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Technical Report

Analysis of Dioxins in Foods and Feeds Using GC-MS/MS

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Abstract:

Until recently, the analysis of dioxins in foods and feeds was performed using magnetic sector GC-MS (GC-HRMS), which provided highly accurate quantitation. In recent years, the quantitative accuracy of GC-MS/MS has improved significantly. Accordingly, this method has become officially recognized in the EU as can be used for analyzing dioxins (EU589/2014, 644/2017). In this investigation, dioxins were analyzed in 44 types and 201 samples of foods and feeds using the GCMS-TQ8050 and the GC-MS/MS method package for dioxins in foods, which is compliant with EU regulations. Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS, to compare the quantitative capabilities of both methods. For the comparison, the TEQ ratio was calculated for various samples. From the comparison of the results, for samples with a higher TEQ than 0.060 pg/uL (TEQ when any of the compounds was detected at a higher concentration than LOQ), GC-MS/MS and GC-HRMS provided similar TEQ values in at least 98 % of the samples. Accordingly, it was evident that analysis with GCMS-TQ8050 and method package provides a quantitative capability equivalent to that from GC-HRMS for samples at the concentration levels required for analysis.

Keywords: GC-MS/MS, foods, feeds, dioxins

1. Introduction

Residual organic compounds (persistent organic pollutants or POPs) in foods and feeds are analyzed using a variety of methods. In particular, dioxins are particularly toxic even for POPs, so quantitative analysis is required down to low concentrations.

Until recently, the analysis of dioxins was performed using magnetic sector (double focusing) GC-MS (hereinafter "GC-HRMS"), which provides highly accurate quantitation. However, triple quadrupole GC-MS (hereinafter "GC-MS/MS") is less expensive and easier to handle than GC-HRMS, so its use is being increasingly investigated. In recent years, the quantitative accuracy of GC-MS/MS has improved significantly. Accordingly, the use of this analysis method has become officially recognized in the EU (EU589/2014, 644/2017).

However, in order to change from GC-HRMS to GC-MS/MS, it is first necessary to compare their respective quantitative capabilities.

The Shimadzu GCMS-TQ8050 combines a high-sensitivity detector, capable of detection at femtogram order concentrations, with noise-reduction technology, enabling the analysis of dioxins in foods and feeds. Additionally, the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods" consists of method files registered with the optimal conditions for the analysis of dioxins, as well as a report creation tool, which can output the items required by EU regulations. As a result, analysis can start without spending time on investigating conditions.

In this technical report, dioxins (polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) only) were analyzed in 44 types and 201 samples of foods and feeds using the GCMS-TQ8050 in combination with the method package. Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS, in order to evaluate the quantitative capabilities of both techniques.

2. EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods

The features of the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods" are shown below.

Method Files Registered with the Optimal Conditions for the Analysis of Dioxins

To perform an analysis with TQ, the transition and collision energy (CE) of each compound must be optimized.

Additionally, when creating method files, it is necessary to calculate the retention times of all the target compounds and then to set up a complicated time program based on those results.

Optimized analytical conditions (including transition and CE) are pre-registered in the method files in this product. Additionally, the files are registered with retention times and retention indices, and the retention times can be adjusted automatically using the retention time adjustment function (AART: Automatic Adjustment of Retention Time), allowing analysis to start immediately.

The retention times and time programs can be adjusted automatically, even if the retention times for the measured compounds change, such as when conducting maintenance of the column tip.

Report Creation Tool, Capable of Outputting Items Required by EU Regulations

Complicated calculations are required in analysis reports for dioxins in foods. A report creation tool is included in this product. It can automatically create reports showing items required by EU regulations.

In the analysis of dioxins, a single sample is fractionated into a dioxin (DXN) analysis sample and a polychlorinated biphenyl (PCB) analysis sample. However, depending on the pretreatment method, some of the PCBs can be eluted in the fraction for DXNs, so the analysis results for PCBs are sometimes divided into two parts (the analysis sample for both DXNs and PCBs, and the analysis sample for PCBs only).

With the report creation tool in this product, even if the analysis results for PCBs are divided into two parts, they can be combined, enabling support for a variety of samples and pretreatment methods.

3. Experiment

For the various food samples, pretreatment was performed using an automatic pretreatment unit (extraction: SpeedExtractor (BUCHI); purification: GO-xHT (Miura Co., Ltd.)). Nonane was used as the final solvent for the sample, and the amount of final solvent for the samples was 10 uL. For the STD, a mixture of DF-ST and DF-LCS from Wellington Laboratories was used.

