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Evaluation of multiwavelength chromatograms for the quantification of mixtures of pesticides by high-performance liquid chromatography–diode array detection with multivariate calibration

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Abstract

Three multivariate calibration methods, partial least squares (PLS-1 and PLS-2) and principal component regression, were applied to the simultaneous determination of the five pesticides iprodione, procymidone, chlorothalonil, folpet and triazophos by high-performance liquid chromatography with diode array detection. Such detection gives multiwavelength chromatograms from a single analysis of one sample. In this paper, calibration models at two different wavelengths were developed to resolve mixtures of five pesticides with overlapping chromatographic peaks. The first model, carried out at 220 nm as detector compromise wavelength, yielded satisfactory sensitivity for accurate estimation of the concentration of iprodione, procymidone, chlorothalonil and folpet and the second model, at 200 nm, was used for accurate estimation of triazophos. Both calibration models were evaluated using the chromatograms and first-derivative (^{1}D) chromatograms by predicting the concentrations of independent test set samples. Finally, the proposed ^{1}D calibration models were successfully applied to the determination of these pesticides in groundwater and soil samples. In all cases, the PLS-1 calibration method showed superior quantitative prediction ability than the PLS-2 or principal component regression methods. © 1997 Elsevier Science B.V.

Keywords: Chemometrics; Multivariate calibration; Least-squares analysis; Principal component regression; Environmental analysis; Pesticides

1. Introduction

The chromatographer in high-performance liquid chromatography (HPLC) is typically faced with three basic questions: (1) peak purity, how complete is my separation?; (2) peak identity, which peak is which? and (3) quantitation, how much of it is there?. Obviously, there are several techniques to answer these questions, depending on the characteristics of the information that is being searched for as well as the information that is available.

In HPLC systems the most widely used detectors have been the ultraviolet–visible (UV–Vis) and fluorescence spectrometers. With the information available from these single-channel services, limited to the detection of only a single wavelength element at a time, the first two queries can be answered in a somewhat limited fashion. On one hand, peak shape parameters such as tailing, width, efficiency and resolution can be used to assess purity. Peak position and, to some degree, peak response are employed for peak identification. On the other hand, quantitation is based on peak area or height by comparison with the same parameters for an appropriate standard. It can be accurate only if peak identification is successful and there are no overlapping peaks. In addition, a

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major limitation in the analysis of multiple components separated by HPLC is the practical fact that a single compromise detector wavelength must often be selected with loss of detection sensitivity, at least for some analytes.

When two detectors are utilized in HPLC, either in series or in separate runs, additional information can be derived from the correlation of the two response functions. Typical examples would be UV detection at two different wavelengths or UV detection in conjunction with another detector, such as fluorescence or refractive index. The relative ratio of one detector wavelength signal to the other is employed as criterion of peak purity [1]. For peak identify, comparison of absolute ratio values to those of a standard increases confidence in the identification process and thus in quantification.

The introduction in 1979 of rapid scanning detectors, as diode array detection (DAD), presents an alternative technology for rapid, multiwavelength detection in HPLC [2,3]. The full UV–Vis spectrum became accessible as a three-dimensional (3D) data matrix (A, λ, t). Data are available in the time, concentration and wavelength domains. This allows the simultaneous use of more than two wavelengths for detection or for the full application of detector information to the analytical problem by means of available chemometric techniques to data from second-order bilinear instruments, as chromatographic and excitation–emission data [4–7]. Thus, peak homogeneity, peak purity and peak identity can be investigated, on the base of the comparison of peak spectra with target spectra. The use of target factor analysis (TFA) [8,9] provides information on whether a given spectrum may be present in a peak. Multicomponent analysis or partial least squares (PLS) analysis can be used to determine the relative concentrations of several target spectra across a chromatographic peak, and, in the absence of any prior information, principal component analysis (PCA) can give an approximation of the number of components in a peak. Iterative target factor analysis (ITFA) [10,11] can then be applied to arrive at the relative concentration as well as spectral estimates for each component. A simpler technique for the validation of homogeneity of a chromatographic peak has also been proposed in several model systems of overlapping drugs [3,12].

The PLS calibration method [13,14] already mentioned above and others, such as the Kalman filter [15,16], rank annihilation factor analysis and the generalized rank annihilation method [4,16,17], are employed to obtain qualitative and/or quantitative information mainly in the case of chromatographically unresolved peaks. Thus, even when full chromatographic resolution is not possible, approximate quantitation of the analytes can be achieved. In general, it is possible if the elution profile can be estimated and compared to the response of this component in a standard solution, or when a quantitative description of the absorption characteristics is available. Also, a component with unique spectral features can be quantitated without any resolution using a characteristic wavelength. However, this uniqueness in either the time or spectral domain can go unnoticed if the multivariate analysis is initially performed in the other domain.

