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Stability indicating RP-HPLC method for determination of Trandolapril (TP) in pure and pharmaceutical formulation

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Abstract: A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of TP in pure and tablet forms. The method showed a linear response for concentrations in the range of 20-60 µg/mL using Acetonitrile: Phosphate Buffer solution in the ratio (60:4 0) as the mobile phase with detection at 210 nm and a flow rate of 0.8 mL/min and retention time 2.645 min. The value of correlation coefficient, slope and intercept were, 0.999, 48442.82 and 69569.6, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability indicating one.

Keywords: TP, RP-HPLC, Degradation studies.

Introduction

Trandolapril is chemically (2S, 3aR, 7aS) – 1 - [(S) – 2 -[[1 – Ethoxy carbonyl – 3 - phenylpropyl] amino] propanoyl] octahydro -1 H – indole – 2 - carboxylic acid. It is a potent non-sulfhydryl and dicarboxyl containing angiotensin converting inhibitor. Trandolapril is a mono ester prodrug and hydrolysed by esterases to its active dicarboxylic acid metabolite namely, trandolaprilat. The structures for both trandolapril and trandolaprilat are shown in Figure 1. It is a white to off white crystalline, odourless powder which melts in the range of 130-135◦C [1].ACE is a peptidyl dipeptidase catalyzing the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Inhibition of conversion of the angiotensin I to the angiotensin II leads to a reduction in vasopressor activity and a decrease in peripheral vascular resistance [2, 3]. Trandolapril is approved for the management of hypertension, left ventricular systolic dysfunction and chronic heart failure [4]. Some of the undesirable effects very commonly reported for trandolapril include, dizziness, cough and headache.

Approximately 10% and 70% of oral dose of trandolapril is bioavailable as trandolapril and trandolaprilat respectively. Following absorption, oral trandolapril is rapidly and extensively hydrolysed to trandolaprilat. The mean *t*1/2of trandolapril is less than 1 h, and that of Trandolaprilat is, approximately, 75 h [5,6] .After single oral dose, the Cmax of trandolapril appeared to be dose proportional (0.83–0.86 ng/ml/mg) and occurred at 0.5–1 h. Trandolaprilat binding to human plasma proteins exceeds80%. The Cmax of trandolaprilat, after single oral doses,

was also dose proportional (1.40–1.92 ng/ml/mg) with a Tmax of 4–8 h [7].

A literarure survey shows various methods developed with different detectors for quantification of trandoalpril. The methods for determination of trandolapril has been carried out by analytical methods such as HPLC [8,9] by Gumieniczek and Hopkala,LC-Mass spectrophotometry[10,11] by Constantinos Pistos et. al.,HPTLC [12] by D. Kowalczuk group, radioimmunoassay, spectrophotometry and potentiometry. But all the previous methods used were quite costly and complicated. The aim of present work was to develop a simple, economical and rapid HPLC stability indicating method with better detection range for estimation of trandolapril in bulk and formulations. Developed method was validated as per ICH guidelines [13].

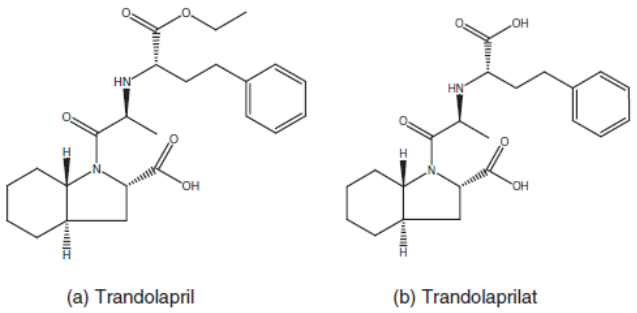

Figure 1: Structures of Trandolapril (a) and its active metabolite, Trandolaprilat (b)

EXPERIMENTAL

MATERIALS AND METHODS

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (100 mmx4.6mm; 3.5µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and Solvents: The reference sample trandolapril of was supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai.

Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer: Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethylamine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents 300 ml of the phosphate buffer was mixed with 700 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum.

