

Short communication

Method for reducing the ambiguity of comprehensive two-dimensional chromatography retention times

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Abstract

Comprehensive two-dimensional chromatography generates a two-dimensional chromatogram from a one-dimensional signal array. This process can only be done unambiguously when the range of secondary retention times is less than the modulation period. However, complex samples often produce wider ranges of secondary retention times. Peaks with retention times that exceed the modulation period are said to be “wrapped-around”. A simple algorithm has been developed that determines absolute retention times when wrap-around occurs. A sample is first analyzed under standard modulation conditions and then re-analyzed with a modulation period that is increased by an integer fraction of the original modulation period. Retention shifts along the secondary axis are used to determine absolute retention times. A theoretical analysis has been performed to optimize the implementation conditions and characterize the technique limitations. The efficacy of this algorithm has been tested through a series of isothermal GC × GC separations. This method has been found to be particularly useful during the initial stages of method development.

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1. Introduction

Assigning peak retention times is an important part of chromatographic analysis. This process is straightforward with conventional one-dimensional chromatography as the injection time is known and the elution time is measured. Comprehensive two-dimensional chromatography attempts to assign a pair of retention times to each component. Four quantities are needed to do this exactly: the primary injection time, the primary elution time, the secondary injection time, and the secondary elution time. However, only two of these quantities are determined: the primary injection time and the secondary elution time. The nature of the modulation process constrains the secondary injection time to an integer multiple of the modulation period and the primary elution time to the modulation cycle immediately preceding secondary injection. However, a degree of ambiguity still exists. For instance,

consider the chromatogram shown in Fig. 1A of a component that is sampled with a modulation period $P_M = 1.5$ s. Peaks are observed 0.80 s after the most recent secondary injection. A list of the possible secondary retention times can be generated using the following equation

$${}^2t_R = nP_M + {}^2t_{rel} \quad (1)$$

where 2t_R is the absolute secondary retention time, n is an integer that is greater than or equal to zero, and ${}^2t_{rel}$ is the relative secondary retention time (i.e. the difference between the arrival time of the peak and the time of the immediately preceding secondary injection). The variable n , called the wrap-around factor, represents the number of full modulation cycles that occur while a peak travels through the secondary column. Using Eq. (1) with $P_M = 1.5$ s and ${}^2t_{rel} = 0.8$ s, a list of possible absolute secondary retention times includes 0.8, 2.3, 3.8, and 5.3 s.

It is impossible to determine the correct secondary retention time, and hence the correct primary retention time, without additional information. To date, a variety of empirical

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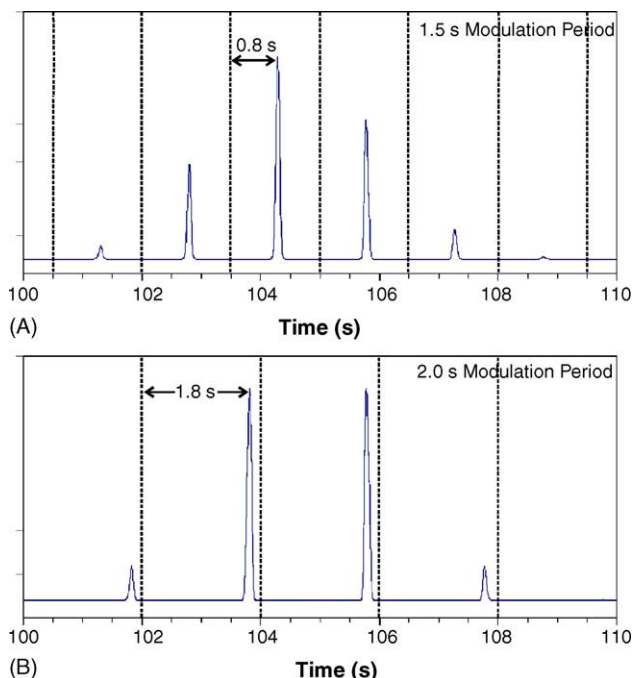


Fig. 1. The 1D signal resulting from the analysis of a component with two different modulation periods: (A) $P_{M,A} = 1.5$ s and (B) $P_{M,B} = 2.0$ s. The dotted lines represent the secondary injection events. The peaks in A have a relative secondary retention time ${}^2t_{rel,A} = 0.8$ s, whereas the peaks in B have ${}^2t_{rel,B} = 1.8$ s.

and theoretical approaches have been employed to gain such information including retention time prediction [1], peak width analysis [2], the use of large modulation periods [3], and the use of continuously changing modulation periods [4]. This article describes a systematic approach for determining absolute secondary retention times. The algorithm is based largely on a protocol that was implemented nearly 30 years ago for automatic range estimation in multiple pulse radar [5].

