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Cost Effective, Efficient and Stability indicating analytical method validation for Ranolazine related by Reverse Phase High Performance Liquid Chromatography in drug substances

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Abstract

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Ranolazine in drug substances. Isocratic elution at a flow rate of 1.4ml/min was employed on Hypersil BDS C18, 150 x 4.6 mm, 5µm or Equivalent at 40°C column temperature. The mobile phase consisted of Mobile phase-A: Mobile phase-B (55:45) (Disodium hydrogen orthophosphate buffer with pH 7.0 and Acetonitrile). The UV detection wavelength was at 205 nm. Linearity was observed in concentration range of 0.07-0.82 ppm for ranolazine impurity-I and concentration range of 0.07-0.78 ppm for ranolazine impurity-II. The retention time for Ranolazine was 7.6 min. The method was validated for validation parameter like specificity, force degradation, linearity, accuracy, precision and robustness as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Ranolazine in pharmaceutical dosage forms.

Keywords: Ranolazine, Method Validation, Drug Substances, HPLC

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INTRODUCTION:

Ranolazine is - (2, 6-dimethylphenyl)- 2 {4- [2- hydroxyl -3- (2-methoxy -phenoxy) propyl piprazine-1-yl} acetamideis piprazine derivative appears as white to off white crystalline powder. The drug is freely soluble in Methanol. Ranolazine is a strong base with pKa values of 13.6, Six-membered Piprazine Ring. Ranolazine melts at 122-124 degree C. Ranolazine is known to increase the QT interval on the electrocardiogram. While the mean increase in the corrected QT interval (QTc) is approximately 6 msec, about 5 percent of individuals may have QTc prolongations of 15 msec or longer. Extended QT intervals increase the risk of sudden cardiac death (SCD). The increase was 60% in adults, independently of other known risk factors, in an analysis of the Rotterdam Study[1-3].Ranolazine is not official in Pharmacopoeia. The high pressure liquid chromatography (HPLC) for Ranolazine estimation. GC method for residual solvent determination in Ranolazine drug substances. HPLC methods are widely used chromatographic methods in the analysis of Ranolazine in Formulation. LC-MS/MS, LC-MS and UHPLC use for estimation of Ranolazine in Plasma. RP HPLC method also developed for determination of concentration of Ranolazine in human serum and also for simultaneous determination of Ranolazine and Dronederone.

Objective of Study:

Literature survey revealed that methods for the determinations of ranolazine include HPLC, Gas chromatography, simultaneous spectrophotometric determination and other methods. Literature survey reveals that different assay methods like spectrophotometry, spectrofluorometry, oxidimetry and HPLC are available for the validation of ranolazine in drug substances, But none of these methods are found suitable for routine quality control studies due to the following reasons like poor sensitivity, longer run time, using costly solvent, suitable at higher concentration only, extraction procedure involved in sample preparation. Based on this, it was felt necessary to develop a validated simple, selective and sensitive HPLC method for the determination of ranolazine in drug substances. The proposed method has been demonstrated superior to the existing procedures due to its sensitivity, speed, accuracy and it is suitable for routine quality control analysis. This proposed method can be successfully employed for quality control during manufacture and for assessment of the stability of drugs in drug substances[4-6].

Table 1: Summary of Chromatographic Method of Ranolazine

Title	Method	Mobile phase	Stationary phase	Wave Length
Ranolazine in bulk & marketed formulation	HPLC & UV	Methanol : 0.5% tri ethyl amine pH 6 with orthophosphoric acid (75:25)	-	271
Estimation of Ranolazine HCL in Tablet Dosage Form	RP-HPLC	Buffer : Acetonitrile(60:40),(pH adjust with triethylamine	Inertsil ODS C18	224 nm
Determination of Ranolazine HCL in bulk and dosage form	LC	Methanol : water (99:1 %,V/V)	HiQ Sil C_{18}HS	273 nm
Quantitation of Ranolazine in rat plasma	LC	Acetonitrile : water : formic acid : 10% *n*-butylamine (70:30:0.5:0.08, v/v/v/v)	Nova-Pak C_{18} column	-
Determination of Ranolazine in human plasma	HPLC	Acetonitrile: 0.1% formic acid(90?10)	Agilent-ZORBAX C_{18} column	-
Estimation of Ranolazine in Human Plasma	LC	methanol–10mM ammonium acetate (60:40 v/v, pH 4.0)	Zorbax extend C_{18} column	-
Ranolazine HCL in bulk and tablet dosage form	HPTLC	Chloroform: methanol : toluene (5 : 1 : 1 v/v/v)	silica gel aluminium plate 60 F – 254	273 nm
Determination of residual solvents in Ranolazine	GC	-	HP-INNOWAX column	-

METHODOLOGY:

Materials, Chemical, Reagents, Equipment's and Column used: The details of the standards, chemicals/Reagents, Instruments and Accessories used in the method validation study are reported hereunder.

