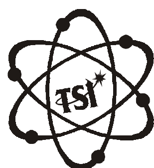

Trade Science Inc.

October 2006

Volume 3 Issues 2-3

Analytical CHEMISTRY

An Indian Journal

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ACAIJ, 3(2-3), 2006 [78-83]

A Stability Indicating Assay Method For Captopril Tablets By High Performance Liquid Chromatography For Stability Studies

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Received: 26th August, 2006
Accepted: 31st August, 2006

Web Publication Date : 11th October, 2006

ABSTRACT

A simple and stability indicating HPLC assay procedure had been developed and validated for captopril tablets stability samples. The HPLC conditions were as follows, column: Luna C8, 5μm packing, 4.6 mm x 250 mm: UV at 220 nm: injection volume:20 μl: mobile phase water : acetonitrile : tetrahydrofuran :methane sulfonic acid in the ratio of (80:10:10:0.1);isocratic elution under ambient condition at flow rate of 1.0 ml min^{1}. The procedure separated captopril and potential degradation product captopril disulphide. The retention time of captopril is 6.4 min and asymmetry of 0.99. Whereas pharmacopical and various available methods shows captopril around 3.0 min(dead volume) and asymmetry about 2.0. The instrument precision obtained was 0.61 %.The procedure provided a linear response in the range of 25 – 75 μg/ml (r = 1.000). Forced degradation study shows, response of main drug is reduced in acid, alkali and peroxide degradation. The method was validated for accuracy, robustness and solution stability was obtained up to 19 hrs. © 2006 Trade Science Inc. - INDIA

KEYWORDS

Captopril;
Cardiovascular drug;
Force degradation;
Validation;
Solution stability;
Stability sampels.

INTRODUCTION

Captopril is angiotension converting enzyme inhibitor, used in the treatment of hypertension and congestive cardiac failure[1]. Captopril is chemically (1-(3-Mercapto-2 methyl-1-oxopropyle-) L-proline(Figure 1). Literature survey reveals that it is official in U.S.P[2], B.P[3] and several techniques using fluorescence detector[4], spectrophotometry[5], derivatisation [6] and gas chromatography[7] have been

reported for estimation of captopril in pharmaceutical formulation and also in biological samples. In USP titremetry method is suggested for captopril tablets, whereas in BP the HPLC method for captopril tablets shows asymmetry of more than 2.0 and retention time of 3.0 min which is very early, either with or very close to the solvent front, this renders quantitation difficult specially for stability samples. Other reported HPLC methods[8] also have same problems.

Hence to overcome the problem for estimation of captopril in stability samples (as titremetry method suggested in USP is not stability indicating and in BP method the early retention of main drug and degradation products makes difficult to quantify captopril tablets in stability samples). In the present study we reported a simple, rapid and accurate stability-indicating HPLC assay procedure that can quantitate captopril tablets in stability samples and identify's its main degradation product simultaneously having a good peak shape with an asymmetry factor of 0.99.

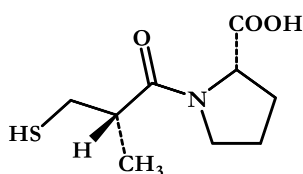

Figure.1: Chemical structure of captopril

EXPERIMENTAL

The separation was carried out under isocratic condition with mobile phase prepared by mixing water:acetonitrile:tetrahydrofuran:methane sulfonic acid in the ratio of (80:10:10:0.1) at a flow rate of 1.0 ml min^1 with UV detection at 220 nm. The column temperature was ambient and an injecton volume of 20μl was used. A Luna (Phenomenex,U.K) C8 column, 5μ, 250 X 4.6 mm was used.A working standard solution containing 50 μg ml 1 captopril was prepared by dissolving captopril reference standard in mobile phase.

A blend of captopril tablets equivalent to 5 mg of captopril is transferred to 100 ml volumetric flask. 20 ml of mobile phase was added and sonicated for 5 minutes with immediate shaking and diluted with mobile phase to volume and mix. This solution was centrifuged at about 2000 RPM for 10 minute, and upper clear solution was used for injection.

