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Resolution of multicomponent peaks by orthogonal projection approach, positive matrix factorization and alternating least squares

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Abstract

The application of orthogonal projection approach (OPA), alternating least squares (ALS), and positive matrix factorization (PMF) to resolve HPLC-DAD data into individual concentration profiles and spectra is discussed. OPA was initially described as a purity method but the inclusion of an ALS procedure allows its application as a curve resolution method. PMF is a least square approach to factor analysis that in this study has been used as a tool to tackle the problem of curve resolution. OPA, ALS and PMF have been applied using a single matrix (two-way data) or an augmented matrix containing several data matrices simultaneously. The results obtained with the different resolution methods are compared and evaluated using measures of dissimilarity between the real and the estimated spectra. The study is performed in three data subsets, obtained by segmentation of the original data matrix. Within each data subset, there is a reduced number of species present which makes the resolution easier.

Keywords: HPLC-DAD; Two and three-way data; Multivariate curve resolution; OPA; PMF; ALS

1. Introduction

Chromatographic techniques that employ multichannel detection, such as high performance liquid chromatography with diode array detection (HPLC-DAD), are among the most powerful techniques for the qualitative and quantitative analysis of mixtures. The ideal situation is that each chromatographic signal corresponds to a single analyte but these conditions are not always achieved. Yet, these techniques have limitations, particularly when the signal from an analyte overlaps with signals from neighbouring compounds in both the time and spectral dimensions.

The result of HPLC-DAD is a data matrix $X(m \times n)$, with m rows formed by the absorption spectra measured at regular time intervals, and n columns formed by the chromatograms measured at successive wavelengths. The combination of these two dimensions enables the application of techniques of factor analysis for the resolution of overlapping chromatographic peaks.

Curve resolution methods are a group of chemometrical approaches suited for the treatment of multicomponent data matrices. Their main purpose is the correct determination of the response profiles (spectra

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and concentration profiles) of the components present in unresolved mixtures. All of these methods assume that Beer's law holds, in which case the original data matrix can be represented by the product of two smaller matrices, that contain the concentration profiles and the pure spectra of each individual compound present in the mixture. The methods can be divided in two groups: (a) Two-way resolution methods applicable to the resolution of single matrices, e.g. orthogonal projection (OPA) [1–3], simple to use interactive self-modelling mixture analysis (SIMPLISMA) [1,2,4–6], iterative target transformation factor analysis (ITTFA) [7–9] or heuristic evolving latent projection (HELP) [10–12] and (b) three-way resolution methods applicable to the resolution of two or more matrices together, e.g. trilinear decomposition (TLD) [13–15], parallel factor analysis (PARAFAC) [16] and restricted Tucker models [17,18].

Positive matrix factorization (PMF) [19–22] and alternating least squares (ALS) [13–15,23–26] methods, in contrast to most of the other methods, are equally applicable to both kinds of data sets (two and three-way data). PMF is a chemometrical approach that solves the factor analysis problem by a weighted least square fit where the individual error estimates for the data points are used to develop the weights. In addition, all the elements of the resulting factors can be constrained to be non-negative. PMF has been successful applied in mixture resolution problems [20,27].

ALS is an iterative process that needs a matrix of initial estimates, either spectra or concentration profiles, to start the optimisation procedure. The initial estimates are usually built by approaches that take into account the information in the original data set, such as Needle Search [9], EFA [28–31], SIMPLISMA or OPA. Due to its iterative character, errors made in the initial estimates can be corrected in successive calculation steps. In addition, some features of the concentration profiles and/or spectra can be introduced in the resolution procedure by the application of suitable constraints [22–26].

In this paper the potential of OPA, PMF and ALS for curve resolution of HPLC-DAD data is compared.

2. Theory

HPLC-DAD instruments yield for each chromatographic run i a bilinear data matrix $X_i(m\times n)$, that can be decomposed into the product of two matrices, one containing the concentration profiles in the chromatogram, $C_i(m\times p)$, and the second the individual spectra, $S_i(n\times p)$, where p is the number of components. The corresponding two-way factor model can be written as

$$X_i = C_i S_i^{\mathrm{T}} + E_i \quad i = 1, 2, \ldots, N \tag{1}$$

where E_i represents the part of the data variance unmodeled by the p factor model.

The methods used in this work belong to the category of iterative curve resolution methods. They follow a general working procedure that involves two steps. In a first step initial estimates of C and/or S matrices are built, and in a second one the initial estimate(s) are improved by an iterative constrained least–squares fit until convergence is achieved. However, differences, such as the way in which the initial estimates are obtained, convergence criteria or constraints applied, can be encountered between them.

