Stability Indicating Methods for the Determination of Some Fluoroquinolones in the Presence of Their Decarboxylated Degradates

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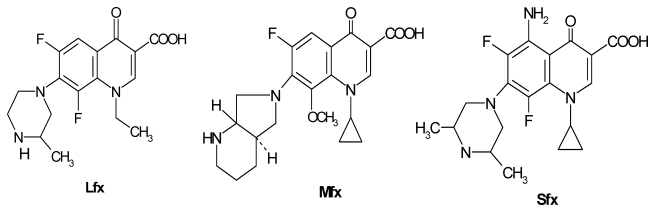
Two stability-indicating methods, namely densitometric TLC and derivative spectrophotometry for the determination of the fluoroquinolone antibacterials lomefloxacin (Lfx), moxifloxacin (Mfx), and sparfloxacin (Sfx) in the presence of their acid degradates are described. Acid degradation was adopted and the main decarboxylated product separated by TLC. Degradation products were identified confirming a previously mentioned degradation scheme. The densitometric method is based on the separation of the intact drug from its acid degradation product on silica gel G plates using different mobile phases and the spots of the intact drugs were scanned at 288, 290, and 292 nm for Lfx, Mfx, and Sfx, respectively. The derivative spectrophotometric method utilizes first derivative D_1 UV spectrophotometry with zero crossing points at 295.2 nm for Lfx, 280.4 and 303.4 nm for Mfx, and 280.8 nm for Sfx. Regression analysis of Beer's plots showed good correlation in the concentration ranges 0.2—1.2, 0.1—1.4, and 0.5—2.0 μg/spot for Lfx, Mfx, and Sfx, respectively, in the densitometric method and 2—16 μg/ml for all drugs in the derivative spectrophotometric method. The proposed methods were successfully applied for the determination of the investigated drugs in bulk powder with mean percentage accuracy ranges from 98.93 to 101.25% for the TLC method and from 98.18 to 100.35% for the D_1 method. The proposed methods were also applied for the determination of the investigated drugs in their pharmaceutical dosage forms and their validity was assessed using the standard addition technique with mean percentage recovery ranging from 100.25 to 101.70% in the TLC method and from 99.27 to 102.12% in the D_1 method. The selectivity of the proposed methods was tested by the analysis of laboratory-prepared mixtures containing different percentages of the studied drugs and their acid degradates. The proposed methods were found selective for the determination of the intact drugs in the presence of up to 90% of their degradates in the TLC method and 70% for Lfx and 90% for Mfx and Sfx in the D_1 method.

Key words lomefloxacin; moxifloxacin; sparfloxacin; stability

Lomefloxacin hydrochloride (Lfx), moxifloxacin hydrochloride (Mfx), and sparfloxacin (Sfx) are 4-fluoroquinolone carboxylic acid antibacterials having the following structures.



The 4-quinolone antibacterials are a group of synthetic antibacterials structurally related to nalidixic acid.[1] They act through the inhibition of DNA gyrase (in G−ve bacteria) or topisomerase (in G+ve bacteria).[2] Several methods have been reported for their analysis including UV–visible spectrophotometry,[3,4] spectrofluorometry,[5—7] atomic absorption spectrophotometry,[8] chromatography,[9—13] electrophoresis,[14—16] flow injection analysis,[17,18] and electrochemical methods.[19—21]

Structure activity studies have shown that the 1,4-dihydro-4-oxo-3-pyridine carboxylic acid moiety is essential for antibacterial activity of the fluoroquinolones. Therefore the detection of the decarboxylated degradates is of particular importance since the carboxylic acid group is required for their pharmacological activity.[22,23]

Thus the aim of this work is to develop efficient, simple TLC densitometric and derivative spectrophotometric methods for the selective determination of Lfx, Mfx, and Sfx in their pure form or in pharmaceutical dosage forms without interference from their decarboxylated degradates.

Experimental

Apparatus Labomed Spectro UV–Vis Double Beam PC 8 scanning auto cells spectrophotometer, with matched 10 mm path-length quartz cells. The derivative conditions were delta lambda of 5 nm and scaling factor=10.

Shimadzu dual wavelength flying spots CS9301 densitometer. The experimental conditions of the measurement were as follow: wavelength=288, 290, and 292 nm for Lfx, Mfx, and Sfx, respectively; photomode=reflection; scan mode=zigzag, and swing width=16.

UV lamp, short and long wavelength 254 and 365 nm, spectroline (U.S.A.).

Glass jar for TLC with lid 22×25×10 cm.

TLC plates 20×20 cm, 0.25 mm thickness silica gel 60 GF254 (E. Merck).

HPTLC plates 20×20 cm, silica gel nanoplates F254, sprayed with 1% EDTA solution in 5% dipotassium hydrogen phosphate then heated for 1 h at 120 °C (E. Merck).

10 μl Hamilton microsyringe.

Bruker IR spectrophotometer.

Materials and Reagents All solvents and chemicals used are of analytical grade.

