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Stability-indicating methods for the spectrophotometric determination of norfloxacin

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Abstract

Two spectrophotometric procedures for the selective determination of norfloxacin (NF) in the presence of its decarboxylated degradant are described. The first depends upon measurement of the pH-induced absorbance difference (ΔA) of the drug solution between 0.1 N HCl and 0.1 N NaOH at 280 nm. The second involves chelation of the intact drug with iron(II) in acetate buffer solution (pH 5.7 ± 0.1) to form a yellow-coloured chelate which absorbs at 358 nm. The two procedures are applied successfully for the determination of the intact drug both in pure form and in tablet form. The two methods retain their accuracy in the presence of up to 62% and 76% degradants, respectively.

Keywords: ΔA; Fluoroquinolone; Iron(II) chelation; Norfloxacin; Spectrophotometry; Stability studies

1. Introduction

Norfloxacin [NF, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid] is a fluoroquinolone carboxylic acid that is currently used as a broad-spectrum antibiotic [1,2]. Several methods have been reported for the analysis of NF, including chromatography [3–5], spectrophotometry [6–8] and electrochemistry [9]. The USP 23 recommended a non-aqueous titration procedure [10]. Concerning its stability, only the photodegradants have been identified and assayed by chromatography [3,4]; no attempts were made to quantitate the drug in the presence of its decarboxylated analogue.

NF is a photosensitive drug which decomposes upon exposure to light, yeilding three photodecomposition derivatives, which have been identified as the amino, ethylenediamine and formylpiperazine derivatives [2,3]. In addition, prolonged heating of an acidic solution of NF results in hydrolysis of the drug into the decarboxylate analogue [2,3] (Scheme 1).

The decarboxylated degradant is of particular significance compared with the three photodegradants since the carboxylic group is required for the pharmacological activity of the drug [11]. Moreover, the decarboxylated form has been identified as a precipitate in NF injections [12] and as an impurity in NF powder [13].

The object of this investigation is to develop efficient, simple spectrophotometric procedures

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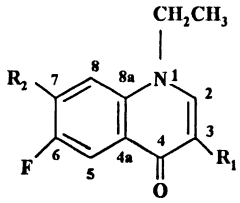

	R_1	R_2
Norfloxacin	- COOH	
Amino	- COOH	H_2N-
Ethylenediamine	- COOH	H_2N-CH_2CH_2-NH-
Formylpiperazine	- COOH	
Decarboxylate	H	

Scheme 1.

for the selective determination of intact NF, either in its pure form or in pharmaceutical formulations without interference from its decarboxylated degradant.

2. Experimental

2.1. Apparatus

UV–Vis spectrophotometers (Shimadzu UV 1601 PC and Beckman DU 7), a Digital pH/MV/Temp. ATC Meter with double junction glass electrode (Jenco Model 5005), an IR spectrometer (Shimadzu 435) and an NMR spectrometer (200 MHz; Varian Chemini) were used.

2.2. Materials and reagents

All chemicals used were of analytical grade and solvents were of spectroscopic grade.

Norfloxacin, reference standard, was kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO), Cairo, Egypt. Its purity was checked by a modified thin-layer chromatography (TLC) procedure [2] and was found to be $100.1 \pm 0.86\%$ using the non-aqueous procedure of the USP 23 [10].

Noroxin tablets (EIPICO), 400 mg NF per tablet, were obtained from the local market. NF solutions prepared were: 0.2 mg ml^{-1} in ethanol and 0.16 mg ml^{-1} in sodium acetate buffer solution (pH 5.7 ± 0.1, 0.2 M) [14]. Other solutions prepared were: hydrochloric acid (HCl), 0.1 N and 2 N; sodium hydroxide (NaOH), 0.1 N; ferrous sulphate ($FeSO_4 \cdot 7H_2O$), 2×10^{-3} M, prepared by dissolving the salt in acetate buffer (pH 5.7 ± 0.1) at about 0.56 mg ml^{-1} and standardising against standard $KMnO_4$ [14].

2.3. Procedures

2.3.1. Preparation of degraded norfloxacin [2,3]

2.3.1.1. Stock degradant solution. Reflux 0.4 g of pure NF powder with 75 ml of 2 N HCl solution on a heating mantle and heat at 150°C for 36 h while protecting the solution from light. Cool, adjust to pH 7–8 with 2 N KOH solution, using universal indicator test paper, and evaporate to

