Application Note

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Determination of Ultratrace Polychlorinated Dibenzo-*p*-Dioxins and Dibenzofurans Using GC/MS/MS

Authors

Xu Chi, Gao Lirong, and Zheng Minghui
State Key Laboratory of Environmental Chemistry and Ecotoxicology,
Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences,
Beijing, China

Wang Wenwen
Agilent Technologies (China) Co., Ltd., Beijing, China

Abstract

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are highly toxic persistent organic pollutants (POPs). Analysis of these toxic PCDD/F congeners is very challenging because they are difficult to detect at ultratrace levels in complex samples. This study developed a gas chromatography triple quadrupole mass spectrometry (GC/MS/MS) method for the analysis of seventeen 2,3,7,8-substituted PCDD/F congeners. It was performed using an Agilent 7010 GC/MS/MS with a novel high-efficiency electron ionization source that can detect trace and ultratrace levels of analytes with higher sensitivity and confidence.

Incineration is the main source of dioxins in the environment. Therefore, the analysis of dioxins in waste incineration fly ash samples is of great significance for controlling the emission of dioxins. Six fly ash samples with varied concentrations between 2.1 and 32.6 pg TEQ/g were analyzed using GC/MS/MS. The GC/MS/MS results were in agreement with values obtained using GC/HRMS. The method was also validated through the analysis of a certified reference material of fish tissue with five injections. For all of the congeners, the average results from GC/MS/MS were in the range of the certified reference values. The relative standard deviations (RSDs) of all the congeners were less than 10.0%. Therefore, this GC/MS/MS method provides a viable and economical alternative to the GC/HRMS method.

Introduction

PCDD/Fs are of great concern because of their toxicity and persistence in the environment.[1] Analysis of these toxic PCDD/F congeners is challenging because they are difficult to detect at ultratrace levels in complex environmental samples. GC/MS/MS has a specific multiple reaction monitoring (MRM) mode that creates specific fragmentation of the PCDD/Fs. This selective reaction can decrease the interference in mass chromatograms and improve sensitivity. Because of this, gas chromatography coupled with tandem mass spectrometry has been applied to the analysis of PCDD/Fs. The European Union (EU) has instituted regulation (709/2014) governing the levels of PCDDs and PCDFs in food and feed that enables the use of GC/MS/MS systems in confirmatory testing for compliance with EU MLs. This means that triple quadrupole mass spectrometers could provide performance similar to HRMS systems.[2] This Application Note describes the sensitivity, selectivity, and precision of the methods for analyzing 17 toxic PCDD/Fs using an Agilent 7010 triple quadrupole GC/MS system. Table1 shows the specific compounds, along with the assigned Toxicity Equivalency Factor (TEF), International Toxicity Equivalency Factor (I-TEF), and World Health Organization TEF (WHO_{2005}-TEF) to calculate toxic equivalency (TEQ). This Application Note also shows that the GC/MS/MS results agree with values obtained using GC/HRMS, thus providing a viable and economical alternative to the GC/HRMS approach.

Experimental

Reagents and standards

Residue grade *n*-hexane, dichloromethane, and toluene were purchased from J.T. Baker (Phillipsburg, NJ, USA). Standard solutions for 2,3,7,8-PCDD/Fs specified by EPA Method 1613, including those for EPA-1613 CVS, LCS, ISS, and certified reference material WMF-01(reference fish tissue) were supplied by Wellington Laboratories Inc (Ontario, Canada).

Instruments

The analyses were performed on an Agilent 7890 GC and an Agilent 7010 triple quadrupole GC/MS system. Table 2 lists the instrument conditions. The methods used MRM mode for data acquisition. For each target, two specific precursor ions as well as two corresponding product ions and collision energies were adapted from Agilent Food and Feed Analyzer.[4] Table 3 gives a full list of MRM transitions.

Table 1. Various toxic equivalent factors (TEF) of PCDD/Fs.[3]

PCDD/Fs	I-TEF	WHO_{2005}-TEF
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	0.5	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.001	0.0003
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-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	0.001	0.0003

Table 2. Instrument conditions.

