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Simultaneous fluorimetric determination of glyphosate and its metabolite, aminomethylphosphonic acid, in water, previous derivatization with NBD-Cl and by partial least squares calibration (PLS)

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Abstract

The reactions of 4-chloro-7-nitrobenzofurazan (NBD-Cl) with glyphosate (GLY) and with its main metabolite, aminomethylphosphonic acid (AMPA), have been studied. The resolution of binary mixtures of glyphosate and aminomethylphosphonic acid has been accomplished by partial least squares (PLS) multivariate calibration. The method of determination is based on the fluorescence emission of the derivatives formed in presence of NBD-Cl at 90 °C, in methanol and in basic medium. The dynamic ranges of the methods were comprised between 10 and 150 $\mu g\,l^{-1}$ for GLY and between 10 and 200 $\mu g\,l^{-1}$ for AMPA, being the detection limits 2 and 5.4 $\mu g\,l^{-1}$ for GLY and AMPA, respectively. The total luminiscence information of the derivatives has been used to optimize the spectral data set to perform the calibration, by analysis of the three-dimensional excitation–emission matrices. A comparison between the predictive ability of the multivariate calibration method, partial least squares type 1 (PLS-1), on two spectral data sets, emission and synchronous spectra, has been performed. The PLS-1 method, applied to the emission spectra, has been selected as optimum. The proposed method has been applied to the simultaneous determination of GLY and AMPA in river water. For concentrations ranging from 100 to 600 $\mu g\,l^{-1}$ of each compound in the samples, analytical recoveries range from 83 to 94% for GLY and from 104 to 120% for AMPA.

Keywords: Glyphosate; Aminomethylphosphonic acid; NBD-Cl; Spectrofluorimetry; PLS-1

1. Introduction

Glyphosate (*N*-(phosphonomethyl)glycine) (GLY) is a widely used, broad-spectrum, foliar-applied herbicide for vegetation control, introduced by Monsanto Company, US in the early 1970s. It is absorbed into the leaves and translocated through the plant to the roots and rhizomes via the phloem. The most common uses include control of broadleaf weeds and grasses in: hay/pasture, soybeans, field corn, ornamentals, lawns, turf, greenhouses, rights-of-way. It is known that glyphosate is rapidly degraded into aminomethylposphonic acid (AMPA). The physical, chemical and toxicological properties of GLY, have been well-documented. It is resistant to volatilisation and sunlight-mediated degradation. It is a very polar and amphoteric compound with pK_a values of 0.78, 2.29, 5.96, and 10.98. Both, GLY and AMPA are well-soluble in water, poor absorbents, and they are not fluorescent. These features make that existing analytical methodology always includes a derivatization step, prior to instrumental analysis.

A very complete revision about the proposed methods for the determination of GLY and AMPA, has been recently published [1]. The majority of the methods for their determination involved the employment of gas or liquid chromatographic techniques which require derivatization of the analytes. By HPLC, three different procedures of derivatization are generally used: (a) post-column ninhydryn derivatization and UV detection [2,3]; (b) post-column fluorogenic labeling with *o*-phtalaldehyde (OPA) and fluorescence detection [4–7], and (c) pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) and fluorescence detection [8–11]. A method based on a pre-column

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derivatization with 4-chloro-7-nitrobenzofurazan (NBD-Cl) has been proposed [12].

In the GC methods, the derivatization reactions are in order to conveniently render less polar and sufficiently volatile derivative to be chromatographed. Several derivatization reactions have been used such as: *O*- and *N*-silylation with MTBSTFA [13]; *N*-acylation with chloroformates, followed by *P*- and *C*-esterification by means of diazomethane [14,15]; simultaneous *N*-acylation and *O*-esterification in presence of perfluorinated anhydrides and halogenated alcohols and/or by treatment with acetic acid and trimethyl orthoacetate (TMOA) [16–18]; derivatization to *N*-acylaminoalkanephosphonates with trifluoroacetic acid-trifluoroacetic anhydride and TMOA [19].

