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Application of matrix solid-phase dispersion to the determination of polychlorinated biphenyls in fat by gas chromatography with electron-capture and mass spectrometric detection

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Abstract

A one-step extraction–purification method for the determination of polychlorinated biphenyls (CBs) in fat samples was developed. Matrix solid-phase dispersion using different combinations of normal phase sorbents and elution solvents was evaluated, in terms of extraction yield and lipids removal efficiency, for the isolation of CBs from butter, chicken and beef fat. Under optimal conditions, 0.5 g of sample was dried with anhydrous sodium sulphate, dispersed on 1.5 g of Florisil and transferred to the top of a polyethylene solid-phase extraction cartridge which already contain 5 g of Florisil. Non-coplanar CBs were quantitatively eluted with 15 ml of *n*-hexane. The lipid percentage in this extract remained below 0.06% of the sample mass. As coplanar congeners show a higher affinity for Florisil, 20 ml of hexane–dichloromethane (90:10) were necessary for the quantitative recovery of coplanar and non-coplanar CBs. The potential of the procedure to fractionate non-coplanar and coplanar congeners is discussed. After extract evaporation to 0.2 ml, quantification limits of 0.4 ng of each CB per g of fat were achieved, using gas chromatography with tandem MS or electron-capture detection (ECD).

Keywords: Matrix solid-phase dispersion; Fats; Food analysis; Polychlorinated biphenyls

1. Introduction

The past industrial applications of polychlorinated biphenyls (CBs), together with their chemical stability and persistence have converted them in ubiquitous pollutants in the environment. Because of their low water solubility and relatively high octanol–water partition coefficients (K_{ow}), CBs are easily bio-accumulated in fatty tissues [1]. Particularly, high CB concentrations have been detected in mammals living in artic regions [2], in humans and in general, in animals placed at the top of the alimentary chain [3-6]. Therefore, levels of CBs in fatty samples, such as whale blubber and fish liver oil, can be used as an estimation of the environment exposition to these compounds; moreover, unusual high levels of CBs in cheese, butter, eggs and fatty tissues from farm animals, are a clear indication of the fraudulent use of polluted raw materials, or even industrial oils, in the feed of animals. In this sense, with occasion of the Belgian dioxin crisis, the European authorities set the maximum allowable concentration of CBs in food products in 200 ng per g of fat (defined as the sum of the most abundant congeners 28, 52, 101, 118, 138, 153 and 180) [7].

Determination of CBs in food samples is normally performed using gas chromatography in combination with electron-capture detection (ECD) or MS detection. The critical step in the analytical procedure is normally the sample preparation: Soxhlet [8], microwave-assisted extraction [9,10], carbon dioxide in supercritical conditions (SFE) [11] and pressurized organic solvents (PLE) [12] have been used to extract CBs from different food products. Apart from CBs, the obtained extracts also contain a high amount of lipids; therefore, further clean-up steps [13,14], using size exclusion chromatography or normal phase sorbents [7,15–17], are necessary previously to injection in the chromatographic system. Recently, several authors have described the use of

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SFE and PLE to obtain lipid-free extracts from food samples. In these simultaneous extraction and clean-up procedures, an appropriate amount of one or several normal phase sorbents is placed in the bottom of the extraction cell [18–22].

Alternatively to those techniques, matrix solid-phase dispersion (MSPD) has been proposed as a low cost and simple technique which allows the simultaneous disruption, extraction and purification of polar and non-polar analytes from solid and semi-solid biota samples. Up to now, several authors have used this technique for the determination of CBs in freeze-dried, fresh fish and seafood samples [21,23–25]. Applications to fat samples have been focussed in the extraction of hexachlorobenzene and other chlorinated pesticides [26,27]; however, from our knowledge, the one-step extraction–purification of CBs from pure fat samples, using matrix solid-phase dispersion, has not been reported yet.

The aim of this work is the optimisation of a low cost and simple procedure to isolate CBs from animal fatty samples, which allows the gas chromatographic determination of the analytes at the low ng, or even at the sub ng per g of sample, using matrix solid-phase dispersion as the sample preparation technique. Moreover, the possibility of fractionating non-coplanar and coplanar congeners, in the MSPD cartridge, is also discussed. To achieve both aims, different combinations of normal phase sorbents and organic solvents were evaluated for the extraction of CBs from butter, beef and chicken fat samples.

