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Measurement of retention in comprehensive two-dimensional gas chromatography using flow modulation with methane dopant

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ABSTRACT

Flow modulation of methane-doped carrier gas is used to visualize the second dimension hold-up time in GC × GC continuously throughout the run. This provides an internal reference of hold-up time and presents a straightforward means of examining retention in each dimension of GC × GC. Retention factors on similar and dissimilar column pairs are examined. Stationary phase bleed is shown to be retained by the second dimension column.

1. Introduction

Invented in the early 1990s [1], comprehensive two-dimensional (2D) GC is a relatively new technique. Many aspects regarding optimal setup and operation of GC × GC are still not well understood. Investigations of retention phenomena in the second dimension are limited by the ability to measure hold-up time accurately throughout the run as temperature changes. Instrumental imprecision or inaccuracy in the timing of actual transfer to the second dimension column (the "time 0" of the second dimension time axis) can stem from electrical, mechanical, pneumatic and/or thermal factors (in the case of thermal modulators). This can lead to errors in calculation of hold-up times and second dimension retention factors and makes it difficult to compare experimental results with theoretical predictions. This is a contributing factor to why second dimension retention times in GC × GC chromatograms are typically presented with arbitrary, often unstated shifts (offsets) in the second dimension axis.

To gain insight into chromatographic retention in GC × GC, it would be highly beneficial, therefore, to be able to have a continuous measure of second dimension hold-up time. This can be accomplished by adding a small amount of methane to the first dimension carrier gas and by using a flow modulator (flow modulation).

Two types of modulation devices—thermal (with or without cryogenic focusing) [1,2] and fluidic [3,4]—are known in contemporary GC × GC. Each type has its own advantages and disadvantages. What is important for this report is that even though thermal modulation of methane has been considered to be possible [5], the practical difficulties of doing so are known [6] and there are no published demonstrations of effective thermal modulation of methane over a wide oven temperature range. Flow modulators do not have this limitation. Flow modulators work by isolating primary column effluent for most of the cycle time period (accumulation), and then transferring the isolated portion into the secondary column (transfer) quickly by flow switching. Thermal processes are not involved, so flow modulators can work well with highly volatile analytes.

Methane is a favored gas for determining hold-up times in columns because it is detected by most GC and MS detectors and is not retained at standard operating temperatures. The use of methane for measurement of second dimension hold-up time in GC × GC is known [6–10]. However, measurements were not continuous during the runs in most cases. LaClair et al. [7] used a syringe pump to continuously add methane to the primary column carrier gas which was then effectively modulated with a flow modulator yielding regularly shaped peaks. The technique was used to examine and tune modulation parameters. Use of modulated methane for measurement of chromatographic parameters (hold-up time, retention times, retention factors, etc.) was not explored.

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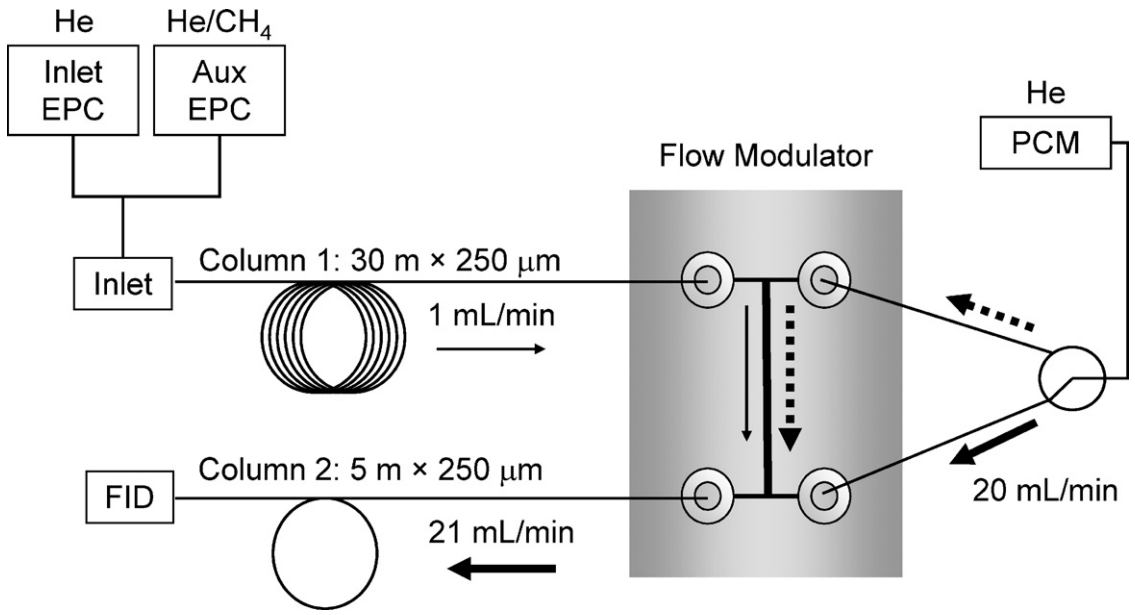

Fig. 1. Configuration used for methane-doped carrier gas. Methane is doped into the primary column carrier gas flow ahead of the inlet. Flow exits the primary column and enters the flow modulator and fills the sample channel at low flow rate (1 mL/min). Meanwhile, a high flow of methane-free carrier gas feeds the secondary column (21 mL/min). During sample transfer, a solenoid is briefly switched to direct the high flow through the sample channel to sweep it to the secondary column. Compression of the peak in time occurs due to the ratio and of secondary column flow to primary (approximately 20:1).