In terms of the analytical conditions for GC-MS/MS, the conditions registered in the method package were used. The analytical conditions in detail are shown in Table 1. Additionally, the transition and collision energies for the compounds measured in this investigation are shown in Table 2.

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Table 1 GC-MS/MS Analytical Conditions

System Configuration

Pretreatment Unit (Extraction)	: SpeedExtractor (BUCHI)
Pretreatment Unit (Purification)	: GO-xHT (Miura Co., Ltd.)
Autosampler	: AOC-20i/s
GC-MS/MS	: GCMS-TQ8050
Software	: GCMSsolution™ Ver. 4.45 SP1
	LabSolutions Insight™ Ver. 3.2 SP1
	GC-MS/MS method package for dioxins in foods

Analytical Conditions (AOC-20i/s)

# of Rinses with Solvent (Pre-run)	: 3
# of Rinses with Solvent (Post-run)	: 3
# of Rinses with Sample	: 0
Plunger Speed (Suction)	: Low
Viscosity Comp. Time	: 0.2 sec.
Plunger Speed (Injection)	: High
Syringe Insertion Speed	: High
Pumping Times	: 5
Inj. Port Dwell Time	: 0.3 sec.
Terminal Air Gap	: No
Plunger Washing Speed	: High
Washing Volume	: 6 uL
Injection Volume	: 2 uL

Analytical Conditions (GC)

Insert Liner	: Topaz® single gooseneck liner, with wool (Restek Corp., P/N: 23336)
Column	: SH-Rxi™-5Sil MS (60 m, 0.25 mm I.D., 0.25 µm), (SHIMADZU, P/N: 227-36036-02)
Injection Mode	: Splitless
Sampling Time	: 1.00 min.
Injection Temp.	: 280 °C
Column Oven Temp.	: 150 °C (1min.) → (20 °C/min.) → 220 °C → (2 °C /min.) → 260 °C (3 min.) → (5 °C /min.) → 320 °C (3.5 min.)
High Pressure Injection	: 450 kPa (1.5 min.)
Flow Control Mode	: Linear Velocity (45.6 cm/sec.)
Purge Flow	: 20 mL/min.
Carrier Gas	: Helium

Analytical Conditions (MS)

Ion Source Temp.	: 230 °C
Interface Temp.	: 300 °C
Detector Voltage	: 1.8 kV (Absolute)
Loop Time	: 0.8 sec. (for native compounds) 0.2 sec. (for labeled compounds)
Transitions	: Refer to Table 2

Table 2 Transition and Collision Energies for the Measured Compounds

I.D.	Compound Name	Retention Index	Quantitative Ion	CE	Reference Ion	CE
1	2,3,7,8-Tetrachlorodibenzo-p-dioxin	2383	319.9>256.9	20	321.9>258.9	20
2	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	2567	355.9>292.9	20	353.9>290.9	20
3	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	2742	389.8>326.9	22	391.8>328.9	22
4	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	2748	389.8>326.9	22	391.8>328.9	22
5	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	2762	389.8>326.9	22	391.8>328.9	22
6	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	2936	423.8>360.8	22	425.8>362.8	22
7	Octachlorodibenzo-p-dioxin	3128	457.7>394.7	22	459.7>396.7	22
8	2,3,7,8-Tetrachlorodibenzofuran	2357	303.9>240.9	28	305.9>242.9	28
9	1,2,3,7,8-Pentachlorodibenzofuran	2513	339.9>276.9	30	337.9>274.9	30
10	2,3,4,7,8-Pentachlorodibenzofuran	2553	339.9>276.9	30	337.9>274.9	30
11	1,2,3,4,7,8-Hexachlorodibenzofuran	2694	373.8>310.9	30	375.8>312.9	30
12	1,2,3,6,7,8-Hexachlorodibenzofuran	2701	373.8>310.9	30	375.8>312.9	30
13	2,3,4,6,7,8-Hexachlorodibenzofuran	2732	373.8>310.9	30	375.8>312.9	30
14	1,2,3,7,8,9-Hexachlorodibenzofuran	2778	373.8>310.9	30	375.8>312.9	30
15	1,2,3,4,6,7,8-Heptachlorodibenzofuran	2867	407.8>344.8	30	409.8>346.8	30
16	1,2,3,4,7,8,9-Heptachlorodibenzofuran	2965	407.8>344.8	30	409.8>346.8	30
17	Octachlorodibenzofuran	3137	441.8>378.8	30	443.8>380.8	30
18	1,2,3,4-Tetrachlorodibenzo-p-dioxin-13C12	2287	331.9>268.0	20	333.9>270.0	20
19	2,3,7,8-Tetrachlorodibenzo-p-dioxin-13C12	2382	331.9>268.0	20	333.9>270.0	20
20	1,2,3,7,8-Pentachlorodibenzo-p-dioxin-13C12	2567	367.9>303.9	20	365.9>301.9	20
21	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin-13C12	2742	401.8>337.9	22	399.9>335.9	22
22	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin-13C12	2747	401.8>337.9	22	399.9>335.9	22
23	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin-13C12	2762	401.8>337.9	22	399.9>335.9	22
24	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin-13C12	2935	435.8>371.8	22	437.8>373.8	22
25	Octachlorodibenzo-p-dioxin-13C12	3127	469.8>405.8	22	471.8>407.8	22
26	2,3,7,8-Tetrachlorodibenzofuran-13C12	2357	315.9>251.9	28	317.9>253.9	28
27	1,2,3,7,8-Pentachlorodibenzofuran-13C12	2513	351.9>287.9	30	349.9>285.9	30
28	2,3,4,7,8-Pentachlorodibenzofuran-13C12	2553	351.9>287.9	30	349.9>285.9	30
29	1,2,3,4,7,8-Hexachlorodibenzofuran-13C12	2694	385.8>321.9	30	387.8>323.9	30
30	1,2,3,6,7,8-Hexachlorodibenzofuran-13C12	2701	385.8>321.9	30	387.8>323.9	30
31	2,3,4,6,7,8-Hexachlorodibenzofuran-13C12	2732	385.8>321.9	30	387.8>323.9	30
32	1,2,3,7,8,9-Hexachlorodibenzofuran-13C12	2778	385.8>321.9	30	387.8>323.9	30
33	1,2,3,4,6,7,8-Heptachlorodibenzofuran-13C12	2867	419.8>355.9	30	421.8>357.9	30
34	1,2,3,4,7,8,9-Heptachlorodibenzofuran-13C12	2965	419.8>355.9	30	421.8>357.9	30
35	Octachlorodibenzofuran-13C12	3137	453.8>389.8	30	455.8>391.8	30

