Surface fitting for calculating the second dimension retention index in comprehensive two-dimensional gas chromatography mass spectrometry

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ABSTRACT

Comprehensive two-dimensional gas chromatography mass spectrometry (GC × GC–MS) has been widely used for analysis of volatile compounds. However, the second dimension retention index (I) of each compound is not widely used to aid compound identification owing to the limited accuracy of I calculation. We report a surface fitting approach to the calculation of I using n-alkanes ($C_7–C_{30}$) as references, where the second dimension retention time (^2t_R) and the second dimension column temperature (^2T_e) formed the X-Y plane and the I was the Z-axis to form the I surface. Compared to the conventional approach for calculating I using isovolatility curves, the surface fitting approach eliminated the construction of isovolatility curves for the reference compounds and gives better reproducibility. The goodness of the proposed surface fitting achieved $R^2 = 0.9999$ and $RMSE = 6.1$ retention index units (iu). Ten-fold cross validation demonstrated the surface fitting approach had a good predictability with average $R^2 = 0.9999$ and $RMSE = 6.6$ iu. The developed method was also applied to calculate the second dimension retention indices of compound standards in two commercial mixtures MegaMix A and MegaMix B. The mean standard deviation of the calculated I was only 1.6 iu for compounds in MegaMix A and 3.4 iu for compounds in MegaMix B. Compared with the literature results, the small value of standard deviation in the calculated retention index using surface fitting method shows that the surface fitting method has less measurement variability than the conventional isovolatility curve approach.

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1. Introduction

While mass spectrometry (MS) coupled with a one-dimensional separation technique, such as gas chromatography (GC) or liquid chromatography (LC) has been widely used in metabolomics, the limited separation power of the one-dimensional GC or LC contributes to the poor performance of GC–MS and LC–MS in detecting low abundance compounds from complex samples such as human body fluids and tissues. Comprehensive two-dimensional gas chromatography mass spectrometry (GC × GC–MS) uses two GC columns with different stationary phases to increase the separation power. The first dimension column, i.e. the primary column, is usually a 10–60 m long column while the second dimension column is a short column about 1–2 m [1–5]. The two columns are connected by a thermal modulator. Compounds eluted from the first dimension column within a modulation period P_M are collected by the thermal modulator and subjected to the second dimension column for further separation at an increased temperature. Depending on the system configuration, the modulation period is usually set to 2–10 s and the temperature gradient on both GC columns is usually programed to 5–10 °C/min. While the two columns are operated

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in linear temperature-programmed condition, the fast separation and slow temperature gradient on the second dimension column results in a small temperature change within a P_M, making a condition such that the second dimension column actually functions under a pseudo-isothermal condition [6–12].

GC × GC–MS provides three pieces of information for each compound, the first dimension retention ^1t_R, the second dimension retention time ^2t_R, and an electron ionization (EI) mass spectrum, if an EI source is used [13,14]. To reduce the dependence of retention time on GC × GC–MS experimental conditions, ^1t_R and ^2t_R of a compound u are respectively converted into linear retention index I^T and Kováts retention index I as follows: [15,16]

$$I_u^T = 100 \times \left[n + \frac{^1t_{R(u)} - ^1t_{R(n)}}{^1t_{R(n+1)} - ^1t_{R(n)}} \right] \qquad (1)$$

$$I_u = 100 \times \left[n + \frac{\log\left(^2t'_{R(u)}\right) - \log\left(^2t'_{R(n)}\right)}{\log\left(^2t'_{R(n+1)}\right) - \log\left(^2t'_{R(n)}\right)} \right] \qquad (2)$$

where n is the number of carbon atoms in a n-alkane. $^1t_{R(u)}$, $^1t_{R(n)}$, and $^1t_{R(n+1)}$ are the first dimension retention times of the compound u, the n-alkane with n carbon atoms, and the n-alkane with $n + 1$ carbon atoms, respectively. $^2t'_R$ is the adjusted second dimension retention time, i.e. the second dimension retention time of a chromatographic peak minus hold-up time (^2t_M) that is the retention time of a unretained chromatographic peak.

During GC × GC–MS analysis, compounds eluted within the same P_M from the first dimension column are collected and subjected to the second dimension column for further separation at a different temperature, as well as on a different stationary phase. While the separation of the compounds on the second dimension column within one P_M can be considered as in pseudo-isothermal condition, the overall temperature of the second dimension column ^2T_e changes during the entire GC × GC–MS analysis. The retention time ^2t_R of a compound in the second dimension column decreases with the increase of column temperature ^2T_e.

