ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 723–730

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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A comparative study of the ratio spectra derivative spectrophotometry, Vierordt's method and high-performance liquid chromatography applied to the simultaneous analysis of caffeine and paracetamol in tablets

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Received 30 December 1998; received in revised form 21 April 1999; accepted 6 May 1999

Abstract

Two spectrophotometric methods and high-performance liquid chromatography were proposed for the simultaneous analysis of caffeine and paracetamol in a tablet formulation. The ratio spectra derivative method is based on the use of the analytical signals obtained by measuring at 267.9 and 291.0 nm for caffeine and 237.0 and 251.8 nm for paracetamol in the first derivative of the ratio spectra. Calibration graphs were prepared in the range 4–40 μg/ml for caffeine and 8–48 μg/ml for paracetamol. In Vierordt's method, A_1^1 (1%, 1 cm) values of paracetamol and caffeine were determined at 242.9 and 273.0 nm in zero-order spectra. The matrix for A_1^1 (1%, 1 cm) values was written and the amounts of both drugs were calculated by means of the program 'Matlab' software. The results obtained by these spectrophotometric methods were compared with the results of HPLC method.

Keywords: Paracetamol; Caffeine; Tablet formulation; Ratio spectra derivative spectrophotometry; Vierordt's method; High-performance liquid chromatography

1. Introduction

The use of the mixture of paracetamol and caffeine as an analgesic and antipyretic is well established in pharmaceutical formulations.

Quantitative determination of active ingredients in pharmaceutical formulations containing caffeine and paracetamol with other active compounds using various methods including spectrophotometry [1–9] and chromatographic [10,11] methods and have been demonstrated in pharmaceutical preparations.

Salinas et al. [12] developed a new method for the analysis of binary mixtures of compounds with overlapped spectra and Berzas Nevado et al. [13–16], applied same method to determine the active compounds in different mixtures. This same

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0731-7085/99/$ - see front matter
PII: S0731-7085(99)00186-7

method was applied by us for the drugs analysis [17–19].

In this paper, the ratio spectra derivative spectroscopy, Vierordt's method and high-performance liquid chromatography (HPLC) were proposed for simultaneous determination of both drugs in a commercial preparation marketed in Turkey. The spectrophotometric methods were compared with the HPLC method (as a comparison method).

2. Material and methods

2.1. Apparatus

A Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC Software which was equipped with an HP 600 printer was used for all the absorbance measurements and treatment of data.

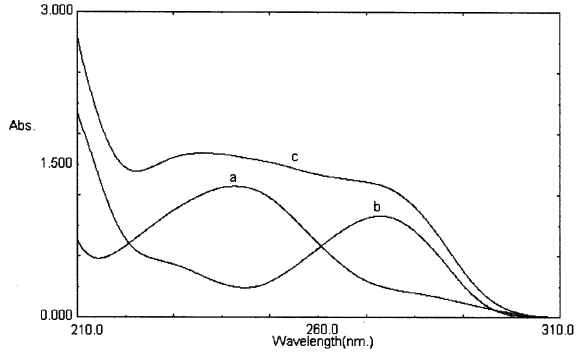

Fig. 1. Zero-order spectra of (a) 20 μg/ml paracetamol, (b) 20 μg/ml caffeine, and (c) their mixture in 0.1 M HCl.

Table 1
Experimental parameters for Vierordt's method used for the simultaneous determination of paracetamol and caffeine

λ nm	Paracetamol		Caffeine	
	α_1	α_2	β_1	β_2
$\lambda_1 = 242.9$	643.8		145.0	
$\lambda_2 = 273.0$		153.2		497.6
Linearity range μg/ml	8–48		4–40	

The HPLC equipment consisting of a Jasco model PU-980 pump, with a Jasco model UV-975 dedector connected to a computer loaded with Borwin Software which was equipped with a HP 600 printer were used. All the chromatograms were plotted and stored in the above computer.

2.2. Tablet formulation

A commercial tablet product (Remidon® tablet, Deva Pharm. Ind., Turkey, Batch no. 706-1770), containing 65 mg caffeine (CAF) and 500 mg paracetamol (PAR) per tablet, was studied.

CAF and PAR were kindly donated by Deva Pharm. Ind., Turkey.

2.3. Reagents

All the solvents were of analytical reagent grade. In HPLC procedure, HPLC methanol (E.Merck, Germany) and double distilled water was used.

2.4. Standard solutions

Stock solutions of 100 mg/100 ml of CAF and PAR were prepared in 0.1 M HCl for spectrophotometric procedures and in methanol for HPLC procedure.

