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A stability indicating RP-UPLC method for estimation of dronedrone related impurities in bulk drugs and its pharmaceutical dosage forms

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ABSTRACT

The objective of the current study was to develop a validated, specific and stability-indicating reverse phase UPLC method for the quantitative determination of Dronedrone related substances. The determination was done for active pharmaceutical ingredient and its pharmaceutical dosage forms in the presence of degradation products, and its process-related impurities. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation per International Conference on Harmonization (ICH) prescribed stress conditions to show the stability-indicating power of the method. Significant degradation was observed during acid, oxidative and photo stress studies. In the developed UPLC method, the resolution between Dronedarone and its process-related impurities was found to be greater than 2.0. Regression analysis shows an r^2 value (correlation coefficient) of greater than 0.999 for all the four impurities. The chromatographic separation was achieved on a C8 stationary phase. The method employed a Isocratic elution and the detection wavelength was set at 290 nm. The stress samples were assayed against a qualified reference standard and the mass balance was found to be close to 99.7%. The developed UPLC method was validated with respect to linearity, accuracy, precision and robustness.

Keywords: Dronedarone, UPLC, Forced Degradation, Validation, Stability Indicating.

INTRODUCTION

Dronedarone is a drug mainly for the indication of cardiac arrhythmias, Chemically as N-(2Butyl-3-(p-(3-(dibutylamino)propoxy)benzoyl)-5-benzofuranyl)Methanesulfonamide and its structural formula is C31H44N2O5S. Multaq is generic name for Dronedarone, is 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 to maintain normal rhythm [1].

In atrial fibrillation, atria beat more than 300 times per minute. The arrhythmatous condition needs to be controlled, as humans cannot withstand this rapid and chaotic beating of the heart. With regards to management of atrial fibrillation five major classes (I,II,III,IV,V) drugs and their analytical methods available[2-4]. Dronedarone is the most recent class III anti arrhythmic drugs (AAD). It was approved by US-FDA and is available in the USA as Multaq tablets (400 mg). Dronedarone falls under the category of multiple ion channel blocker. It mainly targets the repolarisation currents, making them less active and hence pro- longing the action potential duration (APD). Dronedarone also exhibits antiadrenergic activity, thus reducing the pace of the pacemaker. Dronedarone has been proven to be a safe and efficacious AAD, evidenced by both animal and human studies. These studies showed that there was prolongation of the APD and absence of QT interval prolongation with long term administration of the drug. Also there was reduced thyroid hormone receptor expression. Dronedarone is significantly safer and effective

in maintaining the sinus rhythm and reducing the ventricular proarrhythmias, justifying it for the long term treatment of atrial fibrillation compared to other anti arrhythmic drugs [5-7].

Few HPLC methods were available in literature for the analysis of Dronedarone includes simultaneous determination of Dronedarone and its active metabolite debutyldronedarone in human plasma by liquid chromatography tandem mass spectrometry: Application to a pharmacokinetic study [8], Determination of the class III anti arrhythmic drugs Dronedarone and amiodarone, and their principal metabolites in plasma and myocardium by high-performance liquid chromatography and UV-detection [9], RP-HPLC method development and validation of Dronedarone HCl in its Pure form and tablet dosage form-that speaks about the content of Dronedarone in bulk and pharmaceutical dosage forms [10-11]. No HPLC methods were reported in major pharmacopeia like USP, EP, JP and BP.

Extensive literature survey reveals there is no rapid stability-indicating UPLC method for determination of related substances in bulk drugs and pharmaceutical dosage forms. The purpose of the present research work was to develop a suitable, single and rapid stability-indicating UPLC method for the determination of Dronedarone related substances.

Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the de termination of Dronedarone and all the four impurities in bulk drug samples and in pharmaceutical dosage forms along with method validation as per ICH norms. The stability tests were also performed on both drug substances and drug product as per ICH norms [12-14].

