Meeting the Upcoming European Commission Performance Criteria for the Use of Triple Quadrupole GC-MS/MS as a Confirmatory Method for PCDD/Fs in Food and Feed Samples

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Key Words
Dioxins, food and feed, GC-MS/MS, quantification, TargetQuan, isotope dilution

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) constitute a group of highly toxic organic compounds formed unintentionally, mainly in waste combustion processes or as by-products of industrial manufacturing of certain chemicals, such as chlorinated pesticides. PCDD/Fs can enter the food chains where they persist and bioaccumulate. Human exposure to dioxins occurs mostly from ingesting contaminated food. PCDD/Fs have been characterized by the US Environmental Protection Agency as likely to be carcinogenic to humans even at background levels of exposure. Consequently, accurate detection and quantification of PCDD/Fs in the environment, particularly in food and animal feed, is important.

Legislation in the European Union has required the confirmation and quantification of PCDD/Fs in contaminated samples by gas chromatography/high resolution mass spectrometry (GC-HRMS) instruments, considered the "gold standard" approach. However, recent technological 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 PCDD/Fs in food and feed as a full confirmatory method.[4]

According to the upcoming EU regulation, when using GC-MS/MS, the following specific performance criteria for dioxin confirmation with GC-MS/MS technology should be fulfilled in addition to the criteria described previously by the European Commission,[1,3] except the obligation to use GC-HRMS:[4]

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 PCDD/Fs was assessed. For this, both solvent standards, and food and feed samples were used to evaluate the instrument performance against the upcoming criteria for dioxin 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

PCDD/Fs 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 listed in Table 1 and Table 2.

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Table 1. GC and injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	2
Liner	SSL single taper P/N: 453A2342
Inlet (°C):	260
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	100
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min)	25
Temperature 3 (°C):	285
Rate (°C/min)	2.5
Temperature 4 (°C):	330
Rate (°C/min)	10
Hold Time (min):	5

Resolution of each quadrupole was set to unit mass as specified in the upcoming 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 of dioxins. 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, and the results of this are listed below (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 ^{13}C-labelled dioxins and furans.

SRM Compound Information				
Compound Name	**Quant /Qual**	**Precursor Ion [Da]**	**Product Ion [Da]**	**Collision Energy [eV]**
^{13}C-TCDF	Qual	315.94	251.97	26
^{13}C-TCDF	Quant	317.94	253.97	26
TCDF	Qual	303.89	240.94	26
TCDF	Quant	305.89	242.94	26
^{13}C-TCDD	Qual	331.94	267.97	20
^{13}C-TCDD	Quant	333.93	269.97	20
TCDD	Qual	319.89	256.93	20
TCDD	Quant	321.89	258.93	20
^{13}C-PeCDF	Qual	351.89	287.93	26
^{13}C-PeCDF	Quant	353.89	289.93	26
PeCDF	Qual	339.86	276.89	26
PeCDF	Quant	341.86	278.89	26
^{13}C-PCDD	Qual	367.89	303.93	22
^{13}C-PCDD	Quant	369.89	305.89	22
PeCDD	Qual	355.85	292.89	20
PeCDD	Quant	357.85	294.89	20
^{13}C-HxCDF	Qual	383.86	319.89	26
^{13}C-HxCDF	Quant	385.86	321.89	26
HxCDF	Qual	371.82	308.86	28
HxCDF	Quant	373.82	310.86	28
^{13}C-HxCDD	Qual	399.86	335.89	20
^{13}C-HxCDD	Quant	401.86	337.89	20
HxCDD	Qual	387.82	324.86	20
HxCDD	Quant	389.82	326.85	20
^{13}C-HpCDF	Qual	419.82	355.86	28
^{13}C-HpCDF	Quant	421.82	357.85	28
HpCDF	Qual	407.78	344.82	26
HpCDF	Quant	409.78	346.82	26
^{13}C-HpCDD	Qual	435.82	371.85	20
^{13}C-HpCDD	Quant	437.81	373.85	20
HpCDD	Qual	423.78	360.81	20
HpCDD	Quant	425.77	362.81	20
^{13}C-OCDD	Qual	469.78	405.81	20
^{13}C-OCDD	Quant	471.78	407.81	20
OCDD	Qual	457.74	394.77	20
OCDD	Quant	459.74	396.77	20
OCDF	Qual	441.76	378.79	26
OCDF	Quant	443.76	380.79	26

Sample Preparation

PCDD/Fs standards 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: 3x dry fish samples (previously used in inter-laboratory studies), one feed sample (internal reference material), and one milk powder sample (certified reference material).

