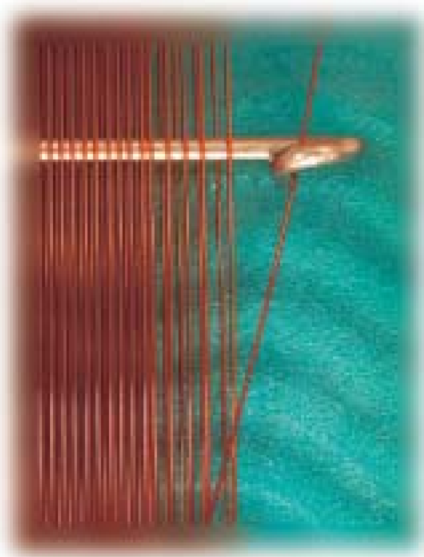
On the Interpretation of GC × GC Data



Comprehensive two-dimensional gas chromatography (GC × GC) is relatively new, and although many people might have heard of the technique, few are familiar enough with it to evaluate GC × GC chromatograms with the same skill they bring to the data interpretation of one-dimensional separations. This article describes the basic operation of GC × GC systems and explains how data are manipulated as they are transformed from raw signals to the more familiar contour plots. The authors present their observations from developing GC × GC data-handling software to demonstrate the challenges associated with accurately conveying GC × GC data.

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Comprehensive two-dimensional gas chromatography (GC × GC) is a relatively new and exciting separation technique. Many researchers have published papers focusing on the principles and applications of the technique (1–4), and the technique received much attention at the 24th International Symposium on Capillary Chromatography and Electrophoresis in Las Vegas, Nevada, in May 2001 (5). Although many people might have heard of the technique, few are familiar enough with it to evaluate GC × GC chromatograms with the same skill as they would one-dimensional chromatograms. To make matters even more difficult, it is almost impossible to accurately convey all of the information contained in a GC × GC chromatogram.

Much of what we will present in this article might seem obvious to those who work with GC × GC and have faced the challenges of interpreting and presenting their own data, but this situation is not the case for other chromatographers who see the material presented in the literature but do not use the method themselves. This article is targeted at the latter group. After providing a brief overview of the requirements of a GC × GC system and its operation, we will shift our focus to the interpretation of the data. We will use a series of plots to demonstrate what can happen to data as they are transformed into the contour plots, which should help average chromatographers in evaluating data published in the literature.

How GC × GC Separation Works

Conceptually, a comprehensive two-dimensional (2-D) GC separation is similar to other 2-D separations such as 2-D thin-layer chromatography (TLC), and the basic requirements are similar. The separation mechanisms used in the two dimensions should be independent of each other, and the entire sample should be subject to separation in both dimensions. In the case of 2-D TLC, this operation is a simple matter of spotting the sample in one corner of the plate, eluting it with one solvent, and then eluting it with a second solvent after rotating the plate 90°.

With GC × GC, the task is not as simple. Two columns with different stationary phases are used to perform the separation, but the second-dimension separations are performed on samples of the primary column effluent as it is eluted from it. This procedure is accomplished by using the GC × GC interface that connects the two columns (Figure 1).

Before describing the functioning of the interface, we should describe what is required to achieve a comprehensive GC ×

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GC separation. First, the entire sample must pass through both dimensions of separation. Second, the two modes of separation must be orthogonal or, in other words, independent in the two dimensions. Third, as the second-dimension separations are performed on portions of the primary column effluent simultaneously with the primary separation, each second-dimension separation should be finished before peaks from a subsequent injection reach the detector.

The requirement of independent separation mechanisms usually is achieved by using a nonpolar 100% polydimethylsiloxane or 95:5 (v/v) methyl–phenyl siloxane phase for the primary column and a polar phase such as a wax or trifluoropropyl phase for the second-dimension column. Although the separation mechanisms in these two phases are not completely independent themselves, they are independent under the conditions of the GC × GC analysis. The compounds that are eluted from the primary column already have been separated by properties that do not depend heavily on polarity when they are introduced to the second-dimension column in which the separations are based heavily upon polarity. Combining this situation with the approximately 3–5 °C/min oven programming rates and the 10-s or less separation times in the second dimension, each second-dimension separation is performed effectively under isothermal conditions at the temperature at which the compounds were eluted from the primary column. These two factors combine to create the independence of the two separation dimensions.

The third requirement — finishing one second-dimension separation before starting the following one — ensures that compounds with higher retention on the secondary column in one injection are not coeluted with poorly retained compounds in the following second-dimension injection. This requirement avoids what are called *wraparound* peaks and allows the correct construction of the GC × GC contour plot. We will describe how data from a GC × GC system are collected, handled, and interpreted below.

The GC × GC Compromise

If chromatographers consider what is happening during a GC × GC separation, it becomes obvious that a compromise must occur between the two separation dimensions and the timing of the injections to the secondary column. To preserve the separation achieved in the first dimension, chromatographers must sample the effluent from the first column as often as possible (approximately every 2 s). However, because the two columns are connected in series, this sampling period defines the amount of time permitted for the second-dimension separation. To avoid wraparound peaks and have enough time to perform a useful separation in the second dimension, analysts usually operate with a sampling period of 4–6 s, although sampling periods of as long as 12 s occasionally are used. This sampling period leaves enough time to perform a separation in the second dimension but sacrifices resolution in the primary dimension. To aid in the fast second-dimension separations, the second-dimension column usually is as short as 1 m and has a diameter of 0.25 mm or less. The column often is operated at linear velocities greater than 70 cm/s — sometimes as much as 150–200 cm/s — velocities that are well beyond optimal.