Table 2: Details of the Materials, Chemical, Reagents, Equipment's and Column used

Name	Chemical name	% Potency	Batch no
Reference Standard	Ranolazine	99.6	RNZ/024/19
Impurity I	6,7-dimethoxy-3,4-dihydro isoquinoline hydrochloride	97.4	RNZ/IMP-I/19
Impurity II	Trans rac-3-isobutyl-9,10-dimethoxy-2,3,4,6,7,11b-hexahydro-1H-pyrido[2,1-a]isoquinolin-2-ol	95.7	RNZ/IMP-II/19
Disodium hydrogen orthophosphate	NA	NA	DG0D701542
Acetonitrile	NA	NA	R072G20
Water	NA	NA	-
Ortho phosphoric acid	NA	NA	R045C20

Chromatographic Conditions:

HPLC	Waters Alliance
Column	Hypersil BDS C18, 150 x 4.6 mm, 5µm or Equivalent
Flow rate	1.4 ml/min.
Wavelength	205 nm
Column Temperature	40°C
Injection volume	10 µl
Run time	20 minutes for Blank, System suitability and Sample solutions and 10 minutes for Diluted standard solution.
Sample cooler temperature	10°C
Mobile phase	Mobile phase-A: Mobile phase-B (55:45)
Rinse/wash solvent	Mixture of 20 volumes of water and 80 volumes of acetonitrile.
Diluent	Acetonitrile

Preparation of Buffer solution: Weighed accurately and transferred 1.41g of disodium hydrogen orthophosphate in 1000 ml water, mixed. Adjust pH to 7.0 with diluted O-phosphoric acid solution. Filtered through 0.45 µ nylon filter and degassed it.

Mobile phase A: Buffer solution.

Mobile phase B: Acetonitrile

Impurity Stock solutions:

Impurity I stock solution: Weighed accurately 5 mg of impurity I reference standard and transferred into 100.0 ml of clean, dry volumetric flask, added 45 mL of diluent and sonicated to dissolve.

Impurity II stock solution: Weighed accurately 5 mg of impurity II reference standard and transferred into 100.0 ml of clean, dry volumetric flask, added 45 ml of diluent and sonicated to dissolve.

System suitability solution: Weighed accurately about 25 mg of Ranolazine reference/working standard and transferred into 50.0 ml of clean, dry volumetric flask, added 25 ml of diluent and sonicated to dissolve and transferred 0.25 ml of each Impurity I and Impurity II stock solution into it and make up to the mark with diluent.

Diluted standard solution: Weighed accurately about 25 mg of Ranolazine reference/working standard and transferred into 50.0 ml of clean, dry volumetric flask, added 25 ml of diluent and sonicated to dissolve and dilute volume with diluent (Stock solution-I).Transfer 1.0 ml of this solution into 100.0 ml of clean, dry volumetric flask and made up to the volume with diluent (Stock solution-II). Further dilute 1.0 ml of this solution into 10.0 ml of clean, dry volumetric flask and made up to the volume with the diluent (Stock solution-III).

Sample preparation: Weighed accurately about 25 mg of sample and transferred into a 50.0 ml of clean, dry volumetric flask, added 25 ml of diluent and sonicated to dissolve. Allowed to equilibrate to room temperature and diluted up to the mark with diluent.

S. No	Name of the impurity	RRT (at about)
1	Impurity I	0.30
2	Impurity II	0.40
3	Ranolazine	1.00

System suitability Criteria: The resolution between impurity I and Impurity II peak should not be less than 1.5 from system stability solution. The % RSD of area of Ranolazine peak for five replicate injections of diluted standard solution should not be more than 2.0 and the retention time of Ranolazine peak is about 7.5 min.

RESULT AND DISCUSSION:

Specificity: A blank, system suitability solutions and diluted standard solution, all individual impurities at specification, impurity spiked solutions and sample of Ranolazine were prepared and injected. A system suitability criterion meets as per test method. The system suitability criteria, relative retention time of known impurities in spiked solution were observed and recorded in the below table.

