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Enhanced mass resolution tandem mass spectrometry method for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin detection with ion trap mass spectrometry using high damping gas pressure

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Abstract

Damping gas flow was optimized for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) determination using ion trap mass spectrometer. A tandem mass spectrometry (MS–MS) method with better than unit-mass resolution (mass width, 0.3 u) was developed at a damping gas flow of 1.5 ml/min and a collision-induced dissociation (CID) voltage of 3.30 V. The relative standard deviation (R.S.D.) at the enhanced resolution was 2.9% in 24 h of consecutive injections. The detection limit was significantly improved because the efficiency of both precursor ion trapping and fragmentation increased with the damping gas flow. Product ion yield was 4.5 times higher and limit of detection was 3.2 times lower than at the default flow (0.3 ml/min and 1.65 V).

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1. Introduction

Dioxins analysis is currently used for general environmental monitoring. A key challenge in the analysis is to improve productivity. Plomley et al. [1] and March et al. [2] described the versatility of quadrupole ion trap mass spectrometry (QIT) for dioxin determination. The biggest disadvantage of QIT is its relatively low sensitivity. QIT is 10–20 times less sensitive than high resolution mass spectrometer. Thermo Finnigan, a QIT manufacturer, reported a significant im-

provement in sensitivity with using a higher damping gas pressure [3]. Jemal and Ouyang used triple-stage type equipment to demonstrate that quadrupole mass spectrometer (QMS) provides better than unit-mass resolution [4]. Instrument stability is important for enhanced resolution because the mass width is narrower than 1 atomic mass unit (u).

The aim of the present study was to improve 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) detection for robust routine dioxin analysis. First, we investigated the QIT mass resolution and established an enhanced resolution tandem mass spectrometry (MS–MS) method. We then examined the fragmentation changes and optimized damping gas flow and collision-induced dissociation (CID) voltage.

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2. Experimental

2.1. Materials and equipment

NK-CVS-B20 CS-5 dioxins–furans standard mixture (Wellington Labs., Ontario, Canada) was di-

luted with toluene and used for the present study. The 2,3,7,8-TCDD concentration was approximately 10 pg/ μ l. Fly ash extract [containing 5.5 ng Toxic Equivalent Quantity (TEQ)/g of polychlorinated dibenzo-f-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs)] was put through a sulfuric acid

