Application note | 004504

Environmental

EPA Method 1613 performance-based dioxin analysis in environmental samples using the Orbitrap Exploris GC S mass spectrometer

Authors

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Keywords

PCDD/F, dioxins, EPA Method 1613, environmental, soil, isotope dilution, GC-HRAM, Orbitrap, tSIM, ion ratio, NeverVent Advanced Electron Ionization (NV-AEI), Chromeleon CDS

Goal

The goal of this application note is to demonstrate the suitability of the Thermo Scientific™ Orbitrap Exploris™ GC S mass spectrometer for EPA Method 1613 performance-based dioxin analysis in environmental samples, with emphasis on ultra-trace sensitivity, calibration and response factor stability, and confirmatory ion ratio agreement for both native and isotopically labeled congeners.

Introduction

Dioxins and furans (polychlorinated dibenzo-*p*-dioxins and dibenzofurans, PCDD/F) are a class of persistent organic pollutants (POPs) that exhibit extreme toxicity even at picogram-per-gram levels. They are not manufactured for commercial use, but form as unwanted by-products of combustion and certain industrial and thermal processes. Their chemical stability, lipophilicity, and tendency to bioaccumulate have led to strict regulation of PCDD/F under national and international frameworks. Therefore, monitoring programs focus on foodstuffs, environmental matrices, and biota to protect public health. Regulatory test methods require both ultra-trace sensitivity and unambiguous compound confirmation to ensure legally robust reporting and to distinguish true positives from matrix interferences.

EPA Method 1613[1] was developed to provide a standardized, reproducible approach for the determination of PCDD/F congeners in environmental matrices using isotope dilution quantitation and stringent confirmation criteria. The method is applied to a wide range of sample types encountered in environmental and compliance testing, for example, soils, sludge and sediments, animal tissues (biota), food and feed items, and certain waste and industrial matrices. The combination of low regulatory action levels and the complexity of many matrices drives the need for robust sample preparation and highly selective instrumental analysis, typically using high resolution mass spectrometry.

Analytical challenges for PCDD/F include extremely low regulatory limits (often in the low pg/g wet weight range), co-extraction of matrix components that produce isobaric or near-isobaric interferences, and the frequent need to analyze a broad congener suite with high precision. Conventional technologies used for regulatory confirmatory PCDD/F analysis include gas chromatography–magnetic sector high-resolution mass spectrometry (GC-HRMS), widely regarded as the gold standard due to its high resolving power, and tandem quadrupole GC-MS/MS, which can provide high sensitivity and throughput but may offer reduced selectivity when complex co-eluting interferences are present. Limitations of the GC-HRMS instrumentation include the high capital and operational cost and specialized maintenance of magnetic sector instruments, flexibility to adapt to changing laboratory demands, potential throughput constraints, and full-scan data capability for retrospective or non-targeted investigations for emerging contaminants.

High-resolution accurate-mass (HRAM) Thermo Scientific™ Orbitrap™ GC-MS combines full-scan or selected ion monitoring (SIM) detection with modern source and maintenance designs that can address many of these challenges. At appropriate resolving power settings, Orbitrap mass analyzers provide narrow mass extraction windows and sub-ppm mass accuracy that improve selectivity against isobaric interferences and increase confidence in congener identification while retaining the quantitative robustness required by isotope dilution procedures. Where full-scan HRAM acquisition is deployed, it also enables retrospective data mining and non-targeted screening alongside targeted regulatory workflows, expanding analytical scope without additional analysis. When integrated with maintenance features that reduce downtime and with software that enables fast implementation, the Orbitrap Exploris GC S mass spectrometer can deliver a practical performance-based alternative for laboratories seeking the combination of sensitivity, selectivity, and operational flexibility needed to meet EPA Method 1613 confirmation criteria across diverse matrices. This study demonstrates those analytical performance elements that are required to meet those criteria.

Experimental

Standards and samples

Solvent standards corresponding to the EPA Method 1613 calibration system were analyzed together with spiked soil and sludge sample extracts.

Standard calibration design

An 11-level calibration series was prepared and analyzed using EPA Method 1613 isotopically labeled PCDD/F standard mixtures purchased from Wellington Laboratories (Canada). The series started at 10 fg on-column for the 2,3,7,8-TCDD and comprised CS4, CS3, CS2, CS1, and CSL (Calibration Solution Lowest) followed by lower-level points prepared as 10 times serial dilutions of the low-level standards (CS3, CS2, CS1 and CSL), together with a CSL check level and a solvent blank to verify background and carryover.

