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Resolution of folpet, procymidone and triazophos in high-performance liquid chromatography–diode array detection by using partial least squares calibration to cross-sections of spectrochromatograms

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Abstract

Multi-wavelength detectors offer improved detection capabilities for liquid chromatographic methods, but require improvements in data analysis methodology to utilize all the available information. In this work, sections through the data matrix are carried out in order to obtain the highest sensitivity of the overlapping peaks produced on a HPLC–diode array detection multi-residue method when a new analyte has to be monitored. After three multivariate calibration methods, partial least squares (PLS-1 and PLS-2) and principal component regression, full spectrum calibration methods were applied to the simultaneous determination of the three pesticides, folpet, procymidone and triazophos. Hence, we resolved the interference that produces procymidone on the determination of folpet and triazophos pesticides with the maximum sensitivity for these pesticides. The approaches were applied satisfactorily to the determination of these pesticides in groundwaters at $\mu\text{g l}^{-1}$ levels, after solid-phase extraction with C_{18} cartridges. © 1997 Elsevier Science B.V.

Keywords: Chemometrics; Multiresidue methods; Least-squares analysis; Pesticides; Folpet; Procymidone; Triazophos

1. Introduction

HPLC has become a powerful separation technique widely used for numerous complex pesticide mixtures. Reversed-phase high-performance liquid chromatography (RP-HPLC) has further increased its usefulness in this field due to great column efficiency and stability, low toxicity of common solvents, facility of gradient elution and broad applicability. RP-HPLC is suitable for the determination of thermally labile and polar pesticides [1–3], which require derivatization prior to gas chromatographic analysis, and is also compatible with on-line pre-

column and post-column systems [4–8] and with the most powerful mass spectrometer detectors [9–11].

However, as samples become more complex, several hundreds of pesticides are currently used, the ability of a particular separation method to resolve all components decreases. Several factors may lead to insufficient resolution for complex samples, including inadequate column efficiency and/or gradient optimization. In other cases, the separation may still be unsuitable due to limitations of selectivity and/or peak capacity. Even, in occasions where the above factors are optimized, problems of overlapped peaks can occur if new pesticides have to be checked within an established multi-residue method (MRM). In this instance, it is possible to modify the MRM, or to apply chemometric techniques in order to extract

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useful information from the overlapped region [12–14]. Perhaps, the first solution is not the most adequate because of the large cost of the new method development. Therefore, the second possibility would be an advantageous option.

The advent of multichannel detection methods such as photodiode array detection (DAD), for monitoring chromatographic processes, has opened up new prospects for the resolution of overlapping peaks [15]. This type of detector expands the available information on the mixture components that yield the chromatographic peaks [16–18]. One advantage of RPLC–DAD is that the full UV–Vis spectrum became accessible as a three-dimensional data matrix, $\mathbf{X}(I \times J)$, where the I rows are absorption spectra measured at regular intervals and the J columns are chromatograms measured at different wavelengths.

However, this multi-wavelength information is often not used for quantification, but instead a single wavelength is selected to obtain the spectroscopic or chromatographic data and apply calibration models. Much of the quantitative information available from these three-dimensional multi-wavelength (A , λ , t) data matrices is therefore wasted.

To make use of the qualitative and quantitative information enclosed in the spectrochromatographic data some form of data elimination is needed. Usually, cross-sections through matrices give very useful information [15], thus a vertical cross-section, i.e. at constant wavelength, is equivalent to a chromatogram and a horizontal cross-section, i.e. at constant time, is equivalent to a spectrum. Later, derivative [19–22] or chemometrical [23–28] techniques can be applied to extract the quantitative information from overlapped analytical data.

It is also possible to select cross-sections, based on linear trajectories, with variable gradient through the three-dimensional matrix data. These sections, function of wavelength and time, produce a new profile where the sensitivity and/or selectivity of the determination are maximized. In this way, the analytical information generated from HPLC–DAD is optimised.

In this work, cross-sections through the data matrix are selected in order to obtain the highest sensitivity of the overlapping peaks produced on a HPLC–DAD multiresidue method [29,30] when a

new analyte has to be monitored. Data treatment using partial least squares (PLS) and principal component regression (PCR) multivariate calibration methods [15,28,31] were applied for simultaneous multi-analyte determination of the components of a mixture of folpet, procymidone and triazophos. Herewith we resolved the interference that produces procymidone on the determination of folpet and triazophos pesticides with the maximum sensitivity for these pesticides.