Lomefloxacin hydrochloride reference standard was supplied by Alkan Pharmaceutical Company, Cairo, Egypt; its potency was 99.5±0.91% by UV spectrophotometric method.[24]

Moxifloxacin hydrochloride reference standard was supplied by Dr. Reddy's Laboratories Ltd, Hyderbad, India; its potency was 98.4±0.62% by UV spectrophotometric method.[25]

Sparfloxacin reference standard was supplied by Jedco Company, Cairo, Egypt; its potency was 98.7±0.85% by UV spectrophotometric method.[26]

Lomeflox tablets (Alkan), labeled to contain 400 mg of Lfx/tablet BN:

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102, Avalox tablets (Bayer, Germany), labeled to contain 400 mg Mfx/tablet, BN: 131, Spara tablets (Global Napi Co, Egypt), labeled to contain 200 mg Sfx/tablet were obtained from commercial sources.

2.0 M hydrochloric acid aqueous solution (Aldrich).

2.0 M potassium hydroxide aqueous solution (El-Nasr, Cairo, Egypt).

Methanol (Riedel-de Haen, Hanover, Germany).

Ethanol absolute (Lab Scan, Dublin, Ireland).

n-Propanol (Lab Scan).

0.3 M ammonium chloride aqueous solution (El-Nasr).

0.3 M ammonium acetate aqueous solution (El-Nasr).

Conc ammonia solution 33% (El-Nasr).

EDTA (Aldrich, Gillingham, England), 1% solution in 5% dipotassium hydrogen phosphate.

Dipotassium hydrogen phosphate 5% aqueous solution (El-Nasr).

Butyl acetate (Aldrich).

0.3 M hydrochloric acid in distilled water (Aldrich).

Developing solvents: for Lfx: 0.3 M ammonium chloride solution : conc ammonia : *n*-propanol (1 : 1 : 8 v/v/v); for Mfx: 0.3 M ammonium acetate solution : conc ammonia : *n*-propanol (1 : 1 : 8 v/v/v); for Sfx: butyl acetate : ethanol : water : conc ammonia (4/4/1/0.25 v/v/v/v).

Preparation of Stock Standard Solutions Solutions (1 mg/ml) of Lfx, Mfx, and Sfx for the densitometric method and (0.2 mg/ml) for the derivative spectrophotometric method using water for Lfx and Mfx and ethanol for Sfx as solvents were prepared.

Preparation of Degradation Products Reflux 100 mg of each Lfx, Mfx, and Sfx separately with 25 ml 2 M HCl solution on a heating mantle at 100 °C for 10 h while protecting the solution from light. Cool the solutions then adjust pH to 7—8 with the aid of 2 M KOH solution (using a universal indicator test paper), then evaporate the solutions just to dryness. Extract the residues using 50 ml ethanol then filter the extracts and concentrate to few milliliters, apply as bands onto TLC plates, and develop using the previously mentioned solvent systems and examine under UV lamp (254 nm). Scratch the spots corresponding to the major degradation product and extract with ethanol, filter, and evaporate just to dryness. Use part of each residue separately for structure elucidation by IR as potassium bromide disc. Weigh another part of the residues and dissolve in methanol so as to obtain solutions with final concentration of 1 mg/ml for the densitometric method or 0.2 mg/ml for the spectrophotometric method.

Preparation of Laboratory-Prepared Mixtures Densitometric Method: Accurately transfer aliquots equivalent to 0.2—1.2 mg of Lfx, 0.1—1.4 mg Mfx, and 0.5—2 mg Sfx from their stock solutions (1 mg/ml) into a series of 10-ml volumetric flasks, then add aliquots equivalent to 10—90% of the corresponding degradation products stock solutions (1 mg/ml) and complete the volume with ethanol.

Derivative Spectrophotometric Method: Accurately transfer aliquots equivalent to 100—300 μg of each drug from its stock solution (0.2 mg/ml) into a series of 25-ml volumetric flasks, then add different percentages ranging from 10—90% of the corresponding degradation product stock solutions (0.2 mg/ml) and complete the volume using 0.3 M HCl.

Preparation of Test Solution for Pharmaceutical Preparations Accurately weigh and powder 10 tablets of Lomeflox tablets, Avalox tablets, or Spara tablets. Weigh and transfer quantitatively an amount of the mixed powdered tablets equivalent to 25 mg of each drug to a 25-ml volumetric flask and extract using 20 ml distilled water for Lomeflox and Avalox tablets or absolute ethanol for Spara tablets. Sonicate the solutions for 5 min, adjust the volume using the same solvent, then centrifuge. These solutions are used as stock test solutions for the densitometric method. Carry out further dilution separately for each drug using 0.3 M HCl to obtain a final concentration of 0.2 mg/ml for the D_1 method.

Procedures. Densitometric Method Construction of Calibration Curves: Accurately transfer aliquots equivalent to 0.2—1.2 mg Lfx, 0.1—1.4 mg Mfx, and 0.5—2 mg Sfx from their stock solutions (1 mg/ml) into a series of 10-ml volumetric flasks and complete the volume with ethanol. Apply 10 μl—in triplicate—of each solution onto a TLC plate for Lfx and Mfx or to an HPTLC plate treated with EDTA and potassium hydrogen phosphate for Sfx. Spots are spaced 1.5 cm from each other and from the bottom edge of the plate. Develop the plates ascendingly for a distance of about 17 cm, remove the plate from the jar, dry at room temperature, and detect under UV lamp at 254 nm. Scan the spots of the drugs at 288, 290, and 292 nm for Lfx, Mfx, and Sfx, respectively, by reflection mode. Plot the calibration curves representing the relationship between the recorded areas and the corresponding concentrations and compute the regression equations. Results obtained were subjected to USP[27] validation scheme.

