Determination of dioxin-like polychlorinated biphenyls in feed and foods of animal origin by gas chromatography and high resolution mass spectrometry

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Summary

The authors describe a method to determine 12 dioxin-like polychlorinated biphenyls (dl-PCBs) in feed and foods of animal origin using high resolution gas chromatography and mass spectrometry (HRGC-HRMS). The use of semi-automated apparatus such as an accelerated solvent extraction device and the Power-Prep purification system significantly reduced the sample treatment time necessary before instrumental analysis in comparison with the Soxhlet extraction method or manual purification on columns traditionally used. This method demonstrated good specificity (mild interference limited to PCB 81 and 123 in highly contaminated matrices, such as fish and fish feed), with limits of detection of 0.2-1.3 pg/g of fat. The coefficients of variation for repeatability tests were in the range of 2.6-18.3%, with recoveries of over 80%. The method was successfully applied to the analysis of 177 samples of various foodstuffs specified in the 2005 Italian National Residues Plan: this is the first data on dioxin-like PCB contamination levels in Italy to emerge under this monitoring plan.

Keywords

Dioxins, Feed, Foodstuffs, High resolution gas chromatography, High resolution mass spectrometry, Dioxin-like polychlorinated biphenyls.

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), commonly known as 'dioxins' and poly-chlorinated biphenyls (PCBs) are a group of widespread organic micro-pollutants which persist in the environment where they can be transported for long distances (18). These substances tend towards bioaccumulation in animal organisms and biomagnification in the food chain, reaching concentrations with potentially serious toxicological effects (11, 12, 13, 14, 19, 23). Dioxins and PCBs have similar chemical and toxic properties, although their sources may be different. In contrast with dioxins, which are unwanted side products of chemical and combustion processes, PCBs were produced deliberately as components of paints, plasticisers, insulating materials, dielectric fluids, etc. until 1985, at which time their sale and use was prohibited, due to their proven toxic effects on the reproductive system (6).

PCBs consist of a group of 209 aromatic compounds derived from biphenyl through

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the substitution of hydrogen atoms with chlorine atoms. Of all the possible compounds, only twelve, with chlorine atoms in a non-ortho position (so-called 'coplanar PCBs') or with a single chlorine atom in one of the four ortho positions (mono-ortho chlorinated PCBs) show a level of toxicity similar to that of the dioxins and, for this reason, are known as 'dioxin-like PCBs' (dl-PCBs). Various studies in European countries have revealed that the contribution of dl-PCBs to the overall toxicity equivalents (TEQ) in foodstuffs is greater than that of dioxins (7, 15). In consequence, European legislation on the maximum dioxin content in food and feed products has been recently updated to include maximum levels for the sum of dl-PCBs, PCDDs and PCDFs (3, 4). However, the determination of dl-PCBs in food products has been hindered by a number of factors, such as the low concentration of the analytes detected (typically at concentrations of pg/g), the presence of interfering substances in the test matrices and the risk of sample contamination during analysis. The essential requisites for an analytical protocol to determine these substances are therefore high sensitivity and specificity. These requisites have been achieved through a combination of different purification techniques and use of high resolution mass spectrometry (HRMS) (21). However, sample preparation involves lengthy extraction and purification processes, which are incompatible with the requirements of routine test laboratories to analyse numerous samples rapidly. This study involved the refinement and validation of an analytical method for the determination of dl-PCBs in food products, based on a semi-automated extraction and purification process with the aim of reducing sample preparation times and the use of manpower. The method was used to determine dl-PCB levels in 177 food samples taken under the 2005 Italian National Residues Plan (NRP).

Materials and methods

Reference standards

All standard solutions in nonane were supplied by Wellington Laboratories (Toronto, Ontario) and stored at 4°C. The six standard solutions reported in Table I (CS1 to CS6) were used for instrument calibration. The following internal standards were used, at a concentration of 50 ng/ml: $^{13}C_{12}$-PCB 77, $^{13}C_{12}$-PCB 81, $^{13}C_{12}$-PCB 126, $^{13}C_{12}$-PCB 169, $^{13}C_{12}$-PCB 105, $^{13}C_{12}$-PCB 114, $^{13}C_{12}$-PCB 118, $^{13}C_{12}$-PCB 123, $^{13}C_{12}$-PCB 156, $^{13}C_{12}$-PCB 157, $^{13}C_{12}$-PCB 167 and $^{13}C_{12}$-PCB 189. A mixture of the compounds $^{13}C_{12}$-PCB 70, $^{13}C_{12}$-PCB 111, $^{13}C_{12}$-PCB 138 and $^{13}C_{12}$-PCB 170 at a concentration of 50 ng/ml was used as the injection standard. Perfluorobutylamine (FC43) (Fluka, Buchs) was used for mass scale calibration of the detector.

Materials and reagents

Analytical grade solvents (hexane, ethyl ether, absolute ethanol, petroleum ether, dichloromethane and nonane) were supplied by J.T. Baker (Deventer). Ultra pure water was produced by a Milli-RO/Milli-Q Millipore system (Bedford, Massachusetts). Anhydrous sodium sulphate, potassium hydroxide, concentrated sulphuric acid and sodium chloride, all at analytical grade, were obtained from Carlo Erba (Milan). Diatomaceous earth was obtained from Varian (Walnut Creek, California). Samples were purified using silica and alumina chromatography columns (Fluid Management System Inc., Waltham, Massachusetts).