4. Analysis Results

4-1. Analysis Results for the STD

In the analysis of dioxins in foods, the maximum permitted concentrations (Maximum Levels, hereinafter "ML") are prescribed for each food and feed. With the food and feed samples in this investigation, the ML for pig's fat and pig's meat were the lowest at 1 pg/g of fat (sum of dioxins (WHO-PCDD/F-TEQ)). Additionally, the limit of quantitation (hereinafter "LOQ") required for each compound in the analysis depends on the food or feed sample's ML, the pretreatment method, and the TEF (toxic equivalence factor) of each compound. The compounds 2,3,7,8-Tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-Pentachlorodibenzo-p-dioxin have the highest TEF (TEF=1), so their LOQ are lower than for other compounds. In this investigation, the LOQ for 2,3,7,8-Tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-Pentachlorodibenzo-p-dioxin in pig's fat and pig's meat was 0.060 pg/uL at the concentration in the final vial.

In the EU regulations, for each compound, at least one of the criteria shown below (a partial excerpt from EU589/2014 and 644/2017) must be satisfied at the LOQ.

1. S/N Ratio (Hereinafter "Method 1")

The concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with a S/N (signal/noise) ratio of 3:1 for the less intensive raw data signal.

2. Lowest Concentration Point on the Calibration Curve (Method 2)

The lowest concentration point on a calibration curve that gives an acceptable (≤ 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.

In this technical report, for the purposes of confirmation, an evaluation was performed using both criteria.

As noted above, for 2,3,7,8-Tetrachlorodibenzo-p-dioxin, it is necessary to set the LOQ to 0.060 pg/uL or less. Accordingly, before analysis, the STD was prepared so that the concentration of each compound was 0.050 pg/uL. (The concentration was double however for Octachlorodibenzo-p-dioxin and Octachlorodibenzofuran.) From the results of the analysis, it was evident that the criteria for Method 1 were satisfied for all compounds. The S/N ratios for each compound are shown in Fig. 1.

Additionally, with Method 2, a calibration curve was created with all six points used, including the two at concentrations less than 0.060 pg/uL (0.025 pg/uL and 0.050 pg/uL). The concentrations for each compound at each calibration curve point (level) are shown in Table 3. For each compound, when the level 1 RRF and average RRF were compared, it was found that all compounds satisfied the criteria for Method 2. The RRF deviations for each compound are shown in Table 3.

From the above-mentioned results, it was evident that at the LOQ, the criteria were satisfied for all compounds.

