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Validated HPLC Method for Determining Related Substances in Compatibility Studies and Novel Extended Release Formulation for Ranolazine

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Abstract

HPLC method has been developed for determination of Ranolazine together with its related substances in a laboratory mixture for drug and excipient compatibility studies as well as in a novel extended release tablet developed in-house. Efficient chromatographic separation was achieved on a Supelcosil C 18, (250×4.6 mm, 5 µm) column with mobile phase containing combination of Phosphate buffer pH 7.0 and Methanol in ratio of 350:650 at flow rate of 1.0 ml/minute and eluent was monitored at 220 nm. Linearity, regression value, recovey, % RSD of method precision, LOD and LOQ values were found with in the limits. In this method impurities were well seperated from the main peak. This method was found to be satisfactory. Limits for reporting threshold and total impurities were 0.1% and 2.0%, respectively, as per Q3B(R) Impurities in New drug Products. Compatibility studies are essential for preformulation studies of formulation development. In the present study, the possible interactions between Ranolazine and some excipients (Hypromellose phthalate grade HP-55, Ethocel 7 FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel PH 101 and Magnesium Stearate) were evaluated by examining the pure drug or drug-excipient powder mixtures which were stored under different conditions (55°C after 15 days and 40°C/75% RH after 30 days) using High Performance Liquid Chromatography (HPLC). The results demonstrate the suitability of Ranolazine with Hypromellose phthalate grade HP55, Ethocel7FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel PH101 and Magnesium Stearate. Same method also used in novel extended release tablet for determination of related substances. Based upon obtained results shows developed formulation was stable at 40°C/75% RH for 3 months.

Keywords: HPLC method; Ranolazine; Chromatographic separation

Introduction

Ranolazine is used for the treatment of Cardiac ischemia it affects sodium dependent calcium channels during myocardial ischemia [1]. The International Conference on Harmonization (ICH). Recommends Regulatory requirements for the identification, qualification and control of impurities in drug substances and their formulated products. As recommended by ICH all routine impurities at or above 0.1% level, should be identified through suitable analytical methods. Ranolazine is not cited in the any pharmacopoeia and contamination by impurities as per supplier specifications [2]. Compatibility studies thus allow in systematic selection of excipients, for formulation development. Excipients may contribute to incompatibility by altering the moisture content, altering the micro-environment pH, and acting as a catalyst for degradation or contributing an impurity that causes degradation [3-7]. After through literature search, it was observed that no HPLC method was found for estimation of impurities in Ranolazine. Therefore, it was thought worth determining the impurities of Ranolazine to ensure the quality, efficacy and safety of the final pharmaceutical formulation. The purpose of the present study was to validate the HPLC method for determining the related substances to evaluate the physical & chemical stability of Ranolazine when mixed with Excipients and in a novel extended release tablet at different storage conditions in accordance with the ICH guidance document.

Experimental

Reagents and chemicals

The following materials where used in the present investigation Ranolazine,Hypromellose phthalate grade HP 55, Ethocel standard 7 FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel pH101 and Magnesium Stearate, HPLC grade chemicals were gifted by Natco Pharma Limited (Hyderabad, India). Novel extended release tablets of Ranolazine and a placebo were formulated in Formulation research and development, Natco Pharma Limited, Kothur, India.

Chromatography apparatus and conditions

The chromatograph consisted of an Water alliance HPLC system with 2489 detector.

Column	: Supelcosil C18, (250×4.6 mm), 5 µm
Flow rate	: 1.0 mL/minute
Wavelength	: UV-220 nm
Column temperature	: 40°c
Sample temperature	: 5°c
Injection Volume	: 20 µL
Runtime	: 60 minutes

Preparation of solutions

Mobile phase preparation: Weighed and transferred about 0.5 g of Disodium hydrogen ortho phosphate (Na_2HPO_4) into 1000 mL beaker

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and 350 mL of Purified water was added and mixed well. pH of the solution was adjusted to 7.0 with diluted orthophosphoric acid. This solution and methanol were mixed in the ratio of 350:650 respectively. Above solution was filtered through 0.45 µ membrane filter and degasify.

