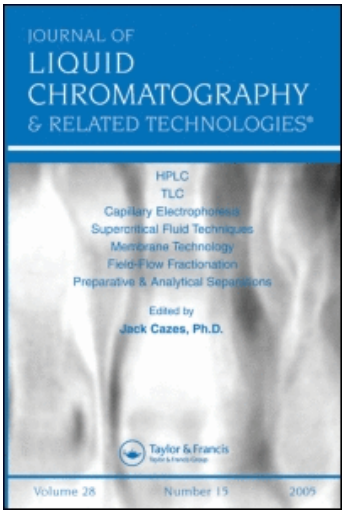

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RESOLUTION OF HPLC-DAD HIGHLY OVERLAPPING ANALYTICAL SIGNALS FOR QUANTITATION OF PESTICIDE MIXTURES IN GROUNDWATER AND SOIL USING MULTICOMPONENT ANALYSIS AND NEURAL NETWORKS

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ABSTRACT

Along with the development of hyphenated chromatography techniques, such as high performance liquid chromatography (HPLC) with diode array detection (DAD), three-dimensional data matrix for each sample can be easily obtained. In this paper, a comparative study of three methods using this type of data is presented. The capabilities of inverse calibration through ordinary least squares (OLS), partial least squares (PLS), and an artificial neural network (ANN) model, have been investigated for the

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simultaneous multicomponent analysis of synthetic mixtures of iprodione, procymidone, chlorothalonil, folpet, and triazophos when highly overlapping analytical signals are present.

Also, the methods have been satisfactorily applied to the determination of the pesticides in groundwater and soil samples, although the ANN gave the best results.

INTRODUCTION

The rapid development of multivariate data analysis techniques is. nowadays, a paramount aspect in analytical chemistry. Within these techniques, the use of multivariate calibration methods has been growing in a significant way and numerous applications are appearing in different instrumental methods of analysis.

Although most of them utilize data from one-order instruments, i.e., the variables are characterized by a vector of spectral measurements for each sample, with the great development of analytical instrumentation, second order data can be easily generated in analytical laboratories. Examples could be the output from a high performance liquid chromatography (HPLC) with multiwavelength detector, the output from a gas chromatography (GC) with mass spectroscopic detection, or an excitation emission fluorescence matrix. In this way, the development of methods that can be applied to this type of data has become mandatory.

Several advancements in higher order calibrations, especially those using second order data, have been cited in reviews.[1-4] Different algorithms[1-10] have been already proposed to estimate the concentration and spectral profile of each component and some analytical applications have begun to appear.[9, 11-15]

Recently, we have developed a new methodological approach[16-20] in order to extract greater analytical information from a complex three dimensional matrix, such as that generated by HPLC with diode array detector (DAD), obtaining new bidimensional signals, different from conventional spectra or chromatograms, with a lower degree of overlapping. The method allows the combination of chromatographic and spectral information, avoiding the use of a three-dimensional matrix to solve the problem.

In this paper, we present a comparative study of the capabilities of three methods that use all the information coming from a HPLC-DAD, when the analytical signals show a high degree of overlapping to develop reliable calibration models and, subsequently, to obtain accurate quantitative information. The methods under investigation are inverse linear regression based on the ordinary least squares with QR-Householder transformation (OLS-QRHT) and on the partial least squares with the general "nipals" algorithm (GPLS), as well as an artificial neural network (ANN) model, the Generalized Regression Neural Network (GRNN).

All three methods are applied for simultaneous multicomponent analysis with two-variable function data $A(\lambda, t)$ generated by HPLC-DAD. A reduction of the λ-t region, aimed to eliminate irrelevant A function data was made by adding up the chromatograms of the calibration set samples and visualizing it using the graphic facilities of the MATLAB system.[21] The reduced data matrix was then vectorized, collecting in one single row all the valuable information for each sample.

In the inverse statistical linear calibration with OLS, an orthogonal Householder transformation[22] is used for factorization of matrix **X** in the under determined linear system $\mathbf{X}\beta=\mathbf{Y}$ obtained, where **X** represents the matrix of absorbances vectorized, and **Y**, the concentration matrix.