MATERIALS AND METHODS

3.1 Chemicals:

Samples of Dronedarone and its related impurities were obtained sample from Unimark remedies (Mumbai, India) (Figure 1). Commercially available 400 mg of Dronedarone tablets (Multaq®) were purchased from Korean market. HPLC grades Acetonitrile, Methanol, analytical reagent grade Potassium dihydrogen phosphate were purchased from Merck.

Dronedrone: N-[2-butyl-3-[4-[3-(dibutylamino) propoxy] benzoyl]-5Benzofuranyl] Methanesulfonamide hydrochloride, CAS Number: [141625-93-6]. Molecular formula : C31H45ClN2O5S, Molecular weight : 593.22.

DRN Amino impurity(Impurity-1): 2-butyl-3-(4-(3-dibutylamino)propoxy)benzoyl)-5-amino benzofuran. This is one of the intermediate which can be carried forward to finish API if it remains Unreacted during mesylation reaction.

DRN Nitro impurity(Impurity-2): 2-butyl-3-(4-(3-dibutylamino)propoxy)benzoyl)-5-nitrobenzofuran. This is one of the intermediate which can be carried forward to finish API if it remains unreacted during hydrogenation of nitro group.

4-HNBF impurity(Impurity-3): 2-butyl-3-(4-hydroxybenzoyl)-5-nitrobenzofuran. This is a starting material, which can be carried forward to finish API if remains unreacted during condensation with DRN Chloro.

Dimesylate impurity(Impurity-4): 2-butyl-3-(4-(3-dibutylamino) propoxy) benzoyl) -5-benzo furanyl) dimethane sulfonam. This impurity is a process related impurity.

3.2 Equipments:

The Acquity UPLC system with Empower software used for method development, forced degradation studies (Waters Corporation, MA, and USA). The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co). Water bath equipped with temperature controller was used to carry out degradation studies for all solution. Photo stability studies were carried out in a photo stability chamber (Newtronic, Mumbai, India). Thermal stability studies were performed in a dry air oven (Biotechnics Mumbai, India).

3.3 Chromatographic conditions:

The chromatographic column used was Acquity UPLC HSS C8 column (100 × 2.1) mm with 1.7 µm particles. Buffer consists of a mixture of 5.44 Grams of Potassium dihydrogen phosphate pH adjusted to 2.5 using Diluted phosphoric acid. The mobile phase consists of buffer and acetonitrile at 1:1 ratio. The flow rate of the mobile phase was 0.6 mL·min–1. The column temperature was maintained at 45°C and the detection was monitored at a

wavelength of 290 nm. The injection volume was 0.5µL. Methanol was used as diluent. The concentration is 2000 ppm .

3.4. Preparation of Solutions

3.4.1. Preparation of Standard Solutions

A stock solution of Dronedarone (2.5 mg·mL–1) was prepared by dissolving appropriate amount in the methanol. Working solutions were prepared from above stock solution for related substances and stock solution of impurities (mixture of imp-1, imp-2 imp-3 and imp-4) at a concentration of 250 µg·mL–1 was also prepared in methanol.

3.4.2. Preparation of Sample Solutions

Multaq® tablets contain 400 mg of Dronedarone. The inactive ingredients present in Multaq® were hypromellose, starch maize, crospovidone, poloxamer, lactose, silica—colloidal anhydrous, magnesium stearate, titanium dioxide, macrogol 6000 and carnauba wax. Twenty Multaq tablets (400 mg) were weighed and the average weight was calculated. The tablets were powdered in a mortar and a sample of the powder equivalent to 400 mg of the active pharmaceutical ingredient (Dronedarone) was transferred to 200 mL volumetric flask. Approximately 150 mL methanol was added and the flask was placed on rotatory shaker for 10 min and sonicated for 30 min to dissolve the material completely. The solution was then diluted to 200 mL and centrifuged at 3000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm pore size Syringe filter. The filtrate was used as sample solution.

