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Journal of Chromatography A, 730 (1996) 177–183

JOURNAL OF CHROMATOGRAPHY A

Application of multivariate self-modeling curve resolution to the quantitation of trace levels of organophosphorus pesticides in natural waters from interlaboratory studies

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Abstract

Multivariate self-modeling curve resolution is applied to the quantitation of coeluted organophosphorus pesticides: fenitrothion, azinphos-ethyl, diazinon, fenthion and parathion-ethyl. Analysis of these pesticides at levels of 0.1 to 1 μg/l in the presence of natural interferences is achieved using automated on-line liquid–solid extraction (Prospekt) coupled to liquid chromatography and diode array detection followed by a recently developed multivariate self-modeling curve resolution method. The proposed approach uses only 100 ml of natural water sample and has improved resolution of the coeluted organophosphorus insecticides and their quantitation at trace level. The results have been compared with those obtained by different laboratories participating in the Aquacheck interlaboratory exercise (WRC, Medmenham, UK) where more conventional analytical techniques are being used.

Keywords: Water analysis; Environmental analysis; Interlaboratory validation; Multivariate techniques; Curve resolution; Chemometrics; Pesticides

1. Introduction

Participation in interlaboratory exercises is a key issue when validating analytical methods. By participating in such exercises, it is possible to enhance precision, accuracy and a general performance of the participating laboratories. It is also true that in order to certify or compare given values prepared by a central laboratory, it is recommended that the participating laboratories use different analytical techniques to determine the final concentration of an analyte [1].

Our laboratory has wide experience in participating in different interlaboratory exercises, such as the ones organized by the Measuring and Testing Program of the Commission of the European Communities (CEE) [1] and the Water Research Center (Aquacheck) [2,3]. Our major interest is the analysis of pesticides in water samples at the levels required by the CEE Drinking Waters Directive of 0.1 μg/l. To solve this problem, we currently use on-line liquid–solid extraction (LSE) techniques followed by diode array detection as a routine method of analysis. In this way, we have been able to participate and solve many analytical problems concerning the determination of a variety of organophosphorus and triazine pesticides spiked in various types of water, such as distilled, ground and waste waters [2,3].

However, often problems arise in the LC sepa-

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0021-9673/96/$15.00
SSDI 0021-9673(95)01206-0

ration with respect to coelution of analytes. This is particularly critical in environmental analysis since the pesticide mixtures contain large number of analytes at low concentration and often coelution problems arise, as shown for alachlor and metolachlor [4]. One way to solve this problem is by using another LC column with different polarity and by optimizing all the parameters for separation. This will probably improve a certain separation of analytes, but can affect other mixtures and new coelution of analytes may appear.

Multivariate self-modeling curve resolution (MSCR) [4–6] is proposed to improve the resolution and quantitation of strongly coeluted compounds in liquid chromatography with diode array detection. In the present work, the MSCR method is extended to the determination of coeluted organophosphorus pesticides (azinphos-ethyl and fenitrothion on one side and diazinon, fenthion and parathion-ethyl on the other side) in interlaboratory analysis of Aquacheck water samples using liquid chromatography. The goal of the study is to attempt the resolution and quantitation of such pesticides in low concentration (μg/l) samples, to investigate the limits of the proposed approach in the analysis of real samples, and to compare the obtained results with previous interlaboratory studies reported using other techniques.

2. Experimental

2.1. Equipment

The LC analysis were performed with a Waters 600-MS solvent delivery unit with a 20-μl injection loop and equipped with a Waters 996 photodiode array detector (Waters, Millipore, MA, USA). A 150×3.9 mm I.D. cartridge column packed with 4 μm C_8 (Waters) was used. A 20×3.9 mm I.D. guard column of C_8 (Waters) was used to protect the analytical column. The automated LSE device (Prospekt) used in this work consisted of a cartridge exchange module, a pump (or solvent delivery unit, SDU) and an electrically operated low-pressure six-port valve, which is connected to the gradient pumps. Samples were preconcentrated on 10×2 mm I.D. disposable Prospekt precolumns (Spark Holland, Emmen, Netherlands) prepacked with 40 μm C_{18} (Baker, Deventer, Netherlands). The precolumns were conditioned via a solvent delivery unit from Spark Holland.

