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# GENERALIZED RANK ANNIHILATION METHOD

## I. APPLICATION TO LIQUID CHROMATOGRAPHY–DIODE ARRAY ULTRAVIOLET DETECTION DATA

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SUMMARY

Qualitative and quantitative analysis of complex mixtures in fast, low-resolution liquid chromatography (LC) with a diode array-UV (DA-UV) spectrometer as a detector, is presented as a direct application of the recently developed "generalized rank annihilation method" (GRAM). GRAM operates on bilinear data, such as LC–DA-UV, and requires the analysis of two samples, *e.g.*, an unknown and a calibration. The samples may have several components in common, which are the components of interest for the analysis. The chromatograms of the two samples are obtained on the same column, under the same conditions, to insure the best possible match in retention times. A reference compound should be included in both samples to verify reproducibility of the relative retention times.

For quantitative analysis, one sample is defined as a calibration mixture. From the two data matrices corresponding to the two samples, GRAM will generate the following information about each shared component, *i.e.*, compounds present in both samples: an extracted spectrum, a resolved chromatogram, and the relative concentration (unknown:calibration).

## INTRODUCTION

Quantitative and qualitative analysis of a mixture with chromatography and a single-channel detector, *e.g.*, a flame ionization detector, usually requires that the components must be totally resolved into separate peaks[1]. When two or more peaks overlap, the quality of the results declines, and if the overlap is very significant, both quantitative and qualitative analysis become nearly impossible. Identification is usually accomplished using retention time information by comparison of an unknown to an earlier analysis of pure standards.

When using a multichannel detector, *e.g.*, a diode-array UV–visible (DA-UV) spectrometer, less chromatographic resolution is required than for a single-channel detector[2]. Qualitative analysis is possible for moderately overlapped components, even when several components overlap, if we know the pure spectrum of the analyte

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of interest and use target factor analysis to verify its presence (*cf.* refs. 3 and 4). Both qualitative and quantitative analysis are possible with curve resolution, if no more than three peaks overlap at a time, the overlap is not too severe and the spectra are not very similar[5–9]. Curve resolution for more than three components has recently been presented in the literature, for cases with little overlap[9].

The method of rank annihilation (RA) is the only method reported in the literature that allows for quantitative analysis in the presence of several unknown interfering components[10–12]. McCue and Malinowski[13] applied RA to liquid chromatography (LC) with UV detection in mixtures of a few components. A disadvantage of the RA method is that it can quantitate only a single analyte at a time. Therefore, multicomponent analysis requires obtaining a separate calibration chromatogram for each overlapped analyte under exactly the same conditions as for the unknown mixture. Furthermore, if the absolute retention times of the unknown sample are not accurately reproduced in the calibration runs, RA produces erroneous results[14].

The *generalized* rank annihilation method (GRAM) is a calibration and curve

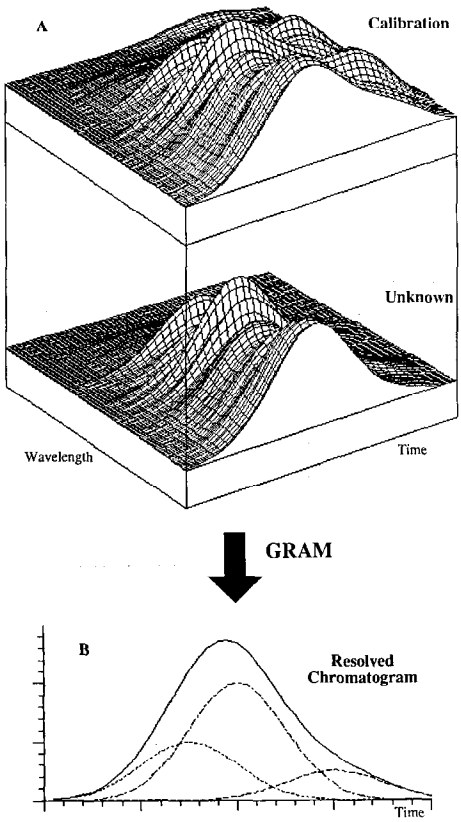



Fig. 1. Illustration of GRAM. (A) Given the bilinear data matrices of the calibration sample and the unknown sample, (B) the resolved profiles can be determined, as well as the corresponding spectra and concentration ratios.

resolution method for multicomponent, bilinear data arrays[15]. Fig. 1 illustrates GRAM as applied to chromatography. Here, bilinear data arrays are defined as two-dimensional data arrays where the contribution from each chemical component to the data can be expressed as the outer product of two vectors. Examples include chromatography–spectroscopy combinations, emission excitation fluorescence[10] and certain other combination methods. Not every two-dimensional technique is bilinear, however; two-dimensional mass spectrometry and two-dimensional nuclear magnetic resonance spectroscopy do not satisfy the bilinear definition. For quantitative analysis, an advantage of GRAM over other calibration methods is that *the unknown sample can contain components not present in the calibration sample*, or *vice versa*, yet accurate concentration estimations are still possible. Another advantage of GRAM is that, for each component present in both calibration and unknown samples, the pure bilinear spectrum can be obtained from the calculation[15].