2. Experimental

2.1. Apparatus

Determinations of CBs in spiked and non-spiked fat samples were performed using two gas chromatographic systems equipped with split/splitless injection ports and ECD or MS detection, respectively. In both systems, injections were done using an autosampler device. The GC–ECD system was a HP 5890 series II gas chromatograph (Hewlett-Packard, Avondale, MA, USA) with a ^{63}Ni electron-capture detector. Separations were carried out using a BP-5 type capillary column (30 m × 0.32 mm i.d., d_f = 0.25 μm) purchased from Supelco (Bellefonte, PA, USA). Nitrogen was employed as column carrier gas at a constant pressure of 45 kPa, and also as auxiliary gas in the detector. Standards and sample extracts (2 μl) were injected in the splitless mode (purge time, 2 min) and analytes were separated using the following oven program: 2 min at 90 °C, first ramp at 20 °C/min to 170 °C (held for 7.5 min), second ramp at 3 °C/min to 250 °C (held for 5 min).

The GC–MS system consisted of a Varian CP 3900 gas chromatograph (Walnut Creek, CA, USA) connected to an ion-trap mass spectrometer (Varian Saturn 2100), and equipped with a VF-5 ms capillary column (30 m × 0.25 mm i.d., d_f = 0.25 μm) obtained from Varian. Helium (99.999%) was used as carrier gas at constant column flow of 1.0 ml/min. Standards and sample extracts (1 μl) were injected in the pulse splitless mode (207 kPa for 2.1 min; purge time, 2 min). CBs were separated using the following oven program: 2 min at 90 °C, first ramp at 20 °C/min to 170 °C (held for 2 min), second ramp at 5 °C/min to 300 °C (held for 5 min). The separation efficiency of the VF-5ms column was evaluated using a test solution containing 62 CBs (Wellington Labs, Ont., Canada). Six pairs of congeners produced overlapped peaks, for the rest resolution factors were higher than 1.3. In the case of CBs 77 and 110, a resolution factor of 1.4 was achieved. The GC–MS transfer line and the ion-trap temperature were set at 300 and 200 °C, respectively. Mass spectra were obtained in the electron impact mode (70 eV) in the range from 100 to 550 *m*/*z*. MS–MS spectra were obtained using non-resonant dissociation. Retention times of CBs in both columns and *m*/*z* ratios used for quantitative purposes in MS and MS–MS detection modes are given in Table 1.

2.2. Reagents and materials

Sulphuric acid and organic solvents for trace analysis (*n*-hexane, dichloromethane and isooctane) were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate, Florisil (60–100 mesh), basic alumina activated to

Table 1
Retention times, quantification ions and MS–MS fragmentation conditions for the CB congeners

Congener	Retention time (min)		MS detection		MS–MS detection	
	ECD	MS and MS–MS	Quant. ion (*m*/*z*)	Parent ion	Quant. ion (*m*/*z*)	Ex. amplitude (v)
30 (IS)	14.8	10.7	256 + 258	258	186	0.9
28	18.7	12.7	256 + 258	258	186	0.9
52	21.0	13.8	290 + 292	292	257	0.9
77	29.5	18.0	290 + 292	292	290	0.9
118	31.1	18.8	326 + 328	326	324	1.10
153	32.6	19.5	360 + 362	360	325	1.40
138	34.3	20.5	360 + 362	360	325	1.40
126	35.0	20.8	326 + 328	326	324	1.40
180	38.7	22.7	394 + 396	396	359 + 361	1.40
169	40.0	23.5	360 + 362	360	290	2.30
209	48.3	27.9	498 + 500	500	463 + 465	2.20

grade I (150 mesh) and neutral silica were obtained from Aldrich (Milwaukee, WI, USA). Sodium sulphate, alumina and Florisil were heated in an oven at 140 °C for at least 1 week and then allowed to cool down at room temperature in a desicator. Acidic silica gel (44% sulphuric acid) was prepared in the laboratory mixing neutral silica with concentrated sulphuric acid.

Polyethylene solid-phase extraction (SPE) cartridges (15 ml capacity) and 20 μm frits were purchased from International Sorbent Technology (Mid Glamorgan, UK).

