Research Article

Drug Testing and Analysis

Received: 12 October 2009 Revised: 14 January 2010 Accepted: 14 January 2010 Published online in Wiley Interscience: 9 February 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.118

Application of some chemometric methods in conventional and derivative spectrophotometric analysis of acetaminophen and ascorbic acid

H. Khajehsharifi,[a]* Z. Eskandari[a] and A. Asadipour[b]

The multivariate methods, principal component regression (PCR) and partial least squares (PLS) were tested as a calibration procedure for simultaneous ultraviolet spectrophotometric determination of acetaminophen (AC) and ascorbic acid (AA). Determination of these compounds is important because of their pharmacotherapeutic advantages. Due to spectral overlapping of AC and AA, PCR and PLS were used for construction of the calibration sets. The concentration linear range of AC and AA were 1.5–24.2 and 1.8–21.1 μg mL^{-1} respectively. The absorption spectra were recorded from 215–310 nm. The minimum root mean square error of prediction (RMSEP) was 1.3507 and 0.4088 for AC and AA, by PLS, 0.7525 and 0.4015 by PCR in original data and 0.9454 and 0.2875, by PLS and 1.0386 and 0.4000 by PCR in derivative data. The procedure allows the simultaneous determination of AC and AA in synthetic mixtures and real sample solutions made up from pharmaceutical products, human serum and urine.

Keywords: acetaminophen; ascorbic acid; derivative spectrophotometry; partial least squares; principal component regression

Introduction

Acetaminophen (AC) (N-acetyl-*p*-aminophenol), also known as paracetamol, is an extensively employed antipyretic analgesic frequently prescribed on its own or combined with other related drugs.[1] AC is the component of a number of analgesic pharmaceutical preparations, both single drug and multidrug formulations. Although it is known that AC has no anti-inflammatory properties, its action is similar to acetylsalicylic acid.[2] AC represents an attractive alternative for patients who are sensitive to aspirin and it is the analgesic of choice for people with asthma.[3] AC is metabolized predominantly in the liver and excreted in the urine mainly as the glucuronide and sulfate conjugates.[4] However, many studies have shown that an overdose of AC is associated with hepatic toxicity and renal failure despite its apparently innocuous character.[5] Paracetamol is an effective alternative to aspirin as an analgesic and antipyretic agent. It is often self-prescribed for relief of moderate pain, fever, lumbar pain, migraine or non-specific indications without any medical control.[6] It has been reported as a useful drug in osteoarthritis therapy[7] as well.

AA is a compound that takes part in many important life processes.[8,9] AA has been used for the prevention and treatment of the common cold, mental illness, infertility and cancer.[11] The synergetic and protective effects conferred during the use of AC for therapeutic purposes by its combination with AA and/or other pharmacological and biologically active compounds can also be mentioned.[12,13] Overdose ingestions of AC lead to accumulation of toxic metabolites, which cause severe and sometimes fatal hepatotoxicity and nephrotoxicity.[14,15]

The overlapping between AC and AA is well known in the UV spectrophotometry. Derivative techniques are useful for the reduction of band overlapping errors in quantitative analysis, if the systematic error caused by the overlap is larger than the random noise error and if the interfering band is either known or constant.[16] The combined use of derivative spectroscopy and chemometric techniques has been demonstrated to be a highly convenient choice in the determination of multicomponent matrices presenting serious spectra overlapping, thanks to their common potential ability to exploit minor spectral features.[17–20]

The application of multivariate calibration techniques on spectral data offers a main advantage which consists of speeding up complex systems resolution. Among the various chemometric approaches applied to multicomponent analysis, principal component regression (PCR) and partial least squares regression (PLS) have been successfully adopted in many quantitative assays of pharmaceutical formulations.[21–25] PCR and PLS are factor analysis methods which allow to establish a relationship between matrices of chemical data.[26]

Several methods have been used for the simultaneous determination of AA and AC, such as differential pulse voltametry,[27] spectrophotometry[28,29] pulse amperometry[30] chronoamperometry [31] and chromatography[32,33] but this is the first time that a chemometrical simultaneous spectrophotometric analysis is presented. A spectrophotometric technique is always an acceptable alternative chemical analysis method, due to its acceptable pre-

* *Correspondence to: H. Khajehsharifi, Department of Chemistry, Yasouj University, Yasouj, Iran. E-mail: khajeh_h@yahoo.com, haka@mail.yu.ac.ir*

a *Department of Chemistry, Yasouj University, Yasouj, Iran*

b *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*

cision and accuracy, associated with its lower cost compared to other techniques. This work investigated a good strategy for simultaneous determination of these drugs in Efferalgan tablets and environment of human's serum, urine and amount of AC in CONTAC powder.