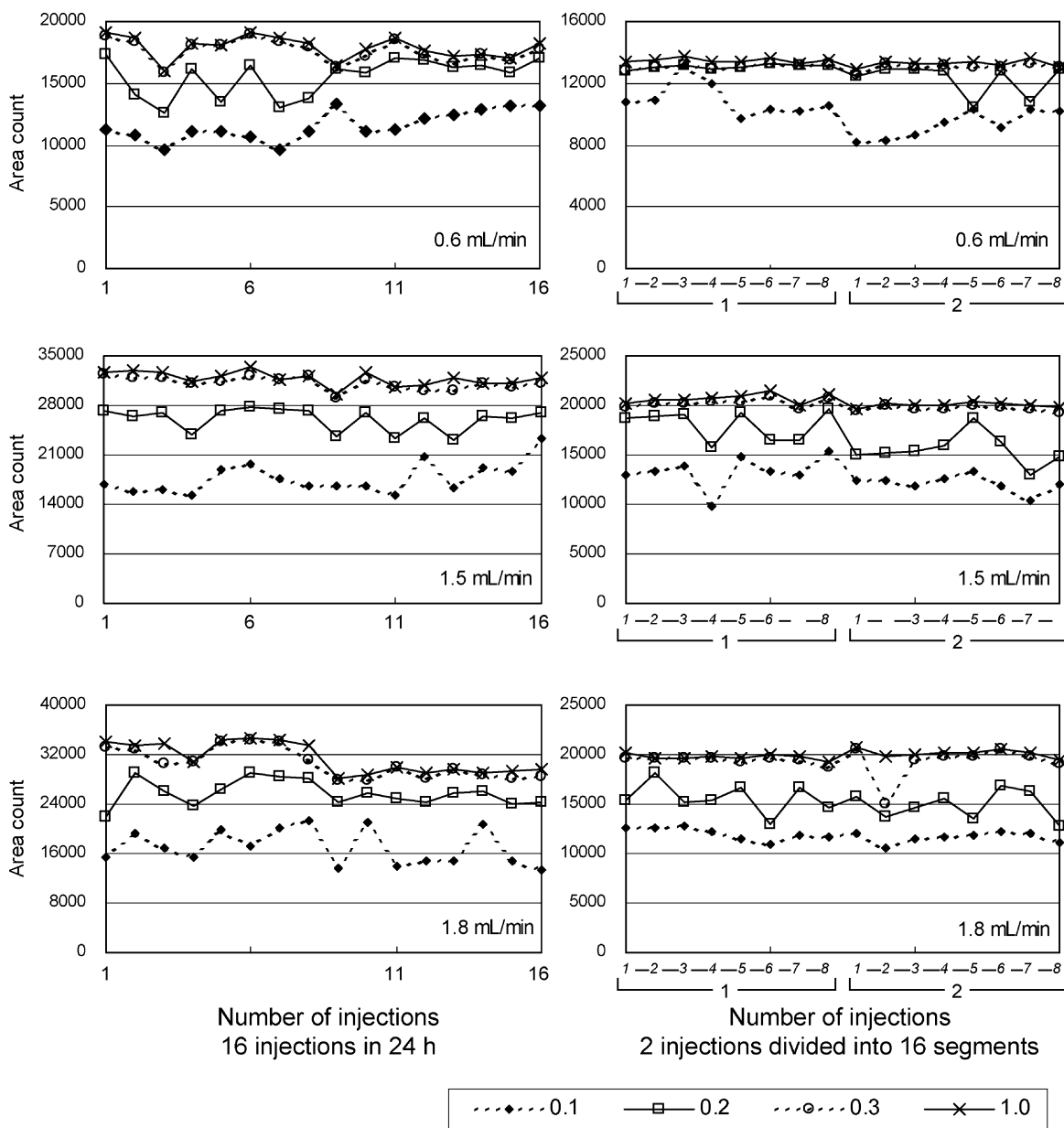


Fig. 1. Effect of damping gas flow on mass resolution stability.

coated diatomaceous column, followed by florisil. The extract was then analyzed to demonstrate the effect of the enhanced resolution MS–MS method. Wastewater extract (containing 0.12 pg/l of 2,3,7,8-TCDD) was put through the acid column, followed by activated carbon according to a method described earlier [5]. The extract was then analyzed to evaluate the improvement of limit of detection (LOD). A PolarisQ ion trap mass spectrometer and a TRACE 2000 gas chromatograph (Thermo Finnigan, Austin, TX, USA) equipped with a BPX-50 (SGE, Melbourne, Australia) capillary column (30 M \times 0.25 mm, 0.25 μ m) were used. Concurrently, both extracts were analyzed at an independent laboratory to ensure the dioxin concentration.

2.2. Mass resolution determination

2,3,7,8-TCDD was analyzed in the electron impact ionization (EI) MS–MS mode. Jemal and Ouyang narrowed the mass width in the precursor ion uptake with triple-stage QMS [4]. Our preliminary studies, however, indicated that a narrow precursor mass width caused a significant decline in sensitivity with QIT, compared to the previous report [6]. Thus, the precursor ion mass width was set at 5.0 u, so that $[M]^+$, $[M+2]^+$, and $[M+4]^+$ were introduced as a cluster. Product ion mass widths were also set at 5.0 u. Then, undissociated ions (i.e. $[M]^+$, $[M+2]^+$, and $[M+4]^+$) were monitored in chromatograms at the mass ranges of 0.1, 0.2, 0.3, and 1.0 u, respectively. The injector temperature was 300 °C and 1 μ l was injected in a splitless mode. The ion source was set at 246 °C, and oven temperature was programmed as follows: 142 °C (0.5 min) \rightarrow 40 °C/min \rightarrow 222 °C \rightarrow 1 °C/min \rightarrow 250 °C \rightarrow 2 °C/min \rightarrow 290 °C \rightarrow 5 °C/min \rightarrow 300 °C (12 min). Helium flow was 1.1 ml/min. Ion gauge pressure was 0.9×10^{-5} Torr at a damping gas flow of 0.3 ml/min, 1.5×10^{-5} Torr at 1.2 ml/min, and 1.9×10^{-5} Torr at 1.8 ml/min (1 Torr = 133.322 Pa). The collision-induced dissociation (CID) voltage was set at 0.5 V to simulate the actual MS–MS analysis as much as possible, and the q -value was set at 0.45 for the center of the 5.0 u ($[M+2]^+$). Mass resolution was determined based on the variation of the ion ejection. In practice, area counts of the 2,3,7,8-TCDD peaks were used for the mass resolution calculation. First, six series of 16 injections were performed at damping gas flows of 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 ml/min helium.