2.3. Standards and samples

A mixture containing several CBs in isooctane (2,4,4′-trichlorobiphenyl CB 28; 2,2′,5,5′-tetrachlorobiphenyl CB 52; 2,2′,3,4,4′,5′-hexachlorobiphenyl CB 138; 2,2′,4,4′,5,5′-hexachlorobiphenyl CB 153; and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl CB 180, 10 μg/ml per congener) was obtained from Supelco (Bellefonte, PA). In addition, individual standards of 2,3′,4,4′,5-pentachlorobiphenyl CB 118, 2,2′,4,5,5′-pentachlorobiphenyl CB 101, 3,3′,4,4′-tetrachlorobiphenyl CB 77, 3,3′,4,4′,5-pentachlorobiphenyl CB 126, 3,3′,4,4′,5,5′-hexachlorobiphenyl CB 169 and decachlorobiphenyl CB 209, were purchased from Dr. Ehrensdorfer (Augsburg, Germany). Standard mixtures of the 10 congeners were prepared in isooctane and *n*-hexane. Calibration solutions in the concentration range from 0.3 to 200 ng/ml were prepared in isooctane. All solutions were conserved in the dark and refrigerated at 4 °C. Moreover, 2,4,6-trichlorobiphenyl (CB 30) was obtained from Dr. Ehrensdorfer and used as internal standard to compensate any variation in the final volume of organic extracts obtained from fat samples.

Unsalted butter and purified abdominal beef fat were purchased in the local supermarket. Chicken fat was directly obtained from the dorsal part of the animals, cut in small pieces and homogenized with a blender. The percentage of fat in beef and chicken samples was determined using Soxhlet extraction for 24 h with a mixture of hexane–acetone (1:1), followed by evaporation of the extract and gravimetrical analysis of the residue. Values around 100% and 70% ($n = 2$ extractions) were obtained for purified beef and chicken fat, respectively. The percentage of fat in butter, according with the producer information, was around 85%. Portions of 10 g of these samples were heated at 50 °C, spiked with 2 ml of different CB mixtures in *n*-hexane (congener 101 was not included in these experiments), and the organic solvent allowed to evaporate for 12 h with periodical stirring. Using this procedure spiked samples, at three concentration levels: 5, 50 and 200 ng/g per congener, were prepared. Spiked and blank samples (non-spiked) were stored for at least 1 week at −18 °C before being submitted to the proposed extraction procedure.

A reference material of pork fat (IRMM 446) with a certified concentration of seven CBs (28, 52, 101, 118, 138, 153 and 180) and a total CBs content around 207 ng/g was obtained from the Institute for Reference Materials and Measurements (Geel, Belgium).

2.4. Sample preparation

Samples, around 0.5 g, were weighed in capped 25 ml glass tubes and thoroughly dried with 2 g of anhydrous sodium sulphate, 1.5 g of Florisil were added, and the mixture was mechanically shaken using a vortex agitator for 5 min. After that, it was passed to the top of a SPE cartridge containing 5 g of Florisil. Analytes were isolated from the fat sample using different solvents. Under optimal conditions non-coplanar CBs, were extracted with 15 ml of *n*-hexane. Coplanar and non-coplanar congeners could be eluted with 20 ml of *n*-hexane–dichloromethane (90:10). In this case, 1 g of acidic silica was also placed in the bottom of the SPE cartridge, in addition to the 5 g of Florisil.

Organic extracts from fat samples were spiked with 100 μl of the internal standard solution in isooctane and evaporated to approximately 0.5 ml using a Turbo Vap concentrator. They were transferred to a GC autosampler vial, 0.1 ml of isooctane were added, and the extract additionally reduced, with a gentle stream of nitrogen, to approximately 0.2 ml. In preliminary experiments, working with samples spiked at high concentration levels (200 ng/g per congener), the last evaporation step was not used. Concentrated extracts were stored at 4 °C until their injection into the chromatographic system.

Fat content in organic extracts was gravimetrically determined after dryness evaporation of the extracts.

3. Results and discussion

3.1. Optimisation of extraction conditions

Experiments were carried out in order to assess the capabilities of Florisil, acidic silica and basic alumina to isolate CBs from fat. Obviously, the goal of the study was to achieve the quantitative extraction of the analytes from the sample, keeping the percentage of co-extracted lipids at the lowest possible level. In all cases, sample dispersion was performed in the same sorbent used as fat retainer.

