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Overloading of the second-dimension column in comprehensive two-dimensional gas chromatography

James Harynuk[a], Tadeusz Górecki[a,*], Jaap de Zeeuw[b]

[a] *Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ont., Canada N2L 3G1*
[b] *Varian Inc., Herculesweg 8, 4330 EA Middelburg, The Netherlands*

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Abstract

Comprehensive two-dimensional gas chromatography (GC × GC) is based on a coupling of two GC columns of different characteristics by means of a device that allows portions of the effluent from the primary column to be injected onto the second dimension column for an additional separation. The time available for the separation in the second-dimension column is very short. Thus, this separation should be very efficient. The vast majority of GC × GC practitioners use very narrow bore columns for the second dimension. While this approach is justified in principle, if peaks in the second dimension overload this column, its peak capacity is severely reduced. A series of second-dimension columns of varying internal diameters, but similar phase ratios, were used to study these effects. The results indicate that 250 μm columns often provide comparable second dimension peak widths to 100 μm columns, while at the same time being less prone to overloading, indicating that they may often be a better choice than smaller diameter columns in the second dimension of GC × GC systems.

Keywords: Comprehensive two-dimensional gas chromatography; GC × GC; Overloading; Column diameter

1. Introduction

Comprehensive two-dimensional gas chromatography (GC × GC) is receiving more and more attention recently owing to its vastly improved separation power over conventional GC. The improvement is accomplished through coupling of two GC columns coated with different stationary phases by a special GC × GC modulator, which helps preserve the separation achieved in the first column while enabling additional separation in the second column. The technique has been reviewed recently in a number of contributions (see e.g. [1,2]).

In order to maintain the "comprehensive" nature of a GC × GC separation, the second dimension must operate fast enough for the separation accomplished in the first-dimension to be preserved. To achieve the most faithful representation of this primary separation, each peak eluting from the first dimension column should be sampled as often as possible. However, in practice, the sampling rate in the first dimension is limited by the duration of a single separation cycle in the second dimension. Thus, it would be advantageous to use as short a time for second-dimension separation as possible in order to achieve high sampling frequency. On the other hand, for the separation in the second dimension to be efficient, it is advantageous to use longer time. Consequently, a compromise usually has to be struck between the first dimension sampling frequency and the second dimension separation time. Theoretical studies indicated that the optimum primary dimension sampling frequency is achieved when each primary dimension peak is sampled three to four times [3].

With typical peak widths for 1-D GC being about 6 s at the base, either a modulation period of at most 2 s must be used, or the analysis must be carried out under conditions that broaden the primary peaks to 12–18 s (or more) so that modulation periods of 4–6 s may be used. To accomplish this, thick-film primary columns, slow oven temperature programming rates, slow carrier gas linear velocities in the primary column, or a combination of these are used. The net result is an analysis time that is usually increased substantially over

* Corresponding author. Tel.: +1 519 888 4567x5374; fax: +1 519 746 0435.
E-mail address: tgorecki@uwaterloo.ca (T. Górecki).

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that for a 1-D GC analysis. If one wishes to shorten analysis times, the second-dimension separation must be made faster. This means shortening the secondary column, which decreases its separation power, or using narrower secondary columns, which allow for higher linear flow rates, greater speed, and lower loss of efficiency with the increased speed. This allows for very rapid separations to be performed in the second dimension, with the losses in separation efficiency from choosing a short secondary column and a very fast linear flow rate being compensated for by the gains in efficiency from the narrow column diameter. The most extreme example of the narrow second-dimension column found in the literature is that presented by Adahchour et al., who used a 50 μm diameter second-dimension column [4].

