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DEVELOPMENT AND COMPARISON OF THE VALIDATION METHODS OF HPLC AND UV FOR DETERMINATION OF RANOLAZINE IN PURE FORM AND PHARMACEUTICAL FORMULATION

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Keywords:
Ranolazine, Reverse phase HPLC, Validation, Degradationstudies, UV Spectrophotometry.

Abstract
A new method was developed for the determination of ranolazine using HPLC and UV and their validation by comparing their efficacy in pure and pharmaceutical formulations.The U.V method used different solvent systems and the most solubilizing solvent for ranolazine was found to be methanol and the stock solution were prepared and plotted standard graph and compared with pure drug.The validation were also carried out by determining the precision, accuracy, robustness, specificity, limit of detection and quantitation and reproducibility. The HPLC determination of ranolazine in pure and pharmaceutical formulation was developed and best detected at 273 nm. It utilized Kromasil C 18 column with 5 µm particle size as the stationary phase. The mobile phase consist of methanol and water containing 1% formic acid in the ratio 60:40.The flow rate was 1ml/min and utilized 10 min run time. The calibration curves were also plotted. The method was validated using various parameters.The results from both the UV and HPLC detection showed good agreement with the pure drug and it can be used for the quantitative analysis of ranolazine due to it's sensitivity and reproducibility.The method was found to be precise, rapid and accurate for determination of ranolazine.

Introduction

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention or treatment of disease in human beings or animals, which act by altering any structure or function of the body of human beings or animals[1]. Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. The quality of a drug can be determined after establishing it's authenticity by carrying out purity test and by comparing the quality of pure substance in the drug and it's formulation.The number of drugs introduced into market have been increasing at an alarming rate.The drug can be either new entities or partial structural modifications of the existing drugs. So it is necessary to develop newer analytical procedures for these drugs.So pharmaceutical analysis and the various spectrophotometric methods play an important role in the quality assurance and quality control.

The article mainly deals with the determination and validation of ranolazine by UV and HPLC in pure drug and pharmaceutical formulation. The UV spectroscopyis based on the principle that when a light of definite wavelength is passed through a cell containing solution or solvent,some of the light is absorbed by the solution and the remaining light get transmitted.The transmitted light falls on the detector that transforms the radiant energy to electrical energy. The purpose of HPLC method is to enable quantification of a drug compound under a variety of physical, chemical and photochemical stress conditions. The advantage of the method is that the drug peak can be prepared from all degradation product peaks[2,3,4,5].

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Drug Profile

Ranolazine {N-(2, 6-dimethyl; phenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazin1-yl] acetamide is indicated for the treatment of chronic angina and also act as an anti-ischemic agent. The drug may be used with beta blockers, anti-platelet therapy, nitrates, calcium channel blockers, lipid lowering therapy and ACE inhibitors. They decrease angina episodes, increase exercise tolerance in individuals with coronary artery therapy. The drug is freely soluble in alcoholic solution and insoluble in water [6]

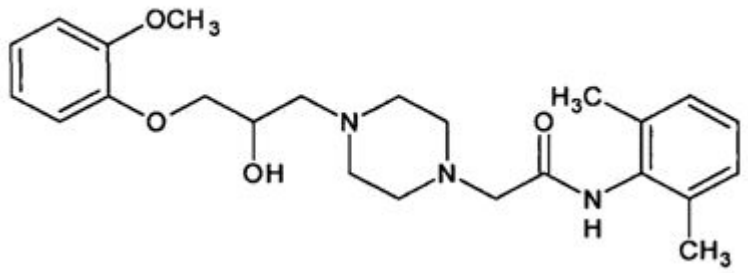

Fig 1: Structure of Ranolazine

Materials and methods

In the present experimental work all the works were performed at room temperature. Spectral and absorbance measurements were made on a shimadzu spectrophotometre by using 1 cm quartz cells.Shimadzu electronic balnce was used for weighing the samples.Commercially available tablets of Ranolazine(Ranexa) containing 500 mg of ranolazine was procured from the local market and estimated.

