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Cost Effective, Efficient and New Reverse Phase High Performance Liquid Chromatographic Method Validation for the Determination of Assay of Ranolazine in Drug Substances

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ABSTRACT: A simple, selective, linear, precise and accurate RP-HPLC method was validated for rapid assay of Ranolazine in drug substances. Isocratic elution at a flow rate of 1ml/min was employed on a BDS Hypersil C18, 150 x 4.6 mm, 5µm or Equivalent column at 40°C temperature. Sample cooler temperature was 10°C and runtime for the analysis of assay method is 10.0 minutes. The mobile phase consisted of phosphate buffer with pH 7.0 as mobile phase A and Acetonitrile as mobile phase B with ratio Mobile phase-A: Mobile phase-B (40:60). The UV detection wavelength was at 205 nm. Linearity was observed in concentration range of 25-75 ppm. The retention time for Ranolazine was about to 5.0 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Ranolazine in pharmaceutical drug substances in routine analysis.

KEY WORDS: Ranolazine, Method Validation, Assay, Drug substances.

I. INTRODUCTION:

This research manuscript is based on the study of analytical method validation of assay of the Ranolazine in drug substances by HPLC. Ranolazine, a piperazine derivative sold under the trade name Ranexa, is a well- tolerated medication that selectively inhibits the late sodium current. Additionally, ranolazine has beneficial metabolic properties and does not affect heart rate or blood pressure. Ranolazine is currently approved in the United States and Europe as a second- line agent in the management of chronic stable angina pectoris (CSAP). It is not currently approved for use by Health Canada and requires an application through the Special Access Programme. In the European Society of Cardiology (ESC) guidelines on the management of stable angina, ranolazine is given a class IIa (level of evidence B) recommendation as a second-line agent for the relief of angina and ischaemia [1-3]. Chronic stable angina pectoris (CSAP) is estimated to affect >7 million North Americans, and is associated with significant morbidity. 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 $C_{24}H_{33}N_3O_4$, Molecular weight is 427.537g/mol. Ranolazine is known to increase the QT interval on the electrocardiogram[4-6].

The scope of this paper is to conclude the suitability of the method for routine analysis, to prove that the method is stability indicating and meeting the requirements of ICH guidelines. The method is a non-compendia method and is developed in-house.

II. METHODOLOGY:

Chromatographic Conditions:

Column : BDS Hypersil C18, 150 x 4.6 mm, 5µm or Equivalent
Flow rate : 1.0 ml/min.
Wavelength : 205 nm
Column Temperature: 40°C
Injection volume : 10 µl
Run time: 10 minutes.
Sample cooler temperature: 10°C
Mobile phase: Mobile phase-A: Mobile phase-B (40:60)
Rinse/wash solvent: Mixture of 20 volumes of water and 80 volumes of Acetonitrile.

Preparation of Buffer solution: Weighed accurately and transferred 1.41g of disodium hydrogen orthophosphate in 1000 ml water, mixed. Adjusted pH to 7.0 with diluted O-phosphoric acid solution. Filtered through 0.45 µ nylon filter and degassed it.

Mobile phase A: Buffer solution.

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Mobile phase B: Acetonitrile.
Diluent: Acetonitrile

Standard preparation: Weigh accurately 50 mg of working standard and transfer into 100.0 ml of clean, dry volumetric flask, add 50 ml of diluent and sonicate to dissolve. Allow to equilibrate to room temperature and makeup to the volume with the diluent. Transfer 5.0 ml of this solution to 50.0 ml of clean, dry volumetric flask and dilute up to the mark with diluent.

Sample preparation: Weigh accurately 50 mg of sample and transfer into 100.0 ml of clean, dry volumetric flask, add 50 ml of diluent and sonicate to dissolve. Allow to equilibrate to room temperature and makeup to the volume with the diluent. Transfer 5.0 ml of this solution to 50.0 ml of clean, dry volumetric flask and dilute up to the mark with diluent.

System suitability Criteria: The % RSD of five replicate injection of standard preparation, should not be more than 2.0 and the cumulative %RSD for five replicate injection of standard preparation including bracketing standard should not be more than 2.0**.** The retention time of Ranolazine is about 5.0 min.