The idea that a narrower column should be used in the second dimension has become the conventional wisdom when choosing column sets for GC × GC separations, as evidenced by the experimental sections of the vast majority of papers published in the literature. However, relatively little work has been done to prove that it is in fact the best choice under any and all circumstances. While the line of reasoning presented above is generally correct, problems may arise when the second dimension column becomes overloaded, which happens frequently in GC × GC with thermal modulation. Modulators of this type are currently the most widely used. They collect and focus portions of the effluent from the primary column into very narrow, concentrated bands. It has been shown that under such circumstances, second dimension column can easily become overloaded, even when the primary dimension column is not overloaded [5]. This results in reduced peak capacity in the second dimension. Since the amount of second dimension separation space is highly limited, any losses here, including those due to overloading, could seriously impair the performance of the system.

The goal of this study was to compare the performance of second-dimension columns of different internal diameters under the conditions of different mass loadings in order to assess how easily they become overloaded, and to evaluate whether this phenomenon is of any significant importance in GC × GC separations. To the best of our knowledge, this is a first comprehensive study of this kind. Presented in this paper are preliminary results obtained for four different second dimension column diameters and the same primary column.

2. Experimental

The separations were performed using an Agilent 6890 GC (Agilent Technologies, Mississauga, ON) fitted with a liquid nitrogen-based single cryojet modulator described elsewhere [6].

The samples analyzed included a linear *n*-alkane test mix consisting of *n*-pentane through *n*-tridecane, and unleaded gasoline. Pentane was obtained from Sigma–Aldrich (Oakville, ON). Hexane was obtained from Fisher Scientific (Toronto, ON), and the remaining linear alkanes were obtained from PolyScience Corporation (Niles, IL). Regular unleaded gasoline was obtained from a local gas station. The linear alkane test mix was prepared as a neat mixture with the following concentrations: pentane (65.9 μg/μl), hexane (72.4 μg/μl), heptane (76.0 μg/μl), octane (78.5 μg/μl), nonane (79.8 μg/μl), decane (81.6 μg/μl), undecane (82.8 μg/μl), dodecane (83.2 μg/μl), and tridecane (84.6 μg/μl). This mixture was then diluted from 10 to 100,000 times in CS_2 (Fisher Scientific).

The primary dimension column was a 30 m × 0.25 mm × 0.25 μm VF-5MS, an arylene stabilized equivalent of 5% phenyl/95% methyl polydimethylsiloxane (Varian Inc., Middelburg, The Netherlands). The second-dimension columns that were tested had diameters (and stationary phase film thicknesses) of 0.32 mm (0.25 μm), 0.25 mm (0.15 μm), 0.15 mm (0.1 μm) and 0.10 mm (0.1 μm). All these columns were one metre in length and were coated with VF-23MS stationary phase, which is a stabilized >70% cyanopropyl polysiloxane (Varian Inc.). This combination of columns can be used up to 290 °C. Connections between the columns were made with press-fit connectors (Chromatographic Specialties, Brockville, ON). The trapping capillaries were made of 0.10 mm i.d. deactivated fused silica tubing (Polymicro Technologies, Phoenix, AZ), and the delay loop between the two trapping stages was a segment of 0.25 mm i.d. deactivated fused silica tubing (Chromatographic Specialties, Brockville, ON).

The separations were conducted with hydrogen carrier gas under conditions of constant flow of 2.0 ml/min measured with a flow meter at 45 °C. The oven was programmed from 45 to 180 °C at 3 °C/min. The detection was performed by FID at 100 Hz, and the samples were injected in split mode with a split ratio of 1:100 to 1:200 for gasoline.

3. Results and discussion

The study was carried out by comparing the widths and the symmetry of peaks eluting from the second dimension columns of different diameters for progressively lower amounts of the analytes injected on column. The columns used in the study were custom-made for the project to have as similar phase ratios as possible. For the 0.1 mm column, a minimum film thickness of 0.1 μm had to be used, as thinner films result in surface interactions changing the polarity of the column. All other parameters, including the carrier gas volume flow rate and the second dimension column length, were kept constant in all experiments. While certain important parameters (e.g. elution temperatures of the analytes) differed somewhat from one column to another under these conditions, this approach was chosen because in our opinion it reflected everyday practice in the best way. The experiments allowed the evaluation of the useful range of mass loadings for each column, resulting in little or no overloading. Normal alkanes were chosen as model compounds for the study. The results were verified by analyzing gasoline in the GC × GC

mode with the second dimension columns used in the study.

