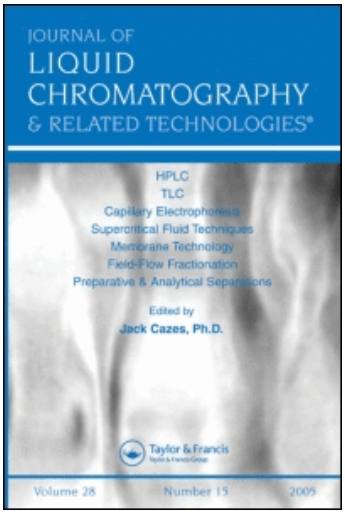

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Determination of Folpet, Procymidone, and Triazophos in Groundwater by HPLC Using Partial Least Squares and Principal Component Regression

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DETERMINATION OF FOLPET, PROCYMIDONE, AND TRIAZOPHOS IN GROUNDWATER BY HPLC USING PARTIAL LEAST SQUARES AND PRINCIPAL COMPONENT REGRESSION

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ABSTRACT

Three multivariate calibration methods, partial least squares (PLS-1 and PLS-2) and principal component regression (PCR), full spectrum calibration methods, were applied to the simultaneous determination of the three pesticides folpet, procymidone and triazophos, in mixtures, by high performance liquid chromatography with photodiode array detection (HPLC-DAD). The effects of several preprocessing techniques are discussed in order to optimize the calibration matrices by the PLS and PCR methods. The use of mean-centering and smoothing function chromatograms allows better prediction of the samples. The average recoveries from the different mixtures assayed ranged between 79.6 % and 114.0 %. No advantages were found for the prior differentiation step.

The results obtained by the application of the different chemometric approaches are discussed and compared. The methods were applied with satisfactory results in the determination of folpet, procymidone and triazophos in groundwater at ppb levels, having previously employed a solid-phase extraction with C_{18} cartridges.

INTRODUCTION

Pesticides are of major importance in modern agriculture. Their application over a number of decades has led to the development of multirresidue analysis methods by HPLC among others, for their detection and control in the sustracts where they are applied. The development of reliable methods to cover a broad spectrum of relevant substances, in one analytical run, for systematic environmental analysis is an important field of research. The analysis of pesticides in environmental samples by HPLC techniques requires the elution of a wide variety of analytes under conditions as optimum as possible. One way to reach this objective is to apply solvent programming or gradient elution techniques.[1-4]

HPLC using DAD provides an opportunity for chromatographers to explore all wavelengths in the UV-vis, improving the selectivity of HPLC and allowing the identification of compounds not only from their retention behaviour but also from their UV spectral properties. The use of DAD allows choice of the monitoring wavelengths which maximize the instrumental sensitivity.[5-7]

In previous papers we studied the optimization of the separation, isolation and determination of nine pesticides by a new sequential procedure for the automated location of the mobile phase composition optimum[8] and the proposed method was extended to the determination of 21 pesticides in water samples.[9,10] However, it is difficult to avoid the overlapping of peaks in the analysis of complex mixtures owing to similar retention times obtained, either for different analytes or for analytes and interferences.

In this situation, where the overlapping signals do not permit the analysis of all analytes in a single chromatographic run, it is possible to modify the multirresidue method, or apply chemometric techniques in order to extract useful information from the overlapped region[3,4,11-13] The first solution is not the most adequate due to the great cost involved in developing a new method.

Therefore, the second solution can be selected. Moreover, the advent of multi-dimensional data systems used in conjunction with modern computer technology has allowed the development of new experimental procedures for the characterization of unresolved chromatographic peaks.[14-19]

Direct calibration methods, such as multiple linear regression[20] or Kalman filter[21-23] have also been applied to HPLC data. Direct calibration methods assume that chromatograms are available for all chemical species existing in the mixture. The major advantage of these methods is their simplicity to resolve linear analytical systems that obey the Beer-Lambert law. If some species interact, indirect calibration methods present a better alternative than direct methods, since we can design and measure the response of a training set of mixtures with known concentrations (standards), and thus accomodate moderate effects of nonadditivity, which are normally not accounted for with direct methods.

