

## Food and beverage

## Trace analysis of polychlorinated dibenzo-p-dioxins/ dibenzofurans using GC-MS/MS in accordance with EU Regulations 2017/644 and 2017/771 for food and feed

### Authors

Nicholas Warner<sup>1</sup>, Adam Ladak<sup>2</sup>, Daniel Kutscher<sup>1</sup>, Alexander Schächtele<sup>3</sup>, and Kerstin Krätschmer<sup>4</sup>

<sup>1</sup>Thermo Fisher Scientific, Germany

<sup>2</sup>Thermo Fisher Scientific, UK

<sup>3</sup>European Union Reference Laboratory (EURL) for Halogenated POPs in Feed and Food, Freiburg, Germany

<sup>4</sup>Wageningen Food Safety Research, Part of Wageningen University & Research, Wageningen, The Netherlands

### Keywords

GC-MS/MS, timed-SRM, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo-p-furans, sensitivity, Regulation (EU) 2017/644, Regulation (EU) 2017/771, feed, food

### Goal

To demonstrate the suitability of the Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for the routine and regulatory compliance testing of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs) in food and feed samples in accordance with Commission Regulations (EU) 2017/644 and 2017/771.

### Introduction

Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/F), or dioxins as they are commonly referred to, are classified as persistent organic pollutants (POPs) under the Stockholm Convention. Due to their chemical stability and high fat solubility, these chemicals can accumulate within food chains and pose exposure/health risks to humans through consumption of food items (i.e., dairy, meat, and fish). Regulations are in place to monitor food and feed for the presence of dioxins to protect the population.<sup>1</sup>

Current maximum levels allowable for PCDD/Fs in food and feedstuffs are at the  $\text{pg}\cdot\text{g}^{-1}$  concentration range due to the toxicity risk they pose.<sup>2,3</sup> However, the European Commission has announced plans to lower the current maximum levels in certain feedstuffs in 2024 based on an updated risk assessment.<sup>4</sup> As of 2014, a change of EU regulations permitted the use of gas chromatography-triple quadrupole mass

spectrometry (GC-MS/MS) for the analysis of food and feed as an alternative to GC-high-resolution mass spectrometry (GC-HRMS).<sup>5,6</sup> With the proposed lowering of some maximum levels, performance of current instrumentation to achieve these targeted limits must be assessed.

Achieving these targeted limits places further stress on laboratories in maintaining consistent sensitivity to detect the trace levels of dioxins in difficult food matrices. Another challenge for analysts is the time-consuming data assessment based on regulatory requirements for PCDD/F analysis. Often the necessary tools for PCDD/F quantification (i.e., isotopic dilution quantification) and reporting of regulatory requirements (ion ratio, calibration, and response factor deviation)<sup>7,8</sup> do not exist within a single software. This requires analysts to export to external packages (i.e., Microsoft<sup>™</sup> Excel<sup>™</sup>) to perform critical calculations leading to transcription errors and added time for reporting. In addition, laboratories must ensure they produce consistent data throughout the analytical run to avoid reanalysis or re-extraction when sample/extract material is limited.

In this analysis, the performance of the TSQ 9610 triple quadrupole GC-MS/MS system equipped with the Thermo Scientific<sup>™</sup> NeverVent<sup>™</sup> advanced electron ionization (AEI) source was used to demonstrate compliance for regulatory limits for PCDD/F in food and feed samples in accordance with EU Regulations. Food and feed samples were provided by the European Union Reference Laboratory for halogenated Persistent Organic Pollutants in feed and food (EURL POPs) (Germany) and Wageningen Food Safety Research, in their capacity as National Reference Laboratory for POPs (NRL POPs, The Netherlands). These samples contained known trace levels of dioxins previously confirmed at the respective institutes or in international proficiency tests. The aim of this analysis was to ensure results were consistent with the previously determined concentrations in food and feed matrices and meet current compliance with the EU regulations.

## Experimental

### Sample preparation

Samples analyzed consisted of a range of food materials including various animal fats and oils, baby food, and herbs. A nominal sample intake weight of approximately 2 grams (lipid weight) was used for the samples provided the EURL. Similar sample intake was used for feed fat samples from Wageningen Food Safety Research. For samples with low fat content, a sample intake weight of approximately 10 grams was used.

At the EURL, sample extraction was performed with a Twisselmann hot extractor after drying the samples using respective extraction solvent mixtures. At Wageningen Food Safety Research, sample extraction was performed by

