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Technical Report

Determination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans (PCDD/Fs) in Foodstuffs and Animal Feed Using a Triple Quadrupole GCMS-TQ8040 System with Smart MRM Transforms Laboratory Analysis

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Abstract:

A method was developed on a high selectively triple quadrupole GCMS-TQ8040 system for screening trace levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in foodstuffs and animal feed. Smart MRM technology was employed for method development and GC-MS/MS analysis. The results showed good sensitivity and repeatability for PCDD/Fs at low levels, as well as a good linear response over the required concentration range. The performance on real sample analysis indicated the feasibility of Shimadzu GCMS-TQ8040 system for PCDD/Fs measurement in food and feed, as required by European Union legislation.

Keywords: GC-MS/MS, MRM, PCDDs, PCDFs, dioxins, food, feed

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are of special concern, due to their harmful health effects. They have neurotoxic potential and are linked to causing cancer, endocrine disruption, and reproductive disorders. PCDD/Fs were never produced intentionally as marketable products, while they are widespread around the world. They can also bioaccumulate and biomagnify through the food chain, and finally pose a threat to human body. Most dioxin exposure to human derives from food (>90%), 90 % of which is of animal origin [1]. Consequently, incidents of dioxin contamination involving food and feed have generally raised great public concern in the world. For example, Germany's dioxin-tainted food scandal in 2011 led to a shutdown of more than 4700 farms and tremendous economic losses in Germany.

European regulations specifying official sampling and analysis methods for controlling PCDD/F levels and polychlorinated biphenyls (PCBs) in food and feed were initially issued in the early 2000s, followed by several amendments thereafter. The latest amendment was Commission Regulation (EU) Nos. 589/2014 [2] and 709/2014 [3], where a major update is that gas chromatography–triple quadrupole mass spectrometry (GC-MS/MS) was recognized as an appropriate confirmatory method for checking compliance with the maximum levels of PCDD/Fs and PCBs in food and feed control. This means GC-MS/MS can be used to provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or in case of need at the action threshold.

This Technical Report describes a highly sensitive and reproducible method for determining PCDD/Fs (Table 1) in food and feed using a triple quadrupole GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The results show performance comparable to using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) for quantitation of PCDD/Fs in food and feed.

Table 1 PCDD/Fs Specified in EU Legislation and TEF Values Established by WHO

Congener	WHO_{1998}-TEF	WHO_{2005}-TEF	Congener	WHO_{1998}-TEF	WHO_{2005}-TEF
2,3,7,8-TCDD	1	1	2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDD	1	1	1,2,3,7,8-PeCDF	0.05	0.03
1,2,3,4,7,8-HxCDD	0.1	0.1	2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,6,7,8-HxCDD	0.1	0.1	1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1	1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01	2,3,4,6,7,8-HxCDF	0.1	0.1
OCDD	0.0001	0.0003	1,2,3,7,8,9-HxCDF	0.1	0.1
			1,2,3,4,6,7,8-HpCDF	0.01	0.01
			1,2,3,4,7,8,9-HpCDF	0.01	0.01
			OCDF	0.0001	0.0003

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2. Experimental

2-1. Sample Preparation and Instrumental Analysis

The sample preparation of food and feed was mainly followed US EPA method 1613B [4] with proper modification [5]. The samples were extracted using an accelerated solvent extraction (ASE) system, followed by purification steps (including acidic/basic silica gel, alumina, and carbon columns). Samples were spiked with ^{13}C-isotope labeled surrogate standards (1613-LCS) and internal standards (1613-IS) prior to extraction and instrumental analysis, respectively.

Samples were analyzed in a Shimadzu GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The GC unit was equipped with a split/splitless injection port. The capillary column was a 60 m DB-5MS fused silica capillary column (J&W, Scientific, 0.25 µm film thickness, 0.25 mm I.D.). Table 2 showed the selected instrumental conditions for PCDD/Fs detection. Measurements were performed in EI-MS/MS Multiple Reaction Monitoring (MRM) mode. The MRM method for PCDD/Fs was developed using the Shimadzu GCMS-TQ8040 MRM optimization tool, which automatically determines the optimum transitions and collision energies in a single sequence, and then seamlessly incorporates them into the Smart Database series for full method development (Fig. 1). At that point, the two most intense ions of the molecular chlorine isotope cluster of each congener and internal standard were chosen. Using flexible MS event, the Smart MRM function routinely determines the best dwell and loop times for the two transitions in a single method, providing optimum precision and sensitivity. In order to improve the peak shape for 2,3,7,8-TCDD/F at very low concentration levels (10 fg/µL), the event times were manually increased to 0.35 seconds for the native 2,3,7,8-TCDD/F and decreased to 0.20 seconds for the ^{13}C-labeled 2,3,7,8-TCDD/F to maintain the same loop time as other congeners. Information on the MRM settings and analyte retention times are given in Table 3.

