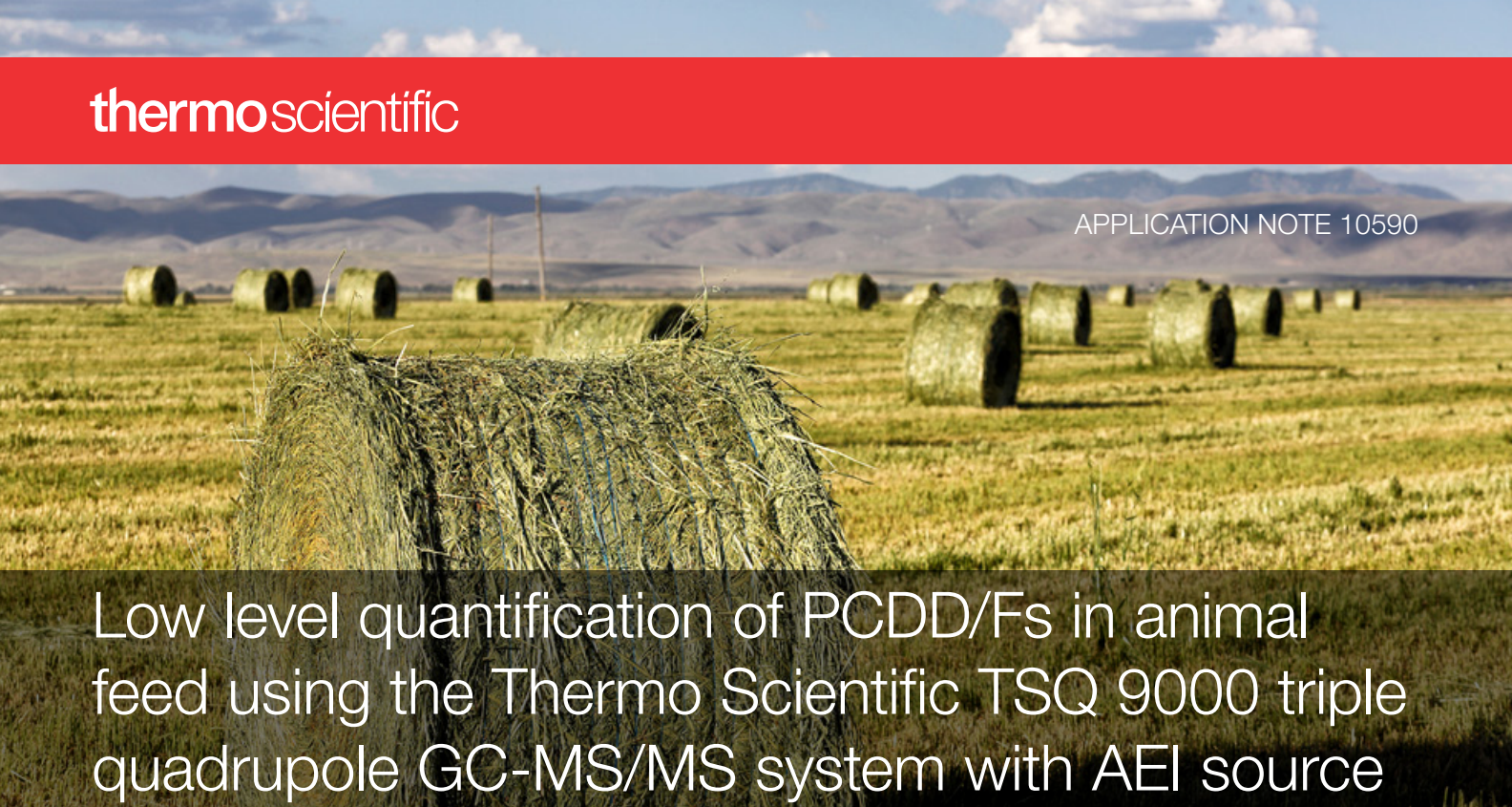




Low level quantification of PCDD/Fs in animal feed using the Thermo Scientific TSQ 9000 triple quadrupole GC-MS/MS system with AEI source

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Keywords

GC-MS/MS, persistent organic pollutants (POPs), sensitivity, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzo-*p*-furans, confirmatory analysis, TSQ 9000, advanced electron ionization, AEI, Dionex ASE 350 Accelerated Solvent Extractor

Goal

To demonstrate that a sensitive and robust quantitative analysis of PCDD/Fs, compliant with EU regulations—specifically related to ion ratios (IR) and response factor (RF) thresholds—was achievable in a routine environment using the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system.

Introduction

Recent developments in gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) technologies have allowed their use for confirmatory analysis and control of maximum levels (MLs) and action levels (ALs) of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in certain food and feed samples, according to current EU regulations 644/2017 and 771/2017.^{1,2}

Gas chromatography – high-resolution mass spectrometry (GC-HRMS) employs high mass resolution to provide the high selectivity required for the separation of toxic congeners from any interfering matrix components. By contrast, triple quadrupole tandem mass spectrometry (GC-MS/MS) utilizes highly selective, compound-dependent precursor-to-product transitions to obtain total ion chromatograms (TICs) with little or no interference from non-related matrix components.

Since the regulatory changes in Europe in 2014,¹ sensitivity has often been the primary focus when demonstrating GC-MS/MS performance for confident (and regulatory compliant) confirmation of the presence of dioxins and dioxin-like compounds. Sensitivity is an important performance criterion, but other factors have to be taken into account for the evaluation of instrument performance for routine work. For example, sample weight to solvent volume ratio and injection volume will influence whether a method (and system) is fit for purpose in a routine environment.

In this study, the performance of the TSQ 9000 triple quadrupole GC-MS/MS system equipped with the new Advanced Electron Ionization (AEI) source was evaluated for the analysis of PCDD/Fs in solvent standards and real food/feedstuff samples. Suitably set LOQs³ were employed to show compliance to one-fifth maximum levels and demonstrate the sensitivity, selectivity, and robustness required to operate in a routine environment.

Experimental Samples

Animal feedstuff samples, relevant quality control samples, and procedural blanks were provided by the University of Liege. Sample types, weights, and maximum levels allowed (ML)⁴ are given in Table 1. European Method EN:1948 standard solutions (Wellington Laboratories Inc., Canada) were utilized for initial performance tests, and for calibration and quantitation.

