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Estimation of Ranolazine Using Reverse Phase High Performance Liquid Chromatography Technique

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ABSTRACT

A simple, precise, rapid and accurate reverse phase high performance liquid chromatography method (RP-HPLC) in isocratic mode has been developed for the estimation of ranolazine in pure form and in its tablet dosage form. An Agilent Eclipse XDB C18, 150 x 4.6 mm, 5µm particle size column, with mobile phase consisting of phosphate buffer pH 3.5 and acetonitrile in the ratio of 65:35 % v/v was used. The flow rate was 1.0 mL/min and the column effluent was monitored at 272 nm. The retention time was 4.7 min. The detector response was linear for ranolazine in the concentration range of 10-150 µg/mL. The limit of detection (LOD) was found to be 0.034 µg/mL. The limit of quantification (LOQ) was 0.102 µg/mL. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of ranolazine in pure drug and in its tablet dosage form.

Keywords: Ranolazine, RP-HPLC estimation, tablets.

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INTRODUCTION

Ranolazine (figure 1), is chemically N-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]piperazin-1-yl}acetamide. Clinical trials show that ranolazine reduces the frequency of anginal attacks and increases exercise capacity in patients with

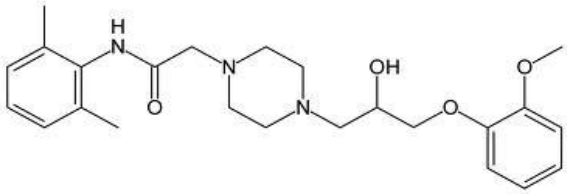

Figure 1. Chemical structure of Ranolazine

chronic angina[1]. Literature survey reveals that the drug can be estimated in human plasma by LC-tandem MS[2], LLE-HPLC[3], U-HPLC-MS/MS[4], in formulation by RP-HPLC methods[5,6], HPTLC[7], LC[8], in rat plasma by LC-MS[9], Stability indicating RP-HPLC method[10] and novel spectrophotometric method[11]. Present study aims to develop a simple, sensitive and accurate reverse phase high performance liquid chromatography technique for estimation of ranolazine in its tablets.

MATERIALS AND METHODS

Ranolazine was obtained as a gift sample from Aurobindo pharma Ltd, Hyderabad, India. Potassium dihydrogen orthophosphate was of analytical grade and supplied by s.d. Fine Chem. Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available ranolazine tablets were procured from local market. Quantitative estimation was performed on Waters 2996 HPLC system, PDA detector module equipped with 2693 pump and 25 µL fixed loop. Agilent Eclipse XDB C18, 150 x 4.6 mm, 5µm column was used. The HPLC system was equipped with Empower software.

Chromatographic conditions

The contents of the mobile phase were phosphate buffer (pH adjusted to 3.5 with orthophosphoric acid) and acetonitrile in the ratio of 65:35 v/v. It was filtered before use through a 0.22 µm membrane filter and pumped to the column at a flow rate of 1 mL/min. The run time was set at 10.0 min at the ambient column temperature. Water: Methanol (50:50) was used as diluent.

Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving 100 mg of ranolazine in 70 mL HPLC grade water in a 100 mL volumetric flask and volume was made up to the mark with

diluent, this standard stock solution contains 1000 µg/mL of ranolazine.

Linearity

10-150 µg/mL solutions of ranolazine were prepared by diluting suitable aliquots of standard stock solution by using mobile phase. From this 25 µL of each solution was injected in to the HPLC system. Chromatograms were recorded and peak areas were noted. The linearity plot was constructed by taking concentration (µg/mL) in X axis and area of the peak in Y axis.

Estimation in formulation

Twenty tablets were weighed accurately, average weight was calculated, powdered and powder equivalent to 100 mg of ranolazine was transferred to a 100 mL volumetric flask. It was dissolved in mobile phase and filtered through a 0.2 µm membrane filter. The filtered solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 25 µL fixed sample loop of the injection port. The sample solution was injected six times and chromatograms were recorded.

Recovery studies

Recovery studies used to find the accuracy of the method. This was carried out by adding known quantity of standard to the previously analysed sample and area of peak was noted. This study was carried out in 50, 100 and 150 % level of 50 µg/mL of standard.

