High-throughput GC-ECD analysis of PCBs in food by Accelerated Solvent Extraction: method validation.

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Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants widespread in the environment. The hazard associated with them is mainly due to their toxicity along with their hydrofobicity and ability to bio-accumulate. The main way of exposure for humans is food of animal origin.

Since the Belgian incident occurred (1999), when a stock of recycled fat contaminated with PCB and dioxins was used for animal feeds production, more attention is posed in monitoring the presence of these contaminants in food.[1]

In the year 2000 the determination of the PCBs in food commodities was introduced in the Italian national residue control plan in which government labs were requested to estimate the total PCB content as sum of seven more representative congeners. Later on, in 2001, it was decided that a more appropriate estimation of the total PCBs was possible through analysis of eighteen rather than seven congeners. Therefore the need for simple and validated analytical methods arose.[2]

In this work a method for the analysis of the PCBs 18-congeners (T_3CB-28, T_4CB-52, P_5CB-95, P_5CB-99, P_5CB-101, P_5CB-105, P_5CB-110, P_5CB-118, H_6CB-138, H_6CB-146, H_6CB-149, H_6CB-151, H_6CB-153, H_7CB-170, H_7CB-177, H_7CB-180, H_7CB-183, H_7CB-187) is reported.[3]

This has been set up taking in account the advantages of the automated and high efficient Accelerated Solvent Extraction together with good purification achieved by a one-step acidic-extrelut/silica chromatography. The instrumental analysis is performed by capillary-GC equipped with an ECD detector.

An in-house validation study has been made on swine muscle assessing the method performances in terms of limit of detection, response linearity range, trueness and precision.

Methods and Materials

Reagents and materials

Acetone, *n*-Hexane and *iso*-octane were pesticide grade (Carlo Erba, Milano, Italy); Hydromatrix and Extrelut were purchased from Varian and Merck respectively; concentrated sulfuric acid (96-98%) was analytical reagent grade (Carlo Erba, Milano, Italy); silica SPE cartridges (1g/6mL) were supplied by International Sorbent Technology.

Standard Solutions

The 20 PCB congener (T_3CB-28, T_4CB-52, P_5CB-95, P_5CB-99, P_5CB-101, P_5CB-105, P_5CB-110, P_5CB-118, H_6CB-138, H_6CB-146, H_6CB-149, H_6CB-151, H_6CB-153, H_7CB-170, H_7CB-177, H_7CB-180, H_7CB-183, H_7CB-187 and H_6CB-155, O_8CB-198 as internal standards) reference materials were purchased as solutions at 10 µg/mL in *iso*-octane from Dr. Ehrenstorfer reference materials. Stock solutions (0.5 µg/mL) were prepared in *iso*-octane and stored at 4°C. Calibration standards were prepared at concentrations of 1, 50, 100, 150, 200 ng/mL for each congener, the internal standards only (I.S.: H_6CB-155, O_8CB-198) were always at 50 ng/mL.

Apparatus

An HETO freeze drying system (Analitica De Mori, Milan, Italy), a rotary evaporator (Büchi, Flawil, Switzerland) and a nitrogen evaporation system, with thermostated heating plate (Stepbio, Bologna, Italy) were used. Extraction was achieved with a Dionex ASE 200 (Dionex, Idstein, Germany) equipped with 33 mL stainless steel cells. The clean up procedure was carried out using an SPE vacuum system (J. T. Baker). Samples were analysed with a GC Agilent-Technology (6890N) equipped with a 7683 series automatic injector, a PTV injector and an µ-ECD. 1 µL of sample was injected in splitless mode and the injector temperature was programmed as follows: 0.08 min at 80°C, increased to 270°C at 700°C/min, kept at 270°C for 20 min. The chromatographic separation was achieved in temperature programmed mode using a 30m x 0.25mm I.D. Rtx-5MS/Integra Guard column (5% diphenyl - 95% dimethyl polysiloxane, film thickness 0.25 µm, Restek Corporation, Bellefonte, PA, USA) as follows: 1 min at 60° C, increased to 190°C at 10°C/ min, kept at 190°C for 13 min, increased to 270°C at 3°C/min, kept at 270°C for 6.33 min. A column with a different polarity was used for confirmation of positive samples (30m x 0.25mm I.D., Rtx-

1701/Integra Guard: 14% cyanopropylphenyl – 86% dimethyl polysiloxane, film thickness 0.25 μm).

