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A method of calculating the second dimension retention index in comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry

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ABSTRACT

A method was developed to calculate the second dimension retention index of comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC/TOF-MS) data using *n*-alkanes as reference compounds. The retention times of the C_7–C_{31} alkanes acquired during 24 isothermal experiments cover the 0–6 s retention time area in the second dimension retention time space, which makes it possible to calculate the retention indices of target compounds from the corresponding retention time values without the extension of the retention space of the reference compounds. An empirical function was proposed to show the relationship among the second dimension retention time, the temperature of the second dimension column, and the carbon number of the *n*-alkanes. The proposed function is able to extend the second dimension retention time beyond the reference *n*-alkanes by increasing the carbon number. The extension of carbon numbers in reference *n*-alkanes up to two more carbon atoms introduces <10 retention index units (iu) of deviation. The effectiveness of using the proposed method was demonstrated by analyzing a mixture of compound standards in temperature programmed experiments using 6 different initial column temperatures. The standard deviation of the calculated retention index values of the compound standards fluctuated from 1 to 12 iu with a mean standard deviation of 5 iu.

1. Introduction

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC/TOF-MS) has increasingly been applied to analyze the chemical compositions of complex organic mixtures such as drug, food, fat, oil, and biological samples [1–6]. The two-dimensional gas chromatography separates compounds using two distinctly different capillary columns of different polarities. The interface between the two columns is called a modulator [7,8]. Compared to the 15–30 m length of the first dimension column, the length of the second dimension column is very short, in general only 0.5–2 m [7,9,10]. This means that the second dimension column's separation is a very fast process and can be considered to be under pseudo-isothermal conditions before the next modulator pulse [11].

Spectrum library searching is widely used for compound identification in analyzing experiment data generated on GC × GC/TOF-MS instruments by finding the best mass spectrum matched compound recorded in the mass spectrum library [12]. However, the mass spectrum represents only partial information of the molecular structure of a compound. Identifying compounds based on spectrum matching only, therefore, may introduce a high rate of false-positive identifications, especially for the analysis of highly complex mixtures. For instance, it is likely that structurally similar molecular homologous compounds may have similar mass spectra and therefore, increase the chance of misidentifications. Additional molecular information may be employed to increase the identification confidence of a spectrum library match. One approach is to combine compound separation information with the mass spectrum matching. The *n*-alkane-based retention index (*I*) has been playing an important role in one-dimensional gas chromatography to identify complex compounds [13,14], and several retention index libraries have been developed [12,15–18]. It is possible that the retention index of the second dimension column in a GC × GC/TOF-MS system can also enhance the confidence of compound identification.

The retention index values of compounds in the second dimension column of a GC × GC/TOF-MS system can be calculated from a second dimension retention map, which composes a set of curves

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generated by fitting the retention time values of individual reference compound using an empirical column temperature–second dimension retention time function ($^2t_R - {}^2T_e$ function). The second dimension retention index of any target compound, whose second dimension retention time falls between the second dimension retention times of two consecutive reference compounds, can be deduced from the retention index values of the two reference compounds via linear interpolation. Beens and co-workers first constructed a second dimension retention map from a continuous injection of *n*-dodecane [19], and then introduced five alkanes, *i.e.*, C_9–C_{13} [20]. Western and Marriott developed different second dimension retention map with repetitive, timed, and sequential injections and with injections at different temperatures and different temperature ramp rates using mixtures of reference compounds such as *n*-alkanes, 2-methyl ketone, fatty acid methyl ester, and alcohol standards [11,21]. Bieri and Marriott proposed solid-phase microextraction (SPME) [22] and a dual-injection system to extend the range of the second dimension map from C_{10} to C_{21} [23]. Meanwhile, some researchers applied the second dimension retention map to identify the unknown compounds in tobacco leaves [24] and cigarette essential oil [25].

In most published studies, multiple injections of the reference compounds were accomplished in one chromatographic run, which would limit the reference compound identification and therefore the second dimension retention map would only cover a small portion of the two-dimensional retention time space. For example, using the *n*-alkanes from C_9 to C_{22}, the second dimension retention map would not cover the retention time space in which the more polar target compounds elute off the GC columns [21]. Covering a limited retention time space by the retention times of the reference compounds makes the accurate calculation of the retention index values of all target compounds a significant challenge in applying the GC × GC/TOF-MS system to analyze mixtures, like for example in metabolomics, where the target compounds in samples can range from being polar to non-polar.

