

Development, Validation, and Forced Degradation Studies of a Stability-Indicating RP-HPLC Method for Ranolazine in Bulk Drug and Tablets

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Abstract: Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Estimation of Ranolazine was achieved by using UV visible spectrophotometer (SHIMADZU 1800). The normal spectrum of Ranolazine was recorded using the solvent methanol and distilled water. Then the zero order spectra of Ranolazine was recorded, showed maximum absorbance at 274nm. Methanol was selected as solvent. The linearity and range were found to be in the range of 10-60 mcg/ml. The correlation coefficient of Ranolazine at 274nm in bulk was found to be 0.999, showed good linearity between above range. For HPLC study; the mobile phase Acetonitrile: Methanol: Water (50:30:20%v/v) was selected. Run time was selected to be 10.0 min because analyze gave peak around 5.462±0.02 min respectively and also to reduce the total run time. The percent recovery was found to be 98-102% was linear and precise over the same range. The sample exposed to condition like hydrolytic and oxidative degradation in 20% H₂O₂. The reaction was carried out at room temperature for 30 min at 60°C. UV chamber was used for photo degradation studies. The analytical method as developed by studying different parameters. Simple, precise and accurate method was developed for the estimation of Ranolazine.

Keywords: Ranolazine, UV spectrophotometer, Absorbance maxima, linearity, correlation coefficient, HPLC chromatography.

Introduction:

Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package. In addition, the regulatory guidance is very general and does not explain about the performance of forced degradation studies¹. The FDA and ICH guidance's state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used².

High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC methods should be able to separate, detect, and quantify the various drug-related degradates that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. Forced degradation studies (chemical and physical stress testing) of new chemical entities and drug products are essential to help develop and demonstrate the specificity of such stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products of the APIs that could form during storage, and facilitate formulation development, manufacturing, and packaging. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies. For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability-indicating nature of the analytical procedures, and the degradation pathways of the API in solid state, solution, and drug product. The chemical structures of significant degradation products and the associated procedures for their isolation and/or characterization are also expected to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. In general, a target of approximately 10% degradation of the API during forced degradation, or exposure to energy in slight excess of what is typically used in accelerated storage is recommended. In this way, the “worst-case” degradation products can be studied. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA³.

Ultraviolet and visible spectroscopy deals with the recording of the absorption of radiations in the ultraviolet and visible regions of the electromagnetic spectrum⁴. The ultraviolet region extends from 10 to 400 nm. It is subdivided into the near ultraviolet (quartz) region (200–400 nm) and the far or vacuum ultraviolet region (10–200 nm). The visible region extends from 400 to 800 nm⁵.

Methods

Materials:

Ranolazine was supplied as a gift sample by Sura labs Pvt. Ltd; India. Water, Acetonitrile and Methanol for HPLC were purchased from LICHROSOLV (MERCK, Mumbai (India)).

Methods

UV-Visible Spectrophotometric Method Development and Validation.

Preparation of stock solution

Standard stock solution of Ranolazine was prepared by dissolving 100 mg of drug in 100 ml 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 solution⁶.

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 274nm⁷.

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-axis⁸.

Preparation of sample solution

Twenty tablets 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 274nm⁹.

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 guidelines¹⁰.

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.

$$LOQ = 10 \sigma/S$$

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 observation¹¹.

RP-HPLC method development and validation of Ranolazine¹²

HPLC was selected as analytical technique for estimation of ranolazine.

Chromatographic condition (initial):

Column : Develosil C18 (4.6mm x 150mm, 5 μ m)

Column temperature : 45°C

Wavelength : 274 nm

Mobile phase ratio : Methanol: Water (65:35%) V/V

Flow rate : 0.9 ml/min

Injection volume : 20 μ l

Run time : 10 mi

Standard Stock solution of Ranolazine (1000 μ g/ml):

Accurately weighed 100 mg Ranolazine and transferred in to 100 ml volumetric flask. Methanol was added about 30 ml to same flask and sonicated for about 10 min. Mixture was allowed to cool to room temperature and final Volume was marked with Methanol. Concentration of Ranolazine solution: 1000 μ g/ml.

Selection of detection wavelength:

0.1 ml of Stock solution of Ranolazine was diluted with 10 ml of methanol. The standard solution of Ranolazine (10 μ g/ml) in methanol was scanned in the range of 200nm-400nm record the spectra. Detection wavelength selected is 274nm.

Selection of mobile phase and column:

Procedure: Used standard solution (100 μ g/ml) of Ranolazine for method development of HPLC method.

