

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

Persistent Organic Pollutants (POPs) in food and animal feed products are analyzed by a variety of methods. In particular, quantitative analysis of dioxins to low concentration levels is necessary because dioxins have high toxicity, even among POPs. Until now, magnetic sector-type (double focusing) GC-MS (GC-HRMS) had been used in analysis of dioxins, as this method provides high quantitation accuracy. However, because the quantitation performance of triple quadrupole GC-MS (hereinafter, GC-MS/MS) has improved remarkably in recent years, the EU issued Commission Regulations (EU) 589/2014 and 644/2017 establishing analysis methods using GC-MS/MS as official test methods with the same positioning as methods using GC-HRMS. For this reason, analysis of dioxins by GC-MS/MS has attracted interest.

In ionization by the EI method, Boost Efficiency Ion Source (BEIS) is an ion source that maximizes ion efficiency by optimizing the focal point of the electron beam. BEIS realizes sensitivity approximately four times* higher than that of conventional ion sources.

In this article, we analyzed dioxins in about 250 samples of approximately 40 types of food and animal feed products using a GC-MS/MS with BEIS. (However, the dioxins were limited to polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF).) 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.

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■ 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.

■ 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). This study confirmed that both criteria can be satisfied at the lowest point of the calibration curve. The results are shown in Table 2 on the following page.

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,

2. Lowest concentration point on a calibration curve

The lowest concentration point on a calibration curve that gives an acceptable ($\leq 30\%$) 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.

Table 1 GC-MS/MS Analysis Conditions

| Instrument composition | | Detailed analysis conditions (GC) | |
|--|---|-----------------------------------|---|
| Sample preparation (extraction): | SpeedExtractor (BÜCHI Labortechnik AG) | Inlet liner: | Topaz Single Gooseneck Inlet Liner, w/ Wool (Restek Corp., P/N: 23336) |
| Sample preparation (clean-up): | GO-xHT (Miura Co., Ltd.) | Column: | SH-Rxi™ -5Sil MS (60 m, 0.25 mm I.D., 0.25 µm), (SHIMADZU, P/N: 227-36036-02) |
| Auto sampler: | AOC-20i/s | Injection Mode: | Splitless |
| GC-MS/MS: | GCMS-TQ™ 8050 | Sampling Time: | 1.00 min |
| Software: | GCMSsolution™ Ver. 4.50SP1 LabSolutions Insight™ Ver. 3.6 EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods | 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 |
| Detailed analysis conditions (AOC-20i/s) | | Analysis conditions (MS) | |
| # of Rinses with Solvent (Pre-run): | 3 | Ion Source Temp.: | 230 °C |
| # of Rinses with Solvent (Post-run): | 3 | Interface Temp.: | 300 °C |
| # of Rinses with Sample: | 0 | Detector Voltage: | 1.8 kV (Absolute) |
| Plunger Speed (Suction): | Low | Loop time: | 0.8 sec. (for native compounds) 0.2 sec. (for labeled compounds) |
| Viscosity Comp. Time: | 0.2 sec. | Transition: | Conditions of Method Package |
| Plunger Speed (Injection): | High | Ion source: | BEIS |
| Syringe Insertion Speed: | High | Emission current: | 150 µA |
| 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 | | |

* The increase in sensitivity is dependent on the compound.

Table 2 Evaluation Results of LOQ in Analysis of Standard Samples

| I.D. | Compound name | Average RRF | RRF (Level 1) | RRFDev (%) (Level 1) | S/N ratio (Level 1) | LOQ |
|------|---|-------------|---------------|----------------------|---------------------|-------------|
| 1 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 1.07 | 1.15 | 8.10 | 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 | 1.10 | 1.05 | 4.66 | 793 | 0.025 pg/μL |
| 9 | 1,2,3,7,8-Pentachlorodibenzofuran | 1.04 | 1.00 | 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 | 16.10 | 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 | 1.00 | 0.84 | 15.80 | 689 | 0.050 pg/μL |