Initially, the peak widths at the base and the asymmetries were measured and calculated manually. This was overly time consuming, and the results were poorly reproducible with peak start and stop points assigned arbitrarily. To solve this problem, software was written in Matlab (Mathworks, Natick, MA) that would import the raw chromatographic data from a .CSV file and allow the user to zoom in on the major slice for each ID peak. The user would have to select a point near the apex of the peak, and a point on the baseline away from the peak. The software would then find the apex of the peak and calculate its height above the baseline noise. The width of the peak at 10% of its height above the noise was then measured, and the asymmetry of the peak was calculated based on this width. While the peak widths reported in this manner are smaller than widths at the base, the method described produces much more robust results in a significantly shorter time. The code for this piece of software is available from the authors upon request.

Even though it was a vast improvement, the approach proposed encountered problems sometimes due to the insufficient data acquisition rate, as illustrated in Fig. 1 By choosing the highest data point, some of the narrower peaks may have been reported to be fronting or tailing unfairly. The error of the determination of the peak apex position may be as large as ±5 ms with data collected at 100 Hz. Thus, a peak with a width of 100 ms that is perfectly symmetrical could be reported to have an asymmetry of 0.8–1.2. Ideally, the software would fit a curve across the top of the peak to estimate where the apex is, but to do this one should apply a non-symmetrical Gaussian model, which requires the asymmetry of the peak – precisely what is being measured. A solution to this problem is being developed for use in future studies of these phenomena.

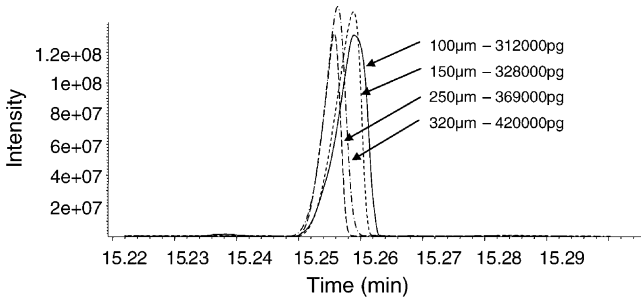

Fig. 2. Major undecane slices from the experiments with the neat mixture. The peaks eluting from the 100 and 150 μm columns were much broader than peaks eluting from the larger diameter columns. The masses refer to the mass of analyte in that particular slice, estimated by comparing the fraction of the total peak area (sum of all the slices) contained in the single major slice and the mass of the compound on column.

Once the software for peak evaluation was developed, the study commenced as described above. When the neat mixture was analyzed, several of the slices for each analyte were overloaded. This is exemplified in Fig. 2, which shows the major undecane slice for each of the columns tested with the neat mixture. While all of the columns were overloaded, the effect was much smaller for the 0.32 and 0.25 mm columns than for the 0.15 and 0.10 mm columns, for which the peak widths at the base approached 1 s. As the experiments proceeded to lower and lower concentrations of analyte on column, the peak shapes improved as they became less overloaded. At a dilution of 10,000×, the peaks were no longer overloaded and had a narrow, Gaussian shape, with the exception of the peak from the 0.32 mm secondary column, which was quite broad (Fig. 3). Though the peak eluting from the 0.10 mm column was slightly narrower than the others, the peak from the 0.25 mm column was only 120 ms at the base, which is usually considered more than adequate for GC × GC work. Peaks eluting from the 0.32 mm column were significantly