Table 2 Conditions for Gas Chromatograph and Mass Spectrometer

GC Conditions	
Column	: DB-5MS (60 m × 0.25 mm × 0.25 µm)
Injection Mode	: Splitless
Injection Volume	: 2 µL
High Pressure Injection	: 300 kPa (2 min)
Sampling Time	: 1 min
Injection Temperature	: 290 °C
Column Oven Temperature	: 150 °C (3 min) → (20 °C/min) → 230 °C (18 min) → (5 °C/min) → 235 °C (10 min) → (4 °C/min) → 320 °C (1 min)
Flow Control Mode	: Linear Velocity
Column Flow	: 1.03 mL/min

MS Conditions	
Ionization Mode	: EI
Ion Source Temperature	: 250 °C
Interface Temperature	: 270 °C
Acquisition Mode	: MRM Mode
Collision Gas	: Argon
Emission Current	: 250 µA
Loop Time	: 1.1 sec
Pressure of CID Gas	: 150 kPa
Detector Voltage	: 1.8 kV

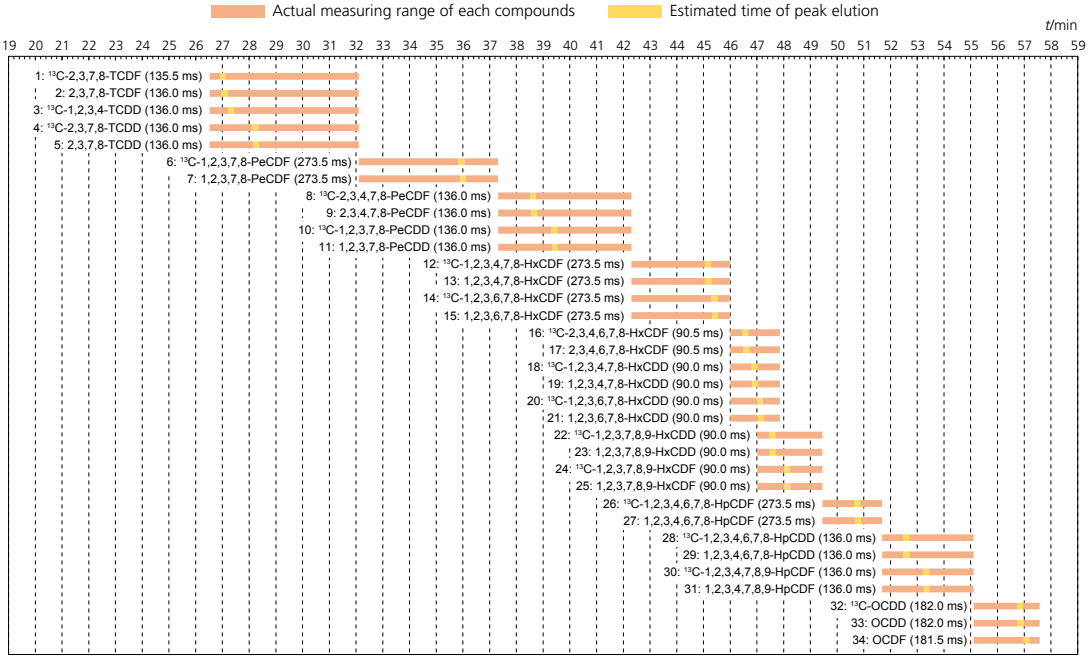


Fig. 1 Optimized MS Event Times for PCDD/F Congeners with Smart MRM Function

3. Results and Discussion

3-1. Chromatography

The chromatographic separation of the 17 native 2,3,7,8-substituted PCDD/F congeners is shown in Fig. 2 (EPA 1613 CS3). The sample was completely separated in a total run time of 60 minutes.

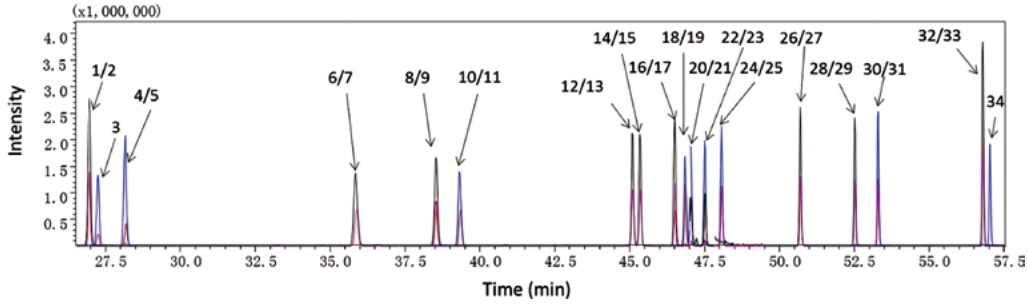


Fig. 2 Mass Chromatogram of PCDD/Fs in EPA 1613 CS3 (10 – 100 pg/µL) (Peak numbers refer to analytes listed in Table 3.)

