ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 837–844

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Simultaneous spectrofluorimetric determination of (acetyl)salicylic acid, codeine and pyridoxine in pharmaceutical preparations using partial least-squares multivariate calibration

N. Ramos Martos [a], A. Molina Díaz [a,*], A. Navalón [b], I. De Orbe Payá [b], L.F. Capitán Vallvey [b]

[a] *Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, E-23071 Jaén, Spain*
[b] *Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain*

Received 31 December 1999; received in revised form 7 April 2000; accepted 27 April 2000

Abstract

A partial least-squares calibration (PLS) method for the simultaneous spectrofluorimetric determination of salicylic acid (SA), codeine (CO) and pyridoxine (PY) is proposed. The determination of SA, CO, and PY has been carried out in mixtures of up to three components by recording the emission fluorescence spectra between 300 and 500 nm ($\lambda_{exc} = 220$ nm). Due to the fact of the strong spectral overlap among the excitation and also among the emission spectra of these compounds, a previous separation should be carried out in order to determine them by conventional spectrofluorimetric methodologies. Here, a full-spectrum multivariate calibration PLS method is developed. The experimental calibration matrix was constructed with 14 samples. The concentration ranges considered were 0.1–2.0 (SA), 0.25–3.0 (CO) and 0.10–2.0 (PY) $mg \cdot l^{-1}$. The optimum number of factors was selected by using the cross-validation method. The method also allows the simultaneous determination of acetylsalicylic acid (ASA), CO and PY by previous alkaline hydrolysis of ASA to SA. To check the accuracy of the proposed method, it was applied to the determination of these compounds in synthetic mixtures and in pharmaceuticals.

Keywords: (Acetyl)salicylic acid; Codeine; Pyridoxine; Fluorimetry; Partial least-squares calibration; Multicomponent analysis

1. Introduction

Fluorescence spectroscopy is widely used in different fields of the chemical analysis owing to its high sensitivity and selectivity, and relativity low cost. However, as is usual in other spectroscopic

* Corresponding author. Tel.: +34-95-3212147; fax: +34-95-3212141.
E-mail address: amolina@ujaen.es (A. Molina Díaz).

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00388-5

techniques, when analyzing mixtures of components that show overlapping spectra, they often cannot be successfully resolved and a previous separation step is usually required. As a consequence, analysis costs are increased and the procedure moreover, becomes more time consuming.

In recent years multicomponent analysis has become an important tool in resolution of mixtures of components in many different fields including biomedical, clinical, environmental and drugs analysis. Increasing attention has been paid in the last few years to multivariate calibration methods, specially to those using the partial least squares (PLS) method with decomposition into latent variables [1–3]. The basic concept PLS regression was originally developed for Wold [4] and its first chemical application carried out by Wold et al. [5]. PLS has been used in combination with various spectroscopic techniques such as UV-VIS [6–9] (including UV optosensing devices [10,11]), NIR [12,13] and fluorescence spectroscopy [14,15]. Assuming a linear relationship between concentration and fluorescence intensity, PLS approaches have been successfully applied in the quantitative analysis of multicomponent mixtures that cannot be resolved by conventional spectrofluorimetry [16–18].

Some vitamins of the B-group (e.g. thiamine and pyridoxine) are found along with analgesics or central nervous system stimulants in pharmaceuticals. Therefore, a rapid, one step procedure allowing the determination of ternary mixtures, as weel as binary ones is useful in pharmaceutical analysis.

PY, ASA and CO are respectively, representative active principles of these groups of compounds; all of them showing native fluorescence. In this paper, a rapid and very simple procedure for determination of ternary an binary mixtures of SA, CO and PY is developed based in PLS-1 regression applied to their intrinsic fluorescence spectra. In addition, the procedure also allows indirectly the determination of ASA, CO and PY mixtures previous hydrolysis of ASA to SA.