The quality of the results obtained with GRAM is a function of several factors. The most important factors are noise level, number of overlapped components, similarity of the concentration ratios, similarity of spectra and degree of chromatographic overlap (resolution). A study characterizing the effects of these parameters and the limitations of GRAM will be forthcoming[16].

In order to apply GRAM to LC–UV data, chromatographic data from two samples must be generated on the same column, under the same conditions. These samples should have in common the set of analytes that are of interest. It is necessary that the retention times ($t_R$) in both analyses be the same, as accurately as possible. The more different are the $t_R$ values, the less accurate are the GRAM results. The problems of $t_R$ non-reproducibility demand special data treatment and will be discussed in a separate publication[14].

In this paper, we demonstrate the applicability of GRAM to chromatographic data of multicomponent samples. The complex mathematical formulation of GRAM (see ref. 15) has been avoided to give more emphasis to the results which it produces. The possibilities of GRAM are presented through examples of its application to simulated and real multicomponent samples.

## THEORY

Only a brief description of GRAM as applied to LC–DA-UV data will be presented here. First, a detailed presentation of the bilinear nature of the LC–DA-UV data is given. It can be shown that, if Beer's law can model the absorption of light in a chromatographic detector, the data can be factored as the product of three matrices, which separate the spectral, concentration and chromatographic information. Because GRAM works with bilinear data, LC–DA-UV data are suitable for GRAM. Some of the limitations imposed by the equations are described in practical terms.

In the following, boldface capital letters are used for matrices, *e.g.*, $\mathbf{X}$; superscript T for transposed vectors and matrices, *e.g.*, $\mathbf{Y}^T$; superscript $-1$ for the inverse of a matrix, *e.g.*, $\boldsymbol{\lambda}^{-1}$; superscript $+$ for the pseudoinverse of a matrix, *e.g.*, $\mathbf{M}^+$; boldface lower case characters for column vectors, *e.g.*, $\mathbf{y}_k$; and plain, lower case characters for scalars, *e.g.*, $\beta_k$.

*Bilinear data*

Beer's law assumes a *linear* relation between the concentration of a single analyte in a homogeneous solution and the absorption of light by that solution at a given wavelength, $\lambda_0$:

$$A = a\,b\,c \tag{1}$$

where $A$ is the absorption of light at wavelength $\lambda_0$, $a$ is the absorptivity constant at that wavelength, $b$ is a proportionality constant, and $c$ is the concentration of the analyte in the solution. In general, we can express eqn. 1 for every wavelength $\lambda$ as

$$A(\lambda) = a(\lambda)\,b\,c \tag{2}$$

where $a(\lambda)$ is a continuous function of the wavelength, and represents the spectrum of the analyte.

Since the concentration $c$ of an analyte at the output of a chromatographic column is not a constant, but a function of time, the absorption of light is therefore a function of both the wavelength and time ($t$):

$$A(\lambda,t) = a(\lambda)\,b\,c(t) \tag{3}$$

$A(\lambda,t)$ is a function of $\lambda$ and $t$, independently, *i.e.*, it is a *bilinear* function. In practical terms, this means that the spectrum $a(\lambda)$ is the same (within the noise level) at all times within the chromatographic peak. Similarly, the shape of the chromatographic peak is the same at every wavelength, $\lambda$, only differing by a proportionality factor.

The data collected from a liquid chromatograph with a DA-UV spectrometer as a detector can be modeled by eqn. 3. But, the data are not collected in a continuous fashion. The absorption is measured at certain wavelengths ($\lambda_1, \lambda_2, \cdots, \lambda_m$), producing a spectrum vector, $\mathbf{a} = (a_1, a_2, \cdots, a_m)$, for every scan. Similarly, a full spectrum is measured at certain times ($t_1, t_2, \cdots, t_n$), yielding a concentration profile vector, $\mathbf{c} = (c_1, c_2, \cdots, c_n)$, for every wavelength. Thus, eqn. 3 takes the form

$$A(\lambda_i,t_j) = a(\lambda_i)\,b\,c(t_j) = a_i\,b\,c_j \tag{4}$$

The data can be assembled into a matrix, $\mathbf{A}$ ($m \times n$). The rows are assigned to the different wavelengths and the columns to the different times corresponding to the acquired spectral scans. In matrix notation, eqn. 4 can be expressed as an outer product,

$$\mathbf{A} = \mathbf{a}\,b\,\mathbf{c}^{\mathrm{T}} \tag{5}$$

In general, any analytical technique for which the data of a single component can be factored as in eqn. 5, is defined as a bilinear technique,

$$\mathbf{M} = \mathbf{x}\,\beta\,\mathbf{y}^{\mathrm{T}} \tag{6}$$

where $\mathbf{M}$ is the data matrix, $\mathbf{x}$ and $\mathbf{y}$ are data vectors in the two different orders (*e.g.*, wavelength and time), and $\beta$ is a proportionality constant. For chromatography–spectroscopy combinations, the vector $\mathbf{x}$ corresponds to the normalized *spectrum* of

the analyte (normalized **a**) and **y** corresponds to the normalized *concentration profile* or peak shape (normalized **c**). By defining **y** as normalized, its absolute concentration information is lost (in **y**), but is retained in the constant $\beta$.

