Available online at www.sciencedirect.com
SCIENCE DIRECT®
Spectrochimica Acta Part A 60 (2004) 1769–1774
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Spectrophotometric microdetermination of nefopam, mebevrine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins

S.A. Shama*, A.S. Amin

Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

Received 16 June 2003; accepted 31 July 2003

Abstract

Simple and rapid spectrophotometric procedures have been established for quantitation of nefopam hydrochloride (NF) mebevrine hydrochloride (MB) and phenylpropanolamine hydrochloride (PP). The procedures are based on the reaction between the examined drugs (NF, MB and PP) and alizarin (I), alizarin red S (II), alizarin yellow G (III) and quinalizarin (IV) producing ion-pair complexes which can be measured at the optimum wavelength. The optimization of the reaction conditions is investigated. Beer's law is obeyed in the concentration ranges 0.5–30.0 $\mu g\,ml^{-1}$. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. The correlation coefficient was $\geq$0.9988 ($n = 6$) with a relative standard deviation (R.S.D.) of $\leq$1.3, for six determinations of 20 $\mu g\,ml^{-1}$. The methods are successfully applied to the determination of NF, MB and PP in their pharmaceutical formulations.

Keywords: Alizarins; Ion-pair complexes; Spectrophotometry; Dosage forms

1. Introduction

Nefopam hydrochloride (NF) (3,4,5,6-tetrahydro-5-methyl-1-phenyl-1-H-2,5-benzoxazoncine hydrochloride) (13669-70-0) is analgesic and anti-inflammatory agents [1]. It has some antichlolinergic and sympathomimetic actions [2]. It is used for the relief of acute and chronic point [3]. Several methods have been applied in the literature for the determination of nefopam hydrochloride in dosage forms and in biological fluids. The different techniques used in this action include gas chromatography [4,5], spectrophotometrically [6] and colorimetry [7].

Mebevrine hydrochloride (MB) acid 3,4-dimethoxybenzoic acid 4-(ethyl-2-(4-methoxyphenl)-1-methylethyl)amino) butylveratrate hydrochloride (2753-45-9) is used as a gastro-intestinal antispasmodic in conditions such as the irritable bomel ayndrme [8]. Many procedures are known for the qualitative detection and for quantitative determination of mebevrine hydrochloride. Among the several analytical methods are spectrophotometrically [9], colorimetry [10], HPLC [11,12], TLC-denstitometry [13] and gas chromatographic mass spectrometric methods [14].

Phenylpropanolamine hydrochloride (PP), 2-amino-1-phenyl-1-ol (14838-15-4) is a largely indirect acting sympathomimetic agent and less active as a central nervous stimulant [15]. Several methods have been reported for the quantitative determination of phenylpropanolamine hydrochloride in pure and in dosage forms, including spectrophotometry [16,17], capillary electro-phoresis [18], HPLC [19], MC [20] and fluorimetry [21].

In order to continue our work for using different alizarin compounds for drug analysis [22,23], a simple accurate, economic, sensitive, more essential and less-time consuming spectrophotometric method for the determination of the drugs under investigation in pure and in their dosage forms are performed.

2. Experimental

2.1. Apparatus

A JASCO 530 V spectrophotometer with a 10 mm quartz cell was used for all spectrophotometric measurements

* Corresponding author. Fax: +20-13-222978.
E-mail address: atefab@yahoo.com (S.A. Shama).

1386-1425/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2003.07.012

and an Orion research model 601 A/digital ionalyzer was used for checking the pH of phosphate buffer solutions of pH values 2.0–12.0 prepared by the recommended method [24].

2.2. Reagents

Alizarin, 1,2-dihydroxyanthraquinone (I), alizarin red S, 3,4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (II), alizarin yellow G, 5-(4-nitrophenylazo) salicylic acid (III) and quinalizarin 1,2,5,8-tetrahydroxy-anthraquinone (IV) were Aldrich products and used without further purification. A stock solution (2×10^{-3} M) was prepared by dissolving the appropriate weights of II and III in doubly distilled water, while that for I and IV were dissolved [22,23] in slightly alkaline media (0.001 M NaOH).

Egyptian International Pharmaceutical Industrial Company, Egypt, supplied NF and MB and their pharmaceutical formulations (i.e. Acupan injection and tablets (20 mg) and Colospasmin tablets (135 mg)). Glaxowellcome Company, Egypt supplied PP and its pharmaceutical formulations (i.e. Flurest tablets (24 mg) and syrup (10 mg)).

