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Short communication

Determination of norfloxacin spectrophotometrically using 2,4-dinitrofluorobenzene

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1. Introduction

Norfloxacin,1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid, is one of the broad-spectrum fluoroquinolone synthetic antibiotics [1] which was developed during the last decade. Quinolones are active against many gram-positive and gram-negative bacteria [2]. Norfloxacin finds widespread use in the treatment of urinary tract infections [3].

Norfloxacin (NOR) and its tablets are listed in the USP XXIII [4], and the European Pharmacopoeia 1997 [5]. The USP XXIII described an HPLC method for norfloxacin determination in its tablet form, while the European Pharmacopoeia used a non-aqueous titration procedure using perchloric acid as titrant. The analytical profile of norfloxacin and a significant number of references for its determination in pharmaceutical dosage forms and biological fluids have been published [6]. Norfloxacin is generally determined in biological fluids by high performance liquid chromatographic (HPLC) [7–10] and by microbiological methods [11]. Several analytical methods were reported for the determination of norfloxacin in pharmaceutical dosage forms. These methods include direct spectrophotometry [12], ion pair formation [13]and after reaction with ferric ion [14] or *p*-benzoquinone [15]. Other methods for quantitating norfloxacin comprise HPLC [16–18], fluorimetry [19–21], titrimetry [22] and differential scanning potentiometry [23].

In aqueous borate buffer, the 2,4-dinitrofluorobenzene (DNFB) or Sanger's reagent, as an activated halide, has been used for the determination of amines, phenols and thiols to yield intensely yellow colored substances, which permits their spectrophotometric determination [24]. At the same time and in dipolar aprotic solvent, the DNFB reagent, as a dinitrophenyl derivative, has

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been found to react with different amines to produce a Meisenheimer-type complex [25].

The norfloxacin molecule features a piperazine ring, at the 7th position of the quinolone carboxylic acid, which contains tertiary and secondary amine groups. This structure suggests the possibility of utilizing the DNFB reagent as a chromogenic agent, for the determination of norfloxacin in its pharmaceutical tablets, in aqueous borate buffer and in dimethyl sulphoxide (DMSO) as an aprotic polar solvent. The objective of the present work was, therefore, to develop a fast and direct spectrophotometric method for the determination of norfloxacin in tablets. The experimental conditions were studied and incorporated into the procedures. The proposed procedures have been successfully applied to the analysis of pharmaceutical tablets and the results obtained have been statistically compared with those obtained using the official method (USP XXIII [4]).

2. Experimental

2.1. Materials and methods

Norfloxacin (Chem Iberica, Barcelona, Spain) was obtained from Pharco Pharmaceutical (Alexandria, Egypt) and has been certified to contain 98.50%. The powder was used without further purification. The commercial tablets containing norfloxacin (400 mg per tablet) were bought from the local market. Acetone, DMSO, and sodium tetraborate (borax) were analytical reagent grade and were purchased from BDH (Poole UK). The DNFB (Hopkin & Williams, Essex, UK) was prepared as a 1.3% v/v stock solution in acetone for procedure I and as a 0.2% v/v working solution in DMSO for procedure II. The aqueous working reagent was prepared by diluting the stock acetone solution in a ratio of 1:10 with borate buffer of pH 9. The reagents were protected from light and stored in a refrigerator. Norfloxacin standard solutions were prepared as 500 μg ml^{-1} solutions in distilled water (procedure I) and as a 100 μg ml^{-1} solution in DMSO (procedure II). The decolorizing solution was prepared as 0.5 M solution from a saturated solution of hydrochloric acid gas in dioxan.

Spectral measurements were taken with a Perkin-Elmer Model 550S spectrophotometer (Norwalk, CT) using 1-cm pathlength quartz cuvettes. The spectrophotometer was attached to a Hitachi Model 561 recorder with a scan speed of 60 nm min^{-1}. A thermostated water-bath, accurate to ± 0.5°C, was utilized throughout.

2.2. Treatment of analytical data

Sandell's sensitivity is the concentration of the drug (μg ml^{-1}) which gives 0.001 absorbance [26].