Table 3 MS/MS Settings for Native PCDD/F Congeners and ^{13}C-Internal Standards

Peak No.	Analyte	RT (min)	Precursor > Product	CE (V)	Precursor > Product	CE (V)
1	^{13}C-2,3,7,8-TCDF	27.024	315.90 > 251.90	31	317.90 > 253.90	31
2	2,3,7,8-TCDF	27.076	303.90 > 240.90	31	305.90 > 242.90	31
3	^{13}C-1,2,3,4-TCDD	27.323	331.90 > 267.90	25	333.90 > 269.90	25
4	^{13}C-2,3,7,8-TCDD	28.235	331.90 > 267.90	25	333.90 > 269.90	25
5	2,3,7,8-TCDD	28.253	319.90 > 256.90	25	321.90 > 258.90	25
6	^{13}C-1,2,3,7,8-PeCDF	35.953	351.90 > 287.90	34	349.90 > 285.90	34
7	1,2,3,7,8-PeCDF	36.01	339.90 > 276.90	34	337.90 > 274.90	34
8	^{13}C-2,3,4,7,8-PeCDF	38.625	351.90 > 287.90	37	349.90 > 285.90	37
9	2,3,4,7,8-PeCDF	38.673	339.90 > 276.90	37	337.90 > 274.90	37
10	^{13}C-1,2,3,7,8-PeCDD	39.419	367.90 > 303.90	25	365.90 > 301.90	25
11	1,2,3,7,8-PeCDD	39.441	355.90 > 292.90	25	353.90 > 290.90	25
12	^{13}C-1,2,3,4,7,8-HxCDF	45.168	385.80 > 321.90	37	387.80 > 323.90	37
13	1,2,3,4,7,8-HxCDF	45.183	373.80 > 310.90	37	375.80 > 312.90	37
14	^{13}C-1,2,3,6,7,8-HxCDF	45.413	385.80 > 321.90	37	387.80 > 323.90	37
15	1,2,3,6,7,8-HxCDF	45.438	373.80 > 310.90	37	375.80 > 312.90	37
16	^{13}C-2,3,4,6,7,8-HxCDF	46.568	385.80 > 321.90	37	387.80 > 323.90	37
17	2,3,4,6,7,8-HxCDF	46.604	373.80 > 310.90	37	375.80 > 312.90	37
18	^{13}C-1,2,3,4,7,8-HxCDD	46.911	401.80 > 337.90	25	403.80 > 339.80	25
19	1,2,3,4,7,8-HxCDD	46.935	389.80 > 326.90	25	391.80 > 328.80	25
20	^{13}C-1,2,3,6,7,8-HxCDD	47.1	401.80 > 337.90	25	403.80 > 339.80	25
21	1,2,3,6,7,8-HxCDD	47.139	389.80 > 326.90	25	391.80 > 328.80	25
22	^{13}C-1,2,3,7,8,9-HxCDD	47.577	401.80 > 337.90	25	403.80 > 339.80	25
23	1,2,3,7,8,9-HxCDD	47.591	389.80 > 326.90	25	391.80 > 328.80	25
24	^{13}C-1,2,3,7,8,9-HxCDF	48.126	385.80 > 321.90	34	387.80 > 323.90	34
25	1,2,3,7,8,9-HxCDF	48.145	373.80 > 310.90	34	375.80 > 312.90	34
26	^{13}C-1,2,3,4,6,7,8-HpCDF	50.755	419.80 > 355.80	37	421.80 > 357.80	37
27	1,2,3,4,6,7,8-HpCDF	50.788	407.80 > 344.80	37	409.80 > 346.80	37
28	^{13}C-1,2,3,4,6,7,8-HpCDD	52.584	435.80 > 371.80	25	437.80 > 373.80	25
29	1,2,3,4,6,7,8-HpCDD	52.596	423.80 > 360.80	25	425.80 > 362.80	25
30	^{13}C-1,2,3,4,7,8,9-HpCDF	53.348	419.80 > 355.80	37	421.80 > 357.80	37
31	1,2,3,4,7,8,9-HpCDF	53.357	407.80 > 344.80	37	409.80 > 346.80	37
32	^{13}C-OCDD	56.85	469.70 > 405.80	25	471.70 > 407.80	25
33	OCDD	56.867	457.70 > 394.80	25	459.70 > 396.80	25
34	OCDF	57.081	441.70 > 378.80	34	443.70 > 380.80	34

3-2. Sensitivity

To verify the performance of the GC-MS/MS system in the low concentration range, 1:50 dilutions of the calibration standard EPA-1613CS1 were also measured (10 fg/µL 2,3,7,8-TCDD). Based on the 2 µL injection volume, the dilutions of the calibration standard EPA-1613CS1 indicate an absolute amount of 20 fg 2,3,7,8-TCDD in the column. The MRM chromatograms for native PCDD/Fs are shown in Fig. 3.