The physical–chemical properties of these compounds may be suitable for their determination by CE methods. Several methods, with photometric detection (direct or indirect) at wavelengths near at 240 nm, have been proposed [20–23]. Methods with indirect fluorescence [24] and laser-induced fluorescence [25] detection, have been also proposed. Other proposed methods have used hyphenated techniques of CE interfaced with mass spectrometry [21,26] and with an electrospray condensation nucleation light scattering detector (ESI–CNLSD) [27].

Other type of methods, as enzyme-linked immunosorbent assays (ELISA), have been used for the determination of this herbicide. Two of the methods compare the obtained results with HPLC data [28,29] and other method proposes a novel linker-assisted enzyme-linked immunosorbent assay [30].

On the other hand, in the recent years, several multicomponent analysis methods, based on luminiscence spectral properties, have been demonstrated as powerful tools in diverse analysis fields [31]. Partial least squares (PLS) methods have been applied to resolve complex mixtures of organic compounds by using conventional, derivative, and also kinetic, fluorimetric signals [32,33]. Lindberg et al. [34] applied, for the first time, PLS calibration to emission fluorescence data for the determination of humic acid and lignin-sulfonate. More recently, we reported on the resolution of ternary mixtures of salicylic acid and two of its main urinary metabolites, by PLS [35,36], and on the analysis of binary mixtures of salicylic acid and diflunisal in serum samples, by combination of synchronous fluorimetry with PLS calibration [37].

We have not found determination methods of these herbicides based on the spectrofluorimetric analysis.

In the present work, the fluorescence properties of the derivatives formed by reaction of NBD-Cl with glyphosate and with aminomethylphosphonic acid (AMPA), have been studied. The previous studies have as objective the developing of a sensitive and selective procedure for the simultaneous determination of binary mixtures composed by glyphosate and aminomethylphosphonic acid by means of previous derivatization with NBD-Cl and by combination of fluorescence emission spectra of the derivatives and partial least squares type 1 (PLS-1) multivariate calibration.

2. Experimental

2.1. Apparatus

Fluorescence measurements were made on an SLM Aminco-Bowman, Series 2, spectrofluorimeter, equipped with a 150 W continuous Xenon lamp, interfaced by a GPIB card and driver with a Pentium PC microcomputer. Data acquisition and data analysis were performed by the use of AB2 software, Version 5.00, running under Windows 98. The excitation and emission slits were 4 nm for both. The scan rate of the monochromators was maintained at $10\,\mathrm{nm\,s^{-1}}$. All measurements were made at $10.0 \pm 0.1\,^{\circ}\mathrm{C}$, by use of a thermostatically controlled cell holder, and a Selecta Model 382 thermostatically controlled water-bath.

2.2. Softwares

The GRAMS-386 Level I Version 2.0 software package, with the PLS plus Version 2.1G application software [38], were used for the statistical treatment of the data, and the application of the PLS and PCR factor analysis based multivariate calibration methods. The spectra, acquired with the Series 2 luminescence instrument, were converted to ASCII XY format with the converter included in the AB2 software, and imported to the GRAMS 386 program through the included ASCII XY converter. A converter program, running in BASIC, developed by us, was used to transform the bi-dimensional files, in ACSII XY, in the format appropriate to the software package SURFER for Windows [39]. This software was used to obtain the three-dimensional excitation–emission matrices presented as contour plots. The contour plots, in the two dimensions of excitation and emission, are generated by linking points of equal fluorescence intensity to form the contour map.

2.3. Chemicals

All experiments were performed with analytical-reagent grade chemicals. Purified liquid chromatographic grade water (Milli-Q system) was used. Glyphosate was obtained from Greyhound Chromatography and Allied Chemicals (UK), aminomethylphosphonic acid from Sigma–Aldrich Chemical (Spain), 4-chloro-7-nitrobenzofurazan was obtained from Across Organics and sodium tetraborate decahydrate (borax) from Aldrich (Spain). All products were used as received.

Stock standard solutions, $100\,\mu\mathrm{g\,ml^{-1}}$, of GLY and AMPA were prepared in water. A 3.80×10^{-3} M stock solution of NBD-Cl was prepared in methanol. Solutions of lower concentrations were prepared by appropriate dilution of the stock solutions. A 0.2 M solution of borax, pH 9.5, was used.