2.1. OPA

OPA is a stepwise approach based on the selection of a 'representative' set of the pure or purest spectra from the data matrix X to be used as initial estimates in the iterative process. The principle of the method is as follows:

1. The 'representative' set of spectra is selected using a dissimilarity (dis) criterion by means of an iterative determinant calculation. The dis_i of spectrum is defined as the determinant of a dispersion matrix Y_i:

$$\mathrm{dis} = \det(Y_i^{\mathrm{T}} Y_i) \quad \text{for } i = 1, \ldots, m \tag{2}$$

   where Y is the $(n\times p)$ matrix of the selected key or representative spectra [27]. The representative spectra are those that are the purest possible for each compound. The selection process consists of: (i) compare each spectrum in X with all spectra already selected by applying the Eq. (2). Initially, the spectra are compared with the average spectrum of matrix X; (ii) plot of the dis_i values as a function of the retention time and (iii) select the spectrum with the highest dis_i value by including it as a reference in matrix Y_i. The selection of the spectra is finished when the dis plot shows a random

profile. In principle one expects that the number of representative spectra is equal to the number of compounds.

2. Estimation of the concentration profiles by least squares, using the matrix Y as input, after normalisation

$$y_{Nh} = \frac{y_h}{(t_h^{\mathrm{T}} y_h)} \quad \text{for } h = 1, \ldots, p$$

i.e. $Y{=}S$ in Eq. (1),

$$C = XS(S^{\mathrm{T}}S)^{-1} \tag{3}$$

The estimated concentration profiles are constrained to have non-negative values and unimodal shape. The non-negativity constraint is achieved by setting all negative values equal to the mean background.

3. New estimates for the pure spectra are generated by least squares, using the previously estimated concentration profiles

$$S = X^{\mathrm{T}}C(C^{\mathrm{T}}C)^{-1} \tag{4}$$

4. Steps 2–3 are repeated until convergence is achieved. Convergence is reached when the relative difference (RD), between the original and the reproduced data matrix, between two consecutive iterations is lower than 0.1%

$$\mathrm{RD} = \left[\sum_{ij}(x_{ij} - x_{ij}^*)^2\right]^{0.5} \tag{5}$$

where x_{ij} represent the experimental absorbances at elution time i and wavelength j, and x_{ij}^* the reproduced absorbances at elution time i and wavelength j.

2.2. OPA-A

OPA-A allows the simultaneous analysis over several chromatographic runs. The resulting row-wise data matrix X has a number of rows equal to the total number of acquired spectra in the different chromatographic runs, and has a number of columns equal to the number of wavelengths. This new data matrix X is the product of the augmented concentration matrix C and the unit spectra matrix S^{T}. Including pure spectra in the analysis reduces the rotational indeterminacy that would otherwise exist in the solution.

2.3. ALS

ALS also called alternating regression was described by Tauler et al. [24]. In this study, the method uses initial estimates obtained with OPA. Then an identical iterative process to that previously described for OPA (steps 2–3) accomplishes the resolution of X. Convergence is reached when the relative change of the lack of fit between the original data matrix and the ALS-reproduced data matrix, between two consecutive cycles is lower than 0.1%.

$$\text{Lack of fit} = \left[\frac{\sum_{ij}(x_{ij} - x_{ij}^*)^2}{\sum_{ij}(x_{ij})^2}\right]^{0.5} = \frac{\mathrm{RD}}{\sum_{ij}x_{ij}} \tag{6}$$

However, ALS provides an important advantage in relation to the OPA. This one is the great flexibility in the introduction of constraints [22–26] in both the concentration profile and the spectra matrices.

The constraints that were applied include: (a) non-negativity for all the concentration profiles and spectra; (b) unimodality for concentration profiles, i.e. the presence of only one maximum in each chromatographic profile is allowed (in two versions, the vertical and the horizontal [26]) and (c) selectivity in the input values where only one component is present, that forces the concentration of the other components to be zero after each iterative cycle. Another constraint applied, in some cases, is keeping the spectrum of a certain component constant during the ALS optimisation and only the spectra of the remaining components may be modified. This constraint is applied to improve the resolution in those cases where the spectrum of a particular specie is previously well known.

ALS has been applied to the augmented data matrix in the same manner as OPA-A outlined above. This approach will be designated as ALS-A.

