Multivariate curve resolution applied to liquid chromatography–diode array detection

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Multivariate curve resolution methods can be used for the quantitation of the overlapping components in a chromatographic peak. Initial qualitative solutions obtained by self-modelling curve resolution methods, such as evolving factor analysis, can be further optimized by simultaneous analysis of multiple chromatographic runs with alternating least-squares regression. Quantitation is achieved by constraining the pure unit spectra and elution profiles of the common analytes in the different chromatographic runs to be equal. The relationship between the proposed method and other higher-order calibration and resolution methods is examined.

Introduction

Curve resolution methods have been proposed for the analysis of unresolved peaks in liquid chromatography with diode array detection (LC–DAD) [1] and particular attention has recently been focused on the use of evolving factor analysis [2]. In general, these methods are used as qualitative tools and for peak purity, and they are not intended to provide full quantitative information about the overlapping components. When only one sample is analyzed the quantitation can only be achieved if some additional external information is explicitly provided [3]. Multivariate curve resolution methods, such as the iterative target transformation method (ITTFA) [4] evolving factor analysis (EFA) [5], window factor analysis [6], the SIMPLISMA approach [7] or the HELP method [8] only use one data matrix from one sample in the numerical analysis and they cannot provide quantitative information directly. Conversely, higher order calibration and resolution methods [9] such as the generalized rank annihilation method (GRAM) [10], three-way data analysis [11], and residual bilinearization [12], take advantage of the higher structure order of the data obtained when several chromatographic samples (each giving a data matrix) are simultaneously analyzed. Higher order methods directly provide the quantitative information desired. These methods, however, are not intended to recover the real qualitative responses.

A new approach has been developed recently [13–15] which has some of the advantages of each of the previous approaches. It has been applied to the solution of other analytical problems, such as the speciation in multi-equilibria systems using spectroscopic titrations [13], chemical changes in process monitoring and control [14] and in speciation and quantitation in kinetic reaction-based chemical sensors [15]. In the present work, the same method is applied to LC–DAD. Concurrently with the present work, the DATAN [16] approach has been published which calculates the number of components, the spectral profiles, their concentrations, and the ratio of their responses to two spectroscopic measurements in a similar way to that presented here. The main difference between the DATAN approach and our approach is that DATAN is a latent-variable based method and does not take advantage of the qualitative and selective information gathered from local rank-based methods [4–8,13–15].

When several samples are analyzed simultaneously using a self-modelling curve resolution approach, the relative amounts of the common components in the different chromatographic runs can be determined, and quantitation can be achieved. The two key aspects for quantitation using the proposed multivariate curve resolution method are the assumptions that the same chemical components have the same unit spectra and that they have the same elution profile (same shape) in the different chromatographic runs. This new multivariate curve resolution approach requires that only one

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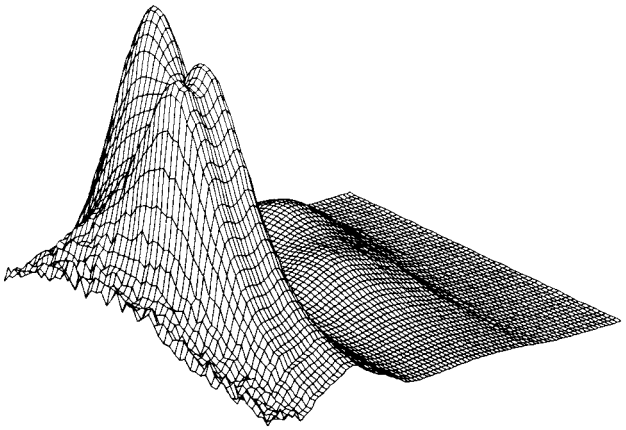

Fig. 1. Three-dimensional plot of the LC–DAD peak if the unresolved mixture naphthol–pirimicarb.

of the two orders (usually the spectral order) matches between the data matrices acquired for each of the samples analyzed in each chromatographic run. This avoids the practical limitations associated with synchronization of the elution profiles of the same component. In case that synchronization exists, the proposed method also takes advantage of the optimization of the elution profiles. If synchronization does not exist, or if it is not reasonable to assume that one component has exactly the same elution profile in the different chromatographic runs, multivariate curve resolution can still be used to solve the overlapped components which are common in the different chromatographic runs and to approximately quantitate them.