For the calibration plots in Figure 2, the highest calibration level was excluded for all congeners in accordance with the study design, and linearity and fit were evaluated using the remaining calibration points.

Instrumental method and data processing

Injection and chromatographic conditions for the Thermo Scientific™ TRACE™ 1610 GC coupled to an Orbitrap Exploris GC S mass spectrometer equipped with a Thermo Scientific™ NeverVent™ Advanced Electron Ionization (NV-AEI) Source are summarized in Appendix Tables A1 and A2. Chromatographic separation was performed using a Thermo Scientific™ TRACE™ TR-Dioxin Capillary Column, 60 m × 0.25 mm i.d. × 0.10 µm film (Cat. No. 26AF059P). Automated liquid injection was accomplished using a Thermo Scientific™ TriPlus™ RSH SMART Autosampler. Data acquisition was performed in timed single ion monitoring (tSIM) at 60,000 resolving power (FWHM at *m/z* 200), monitoring two exact-mass ions (quantifier and qualifier) for each native and corresponding ^{13}C-labeled target to support confirmatory ion-ratio evaluation consistent with EPA Method 1613 requirements. Additional mass spectrometer and acquisition parameters are summarized in Appendix Figure A1. External mass calibration was performed prior to batch analysis; where applicable, characteristic background/column-bleed ions were used as lock masses for internal mass correction during acquisition. All accurate masses were extracted with a 5 ppm window.

Data were acquired and processed in Thermo Scientific™ Chromeleon™ 7.4 Chromatography Data System (CDS). Confirmation was performed by evaluating:

- Presence of the target peak at the expected retention region
- Ion ratio agreement between quantifier and qualifier ions using a ±15% relative tolerance (reported as "Confirmed" in sample tables). Data processing and isotopic dilution quantification were performed using the workflow in Chromeleon software.