Fig. 1 shows the basic concept of flow modulation with methane-doped carrier gas used for this study. As a direct result of flow modulation, the continuous background of methane emanating from the primary column appears as an unretained peak in each cycle from the secondary column.

The continuous presence of a peak marking the actual hold-up time in each second dimension slice makes it possible to visualize the second dimension hold-up time in GC × GC chromatograms and provides an internal reference for flow changes during the run. Having a marker for each second dimension hold-up time, allows one to more accurately investigate retention phenomena and to test theory. This approach, for example, clearly demonstrates that, contrary to common impression, stationary phase bleed can be retained by the second dimension column.

Another benefit is that methane greatly helps visualize hold-up time trends as the analytical run progresses. By having a continuous hold-up time marker one can visualize retention trends and anomalies better. For example, one can see the trend of change in hold-up time as a function of run time. Having an internal reference of hold-up time, ${}^{2}t_{M}$, would also allow determination of retention factors ${}^{2}k$ throughout the analysis.

A further benefit of using methane-doped carrier gas is that it can help in system validation, diagnosing problems and tuning instrumental parameters [7]. Having a peak at ${}^{2}t_{M}$ can speed the modulator tuning process considerably compared to the usual process of using a retained peak in flow modulated systems. In addition, the width and shape of the methane peak are good indicators of initial band width and shape for all components in the cycle. Further, one can compare the retention time of the methane peak to the theoretical one to highlight potential system issues or column dimension inaccuracies.

Not all potential advantages of having a continuous ${}^{2}t_{M}$ line in GC × GC chromatograms were explored in this report. Our focus was only on using the line for measurement of the secondary column hold-up and retention times as well as for measurement of retention factors in second dimension column. Improvement of separation was not our goal. In fact, quite contrary to that, our method of measurement of retention factors in a primary column required using the same stationary phase type and phase ratio in both columns—an approach that was unfavorable for the peak separation in the secondary column. Poor peak separation in the secondary column, therefore, was a consequence of better visualization of the retention trends.

2. Methods and materials

The basic configuration of the GC system is illustrated in Fig. 1. An Agilent 7890A GC was used with Capillary Flow Technology Flow Modulator accessory (Agilent 3486A) with associated PCM (pressure control module), split/splitless inlet and FID. A 200 ppm methane in He (Scott Specialty Gases, Plumsteadville, PA) gas mixture was plumbed to an Aux EPC channel with low-flow internal restrictor (high restriction frit, G3430-80063). The output line of Aux EPC was teed with the carrier gas line going to the inlet. Pressure of the Aux channel was adjusted to yield a target FID baseline offset (measured with no modulation). The target offset was such that the modulated methane peak was of similar height to mid-level peaks in samples. The corresponding EPC pressure ended up equal to inlet pressure plus approximately 23 psig. Any concentration of methane, including pure methane, can be used with this configuration. By adjusting the combination of EPC internal restrictor and pressure, the target level of methane in carrier gas can be achieved.

A reference mixture (Agilent Technologies, Inc. part 5080-8768) of n-alkanes from pentane to octadecane (minus n-C_{13}) at 2–20% each was used to demonstrate retention behavior. A diesel fuel sample from a local gas station was used (no dilution) to illustrate compound grouping. 0.1 μL injections at 50:1 split were done using fast autoinjection (Agilent 7683B ALS) into the S/Sl inlet at 250 °C.

The primary column was an apolar Agilent 30 m × 250 μm × 0.25 μm HP-1MS (19091S-933). The flow rate was 1.0 mL/min (constant flow mode) of He + CH_4 (${}^{1}t_{M}$ = 4.364 min). The secondary column was either an apolar 5 m × 250 μm × 0.25 μm HP-1MS (cut from a longer column) or a polar 5 m × 250 μm × 0.25 μm Innowax column (cut from a longer column). Each secondary column was operated with pure He at 21 mL/min in constant flow mode.

The temperature program for apolar/apolar column set was 50 °C (1 min) → 280 °C (5 min) at 2, 4, or 8 °C/min. The temperature

