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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RANOLAZINE AND DRONEDARONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Accurate, Precise method was developed for the simultaneous estimation of Ranolazine and Dronedarone in pharmaceutical dosage form. Chromatogram was run through Water symmetry, 3.5 mm; 150 x 4.6 mm ID. Mobile Phase containing trifluoroacetic acid and acetonitrile (24:76) as a mobile phase was pumped through column at a flow rate of - 1.2 ml/min. temperature was maintained 30^0C. Optimized wavelength of Ranolazine and Dronedarone was 282 nm. Retention time of Ranolazine and Dronedarone were found to be 3.39min and 7.92min. % RSD of the Ranolazine and Dronedarone were found to be 1.11 and 1.66 respectively. LOD, LOQ values are obtained from equation of Ranolazine and Dronedarone were 2.02, 6.76 & 0.23, 0.76 respectively. linear regression observed for RZN and DRD. As resulted, they were y = 36923x + 52025 and y = 46667x - 68266, respectively.

KEYWORDS: Method Development, Ranolazine, Dronedarone, RP-HPLC.

INTRODUCTION

An analytical procedure is developed to test a defined characteristic of the substance against acceptance criteria for that the characteristics. In the development of a new analytical procedure, the choice of analytical instrumentation and methodology should be based on the and scope of the analytical method. Analytical procedures developments are primarily

intended purpose based on a combination of mechanistic understanding of the basic methodology and prior experiences.

Ranolazine:- Ranolazine, is an antianginal medication, is a medication used to treat heart related chest pain. Ranolazine was approved for medical use in the United States in 2006. In 2021, it was the 202nd most commonly prescribed medication in the United States, with more than 1 million prescriptions.

Dronedarone:- Dronedarone is a drug it is a antiarrhythmic medication developed by Sanofi-Aventis. It was approved by the US Food and Drug Administration (FDA) in July 2009. It was recommended as an alternative to amiodarone for the treatment of atrial fibrillation and atrial flutter in a people whose hearts have either returned to normal rhythm or who undergo drug therapy or electric shock treatment i.e. direct current cardioversion (DCCV) to maintain normal rhythm.

EXPERIMENTAL WORK

Instrumentation

The High-Performance liquid chromatography (HPLC) of Shimadzu SCL-10A$_{VP}$ inbuilt with binary pump (LC-10AT$_{VP}$), UV detector (SPD-10A$_{VP}$), Rheodyne 20 ml loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC –Solution software was used to interpret the HPLC reports. Water symmetry, 3.5 mm; 150 x 4.6 mm ID, HPLC column purchased from Newcastle (UK) was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler –Toledo (USA), Ultra sonicator labman purchased from ultrachrom Ltd, India.

Table No. 1: Instrumentation.

Sr.No.	Instruments	Make	Model	Place
1.	HPLC system	Shimadzu Class A10$_{VP}$	HPLC 3000 Series	Made in Japan
2.	UV spectrometer	Shimadzu UV 1800	2012	Made in USA
3.	Weighing balance	Mettler Toledo	ME 203	Made in USA
4.	pH meter	Equip-Electronics	EQ- 610	Made in India Mumbai
5.	Ultra-sonicator	Labman	LMUC-3	Made in India, Chennai
6.	Hot air oven	Bionic Scientific Technologies Pvt.Ltd.	250 BSS	Delhi
7.	Double distilled assembly	Lab Sil Instrument		Bangalore

REAGENTS AND CHEMICALS

Table No. 02: Reagents and chemicals.

Sr. No.	Chemicals	Grade	Manufacturer
1.	Methanol	HPLC	Merck, India
2.	Deionized water	HPLC	Merck, India
3.	Ammonium acetate	AR	Merck, India
4.	Acetonitrile	HPLC	Merck, India

Standard stock solutions

Standard stock solutions of RZN and DRD (1.2 mg/ml^{-}) were prepared separately by dissolving 10 mg of the drug in using in 20 ml volumetric flask and completing final volume adjusted with trifluoroacetic acid– acetonitrile (24:76) based on the solubility of drugs in particular eluents. Furthermore, freshly prepared sample solution was sonicator for 10 min and later filter through 0.20 Micro nylon filters. Required serial dilution was made for evaluating the validation studies. And concentration of drug is 100 ppm.