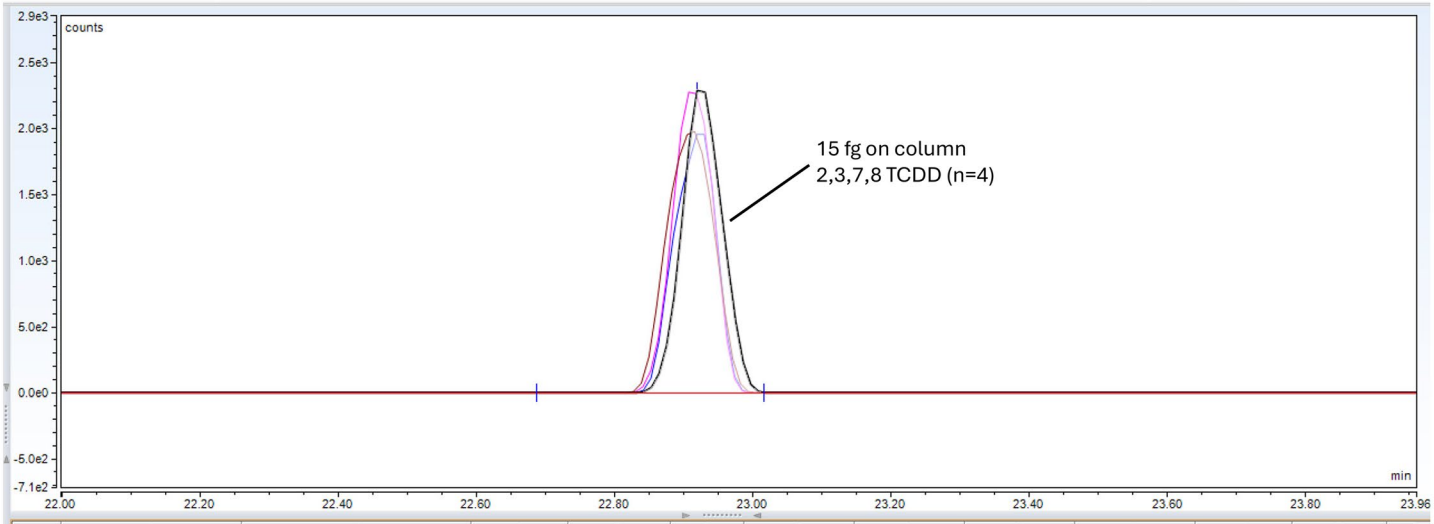
Results and discussion

Compliant sensitivity and confirmation at ultra-trace levels

Consistent trace-level sensitivity is critical for compliant and accurate dioxin analysis. Here, sensitivity was demonstrated using 2,3,7,8-TCDD, a key congener commonly used as a benchmark due to its toxicity and stringent reporting needs. EPA Method 1613 requires that, at the lowest calibration level (CS1), the native target congeners and their corresponding isotope-labeled analogs are detectable with a signal-to-noise ratio (S/N) ≥ 10, providing a practical sensitivity check at the method's minimum levels. In HRAM workflows from the GC Orbitrap MS, S/N is improved, not because instrumental noise is entirely eliminated, but because extracting the analyte at its exact accurate mass using a narrow mass window (typically ±5 ppm) substantially reduces contributions from co-eluting, near-isobaric background ions that can dominate baseline in mass traces. This reduction in background interference leads to cleaner extracted ion chromatograms and more reliable sensitivity assessment at ultra-trace levels, supporting the intent of the EPA Method 1613 compliant-sensitivity requirement. Figure 1 shows extracted ion chromatograms for 2,3,7,8-TCDD at 15 fg on-column (extracted mass: *m/z* 321.8936 in CSL standard diluted ten times, 1.5 µLinjection volume), with four replicate injections overlaid, demonstrating repeatable signal at ultra-trace levels and exceeding the requirements. Furthermore, mass resolution is reported in the Chromeleon CDS results table at average 23,624 resolving power at 5% peak height for *m/z* 321.8936, exceeding the requirements in EPA Method 1613 of 10,000.

Calibration performance across 17 congeners

EPA-style dioxin workflows require robust calibration performance across all regulated congeners. A 10-point calibration was generated and evaluated for all 17 2,3,7,8-substituted PCDD/F congeners. The second lowest calibration point was injected in triplicate.



	A	B	C	D	E	F	G	H	I	J	K
1	Inj.	Injection Name	Area	Resolution							
2	No.	Selected Peak:	counts*s	5% peak height							
4			2,3,7,8-TCDD	2,3,7,8-TCDD							
5	*19*	*CSL dil10 - 15 fg OC*	*9383*	*22680*							
6	20	CSL dil10 - 15 fg OC	8490	23902							
7	21	CSL dil10 - 15 fg OC	9597	23525							
8	22	CSL dil10 - 15 fg OC	9681	24392							
9	Maximum										
10	**Average**		**9288**								
11	Minimum										
12	Standard Deviation		546								
13	**Relative Standard Deviation**		**5.88%**								

Figure 1. Chromeleon CDS results view of extracted ion chromatograms for 2,3,7,8-TCDD at 15 fg on-column in solvent (extracted mass: *m/z* 321.8936 ± 5 ppm), with four replicate injections overlaid, demonstrating repeatable signal at ultra-trace levels. Mass resolution is reported in the table at average 23,624 exceeding the requirements in EPA Method 1613.

Linearity for accurate quantitation

Linearity across the working calibration range is essential for dioxin analysts because it supports accurate isotope-dilution quantitation at ultra-trace levels while maintaining robustness for higher-level samples. In this study, linearity was evaluated from the lowest-level point (CSL prepared as a 10× dilution) through CS4, providing coverage from near the low-end reporting region to the upper end of the routine range.

This calibration approach aligns with EPA Method 1613 expectations for multi-level calibration and verification of calibration performance across the range. EPA Method 1613 specifies that, where the relative response is sufficiently constant (≤20% CV) across the multi-point (5 points at least) calibration, an average response factor may be applied; otherwise, a full calibration curve is required. Demonstrating consistent response behavior from the 10× diluted low-end level through CS4 provides confidence that quantitation near the method's lowest levels is not compromised, while still meeting routine dynamic range needs in compliance workflows.