The example chosen in the present work to show the power of the proposed multivariate curve resolution method is the co-eluted mixture of two pesticides, the carbamate pirimicarb and 1-naphthol [17]. The apparatus, reagents, chromatographic system, samples analyzed, and other experimental details have been described previously [18]. Fig. 1 shows the three-dimensional plot of the data matrix obtained in the study of a naphthol–pirimicarb mixture.

Principles

Suppose a number, K, of chromatographic runs of different mixtures of the analytes, at different concentrations, are analyzed. In each chromatographic run a data matrix $\mathbf{D}_k$ is obtained:

$$\mathbf{D}_k = \mathbf{C}_k\mathbf{B} + \mathbf{D}_{k0}, \text{ with } k = 1,2,...,K$$

where $\mathbf{C}_k$ is the matrix of the elution profiles of the chemical components eluted during a particular chromatographic run in the analysis of sample k, $\mathbf{B}$ is the matrix of the unit or pure spectra of these components, and $\mathbf{D}_{k0}$ is the background absorption not caused by the components considered. Once the samples have been analyzed, the analysis can be performed simultaneously over all of them, by setting the respective data matrices $\mathbf{D}_k$ one in top of each other and keeping the number of columns (wavelengths) the same for all of them as follows:

$$\mathbf{D} = \begin{bmatrix} \mathbf{D}_1 \\ \mathbf{D}_2 \\ \cdot\cdot \\ \cdot\cdot \\ \cdot\cdot \\ \mathbf{D}_k \end{bmatrix} = \begin{bmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \cdot\cdot \\ \\ \cdot\cdot \\ \mathbf{C}_k \end{bmatrix} \quad \mathbf{B} + \mathbf{D}_0 = \mathbf{CB} + \mathbf{D}_0$$

The new augmented data matrix $\mathbf{D}$ has a number of rows equal to the total number of acquired spectra in the different chromatographic runs (elution times). In the case of simultaneous analysis of different samples, the new augmented data matrix will be the product of an augmented concentration matrix times the unit spectra matrix. The augmented concentration matrix will contain the different submatrices $\mathbf{C}_k$ corresponding to the elution of the components present in each of the data matrices $\mathbf{D}_k$ analyzed. As before, $\mathbf{B}$ contains the unit pure spectra of the components and $\mathbf{D}_0$ the background absorption. Initially, the proposed method will not force the data to have a complete second-order structure [9], although it will be a useful test and will make use of it if it is really present. In case the data are found to have a full second-order structure, the additional constraint of synchronization and equal shape of the concentration–elution profiles can be set optionally (see later). In any case, the spectra of the common components in the different chromatographic runs are always considered to be equal, and described in a unique matrix (see below for the case of non-common components). The later assumption is true whenever the external physical conditions such as temperature and solvent composition are held constant and the unit spectrum of every component is assumed to be unique.

In Fig. 2 is shown a scheme of the matrix arrangement for the particular case of the simultaneous analysis of three chromatographic samples,