After every eighth injection, toluene was injected in a split mode at an injector temperature of 320 °C and an ion source temperature of 300 °C to simulate an actual sample injection sequence. A series of injections required approximately 24 h. Second, two injections were made and the 2,3,7,8-TCDD, ionized at the external ion source, was divided in time at the ion trap into eight segments and each data was processed individually. Due to the difference in scanning interval of time, a dioxin peak in the two-injections test had half as much data (10–15 points) as a peak in the 24 h test had (20–30 points). Other scanning conditions were the same in both experiments. Fly ash extract was used for the mass resolution confirmation in the MS–MS mode. $[M - \text{CO}^{35}\text{Cl}]^+$, $[M + 2 - \text{CO}^{35}\text{Cl}]^+$, $[M + 2 - \text{CO}^{37}\text{Cl}]^+$, and $[M + 4 - \text{CO}^{37}\text{Cl}]^+$ ions were monitored. Product ion mass widths were set to 0.3, 0.6, and 1.0 u. The effect of the enhanced resolution was demonstrated with fly ash chromatograms.

2.3. Optimum collision energy

2,3,7,8-TCDD was analyzed in the EI-MS–MS mode. The CID voltage was set from 0 to 3.95 V in every 0.05 V increment (e.g. 0.00, 0.05, 0.10, . . . , 3.95 V). Mass width was set at 0.3 u. Other conditions were the same as described above.

3. Results and discussion

3.1. Mass resolution determination

Fig. 1 and Table 1 show the effects of damping gas flow on stability. The 24 h test indicated that the area counted within 0.2 u was 87% at 0.6 ml/min.

Table 1
Effect of damping gas flow on stability at a mass width of 0.3 u

Damping gas flow (ml/min)	R.S.D. (%)	
	24 h/16 injections	Two injections/16 segments
0.3	4.3	–
0.6	5.2	1.5
0.9	6.7	2.3
1.2	4.9	3.4
1.5	2.9	2.3
1.8	8.0	6.5

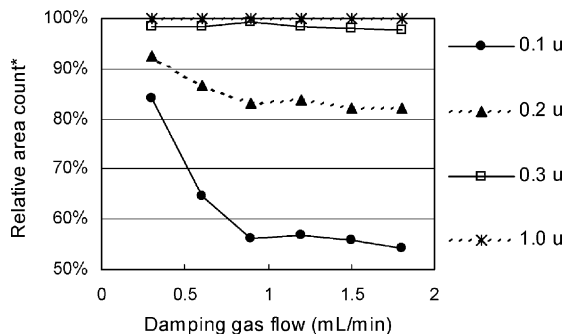


Fig. 2. Effect of damping gas flow on mass resolution.

On the other hand, the two-injection test indicated that the area counted within 0.2 u was 95%. At the two-injection test, the injections were performed just after MS tuning. Interval of time between two injections was <5 min. Thus, the center of mass axis did not likely change much. On the contrary, the relative standard deviation (R.S.D.) of the 24 h test included the mass axis shift. This was why the two-injection test had better mass resolution stability. The 24 h test integrated variance caused by gas chromatography as well. For the same reason, the two-injection test was more stable at a mass width of 0.3 u.

