EU Compliant Routine Quantitative Dioxin, Dioxin-like PCB and Marker PCB Analysis by GC-MS/MS Using an Advanced Electron Ionization Source

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ABSTRACT

In this study, the performance of a triple quadrupole GC-MS/MS system equipped with an advanced electron ionization (AEI) source was evaluated. Data was acquired on two different Thermo Scientific™ 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).

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.

MATERIALS AND METHODS

Sample Preparation

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 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).

Test Method(s)

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.

Data Analysis

Data were acquired using timed-SRM mode, processed and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2, which allows instrument control, quantitative/qualitative analysis, and customizable.

RESULTS

Chromatography

The proprietary phase of the TG-Dioxin capillary GC column (P/N: 26066-1540) provided excellent separation of PCDD/F and PCB congeners, particularly the tetra (figure 1) and penta-substituted PCDD/Fs. All chromatographic criteria stated in regulation were met using the TG-Dioxin column in this study.[1,2,3]

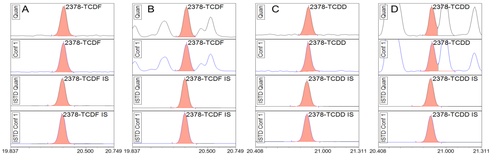

Figure 1. TeCDD/F congener separation in solvent standard and palm fatty acid distillate (PFAD) PT sample. Associated 13C-labelled congeners are displayed below. A - 20fg on-column 2,3,7,8-TCDF; solvent standard, B - ~180fg on column 2,3,7,8-TCDF; PFAD sample, C - 20fg on-column 2,3,7,8-TCDD; solvent standard and, D - ~55fg on column 2,3,7,8-TCDD; PFAD sample. All quantification and confirmation ions are labelled.

Determination of Limits of Quantitation (LOQs)

As previously described (AN-10590), calculation of LOQs based on signal to noise ratios obtained using GC-MS/MS systems is unreliable, hence, it is more appropriate to use a calibration-based approach.[3, 4] Employing calibration standards at the LOQ, and subsequent check standards at this level, allows the user to demonstrate continual method performance throughout the analytical sequence (figures 2,3 & 4). It also allows for a simple calculation to determine the LOQ which will be achieved for PCDD/Fs using a fixed sample weight:

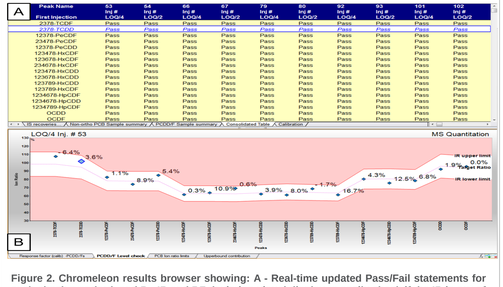

Figure 2. Chromeleon results browser showing: A - Real-time updated Pass/Fail statements for each check standard, and B - IR and RF deviation visual display to easily check if the IR is out of the allowable range (±15%) and if the congener has an RF within acceptable deviation (<30%)

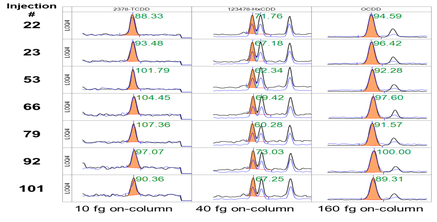

Figure 3. LOQ repeatability during the UK based PCDD/F & non-ortho PCB sequence. Overlaid extracted ion chromatograms (XICs) are displayed (quantification and confirmation ions) for selected TCDD, HxCDD and OCDD congeners, all IRs (as displayed in green) and RFs were within the allowable tolerances (IR ±15% from theoretical or average value; RF <30% deviation from average value) as defined by EURL guidance4 throughout the sequence.

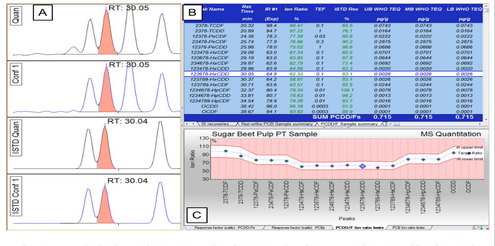

Figure 4. Typical Chromeleon processing browser showing A - native quantification and confirmation peak with associated 13C-labelled quantification and confirmation peaks, B - interactive sample results browser showing upper, middle and lowerbound, WHO-PCDD/F-TEQ values, flagged IRs and 13C-labelled congener recovery, C – IRs and LOQs visual display to easily check if the IR is outside the allowable range and if the peak amount is below the LOQ. Similar displays are available for PCBs. Sugar beet pulp PT sample shown; WHO-PCDD/F-TEQ 0.715 pg/g.

