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RAPID RP – HPLC METHOD FOR ESTIMATION OF CAPTOPRIL FROM TABLET DOSAGE FORM

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ABSTRACT

A HPLC method was developed, validated and applied for determination of Captopril in pharmaceutical formulations. A LiChrosorb® RP-18 (10 µm, 250x 4 mm) column was used with a mobile phase consisting of methanol: water (60: 40% v/v, pH adjusted to 3.0 with 85%, w/v phosphoric acid.), a quantitative evaluation was performed at 220 nm with flow rate of 2 ml/min, and column cooler temperature was maintained at 30 ºC. The retention time was about 8 min. Suitability of this method for the quantitative determination of the drug was proved by validation in accordance with the requirements by the International Conference of Harmonization (ICH) guidelines. The method is selective, precise, accurate and can be used for analysis of pharmaceutical preparations in quality control.

Keywords: Captopril, ACE-Inhibitors, Validation, Estimation, Tablets, RP-HPLC

INTRODUCTION

Angiotensin converting enzyme (ACE) plays a major physiological role in the renin-angiotensin system for increasing blood pressure, which removes a dipeptide from the C terminus of angiotensin I to form angiotensin II, a potent vasoconstrictor, and inactivates the vasodilator nonapeptide bradykinin **[1-4]**. ACE inhibitors can be classified as Sulfhydryl containing ACE inhibitors structurally related to captopril (eg. Fentiapril, Pivalopril, Zefenopril, Alacepril); Dicarboxyl-containing ACE inhibitors structurally related to enalapril (eg. lisinopril, benazepril, quinapril, moexipril, ramipril, spirapril, perindopril, spirapril, pentopril, cilazapril); phosphorous containing ACE inhibitors structurally related to fosinopril. Many of these ACE

inhibitors are ester containing prodrugs that are 100-1000 times less potent than the active metabolites, but have a much better oral bioavailability than the active molecules **[5]**. Many synthetic ACE inhibitors such as benazepril, captoril, enlapril, alacepril and so on are currently used in the treatment of hypertension and heartfailure. So far, many methods such as spectrophotometry **[6-8]**, HPLC **[9-11]**, fluorimetry **[12, 13, 20]**, micellar electrokinetic chromatography **[14]** and biochemical detection **[15, 21]** have been known to determine ACE inhibitory separately or mixed with other drugs **[16-22]**.

Captopril (CPL) **(Figure 1)** is (2S)-3-mercapto-2-methyl-1-oxo-proptonyl]-L-proline, the first orally active and specific inhibitor of angiotensinconverting enzyme.

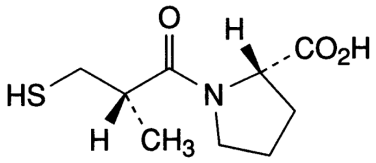

Figure 1: Chemical Structure of Captopril

For the quantity determination of captopril in pharmaceutical preparations and biological fluids, various instrumental methods have been reported, including gas chromatography **[23]**, high performance liquid chromatography **[24-25]**, capillary electrophoresis **[26]**, flow injection analysis **[27]**, titrimetry **[28-29]**, potentiometry **[30]** and amperometry **[31]**.

The aim of the present study is to apply simple, rapid, accurate, selective and reproducible HPLC method of the determination of captopril in pure form and pharmaceutical formulations.

MATERIALS AND METHODS

Reagents

All chemicals and reagents were used of HPLC. Captopril used was standard reference compound according to European Pharmacopoeia. Tablet formulation containing Captopril 25 mg was obtained commercially. HPLC grade Methanol was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: quaternary pump LC – 10 AD, vacuum degasser unit DGU – 3A and a DAD – SPD – M10 A. Separation was carried out on a LiChrosorb C18 column (250 x 4 mm, particle size 10 µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC with properly installed chromatographic software.

Chromatographic Conditions

The mobile phase was a 60:40 % v/v Methanol:Water, pH adjusted to 3.0 with 85%, w/v phosphoric acid. The mobile phase was filtered through 0.45 µm membrane filter and degassed by using a

sonicater for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 2.0 ml/min. The column was maintained at 30ºC temperature. The injection volume was a 20 µl and the total run time was 9 minutes. The detection was carried out at 220 nm. Typical chromatogram of Captopril is given in **Figure 2**.

Preparation of the Standard Stock Solution

Accurately weighed quantity of 50 mg Captopril is transferred in 50 ml volumetric flask, dissolved with 25 ml mobile phase and made up with mobile phase having the concentration of 1000 µg/ml of Captopril.

Sample Preparation

Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finally powdered and weight equivalent to 25 mg of Captopril was weighed and transferred into a 25 ml volumetric flask. Approximately 15 ml of mobile phase was added and the mixture was sonicated for 15 minutes. The mixture was then diluted to volume with mobile phase. The solution was then filtered off through a 0.45 µ filter paper discarding the first few ml of filtrate.

