Performance evaluation of GC-MS/MS for Dioxin analysis with amendments to EU Regulations 644/2017 and 771/2017 for food and feed

Thermo Fisher SCIENTIFIC

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Overview

- Demonstration of compliance of GC-MS/MS with the newly proposed regulatory limits for PCDD/F in food and feed samples
- Demonstrating good sensitivity using a mixed standard containing six different of Tetrachlorodibenzodioxin (TCDD) was used at concentrations ranging between 2 to 100 fg•µL-1.
- Confirming results of previously analyzed food samples from by the European Union Reference Laboratories (EURL) in Germany and Wageningen Food Safety Research in the Netherlands showing consistent results with magnetic sector analysis.
- Demonstration full compliance with EU Regulations 644/2017 and 771/2017 for food and feed.

Introduction

Polychlorinated dibenzo-p-dioxins/furans (PCDD/F), or dioxins, are persistent organic pollutants (POPs) that can accumulate within food chains due to their chemical stability and high fat solubility, posing health risks to humans through food consumption[1]. Current regulations monitor food and feed for dioxins, with maximal allowable levels for PDDC/Fs in feedstuffs at the pg•g-1 concentration range[2]. However, a recent review process has proposed a reduction of up to 40% in these levels by 2024 for certain feed items, such as animal/milk/egg fats (1.0 pg•g-1), fish oil (3.5 pg•g-1), and fish feed (1.0 pg•g-1)[3].

Since 2014, the European Commission has permitted the use of gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) for food and feed analysis as an alternative to GC-high resolution mass spectrometry (HRMS)[4,5]. With the proposed reduction in maximum limits, the performance of current instrumentation to achieve these targeted limits must be assessed.

Laboratories face challenges in maintaining consistent sensitivity to detect trace levels of dioxins in complex food matrices and in reporting various regulatory requirements for PCDD/F analysis[6,7]. This study used the Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system with the NeverVent™ advanced electron ionization (AEI) source to demonstrate compliance with the proposed regulatory limits for PCDD/F in food and feed samples. The aim was to ensure consistency with previously determined concentrations of PCDD/F in food and feed matrices and meet current compliance with the EU regulations.

Materials and methods

Food and feed samples were provided by the European Union Reference Laboratories (EURL) (Germany) and Wageningen Food Safety Research (Netherlands). Samples consisted of a range of food materials including various animal fats and oils, baby food and herbs. Sample preparation details can be found by scanning the QR code to view the detailed application note.

For the full application note scan here
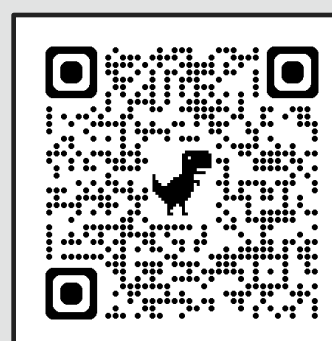
Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3. Integrated instrument control ensures full automated setup of the Dioxin analytical workflow through the eWorkflow which provides optimized instrument / quantification methods, view settings for time efficient data evaluation and customizable report templates. Isotopic dilution quantification is performed automatically in real time with the incorporated custom variable and formula functionality eliminating the need for exporting to external software packages.