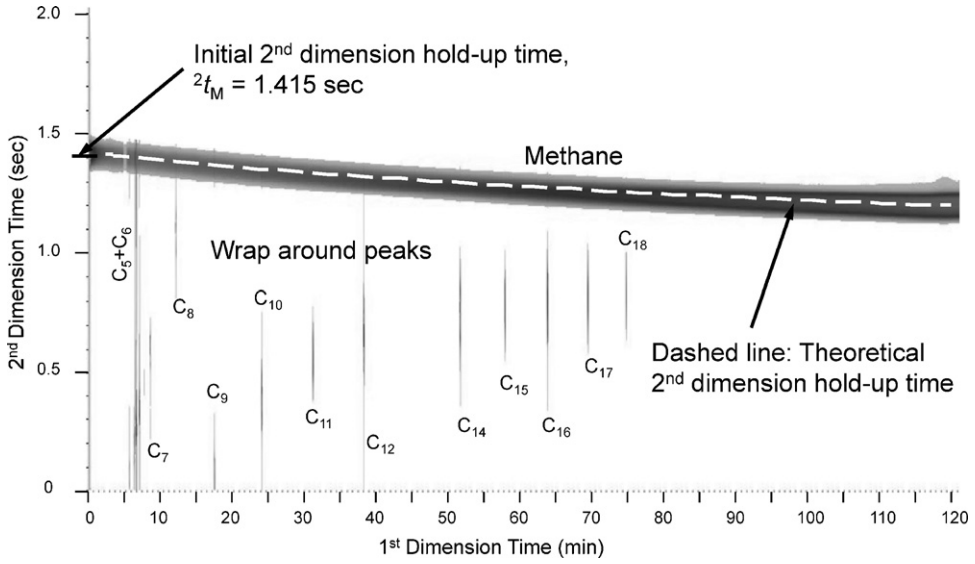

Fig. 2. 2D plot of data from analysis of an n-alkane mix on apolar/apolar HP-1MS column combination at 2 °C/min heating rate (close to optimal 10 °C/^1t_M). Data was processed with a 3.0 s (2 cycles) time second dimension range and then zoomed to 0–2 s range with no offset. The dark line is from the modulated methane and marks hold-up time. The dashed line represents the theoretical hold-up time for the 5.0 m × 250 μm i.d. second dimension column run in constant flow mode. One can see peaks that wrap-around to subsequent periods being displayed below the methane line.

program for apolar/polar column set was 50 °C (1 min) → 260 °C (20 min) at 2, 4, or 8 °C/min.

The FID was operated at 275 °C with 45 mL/min H_2, 450 mL/min instrument air, and 5 mL/min N_2 makeup gas.

A modulation period (cycle time) of 1.500 s (after 0.1 min initial delay; exactly 4 cycle times) was used. Each modulation period consisted of 1.397 s of accumulation time and 0.103 s of transfer time.

GC Image software (Zoex Corp., Pasadena, TX, USA) was used for plotting data. The software allows one to enter cycle time (the second dimension time axis), offset (a fixed shift in the second dimension time axis), cycle time adjustment (incremental offset for each cycle time) and scaling parameters (color, intensity threshold, range, zoom, etc.).

3. Results and discussion

To investigate general retention phenomena and the utility of having a continuous measure of second dimension hold-up time, initial experiments were conducted using a secondary column with the same i.d., stationary phase type (apolar–apolar), and film thickness as the primary column (HP-1MS; polydimethyl silicone). The reference standard of n-alkanes was initially analyzed at a heating rate of 2 °C/min (close to optimal 10 °C/^1t_M [11,12]). Initial results were plotted with the second dimension axis equal to twice the sampling period and then zoomed to a second dimension time range from 0 to 2 s as shown in Fig. 2. Data in Fig. 2 were plotted with no offset or cycle time adjustment. The initial delay time before onset of the modulation was 0.1 min (6 s, 4 exact cycles).

3.1. Hold-up time, offsets, sampling period stability

The first thing to notice in Fig. 2 is the considerable wrap-around (retention of solutes into subsequent cycles) [13]. The second thing to notice is the line generated by the modulated methane. It overlaps with the theoretical line for ^2t_M calculated with the actual column dimensions, carrier gas type, and run time oven temperatures. Calculations predicted that the hold-up time of the second column should be 1.415 s at the initial oven temperature (50 °C), which agrees quite well with the observed retention time. Not only that, but the methane line overlaps with theoretically predicted second dimension retention times for the entire analysis. These observations together with known accuracy of oven temperature allow one to conclude that the timing of the modulation is accurate throughout the entire analysis.

Another observation is that the hold-up time is close to the sampling period. That means that retained peaks will elute in at least the next cycle (i.e., they all will be "wrapped around" at least once). That is supported by the presence of later-eluting peaks that appear below the methane line—the plotting software cannot determine from which cycle a peak emanated.

Finally, one can easily see the gradual decline in ^2t_M as the run progresses and temperature increases. This is as expected for a system in constant flow mode. This mode maintains constant mass flow causing gradually increasing average velocity of the carrier gas and, therefore, gradually declining hold-up time. If constant pressure mode were used, the line would increase (hold-up time increasing) with increasing oven temperature. This clearly illustrates the advantage of using modulated methane throughout the run to visualize the actual trend of hold-up time change for any mode of pressure/flow control.

3.2. Retention factors

Measurement of the elution retention factors (the retention factor at the moment of elution) of chromatographic peaks in one-dimensional temperature-programmed analysis is not a simple task. One can significantly simplify the measurement by serially connecting a secondary column of the same stationary phase and phase ratio, after the primary column in the same oven, and by running the combination as a temperature-programmed GC × GC analysis (illustrated in Fig. 1).