Sample preparation and extraction

All samples were homogenised and a representative aliquot, of a sufficient quantity to obtain 1 to 5 g fat after extraction, was fortified with 20 µl of the internal standard solutions at a concentration of 50 ng/ml. Samples with a high water content (except milk) were mixed with diatomaceous earth at a ratio from 1:3 to 1:2 (w/w) and left to equilibrate for 6-12 h.

The samples was placed in an accelerated solvent extraction (ASE) cell and underwent extraction with hexane:acetone 80:20 (v/v) using the Dionex (Sunnyvale, California) ASE 200 system using the following programme: temperature 125°C, pressure 1 500 psi, pre-heating time 5 min, heating time 6 min, holding time 6 min, flush volume 60%,

purge time 60 s and three extraction cycles. The extract was then filtered through anhydrous sodium sulphate into a volumetric flask and evaporated to dryness on a rotary evaporator at 45°C.

Table I
Calibration solutions (CS) (concentrations in ng/ml)

Analyte	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6
Native standards						
PCB 77	0.5	2	10	40	200	800
PCB 81	0.5	2	10	40	200	800
PCB 126	0.5	2	10	40	200	800
PCB 169	0.5	2	10	40	200	800
PCB 105	0.5	2	10	40	200	800
PCB 114	0.5	2	10	40	200	800
PCB 118	0.5	2	10	40	200	800
PCB 123	0.5	2	10	40	200	800
PCB 156	0.5	2	10	40	200	800
PCB 157	0.5	2	10	40	200	800
PCB 167	0.5	2	10	40	200	800
PCB 189	0.5	2	10	40	200	800
Internal standards						
$^{13}C_{12}$-PCB 77	50	50	50	50	50	50
$^{13}C_{12}$-PCB 81	50	50	50	50	50	50
$^{13}C_{12}$-PCB 126	50	50	50	50	50	50
$^{13}C_{12}$-PCB 169	50	50	50	50	50	50
$^{13}C_{12}$-PCB 105	50	50	50	50	50	50
$^{13}C_{12}$-PCB 114	50	50	50	50	50	50
$^{13}C_{12}$-PCB 118	50	50	50	50	50	50
$^{13}C_{12}$-PCB 123	50	50	50	50	50	50
$^{13}C_{12}$-PCB 156	50	50	50	50	50	50
$^{13}C_{12}$-PCB 157	50	50	50	50	50	50
$^{13}C_{12}$-PCB 167	50	50	50	50	50	50
$^{13}C_{12}$-PCB 189	50	50	50	50	50	50
Injection standards						
$^{13}C_{12}$-PCB 70	50	50	50	50	50	50
$^{13}C_{12}$-PCB 111	50	50	50	50	50	50
$^{13}C_{12}$-PCB 138	50	50	50	50	50	50
$^{13}C_{12}$-PCB 170	50	50	50	50	50	50

For milk samples, 200 ml of homogenised sample were added to 200 ml ethanol, 250 ml ethyl ether and 250 ml petroleum ether. After vigorous shaking in a separatory funnel, the organic phase of the mixture was recovered by filtering through anhydrous sodium sulphate into a volumetric flask and evaporated to dryness on a rotary evaporator at 45°C.

The fat obtained from the extraction process was weighed on an analytical balance.

Sample purification

The extracted fat was solubilised with 40 ml hexane and transferred to a separatory funnel, after which 40 ml of concentrated sulphuric acid was added and the mixture shaken for several minutes. After removing the acid phase, the partition was repeated until the acid phase was no longer coloured.

After washing the organic phase with 40 ml of 5% (w/v) aqueous sodium chloride solution, a second partition with 40 ml of 20% (w/v) aqueous potassium hydroxide solution was conducted, followed by a further washing with sodium chloride solution. The extract was concentrated on a rotary evaporator at 45°C to a volume of approximately 1 ml and purified by the PowerPrep automated system (Fluid Management System Inc., Waltham, Massachusetts). The system is equipped with two types of column pre-packed with silica (multi-layer column with acid, neutral and basic silica) and alumina. Purification columns were activated with 155 ml hexane at a flow rate of 10 ml/min. The extract from the liquid-liquid partition was then loaded into the top of the silica column and eluted with 80 ml hexane at a flow rate of 5 ml/min through the silica column followed by the alumina column, connected in series. Using 140 ml of dichloromethane/hexane mixture (95:5 v/v), dl-PCBs were eluted from the alumina column at a flow rate of 5 ml/min. The final eluate was concentrated on a rotary evaporator at 45°C to a volume of approximately 0.5 ml and transferred into a 1 ml conical-bottom vial. After the solvent had completely evaporated, 20 µl of the standard injection solution (50 ng/ml) was added.

Instrumentation and chromatographic conditions

The instrumental analysis was conducted using high resolution capillary gas chromatography/high resolution mass spectrometry (HRGC/HRMS) in the single ion monitoring mode at a resolution of 10 000. The masses used for determination of dl-PCBs are reported in Table II. The HRGC/HRMS system consisted of a MAT 95 XL mass spectrometer (Finnigan, Bremen) coupled to a Trace Series 2000

capillary gas chromatograph (Thermoquest, Milan), fitted with an A200S autosampler (Finnigan, Bremen).