The objectives of the current research was to employ *n*-alkanes as the reference compounds to create a second dimension retention map to cover most of the second dimension retention time space at various temperatures of the second dimension column in a GC × GC/TOF-MS system. An empirical $^2t_R - {}^2T_e$ function was developed using the experimental retention times of the reference *n*-alkanes. The capability of the developed $^2t_R - {}^2T_e$ function was explored for the prediction of retention index of target compounds whose retention times fall outside of the second dimension retention map, *i.e.*, the retention time space that was not covered by the reference *n*-alkanes. In addition, a mixture of compound standards was used to demonstrate the accuracy of converting the second dimension retention time values to the retention index values in different experimental conditions.

2. Experimental

2.1. Materials

Two standard solutions were used in this study: (A) a mixture containing the C_7–C_{40} *n*-alkanes (Sigma–Aldrich Corp., St. Louis, MO); (B) a mixture containing 76 compounds (8270 MegaMix, Restek Corp., Bellefonte, PA). All of the compounds were present at a purity of between 95% and 99% and prepared in dichloromethane at a concentration of 2.5 μg/mL per compound.

2.2. GC × GC/TOF-MS experiments

A LECO Pegasus® 4D GC × GC/TOF-MS instrument was equipped with an Agilent 6890 gas chromatography, featuring a LECO two stage cryogenic modulator (a cold pulse of liquid N_2-cooled N_2 gas and a hot pulse of heated air) and a secondary oven, and a Gerstel MPS2 autosampler. The first dimension column was a non-polar DB-5ms (phenyl arylene polymer virtually equivalent to a (5%-phenyl)-methylpolysiloxane) 30 m × 0.25 mm ^1d_c × 0.25 μm ^1d_f column, and the second dimension column was a medium-polar BD-17 ((50%-phenyl)-methylpolysiloxane) 1.79 m × 0.1 mm ^2d_c × 0.1 μm ^2d_f column. Both columns were obtained from Agilent Technologies (Agilent Technologies, Santa Clara, CA), and were connected by means of a press-fit connector before the modulator. The flow rate of ultra-high purity helium carrier gas (99.999%) was set to 1.0 mL/min in a constant flow with an initial head pressure of 65 psi.

A 2 μL liquid sample was injected into the liner using the splitless mode with the injection port temperature set at 260 °C. The ion source chamber was set to 230 °C, the detector voltage was 1700 V, and the electron energy was 70 eV. The thermal modulator was set to +20 °C relative to the primary oven. The second oven was set to +5 °C with respect to the primary oven. The mass spectra were acquired at a rate of 200 spectra per second with mass range set to m/z = 45–800. The modulation period for the temperature programmed experiments was set as 6 s, while it was set at 9 s for the isothermal experiments. Using the standard solution A, the first dimension column was set to isothermal at 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 265, 270 and 280 °C, respectively, with a 60 min analysis time. For the analysis of a mixture of standard solutions A and B, the first dimension column was programmed from 60, 80, 100, 120, 140, and 160 °C ramped to 280 °C at 5 °C/min with a hold time of 0.5 min at the initial temperature and 20 min at the end temperature, respectively.

2.3. Raw data reduction

The LECO ChromaTOF software (version 4.21), equipped with the National Institute of Standards and Technology (NIST) MS database (NIST MS Search 2.0, NIST/EPA/NIH Mass Spectral Library; NIST 2002), was used for instrument control, spectrum deconvolution, and compound identification. By manually visualizing the raw data, we estimated the peak widths on the first dimension column ranges from 2 to 3 modulation periods, while the peak width on the second dimension column ranges from 0.2–0.5 s. Manufacturer recommended parameters for ChromaTOF were used to reduce the raw instrument data into a compound peak list. These parameters are: baseline offset = 0.5; smoothing = auto; peak width in first dimension = 6 s; peak width in the second dimension = 0.1 s; signal-to-noise ratio = 100; match required to combine peaks = 500; R.T. shift = 0.08 s; minimum forward similarity match = 600. The true peak spectrum was also exported as part of the information for each peak in absolute format of intensity values.

