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RP-HPLC Method for Simultaneous Determination of Captopril and Diuretics: Application in Pharmaceutical Dosage Forms and Human Serum

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Abstract

An isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of captopril and diuretics (furosemide and hydrochlorothiazide) in API, dosage formulations and human serum. Chromatographic separation was achieved on Purospher Start C18 (250 mm x 4.6 mm, 5 µm) and Hypersil ODS C18 (150×4.6mm, 5micron) columns using mobile phase, methanol: water (70:30 v/v) adjusted to pH 3.0 via phosphoric acid 85% having flow rate of 1.0 mL min^{-1} at ambient temperature with detector set at 225 nm. Calibration curves were linear over range of 5-25 µg mL^{-1} with a correlation coefficient ± 0.999. LOD and LOQ were in the ranges of 0.4-2.3 µg mL^{-1}. Intra and inter-run precision and accuracy results were 98.0 to 102%.

Keywords: Captopril; Diuretics; RP-HPLC

Introduction

Captopril (CAP) belongs to angiotensin II receptor antagonist effective in lowering blood pressure in hypertensive patients. Chemically it is known as (2S)-3-mercapto-2-methyl-1-oxo-proptonyl-L-proline [1]. It has a relatively poor pharmacokinetic profile, while the adverse drug reaction profile of captopril is similar to other ACE inhibitors [2]. In the presence of stenosis, captopril abrogates the protective actions of the high levels of angiotensin II on the autoregulation of the glomerular filtration rate [3]. Captopril does not have a diuretic sparing effect in patients with severe or moderate chronic heart failure. Those treated need the original dose of diuretics for maximal symptomatic benefit. Captopril may be administered with diuretics to manage renal clearance. There have been number of studies using captopril with diuretics [4-13]. Hydrochlorothiazide (HCT) and furosemide (FRS) have widely been used in the treatment of congestive heart failure and hypertension [14,15] (Figure 1).

There are very few methods reported in literature for the simultaneous estimation of angiotensin II receptor antagonist and hydrochlorothiazide in tablets [16-18]. Since these methods were based on HPLC, Capillary electrophoresis and UV derivative spectrophotometry [19,20], the procedures were inconvenient for determination and run time were rather long. Thus, an attempt was made to develop a simple, precise, accurate and economical RP-HPLC method for the simultaneous estimation of captopril furosemide and hydrochlorothiazide in API, pharmaceutical dosage formulations and human serum. Hydrochlorothiazide and furosemide are thiazide and loop diuretics, respectively.

Our research group earlier reported simultaneous quantitation of hydrochlorothiazide with number of other angiotensin II receptor blockers [21]. In present paper, we report a simple, easy, quick and inexpensive isocratic RP-HPLC method with ultraviolet detection at 225 nm for the simultaneous determination of CAP and two diuretics i.e. HCT and FRS. Simultaneous determination of both drugs is desirable as this would allow more efficient generation of clinical data and could be performed at more modest cost than separate assays. The method is equally valid for the determination in bulk materials, pharmaceutical dosage formulations and human serum. This method can be used for the quantitative analysis of diuretics and captopril alone or in combination. The low LOD and LOQ values merit the method for the determination of these drugs in clinical samples.

Materials and Reagents

Materials

All chemicals and reagents were of analytical grade. Captopril was a kind gift from BMS (Pvt) Limited, Pakistan. Hydrochlorothiazide and furosemide were gifts from Zafa Pharmaceutical Laboratories (Pvt) Ltd and Sanofi Aventis (Pvt) Ltd Pakistan. HPLC grade methanol and phosphoric acid were obtained from Tedia (USA) and Merck Darmstadt, Germany.

Pharmaceutical dosage form

Capoten™ (Captopril 25 mg tablets by BMS Pharmaceuticals (Pvt) Ltd), Diuza™ (Hydrochlorothiazide 25 mg tablets by Zafa Pharmaceutical Laboratories (Pvt) Ltd) and Lasix™ (40 mg tablets from Sanofi Aventis Pakistan Limited), were purchased from the local pharmacies (Figure 1). All these drugs had an expiry of not less than 1 year at the time of study.

