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Research Article

Fast Stability Indicating UPLC Method for Quantitative Analysis of Dronedarone in Pharmaceutical Dosage Form: Force Degradation Study

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A simple, precise, rapid reproducible, selective, and stability indicating reverse phase UPLC method has been developed for the estimation of dronedarone in pharmaceutical dosage form. Estimation of dronedarone hydrochloride was achieved on Acquity BEH C_{18} (100 mm∗2.1 mm) 1.7 μm column using buffer [20 mM KH_2PO_4 + 1 mL Triethylamine (pH = 2.5 by orthophosphoric acid)] and methanol in ratio of 40 : 60 as mobile phase at 30°C. The flow rate was 0.4 mL/min and effluents were monitored at 290 nm. The method was validated with respect to linearity, accuracy, precision, LOD, LOQ, and robustness. The method was linear over the concentration range 0.38–90 μg/mL (r^2 = 0.999), with a limit of detection and quantification of 0.1 and 0.38 μg/mL, respectively. Dronedarone was subjected to acid and alkali hydrolysis, chemical oxidation, dry heat degradation, and photo (sunlight) degradation. The degraded product peaks were well resolved from the drug peak with significant difference in their retention time value.

1. Introduction

Dronedarone (DRO), N (2Butyl3 (p (3(dibutylamino) propoxy)benzoyl)-5-benzofuranyl) methane sulfonamide (Figure 1). DRO is used 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 to maintain normal rhythm [1]. A literature review revealed that DRO has been determined and studied by several procedures; quantitative analysis of DRO in bulk drug and pharmaceutical dosage is formed by HPLC [2–4] and by HPTLC [5]. The HPLC method for determination of DRO in plasma and myocardium has also been reported [6]. There is a report on the determination of the DRO in human plasma by liquid chromatography-tandem mass spectrometry [7]. There is no method reported for quantification of dronedarone by UPLC.

2. Experimental

2.1. Material and Reagents. Pure DRO was procured as gift sample from Sanofi Aventis, India. DRO tablet was purchased from local commercial sources. HPLC grade methanol was procured from Merck India, Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid (S.D. Fine Chemicals, Mumbai, India) were of analytical grade used for the preparation of mobile phase. HPLC grade water acquired from Millipore Milli-Q water system.

2.2. Chromatographic Conditions. Analysis of dronedarone was carried out on a Waters Acquity UPLC with PDA detector. The output signal was monitored and processed using empower software. The chromatographic column used was Acquity BEH C18 (100 × 2.1) mm and 1.7 μm particle size. Isocratic elution analysis was used throughout the analysis.

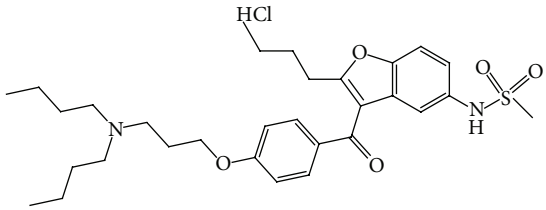

Figure 1: Structure of dronedarone hydrochloride.

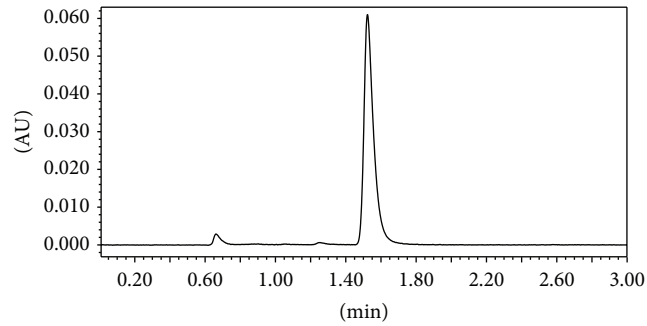

Figure 2: Chromatogram of DRO reference standard.

2.3. Mobile Phase. [20 mM KH_2PO_4 + 1 mL triethylamine (pH = 2.5 by orthophosphoric acid)] and methanol one in ratio of 40 : 60.

