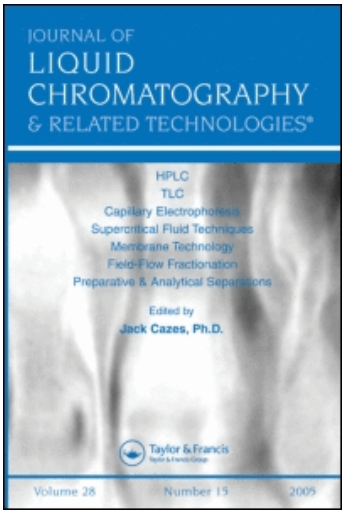

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EFFECT OF USING SELECTED INFORMATION FROM HPLC-DAD AND PLS IN THE ELIMINATION OF INTERFERENCES FOR THE RESOLUTION OF A COMPLEX PESTICIDE MIXTURE

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EFFECT OF USING SELECTED INFORMATION FROM HPLC-DAD AND PLS IN THE ELIMINATION OF INTERFERENCES FOR THE RESOLUTION OF A COMPLEX PESTICIDE MIXTURE

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ABSTRACT

Partial Least Squares (PLS) and Principal Component Regression (PCR) methods were applied to the simultaneous determination of a mixture of twelve pesticides by high performance liquid chromatography (HPLC). Calibration models at two different wavelengths were developed to resolve mixtures of the pesticides with overlapping chromatographic peaks. The first model carried out at 205 nm, as first detector compromise wavelength, yielded satisfactory sensitivity and selectivity for estimation of the concentration of iprodione, procymidone, triadimefon, and vinclozolin. The other model at 250 nm, as second detector compromise wavelength, was used for estimation of chlorothalonil, clorfenvinphos, fenamiphos, parathion-methyl, parathion-ethyl, and triazophos. However, two pesticides of the mixture, malathion and tebuconazole, showed bad prediction ability and were not determined, perhaps owing to their low signal relative to the other compounds. Both calibration models were evaluated by predicting the concentration of independent test set samples, and were successfully applied to the determination of these pesticides in groundwater samples. In all cases the PLS calibration method showed superior quantitative prediction ability than the PCR method.

INTRODUCTION

Quantitative analysis of individual components in complex mixtures represents a considerable portion of the daily routine in laboratory analysis. For these mixtures HPLC has emerged as the ideal and most commonly used separation method.[1-4] As samples become more complex, the ability of a particular separation method to resolve all components decreases. Under isocratic conditions the initial peaks are likely to be poorly resolved and the final peaks will probably be broad and flat and may be swamped by background noise. If a weaker solvent is used, the initial peaks show improved resolution but the final ones are not eluted at all. A stronger solvent compresses the early peaks together more, so that some components can no longer be distinguished. This is, then, the general elution problem and, at present, there are several techniques of programming gradient formation for dealing with it:[5-13] solvent gradient, column switching, temperature gradient, and flow gradient. All the above alternatives aim to create a greater distance between the early peaks and to speed up the elution of the later ones so that they become closer together. The main drawback of the mentioned gradients is that they require reconditioning, which takes a certain amount of time.

However, there are cases where chromatographic conditions are optimized and 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 overlapping peaks can occur if new pesticides have to be checked within an established multi-pesticide method. In these instances, it would be an advantageous option to apply chemometric techniques in order to extract useful information from the overlapped region, basically due to both the high cost of developing a new multi-residue method and also the difficulty of applying a clean-up procedure.

In recent years, the use of multicomponent data, that is, of the analytical signal depending on two or more variables, has become more general owing to the increase in the resolving power of analytical instrumentation and easier access to the microcomputer with appropriate software. Multivariate calibration[14-17] allows the simultaneous inclusion of multiple variables in the analysis that can greatly improve the precision and applicability of quantitative determinations and, in addition, with its use is often possible to reduce interference problems. There are two types of multivariate methods, direct and indirect calibration methods. The most serious limitation of direct multicomponent analysis[18-21] is that correct chromatograms must be known for all chemical species existing in the sample. However, indirect multivariate analysis is based on statistical analysis of empirical data, which allows correct prediction even in the presence of unidentified interferences, provided that a sufficiently heterogeneous sample set is available for calibration. Methods such as PCR and PLS, that belong to the second category, have frequently been used in quantitative

analysis to obtain very selective information from unselective chromatographic data.[22-25]

On the other hand, HPLC using diode array detection (DAD) provides an opportunity for the chromatographer to explore all wavelengths in the UV, in order to confirm the presence of tentatively identified peaks and choose the monitoring wavelength which maximize instrumental sensitivity and/or minimize possible interferent signals. Unfortunately, to reach these goals in the case of complex mixtures it will be necessary to select more than one wavelength to carry out a successful determination of each component. In this way one of the most significant advantages of HPLC-DAD is being used.

