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Der Pharmacia Sinica, 2016, 7(4):1-6

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Validation of the RP-HPLC method for analysis of captopril in pharmaceutical tablets

B. Jebaslinhepzybai[1], C. Velmurugan[2] and A. Chenthilnathan[1*]

[1]*Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli – 627 012, Tamil Nadu, India*
[2]*Bafna Pharmaceuticals Limited, Madhavaram, Chennai – 600 060, Tamil Nadu, India*

ABSTRACT

A simple and sensitive reversed-phase liquid chromatographic method has been developed and validated for the analysis of Captopril in bulk and its tablet dosage form. The separation was carried out on LC1(C_{18}) coloum (250 × 4.6mm; 5µm) column at ambient temperature using methanol and water (55:45) as eluent. The flow rate was 1.0 ml/min and Captopril was quantified by absorbance at 220 nm. The retention time of Captopril was 7.20 min. The percentage recovery was within the range between 100.05 % and 100.79% for Captopril. The linear ranges were found in the range of 80µg/ml – 120µg/ml (r^2 = 0.999) of Captopril. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the proposed method could be successfully employed for routine analysis of Captopril in pharmaceutical formulations according to ICH guidelines.

Keywords: Captopril, RP-HPLC, Tablets, Analysis

INTRODUCTION

Captopril (Fig.1), chemically (2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure[1]. 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 rennin -angiotensin system and simulating action on kallikerin –kinin [2].

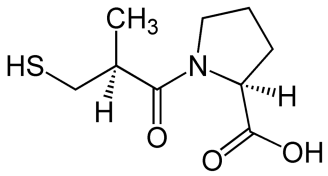

Figure 1: Structure of Captopril

Literature survey revealed that some methods had been developed for determination of Captopril by HPLC [3-5] and Spectrophotometric method [6-8]. 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. Therefore, this study focused on the development of simple and rapid RP- HPLC method which can be employed for the routine analysis of captopril in bulk drug and pharmaceutical formulations.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Methanol of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Captopril was a gift sample by Bafna Pharmaceuticals Ltd., Chennai – 600 060, Tamil Nadu, India. The commercially available tablets containing Captopril were procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu 1100 Series, Germany) with UV-Visible dual absorbance detector (PDA), LC1(C_{18}) (250 × 4.6mm; 5µm). The mobile phase consisting of methanol and water was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 55:45 v/v was pumped into the column at a flow rate of 1.0 ml/min. The detection was monitored at 220nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

50 mg of Captopril working standard was weighed accurately and transferred carefully in 50 ml volumetric flask. About 25 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5ml of above solution was diluted to 50 ml with mobile phase. The resulting solution was mixed and filtered through 0.45 µm filter.

Analysis of Sample Preparation

Twenty tablets containing Captopril were accurately weighed and crushed to fine powder using a glass mortar and pestle. A portion of the power equivalent to about 50 mg of Captopril was weighed and transferred to 50 ml volumetric flask. 30 ml of mobile phase was then added and sonicated to dissolve the power completely and the volume was made up with mobile phase. 5 ml of the above stock solution was taken in a 50 ml volumetric flask and diluted up to the mark with mobile phase. Finally the solution was mixed well and filtered through 0.45 µm filter.

Procedure: About 20 µl each of the test and the standard solutions were injected separately into the chromatograph and the chromatograms were recorded and the responses for the major peaks were then measured. The quantity of Captopril in mg/ tablet was calculated by using the formula:

$$\frac{\text{Test area}}{\text{Std. area}} \times \frac{\text{Std. weight in mg}}{50} \times \frac{5}{50} \times \frac{50}{TW} \times \frac{5}{100} \times P \times \text{Average weight in mg}$$

Where, P= Purity of Captopril working reference standard.

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [9].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Six injections of standard drug solutions were given separately to the system. The system suitability parameters such as retention time, peak area response, number of theoretical plates, tailing factor and their respective mean, standard deviation & %RSD were calculated for the standard drug solutions and mentioned in Table 1. It was observed that all the values are within the limits.

Table 1: System suitability for Captopril

S.No.	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	7.200	1792273	4670	1.074
2.	Standard -2	7.198	1792180	4763	1.081
3.	Standard -3	7.196	1800708	4708	1.086
4.	Standard -4	7.195	1801812	4630	1.088
5.	Standard -5	7.195	1796809	4606	1.086
6.	Standard -6	7.194	1800663	4601	1.088
Mean		**7.196**	**1797407**	**4663**	**1.083**
Standard deviation		**0.002**	**4357.5**	**63.64**	**0.005**
RSD in %		**0.03**	**0.24**	**1.36**	**0.50**

Specificity

The specificity of the HPLC method is illustrated in Fig. 2, where complete separation of Captopril was noticed in presence of other inactive excipients used in tablet dosage form. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 2.

