Mass Spectrometry & Spectroscopy

Ultra-low Level Analysis of Dioxins in Food using a triple quadrupole mass spectrometer (MS/MS) with Boosted Efficiency Ion Source

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Dioxins are a class of very toxic compounds found throughout the world in the environment. Equipment sensitivity is of great importance for the analysis of low concentrations of these highly-toxic compounds. Historically, analysis and detection of dioxins was done with magnetic sector-type high-resolution mass spectrometers (HRMS). However, in recent years, the performance of triple quadrupole mass spectrometers (MS/MS) has improved significantly. In addition, the development of the Boosted Efficiency Ion Source (BEIS) offers compound-specific sensitivity up to 4 times greater than previous ion sources and provides accurate quantitation of dioxins at levels comparable to HRMS. Detection limits as low as 20 fg for Tetrachlorodibenzo-p-dioxin (TCDD) were achieved. In this study, we analysed dioxins in about 250 samples of approximately 40 types of food and animal feed products using a GC-MS/MS with BEIS. Quantitation performance was evaluated by comparing the analysis results obtained by GC-HRMS and GC-MS/MS. We also evaluated the number of analyses possible while maintaining sensitivity at low concentrations in order to verify the durability of the GC-MS/MS instrument.

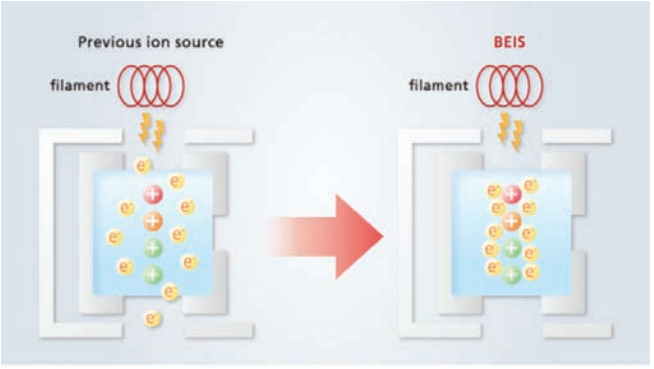
Introduction

Dioxin and Furan are the frequently used short names for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) [1]. They belong to a group of toxic compounds known as Persistent Organic Pollutants (POPs) because they take a long time to break down in the environment. As such, dioxins are highly toxic to humans [2]. They are unwanted by-products of a wide range of manufacturing processes, including smelting, chlorine bleaching of paper pulp, and waste incineration. In nature, dioxins can be created by forest fires and volcanic eruptions.

PCDD/Fs have long been an environmental concern. Due to their tendency to accumulate in biological tissues and their toxicity to biota, PCDD/Fs have received more widespread concern and have recently fallen under the scrutiny of the global food community.

At the same time, because of their extremely low level, they have been used as one of the benchmarks for evaluation the performance of the analytical instruments. In this study, seventeen congeners of dioxin were analysed by GC-MS/MS (Model GCMS-TQ8050 NX, (Shimadzu Corporation) in combination with Boosted Efficiency Ion Source (BEIS). The developed instrument method was applied to real sample analysis, and the results (on a TEQ level basis) from the GC-MS/MS were consistent with results from GC-HRMS. The latter is the traditional method for analysis of dioxins [3, 4]. Durability of the instrument and ruggedness of the method was also evaluated with no decrease in sensitivity observed after more than 500 samples at low concentrations.

BEIS was developed (Shimadzu Corporation) to maximise ionisation efficiency through optimising the focal point of the electron beam in EI ionisation. This image illustrates the principle of BEIS:



By optimising the focal point of the electron beams, the rate at which electron collide with the molecule is increased. Although the same number of electrons are produced by the filament, the ionisation rate is increased. This enables up to four-times higher sensitivity compared to previous ion sources. However, depending on the actual usage, the lifetime of the filament may be slightly shortened.

Materials and Methods

Samples and Analysis Conditions

All food and feed samples were prepared using automatic

pretreatment devices (extraction: SpeedExtractor (BÜCHI Labortechnik AG), sample clean-up: GO-xHT (Miura Co, Ltd)). Nonane was used as the final solvent of the samples, and the final solvent amount was 10 µL. Standard samples were prepared by mixing DF-ST and DF-LCS (Wellington Laboratories Inc).

