



Development and validation of stability indicating RP-LC method for estimation of ranolazine in bulk and its pharmaceutical formulations

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of Ranolazine in Bulk and its pharmaceutical formulation. Separation was achieved with a Symmetry RP-18 ((Make: Waters Corporation; 150 mmx4.6 mm I.D; particle size 3 μ m)) Column and Potassium dihydrogen phosphate monohydrate buffer (pH adjusted to 3.0 with diluted orthophosphoric acid): methanol: acetonitrile (400:400:200) v/v as eluent at a flow rate of 1.2 mL/min. UV detection was performed at 225nm. The method is simple, rapid, and selective. The described method of Ranolazine is linear over a range of 11.98 μ g/mL to 37.92 μ g/mL. The method precision for the determination of assay was below 1.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.1 to 100.9%. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Ranolazine in bulk, its capsule dosage forms.

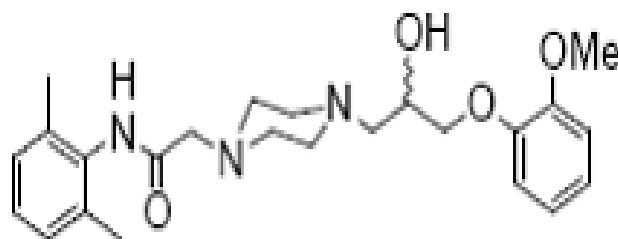
Key words: Ranolazine, RP-LC, Validation, Dosage form.

INTRODUCTION

Ranolazine¹⁻³ (Fig.1) a piperazine derivative is a new anti-anginal agent approved for the treatment of chronic stable angina pectoris. Ranolazine has anti-anginal and anti-ischemic effects that do not depend upon reductions in heart rate or blood pressure. Ranolazine reduces the late sodium current and, is expected to decrease sodium entry into ischemic myocardial cells. As a consequence, ranolazine is proposed to reduce calcium uptake indirectly via the sodium/calcium exchanger. Ranolazine, chemically is (RS)-N-(2, 6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazin-1-yl]acetamide, which was initially known to prolong the QT interval. The drug can be used in combination with other anti angina drugs for patients who have not achieved an adequate response

The authors have developed a new, simple and fast analytical method by RP-LC to quantify Ranolazine in bulk and its dosage forms. This validation study is carried out as per ICH guidelines.

Figure-1: Chemical Structure of Ranalozine



EXPERIMENTAL SECTION

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695 pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Symmetry RP-18 ((Make: Waters Corporation, Ireland); 150 mmx4.6 mm I.D; particle size 5 μ m)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Chemicals and solvents

Milli-Q Water, Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Orthophosphoric acid (GR Grade), Potassium dihydrogen phosphate monohydrate (GR Grade) were obtained from Qualigens Ltd., Mumbai.

Buffer preparation

Accurately weigh and transfer about 2.72 grams of Potassium di-hydrogen phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 3.0 (± 0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45 μ m membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer, Methanol and Acetonitrile in the ratio of 400:400:200 v/v respectively.

Diluent preparation

Mobile phase is used as diluent.

Standard preparation: (For Ranalozine Tablets 500mg)

Accurately weigh and transfer about 100.0mg of Ranalozine into a 200 mL volumetric flask, add 160 mL of mobile phase and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent (Mobile Phase).

Sample preparation: (For Ranalozine Tablets 500mg)

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 100 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of mobile phase, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with mobile phase and mix. Filter the solution through 0.45 μ m membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Chromatographic conditions

An Symmetry RP-18 ((Make: Waters Corporation (Ireland); 150 mmx4.6 mm I.D; particle size 3 μ m)) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.2mL/min. The sample injection volume was 20 μ L. The photodiode array detector was set to a wavelength of 225nm for the detection and Chromatographic runtime was 10minutes.

RESULTS AND DISCUSSION

Method development⁵⁻⁷

To develop a suitable and robust LC method for the determination of Ranalozine, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Symmetry RP-18 ((Make: Waters Corporation (Ireland); 150 mmx4.6 mm I.D; particle size 3 μ m)) with the following mobile phase. Accurately weigh and transfer about 2.72 grams of Potassium di-hydrogen phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 3.0 (\pm 0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45 μ m membrane filter. Prepare a filtered and degassed mixture of Buffer and methanol in the ratio of 500:500 v/v respectively.