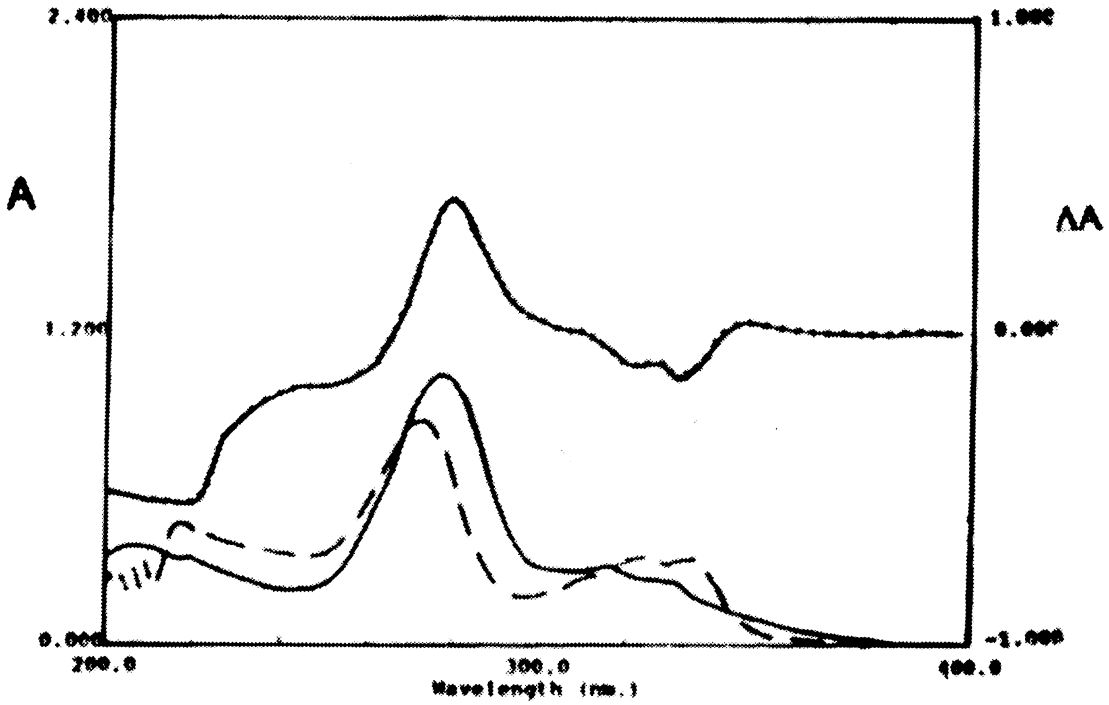

Fig. 1. The UV spectra of intact norfloxacin (8 μg ml^{-1}) in 0.1 N HCl (——), 0.1 N NaOH (– – –) and their ΔA spectrum (- - -).

dryness under vacuum. Extract the residue three times, each with 70 ml of ethanol. Filter into a 250-ml volumetric flask and make to volume with ethanol.

Analysis of this stock solution by the chelation procedure after the appropriate treatment (see Section 2.3.1.3) revealed the presence of 5% of undegraded NF. Thus, each millilitre of this stock solution corresponds to the degradant from 1.52 mg of NF.

2.3.1.2. Preparation of degradant solution for ΔA procedure. Dilute 12.5 ml of the stock solution (as in Section 2.3.1.1) with ethanol up to 100 ml; each millilitre of this diluted solution corresponds to the degradant from 0.19 mg of NF, to be used in the ΔA procedure.

2.3.1.3. Preparation of degradant solution for chelation procedure. Pipette 10 ml from the prepared stock solution (as in Section 2.3.1.1) into a 100-ml beaker, evaporate almost to dryness at $\approx$70°C, cool and dissolve in about 70 ml of the acetate buffer solution (pH 5.7 ± 0.1). Transfer quantitatively to a 100-ml volumetric flask, wash the beaker twice, each with 10 ml of the buffer solution, and add to the flask, then adjust to volume with the same buffer. Each millilitre of this solution corresponds to the decarboxylated degradant from 0.152 mg of NF.

2.3.2. Determination of NF in pure powder

2.3.2.1. ΔA procedure. Transfer aliquot volumes of the pure NF ethanolic solution (0.2 mg ml^{-} l), accurately measured to correspond to 0.2–0.8 mg of the drug, into two sets of 50-ml volumetric flasks. Dilute to volume in one set with 0.1 N HCl and in the other set with 0.1 N NaOH. Compute the ΔA for each concentration at 280 nm by placing the acid solution in the sample beam and the alkaline one in the reference beam.

Calculate the concentrations of NF either from a simultaneously prepared calibration curve or from the regression equation:

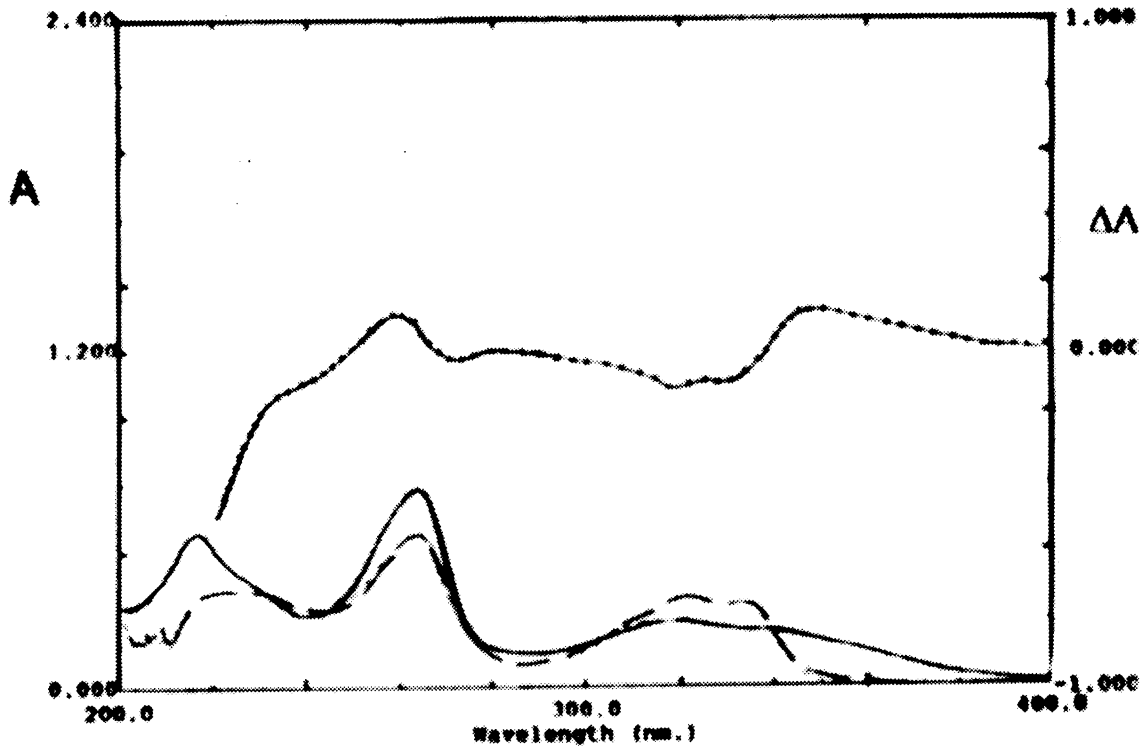

Fig. 2. The UV spectra of decarboxylated norfloxacin (derived from 8 μg ml^{-1} NF) in 0.1 N HCl (——), 0.1 N NaOH (– – –) and their ΔA spectrum (- - -).

$$\Delta A = -0.021 + 0.053C \qquad r = 1$$

where ΔA denotes the difference in absorbances at 280 nm, C is the concentration (in μg ml^{-1}) and r is the correlation coefficient.

2.3.2.2. The chelation procedure. Into a series of 10-ml volumetric flasks, transfer volumes of the pure NF solution in acetate buffer (pH 5.7 ± 0.1), accurately measured to be equivalent to 0.16–0.64 mg of NF. Add 1 ml of 2×10^{-3} M ferrous sulphate solution and dilute to volume with the same buffer. Mix well and, after standing for 30 min, measure the absorbances at 358 nm against blanks similarly prepared but omitting the ferrous ion solution.

Calculate the concentration of NF either from a simultaneously prepared calibration curve or from the regression equation:

$$A = -0.01 + 0.013C \qquad r = 0.999$$

where A is the absorbance at 358 nm, C is the concentration (in μg ml^{-1}) and r is the correlation coefficient.

2.3.3. Determination of NF in mixtures of intact and degraded drug

2.3.3.1. The ΔA procedure. Mix volumes of the ethanolic pure drug solution, accurately measured to contain 0.7–0.2 mg of the intact NF, with volumes of its degradant solution (see Section 2.3.1.2), accurately measured to contain the degradant derived from 0.095–0.57 mg of NF, in each of two sets of 50-ml volumetric flasks. Dilute to volume with acid or alkali and proceed as described in Section 2.3.2.1.

2.3.3.2. The chelation procedure. Transfer volumes of the drug solution in acetate buffer, accurately measured to contain 0.64–0.16 mg of intact NF, into a series of 10-ml volumetric flasks. Mix with

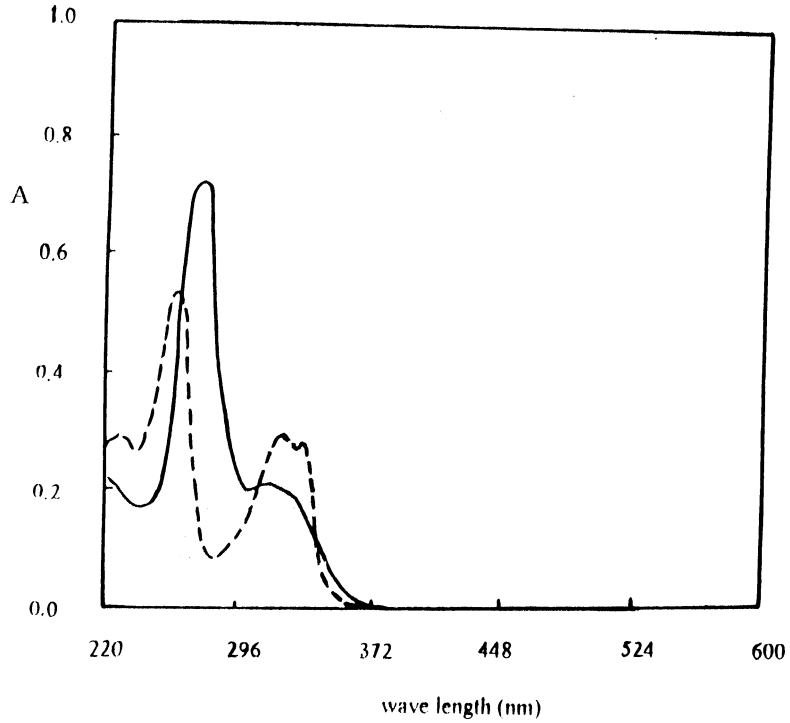

Fig. 3. The UV–Vis spectra of 6.4 μg ml^{-1} each of intact (——) and decarboxylated (– – –) norfloxacin in acetate buffer solution, pH 5.7 ± 0.1.

volumes of its degradant solution in acetate buffer (see Section 2.3.1.3), accurately measured to contain the decarboxylated degradant derived from 0.076–0.608 mg of NF. Add 1 ml of 2×10^{-3} M ferrous sulphate solution and proceed as described in Section 2.3.2.2.

