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"DEVELOPMENT AND VALIDATION OF INDICATING INSTRUMENTAL METHOD FOR ESTIMATION OF RANOLAZINE IN BULK AND TABLET DOSAGE FORM"

Vishal Rathod [1*], Alok Kadam[1], Arjun Bembade[1], .O.G. Bhusnure[1]

Channabasweshwar Pharmacy College (Degree), kava road, basweshwar chowk, Latur-413512

ABSTRACT

Ranolazine Hydrochloride (RAN) chemically is a piperizine derivative used as an anti-aginal drug. Ranolazine is used for the treatment of cardiac ischemia and it effects sodium dependent calcium channel during myocardial ischemia. Ranolazine indirectly prevents the calcium overload that causes cardiac ischemia. Ranolazine Hydrochloride is indicated for the treatment of chronic angina. Ranolazine may be used with beta-blockers, nitrates, calcium channel blockers, antiplatelet therapy, lipid-lowering therapy, ACE inhibitors, and angiotensin receptor blockers. This review article represents the various analytical methods which have been reported for estimation of ranolazine in synthetic mixture. Chromatographic methods like HPLC, RP-HPLC, HPTLC, GC, LC-MS, LCMS/MS were reported.

KEY WORDS: Ranolazine hcl, anti aginal drug,Hplc, Rp hplc,GC, ace inhibitor

INTRODUCTION

Analytical chemistry

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the chemical composition of samples of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation. Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species

are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography .

Structure of Ranolazine

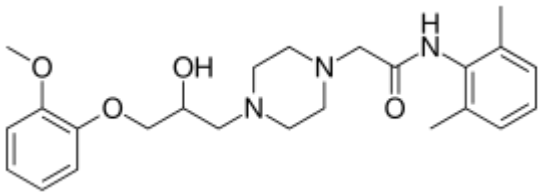


Figure 1 structue of ranolizine

MATERIALS AND INSTRUMENTS

Table No. 1.1: Instruments Used

Sr. No	Instruments And Glass wares	Model
1	Hplc	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	PH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man
8	uv visible spectrophotometer	Shimadzu 1800

CHEMICALS USED:

Table No. 1.2: Chemicals used

S.NO	CHEMICAL	BRAND NAME
1	Ranolazine (pure)	sura labs
2	methanol and water for hplc	LICHROSOLV (MERCK)
3	Acetonitrile for hplc	Merck

EXPERIMENTAL WORK

UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION.

Method Development

Preparation of stock solution

Standard stock solution of Ranolazine was prepared by dissolving 100mg of drug in 100ml of Methanol solvent system to get a concentration of 1mg/ml or 1000 µg/ml (Stock I). From the above solutions, 1ml was taken in 10 ml volumetric flask and volume was made up with 10 ml of Methanol solvent system to get a concentration of 100µg/ml (Stock II). This solution was taken as stock solution.

Determination of wavelength of maximum absorption

From the above stock solution, 1 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted to 10 ml with Methanol solvent system to give concentration of 10µg/ml, it was used for spectral scan in the UV range of 400-200 nm, and the wavelength corresponding to maximum absorbance was noted at 274nm.

Preparation of Calibration Curve

For the preparation of standard calibration curve, concentration of 10-60µg/ml was prepared by pipetting out 1, 2, 3, 4, 5, 6 ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with methanol solvent system. The absorbance of each solution was measured at 274 nm against methanol solvent system as blank. Calibration curve of the Ranolazine was plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis. The calibration curve is shown in Fig.8.2.

Preparation of sample solution

Twenty tablets of sun pharma each containing 500 mg of Ranolazine were weighed accurately and made into a fine powder. The tablet powder equivalent to 10mg of Ranolazine was weighed accurately and transferred into a 10 ml volumetric flask, 4ml of Methanol solvent system was added, mixed well and sonicated for 10 min using ultra sonicator. The volume was made up to the mark with the same solvent to get 1000µg/ml. Ranolazine 100µg/ml sample solution was prepared by diluting 1ml of 1000µg/ml of the stock solution with Methanol solvent system. Accurately 1ml of 100µg/ml solution was transferred to 10ml volumetric flask and made up to the mark with Methanol solvent system to get 10µg/ml of Ranolazine and the absorbance of the prepared solution was measured at 274nm.

Validation of the Developed Method

The developed method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Precision

The precision of proposed method was determined by Intra-day and Inter-day precision. Three different solutions of three different concentrations (5, 10,15µg/ml) were analyzed, and it was expressed in terms of percent relative standard deviation (%RSD). For Inter-day and Intra-day %RSD were found in the range of 0.19493 and 0.15414 respectively.

Accuracy

Accuracy of the present method was carried out by using the drug substance 10ppm as standard solution and spiked solution at three different concentration levels of 50%, 100% and 150% in triplicates. Absorbance was measured at 274nm and results were expressed in terms of % recoveries. Standard deviation and % RSD were calculated.

