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SIMULTANEOUS DETERMINATION OF MOXIFLOXACIN AND H_2 RECEPTOR ANTAGONIST IN PHARMACEUTICAL DOSAGE FORMULATIONS BY RP-HPLC: APPLICATION TO *in vitro* DRUG INTERACTIONS

Najma Sultana*, Mahwish Akhtar, Sana Shamim and Somia Gul
Research Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan
Muhammad Saeed Arayne
Lab 9, Department of Chemistry, University of Karachi

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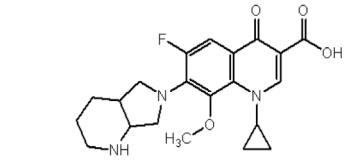
Simultaneous determination of moxifloxacin (MOX) and H_2-antagonists was first time developed in bulk and formulations. Purospher STAR C_{18} (250 x 4.6 mm, 5 µm) column was used. The mobile phase (methanol: water: ACN, 60:45:5 v/v/v, pH 2.7) was delivered at a flow rate of 1.0 mL min^{-1}, eluent was monitored at 236, 270 and 310 nm for cimetidine, famotidine and ranitidine, respectively. The proposed method is specific, accurate (98-103%), precise (intra-day and inter-day variation 0.098-1.970%) and linear (r>0.998). The LOD and LOQ were 0.006-0.018 and 0.019-0.005 µg mL^{-1}, respectively. The statistical parameters were applied to verify the results. The method is applicable to routine analysis of formulations and interaction of MOX with H_2-antagonist.

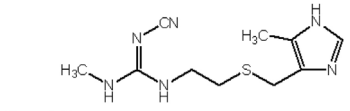
Keywords: moxifloxacin; H_2 receptor antagonists; ANOVA.

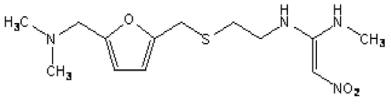
INTRODUCTION

Moxifloxacin (l-cyclopropyl-6-fluoro-l, 4-dihydro-8-methoxy-7-[(4aS, 7aS)-octahydro-6H-pyrrolo-[3, 4-b] pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid hydrochloride) (Figure 1) is a new generation, 8-methoxyquinolone derivative of fluoroquinolone antibacterial agent, synthetic, active against a broad spectrum of pathogens, encompassing Gram-negative and Gram-positive bacteria.[1-10] However, most of fluoroquinolones show miner side effect one of these is skin reaction including photosensitivity. This response is inhibited by co-administration with H_2 receptor antagonist (ranitidine, cimetidine or famotidine).[11] Deppermann *et al.*[12] published interaction of quinolones with H_2 receptor antagonist.

Literature survey assembled a number of HPLC methods,[13-16] which have been used for analysis of moxifloxacin in bulk and in pharmaceutical preparations. Ocane *et al.*[14] discovered the simultaneous determination of cephalosporin, cefepime and quinolones (garenoxacin, levofloxacin and moxifloxacin). The method was applied to the determination of the four molecules in spiked samples of human urine. Nguyen *et al.*[17] reported simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in human serum. Srinivas *et al.*[18] also described HPLC method for simultaneous determination of moxifloxacin, sparfloxacin and gatifloxacin using levofloxacin as internal standard in human plasma. Lorena *et al.*[16,19] determined simultaneous quantification of linezolid, rifampicin, levofloxacin and moxifloxacin in human plasma using HPLC-UV. The use of quinoxaline as internal standard and Nemutlue *et al.*[20] published simultaneous separation and determination of seven quinolones using HPLC: analysis of levofloxacin and moxifloxacin in plasma and amniotic fluid. While Diane *et al.*[21] reported simultaneous determination of H_2 receptor antagonists in urine sample.