UV Spectrophotometric determination of ranolazine in Pure and Tablet formulation[9,10]

Solubility determination

The solubility of ranolazine in different solvent systems were carried out and compared.

Source of pure Drug

Pure drug ranolazine was obtained from Ajanta Pharmaceuticals limited, Kalibery, Mubai

Preparation of Stock solution

Standard stock solution of ranolazine was prepared by dissolving 10 mg of drug in 100 ml of methanol and prepare a 100 µg/ml stock solution

Scanning and Determination of maximum wavelength

Preparation of standard solution

The prepared stock solution is diluted with methanol to get a standard solution of concentration 10 µg/ml of ranolazine

To determine the wavelength of maximum absorption (λ_{max}) of the drug, the standard solution of concentration 10 µg/ml was scanned using spectrophotometre within the wavelength of 200-400 nm against methanol as a blank.

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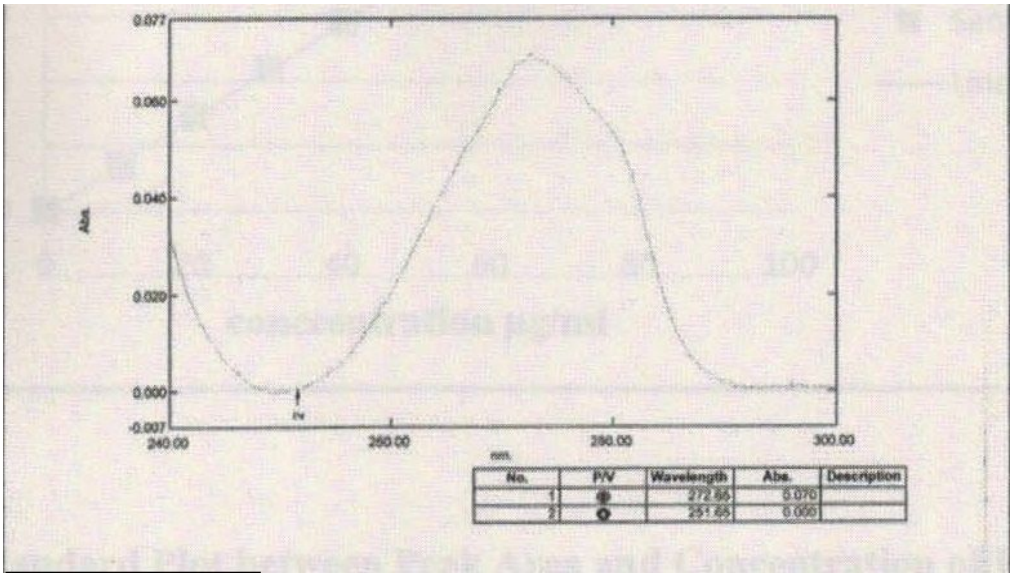

Figure 2: Spectra of ranolazine in methanol.

Verification of linearity of analyte by Beer's Lambert's Law (Construction of standard graph)
The standard graph was plotted by preparing a series of diluted solutions of ranolazine made in methanol and scanned for linearity curve response on the selected wavelength. This procedure is done for the verification of Beer's Lambert's law. The % recovery of ranolazine at different time were also conducted.

Estimation of Pure drug
For the estimation of pure drug of ranolazine weigh accurately the drug to get a solution of concentration 50 µg/ml and the absorbance of the solution was measured at 273 nm . From the absorbance values obtained the absorptivity were determined using the equation A = € c t where
€ = Absorptivity
A = Absorbance/Optical density € = A/c
c = concentration
t = Path length (1 cm)

Estimation of marketed tablets
The marketed ranolazine tablets like Razine(Ajanta Pharmaceuticals) and Ranozex (Sun Pharma) wereselected for the estimation purpose.