Instrumentation

The HPLC system consisted of an LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector, a Purospher Start C18 (250 x 4.6 mm, 5µm) and Hypersil ODS C18 columns were used for separation. The chromatographic system was integrated using a CBM-102 communication BusModule Shimadzu with a Pentium™ IV PC loaded with Class GC software for data acquisition. Separation was carried out under isocratic elution with methanol:water (70:30) as mobile phase. The pH of the mobile phase was adjusted to 3.0 with phosphoric acid (85%), sonicated by DGU-14 AM on-line degasser, and filtered through 0.45-micron membrane filter. The flow rate was 1.0 mL min^{-1}, the elution was monitored at 225 nm, and the injection volume was 20 µL.

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Preparation of standard and sample solutions

Standard preparation: Stock standard solutions 100 ppm of CAP, HCT and FRS were prepared in 100 mL mobile phase as solvent. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain concentration between 5-25 μg mL^{-1} for CAP, HCT and FRS, respectively. These solutions were stored at 20°C. Once prepared, analyzed daily for inter and intra-day variations of the method. 20μL of these solutions were injected into LC system and chromatographed.

Procedure for tablets: Twenty tablets of each formulation were powdered finely and an amount equivalent to 10 mg of CAP, HCT and FRS was weighed and then dissolved in the mobile phase. Solutions were then filtered through ordinary filter paper. The desired concentrations 2.5, 5, 10, 15, 20 and 25μg mL^{-1} for CAP, HCT and FRS, respectively were obtained by accurate dilution, solutions were then sonicated. Finally, all the solutions were filtered through 45-μm Millipore™ filter, in order to separate out the insoluble excepients before chromatographed.

Procedure for human serum: Plasma samples, obtained from healthy volunteers, were collected and stored. To 1.0 mL of plasma, 9.0 mL of acetonitrile was added; the mixture was vortexed for 1 min and than centrifuged for 10 min at 10,000 rpm and the supernatant was filtered by 0.45-micron membrane filter. An aliquot of serum sample was fortified with CAP, HCT and FRS to achieve final concentration.

Results and Discussion

Development and optimization of isocratic HPLC conditions

The aim of the present study was to develop a simple, isocratic, accurate and sensitive HPLC method for the simultaneous determination of CAP, HCT and FRS. A UV scan showed a maximal absorbance at near 225 nm. Initial method development was conducted on a Purospher Start C18 (250 x 4.6 mm, 5 μm) column for separation at ambient temperature. This column provides efficient and reproducible separations of non-polar compounds while minimizing solvent usage. Initially various mobile phases were tested to obtain the best separation and resolution. The mobile phase consisting of methanol and water in the ratio of 70:30 v/v was found to have good resolution. The chromatographic conditions were optimized to achieve best separation and to get best resolution between analytes and to optimize chromatographic parameters like resolution, tailing factor and retention time. The optimized conditions were reached at pH 3.0,

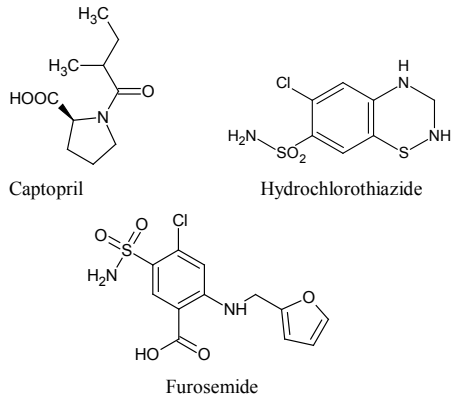

Figure 1: Chemical structures of Captopril, Hydrochlorothiazide and Furosemide.

Analytes	Retention time (T_R) (min)	Capacity factors (K')	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Separation factor
CAP	3.3	2.13	3200	1.23	3.4	2.48
HCT	3	2.25	3500	1.23	3.5	2.36
FRS	4	2.36	3200	1.36	3.6	2.59

CAP: captopril ;HCT : hydrochlorothiazide; FRS: furosemide

Table 1: System suitability parameters of the proposed method for captopril, hydrochlorothiazide and furosemide.