Experimental

Chemicals and Reagents

All the used chemicals were of analytical reagent grade. Throughout the experiments, doubly distilled water was used. AC was purchased from Darou Pakhsh, Tehran, Iran, while AA and trichloroacetic acid were supplied from Merck, Darmstadt, Germany. The Efferalgan tablets and CONTAC powder were purchased from Laboratories UPSA a Bristol-Myers Squibb Company, Paris, France and GlaxoSmithKline, Germany, Buhl/Baden, respectively. The stock solutions of AC and AA were prepared daily, by dissolving them in a buffer solution (pH = 7.0) that was prepared by KH_2PO_4 and NaOH from Merck, Darmstadt, Germany.

Apparatus and software

Electronic absorption measurements were carried out on a Jasco v-570 spectrophotometer (slit width: 1.0 nm, scan rate: 2000 cm/min) using 1.00 cm quartz cells. Measurements of pH were made with a Metrohm 692 pH meter using a combined electrode. All spectra were digitized and stored at wavelengths from 215 to 310 nm in steps of 1 nm and then transferred in TXT format to an Pentium 4, 2.4 GHz computer using MATLAB software, version 7 (The Math Works). PCR and PLS calculus were carried out in the PLS Tool box (Eigenvector Company, version 2.1).

Procedure

Standard calibration set

A mixture design for two component mixtures was used for calibration set in order to provide good prediction in PCR and PLS methods. A training set of 25 samples was taken (Table 1). As shown in Table 1, the concentration of AC and AA were varied between 1.5–24.2 and 1.8–21.1 μg mL^{-1}, respectively. The mixed standard solution were placed in a 10 ml volumetric task and completed to final volume with buffer solution (pH = 7.0). Finally the absorption spectra of all prepared solutions were recorded between 215 and 310 nm against a blank of buffer.

Application of models to the prediction set

For prediction set, 8 mixtures were prepared randomly, but due to employing as an independent test concentrations were not present in the previous set. Table 2 shows the solutions that were used for the prediction set. The added amounts were in linear of AC and AA.

Real sample preparation

Serum sample

The serum sample was homogenized. For the deproteinization, 1 ml of 24% w/v trichloroacetic acid was added to 1 ml of serum, and after 15 min, the resulting mixture was centrifuged at 3000 rpm.[34]

Table 1. Concentration data of mixtures that used in the calibration set for the determination of AC and AA

Mixtures	AC(μg/ml)	AA(μg/ml)
M1	1.5	1.8
M2	7.1	1.8
M3	12.9	1.8
M4	18.5	1.8
M5	24.2	1.8
M6	1.5	6.5
M7	7.1	6.5
M8	12.9	6.5
M9	18.5	6.5
M10	24.2	6.5
M11	1.5	11.4
M12	7.1	11.4
M13	12.9	11.4
M14	18.5	11.4
M15	24.2	11.4
M16	1.5	16.2
M17	7.1	16.2
M18	12.9	16.2
M19	18.5	16.2
M20	24.2	16.2
M21	1.5	21.1
M22	7.1	21.1
M23	12.9	21.1
M24	18.5	21.1
M25	24.2	21.1

The pH of supernatant solution was fixed on pH = 7.0 by amount of NaOH solution. Afterwards, the appropriate amount from the stock solution of AC and AA was added to 0.5 ml of the final prepared serum, and then filled to the final volume (10 ml) with buffer solution to obtain the desired concentration. The electronic absorption spectra were recorded in the range of 215–310 nm against a blank solution of serum.

Urine sample

The urine sample was diluted 1 : 3 with doubly distilled water. Then cell debris and the particulate matter were removed from the urine using low-speed centrifugation for 5 min at 1500 rpm.[35]

Afterwards the pH of sample was fixed on pH = 7.0 by amount of NaOH solution. Then appropriate amount of the stock solution of AC and AA was added to 0.5 ml of the final prepared urine and completed to the final volume (10 ml) with buffer solution to obtain the desired concentration. The electronic absorption spectra were recorded in the 215–310 nm against a blank of urine.