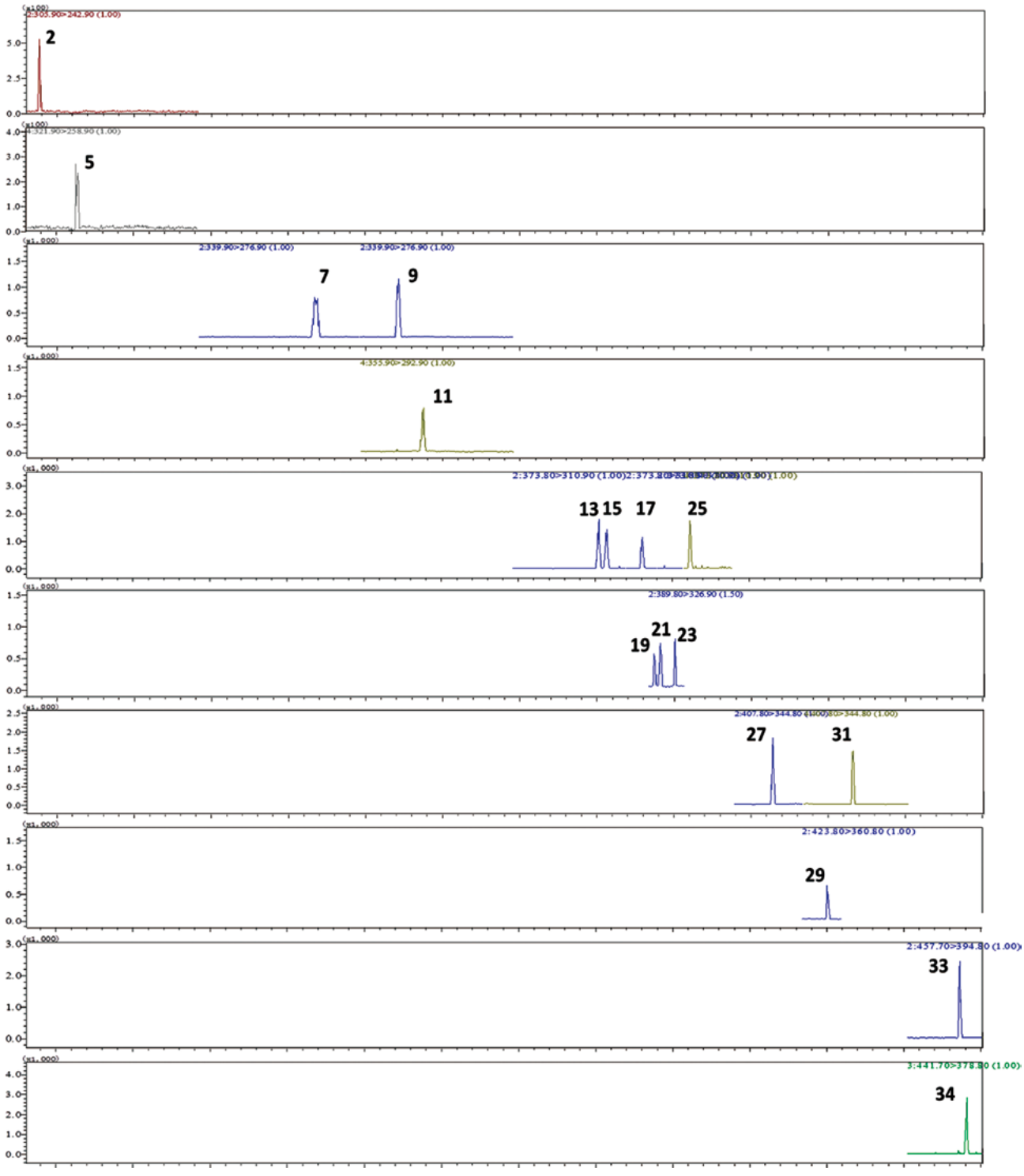


Fig. 3 MRM Chromatograms of Native PCDD/F Congeners (10 – 100 fg/µL, 2 µL injection volume) (Peak numbers refer to analytes listed in Table 3.)

3-3. Linearity of Response

A seven-point calibration curve was prepared based on US EPA method 1613, defined for calibration verification solutions (CS1-CS5), together with EPA-1613CSL and EPA-1613CS0.5. Excellent linearity was obtained for the calibration standards over the concentration range from 0.1 to 200 ng/mL (TCDD), with R^2 values >0.999 for an injection volume of 1 µL (Table 4). The mean response factor for each congener is also given in Table 4. The calibration curves for 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDD are shown in Fig. 4 to 6.