QMS had been reported to have unit-mass resolution [7]. Jemal and Ouyang used triple-stage type equipment (TSQ Quantum, Thermo Finnigan, CA, USA) to demonstrate that QMS had capability to provide better than unit-mass resolution [4]. This

equipment, however, depends its accurate mass measurements on an internal lock mass system. It is not possible to apply the system in QIT because excess amount of ions in the trap prone to cause space charge effect and deteriorate the mass determination [8]. Fig. 2 shows the effect of damping gas on mass resolution. The m/z of 2,3,7,8-TCDD $[M]^+$ ion is 319.897. Thus, 0.1 u is equivalent to mass resolution (R) 3200, 0.3 u to $R = 1070$, and 1 u to $R = 320$. This result indicated that QIT with the MS–MS mode obtained better than unit-mass resolution without having the internal lock mass system. The mass resolution and its stability were, however, inferior to the triple stage QMS. By assuming that the area ratio in Fig. 2 had a normal distribution, the standard deviation (σ) of mass resolution was calculated using a sequential selection method [9]. σ was 0.11 u at 0.6 ml/min, and 0.14 u at 1.8 ml/min.

As the flow increased from 0.3 to 0.9 ml/min, the relative area count within the narrower mass width decreased (Fig. 2). The reduction of the count was due to the degradation of the mass resolution. The area count, however, was rather stable between 0.9 and 1.8 ml/min. In the meantime, the R.S.D. at 1.8 ml/min was relatively high in both 24 h and two injections tests. The results indicated that the higher damping gas flow affects mass axis stability. The difference of the mass resolution degradation and the mass axis shift are shown in Fig. 3. In our study, the center of the mass axis was likely to shift to lower than its theoretical position.