Extraction and clean-up of the matrix samples were 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 and fish samples).

Data Processing

Data processing was performed using TargetQuan software, which is specifically designed for the analysis of POPs using isoptope 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.

Results and Discussion

Timed-SRM uses a completely different analytical strategy than the "classical" segmented setup, allowing data acquisition for a target compound in a defined window around the known compound's retention time, and not in a wide retention time segment. As a result, dwelling on the target compounds is very efficient, ultimately improving the sensitivity and lowering the method detection limit. Additionally, using timed-SRMs, the compound's acquisition window can be individually set to cover closely eluting isomers, such as HxCDD/Fs. An example of sensitivity gain when using t-SRM versus segmented SRM is shown in Figure 1.

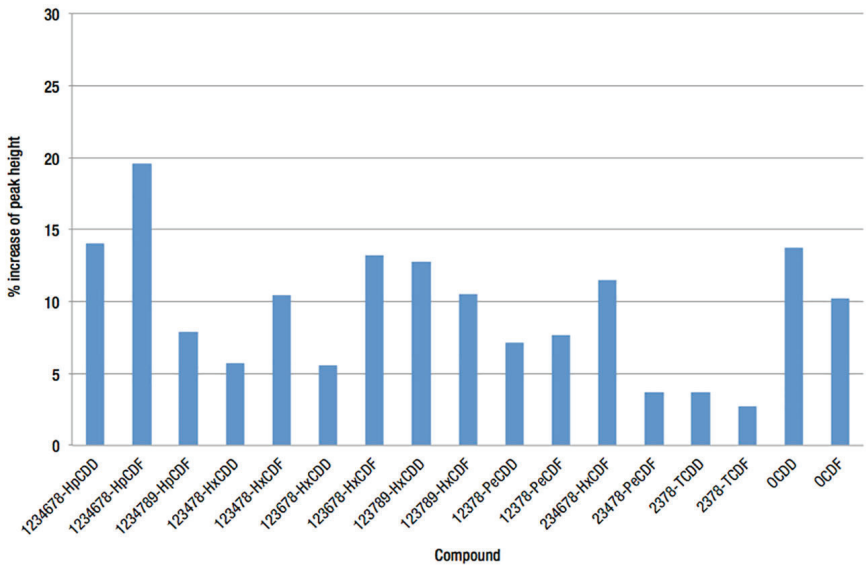

Figure 1. Sensitivity increase (%) for data acquired in timed-SRM versus segmented SRM. Peak height (counts per second) of each PCDD/F congener was compared.

Chromatography

Achieving sufficient chromatographic separation of the PCDD/F isomers is critical "sufficient" defined as <25% peak to peak between 123478-HxCDF and 123678-HxCDF).[1] Chromatography of PCDD/Fs was assessed with the lowest calibration standard (EPA 1613-CLS) containing 0.1 pg/µL TCDD/F, 0.5 pg/µL PeCDD/F - HpCDD/F and 1.0 pg/µL OCDD/F. All the native congeners and their corresponding ^{13}C-labelled internal standards were easily detected, excellent peak shape was obtained for all compounds (Figure 2), and 5% valley separation was achieved for HxCDF isomers (Figure 3).