2.4. Instrument Parameter. The isocratic flow rate of mobile phase was maintained at 0.4 mL/min. The column temperature was adjusted to 35°C. The injection volume was set as 1 μL. Eluted sample was monitored at 290 nm and the run time was 3.0 min. The retention time of the DRO was about 1.5 min.

2.5. Preparation of Tablet Extract. 10 tablets (each tablet contained 400 mg DRO) were weighed, transferred into a clean and dry mortar, and powdered. Tablet powder equivalent to 25 mg of DRO was transferred into a 50 mL volumetric flask and 30 mL of the methanol was added. The solution was sonicated for 15 min to achieve complete dissolution of DRO and then filtered through a Whatman filter paper number 42. Wash the residue with small amount of methanol and the volume was made up to the mark with the methanol.

Appropriate volume of the aliquot was transferred to a 50 mL volumetric flask, and the volume was made up to the mark with the mobile phase to obtain a solution containing 50 μg/mL of DRO.

3. Validation

3.1. Accuracy. To determine the accuracy, DRO solutions were analysed at three different concentrations (50%, 100%, and 150%). Mobile phase was used as a blank and injected before sample injection and the RSD values of peak area and retention time were calculated.

3.2. Precision. Precision study was performed by injecting the solution containing DRO (50 μg/mL) repeatedly, and peak area was measure. The results were reported in terms of relative standard deviation (Figure 2). The intraday and interday precision study of DRO was carried out by estimating the corresponding responses on the same day and on different days and the results were reported in terms of relative standard deviation (RSD).

3.3. Limit of Detection (LOD) and Quantification (LOQ). The LOD and LOQ were determined at a signal-to-noise ratio of 3 and 10, respectively. LOQ and LOD were achieved by injecting a series of dilute solution of DRO. The precision and accuracy for DRO at LOQ were checked.

3.4. Linearity. Linearity was prepared from LOQ level to 160% of the actual sample concentration (50 μg/mL). A total of eight concentrations of the solutions were made separately and injected (LOQ: 40%, 60%, 80%, 100%, 120%, 140%, and 160% of the actual sample concentration). Calibration curve constructed by plotting peak area versus concentrations of the drug and regression equations was computed for DRO.

3.5. Robustness and Ruggedness. Robustness of the method was studied by deliberately changing the experimental conditions. The experimental conditions changed like flow rate (0.4 ± 0.04 mL/min), column oven temperature (30 ± 5°C), mobile phase composition (buffer: methanol = 38 : 62 and 42 : 58, v/v), and wavelength (290 ± 1 nm). Different columns of same dimensions were used for the analysis. The study was performed on the same day and on three different days by three different analysts. In each case, the RSD values were calculated for the peak area and retention time. The number of theoretical plates and tailing factors was compared with those obtained under the optimized conditions (Table 10).

3.6. Solution and Mobile Phase Stability. Stability of solution was investigated by injecting the sample into the chromatographic system in the time intervals of 0, 12, and 24 hrs. The mobile phase stability, was studied by injecting a freshly prepared sample solution at the same time intervals (0, 12, and 24 hrs) with the same mobile phase. The peak area was recorded and the RSD values were calculated.

3.7. Force Degradation Study. Stress degradation study using acid and alkali hydrolysis, chemical oxidation, dry heat degradation, and photo (sunlight) degradation was carried out and interference of the degradation products was investigate. All stress decomposition studies were performed at an initial drug concentration of 500 μg/mL in mobile phase and 5 mL of 1 M HCl, 1 M NaOH, and 5% H_2O_2 were added separately. The flasks were heated for 1 hr on a water bath at 80°C. The solutions were cooled and neutralized by adding acid or base, and then the solution was diluted up to the mark with diluent.

Table 1: HPLC and UPLC method comparison.

Parameter	HPLC [3]	UPLC
Elution time	3.02	1.52
Flow rate (mL/min)	1	0.4
Injection volume (μL)	20	1
LOD	0.87	0.38
LOQ	2.65	0.1
Run time	10 min	3 min

Solid state thermal degradation was carried out by exposing drug to dry heat at 80°C for 48 hrs. For photolytic degradation studies, drug in solid state was exposed to sunlight for 48 hrs. The sample after stress degradation was used to prepare 50 μg/mL.

