CONFIRMATORY MEASUREMENT OF PCDD/Fs AND (N)DL-PCBs IN FOOD/FEED USING A NEW SHORT COLLISION CELL TRIPLE QUADRUPOLE GC-MS/MS SYSTEM

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Introduction

In 2014, European Regulations laying down methods of sampling and analysis for the EU official control of levels of polychlorodibenzo-p-dioxins (PCDDs), polychloro-dibenzofurans (PCDFs), dioxin-like (DL) and non-dioxin-like (NDL) PCBs in food and feed have been amended by EU Regulations No 589/2014[1] and 709/2014[2]. As a direct consequence, based on validations studies[3,4], gas chromatography (GC) coupled to triple quadrupole mass spectrometry (GC-QQQMS/MS) was recognized as a confirmatory tool for checking compliance with maximum levels (ML) following specific analytical criteria[5]. Later EU Commission Regulations (2017/644[6] and 2017/771[7]) further confirmed the use of GC-QQQMS/MS and a significant number of laboratories have nowadays implemented QQQ approaches to replace, or in parallel to, their classical high resolution (HR)MS approaches based on the use of sector instruments.

In this study, the performance of a novel triple quadrupole GC-QQQMS/MS system equipped with a programable temperature vaporization (PTV) injector was evaluated for the analysis of PCDD/Fs and PCBs in food and feed. The MS analyzer was equipped with a titanium ionization chamber and a new short collision cell capable to accumulate and eject ions by means of very narrow pulses that allow to minimize the noise and to adapt accumulation times for sensitive selected reaction monitoring (SRM). The analytical capability of the system was confronted by the strict requirements set by the EU Regulation for a range of standards, quality control (QC) and food/feed samples.

Materials and methods

Standards and Chemicals. For PCDD/Fs and non-ortho (NO-)PCBs a six-point calibration curve (Cambridge Isotope Laboratories CIL, USA) ranging from 0.05 to 50 pg.µL^{-1} was used. For mono-ortho (MO-)PCBs and non-dioxin like (NDL-)PCBs, a nine-point calibration curve (CIL) ranging from 0.5 to 500 pg.µL^{-1} was used. All congeners were quantified against their own ^{13}C-labeled internal standards using isotope dilution. Solutions of standards and purified extracts were made of nonane (Fluka, Germany) tested to be contamination free.

Sample preparation. Samples were prepared following our accredited ISO17025 procedure. Briefly, sample extractions were performed using accelerated solvent extraction (ASE^{TM} 350, Dionex, Thermo Fisher Scientific). A multistep automated clean-up and fractionation procedure was used (PowerPrep^{TM} system, FMS Inc, Waltham, USA) to produce two separate fractions (MO/NDL-PCBs in hexane/dichloromethane 50:50, PCDD/Fs and NO-PCBs in toluene). The method has previously been described in details[8].

Instrumentation and measurements. A Jeol (Tokyo, Japan) JMS-TQ4000GC triple quadrupole system equipped with a programmable temperature vaporization (PTV) inlet (Optic-4, GL Sciences, The Netherlands) was used. The volume of injection was set at 4 µL for PCDD/Fs and NO-PCBs standards solutions, and 2 µL for MO and NDL-PCBs, injection at 45 °C (5 sec), then ramp of 8 °C.sec^{-1} until 325 °C. The vent time was set at 80 sec with vent flow of 100 mL.min^{-1}, transfer time of 4 min, split flow at 25 mL.min^{-1} until the end of the analysis. All separations were performed with a VF-5ms 50 m x 200 µm x 0.33 µm (Agilent, USA) using a temperature program starting at 60 °C (5 min), ramp at 70 °C.sec^{-1} until 200 °C, 3.2 °C.sec^{-1} until 235 °C (1.5 min), 3.2 °C.sec^{-1} until 270 °C (10 min), 15 °C.sec^{-1} until 310 °C (10 min), for a total run of 56 min. Transfer line and ion source (EI, 70 eV) temperature were held at 280 °C. Helium was used as carrier gas at a flow rate of 1 mL.min^{-1}. Quadrupoles were held at 100 °C and nitrogen was used as collision gas. The MS was operated in multiple reaction monitoring (MRM) mode, with collision energy and transitions optimized for native PCDD/Fs and PCBs congeners using an AutoSRM function. Two MRM transitions (Quant. and Qual.), were extrapolated and optimized for each target. Each transition was derived from two specific precursor ions and two distinct product ions. The quantification was performed exclusively by Quant. transitions and the ratio between Qual./Quant. transitions was used for qualitative purpose. All criteria for the method validation followed the requirements of the recent EU regulation 2017/771.

