Application note | AN004451

Food safety

Polychlorinated dibenzo-*p*-dioxins/furans analysis in food and feed at regulatory limits using the Orbitrap Exploris GC S mass spectrometer

Authors

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Keywords

Dioxin analysis, PCDD/F, EU 2017/644, isotopic dilution, gas chromatography high resolution, accurate mass

Goal

The goal of this application note is to demonstrate the enhanced analytical performance of the Thermo Scientific™ Orbitrap Exploris™ GC S Mass Spectrometer for the determination of polychlorinated dibenzo-*p*-dioxins in food and feed with consideration of regulatory criteria.

Introduction

The analysis of polychlorinated dibenzo-*p*-dioxins/furans (PCDD/F) is performed under strict analytical criteria, where ultra-high sensitivity and selectivity are critical due to the toxicity of these compounds at low concentrations. For many years, magnetic sector high-resolution mass spectrometers (GC-HRMS) have been considered the gold standard for regulatory compliance with mass resolution (R) capabilities to avoid isobaric interferences (R ≥ 10,000 at 5% peak height) at picogram levels. However, advancements in mass spectrometry have seen laboratories moving towards alternative technologies that offer equivalent or improved performance, but at a reduced cost and with less operational complexity.

The Orbitrap Exploris GC S mass spectrometer has been designed to address the needs of laboratories providing regulatory-compliant sensitivity and enhanced selectivity. Incorporation of the Thermo Scientific™ NeverVent™ Advanced Electron Ionization Source (AEI) from the Thermo Scientific™ TSQ™ 9610 GC-MS/MS enhances the Orbitrap Exploris GC platform to deliver low-femtogram dioxin sensitivity.

The high-resolution accurate mass capabilities of Thermo Scientific™ Orbitrap™ technology enable routine analysis with mass resolution 60,000 (full-width half-maximum (FWHM) at *m/z* 200) and sub-ppm mass accuracy (<1 ppm, internal correction), readily meeting the analytical requirements outlined in Commission Regulation (EU) 2017/644 for dioxin analysis in food and feed[1]. These requirements include:

- Mass resolution ≥10,000 at 5% peak height
- Isotopic dilution mass spectrometry
- Detection at low pg/g (part per trillion) levels with LOQ ≤1/5 maximum levels (MLs) allowed in food/feed
- Ion ratio deviation from the theoretical value ±15%
- Measurement uncertainty ≤25% from calibration response

In this study, the performance of the Orbitrap Exploris GC S mass spectrometer in delivering precise and accurate analysis of dioxin in food and feed previously determined by GC-HRMS was investigated in view of compliance criteria defined within EU 2017/644.

Experimental

Sample and standard preparation

Various types of food (butter, laying hen fat, sunflower and fish oils) and feed (edible flowers and dill tips), along with a laboratory control spike, were extracted at Wageningen Food Safety Research (WFSR) using accelerated solvent extraction (Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor) for feed material, while fats and oils were treated with sulfuric acid for lipid degradation. The organic supernatant was then back extracted into hexane with further purification using an automated solid phase multi-column cleanup (acidic silica, alumina, and carbon) setup on the DEXTech™ Plus system (LCTech GmbH). The first fraction collected containing ortho-PCBS and PCDD/Fs was concentrated to a final volume of 50 µL in nonane. All extracts were spiked with ^{13}C-isotopically labeled standards prior to extraction with ^{13}C-1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TCDD) and ^{13}C-1,2,3,7,8,9 hexachlorodibenzo-*p*-dioxin (HxCDD) added as syringe standard prior to shipment for analysis.

A six-level calibration standard curve was prepared using the EN1948 isotopically labeled standard mixtures from Wellington Laboratories (Canada). All standards (EN1948 CSL, CS1-4) were diluted by a factor of 10. The 1/10 dilution of the EN1948 CSL standard was diluted by another factor of 2 to produce to produce a 6-point calibration ranging for tetra- (0.002–1.6 pg/µL), penta- and hexa- (0.004–3.2 pg/µL), and hepta- and octa- (0.008–6.4 pg/µL) chlorinated congeners.

Instrumental method

The instrumental setup consisted of the Orbitrap Exploris GC S mass spectrometer connected to Thermo Scientific™ TRACE™ 1610 GC equipped with a Thermo Scientific™ TraceGOLD™ TG-Dioxin Film Capillary Column, 60 m x 0.25 mm I.D. x 0.25 µm (Cat. No. 26066-1540) for chromatographic separation. Sample injection was carried out using a Thermo Scientific™ TriPlus™ RSH Smart Autosampler with large volume injection into a Thermo Scientific™ iConnect™ Programmable Temperature Vaporizing (iConnect-PTV) Injector. Injection and oven parameters are summarized in Appendix Table A1. MS acquisition was carried out using timed single ion monitoring (tSIM) with acquisition parameters outlined in Appendix Table A2.