accelerated solvent extraction (Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> ASE<sup>™</sup> 350 Accelerated Solvent Extractor) for feed material after mixing with diatomaceous earth. Feed fat sample extracts were treated with concentrated sulfuric acid for one hour for lipid degradation. The organic supernatant was back extracted into hexane using liquid-liquid extraction. Automated clean-up of all extracts was performed using a three column (multi-layered acidic silica, alumina, and carbon columns) setup on the DEXTech Plus system (LCTech GmbH). Only the first fraction containing non-ortho-PCBs and PCDD/Fs was provided for each sample, with a final volume of 30  $\mu$ L nonane split in three vials. All samples were spiked with <sup>13</sup>C isotopically labeled standard prior to extraction to perform isotopic dilution quantification. A 30  $\mu$ L mixture of <sup>13</sup>C-1,2,3,4 tetrachlorodibenzo-p-dioxin (TCDD) and <sup>13</sup>C-1,2,3,7,8,9 hexachlorodibenzo-p-dioxin (HxCDD)\* at 2  $\mu$ g· $\mu$ L<sup>-1</sup> was spiked into each sample after clean-up was complete and shipped to Thermo Fishers Scientific's facility for mass spectrometry in Bremen, Germany. In addition to samples, external standards were provided by each laboratory for method performance evaluation. All standards were obtained from Wellington Laboratories Inc., Canada.

\* This is the standard mixture of Method EN-1948. During routine analysis, all 17 2,3,7,8-substituted congeners would be used as internal standards.

### GC-MS parameters

In the experiments described here, a TSQ 9610 triple quadrupole mass spectrometer equipped with the NeverVent AEI ion source was coupled to a Thermo Scientific<sup>™</sup> TRACE<sup>™</sup> 1610 gas chromatograph equipped with a Thermo Scientific<sup>™</sup> iConnect<sup>™</sup> Programmable Temperature vaporization (iConnect-PTV) injector and a Thermo Scientific<sup>™</sup> TriPlus<sup>™</sup> RSH SMART autosampler. The TriPlus RSH SMART autosampler was equipped with the large volume wash station (P/N 1R77010-1030) to perform multiple syringe cleaning cycles. Prior to sample aspiration, a pre-cleaning with nonane (5  $\times$  8  $\mu$ L) was performed followed by toluene (10  $\times$  8  $\mu$ L) after sample injection was complete. Chromatograph separation was carried out using a Thermo Scientific<sup>™</sup> TRACE<sup>™</sup> TG-Dioxin capillary column (60 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) (P/N 26AF154P). The remaining injection, oven, and MS parameters can be found in Table 1.

### Instrument control and data processing

Data was acquired, processed, and reported using Chromeleon CDS, version 7.3. Integrated instrument control ensures full automated setup of the Dioxin analytical workflow through the eWorkflow<sup>™</sup> panel (Figure 1). Within seconds, users have full access to optimized instrument / quantification methods, view settings for time efficient data evaluation, customizable report templates, and storage in compliance with the Federal Drug Administration Title 21 Code of Federal Regulations Part 11 (Title 21 CFR Part 11). Isotopic dilution quantification is performed

Table 1. TRACE 1610 GC system and TSQ 9610 triple quadrupole GC-MS parameters

Injection		Oven temperature program	
Injector	iConnect PTV	Initial temperature (°C)	140
Liner	PTV liner with concentric baffle (P/N 453T2845-UI)	Hold time (min)	2
Injection mode	Large volume	Rate 1 (°C·min <sup>-1</sup> )	25
Injection volume (µL)	5	Temperature 1 (°C)	250
Split flow (mL·min <sup>-1</sup> )	100	Rate 2 (°C·min <sup>-1</sup> )	2.5
Initial injector temperature (°C)	75	Temperature 2 (°C)	260
Injection time (min)	0.4	Hold time (min)	5
Transfer rate (°C·s <sup>-1</sup> )	2.5	Rate 3 (°C·min <sup>-1</sup> )	2.5
Final temperature (°C)	300	Temperature 3 (°C)	285
Cleaning phase rate (°C·s <sup>-1</sup> )	14.5	Rate 4 (°C·min <sup>-1</sup> )	10
Cleaning phase temperature (°C)	330	Temperature 4 (°C)	320
Cleaning phase hold time (min) / flow rate (mL·min <sup>-1</sup> )	5 / 200	Hold time (min)	15
Carrier gas flow rate (mL·min <sup>-1</sup> )	1.2		

**TSQ 9610 triple quadrupole GC-MS parameters**

Transfer line temperature (°C)	300
Advanced Electron Ionization (AEI) ion source temperature (°C)	350
Electron energy (eV)	50
Acquisition mode	Timed-SRM with dwell time prioritization 10 x (native (High), labeled (Low))
Emission current (µA)	50
Collision gas and pressure (psi)	Argon, 70

**Dioxin Analyzer eWorkflow allows full method setup for PCBs and PCDD/Fs including:**

- Instrument methods for TriPlus RSH Smart and AI-AS 1610 autosamplers
- Isotopic dilution quantification methods
- Reporting layouts/templates
- Supporting compliance documentation

