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Comprehensive Two-Dimensional High-Speed Gas Chromatography with Chemometric Analysis

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High-speed comprehensive two-dimensional gas chromatography (GC×GC) is performed, in which a polar second column performs separations every half second on portions of the effluent from a nonpolar first column. Chemometric techniques that are traditionally used on chromatographic separations with multichannel detection are applied to two-dimensional chromatographic data, for the purpose of quantifying incompletely resolved peaks. Generalized rank annihilation method (GRAM) is evaluated in the quantification of varying amounts of selected overlapped analytes in a GC×GC analysis of modified white gasoline. GRAM requires a sample and standard data set for quantification, and the high retention time precision arising from use of shortened GC columns aids in the analysis. Results from GRAM analysis of GC×GC data are compared with a reference GC method. The test analytes ethylbenzene and *m*-xylene, existing in various proportions in white gasoline samples, were successfully deconvoluted despite having resolutions of 0.46 and 0.20 on the first and second dimensions of separation, respectively. Like other second-order techniques, GRAM was able to reliably quantify *m*-xylene despite the presence in the analytical sample of an overlapping compound not present in the calibration standard. Because GRAM can be successfully applied to GC×GC data, full resolution of all the analytes of interest is not necessary. As a result, GC×GC run times can be dramatically shortened, which has significant implications for analyses in which short cycle times are critical, such as in process analysis.

Gas chromatographic (GC) analysis of multicomponent samples can be a time-consuming process. To reduce analysis times, many separations are done using multicolumn GC. In traditional multicolumn GC (GC−GC), the first column performs a partial separation of a sample, and a fraction, or heartcut, of the eluent is injected into the second column for final separation. The first column effectively performs sample preparation, reducing the number of compounds injected into the second column. The second column must have a different chemical selectivity than the first column in order to optimize the GC−GC resolving power. The difference in the chemical selectivity between the two columns allows compounds that are poorly separated in the first dimension to be more fully resolved in the second dimension. Although this technique is proven to have enormous practical value,[1] it can be a time-consuming process. Heartcuts from the first column range from seconds to minutes in length, and the separation that the first column has performed on the compounds within that heartcut is generally undone during the injection onto the second column. The lengthy run times of the second column limit the number of heartcuts that can be taken from the first column.

Traditional GC−GC is not a true "hyphenated" technique. Hyphenated chromatographic techniques, such liquid chromatography with diode array absorbance detection, offer the advantage of being able to identify eluting compounds through inspection of the spectral information. For hyphenated techniques, a vector of data is obtained at short time intervals during a separation, producing a two-dimensional data array for each run. The ideal GC−GC instrument is one that can function as a hyphenated instrument, for it would preserve all the chromatographic information from both dimensions of separation. This ideal system would perform a second dimension separation of an infinite number of infinitely thin heartcuts from the first column. Under these circumstances, the separating power of the ideal system is proportional to the product of the separating powers of the individual columns.[1,2] There is a practical limit to how frequently a sample can be cut to the second column, so the actual separating power is less than that theoretically calculated. This mode of operation is termed comprehensive GC−GC, or GC×GC,[3,4] with the multiplicative term emphasizing the enhanced separation power of this technique. Because GC×GC can provide much larger peak capacities, difficult separation problems are more easily handled with GC×GC. In addition, if a separation problem requires a given peak capacity, GC×GC is capable of generating it much more rapidly than an existing multiple-column GC using a few heartcuts. This article investigates how chemometric techniques can be applied to GC×GC to get the most information from the data and reduce run times, thus broadening the scope of GC×GC.

In fast GC, peak width, and thus separation efficiency, is frequently limited by the performance of the injector. To reduce separation times, the injected sample pulse width must be as short in time as possible.[5] GC×GC is most useful when the second

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column generates chromatograms faster than the first column generates peaks. This requirement places stringent demands on the second dimension of separation. Sampling that is both fast and reproducible between the two columns is, therefore, critical. Various sample switching mechanisms have been evaluated in the literature, ranging from modified rotary valves[6] and pressure-based flow switching[7] to thermal modulation of the stage connecting the columns.[8,9] Currently, thermal modulation is the most commonly used sample switching technique in GC×GC.[2,3,10−17] Thermal modulation functions by repeatedly focusing the first GC column effluent on a cooled stage, followed by rapidly heating to inject narrow plugs onto the second column. This thermal focusing technique is effective at reinjecting narrow plugs as long as sample compounds are not highly volatile and has been reported to inject plugs to the second column as frequently as once every 2 s.[14] In this work, a commercial diaphragm valve was modified for use as an interface between the two columns of a GC×GC system. This valve does not inject the entire first column effluent onto the second column, as can be the case with thermal modulation. As a result, some detection sensitivity is lost. However, the diaphragm valve is capable of repeatably transferring 50-ms-wide sample pulses to the second column several times a second, which means that a significantly larger portion of the first column chromatographic information can be retained.

Analysis cycle times can be shortened not only by switching from traditional GC−GC to GC×GC but also by doing less chromatography on both columns and by completing the separation mathematically with chemometric tools. For single-column chromatography, classical least-squares (CLS) is one technique that has been used to fit standard compound chromatograms to an overlapped mixture chromatogram for peak quantification. Using CLS, it was previously demonstrated that the quantification precision of single-channel detector chromatographic data could be maintained as column length is shortened, even in the case of severe peak overlap, since absolute retention time precision improves.[18] This work is significant because it indicates that chromatographic separation times can be shortened without a significant loss of analytical information, which has positive implications for the design of the GC×GC system.