In the present paper, PLS and PCR multivariate calibration methods were applied to resolve highly overlapped chromatographic peaks. Both are examples of indirect calibration methods, i.e. they do not require individual chromatograms for each analyte and interferent to be known in advance, but all expected phenomena must be spanned in the calibration set. They offer full spectrum advantages.

Each method requires a calibration step where the relationship between the chromatograms and the component concentrations is deduced from a set of reference samples by means of a least-squares procedure, followed by a prediction step in which the results of the calibration are used to determine the component concentrations from the sample chromatogram.

Biased regression methods such as PLS and PCR are based on the regression of chemical concentrations on latent variables or factors. PLS differs from PCR in that it uses the concentration data from the training set and the chromatographic data in modelling, whereas PCR only uses the chromatographic data. Hence PLS can reduce the influence of dominant but irrelevant factors, and in some cases yields models of lower dimensionality, in order to achieve better correlations with concentrations during prediction.

PLS also has the advantage of being able to model a number of analytes simultaneously, the so-called PLS-2 approach. These chemometric techniques have been discussed in more detail elsewhere.[24,25]

Table 1

Retention Times of Each Pesticide in the Multirresidue Method

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 sulftate	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

This paper describes the development of a combined HPLC-DAD system and direct data treatment using PLS and PCR for simultaneous multi-analyte determination of the components of a mixture of folpet, procymidone and triazophos.

The procedures were applied in order to determine these pesticides in groundwater at ppb levels after a solid-phase extraction (SPE) with C_{18} cartridges.

EXPERIMENTAL

Instrumentation

A Waters (Milford, MA, USA) Model 990 liquid chromatographic system, equipped with a Model 600E constant flow pump, a Rheodyne six-port injection valve with a 20 μL sample loop and a Model 990 photodiode array detector, was used. The detector was interfaced with an Olivetti PCS-386 personal computer using a Waters Model 991 software and a Waters Model 990 plotter. The absorbance (A), wavelength (λ), and time (t) were digitized using the Waters Model 991 software, which allows representation and storage of absorption spectra obtained at the same time. An IBM 486-DX microcomputer, provided with a Grams/386 software package and PLSplus V2.1G,[26] was used for treatment of data. A conversion program written in Array-Basic was used with the object of transferring the files obtained with the Waters Model 991 software to an ASCII XY format, which allows the manipulation of these files with the Grams/386 software.

HPLC separations were carried out using a Hypersil Shandon Green Env. 3 x 150 mm (5 μm particle size) C_{18} column.

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 (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 during and before use. Distilled water was obtained from a Millipore water purification Milli-Q system. All solvents and samples were filtered through Millipore membrane filters before injection into the column. Prepacked Sep-Pak C_{18} cartridges containing 360 mg of C_{18} chemically bonded silica (Waters) were used.

HPLC Operating Conditions

Flow rate: 1 mL. min^{-1}; chart speed: 0.5 cm. min^{-1}; detector sensitivity: 0.02 a.u.f.s. ; column at room temperature; wavelength: 210 nm.