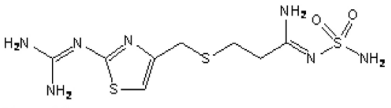


Figure 1. *Chemical structures of moxifloxacin, cimetidine, ranitidine and famotidine*

There is no method reported for the simultaneous determination of moxifloxacin and H_2 receptor antagonists using HPLC-UV. The main purpose of our study was to develop a simple, reliable and economical method to the simultaneous determination of moxifloxacin (MOX), cimetidine, famotidine and ranitidine (Figure 1) in short run time (< 8 min) with high linearity. Therefore, this study is focused on the development of simple and rapid isocratic RP-HPLC method which may be employed for the routine analysis of moxifloxacin in

*e-mail: dr.najma.sultana@gmail.com

bulk drug and pharmaceutical formulations. Simultaneous determination of both drugs was desirable as this would allow more efficient generation of clinical data and could be performed at more modest cost than separate assays.

EXPERIMENTAL

Reagents

Standard bulk drug sample of moxifloxacin was supplied by Getz Pharma Pakistan (Pvt) which was 99.50% pure while the H_2 receptor antagonists were supplied by different industries were also > 98% pure. Moxifloxacin (moxiget 400 mg tablet), cimetidine (cimet 400 mg tablet), ranitidine (H_2–Rec 150 mg tablet) and famotidine (Hiler 40 mg tablet) were purchased by Getz Pharma Pakistan (Pvt) Ferozsons laboratories Ltd., Nowshera-Pakistan, Zafa Pharmaceuticals (Pvt.) Ltd. and Getz Pharma Pakistan (Pvt.) Ltd., respectively. Each product was labeled and expiry dates were not earlier than two years, at the time of study. HPLC grade methanol and acetonitrile were supplied by Tedia company, Inc (USA). Ultrapurified water was used throughout the experiments.

Instrumentation

Two identical instrument utilized in the method development contained Shimadzu HPLC system equipped with LC-20 AT VP Pump, SPD-20AV Shimadzu UV visible detectors and Purospher STAR C_{18} (250 x 4.6 mm, 5 µm) column used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu to Intel Pentium 4 machine with Shimadzu CLASS-GC10 software. Mobile phase was sonicated by DGU-14 AM on-line degasser, and filtered through 0.45-micron membrane filter. Rheodyne manual injector fitted with a 20 µL loop. Calibrated Pyrex glassware was used for the solution and mobile phase preparation.

Preparation of solutions

Standard solution of each drug was prepared in mobile phase to produce a concentration of 100 µg mL^{-1} and injected onto the HPLC column to determine the individual retention times of the analytes. Working solutions were prepared by serial dilution of stock solution with the same solvent to contain desired concentrations that were 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, 5.000 µg mL^{-1} in 50 mL volumetric flask.

Analysis of formulation

Twenty tablets of each formulation were accurately weighed, crushed to make a fine powder. Calculated amount of powder was weighed and found to be equivalent to 10 mg and transferred to a separate 100 mL volumetric flask. It was dissolved in the mobile phase and filtered through a membrane filter (0.45 µm). The sample solution was suitably diluted and used for the analysis.

Procedure of interaction studies

The stock solutions (100 µg mL^{-1}) of moxifloxacin and interacting drugs (H_2 receptor antagonists) were prepared in simulated gastric juice, buffers of pH 4, 7.4 and 9 individually. These solutions were mixed in 1:1 ratio in Erlenmeyer flasks individually to get 50 µg mL^{-1} concentration; these were heated on a water bath at 37 ± 5 °C with constant stirring at 100 rpm speed. 5 mL of solution was withdrawn after every 30 min of interval for 3 h. The sample was diluted in suitable dilution then filtered through 0.45 µm filter membrane. Three replicates of filtered samples were injected to HPLC system. The concentration of each drug was determined using linear equation and percent availability was calculated. We also monitored the pH affect on the availability of MOX in presence of interacting drugs.

Statistical calculations

The standard curve, slope and intercept were determined by software STATISTICA 7. Regression curve analysis was carried out by using of Microsoft Excel 2007 software, without intersecting through zero. Means, standard deviations, ANOVA, Student's *t*-test, and homoscedasticity for the calibration plots were calculated by Kendall's test and Friedman's test using SPSS software version 10 (SPSS, Cary, NC, USA).