3.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the Dronedarone in the presence of its impurities namely imp-1, imp-2, imp-3, imp- 4 and degradation products was determined by developed UPLC method. Forced degradation studies were also performed on Dronedarone to provide an indication of the stability indicating property and specificity of the proposed method [9-12]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60°C), acid hydrolysis (1 N HCl), base hydrolysis (1 N NaOH) and oxidation (10% H2O2). For heat study period was 1 day and for light studies, study period was to illuminate the sample for 1.2 million Lux hours, where as for acid, base and peroxide hydrolysis the test period was 24 h. Peak purity of stressed samples of Dronedarone was checked by using Photo diode array detector of Waters Corporation, MA, USA.

3.6. Analytical Method Validation

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and Solution stability.

3.6.1. Precision

The precision of the related substance method was checked by injecting six individual preparations of (250 mg·mL–1) Dronedarone spiked with 0.30% each imp-1, imp-2, imp-3, and imp-4. The %RSD area of each imp-1, imp-2, imp-3, and imp-4 was calculated. Precision study was also determined by performing the same procedures on a different day (intraday precision).

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

3.6.2. Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for imp-1, imp-2, imp-3, and imp-4 estimated By using the linearity slope calculations of imp-1, imp-2, imp-3, and imp-4.

3.6.3. Linearity and Range

A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels. From 10% to 400% of the permitted maximum level of the impurity was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses.

Upper and lower levels of range were also established.

3.6.4. Accuracy
The accuracy of the related substance method was evaluated in triplicate sample preparations at 10% to 400% of the analyte concentration (5 ppm). The percentage of recoveries for imp-1, imp-2, imp-3and imp-4 were calculated.

3.6.5. Robustness
To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (*Rs*) between Dronedarone imp-1, imp-2, imp-3, and imp-4 were evaluated. The flow rate of the mobile phase was 0.6 mL·min–1. To study the effect of flow rate on the developed method, 0.05 units of flow was changed (*i.e.* 0.55 and 0.65 mL·min–1). The effect of column temperature on the developed method was studied at 40°C and 50°C instead of 45°C. The effect of % Acetonitrile on resolution of impurities was studied by varying ±5% (*i.e.* buffer % altered from 50% to 45% and 55%). In the all above varied conditions, the components of the mobile phase were held constant.

3.6.6. Solution Stability and Mobile Phase Stability
The solution stability of Dronedarone and its related impurities were carried out by leaving both spiked sample solution in tightly capped volumetric flask at room temperature for 48 h.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions, at 24 hrs and 48 Hrs. Content of imp-1, imp-2, imp-3, and imp-4 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

4.1. Method Development and Optimization
The UPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Dronedarone from its process related impurities and degradation products that comply with the general requirements for system suitability. Initial trials were done with 0.01M KH_2PO_4 Buffer concentration at flow rate 0.6 mL·min–1. Longer retention times and poor peak shape of Dronedarone was problem with the above method.

Different columns such as HSS C18, BEH C18 and different buffers such as potassium dihydrogen phosphate, Trifluoroacetic acid were also tried with different isocratic and gradient methods to achieve the best chromatographic separation. But long retention times and poor peak shapes were still unavoidable. With 0.1% trifluoroacetic acid, impurity-4 and Main peak are co-eluting and long retention times are seen. Studied the separation and peak shape by varying pH from 2.5 to 7.0 with phosphate buffer, and observed that, as the pH is increasing towards 7.0, peaks were strongly retaining. Also at higher pH, Dronedarone and impurity-4 are co eluting. Added triethylamine to the mobile phase to study the separation on a HSS,C18 column at 6.5 pH. The peak shapes significantly improved but Dronedarone and impurity-4 are still co-eluting. Changed the column to BEH, C-8,2.1x100 mm,1.7 µm and obtained better separations and peak shapes with 1:1 Buffer and acetonitrile. The % of Acetonitrile played a key role in the retention times and resolution between impurities.

After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug degradation products and four known impurities.

