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Der Pharma Chemica, 2013, 5(1):189-195
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Forced degradation study on dronedarone and application of validated stability-indicating HPLC-UV method in stability testing of dronedarone tablets

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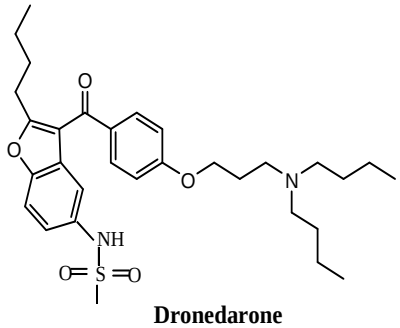
ABSTRACT

A stability indicating reverse phase high performance liquid chromatography method has been developed for the estimation of Dronedarone in tablet dosage form. A symmetry C18 column having dimensions of 150×4.6mm and 3µm particle size, with mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 60:40 v/v was used. P^H of mobile phase was adjusted to 6.8 with sodium hydroxide. The flow rate was 1ml/min and the column effluents were monitored at 290 nm. The retention time for Dronedarone was found to be 2.65min. The proposed method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness. The LOD and LOQ were found to be 0.018µg/ml and 0.06µg/ml respectively. The method was found to be linear in the range of 10 – 50 µg/ml with regression coefficient (r = 0.9996). The % recovery for the Dronedarone was found to be 99.6 and the forced degradation studies were also carried out as per ICH guidelines. There was complete separation of degradation peak and Dronedarone peak, which demonstrate the specificity of assay method for estimation of Dronedarone in the presence of its degradation products; it can be employed as a stability indicating one. Due to simplicity, rapidity and accuracy of the proposed stability indicating HPLC method is useful for quality control analysis.

Keywords: Dronedarone, stability indicating HPLC, forced degradation, validation, Formulation.

INTRODUCTION

Chemically Dronedarone is a benzofuran derivative related to amiodarone, a popular antiarrhythmic [1]. Dronedarone is a drug by sanofi-aventis, mainly for the indication of cardiac arrhythmias. In Dronedarone, the iodine moieties are not present so reducing toxic effects on the thyroid and other organs. A methyl sulfonamide group is added to reduce solubility in fats and hepatic impairment. Dronedarone displays amiodarone like class III antiarrgythmic activity in vitro and in clinical trials. The drug also appears to exhibits activity in each of the four Vaughan-Williams antiarrhythmic classes. Synthetic name is N-(2-butyl – 3 – (p – (3-(dibutyl amino) propoxyl) benzoyl)-5-benzofuranyl) methane sulfonamide.



Dronedarone

Literature survey reveals a very few HPLC methods for the analysis of Dronedarone includes, simultaneous determination of Dronedarone and its active metabolite debutyl Dronedarone in human plasma by liquid chromatography-tandem mass spectrometry application to a pharmacokinetic study [2], hplc chromatographic methods [3] for the determination of Dronedarone in its pure form and tablet dosage form and one method for Spectrophotometric [4] estimation and stability indicating HPLC method for Dronedarone in bulk drugs and Pharmaceutical dosage forms [5]. The existing stability indicating method requires much time for analysis. So it is needed to develop stability study with less time of analysis. This paper deals with the forced degradation of Dronedarone under stress conditions like acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic stress and also deals with validation of the developed method for the assay of Dronedarone from its bulk drug and in pharmaceutical dosage forms. This proposed method shows good linearity, accuracy, sensitivity and less time for the analysis of the existing methods. So the proposed method is rapid stability-indicating one for the determination of Dronedarone in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on liquid chromatography water separation 2695 DAD or UV detector module equipped with automatic injector with injection volume 20µl and 2487 pump. The RP C18 symmetry column 150×4.6mm and 3µm particle size was used. The HPLC system was equipped with empower software.

Chemicals and reagents

Dronedarone was obtained as a gift sample from Pharmatrain, an analytical testing centre, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens) sodium dihydrogen phosphate, sodium hydroxide were of analytical grade and supplied by M/S S.D Fine chem. limited, Mumbai. Commercially available Dronedarone tablets were procured from local market.

