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Simultaneous interaction, degradation, and kinetic study of sparfloxacin with H_2 receptor antagonist

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Abstract: Sparfloxacin is a quinolone carboxylic acid derivative that shows activity as an antimicrobial agent, against a wide range of Gram-negative and Gram-positive organisms. It is clinically useful for the treatment of urinary tract infections, respiratory tract infections and gynecological infections. In this study *in vitro* drug-drug interaction of sparfloxacin has been carried out with famotidine and ranitidine. For these studies a two-component spectrophotometric process has been developed for sparfloxacin assay in the presence of famotidine or ranitidine. The reproducibility of the method is within ±5%. The technique has been applied to the development of sparfloxacin in methanol. The interaction studies of sparfloxacin with ranitidine and famotidine were carried out in methanol and methanol: Water mixtures (30:70, v/v; 50:50, v/v) and the kinetics of sparfloxacin degradation were evaluated in the presence and absence of famotidine and ranitidine. The decrease in the rate of degradation of sparfloxacin in the presence of famotidine or ranitidine, compared to that of sparfloxacin alone, indicated the possibility of interaction between the sparfloxacin and famotidine or ranitidine. The Thin layer chromatography (TLC) of the degraded solution showed the presence of a degradation product of sparfloxacin. The studies show that complexation with famotidine or ranitidine may affect the bioavailability of sparfloxacin.

Keywords: Sparfloxacin, famotidine, ranitidine.

INTRODUCTION

Chemically, sparfloxacin is 5-amino-1-cyclopropyl-7-[(3R, 5S) 3, 5-Dimethylpiperazine-1-yl] Acid -6,8-difluro-4oxoquinoline-3-carboxylic (fig.1.a.). A synthetic fluoroquinolone antibacterial drug with a broad spectrum of activity, sparfloxacin is effective against a variety of Gram-positive and Gram-negative pathogens. The bacterial topoisomerase DNA gyrase is inhibited by sparfloxacin, which then exhibits its antibacterial effect (Shen *et al*., 1989). The antibiotic sparfloxacin is effective against common respiratory pathogens as well as increasingly common atypical pneumonia pathogens. It is a successful treatment for respiratory tract infections (Brar *et al*., 2006, Goa *et al*., 1997, Shimada *et al*., 1993). A drawback of sparfloxacin is their photoreactivity occurs more frequently than with other fluoroquinolones (Engler *et al*., 1998, Marutani *et al*., 1993, Phillips *et al*., 1990, Marona and Schapoval, 2001, Argekar and Shah, 1999). Clinical experience to date has demonstrated that fluoroquinolone antibiotics are safe and effective in the management of a variety of infections (Rahm and Schacht, 1989). Sparfloxacin unwanted reactions involving the gastrointestinal tract such as gastrointestinal disturbances and CNS issues when treating patients with respiratory disease (Schentag, 2000, Hooper and Wolfson, 1991). For the treatment of gastrointestinal disturbances famotidine and ranitidine (figs. 1.c., fig.1.b), a drug from an H_2 receptor antagonist, is commonly used (Azizollahi and Rafeey, 2016). H_2 antagonists were the first successful peptic ulcer medications discovered. These medications are currently widely used to treat GERD. Ranitidine, famotidine, nizatidine, and cimetidine are examples of H_2 blockers (Mo *et al*., 2015). Co-administration of quinolones with cyclosporin shows drug interactions as cyclosporin nephrotoxicity enhanced (Avent *et al*., 1988, Elston and Taylor, 1988, FN, 1988). Further, drug interactions show with reductions in the gastrointestinal absorption of quinolones by concurrent use of quinolones with antacids (Höffken *et al*., 1985, Nix *et al*., 1989b, Fleming *et al*., 1986, Preheim *et al*., 1986). Moreover, decrease in quinolone bioavailability due to drug interaction between quinolone and sucralfate as aluminum ions are released by sucralfate and which formed chelate with the quinolones (Parpia *et al*., 1989, Nix *et al*., 1989a) while serum concentration of theophylline increases and inhibition of hepatic metabolism of caffeine also reported by co-administration quinolones and theophylline or caffeine as reduced theophylline clearance may persist for as long as five days following discontinuation of quinolones (Healy *et al*., 1989, Raoof *et al*., 1987, Schwartz *et al*., 1988, Niki *et al*., 1987, Prince *et al*., 1988). As sparfloxacin also shows drug interaction with cisapride and antacid (Zix *et al*., 1997, Hussain *et al*., 2006, Sohail *et al*., 2021). The main object of the present work is to investigate the possibility of the interaction between sparfloxacin with famotidine

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and ranitidine (Rubinstein and Segev, 1987, Stahlmann and Lode, 2000) in methanol-water medium and the detection of the degradation product of this compound in the medium during UV degradation at room temperature. Further studies were considered regarding the development of a two-component spectrophotometric method for the simultaneous determination of sparfloxacin with famotidine or sparfloxacin with ranitidine in photodegraded solution using appropriate analytical wavelengths and validation of the assay method under the present experimental conditions. It is intended to study the kinetics (Vaid *et al*., 2019) of degradation of sparfloxacin alone and in the presence of famotidine or ranitidine, in methanol and methanol: water mixtures to evaluate the possibility of an interaction between sparfloxacin with famotidine and ranitidine. The determination of rate constants for these reactions were take place by using UV light on the influence of famotidine and ranitidine on the rate of degradation of sparfloxacin. Any variation in the spectral characteristics of sparfloxacin and the individual famotidine and ranitidine and their mixtures could indicate the interaction between these compounds. The kinetics data could suggest whether the bioavailability of sparfloxacin is influenced by famotidine and ranitidine and whether any precaution should be taken in the administration of these compounds together.

MATERIALS AND METHODS

Reagents and materials

Sparfloxacin was a gift from Abbott Laboratories Pakistan Ltd, famotidine used was in the form of raw material which was provided by Platinum Pharmaceutical (Pvt) Ltd. Analytical grade methanol was used. To prepare deionized water, double distilled water was passed through the deionizer (Stedec CSW 300), and the conductance of the effluent was monitored. UV/ Visible Shimadzu double beam spectrophotometer CE 7200 series (1 cm rectangular quartz cells), Philips TUV 40 W UV tube, silica gel TLC plate GF 254, UV light lamp 254nm.

