1. INTRODUCTION:

Dronedarone is a drug mainly for the indication of cardiac arrhythmias. It was recommended as an alternative to amiodarone for the treatment of atrial fibrillation and atrial flutter in people whose hearts have either returned to normal rhythm or who undergo drug therapy or electric shock treatment i.e. direct current cardioversion to maintain normal rhythm[1].

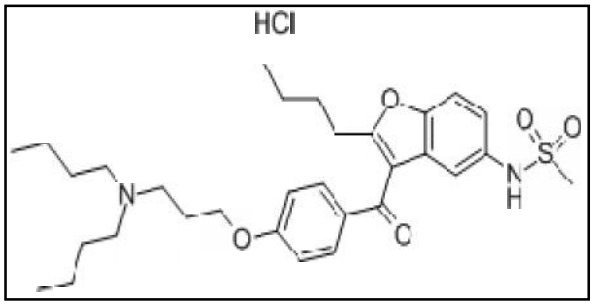


Figure 1: Structure of Dronedarone Hydrochloride

Chemically dronedarone is a benzofuren derivative related to aminodarone, a popular antiarrhythmic. The use of amiodarone is limited by toxicity due its high iodine content as well as by liver disease. In dronedarone, the iodine moieties are not present, reducing toxic effects on the thyroid and other organs. A methyl sulfonamide group is added to reduce solubility in fats and thus reduce neurotoxic effects [2]. Dronedarone displays amiodarone-like class III antiarrhythmic activity in vitro[3] and in clinical trials[4]. The drug also appears to exhibit activity in each of the 4 vaughan-williams antiarrhythmic classes[5].

Dronedarone has been termed a “multichannel blocker” however it is unclear which channel play a pivotal role in its success[6]. Thus, dronedarone’s action at the cellular level are controversial with most studies suggesting an inhibition in multiple outward potassium currents including rapid delayed rectifier, slow delayed rectifier and Ach-activated inward rectifier[7]. It is also belived to reduce inward rapid Na current in some studied was shown to be due to inhibition of K-Ach channel or associated GTP- binding proteins[6]. Reduction of K+ current by 69% led o increased AP duration and increased effective refractory periods, thus shown to suppress pacemaker potential of the SA node and return patients to a normal heart rhythm[7]. Clinical trials have compared dronedarone to placebo and to amiodarone, for its ability to reduce atrial fibrillation, to reduce mortality overall and from cardiac causes, and for its adverse effects, including excess mortality[3][6]. Dronedarone is a non-iodinated class III anti-arrhythmic drug

which helps patients return to normal sinus rhythm. This treatment of AF is also known to reduce associated mortality and hospitalizations compared to other similar antiarrhythmic agents[8].

Pharmacokinetics:

Dronedarone is less lipophilic than amiodarone, has a much smaller volume of distribution, and has an elimination half-life of 13-19 hours- this stands in contrast to amiodarone's half-life of several weeks[9][10]. As a result of these pharmacokinetic characteristics, dronedarone dosing may be less complicated than amiodarone.

2. LITERATURE REVIEW:

The literature review shows that various analytical methods were reported for its determination as API, pharmaceutical formulation. Brief details for the same are as under.

Xie C, Yang S, Zhong D, Dai X, Chen X. describe simultaneously determination of dronedarone and debutyldronedarone in human plasma using aminodarone as internal standard and CAPCELL PAK C18MG (100mm x 4.6mm,5µm) column with gradient elution (5 mmol/L ammonium acetate- acetonitrile, with each phase containing 0.2% acetic acid) at a flow rate of 0.7mL/min using tandem mass spectrometer in multiple reaction monitoring mode using a positive atmospheric pressure chemical ionization interface[12].

Bolderman RW, Hermans JJR, Maessen JG. Developed a method for determination of dronedarone and debutyldronedarone in both plasma and myocardial tissue by HPLC coupled with UV detection using pathfinder PS polymeric C18 column (50mm x 4.6mm, 2.5 µm) with mobile phase of acetonitrile, isopropanol, water and ammonia (80/10/10/0.025, v/v/v/v) at a flow rate of 1ml/min[13].

Arpan P, Jawed A. reported a isocratic RP-HPLC method for determination of dronedarone in pure and tablet dosage form using Hypersil ODS 3V (250mm x 4.6mm, 5µm) colulmn and mobile phase buffer (potassium dihydrogen phosphate pH=3.0):acetonitrile (42:58) at flow rate of 1.1 ml/min[14].

Naresh T, Shakil S. Sait, Surendranath KV, Ravi KK, Kumar S. developed a stability indicating RP-HPLC method quantitative determination of dronedarone and related substance using Agilent zorbaz RX C8 (150 x 4.6mm) 5µm column and stationary phase

and mobile phase (A) 10mM potassium dihydrogen phosphate and 10 mM Tetra n-butyl ammonium hydrogen sulfate pH=3.2 (B) Acetonitrile in gradient mode at flow rate of 1ml/min[15].

3. AIM OF PRESENT WORK:

The above literature review reveals that there were many methods for the quantitative analysis of dronedarone as a drug saubstance as well as pharmaceutical dosage form, few methods are there which deals with bionalytical study and stability study. The aim of present work is to developed a shortest and optimized method for quantitative analysis of dronedarone formulation development and stability testing as well as for routine analysis. The aim and scope of the proposed work are as under.

3.1. To develop rapid RP-HPLC method for quantification of the drug substance with highest selectivity, precision and accuracy.

3.2. Forced Degradation Study to confirm the stability of the drug substance.

3.3. Perform analytical method validation for the proposed method as per ICH guideline[16].

4. METHOD DEVELOPMENT:

4.1 Mobile phase and column selection:

Based on literature review and drug information (pKa value 9.4), acetate and phosphate buffer (pKa = 2.1, 7.2 and 12.3) were selected. Many experimental trials were performed using selected buffer to obtained best chromatographic condition for assay analysis of dronedarone in tablet formulation. During experimental trails different column and organic solvent (i.e. methanol, acetonitrile etc.) used and at last accepted result obtained using following chromatographic condition.

Chromatographic condition:

- Buffer : 50mM potassium phosphate in water + 1mL TEA pH=2.5 by OPA
- Mobile phase : Buffer:Methanol (40:60)
- Column : Waters symmetry C8(100 x 4.6mm), 5µm
- Temperature : 30°C

- Flow rate : 1mL/min
- Wavelength : 290 nm
- Runtime : 12 minute

4.2 Detection wavelength Selection:

Based on literature and drug information uv analysis performed using methanol as a blank. Uv analysis performed from 200nm to 400nm wavelength. From the spectrum maximum absorbance observed at 216 nm, 251nm and at 289.9 nm. From the literature review and theoretical information wavelength λ =290 selected for proposed method to reduce interference of other impurityor compound which were not shown any absorbance at this wavelength. it was observed that most of organic compound absorb UV light near 216 nm.

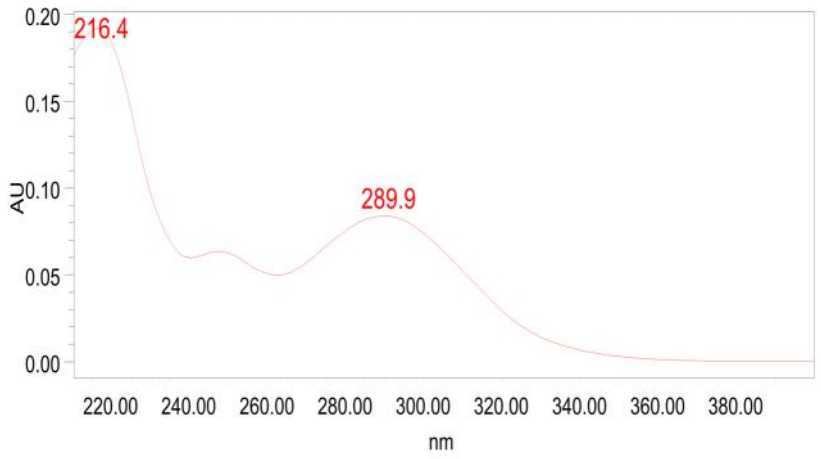


Figure 2:- UV spectrum of dronedarone hydrochloride

5. ANALYTICAL METHOD VALIDATION:

Materials:

The pharmaceutical dosage form of dronedarone hydrochloride was procured from local pharmacy with 400mg label claim, manufactured by Sanofi Aventis pharmaceuticals. HPLC grade methanol and Ortho Phosphoric Acid (OPA) were purchase from Merck india Limited, Mumbai, India. . Analytical Reagent grade hydrochloric acid, sodium hydroxide pellets and hydrogen peroxide solution 30 % (v/v) were purchase from Ranbaxy Fine Chemicals, New Delhi, India. Nylon syringe filters 0.45 µm were purchase

from Millex-HN, Mumbai, India. HPLC grade water was obtained from Milli-Q water purification system.

Instrumentation:

A Waters HPLC system equipped with quaternary pump (TM 600), UV/Vis detector (Waters 2489), controller (Waters 600), Waters inline degasser AF, thermostatic compartment and manual injector with 20 µL loop was utilized for method development and validation. The out put signal was monitored and processed using Empower software.

Chromatographic Conditions:

The column used for the separation was (100 x 4.6mm), Symmetry C_8 (Waters make) packed with 5µm particles. The mobile phase was potassium di hydrogen phosphate buffer and 1mL Tri Ethyl Amine (TEA) (pH 2.5 by OPA, 50mM): Methanol (40:60, v/v). The flow rate was set at 1.0mL/min. The column was maintained at 30° C and the detection was carried out at a wavelength of 290 nm. The injection volume and run time were 20µL and 12min respectively.

Mobile Phase Preparation:

The mobile phase was 50mM potassium dihydrogen phosphate + 1mL Tri ethyl amine (pH=2.5 by Ortho phosphoric acid)- Methanol: (40:60). The buffer solution was prepared by dissolving 6.8gm Potassium dihydrogen phosphate in 1000 mL HPLC grade water. Add about 500 mL of water, 1mL of tri ethyl amine and dilute it up to the mark with water. pH was set 2.5 by ortho phosphoric acid. The buffer solution was filtered through a 0.45 µm nylon membrane (Millipore Pvt. Ltd. Banglore, India). The final mobile phase was prepared by mixing 400mL of buffer solution and 600 mL of methanol in 1000 mL mobile phase bottle and degassed in an ultrasonic bath (Spincotech Pvt. Ltd. Mumbai).

Diluent Preparation:

Water: Methanol (50:50) used as a diluents.

Blank Preparation:

Diluent is used as a blank

Standard Preparation:

To prepare a stock solution (500 µg/mL) for assay, weigh accurately about 25 mg dronedarone hydrochloride reference standard and transfer into 50 mL volumetric flask.

Add 20 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Test Preparation:

To prepare a stock solution (500 µg/mL) for assay, 5 tablets were weighed, crushed and mixed. An aliquot of powder equivalent to the weight of 50 mg dronedarone hydrochloride was accurately weighed and transferred to 100 mL volumetric flask and dissolved in 50 mL of diluents and the mixture was sonicated for 15 min. The content of the flask were than left to return to room temperature and volume was adjusted with the diluents. A 10 mL of this solution was filtered through a 0.45 µm nylon syringe filter.

Pipette out 5 mL of above test stock solution, transrer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Procedure:

Inject blank followed by five replicated injection of standard preparation. Asymmetry of first injection of standard should not more than 2.0. Related standard deviation of replicate standard preparation should not more than 2.0 %. If system suitability pass than make duplicate injection of sample preparation.

Average weight of the tablets:

Randomly selected 5 tablets were weighed accurately (3276.43 mg) and the mean weight was calculated for the same. This mean weight (655.29 mg) was used as average weight through out all experiments**.**

5.1 Specificity:

The evaluation of the specificity of the method was determined against placebo and stress (forced) degradation. The interference of the excipients of the claimed placebo present in the pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of the interference of the degradation products against drug during the forced degradation study.

Blank Preparation:

Diluent is used as a blank.

Standard Preparation:

Weigh accurately about 25.06 mg dronedarone hydrochloride reference standard and transfer into 50 mL volumetric flask. Add 20 mL of diluents to dissolve the substance by sonication and dilute it up to the mark with diluents. The concentration obtained is 501.2 µg/mL of dronedarone hydrochloride.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50.12 µg/mL of dronedarone hydrochloride.