Name	Date Modified	Type
eur 28099_en_loq guidance document.pdf	3/28/2019 3:50:19 PM +01:00	Attachment
PCB review	5/29/2019 12:54:44 PM -05:00	View Settings
PCBs	3/29/2019 2:32:45 PM +00:00	Report Template
PCBs	3/29/2019 2:50:50 PM +00:00	Processing Method
PCBs - PTV(bk) - AI-AS1610 - 1µL	5/31/2024 4:17:24 PM +02:00	Instrument Method
PCBs - PTV(bk) - TriplusRSHSmart - 1µL	5/31/2024 4:20:13 PM +02:00	Instrument Method
PCBs - PTV(fr) - AI-AS1610 - 1µL	5/31/2024 4:17:40 PM +02:00	Instrument Method
PCBs - PTV(fr) - TriplusRSHSmart - 1µL	5/31/2024 4:20:01 PM +02:00	Instrument Method
PCBs - SSL(bk) - AI-AS1610 - 1µL	5/31/2024 4:17:47 PM +02:00	Instrument Method
PCBs - SSL(bk) - TriplusRSHSmart - 1µL	6/3/2024 9:54:57 AM +02:00	Instrument Method
PCBs - SSL(fr) - AI-AS1610 - 1µL	5/31/2024 4:19:53 PM +02:00	Instrument Method
PCBs - SSL(fr) - TriplusRSHSmart - 1µL	6/3/2024 9:55:06 AM +02:00	Instrument Method
PCDDF	4/25/2019 11:45:45 PM -05:00	Report Template
PCDDF review	5/29/2019 12:51:55 PM -05:00	View Settings
PCDDFs	3/29/2019 3:07:36 PM +00:00	Processing Method
PCDDFs - PTV(bk) - AI-AS1610 - 1µL	5/31/2024 4:18:10 PM +02:00	Instrument Method
PCDDFs - PTV(bk) - TriplusRSHSmart - 1µL	5/31/2024 4:19:21 PM +02:00	Instrument Method
PCDDFs - PTV(fr) - AI-AS1610 - 1µL	5/31/2024 4:18:30 PM +02:00	Instrument Method
PCDDFs - PTV(fr) - TriplusRSHSmart - 1µL	5/31/2024 4:19:15 PM +02:00	Instrument Method
PCDDFs - SSL(bk) - AI-AS1610 - 1µL	5/31/2024 4:18:37 PM +02:00	Instrument Method
PCDDFs - SSL(bk) - TriplusRSHSmart - 1µL	5/31/2024 4:19:08 PM +02:00	Instrument Method
PCDDFs - SSL(fr) - AI-AS1610 - 1µL	5/31/2024 4:18:47 PM +02:00	Instrument Method
PCDDFs - SSL(fr) - TriplusRSH - 1µL	4/25/2019 7:22:55 PM -05:00	Instrument Method
TSQ ion ratio intensity calculator PCB.xlsx	3/4/2019 10:16:46 AM +01:00	Attachment
TSQ ion ratio intensity calculator PCDD.xlsx	3/5/2019 11:44:09 AM +01:00	Attachment
TSQ ion ratio intensity calculator PCDF.xlsx	3/1/2019 12:17:38 PM +01:00	Attachment
WHO-TEQ_Upperbound calculator - standards.xlsx	3/28/2019 3:42:37 PM +01:00	Attachment

Figure 1. The Dioxin Analyzer eWorkflow automated method setup within Chromeleon CDS 7.3.2

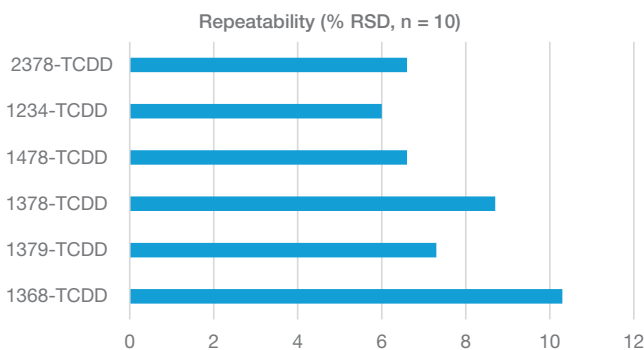
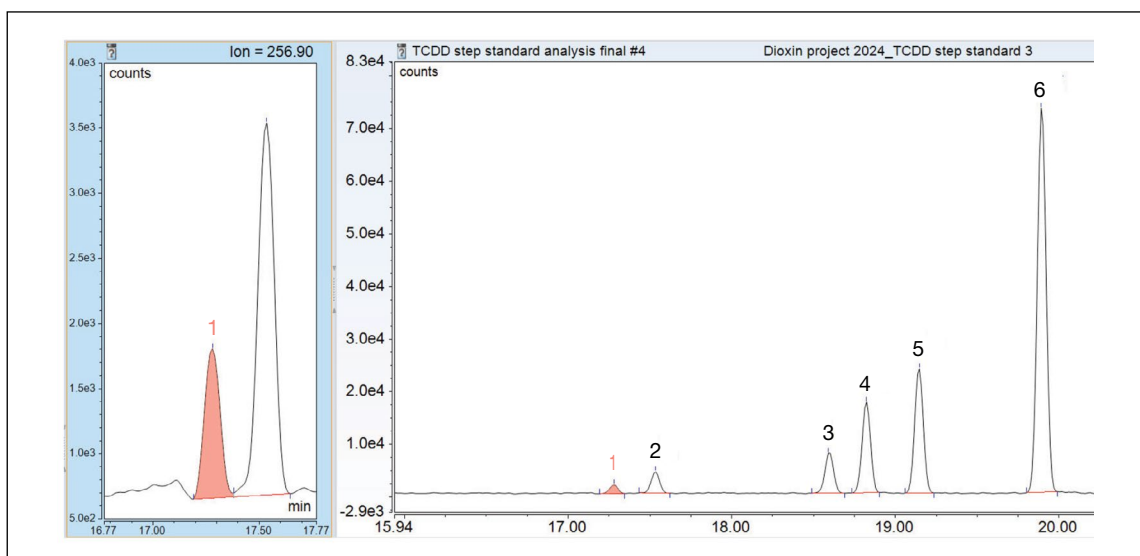
automatically in real time with the incorporated custom variable and formula functionality. This eliminates the need for exporting to external software packages for additional data processing. For new users not familiar with dioxin analysis, additional worksheets are provided for QA/QC, PCDD/F upper boundary calculation, and information surrounding regulatory guidance.