4. Result and Discussion

UPLC becomes very famous in the recent year due to its fast approach for method development and validation of pharmaceutical drug. The smaller particles in column increase the efficiency, resolution, and speed in analysis (Table 1). It is observed that the run time of the UPLC method is very short (3 min) to that of HPLC methods (Table 12). The present method discusses the development and validation of a method for quantitative analysis of DRO with degradation study under different stress conditions.

4.1. Method Optimization. Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Choice of retention time, tailing, theoretical plates, and run time was the major task while developing the method. Several proportions of buffer and solvents were evaluated in order to obtain suitable composition of mobile phase. In order to achieve symmetrical peak of DRO, various stationary phases like C_8, C_{18} and phenyl column with different lengths (50 and 100 mm) were studied. From the summary, it is concluded that Acquity BEH C_{18} (100∗2.1) mm, 1.7 μm column has the ideal stationary phase for the determination of DRO. UV spectra of DRO showed that the drug was absorbed appreciably at 290 nm, so the same was selected as the detection wavelength during the studies. Summary of mobile phase and stationary phase optimization is represented in Tables 2 and 3, respectively.

4.2. Accuracy. The accuracy of the method was determined by calculating recoveries of DRO. The recoveries for DRO obtained were 99.31%–99.98%. The results of accuracy study and RSD illustrate that the method is accurate. The summary of accuracy study is revealed in Table 4.

4.3. Precision. The summary of precision study is illustrated in Table 5. In the intermediate precision, study results demonstrate that RSD values were in the same order of magnitude as those obtained for precision study. Overall, the data for the precision study suggested that assay value for each test preparation was between 98 and 102% and RSD of all results was less than 2%. Therefore, from all the observations, it can be concluded that the proposed method is highly precise.

4.4. Linearity. A calibration curve was obtained for DRO from LOQ to 180% of its actual sample concentration (50 μg/mL). A linear correlation was obtained between the mean peak area and the concentration in the range of 0.38–90 μg/mL, from which the linear regression equation was

Table 2: Summery of mobile phase optimization.

Solvent A	Solvent B	Remarks
Water	Acetonitrile	Very broad peak with peak splitting
5 mM ammonium acetate	Methanol	Broad peak with peak tailing
5 mM ammonium acetate	Acetonitrile	Broad peak with peak tailing
1% orthophosphotic acid (OPA) in water	Acetonitrile	Peak eluted early with peak tailing
5 mM potassium dihydrogen phosphate (pH = 2.5 by OPA)	Methanol	Broad peak with peak tailing
5 mM potassium dihydrogen phosphate (pH = 2.5 by OPA)	Acetonitrile	Broad peak with peak tailing
5 mM potassium dihydrogen phosphate + 1 mL triethylamine (TEA) (pH = 2.5 by OPA)	Methanol	Good peak shape but retention time precision fail
20 mM potassium dihydrogen phosphate + 1 mL triethylamine (TEA) (pH = 2.5 by OPA)	Methanol	Good peak shape with good precision

Table 3: Summery of stationary phase optimization.

Stationary phase	Dimension	Remarks
Acquity BEH C_8	(50 × 2.1) mm, 1.7 μm	No retention of DRO
Acquity BEH Phenyl	(50 × 2.1) mm, 1.7 μm	Peak eluted at dead peak with tailing
Acquity BEH C_8	(100 × 2.1) mm, 1.7 μm	Peak eluted at dead peak volume
Acquity BEH Phenyl	(100 × 2.1) mm, 1.7 μm	Peak tailing observed
Acquity BEH C_{18}	(100 × 2.1) mm, 1.7 μm	Satisfactory peak shape

Table 4: Accuracy study of dronedarone hydrochloride.