Assay

Twenty tablets of Ranolazine were weighed accurately and powdered. Powder equivalent to 50 mg of was weighed and transferred to a 50 ml volumetric flask and make up the volume up to 50ml with Methanol solvent system, which gives 1000µg/ml solution and sonicated for 15 minutes to get homogeneous solution. Then it was filtered through a Whatman filter paper. A final concentration of 100 mg/ml of Ranolazine was prepared. From this 1 ml was taken and diluted to 10 ml with Methanol solvent system which gives 10µg/ml solution and the absorbance of the solution was measured at 274 nm.

Percent Recovery Study

Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 50%, 100% and 150%. The final concentration of Ranolazine determined. The percentage recovery was calculated as mean± standard deviation and %RSD.

Limit of detection and Limit of Quantification

Limit of detection (LOD) and Limit of quantification (LOQ) of Ranolazine was calculated by using equation given in the ICH guidelines.

Limit of Detection

Limit of Detection was determined on the basis of slope and standard deviation of the calibration curve.

$$LOD = 3.3\ \sigma/S$$

Where,

σ = standard deviation of Y intercept of regression lines

S = slope of the calibration curve Limit of Quantitation Limit of Quantitation was determined on the basis of slope and standard deviation of the calibration curve

LOQ = 10 σ/S Where

σ = standard deviation of Y intercept of regression lines

S = slope of the calibration curve.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Identification of standard dug by RP-HPLC

Solubility study:

Ranolazine API sample was taken in separate glass test tube and observed for solubility in various solvents.

Melting Point Study:

Melting point study of Ranolazine was performed by melting point apparatus sample was taken in separate capillaries and kept in to the apparatus for observation.

RP-HPLC method development and validation of ranolazine

Method development:

HPLC was selected as analytical technique for estimation of ranolazine.

Chromatographic condition (initial):

Column	Develosil C18 (4.6mm x 150mm, 5µm)
Column temperature	45%
Wavelength	274nm
Mobile phase ratio	Methanol: Water (65:35%) V/V
Flow rate	0.9 ml/min
Injection volume	20 µl
Run time	10min

Diluent: Use as such Methanol as diluents

FORCED DEGRADATION STUDIES

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.]

Acid Degradation Studies: To 1 ml of Ranolazine stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Oxidation Degradation Studies: To 1 ml of stock solution of Ranolazine 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solution was kept for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Studies Dry Heat Degradation: The 1 ml of Ranolazine drug solution was placed in the oven at 60ºC for 6h to study dry heat degradation. for HPLC study, the resultant solution was makeup to final volume to obtain (100µg/ml) solution. Cool the solution to room temperature and filtered through a 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Photo Degradation Studies: The photo stability of the Ranolazine was studied by exposing the stock solution to UV light for 1 day or 200Watt-hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (100µg/mL) solution and filtered with 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability

RESULTS AND DISCUSSION

UV-VISIBLE SPECTROPHOTOMETRICMETHODDEVELOPMENT AND VALIDATION

This study was focused on development of a new spectrometric method for theanalysis of ranolazine in bulk drug and tablet dosage form. Spectrophotometric analysis was performed using double beam UV-Visible spectrophotometer (SHIMADZU 1800) with 1cm path length supported by UV-WIN Software

Method Development

The standard solution of ranolazine in Methanol solvent system was found to exhibit maximum absorption at 274 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and the procured drug sample of ranolazine complies with the reference spectra .

From the standard stock solution, 1 ml of standard stock II solution was transferred into 10 ml was used for spectral scan in the UV range of 400-200 nm, and the wavelength corresponding to maximum absorbance was noted at 274nm.

Determination of wavelength of Ranolazine in Methanol.

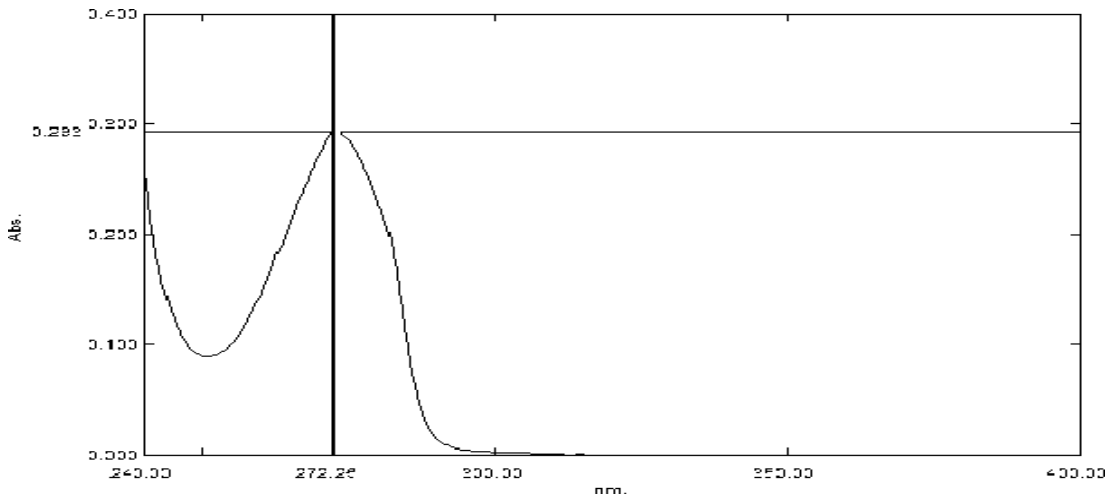


Fig no1 **UV Spectrum of Ranolazine**

Observation: the wavelength of ranolazine is found to be 274 nm when scannedfrom 200-400nm spectrophotometrical.