GC Conditions	
Column	Agilent J&W DB-5ms UI, 60 m × 0.25 mm, 0.25 µm
Injection Volume	1 µL
Oven Temperature	150 ℃ held for 3 minutes, at 20 ℃ /min to 230 ℃ held for 18 minutes, at 5 ℃/min to 235 ℃ held for 10 minutes, at 4 ℃ /min to 320 ℃ held for 1 minute
Injection Mode	Splitless, purge on after 1.5 minutes
Injection Port Temperature	290 ℃
Carrier Gas	Helium
Flow Rate	1.0 mL/min
MS Conditions	
Operation Mode	Electron ionization (EI), MRM
Ionization Voltage	70 eV
Ion Source Temperature	320 ℃
Interface Temperature	320 ℃
Quadrupole Temperature	150 °C
Solvent Delay	10 minutes
MS1 Resolution	Unit
MS2 Resolution	Unit
Collision Cell Gas Flows	Nitrogen at 1.5 mL/min, helium at 4.0 mL/min

Table 3. Main parameters for MS/MS analysis of PCDD/Fs.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)
TCDF	303.9	240.9	40
	305.9	242.9	40
$^{13}C_{12}$-TCDF	315.9	251.9	40
	317.9	253.9	40
TCDD	319.9	256.9	26
	321.9	258.9	26
$^{13}C_{12}$-TCDD	331.9	267.9	26
	333.9	269.9	26
PeCDF	337.9	274.9	40
	339.9	276.9	40
$^{13}C_{12}$-PeCDF	349.9	285.9	40
	351.9	287.9	40
PeCDD	353.9	290.9	26
	355.9	292.9	26
$^{13}C_{12}$-PeCDD	365.9	301.9	26
	367.9	303.9	26
HxCDF	373.8	310.9	40
	375.8	312.9	40
$^{13}C_{12}$-HxCDF	385.8	321.9	40
	387.8	323.9	40

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)
HxCDD	389.8	326.9	26
	391.8	328.8	25
$^{13}C_{12}$-HxCDD	401.8	337.9	26
	403.8	339.9	25
HpCDF	407.8	344.8	40
	409.8	346.8	40
$^{13}C_{12}$-HpCDF	419.8	355.8	40
	421.8	357.8	40
HpCDD	423.8	360.8	24
	425.8	362.9	24
$^{13}C_{12}$-HpCDD	435.8	371.8	24
	437.8	373.8	24
OCDF	441.7	378.8	40
	443.7	380.8	40
$^{13}C_{12}$-OCDF	455.8	391.8	40
	453.8	389.8	40
OCDD	457.7	394.8	24
	459.7	396.8	24
$^{13}C_{12}$-OCDD	469.7	405.8	24
	471.7	407.8	24

Sample preparation

Samples required elaborate extraction and cleanup procedures before instrumental analysis. Before extraction, samples were spiked with known amounts of the $^{13}C_{12}$-labeled standards for EPA-1613 LCS, then equilibrated for 12 hours. After that, samples were extracted with *n*-hexane and dichloromethane (1:1, V/V), using Soxhlet or ASE. After concentration, the extractions were cleaned by three columns: an acidified silica gel column, a multilayered acid/base/$AgNO_3$/neutral silica gel, and an active carbon column. The final extracts were eluted with toluene, then reduced under a gentle stream of purified nitrogen to an appropriate volume. For recovery quantification, the $^{13}C_{12}$-labeled standards for EPA-1613 ISS were added immediately before instrumental analysis. Figure 1 shows a flow diagram summarizing the sample preparation steps.

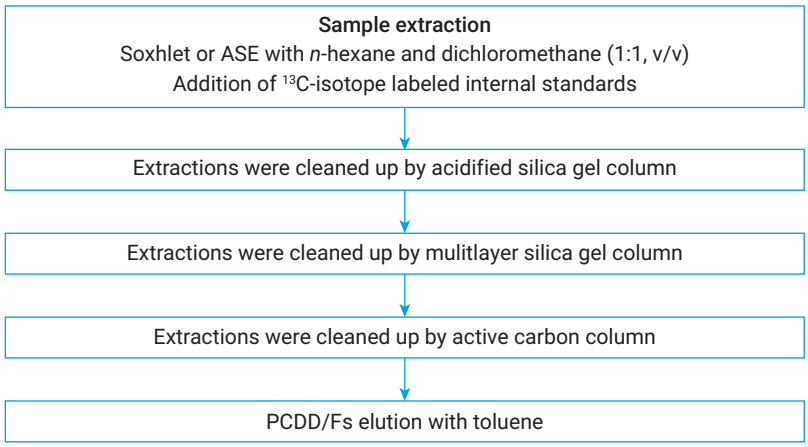

Figure 1. Flow diagram of the sample extraction and cleanup procedures.