Level %	Number	Amount of drug added (μg/mL)	Amount of drug found (μg/mL)	Recovery (%)	Mean recovery (%)	RSD (%)
50	1	25.01	24.94	99.72	99.31	0.40
	2	25.14	24.87	98.92		
	3	25.26	25.08	99.28		
100	1	50.17	50.31	100.27	99.98	0.52
	2	50.02	50.18	100.31		
	3	50.11	49.80	99.38		
150	1	75.02	74.73	99.61	99.87	0.65
	2	75.13	74.68	99.40		
	3	75.16	75.63	100.62		

Table 5: Result of precision, linearity, LOD, and LOQ study.

Parameter	UPLC
Linearity (μg/mL)	0.38–90
Correlation coefficient (r^2)	0.999
Slope (m)	2303
Intercept (c)	2536
LOD (μg/mL)	0.1
LOQ (μg/mL)	0.38
Accuracy (% RSD)	0.40–0.65
Interday precision (RSD, $n = 6$)	0.59
Intraday precision (RSD, $n = 6$)	0.42

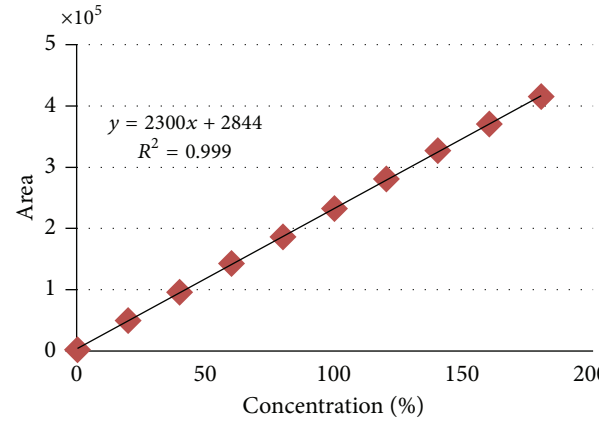

Figure 3: Linearity study of DRO.

Table 6: Result of robustness study.

Parameter	Mean R_t	% RSD	Mean area	% RSD
Flow rate				
0.36	1.56	0.10	240218	0.27
0.40	1.52	0.14	232437	0.24
0.44	1.49	0.12	220445	0.28
Mobile phase composition (buffer : methanol)				
(38 : 62)	1.41	0.14	217352	0.34
(40 : 60)	1.52	0.24	232416	0.27
(42 : 58)	1.58	0.11	245938	0.26
Column oven temperature				
25°C	1.61	0.21	248475	0.39
30°C	1.52	0.28	232387	0.32
35°C	1.39	0.17	212868	0.21

computed and found to be $Y = 2300x + 2844$, $r^2 = 0.999$ (Figure 3). Where y is the mean peak area, x is the concentration of DRO in μg/mL and r is the correlation coefficient. These results confirm the linearity relation between the mean peak area and concentration as well as the sensitivity of the method. The summary of linearity study is represented in Table 5.

4.5. Robustness and Ruggedness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate change in method parameters and provides an indication of its reliability during normal usage. On deliberate variation in chromatographic condition (flow rate, temperature, and mobile phase composition) of DRO analysis, the value of assay (with %RSD < 2), tailing factor, and theoretical plates remained near to those of the result obtained by the optimized chromatographic condition. The summaries of robustness and ruggedness studies are represented in Tables 6 and 7, respectively.

4.6. Solution and Mobile Phase Stability. At the specified time interval, % assay of DRO obtained from drug solution stability and mobile phase stability was within accepted criteria. The results also confirmed that the standard solution of drug and mobile phase were stable at least for 24 hours

Table 7: Ruggedness study of DRO.

Variable	R_t	% RSD	Area	% RSD	Theoretical plate	% RSD	Asymmetry	% RSD
Analyst	1.52	0.87	242374	1.48	3447	1.69	1.32	1.36
Column	1.52	0.42	242463	1.26	3559	1.82	1.28	1.22

Table 8: Solution and mobile phase stability study.