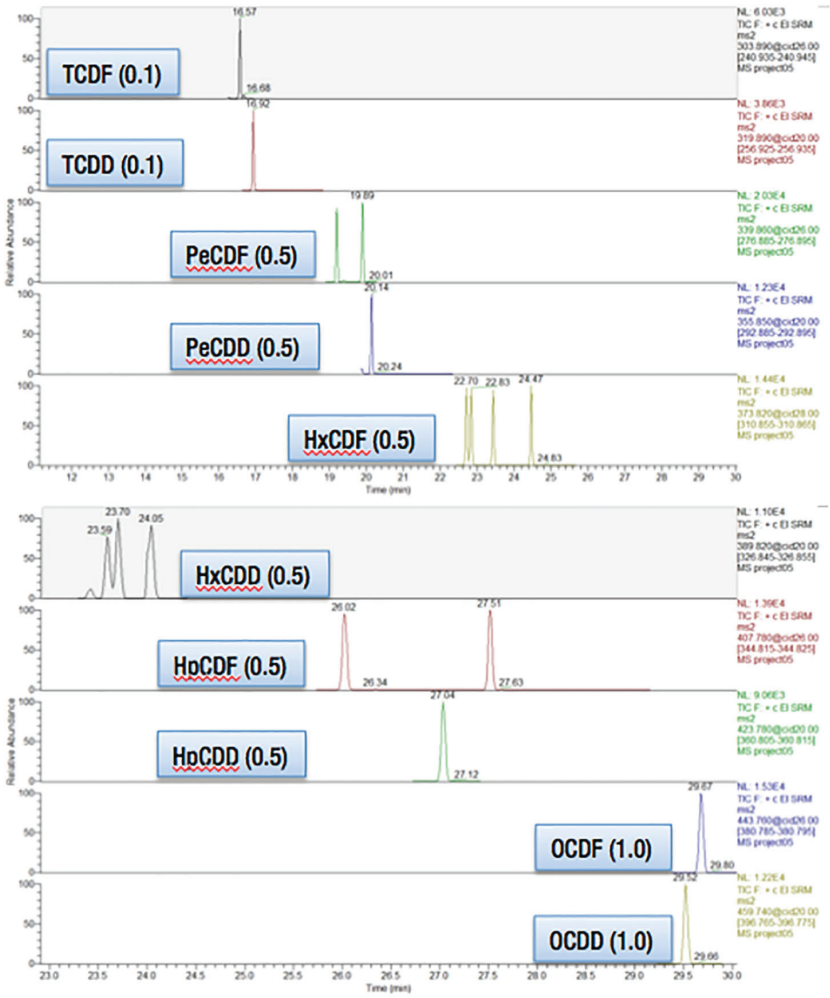

Figure 2. Chromatographic separation of native PCDD/Fs in the lowest standard (in brackets concentration in pg/µL). One SRM transition (quantification ion) per compound is shown.