Results

Sensitivity assessment

Before performing analysis of the food and feed samples, an assessment was made to ensure the instrument was performing at sufficient sensitivity for the analysis. To perform this assessment, a mixed standard containing six different congeners of Tetrachlorodibenzodioxin (TCDD) step standard was used. Each TCDD congener possessed a different chlorine substitution, enabling chromatographic separation (Figure 1). Each of these compounds are at a different concentration ranging from 2 to 100 fg•µL-1. At a 5 µL injection volume, the lowest level TCDD congener (1,3,6,8-TCDD) with an on-column concentration of 10 fg was easily detected. Furthermore, repeated injection (n = 10) of the step standard showed acceptable %RSD for all congeners, including the 1,3,6,8-TCDD with a %RSD < 11% at a level of 10 fg on column.
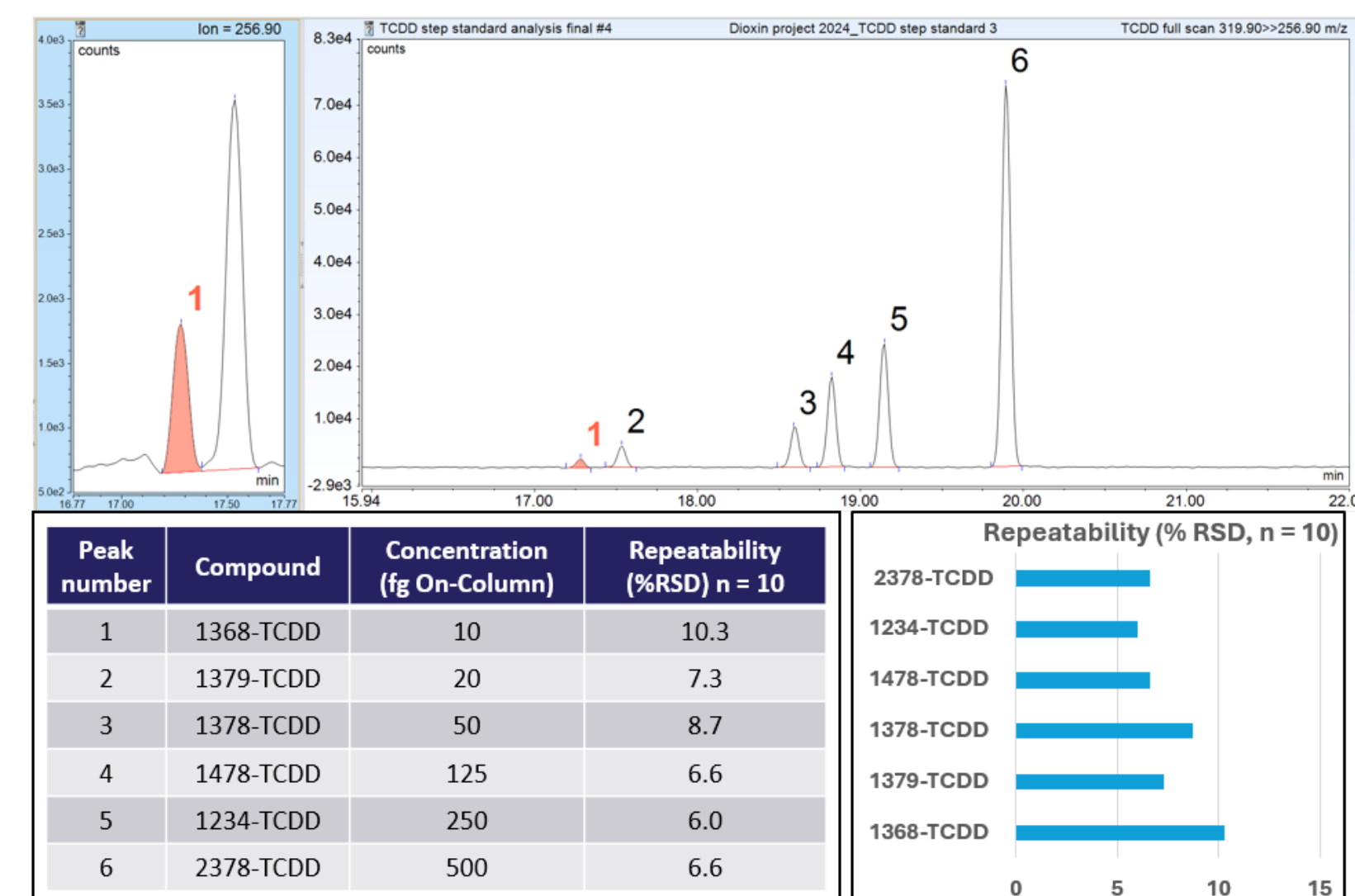

Peak number	Compound	Concentration (fg On-Column)	Repeatability (%RSD) n = 10
1	1368-TCDD	10	10.3
2	1379-TCDD	20	7.3
3	1378-TCDD	50	8.7
4	1478-TCDD	125	6.6
5	1234-TCDD	250	6.0
6	2378-TCDD	500	6.6

Figure 1. Assessment of the dioxin sensitivity using TCDD step standard ranging from 2 to 100 fg•µL-1 with a 5 µL injection and reproducibility over 10 injections.