Table 3 Each Calibration Point Concentration and RRF for the Measured Compounds

I.D.	Compound Name	TEF	Calibration Point Concentration						Average RRF	RRF (level 1)	RRFDev (%) (Level 1)
			Level 1	Level 2	Level 3	Level 4	Level 5	Level 6			
1	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1	0.025	0.050	0.100	0.250	0.500	1.000	1.07	1.15	8.10
2	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	1	0.025	0.050	0.100	0.250	0.500	1.000	1.09	0.97	10.56
3	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.14	1.39	22.26
4	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.1	0.025	0.050	0.100	0.250	0.500	1.000	0.95	0.92	2.72
5	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.03	1.25	21.44
6	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.01	0.025	0.050	0.100	0.250	0.500	1.000	0.92	0.82	11.46
7	Octachlorodibenzo-p-dioxin	0.0003	0.050	0.100	0.200	0.500	1.000	2.000	1.19	1.04	12.21
8	2,3,7,8-Tetrachlorodibenzofuran	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.10	1.05	4.66
9	1,2,3,7,8-Pentachlorodibenzofuran	0.03	0.025	0.050	0.100	0.250	0.500	1.000	1.04	1.00	3.23
10	2,3,4,7,8-Pentachlorodibenzofuran	0.3	0.025	0.050	0.100	0.250	0.500	1.000	0.97	0.89	7.59
11	1,2,3,4,7,8-Hexachlorodibenzofuran	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.03	0.82	20.72
12	1,2,3,6,7,8-Hexachlorodibenzofuran	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.09	1.36	24.62
13	2,3,4,6,7,8-Hexachlorodibenzofuran	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.09	1.39	27.83
14	1,2,3,7,8,9-Hexachlorodibenzofuran	0.1	0.025	0.050	0.100	0.250	0.500	1.000	1.06	1.23	16.10
15	1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.01	0.025	0.050	0.100	0.250	0.500	1.000	1.17	1.05	10.37
16	1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.01	0.025	0.050	0.100	0.250	0.500	1.000	1.02	0.97	4.97
17	Octachlorodibenzofuran	0.0003	0.050	0.100	0.200	0.500	1.000	2.000	1.00	0.84	15.80