2.4. Hold-up time of the second dimension column

The hold-up time in the second dimension column ^2t_M varies with different column temperatures and head pressure. The head pressure was set constant as 65 psi for all experiments in this study. To a fixed temperature of the second dimension column ^2T_e, ^2t_M can be calculated from the retention times of three homologues with equidistant carbon number as follows [26]:

$$^2t_M = \frac{t_{R1}t_{R3} - t_{R2}^2}{(t_{R3} - t_{R2}) - (t_{R2} - t_{R1})} \tag{1}$$

where t_{R1}, t_{R2} and t_{R3} are the continuous retention times of three homologues such as C_{n-1}, C_n and C_{n+1}-alkanes. In the current study, the isothermal experiments were carried out at 24 different temperatures of the second dimension column. Multiple *n*-alkanes were analyzed at the same column temperature of the second dimension column. Therefore, we can obtain more than one hold-up times in one temperature using Eq. (1). Using the mean hold-up time values at different temperatures, the hold-up time at different column temperatures of the second dimension column was fitted as follows:

$$^{2}t_M = 0.000004(^{2}T_e)^2 - 0.0025(^{2}T_e) + 1.6184 \tag{2}$$

where t_M is the fitted hold-up time in the second dimension column and $^{2}T_e$ is the second dimension column temperature in °C. The fitting accuracy R^2 was 0.9773.

2.5. Selection of the $^{2}t_R - {}^{2}T_e$ function

The sample of the *n*-alkanes was analyzed multiple times on the GC × GC/TOF-MS system by setting both the first and the second dimension columns in isothermal mode. After correcting the hold-up time of the second dimension column using Eq. (2), a second dimension retention map of all *n*-alkanes could be constructed with the relationship of column temperature and retention time.

In order to make it possible to extend the second dimension retention map beyond the reference compounds, it is better to incorporate the carbon number of the *n*-alkanes reference compounds into the $^{2}t_R - {}^{2}T_e$ function. The carbon number in each *n*-alkane (n) and temperature of the second column ($^{2}T_e$) were first transformed by a logarithmic function. Then a multiple dimension polynomial fitting method was used to get a comprehensive $^{2}t_R - {}^{2}T_e$ function as follows:

$$^{2}t_R = \exp^{(p_1 * n'^3 + p_2 * n'^2 * T' + p_3 * n'^2 + p_4 * n' * T'^2 + p_5 * n' * T' + p_6 * n' + p_7 * T'^3 + p_8 * T'^2 + p_9 * T' + p_{10})} \tag{3}$$

where n' is log(n), T' is log($^{2}T_e$), and each p_i is the parameter of the $^{2}t_R - {}^{2}T_e$ function. The numeric values of p_i can be generated by fitting the retention time values of all *n*-alkanes reference compounds.

2.6. Evaluating the performance of the proposed $^{2}t_R - {}^{2}T_e$ function

The retention index of a C_n-alkane, an alkane with n carbons, is defined as 100 × n. Since the second dimension column can be considered under pseudo-isothermal conditions [11], the Kovats retention index for isothermal experimental conditions can be used to calculate the retention index value of a target compound in the second dimension column as follows [27]:

$$I = 100n + 100\left(\frac{\log(t_t) - \log(t_n)}{\log(t_{n+1}) - \log(t_n)}\right) \tag{4}$$

where I and t_t are the retention index and adjusted retention time of the target compound in the second dimension column, respectively, and t_n and t_{n+1} are the adjusted retention times of two *n*-alkanes between which the target compound eluted off the second dimension column.

Two methods were employed to evaluate the performance of the proposed $^{2}t_R - {}^{2}T_e$ function. In the first approach, the fitted $^{2}t_R - {}^{2}T_e$ curves of the C_{n-1}-alkane and C_{n+1}-alkane were used to predict the retention index of the C_n-alkane at different temperatures of the second dimension column by linear interpolation using Eq. (4). The distribution of the absolute retention index deviations (d_{abs}) between the predicted values and the true values, and the root mean square error (RMSE) were then used as evaluation criteria:

$$d^i_{abs} = \left|I^i_p - I\right| \tag{5}$$

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(I^i_p - I)^2} \tag{6}$$

where I^i_p is the predicted value of retention index for the ith data point, I is true retention index value of a given *n*-alkane by definition, d^i_{abs} is the absolute deviation of the ith data point, and N is the total number of the data points used in calculation.

For the temperature programmed data of MegaMix compounds, the mean standard deviation of all compounds ($\overline{STD}$) was used to evaluate the performance of $^{2}t_R - {}^{2}T_e$ function. A larger value of $\overline{STD}$ indicates the instability of the testing $^{2}t_R - {}^{2}T_e$ function for converting the retention time values of all compounds into the retention index values. The $\overline{STD}$ of all compounds is defined as follows:

$$\overline{I_j} = \frac{1}{M_j}\sum_{i=1}^{M_j} I^i_p \tag{9}$$

$$STD_j = \sqrt{\frac{1}{M_j}\sum_{i=1}^{M_j}(I^i_p - \overline{I_j})^2} \tag{10}$$

$$\overline{STD} = \frac{1}{N}\sum_{j=1}^{N} STD_j \tag{11}$$

where M_j is the number of retention index values for the jth compound, $\overline{I_j}$ is the mean of calculated retention index values of the jth compound, STD_j is the standard deviation of the jth compound, and N is the number of identified compounds in the MegaMix.