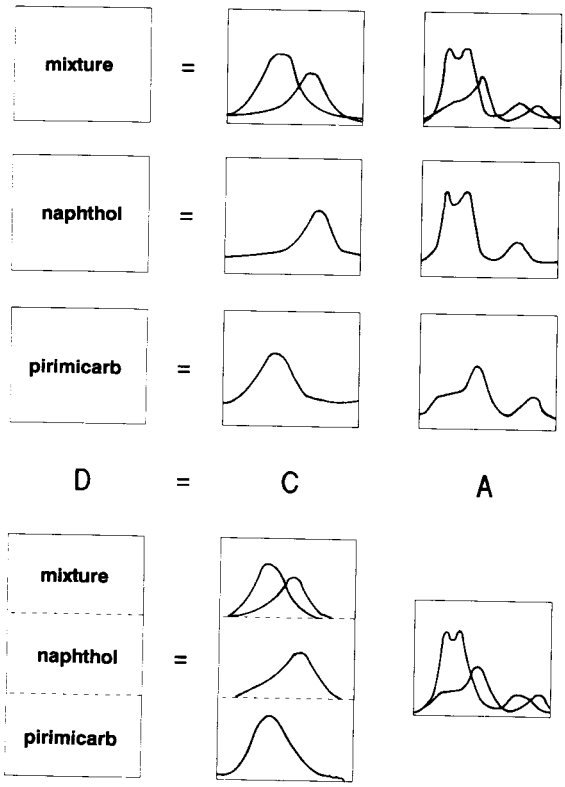

Fig. 2. Scheme showing the advantages of the simultaneous analysis of several chromatographic runs. First, the unknown mixture and the pure analyte samples are analyzed individually. Second, the unknown mixture is analyzed simultaneously with the pure analyte samples; in this case two additional constraints can be applied: equal unit pure spectra and equal elution profiles.

one of them containing the unknown co-eluted mixture and the other two containing the two pure analyte samples of both pesticides. This arrangement involves three data matrices having the same number of columns (wavelengths) but it is not forced to have the same number of rows (elution times). In the case where the number of rows is the same in the three data matrices, the augmented data matrix arrangement shown in Fig. 2 is similar to those found in three-way data analysis [11].

Principal component analysis

The data matrix **D** (augmented or not) is decomposed using principal component analysis

$$\mathbf{D} = \mathbf{USV}^T + \mathbf{E} = \mathbf{D}^* + \mathbf{E}$$

where now **U**, **S**, $\mathbf{V}^T$ are respectively the score, singular value and loading matrices of **D** for the preselected number of components, **E** is the residual error matrix containing the variance not explained by **U**, **S** and $\mathbf{V}^T$ and $\mathbf{D}^*$ is the reproduced data matrix using them. Under the assumption of linearity, when the correct number of components is chosen the residual error matrix **E** is close to the noise, or experimental error. The transformation of the **U**, **S** and $\mathbf{V}^T$ matrices in the **C** and **B** matrices cannot be achieved directly if no additional information is provided. As **C** and **B** are unknown the equation

$$\mathbf{D}^* = \mathbf{USV}^T = (\mathbf{UST})(\mathbf{T}^{-1}\mathbf{V}^T) = \mathbf{CB}$$

has an infinite number of solutions for any arbitrary transformation-rotation matrix **T**. There is an intrinsic ambiguity in the factor analysis solutions to solve for **C** and **B** if no more information is provided. The general task of the curve-resolution methods, and in particular of the proposed method, is to constrain the number of possible solutions which can eventually give the real physically and chemically meaningful solutions for **C** and **B**.

Determination of the number of components

As the goal of the study is the determination of the concentration of the chemical components present in the system, as well as their nature, the first thing to consider is the estimation of how many different components are in the data-set. The determination of this number is related to the level of variance which is caused by other sources (not the components of interest). Methods traditionally used for the determination of the number of components, such as cross-validation [19] or the theory of error in factor analysis [20] will not work here. This is because they provide the total number of contributions to the data variance, chemical and non-chemical, and including systematic noise, the baseline changes, and the background absorption. There are difficulties associated with the determination of the number of co-eluted species using these approaches for the particular case of the data, and neither Malinowski's functions [6,20] nor cross-validation [19] allow the correct estimation of the number of chemical components.

If it is assumed that the chemical components have a larger contribution to the data-variance than do the noise, background, or baseline changes, the number of chemical components can initially be

estimated, simply from comparison of their respective singular values. Singular values related to the background absorption and baseline changes are obtained from spectral regions which show no absorption bands. Singular values related with the chemical components of interest are obtained from the spectral regions which do show absorption bands. The number of components initially estimated in this way is tested afterwards, using evolving factor analysis, and during the alternating least-squares regression optimization (see below). One looks always for those solutions which better fit the data and have a physical meaning, *i.e.*, give reasonable shapes in the recovered concentration-profiles and unit spectra.

