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SPECTROPHOTOMETRIC AND STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RANOLAZINE IN TABLET DOSAGE FORM

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Keywords:

Ranolazine, RP-HPLC, UV Spectroscopy, tablet dosage forms

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ABSTRACT: A spectrophotometric and reverse phase high performance liquid chromatography methods has been developed for the estimation of Ranolazine in bulk and pharmaceutical dosage form. For UV spectrophotometric method Ranolazine shows maximum absorbance at 273 nm with the concentration ranges of 20-150 µg/mL. The detection limit and quantification limit were found to be 1.23 and 5.21 µg/mL, respectively. Accuracy was in the range of 99.8-100.2%. Intraday and interday precision was in the range 0.16- 0.38% and 0.50-0.82% respectively. The RP-HPLC separation was achieved by using Phenomenex Gemini C_{18} (100mm X 4.6mm), 3µm column and buffer: acetonitrile (60:40, v/v), (1.0 mL of triethylamine in 1000 mL milli- Q water, pH adjusted to 6 using dilute o-phosphoric acid solution) as mobile phase. The flow rate was 1.0 mL/min and effluents were monitored at 224 nm. The retention time was 5.08 min. The linearity was in the range of 20-150 µg/mL. The detection limit and quantification limit were found to be 1 and 4 µg/mL, respectively. Accuracy was in the range of 99.9-100.1%. Intraday and interday precision was in the range 0.29- 0.75% and 0.51-0.95% respectively. Ranolazine were subjected to acidic, alkali, oxidative, photolytic and dry heat degradation. The degraded product peaks were well resolved from the pure drug peak. The proposed methods were validated and successfully applied to the estimation of Ranolazine in tablet formulation.

INTRODUCTION: Ranolazine (RS)–N-(2,6-di methylphenyl) - 2 - [4 - [2-hydroxy - 3 -(2-methoxy phenoxy)-propyl] piperazin-1-yl] acetamide (**Fig. 1**) is an Antianginal class. Ranolazine is available as tablet dosage form [1 - 2]. Ranolazine is not official in pharmacopoeia. A few methods in literature were reported for the determination of Ranolazine by UV-visible spectroscopy, HPLC, HPTLC method [3 - 10]. Although these techniques are sufficiently sensitive, but they use expensive instrument and time consuming. The present LC method uses a simple mobile phase combined with UV detection and does not require complicated sample preparation. Therefore, this study aimed to develop and validate simple, rapid, accurate and specific, fast, low-cost, and selective methods for routine quality control analysis of pharmaceutical product containing Ranolazine. UV spectrophotometry is an easy-to-use and robust method for the quantification of drugs in formulation when there is no interference from excipients.

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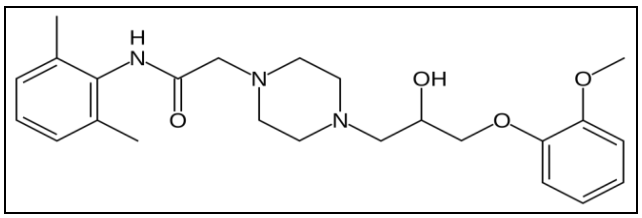


FIG. 1: CHEMICAL STRUCTURE OF RANOLAZINE

MATERIALS AND METHODS:

Chemicals and reagents: Ranolazine used as reference substance assigned purity 99.86% (Zydus Cadila Pharmaceuticals Ltd., Ahmedabad, India). Ranolazine is a single component film-coated tablet formulation of Caroza (500 mg). HPLC Grade Acetonitrile and methanol (E. Merck, Mumbai, India), AR Grade Triethylamine and o-phosphoric acid (Spectrochem, Mumbai, India) were used for preparation of mobile phase. The water for HPLC used for mobile phase preparation was of HPLC grade by Milli Q.

Methods:

UV Spectrophotometric Method:

Instrumentation: A Shimadzu Model 1600 double beam UV-Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm, and a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions.

Preparation of Stock Solution: A stock solution of 500 µg/mL ranolazine was prepared by dissolving 50 mg of ranolazine in 100 mL volumetric flask in methanol.