2.4. General procedure for the individual fluorescent determination of glyphosate and aminomethylphosponic acid, previous derivatization with NBD-Cl

Transfer a suitable aliquot containing 0.10–1.60 μg of glyphosate or aminomethylphosphonic acid into a 10 ml calibrated flask and add desionized water to complete 1 ml. Add 1 ml of 0.2 M solution of borax, pH 9.5 and 7 ml of 3.8×10^{-3} M methanolic solution of NBD-Cl. Heat the solution at 90 °C during 5 min, cool in ice-water, add 0.6 ml of HCl 12 M and dilute to the mark with methanol. Measure the fluorescent intensity at 549 nm, with excitation at 468 nm for the determination of glyphosate and at 545 nm with excitation at 478 nm for the determination of aminomethylphosphonic acid, against a reagent blank prepared in a similar way but without herbicides.

2.5. Procedure for the simultaneous determination of GLY and AMPA by PLS-1 calibration in river water

In a 10 ml volumetric flask, introduce 1 ml of water containing between 0.225 and 1.5 μg of glyphosate and of AMPA, add 1 ml of 0.2 M borax solution, pH 9.5, and 7 ml of 3.8×10^{-3} M methanolic solution of NBD-Cl. Heat the solution at 90 °C during 5 min, cool in ice-water, add 0.6 ml of HCl 12 M and dilute to the mark with methanol. Record the emission spectrum between 490 and 700 nm with λ_{ex} of 475 nm. Use the emission spectra as the analytical signal to make the calibration. Perform the PLS-1 calibration with a calibration set of 10 samples, containing between 0.225 and 1.50 μg of both analytes in 1 ml of river water, following a central composite design. Apply the optimized calibration matrix, calculated by application of the PLS-1 method, to analyze the emission spectra of the problem samples, and determine the concentrations of GLY and AMPA in river water samples.

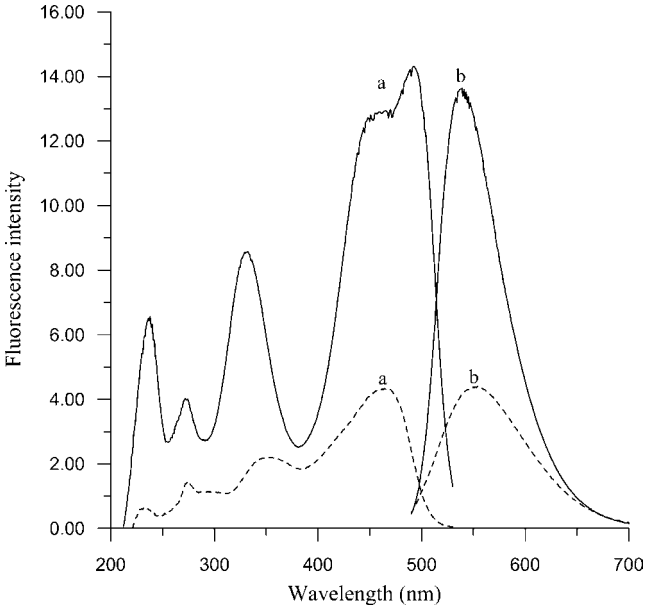
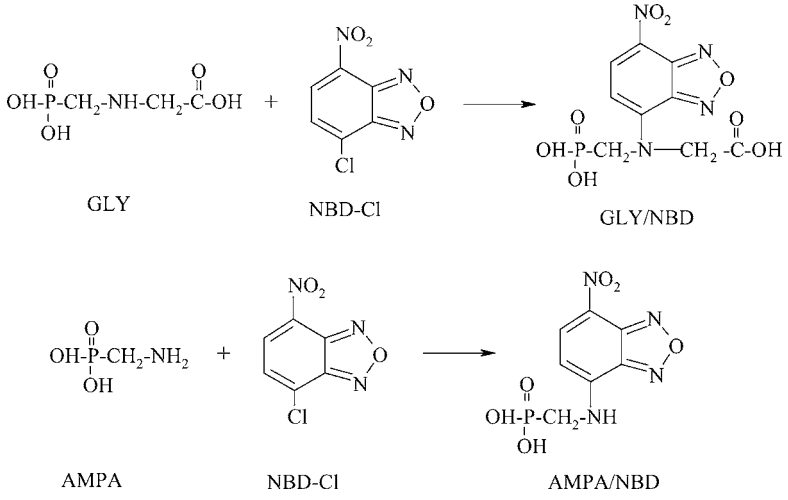


Fig. 1. Fluorescence excitation (a) ($\lambda_{em} = 549$ nm) and emission spectra and (b) ($\lambda_{ex} = 468$ nm) of the GLY/NBD reaction product and of the NBD-Cl alone.

