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Determination of traces of herbicide mixtures in water by on-line solid-phase extraction followed by liquid chromatography with diode-array detection and multivariate self-modelling curve resolution

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Abstract

Multivariate self-modelling curve resolution was applied to the determination of co-eluted alachlor and metolachlor mixtures. Mixture analysis of these two pesticides in the presence of other interferents in real water mixtures was achieved using automated on-line solid-phase extraction coupled with liquid chromatography and diode-array detection (DAD) followed by a recently developed multivariate self-modelling curve resolution method. Two different kinds of water samples were analysed (Milli-Q-purified water and surface river water) using two different analytical columns (Merck and Waters). The proposed approach permitted an improvement in the resolution of the co-eluted herbicides after preconcentration of 100 ml of water samples spiked at the 1.5 μg/l concentration level and also allowed their simultaneous determination independently of the water matrix. Recommendations about further incorporation of the proposed method in liquid chromatographic–diode-array detection methods are given.

1. Introduction

Solid-phase extraction (SPE) coupled on-line with liquid chromatography (LC) is being used for monitoring pesticides in surface and drinking water samples at trace levels (0.1–5 μg/l) in various monitoring programs through Europe [1–5]. The use of automated systems [4–6] or fully automated systems [control of SPE, gradient elution and diode-array detection (DAD) with unique software] [7,8] offers event more advantages for the routine measurement of pesticides at trace levels, since it guarantees good reproducibility and the possibility of analysing a large number of samples routinely. Recently, such systems were validated [6] in interlaboratory exercises in which groundwater samples containing pesticides were analysed and the results were compared with those given by conventional gas chromatographic techniques.

One of the major problems in the on-line determination of pesticides at low levels in different types of waters is the presence of interfering substances, namely humic substances, in the early eluting peaks of the chromatogram. Such interferences are more relevant when analysing surface and estuarine river waters [1,3–5]. The water type is a relevant parameter in the

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determination of pesticides at these low levels, and differences in calibration and quantification have been found depending on the water type [1,5]. A second problem that arises when determining pesticides at these low levels is derived from the co-elution of two compounds in a mixture. Since the LC runs usually involve a mixture of about 30 compounds, is difficult to achieve baseline separation between all the analyte peaks and matrix interferences which appear at this low level of concentration. One co-elution problem in the determination of pesticides in water samples is the co-elution of alachlor and metolachlor [1,9], which is also enhanced by the fact that both compounds elute approximately at 100% organic modifier and also by the presence of interfering compounds.

Recently, our groups [10,11] have been developing software systems for the quantification and deconvolution of chromatographic peaks in different mixtures. Such methods have already been applied to the determination of naphthol and pirimicarb in different eluting mixtures [12]. In the case of alachlor and metolachlor, multivariate self-modelling curve resolution (MSCR) [10–16] is proposed for the resolution and quantification of co-eluted compounds in LC–DAD. In this work, the MSCR method was extended to handle real LC data for herbicides at low concentrations (μg/l) using automated preconcentration techniques.

MSCR will be applied to improve the resolution of co-eluted herbicides metolachlor and alachlor, which have very similar spectra. The determination of these compounds is even more problematic in the case of mixtures at low concentrations (μg/l) when on-line solid-phase preconcentration techniques are used [17]. Then, interferents at very low concentrations and mobile phase effects become more significant than for direct injection analysis of more concentrated samples (mg/l level).

The aim of this work was the application of the most recent chemometric approaches developed by us for the resolution and determination of alachlor and metolachlor at trace levels (1–5 μg/l) in various water samples. The chemometric system developed will be applied in the first instance to (i) direct injection of standard samples of both compounds, (ii) the preconcentration of clean water samples and (iii) the preconcentration of surface river water samples containing both pesticides and the natural interfering substances. The development of such a software system that allows the quantification of peaks at trace levels is particularly relevant since this problem has not been solved in the automated routine measurement of pesticides in water samples by automated SPE methods. The incorporation of such software systems into the DAD software will be a consideration for the future and would be of help for laboratories involved in the routine measurement of pesticides at trace levels using automated systems.

2. Experimental

2.1. Chemicals and reagents

The herbicides alachlor and metolachlor were obtained from Promochem (Wesel, Germany). Pesticide-grade acetonitrile, methanol and water obtained from a Milli-Q purification system (Milli-Q water) were purchased from J.T. Baker (Deventer, Netherlands).

2.2. Chromatographic analysis

Standard solutions of alachlor, metolachlor and a mixture of alachlor and metolachlor were prepared in acetonitrile at a concentration of 20 μg/ml. Each standard was analysed by direct injection using a 20-μl loop and by "on-line" SPE coupled to LC–DAD. When the "on-line" method was used, Milli-Q water was spiked with each standard at a level of 1.5 μg/l. Further, estuarine water samples from the Ebre delta were spiked with the solution containing alachlor and metolachlor.

