Applying EU LOQ guidance for the low level quantification of PCDD/Fs in animal feed using a triple quadrupole GC-MS/MS system with an advanced EI source

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Introduction: Developments in gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) technologies have allowed their use for confirmatory analysis and control of maximum levels (MLs) and action levels (ALs) of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-p-furans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in certain food and feed samples, according to current EU regulations 644/2017 and 771/2017.

Since the regulatory changes in Europe in 2014, sensitivity has often been the primary focus when demonstrating GC-MS/MS performance for confident, and regulatory compliant, confirmation of the presence of dioxins and dioxin-like compounds. Sensitivity is an important performance criterion, but other factors have to be taken into account for the evaluation of instrument performance for routine work. For example, sample weight to solvent volume ratio, injection volume and stability of the ion ratio (between the primary and secondary precursor/product selected reaction monitoring (SRM) ion transitions) at the defined limit of quantitation (LOQ) will influence whether a method (and system) is fit for purpose in a routine environment. Recent guidance from the European reference laboratory for Dioxins and PCBs in Feed and Food[1], suggest a difference in determination of the LOQ from the standard, widely accepted GC-HRMS method which is not suitable for GC-MS/MS.

In this study, the performance of a triple quadrupole GC-MS/MS system equipped with the new Advanced Electron Ionization (AEI) source was evaluated for the analysis of PCDD/Fs in solvent standards and real food/feedstuff samples. Suitably set LOQs were employed to show compliance to one-fifth maximum levels and demonstrate the sensitivity, selectivity, and robustness required to satisfy both guidance and regulation.

Materials and Methods: Animal feedstuff samples, relevant quality control samples, and procedural blanks were provided by the University of Liege. European Method EN:1948 standard solutions (Wellington Laboratories Inc., Canada) were utilized for initial performance tests, and for calibration and quantitation. Extraction (where required) was performed by the accelerated solvent extraction technique, with cleanup using an offline multi-layered acidic silica column step and the PowerPrep™ automated system (FMS Inc.) with a three-column set (multi-layered ABN silica, basic alumina, and carbon columns). The final extract volume and solvent was 10 µL of nonane. A TSQ 9000 triple quadrupole mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph. Injection of liquid samples was performed automatically using a Thermo Scientific™ TriPlus™ RSH autosampler. Chromatographic separation was performed using a TG-Dioxin (60 m × 0.25 mm, 0.25 µm) capillary GC column.

Acquisition, processing, and reporting of the data were performed using Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software.

Results: Chromatographic resolution was compliant with current EU regulations (figure 1) with excellent separation for the TCDD/F and PeCCD/F congeners in sample (figures 2 and 3 respectively).

Figure 1. Separation between 123478 and 123678 HxCDF congeners. 25% peak height of the Quantifier SRM transition is marked in green, with $_{12}C^{13}$ labelled congeners displayed below, EN:1948 CSL standard is shown (160fg on column).