	Initial	6 hr	12 hr	24 hr	48 hr
% assay for solution stability	99.82	100.11	99.52	99.67	99.38
% assay for mobile phase stability	100.17	99.83	100.06	99.32	99.74

Table 9: Stress degradation study.

Stress condition	Time	Purity angle	Purity threshold	% degradation
Acid hydrolysis	5 hr	0.404	0.978	3.24
Base hydrolysis	1 hr	1.045	12.715	24.07
Oxidation	1 hr	3.297	11.91	18.56
Thermal	5 days	0.573	0.944	2.37
Photo	5 days	0.764	1.363	0.48

Table 10: System suitability parameter.

Parameter	UPLC
Retention time	1.52
Asymmetric factor	1.18
Theoretical plate	3482

Table 11: Assay results of tablet dosage form using proposed method.

Tablet	Labeled amount	% amount recovered	% recovery
DRO	400	399.68	99.92%

during the assay analysis. The summary of solution and mobile phase stability is illustrated in Table 8.

4.7. Force Degradation Study. Force degradation study was carried out by subjecting the drug to acid, alkali, chemical oxidation, dry heat degradation, and photo (sunlight) degradation condition. The DRO was found to be stable to acid hydrolysis, dry heat degradation, and photolytic condition. Degradation was observed in alkali hydrolysis and oxidative stress condition. The summary of force degradation study is represented in Table 9. The figure of degradation study is represented in Figures 4, 5, 6, 7, 8, 9, and 10.

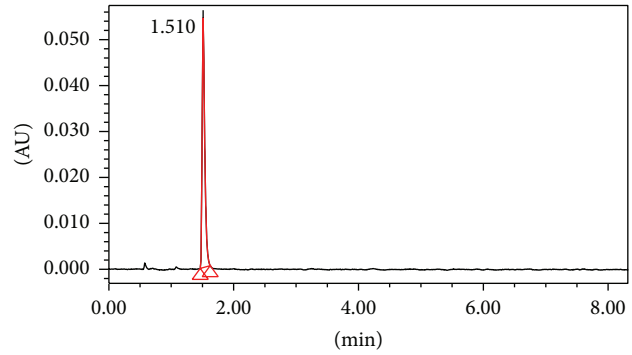

Figure 4: Acid degradation study of DRO.

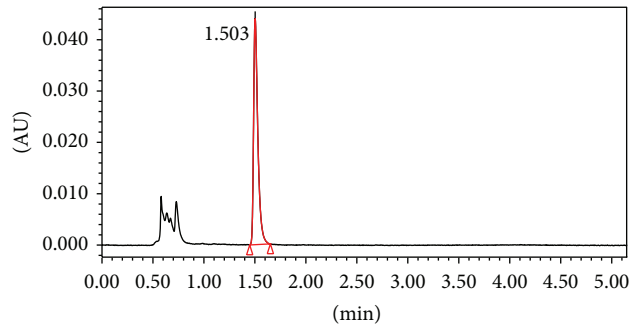

Figure 5: Alkali degradation study of DRO.

5. Conclusion

A stability-indicating reverse phase UPLC method has been developed and validated for the determination of DRO in bulk and their pharmaceutical dosage form. Compared to reported method [2–5], the proposed method is very short and sensitive. The result of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability indicating. Apart from alkali hydrolysis and oxidative stress degradation condition, the drug was found to be stable in the rest of forced degradation conditions. The proposed method was validated and found to be simple, accurate, precises and specific. The method was successfully applied for the estimation of tablet dosage form (Table 11). The proposed UPLC method can be applied for the analysis of sample obtained during stability study to predict expiration dates of pharmaceuticals. Hence, the method can be used in routine analysis of DRO drug in quality control laboratories.

Table 12: Comparison of proposed UPLC method with published HPLC method.

Method	Run time (Min)	Elution time (Min)	LOD	LOQ	Reference
HPLC	10	3.02	0.87 μg/mL	2.65 μg/mL	[3]
HPLC	25	7.3	NA	NA	[4]
HPTLC	NA	$R_f = 0.57$	34.6 ng/band	75.2 ng/band	[5]
UPLC	3	1.5	0.1 μg/mL	0.38 μg/mL	Proposed method

NA: not available.