Sample Preparation

An amount of sample containing between 250 and 500 mg of fat was weighted on a Petri glass plate, frozen at –80 °C overnight and submitted to freeze drying (from 5 to 13 hrs depending on the moisture content of the sample). Between 3-5 g of Hydromatix (dispersing agent) were added to the dried minced material and the whole was loaded in the ASE cells and submitted to pressurised extraction with a mixture of Hexane/Acetone (1:1 v/v), after which the extract was brought to dryness under reduced pressure and the fat obtained was cleaned-up after the addition of a solution of the two internal standards (25 ng for each I.S.: H_6CB-155 and O_8CB-198).

The Extrelut NT-3 column was loaded with 3 mL of concentrated sulfuric acid, kept standing for about one hour and connected on top of the preconditioned (4 mL *n*-Hexane by gravity) silica cartridge.[4] Both columns were washed with 10 mL *n*-Hexane. The extracted fat was dissolved in 1mL of *n*-Hexane and loaded on the Extrelut column allowing adsorption of the solution. The sample loading was repeated two more times. After ten minutes, the analytes were eluted with 13 mL *n*-Hexane. The solvent was removed with nitrogen stream, and the sample re-dissolved in 0.5 mL *iso*-octane and injected in GC- ECD.

Method Validation

Linearity

To investigate the instrumental response linearity, three calibration graphs for each PCB were built in three different days on five different concentration levels (1, 50, 100, 150 and 200 ng/mL in *iso*-octane), keeping the concentrations of two internal standards at 50 ng/mL constant.

Trueness and precision

To investigate the precision and trueness of the proposed GC-ECD method, six replicate analyses on a swine muscle spiked with all the eighteen congeners at three different concentration levels were performed (precision). Thereafter, a certified reference material (Cod Liver Oil – CRM349 - Community Bureau of Reference, Commission of the European Communities), in which the amounts of six PCB congeners representing all the homologues classes (tri- to epta-chlorinated) were certified, was analysed in six replicates (trueness).

Spiked muscle. The three concentration levels were chosen to cover the application field of the method, considering swine muscle as representative of all muscle types (bovine, poultry, ovine, horse, etc…). To roughly 7.5 g of swine muscle, homogeneously distributed on a Petri glass plate, an appropriate volume of the spiking solutions (500 ng/mL for the higher level and 50 ng/mL for the lower two) was added, and the test samples were let to stand in order to remove the exceeding solvent. All samples were processed as previously described.

Certified Reference Material (CRM349). About 500 mg of cod liver oil were dissolved in 1 ml *n*-Hexane, submitted to clean-up and GC-ECD analysed as previously described.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was estimated according to the U.S. EPA guidelines by multiplying the appropriate one-sided 99% *t*-statistic by the standard deviation.[5] This was obtained from a minimum of three analyses of a spiked matrix containing the analytes at a concentration three to five times higher than the estimated LOD. The LOQ was calculated multiplying the LOD value by 3.3. In our validation approach, the suitable standard deviations were those obtained from the experiments carried out at 0.39 ng/g.

Results and Discussion

The regression equations, calculated with the least square method, were obtained by plotting peak area ratios (congener area/internal standard area) versus the relative concentration (Table 1). Good correlation coefficients r^2 were obtained for each congener (>0.999). The compliance to linearity was also investigated by calculating the response factors (peak area ratio/concentration) and verifying that each of the five values obtained for each congener were included in the mean value ± 10%. Finally, all calibration graphs pass through the origin of the axes as demonstrated with the *t*-test, comparing the intercept "*a*" with zero.[6] In Table 2 the mean concentration, the coefficients of variation (CV%) and the recoveries obtained for the three fortification levels are shown. The precision (repeatability) is generally very good for the two higher spiking-levels, unlike, for the lowest validation level (0.39 ng/g) several CV values are higher because, the concentration falls between the LOD and the LOQ of the method for most of the congeners.

The recoveries reported in Table 2 are automatically corrected for bias due to the use of internal standards for quantification (corrected recoveries). CRM349 was used to investigate the trueness of the method. In Table 3 the mean concentrations (fat basis) measured with their standard deviations and the certified values for the six congeners are reported. The statistic control of trueness was achieved performing a *t*-test: for all the six molecules results demonstrate that the measured values were not significantly different from those certified.

Table 1: Regression equations of calibration graphs.

