RP HPLC Method Development & Validation for Ranolazine in Pharmaceutical Formulation

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INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.

FACTOR AFFECTING

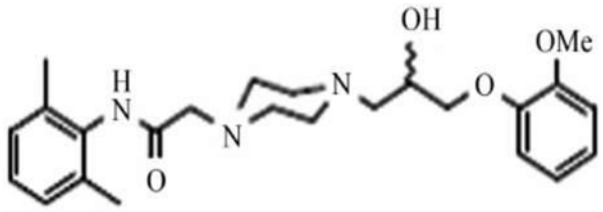
Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. An important task for the analyst is to select best procedure for a given determination this will require careful consideration of the following criteria:

a) The type of analysis required: elemental or molecular, routine or occasional.
b) Problem arising from the nature of the material to be investigated, e.g. radio- active substance, corrosive substance, substances affected by water.
c) Possible interference from components of the material other than those of interest.
d) The concentration range to be investigated.
e) The accuracy required.
f) The facilities available, particularly the instrument.
g) The time required to complete the analysis.

The various components of HPLC are

1. Mobile Phase Reservoir and solvent System treatment
2. Pumps (Displacement Pump, Reciprocating Pump, Pneumatic Pump)
3. Sample Injectors
4. Pre columns
5. Liquid chromatographic column (Analytical Column)
6. Detectors

DRUG PROFILE- Ranolazine:
Molformula-C24H33N3O4 Mol weight-427.53g/mol



- IUPAC Name-
- N-(2, 6 -dimethylphenyl)-2-{4-[2-hydroxy- 3-(2-methoxyphenoxy)propyl]- 1piperazin-1-yl} acetamide.
- Solubility-soluble in dichloromethane and methanol, sparingly soluble in ethanol and acetone, very slightly soluble in water.
- PKa-7.2
- Description- White amorphous powder.
- Category- Anti anginal drug.
- Mechanism of action: The drug act by inhibiting sodium and potassium ion channel currents. This effect is obtained as a result of the inhibition of peak and late sodium channel which is in order increases myocardial function.

INSTRUMENT & CHEMICAL USED

AGILENT HPLC SYSTEM
Make: Agilent.
Specification: Gradient system with auto injector.
Pump: 1100- Reciprocating pump.
UV detector: UV (DAD).
Column: Fortis C18 (4.6 mm. x 100 cm.).
Flow rate range: 0.7 mL/min.

REAGENTS AND CHEMICALS USED:

In UV spectrophotometer method, methanol of AR grade was used.

In RP-HPLC method, Sodium citrate in citric acid of AR grade, where as methanol and buffer of HPLC grade were used (Research Laboratories Pvt. Ltd., Mumbai). Double distilled water was used during the project work, Whatmann filter paper Nylon 6, 6 was used for filtration.

For the purpose of project pure samples of drugs were obtained as gift sample from manufacturer and marketed formulation was procured from local market. They are listed in Table No. 03 and 04.

Name of Drug	Supplied by
Ranolazine	Unichem Labs Ltd.Mumbai.

Table No. 03: List of Pure Drugs and Suppliers

Brand Name	Combination Content (mg/tablet)	Mfg. Company	Batch No.	Mfg. Date	Exp. Date
RANX	500mg	Unichem Labs Ltd.India	BRXL18001	Feb 19	ept 22

Table No. 04: Marketed formulation selected for analysis

EXPERIMENTAL WORK AND RESULT

Selection of Analytical Wavelength

The maximum absorbance was found to be at 272 nm (Fig. 4) while scanned in UV spectrometer.

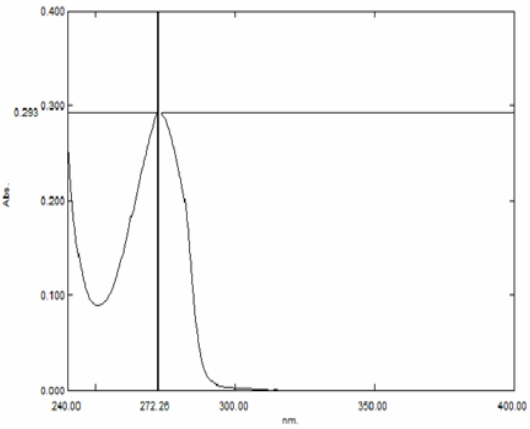


Fig.No.2: UV spectrum of standard Ranolazine

SELECTION OF EXPERIMENTAL CHROMATOGRAPHIC CONDITION

System: The Agilent 1100 Infinity LC Gradient System VL

Column: id 4.6 ×100 mm length

Detector: Variable Wavelength Detector(VWD)

