ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 291–298

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Comparison of derivative spectrophotometric and liquid chromatograpic methods for the determination of omeprazole in aqueous solutions during stability studies

D. Castro *, M.A. Moreno, S. Torrado, J.L. Lastres

Dpto. Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Pza. Ramón y Cajal s/n, Madrid 28040, Spain

Received 29 September 1998; received in revised form 12 February 1999; accepted 28 February 1999

Abstract

A first derivative spectrophotometric method was developed for the determination of omeprazole in aqueous solutions during stability studies. The derivative procedure was based on the linear relationship between the omeprazole concentration and the first derivative amplitude at 313 nm. The first derivative spectra was developed between 200 and 400 nm ($\Delta\lambda = 8$). This method was validated and compared with the official high-performance liquid chromatography (HPLC) method of the USP. It showed good linearity in the range of concentrations studied (10–30 μg ml^{-1}), precision (repeatability and inter-day reproducibility), recovery and specificity in stability studies. It also seemed to be 2.59 times more sensitive than the HPLC method. These results allowed to consider this procedure as useful for the rapid analysis of omeprazole in stability studies since there was no interference with its decomposition products.

Keywords: Derivative UV spectrophotometry; High-performance liquid chromatography; Omeprazole; Validation; Stability studies

1. Introduction

Omeprazole, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, is a substituted benzimidazole that inhibits gastric secretion by altering the activity of H^+/K^+ ATPase, which is the final common step of acid secretion in parietal cells [1–3]. It is used in the treatment of peptic ulcers [4,5], reflux oesophagitis [6] and the Zollinger–Ellison syndrome [7,8]. It is a lipophilic, weak base with $pK_{a1} = 4{,}2$ and $pK_{a2} = 9$ and will be degraded unless it may be protected against acid conditions [2]. The number of decomposed products of omeprazole have been elucidated and characterised by Brändström et al. at different conditions [9]. Several methods such as HPLC [9,10], spectrophotometry [11], radioactivity [12], polarography [13], voltammetric method [14] and high-performance thin layer chromatography (HPTLC) [15] have

* Corresponding author. Tel.: +34-913-941727; fax: +34-913-941736.

E-mail address: marco@eucmax.sim.ucm.es (D. Castro)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00136-3

been developed for the determination of omeprazole.

In the last years, derivative techniques in UV spectrophotometry have been used as separative methods for the analysis of different commercial preparations [17–19] as well as in stability studies. Özaltin and Koçer [20] developed a method using second derivative UV spectrophotometry for the determination of omeprazole in pharmaceutical preparations, which has been used as a reference work to develop a derivative spectrophotometric procedure.

This paper describes derivative spectrophotometry and HPLC methods. The HPLC method is the official one proposed by the USP 23 [16], for the determination of omeprazole and for its quantification within bulk material. This procedure can be used in stability studies as there is no interference between the drug and its decomposition products. The derivative spectrophotometric procedure has been applied to quantify omeprazole in the presence of its decomposition products during stability studies unlike other methods such as conventional spectrophotometric methods [12] that can not be used in studies of this kind. Also, with the use of derivative spectroscopy, a simple, quick and accurate technique without preliminary separation procedures could be developed compared to some of the aforementioned methods, which were not suitable for routine analysis in laboratories, as they always require long term pre-treatment of the samples and expensive equipment that is not available in most control laboratories. This spectrophotometric derivative method will be compared with the HPLC one of the USP 23 [16].

2. Experimental

2.1. Materials

Omeprazole and dibasic anhydrous sodium phosphate were purchased from Merck (Darmstadt, Germany). HPLC grade methanol and acetonitrile were purchased from Symta (Madrid, Spain). Monobasic sodium phosphate, sodium borate, boric acid and sodium hydroxide were purchased from Panreac (Barcelona, Spain). Distilled, deionized water was obtained from Milli-Q water purification system (Millipore, USA) and used for the preparation of all aqueous solutions.