In the present work, the capability of PCR and PLS methods to model interference problems in the resolution of a complex pesticide mixture, with highly overlapped chromatographic peaks, is researched. Hence, our interest is in using the multi-wavelength information from HPLC-DAD to construct reliable calibration models and to obtain selective and accurate quantitative information for each analyte. Models using chromatograms at two wavelengths have been optimized. The quantitative ability predictions of both models are compared, discussed, and applied to the simultaneous determination of the analytes in groundwater.

EXPERIMENTAL

Chemicals and Solvents

Pesticide standards (Pestanal quality) of iprodione, procymidone, chlorothalonil, chlorfenvinphos, fenamiphos, malathion, parathion-methyl, parathion-ethyl, tebuconazole, triadimefon, triazophos, and vinclozolin were obtained from Riedel-de Haën (Seelze, Germany). Solid standards were dissolved in acetonitrile (AcN) and diluted in this solvent, where they were stable for several months. Analytical-reagent grade solvents, AcN, acetone, and methylene chloride, obtained from Merck (Darmstadt, Germany) were also used. HPLC-grade water provided by a Milli-Q water filtration/purification system from Millipore (Bedford, MA, US) was used.

Instrumentation and Software

A Waters (Milford, MA, US) model 990 liquid chromatographic system was used, equipped with a Model 600 E constant-flow pump, a Rheodyne six-port injection valve with a 20 μL sample loop; a Model 990 UV-visible photodiode-array detector, a printer/plotter, and a microcomputer using the Waters 991 software package.

Grams/386 software package version 1.01 and the PLSplus V2.1 G[26] were used for the application of the PLS and PCR methods.

HPLC Procedure

HPLC separations were performed on a Hypersil C_{18} column (100 x 0.46-mm I.D., 5 μm particle size). The mobile phase, under isocratic conditions, was AcN:water (60:40) v/v. This composition mobile phase was used to reduce the time of analysis and avoid too much dispersion of peaks.

The solvents were filtered daily through a 0.45 μm cellulose membrane filter before use and degassed with helium before and during use. 20 μL samples were injected with the solvent flow-rate maintained at 1 mL min^{-1}. Photometric detection was performed in the range 200 - 280 nm, with a spectral resolution of 1.4 nm. Data was obtained over an integration period of 1.4 seconds per spectrum.

Procedure for Analysis of the Pesticide Mixtures

A calibration matrix with mixtures of the twelve pesticides was prepared, using a thirty-five-sample set, in the range 0-10 μg mL^{-1} for each. 20 μL volumes were injected into the HPLC system and the spectrochromatographic data was collected. Two optimized PLS-1 models were applied to analyze synthetic mixtures and to determine the concentrations of the pesticides. The first model built up with chromatograms at 205 nm was used to determine the concentrations of iprodione, procymidone, triadimefon, and vinclozolin, while the optimized model at 250 nm was used to determinate the concentrations of chlorothalonil, chlorfenvinphos, fenamiphos, parathion-methyl, parathion-ethyl, and triazophos.

Procedure for Determining Pesticides in Groundwater

Water samples (500 mL) containing 50 mL of acetone, were shaken with 50 mL of methylene chloride for 2 min each. Three extractions with methylene chloride were carried out. The combined organic phases were dried, by passing them through anhydrous Na_2SO_4, and evaporated using a rotary vacuum evaporator. The samples thus concentrated were diluted with 1 mL of AcN and the pesticides were determined as described above.

RESULTS AND DISCUSSION

Mixture resolution with a large number of homologous chemicals compounds, in most cases, present separation problems. Normally, multivariate cal-

ibration methods give good results when there is not too much analytes or not strongly overlapped profiles. It is, however, of interest to evaluate the capacity of these methods to resolve more complex samples. With this aim a quite complex HPLC-DAD pesticide mixture has been studied.