Assay of Pharmaceutical Dosage Forms: Accurately transfer measured aliquots equivalent to 0.4—0.8 mg of Lfx, 0.4—0.9 mg Mfx, and 0.8—1.2 mg Sfx from their stock solutions (1 mg/ml) into a series of 10-ml volumetric flasks, complete to volume using ethanol, and apply 10 μl of the solutions onto chromatographic plates proceeding as under construction of calibration curves. Scan the spots, record the areas, then calculate the corresponding concentrations of the recovered drugs from the regression equations.

Assay of Laboratory-Prepared Mixtures: Apply 10 μl of the laboratory-prepared mixtures onto the TLC plates proceeding as under construction of calibration curves. Scan the spots then record the areas and calculate the corresponding concentrations of the intact drugs from the regression equations.

Derivative Spectrophotometric Method Construction of Calibration Curves: Transfer accurately measured portions equivalent to 50—400 μg of each drug from their stock solutions (0.2 mg/ml) into a series of 25-ml volumetric flasks and complete the volume to the mark using 0.3 M HCl. Record the zero-order curves using 0.3 M HCl as blank. Manipulate the first derivative curves at 5-nm intervals and scaling factor 10. Measure D_1 value at 295.2 nm for Lfx, 280.4 and 303.4 nm for Mfx, and 280.8 nm for Sfx. Construct the calibration curves between the recorded peak amplitudes and the corresponding concentrations then compute the regression equations. Results obtained were subjected to USP validation scheme.[27]

Assay of Pharmaceutical Dosage Forms: Transfer accurately measured portions equivalent to 100—400 μg of each drug from their stock solutions (0.2 mg/ml) into a series of 25-ml volumetric flasks, complete the volume using 0.3 M HCl, then proceed as under construction of calibration curves. Record the zero-order spectra of the solutions, manipulate the D_1 spectra, and record D_1 values at the selected wavelengths. Calculate the corresponding concentrations of the recovered drugs from the regression equations.

Assay of Laboratory-Prepared Mixtures: Record the zero-order spectra of the laboratory-prepared mixtures containing different ratios of the intact drugs and their decarboxylated degradates. Manipulate the D_1 spectra and record the D_1 values at the selected wavelengths. Calculate the corresponding concentrations of the intact drugs from the regression equations.

Results and Discussion

Lfx, Mfx, and Sfx are fluorinated quinolones widely used for the treatment of a variety of serious systemic infections. All members of this drug class have a 6-fluoro substituent in common.[22] They were expected to decompose into their decarboxylated degradates when heated with 2 M HCl while protected from light.[23] In the present work the studied drugs were determined in the presence of their decarboxylated degradates since the carboxylic acid group is required for the pharmacological activity.[22,23]

Densitometric Method Shah *et al.*[10] determined Lfx in urine using HPTLC plates and butanol/methanol/ethyl acetate/6 M ammonia (4/2/3/2, v/v/v/v) and scanning at 292 nm. Meanwhile, Zheng and Feng[12] determined Sfx using 0.27 M EDTA-treated silica gel plates and ethanol/ethyl acetate/dichloroethane/10% ammonia (4/3/2/1, v/v/v/v) as developing solvent. Although these methods can determine the intact drugs, they were not used as stability-indicating ones for the selective determination of the studied drugs. The present work is concerned with the application of a densitometric TLC technique for the determination of Lfx, Mfx, and Sfx. The method is based on the difference in the *Rf* values of the 3 studied drugs and their main acid degradation products. Many developing solvents in different ratios were tried such as chloroform : methanol : ammonia (2 : 2 : 1, v/v/v), butanol : acetic acid : water (8 : 1 : 1, 8 : 1 : 3, 8 : 3 : 1, v/v/v), acetonitrile : ammonium chloride (9 : 1, 8 : 2, 5 : 5, v/v), *n*-propanol : ammonium chloride (9 : 1, 8 : 2, 5 : 5, v/v), but good and complete separation of the intact drugs from their decarboxylated degradates was achieved using the stated developing solvents and the *Rf* values were 0.47, 0.50, and 0.43 for Lfx, Mfx, and Sfx, respectively, and 0.83, 0.90, and 0.78 for their decarboxylated degradates.