The GC × GC Interface

The key to the GC × GC system is the interface between the two columns. This interface must be capable of trapping the analytes that are eluted from the primary column and compressing them into a narrow band. Periodically, it also must inject all

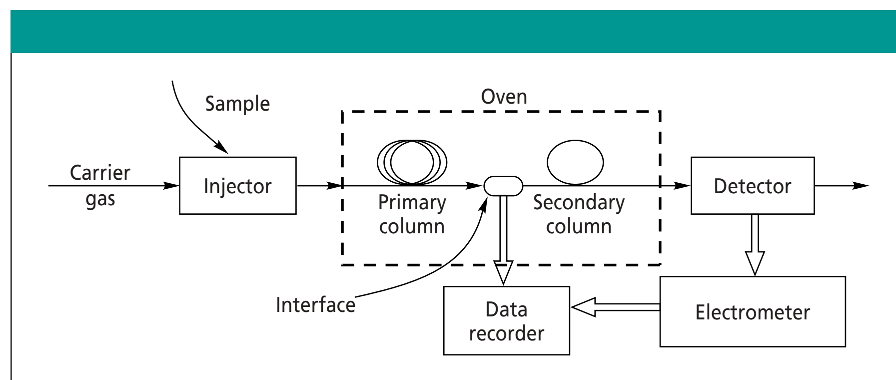

Figure 1: Schematic of a GC × GC system showing how the components are connected.

of the material that it has trapped onto the secondary column and then begin trapping again as soon as possible after it performs an injection. The final task of the interface is to perform these injections at times that can be recorded precisely, so the GC × GC plot can be constructed properly. Several interface technologies have been developed. Readers who desire explanations more detailed than the brief description presented here can find more information in the literature (1–4).

The most widely used interfaces include the rotating thermal modulator developed by the late John Phillips and co-workers (3). This interface uses a rotating heater that passes over a segment of thick-film fused-silica capillary, which traps analytes eluted from the primary column (Figure 2). As the heater passes over the capillary it heats it locally, desorbs the contents, and sweeps the analytes onto the second column in a narrow band. It is commercially available from Zoex Corp. (Lincoln, Nebraska), which more recently has modified the instrument into a thermal jet interface that uses jets of hot and cold air for desorption and trapping (2).

The longitudinally modulated cryogenic system was developed by Marriott and his research group (4). This interface uses a small cryogenic chamber that cools a short segment of the column to trap the analytes (Figure 3). To release the analytes, the chamber is moved from position T to position R (Figure 3), the previously cooled segment of the column heats up, and the analytes are desorbed. Restoring the trap to the initial position permits trapping and focusing of the subsequent portion of the primary-column effluent. Other GC × GC interfaces based upon thermal modulation have been developed in other laboratories, including several in our laboratory (6,7).

Interfaces based upon a series of valves that produce similar results also have been developed (8). Because these valve-based interfaces vent primary-column effluent to a certain extent, they violate the first rule of a

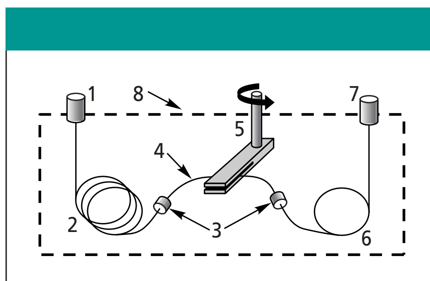

Figure 2: Rotating thermal modulator system designed by Phillips and co-workers comprising the injector (1), primary column (2), connectors (3), transfer capillary (4), rotating slotted heater (5), secondary column (6), detector (7), and oven (8).

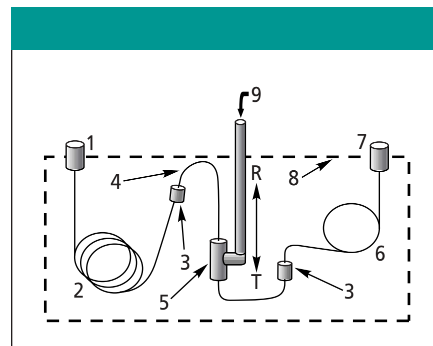

Figure 3: Longitudinally modulated cryogenic system designed by Marriott and Kinghorn comprising the injector (1), primary column (2), connectors (3), transfer capillary (4), cryogenic chamber (5), secondary column (6), detector (7), oven (8), and cryogen inlet (9). Analytes are trapped in the cooled segment of capillary when it is in the T position and released when the trap is moved to the R position.

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comprehensive 2-D GC separation — having the entire sample undergo separation in both dimensions and reach the detector. Thus, strictly speaking, they are not comprehensive 2-D GC interfaces.

The Handling and Interpretation of GC × GC Data

The data generated by a GC × GC system are collected at a detector as a linear signal with dimensions of retention time and signal amplitude, as any other chromatogram. What is actually seen at the detector is a series of the short second-dimension separations plotted continuously, one after another in succession (Figure 4a). The raw data file must be transformed from its linear form to a three-dimensional (3-D) surface with dimensions of primary retention time, secondary retention time, and signal amplitude by data-handling software for interpretation.

Although most research groups in the field use their own custom-written software, the essential tasks performed by these software packages are the same. The software scans the raw data signal and slices it into the individual second-dimension chromatograms (Figure 4b). This function is performed either by entering a specific modulation period and using it to indicate when an injection occurred or by using timestamp data collected along with the chromatogram that indicate when injec-