Preparation of solutions

Stock solution of sparfloxacin (5 x 10^{-4} M)

0.0196 g of sparfloxacin was accurately weighed induced by the funnel in a 100ml volumetric flask, dissolved in methanol, and volume was made up with the same solvent to the mark. The concentration of this stock solution was $5x10^{-4}$M.

Solution of sparfloxacin (5 x 10^{-5} M)

10ml of stock solution was further diluted in a 100ml volumetric flask with distilled methanol to make the concentration of $5x10^{-5}$M. This solution was used in all methods of analysis and degradation.

Stock solutions of famotidine and ranitidine ($5x10^{-4}$M)

For the preparation of $5x10^{-4}$M, famotidine (0.0168g), and ranitidine (0.0157g), accurately weighed induced by the funnel in 100ml volumetric flask separately, dissolved in methanol and the volume was made up to the mark with the same solvent.

Solutions of famotidine and ranitidine ($5x10^{-5}$M)

Take 5ml of stock solution of famotidine/ranitidine in a 50ml volumetric flask and make up the volume to the mark with the same solvent to get $5x10^{-5}$ M.

Solutions of famotidine and ranitidine solution ($2.5x10^{-5}$M)

Take 2.5 ml of stock solution of famotidine/ranitidine in a 50 ml volumetric flask and make up the volume to the mark with the same solvent to get $2.5x10^{-5}$M.

Solutions of famotidine and ranitidine ($1.5x10^{-5}$M)

Take 1.5 ml of stock solution of famotidine/ranitidine in a 50 ml volumetric flask and the volume was made up to the mark with the same solvent to get $1.5x10^{-5}$ M.

Preparation of mixtures

Methanol: water mixture (50:50, v/v) of sparfloxacin

10 ml of stock solution of sparfloxacin is taken in a 100 ml volumetric flask; add 40 ml methanol and make up the volume to the mark with distilled water.

Methanol: Water mixture (30:70, v/v) of sparfloxacin

10 ml of stock solution of sparfloxacin is taken in a 100 ml volumetric flask; add 20 ml methanol and make up the volume to the mark with distilled water.

Methanol: Water mixture (50:50 v/v) of different strengths of famotidine/ranitidine

5ml solution of famotidine/ranitidine ($5x10^{-5}$M, $2.5x10^{-5}$M and $1.5x10^{-5}$M) is taken in 50 ml volumetric flasks; add 20 ml methanol and makes up the volume to the mark with distilled water.

Table 1: R_f value of sparfloxacin and degraded product

Component	R_f value
Sparfloxacin	0.676
Degraded product	0.842

Methanol: Water mixture (30:70 v/v) of different strengths of famotidine/ranitidine

5ml solution of famotidine/ranitidine ($5x10^{-5}$M, $2.5x10^{-5}$M, and $1.5x10^{-5}$M) is taken in 50 ml volumetric flasks; add 10 ml methanol and makes up the volume to the mark with distilled water.

UV light irradiation

UV light irradiation of sparfloxacin (50:50 v/v) with famotidine/ranitidine (50:50v/v)

10ml of sparfloxacin (50:50, v/v) solution was taken with each 10ml of famotidine and ranitidine (50:50) in the beakers and irradiated with UV light using Philips a TUV 40W, UV tube.

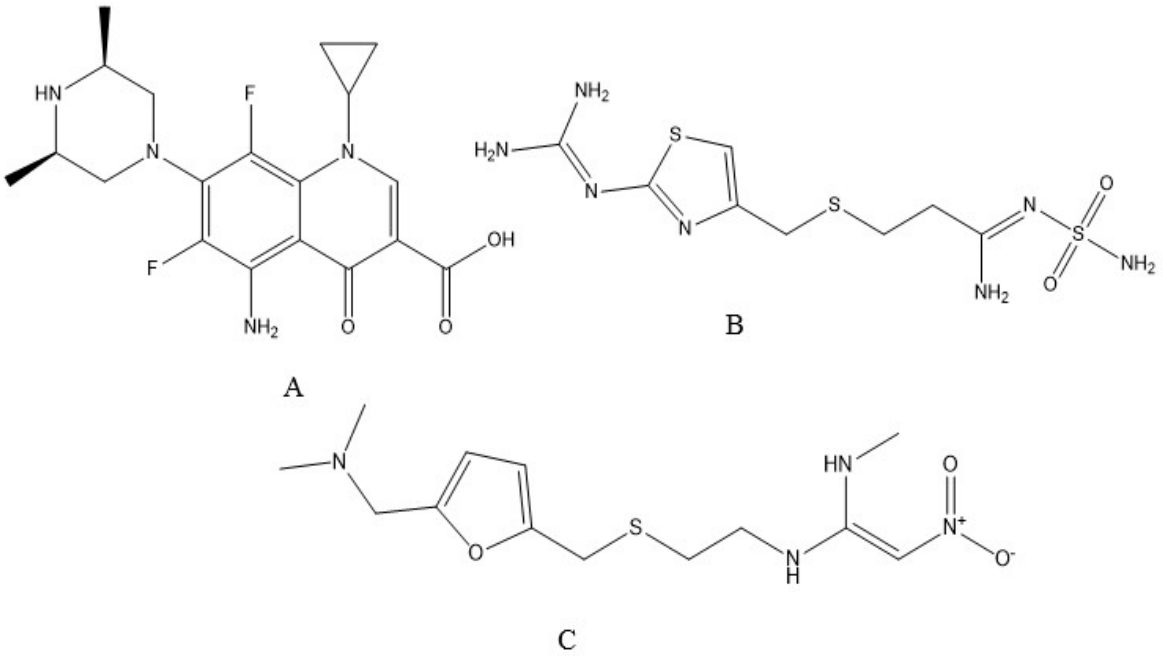

Fig. 1: a. structure of sparfloxacin, b. structure of famotidine, c. structure of ranitidine

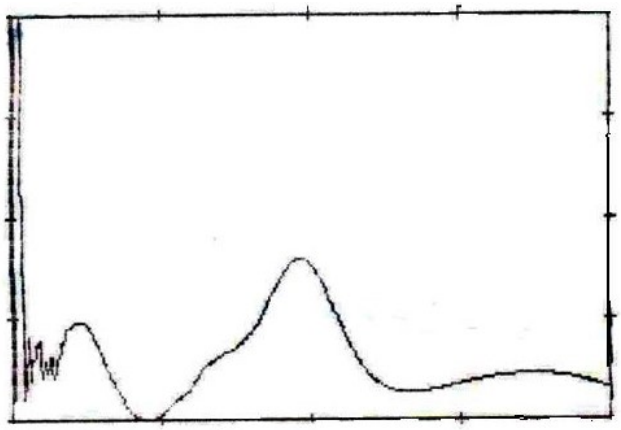

Fig. 2: Absorption spectrum of 5x10^{-5}M sparfloxacin

UV light irradiation of sparfloxacin (30:70 v/v) with famotidine/ranitidine (30:70v/v)

10ml of sparfloxacin (30:70, v/v) solution was taken with each 10ml of famotidine and ranitidine (30:70) in the beakers and irradiated.