Extraction

Sample preparation was performed following previously described extraction and cleanup methods.⁶ Extraction (where required) was performed by the accelerated solvent extraction technique, with cleanup using an offline multi-layered acidic silica column step and the PowerPrep™ automated system (FMS Inc.) with a three-column set (multi-layered ABN silica, basic alumina, and carbon columns). The final extract volume and solvent was 10 µL of nonane.

In the experiments described here, a TSQ 9000 triple quadrupole mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph. Injection of liquid samples was performed automatically using a Thermo Scientific™ TriPlus™ RSH autosampler with bottom sensing enabled. The mass spectrometer was tuned automatically and calibrated using perfluorotributylamine (PFTBA). The mass spectrometer was operated in Selected Reaction Monitoring (SRM) mode, with dwell time priority given to the specific transitions of native dioxin and furan congeners. Collision energies were optimized for both labeled and native PCDD/F congeners using the AutoSRM function. Additional details regarding the GC and MS/MS conditions are given (Table 2 and Appendix A). Acquisition, processing, and reporting of the data were performed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

Table 1. Sample details and maximum levels for each commodity.

	Alfalfa	Pork Fat	Premix 1	Premix 2	Sheep	QC – Pork Fat	QC – Feed (grass)
Sample intake (g)	32.13	4.57	10.17	11.1	2.55	6.8	20.71
Regulatory ML (WHO-PCDD/F-TEQ/g)*	0.75	1	1	1	2.5	1	0.75**
1/5th ML (WHO-PCDD/F-TEQ/g)*	0.15	0.2	0.2	0.2	0.5	0.2	0.15

* Maximum limits taken from European Directive 2002/32/EC⁴

** Lower limit applied

Table 2. GC and MS conditions.

TRACE 1310 GC System Parameters	
Injection Volume (μL):	2
Liner:	Thermo Scientific™ LinerGOLD™ single taper with wool (P/N: 453A0924-UI)
Inlet (°C):	280
Carrier Gas (mL/min):	He, 1.2
Inlet Mode:	Splitless (split flow 120 mL/min after 2 min)
Column:	Thermo Scientific™ TraceGOLD™ TG-Dioxin (60 m × 0.25 mm, 0.25 μm) (P/N: 26066-1540)
Oven Temperature Program	
Temperature 1 (°C):	120
Hold Time (min):	2
Rate 1 (°C/min):	25
Temperature 2 (°C):	250
Hold Time (min):	0
Rate 2 (°C/min):	2.5
Temperature 3 (°C):	285
Hold Time (min):	0
Rate 3 (°C/min):	10
Temperature 4 (°C):	320
Hold Time (min):	15
Total Run Time (min):	39.7
TSQ 9000 Mass Spectrometer Parameters	
Transfer Line (°C):	300
Ionization Type:	EI
Ion Source (°C):	300
Electron Energy (eV):	50
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (×10 – natives HIGH, labeled LOW)
Tuning parameters:	AEI Full Tune (Default)
Collision gas and pressure (psi):	Argon, 70
Resolution:	0.7 Da @ FWHM (both Q1 and Q3)

The dwell time given to the native congeners is longer than for the relevant labeled congeners used as internal standards for syringe/recovery correction. This is because labeled congeners are generally added in the samples at much higher levels than their corresponding natives. In this study, a dwell time prioritization was used for the native congeners, providing maximum sensitivity, while retaining the scan rate needed for accurate quantitation of the labeled congeners.

All comparisons made between GC-MS/MS and GC-HRMS contrast a 2 μL SSL injection made on the TSQ 9000 GC-MS/MS system with a 5 μL PTV injection made on a Waters™ Autospec Premier™ (Milford, USA).

Results and discussion

Chromatography

The TraceGOLD TG-Dioxin column provided excellent separation for TCDD/F (Figure 1) and PeCDD/F congeners and chromatographic resolution compliant with current EU regulations⁸ (Figure 2).