PCB	Slope (*b*)	Intercept (*a*)	r^2	s_b[1]	s_a[1]
28[2]	0.0234	-0.0038	0.9993	0.0003	0.0351
52[2]	0.0175	0.0177	0.9993	0.0002	0.0261
95[2]	0.0233	0.0238	0.9993	0.0003	0.0338
101[2]	0.0241	-0.0007	0.9994	0.0003	0.0322
99[2]	0.0260	0.0129	0.9994	0.0003	0.0343
110[2]	0.0306	0.0015	0.9994	0.0004	0.0417
151[2]	0.0300	0.0216	0.9997	0.0002	0.0276
149[2]	0.0250	0.0238	0.9993	0.0003	0.0366
118[2]	0.0268	0.0043	0.9995	0.0003	0.0329
146[2]	0.0285	0.0205	0.9993	0.0004	0.0413
153[2]	0.0274	-0.0171	0.9991	0.0004	0.0471
105[2]	0.0350	-0.0114	0.9991	0.0005	0.0592
138[2]	0.0333	0.0025	0.9993	0.0004	0.0480
187[3]	0.0185	0.0002	0.9994	0.0002	0.0251
183[3]	0.0205	-0.0086	0.9993	0.0003	0.0302
177[3]	0.0190	0.0179	0.9994	0.0002	0.0264
180[3]	0.0224	-0.0184	0.9991	0.0003	0.0371
170[3]	0.0248	-0.0251	0.9990	0.0004	0.0436

[1] s_b and s_a are the standard deviations for the slope (*b*) and the intercept (*a*) respectively.
[2] quantified using H_6CB-155 as internal standards
[3] quantified using O_8CB-198 as internal standards

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Table 2: LOD, precision and recoveries.

PCB	LOD (ng/g)	Spiking level[1]								
		0.39 ng/g			1.66 ng/g			3.32 ng/g		
		Mean[2] (ng/g)	CV (%)	R (%)	Mean[2] (ng/g)	CV (%)	R (%)	Mean[2] (ng/g)	CV (%)	R (%)
28	0.20	0.38	16	97	1.11	6	67	1.91	9	57
52	0.12	0.44	8	112	1.33	6	80	2.75	7	83
95	0.10	0.42	7	106	1.51	2	91	2.51	3	76
101	0.18	0.30	18	77	1.63	2	98	2.73	2	82
99	0.12	0.42	9	106	1.57	1	95	2.68	3	80
110	0.13	0.38	10	97	1.52	1	92	2.66	2	80
151	0.20	0.40	15	102	1.62	1	97	2.77	1	83
149	0.19	0.33	17	84	1.68	1	101	2.77	1	83
118	0.23	0.41	17	104	1.64	1	99	2.83	1	85
146	0.14	0.40	10	102	1.66	1	100	2.91	1	88
153	0.15	0.38	12	96	1.71	1	103	3.01	1	91
105	0.13	0.39	10	100	1.52	1	92	2.79	2	84
138	0.16	0.38	13	96	1.63	1	99	2.93	1	88
187	0.09	0.40	7	101	2.03	2	122	3.31	1	100
183	0.12	0.38	10	96	1.92	1	116	3.22	1	97
177	0.27	0.47	17	118	1.65	2	100	2.83	2	85
180	0.11	0.35	9	88	1.71	1	103	2,94	1	89
170	0.37	0.41	27	103	1.74	1	105	3.02	1	91

[1]For each level six replicate analysis (n=6) were carried out; [2]All concentrations are referred to wet weight

Table 3: Results obtained for CRM349.

PCB	28 (T_3-Cl)	52 (T_4-Cl)	101 (P_5-Cl)	118 (P_5-Cl)	153 (H_6-Cl)	180 (H_7-Cl)
Observed value (ng/g lipid)	62	133	310	379	811	317
SD (ng/g lipid)	5	6	9	12	23	23
Certified value (ng/g lipid)	68	149	370	454	938	280
S_{CRM}[1] (ng/g lipid)	± 7	± 20	± 17	± 31	± 40	± 22

[1] Standard deviation of CRM349 reproducibility

Conclusions

The developed procedure is rapid, inexpensive and fit to purpose for a laboratory with high sample throughput. The method is characterized by high degree of automation which results in operators time saving with minimization of human errors; many PCB determinations can be performed simultaneously by one single operator giving highly reproducible results. Besides, the obtained data are automatically corrected for bias as recommended by the international organisations.[7]

We are currently engaged in experiments to extend the applicability of the method to other food commodities of animal origin such as milk and eggs.

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