The procedure was applied to the determination of these pesticides in groundwater at $\mu\text{g l}^{-1}$ levels after a solid-phase extraction (SPE) with C_{18} cartridges.

2. Experimental

2.1. Chemicals and solvents

HPLC-grade solvents were used. The pesticide standards (pestanal quality), summarized in Table 1, were obtained from Riedel-de Haën (Seelze, Germany). Solid standards were dissolved in acetonitrile

Table 1
Retention times

Peak no.	Pesticide	Retention time (min)
1	Metomyl	2.3
2	Dimethoate	3.1
3	Aldicarb	4.4
4	Diclorvos	5.6
5	Carbofuran	6.2
6	Atrazine	7.3
7	Diuron	8.6
8	Dichloran	9.9
9	Methiocarb	11.2
10	Folpet	13.1
11	Procymidone	13.4
12	Triazophos	13.7
13	Iprodione	13.9
14	Vinclozolin	14.7
15	Chlorfenvinphos	14.9
16	Chlorpyrifos methyl	16.4
17	Endosulfan sulfate	16.7
18	Tetradifon	17.8
19	β -endosulfan	18.0
20	α -endosulfan	18.4
21	Chlorpyrifos ethyl	18.7
22	Carbophenothion	19.4

(ACN) and stored at 4°C in the dark, where they were stable for several months. Working solutions were prepared daily by appropriate dilution with ACN. Mobile phases were degassed with helium prior to use. Distilled water was obtained from a Millipore (Bedford, MA, USA) Milli-Q water purification system. All solvents and samples were filtered through Millipore membrane filters before injection into the column.

Prepacked Sep-Pak C₁₈ cartridges containing 360 mg of C₁₈ chemically bonded silica (Waters) were used.

2.2. Apparatus

A Waters (Milford, MA, USA) Model 990 liquid chromatographic system was used, equipped with a Model 600E constant-flow pump, a Rheodyne six-port injection valve with a 20- μ l sample loop and a Model 990 photodiode-array detector. The spectral resolution used was 1.4 nm per diode in the range 200–280 nm. HPLC separations were carried out using a Hypersil Shandon Green Env 150 \times 3 mm (5- μ m particle size) C₁₈ column.

2.3. Software

An IBM 486 DX personal computer was used for acquisition and treatment of the data. The liquid chromatographic system allows the acquisition of a series of chromatograms at different wavelengths (λ). The Waters 991 software controlling the instrument, generates a three-dimensional file (A , λ , t) in binary format. Then, the three-dimensional file is converted in a series of n individual spectra, each of them corresponding to an absorption spectrum, acquired at a different time, with the ASCII converter included in the Waters 991 program. The resolution used in the time domain is 1.4 s.

A converter program written in BASIC was used to transform the bidimensional files in ASCII format, to the software packages SURFER and GRAPHER [32]. The three-dimensional spectrochromatograms are obtained and presented as isometric plots (A , λ , t). Alternatively, the data are presented as a contour plot in the two dimensions of time and wavelength, by linking points of equal intensity to form the contour map. The SURFER program allows generation of cross-

sections, and shows the trajectory followed in the contour or isometric plot. Using GRAPHER software, cross-section data are plotted to produce a profile from the bidimensional data projection [$A=f(\lambda, t)$]. When the data are plotted, the absorbance value is plotted as the y coordinate.

Finally, GRAMS/386 software package and PLSplus V2.1G [33], were used for treatment of data.

2.4. HPLC operating conditions

Flow rate: 1 ml min⁻¹; chart speed: 0.5 cm min⁻¹; detector sensitivity: 0.02 AUFS; column at room temperature; the solvent programme was as follows. Initial: water-ACN-MeOH (56:27:17, v/v) 2 min in isocratic mode: water-ACN-MeOH (56:27:17, v/v) linear gradient for 20 min until: water-ACN-MeOH (5:90:5, v/v). An additional 10 min of gradient programme was enough to return the system to the initial conditions for subsequent analysis runs. The solvents were filtered daily through a 0.45- μ m cellulose acetate (water) or polytetrafluoroethylene (ACN) membrane filter before use, and degassed with helium during and before use.