Test Preparation:

Weigh accurately 5 tablet (3282.1 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 82.05 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of dronedarone hydrochloride.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Placebo preparation:

Stock solution: Placebo equivalent to 5 times of average placebo weight was weighed and transferred into 100 ml volumetric flask.70 ml of diluent was added into the volumetric flask and sonicate for 30 minutes with normal hand-shaking. The volumetric flask was cooled to room temperature and diluted to volume with diluent. 25 ml of this solution was filtered through 0.45 µm nylon syringe filter. 5 ml of above placebo stock solution was pipette out and transferred into 50 ml volumetric flask followed by dilute up to volume with diluent.

5.1.1 Forced degradation study:

Stress study was carried out by application of chemical and physical forced degradation. To perform forced degradation study, the drug content equivalent to 25 mg was employed

for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content were allowed to equilibrate to room temperature and diluted with diluent to attain 50 µg/ml concentrations. Pattern of stress (degradation) conditions and preparation for same was described as under:

Acid Degradation:

Acidic degradation study was performed by heating the drug content in 30 ml of 1 N HCl at about 80° C for 1 hour and after cooling to room temperature it was neutralized with 1 N NaOH solution. Further solution was diluted to achieve concentrations 50 µg/ml with diluent.

Alkali Degradation:

Alkaline degradation was performed by heating the drug content in 1 M NaOH at around 80˚ C for 1 hour and then the mixture was neutralized with 1 M HCl. It was further diluted with diluent to achieve 50 µg/ml concentrations.

Oxidative Degradation Study:

Oxidative degradation study was performed by heating the drug content in 3% v/v H2O2 at 80°C for 1 hour then diluted to 50µg/ml with diluent.

Thermal Degradation Study:

Thermal degradation study was performing by keeping powdered drug content at around 80˚C for 72 hour. After this it was allowed to come at room temperature.

Photolytic Degradation Study:

Photolytic degradation study was performed by exposing drug content in sun-light for 72 hour, further it diluted to 50 µg/ml using diluent.

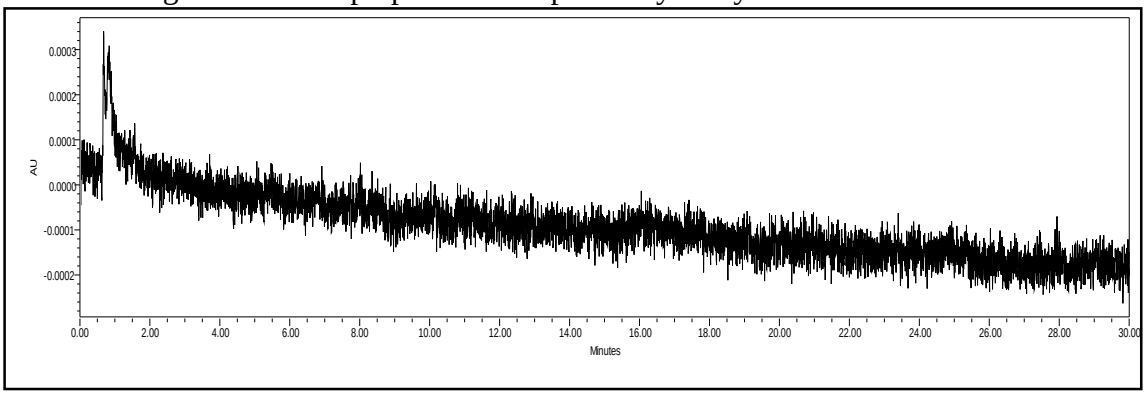
Placebo and Blank preparation:

Placebo preparation and blank preparation was also performed for all degradation to identify the peaks which arise due to placebo and blank. Placebo and blank was subjected under same all stress condition as that of sample.

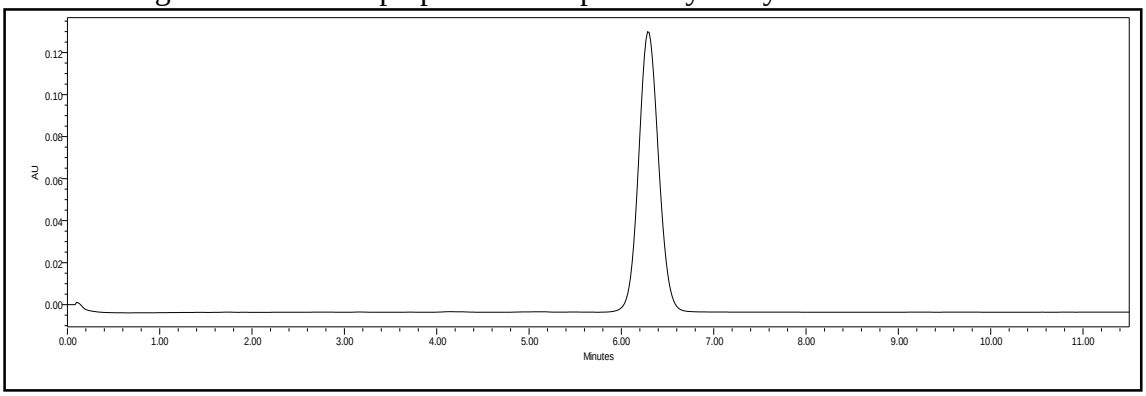
Table 1:- Chromatographic sequence for Specificity study

No.	Description	Injection Replicate	Remarks
1	Blank	1	As such
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank preparation of acidic stress	1	Acidic Forced Degradation
6	Placebo preparation of acidic stress	1	
7	Test preparation of acidic stress	2	
8	Bracketing Standard	1	
9	Blank preparation of alkali stress	1	Alkali Forced Degradation
10	Placebo preparation of alkali stress	1	
11	Test preparation of alkali stress	2	
12	Bracketing Standard	1	
13	Blank preparation of oxidative stress	1	Oxidative Forced Degradation
14	Placebo preparation of oxidative stress	1	
15	Test preparation of oxidative stress	2	
16	Bracketing Standard	1	
17	Blank preparation of thermal stress	1	Thermal Forced Degradation
18	Placebo preparation of thermal stress	1	
19	Test preparation of thermal stress	2	
20	Bracketing Standard	1	
21	Blank preparation of photolytic stress	1	Photolytic Forced Degradation
22	Placebo preparation of photolytic stress	1	
23	Test preparation of photolytic stress	2	
24	Bracketing Standard	1	

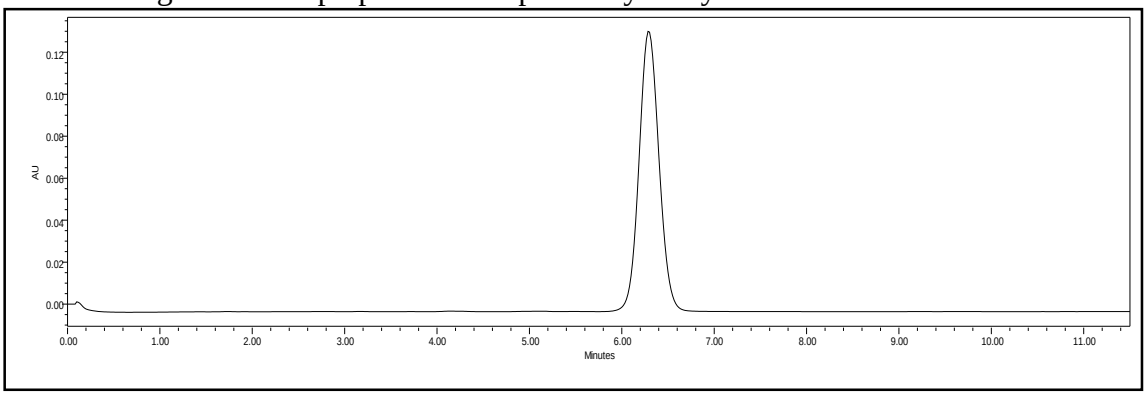
Chromatogram of blank preparation of specificity study:



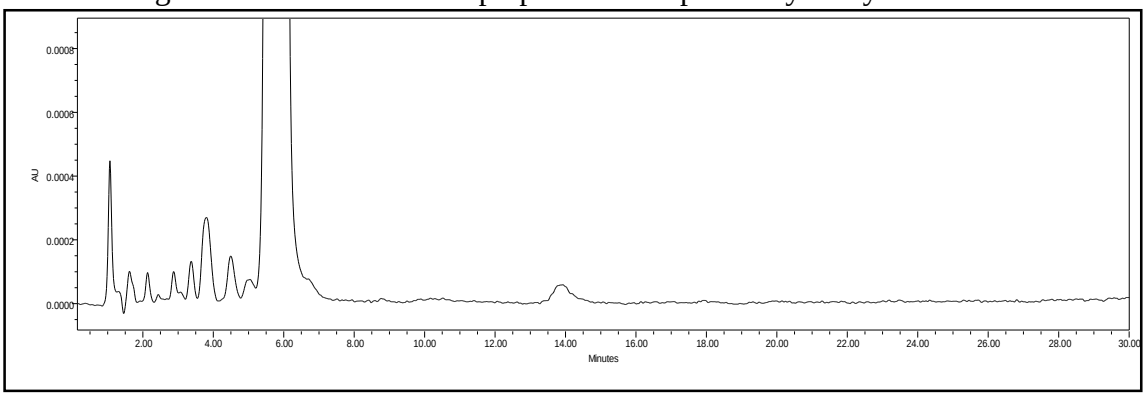
Chromatogram of standard preparation of specificity study:



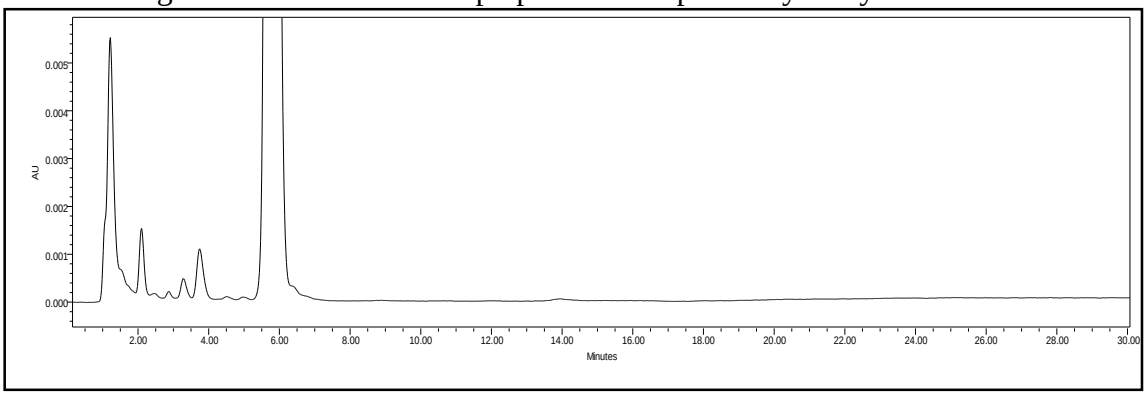
Chromatogram of test preparation of specificity study:



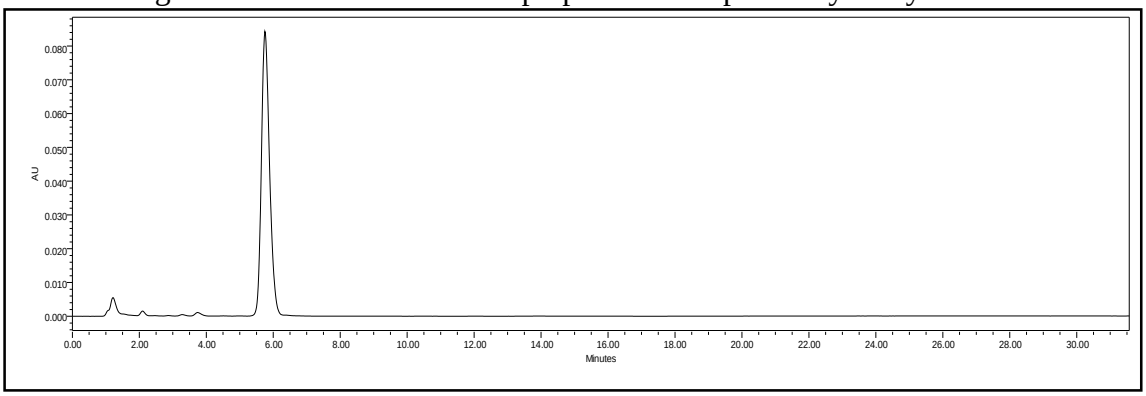
Chromatogram of acidic stress test preparation of specificity study:



