Routine, regulatory analysis of dioxins and dioxin-like compounds in food and feed samples using a triple quadrupole GC-MS/MS 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 must be considered 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 triple quadrupole GC-MS/MS system equipped with an advanced electron ionization (AEI) source was evaluated. Data was acquired on two different TSQ 9000 AEI systems located in two different laboratories and operated by different chemists (UK and USA). Commercially available solvent standards, food/ feedstuff, and proficiency test (PT) samples were used to evaluate the performance of each system for the analysis of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-p-furans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs), and non-dioxin-like (indicator) PCBs. Guidance from the European Union Reference Laboratories (EURL) on the use of a calibration approach was followed to set suitable LOQs essentially, to demonstrate sufficient sensitivity to enable reporting at 1/5th of the maximum level (ML) upper bound sum toxic equivalences (TEQs). To demonstrate the robustness required to operate in a routine environment an experiment involving continuous analysis of extracts over a period of two weeks was also performed.

Materials and Methods:

Food and feedstuff samples (including PT samples) were provided by the EURL for Halogenated POPs in Feed and Food, Freiburg, Germany. A nominal sample intake weight of 2 grams (fat) was used for the samples unless indicated otherwise (Table 3). European method EN:1948 standard solutions; EN-1948CVS, WM48-CVS (calibration and quantitation), EN-1948ES, EN-1948IS, P48-W-ES, P48-M-ES, and P48-RS (extraction) were utilized for the extraction, calibration, and quantitation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs. All standards were obtained from Wellington Laboratories Inc., Canada.

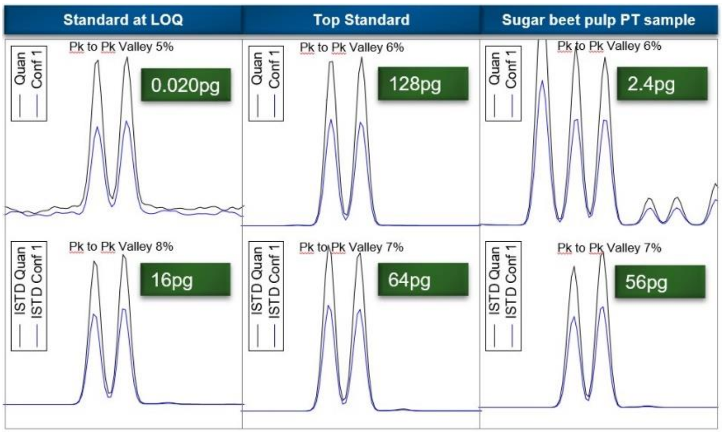
Extraction (where required) was performed by Twisselmann hot extraction (comparable with Soxhlet extraction) or pressurized liquid extraction. Automated clean-up of extracts was performed using a three column (multi-layered acidic silica, alumina, and carbon columns) setup on the DEXTech™ Plus system (LCTech GmbH). Two extract fractions were provided per sample, the first containing the non-ortho PCBs and PCDD/Fs (final volume 20 µL nonane) and the second containing the mono-ortho and di-ortho PCBs and indicator PCBs (final volume 100 µL nonane). Due to the absence of a nonortho syringe standard in the calibration and extraction solutions, recoveries were not calculated for the four ^{13}C-labeled non-ortho PCBs. As all the non-ortho PCBs were found in all samples at values greater than the LOQ this does not impact the validity of the results obtained. 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:

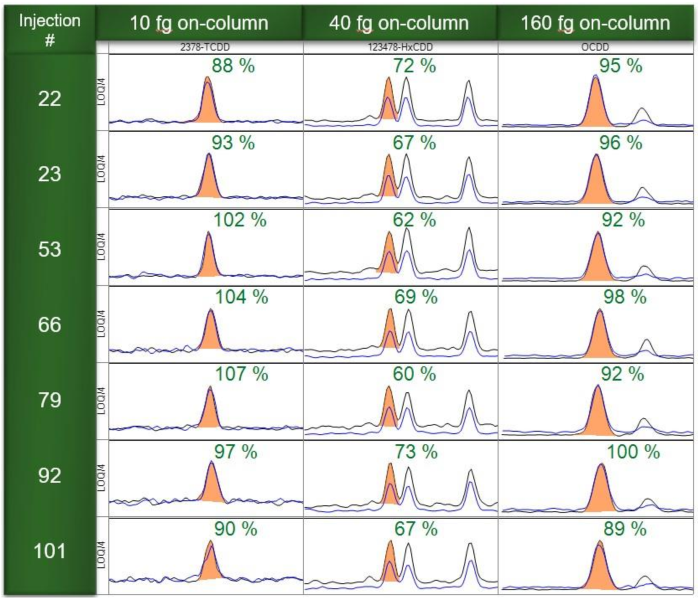
Chromatographic resolution was compliant with current EU regulations with excellent separation for the TCDD/F and PeCCD/F congeners in standards at the LOQ, highest calibration level and in a sugar beet pulp sample (figure 1).