Results and discussion

The system was subjected to series of calibrations using dedicated sets of standard solutions and iLOQ values were calculated. Maximum permitted tolerance of relative ion intensities of ± 15 % for selected transition product ions in comparison to calculated or measured values (average from calibration standards), applying identical MS/MS conditions for each transition of an analyte were successfully measured over the entire calibration range (Figure 1) and demonstrated the absence of interferences. Calculated relative response factors (RRFs) also demonstrated acceptable (≤30%) deviation from the average RRF throughout the analytical sequence (Table 1).

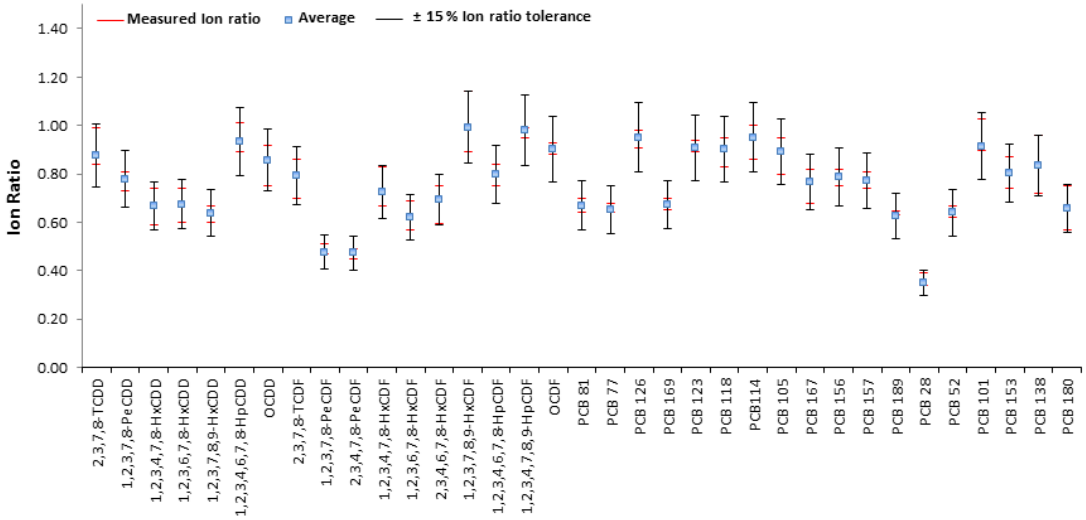

Figure 1. Ion ratio average values for native congeners for all calibration standards (one week of injections, n=25). Red bars: range of minimum/maximum ion ratios; Black bars: 15% tolerance allowed.

Table 1. Calibration curve data and instrumental limits of quantitation (iLOQs) for PCDD/Fs and PCBs.