The R.S.D. of the slope [27] is:

$$S_{b\ \mathrm{rel}}\ (\%) = (S_b/b) \times 100$$

The detection limit [27] (DL) is:

$$\sqrt{(S_o^2(n-2/n-1))}tp/b$$

where n is the numbers of values, tp is the value of the Student t-test at $P = 0.05$ level of significance and $n-2$ degree of freedom; b is the slope, and S_o^2 is the variance characterizing the dispersion of the points with respect to the regression line.

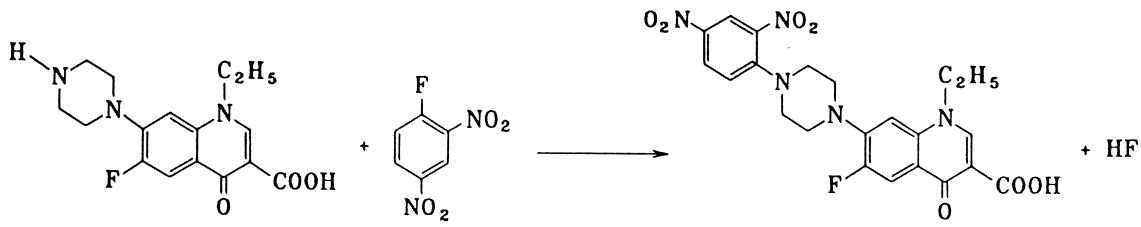
2.3. General procedures

2.3.1. Procedure I

From the aqueous norfloxacin stock solution, 0.2–0.8 ml (in 0.1-ml increments) was pipetted into a 10-ml volumetric flask and the volume was brought to 1 ml with distilled water. Then 0.6 ml of DNFB working solution in borate buffer was added. The mixture was heated for 20 min at 60°C in a water-bath. After cooling, 2 ml of acidic dioxan was added to all the flasks. The volume was completed using dioxan. The absorbance of the yellow solution was measured at 365 nm against a similarly treated DNFB blank solution, where distilled water was used instead of norfloxacin solution.

2.3.2. Procedure II

From the DMSO stock norfloxacin solution, 0.2–0.8 ml (in 0.1-ml increments) was pipetted into a 10-ml volumetric flask. Then 0.5 ml of DMSO stock solution of DNFB was added. The



Scheme 1.

mixture was diluted to the flask mark with DMSO. The absorbances of the developed color were measured at 410 nm against DNFB blank and replacing norfloxacin with DMSO.

2.3.3. Preparation of sample solution

Ten tablets each labeled to contain 400 mg substance were carefully weighed and powdered. A quantity of the powder equivalent to 25 or 5 mg norfloxacin was transferred to a 50-ml volumetric flask using distilled water or DMSO for procedure I and II, respectively. The volume was adjusted to 50 ml with either distilled water or DMSO, shaken mechanically for 30 min and filtered through analytical filter paper. The first 10 ml was rejected. It is further treated as described above for both procedures.