For multicomponent samples, with $p$ components, the resultant data matrix can usually be approximated by the sum of the $p$ individual bilinear contributions,

$$\mathbf{M} = \sum_{k=1}^{p} \mathbf{x}_k \, \beta_k \, \mathbf{y}_k^{\mathrm{T}} \tag{7}$$

or, in matrix form

$$\mathbf{M} = \mathbf{X} \, \boldsymbol{\beta} \, \mathbf{Y}^{\mathrm{T}} \tag{8}$$

where the $k$th column of the matrix $\mathbf{X}$ ($m \times p$) corresponds to the spectrum $\mathbf{x}_k$; the $k$th row of the matrix $\mathbf{Y}^{\mathrm{T}}$ ($p \times n$) corresponds to the chromatogram $\mathbf{y}_k^{\mathrm{T}}$; $\boldsymbol{\beta}$ is a diagonal matrix with $\boldsymbol{\beta}_{kk} = \beta_k$, which are proportional to the concentrations; $m$ is the number of wavelengths and $n$ is the number of scans in the chromatogram.

*Generalized rank annihilation*

The **M** matrix is generated as the output data from the LC–UV instrument. The matrices **X**, $\boldsymbol{\beta}$ and **Y** are usually unknown. It is the goal of GRAM to estimate these matrices.

A minimum of two samples are necessary to perform a GRAM determination. For quantitation purposes, one is defined as the calibration sample, and the other as the unknown, or test, sample. Thus, two data matrices are obtained from the chromatographic–spectroscopic data: define **M** as the unknown sample, and **N** as the calibration sample.

The matrix **M** can be modeled as in eqn. 8. The calibration matrix **N** can be modeled with a similar equation,

$$\mathbf{N} = \mathbf{X} \, \boldsymbol{\xi} \, \mathbf{Y}^{\mathrm{T}} \tag{9}$$

where $\xi$ is a diagonal matrix, similar to $\boldsymbol{\beta}$. For simplicity, assume that the matrices **X** and **Y** are the same for **M** and **N**. These equations are still valid even if the samples do not have exactly the same components, as long as the matrices **X** and **Y** include all the components present in both samples. If some components are not present in the unknown sample (or the calibration sample), eqn. 8 (or 9) still models the data if the corresponding diagonal elements of $\boldsymbol{\beta}$ (or $\xi$) are zero.

Eqn. 9 implies that the retention times and peak shapes (**Y** matrix) are the same in the two samples; in fact, if they are not equal, GRAM will not work. The following discussion assumes that this condition is valid. When retention times are not reproduced, a preliminary transformation step is necessary to use GRAM (see ref. 14).

Eqns. 8 and 9 comprise a system of two matrix equations with four unknowns: **X**, $\mathbf{Y}^{\mathrm{T}}$, $\xi$ and $\boldsymbol{\beta}$. Under certain conditions, these equations can be rearranged to a form solvable by eigenanalysis[15]. Briefly, the conditions necessary to solve the equations are:

(1) The spectra $\mathbf{x}_k$ and the chromatograms $\mathbf{y}_k$ of all components present in both **M** and **N** must be linearly independent. Therefore, if the spectra of overlapped components are identical, a solution can not be found. Similarly, total overlap (resolution $R_s = 0.0$) is also unsolvable[17].

(2) $\xi_k/\beta_k \neq \xi_j/\beta_j$ for every $j \neq k$. This means that the ratio of concentrations calibration/unknown must be different for different analytes.

These limitations are serious if we consider a complex mixture with hundreds of analytes and experimental noise. In practice, however, the data are subdivided in narrow windows with a few components, where these conditions are easily met, and each window is solved separately.

## EXPERIMENTAL

### *Equipment*

The chromatography hardware consisted of two Beckman (Berkeley, CA, U.S.A.) 114M pumps, a Beckman 340 $\mu$flow mixer, a Valco (Houston, TX, U.S.A.) 10-port injection valve fitted with a 10-$\mu$l injection loop, and a Hewlett-Packard (Palo Alto, CA, U.S.A.) 1040A DA-UV detector. Program control was provided by a Beckman 421A LC controller, while data acquisition and storage were accomplished by a Hewlett-Packard 85B computer and 9121 dual floppy disk drive.

### *Reagents*

The mobile phase solvents, UV-grade acetonitrile and water, were obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). Polynuclear aromatic hydrocarbon standards were purchased from Chem Services (West Chester, PA, U.S.A.), and included: acenaphthylene (Acen), phenanthrene (Phen), benz[*a*]anthracene (B*a*A), anthracene (Anth), chrysene (Chry), benzo[*a*]pyrene (B*a*P), benzo[*e*]pyrene (B*e*P), benzo[*b*]fluoranthene (B*b*Fl), benzo[*k*]fluoranthene (B*k*Fl) and perylene (PER).

### *Procedures*

A 5-$\mu$m $C_8$, 150 × 4.6 mm column (Brownlee, Santa Clara, CA, U.S.A.) was used in the LC analyses. The LC analyses followed a basic procedural outline: an events table was first created in the 421A controller, including % of solvent B, mobile phase flow-rate, time of injection and time for initiating data acquisition. Calibration and test samples were analyzed in exactly the same manner, one immediately after the other, to minimize error in retention time reproducibility.