However, a vast amount of data is available when HPLC–DAD is used. Therefore, for most multivariate calibration methods some form of data reduction is essential to make optimal use of the information enclosed in the collected spectrochromatograms. The more practical approach is to select a single compromise detector wavelength to obtain calibration models [18]. However, the selection of this single wavelength is a difficult task, mainly in the case of multicomponent mixtures with individual spectra that differ significantly. In this case, it will be necessary to perform calibration models at the wavelengths that maximize the sensitivity in the determination of each analyte.

Hence, our interest is in using the multi-wavelength information from HPLC–DAD to construct reliable calibration models, at different wavelengths, and to obtain accurate quantitative information for each analyte.

In the present paper, PLS-1 calibration models, at two different wavelengths have been performed to resolve mixtures of iprodione, procymidone, chlorothalonil, folpet and triazophos pesticides. Two models at 200 and 220 nm have been studied using in both cases the chromatograms and their first derivative (^{1}D). The quantitative abilities predictions of both optimized models are compared, discussed and applied to the simultaneous determination of the analytes in groundwater and soil samples.

2. Experimental

2.1. Chemicals and solvents

Analytical standards (Pestanal quality) of iprodione, [3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide], procymidone, [N-(3,5-dichlorophenyl)-1,2-dimthylcyclopropane-1, 2-dicarboximide), chlorothalonil, [tetrachloroisophthalonitrile], folpet, [N-(trichloromethylthio)-phthalimide] and triazophos, [O,O-diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate] were obtained from Riedel-de Haen (Seelze, Germany). Analytical-reagent grade solvents, acetonitrile (ACN), acetone and methylene chloride, were obtained from Merck (Darmstadt, Germany). Distilled water provided by a Milli-Q water filtration/purification system from Millipore (Bedford, MA, USA) was used. All solvents and samples were filtered through Millipore membrane filters.

2.2. Instrumentation

The high-performance liquid chromatograph was a Waters (Milford, MA, USA) model 990, composed of a Model 600 E constant-flow pump; a Rheodyne six-port injection valve with a 20-μl sample loop; a Model 990 UV–Vis DAD system; a printer/plotter and an Olivetti PCS-386 personal computer using a Waters 991 software.

A Model 461 rotary vacuum evaporator (Büchi, Flavil, Switzerland) thermostated by water circulation with an A-35 vacuum pump (Eyela, Tokyo, Japan) was used.

2.3. Data handling and analysis

An IBM PCS-486 425 DX microcomputer provided with the Grams/386 software package and PLS-plus V 2.1G [19] was used for treatment of HPLC data and the generation of the calibration models by PLS and principal component regression methods.

2.4. HPLC procedure

HPLC determinations were conducted in a RP-C_{18} (250×4 mm I.D., 5-μm particle size) column from Merck. The mobile phase was ACN–water (70:30, v/v) in isocratic conditions for 7 min. The solvents were filtered daily before use through a 0.45-μm cellulose acetate (water) or politetrafluoroethylene (ACN), and degassed with helium prior and during use. Samples of 20 μl volume were injected at a solvent flow-rate of 1 ml min^{-1} and the photometric detection was performed at 200 and 220 nm.

2.5. Procedure for determining the pesticides in synthetic mixtures

A calibration matrix with mixtures of the five pesticides was prepared, using a 24-sample set, in the range 0–8 μg ml^{-1} for each of them (Table 1). Volumes of 20 μl were injected into the HPLC system and the spectrochromatographic data were collected. The optimized models, obtained with the chromatograms and their ^1D, at 220 nm were applied to analyze synthetic mixtures and to determine the concentrations of iprodione, procymidone, chlorothalonil and folpet, while the optimized models at 200 nm were used to analyze the same samples and to determine the concentration of triazophos.

2.6. Procedure for determining the pesticides in groundwater

Three extractions with methylene chloride were carried out. Water samples (500 ml) were shaken with 50 ml of methylene chloride for 2 min each. 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 eluted with 1 ml of ACN and the pesticides iprodione, procymidone, chlorothalonil and folpet were determined by the ^1D-PLS-1 model obtained at 220 nm and triazophos by the ^1D-PLS-1 model evaluated at 200 nm.