For spectrophotometry: Working standard solutions were prepared in 25 ml volumetric flasks containing 4–40 μg/ml CAF, 8–48 μg/ml PAR and their synthetic mixtures by using their stocks solutions. The zero-order spectra were recorded with a sampling interval of $\Delta\lambda = 0.1$ nm and a medium level of scanning speed against a reagent blank (0.1 M HCl) and stored in the IBM PC computer.

For HPLC method: Synthetic mixtures were prepared containing CAF and PAR in the range 2–28 and 8–48 μg/ml of CAF and PAR, respectively with a constant concentration of cetrimide (0.6 mg/ml) as internal standard (IS) in methanol. These solutions were filtered through a 0.45 μm membrane filter before injection into the chromatograph.

Table 2
Recovery data obtained for different mixtures by using the Vierordt's method

Mixture number	Paracetamol			Caffeine		
	Added (μg)	Found (μg)	Recovery[a] (%)	Added (μg)	Found (μg)	Recovery[b] (%)
1	32.0	32.2	100.6	4.0	4.0	100.0
2	32.0	32.5	101.6	8.0	7.9	98.8
3	32.0	31.9	99.7	12.0	12.1	100.8
4	32.0	31.8	99.4	20.0	19.8	99.0
5	32.0	32.2	100.6	32.0	31.8	99.8
6	32.0	32.4	101.3	40.0	39.8	99.5
7	8.0	8.1	101.3	12.0	12.0	100.0
8	16.0	16.0	100.0	12.0	11.8	98.3
9	20.0	20.5	102.5	12.0	12.1	100.8
10	32.0	32.0	100.0	12.0	11.4	98.3
11	40.0	40.3	100.8	12.0	11.9	99.2
12	48.0	48.9	101.9	12.0	12.0	100.0

[a] $\bar{x} = 100.8$, Relative Standard Deviation (RSD) = 0.89
[b] $\bar{x} = 99.5$, RSD = 0.82

2.5. Samples solutions

In spectrophotometric methods, 20 tablets were accurately weighed and powdered in a mortar. A mass corresponding to a tablet, was dissolved in 0.1 M HCl in 100 ml calibrated flasks. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml calibrated flask through Whatman no:42 filter paper. The residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with 0.1 M HCl (solution 1). The solution 1 was dilued 1:500 with the same solvent.

In HPLC method, the same procedure was realized by using methanol as solvent instead of 0.1 M HCl (solution 2). The solution 2 was diluted with 1:500 with methanol.

3. Application of methods

3.1. Spectrophotometric procedures

3.1.1. Vierordt's method

This method [20–22] is based on the solving of equations with two unknowns using $A\,{}^{1}_{1}$ (absorbance value of the 1% solution in 1 cm cell) values calculated from absorbances measured at two suitable wavelengths for two compounds in the mixture have an absorption minimum and maximum inversely. The concentration of ingredients of the pharmaceutical preparation are then evaluated from a pair of simultaneous equations of the following form:

$$A = \alpha lC \quad \text{(pathength } (l) \text{ is equal to 1)}$$

$$A_1 = \alpha_1 \cdot C_1 + \beta_1 \cdot C_2 \quad \text{for } \lambda_1$$

$$A_2 = \alpha_2 \cdot C_1 + \beta_2 \cdot C_2 \quad \text{for } \lambda_2$$

where A_1, and A_2 denotes the absorbances of a mixture solutions of CAF and PAR. C_1, and C_2 are the concentrations of CAF and PAR, whilst α and β are their respective $A\,{}^{1}_{1}$ (%1, 1 cm) values. The subscripts 1 and 2 refer to wavelengths.

Matrix notation greatly simplifies the matters and solves system of equations with two unknowns, easily as shown below:

$$|A_1| = |\alpha_1 \beta_1| \ |C_1|$$

$$|A_2| = |\alpha_2 \beta_2| \ |C_2| \qquad \text{or } A = E.C$$

This matrix can be solved by means of the program 'Matlab' in the computer and the concentrations of each compound in the mixture were determined.

3.1.2. Ratio spectra first derivative spectrophotometry

To determine CAF, the recorded zero-order spectra of samples were divided by a spectrum of the standard solution of PAR. From the ratio spectra thus obtained, first derivative were calculated. The concentration of CAF is proportional to the amplitude at 267.9 nm and 291.0 nm. Also, to determine PAR, the stored zero-order spectra of the samples were divided by a spectrum of the standard solution of CAF. The first derivative of the ratio spectra of PAR were obtained by an analogous procedure, when a spectrum of the standard solution of CAF used as divisor. The concentration of PAR is proportional to the amplitude at 237.0 nm and 251.8 nm.