For the pair butter: Florisil, using 10 ml of *n*-hexane as elution solvent, the percentage of lipids in the extract decreased from 34 to 0.06% when the amount of retainer sorbent, raised from 1 to 5 g. Experiments were repeated for chicken and beef fat using 5 g of Florisil. In both cases, extracts with relatively low fat contents were obtained (Table 2). On the other hand, the use of hexane–dichloromethane mixtures, as CBs elution solvent, increased the amount of fat in the extracts from butter samples. The higher the percentage of dichloromethane in the mixture, the higher the amount of fat in the extract, Table 2. Results in Table 2 clearly show that considering butter samples, Florisil and acidic silica are more effective as fat retainers than basic alumina.

Table 2
Percentage of lipids in organic extracts obtained from different fat samples, as function of the type of sorbent and elution solvent

Sorbent	Sample	Percentage of fat in the extract (g of lipids/100 g of sample)			
		n-Hexane	Hexane–Cl_2CH_2 (90:10)	Hexane–Cl_2CH_2 (85:15)	Hexane–Cl_2CH_2 (80:20)
Florisil	Butter	0.06	0.31	4.67	7.04
Florisil	Beef fat	<0.01	0.02		
Florisil	Chicken fat	0.02	0.02		
Basic alumina	Butter	4.00			
Acidic silica	Butter	0.03			

0.5 g of fat sample (previously dispersed on 2 g of sodium sulphate and 1.5 g of the same sorbent used as fat retainer), 5 g of fat retainer and 10 ml of elution solvent were considered in all cases.

Normalized peak areas of CBs in successive 10 ml extracts obtained from butter, using different sorbents and elution solvents, are shown in Table 3. In all cases, spiked samples at the 200 ng/g were used. The elution profile of the CBs depended on their chemical structure and on the pair solvent–fat retainer. Using *n*-hexane and Florisil, the non-coplanar congeners were quantitatively extracted with 10 ml of solvent; whilst, 20 ml and up to 30 ml were necessary to elute the same congeners from alumina and acidic silica, respectively. On the other hand, the behavior of the coplanar congeners (CBs 77, 126 and 169) was sorbent dependent: in the case of basic alumina and acidic silica, they eluted together with the non-coplanar congeners; however, they showed a stronger interaction with Florisil than the non-coplanar species. For this sorbent, as shown in last three rows of Table 3, the use of hexane–dichloromethane (90:10) was necessary to allow their quantitative elution within an acceptable volume. Obviously, the non-coplanar congeners were also recovered with this mixture.

The need of using hexane–dichloromethane (90:10) to elute the coplanar congeners form MSPD Florisil cartridges was confirmed using beef and chicken fat samples. Again, non-coplanar congeners were eluted in more than 95% using only 10 ml of *n*-hexane (results not given); however, as shown in Fig. 1, at least 20 ml of hexane:dichloromethane (90:10) were necessary to recover the coplanar congeners 77, 126 and 169.

In view of these results, Florisil was chosen as dispersant and fat retainer for the extraction of CBs from fat samples. It led to extracts with a lower lipid content than alumina; and moreover, a lower solvent volume than in case of alumina and silica was necessary for the quantitative recovery of the non-coplanar congeners. On the other hand, the different affinity of coplanar and non-coplanar congeners (less abundant but more toxic) for the Florisil sorbent suggested the possibility of fractionating both groups of CB congeners.

3.2. Performance of the procedure

Recoveries of the proposed method were evaluated using fat samples spiked with the selected CBs at two different concentration levels, 50 and 5 ng/g. After sample dispersion, cartridges were eluted well using 15 ml of *n*-hexane, well with 20 ml of hexane–dichloromethane (90:10). Obviously, in the first situation, only the recoveries for the indicative non-coplanar congeners were evaluated. In both cases, extracts were spiked with 100 μl of a CB 30 standard solution in isooctane and concentrated to 0.2 ml, as described in Section 2. Using 20 ml of hexane–dichloromethane (90:10),