Pump: Gradient with degasser

Mobilephase: Acetonitrile: Buffer ph3 with opa

Detection wavelength: 250 nm

Mode: Gradient (Auto sampler)

Sample size: 20µl

Flow rate: 0.7 ml/min

Type of injector: Loop injector

Temperature: 25°C Column equation

Time: 30 min. (between each run)

Software: Open LAB CDS, Chemo station Workstation.

Preparation of Standard StockSolutions:

Stock Solution A: Accurately weighed quantity (10 mg) of RNZ was transferred to 25.0 mL volumetric flask, dissolved and diluted up to the mark with methanol. From this solution,

0.2 mL was diluted to 10.0 mL with methanol (Concentration 20 □g/mL). The Solution was mixed and filtered through 0.2□ membranefilter.

Stock Solution B: Accurately weighed quantity (~15.42 mg) of RNZ was transferred to 25.0 mL volumetric flask, dissolved and diluted up to the mark with methanol. From this solution, 0.2 mL was diluted to 10.0 mL with methanol (Concentration 20 □g/mL). The Solution was mixed and filtered through 0.2□ membranefilter.

5mMBuffer Solution:- 12.03 gm sodium citrate AND 1.72 gm Citric acid in 1000 ml water and adjust pH 3.2 with ortho Phosphoric acid.

PREPARATION OF MOBILE PHASE

5 mM Sodium citrate in citric acid buffer (5m M) was prepared by dissolving accurately weighed quantity 12.03 g of Sodium citrate and 1.72gm citric acid in a 1000.0 mL of double distilled water. Mobile phase was prepared by mixing 100.0 mL of 5m M Phosphate buffer with 100.0 mL of methanol. This mobile phase was ultrasonicated for 10 minutes and then it was filtered through 0.45 µ membrane filter.

SELECTION OF WAVELENGTH

Standard stock solution A and B were diluted separately with mobile phase to obtain final concentration of 20 µg/mL of RNZ. Each solution was scanned using double beam UV- Visible Spectrophotometer-1800 in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid. The wavelength selected was 274.0 nm as the drug showed significant

absorbance at this wavelength

OPTIMIZED CHROMATOGRAPIC CONDITION

Mobile phase	5Mm buffer: methanol (45:55v/v)
Column	FORTIS C18
Flow rate	0.7ML/MIN
Detection wavelength	274 nm
Injection volume	20 µL
Run –time	10MIN

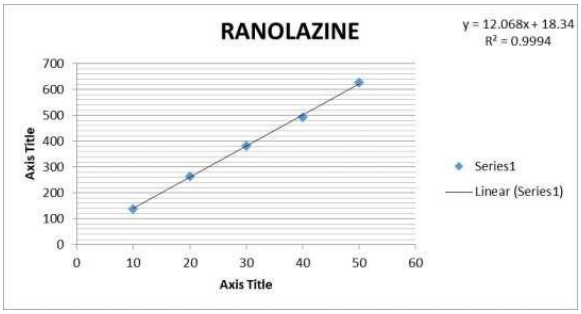


Fig. No. 4: Standard Calibration Curve for Ranolazine Coefficient of correlation values (R^2) for Ranolazine indicated that a linear relationship exists between the peak area and concentration.

PREPARATION OF SAMPLE SOLUTION

Six sample solutions were prepared and analyzed in following manner:

An accurately weighed quantity of 15.42mg of RNZ was transferred to 50.0 mL volumetric flasks, dissolved and diluted to the mark with mobile phase. From this solution, 1.0 mL was transferred to 10.0 mL volumetric flask and diluted to the mark with mobile phase. Further, 2.0 mL of above solution was diluted to 10.0 mL with mobile phase to obtain final concentration of32 µg/mL RNZ, respectively. The solution was mixed and filtered through 0.2 µ membrane filter.

Equal volume of standard and sample solution (20 µL) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for RNZ was measured at 274.0 nm. Amount of RNZ in sample (mg) was calculated by comparing the mean peak area of standard and sample solution.