Table 2: Specificity for Captopril

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1792273
4.	Sample	1	1823878

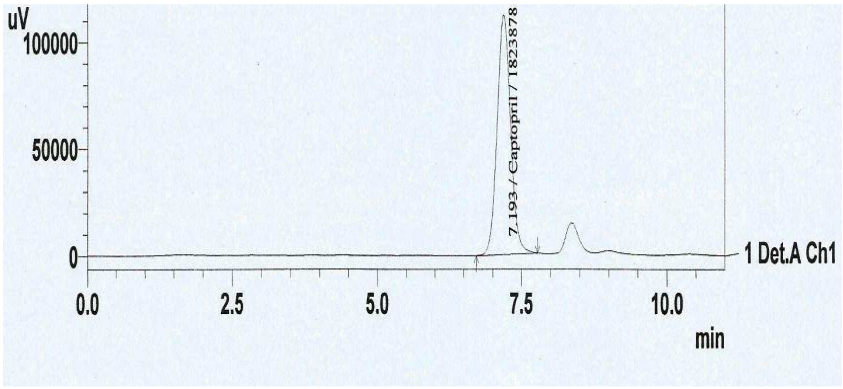

Figure 2 : Typical HPLC Chromatogram of Captopril Tablets

Linearity and Range

The Linearity of this method was determined at five levels from 80%– 120% of operating concentrations for Captopril and it was shown in Table 3. The plot of peak area of each sample against respective concentration of Captopril was found to be linear (Figure 3) in the range of 80%– 120% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 17728x + 57716 for Captopril and correlation coefficient of the standard curve was found to be 0.999 for Captopril. It observed that correlation coefficient and regression analysis are with in the limits.

Table 3: Linearity of response for Captopril

S.No	Linearity Level (%)	Concentration (µg/ml)	Area**
1.	80	80	1476197
2.	90	90	1652374
3.	100*	100	1834120
4.	110	110	2002343
5.	120	120	2187620

** Operating concentration*
***Mean area of three replicate injections*

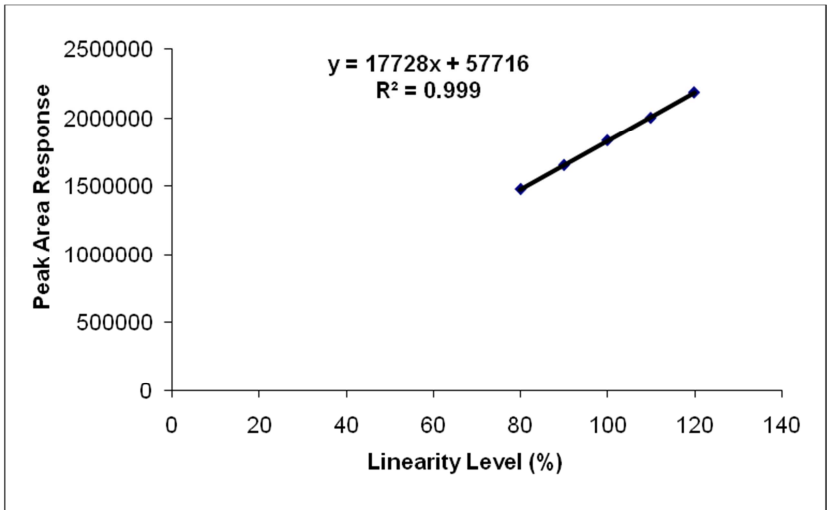

Figure 3: Linearity of response for Captopril

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, Captopril was added to pre-analyzed samples at a level from 50% up to 150% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recovery was found to be for Captopril which demonstrated that the method was highly accurate.

Table 4: Accuracy for Captopril

S.No.	Spike Level (%)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
1.	50	2.44	2.453	100.534
2.	50	2.44	2.455	100.602
3.	50	2.44	2.451	100.467
4.	100	4.88	4.963	101.706
5.	100	4.88	4.968	101.799
6.	100	4.88	4.954	101.514
7.	150	7.32	7.324	100.050
8.	150	7.32	7.330	100.131
9.	150	7.32	7.334	100.196
Mean				**100.77**
Standard deviation				**0.6999**
RSD in %				**0.69**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Captopril was shown in Table 5. This indicated that method was highly precise.

Table 5: Precision - Reproducibility for Captopril

S.No.	Name	Amount (µg/ml)	Area	Drug Content (%)
1.	Standard -1	100	1796623	97.757
2.	Standard -2	100	1799619	97.920
3.	Standard -3	100	1801255	98.009
4.	Standard -4	100	1802614	98.083
5.	Standard -5	100	1802989	98.104
6.	Standard -6	100	1802681	98.087
Mean				**97.993**
Standard deviation				**0.134**
RSD in %				**0.14**

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Captopril was shown in Table 6. This indicated that method was highly precise.