Intensity and rotational ambiguities

There are two classes of ambiguities associated with curve-resolution methods, the intensity and the rotational ambiguities. The intensity ambiguity is always present in factor analysis and curve resolution solutions, since for any scalar, m

$$d_{ij} = \sum_n c_{in} b_{nj} + e_{ij}$$

$$c_{in} b_{nj} = (c_{in} m)\ (1/m\ b_{nj}) = c'_{in} b'_{nj}$$

This means that the estimated concentrations and spectra will be scaled by some unknown factor, m. This is not a serious problem in qualitation (spectral identification, fingerprinting) but it is a serious problem in quantitation. This intensity ambiguity can be solved when several samples are analyzed simultaneously [13–15].

More important still is the rotational ambiguity, inherent also to factor analysis and curve resolution solutions, which generally occurs when there are two or more linearly independent overlapped components. The estimated spectrum for any of these components will be an unknown linear combination of the true components

$$\mathbf{b}' = \Sigma\ t_k \mathbf{b}_k$$

where t_k are unknown rotation constants; $\mathbf{b}_k$ are the true unit pure spectra of the components and $\mathbf{b}'$ are their estimated pure unit spectra.

Conversely, for those measured spectra where only one component is present, selectivity is present and there is no rotational ambiguity. This means that, in this particular situation, the principal component analysis solution yields the correct shapes of the unit spectra. Several methods can be used to detect selective regions, including local rank analysis [22], fixed-size moving window evolving factor analysis [23], and the recently proposed HELP method [8]. Fig. 3 shows the results of evolving factor analysis with a fixed-size moving window [23] along the time order of the data matrix of Fig. 1. Two major components are clearly detected in the system. In the ranges between 0.44 and 0.45 min and between 0.51 and 0.54 min only one species exists; whereas in the time interval between 0.45 and 0.50 min, two components are overlapped. The application of evolving factor analysis [23–27] to the same data matrix provides good initial estimates of the elution–concentration profiles which will eventually give the correct shapes of the unit spectra and the concentration profiles. However the intensity ambiguity is still present in the concentration profiles, and quantitation is not possible yet.

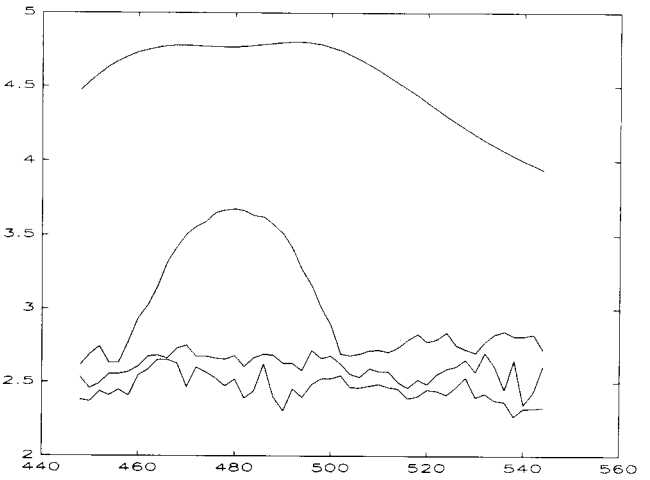

Fig. 3. Evolving factor analysis with a fixed-size moving window of the data given in Fig. 1. On the *x*-axis are the elution times ($\times 10^{-3}$ min); on the *y*-axis is the log of the singular values obtained with a window of five spectra. Along the experiment, two distinctive components are clearly distinguished with an overlapped range between 450 and $510 \cdot 10^{-3}$ min.

Constrained alternating least squares regression of the elution profiles and unit spectra

In the analysis of a single chromatographic run (even if the rotational ambiguity is solved) quantitation is only possible if some external informa-