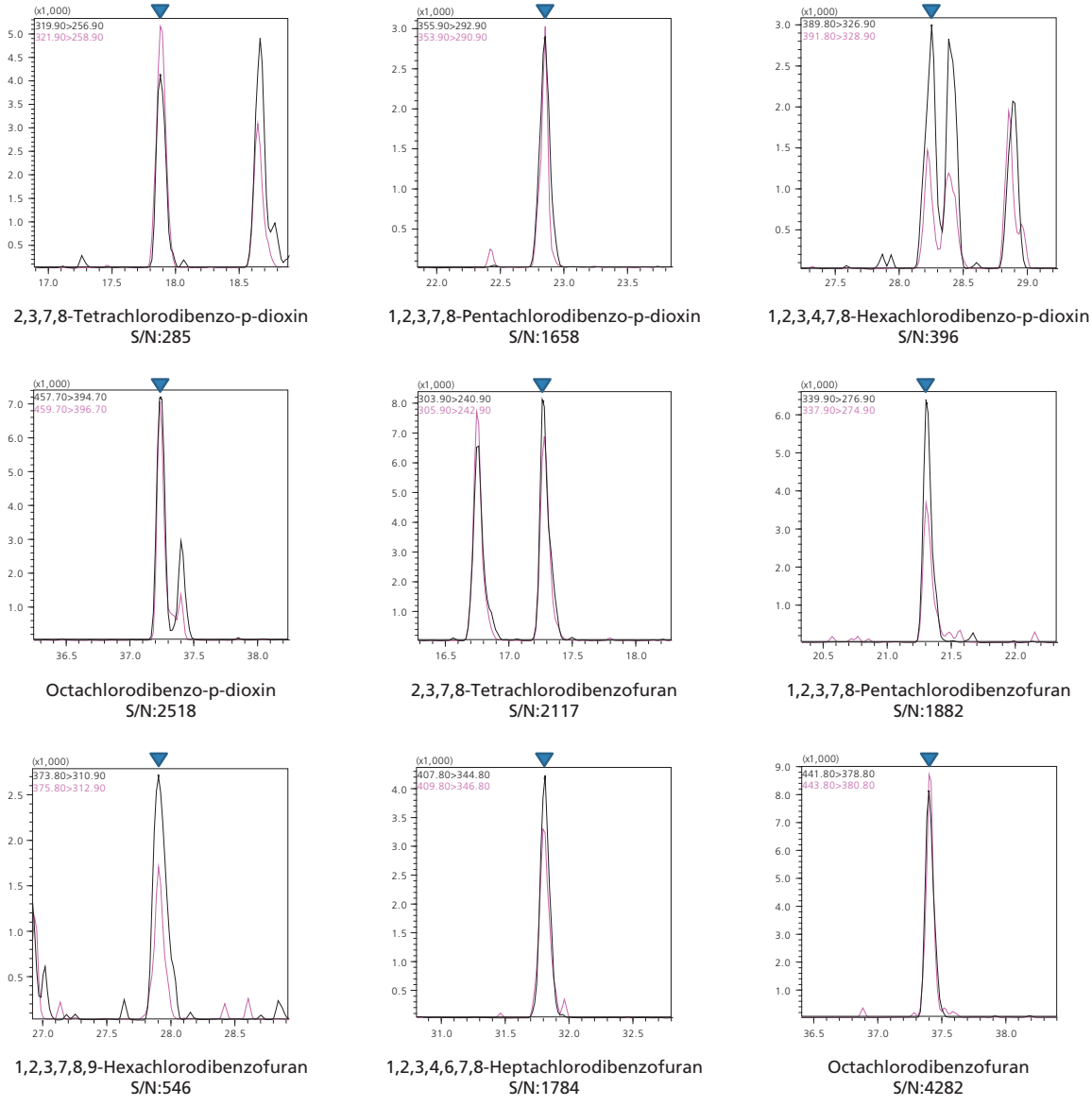

Fig. 1 Chromatograms for a Concentration of 0.050 pg/uL

4-2. Analysis Results for the Test Samples

As previously noted, the strength of toxicity differs for each dioxin compound. The TEF, which is calculated for each compound by taking the toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin as 1, is used as an index of strength. Note that the TEF values for each compound are as shown in Table 3.

The ML for the dioxins in foods and feeds are prescribed by their toxic equivalents (TEQ). The TEQ is calculated by multiplying the concentration of each compound by the TEF, and then calculating the total TEQ for all compounds.

In this investigation, 44 types and 201 samples of foods were analyzed using GC-MS/MS. Additionally, the same samples were analyzed with GC-HRMS, and the results were compared with the GC-MS/MS analysis results. For this comparison, the TEQ was calculated by multiplying the concentration in the final vial for each compound by the TEF, and then calculating a total TEQ for all compounds. The results were tallied separately for each food and feed.

The results for typical foods and feeds are shown in Fig. 2. The results for all foods and feeds are shown in Fig. 3. Additionally, typical chromatograms for each compound are shown in Fig. 4.

Fig. 2 shows a comparison of the TEQ values for GC-MS/MS and GC-HRMS by food and feed. The sample is indicated on the horizontal axis, and the TEQ for each sample is indicated on the vertical axis.

Fig. 3 shows the GC-HRMS TEQ on the horizontal axis, and the GC-MS/MS TEQ on the vertical axis. If they were correlated, the values would approach a straight line with a slope of 1 (the blue dashed line in the figure).

A TEQ of 0.060 pg/uL and a TEQ of 0.025 pg/uL are marked as indicators for the samples.

TEQ 0.060 pg/uL: If even one compound is detected at a concentration higher than the LOQ, the total TEQ value will be higher than 0.060 pg/uL. Accordingly, a straight line (red dashed line in the figure) is drawn on the vertical axis in Fig. 2, and on the horizontal axis and vertical axis in Fig. 3 to mark 0.060 pg/uL.

TEQ 0.025 pg/uL: If a compound with the highest TEF (2,3,7,8-Tetrachlorodibenzo-p-dioxin or 1,2,3,7,8-Pentachlorodibenzo-p-dioxin) is detected at a higher concentration than the lowest point in the calibration curve, the total TEQ value will be higher than 0.025 pg/uL. Accordingly, a straight line (green dashed line in the figure) is drawn on the vertical axis in Fig. 2, and on the horizontal axis and vertical axis in Fig. 3 to mark 0.025 pg/uL.

In order to check the correlation between GC-MS/MS and GC-HRMS, a regression line was calculated with respect to Fig. 3, and a t-test was performed for the slope and intercept. The calculated results are shown in Table 4. The 95 % confidence limits for the intercept and slope are extremely close to 0 and 1, respectively.

Next, the distribution of the TEQ ratios for GC-MS/MS and GC-HRMS was calculated and checked in detail.