2.2. Apparatus and conditions

Absorption and derivative spectra were recorded over the wavelength range 200–400 nm in 1 cm quartz cells using a Beckman DU®-7 spectrophotometer. The derivative spectra were obtained at a slit width ($\Delta\lambda$) of 8 and 16 nm for the first and second derivative, respectively. The scan speed was of 300 nm min^{-1}.

The official HPLC method described in the USP 23 [21] allowed the separation of omeprazole from its decomposition products as well as the quantitation of the drug and so it was used to compare the results obtained with the derivative spectrophotometric method. For this HPLC technique, a Hewlett-Packard system consisting of a quaternary pump, with a HP 1050 programmable multiple wavelength detector set at 280 nm, was used. The chromatograms were recorded and the peak area responses were measured using a HP 3396 Series II Integrator. The separation was carried out at room temperature, on a reverse-phase C_8 Spherisorb Column of 200 × 4.6 mm ID and 10 μm particle size (Teknokroma Madrid, Spain). The mobile phase was a mixture of Phosphate buffer (6.04×10^{-3} M monobasic sodium phosphate and 3.15×10^{-2} M anhydrous dibasic sodium phosphate) and acetonitrile (75:25, v/v), filtered through 0.45 μm nylon filters, degassed and pumped at a constant flow rate of 2 ml min^{-1}. The injection volume was 100 μl for all standards and samples.

2.3. Standard solutions

For the derivative measurements, a stock solution was prepared by accurately weighing 50 mg of omeprazole into a 50 ml volumetric flask, dissolved and diluted to volume with methanol to obtain a concentration of 1 mg ml^{-1}. The stock solution was further diluted with methanol to reach a concentration range of 10–30 μg ml^{-1}.

For the HPLC measurements, a stock standard solution was prepared by dissolving the drug in sodium borate (0.01 M) and acetonitrile (75:25, v/v), to obtain a concentration of 1 mg ml^{-1}. Further dilutions were made by using the same diluent to give final concentrations in the range 10–30 μg ml^{-1}.

2.4. Sample solutions

In order to obtain 10%, 25% and totally composed omeprazole from 20 μg ml^{-1} sample solutions, 50 mg of omeprazole were accurately weighed for each sample solution, stirred and dissolved by adding 10 ml NaOH 0.1 N to obtain a concentration of 5 mg ml^{-1}. Four ml of these solutions were transferred to 100 ml volumetric flasks, diluted to volume with Palitzsch Borate buffer (pH 8; 0.2 M boric acid and 0.05 M borax) to reach a concentration of 200 μg ml^{-1} and stored at 37° for 6, 20 and 96 h to obtain the 10%, 25% and totally decomposed omeprazole, respectively. Finally, the 10%, 25% and totally decomposed omeprazole from 20 μg ml^{-1} sample solutions were prepared by appropriate dilutions with methanol and the mixture of sodium borate (0.01 M) and acetonitrile (75:25, v/v) for the derivative and HPLC measurements, respectively.

2.5. Determination of analytical parameters

2.5.1. Linearity

Linearity was evaluated by preparing five standards of omeprazole at different concentrations in the range 10–30 μg ml^{-1} for both derivative spectrophotometry and HPLC methods. Each measurement was carried out in triplicate. The relationship between the concentration of omeprazole and the variable measured, peak area in HPLC and absorbance in derivative spectrophotometry, was adjusted by means of least squares regression.

The precision of the slope relative to its size was also evaluated by calculating the relative standard error of the slope ($Sb_{rel}\%$) according to the following equation:

$$Sb_{rel}\% = Sb/b \times 100 \quad (1)$$

where Sb was the standard deviation of the slope.

The confidence limits for the slope (b) and the intercept (a) of the regression line were calculated from the following equations:

$$a \pm tS_a$$

$$b \pm tS_b$$

where t was the value of Student's t-test at $P = 0.05$ for n-2 degrees of freedom and S_a and S_b were the standard error of the intercept and the slope, respectively. Proportionality was achieved if zero was between the confidence limits for a.The resolution between two chromatographics peaks (R) was calculated from:

$$R = 2(t_2 - t_1)/W_1 + W_2 \quad (2)$$

where t_1 and t_2 were the retention times and W_1 and W_2 the respective widths of the peaks.