The GC-MS/MS analysis conditions registered in EU Regulations Compliant GC-MS/MS Method Package for Dioxins in Foods were used as the GC-MS/MS analysis conditions. *Table 1* shows the detailed conditions.

Table 1: GCMSMS Analysis Conditions.

Instrument composition	
Sample preparation (extraction):	SpeedExtractor
	(BÜCHI Labortechnik AG)
Sample preparation (clean-up):	GO-xHT (Miura Co., Ltd.)
Auto sampler:	AOC-20i/s
GC-MS/MS:	GCMS-TQ™ 8050 (Shimadzu Corporation, Japan)
Software:	GCMSsolution™ Ver. 4.50SP1
	LabSolutions Insight™ Ver. 3.6
	EU Regulation Compliant GC-MS/MS Method
	Package for Dioxins in Foods
Detailed analysis 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 µL
Injection Volume:	2 µL
Detailed analysis conditions (GC)	
Inlet liner:	Topaz Single Gooseneck Inlet Liner, w/ 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 (1 min)"(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

Analysis 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 labelled compounds)
Transition:	Conditions of Method Package
Ion source:	BEIS
Emission current:	150 µA

Results and Discussion

Analysis Results of Standard Sample

As the concentration range of the calibration curve, standard samples were prepared for concentrations from 0.025 pg/µL to 1 pg/µL (double concentration for Octa-PCDD/PCDF).

In the EU Regulations, all compounds must satisfy the two criteria shown below (partially excerpted from EU 589/2014 and 644/2017) at the LOQ (limit of quantitation):

Criterion 1. S/N ratio

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.

or, if for technical reasons the signal-to-noise calculation does not provide reliable results,

Criterion 2. Lowest concentration point on a calibration curve

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.

This study confirmed that both criteria can be satisfied at the lowest point of the calibration curve. The results are shown in *Table 2* (See next page).

Evaluation of Sensitivity in Analysis of Actual Samples

Sensitivity at low concentration levels in the analysis of actual samples was verified. *Figures 1a* and *1b* (see next page) show the chromatograms of representative individual compounds of the standard and of actual samples, respectively. Satisfactory sensitivity near the limit of quantitation (LOQ) was also obtained in analysis of the actual samples.

Evaluation of Quantitation Accuracy in Analysis of Actual Samples

More than 250 samples of approximately 40 kinds of food and animal feed products were analysed using GC-MS/MS. The quantitation accuracy of GC-MS/MS was evaluated by analysing the same GC-MS/MS samples by GC-HRMS and comparing the

Table 2: Evaluation Results of LOQ in Analysis of Standard Samples.

I.D.	Compound name	Average	RRF	RRFDev (%)	S/N ratio	LOQ
		RRF	(Level 1)	(Level 1)	(Level 1)	
1	2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.07	1.15	8.1	552	0.025 pg/µL
2	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	1.09	0.97	10.56	411	0.025 pg/µL
3	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	1.14	1.39	22.26	269	0.025 pg/µL
4	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.95	0.92	2.72	254	0.025 pg/µL
5	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	1.03	1.25	21.44	260	0.025 pg/µL
6	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.92	0.82	11.46	421	0.025 pg/µL
7	Octachlorodibenzo-p-dioxin	1.19	1.04	12.21	915	0.050 pg/µL
8	2,3,7,8-Tetrachlorodibenzofuran	1.1	1.05	4.66	793	0.025 pg/µL
9	1,2,3,7,8-Pentachlorodibenzofuran	1.04	1	3.23	483	0.025 pg/µL
10	2,3,4,7,8-Pentachlorodibenzofuran	0.97	0.89	7.59	474	0.025 pg/µL
11	1,2,3,4,7,8-Hexachlorodibenzofuran	1.03	0.82	20.72	447	0.025 pg/µL
12	1,2,3,6,7,8-Hexachlorodibenzofuran	1.09	1.36	24.62	446	0.025 pg/µL
13	2,3,4,6,7,8-Hexachlorodibenzofuran	1.09	1.39	27.83	286	0.025 pg/µL
14	1,2,3,7,8,9-Hexachlorodibenzofuran	1.06	1.23	16.1	315	0.025 pg/µL
15	1,2,3,4,6,7,8-Heptachlorodibenzofuran	1.17	1.05	10.37	705	0.025 pg/µL
16	1,2,3,4,7,8,9-Heptachlorodibenzofuran	1.02	0.97	4.97	650	0.025 pg/µL
17	Octachlorodibenzofuran	1	0.84	15.8	689	0.050 pg/µL