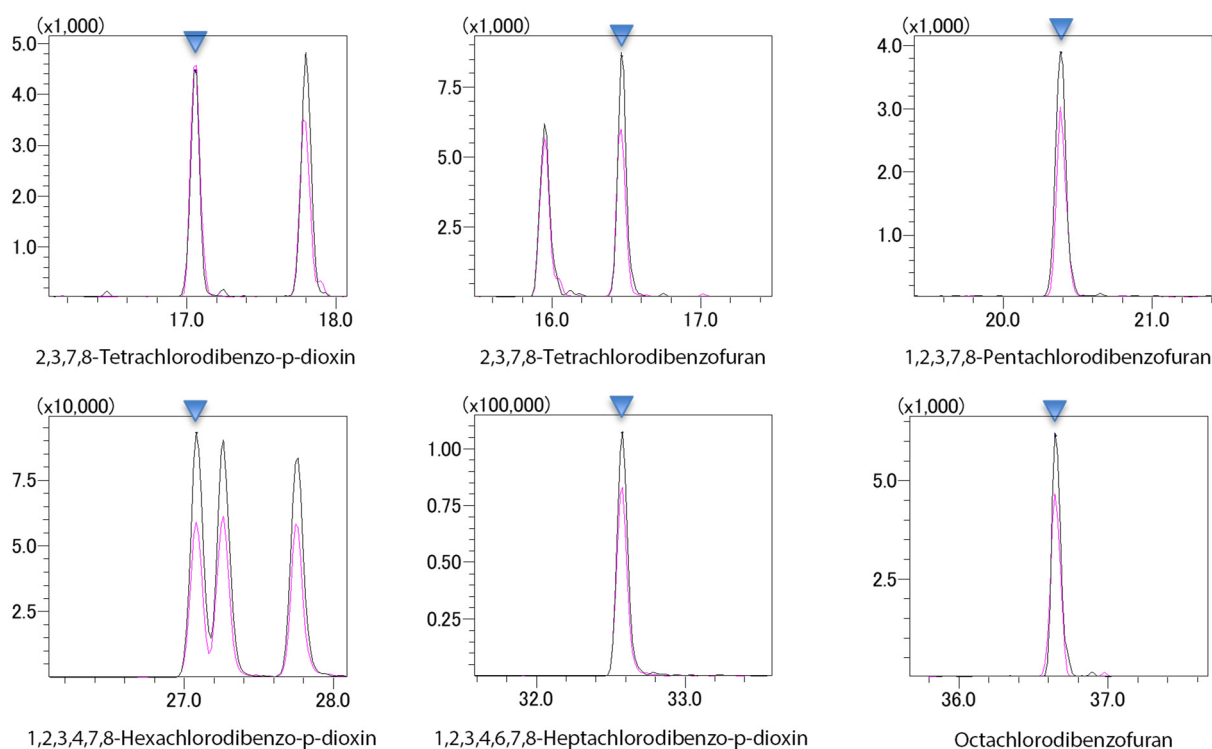


Fig. 1 Chromatograms of Representative Compounds in Analysis of Standard Samples (Concentration: 0.025 pg/μL)

Evaluation of Sensitivity in Analysis of Actual Samples

Sensitivity at low concentration levels in analysis of actual samples was verified. Fig.2 on the next page shows the chromatograms of representative compounds. 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 analyzed using GC-MS/MS. The quantitation accuracy of GC-MS/MS was evaluated by analyzing the same GC-MS/MS samples by GC-HRMS and comparing the results. The results were compared by converting the quantitation values of each sample to TEQ (Toxicity Equivalent Quantity). The results are shown in Fig. 3 on the next page.

In the figure on the left in Fig. 3, the horizontal and vertical axes are shown by linear scales. In the figure on the right, logarithmic scales are used to enable detailed confirmation of the results of samples with small quantitation values. Both

figures show the quantitation values by GC-HRMS on the horizontal axis, and those by GC-MS/MS on the vertical axis. In both figures, 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 figure 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/kgTEQ or more.

The figure 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/kgTEQ 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/kgTEQ. 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.

