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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TRANDOLAPRIL IN TABLET DOSAGE FORM

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INTRODUCTION

Trandolapril is the ethyl ester prodrug of a nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor, trandolaprilat. Trandolapril is chemically described as (2S, 3aR, 7aS)-1-[(S)-N-[(S)-1-Carboxy-3phenylpropyl]alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl ester. Its empirical formula is $C_{24}H_{34}N_2O_5$. Trandolapril is deesterified to the diacid metabolite, trandolaprilat, which is approximately eight times more active as an inhibitor of ACE activity. 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.

Various UV Spectroscopy[2-7], Spectrofluorometric[8], GC[9], UPLC-MS[10], HPLC with ampherometry[11] and Raman spectroscopic[12] assay methods are reported in the literature for the estimation of Trandolapril. According to literature survey there is no official method for the estimation of Trandolapril by RP-HPLC in tablet dosage forms. Hence, an attempt has been made to develop new method for the estimation and validation of Trandolapril in tablet formulation in accordance with the ICH guidelines[13].

EXPERIMENTAL

Instrumentation

Chromatography was performed with Alliance waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and convenient for LC with class Empower-2 software.

REAGENTS AND CHEMICALS

The reference sample of Trandolapril was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (NIMOTOP-30mg) were purchased from the local pharmacy.

Chromatographic condition

The mobile phase consisted of phosphate buffer and acetonitrile was taken in ratio of 35:65 at a flow rate of 1.0 mL/min. Altima, C18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase. 220 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution

Accurately Weighed and transferred 20 mg of Trandolapril working Standard into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents.

Preparation of Working Standard Solutions

Aliquot of 0.3, 0.6, 0.9, 1.2, 1.5 & 1.8 mL were pipette out from stock solution into 10 mL volumetric flask and volume was made up to 10 mL with diluent. This gives the solutions of 6, 12, 18, 24, 30 and 36µg/mL for Trandolapril.

Preparation of phosphate Buffer

Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a

1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degassed to sonicate and finally make up the volume with water, then pH adjusted to 3.6 with dil. Ortho phosphoric acid solution.

Sample preparation
20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to one tablet was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation
Parameters such as systems suitability, Linearity, accuracy, specificity, LOD & LOQ and robustness were performed according to the ICH guidelines.

RESULTS AND DISCUSSION
Method development
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which the drug did not responded properly. The organic content of mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, phosphate buffer: acetonitrile were taken in isocratic ratio: 35: 65 and with flow rate of 1.0 mL/min was employed. Altima C18 column (4.6 x150mm, 5µ particle size) was selected as the stationary phase to reduce the tailing of the peak. 220 nm was selected as the detection wavelength for PDA detector. The retention times was found to about 2.9 min and the results were shown in Table 1 and Figure 2.

Method Validation
System suitability
A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 3. The analytical method validation was carried out as per ICH method validation guidelines.

Linearity
The linearity range was found in the range of 6-36µg/mL. The response for the drug was linear and the regression equation was found to be y=34750x-792.38 and correlation coefficient was found to be 0.9999 and the results are given in Table 2 and Figure 3.

Precision
Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intra-day precision and inter-day precision.

Intra-day precision
To study the intra-day precision, six replicate standard solutions of Trandolapril were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.45 which are well within the acceptable criteria of not more than 2.0.

Inter-day precision
To study the inter-day precision, six replicate standard solutions of Trandolapril were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.52 which are well within the acceptable criteria of not more than 2.0.

Specificity
The effect of wide range of excipients and other additives usually present in the formulation of Trandolapril in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Trandolapril.

Limit of detection and limit of quantification
A calibration curve was prepared using concentrations in the linearity range (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Trandolapril were 0.28 and 0.85 µg/mL, respectively (Table 3).

Accuracy
The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD was found to be 1.39. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Tablet Analysis

The Content of Trandolapril in the tablets was found by the proposed method. RSD values for Trandolapril are found to be 0.46 and results were shown in table.4.

