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Stability Indicating RP-HPLC Assay Method for Estimation of Dronedarone Hydrochloride in Tablet

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ABSTRACT

A sensitive and precise high performance liquid chromatographic method has been developed and validated for determination of Dronedarone Hydrochloride. The proposed method was carried out on Analytical HPLC system consisting of Hyperchrome ODS C18 column (250 mm × 4.6 mm, 5µ). The chromatographic separation was achieved using a mobile phase containing acetonitrile: triethylamine buffer (pH-2.3) in the ratio of 70:30 v/v at flow rate of 1.0 mL/min using UV detection at 290 nm. The linear regression analysis data showed good linearity over the concentration range of 10-60µg/mL of dronedarone hydrochloride. The percent assay of dronedarone hydrochloride from tablet was found to be 99.75. The determination of intrinsic stability of the drug was assessed under acidic, alkaline, peroxide, thermal and photolytic stressed conditions. The drug was estimated in presence of its degradation products without interference. The method was validated for accuracy, precision, robustness and recovery studies as per ICH guidelines. The method can be adopted for routine analysis of drug in its tablet formulation.

INTRODUCTION

Dronedarone, N- (2-Butyl-3-(p-(3-(dibutylamino) propoxy) benzoyl)-5-benzofuranyl) methane sulfonamide is a anti-arrhythmic agent (Fig 1).

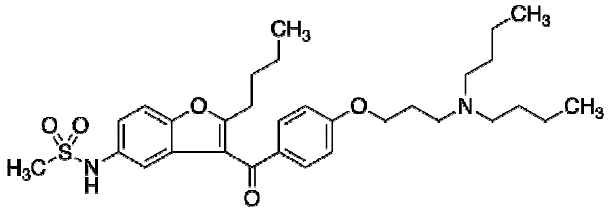

Fig. 1: Structure of Dronedarone Hydrochloride

Chemically, dronedarone is a benzofuran derivative related to amiodarone, used is limited by toxicity due its high iodine content (pulmonary fibrosis, thyroid disease) as well as by liver disease (Maryadele, 2013). A survey of pertinent literature indicates that HPTLC (Dabhi *et al.*, 2012), HPLC (Bhatt *et al.*, 2013; Chhabra and Banerjee, 2013; Tondepu *et al.*, 2012; Patel and Akhtar, 2012; Patel and Choudhury, 2012; Bolderman et al, 2009; Landge *et al.*, 2013), RP-UPLC (Molleti *et al.*, 2013) and Spectrophotometric (Pravalika *et al.*, 2013) methods have been reported on quantification of dronedarone in pharmaceutical dosage forms and in biological samples. The present research work represents development of validated stability indicating P-HPLC method using milder stress conditions and study of degradation kinetics.

MATERIALS AND METHODS

Chemicals and reagents

Dronedarone Hydrochloride was obtained as a gift sample from Sanofi Winthrop Industries, Pvt Ltd, Mumbai. The marketed formulation containing Dronedarone Hydrochloride 400 mg was purchased from a local pharmacy. All chemicals and solvents used throughout experimentation were HPLC and GR Grade.

Instrumentation

Analytical Technological Limited® HPLC isocratic mode, UV-visible detector with manual rheodyne injector (20 µL loop) and a reversed phase, Hyperchrome ODS C18 column (250 x 4.6 mm, 5µm) with pore size of 100 A^0.

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The mobile phase comprised of Acetonitrile: Triethylamine buffer pH 2.3 (70:30 v/v), at flow rate 1.0 mL/min. The mobile phase was sonicated and filtered through a 0.45 µ membrane filter. Analysis was performed at ambient temperature. The detection was monitored at 290 nm.

Optimized chromatographic conditions

Column - Hyperchrome ODS C18 column (250 X 4.6mm, 5µ) Mobile Phase-Acetonitrile: Triethylamine buffer pH 2.3 (70:30%v/v)

Detection Wavelength - 290 nm
Flow rate - 1.0 mL/min
Temperature (Ambient) - 25°C
Injection volume - 20 µL

Preparation of Triethylamine Solution (pH-2.3)

2.0 mL of triethylamine was diluted with double distill water to 1000.0 mL and pH was adjusted to 2.3 with ortho-phosphoric acid.