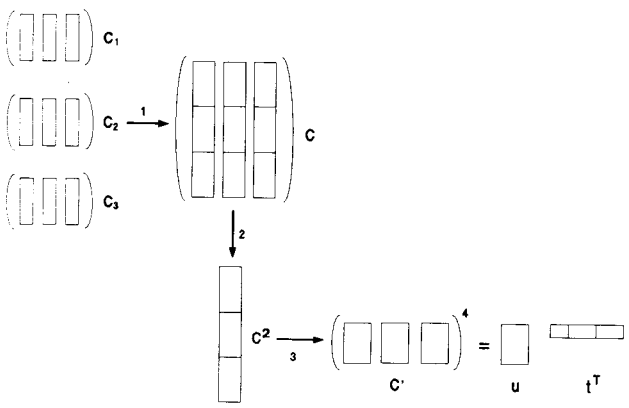

Fig. 4. Scheme showing an example of the matrix implementation of the constraint (iv) in the alternating least-squares optimization of the unit concentration profiles. $\mathbf{C}_1$, $\mathbf{C}_2$, $\mathbf{C}_3$ are the current estimates of the concentration matrices related to each of the analyzed samples; **C** is the augmented concentration matrix; $\mathbf{C}^2$ is the second column of matrix **C** which contains the concentration profiles of the second component in the three samples analyzed; **C'** is formed by folding $\mathbf{C}^2$ in three columns corresponding to the three samples; **u** and **t** are respectively the unit concentration profile and the (total) analyte concentrations for that particular component in the three samples.

tion is provided explicitly [18]. In the present work it is shown, however, that direct quantitation is possible if several samples are analyzed simultaneously by a constrained alternating least-squares regression procedure.

As a first step, the augmented matrix of the concentration profiles **C** is built up from the initial estimate of the $\mathbf{C}_1$, $\mathbf{C}_2$, ... $\mathbf{C}_K$ submatrices obtained by evolving factor analysis of the $\mathbf{D}_1$, $\mathbf{D}_2$, ... $\mathbf{D}_K$ submatrices, as described before [3,5,23–27]. The augmented **C** data matrix is then obtained simply by setting the estimate of the $\mathbf{C}_i$ matrices on top of each other in the same order as they are in **D**. At each iteration of the alternating least-squares regression a new estimation of the matrix of spectra **B** and of the concentration profiles **C** is obtained. At each iteration a set of constraints is applied:

(i) Non-negativity. Both concentration and unit pure spectra of the resolved components must be positive. Any initial negative absorbances in the experimental spectra caused by solvent subtraction are also set to zero.

(ii) Unimodality. Elution profiles have an unimodal shape. This constraint is applied using an algorithm which does not allow the appearance of second peaks for the same component.

In the simultaneous analysis of multiple data-matrices the following constraints can are added:

(iii) Common components have the same spectrum in all the chromatographic runs. When the sets of unit spectra in **B** are obtained for the different runs, the components in common in the different runs are forced to have the same unit spectra. This constraint has an important effect on the quantitation. The scaling and intensity ambiguities can be removed in this way (see later).

(iv) Common components have concentration profiles with the same shape in all the chromatographic runs. The shapes of the elution–concentration profiles for a particular component which is present in different samples can be forced to be identical. This is a desirable situation in chromatography when synchronization exists between experiments. In this case, the concentration profile for each common component in the different experiments will differ only in intensity and not in synchronization or shape, thus allowing the direct extraction of quantitative information from these profiles. The proposed method is based on the following equations.

Let $\mathbf{C}^n$ be the nth column of the augmented matrix, **C**, containing the current estimation of the concentration profiles of species n in the **K** different samples analyzed (see Fig. 4 for a graphical explanation). This column can be folded in a matrix $\mathbf{C}'$ (I,K) where I is the number of spectra acquired in the analysis of every sample (when synchronization exists between experiments). Under the assumption of equal shape, this matrix can be decomposed as the product of two vectors:

$$\mathbf{C}' = \mathbf{u}\mathbf{t}^{\mathrm{T}}$$

where $\mathbf{u}(I)$ is the unit concentration profile containing the common shape information of $\mathbf{C}'$, and $\mathbf{t}^{\mathrm{T}}(\mathrm{K})$ contains the relative amounts of this unit profile in $\mathbf{C}'$. Then u and $\mathbf{t}^{\mathrm{T}}$ can be calculated easily from the first singular value decomposition of $\mathbf{C}'$:

$$\mathbf{C}' = \mathbf{u}_1 s_{11} \mathbf{v}_1^{\mathrm{T}} + \mathbf{E}$$

$$\mathbf{u} = \mathbf{u}_1$$

$$\mathbf{t}_{\mathrm{T}} = s_{11} \mathbf{v}_1^{\mathrm{T}}$$

The unit profile, **u**, is equal to the first-score vector $\mathbf{u}_1$, and the relative analyte concentrations are obtained from the first loading $\mathbf{v}_1^T$ scaled by its first singular value s_{11}.