The typical second dimension analysis time is so short, relative to the overall time of the temperature ramp, that the temperature of each second dimension cycle can be considered isothermal (at the examined heating rates), with the temperature equal to the elution temperature of solutes from the first dimension column. As a result, each component re-injected from the primary column into the secondary column migrates through the secondary column with a retention factor equal to the retention factor with which that component eluted from the primary column. In other words, at any

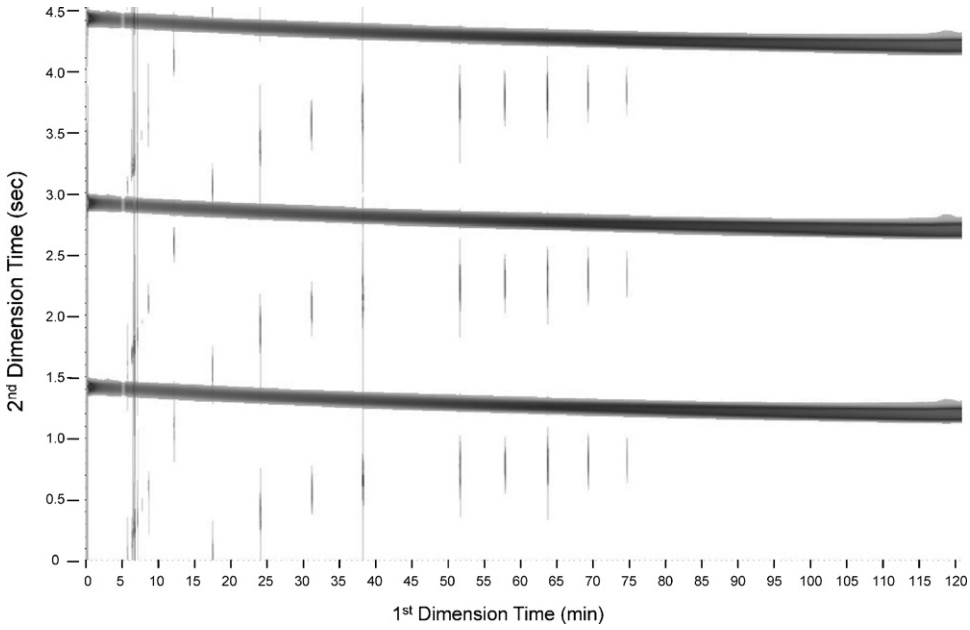

Fig. 3. The same n-alkane data as in Fig. 2 plotted with the second axis time scale equal to three cycles (4.5 s). As a consequence of data processing and plotting peaks repeat in 1.5 s segments on the plot, making it more difficult to visualize retention behavior.

given time, retention factors in the secondary column are equal to elution retention factors from the primary column. This is a convenient and effective means (actually, the only way known to us) of measuring elution retention factors in temperature-programmed GC.

In the remaining figures, a multiple of the actual cycle time was used to expand the plotted time axis of the second dimension further. The resulting plots show reflections (repetitions) in each multiple of the actual cycle time, as illustrated in Fig. 3 where the data were replotted with a 4.5 s axis (3 cycle times). In order to visualize the retention parameters of n-alkanes more clearly, peak reflections and methane line multiples were graphically removed, reference lines representing multiples of ${}^2t_M$ and integer values of ${}^2k$ were added. The result of cleaning Fig. 3 is shown in Fig. 4. The process of cleaning up the 2D chromatograms in this way would be complicated if not impossible to automate, especially for samples of unknown composition and/or retention characteristics.

One can see in Fig. 4 that for nearly the first 60 °C or so increase in the column temperature, elution retention factors of n-alkanes rapidly increase to the level of approximately ${}^2k = 2$ whereafter the rate of increase in ${}^2k$ significantly declines.

Fig. 5 repeats the above experiments using diesel fuel as a sample, at heating rates of 2, 4 and 8 °C/min (10 °C/t_M, 20 °C/t_M, and 40 °C/t_M).

The chromatograms shown in Fig. 5 (the apolar–apolar column configuration) show several common features regarding