Chromatographic conditions:

Mobile phase consist Conditions of ACN-Phosphate buffer in the ratio of70:30 at a flow rate of 08 ml/min and pH of buffer was adjusted to 3.0 UV detection was performed at 225nm. The mobile phase was degassed by an ultrasonic water bath for 5 min. Filter through 0.45µ filter under vacuum filtration. The column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of the Trandalopril Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10mg of Trandalopril Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the

mark with diluent. Mix well and filter through 0.45µm filter.

Sample Solution Preparation:

Weigh 5 Trandalopril Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Trandalopril into a 10 ml volumetric flask. Add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of calibration graph:

The linearity of response for Trandalopril assay method was determined by preparing and injecting solutions with concentrations of about 20,30,40,50,60µg/ml of Trandalopril.

Validation of the Proposed Method

After chromatographic method development and optimization it was validated. The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated according to ICH guidelines for linearity, precision, sensitivity, and recovery. For linearity studies, working standard solutions equivalent to 20 to 60 µg/ml of Trandalopril were prepared with the mobile phase.

Precision & Accuracy

According to ICH, precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions and may be considered at three levels: repeatability, intermediate precision and reproducibility. The intra-day and inter-day variations of the method were determined using five replicate injections and analyzed on the same day and different days.

The accuracy of an analytical method expresses the closeness between the theoretical value and experimental value. To ensure the reliability and accuracy of the method, the recovery studies were carried out to ensure the reliability and accuracy of the method. Accuracy was evaluated by injecting the Trandalopril about five times, at three different concentrations equivalent to 50, 100, and 150% of the active ingredient, by adding a known amount of Trandalopril standard to a sample of known concentration and calculating the recovery Trandalopril of for each concentration.

Detection and Quantification Limits

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (*S*) of the calibration plot using the formula LOD = 3.2 σ/*S* and LOQ =9.9 σ/*S*.

Specificity

The specificity test of proposed method demonstrated that excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

Assay

Twenty tablets were weighed and powdered equivalent to 10 mg Trandalopril of was accurately weighed and diluted up to 10 ml. Working dilution was prepared using same diluents and used for analysis.

Forced degradation studies

Acid Degradation:-

Accurately weigh and transfer 10mg Trandalopril of working standard into a 10mL volumetric flask add about 3 ml of 0.5N HCl and sonicated for 5minutes. Reflux under heat at 60 degrees in a

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heating mantle for 2 hours. Neutralize the sample solution using 0.5N NaOH and diluted up to the mark with diluents (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Base Degradation:

Accurately weigh and transfer 10mg of Trandalopril working standard into a 10ml volumetric flask add about 3ml of 0.5N NaOH and sonicated for 5 minutes. Reflux under heat at 60 degrees in a heating mantle for 2 hours. Neutralized the sample solution using 0.5N HCl and diluted up to the mark with diluents (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Thermo Degradation:

Accurately weigh and transfer 10mg of Trandalopril working standard into a 10mL volumetric flask and oven under heat at 105 degrees for 12 hours (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Trandalopril was found to be 2.645 min. Quantification was linear in the concentration range of 20-60µg/ml. The regression equation of the linearity plot of concentration of Trandalopril over its peak area was found to be Y=69569.6+48442X (r2=0.999), where X is the concentration of Trandalopril (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 2094.9, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.024µg/ml and 0.08 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 40:60v/v resulted in peak with good shape and resolution.

Calibration curve

These results indicate that the response is linear over the range of 20, 30, 40,50and 60µg/ml of Trandalopril with coefficient of regression, R2, value as 0.999 as shown in Table: 1. the value of correlation coefficient, slope and intercept were 0.999 48442.82 and 69569.6 respectively.