Calibration Curve for Ranolazine: Appropriate aliquots of Ranolazine stock solution were taken in different 25 mL volumetric flasks and volume was made up to the mark with methanol to obtain final concentrations of 20, 50, 80, 100 120, 150 µg/mL. One of the solutions was scanned in UV range using methanol as blank and λ_{max} was found to be 273 nm. The absorbances of solutions were measured at 273 nm against blank and calibration curve of Ranolazine was constructed.

Analysis of Marketed Formulation: Twenty intact tablets of Ranolazine were weighed and powdered. Amount equivalent to 500 mg of Ranolazine was transferred in to a 100 mL volumetric flask, and dissolved in 50 mL methanol. The flask was sonicated for 20 minutes with intermediate shaking and the volume was made up to the mark with methanol. This solution was then filtered through 0.45 µm HVLP (whatman filter paper No.41) filter. From this solution 2 mL was further diluted to 100 mL with methanol to obtain solution of 100µg/mL. The absorbance of sample solution was measured and the quantification was carried out by keeping these values to the linear equation of calibration curve.

Validation: The method was validated with respect to parameters such as linearity, accuracy, precision, specificity, detection limit, quantitation limit and robustness, as required under ICH guideline [11].

The linearity was obtained at 6 concentration levels of Ranolazine standard solution. The absorbances of all solutions were measured using above method. The linearity was evaluated by least square regression method. The accuracy of the method was determined by calculating recoveries of Ranolazine by method of standard additions. Known amount of drug (25, 50, 75 µg/mL) were added to a pre-quantified sample solution and the amounts were estimated.

The instrument precision was evaluated by taking absorbance of the solution six times repeatedly. The results are reported in terms of relative standard deviation. The intra-day and inter-day precision study of Ranolazine was carried out by estimating the corresponding absorbances 3 times on the same day and on 3 different days and the results are reported in terms of relative standard deviation (RSD). The specificity was estimated by spiking commonly used excipient (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The absorbance was taken by appropriate dilutions and the quantities of drugs were determined.

The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD was the concentration that yielded signal to noise ratio (S/N) 3:1 and LOQ was the concentration that yielded signal to noise ratio (S/N) 10:1. Robustness of the method was studied by deliberately changing the experimental conditions like analytical wavelength.

RP-HPLC Method:

Instrumentation: The LC system consisted of a Shimadzu (LC- 2010CHT) equipped with UV-Visible detector having auto sampler.

Chromatographic Conditions: Phenomenex Gemini C_{18} (100mm X 4.6mm, 3µm particle size) column equilibrated with mobile phase comprising of buffer: acetonitrile (60:40, v/v, 1.0 mL of triethylamine in 1000 mL milli- Q water**)** was used. Mobile phase flow rate was maintained at 1.0 mL/min with the sample injection volume 10 µl and eluent were monitored at 224 nm. All the chromatographic separations were carried out at controlled room temperature (25 ± 2 ^{o}C).

Preparation of Mobile Phase and Stock Solution: Buffer (1.0 mL of Triethylamine in 1000 mL water. pH was adjusted to 6.0 with dilute o-Phosphoric acid) : Acetonitrile in ratio of 60:40 mixed and filtered through 0.45 µm filter paper with vacuum filtration assembly. Mobile Phase was transferred to mobile phase bottle and sonicated for 15 min prior to use. Methanol was used initially for extracting the drug. Buffer: Acetonitrile (50:50) was used as diluent for further dilution.

A stock solution of 500µg/mL Ranolazine was prepared by dissolving 50 mg of Ranolazine in 100 mL volumetric flask in methanol.

Calibration Curve for Ranolazine: Appropriate aliquots of Ranolazine stock solution were taken in different 25 mL volumetric flasks and volume was made up to the mark with diluent to obtain final concentrations of 20, 50, 80, 100 120, 150 µg/mL. 10µL of each solution were injected and chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentration and regression equation was computed.