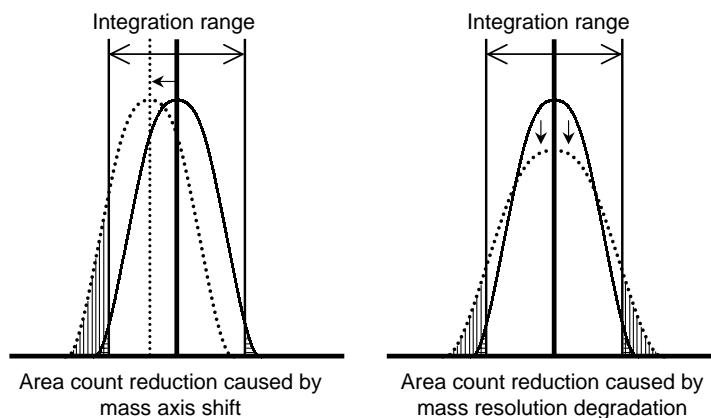
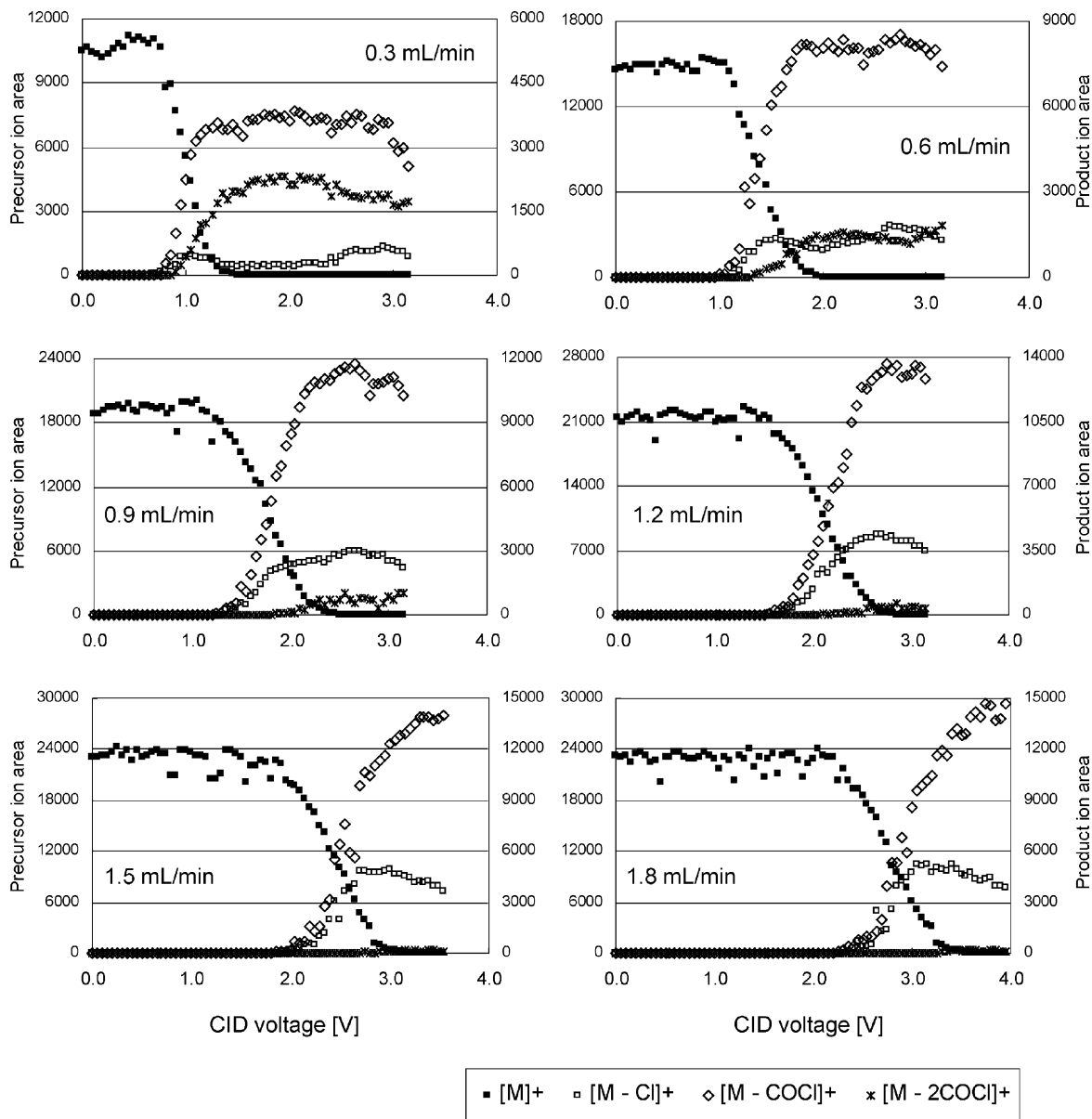


Fig. 3. Mass resolution degradation and mass axis shift.



*: “[M]⁺” is the sum of [M]⁺, [M+2]⁺, and [M+4]⁺. “[M - Cl]⁺” is the sum of [M - ³⁵Cl]⁺, [M+2 - ³⁵Cl]⁺, [M+2 - ³⁷Cl]⁺, [M+4 - ³⁵Cl]⁺, and [M+4 - ³⁷Cl]⁺. “[M - COCl]⁺” is the sum of [M - CO³⁵Cl]⁺, [M+2 - CO³⁵Cl]⁺, [M+2 - CO³⁷Cl]⁺, [M+4 - CO³⁵Cl]⁺, and [M+4 - CO³⁷Cl]⁺. “[M - 2COCl]⁺” is the sum of [M - 2CO³⁵Cl]⁺, [M+2 - 2CO³⁵Cl]⁺, [M+2 - ³⁵Cl³⁷Cl₂CO]⁺, [M+4 - 2CO³⁵Cl]⁺, [M+4 - ³⁵Cl³⁷Cl₂CO]⁺, and [M+4 - 2CO³⁷Cl]⁺.

Fig. 4. Effect of damping gas and CID voltage on area count and on fragmentation.