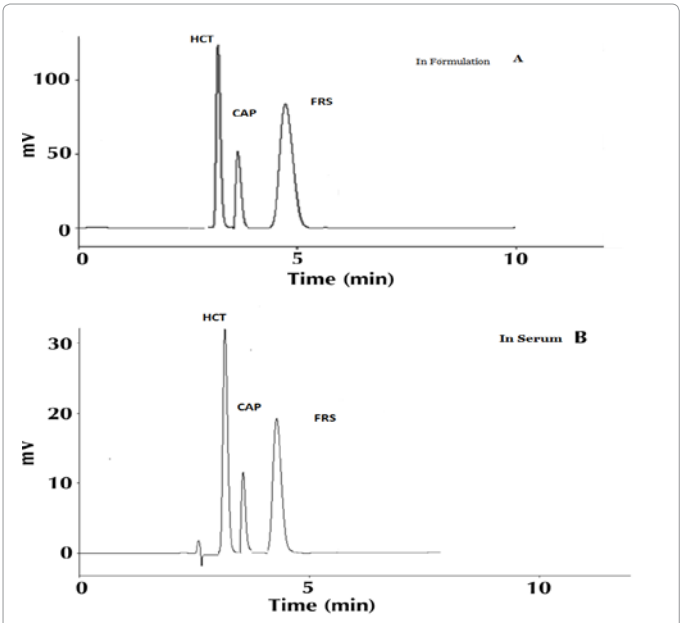

Figure 2: Chromatograms of CAP, HCT and FRS (A) in formulation and (B) human serum at 225 nm.

producing well resolved and sharp peaks for all drugs. The specificity of the method was established through the study of resolution factor of captopril peak. 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 and USP 2002 were followed for the accuracy tests, precision, specificity, linearity, work strip and robustness of the method. Retention time of HCT 3.0 min, CAP was 3.3 min and FRS was 4.0 min, at a flow rate of 1.0 mL min^{-1}.

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 6 injections of the same standard. These 6 consecutive injections were used to evaluate the system suitability on each day of method validation. Parameter of system suitability, peaks symmetry (symmetry factor), theoretical plates of the column, mass distribution ratio (capacity factor), and resolution are summarized in Table 1.

Specificity and selectivity

The specificity of the chromatographic method was determined to ensure separation of captopril and diuretics as shown in Figure 2. Specificity was also determined by screening four different samples of controlled human serum, which were free from interfering endogenous plasma components. Solutions of placebo, captopril and diuretics were

prepared and then injected to check for interference from common excepients.

Linearity

Linearity is generally reported as the variance of the slope of the regression line. Linearity was tested with known concentrations of CAP, HCT and FRS i.e. 5, 10, 15, 20 and 25 µg mL^{-1}, respectively. Six runs were performed for every concentration [22,23]. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in Table 2.

Accuracy

The accuracy of an analytical procedure measures the closeness of measured values to the true values. It was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. Minimal of 3 concentration levels covering the specified ranges were selected and five runs were performed for every concentration and then peak area was calculated as given in Table 3.

Precision

For intra-day and inter-day precision, ten samples of five concentrations were analyzed on the same day and after one day (Table 4). Generally acceptable repeatability of the results with in one day and day-to-day was observed. The precision of the method was analyzed as % RSD throughout the linear range of concentrations.

Drugs	Conc.	Regression equation	r^2	LOD	LOQ
	µgmL^{-1}			µgmL^{-1}	
CAP	2.5-25	A= 2501.7x + 3073.7	0.9995	0.7	2.3
HCT	2.5-25	A= 3841.3x + 4744.2	0.9998	0.4	1.9
FRS	2.5-25	A = 2419.8x +2988.8	0.9995	0.7	2.2

CAP: captopril; HCT: hydrochlorothiazide; FRS: furosemide; LOD: Limit of Detection; LOQ: Limit of Quantification

Table 2: Regression characteristics of the proposed method for captopril, hydrochlorothiazide and furosemide.