The method was validated by application to resolution of synthetic samples of SA, CO and PY as well to the resolution of ASA, CO and PY in pharmaceutical preparations. Quite good results were found in all cases.

2. Experimental

2.1. Reagents

Stock solutions with 100 mg l^{-1} of Salicylic acid (Fluka, Madrid, Spain), Codeine (Abelló, Barcelona, Spain) and Pyridoxine hydrochloride (Fluka, Madrid, Spain) were prepared by dissolution of the appropriate amount in deionized water. The stock solution of vitamin was stored at 0°C. At this temperature it is stable at least for one week. CO and SA were stable for at least one month at 5°C. Working solutions were daily prepared by suitable dilution. A NaH_2PO_4/Na_2HPO_4 buffer solution pH 7.0 at $C_t = 0.01$ M was also employed. All solutions were prepared in deionized water.

2.2. Apparatus

Spectrofluorimetric measurements were made on a Perkin-Elmer LS 50 spectrofluorimeter (Beaconsfields, Buckinghamshire, UK) equipped with a xenon discharge lamp (20 kw), Monk–Gillieson mono chromators, a Quantic Rhodamine 101 counter to correct the excitation spectra and a Gated photomultiplier. The luminescence spectrometer was connected via an RS232C interface to a MITAC 386 mpc 3000F-computer running Fluorescence Data Manager (from Perkin Elmer) v. 2.50 software for controlling the instrument. The computer was also connected to an Epson LX-800 printer for delivery of results. The excitation and emission slits were both maintained at 5 nm and the scan rate of the monochromators at 240 nm min^{-1}. All measurements were made with a quartz cell of 1 cm pathlength at 20°C, the temperature was controlled to within ± 0.1°C with aid of a Selecta (Barcelona, Spain) Frigiterm 6000 382 thermostat.

A pH Crison 2000 digital pH meter (Barcelona, Spain) furnished with a combined glass/ saturated calomel electrode was used for pH measurements. The pH meter was calibrated with two buffers at pH 4.00 and 7.02. An Ultrasons Selecta ultrasonic bath (Barcelona, Spain) was also employed.

2.3. Software

Excitation and emission spectra were acquired and processed by using the spectrofluorimeter's bundled software. The Grams/386, version 2.02 level 1 and Grams/32 version 4.0 software package, with the PLS plus/IQ application [19], was used for the statistical treatment of the data and the application of the PLS multivariate calibration method. The digitized spectra of samples and standards acquired from the spectrofluorimeter were converted by the software to its own format.

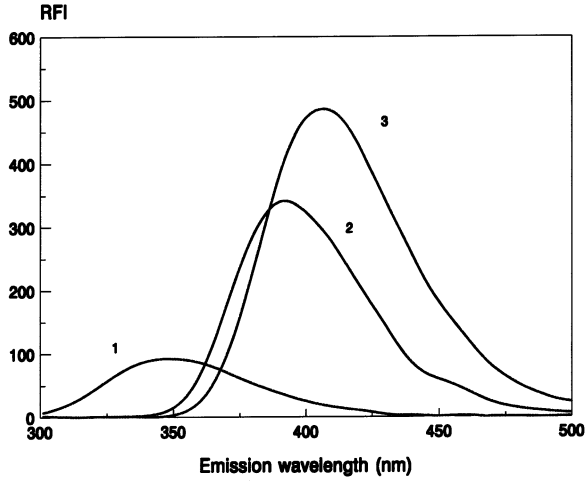

Fig. 1. Fluorescence emission ($\lambda_{exc} = 220$ nm) spectra: CO 2.00 mg l^{-1} (1), PY 0.45 mg l^{-1} (2) and SA 0.25 mg l^{-1} (3). pH = 7.0 (NaH_2PO_4/Na_2HPO_4 $C_t = 0.01$ M buffer). (RFI, relative fluorescence intensity).