2.2. Chemicals and reagents

HPLC-grade acetonitrile and methanol were obtained from J.T. Baker. Pesticide standards fenitrothion, azinphos-ethyl, diazinon, fenthion and parathion-ethyl were obtained from Promochem (Wesel, Germany).

2.3. Procedures

The experimental set-up was as follows: a certified standard solution containing an unknown concentration of pesticides and a 2-l bottle of waste water with 0.5% HNO_3 were provided by Aquacheck. The aim was to spike the water sample with the solution provided in order to determine the levels of these pesticides in water using on-line LSE–LC–DAD. Prior to LC analysis, Aquacheck water samples were filtered through 0.45-μm filters (Millipore, MA, USA) to remove suspended particles.

Precolumns were conditioned with 10 ml acetonitrile, 10 ml of methanol and 10 ml of Milli-Q water at 2 ml/min, before percolating the water sample. A 100-ml volume of sample was percolated through the precolumn at a flow-rate of 5 ml/min. Desorption was carried out by coupling the precolumn on-line with the analytical column and starting the gradient.

Gradient elution was carried out with acetonitrile–water as follows: the gradient started with 10% acetonitrile and 90% water, linearly to 20% acetonitrile and 80% of water in 7 min, to 40% acetonitrile and 60% of water in 10 min, to 53% acetonitrile and 47% of water in 16 min, and to 100% acetonitrile in 8 min. The flow-rate was set at 1 ml/min and the total run time was of 40 min. Back to initial conditions in 5 min.

2.4. Calibration curve

A calibration curve was constructed for all the compounds analyzed over a concentration range 0.4–2 μg/l. Moreover, two other calibration curves containing the pure standards, fenitrothion and azin-

phos-ethyl respectively were performed over a concentration range 0.4–1 μg/l. Analytical conditions were as described above.

2.5. MSCR

The method for data treatment has been described previously in Refs. [5,6] for chromatographic data systems and in Refs. [7–9] for reaction-based data systems. Only particular aspects concerning the data treatment are given. In a recent study, the conditions under which resolution and total recovery of the true profiles are achieved have been examined in detail [10,11].

Suppose K chromatographic runs of different mixtures of the analytes at different concentrations are analyzed. For each chromatographic run a data matrix $\mathbf{D}_k$ is obtained. Assuming linear behaviour of the experimental data:

$$\mathbf{D}_k = \mathbf{C}_k\mathbf{S} + \mathbf{D}_{ko},\ k = 1,2,\ldots,N \qquad (1)$$

where $\mathbf{C}_k$ is the matrix of the concentration profiles of the chemical components eluted during a particular chromatographic run in the analysis of sample k, $\mathbf{S}$ is the matrix of the unit or pure spectra of these components, and $\mathbf{D}_{ko}$ is the background absorption.

The data analysis can also be performed simultaneously over several chromatographic runs, setting the corresponding data matrices $\mathbf{D}_k$ one in top of each other keeping their columns (wavelengths) the same for all of them:

$$\mathbf{D} = \begin{bmatrix} \mathbf{D}_1 \\ \mathbf{D}_2 \\ \vdots \\ \mathbf{D}_N \end{bmatrix} = \begin{bmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \vdots \\ \mathbf{C}_N \end{bmatrix} \mathbf{S} + \mathbf{D}_o = \mathbf{CS} + \mathbf{D}_o \qquad (2)$$