Table No. 9: Results of Analysis of Standard Laboratory Mixture

Sr. No.	Weight of std.(mg)	Weight of sample	Peak area of std	Peak area of sample	% Drug estimation
	RNZ	RNZ	RNZ	RNZ	RNZ
1.	500	496.9	625.61	619.32	98.99
2.		502.1		624.51	99.97
3.		497.5		622.02	99.81
				Mean	99.59
				S.D	0.5257
				C.V	0.5278

Preparation of Tablet Solutions:

Six tablet sample solutions were prepared and analyzed in following manner:

Twenty tablets were weighed, average weight was calculated and crushed to obtain fine powder. An accurately weighed quantity of tablet powder equivalent to about 80 mg RNZ was transferred into 50.0 mL volumetric flasks, 30 mL of mobile phase was added and content of the flask were ultrasonicated for 20 minutes, volume was then made up to the mark with mobile phase. The solution was mixed and filtered through Whatman filter paper No. 42. From the filtrate, 1.0 mL was diluted to 10.0 mL with mobile phase. Further diluted 2.0 ml of this solution to 10.0 mL with mobile phase. The diluted solution was filtered through 0.2 µ membrane filter. Equal volume of standard and sample solution (20 µL) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for RNZ was measured at 274.0 nm. Amount of RNZ in sample (mg) was calculated by comparing the mean peak area of sample with that of standard. Amount of drug estimated in mg/tablet and percent label claim was calculated using following formula:

$$\text{Content of drug in sample (mg/tab)} = \frac{PA_{Spl}}{PA_{Std}} \times \frac{\text{Weight of std. (mg)}}{df\ std} \times \frac{df\ spl}{\text{Weight of tablet powder taken (g)}} \times \text{Avg. weight of tablet (g)}$$

Where,

PASpl- Peak area of sample,

PAStd- Peak area of standard,

df std- Dilution factor for standard,

df spl- Dilution factor for Sample.

Results of analysis of tablet formulation and its statistical evaluation is given in the Table No. 10.

Table No. 10: Results of Analysis of Tablet Formulation

Sr. No.	Weight of std.(mg) RNZ	Weight of sample(mg) RNZ	Peak area of std RNZ	k area of sample RNZ	Label Claim RNZ
1.	500	500.2	625.61	618.99	99.99
2.		500.3		622.89	100.2
3.		500.4		624.79	99.18
				Mean	99.79
				S.D.	0.5386

VALIDATION OF PROPOSED METHOD

The proposed method was validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Accuracy:

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Preparation of sample solution:

An accurately weighed quantity of pre-analysed tablet powder equivalent to about 15.42 mg RNZ was transferred individually in six different 50.0 mL volumetric flasks. To each of the flask following quantities of RNZ were added:

Flask No.1 0.1 ml TAB sol.+0.08ml STD sol Flask No.2 0.1 ml TAB sol.+0.08ml STD sol Flask No.3: 0.1 ml TAB sol.+0.1ml STD sol Flask No.4: 0.1 ml TAB sol.+0.1ml STD sol Flask No.5: 0.1 ml TAB sol.+0.12ml STD sol Flask No.6: 0.1 ml TAB sol.+0.12ml STD sol

Then 30 mL mobile phase was added to each flask and content of the flask were ultrasonicated for 20 minutes, volume was then made upto the mark with mobile phase. The solution was individually mixed and filtered through Whatman filter paper No. 42. From the filtrate, 5.0 mL solution was diluted to 50.0 mL with mobile phase. Further diluted 1.0 mL of this solution to 10.0 mL with mobile phase. The diluted solution was filtered through 0.2 µ membrane filter.

Equal volume of standard and sample solution (20 µL) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for RNZ was measured at 274 nm.

Amount of RNZ in sample was calculated by comparing the mean peak area for standard and sample solution by Equation No. 37 (Page No. 86)

Amount of the drug recovered (mg) and % recovery was calculated by using Equation No. 35 and 36, respectively (Page No. 72). Results of recovery studies are shown in Table No. 11

Level of Recovery (%)	Weight of tablet powder taken (g)	Mean Peak Area* RNZ	Amount of drug added (mg) RNZ	Amount of drug recovered (mg) RNZ	% Recovery RNZ
80	0.3191	235.59	8	8.01	100.17
80	0.3191	235.9	8	8.03	100.49
100	0.3190	260.17	10	10.05	100.52
100	0.3190	260.12	10	10.04	100.40
120	0.3190	282.73	12	11.87	98.00
120	0.3190	283.13	12	11.93	99.49
				mean	99.74
				S.D	±0.4871
				C.V	0.4883

RESULTS OF RECOVERY STUDIES

Precision:

a) Intra-day Precision:

Intraday precision was determined by analyzing tablet sample solutions at different time intervals on the same day. Tablet sample solution was prepared and analysed in the similar manner as described under analysis of the tablet formulation.

b) Inter-day Precision:

Inter-day precision was determined by analyzing tablet sample solutions on three different days. Tablet sample solution was prepared and analysed in the similar manner as described in analysis of the tablet formulation.