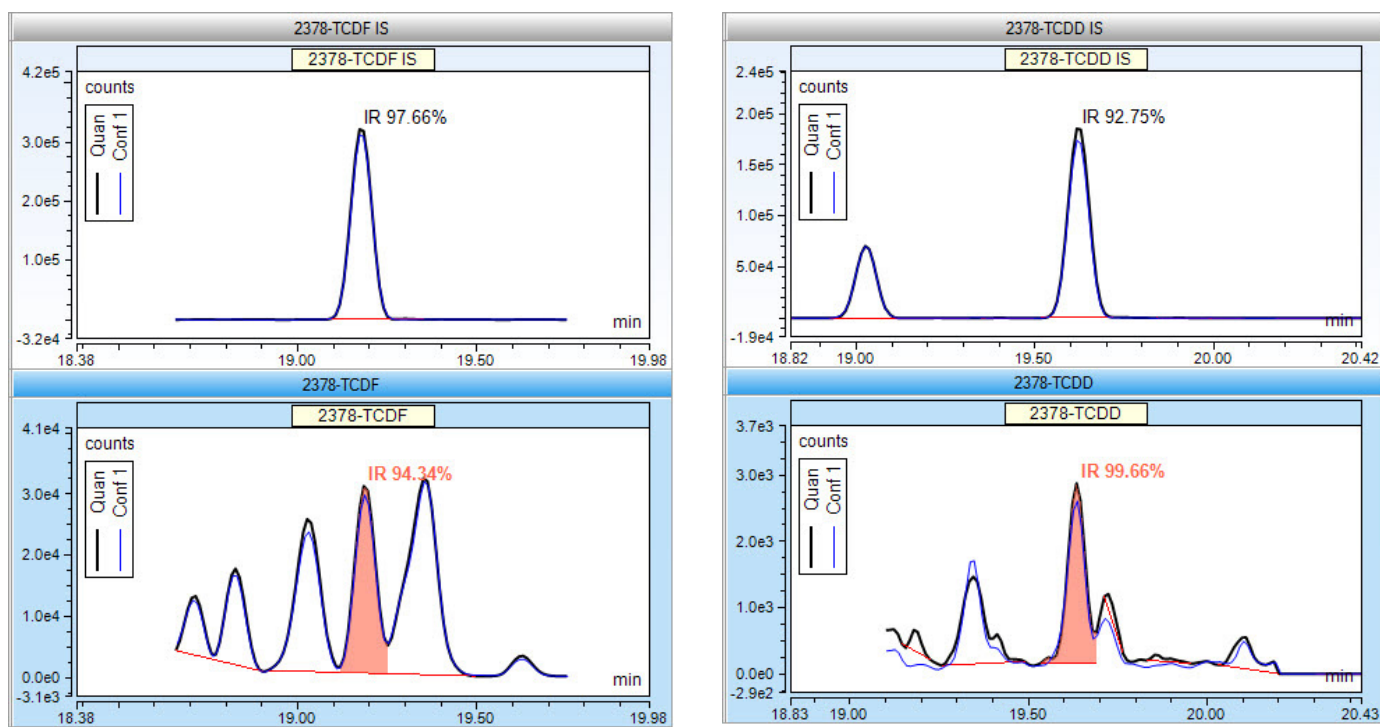


Figure 1. TCDD/F congener separation on the TraceGOLD TG-Dioxin (60 m × 0.25 mm, 0.25 μm) column (QC feed sample: 0.65 pg TCDF and 0.08 pg TCDD [on-column amounts]). Top: quantifier and qualifier ion overlaid chromatograms for ¹³C labeled 2,3,7,8-TCDF and 2,3,7,8-TCDD. Bottom: quantifier and qualifier ion overlaid chromatograms for native 2,3,7,8-TCDF and 2,3,7,8-TCDD, potentially interfering congeners are well resolved.

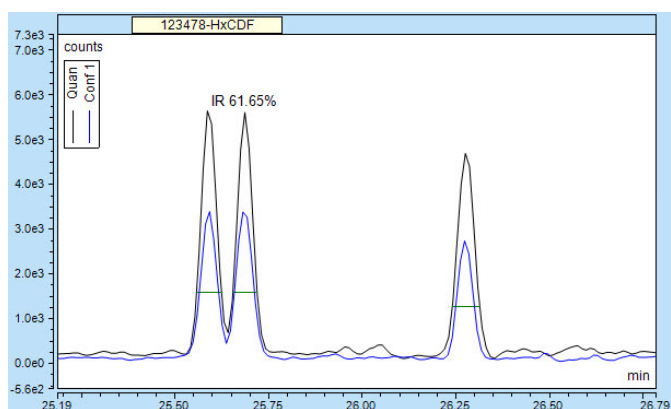


Figure 2. HxCDD/F congener separation on the TraceGOLD TG-Dioxin (60 m × 0.25 mm, 0.25 μm) column (CSL standard 0.08 pg/μL, on column concentration 0.16 pg). Quantifier ion peak heights of 25% are marked in green.

Sensitivity

Instrumental detection limits (IDLs) are typically determined in two ways: by calculating the signal-to-noise ratio of a peak, or statistically, using a series of repeat injections. In the absence of noise generated for many transitions in GC-MS/MS analysis, the latter is usually performed. In this way, IDL is focused on a single compound using a limited number of transitions. Figure 3 shows the IDL performance of the TSQ 9000 GC-MS/MS system for 2,3,7,8-TCDD using repeated injections (n=8) of a solvent standard equivalent to 5 fg on-column.

However, this approach is not ideal for comparison of real-world methods used in routine control, as several transitions must be used (for confirmation of both native and labeled congeners) and ion ratio and response factor criteria must be adhered to. Due to measurement uncertainty at such low levels, these criteria are difficult to meet at the IDL, therefore, it is more reasonable to define a method limit of quantitation (LOQ).

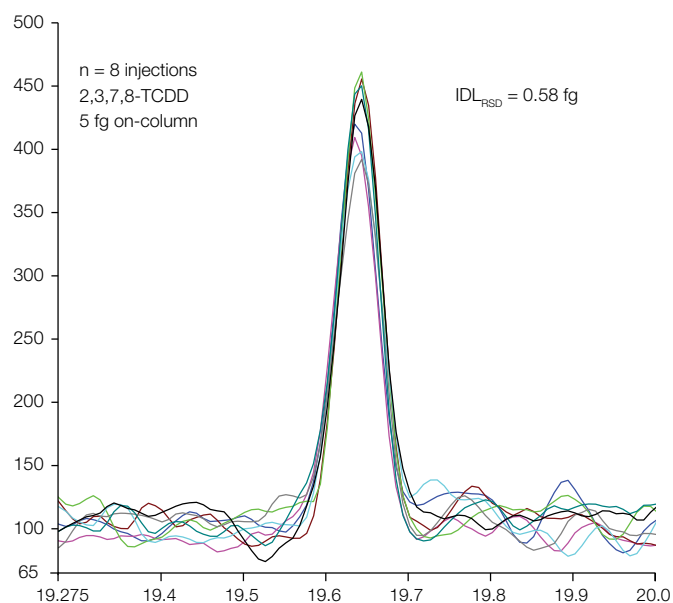


Figure 3. Overlaid SRM chromatogram of 2,3,7,8-TCDD (321.9 → 258.9 @ 20V collision energy) showing 5 fg on-column. Calculated instrument detection limit from n= 8 consecutive injections is annotated.

In order to demonstrate compliance for the analysis of PCDD/Fs using a triple quadrupole mass spectrometer, guidance from the EU³ states that:

“The limit of quantification of an individual congener may be defined as... the lowest concentration point on a calibration curve that gives an acceptable ($\leq 30\%$) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor calculated for all points on the calibration curve in each series of samples (The LOQ is calculated from the lowest concentration point taking into account the recovery of internal standards and sample intake.).”

Considering the EU requirements, for the analytical method to be deemed suitable, the required level of sensitivity (LOQ) needs to be 1/5 ML for that food or feedstuff commodity or lower.^{1,2} The other specific criteria

for confirmatory methods (suitable chromatography, ion ratio tolerance ($\pm 15\%$), number of transitions (2), and quadrupole resolution (equal or better than unit mass))^{1,2} must also be met.

This makes it essential that a suitably low standard is used in a calibration curve to satisfy these requirements. For this reason, the CSL (Extended Calibration/Low Level, see Table 3) standard was serially diluted by factors of two and four and added to the calibration curve. Concentrations used for the LOQ are referenced in Table 3, with example chromatograms of the most relevant native congener's quantifier and qualifier ions shown for reference in Figure 4. LOQ values reported in Table 3 are absolute amount expressed in pg as WHO-TEQ values are normally expressed as pg/g values. Matrix LOQs are calculated from absolute amounts, taking into account congener TEFs and sample amount in g.

Table 3. LOQ—absolute amount on column in pg, shown in bold—was set at the lowest calibration curve point that demonstrated ion ratios (IR) and response factors (RF) repeatedly within permitted EU tolerances (IR $\pm 15\%$, RF RSD $< 30\%$).