For samples with a TEQ of at least 0.060 pg/uL, when the TEQ ratio (%) was calculated, for at least 98 % of the samples, the ratio was between 50 % and 200 %, indicating a similar TEQ value for both systems. (Table 5)

In contrast, for samples with a TEQ less than 0.060 pg/uL, 79 % of the samples had a ratio between 50 % and 200 %, indicating a significant difference for 21 % of the samples. For many of the samples, the lower the TEQ, the greater the difference. 92 % of the samples with a ratio less than 50 % or more than 200 % had a TEQ less than 0.025 pg/uL. (Table 6)

From the above-mentioned results, it was evident that GC-MS/MS and GC-HRMS provide similar TEQ values for samples with a TEQ higher than 0.060 pg/uL. Additionally, it was evident that the lower the TEQ, the greater the number of samples with a significant difference in TEQ values.

Table 4 Results of the t-Tests for the Intercept and Slope

	Coefficient	Standard Error	t	95 % Confidence Interval	
				Lower Limit	Upper Limit
Intercept	-0.005	0.001	-3.235	-0.008	-0.002
Slope	1.049	0.001	741.500	1.046	1.051

Table 5 Distribution of the Ratio of Total TEQ Values for Samples with a TEQ of at Least 0.060 pg/uL

	TEQ Ratio (TQ/Sector) (%)		
	<50	50 - 200	200<
Number of Samples (pc)	2	87	0
Distribution (%)	2	98	0.00

Table 6 Distribution of the Ratio of Total TEQ Values for Samples with a TEQ Less Than 0.060 pg/uL

	TEQ Ratio (TQ/Sector) (%)		
	<50	50 - 200	200<
Number of Samples (pc)	21*	92	4
Distribution (%)	18	79	3

* 19 of the 21 samples with a ratio under 50 % had a TEQ less than 0.025 pg/uL. It was evident that the lower the total TEQ value, the greater the tendency for a difference to arise.