On-line SPE–LC–DAD was performed with a Prospekt automated preconcentration system (Spark Holland, Emmen, Netherlands). Samples were preconcentrated on 10 × 2 mm I.D. disposable precolumns prepacked with 15–25-μm PLRP-s styrene–divinylbenzene copolymer,

(Spark, Holland). Precolumns were conditioned via a solvent-delivery unit (SDU) from Spark Holland with 10 ml of acetonitrile, 10 ml of methanol and 10 ml of water at a flow-rate of 2 ml/min. A 100-ml volume of Milli-Q water was percolated through the precolumn at a flow-rate of 2 ml/min. The analytes were desorbed by coupling the precolumn "on-line" with the analytical column and starting the gradient. This method has been validated for some organophosphorus pesticides and was described in a recent paper [6].

The LC analysis was performed with a Waters model (Milford, MA, USA) 600-MS solvent-delivery unit equipped with a Waters model 996 photodiode-array detector. Two cartridge columns were used: (a) a Superspher column (250 × 4 mm I.D.) packed with 4-μm Superspher C_8 (Merck, Darmstadt, Germany) with gradient elution from acetonitrile–water (40:60) to (75:25) in 22 min at a flow-rate of 1 ml/min and then returning to the initial conditions in 3 min and (b) a Novo-Pak cartridge column (15 × 4 mm I.D.) packed with 4–6-μm C_8 (Waters) with gradient elution from acetonitrile–water (30:70) to (80:20) in 8 min, then isocratic for 2 min, at a flow-rate of 1 ml/min, with a return to the initial conditions in 3 min.

The detector was operated at the maximum resolution of 1.2 nm and at one spectrum per second. These conditions were necessary to attain the best spectral resolution.

2.3. Data pretreatment

When the Waters column was used, eight different conditions were studied (matrices W1–W8). When the Merck column was selected, five conditions were studied (matrices M1–M5). A description of each data matrix is given in Table 1.

The selection of the particular elution time

Table 1
Experimental details of different data matrices

Analytical column[a]	Data matrix[b]	Composition[c]		Sample type[d]	Method[e]
		Ala (ng)	Met (ng)		
Waters	W1 (61,51)	404	–	Pure	1
Waters	W2 (41,51)	–	400	Pure	1
Waters	W3 (41,51)	404	400	Pure	1
Waters	W4 (41,51)	–	–	Milli-Q	2
Waters	W5 (41,51)	150	–	Milli-Q	2
Waters	W6 (41,51)	–	151	Milli-Q	2
Waters	W7 (41,51)	150	151	Milli-Q	2
Waters	W8 (34,51)	150	151	River	2
Waters	W9 (34,51)	–	–	River	2
Merck	M1 (81,51)	404	–	Milli-Q	1
Merck	M2 (51,51)	–	400	Milli-Q	1
Merck	M3 (101,51)	404	400	Milli-Q	1
Merck	M8 (66,51)	150	151	River	2
Merck	M9 (66,51)	–	–	River	2

[a] Columns used in each analysis.
[b] Data matrices used in the analysis. In parentheses, the number of rows (elution times every 1 s) and the number of columns (51 wavelengths, between 190 and 251 nm) are given.
[c] Composition of the samples analysed given as total amount of the analytes input to the column; Ala = ng of alachlor and Met = ng of metolachlor injected.
[d] Sample type: Pure = standards prepared in acetonitrile at a concentration of 20 μg/ml; Milli-Q = Milli-Q water spiked with the analytes at a concentration of 1.5 μg/l; River = real water samples taken from the Ebre river spiked or not (see Experimental).
[e] Analytical method used: 1 = direct injection of the standard without preconcentration; 2 = on-line SPE–LC–DAD with the Prospekt preconcentration system.

ranges included in each data matrix is done empirically, choosing those elution times having the desired cluster of peaks (identified from the spectra). Each chromatographic run provides a data array of numbers which are ordered in a data matrix with a number of rows equal to the number of selected elution times and a number of columns equal to the number of wavelengths. Whereas for all the runs the same spectral range is selected (190–251 nm, 51 wavelengths, R_s = 1.2 nm), a different number of elution times is selected for each data matrix depending on the elution of the components of interest. Thus, all data matrices will have the same number of columns but they can have different number of rows (see Table 1). The time resolution in the data analysis is one spectrum per second.