RESULTS AND DISCUSSION

Development and optimization of isocratic HPLC conditions

A reversed-phase high performance liquid chromatography was used for the development of simultaneous determination of moxifloxacin and H_2 receptor antagonists. Initial different C_{18} columns were used to develop method. H_2 receptor antagonist and MOX cannot separate properly by Discovery C_{18} (250 x 4.6 mm, 5 µm) (Supelco, USA) while another C_{18} column was successfully used for method development, that was C_{18} Hiber RT 250-4.6 Purospher STAR RP-18 (5 µm) (Merck, Germany), of each analyte. This column provides efficient and reproducible separations of nonpolar compounds even as minimizing solvent usage with typical peak symmetry. To investigate appropriate wavelength for determination of MOX and H_2 receptor antagonists, we scanned solution of all drugs individually by UV-visible spectrophotometer. The isosbestic points were calculated by comparing UV spectra of H_2 receptor antagonists with MOX individually. The selected wavelengths for simultaneous determination of MOX were 236 nm for cimetidine, 270 nm for famotidine and 310 nm for ranitidine (Figure 2). Initially different combination of methanol and water were tried, the best separation was achieved at methanol: water: ACN in ratio of 60:45:5, v/v/v. these solvents are easily available and commonly used solvents for RP-HPLC little quantity of ACN used to refine peak symmetry and shape. To select the optimum mobile phase pH range 2.5 to 4.0 were investigated, excellent performance was achieved at pH 2.7 adjusted with phosphoric acid. Analysis was completed within seven min with a flow rate of 1.0 mL min^{-1} at ambient temperature with isocratic elution.

The developed method was validated by AOAC guidelines CDER, US Pharmacopeia and ICH guidelines.[23-26] Two laboratory analysts performed the method validation work (using two HPLC systems with same configuration) with respect to parameters such as linearity, assay accuracy, limit of quantification (LOQ), limits of detection (LOD), ruggedness, precision, specificity, robustness and sample stability in solution. The seven different concentrations were prepared in mobile phase to evaluate the method. The low concentration was prepared to investigate the limit of detection and quantification.

System suitability

The HPLC system was equilibrated with the initial mobile phase composition followed by 10 injections of the same standard to evaluate the system suitability on each day of method validation. Parameters of system suitability are peaks symmetry (symmetry factor); theoretical plates of the column, resolution, mass distribution ratio (capacity factor) and relative retention as summarized in Table 1.[22-24]

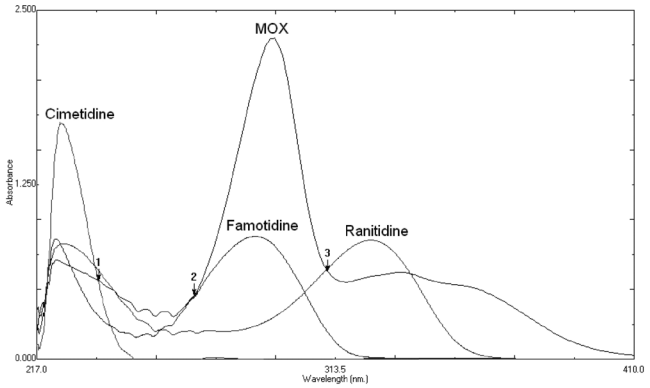


Figure 2. *Uv-visible spectra of moxifloxacin and H_2 receptor antagonists*

Linearity

The reason of the check for linearity was to demonstrate that the entire analytical system (including detector and data acquisition) presents a linear response and it is directly proportional over the relevant concentration range of analytes.[25] To determine the linearity of moxifloxacin and H_2 receptor antagonists in the mobile phase at each of the seven concentration levels were carried out. The sample concentrations were range from 0.078-5.000 μg mL^{-1} for moxifloxacin and H_2 receptor antagonists (Table 2). Analytical curves were constructed. The peak areas of each individual compound were plotted against corresponding concentrations. Excellent linearity was obtained in all cases with determination coefficient (r) > 0.998: moxifloxacin (0.9993), cimetidine (0.9998), famotidine (0.9993) and ranitidine (0.9985). The standard curve, slope, *Y*-intercept, and determination coefficient (r) were obtained from linear regression analysis performed by statistical software. The homoscedasticity of the calibration plots, tested by Kendall's and Friedman's tests were found to be significantly linear over the tested ranges.