Working stock solutions

Working stock solution of RNZ (200ug ml^{-1}) was prepared by serial dilution of 37.5 ml of its solution of 37.5 ml of its stock solution in 100 ml volumetric flask by completing to volume with mobile phase. Working solution of DRD (200ug ml^{-1}) was prepared by serial dilution of 12.5 ml of its stock solution in a 100 ml volumetric flask by completing to volume with the mobile phase.

Chromatographic Conditions

Chromatographic separation was achieved on a water symmetry C18 column (150mm x 4.6mm) and ultrasil-MCX column (100 mm x 2.1 mm) applying an isocratic elution based on trifluoroacetic acid and acetonitrile (24:76) as a mobile phase.

The ultra violet detector was operated at 223 nm. The solution was filtered through 0.2 micro nylon membrane filter and degassed for 30 min in an ultrasonic bath prior to its use.

The mobile phase was pumped through the column at a flow rate of 1.2ml/min. the column temperature was adjusted to 28^{0}C and the injection volume was 20 microliter.

Marketed Sample Preparation

Exactly 20 Tablets of Ranexa-500 containing 5 mg RZN and 400 mg of DRD were weighed separately, powder and mixed in a mortar. And accurately weighed 10 mg amount of the

finely powdered Ranexa-500 tablets were transfer into 100 ml volumetric flask and volume was adjusted with 10 ml of trifluoroacetic acid-acetonitrile (24:76) and sonicated until completely dissolved. The solution was filtered with 0.2 micro nylon filters, followed by serial dilutions to the required concentrations using the same mobile phase for experiment with standard addition techniques.

Table No. 03: Marketed Sample Preparation.

Brand name	Mfg. by	Content	Quantity
Multaq	Sanofi India Ltd	Dronedarone	400mg

Brand name	Mfg. by	Content	Quantity
Ranexa	Gilead	Ranolazine	500 mg

Study of overlain spectra and determination of ƛ max of RNZ and DRD

Accurately 500 mg of Ranolazine and 400 mg of Dronedarone reference standard was weighted and transferred to 100 ml of volumetric flask and make up the volume with diluents that is methanol the solution is further diluted to get final concentration of (100 micro gram / ml of Ranolazine and 100 micro gram/ ml of DRD). The solution was scanned separately in the range of 400 to 200 nm against methanol as blank. The RZN and DRD shows their maximum absorbance at 271 to 288 nm respectively. While 223 nm was the point of equal absorbance i.e. isosbestic point. The resulting overlain spectrum is shown in fig.]

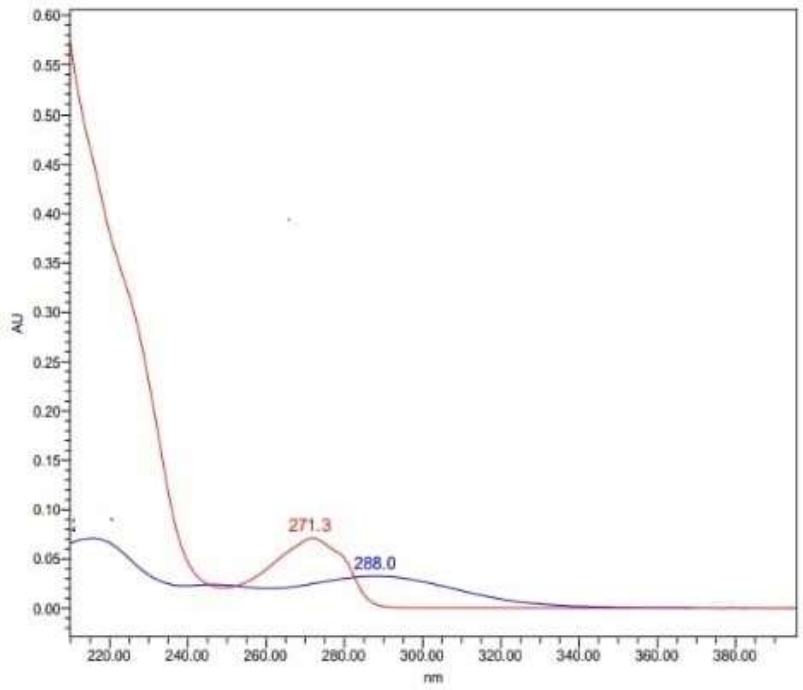

Fig. 1: Overlain Spectra of RZN and DRD.