2. Algorithm

- (1) Analyze the sample with the desired modulation period, $P_{M,A}$. The value of $P_{M,A}$ is often selected to be small enough to maintain primary resolution and large enough to generate additional secondary resolution.
- (2) Repeat the analysis with a modulation period $P_{M,B}$ that exceeds $P_{M,A}$ by the increment δ :

$$P_{M,B} = P_{M,A} + \delta$$

The value of δ should be an integer fraction of the original modulation period. That is, $\delta = P_{M,A}/c$ where c is an integer that is greater than 0.

- (3) Determine the relative secondary retention times ${}^2t_{rel}$ from both chromatograms. This is most conveniently done by plotting the two-dimensional chromatogram obtained in Step 1 with a secondary axis scaled from 0 to $P_{M,A}$ and by plotting the two-dimensional chromatogram

obtained in Step 2 with a secondary axis scaled from 0 to $P_{M,B}$.

- (4) Calculate the displacement factor d with

$$d = \text{Int} \left(\frac{{}^2t_{rel,A} - {}^2t_{rel,B}}{\delta} \right)$$

Note that the “Int” operator represents rounding to the nearest integer and not simply truncating the digits to the right of the decimal point. The argument of the “Int” operator should be close to an integer value prior to rounding. Large deviations from integer values indicate a change in the absolute secondary retention time.

- (5) Use the following rule to determine the wrap-around factors for both runs

if $d \geq 0$ then

$$n_A = d$$

$$n_B = d$$

if $d < 0$ then

$$n_A = c + d + 1$$

$$n_B = c + d$$

- (6) Calculate the absolute retention times using

$${}^2t_{R,A} = n_A P_{M,A} + {}^2t_{rel,A}$$

$${}^2t_{R,B} = n_B P_{M,B} + {}^2t_{rel,B}$$

3. Example application

Fig. 1 shows two chromatograms of the same component. Chromatogram A has $P_{M,A} = 1.5$ s and ${}^2t_{rel,A} = 0.8$ s. Chromatogram B has $P_{M,B} = 2.0$ s and ${}^2t_{rel,B} = 1.8$ s. The modulation period was increased when going from Run A to Run B by 0.5 s so $\delta = 0.5$ s and $c = 1.5/0.5 = 3$. The value of d is calculated by

$$d = \text{Int} \left(\frac{0.8 \text{ s} - 1.8 \text{ s}}{0.5 \text{ s}} \right) = \text{Int}(-2.0) = -2$$

The value of d is less than 0 so n_A and n_B are calculated by

$$n_A = 3 - 2 + 1 = 2$$

$$n_B = 3 - 2 = 1$$

The values of ${}^2t_{R,A}$ and ${}^2t_{R,B}$ are determined by

$${}^2t_{R,A} = 2 \times 1.5 \text{ s} + 0.8 \text{ s} = 3.8 \text{ s}$$

$${}^2t_{R,B} = 1 \times 2.0 \text{ s} + 1.8 \text{ s} = 3.8 \text{ s}$$

4. Algorithm alterations and limitations

There are two important points that should be considered when implementing the algorithm. First, alternate forms of the algorithm are possible. For instance, Steps 4–6 can be

replaced by a “trial and error” approach where Eq. (1) is used to generate a list of possible retention times for Run A and a list for Run B. The lists are then compared and the true retention time will be represented in both lists (or at least there should be a retention time in List A that is very similar to a retention time in List B). For example, the chromatogram in Fig. 1A has a list of retention times of 0.8, 2.3, 3.8, and 5.3 s and the chromatogram in Fig. 1B has a list of retention times of 1.8, 3.8, and 5.8 s. Only the secondary retention time of 3.8 s appears in both lists, so it is chosen as the absolute secondary retention time. While the “trial and error” approach is more intuitive than Steps 4–6, it is more difficult to implement with a spreadsheet program.