Precision

ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) states that method precision may be considered at three levels: repeatability, intermediate precision and reproducibility.[23] Only repeatability (intra-day precision) and intermediate precision (intra-day precision) were evaluated in this validation. It is expressed as percent relative standard deviation (RSD). Different concentrations of analytes in the linear range were analyzed on the same day (intra-day precision) and two consecutive days (inter-day precision); every sample was injected five times. Both intra- and inter-day RSD range from 0.098-1.970% for all analytes, confirming good precision. It is verified by performing Student *t*-test to evaluate the difference in results. The *t*-stat value was lower than the *t*-two-tailed value indicating no significant difference between intra-day and inter-day precision. The results were insignificant and indicated no remarkable deference in intra-day and inter-day precision (Table 3).

Accuracy

Accuracy was determined by spiking the known amounts of the analytes and it was evaluated as the percentage of recovery of analytes to the pharmaceutical formulation and human serum (Figure 3). Each sample was injected five times and accuracy was determined in range of 98-103 at 80%, 100 and 120% for all investigated analytes. It is very acceptable values according to the AOAC guidelines.[26] One-way ANOVA was performed to check differences between amounts added

Table 1. System suitability parameters of MOX and H_2 receptor antagonists

Analytes	Retention time (t_R)	Capacity factors (K')	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Separation factor
			pH ± 0.05			
MOX	6.75 ± 0.05	2.18 ± 0.16	3717 ± 166.3	1.40 ± 0.06	3.50 ± 0.06	2.48 ± 0.26
Cimetidine	5.29 ± 0.06	-	3108 ± 319.0	1.60 ± 0.03	-	-
Famotidine	4.70 ± 0.09	-	2401 ± 169.6	1.69 ± 0.01	-	-
Ranitidine	4.70 ± 0.29	-	2497 ± 313.0	1.81 ± 0.04	-	-
			Flow rate ± 0.2			
MOX	6.76 ± 0.01	2.11 ± 0.21	3189 ± 198	1.50 ± 0.03	3.50 ± 0.12	2.5 ± 0.09
Cimetidine	5.34 ± 0.01	-	3099 ± 386	1.60 ± 0.04	-	-
Famotidine	4.70 ± 0.12	-	2426 ± 159	1.72 ± 0.03	-	-
Ranitidine	4.70 ± 0.30	-	2541 ± 265	1.79 ± 0.03	-	-
			Methanol percentage ± 2			
MOX	6.71 ± 0.06	2.12 ± 0.15	3789 ± 126	1.42 ± 0.04	3.50 ± 0.13	2.42 ± 0.31
Cimetidine	5.21 ± 0.08	-	3089 ± 291	1.60 ± 0.02	-	-
Famotidine	4.70 ± 0.09	-	2475 ± 173	1.65 ± 0.02	-	-
Ranitidine	4.69 ± 0.31	-	2501 ± 332	1.85 ± 0.05	-	-

Table 2. Regression characteristics of MOX and H_2 receptor antagonists

Analytes	Conc. range (μg mL^{-1})	Correlation coefficient (r)	Standard error of stimate	Standard error	Intercept	Slope
MOX	0.078-5.000	0.9993	0.0500	0.0257	-0.1199	15357
Cimetidine	0.078-5.000	0.9998	0.2058	0.1093	-2.1979	67071
Famotidine	0.078-5.000	0.9993	0.5033	0.2511	-0.1229	6043
Ranitidine	0.078-5.000	0.9985	0.7590	0.3977	-1.7460	15968