Table 3: System Suitability in Specificity

S. No	Identification	RRT		Resolution		%RSD	
		Observed	As per method	Observed	As per method	Observed	As per method
1	Impurity I	0.34	0.30	1.93	NLT 1.5.	0.33%	NMT 2.0 %
2	Impurity II	0.39	0.40				

Forced degradation study: Ranolazine is subjected to stress degradation at the analyte concentration using 1N hydrochloric acid, 1N sodium hydroxide, 5% hydrogen peroxide and the thermal condition at 105°C for 24 hours to obtain required degradation. All Impurities are separated from target analyte peak and the resolution between analyte peak and closely eluting peak is well within acceptance criteria. Therefore, the method can be termed as specific and stability indicating method. Peak purity of known impurities and Ranolazine peak in the spike solution, and the degraded sample solution were observed and tabulated here under Table 4.

Table 4a: Force degradation condition of Ranolazine

Parameters			Acid degradation			Base degradation		
Condition			1N HCl_0 Hrs.			1N NaOH_0 Hrs.		
S.No	Impurity Name	RRT	% Area	PA	PT	% Area	PA	PT
1	Unk	0.16	13.7	5.46	0.23		-	-
2	Unk	0.24	2.47	4.559	0.274	ND	-	-
3	Unk	0.30	ND	-	-	ND	-	-
4	Unk	0.37	ND	-	-	0.05	5.675	5.842
5	Impurity-II	0.39	ND	-	-	ND	-	-
6	Unk	0.48	ND	-	-	ND	-	-
7	Unk	0.53	ND	-	-	ND	-	-
8	Unk	0.57	ND	-	-	ND	-	-
9	Unk	0.76	0.04	9.199	20.756	ND	-	-
10	Unk	1.66	ND	-	-	ND	-	-
11	Unk	1.68	0.02	31.398	54.211	ND	-	-
12	Ranolazine	1.00	83.76	1.481	1.66	99.95	0.745	0.879

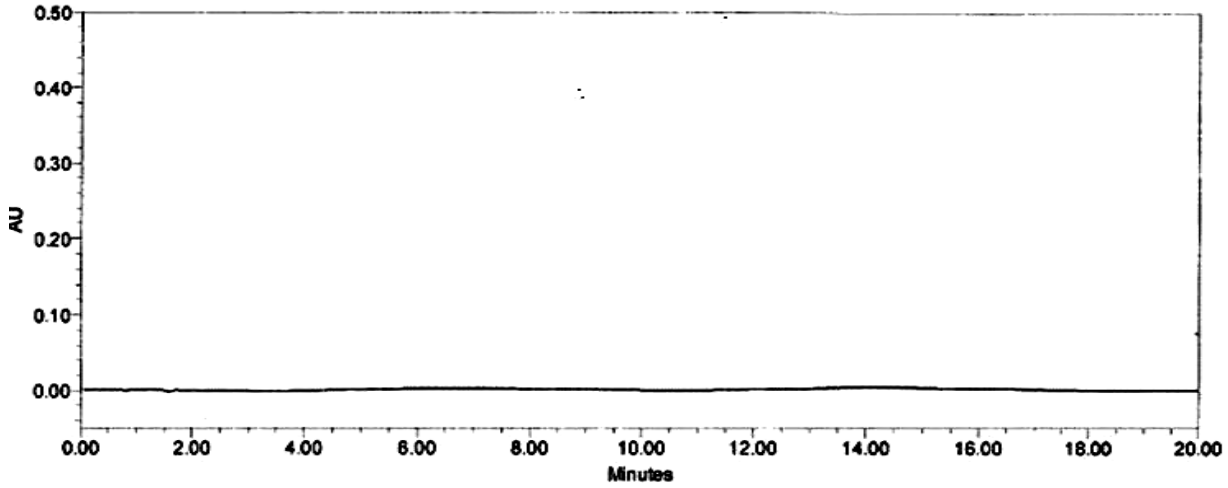
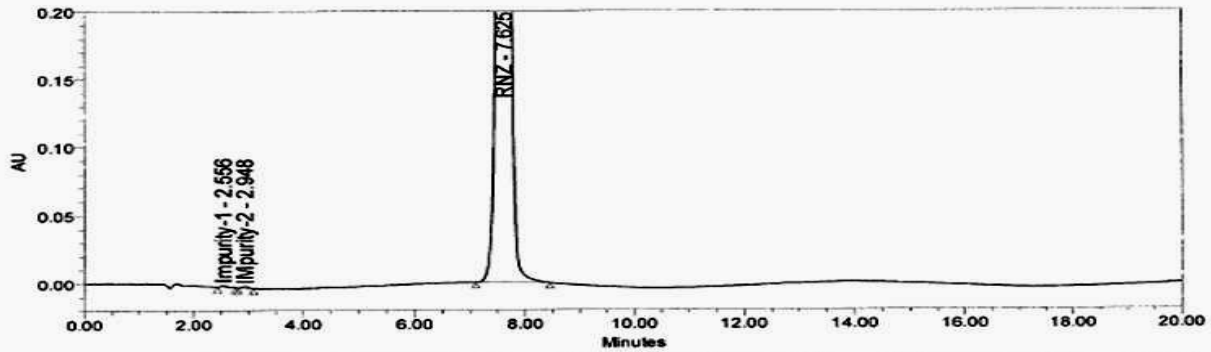