Preparation of Calibration CurveLinearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 10–60 µg/ml was linear with a correlation coefficient (R^2) greater than 0.999.

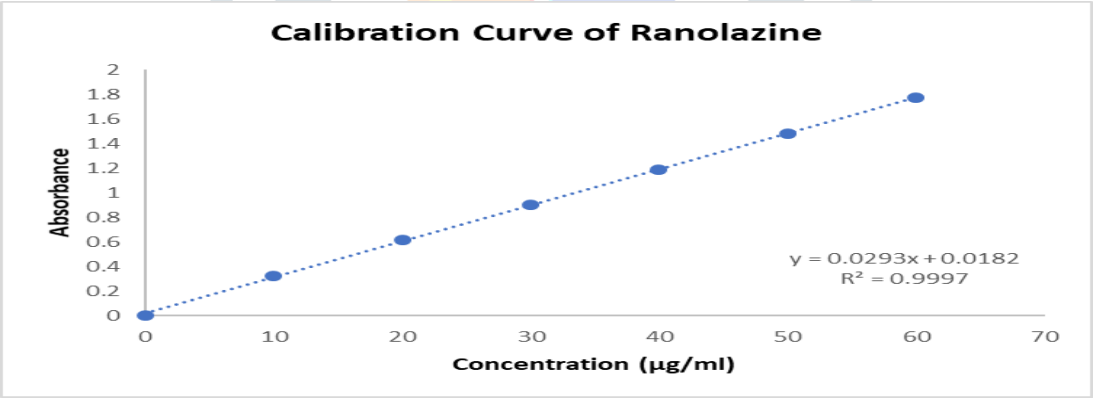


Fig no 2: Calibration Curve of Ranolazine

Result & Discussion

Linearity range was found to be 10-60 µg/ml for ranolazine at 274 nm. The correlation coefficient was found to be 0.999, which shows good linearity between above range. The slope was found to be 0.029 and intercept was found to be 0.018 which was close to zero intercept.

Validation of the Developed Method

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), Assay, Percent recovery and specificity according to ICH Q2 (R1) guideline and USP guidelines.

Repeatability

Table No. 1.3: Repeatability Data of Ranolazine

Concentration (µg/ml)	Absorbance (nm)	Mean	Standard Deviation	%RSD
10	0.335	0.337	0.002828	0.839296
10	0.342			
10	0.334			
10	0.337			
10	0.338			
10	0.336			

Precision

The precision (measurement of intraday, inter-day) results showed good reproducibility with percent relative standard deviation (%RSD) was below 2.0%. This indicated that method is highly precise.

Intermediate Precision:

Table no 1.4 Inter-day Precision of Ranolazine

Concentration (µg/ml)	Absorbance (nm)			Mean	Standard Deviation	%RSD	Average of % RSD
	1	2	3				
5	0.373	0.374	0.373	0.373333	0.000577	0.154647	0.15414
10	0.375	0.374	0.375	0.374667	0.000577	0.154097	
15	0.376	0.375	0.376	0.375667	0.000577	0.153687	

Accuracy

The accuracy of analytical method results found within the range of 99–100.90%,which indicate that the method is accurat

Table no1.5 Accuracy Data of Ranolazine

Name of Drug	No. of Preparation	Conc. on {Amount Added} (µg/ml)	Amount Found (µg/ml)	Percent Recovery	Mean % Recovery ± SD	% RSD
Ranolazine	1	5	5.034	100.680	99.99333 ± 0.690024	0.69007
	2	5	4.965	99.300		
	3	5	5.000	100.00		
Ranolazine	4	10	9.931	99.310	99.76667 ± 0.524817	0.526045
	5	10	10.034	100.340		
	6	10	9.965	99.650		
Ranolazine	7	15	14.931	99.540	99.69067 ± 0.478148	0.479631
	8	15	15.034	100.226		
	9	15	14.896	99.306		

* Each value corresponds to the mean of three determinations

Robustness

The result of robustness study of the developed assay method was established inTable No. 8.5. The result shown that during all variance conditions, assay value of thetest preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical would be concluded as robust.

Table No.1.6: Evaluation Data of Robustness Study (Temperature-25°C)

Concentration (µg/ml)	Absorbance	Statistical Analysis
10	0.333	Mean = 0.337 SD = 0.00228 % RSD = 0.676662
10	0.336	
10	0.338	
10	0.339	
10	0.337	
10	0.339	

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD.