2.4. PMF

PMF is a variant of factor analysis with factors constrained to be non-negative. The method assumes that X_{ij}, σ_{ij} (standard deviation) and the number of factors h are known. The bilinear model (Eq. (1)) is based on a weighted least squares fit, where the sum of the squares of the residuals weighted inversely with

the standard deviation (σ_{ij}) of the individual data elements of X_{ij} is minimised,

$$Q=\sum_{i=1}^{m}\sum_{j=1}^{n}\left(\frac{X_{ij}-C_{ih}S_{hj}^{T}}{\sigma_{ij}}\right)^{2}=\sum_{i=1}^{m}\sum_{j=1}^{n}\left(\frac{E_{ij}}{\sigma_{ij}}\right)^{2} \quad (7)$$

The factors are optimised simultaneously by an iterative algorithm that starts using pseudorandom initial values of C and S. The process continues until convergence (typically less than 100 iterations). The PMF2 method uses penalty terms to force the factors to be non-negative and regularisation terms to eliminate singularity from the model. The details of the algorithm are provided elsewhere [19–21].

The correct number of factors (h) is found trying different values. If h is too small, the convergence is rapid, but the Q remains large. If h is too large, the process converges slowly and gives several different results when repeated. Theoretical Q values should approach the number of data points in the data matrix X if the true standard deviations are available, i.e. if the experimental error estimates are good approximations to the actual values. The associated factors are then the final solutions.

PMF2 has been applied to the augmented data matrix in the same manner as OPA-A and ALS-A outlined above. This approach will be designated as PMF2-A.

3. Experimental

3.1. Data

The data were acquired on a Waters (Milford, MA, US) model 990 DAD liquid chromatographic system, equipped with a Hypersil C_{18} column (100×0.46 mm I.D., 5 μm particle size). A mobile phase acetonitrile:water (60:40 v/v) under isocratic conditions was used. Injections were made through a Rheodyne six-port injection valve with a 20 μl sample loop and a flow rate of 1 ml min^{-1}. Photometric detection was performed in the 200–280 nm range, with a spectral resolution of 1.4 nm. Data was obtained over an integration period of 1.4 s per spectrum.

Analytical-reagent grade solvents from Merck (Darmstadt, Germany) and purified Milli-Q water (Bedford, MA, US) were used. Pesticide standards (Pestanal quality) of iprodione (Ip), procymidone (Pr), chlorothalonil (Ct), chlorfenvinphos (Cf), fenamiphos (Fe), malathion (Ma), parathion-methyl (P-m), parathion-ethyl (P-e), tebuconazole (Te), triadimefon (Td), triazophos (Tz) and vinclozolin (Vi) were obtained from Riedel-de Haën (Seelze, Germany) and used without further purification.

3.2. Software

The analysis of the data using OPA and ALS was performed using in-house routines written in Matlab 5.0 and 4.0. PMF2 was performed using the routines of Paatero [20,21].

4. Results and discussion

The performance of OPA, PMF2, ALS, OPA-A, ALS-A and PMF2-A methods was tested on a twelve component HPLC-DAD system. The study was carried out in three different submatrices of peak clusters, obtained by segmentation of the complex chromatographic data set, on the basis of previous results [32]. In Fig. 1, the baseline-corrected chromatograms of the individual pure standards of the 12 pesticides are superposed. Baseline correction was performed by subtracting the linear interpolation in regions before and after the elution of the peaks.

The quality of the final results, in all methods, is assessed by using measures of dissimilarity between the real and the estimated spectra obtained:

$$\mathrm{dis}(s_{\mathrm{est}}, s_{\mathrm{real}}) = (1 - r_{(s_{\mathrm{est}}, s_{\mathrm{real}})})^{0.5} \quad (8)$$

where s_{est} and s_{real} are the estimated and real spectrum, respectively, and r is the correlation coefficient between these two vectors. A dissimilarity value equal to zero means a total agreement. In addition, the results were evaluated qualitatively by plotting the resolved and the true spectra in the same plot. The true absorption spectra of all compounds are shown in Fig. 2.

4.1. Region 1

Three compounds are eluting, Fe, Td and P-m, although some part of the data variation is explained by the following eluting component, Ip, that starts

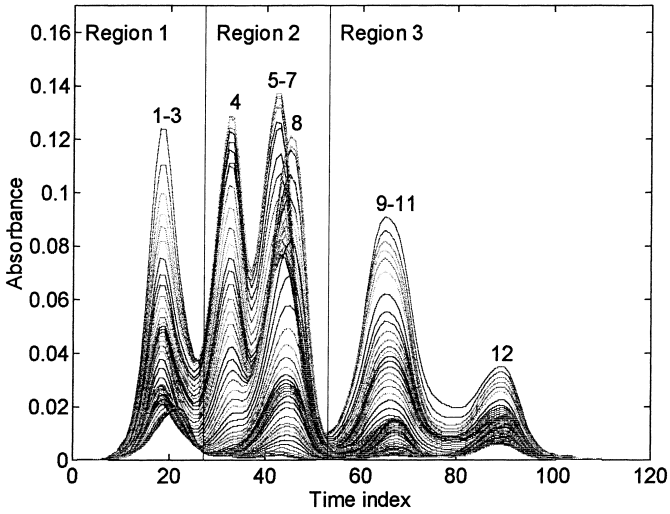

Fig. 1. Superposed baseline-corrected chromatograms of the 12 standards of the pesticides: (1) Fe, (2) Td, (3) P-m, (4) Ip, (5) Ma, (6) Tr, (7) Pr, (8) Ct, (9) Vi, (10) Cf, (11) Te and (12) P-e.