The plates were scanned at 288, 290, and 292 nm for Lfx, Mfx, and Sfx, respectively, because these wavelengths are around the λ_{max} of the studied drugs. By applying this technique, linear correlations were obtained between the recorded areas in the concentrations of 0.2—1.2, 0.1—1.4, and 0.5—2.0 μg/spot for Lfx, Mfx, and Sfx, respectively. Re-

Table 1. Regression Equation Parameters and Determination of Pure Samples of Lfx, Mfx, and Sfx by the Proposed Methods

Parameters	TLC method								
	Lfx			Mfx			Sfx		
Range	0.2—1.2 μg/spot			0.1—1.4 μg/spot			0.5—2.0 μg/spot		
Slope	3671.4			3981.6			3471.0		
Intercept	573.67			268.7			187.96		
R	0.9999			0.9987			0.9958		
Mean accuracy	101.25			99.55			98.93		
S.D.	1.21			0.53			0.79		
Intraday determinations	Concentration μg/spot	*n*	% age recovery	Concentration μg/spot	*n*	% age recovery	Concentration μg/spot	*n*	% age recovery
	0.4	3	102.20	0.4	3	101.11	1.0	3	99.57
	0.6	3	101.66	0.6	3	99.80	1.2	3	98.45
	0.8	3	99.88	0.8	3	99.91	1.4	3	99.64
Mean	101.25			100.27			99.22		
RSD[a)]	1.21			0.73			0.67		
Interday determinations	Concentration μg/spot	*n*	% age recovery	Concentration μg/spot	*n*	% age recovery	Concentration μg/spot	*n*	% age recovery
		3	101.66		3	99.80		3	98.45
	0.6	3	100.33	0.6	3	98.19	1.2	3	99.13
		3	100.98		3	101.56		3	100.38
Mean	100.98			99.85			99.32		
RSD[b)]	0.68			1.69			0.98		

Parameters	D_1 method								
	Lfx 295.2			Mfx 280.4		303.4	Sfx 280.8		
Range	2—16 μg/ml								
Slope	0.0509			0.039		0.0599	0.0287		
Intercept	0.003			0.0032		0.0099	0.0066		
R	0.9995			0.9999		0.9999	0.9995		
Mean accuracy	98.18			100.35		99.86	99.19		
S.D.	0.83			0.91		1.08	0.64		
Intraday determinations	Concentration μg/ml	*n*	% age recovery	Concentration μg/ml	*n*	% age recovery 280.4	303.4	Concentration μg/ml	*n*	% age recovery
	4	3	98.10	4	3	100.89	100.66	6	3	99.45
	5	3	98.14	6	3	100.07	100.29	8	3	98.46
	8	3	98.31	8	3	100.01	100.09	10	3	99.66
Mean	98.18			100.32			100.35	99.19		
RSD[a)]	0.11			0.49			0.29	0.64		
Interday determinations	Concentration μg/ml	*n*	% age recovery	Concentration μg/ml	*n*	% age recovery 280.4	303.4	Concentration μg/ml	*n*	% age recovery
		3	98.14		3	100.01	100.09		3	99.16
	5	3	99.26	8	3	99.07	99.70	8	3	97.89
		3	98.89		3	100.33	101.01		3	99.16
Mean	98.76			99.80			100.26	98.74		
RSD[b)]	0.57			0.65			0.67	0.73		

Where *a*) is the intraday $n=9$ and *b*) is the interday $n=9$ relative standard deviation of sample concentrations 0.4, 0.6, and 0.8 μg/spot for Lfx and Mfx and of 0.8, 1.0, and 1.2 μg/spot for Sfx in the TLC method and 4, 6, and 8 μg/ml for Lfx and Mfx and 6, 8, and 10 μg/ml Sfx in the D_1 method.

gression parameters and correlation coefficients are shown in Table 1.

Derivative Spectrophotometric Method Zero-absorption spectra of the investigated drugs and their decarboxylated degradates show considerable overlapping, which interferes with the direct determination of the intact drugs. (Figs. 1—3). The first derivative technique was suggested to overcome this overlapping. Many diluting solvents were tried, but 0.3 M HCl was chosen as a solvent because it gave the best D_1 spectra. It should be noted that the stability of the studied drugs in this molarity away from light and at room temperature for the duration required for measurement was investigated and no degradation was detected under these conditions. By applying the D_1 method a zero crossing was obtained at 270.2 and 295.2 nm for Lfx, 280.4 and 303.4 nm for Mfx, and 280.8 and 311 nm for Sfx for the direct determination of the intact drugs in the presence of their decarboxylated degradates (Figs. 4—6). However, zero-crossing values at 270.2 nm for Lfx and at 311 nm for Sfx were excluded due to poor specifity and accuracy. A linear correlation was ob-

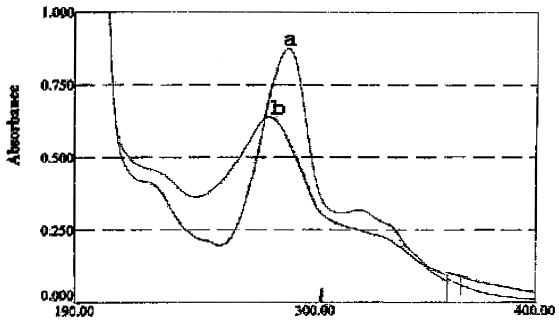


Fig. 1. Zero Absorption Spectra

a) Intact lomefloxacin in 0.3 M HCl (8 μg/ml). b) Lomefloxacin acid degradate in 0.3 M HCl (8 μg/ml).