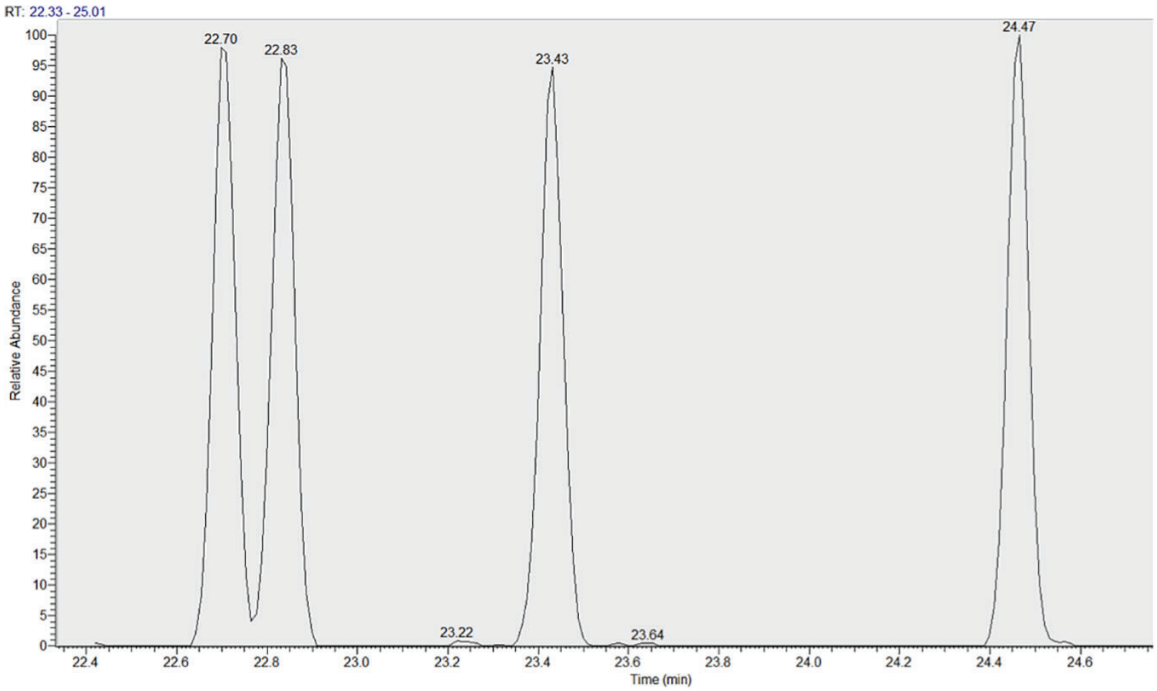

Figure 3. GC separation of HxCDF isomers showing 5% valley separation between 123478-HxCDF and 123678-HxCDF.

Reaching the level of interest

For the analysis of PCDD/Fs, reaching the expected sensitivity is critical. Limit of quantification (LOQ) for a confirmatory method should be about one-fifth of the maximum level.[1,3,4] Traditionally, sensitivity of an instrument for dioxin analysis took into account the limit of detection (LOD) and the limit of quantification (LOQ), and on sector instruments they were calculated from the S/N values (LOD corresponding to 3x the noise level, whereas the LOQ corresponds to 10x the noise level).

Often, data acquired on GC-MS/MS quadrupoles operated in MS/MS have little or no noise therefore calculation of LOD/LOQ required a rethinking of how these parameters should be determined. If, for technical reasons the S/N calculation does not provide reliable results, the LOQ of an individual congener may be calculated from the lowest concentration point (i.e., CSL) taking into account the recovery of internal standards (should be between 60–120%), ion ratio abundance, and chromatography of the sample.[4] Consequently, the instrument LOQ was assessed by repeatedly (n = 10) injecting the lowest calibration standard (CSL) and three subsequent serial dilutions of this standard.

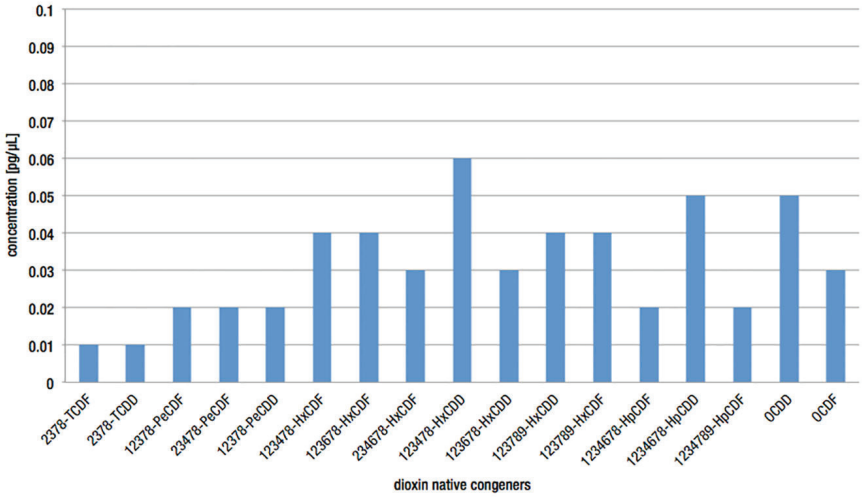

Figure 4. LOQ calculation for PCDD/Fs from repeat injections of a serial dilution. Data indicate the LOQ for each congener with ion ratios and response factors values within the expected limits.

Calculation of the LOQ for each native compound took into account Student's t-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 PCDD/Fs analyzed were between 0.01–0.05 pg/µL, corresponding to CSL 1:5 and CSL 1:10 diluted (ion ratios and response factors RF at these levels still within ±15% limit, % recovery of ^{13}C-labelled within the 60–120% limit) (Figure 4). The results of this experiment demonstrate that the TSQ 8000 Evo GC-MS/MS can detect and confirm PCDD/Fs at low femtogram levels, thus meeting the detection limit requirements.[3]

Linearity of Response

Dioxin quantification is based on isotope dilution and uses RF type of calibration where the average response factor of all the standards from an external calibration curve are taken into account to quantify the 17 toxic congeners.[1,5] Average RF %RSD values were calculated from duplicate measurements of a six point calibration curve measured at the beginning and at the end of the sample batch. The results of this experiment show excellent %RSD for all measured compounds with values between 1.2–8 %, well within the 15% limits established by EPA[5] (Table 4).