Table II
Diagnostic ions (m/z) for the reference standards

Analyte	m^1/z	m^2/z
Native standards		
PCB 77	289.9224	291.9194
PCB 81	289.9224	291.9194
PCB 126	325.8804	327.8775
PCB 169	359.8415	361.8385
PCB 105	325.8804	327.8775
PCB 114	325.8804	327.8775
PCB 118	325.8804	327.8775
PCB 123	325.8804	327.8775
PCB 156	359.8415	361.8385
PCB 157	359.8415	361.8385
PCB 167	359.8415	361.8385
PCB 189	393.8025	395.7995
Internal standards		
$^{13}C_{12}$-PCB 77	301.9626	303.9597
$^{13}C_{12}$-PCB 81	301.9626	303.9597
$^{13}C_{12}$-PCB 126	337.9207	339.9178
$^{13}C_{12}$-PCB 169	371.8817	373.8788
$^{13}C_{12}$-PCB 105	337.9207	339.9178
$^{13}C_{12}$-PCB 114	337.9207	339.9178
$^{13}C_{12}$-PCB 118	337.9207	339.9178
$^{13}C_{12}$- PCB 123	337.9207	339.9178
$^{13}C_{12}$-PCB 156	371.8817	373.8788
$^{13}C_{12}$-PCB 157	371.8817	373.8788
$^{13}C_{12}$-PCB 167	371.8817	373.8788
$^{13}C_{12}$-PCB 189	405.8428	407.8398
Injection standards		
$^{13}C_{12}$-PCB 70	301.9626	303.9597
$^{13}C_{12}$-PCB 111	337.9207	339.9178
$^{13}C_{12}$-PCB 138	371.8817	373.8788
$^{13}C_{12}$-PCB 170	405.8428	407.8398

FC43 was used to calibrate the mass spectrometer. The analysis was conducted using a Varian VF-5MS capillary column (60 m × 0.25 mm × 0.25 µm) (Walnut Creek, California). The oven temperature control programme was as follows: initial hold at 140°C for 2 min, ramped to 230°C at 13°C/min, held at 230°C for 20 min, ramped to 310°C at 6°C/min, held at 310°C for 5 min. Helium (1 ml/min) was used as the carrier gas. The injector temperature was set at 280°C. Injections were performed in splitless mode (for 1 min) with a 1 µl volume and split rate of 140 ml/min. The interface temperature was set at 290°C. Electron impact ionisation (EI) was conducted at an ion source temperature of 270°C and voltage 40-50 eV.

Instrument calibration and analyte identification

The concentration of the various congeners was determined from a six-point calibration curve (standard solutions CS1 to CS6 as indicated in Table I). Relative response factors for unlabelled analytes (RFn) referred to the appropriate internal standards (Table III) and were calculated according to the following formula:

$$RF_n = \frac{(A_n^1 + A_n^2) \times Q_{is}}{(A_{is}^1 + A_{is}^2) \times Q_n}$$

where:
A_n^1 and A_n^2 = integrated areas of the two native congener diagnostic ions (Table II)
A_{is}^1 and A_{is}^2 = integrated areas of the two internal standard diagnostic ions (Table II)
Q_n = concentration in ng/ml of native congener
Q_{is} = concentration in ng/ml of internal standard.

The same procedure was followed to calculate the relative response factors for the labelled internal standards (RFis) referred to in the appropriate injection standards (Table III), using the following formula:

$$RF_{is} = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times Q_{is}}$$

where:
A_{rs}^1 and A_{rs}^2 = integrated areas of the two injection standard diagnostic ions (Table II)
Q_{rs} = concentration in ng/ml of injection standard.

The mean response factor and coefficient of variation (CV) were calculated for each native and labelled dl-PCB. The calibration was considered valid when the CV values did not exceed 25%. The following criteria were adopted for dl-PCB identification:

- retention time: maximum analyte peak within –1 to +3 secs of the corresponding labelled standard
- signal-noise ratio (S:N): ion fragment intensity ≥3 times background noise
- ion abundance ratios: respecting the relative abundance ratios listed in Table IV.

Table III
Relations of analytes with internal and injection standards

Analyte	Internal standard	Injection standard
PCB 77	$^{13}C_{12}$-PCB 77	$^{13}C_{12}$-PCB 70
PCB 81	$^{13}C_{12}$-PCB 81	$^{13}C_{12}$-PCB 70
PCB 126	$^{13}C_{12}$-PCB 126	$^{13}C_{12}$-PCB 111
PCB 169	$^{13}C_{12}$-PCB 169	$^{13}C_{12}$-PCB 138
PCB 105	$^{13}C_{12}$-PCB 105	$^{13}C_{12}$-PCB 111
PCB 114	$^{13}C_{12}$-PCB 114	$^{13}C_{12}$-PCB 111
PCB 118	$^{13}C_{12}$-PCB 118	$^{13}C_{12}$-PCB 111
PCB 123	$^{13}C_{12}$-PCB 123	$^{13}C_{12}$-PCB 111
PCB 156	$^{13}C_{12}$-PCB 156	$^{13}C_{12}$-PCB 138
PCB 157	$^{13}C_{12}$-PCB 157	$^{13}C_{12}$-PCB 138
PCB 167	$^{13}C_{12}$-PCB 167	$^{13}C_{12}$-PCB 138
PCB 189	$^{13}C_{12}$-PCB 189	$^{13}C_{12}$-PCB 170

Expression of results

The most recent toxicity evaluation method for dl-PCB isomers is based on the results by Van den Berg *et al.* (22), who defined toxicity equivalency factors (WHO-TEF = World Health Organization toxicity equivalency factors).

The WHO-TEF value for each congener was calculated as a ratio of its toxicity against that of 2,3,7,8-TCDD, the most toxic dioxin.

The concentration of each dl-PCB congener in a sample was multiplied by its WHO-TEF; concentrations were then summed to obtain the total concentration of dioxin-like compounds expressed in WHO-TEQ (toxic equivalent concentration).

