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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RANOLAZINE IN BULK AND TABLET DOSAGE FORM

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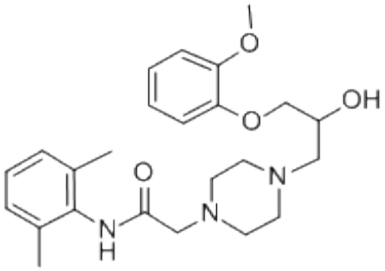
Abstract: An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Ranolazine in Bulk and its pharmaceutical formulation. Separation was achieved with a X-terra c_8 (Make: Waters Corporation; 150 mm × 4.6 mm I.D.; particle size 5 µm) Column and Sodium di-hydrogen phosphate buffer in water (pH adjusted to 5.0 with diluted orthophosphoric acid): Acetonitrile (600:400) v/v as eluent at a flow rate of 1.0 mL/min. UV detection was performed at 210 nm. The method is simple, rapid, and selective. The described method of Ranolazine is linear over a range of 50-150 µg/mL The method precision for the determination of assay was below 2.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 98% to 101%. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Ranolazine in bulk, its Tablets dosage forms.

Key words: RP HPLC, Ranolazine, Method development.

INTRODUCTION

IUPAC name of Ranolazine is (RS)-N-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl]piperazin-1-yl]acetamide, Molecular formula is C24H33N3O4, Molecular weight is 427.537g/mol. Ranolazine is known to increase the QT interval on the electrocardiogram.[1-2] While the mean increase in the corrected QT interval (QTc) is approximately 6 msec, about 5 percent of individuals may have QTc prolongations of 15 msec or longer. Extended QT intervals increase the risk of sudden cardiac death (SCD). The increase was 60% in adults, independently of other known risk factors, in an analysis of the Rotterdam Study.



Structure of Ranolazine

Literature reveals the methods like HPLC[3], LC-Mass[4-6] are available to estimate in dosage form and in biological fluid. Here, we tried to develop and validate a simple RP-HPLC method to estimate Ranolazine in bulk and tablet dosage form.

INSTRUMENTATION:

The analysis of drug was carried out on a waters HPLC system equipped with 2695 pump and 2998 photo diode detector was used and a Reverse phase HPLC column X-Terra c_8 ((Make: Waters Corporation, Ire-land); 150 mm × 4.6 mm I.D.; particle size 5 µm)) was used. The output of signal was monitored and integrated using Waters Empower 2 software.

MATERIALS AND METHODS:

Ranolazine were obtained as a memento sample from Dr. Reddy's, Hyderabad. Acetonitrile HPLC grade rankem New Delhi, milli-Q water it was purified by miili Pore Corporation's system mfg Barnstead, Methanol HPLC grade rankem, Ortho phosphoric acid AR grade, Fisher scientific pvt ltd. The analysis was carried out on HPLC Waters 2695 connected with PDA detector 2998 and Empower2 soft ware.

Chromatographic Conditions:

An X-terra ((Make: Waters Corporation (Ireland); 150 mm × 4.6 mm I.D.; particle size 5 µm)) Column was used for analysis at 25° c column temperature. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wave-length of 210 nm for the detection and Chromatographic runtime was 6 minutes.

Buffer Preparation:

Weigh accurately 2.725 Gms of NaH_2PO_4 in 1000ml of purified water and mix. Adjust pH to 5.0 with dilute ortho phosphoric acid solution. Filter the solution through 0.45µm membrane filter.

Preparation Of Mobile Phase:
Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 600:400(v/v) respectively.

Diluent Preparation:
Methanol is used as a diluent.

Preparation of standard solution:
Accurately weighed quantities, 50.8 mg of Ranolazine was transferred into 200ml of volumetric flask and add 50ml of methanol and sonicate for 20 mins make up the volume with methanol. Transfer above solution 5ml into 50ml volumetric flask and make up the volume with methanol.

Preparation of sample preparation:
An accurately weigh 20 tablets and calculate average weight of those tablets and crushed. Transfer the tablet powder equivalent weight of 135.1mg of Ranolazine. Into 200ml of volumetric flask add 30ml of methanol and sonicate for 20mins and shake 10mins and filter through the 0.45µm filter paper and make up the volume with methanol. Transfer the above solution 5 ml in to 50 ml volumetric flask dilute to volume with methanol.

METHOD VALIDATION [7-9]

Specificity:
To determine the specificity of the drug carried out by inject the blank, excipients standard one by one and same manner blank ,excipients and sample at this time blank and excipients peaks are not interference with standard and sample peaks. It proves method is highly selective.

Linearity:
Method linearity is performed by prepare 5 replicate samples in different concentration levels (50%, 75%, 100%, 125%, 150%) inject into HPLC system. Plot the graph concentration versus area and caluculate correlation co efficient.