## Results and discussion

### 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) at different concentrations (step standard) was used. Each TCDD congener possessed a different chlorine substitution, enabling chromatographic separation (Figure 2). Each of these compounds are at a different concentration ranging from 2 to 100  $\text{fg}\cdot\mu\text{L}^{-1}$ . At a 5  $\mu\text{L}$  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 2. Assessment of the dioxin sensitivity using TCDD step standard ranging from 2 to 100  $\text{fg}\cdot\mu\text{L}^{-1}$  with a 5  $\mu\text{L}$  injection and repeatability over 10 injections

## Linearity and trace level quantitation

The TSQ 9610 NeverVent AEI ion source is equipped with the Thermo Scientific™ XLXR™ detector, which is an electron multiplier that offers extended detector lifetime and dynamic range. 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<sup>-1</sup>), pent-, hexa-, hept- (0.05–100 pg·μL<sup>-1</sup>) and octa- (0.1–200 pg·μL<sup>-1</sup>) substituted PCDD/F congeners. The 17 target analytes showed a linear response over the investigated calibration ranges (Figure 3A, B) with coefficient of determination ( $R^2$ ) > 0.990 and residual values (measured as % RSD of average

response factors, AvCF %RSD) < 10% over six calibration points (Figure 3B).

To ensure good performance for dioxin analysis, laboratories often follow criteria set out in EPA Method 1613B, which states the response factor across all standards must be within 20% of the AvCF over the course of the 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 CDS 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  $\pm 30\%$  (Figure 4).

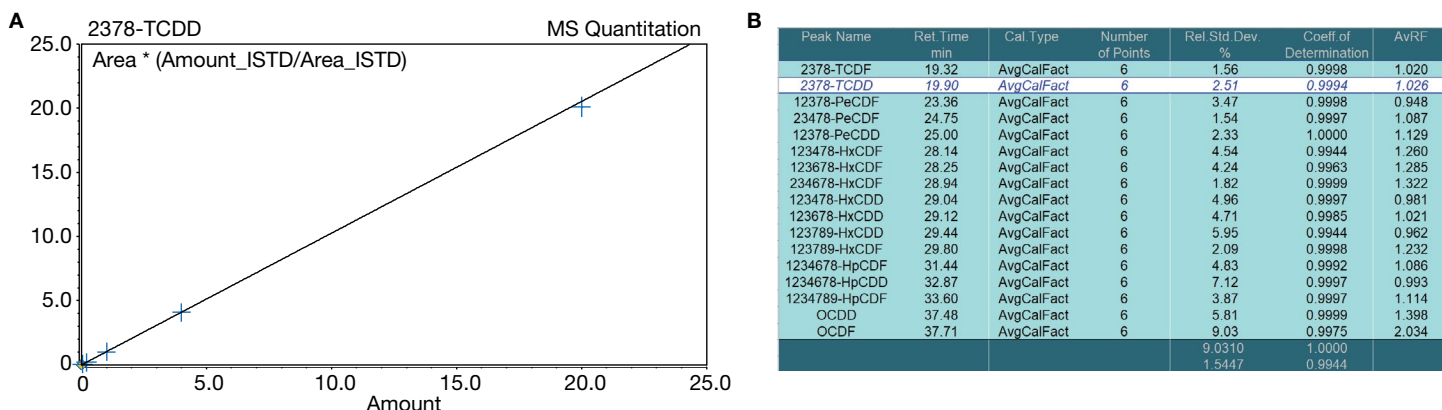


Figure 3. (A) Calibration curve for 2378-TCDD (0.01–20 pg·μL<sup>-1</sup>); (B) calibration summary of % relative standard deviation, coefficient of determination, and average response factor results for all PCDD/F analytes