The assay method of sparfloxacin

In comparison to a reagent blank, the UV/visible area absorbance maxima for these solutions were scanned. The maxima were observed at 300 and 377 nanometers. Graphs were plotted for absorbance against concentration and molar absorptivity was calculated from these observations. Measurement of this absorbance band has been employed for the assay of sparfloxacin which followed Beer Lambert's law in the concentration range of 2 to 5x10^{-5} M.

TLC for degradation product of sparfloxacin

TLC of the degraded solution of sparfloxacin was performed in the following system

Adsorbent:	Silica gel GF 254
Spot detection:	UV light (254 nm)
Solvent system:	Methanol: water (50:50, v/v)

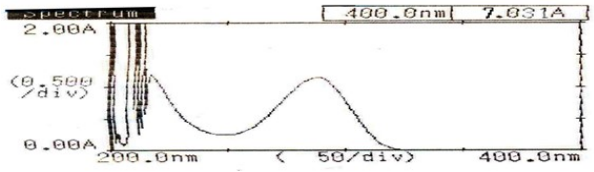

Fig. 3: Absorption spectrum of 5x10^{-5}M famotidine

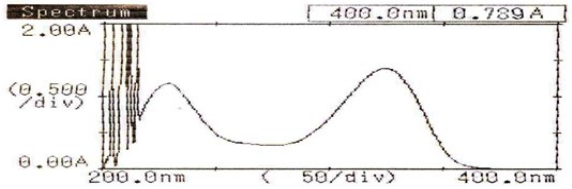

Fig. 4: Absorption spectrum of 5x10^{-5}M Ranitidine

Reproducibility of method

Mixtures of sparfloxacin with famotidine or ranitidine in different concentrations of two compounds were prepared in methanol (tables 3 and 4). The solutions' absorbance was measured at 377 nm and 288 nm or 377 nm and 324 nm and the concentration of sparfloxacin was determined by a two-component spectrophotometric method, The reproducibility of the method was determined from analytical data.

Table 2: Molar absorptivities ($M^{-1}cm^{-1}$)

Molar absorptivities (M^{-1} cm^{-1}) of sparfloxacin, famotidine and ranitidine						
Medium	wavelength nm	Sparfloxacin Є	wavelength nm	Famotidine Є	wavelength nm	Ranitidine Є
methanol	288	20376	288	15395	324	16324
	300	20500	377	150	377	192
	324	18855				
	377	9467				
methanol: water (50:50,v/v)	288	20311	288	15310	324	16298
	300	20355	377	178	377	189
	324	18805				
	377	9350				
methanol: water (30:70,v/v)	288	20097	288	15308	324	16280
	300	20300	377	145	377	185
	324	18822				
	377	93335				

Table 3: Absorbance values for two-component spectrophotometric analysis.

S. No	Sparfloxacin Mx10^{-5}	Famotidine Mx10^{-5}	Absorbance at 377nm	Absorbance at 288nm
1	9.0	1.0	0.430	1.000
2	8.0	2.0	0.391	0.999
3	7.0	3.0	0.336	0.960
4	6.0	4.0	0.295	0.950
5	5.0	5.0	0.245	0.908
6	4.0	6.0	0.200	0.900
7	3.0	7.0	0.151	0.850
8	2.0	8.0	$0.^{105}$	0.858
9	1.0	9.0	0.055	0.799

Table 4: Analysis of synthetic mixtures of sparfloxacin and famotidine.

Sparfloxacin				Famotidine			
Added M x 10^{-5}	Found M x 10^{-5}	%Recovery	%RSD	Added M x 10^{-5}	Found M x 10^{-5}	%Recovery	%RSD
4.5	4.534	100.75	0.143	0.5	0.494	98.800	2.150
4.0	4.113	102.820	0.267	1.0	1.044	104.400	1.340
3.5	3.524	100.685	0.340	1.5	1.571	104.733	1.749
3.0	3.082	102.733	0.040	2.0	2.092	104.600	0.195
2.5	2.547	101.880	0.023	2.5	2.525	101.000	1.990
2.0	2.063	103.150	0.224	3.0	3.115	103.833	0.633
1.5	1.539	102.600	0.394	3.5	3.483	99.514	0.671
1.0	1.043	104.300	1.16	4.0	4.190	104.750	0.508
0.5	0.509	101.800	1.256	4.5	4.515	100.333	0.165

Table 5: Absorbance values for two-component spectrophotometric analysis.

S. No	Sparfloxacin M x10^{-5}	Ranitidine M x10^{-5}	Absorbance at 377nm	Absorbance at 324nm
1	9.0	1.0	0.430	0.935
2	8.0	2.0	0.390	0.932
3	7.0	3.0	0.334	0.915
4	6.0	4.0	0.296	0.910
5	5.0	5.0	0.240	0.880
6	4.0	6.0	0.200	0.885
7	3.0	7.0	0.151	0.860
8	2.0	8.0	0.103	0.840
9	1.0	9.0	0.055	0.818

Table 6: Analysis of synthetic mixtures of sparfloxacin and ranitidine.