2.7. Procedure for determining the pesticides in soil

A 25-g amount of ground soil was weighed, spiked with the pesticides and passed through a 55-mesh sieve. The soil sample was stirred for 4 h with 50 ml of acetone, filtered through a Büchner funnel and washed thoroughly with two 25-ml

Table 1
Concentration data of the training set for the five component system ($\mu g\ ml^{-1}$)

Training set	Iprodione	Procymidone	Chlorothalonil	Folpet	Triazophos
T1	0.0	3.0	4.0	4.0	6.0
T2	3.0	0.0	4.0	4.0	6.0
T3	4.0	6.0	0.0	3.0	3.0
T4	4.0	2.0	2.0	0.0	4.0
T5	5.0	4.0	6.0	6.0	0.0
T6	2.0	5.0	2.0	2.0	5.0
T7	2.0	2.0	5.0	5.0	3.0
T8	6.0	3.0	2.0	3.0	3.0
T9	2.0	2.0	4.0	4.0	7.0
T10	3.0	5.0	3.0	2.0	5.0
T11	5.0	4.0	5.0	4.0	2.2
T12	2.0	6.0	6.0	2.2	6.0
T13	4.0	2.0	4.0	2.2	2.4
T14	2.0	2.0	2.0	4.0	4.0
T15	3.0	5.0	3.0	2.0	6.0
T16	5.0	6.0	4.0	2.0	3.0
T17	3.0	2.0	4.0	6.0	5.0
T18	4.0	5.0	2.0	3.0	6.0
T19	7.0	2.0	5.0	3.0	5.0
T20	3.0	7.0	5.0	4.0	8.0
T21	5.0	5.0	3.0	7.0	2.2
T22	1.0	4.0	1.0	5.0	5.0
T23	2.0	1.0	2.0	2.0	4.0
T24	6.0	3.0	6.0	1.0	2.2

portions of acetone. The resulting extract was evaporated to dryness using a rotary vacuum evaporator. The residue was dissolved in 5 ml of ACN and the pesticides were determined as described above.

2.8. Safety

Normal laboratory procedures should be observed when handling volatile solvents, compressed gases, HPLC equipment and analytical standards of iprodione, procymidone, chlorothalonil, folpet and triazophos. Their acute oral medium lethal dose (LD_{50}) are 3500 $mg\ kg^{-1}$ for iprodione in rats, 6.8 $mg\ kg^{-1}$ for procymidone in female rats, 10 000 $mg\ kg^{-1}$ for chlorothalonil and folpet in rats and 57–68 $mg\ kg^{-1}$ for triazophos in rats depending on carrier and sex.

3. Results and discussion

The pesticides studied are highly absorbing substances in the UV region of the spectrum, with absorption maxima at 206 nm for iprodione, 207 nm for procymidone, 233 nm for chlorothalonil, 225 nm for folpet and 200 nm and 245 nm for triazophos (Fig. 1). The overlapping of spectra prevent the detection of each analyte at its absorption maximum wavelength by HPLC–DAD analysis.

Fig. 2a shows a spectrochromatogram of a mixture of iprodione, procymidone, chlorothalonil, folpet and triazophos and Fig. 2b a representative chromatogram at 220 nm from the same mixture. The simultaneous elution of the five component peaks under isocratic conditions can be observed. A satisfactory separation was not investigated in order to reduce the analysis time, avoiding the dispersion of signals or the time necessary for the regeneration of the system between analysis if gradient conditions are used.

With the aim of improving the analysis for these commonly used pesticides in environmental samples, PLS and principal component regression multivariate calibration methods were evaluated. To take advantage of the better information available for each analyte from HPLC–DAD, it is necessary to select the wavelength of maximal absorbance for each one.