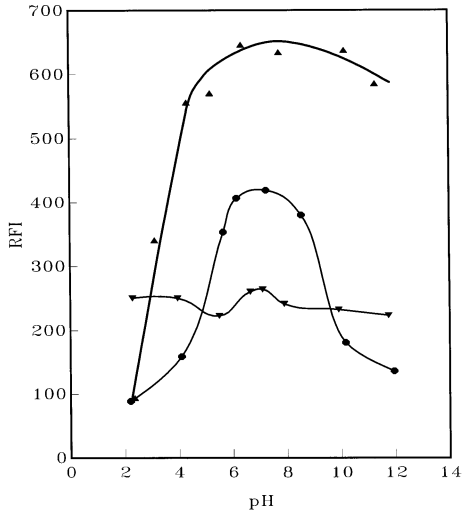

Fig. 2. Effect of the pH: CO (-▼-) 5 mg l^{-1}, PY (-●-) 2 mg l^{-1} and SA (-▲-) 1 mg l^{-1}. (RFI: relative fluorescence intensity).

2.4. Treatment of samples

The content of either capsule, accurately transferred and the tablets were crushed to a fine powder and dissolved in water by sonication, the solution was filtered through a 0.45 μm pore size Millipore filter, and diluted to an appropriate volume with deionized water. In order to obtain the complete transformation to SA, a previous alkaline hydrolysis as usually was performed in those pharmaceuticals containing ASA, by means of a sample treatment with 1 M NaOH solution, by heating 30 min to 60°C.

2.5. Procedure

SA, CO and PY binary and ternary mixtures, were prepared as follows: 10 ml phosphate buffer solution at pH 7.0 were placed into a 100 ml volumetric flask and appropriate volumes of the drugs standard solutions were then added and mixed, making finally to the mark with deionized water. The final concentrations were in the ranges 0.10–2.00 mg l^{-1} SA, 0.25–3.00 mg l^{-1} CO and 0.10–2.00 mg l^{-1} PY. The excitation and emission slit widths were 5 nm. The mixed solutions were thermostated at 20 ± 0.1°C and their emission spectra (300–500 nm) recorded at $\lambda_{exc} = 220$ nm and processed with the aid of the spectrofluorumeter's bundled software. The optimized calibration model for PLS-1 method was applied to the spectra of the samples to calculate the concentration of each chemical in the mixture.

3. Results and discussion

The spectral region for emission spectrum between 300 and 500 nm was recorded for the following excitation wavelengths: 215, 220, 225, 230, 240, 250, 260 and 293 nm. SA and PY show highly overlapping fluorescence maxima at 405

Table 1
Calibration matrix

Sample	Salicylic acid (mg l^{-1})	Codeine (mg l^{-1})	Pyridoxine (mg $^{-1}$)
1	–	1.00	1.00
2	–	0.25	1.00
3	0.25	0.25	–
4	1.00	–	1.00
5	0.50	–	1.00
6	0.25	–	1.00
7	2.00	1.00	0.10
8	0.10	2.00	0.25
9	1.00	0.50	0.50
10	0.25	0.25	2.00
11	0.50	2.00	0.50
12	0.10	1.00	0.25
13	0.25	3.00	0.10
14	0.50	2.00	1.00

Table 2
Selection of the optimum number of factors

Number factors	Salicylic acid		Codeine		Pyridoxine	
	PRESS	*P*	PRESS	*P*	PRESS	*P*
1	0.57308	0.9999	8.00737	0.9999	1.91018	0.9999
2	0.07385	0.9995	1.56206	0.9999	0.24338	0.9992
3	0.00775	0.5000	0.03436	0.5000	0.03893	0.6784
4	0.01392	0.0000	0.05326	0.0000	0.03061	0.5216
5	0.01080	0.0000	0.10821	0.0000	0.03769	0.6585
6	0.00945	0.0000	0.17156	0.0000	0.03746	0.6546
7	0.00948	0.0000	0.16278	0.0000	0.03029	0.5144
8	0.00849	0.0000	0.16064	0.0000	0.02983	0.5043

and 395 nm, respectively. The overlapping between the spectrum of codeine and those ones from the other two analytes is lower. Therefore, conventional spectrofluorimetry cannot be applied satisfactorily to the determination of the mixture. We selected 220 nm as optimum wavelength that is more appropriate for the signal from codeine, which shows lower fluorescence than the other two analytes.