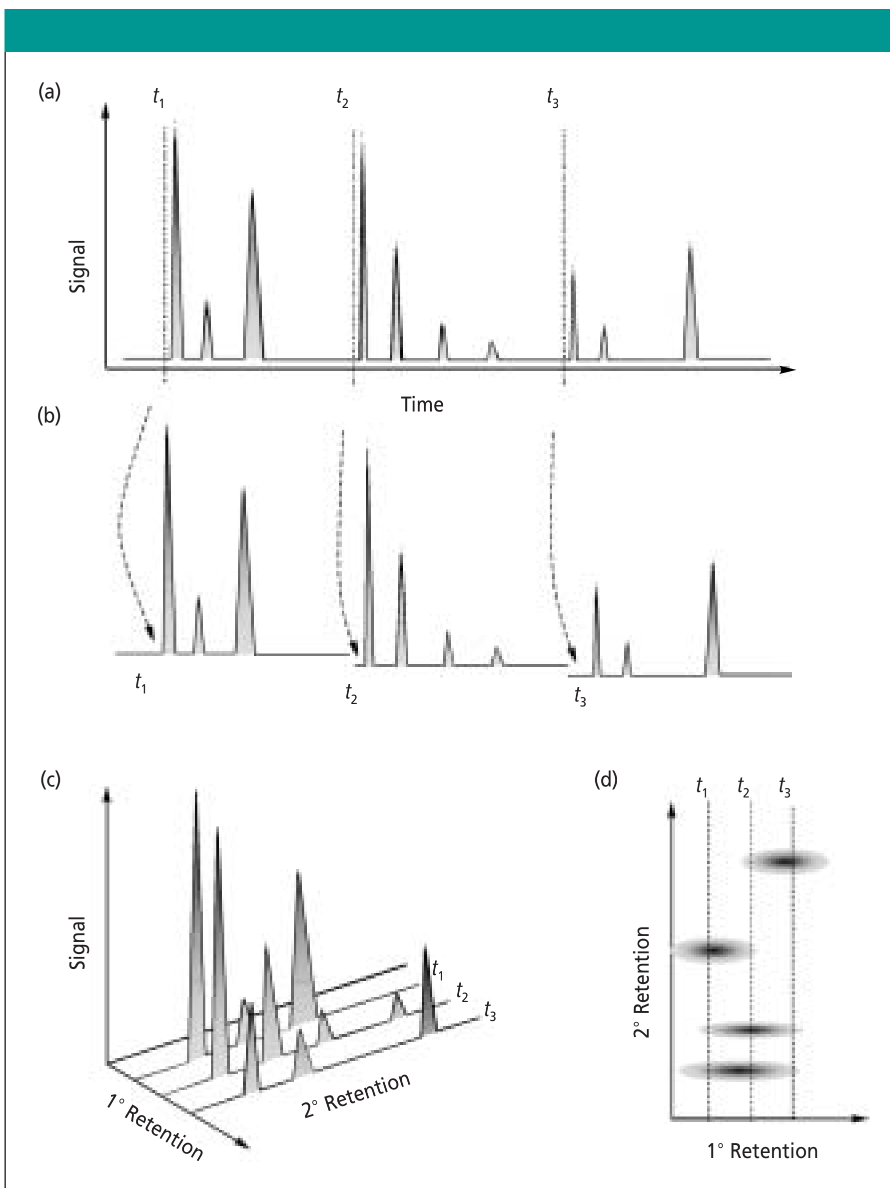

Figure 4: The transformation of GC × GC data. Shown are (a) the raw linear chromatographic signal with t_1, t_2, and t_3 denoting when injections onto the second dimension occurred, (b) the individual slices of the raw data that represent each individual second-dimension chromatogram, (c) the 3-D space into which the computer software places the individual slices of chromatographic data, and (d) the final contour plot of the data viewed top-down.

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tions to the second-dimension column occurred. In our experience, having an exact indication of when the injection occurred is better than simply using a nominal modulation period, because the timestamps will account for any fluctuations in the modulation period. These mini-chromatograms then are aligned side by side, with the primary retention plotted on the *x* axis, the secondary retention plotted on the *y* axis, and the signal intensity plotted on the *z* axis, as in Figure 4c. The primary retention time for all data points in a single second-dimension chromatogram is identical, and it corresponds to the time at which the sample was injected onto the secondary column (the recorded timestamp). The secondary retention time is equal to the difference between the raw retention time and the timestamp for that particular secondary chromatogram. The data usually are viewed from a top-down perspective, plotted as a contour plot (Figure 4d). This contour plot makes rapid interpretation of the results possible, assuming that the plots were constructed in such a way as to accurately convey the information and that chromatographers have the required skills for evaluating the data.

Below, we will present observations from our GC × GC data-handling software to demonstrate some of the challenges in presenting GC × GC data.

Experimental

To perform the GC × GC separations presented here, we used an Agilent 6890 gas chromatograph (Agilent Technologies Canada Inc., Mississauga, Ontario) that was customized to accommodate the custom-built cryotrap GC × GC interface, which we introduced at Pittcon 2001 (6). The interface has undergone several modifications to improve its performance since its introduction (7). We used a 30 m × 0.25 mm, 1.0-μm d_f ZB-1 column (Phenomenex, Torrance, California) as the primary column and a 0.9 m × 0.25 mm, 0.25-μm d_f Innowax column (Agilent Technologies) as the secondary column. The carrier gas was hydrogen at a constant flow rate of 45 cm/s.

The sample analyzed was regular, unleaded 87-octane gasoline, 1 μL split approximately 200:1. We chose this sample because gasoline is the most commonly analyzed material for testing GC × GC systems. It provides a well-known pattern of peaks, and analysts can tell at a glance whether a system is working properly. A closer inspection of the data is required to determine how well a system is performing.

The data were processed by using the export.csv function in the commercial MS Chemstation software (Agilent Technologies), and the file was processed with a program written in-house using Matlab

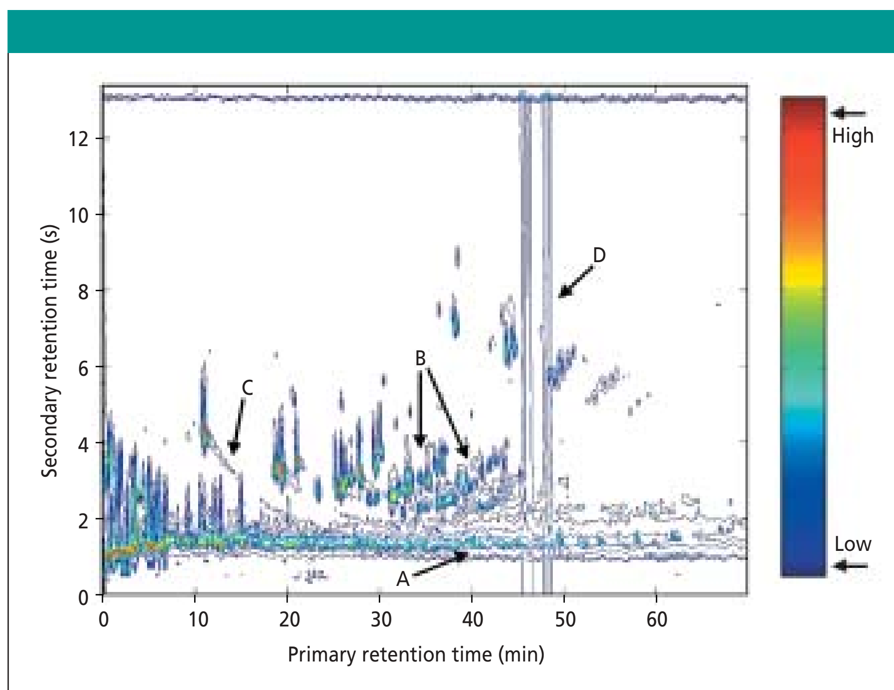

Figure 5: GC × GC chromatogram of gasoline. Logarithmic scale (4.97–7.97, 15 contour levels). A = the hydrocarbon band, B = families of compounds, C = curving tail from the major component peak, D = vertical bands from earlier injections left on the primary column.