Preparation of mobile phase

The mobile phase was prepared by mixing Acetonitrile and Triethylamine buffer (pH 2.3) in the ratio of 70:30 v/v. Each time mobile phase was sonicated and filtered through 0.45 µm membrane filter paper.

Diluent

The mix solution of Acetonitrile and double distill water (50:50) was used as diluent.

Preparation of working standard drug solution (DDH)

About 25 mg of Dronedarone Hydrochloride was weighed and transferred to 50 mL volumetric flask and volume made up to mark with diluent. A portion 5.0 mL of stock solution was further diluted to 50 mL with diluent to get final solution having 50µg/mL of DDH.

Study of system suitability parameters

The system suitability test is an integral part of chromatographic analysis. It is used to verify the resolution and reproducibility of the system is suitable for analysis. Standard solution of DDH was prepared following the above procedure and used for system suitability study.

Table 1: Observations of system suitability parameters.

Sr. No	Wt. of Std. DDH taken	AUC of DDH (µV)
1	~ 25 mg	4108020
2		4118308
3		4229317
4		4200101
5		4089541
6		4112342
Mean		4142938.16
% RSD		1.37
Theoretical plate		7445
Retention time		4.14
Asymmetry		1.10

The mobile phase was allowed to equilibrate with stationary phase, as indicated by steady baseline. A 20µL solution was injected through manual injector and chromatographed. The chromatogram so obtained is shown in Fig 2. The results obtained with six replicate injections of the standard solution are summarized Table 1.

Force degradation study

Solution state stability: The study was carried on standard drug as well as marketed formulation.

Preparation of Stress standard and sample

An accurately weighed quantity of about 25 mg standard DDH and tablet powder equivalent to standard was transferred to a series of 50 mL of volumetric flasks and then 25 mL of 0.1N acid/0.1N base/ 3% H_2O_2/Neutral medium (Water) were added to each flask and kept at 80°C for a period of 3h. The standard solutions were withdrawn at the end of 3h and sample solutions were withdrawn at interval of 30, 60, 90, 120, 150, 180 min respectively for all stress conditions.

Sample preparation of stressed samples

At specified intervals, acid and base stressed standard as well as samples were neutralized and the solutions were diluted to volume with diluent. The peroxide and neutral stress standard and samples were diluted to volume with diluent at the end of specified periods. The content of each flasks were sonicated for 15 min and samples were filtered separately. A 5.0 mL portion of the above standard and sample solutions were further diluted up to 50 mL with diluent. A 20µL volume of each solution was injected through manual injector and chromatographed separately. The area of each sample obtained was recorded and compared with that of the unstressed standard to calculate the concentration of the drug (undegraded). The chromatograms for stressed standard and sample at 80°C recorded under basic, acidic, oxidative and neutral hydrolysis are shown in Fig 3-6 and Fig 7-10 respectively. The results are shown in Table 2a.

Table 2a: Results of solution state stability.

Stress conditions At 80°C	% Undegraded DDH, after 180 min	
	Standard	Sample
0.1N HCl	92.80	88.16
0.1N NaOH	95.61	85.81
3% H_2O_2	90.44	85.73
H_2O	91.74	83.64

Solid state stability

The study was carried on marketed formulation.

Humidity studies

Tablet powder was spread on petridish and exposed to 40°C/75% RH for 15 days.

Photostability studies

Tablet powder was spread on petridish and exposed to UV light at 254nm, for 15 days

Thermal studies (Dry Heat)

Tablet powder was spread on petridish and kept in oven at 60°C, and expose for 15 days. In solid state stability, samples were analysed on 1st, 3rd, 5th, 7th and 15th day. On the day of analysis samples were appropriately diluted using diluents followed by sonication for 15 min and filtration. A chromatogram for each sample was recorded and AUC noted. The results are shown in Table 2b.

Table 2b: Results of solid state stability.