Multichannel detection of a GC separation is inherently better than single-channel detection due to the enhanced selectivity. Compounds partially overlapped on the first chromatographic axis are mathematically more easily separated, because a multichannel detector can provide selective detection for each compound.[19] A mass spectrometer is the most common multichannel detector used in GC. Successful deconvolution can only occur, however, if the multichannel detector provides repeatable "spectra". For a mass spectrometer, this means that the fragmentation pattern for a given compound should not change. The second column of the GC×GC system can be treated as a multichannel detector, because it frequently generates chromatographic profiles of compounds as they elute from the first column. As long as retention times and peak shapes are repeatable on the second column, overlapped compounds on the first column can be successfully quantified on a GC×GC system. A key issue in the design of the GC×GC for coupling to chemometric analysis is that a shortened second column, combined with a precise mechanism for sample transfer to the second column, can provide the necessary analogous "spectral" repeatability.[18]

Many peak deconvolution algorithms exist for chromatography with multichannel detection. Evolving factor analysis (EFA) is a multivariate curve resolution technique that makes the assumption that, in chromatographic data, the concentration profiles are well defined: they are unimodal and nonnegative, and they reach zero asymptotically on both sides of the maximum. Once each compound's peak boundaries are determined by a moving window form of factor analysis, the compound concentrations outside the estimated peak region are set to zero. This restricts the set of possible peak shapes that factor analysis can predict. A noniterative EFA approach has been developed[20] to further refine the estimates of the pure compound's concentration and detector profiles. EFA has been applied to the resolution of peaks using LC/diode array UV data[21] and GC/MS data.[22] EFA can resolve more than three overlapped peaks. However, EFA encounters problems in profile estimation when chromatographic peaks are tailed, since estimating the peak boundaries is more difficult.

More information can be incorporated into peak deconvolution by comparing multiple data sets when the relative concentrations of the overlapped compounds differ. Generalized rank annihilation method (GRAM) compares two multidimensional data sets to determine the concentration ratios of compounds in one sample relative to a second, as well as their pure peak profiles.[23] If standard data contain compounds of known concentration, those compounds can then be quantified in the unknown sample. A key advantage of GRAM over other analysis methods is that the unknown sample can contain overlapped peaks not present in the calibration standard. Thus, for quantification purposes, the standard need only contain compounds of interest.[24] The GRAM algorithm is not iterative, as many curve resolution techniques can be, and so it is possible to track experimental error through a GRAM calculation, which can be used to provide error estimates

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of the results.[25] GRAM has been applied to the deconvolution of overlapped peaks in LC/diode array UV,[19,24] LC/diode array fluorescence,[26] and GC/MS.[27] We previously mentioned that the second column of a GC×GC system can be treated as a multichannel detector. As a result, GRAM can be used to quantify overlapped GC×GC peaks as well. A more detailed discussion of GRAM will be given in the Theory section.

In this article, the high retention time reproducibility for the second dimension of fast GC×GC is evaluated and substantiated. In addition, GRAM analysis is performed on significantly overlapped analytes in test white gasoline mixtures analyzed by a GC×GC system.[28] This is the first time a detailed description is given of chemometric analysis of GC×GC data. A concurrent publication from this group[27] applied an objective retention time standardization technique[19] to fast, short-column GC×GC data and demonstrated that retention times on the first column were sufficiently reproducible between runs that standardization was not usually necessary. This article also presents analyte quantification when an overlapping interference is present in the sample that is not accounted for in the standard mixture. The precision and accuracy of analyte quantification will be determined by comparing the GC×GC analyses to simultaneous full separations of the test mixtures on a standard, long single-column GC. An analytical figure-of-merit is introduced that can be used to evaluate the quality of a given peak deconvolution. It will be shown that reasonable and useful quantification accuracy and precision can be achieved even when chromatographic resolution is substantially less than 1 in both GC axes for a given overlapped group of analytes.

THEORY

Quantification of specific analytes in white gas samples was performed using generalized rank annihilation method (GRAM). Currently there are several algorithms for GRAM.[23,29−31] In this work, the standard eigenvalue method described by Faber et al. is used.[29] In order for GRAM to produce meaningful results, GC×GC data must meet certain requirements. First, detector response must be linear with concentration, a condition easily met with a flame ionization detector. Second, peak profile shapes must not change. In chromatographic terms, this means that peaks must not overload the columns, and the retention times of the compounds in the chromatograms of the analytical sample and its standard must be the same. For the experiments reported here, these conditions were met.

GRAM analysis also requires that overlapped peaks have some resolution on both GC columns. In the cases where overlapped peaks are somewhat resolved on only one column, GRAM is likely to predict deconvoluted peak profiles that have multiple maxima. These results are incorrect and are a consequence of GRAM not making any assumptions on the nature of chromatographic peak shapes. Evolving factor analysis (presented in the introduction) will also have this difficulty. Model-based peak-fitting algorithms may be used. The chromatographic models, however, may not represent the data well. In addition, "best-fit" solutions are susceptible to the initial estimates of peak number and location.[32] One possible solution to total overlap in one dimension of separation is to require full separation on the other dimension and use basic peak integration techniques for quantification.

The final requirement for appropriate use of GRAM is that no two compounds within the window of data analyzed can perfectly covary in concentration from the standard to the sample. This covariance possibility is minimized by performing GRAM on subsections of the entire GC×GC data set, which reduces the number of peaks analyzed at one time. A variant of GRAM, direct trilinear decomposition (DTD), removes this covariance restriction by comparing multiple standard data sets with the unknown.[33,34] As long as no two compounds covary between the unknown sample and at least one of the standard samples, successful deconvolution is possible. Precautions need to be taken that retention times are constant for each compound over the larger number of data sets used by DTD.

