Application Note 10478

Using GC-MS/MS as a Confirmatory Method for Dioxin-Like PCBs in Food and Feed

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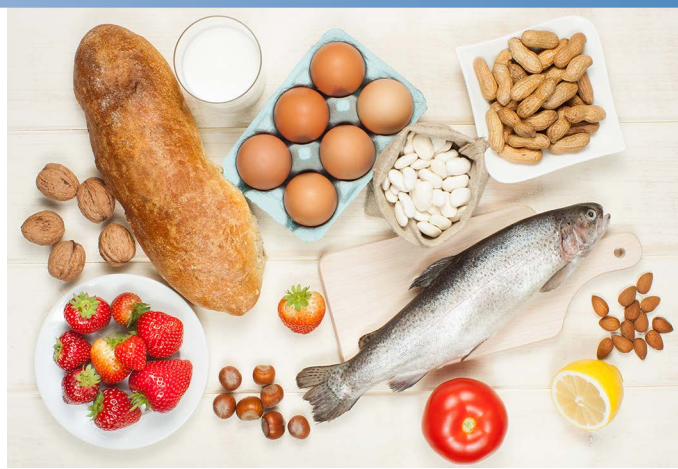
Key Words
Confirmation, DL-PCBs, Food and feed, GC-MS/MS, Isotope dilution, Quantification, TargetQuan

Goal
To assess the performance of the Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS/MS for the analysis of DL-PCBs.



Introduction
Polychlorinated biphenyls (PCBs) are a group of highly toxic organochlorine compounds and, depending upon the number of chlorine atoms available, there are 209 possible congeners.[1] These congeners can be divided into two groups based on their structural and toxicological characteristics. The first group comprises of 12 congeners that have a structure and toxicity similar to dioxins. These are termed "dioxin-like PCBs" (DL-PCBs) and are the focus of this study. The other group, forming the majority, do not exhibit the same degree of toxicity and these are called "non dioxin-like PCBs" (NDL-PCBs).

DL-PCBs have been classified as persistent organic pollutants (POPs) and have been regulated under the Stockholm convention for POPs since 2001, with the aim of protecting both human health and the wider environment.[2] The major source of these compounds is from industrial processes where they are manufactured for numerous applications including flame retardants, sealants, and lubricants.[3] DL-PCBs are of particular concern because of their resistance to degradation and their ability to be transported long distances. These properties enable DL-PCBs to bioaccumulate within organisms, with those organisms at the higher trophic levels receiving the highest exposure. Humans are primarily exposed to these compounds via the ingestion of foods, and in particular high animal-fat-containing foods.[4] High levels of DL-PCBs in animal fat are due to the high solubility of DL-PCBs in adipose tissue and low solubility in water, which prevents them from being excreted from the body.

The potential for human exposure to these compounds makes accurate detection and quantification of DL-PCBs in the environment, particularly in food and animal feed, very important. The European Commission established a monitoring program in 2001 with the aim of limiting, and ideally eliminating, the emission of both dioxins and DL-PCBs into the environment.[5] They set out maximum levels (ML) for the sum of dioxins, DL-PCBs, and the six non-dioxin-like PCBs (NDL-PCB) in food, and feed and these are laid down in Commission Regulation 1883/2006. The Commission also established an "action level" (AL) to be used as an early warning level to identify a particular source of contamination. Both ML and AL are expressed as toxic equivalence quotients (TEQ). The toxic equivalent quotient compares the toxicity of less toxic compounds relative to the toxicity of the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Each compound is given a specific toxic equivalence factor (TEF) relative to TCDD, which has a value of 1. For DL-PCBs the TEF values range from 0.0001 (PCB 77) to 0.1 (PCB 126).

