Method for the Determination of Captopril in Bulk, Pharmaceutical Formulations and Serum by HPLC using two different System.

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Abstract:*A simple reversed phase HPLC method have been successfully developed and validated for the quantitative determination of captopril (CAP) in bulk material, pharmaceutical formulation and serum. Purospher Start C_{18} (250cm x 4.6mm, 5µm) and Hypersil ODS C18 (150×4.6mm, 5micron) columns were used. The mobile phase, (methanol-water 50:50(v/v) pH 3.0 adjusted by phosphoric acid), was delivered at a flow rate of 1mLmin^{-1}, eluent was monitored using UV detector at 215, 220, 225 nm. The proposed method is specific, accurate (99-102%), precise (intra-day variation 0.04-0.98 % and inter-day variation 0.06-1.5%) and linearity (R^2>0.999) within the desired range 1.25-50 µgmL^{-1} concentration. The detection limit and quantification limit was 2.0 ngmL^{-1} and 8.0 ngmL^{-1} respectively. The anticipated method is applicable to routine analysis of CAP in pharmaceutical formulations as well as in human serum samples.*

Keywords: Captopril, HPLC, Serum,

Introduction:

Captopril (fig 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[3-8] and spectrophotometry[9] but these methods were not use different column and different wave lengths. Almost all previously reported methods used acetonitrile in their mobile phase which may increase the cost of the method. The main purpose of our study was to develop a simple, reliable and economical method to determine captopril in a relatively short time with high linearity using atorvastatin as internal standard. Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of captopril in bulk drug, pharmaceutical formulations and in serum.

Experimental

Material and reagents

Standard bulk drug sample of captopril were supplied by Squib Pharmaceutical Laboratories Atorvastatin was obtained from Atco Pharma (Pvt.) Ltd. HPLC grade methanol was obtained from Merck , Germany.

Instrumentation

HPLC system equipped with Shimadzu LC-20 AT VP Pump, SPD-20AV VP Shimadzu UV visible detectors and second HPLC system consisted of an LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector, both connected by CBM-102 communication Bus Module Shimadzu to Intel Pentium 4 machine with Shimadzu CLASS-VP software (Version 5.03) and Rheodyne manual injector fitted with a 20 µL loop. Separation was achieved on a Purospher Start C_{18} (250cm x 4.6mm, 5µm) column and Hypersil ODS C18 . The chromatographic analysis was integrated using a Mobile phase, which was methanol- -water (50:50, v/v pH 3.0 adjusted by phosphoric acid). The mobile phase was sonicated by DGU-14 AM on-line degasser, and filtered through 0.45-micron membrane filter, calibrated Pyrex glassware was used for the solution and mobile phase preparation.

Preparation of solutions

To produce a concentration of 100 µgmL^{-1}, 10 mg of the captopril and internal standard (atorvastatin) were diluted to 100 mL with mobile phase. This solution was used for preparation of working solutions which were prepared by diluting the stock solutions with the same solvent to contain 1.25-50 µgmL^{-1} for captopril and for IS then filtered with 0.45-micron membrane filter. These solutions were ready to inject.

Analysis of formulation

Twenty tablets of four different brands were accurately weighed, grinded to make a fine powder. Calculated quantity of powder of each brand was weighed, which was corresponding to 10 mg of captopril and shifted to separate 100 mL volumetric flask. Each was dissolved in the mobile phase and filtered through a membrane filter (0.45µ). The sample solutions were further diluted to desired concentrations and then used for the analysis.

Procedure for human serum

Plasma sample, obtained from healthy volunteers, was collected and stored at -20 ˚C. Then, 1.0 mL of frozen plasma mixed with 10 mL of acetonitrile. The mixture was vortexed for one minute and then centrifuged for 10 minutes at 10,000 rpm. It was then alienated supernatant by filtration (0.45µ pore size membrane filter). An aliquot serum sample was fortified with captopril to get final concentrations of 50, 25, 12.5, 6.25, 3.125 ,and 1.25 μgmL^{-1}.