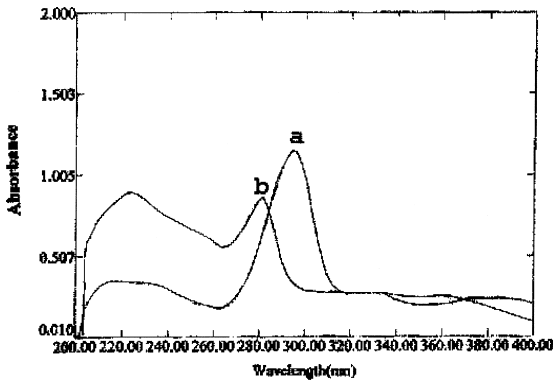


Fig. 2. Zero Absorption Spectra

a) Intact moxifloxacin in 0.3 M HCl (8 μg/ml). b) Moxifloxacin acid degradate in 0.3 M HCl (8 μg/ml).

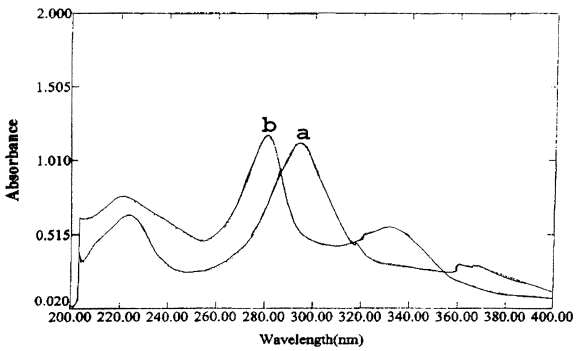


Fig. 3. Zero Absorption Spectra

a) Intact sparfloxacin in 0.3 M HCl (10 μg/ml). b) Sparfloxacin acid degradate in 0.3 M HCl (10 μg/ml).

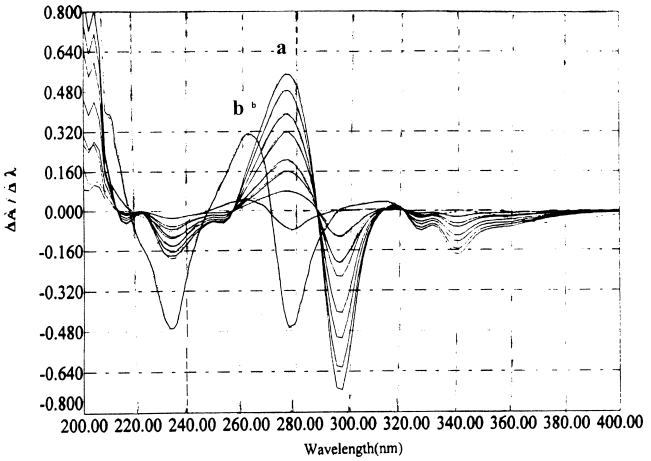


Fig. 4. D_1 Absorption Spectra

a) Intact lomefloxacin in 0.3 M HCl (2—16 μg/ml). b) Lomefloxacin acid degradate in 0.3 M HCl (2, 16 μg/ml).

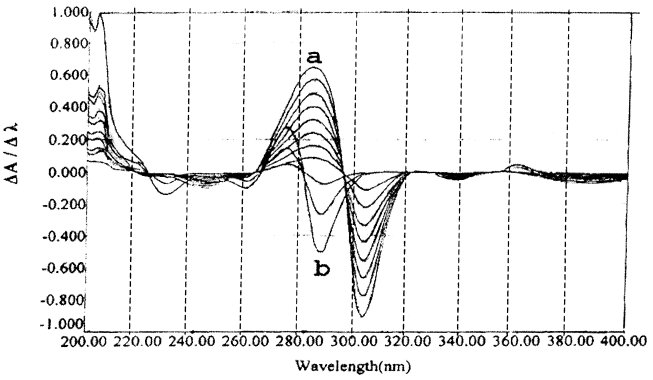


Fig. 5. D_1 Absorption Spectra

a) Intact moxifloxacin in 0.3 M HCl (2—16 μg/ml). b) Moxifloxacin acid degradate in 0.3 M HCl (2, 8, 16 μg/ml).

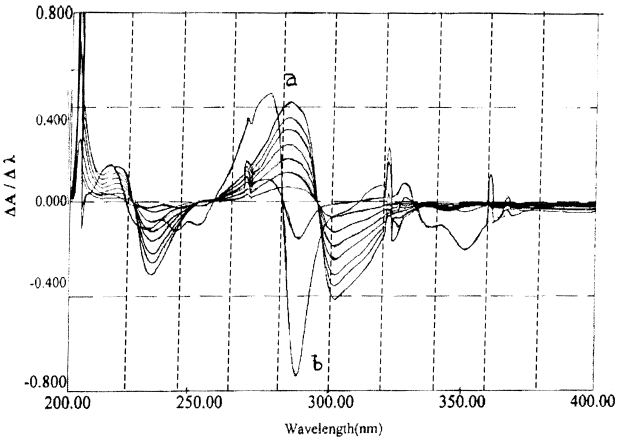


Fig. 6. D_1 Absorption Spectra

a) Intact sparfloxacin in 0.3 M HCl (2—16 μg/ml). b) Sparfloxacin acid degradate (2, 16 μg/ml).

tained between the amplitude of the peak or the trough and the concentration of the intact drugs in the range of 2—16 μg/ml from which linear regression equations were calculated. Regression parameters and correlation coefficients are shown in Table 1.