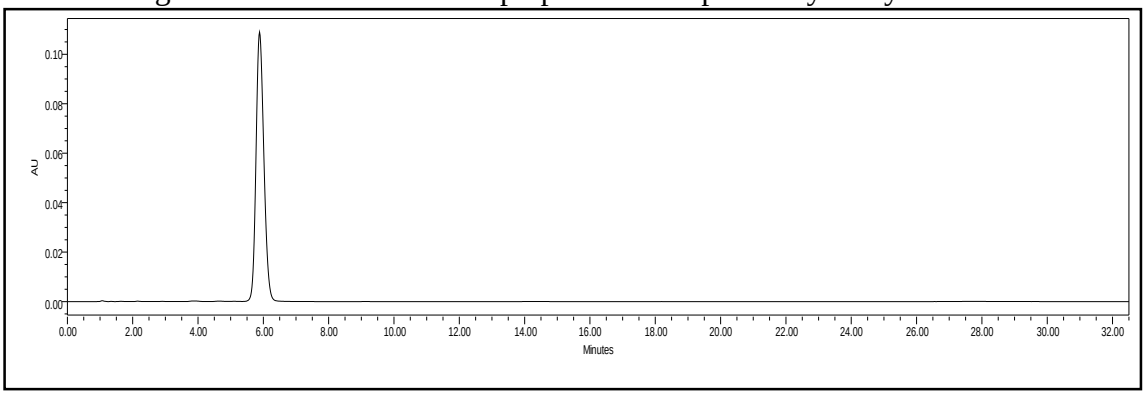
Chromatogram of alkali stress test preparation of specificity study:



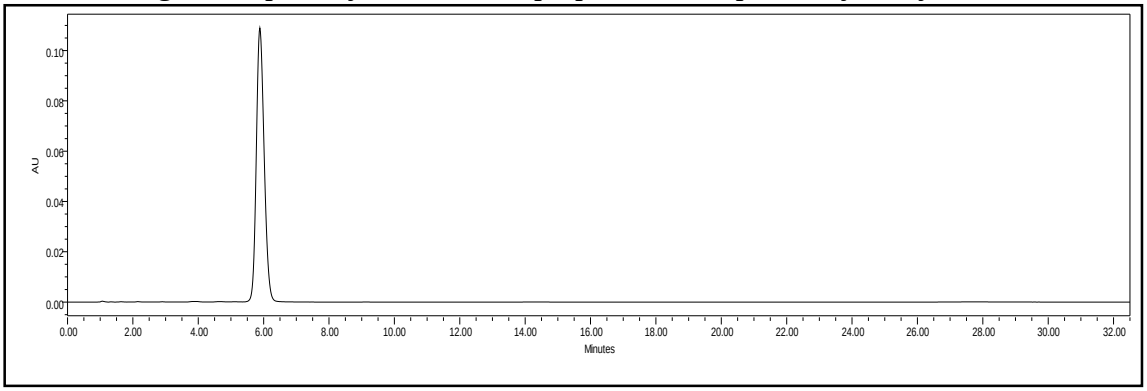
Chromatogram of oxidative stress test preparation of specificity study:



Chromatogram of thermal stress test preparation of specificity study:



Chromatogram of photolytic stress test preparation of specificity study:



Observations:

(1) Any interference was not observed from blank or placebo to the peak of interest, in addition to this peak purity was also within the acceptance criteria proved by the photo diode detector.

(2) From the above chromatogram it can be conclude that there is no interference of any degradation product to the peak of interest and impurity has been generated by each stress condition.

Table-2: Degradation result of stress condition

Degradation Condition	Acidic	Alkali	Oxidative	Thermal	Photolytic
%Degradation	12%	32%	16%	2.8%	0.3%

5.2 Linearity and Range:

The linearity plot was prepared with 8 concentration levels (40, 60, 80, 100, 120, 140, 160 and 180 µg/ml of Dronedarone). These concentration levels were respectively corresponding to 40, 60, 80, 100, 120, 140, 160 and 180% of standard solution concentration. The peak areas vs. concentration data were evaluated by linear regression analysis.

Standard solution preparation:

25.02 mg of Dronedarone working standard was accurately weighed and transferred into 50 ml volumetric flask. 20 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was made up with diluent. The concentration obtained was 500.4 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Linearity Standard Solution Preparation:

Stock Solution:

25.04 mg of Dronedarone working standard was accurately weighed and transferred into 50 ml volumetric flask. 20 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was made up with diluent. The concentration obtained was 500.8 µg/ml of Dronedarone.

Linearity Level 1 (40%):

2 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 20.03 µg/ml of Dronedarone.

Linearity Level 2 (60%):

3 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration is 30.05µg/ml of dronedarone.

Linearity Level 3 (80%):

4 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 40.06 µg/ml of Dronedarone.

Linearity Level 4 (100%):

5 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 50.08 µg/ml of Dronedarone.

Linearity Level 5 (120%):

6 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 60.10 µg/ml of Dronedarone.

Linearity Level 6 (140%):

7 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 70.11 µg/ml of Dronedarone.

Linearity Level 7 (160%):

8 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 80.13 µg/ml of Dronedarone.

For each linearity level, the solution was injected in duplicate. Linearity was evaluated by linear regression analysis.

Linearity Level 8 (180%):

9 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 90.14 µg/ml of Dronedarone.

For each linearity level, the solution was injected in duplicate. Linearity was evaluated by linear regression analysis.

Table 3: Sequence of Linearity and range study:

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Linearity level-1 (40%)	2
4	Linearity level-1 (60%)	2
5	Linearity level-1 (80%)	2
6	Linearity level-1 (100%)	2
7	Linearity level-1 (120%)	2
8	Linearity level-1 (140%)	2
9	Linearity level-1 (160%)	2
10	Bracketing Standard	1

Table 4:-Evaluation of linearity of dronedarone:

Linearity Level	% of Level	Concentration (µg/ml)	Mean Area
1	40	20	824044.5
2	60	30	1250170
3	80	40	1658594
4	100	50	2072383
5	120	60	2464944
6	140	70	2902003
7	160	80	3316185
Correlation Co-efficient			0.999
Slope			22511
Intercept			24070

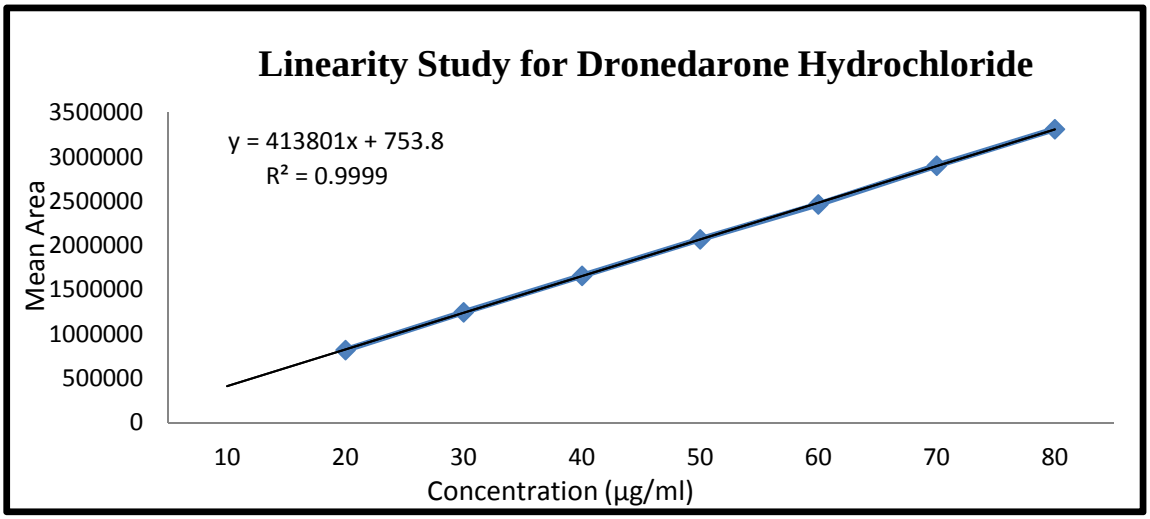
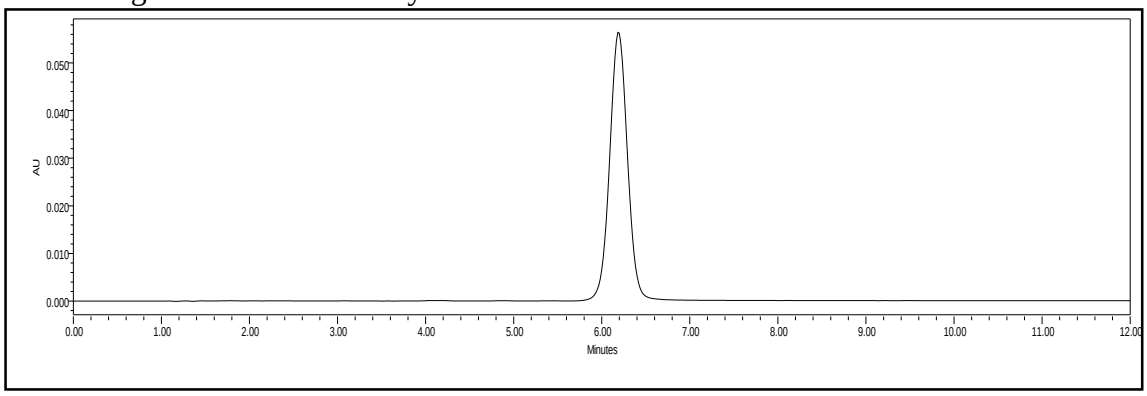


Figure 3: Evaluation of linearity of dronedarone

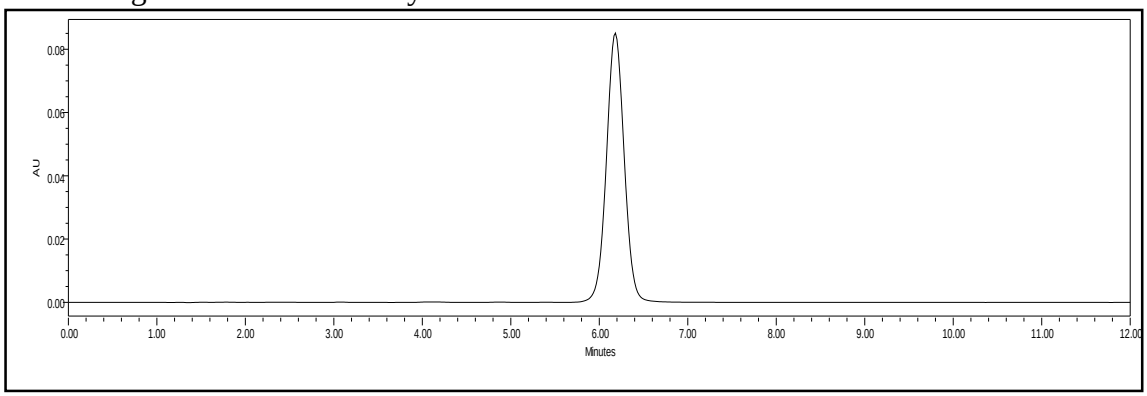
Table 5: Summary of Linearity and range study:

Observation				
Data for Standard Preparation				
Replicate	**Area**		**Standard Weight**	**25.02**
1	2072387		Standard Potency	99.48
2	2073202			
3	2075324			
4	2073489			
5	2074321			
Average	2073744.6			
Stdev	1121.33			
%RSD	0.05			
Data for Leniarity Level Preparation				
Linearity Level	Replicate	Area	Mean Area	
Level-1 (40%)	1	824058	824044.5	
	2	824031		
Level-2 (60%)	1	1250203	1250170	
	2	1250137		
Level-3 (80%)	1	1658467	1658594	
	2	1658721		
Level-4 (100%)	1	2072489	2072383	
	2	2072277		
Level-5 (120%)	1	2464823	2464944	
	2	2465065		
Level-6 (140%)	1	2902037	2902003	
	2	2901969		
Level-7 (160%)	1	3316114	3316185	
	2	3316256		