The relation ^2t_R–^2T_e of a compound can be captured by an isovolatility curve. Several methods have been proposed to construct the isovolatility curves for the reference compounds such as n-alkanes, including Beens et al. [17,18], Marriot et al. [19–22], and Zhao et al. [23]. In these reported methods, the hold-up time ^2t_M at different temperatures of the second dimension column must be first estimated to get the adjusted retention time $^2t'_R$. The $^2t'_R$ values of the same reference compound at different column temperatures were then used to construct the isovolatility curve of a reference compound. The isovolatility curves of all reference compounds formed the second dimension retention index map, and the I value of a compound was finally calculated from the two adjacent isovolatility curves of two reference compounds.

The objective of this study was to develop a method to calculate I value of each compound without the construction of isovolatility curves for the reference compounds. We developed a surface fitting method for the calculation of I values for all compounds detected by GC × GC–MS. The developed surface fitting method used n-alkanes as reference compounds, where the second dimension retention time ^2t_R and the second dimension column temperature ^2T_e formed a X-Y plane and the I was the Z-axis to form the I surface. The robustness of the surface fitting method was validated using 10-fold cross validation. The method was then applied to calculate the retention indices of 152 compound standards in two commercially available mixtures, MegaMix A and MegaMix B.

2. Experimental

2.1. Chemicals

A mixture containing C_7–C_{30} n-alkanes was purchased from Sigma-Aldrich Corp., St. Louis, MO. MegaMix A (Cat. No. 8270) and MegaMix B (Cat. No. 8260) were purchased from Restek Corp., Bellefonte, PA. All of the compounds were present at a purity of between 95 and 99%. Two solutions were prepared from these three commercial mixtures. One solution contained only C_7–C_{30} n-alkanes, while the other contained MegaMix A and MegaMix B. These two solutions were prepared in dichloromethane at a concentration of 25 μg/mL per compound.

2.2. GC × GC–MS experiment

The LECO (St. Joseph, MI) Pegasus® 4D GC × GC–MS instrument was equipped with a Gerstel MPS2 autosampler and an Agilent 6890 gas chromatograph featuring a LECO two-stage cryogenic modulator and a secondary oven. The first dimension column was a non-polar DB–5 ms (phenyl arylene polymer virtually equivalent to a (5%-phenyl)-methylpolysiloxane) 60.0 m × 0.25 mm ^1d_c × 0.25 μm ^1d_f column, and the second dimension column was a medium polar DB–17 ms ((50%-phenyl)-methylpolysiloxane) 1.0 m × 0.1 mm $2d_c$ × 0.1 μm $2d_f$ column. Both columns were obtained from Agilent Technologies (Santa Clara, CA), and were connected by means of a press-fit connector before the thermal modulator. The flow rate of ultra-high purity helium carrier gas (99.999%) was set to 2.0 mL/min at a corrected constant flow via pressure ramps with an initial head pressure of 36.9 psi.

A 1 μL sample was injected into the inlet chamber of the GC × GC–MS system and split ratio was set to 20:1. The inlet temperature was 280 °C. The temperature of thermal modulator was set to +15 °C relative to the secondary oven temperature. The MS parameters were: MS range 45–800 m/z; data acquisition rate 200 spectra/s; temperature of ion source chamber 230 °C; temperature of MS transfer line 280 °C; detector voltage 1641 V; electron energy 70 eV. The acceleration voltage was turned on after a solvent delay of 220 s.

To test the variability of the GC × GC–MS system, the mixture of n-alkanes was analyzed 25 times in five days in temperature-programmed mode, where the primary column temperature was held at an initial temperature 60 °C for 1 min and then ramped at 5 °C/min to 285 °C, and kept at 285 °C for 13 min. The secondary column temperature program was identical to that of the first dimension column but temperature was set to +5 °C higher than that of the first dimension column. P_M was set as 10 s.

To construct the second dimension retention index surface, the mixture of C_7–C_{30} n-alkanes was analyzed by GC × GC–MS 46 times under isothermal conditions. Temperature of the first dimension column $1T_e$ was set to 60 °C in the first injection, and increased +5 °C in each of the following 45 injections with $^1T_e = 285$ °C in the last injection. The second dimension column was also set under isothermal conditions but at +5 °C with respect to the temperature of the first dimension column. P_M was set as 10 s.

The mixture of MegaMix A and MegaMix B was analyzed four times in temperature-programed conditions with the first dimension column temperature initially set to 60, 80, 100, and 120 °C, respectively. The column temperature was held at the initial temperature for 1 min and then ramped at 5 °C/min to 285 °C, and kept at 285 °C for 13 min. The secondary column temperature program was identical to that of the first dimension column but temperature was set +5 °C higher than that of the first dimension column. P_M was set as 4 s.

To study the robustness of surface fitting method, the mixture of MegaMix A and MegaMix B was also analyzed under different