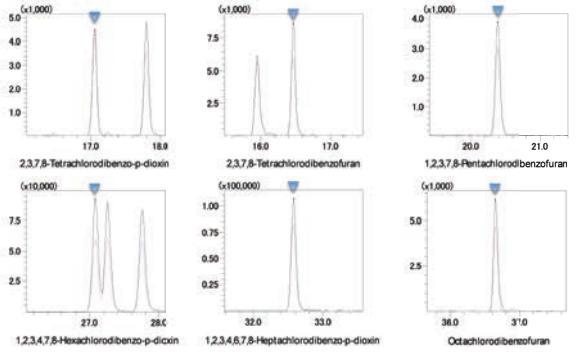


Figure 1a: Chromatograms of Representative Compounds in Analysis of Standard Samples.

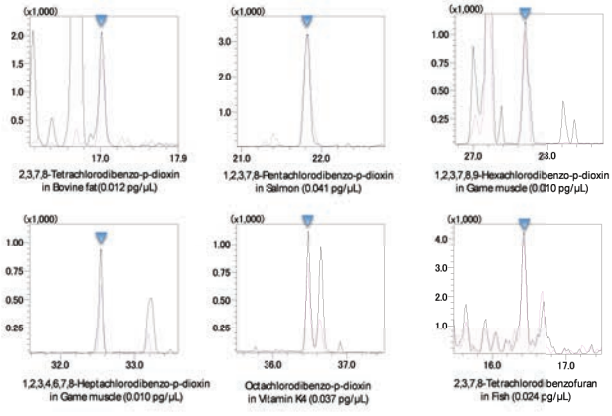


Figure 1b: Chromatograms of Representative Compounds in Analysis of Actual Samples.

results. The results were compared by converting the quantitation values of each sample to Toxicity Equivalent Quantity (TEQ). The results are shown in *Figure 2*.

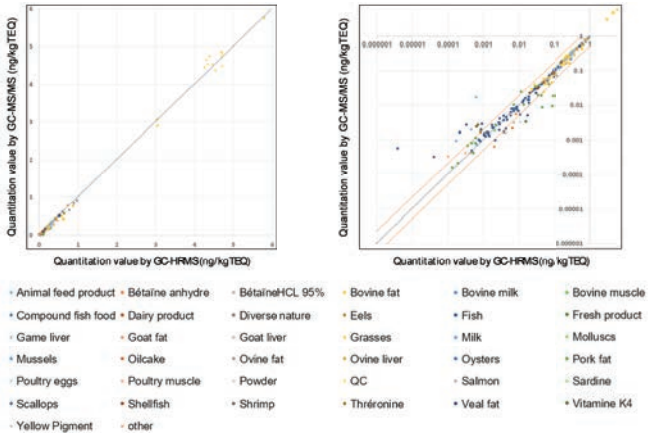


Figure 2: Comparison of Quantitation values of GCMSMS and GCHRMS.

In the graph on the left in *Figure 2*, the horizontal and vertical axes are shown by linear scales. In the graph on the right, logarithmic scales are used to enable detailed confirmation of the results of samples with small quantitation values. Both graphs show the quantitation values by GC-HRMS on the horizontal axis, and those by GC-MS/MS on the vertical axis. In both graphs, when a correlation exists between the GC-HRMS and GC-MS/MS values, the values are close to a line with a slope of 1 (blue broken line).

In the graph on the left, the quantitation values by GC-MS/MS and GC-HRMS were similar in all samples in which quantitation values were detected at the level of 1 ng/kg TEQ or more.

The graph on the right shows lines (orange broken lines) where the ratio of the quantitation values by GC-MS/MS and GC-HRMS were 50% or 200% as a standard for difference between the quantitation value obtained by the two methods. In samples in which the quantitation value by GC-HRMS was 0.1 ng/kg TEQ or less, some scattered results in which the quantitation value ratio fell outside the range of 50% to 200% were seen.

The sample with the smallest maximum permissible level (ML) was pork fat, with ML of 1.0 ng/kg TEQ. For this reason, a large difference in the quantitation values (i.e., a quantitation value ratio outside the 50% to 200% range) is possible at concentration levels at least 10 times lower than ML. However, no significant difference could be seen in the quantitation performance of GC-MS/MS and GC-HRMS at the concentration level required in analyses.