Table No. 1.7 Evaluation Data of Ruggedness (Analyst-I)

Concentration (µg/ml)	Absorbance	Statistical Analysis
10	0.342	Mean = 0.341 SD = 0.001414 % RSD = 0.414725
10	0.341	
10	0.339	
10	0.344	
10	0.343	
10	0.341	

Limit of Detection:

The limits of detection (LOD) which represent the sensitivity of the proposed method were determined. The LOD value obtained was 0.68µg/ml. It indicates the highsensitivity of the proposed method.

Limit of Quantitation:

The limits of Quantitation (LOQ), which represent the sensitivity of the proposed method, were determined. The LOQ value obtained was 2.15µg/ml. It indicates the high sensitivity of the proposed

HPLC METHOD DEVELOPMENT AND VALIDATION

Identification of Standard Drug By HPLC Solubility of Drug

Sr. No.	Solvents	Ranolazine
1	Distilled water	Slightly soluble
2	Acetonitrile	Soluble
3	Methanol	Soluble

Result of Melting Point Study

Table No. 1.8: Melting Point of Drug

Sr. No.	Drug	Observed melting point
1	Ranolazine	120-124 ^{0}C

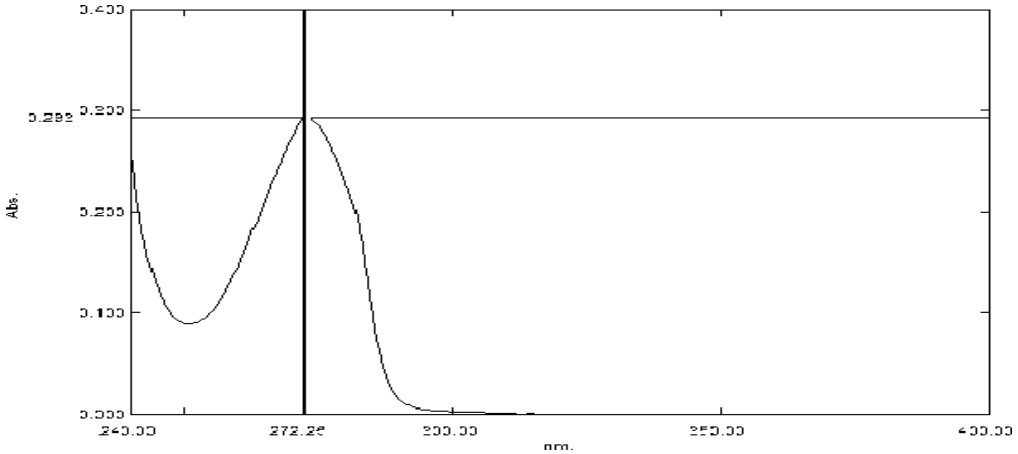


Fig 3 Spectrum of Ranolazine

RP-HPLC Method Development and Validation of Ranolazine

Selection Detection Wavelength of Ranolazine 274 nm

METHOD DEVELOPMENT TRIAL

Trail 1:

Column	: Develosil C18 (4.6mm x 150mm, 5µm)
Column temperature	: 45ºC
Wavelength	: 274 nm
Mobile phase ratio	: Methanol: Water (65:35%) V/V
Flow rate	: 0.9 ml/min
Injection volume	: 20 µl
Run time	: 10 min