Figure 1 shows the spectrochromatogram of a mixture in which the simultaneous elution of the twelve components can be observed. In addition, the pesticides under research are absorbing substances in the UV region of the spectrum. The overlapping of the spectra prevents the selective detection of each analyte at its absorption maximum wavelength by HPLC-DAD analysis (Figure 2a and 2b). In consequence, PLS and PCR methods were evaluated in order to perform the analysis for these commonly used pesticides in environmental samples.

To take advantage of the better information available for each analyte from HPLC-DAD, it would be necessary to select the wavelength of maximal absorbance for each one. However, this will involve building a great number of PLS or PCR models. To avoid this, an established practice for simple multicomponent determinations, was the selection of a single compromise detector wavelength to develop calibration models. Here, owing to the complexity of the mixture, two different wavelengths, 205 and 250 nm, were selected for the function of the location of the absorption maxima to carry out the determination of the mixture.

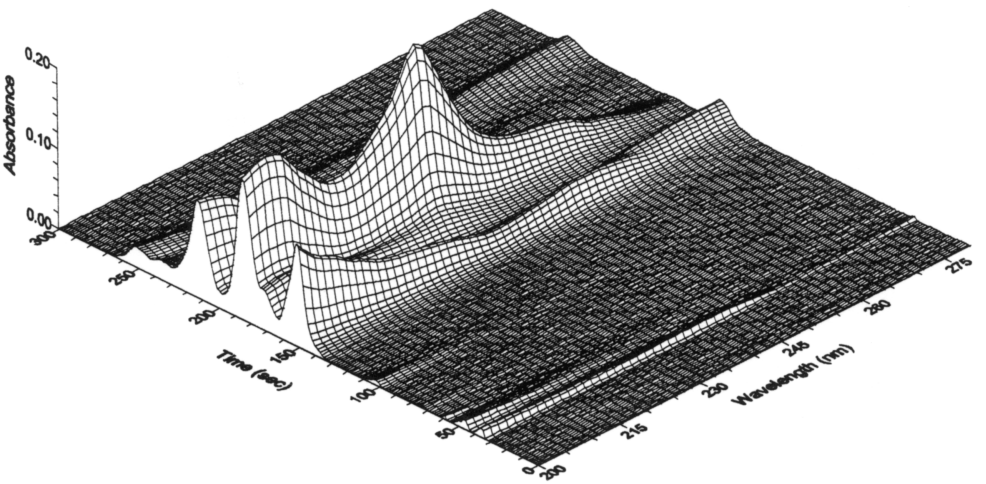

Figure 1. Three-dimensional plot of absorbance, wavelength and time corresponding to a twelve pesticide mixture of the calibration set.