Linearity and trace level quantitation

Calibration curves were prepared using the isotopically labelled EPA 1613 calibration mixtures from wellington standards (Canada). Each calibration standard was diluted by a factor of 10 in nonane to investigate the linear dynamic range for the tetra- (0.01 – 20 pg•µL-1), pent-, hexa-, hept- (0.05 – 100 pg•µL-1) and octa- (0.1 – 200 pg•µL-1) substituted PCDD/F congeners. The 17 target analytes showed a linear response over the investigated calibration ranges (Figure 2A,B) with Coefficient of determination (R2) > 0.990 and residual values (measured as % RSD of average response factors, AvCF %RSD) < 10% over 6 calibration points (Figure 2b).

The calibration curve was utilized to quantify the food and feed extracts provided by the EURL and Wageningen Food Safety Research. Figure 3 and 4 show the Toxic Equivalents or TEQ results obtained on the TSQ 9610 GC-MS/MS system compared to the values obtained at the respective institutes. The calculated results on the TSQ 9610 GC-MS/MS system are in line with the results obtained from both EURL and Wageningen Food Safety Research institute laboratories
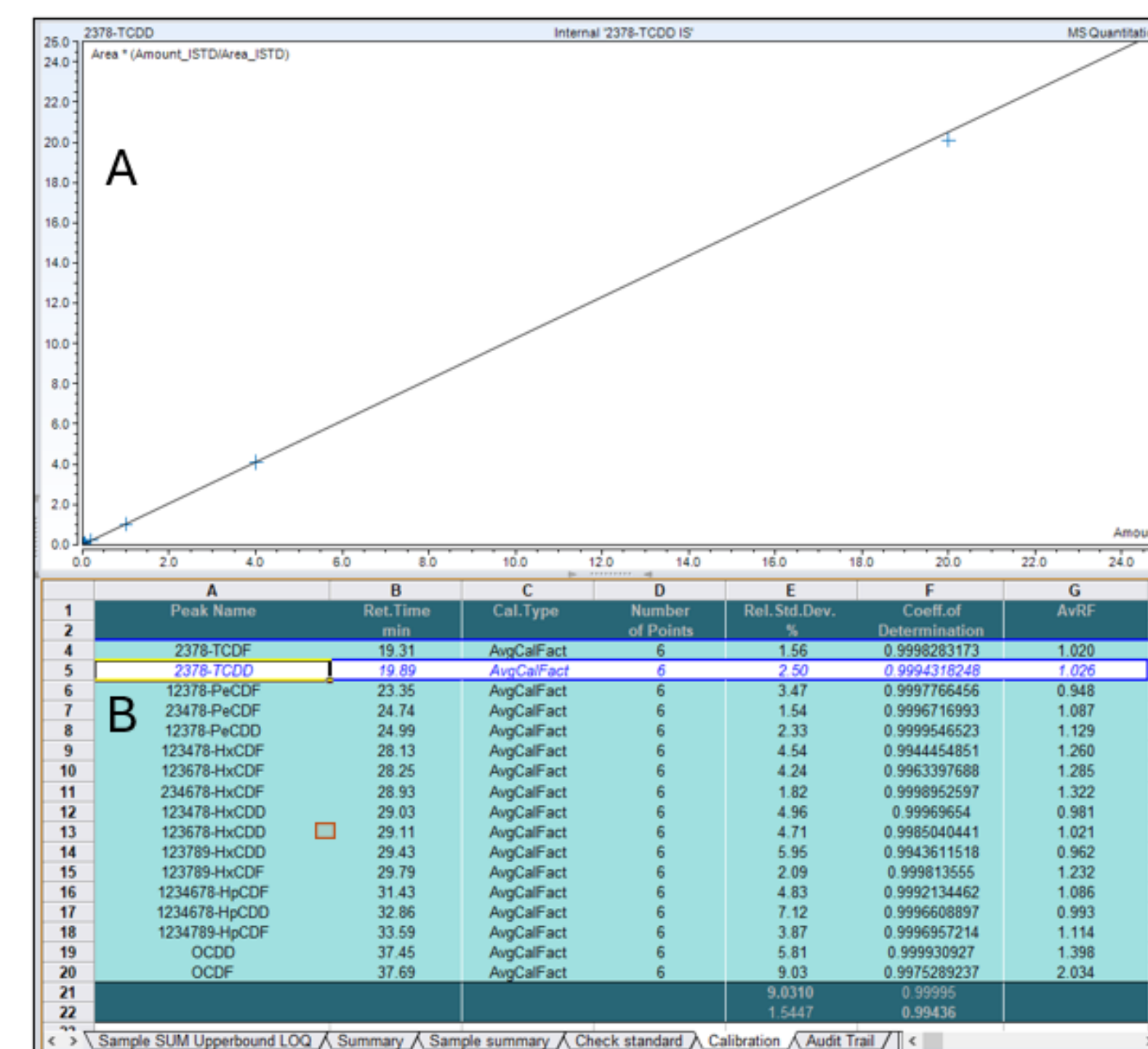