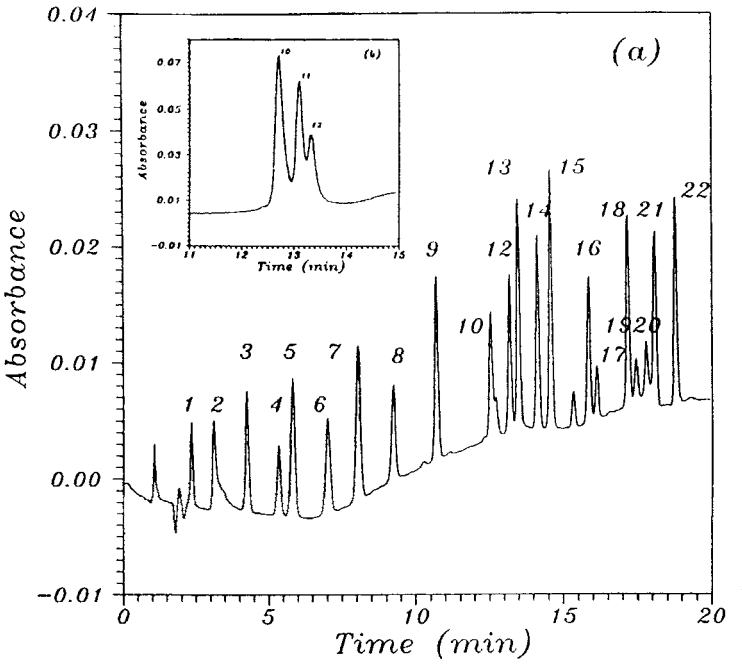

Figure 1. a) Chromatogram obtained by injection of 20 µl of pesticide standard solution with a 20 min gradient, (2 µg. mL^{-1} of each pesticide at 210 nm). Numbers above the peaks correspond with those given in Table 1. b) Chromatogram with a new analyte, procymidone (peak number 11), is observed with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

Solvent Programming

The solvent program was as follows: Initially 2 min isocratic with 56 % Water, 27 % AcN, 17 % MeOH, 20 min linear gradient to 5 % Water, 5 % MeOH, 90 % AcN. An additional period of 10 minutes of gradient program was sufficient to return the system to the initial conditions for subsequent analysis runs.

Procedure for Analysis of Mixtures of Folpet, Procymidone and Triazophos

A calibration matrix for folpet, procymidone and triazophos using a fifteen sample set in the range 0-10 µg. mL^{-1} was performed. Volumes of 20 µL were injected into the chromatographic system and the chromatographic

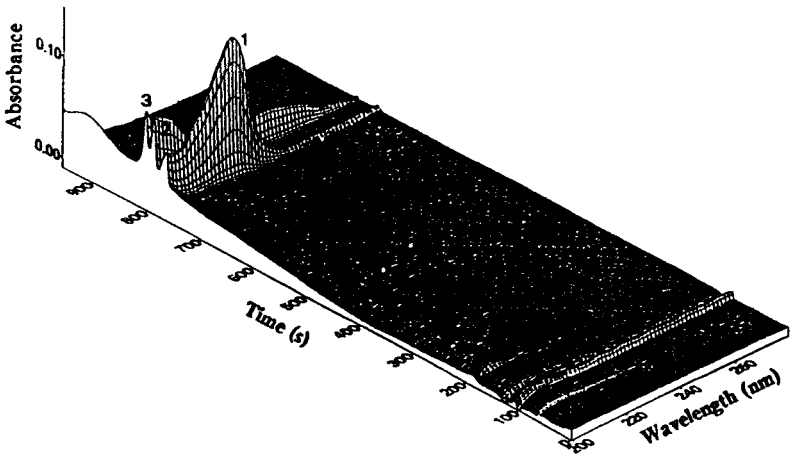

Figure 2. Three-dimensional plot of absorbance, wavelength and time for (1) folpet, (2) procymidone and (3) triazophos at concentrations of 9 μg. mL^{-1} for folpet, 4 μg. mL^{-1} for procymidone and 6 μg. mL^{-1} for triazophos.

separations were performed on a C_{18} column with the solvent programming described above. A mean-centering and smoothing pretreatments of data were applied. The optimized calibration matrices, in the chromatographic region between 11.0 and 14.5 min, calculated by application of PLS and PCR methods, were used to determine folpet, procymidone and triazophos in the prediction set.

Procedure for the Determination of Folpet, Procymidone and Triazophos in Groundwater

The 360 mg C_{18} Sep-Pak cartridges were conditioned with 5 mL of AcN followed by 5 mL of ultrapure water without allowing the cartridges to dry out. 400 mL water samples previously filtered through a 0.4 μm filter were passed at a flow rate of 8-10 mL. min^{-1} through PTFE tubes fitted with the conditioned cartridges; the cartridges were then sucked dry for 5 minutes.

The sample was eluted with 1 mL of AcN and 20 μl was injected into the system. Finally, folpet, procymidone and triazophos were determined as described above.

