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Development and Validation of RP-HPLC Method for Simultaneous Determination of Ranolazine In Bulk and Pharmaceutical Dosage Form

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ABSTRACT

This present study was undertaken with an objective to develop & validate a simple, precise, cost effective, sensitive & fast RP- HPLC method for the analysis of Ranolazine. A Shimazu HPLC system with Luna 5µm C_{18} is employed for the analysis using Methanol: Acetonitrile(50:50, v/v) as mobile phase. Signal from Ranolazine is detected at 227nm by UV Spectrophotometer. The total retention time was 5 min with a flow rate of 1.0 ml/min. % 0f RSD values for precision is found to be 0.798%.The limits of detection (LOD) and quantification (LOQ) were 0.616 and 1.86, respectively. As per ICH guidelines the proposed method is fully validated and found to be linear over a workable drug concentration, accurate, precise and robust. This fast, simple and inexpensive method is suitable for research laboratories & also for quality control analysis in pharmaceutical industries.

Keywords: Ranolazine, RP-HPLC, Acetonitrile, Linearity, Validation.

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INTRODUCTION

Ranolazine is an anti angina medication[1]. On January 31, 2006, Ranolazine was approved for use in the United States by the FDA for the treatment of chronic angina[2].Its chemical formula is $C_{24}H_{33}N_3O_4$. Its parent compound is Alpha amino acid amides[3]. Ranolazine is soluble in dichloromethane, methanol[4]. It is sparingly soluble in tetrahydrofuran, ethanol, acetonitrile, and acetone[5]. It is slightly soluble in ethyl acetate, isopropanol, toluene, ethyl ether[6]. It is very slightly soluble in water[7]. It is used for the treatment of chronic angina. It should be used in combination with amlodipine, beta-blockers or nitrates[8].

The objective of analytical method validation is to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample[9]. The specific aim of the work undertaken was to develop validated analytical method based on HPLC for Ranolazine as a single component in bulk and in a commercial tablet formulation[10].

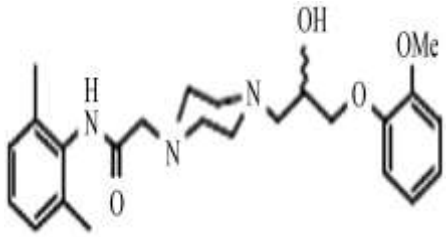

Figure 1: Chemical structure of Ranolazine

MATERIALS AND METHOD

Reagents and chemicals

The API used in this method validation is Ranolazine a gift sample from Incepta Pharmaceuticals Ltd. Bangladesh. Other reagents used in this method are reagent grade Methanol (Pharmacy lab, Noakhali Science & Technology University), HPLC grade Methanol (Active Fine Chemicals ltd. Bangladesh), Acetonitrile (Pharmacy lab, NSTU), Acetone (Pharmacy lab, NSTU), distilled water (Pharmacy lab NSTU), Water For Injection (WFI) (gift from Glob Pharmaceutical Ltd. Bangladesh), Ranolazine 500mg (INN) ER tablet (Mfg. by GENERAL Pharmaceuticals Ltd).

Instruments

High Performance Liquid Chromatography (HPLC), The UV detector model was SPD-20A, Shimadzu brand. Japan. The DGU model was DGU-20A3R, Shimadzu brand, Japan. The LC model was LC-20AT, Shimadzu brand, Japan. The CO model was CTO-10ASVP, brand Shimadzu, Japan. The software was LC solution software.

Other Instruments

UV Spectrophotometer (Model: UV-1800 240V, Shimadzu Corporation, Made In Japan), Analytical Balance (*II*AXIS, Model: AGN220C, Spolkazoo, Made in Poland), Oven (Model: Binder, Made in Germany), Volumetric flask, Pipette, Syringe, Syringe filter, Meta filter, Filter paper, Beaker, HPLC vial, Mortar, pestle, Measuring cylinder, Spatula, Funnel, Glass rod etc. are used for this analysis.

Standard and Sample Preparation

The standard and sample stock solutions of Ranolazine are prepared separately. 50mg Ranolazine (active) was transferred in to a 100ml volumetric flask. Then 100ml methanol was added as a solvent. The concentration was 2mg/ml. Sonicate it for 10 min and then filter it with vacuum filter. The working standard solutions were prepared by diluting the 1ml standard mother solution with 50ml methanol to get solutions of concentration in the .01/ml.

For preparation of Ranolazine (market sample) following procedure was followed.

