0	81	4.7	66	5.6	63	8.9	81	6.4	79	1.2
52	149	98	2.6	91	0.1	73	4.2	70	8.6	84	3.3	81	2.2
101	370	87	0.9	81	5.0	70	6.5	67	5.1	84	1.3	83	1.8
118	454	87	2.0	81	11.7	71	7.5	71	6.1	92	1.2	89	3.8
153	938	94	2.3	82	15.6	76	10.1	73	3.0	97	4.4	100	1.6
180	280	88	2.0	82	7.2	75	10.2	74	2.7	103	1.0	104	1.8
Average		90		83		72		70		89		90	
Extracted fat		–		0.01 g		–		<0.001 g		–		<0.001 g	

The cell was packed according to procedure C in Fig. 1. The coextracted fat was removed by external clean-up according to the procedure described in the Experimental section, or in some cases the raw PLE extracts were directly injected into the GC–MS system. All measurements were made in triplicate.

The amount of recovered fat was, in this case, 2% (0.01 g) and the PLE extracts were injected both raw and after external sulfuric acid clean-up. The results once again demonstrated that the presence of co-extracted lipids at this level suppressed the PCB recoveries during the analysis giving an average of the indicator PCBs of 83% for the raw extract as compared to 90% for the externally cleaned extract. Therefore the FFR ratio was decreased to 0.025 by extracting 250 mg of cod-liver oil. In this case no coextracted fat could be determined, and the obtained recoveries after injection of PLE extracts were equal to those obtained after external clean-up, with average recoveries of 70 and 72%, respectively. However, when using this small amount of cod-liver oil, the recoveries were not quantitative. In an attempt to improve the recoveries while still obtaining a fat free extract, 250 mg of cod-liver oil was extracted for 5 min in two cycles. This reestablished the recoveries to levels close to certified values. No difference between externally cleaned extracts and injection of PLE extracts were observed since no coextracted fat was present.

3.2.2. Milk powder, CRM 450

The same experimental set-up as that performed for the cod-liver oil in Table 4 was also done on milk powder (Table 5). Quantitative recoveries were obtained, when 2 g of milk powder containing 0.5 g of fat was extracted for 1 min in two cycles through 10 g of fat remover (FFR ratio of 0.05). In this case the raw PLE extracts could not be injected since they contained 8% (0.04 g) of the total fat. No coextracted fat occurred in the PLE extracts, when the amount of milk powder was decreased to 1 g to obtain a FFR ratio of 0.025. No difference in PCB recoveries could be observed between raw PLE extracts and externally cleaned extracts which was in accordance with the data obtained for the cod-liver oil. Additionally, decreasing the sample amount from 2 to 1 g (fat decrease from 0.5 g to 0.25 g) showed somewhat lowered recoveries due to too short extraction time (compare to Table 4). In order to achieve quantitative recoveries an extraction time of 5 min in two cycles was necessary with no differences in recoveries after external clean-up and injection of raw PLE extracts.

4. Conclusions

On-line clean-up of fat containing food and feeding stuff matrices is possible in PLE using sulfuric acid impregnated silica gel.

Table 5
Recoveries of extracted PCBs calculated versus certified values for milk powder CRM 450

PCB congener	Certified value (ng/g)	1 min, 2 cycles, 2 g milk powder containing 500 mg fat, 10 g fat remover, external clean-up		1 min, 2 cycles, 1 g milk powder containing 250 mg fat, 10 g fat remover				5 min, 2 cycles, 1 g milk powder containing 250 mg fat, 10 g fat remover			
				External clean-up		Injection of raw extract		External clean-up		Injection of raw extract	
		Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)	Recovery (%)	RSD (%) (n=3)
52	1.16	92	14.3	101	4.9	105	2.4	83	17.7	99	17.5
118	3.3	105	10.9	83	3.6	86	4.1	107	8.8	104	12.5
153	19.0	101	12.3	73	4.0	72	3.6	99	8.9	100	14.1
180	11.0	95	10.2	76	1.8	78	1.8	97	9.1	97	17.9
Average		98		83		86		97		100	
Extracted fat		–		0.04 g		<0.001 g		–		<0.001 g	

The cell was packed according to procedure C in Fig. 1. The coextracted fat was removed by external clean-up according to the procedure described in the Experimental section, or in some cases the raw PLE extracts were directly injected into the GC–MS system. All measurements were made in triplicate.

In order to accomplish completely fat free extracts, an FFR ratio of 0.025 is needed and the total amount fat present in the extract should be below 0.001 g. In this case no differences can be observed in PCB recoveries between injected raw PLE extracts and extracts that have passed an additional external clean-up. However, in cases where too much fat is present due to too high fat/fat retainer ratio, the PCB recoveries are suppressed when injecting raw PLE extracts. In order to assure that quantitative recoveries are obtained in all cases, the preferable extraction time is 5 min using two cycles.

Applying the developed methodology gives increased possibilities of automation with no extra clean-up step needed, leading to substantial time savings as compared to classical methodologies. This will also be of great use whenever there are high demands on routine laboratories such as in the previous Belgian dioxin crisis where numerous samples had to be analyzed in a very limited time.

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