Result and Discussion

Electronic absorption spectra

The original and derivative electronic absorption spectra of AC and AA are shown in Figure 1. As can be seen, the spectrum of each component overlap. Thus, these compounds cannot be measured in the presence of each other by a simple calibration procedure without prior separation. Therefore multivariate calibration was used to resolve the spectra and for determination of each

Table 2. Added and found results of the synthetic mixture of AC and AA

Mixtures	Add(μg/ml) AC	Add(μg/ml) AA	Found(μg/ml) AC	Found(μg/ml) AA	Recovery (%) AC	Recovery (%) AA
Original PLS						
1	4.5	17.6	4.4	17.2	97.8	97.7
2	6.1	14.1	5.6	14.7	91.8	104.1
3	7.6	12.3	6.9	12.4	90.8	100.7
4	18.1	7.0	16.4	6.8	90.6	95.9
5	21.2	6.2	19.8	6.1	93.4	99.3
6	23.4	6.2	21.8	6.3	93.2	102.5
7	24.2	5.3	22.3	5.5	92.1	104.3
8	12.9	8.1	11.9	7.4	92.2	102.8
Original PCR						
1	4.5	17.6	4.5	17.1	100.0	97.2
2	6.1	14.1	6.6	14.6	108.2	103.5
3	7.6	12.3	7.9	12.3	103.9	100.0
4	18.1	7.0	17.3	6.7	95.6	95.7
5	21.2	6.2	20.0	6.0	94.3	96.8
6	23.4	6.2	22.5	6.1	96.2	98.4
7	24.2	5.3	23.1	5.4	95.5	101.9
8	12.9	8.1	13.2	7.3	102.3	90.1
Derivative PLS						
1	4.5	17.6	4.3	17.2	95.6	97.7
2	6.1	14.1	5.7	14.7	93.4	104.4
3	7.6	12.3	6.9	12.4	90.8	100.6
4	18.1	7.0	16.8	6.8	92.8	96.6
5	21.2	6.2	20.2	6.1	95.3	99.0
6	23.4	6.2	22.3	6.3	95.3	102.3
7	24.2	5.3	22.6	5.5	93.4	104.2
8	12.9	8.1	11.9	7.5	92.2	92.6
Derivative PCR						
1	4.5	17.6	4.3	16.8	95.6	95.5
2	6.1	14.1	5.7	14.1	93.4	100.0
3	7.6	12.3	6.9	11.9	90.8	96.7
4	18.1	7.0	16.7	7.2	92.3	102.9
5	21.2	6.2	20.1	6.4	94.8	103.2
6	23.4	6.2	22.3	6.0	95.3	96.8
7	24.2	5.3	22.6	5.3	93.4	100.0
8	12.9	8.1	11.9	7.5	92.3	92.6

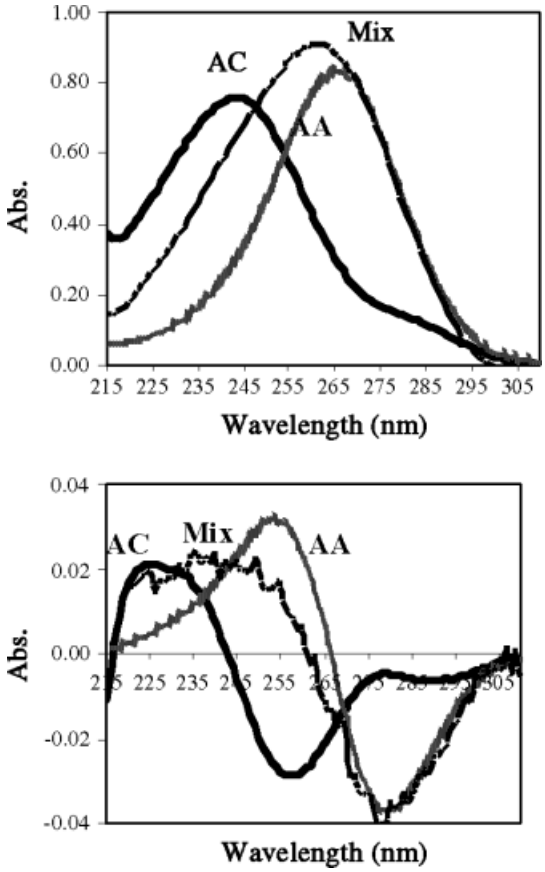

Figure 1. (A) Original UV spectrum of AC (12.1 μg/ml) and AA (10.6 μg/ml) and their mixtures. (B) Derivative UV spectrum, at pH = 7.0 and T = 273 K.

compound in the mixtures. Regarding spectral data (Figure 1), spectra were recorded in the region between 220 and 320 nm (1.00 nm steps). The same way was preformed for prediction, artificial and unknown samples.

