ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TRANDOLAPRIL IN TABLETS BY RP-HPLC

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ABSTRACT:

A High performance liquid chromatography method for quantification of Trandolapril using UV detection was developed and validated using ODS INERTSIL C18 column (250×4.6mm, 5µm) and the mobile phase composition was phosphate buffer and acetonitrile (1: 1) at a flow rate of 1.0ml/min. The monitoring wavelength used was 210nm with UV detection and the method was validated as per ICH guidelines for various parameters and they are found to be within accepted limits.

KEYWORDS: Liquid chromatography, Trandolapril, Validation.

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 125-130º C. 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\(^4\),\(^5\) and a decrease in peripheral vascular resistance. Trandolapril is approved for the management of hypertension, left ventricular systolic dysfunction and chronic heart failure\(^6\).

Some of the undesirable effects very commonly reported for trandolapril include, dizziness, cough and headache. From the literature survey\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) trandolopril was reported only in biological sample.

**MATERIALS AND METHODS**

Trandolapril was provided by Orchid Chemicals and Pharmaceuticals Ltd, Chennai. HPLC grade acetonitrile were obtained from RANKEM Laboratories. Analytical grades 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.

**INSTRUMENTS AND CHROMATOGRAPHIC CONDITIONS:**

The chromatographic system SHIMADZU LC 20AT and a UV-visible detector equipped with LC-Solutions Software for data collection and peak integration. The chromatographic separations were performed through injection of 20 µl samples on ODS INERTSIL C18 column (250×4.6mm, 5µm), which were detected at 210nm. The mobile phase was pumped into the column at a flow rate of 1.0 ml/min. Mobile phase composition consisted of 1:1 ratio of acetonitrile & phosphate buffer. The mobile phase 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 with the ease of application.

**Preparation of Stock and working standard solutions**

The stock solution was prepared by dissolving 50mg of Trandolapril in 70mL of diluent and the volume was made to 100mL with phosphate buffer pH 3.0, sonicated to dissolve the material completely, diluted to volume with diluent and mixed. A series of working standards was prepared from this stock to prepare concentrations after proper dilution 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 $\sigma$ of proposed method was determined using calibration curve data. LOD and LOQ were calculated as $3.3 \sigma/S$ and $10\sigma/S$, respectively, where $S$ is the slope of the calibration curve and $\sigma$ is the standard deviation of y-intercept of regression equation.

![Figure 1: Calibration Curve of Trandolapril](image)

![Figure 2: Linearity of Trandolapril](image)

Fig. 2 calibration curve of Trandolopril
RESULTS AND DISCUSSION

Initially, proper media for preparation of stock and its corresponding dilutions were investigated. Based on this, phosphate buffer pH 3.0 was found to be optimum. The phosphate buffer used 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 and phosphate buffer in ratio of 1:1 was based on criteria like peak shape, retention time, sensitivity of the method, cost of method and ease of application. The λmax used for UV detection was 210nm. The retention time of trandolapril by proposed method was found to be 5.8 min. In above mentioned conditions, each concentration in range of 6 - 14 µg/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 µg/ml) on the abscissa and peak area on ordinate axis in the range of 6-14µg/ml. The Limit of detection and Limit of quantitation was calculated according to the ICH guidelines and found to be 0.36 and 1.21. The results are given below.

Analytical Method Validation

Linearity

To prove linearity of the proposed method, five separate determinations of solutions of drug (in the range 6 to 14 µg/mL) were done from stock solutions. The data obtained was subjected to least square regression analysis was supported by good correlation coefficient values of 0.9995.
Fig.3. chromatogram of Trandolopril

Accuracy

To determine the accuracy of proposed method, three different drug concentrations were prepared from separate stock solution and analysed (N=9). Accuracy was determined as the
percentage bias and mean percentage recovery. The percentage recovery varied from 100.38% to 99.18%. Thus proposed method possesses excellent accuracy.

**Precision**

Precision was determined by using same concentration level as in accuracy, prepared from independent stocks and were analysed (N=9). Precision was assessed by calculating % R.S.D. Precision was determined by studying repeatability and intermediate precision. 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 NMT 2% at all concentrations.

**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 were prepared in each media with different pH and mean percentage recovery was determined. The results obtained were assessed by calculating %RSD. Variation in the pH of media used by ±0.2 did not have any significant effect on the outcome of results.

**CONCLUSION**

The HPLC method for determination of Trandolapril in tablets 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 8min. The solvent system is more economical and eco-friendly than the previously reported methods. It is possible to quantify
concentrations down to 0.30µg/ml with detection limit of 0.1µg/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 also be applied for quality control and routine analysis of Trandolapril.

REFERENCES