Data acquisition and processing was performed using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). Within Chromeleon CDS, the HRAM Dioxin eWorkflow v1.0 was used to automate acquisition method setup (Figure 1). In addition, data processing methods using isotopic dilution quantitation as required by EU 2017/644 are preinstalled along with templates for both data evaluation and reporting to streamline data processing. An example of this is shown in Figure 2 for a sample extract of edible flowers. An integrated data reviewing template provides extracted exact mass windows of both quantification and qualifier ions of 2,3,7,8 TCDD detected at 0.019 WHO-TEQ pg/g and its mass-labeledinternal standard, calibration curve, and ion ratio deviation for all congeners detected within the sample extract.

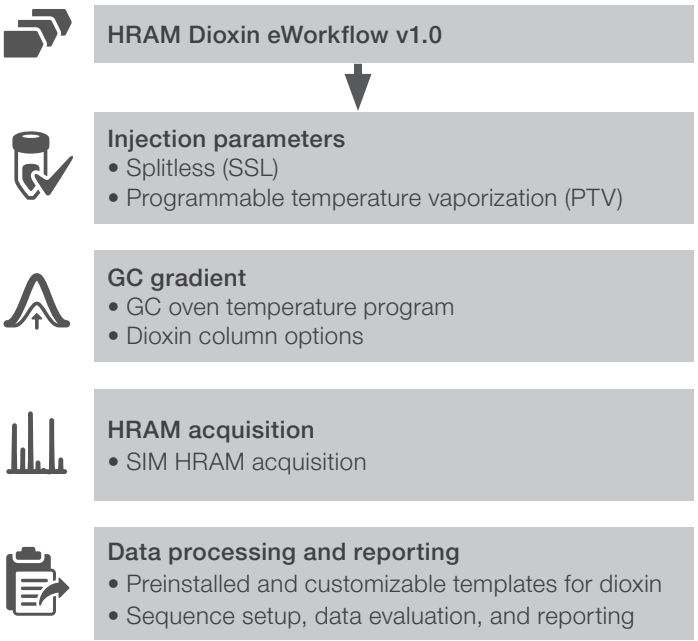
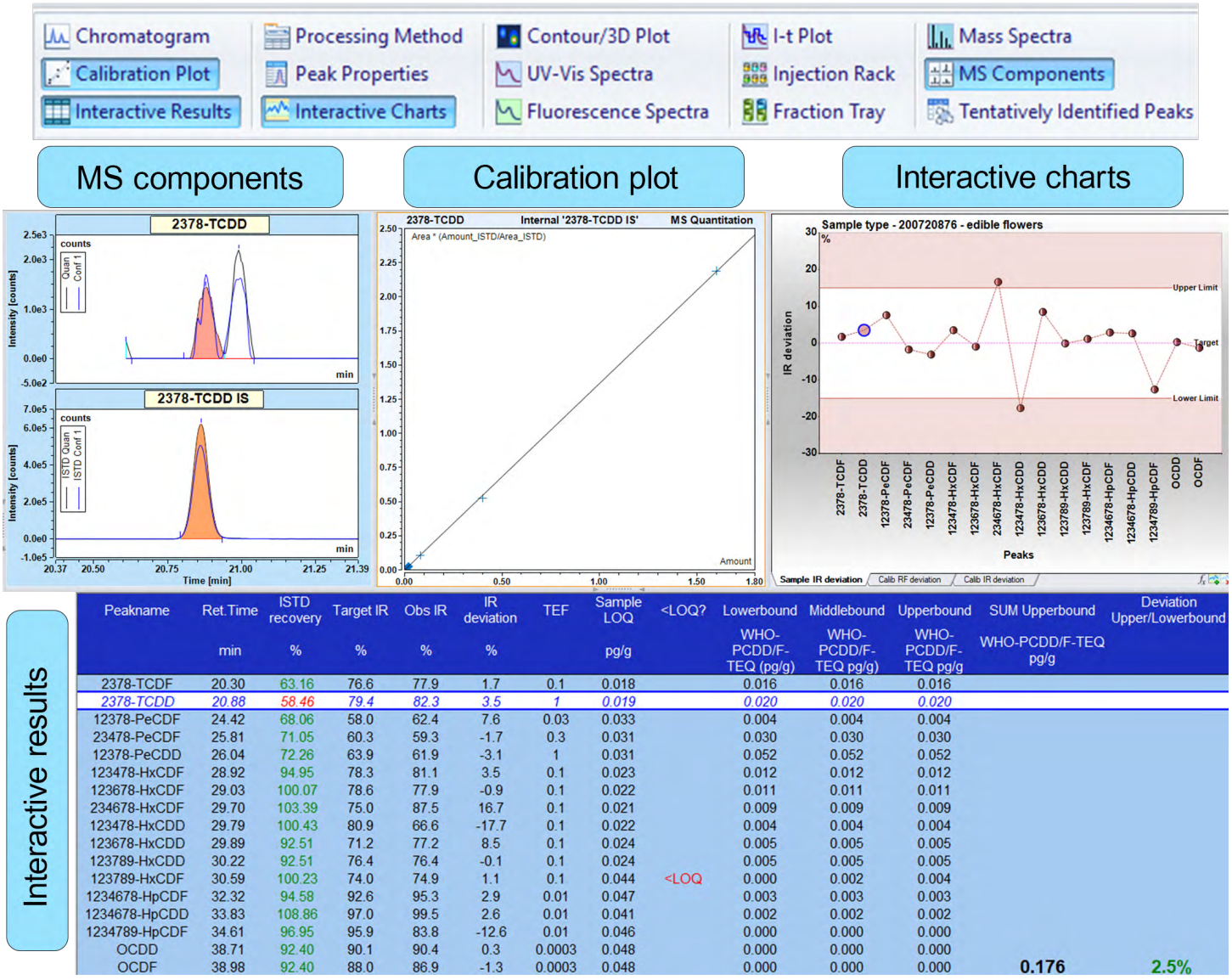