Results and discussion

This work has been designed to develop a simple, rapid, precise and accurate HPLC method with UV detection for captopril in bulk, dosage form and human serum by using two different column. RP-HPLC was used for the development and validation of captopril in bulk, pharmaceutical formulations and human serum.Developed method was conducted on Purospher Start C_{18} (250cm x 4.6mm, 5µm) and Hypersil ODS C18 (25cm x 4.6mm, 5µm) columns for separation at ambient temperature. Mobile phase contained methanol-acetonitrile-water (50:50 v/v), commonly used solvents for RP-HPLC and non toxic for column, pH 3.0 adjusted with phosphoric acid then degassed by sonicator and filtered by 0.45-micron membrane filter. Flow rate adjusted was $1mLmin^{-1}$ at ambient temperature with isocratic elution. The work was carried out at 215, 220, 325 nm because UV scan of captopril produced maximal absorption around these wavelengths. The samples were injected by a 20 µL sample loop.

Method validation

The developed method was validated by various parameters which include system suitability, selectivity, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification, according to FDA, EMEA and ICH guidelines.

Linearity

Linearity was determined in the range 1.25-50 μgmL^{-1}. Concentration of CAP versus peak area was subjected to least square linear regression analysis. A linear regression line was obtained with correlation coefficient ($R^2 > 0.999$). The standard curve, slope and intercept are displayed in table 1.

Accuracy

Method accuracy was evaluated as the percentage of recovery of known amounts of CAP to the pharmaceutical formulation and serum . It is performed at spike concentration that was 80, 100 and 120%. Each sample was injected five times and result range was 99.4-100.8%, (table 2), high recovery indicated that the method has a high degree of accuracy.

Precision

Precision of the proposed method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). It was noted as relative standard deviation (RSD). Seven different concentrations of CAP in the linear range were analyzed on the same day (intra-day precision) and two consecutive days (inter-day precision); each sample for precision was injected six times. Both intra- and inter-day RSD values were in the range 0.04-1.50% confirming good precision (table 3). The results were insignificant and indicated no remarkable difference in interday and intraday precision.

Limit of detection and limit of quantification

The limit of detection was calculated by lowest concentration of CAP and quantification (LOQ) was determined from the calibration curve. LOD and LOQ were 2.0 $ngmL^{-1}$ and 8.0 $ngmL^{-1}$ respectively

Specificity and selectivity

The selectivity and specificity of the method was found during whole study. Resolution factor of the peak of CAP was calculated from internal standard. The method confirmed good resolutions . It was found to be free of interference from the excipient used in pharmaceutical formulation and it indicated the specificity of the system.

Robustness

Robustness was performed by making minor changes in the percentage of mobile phase (methanol or water) wave length, pH and flow rate. Therefore, five repeated samples were injected under small variations of each parameter. When a parameter was changed ± 0.2 % (in in flow rate), ± 0.2% (pH 3.5), and ±5 % wave length from its optimum condition, the shifting in retention time of ± 0.2% was observed that assessed as inconsequentiality. The method proved to be quite stable (table 4).

Ruggedness

Ruggedness of our method was determined using two different labs, two different instruments (LC 10 and LC 20) and two different columns (Purospher STAR C_{18} and Hypersil,ODS) fig 2 and 3. All parameters were compared. The developed method did not show any remarkable difference in calculated results from acceptable limits in precision, but the area under curve of peak was affected with change of wavelength.

Conclusion

A simple and reliable HPLC method for determining captopril in human serum and pharmaceutical dosage formulation has been developed. The limit of quantification, small sample volume and short chromatographic time 2 min for CAP of this method are particularly adapted for routine assay. Atorvastatin could be successfully used as an internal standard. The intra-run and inter-run variability and accuracy results were in acceptable limit.