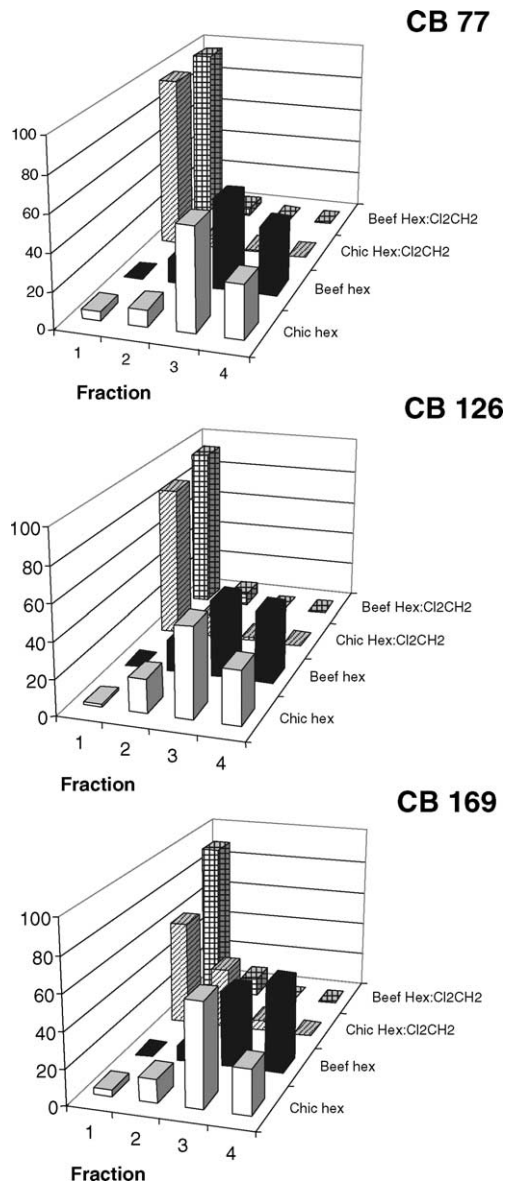

Fig. 1. Normalised peak areas for coplanar congeners in 10 ml fractions of spiked chicken and beef fat samples.

Table 3
Normalized peak areas for each CB congener in consecutive 10 ml fractions obtained from butter samples

Congener	Sorbent solvent	Fraction number				
		1st	2nd	3rd	4th	5th
28	Flo *n*-hexane	99	1			
	Sil *n*-hexane	90	8	2		
	Alu *n*-hexane	63	27	10		
	Flo hexane (90:10)	100				
52	Flo *n*-hexane	100				
	Sil *n*-hexane	90	8	2		
	Alu *n*-hexane	80	20			
	Flo hexane (90:10)	100				
118	Flo *n*-hexane	98	1	1		
	Sil *n*-hexane	85	13	2		
	Alu *n*-hexane	76	24			
	Flo hexane (90:10)	100				
153	Flo *n*-hexane	100				
	Sil *n*-hexane	86	11	3		
	Alu *n*-hexane	100				
	Flo hexane (90:10)	100				
138	Flo *n*-hexane	99	1			
	Sil *n*-hexane	83	14	3		
	Alu *n*-hexane	69	27	4		
	Flo hexane (90:10)	100				
180	Flo *n*-hexane	98	1	1		
	Sil *n*-hexane	83	14	3		
	Alu *n*-hexane	100				
	Flo hexane (90:10)	100				
209	Flo *n*-hexane	100				
	Sil *n*-hexane	87	13			
	Alu *n*-hexane	100				
	Flo hexane (90:10)	100				
77	Flo *n*-hexane	32	52	13	3	
	Sil *n*-hexane	85	13	2		
	Alu *n*-hexane	56	23	21		
	Flo hexane (90:10)	100				
126	Flo *n*-hexane	11	38	32	12	7
	Sil *n*-hexane	77	18	5		
	Alu *n*-hexane	40	32	28		
	Flo hexane (90:10)	100				
169	Flo *n*-hexane	3	19	29	23	26
	Sil *n*-hexane	73	21	6		
	Alu *n*-hexane	57	25	18		
	Flo hexane (90:10)	86	14			

Flo, Florisil; Sil, silica; Alu, alumina.

the presence of fat (up to 0.5% of the sample intake) was easily observed when final extracts were cooled down to –18 °C, particularly in the case of butter. To avoid this problem, 1 g of acidic silica was placed in the bottom of the MSPD cartridge under the Florisil clean-up layer. With this combination of sorbents, the small amount of fat which elutes from Florisil was oxidized by the silica layer, leading to cleaner extracts with a fat content under 0.05% of the sample intake weight. A GC–ECD chromatogram for chicken fat spiked with the analytes at the 50 ng/g level is given in Fig. 2. Interferences,