3. Results and discussion

Fig. 1a shows a chromatogram corresponding to 21 pesticides selected for their agricultural interest. The mixture contains organochlorines, triazines, organophosphorus compounds, carbamates and ureic and imidic derivatives with very different polarities. The composition of the mobile phase was optimized by an automated sequential procedure [30,34]. However, overlapping of peaks occurred if the number of analytes increased. Fig. 1b shows a chromatogram containing a new analyte, procymidone (peak 11), and overlapping among the peaks of folpet, procymidone and triazophos can be observed. Taking into account the absorption data of the mixture of interest, 210 nm was first selected as a compromise monitoring wavelength, for the detection of the three compounds. Table 1 summarizes the retention times of each pesticide. The R_s values are 0.9 for folpet and procymidone and 0.7 for procymidone and triazophos.

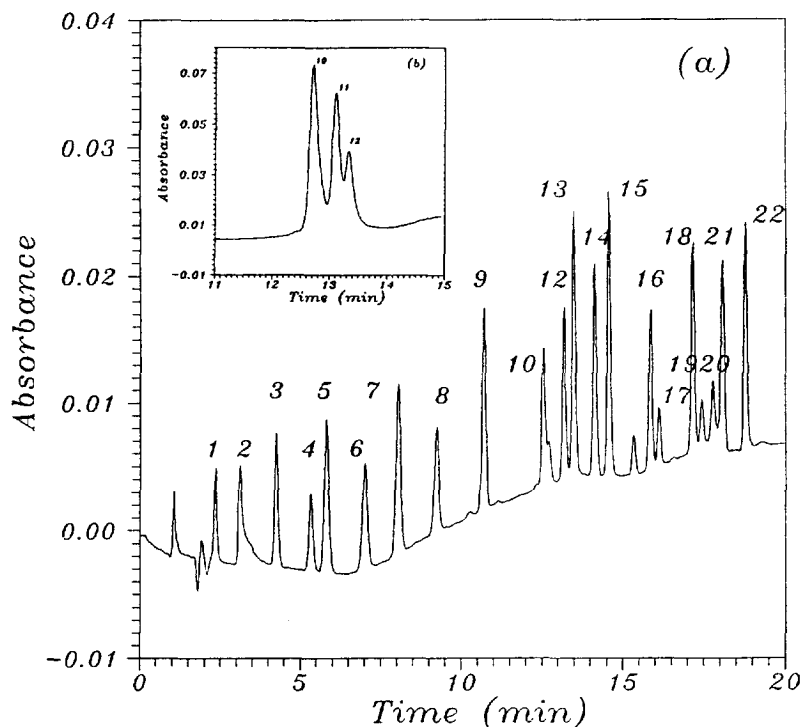


Fig. 1. (a) Chromatogram obtained by injection of 20 μl of pesticide standard solution with a 20 min gradient. (2 $\mu\text{g ml}^{-1}$ of each pesticide at 210 nm). Numbers above the peaks correspond with those given in Table 1. (b) Chromatogram with a new analyte, procymidone (peak number 11), is observed with 20 min gradient (9 $\mu\text{g ml}^{-1}$ of folpet, 4 $\mu\text{g ml}^{-1}$ of procymidone and 6 $\mu\text{g ml}^{-1}$ of triazophos).

3.1. Three-dimensional spectrochromatograms

DAD allows the collection of full spectral data at rates of up to several scans per second. With these data it is possible to construct three-dimensional plots of absorbance, wavelength and time. Moreover, these plots can be manipulated to allow the data to be viewed from different angles, including from the end of the chromatogram towards the beginning. Such plots depict an incomplete separation of the folpet, procymidone and triazophos when the optimized chromatographic method is used (Fig. 2) and it is difficult to extract quantitative data from them. The corresponding absorption maxima are located at 226 nm for folpet, at 206 nm for procymidone and at 200 and 245 nm for triazophos. From the observation of the corresponding absorption spectra, it is evident that folpet and procymidone have their absorption maxima at very close wavelengths, while triazophos has a second maximum absorption at a longer

wavelength, but its absorption spectrum overlaps in part with that of procymidone.

