

Environmental

Orbitrap Exploris GC mass spectrometer performance-based analysis of polychlorinated dibenzo-*p*-dioxins/furans (PCDD/F)

Authors

Nicholas A. Warner¹, Paolo Benedetti²,
Xin Zheng³, Dominic Roberts⁴,
Daniel Kutscher¹

¹Thermo Fisher Scientific,
Bremen, Germany

²Thermo Fisher Scientific, Milan, Italy

³Thermo Fisher Scientific,
Austin, Texas, United States

⁴Thermo Fisher Scientific,
Hemel Hempstead, United Kingdom

Keywords

Dioxin analysis, PCDD/F, EPA 1613,
isotopic dilution, gas chromatography-
high resolution mass spectrometry,
sensitivity, selectivity, precision

Goal

The goal of this technical note is to demonstrate the optimization and performance of the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometer for the trace analysis of polychlorinated dibenzo-*p*-dioxins using high-resolution accurate-mass spectrometry.

Introduction

Polychlorinated dibenzo-*p*-dioxins/furans (PCDD/F) and their severe toxicity have been a global issue for over six decades. Although not intentionally produced, these chemicals can be formed through industrial/thermal processing of materials containing chlorinated organic compounds under certain conditions (i.e., high temperature, low oxygen content, and presence of a metal catalyst). Due to their inherent chemical stability and toxic nature, PCDD/F are classified as persistent organic pollutants (POP) with their production banned under the Stockholm Convention in 2001. Despite these regulations, exposure to PCDD/F continues due to their environmental stability and persistence, with the most recent incident occurring in 2011, when contaminated animal feed caused the closure of poultry farms in Germany and the subsequent emergency slaughtering of wildlife.

Due to their extreme toxicity, maximum allowable limits are constantly being revised. For example, on October 21, 2022, the maximum allowable limits of PCDD/F were lowered by the European Union to 0.02–3.5 pg/g wet weight within various food items,¹ placing greater emphasis on instrument sensitivity and selectivity to assure accurate and reliable results.

As soil contamination is a key vector in the transport of contaminants into food, its analysis for PCDD/F is of importance. [Gas chromatography-magnetic sector mass spectrometry](#) is recognized as the gold standard in the analysis of environmental

samples for PCDD/F, providing mass resolution (i.e., >10,000 resolving power at 10% valley) and mass accuracy (i.e., <5 ppm) required for worldwide compliance, including the U.S. Environmental Protection Agency (EPA) Method 1613.² However, with the current challenges faced by some laboratories, their instrumental needs have changed where higher mass resolution and system flexibility are needed to increase scope. A greater emphasis is being placed on non-targeted analysis and the identification/quantification of unknown exposure risks.

The [Orbitrap Exploris GC mass spectrometer](#) can help laboratories address these needs by providing unsurpassed mass resolution up to 60,000 full width half maximum (FWHM) at *m/z* 200 (or 240,000 mass resolution with the Orbitrap Exploris GC 240 mass spectrometer) and sub ppm mass accuracy while providing an alternative tool for PCDD/F analysis that fulfills strict criteria such as that laid out in EPA Method 1613.

In this technical note, the performance and suitability of the Orbitrap Exploris GC mass spectrometer at a mass resolution of 60,000 for the trace analysis of PCDD/F are demonstrated at current maximum allowable limits in soil samples.

Experimental

Standard and sample preparation

A six-level calibration standard curve was prepared using isotopically labeled standard mixtures for EPA Method 1613 purchased from Wellington Laboratories (Canada). The lowest calibration standard was diluted by a factor of 10 to produce a check standard (0.01–0.1 pg·μL⁻¹ according to the degree of chlorination) to assess sensitivity of the system.

A two-gram soil sample was used to demonstrate the suitability of the Orbitrap Exploris GC mass spectrometer for PCDD/F analysis. The sample was extracted in a bi-phasic mixture of acetonitrile/hexane (4 mL:4 mL) followed by centrifugation at 3,000 rpm. A 100 μL aliquot of the hexane layer was then evaporated to dryness, spiked with 30 μL of the lowest calibration standard, and reconstituted in 100 μL nonane.