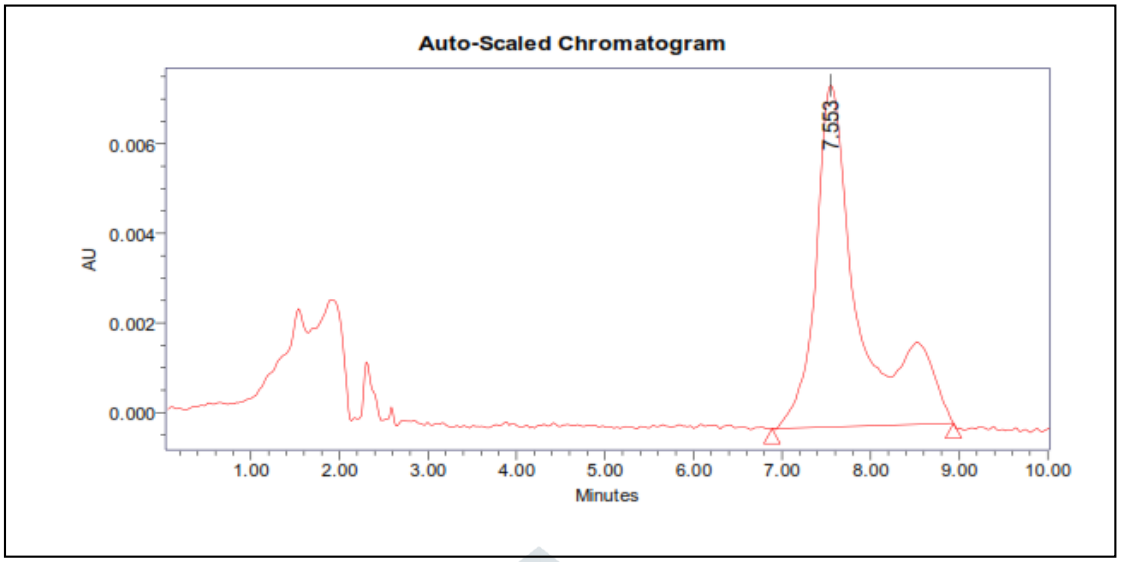


Table No.1.9: Peak Results for Trail 1

Sr. No	Peak Name	Rt	Area	Height
1	Ranolazine	7.553	325645	5874

ObSERVATION: chromatogram. So, more trials required to obtain good peak

Trail 2:

Column	: Phenomenex Gemini C18 (4.6mm × 150mm) 5µm
Column temperature	: 40°C
Wavelength	: 274 nm
Mobile phase ratio	: Methanol: Water (75:25) V/V
Flow rate	: 0.8 ml/min
Injection volume	: 20 µl
Run time	: 7.5 min

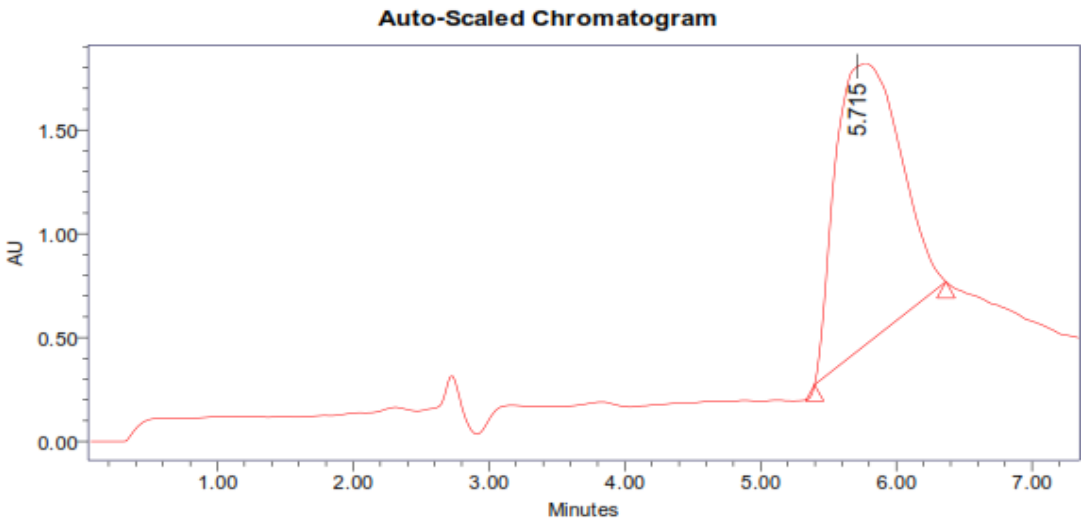


Fig.4 : Chromatogram for Trail 2 Table No. 8. Peak Results for Trail 2

Sr. No.	Peak name	Rt	Area	Height
1	Ranolazine	5.715	584587	36582

Observation: This trial show very less plate count and improper baseline in thechromatogram, so more trials were required for obtaining good peak

Trail 3:

Column	: Symmetry ODS C18 (4.6mm×250mm) 5µm
Column temperature	: 35ºC
Wavelength	: 274 nm
Mobile phase ratio	: Methanol: Acetonitrile (60:40) V/V
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Run time	: 10 min