Analysis of Marketed Formulation: Twenty intact tablets of Ranolazine were weighed and powdered. Amount equivalent to 500 mg of Ranolazine was transferred in to a 100 mL volumetric flask, and dissolved in 50 mL methanol. The flask was sonicated for 20 minutes with intermediate shaking and the volume was made up to the mark with methanol. This solution was then filtered through 0.45 µm HVLP (whatman filter paper No.41) filter. From this solution 2 mL was further diluted to 100 mL with diluent to obtain solution of 100µg/mL. It was injected as per the above chromatographic conditions and peak area was recorded. The quantification was carried out by keeping these values to the linear equation of calibration curve.

Validation: The analytical method was validated with respect to various parameters such as linearity, accuracy, precision, specificity, detection limit, quantitation limit and robustness, as required under ICH guideline [11]. The accuracy of the method was determined by calculating recoveries of Ranolazine by method of standard additions. Known amount of drug (25, 50, 75 µg/mL) were added to a pre-quantified sample solution and the amounts were estimated.

The instrument precision was evaluated by injecting the solution six times repeatedly and peak area was measured. The results are reported in terms of relative standard deviation. The intra-day and inter-day precision study of Ranolazine was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days and the results are reported in terms of relative standard deviation (RSD).

The specificity was estimated by spiking commonly used excipients (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined. The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantitation of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD was the concentration that yielded signal to noise ratio (S/N) 3:1 and LOQ was the concentration that yielded signal to noise ratio (S/N) 10:1.

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, percentage of organic phase, column temperature and pH of mobile phase and observing the stability of the solution at 25 ± 2 ºC for 24 hrs.

Forced Degradation Study: Stress degradation study using acid and alkali hydrolysis, chemical oxidation and thermal degradation was carried out and interference of the degradation products was investigated.

50 mg of ranolazine and tablet powder equivalent to 50 mg of ranolazine dissolved in separate 50 mL volumetric flask, 25 mL of methanol was added, sonicated it for 20 min with intermediate shaking and volume was made up to the mark with methanol. These stock solutions were used for acidic, alkali and oxidative degradation.

Acidic Condition: 5 mL of each stock solution was taken in separate 50 mL volumetric flask and 5 mL of 5 N HCl was added to both flasks to perform acid hydrolysis. The flasks were boiled at 80 ºC for 2 hours and allowed to cool to room temperature. Solutions were neutralized with 5 N NaOH and diluted up to mark with diluent. The solutions were filtered through 0.45µm filter.

Alkaline Condition: 5 mL of each stock solution was taken in separate 50 mL volumetric flask and 5 mL of 5N NaOH was added to both flasks to perform alkali hydrolysis. The flasks were boiled at 80 ºC for 2 hours and allowed to cool to room temperature. Solutions were neutralized with 5 N HCl and diluted up to mark with diluent. The solutions were filtered through 0.45µm filter.

Oxidation Condition: 5 mL of each stock solution was taken in separate 50 mL volumetric flask and 5 mL 30% hydrogen peroxide was added to each flask to perform chemical oxidation The flasks were boiled at 80 ºC for 2 hours and allowed to cool to room temperature. The volume was made up to mark with diluent. The solutions were filtered through 0.45µm filter.

Thermal Degradation:

Dry Heat: Analytically pure sample of Ranolazine and tablet powder were exposed in oven at 80 ºC for 5 days. The solids were allowed to cool. 50 mg of ranolazine standard and tablet powder equivalent to 50 mg of ranolazine dissolved in separate 50 mL volumetric flask, 25 mL of methanol was added, sonicated it for 20 min with intermediate shaking and volume was made up to the mark with methanol.

5 mL of both solutions were taken in separate 50 mL of volumetric flask, dissolved in 5 mL of diluent and mix it. Then volume was made up to mark with diluent. The solutions were filtered through 0.45µm filter.

Exposed to UV Radiation: Analytically pure sample of Ranolazine and tablet powder were exposed to UV radiation for 24 hrs. After removal from exposure, 50 mg of API and tablet powder equivalent to 50 mg of ranolazine were dissolved in separate 50 mL volumetric flask containing 25 mL of methanol, sonicated it for 20 min with intermediate shaking and volume was made up to the mark with methanol. 5 mL of both solutions were taken in separate 50 mL of volumetric flask containing 5 mL of diluent and mix it. Then volume was made up to mark with diluent. The solutions were filtered through 0.45µm filter.