For all data matrices, especially those obtained using preconcentration techniques and for chromatograms at lower wavelengths, a certain amount of background absorption is detected. Most of this background contribution is caused by absorption of the mobile phase, whose composition and relative concentration change during the chromatographic run. To subtract the initial background absorption caused by the mobile phase, the spectrum at the first initial elution time is subtracted from subsequent spectra at all other elution times. Fig. 1A and B show data matrix W8 and W3 after background subtraction was done. In this way all the chromatograms start at zero, but owing to gradient elution a small background contribution is still present.

2.4. Data structure

We assume that K chromatographic runs of different analyte mixtures at different concentrations are analysed. For each chromatographic run a data matrix $\boldsymbol{D}_k$ is obtained:

$$\boldsymbol{D}_k = \boldsymbol{C}_k\boldsymbol{S} + \boldsymbol{D}_{k0}, \qquad k = 1, 2, \ldots, N \tag{1}$$

where $\boldsymbol{C}_k$ is the matrix of the concentration profiles of the chemical components eluted during a particular chromatographic run in the analysis of sample k, $\boldsymbol{S}$ is the matrix of the unit or pure spectra of these components and $\boldsymbol{D}_{k0}$ is the background absorption. The analysis can also be performed simultaneously over several chromatographic runs, setting the corresponding data matrices $\boldsymbol{D}_k$ one on top of each other keeping their columns (wavelengths) the same for all of them:

$$\boldsymbol{D} = \begin{bmatrix} \boldsymbol{D}_1 \\ \boldsymbol{D}_2 \\ \vdots \\ \boldsymbol{D}_N \end{bmatrix} = \begin{bmatrix} \boldsymbol{C}_1 \\ \boldsymbol{C}_2 \\ \vdots \\ \boldsymbol{C}_N \end{bmatrix} \boldsymbol{S} + \boldsymbol{D}_0 = \boldsymbol{C}\boldsymbol{S} + \boldsymbol{D}_0 \tag{2}$$

$$\boldsymbol{D} = \boldsymbol{C}\boldsymbol{S} + \boldsymbol{D}_0 \tag{3}$$

The new augmented data matrix $\boldsymbol{D}$ has a number of rows equals to the total number of acquired spectra in the different chromatographic runs (elution times) and has a number of columns equals to the number of wavelengths. In the case of the simultaneous analysis of different samples, the new augmented data matrix will be the product of an augmented concentration matrix $\boldsymbol{C}$ times the unit spectra matrix $\boldsymbol{S}$. The augmented concentration matrix $\boldsymbol{C}$ includes the different concentration submatrices $\boldsymbol{C}_k$ related to each of the data matrices $\boldsymbol{D}_k$ analyzed. $\boldsymbol{S}$ has the unit pure spectra of the components and $\boldsymbol{D}_0$ the background absorption. Multivariate self-modelling curve resolution is applied to this new augmented data matrix using the method previously described [10–16] and also based on other methods previously developed by other workers [18–24].

In the analysis of a single chromatographic run, quantification is possible only if some external information is provided explicitly [25]. When several samples are simultaneously analysed (Eq. 2) using the constrained alternating least squares [10–12], the area of the concentration profiles of the different components in the matrices give the relative amounts of the analytes in each individual data matrix. As under conditions of linearity, the area under the concentration profile of a certain component is proportional to the amount of the analyte, and the ratio between these areas for a particular analyte gives the ratio between the amounts of that particular analyte in the different samples. Absolute concentrations or absolute amounts can be then estimated if for one of the individual data matrices included in