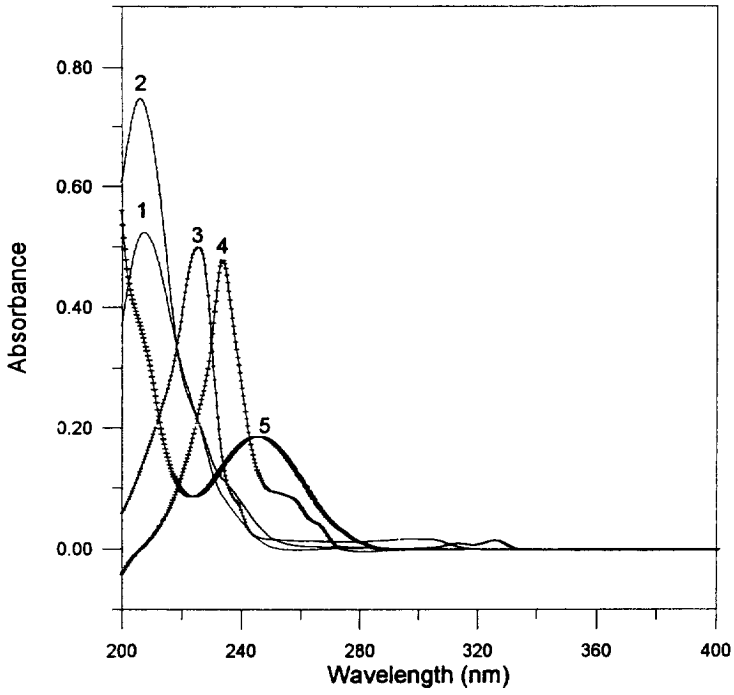

Fig. 1. Absorption spectra of: (1) 4 μg ml^{-1} of iprodione, (2) 5 μg ml^{-1} of procymidone, (3) 3 μg ml^{-1} of folpet, (4) 2 μg ml^{-1} of chlorothalonil and (5) 6 μg ml^{-1} of triazophos.

However, a well established practice, for simple multicomponent mixtures, involves the selection of a single compromise detector wavelength to develop calibration models. In this work 220 nm was selected to evaluate the PLS and principal component regression methods. A comparative study of the prediction capabilities of both chemometric approaches was undertaken.

3.1. Calibration

A training set of 24 samples was taken. The concentrations of all pesticides were between 0 and 8 μg ml^{-1}. The composition of the mixtures of the five pesticides used in the calibration matrix is summarized in Table 1.

The chromatographic region between 240 and 408 s was selected for analysis, because this is the zone with the maximum information from the mixture components under study. The number of factors was estimated by cross-validation [20,21] using the first predicted residual sum of squares (PRESS) value the F-ratio probability of which drops below 0.75, as Haaland and Thomas empirically determined [22].

The PRESS obtained by optimizing the calibration matrix with the PLS-1 method is shown in Fig. 3. The optimum number of factors was found to be 5 for iprodione and folpet, 9 for procymidone, 7 for chlorothalonil and 13 for triazophos. In PLS-2 and principal component regression methods, cross-validation was performed with respect to the number of factors affecting the prediction of all compounds simultaneously and 11 and 4 factors, respectively, were found.

A commonly used measure of the ability of different calibration models to predict concentrations in future samples is the root mean squared prediction error of cross-validation, RMSCV:

$$\mathrm{RMSCV}(n) = \left[\frac{1}{I}\sum_{i=1}^{I}[\hat{x}_i(n) - x_i]^2\right]^{0.5} = \left[\frac{\mathrm{PRESS}}{I}\right]^{0.5}$$