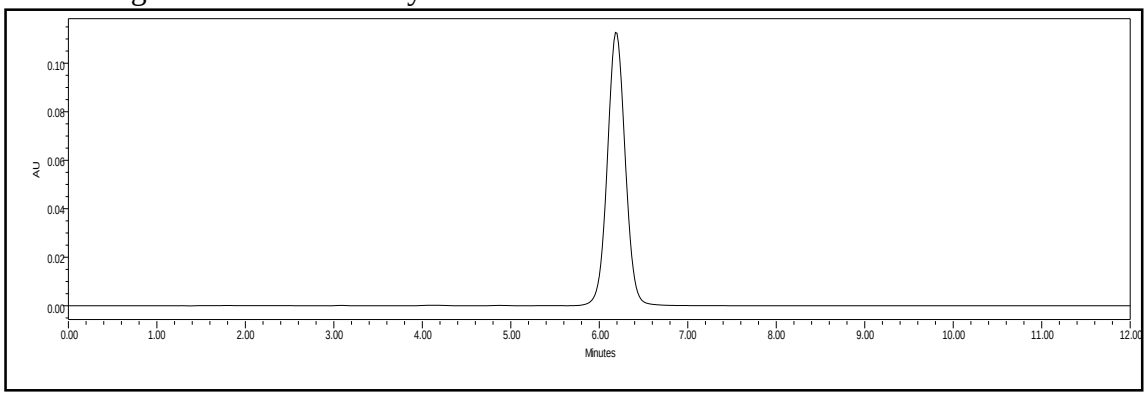
Chromatogram of 40% Linearity level:



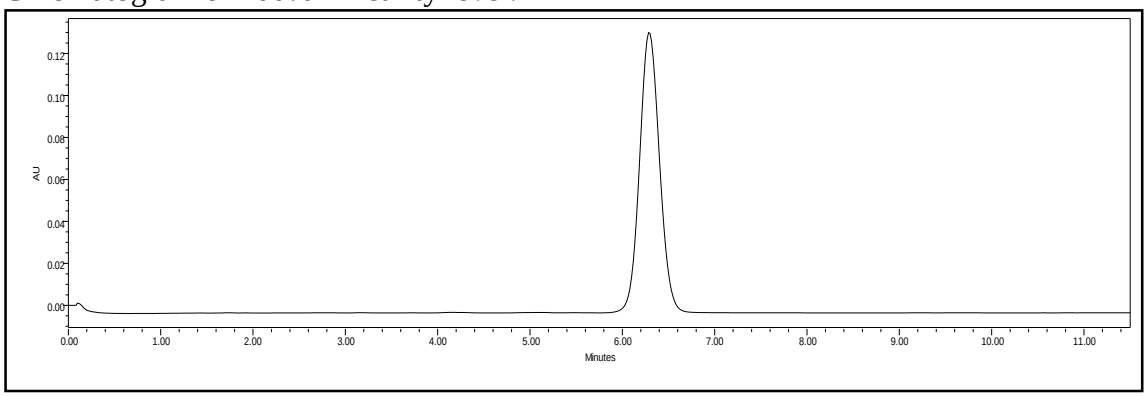
Chromatogram of 60% Linearity level:



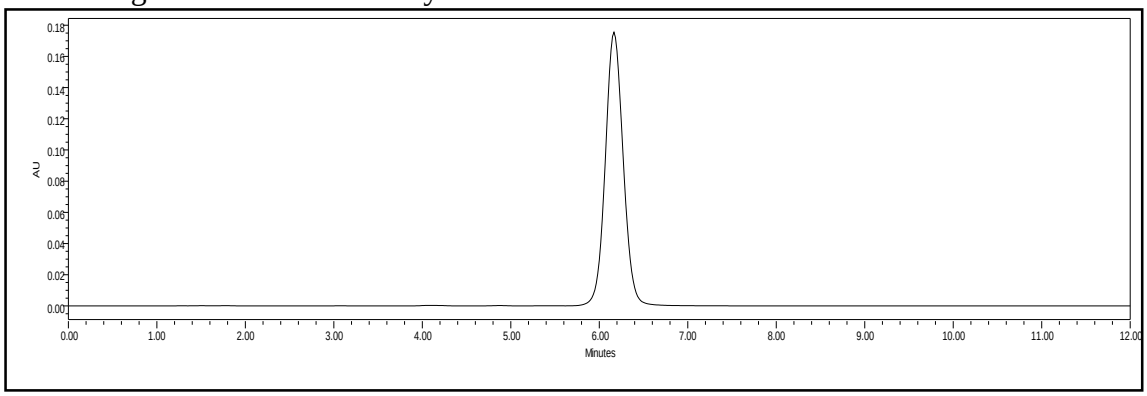
Chromatogram of 80% Linearity level:



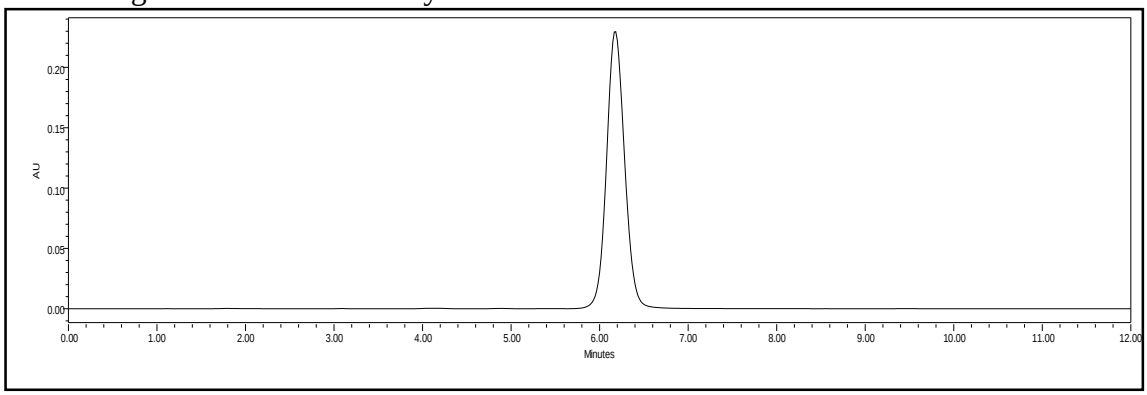
Chromatogram of 100% Linearity level:



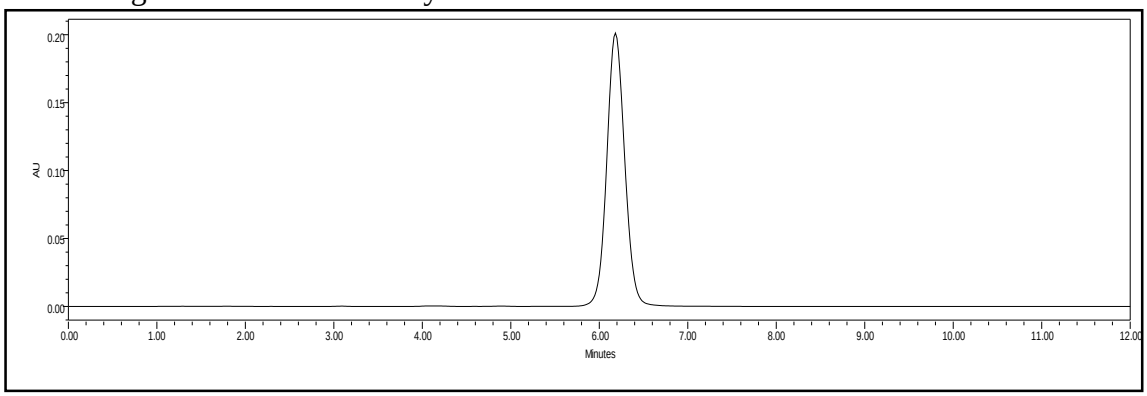
Chromatogram of 120% Linearity level:



Chromatogram of 140% Linearity level:



Chromatogram of 160% Linearity level:



Correlation coefficient of the linearity study was found to R^2 = 0.999 with linear regression equation y = 41380x + 753.8, which proves the method is highly linear over the working range 20 – 160 µg/ml.

5.3 Limit Of Detection and Limit of Quantitation:

LOD is the lowest amount of the drug content which can be detected by the proposed method while LOQ is the lowest amount which can be quantified by the method. The guideline suggest minimum signal to noise ratio (S/N) more than 3.3 for LOD and more

than 10 for LOQ. On the basis of linearity data theoretically it can be also calculated by the given formula,

$$LOD = 3.3\ \sigma/S \qquad LOQ = 10\ \sigma/S$$

Where σ = Residual Standard Deviation of regression line and S = Slope of regression line.

LOQ value is precised by six replicate injections and checked for linear response with respect to other linearity levels by extended linearity curve.

For LOD and LOQ study, blank, standard preparation, LOD preparation and LOQ preparation was prepared as under:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.07 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.4 µg/ml of Dronedarone.

LOD and LOQ solution:

Solution-A: 25.00 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 500 µg/ml of Dronedarone. 5 ml of this solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.0 µg/ml of Dronedarone. 5 ml of this solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 5.0 µg/ml of Dronedarone. This solution is designated as Solution-A.

LOD preparation:

1 ml of above Solution-A was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 0.1 µg/ml of Dronedarone. 5 ml of this solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 0.01 µg/ml of Dronedarone.

LOQ preparation:

3 ml of above Solution-A was pipette out and transferred into 50 ml volumetricflask followed by diluted to volume with diluent. The concentration obtained is 0.3 µg/ml of Dronedarone. 5 ml of this solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 0.03 µg/ml of Dronedarone.

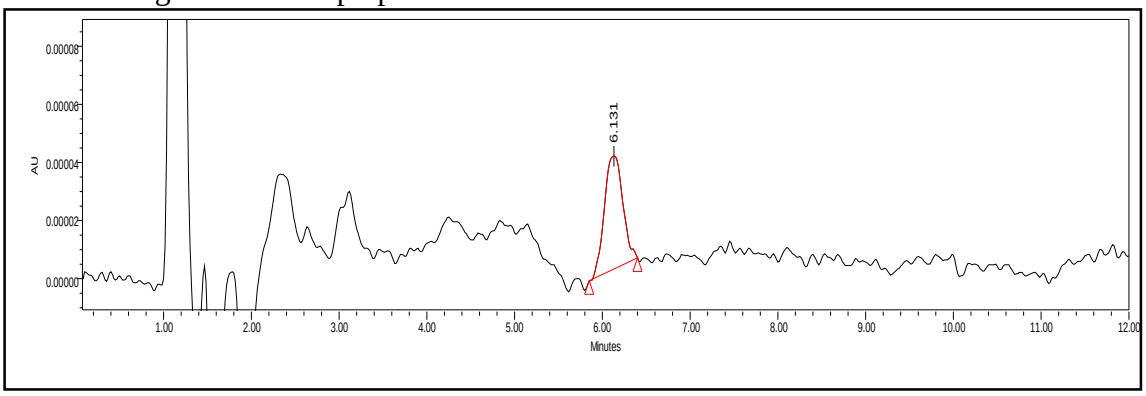
Table 6: Sequence of LOD and LOQ study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Blank	1
4	LOD	2
5	LOQ	6
6	Bracketing Standard	1

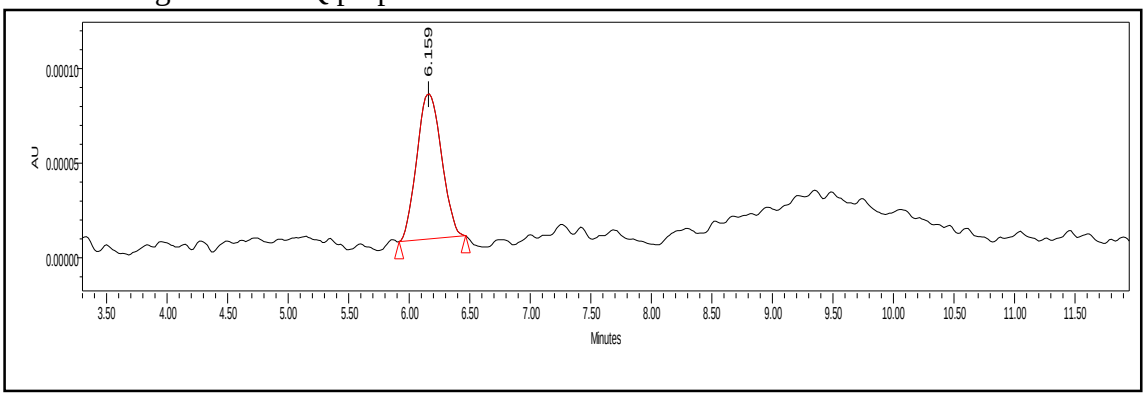
Table 7: Summary of LOQ study

Observation				
Data for Standard Preparation			***Data for LOQ Preparation***	
Replicate	**Area**		**Replicate**	**Area**
1	2072423		1	1132
2	2073467		2	1090
3	2072285		3	1108
4	2073552		4	1048
5	2076288		5	1120
Average	2073603		6	1042
Stdev	1609.34		Average	1090
%RSD	0.08%		STDEV	37.57%
			%RSD	3.45%

Chromatogram of LOD preparation:



Chromatogram of LOQ preparation:



LOQ of the analytical method can evaluated by establish linearity up to LOQ value. Hence linearity study is extended to LOQ value.