Validation tests

Specificity was evaluated taking into account factors such as the matrix and interference from any non-dl-PCBs or dioxins. The precision of the method was evaluated by analysing 18 pork fat samples spiked at three concentrations levels (10, 20 and 40 pg/g) of PCB 77, 81, 126, 169, 114, 123, 157 and 189 (six repetitions per concentration). Accuracy was expressed in terms of percentage recovery of dl-PCB with respect to the quantity added. Given the impossibility of obtaining a fat sample negative for PCB 118, 105, 167 and 156, repeatability for these congeners was estimated from the analysis of three naturally contaminated pork fat samples, repeated six times. The limit of detection (LOD) is not a constant value but varies with the procedure used (dilutions, weight of sample processed, analyte recovery). For each congener, when the response for both diagnostic ions of the native analyte was less than three times the level of

Table IV
Criteria for dioxin-like polychlorinated biphenyls isotype ratio measurements

Analyte	Selected ion (approximate mass)	Theoretical ion abundance	Control limits
TeCB[a]	290/292	0.77	0.62-0.92
PeCB[b]	326/328	1.55	1.24-1.86
HxCB[c]	360/362	1.24	0.99-1.49
HpCB[d]	394/396	1.05	0.84-1.26
$^{13}C_{12}$-TeCB	302/304	0.77	0.62-0.92
$^{13}C_{12}$-PeCB	338/340	1.55	1.24-1.86
$^{13}C_{12}$-HxCB	372/374	1.24	0.99-1.49
$^{13}C_{12}$-HpCB	406/408	1.05	0.84-1.26
$^{13}C_{12}$-PCB 70	302/304	0.77	0.62-0.92
$^{13}C_{12}$-PCB 111	338/340	1.55	1.24-1.86
$^{13}C_{12}$-PCB 138	372/374	1.24	0.99-1.49
$^{13}C_{12}$-PCB 170	406/408	1.05	0.84-1.26

a) TeCB: PCB 77, PCB 81
b) PeCB: PCB 105, PCB 114, PCB 118, PCB 123, PCB 126
c) HxCB: PCB 156, PCB 157, PCB 167, PCB 169
d) HpCB: PCB 189

the noise, the LOD was calculated from the ratio of the labelled signal to the signal (noise) of the native. The linearity of response was evaluated by constructing a calibration curve of the six concentrations (0.5-800 ng/ml) for each dl-PCB and calculating the coefficient of determination. To comply with ISO/IEC 17025, the measurement uncertainty was estimated according to EURACHEM/CITAC guidelines (1).

Samples examined

In 2005, the validated method was used to analyse 177 samples of foods of animal origin (62 meat and fat, 55 milk, 24 egg and 36 fish samples) taken under the Italian NRP, with the aim of determining background contamination levels of dl-PCBs. Samples were taken in all areas of Italy from slaughterhouses, breeders, farms, and production and processing sites.

Results

Figures 1-4 show example mass chromatograms for each reference standard, a 'negative' sample and a naturally contaminated fish oil sample for all test analytes. In order to identify any signals from dioxin interference, a standard mixture containing the seventeen 2,3,7,8-substituted PCDD and PCDF congeners (concentration 0.4 to 4 ng/ml) and the relative sixteen labelled congeners (20 ng/ml) were injected into the HRGC-HRMS system with the dl-PCBs heating and mass acquisition programme. No chromatographic overlap was seen between the dioxins and dl-PCBs, except for slight interference with 1,2,3,7,8-PCDD fragment ions at low intensity, which co-elute with PCB-169. In samples with relatively high contamination levels, such as fish and fish feed, two interferents made PCB 81 and 123 quantification particularly difficult; this problem has also been encountered by other researchers (2, 9). In order to identify the nature of these interferents, a standard mixture containing sixteen non-dl-PCBs (PCB 28, 52, 95, 99, 101, 110, 138, 146, 149, 151, 153, 170, 177, 180, 183 and 187) (concentration 100 ng/ml) was injected into the HRGC-HRMS system with the dl-PCB heating and mass acquisition programme. Corresponding with the PCB 123 peak was a signal from one of the minor ion fragments of the hexachlorobiphenyl PCB 149, typically present at higher concentrations than PCB 123 in food matrices, with a consequent overlap of the two signals. The PCB 81 interferent could not be identified, although it is probably a pentachlorobiphenyl (9). This interference was partially resolved by using a 5% phenyl, 95% methyl silicone stationary phase of greater thickness (0.25 μm) than that originally used (0.1 μm).

This adjustment enabled clearer identification of the peak, to the detriment of instrumental sensitivity, which was reduced by approximately half.

Figures 5 and 6 show the chromatograms for an extract of the same sample (fish oil) injected in different columns.