3. Results and discussion

Glyphosate and its main metabolite, aminomethylphosphonic acid, do not present native fluorescence and it is necessary a derivatization reaction to their determination. These analytes, in methanolic and basic medium, react with 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) giving rise to fluorescent derivatives (Scheme 1). The excitation and emission spectra obtained for samples of both GLY/NBD and NBD-Cl, treated as described in Section 2.4, are shown in Fig. 1. The excitation spectrum of the derivative GLY/NBD shows maxima located at 221, 353, and 466 nm, and the emission spectrum shows only a maximum



Scheme 1.

located at 549 nm. The longest excitation wavelength has been used for all the spectrofluorimetric studies, because the emission intensity obtained is higher, so the wavelengths chosen for the measurements were 468 and 549 nm, for excitation and emission, respectively. In the excitation spectra of a solution of NBD-Cl, we can observe maxima located at 221, 353, and 455 nm. The emission spectrum shows only one maximum at the same wavelength that the derivative product. For AMPA/NBD derivative, similar spectra have been obtained and the wavelengths chosen for the measurements were 478 and 545 nm for excitation and emission, respectively.

3.1. Optimization of chemical and physical conditions for the derivatization of GLY and AMPA in presence of NBD-Cl

3.1.1. Influence of methanol

The methanol concentration in the medium was varied between 40 and 80%. The fluorescence intensity increases with the percentage of methanol in all of the range assayed, for both derivatives. A 74% of methanol was therefore chosen as the optimum for better compatibility for the determination of these compounds in river water samples.

3.1.2. Influence of NBD-Cl concentration

The effect of NBD-Cl concentration on the fluorescence intensity of the derivative products was studied in the range between 3.5×10^{-5} and 2.6×10^{-3} M. The fluorescence intensity, of both derivatives, remains constant between 1.0×10^{-3} and 2.5×10^{-3} M. Hence, a 2.5×10^{-3} M NBD-Cl concentration was chosen as the optimum to ensure that a sufficient excess of reagent was present. This was taken in 7 ml of a 3.8×10^{-3} M solution of NBD-Cl, prepared in methanol, to maintain the previously selected percentage of methanol.

It is noticeable that NBD-Cl shows fluorescence in these conditions against to bibliographic data [40], whilst their fluorescence is weaker than in basic medium.

3.1.3. Influence of heating time

The formation of the fluorescence products between GLY or AMPA and NBD-Cl depends of the reaction temperature. The effect of the heating time was studied at 70 and 90 °C. In both cases, the fluorescence intensity increases with increasing the heating time. At 70 °C, they are necessary 25 min for the formation of the fluorescent derivatives. When derivatization reactions take place at 90 °C they are completed in only 5 min. These conditions were selected as optima.

3.1.4. Influence of HCl concentration

Before the addition of HCl, the reaction mixture was cooled in ice-water to stop the reaction. The addition of HCl, after cooling the mixture, has as objective to increase the fluorescence signal of the derivative products because these products are non-fluorescent in basic media. The HCl concentration in the medium was varied between 0 and 0.96 M. The fluorescence intensity reaches a maximum, and is independent of the hydrochloric acid concentration, between 0.24 and 0.72 M. A concentration of 0.72 M (0.6 ml of HCl 12 M) was therefore chosen as optimum.

Table 1
Analytical and statistical parameters for the fluorimetric determination of GLY and AMPA previous derivatization with NBD-Cl

	GLY	AMPA
Dynamic range ($\mu g\,l^{-1}$)	10–150	10–200
Slope, m	0.09278	0.08293
Intercept, b	0.0371	0.464
Standard deviation of slope, S_m	3.35×10^{-4}	1.17×10^{-3}
Standard deviation of intercept, S_b	0.0289	0.1423
Regression standard deviation, $S_{y/x}$	0.0961	0.3938
Coefficient regression, R	0.9999	0.9981
Percentage error, $n = 11$	0.14	0.20
LOD[a] ($\mu g\,l^{-1}$)	2.0	5.4
Analytical sensibility[b], $S_{y/x}/m$ ($\mu g\,l^{-1}$)	1.03	4.75

[a] Clayton et al. [41].
[b] Cuadros et al. [42].