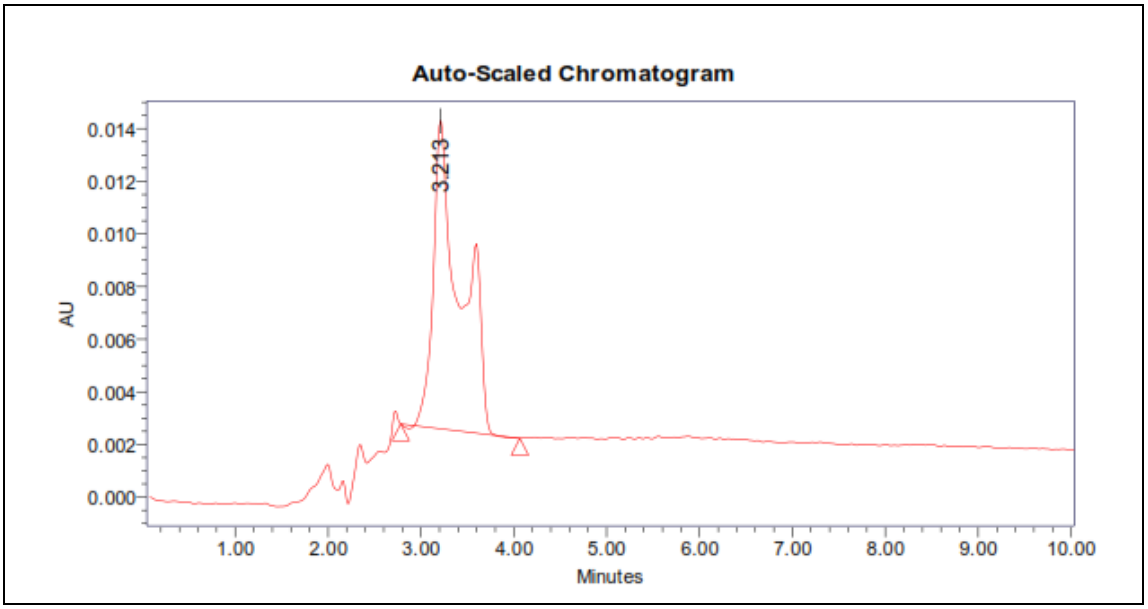


Fig. 5: Chromatogram for Trail 3

Table No. 2.1 Peak Results for Trail 3

Sr. No.	Peak name	Rt	Area	Height
1	Ranolazine	3.213	258455	4589

Observation: This trial does not show Proper base line and plate count in thechromatogram. So go for further trails to obtain proper peak .

Finalized Parameters

Mobile phase ratio	: Acetonitrile: Methanol: Water (50:30:20% v/v)
Column	: Zorbax C18 (4.6mm x 250mm, 5□m, Make: X terra)
Column temperature	: 35^0C
Wavelength	: 274 nm
Flow rate	: 1 ml/min
Injection volume	: 20 µl
Run time	: 10 min

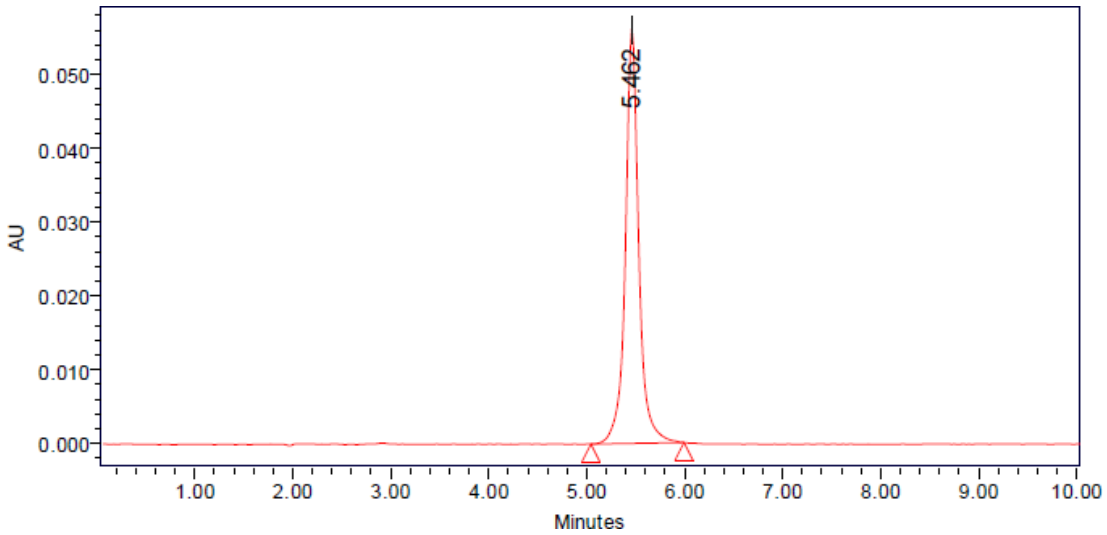


Fig.6 Finalized Parameters Chromatogram

Table No. 8.16: Peak Results for Finalized Parameters

Sr. No.	Name	RT	Area	Height
1	Ranolazine	5.462	1052689	75421

Observation: In this trial it shows proper peak, tailing, plate count and baseline in thechromatogram. So, it's Finalized chromatogram.

Optimized Chromatogram (Sample)