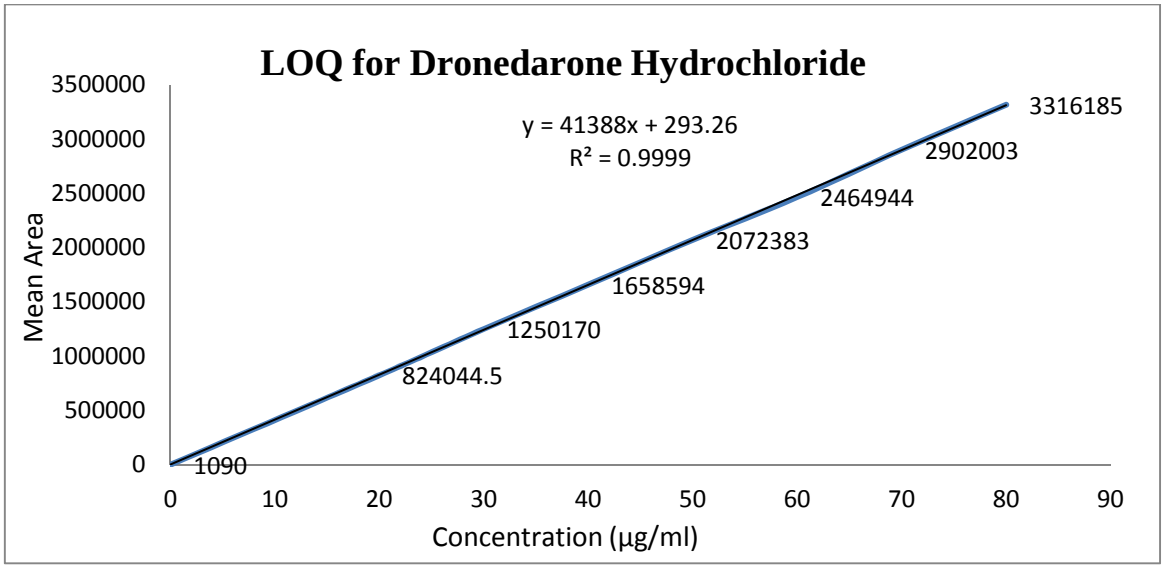


Figure 4: Extended linearity study up to LOQ level

Table 8: Summary of LOQ study by evaluating linearity up to LOQ concentration

Linearity Level	% of Level	Concentration (µg/ml)	Mean Area
1	LOQ	0.03 µg/ml	1090
2	40	20	824044.5
3	60	30	1250170
4	80	40	1658594
5	100	50	2072383
7	120	60	2464944
8	140	70	2902003
9	160	80	3316185
Correlation Co-efficient			0.999
Slope			41388
Intercept			293.2

All the results of LOD and LOQ data were within the acceptance criteria, hence it can be conclude that the LOD and LOQ of the method was 0.01 µg/ml and 0.3 µg/ml respectively which correspond to 0.02% and 0.05% of working concentration.

5.4 Precision:

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated.

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different make of raw materials under same experimental condition. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated. Overall assay value of method precision and intermediate precision was compared and % difference and overall % relative standard deviation was calculated.

For method precision, blank, standard preparation and six sets of test preparations was prepared as per method as under:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.02 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 500.4 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Test preparation:

Weigh accurately 5 tablet (3268.4 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 81.86 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of dronedarone.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

The same procedure was used for preparing the six Test preparation Sets. Same approach was applied for the intermediate precision study on the second day with different analyst.

Table 9: Sequence of precision study:

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Test preparation (Set-1)	2
4	Test preparation (Set-2)	2
5	Test preparation (Set-3)	2
6	Test preparation (Set-4)	2
7	Test preparation (Set-5)	2
8	Test preparation (Set-6)	2
9	Bracketing Standard	1

Table 10: Summary of precision study

Observation					
Data for Standard preparation					
Replicate	**Area**		Standard Weight	25.02	
1	2094365		Standard Potency	99.48	
2	2082248				
3	2078842				
4	2086533				
5	2073255				
Average	2083048.6				
Stdev	7975.79				
%RSD	0.38				
Data for Test preparation					
Set No.	Replicate	Area	Mean Area	Weight of Sample	%Assay
1	1	2096441	2093052	81.86	100.04
	2	2089663			
2	1	2078114	2076246	81.38	99.83
	2	2074378			
3	1	2085568	2086294.5	81.64	99.99
	2	2087021			
4	1	2096511	2095499	81.78	100.26
	2	2094487			
5	1	2079338	2078807.5	81.34	100.00
	2	2078277			
6	1	2092588	2092030.5	81.88	99.97
	2	2091473			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 11: Summary of Intermediate precision study

Observation

Data for Standard preparation

Replicate	**Area**
1	2088357
2	2089224
3	2081366
4	2092451
5	2094787
Average	2089237
Stdev	5095.57
%RSD	0.24

Standard Weight	**25.08**
Standard Potency	**99.48**

Data for Test preparation

Set No.	**Replicate**	**Area**	**Mean Area**	**Weight of Sample (mg)**	**%Assay**
1	1	2084554	2084171	81.48	100.08
	2	2083787			
2	1	2085366	2085056	81.50	100.10
	2	2084746			
3	1	2082986	2082915	81.32	100.22
	2	2082843			
4	1	2087478	2087200	81.63	100.04
	2	2086921			
5	1	2084169	2084246	81.41	100.17
	2	2084322			
6	1	2085264	2084840	81.47	100.13
	2	2084416			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

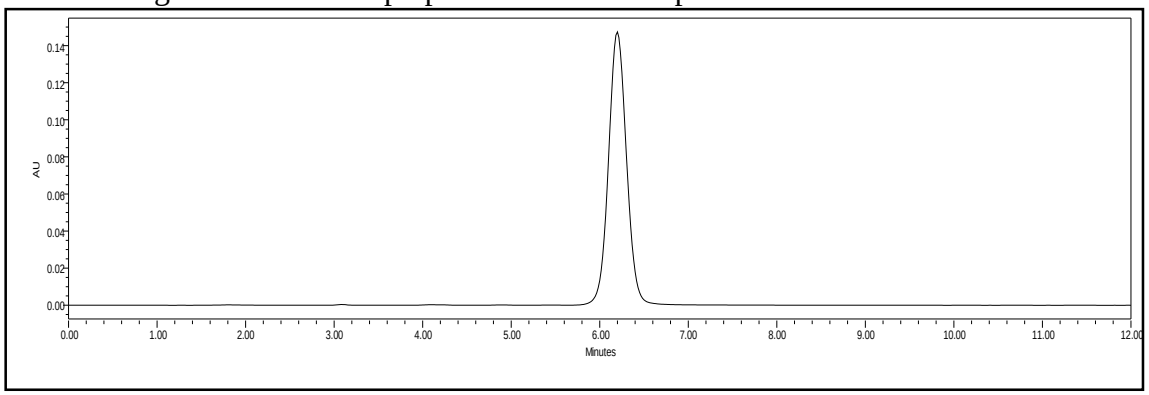
W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

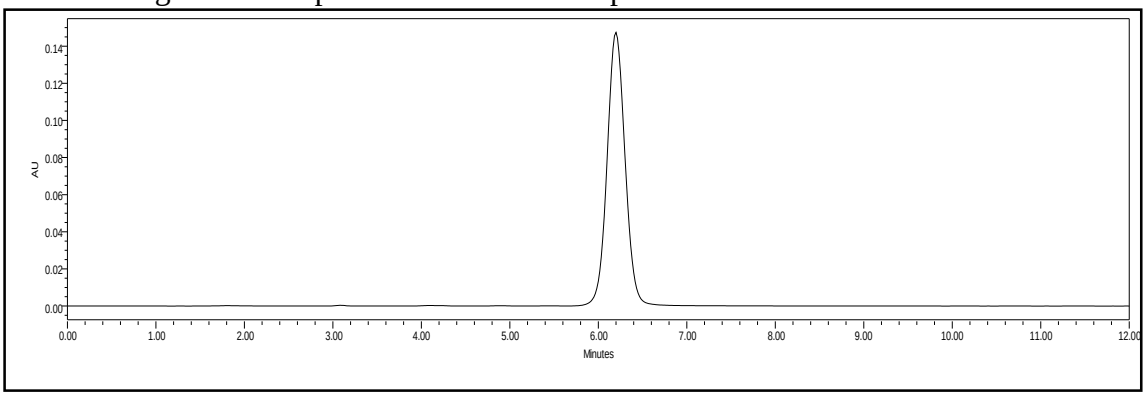
LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

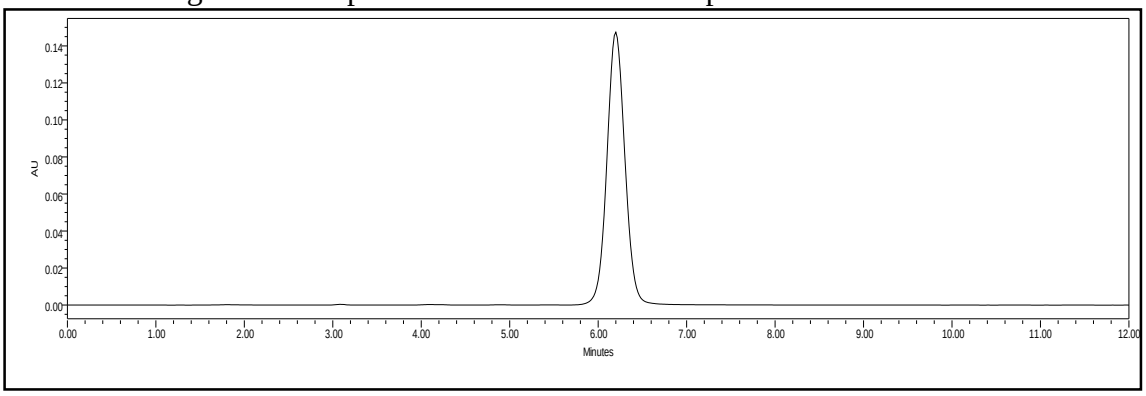
Chromatogram of Standard preparation in method precision:



Chromatogram of sample solution in method precision:



Chromatogram of sample solution in intermediate precision:



Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence from all the observation it can conclude that the proposed method is highly precise.

5.4 Accuracy:

This Experiment can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration level, 3 sets were prepared and injected in duplicate.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.02 mg of dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 500.4 µg/ml of dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Sample preparations for accuracy levels are as under:

Accuracy level 1 (50%):

Test stock solution:

25 mg of dronedarone reference standard was accurately weighed and transferred into 100 ml volumetric flask. 1082.1 mg placebo (equivalent of 5 average placebo weights) was weighed and transferred into the same 50 ml volumetric flask. Around 40 ml of diluent added into the volumetric flask. The volumetric flask was proceeding for sonication of 30 minutes with normal handshaking. Then, the flask was cooled to room temperature and diluted to volume with diluent. 10 ml of this solution was filtered through 0.45 µm nylon syringe filter. The concentration obtained was 500 µg/ml of dronedarone.

5 ml of above test stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50 µg/ml of Dronedarone. The same procedure was applied for preparing the three sets.

Accuracy level 2 (100 %):

Test stock solution:

50 mg of Dronedarone reference standard was accurately weighed and transferred into 100 ml volumetric flask. 1082.1 mg placebo (equivalent of 5 average placebo weights) was weighed and transferred into the same 50 ml volumetric flask. Around 40 ml of diluent added into the volumetric flask. The volumetric flask was proceeding for sonication of 30 minutes with normal handshaking. Then, the flask was cooled to room temperature and diluted to volume with diluent. 10 ml of this solution was filtered through 0.45 µm nylon syringe filter. The concentration obtained was 500 µg/ml of Dronedarone.

5 ml of above test stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50 µg/ml of Dronedarone. The same procedure was applied for preparing the three sets.