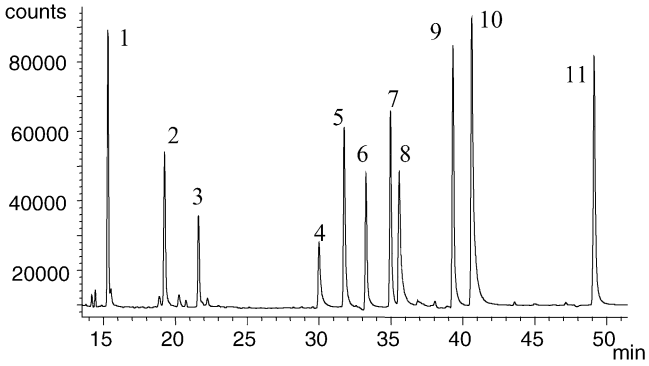

Fig. 2. Chromatogram obtained for spiked chicken fat (50 ng/g) using ECD detection and hexane–dichloromethane (90:10) as elution solvent. (1) CB 30 (IS); (2) CB 28; (3) CB 52; (4) CB 77; (5) CB 118; (6) CB 153; (7) CB 138; (8) CB 126; (9) CB 180; (10) CB 169 and (11) CB 209.

which could potentially disturb the CB signals, were not observed in the chromatogram.

Concentrations of each congener in the final extracts were determined using ECD and MS detection, in the case of samples spiked at the high level (50 ng/g), and using ECD and tandem MS detection for samples spiked at low level (5 ng/g). Quantification, using the internal standard method, was based on five- or six-points calibration curves in the concentration interval from 5 to 200 ng/ml for MS detection, and from 0.3 to 200 ng/ml in case of ECD and tandem MS detection. A good linearity (correlation coefficients higher than 0.998) was obtained for all congeners using the three considered detection techniques.

Table 4 shows the percentage of recovery for the spiked samples considered in this study. Non-spiked samples (blanks) were also processed and taken into account for calculations. Only congeners 153, 138 and 180 were detected at low concentration levels (below 1 ng/g) in butter and beef fat (Fig. 3). Therefore, blank contributions were only significant

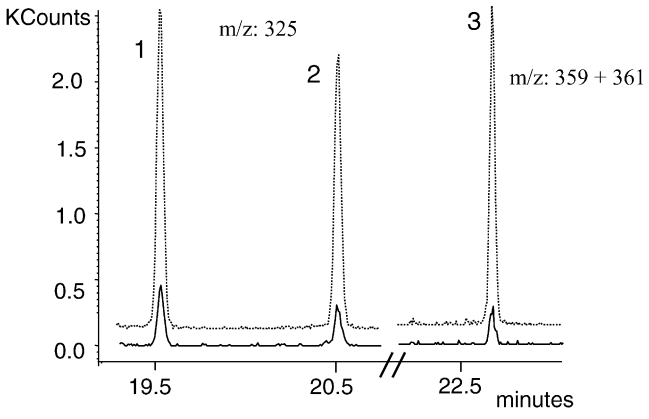

Fig. 3. GC–MS–MS chromatograms (traces at *m*/*z* 325 and 359 + 361) corresponding to a non-spiked (solid line) and a low level spiked (5 ng/g), butter sample (dotted line). *n*-Hexane (15 ml) was used to extract the compounds. (1) CB 153; (2) CB 138 and (3) CB 180.

Table 4
CB recoveries from spiked fat samples (n = 3)

Congener	Mean recovery (%), chicken fat				Mean recovery (%), beef fat			
	ECD (hex)	MS (hex)	ECD (hexane–Cl_2CH_2)	MS (hexane–Cl_2CH_2)	ECD (hex)	MS (hex)	ECD (hexane–Cl_2CH_2)	MS (hexane–Cl_2CH_2)
(A) Spiking level, 50 ng/g								
28	90	98	113	111	97	96	108	104
52	91	98	109	104	104	100	107	100
118	95	90	106	100	99	99	106	88
153	102	106	101	101	105	111	102	93
138	100	105	107	108	111	114	107	104
180	102	112	103	106	111	109	102	95
209	105	110	108	111	108	100	108	77
77			104	100			101	94
126			99	96			97	86
169			101	97			99	74
	Mean recovery (%), chicken fat				Mean recovery (%), butter			
	ECD (hex)	MS–MS (hex)	ECD (hexane–Cl_2CH_2)	MS–MS (hexane–Cl_2CH_2)	ECD (hex)	MS–MS (hex)	ECD (hexane–Cl_2CH_2)	MS–MS (hexane–Cl_2CH_2)
(B) Spiking level, 5 ng/g								
28	102	113	97	98	92	88	98	102
52	99	104	80	86	78	86	79	91
118	98	102	83	92	91	89	92	100
153	101	95	82	83	98	107	109	99
138	106	99	90	86	97	100	96	97
180	109	99	94	84	95	103	107	97
209	100	95	92	76	86	98	87	79
77			83	93			76	96
126			87	87			82	88
169			86	77			84	81

