

A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR QUANTIFICATION OF TRANDOLAPRIL USING UV SPECTROMETRIC DETECTION

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ABSTRACT

A high performance Liquid chromatographic method for quantification of trandolapril using UV detection was developed and validated. Trandolapril samples were analysed on Merck LiChroCART -RP C18 column (250x4.0, i.d.5 μm) and the mobile phase composition used for detection was combination of acetonitrile: methanol: phosphate buffer (0.025mM) pH3.0 (40:35:25) at a flow rate of 1 ml/min. The λ_{max} used was 220nm with UV detection. The retention time of trandolapril by proposed method was found to be 2.750 ± 0.008 min. Peak area obtained were linearly related to concentration of drug in samples in range of 2.5-17.5 $\mu\text{g/mL}$ having correlation coefficient of 0.999. The LOD and LOQ of trandolapril by proposed method was found to be 0.099 $\mu\text{g/mL}$ and 0.300834 $\mu\text{g/ml}$ respectively. The method was validated as per ICH guidelines for various parameters. The results for accuracy, precision and robustness were found to be within accepted limits.

KEY WORDS: Liquid chromatography, Trandolapril

INTRODUCTION

Trandolapril is chemically (2S, 3aR, 7aS)-1-[(S)-2-[[1-Ethoxycarbonyl-3-phenylpropyl] amino] propanoyl] octahydro -1H-indole-2-carboxylic acid. It is a potent nonsulfhydryl and dicarboxyl containing angiotensin converting inhibitor. Trandolapril is a monoester prodrug and is 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 trandoalpril and trandolaprilat respectively. Following absorption, oral trandolapril is rapidly and extensively hydrolysed to trandolaprilat. The mean $t_{1/2}$ of trandolapril is less than 1 h, and that of Trandolaprilat is, approximately, 75 h^[5, 6]. After single oral dose, the C_{max} 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 exceeds 80%. The C_{max} of trandolaprilat, after single oral doses, was also dose proportional (1.40–1.92 ng/ml/mg) with a T_{max} of 4–8 h^[7].

A literature 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 method with better detection range for estimation of trandolapril in bulk and formulations. Media used was a combination of acetonitrile: methanol: phosphate buffer (40:35:25). Developed method was validated as per ICH guidelines^[13].

EXPERIMENTAL

MATERIALS AND METHODS

Trandolapril was provided by Ranbaxy Laboratories, India. HPLC grade methanol and acetonitrile were obtained from Merck, India. Analytical grade Potassium Phosphate monobasic, Sodium hydroxide, ortho-phosphoric acid were supplied by SD fine chemicals Ltd, India. All other chemicals used were of HPLC grade or grade equivalent in purity. TDW (triple distilled water) was obtained in-house from milli-Q assembly (milipore, USA).

Instruments and Chromatographic conditions

The chromatographic system Jasco HPLC (Japan) consisted of binary pumps (Jasco-PU-1580), auto sampler (Jasco-AS-1559) and a UV-visible detector (JascoUV-1575) equipped with Borwin 1.0 software, window XP for data collection and peak integration. The chromatographic separations were performed through injection of 20 μ l samples on Merck LiChroCART -RP C18 column (250x4.0, i.d.5 μ m), which were detected at 220nm

The mobile phase was pumped in isocratic mode into the column at a flow rate of 1 ml/min. Mobile phase composition consisted acetonitrile: methanol: phosphate buffer (0.025mM) pH3.0 (40:35:25). The mixture was sonicated for 30 min.

Analytical method development

Different mobile phase compositions were investigated to develop a suitable HPLC method for detection of trandolapril. While selecting appropriate mobile phase, the criteria employed were peak shape, retention time, sensitivity of method, cost of solvents and ease of application.

Preparation of Stock and working standard solutions

The stock solution was prepared by dissolving 10 mg of trandolapril in 5mL of methanol and the volume was made to 100mL with phosphate buffer pH 3.0. A series of working standards was prepared from this stock by pipetting volumes of 0.25-1.75 mL to prepare concentrations of 2.5, 5, 7.5, 10, 12.5, 15, 17.5µg/ml. so as to obtain calibration curve .

Calibration Curve

Calibration curve was obtained from above mentioned working standards. Peak area obtained were linearly related to concentration of drug in samples and Least-squares linear regression was used to fit the measured signal versus the theoretical concentration. The LOD and LOQ of proposed method was determined using calibration curve data. LOD and LOQ were calculated as $3.3r/S$ and $10r/S$, respectively, where S is the slope of the calibration curve and r is the standard deviation of y-intercept of regression equation (9).

ANALYTICAL METHOD VALIDATION

Linearity

To prove linearity of the proposed method, nine separate determinations of solutions of drug (in the range 2.5 to 17.5 µg/mL) were done from stock solutions. The data obtained was subjected to least square regression analysis.