Methods Validation The suggested procedures were subjected to the validation scheme according to USP[27] and showed good specifity and reproducibility (Table 1).

Linearity The calibration curves for the 3 drugs in both methods were obtained by plotting the recorded area in the densitometric method or the amplitude of the peak or the trough in the D_1 method *versus* the corresponding concentration of the drug. The obtained high correlation coefficients indicate great obedience to Beer's law (Table 1).

Precision The intraday and interday precision was evaluated by assaying freshly prepared samples of the 3 drugs in triplicate in concentrations 0.4, 0.6, and 0.8 μg/ spot for Lfx and Mfx and 0.8, 1.0, and 1.2 μg/spot for Sfx for the TLC method and 4, 6, and 8 μg/ml for Lfx and Mfx and 6, 8, and 10 μg/ml for Sfx for D_1 method. The intraday and interday relative standard deviation (RSD) values obtained are shown in Table 1.

Accuracy The suggested procedures were also successfully applied to quantify the drugs in their pharmaceutical dosage forms (Table 2) and the validity of the obtained results was assessed by the recovery of the added standards (Table 3).

Selectivity To assess the efficacy of the suggested meth-

Table 2. Determination of Lfx, Mfx, and Sfx in Their Dosage Forms Using the Proposed Methods

	TLC method			D_1 method			
	Lomeflox tablets	Avalox tablets	Spara tablets	Lomeflox tablets	Avalox tablets		Spara tablets
					280.4	303.4	
Concentration	0.4—0.8 μg/spot	0.4—0.9 μg/spot	0.8—1.2 μg/spot	8—16 μg/ml	6—14 μg/ml		4—8 μg/ml
n	3	3	3	3	3	3	3
Mean percentage found	101.37±1.45	99.50±1.25	99.73±0.88	99.95±1.60	100.61±2.06	101.43±1.15	98.84±1.79

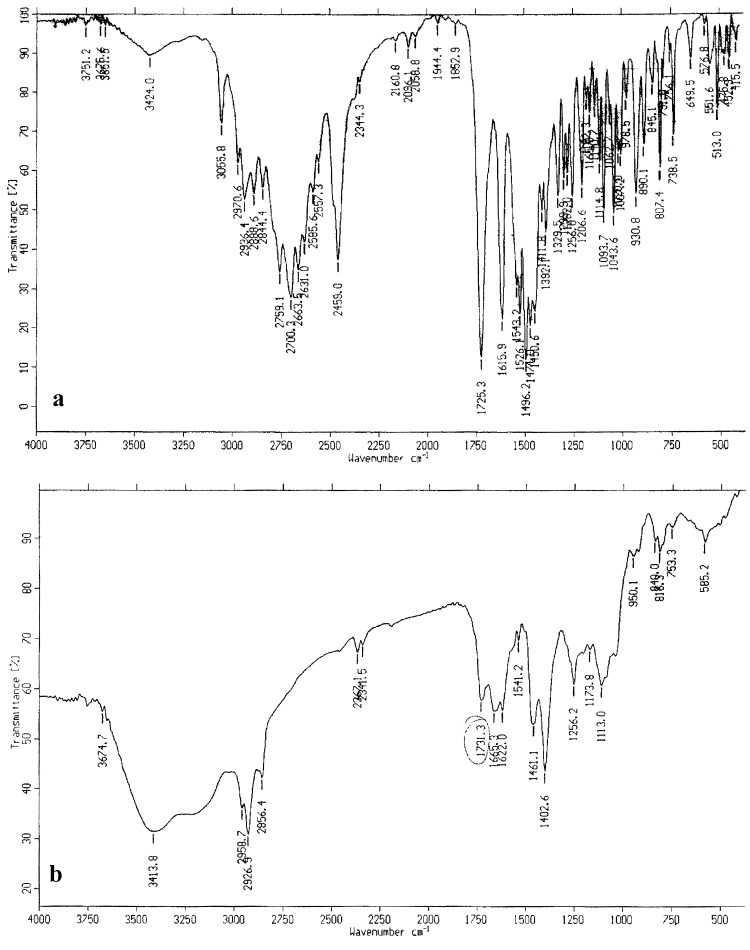


Fig. 7. IR Spectra

a) Intact lomefloxacin as KBr disc. b) Lomefloxacin acid degradate as KBr disc.