2.5.2. Precision

Repeatability was calculated by assaying six samples of the 100% standard concentration (20 μg ml^{-1}).

Reproducibility was calculated by comparing the results obtained in triplicate from three different omeprazole concentrations in triplicate within 3 different days.

2.5.3. Limit of detection (DL)

The calculated limits of detection were obtained from the following equation [22]:

$$DL = (S_0^2 n - 2/n - 1)^{1/2} x t_p/b \quad (3)$$

where n was the number of samples, t_p was the value of Student's t-test at $P = 0.05$ level of significance and (n-2) degrees of freedom, b was the slope and S_0^2 was the variance characterizing the dispersion of the points regarding the regression line.

The experimental detection limit was established as the concentration where a significant difference could be seen between a 20 μg ml^{-1} standard solution and a spiked sample (paired Student's t-test, $P > 0.05$) [23]. For its determination, the concentration of 20 μg ml^{-1} was

selected as this concentration was the 100% of omeprazole standard sample concentration used to assay the recovery and the precision of both methods.

2.5.4. Recovery

The accuracy of the method was assessed by spiking placebos in triplicate with known amounts of omeprazole at 75% (15 μg ml^{-1}), 100% (20 μg ml^{-1}) and 125% (25 μg ml^{-1}) of the standard solution concentration.

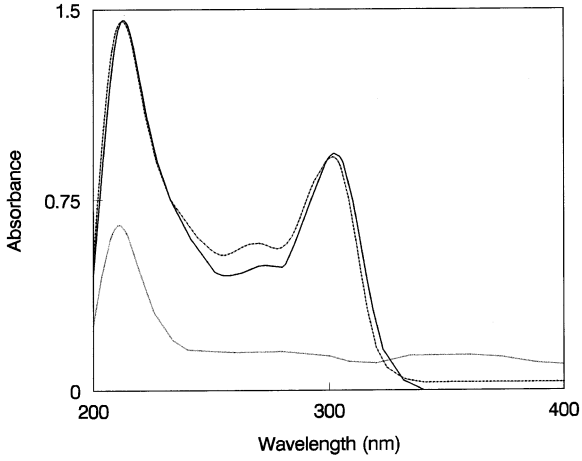

Fig. 1. Zero-order spectra of omeprazole (—), 10% decomposed omeprazole (– • –) and totally decomposed omeprazole (• • •) in 20 μg ml^{-1} methanol solutions.

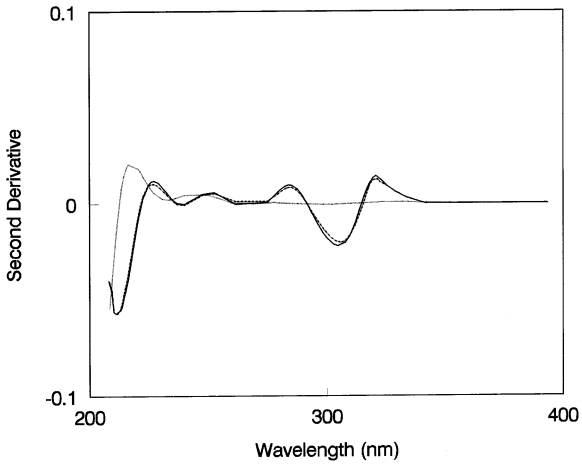

Fig. 2. Second derivative spectra of omeprazole (—), 10% decomposed omeprazole (– • –) and totally decomposed omeprazole (• • •)in 20 μg ml^{-1} methanol solutions.