3. Results and discussion

In the current study, *n*-alkanes (C_7–C_{40}) were chosen as the reference compounds to create a second dimension retention map to cover most of the retention time space defined by the two dimension chromatogram of a GC × GC/TOF-MS system. An empirical $^{2}t_R - {}^{2}T_e$ function was further developed to construct the second dimension retention map from the isothermal data of the *n*-alkanes. The constructed second dimension retention map was extended into the retention time area that was not covered by the reference *n*-alkanes for the calculation of the compound retention index in the second dimension column. A mixture of *n*-alkanes and MegaMix was analyzed on GC × GC/TOF-MS, from which C_7–C_{31} alkanes and 47 MegaMix compounds were identified.

3.1. Experiment repeatability

A mixture of solution A (*n*-alkanes) and solution B (MegaMix) were analyzed with 6 replicate injections every day for five continuous days. For the experiments performed within one day, the standard deviation of the second dimension retention time of each identified compound ranges from 0 to 0.0225 s. Fig. 1 displays the cumulative probability curves of the day-to-day experiments, *i.e.*, a total of 30 replicate analyses in five days. The standard deviation of the second dimension retention time of the *n*-alkanes fluctuated from 0.0015 to 0.0046 s and of compounds detected from the MegaMix it fluctuated from 0.0037 to 0.0059 s, with mean standard deviation values of 0.0022 and 0.0043 s, respectively. The standard deviation of 90% of the second dimension retention time values in all 30 experiments were <0.005 and 0.01 s for the *n*-alkanes and the compounds in the MegaMix, respectively. Such a small standard deviation of the second dimension retention time indicates that the instrument has a very good repeatability in measuring the retention times of compounds separated on the second dimension column during the experiment period.

Fig. 2 shows the relation between the second dimension retention time and the first dimension retention time of all *n*-alkanes detected in the 6 temperature programmed experiments performed in one day. The retention times of all *n*-alkanes measured

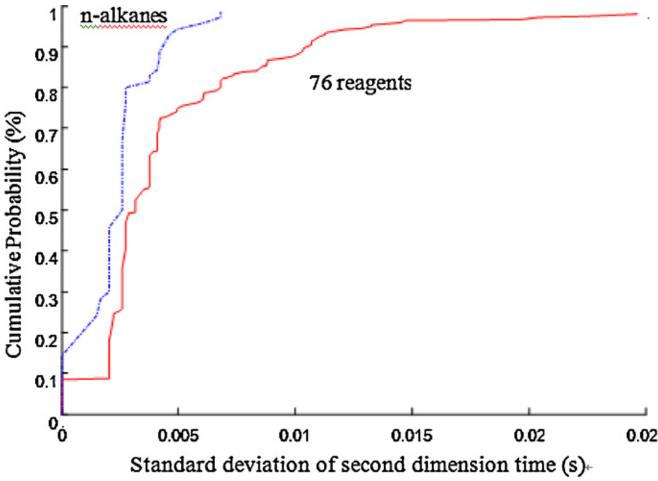

Fig. 1. Cumulative probability curves of standard deviation of the second dimension retention times of the *n*-alkanes (in blue) and compounds detected in MegaMix (in red). A mixture of *n*-alkanes and MegaMix was analyzed on a GC × GC/TOF-MS system operated in temperature programmed mode with 6 replicate injections every day for five continuous days (For interpretation of references to colour in this figure legend, the reader is referred to the web version of this article.).