Figure 1. Schematic overview of Dioxin HRAM eWorkflow contents within Chromeleon CDS.



Interactive results

Peakname	Ret.Time	ISTD recovery	Target IR	Obs IR	IR deviation	TEF	Sample LOQ	<LOQ?	Lowerbound	Middlebound	Upperbound	SUM Upperbound	Deviation Upper/Lowerbound
	min	%	%	%	%		pg/g		WHO-PCDD/F-TEQ (pg/g)	WHO-PCDD/F-TEQ pg/g)	WHO-PCDD/F-TEQ pg/g	WHO-PCDD/F-TEQ pg/g	
2378-TCDF	20.30	63.16	76.6	77.9	1.7	0.1	0.018		0.016	0.016	0.016		
2378-TCDD	*20.88*	*58.46*	*79.4*	*82.3*	*3.5*	*1*	*0.019*		*0.020*	*0.020*	*0.020*		
12378-PeCDF	24.42	68.06	58.0	62.4	7.6	0.03	0.033		0.004	0.004	0.004		
23478-PeCDF	25.81	71.05	60.3	59.3	-1.7	0.3	0.031		0.030	0.030	0.030		
12378-PeCDD	26.04	72.26	63.9	61.9	-3.1	1	0.031		0.052	0.052	0.052		
123478-HxCDF	28.92	94.95	78.3	81.1	3.5	0.1	0.023		0.012	0.012	0.012		
123678-HxCDF	29.03	100.07	78.6	77.9	-0.9	0.1	0.022		0.011	0.011	0.011		
234678-HxCDF	29.70	103.39	75.0	87.5	16.7	0.1	0.021		0.009	0.009	0.009		
123478-HxCDD	29.79	100.43	80.9	66.6	-17.7	0.1	0.022		0.004	0.004	0.004		
123678-HxCDD	29.89	92.51	71.2	77.2	8.5	0.1	0.024		0.005	0.005	0.005		
123789-HxCDD	30.22	92.51	76.4	76.4	-0.1	0.1	0.024		0.005	0.005	0.005		
123789-HxCDF	30.59	100.23	74.0	74.9	1.1	0.1	0.044	<LOQ	0.000	0.002	0.004		
1234678-HpCDF	32.32	94.58	92.6	95.3	2.9	0.01	0.047		0.003	0.003	0.003		
1234678-HpCDD	33.83	108.86	97.0	99.5	2.6	0.01	0.041		0.002	0.002	0.002		
1234789-HpCDF	34.61	96.95	95.9	83.8	-12.6	0.01	0.046		0.000	0.000	0.000		
OCDD	38.71	92.40	90.1	90.4	0.3	0.0003	0.048		0.000	0.000	0.000		
OCDF	38.98	92.40	88.0	86.9	-1.3	0.0003	0.048		0.000	0.000	0.000	0.176	2.5%

Figure 2. Interactive data processing layout of the Dioxin HRAM eWorkflow within Chromeleon CDS with full overview of exact mass windows (MS components), calibration plots, and ion ratio performance (Interactive charts) for 2,3,7,8 TCDD detected in edible flowers at 0.019 pg/g. The interactive results table provides complete analysis overview on internal standard recovery, ion ratio deviation, sample LOQ, Sum Upperbound concentration, and Upper/Lowerbound deviation.

