Evaluation of the capabilities of atmospheric pressure chemical ionization source coupled to tandem mass spectrometry for the determination of dioxin-like polychlorobiphenyls in complex-matrix food samples.

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ABSTRACT

The use of the novel atmospheric pressure chemical ionization (APCI) source for gas chromatography (GC) coupled to triple quadrupole using tandem mass spectrometry (MS/MS) and its potential for the simultaneous determination of the 12 dioxin-like polychlorobiphenyls (DL-PCBs) in complex food and feed matrices has been evaluated. In first place, ionization and fragmentation behavior of DL-PCBs on the APCI source under charge transfer conditions has been studied followed by their fragmentation in the collision cell. Linearity, repeatability and sensitivity have been studied obtaining instrumental limits of detection and quantification of 0.0025 and 0.005 pg μL^{-1} (2.5 and 5 fg on column) respectively for every DL-PCB. Finally, application to real samples has been carried out and DL-PCB congeners (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) have been detected in the different samples in the range of 0.40 to 10000 pg g^{-1}. GC-(APCI)MS/MS has been proved as a suitable alternative to the traditionally accepted confirmation method based on the use of high resolution mass spectrometry and other triple quadrupole tandem mass spectrometry techniques operating with electron ionization. The development of MS/MS methodologies for the analysis of dioxins and DL-PCBs is nowadays particularly important, since this technique was included as a confirmatory method in the present European Union regulations that establish the requirements for the determination of these compounds in food and feed matrices.

Key words

Dioxin-like polychlorinated biphenyls, persistent organic pollutants, atmospheric pressure chemical ionization, food and feed samples.

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) belong to a broad family of anthropogenic organic chemicals known as chlorinated hydrocarbons. Due to their non-flammability, chemical stability and electrical insulating properties, PCBs were commonly used in the past in hundreds of industrial and commercial applications [1]. PCBs have been demonstrated to cause a variety of adverse effects on the immune, reproductive, nervous and endocrine systems of the living organisms, as well as other health effects [2]. As a result of their structure, PCBs are lipophilic and persistent, expected to be bioaccumulated (specially the coplanar ones) in the environment and biological matrices. Recently, a Working Group of the International Agency for Research on Cancer (IARC) has evaluated PCBs as carcinogenic for humans (Group 1) [3].

Among the total number of 209 PCB congeners, there are congeners that can take a planar conformation, which could confer on them toxicological properties, the same as those observed for dioxins (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs)) These PCBs congeners are called dioxin-like PCBs (DL-PCBs). Even though DL-PCBs are usually present at levels quite lower than other PCBs, they have demonstrated to be harmful to living organism at these very low levels like PCDD/Fs [4]). Consequently sensitive and selective analytical methodologies are needed, to demonstrate foods are safe. The analysis of DL-PCBs has been traditionally close related to that of dioxins. These PCBs have been assigned with toxicity equivalency factors (TEFs), taking the toxicity of the 2,3,7,8-tetraclorodibenzo-*p*-dioxin as a reference, similarly to what was previously done for all the toxic PCDD/Fs. Since 2006, maximum levels for the sum of PCDD/Fs and DL-PCBs in food and feedstuff products are listed, together with the maximum levels for PCDD/Fs in the same matrices, in the corresponding European Union (EU) regulations and directives.

Furthermore, in 2002 the European Commission had already laid down methods of analysis for the official control of dioxins that also referred to the determination of DL-PCBs.

In these EU regulations and directives, confirmatory methods were based on high resolution gas chromatography coupled to high resolution mass spectrometry (GC-HRMS). The HRMS technique allowed to totally fulfill most of the basic requirements (i.e. high sensitivity and low detection limits, high selectivity and specificity and high accuracy). Alternatively, other techniques have been explored, in particular for the analysis of DL-PCBs: GCxGC-μECD [5], ECNI–LRMS (for non-ortho PCBs) [6,7] and ITMS/MS [8]. For real samples, accuracy, precision and LOQs obtained with these techniques in the analysis of food samples are in the same range (fish oil and fish), or slightly worse (milk and pork) compared to GC–HRMS results [8–10], confirming their potential for DL-PCB determination.

To complete the scenario, GC-MS/MS techniques have recently been approved as valid techniques for confirmatory methods for the determination of PCDD/Fs and DL-PCBs, according to EU Regulations No 589/2014 and 709/2014 of June 2014 [11,12]. However, specific criteria are applied to these techniques; in particular it is mandatory to monitor at least 2 specific precursor ions. Although from a theoretical point of view with ITMS/MS it is possible to monitor the product ions coming from a precursor cluster, from a practical perspective this would lead to an increment of the scan time and quite compromised sensitivity and peak shape. This would be even worse when monitoring the isotopically labelled internal standards. On the contrary, mass spectrometry instruments with a triple quadrupole configuration (QqQ) can perform multiple reaction monitoring, allowing to acquire various specific transitions (with different precursor ions) simultaneously. In addition, other criteria has to be fulfilled

both for GC-HRMS and GC-MS/MS, such as those related to the sensitivity of the method. It is important to have appropriate LOQs since most food and feed samples showed low levels of PCDD/Fs and DL-PCBs, far below the maximum established. Quantitation at around one fifth of the level of interest has to be feasible.