to elute at the end of this region. The analysis of the data by OPA and PMF indicates the presence of this fourth component. Results obtained by OPA, PMF2, ALS and PMF2-A are presented in Fig. 3.1 and Table 1.

Similar concentration profiles were estimated with PMF2 and ALS (with both horizontal and vertical unimodality constraints), and these were in agreement with their real location Figure 3.1. However, the top of the chromatographic profile obtained with OPA for the second component was not correct. The recovered spectra with OPA for the two first components were quite similar, but very different from the real ones Figure 3.2a. With PMF2, the estimated spectrum for the second component (Td) matches the real one very well, and the recovered spectrum of the first component is better than with OPA Figure 3.2b. A slightly better performance was obtained for the estimated spectrum of the third component, P-m.

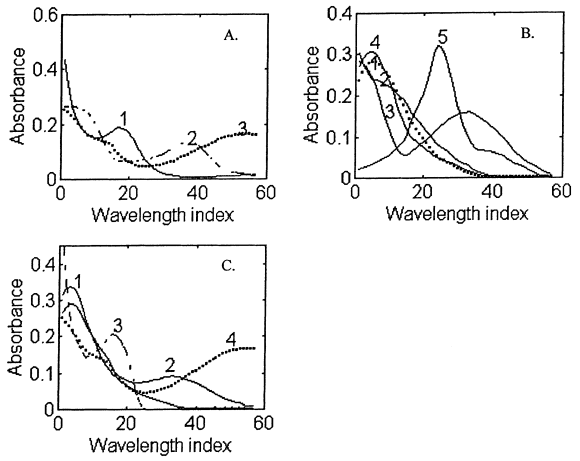

Fig. 2. Absorption spectra of: (a) (1) Td, (2) Fe and (3) P-m; (b) (1) IP, (2) Ma, (3) Tz, (4) Pr and (5) Ct; (c) (1) Vi, (2) Cf, (3) Te and (4) P-e.

OPA-A, ALS-A and PMF2-A were also applied. Two augmented data matrices with five samples each were evaluated. The first matrix contained the individual standards of the Fe, Td, P-m and Ip components and a quaternary mixture of them, and the second matrix was a five quaternary mixture of the components. Table 1 summarises the results obtained, finding smaller or similar dissimilarity values using the first augmented matrix compared to the second. In addition, ALS-A and PMF2-A yielded better results than the other methods tested in this region. Fig. 3.1d shows the resolved concentration profiles while Fig. 3.2c shows the spectra. In Fig. 3.2c almost perfect matches can be seen for all components. Similarly excellent results were observed for the ALS-A derived spectra.

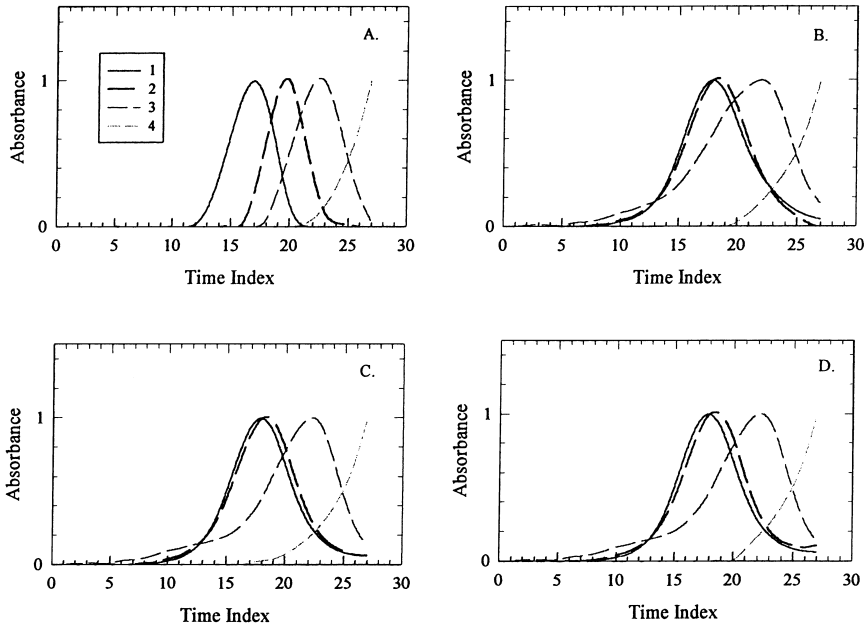

Fig. 3.1. Estimated concentration normalised profiles in Region 1 by: (a) OPA, (b) PMF2, (c) ALS (horizontal unimodality), and (d) PMF2-A of (1) Fe, (2) Td, (3) P-m and (4) Ip.