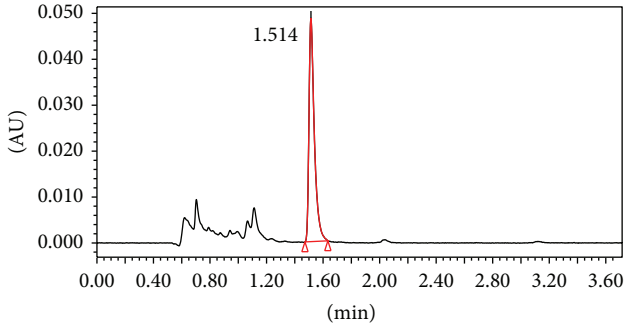

Figure 6: H_2O_2 degradation study of DRO.

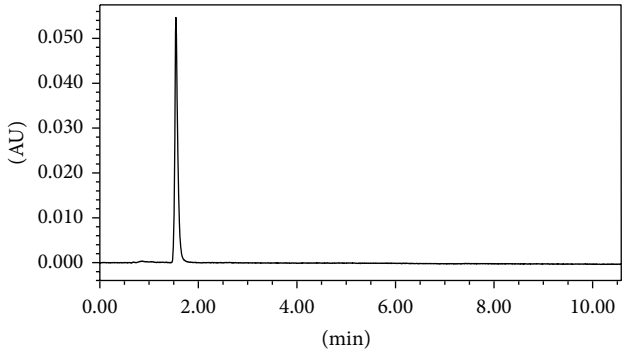

Figure 7: Thermal degradation study of DRO.

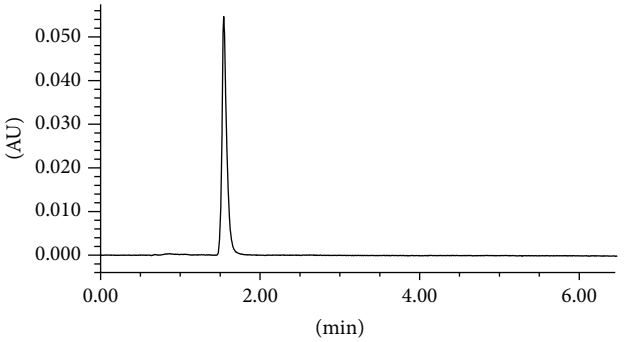

Figure 8: Photo degradation study of DRO.

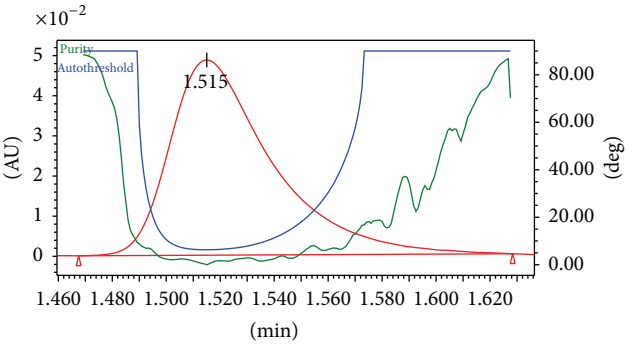

Figure 9: Peak purity result of alkali degradation.

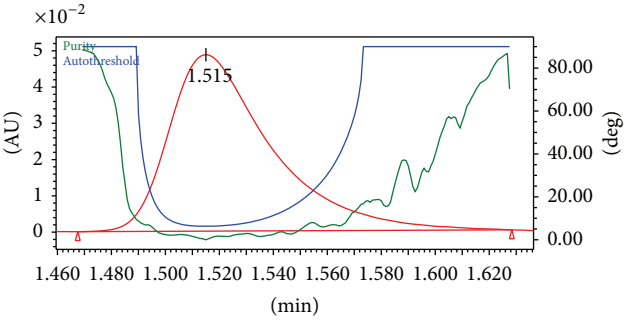

Figure 10: Peak purity result of H_2O_2 degradation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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