Multivariate Curve Resolution of Hyphenated and Multidimensional Chromatographic Measurements: A New Insight to Address Current Chromatographic Challenges

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Multivariate Curve Resolution of Hyphenated and Multidimensional Chromatographic Measurements: A New Insight to Address Current Chromatographic Challenges

In this Feature, the capabilities and versatility of multivariate curve resolution methods are discussed in light of the current challenges in chromatographic measurements, with special emphasis on hyphenated and multidimensional chromatographic analysis. This Feature provides insights and perspectives on recommended chemometric strategies to improve the qualitative and quantitative chromatographic information gathered from analytical determinations of complex natural samples.

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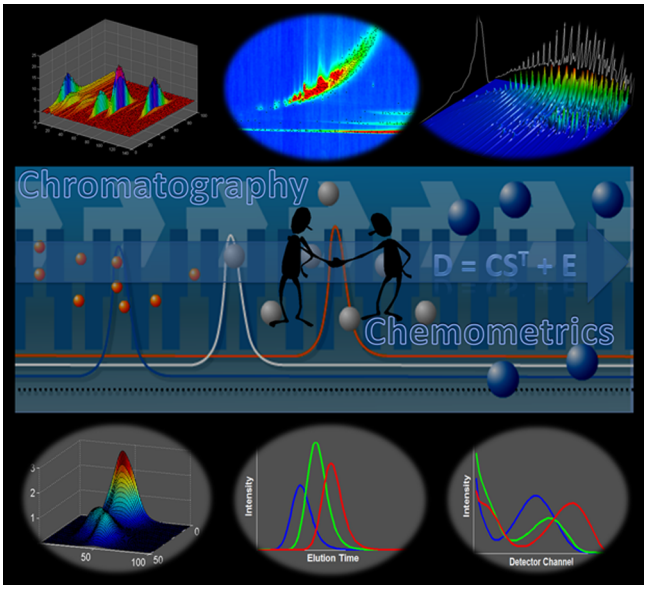

CHROMATOGRAPHIC CHALLENGES

Current chromatographic analyses are faced with some fundamental chromatographic challenges that can directly affect qualitative and quantitative chromatographic results.[1] Development and evolution of new chromatographic systems for separation, identification, and quantification of chemical compounds in complex samples matrices has become one of the most important developments in analytical chemistry in the recent years. Chromatographic science is now focused on the analysis of trace and ultratrace constituents in natural complex samples with minimum sample preparation and on reducing its time scales and sample size requirements.[2−4]

The development of several one-dimensional (1D, one column) chromatographic techniques, such as gas chromatography (GC) and liquid chromatography (LC), led to the generalized idea that these techniques could be just finely tuned in order to solve all practical problems in analytical chemistry. However, the need for analysis of increasingly complex samples with a large number of compounds highlighted the limitations of such techniques and prompted the development of technologies with a much higher separation capacity. For many years already, great efforts have been concentrated on online combinations of chromatographic methods and different spectroscopic detection methods, in order to provide different hyphenated chromatographic methods, such as gas and liquid chromatography−mass spectrometry (GC/MS and LC−MS). The need for improving analytical figures of merit associated with the research explosion in proteomics, foodomics, metabolomics, and petroleomics and the ever increasing requirements for adequate identification and quantification of proteins, glycoproteins, metabolites, and petrochemical products has prompted a need to push separation techniques to their limits.[2−4] Furthermore, even when 1D chromatographic methods can produce acceptable results, they do not have the separation power to deal with natural complex samples, and their use in such cases represents spending a lot of time for every analysis. Therefore, in spite of the advancement in hyphenated chromatographic systems, frequent incomplete separation issues along with other chromatographic challenges still exist in the analysis of complex samples.[1] One possible solution for these limitations was the advent of multidimensional chromatographic systems.[5−7] Multidimensional chromatographic techniques have emerged as powerful techniques suitable for the separation of very complex mixtures because of their higher resolution and higher peak capacity.[5,8−11] Multidimensional chromatography can be understood as a chromatographic process capable of resolving the components from a mixture, using different separation chromatographic dimensions and mechanisms which are connected but do not interact among themselves, that is, they should be completely independent from each other. The development of comprehensive multidimensional chromatography can be viewed as a cornerstone development in chromatographic history.[6] When comprehensive two-dimensional chromatographic techniques are coupled with a high acquisition rate detector, like a time-of-flight mass spectrometer (TOFMS), the result is a extremely powerful instrument (e.g., GC × GC−MS and LC × LC−MS) for the analysis of very complex natural samples having

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thousands of chemical components and its performance has been confirmed in analyzing petrochemical products, environmental pollutants, and biological metabolites.[5,9] However, one of the main challenges in multidimensional chromatography is related to the difficulty of the analysis and interpretation of the enormous amount of data obtained in these cases. Additionally, complete separation of all detectable components still cannot be achieved because of the extremely high complexity of natural samples and limitations in experimental and instrumental conditions. This incomplete separation directly reflects the lack of selectivity of chromatographic separations in the analysis of complex natural samples.[12,13]

In this context, resolution, identification, and quantification of target compounds in the presence of interferences (i.e., known and/or unknown) in natural samples are still challenging problems which are not totally solved yet with the current available methods. Therefore, it is clear that both types of chromatographic analyses (i.e., hyphenated and multidimensional) suffer from artifacts that directly affect their performance in many circumstances.[1,14−17] These challenges are not different from those derived from any other instrument, being based on both chemical and nonchemical aspects of the analysis. In general these challenges can be divided into three different classes of (1) baseline/background contributions and noise, (2) retention time (RT) shifts, and (3) coelution or peak overlap (overlapped and embedded peaks).

Figure 1 schematically shows these types of chromatographic challenges occurred in practice during chromatographic analyses for two chemical components with retention times, p_1 and p_2 in four different chromatographic runs.[1] In this figure, the more common chromatographic problems are shown: (a) baseline/background drift, (b) low signal-to-noise ratios (S/N), (c) RT shifts, (d) coelution or peak overlap, and (e) combination of all problems.

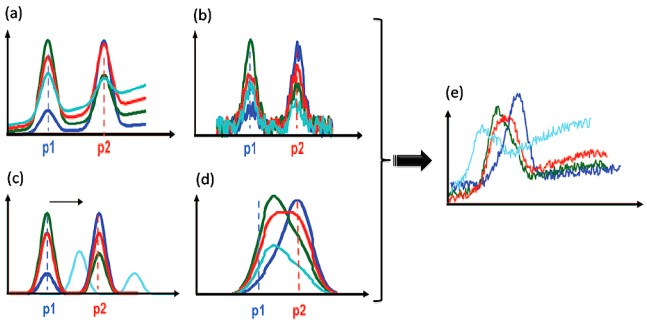

Figure 1. Schematic representation of more common chromatographic challenges for a two-component chromatographic system; (a) baseline drift, (b) low S/N ratio, (c) RT shift, (d) coleution, and (e) combination of all chromatographic challenges. Modified from ref 1. Copyright 2010 American Chemical Society.