Accuracy:
The method accuracy was determined by recovery studies using method of standard addition to pre analyzed formulation of Ranolazine. Known amount of three spike levels (50%,100%,150%) six replicate samples are inject into HPLC then accuracy was calculate as per test method assay results.

Precision:
Method precision was carried out by prepare six replicate samples from single formulation and samples run by on the same day and on three different days over a period of one week.

Robustness:
Method robustness was evaluated by carrying deliberate changes in flow rate ±0.2, temperature ±5 run the samples as per test method. Those results are compared to other trails they were not observed significant changes.

Limit of detection (LOD) and Limit of quantification (LOQ):
LOD and LOQ were calculated for the sensitivity of the method they were qualified based on the signal to noise ratio. LOD is lowest detectable concentration of analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines

LOD= 3.3× SD/slope

LOQ= 10×SD/slope

RESULTS AND DISCUSION:
System suitability results were given by table1 and table 2 six replicate standards system suitability parameters are retention time, tailing and plate count were shown uniformity and %RSD was less than2 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Ranolazine standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The method accuracy was evaluated by recovery studies those values are given by table3. Ranolazine recovery was founded 98-101% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate. Linearity calibration curve was given below fig: 3 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co efficient. Linear correlation was found to be (r=0.999). Precision results were shown by table 4. The intraday and inter day variations was calculated in terms of %RSD and results was found to be intraday and inter day respectively. Method robustness results was given by table5 they were not observed marked changes of those trails compared to other trails so it proves method was robust.Stability studies are given in table 7.

FORCED DEGRADATION STUDIES:
Control Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranolazine into a 100 ml volumetric flask add about 70 ml of methanol, and sonicate for 30 minutes with shaking at controlled temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5.0ml 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 Ranolazine into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with inter-mitten shaking at controlled temperature. Then add 10 mL of 5 N acid, refluxed for 30 min at 60˚C, then cooled to room temperature, neutralize with 5 N NaOH and di-lute to volume with methanol 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. (Refer fig 4)

Base Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranolazine into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 10 mL of 5 N Base (NaOH), refluxed for 30 min at 60˚C, then cooled to room temperature, neutralize with 5 N Acid (HCl) and dilute to volume with methanol 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. (Refer fig 5)

Peroxide Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Ranolazine into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with shaking at controlled temperature. Then add 2 ml of 30% Peroxide, refluxed for 30 min at 60^{o} c, then cooled to room temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5.0ml of the above solution into a 50ml volumetric flask and dilute to volume with diluent. (Refer fig 6)

Thermal Degradation Sample:

Powders collected from 20 tablets are exposed to heat at 105^{o}C for about 5 days. Then Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer equivalent to 50 mg of Ranolazine into a 100 ml volumetric flask add about 70 ml of methanol, and sonicate for 30 minutes with shaking at controlled temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5ml 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. (Refer fig 7 & 8)

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

Table 1: System Suitability Test Parameters for the proposed method

Parameters	Ranolazine
Retention Time (min)	2.50
Theoretical plates	4660
Tailing factor	1.15

Table2: Standard 2 result of Ranolazine

	Sample name	inj	Name	RT	Area	Tailing	Plate count
1	STD2	1	Ranolazine	2.530	701835	1.15	4738
2	STD2	2	Ranolazine	2.531	698249	1.15	4736
3	STD2	3	Ranolazine	2.532	699522	1.16	4713
4	STD2	4	Ranolazine	2.532	705600	1.17	4759
5	STD2	5	Ranolazine	2.531	719990	1.17	4745
Mean					705039		
Std.dev					8813		
%RSD					1.2		

Table3: Accuracy results of Ranolazine

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	65.40	327219	23.2585	23.3413	100.36	101
50%	65.60	328213	23.3296	23.4122	100.35	
50%	65.50	335589	23.294	23.9383	102.77	
50%	65.40	336037	23.2585	23.9703	103.06	
50%	65.40	321730	23.2585	22.9497	98.67	
50%	65.30	329275	23.2229	23.4879	101.14	
100%	135.30	660616	48.1173	47.1232	97.93	98
100%	135.30	654528	48.1173	46.6889	97.03	
100%	135.30	661256	48.1173	47.1689	98.03	
150%	215.40	1076738	76.6035	76.8061	100.26	100
150%	215.40	1080536	76.6035	77.077	100.62	
150%	215.40	1067415	76.6035	76.1411	99.40	
150%	215.30	1069186	76.568	76.2674	99.61	
150%	215.30	1064157	76.568	75.9087	99.14	
150%	215.20	1072985	76.5324	76.5384	100.01	