A second important point is that a unique retention time cannot be determined if the secondary retention time has the possibility of exceeding ${}^2t_{\max}$ given by

$${}^2t_{\max} = (c + 1)P_{M,A} \quad (2)$$

If secondary retention times greater than ${}^2t_{\max}$ are possible, then a series of values separated by ${}^2t_{\max}$ must be considered. For example, Eq. (2) demonstrates that the analysis shown in Fig. 1 has a ${}^2t_{\max}$ of 6.0 s. A unique secondary retention time can be determined provided the absolute retention time is less than 6.0 s. If greater retention times are possible, then a series of values separated by 6.0 s must be

considered; i.e., 3.8, 9.8, 15.8 s, etc. This conclusion can be verified by extending the lists of possible secondary retention times out to much larger values.

The presence of a retention time limit makes the proper selection of the modulation periods critical. Eq. (2) shows that large values of c will lead to a large ${}^2t_{\max}$; however, large values of c also lead to small retention time shifts. So it is advantageous to first estimate the range of possible retention times and then choose the minimum value of c that covers that range.

5. Experimental validation

A mixture of C_5 – C_7 *n*-alkanes, C_3 – C_5 2-ketones, and C_3 – C_5 1-alcohols was analyzed with GC \times GC. The experimental platform has been described previously in detail [6]. Isothermal conditions (50 °C) were employed to generate a variety of wrap-around factors. The two-dimensional chromatograms obtained with $P_{M,A} = 1.75$ s and $P_{M,B} = 2.0$ s are shown in Fig. 2. The alcohol peaks were particularly broad along the secondary dimension due to high levels of retention on the DB-Wax secondary column. Analysis with $P_{M,A} = 1.75$ s, $P_{M,B} = 2.0$ s, leads to $\delta = 0.25$ s and $c = (1.75 \text{ s}/0.25 \text{ s}) = 7$. It can be shown using Eq. (2), that this

