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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Introduction

Until recently, legislation in the European Union 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 GC-MS/MS technology has allowed high sensitivity and selectivity to be achieved. These have led to GC-MS/MS being permitted for use in the EU to control the maximum levels for PCDD/Fs in food and feed as a full confirmatory method[4]. 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 defined by European Commission in the regulations[1,3], except the obligation to use GC-HRMS[4]. In this work, the performance of the Thermo Scientific™ TSQ8000™ 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 new 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.

Materials and 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. 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. Resolution of each quadrupole was set to unit mass as specified in the new EC criteria for dioxin confirmation using GC-MS/MS. 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. Data processing was performed with Thermo Scientific™ TargetQuan 3.1 software.

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 was performed either by PowerPrep system (FMS) for the feed sample or using a manual clean-up with multilayer silica, followed by basic alumina and a final carbon column (milk and fish samples).

Results and Discussion

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 ^{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).

For the analysis of PCDD/Fs, reaching the expected sensitivity is critical. 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 (CSL) and three subsequent serial dilutions of this standard. Calculation of the LOQ for 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 show that the LOQs were between 0.01 – 0.07 pg/µL, corresponding to CSL (x5) diluted (ion ratios and response factors RF at these levels within ±15% limit, % recovery of 13-labelled within the 60 - 120% limit). 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].

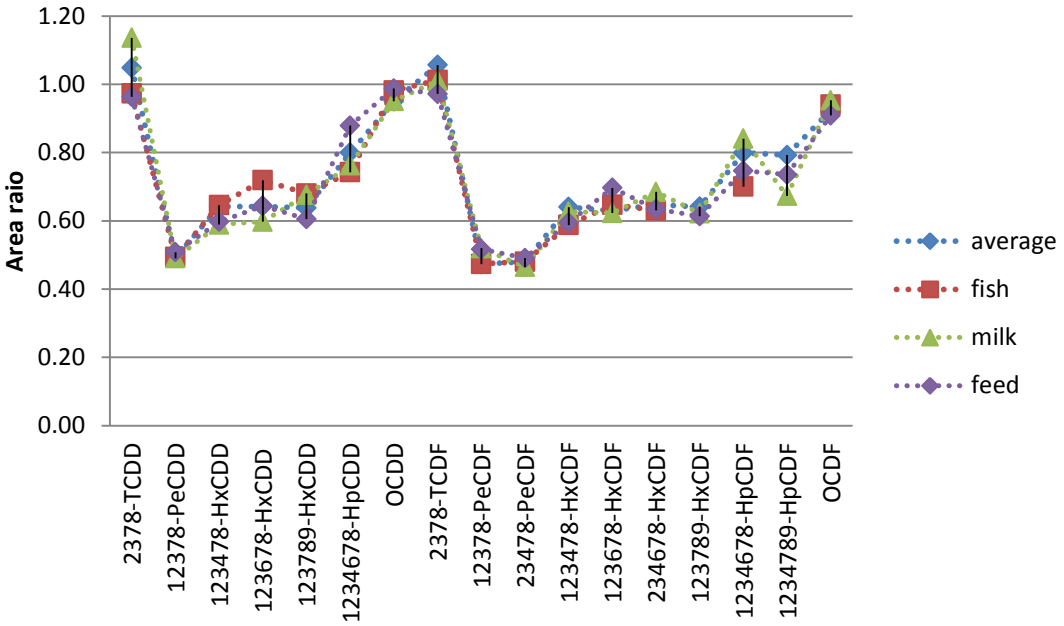
Linearity of response and repeatability of peak area

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[1,6]. 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 1.2 - 8 %, well within the 15% limits established by EPA[6]. 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%with the highest value observed for 2,3,7,8-TCDD (8.3 %) and the lowest for 12378-PeCDD (3.2%)

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[4](Figure 1).

FIGURE 1. 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).