Sparfloxacin				Ranitidine			
Added M x 10^{-5}	Found M x 10^{-5}	Recovery %	RSD %	Added M x 10^{-5}	Found M x 10^{-5}	Recovery %	RSD %
4.5	4.532	100.711	1.337	0.5	0.493	98.600	0.000
4.0	4.099	102.475	0.538	1.0	0.974	97.400	0.405
3.5	3.496	99.885	0.000	1.5	1.566	104.400	0.787
3.0	3.085	102.833	0.349	2.0	2.010	100.500	0.122
2.5	2.484	99.360	0.240	2.5	2.522	100.880	1.230
2.0	2.050	102.500	0.000	3.0	3.053	101.766	0.379
1.5	1.523	101.533	0.289	3.5	3.508	100.228	0.087
1.0	1.007	100.700	0.340	4.0	3.982	99.550	0.744
0.5	0.490	98.000	0.014	4.5	4.444	98.755	0.088

Table 7: Concentrations of sparfloxacin degraded in UV radiation with famotidine ($5x10^{-5}$ M) in methanol and methanol-water mixtures (50:50, v/v and 30:70, v/v).

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of famotidine M x 10^{-5}	Concentration of sparfloxacin (log)
0	Methanol	4.882	Famotidine	5.0	-4.311
60		4.775			-4.321
120		4.634			-4.334
180		4.508			-4.346
240		4.355			-4.361
0	Methanol: water mixture (50:50, v/v)	4.884	Famotidine	5.0	-4.311
60		4.808			-4.318
120		4.709			-4.327
180		4.613			-4.336
240		4.508			-4.346
0	Methanol: Water mixture (30:70, v/v)	4.882	Famotidine	5.0	-4.311
60		4.841			-4.315
120		4.764			-4.322
180		4.698			-4.328
260		4.634			-4.334

Table 8: Concentrations of sparfloxacin degraded in UV radiation with famotidine ($2.5x10^{-5}$M) in methanol and methanol-water mixtures (50: 50, v/v.)

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of famotidine M x 10^{-5}	Concentration of sparfloxacin (log)
0	Methanol	4.883	Famotidine	2.5	-4.311
60		4.720			-4.326
120		4.518			-4.345
180		4.345			-4.362
240		4.120			-4.385
0	Methanol: Water mixture (50:50, v/v)	4.883	Famotidine	2.5	-4.311
60		4.753			-4.323
120		4.634			-4.334
180		4.508			-4.346
240		4.375			-4.359

Table 9: Concentrations of sparfloxacin degraded in UV radiation with famotidine ($1.5x10^{-5}$M) in methanol and methanol-water mixtures (50:50, v/v).

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of famotidine M x 10^{-5}	Concentration of sparfloxacin(log)
0	Methanol	4.884	Famotidine	1.5	-4.311
6		4.666			-4.331
120		4.436			-4.353
180		4.236			-4.373
240		3.999			-4.398
0	Methanol: water mixture (50:50,v/v)	4.881	Famotidine	1.5	-4.311
60		4.720			-4.326
120		4.591			-4.338
180		4.456			-4.351
240		4.305			-4.366

Table 10: Concentrations of sparfloxacin degraded in UV radiation with ranitidine ($5x10^{-5}$M) in methanol and methanol-water mixtures (50:50, v/v and 30:70, v/v)

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of famotidine M x 10^{-5}	Concentration of sparfloxacin (log)
0	Methanol	4.881	Ranitidine	5.0	-4.311
60		4.775			-4.321
120		4.645			-4.333
180		4.528			-4.344
240		4.395			-4.357
0	Methanol: water mixture (50:50,v/v)	4.883	Ranitidine	5.0	-4.311
60		4.819			-4.317
120		4.742			-4.324
180		4.645			-4.333
240		4.560			-4.341
0	Methanol:water mixture (30:70,v/v)	4.886	Ranitidine	5.0	-4.311
60		4.830			-4.316
120		4.775			-4.321
180		4.720			-4.326
240		4.655			-4.332

Table 11: Concentrations of sparfloxacin degraded in UV radiation with ranitidine ($2.5x10^{-5}$ M) in methanol and methanol-water mixtures (50:50, v/v).

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of ranitidine M x 10^{-5}	Concentration of sparfloxacin (log)
0	Methanol	4.883	Ranitidine	2.5	-4.311
60		4.742			-4.324
120		4.522			-4.344
180		4.340			-4.362
240		4.149			-4.382
0	Methanol: water Mixture (50:50, v/v)	4.883	Ranitidine	2.5	-4.311
60		4.775			-4.321
120		4.655			-4.332
180		4.528			-4.344
240		4.425			-4.354

Table 12: Concentrations of sparfloxacin degraded in UV radiation with ranitidine ($1.5x10^{-5}$M) in methanol and methanol-water mixtures (50:50, v/v).

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of ranitidine M x 10^{-5}	Concentration of sparfloxacin (log)
0	Methanol	4.884	Ranitidine	1.5	-4.311
60		4.709			-4.327
120		4.477			-4.349
180		4.275			-4.369
240		4.036			-4.394
0	Methanol:water mixture (50:50, v/v)	4.881	Ranitidine	1.5	-4.311
60		4.753			-4.323
120		4.623			-4.335
180		4.497			-4.347
240		4.335			-4.363

Table 13: Concentrations of sparfloxacin degraded alone in UV radiation without ranitidine or famotidine in methanol and methanol-water mixtures (50:50, v/v).

Time (min)	Medium	Concentration of sparfloxacin M x 10^{-5}	H_2-receptor antagonist	Concentration of sparfloxacin (log)
0	Methanol	4.884		-4.311
60		4.591		-4.338
120		4.355		-4.361
180		4.120		-4.385
240		3.881		-4.411
0	Methanol: Water mixture (50:50,v/v)	4.881	-	-4.311
60		4.698		-4.328
120		4.560		-4.341
180		4.405		-4.356
240		4.255		-4.371

Interaction studies of sparfloxacin and famotidine/ranitidine

Interaction of $5.0x10^{-5}$M sparfloxacin and $5x10^{-5}$M, $2.5x10^{-5}$M and $1.5x10^{-5}$M famotidine/ranitidine was studied in these ratios of the two compounds in methanol and water: methanol mixtures (50:50 and 30:70, v/v) on exposure to UV light. The concentration of sparfloxacin in the mixtures was calculated by a two-component spectrometric method. Aliquots were taken at 1hr intervals and the absorbance was noted, at 0min, 60min, 120min, 180min and 240min.

