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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR DETERMINATION OF DRONEDARONE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Dronedarone in bulk formulation. The λ_{max} of the Dronedarone was found to be 290nm in Acetonitrile: water [70:30(v/v)].The method shows high sensitivity with linearity 5 to 30µg/ml (regression equation: Y=14163x+ 15315; r^2 = 0.999). The apparent molar absorptivity was found to be lmol–1 cm–1 in Acetonitrile: water [70:30 (v/v)]. This method was tested and validated for various parameters according to ICH guidelines and USP. The Detection limit and quantitation limit were found to be 25 µg ml–1 and 150 µg ml–1 in Acetonitrile: water [70:30(v/v)] respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Dronedarone in bulk and pharmaceutical formulation.

KEY WORDS

Dronedarone, Acetonitrile: water [70:30 (v/v). RP-HPLC.

1. INTRODUCTION

Dronedarone belongs to a class –III of antiarrhythmic drug and is used to treat cardiac arrhythmias and is chemically N-(2-butyl-3-(p-(3-(dibutylamino) propoxy) benzoyl)-5- benzofuranyl) methanesulfonamide. It prolongs the duration of action potential and refractory period in myocardial tissue via inhibition of sodium and potassium channels. Via inhibition of calcium channels and blockage of beta1-adrenergic receptors, a decrease in AV conduction and sinus node function can be observed. Dronedarone can also cause an increase in blood pressure by inhibition of alpha1-adrenergic receptors. Chemically dronedarone is a benzofuran derivative related to amiodarone, a popular antiarrhythmic; the use of which is limited by toxicity due to its high iodine content (pulmonary fibrosis, thyroid disease) as well as by liver disease. In Dronedarone the iodine moieties are removed, to reduce toxic effects on the thyroid and other organs; and a methylsufonamide group was added, to reduce solubility in fats (liphophilicity) and thus reduce neurotoxic effects. Literature survey revealed that few new and economic RP-HPLC methods have been established for estimation of Dronedarone and precise UV spectrophotometric methods with multivariate calibration technique have been developed and validated for the quantitative analysis of Dronedarone HCl in tablet form [9-16]. 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 Acetonitrile: water [70:30 (v/v)]. The method developed is specific, precise, accurate and well validated. This method is economical. The developed method was validated as per ICH guidelines and USP requirements suitable statistical tests were performed on validation data [1-7]

2. MATERIALS AND METHOD

2.1 Instrument

The liquid chromatographic system consisted of following components: Waters 2998 HPLC model (automated) containing variable wavelength programmable PDA (Photo diode array) detector. The chromatographic separation for Dronedarone was carried out on a phenomenex, kinetex C_{18} (75 x 4.6mm i.d.) with particle size of 2.6 µ, 100A^0.

2.2 Reagents and materials

Dronedarone pure drug was obtained from MSN Laboratories Limited (Hyderabad) as a gift sample with 99.25% (w/w) assay value and was used without further purification. All chemicals and reagents used were of analytical grade (Rankem, India). Dronedarone tablets were purchased from local market and used with in self-life period. Each tablet was labeled contain 400 mg of Dronedarone.

2.3 Selection of wavelength

. For HPLC, mobile phase Acetonitrile: Phosphate Buffer (pH 6.5) (80:20 v/v) was filtered and degassed. The injection volume was injected 10µl with a flow rate of 1.5ml/min. Detection was carried out at 290 nm at column temperature 40°C and run time set at 5 minutes.

2.4 Preparation of stock solution

Weigh accurately Dronedarone HCl standard equivalent to 20 mg of Dronedarone and transfer into 200 ml volumetric flask ,and dissolved in 120 ml of diluent i.e. ACN: Water [70:30(v/v)], sonicate to dissolve and made up to the volume with diluent to get the final concentration of 100 µg/ml.