A potentially more informative way of presenting the chromatograms is to use the cartographic technique of a contour plot, a map of signal intensity in the wavelength–time domain (Fig. 3). From this plot it is easier to see the incomplete resolution of folpet, procymidone and triazophos. Because of the highly overlapping peaks, conventional measures of the different analytical signals (area or height of chromatographic peaks) cannot be used. With the aim of resolving the ternary mixture, a chemometric approach was evaluated.

3.2. Cross-sections through the three-dimensional data matrix

The contour plots are specially useful to make cross-sections through the data matrix, in a way allowing to pass as close as possible to the wave-

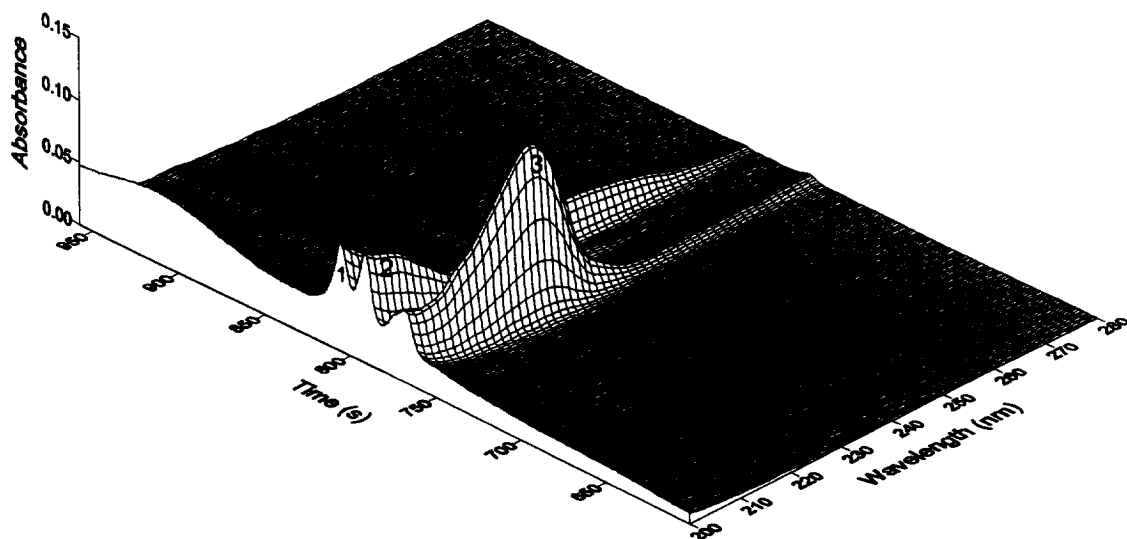


Fig. 2. Three-dimensional plot of absorbance, wavelength and time for (1) $6 \mu\text{g ml}^{-1}$ of triazophos, (2) $4 \mu\text{g ml}^{-1}$ of procymidone and (3) $9 \mu\text{g ml}^{-1}$ of folpet.

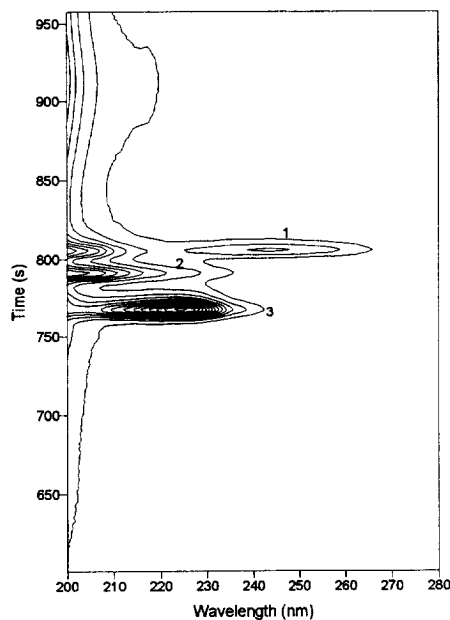


Fig. 3. Contour plot of (1) $6 \mu\text{g ml}^{-1}$ of triazophos, (2) $4 \mu\text{g ml}^{-1}$ of procymidone and (3) $9 \mu\text{g ml}^{-1}$ of folpet.

length maxima, and elution times of the different peaks, with the object of optimizing the resolution of the overlapping peaks in both, selectivity and sensitivity.