Mobile phase preparation

To prepare mobile phase added 500ml Methanol with 500ml Acetonitrile at ratio 50:50. Sonicate it for 5 min and then filter it with vacuum filter where pore size of filter paper is 4.5 micron.

Chromatographic Condition

Chromatographic analysis was performed on a Thermo Hypersil reversed phase C-18 column ODS (with 250 cm x 4.6 mm and 4.6 µm particle size). The mobile phase consisted of Methanol and Acetonitrile (50: 50 v/v) and was set at a flow rate of 1.0 ml/min. Diluents was methanol. UV detector was used at a wave length 237nm. Injection volume was 20µl. and then retention time was 5 min.

Method Development and optimization

To develop a suitable and robust HPLC method for the determination of Ranolazine, different mobile phases were employed to achieve the best separation and resolution. Ranolazine was soluble in methanol, acetonitrile, and ethanol. It is slightly soluble in water. So the method development was started with the following mobile phase. Accurately weigh and transfer about 133.2mg of sodium hydroxide and 1.02gm potassium dihydrogen phosphate in 150mL of WFI and mix. Adjust pH to 6.8. Filter the solution through 0.45µm membrane filter. Prepare a filtered and degassed mixture of buffer and acetonitrile and methanol in the ratio of 30:35:35 (v/v) respectively. Ranolazine peak was eluted at void volume. For next trial the mobile phase composition was changed slightly. The mobile phase composition was buffer and acetonitrile and methanol in the ratio of 10:45:45 (v/v). Above trail the separation was little. Again the mobile phase composition changed slightly to WFI and acetonitrile and methanol in the ratio of 20:30:50

(v/v) respectively as eluent at flow rate 1.0mL/min. UV detection was performed at 225 nm. The separation was not so good. Then the final mobile phase was selected acetonitrile and methanol in the ratio of 50:50 (v/v). Ranolazine shows significant UV absorbance at Wave- length 237 nm. Hence this wavelength has been chosen for detection in analysis of Ranolazine. The chromatogram of Ranolazine presented on figure 2.

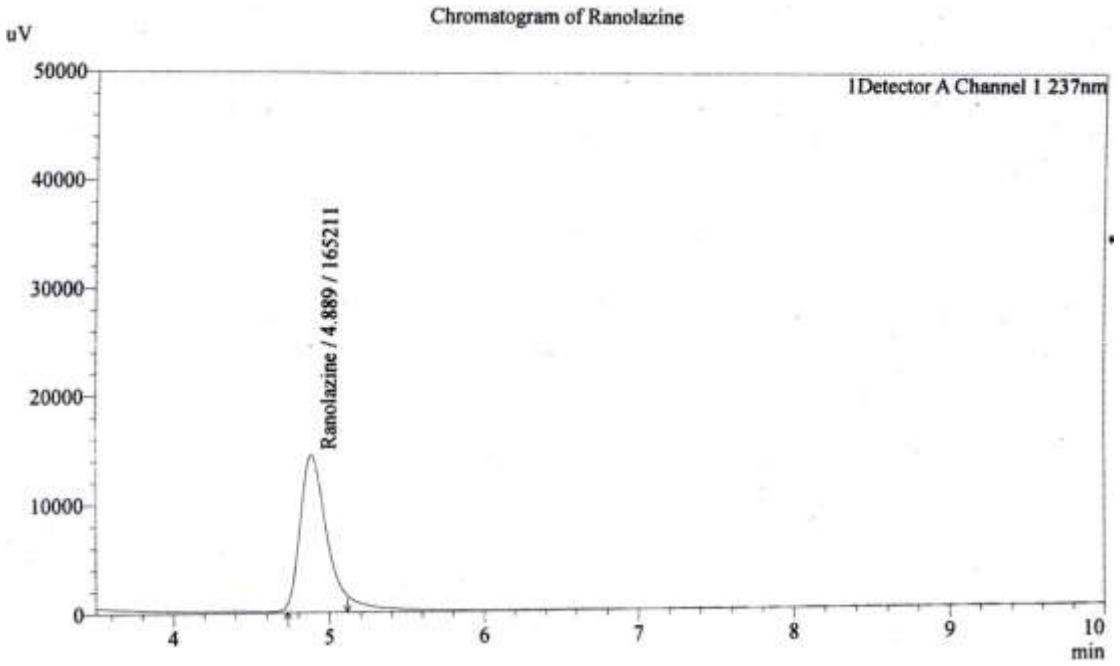

Figure 2: Chromatogram of Ranolazine

RESULTS AND DISCUSION

A simple, rapid and accurate HPLC analytical method has been developed and validated for the analysis of Ranolazine in bulk and tablet dosage form.