This method assumes that the concentration profiles of the same species have the same shape in the different experiments and consequently that the matrix $\mathbf{C}'$ has rank one and **E** is at the level of the experimental error. An easy way to test this assumption during the analysis is to compare the magnitude of the next singular values with the first singular value.

(v) Zero concentration components. When a component is known not to be present in a particular experiment, the concentration of that component in **C** is forced to be equal to zero. The question about the presence or absence of a certain component in an experiment can be solved by looking at the pseudo-rank of the associated data matrix, and at the results of the individual alternating least-squares regression applied to single data matrices, to see whether it is reasonable to assume the presence of that component in the different experiments. If the unit spectra of two resolved components in the different experiments are similar (fingerprint matching) then they probably correspond to the same component and will be postulated in the simultaneous analysis. Conversely, components with different spectra in different chromatographic runs correspond to different components in the simultaneous analysis.

It is important to note that the use of the constraints (iii), (iv) and (v) leads to the second-order advantage [9] and that the solutions obtained with the proposed method are very close to those obtained by using the second-order resolution methods [10–12].

In Figs. 5 and 6 are given the unit (pure) spectra and concentration profiles obtained by the simultaneous analysis of the three data-matrices arranged as in Fig. 2. They were obtained using the set of constraints (i) to (v) described in the constrained alternating least-squares regression. In both figures, Component 1 refers to pirimicarb, and Component 2 refers to naphthol which elutes second. Each component is characterized by the same pure unit spectrum, in all the data matrices where it is present. The unit spectra recovered from the numerical treatment have the same shape as those obtained from individual chromatographic analyses of the pure analyte samples, for each of the two eluted compounds. If a component is present in a data matrix, a concentration profile is deduced for it. In Fig. 5, two concentration profiles are drawn for each of the two components, one for the component in the pure analyte sample and the other for it in the unknown mixture. Both the synchronization and shapes of the elution profiles match for each component.

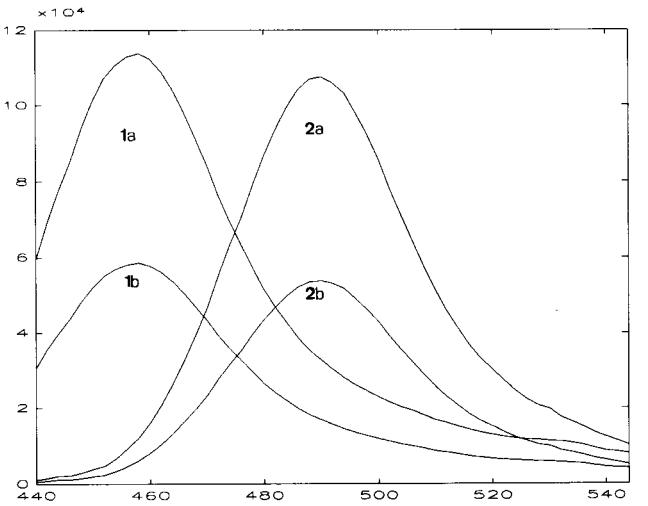

Fig. 5. Resolved elution–concentration profiles of the components in the three samples simultaneously analyzed (Fig. 3): **1a**, pirimicarb in the pure analyte sample; **1b**, pirimicarb in the mixture (Fig. 1); **2a**, naphthol in the pure analyte sample; **2b**, naphthol in the mixture (Fig. 1).

The simultaneous analysis of several chromatographic samples by the proposed method also helps to solve the rotational ambiguities where there is very high overlapping of both the spectral