It should be pointed out that chromatographic signals can be divided into three major parts of analytical signal, baseline/background signal, and noise. The analytical signal gives the chromatographic response of the analyte for a particular set of experimental conditions. The baseline/background signal is associated with the systematic response from the chromatographic systems not related to the analyte. Baseline/background contribution (as it is shown in Figure 1) can result from changes in mobile phase composition which influence detector signals in LC or chemical bleed signatures from stationary phases as they degrade in GC. Moreover, variations of pressure and temperature and fluctuations caused by the injection valve can bring additional baseline/background contributions in chromatographic measurements. On the other hand, noise contributions are associated with unknown random variations and they are usually related to the sensitivity of the detector. Changes in detector responses as their components age, instrumental noise (e.g., thermal and electronic noise), and contamination of solvents or gases can also be the sources of noise and drift in chromatographic signals. Baseline/background contributions, particularly associated with noisy signals, may cause problems, especially for quantification, since they can change both the shapes and retention times of chromatographic peaks. When the S/N is low, separation between noise and baseline/background contribution becomes difficult in practice, but in order to achieve a clear analytical signal, it is necessary to identify and eliminate the interferences caused by noise and baseline/background contributions.[18]

Another important challenge in chromatographic measurements is RT shifts. Possible reasons for RT shifts between chromatographic runs in 1D and 2D chromatography are associated with control fluctuations in temperature, flow rate, and pressure, matrix effects, stationary phase degradation, changes in mobile phase composition in LC, and errors in the timing of manual injections.[1,15,16] However, there is another type of RT shift in GC × GC when temperature programming is used to improve resolution and to avoid general coelution problems in the multicomponent analysis of very complex natural samples, such as fuels. In most practical purposes, the two GC dimensions are normally temperature programmed at about the same rate or nearly so. Therefore, the temperature increase per time for the first GC dimension will simultaneously correlate to a temperature increase on the second GC dimension. The result of this can be observed as a change in second dimension retention time for a specific analyte found in succeeding first dimension fractions (i.e., for each subsequent modulation period) within a run.[7,19−22] In these situations the RT shift is progressive and it can make the data structure more complex.[23,24]

The peak overlap (coelution) problem is perhaps the most important challenge in chromatographic analysis which arises mainly due to the lack of peak capacity (resolving power) and selectivity of chromatographic columns. In spite of the significant increase and improvement of peak capacity in comprehensive multidimensional chromatographic systems, the aforementioned problems still exist, especially in the analysis of complex natural samples.[1,15,16]

Great efforts have been made by chemometricians to present new strategies and mathematical models to extract the required chemical information from hyphenated and multidimensional chromatographic signals in difficult chromatographic scenarios. Fortunately, during the past decade, different multivariate curve resolution methods have matured and have been proposed to compensate for the lack of total selectivity in chromatography.[1,8,15,16,25−27] Multivariate curve resolution techniques attempt to overcome different fundamental chromatographic challenges during hyphenated and multidimensional chromatographic separations and to gain pure qualitative and quantitative chromatographic information about the components in the analyzed samples, compensating (or complementing) for the possible lack of total selectivity of chromatographic separations and of spectral detection systems. This is achieved by mathematical resolution means based on exploiting the spectral

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and chromatographic differences (even if they are very small) between all the components present in a particular unresolved mixture. However, according to the type of chromatographic data and to the degree of data complexity, different strategies can be recommended. The potential of multivariate curve resolution methods as the most effective strategies to overcome chromatographic challenges in hyphenated and multidimensional chromatography[1,25,28] will be briefly shown and discussed in the following sections.

MULTIVARIATE CURVE RESOLUTION: DATA STRUCTURES AND MATHEMATICAL MODEL

Multivariate resolution methods (also called multivariate curve resolution methods, MCR, or also self modeling curve resolution methods, SMCR) belong to a family of chemometric methods used in many different chemical fields, which have been proposed for handling different types of chromatographic challenges and for improving the selectivity and sensitivity of chromatographic measurements.[25,29−31] In general, modern spectrometric detection methods in chromatographic instrumentation provide rich analytical information, which in some circumstances, however, is not sufficiently selective. Chemometric resolution methods attempt to resolve simultaneously the chemical constituents of unresolved mixtures by mathematical means, in particular in hyphenated and multidimensional chromatography, without their physical separation, i.e., by means of mathematical resolution of their respective signal contributions. Coupling existing chromatographic systems with multivariate curve resolution methods is an extension of current chromatographic tools to resolve chromatographic challenges in an economical way, and it may represent an interesting and powerful alternative in many analytical laboratories.[32] In the literature, different applications of chemometric resolution methods have been described for the analysis of complex mixtures and are increasingly accepted in chromatography.[1,25,31]

The main goal of all multivariate curve resolution methods is the decomposition of a mixed signal into the contribution of the pure component profiles of the constituents, by means of a simple bilinear data decomposition, which is defined as follows

$$\mathbf{X} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E} \tag{1}$$

where $\mathbf{X}(I \times J)$ is the raw experimental data matrix containing the chromatographic section subjected to analysis, $\mathbf{C}(I \times N)$ is the factor matrix which contains the resolved elution (concentration) profiles of the N components present in this chromatographic section, and $\mathbf{S}^{\mathrm{T}}(N \times J)$ is the factor matrix of their corresponding pure spectral profiles. The part of data which is not explained by the model is in the error $\mathbf{E}(I \times J)$ matrix. Indices I and J are the numbers of row and column variables, respectively, spectral channels (e.g., wavelengths or m/z ratios), and chromatographic retention times. In addition, N is the number of eluted components in the analyzed chromatographic section of data matrix $\mathbf{X}$.

In the case of simultaneous analysis of multiple chromatographic runs of the same sample or of multiple samples having some components in common (e.g., different unknown mixtures of them containing possible interferences, with or without additional known analyte mixture samples used for calibration),[30] this general bilinear model given in eq 1 can be easily extended to their simultaneous analysis as given in

$$\begin{pmatrix} \mathbf{X}_1 \\ \mathbf{X}_2 \\ \cdots \\ \mathbf{X}_K \end{pmatrix} = \begin{pmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \cdots \\ \mathbf{C}_K \end{pmatrix} \mathbf{S}^{\mathrm{T}} + \begin{pmatrix} \mathbf{E}_1 \\ \mathbf{E}_2 \\ \cdots \\ \mathbf{E}_K \end{pmatrix} \tag{2}$$

where now, $\mathbf{X}_1$, $\mathbf{X}_2$, ..., $\mathbf{X}_K$, are, respectively, the data matrices corresponding to the k = 1,...,K chromatographic runs having components in common to be analyzed simultaneously, $\mathbf{C}_1$, $\mathbf{C}_2$, ..., $\mathbf{C}_K$ are the concentration matrices with the elution profiles of the resolved components in the k = 1,..., K runs, $\mathbf{S}^{\mathrm{T}}$ is the matrix of spectral profiles of these eluted components, and $\mathbf{E}_1$, $\mathbf{E}_2$, ..., $\mathbf{E}_K$ are the corresponding error matrices containing the part of the measured data unexplained by the proposed bilinear model. In a more compact form this equation can be written as

$$\mathbf{X}_{\mathrm{aug}} = [\mathbf{X}_1; \mathbf{X}_2; \ldots; \mathbf{X}_K] = [\mathbf{C}_1; \mathbf{C}_2; \ldots; \mathbf{C}_K]\mathbf{S}^{\mathrm{T}} + [\mathbf{E}_1; \mathbf{E}_2; \ldots; \mathbf{E}_K] = \mathbf{C}_{\mathrm{aug}}\mathbf{S}^{\mathrm{T}} + \mathbf{E}_{\mathrm{aug}} \tag{3}$$

In which $\mathbf{X}_{\mathrm{aug}}$, $\mathbf{C}_{\mathrm{aug}}$, and $\mathbf{E}_{\mathrm{aug}}$ stand for the column-wise augmented matrices containing, respectively, the chromatographic data of the different runs, the elution profiles of the resolved components in the different runs, and the error or unexplained modeled data in the different runs. In these equations, the notation ";" is used to indicate column-wise augmentation.

Multivariate curve resolution-alternating least-squares (MCR-ALS)[33−35] is one of the most popular multivariate curve resolution methods which solves eq 1 and 2 for $\mathbf{C}$ and $\mathbf{S}^{\mathrm{T}}$, using an iterative algorithm based on two constrained linear least-squares steps. It requires an initial estimation of the elution, $\mathbf{C}$, or of the spectral, $\mathbf{S}^{\mathrm{T}}$, profiles.