Table 4. Linearity of PCDD/Fs across six-point calibration curve. The precision on the average response factor (%RSD) for each native compound is shown. Values represent duplicate measurements of each calibration point, measured at the beginning and end of a batch.

Linearity / Calibration				
Compound	**Concentration range (pg/µL)**	**Average RF**	**stdev**	**RF % RSD**
2378-TCDF	0.1 - 40	1.04	0.02	1.9
2378-TCDD	0.1 - 40	1.12	0.02	2.2
12378-PeCDF	0.5 - 200	1.01	0.02	1.5
23478-PeCDF	0.5 - 200	1.03	0.02	1.6
12378-PeCDD	0.5 - 200	1.08	0.01	1.4
123478-HxCDF	0.5 - 200	1.03	0.01	1.2
123678-HxCDF	0.5 - 200	1.02	0.01	1.4
234678-HxCDF	0.5 - 200	1.06	0.03	3.2
123478-HxCDD	0.5 - 200	0.96	0.01	1.6
123678-HxCDD	0.5 - 200	1.21	0.04	3.6
123789-HxCDD	0.5 - 200	1.21	0.04	3.6
123789-HxCDF	0.5 - 200	1.36	0.01	0.8
1234678-HpCDF	0.5 - 200	1.06	0.02	1.7
1234678-HpCDD	0.5 - 200	1.07	0.02	2.1
1234789-HpCDF	0.5 - 200	1.12	0.02	2.2
OCDD	1.0 - 400	1.54	0.04	2.4
OCDF	1.0 - 400	1.09	0.03	3.2

Repeatability of Peak Area

Peak area precision of the 17 PCDD/Fs congeners was calculated from a series of repeat injections (n = 16) of the lowest standard (CSL). The results of this experiment showed %RSD values for all compounds below the maximum limit of 15%[1] with the highest value observed for 2378-TCDD (8.3 %) and the lowest for 12378-PeCDD (3.2%) (Figure 5).

Ion Ratio Abundance

The ion ratio (IR) abundance for selected transitions of each of the 17 PCDD/F congeners was measured in each of the samples analyzed, and the values compared with the measured ion ratio values (average from calibration standards CSL-CS4). The results of this experiment show that all the IR for the compounds analyzed were within the 15% tolerance meeting the upcoming EU criteria for dioxin confirmation[4] (Figure 6).

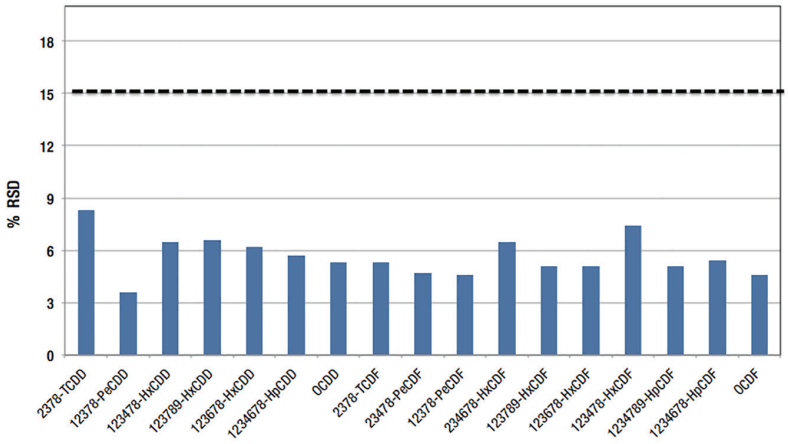

Figure 5. Peak area (quantification trace) repeatability from n=16 injections of the lowest standard (concentration range 0.1 – 1 pg/µL). The dotted line represents the 15% maximum tolerance.