Accuracy

To determine the accuracy of proposed method, three different drug concentrations (LQC=4, MQC=12, HQC=16 µg/mL) were prepared from separate stock solution and analysed (N=9). Accuracy was determined as the percentage bias and mean percentage recovery.

Precision

Precision was determined by using same concentration levels (LQC=4, MQC=12, HQC=16 µg/mL) as in accuracy, prepared from independent stocks and were analysed (N=9). Intra-day and inter-day variation were considered to determine intermediate precision of the proposed method. Different levels of drug concentrations (LQC, MQC and HQC) were prepared at three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation. Precision was assessed by calculating % R.S.D.

Robustness

Robustness of the analytical method was established by changing pH of phosphate buffer, by ± 0.2 units, used to prepare stock and series of dilutions. Three different concentrations (LQC=4, MQC=12, HQC=16 µg/mL) were prepared in each media with different pH and mean percentage recovery was determined . The results obtained were subjected to studentized t-test to find any significant difference.

RESULTS AND DISCUSSION

Initially, proper media for preparation of stock and its corresponding dilutions were

investigated. Based on this, 5% methanol in phosphate buffer pH 3.0 was found to be optimum. The phosphate buffer used was 0.025 mM and was maintained at pH of 3.0 using orthophosphoric acid. Then, for mobile phase composition optimisation, various runs were given. The final decision of using a combination of acetonitrile: methanol: phosphate buffer (40:35:25) was based on criteria like peak shape, retention time, sensitivity of the method, cost of method and ease of application. The λ_{max} used for UV detection was 220nm. The retention time of trandolapril by proposed method was found to be 2.750 ± 0.008 min. The chromatogram for trandolapril solution of concentration 17.5 $\mu\text{g/mL}$ is shown in Figure 2.

Calibration curve

In above mentioned conditions, each concentration in range of 2.5-17.5 $\mu\text{g/mL}$ were injected ($n=9$) and data obtained was subjected to linear regression analysis to obtain calibration curve. The calibration curve was prepared by plotting concentration (in $\mu\text{g/ml}$) on the abscissa and peak area on ordinate axis in the range of 2.5-17.5 $\mu\text{g/ml}$. The Limit of detection and Limit of quantitation was calculated according to the ICH guidelines and found to be 0.099 and 0.301 $\mu\text{g/ml}$. The results are given in table1.

Analytical Method Validation

Linearity

In the proposed method, the linearity was found to be 2.5-17.5 $\mu\text{g/mL}$. The best fit line obtained by least square regression analysis was supported by good

correlation coefficient values of 0.9996 (Table1).

Accuracy

Accuracy of the method was determined through recovery studies. The percentage recovery varied from 100.38% to 99.18% while the percent bias for LQC was found to be -0.81%. Data for accuracy studies is presented in Table 2. Thus proposed method possesses excellent accuracy.

Precision

Precision was determined by studying repeatability and intermediate precision. Repeatability (%R.S.D.) was found to be in the range 0.396686 to 1.21089 (Table 2) at all levels of concentrations. Repeatability results represent the precision under the same operating conditions over a short period of time while, intermediate precision represents within-laboratory variations on different days. In intermediate precision study, % R.S.D. values were below 1.3836 % at all concentrations (Table 3). % R.S.D. values obtained were found to be well within the acceptable range. This proves excellent repeatability and intermediate precision of the proposed method.

Robustness

Variation in the pH of media used by ± 0.2 did not have any significant effect on the outcome of results. Mean percentage recovery was found to be 100.82 ± 1.292 for LQC. Thus robustness of the method was established by applying t-test to compare pH variation in which

experimental values posed no significant difference at $p > 0.05$.

CONCLUSION

The HPLC method for determination of trandolapril in bulk samples was developed and validated. The method described is simple, reproducible and sensitive with adequate accuracy and precision. In addition the method is rapid as the retention time is 2.75min. The solvent system is more economical and eco-friendly than the previously reported methods. It is possible to quantify concentrations down to 0.30 μ g/ml with detection limit of 0.1 μ g/ml thus demonstrating very high sensitivity. The validation was done in accordance with ICH and results obtained were within acceptance limits. Thus the method developed can be applied for quality control and routine analysis of enalapril.

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Table1. Analytical parameters of the calibration equations for the determination of trandolapril by high performance liquid chromatography.