2.3. General procedure

An aliquot containing from 2.0 to 30 μg of NF, MB and PP were transferred into 10 ml calibrated flask 1.0 ml of 2×10^{-3} M of reagents I, II and IV for NF, PP and MB. 0.5 ml of III for MB was added to each flask. Four milliliters of the optimum buffer media (phosphate) of the optimum pH value for each system as recorded in Table 1 and 2.0 ml of acetone was added in case of NF with reagent II and 2.0 ml of methanol was added in case of PP with reagents I and II to achieve 20% (v/v) in the final assay solution. For each system, the optimum time and temperature as recorded in Table 1 is attained and completed each flask to 10 ml with water. The absorbance was measured for each system at the optimum wavelength (Table 1) against a reagent blank prepared in the same way without addition of the examined drug.

2.4. Application to various dosage forms

At least 10 tablets of the drug were weight into a small dish, powdered and mixed well. A portion equivalent to 100 mg was weight and dissolved in 100 ml water, shaken well and filtered through a sintered glass crucible G_4. The clear solution was diluted to 250 ml with water in a 250 ml calibrated flask. The drug content of this solution was obtained by applying the general procedure to aliquot containing 30 μg ml^{-1} of the drug as described above. For injection, the contents of five ampoules (100 mg of NF) were quantitatively transferred into 250 ml calibrated flasks completed to the mark with water. For syrup (PP), the same procedures were done.

2.5. Stoichiometric relationship

Job's method of continuous variation was employed, a 2×10^{-3} M standard solution of NF, MB or PP and 2×10^{-3} M solution of reagent (I–IV) were used. A series of solution were prepared in which the total volume of drug and reagent was kept at 2.0 ml. The reagents were mixed in various proportions and diluted to volume in a 10 ml calibrated flask with the appropriate solvent following the above mentioned procedures.

3. Results and discussion

3.1. Optimization

Careful investigations were carried out to establish the most favourable conditions to achieve maximum colour intensity in the quantitative determination of the examined drug (NF, MB and PP). The absorption spectra of NF, MB and PP and their complexes with alizarin derivatives (I–IV) under the optimum conditions are shown in Figs. 1–3 and recorded in Table 1. The absorption band of NF, MB and PP complexes are located at 566, 525, 410 and 582 nm, 553, 483, 391 and 586 nm and 606, 576, 427 and 624 nm with reagents (I–IV), respectively. However, in all instances, the

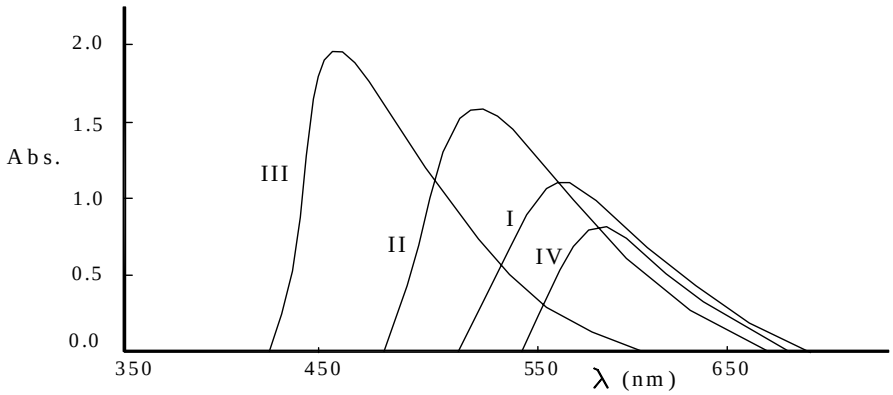

Fig. 1. Absorption spectra of the ion-pair of NF complexed with reagents (I–IV) against blank.

