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RESEARCH ARTICLE

STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF DRONEDARONE HYDROCHLORIDE IN ITS BULK FORM BY RP-HPLC

Gurmeet S. Chhabra* and Saurabh Kumar Banerjee

Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, SVKM's NMIMS, Shirpur Campus, Dhule-425 405, Maharashtra, India

**E-mails*: gurmeetchhabra@gmail.com, saurabhbanerjee15@gmail.com
Tel.: +91 9764442450, +91 9765362007.

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This study describes the development and validation of stability indicating HPLC method for dronedarone hydrochloride in its bulk form. Dronedarone was subjected to stress degradation under different conditions recommended by International Conference on Harmonization. The sample so generated was used to develop a stability indicating high performance liquid chromatographic method for dronedarone·HCl. The peak for dronedarone·HCl was well resolved from the peaks of degradation products, using a kromasil C18 (250 mm × 4.6 mm, 5 μm) column and mobile phase comprising of buffer:methanol (buffer:30 mM KH_2PO_4 + 1 ml triethylamine in 1 litre water, pH=3.6 adjusted with ortho-phosphoric acid) using the gradient method at a flow rate of 1 ml/min. Detection was carried out using a UV detector at 291 nm. The degradation product peak was well resolved from drug peak. The method proved to be specific to the drug and its degradation products. The developed HPLC method was validated with respect to linearity, accuracy, precision and robustness. All the results were found to be within the specification limit.

Key words: Dronedarone hydrochloride, HPLC, Validation, Forced degradation, Stability indicating.

INTRODUCTION

Dronedarone hydrochloride is a class III anti-arrhythmic drug that is mainly used for treatment of atrial fibrillation and atrial flutter of cardiac arrhythmia. Dronedarone is a benzofuran derivative and is chemically *N*-(2-Butyl-3-(4-(3-(dibutylamino)propoxy) benzoyl)-5-benzofuranyl)-methanesulfonamide (Mol. formula: $C_{31}H_{44}N_2O_5$·HCl, Mol. wt.: 556.76 g/mole) (**Figure 1**).

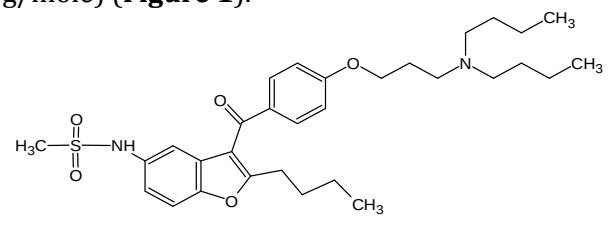

Fig. 1. Structure of dronedarone·HCl

Dronedarone is recommended as an alternative drug of amiodarone for treatment of atrial fibrillation and atrial flutter in cases where heart requires drug therapy or electric shock treatment to maintain normal rhythm of heart. Dronedarone is the most recent anti-arrhythmic drug (AAD) which is approved by USFDA and is available in the USA as Multaq tablets (400 mg). It mainly targets the repolarization currents, making them less active and hence prolonging the action potential duration (APD). Dronedarone also exhibits anti-adrenergic activity. Dronedarone is significantly safer and effective in maintaining the sinus rhythm and reducing the ventricular pro-arrhythmic, justifying it for the long term treatment of atrial fibrillation compared to other anti-arrhythmic drugs. HPLC is a well-known and widely used

analytical technique for the analysis of drug products and drug substances (Prasanthi *et al* 2011; Bhimavarapu *et al* 2011; Basaveswara Rao *et al* 2012a; 2012b; Banerjee and Vasava, 2013; Bindaiya and Argal, 2013). Few reports exist in literature about the analysis of dronedarone hydrochloride in human plasma by liquid chromatography-tandem mass spectrometry, the combination with amiodarone and their principle metabolites in plasma and myocardium by HPLC and UV-Detection, in bulk drugs by HPLC, and for the stability-indicating analysis by HPLC (Patel *et al* 2012; Dabhi *et al* 2012; Bhatt *et al* 2013; Rajyalakshmi *et al* 2013).

Extensive literature survey revealed that there is no rapid stability-indicating HPLC method for determination of related substances and for quantitative estimation of dronedarone in bulk drug form. Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of dronedarone and all the five impurities in bulk drug sample along with method validation as per ICH norms.