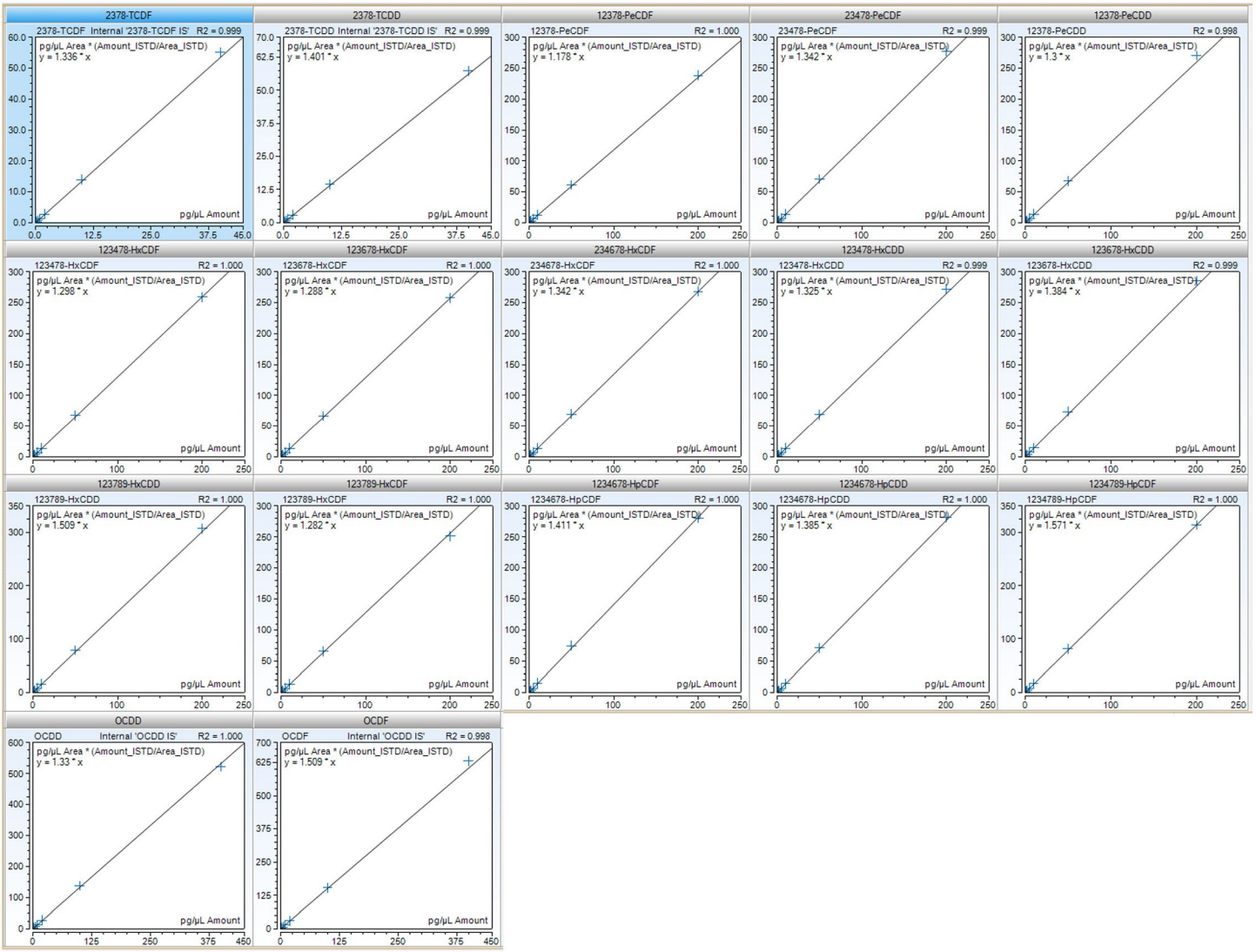


Figure 2. Chromeleon CDS report view of the ten point calibration graphs for the 17 congeners in nonane. All show excellent linearity across the range from 10× diluted CSL to CS4.

Response factor stability

Response factor stability and ion ratio compliance (±15%) were evaluated across the calibration series because these are the primary checks used to verify that isotope dilution quantitation and confirmatory identification criteria are being met prior to sample analysis. Figure 3 summarizes the Chromeleon CDS response factor results across the full calibration range (CSL, prepared as a 10× dilution, through CS4), including the %RSD calculated for each of the 17 congeners. All met the 15% RSD criterion, indicating stable response across the working range and supporting reliable quantitation using isotope dilution.