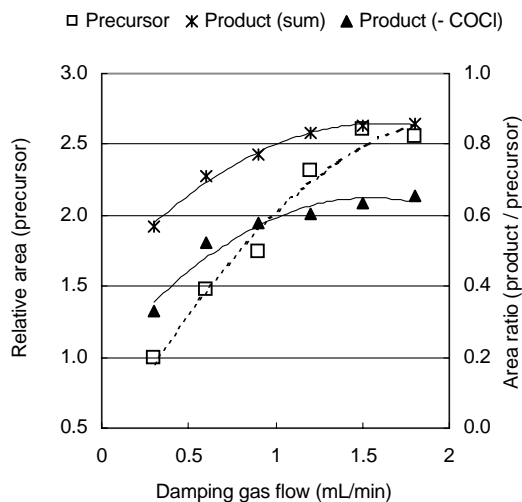


Fig. 5. Effect of damping gas flow on both sensitivity and total product ions ($[M - Cl]^+$, $[M - COCl]^+$, and $[M - 2COCl]^+$).

3.2. Fragmentation efficiency

Fig. 4 shows the effect of damping gas and CID voltage on area count and fragmentation. Charles and Marbury [10] and Charles [11] reported using tandem-in-space MS that higher collision gas pressure increased both the fragmentation efficiency and the area count of the $[M - 2COCl]^+$ ion. The ratio of total product ions to precursor ion was increased as shown in Fig. 5. The results indicated that the overall fragmentation efficiency improved by the higher collision gas pressure. The fragmentation efficiency of the $[M - 2COCl]^+$ ion, however, decreased as helium pressure increased with QIT. In contrast, the fragmentation efficiency of the $[M - COCl]^+$ ion increased with damping gas flow. It was beneficial for dioxin quantification since $[M - COCl]^+$ was the most desirable ion for the dioxin detection in the MS–MS mode [1,2,10–13]. As the flow rate was increased, a higher amplitude of the CID voltage was required in order to dissociate fully the precursor ions.

3.3. Chromatograms

Fig. 6 shows the effect of mass resolution enhancement. Cleaner chromatograms were obtained using the enhanced resolution MS–MS method. Hex-

achlorodibenzofuran is likely to be affected by phenyl polysilphenylene siloxane bleeding from BPX-50 column. The interference caused by the column bleeding was removed by using enhanced mass resolution method.

The advantage of BPX-50 use is its capability to separate 1,2,3,7,8-PCDF from 1,2,3,4,8-PCDF. Conventional SP-2331 column cannot separate these two isomers. Unidentified peak from fly ash extract, however, still interfered the dioxin quantification in case the unit-mass resolution method was used. Chromatogram of 1,2,3,4,6,7,8-H7CDD also showed the interference of fly ash matrix with the conventional unit-mass resolution. Enhanced mass resolution method removed the interference originated by the fly ash extract.

3.4. Optimum condition

Higher damping gas enhanced the trapping efficiency [3]. Consequently, the precursor ion area counts increased as the flow increased (Fig. 5). No significant improvement in area count was, however, observed by raising the flow from 1.5 to 1.8 ml/min. On the other hand, R.S.D. was relatively high at 1.8 ml/min. Considering both sensitivity and stability, 1.5 ml/min was optimum for dioxin analysis. The required CID voltage at this flow was 3.30 V. In comparison with default, 0.3 ml/min and 1.65 V, precursor ion trapping efficiency was 2.6 times higher and $[M - COCl]^+$ ion ratio to precursor was 1.8 times higher at the optimum condition. Ion loss by narrowing the mass width from unit-mass resolution to 0.3 amu was 3%. As total, product ion yield was 4.5 times higher than at the default condition. 2,3,7,8-TCDD determination in the MS–MS mode was significantly improved because the efficiency of both precursor ion trapping and fragmentation increased. LOD was calculated according to Japanese Industrial Standard method for dioxin analysis (3σ was regarded as LOD) [14]. Wastewater extract was used for the LOD determination. Injections were repeated five times with both default and optimized conditions, and each σ was used for the LOD calculation. As shown in Table 2, LOD at the optimum condition was 3.2 times lower than at the default condition.