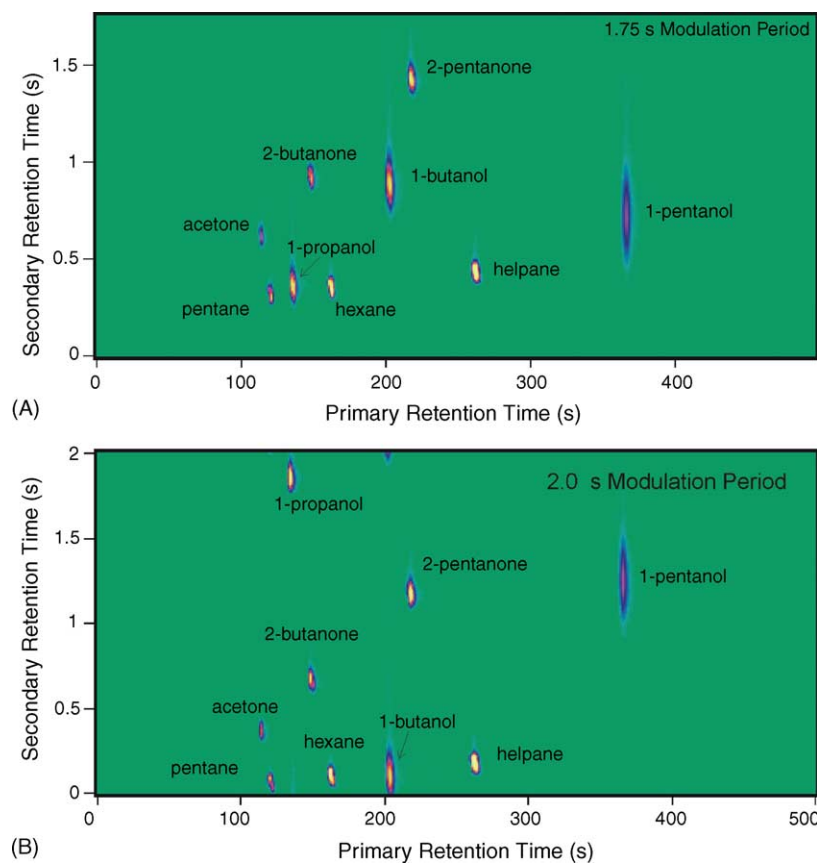


Fig. 2. Two GC \times GC chromatograms of a mixture of *n*-alkanes, 2-ketones, and 1-alcohols. The mixture was analyzed with two different modulation periods: (A) 1.75 s and (B) 2.00 s. Isothermal conditions at 50 °C were employed with a 15 m DB-1 primary column and a 5 m DB-Wax secondary column.

Table 1
Algorithm results for the data shown in Fig. 2

Compound	${}^2t_{\text{rel,A}}$	${}^2t_{\text{rel,B}}$	$({}^2t_{\text{rel,A}} - {}^2t_{\text{rel,B}})/\delta$	d	n_A	${}^2t_{\text{R,A}}$	n_B	${}^2t_{\text{R,B}}$
Acetone	0.615	0.370	0.98	1	1	2.365	1	2.370
Pentane	0.300	0.075	0.90	1	1	2.050	1	2.075
1-Propanol	0.370	1.855	-5.94	-6	2	3.870	1	3.855
2-Butanone	0.910	0.675	0.94	1	1	2.660	1	2.675
Hexane	0.360	0.115	0.98	1	1	2.110	1	2.115
1-Butanol	0.880	0.110	3.08	3	3	6.130	3	6.110
2-Pentanone	1.430	1.170	1.04	1	1	3.180	1	3.170
Heptane	0.435	0.190	0.98	1	1	2.185	1	2.190
1-Pentanol	0.725	1.250	-2.10	-2	6	11.225	5	11.250

All retention times have units of seconds.

set of conditions provides unique results for secondary retention times that are less than 14 s.

The relative retention times obtained from the chromatograms are listed in Table 1. Algorithm Steps 4–6 were performed with Microsoft Excel. The results from each step are listed in Table 1. It is important to note that the values that were rounded to determine the displacement factor d , listed under “ $({}^2t_{\text{rel,A}} - {}^2t_{\text{rel,B}})/d$ ” in Table 1, are all within 0.1 of integer values. In addition, the final estimates of ${}^2t_{\text{R,A}}$ and ${}^2t_{\text{R,B}}$ all agree to within 25 ms. Both of these observations indicate that the absolute retention times do not change significantly between Run A and Run B.

One-dimensional chromatography was used to verify the accuracy of the absolute secondary retention times. The nine-component test mixture was injected into a standard gas chromatograph fitted with a 10 m DB-Wax column. The mixture was separated at 50 °C. Retention factors were determined for all nine components using methane to measure the dead time. Retention factors were also determined from the calculated secondary retention times and the experimentally determined secondary retention time of methane (${}^2t_{\text{R}} = 2.050$ s). The GC \times GC retention factors were plotted as a function of the 1D retention factors. A linear regression of the data had a slope of 1.02, an intercept of 0.003, and an R^2 value greater than 0.9999. The excellent agreement between the two data sets confirms that the algorithm produces accurate secondary retention times for a wide range of wrap-around values. Equally accurate results were found when the algorithm was used with different modulation periods, oven temperatures, and secondary columns.

6. Conclusions

A simple experimental algorithm has been developed that allows absolute secondary retention times to be determined with much greater certainty. Accurate values of absolute

secondary retention times are vital for proper compound identification and also helpful in optimizing experimental conditions. Once an absolute retention time is known, the relative retention time can be predicted for any modulation period using

$${}^2t_{\text{rel}} = \text{Mod}({}^2t_{\text{R}}, P_M)$$

where Mod is the modulus operator that returns the remainder of ${}^2t_{\text{R}}/P_M$.

The validity of the algorithm has been confirmed with a series of experimental measurements. The main strength of this method is that small changes in modulation period can be used to determine the retention times of peaks having high wrap-around factors. For instance, the experimental study showed how a 14% increase in modulation period allowed the retention time of a peak that was wrapped-around six times to be determined accurately. This method could be adopted easily as a routine technique for verifying secondary retention times.

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