4.2. Region 2

This is the more complex region where five compounds elute: Ip, Ma, Tz, Pr and Ct. The Ma, Tz and Pr components have very similar retention times. Ma has a low signal relative to the other compounds, and its absorption spectrum does not present selectivity with respect to that of Pr. In this region, the P-m component which is the third component eluting in the first region, and the Vi, the first eluting component in the Region 3 are detected by OPA and PMF2. However, when ALS was applied, the position of the estimated concentration profile for the Vi component was not correct, so that Vi was not included in the ALS studies performed in this region.

The estimated concentration profiles show a large overlap (Fig. 4). The tops of the recovered peaks, by all three methods, are located at very close time index values in agreement with their real elution times, although there are some differences in the peak shapes. OPA gives narrowest peaks, despite sharp peak obtained for the Tz component with PMF2. However, the estimated concentration profiles by PMF2 and ALS for Pr and Ct show peak bases that are too wide. The Ma component shows an abrupt cut off in the concentration profile generated with PMF2. ALS using horizontal or vertical unimodality performed similarly.

The dissimilarity of the estimated and reference spectra is given in Table 2. The calculated spectra for Ip with all the multivariate curve resolution methods

Table 1
Resolution of the Region 1 by OPA, PMF and ALS: dissimilarity between the individual estimated and reference spectra[a]

Component	OPA	PMF2	ALS	OPA-A	PMF2-A	ALS-A
Fe	0.3240	0.2983	0.2642	0.0033 (0.3967)	0.0074 (0.0077)	0.0100 (0.1100)
Td	0.3130	0.0710	0.0775	0.0141 (0.4231)	0.0060 (0.0064)	0.0245 (0.0245)
P-m	0.2385	0.2086	0.0316	0.0100 (0.6753)	0.0033 (0.0034)	0.0141 (0.0316)

[a] Values within parentheses are the dissimilarity values obtained with the augmented data matrix build with mixtures.

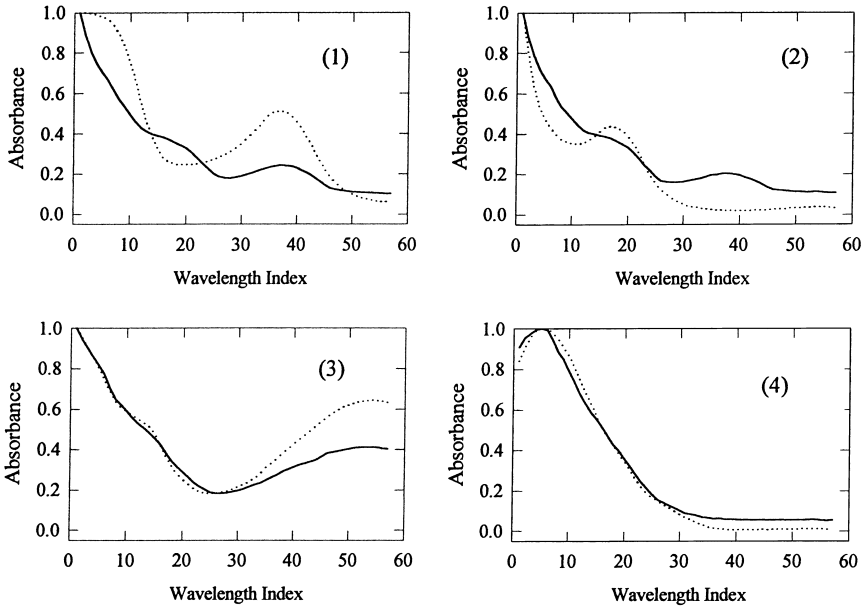

Fig. 3.2a. Comparison of the estimated normalised spectra (dashed lines) and reference spectra (single lines) in Region 1 by OPA, of (1) Fe, (2) Td, (3) P-m and (4) Ip.