Congener	Concentration (pg/ μ L)		TSQ 9000 LOQ - pg	EU Criteria Compliant?
	EN:1948 CSL	EN:1948 CSL/4		
Native PCDDs				
2378- TCDD	0.04	0.01	0.02	✓
12378- PeCDD	0.08	0.02	0.04	✓
123478- HxCDD	0.08	0.02	0.08	✓
123678- HxCDD	0.08	0.02	0.08	✓
123789- HxCDD	0.08	0.02	0.08	✓
1234678- HpCDD	0.16	0.04	0.08	✓
OCDD	0.16	0.04	0.32	✓
Native PCDFs				
2378-TCDF	0.04	0.01	0.02	✓
12378-PeCDF	0.08	0.02	0.04	✓
23478-PeCDF	0.08	0.02	0.04	✓
123478-HxCDF	0.08	0.02	0.04	✓
123678-HxCDF	0.08	0.02	0.04	✓
234678-HxCDF	0.08	0.02	0.04	✓
123789-HxCDF	0.08	0.02	0.04	✓
1234678-HpCDF	0.16	0.04	0.08	✓
1234789-HpCDF	0.16	0.04	0.08	✓
OCDF	0.16	0.04	0.32	✓

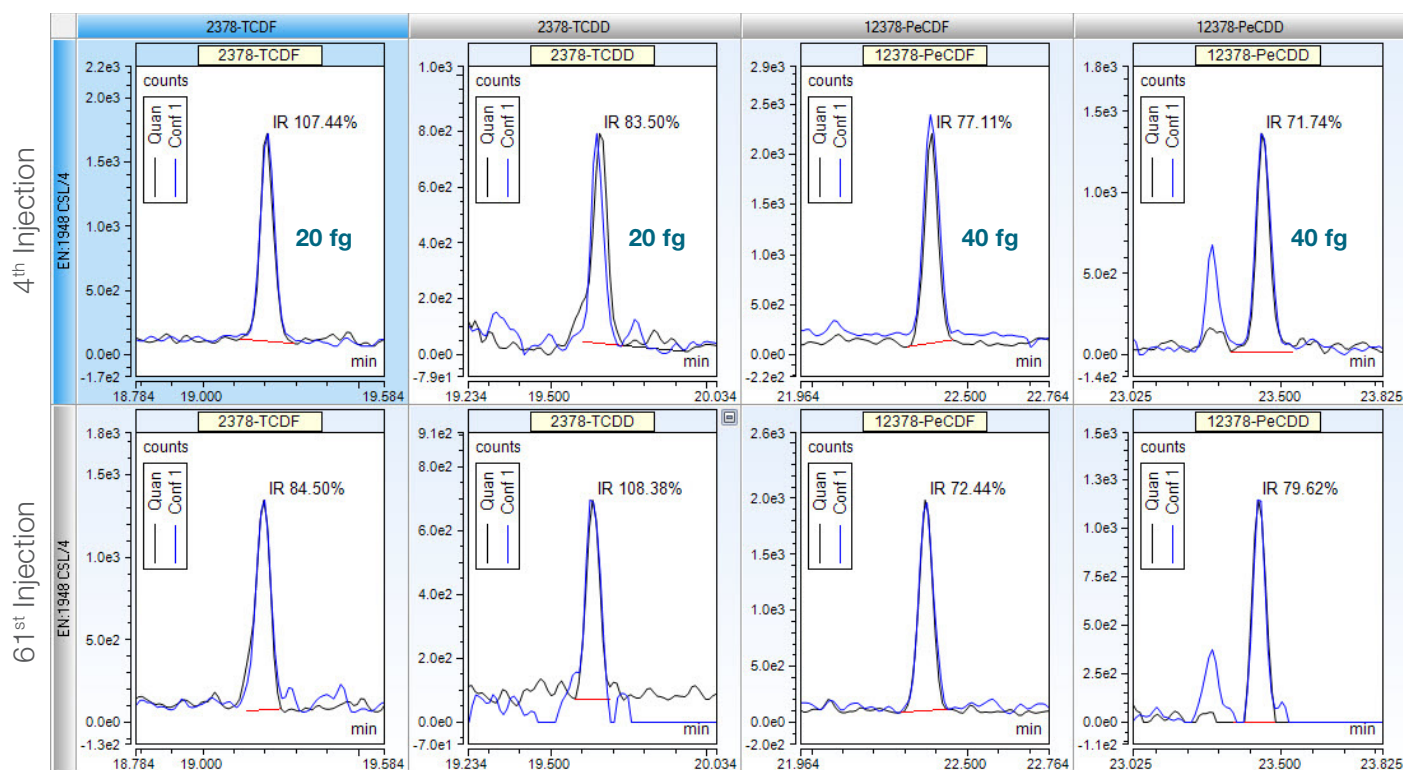


Figure 4. Tetra and penta CDD/F example chromatograms at LOQ (CSL/4) at beginning (4th injection) and end (61st injection) of analytical sequence. Inset absolute concentration on column.

Calibration, robustness, and repeatability

Dioxin quantification is based on isotope dilution and uses a response factor (RF) type of calibration, where the average RFs (ARFs), determined from an external calibration curve, are taken into account to quantify the 17 toxic PCDD/Fs congeners. For each

congener, the relative standard deviation (%RSD) of RFs was calculated from measurements of an eight-point calibration curve. Calculated %RSD values were excellent for all congeners, with values between 1.8% and 7.3%, well within the 15% limits established by EU Regulation^{1,2} and the EPA⁵ (Table 4).

Table 4. Response factors and RF RSD determined as average values from an eight-point calibration curve. Calibration range data is also annotated.

	Congener	Average RF from calibration	RF standard deviation	RF RSD (%)	Calibration range (pg/μL)
Response factor	2378-TCDF	1.06	0.06	5.6%	0.01–80
	2378-TCDD	1.13	0.06	5.6%	0.01–80
	12378-PeCDF	1.02	0.02	2.3%	0.02–160
	23478-PeCDF	1.08	0.02	1.9%	0.02–160
	12378-PeCDD	1.11	0.03	2.4%	0.02–160
	123478-HxCDD	1.08	0.03	3.1%	0.04–160
	123678-HxCDD	1.04	0.04	3.8%	0.04–160
	123789-HxCDD	1.05	0.04	3.7%	0.04–160
	123478-HxCDF	1.04	0.02	2.3%	0.02–160
	123678-HxCDF	1.04	0.03	2.4%	0.02–160
	234678-HxCDF	1.04	0.04	3.9%	0.02–160
	123789-HxCDF	0.98	0.04	4.4%	0.02–160
	1234678-HpCDF	1.09	0.02	1.8%	0.04–320
	1234789-HpCDF	1.13	0.03	2.5%	0.04–320
	1234678-HpCDD	1.16	0.08	7.3%	0.04–320
	OCDD	1.15	0.05	4.5%	0.16–320
	OCDF	0.99	0.06	5.8%	0.16–320

Throughout the analytical sequence, additional EN:1948 CSL/4 standards were injected to assess instrumental performance across the sample batch. Response factors and ion ratios for all the native congeners over the full, two-day, analytical sequence (63 injections comprising 21 matrix samples, 11 blanks, and 31 standard injections) demonstrated consistent compliance with both EU regulations¹ and EU guidance,³ maintaining the sensitivity to comfortably meet the required LOQs (Figures 5 and 6).