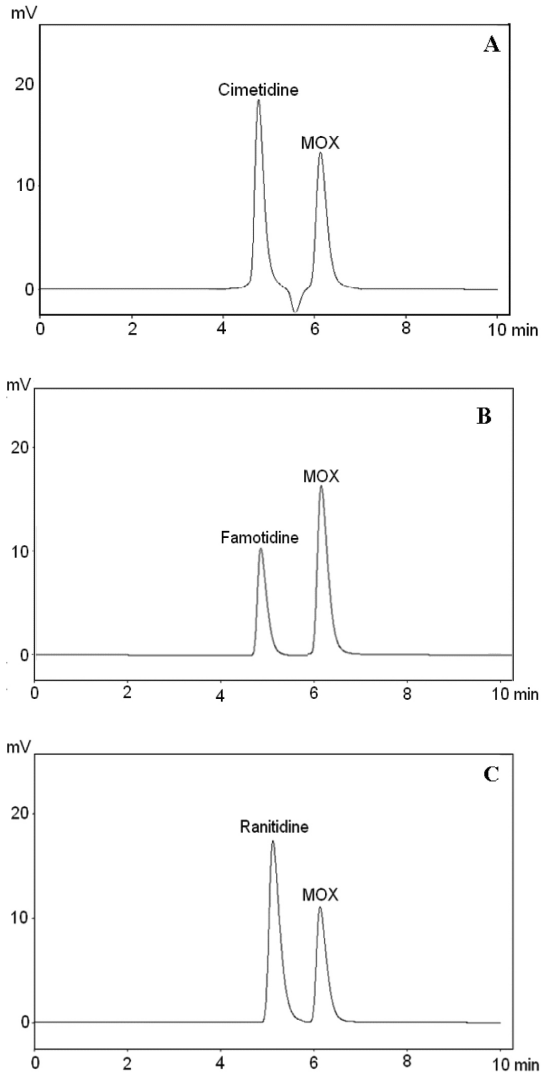


Figure 3. *Chromatogram of moxifloxacin with ranitidine, cimetidine, and famotidine*

and recovered which was insignificant (F-2.212, p>0.1). Thus, used excipients did not interfere with active present in tablets. Also, the filtration medium did not absorb the drug to any extent. The results are presented in Table 4; high recovery indicated that the method has a high degree of accuracy for the simultaneous determination of moxifloxacin and H_2 receptor antagonists.

Specificity and selectivity

The selectivity and specificity of the method was established by studying resolution factor of the peak of moxifloxacin from that of H_2 receptor antagonists. The method confirmed good resolutions ≥ 2 (Table 1) and was found to be free of interference from the excipients used in pharmaceutical formulation. The results indicate the specificity of the system.

Limit of detection (LOD) and quantification (LOQ)

Limit of detection (LOD) and quantification (LOQ) are determined for all impurity tests (including residual analysis during cleaning verification).[23,25] The limit of quantification refers to the lowest amount of an analyte in a sample. There are different approaches to determine the LOQ and LOD. Typically the concentration level that generates a signal-to-noise (S/N) of 10 is regarded as the LOQ and the concentration level that generates S/N = 3 is regarded as the LOD.[27,28] The

Table 3. Precision of moxifloxacin and H_2 receptor antagonist

Analytes	Conc. (μg mL^{-1})	RSD (%)		*t*-stat	P (T<t) two-tail
		day 1	day 2		
MOX	0.078	0.325	1.299	-1.865	0.111
	0.156	0.516	0.782		
	0.313	0.463	1.970		
	0.625	0.279	1.473		
	1.250	0.739	0.524		
	2.500	0.818	0.458		
	5.000	0.714	0.571		
Cimetidine	0.078	0 .620	0.490	0.368	0.725
	0.156	0.760	0.520		
	0.313	0.460	0.460		
	0.625	0.350	0.280		
	1.250	0.440	0.740		
	2.500	0.380	0.420		
	5.000	0.570	0.510		
Famotidine	0.078	0.129	0.409	-0.412	0.694
	0.156	0.828	0.098		
	0.313	0.501	0.467		
	0.625	0.571	0.642		
	1.250	0.182	0.535		
	2.500	0.255	0.486		
	5.000	0.124	0.358		
Ranitidine	0.078	0.414	0.665	-0.749	0.482
	0.156	0.154	0.647		
	0.313	1.211	0.783		
	0.625	0.432	0.986		
	1.250	0.712	0.319		
	2.500	0.194	0.391		
	5.000	0.258	0.357		