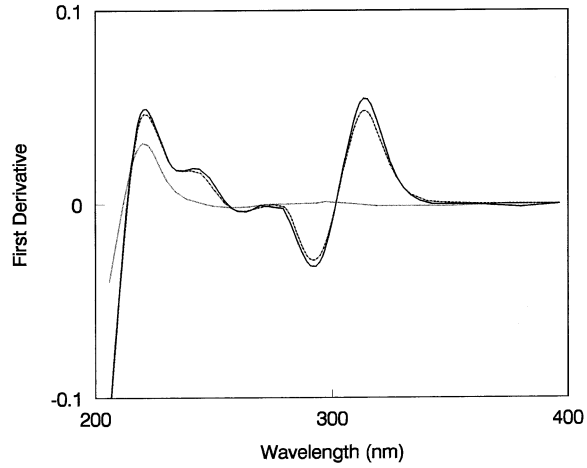

Fig. 3. First derivative spectra of omeprazole (—),10% decomposed omeprazole (– • –) and totally decomposed omeprazole (• • •) in 20 μg ml^{-1} methanol solutions.

3. Results and discussion

A comparative study of the different spectrophotometric procedures used to assay omeprazole solution recently prepared and partially decomposed omeprazole sample solutions, was carried out. For the conventional spectrophotometric procedure, the absorption spectra of omeprazole (20 μg ml^{-1}), 10% decomposed omeprazole and totally decomposed omeprazole in the 200–400 nm wavelength region are reported in Fig. 1. The spectra clearly displayed considerable overlap. Omeprazole showed a maximum at 300 nm while the decomposition products of omeprazole exhibited absorbance over the wavelength range 200–400 nm. The overlapping displayed by zero-order absorption spectra of 10% decomposed omeprazole in the wavelength range, was due to the absorptivity of the decomposition products at the working wavelength ($\lambda = 300$ nm). The absorbance values obtained were similar to the ones observed for the recently prepared omeprazole solution with a concentration of 20 μg ml^{-1} and hence no differences could be found between absorbances of omeprazole without decomposition and the 10% decomposed omeprazole ones at 300 nm. It was, therefore, impossible to determine omeprazole in the presence of its degradation products by reading the absorbances

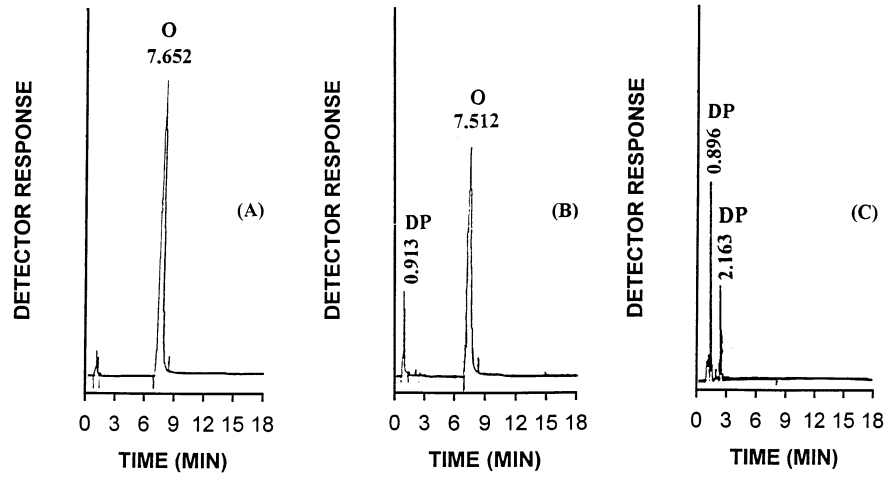

Fig. 4. HPLC chromatograms of 20 μg ml^{-1} omeprazole solution (A), 10% decomposed omeprazole solution (B) and totally decomposed omeprazole solution (C). Relative retention times for decomposition products (DP) and omeprazole (O).

