Automatic Isomer Identification Method in HRGC/HRMS Analysis of PCDDs/PCDFs

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1. Introduction

In quantitative analysis of environmentally toxic compounds such as polychlorinated dioxins and furans (PCDDs/PCDFs), high resolution gas chromatography (HRGC) and high-resolution mass spectrometry (HRMS) are routinely employed.[1-3] Isomers in PCDDSs/PCDFs are separated by HRGC and detected by HRMS with high sensitivity and selectivity using selected ion monitoring (SIM) method. In the analysis of PCDDs/PCDFs, not only the amounts of seventeen toxic 2,3,7,8 substituted isomers but also the total amounts of all isomers in each homologue have to be quantified. In the quantitation process, it is necessary to identify each isomer to the respective peak in SIM chromatogram. This is however a labor-intensive and time-consuming work and is desirable to be automated by a computer program. Retention time windows can be used for the automation, but accurate retention times and narrow ranges should be set since isomer peaks elut close to one another.[4] Moreover, peaks could be shifted by shortening GC column (column performance can often be recovered by cutting off the degraded and/or contaminated portion) or replacing a column with the new one. In these cases, narrow retention time windows may lead misidentifications. In order to compensate these retention time shifts and automate the isomer identification process, we examined retention time shifts and developed a new method to precisely predict isomer retention times of target compounds.

2. Experimental

In accordance with the analytical manual[3], fly ash samples were prepared and measured by JMS-700 mass spectrometer (JEOL Ltd. Japan) connected with Model 6890 gas chromatography (Hewlett-Packard). GC columns used were SP-2331 (Supelco Inc.) with 60m x 0.32 mm i.d. and 0.20 μm film thickness. Prepared Samples were injected with column temperature maintained at 100℃, held for 1 minute, then increased at 20℃/min to 150℃, 2℃ /min to 240℃ and 1℃/min to 260℃.

3. Retention Time Shift

The retention time T_i of a specific isomer in a target sample is expressed with the retention time

To_i of the same isomer in a reference sample and the retention time shift Δ_i caused by cutting off column, or replacing column.

$$T_i = To_i + \Delta_i \qquad (1)$$

From the mathematical examination of the relationship between retention characteristics and molecular structure[5,6], Δ_i can be expressed by a retention time shift function $\Delta(To)$.

$$\Delta_i = \Delta(To) \qquad (2)$$

$\Delta(To)$ can be expanded to the Taylor series.

$$\Delta(To) = \Delta(0) + \Delta'(0)\, To + \Delta''(0)/2!\, To^2 + ... \qquad (3)$$

We assume that higher order terms are negligible when shift is small,

$$\Delta(To) \fallingdotseq b + a\, To \qquad (4)$$

where $a = \Delta'(0)$, $b = \Delta(0)$. The retention time of a isomer in a target sample can thus be predicted as

$$Tc_i = (1+a)\, To_i + b \qquad (5)$$

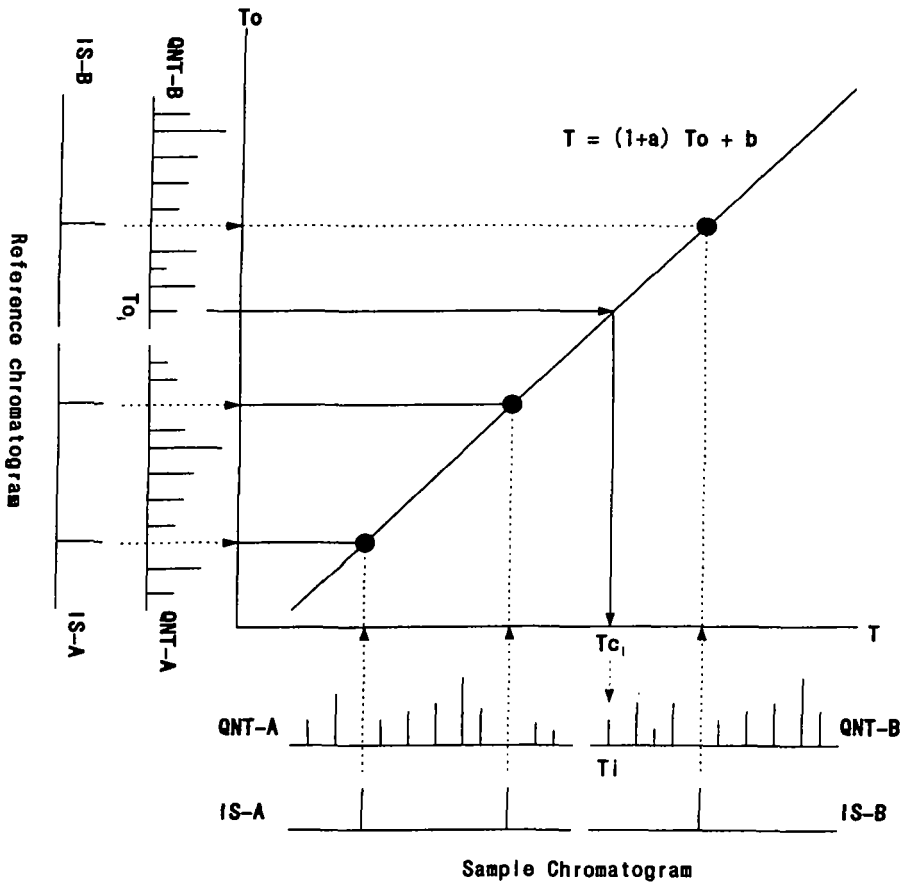

Figure 1. Schematic diagram of isomer identification.

where Tc_i is the calculated retention time of a specific isomer. A linear line expressed by a and b is called a compensation line.