Standard preparation: Accurately weighed and transferred about 20 mg of Ranolazine working standard into a 100 mL volumetric flask. Then added about 30 mL of mobile phase and sonicate to dissolve then cooled toroom temperature and diluted to volume with mobile phase. 1.0 mL of the solution was transferred into a 200 mL volumetric flask, and make up the volume with mobile phase.

Placebo Preparation: Accurately weighed placebo powder (without drug but having same composition) equivalent to formulation sample containing 500 mg Ranolazine was transferred into 100 mL volumetric flask, 50 mL of mobile phase was added and sonicate for 20 min. Cooled to room temperature and diluted to volume with mobile phase and mixed well. The solution was filtered through a 0.45 µm membrane filter. 5.0 mL of the solution was transferred into a 25 mL volumetric flask, and made up the volume with mobile phase.

Sample preparation: Not fewer than 5 tablets were grounded to fine powder and transferred an accurately weighed portion of the powder, equivalent to 500 mg of Ranolazine, to a 100 mL volumetric flask. Then added about 50 mL of mobile phase, and sonicate for 20 minutes with occasional shakings. Cooled to room temperature and diluted to volume with mobile phase and mixed well. The solution was filtered through a 0.45 µm membrane filter.

5.0 mL of the solution was transferred into a 25 mL volumetric flask, and made up the volume with mobile phase.

System suitability: Chromatograph the standard preparation (six replicate injections), measured the peak area responses for the analyte peak and evaluated the system suitability parameters as directed.

Acceptance criteria:

• %RSD for replicate injections of peak area response for Ranolazine peak from the standard preparation should be not more than 2.0.

• The Tailing factor for Ranolazine peak should be not more than 2.0.

• Theoretical plate for Ranolazine peak should be not less than 2000.

Procedure: Separately injected equal volumes (about 20 µL) of mobile phase as blank, placebo and sample preparations into the chromatograph and recorded the peak area response for the analyte peaks and calculated by using formula as mentioned in below formula.

$$\text{UASW}_{110025}\text{PAvg.wt} \quad \text{-----x------- x ------- x ------- --------x---------x---------- x 100}$$

$$\text{SA}_{100200}\text{TW}_{5100}\text{LA}$$

Total Impurities: Sum of Unknown impurities

Where,

SA : Peak area response due to Ranolazine from sample preparation

UA : Peak area response due to Un-known impurity from sample preparation

SW : Weight of Ranolazine working standard taken, in mg

TW : Weight of sample taken in, mg

P : Purity of Ranolazine working standard taken on, as is basis

Preparation of physical powder mixture: In order to evaluate Ranolazine drug excipient interactions, The excipients and drug were taken in different ratios as reported in Table 1, required to prepare extended release tablets [Ranolazine with Hypromellose phthalate grade HP 55, Ethocel standard 7 FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel pH 101 in (1:0.5) and Magnesium Stearate in (1:0.2)] and homogeneously mixed with a mortar and pestle for 10 min, then powder mixture was placed in glass vials with a rubber stoppers. These vials were stored in at 55°C for 14 days and 40°C/75% RH for 28 days and evaluated periodically using HPLC.

Preparation of extended release tablets: The tablets were prepared by wet granulation technique. Drug and other excipients were accurately weighed, mixed and sifted through ASTM (American society of Testing and Materials) 40 mesh. The wet mass was passed through ASTM 12 mesh and granules were dried. Dried granules were further passed through ASTM 18 mesh. The granules were lubricated and compressed into oblong shaped (16.5×8.0 mm for 500 mg) tablets using 12-station rotary compression machine (Rimekminipress–II MT).

Analytical method validation

Specificity: Specificity is to validate the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present such as matrix components, impurities and degradation products.

Acceptance criteria: The interference of the placebo and diluent is considered insignificant, if the chromatogram of the diluent and placebo shows no peak at the retention time of Ranolazine and impurity peaks.

Limit of detection and Limit of Quantification of Ranolazine (un-known impurity): For calculating the LOD and LOQ values, solutions with known decreased concentrations of analyte were injected into the HPLC system. The limit of detection (LOD) and quantification (LOQ) were then measured by calculating the minimum level at which the analyte can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1).

