



OPTIMIZED AND VALIDATED REVERSE PHASE HPLC METHOD FOR THE DETERMINATION OF TRANDOLAPRIL IN BULK AND FORMULATIONS

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ABSTRACT

A simple, sensitive and accurate reverse phase liquid chromatographic method has been developed to determine the amount of active pharmaceutical ingredient trandolapril and applied for the analysis pharmaceutical formulation. Chromatographic determination is performed on a Hypersil gold C18(100mm, 4.6mm-ID; 5 μ m) with mobile phase consisting of 50% buffer and 50% acetonitrile v/v, and flow rate at 1.0 ml/min, keeping the column in thermostat at a constant temperature of 30^oC. The response of the instrument is recorded at maximum wavelength of absorption 215nm. The standard curve is linear over a concentration range of 25.0-150 μ g/ml ($r^2=0.9999$). The limit of detection and limit of quantification are calculated and found to be 1.149 and 3.832 μ g/ml. The developed method is validated statistically. The low percent of relative standard deviation (0.297) and high percent of recovery of the drug (99.78%-100.23) indicate that the developed method is precise and accurate.

Keywords: Trandolapril, Liquid Chromatography, Mobile Phase, Chromatogram, Limit of Detection, Assay.

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INTRODUCTION

Trandolapril is a colorless and crystalline solid soluble in chloroform, methanol and dichloromethane. It is available in the brand names Zetpril (Hetero HC (Genx)), Mavik (Abbott), Tarka (Abbott) Trandolaprilum (Halo). Molecular formula and molecular weight of the drug are C₂₄H₃₄N₂O₅ and 430.537 grams/mol respectively. The IUPAC name of the drug is (2S,3aR,7aS)-1-[(2S)-2-[[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2yl]amino]propanoyl]2,3,3a,4,5,6,7,7a octahydroindole-2-carboxylic acid. It is an ACE inhibitor used to treat high blood pressure. It is believed to exert its antihypertensive effect through the renin-angiotensin-aldosterone system. The effect of trandolapril in hypertension appears to result primarily from the inhibition of circulating and tissue ACE activity thereby reducing angiotensin II formation, decreasing vasoconstriction, decreasing aldosterone secretion, and increasing plasma renin. Decreased aldosterone secretion leads to diuresis, natriuresis, and a small increase of serum potassium. Literature survey reveals that several workers developed different chromatographic methods to determine the amount of trandolapril present human plasma, in bulk, dosage forms, and in combination with verapamil.

Bulk drug and pharmaceutical dosage forms of trandolapril are qualitatively and quantitatively analyzed¹⁻³ by reverse phase HPLC. The quantity of trandolapril present in combination with verapamil is determined by several workers⁴⁻⁵. Some workers⁶⁻⁸ developed a liquid chromatography/tandem mass spectrometry using solid phase extraction to determine the amount of trandolapril and its metabolite trandolaprilat in human plasma, the analytes are separated using isocratic mobile phase on a reversed phase column. Stability-indicating high-performance thin-layer chromatographic (HPTLC) method for analysis of trandolapril in pharmaceutical dosage forms has been reported in the literature⁹. But a little attention is paid on the analysis of trandolapril in pure form and present in formulations. The existing methods are costly, but the developed method is low cost and the time for the analysis is minimized.

EXPERIMENTAL

Instrumentation

Shimadzu LC- 20AT Prominence liquid chromatographic system is used for the analysis. Shimadzu LC-20AT system equipped with binary gradient pump, UV-VIS SPD 20A detector, Column Oven and controlled by Spinchrom software.

Materials and Reagents

All the Chemicals and reagents used in the analysis are of HPLC grade. HPLC grade water is used to prepare the mobile phase. Stock solutions of trandolapril and sample solutions are prepared in the mobile phase. Fresh working solutions are prepared daily. All solutions are filtered through 0.45 μ membrane filter and degassed using a sonicator.

Preparation of buffer solution of pH=2.8

About 0.544g of ammonium dihydrogen orthophosphate is accurately weighed, transferred into 500ml volumetric flask and dissolved in HPLC grade water, made up to the mark by adjusting the pH =2.8 by adding a few milliliters of o-phosphoric acid, filtered through 0.45 μ filter and degassed by sonication.