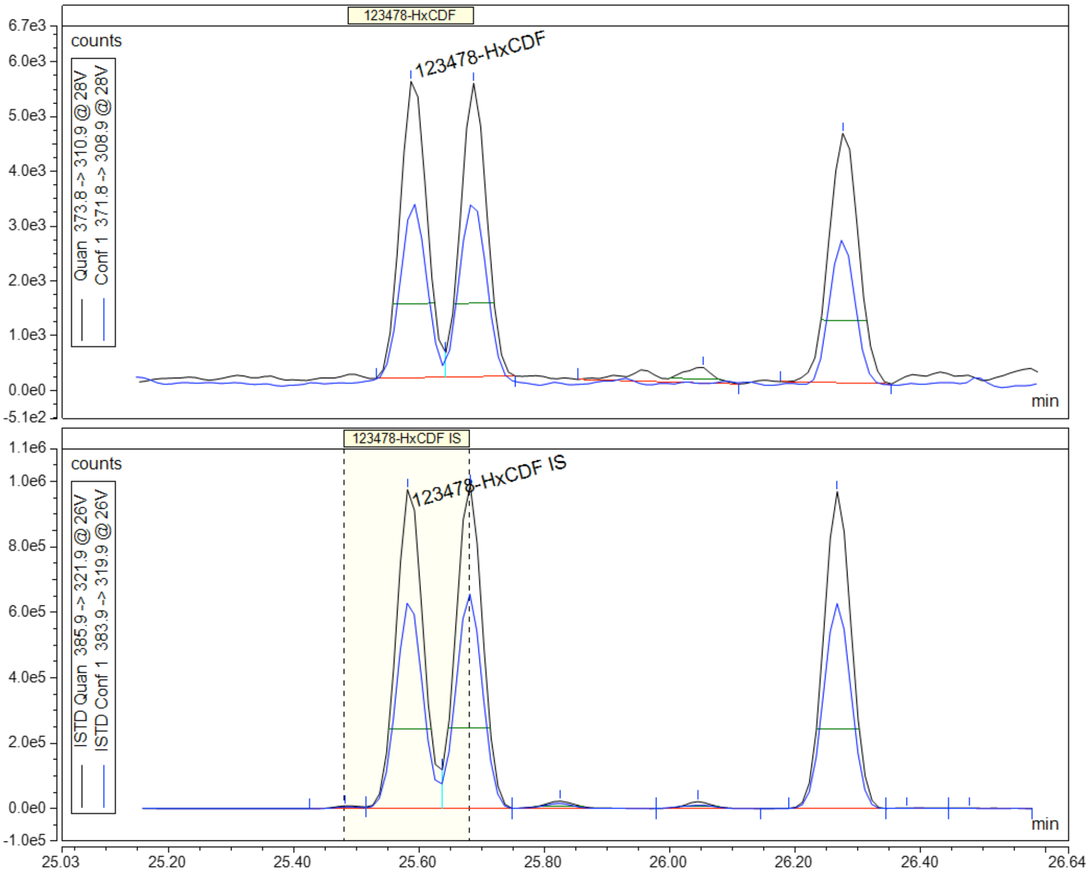


Figure 2. Tetra furan and dioxin congener separation on the TG-Dioxin type column with $_{12}C^{13}$ labelled congeners displayed below. Sample type shown is a grass feed QC.