In some circumstances the extended bilinear model for simultaneous analysis of multiple chromatographic runs can be extended to the trilinear model, which can be defined as follows

$$\mathbf{X}_{\mathbf{k}} = \mathbf{C}\mathbf{D}_{\mathbf{k}}\mathbf{S}^{\mathrm{T}} + \mathbf{E}_{\mathbf{k}} \tag{4}$$

where $\mathbf{X}_{\mathbf{k}}$ refers to the single data matrix obtained in the chromatographic run k, $\mathbf{C}$ is the matrix of pure elution profiles, $\mathbf{D}_{\mathbf{k}}$ is the diagonal matrix of composition variations of the pure components from sample to sample, and $\mathbf{S}^{\mathrm{T}}$ is the matrix of pure spectral profiles. The unmodeled part of $\mathbf{X}_{\mathbf{k}}$ is present in the $\mathbf{E}_{\mathbf{k}}$ matrix. Other higher multilinear models (e.g., quadrilinear and so forth) can be defined in a similar way. Observe that in this case of the trilinear modeling, we are assuming that all $\mathbf{C}_{\mathbf{k}}$, k = 1...K, of eq 4 can be described by the equation $\mathbf{C}_{\mathbf{k}}$ = $\mathbf{C}$ $\mathbf{D}_{\mathbf{k}}$, which implies that profiles in $\mathbf{C}_{\mathbf{k}}$ only differ in a scalar (intensity) factor defined in the diagonal matrix $\mathbf{D}_{\mathbf{k}}$ corresponding to chromatographic run k. This implies necessarily that elution profiles of the common components in different runs appear exactly at the same retention time (no shifting) and that they have exactly the same shape (the same profile). This is a very challenging constraint in chromatography, where very commonly between run shifts are the rule more than the exception and where also peak shapes can change especially in the case of component coelution (peak overlap), since column sample capacities change over time and sample overloading can occur. It is however worthwhile to introduce this trilinear model here, both to be aware of these limitations and also in the context of current applications of chemometric methods to solve chromatographic problems.

Parallel factor analysis (PARAFAC)[36] has a central role in multiway data processing methods because of the exceptional

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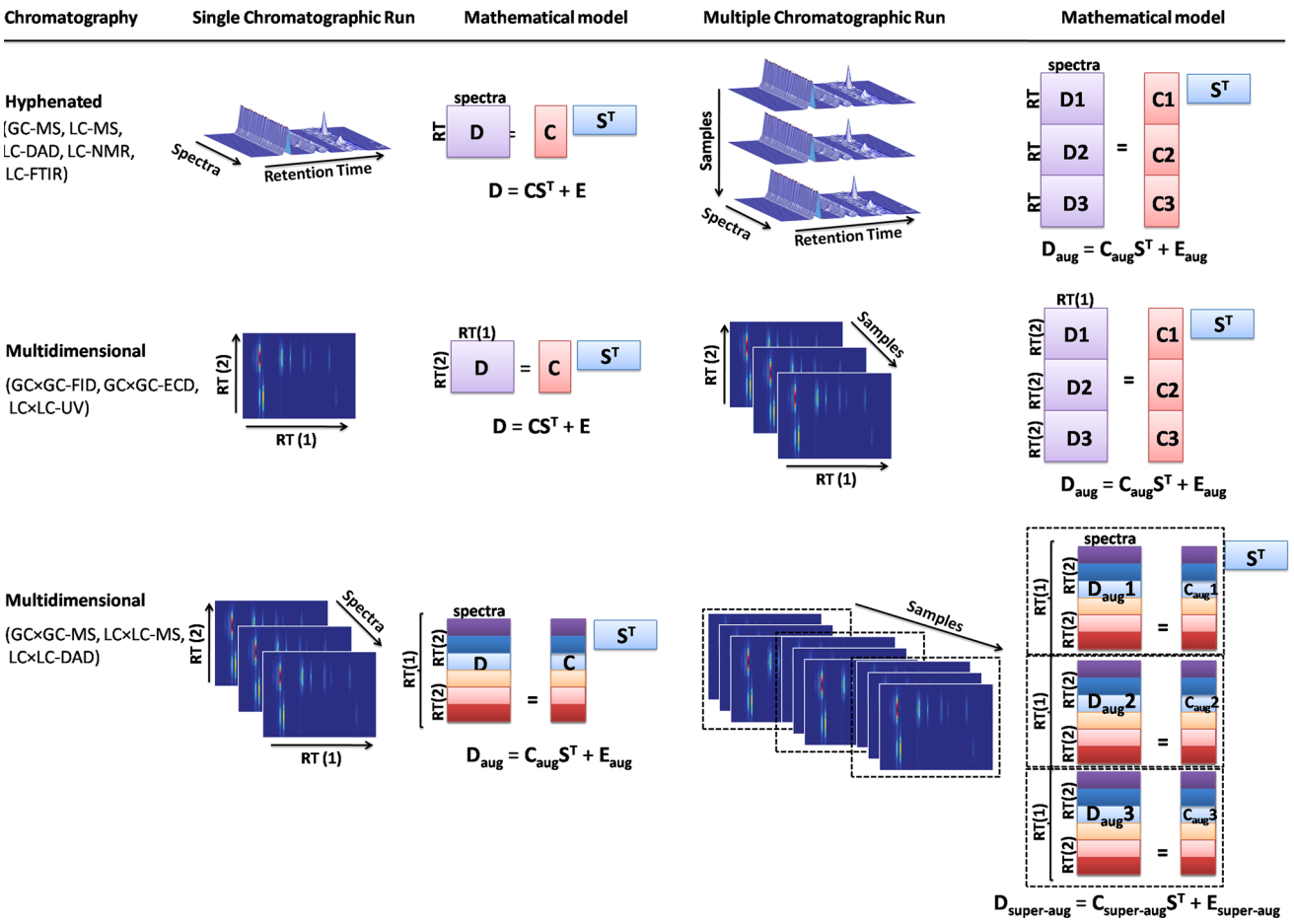

Figure 2. Data structures, data arrangement, and mathematical model for different types of individual and simultaneous hyphenated and multidimensional chromatographic runs coupled to single- and/or multichannel detectors.

uniqueness property for resolution purposes and it has been frequently used for chromatographic data analysis.[37−48] However, this method is based on the fulfillment of the trilinear model. Multirun hyphenated chromatographic data can be arranged in a three-way data structure (in a data cube where the three directions of the data are elution times, spectroscopic channels, and chromatographic runs), but this does not imply that the trilinear model be fulfilled by these data, in particular in terms of between run peak shifts and peak shape changes, due to changes in the chromatographic conditions, especially in the chromatographic column.[1,32]

PARAFAC2 is a variant of the PARAFAC technique which has been applied to chromatographic data.[1,14,49−52] Strict trilinearity in the PARAFAC2 model is not necessary, although it does not allow for significant shape changes in the elution peak across different runs (the extent of the allowed peak deformation in practice is still unknown). PARAFAC2 allows for analysis of data where there are moderate RT shifts in one chromatographic dimension due to, for example, temperature programming or misalignment across samples. However, PARAFAC2 is computationally more complex and expensive, and it does not allow for constraints (e.g., non-negativity and/or unimodality) in the chromatographic direction and therefore negative values and multimodal peaks may appear in the results. Also, applying constraints selectively only to some selected components is not possible.

Our point of view in this work is that in the case of the occurrence of chromatographic challenges, it is generally more appropriate to use the extended bilinear model expressed by eq 2 for chromatographic multirun data rather than the trilinear model described by eq 4.

It is clear that the general goal of all multivariate resolution methods is, thus, passing from the mixed nonselective information that comes from the instrument ($\mathbf{X}$) to the unmixed contribution of the pure components in the systems represented by $\mathbf{C}$ and $\mathbf{S}^T$ needing a minimum of prior knowledge from fundamental or deterministic concepts governing shapes of the resolved elution and spectra profiles, apart from soft constraints that will be discussed in the next paragraphs.