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software (The MathWorks, Inc., Natick, Massachusetts).

Results and Discussion

As every second-dimension separation should be finished before unretained peaks from a subsequent injection reach the detector, each peak should reside in a very characteristic space on the chromatogram, well separated from its neighbors. However, this situation is not always the case, especially in systems in which both columns are housed in the same oven and have no independent temperature control. Often, peaks require more than one injection period to be eluted from the second-dimension column. These wraparound peaks are broader than typical second-dimension peaks because of their longer retention time in the second column. This result greatly increases the potential for overlap in the second dimension, wastes potentially valuable separation space, and can cause identification difficulties, even with two-dimensional retention times, because these peaks reside in a separation space that can be shared with other, fast-eluted peaks from subsequent injections. If a broad peak appears in the second dimension surrounded by narrower peaks, it probably is a peak that has wrapped around.

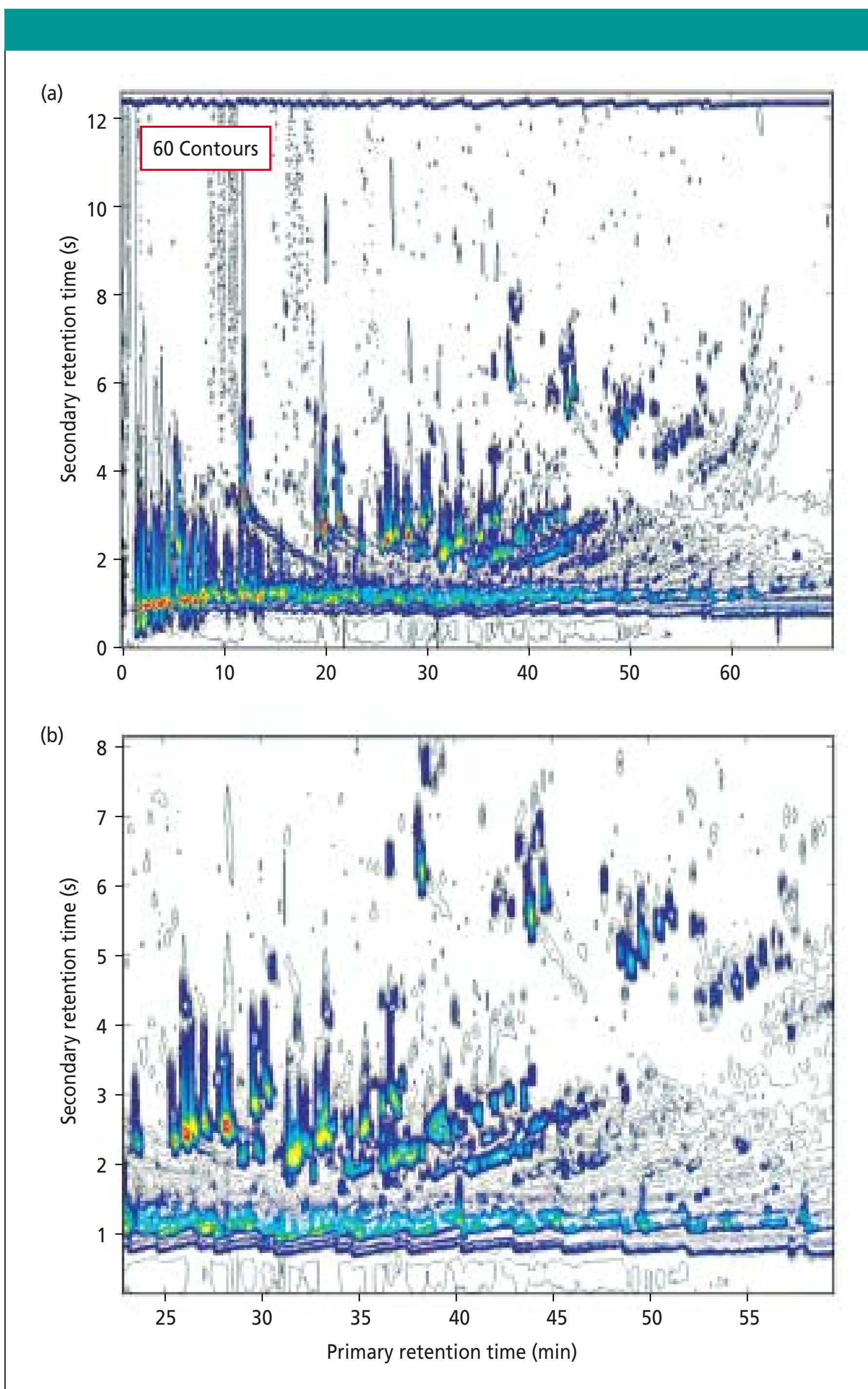

Figure 6: GC × GC chromatogram of gasoline. Logarithmic scale (4.97–7.97, 60 contours). Most peaks are displayed in their entirety; much emphasis is put on tails and base width due to more intervals falling at lower levels.

How the chromatograms are presented is another issue. Analysts can do many things to alter the appearance of the data when converting them from the 2-D raw signal (retention time and amplitude) to their final 3-D form — primary retention time, secondary retention time, and amplitude. Sometimes the chromatograms are presented as a simple plot of the peak maxima; more often, the data are presented as a contour plot.

Figure 5 is a contour plot for the analysis of gasoline. The chromatogram exhibits a few familiar features. First, a horizontal band of peaks runs along the primary axis (Figure 5a). This band comprises mainly linear hydrocarbons and, at sufficient oven temperature, primary column bleed. Other features of note are the diagonal lines of compounds (Figure 5b). Each of these lines of peaks represents a homologous series of compounds. The final feature of note is the curved tails on some peaks (Figure 5c). These features arise due to large, tailing peaks in the primary dimension that are eluted from the primary column throughout many cycles of the interface. In this figure, the temperature of the oven is slowly increasing throughout the run so that these separations occur at higher and higher temperatures as the run progresses, although the oven temperature effectively is constant for each individual second-dimension separation. Consequently, the second-dimension retention time of each segment of the tail decreases as the run proceeds, and the curved tail results. We chose this chromatogram in part because of the two vertical bands that appear toward the end of the chromatogram (Figure 5d). These bands are the results of components with low volatility that remained on the first-dimension column from a previous run and caused a pair of broad humps in the baseline as they were eluted from the column. The colored bar represents the scale used in the remaining contour plots; the values for high and low are specified in the figure caption.