Results and discussion

The separation of the dioxin/furan isomers

Figure 2 shows the MRM chromatograms for 17 PCDD/F congeners, with an analysis time of 58 minutes. It shows the excellent separation of 17 PCDD/F congeners and it also zooms in on the hexa-dioxin/furan isomers that were difficult to separate.

Calibration and average relative response factor

The optimized GC/MS/MS method was applied to analyze the calibration standard solutions EPA-1613 CVS (CS1 to CS5) (Table 4). According to EPA Method 1613,[5] the relative response factor (RRF) of each individual 2,3,7,8-chloro-substituted PCDD/F congener was obtained from a five-point calibration curve. The RSDs of all the congeners were below 3.0 %, which fully complies with the EPA's requirement of less than 15% (Table 5).

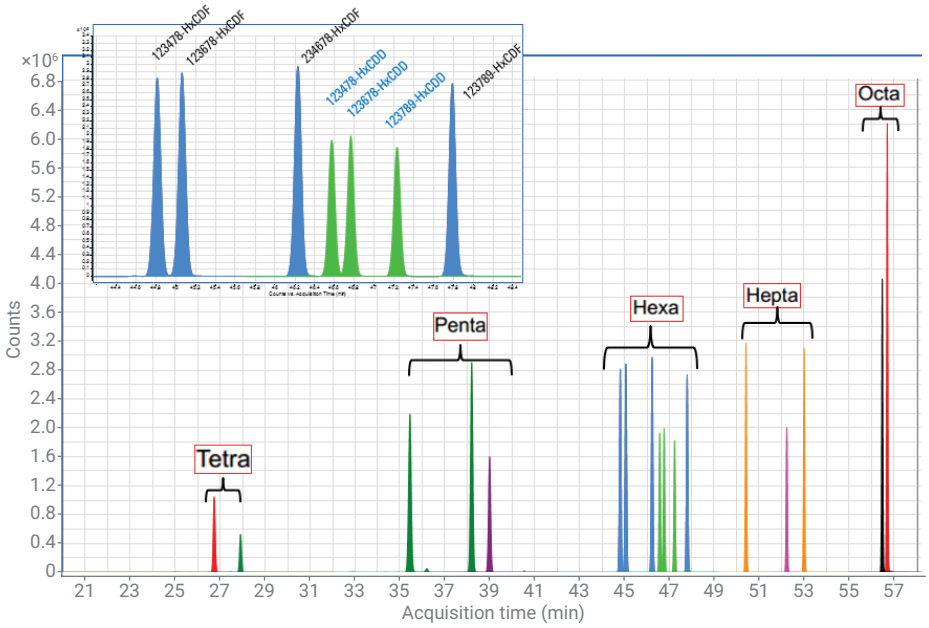

Figure 2. MRM chromatograms of 17 PCDD/F congeners.

Table 4. Concentrations of congeners in calibration solutions (ng/mL).

Native PCDD/Fs	1613 CS1	1613 CS2	1613 CS3	1613 CS4	1613 CS5
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDF	2.5	10	50	200	1,000
2,3,4,7,8-PeCDF	2.5	10	50	200	1,000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1,000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1,000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1,000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1,000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1,000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1,000
OCDF	5.0	20	100	400	2,000
2,3,7,8-TCDD	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1,000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1,000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1,000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1,000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1,000
OCDD	5.0	20	100	400	2,000

Limits of detection (LODs)

The LODs for PCDD/Fs were determined from seven replicate analyses of a standard solution containing low concentrations (diluted 10 times of CS1) of the PCDD/Fs according to the US EPA method. The obtained LODs ranged from 0.008 to 0.08 pg/µL for the 17 PCDD/F congeners, which suggested that the GC/MS/MS method was sensitive enough for ultratrace analysis of PCDD/Fs. Table 5 shows the results. Figure 3 shows MRM chromatograms of 17 PCDD and PCDF congeners. (Diluted 10 times of CS1, from 0.05 to 0.5 pg/µL).