One trajectory has been selected through the contour plots to define the corresponding cross-section. This trajectory has been defined by the initial and final coordinate (λ , t) pairs, that describe the different linear paths, of the corresponding cross-section. The contour plot of a mixture containing $9 \mu\text{g ml}^{-1}$ of folpet, $4 \mu\text{g ml}^{-1}$ of procymidone and $6 \mu\text{g ml}^{-1}$ of triazophos, in which the trajectory of the selected cross-section is indicated, is presented in Fig. 4. In the present case, the cross-section selected is defined by two linear paths. In the first path, the initial and final coordinates (λ , t) are (200, 810)–(240, 740) and in the second path, the initial and final coordinates (λ , t) are (240, 740)–(247, 900). The cross-section tested has been selected in order to get bidimensional projections with the best analytical sensitivity.

The bidimensional projection on the wavelength domain, generated by the selected cross-section through the data matrix, is represented in Fig. 5. The analytical signals obtained after this process are more sensitive than those of the original chromatograms

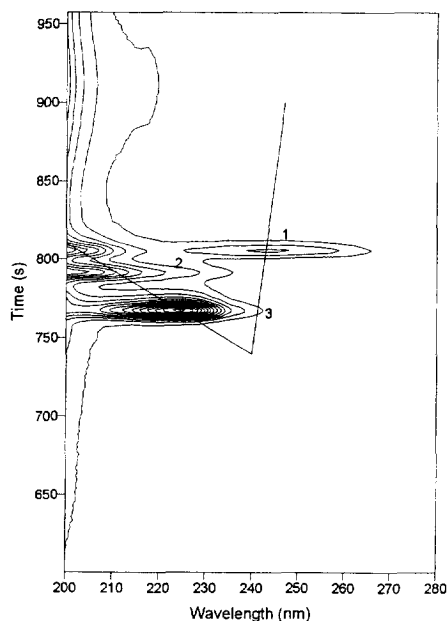


Fig. 4. Trajectory selected for the analysis, plotted across the contour plot of the spectrochromatogram of a mixture containing (1) $6 \mu\text{g ml}^{-1}$ of triazophos, (2) $4 \mu\text{g ml}^{-1}$ of procymidone and (3) $9 \mu\text{g ml}^{-1}$ of folpet.

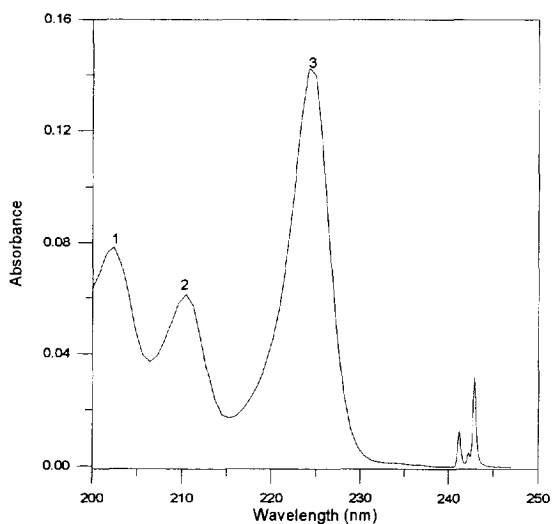


Fig. 5. Two-dimensional projection of the cross-section produced from the three-dimensional data, by plotting absorbance versus a function of wavelength. Numbers above the peaks correspond with: (1) triazophos, (2) procymidone and (3) folpet.

obtained at 210 nm (Fig. 1). Under these conditions, where the higher sensitivity on the determination is got, the separation of the three peaks, corresponding to each one of the mixture components, is not possible.

In Fig. 6, the isometric projection of the complete spectrochromatogram of the mixture analyzed, in which the trajectory of the selected cross-section is marked, is presented.