Preparation of mobile phase

Mobile phase is prepared by mixing accurately measured volumes of 250ml of buffer and 250 ml of acetonitrile in a 1000ml beaker, stirred well, filtered, sonicated and used for the analysis.

Preparation of standard drug solution

About 50.0mg of trandolapril is accurately weighed, transferred into a well cleaned, dried 50ml volumetric flask, 30ml of mobile phase is added and sonicated to dissolve the drug. The solution is made up to the mark with mobile phase. Working standard solution (100 μ g/ml) is prepared daily by accurately measuring 10.0ml of the stock solution into 100ml volumetric flask and made up to the mark with mobile phase.

Preparation of test solution

Fifty tablets of zetpril are powdered and mixed thoroughly. An amount of the powder equivalent to 50mg of the drug is dissolved in acetonitrile by sonication, and filtered through 0.45 μ filter. The filtrate is diluted to 100ml with mobile phase. The resulting solution is again sonicated and filtered through 0.45 μ filter and used for the analysis.

Method Development

The chromatographic conditions are fixed for the Shimadzu HPLC system. The mobile phase is allowed to pump from the mobile phase reservoir into the column Hypersil BDSC18(100mm, 4.6mm-ID; 3 μ m particle size) at a flow rate of 1.0 ml/min., keeping the column in thermostat at a temperature of 30^oC about 30minutes. The response of the system is recorded against time at maximum wavelength 215nm. Now 20 μ l of the standard solution of trandolapril of (100 μ g/ml) is injected into the system, the mobile phase is allowed to pass through the column for 20minutes and the chromatogram is recorded and is presented by Fig-1. The chromatogram consists single sharp peak, having tailing factor less than 2.0, number of theoretical plates greater than 2000 and retention time 6.00minutes.

RESULTS AND DISCUSSION

Working standard solution of trandolapril is injected into the HPLC system and the chromatogram is recorded. The system suitability parameters and system precision are evaluated based on the area of the peak. The USP tailing factor and number of theoretical plates are found to be 1.742 with 4272 respectively. In order to predict the linearity of detector response to the concentration of the drug a series of six different concentration solutions of trandolapril standard are prepared each in duplicate in the concentration range of about 25.0 μ g/ml to 150 μ g/ml corresponding to 25% to 150% of target concentration. Each solution is injected into the system and chromatograms are recorded under the standard chromatographic conditions. A graph is plotted to concentration in μ g/ml on X-axis versus mean response of the detector on Y-axis. The detector response is found to be linear (Fig.2). The slope, intercept of the straight line are found to be 19532 and 2671 respectively. The correlation coefficient

between the concentration of the drug and detector response is found to be 0.9999. The results are summarized in Table-1

The precision of the test method is evaluated by assaying six samples of zetpril tablets of 2.0mg and 4.0mg. The average percent of assay of zetpril in tablets is found to be 100.06%, 100.09% and %RSD is found to be 0.148 and 0.135 respectively for 2.0mg and 4.0mg tablets respectively. The results are summarized in Table-2. Accuracy of the method is determined at three different concentrations of the test solution equivalent to about 75%, 100%, and 125% of the target concentration. Each solution is prepared in triplicate and assayed as per test method. The percent RSD and percent of recovery of the active pharmaceutical ingredient is calculated. The percent RSD values are found to be 0.595, 0.244 and 0.332 at 75%, 100%, and 125% of the target concentrations respectively and the percent of recovery of the drug is found to be 99.95%, 100.03% and 100.26% corresponding to 75%, 100% and 125% spike levels. These calculated values indicate that the method is highly precise and accurate. The results are expressed in Table-3.

To determine ruggedness of the developed method, a study is conducted between two different analysts, systems, and columns at same experimental conditions. The values of the precision at each stage are compared with method precision statistically. The average % assay and relative standard deviation are calculated and found to within the limits (Table-4). Comparison of the results thus obtained shows that the proposed method is rugged. Robustness of the method is studied by determining the system suitable parameters by studying the effect of small change in chromatographic conditions such as composition of the mobile phase, flow rate, pH of the buffer solution, wavelength and column temperature and found to be acceptable limits.

