Abstract

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 analyzed dioxins in about 250 samples of approximately 40 types of food and animal feed products using a GC-MS/MS instrument. 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.

Keywords

Dioxin, GCMS, Sensitivity, Food safety

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 analyzed by GCMS-TQ8050 NX 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 GCMS-TQ8050 NX were consistent with results from GCHRMS. 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.

Shimadzu developed the BEIS to maximize ionization efficiency through optimizing the focal point of the electron beam in EI ionization. Figure 1 illustrates the principle of BEIS. By optimizing 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 ionization 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 (BUCHI 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.

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

| Instrument Composition | Value |
|------------------------|-------|
| SpeedExtractor         |       |
| GO-xHT (Miura Co., Ltd.) |     |
| AOC-20/s               |       |
| GCMS-TQ8050 NX         |       |
| GCMSolution Ver. 4.50SP1 |      |
| LabSolutions Insight Ver. 3.6 | |
| EU Regulation Compliant GC-MS/MS Method | |
| Package for Dioxins in Foods | |

| Detailed analysis conditions (AOC-20/s) | Value |
|----------------------------------------|-------|
| # 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)     | Value |
|---------------------------------------|-------|
| Topaz Single Gooseneck Inlet Liner, w/ Wool | |
| SH-Rxi™ -5Sil MS (60 m, 0.25 mm I.D., 0.25 μm) | |
| SHIMADZU, P/N: 227-36036-02 | |
| Splitless                             |       |
| 1.00 min                              |       |
| 280 °C                                |       |
| 150 °C (1 min)/(20 °C/min)“220 °C"(2 °C/min) | |
| “260 °C (3 min)/(5 °C/min)/320 °C (3.5 min) | |
| 450 kPa (1.5 min)                     |       |
| Linear Velocity (45.6 cm/sec.)        |       |
| 20 mL/min                             |       |
| Helium                                |       |
| 230 °C                                |       |
| 300 °C                                |       |

Table 1: GCMS/MS analysis conditions.
Analysis of Dioxins in Food by GCMS/MS Coupled with Boosted Efficiency Ion Source (BEIS) Kuhn and Takakura.

Evaluation of sensitivity in analysis of actual samples

Sensitivity at low concentration levels in the analysis of actual samples was verified. Figures 2 and 3 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 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 figure 4.

Figure 1: Principle of operation of boosted efficiency ion source (BEIS).

Table 2: Evaluation results of LOQ in analysis of standard samples.

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

In the graph on the left in figure 4, 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 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 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. Figure 5 shows the results.

In figure 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.

Conclusions

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. 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.
Table 3: Average peak area and repeatability for standard samples in durability test (concentration: 0.05 pg/μl).

| 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        |

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References

1. Dioxin. [https://www.epa.gov/dioxin/learn-about-dioxin]
2. WHO. [https://www.who.int/news-room/fact-sheets/detail/dioxins-and-their-effects-on-human-health]
3. Recommended toxicity equivalence factors (TEFs) for human health risk assessments of 2,3,7,8- tetrachlorodibenzo-p-dioxin and dioxin-like compounds.
4. Method 1613: Tetra- through octa-chlorinated dioxins and furans by isotope filution HRGC/HRMS.