In this situation, where because of the overlapping signals, conventional measures of the different analytical signals (area or height) cannot be used for quantification we apply three multivariate calibration methods, PLS-1 and PLS-2 and PCR, with the aim of resolving the ternary mixture with the highest sensitivity. In this way, we have also avoided building a calibration model at the absorption maximum wavelength for each pesticide in order to carry out the determination at the optimum wavelength.

3.3. Calibration

A trial set of fifteen samples (C1–C15) was taken, whose concentrations are given in Table 2; these concentrations belong to linear range for the analytes, which values were in the ranges: 0.2–12.0, 0.2–12.0 and $0.3\text{--}12 \mu\text{g ml}^{-1}$ for folpet, procymidone and triazophos respectively. The cross-section selected was applied to all trial set samples. The new signals obtained, by projection the cross-section data on the wavelength domain, were used to apply the multivariate methods (PLS and principal component regression).

The optimum dimensionality of the PLS and principal component regression methods was selected as that with the fewest number of factors such that the PRESS (prediction error sum of squares) is not significantly greater than the PRESS from the model that yields a minimum PRESS. The F -statistic was used to make the significance determination. Empirically it was determined that an F -ratio probability of 0.75 was a good choice [35].

For each number of factors, ' f ', an appropriate value of PRESS is obtained. Thus, the PRESS is defined as

$$\text{PRESS}(f) = \sum_{i=1}^N \sum_{j=1}^M (\hat{c}_{i(f)} - c_i)^2$$

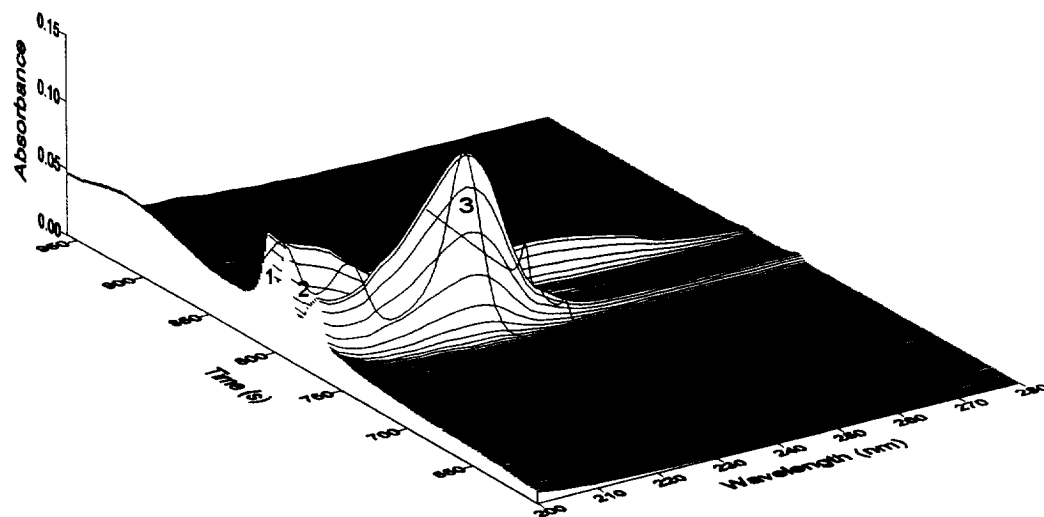


Fig. 6. Isometric projection of the spectrochromatogram of the mixture analysed, in which the trajectory of the selected cross-section, is marked.

where N is the number of samples, M is the number of analytes, c_i is the true concentration of sample i and $\hat{c}_{i(f)}$ is the predicted concentration of sample i using a model with f factors. The PRESS was calculated in all cases using a cross-validation method, leaving out one sample at a time, in order to model the system without overfitting the concentration data [36,37]; thus the concentration of the sample left out was predicted using the $N-1$ model for all N samples. The prediction ability of the

methods for each analyte is expressed in terms of the square of the correlation coefficient (r^2), which is an indication of the quality of fit of all the data to a straight line and of the root mean square difference (RMSD):

$$\text{RMSD}(f) = \left(\sum_i^N \frac{(c_i - \hat{c}_{i(f)})^2}{N} \right)^{0.5}$$

Often, software computes RMSD (0), i.e. RMSD value calculate with $\hat{c}_{i(0)}$, which is defined as the average analyte concentration in the set of all calibration samples when the i th sample is left out. Therefore, RMSD (0) provides an indication of how well we would predict the average analyte concentration in the trial set rather than instrumental measurements.