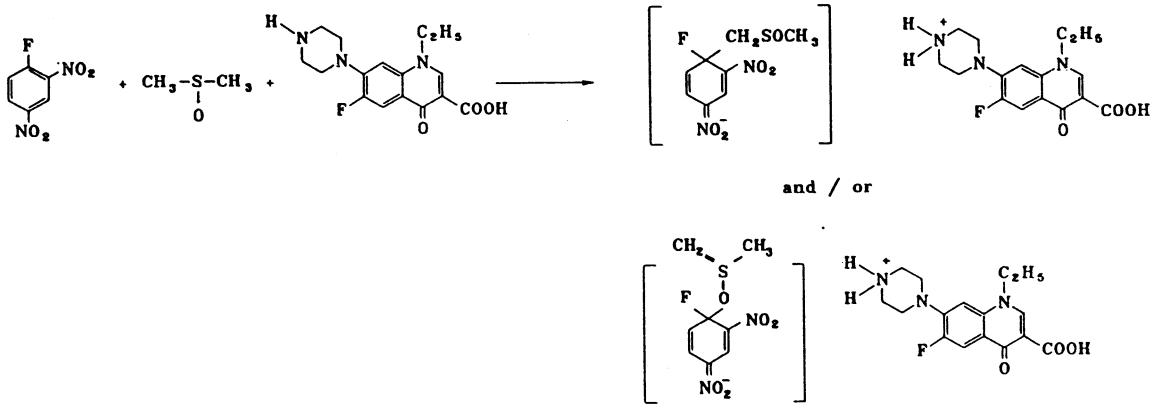
3. Results and discussion

The analytical applications of 2,4-dinitro-1-fluorobenzene (DNFB) in the assay and characterization of amines have been established by Conner [24]. The reagent had been used to quantitate primary and secondary amines [28–32]. The DNFB, or Sanger's reagent, as an active aryl halide reacts with primary or secondary amines in aqueous alkaline medium to form a yellow colored product and this type of reaction was considered a nucleophilic aromatic substitution reaction [24]. In addition to such 'end group' reaction analysis, the DNFB forms a colored Meisenheimer complex upon the interaction with basic compounds (amines) in dipolar aprotic solvent (DMF or DMSO) [33–35].

3.1. Method I

The fact that the norfloxacin molecule features a piperazine ring in the 7th position of its pyridone carboxylic acid structure and two tertiary amines suggested the possibility of using DNFB to increase the sensitivity of NOR spectrophotometric measurements. As the NOR molecule contains both a secondary and tertiary amino group, it was thought useful to analytically compare the use of the reagent in both aqueous alkaline or in aprotic solvent medium. Secondary amines react with excess DNFB to form a yellow product of DNB-amine. In the case of NOR, the end product is 2,4-dinitrobenzene-NOR (Scheme 1).

According to the literature, the substitution reaction of DNFB with amines was carried out in alkaline medium. So, the reaction was investigated over the pH range 8–10 in either bicarbonate or borate medium. The study showed that the reaction product possesses the highest absorption in borate buffer of pH 9 (Fig. 1). The wavelength of maximum absorption of 2,4-DNB-NOR was 365 nm (Fig. 1). The wavelength of maximum absorbance agrees with values reported in the literature for the formation of such product between amines and DNFB. To get ride of the excess reagent interference in the absorbance measurements of the reaction product, the excess reagent is hydrolyzed to the colorless 2,4-dinitrophenol using hydrochloric acid. The exact volume of the acid necessary to discolor the excess of DNFB was determined. The volume was established as 2 ml of 0.5 M hydrochloric acid, and larger volumes were found to have no effect on the absorbance. In order to obtain the highest and most stable absorbance, the effects of the reaction time and heating temperature on the absorbance



Scheme 2.

of the reaction product were studied. The reaction was carried out at different temperatures (ambient (20°C), 60 and 70°C) using a thermostated water-bath for periods ranging from 10 to 45 min. Maximum and constant absorbance was obtained at 60°C after 20 min. The effect of the reagent concentration on the developed color was also investigated and 0.6 ml of the reagent was found sufficient to develop full color intensity. A reagent/substance ratio of 20:1 was found to be suitable. The colored product was stable for at least 30 min. Acetone, acetonitrile, methanol and dioxan were tried as diluting solvents. With the first two solvents the reproducibilities were poorer. Methanol and dioxan gave good results. Dioxan was preferred because the decolorizing solution was prepared in dioxan.