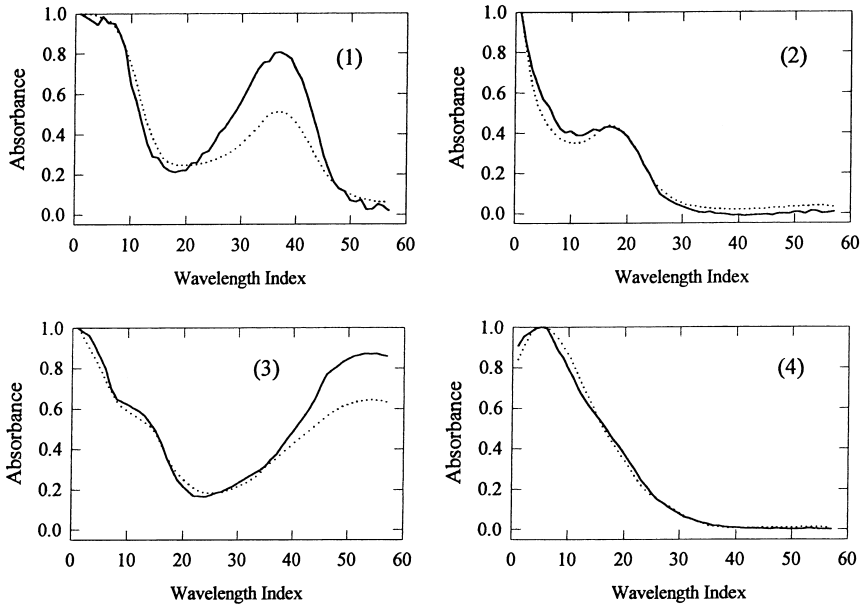

Fig. 3.2b. Comparison of the estimated normalised spectra (dashed lines) and reference spectra (single lines) in Region 1 by PMF2 of Fe(1), Td(2), P-m (3) and Ip (4).

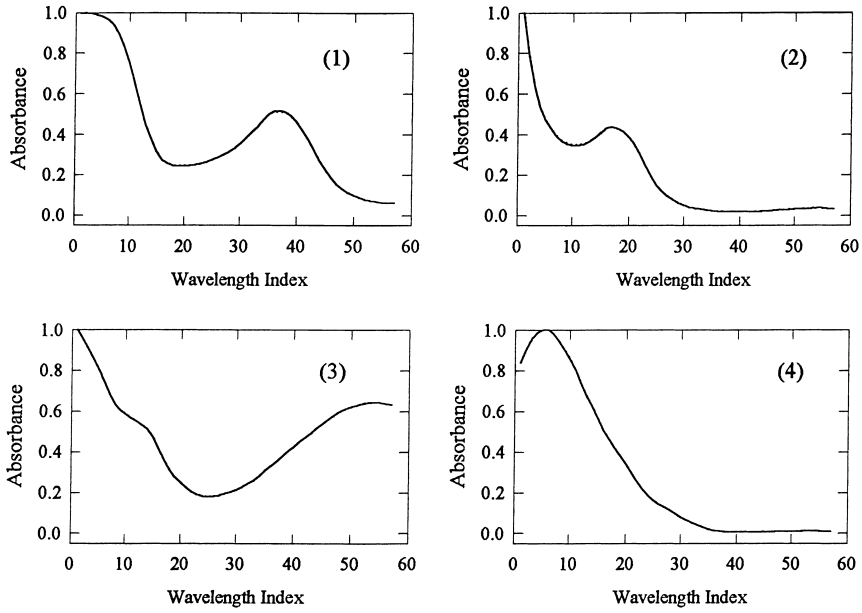

Fig. 3.2c. Comparison of the estimated normalised spectra (dashed lines) and reference spectra (single lines) in Region 1 by PMF2-A of Fe(1), Td(2), P-m (3) and Ip (4).