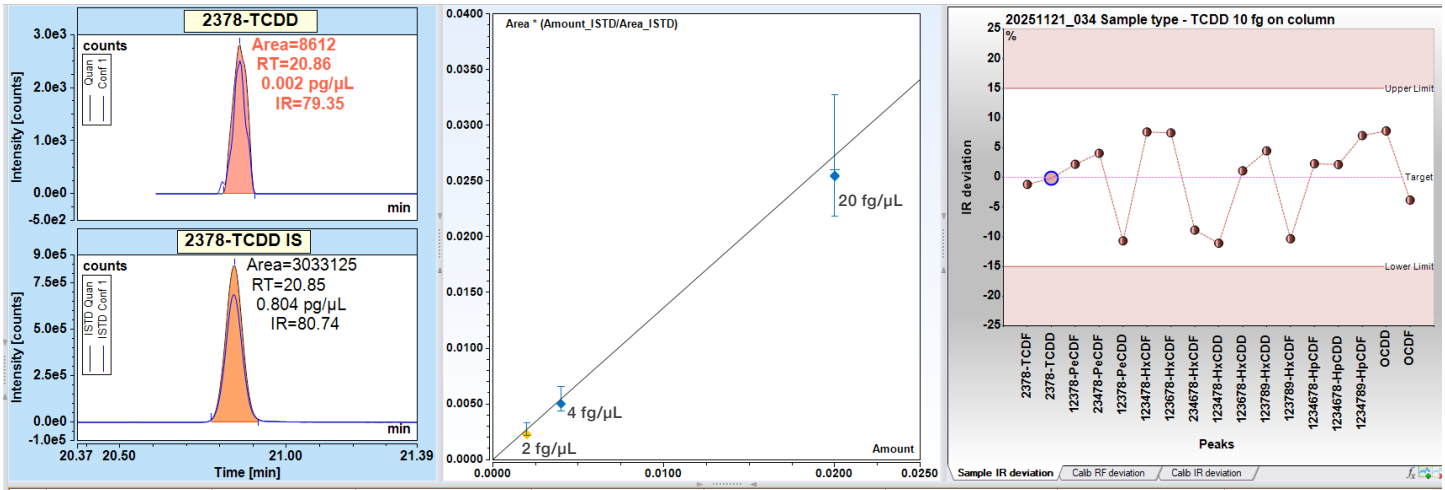
Additional information surrounding the internal standard recovery, sample LOQ, Sum Upperbound concentration, and Upper/Lowerbound deviation are automatically calculated and provided in the summary table.

Results and discussion

Compliant sensitivity and quantitation

Consistent trace level sensitivity is critical for instrumentation to deliver compliant and accurate results in dioxin analysis. This criterion is often evaluated based on detection of 2,3,7,8 TCDD due to its extreme toxicity. Accurate quantitation was achieved down to 2 fg/µL (10 fg on column with 5 µL injection) and within 25% of the calibration response. Check standards at 4 fg/µL (20 fg on column) and 20 fg/µL (100 fg on column) (Figure 3) were also within 25% tolerance limits, as required by EU 2017/644 for measurement uncertainty. Ion ratios between quantifier and qualifier ions were within the ±15% criterion of the target ion ratio for all calibration and check standards, demonstrating compliance at fg level concentrations. Sample LOQs on a congener basis were defined at the lowest concentration in which the above-mentioned criteria were met and are provided in Appendix Table A3.

Linear calibration response was observed for all congeners across the calibration range investigated with all compounds showing correlation coefficients >0.999 with % RSD ≤2.25%. Laboratories are required to show that the response factor across all standards must be within 20% of the average response factor (AvRF) over the course of the analysis. This can also be viewed in the interactive charts function of the data processing layout, which has been preformatted based on method criteria to show compliance (Figure 4).