In a more abbreviated form, the same equation is written

$$\begin{aligned} \mathbf{D} &= [\mathbf{D}_1;\mathbf{D}_2;\cdots;\mathbf{D}_N] \\ &= [\mathbf{C}_1;\mathbf{C}_2;\cdots;\mathbf{C}_N]\mathbf{S} + \mathbf{D}_o \\ &= \mathbf{CS} + \mathbf{D}_o \end{aligned} \qquad (3)$$

The new augmented data matrix $\mathbf{D}$ has a number of rows equal to the total number of acquired spectra in the different chromatographic runs (elution times) and has a number of columns equal to the number of wavelengths. In the case of simultaneous analysis of different samples, the new augmented data matrix will be the product of an augmented concentration (elution) matrix $\mathbf{C}$ times the unit spectra matrix $\mathbf{S}$. The augmented concentration matrix $\mathbf{C}$ includes the different concentration submatrices $\mathbf{C}_k$ related to each of the data matrices $\mathbf{D}_k$ analyzed. Elution profiles of the components in the different data concentration matrices are allowed to have different shapes and retention times. $\mathbf{S}$ is the matrix of the pure spectra of the all the components and $\mathbf{D}_o$ the background absorption.

The following steps summarize the proposed approach:

(A) Individual analysis of chromatographic runs by multivariate curve resolution.

1. Each chromatographic run gives a experimental data matrix $\mathbf{D}_i$.
2. Data pretreatment. Selection of the elution times and wavelengths of interest. Subtraction of the solvent contribution (substraction of the first spectrum before the peak [4,7]
3. Estimation of the number of coeluted components in $\mathbf{D}_i$ (number of larger eigenvalues, factor analysis [12]. Reproduction of experimental data matrix for the selected number of components.
4. Initial estimation of elution profiles by evolving factor analysis (EFA) [13] or of pure spectra by purest variable detection methods [14]. Estimation of selective chromatographic regions [15,16].
5. Optimization of the elution profiles and of pure spectra of all the coeluted compounds present in the data matrix by alternating least squares (ALS) [7,17,18]. Constraints applied during the optimization are: (i) non-negative pure spectra; (ii) non-negative elution profiles; (iii) unimodal elution profiles; (iv) selectivity.Estimation of the concentration $\mathbf{C}_i$ and pure spectra $\mathbf{S}$ ($\mathbf{C}_i = \mathbf{D}_i\mathbf{S}^+$ and $\mathbf{S} = \mathbf{C}^+\mathbf{D}_i$, where $\mathbf{S}^+$ and $\mathbf{C}^+$ are the pseudo-inverses [19] of $\mathbf{S}$ and $\mathbf{C}$ respectively) of the coeluted compounds.
6. Calculation of the lack of fit between experimental data matrix and ALS reproduced data matrix.

$$\%\text{lack of fit} = ((d_{ij} - d^*_{ij})^2 / d^2_{ij}) \times 100$$

where d^{ij} are the experimental absorbance at

elution time i and wavelength j and d_{ij}^{*} are the ALS reproduced absorbance at the same elution time i and wavelength j.

(B) Simultaneous analysis of several chromatographic runs (several data matrices) by multivariate curve resolution.

1. Set-up of the augmented data matrix including different data matrices obtained in different chromatographic runs (including standard samples and unknown samples).

$$\mathbf{D} = [\mathbf{D}_1\ \mathbf{D}_2;...;\mathbf{D}_N] \qquad (4,5,7,8,9)$$

2. Set up of the correspondence between species in the different data matrices based on the elution order of the coeluted compounds discovered in the individual analysis or from previous knowledge of the system [4,9]
3. Alternating least squares (ALS) optimization of elution profiles and pure spectra of all the coeluted compounds present in the different data matrices simultaneously analyzed. Apart from constraints used in individual analysis, the following constraints are applied in the simultaneous analysis: (i) common coeluted components in different runs have the same pure spectrum (elution profiles are allowed instead to have different shapes and retention times); (ii) correspondence between common coeluted components and elution order in the different runs (individual data matrices). Estimation of the augmented concentration matrix $\mathbf{C}=[\mathbf{C}_1;\mathbf{C}_2;...;\mathbf{C}_N]$ and of pure spectra $\mathbf{S}$ matrix ($\mathbf{C}=\mathbf{DS}^{+}$ and $\mathbf{S}=\mathbf{C}^{+}\mathbf{D}$) [19].
4. Calculation of the lack of fit between experimental data matrix and ALS reproduced data matrix. Same equation as previously for individual analysis (A6).
5. Built up of calibration curves for each analyte of interest from standard samples and estimation of figures of merit of analytical procedures. Estimation of concentrations in unknown samples.