Native Standards	Retention time (min)	Lowest cali. point (pg.µL^{-1})	Lowest cali. point RSD (%)	Highest cali. point (pg.µL^{-1})	Correlation coef. (R^2)	Average RRF	Difference (%) RF (lowest point)-RF (all)	iLOQ (pg.µL^{-1})
PCDDs								
2,3,7,8-TCDD	29.41	0.05	1.9	10	0.99998	1.35	8.7	0.028
1,2,3,7,8-PeCDD	34.61	0.05	15.0	10	0.99990	1.07	4.5	0.046
1,2,3,4,7,8-HxCDD	41.11	0.10	11.6	20	0.99996	1.16	22.7	0.168
1,2,3,6,7,8-HxCDD	41.30	0.10	15.4	20	0.99930	1.18	-3.6	0.094
1,2,3,7,8,9-HxCDD	41.76	0.10	8.3	20	0.99999	1.19	6.3	0.103
1,2,3,4,6,7,8-HpCDD	45.50	0.10	15.1	20	0.99998	1.38	-25.5	0.060
OCDD	49.97	0.25	12.3	50	0.99990	1.04	8.3	0.164
PCDFs								
2,3,7,8-TCDF	28.81	0.05	11.4	10	0.99980	1.24	-12.7	0.032
1,2,3,7,8-PeCDF	32.99	0.05	10.9	10	0.99999	1.09	1.8	0.051
2,3,4,7,8-PeCDF	34.24	0.05	10.2	10	0.99997	1.10	6.4	0.033
1,2,3,4,7,8-HxCDF	39.33	0.10	9.9	20	0.99999	1.03	-5.2	0.090
1,2,3,6,7,8-HxCDF	39.61	0.10	10.2	20	0.99998	1.07	-3.0	0.053
2,3,4,6,7,8-HxCDF	40.84	0.10	6.0	20	0.99999	1.23	7.6	0.066
1,2,3,7,8,9-HxCDF	42.33	0.10	8.0	20	0.99900	1.03	-8.5	0.110
1,2,3,4,6,7,8-HpCDF	44.10	0.10	11.9	20	0.99990	1.29	3.9	0.075
1,2,3,4,7,8,9-HpCDF	46.30	0.10	9.5	20	0.99996	1.20	7.1	0.100
OCDF	50.33	0.25	8.2	50	0.99880	1.13	1.6	0.273
NO-PCBs								
PCB 77	24.76	0.50	4.8	20	0.99970	1.10	-5.2	0.118
PCB 81	25.33	0.50	6.5	20	0.99970	1.17	-7.5	0.057
PCB 126	29.72	0.50	4.3	20	0.99990	1.37	-0.3	0.253
PCB 169	34.41	0.50	8.0	20	0.99990	1.12	-3.2	0.397
MO-PCBs								
PCB 123	26.37	0.40	3.7	140	0.9913	1.20	14.1	0.774
PCB 118	26.57	0.40	2.5	140	0.9908	1.26	15.8	0.233
PCB114	27.16	0.40	6.3	140	0.9950	1.19	6.6	0.756
PCB 105	27.98	0.40	6.8	140	0.9976	1.25	14.6	0.226
PCB 167	30.68	0.40	5.1	140	0.9926	1.15	15.1	0.121
PCB 156	32.01	0.40	9.2	140	0.9928	1.13	15.5	0.255
PCB 157	32.31	0.40	13.7	140	0.9998	1.21	11.7	0.494
PCB 189	37.16	0.40	10.1	140	0.9998	1.14	14.1	0.119
NDL-PCBs								
PCB 28	17.95	0.40	7.5	500	0.9963	0.78	17.0	0.210
PCB 52	19.23	0.40	8.0	500	0.9914	1.21	14.3	0.176
PCB 101	23.29	0.40	7.8	500	0.9961	1.31	19.8	0.130
PCB 153	27.64	0.40	14.9	500	0.9906	1.05	-4.9	0.434
PCB 138	29.13	0.40	4.5	500	0.9908	0.98	2.5	0.235
PCB 180	32.92	0.40	12.0	500	0.9927	0.99	-12.5	0.249

iLOQs were calculated using 8 replicate injections of the lowest acceptable calibration point, and iLOQs were further defined as 10 times the standard deviation (SD) associated to these replicates. The lowest acceptable calibration point was determined according to the two following criteria. First, the calculated RSDs of the lowest level for all congeners must be ≤15%. Second, the relative difference between the average RRF obtained for all

points (including replicates) and the average response factors obtained for only the lowest point must be ≤30%, according to the Regulation (this is the 'acceptable deviation to the relative response factor').When these criteria were met, the linearity was acceptable in the calibration range and the resulting lowest calibration level was eventually used to determine iLOQ as explained before.

RRF stability over time was followed for each congener. Figure 2 illustrates such stability for two congeners over a two-month period.