3.2. Method II

Nitro compounds are known to give interesting colors with amines in different polar media. The amine acts as base when it is added to polynitro-aromatic compounds. A proton transfer from the polynitro compound (DNFB) to the base was apparently responsible for the color formation. In aqueous and alcoholic solutions, DNFB does not react instantaneously with the amine (NOR), although it would presumably be quite an efficient proton acceptor. An early study has shown that dipolar aprotic solvents (DMSO) stabilize the conjugate base of the polynitro derivatives. The studies showed that a Meisenheimer-type complex was proposed for this color formation (Scheme 2). Preliminary studies were carried out using 2,4-dinitrophenol, 3,5-dinitro-benzoic acid and 2,4-dinitrofluorobenzene. The best results were obtained using the 2,4-DNFB. The visible spectra of NOR and the polynitro aromatic derivatives in DMSO are shown in Fig. 2. The color was formed instantaneously between NOR and DNFB, and a large excess of the reagent, 5×10^{-4} M in the final solution, was used. The large excess was essential to force the equilibrium between the drug and the reagent to the right. Moreover, the stoichiometry of DNFB to NOR using Asmus method [36] showed a ratio of 1:1. The stability of the Meisenheimer complex was mainly due to the higher solvation of the complex in DMSO than in any other polar solvent (water or alcohol). The formation of a stable Meisenheimer complex [NOR-DNFB] was used to quantitate NOR in tablets.

As the complex has been formed instantaneously after the addition of the reagent and at ambient temperature, so the formation of the

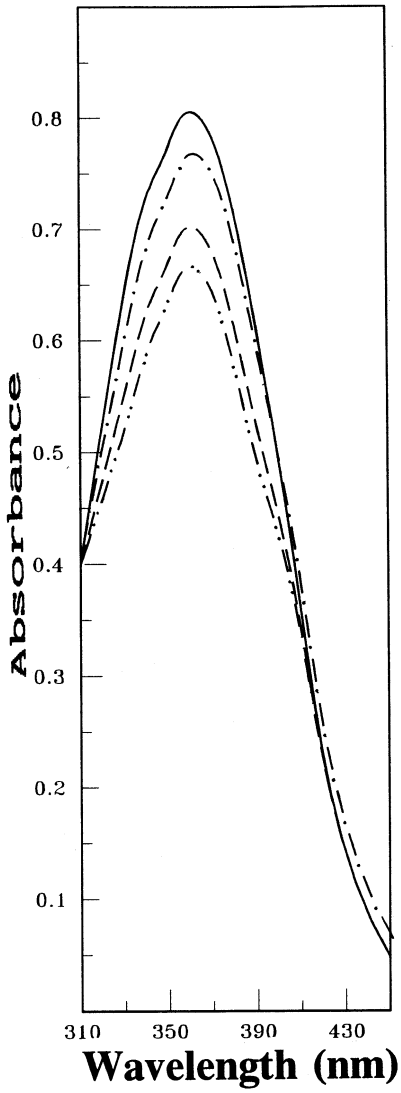


Fig. 1. Absorption spectra of the colored product formed through either the reaction of NOR (35 μg ml^{-1}) and DNFB in bicarbonate buffer (_.._..) and in borate buffer pH 8 (- - -), 9 (——), and 10 (_._._.).

complex will only be affected by the reagent concentration. It was found that 0.5 ml of 0.2% v/v DNFB in DMSO gave the highest absorbance. The Meisenheimer colored complex was found to be stable for 45 min.

In the above experimental conditions, the calibration graphs obtained from both methods, by plotting absorbance at the specified wavelengths against concentrations, were found to be linear over the Beer's law ranges given in Table 1. The molar absorptivities, limits of detection, variances, slopes, intercepts and correlation coefficients obtained by linear least squares treatment of the results are also given in Table 1. The excellent linearity of the calibration graphs is clear from the correlation coefficients and at the same time, the intercepts are close to zero. The values of the correlation coefficients were not sufficient to evaluate the linearity of the calibration graphs. The linearity was evaluated by the R.S.D. (%) of the slope ($S_{b\ \mathrm{rel}}$ (%)) [27] (Table 1).The values of the detection limits and variances are evidence of the sensitivity of both methods and the negligible scatter of the points with respect to the line of regression.