Table 1
Quantitative parameters for the complexation of NF, MB and PP with alizarin derivatives (I–IV)

Parameters	NF				MB				PP			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
pH	7.5	6.5	6.5	7.5	75	6.5	8.2	8.2	5.88	6.5	6.5	8.2
λ_{max} (nm)	566	525	410	582	553	483	391	586	606	576	427	624
Reagent (ml) (2×10^{-3})	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Solvent ratio (%)	H_2O	Acetone	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O	Methyl	Methyl	H_2O	H_2O
Time (min)	1.0	20	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Temperature (°C)	25	40	25	25	25	25	25	25	25	25	25	25
Beer's law limits ($\mu g\,ml^{-1}$)	0.5–20	0.5–25	0.5–30	0.5–25	0.5–28	0.5–28	0.5–30	0.5–25	0.5–20	0.5–20	0.5–25	0.5–18
Ringbom concentration ($\mu g\,ml^{-1}$)	1–18	1–20	1–28	1–20	1–25	1–25	1–27	1–20	1–16	1–17	1–20	1–15
Molar absorbance ($l\,mol^{-1}\,cm^{-1}$) $\times 10^3$	4.2	4.6	8.2	5.1	5.9	8.6	19.3	5.6	1.2	3.2	4.6	1.1
Sandell sensitivity ($\mu g\,cm^{-2}$)	0.060	0.065	0.031	0.049	0.043	0.029	0.027	0.045	0.211	0.079	0.059	0.225
Detection limit ($\mu g\,ml^{-1}$)	0.25	0.36	0.49	0.39	0.22	0.45	0.5	0.44	0.21	0.26	0.24	0.36
Quantification limit ($\mu g\,ml^{-1}$)	1.02	1.54	1.96	1.59	0.89	1.69	2.01	1.72	0.91	1.05	1.03	1.49
Regression equation[a]												
Slope	0.032	0.011	0.013	0.016	0.019	0.011	0.011	0.021	0.030	0.026	0.014	0.011
Intercept	−0.001	0.0013	0.0013	0.0022	0.0016	0.0031	0.0022	0.0016	0.0031	0.0011	0.0012	0.0012
Correlation coefficient (r)	0.9969	0.9849	0.9990	0.9980	0.9989	0.9969	0.9869	0.9990	0.9981	0.999	0.9981	0.9990
R.S.D.(%)[b]	1.00	0.90	1.20	1.10	0.80	0.90	1.20	1.10	1.20	1.3	1.20	1.10
Range of error (%)	1.40	1.20	1.60	1.50	1.00	1.20	1.60	1.80	1.60	1.90	1.60	1.40

[a] $A = a + bc$, where c is the concentration of drug in $\mu g\,ml^{-1}$.
[b] Average of six determinations.

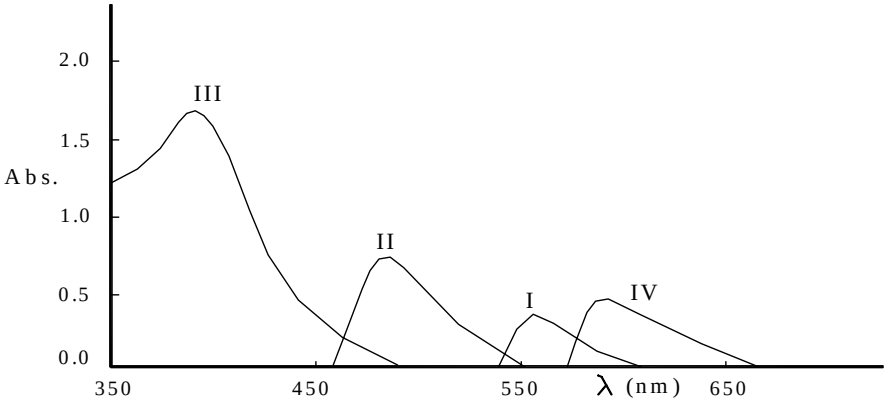

Fig. 2. Absorption spectra of the ion-pair of MB complexed with reagents (I–IV) against blank.

absorbance was measured at those λ_{max} against a reagents blank prepared under identical conditions. The influence of each of the following variables on the reaction was tested.

3.1.1. Effect of pH

Different buffer media (universal, thiel, phosphate, borate and acetate buffer solutions) [24] were examined to achieve maximum colour intensity. Phosphate buffer proved to be the most favourable one due to its highly absorbance values in addition to instantaneously formation of the ion-pair complex without affecting the absorbance in the pH range 5.5–8.5. Moreover, the optimum volume of buffer solution added to 10 ml to give constant absorbance value was also studied and found to be 4.0 ml. For NF complexes, the optimum pH were 7.5, 6.5, 6.5 and 7.5, for MB complexes the optimum pH were 7.5, 6.5, 8.2 and 8.2 and for PP complexes, the optimum pH were 5.88, 6.5, 6.5 and 8.2 for I, II, III and IV reagents, respectively.