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Table I. Regression statistics

Systems	Column	Drug	Conc. μgmL^{-1}	Regression equations	r2
LC 10	Hypersil, ODS	Active	1.25-50	y=5311.8x+6011.6	0.9992
		Serum	1.25-50	y= 3140.1x+6049.8	0.9992
	Purospher STAR	Active	1.25-50	y=5505.8x+ 720.8	0.9996
		Serum	1.25-50	y=5697.5x+ 391.9	0.9994
LC 20	Hypersil, ODS	Active	1.25-50	y=5311.8x+6011.4	0.9992

		Serum	1.25-50	y=5314.1x+ 049.8	0.9992
	Purospher STAR	Active	1.25-50	y=5505.8x+ 720.7	0.9996
		Serum	1.25-50	y=5697.5x+ 391.8	0.9995

Table II. Accuracy of captopril

Columns	Con. μgmL^{-1}	LC 10	LC 20	LC 10	LC 20
		Found Conc.		%Rec.	
Hypersil ODS	5	5.03	7.99	100.4	99.88
	10	10	10.03	100	100.3
	15	15.1	12.03	100.8	100.25
Purospher STAR	5	5.98	5.99	99.8	99.88
	10	9.99	9.99	99.9	99.9
	15	15.93	15.99	99.4	99.92

Table III. Precision of captopril

Column		Purospher STAR			Hypersil ODS		
Systems	Conc. μgmL^{-1}	Formulation %RSD		Serum %RSD	Formulation %RSD		Serum %RSD
		Intra-day variation	Inter-day variation	Intra-day variation	Intra-day variation	Inter-day variation	Intra-day variation
LC 10	1.25	0.69	0.78	0.56	0.98	1.02	0.98
	2.5	0.59	0.75	0.59	0.96	2.02	0.98
	5	0.056	0.78	0.52	0.98	1.36	0.95
	10	0.69	0.79	0.89	0.98	1.5	0.89
	25	0.059	0.75	0.69	0.97	1.02	0.86
	50	0.58	0.76	0.57	0.96	1.02	0.86
LC 20	1.25	0.04	0.066	0.39	0.36	1.03	0.59
	2.5	0.45	0.069	0.59	0.96	0.99	1.02
	5	0.43	0.69	0.93	0.95	0.96	0.36

	10	0.42	0.63	0.36	0.98	0.96	1.02
	25	0.49	0.62	0.33	0.93	0.98	1.03
	50	0.59	0.62	0.35	0.36	0.61	1.06

Table IV. Robustness of the method (n=6)

	Level	tR	K'	T	(R_s)
A: pH of mobile phase					
2.70	-0.20	2.13	2.90	1.39	3.37
2.90	0.00	2.10	2.30	1.33	3.36
3.10	0.20	2.15	2.79	1.48	3.35
S.D (n=6)		0.03	0.32	0.08	0.01
B: Flow rate ($mLmin^{-1}$)					
0.80	-0.20	2.23	2.00	1.46	3.34
1.00	0.00	2.10	2.3	1.33	3.36
1.20	0.20	1.90	2.50	1.12	3.37
S.D (n=6)		0.17	0.35	0.17	0.02
C: Percentage of water in mobile phase (V/V)					
60/40	-10.00	2.30	2.50	1.42	3.38
50/50	0.00	2.10	2.3	1.33	3.36
70/30	-20.00	2.25	2.40	1.45	3.32
S.D (n=6)		0.10	0.07	0.06	0.03
C: Wavelength (nm)					
215.00	-5.00	2.15	2.70	1.42	3.39
220.00	0.00	2.10	2.3	1.33	3.36
225.00	5.00	2.02	2.40	1.49	3.35
S.D (n=6)		0.07	0.21	0.08	0.02
tR = Retention time K'= Capacity factors, T = Tailing factor, R_s = Resolution.					