All the solutions were filtered through 0.45 µm filter and solutions were injected in the liquid chromatographic system and chromatograms were recorded.

RESULTS AND DISCUSSION:

UV Spectrophotometric Method: In order to choose optimum condition for the spectro-photometric analysis, the influences of various solvents were studied, such as methanol and ethanol. Ranolazine is soluble in methanol, ethanol and poorly soluble in water. Ranolazine in methanol showed absorption maxima at 273 nm (**Fig. 2**). Beer's law was obeyed over the concentration range of 20-150 µg/mL. Significant linear regression: the r^2 value was found to be 0.999, indicating that a linear relation existed between absorbance and concentration of the drug (**Table 1**). No significant differences were observed between the amount of Ranolazine added and the amount found, which indicated the accuracy of the method (**Table 2**). The obtained RSD values for the intraday and inter day precision were <2%, indicating satisfactory results (**Table 2**).

The specificity was studied by comparing spectra of Ranolazine standard and placebo spiked with ranolazine. The same shapes and wavelength maxima of the both the spectra revealed that the method was specific at 273 nm. Only Ranolazine was detected and not the excipients in the formulation. The LOD and LOQ values were 1.23 and 5.21 µg/mL respectively. The robustness of the method was assessed by changing analytical wavelength (271 and 275 nm) which did not have any significant effect on the absorbance (**Table 3**).

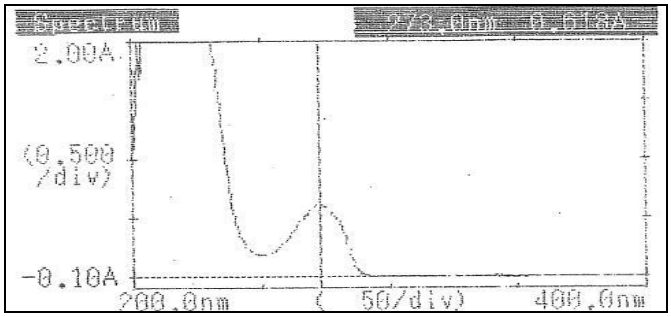


FIG. 2: UV SPECTRUM OF RANOLAZINE STANDARD SOLUTION (100 µG/ML)

RP-HPLC Method: Optimization of mobile phase was carried out by taking different proportions of aqueous and organic phases to obtain rapid, simple assay method for Ranolazine with appropriate run time, asymmetric factor and theoretical plates. Mobile phase consisting of Buffer: Acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min was found to be satisfactory which gave symmetric and sharp peak. The UV spectrum of the drug shows absorption bands at 224 nm therefore, the wavelength of detection was fixed at 224 nm. The retention time was found to be 5.09 ± 0.01 min. The calibration curve for Ranolazine was obtained by plotting the peak area versus concentration. Result of regression analysis was found by plotting peak area (y) versus the concentration (x) expressed in µg/mL. The correlation coefficient (r^2) values was found to be 0.999, indicating that a linear relation existed between peak area and concentration of the drug (**Table 1**). The obtained recovery values ranged from 99.9 to 100.1 %, showing the accuracy of the method (**Table 2**). The obtained RSD values for the intra-day and inter-day precision were <2%, indicating satisfactory results (**Table 2**). The interference of excipients was studied by comparing chromatogram of Ranolazine standard and placebo spiked with ranolazine (**Fig. 3** and **4**). The same shape and retention time of the peaks in both of the chromatograms revealed that there was no interference from excipients. The LOD and LOQ values calculated was 1.0 and 4.0 µg/mL, respectively, indicating high sensitivity of the method. The robustness of the method was assessed by analysis under variable chromate-graphic conditions. The change in organic phase ratio in the mobile phase did not have any significant effects on the response. Variation of the pH (5.8 and 6.2) of the mobile phase and column temperature (± 5°C) did not have any significant effects on the results (**Table 3**).