Table 5. Average RRFs for individual congener calibrations and their corresponding LODs.

Compound name	Average RRF	RSD (%)	LOD (pg/µL)
2,3,7,8-TCDF	1.07	1.07	0.01
1,2,3,7,8-PeCDF	1.03	1.27	0.049
2,3,4,7,8-PeCDF	1.06	1.39	0.039
1,2,3,4,7,8-HxCDF	1.02	1.81	0.04
1,2,3,6,7,8-HxCDF	1.01	1.89	0.04
2,3,4,6,7,8-HxCDF	1.08	1.36	0.04
1,2,3,7,8,9-HxCDF	0.95	1.46	0.04
1,2,3,4,6,7,8-HpCDF	0.99	1.56	0.04
1,2,3,4,7,8,9-HpCDF	0.98	1.17	0.04
OCDF	1.56	1.48	0.08
2,3,7,8-TCDD	1.15	0.86	0.008
1,2,3,7,8-PeCDD	1.07	1.96	0.04
1,2,3,4,7,8-HxCDD	1.05	2.04	0.04
1,2,3,6,7,8-HxCDD	0.96	1.39	0.05
1,2,3,7,8,9-HxCDD	0.88	2.46	0.04
1,2,3,4,6,7,8-HpCDD	0.98	1.18	0.04
OCDD	1.00	0.95	0.08