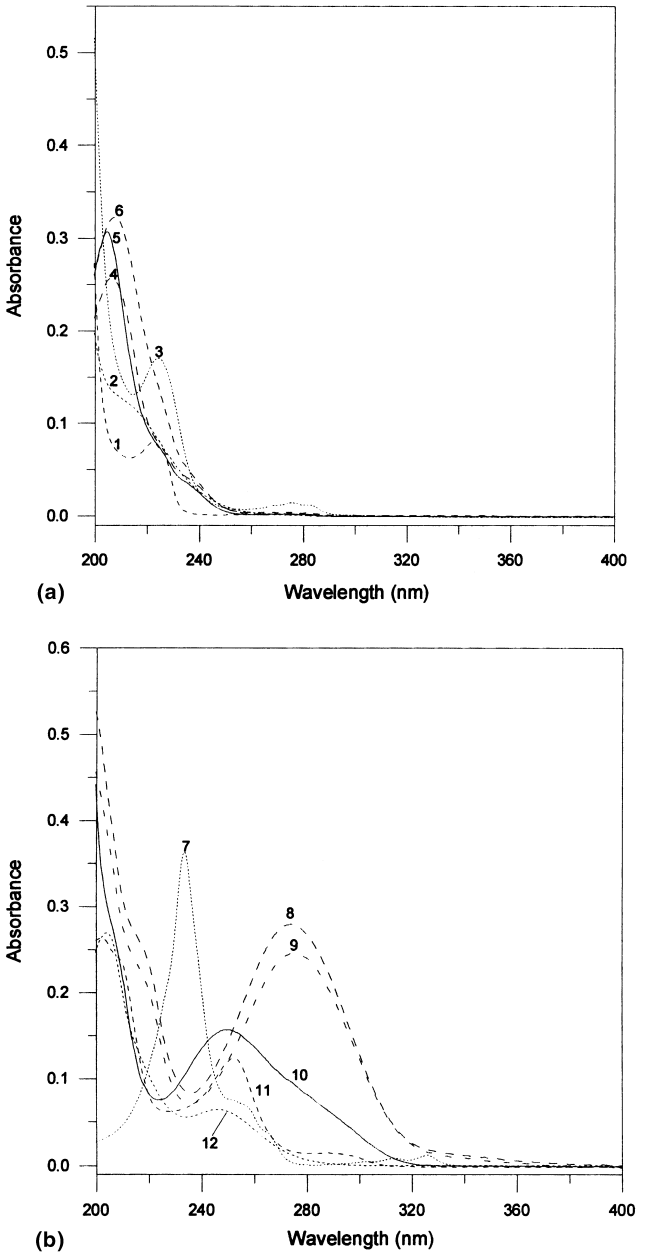

Figure 2. Absorption spectra of 4 μg ml^{-1} of: **(a)** (1) tebuconazole, (2) malathion, (3) triadimefon, (4) procymidone, (5) vinclozolin and (6) iprodione; **(b)** (7) chlorothalonil, (8) parathion-methyl, (9) parathion-ethyl, (10) triazophos, (11) fenamiphos and (12) clorfenvinphos.

The first model was built with chromatograms at 205 nm (Figure 3a) to determine the pesticides from subset 1: iprodione, procymidone, malathion, tebuconazole, triadimefon, and vinclozolin. The other model, evaluated with chromatograms at 250 nm (Figure 3b), was used to determine the pesticides from subset 2: chlorothalonil, chlorfenvinphos, fenamiphos, parathion-methyl, parathion-ethyl, and triazophos. Obviously, all the pesticides belonging to subset 2, with the exception of chlorothalonil, show also absorption maxima about 200 nm. It was decided to determine these in the subset 2 because of the lower overlapping spectra of the pesticides in this region. Calibration models were built applying the mean centering of the data as the only pre-processing algorithm, and using the chromatographic region between 100 and 280 seconds for the analysis, because this is the zone with the maximum information from the pesticide mixture under study.

Determination of Subset 1 Pesticides in Presence of the Interferents Originated by Subset 2 Pesticides

The absorption maxima of the pesticides from this subset were: iprodione 207 nm, procymidone 206 nm, vinclozolin 205 nm; malathion 200 nm, tebuconazole 200 nm and 223nm, and triadimefon 200 nm and 223 nm. So, 205 nm was selected as a compromise wavelength for carrying out the determination using multivariate methods.

A training set of 35 samples was taken whose concentrations for all pesticides varied between 0 and 10 μg ml^{-1} (Table 1). The pesticide concentrations from subset 2 were not included in the calibration matrix to build up the PLS and PCR algorithms. The number of factors was estimated by cross-validation[16,27] using the first predicted residual sum of squares (PRESS) value, the F-ratio probability of which drops below 0.75, as Haaland and Thomas determined.[14]

The statistical parameters R^2 (square of the correlation coefficient), which shows how the plots of actual versus predicted concentrations fit to a straight line, and RMSD (root mean squared prediction), which is an indication of the average error in the analysis for each component, were used to evaluate the different methods:

$$R^2 = \left(\frac{\sum_{i=1}^{N} (\hat{y}_i - \bar{y}_i)^2}{\sum_{i=1}^{N} (y_i - \bar{y}_i)^2} \right) \qquad RMSD = \left[\sum_{i=1}^{N} \frac{(y_i - \hat{y}_i)^2}{N} \right]^{0.5}$$