Pharmaceutical formulations are analyzed by calculating the percent of recovery of the standard drug added to preanalysed formulations. Known amount of the standard drug equivalent to 25% to 150% of the target concentration is added to same amount of test sample. Chromatogram is recorded under the test chromatographic conditions for each concentration; a graph is drawn between the amount of drug added and the amount of drug recovered. The plot is linear (Fig.3) and regression equation is given by $y=1.0038x+0.1873$ with $r^2=0.9999$. The results are given in Table-5.

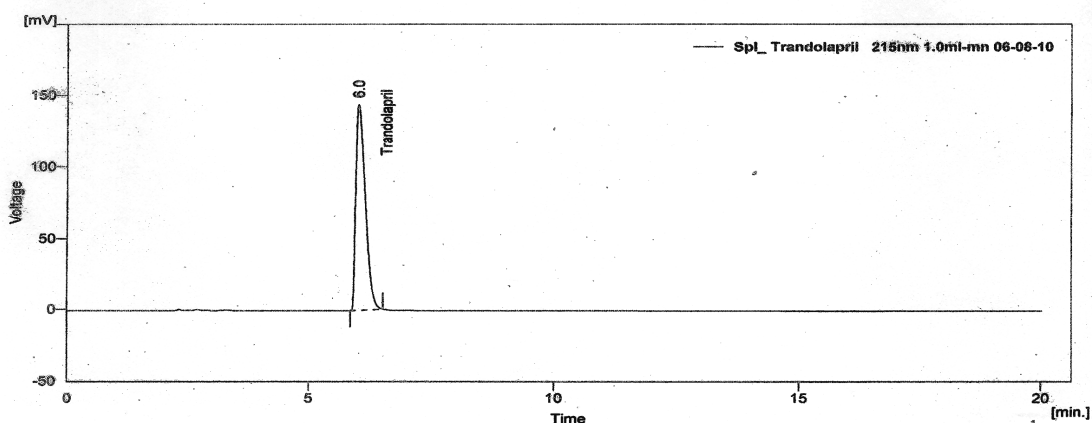


Fig.-1: Chromatogram of Trandolapril Standard (100µg/ml)