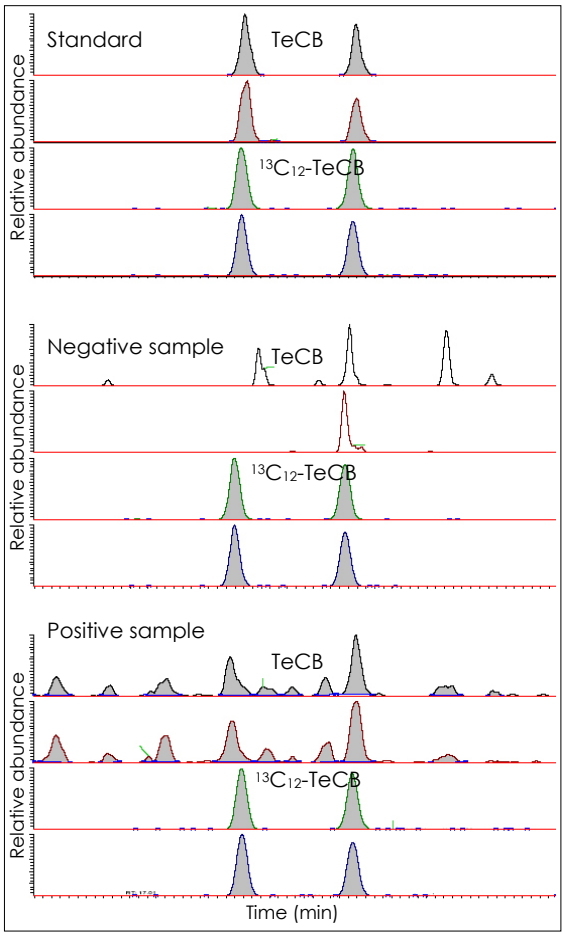

Figure 1
Chromatograms for standard mixtures (2 ng/ml of each TeCB and 50 ng/ml of each $^{13}C_{12}$-TeCB), negative sample (200 pg/g of each $^{13}C_{12}$-TeCB) and naturally contaminated fish oil sample (0.5-3 pg/g of TeCB and 200 pg/g of each $^{13}C_{12}$-TeCB)

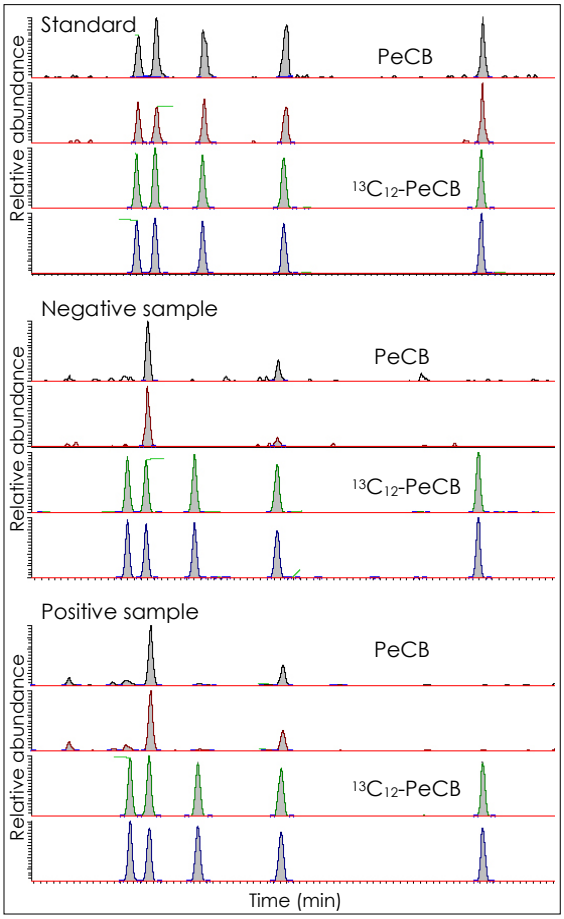

Figure 2
Chromatograms for standard mixtures (2 ng/ml of each PeCB and 50 ng/ml of each $^{13}C_{12}$-PeCB), 'negative' sample (presence of 3 pg/g PCB-118 and 1 pg/g PCB-105 and 200 pg/g of each $^{13}C_{12}$-PeCB) and naturally contaminated fish oil sample (1-60 pg/g of PeCB and 200 pg/g of each $^{13}C_{12}$-PeCB)

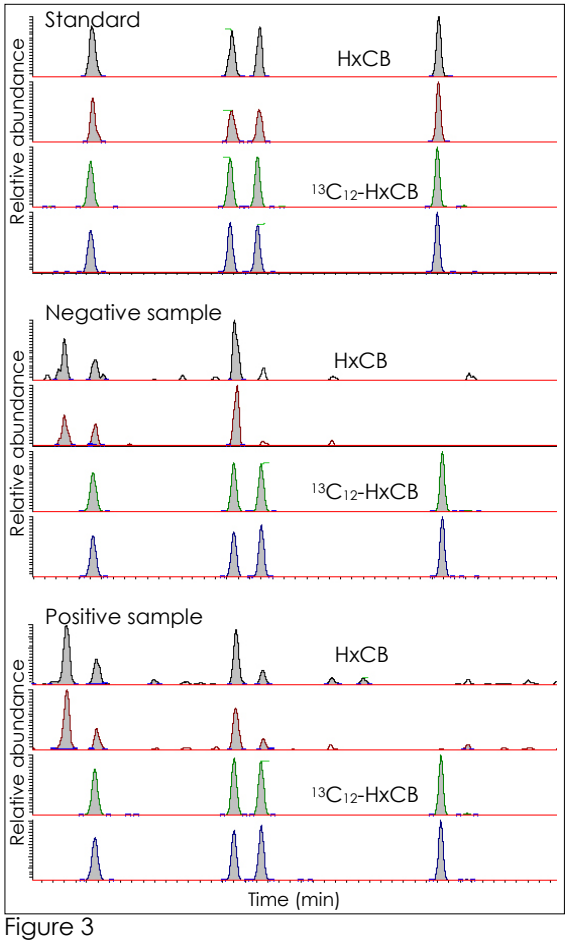

Figure 3
Chromatograms for standard mixtures (2 ng/ml of each HxCB and 50 ng/ml of each $^{13}C_{12}$-HxCB), 'negative' sample (presence of 0.5 pg/g PCB-167 and 1.5 pg/g PCB-156 and 200 pg/g of each $^{13}C_{12}$-HxCB) and naturally contaminated fish oil sample (0.3-4 pg/g of HxCB and 200 pg/g of each $^{13}C_{12}$-HxCB