Calculation:

% Assay (as such)

$$\frac{A \times W1 \times 5 \times 100 \times 50 \times P}{B \times 100 \times 50 \times W2 \times 5}$$

A = Area response of Ranolazine peak in the sample preparation chromatogram
B = Area response of Ranolazine peak in the standard preparation chromatogram
W1 = Weight of Ranolazine reference/ working standard taken in mg
W2 = Weight of sample taken in mg
P = Potency of Ranolazine reference/ working standard used (as such)

Materials, Chemical, Reagents, Equipment's and Column use: The details of the standards, chemicals/Reagents, Instruments and Accessories used in the method validation study are reported here under.

Table-1a: Details of Reference standard, chemicals and reagents used

S. No	Chemicals/Reagents	Grade/potency	Make	B. No
1	Disodium hydrogen orthophosphate	GR	Merck	DG0D701542
2	Acetonitrile	HPLC	Rankem	R072G20
3	Water	Milli Q	-	-
4	Ortho phosphoric acid	AR	Rankem	R045C20
5	Reference Standard	99.6	-	RNZ/024/19

Table-1b: Details of the instruments

S. No	Equipment	Make	Model	Identification No.
1	HPLC	Waters	Alliance	AR/HPLC/018
2	HPLC	Waters	Alliance	AR/HPLC/022

III. RESULT AND DISCUSSUION

Specificity/Selectivity: A blank, standard preparation, sample solution injected as per method. System suitability criteria meet as per method. There is no interference from the blank at the retention time of Ranolazine peak. All the observed results are well within the acceptance criteria. Therefore, the method can be termed as specific. The system suitability criteria is observed and recorded in the below table.

Table-2: Details of the system suitability

Identification	%RSD of standard solution	
	Observed	Limit
Standard preparation	0.18 (for five replicate injection) 0.22 (five replicates and including bracketing standard)	Not more than 2.0

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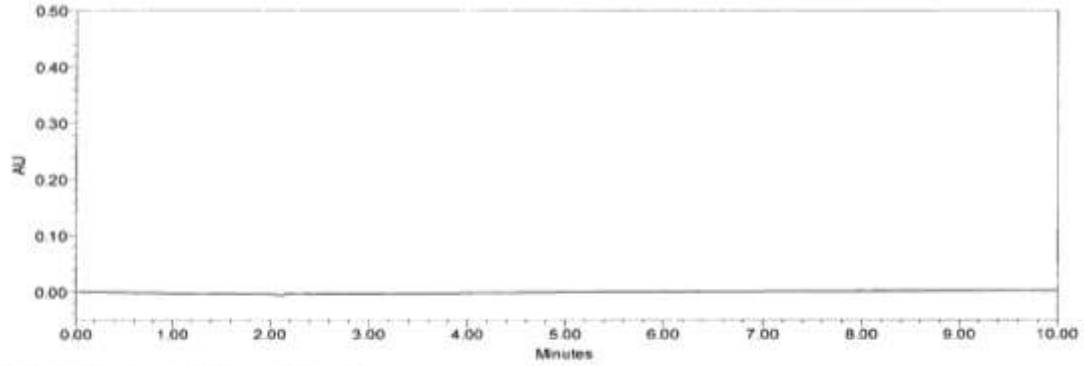
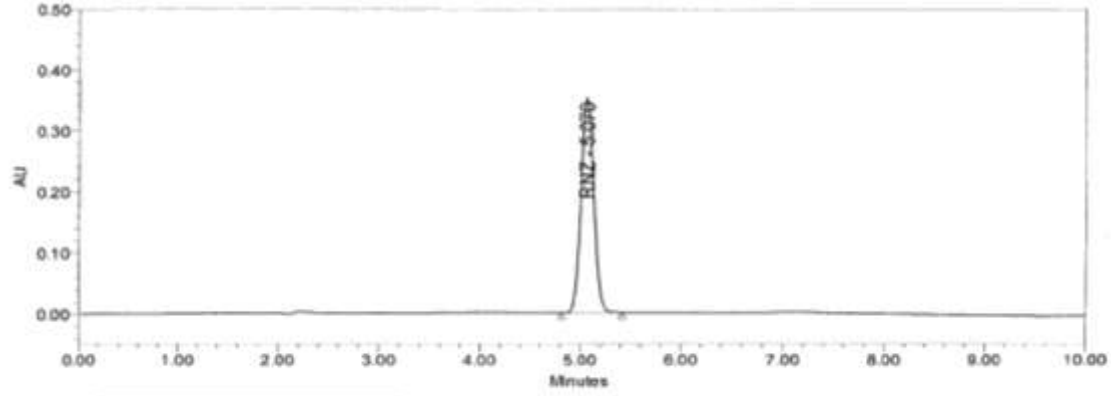