Instrument and method setup

Injection and chromatographic conditions for the Thermo Scientific™ TRACE™ 1610 GC equipped with a Thermo Scientific™ TraceGOLD™ TG-Dioxin 60 m × 0.25 mm i.d. × 0.25 μm film capillary column (P/N 26266-1540) are summarized in Table 1. Analysis run time on the TraceGOLD TG-Dioxin column is drastically reduced compared to conditions described in EPA Method 1613 on a DB-5 stationary phase without loss of chromatograph resolution efficiency. Automated liquid injection was performed using a Thermo Scientific™ TriPlus™ RSH autosampler. Data acquisition was performed in timed single ion monitoring (tSIM) with the Orbitrap Exploris GC mass

spectrometer with additional details summarized in Table 2. External mass calibration was performed prior to batch analysis, while characteristic ions representing column bleed were used as lock masses to perform internal mass calibration. Quantification was performed using isotopic dilution using the Dioxin analyzer workflow within the Thermo Scientific™ Chromeleon™ version 7.3.1 Chromatography Data System (CDS) software.

Table 1. GC injection and column conditions

TRACE 1610 GC system parameters	
Injector	Thermo Scientific™ iConnect™ Split/Splitless (SSL)
Injection volume (μL)	1.5
Liner	Thermo Scientific™ Straight liner with quartz wool (P/N 453A2265-UI)
Injection mode	Splitless (split flow 120 mL·min ⁻¹ after 2 min)
Split flow (mL·min ⁻¹)	120
Injector temperature (°C)	280
Carrier gas, (mL·min ⁻¹)	He, 1.2 (constant flow)
Oven temperature program	
Initial temperature (°C)	120
Hold time (min)	2
Rate 1 (°C·min ⁻¹)	25
Temperature 1 (°C)	250
Hold time 1 (min)	0
Rate 2 (°C·min ⁻¹)	2.5
Temperature 2 (°C)	260
Hold time 2 (min)	5
Rate 3 (°C·min ⁻¹)	2.5
Temperature 3 (°C)	285
Hold time 3 (min)	0
Rate 4 (°C·min ⁻¹)	10
Temperature 4 (°C)	320
Hold time 4 (min)	15
Total run time (min)	44.7

Table 2. Ion source and mass spectrometer conditions

Orbitrap Exploris GC MS parameters	
Transfer line (°C)	300
Extractabrite ion source temperature (°C)	350
Electron energy (eV)	40
Acquisition mode	tSIM
Isolation window width (<i>m/z</i>)	10
Resolving power (at 200 <i>m/z</i>)	60,000
Emission current (μA)	50
C-Trap offset (V)	-5
Mass accuracy on lock mass	5 ppm
Internal lock mass calibration (column bleed, <i>m/z</i>)	207.0324, 281.0511, 355.0699

Results and discussion

Source optimization for sensitivity

An electron energy of 70 eV is commonly used in GC-MS for identification/comparison purposes with mass spectral databases. However, significant fragmentation of the analyte molecular or targeted ion of interest and lower number of ions produced may occur at high electron energies, decreasing the overall abundance. Applying lower electron energies often provides a softer ionization process, reducing fragmentation and boosting higher masses for targeted quantification. As such, an electron energy between 28–40 eV is listed as a criterion in EPA Method 1613 to obtain the sensitivity needed to reach regulatory limits for dioxin analysis. The Orbitrap Exploris GC mass spectrometer dioxin method template (Figure 1) provides a default setting of 35 eV, although small fluctuations can occur between instruments and should be optimized/confirmed prior to analysis.

In addition, the instrument control software, including tune application and method editor, are built into Chromeleon CDS for compliance purposes. The ion source can be optimized easily at the user-defined electron energy (eV) with mass calibration within minutes in the Tune application. Results of eV optimization can be seen for 5 $\text{pg}\cdot\mu\text{L}^{-1}$ calibration standard of 2,3,7,8 – Tetrachlorodibenzodioxin (TCDD), the most toxic PCDD congener with the lowest detection limit requirements (Figure 2A). Of the various electron energies tested, the optimal response was observed at 40 eV. This drastically improved analysis quality at the low fg range as can be seen in the overall response and peak shape of 2,3,7,8-TCDD in a spiked raw (no clean-up) soil extract (45 fg on column) at 40 eV compared to 70 eV using a 1.5 μL injection volume (Figure 2B).