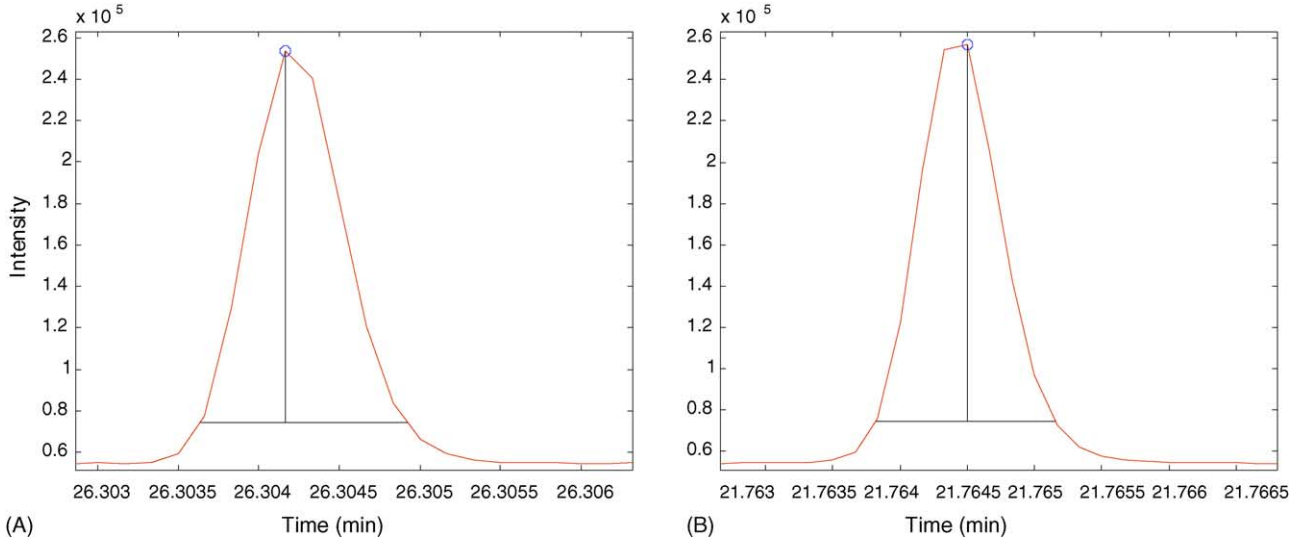

Fig. 1. Peaks showing (A) where the apex is reported early by the software (the peak is reported to be more tailing than it actually is), and (B) where the apex is reported later than it actually is (the peak is reported to be less tailing than it actually is).

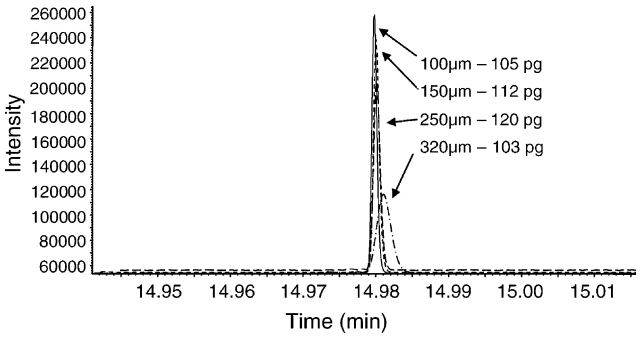

Fig. 3. Major undecane slices from the experiments with the 10,000× dilution of the neat mixture. None of the columns were overloaded, and with the exception of the 320 μm column, they all produced peaks of comparable widths. The masses refer to the mass of analyte in that particular slice, estimated by comparing the fraction of the total peak area (sum of all the slices) contained in the single major slice and the mass of the compound on column.

broader, mainly because the separation was done with a primary column that was narrower than the secondary column. Consequently, the carrier gas linear velocity in the secondary column was reduced substantially. The increased dead time combined with the small number of theoretical plates available in a 1 m segment of a 0.32 mm i.d. column resulted in broad peaks.