The core of all multivariate curve resolution methods is the application of constraints during the resolution of elution and spectral profiles.[25,30,53,54] This can be achieved using alternating least-squares optimization, where constraints are applied to $\mathbf{C}$ and $\mathbf{S}^T$ matrices at each iteration of the two separate linear regression steps (see refs 25, 29 30, and 54 for more details about how constraints are implemented in the MCR-ALS method).

Constraints are defined as chemical or mathematical properties that concentration (in $\mathbf{C}$ matrix) and/or spectral ($\mathbf{S}^T$) profiles should fulfill. Therefore, when a particular profile is constrained, its shape is modified accordingly to fulfill a preselected property. A suitable choice of constraints customizes the resolution method according to the chromatographic problem to be solved. It is worth mentioning that it may not be appropriate to apply nonnegativity to the spectral profiles in the

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case of DAD spectra, depending on how the instrument applies the autozeroing. Apart from the application of non-negativity to both elution (concentration) profiles and pure spectra, as imposed also in other chemical data sets, the particular properties of the elution direction allow for the introduction of constraints related to the peak shape or to the sequential elution pattern of compounds. Thus, unimodality can be selected to preserve the presence of only one maximum in each chromatographic profile. On the other hand the knowledge of the time windows where the components elute can provide very useful selective information (time channels where a particular component elutes and where it does not), which can be used to introduce local rank information (time channels where the different components elute, coelute, or are absent). Finally, when multiple chromatographic runs are simultaneously analyzed, correspondence among the eluted components in the different runs fixes the sequence and presence, absence, and correspondence of each component in the different simultaneously analyzed data submatrices. All these constraints are extremely helpful to guarantee the proper resolution of the coeluted components in the analyzed samples and increase the reliability of the quantitative determinations derived from the corresponding quantitative analysis.

The results from bilinear multivariate curve resolution methods when applied to chromatographic data analysis are the pure elution and spectral profiles of the constituents of the analyzed samples. Resolved spectral profiles can be used to identify these components by comparing the resolved spectra with those of authentic standards or standard spectra in available libraries. On the other hand, to obtain quantitative chromatographic information, the areas of the resolved elution profiles can be exploited for quantification, especially in the case of simultaneous analysis of several chromatographic runs.

Figure 2 shows different types of data set arrangements for hyphenated and multidimensional chromatographic data and the corresponding bilinear models (eqs 1 and 2) to analyze them. As it can be seen, data from spectroscopic hyphenated chromatographic analysis for a single sample can be arranged in a rectangular data matrix with elution times as rows and spectroscopic variables (e.g., m/z for GC/MS/LC−MS, λ for LC−DAD, δ for LC−NMR, and ν for GC-FTIR/LC-FTIR) as columns. In the case of simultaneous analysis of multiple hyphenated chromatographic analyses (multiset data), column-wise data arrangement is recommended. Moreover, simultaneous row- and column-wise augmentation is also possible for multiset data fusion, which means for instance when combination of different spectroscopic data for the same chromatography (i.e., LC−DAD-MS) is possible.[55]

In the particular case of data structures for multidimensional chromatographic techniques, such as GC × GC and LC × LC, relatively more complicated data structures due to the presence of two chromatographic dimensions and therefore, larger amounts of raw data arranged in multiset data structures are obtained. For two-dimensional chromatographic data with univariate detection systems, like in GC × GC-FID or in LC × LC−UV, data from a single analysis gives a data matrix with retention time along the two measurement axes. In the case of multiple analyses, two- and three-way data arrangements similar to hyphenated chromatographic analysis can be used. However, more importantly, in the case of multivariate spectroscopic detectors, such as MS or DAD, data amounts increase significantly and they should be arranged in more involved data structures. In this case, three- (for a single run (sample) chromatographic analysis) and four-way (for multiple run (samples) chromatographic analyses) data will be obtained. Column-wise superdata augmentation approaches are the regular way of chromatographic data arrangement in the case of multiple run (samples) analyses using multivariate resolution methods. For comprehensive three-dimensional GC (GC × GC × GC) with FID, a similar column-wise data arrangement can be used, even if the number of data axes (directions) is increased.[56]

For resolution of the correct elution and spectral profiles of the components of the analyzed samples, the data analysis and interpretation steps require the development and application of appropriate data analysis models which are adapted to the true data structure and particularities. Thus, multiset data structures can be easily processed using a method that is easily adapted to the chromatographic challenges described above and discussed in more detail in the next section. In particular, the popular method of multivariate curve resolution-alternating least-squares (MCR-ALS) has been shown to adapt very easily to multiset chromatographic analysis in hyphenated[17,25,57] and multidimensional chromatography[16,22,24,58] and also for multi-detection fused data scenarios.[55] Rotational ambiguities and rank deficiency problems associated to the application of MCR methods make the recovery of a unique and chemically valid result difficult especially in the case of the analysis of a single data set. This difficulty can be significantly reduced or even eliminated when multiset data are analyzed using the procedures described in this work (see refs 25, 29, and 30 for a more complete discussion of these aspects).

MULTIVARIATE CURVE RESOLUTION AND CURRENT CHROMATOGRAPHIC CHALLENGES IN HYPHENATED AND MULTIDIMENSIONAL CHROMATOGRAPHY

In this section, possible solutions for the more common chromatographic challenges encountered in hyphenated and multidimensional chromatographic analyses using multivariate curve resolution methods will be presented and will be discussed with some examples in more detail.

Baseline/Background Contribution and Noise. As it has already been mentioned, baseline/background contribution and noise are aspects of chromatographic analyses which can significantly affect qualitative and quantitative chromatographic results. The aim of baseline correction is to separate the analyte signal from the signal which arises due to changes in mobile phase composition or stationary phase bleed and to signal changes due to electronic drift and random noise. Quantitative data evaluation is based on the assumption that the baseline is stable during analysis. If this prerequisite is violated, major concentration errors can result. In addition, the presence of spectral background in the DAD or MS dimension can cause identification problems of target compounds. Furthermore, baseline/background can change both the shape and the retention times of peaks.

In the literature, there are two general approaches to handle baseline/background contributions.[1,8,15−17,25] The first approach is based on curve fitting, which means fitting a certain polynomial to a background section of the chromatographic profile and then subtract it from the overall signal. However, the curvature in the baseline is not readily described by higher order polynomials, and thus, artifacts are introduced if these methods are applied. In addition, in case of the simultaneous

