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New validated ultra high performance liquid chromatographic method for estimation of Ranolazine hydrochloride

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Abstract
In present work attempt has been made to develop and validate new Ultra High Performance Liquid Chromatographic (UHPLC) method for estimation of Ranolazine hydrochloride. A simple, selective, specific and precise UHPLC method was developed for estimation of Ranolazine Hydrochloride in bulk powder. Method was developed by using mobile phase composition Methanol: 0.1% Orthophosphoric Acid (50:50 v/v) and the separation were achieved using BDS Hypersil C18 (250mm x 4.6mm, 5µm) column. The flow rate was adjusted to 1.5 ml/min and the temperature was maintained at 20^0 C. UV detection was monitored at 224 nm. The volume of the sample injected was 10 µl. The retention time of Ranolazine was found to be at 8.9 min. It showed linear response between the concentration ranges of 20-100 µg/ml with correlation coefficient was found to be 0.999. The method was validated for accuracy, precision, robustness as per the ICH Guidelines and all the values of validation was found to be within the acceptance. A new UHPLC method was developed for estimation of Ranolazine and it was validated as per ICH Guidelines. Hence it can be concluded that method was new, simple, selective, specific, precise and found to be economic for estimation of Ranolazine in bulk powder.

Keywords: Ranolazine HCl, UHPLC, ICH guidelines, precise, hypersil C-18

1. Introduction
Ranolazine (Fig 1) is derived from piperazine and an anti-anginal which acts as a sodium channel blocker and also prevents the sodium dependent calcium channels which are responsible for myocardial ischemia [1]. Many branded formulations containing Ranolazine were available in market. Hence the quality control analysis of Ranolazine in bulk and its formulation play an important role. Literature survey revealed very few analytical methods for the estimation of Ranolazine HCl have been developed and validated. Methods such as UV-Spectrophotometric [2], HPLC [2, 4-9] were reported. In the present research an attempt has been made to develop a new, simple, precise and accurate UHPLC method foe estimation of Ranolazine HCl which will be helpful for the routine analysis of Ranolazine HCl.

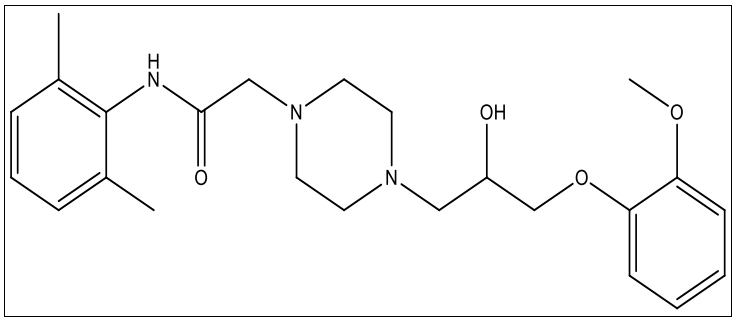

Fig 1: Structure of Ranolazine HCl

2. Material and Methods
2.1 Drug Sample
2.2 Chemical and Reagents: All the chemicals and reagents used for analysis were analytical grade and obtained from Merck laboratories. Milli-Q water was used and it was obtained from Basic Science Research Laboratory from KLE College of Pharmacy, Belagavi.

2.3 Instruments used: The UHPLC used was of Shimadzu Prominence LC-30AD. The software used was Lab Solutions. The column used was BDS Hypersil C18 (250 x 4.6mm, 5µ). Detection was done using a PDA Detector. Analytical balance used was of Uni Bloc of Shimadzu make. Eutech Instruments pH meter was used and the sonication was done using Branson 1800 sonicator.

2.4 Method Development
Development of UHPLC method was started with the selection of mobile phase system and stationary phase selection. Literature survey has been done and solubility's of Ranolazine in various solvents were checked and trials were carried out using different mobile phase composition.

2.4.1 Preparation of 0.1% Orthophosphoric Acid
Transfer 500 ml of Millipore water in a 500 ml beaker. With the help of a calibrated digital pipette, pipette out 0.1 ml of Orthophosphoric acid and transfer into a beaker containing water. Filter the mobile phase by using 0.2 µ Nylon syringe filter.

2.4.2 Preparation of mobile phase
Methanol filtered through 0.2 µ Nylon syringe filter and degassed and 0.1% Orthophosphoric acid degassed.