The results obtained for three of the compounds used in the study are summarised in Tables 1 and 2, which show the peak widths at all of the loading levels and their asymmetries, respectively. Peaks which were overloaded are highlighted in the tables. In order to determine which peaks were overloaded, the asymmetry and the change in peak width in going to the next lower concentration level were considered. If a peak was clearly fronting (as evidenced by its asymmetry) and became significantly less broad at the next dilution level (a decrease in width by more than 30%), it was marked as overloaded. If the peak became less broad, but its asymmetry was within the bounds of uncertainty given the data rate, it was marked as possibly overloaded.

Table 1
Peak widths at 10% height for the major slices of selected alkanes

	Peak width at 10% H (ms)			
	320 μm	250 μm	150 μm	100 μm
Hexane				
Neat	241	178	169	281
10×	205	168	142	132
100×	177	116	86	88
1000×	179	99	68	66
10k×	215	112	71	67
100k×	203	109	74	81
Undecane				
Neat	436	357	497	589
10×	334	221	321	289
100×	266	138	160	191
1000×	240	116	99	94
10k×	248	119	103	88
100k×	251	126	112	95
Tridecane				
Neat	419	331	458	689
10×	412	218	336	259
100×	206	123	146	154
1000×	181	94	91	84
10k×	190	98	88	78
100k×	229	118	97	89

Overloaded peaks are shaded; possibly overloaded peaks are hatched.

Table 2
Asymmetries of the major slices of selected alkanes (calculated based on width at 10% height)

	Asymmetry			
	320 μm	250 μm	150 μm	100 μm
Hexane				
Neat	0.87	0.76	0.39	0.52
10×	1.25	0.80	0.70	0.40
100×	1.47	1.25	0.94	0.78
1000×	1.78	1.61	1.29	1.37
10k×	1.44	1.41	1.29	1.00
100k×	1.83	1.09	1.5	1.58
Undecane				
Neat	0.54	0.43	0.43	0.52
10×	0.91	0.80	0.52	0.31
100×	1.02	1.00	0.52	0.36
1000×	1.27	1.42	0.97	1.09
10k×	1.28	1.17	1.36	1.00
100k×	1.24	1.34	1.03	1.18
Tridecane				
Neat	0.39	0.37	0.28	0.54
10×	1.36	0.68	0.62	0.23
100×	1.02	0.85	0.51	0.43
1000×	1.25	1.39	0.92	1.13
10k×	1.12	1.41	1.23	1.43
100k×	0.82	1.30	1.18	1.07

Overloaded peaks are shaded; possibly overloaded peaks are hatched.

It should also be pointed out that the results for the 100,000× dilution were likely characterized by greater uncertainty than the remaining results, because they were close to the limits of detection of the system. Worth emphasizing is the fact that the concentrations of all the analytes in this solution were at sub-ppm levels, yet the analytes could still be detected easily with a simple FID detector at a split ratio of 1:100, resulting in the absolute mass of the analyte in a single slice on the order of single pg. This in itself is a great testament to the improved detectability offered by modern GC × GC systems.

Fig. 4 demonstrates what happens in the two-dimensional retention plane when chromatographic peaks are overloaded. The overloaded peaks can be seen to occupy a much larger area of the retention plane, but the overloaded peak from the 100 μm secondary column has a much larger footprint than the overloaded peak from the 250 μm secondary column. The different retention times of the peaks seen in Fig. 4A and B versus Fig. 4C and D can be easily explained by the different diameters of the second dimension columns. With the 100 μm i.d. column (A and B), the linear velocity of the carrier gas in the first dimension column was lower than with the 250 μm i.d. column for the same volumetric flow rate, and