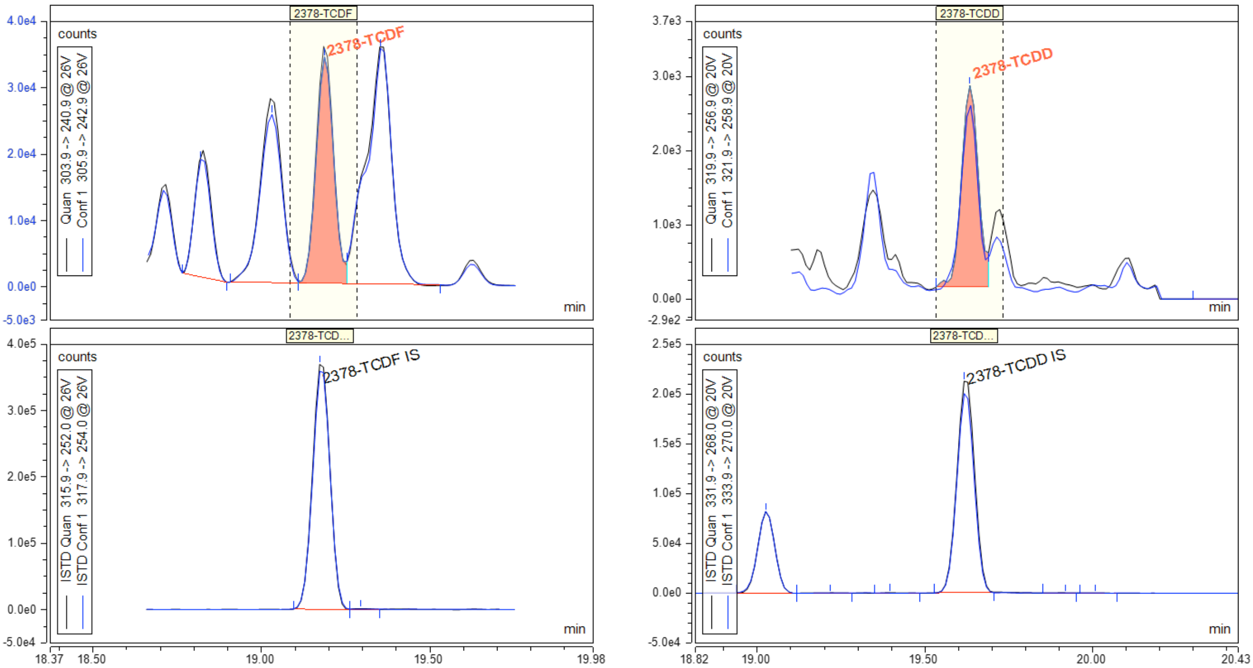
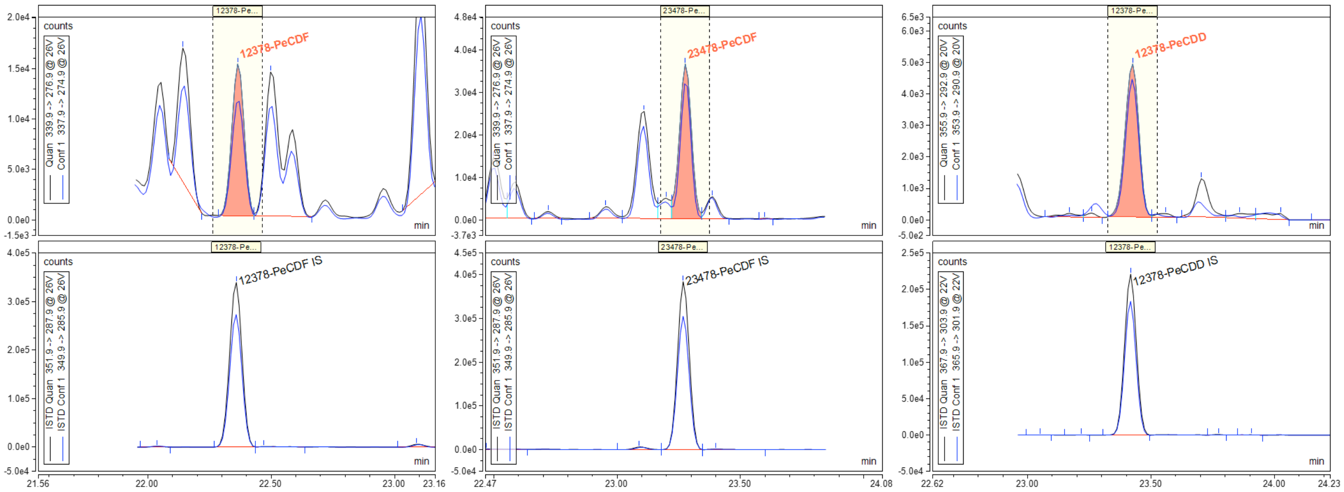
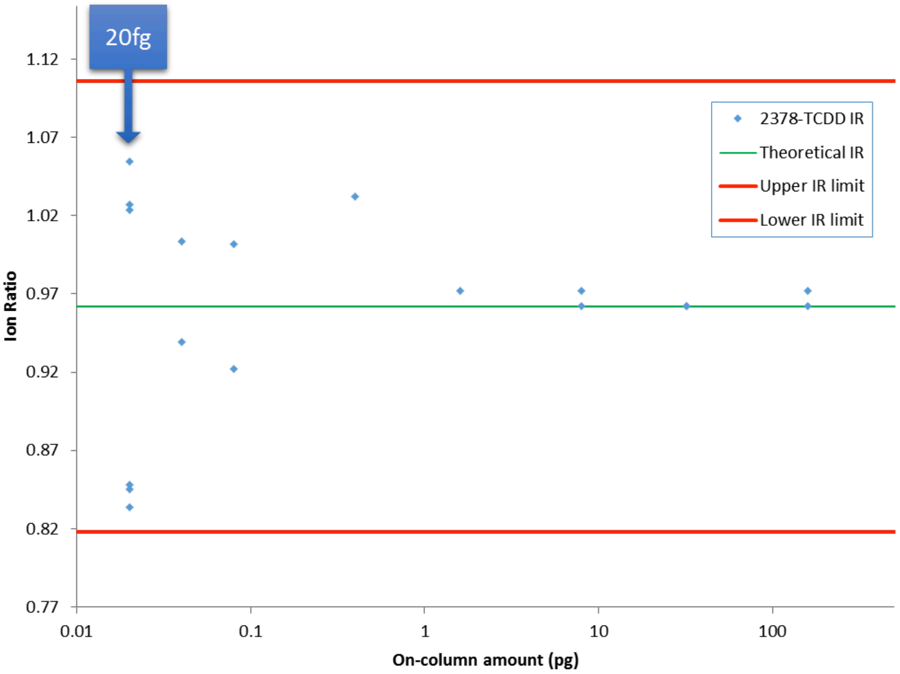


Figure 3. Penta furan and dioxin congener separation on the TG-Dioxin type column with $_{12}C^{13}$ labelled congeners displayed below. Sample type shown is a grass feed QC.