Point 5 of the proposed procedure for simultaneous analysis of several chromatographic runs is a new implementation in the present work and deserves a more detailed explanation. Once resolution of the elution profiles of the coeluted compounds in the different chromatographic runs is achieved, the relative quantitation of these compounds is possible [4,5,9–11]. In particular for an analyte of interest, the area of its elution profile in a certain run (matrix) is compared with the area of the same compound in the other runs (matrices) simultaneously analyzed. The relative areas of the same compound in the different chromatographic runs gives their relative concentrations. In the case that one or more of the samples included in the analysis have known concentrations of the analytes, the absolute amounts can be also estimated. In the present work this method has been extended to built a calibration curve for each analyte within a certain range of concentrations: a set of standards (samples with a known spiked amount of analytes) are analyzed under the same chromatographic conditions of preconcentration as the unknown samples. A calibration curve is built for each analyte from the area of their resolved peaks and known spiked concentrations on each standard sample. Least squares linear regression of these data provides an estimation of the best linear equation explaining the observed data variation and figures of merit of the analytical determinations. The limit of detection and sensitivity of the proposed method under the experimental conditions (amount of water preconcentrated) and for each coeluted analyte can be easily derived from the intercept and slope of the fitted straight line to the experimental data [20]. The amount of any of the analytes in an unknown sample for which a calibration curve has been derived can be then easily obtained from the peak area of its resolved elution profile by inverse calibration [20].

3. Results

Initial estimates for the ALS procedure obtained from pure variable detection methods [14] gave in this case better results (less number of iterations) than initial estimates obtained from evolving factor analysis [13].

3.1. Calibration of azinphos-ethyl and fenitrothion

Although azinphos-ethyl and fenitrothion strongly coelute at the experimental conditions (chromatographic resolution is lower than 0.1 units in all

cases), simultaneous analysis of ten HPLC water samples spiked with standard solutions of these two pesticides with the proposed multivariate curve resolution method allowed the resolution and recovery of their elution and spectra profiles (Fig. 1A). Good linearity is observed between the spiked concentrations on calibration samples and the peak areas of the resolved elution profiles for the two compounds, azinphos-ethyl [peak area=1.73 (conc. azinphos ethyl)+0.11] and fenitrothion [peak area= 1.02 (conc. fenitrothion)+0.03]. From these calibration curves, detection limits of the two pesticides at the experimental conditions and for the amount of preconcentrated HPLC water (100 ml) are estimated. These limits of detection for azinphos-ethyl and fenitrothion, are respectively 0.13 and 0.17 μg/l.