TABLE 1: RESULTS OF REGRESSION ANALYSIS OF DATA FOR THE QUANTIFICATION OF RANOLAZINE BY THE PROPOSED METHODS

Parameters	UV method	HPLC method
Concentration range (µg/mL)	20-150	20-150
Slope	0.006	24849
Intercept	0.007	46528
Correlation coefficient (r^2)	0.999	0.999

TABLE 2: SUMMARY OF VALIDATION PARAMETER

Parameters	UV method	HPLC method
Detection limit (µg/mL)	1.23	1
Quantitation limit (µg/mL)	5.21	4
Accuracy (%)	99.8-100.2	99.9 –100.1
Precision (RSD[a], %)		
Intra-day precision (n=3)	0.16-0.38 %	0.29 - 0.75 %
Inter-day precision (n=3)	0.50-0.82 %	0.51 - 0.95 %
Instrument precision (RSD[a] , %)	0.35	0.45

[a] RSD is relative standard deviation and 'n' is number of determinations

TABLE 3: DATA DERIVED FROM ROBUSTNESS OF RANOLAZINE FOR PROPOSED UV AND HPLC METHOD

Parameters	Normal Condition		Change in condition		%RSD	
	UV	HPLC	UV	HPLC	UV	HPLC
Wavelength	273 nm	-	271 nm	-	0.8	-
			275 nm		0.6	
Temperature	-	25°C	-	20° C	-	0.1
				30° C		0.2
Flow rate	-	1 mL/min	-	0.9 mL/min	-	0.1
				1.1 mL/min		0.1
Mobile phase ratio	-	60 : 40	-	61:49	-	0.1
				59:41		0.1
pH	-	6.0	-	6.2	-	0.1

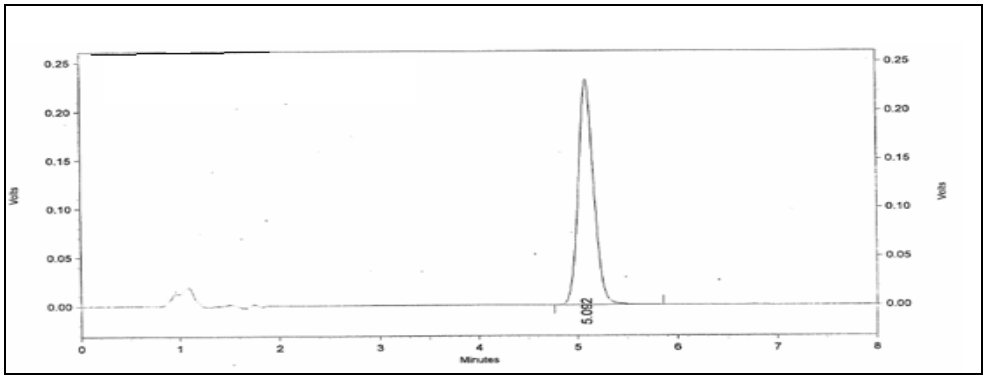


FIG. 3: CHROMATOGRAM OF RANOLAZINE STANDARD SOLUTION (100µG/ML)