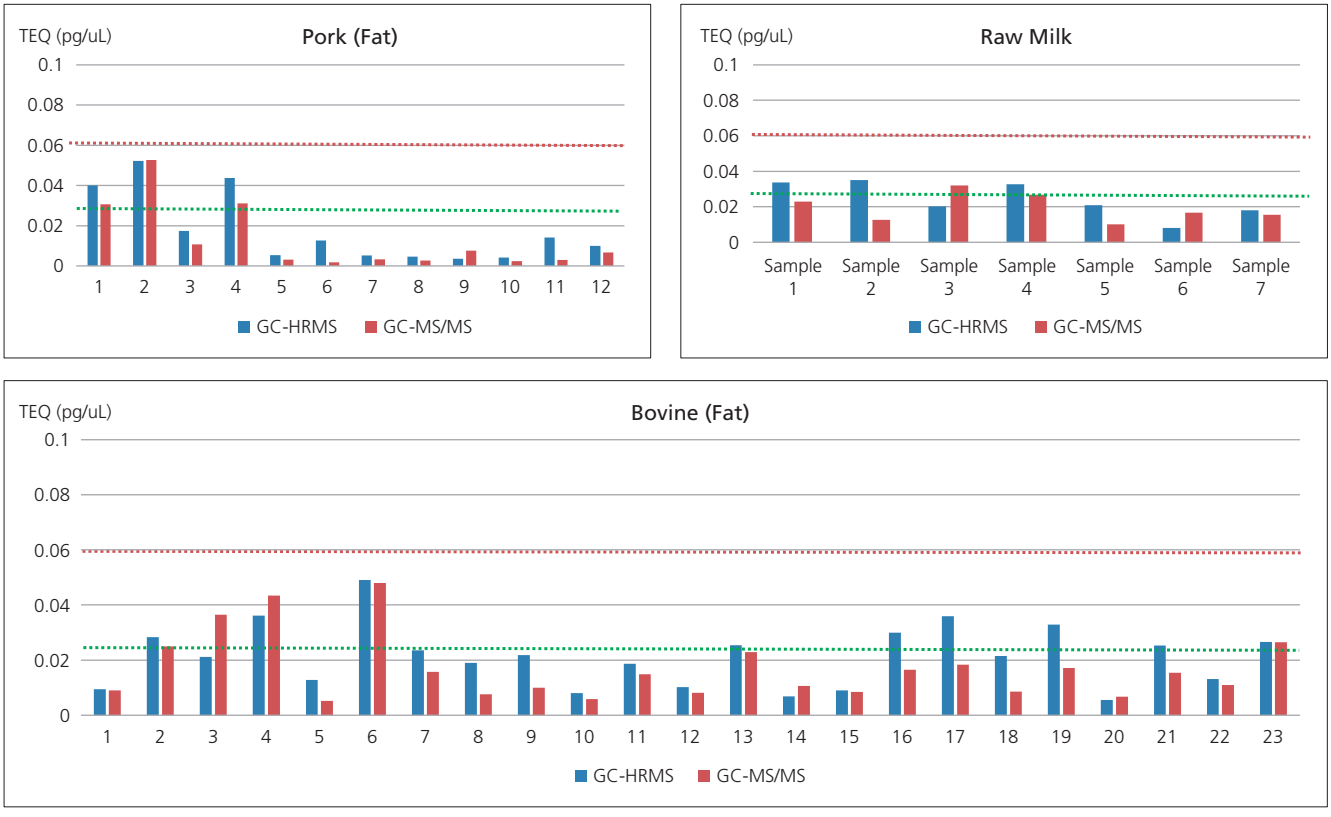

Fig. 2 Comparison of the TEQ Results for Each Food and Feed

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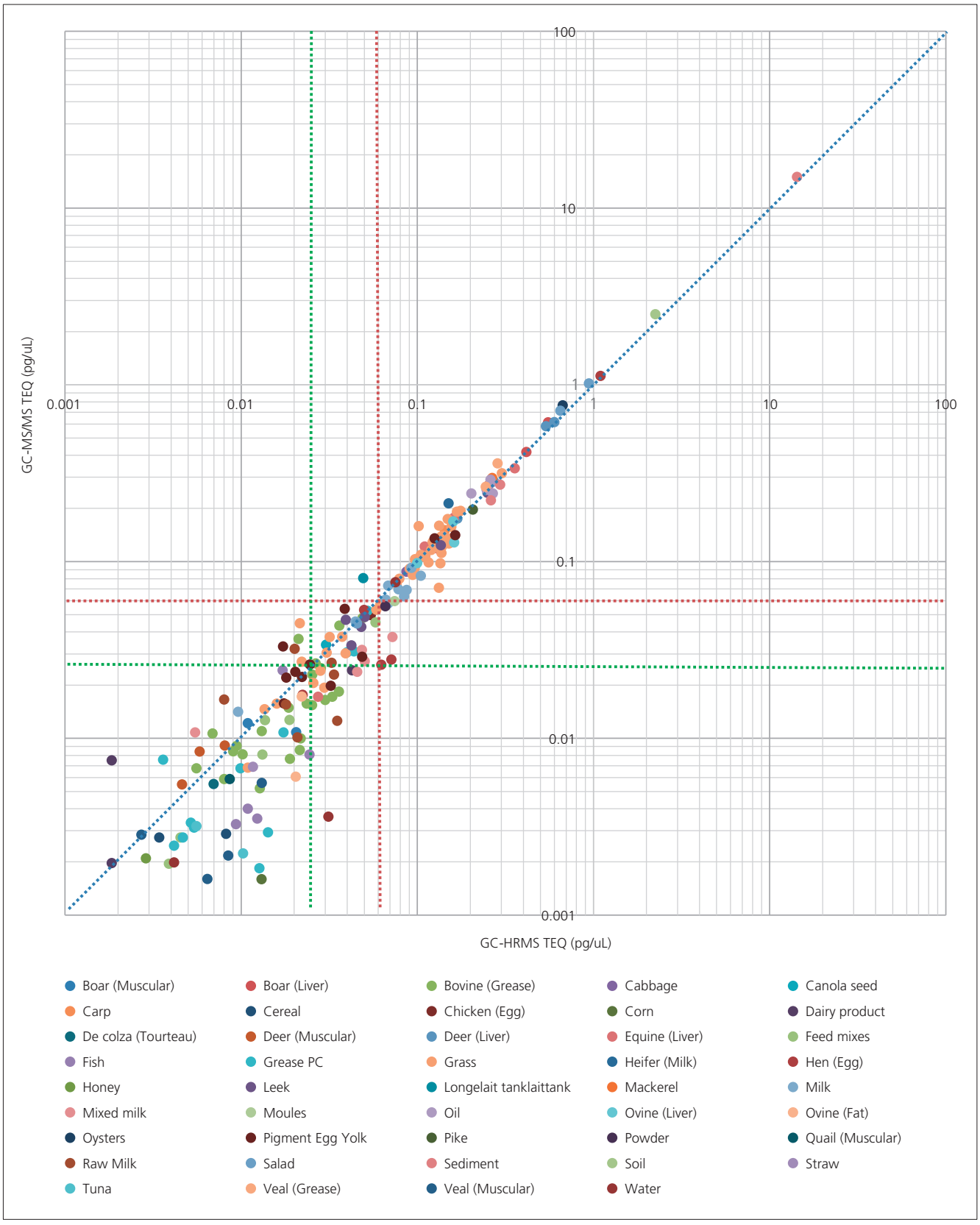