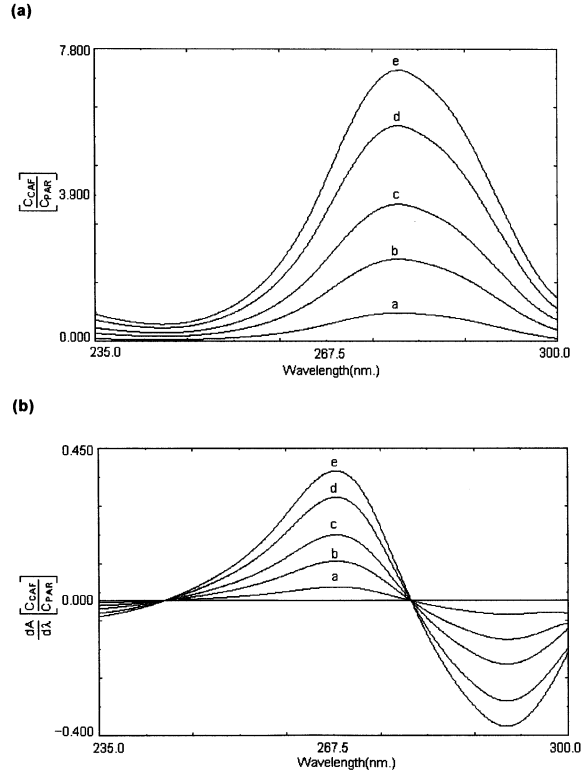

Fig. 2. Ratio spectra (a) and first derivative of the ratio spectra of (b) caffeine of (a) 4 μg/ml, (b) 12 μg/ml, (c) 20 μg/ml, (d)32 μg/ml, (e) 40 μg/ml, when 20 μg/ml paracetamol is used as a divisor in 0.1M HCl ($\Delta\lambda = 8$ nm).

3.2. HPLC procedure

The chromatograms were plotted and stored in the computer. The detector responses were measured in terms of peak area. The data was processed using Borwin software. Separation was carried out at ambient temperature on on Nucleosil 100-5 C_{18} (250 × 4.6 mm I.D. 5μm) column (Macherey–Nagel, Germany) and the mobile phase consisted of water and methanol (20:80). The flow rate was set at 1.0 ml min^{-1} with 10 μl as injection volume. The photometric detection was performed at 254 nm.

4. Results and discussion

4.1. Vierordt's method

The zero-order absorption spectra in Vierordt's method were obtained by a sampling interval of $\Delta\lambda = 0.1$ nm and a medium level of scanning speed in the spectrophotometer. Under these conditions, the original spectra were recorded in the computer. Fig. 1 shows the absorption zero-order spectra of the solutions of CAF and PAR in 0.1 M HCl are overlapped at the region 210.0–310.0 nm. By using Vierordt's method, the determination of the two compounds is possible for direct absorbance measurements in their zero-order spectra. For this procedure, the absorbance values were measured at 242.9 nm and at 273.0 nm, selecting the maximum and the minimum wavelengths of the two compounds in a way that the maximum wavelength of one compound would be corresponding to the minimum wavelength of the second compound. In Vierordt's method, the parameters used, shown in Table 1 and the equations used have been explained in the methods section.

In the method, Beer's law was valid in the concentration range 4–40 μg/ml for CAF and 8–48 μg/ml for PAR. Mean recovery and relative standard deviation of the method was obtained as 99.5 and 0.82%, respectively, for CAF, and also 100.8 and 0.89% for PAR, respectively, in the synthetic mixtures prepared by adding known amounts of CAF and PAR (Table 2).

Table 3
Recovery data obtained for different mixtures by using the first derivative of the ratio spectra

Mixture number	Paracetamol			Caffeine		
	Added (μg)	Found (μg)	Recovery[a] (%)	Added (μg)	Found (μg)	Recovery[b] (%)
1	32.0	31.8	99.4	4.0.	4.1	102.5
2	32.0	32.2	100.0	8.0	7.9	98.8
3	32.0	32.9	102.8	12.0	12.0	100.0
4	32.0	32.0	100.0	20.0	20.1	100.5
5	32.0	31.8	99.4	32.0	32.3	100.9
6	32.0	33.0	103.1	40.0	40.4	101.0
7	8.0	8.0	100.0	12.0	12.1	100.8
8	16.0	16.1	100.6	12.0	12.0	100.0
9	20.0	20.0	100.0	12.0	11.7	95.8
10	32.0	32.3	100.9	12.0	11.5	100.0
11	40.0	40.2	100.5	12.0	12.2	101.6
12	48.0	48.5	101.0	12.0	11.9	99.2

[a] $\bar{x} = 100.6$, RSD = 1.13.
[b] $\bar{x} = 100.1$, RSD = 1.61.