3.1.5. Influence of temperature and stability of the products

The dependence of the fluorescence intensity of the derivative products on the temperature is critical. The fluorescence emission decreases as the temperature increases and a constant temperature of 10 °C is recommended.

The derivative products are stable, at least, 60 min.

3.1.6. Analytical parameters

Under the optimum operating conditions selected, there is a satisfactory linear relationship between fluorescence intensity and GLY or AMPA concentrations in the range 10–200 ng ml^{-1}. The calibration graphs were obtained by preparing samples by triplicate with increasing concentrations of each analyte. Analytical characteristics of the determination of GLY and AMPA are summarized in Table 1, which also includes the detection limits calculated from the standard deviation values of slope and origin intercept, and choosing a false-positive and a false-negative probability value of 0.05 [41]. For a series of 11 measurements on 100 ng ml^{-1} of GLY or AMPA, relative errors of 0.14 and 0.20% and standard deviations of 0.328 and 0.268, respectively, were obtained (95%).

4. Optimization of the procedure for the simultaneous determination of glyphosate and aminomethylphosphonic acid by PLS-1

The spectra of both derivatives, GLY/NBD and AMPA/NBD, are highly overlapped and the resolution of mixtures of these compounds is accomplished by a multivariate calibration technique, such as partial least squares type 1. To perform the determination of binary mixtures, all samples are prepared according with Section 2.

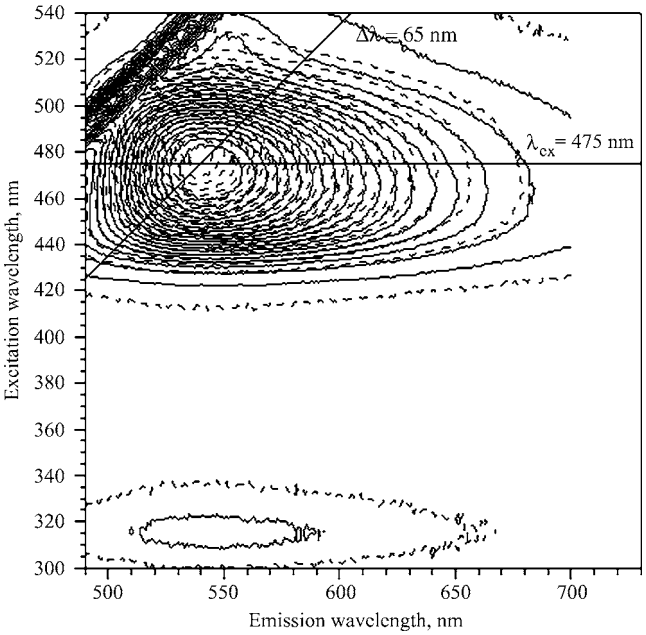


Fig. 2. Contours plots of the total fluorescence spectra of GLY/NBD (—) and AMPA/NBD (- - -) derivatives. The selected paths for scanning the emission ($\lambda_{ex} = 475$ nm) and synchronous ($\Delta\lambda = 65$ nm) spectra are shown by the solid lines slicing the data matrix.

4.1. Contour plots

The overlap between spectra can be better examined by collecting a total luminescence spectrum of each one of the compounds, in the form of an emission–excitation matrix (EEM). The EEMs were collected by scanning the emission spectrum, between 490 and 730 nm, at increments of the excitation wavelength of 4 nm, between 312 and 552 nm, and displayed as contour plots. Fig. 2 shows the contour plots corresponding to the derivatives GLY/NBD and AMPA/NBD. The spectral data sets selected are represented in the contour plots as solid lines slicing the data matrix. They were selected as compromise values to pass as close as it is possible of the maxima of the two compounds of the mixture. The emission spectra were recorded maintaining constantly an excitation wavelength of 475 nm, and the synchronous spectra described by a 45° cut in the excitation–emission matrix were scanned, maintaining a constant interval between the emission and excitation wavelengths $\Delta\lambda = \Delta\lambda_{em} - \lambda_{ex} = 65$ nm, with the objective to pass around the maximum of both compounds without considerable loss of sensitivity.