Figure 2. (A) Calibration curve for 2378-TCDD (0.01 – 20 pg•µL-1), (B) Calibration summary of % relative standard deviation, coefficient of determination and average response factor results for all PCDD/F analytes.
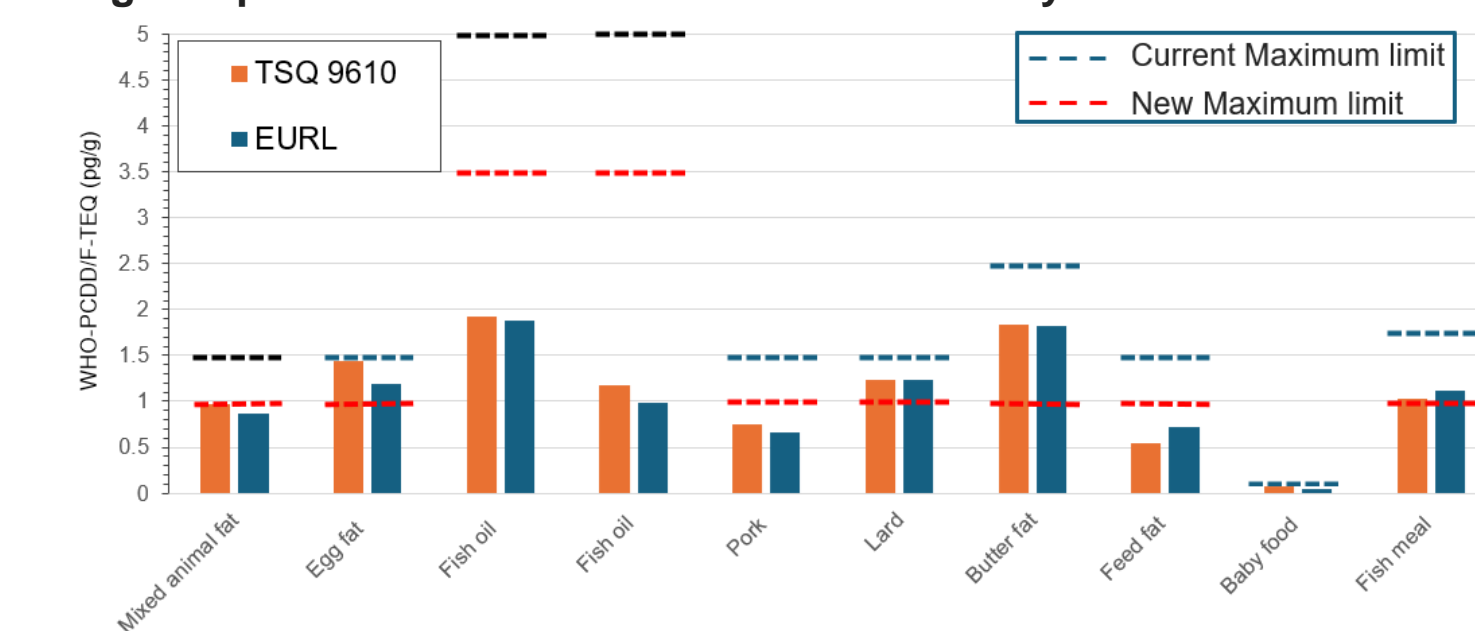