In the inverse calibration with the GPLS,[23,24] an incomplete singular value decomposition is obtained through the "nipals" algorithm, which gives an approximate factorization of **X** for solving $\mathbf{X}\beta=\mathbf{Y}$.

A series of publications have summarized main applications of ANNs in Chemistry and, particularly, in Analytical Chemistry.[25-28] They have been applied to solve a wide array of problems including modeling non-linear calibration curves[29] and data reduction or mapping.[30] In addition, ANNs have been frequently used to tackle the problem of simultaneous identification and/or determination of chemical species when they present overlapped analytical signals.[31-34] All the last cited papers employ the Multilayer Feed Forward Neural Networks (MLFNN) with the back-propagation learning rule (BPLR)[25,35] as training algorithm.

In this ANN model, various parameters have to be optimized for reaching good results. The number of hidden layers (the most common architecture is one hidden layer), number of neurons in the hidden layers, transfer functions in the hidden and output units, learning rate and momentum term of the training procedure, are some of the parameters that have to be determined. Likewise, the BPLR is essentially a gradient based optimization method that takes place along an iteration process.

As a consequence of these facts, the price that should be paid in terms of time of searching of the adequate model is an important drawback of the MLFNN.

The GRNN was first published by Specht in 1991.[36] Based on probability density functions, the GRNN performed a regression that estimates the most probable curve of the observed data without assuming any particular form of the function. Its advantages have been summarized by Caudill.[37] In this sense, three main aspects should be mentioned: a) the architecture of the network is determined by the amount of the elements in the training set; b) only one parameter is necessary to determine, the "smoothing constant", σ, and, once it is selected, c) the training process is a single pass of the training set. In spite of its possibilities, after the publication of the original paper, only one application has been reported in the field of analytical chemistry.[38] Considering the features that exhibit the

GRNN, it was decided to study how it behaves as a calibration tool for quantitative estimation of analyte concentrations under the aforementioned conditions.

In this paper the three methods have been evaluated with a synthetic HPLC-DAD data set, corresponding to a complex mixture of five components with a high degree of overlapping. Finally, the methods have been applied to the simultaneous determination of five pesticides, in environmental groundwater and soil samples at $\mu g\ l^{-1}$ levels, after an extraction with methylene chloride and acetone, respectively.[39]

THEORY

Ordinary Least Squares Method

To solve the linear system $X\beta = Y$, $X_{n\times m}$ and $Y_{n\times p}$, using the least squares method, an estimated matrix $\hat{\beta}_{m\times p}$ of parameters must be found such that the j-th column $\hat{\beta}^{(j)}$ of $\hat{\beta}$ minimizes the sum of squares of the j-th residuals ($1\leq j\leq p$):

$$\left\| Y^{(j)} - X\hat{\beta}^{(j)} \right\|_2^2 = \min_{\beta^{(j)}} \sum_{i=1}^{n} \left[Y_{ij} - \sum_{k=1}^{m} X_{ik}\beta_{kj} \right]^2$$

If a QR-type factorization of X is obtained through a Householder transformation,[22] $X = QR$, then

$$\left\| Y^{(j)} - X\hat{\beta}^{(j)} \right\|_2^2 = \left\| Q' \left(Y^{(j)} - X\hat{\beta}^{(j)} \right) \right\|_2^2$$

and these factors are used to find the j-th minimum-length least squares solution $\hat{\beta}^{(j)}$, solving

$$R\hat{\beta}^{(j)} = Q'Y^{(j)},\ 1\leq j\leq p,$$

with R_{nxm} upper triangular and $Q' = Q_{nxn}$. The prediction of concentration $\bar{Y}$, for unknown samples will then be carried on using the product $X_{unk}\hat{\beta}$.

The ordinary least squares method is the best one when matrix **X** has full column rank (i.e., linearly independent columns), but the presence of collinearity is not so good because the variance of parameters $\hat{\beta}^{(j)}$ can be large. For this reason, other methods are preferred as, for example, the GPLS, which follows.

The General Partial Least Squares Method

If an incomplete singular value decomposition of **X** is obtained through the "nipals" algorithm[23,24]

$$X = USV' \approx s_1u_1v'_1 + s_2u_2v'_2 + ... + s_ru_rv'_r ,$$

where u_i, v_i: singular vectors of matrix X, s_i: dominant singular values of X and r: numerical or effective rank of X. This approximate factorization is used to find $\hat{\beta}$, and knowing $\hat{\beta}$, concentrations of unknown samples can be predicted in a similar way as before.