Regarding available sources for GC-MS/MS, electron impact ionization (EI) have been the most widely used in this field. However, EI sources usually give a considerable fragmentation of the molecules, due to the high energy transferred to them during the ionization process which could affect selectivity in some samples as a consequence of a higher matrix effect. . Considering these limitations related with EI sources, it is clear that MS/MS methods with QqQ could benefit from the use of soft and universal ionization techniques able to provide more abundant molecular ions and less in-source fragmentation, thus allowing to reach higher sensitivity. Atmospheric pressure chemical ionization (APCI) source has already demonstrated its efficacy in obtaining the molecular ion (or the protonated molecule) and enhancing sensitivity in many applications, mainly coupled to HPLC [13–15] or GC-(Q)TOF [16–18]. The GC-(APCI)MS/MS coupling with QqQ has not been fully tested, but it is showing promising results in terms of sensitivity when compared to GC-(EI)MS/MS methods [19,20].

This work follows up the pioneer contribution to the analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs) by GC-(APCI)MSMS which proved the capabilities of this technique as a real alternative to the HRMS instruments [21]. In the present work, a method for the determination of DL-PCBs in different food and feed complex matrices has been optimized and compared with the widely accepted GC-HRMS technique.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Solvents for organic trace analysis (cyclohexane, dichloromethane, *n*-hexane and toluene) were from J.T. Baker (Deventer, The Netherlands), ethanol was from Merck (Darmstadt, Germany) and nonane was purchased from Fluka Chemie (St. Gallen, Switherland). Silica and basic alumina for clean-up and fractionation were obtained from J.T. Baker and MP Biomedicals (Eschwege, Germany), respectively. Sulfuric acid (Merck) and sodium hydroxide (Carlo Erba, Milano, Italy) were also used to prepare modified silica.

Standard solutions of ^{13}C-labelled DL-PCBs for quantification (WP-LCS) and analytical recovery of the samples (WP-ISS), as well as calibration standards (WP-CS1 to WP-CS7), were from Wellington Laboratories Inc. (Guelph, Ontario, Canada).

2.2. Samples

Nine samples archived from Proficiency Test (PT) organized by the European Union Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food (2 pork meat, 2 lard, 1 whole egg and 1 egg yolk powder, 1 milk powder and 1 milk fat, and 1 mineral (sepiolite) together with 3 additional samples (1 fish, 1 spiked feed and 1 milk powder) were used for the evaluation of the applicability of the developed method.

2.3. Sample preparation

Matrices with high water content (pork meat, egg, fish) were freeze-dried as a pre-treatment step. Lyophilized samples and dry samples (milk powder, feed, egg yolk powder, mineral) were then spiked with a working standard solution, containing the 12 ^{13}C-labelled DL-PCBs in nonane, and extracted in a Soxhlet for aprox. 24 h with a

mixture of cyclohexane:toluene (50:50) or ethanol:toluene (70:30) (in the case of milk powder and mineral matrices). Next, extracts were concentrated in a rotary evaporator and transferred to *n*-hexane for purification on a multilayer (acid/basic) silica column. If needed, fat content was gravimetrically determined after keeping the dry residue coming from the rotary evaporator overnight in the oven (105 ºC). Fat samples (lard, milk fat) were directly dissolved in *n*-hexane, spiked with the working standard solution and added to the multilayer silica column without previous extraction. Further fractionation of the extracts was carried out on a basic alumina column (14 g), with all the DL-PCBs been eluted in a 75 mL hexane:dichloromethane fraction (90:10). Finally, this fraction was concentrated to dryness in a vial and a standard mixture in nonane, containing ^{13}C-labelled PCBs for analytical recovery evaluation, was added.

2.4. Instrumentation

2.4.1. GC-(APCI) MS/MS

The chromatographic analysis were performed using an Agilent 7890A gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with an Agilent 7693A autosampler, coupled to a triple quadrupole mass spectrometer, Xevo TQ-S (Waters Corporation, Manchester, UK), with an APCI source. The GC separation was performed using a fused silica DB-5MS capillary column with a length of 60 m x 0.25 mm ID and a film thickness of 0.25 µm (J&W Scientific, Folson, CA, USA) working at a constant flow of 2 mL min^{-1} of Helium (99.999 %; Praxair, Valencia, Spain). The oven program was set as follows: 140 ºC; 20.00 ºC min^{-1} to 200 ºC (1.00 min); 3.00 ºC min^{-1} to 270 ºC; 50.00 ºC min^{-1} to 300ºC (1.33 min) with a total runtime of 30 min. The injections of 1 µL of sample extracts were performed in pulsed splitless

mode with at a temperature of 280 ºC and a pulse time of 1.00 min. The pulse pressure was set to 35.0 psi, with a purge flow of 80.0 ml min^{-1} and purge time of 1.00 min.

The interface temperature was set to 310 ºC using N_2 as auxiliary gas at a flow rate of 250 L h^{-1}, the make-up gas flow rate was set to 320 mL/min and the cone gas flow rate to 170 L h^{-1}. APCI corona pin operated at 1.4 μA. Mass spectrometer was operated in SRM mode, acquiring one quantification transition and one confirmation transition for both, native and ^{13}C-labelled compounds. In the SRM method, automatic dwell time (values ranging from 20 to 60 ms) was applied in order to obtain at least 15 points per peak. Targetlynx (a module of MassLynx) was used to handle and process the acquired data.

2.4.2. GC-(EI)HRMS and GC-(EI)MS/MS

Sample extracts were also analyzed on a 6890N Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) coupled to an Autospec NT high resolution mass spectrometer (EBE geometry) (Micromass, Manchester, UK), using a EI source and operating in the SIM mode.

Additionally, sensitivity and specificity for selected compounds with traditional GC-(EI)MS/MS methodology was tested. For that purpose, standards were injected on a 6890N Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an autosampler (Agilent 7683) coupled to a triple quadrupole mass spectrometer, Quattro Micro GC (Micromass, Boston, MA) operating in EI mode. Chromatographic conditions in both instruments were the same as in GC-(APCI)MS/MS analysis.