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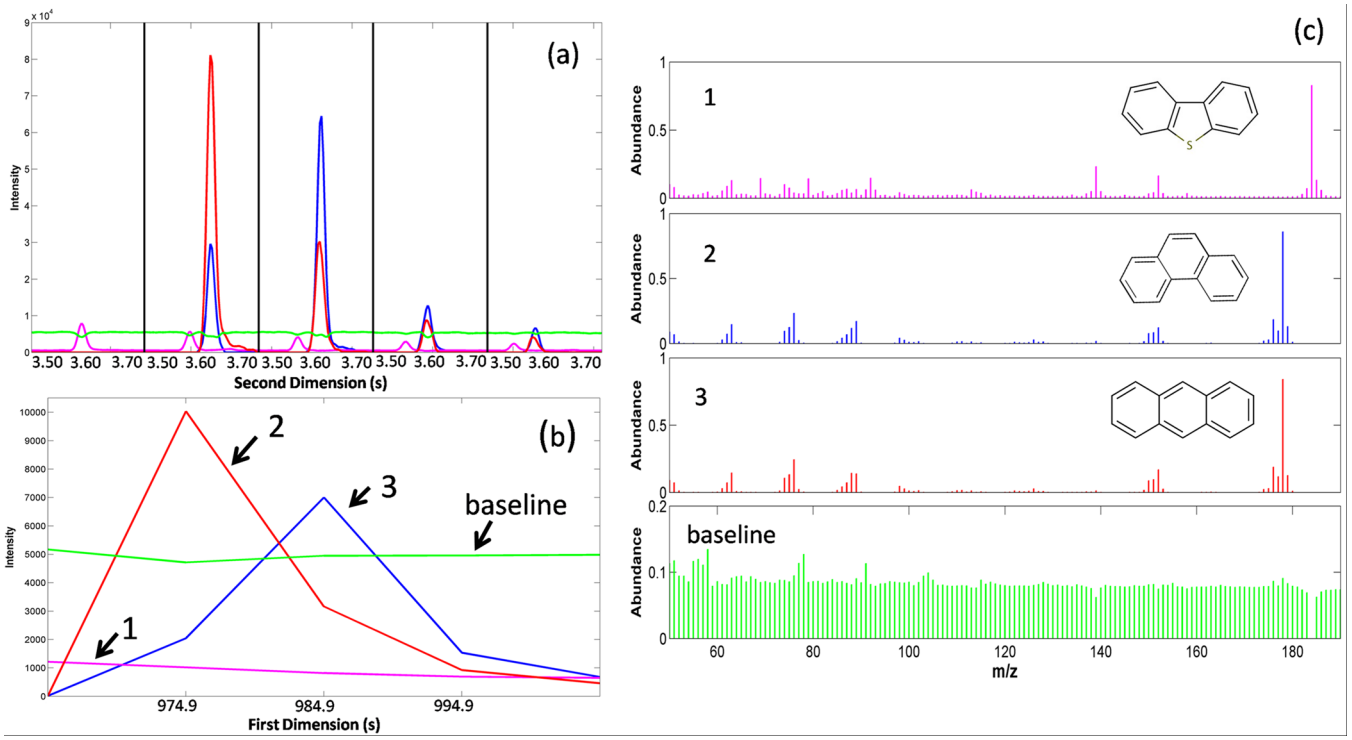

Figure 3. Performance of MCR-ALS for modeling baseline/background contribution in a chromatographic region containing phenanthrene and anthracene taken from the GC × GC-TOFMS analysis of a complex mixture of PAHs. (a) MCR-ALS resolved second dimension elution profiles in five modulations taken from the first dimension, (b) resolved first dimension elution profiles, and (c) resolved mass spectra for (1) dibenzothiophene, (2) phenanthrene, and (3) anthracene. Reprinted from ref 22. Copyright 2011 American Chemical Society.

presence of interferences and strong baseline/background drifts, they can be really difficult to distinguish and resolve separately from the analyte contribution without properly correcting for the presence of a baseline. Because of these limitations of curve fitting based methods, in many circumstances a second approach based on baseline/background modeling using multivariate resolution methods is recommended. In this case, one or more components are added to the MCR model to account for the baseline/background contribution. This approach can produce results of better quality than the curve fitting approach previously mentioned. However, it has been little used compared to curve fitting approaches due to the lack of popularization of multivariate chemometrics resolution software for nonexpert users. In this approach, baseline/background contributions are removed without loss of information in entire chromatographic and spectral regions and without the need for replicates or blank chromatograms.

Among different multivariate curve resolution methods, the MCR-ALS method has clear advantages over other methods to model baseline/background contributions because of the bilinear model assumption properties associated with this method. In simultaneous analysis of multiple chromatographic runs by MCR-ALS, no limitation exists on baseline shape changes among all samples. In other words, when hyphenated or multidimensional chromatographic data are column-wise augmented (retention times in rows and spectral channels in columns), baseline chromatographic profile can change from sample to sample, but it keeps the same spectral characteristics among all samples. This is a very important advantage of MCR-ALS bilinear modeling in multirun chromatographic measurements, since baseline changes from run to run (sample to sample) are common place, either due to changes in sample matrix or in instrumental parameters. It should be pointed out that multiple background components may be needed to properly correct for background/baseline issues.

In MCR-ALS bilinear modeling, baseline/background contribution in the chromatographic time direction (mode, axis) can change without any restriction. It should not change however in the spectral detection. In multidimensional chromatography, due to the presence of two chromatographic dimensions as well as one spectroscopic dimension (if any), modeling baseline/background contributions can be more difficult.

In Figure 3, an example of the analysis of a chromatographic region taken from the analysis of a complex mixture of polycyclic aromatic hydrocarbons (PAHs) using GC × GC-TOFMS combined with bilinear MCR-ALS is shown.[22] In the chromatographic region investigated, three chemical components and one baseline/background contribution (i.e., baseline is present in both chromatographic dimensions and in the MS dimension) were resolved (see ref 22 for more details).

In Figure 3a, the second dimension MCR-ALS resolved elution profiles in five simultaneously analyzed second dimension modulations are shown. In Figure 3b, the corresponding first dimension elution profiles are given. In these results, the strong baseline contributions (especially in the first dimension) can be well resolved by MCR-ALS despite the extreme overlap between two of the components, 2 and 3, in the first and second chromatographic dimensions. This is a direct consequence of the flexibility provided by bilinear model assumptions, which hold for all individual data matrices column-wisely arranged with their mass spectra in the common column vector space.

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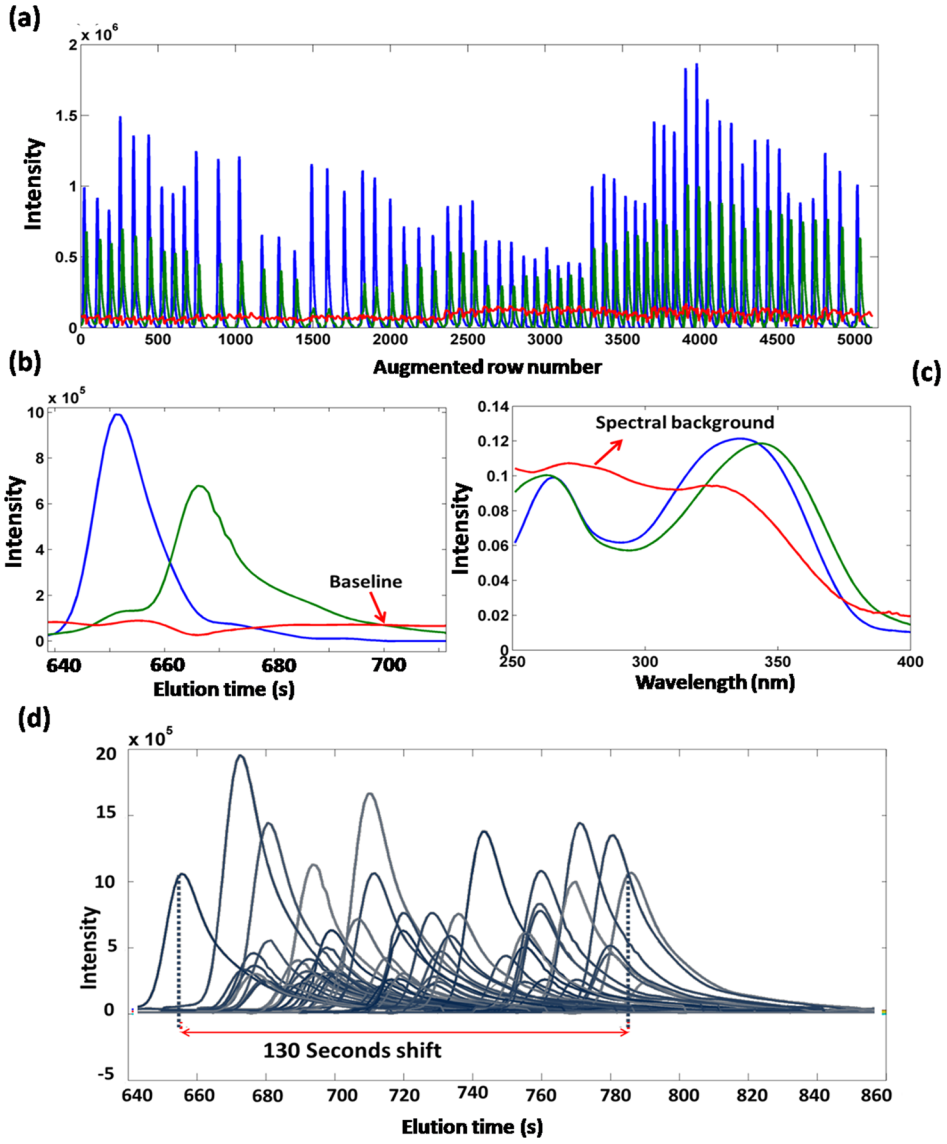

Figure 4. MCR-ALS resolution of a HPLC−DAD chromatographic segment taken from the analysis of 60 *Salvia R.* methanolic extracts in the presence of large amount of RT shifts. (a) Augmented MCR-ALS resolved elution profiles, (b) pure elution profiles for one sample (first sample), (c) resolved spectral profiles, and (d) superimposed MCR-ALS resolved elution profiles of first component in part a (blue profiles) in 60 samples.