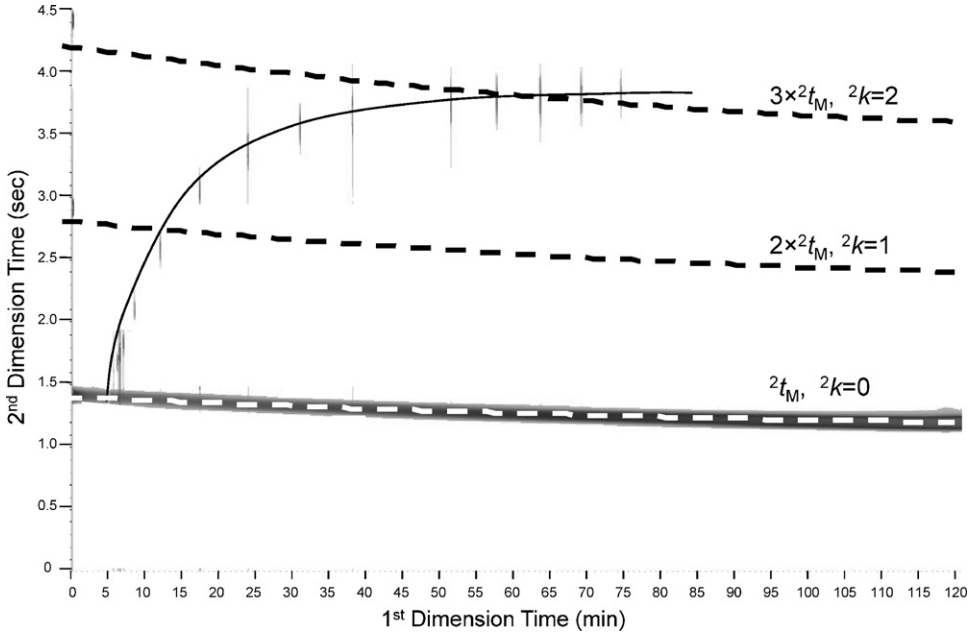

Fig. 4. The same plot as Fig. 3, only cleaned up for better visualization of retention phenomena. Replicate methane lines and reflections of analyte peaks from wrap-around were hidden graphically by overlaying white shapes. Dark dashed lines multiples of hold-up time ($2 \times {}^2t_M$, ${}^2k = 1$) and at ($3 \times {}^2t_M$, ${}^2k = 2$) were calculated and added to the plot for reference. A solid line intersecting n-alkane peak maxima was also added to help show the retention trend.

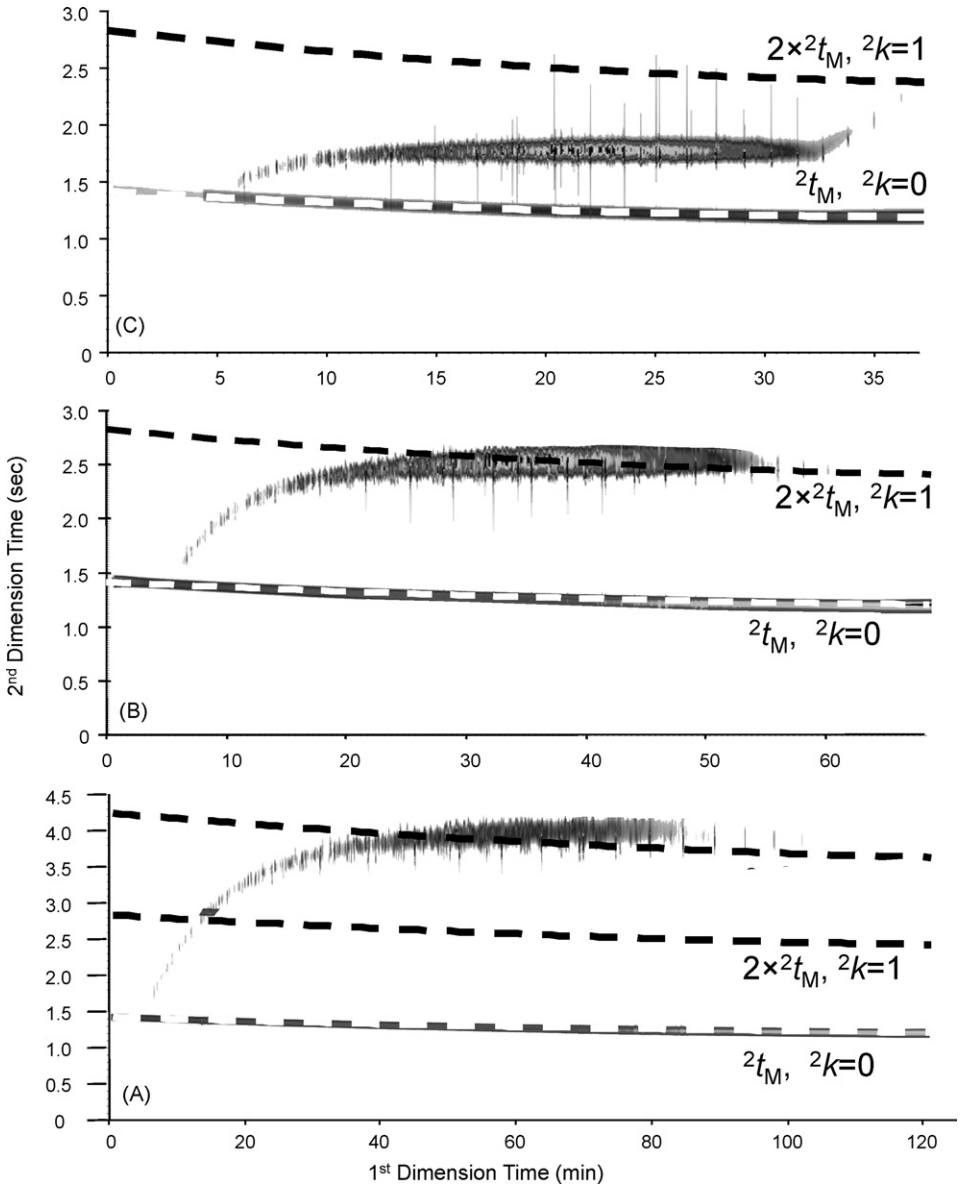

Fig. 5. Comparison of GC × GC plots of diesel sample analyzed on apolar–apolar column set of Fig. 2. (A) 2 °C/min oven ramp rate (10 °C/t_M), (B) 4 °C/min oven ramp rate (20 °C/t_M), and (C) 8 °C/min oven ramp rate (40 °C/t_M).

