POSTER NOTE

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

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Overview

Purpose: 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, both solvent standards and food and feed samples were used to evaluate the instrument performance against the criteria for dioxin-like PCBs (DL-PCBs) 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.

Method: 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 x 0.25 mm I.D. x 0.25 μm film capillary column.

Results: The selectivity, sensitivity and repeatability of the developed method was shown to be more than adequate, meeting or exceeding the requirements stipulated in the legislation.

Introduction

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 mass spectrometry (GC-HRMS) instruments, which is still considered the "gold standard" approach. This technique has 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 [4].

According to the revised EU regulation, when using GC-MS/MS, the following specific performance criteria for confirmation with GC-MS/MS technology should be fulfilled in addition to the criteria described previously by the European Commission [1, 4, 5], 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.

Method

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.

Table 1. Instrument method

TRACE 1310 GC Parameters	
Injection Volume (µL):	1.0
Liner	SSL single taper
Inlet (°C):	280
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven T Program:	
Temperature 1 (°C):	120
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min)	25
Temperature 3 (°C):	285
Rate (°C/min)	2.5
Temperature 4 (°C):	320
Rate (°C/min)	10
Hold Time (min):	8

TSQ 8000 Evo MS Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	300
Electron energy (eV):	40
Acquisition Mode:	SRM
Q2 Gas Pressure (psi)	60 (Argon)
Collision Energy (eV)	See Table 2
Q1 Peak Width (Da)	0.7
Q3 Peak Width (Da)	0.7

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Table 2. SRM transitions used for native and ^{13}C 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

Results

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 the native and their 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).

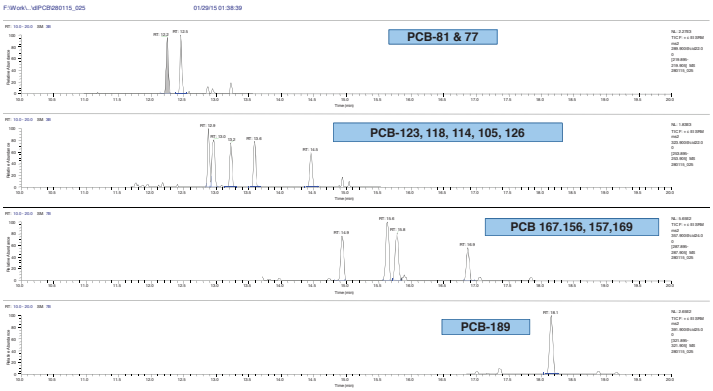


FIGURE 1. Chromatographic separation of native DL-PCBs in the lowest standard (1 pg/µL). One SRM transition (quantification ion) per compound is shown.

Data Analysis: 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 total TEQ.

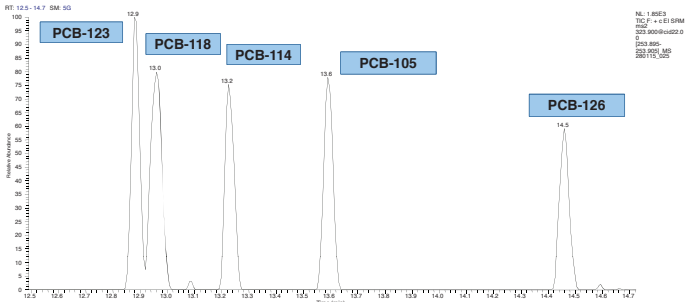


FIGURE 2. Chromatographic separation of the pentachlorobiphenyl congeners in the CS-1 standard (1 pg/µl)

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 [3, 5, 7].

The instrument LOQ was assessed by repeatedly (n = 8) injecting the lowest calibration standard (CS1). The results of this test show that the LOQs for the DL-PCBs analyzed were between 0.1 – 0.41 pg/µL (ion ratios and response factors RF at these levels still within ±15% limit, % recovery of ^{13}C-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 femtogram levels, thus meeting the detection limit requirements [5].

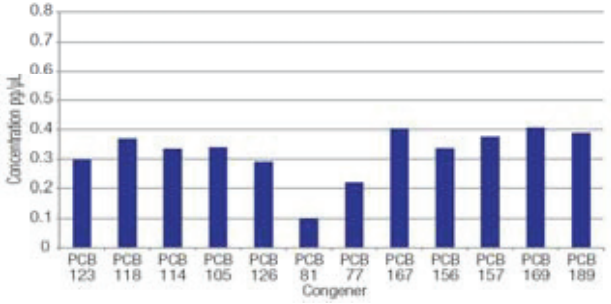


FIGURE 3. LOQ calculation for DL-PCBs from repeat injections of a serial dilution. Data indicate the LOQ for each congener with ion ratios and response factors values within the expected limits.

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 EPA [7] (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

Linearity / Calibration				
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	1.20	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. An example of the chromatography is shown below for PCB 189 (Figure 4).