Figure 1: Reference Blank Chromatogram

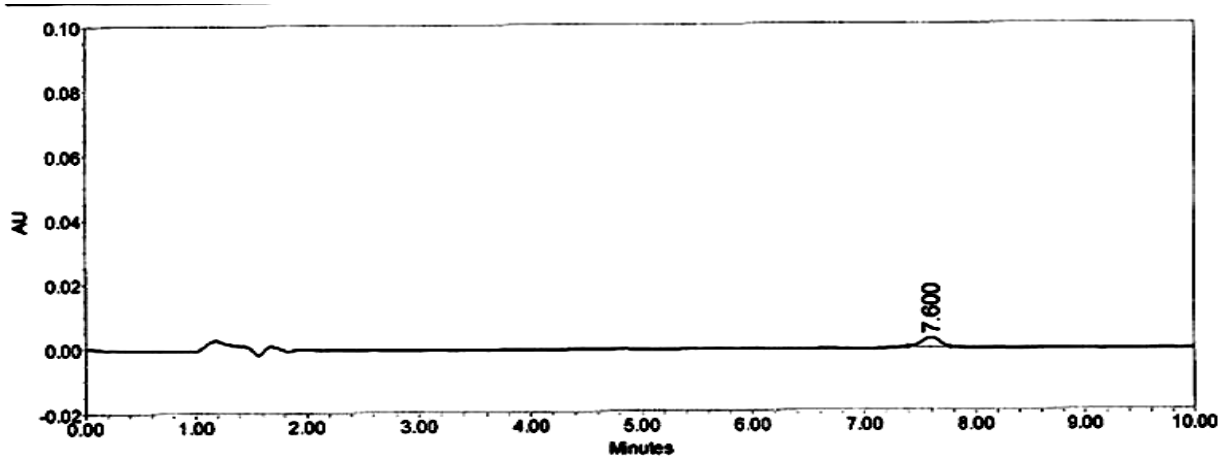
Table 4b: Force degradation condition of Ranolazine

Parameters			Peroxide degradation			Thermal degradation		
Condition			5% H2O2_0 Hrs.			At 105°C for 24 Hrs.		
S.No	Impurity Name	RRT	% Area	PA	PT	% Area	PA	PT
1	Unk	0.16	ND	-	-	ND	-	-
2	Unk	0.24	ND	-	-	ND	-	-
3	Unk	0.30	0.11	2.317	5.77	ND	-	-
4	Unk	0.37	ND	NA	NA	ND	-	-
5	Impurity-II	0.39	13.04	3.039	15.5	ND	-	-
6	Unk	0.48	ND	-	-	0.41	5.23	8.29
7	Unk	0.53	ND	-	-	0.06	11.40	28.38
8	Unk	0.57	ND	-	-	0.05	20.44	50.46
9	Unk	0.76	ND	-	-	ND	-	-
10	Unk	1.66	0.02	14.319	28.4	ND	-	-
11	Unk	1.68	ND	-	-	0.02	63.28	90.00
12	Ranolazine	1.00	86.82	5.161	5.75	99.46	3.18	3.56