	A	B	C	D	E	F	G	H
1	Comment	Quant. Area	Conf. Area	Amount	Target IR	Conf. Ratio	IR deviation	Conf. Result
2		counts*sec	counts*sec	pg/µL		%	%	
3		MS Quantitation Peak	MS Quantitation Peak	MS Quantitation Peak	MS Quantitation Peak	MS Quantitation Peak	MS Quantitation Peak	MS Quantitation Peak
4		2378-TCDD	2378-TCDD	2378-TCDD	2378-TCDD	2378-TCDD	2378-TCDD	2378-TCDD
5	TCDD 20 fg on column	18455	15869	0.004	79.45	85.99	8.24	Confirmed
6	TCDD 100 fg on column	106065	83665	0.019	79.45	78.88	-0.71	Confirmed
7	TCDD 100 fg on column	98534	77459	0.019	79.45	78.61	-1.05	Confirmed
8	*TCDD 10 fg on column*	*8612*	*6833*	*0.002*	*79.45*	*79.35*	*-0.13*	*Confirmed*

Figure 3. Extracted ion chromatogram of 2,3,7,8-TCDD and mass-labeled internal standard together with expanded view of lower calibration range and congener ion ratio performance. Error bars represent 25% tolerance level from the calibration response for check standards at 2 fg/µL (10 fg on column), 4 fg/µL (20 fg on column), and 20 fg/µL (100 fg on column). Complete overview of response, quantified amount and ion ratio performance are provided in the summary table.

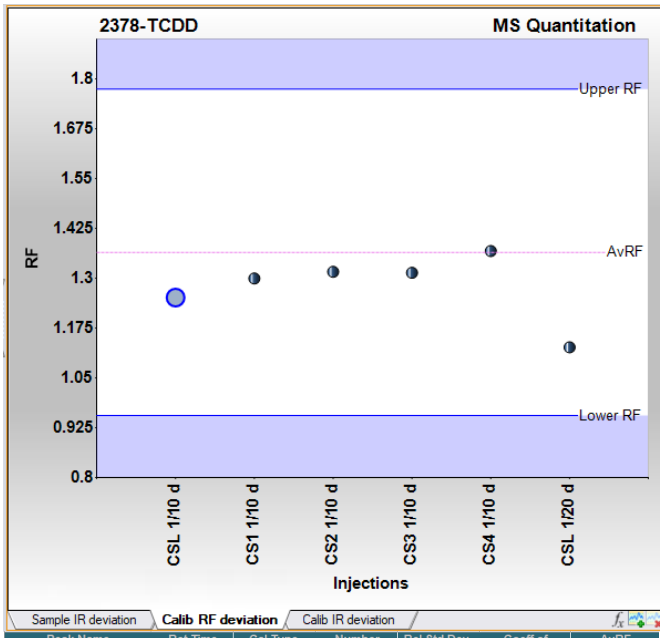
Enhanced mass selectivity for data confidence

Mass resolving power of ≥10,000 at 5% peak height is required by EU 2017/644 to avoid potential isobaric interferences on PCDD/F analysis from other co-extracted contaminants. This criterion is required across the entire mass range. As mass resolution decreases with increasing mass-to-charge ratio with Orbitrap technology, fulfilment of this criterion is 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 fulfil mass resolution criteria for OCDD is approximately 23,000 (Figure 5). Analysis with the Orbitrap Exploris GC S MS is routinely operated at 60,000 mass resolution, providing a mass resolution of approximately 40,000 for OCDD—nearly double the required resolution for compliance.

Accurate determination at and below maximum levels

Maximum levels for dioxins within food and feed are set to strict levels within the European Union to ensure both human and animal safety.[3,4] Limits vary depending on the matrix investigated. Meat and meat-related products are set between 1 to 5 pg WHO-TEQ/g. Levels within feed items, such as edible flowers and dill tips, are set at 0.75 pg WHO-TEQ/g.

The European Commission has planned to lower these limits further within feedstuffs based on recent risk assessment.[5] Although these new levels have not yet been implemented, it highlights the importance for instrumentation to deliver accurate results at current maximum levels and lower. Food and feed samples previously extracted and analyzed with a