and another statistical parameter, closely related to

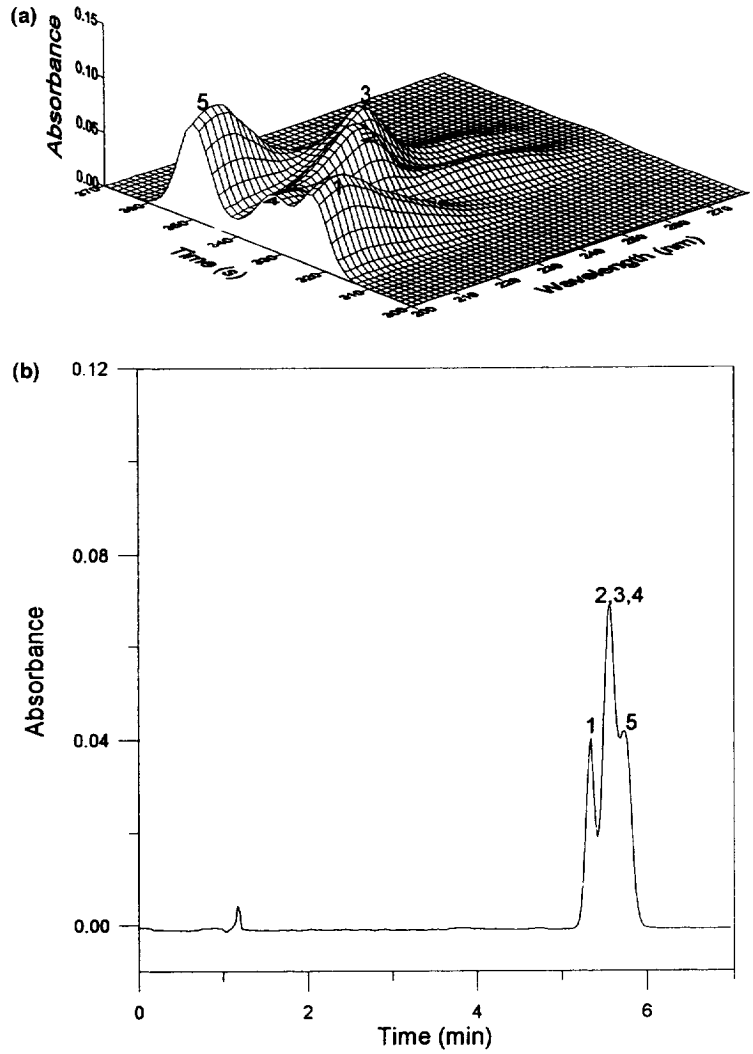

Fig. 2. (a) Isometric plot of a sample containing: (1) 4 μg ml^{-1} of iprodione, (2) 3 μg ml^{-1} of folpet, (3) 2 μg ml^{-1} of chlorothalonil, (4) 6 μg ml^{-1} of triazophos and (5) 5 μg ml^{-1} of procymidone. (b) Chromatogram at 220 nm of the same sample.

the above is the total error of prediction based on cross validation, ET, defined as

$$\mathrm{ET}(n) = \left[\sum_{i=1}^{I} [\hat{x}_i(n) - x_i]^2\right]^{0.5} = [\mathrm{PRESS}]^{0.5}$$

where I is the total number of calibration samples, $\hat{x}_i$(n) represents the estimated concentration of the ith component using a model of n factors and x_i is the reference concentration.

The statistical results obtained for PLS-1, PLS-2 and principal component regression methods are summarized in Table 2. It can be observed that in all cases the best RMSCV and ET values are obtained by PLS-1 method, although much too high results are obtained for triazophos. PLS-2 and principal component regression methods showed less precise results and therefore only the PLS-1 method was applied to the determination of the pesticides.

It is known that the quality of the results obtained in multicomponent analysis from extensively overlapping signals depends on the data set mode (nor-

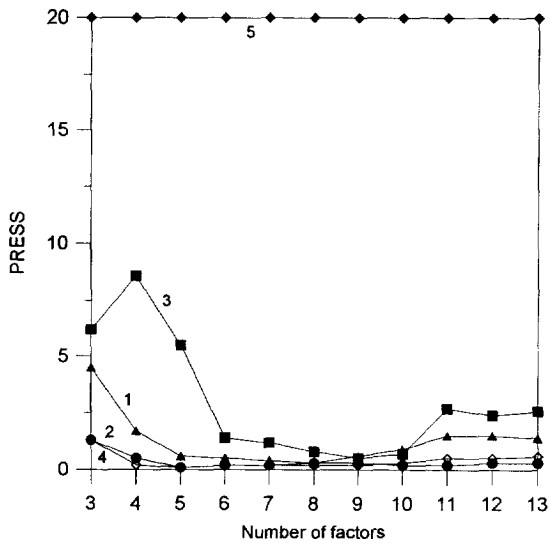

Fig. 3. Representation of PRESS values generated from the prediction of: (1) chlorothalonil, (2) iprodione, (3) procymidone, (4) folpet and (5) triazophos by the PLS-1 method, as a function of the number of factors used in the calibration for chromatogram data set at 220 nm.

mal or derivative) used [23,24]. The differentiation of the recorded data before quantification can be used to identify, estimate and remove unmodeled background constituents in routine analysis of non-cleaned-up samples. Also derivative techniques have been used to eliminate problems associated with baseline shifts and overlapping features.

With the aim of improving the results for triazophos the PLS-1 method was evaluated using the ^{1}D chromatograms of the mixtures. The values found for the RMSCV and ET showed that there is no significant difference between the precision of prediction for the PLS-1 models constructed with chromatograms or ^{1}D chromatograms (Table 2). Hence, the proposed PLS-1 models, at 220 nm, allowed the simultaneous determination of iprodione, procymidone, chlorothalonil and folpet, but did not allow the quantification of triazophos, probably owing to the low absorbance of this pesticide at the proposed wavelength.

Making use of the multi-wavelength information from DAD another wavelength, 200 nm, was selected in order to resolve triazophos. Again, PLS-1 calibration method was applied using two different data sets, chromatograms and ^{1}D chromatograms. The PRESS plot obtained by optimizing the calibration matrix of the chromatograms at 200 nm is shown in Fig. 4. The optimum number of factors, RMSCV and ET values for both PLS-1 models are summarized in Table 3. Analysis of the results revealed significantly more precise predictions for triazophos than with PLS-1 models evaluated at 220 nm. On the other hand, there were no significant differences between the precision of prediction for the PLS-1 models constructed at 200 nm with chromatograms or ^{1}D chromatograms. As expected the prediction ability of iprodione, procymidone, chlorothalonil and folpet was poor.