Univariate calibration

Individual calibration curves were constructed with several points, as absorbance versus AC and AA concentration. The linear regression equation of calibration graph for AC in the concentration range of 1.5–24.2 μg mL^{-1} was $A = 0.1144\ C_{AC} - 0.0715$ ($R^2 = 0.9864$) and $A = 0.0804\ C_{AA} - 0.0046$ ($R^2 = 0.9962$) for the concentration range of 1.8–21.1 μg mL^{-1} of AA. The max wavelength of AC is 244 and AA, 266. The wavelengths used to generated calibration curves were 215–310 nm.

Multivariate calibration and prediction

Multivariate calibration methods such as PCR and PLS require a suitable experimental design of the standard belonging to the calibration set in order to provide good prediction. In this study, the mixture design was used for experimental design. It is important that we use a selection method that does not create an underlying correlation among the concentrations of the components. The calibration matrix used for the analysis is shown in Table 2 for the prediction step; as stated previously, 8 prepared mixtures that were not included in the previous set were employed as an independent test. It should be noted that the PLS and PCR

Table 3. Statistical parameters of the optimized matrix using the PCR, PLS and their first derivative

Method	Component	NPC	PRESS	RMSEP	RSEP (%)
PCR	AC	3	4.53	0.7525	0.2221
	AA	3	1.29	0.4015	0.1504
PCR 1st derivative	AC	2	8.63	1.0386	0.4469
	AA	2	1.28	0.4000	0.1531
PLS	AC	2	12.77	1.3507	8.5870
	AA	3	1.17	0.4088	3.7829
PLS 1st derivative	AC	2	7.15	0.9454	6.0732
	AA	3	0.66	0.2875	2.7505

regressions were applied on the second-order derivative spectra, but no significant results were obtained, so corresponding data were not reported here.

Quality of fit estimations

The selection of the optimal number of factors used to build PCR and PLS models represent a decisive step to improve the prediction power of the methods. The predicted residual error sum of squares (PRESS) was used for cross-validation models using a high number of factors (half the number of total standard +1), which is defined in Eqn (1).

$$PRESS = \sum (c_i - \hat{c}_i)^2 \quad (1)$$

where c_i is the reference concentration for the i th sample and $\hat{c}_i$ represents the estimated concentration. The cross-validation method is employed to eliminate only one sample at a time and then the models calibrate the remaining standard spectra. By using this calibration the concentration of the sample left out was predicted; this process was repeated until each standard had been left out once.

One reasonable choice for the optimum number of factors would be the number that yielded the minimum PRESS. Since there are a finite number of samples in the training set, in many cases the minimum PRESS value causes over fitting for unknown samples that were not included in the model. A solution to this problem has been suggested by Haaland *et al*. in which the PRESS values for all previous factors are compared with the PRESS value at the minimum.[36]

The F-statistical test can be used to determine the significance of PRESS values greater than the minimum.

The maximum number of factored used to calculate the optimum PRESS was selected, and the optimum number of factors obtained by PLS and PCR model on original and derivative data are summarized in Table 3. In all cases, the number of factors for the first PRESS values whose F-ratio probability drops below was selected as the optimum. Plots of PRESS *vs* number of factors by PCR and PLS and their derivatives are shown in Figure 2.