The Generalized Regression Neural Network

The GRNN is a neural network model formed by four neuron layers. The first one, the input layer, has as many neurons as the amount of elements in the input vector and it distributes the input vector to each of the neurons of the second one, the pattern layer. The input layer is fully connected to the pattern layer. The number of neurons of the pattern layer is given by the amount of samples in the training set. Each pattern layer unit will subtract the input of each training element from the corresponding weight. After this operation, it can be taken as either the square of these differences or the absolute values of the differences across all the weights.

The input function of the *j*th unit of the pattern layer will be

$$I_j = \Sigma \left| w_{ij} - x_{ij} \right| \quad \text{or} \quad I_j = \Sigma (w_{ij} - x_{ij})^2$$

where x_i are the input signals and w_{ij} are the connection weights between the *i*th input layer neuron and *j*th pattern layer neuron.

This net input is then passed through a nonlinear activation function, usually an exponential function of the form:

$$f(I_j) = \exp\left(-\frac{I_j}{2\sigma^2}\right)$$

Here σ is the smoothing constant. This parameter plays an important role for an accurate fit of the modeling function. The output of the pattern layer is transmitted to the summation layer. There are two kinds of summation neurons, A and B, that take different values depending on the particular application. The

pattern layer and the summation layer are also fully connected. When each neuron of the pattern layer corresponds to one sample in the training set the B weights are all set to one. The A weights are the expected outputs for each sample of the training set. The summation neurons, A and B, perform a dot product between the weight vector and the output signals from the pattern layer neurons. These products go to the neurons of the output layer where the A summation's neuron outputs are divided by the B summation's neuron outputs to give the network's output.

For the estimation of the optimum σ value, we searched the minimum error of the predictions using all but one sample of the training set to train the network with a given σ value and then we compared the output with the expected concentrations of the sample not included in the training set. This process is repeated for all the samples of the training set, which allow calculating an average prediction error for the given σ value. In this way, the optimization of the smoothing constant is carried out using the Fletcher-Reeves method.[40]

To asses the ability of a calibration model to predict concentrations in future samples, we commonly use the root mean squared prediction error, RMSEP, expressed as:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} \left(y_{ij} - \hat{y}_{ij}\right)^2}{n}}$$

where n is the total number of calibration samples; y_{ij} is the reference concentration of the *jth* component in the sample *i*, and y_{ij} represents the estimated concentration.

This error estimator is used to evaluate the fitness of the calibration methods.

EXPERIMENTAL

Chemicals and Solvents

Pesticide standards (Pestanal quality) of iprodione, procymidone, chlorothalonil, folpet, and triazophos, 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 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, 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 991 software were used.

The programs used for data processing were developed by the authors, implemented in Matlab 5.2, and run on a Pentium II with 64 MB RAM.

HPLC Procedure

HPLC separations were carried out using a RP-C_{18} 150 x 4 mm (5 μm particle size) column from E. Merck (Darmstadt, Germany). The mobile phase, under isocratic conditions, was AcN:water (70:30) v/v. This composition of the mobile phase was used to reduce the time of analysis and avoid the 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. Samples of 20 μL were injected with the solvent flow-rate maintained at 1 $mL.min^{-1}$.

Photometric detection was performed in the range 200 - 250 nm, with a spectral resolution of 1.4 nm. Data were 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 five pesticides was prepared, using a twenty four-sample set, in the range 0-8 $\mu g.mL^{-1}$. Volumes of 20 μL were injected into the HPLC system and the spectrochromatographic data were collected. The proposed methods were applied to analyze synthetic mixtures and to determine the concentrations of the pesticides.

Procedure for Determining 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 diluted with 1 mL of AcN and the pesticides were determined as described above.

Procedure for Determining 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 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.