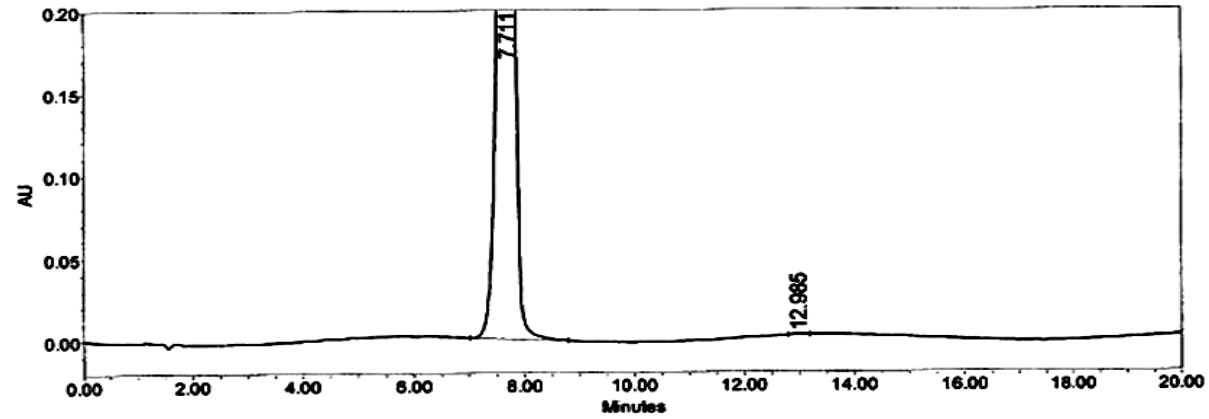
	Peak Name	RT	Area	% Area	Resolution	USP Tailing	RT Ratio	Purity1 Angle	Purity1 Threshold	Purity_criteria
1	Impurity-1	2.556	11178	0.06		1.38	0.34	4.306	6.044	PASS
2	IMpurity-2	2.948	8837	0.04	1.93	1.21	0.39	5.862	6.163	PASS
3	RNZ	7.625	20294896	99.90	20.30	0.96	1.00	1.347	1.816	PASS

Figure 2: Reference System Suitability Chromatogram



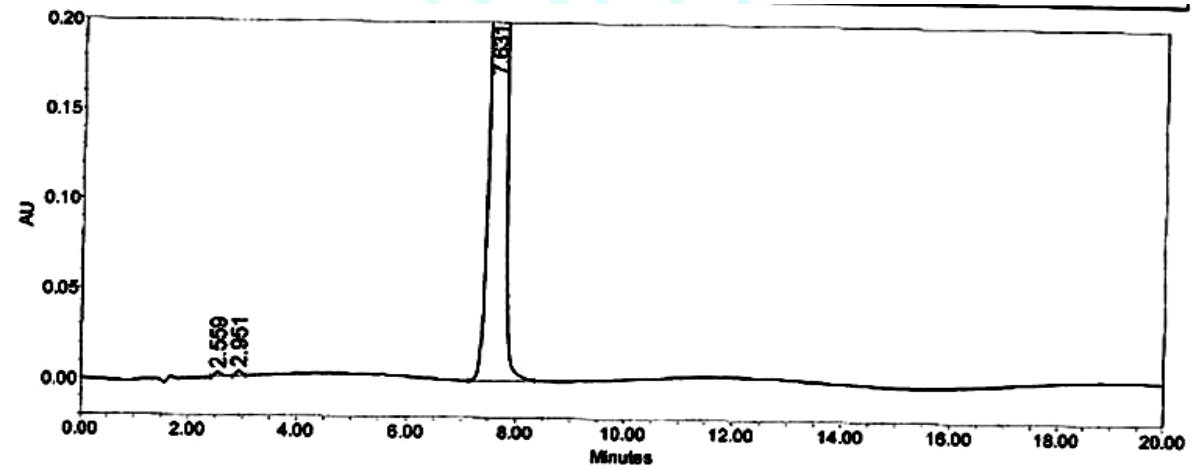
	Peak Name	RT	Area	% Area
1	RNZ	7.600	30635	100.00

Figure 3: Reference Standard Chromatogram



	Peak Name	RT	Area	% Area	RT Ratio
1	RNZ	7.711	22754180	99.98	
2	Peak2	12.985	4689	0.02	1.68

Figure 4: Reference Control Chromatogram



	Peak Name	RT	Area	% Area	RT Ratio
1	Impurity-1	2.559	22163	0.11	0.34
2	Impurity-2	2.951	17848	0.09	0.39
3	RNZ	7.631	20207256	99.80	1.00

Figure 5: Reference Spike Sample Chromatogram

Limit of Detection and Limit of Quantification:

Prediction LOD and LOQ: This experiment was carried out from the lowest concentration of each impurity to Ranolazine, to find out the quantization and detection limit for each impurity on standard deviation of response and slope method. % RSD for each impurity at LOQ level concentration is found less than 10 (with respect to specification limit). The results obtained are well within acceptance criteria.