Statistical parameters

For the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction

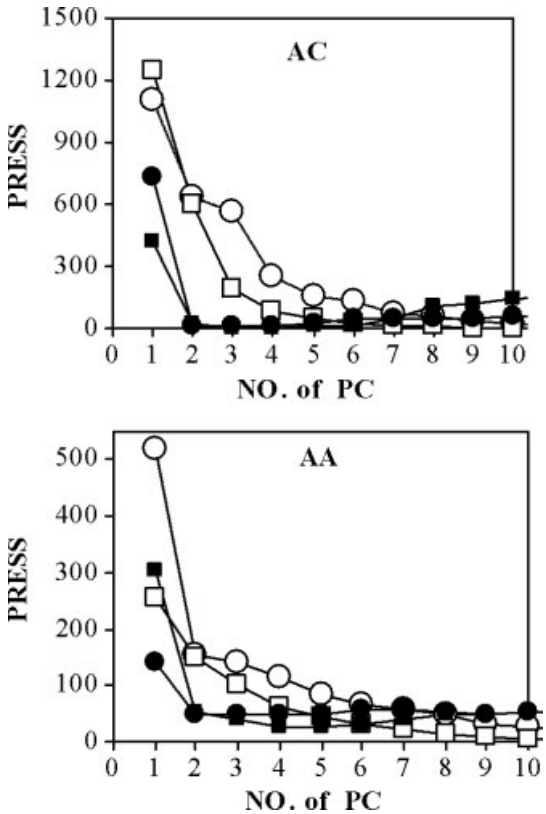

Figure 2. Plots of PRESS *vs* number of factors by PCR and PLS. (Derivative PCR ●, Derivative PLS □, Original PCR ○, Original PLS ■).

(RMSEP), the prediction error of a single component in the mixture as the relative standard error of prediction (RSEP%) can be used.[37]

$$RMSEP = \left[\frac{1}{n}\sum_{i=1}^{n}(c_{pred} - c_{obs})^2\right]^{0.5} \quad (2)$$

$$RSEP(\%) = 100\left[\frac{\sum_{i=1}^{n}(c_{pred} - c_{obs})^2}{\sum (c_{obs})^2}\right]^{0.5} \quad (3)$$

where c_{pred} and c_{obs} are the predicted concentration and the observed value of the concentration in the sample, respectively, and n is the number of samples in the validation set. The values of RMSEP, RSEP (%) for AC and AA are summarized in Table 3.

Determination of AC and AA spiked in real samples

To assess the reliability of the method, 5 prepared real samples were analyzed. Table 4 shows the results as well as the composition of the real sample.

The validation of the method was carried out by comparing with the labelled amounts. As can be seen, the recovery was quantitative and there were no significant differences between the amounts obtained from this method and the labelled amounts. The plots of the prediction concentration versus actual values are shown in Figure 3 for AC and AA by derivative PLS that yielded the best results. (Line equations R^2 values are also shown.)

Table 4. Recoveries of AC and AA in spiked real samples by PLS

Serum samples	Added (μg/ml)		Found (μg/ml)		Recovery (%)	
Mixtures	AC	AA	AC	AA	AC	AA
S1	5.3	15.8	5.8	16.1	109.4	101.9
S2	6.8	12.8	5. 9	12.8	95.6	100.0
S3	9.8	12.0	10.1	11.7	103.1	97.5
S4	15.1	13.2	14.0	14.5	93.4	109.8
S5	18.9	8.8	17.7	8.6	93.7	97.7
Mean recovery					99.0	101.4
Urine samples	Added (μg/ml)		Found (μg/ml)		Recovery (%)	
Mixtures	AC	AA	AC	AA	AC	AA
U1	6.8	15.8	7.6	13.2	111.8	89.9
U2	9.8	12.8	8.8	11.9	89.8	93.0
U3	15.1	12.0	13.6	12.0	90.1	100.0
U4	18.9	8.8	19.9	9.6	105.3	109.1
U5	13.6	4.9	11.9	4.7	87.5	95.9
Mean recovery					96.9	97.6

Analysis of pharmaceutical formulations

In order to assess the applicability of the proposed method to the analysis of real samples, determination of these drugs was conducted in mixtures of their pharmaceutical formulations. Thus, different mixtures of the commercially available Efferalgan tablets and CONTAC powder were prepared and analyzed with the PCR and PLS methods. The recovery values are presented in Table 5. It can be observed that in all mixtures the calculated values are in satisfactory agreement with the declared values.