Figure 3. EURL sample results: Comparison of results obtained on the TSQ 9610 GC-MS/MS vs those obtained by the EURL. Values are TEQ (pg/g). Current and new maximum limit (by end of 2024) to food and feed items represented by black and red dashed lines, respectively.
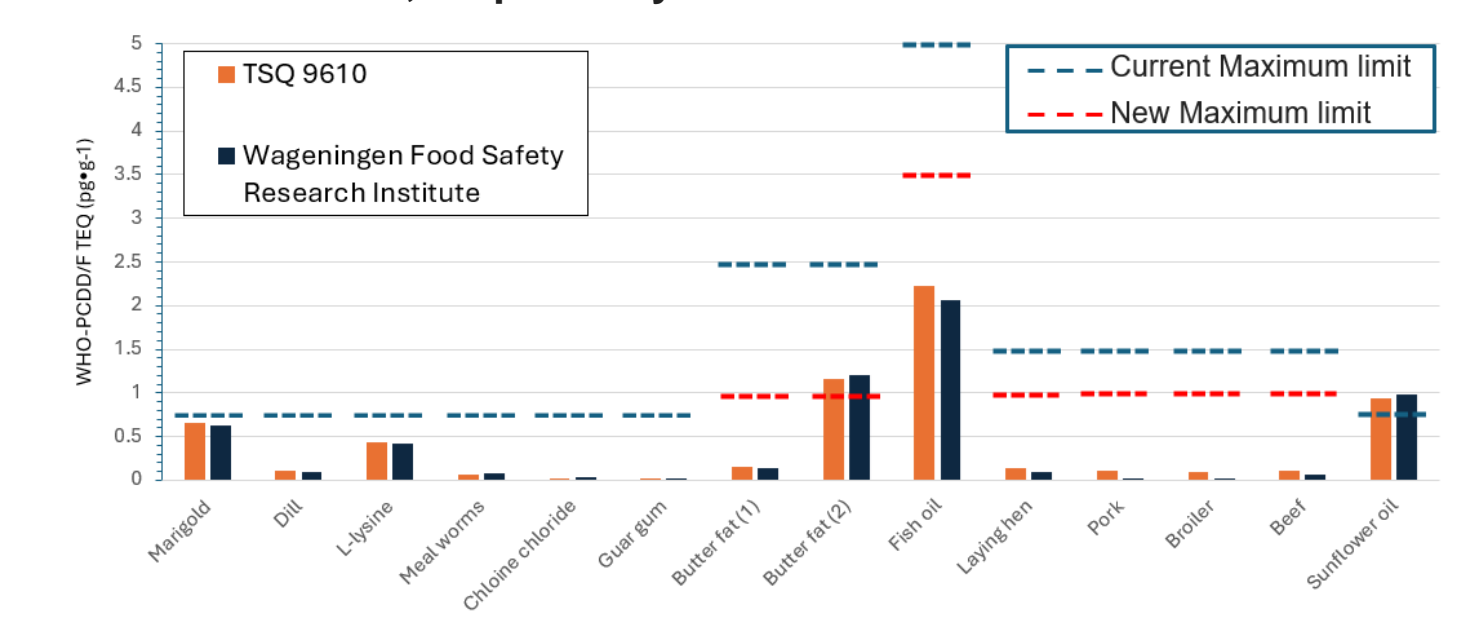

Figure 4. Wageningen Food Safety Research institute sample results: Comparison of results obtained on the TSQ 9610 GC-MS/MS vs those obtained by the Wageningen Food Safety Research institute. Values are TEQ (pg/g). Current and new maximum limit (by end of 2024) to food and feed items represented by black and red dashed lines, respectively.