Table 1
Optical characteristics and statistical data for the regression equations

Parameter	Substitution reaction (Procedure I)	Meisenhiemer complex (Procedure II)
Concentration range (μg ml^{-1})	10–40	2–8
Apparent molar absorptivity (l mol^{-1} cm^{-1})	7.19×10^{3}	3.64×10^{4}
Sandell's sensitivity (μg ml^{-1} per 0.001 A)	4.55×10^{-2}	8.89×10^{-3}
Regression equation		
Intercept (a)	-1.09×10^{-2}	-7.30×10^{-3}
$t\ S_a$[a]	1.29×10^{-2}	3.09×10^{-2}
Slope (b)	2.25×10^{-2}	1.14×10^{-2}
$t\ S_b$[b]	5.09×10^{-4}	4.61×10^{-3}
Correlation coefficient (r)	0.9999	0.9996
Variance (S_o^2)	1.485×10^{-5}	1.286×10^{-4}
Linearity ($S_{b\ \mathrm{rel}}$ %)[c]	0.710	1.455
Detection limit (μg ml^{-1})	0.4711	0.2741

[a] Confidence intervals of the intercepts ($P = 0.05$).
[b] Confidence intervals of the slopes ($P = 0.05$).
[c] Linearity is the R.S.D. of the slopes.

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

Proposed method	Added[a]	Found ± S.D.[b]	R.S.D.%	S.A.E.[c]	Confidence limit[d]
Procedure (I)	20.00	20.01 ± 0.058	0.29	2.59×10^{-2}	0.072
	30.00	30.03 ± 0.074	0.25	3.31×10^{-2}	0.092
	40.00	40.01 ± 0.038	0.09	1.70×10^{-2}	0.047
	Mean	0.057	0.21	2.53×10^{-2}	
Procedure (II)	2.00	2.00 ± 0.021	1.05	9.39×10^{-3}	0.026
	4.00	4.02 ± 0.031	0.77	1.39×10^{-2}	0.014
	6.00	6.01 ± 0.040	0.68	1.83×10^{-2}	0.051
	Mean	0.031	0.83	1.39×10^{-2}	

[a] Concentration in μg ml^{-1} (final concentration).
[b] Mean ± S.D. for five determinations.
[c] S.A.E., standard analytical error.
[d] Confidence limits at $P = 0.05$ and four degrees of freedom.

Table 3
Determination of norfloxacin in commercial tablets using the proposed procedures compared statistically with an official method (USP XXIII)

Name of formulation	Recovery (CV%)[a]		
	Proposed procedures		Official method[b]
	Procedure I	Procedure II	
Spectrama tablets[e] (BN 1626)	99.58 (1.19)	98.55 (1.49)	99.58 (1.19)
	$t = 0.26$	$t = 1.41$[c]	
	$F = 1.08$	$F = 1.27$[d]	
Noroxin tablets (BN 972071)	100.34 (0.71)	99.85 (1.11)	100.89 (1.02)
	$t = 0.98$	$t = 1.53$	
	$F = 2.06$	$F = 1.17$	
Neofloxin tablets (BN 6055009)	97.28 (0.66)	97.52 (0.61)	97.73 (0.48)
	$t = 1.26$	$t = 0.62$	
	$F = 1.83$	$F = 1.55$	
Norbactin tablets (BN 101)	97.83 (1.37)	97.64 (1.29)	97.50 (1.35)
	$t = 0.40$	$t = 0.17$	
	$F = 1.03$	$F = 1.10$	
Noracin tablets (BN 395174)	99.50 (0.83)	99.39 (0.95)	99.45 (1.26)
	$t = 0.07$	$t = 0.08$	
	$F = 2.32$	$F = 1.76$	

[a] Mean ± S.D. of five determinations.
[b] USP XXIII procedure.
[c] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306.
[d] Tabulated F-value for $P = 0.05$ and $f1 = f2 = 4$ is 6.39.
[e] All the norfloxacin tablets were labelled to contain 400 mg per tablet; Spectrama tablets, product of Amoun Pharmaceutical Industries (El Salam City, Egypt); Noroxin tablets manufactured by EIPICO (10th of Ramadan City, Egypt) under licence from Merck, Sharp & Dohme (New York, USA); Neofloxin tablets product of Alexandria Co. for Pharmaceutical and Chemical Industries (Alexandria, Egypt); Norbactin tablets manufactured by CID Pharmaceutical Co. under licence from Ranbaxy, India; Noracin tablets manufactured by Memphis Chemical Co. (Cairo, Egypt).