Table 4 Linear Regression for Seven-Point Calibration Curves over the Range 0.1 to 200 ng/mL (TCDD) and the Mean Response Factor for Each Congener

Congener	Regression line equation	R^2	Mean RF	RF %RSD
2,3,7,8-TCDD	Y = 1.150399X + 3.29953e-004	0.99999	1.22794	8.06
1,2,3,7,8-PeCDD	Y = 1.014733X + 3.009239e-003	1.00000	1.03887	1.96
1,2,3,4,7,8-HxCDD	Y = 1.079761X – 5.260601e-004	0.99997	1.09358	3.65
1,2,3,6,7,8-HxCDD	Y = 0.9705907X + 5.362575e-002	0.99915	1.08710	5.37
1,2,3,7,8,9-HxCDD	Y = 1.024768X + 3.682249e-002	0.99967	1.00394	14.48
1,2,3,4,6,7,8-HpCDD	Y = 0.9429045X + 1.331675e-002	0.99998	1.02985	6.92
OCDD	Y = 1.242978X – 6.145206e-002	0.99929	1.14683	6.10
2,3,7,8-TCDF	Y = 1.15754X + 9.032785e-004	0.99996	1.18104	3.57
1,2,3,7,8-PeCDF	Y = 1.015266X – 5.771587e-003	0.99997	1.07846	7.83
2,3,4,7,8-PeCDF	Y = 1.045151X – 6.304552e-003	0.99998	1.04210	4.49
1,2,3,4,7,8-HxCDF	Y = 1.006328X + 2.605984e-002	0.99988	1.09930	6.04
1,2,3,6,7,8-HxCDF	Y = 0.9307018X + 3.432044e-002	0.99971	1.06611	8.06
2,3,4,6,7,8-HxCDF	Y = 0.9080292X + 3.053454e-002	0.99983	1.00464	6.34
1,2,3,7,8,9-HxCDF	Y = 0.960272X + 2.450491e-002	0.99993	1.03403	9.10
1,2,3,4,6,7,8-HpCDF	Y = 0.9732686X + 4.031919e-002	0.99958	1.08255	4.88
1,2,3,4,7,8,9-HpCDF	Y = 0.9562794X + 3.622056e-002	0.99969	1.06788	5.48
OCDF	Y = 1.424071X + 3.271179e-003	0.99999	1.50245	8.94

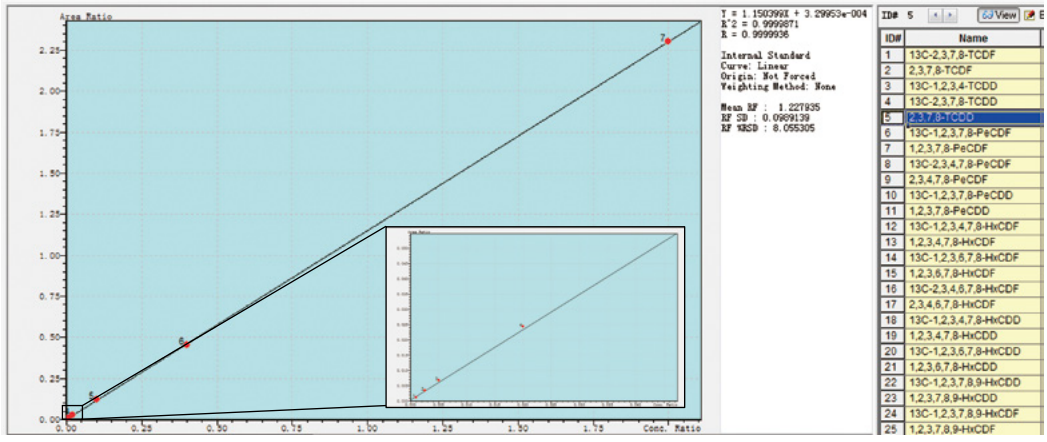


Fig. 4 Seven-Point Calibration Curve for 2,3,7,8-TCDD with Both Linear Fit and Mean Response Factors