Data acquisition was initiated upon a command from the 421A controller and was terminated at the time entered for stop-time in the HP 85B system. The DA-UV detector was operated in the "periodic spectra" mode, in which full spectral scans from 210–400 nm were acquired at a rate of *ca.* 1 scan/s, with a bandwidth of 2 nm.

### *Computation*

Raw data binary files stored on floppy disks were translated to ASCII format, then transferred to a VAX Station II (Digital Equipment Corporation, Marlboro, MA, U.S.A.). The data processing routines were implemented on the VAX station, and included routines for generating simulated chromatograms, a multiple linear regression routine which used to estimate the resolved chromatographic profiles based on a knowledge of the input components, and GRAM[15].

## RESULTS AND DISCUSSION

### *Simulated data*

Two simulations were performed to illustrate the utility of the method for multicomponent analysis. Real spectra and Gaussian chromatographic peak profiles were used for the simulations. In every case, the peak width of the chromatograms was set equal to 20 s.

As mentioned earlier, the quality of the results obtained with GRAM is a function of several factors, the most important being noise level, number of overlapped components, similarity of the concentration ratios, similarity of the spectra and degree of chromatographic overlap (resolution).

Fig. 2 shows the total wavelength chromatogram (TWC) of the first simulation samples. The TWC is defined as the chromatogram resulting from summing the absorptions at all wavelengths for each scan. Table I presents a summary of the details of this simulation. Gaussian-distributed noise was added to the matrices to simulate experimental noise as 1% of the average signal value.

The "unknown", or test, sample and the calibration sample have several components in common (Phen, B*a*A, Chry, B*b*Fl and B*e*P); two components are present only in the test sample, namely Acen and Anth; and two components are present

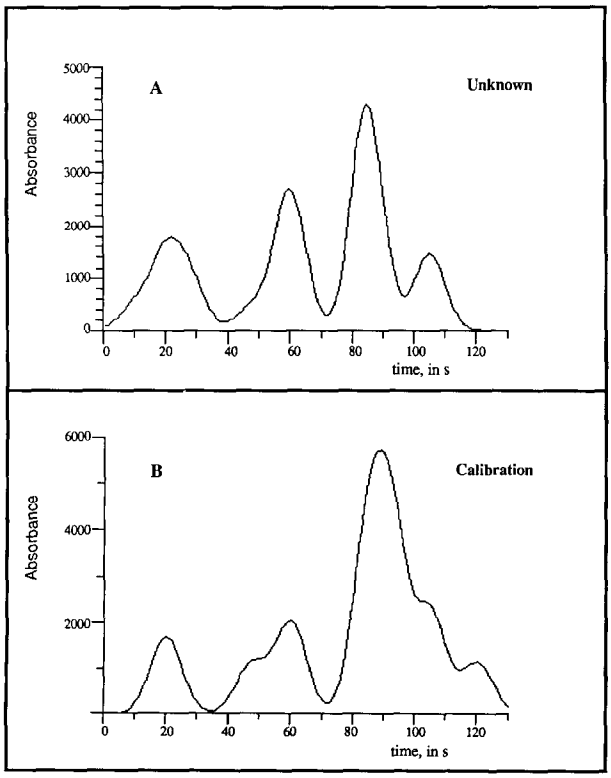



Fig. 2. Total wavelength chromatograms (TWC) for the first simulation: (A) "unknown", or test, sample; (B) calibration sample.

TABLE I

MULTIPLE COMPONENT SIMULATION WITH 1% NOISE

Noise is 1% of the average absorbance. Concentrations in arbitrary units. The groups correspond to peak clusters.

| *Component* | $t_R$ *(s)* | *Input concentrations* | | *Estimated concentration* |
|---|---|---|---|---|
| | | *Calibration* | *Test* | |
| Acen | 10.0 | 0.000 | 0.500 | – |
| Phen | 20.0 | 1.000 | 0.800 | 0.801 |
| Anth | 27.5 | 0.000 | 1.000 | – |
| B*a*A | 47.5 | 0.500 | 0.200 | 0.200 |
| Chry | 60.0 | 1.500 | 2.000 | 2.000 |
| B*b*Fl | 85.0 | 0.900 | 1.000 | 1.000 |
| B*k*Fl | 92.5 | 1.000 | 0.000 | 0.000 |
| B*e*P | 105.0 | 0.600 | 0.400 | 0.400 |
| B*a*P | 120.0 | 0.300 | 0.000 | 0.000 |