Placebo Solution Preparation

The placebo solution is prepared by using 100 mg of placebo, dissolved in 15 ml mobile phase and the mixture was sonicated for 15 minutes. The mixture was diluted to 25.0 ml with mobile phase and mixed well. The solution was then filtered off through a 0.45 µ filter paper discarding the first few ml of filtrate. This solution was injected to HPLC system.

RESULTS AND DISCUSSION

Validation of the Method and Procedures

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines as follows:

Selectivity

The selectivity of the method was evaluated with regard to interference due to the presence of any other excipients. This shows that drug was clearly separated from its excipients. Thus, the HPLC method presented in this study was found to be selective, presented in **Figure 3**.

Limit of Detection and Quantification

The detection limit (LOD) is the lowest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The quantification limit (LOQ) is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ are calculated as given in **Table 1**.

Linearity

Calibration standards at seven levels were prepared by appropriately mixing and

further diluting stock standard solutions in the concentration range of 25 – 200 µg/ml. Samples in triplicates were made for each concentration, and peak areas were plotted against the corresponding concentrations to obtain the calibration graph. The regression equation was derived using mean peak area concentration data, and the concentration of the unknown was computed from the regression equation. Hence, the regression line relating standard concentrations of drug using regression analysis showed linearity in the studied range. The results are given in **Table 1**.

Precision

The precision of the method was determined by repeatability, intermediate precision (intra-day, inter-day) and was expressed as % relative standard deviation (% RSD). Intra-day precision was determined by performing analysis of triplicate injections of two different concentrations of Captopril on the same day at different time intervals and on two different days for inter-day precision. The % RSD of the study was found to be less than 2% as shown in **Table 2**.

Accuracy/Recovery

Accuracy of the developed method was confirmed by performing a recovery study as per ICH norms at three different concentration levels (50%, 100%, 150%) by replicate analysis (n = 3). The results obtained **(Table 3)** indicate that recovery is good, not less than 98% and percentage relative standard deviation is less than 2%.

Table: 1. Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compounds	r^2	Calibration curve equation	LOQ ng	LOD Ng
Captopril	0.9997	Y=19598690X+18453	20	7

Table 2: Results of Precision

Sample	Concentration (µg/mL)	RSD (%) Intra-day (n=3)	RSD (%) Inter-day (n=3)
Captopril	50	1.185	1.322
	100	0.844	1.368

Table 3: Recovery Studies of Captopril

Drug	Concentration added (µg/mL)	Concentration recovered (µg/mL)	Recovery (%)
Captopril		50.12	100.24
	50.00	49.78	99.56
		50.38	100.76
		98.96	98.96
	100.00	10.19	100.15
		100.50	100.50
		148.95	99.30
	150.00	149.20	99.47
		147.88	98.59
Mean			99.73
SD			0.730
% RSD			0.732
% Error			±0.563