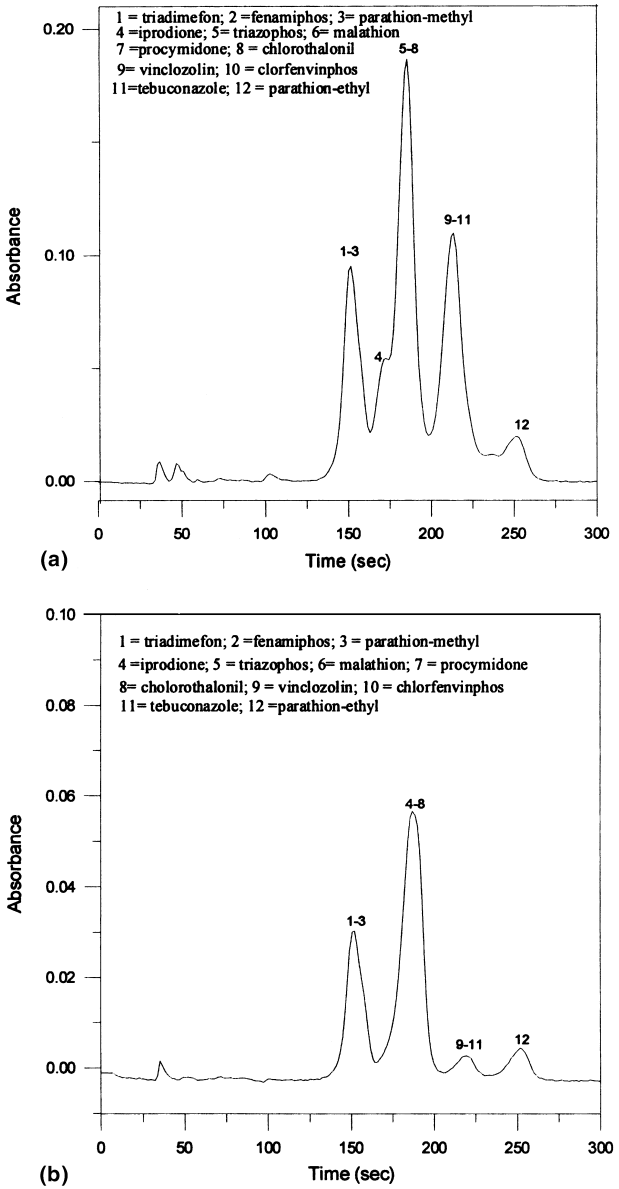

Figure 3. Chromatogram of the same sample from Figure 1: **(**a**)** at 205 nm and **(**b**)** at 250 nm.

Table 1

Concentration Data for the Mixtures in the Calibration Set*

Sample	I	P	Ct	Cr	F	M	P-M	P-e	T	Td	Tz	V
C1	0	2	4	8	3	5	6	3	8	4	5	2
C2	5	0	3.5	7.5	3	5	5	3	8	4	4	2
C3	5	4	0	2	7.5	8	3	8	3	6	3	10
C4	2	4	6	0	7.5	8	3	8	3	6	3	9.5
C5	2	6	6	2	0	4	5	5	5	3	8	5
C6	6	6	3	4	6	0	5	5	5	3	8	5
C7	6	3	3	4	6	4	0	10	4	7	4	4.5
C8	4	3	8	7	5	5	7	0	4	6	4	4
C9	4	8	8	7	5	5	7	10	0	2	6	8
C10	8	8	2	3	2	8	4	8	7	0	6	8
C11	8	4	2	3	3	8	4	8	7	2	0	6
C12	3	5	7	5	4	7	6	4	4	7	5	0
C13	3	10	7	5	4	7	6	4	4	7	5	6.5
C13	2	10	10	2	7	3	8	6	6	5	8	7
C15	2	5	10	2	7	3	8	6	6	5	8	7
C16	7	5	5	6	6	9	4	7	8	6	3	3
C17	7	8	5	6	6	9	4.5	7	8	6	3	3
C18	4	8	4	10	3	5	5.5	3	10	4	4	5
C19	4	2	4	10	3	5	5.5	3	10	4	4	5
C20	6	2	7	3	8	6	8	4	5	10	6.5	4
C21	6	7	7	3	8	6	7.5	4	5	10	9	4
C22	2	7	5	6	1.5	10	3	8	8	6	8	7
C23	2	4	5	6	1.5	10	3	8	8	5	7.5	7
C24	8	4	8	8	5	6	6	3	4	3	4	5
C25	8	3	8	8	5	6	6	3	4	3	4	5.5
C26	5	3	3	5	5	8	10	5	7	3	6	8
C27	5	8.8	3	5	5.5	8	10	5	7	4	6	8
C28	7	6	6	4	6	3	7	7	5	5	5	4
C29	7	4	6	4	6	3	7	7	5	5	5	4
C30	3	4	8	7	7	4	5	4	3	9	3	3
C31	3	2	8	7	7	4	4	4	3	8	3	3
C32	10	2	4	3	4	7	3	3	8	5	4	6
C33	6	5	4	3	4	7	3	3	8	6	4	6
C34	6	5	2	6	8	5	7	6	6	7	5.5	5
C35	6	8	2	6	8	5	7	6	6	7	7	5