RESULTS AND DISCUSSION

Different mobile phase compositions were tried to elute the drug molecule from the column and adequate resolution is achieved with phosphate buffer and acetonitrile in the ratio of 65:35 % v/v at room temperature with Agilent Eclipse XDB C18, 150 x 4.6 mm, 5µ, column and this solvent system was found to be most suitable for method development and validation. The system suitability studies were carried out and results shown in Table 1. In the proposed method, the retention time of ranolazine was found to be 4.7 min. Quantification was linear in the concentration range of 10-150 µg/mL. The linearity datas are given in Table 2. The limit of detection and limit of quantitation were found to be 0.034 µg/mL and 0.102 µg/mL respectively, which indicate the sensitivity of the method. A typical chromatogram of ranolazine is shown in figure 2. A chromatogram for pure mobile phase (blank) is shown in figure 3.

The use of phosphate buffer pH 3.5 and acetonitrile in the ratio of 65:35 % v/v resulted in peak with good shape and resolution. The developed method was used to estimate the drug in formulation and results are given in Table 3. The recovery study of this method indicates that the

proposed method is highly accurate (Table 3). No interfering peaks were found in the chromatogram within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed RP-HPLC method. The precision studies were carried out by repeatability and by intermediate precision; the results are in Table 4.

Table 1. System suitability data

Drug	Theoretical plates (N)	Tailing factor (T)	Retention time (min), (n=6)		Peak area, (n=6)	
Ranolazine 100 µg/mL	4163	1.76	Mean± S.D	%CV	Mean± S.D	%CV
			4.828±0.0380	0.7876	883492.3±206.2	0.0233

Table 2. Linearity Study of Ranolazine

Parameters	Values
Linearity (µg/ml)	10- 150
Slope	8919
Intercept	-18935
Correlation co-efficient	0.999

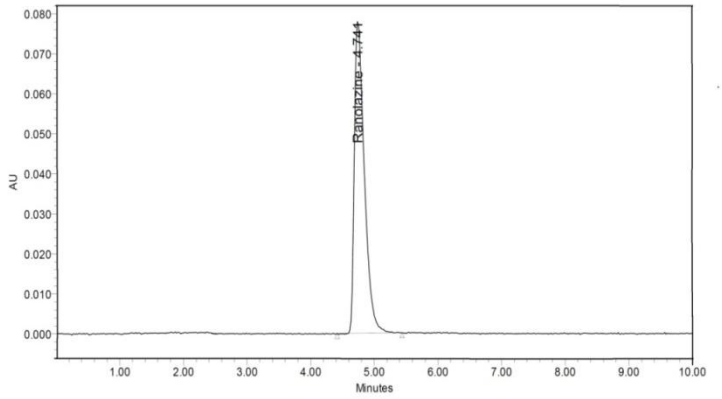

Figure 2. A typical chromatogram for Ranolazine

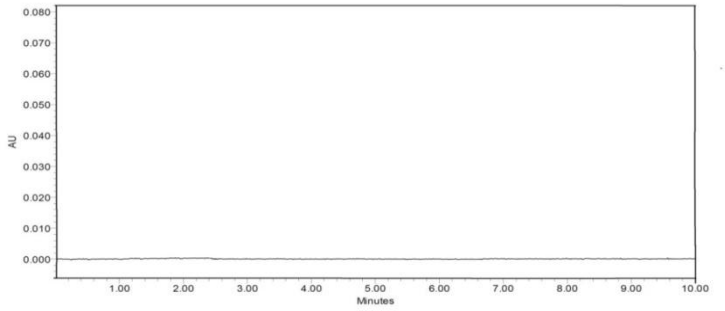

Figure 3. A typical chromatogram for blank

Table 3. Assay results and recovery studies

Formulation	Labeled amount (mg/ tablet)	Amount present (mg/ tablet)* ± SD	(%) label claim* ± S.D	% Recovery
Ranolazine Tablets	500	499.95 ±0.0682	99.99 ± 0.0136	99.88 to 100.16

* Average of six determinations.

Table 4. Precision Study

Repeatability (% RSD) (n=6)	Intermediate precision (% RSD) (n=6)			
	Day 1		Day 2	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
0.0136	0.0266	0.0402	0.2793	0.1771

CONCLUSION

The proposed RP-HPLC method is simple, precise, accurate and rapid for the determination of ranolazine in tablet dosage form. It can be easily and conveniently adopted for routine quality control analysis of the ranolazine.

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