Table4: precision results of Ranolazine

S.NO	Sample weight	Area	%Assay
1	135.1	673050	101
2	134.9	671990	101
3	135.2	679581	102
4	135.1	659243	99
5	135.0	665267	100
6	135.2	674318	101
Mean		670575	101
Std.dev		7209	1.05
%RSD		1.1	1.05

Table5: Robustness of Ranolazine

Sl. No.	Sample name	change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Ranolazine	2.532	671990	1.16	4679
2	Flow2	1.2ml/min	Ranolazine	2.530	679581	1.17	4639
3	Temp1	20^{o} C	Ranolazine	2.530	659243	1.18	4696
4	Temp2	30^{o}C	Ranolazine	2.530	665267	1.17	4639

Table 6: stability studies

	Sample weight	Area	%Assay	%deg
Acid	150.0	1346184	91	10
Base	150.0	788196	53	48
Peroxide	160.0	1543072	98	3
water	150.0	1461190	99	2
light	180.0	1755062	99	2

Fig:1 A typical standard chromatogram of Ranolazine

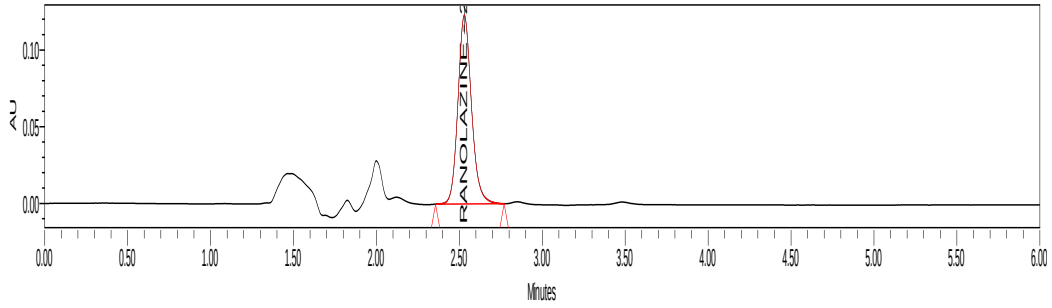


