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RP-HPLC Method for the Simultaneous Determination of Captopril and H_2-Receptor Antagonist: Application to Interaction Studies

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Abstract

Rapid, sensitive, simple and accurate high-performance liquid chromatographic (HPLC) method is developed and validated for the simultaneous determination of captopril with H_2 receptor antagonists as cimetidine, ranitidine and famotidine. Captopril was separated from H_2 receptor antagonist using pre packed Purospher star C18 (5 µm, 25×0.46 cm) column methanol: water (60:40 v/v) were used as the mobile phase, pH 3.0 ± 0.02 was adjusted by orthophosphoric acid. The flow rate was 0.8 $mLmin^{-1}$ at ambient temperature, diluent was 50:50 methanol water while UV detection was performed at 225 nm. The retention time for captopril was found to be 5.2 minute, for ranitidine and famotidine 2.5 minute and cimetidine 2.7 minute. Each drug showed a good resolution from captopril. *In vitro* interaction studies of captopril with commonly administered H_2 receptor antagonists i.e. cimetidine, ranitidine and famotidine were carried out at 37°C using above validated method. These studies clearly indicated that H_2-receptor antagonists bind to captopril causing drastic changes in the availability of the drug.

Keywords: Captopril; H_2-receptor antagonists; RP-HPLC; Interaction studies

Introduction

Captopril (Figure 1) (2S)-3-mercapto-2-methyl-1-oxo-proptonyl]-L-proline [1], the first orally active and specific inhibitor of angiotensin-converting enzyme. It blocks the conversion of angiotensin I to angiotensin II by inhibiting the angiotensin converting enzyme and inactivates bradykinin, a potent vasodilator. The hypotensive activity of captopril probably results both from inhibitory action on renin-angiotensin system and simulating action on kallikerin-kinin system [2]. Various instrumental methods have been developed for the determination of captopril by HPLC [3-6] and Spectrophotometry [7].

Histamine H_2 antagonists cimetidine (CIM), ranitidine (RAN), and famotidine (FAM) classified as class III drugs (high solubility, low permeability) according to the Biopharmaceutics Classification System (BCS) [8,9] are used in the treatment of gastro-esophageal reflux disease and gastric and duodenal ulceration [10]. Several methods have been reported for the determination of ranitidine and/or cimetidine in pharmaceutical preparations using high performance liquid chromatography (HPLC) [11-13]. Number of HPLC-UV methods has been reported for the analysis of individual H_2 antagonist's cimetidine [14,15], famotidine [16,17], and ranitidine[18] in biological samples comprising urine or urine and plasma. But our method use simple methanol water 60:40 and all drugs elute out same mobile phase within 5.5 min without interference of excepients.

However, no simultaneous method for determination of both the drugs in active, in dosage formulations and in human serum has been studied so far. It is reported that Hypertension and gastrointestinal disorders are two common co-existing conditions, therefore H_2-receptor antagonists and antiarrhythmic agents like calcium channel blockers, ACE Inhibitors may be administered to patients to cure hypertension with GIT problems. Co administration of H_2 receptor antagonists and cardiovascular drugs are described in number of cases [19,20]. On this basis, it became apparent to develop and validate for the first time a simultaneous method for the estimation of these drugs in bulk material, dosage formulations and in human serum which can be used for therapeutic monitoring in clinical practice. The method was validated according to ICH guidelines and was found to be reproducible. Further, this validated method was used to study the possible *in vitro* interactions of captopril with H_2-receptor antagonists (cimetidine, ranitidine and famotidine). Proposed method was selective, precise and accurate, therefore can be used for routine, quality control and clinical study and there is no limitation for it is used.

Experimental

Materials

Reference standard of captopril was obtained from Squib Pharmaceutical Laboratories Pakistan. H_2 receptor antagonist cimetidine (Tagamet 200 mg), ranitidine (H_2-REC 150 mg) and famotidine (Famopsin 40 mg) were of Park Davis (Pvt.), Smith Kline Beecham (Pvt.) Ltd. and Remedica Ltd. Karachi Pakistan, respectively. Methanol used was of HPLC grade and all the reagents used were of analytical grade. Solutions of the compounds were prepared in methanol: water in the ratio of 60:40 v/v. These solutions were daily prepared fresh. Double distilled Deionized water was used through out the experiment which was degassed by sonicator and filtered through 0.45 µm filter paper.