2.5 Preparation of calibration curve

From the standard stock solution of Dronedarone (200µg /ml), a series of concentrations were prepared in the range of 25-150 µg/ml by diluting the stock solution with ACN: Water [70:30(v/v)] solvent system and absorbance were found at 290 nm with an overlaid spectrum (**Fig-2**). The standard graph was plotted (**Fig-3**) and the calibration data are presented in **Table -1.**

Table-1: Calibration data of Dronidarone by HPLC

Conc(mcg/ml)	Peak area*(±SD)	%RSD
25	371409(1154.7)	0.3
50	728995(2776.1)	0.3
80	1145845(1873.1)	0.2
100	1415010(2068.3)	0.1
120	1721307(3413.9)	0.2
150	2144719(2520.1)	0.1

***Average of three determinations**

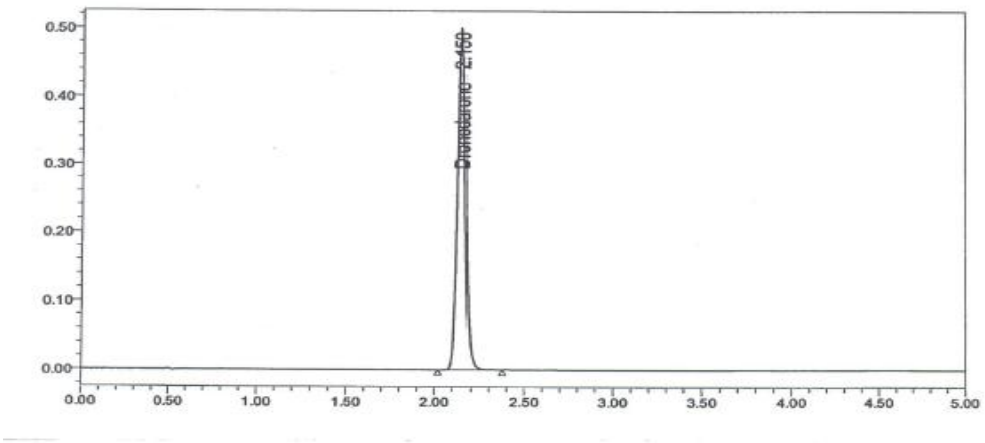

Figure 2: Chromatogram of Dronedarone

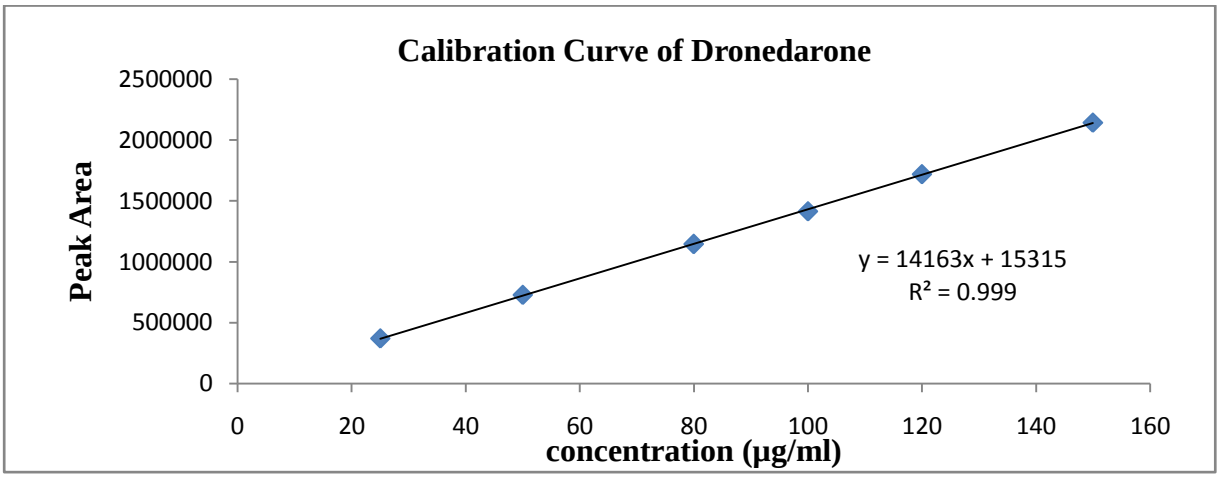

Figure 3: Calibration curve of Dronidarone

3. METHOD VALIDATION

3.1Specificity

The specificity of the method was determined by checking for interference with the drug from placebo components. This method was proven by the no change in absorbance of the drug with and without excipients at respective wavelength. Therefore proposed method was specific and selective for the drug.