The stability of the 2,3,7,8-TCDD ion ratio at the lowest level (20 fg on column) was consistently maintained within the $\pm 15\%$ tolerance throughout the run and entire calibration range (Figure 6a).

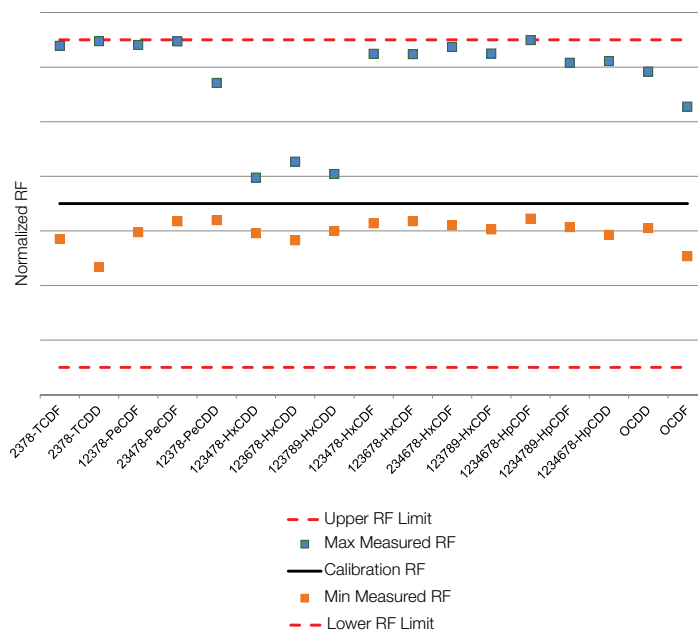


Figure 5. Minimum and maximum normalized response factors values measured for all native congeners in all calibration standards throughout the analytical sequence.

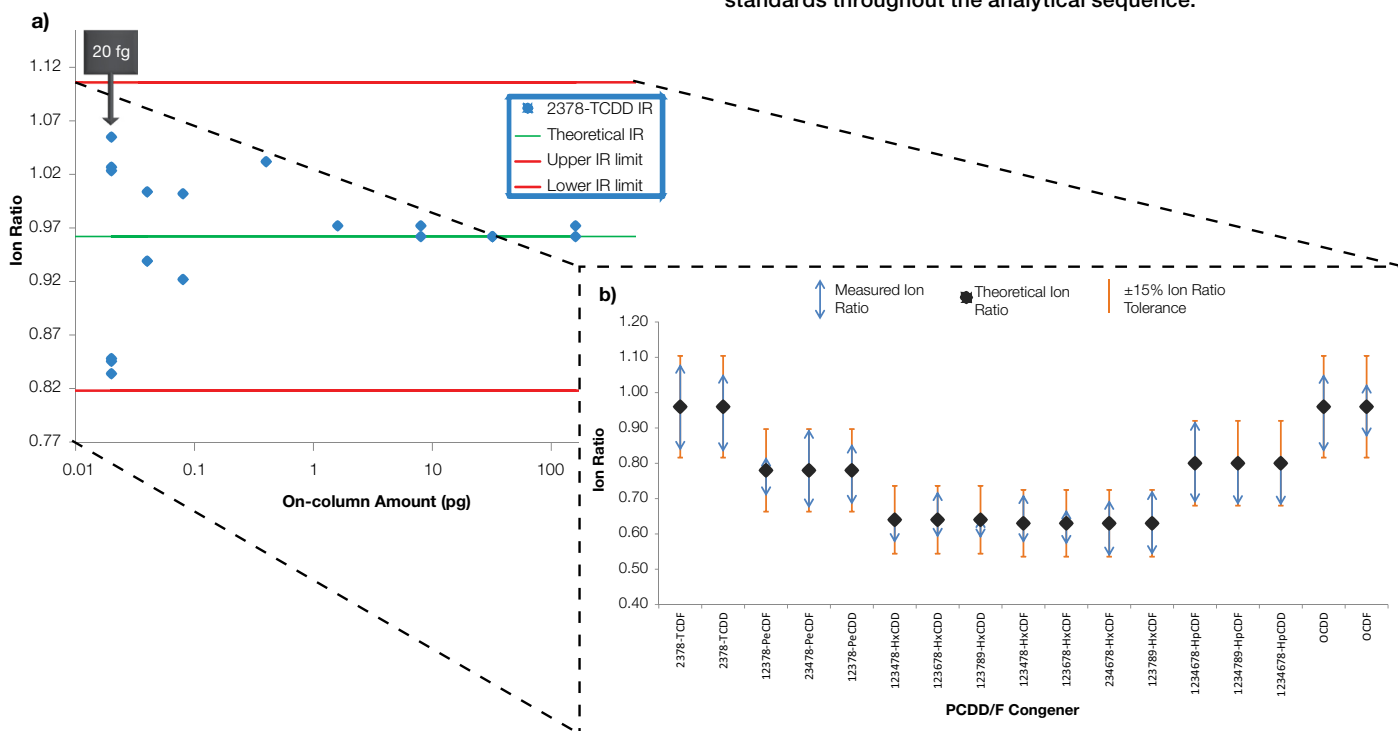


Figure 6a. Ion ratios shown over the full calibration range (0.01–80 pg/ μ L) for the two native 2,3,7,8-TCDD transitions.

Figure 6b. Ion ratio values for all native congeners in all calibration standards throughout the entire 2-day batch.

The range of minimum/maximum IRs (depicted by the blue arrows) shown are within the $\pm 15\%$ tolerance allowed (orange bars).