Meeting the regulatory requirements

In accordance with EU Regulations 644/2017 and 771/2017 for food and feed analysis,the measured response factor across all standards must be within 30% of the AvCF over the course of an analysis. Within the Dioxin Analyzer eWorkflow, users have the option to assess analysis performance against regulated criteria through the interactive chart feature available in Chromeleon 7.3.2. This functionality allows for a visual evaluation of the measured response across all standards in relation to the upper and lower regulatory limits, which are defined as ± 30%. (Figure 5a). The diluted check standards were also plotted on the calibration curve and produced accurate quantitation values (Figure 5B). Threshold criteria for all performance check standards were set to +/- 30% of the true value within the Chromeleon software processing method with all check standards (LOQ/2, LOQ/4 and EURL external standard) within the threshold criteria.
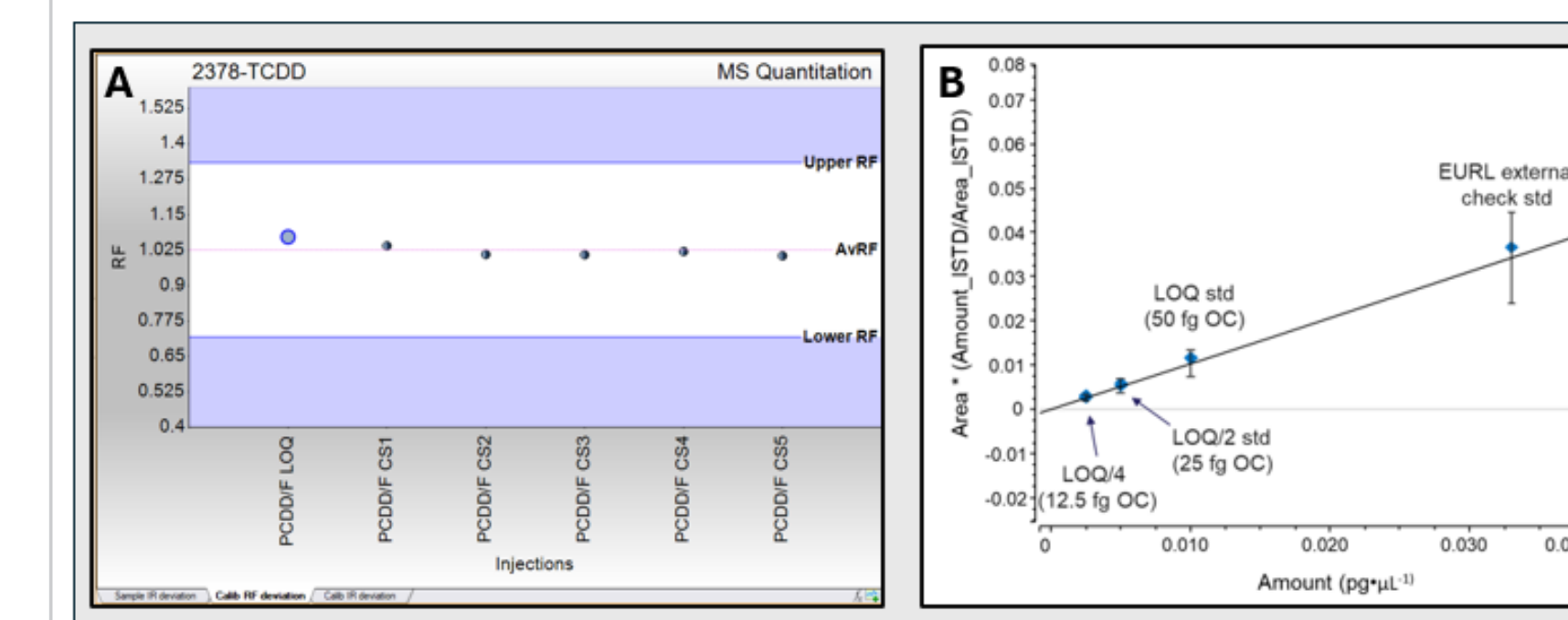

Figure 5. A: Interactive chart functionality within Chromeleon with visual display of the calibration response factor deviation in relation to regulated upper/lower reporting limits (± 30%) as part of the Dioxin eWorkflow. B: Response of performance check standards (LOQ/2, LOQ/4 and EURL external check standard) in relation to calibration curve for 2378-TCDD. Brackets surrounding each check standard represent the 30% tolerance threshold