Accuracy level 3 (150 %):

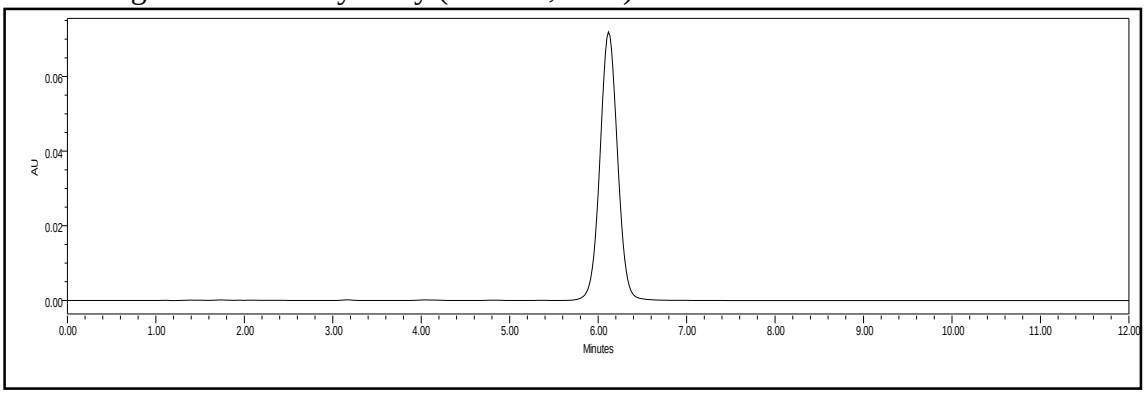
Test stock solution:

75 mg of Dronedarone reference standard was accurately weighed and transferred into 100 ml volumetric flask. 1082.1 mg placebo (equivalent of 5 average placebo weights) was weighed and transferred into the same 50 ml volumetric flask. Around 40 ml of

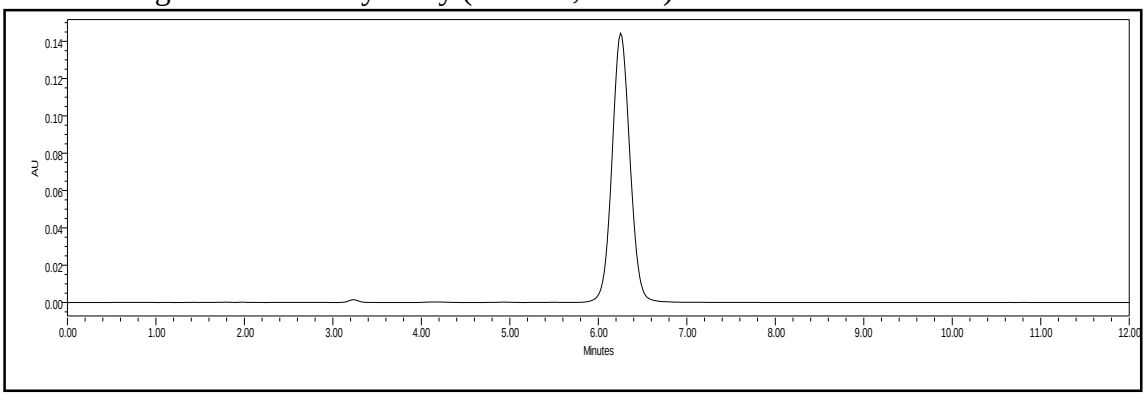
diluent added into the volumetric flask. The volumetric flask was proceeding for sonication of 30 minutes with normal handshaking. Then, the flask was cooled to room temperature and diluted to volume with diluent. 10 ml of this solution was filtered through 0.45 µm nylon syringe filter. The concentration obtained was 500 µg/ml of Dronedarone.

5 ml of above test stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50 µg/ml of Dronedarone. The same procedure was applied for preparing the three sets.

Chromatogram of accuracy study (Level-1, 50%):



Chromatogram of accuracy study (Level-2, 100%)



Chromatogram of accuracy study (Level-3, 150%)

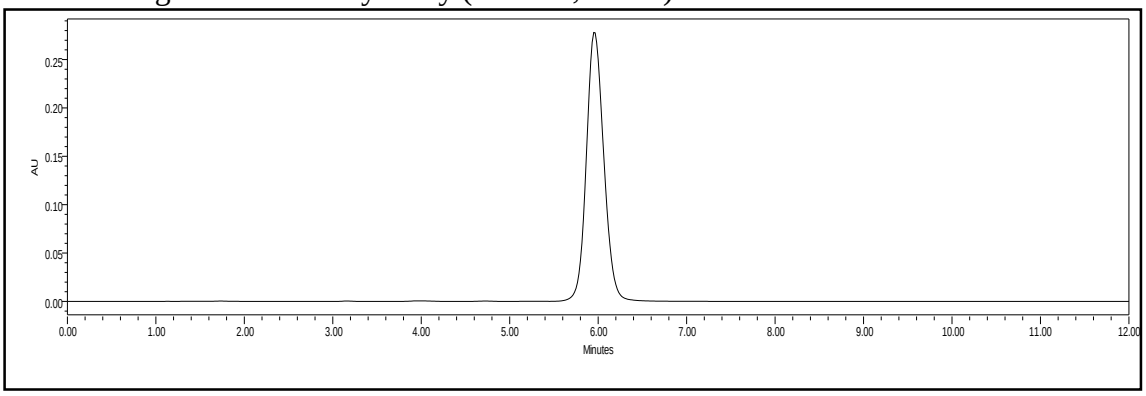


Table 13: Sequence of Accuracy Study

Sr. No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Accuracy level-1 preparation: Set-1	2
4	Accuracy level-1 preparation: Set-2	2
5	Accuracy level-1 preparation: Set-3	2
6	Accuracy level-2 preparation: Set-1	2
7	Accuracy level-2 preparation: Set-2	2
8	Accuracy level-2 preparation: Set-3	2
9	Accuracy level-3 preparation: Set-1	2
10	Accuracy level-3 preparation: Set-2	2
11	Accuracy level-3 preparation: Set-3	2
12	Bracketing Standare	1

Table 14: Summary of accuracy study

Observation				
Data for Standard preparation				
Replicate	**Area**		Standard Weight	25.07
1	2079664		Standard Potency	99.48
2	2081477		Standard Conc.	25.07
3	2081811			
4	2082196			
5	2083785			
Average	2081787			
Stdev	1480.13			
%RSD	0.07%			
Data for Test preparation				
Accuracy Level	Set No	Replicate	Area	Mean Area
1(50%)	1	1	1026833	1026749
		2	1026665	
	2	1	1051260	1051294
		2	1051328	
	3	1	1071762	1071679
		2	1071596	
2(100%)	1	1	2083938	2083867
		2	2083796	
	2	1	2057629	2057658
		2	2057687	
	3	1	2077079	2077210
		2	2077341	
3(150%)	1	1	3166769	3166363
		2	3165957	
	2	1	3106334	3106456
		2	3106578	
	3	1	3147141	3147226
		2	3147311	

Table 15: Summary of accuracy study:

Accuracy (Recovery) Study							
Accuracy Level	**Set No**	**Amount added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
I (50%)	1	24.56	24.68	100.48	100.43	0.39	0.26
	2	25.12	25.27	100.59			
	3	25.18	25.76	101.21			
II (100%)	1	50.46	50.09	99.27	99.22	0.16	0.16
	2	49.79	49.46	99.35			
	3	50.43	49.93	99.04			
II (150%)	1	75.68	76.11	100.58	100.54	0.05	0.08
	2	74.67	74.67	100.56			
	3	75.28	75.65	100.49			

Calculation formulas for recovery study are as under:

$$\text{Amount added (µg/ml)} = \frac{Wt.taken}{Volume\,1} x \frac{Volume\,2}{Volume\,3} x\,1000$$

Where, Volume = Dilution given for preparing the solution.

$$\text{Amount found (µg/ml)} = \frac{Mean\ area\ of\ test\ preparation}{Average\ area\ of\ standard\ preparation} x\ standard\ \text{conc.}$$

$$\%\ \text{Recovery} = \frac{Amount\ found}{Amount\ added} x\,100$$

From the all above data it has been proven that the % recovery is within the limit of 98 to 102 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2 % .Hence this suggest that proposed method is highly accurate.

5.5 Robustness:

Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate, change in proportions of Buffer-Methanol (42:58 and 38:62, v/v), Change in temperature and change in column-lot.

5.5.1 Change in flow rate:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.06 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.2 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Test preparation:

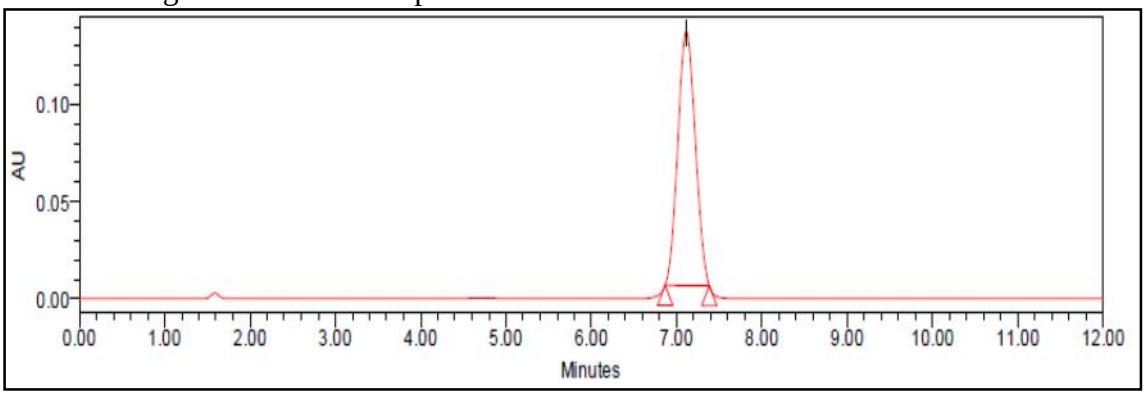
Weigh accurately 5 tablet (3268.4 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 81.82 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Table 16: Sequence for flow rate robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Flow rate : 0.9 mL/min
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Flow rate: 1.1 mL/min
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of robustness parameter flow rate 0.9ml/min



Chromatogram of robustness parameter flow rate 1.1 ml/min:

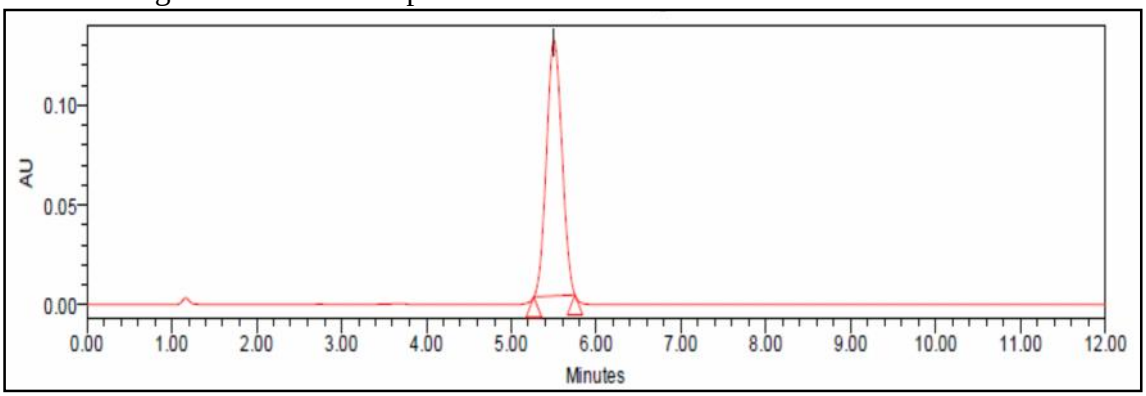


Table 17: Summary for flow change parameter of robustness study:

At 0.9 mL/min flow rate		**At 1.1 mL/min flow rate**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	2201569	1	1923378
2	2201344	2	1927433
3	2202748	3	1928524
4	2201228	4	1920562
5	2201737	5	1920241
Mean	2201725	**Mean**	1924028
Std.dev.	604.8	**Std.dev.**	3827.04
%RSD	0.03%	**%RSD**	0.20%
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	2183624	1	1911044
2	2184098	2	1910580
Mean	2183861	**Mean**	1910812
Standard wt. (mg)	25.06	Standard wt. (mg)	25.06
Test wt. (mg)	81.82	Test wt. (mg)	81.84
Label claim	400	Label claim	400
Average wt. (mg)	655.29	Average wt. (mg)	655.29
%Assay	99.02%	%Assay	99.12%