3.2. Determination of the pesticides in synthetic mixtures

The proposed PLS-1 models, applied to both chromatograms and ^{1}D chromatograms, allow the resolution of synthetic mixtures of the five components. In Table 4 the composition of the mixtures assayed and the recoveries obtained are shown. It can be observed that the results obtained by applica-

Table 2
Statistical parameters of the PLS-1, PLS-2 and principal component regression (PCR) methods with use of chromatogram data set and first-derivative chromatogram data set (^{1}D-PLS-1), at 220 nm

Component	PLS-1		^{1}D-PLS-1		PLS 2		PCR	
	RMSCV	ET	RMSCV	ET	RMSCV	ET	RMSCV	ET
Iprodione	0.0851 (5)	0.38	0.1296 (5)	0.58	0.0900 (11)	0.40	0.2960 (4)	1.32
Procymidone	0.1542 (9)	0.69	0.1214 (9)	0.54	0.1553 (11)	0.69	0.5431 (4)	2.43
Chlorothalonil	0.1428 (7)	0.69	0.1352 (8)	0.60	0.1852 (11)	0.83	0.6978 (4)	3.12
Folpet	0.0735 (5)	0.33	0.0862 (9)	0.38	0.0962 (11)	0.43	0.4637 (4)	2.07
Triazophos	1.4035 (13)	6.28	2.3573 (13)	10.54	1.4005 (11)	6.26	1.9796 (4)	8.85

Values in parentheses correspond to the optimum number of factors used for prediction.

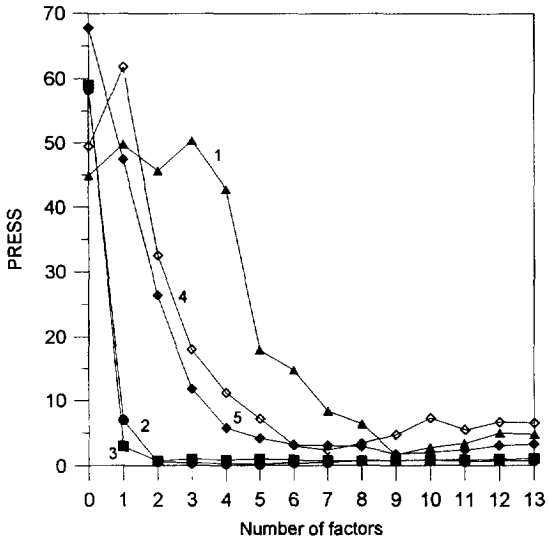

Fig. 4. Representation of PRESS values generated from the prediction of: (1) chlorothalonil, (2) iprodione, (3) procymidone, (4) folpet and (5) triazophos by the PLS-1 method, as a function of the number of factors used in the calibration for chromatogram data set at 200 nm.

tion of PLS-1 models and ^{1}D-PLS-1 models are not significantly different.

3.3. Applications

3.3.1. Determination of the pesticides in groundwater

The proposed models were applied to the determination of the pesticides in groundwater, as described in Section 2.6. Liquid–liquid extraction of the five pesticides was carried out prior to their determination. Samples were spiked at levels between 2 and 12 μg ml^{-1} and the recoveries were calculated. In this case more precise prediction were found by the application of ^{1}D-PLS-1 models. Table 5 shows the results obtained, with recoveries ranging from 85.0–115.0%.

Table 3
Statistical parameters of the PLS-1 method with use of chromatogram and first-derivative chromatogram data set, at 200 nm

Component	PLS-1		^{1}D-PLS-1	
	RMSCV	ET	RMSCV	ET
Iprodione	0.1175 (4)	0.50	0.1455 (5)	0.52
Procymidone	0.1963 (2)	0.88	0.1833 (7)	0.87
Chlorothalonil	0.2953 (9)	1.32	0.3420 (8)	1.38
Folpet	0.3930 (6)	1.76	0.4337 (6)	1.81
Triazophos	0.3012 (9)	1.35	0.4331 (8)	1.38

Values in parentheses correspond to the optimum number of factors used for prediction.

3.3.2. Determination of the pesticides in soil

The proposed PLS-1 models were applied to the determination of the pesticides in soils. A sample soil (AL-08) was collected in a greenhouse in Almería (Spain). Its characteristics and composition were described elsewhere [24]. Samples were spiked at levels between 0.3 and 1.2 mg kg^{-1}.

With complex matrices as soils, significant advantages were also found with the application of the differentiation technique. The composition and the percentage recoveries obtained are summarized in Table 6. It can be observed satisfactory recoveries ranged from 85.7–114.0%.