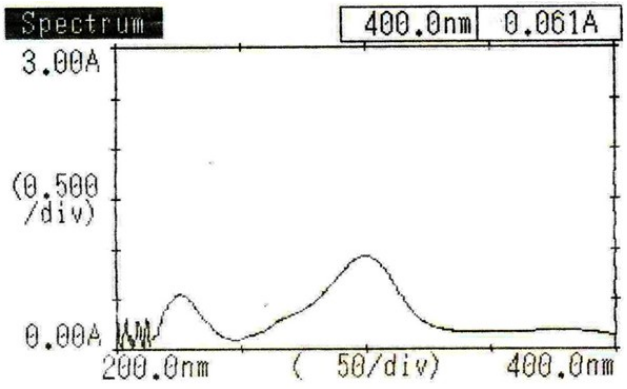

Fig. 5: Absorption spectrum of the mixture of sparfloxacin (5 x 10^{-5} M) and famotidine (5 x 10^{-5} M)

Calculation of concentration in a spectrophotometric assay

The assay of sparfloxacin, famotidine and ranitidine, alone and in mixtures was carried out by one and two-component spectrophotometric assays at the specific wavelength, (tables 3-6). The concentrations of the component were calculated as follows:

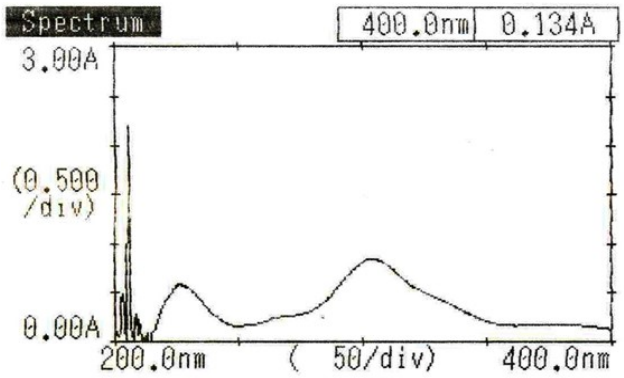

Fig. 6: Absorption spectrum of the mixture of sparfloxacin (5 x 10^{-5} M) and ranitidine (5 x 10^{-5} M)

One component assay

If Beer's law is followed by the solution of a compound, the concentration of the compound can be calculated as follows

Table 14: Concentrations of sparfloxacin degraded by UV radiation in different ratio of methanol and methanol-water mixtures (50:50, v/v and 30:70, v/v).

Time (min)	Medium	H_2-receptors antagonist	Concentration $Mx10^{-5}$	Concentration (log)
0	Methanol	Famotidine	4.882	-4.311
60			4.775	-4.321
120			4.634	-4.334
180			4.508	-4.346
240			4.355	-4.361
0	Methanol	Ranitidine	4.881	-4.311
60			4.775	-4.321
120			4.645	-4.333
180			4.528	-4.344
240			4.395	-4.357
0	Methanol: water mixtures (50:50, v/v)	Famotidine	4.884	-4.311
60			4.808	-4.318
120			4.709	-4.327
180			4.613	-4.336
240			4.508	-4.346
0	Methanol: water mixtures (50:50,v/v)	Ranitidine	4.883	-4.311
60			4.819	-4.317
120			4.742	-4.324
180			4.645	-4.333
240			4.560	-4.341
0	Methanol: water mixture (30:70,v/v)	Famotidine	4.882	-4.311
60			4.841	-4.315
120			4.764	-4.322
180			4.698	-4.328
240			4.634	-4.334
0	Methanol: water mixture (30:70,v/v)	Ranitidine	4.882	-4.311
60			4.830	-4.316
120			4.775	-4.321
180			4.720	-4.326
240			4.655	-4.332

Table 15: Apparent first-order rate constants (k_0) for the degradation of sparfloxacin in methanol and methanol-water mixtures (50:50, v/v and 30:70, v/v).

Medium	H_2-receptors antagonist	$(k)_{obs}\ 10^{-4}$	Correlation coefficient
Methanol	Famotidine	9.190	0.996
Methanol	Ranitidine	10.918	0.998
Methanol: water (50:50, v/v)	Famotidine	4.959	0.996
Methanol: water (50:50, v/v)	Ranitidine	5.595	0.995
Methanol: water (30:70, v/v)	Famotidine	2.494	0.998
Methanol: water (30:70, v/v)	Ranitidine	2.211	0.996

$A_1 = {}_1Є_1\ {}_1C$ (Baghel and Shah, 2023)

Where Є is the molar absorptivity at wavelength λ_1, $_1C$ is the concentration of a component and A is the absorbance at wavelength λ_1. If the same cell is used throughout then $A_1 = {}_1k_1\ {}_1C$, where $_1k_1$ is the absorptivity cell path product.

Two-component assay

In this case, absorbance measurements are carried out on the solution at two suitably. The selected wavelength and the concentration are calculated by solving two simultaneous equations.

$A_1 = {}_1k_1\ {}_1C + {}_2k_1\ {}_2C$ (Behera *et al*., 2012)

$A_2 = {}_1k_2\ {}_1C + {}_2k_2\ {}_2C$ (Behera *et al*., 2012)

Where A_1 = absorbance at wavelength λ_1, A_2 = absorbance at wavelength λ_2, $_1k_1$ = absorptivity-cell path product for component 1 at wavelength λ_1, $_1k_2$ = absorptivity-cell path product for component 1 at wavelength λ_2, $_2k_1$ = absorptivity-cell path product for component 2 at wavelength λ_1, $_2k_2$ = absorptivity-cell path product for component 2 at wavelength λ_2, $_1C$ = is a concentration of component 1, $_2C$ = is the concentration of component. The solution of equation for $_1C$ and $_2C$ is made as: $_1C = (_2k_2\ A_1 - {}_2k_1\ A_2) / (_1k_1.\ {}_2k_2 - {}_2k_1.\ {}_1k_2)$, $_2C = (_1k_1\ A_2 - {}_1k_2\ A_1) / (_1k_1.\ {}_2k_2 - {}_2k_1.\ {}_1k_2)$

Kinetics of degradation of sparfloxacin with famotidine and ranitidine

The values of molar concentration on these compounds alone and in mixtures during degradation are reported in tables 7- 13 and 14. Plotting the values of log concentration versus time both alone and in mixtures for each sparfloxacin gave a straight line indicating that it degrades in these media by first-order kinetics. The apparent first-order rate constants for these reactions are given in table 15.

Table 16: Calculated values of dielectric constant for methanol and methanol: water mixtures.