3. RESULTS AND DISCUSSION

3.1. GC-(APCI)MS/MS optimization

DL-PCBs commercial standard mixture WP-CS5 (40 ng mL^{-1}) was used to study the ionization of the 12 DL-PCB congeners and their corresponding ^{13}C-labelled internal standards under APCI source. As expected, all studied DL-PCBs were ionized under charge-transfer conditions due to the presence of only C, H and Cl atoms in their structure, without any protonable groups. Consequently, $M^{+\bullet}$ was formed as base peak of the spectrum for all the compounds. Additionally, as a consequence of the soft ionization character of the APCI source, low in-source fragmentation was observed if compared with their corresponding EI spectra. These aspects will have important consequences in sensitivity of the obtained transitions. As an illustrative example, **Figure 1** shows the difference between fragmentation degrees generated by EI and APCI sources for the PCB 156 where the fragment ion corresponding to the loss of 2 Cl atoms represents the 60 % of the base peak under EI, while only the 2% under APCI.

For developing the SRM method, up to three different precursor ions were studied, i.e. $M^{+\bullet}$, $[M+2]^{+\bullet}$ and $[M+4]^{+\bullet}$. Values between 20 and 70 V were tested, pursuing the optimal ionization for each DL-PCB. After selecting the precursor ions, daughter scan experiments at different collision energies, (5 to 50 eV) were conducted in order to determine the most sensitive and specific transitions. DL-PCBs commercial standard mixture WP-CS7 diluted to a final concentration of 80 ng mL^{-1} was used. For tetra- and penta-DL-PCBs, the most sensitive transitions were the ones which came from the corresponding $M^{+\bullet}$ ions losing two ^{35}Cl atoms, i.e. $[M-^{35}Cl_2]^{+\bullet}$, so the selected quantification (Q) transitions were 290>220 for tetra-DL-PCBs and 324>254 for the penta-DL-PCBs. On the contrary, the most sensitive transitions for hexa- and hepta-DL-PCBs were the ones which came from the corresponding $[M+2]^{+\bullet}$ ions losing two ^{35}Cl

atoms, i.e. $[M+2-^{35}Cl_2]^{+\bullet}$, so the selected Q transition was 360>290 and 394>324, respectively. Their corresponding ^{13}C-labelled internal standards showed a correlative fragmentation behavior. As a summary, experimental MS/MS parameters for each compound are shown in **Table 1**.

3.2. Linearity, repeatability and limits of detection

In order to test the reliability of the instrumental method, parameters as linearity, repeatability, specificity, limits of detection (LODs) and quantification (LOQs) were evaluated (see **Table 2**). Linearity was tested with calibration curves at 10 different concentration levels ranging from 0.001 pg μL^{-1} to 40 pg μL^{-1} and analyzed by triplicate. The solvent calibration curves generated by plotting relative response versus concentration (pg μL^{-1}) of each standard showed an acceptable correlation coefficient (r^2) > 0.999 for all the DL-PCBs. Repeatability of the response (n=10) of relative peak areas ranged from 2% to 12% for standards at concentration levels as low as 0.005 pg μL^{-1}. Instrumental LOQs were calculated as the lowest calibration point with a response factor (RF) deviation lower than 30% related to the mean response factor of the different standards of the calibration curve. For all the DL-PCBs, concentration level of 0.005 pg μL^{-1} gave RF values with deviations ranging from 3 to 19% analyzed by triplicate so this concentration was set as LOQ (5 fg on column). Additionally, to test the reproducibility of this LOQ level, calibration point at 0.005 pg μL^{-1} was injected at the beginning and at the end of the sequence, showing RSDs below 20 % in all cases. Instrumental LODs were calculated as the lowest concentration level giving a signal-to-noise ratio (S/N) of at least 3. The calibration point at 0.0025 pg μL^{-1} (2.5 fg on column) was estimated as the LOD for every DL-PCB. Instrumental LOQs and LODs reported

in the bibliography for DL-PCBs by GC-(EI)MS/MS range from 0.030 to 2.1 pg μL^{-1} and from 0.05 to 0.6 pg μL^{-1} respectively [22,23].

3.3. Analysis of real samples

Once the method was fully instrumentally validated, analysis of 3 real samples (1 fish, 1 spiked feed and 1 milk powder) and nine samples belonging to proficiency test studies (two replicates of a pork meat sample, two replicates of a lard sample, egg, egg yolk powder, milk powder, milk fat, and one mineral) were performed. All these samples had been previously analyzed by the Laboratory of Dioxins from the Institute of Environmental Assessment and Water Research (IDAEA, CSIC, Barcelona) by using the standard methodology by HRMS [24].

For the first three real samples analyzed, a good agreement was found. Relative errors below 15% were obtained for all the studied DL-PCBs in the different samples. Considering the complexity of these matrices, this kind of errors are assumable, validating the use of the developed GC-(APCI)MS/MS methodology as an alternative to traditional GC-(EI)HRMS. **Figure 2** represents the comparison between HRMS and APCI for the three mentioned matrices in pg g^{-1} of product for every single DL-PCB.