Repeatability in matrix performance

To demonstrate performance in an environmental matrix, soil unprocessed extracts spiked with 2,3,7,8-TCDD at 15 fg on-column were analyzed with ten replicate injections (Figure A2), enabling assessment of repeatability at the lowest evaluated level. The Chromeleon CDS results view (Figure 4) shows peak area results for the ten replicates. For the congeners shown, %RSD values ranged from 3.5% to 6.9%, indicating strong injection-to-injection precision at ultra-trace levels in a complex matrix.

Ion ratio performance was simultaneously evaluated using a ±15% relative tolerance, consistent with confirmatory criteria applied in EPA Method 1613 workflows. All monitored ion ratios met the <15% deviation requirement across the replicate set, demonstrating that both quantitative precision and confirmatory performance were maintained. This level of repeatability and ion ratio stability at 15 fg on-column supports alignment with the intent of EPA Method 1613, where reliable low-level detection and confirmation in environmental samples are essential for compliant reporting.

Peakname	Ret.Time min	Cal.Type	Number of Points	Rel.Std.Dev. %	RSD% check <15%	Actual RF	Average RF	R-Squared %
2378-TCDF	22.23	AvgCalFact	12	3.50	Pass	1.31	1.34	0.999
2378-TCDD	22.94	AvgCalFact	12	3.15	Pass	1.40	1.40	0.999
12378-PeCDF	27.42	AvgCalFact	12	2.63	Pass	1.18	1.18	1.000
23478-PeCDF	29.27	AvgCalFact	12	4.56	Pass	1.40	1.34	0.999
12378-PeCDD	29.77	AvgCalFact	12	3.31	Pass	1.30	1.30	0.998
123478-HxCDF	34.96	AvgCalFact	12	2.28	Pass	1.30	1.30	1.000
123678-HxCDF	35.18	AvgCalFact	12	2.58	Pass	1.30	1.29	1.000
234678-HxCDF	36.28	AvgCalFact	12	2.71	Pass	1.35	1.34	1.000
123478-HxCDD	36.52	AvgCalFact	12	3.61	Pass	1.33	1.32	0.999
123678-HxCDD	36.69	AvgCalFact	12	4.80	Pass	1.42	1.38	0.999
123789-HxCDD	37.09	AvgCalFact	12	4.03	Pass	1.55	1.51	1.000
123789-HxCDF	37.62	AvgCalFact	12	2.90	Pass	1.29	1.28	1.000
1234678-HpCDF	39.79	AvgCalFact	12	2.42	Pass	1.40	1.41	1.000
1234678-HpCDD	41.37	AvgCalFact	12	2.51	Pass	1.38	1.38	1.000
1234789-HpCDF	42.05	AvgCalFact	12	3.08	Pass	1.55	1.57	1.000
OCDD	44.91	AvgCalFact	12	2.12	Pass	1.34	1.33	1.000
OCDF	45.11	AvgCalFact	12	3.76	Pass	1.50	1.51	0.998

Figure 3. Chromeleon CDS response factor summary table from across the calibration series, including %RSD for each of the 17 congeners. Response factor evaluated using a strict ±15% tolerance (typical is 20%). Flagging of tolerance automatically displayed.

Inj. No	Injection Name	Area counts*s					IR deviation % <15%				
		2378-TCDF	2378-TCDD	12378-PeCDF	23478-PeCDF	12378-PeCDD	2378-TCDF	2378-TCDD	12378-PeCDF	23478-PeCDF	12378-PeCDD
23	15 fg OC Soil Matrix rep 1	18204	12815	83520	89365	45529	-5.6	-13.3	-3.3	-3.2	-4.2
24	15 fg OC Soil Matrix rep 2	17173	10398	87731	89611	45407	-2.9	-3.7	-9.5	-5.9	3.2
25	15 fg OC Soil Matrix rep 3	18078	12220	86725	91416	44087	-0.7	-2.4	-6.9	-4.3	-3.9
26	15 fg OC Soil Matrix rep 4	18999	12600	88195	97912	46796	-10.0	-9.9	-3.8	-5.5	-5.8
27	15 fg OC Soil Matrix rep 5	17468	11080	87607	97161	47980	5.5	-0.1	0.7	-9.4	0.9
28	15 fg OC Soil Matrix rep 6	18261	13087	89328	94546	47662	-7.3	-4.0	-0.4	-2.6	-6.4
29	15 fg OC Soil Matrix rep 7	18890	12281	91947	96885	47224	9.3	-12.0	-4.5	-4.2	0.6
30	15 fg OC Soil Matrix rep 8	18325	12060	88546	89546	45421	6.2	3.5	-2.8	2.4	-3.2
31	15 fg OC Soil Matrix rep 9	18666	12631	92138	97611	50318	3.6	-8.3	0.1	-3.9	-6.8
32	*15 fg OC Soil Matrix rep 10*	*19674*	*11638*	*94139*	*101062*	*47665*	*-5.9*	*-0.9*	*-5.3*	*-5.3*	*-0.6*
Maximum											
Average		18374	12081	88988	94512	46809					
Minmum											
Standard Deviation		732	831	3067	4236	1772					
Relative Standard Deviation		3.99	6.88	3.45	4.48	3.79					