2.4.3 Ranolazine HCl Standard Preparation
Weigh accurately 10 mg of Ranolazine HCl and transfer into a 10 ml volumetric flask and make up the volume with methanol to 10 ml. it gives stock solution of 1000 µg/ml. Pipette out 2.5 ml from above stock solution and transfer into a 25 ml volumetric flask and make up the volume to 25 ml with methanol. It gives stock solution of 100 µg/ml.

2.4.4 Working Standard for Ranolazine HCl
Pipette out 6 ml of stock solution and transfer into 10 ml volumetric flask and make up the volume to 10 ml with methanol to give working stock solution of 60 µg/ml.

2.4.5 Determination of retention time of Ranolazine
Working standard solution containing 60 µg/ml of Ranolazine was injected into UHPLC which was supported with Hypersil C-18 column as stationary phase and mobile phase composed of Methanol: 0.1% Orthophosphoric Acid (50:50 v/v). The flow rate was adjusted to 1.5 ml/min and the temperature was maintained at 20^0 C. UV detection was monitored at 224 nm. The volume of the sample injected was 10 µl. The Chromatogram was obtained and Retention time was determined.

2.5 Validation of UHPLC method [10,11]
In order to check the performance of developed UHPLC method, validation was performed as per ICH Guidelines using Specificity, selectivity, linearity, precision, accuracy and robustness parameters.

2.5.1 System Suitability
Six replicates of a solution containing analyte of working concentration were injected to determine the precision and accuracy of the system.

2.5.2 Specificity and Selectivity
It was performed by injecting the triplicates of mobile phase and sample solutions into UHPLC. Standard solutions was prepared and injected into the UHPLC system and the Amount Found, Peak Area, % Label claim were calculated. Mean and %RSD were found within the limits.

2.5.3 Linearity
2.5.3.1 Preparation of linear dilution: From the stock solution of Ranolazine HCl 100 µg/ml, pipette out 2ml, 4ml, 6ml, 8ml, 10ml and transfer each ml into separate 10 ml volumetric flask to get the final concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml. The freshly prepared linear dilutions were injected into UHPLC in triplicates.

2.5.4 Precision
In order to prove precision of method, working standard solution containing Ranolazine HCl were injected into UHPLC in six replicates on two different days and on the same day at different time intervals.

2.5.5 Robustness
It was performed by changing the flow rate and temperature conditions and injecting the working standard solution containing Ranolazine HCl into UHPLC.

2.5.6 Ruggedness
In order to prove the repeatability of developed analytical method six replicates of working standard Ranolazine solution were injected by different analyst on different days.

2.5.7 Limit of Detection and Limit of Quantification
It was calculated from the standard calibration plot and statistical calculations.

2.5.8 Accuracy
Accuracy was performed by the Standard Addition Method at three different levels. Weighed 10 mg Ranolazine Hydrochloride and transfer it into 10 ml volumetric flask and make up the volume to 10 ml with methanol to get stock solution of 1000 µg/ml. pipette out 2.5 ml and transfer it into 25 ml volumetric flask and makeup the volume to 25 ml with methanol to get stock solution of 100 µg/ml. Pipette out 1.5 ml, 3 ml, 4.5 ml from above solution and transfer each ml into individual 10 ml volumetric flask to get 15 µg/ml, 30 µg/ml, 45 µg/ml.

2.5.9 Accuracy of Ranolazine Hydrochloride at three different levels
Level-I (50 %): 30 µg/ml of stock solution of Ranolazine Hydrochloride was spiked with 50 % i.e 15 µg/ml and injected.
Level-II (100 %): 30 µg/ml of stock solution of Ranolazine Hydrochloride was spiked with 100 % i.e 30 µg/ml and injected.
Level-III (150 %): 30 µg/ml of stock solution of Ranolazine Hydrochloride was spiked with 150 % i.e 45 µg/ml and injected.

3. Results and Discussion
3.1 Method Development
UHPLC method was developed for estimation of Ranolazine in bulk powder using mobile phase composition Methanol: 0.1% Orthophosphoric Acid (50:50 v/v) and the separation was achieved using BDS Hypersil C18 (250mm x 4.6mm, 5µm) column. The flow rate was adjusted to 1.5 ml/min and the temperature was maintained at 20^0 C. UV detection was

monitored at 224 nm. The volume of the sample injected was 10 µl. Retention time of Ranolazine HCl was found to be at 8.9 min. The chromatographic conditions were presented in Table 1 and chromatogram was showed in Fig. 2. System Suitability parameter were performed and from the chromatograms obtained Plate count, tailing factor, resolution and reproducibility were analyzed. System suitability data presented in Table 2.