Instrument parameter: Detection Wavelength: 274

Injection Volume: 20 μ l

Finalized Parameters:

Table No.1: Finalized Parameters for Chromatographic condition

Parameters	Specifications
Mobile phase ratio	Acetonitrile: Methanol: water (50:30:20%v/v)
Column	Zorbax C ₁₈ (4.6mm x 250mm, 5 μ m, Make: X terra)
Column temperature	35°C
Wavelength	274 nm
Flow rate	1 ml/min
Injection volume	20 μ l
Run time	10 min

Calibration Curve of Ranolazine

Calibration curves were prepared by talking concentrations 10-50 μ g/ml. Each sample in five replicates was analyzed and peak areas were recorded. The area was plotted against the corresponding concentrations of Ranolazine.

Assay of Ranolazine Marketed Formulation

Accurately measured sample equivalent to 10 mg Ranolazine was transferred to 100 ml of volumetric flask then add 50 ml of diluent and shake to mix and make volume with diluent to give solution containing 100 μ g/ml of Ranolazine. Above solution was filtered through Whatman filter paper¹³.

Method Validation

System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each

parameter. The test includes parameters like Number of Theoretical Plates, Resolution, Retention time and tailing factor.

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 4 different concentration levels (0%, 50%, 100% and 150%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to analysis by the proposed method.

Precision

a) Repeatability Study:

Repeatability studies were carried out by estimating the assay of six different sample preparation of same batch against mixed standard solutions of 100 µg/ml for Ranolazine were prepared and chromatograms were recorded. Assay was measured and %RSD was calculated.

b) Intra- day precision

Solutions of concentration 100 µg/ml of Ranolazine were analyzed three times on the same day and % R.S.D was calculated.

c) Inter-day precision

Solutions of concentration 100 µg/ml of Ranolazine were analyzed three times on the same day and % R.S.D was calculated.

Limit of Detection and Limits of Quantitative

Limit of Detection (LOD)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 \sigma / S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

Limit of Quantitation (LOD)

The limit of quantitation (LOD) of the drug was calculated by using the following equation designed by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10 \times \sigma / S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters.

a) Flow rate: ± 0.2 ml/min.

b) Change in the column oven temperature $\pm 5^\circ\text{C}$.

c) Change in the detection $\pm 2\text{nm}$.

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 ingredient¹⁴.

Acid Degradation Studies:

To 1 ml of Ranolazine stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 °C. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (100 $\mu\text{g/mL}$) solution. Cool the solution to room temperature and filtered with 0.45 μm membrane filter. A sample of 10 μl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample¹⁵.

Oxidation Degradation Studies:

To 1 ml of stock solution of Ranolazine 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain (100 $\mu\text{g/mL}$) solution. Cool the solution to room temperature and filtered with 0.45 μm membrane filter. A sample of 10 μl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample¹⁶.

Dry Heat Degradation Studies:

The 1 ml of Ranolazine drug solution was placed in the oven at 60°C for 6h to study dry heat degradation. for HPLC study, the resultant solution was makeup to final volume to obtain (100 $\mu\text{g/mL}$) solution. Cool the solution to room temperature and filtered through a 0.45 μm membrane filter. A sample of 10 μl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability¹⁷.

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/ m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (100 $\mu\text{g/mL}$) solution and filtered with 0.45 μm membrane filter. A sample of 10 μl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability¹⁸.

RESULTS:

UV -Visible Spectrophotometric Method Development and Validation

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 λ_{max} in the literature and the procured drug sample of Ranolazine complies with the reference spectra. The wavelength of Ranolazine is found to be 274 nm when scanned from 200-400nm spectrophotometric ally.

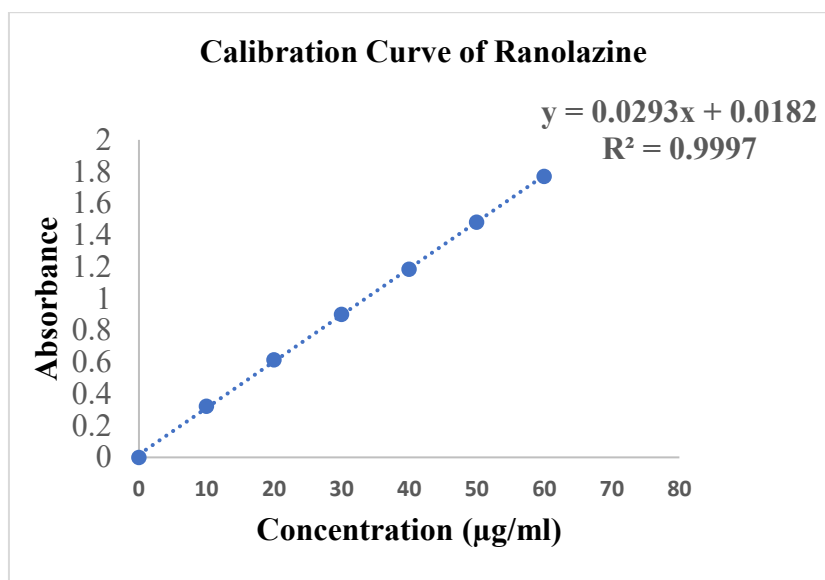


Figure 1: Calibration Curve of Ranolazine

Linearity range was found to be 10-60 µg/ml for Ranolazine at 274 nm. The correlation coefficient was found to be 0.9997, 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. 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 and result was depicted in table no. 1.