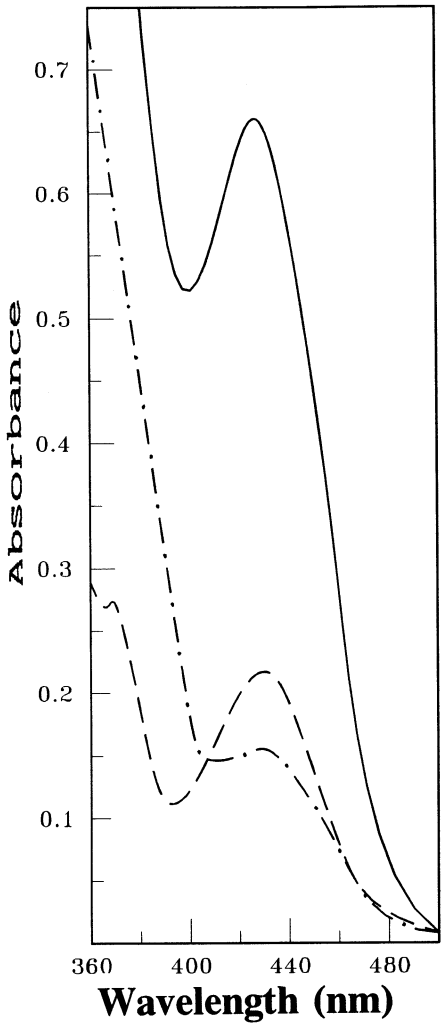


Fig. 2. Absorption spectra of the Meisenheimer complex formed between NOR (6 μg ml^{-1}) and 2,4-dinitrofluorobenzene (——), 2,4-dinitrobenzoic acid (_._._) and 2,4-dinitrophenol (- - -).

In order to determine the accuracy and precision of both procedures, solutions containing three different concentrations of NOR were prepared and analyzed in five replicates. The analytical results obtained from the investigation are summarized in Table 2. The mean S.D., the R.S.D. (%) and the mean standard analytical error (S.A.E.) can be considered to be very satisfactory.

Table 3 shows the results obtained for the determination of NOR in pharmaceutical tablets by both proposed methods and the reference method. For comparison, NOR was determined using the USP XXIII procedure. There were no significant differences between the two sets of results. Tablet excipients, such as starch, methylcellulose, avicel, talc and magnesium stearate, did not interfere.

4. Conclusion

Under the experimental conditions described, the linearity was best in procedure I (substitution reaction) and the second procedure (Meisenhiemer complex) was the most sensitive. The data in Tables 1 and 2 indicate rectilinearity, precision and reproducibility of the proposed procedures. Other techniques such as HPTLC, GLC and HPLC may also give good results but, because of the low cost and ease of carrying out the spectrophotometric methods, the proposed procedures are likely to be very suitable for the analysis of norfloxacin in tablet dosage form.

Norfloxacin produced stable colors, peaking at 365 and 410 nm, respectively. The colors produced obey Beer's law in the range of 10–40 and 2–8 μg ml^{-1}, respectively. The procedures described were applied successfully to the determination of norfloxacin in tablets dosage form. The results showed that the proposed procedures compared favorably with the reference method (USP XXIII [4]) and satisfactory sensitivity, accuracy and precision were noted. Detection limits were 0.47 and 0.27 μg ml^{-1} for each procedure, respectively.

References

[1] K. Grohe, Chem. Br. 1 (1992) 34–36.
[2] J.E.F. Reynolds (Ed.), Martindale—The Extra Pharmacopoeia, 31st edn, Pharmaceutical Press, London, 1996, p. 257.
[3] L.A. Mitscher, Antibiotics, in: W.O. Foye (Ed.), Principles of Medicinal Chemistry, 8th edn, Lea and Febiger, Philadelphia, PA, 1995, p. 768.
[4] United States Pharmacopoeia XXIII, United States Pharmacopoeia Convention, Mack, Easton, PA, 1995, pp. 1104, 2958.