* Concentration data = $\mu g ml^{-1}$. Abbreviations: I: Iprodione; P: Procymidone; Ct: Chlorothalonil; Cf: Chlorofenvinphos; F: Fenamiphos; M: Malathion; P-m: Parathion-methyl; P-e: Parathion-ethyl; T: Tebucanazole; Td: Triadimefon; Tz: Triazophos; V: Vinclozolin.

where $\hat{y}_i$ is the prediction of the concentration of interest in calibration sample i, y_i is the real concentration in calibration sample y, $\bar{y}_i$ is the mean of the concentration matrix Y and N is the total number of samples.

Table 2 summarizes these statistical parameters for the different multivariate algorithms, along with the number of factors found to be significant in the calibration. In general, a number of factors higher than the number of target pesticides in this subset were obtained, because of the presence of the pesticides from the other subset.

However, it is interesting to note that much too high prediction errors and bad correlations were obtained for malathion and tebuconazole. This fact may be a consequence of the low signal relative of them in relation to the other pesticides (Figure 2a). For this reason, as both pesticides show absorption maximum at 200 nm, PLS-1 models were built at this wavelength for carrying out their determination without loss in the sensitivity of the method. Finally, another PLS-1 model was built at 223 nm to evaluate tebuconazole, as it shows a second maximum at this wavelength. Even so, bad results were obtained for both pesticides.

With the aim of improving the results for malathion and tebuconazole, PLS-1 models with standardized data were built up. The prediction errors were slightly better than the ones obtained with the centered models, but the last models continued without having predictive ability.

Table 2

Statistical Parameters of the PLS and PCR Models*

Pesticide	PLS (205 nm) RMSD	PLS (205 nm) R^2	PCR (205 nm) RMSD	PCR (205 nm) R^2	PLS (250 nm) RMSD	PLS (250 nm) R^2	PCR (250 nm) RMSD	PCR (250 nm) R^2
Iprodione	0.12 (10)	0.99	0.13 (14)	0.99	---	---	---	---
Procymidone	0.30 (10)	0.98	0.41 (14)	0.97	---	---	---	---
Triadimefon	0.33 (14)	0.98	0.45 (14)	0.96	---	---	---	--
Vinclozolin	0.23 (4)	0.99	0.57 (14)	0.93	---	---	---	---
Malathion	2.17 (17)	0.34	2.14 (14)	0.26	---	---	---	---
Tebuconazole	1.87 (6)	0.31	1.68 (14)	0.48	---	---	---	---
Chlorothalonil	---	---	---	---	0.27 (16)	0.99	0.63 (6)	0.93
Chlorfenvinphos	---	---	---	---	0.18 (9)	0.99	0.29 (6)	0.98
Fenamiphos	---	---	---	---	0.38 (5)	0.97	0.48 (6)	0.95
Parathion-m	---	---	---	---	0.30 (10)	0.98	1.18 (6)	0.69
Parathion-e	---	---	---	---	0.27 (9)	0.99	0.59 (6)	0.59
Triazophos	---	---	---	---	0.30 (13)	0.98	1.24 (6)	0.61

* Number of optimum factors are given in parentheses.

In summary, four pesticides from subset data 1 have been satisfactorily resolved in the presence of the six pesticides from subset data 2. It is worthwhile to underline the power of these algorithms to modulate the chemical interferences originated by the pesticides from subset 2. Mainly, taking into account that all of them presented a relative intense signal at 205 nm and, in addition, their concentrations did not happen to vary with the concentration of the target pesticides in this subset in a constant way, their concentrations were randomly varied in the range between 0 to 10 μg mL^{-1}.

Determination of Subset 2 Pesticides in the Presence of the Interferents Originated by Subset 1 Pesticides

A similar study was carried out in order to analyze the six pesticides from subset data 2, without including in the calibration matrix, the concentrations of the six pesticides from the subset 1. Here 250 nm was selected as the compromise wavelength because the absorption maxima of these pesticides were located at: 233 nm for chlorothalonil, 250 nm for chlorfenvinphos and triazophos, 252 for fenamiphos, and 275 nm for parathion-methyl and parathion-ethyl.