Method validation was performed as per USP 27-NF22[9]. The following validation parameters were addressed: specificity, precision, linearity, accuracy and solution stability of captopril in mobile phase.

Specificity

Stress testing of the drug substance can help in identifying the likely degradation products, which in turn help's to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used[10]. Stress testing is done by exposing the captopril to following conditions.

Acid degradation: Drug was exposed to 2 ml, 2 M HCl and heated at 70°C for 1 hr. figure 2 A represents acid degradation.

Alkali degradation: Drug was exposed to 2 ml, 2 M NaOH and heated at 70°C for 1 hr. figure 2 B represents alkali degradation.

Peroxide degradation: Drug was exposed to 1 ml, 30% hydrogen peroxide and heated at 70°C for 1 hr. figure 2 C represents peroxide degradation

Thermal degradation: Drug was exposed to heat at 70°C for 1 hr. figure 2 D represents thermal degradation.

Sunlight degradation: Drug was exposed to sunlight for 2 hr. figure 2 E represents sunlight degradation.

The result obtained from degradation study shows peak purity of captopril is 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak.(Figure 2A-E) where as in all the degradation conditions except sunlight, a peak at retention time of 12.7 min is generated as major component, which is confirmed as captopril disulphide. when a mixture of captopril and captopril disulphide is co injected showing the same RRT as observed in degradation graphs.(Figure 3).

Precision

The system precision was determined by perform-

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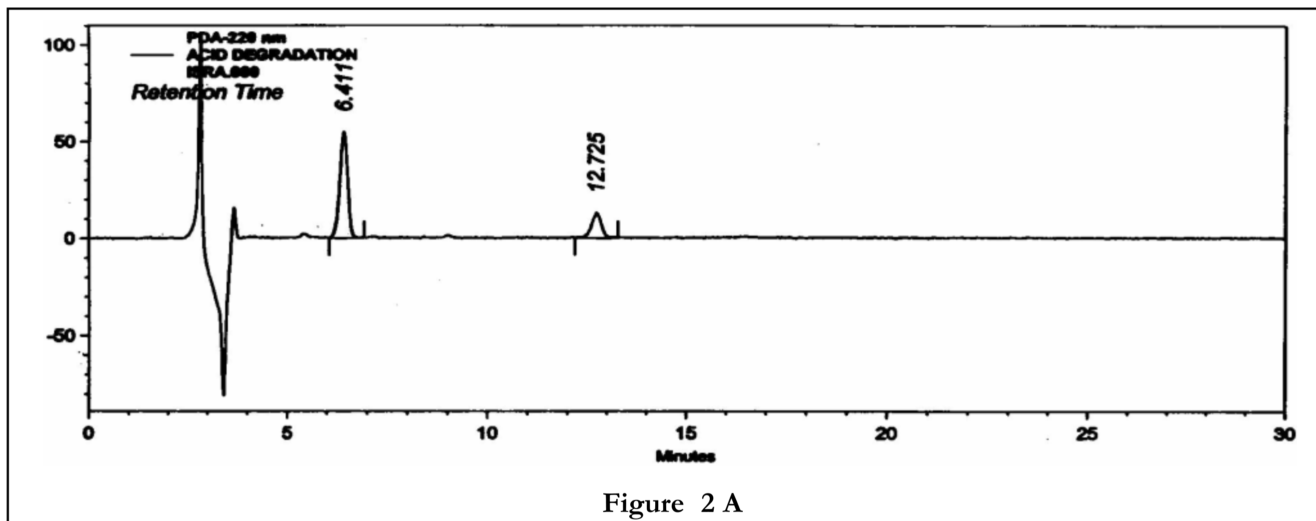