Fig:2 A typical formulation chromatogram of Ranolazine

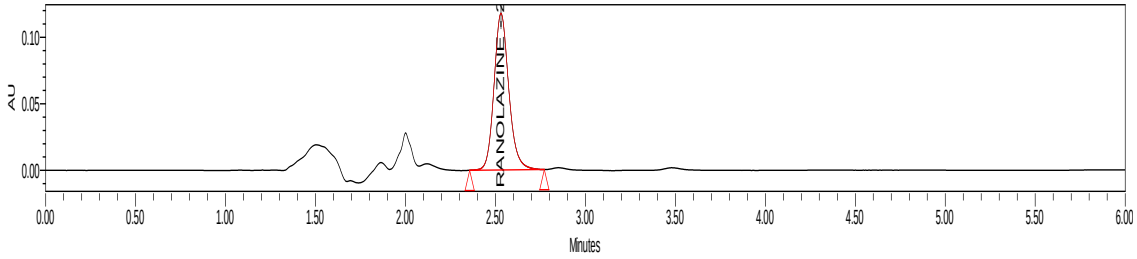


Fig:3 Calibration curve of Ranolazine

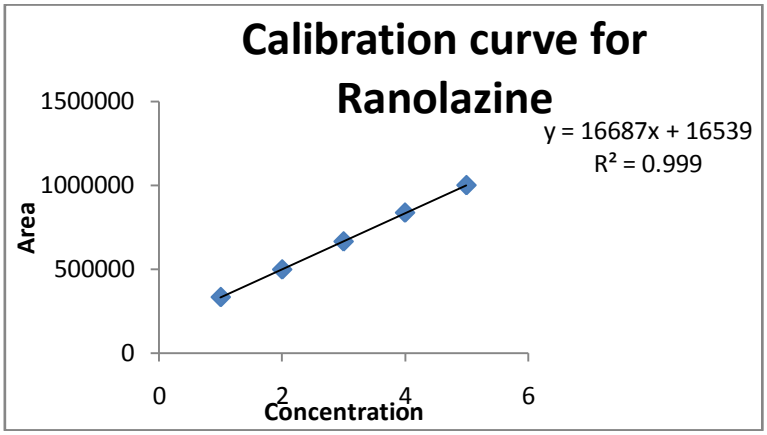


Fig: 4 Acid

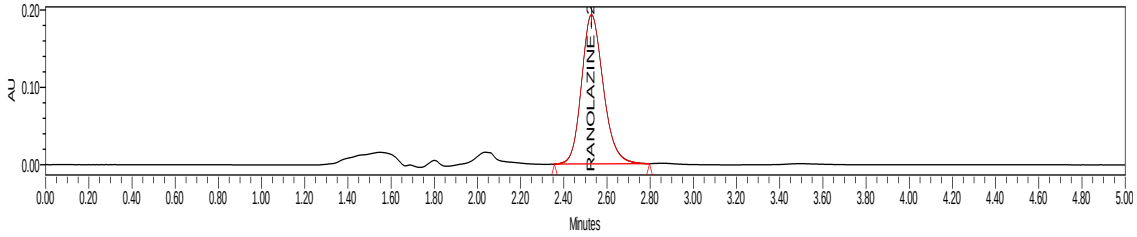


Fig: 5 Base

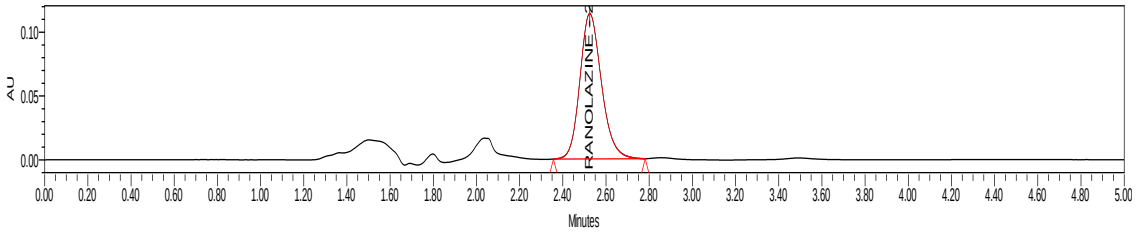


Fig 6: Peroxide

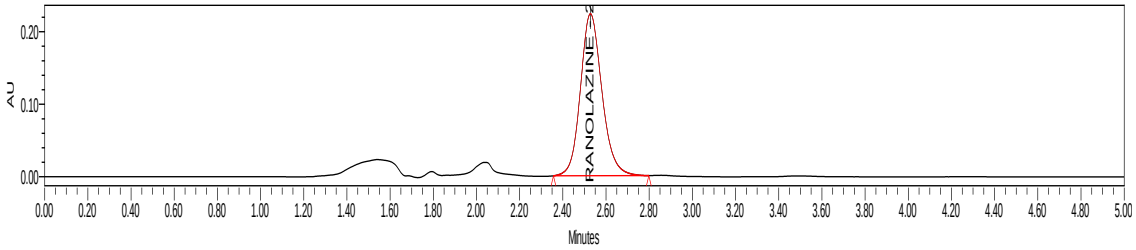


Fig: 7 Light

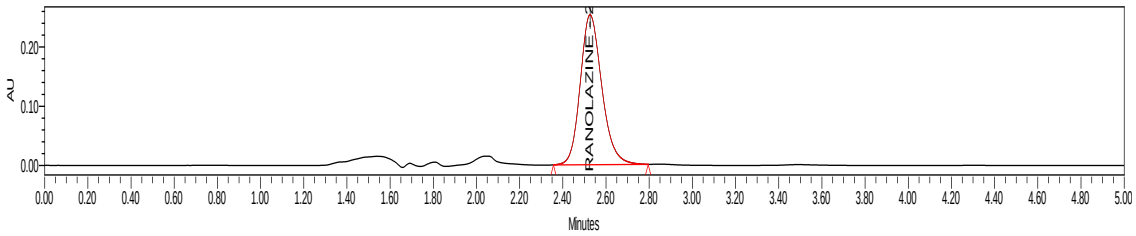
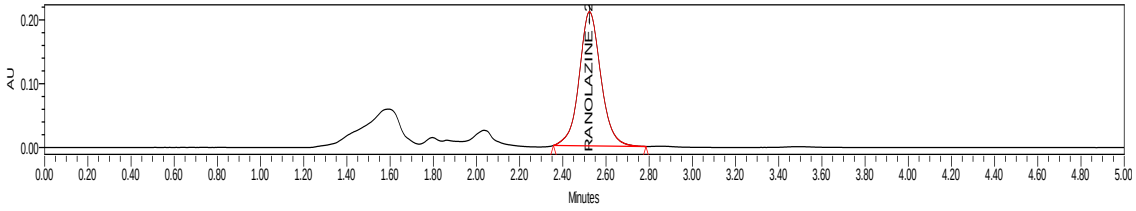


Fig: 8 Water



CONCLUSION:

The %RSD was very low and also standard deviation as required by ICH guidelines it indicates high degree of precision. The accuracy results was found within the limit hence it proves method is highly validated so it use full quality control and stability departments for estimation of Ranolazine bulk and tablet dosage forms.

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