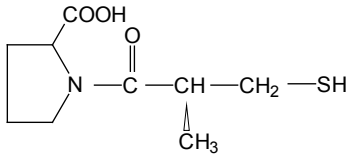

Figure 1: Structure of Captopril.

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Apparatus

Shimadzu HPLC system equipped with LC-10 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 µL loop, and SPD-10 A VP UV–VIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module Separation was achieved on a Purospher Start C_{18} (250 cm × 4.6 mm, 5 µm) column and Supelco C_{18} (25 cm × 4.6 mm, 5 µm) column, for ruggedness studies was used Shimadzu CLASS-GC software (Version 5.03) was used for data acquisition and mathematical calculations.

Preparation of stock and working solutions

Stock solutions of 100 µgmL^{-1} of captopril, cimitidine, famotidine and ranitidine were prepared individually by dissolving 10 mg of each drug in 100 mL volumetric flask using 50:50 methanol water. Aliquots were diluted in the range of 0.7-12.50 µgmL^{-1} for H_2-receptor antagonist and for captopril 9.37-150 µgmL^{-1}. These solutions were stored at 20°C, they were prepared once and analyzed daily for inter-day and inter-operator variations of the method and analyzed each time before drug analysis in biological samples. 20 µL of these solutions were injected into LC system and chromatographed.

Sample preparation

To determine the content of the drugs in the formulations, 20 tablets of each drug were powdered and powder equivalent to 10 mg of captopril, 10 mg of H_2 antagonists (cimetidine, famotidine and ranitidine) were weighed, the solutions were transferred to 100 mL volumetric flask and were diluted with 50:50 v\v aqueous methanol. The resulting solutions were filtered and analyzed for the drug content. A placebo tablet was also subjected to the same process as discussed above.

Drug serum solutions

Blood samples were collected from healthy volunteers and immediately centrifuged at 3,000 rpm for 10 min. The supernatant obtained was stored at -20°C. After thawing, serum was deproteinated by addition of acetonitrile and spiked daily with working solutions to furnish the desired concentrations of captopril and the H_2 receptor antagonist to get the final concentrations ranging from 0.7-12.50 µgmL^{-1} for H_2 receptor antagonist and for captopril 9.37-150 µgmL^{-1}.

In-vitro interaction studies

Procedure: The interaction studies were performed by preparing 200 µgmL^{-1} stock solutions of each drug. 20 mL of stock solution of captopril was added 20 mL of stock solution of cimetidine in a conical flask and heated on a water bath at 37°C for 3 hours. The samples were withdrawn after every half an hour interval and were analyzed on HPLC. Peak areas were recorded and degree of interactions was evaluated.

Results and Discussion

Various ratios (80:20, 70:30, 50:50 v/v) of mobile phase were tested as starting solvent for system suitability studies. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry and retention time. However, the ratio of (60:40 v/v) yielded best results. The pH effect showed that optimized conditions are reached when the pH value is 3 producing well resolved and sharp peaks for all drugs assayed using wavelength 225 nm (isobetic point Figure 2).

The method for the determination of captopril in raw material and dosage formulations was validated for the parameters like specificity, range and linearity, inter-assay accuracy and precision, detection and quantitation limit, robustness according to the guidelines provided by ICH. Moreover, method was also subjected to study the *in vitro* interactions of drugs.

Method validation

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) [21] have recommended the accomplishment of the method.

Range and linearity

To establish linearity of the proposed method, five separate series of solutions of the drug were prepared and analyzed. Standard curves were constructed at concentrations 0.78, 1.56, 3.1, 6.25 and 12.50 µgmL^{-1} for cimetidine, famotidine and ranitidine. Captopril 9.3, 18.8, 37.5, 75 and 150 µgmL^{-1}. The standard calibration curves were shown to be linear in the above mentioned range in human serum. Curves were obtained by plotting the peak area against concentrations of these drugs. Linear calibration curves were generated by linear regression analysis and obtained over the respective standard concentrations ranges. The standard curve, slope, intercept and the correlation coefficient were determined. The regression statistics are shown in table 1.

Limit of detection (LOD) and quantitation (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ values for captopril and H_2-recepter antagonist for bulk material, pharmaceutical preparation and in serum were determined and are presented in table 1.