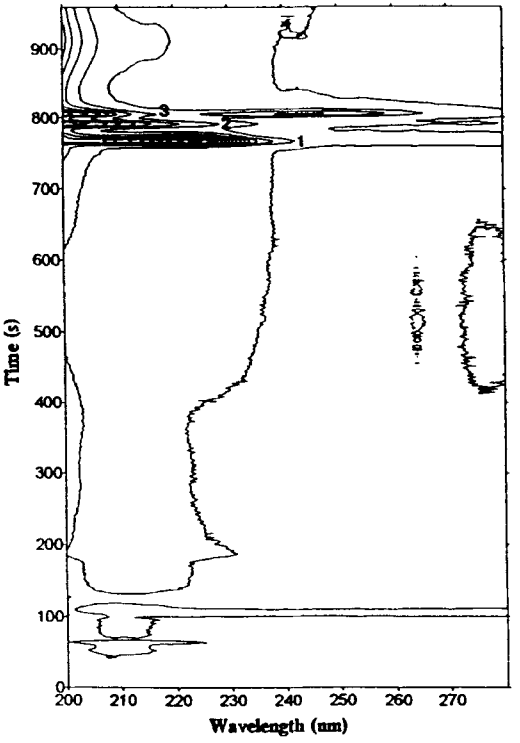

Figure 3. Contour plot of (1) folpet, (2) procymidone and (3) triazophos at concentrations of 9, 4 and 6 μg. mL^{-1}, respectively.

RESULTS AND DISCUSSION

Figure 1(a) 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 greatly differing polarities. The composition of the mobile phase was optimized by an automated sequential procedure.[9,10] However, overlapping of peaks occurs if the number of analytes increases. Figure 1(b) shows a chromatogram containing a new analyte, procymidone (peak 11), and overlapping between the peaks of folpet, procymidone and triazophos can be observed. The optimum detection wavelength for a single-channel detector is 210 nm. The R_s values are 0.9 for folpet & procymidone and 0.7 for procymidone and triazophos. Table 1 summarizes retention times of each pesticide.

The DAD allows the collection of full spectral data at rates of up to several scans per second. With the 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 (Figure 2) and it is difficult to extract quantitative data from them. 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 (Figure 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) can not be realized. With the aim of resolving the ternary mixture, several different chemometric approaches were evaluated.

Calibration

A training set of fifteen samples (C1-C15) was taken; the concentrations are given in Table 2.

The optimum dimensionality of the PCR and PLS methods was selected as that which has 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 carry out the significance determination. Empirically it was determined that an F-ratio probability of 0.75 is a good choice.[24]

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

$$\text{PRESS (f)} = \sum_{i=1}^{N} \sum_{j=1}^{M} (\hat{x}_i(f) - x_i)^2 \qquad (1)$$

where N is the number of samples, M is the number of analytes, x_i is the true concentration of sample i and $\hat{x}_i(f)$ 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

Table 2

Concentration Data for the Calibration Set

Standard	Folpet ($\mu g.\ mL^{-1}$)	Procymidone ($\mu g.\ mL^{-1}$)	Triazophos ($\mu g.\ mL^{-1}$)
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

the system without overfitting the concentration data;[25,27] 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 root mean square difference (RMSD):

$$RMSD(f) = \left(\frac{\sum_{i=1}^{N} (xi - xi(f))^2}{N} \right)^{0.5} \quad (2)$$

and 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:

$$r^2(f) = \frac{\sum_{i=1}^{N} \left(\hat{x}_i(f) - \bar{x}\right)^2}{\sum_{i=1}^{N} \left(x_i - \bar{x}\right)^2} \qquad (3)$$

where $\bar{x}$ is the mean of the true concentrations in the prediction set. Often, softwares compute PRESS (0) and RMSD (0), i. e. the PRESS or RMSD value calculated with $\hat{x}_i(0)$, which is defined as the average analyte concentration in the set of all calibration samples when the ith sample is left out. Therefore, RMSD (0) provides an indication of how well we would predict the average analyte concentration in the training 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 the present study three variables are considered.