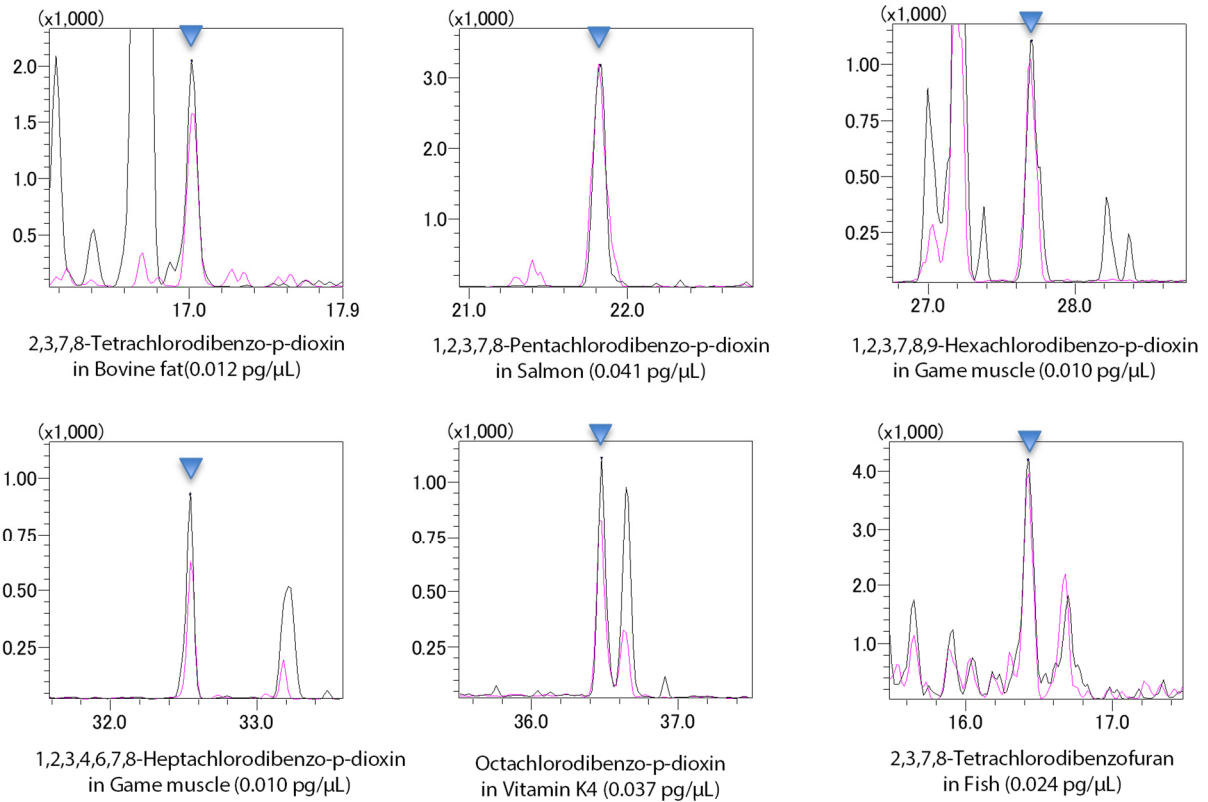
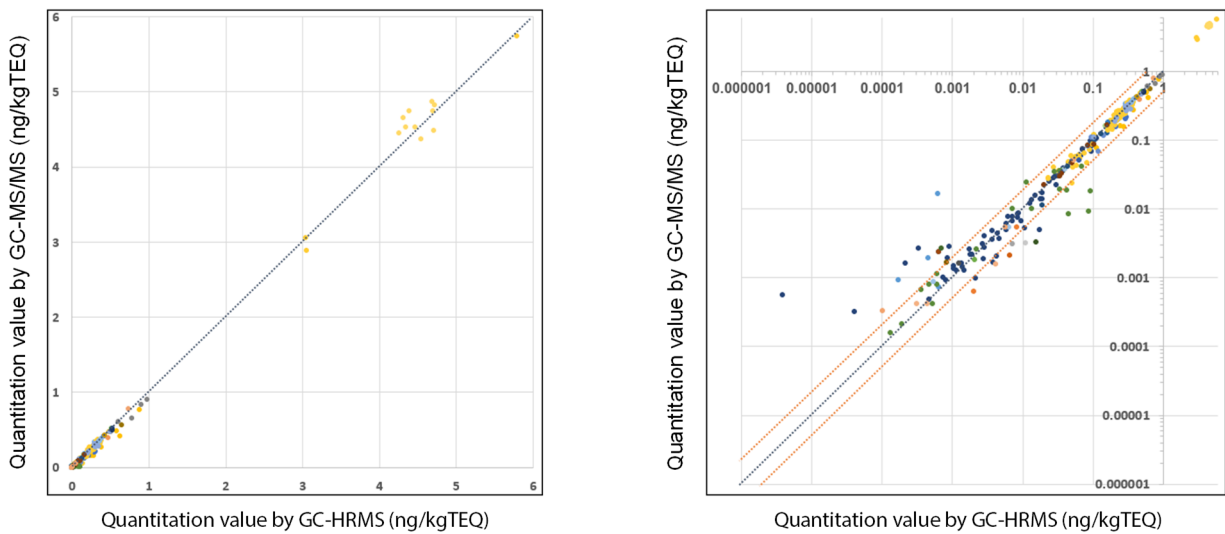