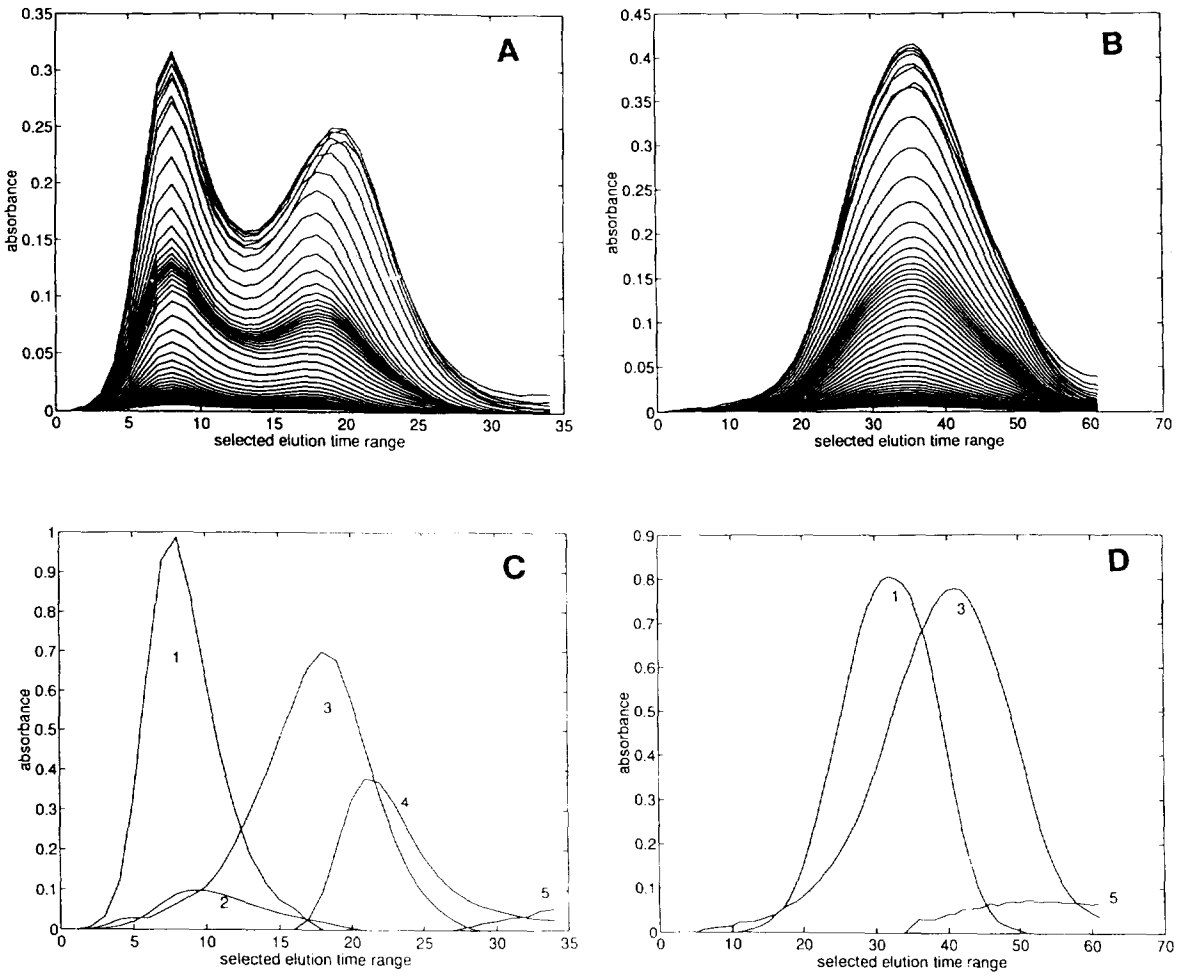

Fig. 1. (A) Elution profile of Ebre delta water sample spiked with alachlor and metolachlor, corresponding to matrix W8 (see Table 1); (B) elution profile of W3 (see Table 1); (C) elution profiles of the five resolved species in the simultaneous analysis of W8 (for identification of species, see Table 2); (D) elution profiles of the three resolved species in the simultaneous analysis of samples W3, W1 and W2 (augmented data matrix W3 + W1 + W2, see Table 4).

the augmented data matrix, the concentration of that analyte, is known in advance, i.e., a standard sample is included in the simultaneous analysis. The amount of this particular analyte in the other data matrices included in the augmented data matrix is easily estimated from

$$m_u = (A_u/A_k)m_k \quad (4)$$

where m_k and m_u are the amounts of the analyte in the known and unknown samples, respectively, and A_u/A_k is the ratio of areas of the resolved elution profiles for the analyte in both known and unknown data matrices which are included in the simultaneous analysis (Eq. 2).

3. Results and discussion

3.1. Individual analysis of each chromatographic run

The number of co-eluted compounds detected in the individual analysis of each data matrix is given in Table 2. A maximum number of five

Table 2
Individual analyses of the eight chromatographic runs

Matrix[a]	No. of components[b]	PCA fit[c]	ALS fit[d]	Species[e]
W1	2	0.34	1.26	3, 5
W2	2	0.19	0.44	1, 5
W3	3	0.16	–	1, 3, 5
W4	3	1.94	3.97	2, 4, 5
W5	4	0.25	0.65	2, 3, 4, 5
W6	4	0.32	0.52	1, 2, 4, 5
W7	5	0.17	1.14	1, 2, 3, 4, 5
W8	5	0.24	2.18	1, 2, 3, 4, 5
W9	3	2.55	2.71	2, 4, 5
M1	2	0.31	0.59	3, 5
M2	2	0.19	0.90	1, 5
M3	3	0.25	0.75	1, 3, 5
M8	4	0.43	4.79	1, 2, 3, 5
M9	4	9.99	1.50	2, 5