Peak Name	Ret.Time min	Cal.Type	Number of Points	Rel.Std.Dev. %	Coeff.of Determination	AvRF
2378-TCDF	20.29	Lin	6	0.99	0.9999700851	1.402
2378-TCDD	*20.87*	*Lin*	*6*	*2.03*	*0.9998746935*	*1.365*
12378-PeCDF	24.41	Lin	6	1.12	0.9999961872	2.030
23478-PeCDF	25.79	Lin	6	1.14	0.9999598958	1.413
12378-PeCDD	26.03	Lin	6	0.41	0.9999947003	1.239
123478-HxCDF	28.91	Lin	6	1.12	0.9999615012	1.615
123678-HxCDF	29.02	Lin	6	1.62	0.9999201549	1.564
234678-HxCDF	29.70	Lin	6	1.71	0.9999108259	2.561
123478-HxCDD	29.78	Lin	6	1.02	0.9999675192	1.320
123678-HxCDD	29.88	Lin	6	1.13	0.9999611174	1.354
123789-HxCDD	30.22	Lin	6	1.84	0.9998964279	1.534
123789-HxCDF	30.60	Lin	6	1.93	0.9998869077	1.536
1234678-HpCDF	32.32	Lin	6	0.36	0.9999960491	1.714
1234678-HpCDD	33.82	Lin	6	1.40	0.9999403028	1.342
1234789-HpCDF	34.61	Lin	6	0.65	0.9999869793	1.747
OCDD	38.72	Lin	6	1.57	0.9999249789	1.308
OCDF	38.99	Lin	6	1.77	0.999905268	1.748
				2.0315 0.3579	1.00000 0.99987	

Figure 4. Calibration response factor (RF) deviation across all calibration points displayed in the interactive charts function of the Dioxin eWorkflow data processing layout. Coefficient of determination, % RSD, and AvRF for each congener are summarized in the calibration tab of the interactive results table.

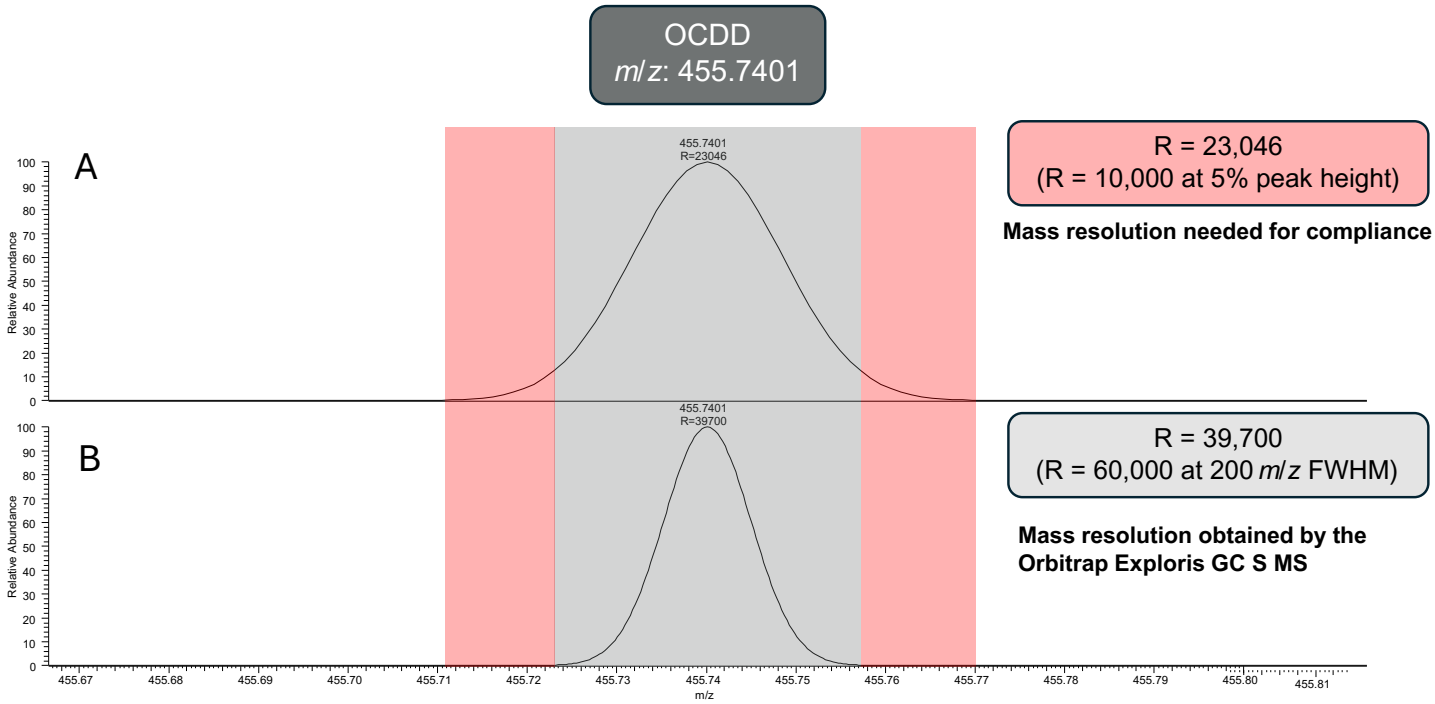


Figure 5. Simulated mass resolution (R) of OCDD at (A) 10,000 at 5% peak height and (B) 60,000 at *m/z* 200 FWHM.

Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS were analyzed using the Orbitrap Exploris GC S MS. Comparison of the results showed good agreement between the two techniques for not only matrices that exceeded regulatory thresholds (i.e., sunflower oil), but also for matrices well below maximum levels (Figure 6). According to Regulation (EU) 2017/644, sample LOQs have to be ≤1/5 ML for the corresponding food matrix. The Sum Upperbound concentration of individual food/feed items investigated in this study are presented in Table 1. All sample LOQs for classified food items are below the 1/5th ML. Feed sample items also show LOQs below the 1/5th ML, demonstrating compliance with the regulation even though it is not mandatory for items classified as feed. These results illustrate the enhanced sensitivity of the Orbitrap Exploris GC S MS delivers compliant trace-level detection needed for PCDD/F under EU 2017/644.

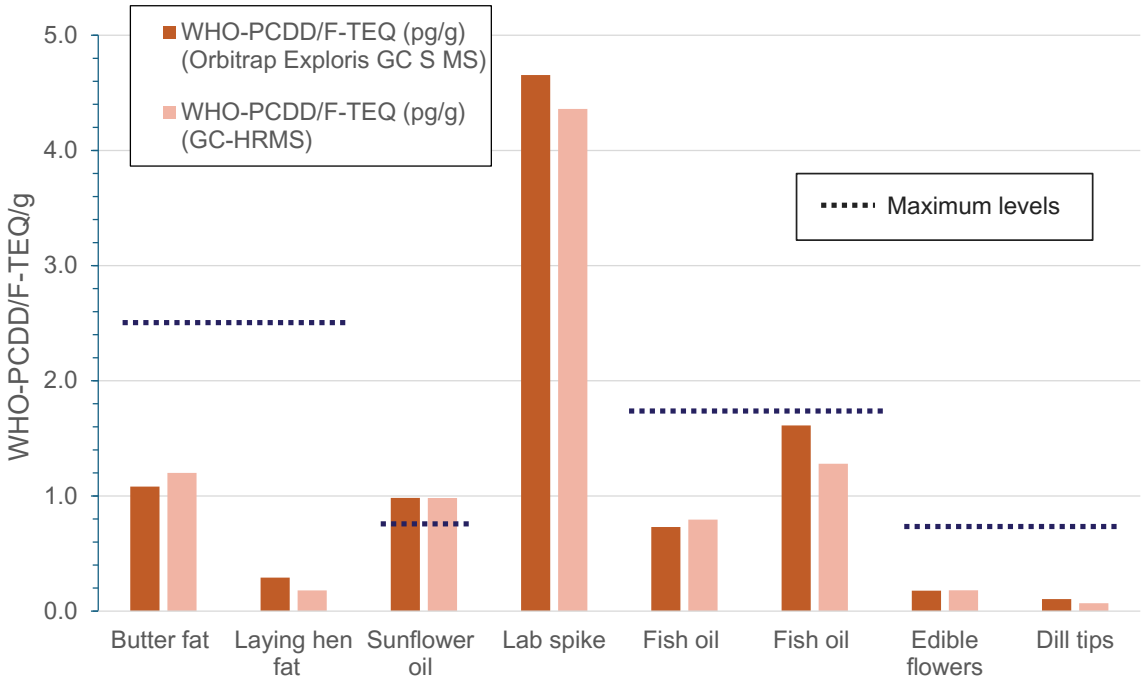


Figure 6. Comparison of concentrations (WHO-PCDD/F TEQ/g) of PCDD/F in various food and feed items determined by HRMS and Orbitrap Exploris GC S MS. Dotted lines represent current maximum levels for PCDD/F.

Table 1. Comparison of sample LOQ for food/feed matrices, maximum levels (ML) according to Regulation (EU) 2023/915 and Directive 2002/32/EC, and 1/5th ML for compliance with Regulation (EU) 2017/644.