3.2 Method Validation

Developed UHPLC method was found to be specific and selective as the mobile phase not eluting any components at the retention time of analyte. The data obtained were presented in Table 3. Linearity was carried out for Ranolazine of working level concentration from 20.0 µg/ml to 100 µg/ml. The linearity regression correlation coefficient was within limits and found 0.999. The % RSD for the peak area and retention time was found within the limit. Data of linearity were showed in Table 4 and calibration graph were showed in Fig.3. Precision was carried out on different time intervals and on different days. Mean and % RSD were found within the limits an data presented in Table 4. Robustness were Analyzed by modifying the method parameters. Variations in temperature, total flow were made and the outcome was analyzed and calculated. Data were presented in Table 5 and Table 6. Ruggedness and Repeatability were carried out by injecting six replicates of the sample solution having target level of the analyte. Retention time, peak area were recorded. The mean, standard deviation and % RSD were found within the limits. Data were presented in Table 7. Samples were spiked at three levels 50 %, 100 %, 150 % of the target concentration. Three replicates for each level were analysed. Theoretical plate, % recovery were calculated. Mean and % RSD were found within the limits. Accuracy data were presented in Table 8. In the proposed method the estimation of Ranolazine HCl has been done using UHPLC and is a new method as compared to previously performed works. The work done using UHPLC shows better peak resolution and faster elution of the peak. The mobile phase that is used is Methanol:0.1% Orthophosphoric acid and it shows a better peak elution and shows a peak retention at 8.9 min. The method shows theoretical plate count more than 2000 and a tailing factor less than 2. The LOD and LOQ was found to be 0.1353 and 0.4198 respectively.

Table 1: Chromatographic Conditions used for UHPLC Analysis

Parameter	Values
Mobile Phase	Methanol: 0.1% OPA (50:50 v/v)
Diluents	Methanol
Column	BDS Hypersil C_{18}(250 × 4.6 mm, 5 µm)
Pressure	65 kgf/c
Temperature	20 ºC
Tot Amount	1.5 ml/min
In. Volume	10 µl
UV Wavelength	224nm

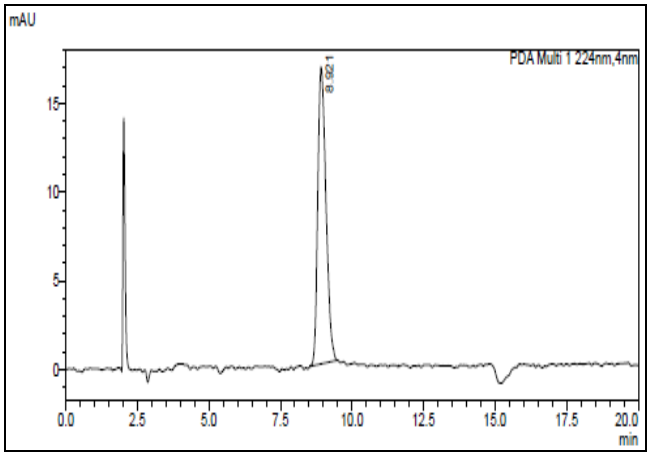

Fig 2: Chromatogram of Ranolazine HCl

Table 2: System Suitability Table of Ranolazine Hydrochloride

Sample	Retention Time	Peak Area	Theoretical Plate	Tailing Factor
1	9.063 min	336306	4376	1.297
2	9.067 min	333943	4319	1.298
3	9.049 min	340312	4395	1.34
4	9.019 min	334830	4385	1.311
5	8.980 min	342291	4343	1.333
6	8.921 min	333442	4421	1.331
Mean	9.016 min	336854	4373.16	1.318
% RSD	0.04	0.2		

Table 3: Linearity of Ranolazine Hydrochloride

Concentration (µg/ml)	Peak Area
20.0 µg/ml	117439
40.0 µg/ml	234754
60.0 µg/ml	337371
80.0 µg/ml	459616
100.0 µg/ml	558103
Mean	341456.6
Equation for Regression Line	Y= 5531 x + 9599.6
Correlation Coefficient (r^2)	0.999