Table 5: Limit of Detection and Limit of Quantification

Test	Imp-I	Imp-II	Ranolazine
LOQ concentration	0.014%	0.0134%	0.0172%
LOD concentration	0.0046%	0.0044%	0.0056%

Precision LOQ: Precision LOQ was performed by injecting six replicate injections of LOQ concentration to find the % RSD.

Table 6: Precision of Limit of Quantification

S. No	Imp-I	Imp-II	Ranolazine
1	3039	2995	3336
2	2966	2998	3495
3	2957	3015	3508
4	2958	3079	3228
5	2922	2977	3297
6	3071	3244	3422
Average	2986	3051	3381
%RSD	1.74	3.01	3.04

Linearity and Range: Linearity was determined at seven levels over the range of LOQ to 150% of specification limit for Impurity I, II and Ranolazine. A standard stock solution was prepared and further diluted to attain concentration at about LOQ, 50%, 80%, 100%, 120% and 150% of the specification limit. Each standard preparation was injected. The area of each level was recorded and a graph of area verses slope of regression line, residual sum of squared were calculated and recorded. The linear correlation co-efficient(r) for each impurity is found greater than 0.99 over the selected range. The correlation coefficient value is found well within acceptance criteria.

Table 7: Linearity and Range of Impurity I

S. No	Linearity level	concentration in ppm	Area observed
1	LOQ	0.07	2986
2	50%	0.27	10994
3	80%	0.43	17321
4	100%	0.54	21881
5	120%	0.65	25829
6	150%	0.82	31028
Correlation coefficient(r)			0.99905
Slope			38109.615

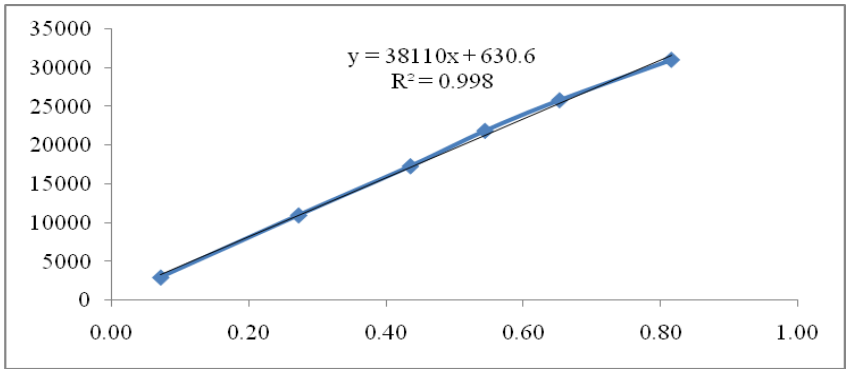


Figure 6: The graphical representation of correlation coefficient curve of Impurity I

Table 8: Linearity and Range of Impurity II

S. No	Linearity level	concentration in ppm	Area observed
1	LOQ	0.07	3051
2	50%	0.26	8305
3	80%	0.42	12788
4	100%	0.52	16796
5	120%	0.63	19759
6	150%	0.78	25267
correlation coefficient(r)			0.99856
Slope			31251.137

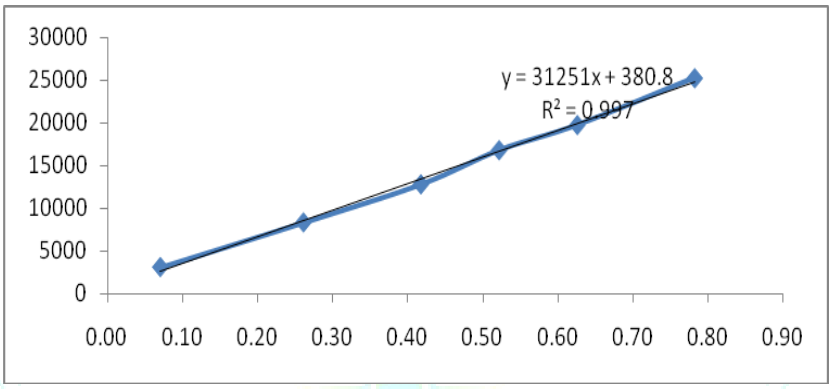


Figure 7: The graphical representation of correlation coefficient curve of Impurity II