S.No	Ingredients	Sample ID	Ratio
1	Ranolazine	RZ01	1:0
2	Hypromellose phthalate grade HP-55	RZ02	1:0
3	Ethocel standard 7FP Premium	RZ03	1:0
4	Natrosol Type 250 HHX	RZ04	1:0
5	Klucel HF pharm	RZ05	1:0
6	Avicel pH101	RZ06	1:0
7	Magnesium Stearate	RZ07	1:0
8	Hypromellose phthalate grade HP-55+ Ethocel standard 7FP Premium+ Natrosol Type 250 HHX+ Klucel HF pharm+ Avicel pH101+ Magnesium Stearate	RZ08	0.5:0.5:0.5: 0.5:0.5:0.2
9	Ranolazine+Hypromellose phthalate grade HP-55	RZ09	1:0.5
10	Ranolazine+Ethocel standard 7FP Premium	RZ10	1:0.5
11	Ranolazine+Natrosol Type 250HHX	RZ11	1:0.5
12	Ranolazine+Klucel HF pharm	RZ12	1:0.5
13	Ranolazine+Avicel pH101	RZ13	1:0.5
14	Ranolazine+Magnesium Stearate	RZ14	1:0.2
15	Ranolazine+Hypromellose phthalate grade HP-55+ Ethocel standard 7FP Premium+ Natrosol Type 250HHX+ Klucel HF pharm+ Avicel pH101+ Magnesium Stearate	RZ15	1:0.5:0.5:0.5: 0.5:0.5:0.2

Table 1: Ranolazine and Excipients Ratio Used In Compatibility Study.

Linearity: Linearity of detector response was established by plotting a graph to concentration versus area of Ranolazine (un-known impurity). Determined the correlation coefficient and Y-intercept/ response at 100% of working concentration. A series of solutions Ranolazine (un-known impurity) solutions in the concentration ranging from about LOQ% level to about 150% (LOQ, 25%, 50%, 100%, 125%, & 150%) of the target concentration were prepared and injected into the HPLC system.

Acceptance Criteria:

• The correlation coefficient (r) is NLT 0.995

• Regression coefficient (r^2), Y-intercept, slope of regression line should be reported.

• %Y-intercept at 100% target concentration should be NMT ± 5.0%

Precision: The method precision of the method is ascertained by injecting 6 replicates of test sample % impurity and % RSD was calculated.

Accuracy: Accuracy of the proposed method was determined by recovery studies using standard addition method. The percentage recovery studies of Ranolazine was carried out in triplicate 3 different levels 50%, 100%, 150% by spikingstandard drug solution to the placebo [8,9].

Acceptance criteria: The mean recovery of the impurities at each level should be between 85.0% and 115.0%.

Results and Discussion

The purpose of the present study was to validate the HPLC method for determining the related substances to evaluate the physical & chemical stability of Ranolazine when mixed with excipients and in a novel extended release tablet at different storage conditions in accordance with the ICH guidance document. The HPLC method used in this investigation was Accurate, Precise, and linear it is validated by using Water Alliance HPLC system with 2489 detector and Supelcosil C 18, (250×4.6 mm, 5 µm particle size).Limits for reporting threshold and total impurities were 0.1% and 2.0%, respectively, as per Q3B(R) Impurities in New drug Products.

Analytical method validation

Specificity: A study was conducted for the interference of diluent and placebo with Ranolazineand impurities. Samples were prepared in triplicate by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and injected into the HPLC system. The results were summarized in Table 2. No peaks are eluted at the retention time of Ranolazine and impurities.

LOD and LOQ: Determined the limit of detection (LOD) of Ranolazine (un-known impurity) by the analysis of samples with known concentrations of analyte by establishing the minimum level at which the analyte can be reliably detected and results tabulated in Table 3. S/N Ratio of 3.10:1 was observed for LOD.

S No.	Sample name	Retention time (minutes)	% Interference (Respective to Ranolazineand Impurities)
1	Diluent	NA	NO
2	Placebo 500 mg	NA	NO
3	Impurity 1	5.565	NO
4	Impurity 2	8.331	NO
5	Unknown impurity	3.113	NO
6	Ranolazine	10.464	NA

Table 2: Specificity results.