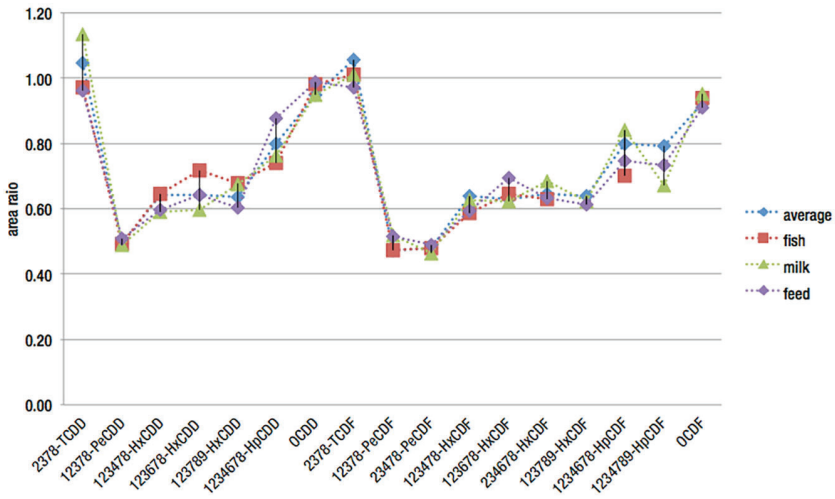

Figure 6. Comparison of the ion ratio abundance of each of the 17 PCDD/F in the samples extracts with the average IR values derived from the calibration standards (CSL-CS4).

Quantification of Dioxins in Sample Extracts

PCDD/Fs were quantified in the sample extracts prepared at CSIC, Barcelona. Excellent chromatographic separation with little matrix interference was observed for all native congeners for all samples analyzed. An example of the chromatography is shown below for 2378-TCDD (Figure 7).

The dioxin content of each sample, expressed as WHO-PCDD/F-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 PCDD/Fs 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 that obtained using GC-HRMS (Figure 8–Figure 10).

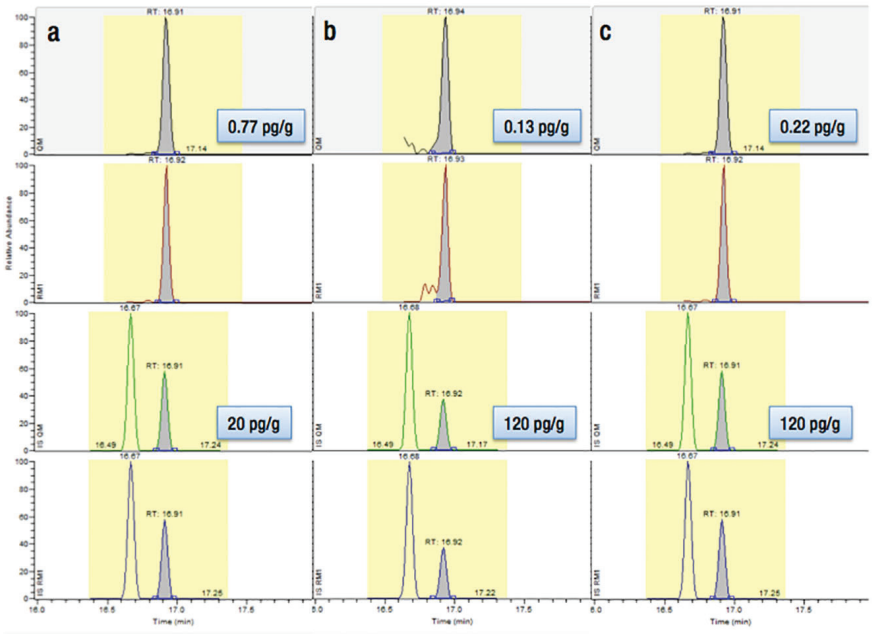

Figure 7. Example of chromatographic separation of 2378-TCDD and its internal standard ^{13}C-2378-TCDD present in the fish (a.), feed (b.) and milk powder (c.) samples. Calculated concentration (pg/g) is indicated.