Table 9: Linearity and Range of Ranolazine

S. No	Linearity level	Concentration in ppm	Area observed
1	LOQ	0.09	3381
2	50	0.25	9570
3	80	0.40	15375
4	100	0.50	20537
5	120	0.60	24436
6	150	0.75	30365
correlation coefficient(r)			0.99931
Slope			41482.935

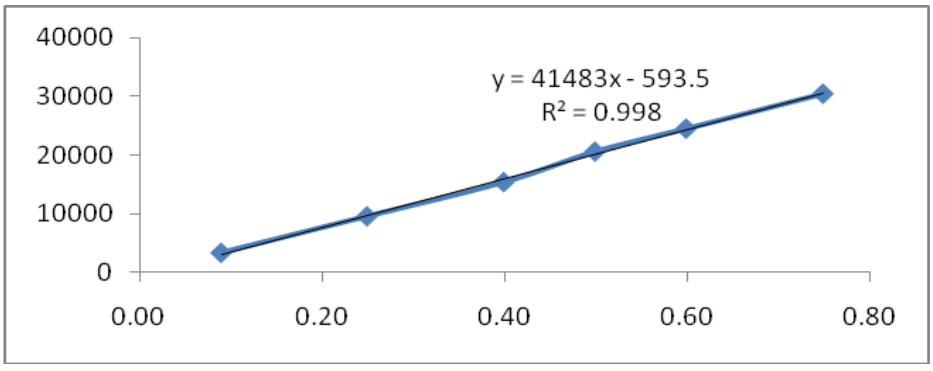


Figure 8: The graphical representation of correlation coefficient curve of ranolazine

Table 10: Correction factor of Ranolazine & Its Impurity (RRF)

Name of Impurity	Slope	Correction factor (RRF)
Impurity-I	38109.615	0.92
Impurity-II	31251.137	0.75
Ranolazine	41482.935	1.00

Precision:

Method precision: Method has been established by analyzing six sample preparations under same conditions. Six replicates of sample were prepared by one analyst and injected on the same equipment and on the same day. Calculate each known impurity Ind single maximum unknown impurity Individual (Ind) total impurities. Mean % impurity value and % RSD were calculated and recorded. The results obtained lies well within acceptance criteria.

Table 11: System Suitability in System Precision

S. No	Identification	RRT		Resolution		%RSD	
		Observed	As per method	Observed	As per method	Observed	As per method
1	Impurity I	0.34	0.30	1.97	NLT 1.5.	1.35%	NMT 2.0 %
2	Impurity II	0.39	0.40				

Table 12: Result of Method Precision & Intermediate precision study of Ranolazine

Sample No	**Method Precision**			**Intermediate Precision**		
	Imp I	Imp II	Total impurities	Imp I	Imp II	Total impurities
1	0.091	0.093	0.184	0.091	0.094	0.183
2	0.090	0.092	0.182	0.090	0.091	0.181
3	0.093	0.092	0.186	0.093	0.092	0.186
4	0.088	0.088	0.176	0.088	0.087	0.175
5	0.089	0.090	0.179	0.089	0.090	0.179
6	0.095	0.096	0.191	0.095	0.093	0.191
Mean	**0.091**	**0.092**	**0.183**	**0.091**	**0.092**	**0.182**
RSD %	**2.863**	**2.875**	**2.792**	**2.616**	**2.507**	**2.793**

Accuracy (Recovery): Accuracy was performed by spiked all known impurities in the test preparation at 50%, 100% and 150% of specification limit. Samples were prepared in triplicate at each level and each preparation is injected separately. The average recovery of known impurities at each level is found between 80% to 120%.The % individual recovery and % mean recovery for each level was calculated and recorded in the below tables.