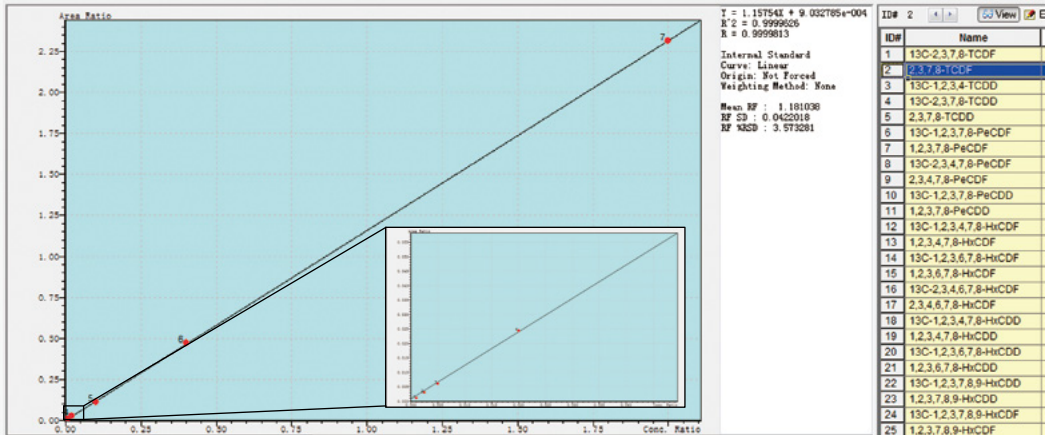


Fig. 5 Seven-Point Calibration Curve for 2,3,7,8-TCDF with Both Linear Fit and Mean Response Factors

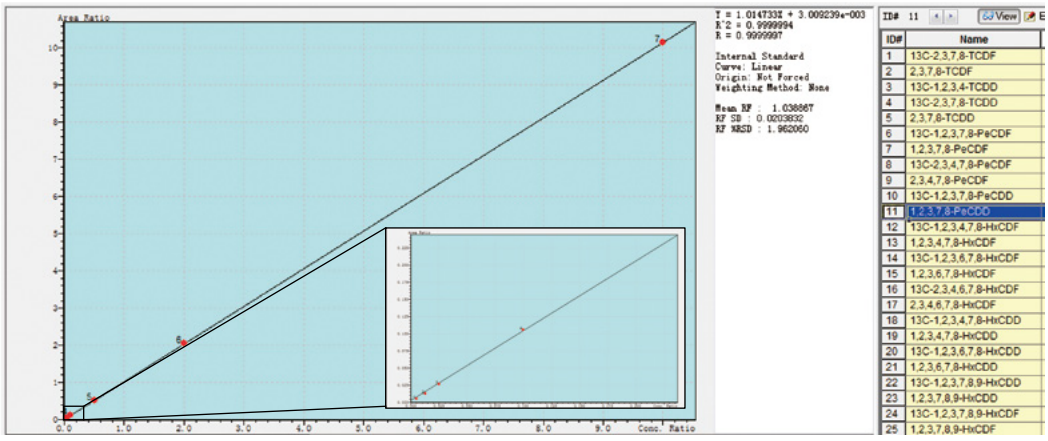


Fig. 6 Seven-Point Calibration Curve for 1,2,3,7,8-PeCDD with Both Linear Fit and Mean Response Factors

3-4. Repeatability

The repeatability was confirmed for within the same day and over different days. Total 12 injections (four 2 µL injections per day for three days) of 1:50 diluted EPA-1613CS1 (10 fg/µL 2,3,7,8-TCDD) were performed. Results exhibited good repeatability for the peak areas of each congener, with a relative standard deviations (RSD) less than 15 % (Fig. 7).

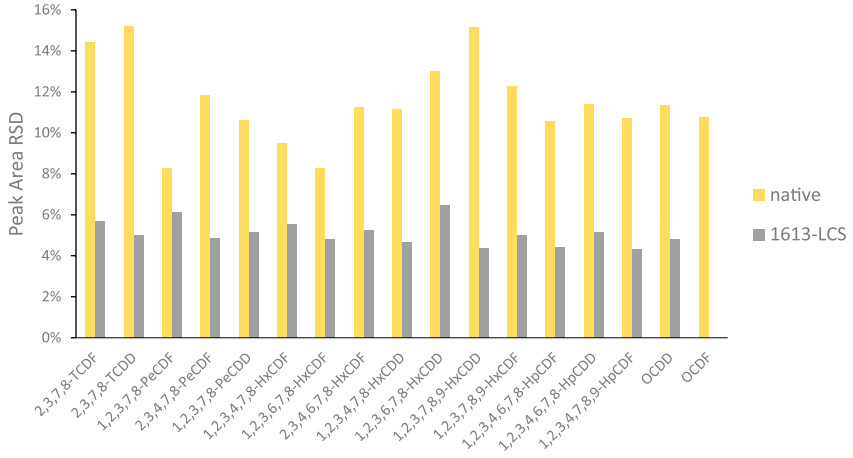


Fig. 7 Repeatability of Peak Areas for Native PCDD/Fs and 1613-LCS (n = 12)