Chromatographic conditions

Mobile phase consists of buffer and acetonitrile in the ratio of 40:60. Buffer was prepared by dissolving 2.5 mg of sodium dihydrogen phosphate in 1000 ml HPLC water and the pH is adjusted to 6.8 with sodium hydroxide and filtered through 0.45µ membrane filter. The mobile phase was pumped from the solvent reservoir in the ratio of 40:60 to the column at a flow rate 1ml/min, whereas runtime was set to 6 min. The column was maintained at ambient and the volume of each injection was 20µl. Prior to injection of the solutions, column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluent were monitored at 290nm.

Standard preparation

10 mg of Dronedarone was weighed and transferred into 10ml volumetric flask containing 7 ml of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of 1 mg/ml solution. Further pipette out 0.3 ml of the above stock solution into 10ml volumetric flask and dilute up to the mark with diluents.

Sample preparation

Weigh about five Dronedarone tablets and calculate the average weight. Accurately weigh and transfer the sample, equivalent to 10mg of Dronedarone into 10 ml volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with mobile phase. Mix well and filter through 0.45µm filter.

Method Validation
The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines

System suitability
A standard solution of Dronedarone was prepared as per procedure and was injected 3times into the HPLC 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. The %RSD for the retention times of principal peak from 3 replicate injections of each standard solution should be not more than 2%. The number of theoretical plates for Dronedarone peaks should be not less than 2000. The system suitability parameters are tabulated in Table-1 and chromatogram was shown in Figure -1

Table-1: System suitability parameters

Retention time	2.65min
USP plate count	2452.6
USP tailing	1.6
LOD	0.018
LOQ	0.06
Peak area	4852847.6
%RSD	0.33

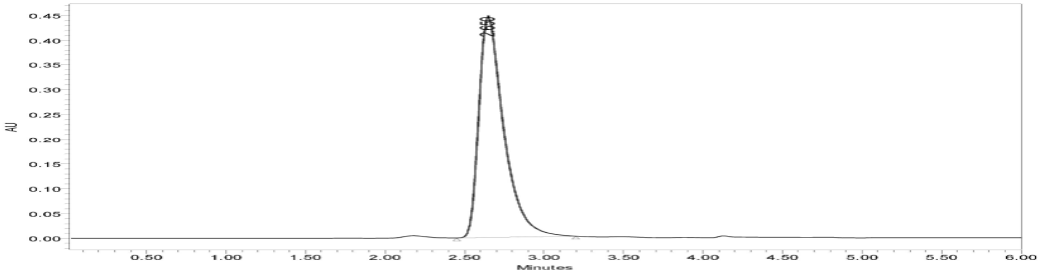


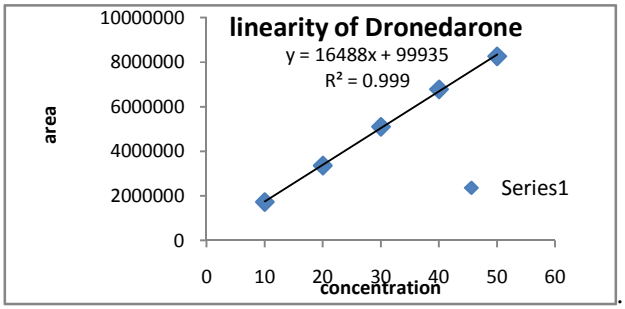
Figure 1: **chromatogram of Dronedarone**

Linearity
Several aliquots of standard solutions of Dronedarone was taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of Dronedarone is 10 -50 µg/ml. Evaluation of drug was performed with DAD detector at 290nm. Peak area is recorded for all the peaks. The slope and intercept value for calibration curve was Y = 164888.8 x + 99935.3 (r^2=0.999). The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range 10-50µg/ml. Regression data was shown in Table-2 and the linearity curve is shown in Figure-2.

Table- 2: Regression data of proposed method

Concentration (µg/ml)	**Peak area**
10	1726565
20	3360963
30	5102798
40	6782358
50	8260305
Concentration range	10-50µg/ml
Slope	164888.8
Intercept	99935.3
Correlation coefficient	0.99963

Figure-2: linearity curve



Precision

The precision of the method was demonstrated by inter day and intraday variation studies. In intraday studies five repeated injections of working standard solutions were made and response factor of drug peaks and % RSD were calculated. In the inter day variation studies, five repeated injections of standard working solutions were made for different day and different make column of same dimensions. The response factor of drug peaks and %RSD were calculated and present in Table-3. From the data, the developed RP-HPLC method was found to be precise.