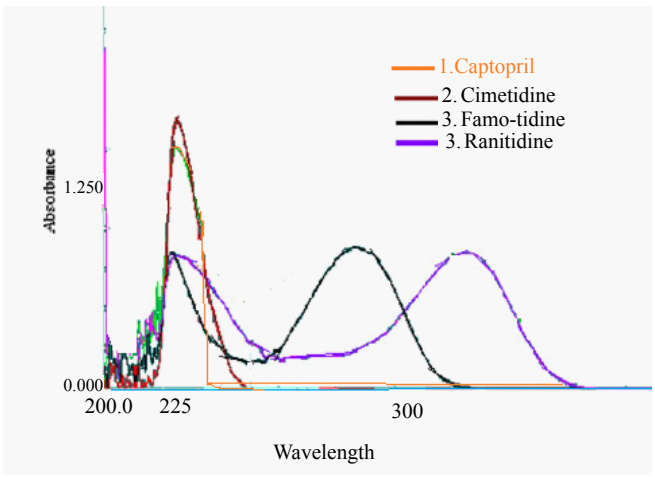

Figure 2: UV-visible spectra of Captopril and H_2 receptor antagonist.

Drugs	Regression equations	R2	LOD* ng/mL	LOQ** ng/mL
Captopril	y = 1883.8x - 1856.6	0.9993	1.75	5.3
Famotidine	y = 21249x + 2854.7	0.9995	0.15	0.47
Cimetidine	y = 27570x - 1123	0.9999	0.12	0.36
Ranitidine	y = 17239x - 1479.9	0.9995	0.3	0.9

Limit of detection, **limit of quantification

Table 1: Regression statistics LOD and LOQ.

Accuracy

The accuracy of the method was calculated at three concentration levels (80, 100 and 120%) by spiking known quantities of the drug analytes. Three injections of each solution were injected to HPLC system and % recovery was calculated in each case.

Precision

For the precision of the method, six replicates of each level were injected to system on two different non-consecutive days in each case and % RSD was calculated. The results of accuracy and precision (Table 2) revealed that the method was accurate for all above purposes.

Specificity and selectivity

Representative chromatograms (Figure 3) were generated to show other components that could be present in the sample matrix are resolved from the parent analytes. No change was observed in the chromatogram of captopril and H_2 receptor antagonist in presence of common excepients. The specificity was also determined by injecting human plasma samples. Therefore, the proposed method is selective and specific for the drugs.

Formulations

Recovery values of all the drugs in formulations (Table 3) indicated that the average recoveries of all drugs did not exceed less than 99% and more than 100% which clearly indicated the suitability of the method.

Serum

Recovery of all the drugs from serum was assessed by calculating the ratio of the peak areas of analyte in serum extract (Figure 4) and diluent. An average recovery of 99 ± 101% (mean ± S.D) was obtained in concentrations studied (n=5). These results (Table 4) clearly indicated the therapeutic application of this method.

Ruggedness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy University of Karachi, while Lab 2 was in the Department of Chemistry, Faculty of Science University of Karachi. The method did not show any notable deviations in results from acceptable limits. The assay results indicated that the method was capable with high precision and it was found that the %R.S.D values did not exceed more than 2% (Table 6).

Analytes	Assay (spiking method)			Assay in serum	
	Conc. μgmL^{-1}	%RSD	%Rec	%RSD	%Rec
CAP	8	0.002	100	0.02	101.3
	10	0.001	100.1	1.02	101
	12	0.005	100	0.36	101.2
CIM	8	0	100	0.36	100.3
	10	0.006	99	0.59	99.8
	12	0.001	100	0.69	100.23
FAM	8	0.002	100	0.36	99.69
	10	0.001	100	0.36	99.98
	12	0.005	101	0.36	101.3
RAN	8	0.003	102	0.63	101.3
	10	1.72	97.9	0.003	101.3
	12	0.49	98	0.003	101.3

Table 2: Accuracy and precision.