Methanol: Water	Dielectric constants
30:70	64.8
50:50	55.5
100:0	32.6

RESULTS

Sparfloxacin and their degraded products

Thin layer chromatography (TLC) of sparfloxacin and their degraded products in methanol: Water mixture (30:70, v/v) was carried out using silica gel GF 254 plates to detect any degradation product formed in the medium. It was observed that the degraded solution of sparfloxacin gives one spot in addition to the parent compound indicating its degradation in this medium. The TLC result has confirmed the presence of degradation product in the degraded solution in this compound, however, the degradation product could not be identified in the absence of any standards of the degraded product**.** The R_f value of sparfloxacin and their degraded product are reported in table 1.

Spectral characteristics of sparfloxacin and famotidine/ranitidine

To observe the spectral characteristics of sparfloxacin and famotidine/ranitidine in methanol, the solvent used in this study, the absorption spectra of this compound were measured in the UV region. Sparfloxacin shows absorption maxima in the UV region at 300 nm and 377 nm. Famotidine and ranitidine exhibit absorption maxima at 288 nm, 324 nm, and 230 nm, respectively fig. 2, 3, 4. These values are in agreement with the value of famotidine/ ranitidine reported (Moffat, 2004), with slight differences due to the change of the medium. The molar absorptivities determined at this wavelength are reported in table 2. Since the present study involves the degradation of these compounds in methanol and methanol-water mixtures. There is a need to develop a suitable method for the analysis of these compounds in presence of each other in the medium used. Sparfloxacin and famotidine/ ranitidine have overlapping spectra in fig. 5 and 6. Therefore it is not possible to assay sparfloxacin directly in the medium. Under these conditions, it is possible to carry out a two-component spectrophotometric assay of sparfloxacin and famotidine or sparfloxacin and ranitidine.

Assay of sparfloxacin and famotidine/ranitidine in degraded solution

A two-component spectrophotometric method has been developed for the simultaneous determination of sparfloxacin and famotidine or sparfloxacin and ranitidine in solutions degraded in methanol-water mixtures in UV radiation. Sparfloxacin and famotidine or sparfloxacin and ranitidine were assayed by two-component analysis using the wavelength corresponding to their absorption maxima. The reproducibility of the method is within ± 5%.

Kinetics of degradation of sparfloxacin with famotidine and ranitidine

It has been found that sparfloxacin degrades faster than that in the presence of famotidine and ranitidine. In the case of ranitidine, the rate constant of degradation is faster than that of famotidine. This could be due to an interaction between sparfloxacin and famotidine or sparfloxacin. The spectral change in UV degradation of sparfloxacin in the presence of famotidine and ranitidine is given in fig. 7, 8 and the first-order rate constant graph is present in fig. 9, 10, 11 and 12. The apparent first-order rate constant graph is given in fig. 13 and 14.

Solvent effect on the rate of degradation

To observe the effect of solvent on the rate of degradation of sparfloxacin in methanol-water mixtures, the apparent first-order rate constant (k_{obs}) for these reactions was plotted as a function of the solvent dielectric constant. A linear relationship between (k) and ϵ indicates the rate of degradation in table 14. The apparent first-order rate constant is given in table 15. As the polarity of the mixture increases, the degradation of sparfloxacin decreases table 16, so there is the inverse effect of dielectric constant on the degradation of the drug given in fig 2.14, 2.15 and their apparent first-order rates constant (k_{obs}) values for the degradation of sparfloxacin ($5x10^{-5}$M) in the presence of famotidine and ranitidine in different ratio of solvent mixtures.

Interaction of sparfloxacin with famotidine and ranitidine

An important aspect of the present study was to observe if any interaction takes place between famotidine/ranitidine in a methanol-water medium and whether famotidine/ ranitidine exerts any effect on the availability of sparfloxacin or vice versa. In the present study, the kinetics data have indicated that the rates of degradation of sparfloxacin in the presence of famotidine/ranitidine are slowed down in the mixture compared to those of sparfloxacin alone. This suggests the possibility of some sort of interaction between these compounds to influences the rates of degradation.

Table 17: Statistical values of sparfloxacin with famotidine.

Famotidine 95% CI		CSFm	CSFmwHalf	CSFmw30	CSF1m	CSF1mwHalf	CSF2m	CSF2mwHalf
		c=5e-5			c=2.5e-5		c=1.5e-5	
0min	t-stat	-3.949	-4.408	-5.210	14.986	23.891	18.921	30.847
60min	Lower	-0.628	-0.481	-0.362	1.643	1.883	2.512	2.812
120min	Upper	-0.109	-0.109	-0.110	2.390	2.378	3.376	3.368
180min	p-value	0.017	0.012	0.006	0.000	0.000	0.000	0.000
240min								

Table 18: Statistical values of sparfloxacin with ranitidine.

Ranitidine 95% CI		CSRm	CSRmwHalf	CSRmw30	CSR1m	CSR1mwHalf	CSR2m	CSR2mwHalf
		c=5e-5			c=2.5e-5		c=1.5e-5	
0min	t-stat	-4.118	-4.648	-5.604	15.306	26.169	19.737	32.670
60min	Lower	-0.594	-0.431	-0.339	1.659	1.924	2.557	2.852
120min	Upper	-0.115	-0.108	-0.114	2.394	2.381	3.394	3.382
180min	p-value	0.015	0.010	0.005	0.000	0.000	0.000	0.000
240min								

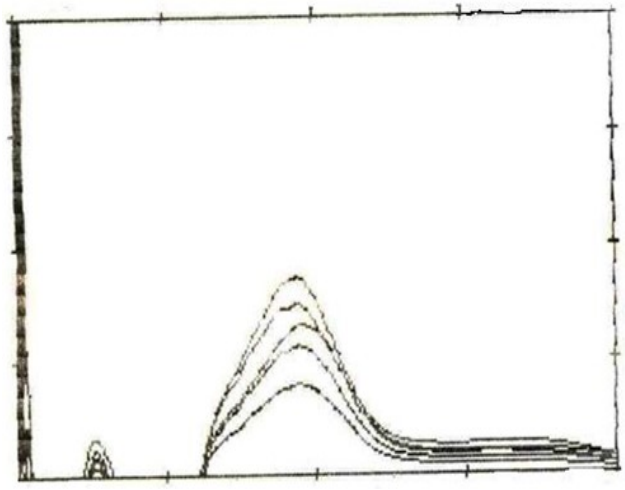

Fig. 7: Absorption spectrum of degradation of sparfloxacin ($5x10^{-5}$ M) with famotidine ($5x10^{-5}$ M)