EXPERIMENTAL

Materials

The dronedarone hydrochloride reference standard was provided by Sanofi-Aventis. HPLC grade methanol and orthophosporic acid were obtained from Merck India Limited, Mumbai, India. Analytical grade hydrochloric acid, sodium hydroxide pellets, and hydrogen peroxide solution 30% (*v/v*) were obtained from Ranbaxy Fine Chemicals, New Delhi, India, and a 0.45 μm membrane filter was obtained from Pall Life Sciences, Mumbai, India. High purity deionised water was obtained from a Milli-Q (Millipore, Milford, MA, USA) purification system. Nylon syringe filters 0.45 μm were from Millex-Hn (Mumbai, India).

Equipment

HPLC system was used for method development, forced degradation studies and method validation. The output signal was monitored and processed using Total Chrome Navigator (TCN). Bath sonicator (Micro clean 103-Oscar); digital pH meter (Mode-Pico, Lab India); analytical balance (Model AUX, Shimadzu, Japan); filtration assembly; filter papers 0.45 μm was used. Water purification system (Elix-3, Millipore, Milford, MA, USA) were used along with the HPLC system. Photo stability studies were carried out in a photo stability chamber. Water bath equipped with temperature controller was used to carry out degradation studies for all solutions. Photo stability studies were carried out in a photo stability chamber.

Chromatographic conditions

The chromatographic system was performed using a kromasil C_{18} (4.6 mm × 100 mm), 5 μm column. Methanol was used as diluent. Separation was achieved using a mobile phase consisting of buffer:methanol (buffer: 30 mM KH_2PO_4 in 1 litre water, pH 3.6 adjusted with ortho-phosporic acid). The HPLC gradient program was set as: time (min)/% solution B: 0.5/85, 7/10, 9/85 and 3/85. The flow rate of the mobile phase was 1.0 ml/min with a short run time (19 min). The eluent was monitored using UV detection at a wavelength of 291 nm. The column temperature was maintained at 24±2°C and the injection volume 20 μl was used. The mobile phase was filtered through a 0.45 μm micron filter prior to use.

Standard solution

Standard stock solution (1000 ppm) was prepared by dissolving the drug in the diluent and standard solution was prepared by further diluting this solution to the desired concentration (100 ppm). Diluent used for preparation was composed of methanol and phosphate buffer pH 3.2 in ratio of 50:50 (*v/v*).

Preparation of stock solution

Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made with diluent. Solution was sonicated for 10 min, centrifuges at 10000 rpm for 10 min, filtered through 0.5 μ filter. From the filtered solution, 1 ml of solution was transferred into a 10 ml volumetric flask and diluted to volume with diluent.

Procedure for forced degradation study

Forced degradation of the drug product was carried out as per the ICH guideline (ICH Q1A (R2), Q2A, Q2B). The forced degradation study of the drug product was performed at photolytic, acid/base hydrolytic and oxidative stress conditions (Tondepu *et al* 2012).

Acidic degradation

A powder equivalent to 20 mg of dronedarone was weighed and transferred to 10 ml volumetric flask. Three ml of diluent and 3 ml of 1 N HCl was added, the mixture was kept at 60°C

for 3 h in water bath. Allow the solution to achieve ambient temperature, neutralized with 1 N NaOH solution to pH 7 and the volume was made up with diluent. The solution was further diluted to achieve the concentration of 100 ppm of dronedarone.

Alkali degradation

A powder sample equivalent to 20 mg of dronedarone was weighed and transferred in 10mL volumetric flask. Three ml of diluent and 2 ml of 0.1 N NaOH was added, the mixture was kept at 60°C for three hours in water bath. The solution was allowed to attend ambient temperature, neutralized with 0.1 N HCl solution to pH 7 and the volume was made up with diluent. The solution was further diluted to achieve the concentration of 100 ppm of dronedarone.

Oxidative degradation

The powder sample equivalent to 20 mg of Dronedarone was weighed and transferred in 10 ml volumetric flask. Three ml of diluent and 2 ml of 30% hydrogen peroxide were added, the mixture was kept at 60°C for 3 h in water bath, the solution was allowed to attend ambient temperature and volume was made up with diluent. The solution was further diluted to achieve 100 ppm concentration of dronedarone.