Although PCR 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[25,28] and this could explain the need of the four factors to describe a three-component system. Nevertheless, some non-linearities may be corrected by external methods (transformation of the data, limiting the span of the regression model) while there are non-linearities that are not compensated. To solve this problem, different algorithms of non-linear expansions of PLS regression have been described[29-31] in addition to a method based on local modelling in PCR.[32-34]

Preprocessing

Different methods for the pretreatment of data, as mean-centering, smoothing and differentiation were applied, the aim of which was to eliminate effects of variations in instrumental conditions, background effects. These methods have been discussed in more detail elsewhere.[35] Smoothing and differentiation were done by the convolution algorithm of Savitzky and Golay.[36]

Table 3

Effect of Several Preprocessing Techniques on the Relative Prediction Errors of PLS-2 Model

Pre-processing Technique	Folpet		Procymidone		Triazophos	
	r^2	RMSD[a]	r^2	RMSD[a]	r^2	RMSD[a]
None	0.9962	0.19 (7)	0.9901	0.19 (7)	0.9865	0.17 (7)
Mean-centering (MC)	0.9966	0.13 (6)	0.9937	0.18 (6)	0.9866	0.17 (6)
MC + smoothing	0.9976	0.11 (7)	0.9937	0.18 (7)	0.9968	0.13 (7)
MC + ^{1}D	0.9614	0.44 (6)	0.9695	0.40 (6)	0.7733	0.60 (6)

[a] The number of factors is given in parentheses.

Table 4

Concentration Data for the Prediction Set

Test N°	Folpet ($\mu g.\ mL^{-1}$)	Procymidone ($\mu g.\ mL^{-1}$)	Triazophos ($\mu g.\ mL^{-1}$)
T1	4	2	7
T2	3	1	4
T3	4	1	7
T4	10	10	10
T5	8	8	8
T6	8	6	7

The effect of these preprocessing techniques on the r^2 and RMSD of the calibration matrix for PLS-2 is shown in Table 3. Mean-centering had a beneficial effect on this data set, because it reduces the PLS-2 model dimensionality and RMSD values for folpet and procymidone. Moreover, r^2 values are higher for folpet and procymidone. Also, it can be seen that the first derivative had a detrimental effect on the r^2 and RMSD of this data set.

Table 5

Recoveries of Folpet, Procymidone and Triazophos in the Prediction Set

Test N°	Recovery (%)[a]								
	Folpet			Procymidone			Triazophos		
	PLS-1	PLS-2	PCR	PLS-1	PLS-2	PCR	PLS-1	PLS-2	PCR
T1	86.5	86.5	86.5	88.8	89.9	85.9	80.5	81.9	79.6
	(3.9)	(3.5)	(4.3)	(4.4)	(4.1)	(4.3)	(5.8)	(5.5)	(6.2)
T2	107.3	107.3	107.3	94.0	94.0	94.0	112.8	112.7	112.9
	(4.1)	(4.1)	(4.5)	(3.9)	(3.8)	(3.9)	(4.8)	(4.6)	(4.8)
T3	98.3	98.5	98.8	114.0	113.0	113.0	100.4	100.4	100.4
	(4.0)	(4.5)	(4.3)	(4.2)	(3.8)	(4.1)	(5.1)	(5.5)	(5.4)
T4	101.7	101.7	101.7	103.6	103.6	103.7	89.3	89.3	82.0
	(4.2)	(4.3)	(4.0)	(3.7)	(4.0)	(3.6)	(5.2)	(5.5)	(5.6)
T5	81.0	82.2	82.5	104.5	104.5	104.8	91.8	92.3	92.0
	(5.0)	(4.7)	(5.0)	(5.3)	(5.7)	(5.2)	(5.3)	(5.1)	(5.3)
T6	103.0	102.6	102.5	101.2	101.2	101.0	90.9	90.4	90.6
	(5.4)	(5.1)	(5.5)	(4.7)	(4.6)	(4.7)	(5.6)	(5.1)	(5.2)

[a] The results are averages of three determinations, with RSDs in parentheses.