Figure 4. Chromeleon CDS results view of ten replicates of soil spiked at 15 fg oc 2,3,7,8-TCDD. RSD% range is 3.5%–6.9% for congeners shown. Ion ratio performance meets the <15% deviation requirement.

Resolution performance at CSL and CS4 levels

Selectivity was further evaluated by assessing measured mass resolving power for each of the 17 target 2,3,7,8-substituted congeners at both the lowest undiluted calibration level (CSL) and at CS4. Figure 5 summarizes peak areas and the corresponding *m/z* resolution measured at 5% peak height for the first injection at each level.

Peak Name	Peak Area counts*s		m/z resolution 5% peak height	
First Injection	CSL	CS4	CSL	CS4
2378-TCDF	111475	48095554	23060	22916
2378-TCDD	71838	31227755	23111	22774
12378-PeCDF	453187	206532509	21818	22904
23478-PeCDF	463519	217386114	22978	22848
12378-PeCDD	238698	112045640	21578	21959
123478-HxCDF	389641	185151997	20894	20821
123678-HxCDF	402773	192522663	21928	20766
234678-HxCDF	387739	187061131	20829	20753
123478-HxCDD	207340	100532029	20794	21138
123678-HxCDD	213487	103987695	20782	21038
123789-HxCDD	230199	111957383	20751	21061
123789-HxCDF	370957	180874690	20889	20801
1234678-HpCDF	318461	155229864	20311	20274
1234678-HpCDD	195174	94364441	20538	20184
1234789-HpCDF	276246	137260521	20141	20400
OCDD	318446	165088774	19756	18943
OCDF	328350	197739465	19776	19754

Figure 5. Chromeleon CDS report view of *m/z* resolution at 5% peak height for all congeners in CSL (calibration solution lowest) and CS4 (calibration solution 4) exceeds regulation target of 10,000 with all at ~20,000.

Across all congeners, measured resolving power at CSL ranged from approximately 19,756 to 23,111, and from 18,943 to 22,916 at CS4. In all cases, the observed resolution exceeded 10,000, the resolving power required with EPA Method 1613. Achieving ~20,000 resolving power at 5% peak height provides a substantial margin above the minimum criterion, supporting effective separation of near-isobaric interferences and stable exact-mass extraction across both low- and mid-level calibration points.

Demonstrating consistent resolution performance at CSL is particularly important for regulatory workflows, as low-level standards represent the most challenging region for confirmation and quantitative reliability. The maintained resolving power at both CSL and CS4 complements the previously demonstrated ion ratio compliance (±15%) and response factor stability, providing evidence of selectivity consistent with the expectations of EPA Method 1613.

Conclusion

The results demonstrate that the Orbitrap Exploris GC S MS delivers performance consistent with the elements of EPA Method 1613 dioxin analysis in environmental matrices.

Performance characteristics relevant to EPA Method 1613 workflows include:

- **Ultra-trace sensitivity**
  2,3,7,8-TCDD was reproducibly detected at 15 fg on-column, with replicate injections demonstrating stable signal at the lowest evaluated level. Sensitivity performance and resolving power at this level support the expectations.
- **High mass resolution exceeding regulatory requirements**
  Measured resolving power at 5% peak height ranged from approximately 19,000–23,000 across all 17 congeners at both CSL and CS4, exceeding the 10,000 resolving power requirement specified for high-resolution dioxin analysis under EPA Method 1613. This provides enhanced selectivity against near-isobaric interferences.
- **Robust multi-level calibration performance**
  A 10-level calibration series demonstrated excellent linearity from the 10× diluted low-level standard through CS4, consistent with EPA Method 1613 expectations for multi-point calibration and response stability assessment.
- **Stable isotope dilution response factors**
  Response factor summaries across the calibration range showed ≤15% RSD for all 17 congeners, supporting reliable quantitative performance using isotope dilution principles.
- **Confirmatory ion ratio compliance**
  Quantifier-to-qualifier ion ratios were evaluated using a ±15% relative tolerance, with automated flagging in Chromeleon CDS. No out-of-tolerance results were observed across calibration or matrix replicate experiments, supporting confirmatory identification criteria.
- **Demonstrated repeatability in environmental matrix**
  Ten replicate injections of soil spiked at 15 fg on-column showed %RSD values of 3.5%–6.9%, with ion ratio confirmation maintained across replicates, demonstrating stable quantitative and confirmatory performance under matrix conditions.

Collectively, these results show that the Orbitrap Exploris GC S mass spectrometer provides the sensitivity, resolving power, calibration robustness, and confirmation control required for performance-based implementation of EPA Method 1613 dioxin analysis, while additionally offering the operational flexibility and HRAM selectivity advantages inherent to Orbitrap technology.

Reference

1. 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.

Appendix

Table A1. TRACE 1610 GC injection and oven program parameters.

Injection parameters	
Injector	SSL
Liner	SSL with quartz wool
Injection mode	Splitless
Injection volume (µL)	1
Split flow (mL/min)	50
Iinjection temperature (°C)	280
Splitless time (min)	1.2

Oven temperature program	
Initial temperature (°C)	140
Hold time (min)	1
Rate 1 (°C/min)	10
Temperature (°C)	220
Hold time (min)	10
Rate 2 (°C/min)	3
Temperature (°C)	235
Hold time (min)	7
Rate 3 (°C/min)	5
Temperature 3 (°C)	310
Hold time (min)	10
Temperature 4 (°C)	330
Total run time (min)	56

Table A2. Orbitrap Exploris GC S MS parameters.

MS parameters	
Transfer line (°C)	300
Ion source type and source temperature (°C)	NeverVent AEI, 350
Ionization type	EI
Tuning parameters	Autotune
C-Trap offset (V)	-5
Acquisition mode	tSIM
Isolation window width (*m/z*) *Native analytes* *Internal standards*	 8 4
Resolving power (at 200 *m/z*)	60,000
Emission current (µA)	50