RSD (%) of the above given recoveries ranged from 2% to 13%.

in the case of the low level spiked samples. A global overview of results given in Table 4 shows that non-coplanar congeners are recovered in an extension around 90% using any of both elution solvents. Only, in a few occasions, slightly lower yields were obtained for CBs 52 and 209. Recoveries of coplanar congeners, using hexane–dichloromethane, were also satisfactory with overall mean values of 95% and 85% for samples spiked at high and low level, respectively. Moreover, independently of the elution solvent, at both spiked levels, GC–ECD led to equivalent recoveries than MS and MS–MS detection, proving the absence of interfering signals or small amounts of fat in the extracts, which could disturb the GC–ECD chromatograms producing significant quantification errors, especially in the case of samples containing low levels of CBs.

Quantification limits of the procedure, defined for a signal to noise ratio of 10, were 3 and 0.4 ng/g per congener, considering MS and MS–MS detection, respectively. In the case of GC–ECD, values of 1.5 ng/g (CBs 28, 52 and 77) and 0.4 ng/g, for the rest of congeners, were achieved. It must be noticed that the low level of fat in the extracts, would allow a further improvement in the sensitivity of the method just by reducing the final extract volume below 0.2 ml.

3.3. Fractionation of coplanar and non-coplanar congeners

Table 5 presents the percentages of recovery in the consecutive elution of chicken fat samples spiked with the considered analytes at the 50 ng/g level. After drying, samples were

Table 5
Recoveries obtained in the fractionation of non-coplanar and coplanar CBs from spiked (50 ng/g) chicken fat samples (n = 4)

Congener	Mean recovery (%) ± S.D.	
	n-Hexane fraction	Hexane–Cl_2CH_2 (90:10) fraction
28	93 ± 3	3 ± 0.3
52	90 ± 5	3 ± 0.2
118	89 ± 2	2 ± 0.2
153	91± 2	n.d.
138	90 ± 5	n.d.
180	92 ± 9	n.d.
209	88 ± 9	n.d.
77	11 ± 0.4	84 ± 10
126	n.q.	84 ± 7
169	n.d.	81 ± 9

n.q., below quantification limit; n.d., below detection limit. Tandem MS was used as detection technique.