Linearity

The standard curve was obtained in the ten concentration of Ranolazine range of 2 - 100µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient of standard curve were calculated and given in Fig 3 to demonstrate the linearity of the method. From the data obtained which given in Table 1. The method was found to be accurate.

Table 1: Linearity studies for Ranolazine

Sr. No	Conc.(µg/ml)	Peak area
1	2	39021
2	4	78042
3	6	148048
4	8	191168
5	10	247445

6	20	549952
7	30	838702
8	40	1087546
9	50	1463590
10	100	2809135
R^2	0.999	
Slop	28549	
Intercept	25566	

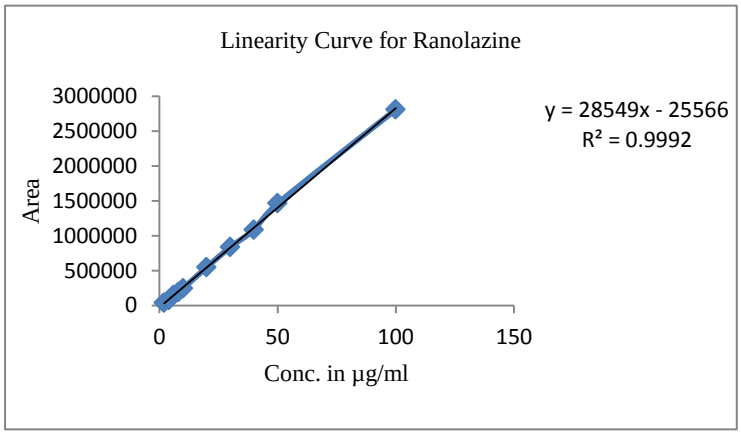

Figure 3: Linearity curve for Ranolazine

Limit of detection (LOD)

Limit of detection was calculated by standard deviation method Ranolazine. LOD for Ranolazine was found to be 0.616.

Limit of Quantification (LOQ)

Limit of Quantification was calculated by standard deviation method Ranolazine. LOQ for Ranolazine was found to be 1.86.

Precision

The precision of the method was demonstrated by variation studies. In the intraday studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. Then six samples were injected. % RSD was calculated as 0.798%. Area of six injected sample are presented in Table 2.

Table 2: Precision result for Ranolazine

Sr. no	Area of sample	Wt. of sample (mg)	Calculated assay value (mg)	Average value (mg)	Std. deviation	%RSD	Retention time
1	166393	105.39	502.17	505.965	4.0416	0.798	5min
2	168091	105.37	507.29				
3	169526	105.35	511.62				
4	169530	105.38	508.95				
5	168424	105.37	505.27				
6	166951	105.36	500.85				

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in temperature 30 to 25 and flow rate .8ml/min to 1ml/min. Concentration of Ranolazine was not changed. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results are shown in Table 3.

Table 3: Robustness result for Ranolazine

Sr. no	Area of sample	Wt. of sample (mg)	Calculated assay value (mg)	Avg.value	Std. deviation	% RSD
1	166162	105.39	505.20	496.25	4.8929	0.98
2	161759	105.37	491.81			
3	161777	105.38	491.87			
4	163398	105.36	496.80			
5	163198	105.35	496.19			
6	163010	105.37	495.62			

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100, and 120% of the standard concentration of Ranolazine (20µg/ml). Recovery was found range 99.86 -99.97%. The results are shown in Table 4.

Table 4: Accuracy result for Ranolazine

Sample	Area of sample	Theoretical value (mg)	Experimental value (mg)	% 0f recover	Mean
80% S -1	153129	401.11	401	99.97	
80% S -2	152930	401.11	399.8	99.94	99.86
80% S -3	152530	401.11	400.9	99.94	
100% S -1	163398	501.38	501.10	99.92	
100% S -2	163198	501.38	500.98	99.92	99.92
100% S -3	163010	501.38	501.00	99.67	
120% S -1	172535	601.66	601.33	99.95	
120% S -2	173515	601.66	601.52	99.98	99.97
120% S-3	173711	601.66	601.61	99.99	

CONCLUSION

A rapid and simple RP-HPLC method for determination of Ranolazine has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including linearity, accuracy, sensitivity, precision and robustness. The chromatographic run time of 5 min allows the analysis of a large number of samples in a short

period of time. Therefore, the method is suitable for analysis of large samples during routine analysis of formulations and raw materials.

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