Figure 1. Separation between 123478 and 123678 HxCDF congeners. 25% peak height of the Quantifier SRM transition in black, with 12 C^{13} labelled congeners displayed below, the amount on column injected is shown in green. The peak to peak valley % value is annotated above each critical pair.



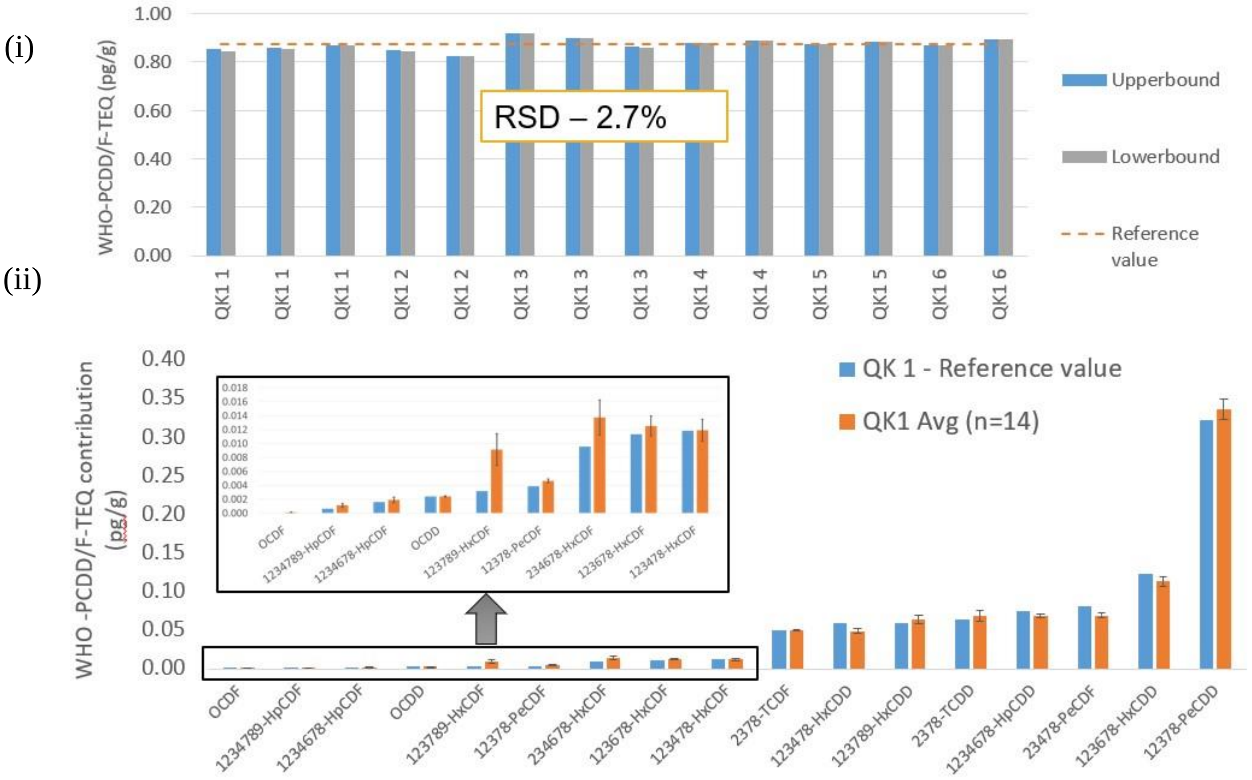
Standards at suitable concentrations demonstrating the defined LOQ were analysed at regular intervals throughout the sequence, displaying ion ratios consistent within the ±15% tolerance required by regulation (figure 2). The relative response factors (RRFs) for all compounds were also within ±30% of the average RRF determined from the calibration which is a further requirement of the regulations.

Figure 2. Ion ratios shown over for the two native 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD and OCDD transitions in the LOQ standard injected throughout the sample sequence. The injection number, amount injected on column, and % ion ratio have been annotated in the figure.