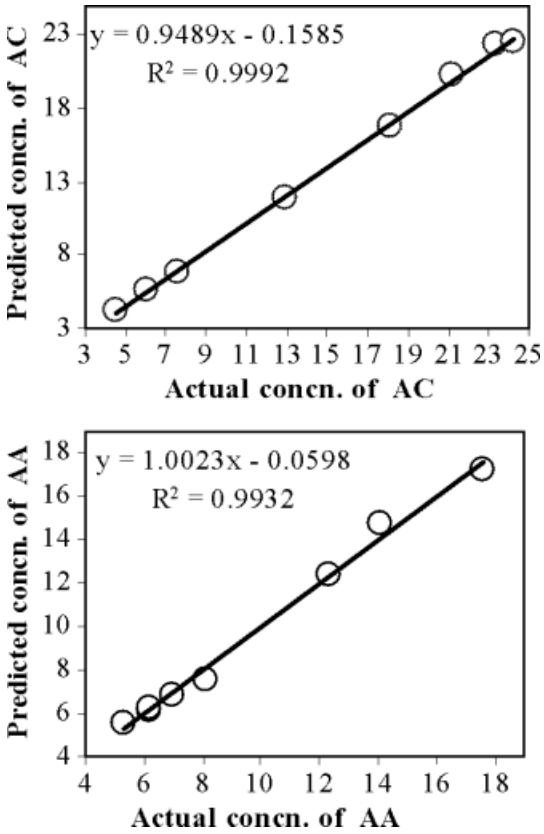

Figure 3. Plots of predicted concentration *vs* actual concentration of AC and AA by derivative PLS.

Conclusion

Multivariate methods by application of spectrophotometric data and their derivative have been valid analytical tools. For overcoming the drawback of spectral interferences between AC and AA, PCR and PLS were applied as two powerful and widely used multivariate calibration methods. The prediction ability of the proposed method was acceptable according to the results obtained on data sets of AC and AA mixtures especially in derivative data that lead to fewer principal components and PRESS in comparison with original data. Satisfactory results demonstrate the utility of these procedures for the simultaneous determination

Table 5. Application of optimized models on the pharmaceuticals

		Nominal		Found				Recovery (%)			
				PCR		PLS		PCR		PLS	
		AC	AA	AC	AA	AC	AA	AC	AA	AC	AA
Efferalgan	Original	33.0	20.0	32.1	13	32.1	13	97.3	67.2	97.3	67.2
		12.2	12.0	12.4	7.9	12.4	7.9	101.6	68.1	101.6	68.3
		14.2	14.0	14.1	8.8	14.1	8.8	99.4	65.0	99.3	65.0
	1st derivative	33.0	20	24.4	22.5	24.4	20.7	73.9	112.5	73.9	103.4
		12.2	7.4	9.2	8.3	9.1	7.6	75.4	112.2	74.5	103.3
		14.2	14	10.4	15.7	10.4	14.5	73.2	112.1	73.3	103.3
Contac	Original	24.0	0	26.0	0	26.0	0	108.3	0	108.3	0
		15.6	0	16.9	0	16.9	0	108.1	0	108.3	0
		19.2	0	21.0	0	20.9	0	108.9	0	109.4	0
	1st derivative	24.0	0	22.2	0	23.2	0	92.5	0	96.7	0
		15.6	0	14.6	0	15.7	0	93.6	0	100.6	0
		19.2	0	17.4	0	18.4	0	90.6	0	95.8	0

of AC and AA in human serum and human urine samples and commercial pharmaceutical mixtures.

Acknowledgment

We are grateful to Yasouj University for supporting the research.