Table No. 1: 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 and result of intraday & Interday was shown in table no. 2 & 3.

Intraday Precision

The intraday precision of Ranolazine was evaluated by analyzing samples at three different concentrations—5, 10, and 15 µg/ml—measured in triplicate on the same day. The absorbance values recorded for each concentration were consistent, reflecting minimal variability. For the 5 µg/ml concentration, the %RSD was 0.15665, while for 10 µg/ml and 15 µg/ml, the %RSD values were 0.15623 and 0.27174, respectively (Table 02). The overall average %RSD was found to be 0.19487, which is well within the acceptable limit, indicating high precision and reliability of the method for same-day analysis.

Table No. 2: Intraday Precision of Ranolazine

Concentration (µg/ml)	Absorbance (nm)			Mean	Standard Deviation	%RSD	Average of % RSD
	1	2	3				
5	0.368	0.369	0.368	0.36833	0.00058	0.15665	0.194873
10	0.37	0.369	0.369	0.36933	0.00058	0.15623	
15	0.368	0.369	0.367	0.368	0.001	0.27174	

Inter-day Precision

The inter-day precision of Ranolazine was assessed by analyzing samples at concentrations of 5, 10, and 15 µg/ml across three different days. The absorbance values for each concentration remained consistent, indicating minimal variation over time. For the 5 µg/ml concentration, the %RSD was 0.15465, while the 10 µg/ml and 15 µg/ml concentrations showed %RSD values of 0.15410 and 0.15369, respectively (Table 03). The overall average %RSD was calculated to be 0.15414, demonstrating excellent reproducibility and confirming the reliability of the analytical method over multiple days.

Table No. 3: 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 accurate (Table No. 4). The accuracy of the developed analytical method was confirmed through recovery studies at three concentration levels: 5, 10, and 15 µg/ml. The percent recovery for Ranolazine ranged from 99.30% to 100.68%, indicating that the method yields result very close to the actual values. At 5 µg/ml, the mean recovery was 99.99% ± 0.69 with a %RSD of 0.69, while the 10 µg/ml level showed a mean recovery of 99.77% ± 0.52 and a %RSD of 0.53. Similarly, for 15 µg/ml, the mean recovery was 99.69% ± 0.48, with a

%RSD of 0.48. All %RSD values were well below 2.0, confirming the method's high level of precision and accuracy. These findings demonstrate that the method is reliable for the quantitative analysis of Ranolazine within the tested range.

Table No. 4: 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

The results obtained for the accuracy study (recovery method) from three sample studies (n = 3) for each level indicated that the mean of the % recovery was 99.99333% and 99.76667% and 99.69067% and %R.SR. D was 0.69007%, 0.526045% and 0.479631% for ranolazine in standard (10 µg/ml). Here the mean % recovery is in between 98-102 % thus showing that the analytical technique has a good recovery study.

Robustness

The result of robustness study of the developed assay method was established in Table No. 5 & 6. The robustness of the analytical method was assessed by evaluating its performance at two different temperatures: 25°C and 28°C, using a 10 µg/ml concentration of Ranolazine. At 25°C, the absorbance values ranged from 0.333 to 0.339, with a calculated mean of 0.337, a standard deviation (SD) of 0.00228, and a %RSD of 0.6767. Similarly, at 28°C, the absorbance values ranged from 0.332 to 0.337, with a mean of 0.3347, SD of 0.00175, and %RSD of 0.5233. In both conditions, the %RSD values remained well below the 2% threshold, indicating that minor variations in temperature did not significantly affect the method's performance. These results confirm that the method is robust and capable of producing reliable data under slight changes in experimental conditions.