[a] Experimental data matrix analysed (see Table 1 for notation).
[b] Number of components found in the principal component analysis (PCA).
[c] Lack of fit by PCA using the selected number of components. It is calculated using the percentage lack of fit by the equation % lack of fit = $[\mathrm{sum}(d_{ij} - d_{ij}^{*})^2)/\mathrm{sum}(d_{ij})^2] \cdot 100$, where d_{ij} and d_{ij}^{*} are, respectively, the experimental absorption at elution time i and wavelength j and the reproduced PCA absorption using the selected number of components at the same elution time i and wavelength j.
[d] Lack of fit by ALS optimization of the concentration and spectra profiles. It is calculated using the percentage lack of fit by the equation % lack of fit = $[\mathrm{sum}(d_{ij} - c_{il}s_{lj})^2/\mathrm{sum}(d_{ij})^2] \cdot 100$, where d_{ij}, c_{il} and s_{lj} are, respectively, the experimental absorption at elution time i and wavelength j, the calculated concentration for special l at elution time i and the unit signal (absorption) contribution of species l at wavelength j.
[e] Identification of the species and their elution order: 1 = metolachlor; 2 = interferent found in preconcentration experiments; 3 = alachlor; 4 = interferent found in preconcentration experiments using the Waters column; 5 = mobile phase contribution (see Fig. 1).

components is detected for matrices W7 and W8 (Fig. 1A). Of these five species, the first is the elution of metolachlor, the second species is an unknown contribution always present when preconcentration techniques are used, the third is the elution of alachlor and the fourth and fifth species are minor contributions which have been related to the mobile phase, the gradient elution and/or the subtraction procedure. Matrices W5 and W6 have a maximum of four components because one of the two analytes is not present. Blank data matrices W4 and W9 have a maximum of three components (unknown interferent and mobile phase contributions). Matrix W3 (Fig. 1B) has three components, the two analytes and a low mobile phase contribution. Matrices W1 and W2 have two components, one of the two analytes and a very low mobile phase contribution. Similar results are obtained using Merck column (data matrices M1, M2, M3, M8 and M9 in Table 1) with a better resolution of the analytes, the interferents and the mobile phase. Matrices M8 and M9 give four components (only a mobile phase contribution is detected for the Merck column). Matrices M1 and M2 have only a major contribution and a very low mobile phase contribution; M3 has three components, the two analytes and the mobile phase.

In Table 2, the principal component analysis (PCA) and alternating least squares (ALS) lack of fit as a percentage of residual error are compared for each species in the individual analysis of all the data matrices. Small residuals are always found except for blank matrices W4, W9 and M9, where the PCA lack of fit per-

centage is higher than for the other data matrices, as a consequence of the much lower signal-to-noise ratio in those blank matrices.

Normalized pure spectra of the two analytes, metolachlor and alachlor, are easily estimated from the individual analysis of matrices W1, W2, M1 and M2 (without preconcentration). They are very similar with a correlation coefficient of 0.9992 (Table 3). These two spectra are used as input values in the analysis of the other data matrices and confirmed in the analysis of matrices W5 and W6 (similarly to W1 and W2 with only one of the two analytes but using the preconcentration method). The analysis of the blank data matrices W4, W9 and M9 provided the initial estimations of the spectra of the interference and mobile phase. Once these components has been identified, multivariate self-modelling curve resolution of co-eluted components in the mixture matrices W3, W7 and W8 was attempted. Resolution for both alachlor and metolachlor is achieved in the individual analysis of data matrices W7, W8, M3 and M8 (see Table 2). For matrix W3 (Fig. 1B) however, resolution fails to converge to a reasonable solution, probably because of the higher overlap of these two compounds in this data matrix. In Fig. 2A, the plots of the estimated normalized pure spectra of the five detached co-eluted compounds in the different chromatographic runs are given. Correlations between these unit spectra are also given in Table 3. More detailed plots of the normalized unit spectra for alachlor and metolachlor are given in Fig. 2B. Using the complete set of pure spectra given in Fig. 2A, a good fit of all the individual data matrices (except for W3) is achieved (Table 2).

3.2. *Quantification of simultaneous chromatographic runs*

By using the proposed method of simultaneous analysis of several data matrices, the resolution power is improved with respect to the individual analysis of the different data matrices, the relative quantification of the different analysis being possible [10]. Results of the simultaneous analysis of different chromatographic runs are given in Table 4, and are grouped in accordance with the combination of matrices that have been used as problems or as standards. Each group is identified with roman numerals.