The estimation of the drug in the marketed preparation were carried out by taking 20 tablets containing 500 mg of ranolazine. The tablets were finely powdered and average weight was taken. The quantity of powder equivalent to 10 mg ranolazine was weighed accurately and transferred to a 100ml volumetric flask and dissolved in methanol and the solution is stirred by magnetic stirrer for 30 min and the volume was made upto the mark with methanol. The solution was filtered through Whatsman filter paper no:1.The solution were diluted to get the concentrations of 50 µg/ml of ranolazine.The solutions were scanned over the range of 200-400 nm. The absorbance of the sample solution was measured at 273 nm and from this value the concentration of drugs in the sample solution was determined using the equation

y = 0.0073x-0.0032 ----linearity equation

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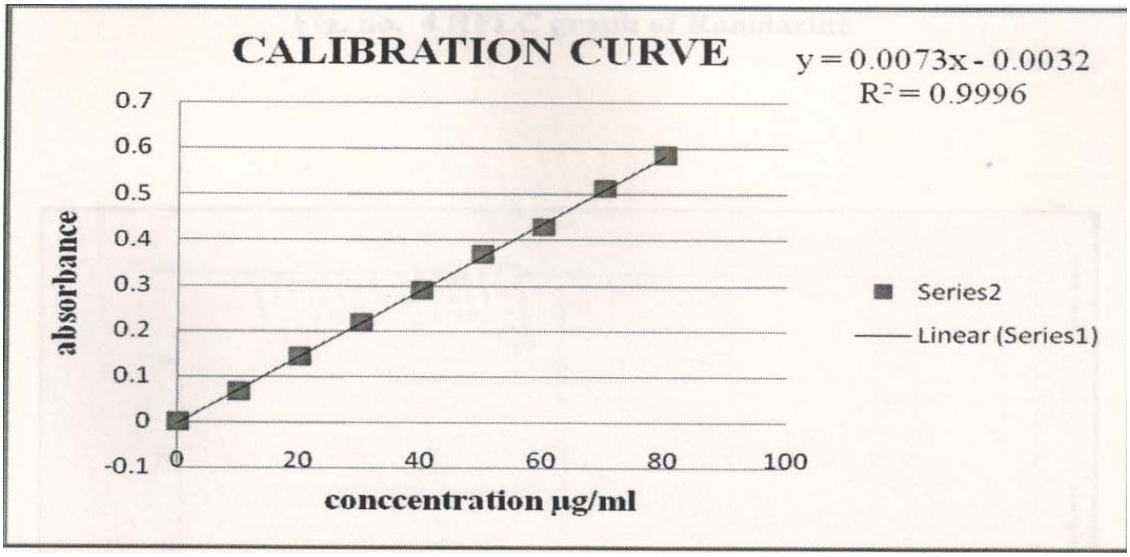

Figure 3: Beer's Standard plot between Absorbance and Concentration of ranolazine by UV spectroscopy

Validation of UV analytivcal data

The validation of analytical procedures helps to develop standard methods and the result of the validation procedure is the assessment of a number of validation parameters like trueness and reproducibility and are determined for a certain scope of application.The various parameters used for validation are precision, accuracy, specificity, robustness, limit of detection and quantitation, reproducibility, optical and regression characteristics.

Precision

The precision of the proposed method was ascertained by actual determination of six replicas of fixed concentration of the drug within the Beer's range and the absorbance was found out. From the analysis the mean, standard deviation and RSD were determined using intraday analysis.

Accuracy

The accuracy of the proposed method were determined and the recovery studies were carried out by adding different amounts of pure drug of ranolazine to the pre-analyzed formulation of concentration 50 µg/ml .The percentage recovery values were calculated.