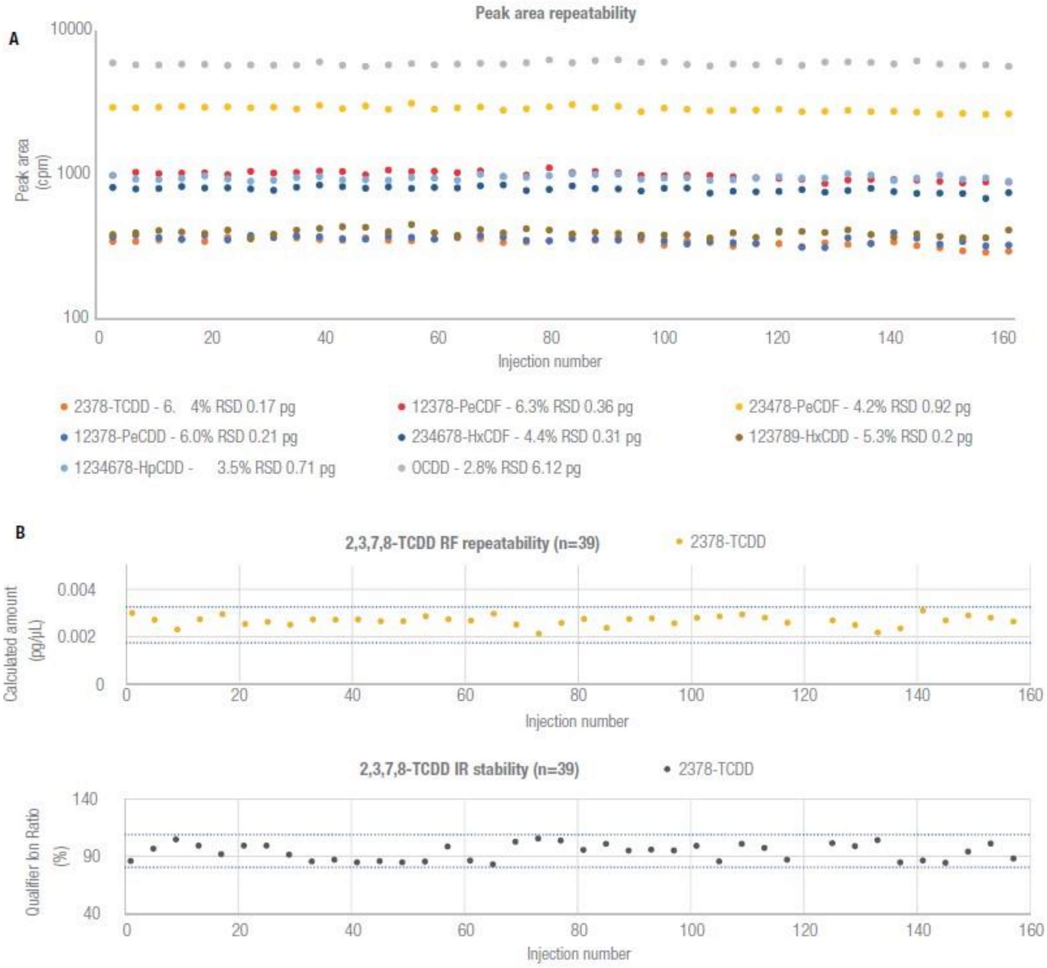
The measured WHO-PCDD/F-TEQ (pg/g) value for each congener was in excellent agreement with the reference value provided by the EURL (Figure 3 ii), with the upper-bound WHO-PCDD/F-TEQ (pg/g) not deviating by more than 6% from the reference value over all 14 measurements. Furthermore, the deviation between the upper-bound and lower-bound WHO-PCDD/F-TEQ (pg/g) for each measurement was consistently less than 1.2%, well below the maximum 20% deviation required for samples that exceed the ML as specified in EU regulation (figure 3 i).

Figure 3. (i) Summary table of results from replicate analysis of the quality control (QK) samples (six extractions in total) analysed a total of 14 times over two testing sites (UK and US) for PCDD/F WHO TEQ (pg/g) including upperbound, lowerbound, reference value (ii) The measured WHO-PCDD/F-TEQ (pg/g) value for each congener (14 replicates with standard deviation added) versus the reference value provided by the EURL.



To further assess the robustness of the analytical system, the remaining extracts from the non-ortho PCBs and PCDD/Fs samples were pooled together into mixed matrix extract. This pooled matrix sample was then analyzed alongside nonane blank and LOQ standard injections. The injection sequence was set up as follows: four injections (LOQ, blank, pooled matrix, blank) were followed by a four-hour hold at the initial oven temperature and repeated, resulting in a total of 161 injection sequence containing n = 40 matrix injections and n = 40 LOQ standards, run over ~2 weeks period. The system maintained its sensitivity throughout delivering excellent robustness, even considering the high matrix complexity and load on column (Figures 4 i and ii). No maintenance (such as source cleaning, liner replacement, tuning, or analytical column trimming) was performed during the sequence.

Figure 4. (i) Absolute peak area repeatability over two weeks of analysis, for selected PCDD/F congeners in pooled matrix sample. Relative standard deviations and amounts on-column (pg) are annotated for each selected congener, (ii) LOQ RF deviation (upper plot, calculated as deviation from target amount) and IR (lower plot) for the 10 fg on-column 2,3,7,8-TCDD congener (2.5 fg/µL, 4 µL injection).



Summary:

The results of this study demonstrate that the GC-MS/MS system configured with the advanced electron ionization source (AEI) satisfy the requirements of the EU commission for the analysis of Dioxins in food and feed. The GC-MS/MS system has been proven to be a viable alternative for routine analysis of dioxins and the work was performed across multiple systems with different users to demonstrate the ease of producing consistent results on the system. The long term robustness study clearly shows that the multiple batches can be run on the system and still produce ion ratios and responses within the regulatory requirements without the need for user intervention. The routine analysis of dioxins is also aided by the automatic calculations performed in a single software, making reporting of the results accessible to all users.

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:10.2787/893