Chromatograms can be presented in many ways. Most commonly, they are presented as contour plots. The intervals cho-

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sen for the contours are important in determining the appearance of the data. The signal amplitude often is transformed before being plotted on the 2-D retention plane because presenting the data with linear contour spacings is impractical due to the wide range of signal amplitudes. Common transformations include plotting either the base-10 logarithm of the signal or the square root of the signal. Figures 6–8 show different representations of data for another analysis of the same sample in which the two vertical bars did not appear. They are presented using different logarithmic contour intervals. Figures 6a, 7a, and 8a show the contours in their entirety; Figures 6b, 7b, and 8b show closeup views of the 25–55 min range.

These figures clearly show marked differences in the appearance of chromatograms

Figure 7: GC × GC chromatogram of gasoline. Logarithmic scale (5.2–8.2, 60 contours). Most of the peaks are displayed in a similar fashion to that in Figure 6; however, most of the small peaks are lost.

as the position of the first contour interval moves from below the baseline (Figure 6) to above the baseline (Figure 7) and when the number of intervals used changes (Figure 8). An obvious advantage of displaying the chromatograms with logarithmically spaced contour lines is that most of the peaks are displayed in their entirety. A disadvantage is that the peaks appear to be broadened in the second dimension because of the increased number of contours at the lower signal intensities. Other observations include the loss of trace-component peaks when the contours start at a higher level and the loss of resolution as the number of contour intervals decreases.

Figure 9 represents the same data set but plotted with linearly spaced contour lines. Rather than spanning the entire signal range ($\sim 10^5$ to 10^8), we set the maximum for the linear contour lines at 10^6. The number of contour intervals placed within the signal

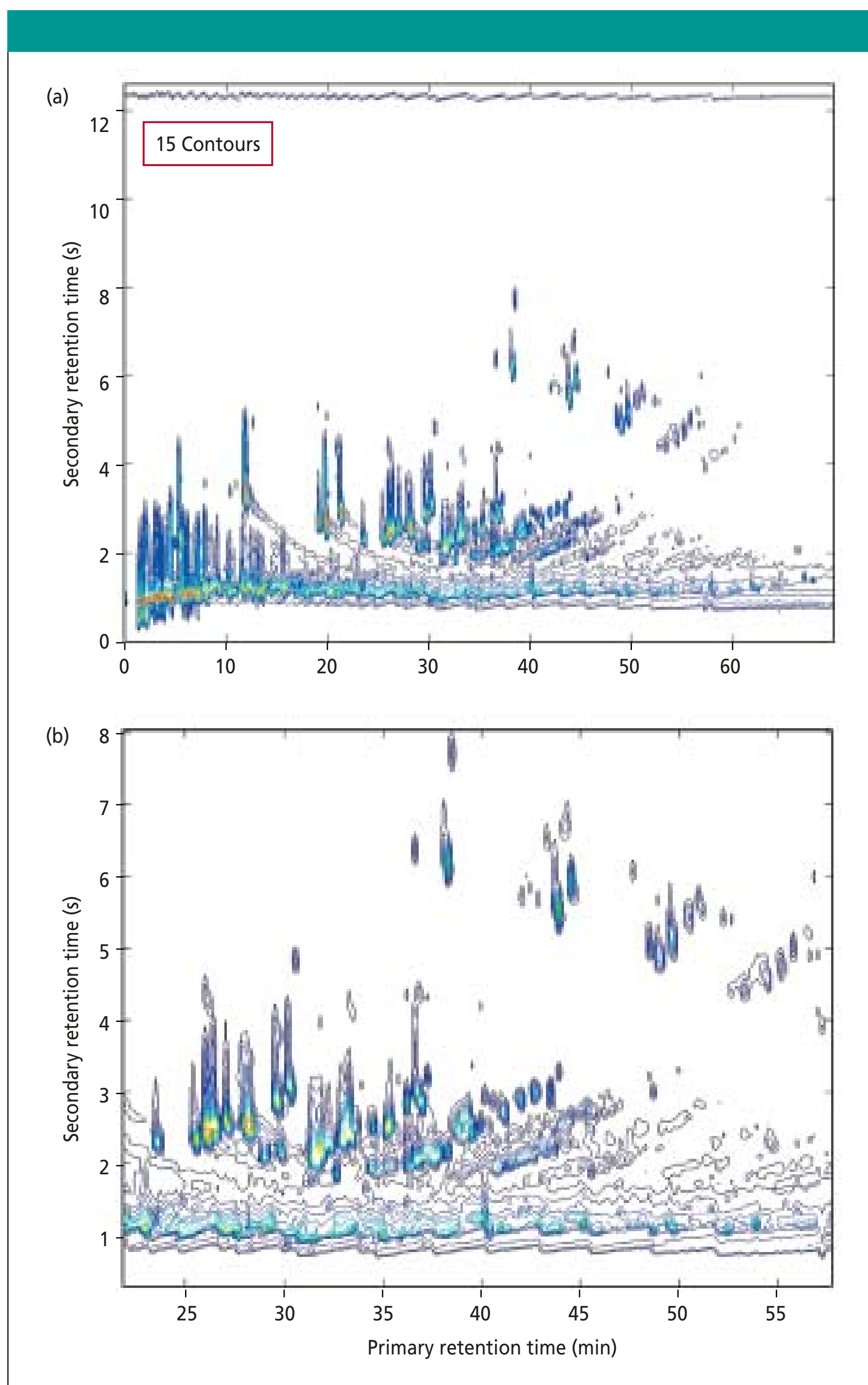

Figure 8: GC × GC chromatogram of gasoline. Logarithmic scale (5.2–8.2, 15 contours). Similar to Figure 7; however, some resolution and details of small or closely spaced peaks are lost.

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range and the lowest contour line are the same as in Figure 7. The linearly scaled plot provides a better description of small and closely spaced peaks than the logarithmically scaled plots, which can be seen in close inspection of the figures. This outcome is true when the peak maxima fall below the maximum of the scale (white areas inside of peaks indicate that the peak maximum has been truncated). However, it is impossible to cover the entire span of the data range with linearly spaced contour intervals, and this limitation is a major disadvantage of using the linear scale.