2.3.4. Determination of NF in noroxin tablets

Weigh 20 noroxin tablets, thoroughly grind them and mix well, until fine and homogeneous. Transfer an accurate weight of the powder equivalent to about 20 mg of NF (for the ΔA procedure) or about 16 mg of NF (for the chelation procedure) into a 100-ml volumetric flask, and dissolve in 80 ml of hot ethanol or hot acetate buffer solution, respectively, by thorough shaking for about 20 min. Dilute to volume with the appropriate solvent. Mix well and centrifuge portions of the solution to remove any undissolved particles. Analyse aliquots of the clear supernatant claimed to contain about 0.2 mg ml^{-1} or 0.16 mg ml^{-1} NF, by the ΔA or chelation procedures, respectively, as in Section 2.3.2.

3. Results and discussion

It has been reported that NF decomposes into the decarboxylated form when heated with 2 N HCl at 100°C [2,3]. However, using such conditions, only 40% degradation was effected after 24 h. Thus, acceleration of hydrolysis was tried by increasing the temperature and time; maximum decarboxylation (95%, as assayed by the chelation procedure) was achieved by heating at 150°C while refluxing the drug solution in 2 N HCl for 36 h, during which it was protected from light. Refluxing for 12 h or 24 h produced 58.1% and 69.4% decarboxylation, respectively.

The UV spectrum of the intact NF showed that the drug is pH sensitive since a small bathochromic shift from 269 nm in alkaline

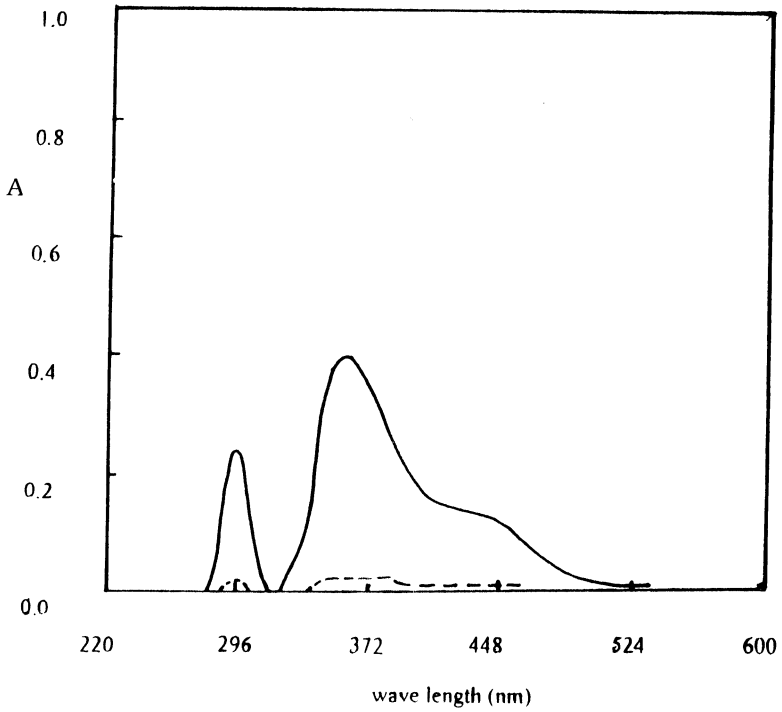

Fig. 4. The UV–Vis spectra of 32 μg ml^{-1} of intact (——) and decarboxylated (– – –) norfloxacin in the presence of 56 μg ml^{-1} iron(II) in acetate buffer solution, pH 5.7 ± 0.1.

medium to 280 nm in acid medium was observed, accompanied by a simultaneous hyperchromic effect (Fig. 1). However, such a bathochromic shift was not observed in the spectrum of the decarboxylated degradate (Fig. 2). These spectral observations called for the proposed pH-induced ΔA measurement of intact NF at 280 nm, whereby the interferences due to its decarboxylated degradate that occur in conventional spectrophotometry can be avoided.

Studies of the chelation of fluoroquinolone antibacterials with metal ions have become particularly important because microbiological studies demonstrated decreased activity of these drugs in the presence of metal ions [6]. Binding of metal ions at the 3,4-carboxylic-carbonyl residues is well documented [7]. These findings were motivational in developing a stability-indicating procedure for the selective determination of intact NF by chelation with iron(II).

In acetate buffer solution (pH 5.7 ± 0.1), NF has a sharp, strong absorption band at 278 nm, in addition to a broad band of much lower intensity at about 320 nm, whereas its decarboxylated degradant has three bands, at 262, 322 and 334 nm (Fig. 3).