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Until recently, legislation in the European Union required the confirmation and quantification of dioxins and DL-PCBs in contaminated samples by gas chromatography/high resolution magnetic sector mass spectrometry (GC-HRMS) instruments, which have the sensitivity and specificity to be used for low level background monitoring as well as maximum and action levels in food and feed. However, recent advances in gas chromatography/triple-quadrupole mass spectrometry (GC-MS/MS) technology have allowed high sensitivity and selectivity to be achieved. These improvements have led to GC-MS/MS being considered a reliable tool that can be used to control the maximum levels for DL-PCBs in food and feed as a full confirmatory method.[5] However, for the determination of low background levels, i.e. below one fifth of the level of regulatory interest, the application of GC-HRMS is required.[6]

According to the revised EU regulation, when using GC-MS/MS, the following specific performance criteria for confirmation should be fulfilled in addition to the criteria described previously by the European Commission[1, 7, 9], except the obligation to use GC-HRMS:

1. Resolution for each quadrupole to be set equal to or better than unit mass resolution (unit mass resolution defined as sufficient resolution to separate two peaks with one mass unit apart).
2. Two specific precursor ions, each with one specific corresponding transition product ion for all labelled and unlabelled analytes should be used.
3. Maximum permitted tolerance of relative ion intensities of ±15% for selected transitions in comparison to calculated or measured values (average from calibration standards), applying identical MS/MS conditions, in particular collision energy and collision gas pressure, for each transition of an analyte.

In this work, the performance of the Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS/MS for the analysis of DL-PCBs was assessed. For this assessment, both solvent standards and food and feed samples were used to evaluate the instrument performance against the criteria for DL-PCB confirmation. Additionally, a direct comparison of the results obtained from food and feed sample extracts using the TSQ 8000 Evo GC-MS/MS with those from a GC-HRMS was made.

Instrument and Method Setup

DL-PCBs were analyzed in the standards and matrix samples using a TSQ 8000 Evo triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1310 GC. Sample introduction was performed with a Thermo Scientific™ TriPlus™ RSH autosampler, and compound separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-5SilMS 60 m × 0.25 mm I.D. × 0.25 µm film capillary column. Additional instrument parameters used to acquire data are displayed in Tables 1 and 2.

Table 1. GC and injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner	SSL single taper P/N: 453A2342
Inlet (°C):	280
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	120
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min)	25
T emperature 3 (°C):	285
Rate (°C/min)	2.5
Temperature 4 (°C):	320
Rate (°C/min)	10
Hold Time (min):	8

Resolution of each quadrupole was set to unit mass as specified in the European Commission criteria for dioxin confirmation using GC-MS/MS (Table 2).

Table 2. Mass spectrometer conditions.

TSQ 8000 Evo Mass Spectrometer Parameters	
Transfer Line (°C):	280
Ionization Type:	EI
Ion source(°C):	300
Electron Energy (eV):	40
Acquisition Mode:	SRM
Q2 Gas Pressure (argon) (psi):	60
Collision Energy (eV):	See Table 3
Q1 Peak Width (Da):	0.7
Q3 Peak Width (Da):	0.7

The TSQ 8000 Evo instrument was operated in MS/MS mode using electron ionization (EI). For data acquisition, two selected reaction monitoring (SRM) transitions per compound were selected, meeting the second EU criteria for GC-MS/MS confirmation. Data was acquired using timed-SRM with a minimum of 12 points/chromatographic peak. Selected SRM transitions and their collision energies were automatically optimized using the AutoSRM software application. The results are shown in Table 3. Data processing was performed with Thermo Scientific™ TargetQuan 3.1 software, designed specifically to comprehensively process MS, MS/MS, or HRMS data for routine quantification of persistent organic pollutants (POPs) in a regulated environment.

Table 3. SRM transitions used for native and 13C-labelled DL-PCBs.

Compound Name	Precursor Ion [Da]	Product Ion [Da]	Collision Energy [V]
PCB - tetrachlorobiphenyl	289.9	219.9	22
PCB - tetrachlorobiphenyl	291.9	221.9	22
^{13}C-PCB - tetrachlorobiphenyl	301.9	231.9	22
^{13}C-PCB - tetrachlorobiphenyl	303.9	233.9	22
PCB - pentachlorobiphenyl	323.9	253.9	22
PCB - pentachlorobiphenyl	325.9	255.9	22
^{13}C-PCB - pentachlorobiphenyl	335.9	265.9	22
^{13}C-PCB - pentachlorobiphenyl	337.9	267.9	22
PCB - hexachlorobiphenyl	357.9	287.9	24
PCB - hexachlorobiphenyl	359.9	289.9	24
^{13}C-PCB - hexachlorobiphenyl	369.9	299.9	24
^{13}C-PCB - hexachlorobiphenyl	371.9	301.9	24
PCB - heptachlorobiphenyl	391.9	321.9	25
PCB - heptachlorobiphenyl	393.9	323.9	25
^{13}C-PCB - heptachlorobiphenyl	403.9	333.9	25
^{13}C-PCB - heptachlorobiphenyl	405.9	335.9	25