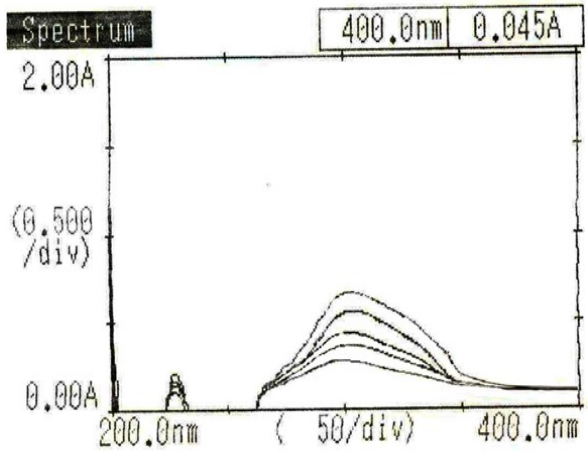

Fig. 8: Absorption spectrum of degradation of sparfloxacin ($5x10^{-5}$ M) with ranitidine ($5x10^{-5}$ M)

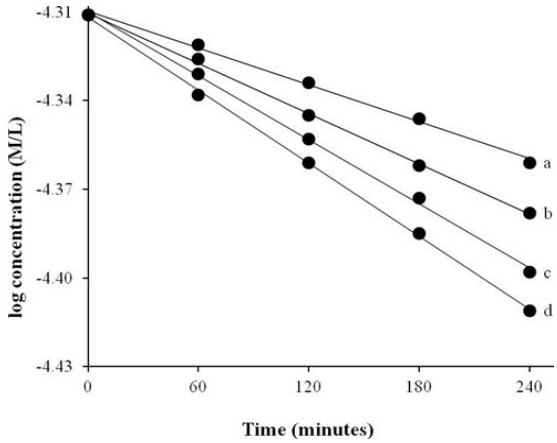

Fig. 9: First-order plot for the degradation of sparfloxacin ($5x10^{-5}$ M) in methanol in presence of famotidine, a: $5x10^{-5}$ M, b: $2.5x10^{-5}$ M, c: $1.5x10^{-5}$ M and d: absent.

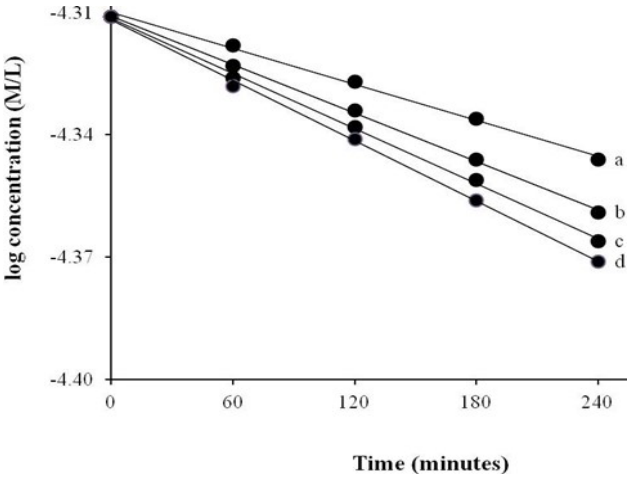

Fig. 10: First-order plot for the degradation of sparfloxacin ($5x10^{-5}$ M) in methanol-water mixture (50:50, v/v) in presence of famotidine, a: $5x10^{-5}$ M, b: $2.5x10^{-5}$ M, c: $1.5x10^{-5}$ M and d: absent.

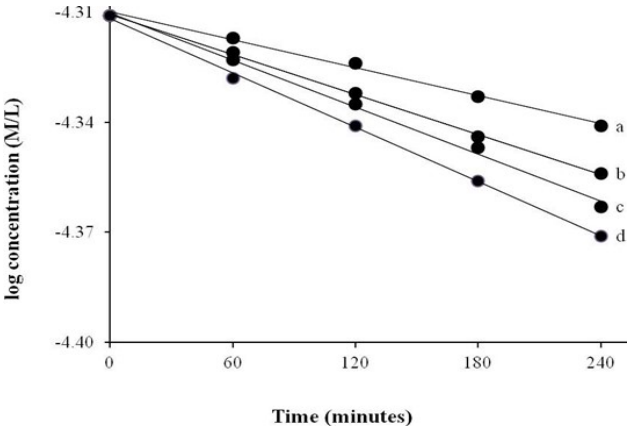

Fig. 11: First-order plot for the degradation of sparfloxacin ($5x10^{-5}$ M) in methanol in presence of ranitidine, a: $5x10^{-5}$ M, b: $2.5x10^{-5}$ M, c: $1.5x10^{-5}$ M and d: absent.

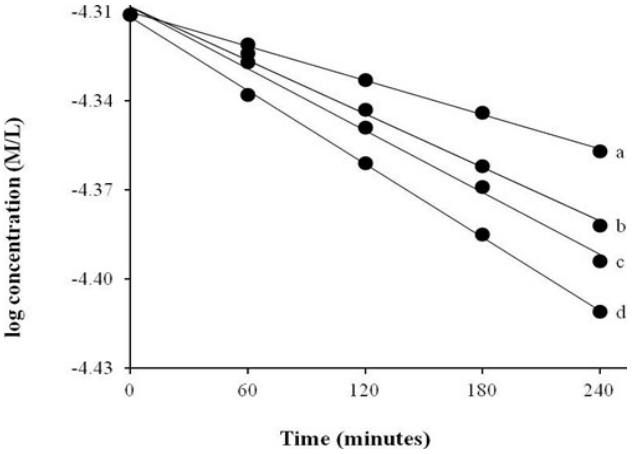

Fig. 12: First-order plot for the degradation of sparfloxacin ($5x10^{-5}$ M) in methanol-water mixture (50:50, v/v) in presence of ranitidine, a: $5x10^{-5}$ M, b: $2.5x10^{-5}$ M, c: $1.5x10^{-5}$ M and d: absent.