Quantification and confirmation of PCDD/Fs in real samples

It is important to have an analytical system that is sensitive enough to allow the user flexibility when preparing the samples. Table 5 shows the calculated LOQs for feed-grass matrix (lowest calibration standard amount adjusted for sample weight and TEF), alongside the contamination values measured for the QC sample. From these results, it is obvious that the method demonstrates exceptional sensitivity, with the LOQ of the method being five times lower than that required by legislation, essentially allowing the analyst to use as little as 5 g feed sample weight if required (although this may not represent the bulk sample).

Regulatory criteria were met for all sample types analyzed and results are shown in Table 6 with example peaks in Figure 7. The majority of the samples analyzed had very low levels of contamination present; this is evidenced by the fact that many of the congeners are described as “<LOQ”, in these instances the result reported can take the form of upper, middle, or lowerbound. Upperbound substitutes the LOQ value (worst case scenario), lowerbound reports a zero value, and middlebound the midpoint between these. The sensitivity demonstrated by the TSQ 9000 GC-MS system ensures that in these cases the Upperbound SUM PCDD/F WHO-TEQ is kept well below the required 1/5 ML.

Table 5. Calculated LOQs for the feed QC sample.

Sample: QC – Feed				
Congener	On-column LOQ (pg)	TEF (WHO 2005)	LOQ - Upperbound WHO-TEQ-PCDD/Fs (2005) pg/g	TSQ 9000 Result Upperbound WHO-TEQ-PCDD/Fs (2005) pg/g
2378-TCDF	0.02	0.1	0.00048	0.02010
2378-TCDD	0.02	1	0.00483	0.01884
12378-PeCDF	0.04	0.03	0.00029	0.00304
23478-PeCDF	0.04	0.3	0.00290	0.06221
12378-PeCDD	0.04	1	0.00966	0.05556
123478-HxCDF	0.04	0.1	0.00097	0.02632
123678-HxCDF	0.04	0.1	0.00097	0.01530
234678-HxCDF	0.04	0.1	0.00097	0.01606
123478-HxCDD	0.08	0.1	0.00193	0.00389
123678-HxCDD	0.08	0.1	0.00193	0.01723
123789-HxCDD	0.08	0.1	0.00193	0.00556
123789-HxCDF	0.04	0.1	0.00097	0.00717
1234678-HpCDF	0.08	0.01	0.00019	0.01185
1234678-HpCDD	0.08	0.01	0.00019	0.00101
1234789-HpCDF	0.08	0.01	0.00019	0.01565
OCDD	0.32	0.0003	0.00002	0.00308
OCDF	0.32	0.0003	0.00002	0.00033
		SUM	0.02844	0.28319
Sample: QC – Feed				
Sample weight (g)				20.71
Final volume (µL)				10
Sample injection volume (µL)				2
Standard injection volume (µL)				2
EU ML (sum WHO-TEQ-PCDD/Fs [2005] pg/g)				0.750
1/5 EU ML				0.150
Expected sum WHO-TEQ-PCDD/Fs [2005] pg/g derived from LOQ				0.028

Table 6. Sample summary results showing the congener specific TEQ pg/g for various feedstuff samples as well as the sum of upper and lower bound etc.

Congener	Alfalfa TEQ (pg/g)	Premix 1 TEQ (pg/g)	Premix 2 TEQ (pg/g)	Pork Fat TEQ (pg/g)	Sheep TEQ (pg/g)	Sheep GCHRMS TEQ (pg/g)
2378-TCDF	0.009	0.001	<LOQ	<LOQ	0.018	<LOQ
2378-TCDD	<LOQ	<LOQ	<LOQ	<LOQ	0.902	0.840
12378-PeCDF	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23478-PeCDF	0.011	<LOQ	<LOQ	<LOQ	0.770	0.793
12378-PeCDD	0.020	<LOQ	<LOQ	<LOQ	2.200	2.246
123478-HxCDF	0.003	<LOQ	0.002	<LOQ	0.167	0.146
123678-HxCDF	0.002	<LOQ	<LOQ	<LOQ	0.093	0.075
234678-HxCDF	0.003	0.002	0.002	<LOQ	0.088	0.072
123789-HxCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.021
123478-HxCDD	<LOQ	<LOQ	<LOQ	<LOQ	0.134	0.083
123678-HxCDD	0.003	<LOQ	<LOQ	<LOQ	0.456	0.339
123789-HxCDD	0.003	<LOQ	<LOQ	<LOQ	0.095	0.092
1234678-HpCDF	0.001	<LOQ	<LOQ	<LOQ	0.013	0.001
1234789-HpCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1234678-HpCDD	0.002	0.001	<LOQ	<LOQ	0.049	0.047
OCDD	0.000	0.000	<LOQ	0.000	0.001	0.001
OCDF	0.000	0.000	<LOQ	<LOQ	<LOQ	<LOQ
Upperbound SUM PCDD/Fs WHO TEQ (pg/g)	0.064	0.059	0.054	0.129	4.999	4.815
Lowerbound SUM PCDD/Fs WHO TEQ (pg/g)	N/A	N/A	N/A	N/A	4.987	4.756
Maximum Level (TEQ pg/g)	0.750	1.000	1.000	1.000	2.500	2.500
1/5th ML	0.150	0.200	0.200	0.200	0.500	0.500