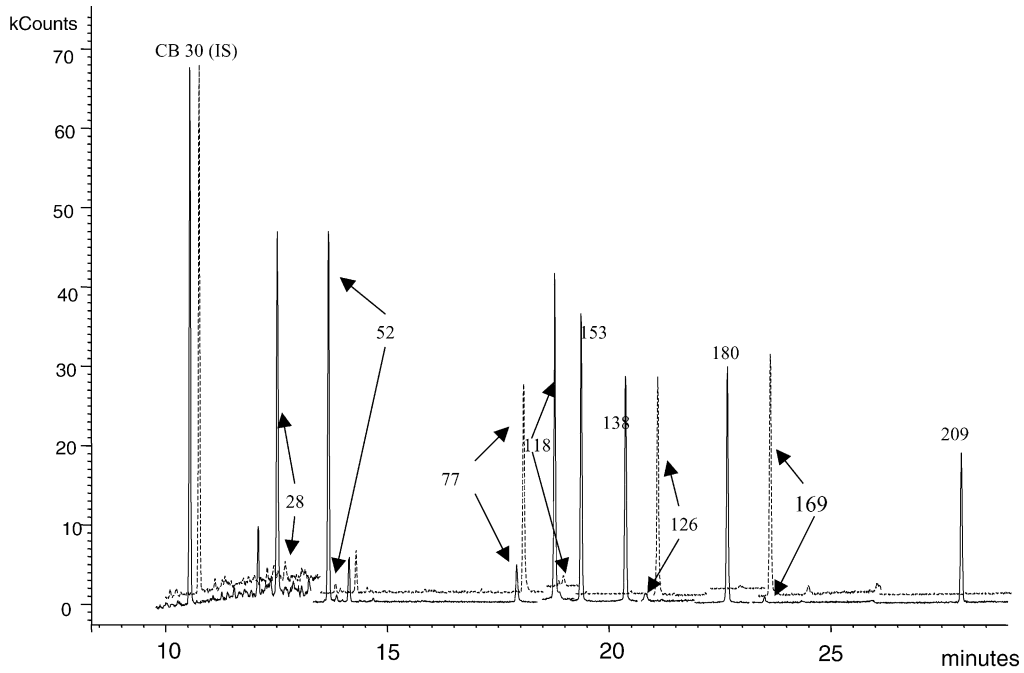

Fig. 4. GC–MS–MS chromatograms (TIC) obtained in the consecutive extraction of spiked chicken samples (50 ng/g), firstly with 15 ml of *n*-hexane (solid line) and secondly with 20 ml of hexane–dichloromethane 90:10 (dotted line). Chromatograms have been slightly displaced to facilitate the observation of a same congener in both records.

dispersed with 1.5 g of Florisil and placed at the top of a cartridge containing 5 g of Florisil and 1 g of acidic silica. The cartridge was eluted firstly with 15 ml of *n*-hexane and then with 20 ml of hexane–dichloromethane. Extracts were reduced to 0.2 ml, and the concentration of each analyte in both fractions determined using tandem MS detection (Fig. 4). Recoveries around 90% were achieved for all non-coplanar congeners in the hexane fraction; moreover, only CBs 28, 52 and 118 were found in the hexane–dichloromethane extracts. Regarding the coplanar CBs, the most toxic congeners: 126 and 169, TEF values of 0.1 and 0.01 [1] were present only in the hexane–dichloromethane fraction. The less toxic coplanar congener 77, TEF 0.0005, was mainly eluted in the hexane–dichloromethane fraction; however, the compound was also found in the *n*-hexane extract. Globally, more than 80% of the coplanar congeners were recovered in the hexane–dichloromethane extract.

3.4. Validation

The proposed procedure was applied to the determination of CBs in the reference material IRMM 446. It corresponds to pork fat with a total CB content around 207 ng/g, and with certified concentration of the seven target congeners. Since, coplanar CBs are not present in this material, *n*-hexane was used as elution solvent; moreover, because of the limited sample amount and the relatively high concentration of the analytes, the sample intake was reduced to 0.25 g. Sample extracts were concentrated to 0.2 ml and analysed using ECD and MS–MS detection. Measured values are shown in Table 6. Globally, an excellent agreement was found between results obtained using both techniques. Moreover, comparison of measured concentrations and certified values (mean values with their standard deviations), shows that all congeners, including CB 101 which had not been considered during the optimisation of the method, were extracted from the material in an extension between 90% and 100%.

Table 6
CB concentrations (ng/g) in IRMM 446, using *n*-hexane as extraction solvent and ECD or MS–MS for detection ($n = 4$)

Congener	Certified concentration ± S.D.	Measured concentration ± S.D.	
		ECD	MS–MS
28	29.6 ± 2.1	29.3 ± 0.8	30.5 ± 1.2
52	25.5 ± 1.8	22.7 ± 0.2	21.7 ± 0.6
101	30.0 ± 4.0	26.1 ± 0.5	25.3 ± 0.8
118	30.2 ± 2.7	27.4 ± 0.6	27.5 ± 1.9
153	30.8 ± 2.4	28.2 ± 0.5	28.4 ± 0.8
138	32.0 ± 4.0	29.0 ± 0.4	30.8 ± 1.4
180	29.8 ± 2.5	30.2 ± 0.9	26.4 ± 0.8

4. Conclusions

A simple method for the determination of non-coplanar and coplanar congeners in fat samples, under concentration limits established by the European regulations, has been optimised. Using a single, inexpensive SPE cartridge filled with Florisil, analytes can be quantitatively extracted from the sample with 15–20 ml of an appropriate organic solvent;

whilst fat, and other potential interferences, were retained on the sorbent material. Relatively clean extracts were obtained; and therefore, GC–ECD, a low cost technique when compared to GC–MS or GC–MS–MS, allowed the reliable quantification of the analytes providing lower detection limits than GC–MS. In addition to its efficiency as fat retainer, Florisil showed certain capacity to discriminate between coplanar and non-coplanar congeners, allowing their partial fractionation without introducing any extra step in the sample preparation protocol.

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