Specificity

The specificity were determined by adding required amounts of pure drug of ranolazine to pre-analyzed formulation of concentration 50 µg/ml. A solution of drug ranolazine and placebo of 50 µg/ml were also prepared.
Placebo = lactose + carbapol 971 + ethyl cellulose binder

Robustness

The analytical method was developed at 273 nm for ranolazine. So the robustness were carried out by making a stoke solution of concentration 50 µg/ml and taking the absorbance at different wavelength and the mean, SD and RSD values were determined

Limit of Detection and Quantitation

The limit of detection and quantitation were carried out by taking the absorbance reading at 273 nm by six time of the solvent methanol and the mean, SD, RSD, LOD, LOQ obtained were calculated using the standard formula as per ICH Guidelines

LOD = 3.3 σ/s σ = wavelength
LOQ = 10 σ/s s = slope of calibration curve

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Reproducibility
The reproducibility, recovery studies of the proposed method were carried out by adding different amount of pure drug of ranolazine to the pre-analyzed formulation of concentration 50 µg/ml. The percentage recovery were calculated and the procedure were repeated next day.

Reverse Phase HPLC estimation of ranolazine in pure and pharmaceutical formulations[11, 12, 13]
A high performance liquid chromatography method was developed for determination of ranolazine in pure and pharmaceutical formulation. Ranolazine exhibited best detection at 273 nm.The detection response was found to be linear in the concentration range of 100 µg/ml for ranolazine.

Instrumentation
A Waters model HPLC double reciprocating pump, D2 UV detector and chromasil C18 column (5µm particle size) was used. The RP-HPLC system was equipped with software for data processing.

Chromatographic Condition
Seperations were carried out on a Kromasil C 18 column packed with 5µm particle size as the stationary phase.The mobile phase consist of methanol and water containing 1% formic acid in the ratio 60:40 % v/v and was pumped at a flow rate 1ml/min.The detection was monitored at 273 nm with a run time of 10 min.

Linearity/Calibration Curves
Appropriate aliquots were pipetted out from each standard stock solution into a series of 10 ml volumetric flasks.The volume was made upto mark with methanol (HPLC Grade) to get solutions having concentration range 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml of drug.Triplicate dilutions of each concentration of drug were prepared separately.From this 40 µl injections of each concentration were injected into the RP-HPLC system separately and chromatographed . Evalation was performed with UV detector at 273 nm. Peak areas were plotted against concentration to obtain the standard calibration curves. The detector response was found to be linear in the concentration range of 10-60 µg/ml.

Preparation of Standard Solution
Standard solutions of pure drug was prepared by dissolving 10 mg of anolazine in a 100 ml of volumetric flask using 25 ml of methanol HPLC grade.The volume was made upto the mark with methanol and appropriate volume of the solution is diluted to get a concentration of 50 µg/ml of ranolazine.

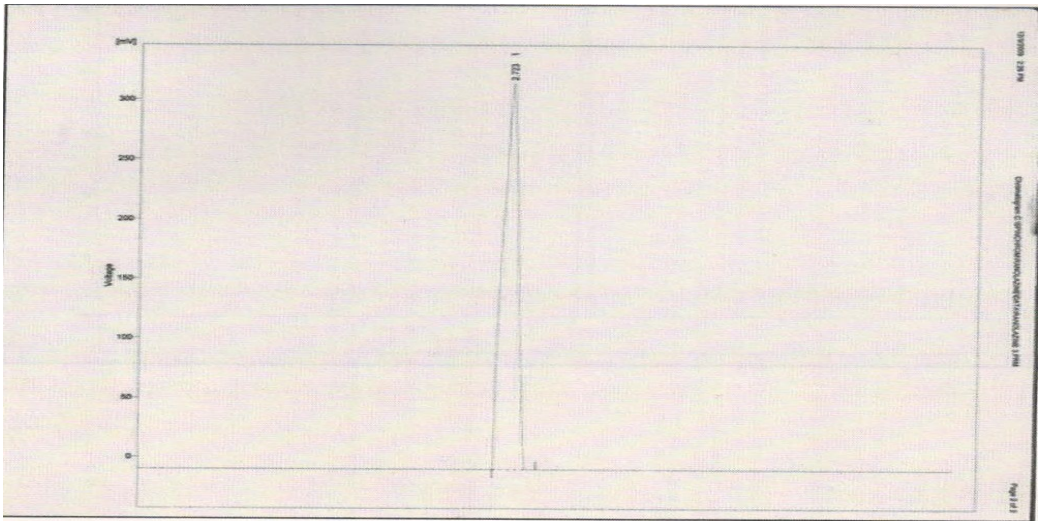

Figure 4 : HPLC Graph of ranolazine

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Analysis of Marketed formulation

