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Research Article

Development and Validation of RP-HPLC assay method for the Estimation of Dronedarone in bulk and Pharmaceutical Preparations

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Abstract: A rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of Dronedarone in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on an ODS C_{18} column (250 x4.6 mm x 5µm length), using a composition of water (0.2% Tri Ethyl Amine):Methanol (20:80v/v) pH adjusted to 3 with Ortho Phosphoric acid as the mobile phase at a Flow Rate of 1 mL/min and the detection was done at 283 nm. The retention time of the drug was 4.707 min. The method produced linear responses in the concentration range of 20-120µg/mL of Dronedarone. The method was found to be reproducible for analysis of the drug in Tablet preparations.
Keywords: Dronedarone, RP-HPLC, tablets dosage forms, validation.

INTRODUCTION

Dronedarone is an Anti-arrhythmic drug IUPAC Name is N-[2-butyl-3-[4-(3-dibutyl-aminopropoxy) benzoyl] methane] sulfonamide, Dronedarone is a white crystalline powder. Dronedarone **(**development code name SR33589 and marketed as Multaq**)** is a drug for the indication of cardiac arrhythmias[1]. It was recommended as an alternative to amiodarone for the treatment of atrial fibrillation and atrial flutter in people whose heart have either returned to normal rhythm or who undergo drug therapy or electric shock treatment i.e. direct current cardio version (DCCV) to maintain normal rhythm[2].

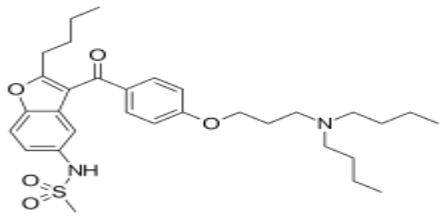

Fig-1: Structure of dronedarone

In literature towards it is found that the development of analytical method for the determination dronedarone hydrochloride and its related impurities in biological samples, bulk drug sample and in pharmaceutical dosage form. A literature survey also revealed liquid chromatography-tandem mass spectrometry [3], HPLC-UV [4], HPTLC [5], RP-HPLC [6,7] for dronedarone in bulk drugs samples and pharmaceutical dosage.

Therefore in the present study, a RP-HPLC method has been developed for the estimation of Dronedarone in the bulk and tablet dosage form using a new method.

MATERIALS AND METHODS

Chromatographic conditions:

A Shimadzu of software LC solution of version 1.2 2SP 510 High pressure liquid chromatographic instrument provided with a SHIMADZU 486 tunable absorbance UV detector of SPD-10A detector model, a 20µL rehodione injection syringe, and isocratic single pump was employed in the study. HPLC grade methanol (Qualigens) and water for HPLC was prepared from milliQ water system were used for preparing the mobile phase. chromatographic separation was achieved on Agilent TC C_{18} (250 x 4.6 mm, 5µ) columns using mobile phase composition of water (0.2% Tri Ethyl Amine): Methanol (20:80 v/v) pH adjusted to 3 with Ortho Phosphoric acid Flow rate was maintained at 1 mL/min with 283 nm UV detection. The retention time obtained for Dronedarone was at 4.707 min with injection volume 20µL and the UV detection was made at 283 nm. Diluents was prepared by mixing 800 mL of Methanol with 200 mL of milliQ water mixed with 0.2% Tri Ethyl Amine filtered through 0.45µ membrane filter and sonicated,

degassed before use. The column temperature was maintained at 25±2^{0}C.

Table-1: Optimized chromatographic conditions of dronedarone

PARAMETER	OBSERVATION
Elution	Isocratic
Temperature	Ambient
Mobile phase	Methanol : Water (80:20) with 0.2% TEA
P^H	3
Column	Agilent Teflon Coated C_{18} (250 x 4.6 mm, 5 µ)
Wave length	283nm
Flow	1ml/min
Run time	7mins

Drug stock solution

Accurately weighed quantity of Dronedarone (10 mg) was transferred to 10mL volumetric flask. Then small amount methanol was added and diluted up to the mark with methanol (Concentration: 1000µg/mL), from that preparing 100µg/mL. It was injected into the C_{18} column using syringe. The working standards of 20-120 µg/mL were prepared by using mobile phase as a solvent and each solution injected into the column at flow rate of 1mL/min. Each dilution was injected six times at 283nm and the corresponding standard chromatograms were obtained in figure 2. From these chromatograms the area under the peak of the drug to that of the Reference standard for each dilution was calculated. The regression of the drug concentrations over the peak areas was computed, Calibration curve of dronedarone was shown in figure no 3. The regression equation was used to estimate the amount of Dronedarone in pharmaceutical dosage forms. The obtained sample chromatograms was shown in Figure 4.