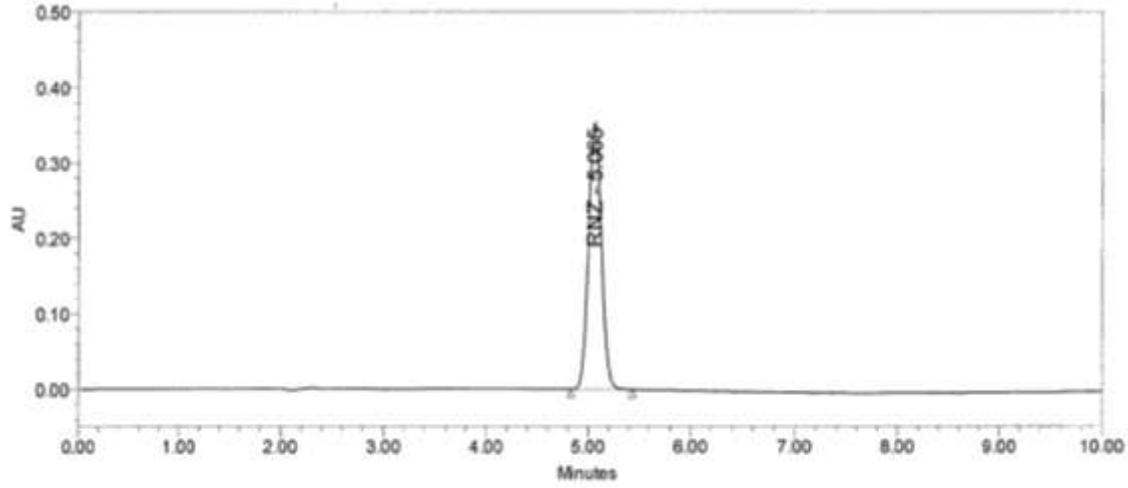
	Peak Name	RT	Area	% Area
1	RNZ	5.065		

Figure 1: Blank Chromatogram of Ranolazine



	Peak Name	RT	Area	% Area
1	RNZ	5.070	3107784	100.00

Figure 2: Standard Chromatogram of Ranolazine



	Peak Name	RT	Area	% Area
1	RNZ	5.065	3107837	100.00

Figure 3: Sample Chromatogram of Ranolazine

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Solution stability at 25°C: Solution stability was performed at the different time intervals like 0hr (Initial) and 12 hours by injecting the same test sample prepared initially while blank, standard preparation prepared freshly and % assay is calculated. The % assay at different time interval is within the limit. The % assay at different time interval is within the limit, hence it is concluded that solution is stable up to 12 hours at 25°C.

Table-3: Details of time intervals and % assay

Time interval	%Assay	Limit
0 hr (Initial)	99.3	98.0 to 102.0
12 hours	99.9	

Linearity and Range: Linearity was determined at five levels over the range of 50% to150% of concentration of sample. A standard stock solution was prepared and further diluted to attain concentration at about 50%, 80%, 100%, 120% and 150% of sample concentration. Each standard preparation was injected in triplicate. The average area of each level was recorded, and a graph of average area verses concentration plotted. The linear correlation co-efficient (r) for assay was found greater than 0.99. The correlation coefficient value is found well within acceptance criteria. Hence the method can be considered as linear over the considered range. The slope of regression line, residual sum of squared was calculated and recorded.