peak retention. There is almost no separation along the second dimension. This suggests that, as predicted by theory [14], (a) all compounds (polar and apolar) simultaneously eluting from the primary column elute with almost the same elution retention factors. Also, as predicted by the same theory, (b) during the first 60 °C or so of the heating ramp, elution retention factors rapidly change from zero to a gradually increasing plateau, and (c) an increase in the heating rate reduces elution retention factors. In the examples in Fig. 5, elution retention factors (^1k_R) decline from a high of approximately $^1k_R = 2.1$ at 2 °C/min (about 10 °C/^1t_M) to approximately $^1k_R = 1$ at 4 °C/min (20 °C/t_M) to approximately $^1k_R = 0.4$ at 8 °C/min (40 °C/t_M).

3.3. Dimensions of secondary column

The secondary column was replaced by a nominal 5 m length of polar (Innowax) column, yielding a more typical column combination for GC × GC. The alkane and diesel samples were again analyzed on this set along with continuously modulated methane to mark ^2t_M.

The observed ^2t_M measured by modulated methane was compared with theoretically predicted ^2t_M. Fig. 6 shows that, at any first dimension time (^1t), the observed ^2t_M is about 17% higher than theoretical ^2t_M (1.65 s instead of theoretical 1.415 s at initial temperature). This deviation of actual from predicted hold-up time would have been missed without the presence of the methane ^2t_M marker, leading to errors in calculation of retention factors. The difference between actual and predicted ^2t_M can be caused by several factors. Inaccurate oven temperature and unknown second dimension time offsets related to system hardware were ruled out based on the results from the apolar–apolar configuration (Fig. 2) wherein observation matched prediction across the full oven temperature ramp.

The fact that the ratio of experimental and theoretical ^2t_M does not change during the analysis suggests that the likely cause of the difference in experimental and theoretical ^2t_M is caused by the difference between nominal and actual dimensions of the secondary

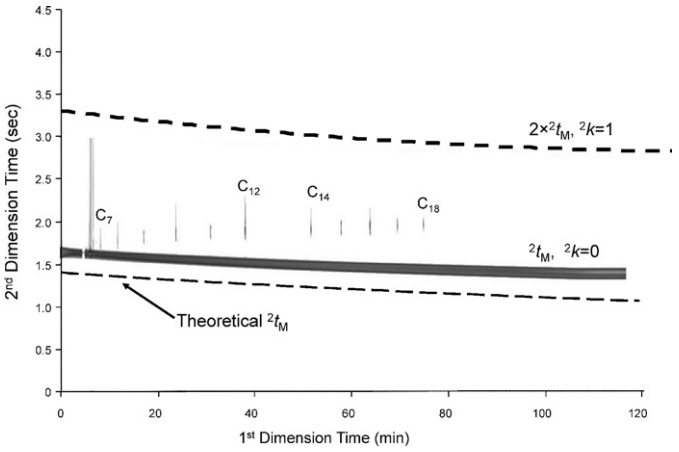

Fig. 6. The same as Fig. 2, but with a 5 m × 250 μm × 0.25 μm Innowax polar secondary column (apolar–polar combination). Modulated methane (dark line) clearly shows that actual hold-up time is slightly higher than the theoretical hold-up time (dark dashed line). This indicates that the length of the column is slightly longer than 5.0 m nominal value, the i.d. slightly less than the 250 μm nominal value or both. n-Alkanes are clearly retained, albeit only slightly.

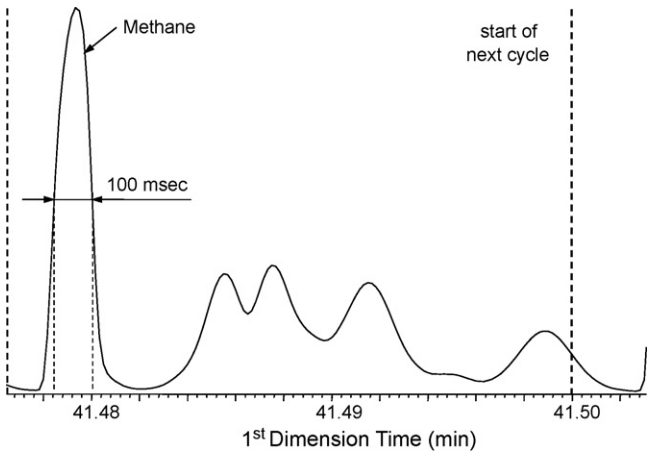

Fig. 8. A 1.5 s section (one cycle time) of detector output from the original data of diesel analysis on apolar–polar column combination and conditions of Fig. 6. The flow modulated methane peak provides not only a direct measure of hold-up time, but also shows initial peak width and shape. In this example, the methane peak is 100 ms wide at half height.

column. Under a given pressure of a given gas, hold-up time (t_M) in a tube with circular cross-section is proportional to $(L/d_c)^2$ [15] where L and d_c are column length and internal diameter, respectively. The fact that experimental ^2t_M is 17% higher than theoretical ^2t_M suggests that the ratio L/d_c is 8% higher than nominal—but consistent with one extra loop of column cut from a 5 in. diameter column cage (40 cm added to expected 5 m column length).