(I) The simultaneous analysis of chromatographic runs W1, W2 and W3 without preconcentration (augmented matrix W1 + W2 + W3) allowed the resolution of the two analytes, alachlor and metolachlor, even for matrix W3 (Fig. 1B), which could not be resolved in the in-

Table 3
Chromatographic resolution between metolachlor and alachlor and correlation between pure spectra of the resolved components

Matrix	Resolution
W3	0.2
W7	0.4
W8	0.5
M3	0.6
M8	0.7

No.[a]	Correlation				
	1	2	3	4	5
1	1.0000	0.9795	0.9992	0.8788	0.8216
2	0.9795	1.0000	0.9876	0.9561	0.9174
3	0.9992	0.9876	1.0000	0.9902	0.9681
4	0.8788	0.9561	0.9902	1.0000	0.9934
5	0.8216	0.9174	0.9681	0.9934	1.0000

[a] Identification of numbers as in Table 2.

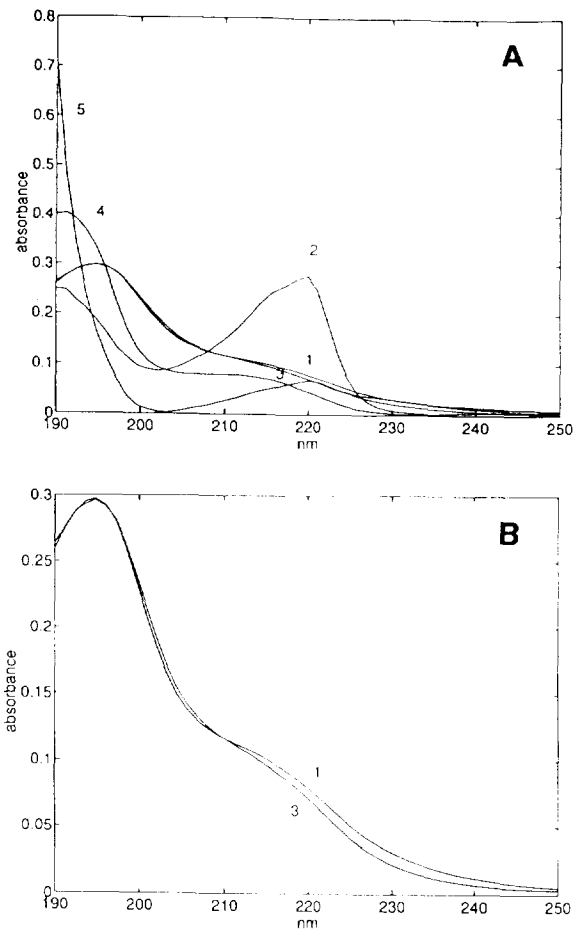

Fig. 2. (A) Normalized spectra of detected species: 1 = metolachlor; 2 = unknown interferent found in preconcentration experiments; 3 = alachlor; 4 = unknown interferent or mobile phase contribution found in the preconcentration experiments mostly with the Waters column; 5 = mobile phase contribution due to gradient and first spectrum subtraction. (B) More detailed plot of the estimated normalized unit spectra of alachlor and metolachlor (the correlation coefficient between these two spectra is 0.9992).

dividual analysis. A small contribution of the mobile phase is also confirmed. In Fig. 1D, the elution profiles of the mixture of alachlor and metolachlor from matrix W3 are given. Even for this unfavourable case of strongly co-eluted (resolution $R_S = 0.2$) and very similar (correlation coefficient 0.9992) compounds (Table 3), a good resolution and quantitative estimation is achieved for the two analytes. The same can be said for matrix M3 when analysed together with matrices M1 and M2 (augmented matrix W3 + W1 + W2), although now (Merck column) the resolution is higher ($R_S = 0.6$). When one of the single analyte matrices is omitted from the analysis (W2 or W1 in the analysis of W3 or M1 or M2 in the analysis of M3) the results permit the determination of both components (see Table 4).

(II) The simultaneous analysis of chromatographic runs of preconcentrated water samples spiked with alachlor and metolachlor at a level of 1.5 μg/l (augmented matrix W7 + W4 + W5 + W6) allowed the resolution of the co-eluted components and their quantification using the set of pure spectra identified in the individual analysis. Samples having only one of the two analytes were taken as standards. Percentage errors in the quantitative estimations are given in Table 4. When one of the single analyte matrices, W5 or W6, is omitted from the augmented matrix, quantitative estimations are still good (see results for the analysis of the augmented matrices W7 + W4 + W5), even when blank information is also omitted (augmented matrices W7 + W5 or W7 + W6).