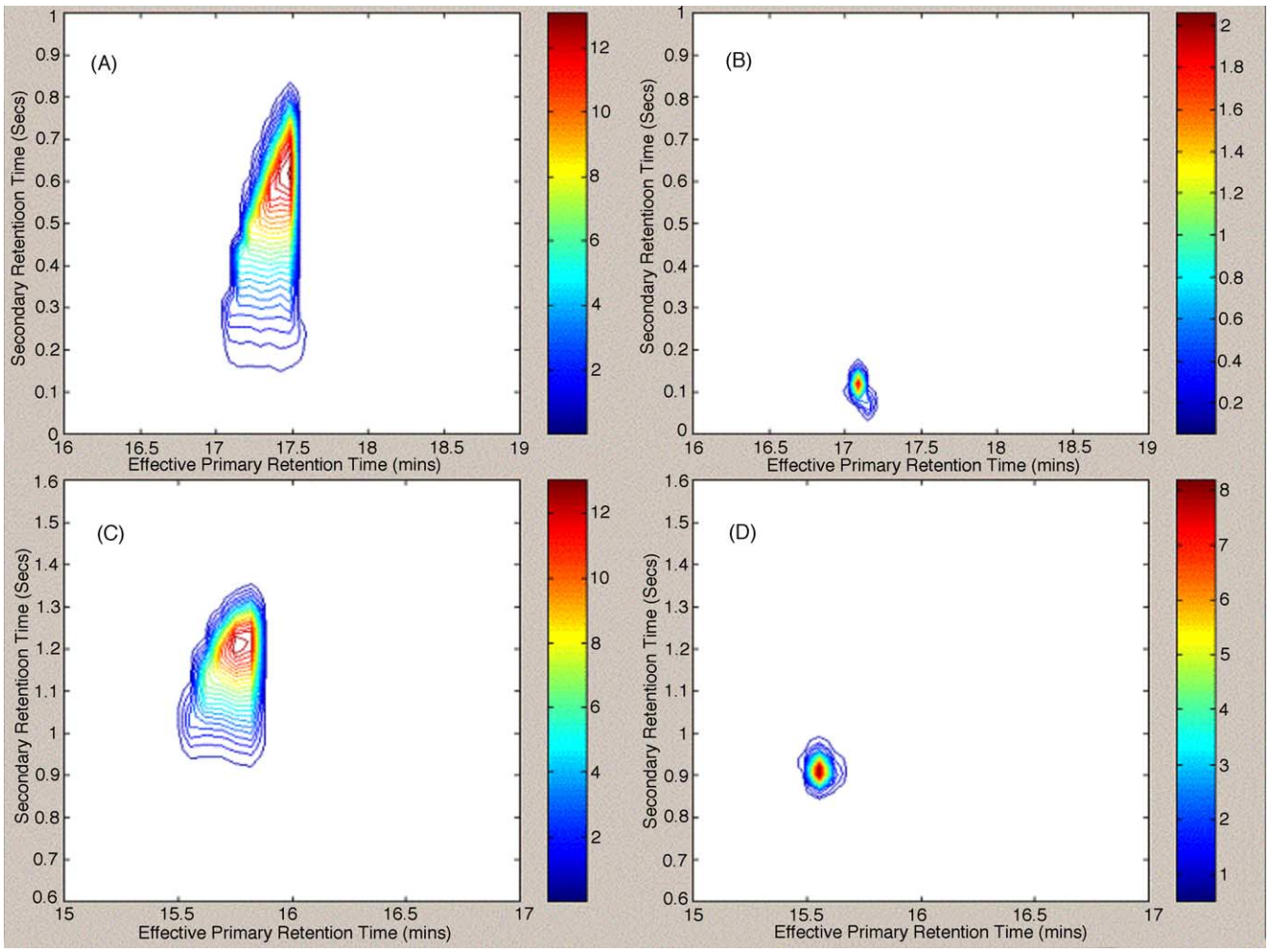

Fig. 4. Undecane peaks from the neat mixture (A, C) and the 1000× dilution (B, D) run on the 100 μm secondary column (A, B) and the 250 μm secondary column, (C, D). Note how the neat mixture overloads both column sets in both dimensions, but the consequences are much more severe for the 100 μm secondary column. When the columns are not overloaded, the 100 μm capillary gives slightly better performance than the 0.25 mm capillary.