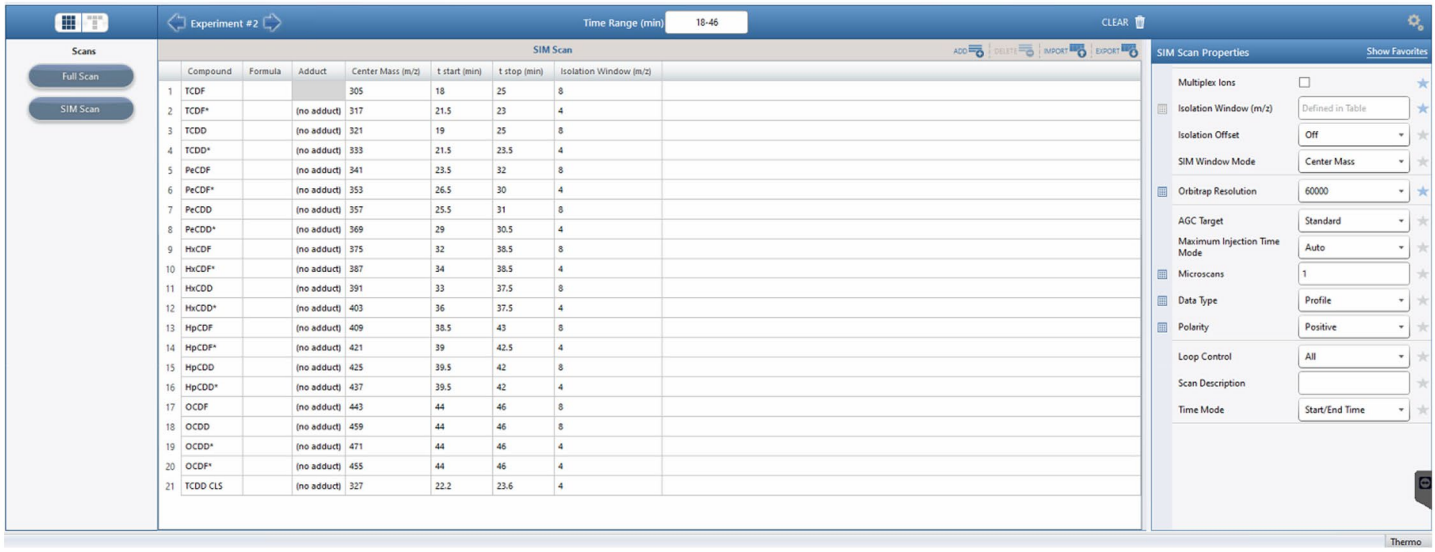


Figure A1. Orbitrap Exploris GC S MS SIM inclusion lists and scan parameters.

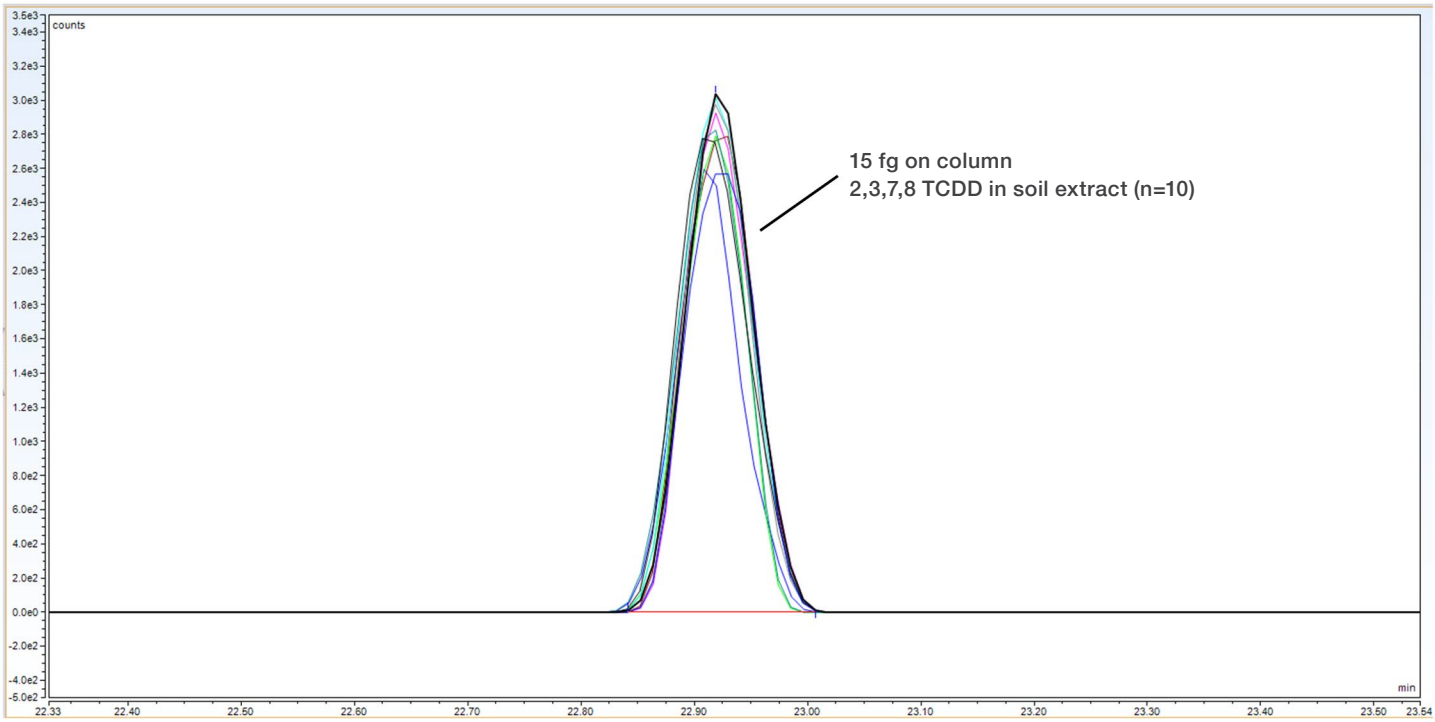


Figure A2. Extracted ion chromatograms for 2,3,7,8-TCDD at 15 fg on-column in soil (extracted mass: *m/z* 321.8936 ± 5 ppm), with ten replicate injections overlaid.

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