The GRAM algorithm requires the sample and standard mixture data to be in the form of a two-dimensional array, or matrix. The GC×GC data fit this form when they are arranged so that each row of the matrix represents a fixed time on the second GC column and each column of the matrix represents a fixed time on the first GC column. A more thorough description on this is given in the Results and Discussion section.

In the first step of the GRAM algorithm used, singular value decomposition (SVD) is first performed on the addition matrix,

$$(\mathbf{M} + \mathbf{N}) = \mathbf{U\Theta V}^{\mathrm{T}} \qquad (1)$$

which is the sum of the sample data **M** and the standard data **N** (matrices are denoted in boldface type). In this decomposition, **Θ** is a diagonal matrix, **U** and **V** are orthogonal matrices, and superscript T denotes a transpose. SVD is a well-known method of decomposing a matrix.[35] Performing SVD on (**M** + **N**) ensures that, if there are different components contained in the sample and standard data, all the components are included in the model.

Next, the decomposed components of the addition matrix, **U**, **Θ**, and **V**, are truncated according to the expected number of chemical components. For the ideal situation, the data contain no noise, and each chemical component has a unique retention time in both dimensions. In this case, the number of significant factors, or rank, of the addition matrix (**M** + **N**) is equal to the number of components. The presence of noise in all data requires that the rank be estimated or assumed. If, for example, the segment of the data analyzed is known to have three species, only the first three columns of the **U** and **V** matrices, and only the first three diagonal components of **Θ**, are retained. Malinowski has shown that a data matrix can be accurately reconstructed

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using only the significant factors found by SVD.[36] The remaining factors will contain only measurement error. The number of significant factors used in the GRAM analyses of the data presented here were easily determined through inspection by SVD, which is a popular method of rank determination.[37] Many other statistical methods for estimating rank have been developed, any one of which could be applied to GC×GC data.[36,38,39]

Third, the eigenvalue problem is solved that contains the truncated SVD components from the addition matrix (**M** + **N**) and the standard matrix **N**. In eq 2, **T** is the resulting matrix of

$$(\bar{\Theta}^{-1}\bar{\mathbf{U}}^{\mathrm{T}}\mathbf{N}\bar{\mathbf{V}})\mathbf{T} = \mathbf{T}\boldsymbol{\Pi} \tag{2}$$

the eigenvectors, and **Π** is the associated diagonal matrix containing the eigenvalues. The overbars denote the truncated versions of the associated matrices, and superscript −1 denotes an inverse. At this point, a similarity transform is used to eliminate any complex solutions that may arise from MATLAB's implementation of the generalized eigenvalue problem.[40]

The vector of analytes' concentrations in the standard, C_N, relative to those in the sample matrix, C_M, are determined from the eigenvalues.

$$\text{diagonal}(\boldsymbol{\Pi}) = \frac{C_N}{C_M + C_N} \tag{3}$$

The pure second GC column chromatographic elution profiles, **X**, and the pure first GC column chromatographic elution profiles, **Y**, of all the components common to both matrices (**M** and **N**) are determined with the eigenvectors and the decomposed components from SVD. The eigenvectors can be thought of as a

$$\mathbf{X} = \bar{\mathbf{U}}\bar{\Theta}\mathbf{T} \tag{4}$$

$$\mathbf{Y} = \bar{\mathbf{V}}(\mathbf{T}^{-1})^{\mathrm{T}} \tag{5}$$

rotation of the ambiguous SVD vectors of the addition matrix (**M** + **N**) into physically meaningful information. Although the signals may not be resolved in the sample or the standard, this is achievable because both the addition matrix and the standard matrix contain signals for the analytes of interest. The comparison of these two data sets allows an otherwise ambiguous decomposition to be rotated into the first and second dimension chromatographic profiles.

EXPERIMENTAL SECTION

Chemicals. White gas (Coleman fuel, Coleman Co., Wichita, KS) was used as a base for all the samples, to which was added between 0% and 3% (w/w) toluene, ethylbenzene, *m*-xylene, and propylbenzene (Aldrich Co., Milwaukee, WI). *o*-Xylene (Aldrich) at 1% (w/w) was added to all samples as an internal standard.

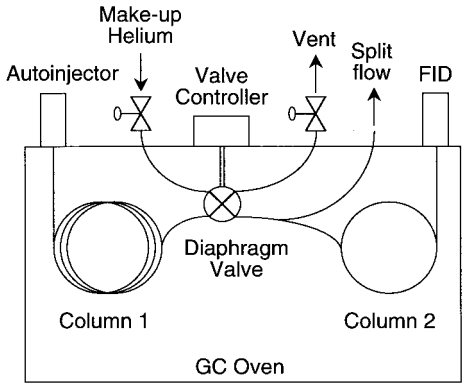

Figure 1. Schematic of GC×GC system. Compounds eluting from the nonpolar first column are diverted with a diaphragm valve twice per second to a polar short second column prior to flame ionization detection. Supply gas lines and system control lines are omitted for clarity.