Results of intra-day precision and inter-day precision are shown in Table No. 12 and 13, respectively.

Table No. 12: Intra-day Precision Data

Drug	% Mean*	S. D.	C. V.
RNZ	381.87	0.37	0.0968

*denotes average of three determinations.

Table No. 13: Inter-day Precision data

Drug	% Mean*	S. D.	C. V.
RAZ	381.46	1.77	0.4640

*denotes average of three determinations

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ were separately determined which is based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ. Results of LOD and LOQ study are shown in Table No. 14.Table No. 14: LOD and LOQ of Ranolazine

Parameter	RNZ
Limit of Detection (µg/mL)	1.881
Limit of Quantification (µg/mL)	0.4726

Repeability:

Repeatability is the closeness of agreement between mutually independent test result obtained with the same method on identical test material in the same laboratory by the same analyst using the same equipment with in short interval of time.

Sr. No	Conc.	Area	Mean	S.D	%RSD
1	30	380.66	381.10	0.62	0.16
2	30	381.53			

TABLE NO: REPEABILITY OF RANOLAZINE

Robustness of Method:

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on retention time and tailing factor were studied. The solution containing 40 µg/mL of RNZ was injected (in twice) into sample injector of HPLC under the varied conditions. Robustness data is given in Table No. 15.

Wavelength change ±1nm		RNZ	RNZ
273	40	492.07	4.057
275	40	484.54	4.057
	Mean	488.30	4.057
	S.D	5.3245	0

Table No. 17: System Suitability Parameters

Sr. No.	Parameter	RNZ
1.	Resolution (R)	-
2.	Retention Time(RT)	4.197
3.	No. of theoretical plates (N)	7597

From above results it is evident that the peaks are almost symmetrical having satisfactory resolution and precision. Efficiency of column is also satisfactory. Linearity of detector response was studied by plotting a graph of concentration vs. mean peak area. Linearity was observed in the concentration range 10-50 µg/ml for RNZ. Coefficient of correlation was found to be 0.9994 for RNZ calibration curves (Fig. No. 3, page no. 85).

The proposed method was first applied to standard laboratory mixture and results indicated that Ranolazine could be estimated accurately and precisely by this method. Results of analysis of the standard laboratory mixture are summarized in Table No. 28. Then, the developed method was employed for the analysis of marketed formulation.

Table No. 18: Summary of Results of RNZ Estimation in Standard Laboratory Mixture and Tablet Formulation

Method	Drug	% Label Claim*	S.D.	C.V.
Laboratory Mixture	RNZ	99.59	0.5257	0.5278
Tablet Formulation (RANX)	RNZ	99.79	0.5386	0.5397

Results of validation studies are summarized in Table No. 29

Table No. 19: Summary of Results of Method Validation

		RP-HPLC Method
		RNZ
Linearity Range (µg/mL)		10-50
Accuracy	Iean Percent Recovery**	99.74
	S.D.	±0.4871
	C.V.	0.4883

Precision	Intra-day Precision	
	% Label Claim*	
	S.D.	± 0.37
	C.V.	0.866
	Inter-day Precision	
	% Label Claim*	
	S.D.	± 1.77
	C.V.	0.533
LOD		1.881
LOQ		0.4726
Robustness		Robust

**denotes average of nine determinations, *denotes average of three determination

CONCLUSION

The methods were found to be sensitive, reliable, reproducible, rapid and economic also. In RP-HPLC method, the analyte were resolved using methanol: sodium citrate in citric acid buffer (5mM), (45:55), pH 3.2 at flow rate of 0.7ml/min, on Agilent 1100 infinity LC Gradient system containing of Uv-1800 Visible C18 column (4.6×100mm length). The detection was carried out at 274 nm. The method gave the good resolution and suitable retention time.

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