Using the optimized conditions, Dronedarone and its known impurities were well separated with a resolution of greater than 2. The Chromatogram was given in figure1

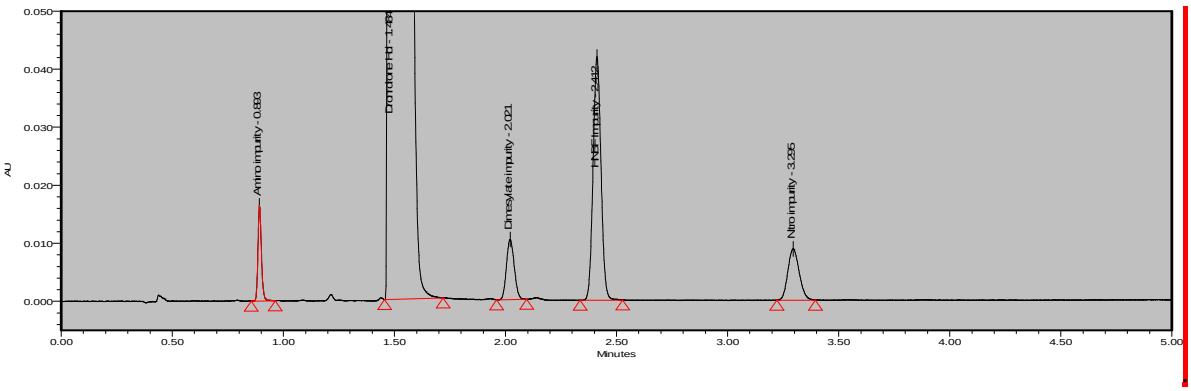

Figure 1: Impurity mixture chromatogram

4.2. Results of Forced Degradation Studies

The forced degradations and the % degradations and Peak purities are given in Table

Table:1 forced degradation conditions and results

1.Conditions	%Degradation	Peak purity
Acid degradation 1N Hcl,60°C,24 Hrs	0.5%	Passed
Base Degradation 1N NaoH,60°C 24 Hrs	81%	Passed
Peroxide degradation 10%,50°C ,24Hrs	0.7%	Passed
Photo degradation 1.2 million Lux hours&200 Whr	0.25%	Passed
Thermal degradation 50°C,48 Hrs	0.25%	Passed
Hydrolysis Water, 50°C,24Hrs	0.25%	Passed

4.3. Method Validation

4.3.1. Precision

The %RSD of Impurities in precision study was Amino(1.23),Dimesylate(3.68),HNBF(2.80) and Nitro(2.59) respectively.

In intermediate precision study was Amino(3.57),Dimesylate(3.46),HNBF(2.80) and Nitro(2.59) in related substance method precision study were within 5.0, confirming the good precision of the developed analytical method.

4.3.2. Sensitivity

The limit of detection and limit of quantification imp-1, imp-2, imp-3, and imp-4 were Listed in below table 3. The precision at LOQ concentration for imp-1, imp-2, imp-3, and imp-4 were below 5%.

Table:2: LOD and LOQ Values of impurities

Impurity Name	LOD (ppm)	LOQ(ppm)
1.Amino impurity	0.20	0.61
2.Dimesylate impurity	0.49	1.50
3.HNBF Impurity	0.23	0.69
4.Nitro impurity	0.11	0.32

4.3.3. Linearity and Range

Linear calibration plot for related substance method was obtained over the calibration ranges tested, *i.e*. LOQ to 10 % to 400 %. The correlation coefficient obtained was greater than 0.999 for all impurities. The result given in table.

Table 3: Linearity concentrations and R^2 Values

Impurity Name	Conc(ppm)	R^2 value
1.Amino impurity	0.8 to 40	0.9996
2.Dimesylate impurity	0.8 to 40	0.9998
3.HNBF Impurity	0.8 to 40	0.9997
4.Nitro impurity	0.8 to 42	0.9998

The range of the method was found from 10% to 400% of the 10 ppm concentration

4.3.4. Accuracy

The percentage recovery of imp-1, imp-2, imp-3, and imp-4 in Formulation samples ranged from 85% to 115% mentioned in the below table(4-5).