Contradictory results about the convenience of applying differentiation techniques prior to the use of multicomponent calibration methods can be found in the literature. Jones et al.[37] applied factor-analysis multicomponent methods to the analysis of a binary mixture, by using several luminiscence analytical signals. They found that for the determination of an analyte, the best choice is the use of the synchronous spectral data whereas for the other analyte, the use of second derivative synchronous spectra was the best choice.

MacLaurin et al.[38] and Durán-Merás et al.[39] applied several multivariate calibration methods to UV-vis spectra to resolve ternary mixtures. They did a comparative study of applying methods based on the use of absorbance and first and second derivative spectral data. Both groups found no significant differences in the predictions from the absorbance and first derivative data with PLS and PCR. The second derivative data yielded much less precise

predictions which can be attributed to the poorer signal-to-noise ratio of the second derivative signal compared with that of the direct absorbance signal. However, derivative techniques have proven to be useful in the resolution of simple binary mixtures and/or turbid background samples.[40-44]

On the other hand, the smoothing had a beneficial effect on the r^2 (>0.99 in all cases) and RMSD of this data set. The PCR and PLS-1 models were also built using the preprocessed data and, as expected, resulted in dimensionality, r^2 and RMSD values very similar to those for PLS-2, in agreement with other authors.[38,39,45]

Selection of the Region for the Analysis

We selected the chromatogram region between 660 and 870 s, which involves working with 210 experimental points (the chromatograms are digitalized every 1 s). This region was taken into account because it is the zone with the maximum analytical information from the mixture components of interest. Moreover, the shorter region is selected because by reducing the size of the regions used the amount of memory and time necessary to perform all the calibration calculations is reduced.

Calibration Design and Prediction

The proposed PLS-1, PLS-2 and PCR methods, applied to the chromatograms (using a fifteen-sample training set) with mean-centering and smoothing pretreatments, allowed the resolution of synthetic mixtures containing between 0 and 10 $\mu g.\ mL^{-1}$ each of folpet, procymidone and triazophos. In Table 4 the composition of the mixtures studied are shown while 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 they do not differ significantly among each other, being in agreement with findings by other researchers.[37-39,45]

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 $\mu g.\ L^{-1}$ of folpet, procymidone and triazophos, were passed through Sep-Pak C_{18} disposable cartridges at flow rates of 8-10 $mL.\ min^{-1}$. It

Table 6

Recoveries in the Preconcentration of 7.5 μg. L^{-1} of Folpet, Procymidone and Triazophos from Groundwater

Method	Recovery (%)		
	Folpet	Procymidone	Tirazophos
PLS-1	103.7 (7.4)	96.3 (5.1)	94.7 (7.9)
PLS-2	103.7 (7.4)	98.2 (5.4)	93.4 (7.7)
PCR	98.6 (8.3)	96.3 (5.1)	91.8 (7.9)

The results are averages of three determinations, with RSDs in parentheses.

was found (Table 6) that all the compounds were removed effectively from their aqueous solutions using SPE in all instances, with recoveries ranging from 91.8 to 103.7 %. The results obtained by the PLS-1, PLS-2 and PCR methods were similar.

The proposed method was applied to the determination of pesticide levels in groundwaters of Almería (Spain) and the chromatograms obtained showed no peaks for the studied pesticides.

CONCLUSIONS

The PLS-1, PLS-2 and PCR methods were successfully applied to the simultaneous determination of folpet, procymidone and triazophos, without a prior separation step, by HPLC. The effect of some preprocessing techniques and the r^2 and RMSD values of the calibration matrix were similar for the PLS-1, PLS-2 and PCR calibration methods. Mean-centering and smoothing (5-points) of the chromatogram to realize the calibration was found to be advantageous, whereas first derivative had a detrimental effect. The results obtained for PLS-1, PLS-2 and PCR were similar.

C_{18} cartridges have shown to be a good adsorbent for SPE of the analytes from groundwater. The method was applied to the determination of folpet, procymidone and triazophos in groundwater samples with satisfactory results.

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