Chelation of NF with iron(II) lead to a bathochromic shift from 278 nm to 296 nm with a simultaneous hypochromic effect, while a new band appeared at 358 nm together with a strong shoulder at 420 nm (Fig. 4). However, upon mixing the degradant with iron, only a new, poor hump around 358 nm appeared, which can be attributed to the remaining undegraded drug. Accordingly, both the ΔA and the chelation procedures can be advantageously used for the selective determination of NF and for evaluating the extent of its degradation, by decarboxylation, in pharmaceutical formulations.

The continuous variation [15] and the molar ratio [16] methods revealed a ratio of 1:3 for the metal ion:ligand. The complex stability constant of the formed chelate was calculated [15–18], and similar values for log β ($\approx$ 12) were obtained by both methods (Table 1).

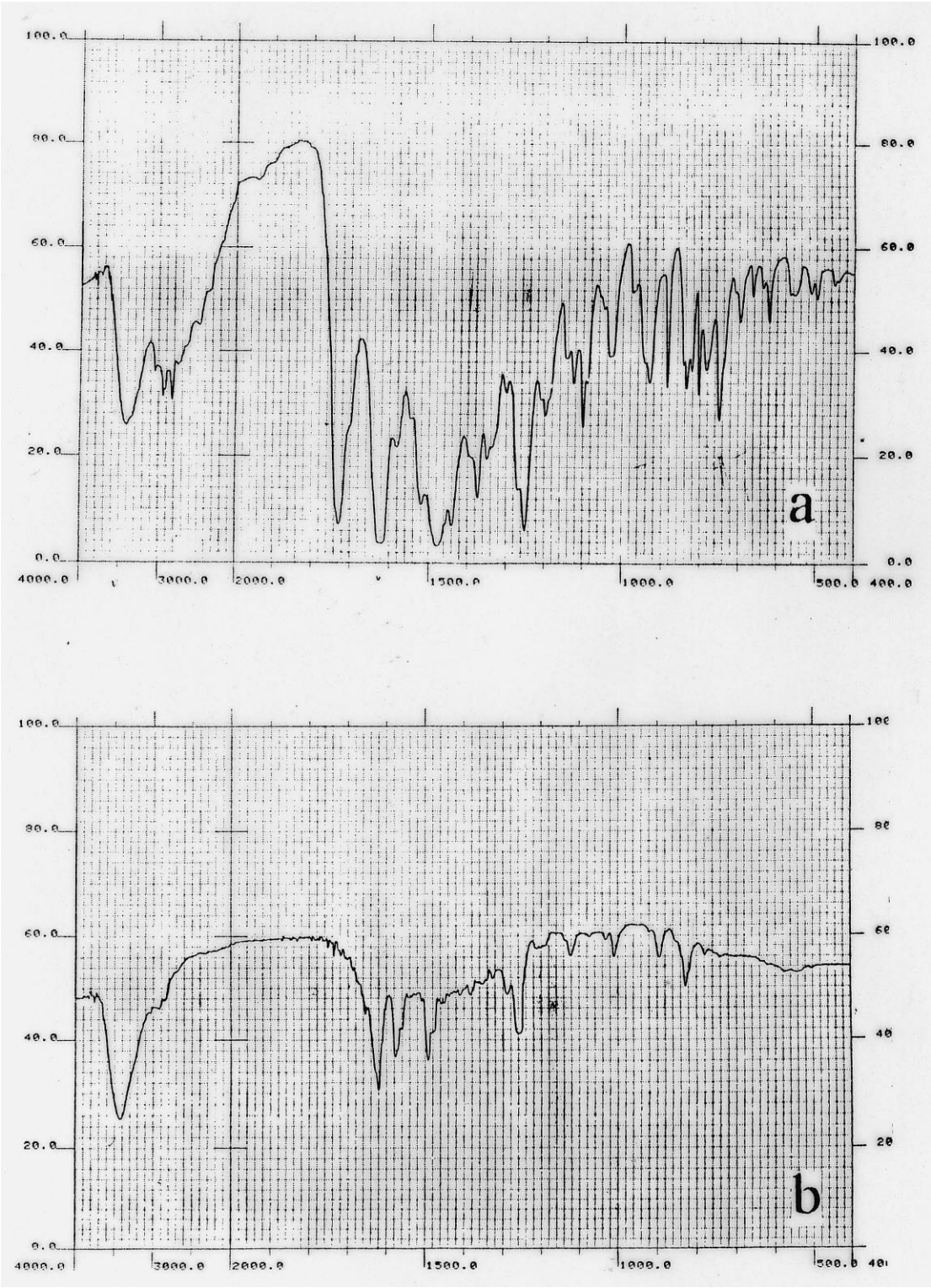

Fig. 5. The IR spectra of intact (a) and decarboxylated (b) norfloxacin, both as KBr tablets.