System performance throughout the analysis sequence was evaluated by diluting the LOQ standard by a factor of 2 (LOQ/2) and 4 (LOQ/4). 2,3,7,8-TCDD represents the lowest level compound within the standard at a concentration of 5 fg•µL-1 (25 fg OC) and 2.5 fg•µL-1 (12.5 fg OC) for LOQ/2 and LOQ/4, respectively. In addition to these, an external standard provided by EURL was analyzed to assess accuracy of calibration. Optimal SRM transitions for detection was facilitated using the AutoSRM software function. Detection of 2378-TCDD in both LOQ/2 and LOQ/4 was easily achieved in both quantification and qualification SRMs, with the calculated ion ratio within 15% of the theoretical value as required in the regulations[4,5]. Figure 6 shows the extraction ion SRM transitions for 2378-TCDD for the LOQ/2 and LOQ/4 check standards together with the ion ratios determined for the 17 toxic congeners for dioxins and furans within those standards. This shows that the system produces consistent results at low concentration level within regulatory requirements.
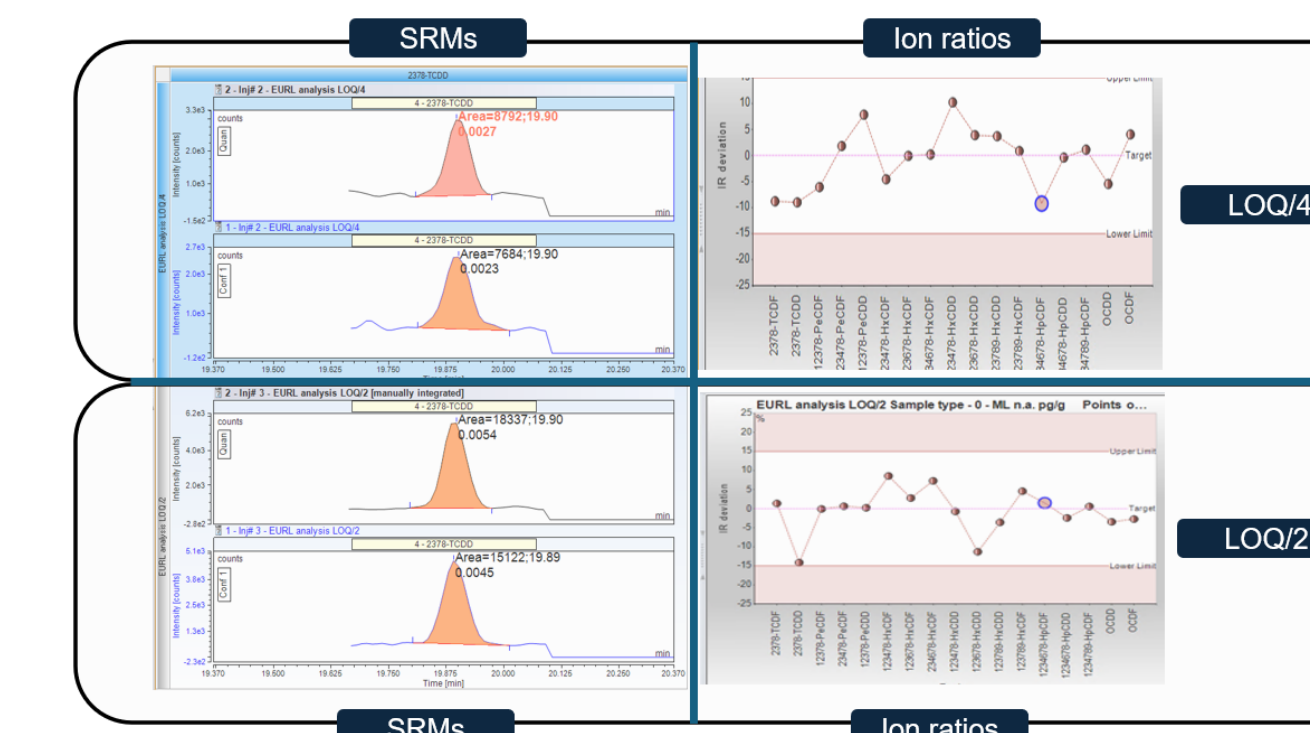

Figure 6. Extracted ion SRM transitions for the 2,3,7,8-TCDD in the LOQ/4 (2.5 fg•µL-1 (12.5 fg OC)) and LOQ/2 (5 fg•µL-1 (25 fg OC)) performance check standards and ion ratios for the 17 toxic congeners for dioxins and furans.

Conclusions

The results of these comprehensive experiments demonstrate that the TSQ 9610GC-MS/MS system, configured and controlled using Chromeleon CDS software together with the Dioxin eWorkflow, can deliver reliable regulatory compliant performance for the quantification and confirmation ofPCDD/Fs food and feedstuffs:

- Fentogram level sensitivity performance for ultra trace level determination of PCDD/F within food and feed samples
- Check standard performance was within quantitation and ion ratio thresholds for regulatory method compliance at femtogram levels.
- Accurate and precise quantitation at both current and newly adopted maximum limits for PCDD/F in food and feed.
- Chromeleon CDS software, version 7.3, 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 programs.
- Chromeleon eWorkflows, available from Thermo Scientific™ also provide error-free execution of each analysis to meet standard operating procedure (SOP) requirements, further simplifying the user

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6. Thermo Fisher Scientific, Application Note 10590: Low level quantification of PCDD/Fs in animal feed using the Thermo Scientific TSQ 9000 triple quadrupole GC-MS/MS system with AEI source. https://assets.thermofisher.com/TFS-Assets/CMD/Application- Notes/an-10590-gc-ms-ms-pcdd-fs-animal-feed-an10590-en.pdf

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