The estimation of drug in the marketed preparation was carried out by taking 20 tablets of Razine(Ajanta Pharmaceuticals) each containing 500 mg of ranolazine was weighed and finely powdered .A quantity of powder equivalent to 10 mg ranolazine and Was transferred into a 100 ml volumetric flask AND dissolved in methanol(HPLC Grade) about 25 ml.The solution was stirred by magnetic stirrer for 30 min and the volume was made upto the mark with methanol and filtered through Whatsmans filter paper no:1.The aliquots were diluted to get the concentration 50 µg/ml of ranolazine and the solution was made upto the mark with methanol and the solution was sonicated for 20 min and then filtered.From the filtrate again 5 ml is taken and diluted to 10 ml with HPLC grade methanol . Then 40 µl of sample solution was injected into sample injector for 3 times under chromatographic condition. Area of each peak was measured at 273 nm. The ratio of peak area of drug was calculated and the retention time was measured.

Method validation [14, 15, 16]

The various HPLC methods were considered for validation including sampling procedure, sample preparation, chromatographic separation, detection and data evaluation.The various parameters includes system suitability, specificity, precision, limit of detection and quantifitation, linearity and accuracy.

System suitability

These test are based on the concept that the equipment, electronics, analytical operation and sample to be analyzed constitute an integral system that can be evaluated.

It was determined by the taking % RSD of the five standards injection by using the same standard method which gives an assay method of ranolazineby HPLC method and the average peak symmetry and theoretical plates.

Specificity

The specificity studies were carried out by adding required amount of pure drug of ranolazine and added to pre-analyzed formulation of concentration 50 µg/ml and make a solution of drug ranolazine and placebo of same concentration with HPLC grade methanol.

Limit of Detection and Quantitation

The limit of detection and quantification were carried out by doing the standardanalysis for six times.The corresponding peak area and slope were obtained .From this the value of LOD and LOQ were determined.

Precision

Precision was determined by multiple injections of a homogenous standard solution/system suitability solution as per the test method which indicate the performance of HPLC instrument under the chromatographic conditions.6 injections of the standard preparation is necessary for the validation purpose.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ∓1 % change in mobile phase ratio or column temperature.

Accuracy (Recovery Studies)

Recovery studies were performed by standard addition method at three levels ie; 80 %, 100%,120%.Ranolazine was added to pre analyzed sample and are subjected to proposed HPLC method.

Results and discussion

Solubility determination

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Table 1: Solubility of Ranolazine

Solvent	Solubility Observed
Methanol	++
Ethanol	+
Water	-
0.1 N HCl	-
Ethyl acetate	-
Acetone	-

++ ------ Freely soluble
+ --------Sparingly soluble
_ --------Practically insoluble

UV analytical data validation

Beer's Lambert's Law

The standard graph showing the different concentration vs absorbance at the respective λ_{max} were plotted and obtained the following readings.

Table 2: Concentration and absorbamce reading of ranolazine at 273 nm

Sl No	Concentration(µg/ml) Ranolazine	Absorbance	Absorptivity
1	0	0	0
2	10	0.0661	66
3	20	0.144	72
4	30	0.2178	73
5	40	0.2884	72
6	50	0.3676	74
7	60	0.4273	71
8	70	0.5121	73
9	80	0.5851	73

From the above graph it was observed that ranolazine obey' s Beer Lambert's law in the range of 10-80 µg/ml thereby it shows good linearity response and stock solution showed good stability
The percentage recovery of ranolazine were also determined

Table 3: % Recovery of ranolazine

Sl No:	Concentration ((µg/ml)	Time (hrs)	Absorbance	% Recovery
1	10	0	0.066	98.5
2	10	2	0.0662	98.8
3	10	4	0.0655	97.85
4	10	8	0.0702	110.45
5	10	16	0.0903	113.32

The observed % of drug in the marketed formulation were determined.