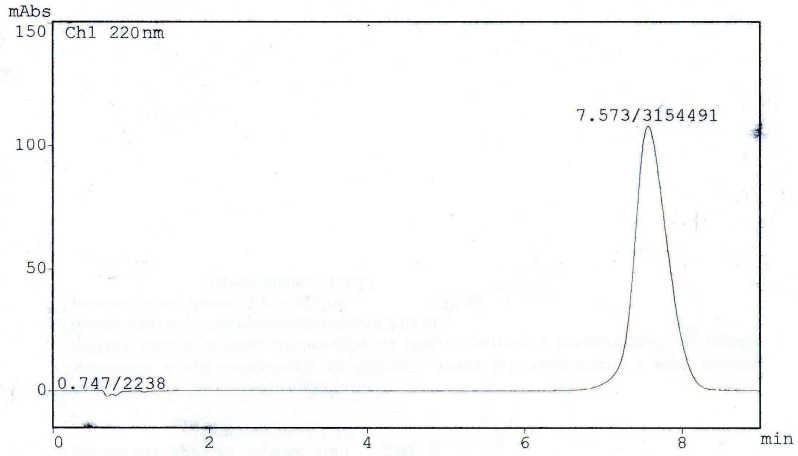

Figure 2: Chromatogram of Captopril

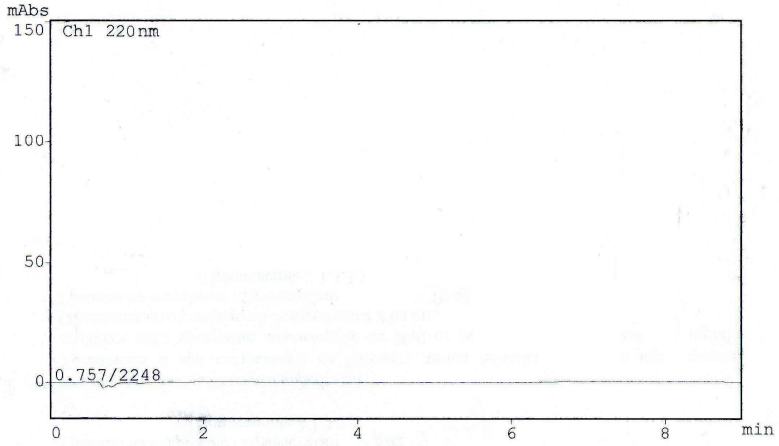

Figure 3: Chromatogram of Placebo

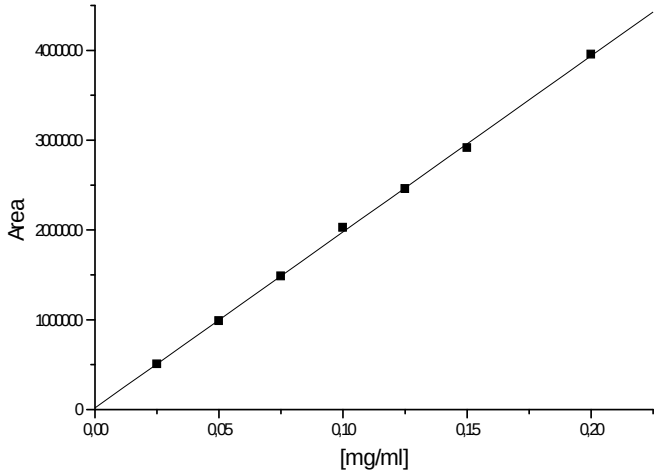

Figure 4: Linearity of Captopril

CONCLUSION

The aim of the present research work was to achieve highest precision in quantitative estimation of Captopril in tablet dosage form. The method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification. The developed method has a simple procedure for the preparation of the samples, shorter run time for chromatographic analysis (less than 10 min). Hence the proposed RP-HPLC method can be considered as simple, rapid, suitable and easy to apply for routine analysis of Captopril in pharmaceutical dosage form.

REFERENCES

[1] Skeggs LT, Kahn JE and Shumway NP, The preparation and function of the hypertension-converting enzyme, J. Exp. Med., 103 (3), 1957, 295-299.

[2] Williams GH, Hypertensive vascular disease. In: Braunwald, Fauci, Kasper et al 'editors'. Harrison's Principles of Internal Medicine, 15th Ed., United States of America: McGraw-Hill 2001, 1414-1430.

[3] Laragh JH, Baer L, Brunner HR, Buhler FR, Sealey JE and Vaughan ED Jr, Renin, angiotensin, aldosterone system in pathogenesis and management of hypertensive vascular disease, Am. J. Med., 52 (5), 1972, 633-652.

[4] Ketnawa S and Rawdkuen S, Enzymatic Method for ACE Inhibitory Peptide Derived from Aquatic Resources: A Review, Int. J. Biol. Pharm. Allied Sci., 2 (2), 2013, 469-494.

[5] Jackson EK, Renin and angiotensin, In: Hardman JG, Limbird LE 'editors', Goodman and Gilman's, The pharmacological basis of therapeutics, 10th Ed., Newyork: McGraw- Hill, 2001, 809-841.

[6] Karlicek R and Solich P, Flow-injection spectrophotometric determination of captopril using on-line solid phase extraction, Pharmazie, 53 (1), 1998, 549-553.

[7] Carlucci EG, Giuseppe ED and Mazzeo Y, Simultaneous determination of enalapril maleate and hydrochlorothiazide in tablets by derivative UV spectrophotometry and high-performance liquid chromatography, Int. J. Pharm., 93 (1-3), 1993, 245-248.

[8] Moldovan Z, A Spectrophotometric Method for Captopril Determination by Using Fluorescein Natrium-Bromine System, Rev. Roum. Chim., 57 (7-8), 2012, 721-727.

[9] Maslarska V and Tencheva J, Development of Analytical Method for Determination of Lisinopril Tablets Using RP-HPLC Method, Int. J. Pharm. Bio. Sci., 4 (4), 2013, 163-167.

[10] Naveed S, Sultana N and Arayne MS, HPLC-UV Method for the Determination of Enalapril in Bulk, Pharmaceutical Formulations and Serum, J. Anal. Bioanal. Techniques, 3 (2), 2012, 130-134.

[11] Russell J, McKeown JA, Hensman C, Smith WE and Reglinski J, HPLC determination of biologically active thiols using pre-column derivatisation with 5,5'-dithio-(bis-2-nitrobenzoic acid), J. Pharm. Biomed. Ana., 15 (11), 1997, 1757-1763.

[12] Cheung HS, Wang FL, Ondetti MA, Sabo EF and Cushman DW, Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. Importance of the COOH-terminal dipeptide sequence, J. Biol. Chem., 255, 1980, 401-407.