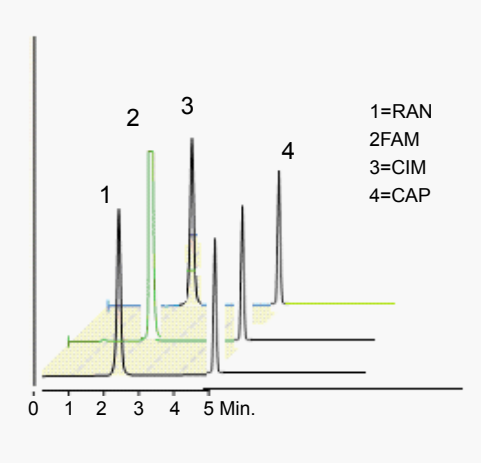

Figure 3: Representative chromatogram of (1) ranitidine (2) famotidine (3) cimetidine and (4) captopril in formulation.

	Conc injected	Conc found	% Recovery
Drugs	$\mu g\ mL^{-1}$	$\mu g\ mL^{-1}$	
Capoten	8	8.9	101.6
	10	10.3	100.3
	12	12.8	101.5
Famopsin	8	8	100.6
	10	10.8	100.9
	12	12.9	101.5
Tagament	8	8	99.9
	10	10.8	100.9
	12	12	100
H_2-REC	8	7.9	99.7
	10	10.2	100.2
	12	12.9	102.1

Table 3: % Recoveries of different brands.

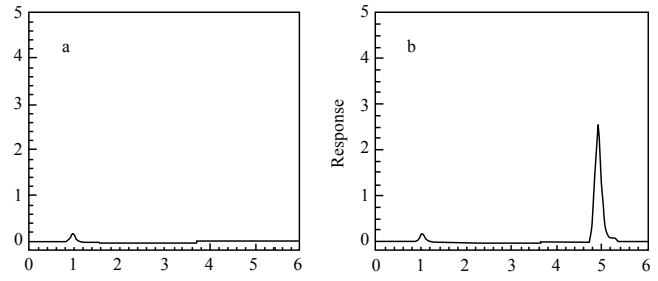

Figure 4: Representative chromatogram of (a) blank plasma samples and (b) spiked plasma samples of captopril.

Time	% Recovery			
(min)	CAP	CAP+CIM	CAP+FAM	CAP+RAN
0	100	102	101	100
30	104	99.8	110	102
60	108	103	115	103
90	104	100	116	115
120	104	101	118	120
150	113	104	120	122
180	113	104	120	122

Table 4: Interaction studies of captopril with H_2 receptor antagonist.

Robustness

Robustness was evaluated by slight changes in pH levels of mobile phase and flow rates. Peak areas of all the drugs were recorded in each case and coefficient of variance was calculated for it which is given in table 6.

In-vitro interaction studies

Due to short analysis time, suitability for routine analysis and for control purposes in pharmaceutical dosage forms and good recovery values in serum as well the present method was employed to study *in vitro* interactions of captopril with H_2-blockers in presence of each other. As concerns the choice of drugs, these drugs have been selected for their importance in therapeutic field. However, remarkable changes in availability values of captopril in presence of different H_2 blockers were observed (Table 5) and shows in figures 5 and 6. Hence forth, present results clearly indicated that captopril may interact with H_2-receptors antagonists. In conclusion availability of captopril increased due to complex formation with H_2-receptor antagonists. Literature survey also reveals the same fact that captopril shows interaction with ranitidine [22]. However, further studies will be conducted to ascertain the chemistry of complexes formed. On the basis of present findings, it is also suggested that these two drugs should not be co administered until extensive *in vivo* studies ensures that the two co administered drugs does not decreases the therapeutic effects of each other.

Conclusion

Our study reveals that the availability of captopril in presence of famotidine and ranitidine increased, which reflects its complexation with these drugs. Moreover, famotidine and ranitidine underwent complexation with captopril more profoundly and intensely compared with cimitidine. It is concluded that coadministration of captopril and H_2-receptor antagonists leads to the formation of charge-transfer

HPLC System			LC 10		LC 20	
Drugs	Columns	Conc. (µg/mL)	Conc.Found (µg/mL)	Recovery (%)	Conc. Found (µg/mL)	Recovery (%)
CAP	Purospher STAR	8	8	100	8.06	100.75
		10	10	100	9.98	99.8
		12	12	100	12	100
	Supelco C18	8	8	100	7.9	98.75
		10	9.9	99	10	100
		12	12	100	12	100
CIM	Purospher STAR	8	8.1	101.25	8.0	100
		10	9.9	99	9.88	98.8
		12	12	100	12	100
	Supelco C18	8	8	100	8	100
		10	10	100	10.1	101
		12	12	100	12.1	100.8
FAM	Purospher STAR	8	7.9	99.7	8.03	100.3
		10	9.99	99.9	9.98	99.8
		12	12	100	12	100
	Supelco C18	8	7.99	99.8	8	100
		10	10	100	10	100
		12	12	100	11.9	99.1
RAN	Purospher STAR	8	7.98	99.7	8	100
		10	9.99	99.9	9.9	99
		12	12	100	11.9	99.1
	Supelco C18	8	7.9	98.7	8.0	100
		10	10	100	10.9	109
		12	12	100	12.2	101.6