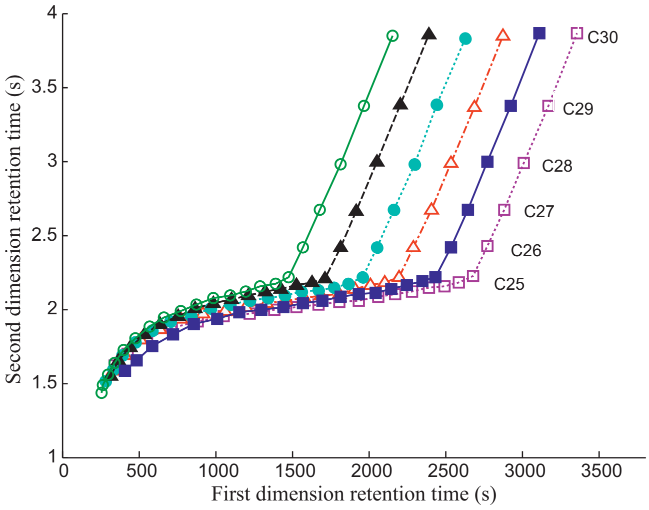

Fig. 2. Retention time map of the *n*-alkanes analyzed in temperature programmed mode with different initial first oven temperatures of 60 (□), 80 (■), 100 (△), 120 (●), 140 (▲) and 160 (○) °C, respectively. There is an inflection point in each curve, at which the temperature of the second dimension column was 285 °C and was held for 20 min.

in the same experiment are connected by a solid line. There is an inflection point in each curve, at which the temperature of the second dimension column was 285 °C and was held for 20 min. This means the second dimension column was operated in isothermal mode during the last 20 min in each of the 6 experiments. There are 6 *n*-alkanes from C_{25} to C_{30} that have the second dimension retention time values at column temperature of 285 °C. The standard deviation of the second dimension retention times of the *n*-alkanes compounds from C_{25} to C_{30} are 0.0032, 0.0038, 0.0055, 0.0069, 0.0058 and 0.0076s, respectively. It can be seen that each of the *n*-alkanes has a very similar retention time value in the second dimension column under different experiment conditions. This indicates that the second dimension retention time of a compound is mainly determined by the column temperature under the current experiment conditions, and the reproducibility of the *n*-alkanes in the isothermal experiments is also quite good.

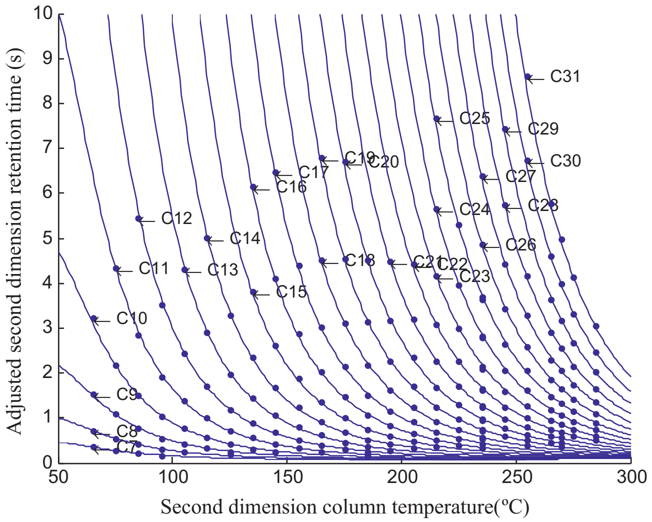

Fig. 3. The second dimension retention map of the *n*-alkanes constructed from the isothermal experimental data. The proposed $^2t_R - {}^2T_e$ function was used to fit the retention time values of the *n*-alkanes. The fitted curves are displayed as solid lines.

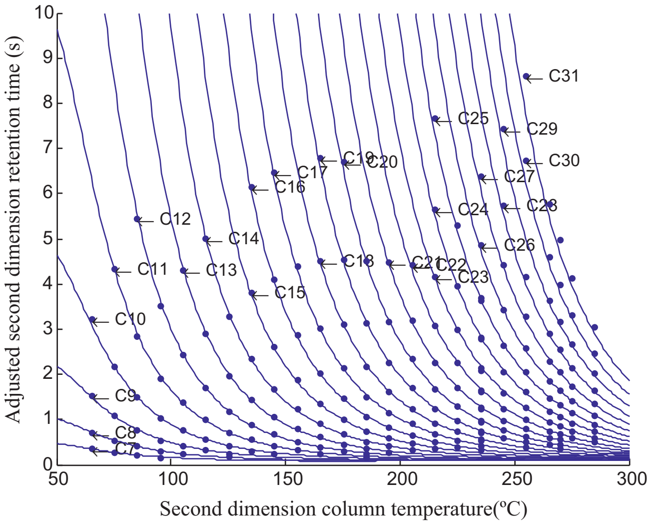

Fig. 4. The second dimension retention map constructed from the isothermal data of the *n*-alkanes from C_7 to C_{26} using the proposed empirical function. The $^2t_R - {}^2T_e$ curves for C_{27}-, C_{28}-, C_{29}-, and C_{30}-alkane were predicted based on the retention time values of *n*-alkanes from C_7 to C_{26} using the proposed $^2t_R - {}^2T_e$ fucntion.

