Meeting the 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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OVERVIEW

Purpose

In this work, the performance of the Thermo Scientific™ TSQ 8000™ Evo Triple Quadrupole GC-MS/MS system for the analysis of Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated dibenzo-p-furans (PCDFs) was assessed. For this, both solvent standards and food and feed samples were used to evaluate the instrument performance against the criteria for PCDD/Fs confirmation. Additionally, a direct comparison of the results obtained from food and feed sample extracts using the TSQ 8000 Evo GC-MS/MS system with those from a GC-HRMS was made.

Methods

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 system. 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 method developed 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 PCDD/Fs 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 PCDD/Fs 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[1,3], 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

PCDD/F standards (EPA Method 1613 1613CSL to 1613CS4) containing the native and the 13C-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 was performed either by PowerPrep™ SPE system (feed sample) or by using a manual clean-up with multi-layer silica, followed by basic alumina and a final carbon column (milk, and fish samples). Final extracts were prepared in nonane.
The GC method setup is described in Table 1 below, while the MS method setup is described in Tables 2 and 3.

Tables 1 & 2. Instrument setup.

Trace 1310 GC Parameters	
Injection Volume (µL):	2
Liner	SSL single Taper 4mm ID 78.5mm
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
Hold Time (min):	2
Temperature 3 (°C):	285
Rate (°C/min)	2.5
Temperature 4 (°C):	330
Rate (°C/min)	10
Hold Time (min):	5

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 (AMU):	0.7
Q3 Peak Width (AMU):	0.7

Table 3. SRM transitions used for native and 13C-labelled PCDD/Fs.

SRM Compound Information				
Compound Name	Quant /Qual	Precursor Ion [Da]	Product Ion [Da]	Collision Energy [V]
^{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**

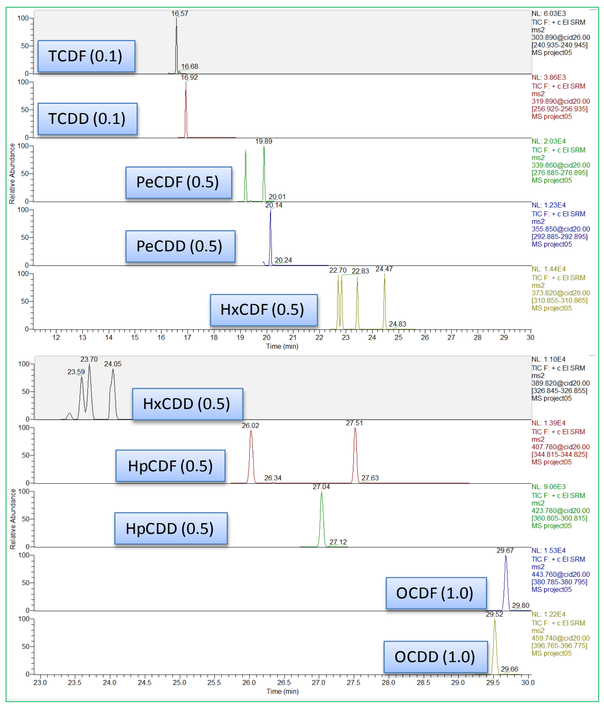
SRM Compound Information				
Compound Name	Quant /Qual	Precursor Ion [Da]	Product Ion [Da]	Collision Energy [V]
^{13}C-HxCDD	Qual	399.86	335.89	**20**
^{13}C-HxCDD	Quant	401.86	337.89	**20**
HxCDD	Qual	387.82	324.86	**20**
HxCDD	Quant	389.82	326.85	**20**
^{13}C-HpCDF	Qual	419.82	355.86	**28**
^{13}C-HpCDF	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**

RESULTS

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 in the lowest calibration standard (EPA1613-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 and their corresponding 13C-labelled internal standards were easily detected, excellent peak shape was obtained for all compounds (Figure 1), and 5% valley separation was achieved for HxCDF isomers (Figure 2).

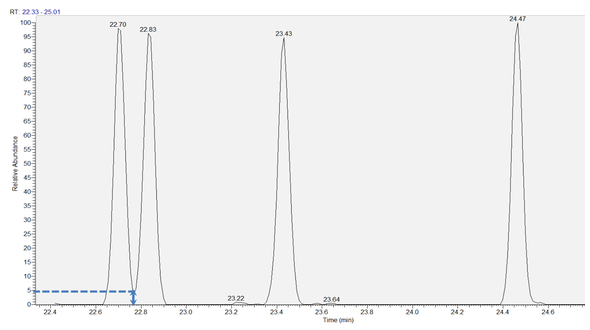
Figure 1. Chromatographic separation of native PCDD/Fs in the lowest standard (in brackets concentration in pg/µL). One SRM transition (quantification ion) per compound is shown.



Data Analysis

Data processing was performed using the Thermo Scientific™ TargetQuan 3 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. 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 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.[1, 3, 5]
The instrument LOQ was assessed by repeatedly (n = 10) injecting the lowest calibration standard (1613CSL) and three subsequent serial dilutions of this standard. The results of this test show that the LOQs for the PCDD/Fs analyzed were between 0.01 – 0.06 pg/µL (ion ratios and response factors RF at these levels still within ±15% limit, % recovery of 13C-labelled within the 60 - 120% limit) (Figure 3). The results of this experiment demonstrate that the TSQ 8000 Evo GC-MS/MS system can detect and confirm PCDD/Fs at low femtogram levels, thus meeting the detection limit requirements.[3]

Figure 3. 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.