4.2. Ratio spectra first derivative spectrophotometry

As could be seen in Fig. 2a, the absorption spectra of the solutions prepared at the increasing concentrations of CAF in 0.1 M HCl, were recorded in the spectral region 235.0–300.0 nm and divided the spectrum of the standard solution of 20 μg/ml PAR in the same solvent and their ratio spectra were obtained. Fig. 2b indicates the first derivative of the ratio spectra which was plotted with the intervals of $\Delta\lambda = 8$ nm from the ratio spectra shown in Fig. 2a. Two calibration graphs of CAF were established by measuring the signals at 267.9 and 291.0 nm corresponding to a maximum and a minimum, respectively, and were tested between 8–40 μg/ml for CAF and its binary mixtures with PAR (Table 3).

Similarly, the absorption spectra of the solutions prepared at increasing concentrations of PAR in 0.1 M HCl, were stored in between 205.0–295 nm and divided the spectrum of the standard solution of 20 μg/ml CAF in the same solvent and their ratio spectra were obtained (Fig. 3a). From the ratio spectra, the first derivative of the ratio spectra was calculated with the intervals of $\Delta\lambda = 8$ nm, as could be seen in Fig. 3b. Two calibration graphs of PAR were established by measuring the signals at 237.0 and 251.8 nm corresponding to a maximum and a minimum, respectively, and were tested between 8–48 μg/ml for PAR and its binary mixtures with CAF, as could be seen in Table 3.

By using the same method for two compounds, the mean recovery and the relative standard deviation of the method were obtained as 100.6 and 1.13%, respectively, for CAF and also 100.1 and 1.61% for PAR in synthetic mixtures prepared by adding known amounts of CAF and PAR (Table 3).

Table 5 summarizes the regression coefficients and the linearity ranges of the calibration graphs, obtained by measuring the signals corresponding to the maximum and minimum wavelengths in the first derivative of the ratio spectra for both active compounds. For the determination of CAF and PAR in their synthetic mixtures and in the tablet formulation, the calibration graphs were only used which were obtained by measuring at 267.9 nm for CAF and 251.8 nm for PAR in the first derivative of the ratio spectra.

The main instrumental parameter conditions were optimized to obtain the most distinct curve of first derivative of the ratio spectra. For selecting a divisor of the appropriate concentration, some divisor concentrations were tested in the

determination. The standard solutions of 20 μg/ml of PAR and CAF for determining PAR and CAF in their binary mixtures were found suitable. The influence of $\Delta\lambda$ for obtaining the first derivative was tested and a value of $\Delta\lambda = 8$ nm was considered as suitable for both determinations.

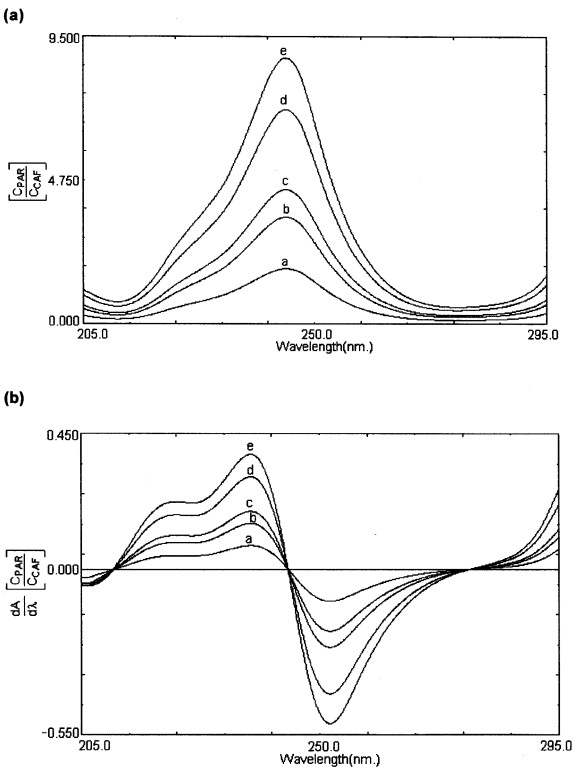

Fig. 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of paracetamol of, (a) 8 μg/ml, (b) 16 μg/ml, (c) 20 μg/ml, (d) 32 μg/ml, (e) 40 μg/ml, when 20 μg/ml caffeine used as divisor in 0.1M HCl ($\Delta\lambda = 8$ nm).