3.2. Construction of the second dimension retention map

Fig. 3 shows the second dimension retention map constructed by applying the proposed $^2t_R - {}^2T_e$ function using the isothermal experimental data of the *n*-alkanes (C_7–C_{31}). The dots are the experimental data and the solid lines represent the curves fitted by the $^2t_R - {}^2T_e$ function. Even though the retention time of each *n*-alkane has limited coverage of the second dimension retention time, the retention time of all reference *n*-alkanes cover all of the retention time area less than 6 s in the second dimension retention time. This makes it possible to calculate the retention indices of target compounds from the corresponding retention time values without the extension of the retention space of the reference compounds.

The proposed $^2t_R - {}^2T_e$ function uses the retention time data and carbon number of all reference compounds for empirical parameter fitting. The variation introduced by each retention time data or the retention time data of an individual reference compound does not significantly affect the fitting, which results a set of evenly dis-

Table 1
The second dimension retention indices of 47 identified MegaMix compounds analyzed in 6 temperature programmed experiments with different initial temperatures of the first dimension column.

Compound name	Second dimension retention index (iu)[a]						Mean	STD
	60 °C	80 °C	100 °C	120 °C	140 °C	160 °C		
N-nitrosodimethylamine	972	986		993			984	11
Aniline	1245	1254	1256	1267			1255	9
Phenol	1204	1209	1210	1222			1212	8
Bis(2-chloroethyl) ether	1207	1212	1211	1218			1212	5
Phenol, 2-chloro-	1213	1224	1227	1242			1226	12
Benzyl Alcohol	1291	1298	1299		1301		1297	5
Phenol, 2-methyl-	1286	1292	1294	1303			1294	7
Phenol, 4-methyl-	1319	1321			1313		1318	4
Phenol, 3-methyl-	1308	1314	1332	1332			1321	12
1-Propanamine, N-nitroso-N-propyl-	1292	1297	1297	1302	1291		1296	5
Ethane, hexachloro-	1283	1290	1294	1305	1303		1295	9
Benzene, nitro-	1373	1379	1381	1390	1391		1383	8
2-Cyclohexen-1-one, 3,5,5-trimethyl-	1361	1365	1366	1372	1375	1367	1368	5
Phenol, 2-nitro-	1409	1416	1417	1425	1430	1434	1422	9
Phenol, 2,4-dimethyl-		1396	1401	1396		1394	1397	3
Methane, bis(2-chloroethoxy)-	1414	1417	1416	1420	1418	1413	1416	3
Phenol, 2,4-dichloro-	1430	1436	1438	1442	1451	1453	1442	9
Naphthalene	1482		1488	1495			1488	7
1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	1425	1428	1429	1433	1437	1438	1432	5
Phenol, 4-chloro-3-methyl-	1584	1586	1586	1588	1592	1597	1589	5
1,3-Cyclopentadiene, 1,2,3,4,5,5-hexachloro-	1578	1579	1579	1582	1587	1595	1583	7
o-Nitroaniline	1836	1837	1838	1840	1846	1856	1842	7
Benzene, 1,4-dinitro-	1851	1852	1851	1853	1858	1865	1855	5
Benzene, 1,3-dinitro-	1867	1868	1868	1870	1873	1882	1871	6
Dimethyl phthalate	1840	1840	1841	1841	1844	1850	1845	4
Benzene, 2-methyl-1,3-dinitro-	1876	1876	1876	1878	1880	1887	1879	4
Benzene, 1,2-dinitro-	1931	1932	1931	1932	1936	1946	1935	6
Acenaphthene	1879	1881	1880	1883	1887.6	1897	1885	7
m-Nitroaniline	1950	1951	1951	1953	1957.3	1966	1955	6
Dibenzofuran	1905	1907	1906	1908	1913	1921	1910	6
Benzene, 1-methyl-2,4-dinitro-	1943	1944	1943	1945	1946	1951	1945	3
Phenol, 4-nitro-				1970	1962	1968	1967	4
Fluorene	1997	1997	1998	1999	2001	2010	2000	5
Benzene, 1-chloro-4-phenoxy-	1969	1970	1970	1971	1972	1978	1972	3
Diethyl phthalate	1977	1978	1978	1977	1977	1980	1978	1
p-Nitroaniline	2138	2139	2139	2140	2144	2150	2142	4
Phenol, 2-methyl-4,6-dinitro-				2010	2011	2016	2012	3
Benzenamine, N-phenyl-	2056	2056	2055	2056	2058	2062	2057	3
Azobenzene	2023	2023	2023	2024	2026	2030	2025	3
Benzene, 1-bromo-4-phenoxy-	2111	2113	2112	2111	2113	2118	2113	3
Benzene, hexachloro-	2097	2097	2097	2097	2100	2105	2099	3
Phenol, pentachloro-					2201	2203	2202	1
Carbazole	2409	2410	2409	2409	2410	2414	2410	2
Fluoranthene	2680	2681	2680	2680	2682	2684	2681	2
Pyrene	2784	2785	2784	2783	2785	2787	2785	2
Benzyl butyl phthalate	2964	2968	2965	2962	2964	2964	2965	2
Hexanedioic acid, bis(2-ethylhexyl) ester	2660	2660	2659	2657	2657	2655	2658	2