In order to fully confirm the goodness of the determination by using the developed methodology, nine additional sample extracts coming from different EU-RL Proficiency Tests [25–28] were also analyzed by the GC-(APCI)MS/MS method after their analysis by GC-(EI)HRMS. As in the other samples, results showed a good concordance between methods. Relative errors were lower than 20% for most of DL-PCBs in every sample, except for PCB 114 in the mineral sample; in that case the highest deviation was obtained (about 50 %) (**Table 3**). The comparison with the HRMS methodology is graphically displayed in **Figure 3**, in which the different box-plots represent the

residuals for each matrix (**Figure 3A**) and DL-PCB congener (**Figure 3B**) considering the HRMS as the reference value. It can be noticed that the matrices which present a larger variability are milk fat and mineral (**Figure 3A**) as their corresponding boxplots are wider than the rest. Milk fat matrix also has the highest median relative error, close to -20%. For individual congeners (**Figure 3B**) PCB 77 presents the largest positive bias, as well as the greatest relative error. This behavior should be further studied to see if the differences in ionization can have this kind of effects in the analysis of DL-PCBs using alternative ionization techniques.

Z-scores were calculated taking into account the congener reference values, included in the corresponding reports of the EU-RL Proficiency Tests [25-28], and a standard deviation of 20%. In general, calculated Z-scores **(Table 4)** are below 2 in most of the cases, with only a few values above 3, but in these specific cases deviations from the reference values could be most likely attributed to problems related to previous steps of the sample treatment (extraction and purification/fractionation) rather to a differences from the instrumental analysis. These findings highlight the good agreement between techniques, as well as the suitability of APCI for the determination of DL-PCBs in these complex samples.

3.4. Dioxins and Furans

Prior to the development of the proposed method, another fraction of some of the samples had been analyzed looking for the presence of dioxins and furans, making use of a recently validated GC-(APCI)MS/MS method [21] in a pioneer work where the potential of this technique was proved in PCDD/Fs analysis field. The obtained results, also compared to HRMS techniques, are summarized in **Table 5** where a concordance in the quantification results with both techniques and the similarity of estimated LODs

can be observed. Taking that method as a reference, the fundamental idea of this work was to test the quantification capabilities of the APCI source not only for the analysis of dioxins and furans but also for DL-PCBs.

The results of the two studies highlight the suitability of the novel atmospheric pressure chemical ionization source coupled to last generation GC-MS/MS instruments for the analysis of these pollutants, which gives comparable results with enhanced limits of detection for the majority of the compounds.

4. CONCLUSIONS

A GC-(APCI)MS/MS with QqQ method has been developed for the determination of DL-PCBs in a wide range of complex matrices which including fish, feed, milk powder, pork meat, lard, egg, egg yolk powder, milk fat and mineral (sepiolite). The comparison with the standard GC-(EI)HRMS methodology demonstrates the capabilities of the method, which relies on the use of the novel APCI with last generation GC-MS/MS instruments, achieving performances almost identical to GC-(EI)HRMS in the determination of the selected POPs. The obtained instrumental LODs and LOQs, are between 6 and 200 times lower than those attained by previously developed analytical methodologies based on GC-(EI)MS/MS with QqQ, and in the same range than those provided by GC-(EI)HRMS. These results are in agreement with the last outputs from APCI-related articles, in which a clear improvement of LODs for POPs has been achieved when analyzing these kind of contaminants by GC-(APCI)MS/MS instead of GC-(EI)-HRMS [29]. Additionally, QqQ instruments are less expensive, and generally easy to use and maintain, making them an interesting addition to commercial laboratories that otherwise would not be able to perform these types of analyses. The low fragmentation provided by the APCI source has demonstrated to be capable of

enhancing the performance of triple quadrupole analyzers applied to the determination of DL-PCBs, dioxins and furans, which may constitute a real alternative to the use of HRMS for the analysis of these compounds in food samples.

The demonstrated capabilities of this revived technique in the field of POPs open a wide range of possibilities for further studies, which could include the analysis of DL-PCBs and dioxins and furans in one single injection.

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FIGURE CAPTIONS

Figure 1. Comparison of the in source fragmentation for the 2,3,3',4,4',5 - Hexachlorobiphenyl DL-PCB in EI (left) and APCI (right) sources.

Figure 2. DL-PCB profile for three different complex matrices in pg g^{-1}. The results for every single DL-PCB are shown comparing the GC-(EI)HRMS determination (blue) and GC-(APCI)MS/MS determination (red).

Figure 3. Box-plots for the comparison of the results given by GC-(APCI)MS/MS and GC-(EI)HRMS by type of matrix (A) and type of analyte (B). The red lines indicate a difference higher than 20% between both techniques.

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[29] K. L. Organtini, L. Haimovici, K. J. Jobst, E. J. Reiner, A. Ladak, D. Stevens, J.W. Cochran, F. L. Dorman, Comparison of Atmospheric Pressure Ionization Gas Chromatography-Triple Quadrupole Mass Spectrometry to Traditional High-Resolution Mass Spectrometry for the Identification and Quantification of Halogenated Dioxins and Furans, Anal Chem 87 (2015), 7902-7908

Table 1. Experimental conditions of the optimized GC-(APCI)-MS/MS method.

Compound	Rt (min)	Cone Voltage (V)	Precursor Ion	Collision Energy (eV)	Product Ion
13C-PCB 81	17.73	50	302	35	232
			304	35	234
PCB 81	17.73	50	290	35	220
			292	35	222
13C-PCB 77	18.22	50	302	35	232
			304	35	234
PCB 77	18.22	50	290	35	220
			292	35	222
13C-PCB 123	19.18	30	336	35	266
			338	35	268
PCB 123	19.17	30	324	35	254
			326	35	256
13C-PCB 118	19.36	30	336	35	266
			338	35	268
PCB 118	19.37	30	324	35	254
			326	35	256
13C-PCB 114	19.86	30	336	35	266
			338	35	268
PCB 114	19.87	30	324	35	254
			326	35	256
13C-PCB 105	20.61	30	336	35	266
			338	35	268
PCB 105	20.61	30	324	35	254
			326	35	256
13C-PCB 126	22.29	30	336	35	266
			338	35	268
PCB 126	22.28	30	324	35	254
			326	35	256
13C-PCB 167	23.22	30	372	35	302
			370	35	300
PCB 167	23.22	30	360	35	290
			358	35	288
13C-PCB 156	24.37	30	372	35	302
			370	35	300
PCB 156	24.36	30	360	35	290
			358	35	288
13C-PCB 157	24.62	30	372	35	302
			370	35	300
PCB 157	24.63	30	360	35	290
			358	35	288
13C-PCB 169	26.37	30	372	35	302
			370	35	300
PCB 169	26.36	30	360	35	290
			358	35	288
13C-PCB 189	28.27	30	406	40	336
			408	40	338
PCB 189	28.28	30	394	40	324
			396	40	326

