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APPLICATION NOTE 10703

Routine, regulatory analysis of dioxins and dioxin-like compounds in food and feed samples

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Keywords

Triple quadrupole GC-MS/MS, persistent organic pollutants, POPs, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzo-*p*-furans, PCDD/Fs, dioxins, polychlorinated biphenyls, PCBs, confirmatory analysis, TSQ 9000, advanced electron ionization, AEI

Goal

To demonstrate the utility of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for the routine and regulatory compliant analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzo-*p*-furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (PCBs), and indicator PCBs in food and feed samples.

Introduction

Dioxins and dioxin-like compounds are highly toxic substances classed as persistent organic pollutants (POPs). Due to their high fat-solubility, dioxins accumulate in the fatty tissues of animals. As a result, more than 90% of human exposure to dioxins is through food, especially meat, dairy, fish, etc. Therefore, accurate monitoring of food and feed is essential to control dioxin uptake from the food chain.[1]

In 2014 a change in European Commission regulations[2,3] permitted gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) to be used as an alternative to gas chromatography-high resolution mass spectrometry (GC-HRMS) for confirmatory analysis and for the control of

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maximum levels (MLs) and action levels (ALs) in certain food and feed samples. Even though the utility of GC-MS/MS for this application has been demonstrated in principle,[4] there is a lack of robust data to validate the suitability of GC-MS/MS, especially for the long-term routine analysis of hundreds of samples. This is further confused by the absence of a clear protocol regarding the setting of appropriate limit of quantification (LOQ) values for GC-MS/MS analysis, with both signal-to-noise (S:N) and calibration-based approaches being used in some validations.

In addition to the deficiencies in validation data, there is a need for software packages to deal with the complexities of the calculations required to process and report data using isotopic dilution. As a consequence many laboratories adopt external software tools to manipulate the data. This practice is not only time-consuming, but can lead to errors in transcription and rounding, and also to an uncontrolled data trail. It is preferable to have the capability to acquire data, process data, and perform calculations and report the required results on a single, compliant software platform.

In this study, the performance of the TSQ 9000 triple quadrupole GC-MS/MS system equipped with an advanced electron ionization (AEI) source was evaluated. Data was acquired on two different TSQ 9000 AEI systems located in two different laboratories and operated by different chemists (UK and USA). Commercially available solvent standards, food/feedstuff, and proficiency test (PT) samples were used to evaluate the performance of each system for the analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs), and non-dioxin-like (indicator) PCBs. Guidance from the European Union Reference Laboratories (EURL) on the use of a calibration approach was followed to set suitable LOQs:[5] essentially, to demonstrate sufficient sensitivity to enable reporting at 1/5th of the maximum level (ML) upper bound sum toxic equivalences (TEQs).

To demonstrate the robustness required to operate in a routine environment an experiment involving continuous analysis of extracts over a period of two weeks was carried out.

Experimental

Instrumental and method setup

In the experiments described here, a TSQ 9000 AEI 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. See Appendix 2 for a list of the consumables used. Mass spectrometer operation was as per AN10590[4] unless otherwise specified. Importantly, acquisition, processing, and reporting of the data were all performed on a single platform using Chromeleon CDS software, version 7.2. Two separate GC-MS/MS methods were used: one for the analysis of non-ortho PCBs and PCDD/Fs (Table 1), and one to capture other dioxin and non dioxin-like compounds such as mono-ortho, di-ortho, and indicator PCBs fraction (Table 2). See Tables 1 and 2 in Appendix 1.

Samples, extraction, and clean-up

Food and feedstuff samples (including PT samples) were provided by the EURL for Halogenated POPs in Feed and Food, Freiburg, Germany. A nominal sample intake weight of 2 grams (fat) was used for the samples unless indicated otherwise (Table 3). European method EN:1948 standard solutions; EN-1948CVS, WM48-CVS (calibration and quantitation), EN-1948ES, EN-1948IS, P48-W-ES, P48-M-ES, and P48-RS (extraction) were utilized for the extraction, calibration, and quantitation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs. All standards were obtained from Wellington Laboratories Inc., Canada.

Extraction (where required) was performed by Twisselmann hot extraction (comparable with Soxhlet extraction) or pressurized liquid extraction. Automated clean-up of extracts was performed using a three column (multi-layered acidic silica, alumina, and carbon columns) setup on the DEXTech™ Plus system (LCTech GmbH). Two extract fractions were provided per sample, the first containing the non-ortho PCBs and PCDD/Fs (final volume 20 µL nonane) and the second containing the mono-ortho and di-ortho PCBs and indicator PCBs (final volume 100 µL nonane). Due to the absence of a non-ortho syringe standard in the calibration and extraction solutions, recoveries were not calculated for the four ^{13}C-labeled non-ortho PCBs. As all the non-ortho PCBs were found in all samples at values greater than the LOQ this does not impact the validity of the results obtained.

Table 3. Sample types and nominal intake weight

Sample type	Matrix	Nominal weight taken (g)	Number of replicates	Basis
PT	Pork sausage	2	2	Fat
PT	Whole egg	2	2	Fat
PT	Milk powder	2	2	Fat
PT	Halibut fillet	13	2	Wet weight
PT	Sugar beet pulp	20	2	Product
QK1	Mixed fat	2	6	Fat
Food	Meat	2	5 (individual)	Fat
Food	Milk	2	4 (individual)	Fat
Food	Fish	25 and 34	2 (individual)	Wet weight
Food	Fish oil	2	2 (individual)	Fat
Food	Eggs	2	5 (individual)	Fat
Feed	Fish meal	12	1	Product
Feed	Grass meal	20	1	Product
Feed	Sepiolite	20	1	Product
Feed	Palm fatty acid distillate (PFAD)	2	1	Product
Feed	Feed fat	2	1	Product

Results and discussion

Chromatography

The proprietary phase of the Thermo Scientific™ TraceGOLD™ TG-Dioxin capillary GC column (P/N 26066-1540) provided excellent separation of all 17 toxic PCDD/F and 18 dioxin-like and non dioxin-like PCB congeners in under 45 minutes, particularly the tetra (Figure 1) and penta-substituted PCDD/Fs.

By contrast, using a 5% phenyl type column, the 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) congener (a major contributor to the WHO-PCDD/F-TEQ) can sometimes co-elute with some of the other non-toxic PeCDF congeners,[6] resulting in an overestimation of the concentration of this important congener. This could ultimately lead to a false TEQ being reported and, in a worst case scenario, false exceedance of MLs. All chromatographic criteria stated in regulation were met using the TG-Dioxin capillary GC column in this study.[2-4]