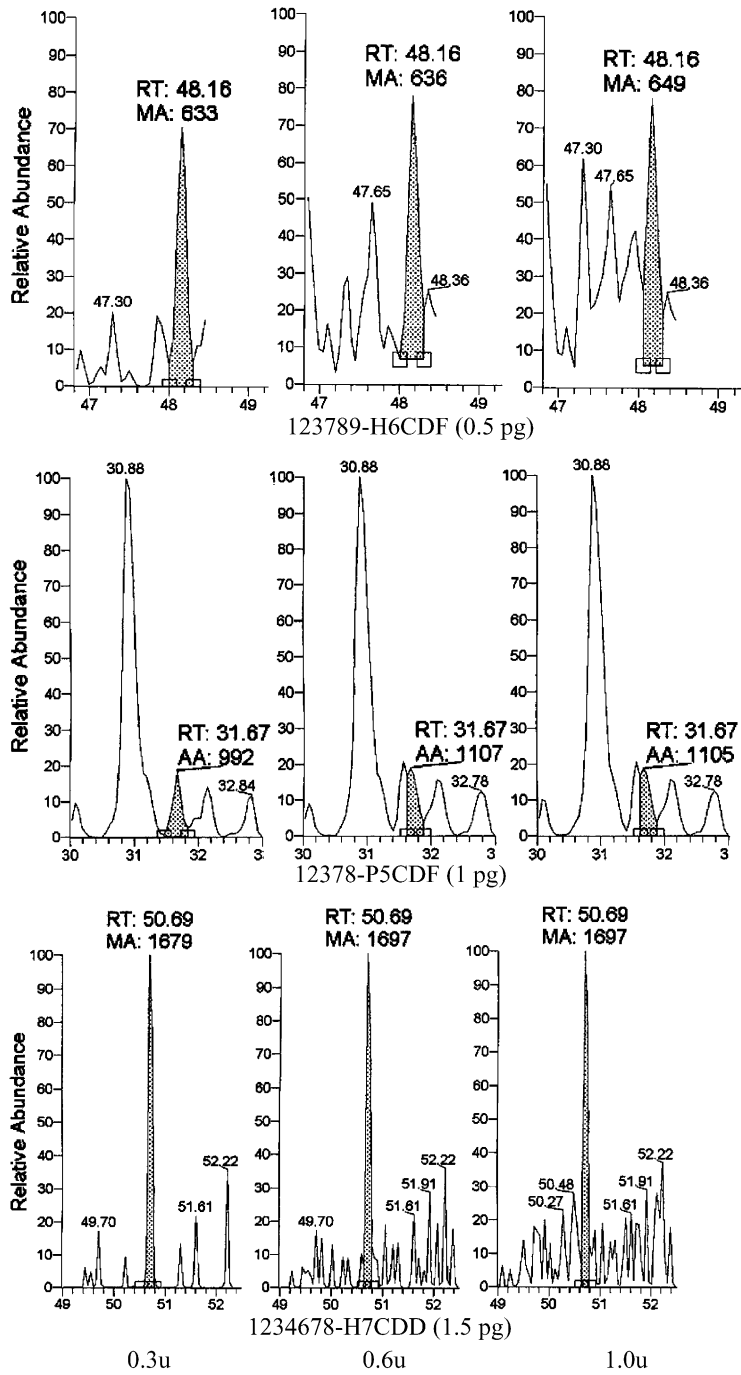


Fig. 6. Fly ash chromatogram with enhanced resolution method.

Table 2
Limit of detection of 2,3,7,8-TCDD

	Injected amount (pg)		R.S.D. (%)	LOD (pg)
	Native	¹³ C IS		
Default (0.3 ml/1.65 V)	0.10	10	50	0.15
Optimized (1.5 ml/3.30 V)	0.05	5.0	31	0.047

4. Conclusion

The effect of damping gas flow change on 2,3,7,8-TCDD determination was evaluated. An MS–MS method using better than unit-mass resolution (mass width, 0.3 u) was developed with 1.5 ml/min gas flow and 3.30 V CID voltage. The R.S.D. at this resolution was 2.9% over 24 h of consecutive injections. The LOD was significantly improved because the efficiency of both precursor ion trapping and fragmentation increased at this damping gas flow. Product ion yield was 4.5 times higher and LOD was 3.2 times lower than at the default flow (0.3 ml/min and 1.65 V).

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