4. Isomer Identification

To calculate Tc_i for each isomer in a target sample, the reference retention time To_i and the compensation line a and b are required. To_i for each isomer is obtained by analyzing a reference sample that contains all PCDDs/PCDFs isomers at relatively high concentration. To estimate the compensation line, we employ internal standard compounds which are spiked into a target sample so that intensities of these peaks are strong and the number of peaks is few in SIM chromatogram. This isomer identification is thus accomplished without difficulty. Our algorithm is that intense peaks are sorted out from detected peaks in the SIM chromatogram, and are assigned to the isomers in order of the retention time of the reference. In PCDDs/PCDFs analysis, several homologues are analyzed in one injection, and thus several internal standard peaks can be used to obtain the compensation line. Tc_i of a specific isomer is then calculated from the equation (5). Considering the difference between T_i and Tc_i, an allowance is introduced. Peaks in the allowance range of Tc_i are picked out, and a peak closest to Tc_i is assigned to a specific isomer. This operation is repeatedly applied to each isomer of PCDDs/PCDFs. A schematic diagram of the isomer identification procedure described is shown in Figure 1.

5. Result and Discussion

A key of the automatic isomer identification is how precisely Tc_i can be predicted for each isomer in a target sample. We examined actual retention time shifts caused by cutting off a column (about 1m) and replacing another column. A analysis before cutting off is used as a reference, and one after cutting off and replacing are treated as targets, respectively. Figure 2 shows plots of $\Delta(To_i)$ versus To_i for 99 isomer peaks of 6 homologues, T_4CDD, P_5CDD, H_6CDD, T_4CDF, P_5CDF and H_6CDF. The lines drawn on Figure-2 are compensation lines calculated from 6 ^{13}C labeled internal standards of 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDD, 1,2,3,6,7,8-H_6CDD, 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF and 1,2,3,4,7,8-H_6CDF. From Figure 2 it is obviously apparent that Δ_i is linearly changed in To and the compensation line expresses the retention time shifts. To evaluate a prediction error of Tc_i, $E = T - To$ is calculated. The root mean square (RMS), minimum and maximum of Es for 99 isomer peaks are shown in Table 1. From the fact that maximum and minimum errors are within the half width of peaks (about 0.1 minutes), Tc_i is predicted for every isomer retention time with high accuracy. As a result, all isomers in these two examples are perfectly identified to respective peaks by the automatic isomer identification method.

The isomer identification method described above enabled us to make a fully automatic quantitative software. Additionally the calculated retention time is useful for validating the isomer identification.

	Cutting off	Replacing
a	-0.00774	-0.00706
b	0.231	0.036
RMS	0.019	0.016
Min	-0.057	-0.047
Max	0.035	0.035

Table 1. Errors of Calculated Retention Times.

6. References

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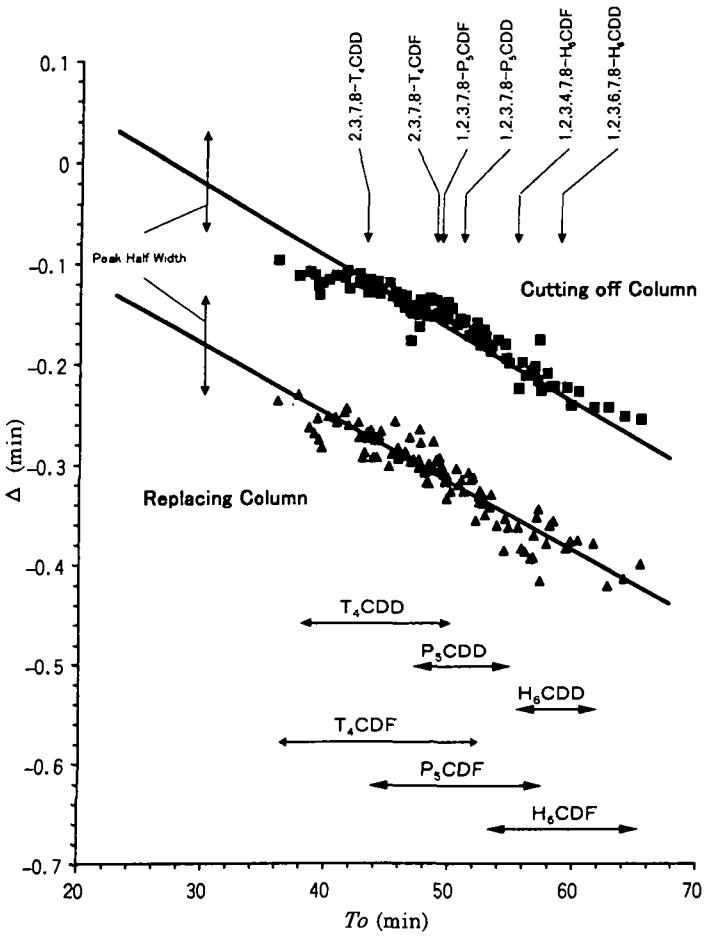

Figure 2. Retention time shifts