4. Conclusions

The paper describes a simple exploration of the possibilities of multivariate techniques in HPLC–DAD. The use of multi-wavelength detectors means that analytical precision can be improved because of the increased number of wavelengths which can be monitored. Such detectors in combination with several multivariate calibration methods can improve and facilitate the determination of complex multicomponent mixtures in many applications of HPLC.

In our particular study the simultaneous determination of the five pesticides with a single calibration model was not possible. Thus, iprodione, procymidone, chlorothalonil and folpet were analyzed with the PLS-1 calibration models evaluated at 220 nm, while triazophos was analysed with the other ones at 200 nm. Superior performance for the analysis carried out with the PLS-1 calibration method has been demonstrated, when comparing the statistical parameters with those found by applying the PLS-2 or principal component regression methods. PLS-1 seems to predict better than PLS-2 and principal component regression in cases when there are multiple components which overlapping peaks or random linear base lines [25].

On the other hand, we did not find significant

Table 4
Recoveries (%) obtained for the five pesticide system in synthetic mixtures by PLS-1 and ^{1}D-PLS-1 models

Added ($\mu g\ ml^{-1}$)					I (220 nm)		P (220 nm)		C (220 nm)		F (220 nm)		T (200 nm)	
I	P	C	F	T	PLS-1	^{1}D-PLS-1	PLS-1	^{1}D-PLS-1	PLS-1	^{1}D-PLS-1	PLS-1	^{1}D-PLS-1	PLS-1	^{1}D-PLS-1
3.5	1.0	4.0	3.5	6.0	98.9 (3.5)	98.6 (3.6)	93.3 (4.2)	100.7 (4.0)	102.7 (4.7)	102.0 (4.5)	100.0 (4.2)	99.7 (4.5)	97.2 (4.2)	97.3 (4.2)
6.0	5.5	4.3	2.2	4.2	101.3 (3.5)	102.2 (3.7)	101.1 (3.7)	104.4 (3.8)	103.0 (4.5)	104.6 (4.7)	101.2 (4.0)	113.2 (4.2)	112.1 (3.6)	112.1 (3.5)
2.0	2.5	1.5	6.0	3.0	103.6 (3.3)	93.5 (3.3)	90.8 (4.7)	92.0 (4.5)	115.3 (4.0)	132.7 (4.0)	97.3 (4.4)	99.0 (4.7)	97.7 (4.2)	99.0 (3.8)
4.4	6.0	3.0	6.0	5.5	97.7 (3.6)	96.6 (3.5)	94.0 (4.1)	96.5 (4.2)	114.0 (3.9)	115.7 (4.0)	91.8 (4.3)	98.3 (4.3)	100.2 (3.9)	101.4 (3.8)
1.5	2.5	1.5	4.0	2.0	90.0 (4.1)	83.3 (4.2)	92.4 (4.4)	91.2 (4.5)	114.3 (4.1)	128.7 (4.2)	98.9 (5.2)	90.5 (5.0)	97.0 (4.4)	98.5 (4.1)
2.4	4.5	2.0	2.0	5.0	97.5 (3.8)	98.3 (4.0)	96.4 (4.0)	99.3 (3.9)	104.0 (4.4)	108.0 (4.5)	99.5 (4.2)	110.5 (4.4)	97.8 (4.2)	98.8 (4.2)
3.0	5.0	4.4	4.5	4.5	114.0 (3.2)	115.3 (3.2)	108.2 (3.5)	111.8 (3.7)	111.1 (4.0)	114.0 (4.1)	102.7 (4.1)	109.1 (4.3)	101.8 (3.8)	100.6 (3.7)
2.0	6.0	3.0	3.5	3.5	92.0 (3.9)	89.0 (4.0)	90.7 (4.7)	87.7 (4.7)	99.7 (4.8)	106.0 (4.6)	103.0 (4.2)	97.7 (4.5)	90.0 (4.8)	91.7 (4.6)
6.0	2.0	5.0	1.0	7.0	92.0 (4.1)	93.0 (4.1)	103.6 (3.8)	113.5 (3.9)	94.0 (5.0)	90.0 (4.8)	115.7 (3.8)	110.0 (4.0)	95.4 (4.4)	95.1 (4.6)
2.0	2.0	5.0	5.0	3.0	93.0 (3.8)	87.4 (3.9)	115.0 (3.4)	119.4 (3.5)	96.0 (4.7)	94.7 (4.9)	95.6 (4.2)	97.8 (4.0)	98.4 (4.2)	98.7 (4.2)

I = Iprodione; P = Procymidone; C = Chlorothalonil; F = Folpet; T = Triazophos.