Table 4. Accuracy of moxifloxacin and H_2 receptor antagonists

Analytes	Conc. (μg mL^{-1})	Conc. Found (μg mL^{-1})	Recovery (%)
MOX	2.00	1.98	99.12
	2.50	2.52	100.76
	3.00	3.01	100.40
Cimetidine	2.00	1.96	98.05
	2.50	2.47	98.88
	3.00	2.99	99.60
Famotidine	2.00	1.95	97.60
	2.50	2.49	99.68
	3.00	2.98	99.17
Ranitidine	2.00	1.93	96.40
	2.50	2.49	99.84
	3.00	2.99	99.53

Table 5. Interaction of MOX with H_2 receptors antagonist

Time (min)	Recovery (%) of MOX and interacting drugs					
	MOX	Cimetidine	MOX	Famotidine	MOX	Ranitidine
			pH 1			
0	100.35	100.13	99.92	100.37	100.03	100.03
30	103.87**	109.66**	100.80**	101.09**	78.22**	102.42**
60	104.54**	110.34**	104.68**	97.23**	81.66**	109.69**
90	97.31**	94.28**	104.20**	90.31**	80.99**	103.97**
120	98.75**	93.58**	99.89**	91.78**	77.92**	98.23**
150	94.74**	92.96**	87.77**	92.74**	67.24**	65.31**
180	79.82**	91.01**	89.57**	86.66**	66.74**	63.08**
ANOVA (df=6, 14)	F-999534, p<0.001	F-2442204, p<0.001	F-1250257, p<0.001	F-763231, p<0.001	F-3904175, p<0.001	F-14542954, p<0.001
			pH 4			
0	100.01	100.55	100.03	100.35	100.01	100.00
30	110.75**	104.34**	107.45**	101.07**	107.22**	120.82**
60	119.39**	108.94**	93.54**	93.53**	104.01**	103.07**
90	95.57**	115.82**	93.22**	86.63**	103.94**	103.27**
120	84.12**	115.05**	96.22**	86.71**	103.59**	84.99**
150	82.97**	118.16**	84.34**	82.59**	94.98**	81.94**
180	82.82**	126.84**	87.10**	71.35**	93.04**	72.04**
ANOVA (df=6, 14)	F-3317152, p<0.001	F-693566, p<0.001	F-281069, p<0.001	F-2061165, p<0.001	F-919742, p<0.001	F-4884931, p<0.001
			pH 7.4			
0	100.11	100.37	99.52	100.24	100.25	100.03
30	78.74**	107.73**	95.72**	104.69**	111.87**	105.01**
60	75.23**	106.72**	96.01**	103.00**	101.28**	100.30**
90	72.38**	91.60**	88.67**	97.73**	94.83**	91.51**
120	66.81**	90.83**	74.63**	95.62**	93.99**	87.52**
150	65.62**	89.48**	71.97**	94.41**	93.41**	88.64**
180	54.32**	87.63**	66.19**	84.19**	89.28**	84.90**
ANOVA (df=6, 14)	F-4249988, p<0.001	F-133021, p<0.001	F-2337639, p<0.001	F-234670, p<0.001	F-682298, p<0.001	F-109912, p<0.001
			pH 9			
0	100.15	103.64	100.09	99.93	100.00	100.00
30	101.93**	97.40**	103.42**	97.58**	100.20**	100.12**
60	102.30**	97.05**	100.33**	95.00**	114.10**	98.16**
90	94.49**	97.71**	86.69**	93.58**	114.84**	99.20**
120	84.61**	96.14**	85.49**	92.66**	106.41**	102.69**
150	81.22**	93.43**	82.17**	92.92**	90.23**	109.36**
180	78.37**	81.46**	79.26**	85.63**	80.32**	110.19**
ANOVA (df=6, 14)	F-1331071, p<0.001	F-652117, p<0.001	F-674.078, p<0.001	F-329194, p<0.001	F-2188303, p<0.001	F-1662.432, p<0.001