Component	Limit of Detection (LOD)		
	Concentration (mcg/mL)	Concentration (%)	S/N Ratio
Ranolazine	0.0273	0.003	3.10 : 1

Table 3: Limit of detection Results.

Component	Limit of Detection (LOD)		
	Concentration (mcg/mL)	Concentration (%)	S/N Ratio
Ranolazine	0.0818	0.008	10.19 : 1

Table 4: Limit of Quantification Results.

Injection	Limit of Quantification (Ranolazine Peak areas)
1	2810
2	2816
3	2754
4	2796
5	2856
6	2896
Average	2821.0
% RSD	1.7

Table 5: Ranolazine Precision results at LOQ.

% Level	Concentration (mcg/mL)	Peak Area (Average)
LOQ	0.0818	2800
25	0.2557	8812
50	0.5115	18623
100	1.0229	37551
125	1.2786	47091
150	1.5344	56320
Correlation coefficient (r)		**1.0000**
Regression coefficient (r^2)		**0.9999**
Slope		37034.797
Y-intercept		-384.55307
Y-Intercept/response at 100% of Concentration×100		-1.0

Table 6: Linearity results.

Determined the limit of quantification (LOQ) of Ranolazine (un-known impurity) by the analysis of samples with known concentrations of analyte by establishing the concentration, which will give a concentration of about 3.3 times of LOD with an RSD of 10% for peak area, with acceptable precision and accuracy at LOQ level and results tabulated in Table 4. S/N Ratio of 10.19:1 was observed for LOD.% RSD for six replicate injections of lower (LOQ level) was 1.7 for Ranolazine precision at LOQ and these results tabulated in Table 5. From the results, it can be concluded that the proposed method can quantify the small quantity of impurities in Ranolazine samples. The Limit of Detection and Limit of Quantification test results met with the acceptance criteria.

Linearity: Linearity of the related substances test method covers from LOQ Level to 150% of target concentration for Ranolazine and results tabulated in Table 6. Hence the test method was found linear for Ranolazine.

Precision: Precision of the test method was determined by injecting test preparation and tested through the complete analytical procedure from sample preparation to final result. Repeatability assessed using a minimum of 6 determinations and calculated% relative standard

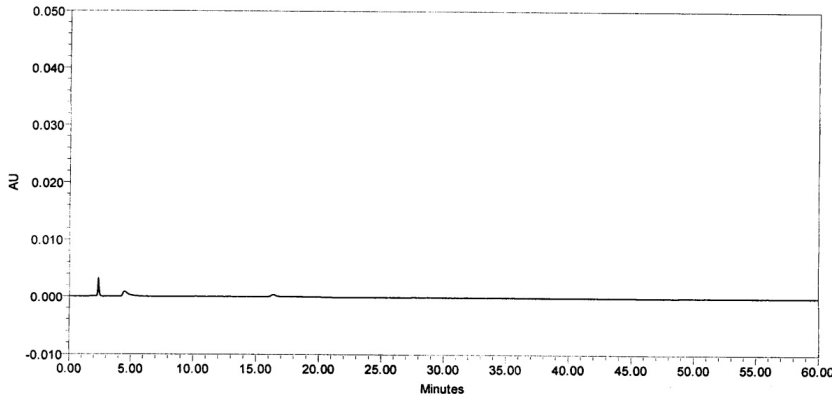

Figure 1: Related Substance Chromatogram for Mobile phase.