It should be noted, moreover, that in all commercial pharmaceutical preparations in which binary mixture of these analytes are found, codeine is the component which shows lower concentration so making more difficult the resolution of the mixture. Fig. 1 shows the emission spectra corresponding to CO, PY and SA obtained in aqueous solutions at concentrations of 2.00, 0.45, and 0.25 mg l^{-1}, respectively.

3.1. Optimization of variables

In order to ascertain whether the simultaneous determination of the mixture components was

Table 3
Statistical parameters

Analyte	R^2	RMSD	REP	AIC
Salicylic acid	0.9956	0.0234	3.04	22.17
Codeine	0.9796	0.0527	4.62	27.05
Pyridoxine	0.9953	0.0495	2.77	26.69

Table 4
Validation set[a]

Sample	Salicylic acid		Codeine		Pyridoxine	
	C_{added}	C_{found}	C_{added}	C_{found}	C_{added}	C_{found}
1	–	0.00	0.30	0.25	–	−0.07
2	–	−0.01	1.00	1.08	–	0.00
3	–	0.00	2.00	2.12	–	−0.05
4	–	−0.03	–	0.17	0.30	0.29
5	–	−0.02	–	−0.09	1.00	0.80
6	–	−0.01	–	−0.04	2.00	1.60
7	0.30	0.33	–	−0.01	–	−0.02
8	0.50	0.43	–	−0.10	–	−0.02
9	1.00	1.08	–	−0.10	–	0.03
10	–	0.03	2.00	2.15	0.75	0.82
11	–	0.02	0.60	0.61	1.50	1.37
12	0.20	0.21	0.60	0.60	–	0.00
13	0.15	0.15	–	−0.1	0.60	0.59
14	0.15	0.18	0.60	0.68	0.60	0.57
15	0.20	0.21	3.50	3.25	0.75	0.77
16	0.20	0.21	3.50	3.27	0.60	0.61
17	0.20	0.18	0.60	0.54	0.30	0.25
18	0.75	0.63	3.50	2.95	0.30	0.32
19	0.30	0.28	2.50	2.20	1.50	1.27
20	0.15	0.14	1.50	1.61	0.15	0.18
21	0.15	0.14	3.50	3.28	0.30	0.36
22	0.30	0.28	0.60	0.51	0.15	0.13

[a] Values are average of three determinations. Concentrations, C_{added}, C_{found} (mg l^{-1}).

feasible, we studied the influence of the variables potentially affecting the fluorescence intensity or the position of the emission maxima in order to optimize the measuring conditions. We initially checked the stability of the drug solutions as a function of the preparation time and pH.

The fluorescence intensity from PY solutions was found not to vary within 7 days after preparation maintained at 5°C. CO and SA solutions were stable from at least one month at 5°C.

Influence of pH on the fluorescence intensity of each drug was studied by adding small volumes of dilute solutions of HCl and NaOH to adjust it. Fig. 2 shows these results. pH values over the range 1–12 had no appreciable effect on the fluorescence intensity of CO; on the other hand the fluorescence of SA increases with pH util pH 7. PY shows maximum fluorescence at pH 7.0. We chose pH 7.0 as optimum value for subsequent experiments in order to ensure the maximum signal for these three analytes. We found NaH_2PO_4/ Na_2HPO_4 buffer solution pH 7.0 to have no effect on the fluorescence signal. Experiments performed using concentrations between 0.005 and 0.025 M revealed the buffer concentration to have no effect on the fluorescence intensity of any of the analytes. Therefore we chose a buffer concentration of $C_t = 0.01$ M as optimum.