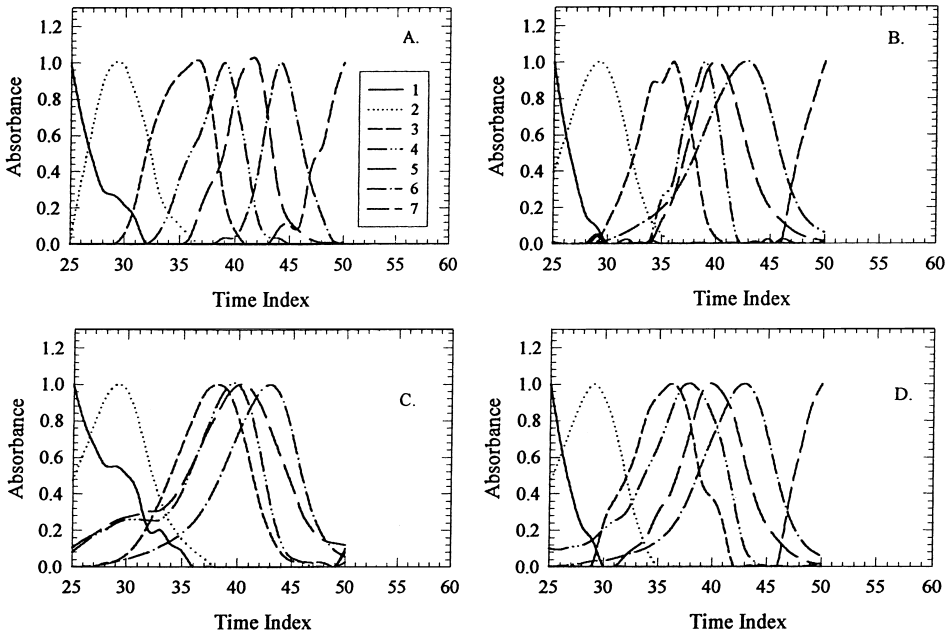

Fig. 4. Estimated concentration normalised profiles in Region 2 by: (a) OPA, (b) PMF2, (c) ALS (horizontal unimodality) and (d) PMF2-A of P-m (1), Ip (2), Ma (3), Tz (4), Pr (5) and Ct (6).

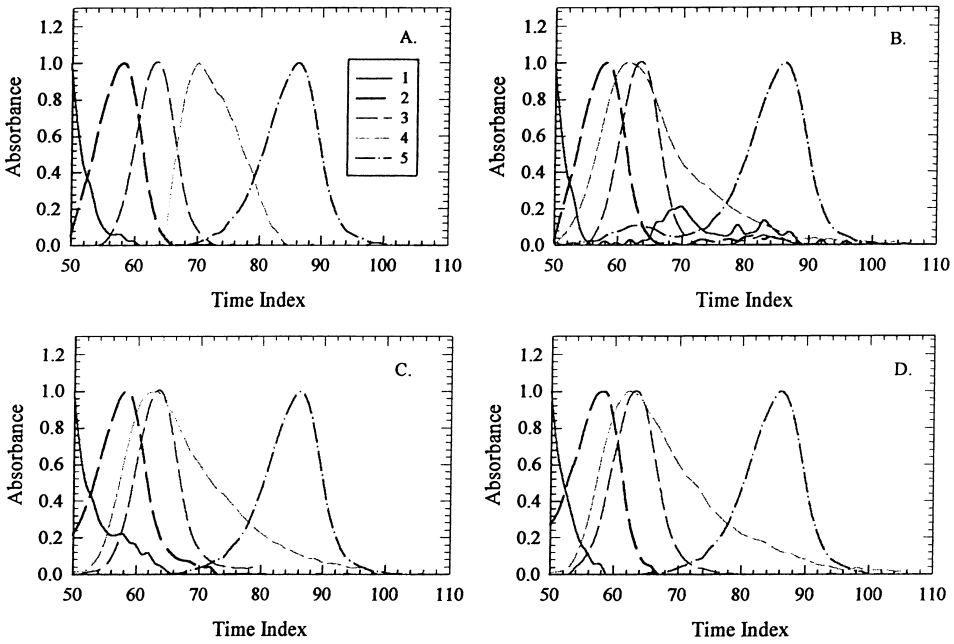

Fig. 5. Estimated concentration normalised profiles in Region 3 by: (a) OPA, (b) PMF2, (c) ALS-A (horizontal unimodality), and (d) PMF2-A of (1) Ct, (2) Vi, (3) Cf, (4) Te and (5) P-e.

are very similar to the real one, since this component is quite well separated from the other four components. The estimated spectra with OPA for Ma, Tz, Pr and Vi are clearly influenced by the Ct component, and large dissimilarity values between these spectra and the reference spectra were obtained.

The calculated spectra by PMF2 are better than those obtained by OPA, except for Ma. In general the results obtained with ALS are not very different to the ones obtained with PMF2, except for Tz and Ma. It is noted that the spectrum of the Ma (with a low relative signal) is wrongly recovered by ALS. To improve the results for this component, ALS was applied, using the standard spectra of Ma in the initial estimates, and keeping its spectrum fixed during the iterative process. However, the ALS procedure diverged in this case.