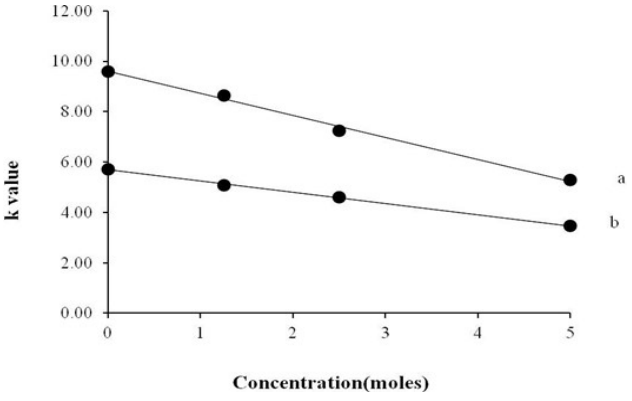

Fig. 13: Apparent first-order rate constants (kobs) for the degradation of sparfloxacin ($5x10^{-5}$ M) in the absence and presence of famotidine ($5x10^{-5}$ M, $2.5x10^{-5}$ M, $1.5x10^{-5}$ M) and second-order rate constants (k_0) for the interaction of sparfloxacin and famotidine, a: methanol, b: methanolwater mixture 50:50, v/v.

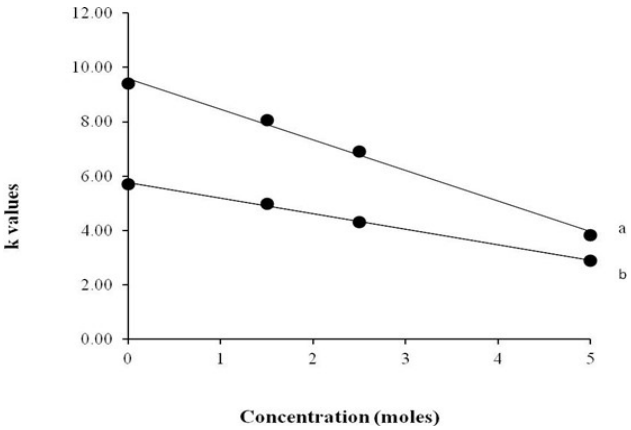

Fig. 14: Apparent first-order rate constants (kobs) for the degradation of sparfloxacin ($5x10^{-5}$ M) in the absence and presence of ranitidine ($5x10^{-5}$ M, $2.5x10^{-5}$ M, $1.5x10^{-5}$ M) and second-order rate constants (k_0) for the interaction of sparfloxacin and ranitidine, a: methanol, b: methanol-water mixture 50:50, v/v.

It appears that the interaction of sparfloxacin and famotidine/ranitidine leads to the formation of a complex that protects sparfloxacin in the presence of famotidine/ ranitidine. The nature of this complex or association is not known, however, a little change in the absorption maxima of these compounds in mixtures compared to the individual compound has been observed indicating some interaction of these compounds (about 2 mm) in the media. Based on the results obtained it was suggested that famotidine or ranitidine may influence the bioavailability of sparfloxacin in the physiological system.

A thin-layer chromatographic study has shown the presence of one spot in a degraded solution of sparfloxacin indicating its degradation in the methanol-water medium. This degradation product could not be identified due to the non-availability of any reference standard of the product. The UV absorption spectra of sparfloxacin, famotidine, and ranitidine were determined in methanol. Sparfloxacin exhibits absorption maxima at 300 nm and 377 nm. Famotidine and Ranitidine showed absorption maxima 288 nm and 324 nm and 230 nm, respectively. These values are in close agreement with the values of absorption maxima of these compounds reported in the literature. The molar absorptivities of these compounds at their respective absorption maxima have been determined. A two-component spectrophotometric method has been developed for the simultaneous determination of sparfloxacin and famotidine or sparfloxacin and ranitidine in solutions degraded in methanol-water mixtures in UV radiation.

STATISTICAL ANALYSIS

The statistical analysis was carried out by SPSS version 22, the statistical calculated values of sparfloxacin with

famotidine are given in table. 17 observations showed that at 0min, 60min, 120min, 180min and 240min results are significant and all p-values are less than 0.05 so we reject H_o means there is interaction between sparfloxacin and famotidine. Readings of CSFmw30, CSF1m, CSF1mwHalf, CSF2m and CSF2mwHalf all are highly significant.

While in table. 18 statistical calculated values of sparfloxacin with ranitidine observations showed that at 0min, 60min, 120min, 180min and 240min results are significant and all p-values are less than 0.05 so we reject H_o means there is interaction between sparfloxacin and ranitidine. Readings of CSRmw30, CSR1m, CSR1mwHalf, CSR2m and CSR2mwHalf all are highly significant.

DISCUSSION

A thin-layer chromatographic study has shown the presence of one spot in a degraded solution of sparfloxacin indicating its degradation in the methanol-water medium. This degradation product could not be identified due to the non-availability of any reference standard of the product. The UV absorption spectra of sparfloxacin, famotidine, and ranitidine were determined in methanol. Sparfloxacin exhibits absorption maxima at 300 nm and 377 nm. Famotidine and Ranitidine showed absorption maxima 288 nm and 324 nm and 230 nm, respectively. These values are in close agreement with the values of absorption maxima of these compounds reported in the literature. The molar absorptivities of these compounds at their respective absorption maxima have been determined.

A two-component spectrophotometric method has been developed for the simultaneous determination of sparfloxacin and famotidine or sparfloxacin and ranitidine in solutions degraded in methanol-water mixtures in UV radiation. It appears that the interaction of sparfloxacin and famotidine/ranitidine leads to the formation of a complex that protects sparfloxacin in the presence of famotidine/ ranitidine. The nature of this complex or association is not known, however, a little change in the absorption maxima of these compounds in mixtures compared to the individual compound has been observed indicating some interaction of these compounds (about 2 mm) in the media. Based on the results obtained it was suggested that famotidine or ranitidine may influence the bioavailability of sparfloxacin in the physiological system.

CONCLUSION

Sparfloxacin and famotidine or sparfloxacin and ranitidine were assayed by two-component analysis using the wavelength corresponding to their absorption maxima. The spectral characteristics of sparfloxacin and famotidine/ ranitidine mixtures and the kinetics of degradation of sparfloxacin in presence of famotidine/ranitidine indicate some form of interaction between sparfloxacin and famotidine and ranitidine. It appears that famotidine and ranitidine inhibit the rate of degradation of sparfloxacin which could be due to the possibility of complexation between these compounds. It has not been possible to investigate the nature of the complex formed by these compounds during the present work however, this complexation may affect the bioavailability of sparfloxacin.

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