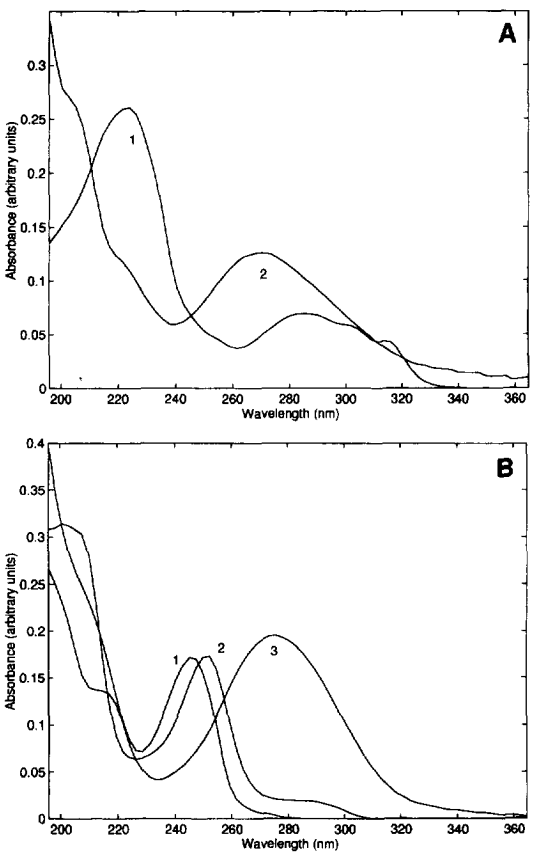

Fig. 1. (A) Normalized spectra of (1) fenitrothion and (2) azinphos-ethyl. (B) Normalized spectra of (1) diazinon, (2) fenthion and (3) parathion-ethyl.

3.2. Calibration of diazinon, fenthion and parathion-ethyl

The simultaneous analysis of five HPLC water samples spiked with standard solutions of the three pesticides allowed their complete resolution. Good linearity is observed between spiked concentrations and peak areas of the three compounds [peak area= 0.59 (conc. diazinon)+0.02, peak area=1.41 (conc. fenthion)+0.03, peak area=0.96 (conc. parathion-ethyl)+0.08], allowing quantitation of all of them within the studied concentration range. The estimated limits of detection for the three compounds, diazinon, fenthion and parathion-ethyl, are respectively 0.11, 0.20 and 0.24 μg/l. In Fig. 1B, the spectra of the three coeluted pesticides resolved using the proposed procedure are given.

3.3. Determination of azinphos-ethyl and fenitrothion in Aquacheck sample

The pure (resolved) elution profiles for the two pesticides, azinphos-ethyl and fenitrothion, resolved in the analysis Aquacheck sample are given in Fig. 2A. Chromatographic resolution in this case was only 0.03 units. Two unknown coeluted interferents were also detected at low concentrations. In Table 1, comparison of the estimated concentrations of the two pesticides, azinphos-ethyl and fenitrothion, are given and compared with those values provided by Aquacheck. Relative errors in quantitation are also compared with mean reported relative errors by other laboratories participating in the interlaboratory study. In the case of fenitrothion, its concentration is lower and close to the limit of detection, giving therefore worse results than azinphos, in agreement also with reported values by other laboratories.

3.4. Determination of diazinon, fenthion and parathion-ethyl in Aquacheck sample

In Fig. 2B the elution profiles of these three compounds resolved using the proposed multivariate curve resolution method in the analysis of the Aquacheck sample are given. Although the three compounds diazinon, fenthion and parathion-ethyl are more resolved than azinphos-ethyl and fenitrothion (Fig. 2A), their quantitation in the Aquacheck