Table 1
The calculated stability constants of norfloxacin–iron(II) chelate at λ_{max} 358 nm [15–18]

Continuous variation method	Molar ratio method
$n = 2.45$	$n = 2.7$
$A/A_{ex} = 0.795$	$\alpha = 0.0875$
$*\beta = 4.938 \times 10^{11}$	$\beta' = 4.724 \times 10^{-13}$
	$\beta = 2.117 \times 10^{12}$
$\log \beta = 11.69$	$\log \beta = 12.33$

$$*\beta = \frac{A/A_{ex}C_X}{(C_M - A/A_{ex}C_X)(C_L - nA/A_{ex}C_X)^n}$$

where $*\beta$ is the stability constant of the formed chelate, M indicates metal, L indicates ligand, $n = X/(1-X)$ where X is the mole fraction of the ligand at the maximum of the 'continuous variation' curve, A/A_{ex} is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength, C_M and C_L are the concentrations of the metal and ligand respectively, and $C_X = C_L/n = C_M$.

$$\beta' = \frac{(\alpha C)(n\alpha C)^n}{C(1-\alpha)}$$

where β' is the instability constant of the formed chelate and $\beta = 1/\beta'$, C is the initial total concentration of the metal ion, n is the ligand:metal concentration ratio, and $\alpha = (A_m - A_s)/A_m$, where A_m is the absorbance when all the metal ion is complexed with the ligand and A_s is the absorbance of the stoichiometric molar ratio of L to M in the chelate.

The optimum parameters for chelation were found to be acetate buffer solution of pH 5.7 ± 0.1 and a standing time of 30–90 min. A concentration of iron(II) of 14 μg ml^{-1} was found to be sufficient to give maximum absorbance at 358 nm upon reaction with 32 μg ml^{-1} of NF. Any excess iron(II) up to 280 μg ml^{-1} did not affect the production, intensity or stability of the formed chelate.

Beer's law was found to hold good in the ranges 4–16 μg ml^{-1} and 16–64 μg ml^{-1} for the ΔA and chelation procedures, respectively. The values of ΔA (1, 1) for the ΔA procedure and A (1,1) for the chelation procedure were calculated as 513.8 and 127.2, respectively.

Table 2 shows good reproducibilities for the two proposed procedures when applied to different, blind experiments of a pure sample of the drug.

To assess the efficiency of the suggested procedures as stability-indicating, they were applied to laboratory-prepared mixtures of intact NF and its decarboxylated degradate in different ratios. It is clear from Table 3 that the proposed procedures are applicable for the selective assay of the intact drug in the presence of up to 62% of its degradant via the ΔA technique and up to 76% via its iron(II) chelate. Both the non-aqueous titration of the USP [10] and second-derivative spectrophotometry at 337 nm in 0.05 N NaOH of a published procedure [8] gave much higher results when applied to similar mixtures (Table 3), indicating their unsuitability as stability-indicating procedures.

Table 2
Determination of a pure sample of norfloxacin by the proposed procedures

ΔA procedure			Iron(II) chelation		
Taken (μg ml^{-1})	Found (μg ml^{-1})	Accuracy (%)	Taken (μg ml^{-1})	Found (μg ml^{-1})	Accuracy (%)
4	3.98	99.5	16	16.05	100.3
6	6.04	100.7	20	20.16	100.8
8	8.02	100.3	28	27.83	99.4
10	9.86	98.6	40	39.84	99.6
12	11.88	99.0	48	48.52	101.1
16	16.12	100.8	60	59.92	99.2
			64	64.42	100.7
Mean ± CV%		99.8 ± 0.92			100.2 ± 0.75

Table 3
Determination of norfloxacin in mixtures of intact and degraded drug by the proposed, USP [10] and published [8] procedures

ΔA procedure			Iron(II) chelation			USP procedure			Published procedure		
Intact[a] (μg ml^{-1})	Degraded[b] (%)	Intact recovery (%)	Intact[a] (μg ml^{-1})	Degraded[b] (%)	Intact recovery (%)	Intact[a] (mg)	Degraded[b] (%)	Intact recovery (%)	Intact[a] (μg ml^{-1})	Degraded[b] (%)	Intact recovery (%)
14.1	11.9	100.5	64.4	10.6	100.2	455.5	9.5	118.3	27.2	9.3	112.8
12.2	23.8	98.5	64.8	19.0	99.2	405.0	19.0	143.7	24.3	19.0	131.9
10.3	35.6	100.2	57.2	28.5	99.5	357.5	28.5	173.0	21.5	28.3	147.5
8.4	47.5	98.8	49.6	38.0	100.1	310.0	38.0	216.8	18.6	38.0	188.9
6.9	57.0	98.2	42.0	47.5	99.8	262.5	47.5	266.7	15.8	47.3	237.4
6.5	59.4	100.4	34.4	57.0	101.2	215	57.0	335.8	12.9	57.0	327.8
6.1	61.8	99.0	26.88	66.5	100.0						
4.6	71.3	112.0[c]	19.2	76.0	101.4						
Mean ± CV		99.3 ± 1.0%			100.2 ± 0.77%						

[a] Taken + undegraded part (5%) in the hydrolysed solution.
[b] Percentage of the total weight.
[c] Rejected.