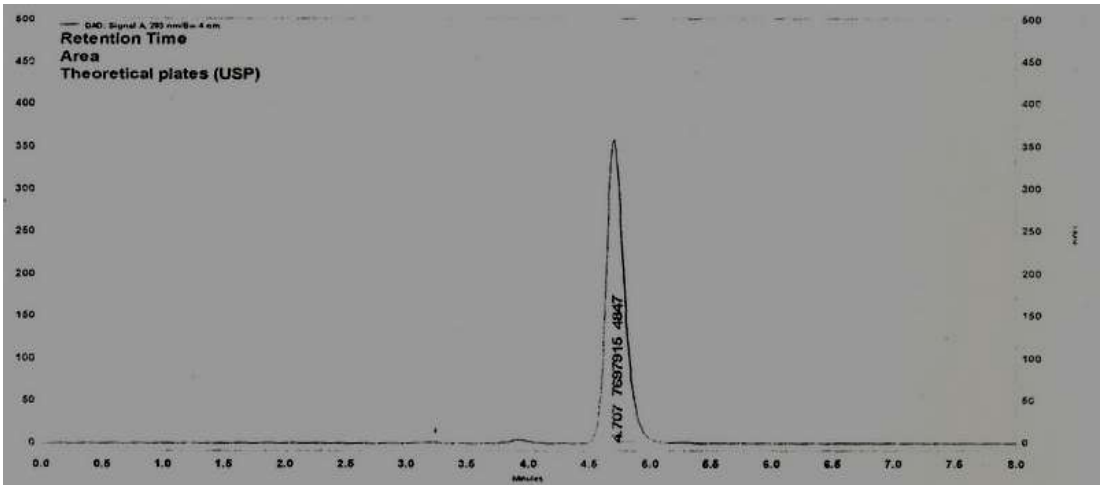

Fig-2: Chromatogram of standard

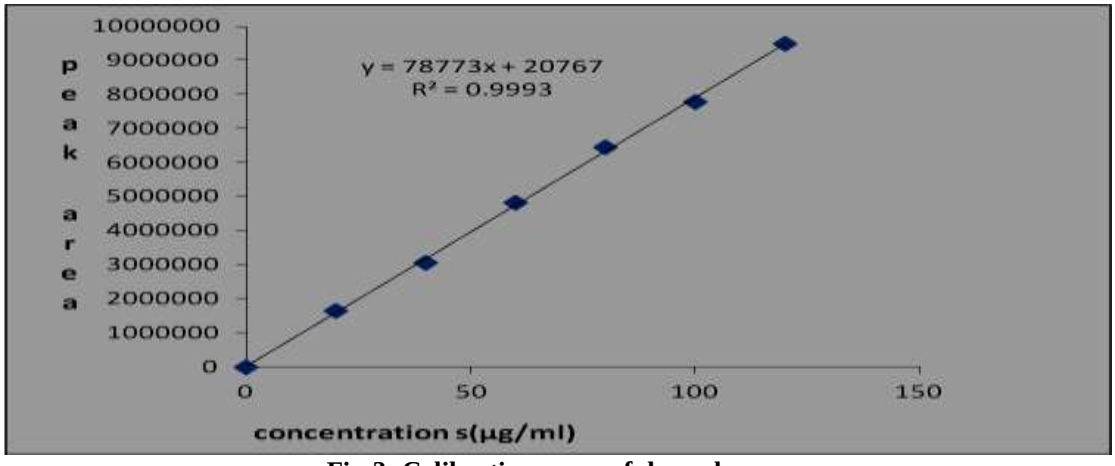

Fig-3: Calibration curve of dronedarone

RESULT

Estimation of Dronedarone in tablet dosage form

The commercially available ten tablets of Dronedarone (400mg) were weighed and the powder equivalent to 10mg of Dronedarone was transferred to 10mL standard flask and small amount methanol was added. The solution was sonicated for 15min, and the final volume was made with same to obtain solution of Dronedarone (1000µg/mL). The mixture was then filtered through a nylon 0.45mm membrane filter. The above solution was suitably diluted with mobile phase to obtain final dilution of Dronedarone (50µg/mL). The obtained result was shown in table no 2.