Table- 3: Precision and intermediate results

Concentration of Dronedarone {30µg/ml}	Peak area	
	Intra day	Inter day
Injection 1	4828752	4869367
2	4822607	4836919
3	4883510	4852257
4	4874044	4862611
5	4748311	4871091
Average	4831445	4858449
S.D	53670.4	14126.3
%RSD	1.11	0.29

Accuracy

The accuracy of the method was evaluated by determination of recovery of Dronedarone at three levels of concentrations. The sample solutions were spiked with Dronedarone standard solutions corresponding to 50%, 100% and 150% of nominal analytical concentrations. The results showed good recovery within limits (99.8% to 101.7%) The results are tabulated in Table-4

Table-4: Accuracy results

Concentration %	Peak area mg	Amount added mg	Amount found	Recovery %	Mean Recovery %
50%	2711428	5	5.08	101.7	100.5%
100%	5319083	10	9.98	99.8	
150%	7999718	15	15.0	100	

LOD and LOQ

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentrations of the standard solutions found to be 0.018µg/ml and 0.06µg/ml respectively. The LOD and LOQ values reveal that the developed method shows very good sensitivity.

Ruggedness and Robustness

Ruggedness test was determined between two columns or two analysts or two instruments. Robustness of the proposed method was determined by small deliberate changes in flow rate, change in composition of mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of RSD indicating that the method was Rugged and Robust. On evaluation of these results, it can be concluded that the variation of flow rate and variation of organic composition in mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in flow rate slightly. The results are tabulated in Table-5

Table -5: Robustness results of the method

Change in flow rate	USP plate count	USP tailing	Retention time
0.8	2447.4	1.7	3.092
1.0	2643.6	1.6	2.650
1.2	2272.9	1.5	2.337
change in organic composition in the mobile phase	**USP plate count**	**USP tailing**	**Retention time**
10% less	3055.6	1.2	2.805
Actual	2643.6	1.6	2.650
10% more	2504.4	1.6	2.600

Application of proposed method to solid dosage form

The assay of commercial Dronedarone tablets showed that the developed method, shown in Table-6 was accurate and reliable with mean drug content of 99.6% of the labeled claim. No interference peaks were found in the chromatogram and indicating that the determination of the drug content was free from interference by excipients.

Table-6: Assay results

Label claim in mg	Amount found in mg	% recovery
400	398.48	99.6

Degradation study

The degradation samples were prepared by transferring powdered tablets, equivalent to 1000µg/ml Dronedarone into a 10ml volumetric flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were allowed to room temperature and diluted with mobile phase up to the mark. Filter the solution with 0.45 microns syringe filters and place in vials. Specific conditions were described below.

Hydrolytic degradation under acidic condition: Acidic degradation study was performed by treating the drug content in 3ml of 0.1 N HCl at normal condition for 90 min and then the mixture was neutralized with 0.1 N NaOH
Hydrolytic degradation under alkaline condition: Alkaline degradation study was performed by treating the drug content in 3ml of 0.1N NaOH at normal condition for 90 min. and then the mixture was neutralized with 0.1N HCl

Thermal induced degradation: Thermal degradation study was performed by reflux the drug content for 1hr at 60 ^{0} C temp

Photolytic degradation: Photolytic degradation was performed by exposing the drug content in UV light for 60min.
Oxidative degradation: Oxidative degradation study was performed by treating the drug content in 3ml of 3%v/v H_2O_2 for 15 min at room temperature.

The results of degradation studies were tabulated in Table 7 and the chromatograms are shown in Figures: 3 to 7

Degradation chromatograms for Dronedarone:

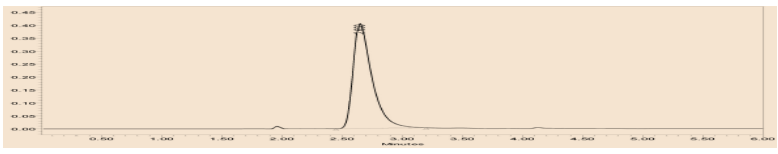


Figure 3: base degradation

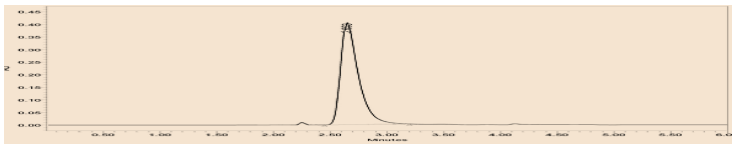


Figure 4: acid degradation

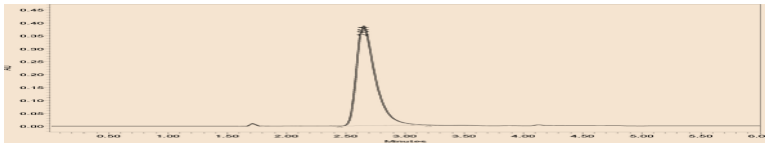


Figure 5: Thermal degradation

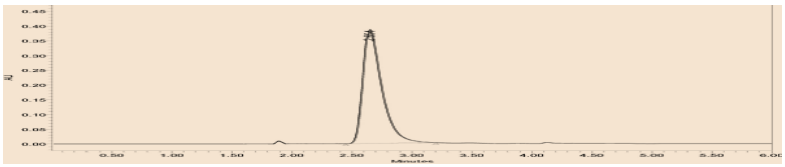


Figure 6: Pholytic degradation

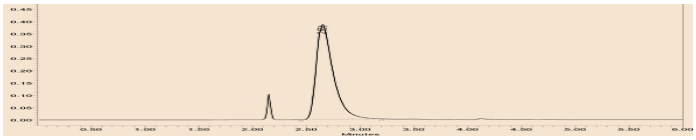


Figure 7: oxidative degradation

Table-7: Degradation characteristics of Dronedarone

Degradation parameter	Degradation Time	Results of Degradation							
		Peak area of degradated product	Peak area of standard	% of recovery	% of Degradation	Purity angle	Purity threshold	Tailing factor	Plate count
Acid Degradation (0.1N HCl)	90 min	4528296	4869136	92.9	7	0.56	0.61	1.5	2426.8
Base Degradation (0.1N NaOH	90 min	4430914	4869136	91.0	9	0.42	0.55	1.4	2513.6
Peroxide Degradation (3% H_2O_2)	15 min.	4090074	4869136	83.9	16	0.12	0.31	1.5	2347.9
Thermal Degradation	60 min.	4284840	4869136	88.0	12	0.39	0.46	1.5	2369.8
Photolytic Degradation	60 min.	4236149	4869136	87.0	13	0.46	0.51	1.5	2486.9

RESULTS AND DISCUSSION

The development of HPLC methods for the determination of drugs has received great attention in analytical research because of their importance in quality control. The technique is unique, versatile, universal, and basic well utilized the researchers because of its ease of operation. The main objective of method development is to determine the drug content of formulations as well as purity. In analytical research, the time and cost of method development and validation are of great importance. The objective of this study was to develop and validate a simple, sensitive, rapid, economic and accurate RP-HPLC method for the estimation of Dronedarone in commercial tablet products. The stability indicating method is a validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanism, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product. Stress testing is a part of development strategy under the ICH requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies. It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values. It is also recommended that analysis of stability sample should be done through the use of a validated stability testing methods.

From the typical chromatogram of Dronedarone shown in Figure1, it was found that the retention time was 2.65 min. The mobile phase acetonitrile and phosphate buffer in the ratio of 60:40 v/v at pH 6.8 was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.999) was observed between the concentration range 10-50µg/ml. Low values of S.D are indicative of high precision of the method. The assay of Dronedarone tablets was found to be 99.6%. From the recovery studies, it was found that about 100.5% of Dronedarone was recovered which indicates high accuracy of the method. The results of LOD and LOQ indicate that the method is reliable and the forced degradation studies were also carried out as per *ICH* guidelines. There was complete separation of degradation and Dronedarone peak, which demonstrate the specificity of assay method for estimation of Dronedarone in the presence of its degradation products; it can be employed as a stability indicating one. This demonstrates that the developed stability indicating HPLC method is simple, linear, accurate, sensitive and reproducible.

CONCLUSION

This study presents a simple and validated stability-indicating HPLC method for estimation of Dronedarone in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability indicating. The method could be applied with success even to the analysis of marketed products of Dronedarone tablet formulation and has no interference was observed due to excipients or other components present.

Acknowledgements
The authors are grateful to the manager, Pharmatrain pharmaceutical training and testing centre, Hyderabad, for providing the necessary facilities to carry out the research work.

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