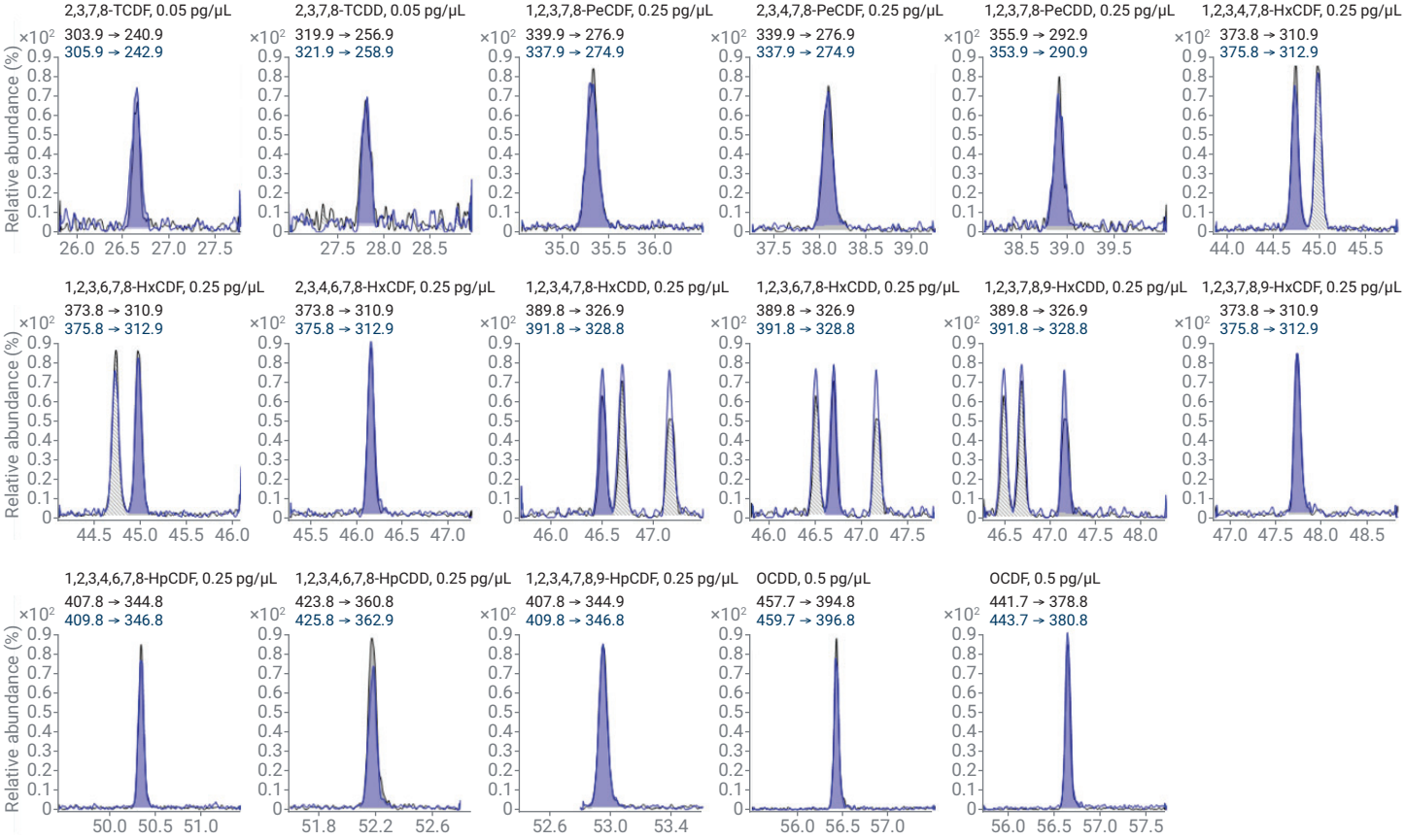

Figure 3. MRM chromatograms of 17 PCDD and PCDF congeners (diluted 10 times of CS1, from 0.05 to 0.5 pg/µL).

The evaluation of the proposed analytical method

To evaluate the performance of the proposed GC/MS/MS method, it was applied to the analysis of certified reference material (CRM) fish tissue with five replications. Table 6 shows that the concentrations of PCDD/F congeners in fish tissue ranged from 0.23 to 13.6 pg/g, and the average results of all the congeners obtained from GC/MS/MS were in the range of the certified reference values. The total I-TEQ result of GC/MS/MS was 19.92 pg/g, which was close to the certified reference value 19.81 pg/g. The RSD of the five injections was less than 10%. The average ion abundance ratio for 17 PCDD/F congeners of CRM (Figure 4), which are all within a ±15% window around the average ion abundance of CS1 to CS5, meet the requirement of EPA 1613. Overall, the proposed analytical method showed good accuracy and precision.

Table 6. Analysis of PCDD/Fs in certified reference material (WMF-01).

	Certified Reference Value (pg/g)	Analyzed Value (n = 5)	
		Average (pg/g)	RSD (%)
2,3,7,8-TCDF	13.1 ± 4.9	12.97	1.0
1,2,3,7,8-PeCDF	1.53 ± 1.4	1.34	8.6
2,3,4,7,8-PeCDF	7.15 ± 2.2	6.43	3.3
1,2,3,4,7,8-HxCDF	0.86 ± 1.0	1.01	6.5
1,2,3,6,7,8-HxCDF	0.51 ± 0.7	0.62	6.0
2,3,4,6,7,8-HxCDF	0.68 ± 1.2	0.67	3.9
1,2,3,7,8,9-HxCDF	0.25 ± 0.4	0.26	8.8
1,2,3,4,6,7,8-HpCDF	1.01 ± 1.9	2.76	2.8
1,2,3,4,7,8,9-HpCDF	0.30 ± 0.5	0.61	3.8
OCDF	1.38 ± 2.1	2.94	8.7
2,3,7,8-TCDD	13.1 ± 4.4	13.6	2.4
1,2,3,7,8-PeCDD	2.72 ± 1.3	2.61	2.9
1,2,3,4,7,8-HxCDD	0.22 ± 0.3	0.27	7.4
1,2,3,6,7,8-HxCDD	0.88 ± 0.4	0.81	9.2
1,2,3,7,8,9-HxCDD	0.27 ± 0.4	0.23	7.6
1,2,3,4,6,7,8-HpCDD	0.59 ± 0.7	0.65	6.5
OCDD	3.91 ± 6.2	2.01	7.9
Total I-TEQ	19.81	19.92	