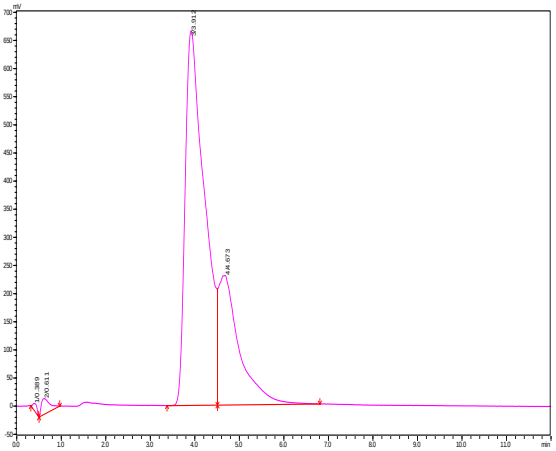

Fig. 2 trial reports of RZN and DRD by RP-HPLC.

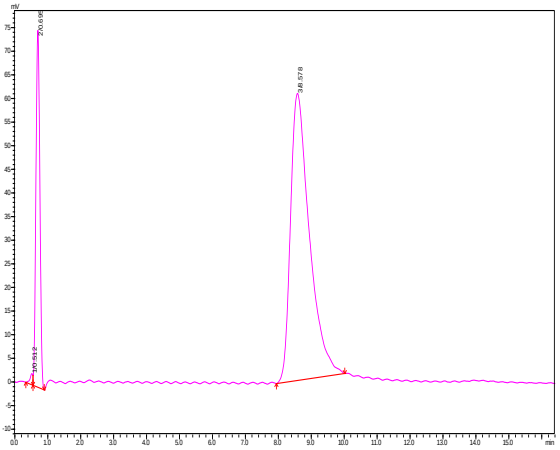

Fig. 3 trial reports of RZN and DRD by RP-HPLC.

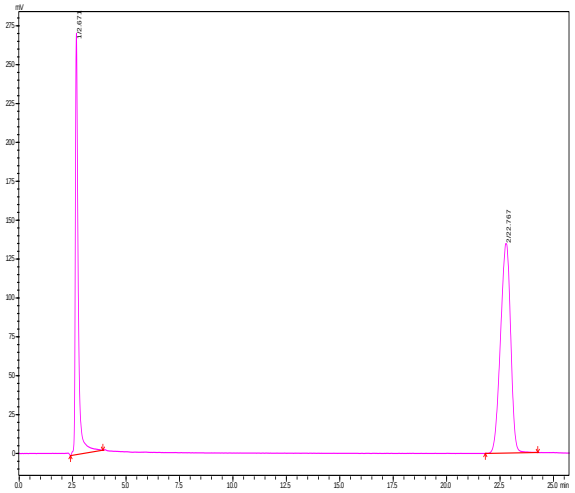

Fig. 4: Trial reports of RZN and DRD by RP-HPLC.

RESULTS AND DISCUSSIONS

System Suitability:- All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Linearity

The linearity of any HPLC method represents its ability to explicit the results that should proportional to the concentration of studied analytes within a selected range (Kharabe and Kadam, 2021). Therefore, over the testing range of 31.5–500 µg.ml−1 for both RZN and DRD, the results were proportional against the peak area with linear regression observed for RZN and DRD. As resulted, they were y = 36923x + 52025 and y = 46667x - 68266, respectively. Moreover, the regression coefficients (*r*2) were almost 0.999 for both drugs; which itself represented a high degree of linearity.

Precision studies of RZN and DRD

The precision of HPLC method reflects its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions (Kharabe and Kadam, 2021). both intra- and inter-day variability for precision studies, this method is significantly precise over the tested range of 100 µg/ml for both RZN and DRD. Moreover, the peak area of the studied samples was also correlated with selected concentration; where the % RSDs were <2%. The RSDs were observed in the range of 0.54%–1.64% for RZN and 0.35%–0.87% for DRD of the intra-day studies; whereas the % RSDs were observed in the range of 0.26%–1.61% for RZN and 1.40% - 1.78%for DRD in the inter-day studies that reflects an acceptable precision with minimum variations of the proposed method.