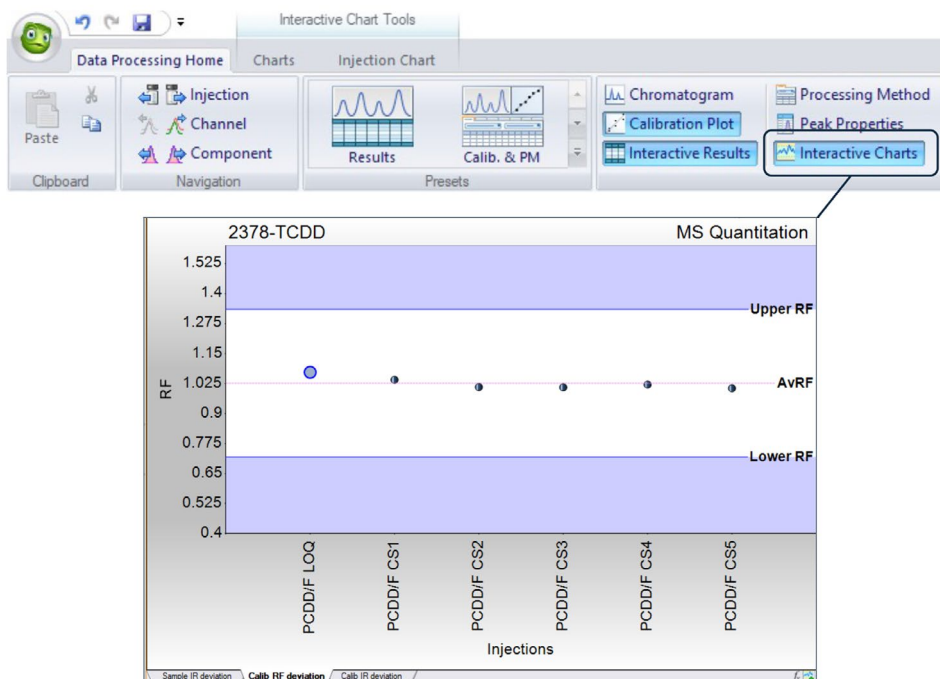
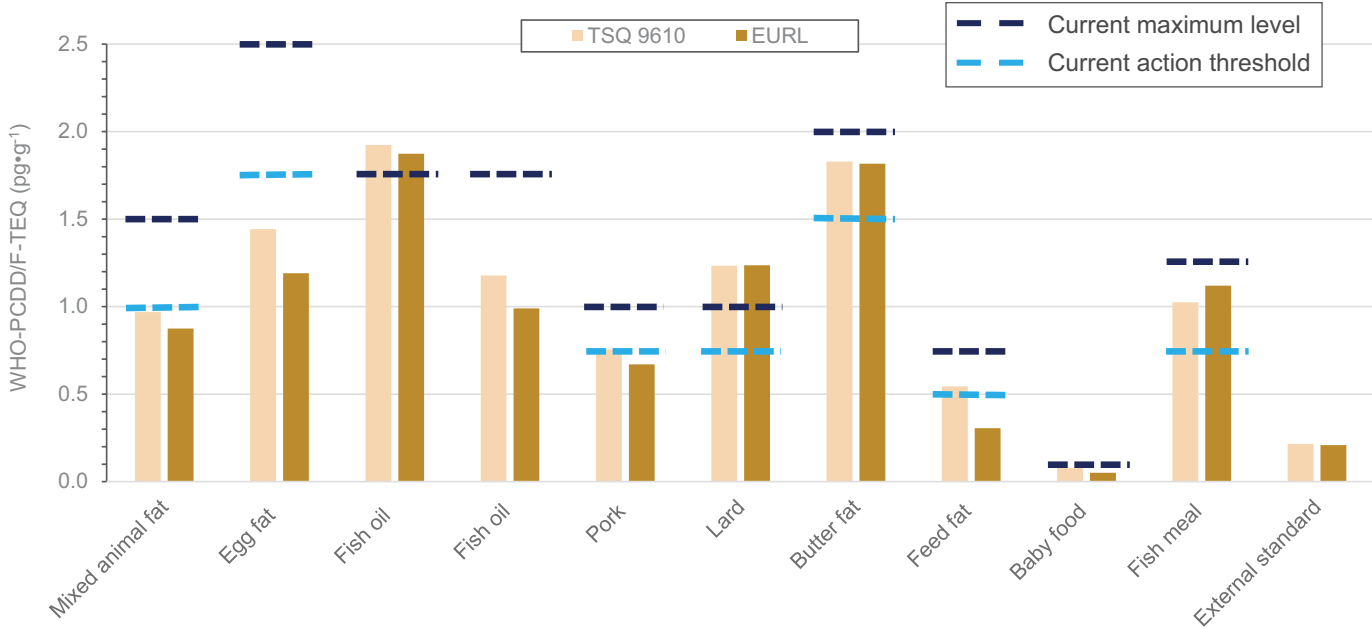