4.2. Calibration matrix and selection of the spectral zones for the analysis

The two different data sets selected, the emission spectra and the synchronous spectra, were evaluated to perform the determination of the mixture by PLS-1, and to establish a quantitative model for the system, 10 calibration samples, containing different amounts of GLY and AMPA, were prepared as described in Section 2.5. The concentration of each compound was varied between 0.00 and 150 $\mu g\,l^{-1}$, through the calibration matrix (Table 2).

Table 2
Composition of the different mixtures used in the calibration set for the determination of GLY and AMPA by PLS-1 method

Calibration set	GLY ($\mu g\,l^{-1}$)	AMPA ($\mu g\,l^{-1}$)
M1	22.5	22.5
M2	22.5	125
M3	125	22.5
M4	125	125
M5	75	0
M6	75	150
M7	0	75
M8	150	75
M9	75	75
M10	0	0

The only pre-processing applied to the data was mean-centering. This pretreatment involves calculating the average spectrum of all training spectra, and subtracting this result from every spectrum. In addition, the mean concentration value for each component is calculated and subtracted from the concentration of every samples. This removes any offset from the data and tends to scale the data such that the mathematics of the spectral decomposition perform better.

The spectral regions between 490 and 710 nm for the emission spectra, and between 350/415 and 560/625 nm for the synchronous spectra, were selected for the analysis, because these are the zones with the maximum spectral information for the mixture components. This implies working with 216 and 211 experimental points per spectra, respectively, as the spectra were digitized each nanometer.

4.3. Selection of the optimum number of factors and calibration

The selection of the number of principal components or factors, to be used in the calibration with the PLS algorithm, is very important in order to achieve the best predictions for to model the system without overfitting the concentration data. The cross-validation procedure was applied. Cross-validation consists of systematically removing one of the observations in turn, and using only the remaining observations for construction of latent factors, and regression on the latent factors. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample, and the predicted error sum of squares (PRESS) was calculated. The PRESS was calculated in the same manner, each time a new factor was added to the PLS model. To select the optimum number of factors, the criterion proposed by Haaland and Thomas was used [43,44]. The model selected is that with the fewest number of factors such that PRESS for that model is not significantly greater than PRESS from the model which yielded

Table 3
Statistical parameters of the PLS-1 method using the two spectral data sets

Component	Emission			Synchronous		
	Factors	RMSD	R^2	Factors	RMSD	R^2
GLY	2	6.95	0.9857	5	8.85	0.971
AMPA	2	7.46	0.9822	3	14.26	0.924

the minimum PRESS. The significance level is attributed by using an F test ($\alpha = 0.25$). When using the emission spectra the optimum number of factors were two for GLY and for AMPA and with the synchronous spectra data set, the optimum number of factors were five for GLY and three for AMPA.

Once the optimum number of PLS-1 factors is determined, it is necessary to perform the final calibration, using all the calibration samples with the optimal number of factors.

4.4. Statistical parameters

Known concentrations of all tested samples, included in the calibration matrix, were compared with the predicted concentrations by cross-validation, for the two spectral data sets employed. The values of the root mean square difference (RMSD), which is an estimate of the absolute error of prediction by cross-validation for each component in the calibration matrix, are summarized in Table 3.

In addition, the values of the determination coefficient (R^2), obtained when plots of actual versus predicted concentration were constructed, are also included in Table 3.

From the cross-validation method and in accordance with the statistical data obtained, it is evident that the synchronous spectra data set cannot be used for the quantification of AMPA, as the calculated RMSD values are very high, specially for AMPA, and the obtained R^2 values indicate a poor relationship between actual and predicted concentrations for the two compounds. The better results for the prediction were obtained when using the emission spectral data set as the analytical signals. In consequence, this spectral data set was selected for the application of the method to river water samples.