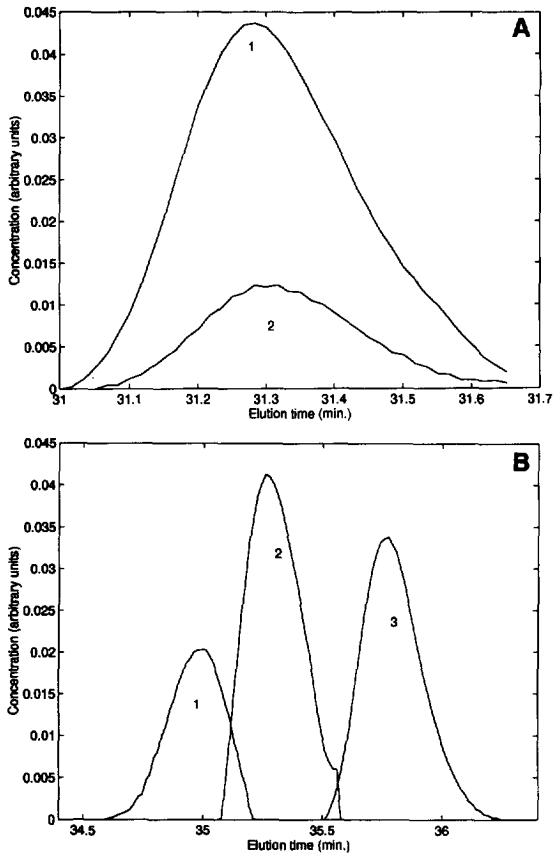

Fig. 2. (A) Elution profile of two resolved species in the coeluted peak in the Aquacheck sample: (1) azinphos-ethyl, (2) fenitrothion. (B) Elution profile of 3 resolved species in the coeluted Aquacheck sample: (1) diazinon, (2) fenthion and (3) parathion-ethyl.

sample was worse than for azinphos-ethyl and fenitrothion (Table 1). Spectral similarities between diazinon and fenthion (Fig. 1B) posed difficulties in the quantitation of both compounds at this low concentration. Moreover, matrix interferences eluting at a high percentage of organic phase interfere with the analytes of interest. In all the cases however, the relative errors in the estimations when compared with Aquacheck spiked values, were comparable with those reported by other laboratories using more selective techniques (GC) and still below the maximum error allowed (22%) in these type of studies.

4. Conclusions

The resolution and quantitation of two sets of coeluted compounds (fenitrothion and azinphos-ethyl and diazinon, fenthion and parathion-ethyl) in waste water was accomplished using on-line LSE–LC–DAD and multivariate curve resolution (MSCR). Standards and water samples were distributed by the European Intercalibration Agency (Aquacheck, Medmenham, UK). These samples were analyzed by different laboratories using different techniques, with the aim to validate the results reported by each of them. The % error between the analyzed samples and the certified values varied from 0.7 to 20%, when analyzing pesticides at levels between 0.27 and 0.61 μg/l. The chemometric approach permitted the quantitation of fenitrothion and azinphos-ethyl, even though strong coelution existed between these two

Table 1
Mean concentration (μg/l) of five OP pesticides from an intercalibration study calculated using MSCR, the Aquacheck concentration, % mean difference of MSCR (simultaneous analysis, approach B) estimated values in relation to reference values (Aquacheck) and % mean difference of all participating laboratories

Compound	Concentration (μg/l)		% Error Aquacheck	% Error mean labs[a]
	Estimated	Aquacheck		
Azinphos-ethyl	0.40	0.41	0.7	0.9
Fenitrothion	0.27	0.23	17.8	17.8
Diazinon	0.60	0.69	14.2	34.6
Fenthion	0.51	0.61	16.4	6.5
Parathion-ethyl	0.61	0.76	20.0	14.8

[a] These results correspond to the mean of 11 laboratories reporting results.

compounds. Matrix interferences at a high percentage of organic phase was the main problem for the quantitation of diazinon, fenthion and parathion-ethyl. However, for these three compounds, errors lower than 20% are reported. In the case of diazinon, the results accomplished using the present method are much better than those obtained by the other participating laboratories, which use more established techniques such as gas chromatography for analyzing organophosphorus pesticides in water.

Moreover, good linearity was observed for all the studied pesticides at a concentration range between 0.4 and 2 μg/l, with complete resolution and recovery. The errors obtained were below 10%.

The overall results obtained indicate that MSCR is a useful and a complementary tool for resolving coelution problems in a chromatographic method at low spiking levels and with the presence of interferences.

Acknowledgements

This work has been supported by the Environment R&D Program (Commission of the European Communities, CEE) contract EV5V-CT92-105, by CICYT (AMB95-0075-C03-1). Research agreement 7978/CF from the International Atomic Energy Agency (IAEA) is also acknowledged. R. Figueras (Waters, Barcelona) is acknowledged for software assistance. S.L. thanks financial support from CICYT (Grant AMB92-0218).

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