Figure 2 A

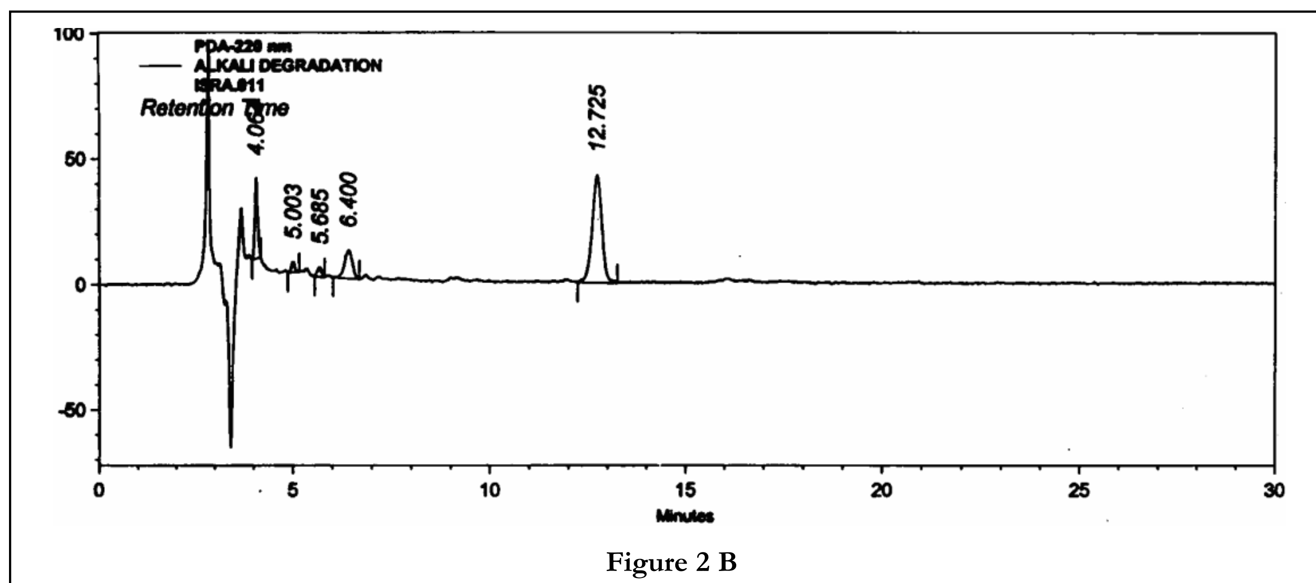

Figure 2 B

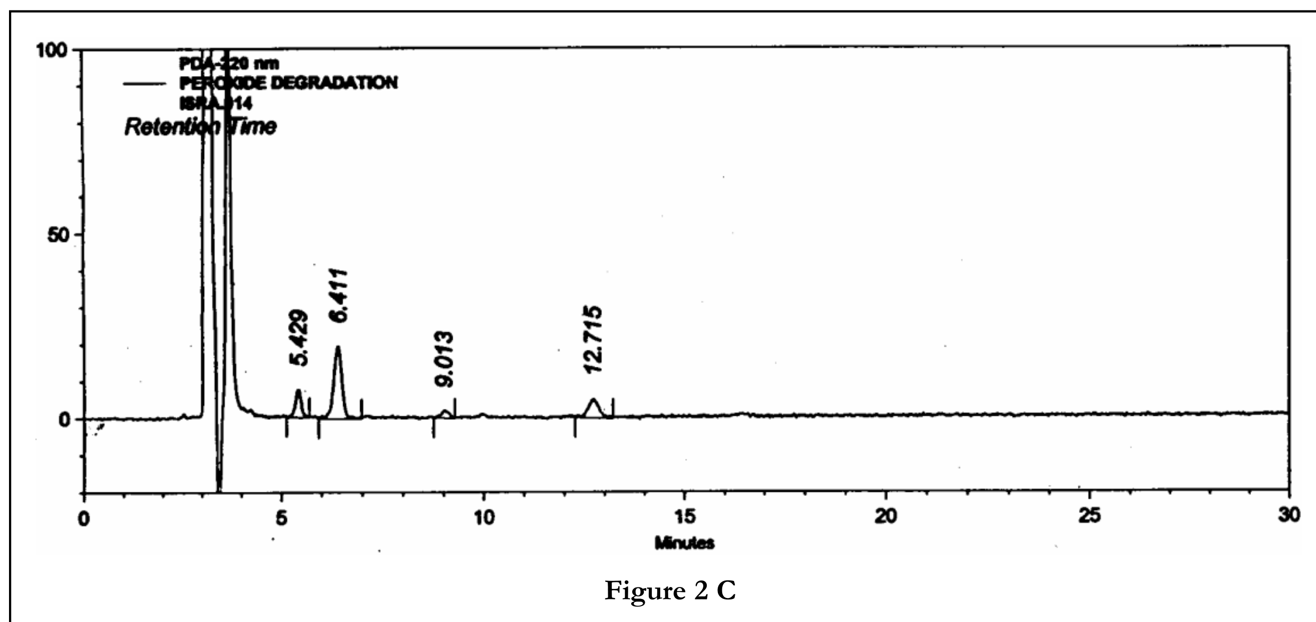