Quantification and confirmation of PCDD/Fs, dioxin-like PCBs and indicator PCBs in food and feed samples

A total 29 different samples were analysed (39 separate sample extractions, with two fractions for each over two sites, on two separate TSQ 9000 AEI GC-MS/MS systems for non-ortho PCBs & PCDD/Fs and di-, mono-ortho PCBs & indicator PCBs. To demonstrate the efficacy of the TSQ 9000 AEI GC-MS/MS systems, 6 replicate extractions of a mixed fat quality control sample (QK1 – reference value: 0.87 pg sum WHO-PCDD/F-TEQ) were prepared, these were split between the sites and analysed at regular intervals throughout the analytical sequences (14 injections in total over the two non-ortho PCBs & PCDD/Fs sequences). An example of the chromatography achieved for a selection of congeners in the non-ortho PCBs & PCDD/Fs fraction is shown in Figure 5.

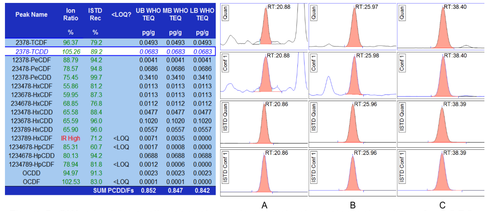

Figure 5. QK1 mixed fat quality control sample example chromatography where, A - 2,3,7,8-TCDD [0.03 pg on-column], B - 1,2,3,7,8-PeCDD [0.14 pg on-column] and C - OCDD [3.1 pg on-column]. The Chromeleon interactive results pane (left) displays IRs and internal standard recoveries, as well as real-time updated WHO-PCDD/F-TEQ (pg/g) values.

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 6), with the upperbound 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 upperbound and lowerbound WHO-PCDD/F-TEQ (pg/g) for each measurement was consistently less than 1.2%, well below the maximum 20% deviation required for samples which exceed the ML as specified in EU regulation (Figure 6). [5]

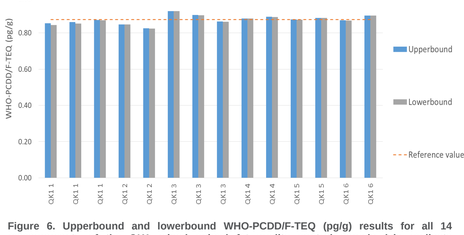

Figure 6. Upperbound and lowerbound WHO-PCDD/F-TEQ (pg/g) results for all 14 measurements of the QK1 mixed animal fat quality control sample (six replicate extractions).

To provide further validation data, an additional certified reference material (CRM) was extracted and analysed on a PTV TSQ 9000 AEI system in Beijing, China. One gram of CRM WMF-01 (Wellington Laboratories Inc., Canada) was extracted and analysed in triplicate (modified oven ramp, 5µL PTV injection). The results obtained were in excellent agreement with the reference values published, with all congeners within the specified tolerance (figure 7). The calculated SUM WHO-PCDD/F-TEQ (pg/g) for the measurements versus the calculated reference SUM WHO-PCDD/F-TEQ (pg/g) is also displayed in figure.

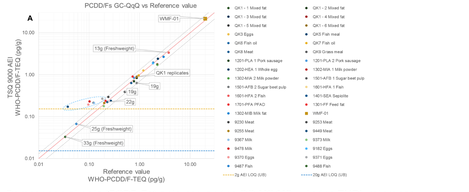

Figure 7. Comparison of data (PCDD/Fs) obtained on the GC-MS/MS with the EURL reference values. The centre red line represents 100% agreement with the value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2g, amount scales are logarithmic to aid comparison.

CONCLUSIONS

- Successful validation of method performance criteria (LOQ, precision, accuracy and calibration) was carried out on two separate TSQ 9000 AEI systems, in two geo-locations.
- The sensitivity achieved with the TSQ 9000 AEI system allowed for Upperbound WHO-PCDD/F-TEQ (pg/g) values as low as 0.15 (for a 2 g sample intake weight), meeting the 1/5th maximum level requirements for all but the most challenging matrices.
- Chromeleon 7.2 CDS software provides an integrated platform, with the ability to automatically setup, easily acquire, process and report compliant data in a fully regulated environment, eliminating the need for using external spreadsheet

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ACKNOWLEDGEMENTS

Thermo Fisher Scientific would like to acknowledge Wellington Laboratories Inc., Canada for the production and supply of the LOQ standards used in this validation.

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PO65504-EN0519S

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