Table No. 5: Evaluation Data of Robustness Study (Temperature-25°C)

Concentration (µg/ml)	Absorbance	Statistical Analysis
10	0.333	Mean = 0.337
10	0.336	

10	0.338	SD = 0.00228 % RSD = 0.676662
10	0.339	
10	0.337	
10	0.339	

Table No. 6: Evaluation Data of Robustness Study (Temperature-28°C)

Concentration (µg/ml)	Absorbance	Statistical Analysis
10	0.335	Mean = 0.334667 SD = 0.001751 % RSD = 0.523264
10	0.334	
10	0.332	
10	0.334	
10	0.336	
10	0.337	

The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The ruggedness of the method was evaluated by performing the analysis using the same procedure but by a different analyst (Analyst-I), to determine its reproducibility under varied operational conditions. Using a concentration of 10 µg/ml, the absorbance values ranged from 0.339 to 0.344. The calculated mean absorbance was 0.341 with a standard deviation of 0.00141 and a %RSD of 0.4147 (Table No. 07). The low %RSD indicates minimal variability, confirming that the method is rugged and yields consistent results even when carried out by different analysts under similar conditions.

Table No. 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	

To further assess the method's ruggedness, the analysis was performed by a second analyst (Analyst-II) using the same experimental conditions. At a concentration of 10 µg/ml, the absorbance values varied slightly between 0.331 and 0.335. The mean absorbance was calculated to be 0.332, with a standard deviation of 0.00179 and a %RSD of 0.5388 (Table No. 08). These values demonstrate that the method maintains good reproducibility across different analysts, with %RSD well within acceptable limits, thus confirming the ruggedness and reliability of the method under varying operator conditions.

Table No. 8: Evaluation Data of Ruggedness (Analyst-II)

Concentration (µg/ml)	Absorbance	Statistical Analysis
10	0.331	Mean = 0.332 SD = 0.001789 % RSD = 0.538812
10	0.332	
10	0.331	
10	0.332	
10	0.333	
10	0.335	

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 high sensitivity 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

RP-HPLC Method Development and Validation of Ranolazine

Selection of Detection Wavelength

The detection wavelength for Ranolazine was optimized and finalized at 274 nm. At this wavelength, the chromatogram displayed a well-defined and symmetrical peak with minimal tailing, an acceptable plate count, and a stable baseline, indicating optimal chromatographic performance. These parameters confirmed the suitability of 274 nm for accurate and reliable detection of Ranolazine. Therefore, this condition was selected as the final method for analysis, and the corresponding chromatographic results are presented in Table No. 9 and illustrated in Figure 2.

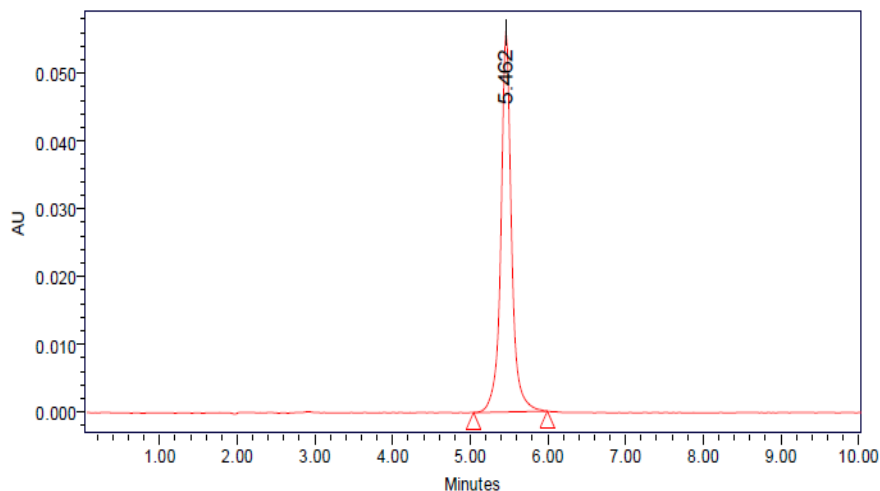


Figure 2: Chromatogram of Ranolazine

Table No. 9: Peak Results for Finalized Parameters

Sr. No.	Name	RT	Peak Area	No. of Theoretical plates
1	Ranolazine	5.462	1052689	75421

Optimized Chromatogram (Sample)

The optimized chromatogram of the Ranolazine sample is presented in Figure 3. Under the finalized analytical conditions, the chromatogram exhibited a sharp and symmetrical peak with an appropriate retention time, minimal tailing, and a stable baseline. These characteristics indicate that the method is both effective and reliable for the quantitative analysis of the sample. The clarity and resolution observed confirm that the optimized parameters are suitable for routine analysis of Ranolazine in pharmaceutical formulations.