3.1.2. Effect of the reagent concentrations

The effect of reagents was investigated by taking various amount of 2×10^{-3} M of reagent added to an aliquot of solution containing 40 μg ml^{-1} of different drugs under investigation (NF, MB and PP), was increased from 0.2 to 2.0 ml. The maximum absorbance was observed with the addition of 1.0 ml of all reagents with different drugs except in case of MB drug, 0.5 ml of reagent III was sufficient to achieve the maximum absorbance value.

3.1.3. Effect of solvent

Various organic solvents were used (methanol, ethanol, propanol, isopropanol, acetone, dioxane and dimethylformamide), 20% acetone gave the maximum colour intensity for NF drug with II reagent and stabilized the formed complex for a long time, 20% methanol was the suitable solvent for PP drug with I and II reagents (Table 1). The higher level of acetone and methanol than 20% caused a decrease of absorbance value. Furthermore, the aqueous solution was the favourable conditions to give the highly intense colour and to achieve maximum colour development (NF with I, III and IV, MB with all reagents and PP with III and IV reagent, respectively) (Table 1).

3.1.4. Effect of time and temperature

The optimum reaction time was determined by following the colour intensity and maximum colour development

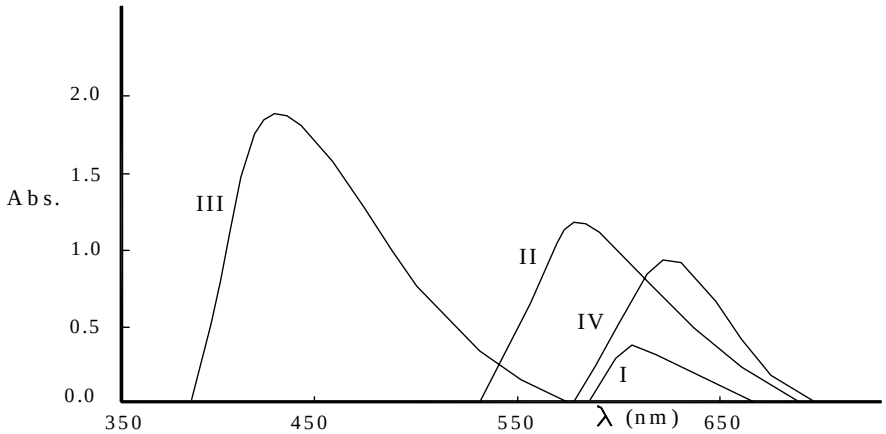

Fig. 3. Absorption spectra of the ion-pair of PP complexed with reagents (I–IV) against blank.

was obtained at 25 ± 2 °C. Also, the colour development was attained after, 1.0 min. For all drugs with different reagents at 25 ± 2 °C. But the colour remains stable with no changes of absorbance for 6.0 h. In case of NF with II reagent the optimum condition was attained after 20.0 min. At 40 ± 2 °C. No change in the absorbance relative to time on raising the temperature up to 40 °C, above which, absorbance begin to fade slowly with increasing the temperature.

3.1.5. Sequence of addition

The optimum sequence was defined by following to colour intensity and maximum absorbance on changing the sequences of addition of drug, reagent and buffer. The best condition was "drug-reagent-buffer-solvent" for the highest absorbance and stability. Other sequences needed longer time in addition to lower stability.

3.2. The stoichiometry of the complex

The stoichiometry of he complex formed between reagents (I–IV) with drugs (NF, MB and PP) was investigated at the pH range 5.88–8.50, by the continuous variation method [25]. The result indicates the existence of 1:1 charge transfer complex at a definite λ_{max} recorded in Table 1. The conditional stability constants ($\log K$), calculated with Harvey and Manning equation [26] applying the data obtained from the continuous variation method (Table 1).

3.3. Interference

No interference was observed in the determination of NF, MB or PP with different reagents (I–IV) from the presence of lactose, glucose, sacarose, glycerol, fructose, sugar, urea, organic acids, proplene glycol, magnesium streate and starch.