Analyte	Assay (spiking method)			Assay in serum	
	Conc. µgmL^{-1}	%RSD	% Rec	%RSD	%Rec
CAP	8	0.01	101	0.002	101
	10	0.33	100.04	0.02	102
	12	0.02	99.97	0.03	101
HCT	8	0.01	100.5	0.002	100.3
	10	0.3	100.02	0.002	101.3
	12	0.3	99.98	0.02	100.3
FRS	8	0.22	99.3	0.5	100.2
	10	0.2	99.98	0.36	100.6
	12	1.03	99	0.9	101.3

CAP: captopril; HCT: hydrochlorothiazide; FRS: furosemide

Table 3: Accuracy of the proposed method for captopril, hydrochlorothiazide and furosemide.

Drugs	Conc. injected	Inter-day		Intra-day	
	µgmL^{-1}	%RSD	%Rec	%RSD	%Rec
CAP	2.5	0.0073	101.11	0.073	101.11
	5	0.0109	102.36	0.009	102.36
	10	0.3261	100	0.361	100.02
	15	0.0009	100	0.09	100.03
	20	0.0005	99.826	0.005	99.26
	25	0.0002	99.998	0.002	99.98
HCT	2.5	0.0047	99.997	0.047	99.9
	5	0.0071	99.988	0.071	99.88
	10	0.0024	100.12	0.024	100.1
	15	0.0006	99.983	0.006	99.93
	20	0.0006	99.968	0.006	99.98
	25	0.0003	99.991	0	99.91
FRS	2.5	0.0075	98.72	0.007	98.72
	5	0.0075	99.98	0.075	99.98
	10	0.0019	99.73	0.019	99.73
	15	0.0012	99.97	0.012	99.87
	20	0.0005	99.97	0.005	99.87
	25	0.0003	100.02	0.003	100.1

CAP: captopril; HCT: hydrochlorothiazide; FRS: furosemide

Table 4: Inter and intra-day precision of the proposed method for captopril, hydrochlorothiazide and furosemide (n=6).

Ruggedness

The ruggedness was established by determining CAP, HCT and FRS in dosage formulation and in human serum using same and different chromatographic system and two different columns on different days. The assay results indicated that the method was capable with high precision (Table 4).

Robustness

Robustness of the method was accomplished by designed modifications made to the method parameters such as mobile phase composition, flow rate, pH and detection wavelength and it was found that the % R.S.D values did not exceed more than 2% (Table 5).

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of this method were determined from the known concentrations of CAP, HCT and FRS. The LOD and LOQ for this assay were calculated which are given in Table 2.

Conclusion

The proposed RP-HPLC method for simultaneous assay of captopril, furosemide and hydrochlorothiazide in combined tablets, dosage forms is simple, precise, specific and highly accurate and less time consumption for analysis. So, it can definitely be employed for the routine analysis. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and also for human serum. The intra-run and inter-run variability and accuracy results

	Level	K'	T	(R_s)
A: pH of mobile phase				
2.8	-0.2	2.5	1.51	2.37
3	0	2.6	1.53	3
3.2	0.2	2.2	1.58	2.35
S.D n=6		0.21	0.04	0.01
B: Flow rate ($mLmin^{-1}$)				
0.8	-0.2	2.1	1.56	2.32
1	0	2.6	1.53	3
1.2	0.2	2.5	1.51	2.37
S.D n=6		0.28	0.03	0.03
C: Percentage of water in mobile phase (V/V)				
90/10	-20	2.7	1.52	2.39
70/30	0	2.6	1.53	3
80/20	-10	2.4	1.57	2.32
S.D n=6		0.21	0.03	0.04
D: Wavelength (nm)				
220	-5	2.7	1.52	2.39
225	0	2.6	1.53	3
230	5	2.5	1.59	2.33
S.D n=6		0.14	0.04	0.03
K'= Capacity factors, T = Tailing factor, R_s = Resolution				

Table 5: Robustness of the proposed method.

were also in acceptable limit. In addition, this method has the potential application to clinical research of drug combination, interactions studies and multi-drug pharmacokinetics.