Table 2. Target compounds with the selected quantification and validation parameters

Compound name	Rt(min)	r^2	RF mean	RF RSD (%)	Instrumental precision RSD (%)	Instrumental LOD (pg μL^{-1})	Instrumental LOQ (pg μL^{-1})
PCB 81	17.73	0.9999	1.14	8	2	0.0025	0.005
PCB 77	18.22	1.0000	1.11	9	6	0.0025	0.005
PCB 123	19.18	0.9995	1.13	12	5	0.0025	0.005
PCB 118	19.36	0.9999	1.09	15	3	0.0025	0.005
PCB 114	19.86	0.9995	1.11	12	8	0.0025	0.005
PCB 105	20.61	0.9998	1.13	6	8	0.0025	0.005
PCB 126	22.29	0.9998	1.14	11	2	0.0025	0.005
PCB 167	23.22	1.0000	1.07	7	4	0.0025	0.005
PCB 156	24.36	0.9995	1.10	9	6	0.0025	0.005
PCB 157	24.63	0.9995	1.09	6	0	0.0025	0.005
PCB 169	26.36	0.9998	1.08	10	6	0.0025	0.005
PCB 189	28.27	0.9997	1.05	11	6	0.0025	0.005

Table 3. Concentrations of DL-PCBs (pg g^{-1} fat) determined in the Proficiency Test (PT) samples analyzed by GC-(EI)HRMS and GC-(APCI)MS/MS together with the Proficiency Test assigned values [25-28]. The relative error (RE) between HRMS and MS/MS is also shown.

Compound	Pork Meat			RE	Pork Meat			RE	Lard			RE	Lard	
	HRMS	APCI	PT		HRMS	APCI	PT		HRMS	APCI	PT		HRMS	APCI
PCB 81	0.61	0.66	n.r.	8%	0.4	0.41	n.r.	2%	6.52	7.54	8.36	16%	7.70	7.82
PCB 77	9.67	7.61	n.r.	-21%	8.02	7.7	n.r.	-4%	185	128	184	-31%	197	133
PCB 123	18.52	14.13	n.r.	-24%	14.54	16.05	n.r.	10%	145	147	131	1%	157	144
PCB 118	656	719	444	10%	630	680	444	8%	7377	7676	7428	4%	7671	8917
PCB 114	10.42	9.63	n.r.	-8%	9.64	9.63	n.r.	0%	235	251	243	7%	241	251
PCB 105	131.26	123.76	79.2	-6%	122.5	112.2	79.2	-8%	3414	3413	3483	0%	3667	3415
PCB 126	7.23	8.32	5.34	15%	6.86	9.26	5.34	35%	6.84	7.9	7.2	16%	7.2	7.2
PCB 167	98,0	89.6	88.7	-9%	100.65	90.6	88.7	-10%	297.5	276	290	-7%	310	281
PCB 156	398	407	382	2%	401	407	382	2%	876	917	906	5%	930	949
PCB 157	68.3	64.4	63.8	-6%	69.5	69.1	63.8	-1%	205	197	202	-4%	212	211
PCB 169	5.75	4.88	4.86	-15%	5.63	5.28	4.86	-6%	< LOQ	< LOQ	n.r.		<LOQ	<LOQ
PCB 189	89.0	85.4	84.6	-4%	89.6	78.3	84.6	-13%	32	38.1	34	16%	35	33.6

Compound	Egg Yolk Powder			RE	Milk Powder			RE	Milk fat			RE	Mineral (*)	
	HRMS	APCI	PT		HRMS	APCI	PT		HRMS	APCI	PT		HRMS	APCI
PCB 81	37.1	22.6	34.6	-39%	8.6	9.2	13.3	7%	14.3	9.6	18.8	-33%	13.2	9.7
PCB 77	821	536	711	-35%	267	182	302	-32%	352	255	404	-28%	297	203
PCB 123	196	238	170	21%	193	191	165	-1%	297	241	232	-19%	35.9	33.8
PCB 118	9541	11167	7960	17%	8451	8701	8670	3%	10501	11651	12100	11%	1267	1559
PCB 114	337	297	304	-12%	248	256	275	3%	341	361	405	6%	62.3	92.8
PCB 105	4776	4670	4160	-2%	4085	3677	4110	-10%	4980	4130	5730	-17%	798	751
PCB 126	18.0	16.8	15.2	-7%	11.0	9.3	12.6	-16%	13	10	16	-26%	3.99	3.09
PCB 167	297	253	256	-15%	354	311	345	-12%	447	383	462	-14%	32.6	29.2
PCB 156	874	853	759	-2%	954	989	1000	4%	1222	1286	1370	5%	96.5	102.5
PCB 157	193	199	167	3%	201	222	224	10%	269	274	310	2%	22.3	22.4
PCB 169	<LOQ	<LOQ	n.r.		0.97	0.95	n.r.	-2%	0.85	0.83	n.r.	-2%	<LOQ	<LOQ
PCB 189	37.39	32.59	32	-13%	36.1	35.2	41.2	-2%	44.7	46.2	52.3	3%	3.6	3.3

n.r. assigned value not reported; (*) pg g^{-1} 12% moisture content

Table 4. Z-scores for the samples analyzed by GC-(EI)HRMS and GC-(APCI)MS/MS relative to the Proficiency Test assigned values [25-28].