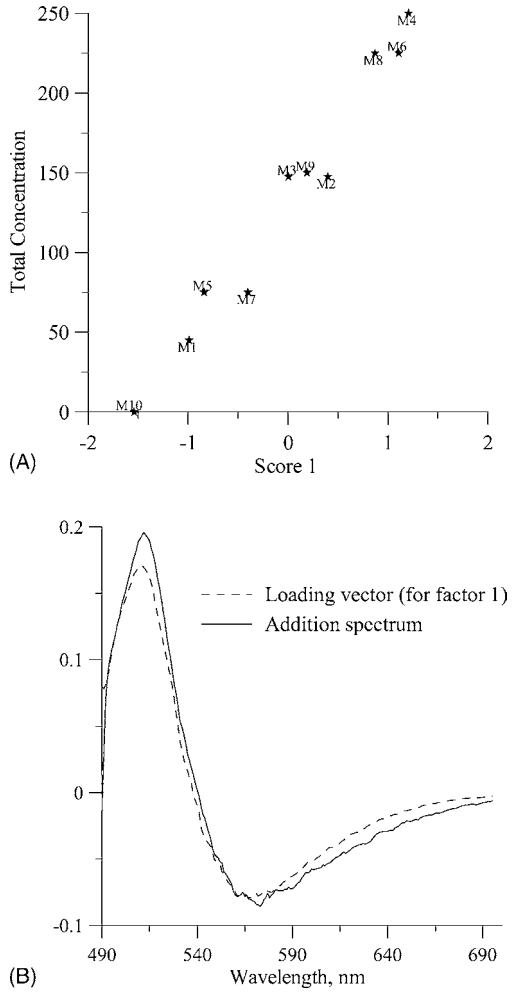


Fig. 3. (A) Plot of score 1 vs. total concentration of both analytes. (B) Loading spectrum for the first factor and the addition spectrum obtained by the addition of the spectrum of each component.

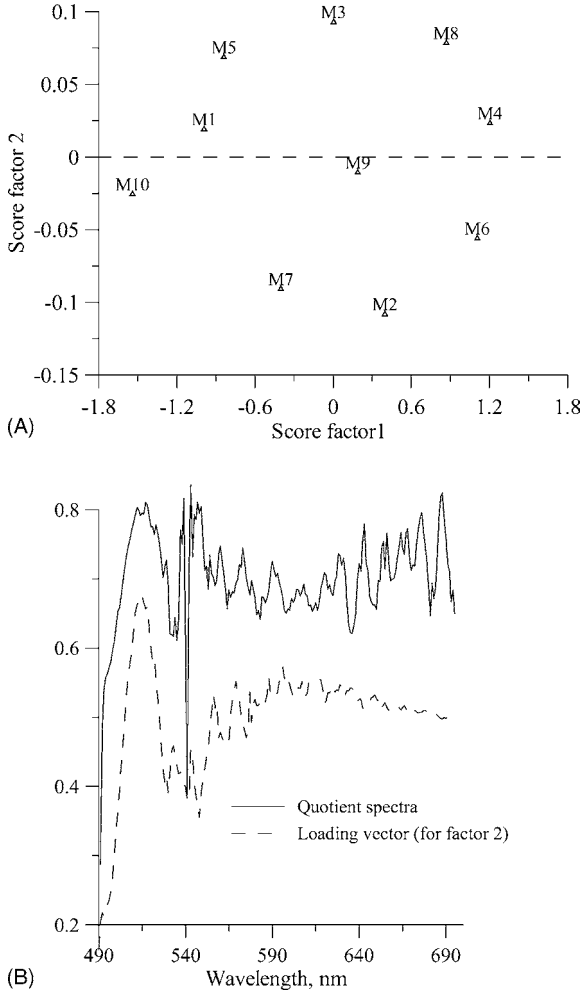


Fig. 4. (A) Plot of score 2 vs. score 1, showing the position of the 10 calibration mixtures (Table 1). (B) Loading spectrum for the second factor 2 and the quotient spectrum between the spectra of both components.

Table 4
Recovery of GLY and AMPA added to river water by PLS-1 calibration

Added ($\mu g\,l^{-1}$)	Found ($\mu g\,l^{-1}$)*				
GLY	AMPA	GLY	% Recovery ± R.S.D.**	AMPA	% Recovery ± R.S.D.**
250	500	226	90.4 ± 3.5	597.5	119.5 ± 4.5
400	400	374	93.6 ± 1.0	416	104.2 ± 2.3
108	602	101	93.9 ± 1.5	669	111.1 ± 6.1
300	500	251	83.7 ± 1.4	547.5	109.5 ± 2.8

* Mean of three determinations.
** R.S.D.: relative standard deviation.