Parameters	Condition	Exposure period	% DDH Undegraded
Humidity	40 °C 75% RH	15 Days	77.79
Thermal	60 °C		82.74
Light	254 nm		79.24

Assay

Weigh and finely powdered 20 tablets and transfer the quantity of tablet powder equivalent to 25 mg of DDH to 50 mL volumetric flask, sonicated for 15 min with sufficient quantity of diluent and volume was made up to mark with diluent. The content of the flask was sonicated and filtered through 0.45 µm membrane filter paper. A 5.0 mL portion of the filtered was further diluted to 50 mL with diluent. After equilibration of stationary phase, five sample solutions were injected separately and chromatograms were recorded.

The content of DDH in each sample was calculated by comparing the peak area of sample with that standard using formula. The results are shown in Table 3.

Table. 3: Results of estimations of DDH in marketed formulation.

AUC of Std. (µV)	AUC of sample (µV)	% Label claim
4048308	4165814	99.46
	4142679	99.14
	4174177	100.4
	4194532	99.69
	4201533	100.07
Mean		99.75
±SD		0.496
% RSD		0.50

Recovery Studies

It was carried out by standard addition method (SAM).

Preparation of sample

An accurately weighed quantity of tablet powder equivalent to 25 mg of DDH was transferred to 50 mL volumetric flask and to it reference standard pure drug were added at five different levels. The sufficient quantity of diluent was added, sonicated for 15 min and volume was made up to the mark. By adopting procedure as described under the estimation of DDH in marketed formulation, chromatogram was recorded and AUC noted. The amount of drug constituted by preanalysed tablet powder was deducted from total amount of the drug estimated and the resultant quantities were assured to be recovered from the pure drug added. Amount contributed by marketed preparation and % recovery were calculated. The results are tabulated in Table 4.

Table 4: Results of recovery studies.

Amount of pure drug added (mg)	Amount recovered (mg)	% Recovery
10.01	9.82	98.20
15.03	14.89	99.26
20.20	19.69	98.47
25.05	24.87	99.48
30.01	29.46	98.20
Mean ±SD% RSD		98.72 0.60

RESULTS AND DISCUSSION

Selection of mobile phase

The mobile phase was selected on trial and error basis. Initially Acteonitrile: phosphate buffer in the ratio of 50:50 v/v was tried, the chromatogram shows tailing. The buffer was replaced by the triethylamine solution of pH 2.3 in the ratio 50:50v/v gave sharp peak but the retention time was too long (tR_13.2min).

In order to reduce the elution time the organic phase was increased which resulted in lesser retention of the drug and finally the mobile phase comprising of Acetonitrile and triethylamine buffer pH 2.3 (70:30v/v) was finalized which gave sharp peak and reasonable retention time (tR_4.1).

Study of system suitability parameters

System suitability was evaluated by injecting six replicate injections of standard solutions of Dronedarone Hydrochloride (50µg/mL). The parameters such as theoretical plate, asymmetry and percent relative standard deviation (RSD) were studied and found satisfactory.

Force degradation studies

The standard and sample were found to degrade around 8-12% in acidic medium, 5-15% in alkaline condition, 9-17% in neutral and 10-16% in peroxide treated at 80°C. Extra peak(s) were seen in the chromatograms for stressed standard and sample solutions.

The relative retention times of degradation products recorded are for alkaline, acidic and neutral stress was found to be 0.77 while in peroxide stress two degradants appeared in the chromatogram with RRT of 0.77 and 1.16 respectively. The drug may be categorized in adequate degradation. The degradation peaks generated after 60min of refluxing and the area under curve increases as the time (180 min) of refluxing increases in all stressed samples.

The RRT of degradant in alkaline, neutral and peroxide condition matches with impurity-4 while a second degradant was observed in the chromatogram of peroxide stress sample with RRT of 1.16 matches with imp-6 (Landge *et al.*, 2013).

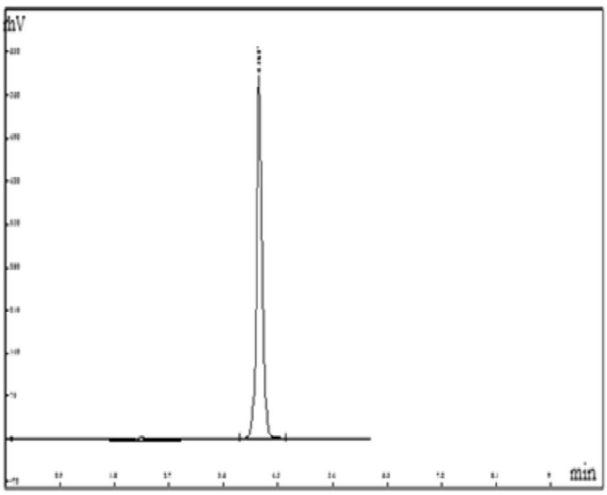

Fig. 2: Chromatogram of DDH (Assay).