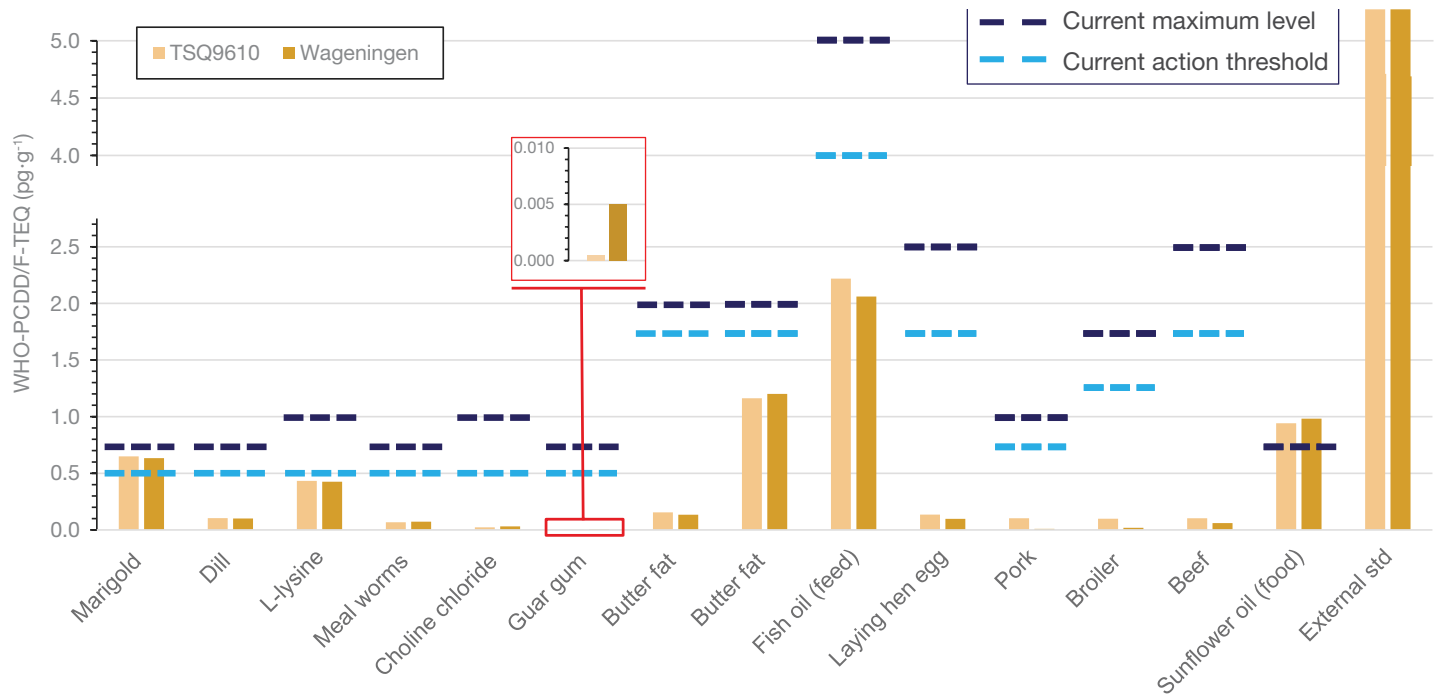
Figure 4. Interactive chart functionality within Chromeleon CDS with a visual display of the calibration response factor deviation in relation to regulated upper/lower reporting limits ( $\pm 30\%$ ) as part of the Dioxin Analyzer eWorkflow

The calibration curve was utilized to quantify the food and feed extracts provided by the EURL POPs and Wageningen Food Safety Research. Figures 5 and 6 show the WHO-TEQs based on WHO-TEFs of 2005 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 on a GC

magnetic sector HRMS from both EURL POPs and Wageningen Food Safety Research laboratories. In addition, good agreement was observed within sample matrices at both maximum levels and action thresholds. This highlights the suitability of the TSQ 9610 GC-MS/MS in meeting sensitivity and accuracy of reporting PCDD/F concentrations with newly implemented maximum levels.



**Figure 5. EURL sample results.** Comparison of results obtained on the TSQ 9610 GC-MS/MS vs. those obtained by the EURL. Values are WHO-TEQ (pg·g<sup>-1</sup>). Current maximum levels and action thresholds in food and feed items represented by dark blue and light blue dashed lines, respectively. Action thresholds defined as outlined under EU regulation 2014/663/EU.<sup>9</sup>



**Figure 6. Wageningen Food Safety Research sample results.** Comparison of results obtained on the TSQ 9610 GC-MS/MS vs. those obtained by the Wageningen Food Safety Research. Values are TEQ (pg·g<sup>-1</sup>). Maximum levels and action thresholds for food and feed items represented by the dark blue and light blue, respectively. Action thresholds defined as outlined under EU regulation 2014/663/EU.<sup>9</sup>

## Check standard accuracy and precision performance

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  $5 \text{ fg}\cdot\mu\text{L}^{-1}$  ( $25 \text{ fg OC}$ ) and  $2.5 \text{ fg}\cdot\mu\text{L}^{-1}$  ( $12.5 \text{ fg OC}$ ) for LOQ/2 and LOQ/4, respectively. In addition to these, an external standard provided by EURL POPs 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.<sup>4,5</sup> Figure 7 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.