Linearity of response

PCDD/F 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 17 toxic congeners.[1, 6] 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 0.8 – 3.6 %, well within the 15% limits established by EPA[6] (Table 4).

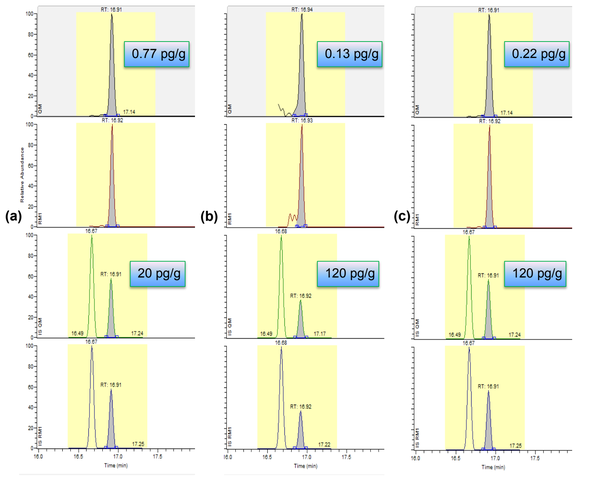
Table 4. Linearity of PCDD/Fs across six point calibration curve. The precision on the average response factor (%RSD) for each native compound is shown. Values represent duplicate measurements of each calibration point, measured at the beginning and end of a batch.

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	0.11	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

Quantification of PCDD/Fs 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 sample analyzed. An example of chromatography is shown below for 2378-TCDD (Figure 4).

Figure 4. Example of chromatographic separation of 2,3,7,8,-TCDD and its internal standard 13C-2,3,7,8-TCDD present in the fish (a) feed (b) milk powder (c) samples. Calculated concentration (pg/g) is indicated.



PCDD/F 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 PCDD/F 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 system and that obtained using GC-HRMS (Figures 5 and 6). The concentrations of the PCDD/Fs 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 PCDD/F congener to the feed sample dioxin content (as TEQ pg/g) and comparison of TSQ 8000 Evo GC-MS/MS system results with the GC-HRMS values.

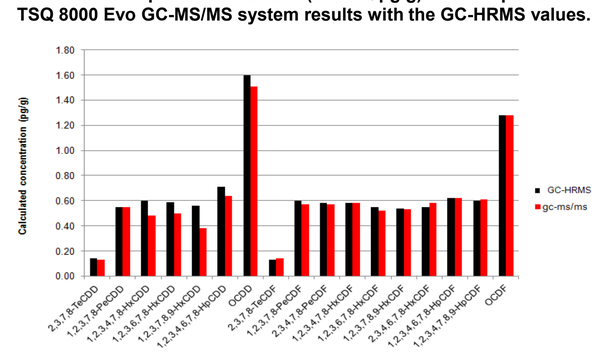
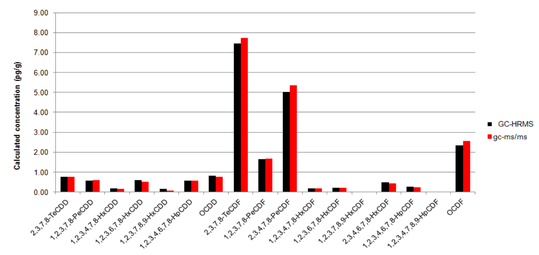


Figure 6. Individual contribution of each PCDD/F congener to the feed sample dioxin content (as TEQ pg/g) and comparison of the TSQ 8000 Evo GC-MS/MS system results with the GC-HRMS values.

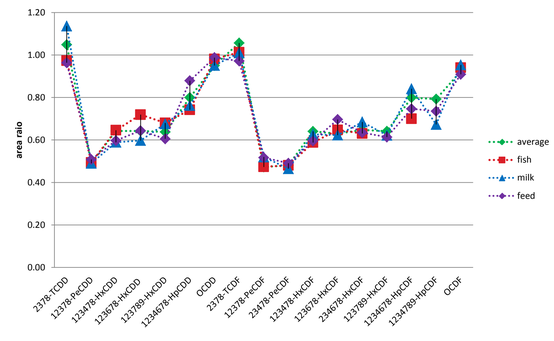


The total PCDD/F content of each sample obtained from the TSQ 8000 Evo GC-MS/MS system analysis was plotted against the sector instrument data, with the calculated deviation not exceeding 5%.

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 EU criteria for dioxin confirmation[2] (Figure 7).

Figure 7. 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).



CONCLUSIONS

Taken together, the results of this evaluation demonstrates that the TSQ 8000 Evo GC-MS/MS system is an extremely effective tool for routine analysis of PCDD/Fs meeting all the 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 PCDDFs detection and confirmation in food and feed samples.

- The TSQ 8000 Evo GC-MS/MS system, together with the TRACE 1310 GC system and the TargetQuan 3 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.
- Moreover, 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.

REFERENCES

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TRADEMARKS/LICENSING