GC×GC Apparatus. All GC×GC experiments were performed with a Varian gas chromatograph (model 3600cx, Varian, Sugar Land, TX) with flame ionization detection (FID). An autosampler (model 7673A, Hewlett-Packard, Palo Alto, CA), modified for use with the Varian GC, injected 1-μL samples to a split injector at 200 °C, which operated at a split ratio of 10:1. The column oven was held at 70 °C and the FID at 200 °C. Column head pressure was set at 13 psi. The first column of the GC×GC system (see Figure 1) was a 4.9-m × 530-μm capillary column with a 3-μm poly(dimethylsiloxane) film (SPB-1, Supelco, Bellefonte, PA). The second column was a 0.85-m × 180-μm column with a 0.15-μm poly(ethylene glycol) stationary phase (Carbowax, Quadrex Corp., New Haven, CT).

The first column effluent was briefly diverted twice per second to the second column, using a gas-actuated six-port diaphragm valve with low dead volume fittings (model 11, Applied Automation, Bartlesville, OK). Four of the ports were used. The diaphragm valve was actuated with nitrogen gas, which was diverted to the diaphragm valve with a three-port normally closed Skinner solenoid valve (model 71315, Honeywell, New Britain, CT), powered by a 24-V dc coil. The nitrogen lines to the diaphragm valve were enlarged to more rapidly deliver actuation gas. A 1-L pressurized reservoir between the house line and the solenoid valve provided buffering to ensure no significant reduction in the nitrogen supply pressure of 55 psi during diaphragm actuation. Makeup helium flow to the diaphragm valve was set using a low-flow controller included with the Varian GC. Helium flow through the vent line was set with a fine metering valve (Nupro, Alltech, Deerfield, IL). Carrier flow from the first column was split after the diaphragm valve between the second column and a 0.5-m length of 180-μm fused silica, so that the high volumetric flow from the large-bore first column would not result in an overly fast linear flow on the narrow-bore second column.

Reference GC. Validation of GC×GC quantification was performed by simultaneously analyzing modified white gas samples with a second Varian GC (model 3400). Samples were injected on-column to a 60-m × 530-μm capillary column with a 1-μm poly(ethylene glycol) stationary phase (Carbowax) with FID detection. The column oven was held at 60 °C for 11 min and then heated at 20 °C/min to a final temperature of 140 °C. Selected analytes of interest were fully resolved, and quantification

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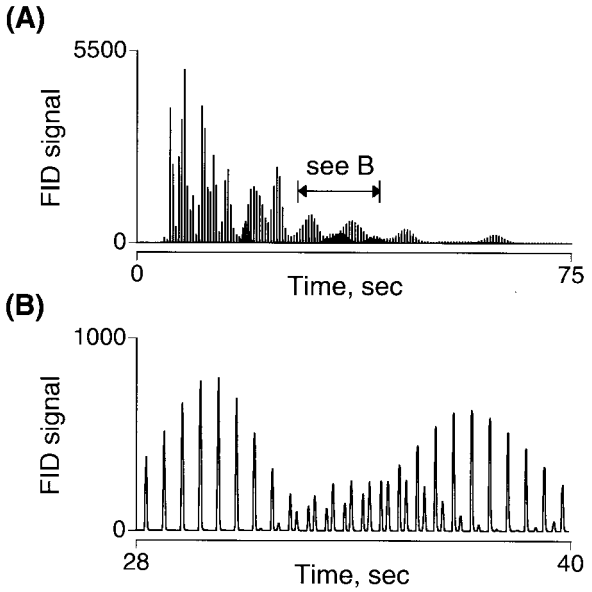

Figure 2. (A) Raw data from a GC×GC analysis of white gas sample 6, as defined in Table 1. (B) Close-up view of (A), highlighting the frequent second GC column modulation of compounds eluting from the first column.

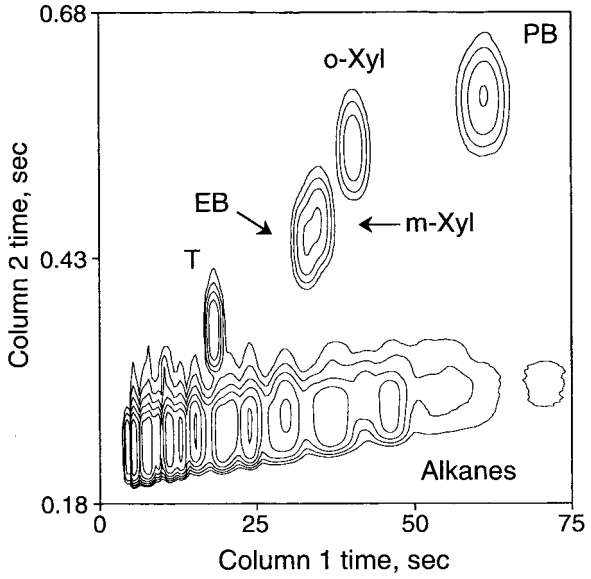

Figure 3. Contour plot of the transformed GC×GC analysis of sample 6, as defined in Table 1. The *x*-axis and *y*-axis represent the separations performed by the nonpolar first column and the polar second column, respectively. The alkanes are not as retained by the polar column as are the aromatics toluene (T), ethylbenzene (EB), *m*-xylene (m-Xyl), *o*-xylene (o-Xyl), and propylbenzene (PB).

was performed using peak area. FID relative response factors of the analytes to the internal standard, *o*-xylene, were the same for both gas chromatographs.

Data Collection and Analysis. Software written in-house controlled a data acquisition board (model AT-MIO-16XE-50, National Instruments, Austin, TX) to monitor the GC×GC FID signal at 500 points/s from the Varian 3600 and control the diaphragm valve. In-house software controlling a second data acquisition board (DACA, IBM, Boca Raton, FL) collected data from the Varian 3400. Conversion of the GC×GC data into a two-dimensional array, and subsequent deconvolution and quantification using the GRAM algorithm, were performed using the MATLAB 4.2c software package (The Mathworks Inc., Natick, MA) on a PowerMac 7200/90 MHz.