Evaluation of Durability in Analysis of Actual Samples

As an evaluation of durability in analysis of dioxins in food products, actual samples and standard samples (concentration: 0.05 pg/µL) were analysed alternately, and the number of analyses possible while maintaining sensitivity was evaluated based on the transition of sensitivity for low concentration standard samples. A total of more than 500 analyses of standard samples and actual samples were carried out. *Figure 3* shows the results.

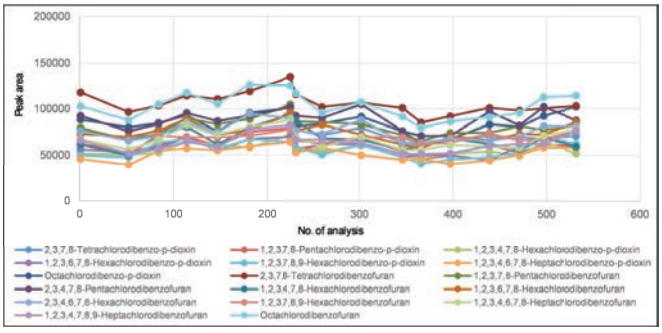


Figure 3: Transition of Peak Area in Repeated Analysis.

In *Figure 3*, the horizontal axis shows the number of analyses and the vertical axis shows the peak area at each analysis number. No large decrease in sensitivity occurred after more than 500 analyses. Next, *Table 3* shows the average peak area and repeatability from the 1st to the 530th analysis. Repeatability was less than 20% RSD for all compounds, indicating that sensitivity could be maintained through the entire test.

Conclusions

In this experiment, dioxins in more than 250 samples of approximately 40 types of food and animal feed products were analysed by GC-MS/MS using BEIS, and the quantitation performance of GC-MS/MS was evaluated by comparing the analysis results by GC-MS/MS and GC-HRMS. The results showed no difference in the quantitation performance of GC-MS/MS and GC-HRMS at the concentration level necessary in analyses.

Durability in analysis of dioxins in actual samples was also evaluated, and no decrease in sensitivity at low concentration levels occurred after more than 500 analyses.

Based on these results, we determined that BEIS with GCMS/MS has the high sensitivity necessary for dioxin analysis comparable to HRMS instruments, while also demonstrating the excellent durability of the GCMS-TQ8050.

Acknowledgement

We wish to take this opportunity to express our sincere thanks to the Laboratoire d'Etude des Résidus et Contaminants dans les Aliments for cooperation in providing samples, guidance, and other assistance in the preparation of this paper.

References

1. https://www.epa.gov/dioxin/learn-about-dioxin

2. https://www.who.int/news-room/fact-sheets/detail/dioxins-and-their-effects-on-human-health

3. https://rais.ornl.gov/documents/dioxin_tef.pdf

4 EPA Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

Table 3: Average Peak Area and Repeatability for Standard Samples Concentration 0.05 pgµL in Durability Test.

I.D.	Compound name	Average peak area	STDEV	%RSD(n = 17)
1	2,3,7,8-Tetrachlorodibenzo-p-dioxin	73596	8321	11.31
2	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	60713	8803	14.5
3	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	55956	7025	12.55
4	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	58167	8034	13.81
5	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	56035	9095	16.23
6	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	51663	7452	14.43
7	Octachlorodibenzo-p-dioxin	84614	10814	12.78
8	2,3,7,8-Tetrachlorodibenzofuran	105930	11847	11.18
9	1,2,3,7,8-Pentachlorodibenzofuran	80339	9592	11.94
10	2,3,4,7,8-Pentachlorodibenzofuran	88317	10631	12.04
11	1,2,3,4,7,8-Hexachlorodibenzofuran	67814	11761	17.34
12	1,2,3,6,7,8-Hexachlorodibenzofuran	74759	9636	12.89
13	2,3,4,6,7,8-Hexachlorodibenzofuran	75794	9605	12.67
14	1,2,3,7,8,9-Hexachlorodibenzofuran	67878	6056	8.92
15	1,2,3,4,6,7,8-Heptachlorodibenzofuran	67665	10199	15.07
16	1,2,3,4,7,8,9-Heptachlorodibenzofuran	62914	9356	14.87
17	Octachlorodibenzofuran	103483	13911	13.44