Fig. 3 Comparison of the TEQ Results for Each Food and Feed

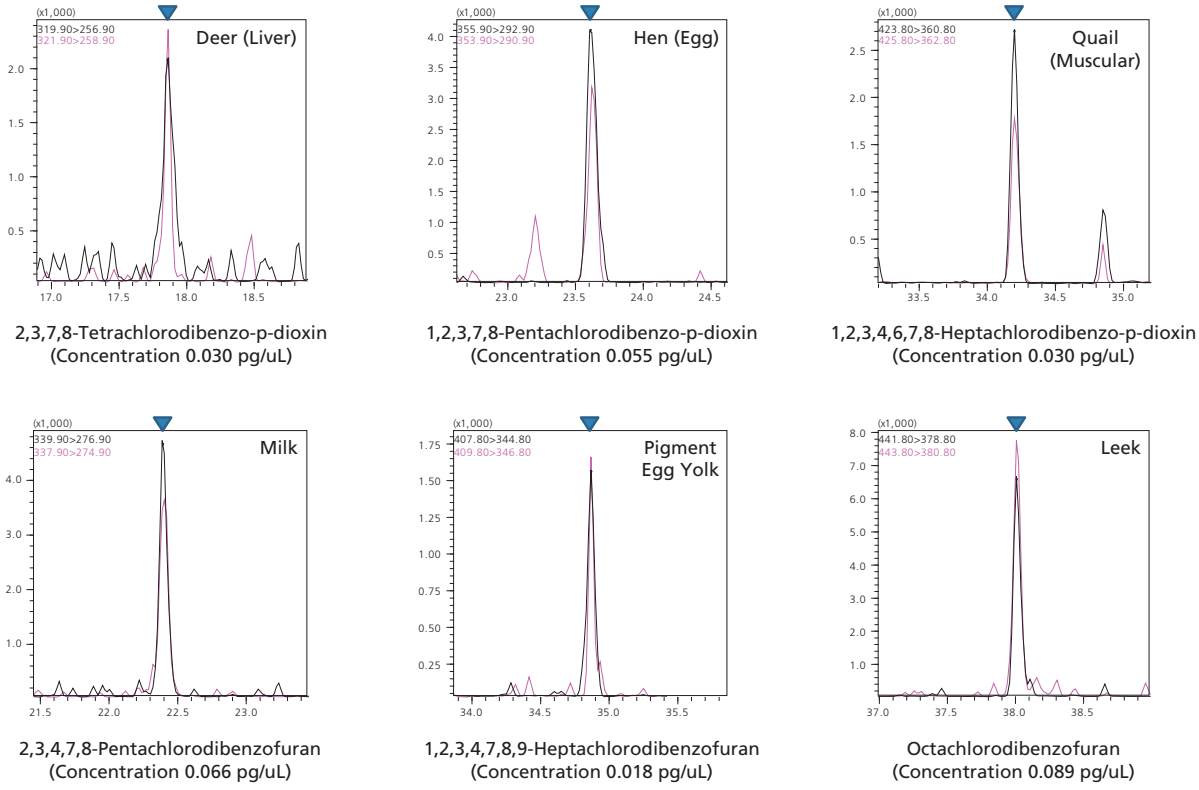

Fig. 4 Chromatograms of Dioxins in Various Samples

5. Conclusion

In this technical report, dioxins were analyzed in 44 types and at least 201 samples of foods and feeds using the GCMS-TQ8050 and the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods". Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS in order to assess the quantitative capabilities of both methods.

Firstly, before analyzing the foods and feeds, a STD was analyzed using GC-MS/MS, and it was confirmed that the criteria were satisfied at the LOQ.

Next, the foods and feeds were analyzed, and the results were compared with those from magnetic sector GC-MS. For the comparison, the TEQ ratio was calculated for GC-MS/MS and GC-HRMS. For samples with a higher TEQ than 0.060 pg/uL (TEQ when any of the compounds was detected at a higher concentration than the LOQ), GC-MS/MS and GC-HRMS provided similar TEQ values in at least 98 % of the samples. Additionally, it was evident that the number of samples with a significant difference in TEQ increases as the TEQ value decreases.

From the above-mentioned results, it is evident that analysis with GCMS-TQ8050 and method package provides a quantitative capability equivalent to that from GC-HRMS for samples at the concentration levels required for analysis. However, at concentrations below the required level, differences in quantitative capability could arise. For this reason, it is necessary to be aware of the system status by confirming quantitative capability at the LOQ, and evaluating whether there has been a decrease in sensitivity.

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