In the process of PLS-1 modelling, the covariance between the chromatographic scores and a single analyte is maximized. This often leads to the loadings of the first PLS-1 factor approximating the pure component chromatogram of the analyte under examination. The PLS-2, however, maximizes the covariance between the spectral scores and a linear combination of a number of variables. In general, the number of variables coincides with the number of components, for this, in our case three variables are considered.

Table 2
Concentration data for the calibration set

Standard	Folpet ($\mu\text{g ml}^{-1}$)	Procymidone ($\mu\text{g ml}^{-1}$)	Triazophos ($\mu\text{g ml}^{-1}$)
C1	3	3	5
C2	5	5	6
C3	2	2	10
C4	10	3	9
C5	5	2	6
C6	4	6	8
C7	2	4	8
C8	4	8	6
C9	3	2	10
C10	6	8	8
C11	8	8	8
C12	5	5	5
C13	0	1	1
C14	1	0	1
C15	1	1	0

Although principal component regression and PLS are linear methods, in a real spectroscopic or chromatographic application there may be sources of non-linearities, e.g., chemical interactions or non-linear responses in the detector at certain wavelengths. If non-linearities are present, they may be modelled by the inclusion of extra latent variables (factors) in the regression model [36,38] and this could explain the need of the four or more factors to describe a three-component system.

A mean-centering data pre-treatment was applied to optimize the calibration matrix. Mean-centering involves the subtraction of the variable mean from the individual variable values. This should almost always be used because it provides better mathematical accuracy in calculating the PLS or principal component regression model. The effect of this preprocessing technique on the r^2 and RMSD of the calibration matrix for PLS-1, PLS-2 and principal component regression is shown in Table 3. Mean-centering had a beneficial effect on this data set, because, in general, it reduces the model dimensionality and RMSD values and r^2 values are higher.

The proposed PLS-1, PLS-2 and principal component regression methods, applied to the bidimensional projection on the wavelength domain generated by the selected cross-section through the data matrix with mean-centering pretreatment, allow the resolution of synthetic mixtures containing between 0 and 10 $\mu\text{g ml}^{-1}$ each of folpet, procymidone and

Table 4
Concentration data for the prediction set

Test no.	Folpet ($\mu\text{g ml}^{-1}$)	Procymidone ($\mu\text{g ml}^{-1}$)	Triazophos ($\mu\text{g ml}^{-1}$)
T1	4	2	7
T2	3	1	4
T3	4	1	7
T4	10	10	10
T5	8	8	8
T6	8	6	7

triazophos. In Table 4 are shown the composition of the mixtures studied and the results obtained by these strategies are summarized in Table 5. It can be observed that the results obtained by all approaches are good and are not significantly different from each other, and this is in agreement with findings by other workers [39–42].

3.4. Simultaneous determination of folpet, procymidone and triazophos in groundwaters

The isolation of the pesticides from groundwater was tested by solid-phase extraction (SPE) with C_{18} cartridges. Samples of 400 ml of groundwater, spiked with 7.5 $\mu\text{g l}^{-1}$ of folpet, procymidone and triazophos, were passed through Sep-Pak C_{18} disposable cartridges at flow-rates of 8–10 ml min^{-1} . The

Table 3
Effect of mean-centering preprocessing technique on the relative prediction errors and the optimum number of factors of PLS-1, PLS-2 and principal component regression models

Pre-processing technique	Folpet		Procymidone		Triazophos	
	r^2	RMSD ^a	r^2	RMSD ^a	r^2	RMSD ^a
	PLS-1					
None	0.9932	0.30 (4)	0.9921	0.20 (5)	0.9831	0.33 (6)
Mean-centering	0.9939	0.29 (3)	0.9920	0.20 (5)	0.9957	0.29 (6)
	PLS-2					
None	0.9931	0.25 (5)	0.9837	0.35 (5)	0.9815	0.37 (5)
Mean-centering	0.9972	0.23 (4)	0.9930	0.33 (4)	0.9911	0.36 (4)
	PCR					
None	0.9940	0.28 (4)	0.9931	0.32 (4)	0.9823	0.32 (4)
Mean-centering	0.9940	0.28 (4)	0.9932	0.30 (4)	0.9882	0.31 (4)

^a The number of factors is given in parentheses.