consequently the first dimension retention time was longer. At the same time, the linear velocity in the second dimension column was much higher for the 100 μm column, and the retention time in this dimension was much shorter. Worth noticing is the fact that overloading of the 100 μm column was more severe than that of the 250 μm column in spite of the fact that the analyte elution temperature from the former column was higher. It should also be pointed out that the 100 μm trapping capillaries used in the modulator helped reduce the differences in the linear velocities in the first dimension column caused by the different restrictions posed by the second dimension columns of different diameters. Without the trapping capillaries, the linear velocities of the carrier gas in the first dimension column would vary to a greater extent.

When gasoline was separated using 0.25 and 0.10 mm second dimension columns, more differences could be seen. Fig. 5 shows the same region (C_3 alkylbenzenes) from the separation of gasoline obtained with the two columns. The peaks were approximately of the same width, but the separation of the major peaks from the band of peaks below them (barely visible in the 0.25 mm plot) was better with the larger diameter column. When individual 1-D slices from the two regions were compared, the resolution obtained for a sample pair of peaks was 2.47 with the 0.25 mm column, but only 1.50 with the 0.10 mm column. Since none of the peaks was large enough to overload either of the two columns, this was exclusively due to the significantly increased linear velocity of the carrier gas in the second dimension when the 0.10 mm column was used. It should be pointed out that the resolution could be easily improved for the smaller diameter column by reducing the volume flow rate of the carrier gas throughout the entire column assembly, which would bring the linear velocity of the carrier gas in the second dimension column closer to optimum. However, this could result in a suboptimal carrier gas linear velocity in the first column and in a significantly longer overall analysis time.