RESULTS AND DISCUSSION

GC×GC System Evaluation. Raw GC×GC data are in the form of a single vector, which is the time-dependent response of the FID to a mixture after being modulated by both GC columns. Figure 2A shows raw data for a GC×GC analysis of a white gas sample modified with several aromatic compounds. A detail view in Figure 2B illustrates how the first GC column separation is modulated by the short second column separation. Since the number of compounds eluting from the first column at any given instant is small, even for complex samples, the second column does not need to have a large peak capacity.

To elucidate the complementary separating powers of the two GC columns, the vector of data is transformed so that each chromatogram resulting from a transfer of effluent from the first GC column onto the second becomes a new column, or vector, in a two-dimensional matrix. Once in this form, the matrix rows reflect the separation performed by the second GC column, and the matrix columns represent the separation performed by the first GC column. Figure 3 shows an example of transformed data, using the data presented in Figure 2. The GC×GC data in Figure 3 are drawn as a contour plot, with each successive contour line reflecting a factor of 3 increase in signal. This representation of the signal axis allows small peaks to be revealed without overemphasizing the large compounds.

The effect of the different chemical selectivities of the GC columns is evident in Figure 3. The nonpolar first column separates primarily on the basis of boiling point, whereas the polar second column separation is more dependent on functional group. Hence, alkane compounds are separated by the first column but are minimally affected by the second column. In contrast, the second column is able to separate the aromatic compounds from the alkanes. The grouping of compound classes into distinct bands has also been seen in GC×GC analyses of more complex samples.[12,17] Compound class grouping simplifies sample classification, as well as individual peak identification.

Retention Time Reproducibility. A key benefit of the two-dimensional data provided by GC×GC is that overlapped compounds can be more easily quantified, allowing an analyst to accept incomplete separation of peaks in the interest of shortened analysis times. As was discussed in the Theory section, peak deconvolution will be accomplished by mathematically comparing two data sets, a calibration standard and a sample. Repeatability of retention times is especially important with fast GC×GC. Successive injections to the second column using the diaphragm valve must generate repeatable retention times, since the two-dimensional "spot" that represents each compound is formed from several second column chromatograms (see Figure 3). Short column retention time repeatability is influenced by the injection precision of the diaphragm valve. The performance of the diaphragm valve was evaluated in a preliminary experiment, prior to the assembly of the GC×GC system. The headspace of a dilute methanol, 1-propanol, and 2-butanol mixture in water was delivered to the diaphragm valve. The valve then repeatedly injected 50-ms pulses of the vapor onto a 1-m × 250-μm column with a 0.5-

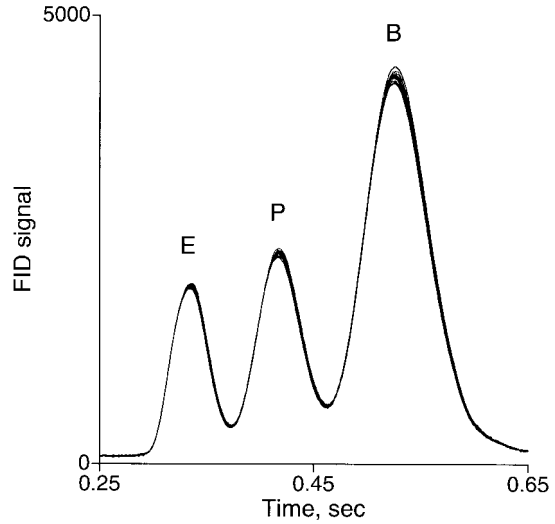

Figure 4. Overlay of chromatograms from 20 successive headspace injections, 0.55 s apart, of ethanol (E), 1-propanol (P), and 2-butanol (B) onto a 1-m column using the diaphragm valve as the injector. This figure demonstrates the high repeatability of chromatograms resulting from a short GC column and a reliable injector.

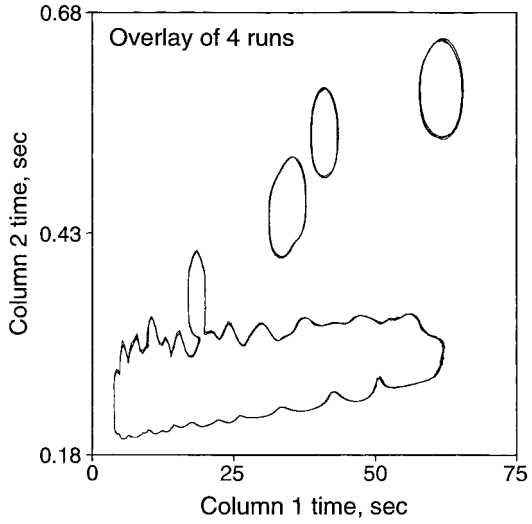

Figure 5. Overlay of contour plots of four GC×GC runs of sample 6, as defined in Table 1. Each run is represented by a single contour line at a fixed detector response. The high repeatability of compound retention times in short column separations, shown by the near total peak overlap, implies that different GC×GC runs can be quantitatively compared using chemometric techniques.