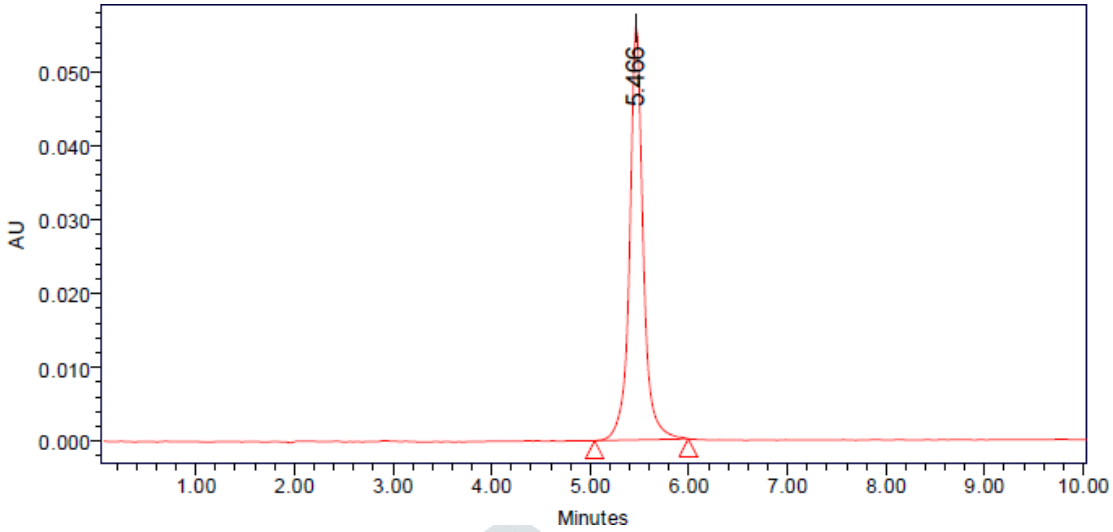


Fig.7 Optimized Chromatogram (Sample)

Finalized Parameters:

Table No. 2.2 Finalized Parameters

Parameters	Specifications
Mobile phase ratio	Acetonitrile: Methanol: water (50:30:20%v/v)
Column	Zorbax C18 (4.6mm x 250mm, 5□m, Make: X terra)
Column temperature	35^0C
Wavelength	274 nm
Flow rate	1 ml/min
Injection volume	20 µl
Run time	10 min

Calibration curve for Ranolazine

Table No.2.3 Calibration Curve for Ranolazine

Ranolazine	
Conc. (µg/ml)	**Area**
0	0
10	110102
20	218757
30	327412
40	436067
50	544722

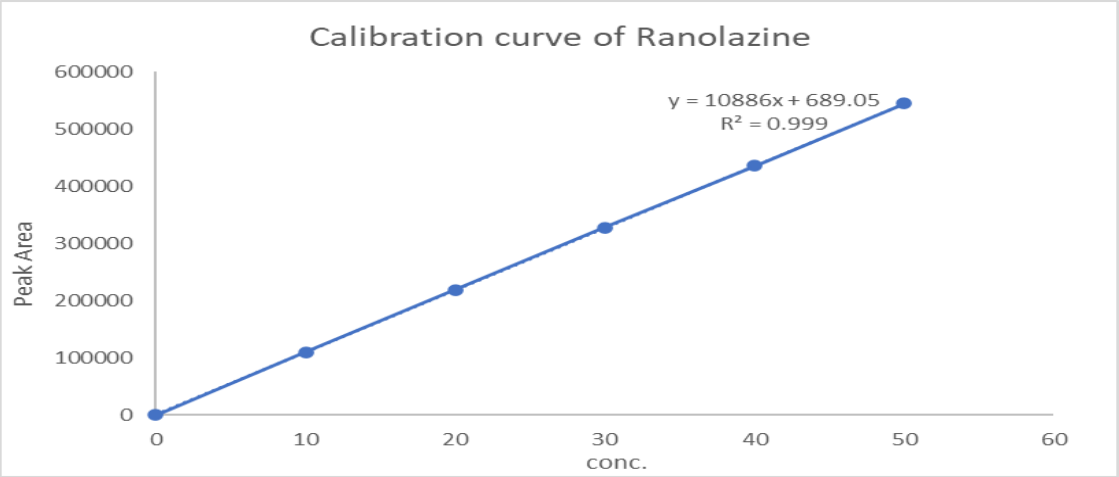


Fig 8. Calibration Curve of RanolazineTable Table no.2.3 Calibration Curve Results

Parameter	**Result**
Linearity range (ug/ml)	10-50 ug/ml
Slope	10886
Intercept	689.05
Regression equation	10886 X + 689.05
Correlation coefficient (R^2)	0.999

Assay Result of Ranolazine in Marketed Formulation:

Table No. 2.4: Assay of Ranolazine

Drug	Actual conc. Of drug (ug/ml)	Conc. Of Drug Found (ug/ml)	% Of Drug found	Avg. of % drug found	SD	% RSD
Ranolazine	100	98.91	98.91	99.37	0.4064	0.4090
	100	99.54	99.54			
	100	99.67	99.67			

METHOD VALIDATION

1. System Suitability Tests

Table No. 2.5 System Suitability Tests

Sr. No	System suitability parameter	Results	Specification
1	Retention time (min)	5.27	-
2	Resolution (R)	30.67	>1.5
3	Theoretical plate number (N)	9674	Not less than 2000
4	Tailing factor (T)	1.63	Not greater than 2.0

2. Accuracy

Table No.2.6 Results of Accuracy

Sample prep. Level	Target conc. (ug/ml)	Spiked conc. (ug/ml)	Final conc. (ug/ml)	Amount recovered conc.(ug/ml)	% Recovery	Mean	% RSD
As such	100	0	100	101.61	101.61	101.22	0.36
	100	0	100	100.88	100.88		
	100	0	100	101.19	101.19		
50 % level	100	50	150	49.95	99.83	99.62	0.94
	100	50	150	50.25	100.44		
	100	50	150	49.33	98.59		
100 % level	100	100	200	99.75	99.68	100.44	0.75
	100	100	200	100.52	100.45		
	100	100	200	101.26	101.19		
150 % level	100	150	250	148.68	99.05	99.61	0.50
	100	150	250	150.11	100.00		
	100	150	250	149.74	99.76		