Parameter	Results
Concentration Range($\mu\text{g/ml}$)	2.5-17.5
Flow rate	1 ml/min
Detection wavelength(nm)	220
Regression equation	
Intercept(a)	- 10577
Slope(b)	15257
Correlation coefficient(r)	0.9996
r^2	0.999
Limit of Detection ($\mu\text{g/ml}$)	0.099
Lower Limit of Quantification ($\mu\text{g/ml}$)	0.301

Table 2: Accuracy data for the developed method (Each value is result of nine separate determinations, n=9)

QC Concentration ($\mu\text{g/ml}$)	Experimental Concentration		Accuracy	
	Mean \pm S.D.	%RSD	Recovery (%)	% bias
4	4.03 \pm 0.049	1.21	99.19	-0.81
12	12.02 \pm 0.048	0.40	99.81	-0.19
16	15.94 \pm 0.074	0.47	100.38	0.38

Table 3: Intermediate Precision data for the developed method

Concentration ($\mu\text{g/mL}$)	Intra-day precision (% R.S.D.)(N=9)			Inter-day precision (% R.S.D.)(N=27)
	Day 1	Day 2	Day 3	
LQC	1.340745	1.228909	1.383642	1.156634
MQC	0.371011	0.354386	0.494408	0.362971
HQC	0.513844	0.551719	0.487255	0.498555

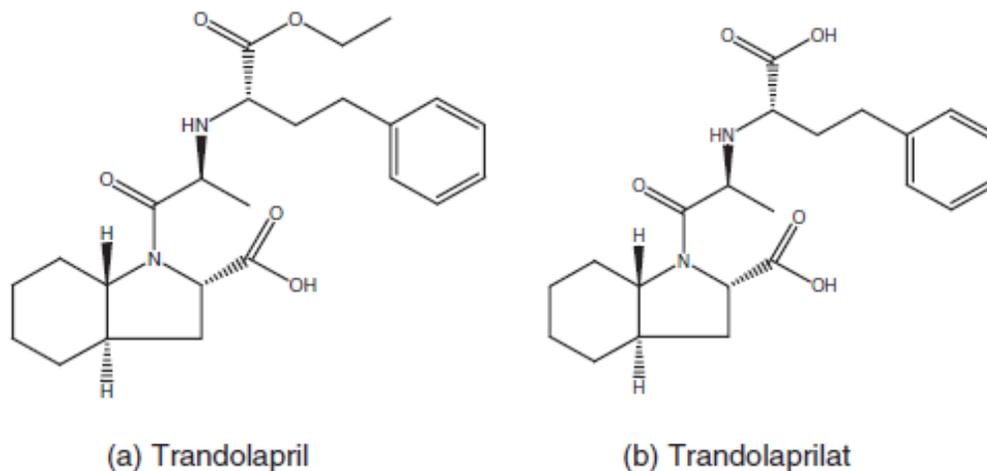


Figure 1: Structures of Trandolapril (a) and its active metabolite, Trandolaprilat (b).

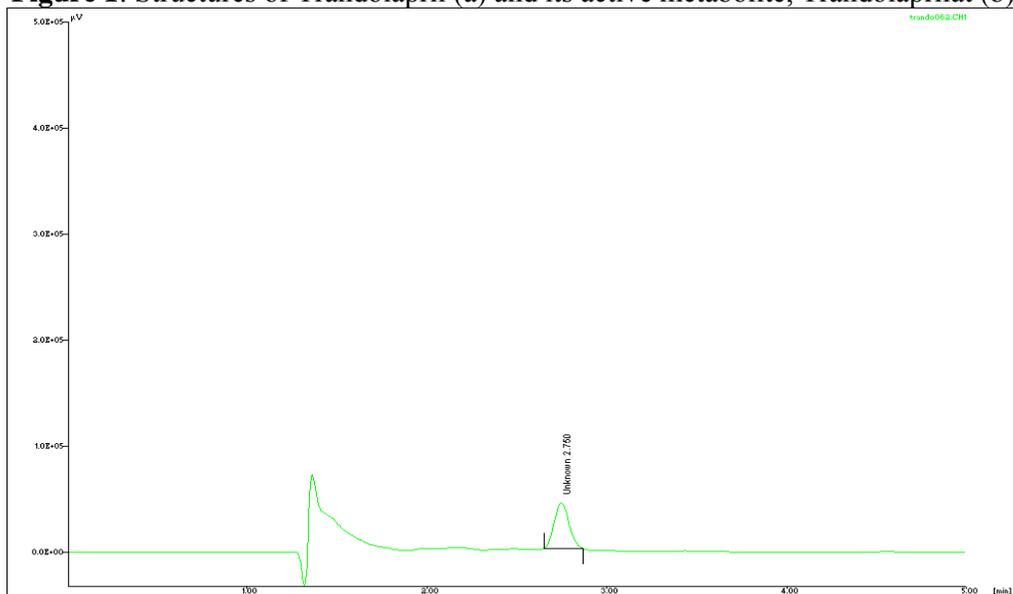


Figure 2 : Chromatogram for trandolapril solution of concentration 17.5 $\mu\text{g/ml}$; .
Chromatographic conditions: reversed-phase HPLC on a C18 column; Mobile phase, acetonitrile: methanol: phosphate buffer(0.025 mM) pH3.0 (40:35:25) , flow rate= 1 mL/min isocratically, $\lambda_{\text{max}} = 220\text{nm}$.