The statistical results obtained are summarized in Table 1. In this case, better predictions were also obtained with the PLS method, but now all pesticides were satisfactorily predicted. At this wavelength, the signals were more selective, i.e., not all pesticides presented an absorption maximum about 250 nm. However, it is important to indicate that always there was some contribution of the pesticides from subset 1.

Probably, better results will be obtained building PLS-1 models at 233 nm for chlorothalonil and at 275 nm for parathion methyl and parathion ethyl. However, in the light of the results obtained, we think that it is more practical to use the previous PLS-1 model at 250 nm to resolve the six pesticides than to perform three different calibration models to determine them. In this case, it is also interesting to print out the robustness of the PCR and PLS algorithms to modulate the non-constant interference levels originated by the pesticides from the subset 1.

Determination of the Pesticides in Synthetic Mixtures

The proposed PLS models, evaluated with chromatograms at 205 nm and 250 nm, were applied to the resolution of synthetic mixtures of the pesticides. The concentrations (Table 3) of the mixtures assayed belonged to the linear range of each analyte. Table 4 gives predictions obtained by using the optimum number of factors for each pesticide. As can be seen, good results were obtained with low relative standard deviations (lower than 5% in every instance).

Table 3

Concentration Data for the Mixtures in the Test Set and in the Spiked Groundwater Samples

Sample	I	P	Ct	Cf	F	P-m	P-e	Td	Tz	V
					Test Set (μg mL^{-1})					
T1	4	8	7	5	5	8	5	4	5	6
T2	4	7	7	5	5	8	5	4	5	6
T3	5	7	4	4	3	4	6	8	10	4
T4	6	4	4	4	3	4	6	8	10	4
T5	6	3	6	7	4	4	6	6.5	7	8
					Groundwater Samples (μg L^{-1})					
G1	2	2.5	4	4	3	3.5	6	5.5	5	3
G2	4	3.5	2	4	5	6	4	4.5	5.5	4
G3	5	4	6	8	2	3.5	5	7	5	8
G4	8	0	8	5	6	4	6	6	6	6
G5	6	5	5	6	4	5	8	6	8	4
G6	4	3.6	3	6	8	8	4	6	8	5
G7	6	4	8	3	5	6	6	8	6	8
G8	8	6	4	5	6	5	9	8	10	9
G9	4	5	3	5	4	8	4	4	6	5

Abbreviations: I: Iprodione; P: Procymidone; Ct: Chlorothalonil; Cf: Chlorfenvinphos; F: Fenamiphos; P-m: Parathion-methyl; P-e: Parathion-ethyl; Td: Triadimefon; Tz: Triazophos; V: Vinclozolin.

Application of the Proposed PLS-1 Models to the Determination of the Pesticide Mixture in Groundwater

The optimized PLS models were applied to the determination of the pesticides in groundwater according to the procedure described in Experimental Section. Samples were spiked at levels between 0 and 10 μg L^{-1} (Table 3) and the recoveries were calculated. Table 5 shows the results obtained, with recoveries ranging from 70.5 to 130.0 % (RSD from 3.9 to 7.3 %).

Table 4

Predictions (%) Obtained in Synthetic Mixtures by the Proposed PLS Models*

Pesticides	T1	T2	T3	T4	T5
Iprodione[1]	97.5(3.5)	99.0(3.6)	94.5(3.2)	104.6(3.6)	102.7 3.6)
Procymidone[1]	101.5(3.2)	100.9(3.9)	122.4(4.6)	109.7(4.2)	110.2(4.8)
Triadimefon[1]	113.5(4.0)	111.0(4.1)	90.6(3.9)	104.0(3.6)	92.3(4.3)
Vinclozolin[1]	99.2(3.4)	93.3(3.6)	83.7(4.3)	93.0(3.9)	101.6(4.0)
Chlorothalonil[2]	101.3(3.2)	98.7(4.3)	87.0(3.5)	98.0(3.8)	114.5(3.8)
Chlorfenvinphos[2]	103.4(4.0)	101.0(4.0)	106.2(3.9)	98.5(4.3)	102.8(3.9)
Fenamiphos[2]	102.2(4.5)	110.0(3.2)	110.4(4.1)	90.0(4.7)	111.2(4.3)
Parathion-m[2]	101.5(3.3)	101.2(3.7)	105.5(3.7)	100.7(3.5)	95.5(3.9)
Parathion-e[2]	99.2(3.5)	102.2(3.9)	101.0(3.4)	99.3(3.9)	102.4(4.0)
Triazophos[2]	116.4(3.9)	103.0(3.8)	116.4(4.0)	101.1(3.5)	91.4(4.1)

*The results are average of three determinations with RSD values in parentheses.
[1]: PLS model at 205 nm; [2]: PLS model at 250 nm.