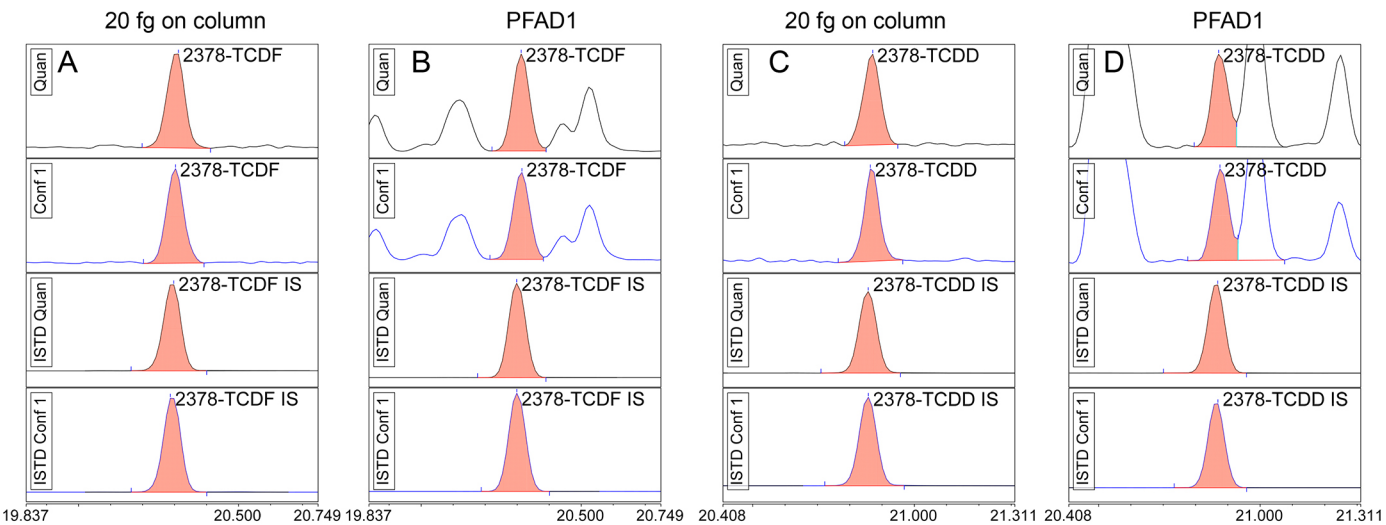

Figure 1. TCDD/F congener separation in solvent standard and palm fatty acid distillate (PFAD) PT sample. Associated ^{13}C-labeled congeners are displayed. (A) 20 fg on-column 2,3,7,8-tetrachlorodibenzofuran (TCDF); solvent standard, (B) ~180 fg on column 2,3,7,8-TCDF; PFAD sample, (C) 20 fg on-column 2,3,7,8-tetrachlorodibenzodioxin (TCDD); solvent standard and, (D) ~55 fg on column 2,3,7,8-TCDD; PFAD sample. All quantification and confirmation ions are labeled.

Determination of limits of quantitation (LOQs)

As previously described (AN10590), calculation of LOQs based on signal-to-noise ratios obtained using GC-MS/MS systems is unreliable; hence, it is more appropriate to use a calibration-based approach.[4,5] Employing calibration standards at the LOQ, and subsequent check standards at this level, allows the user to demonstrate continual method performance throughout the analytical sequence (Figures 3 and 4). It also allows for a simple calculation to determine the LOQ, which will be achieved for PCDD/Fs using a fixed sample weight (Formula 1):

where

Min Conc_n	is the lowest calibration concentration point of congener *n*;
Sample volume	is the final sample volume;
Sample weight	is the sample intake weight;
Recovery I	is the recovery of the associated ^{13}C-labeled congener *I*.

$$\text{Sample LOQ (pg/g)} = \sum_{n=\text{PCDD/F}}^{17} \text{Min Conc}_n\ (\text{pg/µL}) * \left(\frac{\text{Sample volume (µL)}}{\text{Sample weight (g)} * \text{Recovery I (\%)}} \right)$$

Formula 1. Calculation to determine the LOQ for PCDD/Fs

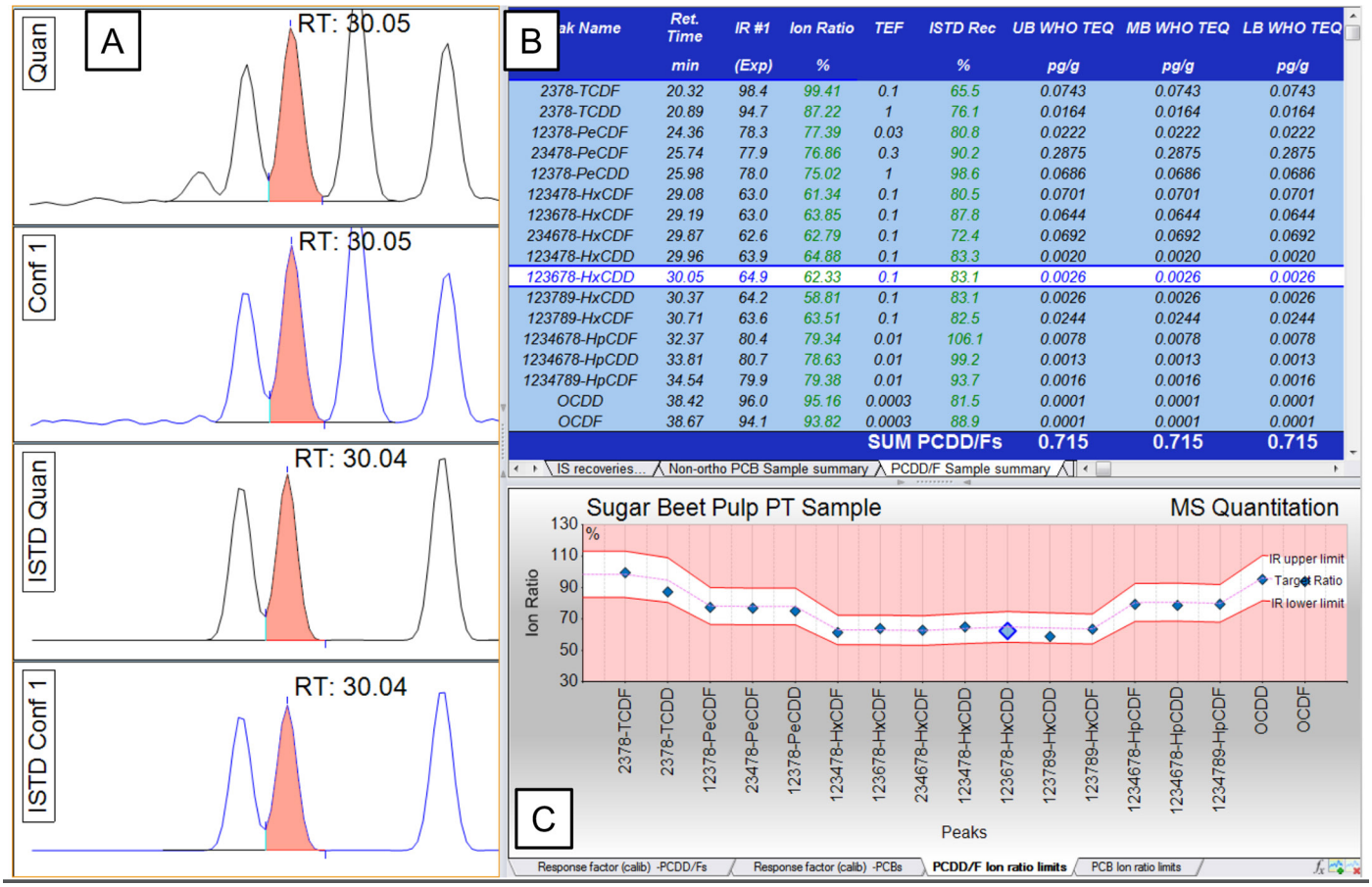

Figure 2. Typical Chromeleon processing browser showing (A) native quantification and confirmation peak with associated ^{13}C-labeled quantification and confirmation peaks, (B) interactive sample results browser showing upper-, middle- and lower-bound, WHO-PCDD/F-TEQ values, flagged ion ratios (IRs) and ^{13}C-labeled congener recovery, (C) IRs and LOQs visual display to easily check if the IR is outside the allowable range and if the peak amount is below the LOQ. Similar displays are available for PCBs. Sugar beet pulp PT sample shown; WHO-PCDD/F-TEQ 0.715 pg/g.