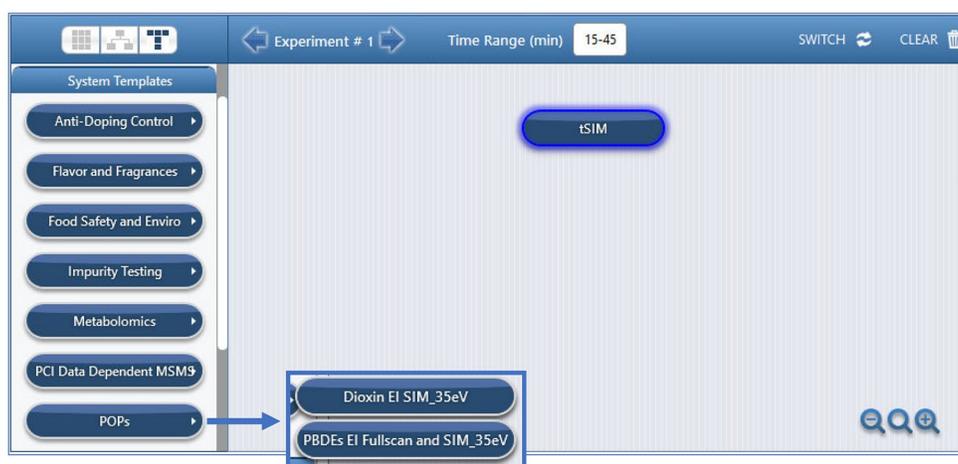


Figure 1. Dioxin method template within method editor built into Chromeleon 7.3.1 CDS

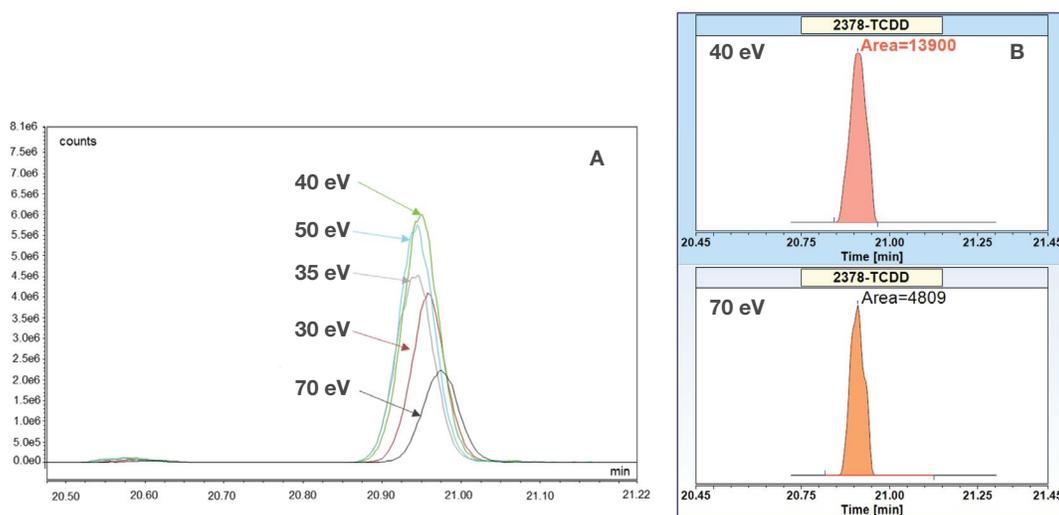


Figure 2. (A) Signal response of 5 $\text{pg}\cdot\mu\text{L}^{-1}$ 2,3,7,8-TCDD calibration standard (extracted mass: m/z 321.8930) at electron energies from 30 to 70 eV; (B) area response (counts·s) of 2,3,7,8-TCDD in spiked soil extract (45 fg on column) at 40 and 70 eV

User friendly performance evaluation

Due to the inherent toxicity of PCDD/F, analytical methodology must be upheld to strict performance criteria to ensure accurate identification and quantification at regulatory limits. Such criteria include:

- Quantification and confirmation ions detected within a ± 0.01 min retention time tolerance
- An ion confirmation ratio between the quantification and confirmation ions is within 15% of the theoretical value
- The average relative response factor (RRF) value deviates no more than 20% (at the start and end of analysis sequence) across all calibration curve points

These criteria are integrated into the Dioxin Analyzer workflow within Chromeleon CDS, with the analysis performance (i.e., ion ratio and RRF deviation) displayed as interactive graphics for easy evaluation, helping to speed up sample processing and performance criteria evaluation analysis. An example of this can be seen in Figure 3, which displays the extracted exact mass chromatogram of 2,3,7,8-TCDD, its calibration curve, and the ion ratio deviation for all PCDD/F congeners within the 0.05 $\mu\text{g}\cdot\mu\text{L}^{-1}$ calibration standard.