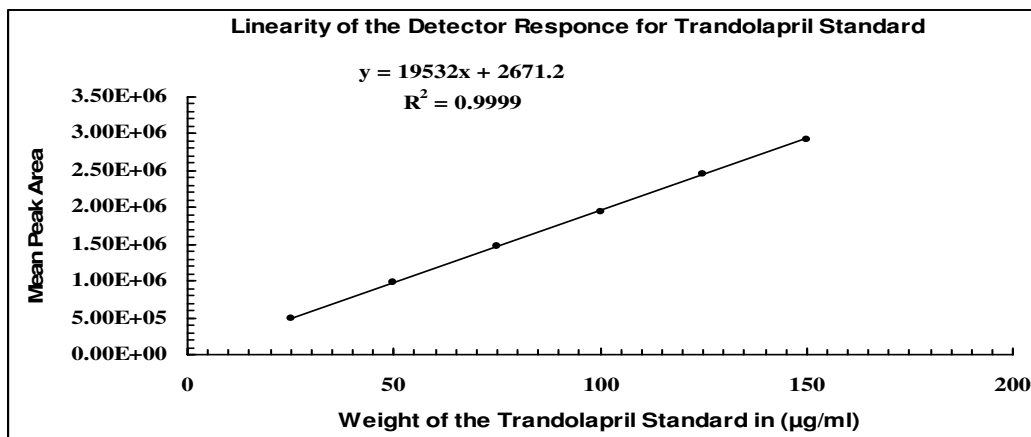


Fig.-2 : Linearity of the detector response to the concentration of trandolapril

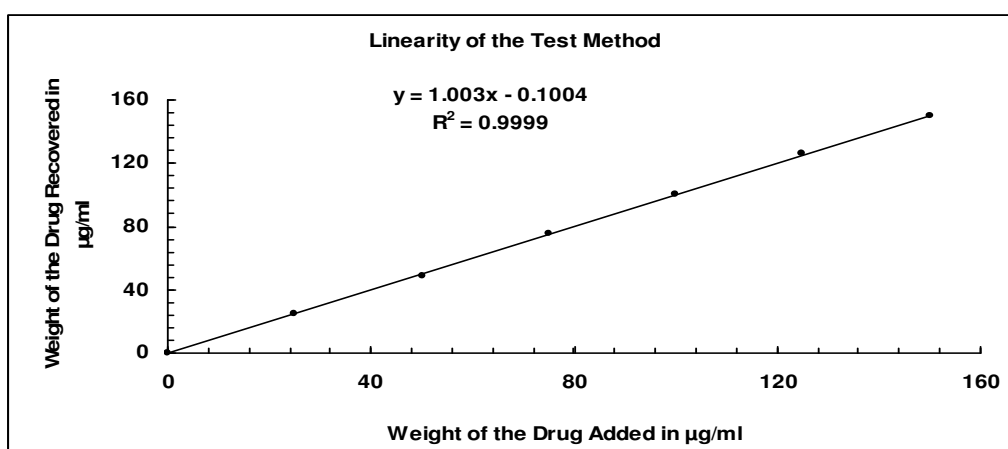


Fig.-3: Linearity of test method

Table-1: Linearity of detector response

S.No.	Concentration µg/ml	Average Area	Regression Parameter	
			Parameter	Value
1	25	489867	Slope	19532
2	50	979724	Intercept	2671
3	75	1466588	Correlation Coefficient	0.9999
4	100	1950445	LOD µg/ml	1.149
5	125	2461326	LOQ µg/ml	3.832
6	150	2922163		

Table-2: Precision of the test method

Sample No.	Percent of Assay Labeled Amount	
	2.0mg/Tablet	4.0mg/tablet
1	99.97	100.21

2	100.3	99.89
3	100.13	99.95
4	100.11	100.19
5	99.94	100.16
6	99.91	100.14
Mean	100.06	100.09
SD	0.148	0.135
%RSD	0.148	0.135

Table-3: Accuracy of the method

Spike level	Sample ID	Amount Added($\mu\text{g/ml}$)	Amount Found($\mu\text{g/ml}$)	% of Recovery	Statistical Analysis	
75%	1	75.00	74.46	99.28	Mean	99.951
	2	75.00	75.31	100.41	SDV	0.595
	3	75.00	75.12	100.16	%RSD	0.595
100%	1	100.00	99.94	99.94	Mean	100.033
	2	100.00	99.85	99.85	SDV	0.244
	3	100.00	100.31	100.31	%RSD	0.244
125%	1	125.00	125.62	100.50	Mean	100.261
	2	125.00	124.85	99.88	SDV	0.333
	3	125.00	125.51	100.41	%RSD	0.332

Table-4: Study of Ruggedness

Statistical Parameter	Analyst-1	Analyst-2	System-1	System-2	Column-1	Column-2
Mean ^a	100.06	100.00	99.95	100.02	99.99	100.13
SD	0.212	0.177	0.183	0.254	0.229	0.264
%RSD	0.212	0.177	0.183	0.253	0.229	0.263
F-test ^b	2.057	1.429	1.532	2.935	2.394	3.176
t-test	0.038	0.876	1.47	0.386	0.749	0.665
F-test ^c	1.435		1.926		1.329	
t-test	0.571		0.662		1.057	

^aMean of six replicate measurements

^bComparison of the precision with method precision. Tabular values are F=5.05 and t=2.571

^cComparison of precision between the two analysts, systems and columns

Table-5: Recovery studies of formulations

Spike level (%)	Drug added($\mu\text{g/ml}$)	Drug Recovered($\mu\text{g/ml}$)	Regression parameters	
25	25.00	25.27	Slope	1.0038
50	50.00	48.94		
75	75.00	75.48	Intercept	-0.1873
100	100.00	100.53		
125	125.00	125.92	Correlation Coefficient	0.9999
150	150.00	149.74		

CONCLUSIONS

The developed procedure for the assay of trandolapril is optimized and validated using the test method and found to be linear, precise, accurate, rugged and robust. The developed method can be applied to determine the assay of the formulations with excellent percent of recoveries.

ACKNOWLEDGEMENTS

The authors are very much thankful to Chandra Labs, an Analytical Testing Laboratory Hyderabad for providing laboratory facilities.

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[RJC-690/2010]

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