RESULTS AND DISCUSSION

Figure 1 shows a spectrochromatogram corresponding to a mixture of iprodione, procymidone, chlorothalonil, folpet, and triazophos, using AcN:H_2O (70:30) v/v as the mobile phase. Great overlapping of the peaks under isocratic conditions can be observed. The analytical conditions were not changed in order to reduce the analysis time, to avoid the dispersion of signals, and to eliminate the time needed for regeneration of the column between analysis, if gradient conditions are used. This simultaneous elution of the pesticides does not permit the resolution of the mixtures by conventional chromatography.

On the other hand, it can also be noticed from Figure 1, that the pesticides are highly absorbing substances in the UV region of the spectrum with maxima absorption at close wavelengths, 200 and 245, 206, 207, 225, and 233 nm for triazophos, procymidone, iprodione, folpet, and chlorothalonil, respectively.

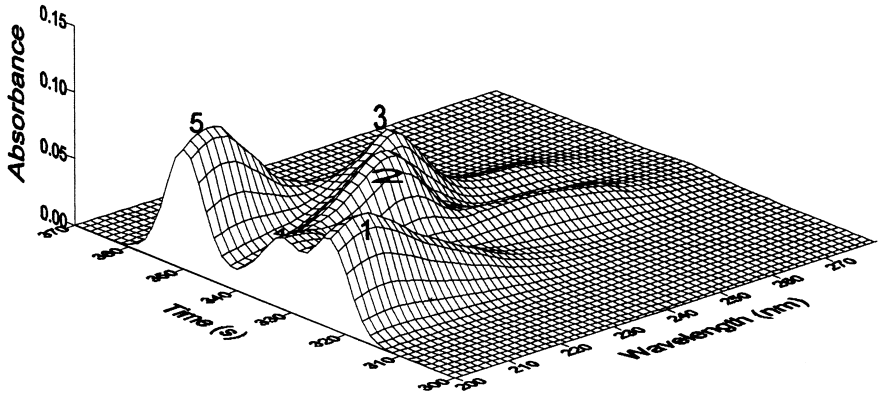

Figure 1. Isometric (A, λ, t) representation of the spectrochromatogram of a mixture of: (1) iprodione (4 μg mL^{-1}), (2) folpet (3 μg mL^{-1}), (3) chlorothalonil (2 μg mL^{-1}), (4) triazophos (6 μg mL^{-1}), and (5) procymidone (5 μg mL^{-1}).

Clearly, the common spectral features prevent discrimination by the traditional techniques of obtaining chromatograms at several wavelengths or absorption spectra at different retention times, to resolve this particular data set.

In consequence, one possible way of tackling the analysis of these pesticides, involves the selection of a single wavelength detector compromise, obtaining overlapped chromatograms, and then applying calibration methods, as PLS1. In a previous paper, two different wavelengths (200 and 220 nm) were selected to evaluate the PLS1 method,[39] but this implies that part of the information from HPLC-DAD is not used for the analysis. To take advantage of the best information, it would be necessary to build a calibration model at the wavelength of maximal absorbance for each analyte, i.e., more than one calibration model.

For solving the analytical problem using all the information enclosed in the collected A(λ,t) data matrix and with a single regression method, a transformation of the data was carried out. This consisted of converting the three-dimensional matrix into bi-dimensional, placing the chromatograms obtained at the different wavelengths in a single row for each sample.

In order to keep only the regions with the maximum information for the analysis, the chromatographic region between 315 and 360 s (which implies working with 46 variables) was selected for the analysis on the time domain, and between 200 and 274.4 nm (which implies to work with 53 variables) on the wavelength domain (Figure 2). So that, the X independent variable matrix had dimensions of 24 x 2438, i.e., 46 x 53 = 2438 columns by 24 rows (number of calibration samples). This matrix was used to apply the OLS-QRHT and GPLS methods.

In the case of the GRNN for building up the calibration model, the input information is composed of the chromatograms contained in the A(λ,t) matrix belonging to each sample ordered as a vector. The vector was formed using the same time and wavelength domain that was selected for the method explained before. To simplify the network and the data processing time, in the input vectors we only included half of the total amount of absorbance values by the elimination of alternate points. As a result of this process, the input vectors had 1218 elements each. No experiments were performed to evaluate the dependence of the RMSEP with the amount of the input information. The smoothing parameter was determined using the method previously described with the samples of the training set.

The training set is given in Table 1. A total of 24 samples were taken in the concentration range of 0.5-8 μg mL^{-1}, which is the linear response range for all the analytes.