References

1. Jaime ND, William AR (1991) Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry. (9th ed), Lippincott, JB Company.
2. Rossi S, Australian Medicines Handbook (2006) Adelaide: Australian Medicines Handbook.
3. Goto A, Kawauchi N (2001) Captopril-Augmented Renal Scan. Engl J Med 2001: 344:430
4. Odemuyiwa O, Gilmartin J, Kenny D, Hall RJC (1989) Captopril and the diuretic requirements in moderate and severe chronic heart failure. Eur Heart J 10: 586-690.
5. Flapan AD, Davies E, Williams BC, Shaw TRD, Edwards CRW (1992) The relationship between diuretic dose, and the haemodynamic response to captopril in patients with cardiac failure. Eur Heart J 13: 971-975.
6. Pacher R, Globits S, Bergler-Klein J, Teufelsbauer H, Wutte M, et al. (1993) Clinical and neurohumoral response of patients with severe congestive heart failure treated with two different captopril dosages. Eur Heart J 14: 273-278.
7. Turini GA, Waeber B, Brunner HR (1983) The renin angiotensin system in refractory heart failure: clinical, hemodynamic and hormonal effects of captopril and enalapril. Eur Heart J 4:189-197.
8. Kayanakis JG, Fauvel JM, Giraud P, Bounhoure JP (1981) Long-term treatment of congestive heart failure by Captopril: hemodynamic, biological and clinical effects. Eur Heart J 2: 75-81.
9. Fouad FM, EL-Tobgi S, Tarazi RC, Bravo EL, Hart NJ, et al. (1984) Captopril in congestive heart failure resistant to other vasodilators. Eur Heart J 5: 47-54.
10. Galcera-Tomas J, Nuño de la Rosa JA, Torres-Martinez G, Rodriguez-Garcia P, Castillo-Soria FJ, et al. (1993) Effects of early use of captopril on haemodynamics and short-term ventricular remodelling in acute anterior myocardial infarction. Eur Heart J 14: 259-266.
11. Mclay JS, Mcmurray J, Bridges A, Struthers AD (1992) Practical issues when initiating captopril therapy in chronic heart failure: What is the appropriate dose and how long should patients be observed?. Eur Heart J 13: 1521-1527.
12. Osterziel KJ, Röhrig N, Dietz R, Manthey J, Hecht J, et al. (1988) Influence of captopril on the arterial baroreceptor reflex in patients with heart failure. Eur Heart J 9: 1137-1145.
13. Schofer J, Hobuss M, Aschenberg W, Tews A (1990) Acute and long-term haemodynamic and neurohumoral response to nisoldipine vs captopril in patients with heart failure: a randomized double-blind study. Eur Heart J 11: 712-721.
14. Briggs JW, Chasseaud LF (1980) Progress in Drug Metabolism.Wiley and Sons: New York, p 7.
15. Ventura R, Nadal T, Alcalde P, Pascual JA, Segura J (1993) Fast screening method for diuretics, probenecid and other compounds of doping interest. J Chromatogr A 655: 233-242.
16. Carlucci G, Palumbo G, Mazzeo P, Quaglia MG (2000) Simultaneous determination of losartan and hydrochlorothiazide in tablets by high-performance liquid chromatography. J Pharm Biomed Anal 23: 185-9.
17. Schoenberger JA (1995) Losartan with hydrochlorothiazide in the treatment of hypertension. J Hypertens 13: 43-7.
18. del Castillo D, Campistol JM, Guirado L, Capdevilla L, Martinez JG, et al. (1998) Efficacy and safety of losartan in the treatment of hypertension in renal transplant recipients. Kidney Int 68: 135-9.
19. Hillaert S, Van den Bossche W (2003) Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis. J Pharm Biomed Anal 31: 329-39.
20. Satana E, Altinay S, Göğer NG, Ozkan SA, Sentürk Z (2001) Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. J pharm Biomed Anal 25: 1009-1013
21. Sultana N, Arayne MS, Ali SS, Sajid S (2008) Simultaneous determination of olmesartan medoxomil and irbesartan and hydrochlorothiazide in pharmaceutical formulations and human serum using high performance liquid chromatography. Se Pu 26: 544-549.
22. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology. The Addendum dated November 1996 incorporated into the core guideline in November 2005.
23. Shabir GA (2003) Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. Journal of Chromatography A 987: 57-66.