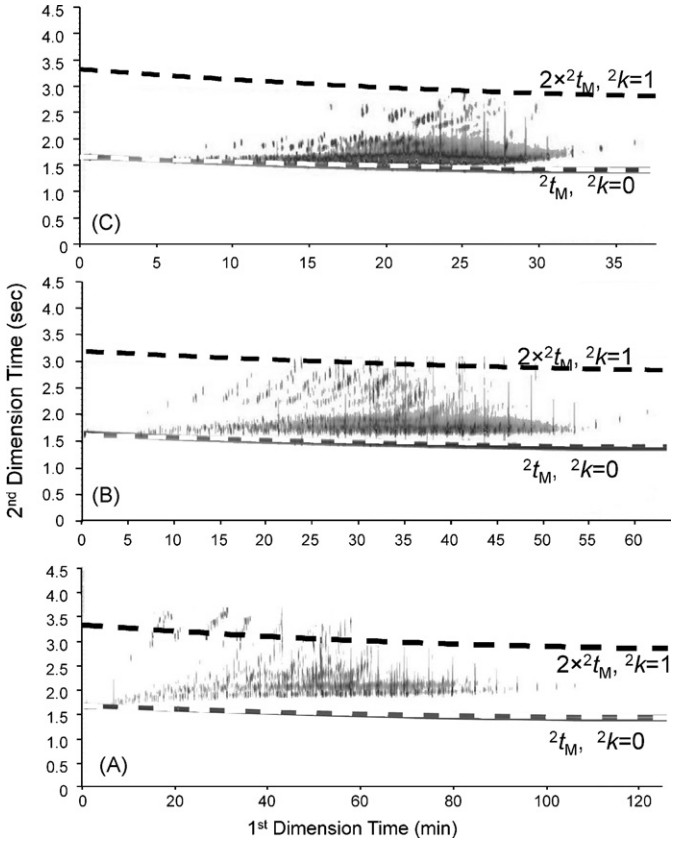

Fig. 7. Comparison of GC × GC plots of diesel sample analyzed on apolar–polar column set of Fig. 6. (A) 2 °C/min oven ramp rate (10 °C/t_M), (B) 4 °C/min oven ramp rate (20 °C/t_M), and (C) 8 °C/min oven ramp rate (40 °C/t_M).

The actual ^2t_M line (whether it coincides with theoretical prediction or not) allows one to directly determine second dimension retention factors as long as the onset of modulation is coincident with system clock time reference (no lag) for that cycle and the timing of each cycle is precise. It should be emphasized that, when the primary and the secondary columns have different polarities, the second dimension retention factor (^2k) of any compound can be (and most likely is) different from its elution retention factor in the primary column.

3.4. Polarity of the second column

From Fig. 6, one can find that, at 2 °C/min, n-alkanes in the wax polar secondary column have $^2k < 0.3$. As follows from the discussion of Fig. 3, this is up to 7 times lower than their elution retention factors from the primary apolar column. This makes sense: the alkanes are more attracted to the apolar primary column and elute at temperatures high enough that there is little retention on the wax column. The presence of the methane line visualizing hold-up time across the oven ramp range allows one to quantify these retention factors and the difference between retention factors in the secondary column and elution retention factors for the primary column.

It is quite easy to see, for example, that not only are the alkanes retained on the polar secondary column, but that they tend to increase in retention factor as a function of run time and column temperature.

More polar compounds are retained more in the polar secondary column, as expected. Fig. 7A shows that, at 2 °C/min, some of the most polar components have $^2k = 1.3$.

The chromatograms compared in Fig. 7 show that, as in the case of apolar columns in both dimensions, retention factors in the apolar/polar column pair decline with increasing heating rate.

3.5. Width and shape of unretained peak

A slice of detector output representing approximately one modulation period (cycle) is shown in Fig. 8. Modulated methane provides an unretained peak in each cycle so one can directly observe its shape and measure its statistical moments throughout the run as temperature and flow might change. This information, as an example, might be valuable for experimental evaluation of