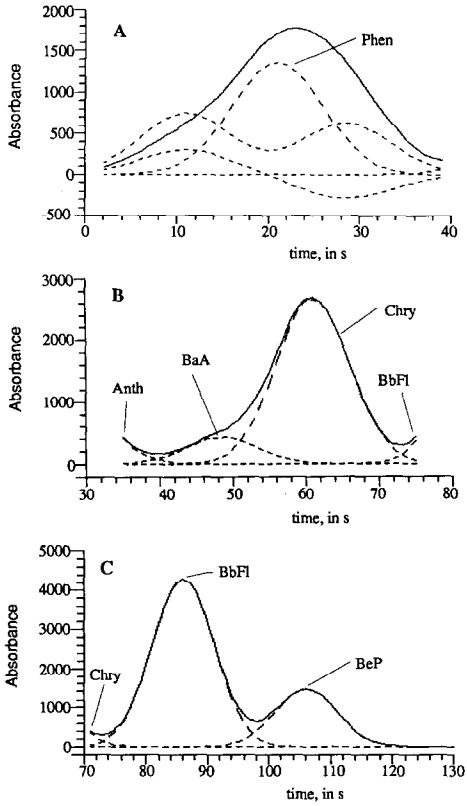



Fig. 3. GRAM-resolved chromatographic profiles for simulated complex test sample, divided by subregions. ——, TWCs; ----------, resolved chromatograms. (A) Only the chromatogram of Phen is resolved, because the other two components (Acen and Anth) are not present in the calibration sample. The other two broken lines are linear combinations of the actual solutions. (B) The chromatograms of Chry and B*a*A are correctly estimated, and also part of the previously unresolved Anth appears resolved in this region. (C) Estimated chromatograms for B*b*Fl and B*e*P.

only in the calibration sample, B*k*Fl and B*a*P. Therefore, these samples represent the most general cases that a chromatographer might face in real conditions.

The data matrices were arbitrarily cut into three workable windows, corresponding to points near the valleys of the TWC. GRAM was applied to each of these windows, and the resultant resolved chromatograms of the test sample are presented in Fig. 3.

Fig. 3A represents the the first peak cluster, in the 0–40-s range. Table I shows that the calibration sample has only one component in this range, Phen, whereas, the test sample has three: Acen, Phen and Anth. Therefore, the only component that is correctly resolved is the one present in both samples, *i.e.*, Phen. The two other curves represent linear combinations of the other two components, and could be resolved using two-component self-modeling curve resolution[5]. This cluster illustrates the most important feature of GRAM: the ability to quantitate an unresolved component, in this case Phen, which is overlapped with other, unknown components in the test sample. The other two components are not resolved because they violate one of GRAM's requirements, *i.e.*, the concentration ratios must be different; for both components the ratio of concentrations calibration/unknown is zero.

Fig. 3B represents the second peak cluster, in the 40–70-s range. Both samples

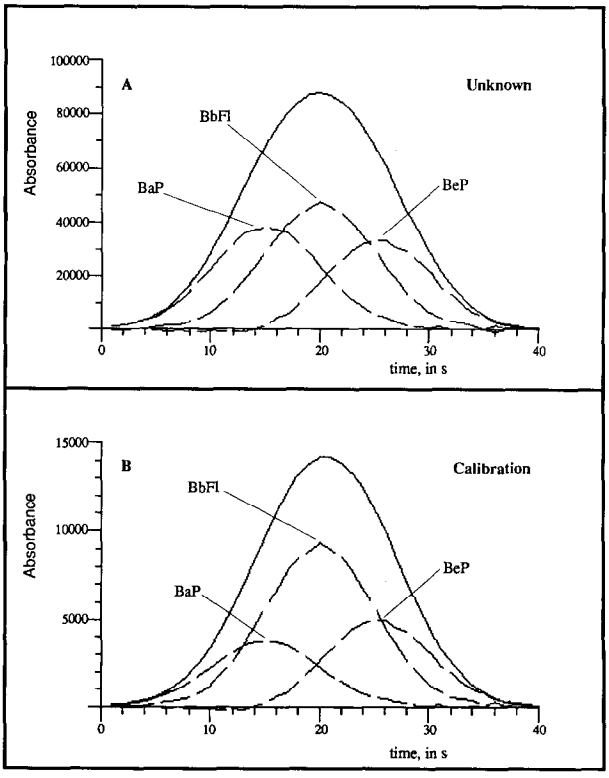



Fig. 4. Results of the second simulation, with 4% noise: GRAM resolved concentration profiles for both the "unknown" and the calibration samples. ——, TWCs; – – –, resolved chromatograms.