Figure 10 shows the effects of a square-root transformation of the signal intensity. The contours span a region from approximately 10^5 to 10^7 in signal intensity, with the same number of intervals and lowest contour level as in Figures 7 and 9. This

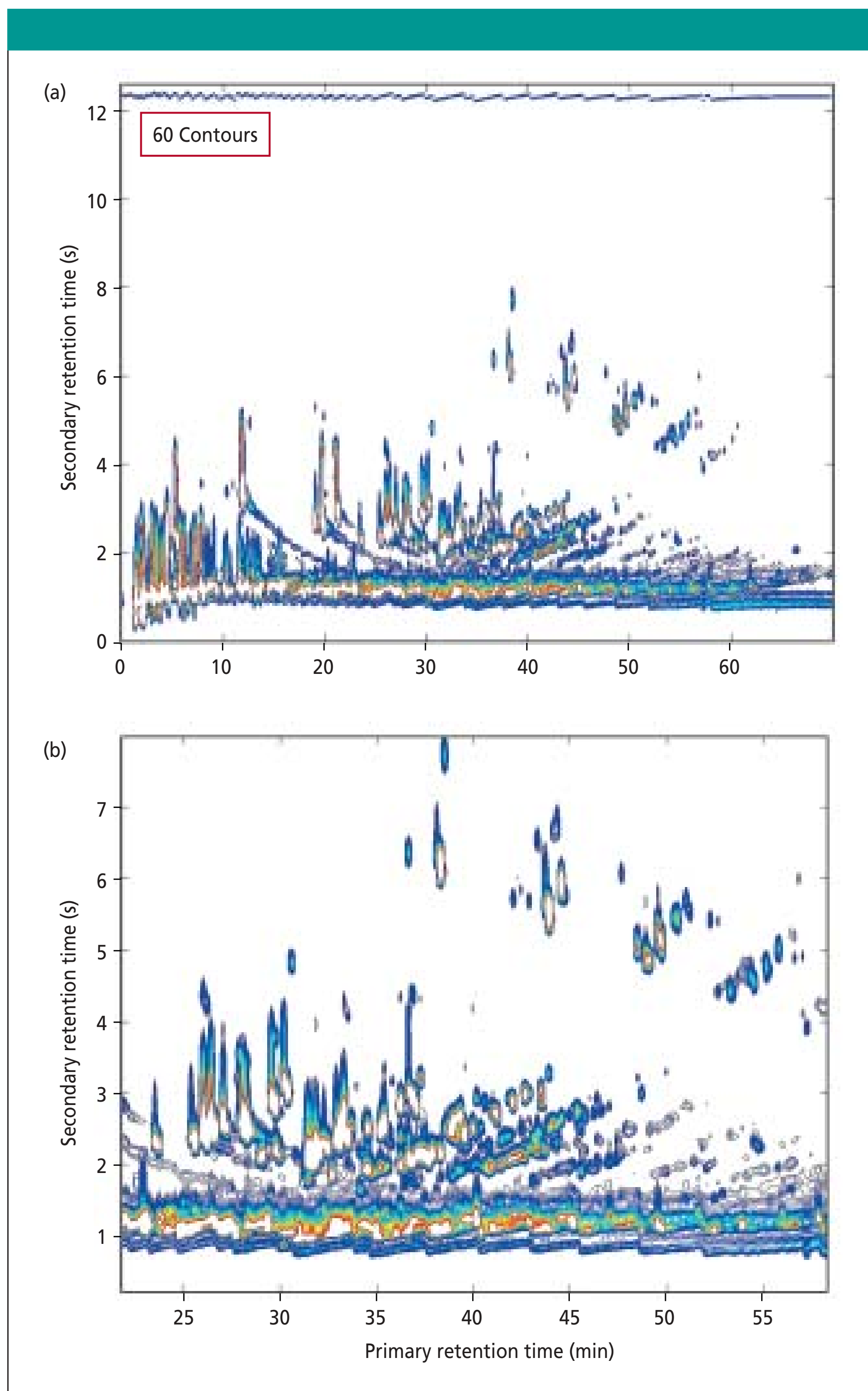

Figure 9: GC × GC chromatogram of gasoline. Linear scale (158,000–1,058,000, 60 contours). Compared with Figure 7, the smaller peaks are more clearly defined, but many of the taller peaks are truncated (white areas inside peaks indicate truncation).

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transformation allows us to display a slightly broader range of signals than the linear contour plot, but, as the regions of truncation show, it does not have the same capacity as the logarithmic scale for displaying small and large peaks. It is better, however, for displaying the smaller, closely spaced peaks than the logarithmic scale and is somewhat of a compromise between linear contours and logarithmic contours.

When a chromatogram is displayed as a contour plot, the first contour line should be placed slightly above the baseline to give an indication of where the baseline lies. Chromatographers should take care that the contour is not so low that small fluctuations in the baseline will cause it to peak above the contour level and add clutter to the contour plot. The plot should include information about any transformations performed