Intraday precision (Repeatability)

Implementing the procedure mentioned under experimental section, the homologous mixture of both DRD and RZN of three replicates of 100 ppm for both RZN and DRD were tested and evaluated within the same day (intra-day precision). The %RSD was calculated and found they are less than 2%. The results have been shown in (Table No. 04).

Table No. 04: Repeatability data of RZN and DR.

Sr. No.	Drug Name: - Ranolazine	Drug Name: - Dronedarone
	Peak Area; Conc.100ppm	Peak Area; Conc.100ppm
1	2424167	2937109
2	2440463	2919863
3	2414360	2818474
4	2434486	2882455
5	2373288	2867434
6	2447621	2944118
Mean	**2422397.5**	**2894908.833**
STD. DEV.	**26788.78348**	**48119.34073**
RSD (%)	**1.11**	**1.66**

Interday precision

Implementing the procedure mentioned under experimental section, the homologous mixture of both DRD and RZN of three replicates of 100 ppm concentration were tested and evaluated in three successive days (interday precision). The %RSD was calculated and found less than 2%. Results were demonstrated in (Table No. 05)

Table No. 05: Interday Precision data of RZN.

Drug Name: Ranolazine (RZN)				
Sr. No.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	100 PPM	2937109	64098.12408	2.22
	100 PPM	2919863		
	100 PPM	2818474		
DAY 2	100 PPM	2882455	38125.6023	1.34
	100 PPM	2867434		
	100 PPM	2810203		
DAY 3	100 PPM	2944118	26545.55074	0.91
	100 PPM	2899222		
	100 PPM	2946211		
	Range of % RSD			**0.91-2.22**

Table No. 06: Interday Precision data of DRD.

Drug Name: Dronedarone (DRD)				
Sr. No.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	100 PPM	2424167	13185.24095	0.54
	100 PPM	2440463		
	100 PPM	2414360		
DAY 2	100 PPM	2434486	23794.02657	0.97
	100 PPM	2480665		
	100 PPM	2447621		
DAY 3	100 PPM	2485814	22656.62225	0.91
	100 PPM	2479524		
	100 PPM	2511227		
Range of %RSD				**0.91-2.22**

Accuracy:- Three Concentration 80%, 100%, 120%, were injected in a triplicate manner and amount recovered. to determine the RZN and DRD were calculated to demonstrate the accuracy in RSD% for the selected pharmaceutical combination.

LOD:- Limit of Detection was calculated by std deviation method Ranolazine & Dronedarone and LOD for Ranolazine and Dronedarone were found to be 2.02 & 0.23 respectively.

LOQ:- Limit of Quantification was calculated by std deviation method Ranolazine & Dronedarone and LOD for Ranolazine and Dronedarone were found to be 6.76 & 0.76 respectively.

Robustness:- Robustness of HPLC method represents its ability to remain unaffected by small but deliberate variations in separation parameters to ascertain its reliability during routine analysis.

In this method, robustness was established by making deliberate changes in flow rate (1.0 ± 0.2 ml/minutes), organic modifier (60% ± 2% ml), and temperature (28°C ± 2°C).

Were almost unchanged which clearly signified that the proposed HPLC method obliged all minimum requirements led by the USP and ICH guidelines.

Table No. 07: System Suitability studies of Ranolazine and Dronedarone method.

Property	Ranolazine (RZN)	Dronedarone (DRD)
Retention time (t_R)	3.39min.	7.92min.
Theoretical plates (N)	2054	10631
Asymmetry/Tailing factor (T)	1.106	0.82

Assay:- Standard preparation are made from the API and Sample Preparations are from Formulations. Both sample and standard are injected six homogenous samples. Drug in the formulation was estimated by taking the standard as a reference.

CONCLUSION

HPLC method for simultaneous determination of Ranolazine and Dronedarone in bulk and pharmaceutical dosage form was determined and validated.

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