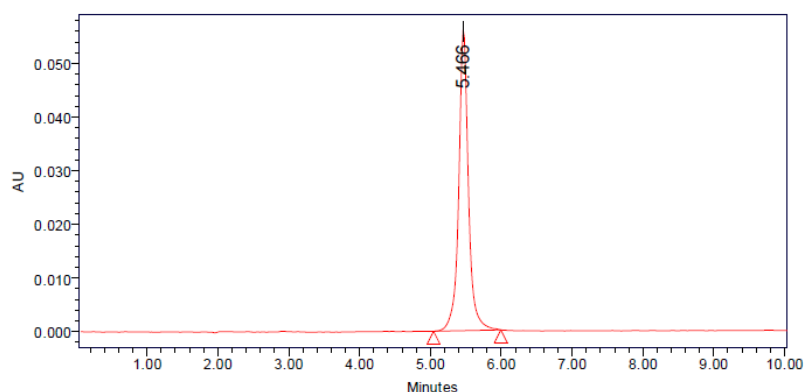


Figure 3: Optimized Chromatogram (Sample)

Calibration curve for Ranolazine

The calibration curve for Ranolazine was developed over a concentration range of 0 to 50 µg/ml, with corresponding peak area responses listed in Table No. 10 and graphically represented in Figure 4. A strong

linear correlation was observed between the concentration and peak area, indicating excellent method linearity. The regression equation obtained from the curve was $Y = 10962X + 57.67$, where Y represents the peak area and X is the concentration in $\mu\text{g/ml}$. The method demonstrated a high correlation coefficient ($R^2 = 0.9999$), confirming the reliability and accuracy of the method for the quantitative estimation of Ranolazine across the tested range.

Table No. 10: Calibration Curve for Ranolazine

Conc. ($\mu\text{g/ml}$)	Area
0	0
10	110102
20	218757
30	327412
40	436067
50	544722

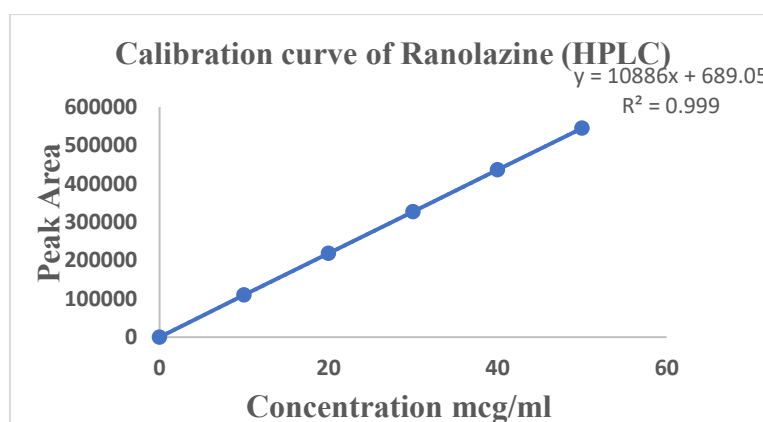


Figure 4: Calibration Curve of Ranolazine

The area responses increased proportionally with concentration, indicating good linearity of the method. The correlation between the variables confirms the suitability of the method for accurate quantitative analysis of Ranolazine within the specified range.

Table No. 11: Calibration Curve Results

Parameter	Result
Linearity range ($\mu\text{g/ml}$)	10-50 mg/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:

The assay of Ranolazine in the marketed formulation was conducted to evaluate the accuracy and reliability of the developed analytical method. As shown in Table No. 12, three samples with an actual concentration of 100 µg/ml were analyzed. The concentrations of Ranolazine found were 98.91, 99.54, and 99.67 µg/ml, corresponding to 98.91%, 99.54%, and 99.67% drug content, respectively. The average percentage of drug found was calculated to be 99.37%, with a standard deviation (SD) of 0.4064 and a %RSD of 0.4090. These results fall well within acceptable limits, confirming the accuracy, precision, and suitability of the method for the routine analysis of Ranolazine in pharmaceutical formulations.

Table No. 12: 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

System Suitability Tests

The results of the system suitability tests are presented in Table No. 13. The chromatographic analysis demonstrated a retention time of 5.27 minutes. The resolution was calculated to be 30.67, which is significantly higher than the minimum required value of 1.5, indicating good separation. The theoretical plate number was found to be 9,674, exceeding the standard criterion of not less than 2,000, thus confirming adequate column efficiency. Additionally, the tailing factor was observed at 1.63, remaining well within the acceptable limit of not more than 2.0, suggesting good peak symmetry. Overall, the system met all the suitability parameters, ensuring reliable and accurate analytical performance.

Table No. 13: 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

Accuracy

The accuracy of the analytical method was evaluated by recovery studies at three different concentration levels: 50%, 100%, and 150%. For the "as such" sample, without any spiking, the percent recovery ranged from 100.88% to 101.61%, with a mean recovery of 101.22% and a %RSD of 0.36, indicating excellent precision. At the 50% spiking level, the recoveries ranged from 98.59% to 100.44%, yielding a mean of 99.62% and %RSD of 0.94, which falls within acceptable limits. Similarly, the 100% level showed recoveries between 99.68% and 101.19%, with a mean of 100.44% and %RSD of 0.75. At the 150% level, the recoveries varied from 99.05% to 100.00%, resulting in a mean recovery of 99.61% and %RSD of 0.50. These findings confirm the method's accuracy and reproducibility across a wide concentration range. The results of the system suitability tests are presented in Table No. 14.