References

[1] J. F. Van Staden, M. Tsanwani, *Talanta* **2002**, *58*, 1095.
[2] M. Espinosa Bosch, A. J. Ruiz Sánchez, F. Sánchez Rojas, C. Bosch Ojeda, *J. Pharm. Biomed. Anal.* **2006**, *42*, 291.
[3] R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commun.* **2005**, *7*, 803.
[4] D. Bose, A. Durgbanshi, A. Martinavarro-Dominguez, M. E. Capella-Peiró, S. Carda-Broch, J. S. Esteve-Romero, M. T. J. Gil-Agusti, *J. Chromatogr. Sci.* **2005**, *43*, 313.
[5] C. Li, G. Zhan, Q. Yang, J. Lu, *Bull. Korean Chem. Soc.* **2006**, *27*, 1854.
[6] A. O. Maria, A. O. Roberto, N. M. Adriana, *Talanta* **2005**, *66*, 229.
[7] K. Brandt, *Drugs* **2003**, *63*, 23.
[8] T. Kleszczewski, E. Kleszczewska *J. Pharm. Biomed. Anal.* **2002**, *29*, 755.
[9] P. J. O'Connell, C. Gormally, M. Pravda, G. G. Guilbault *Anal. Chim. Acta* **2001**, *431*, 239.
[10] W. Zeng, F. Martinuzzi, A. MacGregor, *J. Pharm. Biomed. Anal.* **2005**, *36*, 1107.
[11] D. R. Shankaran, K. Iimura, T. Kato, *Sens. Actuators, B* **2003**, *94*, 73.
[12] H. R. B. Raghavendran, A. Sathivel, T. Devaki, *J. Health Sci.* **2004**, *50*, 42.
[13] J. Guzy, Z. Chovanova, M. Marekova, Z. Chavkova, V. Tomečkova, G. Mojžišová, *J. Kušnir, Biologia, Bratislava*, **2004**, *59*, 399.
[14] F. L. Martin, A. E. McLean, *Drug Chem. Toxicol.* **1998**, *21*, 477.
[15] C. A. Mugford, J. B. Tarloff, *Toxicol. Lett.* **1997**, *93*, 15.
[16] L. Meal, *Anal. chem.* **1983**, *55*, 2448.
[17] M. C. F. Ferraro, P. M. Castellano, T. S. Kaufman, *J. Pharm Biomed. Anal.* **2004**, *34*, 305.
[18] A. Abbaspour, R. Mirzajani, *J. Pharm Biomed. Anal.* **2005**, *38*, 420.
[19] A. Afkhami, M. Bahram, *Spectrochim. Acta Part A* **2005**, *61*, 869.
[20] C. K. Markopoulou, E. T. Malliou, J. E. Koundourellis, *Il Farmaco*, **2004**, *59*, 627.
[21] M. Blanco, J. Coello, F. Gonzales, H. Itturiaga, S. Maspoch, X. Tomas, *J. Pharm. Biom. Anal.* **1994**, *12*, 509.
[22] B. W. Glombitza, P. C. Schimdt, *J. Pharm. Sci.* **1994**, *83*, 751.
[23] R. D. Bautista, F. J. Aberasturi, A. I. Jimenez, *Talanta* **1996**, *43*, 2107.
[24] J. J. Berzas Nevado, J. Rodriguez Flores, G. Castañeda Peñalvo, *Anal. Chim. Acta*, **1997**, *340*, 257.
[25] A. Navalon, R. Blanc, M. del Olmo, J. L. Vilchez, *Talanta* **1999**, *48*, 469.
[26] H. Martens, T. Naes, *Multivariate Calibration*, John Wiley & Sons Ltd: Chichester, **1989**.
[27] C. Radovan, C. Cofan, D. Cinghitaa, *Electroanalysis* **2008**, *20*, 1346.
[28] R. Saňdulescu, S. Mirel, R. Oprean, *J. Pharm Biomed. Anal.* **2000**, *23*, 77.
[29] H. N. Dogan, A. Duran, *Pharmazie* **1998**, *53*, 781.
[30] W. T. P. Santos, E. G. N. de Almeida, H. E. A. Ferreira, D. T. Gimenes, E. M. Richter, *Electroanalysis* **2008**, *20*, 1878.
[31] C. Cofan, C. Radovan, *Sensors* **2008**, *8*, 3952.
[32] H. Okamotoa, T. Nakajimaa, Y. Itoa, T. Aketoa, K. Shimadab, S. Yamatob *J. Pharm Biomed. Anal.* **2005**, *37*, 517.
[33] B. R. Thomas, X. G. Fang, P. Shen, S. Ghodbane, *J. Pharm Biomed. Anal.* **1994**, *12*, 85.
[34] H. Khajehsharifi, M. Sadeghi, E. Pourbasheer, *Monatsh. Chem.* **2009**, *140*, 685.
[35] H. Khajehsharifi, E. Pourbasheer, *J. Chin. Chem. Soc.* **2008**, *55*, 163.
[36] D. M. Haaland, E. V. Thomas, *Anal. Chem.* **1988**, *60*, 1193.
[37] K. Wiberg, A. Sterner-Molin, S. P. Jacobsson, *Talanta* **2004**, *62*, 567.