Photolytic degradation

The powder sample equivalent to 20 mg of dronedarone was weighed and transferred in petridish and was exposed to 1.2 million lux in 24 h. Then, the solution was prepared to achieve 100 ppm of dronedarone.

RESULTS AND DISCUSSION

Selection of λ_{max} for dronedarone

Standard stock solution of dronedarone was diluted with diluent to obtain final concentration of 100 µg/ml. The solution was further diluted to make 10 µg/ml. The solution was scanned using UV-Visible spectrophotometer in the scan mode between the wavelength range of 400 nm to 200 nm and their spectra were overlaid. The wavelength selected was 291 nm (**Figure 2**).

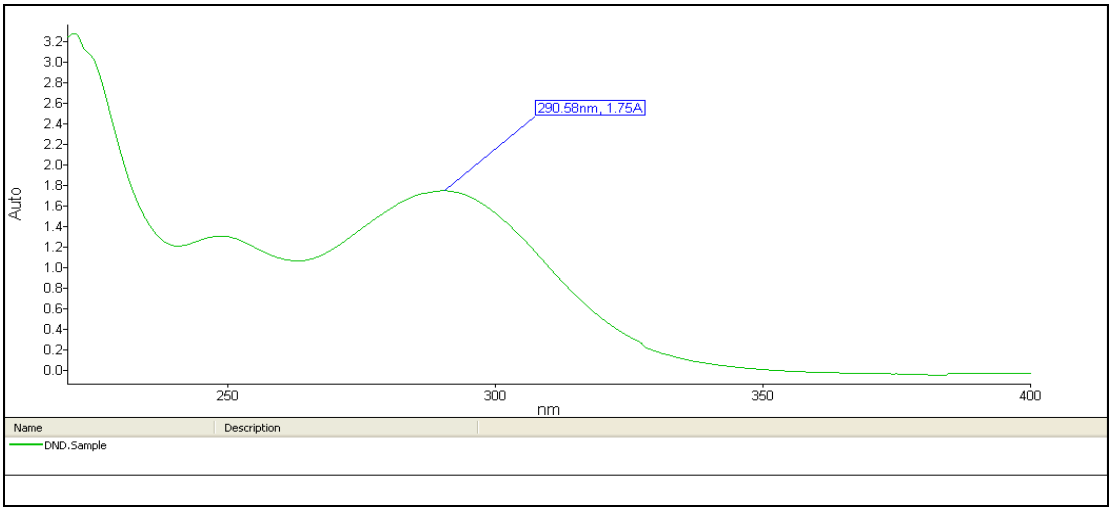

Fig. 2. UV Spectra of dronedarone·HCl

Optimization of the chromatographic conditions

To develop stability indicating method, different stationary phases like C_{18} and different mobile phases containing buffers like phosphate buffer of pH 3.6 were used. Our objective of chromatographic method development was to separate all degrading peaks from drug peak and tailing factor should be less than 2. The chromatographic separation of dronedarone from its degradants was achieved using kromasil C_{18} (4.6 mm × 100 mm) column. Changing the composition of mobile phase, optimization of chromatographic method was achieved. Kromasil C_{18} (4.6 mm × 100 mm) column shows better performance as compared to other C_8 column. System suitability data is shown in **Table 1**.

Development studies confirmed that the aqueous solution of 10 mM phosphate buffer and methanol was in gradient program which was set as time (min)/% solution B: 0.5/85, 7/10, 9/85 and 3/85. The flow rate of mobile phase 1.0 ml/min and column temperature 24±2° C were optimal conditions.

The drug peak has less tailing, resolution

Table 1. Results of system suitability

Parameters	Dronedarone·HCl	Acceptance criteria
Retention time	9.33 min	-
% RSD	0.74	should not be more than 2.0%
USP tailing	1.351	should not be more than 2.0
Theoretical plate count	97988.75	should not be less than 2000

from degradants and the chromatographic analysis time was less than 19 min. In optimized conditions, dronedarone and its degradants were well separated. Typical retention time of dronedarone peak was about 9.33 min.