Ranalozine peak was eluted at void volume. For next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer and methanol in the ratio of 400:600 v/v. above trial the peak shape was little broad. Again the mobile phase composition changed slightly to Buffer: Methanol: Acetonitrile in the ratio of 400:400:200 v/v respectively as eluent at flow rate 1.2 mL/min. UV detection was performed at 225nm. The retention time of Ranalozine is 4.0 minutes (refer Fig-4.) and the peak shape was good.

The chromatogram of Ranalozine standard using the proposed method is shown in Fig-4. System suitability results of the method are presented in Table-1. Ranalozine shows significant UV absorbance at Wavelength 225nm. Hence this wavelength has been chosen for detection in analysis of Ranalozine.

Method validation 5-7

The developed RP-LC method extensively validated for assay of Ranalozine using the following parameters.

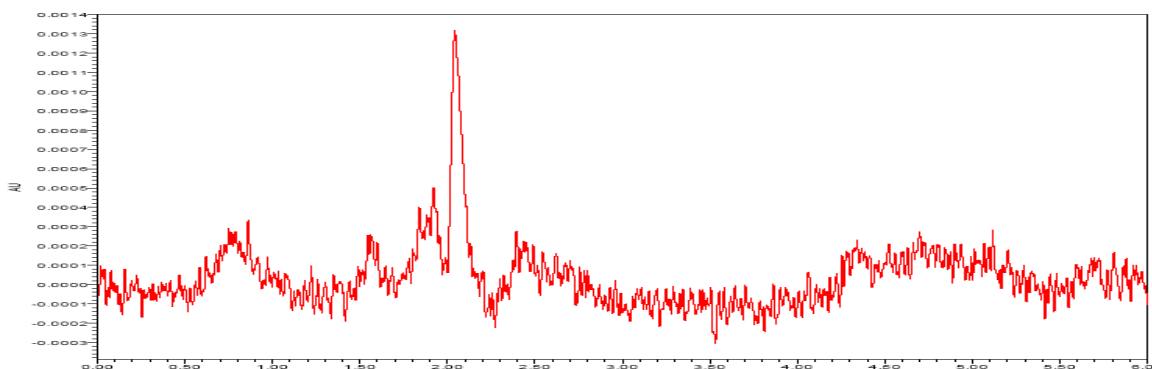
Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solutions showed no peaks at the retention time of Ranalozine peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Ranalozine in Ranalozine tablets.

The chromatogram of Ranalozine Blank using the proposed method is shown in Fig- 2.

Figure 2: A typical HPLC Chromatogram showing the no interference of diluent for Ranalozine



The chromatogram of Ranalozine Placebo using the proposed method is shown in Fig-3.

Figure 3: A typical HPLC Chromatogram showing the no interference of placebo for Ranalozine