Figure 2 C

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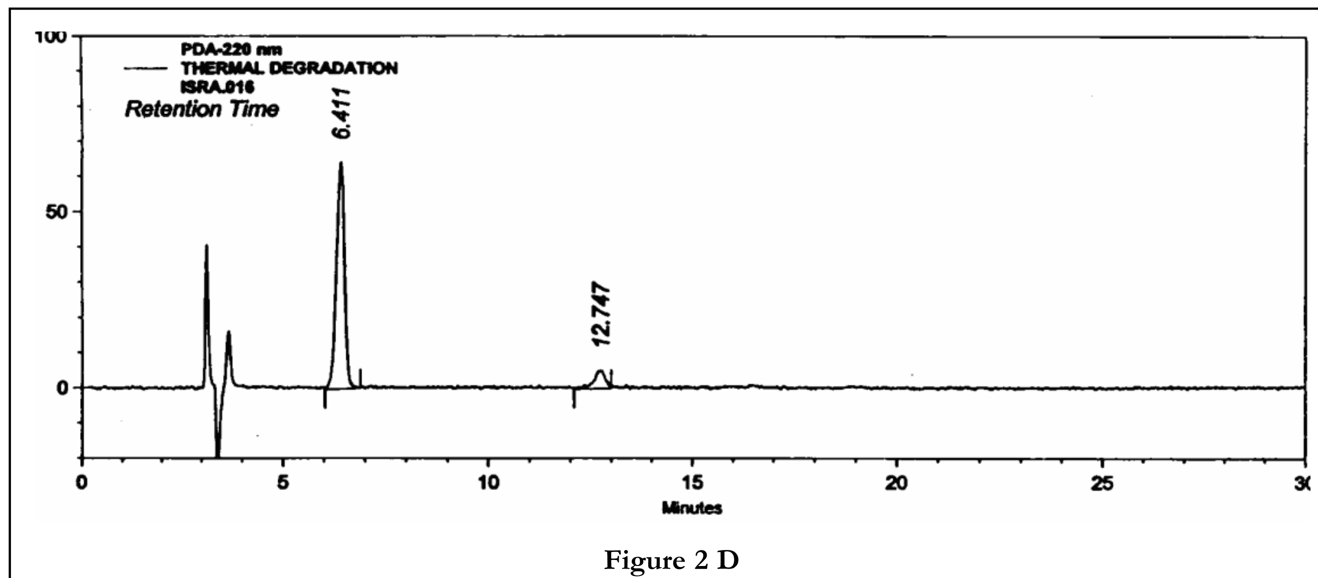