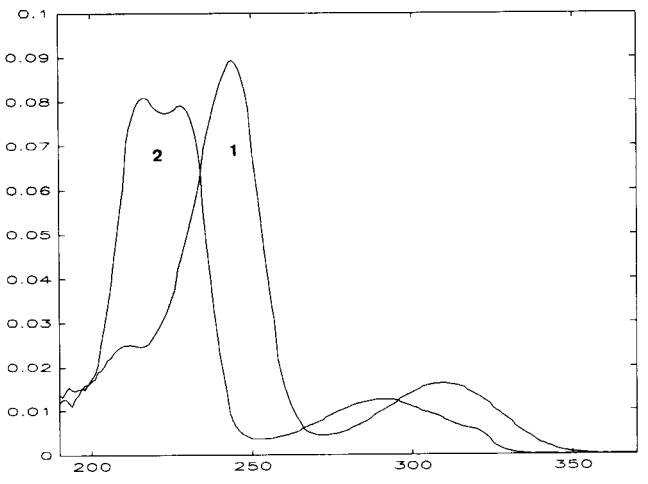

Fig. 6. Resolved unit pure spectra of the two overlapped components. Spectrum 1 corresponds to pirimicarb and spectrum 2 corresponds to naphthol.

and elution orders and there are no selective channels for the eluted components. The number of possible solutions of the linear model in the alternating least-squares optimization is strongly constrained when the unit spectra and the concentration profiles of the common components are forced to be the same in the different chromatographic runs (*i.e.* samples). From the results obtained, the similarities between the two unit spectra and between the two elution profiles (normalized to equal area) are calculated. The inner products of the vectors describing the two unit spectra and the two elution profiles [21] are respectively equal to 0.505 and 0.615. The resolution of the two co-eluted components in the unknown mixture was calculated to be 0.489.

Quantitation of the analytes

Once the concentration profiles of the components in the mixture are recovered using the proposed method, the initial concentrations of the analytes can be estimated. If the conditions of linearity hold, the area under the concentration profile of a certain component (which can be roughly determined from the sum of its concentration values at the different measured times) is proportional to the concentration of the analyte. The ratio between these areas for a particular component gives the ratio between the concentrations of that particular analyte in the different samples, recovering directly the quantitative information. If, in addition, one or more experiments are performed for samples whose concentration of analyte is known, the concentration of analytes in the unknown samples can also be calculated, in absolute values. This method of quantitation is improved when the constraint of equal shape over the concentration profiles is applied (see above). Under completely reproducible and synchronized chromatographic conditions, the intensities of the concentration profiles of the components, at any elution time in the different experiments will only depend on the concentration of the analyte.

In Table 1 is given a summary of the results obtained when different methods of quantitation are applied to the unresolved mixture of pirimicarb and naphthol (Fig. 1). For comparison, the results given first (Method 1), correspond to those obtained by classical least-squares regression, when the pure unit spectra of all the eluted components are given in the input. The analysis is performed

TABLE 1. Results of the quantitation of the unresolved LC–DAD peak mixture of pirimicarb and naphthol[a]

Method[b]	Pirimicarb	Naphthol	N[c]
1	0.267 (4%)[d]	0.039 (17%)	1
2a	0.270 (6%)	0.045 (3%)	3
2b	–	0.043 (8%)	2
2c	0.189 (26%)		2
3a	0.263 (3%)	0.047 (1%)	3
3b	–	0.049 (5%)	2
3c	0.252 (1%)	–	2
4a	–	0.050 (6%)	2
4b	0.256	-	2

[a]Quantitation of the unresolved pirimicarb–naphthol mixture shown in Fig. 1. Correct concentrations are respectively (in ppm), pirimicarb 0.256 and naphthol 0.047
[b]Method used in the quantitation of the unresolved mixture: (1) Classical linear least-squares regression with the pure unit spectra of the two unresolved components given in the input. (2) Simultaneous analysis of multiple chromatographic runs using multivariate curve-resolution and the constraint of equal unit spectra (iii) between runs: (2a) mixture and two pure analyte samples; (2b) mixture and naphthol pure analyte samples; (2c) mixture and pure pirimicarb sample. (3) As in (2) but with the two constraints of equal unit spectra, (iii), and of equal unit elution profiles, (iv). (4) Generalized rank annihilation method (GRAM): (4a) mixture and pure naphthol sample; (4b) mixture and pure pirimicarb sample.
[c]Number of data matrices used in the analysis
[d]Error percentage

only over the unresolved mixture data-matrix. Whereas the results for pirimicarb are rather good, those for naphthol are rather poor.