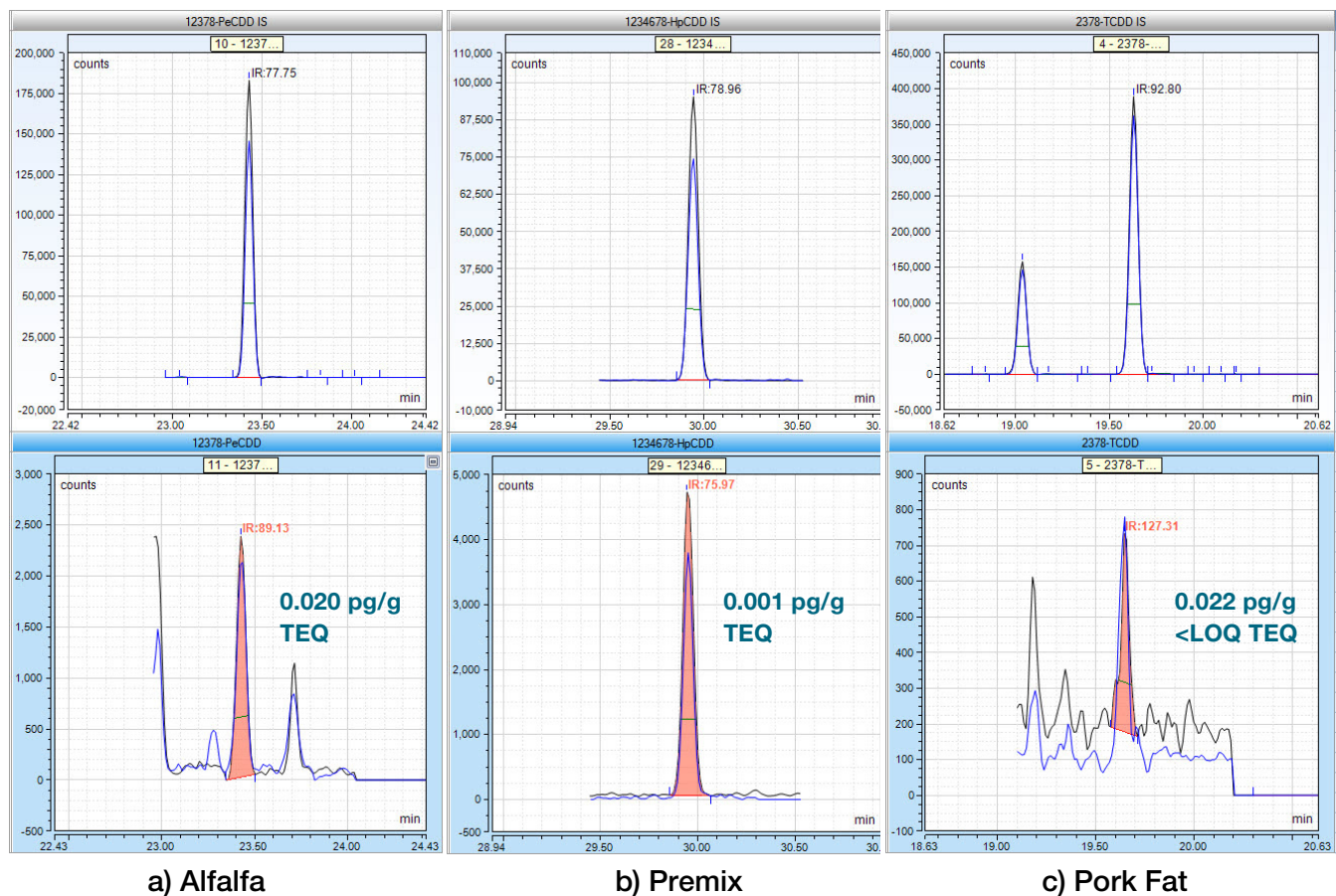


Figure 7. Selected peaks taken from alfalfa, premix, and pork fat samples. The peak shown in the pork fat sample is below the LOQ and therefore the Upperbound TEQ is displayed.

Due to differences in LOQs between the GC-HRMS and TSQ 9000 systems used and the very low levels in many of the samples, a direct comparison of many of the sample results would not be applicable. Comparison of the sheep sample can be made as it is above the ML, with the Lowerbound WHO-TEQ well within the 20% maximum deviation from the Upperbound WHO-TEQ (0.2% difference).¹ Both the “QC- Pork Fat” and “QC – Feed” samples analyzed were in good agreement with the WHO-TEQ results attained by GC-HRMS (Figures 8 and 9) at +1% and –18% deviation, respectively.

It was noted that 2,3,4,7,8-PeCDF showed a higher result in the GC-HRMS data for these QC samples; this can be accounted for by a co-elution of another PeCDF congener on the 5% phenyl-type column used in the GC-HRMS analysis.⁹ Using the TraceGOLD TG-Dioxin column on the TSQ 9000 GC-MS/MS system provided excellent separation of the congeners present. Procedural blanks were used to correct all the results obtained.

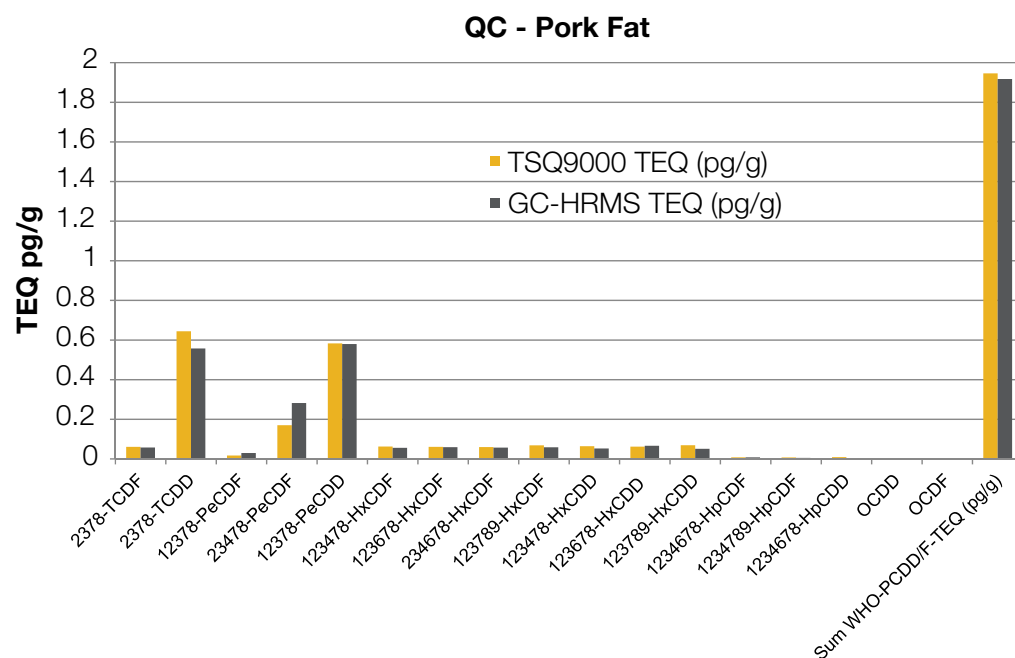


Figure 8. “QC – Pork Fat” WHO-TEQ (pg/g) comparison with GC-HRMS.