Sample Preparation

DL-PCB standards (68C CVS CS 1 to CS 5) containing the native and the ^{13}C-labelled compounds were obtained from Wellington Laboratories Inc. The following food and feed extracted samples were provided by the Institute of Environmental Assessment and Water Research, CSIC Barcelona, Spain: adipose tissue, fish liver, fish oil, dry fish, spiked feed sample and milk powder sample (certified reference material).

Extraction and clean-up of the matrix samples was performed either by PowerPrep™ SPE system (feed sample) or using a manual clean-up with multilayer silica, followed by basic alumina and a final carbon column (milk, tissue and fish samples). Final extracts were prepared in nonane.

Data Processing

Data processing was performed using TargetQuan software, which is specifically designed for the analysis of POPs using isotope dilution. The software streamlines quantitation based upon relative response factors (or optionally average responses), incorporates toxic equivalence factors (TEFs) to automatically calculate toxic equivalence quotients (TEQs), and finally, determines total TEQ.

Results and Discussion

The instrumental method used was based on that described in previous work analyzing PCDD/Fs using GC-MS/MS.[9] Similar to that method, the one used in this study took advantage of timed-SRM which uses a completely different analytical strategy than the "classical" segmented setup. This method allows data acquisition for a target compound in a short retention window around the known compound retention time, and not in a wide retention time segment. As a result, dwelling on the target compounds is very effective, ultimately improving the sensitivity and lowering the method detection limit. Additionally, with timed-SRM the compound acquisition window can be individually set to cover closely eluting isomers.

Chromatography

Achieving sufficient chromatographic separation of all of the DL-PCB isomers is essential for reliable identification and quantification. The chromatography of DL-PCBs was assessed in the lowest calibration standard (CS1) containing 1 pg/µL. All native and corresponding ^{13}C-labelled internal standards were easily detected, excellent peak shape was obtained for all compounds (Figure 1), and <10% valley separation was achieved for the pentachlorobiphenyl isomers (Figure 2).