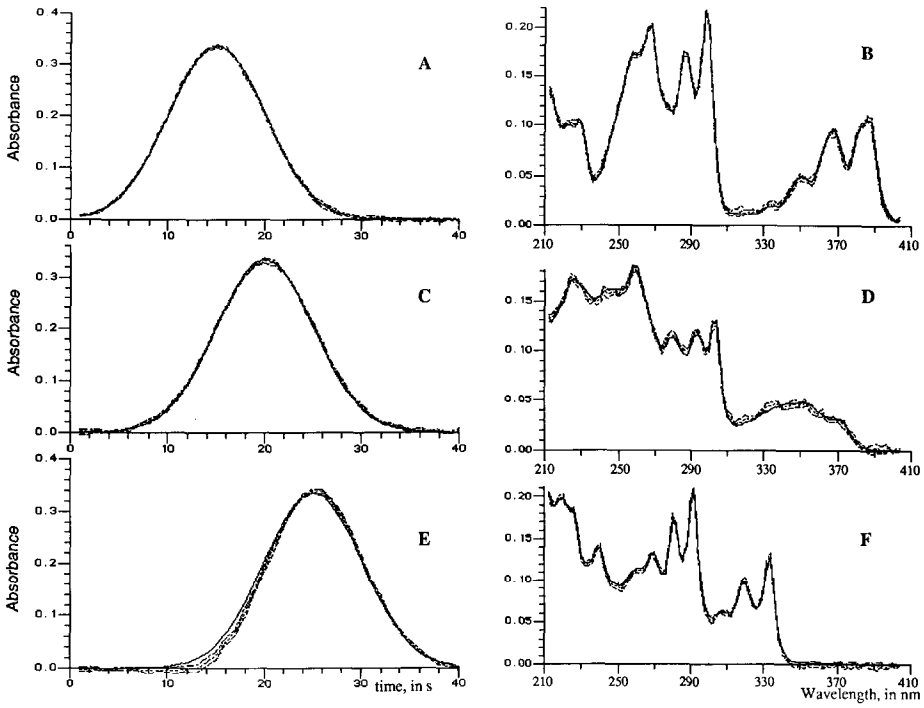



Fig. 5. Second simulation: individual normalized chromatograms and spectra compared with their uncertainty regions. Solid line is the expected result, broken lines represent the estimated spectra and the confidence regions; based on ten simulations. (A) and (B) = B*a*P; (C) and (D) = B*b*Fl; (E) and (F) = B*e*P.

have the same two components with very different concentration ratios (*ca.* 3:1), and both are successfully resolved in the test sample.

Finally, Fig. 3C represents the third cluster of the test sample. This is the opposite case of the first cluster, *i.e.*, the calibration sample has more components than the test sample. Because both components in the test sample are present in the calibration, they are correctly resolved.

Careful observation of Fig. 3 reveals that the arbitrary division into windows occurs at points where two components overlap, and in each case, GRAM uncovered

TABLE II

SIMULATION WITH 4% NOISE

Noise is 4% of the maximum absorbance in the spectrum measured at the apex of the Gaussian peak. Concentrations are relative to the calibration sample. Standard deviation based on ten calculations with the same 4% noise level.

| *Component* | $t_R$ *(s)* | *Expected concentration* | *Estimated concentration* | *Standard deviation* |
|---|---|---|---|---|
| B*a*P | 15 | 2.000 | 1.953 | 0.008 |
| B*b*Fl | 20 | 1.000 | 0.999 | 0.001 |
| B*e*P | 25 | 1.500 | 1.485 | 0.005 |

these borderline components. For example, Fig. 3B shows four resolved components, two of which are the main Gaussian peaks, and the other two, at the beginning and end of the cluster, represent the tailing edges of components in adjacent windows.

Note also that Anth, the trailing component at the beginning of the second cluster, could not be resolved in the first cluster, but its tail portion is correctly solved in the second cluster. This can be explained with the same argument that was used to explain why it could not be resolved in the first cluster. Anth is the only component in the second cluster which has a concentration ratio (calibration:unknown) equal to zero. Therefore, it can be correctly resolved at its trailing edge, where it no longer overlaps with Acen from the first cluster, which was the other component with a zero ratio.

The second simulation was intended to test the effect of a lower signal-to-noise level. An average 4% noise (signal-to-noise ratio = 25) was added to a three-component simulation. The spectra used for the simulation were those of B*a*P, B*b*Fl and B*e*P. Fig. 4 shows the GRAM-resolved chromatograms of both samples. This GRAM calculation was repeated 10 times to estimate the error in the results. Fig. 5 shows the normalized chromatographic and spectral solutions and their respective confidence bands. Table II compares the estimated and expected concentrations and the predicted error in the results.

*Experimental data*

Two sets of samples were used to test the GRAM method with real data. Each contained three components in both the calibration and the "unknown" (test) samples (both sets of samples were prepared from pure standard solutions, therefore, the test samples were actually known). Table III presents the details of the two sets of samples and compares the expected with the estimated concentrations. The samples of the same set were analyzed sequentially, under the same chromatographic conditions, to minimize changes in the relative retention times.

The resolved chromatogram of the first unknown sample is presented in Fig. 6. This sample and its corresponding calibration sample were analyzed with a mobile phase of water–acetonitrile (20:80). The expected solutions were estimated with a multiple linear regression program (MLR) for comparison with the GRAM results;

TABLE III

REAL SAMPLES

Spectral similarities are the dot product of the estimated spectrum and the real spectrum. A value of 1 indicates perfect match and a value of 0 total dissimilarity. A dash (–) indicates data not available.

| *Sample No.* | *Component* | *Expected concentration* | *Estimated concentration* | *Spectral similarity* |
|---|---|---|---|---|
| 1 | B*k*Fl | 4.1 | 4.5 | 0.9998 |
| | PER | 8.6 | 9.0 | 0.9996 |
| | B*b*Fl | – | – | 0.8145 |
| 2 | B*b*Fl | 6.0 | 6.4 | 0.9997 |
| | B*k*Fl | 5.4 | 5.5 | 0.9997 |
| | PER | 4.1 | 4.1 | 0.9980 |