Though conditions used for stress degradation were adjusted to achieve degradation in the range of 5-10%, this was not achieved in case of photolytic degradation even after exposure for prolonged duration. The drug showed extensive degradation (**Table 2**) in acid/alkali hydrolysis and oxidative condition (**Figures 3-6**).

Table 2. Results of forced degradation study

Stress condition	Degradation (%)
Acidic (60°C/3 h)	No degradation
Alkali (60°C /3 h)	No degradation
Oxidative (60°C/3 h)	10.58% and 9%
Photolytic (24 h)	1.03%

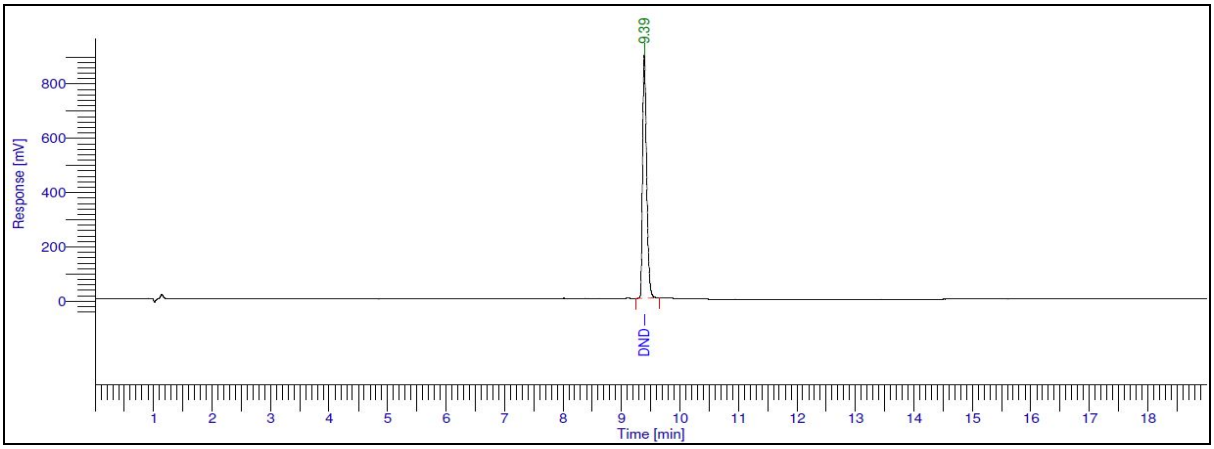

Fig. 3. Acid degradation

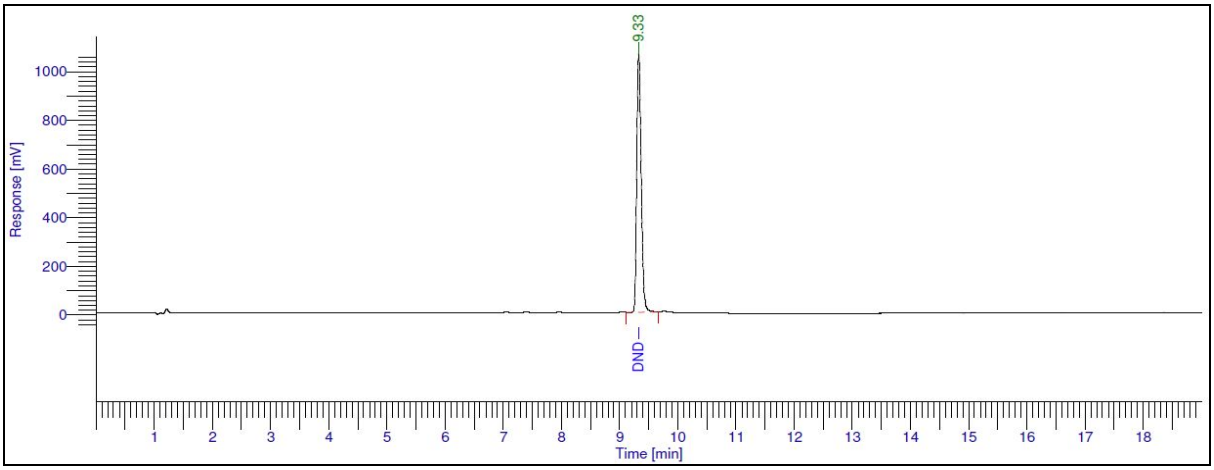

Fig. 4. Alkali degradation

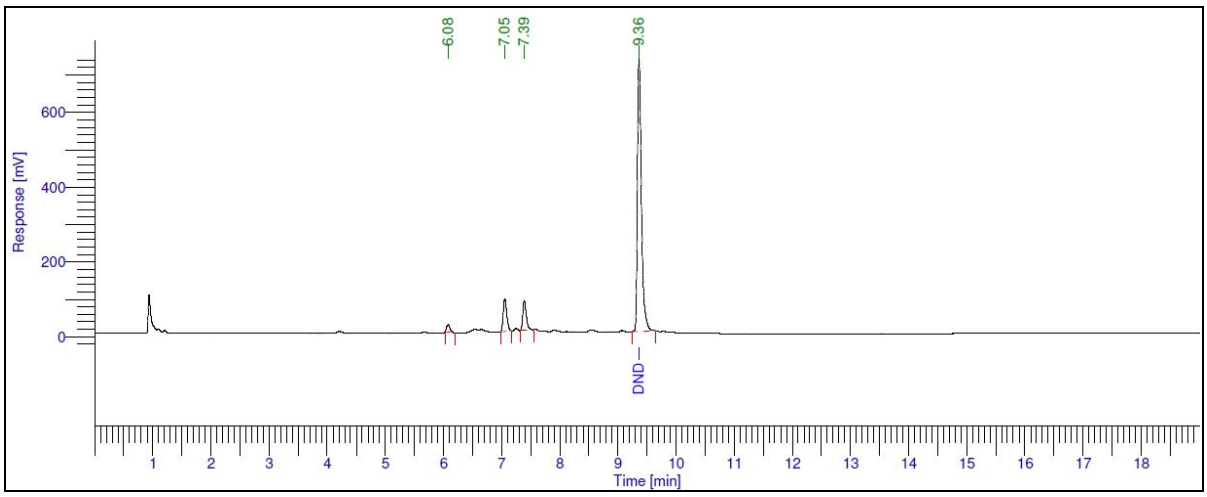

Fig. 5. Oxidative degradation

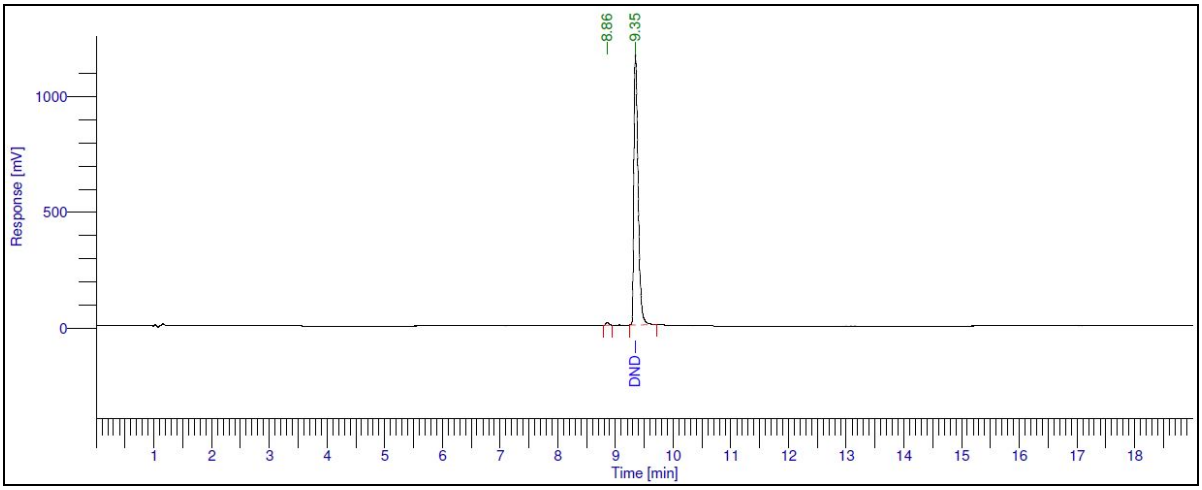

Fig. 6. Photolytic degradation

Method validation

Accuracy, precision, linearity, selectivity, robustness, Limit of Detection (LOD), LOQ (Limit of Quantification), and system suitability were performed as method validation parameters as per ICH guidelines.