Assuming equal injection volume for standards and samples. This formula can also be applied to sum the total 29 PCDD/Fs and dioxin-like PCBs. Individual congener LOQs calculated in this way can be applied to upper-bound, middle-bound, and lower-bound TEQ results by simply replacing the result of any congeners that fall below the lowest calibration point with this value multiplied by the toxic equivalence factor (TEF) of the congener. Figure 2 shows an example of a real-time updated Chromeleon view including upper-, middle- and lower-bound sum values.

To assess the response factor (RF) deviation throughout the analytical sequences, regular standards at the specified LOQ were analyzed at the beginning, during (after every nine sample extracts injections), and end of the sequence. Chromeleon CDS interactive results panes with real-time updates including pass/fail for IR and RF deviation (calculated as deviation from the average calibration factor) are shown in Figure 3.

Using a nominal weight of 2 g and the lowest calibration level to establish the LOQ, a minimum upper-bound value of 0.152 pg/g WHO-PCDD/F-TEQ can be achieved (assuming 100% ^{13}C-labeled standard recovery and all natives are less than the LOQ in sample). This level is sufficient to demonstrate 1/5th ML compliance for all food and feed stuffs with a nominal intake of 2 g with the exception of food *for infants and young children and liver of terrestrial animals,* both with legal limits on fresh weight basis.[6,8] In which case, either a larger sample intake would be required or a magnetic sector instrument, such as the Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS system, should be the technique of choice.

Calibration

Calibration standards (eight levels for PCDD/Fs and seven levels for PCBs) were analyzed for four analytical sequences (PCDD/Fs and non-ortho PCBs and di- and mono-ortho PCBs and indicator PCBs), over the two systems with duplicate injection per level. The results of

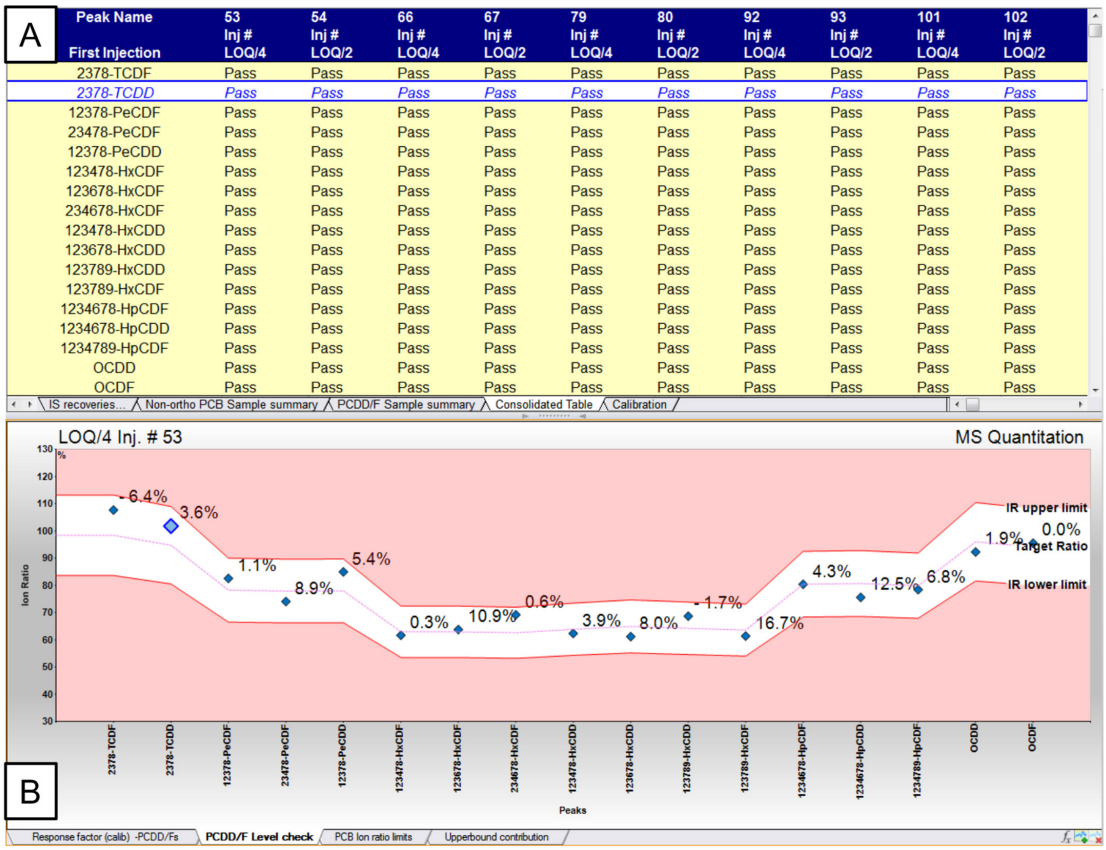

Figure 3. Chromeleon results browser showing (A) interactive results display with real-time updated Pass/Fail statements for each check standard, and (B) IR and RF deviation visual display to easily check if the IR is out of the allowable range (±15%) and if the congener has an RF within acceptable deviation (<30% from calibration average – indicated by the data label). Similar displays are available for PCBs.

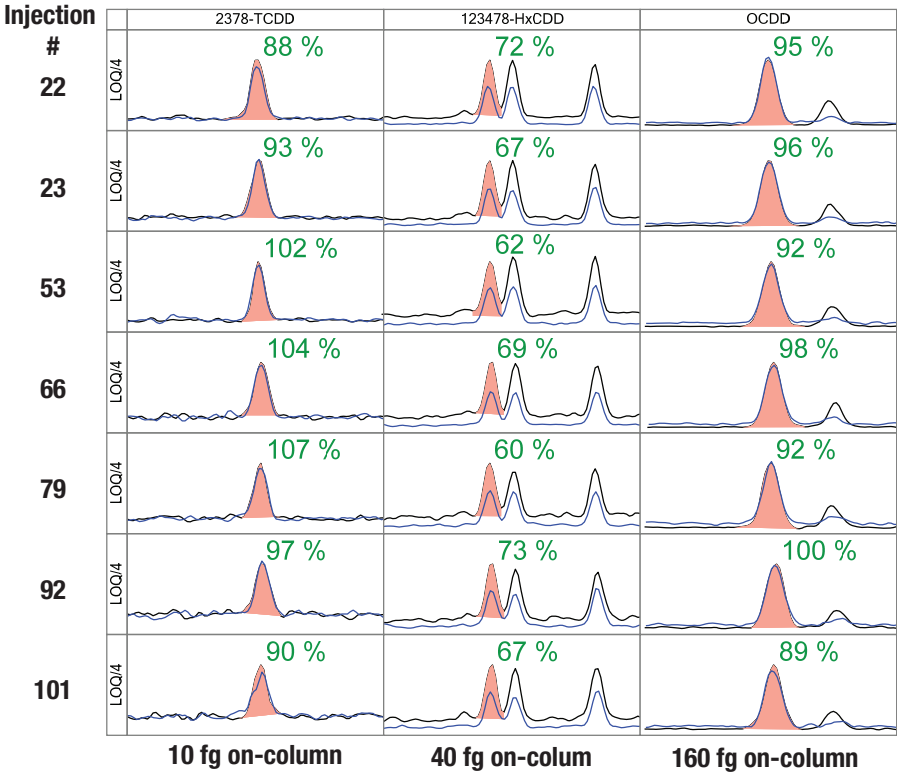

Figure 4. LOQ repeatability during the UK-based PCDD/F and non-ortho PCB sequence. Overlaid extracted ion chromatograms (XICs) are displayed (quantification and confirmation ions) for selected TCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) congeners, all IRs (as displayed in green) and RFs were within the allowable tolerances (IR ±15% from theoretical or average value; RF <30% deviation from average value) as defined by EURL guidance[4] throughout the sequence.

all four calibration sequences demonstrated RF %RSDs well within the EU regulations.[2,3] Table 4 shows examples of the data obtained for the UK-based dioxin-like PCBs and PCDD/Fs. Calibration ranges displayed are absolute amount on-column (pg).