μm film of poly(trifluoropropylmethylsiloxane) at 35 °C. Figure 4 shows an overlay of 20 successive injections, with an injection occurring every 0.55 s. The fluctuation in the amount of sample injected, determined from the integrated area of each chromatogram, was limited to a small 1.4% relative standard deviation. We define relative retention time uncertainty as δ, where δ is calculated as the standard deviation of a peak's retention time from multiple injections, divided by the width of the peak at its base. Based upon our previous work, the smaller the δ, the easier it is to reliably quantify peaks that are poorly resolved.[18] In these experiments, δ was small, with a value of 0.003. This preliminary experiment indicated that fast second column GC separations of compounds eluting from a first column could provide the repeatable, "spectra-like" information necessary for successful peak deconvolution.

Figure 5 shows an overlay of four GC×GC analyses of a white gas sample, each of which is represented as a single-line contour plot. The δ of retention times on the second column was estimated to be 0.01, marginally larger than for the data shown in Figure 4. Nonetheless, the individual peaks do not have jagged contour lines, which is a testament to the repeatable performance of the diaphragm valve and the high precision in retention times on the second column. Additionally, there is a high degree of repeatability in retention times between GC×GC runs, as can be seen from the near total overlap of replicate runs. This repeatability simplifies a mathematical comparison of two data sets when peaks are being deconvoluted. In instances where long-term retention time drift is significant, data pretreatment can be used to standardize the chromatograms. Andersson and Hämäläinen used reference peaks at the beginning and end of single-column GC chromatograms to align the data sets prior to analysis.[41] Prazen et al. developed a standardization technique for multidimensional liquid chromatography/UV−visible absorbance data, which could be used for GC×GC data.[19] Linear interpolation techniques can be used to correct for long-term retention time drift on one or both GC columns of a GC×GC system. However, we observed that the amount of correction necessary for short columns was minimal, and the data presented in this article would not benefit by any retention time correction.

Quantifying Overlapped Peaks. GRAM analysis of the pure propylbenzene peak (see Figure 3) was first performed to establish a baseline level of precision in the quantification of a single resolved analyte. Error in quantification arises from instrumental noise that is propagated through the GRAM analysis. Instrumental noise consists of detector noise, retention time imprecision, split injector sample discrimination, and imprecision in sample transfer between GC columns, among other sources. Given these sources of variation, using a standard with 1.1% (w/w) propylbenzene, GRAM accurately predicted amounts of propylbenzene in white gas samples containing 1%−3% propylbenzene. The predictions varied with a 1.3% relative standard deviation.

To demonstrate the feasibility of GRAM in quantifying overlapped peaks, the overlapped peak group composed of ethylbenzene and *m*-xylene in white gas samples was chosen as a model case for analysis. Figure 6 illustrates the subset of data used. Note that the individual analytes are severely overlapped on both GC dimensions. The deconvoluted profiles shown in Figure 7 are GRAM estimates from a comparison of these two data sets, with the peak magnitudes reflecting the amounts of ethylbenzene and *m*-xylene in the sample. Based on the predicted profiles, the first GC column chromatographic resolution of these peaks is 0.46. Despite this low resolution, the additional information provided by the second GC column (resolution = 0.20) allows these peaks to be successfully deconvoluted. The GRAM-predicted profiles can be used to validate the peak deconvolution. If the profiles do not resemble "chromatographic" peaks, GRAM quantification is suspect.

Deconvolution of the overlapped ethylbenzene and *m*-xylene in varying relative amounts was performed to more extensively examine the model system. Figure 8 summarizes the different

(41) Andersson, R.; Hämäläinen, M. D. *Chemom. Intell. Lab. Syst.* **1994**, *22*, 49−61.

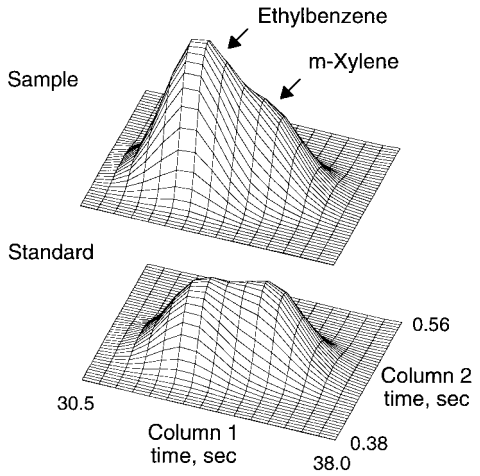

Figure 6. Close-up view of the data for the overlapped ethylbenzene and *m*-xylene peaks from GC×GC runs of sets 4 (top) and 6 (bottom), as defined in Table 1. GRAM uses the calibration standard to determine the amounts of ethylbenzene and *m*-xylene in the sample. For clarity, every other point on the second GC column axis was plotted.

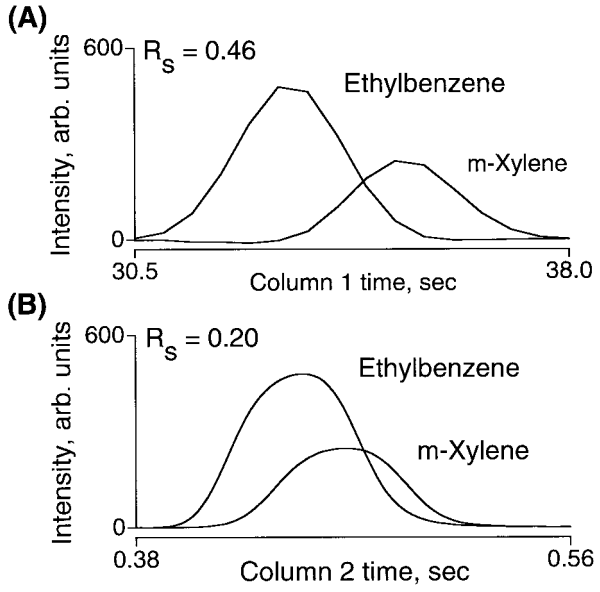

Figure 7. GRAM deconvolution of overlapped peaks in GC×GC, producing pure compound elution profiles showing the separation performed by (A) the first column and (B) the second column. Despite the low resolution, high precision in GC×GC retention times between the sample (set 4) and standard (set 6) GC×GC runs makes successful GRAM deconvolution possible.