[13] Kang DG, Yun YG, Ryoo JH and Lee HS, Antihypertensive effect of water extract of Danshen on renovascular hypertension through inhibition of the renin angiotensin system, Am. J. Chin. Med,, 30 (1), 2002, 87-93.

[14] Watanabe T, Mazumder TK, Nagai S, Tsuji K and Terable S, Analysis method of the angiotensin-I converting enzyme inhibitory activity based on micellar electrokinetic chromatography, Anal. Sci., 19 (1), 2003, 159-161.

[15] Van Elswijk DA, Diefenbach O, van den Berg S, Irth H, Tjaden UR and Van Der Greef J, Rapid detection and identification of angiotensin-converting enzyme inhibitors by on-line liquid chromatography-biochemical detection, coupled to electrospray mass spectrometry, J. Chromatogr. A, 1020 (1), 2003, 45-58.

[16] Maslarska V and Tencheva J, A Validated RP HPLC Method for Simultaneous Estimation of Enalapril Maleate and Hydrochlorothiazide in Combined Dosage Forms, Int. J. Adv. Pharm. Res., 4 (4), 2013, 1629-1634.

[17] Stolarczyk M, Masalanka A, Krzek J and Milczarek J, Application of derivative Spectrophotometry for Determination of Enalapril, Hydrochlorothiazide and Valsartan in Complex Pharmaceutical

Preparations, Acta Poloniae Pharm. Drug Res., 65 (3), 2008, 275-281.

[18] Maslarska V, Peikova L and Tsvetkova B, RP-HPLC Method for the Simultaneous Determination of Lisinopril and Hydrochlorothiazide in Pharmaceutical Formulation, Int. J. Pharm. Sci. Rev. Res., 22 (1), 2013, 253-256.

[19] Tsvetkova B and Peikova L, Development and validation of RP-HPLC method for simultaneous determination of Amlodipine Besylate and Hydrochlorothiazide in pharmaceutical dosage forms by high-performance liquid chromatography, J. Chem. Pharm. Res., 5 (1), 2013, 271-275.

[20] Peikova L, Tsvetkova B,Kostova B and Rachev D, Investigations and HPLC assay of model formulations containing amlodipine besylate and lisinopril, Int. J. Pharmaceut. Sci. Rev. and Res., 20 (1), 2013, 11-15

[21] Chromatographic methods in pharmaceutical analisis, L. Peikova, B. Tsvetkova Macros, 2013

[22] Hillaert S, De Grauwe K and Van den BW, Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis, J. Chromatogr. A, 924, 2001, 439-449.

[23] Ito T, Matsuki Y, Kurihara H and Nambara T, Sensitive method for determination of captopril in biological fluids by gas chromatography-mass spectrometry, J. Chrom., 417 (1), 1987, 79-87.

[24] Huang T, He Z, Yang B, Shao L and Zheng X, Simultaneous determination of captopril and hydrochlorothiazide in human plasma by reversephase HPLC from linear gradient elution, J. Pharm. Biomed. Anal., 41 (2), 2006, 644-648.

[25] Mirza T and Tan HS, Determination of captopril in pharmaceutical tablets by anion-exchange HPLC using indirect photometric detection; a study in systematic method development, J. Pharm. Biomed. Anal., 25 (1), 2001, 39-52.

[26] Pérez-Ruiz T, Martínez-Lozano C and Galera R, Development and validation of a capillary electrophoresis method with laser-induced fluorescence detection for the determination of captopril in human urine and pharmaceutical

preparations, Electrophoresis, 27 (12), 2006, 2310-2316.

[27] Palomeque ME, Band BS, Flow injection biamperometric determination of captopril, J. Pharm. Biomed. Anal., 30 (3), 2002, 547-552.

[28] Mohamed EM, Hassan YAE and Elrasheed AG, Potentiometric and Visual Titrimetric Methods for Analysis of Captopril and Its Pharmaceutical Forms, Anal. Lett., 16 (1), 1983, 45-55.

[29] Ribeiro PRS, Santini AO, Pezza HR and Pezza L, Potentiometric determination of Captopril in pharmaceutical formulations, Eclet. Quim, 28 (1), 2003, 25-30.

[30] Khalilzadeh MA, Karimi-Maleh H, Amiri A, Gholami F and Mazhabi RM, Determination of captopril in patient human urine using ferrocenemonocarboxylic acid modified carbon nanotubes paste electrode, Chin. Chem. Lett., 21 (12), 2010, 1467-1470.

[31] Stefan RI, Van Staden JF and Aboul-Enein HY, An amperometric biosensors/SIA system for the simultaneous determination of S- and R-captopril, Biosensors and Bioelectronics, 15 (1-2), 2000, 1-5.