(III) The simultaneous analysis of preconcentrated Ebre river waters spiked with the two analytes (W8) and blank (W9) together with other water samples (augmented matrix W8 + W9 + W7 + W4 + W5 + W6 + W3 + W2 + W1) gives similar results to those obtained previously. These results are also illustrative of the comparison between preconcentrated water samples and direct injection of standards. In Fig. 1C, the resolved elution profiles of the five co-eluted components present in W8 are given. The chromatographic resolution between metolachlor and alachlor in sample W8 is around 0.5. The error in the determination of the different components is lower than 10%, which is an acceptable value according to the EPA. A relevant question here is whether the area ratios of one specified analyte obtained under the different experimental conditions (direct injection or preconcentration) are reproducible. The results show that the area ratio of the different elution profiles is always between 2.3 and 2.7, showing that the preconcentration factor is independent of the consid-

Table 4
Simultaneous analysis of different chromatographic runs

No. Column	Matrix[a]	ALS fit[b]	Met[c]	Error (%)[d]	Ala[c]	Error (%)[d]
I Waters	W3	1.57	381	5	396	2
	W1		–	–	404	s
	W2		400	s	–	–
	W3	1.60	–		392	3
	W1		–	–	404	s
	W3	0.86	396	1	–	–
	W2		400	s	–	–
II Waters	W7	5.09	159	5	151	1
	W4		–		–	–
	W5			–	150	s
	W6		151	s	–	–
	W7	4.59	–		144	4
	W4		–	–	–	–
	W5		–		150	s
	W7	2.25	–		136	10
	W5			–	150	s
	W7	8.41	156	3	–	–
	W6		151	s	–	–
III Waters	W8	3.26	154	2	158	5
	W9		–		–	–
	W7		146	3	141	6
	W4		–		–	–
	W5		–		150	s
	W6		151	s		–
	W3		420	5	368	9
	W2		–	–	393	3
	W1		410	2	–	–
	W8	2.31	161	7	152	2
	W1		–		404	s
	W2		400	s		–
	W8	3.00	–		144	4
	W1		–	–	404	s
	W8	1.44	172	13		–
	W2		400	s	–	–
	W8	1.85	160	6	154	2
	W3		400	s	404	s
IV Merck	M3	1.54	425	6	400	1
	M1				404	s
	M2		400	s		–
	M3	1.14	–		408	1
	M1		–	–	404	s
	M3	1.23	430	7		–
	M2		400	s		–
	M8	3.09	140	7	149	1
	M9		–	–		–
	M3		437	9	387	4
	M1		–		404	s
	M2		400	s		–
	M8	4.43			135	10
	M1				404	s
	M8	2.51	150	1	–	–
	M2		400	s		–

(Continued on p. 354.)

Table 4 (*continued*)

No. Column	Matrix[a]	ALS fit[b]	Met[c]	Error (%)[d]	Ala[c]	Error (%)[d]
V Merck	W3	1.70	416	4	385	5
	W1		–	–	404	1
	W2		428	7	–	–
	M3		424	6	427	6
	M1		–	–	404	s
	M2		400	s	–	–
VI Merck	W8	1.90	166	10	–	–
	M2		400	s	–	–

[a] Augmented data matrix analysed (see Table 1 and Eq. 2).
[b] Percentage lack of fit in the ALS optimization. See footnote d in Table 2.
[c] Amount of metolachlor (Met) and alachlor (Ala) recovered from the analysis using Eq. 2; s means that this elution profile has been considered as standard for the quantification of the corresponding analyte using Eq. 4.
[d] Relative error in the prediction of the amounts of analyte in the corresponding chromatographic runs.

ered sample, analyte and/or column. The analysis of real samples with preconcentration can be correlated with a standard injected directly on to the column. This is the case, for instance, for the analysis of the augmented data matrix W8 + W1 + W2. Determination of the two analytes alachlor and metolachlor in W8 was also possible (Table 4).

(IV) Similar results were also obtained in the simultaneous analysis of different chromatographic runs using the Merck column (Table 4).

(V) The simultaneous analysis of chromatographic runs obtained using either the Waters or Merck column (augmented matrix W3 + W1 + W2 + M3 + M1 + M2) showed that standards obtained with one of the two columns can be also used in the determination of the analytes in samples analysed with the other column. This is true because the mobile phase composition, the gradient elution and other physical parameters were kept identical in all the experiments.

(VI) Finally, the determination of metolachlor in the Ebre river water analysed using the Waters column (matrix W8) was performed using as a standard metolachlor injected directly onto the Merck column. Even in this case, a reasonable determination of metolachlor is possible with an error of 10% in the prediction of its concentration.

4. Conclusions

The proposed multivariate self-modelling curve resolution method can be used for the simultaneous determination of herbicides with similar elution times and absorption spectra (e.g., alachlor and metolachlor) at a level of few μg/l in real water samples. The error made is less than 10%, even in cases where the determination of metolachlor in Ebre river water samples was performed using as standard direct injection of metolachlor and both samples analysed with different columns. Quantification at these low levels is of environmental importance, as they are similar to those present in the environment.