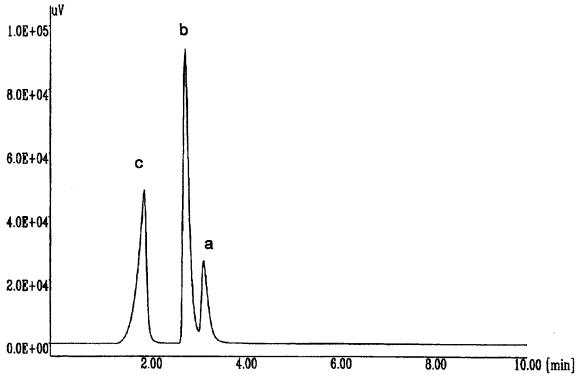

Fig. 4. Typical chromatograms of (a) caffeine and (b) paracetamol, (c) cetrimide as internal standard (IS).

4.3. HPLC method

HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of PAR and CAF, as a referee method for the developed spectrophotometric methods. Several mobile phase systems and different internal standards were tested for separation and determination of the compounds and mobile phase methanol-water (80:20) and cetrimide as internal standard were found suitable. At a flow of 1.0 ml/min, retention times for IS, PAR and CAF were 1.96, 2.84 and 3.22 min, respectively, (Fig. 4). The ratio of the peak areas analyte to IS were plotted against the concentration of PAR and CAF. In this case, a straight line was obtained. By using these calibration graphs, the content of PAR and CAF are determined in the sample containing PAR and CAF.

As could be seen in Table 4, in order to demonstrate the validity and applicability of the HPLC method, recovery studies were performed by analyzing synthetic mixtures of PAR and CAF which were prepared with different composition ratios. The mean recoveries and relative standard deviations of PAR and CAF were found as 100.6 and 1.20%, and 99.8 and 1.48%, respectively. For quantitative analysis, linearity ranges, regression equations and correlation coefficients were summarized in Table 5.

A good coincidence was observed for the assay results of the tablet dosage form by the application of the three methods proposed in this paper (Table 6). Statistical data was obtained by using Student's *t*-test and *F*-tests found no significant difference between performance of both methods as regards to accuracy and precision (Table 6).

5. Conclusion

In this work, although the individual UV absorption spectra of these two compounds overlap in the region of 210–310 nm (Fig. 1), the ratio

Table 4
Recovery data obtained for different mixtures by using HPLC

Mixture number	Paracetamol			Caffeine		
	Added (μg)	Found (μg)	Recovery[a] (%)	Added (μg)	Found (μg)	Recovery[b] (%)
1	32.0	31.8	99.4	2.0	2.0	100.0
2	32.0	32.9	102.8	4.0	4.0	100.0
3	32.0	31.9	99.7	8.0	8.1	101.3
4	32.0	32.0	100.0	12.0	11.8	98.3
5	32.0	31.8	99.4	20.0	20.3	101.5
6	32.0	33.0	103.1	28.0	27.7	98.9
7	8.0	8.1	101.3	4.0	4.0	100.0
8	16.0	15.9	99.4	4.0	4.1	102.5
9	24.0	24.1	100.4	4.0	4.0	100.0
10	32.0	32.0	100.0	4.0	3.9	97.5
11	40.0	40.2	100.5	4.0	4.0	100.0
12	48.0	48.4	100.8	4.0	3.9	97.5

[a] $\bar{x} = 100.6$, RSD = 1.20.
[b] $\bar{x} = 99.8$, RSD = 1.48.