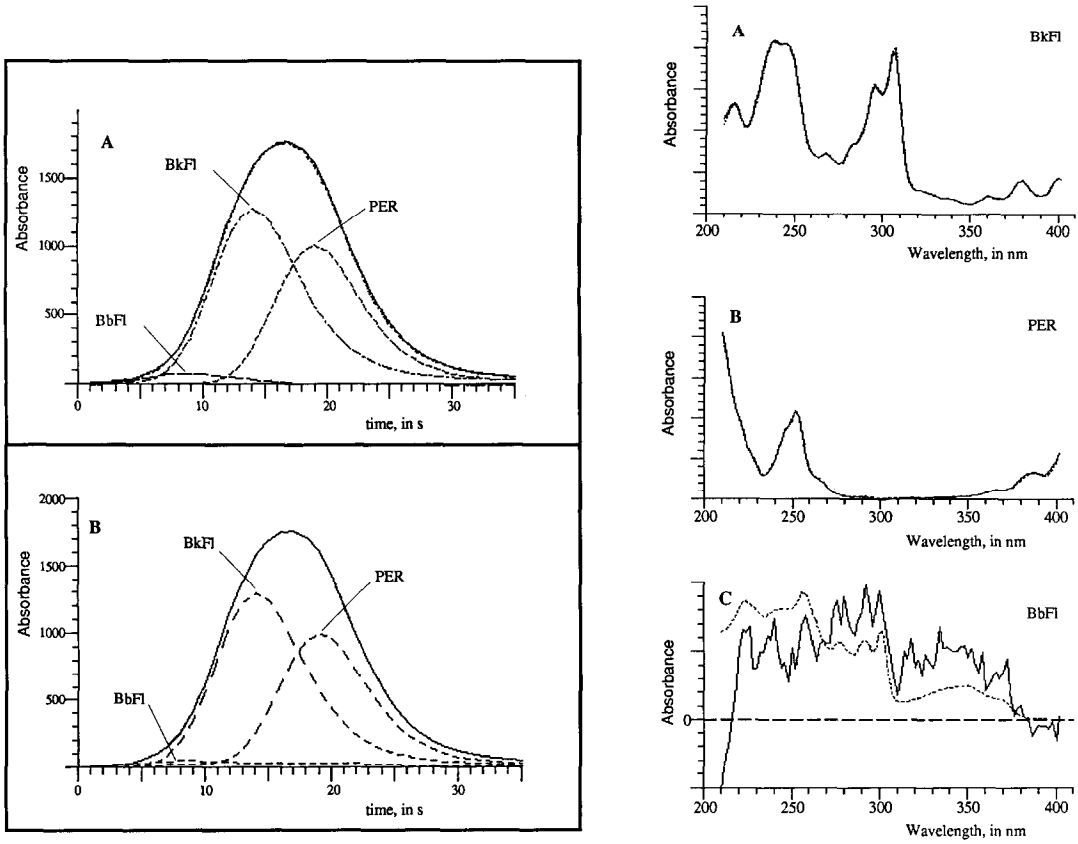
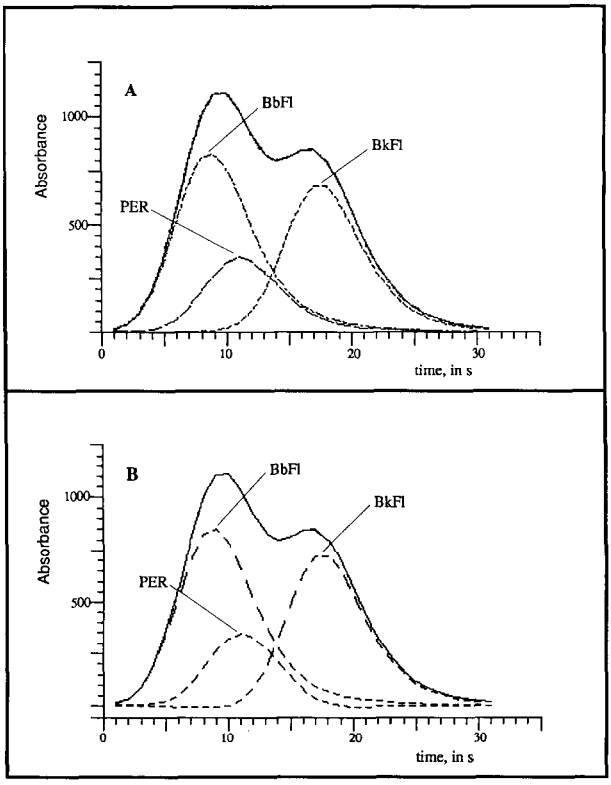



Fig. 6. Comparison of MLR (A) and GRAM (B) estimations of concentration profiles for the real sample 1. GRAM has uncovered the presence of an impurity. The components are B*b*Fl (impurity, $t_R$ = 8.8 s), B*k*Fl ($t_R$ = 14.1 s) and PER ($t_R$ = 19.1 s).