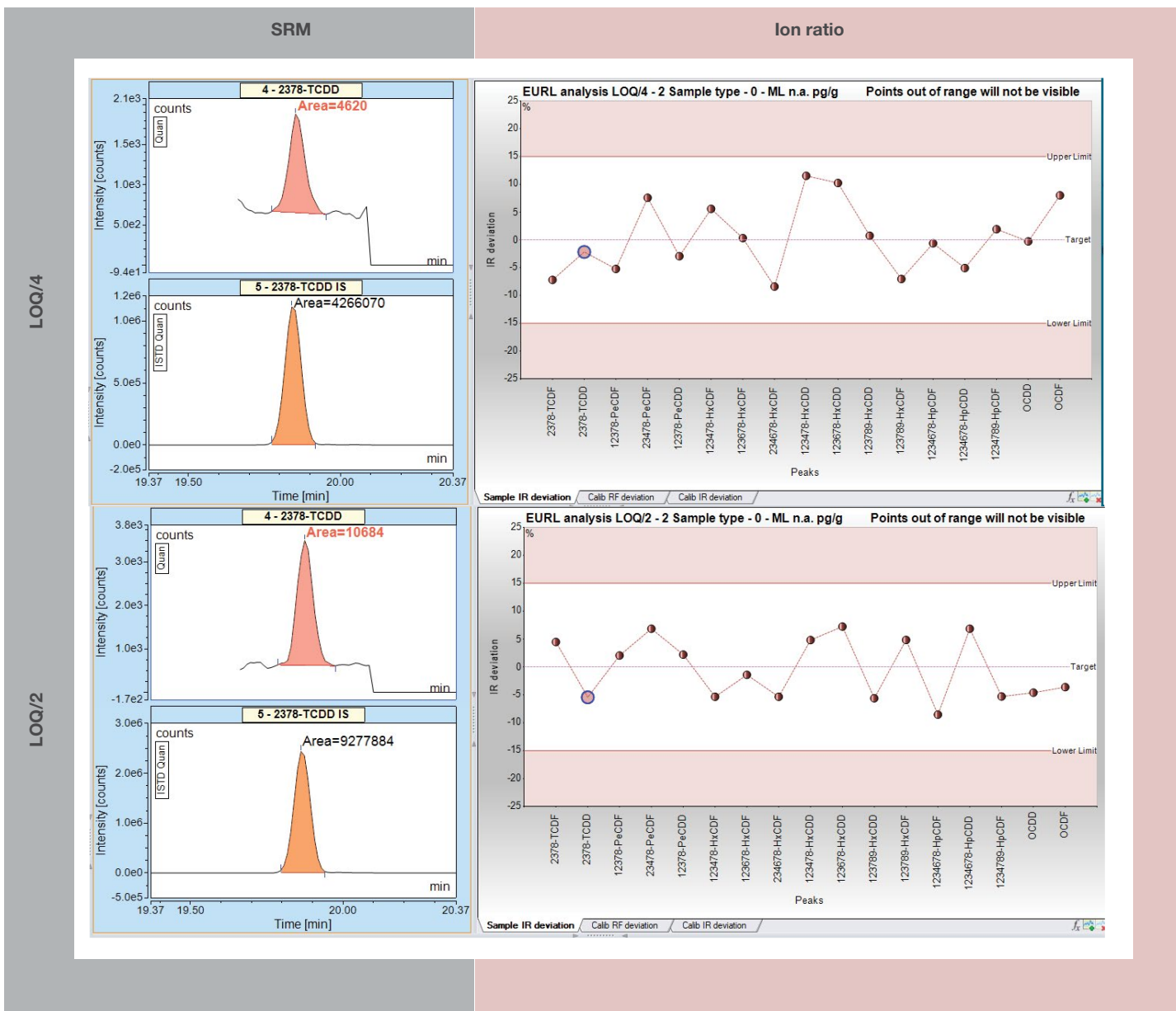


Figure 7. Extracted ion SRM transitions for the 2,3,7,8-TCDD in the LOQ/4 ( $2.5 \text{ fg}\cdot\mu\text{L}^{-1}$  ( $12.5 \text{ fg OC}$ )) and LOQ/2 ( $5 \text{ fg}\cdot\mu\text{L}^{-1}$  ( $25 \text{ fg OC}$ )) performance check standards and ion ratios for the 17 toxic congeners for dioxins and furans

The diluted check standards were also plotted on the calibration curve and produced accurate quantitation values (Figure 8A). Threshold criteria for all performance check standards were set to  $\pm 30\%$  of the true value within the Chromeleon software processing method with all check standards (LOQ/2, LOQ/4, and EURL POPs external standard) within the threshold criteria. Automatic notification if check standards calibration and ion ratio performance is provided in the interactive results table within Chromeleon CDS (Figure 8B).

## Productivity features

The TSQ 9610 NV-AEI ion source is equipped with NeverVent technology. This allows users to perform maintenance on the system without venting it. This includes exchanging the analytical column, changing a dirty ionization source for a new one, and replacing the filaments. The ability to do these maintenance activities without breaking instrument vacuum drastically reduces instrument downtime. Figure 9 shows the estimated time savings on these operations with NeverVent technology.