Figure 2 D

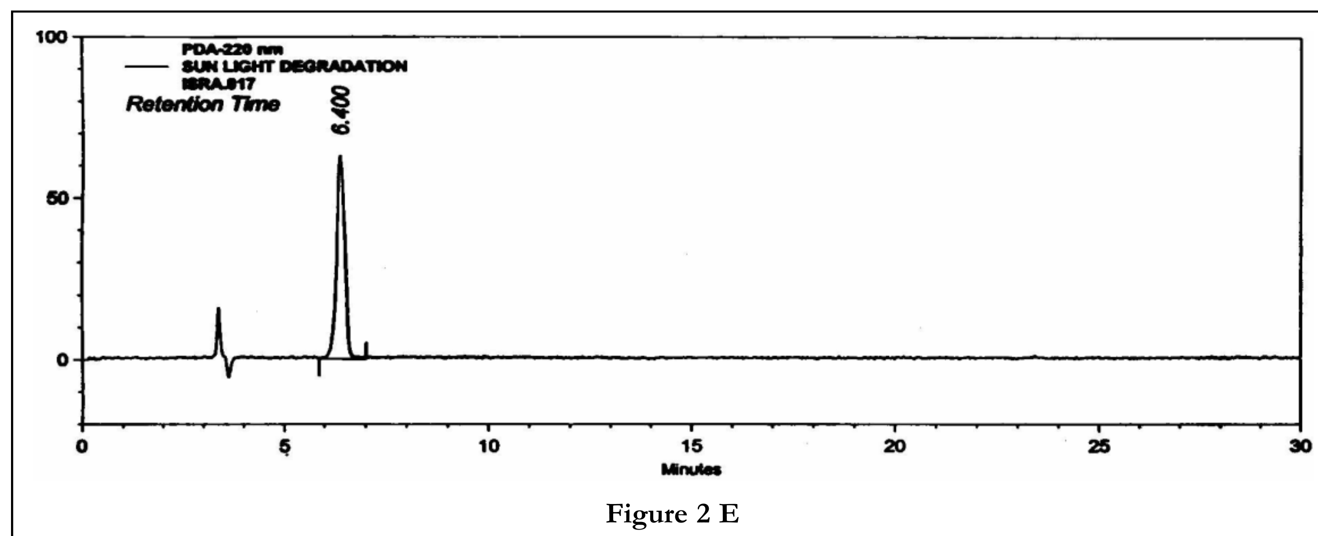

Figure 2 E

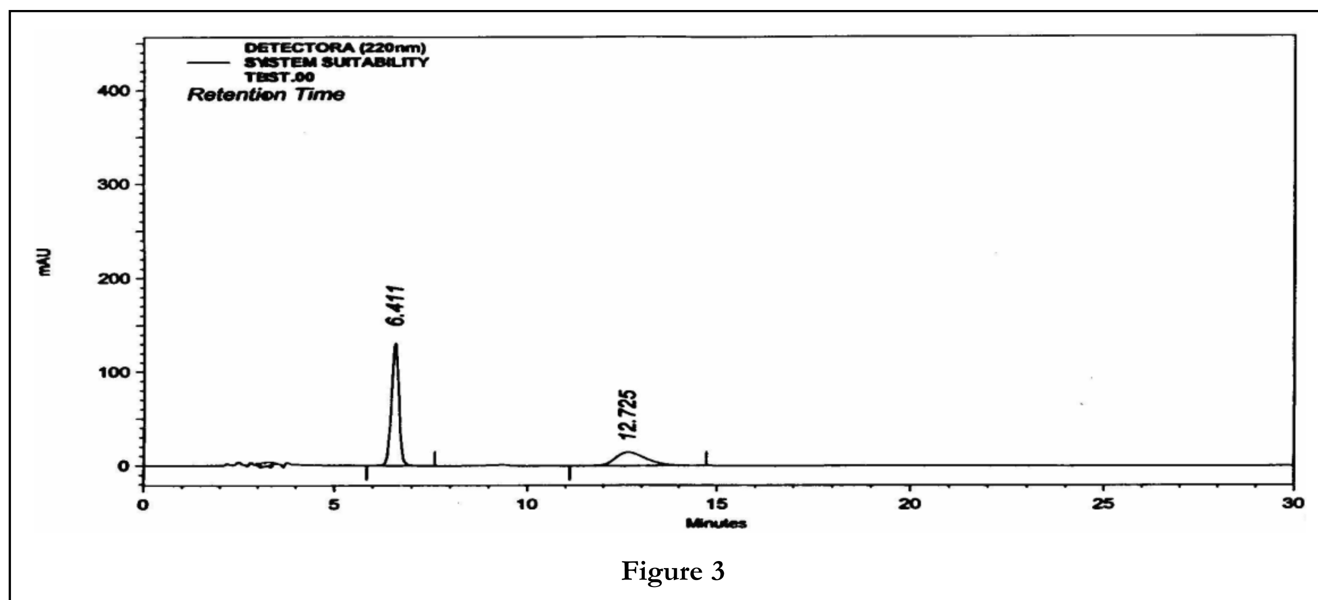

Figure 3

ing six replicate injections of standard solutions, the % RSD of 0.61 is obtained. The method precision was determined by performing six consecutive assays of captopril by preparing six independent samples, the % label claim of 100.19 is obtained. (TABLE-1).