Table 1
Analytical data of the calibration graphs for the determination of omeprazole by HPLC and first derivative spectrophotometry

Analytical methods	Linearity range (μg ml^{-1})	Regression equation ($Y=a+bC$)[a]	r^{2}[b]	S_a[c]	S_b[d]	$Sb_{rel\ (\%)}$[e]
HPLC	(10–30)	$Y=-130.53+70263.33C$	0.995	33272.70	1568.49	2.24
$^{1}D_{313}$	(10–30)	$Y=6.13\times10^{-4}+2.80\times10^{-3}C$	0.999	0.04	2.42×10^{-5}	0.86

[a] Absorbance and peak area values for ^{1}D and HPLC versus concentration (C) of omeprazole in μg ml^{-1}; standard solutions $n=15$.
[b] r^{2}, determination coefficient.
[c] S_a, standard deviation of intercept of regression line.
[d] S_b, standard deviation of slope of regression.
[e] $S_{b,rel\ (\%)}$, relative standard error of the slope.

Table 2
Inter-day reproducibilities ($n=3$) according to the two methods of determination

Analytical method	Concentration (μg ml^{-1})	Found ± SD (μg ml^{-1})[a]	RSD (%)[b]
HPLC	15	14.97 ± 0.23	1.52
	20	20.03 ± 0.33	1.65
	25	25.01 ± 0.34	1.39
$^{1}D_{313}$	15	15.12 ± 0.14	0.93
	20	20.02 ± 0.13	0.64
	25	24.98 ± 0.17	0.67

[a] Mean ± standard deviation of three determinations.
[b] Relative standard deviation.

Table 3
Concentration ranges, detection limits and calculated relative sensitivities of the proposed methods

Analytical method	Concentration range (μg ml^{-1})	Limit of detection (μg ml^{-1}) (calculated/experimental)	Relative sensitivity[a]
HPLC	(10–30)	1.27/1.0	2.59
$^1D_{313}$	(10–30)	0.49/0.5	1.0

[a] Calculated relative to the first derivative spectrophotometric method.

Table 4
Assay of partially decomposed omeprazole in Palitzsch Borate buffer (pH 8) at 37°C

Percentage of decomposed omeprazole	Decomposition time (h)	Omeprazole recovered (mean ± SD) (%)[a]	
		HPLC	$^1D_{313}$
10	6	90.87 ± 1.31	90.64 ± 0.61
25	20	76.14 ± 1.27	74.87 ± 0.63

[a] Mean of five determinations ± SD.

without interference within the 200–400 nm range in the original (zero order) spectra.

Özaltin and Koçer [20] developed a method using the second derivative UV spectroscopy for the determination of omeprazole in pharmaceutical preparations. For the work conditions used in this paper, second derivative spectra of omeprazole in the presence of 10% decomposed omeprazole was poorly resolved (Fig. 2). There were no differences between second derivative absorbance values of omeprazole without decomposition and 10% decomposed omeprazole, and so this fact prevented this method from its use as an analytical technique in stability studies. On the other hand, first derivative spectrophotometry offered an extremely valuable mean for the determination of the drug in partially decomposed omeprazole sample solutions. As shown in Fig. 3, for wavelengths over 250 nm, totally decomposed omeprazole samples did not interfere within the first derivative values at 313 nm, while 10% decomposed omeprazole showed a significant decrease in the first derivative absorbance values for this wavelength.

The first derivative technique was, therefore, used to quantify omeprazole in sample solutions during stability studies. In particular, the wavelength of 313 nm was selected as the optimum working parameter in which the measurements taken gave the best linear response to analyte concentration.

Brändström et al. [9] developed deep investigations on the reactions of omeprazole in different conditions and succeeded in isolating and characterising many of its intermediates and decomposition products. The USP HPLC method [21] did not allow for the separation between these different decomposition products of omeprazole although it was a useful technique to separate omeprazole from its decomposition products and for the quantitation of the drug. It was used as a reference analytical method for the first derivative technique. The mobile phase used in this method allowed for good resolution ($R = 3.30$) between the omeprazole peak and the one due to its decomposition product(s). Fig. 4 shows HPLC chromatograms of omeprazole (A) and 10% decomposed omeprazole (B) with retention times (t_R)of 7.512 min for the drug and 0.913 min for the decomposition product(s). Fig. 4(C) shows the chromatogram of totally decomposed omeprazole in which it was not any notice of the omeprazole peak ($t_R = 7.512$). However, a new peak corresponding to a new decomposition product(s) that appeared ($t_R = 2.163$) in the vicinity of the other decomposition product(s) peak ($t_R = 0.896$). As it can be seen, there is no interference from the decomposition products in the analysis of aqueous solutions of omeprazole during stability studies.