[a] The blank means the compound was not detected during the experiment.

tributed curves. In order to quantitatively analyze the performance of the proposed $^2t_R - ^2T_e$ function, the fitted curves of the C_{n-1}-alkane and the C_{n+1}-alkane were used to predict the retention index of the C_n-alkane at different temperatures of the second dimension column by applying linear interpolation. The mean absolute deviation of the reference compound C_n-alkane was then calculated by comparing the difference between the predicted retention index value and the true retention index value at different column temperatures. The RMSE for all *n*-alkanes reference compounds is 9 iu.

3.3. The predictive capability of the proposed function

In order to assess the predictive capability of the proposed $^2t_R - ^2T_e$ function, isothermal data of the *n*-alkanes only from C_7 to C_{26} were used to get the fitted parameters for the function defined in Eq. (3). The fitted function was subsequently used to create the predicted $^2t_R - ^2T_e$ curves for the *n*-alkanes from C_{27} to C_{31}. Such an extension of the retention time space cannot be achieved using the literature-reported functions because the carbon number of the *n*-alkanes was not incorporated into these $^2t_R - ^2T_e$ functions [11,21,23,24]. Fig. 4 displays the second dimension retention map of the *n*-alkanes from C_7 to C_{31}. The dots are the isothermal experimental data. The curves of C_{27}–C_{31} *n*-alkanes were predicted by the proposed function fitted by the retention time values of the C_7–C_{26} *n*-alkanes. Compared to the curves of the *n*-alkanes from C_{27} to C_{31} in Fig. 3 that were fitted using all the isothermal data of the *n*-alkanes from C_7–C_{31}, it is obvious that the deviation of the predicted curves increased with the increase of the carbon number in an *n*-alkane, especially in the upper area of the retention map.

Fig. 5 shows the mean absolute deviation of the retention index between the predicted values and the true values (defined by the number of carbon atoms in the *n*-alkanes). The predicted values of the retention index using either the entire isothermal data set, *i.e.*, the isothermal retention times of the *n*-alkanes from C_7 to C_{31}, or the isothermal data of C_7–C_{26} *n*-alkanes, are very close to the true values with a mean deviation of less than 8 iu. It can also be noted that the mean absolute deviation is independent in the retention

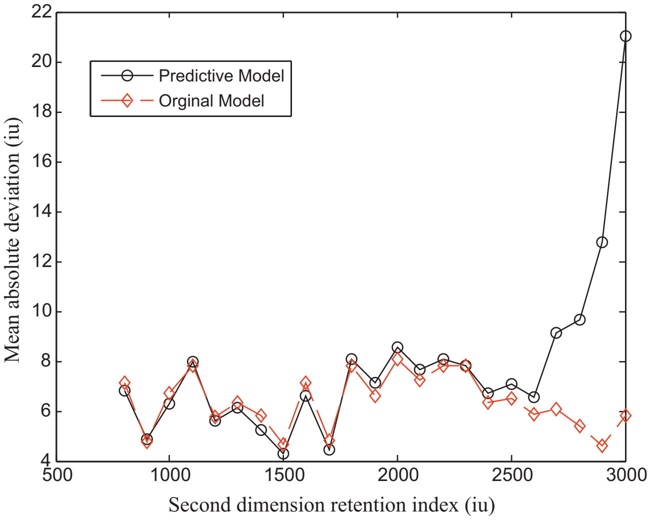

Fig. 5. The relationship of the mean absolute deviation between the predicted retention index values and the true retention index values defined the number of carbon atoms in *n*-alkanes. The retention index was converted from the retention time of *n*-alkanes analyzed in the isothermal mode. The red curve shows the mean absolute deviation obtained using all *n*-alkanes from C_7 to C_{31} to fit the $^2t_R - {}^2T_e$ function. The black curve is the mean absolute deviation using the *n*-alkanes from C_7 to C_{26} to fit the parameters in the $^2t_R - {}^2T_e$ function. The $^2t_R - {}^2T_e$ function with the fitted empirical parameters was then used to predict the column temperature–retention time curves for the *n*-alkanes from C_{27} to C_{31}. (For interpretation of references to colour in this figure legend, the reader is referred to the web version of this article.)