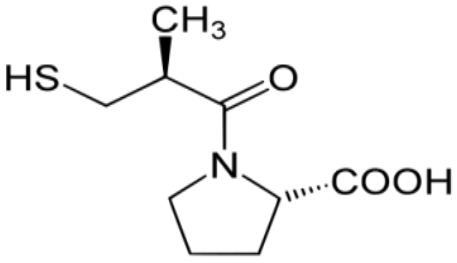

Fig 1.Captopril

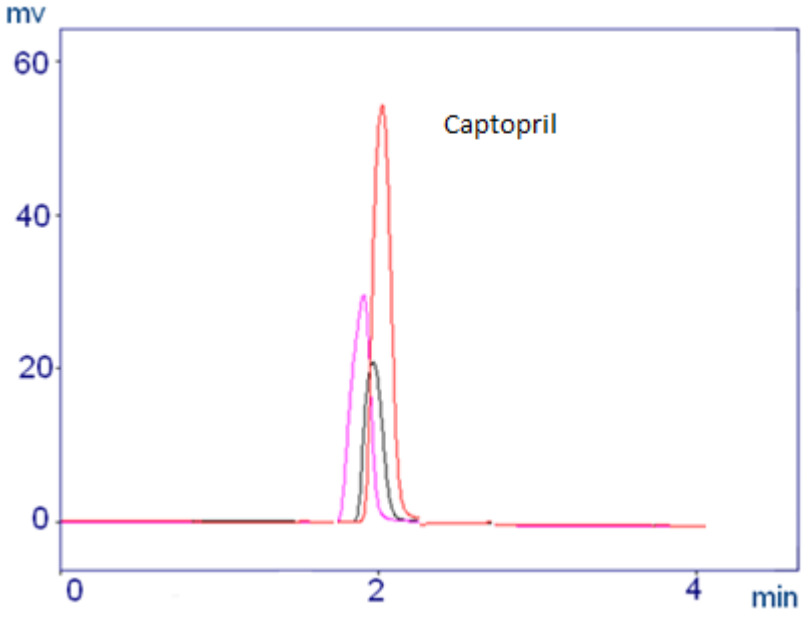

Fig. 2 Representative chromatogram of CAP at three different wavelengths using LC20 operating system, column was Purospher Star, red= 215nm, pink =220nm, black =225nm.

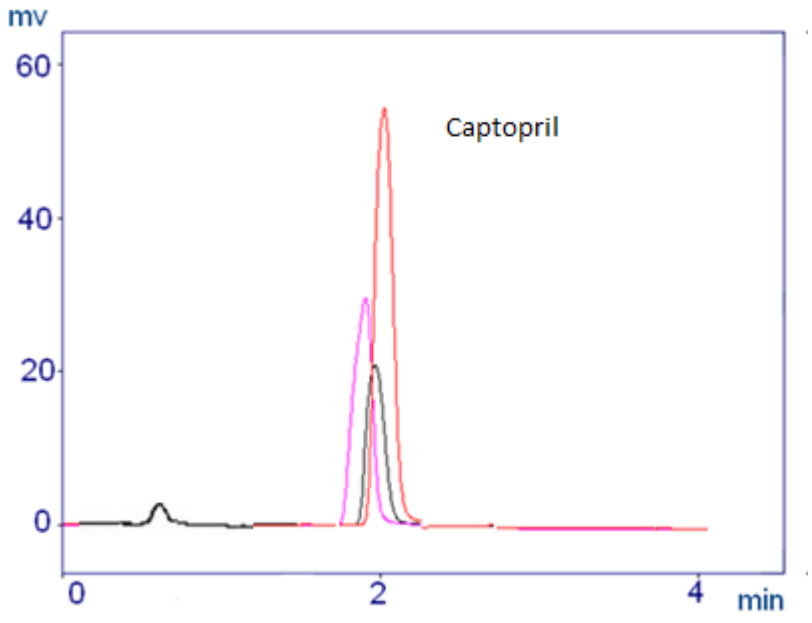

Fig. 3 Representative chromatogram of CAP at three different wavelengths using LC10 operating system, column was Hypersil ODS, red= 215nm, pink =220nm, black =225nm.