Quantification and confirmation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs in food and feed samples

A total of 29 different samples were analyzed [39 separate sample extractions, with two fractions for each (see Table 3)], over two sites, on two separate TSQ 9000 AEI GC-MS/MS systems for non-ortho PCBs and PCDD/Fs and di-, mono-ortho PCBs and indicator PCBs. To demonstrate the efficacy of the TSQ 9000 AEI GC-MS/MS systems, six replicate extractions of a mixed fat quality control sample (QK1 – reference value: 0.87 pg sum WHO-PCDD/F-TEQ) were prepared. These were split between the sites and analyzed at regular intervals throughout the analytical sequences (14 injections in total over the two non-ortho PCBs and PCDD/Fs sequences). An example of the chromatography achieved for a selection of congeners in the non-ortho PCBs and PCDD/Fs fraction is shown in Figure 5.

The measured WHO-PCDD/F-TEQ (pg/g) value for each congener was in excellent agreement with the reference value provided by the EURL (Figure 6), with the upper-bound WHO-PCDD/F-TEQ (pg/g) not deviating by more than 6% from the reference value over all 14 measurements. Furthermore, the deviation between the upper-bound and lower-bound WHO-PCDD/F-TEQ (pg/g) for each measurement was consistently less than 1.2%, well below the maximum 20% deviation required for samples that exceed the ML as specified in EU regulation (Figure 7).[6]

Table 4. Native dioxin-like PCBs and PCDD/Fs calibration data for the UK sequences (as average calibration response factors)

Peak Name	Ret.Time (min)	Number of Points	RF RSD (%)	Coeff. of Determination (R^2)	Average RF (Slope)	Range (pg)
PCB 81	16.38	14	1.49	0.9997	1.06	0.04 – 160
PCB 77	16.86	14	1.08	0.9997	1.00	0.04 – 160
PCB 123	17.40	14	2.66	0.9998	0.92	0.02 – 200
PCB 118	17.64	14	1.46	0.9999	0.96	0.1 – 1000
PCB 114	18.18	14	3.02	0.9989	1.04	0.02 – 200
PCB 105	18.96	14	5.95	0.9947	0.96	0.02 – 200
2378-TCDF	20.30	16	3.87	0.9995	0.96	0.01 – 64
2378-TCDD	20.86	16	4.72	0.9996	1.04	0.01 – 64
PCB 126	20.90	14	5.69	0.9985	0.95	0.04 – 160
PCB 167	21.52	14	1.74	0.9998	1.15	0.02 – 200
PCB 156	22.91	14	1.97	0.9998	1.14	0.02 – 200
PCB 157	23.12	14	2.41	0.9999	1.11	0.02 – 200
12378-PeCDF	24.34	16	1.66	0.9999	0.93	0.02 – 128
PCB 169	25.48	14	4.00	0.9999	1.08	0.04 – 160
23478-PeCDF	25.71	16	5.36	0.9977	1.03	0.02 – 128
12378-PeCDD	25.96	16	3.60	0.9999	1.05	0.02 – 128
PCB 189	27.28	14	1.96	0.9989	0.99	0.02 – 200
123478-HxCDF	29.06	16	2.98	0.9996	1.02	0.02 – 128
123678-HxCDF	29.17	16	1.95	0.9998	1.00	0.02 – 128
234678-HxCDF	29.86	16	2.83	0.9993	1.02	0.02 – 128
123478-HxCDD	29.94	16	2.49	0.9990	1.12	0.04 – 128
123678-HxCDD	30.04	16	2.01	0.9991	1.12	0.04 – 128
123789-HxCDD	30.35	16	3.82	0.9987	1.09	0.04 – 128
123789-HxCDF	30.71	16	3.52	0.9997	0.95	0.02 – 128
1234678-HpCDF	32.35	16	1.78	0.9999	1.03	0.04 – 256
1234678-HpCDD	33.78	16	5.99	0.9968	1.09	0.04 – 256
1234789-HpCDF	34.52	16	1.88	0.9998	1.04	0.04 – 256
OCDD	38.39	16	1.64	1.0000	1.12	0.16 – 256
OCDF	38.64	16	1.34	0.9997	0.94	0.16 - 256
		Max	**5.99**	**1.0000**		
		Min	**1.08**	**0.9947**		

Peak Name	Ion Ratio	ISTD Rec	<LOQ?	UB WHO TEQ	MB WHO TEQ	LB WHO TEQ
	%	%		pg/g	pg/g	pg/g
2378-TCDF	96.37	79.2		0.0493	0.0493	0.0493
2378-TCDD	*105.26*	*89.2*		*0.0683*	*0.0683*	*0.0683*
12378-PeCDF	88.79	94.2		0.0041	0.0041	0.0041
23478-PeCDF	78.57	94.8		0.0686	0.0686	0.0686
12378-PeCDD	75.45	99.7		0.3410	0.3410	0.3410
123478-HxCDF	55.86	81.2		0.0113	0.0113	0.0113
123678-HxCDF	59.95	87.3		0.0113	0.0113	0.0113
234678-HxCDF	68.85	76.8		0.0112	0.0112	0.0112
123478-HxCDD	65.58	88.4		0.0477	0.0477	0.0477
123678-HxCDD	65.59	96.0		0.1020	0.1020	0.1020
123789-HxCDD	65.90	96.0		0.0557	0.0557	0.0557
123789-HxCDF	IR High	71.2	<LOQ	0.0071	0.0035	0.0000
1234678-HpCDF	85.31	60.7	<LOQ	0.0017	0.0008	0.0000
1234678-HpCDD	80.13	94.2		0.0688	0.0688	0.0688
1234789-HpCDF	78.94	81.8	<LOQ	0.0012	0.0006	0.0000
OCDD	94.97	91.3		0.0023	0.0023	0.0023
OCDF	102.53	83.0	<LOQ	0.0001	0.0001	0.0000
			SUM PCDD/Fs	0.852	0.847	0.842

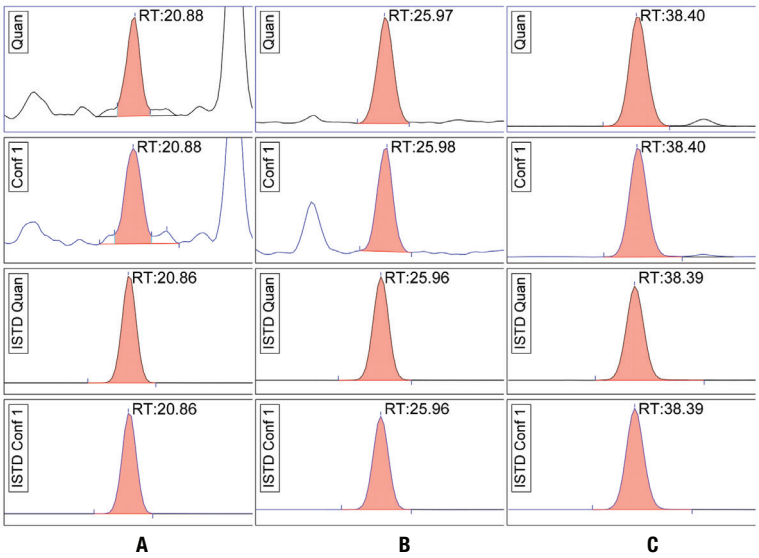

Figure 5. QK1 mixed fat quality control sample example chromatography where (A) 2,3,7,8-TCDD [0.03 pg on-column], (B) 1,2,3,7,8-PeCDD [0.14 pg on-column] and (C) OCDD [3.1 pg on-column]. The Chromeleon interactive results pane (left) displays IRs and internal standard recoveries, as well as real-time updated WHO-PCDD/F-TEQ (pg/g) values.