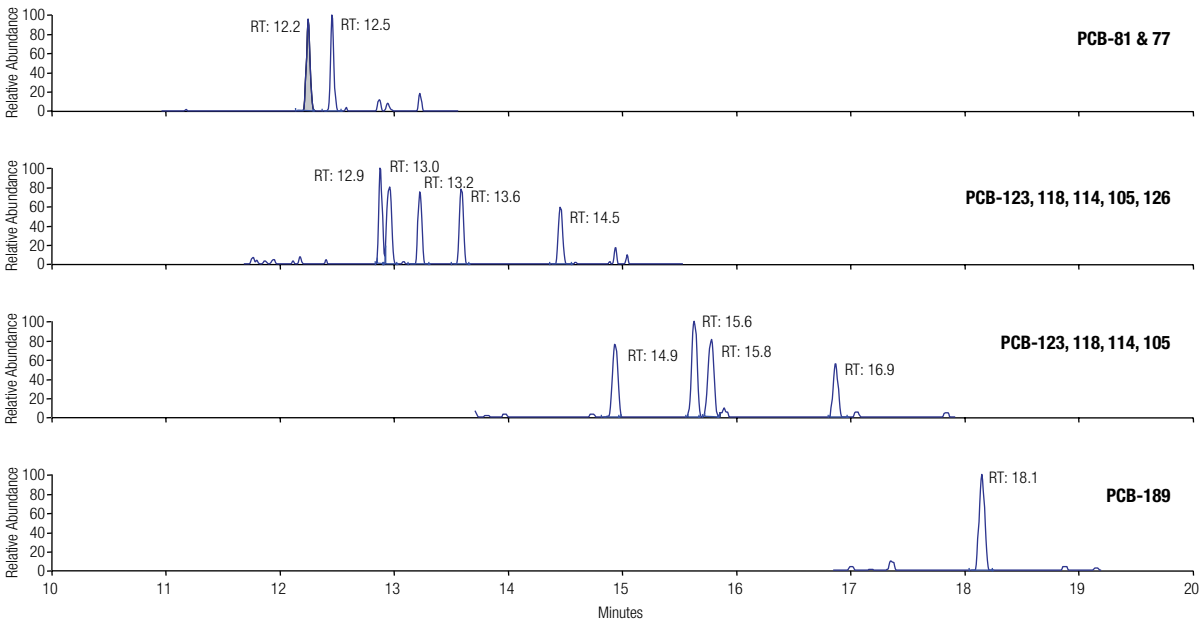

Figure 1. Chromatographic separation of native DL-PCBs in the lowest standard (1 pg/µL). One SRM transition (quantification ion) per compound is shown.

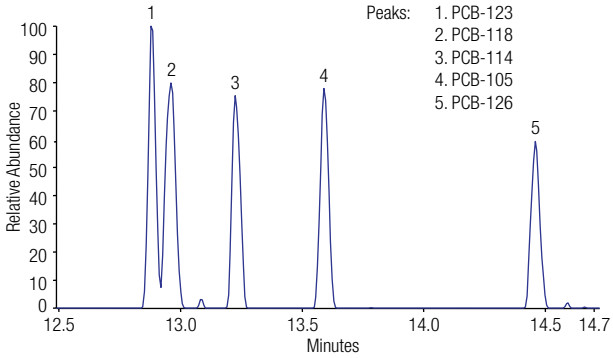

Figure 2. Example of chromatographic separation of the pentachlorobiphenyl congeners in the CS-1 standard (1 pg/µl).

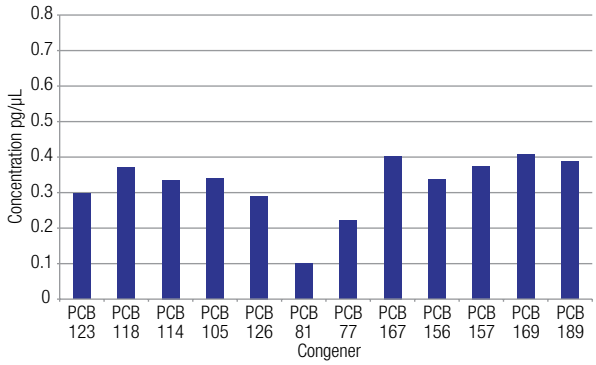

Figure 3. LOQ calculation for DL-PCBs from repeat injections of a serial dilution. Data indicate the LOQ for each congener with ion ratios and response factors values within the expected limits.

Reaching the level of interest

For the analysis of DL-PCBs, reaching the required limits of detection is critical to implementing a routine monitoring method. The limit of quantification (LOQ) for a confirmatory method should be about one fifth of the maximum level.[5, 8, 9] Traditionally, the sensitivity of an instrument for dioxin analysis took into account the limit of detection (LOD) and the limit of quantification (LOQ), and on magnetic sector instruments, these limits were calculated from the S/N values (LOD corresponds to 3× the noise level, whereas LOQ corresponds to 10× the noise level).

Often, data acquired on GC-MS/MS instruments operated in MS/MS have little or no noise, therefore, calculation of LOD/LOQ requires a different approach and rethinking how these parameters should be determined. The LOQ of an individual congener may be calculated from the lowest concentration point (i.e., CS1) taking into account the recovery of internal standards (should be 60–120%), ion ratio abundance, and chromatography of the sample.[8] Consequently, the instrument LOQ was assessed by repeatedly (n = 8) injecting the lowest calibration standard (CS1).