Table 4:Amino,Dimesylate imp.recovery.

Impurity	%Level	%Recovery
Amino impurity	10	111.9
	20	111.8
	50	110.5
	100	108.6
	200	95.6
	400	89.0
Dimesylate impurity	10	88.4
	20	92.1
	50	95.1
	100	95.4
	200	96.6
	400	98.4

Table 5:HNBF, Nitro impurity recovery.

Impurity	%Level	%Recovery
HNBF Impurity	10	99.1
	20	97.8
	50	101.4
	100	100.1
	200	101.2
	400	102.2
Nitro impurity	10	98.5
	20	96.0
	50	102.1
	100	100.5
	200	101.2
	400	102.0

4.3.5. Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, column temperature) revealed that the resolution between closely eluting impurities, namely imp-1, imp-2, imp-3, and imp-4 was always greater than 2.0, illustrating the robustness of the method.

4.3.6. Solution Stability and Mobile Phase Stability

No significant changes were observed in the content of imp-1, imp-2, imp-3, and imp-4 during solu- tion stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions were stable up to the study period of 48 h.

The values are given in table.

Table 6: Solution stability data.

Impurity	Amino	Dimesylate	HNBF	Nitro
Initial	0.26	0.30	0.30	0.27
24Hrs	0.25	0.29	0.29	0.26
%Difference	0.01	0.01	0.01	0.01
48 Hrs	0.25	0.29	0.29	0.26
%Difference	0.01	0.01	0.01	0.01