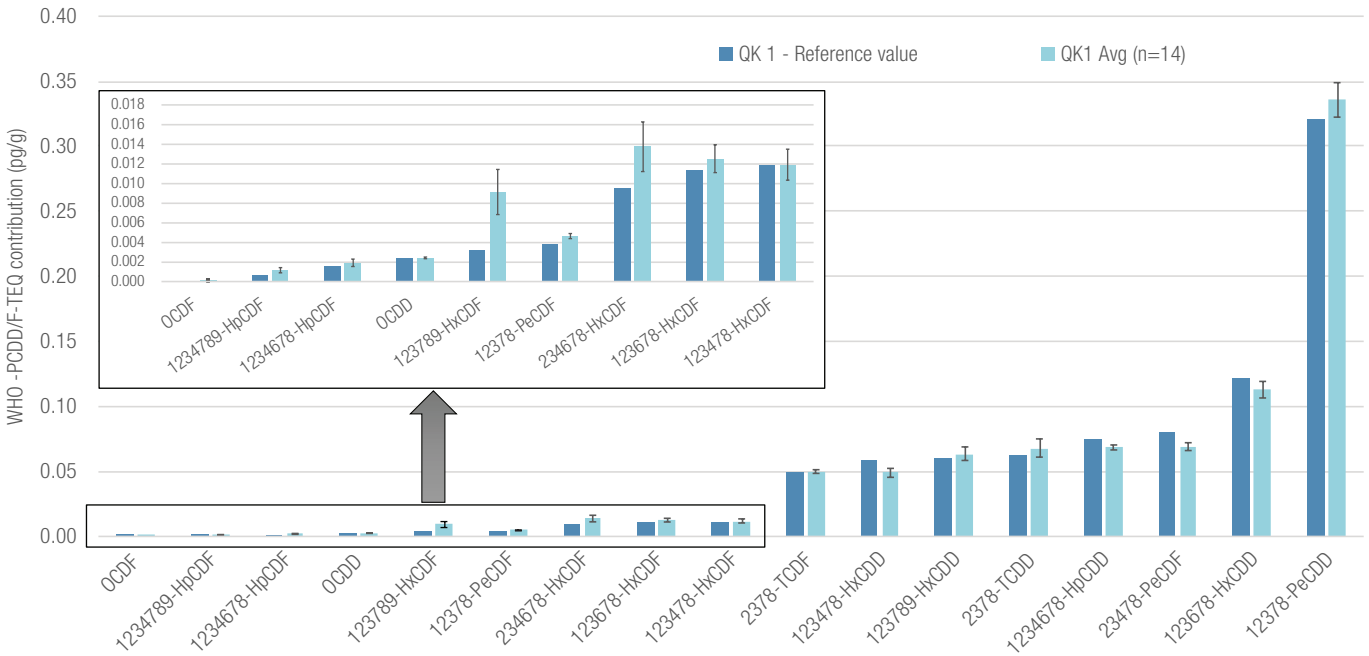

Figure 6. Congener contribution to the WHO-PCDD/F-TEQ (pg/g) for the mixed animal fat quality control sample. Congeners are ranked from left to right in order of contribution. Error bars show ±1σ standard deviation.

The remainder of the samples were analyzed routinely, with eight sample injections bracketed by blanks, LOQ check standards, and quality control samples (QK1). Figures 8A, 8B, and 8C show the correlation of the results obtained on the TSQ 9000 AEI systems with the reference value obtained by the EURL for PCDD/Fs, dioxin-like PCBs, and indicator PCBs, respectively. Where the reference value was below the minimum reportable TSQ 9000 AEI upper-bound WHO-PCDD/F-TEQ (pg/g) value, the samples have been circled with a broken blue line (Figure 8A). These samples all had upper-bound WHO-PCDD/F-TEQ (pg/g) values of less than 0.3 pg/g, which is below 1/5th MLs for these sample types (meat ×2, eggs ×2, and milk).[6] Pearson correlation coefficients were; 0.9902 for PCDD/Fs (Figure 8A), 0.9998 for dioxin-like PCBs (Figure 8B), and 0.9992 for indicator PCBs (Figure 8C), where a value of 1 is total positive linear correlation.

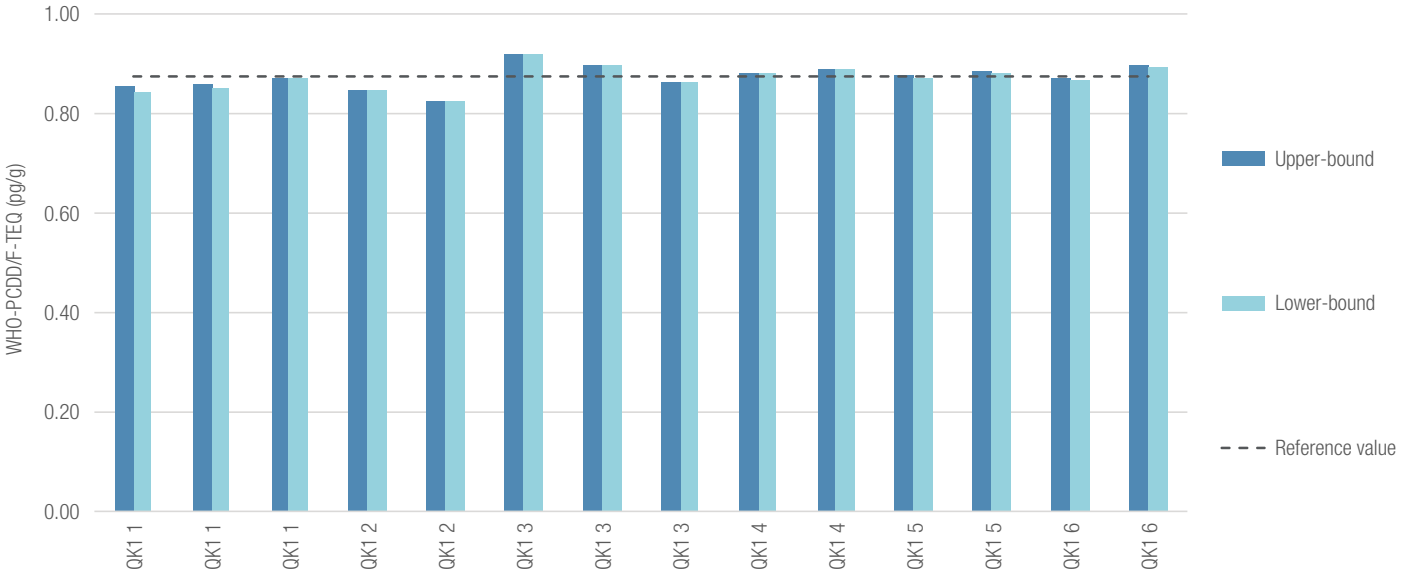

Figure 7. Upper-bound and lower-bound WHO-PCDD/F-TEQ (pg/g) results for all 14 measurements of the QK1 mixed animal fat quality control sample (six replicate extractions)