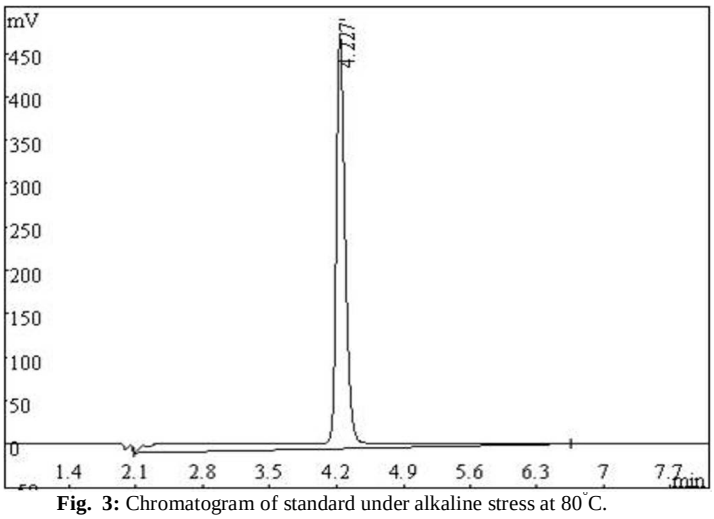

Fig. 3: Chromatogram of standard under alkaline stress at 80˚C.

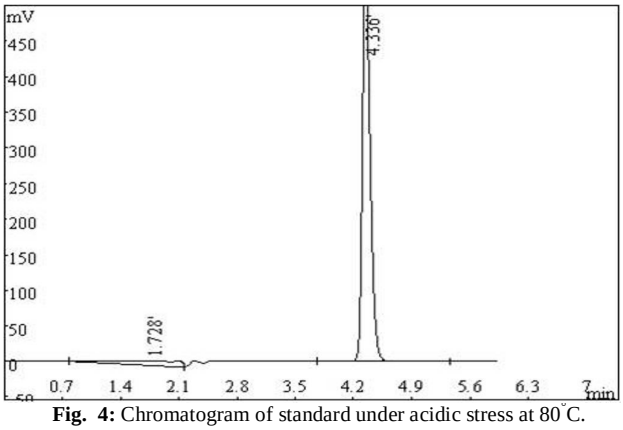

Fig. 4: Chromatogram of standard under acidic stress at 80˚C.

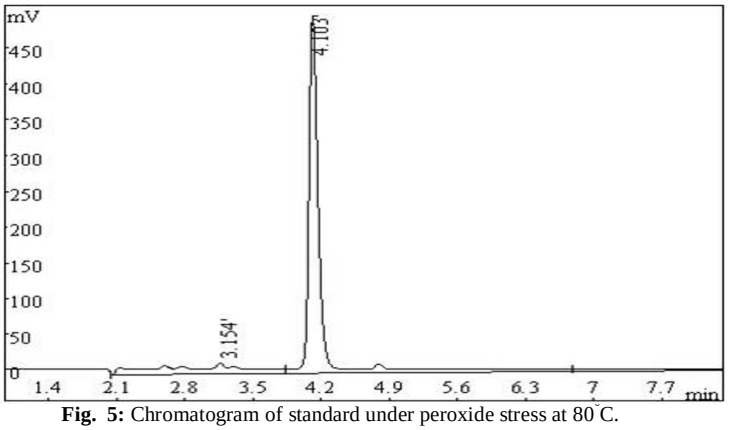

Fig. 5: Chromatogram of standard under peroxide stress at 80˚C.