Table 1: Regression characteristics of the Trandalopril for proposed HPLC method

S. No.	Parameter	Result
1	Range (µg/ml)	20-60
2	Detection wavelength (λ max)	210
3	Mean **'R2'** value	0.999
4	Slope (m).	48442.82
5	Intercept (c)	69569.6
6	Run time(min)	5
7	Retention Time (min)	2.645
8	Theoretical Plates (N)	2094.9
9	Tailing Factor	1.5
10	LOD	0.024
11	LOQ	0.08

Validation of the proposed method

The specificity, linearity, precision, accuracy and limit of detection, limit of quantification, robustness and system suitability parameters were**:** studied systematically to validate the proposed HPLC method for the determination of the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing 40µg/ml

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of Trandalopril was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished. The Solution containing 40 µg/ml of Trandalopril was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2.

The accuracy of the HPLC method was assessed by concentrated levels by the proposed method. The results are furnished in Table-3.

Table 2: Precision of the proposed HPLC method

Concentration of Trandalopril (µg|ml)	Intra day	Inter day
Injection-1	1977823	2020950
Injection-2	2055023	2013520
Injection-3	2007013	2012320
Injection-4	2069758	2014048
Injection-5	2002317	2016497
Average	2022387	2015467
Standard Deviation	38516.9	3421.6
%RSD	1.90	0.17

Table 3: Accuracy studies

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean
50%	1028170	5.1	5.13	100. 0%	99.9 %
100%	1979502	9.9	9.88	99.8%	
150%	2977023	15.0	14.8	99.1%	

Estimation of Trandalopril in tablet dosage forms:

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate Trandalopril tablet formulations 5 tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Trandalopril was transferred into a 10ml volumetric flask and dissolved in 7ml of a40:60 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further amount of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing40µg/ml of as in Trandalopril injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in table-5.

Formulation	Label claim	Amount found	% Amount found
1	5	5.01	100.20
2	5	4.9	

Stability- Indicating property

The stress studies were conducted and the data were depicted in Table: 4 .the chromatogram of no stress treatment of control and sample showed no additional peaks (Figure: 2 & 3)

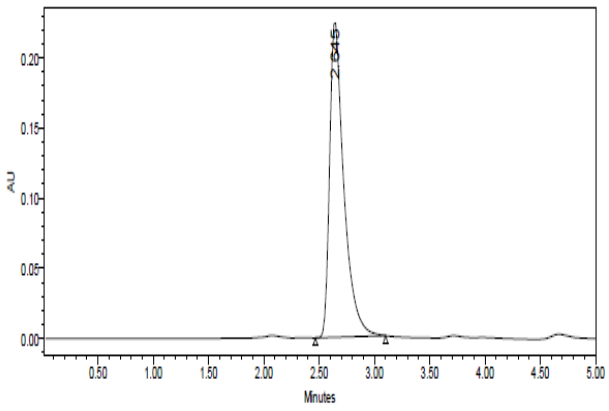

Figure 2: The simple chromatogram of standard Trandalopril

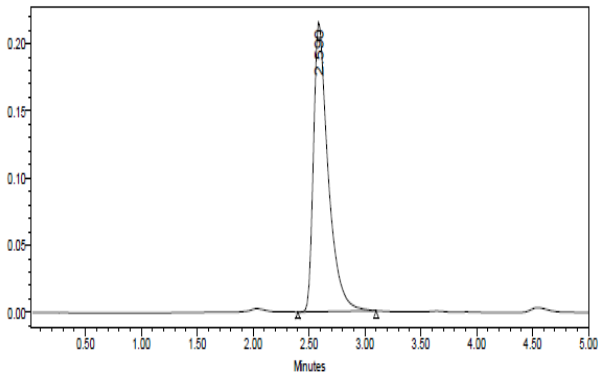

Figure 3: The simple chromatogram of test Trandalopril

The retention time (RT) of standard and sample were 2.287min & 2.318min .The chromatogram of acid degraded sample showed no additional peaks (fig: 4). The chromatogram of alkali degraded sample showed no additional peaks (fig: 5). The chromatogram of thermal degraded sample showed no additional peaks (fig: 6**).** and the values were shown in Table 4.