The internal validation results obtained by the OLS-QRHT and GPLS methods are summarized in Table 2. The best prediction errors were obtained with the OLS-QRHT method, while the GRNN shows slightly better RMSEP values than the GPLS model. The RMSEP values for the GRNN illustrate the effec-

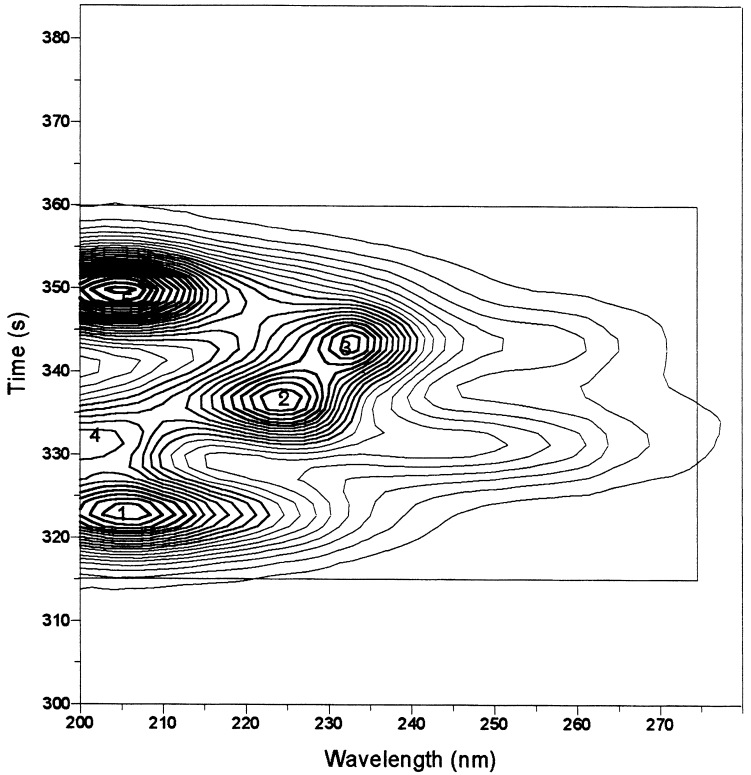

Figure 2. Contour plot representation of the above spectrochromatogram in which the region used in the analysis is marked.

tiveness of the artificial neural network for modeling the system. In a relatively easy and simple way, it was possible to obtain an optimal generalized regression surface.

On the other hand, the prediction error associated to each component will reflect how the given calibration method is capable of identifying and quantifying the particular chemical specie. In our conditions, as can be regarded in Table 2, no significant differences were observed among the errors associated with each component in each method. In addition, similar results were obtained applying the methods to the data obtained placing the spectra registered at the work time range in a single row for each sample, i.e., working at the wavelength domain.

According to the results, it should be pointed out that the three methods were effective for solving systems with high degree of overlapping analytical signals from three-dimensional data.

Table 1. Concentration Data of the Training Set for the Five Component System ($\mu g\ mL^{-1}$)

Sample	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

Table 2. Prediction Error of Internal Validation Using OLS-QRHT (*), GPLS (**) and GRNN (***) Methods

	RMSEP		
Component	*	**	***
Iprodione	$0.61\ 10^{-11}$	$1.2.10^{-1}$	$8.64\ 10^{-3}$
Procimidone	$0.12\ 10^{-10}$	$1.4.10^{-1}$	$8.81\ 10^{-3}$
Chlorothalonil	$0.89\ 10^{-11}$	$1.2.10^{-1}$	$9.85\ 10^{-3}$
Folpet	$0.55\ 10^{-11}$	$1.1.10^{-1}$	$6.49\ 10^{-3}$
Triazophos	$0.43\ 10^{-11}$	$5.8.10^{-1}$	$8.90\ 10^{-3}$

Predictions in Synthetic Mixtures

To check the validity of the proposed models, a set of synthetic mixtures of the five pesticides was prepared. In Table 3, the composition of the mixtures studied is shown. The predictions (expressed as percentage of the expected concentrations) and the precision of the measurements (expressed as relative standard deviation, RSD) obtained by the methods under study are given in Table 4. It can be seen, that the GRNN is better than the other three methods. It can be observed, that the worst predictions with the OLS-QRHT and the GPLS methods, were obtained in samples with the lower analyte concentrations, i.e., V5 for iprodione or V1 for procymidone, while GRNN performed adequately in all cases including these more problematical samples.