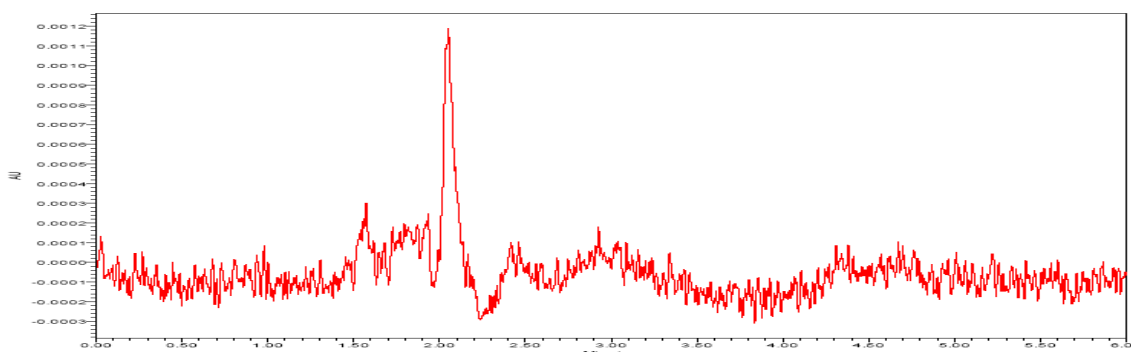


Figure 4: A typical HPLC Chromatogram showing the peak of Ranalozine

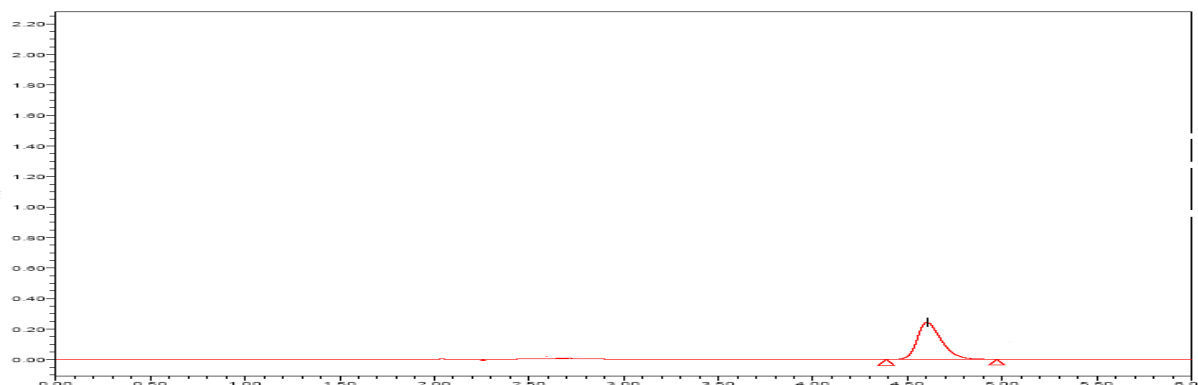


Table 1: System suitability parameters for Ranalozine by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Ranalozine	6821	1.35

Forced Degradation:

Control Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 μm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Acid Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of diluent , and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 μm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Base Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of diluent , and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N Acid (HCl) and dilute to volume with diluent and mix. Filter the solution through 0.45 μm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Peroxide Degradation Sample : Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of diluent , and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 2mL of 30% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 μ m membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Figure 5A: A typical HPLC Chromatogram showing the degradation profile of Ranalozine in Acid hydrolysis by proposed method

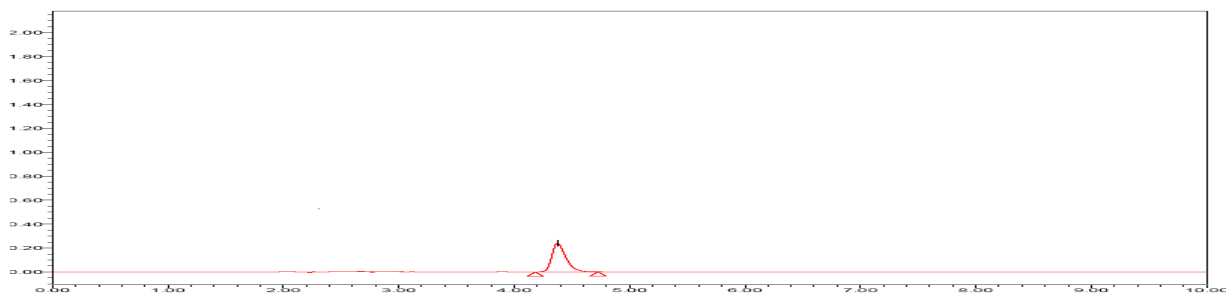


Figure 5B: A typical HPLC Chromatogram showing the degradation profile of Ranalozine in Base hydrolysis by proposed method

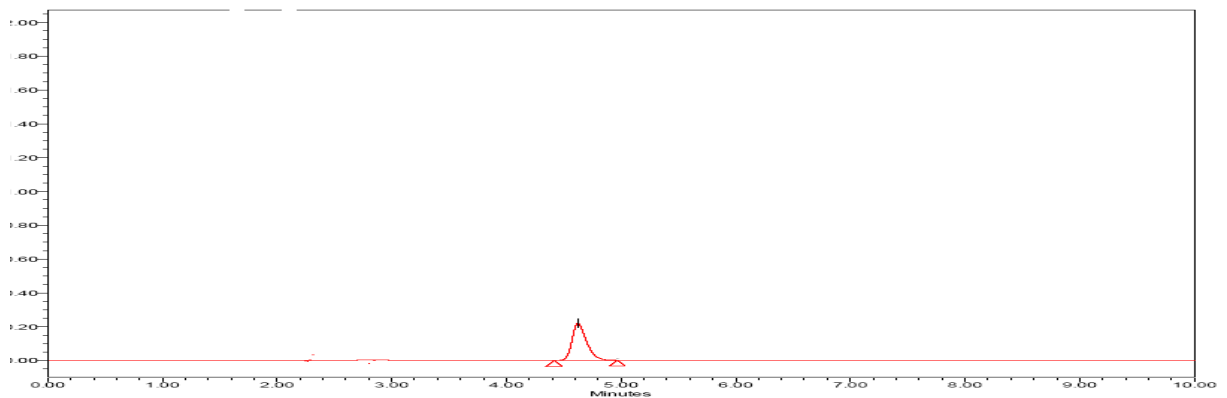


Figure 5C: A typical HPLC Chromatogram showing the degradation profile of Ranalozine in Peroxide hydrolysis by proposed method