Compound name	Pork Meat		Pork Meat		Lard		Lard		Egg	
	HRMS	APCI	HRMS	APCI	HRMS	APCI	HRMS	APCI	HRMS	APCI
PCB 81					-1,10	-0,49	-0,39	-0,32	2,37	0,82
PCB 77					0,04	-1,51	0,36	-1,38	**3,64**	**4,64**
PCB 123					0,55	0,63	1,01	0,51		
PCB 118	2,40	**3,11**	2,10	2,67	-0,03	0,17	0,16	1,00	1,01	1,41
PCB 114					-0,16	0,17	-0,03	0,17		
PCB 105	**3,29**	2,81	2,74	2,08	-0,10	-0,10	0,27	-0,10	0,80	0,49
PCB 126	1,77	2,79	1,42	**3,67**	-0,25	0,51	0,00	0,01	1,01	0,36
PCB 167	0,52	0,05	0,67	0,11	0,13	-0,24	0,35	-0,15	0,81	0,30
PCB 156	0,21	0,33	0,25	0,34	-0,16	0,07	0,13	0,24	0,68	0,54
PCB 157	0,35	0,05	0,45	0,42	0,07	-0,10	0,26	0,24	0,54	0,61
PCB 169	0,92	0,02	0,79	0,43					0,89	0,43
PCB 189	0,26	0,05	0,30	-0,37	-0,18	0,61	0,23	-0,05	0,55	0,45

Compound name	Egg Yolk Powder		Milk Powder		Milk fat		Mineral (*)	
	HRMS	APCI	HRMS	APCI	HRMS	APCI	HRMS	APCI
PCB 81	0,36	-1,73	-1,76	-1,53	-1,19	-2,45	-0,84	-1,94
PCB 77	0,78	-1,23	-0,58	-1,99	-0,64	-1,85	0,07	-1,54
PCB 123	0,77	1,99	0,83	0,80	1,40	0,19	0,23	-0,08
PCB 118	0,99	2,01	-0,13	0,02	-0,66	-0,19	0,07	1,24
PCB 114	0,55	-0,10	-0,49	-0,34	-0,79	-0,54	-0,29	2,01
PCB 105	0,74	0,61	-0,03	-0,53	-0,65	-1,40	0,18	-0,12
PCB 126	0,92	0,54	-0,63	-1,33	-0,88	-1,96	-0,11	-1,21
PCB 167	0,81	-0,07	0,12	-0,50	-0,16	-0,86	0,33	-0,23
PCB 156	0,76	0,62	-0,23	-0,06	-0,54	-0,31	0,13	0,44
PCB 157	0,78	0,97	-0,51	-0,05	-0,67	-0,58	0,36	0,39
PCB 169								
PCB 189	0,84	0,09	-0,62	-0,73	-0,72	-0,59	0,23	-0,28

Table 5. Comparison of quantification results for dioxins and furans in the analyzed samples by GC-(EI)HRMS and GC-(APCI)MS/MS analyses by previously developed method[21]. Comparison of estimated LODs by both techniques.

Congener	Powdered Milk				Fish				Spiked fish feed			
	Conc (pg g^{-1})		LOD (pg g^{-1})		Conc (pg g^{-1})		LOD (pg g^{-1})		Conc (pg g^{-1})		LOD (pg g^{-1})	
	HRMS	**APCI**	**HRMS**	**APCI**	**HRMS**	**APCI**	**HRMS**	**APCI**	**HRMS**	**APCI**	**HRMS**	**APCI**
2,3,7,8-TCDF	0.07	0.05	0.003	0.002	1.50	1.33	0.011	0.001	0.07	0.08	0.001	0.002
1,2,3,7,8-PeCDF	0.07	0.06	0.010	0.002	0.13	0.13	0.013	0.001	0.28	0.26	0.004	0.002
2,3,4,7,8-PeCDF	1.74	1.68	0.011	0.002	0.38	0.37	0.025	0.001	0.26	0.26	0.004	0.002
1,2,3,4,7,8-HxCDF	0.95	0.92	0.006	0.003	0.03	0.03	0.009	0.001	0.25	0.28	0.004	0.001
1,2,3,6,7,8-HxCDF	1.11	1.02	0.007	0.003	0.04	0.04	0.009	0.001	0.26	0.27	0.004	0.001
2,3,4,6,7,8-HxCDF	1.05	0.97	0.007	0.003	0.05	0.04	0.019	0.002	0.24	0.24	0.004	0.001
1,2,3,7,8,9-HxCDF	0.01	0.01	0.009	0.004	-	-	0.015	0.001	0.25	0.26	0.004	0.001
1,2,3,4,6,7,8-HpCDF	0.59	0.54	0.008	0.002	0.02	0.02	0.005	0.001	0.29	0.30	0.003	0.002
1,2,3,4,7,8,9-HpCDF	0.06	0.05	0.011	0.003	-	-	0.008	0.001	0.26	0.26	0.003	0.002
OCDF	0.20	0.16	0.012	0.003	0.04	0.03	0.005	0.001	0.53	0.52	0.004	0.003
2,3,7,8-TCDD	0.27	0.27	0.011	0.001	0.05	0.04	0.005	-	0.05	0.05	0.005	0.001
1,2,3,7,8-PeCDD	0.79	0.82	0.010	0.002	0.07	0.06	0.011	-	0.26	0.26	0.003	0.001
1,2,3,4,7,8-HxCDD	0.42	0.40	0.010	0.002	-	-	0.007	0.001	0.25	0.27	0.003	0.001
1,2,3,6,7,8-HxCDD	0.93	0.93	0.011	0.002	0.06	0.06	0.007	0.001	0.25	0.27	0.003	0.001
1,2,3,7,8,9-HxCDD	0.34	0.36	0.017	0.003	0.01	0.01	0.007	0.001	0.25	0.30	0.002	0.001
1,2,3,4,6,7,8-HpCDD	3.77	3.68	0.021	0.003	0.05	0.04	0.013	0.001	0.37	0.35	0.005	0.002
OCDD	18.03	17.79	0.009	0.007	0.16	0.10	0.013	0.002	0.98	0.73	0.001	0.005