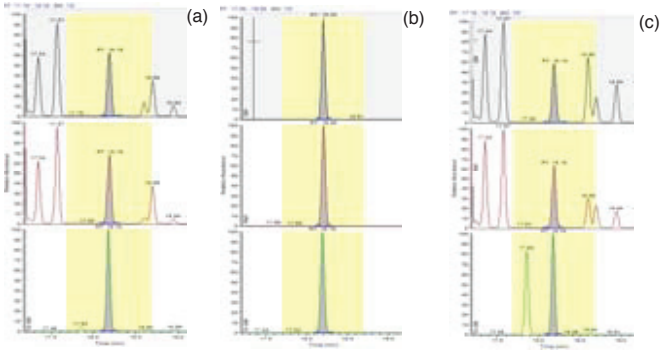


FIGURE 4. Example of chromatographic separation of PCB 189 present in the fish (a) feed (b) milk powder (c) samples

DL-PCB content of each sample, expressed as WHO-TEQ pg/g, was determined for each analyzed sample 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.

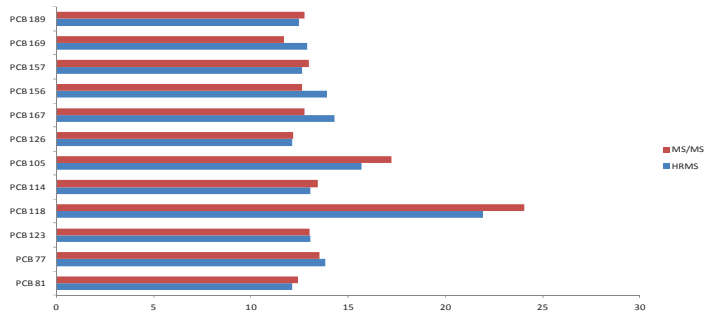
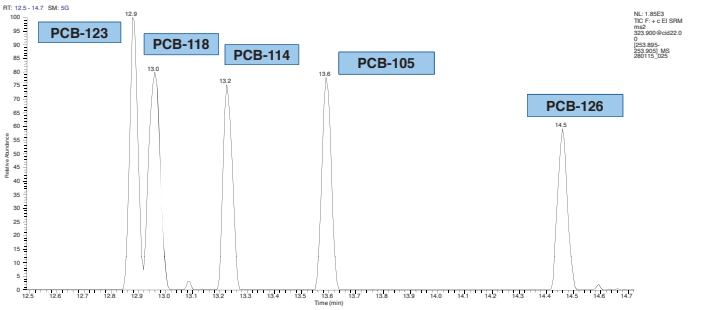


FIGURE 5. Individual contribution of each DL-PCB congener to the feed sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

Data Analysis: 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 total TEQ.



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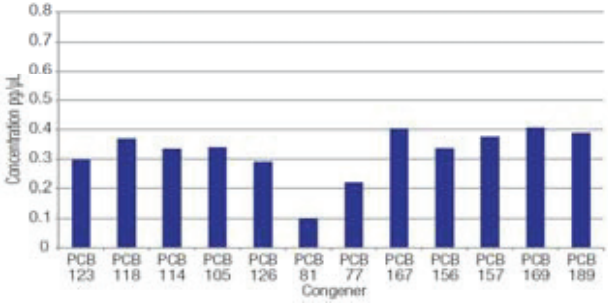

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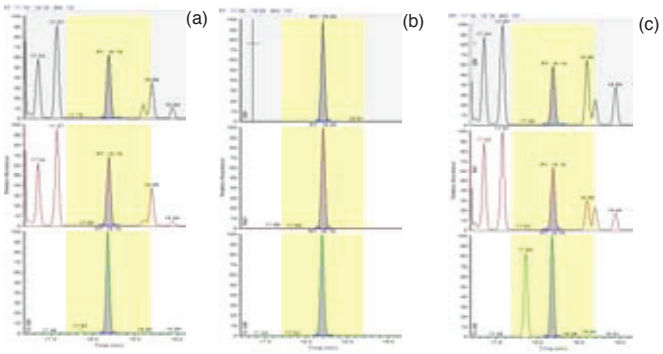

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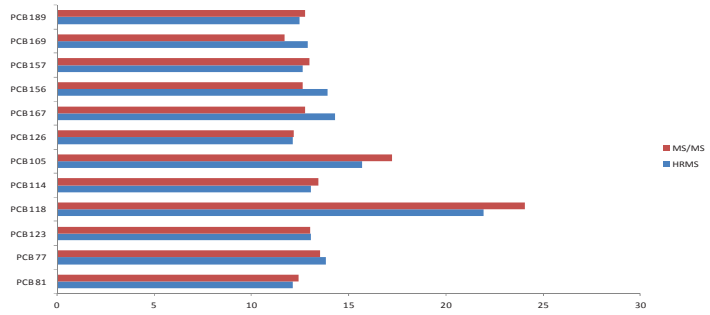

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Conclusion

The results of this evaluation demonstrates 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 the 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 system solution for DL-PCB analysis in complex samples.
- Excellent reproducibility, linearity, sensitivity, and selectivity were obtained in all the experiments performed with standards and sample extracts.
- Moreover, the calculated DL-PCB values for the matrix samples were in very good agreement with those derived from the sector instrument.

References

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