Retention Time Shift. As it has already been mentioned, retention time (RT) shifts are another challenging problem in current chromatographic methods which can cause serious difficulties in peak identification and, therefore, subsequent qualitative and quantitative chromatographic results.[1,14−16,25,31]

RT shifts occur similarly in hyphenated and multidimensional chromatographic measurements. Chromatographic peaks due to the same constituent appear at different time positions along one or two retention time axes. Uncontrollable fluctuations in instrumental parameters such as temperature and pressure, as well as matrix effects and stationary phase degradation, and changes in mobile phase composition in LC can cause random shifts in retention times among chromatographic runs. Random RT shifts among chromatographic runs are commonplace in hyphenated and multidimensional chromatographic determinations. However, in multidimensional chromatography this type of RT shift can occur in both chromatographic dimensions; therefore, it is a much more severe problem than in hyphenated chromatography. In addition, RT shifts do already occur in the second dimension elution for a single GC × GC analysis (within run shifts).[23,24] Very often, to have a faster chromatographic run and a better chromatographic resolution, the temperature of the separation in the fast second dimension is increased throughout the process to improve peak resolution.[23] This second dimension temperature gradient causes a decrease in analyte retention times in the second dimension, from modulation-to-modulation, across the eluting peak from the first dimension. In addition, modulation period, sampling rate, and small deviations in modulation timing can also cause slight changes in second dimension retention times from modulation-to-modulation. Unfortunately, RT shifting problems increase the complexity of the hyphenated and multidimensional chromatographic data analysis. In addition, RT shifts can cause deviation not only from trilinearity but also from bilinearity (see below) and therefore, increase the difficulty of data modeling.[1]

Two different types of approaches to handle RT shifts (within and between chromatographic experiments) have been proposed in the literature, including RT shift correction and RT shift modeling. The first approach is based on correction of RT

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shifts using different data alignment and synchronization pretreatment methods.[59] For this purpose, different methods, such as correlation optimized warping (COW)[60] and dynamic time warping (DTW)[61] have been presented for correction of random RT shifts among hyphenated chromatographic runs. On the other hand, rank alignment,[62] correlation optimized shifting (COShift),[63] piecewise alignment,[64] two-dimensional correlation optimized warping (2D-COW),[65] and DTW[66] have been proposed for correction of random RT shifts among multidimensional chromatographic runs. The basis of these two groups of techniques involves stretching or compressing a target chromatogram until it matches a reference one, with certain objective function indicating the quality of the match, such as the correlation coefficient between chromatograms or a similarity index. These methods are based on selection of a reference chromatogram and the optimization of other parameters which are not trivial to choose in practice. In addition, in the presence of interferences, alignment of chromatograms become much more difficult and therefore, the second alternative strategy based on modeling of RT shifts using multivariate curve resolution methods can be a more efficient and better strategy to correct them.

Bilinear multivariate curve resolution methods, such as MCR-ALS, can handle random RT shifts among hyphenated and multidimensional chromatographic runs and also handle possible progressive RT shifts appearing within the same multidimensional chromatographic run. This is an important feature of bilinear modeling by MCR-ALS. To do this, the corresponding data matrices should be augmented in a column-wise fashion in order to keep the same spectral vector space for all of them. This new column-wise data matrix has a number of rows equal to the total number of recorded spectra at all considered retention times and for all the different chromatographic runs. It is important to note that the same data arrangement is used for multidimensional chromatographic data. However, in this case, the total number of rows in the column-wise superaugmented data matrix will be equal to the total number of recorded spectra in the second chromatographic dimension for all simultaneously analyzed chromatographic runs. This implies that for each chromatographic run, the number of spectral rows will be equal to the number of retention times on the first dimension multiplied by the number of retention times on the second dimension as determined by the modulation time. And this number of spectral rows for each chromatographic run are finally added to give the final total number of spectral rows for the column-wise augmented matrix. Note that the column vector space is the same and it corresponds to the spectral channels, m/z spectral values in MS, and wavelengths in DAD (see Figure 2 for more details regarding the data arrangement). Moreover, whereas, the number of spectral channels is exactly the same for all considered chromatographic runs, the number of retention (or elution) times in each chromatographic run can be different, and different chromatographic time ranges can be analyzed in each case. Allowing the time or chromatographic vector space be different between different chromatographic runs allows each chromatographic run to be described both in peak shape and in retention time by a different set of elution profiles, even if they belong to the same compound in a different chromatographic run. Since bilinear MCR-ALS modeling only requires that the spectral mode (vector space) of the augmented data matrix be the same, the possible presence of RT shifts and peak shape changes of the resolved elution profiles will not affect the performance of the resolution. The correspondence and correct identification of the resolved constituents is indicated by their unique spectra.

As an example, Figure 4 shows the performance of MCR-ALS for multivariate resolution of HPLC−DAD data in the presence of a large amount of random RT shifts, peak shape changes, baseline/background contributions and noise, and coleution among 60 different chromatographic runs in the analysis of a natural product sample. This chromatographic region was taken from the HPLC−DAD analysis of methanolic extracts of 60 *Salvia R.* samples. Because of the complexity of the sample matrix, a large amount of RT shifts can be seen from sample to sample. Figure 4a depicts the resolved augmented elution profiles for 60 different analyzed samples, and Figure 4b shows a zoomed plot of the resolved elution profiles for the first sample. As can be seen, the baseline/background contribution was also successfully modeled by MCR-ALS, even if it varies in different chromatographic segments (red profile in Figure 4a). Figure 4c shows the resolved spectral profiles for this chromatographic segment. As can be seen, the background spectrum is properly resolved in spite of its overlap with two other resolved components. To show the presence of a large amount of RT shifts between 60 HPLC−DAD analyses and the effective role of MCR-ALS to resolve the pure component profiles even in this complex case, Figure 4d shows MCR-ALS resolved elution profiles for the first component in Figure 4a in 60 different chromatographic analyses (i.e., retention times from 640 to 860 s). Although, RT shifts were large (∼130 s), elution profiles were properly resolved by the proposed bilinear MCR-ALS strategy described above even though elution profiles are not symmetrical (eq 2).

The example shown previously in Figure 3 is also a good illustration of how RT shifts can be handled (i.e., random and progressive) in comprehensive multidimensional chromatographic analysis (i.e., GC × GC-TOFMS in this case) using the column-wise superaugmentation strategy of multiple chromatographic runs (see Figure 2 for more details about data arrangement). Again, keeping the spectral vector space and channels the same among different chromatographic runs allows each chromatographic run to be described both in peak shape and in retention time by a different set of elution profiles by the bilinear MCR-ALS method, in comparison to trilinear model based methods.