** p<0.005, n=3

limits of detection (LOD) and quantification (LOQ) were determined from the calibration curve. The LOD and LOQ were 0.006-0.018 and 0.019-0.005 μg mL^{-1}, respectively. At the selected LOQ and LOD concentrations, all the S/N for LOQ standard solutions were larger than 10 and all the S/N for LOD standard solutions were larger than 3. These results suggest that the proposed HPLC method has good sensitivity.

Robustness

The robustness of the method demonstrated by showing the capacity of the method remained unaffected while deliberately changing HPLC parameters. Several parameters including HPLC column batch, flow rate, detector wavelength, temperature, injection volume, mobile phase ratios and gradient conditions were varied around the procedural values to assess the results under each HPLC parameter variation against those obtained under the procedural parameters.[30] In our method, it was performed by making minor variations in the ration of methanol and water in mobile phase and flow rate, which were proved quite stable. When a parameter was intentionally changed ± 2% (in mobile phase), ± 0.1% (in flow rate) and ±0.05% (pH 2.75) from its optimum condition, the shifting in retention time of ± 0.1% was less. These results indicate better robustness of the developed method Table 1.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, different analysis different instruments, different days, etc.[25,29] Ruggedness is an older term that has been replaced by intermediate precision (degree of reproducibility) obtained under a variety of circumstances.[22] Different concentrations of analytes were used in

two different days and two different systems of same configuration indicate suitability of method for all analytes.

Applications of developed method

Developed HPLC method has been effectively employed for the interaction of moxifloxacin with H_2 receptor antagonist (cimetidine, famotidine and ranitidine).

Drug-drug interaction of MOX with H_2-receptor antagonists

The direct interaction method has been used for interaction study,[32,33] stock solutions of active material of selected drugs were prepared individually in simulated gastric juice (pH 1), buffers pH 4, 7.4 and 9. The quantifications were examined by calculating the area under curve (AUC) and percent recovery (Table 5) of interacting drugs. Cimetidine was available up to 91.01-100.13% in simulated gastric juice, 100.55-126.84% in pH 4. However, it was decreased to 12.37% in pH 7.4 and 18.46% in pH 9. Similarly, famotidine was available in simulated gastric juice and pH 7.4 and 9 up to 86.66, 84.19 and 85.63% respectively while decreased up to 28.65% in pH 4. Ranitidine was decreased up to 36.92, 27.96 and 15.10% in simulated gastric juice, buffers of pH 4 and 7.4, respectively while it increased to 10.19% in pH 9.

One way ANOVA was applied to evaluate above interaction results. Individual test verified that significant interaction was observed (Table 5). These results indicated that MOX and H_2 receptor antagonists may affect the efficacy of each other.

CONCLUSION

A simple, reliable, convenient and reproducible HPLC method for simultaneous determination of moxifloxacin, cimetidine, famotidine and ranitidine has been developed in bulk and pharmaceutical dosage formulation. This method indicates high linearity, low limit of detection and quantification, small sample volume and short run time. The intra-run and inter-run variability and accuracy results were also in acceptable limit. This method was than applied for interaction study among H_2 receptor antagonists and MOX. Interaction results were analyzed by one way ANOVA that revealed the significance at studied pH. It may be particularly adapted for routine assay of MOX or H_2-receptor antagonists alone or in combination, in pharmaceutical industries and clinical investigations.

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