Table 6: Precision - Repeatability for Captopril

S.No.	Name	Amount of preparation (mg)	Area	Average area	Drug Content (%)
1.	Sample -1	228.6	1832113	1831837	99.689
2.	Sample -2	228.6	1831564		
3.	Sample -3	228.6	1831835		
4.	Sample -4	227.7	1828635	1826387	99.785
5.	Sample -5	227.7	1821365		
6.	Sample -6	227.7	1829162		
7.	Sample -7	231.1	1843090	1843573	99.242
8.	Sample-8	231.1	1844002		
9.	Sample-9	231.1	1843627		
Mean					**99.57**
Standard deviation					**0.2897**
RSD in %					**0.29**

Robustness

Robustness of the above method was carried out by purposefully varying some chromatographic method parameters. These parameters include changes in the flow rate (0.8 ml and 1.2 ml) and the composition of mobile phase, methanol: water (54.5:45.5 and 55.5:44.5). The results obtained by changing these conditions were obtained in terms of % RSD values. The values % RSDs were given in Table 7. These values were within acceptance a criterion which indicates that the developed method is robust.

Table 7: Robustness data for Captopril

Condition	Level	RSD in %
A: *Change in flow rate*		
0.8 ml/min	-2	0.041
1.0 ml/min	0	0.030
1.2 ml/min	+2	0.021
B: *Change in composition of mobile phase (Methanol: water)*		
54.5:45.5	-0.5	0.963
55:45	0	0.102
55.5:44.5	+0.5	0.015

Ruggedness

Nine sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The Ruggedness data for Captopril was shown in Table 8. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was rugged.

Table 8: Ruggedness data for Captopril -Change of analyst

S.No.	Sample Name	Wt. taken (mg)	Area	Average area	Drug Content (%)
1.	Sample -1	224.9	1823445	1820774	99.863
2.	Sample -2	224.9	1815121		
3.	Sample -3	224.9	1823755		
4.	Sample -4	225.4	1818083	1822870	99.756
5.	Sample -5	225.4	1821365		
6.	Sample -6	225.4	1829162		
7.	Sample -7	224.9	1821195	1822786	99.973
8.	Sample-8	224.9	1824759		
9.	Sample-9	224.9	1822404		
Mean					**99.864**
Standard deviation					**0.108**
RSD in %					**0.11**

Stability

Standard and sample solutions used in the analytical method were scrutinized for their stability. This study was performed by injecting standard and sample solution for the period of 48 hours and the results were presented in Table 9 and 10 respectively. It was found that there were no marked changes in the system suitability parameters.

Table 9: Stability data for Standard solution, Captopril

S.No.	Time point (hrs)	Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	0	7.200	1792273	4670	1.074
2.	12	7.200	1792273	4669	1.074
3.		7.198	1792180	4763	1.081
4.		7.196	1800708	4707	1.086
5.	24	7.195	1801812	4630	1.088
6.		7.195	1796809	4605	1.086
7.		7.194	1800663	4600	1.088
8.	48	7.200	1793921	4666	1.075
9.		7.198	1786009	4768	1.078
10.		7.196	1799322	4709	1.086
Mean		**7.192**	**1795597**	**4678**	**1.081**
Standard deviation		**0.002**	**5101**	**58.81**	**0.005**
RSD in %		**0.03**	**0.28**	**1.26**	**0.54**

Table 10: Stability data for Sample solution, Captopril tablets

S.No.	Time point (hrs)	Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	0	7.196	1823102	4561	1.084
2.	12	7.196	1825469	4559	1.085
3.		7.192	1821507	4579	1.086
4.		7.192	1825947	4566	1.085
5.	24	7.192	1843274	4551	1.086
6.		7.193	1823471	4532	1.078
7.		7.193	1825316	4561	1.077
8.	48	7.196	1819229	4564	1.082
9.		7.196	1821224	4579	1.086
10.		7.192	1828966	4563	1.086
Mean		**7.193**	**1825750**	**4561**	**1.083**
Standard deviation		**0.001**	**6757**	**13.45**	**0.003**
RSD in %		**0.03**	**0.37**	**0.29**	**0.31**

CONCLUSION

The proposed study describes a new, economical and simple reversed phase - HPLC method for the analysis of Captopril in pharmaceutical tablet dosage form. The method was validated as per ICH guidelines and found to be linear, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of Captopril in pharmaceutical dosage form without interference.

Acknowledgements

The authors are thankful to the management of Bafna Pharmaceuticals ltd., Madhavaram, Chennai – 600 060, Tamil Nadu, India for providing the necessary facilities to carry out for the research work.

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