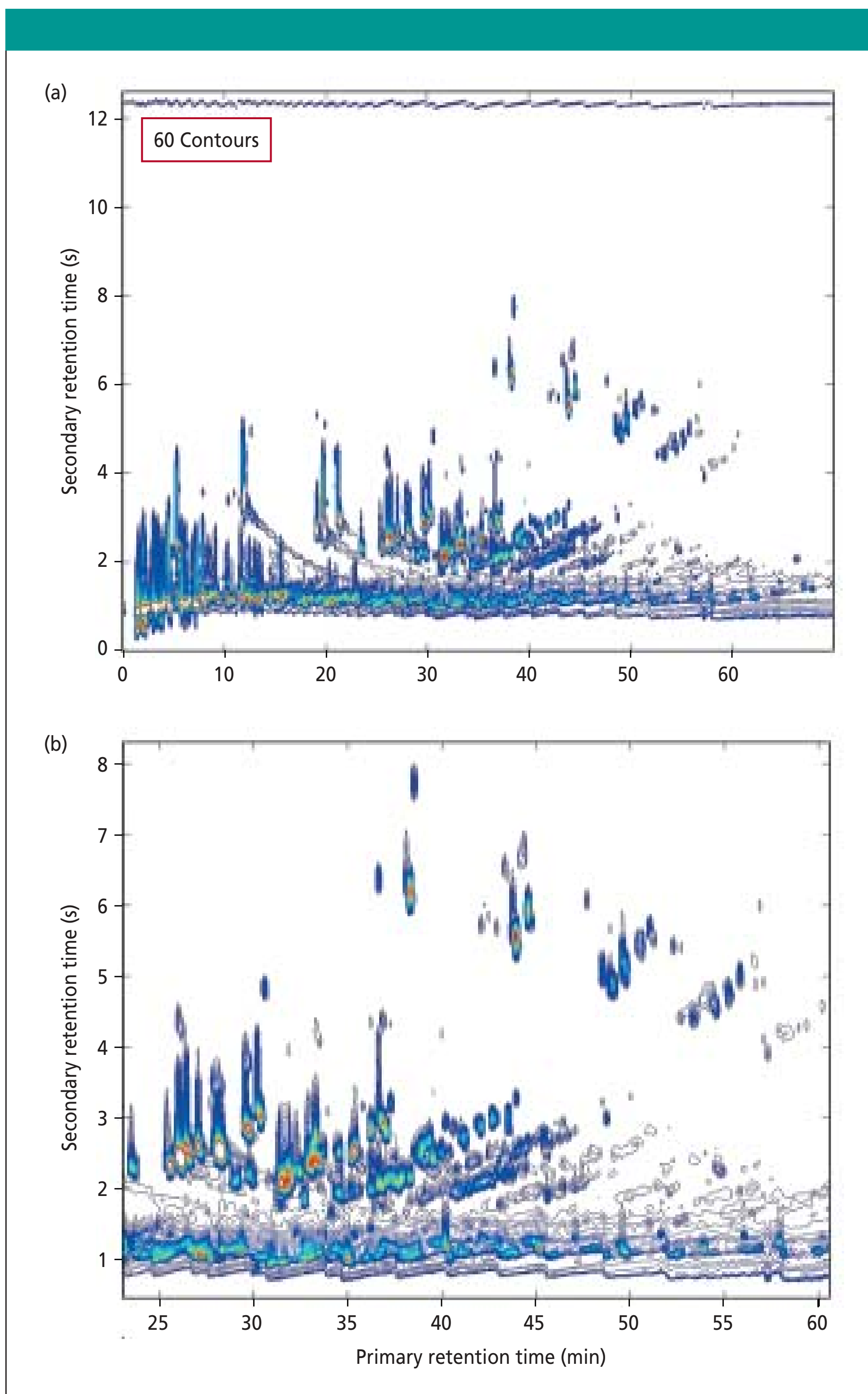

Figure 10: GC × GC chromatogram of gasoline. Square-root scale (398–3162, 60 contours), which allows adequate description of both small and large peaks and can be described as a compromise between logarithmic and linear scales.

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on the raw signal data (square root or logarithm) and some indication of scale.

For contour plots in general, the more contour intervals and the more information about their spacing, the better. Analysts can argue that the number of contour intervals should be increased to avoid masking small peaks in regions with large peaks or truncating large peaks in regions with smaller peaks; however, the demands placed upon the system plotting the data increase quite rapidly as the contours are spaced more closely. Users should remember that data acquisition in GC × GC must be performed at high frequency because of the very small widths of peaks eluted from the second-dimension column, and a single 2-D chromatogram easily can include more than 300,000 data points. Computing power then becomes another practical bar-