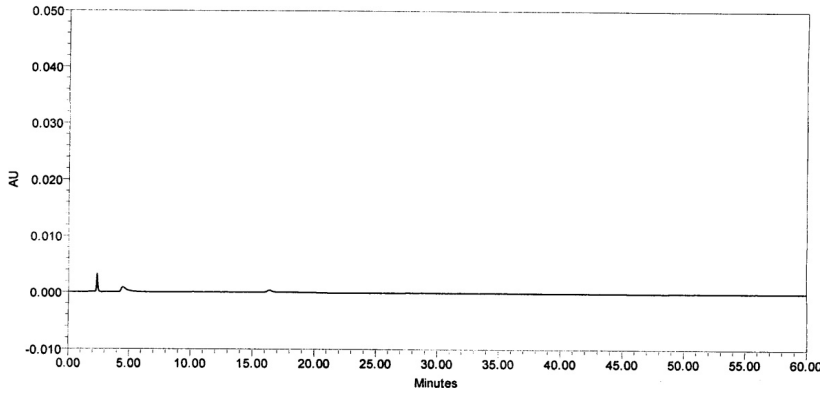

Figure 2: Related Substance Chromatogram for RZ08 sample at 40°C/75%RH for 28 days.

Sample No	% Of Related substances		
	Un-known imp-1	Un-known imp-2	Total impurities
1	0.051	0.042	0.093
2	0.049	0.043	0.092
3	0.052	0.045	0.097
4	0.051	0.044	0.095
5	0.049	0.042	0.091
6	0.051	0.043	0.094
Average	0.051	0.043	0.094
%RSD	**2.43**	**2.71**	**2.31**

Table 7: Precision results for Ranolazine.

deviation of impurities. The results were given in Table 7. Related substances results meet the specification limits.

Accuracy: The Accuracy of the related substances test procedure was determined by Spiking of Ranolazine on placebo and injecting samples in triplicate at LOQ, 50%, 100%, and 150% of the target concentration 0.1% v/v of Ranolazine (Un-known imp). Calculated the% recovery of impurities and the results are given in Table 8. The accuracy test results were met with the acceptance criteria.

Drug-Excipient Compatibility studies

The sample was subjected to above mentioned conditions, chromatograms were shown in Figure 1 for mobile phase, (Figure 2) for RZ08 sample and (Figure 3) for RZ15 sample respectively. System suitability test results were within the USP limits.Same peaks observed in mobile phase and RZ08 sample indicates only blank peaks appeared on chromatogram and no excipient peaks. Chromatograms of Hypromellose phthalate HP55, Ethocel 7FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel PH 101, Magnesium Stearate and mixture of these excipients does not shown the peaks at all storage conditions. In the sample chromatogram for RZ15, drug was eluted at a retention time of 10.491 min and the unknown impurities were observed at a retention time of 5.588, 8.349 min respectively as shown in Figure 3. The percentage of impurities was calculated by dilute standardization method. No significant variation in unknown impurity maximum and total impurities for Pure drug Ranolazine (RZ01), Combination of Ranolazine and excipient (RZ09, RZ10, RZ11, RZ12, RZ13 and RZ14) and combination of Ranolazine and all excipients (RZ15) at different storage conditions. Single unknown impurity were found to be not more than 0.03% and the total impurities were found to be not more than 0.06% in all combinations at different storage conditions shown in Table 9, i.e., impurities are within the limit as per ICH guidelines.

Pure drug Ranolazine or Ranolazine in binary mixtures was stable for 14 days at 55°C and 28 days at 40 ± 2°C/75 ± 5.0% RH. Since there is no change in peak area or change in Rt and the percentage of impurities was within limits as per ICH guidelines It can be deduced that the drug was stable in pure form or in the presence of excipients tested under these elevated temperature and Humidity conditions. Therefore these