Table 5
Calibration data in the determination of caffeine and paracetamol

Methods	λ (nm)	Linearity range (μg/ml)	Equation[a]	Regression coefficient (r)
Ratio spectra	237.0	8–48	$Y = 9.5\times10^{-3}\ C_{PAR} + 2.5\times10^{-3}$	0.9999
First derivative	251.8	8–48	$Y = 1.3\times10^{-3}\ C_{PAR} + 3.3\times10^{-4}$	0.9999
Spectrophotometry	267.9	4–40	$Y = 9.5\times10^{-3}\ C_{CAF} + 2.7\times10^{-4}$	0.9999
	291.0	4–40	$Y = 9.2\times0.10^{-3} C_{CAF} + 4.1\times10^{-4}$	0.9999
HPLC method	254.0	2–28	$Y = 2.2\times0.10^{-2}\ C_{CAF} + 4.7\times10^{-3}$	0.9996
	254.0	8–48	$Y = 1.2\times10^{-1}\ C_{PAR} + 5.0\times10^{-3}$	0.9998

[a] C_{PAR} = μg/ml of paracetamol, C_{CAF} = μg/ml of caffeine.

Table 6
Assay results in commercial product (mg)[a]

	Vierordt's method	Ratio spectra derivative spectrophotometry		HPLC
Paracetamol				
Mean ± SD	501.1 ± 0.6	500.21 ± 1.0		500.62 ± 07
$t_{calculated}$	0.112	0.153	$t_{theoretical}$:	2.26
$F_{calculated}$	0.473	0.376	$F_{theoretical}$:	($P = 0.05$)
Caffeine				
Mean ± SD	64.93 ± 0.4	65.4 ± 0.7		65.21 ± 0.6
$t_{calculated}$	0.108	0.142	$t_{theoretical}$:	2.26
$F_{calculated}$	0.447	0.865	$F_{theoretical}$:	($P = 0.05$) 3.18

[a] Results obtained are average of ten experiments for each, SD = standard deviation.

spectra derivative spectroscopy and Vierordt's method are suitable for the simultaneous determination of both drugs without prior separation from each other.

The HPLC method could be utilized for more specific than the spectrophotometric methods, but it is a more costly method. However, the methods are presently considered more reliable and promising for the routine analysis of CAF and PAR in pharmaceutical dosage forms.

References

[1] L.J. Rogres, M.J. Adams, Anal. Commun. 33 (1996) 401–402.
[2] A. Cerdan Vidal, A.R. Mauri Aucejo, C. Pascual Marti, M. Llobat Estelles, Anal. Lett. 27 (1994) 2317–2330.
[3] E. Dinç, F. Onur, J. Fac. Pharm. Gazi. 12 (1995) 63–72.
[4] E. Dinç, F. Onur, Anal. Lett. 28 (1995) 2521–2534.
[5] E. Dinç, F. Onur, Anal. Chim. Acta 359 (1998) 93–106.
[6] E. Dinç, Talanta, 48 (1999) 1145–1157.
[7] A. Bozdogan, A.M. Acar, G.K. Kunt, Talanta 39 (1992) 977–979.
[8] F. Onur, N. Acar, FABAD Farm. Bil. Der. 14 (1989) 1–8.
[9] R.D. Bautita, A.I. Jimenez, F. Jimenez, J.J. Arias, Anal. Lett. 29 (1996) 2646–2665.
[10] Xu Zhenzihi, Chen Guiliang, Zhhongguo Yiyao Gongye Zazhi Bianjibu 27 (1996) 313–315.
[11] M. Veerabhadrarao, M.S. Narayan, O. Kapur, J. Assoc. Off. Anal. Chem. 70 (1987) 578–582.
[12] F. Salinas, J.J. Berzas Nevado, A. Espinosa Mansilla, Talanta 37 (1990) 347–351.
[13] J.J. Berzas Nevado, J.M. Lemus Gallego, G. Castaneda Pehalvo, Fresenius J. Anal. Chem. 342 (1992) 723–728.
[14] J.J. Berzas Nevado, J.M. Lemus Gallego, G. Castaneda Pahalvo, J. Pharm. Biomed. Anal. 11 (1993) 601–607.
[15] J.J. Berzas Nevado, J. Rodriguez Flores, M.L. De La Morena Pardo, Talanta 38 (1991) 1261.
[16] J.J. Berzas Nevado, J. Rodriguez Flores, M.L. De La Morena Pardo, Analusis 21 (1993) 395–401.
[17] E. Dinç, F. Onur, Analusis 25 (1997) 55–59.
[18] E. Dinç, F. Onur, Anal. Lett. 30 (1997) 1179–1191.
[19] E. Dinç, F. Onur, STP Pharm. 8 (1998) 203–208.
[20] M.E. Mohamed, Anal. Lett. 19 (1986) 1323–1339.
[21] A. Heilmayer, Spectrophotometry in medicine, Adam Hilger, London, 1943, p. 7.
[22] A.L. Glenn, J. Pharm. Pharmacol. 12 (1960) 595–608.