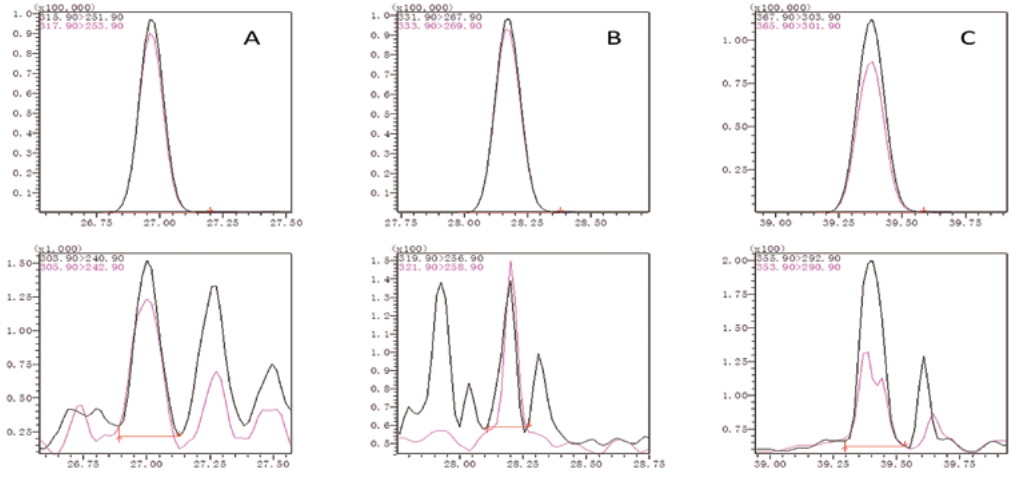


Fig. 8 MRM Chromatograms for ^{13}C-Labeled and Native 2,3,7,8-TCDF (A), 2,3,7,8-TCDD (B), and 1,2,3,7,8-PeCDD (C) in Fish Oil

3-5. Sample Analysis

For comparison, each fish oil and milk sample was analyzed in both GC-MS/MS and HRGC/HRMS systems, using the same GC conditions for the HRGC/HRMS system. The MRM chromatograms for 2,3,7,8-TCDF, 2,3,7,8-TCDD, and 1,2,3,7,8-PeCDD in the fish oil sample are shown in Fig. 8. The congener profiles in both samples are exhibited in Fig. 9 and 10. GC-MS/MS results showed good consistency with HRGC/HRMS results. The toxic equivalents (TEQ) of PCDD/Fs were 29.5 pg WHO-TEQ$_{2005}$/g fat and 1.38 pg WHO-TEQ$_{2005}$/g fat (upper bound values) in the fish oil and milk samples, respectively, which were comparable to the results of 24.9 and 1.37 pg WHO-TEQ$_{2005}$/g fat obtained from the HRGC/HRMS system.

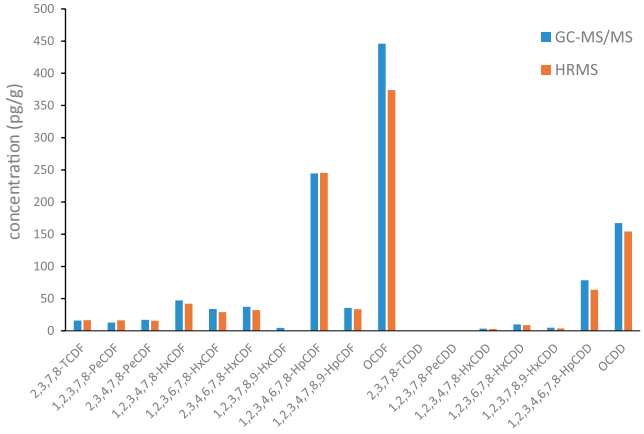


Fig. 9 Congener Profiles of PCDD/Fs in Fish Oil

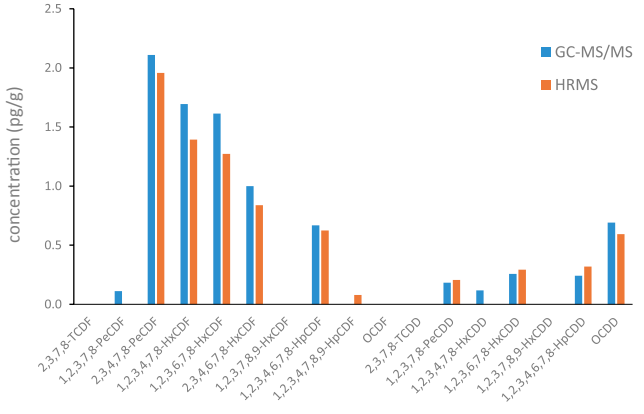


Fig. 10 Congener Profiles of PCDD/Fs in Milk

4. Conclusion

The Shimadzu GCMS-TQ8040 system facilitates the screening and quantitation of low concentration PCDD/Fs in different foodstuffs and animal feed samples. The method showed good linearity, sensitivity, and repeatability. The analytical results from real samples also indicated good precision using this method, when compared with HRGC/HRMS results. This suggests that the Shimadzu GCMS-TQ8040 system provides a substitute solution for routine screening and quantitation of PCDD/Fs in food and feed, as required by European Union legislation.