Next in Table 1 are given the results of the simultaneous analysis of the data matrices obtained in the chromatographic analysis of an unknown mixture, together with one or two pure analyte samples, as shown in the scheme of Fig. 2. When the unknown mixture is analyzed, together with these two samples, using the constraints (i)–(iii) and (v), the results (Method 2a) are better than before, although still with some error in the estimation of the pirimicarb concentration. When one of the two co-eluted components is considered to be an interferent, and the unknown mixture is analyzed together with a pure analyte sample containing the other co-eluted component, the results (Methods 2b and 2c) are worse, especially for

pirimicarb with a 26% error (Method 2c). Conversely, when the constraint of equal shape in the elution profiles is added (Method 3), the results of the quantitation are good in all cases. When naphthol is considered to be an interferent, and only the pure pirimicarb sample is included in the simultaneous analysis, the quantitation of pirimicarb is still very good (1% error).

Although Method 2 has the advantage that it did not require synchronization in the time order, it has the disadvantage of requiring more knowledge about the interferents in order to resolve the overlapped components in quantitative terms. Method 3 is the best choice for quantitation when interferents are present, and is closely related to second-order calibration and resolution methods (see below).

For comparison purposes, the generalized rank annihilation method GRAM [9,10] is also applied in the present work. As the application of this method to chromatography has already been documented [28] only a brief description is given in the present work. In GRAM the data matrix **D** is decomposed into the product of three matrices:

$$\mathbf{D} = \mathbf{XSY}^T$$

where the **X** matrix is related to the pure elution profiles (normalized pure component responses on the time axes), **Y** is related to the pure unit spectra (normalized pure component responses on the wavelength axes) and **S** is a diagonal matrix related to the total amounts of the components in the mixtures. In GRAM the unknown response data matrix $\mathbf{D}_1$ is analyzed simultaneously with the pure analyte response data matrix $\mathbf{D}_2$:

Known analyte sample $\mathbf{D}_1 = \mathbf{XS}_1\mathbf{Y}^T$;

unknown sample $\mathbf{D}_2 = \mathbf{XS}_2\mathbf{Y}^T$,

where $\mathbf{S}_1$ and $\mathbf{S}_2$ are diagonal matrices, whose elements are proportional to the concentrations of the components in the sample (unknown and known) mixtures. GRAM solves the two equations for the set of common components with equal elution profile and unit pure spectra (equal **X** and $\mathbf{Y}^T$ columns). A non-iterative approach based in the QZ algorithm for the generalized singular value decomposition, which spans simultaneously the space described by the columns of both data matrices $\mathbf{D}_1$ and $\mathbf{D}_2$, has been proposed and proved successful for the case of LC–DAD data [28]. From the diagonal values in the $\mathbf{S}_1$ and $\mathbf{S}_2$ matrices the relative concentrations of the components of interest are recovered.

Results obtained by using the GRAM method on the data previously analyzed (Fig. 1) are also given in Table 1. First, the unknown data mixture is analyzed together with the pure naphthol sample (Method 4b) and then with the pure pirimicarb sample (Method 4c). The results obtained with GRAM are very close to those obtained using the proposed multivariate curve resolution method, when the two constraints of equal pure unit spectra and equal shape in the concentration profiles were used (Method 3).

The results in Table 1 show that the multivariate curve-resolution method presented in the present work can be adapted to handle data of various complexities. The method can easily be adapted to the characteristics of the data, and can take advantage of the full second-order structure present in LC–DAD. In cases where the elution of the components is not completely reproducible, because of experimental or instrumental limitations, the method can still be used for quantitation, although the error caused by the presence of unknown interferents, not present in the known analyte samples, will be higher. Work is being done to discover more about the limits of the present method and to find the effects of the starting values, selectivity and constraints.

Conclusions

Multivariate curve-resolution is a useful tool for the resolution and quantitation of overlapped components under a chromatographic peak. Simultaneous analysis of multiple chromatographic runs, including unresolved unknown mixtures and known analyte mixtures, gives accurate quantitative results when the constraints of equal unit pure spectra and equal elution profiles are applied. Compared with other second-order methods, multivariate curve-resolution has the advantage of giving directly the correct shapes of the recovered spectra and unit concentration profiles. Additionally, multivariate curve-resolution is easily adapted to data of varying complexity, from single bilinear data matrices to higher-order data structures.

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