Table 5
Drug compositions

Drug	Composition
Benadom	Pyridoxine hydroclhoride 300 mg
Dolmen	Acetylsalicylic acid 500 mg Codeine phosphate 1/2 H_2O 10 mg Ascorbic acid 250 mg
Dolvirán	Acetylsalicylic acid 400 mg Codeine phosphate 1/2 H_2O 9.6 mg Caffeine 50 mg
Codeisán	Codeine phosphate 1/2 H_2O 28.7 mg Codeine phosphate 1/2 H_2O 50 mg
Codeíne Perduretas	Lactose

Table 6a
Analytical applications

Salicylic acid			Codeine			Pyridoxine	
C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$	C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$	m[a]	$\%R \pm \sigma$
Benadom							
–	–	–	–	–	–	300	110 ± 1
0.50	0.54	108 ± 2	–	–	–	300	116 ± 2
0.50	0.51	102 ± 1	2.00	1.99	99 ± 1	300	116 ± 1
0.50	0.52	104 ± 1	2.00	2.03	102 ± 1	300	108 ± 2
C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$	m[a]	$\%R \pm \sigma$	C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$
Codeisan							
–	–	–	28.7	98.7 ± 0.6	–	–	–
1	1.10	108 ± 4	28.7	103 ± 3	–	–	–
–	–	–	28.7	95.9 ± 0.2	1.00	0.80	80 ± 1
0.50	0.54	108 ± 2	28.7	99 ± 1	0.50	0.45	90 ± 3
0.50	0.54	108 ± 3	28.7	91 ± 2	1.00	0.80	80 ± 3
Codeine peruretas							
–	–	–	50	95.2 ± 0.7	–	–	–
1.00	1.10	110 ± 3	50	99 ± 1	–	–	–
–	–	–	50	96 ± 1	1.00	0.90	90.4 ± 0.7
0.50	0.58	116 ± 1	50	98.3 ± 0.5	0.50	0.50	100.7 ± 0.4
0.50	0.56	112 ± 2	50	94 ± 2	1.00	0.94	94 ± 2
0.50	0.53	106 ± 1	50	106.4 ± 0.6	1.00	1.02	102.3 ± 0.8

[a] m, amount labeled (mg/unit).
[b] Concentrations: C_{added}, C_{found} (mg l^{-1}). Data are mean of three replicate determinations.

Table 6b

Salicylic acid			Codeine			Pyridoxine	
m[a]	$\%R \pm \sigma$	C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$	C_{added}[b]	C_{found}[b]	$\%R \pm \sigma$
Dolmen							
500	113 ± 4	–	–	–	–	–	–
500	105 ± 1	2.00	1.92	96.1 ± 0.2	–	–	–
500	113 ± 1	1.00	1.01	101 ± 4	–	–	–
500	101 ± 2	2.00	2.31	115.3 ± 0.4	0.50	0.59	118 ± 1
500	117 ± 1	2.00	2.09	104.5 ± 0.2	1.00	1.09	109 ± 1
Dolviran							
400	117 ± 2	–	–	–	–	–	–
400	109 ± 1	1.00	1.15	115 ± 2	–	–	–
400	106 ± 1	2.00	2.22	111 ± 1	0.50	0.59	118 ± 1
400	117 ± 2	2.00	1.99	99.2 ± 0.7	1.00	1.01	101 ± 1

[a] m, amount labeled (mg/unit).
[b] Concentrations: C_{added}, C_{found} (mg l^{-1}). Data are mean of three replicate determinations.

Increasing temperatures in the range 0–70°C resulted in decreasing fluorescence intensity, so we chose to thermostat solutions at 20.0 ± 0.5°C in subsequent experiments. Under the optimal con-

ditions above described, the emission fluorescence intensity ($\lambda_{exc} = 220$ nm) was found to be linearly related to the drug concentration over the following ranges: 0.10–2.00, 0.25–3.00, and 0.10–2.00 mg l^{-1} for SA, CO and PY, respectively.