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

5.5.2 Change in mobile phase composition:

In this experiment the test samples were analyzed at the mobile phase proportion of (Buffer:Methanol) 42:58 and 38:62 v/v each and the results were observed in terms of assay value.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.07 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.4 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Test preparation:

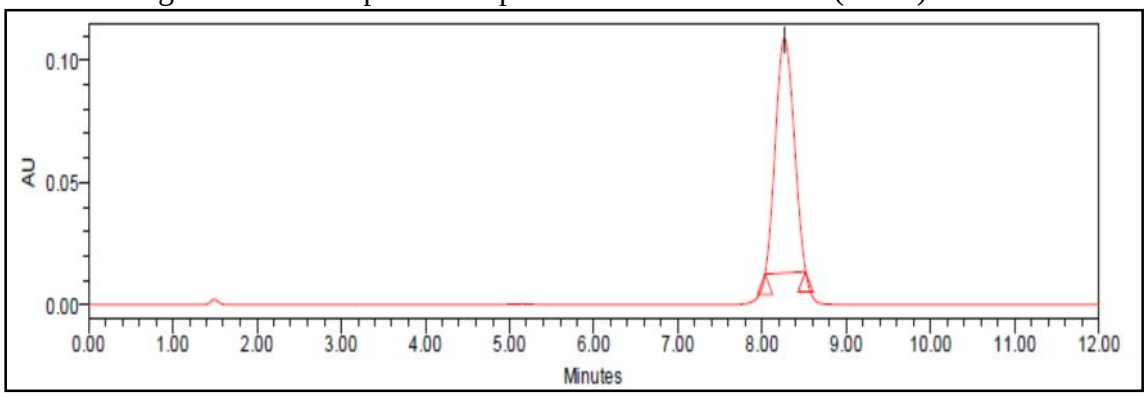
Weigh accurately 5 tablet (3268.4 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 81.84 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of Dronedarone.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Table 18: Sequence for change in composition robustness study:

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Buffer;Methanol (38:62)
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Buffer;Methanol (42:58)
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of mobile phase composition Buffer:Methanol (42:58):



Chromatogram of mobile phase composition Buffer:Methanol (38:62):

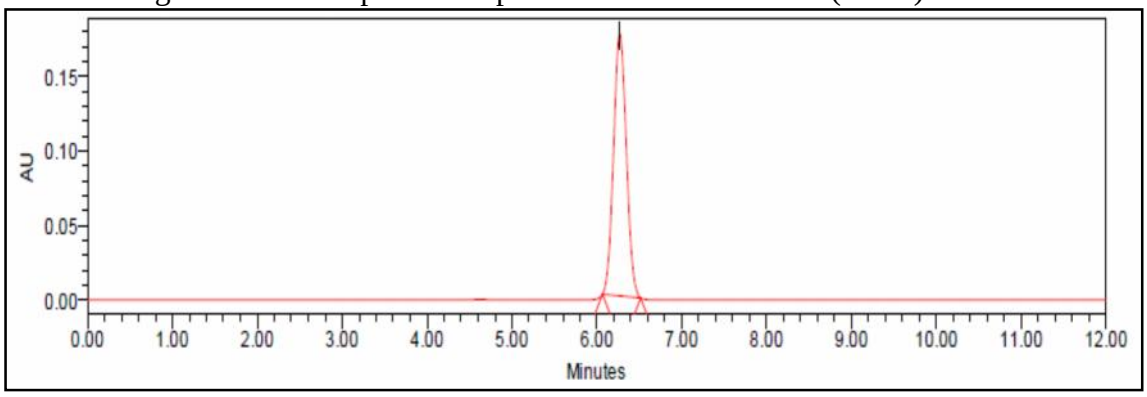


Table 19: Summary for change mobile phase composition parameter

Buffer:Methanol (38:62)		**Buffer:Methanol (42:58)**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	1982262	1	2116855
2	1985889	2	2114229
3	1986395	3	2112474
4	1980256	4	2114569
5	1987303	5	2114877
Mean	1984421	**Mean**	2115601
Std.dev.	3015.2	**Std.dev.**	2854.0
%RSD	0.15%	**%RSD**	0.13%
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	1967765	1	2101967
2	1963101	2	2103157
Mean	1965433	**Mean**	2102562
Standard wt. (mg)	25.07	Standard wt. (mg)	25.07
Test wt. (mg)	81.84	Test wt. (mg)	81.84
Label claim	400	Label claim	400
Average wt. (mg)	655.29	Average wt. (mg)	655.29
%Assay	98.89%	%Assay	99.23%

The calculation formula for th1e determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

5.5.3 Change column lot:

In this parameter, column used in analytical method was changed to different lot. Sample was assayed by changing the lot of column.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.08 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.2 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Test preparation:

Weigh accurately 5 tablet (3268.4 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 81.80 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of Dronedarone.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Table 20: Sequence for change in composition robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Change column lot
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	

Chromatogram of robustness parameter column change

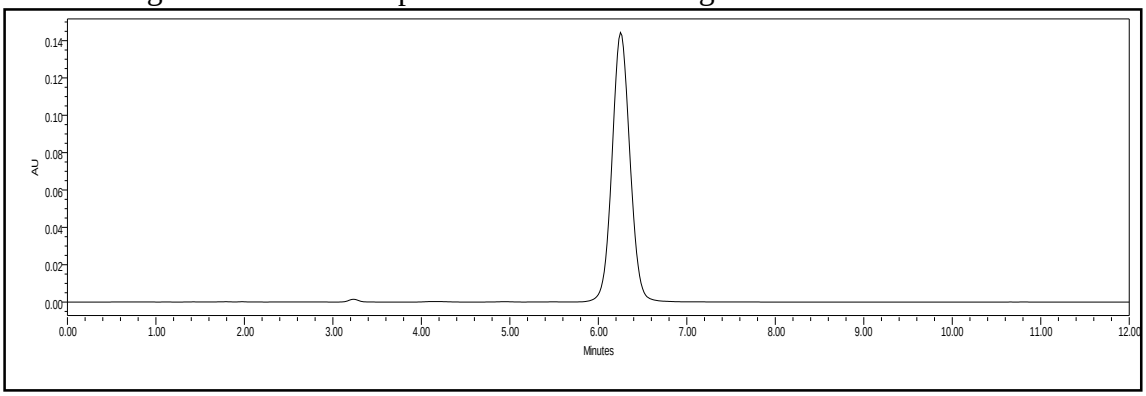


Table 21: Summary for column change parameter of robustness study:

Column lot change	
Data for standard preparation	
Replicate	**Area**
1	2079662
2	2080587
3	2077903
4	2076311
5	2082029
Mean	2079298
Std.dev.	2242.8
%RSD	0.11%
Data for Test preparation	
Replicate	**Area**
1	2064226
2	2068816
Mean	2066521
Standard wt. (mg)	25.08
Test wt. (mg)	81.80
Label claim	400
Average wt. (mg)	655.29
%Assay	99.32%

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{AT}{AS} x \frac{W_1}{50} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 22: Summary of robustness study

Summary of Robustness Study				
Robust Condition	**% Assay**	**Retention time (min.)**	**System Suitability**	
			Theoretical Plates	**Asymmetry**
Flow Change 0.9 ml/min	99.02	5.7	5650	1.29
Flow Change 1.1 ml/min	99.12	7.1	5274	1.25
MP Proportion Change 38:62	99.89	4.9	5284	1.25
MP Proportion Change 42:58	99.23	8.3	5359	1.20
Column Lot Change	99.32	6.2	5642	1.26

The data and the chromatogram given above suggest that there is no considerable influence of the change in flow rate, mobile phase composition and column lot change on the result of the analysis by this method or on chromatographic suitability of this method. Hence, it can be conclude from this experiment that the method is highly robust.

5.6 Solution stability study:

Solution stability period for the solutions of standard preparation and test preparation was evaluated. The solutions were stored at 5° C and ambient temperature without protection against light and tested at interval of 6, 12, 24, 36, and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

5.6.1 Stage of solution stability: Initial

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: 25.02 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric

flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 500.4 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Test preparation:

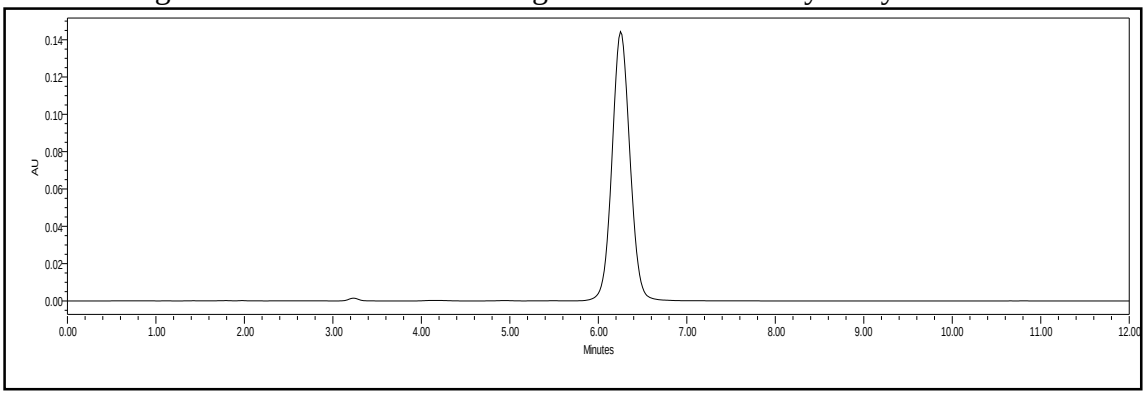
Weigh accurately 5 tablet (3268.4 mg) of dronedarone and crush homogeneously with mortal pistal. Weigh accurately 81.81 mg of crushed powder and transfer into 100 ml volumetric flask. Added 70 ml diluents in volumetric flask. The volumetric flask proceeds for sonication for 30 minutes with normal hand shaking. Then, the flask cooled to room temperature and dilute to volume with diluents. 25 ml of this solution was filtered through 0.45µm nylon syringe filter. The concentration obtained is about 500 µg/ml of Dronedarone.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of dronedarone hydrochloride.

Table 23: Sequence for change in composition solution stability study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Test Preparation	2
4	Bracketing Standard	1

Chromatogram of standard of initial stage of solution stability study:



Chromatogram of sample preparation of initial stage of solution stability study:

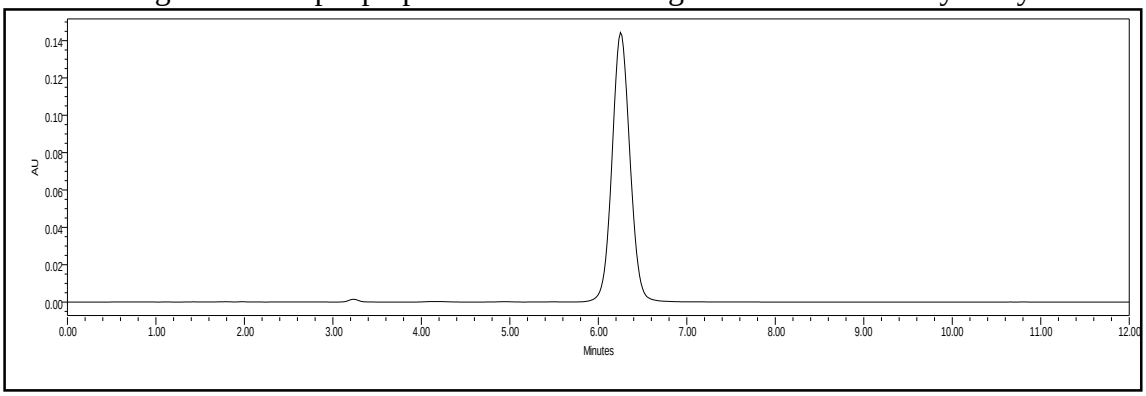


Table 24: Summary of initial stage of stability study

Observation				
Standard detail			**Test detail**	
Data for standard preparation			*Data for test preparation*	
Replicate	**Area**		**Replicate**	**Area**
1	2084557		1	2083048
2	2087441		2	2083161
3	2086919		**Average**	2083105
4	2080437			
5	2087706			
Mean	2085412			
Std.dev.	3046.80			
%RSD	0.15			
Standard weight	25.02		**Test weight**	81.81
Standard potency	99.48%		**Label claim**	400 mg

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

5.6.2 Stage of solution stability: After 6 Hrs

Blank preparation:

Use diluents as a blank.