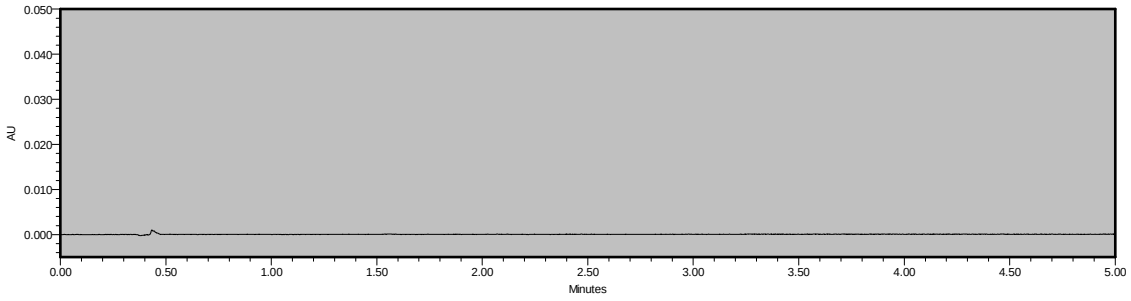

Figure 2: Blank chromatogram

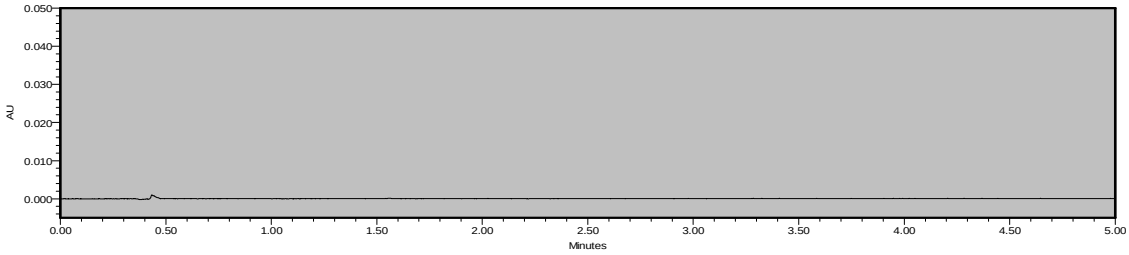

Figure 3: Placebo chromatogram

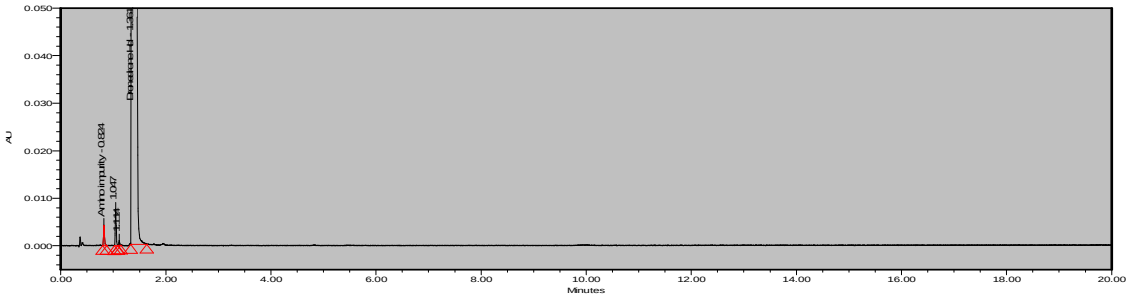

Figure 4: Acid degraded sample .

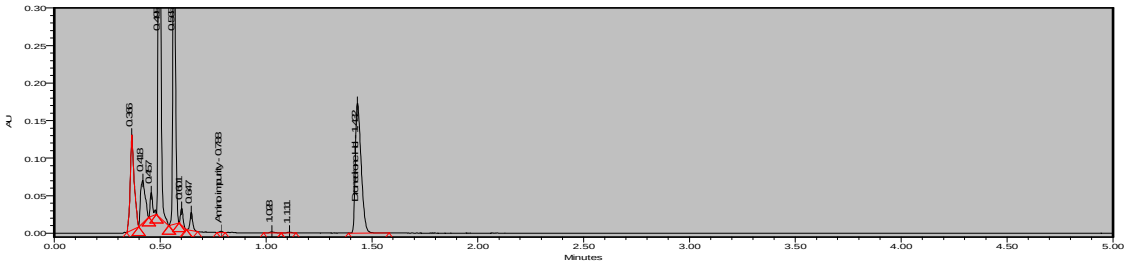

Figure 5: Base degraded sample .

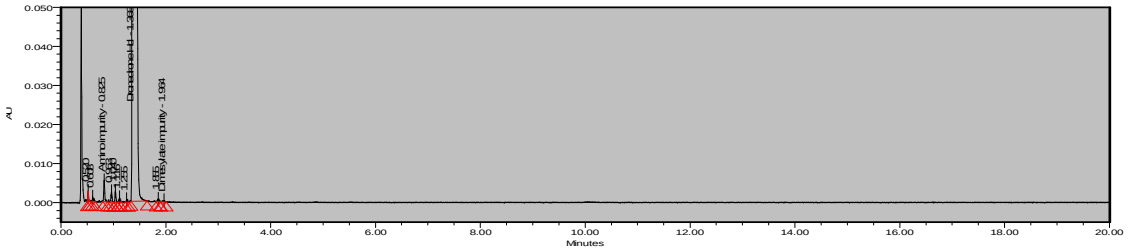

Figure 6: Peroxide degraded sample .

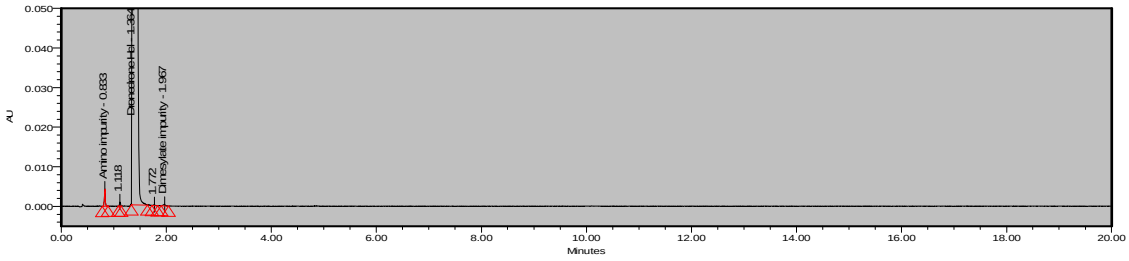

Figure 7: Water degraded sample .