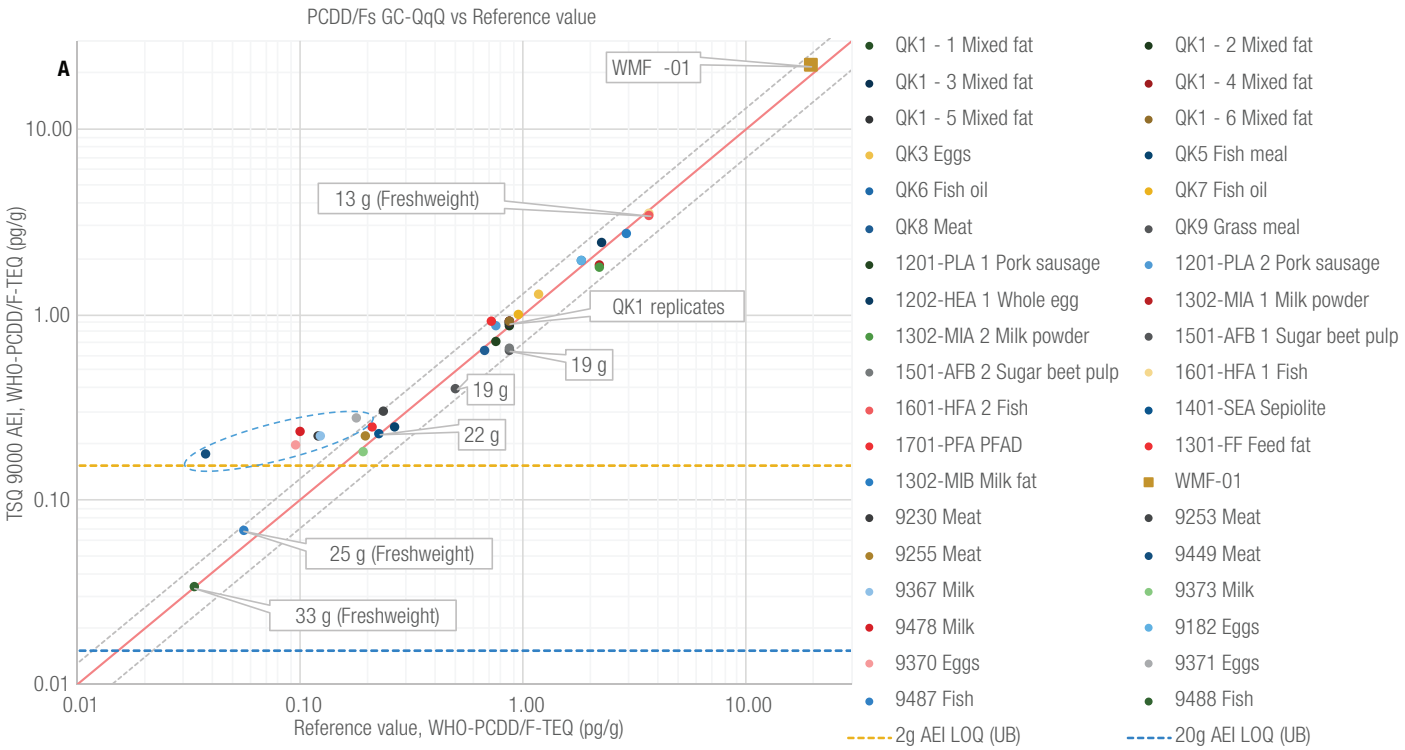

Figure 8 (A). Comparison of data [(A) PCDD/Fs] obtained on the GC-MS/MS with the EURL reference values. The center red line represents 100% agreement with the value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2 g, amount scales are logarithmic to aid comparison.

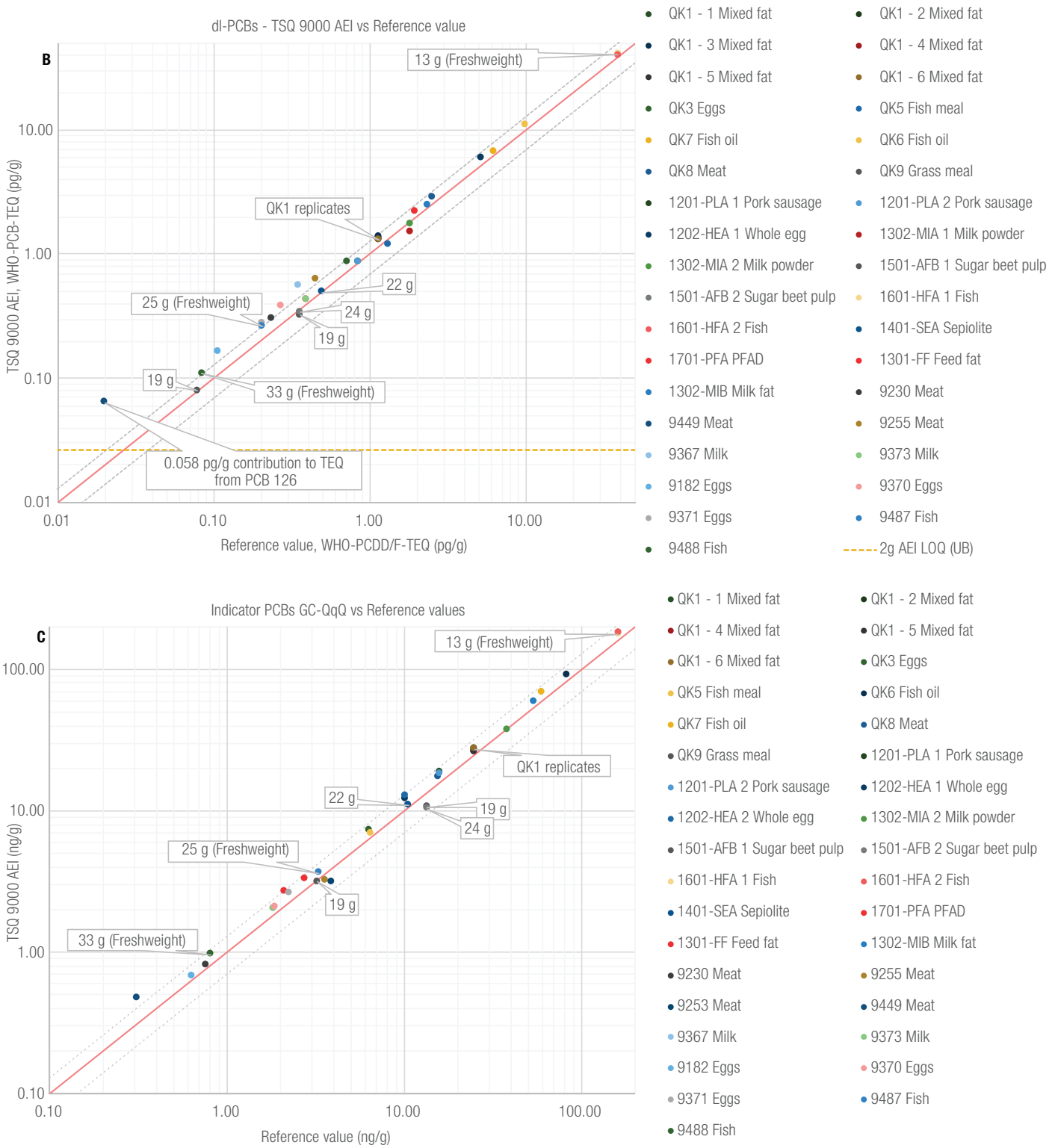

Figure 8 (B and C). Comparison of data [(B) dl-PCBs, and (C) indicator-PCBs] obtained on the GC-MS/MS with the EURL reference values. The center red line represents 100% agreement with the value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2 g, amount scales are logarithmic to aid comparison.