Sample ID	Sample mass (g)	Extract volume (µL)	Sample LOQ (WHO-PCDD/F-TEQ pg/g)	ML (WHO-PCDD/F-TEQ pg/g)	1/5th ML (WHO-PCDD/F-TEQ pg/g)
Butter (food)	2.45	50	0.229	2.5	0.5
Laying hen fat (food)	2.42	50	0.232	2.5	0.5
Fish oil (food)	2.42	50	0.232	1.75	0.35
Edible flowers (feed)	9.04	50	0.062	0.75	0.15
Dill tips (feed)	9.16	50	0.061	0.75	0.15

The analysis by GC-HRMS injected twice as much extract on column compared to the analysis by the Orbitrap Exploris GC S MS. This highlights the enhanced sensitivity to deliver accurate and compliant data to meet both current and future lower regulatory limits.

Conclusion

The results presented here show the enhanced detection capability of the Orbitrap Exploris GC S MS to deliver sensitive and accurate determination of PCDD/F that is compliant with EU Regulation 2017/644.

- The Dioxin HRAM eWorkflow provides preinstalled isotopic dilution quantification processing methods together with comprehensive data review layouts for analysts to assess and report data compliance.
- Enhanced sensitivity of the NeverVent AEI ion source provided accurate quantitation down to 2 fg/µL (10 fg on column) while meeting analytical criteria with respect to deviation in ion ratio (±15%) and calibration response (±25%).
- Good agreement was observed between GC-HRMS magnetic sector and the Orbitrap Exploris GC S mass spectrometer at concentrations near and below current regulatory limits, highlighting the enhanced detection capability of the Orbitrap Exploris GC S MS to deliver accurate results at ultra-trace concentrations.
- Sample LOQs below 1/5th of ML levels in food were achieved as required by EU 2017/644.

References

1. Commission Regulation (EU) 2017/664 (2017). Laying down method of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and no-dioxin-like PCBs in certain foodstuffs and repeal Regulation (EU) No. 589/2014.
2. Commission Regulation (EU) 2017/771 (2017). Amending Regulation (EC) no. 152/2009 as regards the methods for the determination of the levels of dioxins and polychlorinated biphenyls.
3. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.
4. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.
5. World Trade Organization. (2023) Committee on Sanitary and Phytosanitary measures. https://docs.wto.org/dol2fe/Pages/SS/directdoc.aspx?filename=q:/G/SPS/NEU703.pdf&Open=True

Appendix

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

Injection parameters	
Injection	Thermo Scientific™ iConnect™ PTV
Liner	Thermo Scientific™ LinerGOLD™ PTV Liner, Concentric Baffles (Cat. No. 453T2845-UI)
Inlet mode	Large volume
Injection volume (µL)	5
Split flow (mL/min)	100
Iniital injection temperature (°C)	75
Injection time (min)	0.4
Transfer rate (°C/s)	5.0
Final temperature (°C)	300
Splitless time (min)	1
Cleaning phase temperature (°C)	320
Cleaning phase flow rate (mL/min)	200
Cleaning phase hold time (min)	Maintain

Oven temperature program	
Initial temperature (°C)	140
Hold time (min)	2
Rate 1 (ºC/min)	25
Temperature 1 (°C)	250
Rate 2 (ºC/min)	2.5
Temperature 2 (°C)	260
Hold time (min)	5
Rate 3 (ºC/min)	2.5
Temperature 3 (°C)	280
Rate 4 (ºC/min)	10
Temperature 4 (°C)	330
Hold time (min)	15

Table A2. Orbitrap Exploris GC S mass spectrometer parameters.

Orbitrap Exploris GC S mass spectrometer 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
Aquisition mode	tSIM
Isolation window width *(m/z)*	
Native analytes	8
Internal standards	4
Resolving power (at 200 *m/z*)	60,000
Emission current (µA)	50
Mass accuracy on lock mass	5 ppm
Lock masses (*m/z*)	73.0468 133.01356 207.03235 225.04292 281.05114 299.06171 355.06993

Table A3. Limits of quantification for PCDD/F.

Congener	Calculated LOQ (pg/µL)
2378-TCDF	0.002
2378-TCDD	0.002
12378-PeCDF	0.004
23478-PeCDF	0.004
12378-PeCDD	0.004
123478-HxCDF	0.004
123678-HxCDF	0.004
234678-HxCDF	0.004
123478-HxCDD	0.004
123678-HxCDD	0.004
123789-HxCDD	0.004
123789-HxCDF	0.008
1234678-HpCDF	0.016
1234678-HpCDD	0.016
1234789-HpCDF	0.016
OCDD	0.016
OCDF	0.016

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