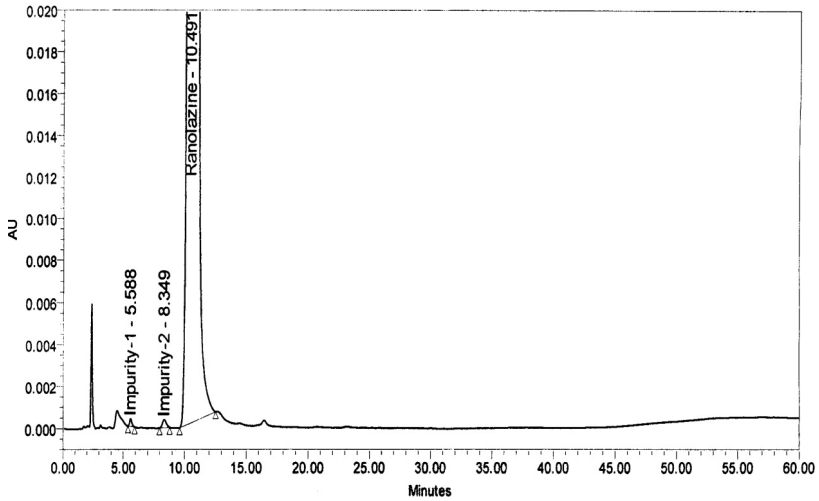

Figure 3: Related Substances Chromatogram of Ranolazine +All Excipients (RZ15) stored at 40ºC/75%RH (after 28 days).

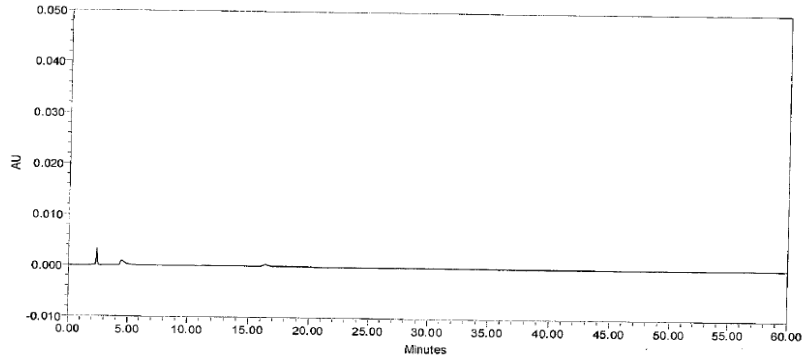

Figure 4: Related Substance Chromatogram for Mobile phase.

S. No.	% Level	mcg/ml Added	mcg/ml Found	%Recovery	Average Recovery (%)
1	LOQ	0.0812	0.0828	101.97	101.9
2			0.0834	102.71	
3			0.0821	101.11	
4	50	0.5121	0.5183	101.20	101.4
5			0.5165	100.90	
6			0.5236	102.20	
7	100	1.0326	1.0395	100.67	101.2
8			1.0496	101.65	
9			1.0456	101.26	
10	150	1.5456	1.5359	99.37	100.4
11			1.5665	101.35	
12			1.5552	100.62	

Table 8: Accuracy results for Ranolazine.

excipients were found to be compatible with Ranolazine.

Extended release tablets

Same method also used in novel extended release tablet for determination of related substances and results were tabulated in Table 4. Same peaks observed in the mobile phase and placebo sample indicates only blank peaks appeared on chromatogram and no excipient peaks as shown in Figures 4 and 5. In the sample chromatogram drug was eluted at a retention time of 10.464 min and the unknown impurities were observed at a retention time of 3.113, 5.565, 8.331 min, respectively as shown in Figure 6. The percentage of impurities was calculated by dilute standardization method. No significant variation was found in unknown impurity maximum and total impurities for novel extended release tablets at different storage conditions. With respect to drug excipient mixtures in compatibility studies, one additional impurity was observed in novel extended release formulation. Single unknown impurity were found to be not more than 0.05% and the total impurities were found to be not more than 0.09% in extended release tablets at different storage conditions shown in Table 10, i.e., impurities are within the limit as per ICH guidelines. Slight trending was observed in tablets when compared with compatibility samples which may be due to additional storage time period. Based on reporting threshold and total impurities results, developed formulation was stable at 40°C/75% RH for 3 months.

Conclusion

The proposed HPLC method for estimation of related substances for Ranolazine is analyzed in bulk drug and Ranolazine extended release tablets as per ICH guidelines. This method is found to be specific for estimation of unknown impurities. Knowledge of drug–excipient interactions is a necessary prerequisite to the development of dosage forms that are stable and of good quality. The results of HPLC