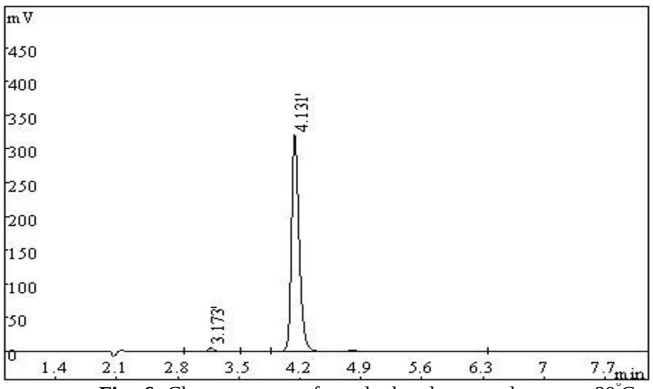

Fig. 6: Chromatogram of standard under neutral stress at 80˚C.

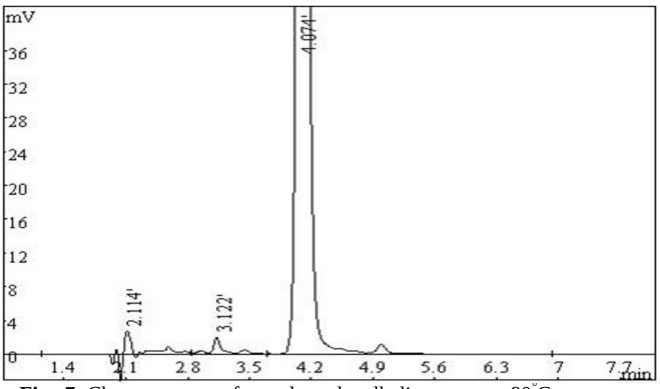

Fig. 7: Chromatogram of sample under alkaline stress at 80˚C.

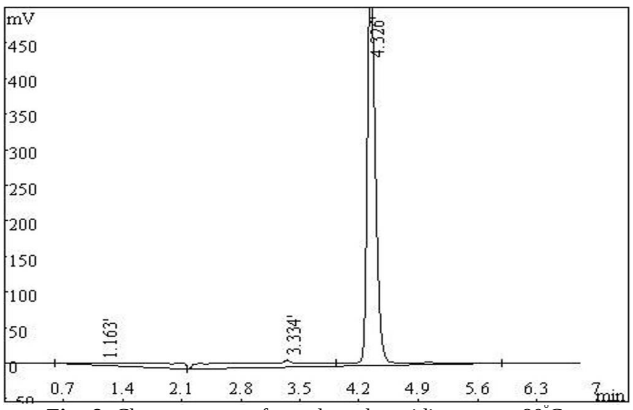

Fig. 8: Chromatogram of sample under acidic stress at 80˚C.

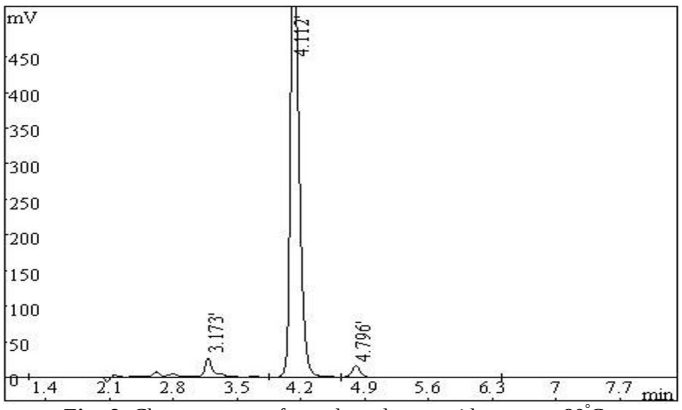

Fig. 9: Chromatogram of sample under peroxide stress at 80˚C.