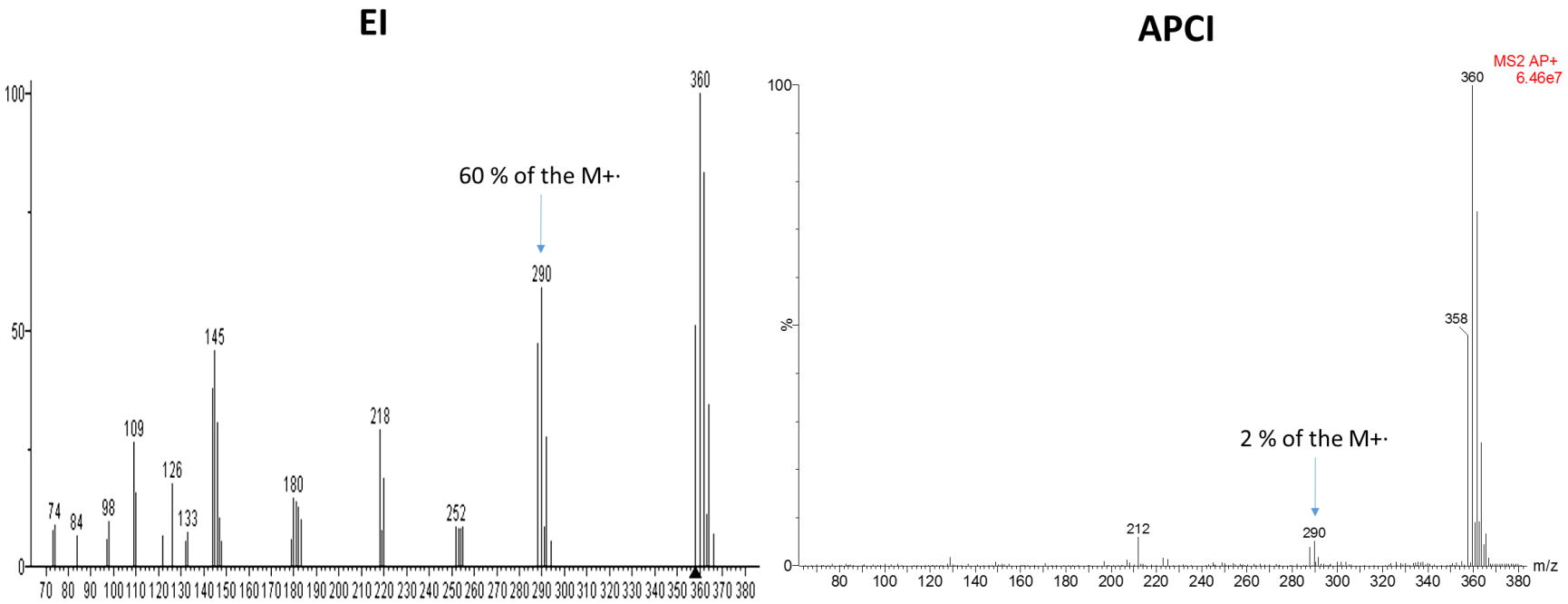
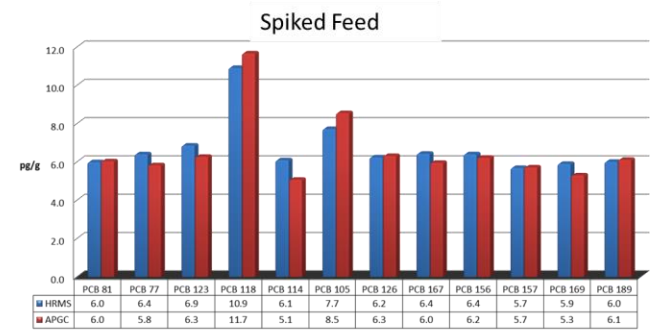
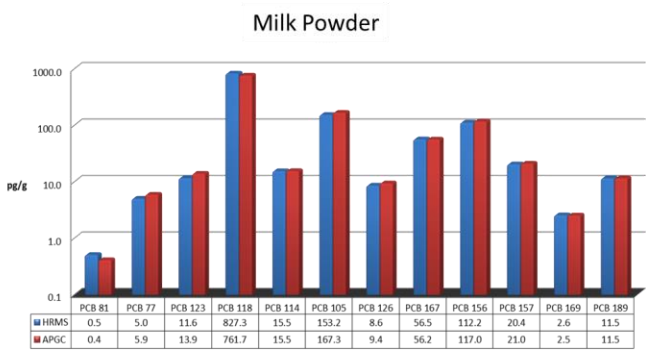

EI
100
50
0
60 % of the M+·
360
290
145
218
109
126
180
74
84
98
133
252
70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380
APCI
MS2 AP+
6.46e7
100
%
0
360
358
2 % of the M+·
212
290
m/z
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380