Calculation of the LOQ for each native compound took into account the student's-*t* critical values for the corresponding degrees of freedom (99% confidence), the concentration of each native compound, and %RSD. The results of this test show that the LOQs for the DL-PCBs analyzed were 0.1–0.41 pg/µL. Ion ratios and response factors (RFs) at these levels still within ±15 % limit, % recovery of 13-labelled within the 60–120% limit (Figure 3). The results of this experiment demonstrate that the TSQ 8000 Evo GC-MS/MS can detect and confirm DL-PCBs at low levels, thus meeting the detection limit requirements.[7]

Linearity of response

DL-PCB quantification is based on isotope dilution and uses RF-type calibration where the average response factor of all the standards from an external calibration curve are taken into account to quantify the 12 congeners.[1] Average RF %RSD values were calculated from measurements of a five-point calibration curve. The results of this experiment show excellent %RSD for all measured compounds with values between 0.8–5.6%, well within the 15% limits established by the U.S. EPA[6] (Table 4).

Table 4. Linearity of DL-PCBs across five-point calibration curve. The precision on the average response factor (%RSD) for each native compound is shown.

Compound	Concentration Range (pg/µL)	Average RF	stdev	RF % RSD
PCB 77	1–2000	1.11	0.01	1.0
PCB 81	1–2000	1.10	0.01	1.0
PCB 105	1–2000	0.98	0.03	2.6
PCB 114	1–2000	1.08	0.04	3.7
PCB 118	1–2000	1.04	0.01	0.8
PCB 123	1–2000	1.01	0.02	1.7
PCB 126	1–2000	1.07	0.03	2.7
PCB 156	1–2000	1.2	0.02	1.4
PCB 157	1–2000	1.15	0.03	2.8
PCB 167	1–2000	1.22	0.07	5.6
PCB 169	1–2000	1.00	0.02	2.5
PCB 189	1–2000	1.01	0.04	4.3

Quantification of DL-PCBs in sample extracts

Following successful validation of the method, DL-PCBs were quantified in the six sample extracts. Excellent chromatographic separation with minimal matrix interference was observed for all native congeners for all samples analyzed. Figure 4 provides an example of the chromatogram for PCB 189.

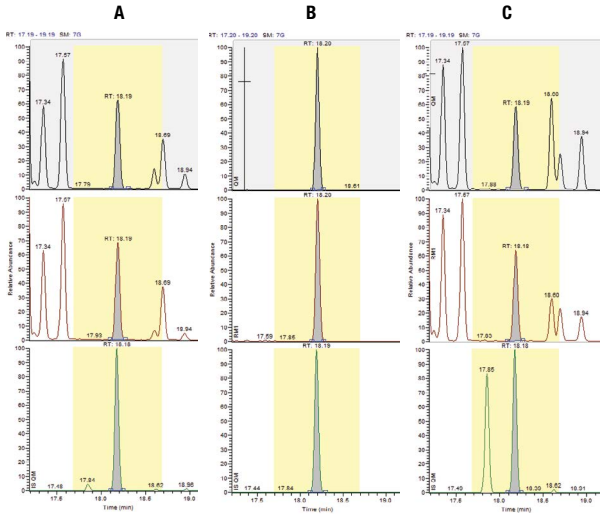

Figure 4. Example of chromatographic separation of PCB 189 present in the fish (A), feed (B), and milk powder (C) samples.

DL-PCB content of each sample, expressed as WHO-TEQ pg/g, was determined for each sample analyzed and the results were compared with the existing data obtained for the same samples from the GC-HRMS. The calculated concentrations of each individual congener (as TEQ pg/g) were compared with the values obtained from the GC-HRMS. The data shows excellent agreement between the results obtained using the TSQ 8000 Evo GC-MS/MS and those obtained using GC-HRMS (Figures 5–10). The concentrations of the DL-PCBs in the samples cover a wide range, but importantly, there is always good agreement with the GC-HRMS results.

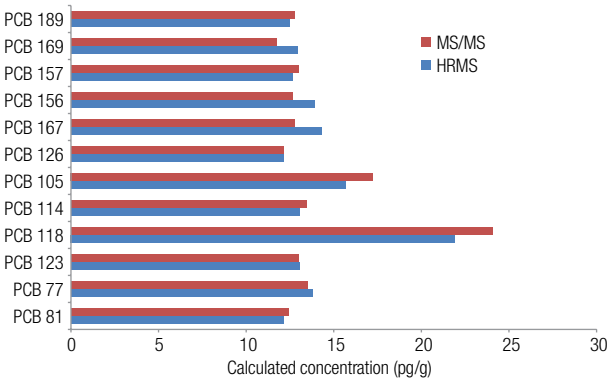

Figure 5. Individual contribution of each DL-PCB congener to the feed sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