[5] European Pharmacopoeia, 3rd edn., Maisonneuve, Sainte-Ruffine, France, 1997, p. S400. [6] C. Mazuel, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 20, Academic Press, New York, 1991, pp. 557–559.
[7] S.C. Wallis, B.G. Charles, L.R. Graham, J. Chromatogr. B Biomed. Appl. 674 (1995) 306–309.
[8] M.S. Hussain, V. Chukwumaeze-Obiajunwa, R.G. Micetich, J. Chromatogr. B Biomed. Appl. 663 (1995) 379–384.
[9] G. Carlucci, P. Mazzeo, Biomed. Chromatogr. 7 (1993) 126–128.
[10] A. Lagana, L. Marino, M. Rotatori, R. Curini, G. D'Ascenzo, L. Miano, J. Pharm. Biomed. Anal. 6 (1988) 221–228.[11] C.J. Eboka, S.O. Aigbavboa, J.O. Akerele, J. Antimicrob. Chemother. 39 (1997) 639–641.
[12] J. Wu, Q. Zhang, Yaowu Fenxi Zazhi 13 (1993) 203–204.
[13] X. He, Yaowu Fenxi Zazhi 12 (1992) 107–108.
[14] F.E.O. Suliman, S.M. Sultan, Talanta 43 (1996) 559–568.
[15] H.A. Al-Khamees, Anal. Lett. 28 (1995) 109–120.
[16] A. Rotar, P. Solmajer-Lamic, Acta Pharm. Yug. 39 (1989) 123–128.
[17] J. Parasrampuria, V. Das Gupta, Drug Dev. Ind. Pharm. 16 (1990) 1597–1604.
[18] R.T. Sane, V.G. Nayak, Indian Drugs 26 (1989) 497–499.
[19] M. Cordoba-Borrego, M. Cordoba-Diaz, M.I. Bernabe, D. Cordoba-Diaz, J. Pharm. Biomed. Anal. 41 (1996) 977–982.
[20] P.T. Djurdjevic, M. Jelikic-Stankov, D. Stankov, Anal. Chim. Acta 300 (1995) 253–259.
[21] M. Stankov, D. Stankov, Z. Milicevic, D. Veselinovic, P. Djurdjevic, Spectrosc. Lett. 26 (1993) 1709–1714.
[22] A.M.C. Baraza, A. Korolkovas, Rev. Farm-Bioquim. Univ. Sao-Paulo 21 (1985) 141–145.
[23] R.H. Manzo, E. Luna, D.A. Allemandi, J. Pharm. Sci. 80 (1991) 80–84.
[24] K.A. Conner, Reaction Mechanisms in Organic Analytical Chemistry, Wiley, New York, 1973, p. 274.
[25] M.J. Strauss, Anionic Complex Chem. Rev. 70 (1970) 667–712.
[26] E.B. Sandell, Colorimetric Determination of Traces of Metals, 3rd edn, Interscience, New York, 1959, pp. 80–84.
[27] S. Torrado, S. Torrado, R. Cadorniga, J. Pharm. Biomed. Anal. 12 (1994) 383–387.
[28] M.R.C. Marques, E.R.M. Hackmann, T. Saito, Anal. Lett. 23 (1990) 1005–1016.
[29] M.R.C. Marques, E.R.M. Hackmann, T. Saito, Anal. Lett. 22 (1989) 621–633.
[30] N. Buyuktimkin, S. Buyuktimkin, Pharmazie 40 (1985) 581–582.
[31] J.A. Ryan, J. Pharm. Sci. 73 (1984) 1301–1302.
[32] D.M. Shingbal, S.D. Naik, J. Assoc. Off. Anal. Chem. 65 (1982) 899–900.
[33] J.P. Heotis, J.W. Cavett, Anal. Chem. 31 (1959) 1977–1978.
[34] P. Baudet, Helv. Chem. Acta 49 (1966) 545–551.
[35] J.A. Vinson, J.F. Evans, H.E. Holets, Mikrochem. Acta III (1983) 301–306.
[36] J.M. Calatayud, P.C. Falco, M.C.P. Marti, Analyst 111 (1986) 1317–1319.