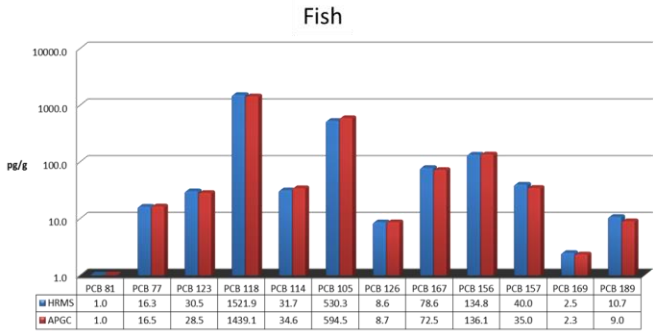
Figure 1



	PCB 81	PCB 77	PCB 123	PCB 118	PCB 114	PCB 105	PCB 126	PCB 167	PCB 156	PCB 157	PCB 169	PCB 189
HRMS	6.0	6.4	6.9	10.9	6.1	7.7	6.2	6.4	6.4	5.7	5.9	6.0
APGC	6.0	5.8	6.3	11.7	5.1	8.5	6.3	6.0	6.2	5.7	5.3	6.1

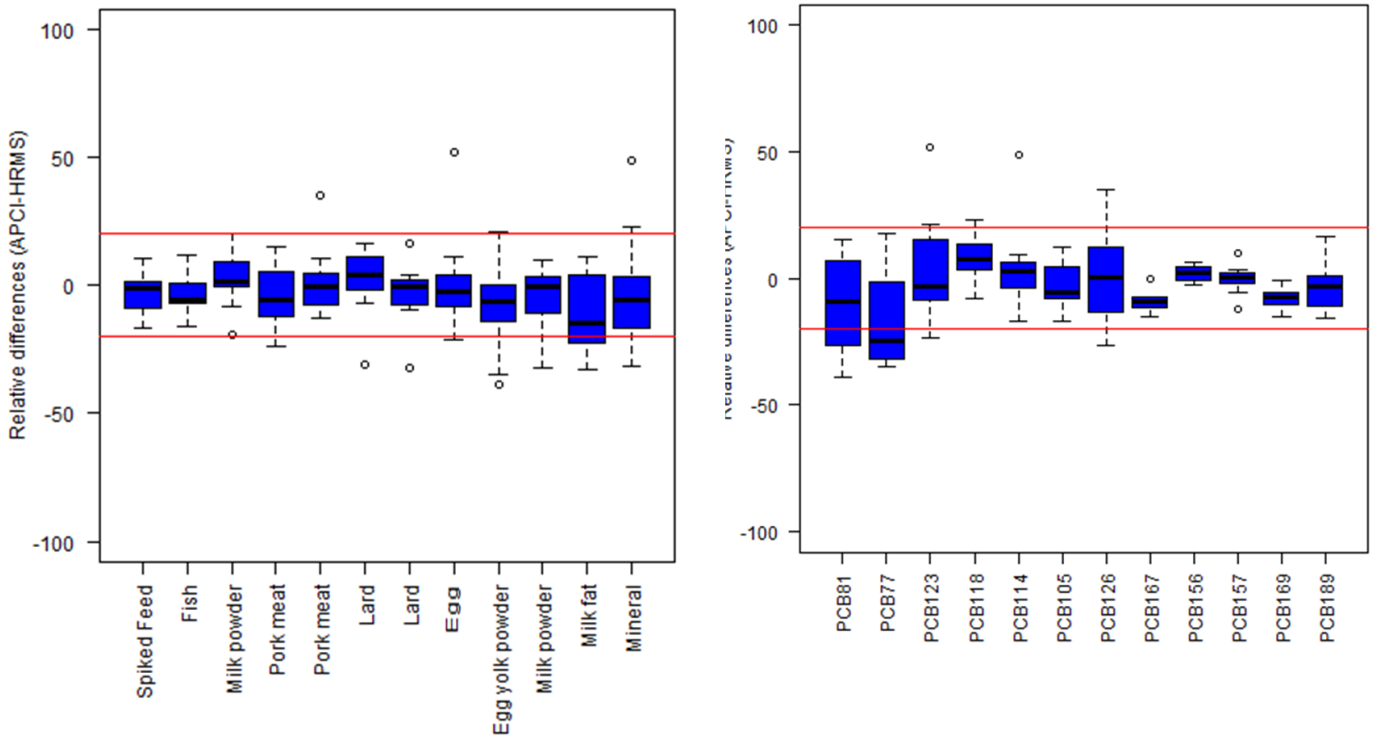


	PCB 81	PCB 77	PCB 123	PCB 118	PCB 114	PCB 105	PCB 126	PCB 167	PCB 156	PCB 157	PCB 169	PCB 189
HRMS	0.5	5.0	11.6	827.3	15.5	153.2	8.6	56.5	112.2	20.4	2.6	11.5
APGC	0.4	5.9	13.9	761.7	15.5	167.3	9.4	56.2	117.0	21.0	2.5	11.5



	PCB 81	PCB 77	PCB 123	PCB 118	PCB 114	PCB 105	PCB 126	PCB 167	PCB 156	PCB 157	PCB 169	PCB 189
HRMS	1.0	16.3	30.5	1521.9	31.7	530.3	8.6	78.6	134.8	40.0	2.5	10.7
APGC	1.0	16.5	28.5	1439.1	34.6	594.5	8.7	72.5	136.1	35.0	2.3	9.0

17 **FIGURE 2**



(A) **(B)**

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FIGURE 3