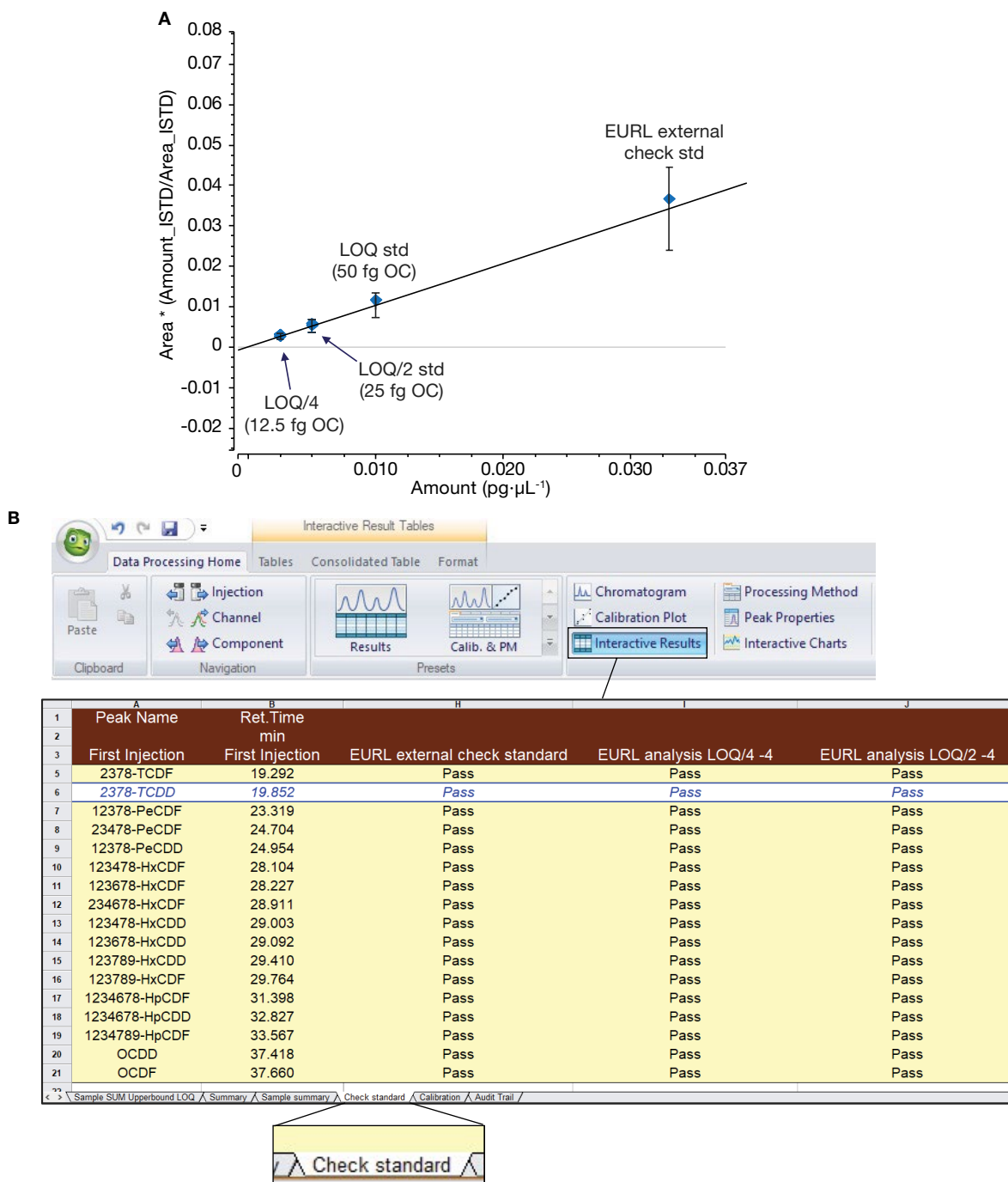








Figure 8. (A) Response of performance check standards (LOQ/2, LOQ/4, and EURL POPs external check standard) in relation to calibration curve for 2378-TCDD. Brackets surrounding each check standard represent the 30% tolerance threshold. (B) Interactive result table of check standard performance within Chromeleon CDS with automatic pass/fail notification for quick evaluation.



		Maintenance activity		
		Column change (hrs:mins)	Exchange ion source (hrs:mins)	Replace filaments (hrs:mins) (only available on NV-AEI)
Standard GC-MS	Requires vacuum system venting and pump down operations	4:35 	4:00 	4:00 
NeverVent GC-MS	Venting and pump down not required	00:35 	00:05 	00:05 
<b>NeverVent time savings</b>		<b>87%</b>	<b>98%</b>	<b>98%</b>




Figure 9. Time savings with NeverVent technology

### Conclusion

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

- Femtogram 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 maximum levels and action thresholds 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 Fisher Scientific also provide error-free execution of each analysis to meet standard operating procedure (SOP) requirements, further simplifying the user experience.

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