The individual analysis of the different data matrices using different columns showed that (1) the analysis was independent of the column used, (2) mobile phase contributions are mostly due to gradient elution and (3) unknown co-eluted contributions can appear when on-line solid-phase preconcentration methods are used.

From all these results, a stepwise recommended chemometric procedure is proposed for data analysis and for the quantification of mixtures of alachlor and metolachlor at low concentrations:

(1) LC analysis of the water sample which has

an unknown amount of alachlor and/or metolachlor. If they are present at very low concentrations, use preconcentration techniques. Storage of data matrix U.

(2) LC analysis of a pure standard of alachlor or metolachlor or of a mixture of both. Storage of data matrix K.

(3) Data pretreatment of matrices U and K. Selection of elution time and wavelength of interest. Background and mobile phase contribution subtraction.

(4) Determination of the number of co-eluted components of matrix U. Multivariate self-modelling curve resolution of matrix U (Eq. 1). Estimation of the elution order.

(5) Multivariate self-modelling curve resolution of the augmented data matrix U + K (Eq. 2). Determination of the concentration profiles and unit spectra of the co-eluted components. Quantification of analytes in the unknown mixture (Eq. 4).

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References

[1] S. Chiron, A. Fernandez Alba and D. Barceló, *Environ. Sci. Technol.*, 27 (1993) 2352.
[2] I. Liska, E.R. Brouwer, A.G.L. Ostheimer, H. Lingeman, U.A.Th. Brinkman, R.B. Geerdink and W.H. Mulder, *Int. J. Environ. Anal. Chem.*, 47 (1992) 267.
[3] R. Reupert, I. Zube and E. Plöger, *LC·GC Int.*, 5 (1992) 43.
[4] J. Slobodnik, E.R. Brouwer, R.B. Geerdink, W.H. Mulder, H. Lingeman and U.A. Th. Brinkman, *Anal. Chim. Acta*, 268 (1992) 55.
[5] V. Pichon and M.C. Hennion, *J. Chromatogr. A*, 665 (1994) 269.
[6] S. Lacorte and D. Barceló, *Anal. Chim. Acta*, 296 (1994) 223.
[7] J. Slobodnik, M.G.M. Groenwegen, E.R. Brouwer, H. Lingeman and U.A.Th. Brinkman, *J. Chromatogr.*, 643 (1993) 359.
[8] U.A.Th. Brinkman, *LC·GC Int.*, 7 (1994) 157.
[9] G. Durand, V. Bouvot and D. Barceló, *J. Chromatogr.*, 607 (1992) 319.
[10] R. Tauler and D. Barceló, *Trends Anal. Chem.*, 12 (1993) 319.
[11] R. Tauler, B.R. Kowalski and S. Flemming, *Anal. Chem.*, 65 (1993) 2040.
[12] R. Tauler, G. Durand and D. Barceló, *Chromatographia*, 33 (1992) 244.
[13] R. Tauler, A.K. Smilde, J.M. Henshaw, L.W. Burgess and B.R. Kowalski, *Anal. Chem.*, 66 (1994) 3337.
[14] R. Tauler, A. Izquierdo-Ridorsa and E. Casassas, *Chemometr. Intell. Lab. Systs.*, 18 (1993) 293.
[15] R. Tauler and E. Casassas, *Anal. Chim. Acta*, 223 (1989) 257.
[16] R. Tauler, E. Casassas and A. Izquierdo-Ridorsa, *Anal. Chim. Acta*, 248 (1991) 447.
[17] M.W.F. Nielen, *Ph.D. Thesis*, Free University, Amsterdam, 1987.
[18] E.V. Dose and G. Guiochon, *Anal. Chem.*, 61 (1989) 2571.
[19] H. Gampp, M. Maeder, Ch. Meyer and A.D. Zuberbuhler, *Talanta*, 32 (1985) 1133.
[20] E.R. Malinowski, *J. Chemometr.*, 6 (1992) 29.
[21] S. Wold, *Technometrics*, 20 (1978) 397.
[22] H.R. Keller, D.L. Massart, Y.Z. Liang and O.M. Kvalheim, *Anal. Chim. Acta*, 263 (1992) 29.
[23] W. Windig and J. Guilment, *Anal. Chem.*, 63 (1991) 1425.
[24] G.H. Golub and Ch.F. Van Loan, *Matrix Computations*, Johns Hopkins, Baltimore, MD, University Press, 2nd ed., 1989.
[25] B.G.M. Vandeginste, M. Gerritsen, J.W. Noor and G. Kateman, *J. Chemometr.*, 1 (1987) 57.