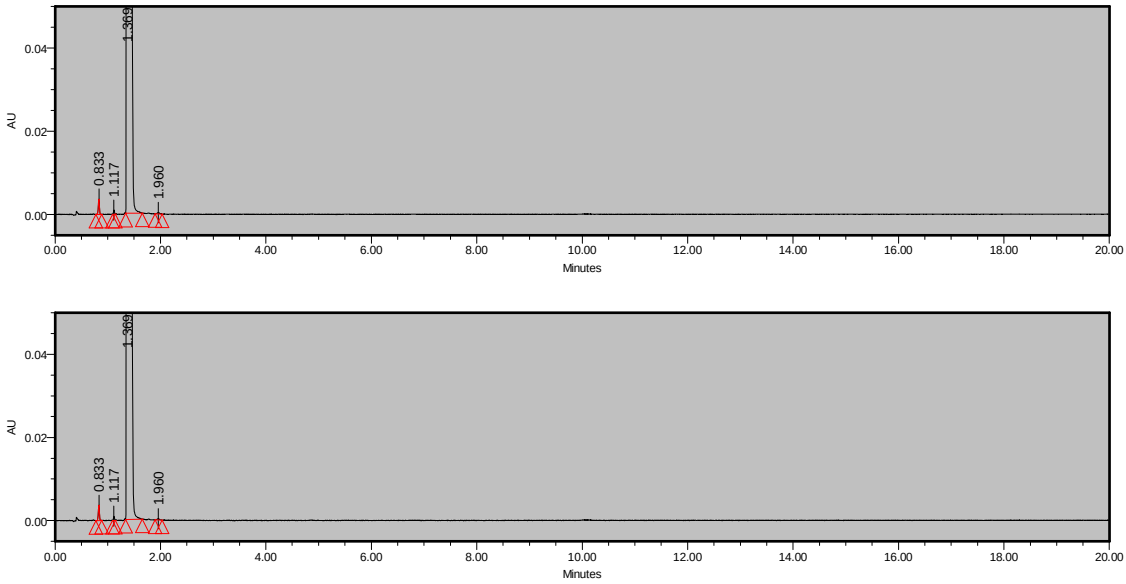

Figure 8: Light degraded sample .

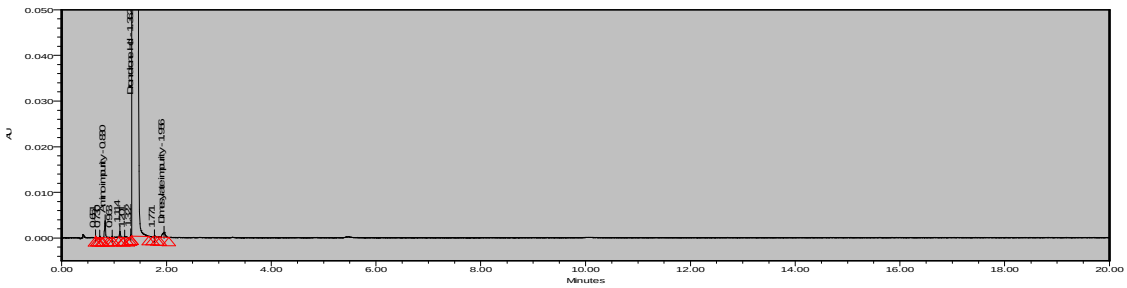

Figure 9: Multaq sample chromatogram.

CONCLUSION

The UPLC method developed for quantitative and related substance determination of Dronedarone in both bulk drugs and pharmaceutical dosage forms are precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Dronedarone samples.

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