Table-2: Results Of Tablet Analysis

Brand name of drug	Labeled amount of drug (mg/Tablet)	Assay Concentration *(µg/ml)	MEAN Amount Found (n=6)	%RSD
MULTAQ	400mg	50µg/mL	98.66%	0.96

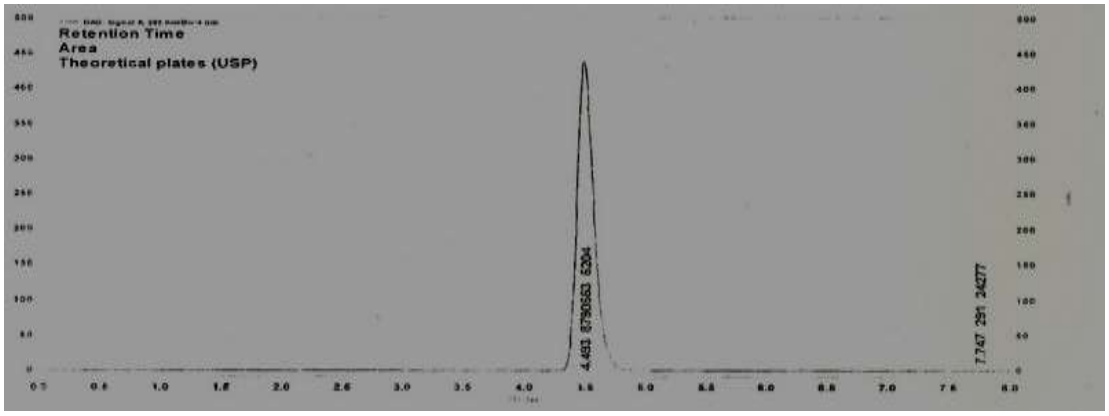

Fig-4:Chromatogram of sample

VALIDATION OF THE DEVELOPED METHOD

The method was validated for its linearity , accuracy, precision, sensitivity ,Robustness, LOD & LOQ and specificity. Method validation is carried out as per ICH guidelines Q2 (R1)[8].

Specificity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by examining blank matrix samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other excipients. Two different samples were injected and studied with respective excipients. HPLC chromatograms recorded for the drug-matrix (mixture of the drug and excipients) showed almost no interfering peaks with in retention time ranges. Fig no 2, 4, show the respective chromatograms for Dronedarone standard and formulation. The figures shows that the selected drugs were cleanly separated. Thus, the HPLC method proposed in this study was selective.

System Suitability Studies

It is to ensure the adequate performance of the chromatographic system. System suitability parameters like Retention time, number of theoretical plates (N), tailing factor (T), and peak asymmetry (As) were evaluated for six replicate injections on assay concentration. The system suitability studies were evaluated from standard chromatogram and obtaining the parameters includes Retention time, Column efficiency, Resolution and Tailing factor. Results are show in table no 3.

Table 3: System Suitability Of The Proposed HPLC Method

Parameters	Results
Relative retention time	4.707 min
Theoretical plates	4847
Tailing factor	1.12
Asymmetric factor	1.07
LOD (mg/mL)	0.45UG/MI
LOQ(mg/mL)	1.55UG/ML

Linearity

In order to find out the linearity range of the proposed HPLC method, studies were carried out by plotting average peak areas of analyte against concentrations of the analyte. A good linear relationship (r^2=0.999) was observed between the concentrations of Dronedarone and the corresponding peak areas. The regression of Dronedarone concentration over its peak area was found to be Y=78773x+76720 (where y is the peak area and x is the concentration of Dronedarone). The slope, intercept and the correlation coefficient of the drug were shown in table no 4.

Table 4: Linearity of Dronedarone

Parameter	Result
Λmax	283nm
Linearity range	20-120µg/mL
Regression equation	Y=78773x+20767
Slope	78773
Intercept	76720
Correlation coefficient	0.999

Accuracy

The accuracy of the methods was determined by calculating recoveries of Dronedarone by the standard addition methods. The accuracy of the method was determined by preparing solutions of different concentrations 50%, 100% and 150% in which the amount of marketed formulation(R was kept constant (20µg/mL) and the amount of pure drug was varied that is 10µg/mL, 20µg/mL and 30µg/mL for 50%, 100% and 150% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery. Results were shown in table no 5.

Table 5: Accuracy Of The Proposed HPLC Method

Concentration taken(pre analysed sample conc) µg/ml	**Recovery level (%)**	**Added amount ‘ (µg/ml)**	**Amount found ‘ (µg/ml) mean*(N=6)**	**%Recovery**
20 µg/ml	50%	10	29.89	99.63
20 µg/ml	100%	20	39.12	97.8
20 µg/ml	150%	30	49.88	99.76

Precision

Precision can be expressed in terms of %RSD. Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra- day) and samples run on three different days (Inter-day).The precision of the method was checked by repeatedly injecting (n=3) solutions of (25µg/mL). Results were shown in table no 6.

Table 6: Precision Of The Proposed Method

Concentration (µg/ml)	**Intra-day Precision** *mean ±SD **(n=3)**	%RSD	**Inter-day Precision *** mean ±SD **(n=3)**	%RSD
20	440982.26±2467.78	0.40	450965.21±4498.23	0.75
40	1019196.5±6772.46	0.62	1028637.16±15298.42	1.1
60	1433845.16±5490.82	0.55	1633845.45±10981.2	0.53

Robustness

To determine the robustness of the method the experimental conditions were deliberately altered slightly. Robustness of the method was determined by carrying out the analysis at three different flow rates (i.e. 1 ± 0.1mL/min), three different wavelengths (280nm+_3) and three different mobile phase ratios (20:80+_2). Results were shown in table no 7.