Table 5
Recoveries of folpet, procymidone and triazophos in the synthetic mixtures

Test	Recovery (%)								
	Folpet			Procymidone			Triazophos		
	PLS-1	PLS-2	PCR	PLS-1	PLS-2	PCR	PLS-1	PLS-2	PCR
T1	93.5 (6.9)	94.3 (6.8)	93.6 (6.5)	103.8 (7.7)	103.1 (7.8)	101.2 (7.5)	80.1 (9.1)	81.7 (9.7)	81.5 (9.5)
T2	95.3 (6.6)	95.9 (6.2)	94.7 (6.4)	97.3 (8.8)	96.1 (8.5)	96.1 (8.8)	82.3 (6.8)	82.4 (6.2)	82.4 (6.9)
T3	83.2 (9.8)	83.7 (9.9)	83.2 (9.5)	109.8 (6.5)	109.8 (6.1)	109.7 (6.8)	79.7 (6.2)	79.7 (6.5)	79.9 (6.1)
T4	105.7 (6.0)	105.7 (5.1)	105.4 (6.2)	96.1 (6.6)	97.2 (6.1)	96.5 (6.0)	85.3 (6.1)	85.4 (6.2)	85.3 (6.2)
T5	91.3 (5.2)	91.4 (5.8)	91.4 (5.3)	92.7 (5.2)	92.5 (5.3)	92.9 (5.8)	86.8 (7.1)	85.3 (7.3)	86.4 (7.8)
T6	103.7 (6.7)	104.1 (6.5)	103.6 (6.6)	95.4 (5.2)	95.4 (5.5)	95.7 (5.4)	83.0 (6.3)	83.7 (6.5)	83.1 (6.2)

The results are averages of three determinations, with R.S.D. values in parentheses.
PCR = principal component regression.

cartridges were previously preconditioned with 5 ml of ACN followed by 5 ml of ultra-pure water. After the preconcentration step, the cartridges were dried on a vacuum source by passing air through them for 10 min. The samples thus concentrated were eluted with 1 ml of ACN and the pesticides were determined. The results obtained are presented in Table 6. It was found that all the compounds were removed effectively from their aqueous solutions using SPE in all instances, with recoveries ranging from 81.5–106.3%. The results obtained by the PLS-1, PLS-2 and principal component regression methods were similar. A blank of water without fortification was also performed in each experiment.

The proposed method was applied to the determination of pesticide levels in groundwaters of

Almería (Spain) and the chromatograms obtained show no peaks of the studied pesticides.

4. Conclusions

The determination of folpet, procymidone and triazophos mixtures has been performed, without a prior separation by HPLC, by means of the coupled cross-sections technique and PLS-1, PLS-2 and principal component regression methods with good reproducibility and sensitivity. The proposed methodology is particularly useful for determining mixtures of analytes in complex samples, as it is the case in MRM pesticide analysis. The usefulness of the proposed methodology lies in the resolution of overlapping chromatographic peaks, maintaining at the same time as much sensitivity in the determination as possible. In conclusion, the combination of advanced computational capability, with the diode array detector technology applied in HPLC, offers a powerful approach for the resolution of highly overlapping peaks, maximizing at the same time the sensitivity of the analysis, as it is demonstrated in the particular case studied.

C₁₈ cartridges have shown to be a good adsorbent for SPE of the analytes from groundwater. The method was applied to the determination of folpet,

Table 6
Recoveries in the determination of 7.5 µg l⁻¹ of folpet, procymidone and triazophos from groundwater

Method	Recovery (%)		
	Folpet	Procymidone	Triazophos
PLS-1	96.2 (6.6)	106.3 (7.5)	81.5 (8.9)
PLS-2	97.6 (7.1)	106.2 (8.7)	82.3 (8.8)
PCR	97.2 (6.6)	105.7 (7.2)	82.3 (9.5)

The results are averages of three determinations, with R.S.D. values in parentheses.

PCR = principal component regression.

procymidone and triazophos in groundwater samples with good results.

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