1.20
1.00
0.80
0.60
0.40
0.20
0.00
Area raio
2378-TCDD
12378-PeCDD
123478-HxCDD
123678-HxCDD
123789-HxCDD
1234678-HpCDD
OCDD
2378-TCDF
12378-PeCDF
23478-PeCDF
123478-HxCDF
123678-HxCDF
234678-HxCDF
123789-HxCDF
1234678-HpCDF
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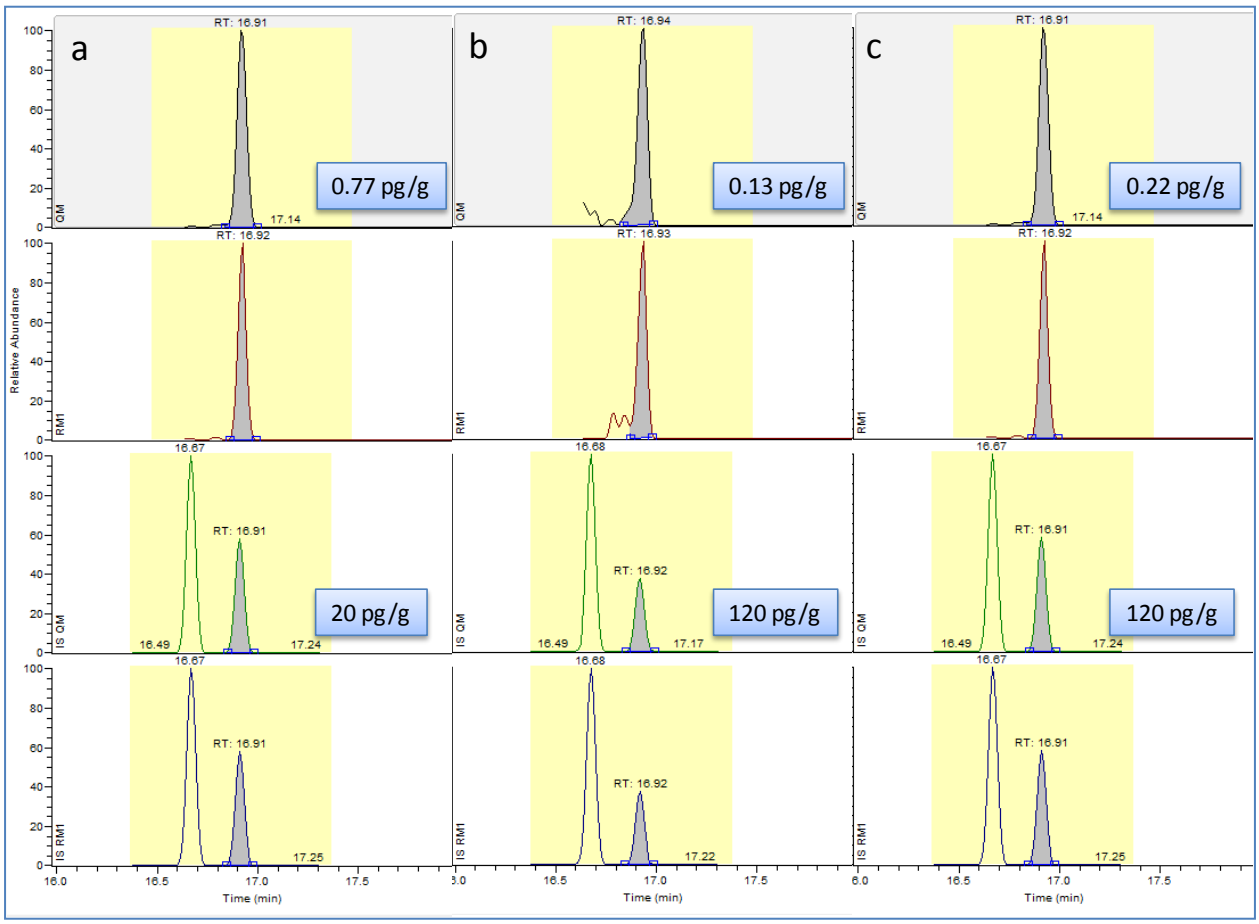
OCDF
average
fish
milk
feed

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 PCDD/Fs for all sample types. An example of chromatography is shown below for 2,3,7,8-TCDD (Figure 2).

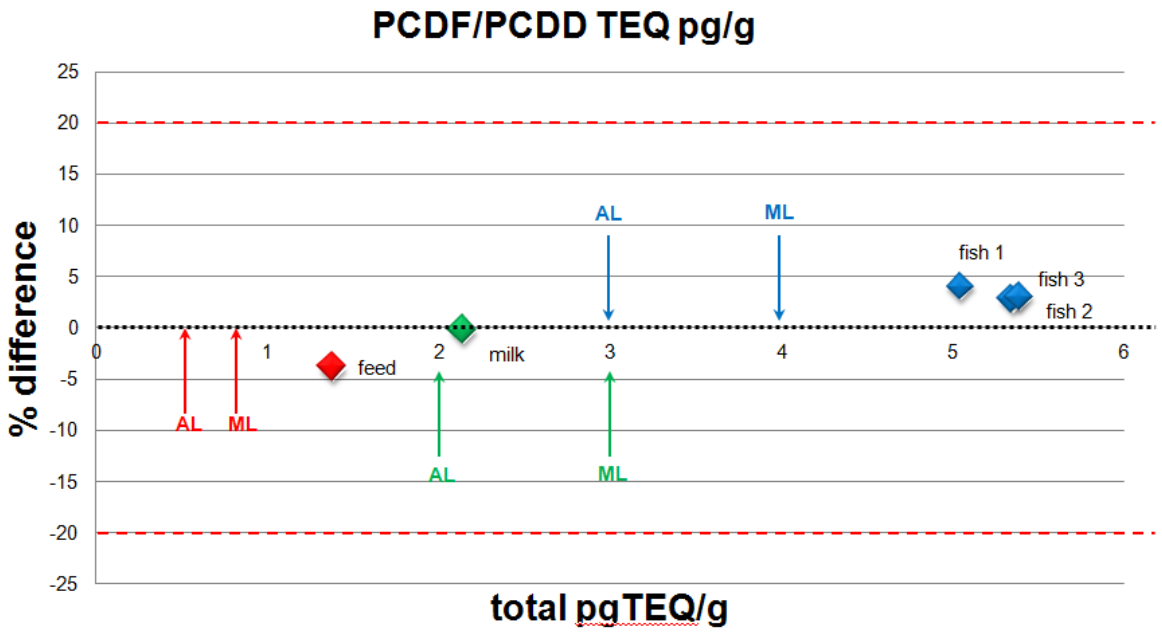
FIGURE 2. Example of chromatographic separation of 2,3,7,8-TCDD and its internal standard ^{13}C-2378-TCDD present in the fish (a), feed (b) and milk powder (c) samples. Calculated concentration (pg/g) is indicated.



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 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.

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%. Maximum limits (ML) and action limits (AL) are indicated for each matrix type[2] (Figure 3).

FIGURE 3. Deviation (%) of the total dioxin concentration (WHO-PCDD/F-TEQ pg/g) measured with the TSQ 8000 EVO from the GC-HRMS results.



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[4].
- 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.
- 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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