index value. For the predictive analysis of extending the carbon number in the *n*-alkanes reference compounds, the mean absolute deviation between the predicted retention index values and the true values increases from 9, 10, 13, up to 21 iu for the C_{27}-, C_{28}-, C_{29}-, and C_{30}-alkanes, respectively. This indicates that the predictive capability of the proposed $^2t_R - {}^2T_e$ function decreases with the extension of the prediction area, *i.e.*, the increase of the carbon number of the *n*-alkanes. But for C_{27}–C_{28} *n*-alkanes, the mean absolute deviation is still less than 10 iu. For this reason, the proposed $^2t_R - {}^2T_e$ function can be used to calculate the retention indices of compounds whose retention times fall outside of the experimental retention time space with limited confidence. The extension of the $^2t_R - {}^2T_e$ function for two carbons in the *n*-alkanes reference compounds can introduce a variation of less than 10 iu.

3.4. Analysis of a mixture of compound standards

MegaMix compounds mixed with the *n*-alkanes were analyzed on the GC × GC/TOF-MS system in temperature programmed mode with 6 different initial temperatures. After data processing, 47 compounds were identified from the MegaMix as listed in Table 1, of which 7 were isomeric compounds. Pyridine is one of the 76 compounds in the MegaMix that was also identified from the experiment data. However, it was excluded from the analysis because it is one of the carry-over compounds frequently detected in our GC × GC/TOF-MS system.

The proposed $^2t_R - {}^2T_e$ function was used to convert the retention time values of the identified MegaMix compounds in the second dimension column into the retention index values. The parameters in each function were obtained during the process of constructing the second dimension retention map from the isothermal retention time data of the *n*-alkanes reference compounds (Fig. 3). Table 1 lists the second dimension retention index values of the 47 compounds identified from MegaMix in the 6 temperature programmed experiments. The values of the retention index were calculated from the isothermal *n*-alkanes data using the proposed function. The standard deviation of the second dimension retention index ranges from 1 to 12 iu with a mean standard deviation of 5 iu, while the standard deviation of the second dimension retention time fluctuates from 0 to 0.5 s (data were not shown). Of the 47 identified compounds, only three compounds have the standard deviations of the second dimension retention index larger than 10 iu. These results show that the compound retention index values in the second dimension column of a GC × GC/TOF-MS system can be obtained by converting the retention time values of these compounds in the second dimension column using the proposed $^2t_R - {}^2T_e$ function, *i.e.*, the second dimension retention mapping.

4. Conclusion

In this study, the homologue *n*-alkanes (C_7–C_{31}) were chosen as the reference compounds to build a second dimension retention map for GC × GC/TOF-MS. Compared to the existing literature reports, the new second dimension retention map covers all the 6 s retention time area in the second dimension retention time space, which makes it possible to calculate the retention index values of the majority of compounds directly from linear interpolation of two consecutive *n*-alkanes. An empirical $^2t_R - {}^2T_e$ function was developed to incorporate the carbon number of the *n*-alkanes and the column temperature of the second dimension column. The proposed function is able to extend the second dimension retention time beyond the reference *n*-alkanes by increasing the carbon number. The predictive analysis demonstrated that the extension of the proposed function for two carbons introduces deviation less than 10 iu.

Forty-seven compounds were identified from the MegaMix containing 76 compound standards on GC × GC/TOF-MS. Using the proposed $^2t_R - {}^2T_e$ function, the standard deviations of the converted retention index values of the 47 identified compounds fluctuated from 1 to 12 iu with a mean standard deviation of 5 iu. This demonstrates that the proposed function can be applied to analyze complex samples for the calculation of retention index values of each compound in the second dimension column with a reasonable accuracy.

It should be noted that the proposed $^2t_R - {}^2T_e$ function is empirical, and was optimized using the data of the 24 isothermal experiments reported in this work. The stability and accuracy of this function on different column configurations of GC × GC system involve other detailed investigations, including its applications to the analysis of various complex samples. In the future, it is possible to build a two dimension retention index database for compounds analyzed on the GC × GC/TOF-MS system. The two dimension retention index values can then be used to assist the spectrum library matching-based compound identification.

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