Table No. 14: 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		

Precision

Repeatability Study (Sample Precision)

The repeatability of the analytical method was evaluated by analyzing six replicate preparations of the same sample. The assay results, as shown in Table No. 15, ranged from 99.55% to 100.69%, with a mean assay value of **100.07%**. The corresponding assay in mg/g varied between 9.95 and 10.07 mg/g. The calculated **%RSD was 0.46%**, which is well within the acceptable limit, indicating excellent precision. These results confirm that the method is highly repeatable and capable of producing consistent and reliable results during routine analysis.

Table No. 15: 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

The precision of the method was further confirmed through intraday and inter-day studies using a 100 µg/ml concentration of Ranolazine. As shown in Table No. 16, the intraday peak areas measured at three different time points (Time-1, Time-2, and Time-3) were 1,052,728, 1,056,854, and 1,052,468 respectively, with %RSD values ranging from 0.2229% to 0.2460%, indicating excellent short-term consistency. In the inter-day study, measurements taken on three consecutive days (Day-1, Day-2, and Day-3) produced peak areas of 1,102,689, 1,102,532, and 1,110,121, with %RSD values between 0.3825% and 0.3986%, confirming high reproducibility over time. All %RSD values remained well below the acceptable limit of 2%, demonstrating that the method maintains robust precision both within the same day and across multiple days.

Table No. 16: 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

Limit of Detection and Limit of Quantitation

The sensitivity of the analytical method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Ranolazine. As shown in Table No. 17, the LOD was found to be 1.6 µg/ml, indicating the lowest concentration of the drug that can be reliably detected but not necessarily quantified. The LOQ was determined to be 4.8 µg/ml, representing the minimum concentration that can be accurately and precisely quantified under the established experimental conditions. These results confirm the method's suitability for detecting and quantifying low levels of Ranolazine in pharmaceutical formulations.

Table No. 17: LOD and LOQ

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

Robustness

The robustness of the developed method was assessed by introducing deliberate variations in key chromatographic parameters, including column temperature, flow rate, and detection wavelength, to evaluate the method's reliability under minor changes. As shown in Table No. 18, when the column temperature was varied between 30°C and 40°C, the detected amount of Ranolazine remained consistent, with %RSD values ranging from 0.120 to 0.372, all within acceptable limits. Similarly, altering the flow rate from 0.8 mL/min to 1.2 mL/min showed minimal effect on the assay results, with %RSD values between 0.142 and 0.414, confirming flow rate robustness. Changes in detection wavelength from 272 nm to 276 nm also produced reliable results, with %RSD values from 0.102 to 0.307. These findings demonstrate that the analytical method is robust, producing accurate and precise results despite small variations in analytical conditions.

Table No. 18: 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 °C	100	99.75±0.372	0.372
	35 °C	100	99.99±0.163	0.162
	40 °C	100	99.78±0.120	0.120
Change in flow 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

Acid Degradation Studies:

The stability of Ranolazine under acidic conditions was assessed through acid degradation studies. As illustrated in Figure 5, the chromatogram reveals the degradation behavior of Ranolazine when subjected to acidic stress. The appearance of additional peaks alongside the primary drug peak indicates the formation of degradation products, confirming that Ranolazine is susceptible to acid hydrolysis. This suggests that the drug is not stable under strongly acidic conditions, highlighting the importance of appropriate formulation and storage measures to ensure product stability and efficacy.

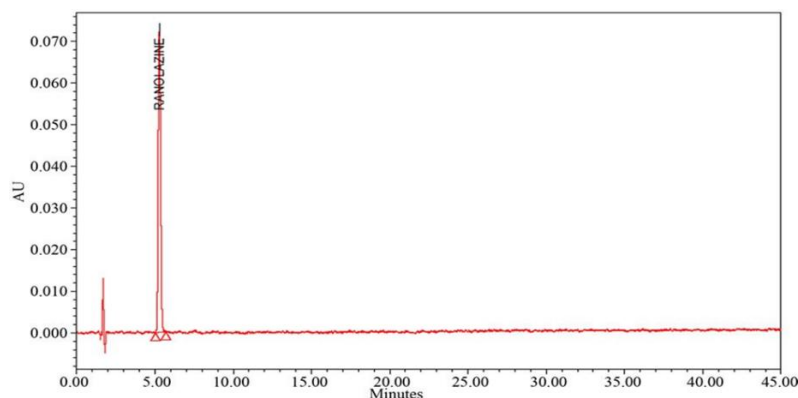


Figure 5: Acid Degradation of Ranolazine Chromatogram

Alkali Degradation Studies:

The alkaline stability of Ranolazine was evaluated by subjecting the drug to basic hydrolytic conditions. As illustrated in Figure 6, the chromatogram reveals the presence of additional peaks apart from the main drug peak, indicating the formation of degradation products. This confirms that Ranolazine undergoes significant degradation under alkaline conditions. The changes observed in the chromatographic profile demonstrate that the drug is not stable in basic environments, emphasizing the need for pH-controlled formulations to maintain its stability and therapeutic efficacy.