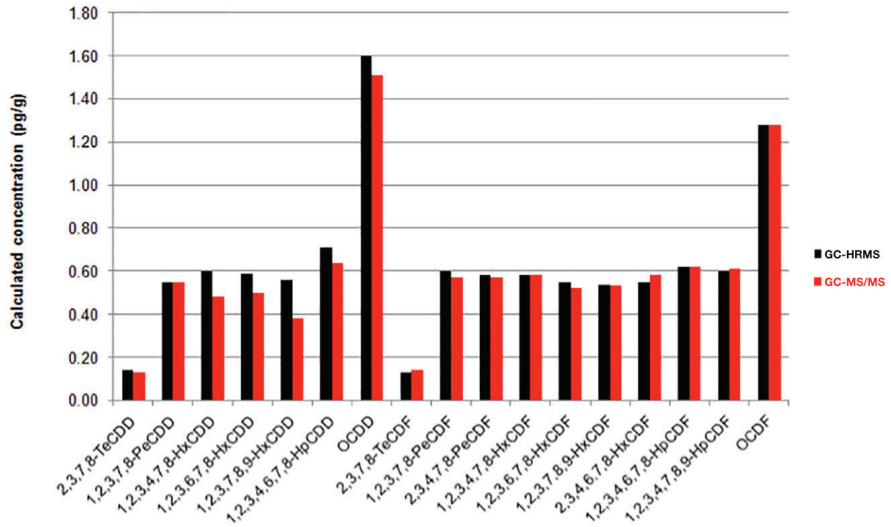

Figure 8. Individual contribution of each PCDD/F congener to the feed sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

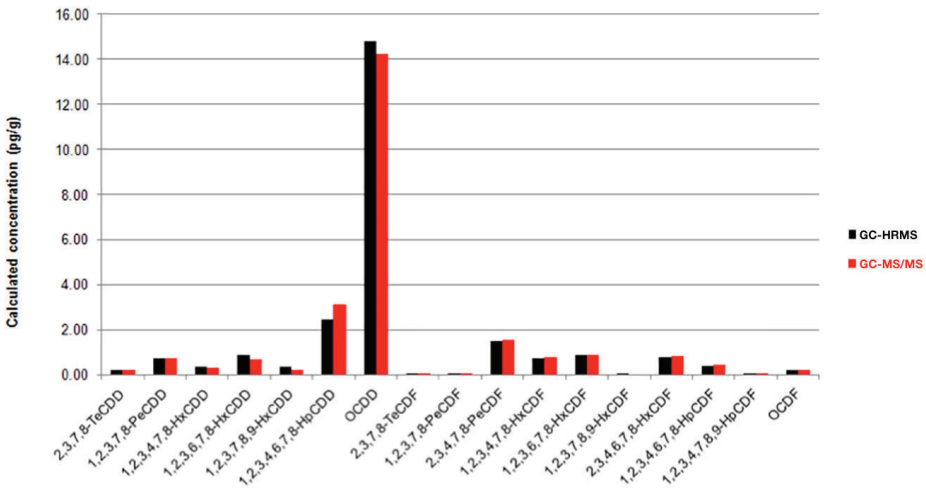

Figure 9. Individual contribution of each PCDD/F congener to the milk sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

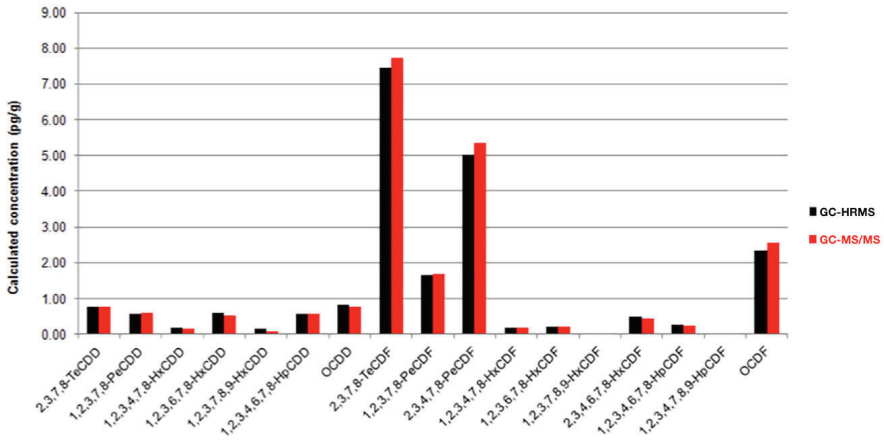

Figure 10. Individual contribution of each PCDD/F congener to the fish sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS results with the GC-HRMS values.