3.2. Matrix of calibration

Fluorescence emission data from a calibration set of 14 standard samples (see Table 1) were taken between 300 and 500 nm. The calibration matrices were established for each individual component their linear ranges being (0.10–2.00), (0.25–3.00) and (0.10–2.00) for SA, CO and PY, respectively. The PLS-1 algorithm that performed the PLS analysis one component at a time has been selected to perform the determination.

3.3. Selection of the optimum number of factors and statistical parameters

To select the number of factors, in order to model the system without overfitting the concentration data, a cross-validation method, leaving out one sample at a time, was used [20]. Cross-validation consists of systematically removing one of the observations in turn and using the remaining observations for construction of latent factors and their regression. PLS calibration was performed on the 14 calibration spectra set: 13 spectra were used and from there, the concentration of the analytes in the sample left out once during this calibration process was predicted. This process was repeated 14 times until each calibration sample had been left out once and its concentration predicted. The predicted concentration of the analytes in each sample was then compared with the known concentration of them in the respective sample and the prediction error sum-of-squares (PRESS) calculated. This parameter (Table 2) was calculated each time adding a new factor to the model. PRESS is a measurement of how well a particular model fits the concentration data.

To select the optimum number of factors, the criterion of Haaland and Thomas was taken into account [20,21]. According to this, rather than the selection of the model which yields a minimum in PRESS that usually leads to some overfitting, the model to be selected is the one with a minimum number of factors that provides a PRESS value not significantly greater than the minimum one. Haaland and Thomas empirically determined that an F-ratio probability of 0.75 was a good choice. Therefore the number of factors for the first PRESS value showing an F-ratio probability below 0.75 was selected as the optimum. The maximum number of factors used was selected at 8 (half of number of standards plus one). It was found that the optimum number of factors for the PLS-1 algorithm was three for all analytes.

The statistical results obtained for the matrix are summarized in Table 3. The values of the root mean square difference (RMSD), the square of the correlation coefficient obtained when plotting actual versus predicted concentration (R^2), the Akaike [22] information criterion (AIC) and the relative error of prediction (REP) for each component are included in order to give an indication both of the average error in the analysis and the quality of fit of all data to a straight line.

To view of the obtained results, it is possible to determine under the conditions of the proposed method the three analytes with an acceptable relative error of prediction ranging from 2.8 to 4.6% depending on the respective analyte.

3.4. Validation set

In order to test the performance of the proposed method, it was applied to the determination of artificial mixtures containing various concentrations of the three compounds. The above mentioned model was used to predict the concentration of the analytes in 22 synthetic mixtures. Results are summarized in Table 4. As can be seen the amounts added and found were consistent for the most of the mixtures tested. The results can be considered satisfactory keeping in mind the strong spectral overlapping between SA and PY and the lower fluorescence signal from CO, compared with the other two analytes of the system.

3.5. Applications

The proposed method has been used in the simultaneous determination of the three analytes in

commercially available pharmaceutical formulations (Table 5). The available pharmaceuticals tested did not contain all the three analytes together. Therefore known amounts of those ones absent were also added. The determination was then carried out by applying the proposed procedure to (a) the pharmaceutical containing only one of the active principles (Benadom, Codeisan and Codeine Perduretas) or two of them (Dolmen and Dolviran), (b) the same pharmaceutical also containing added amounts of some of (an) other analyte(s).

Results are shown in Table 6a and b. All the constituents were predicted with acceptable errors if we take into account the absence, in the calibration standards, of compensation involving the presence of excipients and active principles, which are present in some of the formulations.

4. Conclusions

This paper demonstrates the potential of PLS regression for resolving overlapped fluorescence emission spectra in binary and ternary mixtures of three important active principles: (acetyl)salicylic acid, codeine and pyridoxine. The method firstly allows the simultaneous determination of SA, CO and PY and in addition, the indirect determination of ASA, CO and PY mixtures previous hydrolysis of ASA.