Table 3. Application of Standard Addition Technique to Determination of Lfx, Mfx, and Sfx in Pharmaceutical Dosage Forms Using the Proposed Methods

TLC method									D_1 method									
Lomeflox tablets			Avalox tablets			Spara tablets			Lomeflox tablets			Avalox tablets				Spara tablets		
Taken μg/spot	Standard added μg/spot	Recovery[a] of added standard	Taken μg/spot	Standard added μg/spot	Recovery[a] of added standard	Taken μg/spot	Standard added μg/spot	Recovery[a] of added standard	Taken μg/ml	Standard added μg/ml	Recovery[a] of added standard	Taken μg/ml	Standard added μg/ml	Recovery[a] of added standard		Taken μg/ml	Standard added μg/ml	Recovery[a] of added standard
														280.4	303.4			
0.2	0.4	100.50	0.2	0.4	103.29	1.0	0.5	102.41	8	2	102.99	8	2	102.80	103.67	2	8	97.41
0.4	0.4	98.69	0.4	0.4	99.48	0.8	0.8	103.56	8	4	100.92	8	4	101.58	102.05	4	8	97.41
0.4	0.2	101.55	0.4	0.2	101.80	0.6	1.2	99.13	4	4	101.37	8	8	98.10	98.57	6	6	99.42
									4	8	101.97	4	6	103.68	103.15	6	4	102.41
									2	8	100.99	4	8	102.45	103.15	8	4	99.71
Mean±S.D. 100.25±1.45			Mean±S.D. 101.52±1.92			Mean±S.D. 101.70±2.30			Mean±S.D. 101.65±0.86			Mean±S.D.		101.72 ±2.16	102.12 ±2.07	Mean±S.D. 99.27±2.06		

a) Average of three different experiments.

Table 4. Comparison between Results of Determination of Lfx, Mfx, and Sfx in the Presence of Their Acid Degradates Using the Proposed Methods and Reported Methods

Percentage of added degradates	TLC method			D_1 method				Reported method		
	Lfx	Mfx	Sfx	Lfx	Mfx		Sfx	Lfx[28]	Mfx[29]	Sfx[30]
					280.4	303.4				
10%	98.86	99.22	98.22	100.00	99.07	100.09	98.46	102.30	103.40	104.83
30%	99.65	98.17	97.66	100.00	99.21	100.91	100.00	105.41	107.11	124.87
50%	99.56	97.99	99.01	101.71	99.60	101.20	97.30	112.33	112.31	163.88
70%	99.61	99.01	98.50	102.78	100.00	103.26	97.26	127.99	126.23	170.92
90%	98.90	98.73	100.02	90.30[a]	94.94[a]	103.91	98.41	196.11	202.40	185.06
Mean	99.32	98.62	98.68	101.12	99.47	101.87	98.29			
S.D.	0.4	0.53	0.89	1.37	0.42	1.63	1.12			

a) Rejected.

Table 5. Comparison between Results of Analysis of the 3 Drugs Using the Proposed Methods and Reported Methods in Pure and Pharmaceutical Dosage Forms

	Pure sample										Pharmaceutical dosage form									
	TLC method			D_1 method				Reported method			TLC method			D_1 method				Reported method[23—25]		
	Lfx	Mfx	Sfx	Lfx	Mfx		Sfx	Lfx	Mfx	Sfx	Lfx	Mfx	Sfx	Lfx	Mfx		Sfx	Lfx	Mfx	Sfx
					280.4	303.4									280.4	303.4				
Mean %age	101.25	99.55	98.93	98.18	99.86	100.35	99.19	99.50	98.40	98.70	101.37	99.50	99.73	99.95	100.61	101.43	98.84	100.17	99.55	99.87
S^2	1.46	0.28	0.63	0.69	0.83	1.17	0.41	0.82	0.38	0.72	2.09	1.57	0.77	2.56	4.24	1.33	3.19	0.82	0.93	1.18
S.D.	1.21	0.53	0.79	0.83	0.91	1.08	0.64	0.91	0.62	0.85	1.45	1.25	0.88	1.60	2.06	1.15	1.79	0.91	0.86	1.08
n	3	3	4	3	3	3	3	3	3	3	3	3	3	3	4	4	3	3	3	3
t test	2.01	2.45	0.37	1.86	2.3	2.71	0.80				0.02	0.05	0.17	0.21	0.76	2.13	0.85			
F ratio	1.78	1.36	1.93	1.19	2.18	3.07	1.76				2.55	1.69	1.53	3.12	4.56	1.43	2.70			