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Table 4: Observed % amount of drug in marketed formulation

Formulation	Labelled amout of ranolazine	Observed amout of ranolazine	% amount of ranolazine found
Razine	500	497.55	99.51
Ranozex	500	496.9	99.38

Precision

Table 5: Repeatability of absorbance of ranolazine

Concentration	Absorbance
	0.639
	0.3695
	0.3682
	0.3685
	0.3694
	0.3685
Mean	0.36875
SD	0.000532
RSD	0.1356

Table 6: Intraday analysis of ranolazine

Concentration	Absorbance	Concentration	Absorbance	Concentration	Absorbance
40 µg/ml	0.2872	50 µg/ml	0.3692	60 µg/ml	0.4425
	0.2868		0.3695		0.4419
	0.2862		0.3682		0.4412
Mean	0.2867	Mean	0.3690	Mean	0.4419
SD	0.000503	SD	0.000681	SD	0.000651
RSD	0.17544	RSD	0.1845	RSD	0.1473

The various absorbance of ranolazine and their mean, standard deviation, RSD values were determined and the analysis was confirmed by intraday analysis using various concentrations like 40, 50, 60 µg/ml

Accuracy

Table 7: Recovery studies of ranolazine in tablet formulation

Concentration µg/ml	Reference Std	Conc: before spiking	Conc: after spiking	% Recovery
50	40	49.18	88.62	99.37
50	50	49.26	98.78	99.51
50	60	49.78	109.23	99.49

The recovery studies of ranolazine in tablet formulation were carried out and the % recovery were found to be 99.37, 99.51, 99.49 % respectively for the reference standard concentration 40, 50, 60 µg/ml. The Mean, SD, RSD values were determined and found to be 99.46, 0.0757, 0.761 respectively

Specificity

Table 8: Specificity study of ranolazine

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Conc: (µg/ml)	Abs of drug	Conc: found	Abs of drug+pre analyzed formulation	Conc: found	% Interference
50	0.3629	50.15	0.3596	49.69	0.90
50	0.3645	50.36	0.3634	50.21	0.28
50	0.3639	50.28	0.3611	49.90	0.755

The specificity studies were carried out and the different values were obtained and the % interference were studied.The mean value was found to be 0.645

Robustness

Table 9: Robustness analysis of ranolazine

Conc:	Absorbance at different wavelength						
	270 nm	271 nm	272 nm	273 nm	274 nm	275 nm	276 nm
50 µg/ml	0.3624	0.3654	0.3636	0.3659	0.3620	0.3661	0.3578
	0.3606	0.3627	0.3609	0.3635	0.3697	0.3639	0.3556
	0.3605	0.3626	0.3612	0.3641	0.3699	0.3632	0.3560
Mean	0.3611	0.3635	0.3619	0.3645	0.3638	0.3644	0.3565
SD	0.001069	0.001589	0.00148	0.001249	0.00516	0.001513	0.001172
RSD	0.260	0.4371	0.4089	0.3426	1.4183	0.41520	0.31978

Limit of detection and quantitation

Table 10: Limit of detection and quantitation

Sl No:	Abs of blank at 273 nm	Slope of calibration curve	LOD	LOQ
1	0.0126	S = 0.0073	3.3 σ/s = 0.1292	10 σ/s = 0.391
2	0.0129			
3	0.0123			
4	0.0121			
5	0.0125			
6	0.0127			
Mean	0.01251			
SD	0.000286			
RSD	2.2861			

Reproducibility

Table 11 : Reproducibility data for ranolazine

1		2	
Conc:	**Abs**	**Conc:**	**Abs**
50 µg/ml	0.3670	50 µg/ml	0.3636
	0.3656		0.3630
	0.3640		0.3618
Mean	0.3655	Mean	0.3628
SD	0.001501	SD	0.000917
RSD	0.41067	RSD	0.2527