Additionally, RT shifts (i.e., of both types, random and progressive) can cause a very challenging situation when deviations from bilinear model assumption occur. This is the case for multidimensional chromatographic systems using a univariate detector response like GC × GC-FID, GC × GC-ECD, and LC × LC-UV or in the case of using total signal strategies like in total ion current (TIC) MS detection. In such cases, because of the absence of a highly reproducible spectral mode and therefore to the presence of the two chromatographic dimensions that are not as reproducible, RT shifts and deviations make the bilinear model assumption fail.[24] As has already been discussed, multidimensional chromatographic experiments as GC × GC can suffer not only from the random RT shifts among different independent chromatographic runs but also from within run RT shifts due to (at least in part) temperature gradient strategies to achieve fast and improved resolution of elution profiles on the second dimension.[23,24] When chromatographic data containing within run RT shifts are arranged in a single data matrix and decomposed using eq 1, the number of significant singular values results will be

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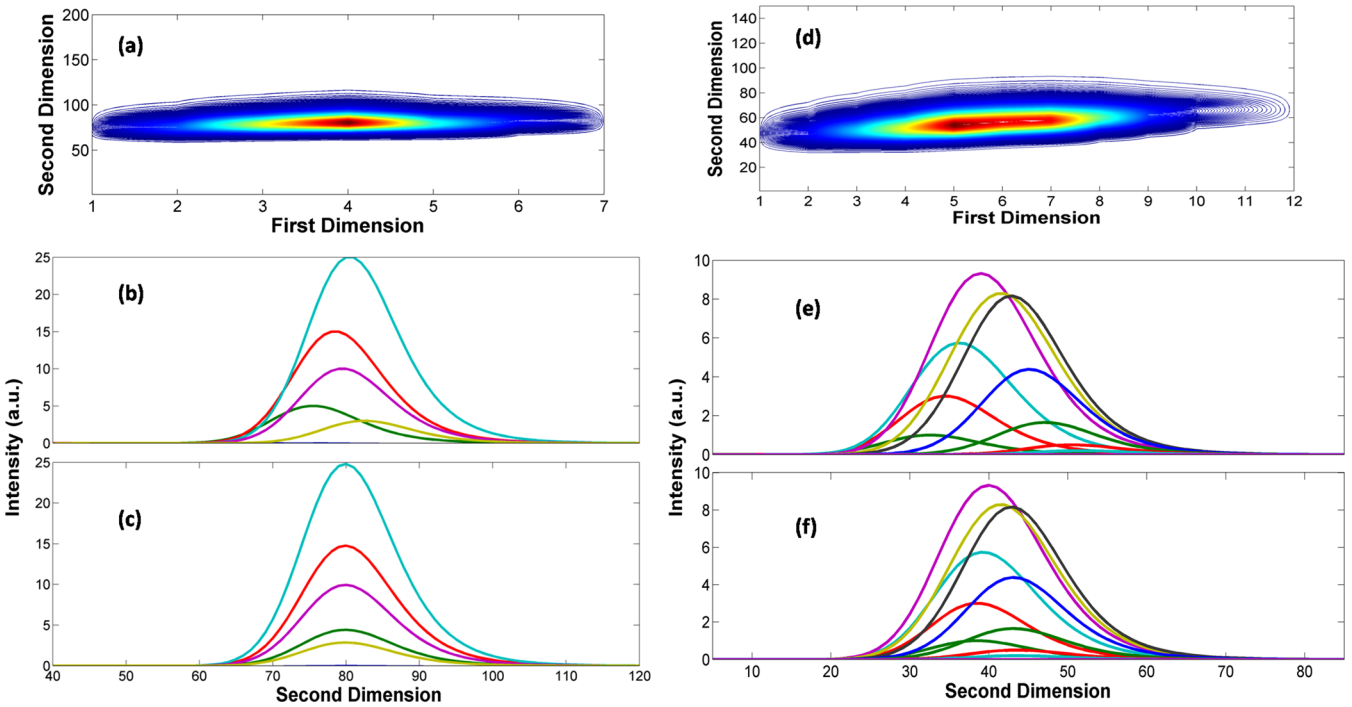

Figure 5. MCR-ALS to correct progressive RT shifts within GC × GC-FID runs for one- and two-component chromatographic systems. (a) 2D contour plot and (b) chromatographic profiles on the second dimension for one-component GC × GC-FID data containing within run RT shift. (c) RT shift corrected data using MCR-ALS. (d) 2D contour plot and (e) second dimension picture for two-component chromatographic data containing the simultaneous presence of progressive RT shift and coleution problems. (f) Corrected data using MCR-ALS bilinear peak alignment method. Modified with permission from ref 24. Copyright 2012 Elsevier.

significantly larger than the number of eluted components. This is due to the deviation from the bilinear model assumption where one component should be strictly described by a single individual elution profile in each of the two chromatographic dimensions. There is no way to describe the RT changes corresponding to the same component with a unique profile on either of the two dimensions. Note that in this case there is no spectral data direction that allows for the fulfillment of the bilinear model. Reproducibility of chromatographic elution profiles of a particular component is considerably lower than the measured pure spectra (either MS or DAD) of the same component. On way to handle this situation is to attempt the transformation of the original measured data to follow the bilinear model, for instance, using multivariate curve resolution methods, such as MCR-ALS and correction for retention time shifts (see ref 24 for more details about this possible approach). However, this strategy can be used only for situations where profile peak shapes do not change much between the different modulations. If the change in peak shape from one modulation to another for a particular component is large, then this method will not work.

Figure 5 shows the performance of the RT shift correction method based on MCR bilinear peak alignment for one- and two-component GC × GC-FID data.[24] It is clear from this figure that the progressive shift in retention times can be correctly corrected when the presence of one and two components is assumed and the bilinear MCR-ALS method is forced. Figure 5a shows the contour plot for the single component chromatographic system with progressive RT shift between the different modulations. As has been discussed previously, because of the increase of the temperature within the same chromatographic run, there is a significant and progressive shift of the second dimension elution peaks to lower retention times in different modulations. Figure 5b depicts this chromatographic region from the second dimension, which demonstrates the progressive RT shift much clearer, and Figure 5c shows the recovered data (i.e., RT shift corrected data) using the MCR-ALS bilinear method.

When these within run RT shifts are accompanied to coelution problem in GC × GC analysis of the same sample, discrimination of shift from coelution effects becomes troublesome and they can severely affect the proper resolution and quantification of the mixture components. Figure 5d shows the 2D contour plot for a two-component chromatographic segment where progressive RT shift and coleution problems occurred at the same time, and due to the absence of a characteristic spectral mode, discrimination between these two problems is very difficult. Figure 5e depicts this chromatographic segment from second dimension which the presence of two above-mentioned problems is clear. RT corrected GC × GC data after MCR-ALS analysis is shown in Figure 5f. As can be seen, the most important feature of MCR-ALS in this case is discrimination between RT shift and coleution which is really interesting. The reader is encouraged to consult ref 24 for more details about this method.

Peak Overlap and Resolution of Multicomponent Peaks. The presence of coeluted or overlapped peaks is frequently observed in any kind of chromatographic systems.[1,15−18,25,28,31,32] As already pointed out in previous sections, multivariate curve resolution methods can be used to solve these situations and they can be able to find the underlying chromatographic and spectral profiles for each component using only the raw chromatographic data plus some constraints (see eqs 1−4 and the method Multivariate Curve Resolution: Data Structures and Mathematical Model).