Table 2
Analysis of NF, MB and PP using alizarin derivatives (I–IV)

Drug taken ($\mu g\,ml^{-1}$)	Reagents											
	I			II			III			IV		
	NF	MB	PP	NF	MB	PP	NF	MB	PP	NF	MB	PP
2												
Found[a] ($\mu g\,ml^{-1}$)	2.01	2.01	2.0	1.99	1.98	1.99	2.02	2.01	1.99	1.98	2.03	2.04
R (%)	100.5	100.5	100	99.5	99.0	99.5	101.0	100.5	99.5	99.0	101.5	102.0
±S.D. (%)	±0.51	±0.99	±0.62	±0.43	±0.63	±0.49	±0.84	±0.63	±0.32	±0.62	±0.33	±0.26
6												
Found[a] ($\mu g\,ml^{-1}$)	5.99	5.96	5.94	6.01	6.01	6.03	5.98	5.97	5.96	5.98	5.96	9.99
R (%)	99.8	99.3	99.0	100.16	100.16	100.5	99.6	99.5	99.3	99.6	99.3	99.8
±S.D. (%)	±0.39	±0.74	±0.62	±0.65	±0.52	±0.33	±0.74	±0.55	±0.43	±0.35	±0.29	±0.70
10												
Found[a] ($\mu g\,ml^{-1}$)	9.99	9.98	9.99	10.02	10.02	10.03	10.01	10.03	10.02	10.00	10.01	10.00
R (%)	99.9	99.8	99.9	100.2	100.2	100.3	100.1	100.3	100.2	100.00	100.1	100.00
±S.D. (%)	±0.49	±0.55	±0.65	±0.44	±0.90	±0.85	±0.69	±0.62	±0.91	±0.26	±0.61	±0.66
14												
Found[a] ($\mu g\,ml^{-1}$)	14.03	14.02	13.91	13.92	13.89	13.96	13.89	13.88	13.89	14.02	14.09	14.5
R (%)	100.2	100.1	99.3	99.4	99.2	99.7	99.2	99.1	99.2	100.1	100.6	103.5
±S.D. (%)	±0.22	±0.63	±0.61	±0.61	±0.32	±0.55	±0.62	±0.61	±0.42	±0.34	±0.43	±0.44
18												
Found[a] ($\mu g\,ml^{-1}$)	18.04	18.05	18.03	18.00	18.01	18.02	17.92	17.89	17.92	17.92	17.91	17.93
R (%)	100.2	100.2	100.2	100.0	100.1	100.1	99.6	99.4	99.6	99.6	99.5	99.6
±S.D. (%)	±0.71	±0.61	±0.33	±0.62	±0.61	±0.43	±0.41	±0.49	±0.63	±0.23	±0.41	±0.46
22												
Found[a] ($\mu g\,ml^{-1}$)	22.06	22.01	22.08	22.07	22.05	22.07	22.00	22.00	22.09	21.86	21.91	21.9
R (%)	100.3	100.0	100.3	100.3	100.02	100.3	100.0	100.0	100.4	99.3	99.5	99.5
±S.D. (%)	±0.29	±0.21	±0.32	±100.3	±0.11	±0.52	±0.44	±0.62	±0.49	±0.36	±0.62	±0.22
26												
Found[a] ($\mu g\,ml^{-1}$)	26.05	26.01	26.09	15.94	25.86	25.89	26.00	26.0	26.05	25.79	25.86	25.91
R (%)	100.2	100.0	100.3	99.6	99.4	99.5	100.0	100.0	100.02	99.2	99.4	99.6
±S.D. (%)	±0.41	±0.32	±0.36	±0.46	±0.66	±0.61	±0.49	±0.44	±0.69	±0.61	±0.43	±0.46
30												
Found[a] ($\mu g\,ml^{-1}$)	30.09	30.1	30.11	29.91	29.81	29.85	29.91	29.92	29.95	29.91	29.92	29.86
R (%)	100.3	100.3	100.4	99.7	99.4	99.5	99.7	99.6	99.8	99.7	99.8	99.6
±S.D. (%)	±0.66	±0.59	±0.49	±0.43	±0.22	±0.44	±0.66	±0.64	±0.29	±0.22	±0.62	±0.24

[a] Average of six determinations.

The results indicate that up to 100-fold excess of them do not interfere (absorbance changes by ±2.0% is non-interference) which may be present in its pharmaceutical preparations. The proposed method is simple, rapid, sufficiently selective and highly sensitive.