3. Precision

a) Repeatability Study (Sample Precision)

Table No.2.7 Repeatability Study

Sample preparation	Assay (mg/g)	Assay (%)	% RSD
1	10.07	100.69	0.46%
2	9.99	99.87	
3	9.95	99.55	
4	10.04	100.40	
5	9.96	99.62	
6	10.03	100.27	
Mean	10.03	100.07	

Intraday and Inter-Day Study

Table No. 2.8: Intraday and Interday Study

Sr. No	Conc. (ug/ml)	Intraday Peak Area. (n=3)	% RSD	Sr. No.	Interday Peak Area (n=3)	% RSD
Time-1	100	1052728	0.2229	**Day-1**	1102689	0.3825
Time-2	100	1056854	0.2334	**Day-2**	1102532	0.3924
Time-3	100	1052468	0.2460	**Day-3**	1110121	0.3986

4. Limit of Detection and Limit of Quantitation

Table No. 2.9 LOD and LOQ

Drug	LOD (ug/ml)	LOQ (ug/ml)
Ranolazine	1.6	4.8

5. Robustness

Table No. 3.1: Results of Robustness Studies for Ranolazine

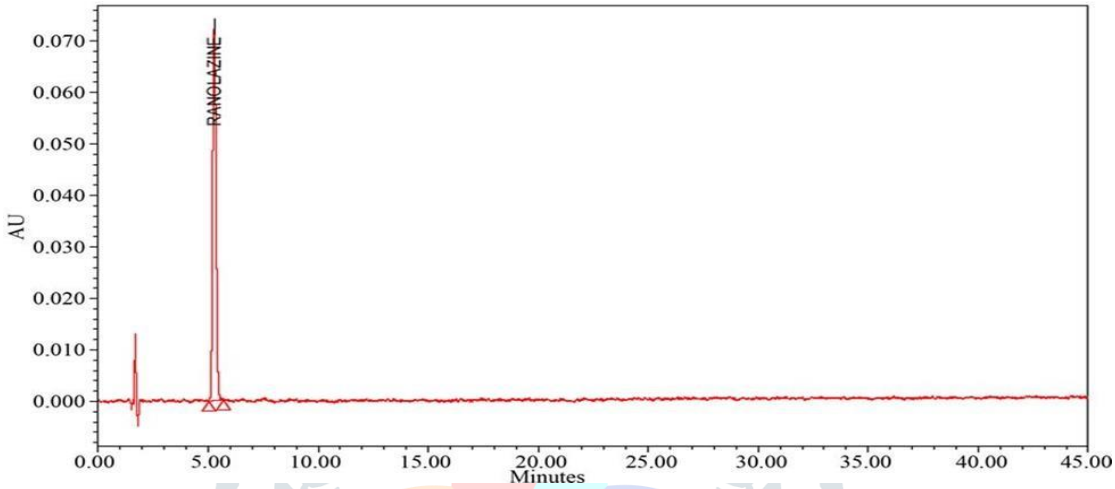
Parameter		Amount of Ranolazine added (umL^{-1})	Amount of Ranolazine detected (Mean±SD) *	%RSD
Change in Column Temperature	30 ^{0}C	100	99.75±0.372	0.372
	35 ^{0}C	100	99.99±0.163	0.162
	40 ^{0}C	100	99.78±0.120	0.120
Change inflow rate	0.8 $mLmin^{-1}$	100	99.92±0.142	0.142
	1.0 $mLmin^{-1}$	100	99.99±0.251	0.251
	1.2 $mLmin^{-1}$	100	100±0.415	0.414
Change in wavelength	272 nm	100	99.70±0.306	0.307
	274 nm	100	99.99±0.102	0.102
	276 nm	100	100.36±0.106	0.106

FORCED DEGRADATION STUDIES

The specificity of the method can be demonstrated by applying stress conditions usingacid, alkaline, peroxide, thermal, UV degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.

Acid Degradation Studies:

To 1 ml of Ranolazine stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60ºC. The resultant solution



was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample

Degradation Studies:

To 1 ml of stock solution of Ranolazine 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCland makeup to final volume to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 10µl wasinjected into the system, and the chromatograms were recorded to an assessment of sample stability

Table No.3.2 forced Degradation Studies for Ranolazine

Sr. No.	Stress Condition	Peak Area	Amount	% Active Amount	Total % Amount
1	Standard	145867.00	0	100%	100%
2	Acidic	112459.12	21.88	78.12	100%
3	Basic	124587.11	17.43	82.57	100%
4	Oxidative	133896.79	**% Degraded** 11.37	88.63	100%
5	Thermal	136341.88	5.81	94.19	100%
6	Photolytic	134762.14	9.48	90.52	100%

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