3.2Accuracy

The accuracy of the method was evaluated by the recovery studies. Recovery studies were carried out at three levels of concentrations i.e. 10, 15, 20 µg/ml by the addition of known amounts of the standard to the placebo preparation within the analytical concentration range of the proposed method. The percentage recovery values were found to be 99.99-100.23 with %RSD of <2% **(Table 3)** which indicates that the proposed method was accurate.

3.3 Precision

Precision study was established by evaluating system precision, method precision and Intermediate precision study. System precision was demonstrated by injecting six replicate injections of standard solution as per test method and % RSD was reported in **Table 4**.

Method precision was determined by analyzing six replicate injections of sample preparation.

Intermediate precision was determined by performing method precision on another day by using different make of raw materials under the same experimental conditions. The method precision done on different days was taken into consideration to determine the intermediate precision of the proposed methods (**Table- 4(A).**

3.4 Linearity

The linearity is established for the proposed method, nine separate series of solutions of the drug (25-250) µg ml^{-1} in ACN: water (v/v) [70:30, v/v] medium) were prepared from the stock solutions and analyzed.

3.6 Robustness

Robustness of the method was carried out by deliberately made small change in the Flow rate by ±10% (1.35 and 1.65 ml/min), Column temperature by ±5^0C, Mobile phase composition by ±5% of ACN: Phosphate buffer (81:19, v/v and 79:21, v/v) and change in pH of Phosphate Buffer 6.5±0.2 units had no significant effect on the retention time and chromatographic response of Dronedarone, indicating that the method was robust and the results are shown in ***Table 5***.

3.7 Estimation from formulations

Twenty tablets were accurately weighed and powdered. The tablet powder equivalent to average weight of the tablet was weighed and transferred into a 200 ml volumetric flask, add 120 ml of diluent i.e. ACN: Water [70:30(v/v)], sonicate for 15 minutes and dilute to the volume with diluent. Filter the solution through 0.45 µm Nylon filter. Pipette out 5 ml of this solution into 100 ml volumetric flask and dilute to the

volume with respective diluent to obtain 100 µg/ml concentration and the sample was analyzed by injecting into HPLC system.

The concentration of Dronedarone in the pharmaceutical formulation was determined by linear regression equation and its percentage purity was calculated.

4. RESULTS AND DISCUSSIONS

The RP-HPLC method was used to quantify the dronedarone in tablet dosage forms. The analyte was detected at 290 nm and the retention time of Dronedarone was found to be 2.1 **(figure 1).** The calibration curve showed linearity over a concentration range from 25-150 µg/mL, which follows the Beer and Lambert's law. The slope and intercept of the calibration line was determined by linear regression. The RP-HPLC method was developed and validated as per designed protocol, based on ICH Q2B guidelines. The calibration curve was developed for peak area Vs concentration (µg/ml) and it was linear over concentration range of 25-150 µg/ml. The regression line equation for the analysis was **y = 14163x + 15315** with regression coefficient of **0.999**. The LOD and LOQ values were shown in **Table 2.** And the calibration curve was shown in **Table 2.**

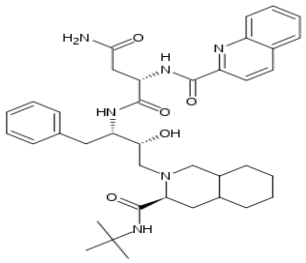

Figure 1: Structure of Dronedarone

Table 2: Optical Characteristics and Regression Equation of Dronedarone

Parameter	HPLC Method
Detector Wavelength (PDA)	290
Linearity range (µg/ml)	25-150
Regression equation	
Intercept (c)	15315
Slope (m)	14163
Regression coefficient (r^2)	0.9999
LOD(µg/ml)	0.85
LOQ(µg/ml)	2.57

4.1. ANALYTICAL VALIDATION

4.2. Specificity and selectivity

The RP-HPLC spectrum of Dronedarone was not changed in the presence of common excipients in both the selected media (**Table 2).** Therefore proposed methods are specific and selective for the drug.

4.3. Accuracy

The excellent % recovery values (nearly 100%) and their low standard deviation values (S.D. < 2) represent accuracy. In ACN: water [70:30, v/v] the mean percentage recoveries (% R.S.D.) for concentrations (**Table 3**) were found to be in the range of 99.99 to 100.23% (%RSD in range of (0.200 to 0.390). This result revealed that any small change in the drug concentration in the solution can be accurately determined by these proposed methods.