Table 4
Determination of norfloxacin in Noroxin tablets by the proposed and by published [8] procedure

Procedure	Noroxin tablets B.N. 30244		Noroxin tablets B.N. 950203	
	Accuracy ± CV (%)	Standard addition recovery ± CV (%)	Accuracy ± CV (%)	Standard addition recovery ± CV (%)
ΔA	102.7 ± 0.61	100.3 ± 0.85	99.5 ± 0.68	100.6 ± 1.04
Iron(II) chelation	102.3 ± 0.64	99.4 ± 0.91	98.7 ± 0.48	99.8 ± 0.90
Published	101.6 ± 0.84		99.1 ± 0.60	

The suggested procedures were successfully applied to quantitate the drug in tablet form, confirming the non-interference by excipients and additives. Compared with a published procedure [8], the results were of equal accuracy when applied to the authentic sample of pure NF or to freshly formulated tablets. The validity of the two suggested procedures when applied to tablets dosage form was assured by the recovery of standard additions (Table 4).

The results obtained by both the ΔA and the chelation procedures were statistically analysed by and compared with those of the USP [10] procedure (Table 5). No significant differences were

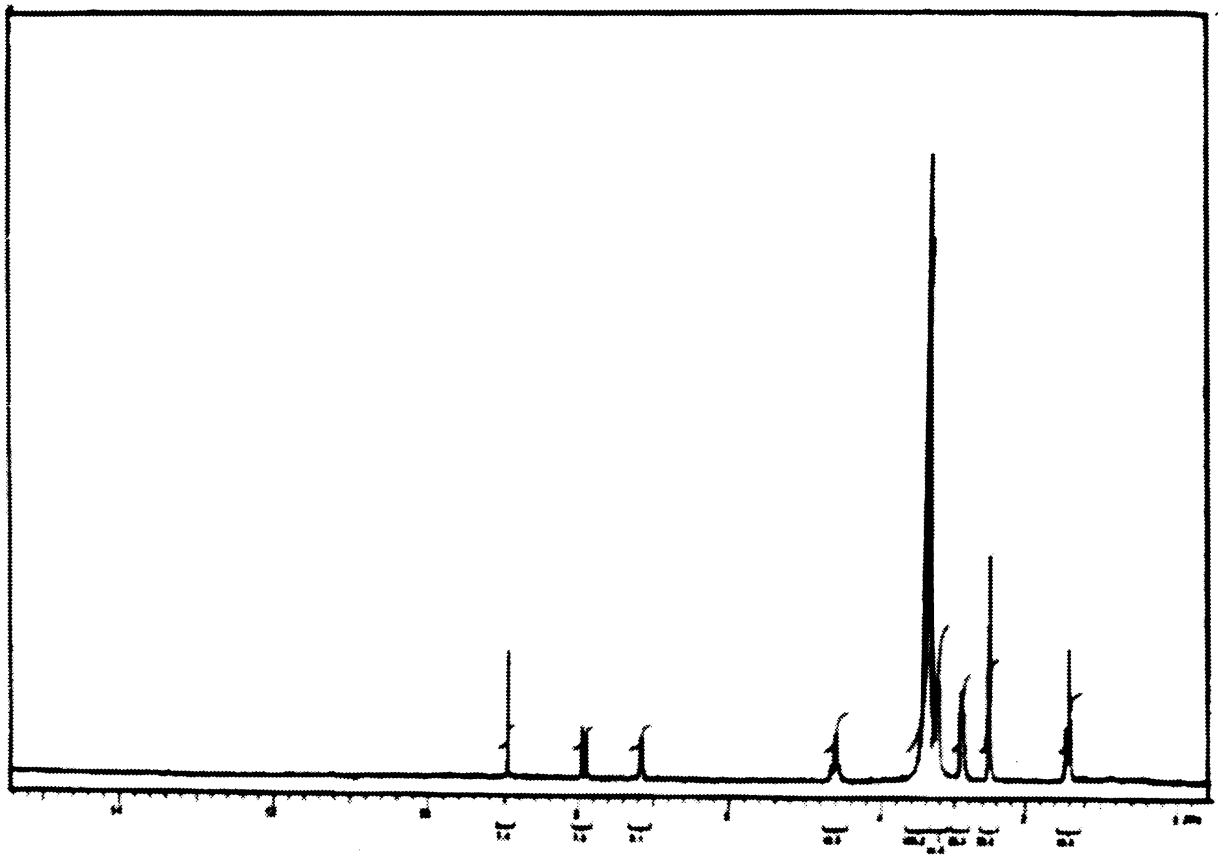

Fig. 6. ^{1}H NMR spectrum of intact norfloxacin in DMSA-d_6.