To provide further validation data, an additional certified reference material (CRM) was extracted and analyzed on a PTV TSQ 9000 AEI system in Beijing, China. One gram of CRM WMF-01 (Wellington Laboratories Inc., Canada) was extracted and analyzed in triplicate (modified oven ramp, 5 µL PTV injection). The results obtained were excellent agreement with the reference values published, with all congeners within the specified tolerance (Figure 9). The calculated SUM WHO-PCDD/F-TEQ (pg/g) for the measurements versus the calculated reference SUM WHO-PCDD/F-TEQ (pg/g) is also displayed in Figure 8A.

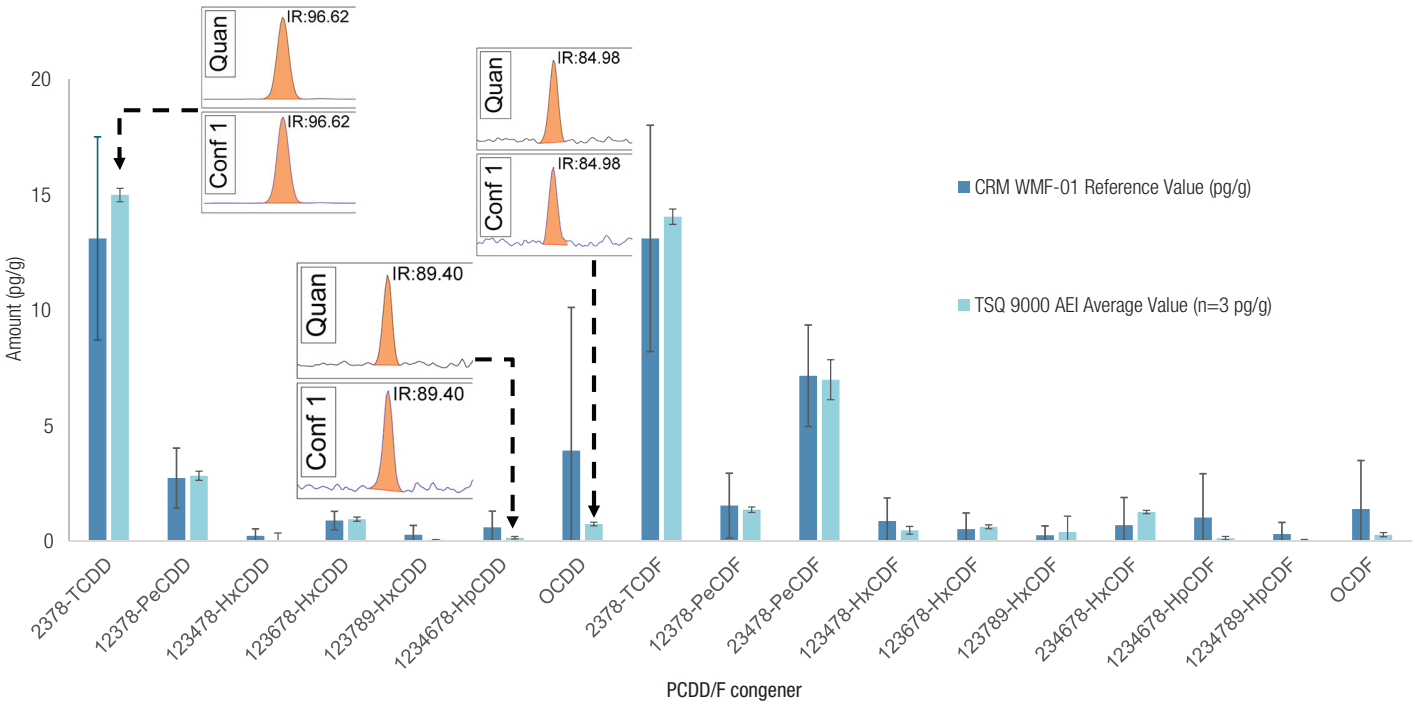

Figure 9. WMF-01 CRM reference value (pg/g) shown in dark blue, average (n = 3) TSQ 9000 AEI value for the WMF-01 CRM (pg/g) shown in light blue. Example XICs for quantification and confirmation ion are inlayed for 2,3,7,8-TCDD (15.13 pg/g), 1,2,3,4,6,7,8-heptachlorodibenzo-P-dioxin (HpCDD) (0.36 pg/g), and OCDD (2.01 pg/g). Error bars show the allowable deviation from the reference value and standard deviation of the TSQ 9000 AEI result.

Robustness

To further assess the robustness of the analytical system, the remaining extracts from the non-ortho PCBs and PCDD/Fs samples were pooled together into mixed matrix extract. This pooled matrix sample was then analyzed alongside nonane blank and LOQ standard injections. The injection sequence was set up as follows: four injections (LOQ, blank, pooled matrix, blank) were followed by a four-hour hold at the initial oven temperature and repeated, resulting in a total of 161 injection sequence containing n = 40 matrix injections and n = 40 LOQ standards, run over ~2 weeks period. The system maintained sensitivity throughout delivering excellent robustness, even considering the high matrix complexity and load on column (Figures 10A and B). No maintenance (such as source cleaning, liner replacement, tuning, or analytical column trimming) was performed during the sequence.