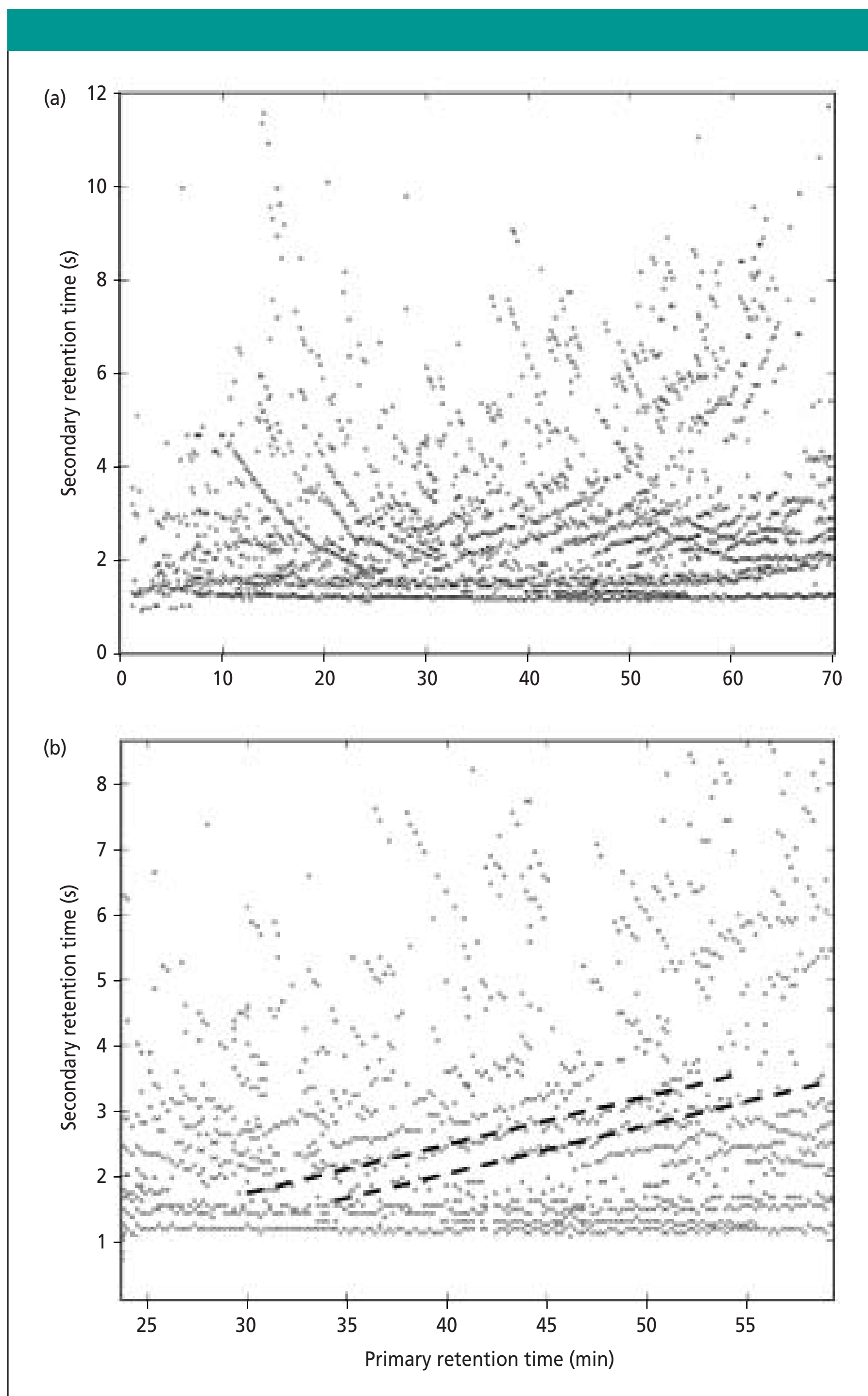

Figure 11: GC × GC chromatogram of gasoline. Scatter plot of peak maxima. Families of peaks run in straight lines (denoted by dashed lines). No information about peak size, shape, or resolution is conveyed.

rier to this approach. Too many closely spaced contour intervals add unnecessary detail and complexity to chromatograms and make data interpretation more difficult.

GC × GC data occasionally are displayed as scatter plots of the peak maxima. Figure 11 is a scatter plot for the same data set presented in previous figures. This plot is quite useful for seeing features such as families of compounds, which line up quite clearly — especially when the plot is viewed at an angle. However, chromatographers cannot glean information about the heights and widths of peaks or how well resolved they are from their neighboring peaks. To increase the value of plotting data as a scatter plot, analysts need to include some indication of scale. This representation could be achieved through the use of points that change color, size, or shape as the signal intensity changes.

The final option for presenting the data discussed here is to plot segments of a linear chromatographic signal as they appear in Figure 12. Although this form of data presentation can convey the information in a form that is more familiar and aids in evaluating the performance of the second-dimension separation, the benefits of the contour plots are lost. Chromatographers cannot easily see which peaks in consecutive second-dimension chromatograms are the same compound and which peaks are members of a homologous series. Additionally, displaying an entire chromatogram in this form is impractical and could be inadequate if major and trace components of interest are eluted in the same second-dimension chromatogram, as a comparison of Figures 12a and 12b shows. Figure 12a shows the entirety of the peaks in the chromatogram, but much of the information about the little peaks is lost. Figure 12b shows the little peaks quite well but not the larger peaks. For crucial parts of the chromatogram, especially those involving trace components, appropriately scaled segments of raw data could be presented in addition to contour or scatter plots.

Conclusion

GC × GC data are very complex. The situation is not helped by the fact that GC × GC researchers can follow no current standard set of rules when they prepare data for presentation, and no standard software package can be used with all GC × GC systems. This situation likely will be remedied as the technique gains popularity and more commercial instruments are used.

Until then, analysts must be aware of the challenges in presenting data and the fact that some information usually will be lost or modified when it is presented. Peaks of major components could be truncated or peaks of minor components could be omitted from the contour plot unless analysts have applied some transformation to their data sets. Plots should include the range spanned by the contour intervals, the interval number, and the interval spacing — linear, logarithmic, or another scale. Researchers presenting GC × GC data also should do their best to make readers aware of what might have been omitted from their chromatograms and why.

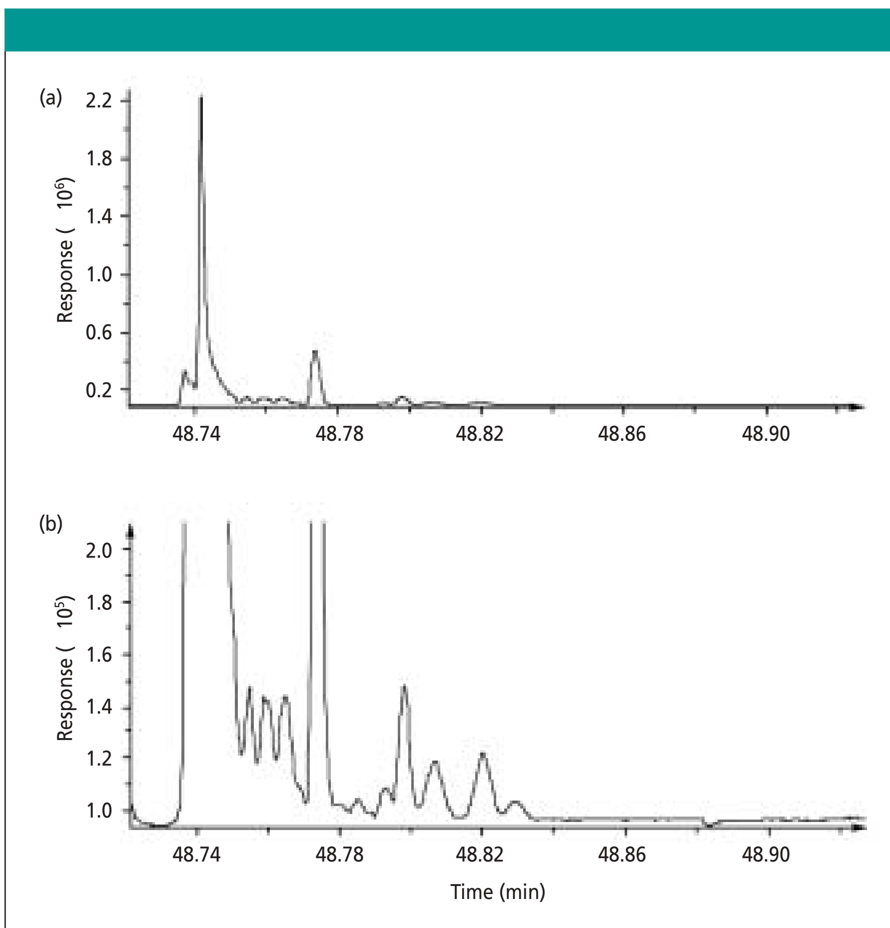

Figure 12: Segment of the linear raw data used in the previous figures. Shown are (a) the entirety of peaks in chromatogram and (b) the small peaks.

Acknowledgments

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