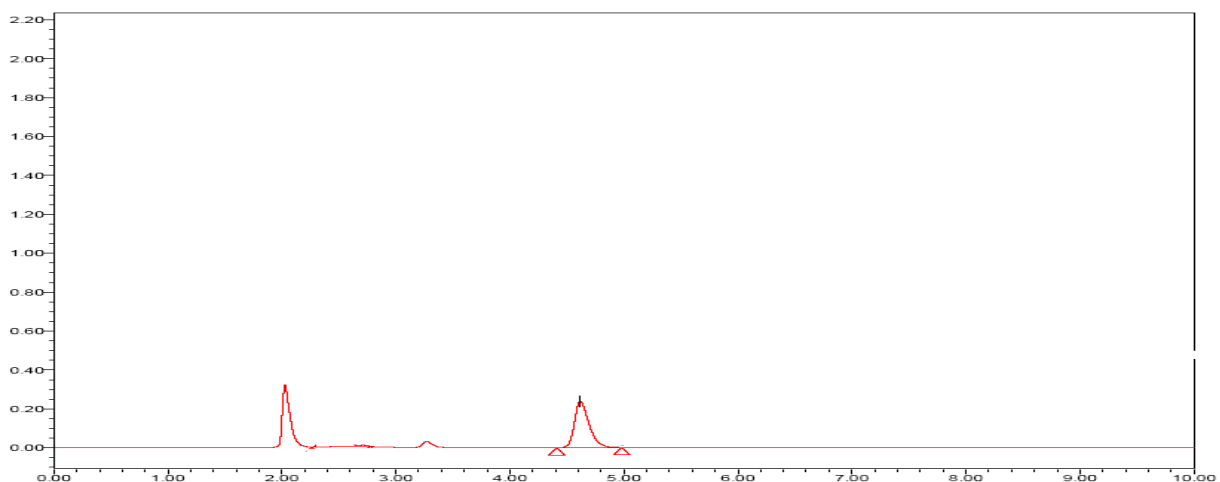
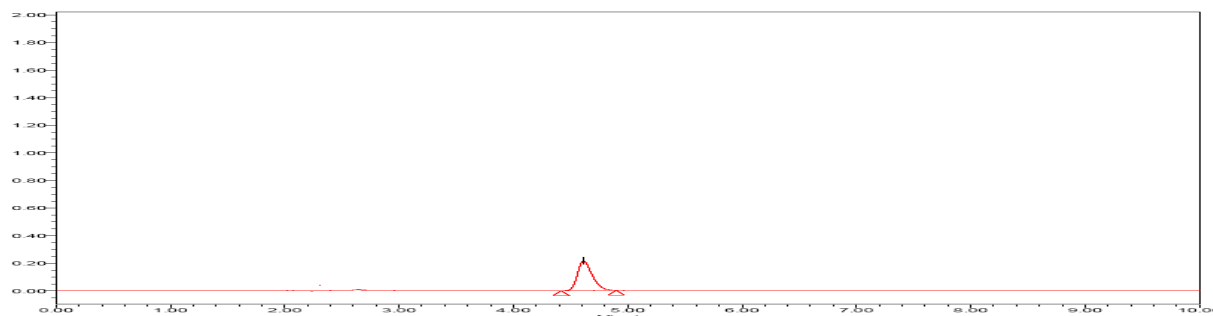


Figure 5D: A typical HPLC Chromatogram showing the degradation profile of Ranalozine in Thermal hydrolysis by proposed method



Thermal Degradation Sample: Powder collected from 20 tablets are exposed to heat at 105°C for about 5 days. Then Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranalozine into a 200 mL volumetric flask add about 160 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Table 2: Summary of the degradation profile of Ranalozine by proposed method