Standard preparation:

Stock solution: 25.05 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.0 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Standard preparation (of 5°C) for stability:

Standard preparation solution which is stored at 5° C for 6 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Standard preparation (of room temperature) for stability:

Standard preparation solution which is stored at room temperature for 6 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Test Preparation (of 5° C) for stability:

Test preparation solution which is stored at 5° C for 6 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Test Preparation (of room temperature) for stability:

Test preparation solution which is stored at room temperature for 6 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Chromatographic sequence for 'After 6 hours' stage of solution stability study is represented through Table as under.

Table 25: Sequence for solution stability study "After 6 hours"

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Standard preparation (at 5°C)	2
5	Standard preparation (at 25°C)	2
4	Test preparation (at 5°C)	2
5	Test preparation (at 25°C)	2
4	Bracketing standard	1

Table 26: Summary of "After 6 hours" stage of stability study

Observation				
Standard detail			**Test detail**	
Data for standard preparation			*Data for test preparation (at 5°C)*	
Replicate	**Area**		**Replicate**	**Area**
1	2082471		1	2082024
2	2087382		2	2081847
3	2083628		Average	2081935
4	2084223			
5	2085824		Data for test preparation (at room temperature)	
Mean	2084706			
Std.dev.	1923.6		**Replicate**	Area
%RSD	0.09%		1	2080774
			2	2080520
	2084557		Average	2080647
Standard Weight	25.05		**Test weight**	81.81 mg
Standard potency	99.48%		**Label claim**	400 mg

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 27: Summary for standard solution stability at 'After 6 hours' stage:

Observation

Data for standard preparation (at 5°C)

Stage	Replicate	Area
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 6 hours	1	2085923
	2	2085715
	Average	2085528
	Std.dev.	2496.3
	%RSD	0.12%

Stage	Mean area
Initial	2085412
After 6 hours	2085819
Absolute difference (%)	0.02%

Data for standard preparation (at room temperature)

Stage	Replicate	Area
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 6 hours	1	2084228
	2	2084349
	Average	2085091
	Std.dev.	2547.6
	%RSD	0.12%

Stage	Mean area
Initial	2085412
After 6 hours	2084289
Absolute difference (%)	0.05%

Calculation:

$$\text{Absolute Difference (\%)} = \left| 100 - \left[\frac{A_R}{A_I} x\, 100 \right] \right|$$

Where by,

A_R= Standard mean area of respective time interval stage

A_t = Standard mean area of initial stage

5.6.3 Stage of solution stability: After 12 Hrs

Blank preparation:

Use diluents as a blank.

Standard preparation:

Stock solution: 25.01 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric

flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 500.2 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Standard preparation (of 5°C) for stability:

Standard preparation solution which is stored at 5° C for 12 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Standard preparation (of room temperature) for stability:

Standard preparation solution which is stored at room temperature for 12 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Test Preparation (of 5° C) for stability:

Test preparation solution which is stored at 5° C for 12 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Test Preparation (of room temperature) for stability:

Test preparation solution which is stored at room temperature for 12 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Table 28: Sequence for Solution stability study "After 12 hours":

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Standard preparation (at 5°C)	2
5	Standard preparation (at 25°C)	2
4	Test preparation (at 5°C)	2
5	Test preparation (at 25°C)	2
4	Bracketing standard	1

Table 29: Summary of "After 12 hours" stage of stability study

Observation				
Standard detail			**Test detail**	
Data for standard preparation			*Data for test preparation (at 5°C)*	
Replicate	**Area**		**Replicate**	**Area**
1	2082862		1	2080977
2	2084664		2	2081213
3	2084797		Average	2081095
4	2083441			
5	2082139		Data for test preparation (at room temperature)	
Mean	2083783			
Std.dev.	999.67		**Replicate**	Area
%RSD	0.05 %		1	2079553
			2	2078934
			Average	2079244
Standard Weight	25.04		**Test weight**	81.85 mg
Standard potency	99.48 %		**Label claim**	400 mg

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 30: Summary for standard solution stability at 'After 12 hours' stage:

Observation

Data for standard preparation (at 5°C)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 12 hours	1	2083686
	2	2083321
	Average	2084438
	Std.dev.	2348.07
	%RSD	0.11 %

Stage	**Mean area**
Initial	2085412
After 12 hours	2083504
Absolute difference (%)	0.09 %

Data for standard preparation (at room temperature)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 12 hours	1	2082447
	2	2082632
	Average	2085412
	Std.dev.	2515.37
	%RSD	0.12 %

Stage	**Mean area**
Initial	2085412
After 12 hours	2082540
Absolute difference (%)	0.14 %

Calculation:

$$\text{Absolute Difference (\%)} = \left| 100 - \left[\frac{A_R}{A_I} x\, 100 \right] \right|$$

Where by,

A_R= Standard mean area of respective time interval stage

A_t = Standard mean area of initial stage

5.6.4 Stage of solution stability: After 24 Hrs

Blank preparation:

Use diluents as a blank.

Standard preparation:

Stock solution: 25.03 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then,

volume of the flask was make up with diluent. The concentration obtained is 500.2 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.06 µg/ml of Dronedarone.

Standard preparation (of 5°C) for stability:

Standard preparation solution which is stored at 5° C for 24 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Standard preparation (of room temperature) for stability:

Standard preparation solution which is stored at room temperature for 24 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Test Preparation (of 5° C) for stability:

Test preparation solution which is stored at 5° C for 24 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Test Preparation (of room temperature) for stability:

Test preparation solution which is stored at room temperature for 24 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Table 31: Sequence for solution stability study "After 24 hours"

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Standard preparation (at 5°C)	2
5	Standard preparation (at 25°C)	2
4	Test preparation (at 5°C)	2
5	Test preparation (at 25°C)	2
4	Bracketing standard	1

Table 32: Summary of "After 24 hours" stage of stability study

Observation				
Standard detail			**Test detail**	
Data for standard preparation			*Data for test preparation (at 5°C)*	
Replicate	**Area**		**Replicate**	**Area**
1	2084554		1	2079668
2	2083677		2	2078541
3	2085649		Average	2079105
4	2086957			
5	2085964		Data for test preparation (at room temperature)	
Mean	2085360			
Std.dev.	1272.88		**Replicate**	Area
%RSD	0.06 %		1	2078874
			2	2078963
			Average	2078919
Standard Weight	25.07 mg		**Test weight**	81.92 mg
Standard potency	99.48 %		**Label claim**	400 mg

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 33: Summary for standard solution stability at 'After 24 hours' stage:

Observation

Data for standard preparation (at 5°C)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 24 hours	1	2082473
	2	2081996
	Average	2084499
	Std.dev.	2938.73
	%RSD	0.14 %

Stage	**Mean area**
Initial	2085412
After 24 hours	2082235
Absolute difference (%)	0.15 %

Data for standard preparation (at room temperature)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 24 hours	1	2080961
	2	2081226
	Average	2084178
	Std.dev.	3261.11
	%RSD	0.16 %

Stage	**Mean area**
Initial	2085412
After 24 hours	2081094
Absolute difference (%)	0.21 %

Calculation:

$$\text{Absolute Difference (\%)} = \left| 100 - \left[\frac{A_R}{A_I} x\, 100 \right] \right|$$

Where by,

A_R= Standard mean area of respective time interval stage

A_t = Standard mean area of initial stage

5.6.5 Stage of solution stability: After 36 Hrs

Blank preparation:

Use diluents as a blank.

Standard preparation:

Stock solution: 25.05 mg of Dronedarone reference standard was accurately weighed and transferred into 50 ml volumetric flask. 30 ml of diluent was added into the volumetric flask and the standard substance was dissolved by sonication for one minute. Then, volume of the flask was make up with diluent. The concentration obtained is 501.0 µg/ml of Dronedarone.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Dronedarone.

Standard preparation (of 5°C) for stability:

Standard preparation solution which is stored at 5° C for 36 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Standard preparation (of room temperature) for stability:

Standard preparation solution which is stored at room temperature for 36 hours was injected (and % RSD for the same solution was measured against initially injected standard).

Test Preparation (of 5° C) for stability:

Test preparation solution which is stored at 5° C for 36 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Test Preparation (of room temperature) for stability:

Test preparation solution which is stored at room temperature for 36 hours was injected (and assay for the same solution was measured against freshly prepared standard followed by comparison of that of initial).

Table 33: Sequence for solution stability study "After 36 hours"

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Standard preparation (at 5°C)	2
5	Standard preparation (at 25°C)	2
4	Test preparation (at 5°C)	2
5	Test preparation (at 25°C)	2
4	Bracketing standard	1

Table 34: Summary of "After 36 hours" stage of stability study

Observation				
Standard detail			**Test detail**	
Data for standard preparation			*Data for test preparation (at 5°C)*	
Replicate	**Area**		**Replicate**	**Area**
1	2084961		1	2072996
2	2084547		2	2073014
3	2083958		Average	2073005
4	2084366			
5	2084732		Data for test preparation (at room temperature)	
Mean	2084513			
Std.dev.	380.61		**Replicate**	Area
%RSD	0.02 %		1	2068809
			2	2068024
			Average	2068417
Standard Weight	25.04 mg		**Test weight**	81.78mg
Standard potency	99.48 %		**Label claim**	400 mg

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{50} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x \frac{AW}{LC} xP$$

Where, A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

W_2 = Weight of Test Sample (mg).

AW = Average Weight of Formulation (mg).

LC = Label Claim Weight of Formulation (mg).

P = Potency of Working Standard (%).

Table 35: Summary for standard solution stability at 'After 36 hours' stage:

Observation

Data for standard preparation (at 5°C)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 36 hours	1	2080365
	2	2079871
	Average	2083899
	Std.dev.	3589.15
	%RSD	0.17 %

Stage	**Mean area**
Initial	2085412
After 36 hours	2080118
Absolute difference (%)	0.25 %

Data for standard preparation (at room temperature)		
Stage	**Replicate**	**Area**
Initial	1	2084557
	2	2087441
	3	2086919
	4	2080437
	5	2087706
After 36 hours	1	2078892
	2	2078774
	Average	2083532
	Std.dev.	4061.44
	%RSD	0.19 %

Stage	**Mean area**
Initial	2085412
After 36 hours	2078883
Absolute difference (%)	0.31 %

Calculation:

$$\text{Absolute Difference (\%)} = \left| 100 - \left[\frac{A_R}{A_I} x\, 100 \right] \right|$$

Where by,

A_R= Standard mean area of respective time interval stage

A_t = Standard mean area of initial stage

Table 36: Summary of solution stability:

Time intervals	**Absolute difference in assay for standard solution %**		**Absolute difference in assay for sample solution %**	
	At 5°C	**At room temperature**	**At 5°C**	**At room temperature**
After 6 hours	0.02	0.05	0.05	0.02
After 12 hours	0.09	0.14	0.02	0.07
After 24 hours	0.15	0.21	0.12	0.17
After 36 hours	0.25	0.31	0.32	0.55

Conclusion:

Solution stability time period for standard solution is 36 hours at 5°C and room temperature. Solution stability time period for test solution is 36 hours at 5°C and at room temperature .

5.7 System Suitability Study:

A system suitability test for the chromatographic system was performed before each validation experiment. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. Only after the system suitability results were in acceptance criteria the experiments were precede further.

The Theoretical plates should be more than 5000, Asymmetry should be less than 2.0 and % RSD should be less than 2.0. As the data suggest the system suitability was within the criteria in each validation experiment. Hence the system was found suitable to perform the validation experiment which confirms the reliability of the data generated during the method validation.

Table 37: Results of System Suitability Test after each Validation Experiment

Summary of System Suitability Test			
Experiment Name	Theoretical Plates	Asymmetry	% RSD
Specificity	5688	1.17	0.22
Linearity and Range	5612	1.18	0.05
LOD and LOQ	5634	1.15	0.08
Method Precision	5647	1.17	0.38
Int. Precision	5619	1.18	0.24
Accuracy	5697	1.19	0.07
Robustness	5274	1.29	0.1
Solution Stability	5633	1.14	0.15

6. CONCLUSION

The surveillance and outcome obtained from each validation experiment including specificity, linearity and range, LOD and LOQ, precision, accuracy, robustness, solution stability and system suitability lies well inside the acceptance criteria. Since, all the results are with-in the limit, the developed Analytical method is considered as validated and suitable for anticipated use.

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