Table 3: Accuracy data of the developed method

Amount added (µg/ml)	Amount found (µg/ml)	% Recovery*± SD	%RSD
50	49.87	99.74±0.389	0.390
100	100.06	100.06±0.259	0.258
150	149.89	99.92±0.2	0.200

S.D- Standard Deviation, % RSD – Relative Standard Deviation

Table 4: Precision data of the developed method

Conc	Standard area	Theoretical plates(N)	Tailing factor
	1457430	12959	1.03
	1463354	12428	1.04
	1457853	12963	1.04
100 µg/ml	1458050	12656	1.18
	1459166	12362	1.08
	1458172	12965	1.12
Average	1459004.167		
SD	2206.95		
%RSD	0.15		

Table- 4(a): Method Precision and Intermediate Precision Results

Study	Replicates	% Assay	Mean %Assay	SD	%RSD
	1	99.57			
Method precision	2	99.83	99.58	0.221	0.222
	3	99.69			
	4	99.19			
	5	99.70			
	6	99.50			
	1	99.71			
Intermediate	2	99.65	99.51	0.170	0.171
precision	3	99.49			
	4	99.35			
	5	99.27			
	6	99.56			
	Mean %assay	99.54			
overall	**SD**	0.1930			
	% RSD	0.1939			

Table 5: Robustness data of the Developed Method

Changed parameter	Peak area± %RSD	R_t	Theoretical plates	Tailing factor
Flow rate (±10%)				
1.35 ml/min	1621068± 0.3	2.270	12423	1.04
1.65 ml/min	1310660± 0.5	1.928	11571	1.06
Column temp(±5^{0}C)				
35^{0}C	1450527± 0.4	2.109	11810	1.03
45^{0}C	1455744± 0.7	2.100	11885	1.08
Mobile Phase (± 5%)				
81:19	1411124± 0.8	1.987	11139	1.12
79:21	1419783± 0.7	2.126	11561	1.09
pH Variation (±0.2 units)				
6.3				
6.7	1384683 ± 0.5	1.967	10222	1.04
	1401130 ± 0.4	2.190	11319	1.18

Table 6: Assay of Dronedarone

Conc of Dronedarone Sample	Replicates	Sample Area	% Mean Assay ± SD	% RSD
	1	1432315		
100 µg/ml			100.20 ± 0.2	0.212
	2	1436579		
	Average area	**1434447**		

Table 7: SYSTEM SUITABILITY PARAMETERS

S.no	Parameter	Dronedarone
1	Peak area	1458134
2	Theoretical plates	12781
3	Retention time	1.09
4	Tailing factor	2.13

4.4 Precision

Precision determined by studying repeatability and intermediate precision .Repeatability (% R.S.D.) In ACN: water [70:30, v/v], at all three levels of concentrations (**Table 4).** Repeatability results indicate the precision under the same operating conditions over a short interval of time and inter-assay precision. Intermediate precision expresses within-laboratory variations in different days and in different instruments. In intermediate precision study, % R.S.D. values were not more than 0.15% in all the cases (**Table 4**). R.S.D. values were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

4.5 Linearity

In ACN: water [70:30, v/v] medium the linearity range was found to be 25-250 µg ml^{-1} at 290 nm. Lower values of parameters like standard error of slope and intercept (**Table 2**) indicated high precision of the proposed methods. (**Table 2).**

4.7 Robustness

Variation of strength in the selected media by ± 2% did not have any significant effect on absorbance. The mean % recoveries (± S.D.) were found to be 100.5±0.080 and 99.9±0.01 in ACN: water [70:30, v/v] respectively (**Table 5).**

4.8 Estimation of formulations

In ACN: water [70:30, v/v] the assay values of Dronedarone for tablet ranged from 100.20 % with

Relative standard deviation not more than 0.1 %. Assay values of formulations were same as mentioned in the label claim; this indicated that the interference of excipient matrix is insignificant in estimation of Dronedarone by proposed methods. The estimated drug content with low values of standard deviation established the precision of the proposed methods **(Table 6).**

5. CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Dronedarone in pure samples and pharmaceutical formulations.

6. ACKNOWLEDGEMENTS

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