Name of the Sample	Condition	Purity angle	Purity threshold	% assay Celecoxib
Control Sample	N/A	0.247	0.321	99.4
Acid Degradation Sample	5mL, 5N HCl, 60°C/60min	0.125	0.129	99.1
Base Degradation Sample	5mL, 5N NaOH, 60°C/60min	0.347	0.367	98.5
Peroxide Degradation Sample	5mL, H ₂ O ₂ , 0°C/30min	0.314	0.363	98.9
Humidity Degradation Sample	@90% RH for 7 days	0.412	0.429	99.3
Thermal Degradation Sample	@105°C for 5 days	0.614	0.687	99.2
Photolytic Degradation Sample	1.2lak Lux units	0.178	0.249	99.4

From the above data of degradation profile it can be conclude that there is no interference found for of Celecoxib peak.

Precision

In the study of the instrumental system precision where, a RSD of 0.1% was obtained for the standard area obtained corresponding to the first day, being 0.3% for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.2% and the 95% confidence interval of 0.2 with the assay range of 99.9-100.4

Table 1: Method Precision (Inter and Intra) studies for Ranalozine by proposed method

Method Precision(Inter &Intra Day)	
100.3	100.2
99.9	100.3
100.3	99.8
100.4	100.4
100.4	100.6
100.1	99.3
Overall Average	100.2
Overage Std Dev	0.35
Over all %RSD	0.3

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 0.3 % (For Standard). The same study was carried out for different analysts ($n = 6$ number of samples per analyst) obtaining a RSD of 0.5 % (Intermediate Precision) and 95% confidence interval of 0.5 with the assay range of 99.8-100.6 The Overall %RSD for $n=12$ is 0.3. Both results

together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Ranalozine and analyzed as per the proposed method. The percentage recoveries with found in the range of 99.1 to 101.1 with an overall %RSD of 0.6. From the data obtained which given in table-2 the method was found to be accurate.

Table 2: Recovery studies for Ranalozine by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	99.1-100.0	0.5	0.6
100	100.9-100.9	0.1	
150	100.4-101.1	0.4	

Linearity of detector response

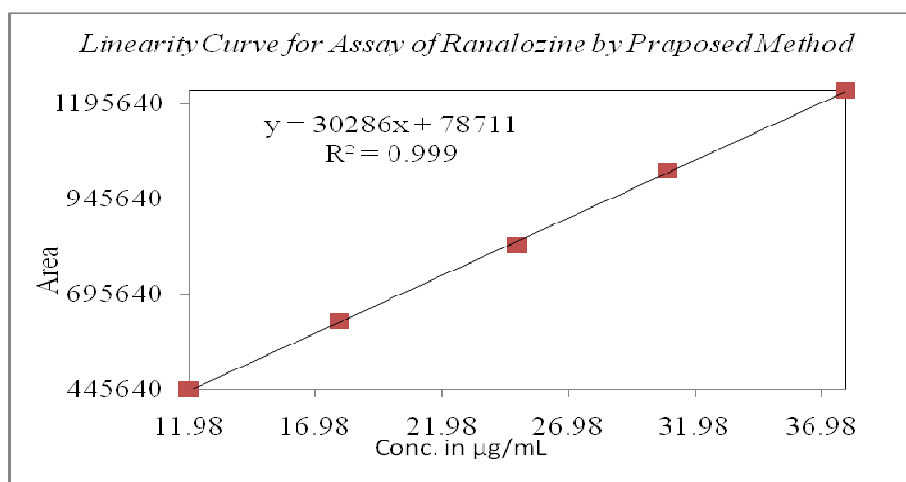
The standard curve was obtained in the concentration range of 12-40µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r²] of standard curve were calculated and given in figure-4 to demonstrate the linearity of the method.

From the data obtained which given in table-3 the method was found to be accurate.

Table 3: Linearity studies for Ranalozine by proposed method

Linearity of Response for Ranalozine		
% Level (Approx.)	Concentration (µg/ml)	Average Abs.
50	11.98	445640
75	17.96	623682
100	24.95	822063
125	30.94	1020352
150	37.92	1229691
Slope		30286
Intercept		78711
% Y-Intercept		7.7
STYEX		8089
CC		0.9997
RSQ		0.9995
Residual sum of squares		8089
LLD		0.34
LLQ		1.03

Figure 5: Calibration curve for Ranalozine



CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of Ranalozine in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Ranolazine. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Ranalozine in bulk, its pharmaceutical dosage forms.

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