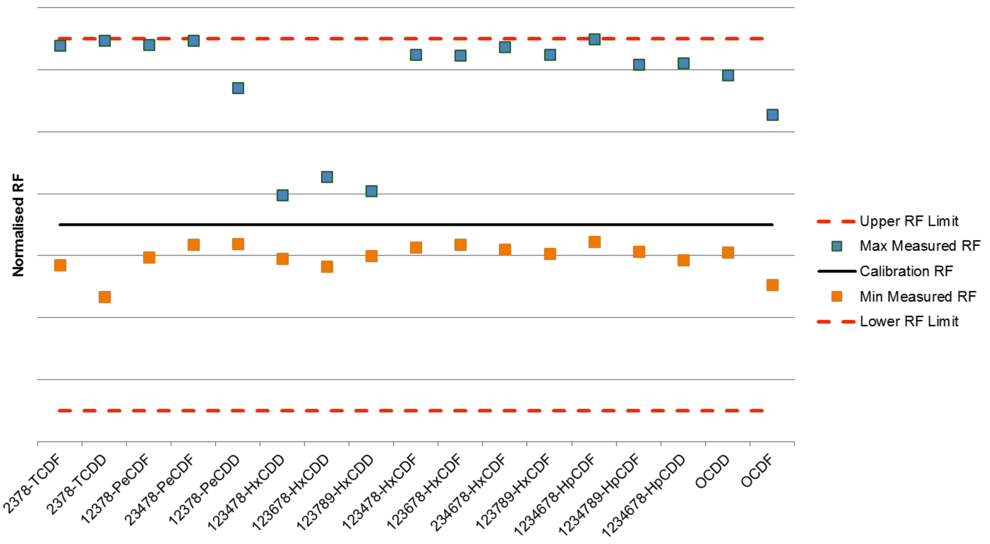
Standards at suitable concentrations demonstrating the defined LOQ were analysed at regular intervals throughout the sequence, displaying ion ratios consistent within the ±15% tolerance required by regulation (figure 4).

Figure 4. Ion ratios shown over the full calibration range (0.01 – 80pg/µL) for the two native 2,3,7,8-TCDD transitions.



Relative response factors (RRFs) also demonstrated acceptable (≤30%) deviation from the average RRF throughout the analytical sequence (figure 5).

Figure 5. Minimum and maximum normalised response factors values measured for all native congeners in calibration standards throughout the analytical sequence.



Calculated upperbound SUM PCDD/Fs WHO TEQ (pg/g) were all significantly lower than the 1/5th ML for the samples analysed with results below the ML, and agreement between the upperbound and lowerbound concentrations for the samples analysed with results above the ML agreed well within the 20% difference allowed and with the GC-HRMS data provided (table 1).

Congener	Alfalfa TEQ pg/g	Premix TEQ pg/g	Premix TEQ pg/g	Pork fat TEQ pg/g	Sheep TEQ pg/g	Sheep GCHRMS TEQ (pg/g)
2378-TCDF	0.009	0.001	<LOQ	<LOQ	0.018	<LOQ
2378-TCDD	<LOQ	<LOQ	<LOQ	<LOQ	0.902	0.840
12378-PeCDF	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23478-PeCDF	0.011	<LOQ	<LOQ	<LOQ	0.770	0.793
12378-PeCDD	0.020	<LOQ	<LOQ	<LOQ	2.200	2.246
123478-HxCDF	0.003	<LOQ	0.002	<LOQ	0.167	0.146
123678-HxCDF	0.002	<LOQ	<LOQ	<LOQ	0.093	0.075
234678-HxCDF	0.003	0.002	0.002	<LOQ	0.088	0.072
123789-HxCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.021
123478-HxCDD	<LOQ	<LOQ	<LOQ	<LOQ	0.134	0.083
123678-HxCDD	0.003	<LOQ	<LOQ	<LOQ	0.456	0.339
123789-HxCDD	0.003	<LOQ	<LOQ	<LOQ	0.095	0.092
1234678-HpCDF	0.001	<LOQ	<LOQ	<LOQ	0.013	0.001
1234789-HpCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1234678-HpCDD	0.002	0.001	<LOQ	<LOQ	0.049	0.047
OCDD	0.000	0.000	<LOQ	0.000	0.001	0.001
OCDF	0.000	0.000	<LOQ	<LOQ	<LOQ	<LOQ
Upperbound SUM PCDD/Fs WHO TEQ (pg/g)	**0.064**	**0.059**	**0.054**	**0.129**	**4.999**	**4.815**
Lowerbound SUM PCDD/Fs WHO TEQ (pg/g)	**N/A**	**N/A**	**N/A**	**N/A**	**4.987**	**4.756**
Maximum Level (TEQ pg/g)	**0.750**	**1.000**	**1.000**	**1.000**	**2.500**	**2.500**
1/5th ML	**0.150**	**0.200**	**0.200**	**0.200**	**0.500**	**0.500**

Table 1. Summary table of results for PCDD/F WHO TEQ (pg/g) including upperbound, lowerbound, Maximum and 1/5th Maximum levels displayed.

The results shown here demonstrate that the GC-MS/MS system with advanced electron ionisation (AEI) source provides a real alternative from GC-HRMS for laboratories looking to control maximum levels. Demonstration of the LOQ throughout the sequence, as per EU guidance, enables simple calculation of upper, middle and lowerbound WHO TEQs ensuring compliance throughout.

References:

1. Wenzl T, Haedrich J, Schaechtele A, Robouch P, Stroka J (2016); *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food*; EUR 28099, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-61768-3; doi:10.2787/8931