With respect to the eight congeners (PCB 77, 81, 126, 169, 114, 123, 157 and 189) for which fortified samples were prepared at three fortification levels (10, 20 and 40 pg/g), recovery percentages ranged from a minimum of 84.4% for PCB 157 to a maximum of 111.8% for PCB 126 (Table V). The coefficients of variation ranged from 4.7% for PCB 81 to 18.3% for PCB 157. For PCB 105, 118, 156 and 167, with which no matrix was found to be uncontaminated, repeatability tests conducted by the analysis of three naturally contaminated samples of pork fat gave the results shown in Table VI. The maximum CV was 12.1% for PCB 118, at a concentration of 52.4 pg/g, while the minimum CV was 2.6% for PCB 105, in a sample containing a concentration of 33.2 pg/g.

The calibration curve for the twelve congeners showed an excellent linearity of response, with coefficients of determination greater than 0.9999 in the working concentration range (0.5-800 ng/ml). Mean LODs were between 0.2 pg/g of fat for PCB 81 and 1.3 pg/g of fat for PCB 123.

Results for measurement uncertainty according to EURACHEM/CITAC guidelines are presented in Table VII. The extended uncertainty was 8.8% to 12.4% at a 95% confidence limit.

The results for dl-PCB contamination levels on 177 samples taken as part of the 2005 Italian NRP are presented in Table VIII. Fish was found to be the most contaminated matrix. On the basis of fat content, milk had the highest

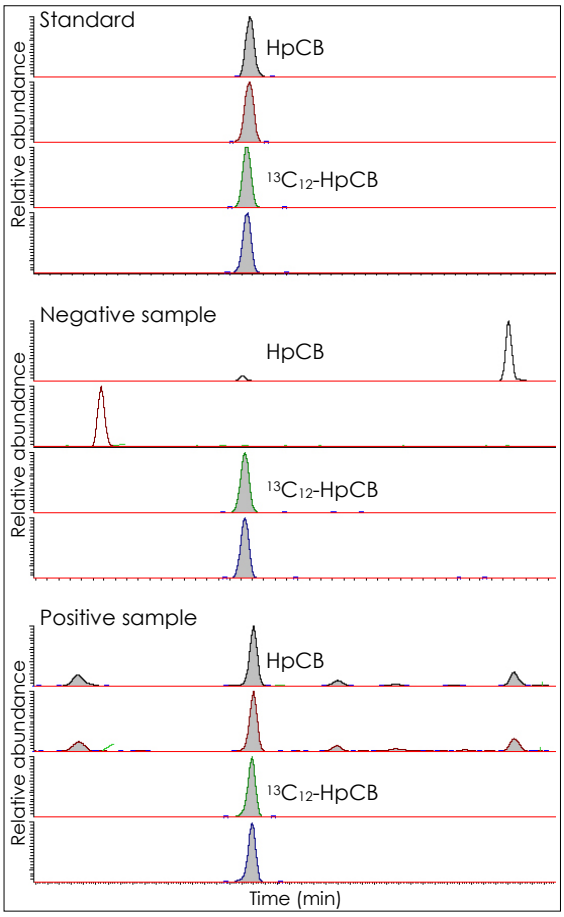

Figure 4
Chromatograms for standard mixtures (2 ng/ml of each HpCB and 50 ng/ml of each $^{13}C_{12}$-HpCB), negative sample (200 pg/g of each $^{13}C_{12}$-HpCB) and naturally contaminated fish oil sample (34 pg/g of HpCB and 200 pg/g of each $^{13}C_{12}$-HpCB)

mean WHO-TEQ (1.24 pg/g fat), followed by meat and fat (0.32 pg/g fat) and finally eggs (0.16 pg/g fat).

Discussion

The starting point for the refinement of an analytical method for the determination of dl-PCBs in food products was EPA method 1668 Revision A, developed mainly for environmental measurements and considering only marginally the matrices studied here (21). A further limit of this method is the rather lengthy sample extraction and purification phases and the considerable level of manpower required to conduct them. The use of automated apparatus (PowerPrep ASE extractor and purification system) presented here significantly reduced analysis times, minimised operator intervention during the process and achieved satisfactory repeatability of results. The use of ASE in the extraction phase reduced both the procedure time and the quantity of solvent necessary to extract the lipid component of the food matrices. In fact, using the traditional Soxhlet method, sample extraction takes 16-18 h and uses 250-500 ml solvent, whereas with ASE, the extraction time is reduced to approximately 45 min and only 30-40 ml of acetone/hexane mixture (20:80 v/v) is required. This mixture ensured a greater