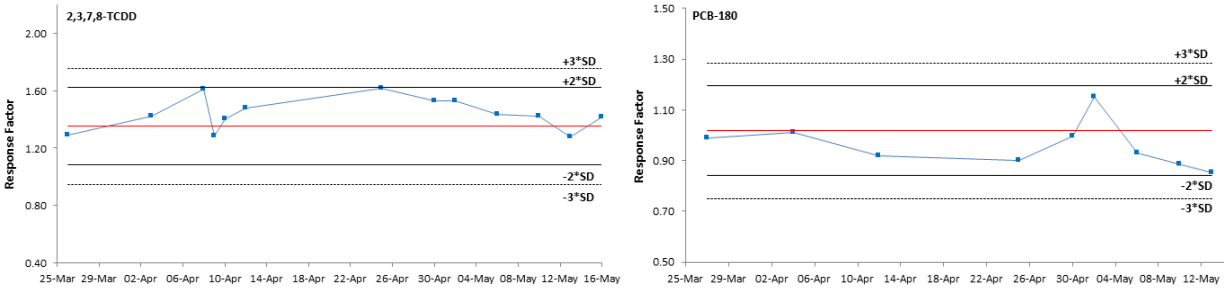

Figure 2. Relative response factor charts for 2,3,7,8-TCDD and PCB-180 over a two-month period.

As part of the investigation of instrument accuracy, and in the line of EU requirements, we estimated the bias of the instrumental part of the method for PCDD/Fs and DL-PCBs using standard solutions at values close to the maximum level (ML) for animal feed of plant and animal origin (Table 2). Good reproducibility and very limited bias values were observed, further demonstrating adequate precision and accuracy at the ML values.

Table 2. Bias of the method for PCDD/Fs and DL-PCBs using standards solutions around maximum level (ML) for animal feed.

		plant origins ng WHO_{2005}TEQ/Kg				
PCDD/Fs	Regulation	**Average**[a]	**SD**	**RSD %**	**Target**	**Bias %**
ML	0.75	0.573	0.018	3.21	0.584	-1.88
ML/2	0.38	0.126	0.003	2.41	0.117	7.69
2ML	1.50	1.166	0.012	1.06	1.168	-0.15
		animal origins (fat) ng WHO_{2005}TEQ/Kg				
PCDD/Fs	Regulation	**Average**[a]	**SD**	**RSD %**	**Target**	**Bias %**
ML	1.50	1.720	0.055	3.21	1.750	-1.95
ML/2	0.75	0.378	0.009	2.41	0.350	7.87
2ML	3.00	3.520	0.037	1.06	3.500	0.34
		plant origins ng WHO_{2005}TEQ/Kg				
DL-PCBs and **PCDD/Fs**	Regulation	**Average**[a]	**SD**	**RSD %**	**Target**	**Bias %**
ML	1.25	1.375	0.014	1.02	1.372	0.28
ML/2	0.625	0.654	0.019	2.85	0.665	-1.77
2ML	2.5	6.152	0.103	1.67	6.247	-1.52
		animal origins (fat) ng WHO_{2005}TEQ/Kg				
DL-PCBs and **PCDD/Fs**	Regulation	**Average**[a]	**SD**	**RSD %**	**Target**	**Bias %**
ML	2.00	1.961	0.056	2.846	1.996	-1.77
ML/2	1.00	0.493	0.010	2.016	0.470	4.82
2ML	4.00	4.126	0.042	1.017	4.115	0.28

(a) calculated using three replicates

A milk certified reference material (BCR-607) was analyzed in independent triplicates and demonstrated the efficiency of the method for a complex fatty matrix. All RSD values were below 15% and the accuracy was good for all certified congeners (Table 3). Careful integration of 1,2,3,7,8-PeCDF signals was necessary to avoid any contribution of close eluting PCB-169 due to the current GC method. In such case, selecting different daughter ions can ensure no contribution from PCB ions. When expressed in TEQ, the accuracy was 96% (1,99 pg TEQ/g fat measured, 2,07 pg TEQ/g fat certified).

Table 3. Performance of the method for the certified PCDD/F congeners present in milk BCR-607 (pg/g fat).