3.1. Linearity

Table 1 shows the statistical analysis of the experimental data: the regression equations from the calibration graphs, along with the standard deviations of the slopes and the intercepts and the relative standard error of the slope for the derivative spectrophotometric and HPLC methods.

The linearity of the first derivative spectrophotometric technique was evaluated within the range of concentrations 0–30 μg ml^{-1}. The same range was selected for the HPLC method in order to compare it with the first derivative spectroscopy.

The intercept values of the determination coefficients (r^2), as seen in Table 1, indicated good linearity of the calibration graphs for both methods.

The high values of the determination coefficients (r^2), as seen in Table 1, indicated good linearity of the calibration graphs for both methods.

3.2. Precision

3.2.1. Repeatability

Omeprazole recoveries ranged from 99.44 to 100.86%, with a mean of 99.87% and 0.53% RSD, for the first derivative spectrophotometric method. For the HPLC procedure, omeprazole recoveries ranged from 98.42 to 102.88% with a mean of 100.57 and 1.14% RSD. Since the first derivative method showed a lower RSD value than the chromatographic one, it was therefore considered to be more repeatable than the HPLC procedure.

3.2.2. Reproducibility

The inter-day reproducibility ($n = 3$) of the different methods for the determination of omeprazole is shown in Table 2. For the concentration range 15, 20, 30 μg ml^{-1}, the first derivative method showed lower RSD values than the ones obtained by the chromatographic method.

The RSD values obtained by the first derivative method for the reproducibility and repeatability were similar to the ones obtained in other derivative spectrophotometric techniques [24].

3.3. Limits of detection

The concentration ranges, quantitation limits and calculated relative sensitivities of the different methods are shown in Table 3. The detection limits for the first derivative spectroscopy and HPLC evaluated statistically were similar to those calculated according to the Eq. (3). The lowest experimental detection limit (0.50 μg ml^{-1}) was found for the first derivative spectroscopic method. In the theoretical studies, first derivative spectrophotometry showed more sensitivity (0.49 μg ml^{-1}) than the HPLC technique (1.27 μg ml^{-1}). The calculated relative sensitivity indicated that the first derivative was 2.59 times more sensitive than the HPLC procedure.

3.4. Recovery

The recoveries of omeprazole from placebo solutions (as indicated in Section 2.5) were 101.92, 100.86 and 101.94%, respectively for the first derivative spectrophotometric method; and 102.90, 101.21 and 99.19%, respectively for the HPLC method. The recovery results indicated that both first derivative and HPLC procedures were able to quantify omeprazole accurately at these concentrations. Similar values were obtained by Montgomery et al. [25].

3.5. Assay of omeprazole in the presence of its decomposition products

First derivative spectrophotometric and HPLC methods were applied for the recovery of partially decomposed omeprazole in aqueous solutions (Palitzsch Borate buffer: pH 8, 0.2 M boric acid and 0.05 M borax). The assays were carried out as described in the Section 2.4. Table 4 shows the recovery of omeprazole from sample solutions with different degrees of decomposed omeprazole. The results obtained by both methods were in good agreement with the real contents of omeprazole without any decomposition like in the sample solutions.