System suitability

A standard solution of Dronedarone was prepared as per procedure and was injected 3 times into the system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak area from 3 replicate injections (**Table 3, Figure 7**).

Calibration and linearity

Linearity experiment was tested from 20 to 120% of the targeted level of assay (60ppm). Linearity solutions were injected in triplicate. The calibration graph was obtained by plotting peak area against the concentration of drug. The equation of calibration curve was found to be y = 56493x - 30348. The calibration graph was found to be linear in the aforementioned concentrations with correlation coefficient 0.995 (**Table 4, Figure 8**).

Precision (repeatability)

The precision of the method was studied by determining the concentration of drug for six times. The area %RSD for Dronedarone was 1.65 (**Table 5**).

Accuracy (estimation test)

Accuracy of the method was studied by estimation experiments. The estimation experiments were performed by adding known amounts of the drugs in the placebo. The estimation was performed at three levels, 80%, 100% and 120% of the standard working

Table 3. System suitability data

Sr. No.	Parameters	Dronedarone·HCl	Acceptance criteria
1	Retention time (min)	9.33 min	-
2	% RSD	0.74	should not be more than 2.0
3	USP tailing	1.351	should not be more than 2.0%
4	Theoretical plate count	97988.75	should not be less than 2000
5	Peak area	5639973.423	-

*Mean of six determinations

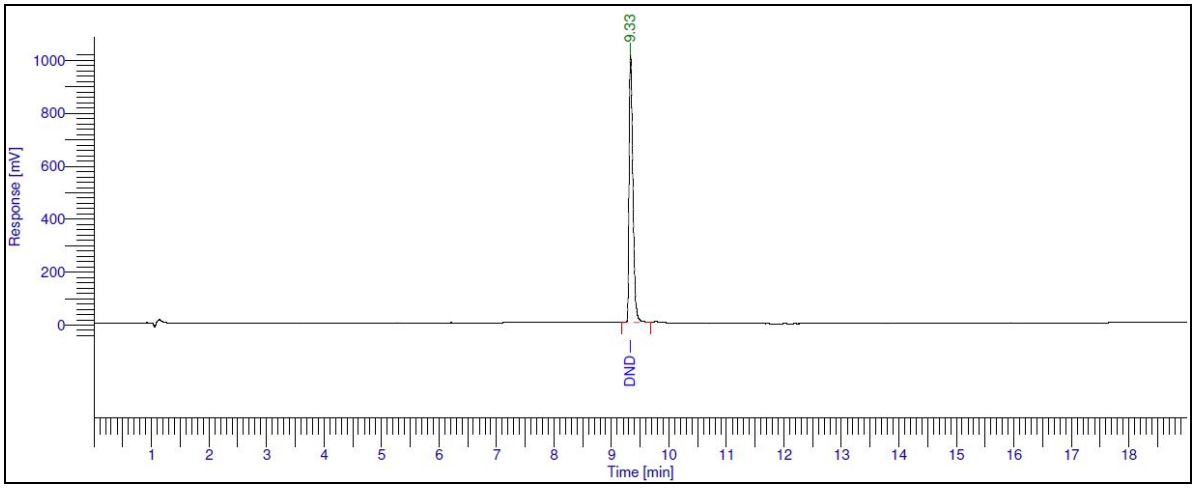

Fig. 7. Chromatogram of optimized method

Table 4. Results of linearity study

Concentrations	Average Area	% RSD
0	0	0
20	1164314.46	0.592396
40	2156245.903	0.911805
60	3321159.567	0.671868
80	4428905.663	0.766127
100	5639973.423	0.77962
120	6804043.983	0.21727

Table 5. Results of the precision study

Concentrations	Average area
80	4639065.83
80	4559448.73
80	4612211.91
80	4429122.25
80	4678943.19
80	4640974.41
Mean	4593294.387
SD	89621.44697
RSD	1.6511365

concentration of drug. The estimation samples were prepared as per different concentration of solution. Three samples were prepared for each estimation level. The solutions were then analysed, and the percentage estimation were calculated. The estimation values for dronedarone for all nine determinations were in range of 99.03 to 100.11%. The average estimation of three levels (nine determinations) was 99.26% (0.34). Estimation results are shown in **Table 6**.