Linear response was observed for all PCDD/F congeners spanning over three orders of magnitude in the concentration range with deviation from the calibration response being less than 6% RSD (Table 3.)

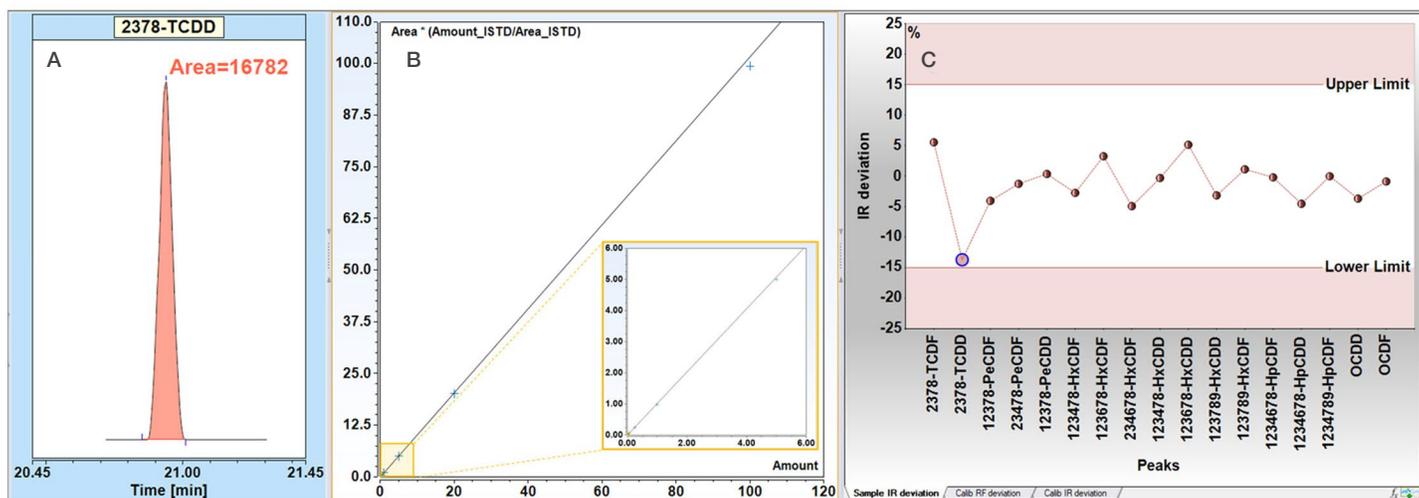


Figure 3. (A) 2,3,7,8-TCDD extracted exact mass (m/z 321.8930) 0.05 $\mu\text{g}\cdot\mu\text{L}^{-1}$ chromatogram; (B) calibration curve (0.05–100 $\mu\text{g}\cdot\mu\text{L}^{-1}$); and (C) ion ratio deviation for all PCDD/F congeners in the 0.05 $\mu\text{g}\cdot\mu\text{L}^{-1}$ calibration standard

Table 3. Relative standard deviation in calibration response (%), coefficient of determination, and average response factor (AvRF) for 6-level calibration curve

Peak Name	Ret. Time min	Cal. Type	Number of Points	Rel. Std. Dev. %	Coeff. of Determination	AvRF
2378-TCDF	20.36	AvgCalFact	6	1.65	0.9997686389	0.970
2378-TCDD	20.94	AvgCalFact	6	5.88	0.9993210905	1.016
12378-PeCDF	24.42	AvgCalFact	6	2.10	0.9994971622	0.955
23478-PeCDF	25.81	AvgCalFact	6	1.58	0.9997365934	1.041
12378-PeCDD	26.07	AvgCalFact	6	1.86	0.9998797744	0.943
123478-HxCDF	29.12	AvgCalFact	6	2.61	0.9975026776	0.968
123678-HxCDF	29.23	AvgCalFact	6	2.94	0.996336479	0.947
234678-HxCDF	29.91	AvgCalFact	6	2.71	0.9963530129	0.967
123478-HxCDD	30.00	AvgCalFact	6	1.69	0.9998097134	1.011
123678-HxCDD	30.10	AvgCalFact	6	2.09	0.9986506808	0.995
123789-HxCDD	30.42	AvgCalFact	6	1.38	0.9995510546	0.919
123789-HxCDF	30.77	AvgCalFact	6	2.34	0.9990108737	0.927
1234678-HpCDF	32.43	AvgCalFact	6	2.49	0.998223791	0.949
1234678-HpCDD	33.88	AvgCalFact	6	1.55	0.9995570502	1.030
1234789-HpCDF	34.61	AvgCalFact	6	2.16	0.9992629519	0.927
OCDD	38.53	AvgCalFact	6	3.51	0.997162704	0.963
OCDF	38.79	AvgCalFact	6	3.45	0.9998981039	1.180
				5.8829	0.99990	
				1.3794	0.99634	