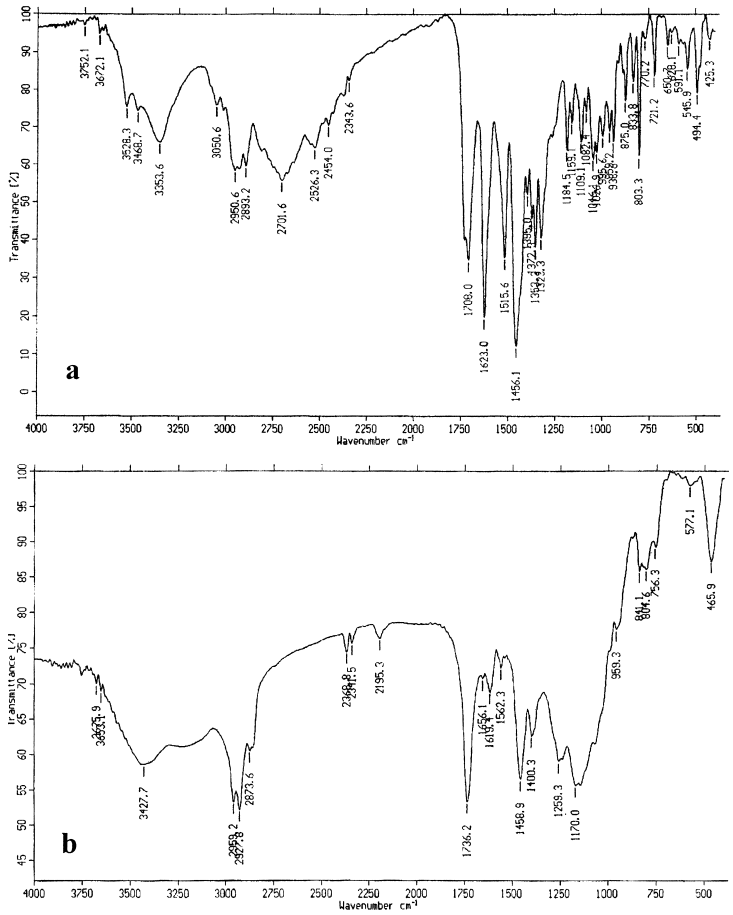


Fig. 8. IR Spectra

a) Intact moxifloxacin as KBr disc. b) Moxifloxacin acid degradate as KBr disc.

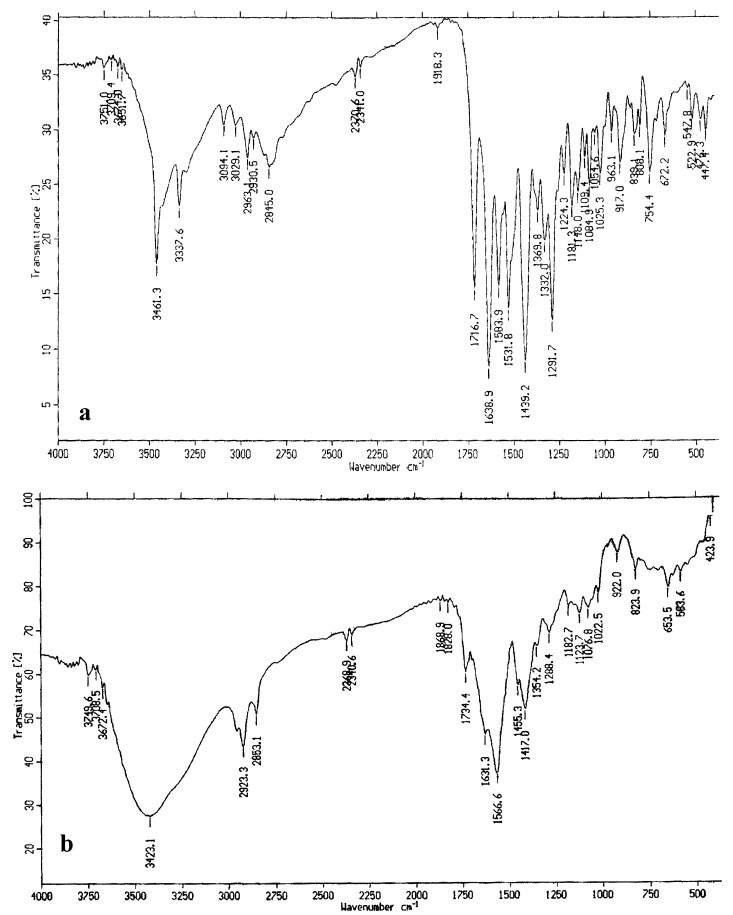


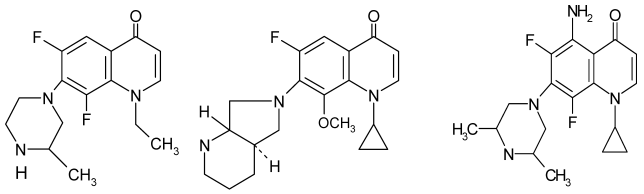
Fig. 9. IR Spectra

a) Intact sparfloxacin as KBr disc. b) Sparfloxacin acid degradate as KBr disc.

ods as stability-indicating methods, the decarboxylated degradate of each drug was prepared and separately mixed with its intact form in different ratios and analyzed by the proposed methods (Table 4). It is clear that the efficacy of the suggested procedures is not affected by the presence of ≤90% of the degradates in the TLC method and 70% of the degradates in the D_1 method for Lfx and ≤90% of degradation products for Mfx and Sfx.

Table 5 shows that there was no significant difference between the results of analysis of the investigated drugs using the proposed methods and reported UV methods that are used for routine analysis of Lfx,[24] Mfx,[25] and Sfx.[26]

The structures of the degradates of the 3 drugs were suggested after performing IR spectroscopy as shown from Figs. 7a, b, 8a, b and 9a, b. The suggested structures of the acid degradates are as follows:



Lfx acid degradate

Mfx acid degradate

Sfx acid degradate

This follows a previously mentioned scheme of degradation.[23]

Conclusion

The proposed TLC and D_1 methods are sensitive, simple, and selective. They permit the determination of Lfx, Mfx, and Sfx in pure form, pharmaceutical dosage forms and in the presence of their acid degradation products. Both methods complied with the validation scheme of the USP 28 and can therefore be used for purity testing, stability studies, quality control, and routine analysis of the 3 drugs in pure form and pharmaceutical dosage forms.

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