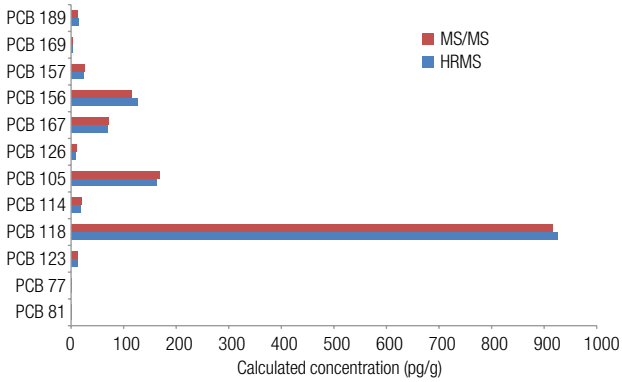

Figure 6. Individual contribution of each DL-PCB congener to the milk sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

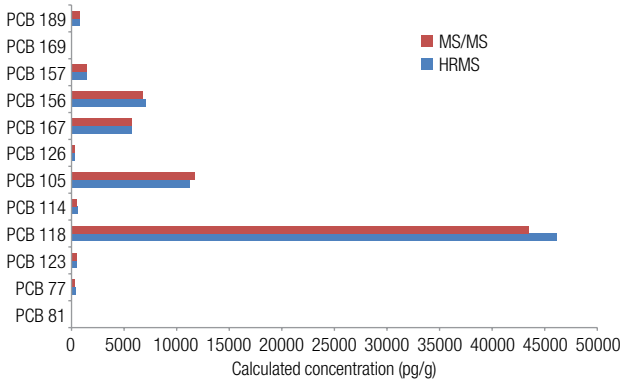

Figure 7. Individual contribution of each DL-PCB congener to the fish liver sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

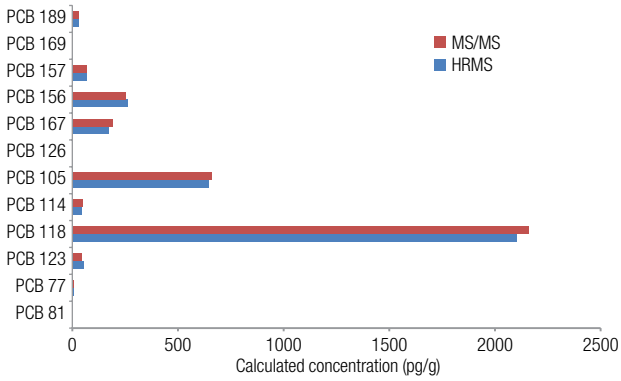

Figure 8. Individual contribution of each DL-PCB congener to the fish oil sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

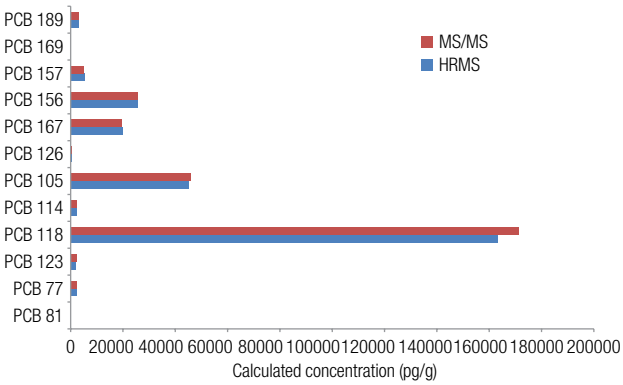

Figure 9. Individual contribution of each DL-PCB congener to the dry fish sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