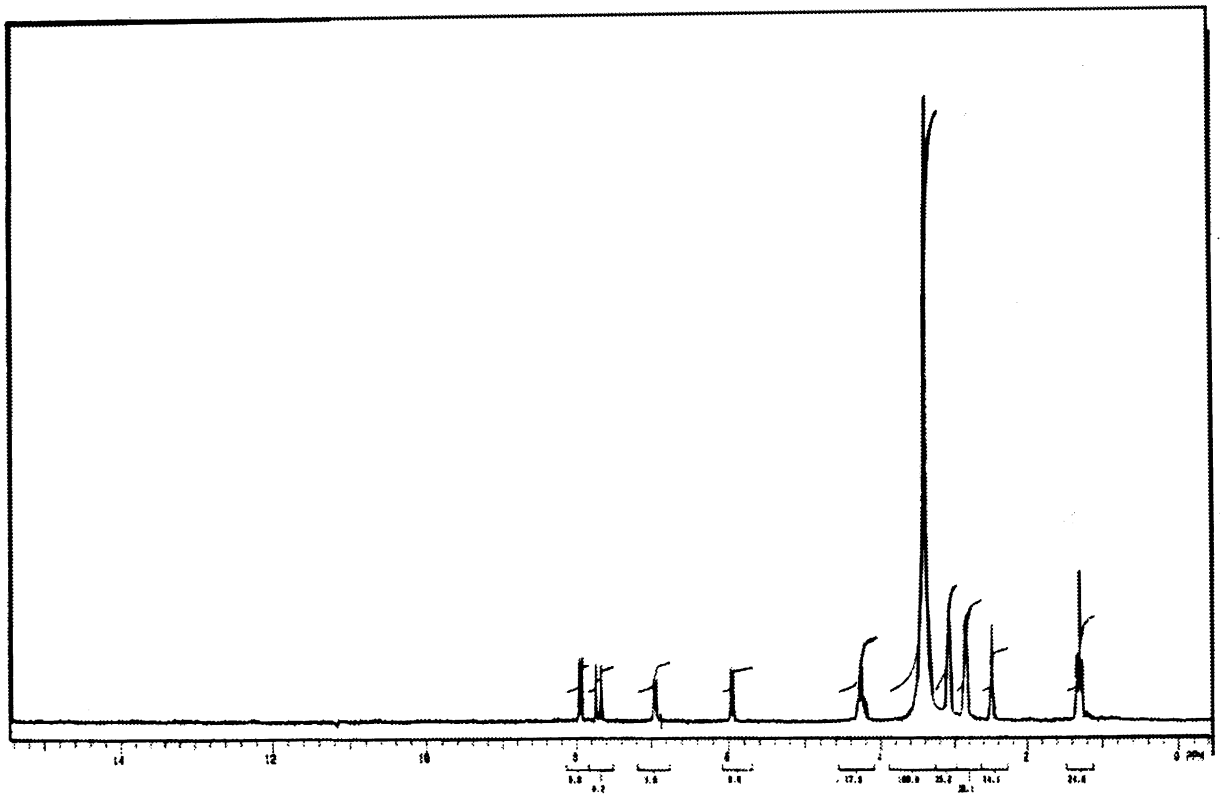

Fig. 7. ^{1}H NMR spectrum of decarboxylated norfloxacin in DMSO-d_6.

found within a probability of 95% of being correct. However, the proposed procedures are far more sensitive than the USP procedure, being suitable for microgram concentrations, while the USP [10] is for milligram amounts. Moreover, the suggested procedures are more selective, as the non-aqueous titration of the USP does not differentiate between the intact drug and its decarboxylated degradant, both behaving as basic compounds in glacial acetic acid.

3.1. Identification of the decarboxylated degradant

The prepared NF degradant was purified by a modified TLC procedure [2] using chloroform–methanol–ammonia (10:10:2, v/v) as developing solvent and UV detection at 254 nm. After hydrolysis, two bands were obtained having R_f values of 0.31 and 0.89, corresponding to the undegraded, intact drug and its decarboxylated degradant, respectively. The degradant band was extracted with chloroform, filtered and evaporated to dryness. Identification was made by scan

Table 5
Statistical analysis of the results obtained by the proposed and USP [10] procedures for the determination of norfloxacin pure powder

	USP	ΔA	Iron(II) chelation
Concentration range	460 mg	4–16 μg ml^{-1}	16–64 μg ml^{-1}
Mean (%)	100.1	99.8	100.2
S.D.	0.86	0.92	0.75
n	5	6	7
Variance	0.740	0.846	0.563
Student's t-test	–	1.314 (1.83)	0.829 (1.812)
F	–	1.14 (6.3)	1.31 (6.2)

Values in parentheses are the corresponding theoretical values of F and t ($p = 0.05$).

ning the IR spectra on KBr discs and ^{1}H NMR spectra in DMSO-d_6 (dimethyl sulphoxide) of both the standard NF and its isolated degradant.

The IR spectrum of pure NF exhibits a strong stretching vibrational band at about 1715 cm^{-1} together with a broad band around 2000–3000 cm^{-1} characteristic of the carbonyl and –OH moieties of the carboxylic group, respectively [2] (Fig. 5a). Complete disappearance of these two bands from the IR degradant spectrum is therefore evidence of decarboxylation (Fig. 5b).

^{1}H NMR evidence for the proposed decarboxylation was seen by the changes in both the chemical shift and the multiplicity of the –CH proton in position 2, using a high-resolution NMR spectrometer (200 MHz) which facilitated interpretation of the charts. In the spectrum of the intact molecule, this proton appeared as a sharp singlet at $\delta = 9$ ppm [2] (Fig. 6), whereas in the degradant spectrum it appeared as a doublet at $\delta \approx 7.85$ ppm (Fig. 7). Such an upfield shift could be explained by the disappearance of the deshielding effect exerted by the carboxylic group in the intact drug molecule. Further evidence was provided by the splitting of its sharp singlet to a doublet by the nearby new proton on the adjacent carbon in position 3, which revealed itself by a new doublet at $\delta = 6$ ppm.

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