Table-4: Details of the system suitability

	%RSD of standard solution	
Identification	Observed	Limit
Standard preparation	0.18 (for five replicate injection) 0.22 (five replicates and including bracketing standard)	Not more than 2.0

Table-5: Details of Linearity levels, concentration, and area response

S. No:	Linearity Levels	Conce. (ppm)	Area	Mean Area
1	50%	25.125	1570152	1571022
			1572647	
			1570268	
2	80%	40.200	2528460	2525952
			2524409	
			2524988	
3	100%	50.250	3099868	3101098
			3097130	
			3106296	
4	120%	60.300	3773672	3774057
			3779846	
			3768653	
5	150%	75.375	4677396	4691909
			4680543	
			4717787	
Correlation coefficient				0.999860

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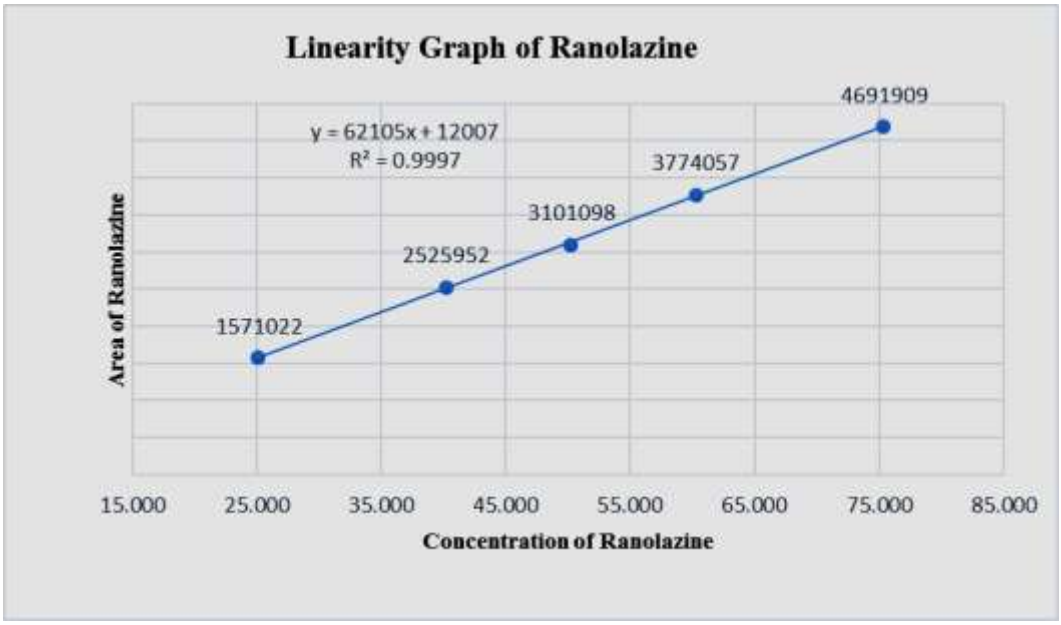

Figure 4: Linearity graph of Ranolazine

Precision:

Method precision: Method has been established by analyzing six sample preparations under same conditions. Six replicates of sample were prepared by one analyst and injected on the same instrument and on the same day.

Intermediate Precision/Ruggedness: Six replicates of sample were prepared by different analyst and injected on the different instrument, different day using different column and on same laboratory. The % RSD of six assay result is found 2.0. The % Assay obtained is found between 98.0-102.0 %. The results obtained are within acceptance criteria. Therefore, method can be termed as precise and rugged. % of assay and % RSD were calculated and recorded in the below table.

Table-6: Details of the system suitability

Identification	%RSD of standard solution	
	Observed	Limit
STD Prep (Method Precision)	0.18 (for five replicate injection) 0.22 (five replicates and including bracketing standard)	Not more than 2.0
STD Prep (Intermediate Precision)	0.25 (for five replicate injection) 0.26 (five replicates and including bracketing standard)	

Table-7: Area of Ranolazine in standard solution

S. No	Method Precision	Ruggedness
	Standard area	
1	3107784	3107574
2	3109736	3109630
3	3114096	3124086
4	3107475	3108411
5	3120886	3120986

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Mean	3111995	3114137
% RSD	0.18	0.25