Analytes	Mean	SD	RSD (%)	Certified values	Accuracy (%)
2, 3, 7, 8 - TetraCDD	0.26	0.025	10	0.25 (0.03)	105
1, 2, 3, 7, 8 - PentaCDD	0.85	0.057	7	0.79 (0.04)	107
1, 2, 3, 4, 7, 8 - HexaCDD	0.34	0.018	5	0.42 (0.07)	81
1, 2, 3, 6, 7, 8 - HexaCDD	0.65	0.071	11	0.98 (0.11)	66
1, 2, 3, 7, 8, 9 - HexaCDD	0.28	0.024	8	0.34 (0.05)	83
2, 3, 7, 8 - TetraCDF	0.04	0.004	10	0.05 (0.03)	82
1, 2, 3, 7, 8 - PentaCDF	0.06	0.005	8	0.054 (0.013)	114
2, 3, 4, 7, 8 - PentaCDF	1.49	0.152	10	1.81 (0.13)	82
1, 2, 3, 4, 7, 8 - HexaCDF	0.91	0.073	8	0.94 (0.04)	97
1, 2, 3, 6, 7, 8 - HexaCDF	1.06	0.074	7	1.01 (0.09)	105
2, 3, 4, 6, 7, 8 - HexaCDF	0.97	0.051	5	1.07 (0.05)	90

*Uncertainties in brackets

Two types of quality control (QC) samples were considered, a beef fat QC and an animal feed QC. Triplicate independent measurements produced data that were included inside the confidence interval calculated over routine measurements using GC-HRMS (Figure 3).

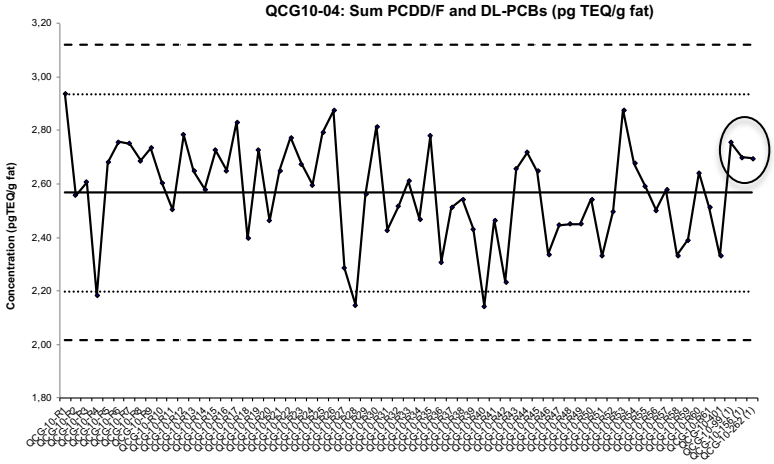

Figure 3. Quality control chart for beef fat routine QC samples. Chart made of routine GC-HRMS measurements, with GC-QQQMS/MS data for the three last points on the right.

As an example of stability of the measurements, the range of RSD % values for NDL-PCB levels was 3-11%, accuracy of 110% (n= 10 QC samples).

Conclusion

The use of a novel triple quadrupole GC-QQQMS/MS system equipped with a programable temperature vaporization (PTV) injector for the analysis of PCDD/Fs and PCBs in food and feed demonstrated to be in line with the EU requirements for food/feed control. The system has been positively tested against a range of standards, quality control (QC) samples and food/feed samples. Further usage of the system will be necessary to demonstrate the stability and robustness over a longer period of time.

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References:

1. Commission Regulation (EU) No 589/2014 (2014) *Offic J Eur Commun* L164: 18-40.
2. Commission Regulation (EU) No 709/2014 (2014) *Offic J Eur Commun* L188: 1-18.
3. L'Homme B, Scholl G, Eppe G, Focant JF, et al. (2015) *J Chromatogr A* 1376: 149-58.
4. Ábalos M, Cojocariu CI, Silcock P, Roberts D, et al. (2016) *Anal Bioanal Chem* 408(13): 3511-25.
5. Kotz A, Malisch R, Focant JF, Eppe G, et al. (2012) *Organohalogen Compd.* 74: 156-159.
6. Commission Regulation (EU) No 2017/344 (2017) *Offic J Eur Commun* L92: 9-34.
7. Commission Regulation (EU) No 2017/771 (2017) *Offic J Eur Commun* L115: 22-42.
8. Focant JF, Eppe G, Massart AC, Scholl G, et al. (2006) *J Chromatogr A* 1130: 97-107.