OPA-A, ALS-A and PMF2-A were also evaluated with the aim of achieving a better performance for Ma. Two augmented data matrices with seven samples each were tested. The first matrix contained the individual standards of the P-m, Ip, Ma, Tz, Pr and Ct components and a mixture with all of them, and the second matrix with seven mixtures of the components. Better results were obtained using the first matrix except for Ma (Table 2). To improve the resolution of Ma, ALS-A was applied also but fixing its spectrum

Table 2
Resolution of the Region 2 by OPA, PMF and ALS: dissimilarity between the individual estimated and reference spectra[a]

Component	OPA	PMF2	ALS	OPA-A	PMF2-A	ALS-A
Ip	0.0447	0.0400	0.0361	0.0141 (0.0586)	0.0124 (0.0226)	0.0100 (0.0332)
Ma	0.1865	0.1931	0.6317	0.02064 (0.0586)	0.0553 (0.0412)	0.3127 (0.2709)
Tz	0.5201	0.2078	0.0935	0.0100 (0.5045)	0.0127 (0.0129)	0.0787 (0.1342)
Pr	0.5311	0.0686	0.0775	0.1039 (0.1005)	0.0127 (0.0129)	0.0100 (0.0735)
Ct	0.1775	0.0656	0.0831	0.0141 (0.2081)	0.0182 (0.0115)	0.0436 (0.1825)

[a] Values within parentheses are the dissimilarity values obtained with the augmented data matrix build with mixtures.

Table 3
Resolution of the Region 3 by OPA, PMF and ALS: dissimilarity between the individual estimated and reference spectra[a]

Component	OPA	PMF2	ALS	OPA-A	PMF2-A	ALS-A
Vi	0.0640	0.0980	0.0458	0.0100 (0.0510)	0.0006 (0.0040)	0.0010 (0.0141)
Cf	0.1490	0.1217	0.0616	0.0100 (0.3723)	0.0148 (0.0017)	0.0100 (0.0458)
Te	0.1470	0.1261	0.1446	0.1628 (0.1278)	0.0440 (0.0103)	0.0671 (0.1131)
P-e	0.2142	0.0825	0.1778	0.0141(0.1010)	0.0147(0.0029)	0.0831 (0.0954)

[a] Values within parentheses are the dissimilarity values obtained with the augmented data matrix build with mixtures.

and using the first augmented data matrix. Now, convergence was achieved and the dissimilarity values for the other components were quite similar to the ones previously obtained (Table 2). In this way the resolution of the Ma component was possible, so that it can be concluded that ALS-A and PMF2-A are better in this region. The capability to fix a number of points in the standard spectra reduces the rotational indeterminacy in the resulting factors and produce better solutions.

4.3. Region 3

In this region the four eluting compounds, Vi, Cf, Te and Pe, do not overlap so severely as in the previous regions, but the Te component presents a poor chromatographic response with a wide and low peak. OPA and PMF2 detected the Ct component, the last component eluting in the second region.

The concentration profiles (Fig. 5) generated by PMF2 and ALS are very similar, with maxima at about the same locations and with similar peak shapes. Slight variations around zero were observed in the concentration profiles obtained by PMF2, mainly for the component that finishes to elute in this region (Ct). On the other hand, the estimated concentration profiles by OPA are narrower and sharper than those obtained by the other methods. Similar conclusions are drawn as in the previous regions with regard to the horizontal and vertical unimodality.

The dissimilarity of the estimated and reference spectra is given in Table 3. The spectrum obtained for Ct matches very badly with the real spectrum. The calculated spectra for Vi, Cf, Te and Pee by PMF2 and ALS are acceptable. As in the previous regions, the estimated spectra by OPA were worse (similar to Vi component) than those obtained by the other two methods.

Despite the good results obtained using a single data matrix, OPA-A, ALS-A and PMF2-A were also evaluated in this region. Two augmented data matrices were built with six samples, the four standards of the components present in this region plus the one of Ct, and one mixture that contained all components, in the first matrix, and six mixtures in the second matrix. The recovered pure spectra show an excellent agreement with the real ones (Table 3).

5. Conclusions

OPA is a useful tool for the assessment of peak purity and for the resolution of not too overlapped components. This method provided the worst estimated spectra for groups with low chromatographic resolutions between components. However, more correct chromatographic profiles were obtained, even in zones with severe overlapping.

In general PMF2 and ALS showed little differences, except for the spectrum of Ma, component with a low relative signal, that was poorly recovered by ALS. PMF2 can be considered a potentially powerful tool for curve resolution analysis of HPLC-DAD data. It does not require a priori information about the concentration profiles or spectra, as is the case in ALS. In addition, the positive nature of the solution in PMF2 is an advantage because instrumental profiles in HPLC-DAD must be positive.

Augmented matrix data analysis showed an improvement in the recovery of the spectral profiles. The inclusion of the standards of the components showed slightly better dissimilarity values than the use of augmented matrices built with mixtures. The application of OPA-A, ALS-A and PMF2-A with adequate constraints allowed the complete resolution of the HPLC-DAD system (dissimilarities lower than 0.09, i.e. correlations higher 0.996).

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