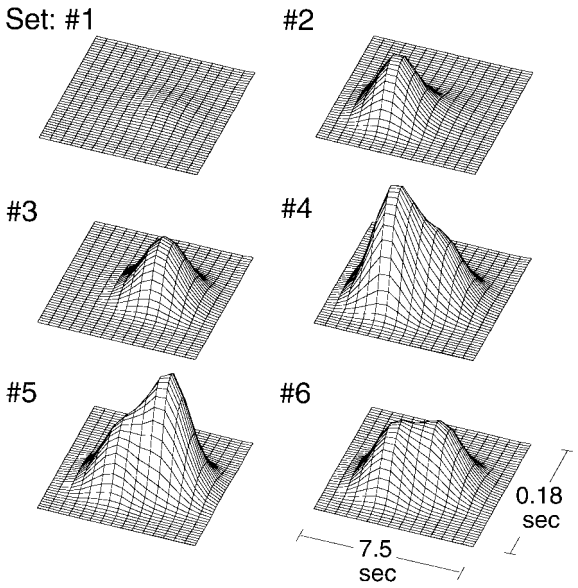

Figure 8. Quantification of various relative amounts of overlapped ethylbenzene and *m*-xylene using GRAM. Table 1 results use GC×GC data from set 6 as the standard to quantify the overlapped components in data sets 1−5. Table 2 results use GC×GC data from set 3 as the standard to quantify the overlapped components in the remaining data sets.

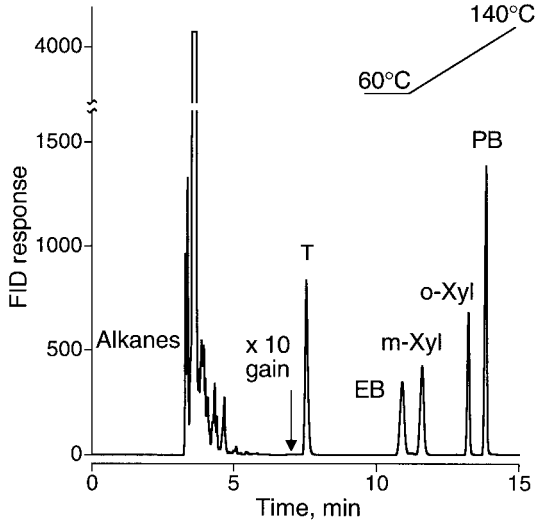

Figure 9. Reference single column chromatogram of a modified white gas sample (data set 6). Compound labels are the same as in Figure 3. Detector sensitivity was increased by a factor of 10 at 7 min. Conditions were adjusted to resolve aromatic compounds. Predicted concentrations using peak area from this GC method are compared to predicted concentrations of overlapped GC×GC peaks in Tables 1 and 2.

data sets examined. In the first case, set 6 was used as the analytical standard to quantify the analytes in sets 1−5. This case represents the situation in which all of the overlapped analytes in the analytical sample are present in the standard. GRAM quantification results for a given sample/standard pair are reported as the mean and standard deviation values of 16 combinations of sample and standard, since each of the white gas samples was run four times. Replicate analyses were performed to determine the confidence intervals in prediction, as well as to minimize bias resulting from any individual outlier runs.

The accuracy of GRAM concentration predictions was evaluated by performing standard single-column GC analysis on the same samples injected onto the GC×GC system. Figure 9 illustrates a chromatogram of a sample in set 6. The chromatographic conditions were chosen to resolve the various aromatic analytes of interest. After using an internal standard to correct for variations in injected sample amounts, peak areas were reproducible to within 0.5% RSD. Sample analyte concentrations were determined by comparing the chromatographic peak areas of the samples to the peak areas of the standard, which had gravimetrically prepared amounts of all the analytes of interest. Predicted concentrations of the resolved ethylbenzene and *m*-xylene analytes using single long-column GC were then compared

Table 1. Quantifying Selected Compounds in Modified White Gas Samples by Reference GC and by High-Speed GC×GC

	ethylbenzene (%, w/w)		*m*-xylene (%, w/w)	
set no.	ref[a]	GRAM[b] (RSD,%)[c]	ref[a]	GRAM[b] (RSD, %)[c]
1	0.031	0.024 (7.8)	0.090	0.094 (2.9)
2	1.10	1.09 (4.1)	0.090	0.097 (5.0)
3	0.031	0.016 (42)	1.11	1.08 (2.7)
4	2.20	2.19 (5.9)	1.11	1.07 (3.0)
5	1.12	1.06 (5.9)	2.13	2.20 (4.7)
6[d]	1.11[d]		1.12[d]	

[a] Quantifying resolved peaks by peak area using reference single-column GC method. [b] Quantifying incompletely resolved components in GC×GC by GRAM (see Figures 6−8). [c] RSD is the relative standard deviation of predictions, expressed as a percentage. [d] Set 6 used as standard. Compound concentrations were determined gravimetrically during sample preparation.