Table 13a: Accuracy for Impurity I

Levels	Area of IMP-I	Conc.mg/ml	ppm	% of IMP-I Added	% Corrected	% Recovery	% Avg.Recovery
50%	10286	0.00024	0.237	0.047	0.043	91.76	93.38
	10543	0.00024	0.237	0.047	0.045	94.02	
	10607	0.00024	0.237	0.047	0.045	94.36	
100%	21589	0.00047	0.473	0.095	0.091	96.18	95.92
	21269	0.00047	0.473	0.095	0.090	94.95	
	21682	0.00047	0.473	0.095	0.091	96.64	
150%	30214	0.00071	0.710	0.142	0.128	90.10	86.71
	27676	0.00071	0.710	0.142	0.120	84.42	
	28206	0.00071	0.710	0.142	0.122	85.62	

Table 13b: Accuracy for Impurity II

Level	Area of IMP-I	Conc.mg/ml	ppm	% of IMP-I Added	% Corrected	% Recovery	% Avg.Recovery
50%	8800	0.00024	0.243	0.049	0.046	93.95	95.78
	8856	0.00024	0.243	0.049	0.046	94.51	
	9288	0.00024	0.243	0.049	0.048	98.88	
100%	18759	0.00049	0.485	0.097	0.097	100.02	98.01
	17679	0.00049	0.485	0.097	0.092	94.45	
	18666	0.00049	0.485	0.097	0.097	99.56	
150%	30995	0.00073	0.728	0.146	0.161	110.61	105.13
	28996	0.00073	0.728	0.146	0.151	103.64	
	28423	0.00073	0.728	0.146	0.147	101.15	

Solution stability at 25°C: The blank, system suitability and Test solution and initial % impurity was determined. As per method sample preparation was stored at 25°C for different time interval like 0 hrs. and 24hrs. All the known impurities are found stable up to 24 hours in the spiked sample (SST) and as such sample. The % of impurity-II is found increasing significantly after 24 hrs.in as such sample. The solution is found stable up to 24 hrs.

Table 14: Solution stability of Ranolazine

Sample Name	Time	Impurity-I	Impurity-II	Unknown impurity	Total impurities
System suitability solution	RRT	0.34	0.39	1.67	-
	Initial (0 hrs)	0.05	0.05	ND	0.10
	24 hrs.	0.05	0.05	ND	0.10
As such sample	Initial (0 hrs.)	ND	ND	0.03	0.03
	24 hrs.	ND	0.01	0.01	0.05

Robustness: The robustness of the method was established by making deliberate minor variations in the following method parameters. Change in flow rate of Mobile phase to 1.3 ml /min and 1.4 ml/min Change in column oven temperature to 39°C to 41°C. A system suitability criterion meets as per test method. Relative retention time of each impurity is found as per test method. All the impurities are well separated from each other and from Ranolazine peak in the changed conditions. The effect of changes was observed on system suitability values and recorded in the below tables.

Table 15: Table of Robustness Study parameter in Ranolazine

Parameter	**Condition**	**Impurity I**	**Impurity II**	**Total impurities**
Change in Column temperature				
Normal condition*	40°C	0.091	0.092	0.183
Deliberate condition	39°C	0.084	0.103	0.187
Difference from normal condition	-1°C	0.007	-0.011	-0.004
Change in Column temperature				
Deliberate condition	41°C	0.082	0.097	0.179
Difference from normal condition	+1°C	0.009	-0.005	0.004
Change in Flow rate				
Normal condition*	1.4 ml/min	0.091	0.092	0.183
Deliberate condition	1.3 ml/min	0.081	0.104	0.185
Difference from normal condition	-0.1 ml/min	0.01	-0.012	-0.002
Change in Column temperature				
Deliberate condition	1.5 ml/min	0.072	0.096	0.168
Difference from normal condition	+0.1 ml/min	0.019	-0.004	0.015

Table 16: System suitability criteria in Robustness

Parameter	Condition	Resolution	%RSD	RRT	
				Impurity-I	Impurity-II
Change in Column temperature					
Normal condition*	40°C	1.97	1.35	0.30	0.40
Deliberate condition	39°C	1.77	0.65	0.35	0.39
Deliberate condition	41°c	1.81	0.97	0.35	0.39
Change in Flow rate					
Normal condition*	1.4 ml	1.97	1.35	0.30	0.40
Deliberate condition	1.3 ml	1.79	0.11	0.35	0.39
Deliberate condition	1.5 ml	1.77	0.14	0.35	0.38

* The initial data taken from method precision.

CONCLUSION:

A validated RP-HPLC method has been developed for the determination of related substance in Ranolazine drug substances. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Ranolazine in pharmaceutical dosage form.

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