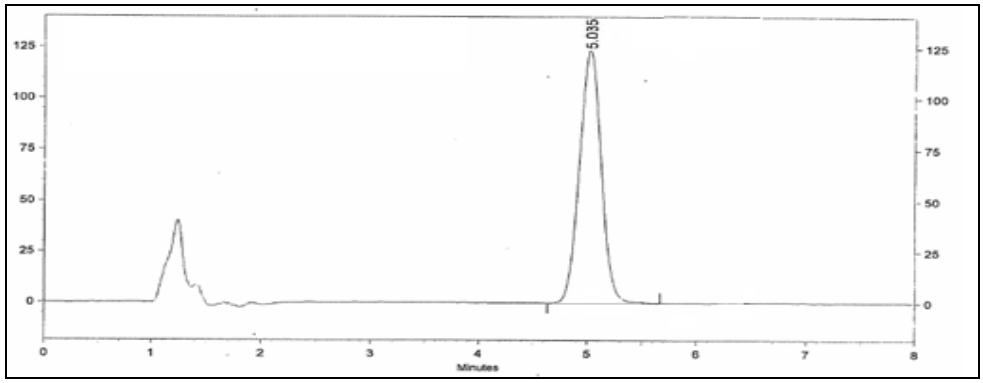
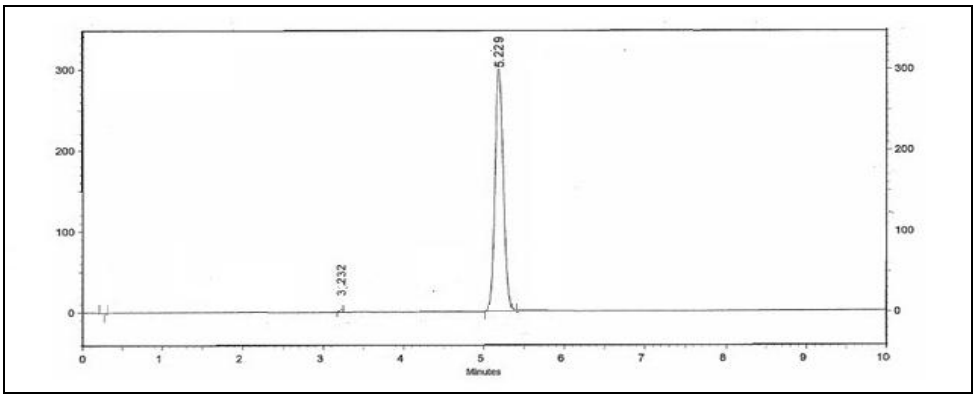
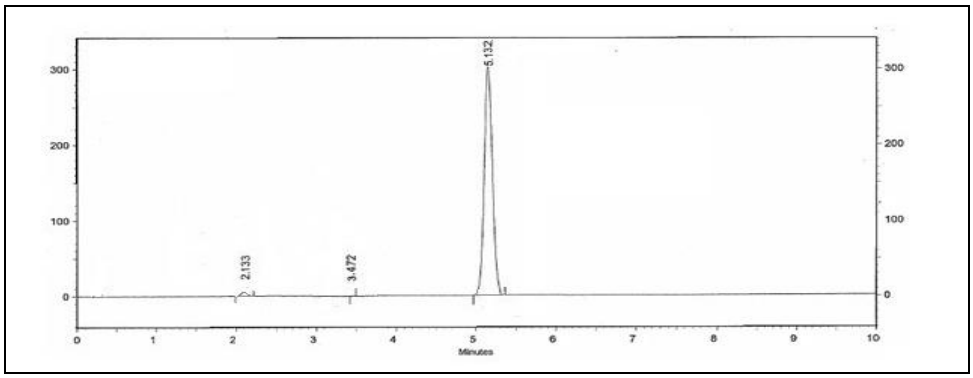


FIG. 4: CHROMATOGRAM OF PLACEBO SPIKED WITH RANOLAZINE (100µG/ML)

Forced Degradation Study: This study showed a reduction in the original drug response in all conditions. From degradation profile it can be concluded that ranolazine is susceptible to acid, alkali and thermal degradation. (**Table 5**)



(a)



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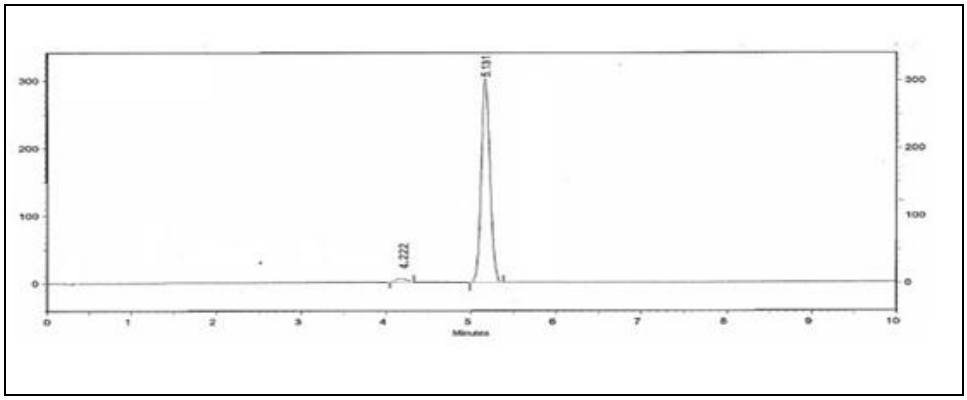