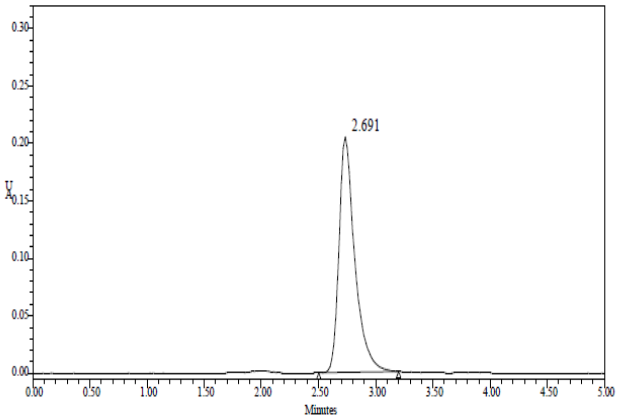

Fig 4: The chromatogram of no stress treatment sample

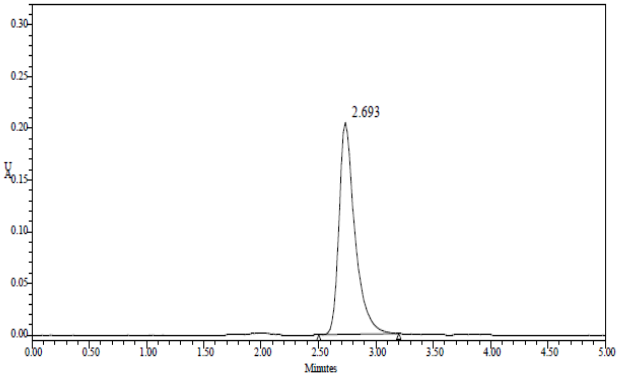

Fig. 5: The chromatogram of acid degraded sample

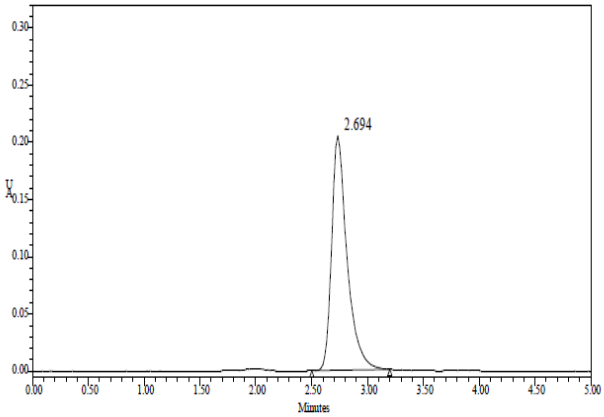

Fig. 6: The chromatogram of alkali degraded sample

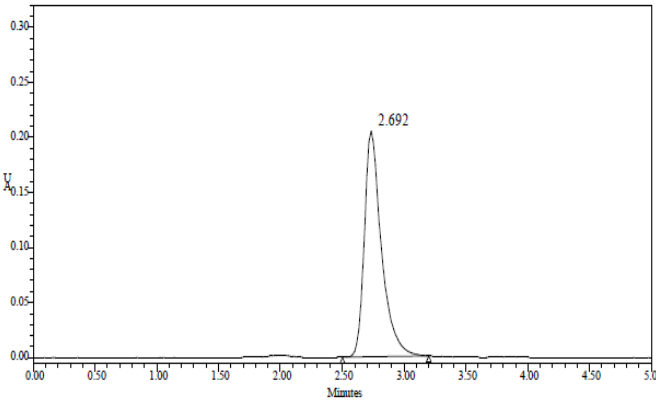

Fig. 6: The chromatogram of thermal degraded sample

Table 4: Stressed study data of Trandalopril

S. No	Condition	Time (hrs)	Assay of Trandalopril	Retention time of Trandalopril	% degradation
1	No stress treatment	-	101.48	2.691	Nil
2	Acid	2	-	-	Nil
3	Alkali	2	-	-	Nil
4	Thermal	12	-	-	Nil

Conclusion

The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis Trandalopril of as bulk drug and in

Pharmaceutical formulation without any interference from the excipients. This study is a

typical example of a stability-indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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