Fig. 7. Reconstructed spectra from GRAM analysis of sample 1. ——, predicted spectra; -------, spectra from pure standards. For (A) and (B) the predicted and the pure spectra are so similar that they can not be distinguished. (C) Impurity spectrum, plotted together with the B*b*Fl standard, the most similar in the data base.

the MLR solutions do not necessarily represent the actual true solutions, but they are a good approximation of the underlying chromatographic profiles. Note that the MLR solutions and the GRAM-resolved solutions are very similar. This sample was originally thought to contain only two components, but GRAM uncovered the presence of a third component, an impurity. Target factor analysis[3,4] was used to test for the presence of several possible impurities, and only B*b*Fl gave a positive test, and its spectrum was used for the MLR estimation. Fig. 7 shows the GRAM-recovered spectra for this sample, together with the expected spectra. Due to the low intensity of the impurity signal, the noise in the recovered spectrum is too high to show clearly its identity. But the estimated spectra of the other two components match very well with their actual spectra.

The solutions for the second set of three components are presented in Fig. 8.



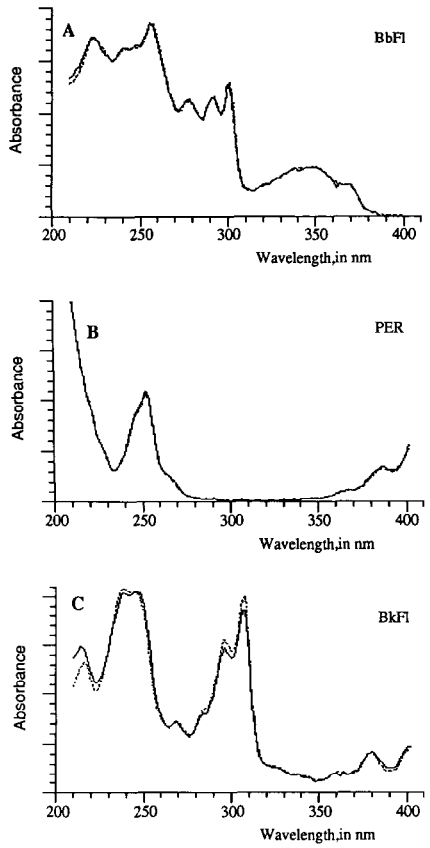




Fig. 8. Comparison of MLR (A) and GRAM (B) estimated concentration profiles for the real sample 2. The components are B*b*Fl ($t_R$ = 8.8 s), PER ($t_R$ = 11.1 s) and B*k*Fl ($t_R$ = 17.5 s).

Fig. 9. Reconstructed spectra from GRAM analysis of sample 2. ——, predicted spectra; -------, spectra from pure standards.

These samples were analyzed using water–acetonitrile (30:70) as the mobile phase. The expected and the estimated spectra can be compared in Fig. 9. The resolution between B*b*Fl and PER is very low ($R_s \approx 0.15$), but the GRAM-resolved chromatograms are a very good approximation of the MLR estimates. The best estimated spectra also correspond to B*b*Fl and PER.

## CONCLUSIONS

The potential for using GRAM in the analysis of complex mixtures in chromatography has been demonstrated. Because GRAM reduces the demand on chromatographic resolution, both analysis and method development time can be considerably reduced. Automation of LC–UV analyses could be possible, because the presence of unexpected overlapping components would not interfere with the GRAM analysis. Work is in progress toward applying the technique to complex environ-

mental samples. Further studies in the potentials of GRAM include application of the method to techniques such as gas chromatography–mass spectrometry and supercritical fluid chromatography–mass spectrometry.

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REFERENCES

1 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979.
2 A. F. Fell, B. J. Clark and H. P. Scott, *J. Chromatogr.*, 316 (1984) 423.
3 E. R. Malinowski and D. G. Howery, *Factor Analysis in Chemistry*, Wiley, New York, 1980.
4 A. Lorber, *Anal. Chem.*, 56 (1984) 1004.
5 D. W. Osten and B. R. Kowalski, *Anal. Chem.*, 56 (1984) 991.
6 B. G. M. Vandeginste, R. Essers, T. Bosman, J. Reignen, G. Kateman, *Anal. Chem.*, 57 (1985) 971.
7 O. S. Borgen and B. R. Kowalski, *Anal. Chim. Acta*, 174 (1985) 1.
8 H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 32 (1985) 1133.
9 B. G. M. Vandeginste, W. Derks and G. J. Kateman, *Anal. Chim. Acta*, 173 (1985) 253.
10 C.-N. Ho, G. D. Christian and E. R. Davidson, *Anal. Chem.*, 53 (1981) 92.
11 A. Lorber, *Anal. Chim. Acta*, 164 (1984) 293.
12 R. R. Kim, *Matrix Algorithms for Bilinear Estimation Problems in Chemometrics, Ph.D. Thesis*, Massachusetts Institute of Technology, Cambridge, MA, 1985.
13 M. McCue and E. R. Malinowski, *J. Chromatogr. Sci.*, 21 (1983) 229.
14 E. Sanchez, L. S. Ramos and B. R. Kowalski, *Anal. Chem.*, (1986) submitted for publication.
15 E. Sanchez and B. R. Kowalski, *Anal. Chem.*, 58 (1986) 496.
16 E. Sanchez and B. R. Kowalski, *J. Chemometrics*, (1986) submitted for publication.
17 L. S. Ramos, E. Sanchez and B. R. Kowalski, *J. Chromatogr.*, 385 (1987) 165.