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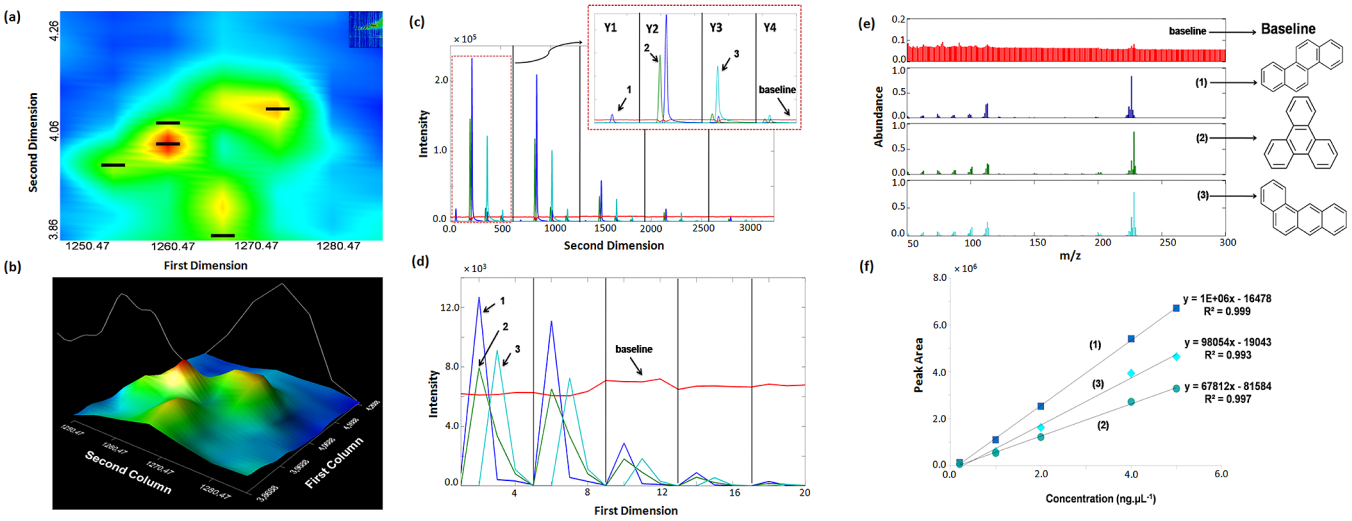

Figure 6. Resolved MCR-ALS pure component profiles for a chromatographic region taken from the GC × GC-TOFMS analysis of North Sea oil sample. (a) 2D contour plot and (b) 3D chromatogram of this chromatographic region. (c) Resolved second dimension elution profiles in five different standard samples. Insert shows the resolved profiles for three components (blue (1), green (2), and light green (3)) as well as baseline/background contribution (red) for four different modulations. (d) Reconstructed first dimension elution profiles of corresponding components in part c. (e) Resolved MCR-ALS mass spectra identified using NIST MS database as chrysene (blue, 1), triphenylene (green, 2), and benz[*a*]anthracene (light green, 3). (f) External calibration curves built up using five different standards for chrysene (blue, 1), triphenylene (green, 2), and benz[*a*]anthracene (light green, 3). Calibration equations and regression coefficients for each component are shown next to the calibration curve. Modified with permission from ref 16. Copyright 2013 Springer.

In this context, although methods based on the assumption of a trilinear model have been frequently proposed for multivariate resolution of hyphenated and multidimensional chromatographic data, limitations in the fulfillment of the trilinear model limits their successful application in a large number of situations.[1,32] In our opinion, the use of multivariate curve resolution methods based on multiset bilinear model strategies is more suitable under these circumstances. The only required assumption for the successful application of multivariate resolution methods when applied to the separation of complex mixtures is the presence of the reproducible spectral mode, i.e., that the spectra of the eluted compounds do not change among chromatographic runs. When natural samples containing hundreds or even thousands of different chemical compounds are analyzed by chromatography hyphenated to spectroscopic detection methods like UV (very little selectivity) or MS (highly selective), the use of multivariate resolution methods provide a powerful extension of their resolution capabilities. Using these methods, total analysis time for routine determination can be reduced by approaches such as fast chromatographic methods where the possible decrease of chromatographic resolution is compensated by the improved resolution achieved mathematically. In the same manner, simultaneous resolution of multiple analytes in complex natural sample matrices can be considerably improved by the use of these methods using the same strategies as with univariate detection chromatographic methods, i.e., using external calibration strategies with pure standards or mixtures, using standard addition and internal standard strategies for more complex situations of strong matrix effects or lack of linear detector response between runs. Examples of these situations can be found in several works.[1,16,18,25,30−32]

As it has already been mentioned, the results from bilinear multivariate curve resolution of chromatographic data are the pure elution and spectral profiles of the constituents of the analyzed samples. Therefore, qualitative chromatographic information can be obtained by comparing the resolved spectra with those of authentic standards or standard spectra in available libraries (e.g., NIST MS database). On the other hand, the areas of the resolved elution profiles especially in the case of simultaneous analysis of several chromatographic runs can be used to obtain quantitative chromatographic information. In this context, multivariate curve resolution methods can be used to extent the analysis of target and nontarget compounds in the presence of peak overlap and coelution with unknown interferences.[28]

Targeted analysis refers to analytical procedures where the aim is to quantify a relatively small number of known analytes of interest. In this case the chromatographer can ignore the remaining interfering agent components in the samples. In contrast, nontarget techniques aim to comprehensively analyze entire complex chromatograms to discover important analytes or chemical fingerprints while requiring a few number of user inputs and minimizing the need of prior information about the sample (for instance in omics studies[31,32]). Nontarget techniques often differ from target techniques in their scope but not in the applied method(s).

An illustrative example of this situation is given in Figure 6. In this case MCR-ALS is applied to the resolution and quantification of a mixture of PAHs in standards and in a North Sea oil sample analyzed by GC × GC-TOFMS.[16] GC × GC-TOFMS data have been arranged in a column-wise super-augmented data matrix in which m/z were in its columns and elution times in second and first chromatographic dimensions in its rows. Because the m/z values were common for all measured spectra in all second dimension modulations, reliable qualitative and quantitative information can be recovered by using bilinear MCR-ALS in the presence of previously described unavoidable chromatographic challenges like RT shifts within and between GC × GC-TOFMS experiments, peak shape changes, and the presence of baseline/background contributions. Another notable aspect of this particular MCR-

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ALS analysis was in the flexibility to consider all samples (standards, unknowns, and replicates) in the same data analysis step using a single superaugmented data matrix. In this way, resolution, identification, and quantification of the sought components can be simultaneously achieved in a fast and reliable way. Figure 6a,b shows the contour and 3D plots for the selected chromatographic region. Figure 6c shows the resolved elution profiles in the second chromatographic dimension for the four different modulations from first dimension and for five different standard samples. MCR-ALS resolved first dimension elution profiles are shown in Figure 6d. Resolved mass spectra for the corresponding components are shown in Figure 6e. In addition, areas of the resolved MCR-ALS elution profiles in the second chromatographic dimension can be used to build calibration curves for three resolved chemical components as shown in Figure 6f. All of these results confirm the power and reliability of MCR-ALS analysis to address the fundamental chromatographic challenges occurring during GC × GC-TOFMS analysis, with proper qualitative and quantitative analytical results.

■ CONCLUSIONS AND FUTURE OUTLOOK

Coupling hyphenated and multidimensional chromatographic measurements with multivariate curve resolution methods is a powerful strategy to solve current challenging problems in chromatography. Some of these challenges are baseline/background contributions, retention time shifts, peak shape changes, and coelution. Indeed, traditional approaches have difficulties in achieving the required analytical selectivity and the simultaneous extraction of the required information about multiple analytes in complex biological and natural samples. Therefore, the complementary use of suitable multivariate curve resolution tools is recommended. The major advantages of these multivariate resolution methods are (1) their increasing resolution power at the time that they decrease expenses of time, chemicals, solvents, and money, (2) allowing at the same time a better understanding of the global chromatographic process, and (3) more flexibility and possible automation of tedious individual steps. Clearly, the combination of hyphenated and multidimensional chromatography together with chemometric resolution methods provides an extremely powerful approach which complements and, in many cases, increases the analytical potential of this new approach. In such a manner that once demonstrated, it should attract the attention of the analytical chemistry community and, in particular, of vendors and people working in chromatography. This view will make application of multivariate curve resolution methods as a common place strategy in solving current more challenging and recalcitrant analytical problems. In this regard, work is still needed to fill the gap between the two fields and communities working in chemometrics and chromatography to take advantages from both sides.

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Notes

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