The total dioxin 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 5% (Figure 11). Maximum limits (ML) and action limits (AL) are indicated for each matrix type.[2]

Z-score Results

In order to check the accuracy of the results obtained with the TSQ 8000 Evo GC-MS/MS, a Z-score test was performed for all the fish samples that were previously used in an inter-laboratory exercise. For this, the consensus values (as total WHO-TEQ pg/g) for each sample, the true values, and corresponding standard deviations for each of the dioxin congeners were used. The results of this test showed that the Z-score values for all the fish samples analyzed by GC-MS/MS were within ±2 limit with an average value of -0.8 (Figure 12).

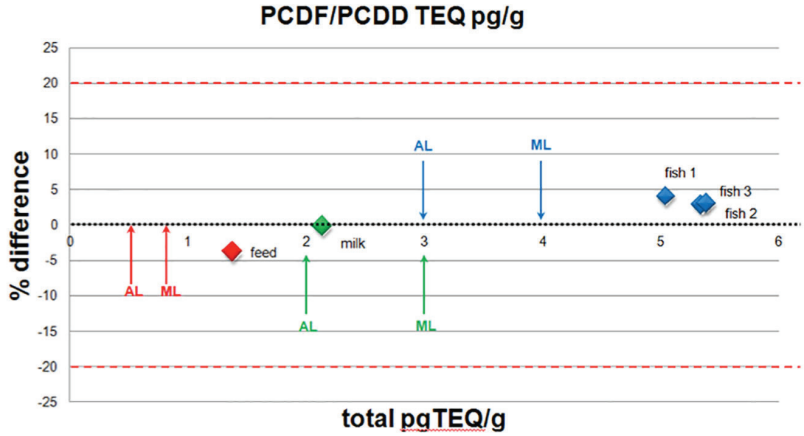

Figure 11. Deviation (%) of the total dioxin concentration (WHO-PCDD/F-TEQ pg/g) measured with the TSQ 8000 Evo from the GC-HRMS results

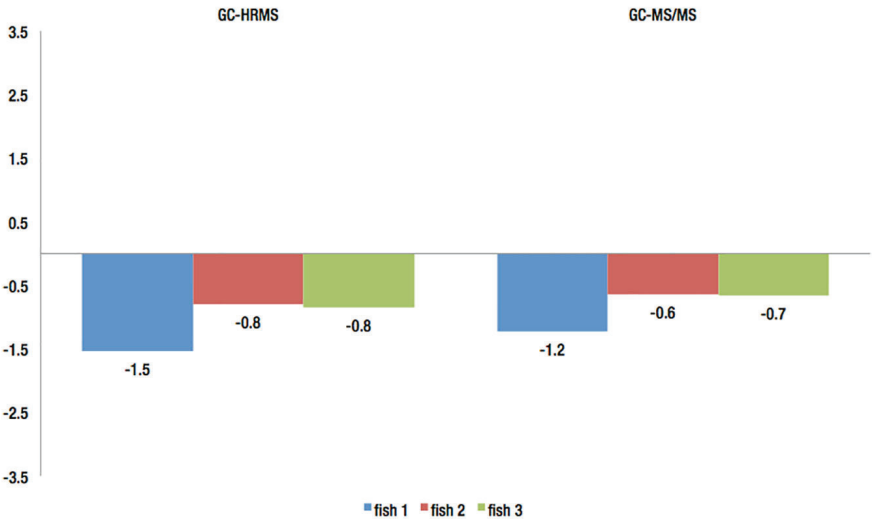

Figure 12. Z-score results calculated for the fish samples using ± 20% of the consensus TEQs (WHO2005-TEQs) as a value for target standard deviation.

Conclusion

Taken together, the results of this evaluation demonstrate that the TSQ 8000 Evo GC-MS/MS system is an extremely effective tool for routine analysis of PCDD/Fs meeting all the upcoming European Commission requirements for the confirmation of dioxins 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 PCDD/Fs 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 dioxin and furan analysis in complex samples.
- Excellent reproducibility, linearity, sensitivity, and selectivity were obtained in all the experiments performed with standards and sample extracts.
- The calculated PCDD/Fs TEQ values for the matrix samples were in very good agreement with those derived from the sector instrument, the results recommending this system for routine and confident analysis of PCDD/Fs.

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