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

Table 5
Recoveries (%) obtained for the five pesticide system in groundwater by the proposed ^{1}D-PLS-1 models

Added ($\mu g\ l^{-1}$)					Recovery (%)				
I	P	C	F	T	I (220 nm)	P (220 nm)	C (220 nm)	F (220 nm)	T (200 nm)
6.0	4.0	12.0	8.0	10.0	95.4 (4.0)	99.2 (4.0)	96.3 (4.3)	90.2 (5.0)	102.8 (3.9)
2.0	8.0	2.0	12.0	4.0	109.0 (3.6)	98.3 (4.1)	111.5 (3.8)	97.8 (4.5)	109.0 (3.5)
8.0	10.0	8.0	10.0	8.0	99.2 (3.8)	101.2 (3.8)	98.5 (4.2)	99.0 (4.4)	108.0 (3.5)
4.0	4.0	6.0	6.0	10.0	106.5 (3.4)	105.5 (3.5)	108.7 (4.0)	93.0 (4.8)	109.5 (3.6)
10.0	8.0	8.0	8.0	2.0	95.2 (3.7)	104.0 (3.6)	95.0 (4.5)	97.0 (4.6)	100.8 (4.1)
6.0	6.0	4.0	10.0	10.0	106.3 (3.4)	85.0 (4.5)	92.5 (5.0)	98.7 (4.7)	113.2 (3.4)
12.0	4.0	8.0	4.0	12.0	96.0 (3.9)	115.0 (3.4)	97.7 (4.4)	93.0 (5.3)	104.5 (3.7)
8.0	4.0	4.0	6.0	8.0	92.2 (4.3)	106.5 (3.6)	91.5 (5.3)	104.0 (3.8)	85.2 (4.7)
8.0	2.0	2.0	8.0	4.0	86.0 (4.5)	115.3 (3.2)	90.4 (5.3)	104.0 (3.8)	96.3 (4.4)
10.0	12.0	8.0	2.0	8.0	98.6 (3.6)	95.5 (4.2)	92.7 (4.8)	96.5 (4.7)	96.7 (4.5)

I = Iprodione; P = Procymidone; C = Chlorothalonil; F = Folpet; T = Triazophos.
The results are averages of three determinations, with R.S.D. values in parentheses.

Table 6
Recoveries (%) obtained for the five pesticide systems in soil by the proposed ^{1}D-PLS-1 models

Added ($mg\ kg^{-1}$)					Recovery (%)				
I	P	C	F	T	I (220 nm)	P (220 nm)	C (22O nm)	F (220 nm)	T (200 nm)
0.8	0.6	1.0	0.5	0.8	93.0 (4.8)	95.7 (5.6)	94.8 (4.9)	87.2 (5.0)	100.7 (4.8)
0.6	0.4	0.8	0.6	0.6	90.0 (5.0)	92.0 (5.8)	89.7 (5.7)	88.3 (4.8)	102.0 (4.6)
1.2	0.8	0.6	0.7	0.8	87.3 (5.3)	100.7 (5.4)	102.7 (4.8)	110.0 (3.6)	112.0 (4.0)
0.4	1.0	0.4	0.8	0.7	107.0 (4.2)	101.4 (5.4)	101.5 (4.8)	95.5 (3.8)	93.1 (5.1)
0.9	0.6	1.1	1.0	0.6	99.8 (4.4)	110.7 (5.0)	91.6 (5.2)	97.4 (3.5)	98.6 (4.7)
0.4	0.6	0.4	0.8	0.4	106.5 (4.3)	98.0 (5.3)	95.0 (5.0)	87.7 (5.0)	111.5 (3.9)
0.6	0.3	0.7	0.8	0.6	93.7 (4.7)	114.0 (4.8)	90.0 (5.4)	90.2 (4.8)	110.3 (3.8)
0.7	0.8	0.6	0.6	1.2	95.4 (4.5)	97.5 (5.4)	107.0 (4.5)	95.7 (4.1)	85.7 (5.7)

I = Iprodione; P = Procymidone; C = Chlotnthalonil; F = Folpet; T = Triazophos.
The results are averages of three determinations, with R.S.D. values in parentheses.

differences in the predictions from chromatograms and ^{1}D chromatograms with the PLS-1 method in the synthetic mixtures studied. However, for groundwater and soil samples, the calculation of the ^{1}D as a prior step in the application of PLS-1 method led to more precise predictions than use of the chromatogram data set, in spite of the statistical analysis performed showing no significant differences in the RMSCV and ET values. In addition, ^{1}D models handled the existence of interferents that are unknown and not included in the calibration data in accordance with other authors' reports.

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