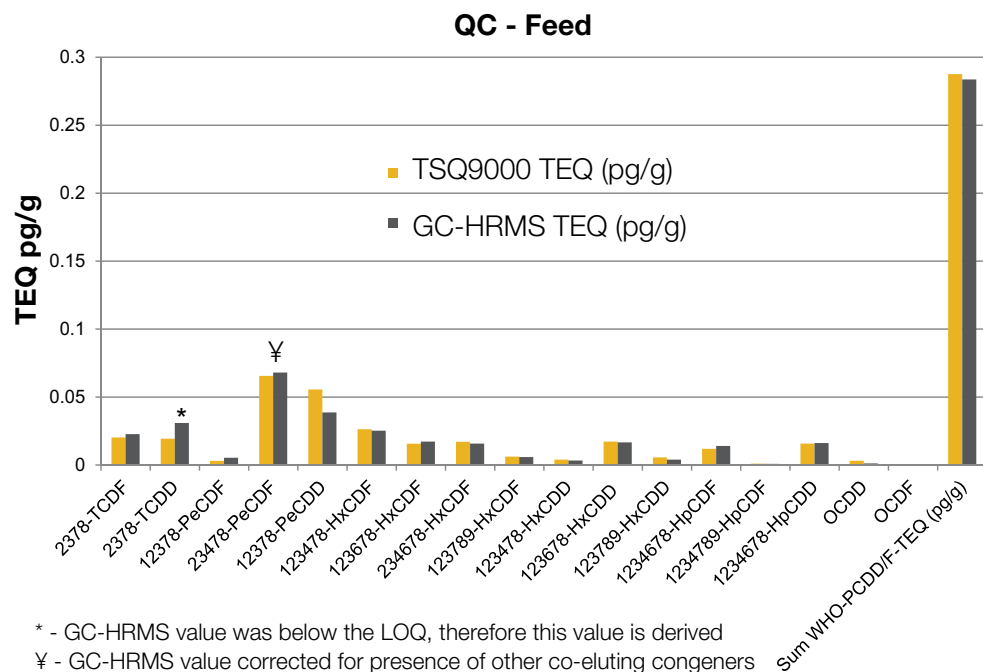


Figure 9. “QC – Feed” WHO-TEQ (pg/g) comparison with GC-HRMS.

Conclusion

The results of this study demonstrate that the TSQ 9000 GC-MS/MS system configured with the AEI source satisfies all of the current EU commission requirements for the detection and confirmation of dioxins in food and feed samples.

- The outstanding sensitivity achieved with the TSQ 9000 GC-MS/MS instrument with the AEI source was proven to be repeatable and robust throughout a continuous two-day analytical sequence. This shows compliance with the regulations for sample intake weights as low as 5 g for feed samples.
- The stability and repeatability of the ion ratios and response factors generated provide confidence in results at the lowest quantifiable levels.
- The reported upperbound WHO-TEQ results reported for low level samples below the MLs were significantly lower than the 1/5 MLs required, giving both reassurance and flexibility when analysing multiple sample types.

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Appendix A

Selected reaction monitoring (SRM) transitions with their corresponding optimized collision energies (CEs) used for the quantitation and confirmation of the target congeners. Dwell time priority was given to the native congeners to increase sensitivity and in turn ion ratio stability at the lowest quantitative limits.

Congener	Standard	Quantifier Precursor → Product (Da)	Qualifier Precursor → Product (Da)	CE (V)	Dwell time priority
2378-TCDF ₁₂ C ¹³	Internal	315.9 → 252.0	317.9 → 254.0	26	Normal
2378-TCDF	Native	303.9 → 240.9	305.9 → 242.9	26	High
12378-PeCDF ₁₂ C ¹³	Internal	351.9 → 287.9	349.9 → 285.9	26	Normal
12378-PeCDF	Native	339.9 → 276.9	337.9 → 274.9	26	High
23478-PeCDF ₁₂ C ¹³	Internal	351.9 → 287.9	349.9 → 285.9	26	Normal
23478-PeCDF	Native	339.9 → 276.9	337.9 → 274.9	26	High
123478-HxCDF ₁₂ C ¹³	Internal	385.9 → 321.9	383.9 → 319.9	26	Normal
123478-HxCDF	Native	373.8 → 310.9	371.8 → 308.9	28	High
123678-HxCDF ₁₂ C ¹³	Internal	385.9 → 321.9	383.9 → 319.9	26	Normal
123678-HxCDF	Native	373.8 → 310.9	371.8 → 308.9	26	High
234678-HxCDF ₁₂ C ¹³	Internal	385.9 → 321.9	383.9 → 319.9	26	Normal
234678-HxCDF	Native	373.8 → 310.9	371.8 → 308.9	26	High
123789-HxCDF ₁₂ C ¹³	Internal	385.9 → 321.9	383.9 → 319.9	26	Normal
123789-HxCDF	Native	373.8 → 310.9	371.8 → 308.9	26	High
1234678-HpCDF ₁₂ C ¹³	Internal	419.8 → 355.9	421.8 → 357.9	26	Normal
1234678-HpCDF	Native	407.8 → 344.8	409.8 → 346.8	26	High
1234789-HpCDF ₁₂ C ¹³	Internal	419.8 → 355.9	421.8 → 357.9	26	Normal
1234789-HpCDF	Native	407.8 → 344.8	409.8 → 346.8	26	High
OCDF ₁₂ C ¹³	Internal	453.8 → 389.8	455.8 → 391.8	26	Normal
OCDF	Native	441.8 → 378.8	443.8 → 380.8	26	High
1234-TCDD ₁₂ C ¹³	Syringe	331.9 → 268.0	333.9 → 270.0	18	Normal
2378-TCDD ₁₂ C ¹³	Internal	331.9 → 268.0	333.9 → 270.0	18	Normal
2378-TCDD	Native	319.9 → 256.9	321.9 → 258.9	18	High
12378-PeCDD ₁₂ C ¹³	Internal	367.9 → 303.9	365.9 → 301.9	18	Normal
12378-PeCDD	Native	355.9 → 292.9	353.9 → 290.9	18	High
123478-HxCDD ₁₂ C ¹³	Internal	401.9 → 337.9	399.9 → 335.9	18	Normal
123478-HxCDD	Native	389.8 → 326.9	391.8 → 328.9	18	High
123678-HxCDD ₁₂ C ¹³	Internal	401.9 → 337.9	399.9 → 335.9	18	Normal
123678-HxCDD	Native	389.8 → 326.9	391.8 → 328.9	18	High
123789-HxCDD ₁₂ C ¹³	Internal	401.9 → 337.9	399.9 → 335.9	18	Normal
123789-HxCDD	Native	389.8 → 326.9	391.8 → 328.9	18	High
1234678-HpCDD ₁₂ C ¹³	Internal	435.8 → 371.9	437.8 → 373.9	18	Normal
1234678-HpCDD	Native	423.8 → 360.8	425.8 → 362.8	18	High
OCDD ₁₂ C ¹³	Internal	469.8 → 405.8	471.8 → 407.8	18	Normal
OCDD	Native	457.7 → 394.8	459.7 → 396.8	18	High

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