Superior mass resolution for regulatory needs

A resolving power of $\geq 10,000$ at 10% valley at the specified mass is required by EPA Method 1613 for the analysis of all PCDD/F target masses. As mass resolution decreases with increasing mass-to-charge ratio on the Orbitrap Exploris mass spectrometer, fulfillment of this criteria will be most critical at the high mass range of our targeted analytes. Octachlorodibenzodioxin (OCDD) is the heaviest targeted analyte at an exact mass of m/z 455.7401. Simulation of the mass resolution needed to fulfill criteria of EPA Method 1613 (10,000 at 10% valley or 5 % peak height) for OCDD

is approximately 23,000 (Figure 4). However, analysis using the Orbitrap Exploris GC mass spectrometer at 60,000 resolution (FWHM at m/z 200) provides a mass resolution of approximately 40,000 (for OCDD, measured at m/z 455.7401), confirming full compliance with EPA Method 1613. For auditing purposes, a short full scan event can be added at GC void time during a run using calibration gas ions as internal lock masses to ensure sub-ppm mass accuracy from injection to injection, which can be easily defined in the instrument method editor (Figure 5).

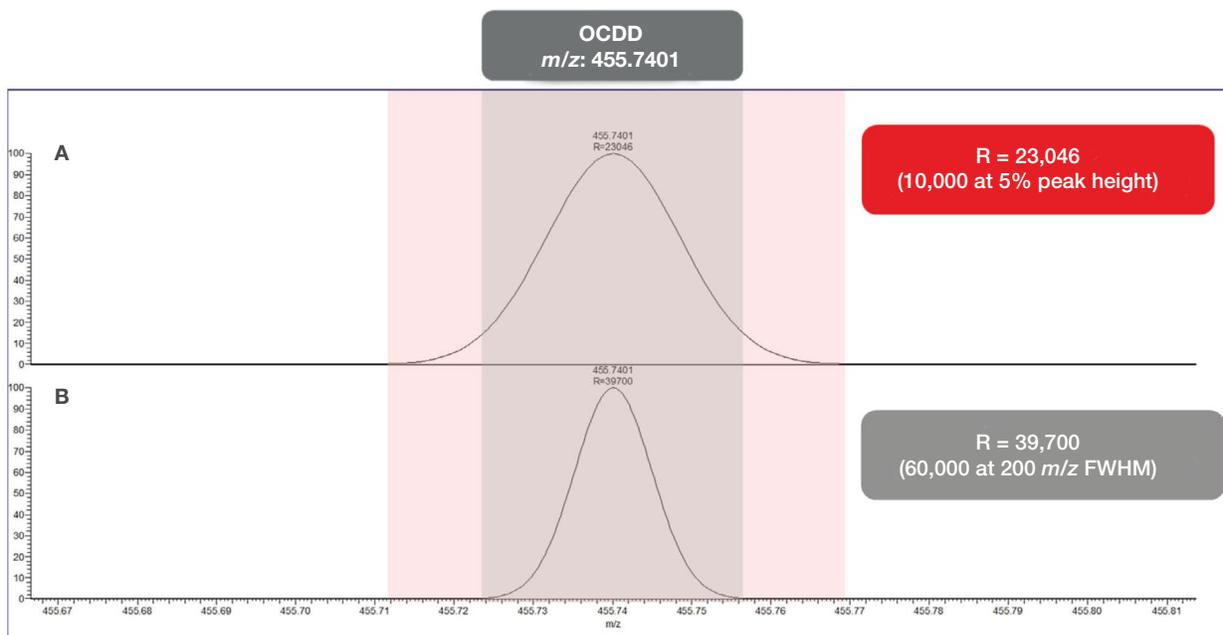


Figure 4. Simulated mass resolution (R) of OCDD at (A) 10,000 at 5% peak height and (B) 60,000 at m/z 200 FWHM