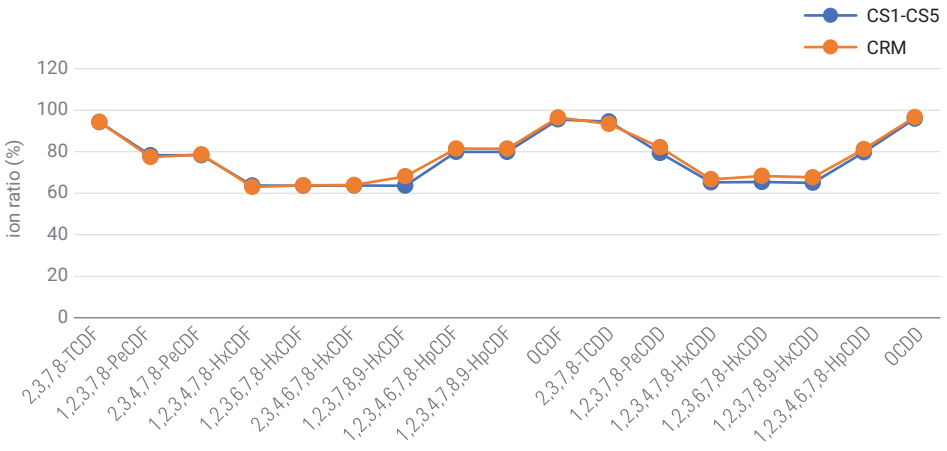

Figure 4. Comparative average ion abundance ratio for 17 PCDD/F congeners of CS1-CS5 and CRM.

The comparison of GC/HRMS and GC/MS/MS for the analysis of PCDD/Fs from fly ash samples

Six fly ash samples were extracted and analyzed using a GC/HRMS. The same sample vials were then transferred to the GC/MS/MS and reanalyzed. Figure 5 shows the comparative sample results (total I-TEQ) of the two sets of measurements between the results obtained by the GC/HRMS and GC/MS/MS analysis of six fly ash samples with varied concentrations between 2.1 and 32.6 pg TEQ/g. The GC/MS/MS results were in agreement with values obtained using GC/HRMS.

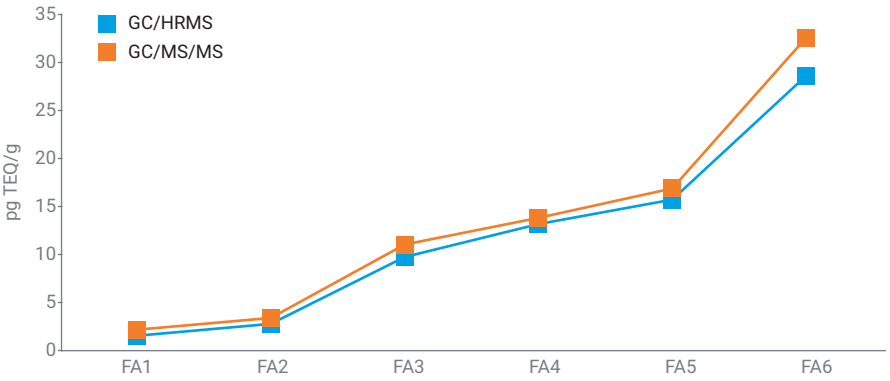

Figure 5. Comparative sample results (total I-TEQ) of the two sets of measurements by the GC/HRMS and GC/MS/MS analyses.

Conclusion

The Agilent 7010 GC/MS/MS system provided reproducible and sensitive detection of 17 toxic PCDD/F congeners. The proposed method was applied to the analysis of certified reference materials to demonstrate its suitability. The results from GC/MS/MS were close to the certified reference values. Comparison of analytical results by GC/HRMS and GC/MS/MS indicated the suitability of the 7010 GC/MS/MS system.

References

1. Zheng, G. J. *et al.* Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans Pollution in China: Sources, Environmental Levels and Potential Human Health Impacts [J]. *Environment International* **2008**, *34(7)*, 1050–61.
2. Union, P. O. of the E. 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 Text with EEA relevance, CELEX1 https://publications. europa.eu/en/ publication-detail/-/ publication/ d626811a-fdcc-11e3831f-01aa75ed71a1/language-en (accessed Nov 16, 2018).
3. Bhavasar, S. P. *et al.* Converting Toxic Equivalents (TEQ) of Dioxins and Dioxin-Like Compounds in Fish From One Toxic Equivalency Factor (TEF) Scheme to Another [J]. *Environment International* **2008**, *34(7)*, 915–21.
4. Riener, J. Validation of a Confirmatory GC/MS/MS Method for Dioxins and Dioxin-Like PCBS. *Agilent Technologies Application Note*, publication number 5991-6590EN, **2016**.
5. Method 1613, Tetra- Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. **1997**. *United States Environmental Protection Agency*.

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