Fig. 3A shows a plot of concentration vs. score 1, obtained with emission spectra. The increase in the score 1 is consistent with an increase in the over all concentration of both components. The spectral variation with the overall concentration was accounted for by this first PLS factor, as we can see in Fig. 3B, where is represented the first loading vector and the addition spectra of the two components and the two are indistinguishable.

Fig. 4A shows a plot of score 2 vs. score 1 for the calibration samples. As can be seen samples with a ratio GLY/AMPA > 1 (M3, M5, M8), present positive values of score 2 and the samples with a ratio of GLY/AMPA < 1 (M2, M6, M7), present negative values of this score. Samples with similar concentrations (M1, M4, M9, M10) present a zero value. Score 2 probably reflect the relation with the GLY/AMPA concentration ratio and it increases with the increase of this ratio. This fact is in accordance with the analysis of the loading vector for factor 2 and the quotient spectra between the individual component, Fig. 4B.

5. Application of the method to river water samples

Initial experiences, devoted to the determination of the analytes in river water samples, they were carried out by utilizing the same previously described model, to predict the concentration of these in fortified river water samples. The analysis of the river water unfortified does not reveal the presence or GLY or AMPA. However, results were not completely satisfactory as consequence, probably, of a quenching effect and we decided to construct another model, based on the use of a calibration set in which river water, without analytes, was present in the samples.

For the recovery experiments, river water samples (Guadiana river, Badajoz, Spain) previously filtered through a 0.45 μm nylon filter, were spiked with different quantities of GLY and AMPA, in such a form that their concentrations were in the range 100–600 $\mu g\,l^{-1}$. Aliquots of 1.0 ml were taken and the proposed method was then applied as described in Section 2.5. The recovery percentages found by application of the PLS-1 calibration method are shown in Table 4. Three spectra of three different samples, at the same concentration, were used for each concentration level included in the table. Analytical recoveries range from 83 to 94% for GLY and from 104 to 120% for AMPA.

Therefore, the utilization of the proposed procedure for the analysis of AMPA and GLY in river water samples, would require to collect samples river water samples to monitorize at different periods of time during the year. Once the absence of AMPA and GLY is proved, these samples would be joined to obtain a pool of river water that, adequately stored, could be used in the later analysis.

6. Conclusions

The results reported here support the formation of derivatives between GLY or AMPA with NBD-Cl in methanolic and in basic medium. These derivative products present fluorescence in acidic medium. The variables influencing the formation of the fluorescence products have been studied. The dynamic ranges of the methods were comprised between 10 and 150 $\mu g\,l^{-1}$ for GLY and between 10 and 200 $\mu g\,l^{-1}$ for AMPA, and the detection limits are 2 and 5.4 $\mu g\,l^{-1}$ for GLY and AMPA, respectively.

The results obtained in this work allow us to conclude that both components of the binary mixture are accurately determined by fluorescence spectroscopy, previous derivatization with NBD-Cl and in combination with PLS-1 calibration. The proposed method allows the direct determination of both analytes simultaneously, without needing a previous separation step. However, the obtained sensitivity is nor sufficient in regulatory practice but the derivatization reaction is very selective and very rapid. In only 5 min, the reaction is completed against 1 h that it is necessary in the method proposed by Colin et al. [12] and the total analysis time per sample is drastically reduced to 10 min. Also, the proposed method is simple with relatively low cost in comparison with the proposed methods by HPLC or GC.

The total luminescence information contained in the three-dimensional excitation–emission matrices allows the selection of the most suitable spectral data set, to perform the multivariate calibration. When comparing the results obtained by PLS-1 with two calibration sets, emission and synchronous spectra, the spectral data set composed of the emission spectrum was found to be the optimum path for the quantification of GLY and AMPA. River water samples

containing different quantities of GLY and AMPA, were analyzed by using the proposed PLS-1 multivariate calibration method, and the recoveries are very acceptable.

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