4. Conclusions

From the different methods studied, direct spectrophotometry and second derivative spectrophotometry were highly influenced by the decomposition products of omeprazole and were not suitable for the quantitation of omeprazole in stability studies. However, the first derivative procedure was successfully applied to quantify omeprazole in such studies. This method was validated in comparison with the official of the USP 23. In the range of concentrations studied (10–30 μg ml^{-1}), both methods showed good linearity values but the spectrophotometric method also had higher repeatability, reproducibility and sensitivity than the chromatographic analytical procedure. The first derivative and HPLC methods showed good recovery and specificity. These results demonstrated that first derivative spectrophotometry is a useful technique for the rapid analysis of omeprazole in an aqueous solution during stability.

References

[1] D. McTavish, M.T. Buckley, R.C. Heel, Drugs 42 (1991) 138–170.
[2] P.N. Maton, N. Engl. J. Med. 324 (1991) 965–975.
[3] B. Wallmark, P. Lorentzon, H. Larsson, Scand. J. Gastroenterol. 20 Suppl. 108 (1985) 37–52.
[4] R.N. Brogden, A.A. Carmine, R.C. Heel, T.M. Speight, G.S. Avery, Drugs 24 (1982) 267–302.
[5] K. Lauritsen, S.J. Rune, P. Bytzer, H. Helbaek, K.G. Jansen, N. Engl. J. Med. 312 (1985) 958–961.
[6] J.G. Hatlebakk, A. Berstad, Clin. Pharmacokinet. 31 (1996) 386–406.
[7] A. Blanchi, J.C. Delchier, J.C. Soule, D. Payen, J.D. Bader, Lancet 2 (1982) 1223–1224.
[8] K. Oberg, H. Lindstrom, Lancet 1 (1983) 66–67.
[9] A. Brändstrom, P. Lindberg, N-K. Bergman, T. Alminger, K. Ankner, U. Junggren, et al., Acta Chem. Scand. 43 (1989) 538–548.
[10] P.O. Langestrom, B.A. Persson, J. Chromatogr. 309 (1984) 347–356.
[11] W. Watanabe, K. Furuno, K. Eto, R. Oishi, Y. Gomita, J. Pharm. Sci. 83 (1994) 1331–1334.
[12] S.N Dhumal, P.M. Dikshit, J. Indian Drugs 28 (1991) 565–567.
[13] J. Naesdol, T. Anderson, G. Bodemer, R. Larsson, C.G. Regardh, I. Skanberg, et al., Clin. Pharmacol. Ther. 40 (1986) 1523–1530.
[14] N. Ozaltin, A. Temizer, Electroanalysis 6 (1994) 799–803.
[15] S. Panzauti, P. Gratteri, S. Furlaneto, P. Mura, E. Dreassi, R. Phan-Tan Luu, J. Pharm. Biomed. Anal. 14 (1996) 881–889.
[16] S. Mangalan, B.P. Ronanbhai, K. Balkriskna, J. Planar Chromatogr. Mod. 4 (1991) 492–493.
[17] F. Onur, N. Acar, S.T.P. Pharma. Sci. 5 (1995) 152–155.
[18] B. Morelli, J. Pharm. Sci. 77 (1988) 1402–1405.
[19] S. Torrado, S. Torrado, R. Cadorniga, J. Pharm Biomed. Anal. 12 (1994) 383–387.
[20] N. Özaltin, A. Koçer, J. Pharm. Biomed. Anal. 16 (1997) 337–342.
[21] USP 23-NF 18, Third Supplement, United States Pharmacopeia Convention, Inc., Rockville, MD, 1995, pp. 2959–2960.
[22] J.N. Miler, Analyst 116 (1991) 3–14.
[23] R. van Gijn, V.M.M. Herben, M.J.X. Hillebrand, W.W. ten Bokkel Huinink, A. Bult, J.H. Beijnen, J. Pharm. Biomed. Anal. 17 (1998) 1257–1265.
[24] S. Ouanês, M. Kallel, H. Trabelsi, F. Safta, K. Bouzouita, J. Pharm. Biomed. Anal. 17 (1998) 361–364.
[25] E.R. Montgomery, S. Taylor, J. Segretario, E. Engler, D. Sebastian, J. Pharm. Biomed. Anal. 15 (1996) 73–82.