Fig. 2 Chromatograms of Representative Compounds in Analysis of Actual Samples



- Animal feed product
- Compound fish food
- Game liver
- Mussels
- Poultry eggs
- Scallops
- Yellow Pigment
- Bétaïne anhydre
- Dairy product
- Goat fat
- Oilcake
- Poultry muscle
- Shellfish
- other
- BétaïneHCL 95%
- Diverse nature
- Goat liver
- Ovine fat
- Powder
- Shrimp
- Bovine fat
- Eels
- Grasses
- Ovine liver
- QC
- Thréronine
- Bovine milk
- Fish
- Milk
- Oysters
- Salmon
- Veal fat
- Bovine muscle
- Fresh product
- Molluscs
- Pork fat
- Sardine
- Vitamine K4

Fig. 3 Comparison of Quantitation Values of GC-MS/MS and GC-HRMS (Left: Linear Scale, Right: Logarithmic Scale)

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 analyzed 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. Fig. 4 shows the results.

In Fig. 4, 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.

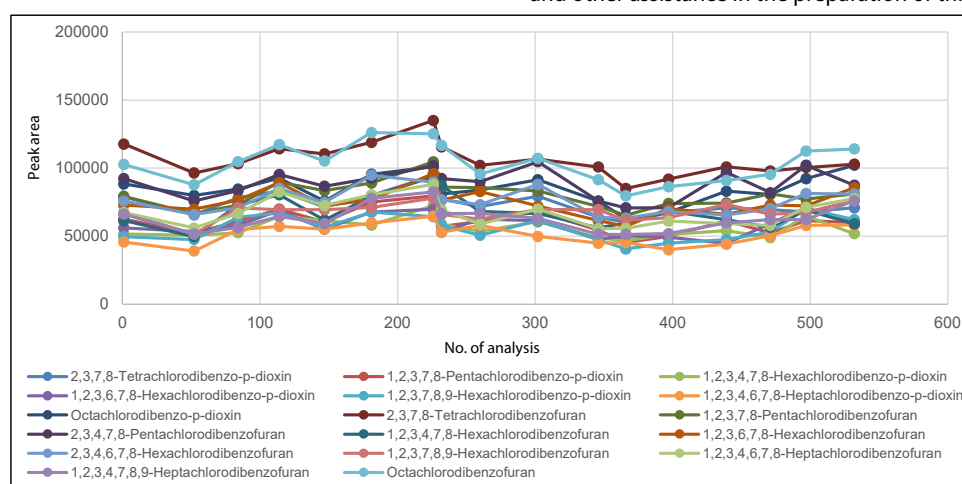


Fig. 4 Transition of Peak Area in Repeated Analysis

Table 3 Average Peak Area and Repeatability for Standard Samples (Concentration: 0.05 pg/μL) in Durability Test

| I.D. | Compound name | Average peak area | SD | %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 | 14.50 |
| 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 |

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Conclusion

In this experiment, dioxins in more than 250 samples of approximately 40 types of food and animal feed products were analyzed 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. As a result of this evaluation, no difference was found 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 found that BEIS has high sensitivity for dioxins and also enables analysis with high durability.

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