Table 3. Concentration Data of the Validation, Groundwater, and Soil Sample Sets for the Five Pesticide System

Sample	Iprodione	Procymidone	Chorothalonil	Folpet	Triazophos
Validation Set (mg mL-1)					
V1	3.5	1.5	4	3.5	6
V2	6	5.5	4.3	2.2	4.2
V3	2	2.5	1.5	6	3
V4	4.4	6	3	6	5.5
V5	1.5	2.5	1.5	4	2
V6	2.4	4.5	2	2	5
V7	3	5	4.4	4.5	4.5
Groundwater Set (mg l-1)					
G1	6	6	12	8	10
G2	8	10	8	10	8
G3	4	4	6	6	10
G4	10	8	8	8	2
G5	6	6	4	10	10
Soil Set (mg l-1)					
S1	4	3	5	2	5
S2	3	2	4	3	3
S3	6	4	3	4	4
S4	2	6	2	4	3
S5	5	3	6	5	3
S6	2	4	3	6	4
S7	3	2	5	5	3
S8	4	5	3	3	6

Table 4. Recoveries Obtained for the Five Pesticides System in Synthetic Mixtures by the ILS-Q RHT (*), GPLS (**) and GRNN (***)

	I			P			C			F			T		
Sample	*	**	***	*	**	***	*	**	***	*	**	***	*	**	***
V1	102.1	91.8	99.1	146.1	64.8	100.0	103.2	96.9	98.5	100.2	100.5	98.5	101.2	95.5	99.6
	(4.1)	(3.8)	(3.0)	(7.9)	(8.1)	(4.2)	(4.0)	(3.8)	(3.6)	(3.3)	(3.0)	(2.9)	(4.3)	(4.5)	(3.2)
V2	100.2	98.9	100.0	95.2	97.5	98.5	96.0	105.2	98.8	92.1	98.7	99.0	97.3	114.2	98.8
	(3.5)	(3.7)	(2.8)	(3.9)	(3.2)	(2.5)	(3.8)	(4.2)	(2.1)	(5.9)	(4.5)	(3.3)	(5.1)	(6.3)	(2.6)
V3	83.5	95.3	99.0	98.4	100.2	100.4	93.4	104.3	100.5	99.3	102.5	100.5	93.4	68.1	99.6
	(6.3)	(6.0)	(4.2)	(4.1)	(3.8)	(2.2)	(6.3)	(6.6)	(2.5)	(4.8)	(4.9)	(3.5)	(7.1)	(6.8)	(3.4)
V4	107.0	91.7	98.6	107.3	90.2	100.5	110.0	99.1	98.6	104.4	95.3	99.7	107.5	80.4	100.3
	(3.1)	(3.3)	(2.4)	(3.8)	(3.9)	(2.0)	(4.7)	(4.2)	(3.1)	(5.1)	(4.5)	(3.8)	(3.9)	(4.8)	(2.5)
V5	128.1	90.5	99.2	118.2	92.6	99.6	133.1	99.2	100.0	110.4	92.7	99.2	106.2	64.9	99.5
	(6.9)	(6.1)	(3.9)	(6.5)	(6.2)	(3.5)	(7.5)	(7.1)	(2.4)	(4.9)	(5.3)	(3.7)	(5.0)	(6.3)	(3.9)
V6	97.4	98.1	98.5	95.1	96.2	98.7	95.2	102.4	99.2	89.1	98.9	99.7	103.1	89.1	98.2
	(4.3)	(3.8)	(3.0)	(4.2)	(3.9)	(2.5)	(5.0)	(4.6)	(2.8)	(6.3)	(5.8)	(2.9)	(4.5)	(5.6)	(2.0)
V7	92.2	114.1	101.1	93.0	103.1	100.1	90.5	111.4	99.7	94.3	104.8	100.2	91.4	93.5	99.3
	(3.5)	(4.5)	(2.7)	(3.9)	(4.2)	(3.1)	(5.1)	(5.5)	(3.5)	(4.2)	(4.7)	(3.4)	(4.8)	(4.2)	(2.3)