Precision

Instrumental precision was determined by six replicate determinations of standard solution. Method precision or intra-assay precision was performed by preparing six different samples from the same sample pool. Each solution was injected in triplicate under same conditions and mean value of peak area response for each solution were taken. The relative standard deviation of the samples in six solutions was calculated.

Intermediate precision was performed by analysing the samples by two different analysts employing different instruments. Standard solution and six different samples at 100 percent target level were prepared by each analyst. Precision results are shown in **Table 7**.

Table 6. Results of accuracy study

Level (%) n=3	Amount of drug used (mg)	Amount estimated (mg)	% Estimation (n=3)
80	8.12	8.09	99.630
100	10.21	10.16	99.545
120	12.25	12.08	98.612
Mean			99.264

Table 7. Results of precision study

Interday						
Concentration	**Area**			**Mean**	**S.D.**	**% RSD**
20	1129644.6	1150573.25	1132683.76	1137633.87	11308.39	0.99
40	2188792.12	2161395.77	2154286.13	2168158.01	18219.82	0.84
60	3391608.59	3448285.50	3399325.49	3413073.19	30737.88	0.90
Intraday						
Concentration	**Area**			**Mean**	**S.D.**	**% RSD**
20	1192647.11	1214785.25	1206475.09	1204635.82	11183.09	0.93
40	2230464.33	2274875.54	2254658.91	2253332.93	22235.28	0.99
60	3244760.7	3188869.46	3199578.76	3211069.64	29664.59	0.92

Precision (% RSD)	Method Precision	0.93
	System Precision	0.74

Robustness

Robustness of a method is the ability of method to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered. Robustness was measured by evaluating peak tailing, theoretical plates and resolution between main peak and oxidation degraded product in all altered conditions.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate it was changed to 0.1 units from 1.1 to 0.9 ml/min. The effect of wavelength±1 nm was studied at 290 to 292, while other mobile phase components were kept constant. The effect of mobile phase composition was studied in aqueous solution of 10 mM phosphate buffer. At all conditions, the sample was assayed for 3 times. Assay % of dronedarone at all deliberate conditions were within the range of 98.21 to 99.60%. Robustness results are shown in **Table 8**.

Limit of quantification and limit of detection (LOQ and LOD)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per ICH guidelines.

Table 8. Results of Robustness study

Sr. No.	Parameter	Variation	% RSD	USP tailing	Theoretical plates
1	Wavelength + 1 nm	290 nm	1.2	1.12	4836.75
		291 nm	0.8	0.93	3541.37
		292 nm	0.5	1.03	4782.65
2	Flow rate + 0.1 ml/min	at 0.9 ml/min	1.1	0.88	3971.07
		at 1 ml/min	0.8	1.32	4394.43
		at 1.1 ml/min	0.7	1.16	3685.04
3	Mobile phase composition	20:80	0.9	0.84	4051.64
		15:85	0.8	1.28	4088.66
		10:90	0.6	0.98	4208.09

$LOD = 3.3 \times \sigma/S$ Eq. 1

$LOQ = 10 \times \sigma/S$ Eq. 2

where σ is standard deviation of the response and S is slope of the regression line.

The LOD and LOQ for dronedarone·HCl were 1.23 and 3.74. Results of LOD and LOQ are depicted in **Table 9**.

Table 9. Results of LOD and LOQ

Sr. No.	Concentration	Peak area	S.D.
1	0	0.00	0.00
2	20	1164314.46	6897.35
3	40	2156245.90	19660.75
4	60	3321159.57	22313.82
5	80	4428905.66	33931.04
6	100	5639973.42	43970.37

The detection and quantification limits were evaluated from calibration curves plotted in range of detection level to 100 μg/ml.

CONCLUSION

The gradient stability indicating HPLC method was developed for study of stress degradation of dronedarone in pharmaceutical preparations. The method is precise, accurate and requires short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method was found to be stability indicating and can be suggested to be conveniently used by quality control department to determine assay of pharmaceutical preparations and also to study stability of dronedarone dosage form.

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