Table-8: Details of the sample area and %assay

Name of Sample	Method Precision			Intermediate Precision		
	Wt.(mg)	Area	% Assay	Wt.(mg)	Area	% Assay
Sample preparation 1	50.22	3112767	99.7	50.20	3112707	99.8
Sample preparation 2	50.25	3105795	99.4	50.21	3106295	99.6
Sample preparation 3	50.12	3097108	99.4	50.25	3098218	99.2
Sample preparation 4	50.18	3103363	99.5	50.21	3103452	99.5
Sample preparation 5	50.11	3097758	99.4	50.11	3088788	99.2
Sample preparation 6	50.18	3111083	99.7	50.12	3111003	99.9
	Average		99.5	Average		99.5
	Std.Dev		0.148	Std.Dev		0.285
	% RSD		0.15	% RSD		0.29
	Overall mean		99.5			
	Std.Dev		0.222			
	% RSD		0.22			

Accuracy: Accuracy was performed at 80%, 100% and 120% of sample concentration. These three different levels were prepared in triplicate and injected. The average recovery of assay in each level is found between 98.0 - 102.0%. The results for % individual recovery and % mean recovery are well within acceptance criteria; therefore, the method can be termed as accurate over the considered range. The % individual accuracy and % mean accuracy for each level was calculated and recorded in the below tables.

Table-09: Details of the system suitability

Identification	%RSD of standard solution	
	Observed	Limit
Standard preparation	0.18 (for five replicate injection) 0.22 (five replicates and including bracketing standard)	Not more than 2.0

Table-10: Details of the accuracy levels, %assay and mean

Accuracy Levels (%)	Wt. in mg	Area	Added in ppm	Recovered in ppm	%Recovery	Mean Recovery (%)
Accuracy-80%-1	40.50	2518683	40.50	40.67	100.4	100.6
Accuracy-80%-2	40.65	2530820	40.65	40.87	100.5	

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Accuracy-80%-3	40.27	2511538	40.27	40.55	100.7	
Accuracy-100%-1	50.32	3106830	50.32	50.17	99.7	99.8
Accuracy-100%-2	50.41	3125995	50.41	50.48	100.1	
Accuracy-100%-3	50.30	3104943	50.30	50.14	99.7	
Accuracy-120%-1	60.07	3776071	60.07	60.97	101.5	100.9
Accuracy-120%-2	60.21	3765281	60.21	60.80	101.0	
Accuracy-120%-3	60.30	3746429	60.30	60.49	100.3	

Robustness: The robustness of the method was established by making small but deliberate variations in the following method parameters. Change in flow rate of mobile phase to 0.9 ml /min and 1.1 ml/min and Change in column oven temperature to 39°C to 41°C. System suitability criteria meet as per method. % Assay in all changed conditions are between 98.0 -102.0 %. All parameters meet the acceptance criteria. Hence the method can be termed as robust. The effect of changes was observed on system suitability values and recorded in the below tables.

Table-11: Details of different parameter of robustness, conditions and % assay

Parameter	Condition	% Assay
Normal condition	1.0ml/min	99.50
Deliberate condition	0.9ml/min	99.40
Difference from normal condition	-0.1ml/min	0.10
Deliberate condition	1.1ml/min	99.70
Difference from normal condition	+0.1ml/min	-0.20
Normal condition	40°C	99.50
Deliberate condition	39°C	99.10
Difference from normal condition	-1°C	0.40
Deliberate condition	41°C	99.1
Difference from normal condition	+1°C	0.40

Table- 12 Comparison of %RSD of different robustness parameters

Parameter	Condition	%RSD Standard solution	Cumulative %RSD
Normal condition*	As per method	0.18	0.22
Deliberate condition	0.9ml/min	0.29	0.29
Deliberate condition	1.1ml/min	0.22	0.20
Deliberate condition	39°C	0.23	0.22
Deliberate condition	41°C	0.06	0.06

* The initial data taken from average % Assay of method precision.

IV. SUMMARY AND CONCLUSION:

A validated RP-HPLC method has been developed for the determination of Assay of Ranolazine in drug substances. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Ranolazine quality control laboratory and stability analysis. Since the results were within acceptance criteria for all validation parameters, the method is considered as validated and suitable for intended use. Hence considered as stability indicating.

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CONFLICT OF INTEREST: Author has no conflict of interest during the preparation of this research manuscript.

ABBREVIATIONS

°C : Degree Centigrade
HPLC: High performance liquid chromatography
Hrs: Hours
ICH: International Conference on Harmonization
µg : Micro gram
NA: Not applicable
ND: Not detected
NLT: Not Less Than
NMT: Not More Than
% : Percentage
RSD: Relative Standard Deviation

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