CONCLUSION

A new precise accurate and simple HPLC method was developed and validated for the estimation of Trandolapril in tablet dosage form. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of Trandolapril tablets in QC laboratories and industries.

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Phosphate Buffer:Acetonitrile (35:65)
2	pH	3.6(+/-0.5)
3	Column, make	Altima, C18 (150 x 4.6 mm, 5µ)
4	Column temperature	30^0C
5	Wave length	220nm
6	Injection volume	10ul
7	Flow rate	1.0ml/min
8	Run time	5mins
9	Retention time	2.9mins

Table 2: Linearity results

S. No.	Concentration in µg/mL	Area
1	6	201012
2	12	416462
3	18	628462
4	24	828288
5	30	1030119
6	36	1245888

Table 3: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (µg/mL)	6-36 µg/mL
2	Correlation coefficient	0.9999
3	Theoretical plates (N)	4526
4	Tailing factor	1.12
5	LOD (µg/mL)	0.28 µg/mL
6	LOQ (µg/mL)	0.85 µg/mL
7	Regression Equation	Y=34570x-782

Table 4: Assay results

S. No.	Formulation	Label claim	Amount found	%Assay
1	MAVIK	2mg	2.002mg	100.13%

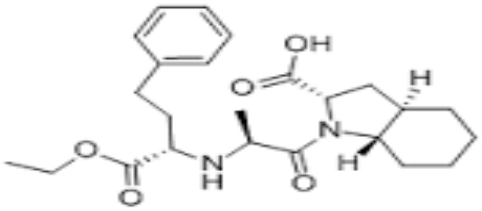

Fig. 1: Structure of Trandolapril

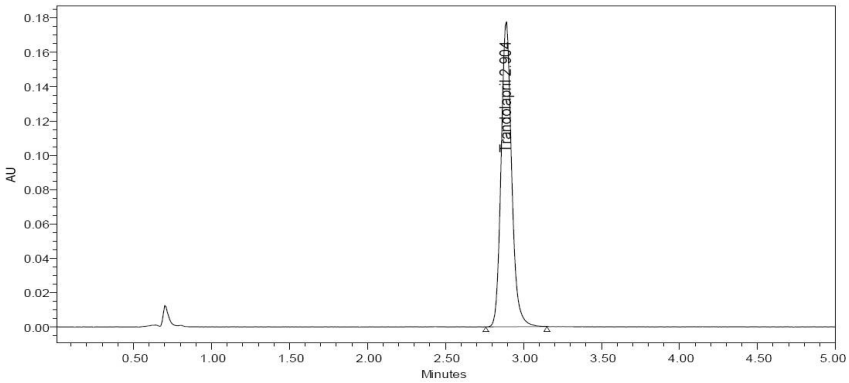

Fig. 2: Chromatogram of Trandolapril Standard

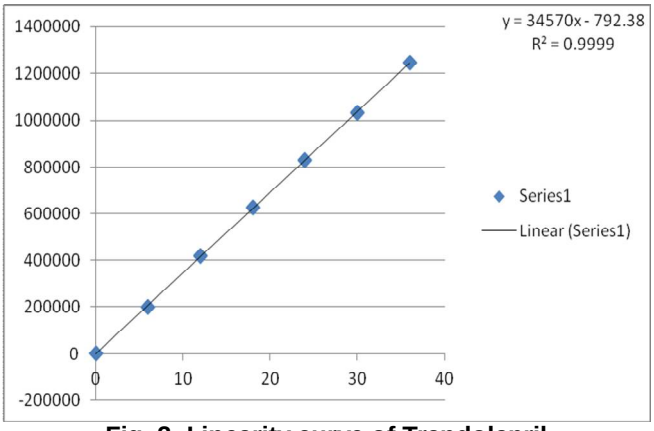

Fig. 3: Linearity curve of Trandolapril

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