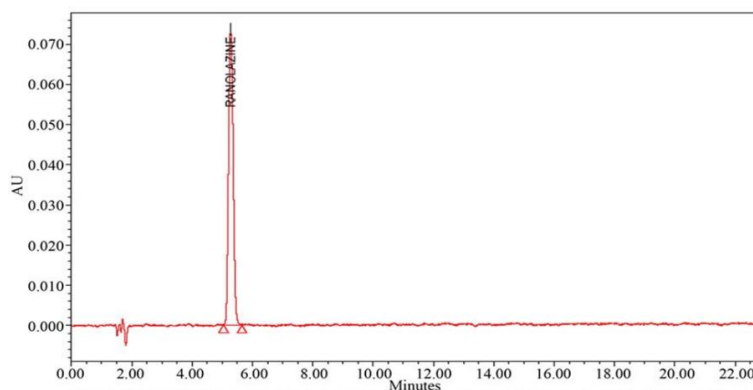


Figure 6: Alkaline Degradation of Ranolazine Chromatogram

Oxidation Degradation Studies:

The oxidative stability of Ranolazine was evaluated by exposing the drug to oxidative stress conditions. As shown in Figure 7, the chromatogram demonstrates notable changes in the drug profile, with the appearance of additional peaks indicating the formation of oxidative degradation products. These results suggest that Ranolazine undergoes degradation when exposed to oxidative agents, confirming its sensitivity to such conditions. This finding underscores the need for protective formulation strategies to minimize oxidative degradation and maintain the drug's therapeutic integrity throughout its shelf life.

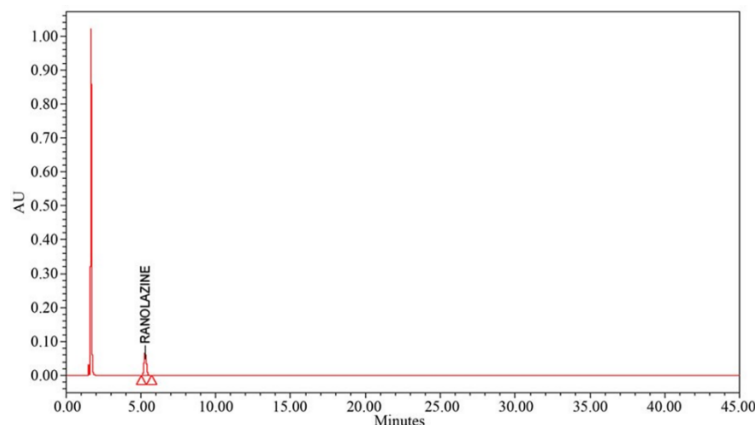


Figure 7: Oxidative Degradation of Ranolazine Chromatogram

Dry Heat Degradation Studies:

The thermal stability of Ranolazine was assessed by exposing the drug to dry heat conditions. As shown in Figure 8, the chromatogram displays the appearance of additional peaks alongside the primary Ranolazine peak, indicating the formation of degradation products due to heat exposure. This suggests that Ranolazine is susceptible to thermal degradation when subjected to elevated temperatures. The findings highlight the importance of protecting the drug from excessive heat during storage and processing to ensure its stability and maintain its therapeutic effectiveness.

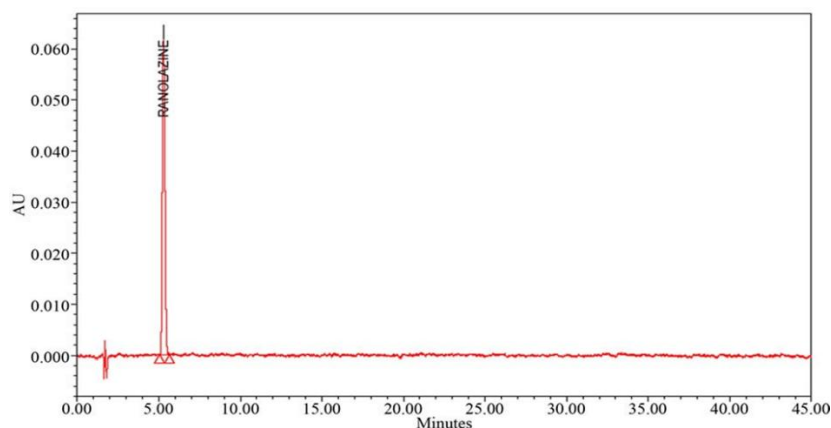


Figure 8: Thermal Degradation of Ranolazine Chromatogram

Photo Degradation Studies:

The effect of light exposure on the stability of Ranolazine was examined through photolytic degradation studies. As depicted in Figure 9, the chromatogram shows the emergence of additional peaks apart from the main drug peak, indicating that Ranolazine undergoes degradation upon exposure to light. These results confirm the drug's sensitivity to photolytic conditions and the formation of light-induced degradation products. Therefore, it is essential to protect Ranolazine from light during storage and handling to preserve its chemical integrity and therapeutic efficacy.

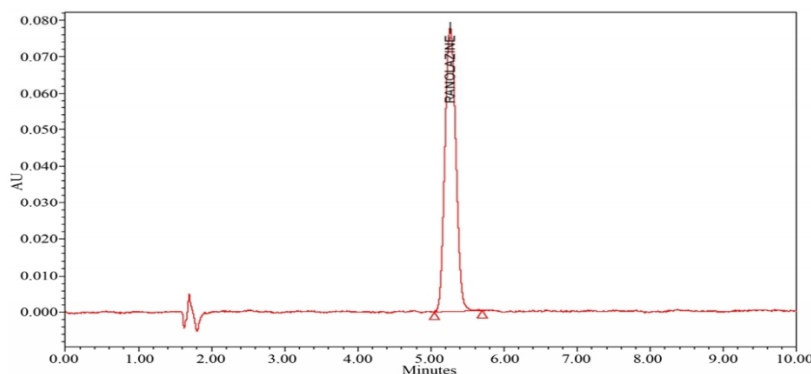


Figure 9: Photolytic Degradation of Ranolazine Chromatogram

Forced degradation studies were conducted under various stress conditions to evaluate the stability profile of Ranolazine. As shown in Table No. 19, the standard sample exhibited 100% active drug content, serving as the control. Under acidic conditions, 21.88% of the drug was degraded, leaving 78.12% of the active component intact. Basic hydrolysis resulted in 17.43% degradation, while oxidative stress led to 11.37% degradation, indicating moderate susceptibility. Exposure to dry heat caused a smaller degradation of 5.81%, and photolytic conditions led to a 9.48% loss of active drug content. These results demonstrate that Ranolazine is most sensitive to acidic and basic environments, while it shows relatively better stability under thermal and photolytic stress. The findings emphasize the need for protective formulation strategies to ensure the drug's stability during manufacturing, storage, and use.

Table No. 19: Results of Forced Degradation Studies for Ranolazine

Sr. No.	Stress Condition	Peak Area	% Degraded 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	11.37	88.63	100%
5	Thermal	136341.88	5.81	94.19	100%
6	Photolytic	134762.14	9.48	90.52	100%

Conclusion

The forced degradation studies of Ranolazine were conducted under various stress conditions—acidic, basic, oxidative, thermal, and photolytic—to assess the stability and degradation behaviour of the drug. The results revealed that Ranolazine is most susceptible to acidic degradation (21.88%), followed by basic degradation (17.43%). Moderate degradation was observed under oxidative stress (11.37%) and photolytic conditions (9.48%), while the drug demonstrated greater stability under thermal conditions, with only 5.81% degradation. These findings indicate that Ranolazine is chemically unstable in strongly acidic and basic environments, with comparatively better resilience to heat and light exposure. Overall, the study highlights the importance of

formulating and storing Ranolazine under controlled conditions to minimize degradation and maintain its therapeutic efficacy throughout its shelf life.

The experimental protocol for degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. A target of the lesser of 10% degradation of the active ingredient or exposure to energy in slight excess of accelerated storage is recommended. A compound may not necessarily degrade under a given stress condition. No further stressing is advised in these cases. The developed methods reported are simple, rapid and precise. From the current work, it was finally concluded that the developed UV spectroscopic and RP-HPLC method were found to be very simple, reliable and selective for providing satisfactory accuracy and precision. The developed UV and HPLC method were found to be linear over wider concentration range. Therefore, the developed HPLC and UV method can be applied for routine quantitative and qualitative analysis of Ranolazine in bulk. The developed HPLC method for the stability studies on Ranolazine within the Industry. The developed Stability indicating HPLC method can be used to monitor Stability and determination of Ranolazine in quality control laboratories. The method has been shown to be specific and selective.

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