Accuracy

The accuracy was evaluated by the recovery of captopril at three different levels (80,100 and 120%) using three preparations for each level tested three times. The mean recovery data for each level is within accepted values (99.93, 99.17, and 99.20 respectively). Therefore, these results indicated a good accuracy of the method for captopril. The mean recovery is 99.43 % (TABLE-1) and % RSD is 0.43.

Linearity

The linearity of detector response for captopril standard was determined by preparing and injecting solutions in the concentration range of 25 – 75 μg/ml (50-150 % of assay conc.) of captopril standard. A calibration curve was constructed using characteristic parameters for regression equation (y=a+bx) of the HPLC method obtained by least squares treatment of the results confirmed the good linearity of the method developed(TABLE-1).

Robustness

The robustness study helps us in demonstrating that transferring the methodology can be done successfully or not. In this study we had compared the results between normal operating conditions and by deliberately changing certain parameters like changing analyst, instrument, column(Inertsil, C 8,250 *4.6 mm,5μ), flow rate(1.1 ml/min).The result obtained shows that by changing deliberately some internal and external parameters of the method does not influence the results obtained.(TABLE-1).

Solution stability

The solution stability study was performed by injecting a standard solution in duplicate at different time intervals, the peak areas were compared with the initial areas, it was found that there was no significant changes in the peak areas up to 19 hrs. Hence the solution is not needed to be injected immediately (TABLE 1).

TABLE 1: Summary of the performance parameters of the HPLC procedure for captopril tablets

Sr. no.	Parameters	Observed value
1.	System suitability	
	a. Theoretical plates	5400
	b. Tailing Factor	0.99
2.	Instrument Precision	RSD 0.61%
3.	Method Precision	Label Claim 100.19%
4.	Linearity and range	Correlation coefficient(r) = 1.0000
5.	Accuracy	Mean recovery 99.43%
6.	Specificity	Peak Purity of captopril peak after degradation is 100%.
7.	Robustness	Difference from original condition 0.44%
8.	Solution stability	19 hrs

RESULT AND DISCUSSION

Captopril is eluted either with or very close to dead volume in all cases when we use previously reported stability indicating HPLC assay procedure. The method reported in B.P 2005 shows peak in dead volume and having tailing factor more than 2.0 which makes quatitation difficult in stability samples. Our effort to modify the procedure by changing ratio of organic phase in mobile phase and column temperature did not improve the results.

The previous procedures mention uses of C18 columns. When we changed to C8 column to reduce hydrophobicity of the column, to increase retention of protonated captopril, it had increased the retention time but still the peak shape is not improved hence we had used ion-pairing reagent methane sulphonic acid. We had chosen methane sulphonic acid and not higher alkyl chain as we were comfortable with retention time only to get sharp peaks to reduce asymmetry factor ion pairing reagent is used.

CONCLUSION

A stability-indicating, rapid and reliable HPLC assay method was developed for the assay of captopril tablets useful for long term and short term

stability samples. This chromatographic assay fulfilled all the requirements such as specificity, precision, accuracy, linearity, robustness and solution stability upto 19 hrs. The peak shape obtained though out the study shows asymmetry of 0.99 as well as the retention time of the captopril is 6.4(±0.1) min, showing a good column life and fast analysis of large numbers of samples in short time period, therefore this method is suitable for routine sample analysis and preferably for samples with short term stability and long term stability studies.

SUPPLEMENTARY INFORMATIONS

The HPLC system used for this study is shimadzu LC-10ATvp solvent delivery pump with SIL-10Avp autoinjector, shimadzu SPD –M10Avp detector (photo diode array), Pentium 4 computer with class VP data integrating software HPLC grade acetonitrile and tetra hydro furan is purchased from Merck India.Analytical grade methane sulfonic acid from National chemicals Ltd.,High quality pure water was prepared by using Millipore milli Q Plus purification system. Captopril formulation was purchased from Wockhard Ltd, India.

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