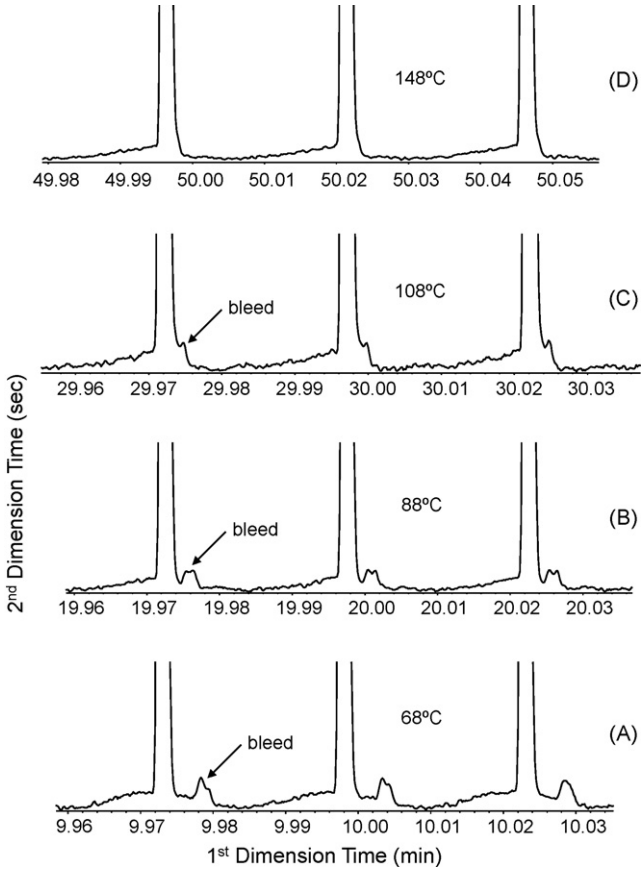

Fig. 9. Comparison of stationary phase bleed retention for the apolar–apolar column combination at 2 °C/min. The internal reference of hold-up time provided by modulated methane (large peaks) shows that bleed retention (small peaks) is decreases with higher temperatures. At 10 min (70 °C), bleed retention is approximately 0.3 ($^2k \cong 0.2$). At 50 min (150 °C) the bleed peak is obscured by the methane peak.

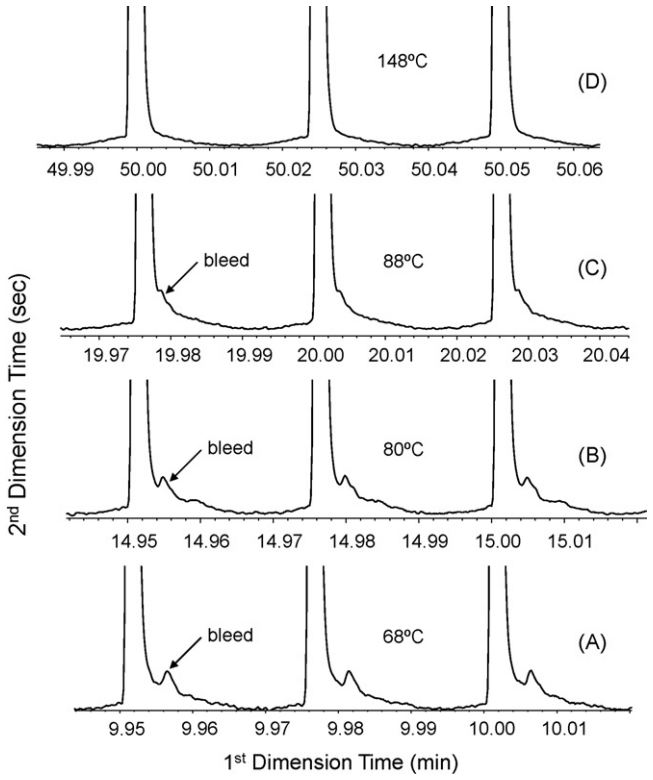

Fig. 10. Stationary phase bleed retention for the apolar–polar column combination at 2 °C/min. Bleed retention at low temperatures is similar to that for the apolar–apolar combination (Fig. 9) but appears to be less at the higher temperatures.

the performance and optimization of modulation devices [7] to improve the performance of the given system or of GC × GC as a whole [16].

3.6. Stationary phase bleed retention

A frequently held misconception is that stationary phase bleed is an accurate measure of second dimension hold-up time. Indeed, stationary phase bleed from the primary column is in many ways the same as methane-doped carrier gas; it is a source of continuous background, although the amount is quite temperature dependent. However, the decomposition products of stationary phases that make up the bleed are larger than and have lower volatility than methane. This difference is easily validated by the fact that thermal modulators can effectively trap and release stationary phase bleed across typical oven temperature ranges whereas they cannot do so for methane. In addition, the amount of stationary phase bleed is temperature dependent; sometimes bleed is undetectable at low oven temperatures and can be very significant at higher temperatures near the end of the run. On the other hand, use of a methane dopant as described herein can provide a relatively constant concentration (peak size) throughout the run.

The important question at hand is, however, is stationary phase bleed retained in the second column? By using modulated methane bleed, one can directly observe the answer. Figs. 9 and 10 demonstrate that stationary phase bleed is indeed retained. The retention is a function of temperature, which is logical. At lower temperatures, retention of the stationary phase decomposition products is highest. As temperatures increase, retention reduces to the point where the bleed peaks are obscured by the methane peaks.

Figs. 9 and 10 clearly show that stationary phase bleed is retained over much of the temperature ramp, so it is not a good measure of hold-up times. Modulated methane is a much better choice in this regard, especially when it is able to be implemented in a straightforward fashion as it is with flow modulation.

4. Concluding remarks

The use of continuous line marking the hold-up time in the secondary column of GC × GC for measurement of the secondary column hold-up and retention times as well as for measurement of retention factors in both columns has been demonstrated. The experimental implementation in GC × GC with flow modulation was straightforward with available hardware. It has also been demonstrated that the column bleed can be retained and, therefore, is not always a reliable marker of the secondary column hold-up time.

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