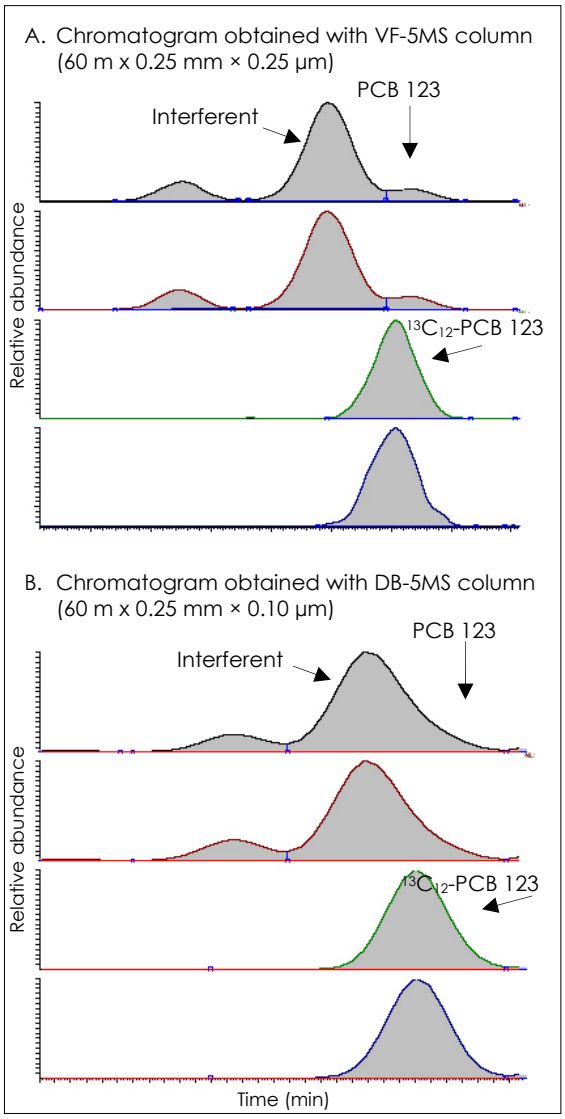

Figure 5
Chromatograms of congeners PCB 123 and $^{13}C_{12}$-PCB 123 relative to a naturally contaminated fish oil extract injected in different GC columns

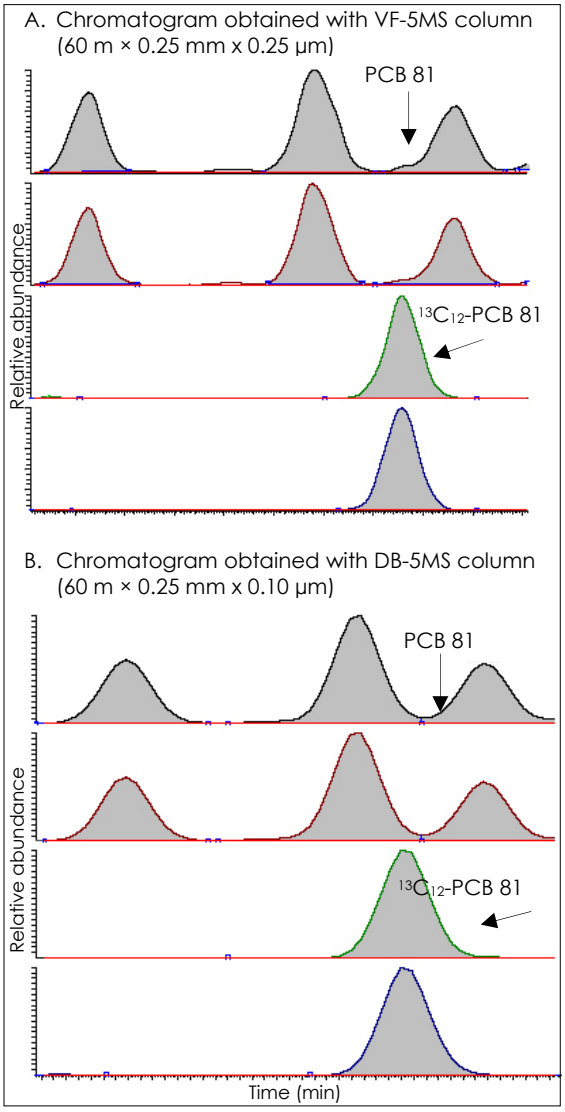

Figure 6
Chromatograms of congeners PCB 81 and $^{13}C_{12}$-PCB 81 relative to a naturally contaminated fish oil extract injected in different GC columns

extraction yield than can be obtained with strongly apolar solvents, due to the presence of acetone, which is able to penetrate water-containing matrices such as animal tissues (10).

Table V
Repeatability tests for PCB 77, 81, 126, 169, 114, 123, 157 and 189
(fortified negative pork fat samples, n=6 for each fortification level)

Analyte	Fortification level (pg/g)			Recovery (%)	SD	CV (%)
PCB 77	10	20	40	105.6	7.7	7.3
PCB 81	10	20	40	111.3	5.2	4.7
PCB 126	10	20	40	111.8	7.5	6.7
PCB 169	10	20	40	106.6	7.4	6.9
PCB 114	10	20	40	104.8	5.6	5.4
PCB 123	10	20	40	94.7	8.8	9.3
PCB 157	10	20	40	84.4	15.5	18.3
PCB 189	10	20	40	94.8	9.9	10.5

SD standard deviation
CV coefficient of variation

The relatively low boiling points of the two solvents also enabled rapid concentration of the extracts by vacuum evaporation at 45°C. To minimise the negative effects caused by the presence of water, samples with a high moisture content (>20%) were mixed with diatomaceous earth to remove the water and homogenise the sample, resulting in a more efficient extraction of lipid component of the sample and the analytes present therein. In the subsequent liquid-liquid partition, samples were treated with concentrated sulphuric acid and then with 20% (w/v) potassium hydroxide. These strong acids and bases removed the vast

Table VI
Repeatability tests for PCB 105, 118, 156 and 167
(analysis in triplicate of three naturally contaminated pork fat samples)