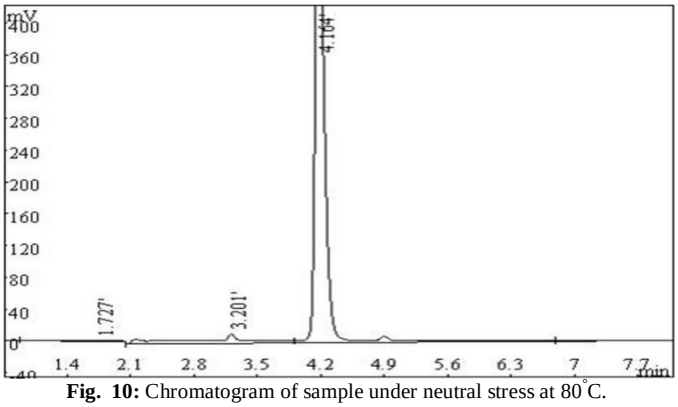

Fig. 10: Chromatogram of sample under neutral stress at 80°C.

Kinetics of solution state degradation studies

The kinetics of degraded samples was evaluated for all the hydrolytic conditions. The plot of regression coefficient (r) obtained and the best fit observed indicates the order of degradation reaction.

1. Values of concentration against time (zero- order kinetics)
2. Log of concentration verses time (first-order kinetics)
3. Reciprocal of concentration verses time (second-order kinetics)

The observation and results of kinetics of degradation are shown in Table 5.

Table. 5: Observation and results of kinetics of degradation studies.

Degradation study	Condition (At 80 °C)	Value of 'r'	Order of reaction
Acid Hydrolysis	0.1M HCl	0.8368	Second
Alkaline Hydrolysis	0.1M NaOH	0.9643	Zero
Oxidative Hydrolysis	3% H2O2	0.7759	Zero
Neutral Hydrolysis	Distill water	0.9543	Zero

Method Validation

The method was validated as per the guidelines in terms of parameters like, precision, accuracy (recovery studies), system suitability parameters, linearity and range etc.

Precision

Precision of proposed method was ascertained by replicate analysis of homogenous samples. The value of percentage relative standard deviation (% RSD) of Dronedarone Hydrochloride was found to be 0.50, indicates proposed method is precise.

Accuracy

The accuracy of the proposed method was evaluated as percent RSD or SD of the drug recoveries using the proposed method. The % RSD was found to be 0.61, which is acceptable.

Linearity and Range

Linearity of DDH was performed using the sample solution in the range of 80-120µg/mL (i.e.80% to 120% of working standard concentration). Linearity curve was constructed by plotting peak area against concentration and regression equation was computed. The correlation coefficient of Dronedarone Hydrochloride was found to be 0.999.

Ruggedness

It is carried out for two parameters:

Different Analyst

The sample was prepared as per the procedure described under assay. The tablet samples were analyzed using proposed method by two different analysts.

Intraday and Interday variation

The sample was prepared as per the procedure described under assay and analyzed at intervals 0 hrs, 5 hrs and 10 hrs for intraday study and on 0^{th}, 1^{st}, 3^{rd} and 7^{th} day for inter-day study. The results of ruggedness study are shown in Table 6.

Table 6: Results of ruggedness parameters.

Parameter*	Mean % label claim	SD	% RSD
Intraday	99.95	0.92	0.92
Interday	99.35	1.37	1.39
Analyst Variation	100.35	0.71	0.72

*mean of three obsevations

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The system suitability parameter was evaluated for each varied condition viz., Change in pH, composition of mobile phase and detection wavelength. Results of robustness study are shown in Table 7, indicating method is found to be robust.

Table 7: Observations of robustness study.

Deliberate changes	DDH		
	RT	Asymmetry	Theoretical plate
Standard condition	4.14	1.10	7445
pH (2.1)	3.92	1.38	7360
pH (2.5)	4.07	1.34	7415
Composition (77:23)	3.89	1.32	7484
Composition (63:37)	4.21	1.40	7501
Wavelength (285 nm)	4.04	1.36	7546
Wavelength (295 nm)	4.18	1.35	7423

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of Dronedarone hydrochloride in bulk and tablet formulation. The method was found to be simple and linear in the concentration range 10-60µg/mL for Dronedarone Hydrochloride. The sample recovery in a formulation was in good agreement with their respective label claims and that suggested non-interference of formulation excipients in the

estimation. The cited literature (Landge *et al.,* 2013) did not report any degradation in alkaline and neutral conditions and moreover the stress conditions applied were milder, even though the degradation was seen with appearance of resolved peaks. Hence the drug can be analyzed in presence of its degradation product indicates that the proposed method is stability indicating.

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