Table 2. Quantifying *m*-Xylene in Modified White Gas Samples by Reference GC and High-Speed GC×GC, in Which an Interference Overlaps *m*-Xylene

	m-xylene (%, w/w)		
set no.	ref[a]	GRAM[b] (RSD, %)[c]	relative amount of interference to *m*-xylene[d]
1	0.091	0.102 (7.1)	1:3
2	0.091	0.101 (4.8)	12:1
3[e]	1.13[e]		1:35
4	1.12	1.11 (2.8)	2:1
5	2.16	2.29 (4.3)	1:2
6	1.13	1.16 (2.7)	1:1

[a] Quantifying by peak area using reference single-column GC method. [b] Quantifying while overlapped with interfering compound in GC×GC using GRAM. [c] RSD is the relative standard deviation of predictions, expressed as a percentage. [d] Ethylbenzene is treated as an interference as it is essentially not present in the standard. Relative amounts based on peak area using reference single-column GC method. [e] Set 3 used as standard. Its *m*-xylene concentration was determined gravimetrically during sample preparation.

with GRAM predictions of the same analytes that were incompletely resolved in fast GC×GC.

A suitably selected single high-efficiency column could have been chosen to separate the compounds of white gas in a time frame comparable to that of the presented GC×GC analysis. The advantage of GC×GC would be realized in the analysis of complex samples such as petroleum, where the extraordinarily high peak capacities that GC×GC can generate are particularly useful.[10,17] The modified white gas analysis detailed here is treated as a test case only, to demonstrate the feasibility of second-order analysis on GC×GC data for the purpose of quantifying overlapped peaks.

Table 1 summarizes the quantification results for the GC×GC test cases. Compared to the reference method, most of the GRAM predictions were accurate within the stated confidence intervals. Relative standard deviations in the GRAM prediction ranged from 3% to 8%, the one exception being ethylbenzene in set 3. However, in this example, ethylbenzene was overlapped with an *m*-xylene peak that was 35 times larger. At some point, a large mismatch in signal sizes will make accurate and precise quantification of overlapped compounds difficult, independent of the analysis method used. In set 1, ethylbenzene was predicted at 0.024%, below the reference value of 0.031%. This result is understandable when one considers that the ethylbenzene concentration of the sample was 35 times smaller than the analytical standard, which had 1.1% ethylbenzene. With any calibration technique, especially a single-point calibration method such as GRAM, the best quantification results are obtained when the standard is similar in composition to the tested sample.

A more challenging analytical case arises when an interfering compound peak overlaps the peaks of interest. The "interference" is a compound not accounted for in the calibration standard. Fortunately, GC×GC, coupled with second-order techniques such as GRAM, is able to handle these situations. In the second case to be examined, data set 3 (see Figure 8) is used as the standard to determine the amount of *m*-xylene in a sample that also contains an overlapped interference, ethylbenzene. Ethylbenzene is not present in the standard at an appreciable level, existing at only 1/35th the concentration of *m*-xylene. Table 2 summarizes the results. In most cases, GRAM accurately predicted the amount of *m*-xylene when overlapped with an interference. The worst case was for data set 2, in which the interference signal was 12 times the signal of *m*-xylene, to the extent that only the interference peak is discernible (see Figure 8). Nevertheless, GRAM is able to predict a concentration that is within 10% of the reference GC value, with a relative standard deviation of only 5%.

Net Analyte Signal. In situations for which short analysis times are critical, the chromatographer can choose to accept reduced resolution separations, on the condition that the requisite information can still be extracted. Successful analysis of overlapped analytes using GRAM or other techniques requires each analyte's chromatographic profile to have a minimum level of distinctiveness. It is not sufficient to set a minimum resolution as the sole criterion for successful analysis. To have a distinct response, the individual peaks require a combination of some resolution, adequate run-to-run retention time precision in the second GC column,[18] a minimum signal relative to the other peaks, and a minimum sampling frequency.[26] Groups of overlapped peaks are considered adequately sampled if enough data points define a given peak on both the first and second dimensions of separation. The distinctiveness of each peak can be described by its net analyte signal (NAS).[42,43] An analyte with a larger NAS is easier to quantify. Additionally, quantification is degraded by noise. Noise consists of instrumental noise and error in run-to-run retention times. We are currently investigating the effectiveness of using an overlapped peak's calculated NAS/noise, also known as second-order S/N, to estimate the confidence intervals in its quantification. Using confidence intervals, a chromatographer will be in a better position to determine the minimum level of resolution that can be accepted for a given analysis, for the sake of reducing run times.

CONCLUSIONS

The field of GC×GC is relatively new. It is proving to be a powerful technique that can quickly analyze complicated samples.[17] The distinct patterns into which different compound classes cluster simplifies sample characterization. Chemometric techniques used in spectroscopic analysis are readily available to allow a chro-

(42) Booksh, K. S.; Kowalski, B. R. *Anal. Chem.* **1994**, *66*, 782A−791A.
(43) Faber, K.; Lorber, A.; Kowalski, B. R. *J. Chemom.* **1997**, *11*, 419−461.

matographer to explore the field of fast, reduced resolution chromatography. These techniques in particular are applicable in process chromatography, where quick analyses are required for effective process control. Since GRAM and other select algorithms can reliably quantify overlapped compounds when an interfering compound is present in the sample, these techniques may provide additional robustness when process upsets occur. This paper is an attempt to bridge the gap between the fields of chromatography and chemometrics. Although there may be obstacles on the road to integrating these fields, the potential rewards are high.

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