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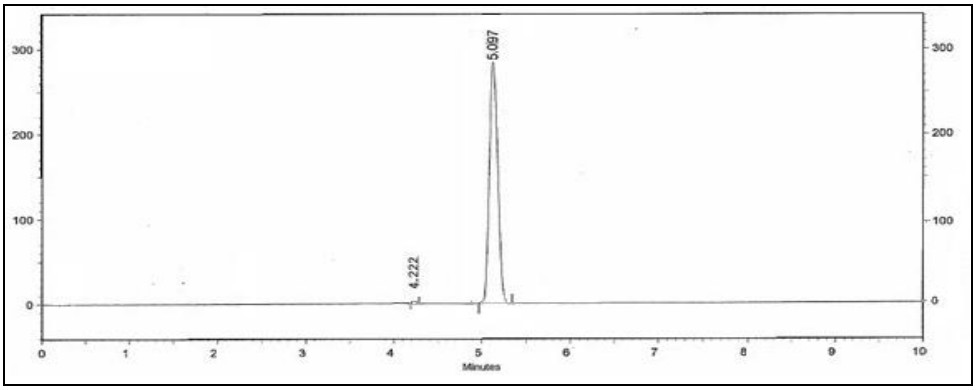

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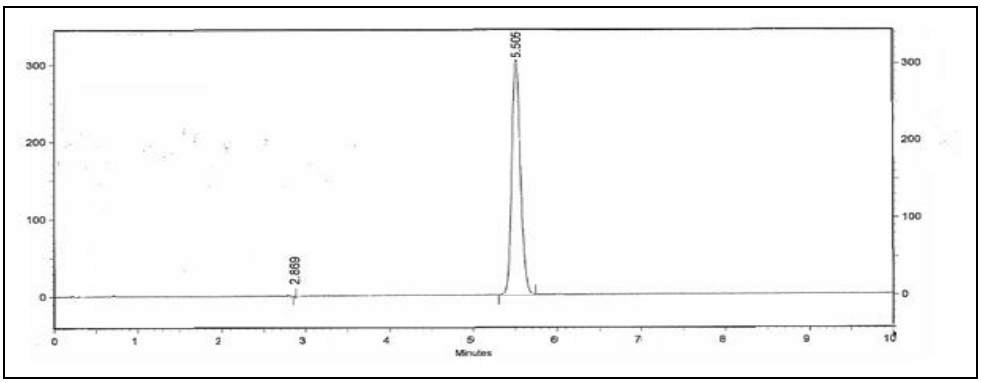

(e)

FIG. 5: TYPICAL CHROMATOGRAMS OBTAINED FROM API DEGRADED BY (a) ACID HYDROLYSIS, (b) ALKALINE HYDROLYSIS, (c) OXIDATION, (d) THERMAL (e) UV LIGHT

TABLE 4: ASSAY RESULTS OF TABLET DOSAGE FORM USING PROPOSED METHOD

Active ingredient	Label claim	Concentration (ppm)	UV		HPLC	
			Amt found (ppm)	% Assay	Amt found (ppm)	% Assay
Ranolazine	500 mg	100	100.1	100.1	100.2	100.2

TABLE 5: DATA DERIVED FROM FORCED DEGRADATION STUDY BY THE PROPOSED HPLC METHOD

S. no.	Condition	% Assay of Degradants	% Assay of active substance
1	As Such	-	100
2	Acidic	1.06	98.74
3	Alkaline	1.78	98.10
4	Oxidation	0.69	99.07
5	UV light	0.23	99.61
6	Thermal	2.49	97.20

CONCLUSION: The proposed UV spectrophotometric and LC methods have the advantages of simplicity, precision, accuracy, and convenience. Both methods are fast and use simple reagents with minimal sample preparation procedure. They are suitable for the routine analysis of Ranolazine in tablets, such as assay and uniformity testing in quality control. The UV spectrophotometric method provides practical and significant economic advantages over other published methods and can be an alternative for developing countries.

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CONFLICT OF INTEREST: Authors do not have any conflict of interest.

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