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Table 7: Robust Values Of Dronedarone

Parameter	Condition	Retention time	Area	%Assay
Flow rate	0.9	5.02	4562879	101%
	1.1	4.1	3752718	94%
Mobile phase	78:22	5.00	4094081	102%
	82:18	4.180	4109899	98%
Wavelength	280	4.507	3883900	101%
	286	4.507	4214292	102.5%

Limit of detection and Limit of quantification

Limit of quantification of an individual analytical method is the lowest concentration/ amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The quantification limit is used particularly for the determination of impurities and/ or degradation products. LOQ is 2-3 times of higher than LOD. The limit of detection (LOD), limit of quantification (LOQ) of the drug was calculated using the following equation as per international conference harmonization (ICH) guidelines and the Results were shown in table no 3.

LOD = 3.3 X SD/S

LOQ = 10 X SD/S

DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of Dronedarone in pharmaceutical dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of water with Tri Ethyl Amine and methanol in different combinations were tested as mobile phase on a C-18 stationary phase. A binary mixture of water with 0.2% Tri Ethyl Amine and methanol in 20:80v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for Dronedarone was 4.707 min**.**

A good linear relationship (r =0.999) was observed between the concentration of Dronedarone and the respective average of peak areas. The regression curve was constructed by linear regression equation and its mathematical expression was y = 78773x+20767 (Where y is the average of peak areas of the drug to that of reference standard and x is the concentration of Dronedarone). When Dronedarone solutions containing 20 40 60 80 100 and 120 µg/mL was analyzed by the proposed method for finding out intra and inter-day variations as low coefficient of variation was observed .This shows that the present HPLC method is highly precise. The amount of Dronedarone obtained from the pre-analyzed samples containing known amounts of added drug are shown in Table 5. About 99.90% of Dronedarone could be recovered from the pre-analyzed samples indicating the high accuracy of the proposed method.

Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were mobile phase ratio ($\pm$2), the flow rate ($\pm$0.1), the detection wavelength ($\pm$3). The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions as shows in Table 7. The system suitability parameters were within the limits as shown in Table 3. The drug content in tablet dosage form preparations was quantified using the proposed analytical method. The tablet dosage form preparations were found to contain an average 98.6% of the labeled amount of the drug, as shown in Table 2.

The low coefficient of variation indicates the reproducibility of the assay of Dronedarone in dosage forms. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of Dronedarone in pharmaceutical dosage forms within a short analysis time. The method was duly validated by evaluation of the required parameters.

CONCLUSIONS

The isocratic RP-HPLC method developed for quantitative determination of Dronedarone is precise, accurate, and selective. The method was completely validated and satisfactory results were obtained for all the method validation data tested.

REFERENCES

1. Australian Public Assessment Report for Dronedarone Hydrochloride," Submission No: PM-2008-3045-3, 2010. http://www.tga.gov.au/pdf/auspar/auspar-multaq.pdf
2. FDA; FDA Approves Multaq to Treat Heart Rhythm Disorder, 2009.
3. Xie C, Yang S, Zhong D, Dai X, Chen X; Simultaneous determination of dronedarone and its active metabolite debutyldronedarone in human plasma by liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study. Journal of Chromatography B, 2011; 879(28):3071-3075.
4. Tondepu N, Sait SS, Surendranath KV, Kaja RK, Kumar S; A stability indicating HPLC method for dronedarone in bulk drugs and pharmaceutical

dosage forms. American Journal of Analytical Chemistry, 2012; 3:544.

5. Dobhi B, Jehaliya H, Patel M, Jadeja Y, Karia D, Shah A; HPTLC method for estimation of dronedarone hydrochloride in both bulk drug and pharmaceutical dosage form. Int J Pharm Sci Rev Res, 2012;17(1):48-51.
6. Bhatt KK, Patelia EM, Amin I; Development of a validated stability-indicating RP-HPLC method for dronedarone hydrochloride in pharmaceutical formulation. J Anal Bioanal Techniques, 2013;4(161):2.
7. Dabhi B, Jadeja Y, Patel M, Jebaliya H, Karia D, Shah A; Method development and validation of a stability-indicating RP-HPLC method for the quantitative analysis of dronedarone hydrochloride in pharmaceutical tablets. Scientia pharmaceutica, 2013; 81(1):115.
8. Guideline I.C.H.; Q2 (R1)(2005) Validation of analytical procedures: text and methodology. In International conference on harmonization, Vol- 6, (1996, November).