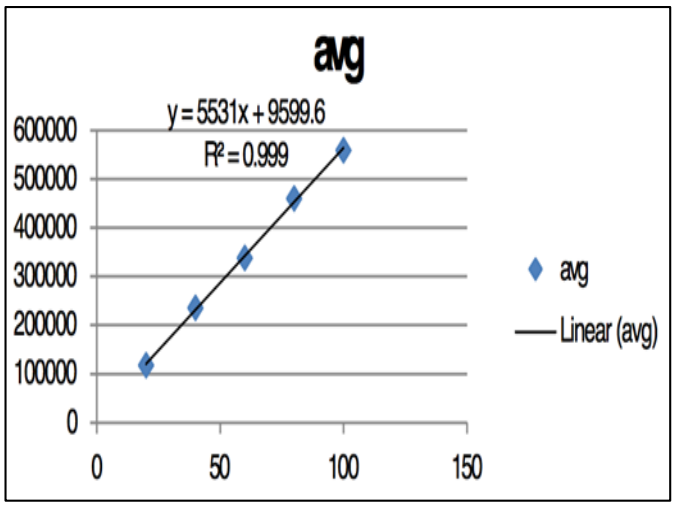

Fig 3: Linearity Graph of Ranolazine Hydrochloride

Table 4: Specificity Table of Ranolazine Hydrochloride

Conc	Peak Area	Amt Found	% Label Claim
60 µg/ml	337371	59.26	98.76
60 µg/ml	342798	60.24	100.40
Mean	340084.5	59.75	99.58
SD	3837.46	0.6929	1.1596
%RSD	1.12	1.15	1.16

Table 5: Precision Data of Ranolazine HCl

	Intraday Precision			Interday Precision		
Sample	Peak Area 1	Peak Area 2	Peak Area 3	Day 1	Day 2	Day 3
1	363324	337371	333943	334830	333442	340350
2	361845	336306	335821	342291	333943	333069
3	362146	334861	340312	337721	338255	342291
Mean	362438.3	336179.3	336692	338280.6	335509	338570
% RSD	0.21	0.37	0.97	1.11	0.78	1.43

Table 6: Robustness Table of Ranolazine Hydrochloride

	At 1.3 ml/min Total Flow		At 1.7 ml/min Total Flow	
Sample	Retention Time	Peak Area	Retention Time	Peak Area
1	9.987 min	383105	7.704 min	300476
2	9.961 min	391352	7.666 min	299470
Mean	9.974 min	387228.5	7.685	299973
% RSD	0.18	1.5	0.34	0.23

Table 7: Robustness Table of Ranolazine Hydrochloride

	At 15^0 C Temperature		At 25^0 C Temperature	
Sample	Retention Time	Peak Area	Retention Time	PeakArea
1	8.867 min	333040	8.013 min	326446
2	9.012 min	329177	7.983 min	332646
Mean	8.939 min	331108.5	7.998 min	329546
% RSD	1.14	0.82	0.26	1.3

Table 8: Ruggedness Table of Ranolazine Hydrochloride

Samples	Retention Time	Peak Area
1	9.063 min	336306
2	9.067 min	333943
3	9.049 min	340312
4	9.019 min	334830
5	8.980 min	342291
6	8.921 min	333442
Mean	9.016 min	336854
SD	0.056	
% RSD	0.04	0.2

Table 9: Accuracy Table of Ranolazine Hydrochloride

Levels	Conc. mcg/ml	Qty Added mcg/ml	Amt Recovered mcg/ml	% Recovered	% RSD
50 %	30	15	22.09	98.1	0.002
50 %	30	15	22.09	98.1	0.06
50 %	30	15	22.09	98.1	0.06
100 %	30	30	30.21	100.7	0.29
100 %	30	30	30.21	100.7	0.081
100 %	30	30	30.21	100.7	0.21
150 %	30	45	37.39	99.7	0.26
150 %	30	45	37.39	99.7	0.61
150 %	30	45	37.39	99.7	0.34

4. Conclusion

A new UHPLC method was developed and validated for estimation of Ranolazine in bulk powder. The use of RP C18 showed compatibility with analyte with good Peak Area, Theoretical Plate Count, Retention Time and Tailing Factor. Validation results of analysis reveals all the parameters were within the acceptable range which indicated method was specific, selective, linear, precise, robust, rugged and accurate for estimation of Ranolazine.

5. Acknowledgement

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