Method Editor | Global Parameters | Scan Parameters | Summary

Application Mode: Default
Method Duration (min): 45

Method Timeline: Experiment #1 | Time Range (min): 0-0.42 | ACTIONS

Settings:

- Internal Mass Calibration: User-defined Lock Mass
- Mode: Timed
- Mass Tolerance (ppm): 5
- Acquisition Delay (min): 1
- Current Lock Mass: Current

m/z	t_start(min)	t_stop(min)	Polarity
130.99147	0	0.4	Positive
413.97698	0	0.4	Positive
451.97488	0	0.4	Negative

Full Scan Properties:

- Orbitrap Resolution: Defined in Table
- Scan Range (m/z): Defined in Table
- Polarity: Positive

Scans:

Scan	Scan Range (m/z)	Orbitrap Resolution
1	50-600	60000

Figure 5. Full scan together with tSIM acquisition setup in the method editor built within Chromeleon CDS to provide full compliance for dioxin analysis

Reliable performance across platforms

For dioxin analysis, it is imperative that laboratories have confidence that the results delivered are accurate. To demonstrate this, continuous analysis of a raw soil extract (no sample clean-up) spiked with PCDD/F (congener concentration range: 30–300 fg·µL⁻¹) was re-analyzed on the Orbitrap Exploris GC mass spectrometer using previously established injection and chromatographic conditions.³

Quantified results from the Orbitrap Exploris GC mass spectrometer were in excellent agreement to spike soil extract (Figure 6), delivering accurate and precise quantitation at fg levels. 1,2,3,4,6,7,8-HpCDD, OCDD, and OCDF were detected above spiking levels. Similar findings were observed in a separate analysis of the soil extract using the Thermo Scientific™ TSQ™ 9610 GC-MS/MS,³ confirming the presence of these congeners within the soil sample prior to spiking.