References

1. IOM (Institute of Medicine). Dioxins and dioxin-like compounds in the food supply: Strategies to decrease exposure. (Washington, DC: The National Academies Press, 2003).
2. Commission Regulation (EU) No. 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No. 252/2012, *Offic. J. Eur. Commun.* 2014, L164/18–40.
3. Commission Regulation (EU) No. 709/2014 of 20 June 2014 amending Regulation (EC) No. 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls, *Offic. J. Eur. Commun.* 2014, L188/1–18.
4. US EPA method 1613B Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. 1997.
5. Wang P, Zhang Q, Wang Y, Wang T, Li X, Ding L, Jiang G. Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. *Anal. Chim. Acta.* 2010, 663(1):43–48.

Triple Quadrupole Gas Chromatograph Mass Spectrometer

GCMS-TQ8040

Finally, a triple quadrupole GCMS *Smart* enough for everyday use in your laboratory.

The Shimadzu GCMS-TQ8040 is the first triple quadrupole with Smart Productivity for high efficiency sample throughput, Smart Operation for quick and easy method development, and Smart Performance for low detection limits and Scan/MRM. These three smart technologies contribute to Smart MRM, and provide the most accurate, cost-effective, and easy-to-use triple quadrupole GCMS you have ever imagined.

Smart Productivity

- Retention time synchronized MRM provides simultaneous sensitivity and precision for hundreds of compounds in one run
- Smart MRM optimizes analysis of 400+ compounds in a single acquisition with maximum sensitivity
- Reduced downtimes with the Twin Line configuration MS system

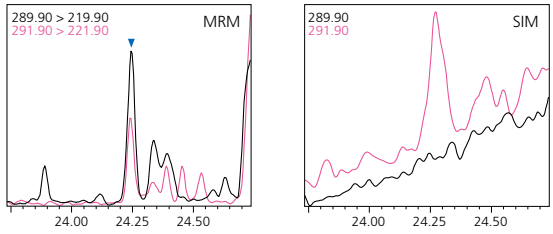
Smart Operation

- MRM Optimization Tool to optimize MRM transitions automatically
- Smart Database Series with fully optimized transitions for hundreds of compounds
- Smart MRM for automatic method creation in a single step

Smart Performance

- The patented ion source design and uniform temperature prevent active spots and boost sensitivity
- The OFF-AXIS ion optics eliminate chemical noise and lower detection limits
- High-sensitivity analysis even in single GC-MS mode

Analysis of PCB in River Water
(2,2',5,5'-Tetrachlorobiphenyl (#52) concentration in water of 0.080 ng/L)



Number of Registered Compounds	Number of Registered Native Compounds	Number of Registered Compounds Labeled with Stable Isotopes
Polychlorinated biphenyls	209	45
Brominated flame retardants	55	28
Dioxins	32	26
Polycyclic aromatic hydrocarbons	38	37
Organochlorine pesticides	32	25

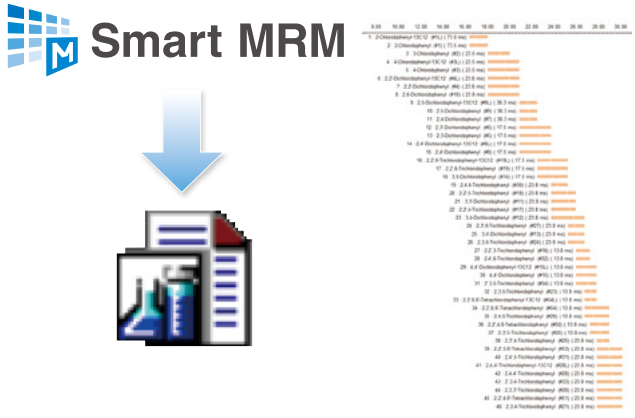
Smart Environmental Database

Create MRM Methods for GC-MS/MS

The Smart Environmental Database contains all the information necessary to create MRM methods for over 500 environmental pollutants, including PCBs, BFRs, dioxins, PAHs, organochlorine pesticides (OCPs), and stable isotopically labeled compounds that are commonly used as Internal and Surrogate Standards.

Smart MRM Optimizes Methods Automatically

The Smart MRM feature allows the user to create fully optimized MRM and Scan/MRM methods automatically. GC-MS/MS Dwell, Event, and Loop times can be difficult to optimize when dozens, or even hundreds of compounds are to be analyzed simultaneously. The Smart MRM feature automatically determines the optimum Dwell, Event, and Loop settings using flexible MRM events, and creates MRM and Scan/MRM methods that provide the best sensitivity for all compounds in a single method.



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