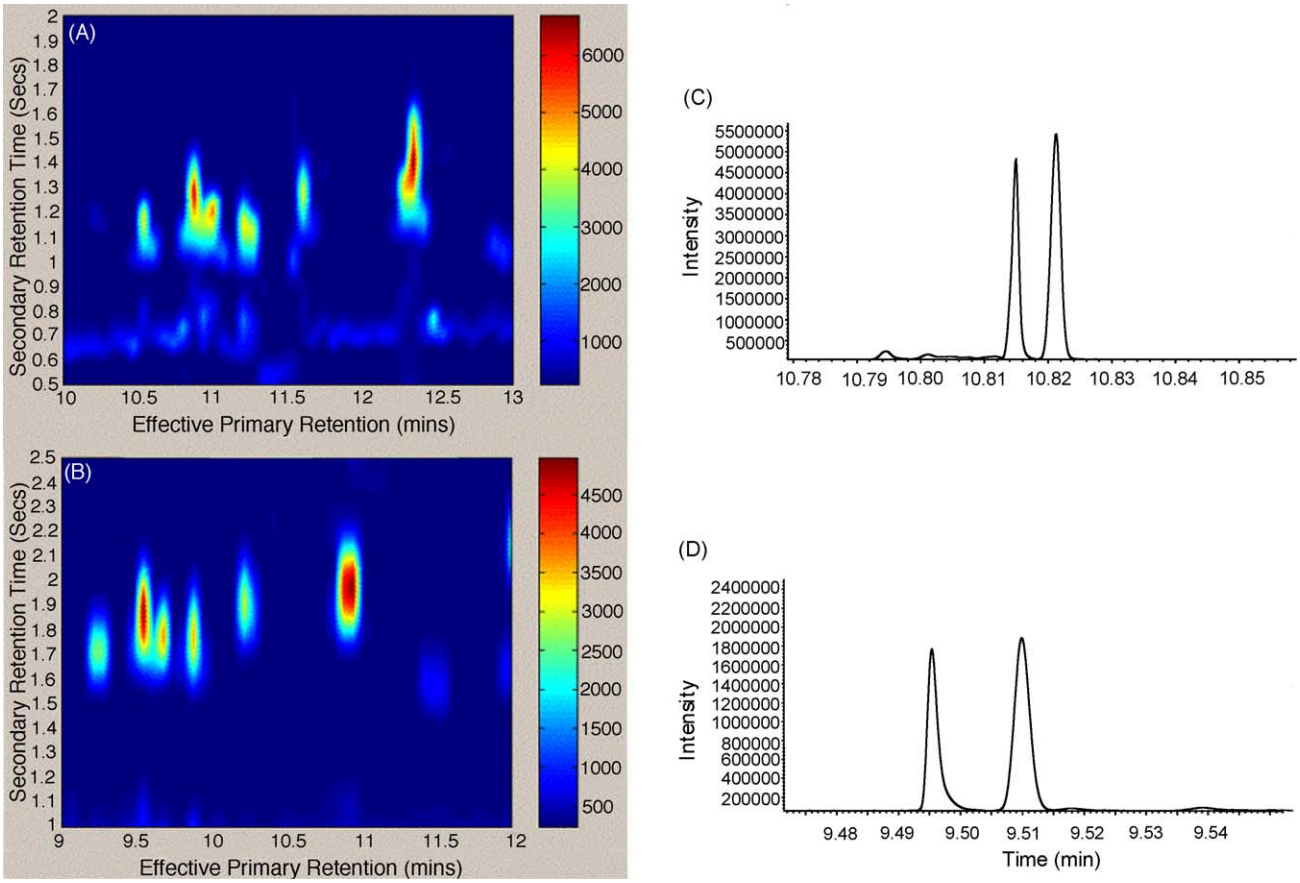

Fig. 5. A portion of the GC × GC chromatogram of gasoline using a 100 μm i.d. second dimension column (A), and a 250 μm column (B). The 1-D traces from these regions are shown for the 100 μm i.d. second dimension column (C), and the 250 μm column (D). The resolution obtained for this pair of compounds was 1.50 for the 100 μm column, but 2.47 for the 250 μm column.

4. Conclusions

Overloading of the second-dimension column in GC × GC separations can have a very significant effect on the available separation space in this dimension. When columns become overloaded, the peaks rapidly get very broad, especially with narrow diameter columns. Though it was possible to overload the 0.25 mm column, overloading was much more likely with the 0.10 mm column. In addition, the effects of overloading were less severe for the larger diameter columns. On the other hand, narrower columns also have significant advantages, most important of which is the separation speed. It is much easier to obtain a very fast separation in the second dimension with a narrow column. They also provide narrower peaks under conditions of no overload. Thus, in situations where ultimate speed is required and there are no large matrix peaks eluting in the vicinity of trace or ultra-trace analytes, a 0.10 mm column would likely be the best choice. However, when analyzing samples in which the concentrations of the analytes or matrix components are unknown and may be high, it may be better to use larger diameter columns in the second dimension, as this will lessen the chances and consequences of overloading in the second dimension. In addition, this may lead to better overall resolution in the second dimension, unless the carrier gas flow rate in a system with the narrower second dimension column is reduced significantly (which lengthens the overall analysis time and might result in less efficient separation in the first dimension). The evidence that it may actually be better to use columns with the same diameter in both dimensions also puts forth a challenge for column manufacturers to produce proper GC × GC columns: a single length of column, with a non-polar coating and a polar coating in two separate regions, without any couplings required.

A downside to using larger diameter second dimension columns is the increased dead time in this dimension, which may easily lead to the undesirable phenomenon of peak “wraparound”. One possible way to deal with this problem is to account for the dead time in the second dimension when plotting the chromatograms, in which case adjusted retention times are used in the second dimension rather than the actual retention times. However, since this may make direct comparisons of GC × GC chromatograms less straightforward, some users may opt against this approach.

It should also be noted that there are numerous other parameters that can affect the separation in GC × GC, such as linear velocity, elution temperature from the primary column, etc. The effects of these parameters will be the subject of future research.

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