Table 5: Ruggedness of the method.

	Level	K'	T	(R_s)
A: pH of mobile phase				
2.8	-0.2	3.8	1.39	2.4
3	0	3.9	1.43	2.3
3.2	0.2	3.6	1.4	2.2
Mean ± S.D (n=6)		3.9 ± 0.2	1.4 ± 0.02	2.3 ± 0.1
B: Flow rate (mL/min)				
0.6	-0.2	3.8	1.45	2.32
0.8	0	3.9	1.43	2.36
1.0	0.2	3.6	1.42	2.37
Mean ± S.D (n=6)		4.3 ± 0.2	1.4 ± 0.01	2.3 ± 0.0
C: Percentage of water in mobile phase (V/V)				
35	-5	4.6	1.42	2.38
40	0	3.9	1.43	2.36
45	5	4.5	1.46	2.33
Mean ± S.D (n=6)		4.3 ± 0.0	1.4 ± 0.02	2.3 ± 0.0
D: Wavelength (nm)				
220	-5	3.5	1.52	2.5
225	0	3.9	1.43	2.36
230	5	3.4	1.55	2.3
Mean ± S.D (n=6)		3.3 ± 0.1	1.5 ± 0.0	2.4 ± 0.1

K'=Capacity factors, N=Theoretical plates, T=Tailing factor Rs=Resolution

Table 6: Robustness of the method for captopril (n=6).

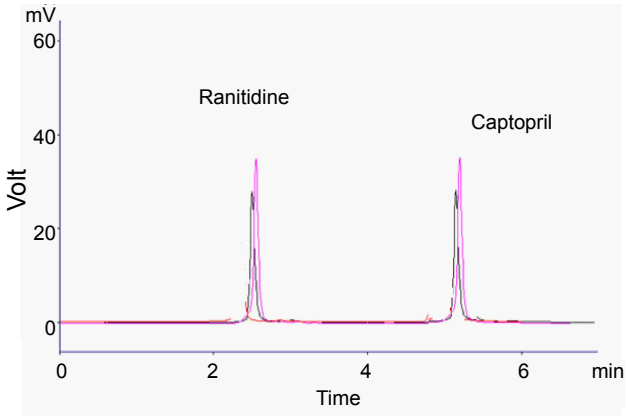

Figure 5: After interaction chromatogram showing drift in retention time as well as change in AUC of drugs (Rantidine and captopril).

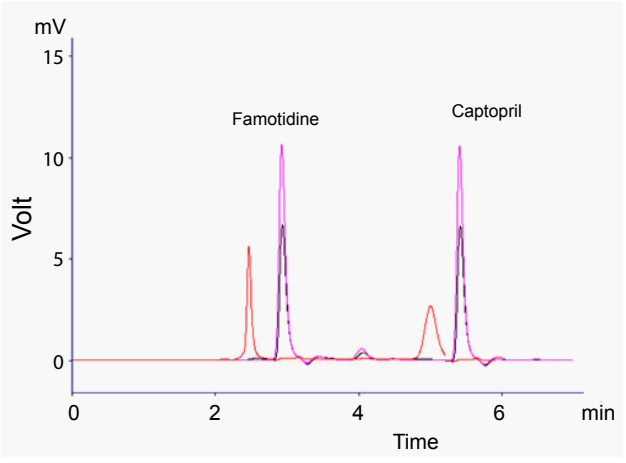

Figure 6: After interaction chromatogram showing drift in retention time as well as change in AUC of drugs (Famotidine and Captopril).

complex depleting both the interacting drugs to bind to their respective receptors. Furthermore, occurrence of such interactions could impair the clinical efficacy of these drugs and reduce their bioavailability further investigations are required for this purpose.

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