Optical and regression characteristics

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Table 12: Quantitative and optical characteristics of the method

PARAMETERS	RANOLAZINE
Beer's Law limit (µg/ml)	10-80
Limit of detection (µg/ml)	0.1292
Limit of quantification (µg/ml)	0.391
Regression equation	y = 0.0073 x-0.0032
Correlation Coefficient	0.9996
Accuracy (% mean recovery)	99.45
Specificity (% mean interference)	0.645
% Relative standard deviation	< 2%

Y= A+ B x where x = conc: of drug A = Intercept B= Slope
Y = absorbance value

Reverse Phase HPLC- Analytical data Validation

Table 13: System Suitability

Injection No:	Peak Area
1	1655.50
2	1656.45
3	1653.4
4	1654.76
5	1656.5
% RSD	0.0782
Avg. Peak Symmetry	0.89
Avg. Theoretical Plate	2213

Specificity

The specificity were noted for the HPLC analytical data and found that in the chromatograms of the formulation additional peaks were not observed which shows that the peak is free from interferences.So the HPLC method is found to be specific.

Table 14: Limit of Detection and Quantification

Sl No:	Peak Area	Slope of calibration curve	LOD	LOQ
1	40.68	S = 31.86	3.3 σ/s = 0.0711	10 σ/s = 0.2153
2	40.12			
3	40.84			
4	40.13			
5	40.16			
6	40.35			
Mean	41.05			
SD	0.6861			
RSD	1.6714			

Precision

Table 15: Reading for precision parameters

Injection of 50 (µg/ml) of drug	Peak Area

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1	1655.50
2	1656.45
3	1653.4
4	1654.76
5	1656.5
6	1655.58
Mean	1655.36
S.D	1.1625
% RSD	0.0702

The precision parameter revealed that the relative standard deviation shall be not more than 2%

Robustness
The robustness study revealed that there is no significant impact on the retention time and tailing factor.

Accuracy (Recovery Studies)

Table 16: Recovery study data

Conc: µg/ml	Reference Std added µg/ml	Peak area before spiking	Peak area after spiking	% Recovery
50	40	1631.648	1482.065	99.57
50	50	1639.295	1639.135	99.49
50	60	1640.251	1794.453	99.31

Optical and regression characteristics

Table 12: Quantitative and optical characteristics of the method

PARAMETERS	RANOLAZINE
Beer's Law limit (µg/ml)	10-60
Limit of detection (µg/ml)	0.0711
Limit of quantification (µg/ml)	0.2153
Regression equation	y = 31.86 x-54.26
Correlation Coefficient	0.997
Accuracy (% mean recovery)	99.46
% Relative standard deviation	< 2%

Y= A+ B x where x = conc: of drug A = Intercept B= Slope
Y = absorbance value

From the quantitative parameters /optical characteristics of the proposed method, it was found that the drug obey linearity within the concentration range of 10-80 µg/ml for UV and 10-60 µg/ml for HPLC method for ranolazine, The % RSD value is also found to be less than 2%, which indicate that the proposed method has good reproducibility. The % recovery values of pure drug from the analyzed formulation were in between for ranolazine, which indicate that the methos is accurate and the commonly used excipients and additives present in formulation were not interfering the proposed method..The system suitability parameters also indicate that the values were within the specified limits. When a known amount of drug solution was added to a powdered sample of tablet dosage form and subjected to estimation of drug by the proposed method, there was a high recovery of ranolazine indicating that the proposed procedure for determination of ranolazine in tablet dosage forms is highly accurate.

Conclusion

The procedures performed in the above study offers a simple and fast method to be practicable in the routine

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analysis of title drug in pharmaceuticals.The development of reliable and affordable procedures for the determination of drug substances either as pure drug or in marketed formulation remains a main research area in today's pharmaceutical care and practice.The selection of brands used in the study was done in a manner to represent the good geographical spread of the different drug markets in India and other parts of Asia.Here the pharmacist play an important role in the optimal quantityof product reaching to consumer.

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