I = Iprodione; P= Procymidone; C = Chlorothalonil, F = Folpet; T = Triazophos; RSD in parenthesis (n=3)

Determination of the Pesticides in Groundwater

The proposed methods were also applied to the determination of the pesticides in groundwater, as was described under Experimental. In Tables 3 and 5, the composition of the mixtures assayed and the recoveries, as well as RSD values obtained, are respectively shown. In all instances, the best results were found with the GRNN, as in the synthetic mixtures. In general, the OLS-QRHT and the GPLS methods did not obtain significant differences, although, for the procymidone component better recoveries were found with the last one.

Determination of the Pesticides in Soil

The methods were also applied to the determination of the pesticides in soil samples, as was described under Experimental. In Tables 3 and 6, the composition of the mixtures assayed and the recoveries (and RSD values) obtained are shown respectively. The best of all results, as in the previous application, were obtained with the GRNN. Now, there are more differences between the predictions obtained with the GRNN with respect to the ones obtained by the OLS-QRHT and GPLS methods. These methods presented significant bad predictions when the pesticides were present at low concentration samples. So, the more predictive capacity of the GRNN over the other two methods in the analysis of complex soil samples is evident.

In short, despite the fact that the best internal validation errors were obtained by the OLS-QRHT method in the analysis of both real and synthetic samples, the best results were found with the GRNN. Perhaps, this fact could be explained considering the multicollinearity appearing in the vectorized absorbance matrix, i.e., the variance of the $\hat{\beta}^{(j)}$ parameters can be very large, and this is not, therefore, advisable for good predictions.

On the other hand, the RMSEP values of the internal validation showed that the proposed methods offer smaller prediction errors than the PLS1[36] and, besides this, they allow the simultaneous determination of the five pesticides with a single calibration model, whereas this was not possible when the PLS1 method was applied using a single wavelength signal.

CONCLUSIONS

In this paper, the OLS-QRHT and GPLS methods and the GRNN were applied, prior to an adequate transformation of three-dimensional data, for the simultaneous determination of five pesticides with overlapped chromatographic and spectral signals.

Table 5. Recoveries Obtained for the Five Pesticide System in Groundwater by the OLS-QRHT (*), GPLS (**) and GRNN(***)

	I			P			C			F			T		
Sample	*	**	***	*	**	***	*	**	**	*	**	***	*	**	***
G1	91.1	104.5	97.6	90.1	97.5	98.1	101.3	89.7	99.2	91.1	111.2	99.3	103.3	95.2	99.1
	(6.3)	(5.2)	(3.1)	(5.3)	(4.7)	(3.8)	(4.2)	(4.5)	(3.5)	(4.1)	(3.2)	(2.6)	(5.2)	(4.7)	(2.5)
G2	102.3	96.1	99.3	86.4	88.5	98.4	95.2	92.4	98.2	114.3	86.8	99.5	106.4	88.3	99.9
	(5.1)	(5.3)	(3.3)	(6.2)	(6.5)	(3.2)	(3.5)	(4.3)	(2.9)	(3.8)	(4.9)	(3.0)	(4.7)	(4.9)	(3.2)
G3	85.5	106.2	98.7	91.3	92.7	98.4	97.4	86.1	98.8	109.2	93.8	99.1	114.1	86.9	99.7
	(6.5)	(6.0)	(2.9)	(6.6)	(5.9)	(3.8)	(5.3)	(4.9)	(3.4)	(5.3)	(6.0)	(3.4)	(5.4)	(5.0)	(3.8)
G4	96.2	95.5	98.5	72.2	91.9	97.4	94.1	88.3	97.8	94.5	98.9	97.4	101.3	112.3	98.3
	(4.1)	(4.7)	(3.3)	(5.2)	(4.7)	(3.5)	(4.3)	(4.8)	(3.1)	(4.2)	(3.6)	(2.5)	(5.5)	(4.9)	(3.3)
G5	80.3	115.3	98.8	62.5	99.4	99.6	85.3	90.5	99.3	90.1	106.7	99.7	100.2	97.1	99.1
	(4.8)	(4.2)	(3.1)	(6.0)	(6.3)	(3.0)	(5.2)	(4.7)	(2.8)	(3.9)	(4.3)	(2.8)	(3.9)	(4.1)	(3.5)

I = Iprodione; P= Procymidone; C = Chlorothalonil, F = Folpet; T = Triazophos; RSD in parenthesis (n=3).