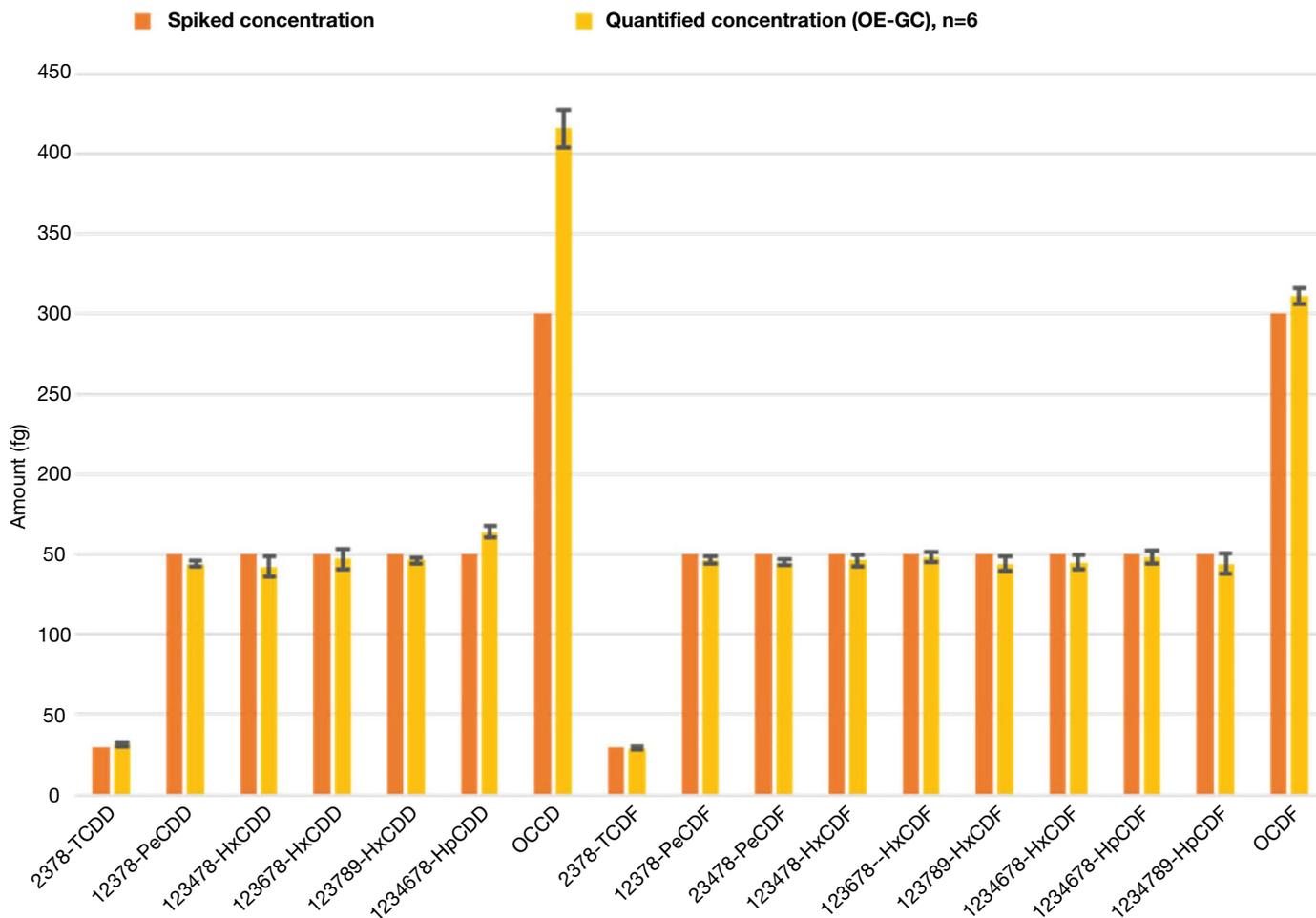


Figure 6. Spike and obtained concentration of PCDD/F quantified from repetitive analysis of raw soil extract (n = sample number)

Conclusion

Thermo Fisher Scientific is the only manufacturer to provide a complete portfolio of GC-MS solutions to meet all different customer requirements globally for PCDD/F analysis. The Thermo Scientific™ DFS™ Magnetic Sector gas chromatography-high-resolution mass spectrometer (GC-HRMS) is the gold standard in dioxin analysis, providing worldwide compliance in environmental samples as outlined in the EPA Method 1613 and in food and feed under EU Regulations 644/2017 and 771/2017 for the analysis of PCDDs/PCDFs. The latter regulation also allows targeted analysis using the TSQ 9610 GC-MS/MS equipped with the advanced electron impact source, providing enhanced sensitivity performance needed for dioxin analysis.

This technical note demonstrates the ability of an alternative performance-based approach using the Orbitrap Exploris GC mass spectrometer to deliver sensitive and accurate trace analysis according to EPA Method 1613 with stable mass accuracy throughout the entire analysis sequence combined with high sensitivity and mass resolving power.

- Femtogram level sensitivity required for dioxin analysis with a 1.5 µL injection volume using software-optimized ion optics at user-defined electron voltage energies
- Optimized and user-friendly workflow within Chromeleon 7.3.1 CDS using isotopic dilution quantification for quick evaluation of PCDD/F analysis performance according to EPA Method 1613 criteria
- Wide linear dynamic range with mass resolution surpassing requirements at 60,000 mass resolution settings (at 200 *m/z* FWHM)
- Accurate and precise performance at fg levels within environmental samples
- Ability to quickly increase the scope of analysis through full-scan high-resolution accurate mass data, thus enabling laboratories to have a flexible system for their analytical services

References

1. European Union Commission Regulation 2022/2002. Amending Regulation (EC) No 1881/2006 as regards maximum levels of dioxins and dioxin-like PCBs in certain foodstuffs. October 21, 2022. <https://eur-lex.europa.eu/eli/reg/2022/2002/oj>
2. Environmental Protection Agency (EPA) Method 1613. Tetra-Through Octa-Chlorinated dioxins and Furans by Isotope Dilution HRGC/HRMS. United States. Revision B, 1994. EPA 821-B-94-005.
3. Warner, N. A.; Ladak, A.; Cavagnino, D.; Kutscher, D. Thermo Scientific Technical Note 001594: Uncompromised sensitivity in polychlorinated dibenzo-*p*-dioxins/furan analysis using triple quadrupole GC-MS with cost-effective helium-saving technology (2022). <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-001594-gc-ms-ms-dioxins-furans-hesaver-h2saver-food-an001594-na-en.pdf>

 Learn more at thermofisher.com/OrbitrapExplorisGC