This is a rapid one step procedure which only requires the dissolution of the sample and to perform its fluorescence spectrum, so it is a simple, inexpensive and very fast procedure which does not need a previous separation of the analytes nor other previous sample treatments.

Although other methods such as chromatographic ones can be used to determine these analytes in pharmaceuticals [23] and usually they can offer more accuracy than the proposed method, they are both more time consuming and expensive than the procedure here developed.

Acknowledgements

The authors are grateful to the Ministerio de Educación y Cultura, Dirección General de Enseñanza Superior (DGES) (Project No. PB98-0301), for financial support.

References

[1] N.A. Kevin, P.J. Worsfold, Analyst 19 (1994) 1541–1546.
[2] V. Baeten, M. Meurens, M.T. Morales, R. Aparicio, J. Agric. Food Chem. 44 (1996) 2225–2230.
[3] J. Saurina, S. Hernández-Cassou, Analyst 120 (1995) 305–312.
[4] H. Wold, in: F. David (Ed.), Research Papers in Statistics, Wiley, New York, 1996, p. 411.
[5] H. Wold, in: H. Joreskog, H. Wold (Eds.), Systems Under Indirect Observation, vol. 2, North Holland, Amsterdam, 1982, p. 1.
[6] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, M. Redon, J. Riba, Anal. Chim. Acta 250 (1992) 219–226.
[7] Z. Bouhsain, S. Garrigues, M. de la Guardia, J. Fresenius, Anal. Chem. 357 (1997) 973–976.
[8] A. Espinosa-Mansilla, F. Salinas, A. Zamoro, Analyst 119 (1994) 1183–1187.
[9] M. de la Guardia, K.D. Khalaf, B.A. Hasan, et al., Analyst 121 (1996) 1321–1326.
[10] M.J. Ayora Cañada, M.I. Pascual Rguera, A. Molina Díaz, L.F. Capitán-Vallvey, Talanta 49 (1999) 691–701.
[11] A. Ruiz Medina, M.L. Fernández de Córdova, A. Molina Díaz, J. Pharm. Biomed. Anal. 21 (1999) 983–992.
[12] Z. Bouhsain, S. Garrigues, M. de la Guardia, Analyst 121 (1996) 1935–1938.
[13] F.J. Ramblas, S. Garrigues, M. de la Guardia, Anal. Chim. Acta 344 (1997) 41–53.
[14] A. Muñoz de la Peña, I. Durán-Meras, M.D. Moreno, F. Salinas, M. Martínez Galera, J. Fresenius, Anal. Chem. 351 (1995) 571–576.
[15] J.J. Berzas Nevado, M.A. Gómez Laguna, J.A. Murillo Pulgarin, J. Amador-Hernández, Analyst 123 (1998) 483–488.
[16] A. Navalón, R. Blanc, M. del Olmo, J.L. Vilchez, Talanta 48 (1999) 469–475.
[17] M. del Olmo, C. Díez, A. Molina, I. de Orbe, J.L. Vilchez, Anal. Chim. Acta 335 (1996) 23–33.
[18] A. Muñoz de la Peña, M.D. Moreno, I. Durán Meras, F. Salinas, Talanta 43 (1996) 1349–1356.
[19] Grams/32 Sofware Package, PLSplus/IQ Add-on Application Software, Galactic Industries, Salem, NH. USA.
[20] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193–1202.
[21] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1202–1208.
[22] M. Meloun, J. Militký, M. Forina, Chemometrics for Analytical Chemistry, Ellis Horwood, Hemel Hempstead, 1994, pp. 41–42.
[23] G. Santoni, L. Fabbri, P. Gratteri, G. Renzi, S. Pinzauti, Intern. J. Pharm. 80 (1992) 263–266.