CONCLUSIONS

In this paper the simultaneous determination of twelve pesticides overlapped in HPLC-DAD using a single calibration model was not possible. So, two calibration models were built up. The first one evaluated with chromatograms at 205 nm was used to analyze successfully four pesticides from the subset 1 modulating, at the same time, the analytes from subset 2. The second calibration model with chromatograms at 250 nm was carried to analyze the pesticides from the subset 2, modulating now the interference caused by the pesticides from subset 1. It is worthwhile to emphasize the robustness of the PCR and PLS algorithms to modulate interferences.

However, two pesticides, malathion and tebuconazole, were not predicted at all. We think that this fact does not demonstrate the incapability of the calibration models tested here to resolve very complex mixtures, but that is important taking into account other factors like relative net signals of the components.

On the other hand, better predictions are obtained with the PLS calibration method than with the PCR method, which agrees with other authors' reports.[17,25] The optimized PLS models were successfully applied to the determination of

Table 5

Recoveries (%) for the Pesticide Mixture in Groundwater by the Proposed PLS Models*

Pesticide	G1	G2	G3	G4	G5	G6	G7	G8	G9
Iprodione[1]	91.5(4.3)	70.5(5.8)	85.0(4.6)	88.6(4.9)	88.8(5.2)	99.0(4.4)	80.0(5.0)	91.2(5.3)	95.0(4.4)
Procymidone[1]	76.0(4.5)	85.7(4.9)	71.0(7.3)	89.7(5.5)	78.0(5.6)	91.1(5.7)	79.1(6.5)	106.1(4.9)	100.8(4.0)
Triadimefon[1]	116.2(6.0)	117.6(6.1)	72.9(6.2)	98.3(4.3)	101.2(5.2)	118.3(6.1)	78.0(6.6)	77.5(6.0)	130.0(7.0)
Vinclozolin[1]	78.7(4.7)	90.1(5.5)	78.9(6.6)	85.0(6.1)	97.2(4.9)	120.0(5.8)	80.5(6.2)	86.7(4.8)	92.4(5.2)
Chlorothalonil[1]	87.0(5.1)	83.5(5.4)	81.7(5.6)	86.2(4.9)	87.2(4.9)	97.3(4.1)	76.5(5.5)	92.5(4.8)	90.0(4.7)
Chlorfenvinphos[2]	95.0(3.9)	75.2(5.7)	82.5(5.8)	98.0(4.2)	91.7(5.0)	95.3(4.4)	91.0(4.9)	92.8(5.1)	102.2(5.5)
Fenamiphos[2]	97.3(5.0)	102.0(5.1)	80.0(5.4)	98.9(4.4)	75.0(7.2)	99.4(5.6)	84.4(6.3)	80.0(5.3)	87.5(6.9)
Parathion-m[2]	101.1(5.4)	81.7(5.6)	82.9(6.5)	95.5(5.1)	105.4(4.9)	93.1(5.2)	80.0(5.6)	96.0(4.2)	108.0(5.3)
Parathion-e[2]	86.7(5.6)	74.2(6.8)	76.4(6.8)	85.3(5.9)	89.5(5.8)	123.7(6.2)	90.7(6.9)	94.4(5.5)	118.2(5.3)
Triazophos[2]	96.4(4.8)	74.9(6.4)	82.8(5.9)	106.7(5.0)	85.1(6.4)	97.4(5.6)	97.2(5.0)	92.5(5.1)	102.7(4.5)

* The results are the average of three determinations with RSD values in parenteses. [1]: PLS model at 205nm; [2]: PLS model at 250 nm.

the pesticides in groundwater samples. We did not observe the matrix effect in the analysis of the groundwater by HPLC because it was previously eliminated (coeluted) with the solvent peak. For that, the potential interferents, that are the main limitation of the application of multicomponent techniques in environmental analysis, were not a problem in the present mixture.

In conclusion, the coupling of the advantageous of both multivariate calibration methods and HPLC-DAD technique offers a powerful way for the resolution of complex mixtures, i.e. with a large number of analytes and which present their peaks with a high overlapping, as well as for the elimination of interferences.

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