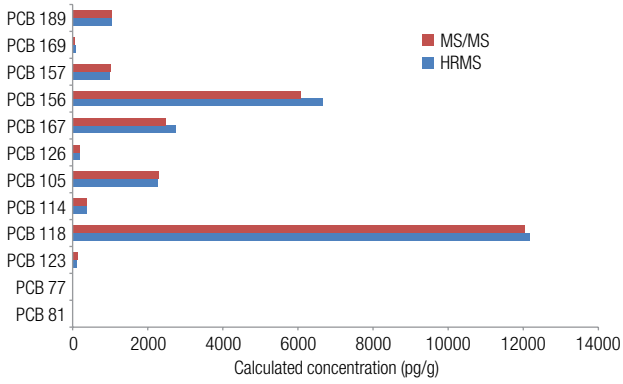

Figure 10. Individual contribution of each DL-PCB congener to the adipose tissue sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

The total DL-PCB content of each sample obtained from TSQ 8000 Evo GC-MS/MS analysis was plotted against the sector instrument data, with the calculated deviation not exceeding 3% (Figure 11). Although the GC-MS/MS system was capable meeting the requirements for confirmation, the magnetic sector GC-HRMS is capable of lower detection levels and is therefore better suited for low level background studies.

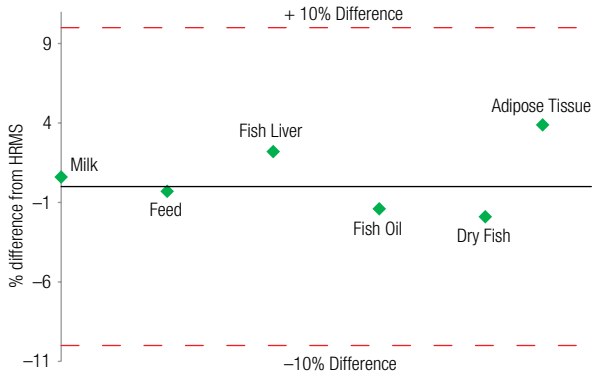

Figure 11. Deviation (%) of the total DL-PCB concentration (pg/g) measured with the TSQ 8000 Evo from the GC-HRMS results for each of the six matrix types. All show excellent agreement with the HRMS results

Ion ratio abundance

The ion ratio (IR) abundance for selected transitions of each of the 12 DL-PCB congeners was measured in each of the samples analyzed and the values compared with the measured ion ratio values (average from calibration standards CS1–CS5). The results of this experiment show that all the IR for the compounds analyzed were within the 15% tolerance, meeting the EU criteria for dioxin confirmation[2] (Figure 12). In addition, the SRM selectivity is high enough for confirmatory analysis.

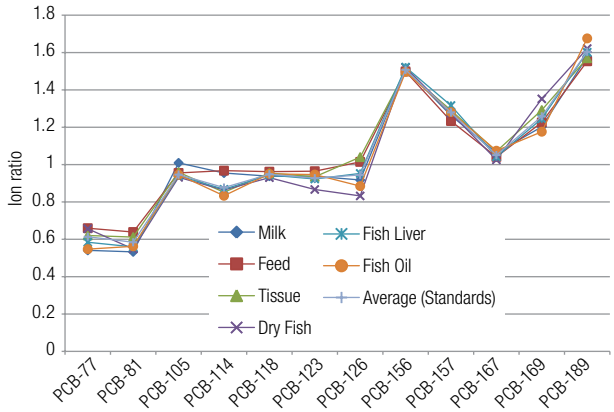

Figure 12. Comparison of the ion ratio abundance of each of the 12 DL-PCB in the samples extracts with the average IR values derived from the calibration standards (CS1-CS5).

Conclusion

The results of this evaluation demonstrate that the TSQ 8000 Evo GC-MS/MS system is an extremely effective tool for routine analysis of DL-PCBs, meeting all the European Commission requirements for DL-PCB confirmation in food and feed samples.

- The results obtained with the TSQ 8000 Evo GC-MS/MS instrument demonstrate that this is a highly sensitive and selective analytical system that can be confidently used for DL-PCB detection and confirmation in food and feed samples.
- The TSQ 8000 Evo GC-MS/MS, together with the TRACE 1310 GC and TargetQuan 3.1 data processing and reporting software, constitute a comprehensive solution for DL-PCB analysis in complex samples.
- Excellent reproducibility, linearity, sensitivity, and selectivity were obtained in all of the experiments performed with standards and sample extracts.
- Calculated DL-PCB values for the matrix samples were in very good agreement with those derived from the magnetic sector instrument.

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