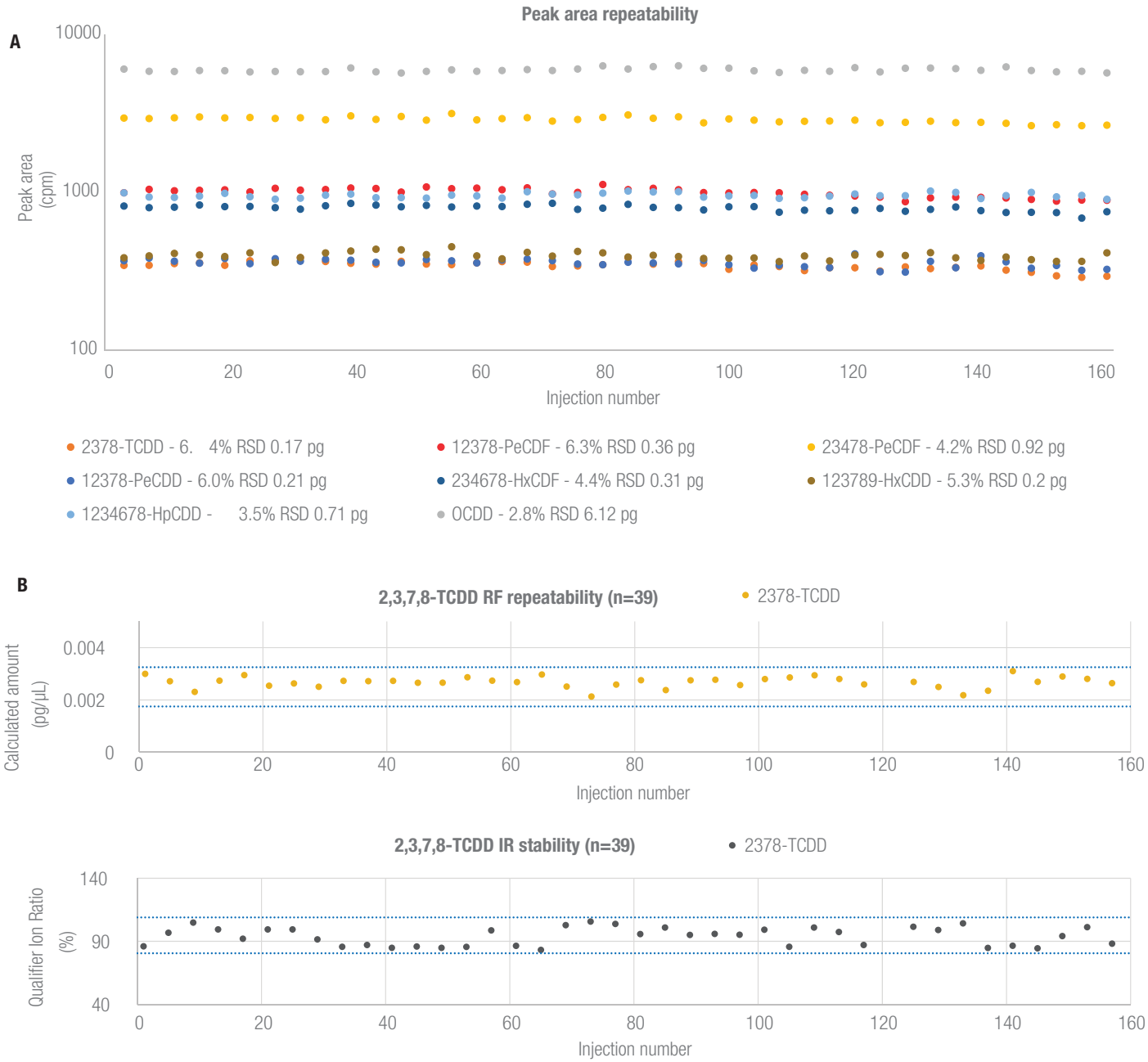

Figure 10. (A) Absolute peak area repeatability over two weeks of analysis, for selected PCDD/F congeners in pooled matrix sample. Relative standard deviations and amounts on-column (pg) are annotated for each selected congener, (B) LOQ RF deviation (upper plot, calculated as deviation from target amount) and IR (lower plot) for the 10 fg on-column 2,3,7,8-TCDD congener (2.5 fg/µL, 4 µL injection).

Conclusions

The results of these comprehensive experiments demonstrate that the TSQ 9000 GC-MS/MS system, configured with the AEI source and controlled using Chromeleon CDS software, can deliver routine-grade performance for the quantification and confirmation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs in food and feedstuffs.

- Successful validation of method performance criteria (LOQ, precision, accuracy, and calibration) was carried out on two separate TSQ 9000 AEI systems, in two geo-locations.
- The sensitivity achieved with the TSQ 9000 AEI system allowed for upper-bound WHO-PCDD/F-TEQ (pg/g) values as low as 0.15 (for a 2 g sample intake weight), meeting the 1/5th maximum level requirements for all but the most challenging matrices.
- The outstanding linear range and accurate quantitative performance generated excellent comparative data to the EURL reference data supplied, with calibration data showing RF %RSD of <6 over more than 4 orders of magnitude for many congeners.
- Minimizing user intervention has been demonstrated by running over two weeks with no maintenance (such as source cleaning, liner replacement, tuning, or analytical column trimming), allowing maximum uptime and sample throughput.
- Chromeleon CDS software, version 7.2, 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 Scientific™ AppsLab Library of Analytical Applications, also provide error-free execution of each analysis to meet standard operating procedure (SOP) requirements, further simplifying the user experience.

Acknowledgement

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Appendix 1. Conditions

Table 1. PCDD/Fs and non-ortho PCBs; Injector/Autosampler and GC-MS/MS conditions

TRACE 1310 GC PTV Parameters					
Operating Mode:	Large Volume				
Injection Volume (µL):	4				
Initial Inlet Temperature (°C):	75				
Carrier Gas, Flow (mL/min):	Helium, 1.2				
Splitless Time (min):	1				
Split Flow (mL/min):	100				
Septum Purge (mL/min):	5 (constant)				
PTV Ramp Settings					
	Pressure (Psi)	*Rate (°C/s)*	*Temp. (°C)*	*Time (min)*	*Flow (mL/min)*
Injection:	-	-	-	0.2	100.0
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0
Autosampler Settings					
Injection Depth (mm):	45				
Penetration Speed (mm/s):	100				
Injection Speed (µL/s):	1				
TRACE 1310 GC Parameters					
Oven Temperature Program					
Temperature 1 (°C):	120 (initial)				
Hold Time (min):	2				
Temperature 2 (°C):	250				
Rate (°C/min):	25				
Hold Time (min):	0				
Temperature 3 (°C):	260				
Rate (°C/min):	2.5				
Hold Time (min):	5				
Temperature 4 (°C):	285				
Rate (°C/min):	2.5				
Hold Time (min):	0				
Temperature 5 (°C):	320				
Rate (°C/min):	10				
Hold Time (min):	15				
Total Run Time (min):	44.7				
TSQ 9000 AEI Mass Spectrometer Parameters					
Transfer Line (°C):	300				
Ionization Type (Source type):	EI with the Advanced EI source				
Ion Source (°C):	350				
Electron Energy (eV):	50				
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (×10 – natives HIGH, labeled LOW)				
Tuning Parameters:	AEI Smart Tune				
Collision Gas:	Argon – 70 PSI				

Table 2. Mono-ortho, di-ortho, and indicator PCBs; Injector/ Autosampler and GC-MS/MS conditions

TRACE 1310 GC PTV Parameters					
Operating Mode:	Splitless				
Injection Volume (µL):	1				
Initial Inlet Temperature (°C):	75				
Carrier Gas, Flow (mL/min):	Helium, 1.2				
Splitless Time (min):	1				
Split Flow (mL/min):	100				
Septum Purge (mL/min):	5 (constant)				
PTV Ramp Settings					
	Pressure (Psi)	*Rate (°C/s)*	*Temp. (°C)*	*Time (min)*	*Flow (mL/min)*
Injection:	-	-	-	0.2	
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0
Autosampler Settings					
Injection Depth (mm):	45				
Penetration Speed (mm/s):	100				
Injection Speed (µL/s):	1				
TRACE 1310 GC Parameters					
Oven Temperature Program					
Temperature 1 (°C):	120 (initial)				
Hold Time (min):	2				
Temperature 2 (°C):	250				
Rate (°C/min):	25				
Hold Time (min):	0				
Temperature 3 (°C):	260				
Rate (°C/min):	2.5				
Hold Time (min):	5				
Temperature 4 (°C):	285				
Rate (°C/min):	2.5				
Hold Time (min):	0				
Temperature 5 (°C):	320				
Rate (°C/min):	10				
Hold Time (min):	15				
Total Run Time (min):	44.7				
TSQ 9000 AEI Mass Spectrometer Parameters					
Transfer Line (°C):	300				
Ionization Type (Source type):	EI with the Advanced EI source				
Ion Source (°C):	350				
Electron Energy (eV):	50				
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (×10 – natives HIGH, labeled LOW)				
Tuning Parameters:	AEI Smart Tune				
Collision Gas:	Argon – 70 PSI				

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Appendix 2. List of consumables used

Part number	Description
	Autosampler
365D0291	10 µL fixed needle syringe, 57 mm, 26s gauge, cone tip
	PTV
453T2845-UI	Thermo Scientific™ LinerGOLD™ PTV Concentric Baffle
29053488	Graphite ferrule for inlet
31303233-BP	11 mm BTO septa
29001318	Liner sealing ring for PTV
290VA191	Graphite/Vespel ferrule for MS
07-CPV (A)	0.7 mL crimp top tapered vial – amber
8-AC-ST101	8 mm aluminum crimp cap silicone/ptfe liner
	Column
26066-1540	GC Column, TG-Dioxin 60 m × 0.25 mm, 0.25 µm

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