3.4. Analytical data

Beer's law limits (0.5–30 μg ml^{-1}) with a correlation coefficient ≤0.9960, molar absorptivity, Sandell sensitivity, regression equation and standard deviation obtained by linear least, square treatment of the results are given in Table 1. For more accurate results, Ringbom [27] optimum concentration recorded in Table 1. Recoveries and range of error percentages are also calculated and recorded in Table 2. Therefore, it can be concluded that the results of the present method are in high agreement with those obtained by the official methods [28,29].

3.5. Analytical applications

The proposed method was successfully applied to various dosage forms, viz. Tablets (nefopam, mebevrine and phenylpropanolamine hydrochloride), injection (nefopam) and syrup (phenylpropanolamine hydrochloride).

The results are recorded in Table 1 compared statistically with the official method [28,29] reveal that the recoveries are in the range (100.5–99.0%) reflecting a high accuracy, in addition to the high precision indicated by very low values of relative standard deviations.

The performance of the proposed method was assessed by calculation of *t* and *f* values compared with the official methods [28,29]. Mean values obtained in students [30] showed the absence of systematic errors in the method.

References

[1] R.C. Heel, Drug Ther. Bull. 17 (1980) 59.
[2] S.J. Wroe, Br. Med. J. 292 (1986) 1672.
[3] D.M. Piercy, Br. Med. J. 283 (1981) 1508.
[4] G.G. Mather, R. Labroo, M.E. Le Guren, F. Lepage, J.M. Gillardin, R.H. Levy, Chirality 3 (2000) 153.
[5] B. Lu, T.S. Shi, Yoown Fenxi Zazhi 15 (1995) 22.
[6] B. Lu, T.S. Shi, Yoown Fenxi Zazhi 5 (1995) 54.
[7] R. Cai, M.M. Ye, C. Xia, Yoown Fenxi Zazhi 14 (1994) 46.
[8] G. Bertaccini, Farmaco. Ed. Sci. 30 (1990) 823.
[9] A.F.M. El-Wality, A. Gindy, M.F. Bedair, J. Pharm. Anal. 21 (1999) 535.
[10] M.N. Reddy, D.G. Sankar, Mikrochim. Acta 126 (1997) 131.
[11] M.N. Reddy, K.V.S. Rao, D.B. Sanrar, K. Sridhar, India Drugs 33 (1996) 604.
[12] O. Al-Deeb, B.M. Hadiya, S.A. Foda, N. H. Chromotographia 441 (1997) 427.
[13] L.J. Tulich, J.L. Rnadall, B.R. Kelm, K.R. Wehmeyer, J. Chromatogr. B Biomed. 682 (1996) 273.
[14] E.M. Osman, A.A. Gazy, M.M. Bedair, Drug Dev. Ind. 21 (1995) 633.
[15] S.M. Mueller, New Engl. J. Med. 308 (1983) 653.
[16] M.F. Zauter, N. Gujib, Y. Tahboub, E. Ghanem, J. Anal. Chem. 54 (1999) 1158.
[17] M. Gil-Agusti, J.R. Laposio, M.C. Garcin-Alvariz-Coque, J. Estervo, J. Chromotogr. 866 (2000) 35.
[18] M.R.S. Fuh, Y.T. Lu, Talanta 48 (1999) 415.
[19] P. Vinas, C. Lopez Erroz, F.J. Cerdan, M. Cordoba, Talanta 47 (1998) 455.
[20] D.H. Khanolkar, V.M. Shinde, India Drugs 36 (1999) 438.
[21] Y. Nakahara, R. Kikura, J. Chromotogr. B Biomed. Appl. 700 (1997) 83.
[22] S.A. Shama, J. Pharm. Biomed. Anal. 30 (2002) 1385.
[23] A.S. Amin, J. Pharm. Biomed. Anal. 29 (2002) 729.
[24] H.T.S. Britton, Hydrogen Ions, fourth ed., Chapman & Hall, London, 1952.
[25] P. Job, Anal. Chim. 9 (1928) 113.
[26] A.E. Harvey, D.L. Manning, J. Am. Chem. Soc. 71 (1950) 4488.
[27] A. Ringbom, Z. Anal. Chem. 115 (1939) 332.
[28] United States Pharmacopia, Twentieth Review, The National Formulary, Ninteenth Review, The United States Pharmacopeial Convention, Rockville, MD, 2000.
[29] British Pharmacopoeia, Her Majasty's, Stationary Office, London, 1998.
[30] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood, Chichester, UK, 1993.