Analyte	Sample 1			Sample 2			Sample 3		
	C (pg/g)	SD	CV (%)	C (pg/g)	SD	CV (%)	C (pg/g)	SD	CV (%)
PCB 105	71.7	3.40	4.7	33.2	0.85	2.6	22.1	2.38	10.7
PCB 118	154.0	14.8	9.6	63.9	4.92	7.7	52.4	6.35	12.1
PCB 156	57.0	3.84	6.7	32.2	1.47	4.6	19.5	1.38	7.1
PCB 167	40.1	3.46	8.6	23.9	1.13	4.7	12.8	0.59	4.6

C concentration
SD standard deviation
CV coefficient of variation

Table VII
Quantification of measurement uncertainties, expressed in relative terms
(p=0.05; k=2)

Analyte	Repeatability uncertainty	Volume uncertainty	Calibration curve uncertainty	Combined uncertainty	Extended uncertainty (%)
PCB 77	0.0171	0.0276	0.0177	0.0470	9.4
PCB 81	0.0110	0.0276	0.0245	0.0482	9.6
PCB 114	0.0127	0.0276	0.0220	0.0474	9.4
PCB 123	0.0219	0.0276	0.0056	0.0459	9.2
PCB 126	0.0159	0.0276	0.0108	0.0443	8.8
PCB 157	0.0432	0.0276	0.0203	0.0622	12.4
PCB 169	0.0163	0.0276	0.0091	0.0441	8.8
PCB 189	0.0247	0.0276	0.0080	0.0476	9.6

Table VIII
Dioxin-like polychlorinated biphenyl contamination levels in various foodstuffs
Concentrations are expressed in pg WHO-TEQ/g of fat for meat and fat, milk and egg, and in pg WHO-TEQ/g for fish

Matrix	No. of samples	Mean	Median	Minimum	Maximum	95th percentile
Meat and fat	62	0.32	0.12	0.01	2.21	0.87
Milk	55	1.24	0.61	0.09	11.17	3.53
Egg	24	0.16	0.13	0.03	0.38	0.36
Fish	36	0.52	0.33	0.05	3.13	1.62

majority of the hydrolysable and saponifiable lipid component which migrated into the aqueous phase and was eliminated, while the hydrophobic analytes remained in the apolar (hexane) phase. The addition of 5% (w/v) sodium chloride solution removed any emulsion formations. As an alternative, glass columns packed with silica gel and Extrelut® saturated with sulphuric acid were used. Processing times with this procedure were longer than for liquid-liquid partition and so the latter was used to validate the method. Automated purification through chromatographic columns using the PowerPrep system reduced the analysis time and increased the number of samples processed simultaneously, compared with manual purification using the columns specified in method EPA 1668. In fact, manual purification requires 8-10 h, against the 1 h approximately necessary for automated purification. The selected eluent mixtures enabled some of the co-extracted interferents to be removed (dioxins and polychlorinated diphenyl ethers), with the opportunity to collect different analyte groups in different eluates (17).

The gas chromatographic separation efficacy was evaluated for the two critical pairs, PCB 123-PCB 118 and PCB 156-PCB 157. A valley to valley separation of ≤2% was found.

The interfering co-extracts can vary depending on the sample type and source. Often, dl-PCBs are associated with other chlorinated compounds such as non-dl-PCBs, dioxins (PCDDs and PCDFs), organochlorinated pesticides and polybrominated diphenyl ethers (PBDEs), which may be present at higher levels than the analytes of interest. The use of high resolution (R≥10 000) mass spectrometry combined with high purity reagents and solvents minimised these interference problems. By adjusting the GC parameters, such as temperature ramping and type of column used, a strong improvement was observed in the separation of peaks co-eluting with PCB 81 and PCB 123.

In samples where interference with PCB 81 was particularly strong, a further purification step through a carbon column was necessary (16). This procedure separated the four coplanar dl-PCBs from the other PCBs, and thus from the interferent.

However, this increases the analysis time and requires a double HRGC-HRMS injection, without a marked increase in the estimated toxicity equivalency, given the low toxicity of the congeners PCB 81 and PCB 123.

With respect to the mass spectrometry (MS) working parameters, the ionisation source was set at the high working temperature of 270°C to minimise the effect of the matrix on the ionisation source. Multiple ion detection (MID) was performed at ionisation voltages of 40 and 70 eV to optimise the instrumental response. The 40 eV gave an instrumental response to dl-PCBs that was approximately three times better than at 70 ev.

Repeatability tests were satisfactory for all twelve dl-PCBs. The concentrations studied were comparable to those found in real samples, both for the eight PCBs (77, 81, 126, 169, 114, 123, 157 and 189) for which negative samples were fortified to overall toxicity equivalency levels of 1.12, 2.24 and 4.48 pg WHO-TEQ/g of fat, and for PCB 105, 118, 156 and 167, examined in naturally contaminated samples containing concentrations of these congeners ranging from 12.8 to 154 pg/g of fat.

The extended measurement uncertainties, calculated at a confidence level of 95% and obtained by determining the potential sources of uncertainty in the method according to international guidelines, were found to be appropriate for the quantification of the test residues with respect to the concentrations examined.

The method was applied to the various matrices with satisfactory results, confirmed by the outcome of inter-laboratory test participation (FAPAS® UK Series 6, Round 20/2004-23/2005), the results of which were within ± 2 z-scores of the permitted values for all the analytes tested.

Mean contamination values for the 177 samples tested under the NRP were expressed as concentration per gram of fat, except for fish samples, where they were expressed as concentration per gram of product in compliance with current legislation (5). Samples from one highly PCB-contaminated area were excluded from the statistical analysis, as they would have altered the background contamination picture which was the main monitoring objective.

In general, contamination levels were found to be in line with those found in other European countries for milk, meat and fat, while they were lower for eggs and fish (8, 20, 24).

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