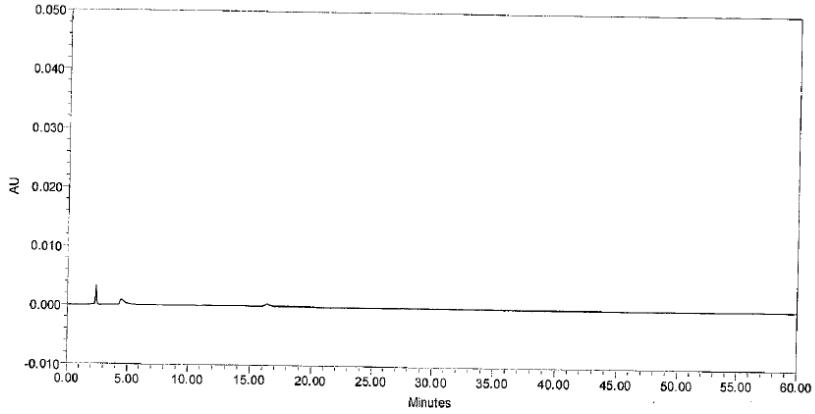

Figure 5: Related Substance Chromatogram for placebo sample.

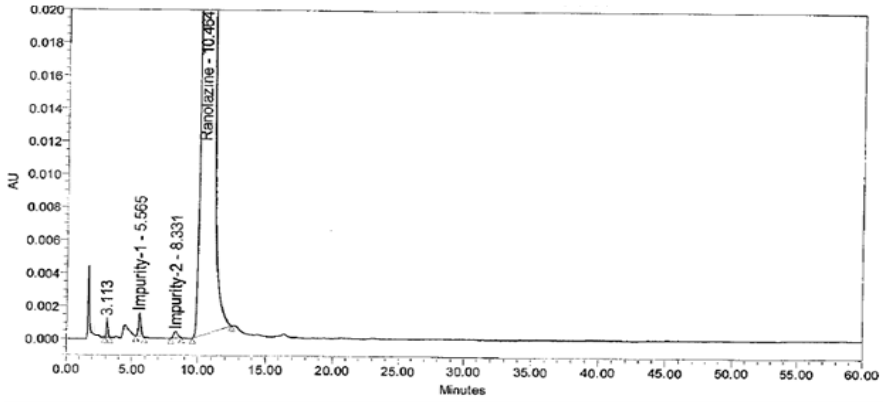

Figure 6: Related Substances Chromatogram of Ranolazine extended release tablets Stored at 40ºC/75% RH (after 3 months).

Sample	Impurities (%) (Initial)		Impurities (%) (550C) 14 days		Impurities (%) (400C/75%RH) 28 Days	
	U_{max}*	Total Impurities	U_{max}*	Total Impurities	U_{max}*	Total Impurities
RZ01	0.02	0.04	0.03	0.05	0.02	0.04
RZ09	0.02	0.04	0.02	0.04	0.02	0.05
RZ10	0.03	0.03	0.03	0.04	0.03	0.04
RZ11	0.02	0.03	0.02	0.04	0.02	0.04
RZ12	0.03	0.04	0.03	0.05	0.02	0.04
RZ13	0.03	0.04	0.02	0.04	0.03	0.04
RZ14	0.03	0.04	0.03	0.04	0.03	0.03
RZ15	0.03	0.04	0.02	0.04	0.03	0.06

Note: Umax* means Unknown Impurity Maximum

Table 9: Related impurities results for Ranolazine with different excipients in compatibility studies.

Impurities (%)							
Initial		400C/75%RH					
		1st Month		2nd Month		3rd Month	
U_{max}*	Total Impurities	U_{max}*	Total Impurities	U_{max}*	Total Impurities	Umax*	Total Impurities
0.03	0.07	0.03	0.07	0.05	0.09	0.05	0.09

Note: U_{max}* means Unknown Impurity Maximum

Table 10: Related impurities results for Ranolazine extended release tablets 500 mg stored at accelerated condition for different time intervals.

studies showed the method is suitable for the assay of Ranolazine in the given Extended release formulation and that there were no possible interactions between drug and selected excipients like Hypromellose phthalate HP55, Ethocel7FP premium, Natrosol type 250 HHX, Klucel HF pharm, Avicel PH 101 and Magnesium Stearate during 14 days at 55°C and 28 days at 40 ± 2°C/75 ± 5.0% RH storage conditions.

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