Table 6. Recoveries Obtained for the Five Pesticide System in Soil by the OLS-QRHT (*), GPLS (**) and GRNN (***)

	I			P			C			F			T		
Sample	*	**	***	*	**	***	*	**	***	*	**	***	*	**	***
S1	83.6	91.2	98.2	71.2	83.3	98.9	96.3	77.6	99.5	72.2	127.2	95.8	112.3	84.9	98.7
	(7.5)	(8.0)	(4.3)	(8.4)	(7.5)	(4.9)	(6.4)	(7.5)	(3.8)	(9.4)	(8.8)	(4.3)	(7.0)	(7.5)	(3.8)
S2	91.1	84.8	100.9	70.3	81.3	103.0	114.4	72.4	102.0	101.2	95.5	101.6	126.3	88.5	102.5
	(6.8)	(7.2)	(3.2)	(9.2)	(8.1)	(3.7)	(7.1)	(6.7)	(4.0)	(8.5)	(9.6)	(4.8)	(9.2)	(9.5)	(4.5)
S3	92.2	85.2	101.1	60.5	81.6	103.1	90.5	72.5	100.9	88.3	87.4	104.1	82.1	94.6	100.8
	(5.4)	(4.3)	(3.1)	(8.4)	(9.5)	(4.8)	(8.5)	(9.2)	(3.3)	(7.5)	(8.2)	(3.9)	(6.7)	(7.8)	(3.9)
S4	117.3	90.8	101.7	93.5	78.9	100.1	135.2	78.6	100.0	110.2	89.6	100.2	125.5	66.6	101.8
	(6.3)	(5.2)	(4.0)	(6.4)	(7.0)	(4.1)	(9.3)	(8.4)	(3.5)	(6.0)	(7.1)	(4.2)	(7.9)	(8.1)	(4.2)
S5	98.5	85.6	100.6	74.4	82.7	104.6	107.3	78.3	101.6	99.4	98.7	100.1	119.2	95.5	102.9
	(7.0)	(7.3)	(3.7)	(9.5)	(8.5)	(3.8)	(8.0)	(8.4)	(4.3)	(7.4)	(8.0)	(4.4)	(8.8)	(9.3)	(3.8)
S6	110.2	87.6	99.4	106.2	65.5	99.7	162.1	60.5	99.9	141.5	71.9	99.9	162.3	63.8	98.8
	(9.1)	(8.3)	(4.2)	(8.0)	(8.6)	(4.2)	(9.2)	(7.4)	(5.2)	(8.5)	(7.0)	(3.8)	(9.5)	(8.8)	(4.3)
S7	105.1	72.9	97.4	82.3	65.7	98.5	151.0	53.5	98.7	129.4	73.1	98.3	199.2	61.4	99.7
	(7.8)	(8.2)	(3.6)	(9.6)	(9.7)	(4.9)	(8.2)	(9.4)	(5.5)	(8.8)	(7.4)	(3.5)	(9.9)	(8.7)	(4.0)
S8	111.4	75.5	99.1	106.5	68.7	99.5	129.4	65.1	98.9	100.2	86.1	99.8	143.0	60.3	98.4
	(5.9)	(6.3)	(3.5)	(6.8)	(8.5)	(3.6)	(7.5)	(8.4)	(5.8)	(6.5)	(7.2)	(4.0)	(7.6)	(8.9)	(4.2)

I = Iprodione; P= Procymidone; C = Chlorothalonil, F = Folpet; T = Triazophos; RSD in parenthesis (n=3)

Although the OLS-QRHT method has the minimum RMSEP for the calibration set when applications in synthetic mixtures, groundwater, and soil samples were carried out, the GRNN was the method with the best performance in the particular conditions in which the experiments were done. Differences are particularly important in groundwater and soil samples due to the complexity of the systems. The GRNN method was demonstrated to be a flexible and powerful model for handling strong overlapped data with advantages in comparison with the other techniques.

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