Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(4):2173-2179

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

RP-HPLC method development and validation of Dronedarone HCl in its Pure form and tablet dosage form

Arpan Patel* and Jawed Akhtar

School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India

---

ABSTRACT

A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for determination of Dronedarone in pure form and tablet dosage form. The method was carried out using Hypersil ODS 3V column (250 mm × 4.5 mm i.d., 5 µm particle size) with mobile phase comprised of Buffer: acetonitrile(42:58%v/v). Buffer use is Potassium dihydrogen phosphate buffer with pH 3.0 . The flow rate was set at 1.1 ml/min and effluent was detected at 220nm. The retention time of Dronedarone was found to be 4. minute. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility stability. LOD and LOQ were found to be 0.87 µg/ml and 2.66 µg/ml respectively. The calibration curve was linear in the concentration range of 10-150 µg/ml with coefficient of correlation 0.9999. The percentage recovery for the Dronedarone was found to be 99.3 and the % RSD was found to be less than 2 %. The proposed method was successfully applied for quantitative determination of Dronedarone in tablet dosage form.

Key words: Dronedarone HCL, HPLC, Validation.

INTRODUCTION

Dronedarone N-(2-Butyl-3-(p-(3-(dibutylamino)propoxy)benzoyl)-5-benzofuranyl)methane-sulfonamide is a antiarrhythmic agent. Chemically, dronedarone is a benzofuran derivative related to amiodarone, a popular antiarrhythmic the use of which is limited by toxicity due its high iodine content (pulmonary fibrosis, thyroid disease) as well as by liver disease.

In dronedarone, the iodine moieties were removed, to reduce toxic effects on the thyroid and other organs; and a methylsulfonamide group was added, to reduce solubility in fats (lipophilicity) and thus reduce neurotoxic effects. Yet it displays amiodarone-like class III antiarrhythmic activity in vitro and in clinical trials. The drug also appears to exhibit activity in each of the 4 Vaughn-Williams antiarrhythmic classes.

EXPERIMENTAL SECTION

Chemicals & Reagent

Dronedarone working standard (purity, 98.70%) used from Cadila Healthcare Ltd., Ahmedabad, India. Dronedarone tablets were obtained from Cadila Healthcare Ltd., Ahmedabad, India. Each tablet was labeled contain 400 mg of Dronedarone. All other reagent required for experimentation was of analytical reagent (AR) grade. Chemicals used for this experiment were, Methanol (HPLC grade) were purchased from Fisher Scientific pvt. Ltd, Acetonitrile (HPLC grade) was purchased from Spectrochem pvt. Ltd, Mumbai, Ortho-phosphoric acid (AR grade) was purchased from Fisher Scientific pvt. Ltd, Potassium Dihydrogen Phosphate (AR grade) was purchased from Merck, pvt. Ltd.

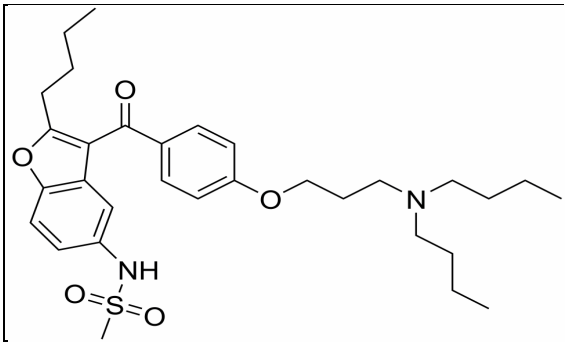

Figure 1: Chemical structure of Dronedarone

Instruments
Analysis was performed on a chromatographic system Shimadzu and Agilent 1200 series equipped with an auto injector, Diode array detector and a single-beam Agilent UV-Visible spectrophotometer, Model 8453. The analytical column used was Hypersil ODS 3V (250 mm × 4.5 mm i.d., particle size 5 µm) UV-Visible spectrophotometer 1700 Shimadzu Limited with 10mm matched pair of quartz cell and spectral band width of ±2nm.

Chromatographic Conditions
For HPLC, mobile phase, Phosphate Buffer(pH 3.0) : Acetonitrile(42:58%v/v) was filtered and degassed. The injection volume was injected 10µl with a flow rate of 1.1ml/min. Detection was carried out at 220 nm at column temperature 30°C and run time set at 7 minutes.

Assay Procedure:
Standard preparation
Accurately 20 mg of Dronedarone HCl was weighed into 200 ml of volumetric flask. The compound was first dissolved in few ml of diluent. The volume was then made up to 200 ml with diluents to obtained final standard solution of 100 µg/ml of dronedarone.

Sample Preparation
Calculate average wt. of 10 tabs. Take wt. equivalent to 100mg. transfer to 100 ml volumetric flask. Then add 50 ml of diluents and sonicate for 30mins. Then makeup with diluents upto mark. Then transfer 5ml of this preparation to 50ml of volumetric flask and makeup with diluent to obtain final concentration of 100 µg/ml. Filter this preparation with 0.45 Millipore filter and fill in HPLC vials. **Note: Diluent use is ACN: Phosphate Buffer pH 3.0 (40:60%v/v)**

Assay of valsartan
Standard and sample solution of dronedarone was injected. Assay was performed as per given chromatographic conditions. The amount of valsartan present in the sample was computed from the linearity curve.

Method Validation:
The method was validated for the parameters like system suitability, range, linearity, accuracy, precision, ruggedness, specificity, limit of detection (LOD), limit of quantification (LOQ), solution stability, and robustness.

System suitability
System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (Symmetry factor), theoretical plates of the column, peak area and retention time. Result of System Suitability data presented in **Table 1.**

Range and Linearity
To evaluate the linearity, serial dilution of analyte were prepared from the stock solution of 1000 µg/ml to get the desired concentrations (10, 25, 50, 100, 120 and 150 µg/ml) for linearity in the range of 10-150 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times

into the column. Absorbance at 220nm was measured and calibration curve for dronedarone was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis) As shown in **Figure 3** and **Table 2 &3**

Accuracy (% Recovery)
The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at 3 different level 50%, 100% and 150% of the target concentration in duplicate. Result of accuracy data presented in **Table 4.**

Precision
The method precision was done by preparing six different sample preparations by one analyst under the same condition. The results were presented in Table 5. The results obtained were within 2% RSD in **Table 5.**

Ruggedness
Ruggedness test was determined between two different analysts, instruments and Columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results were presented in **Table 6**.

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines (41). The LOD and LOQ for Dronedarone are 0.87 & 2.66.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = standard deviation of the response, S= slope of the regression line

The results were presented in **Table 7**

Robustness
Robustness of the method was carried out by deliberately made small change in the flow rate, and organic phase ratio, column oven temperature. Results were presented in **Table 8**.

Solution stability
The standard and sample solutions were found stable up to 24 hours at room temperature. After 4, 8, 12, 16, 20, 24 hours the solutions were analysed. No significant changes (<2%) were observed for the chromatographic responses for the solution analysed, relative to freshly prepared standard. Results related to solution stability are summarized in **Table 9**

RESULTS AND DISCUSSION

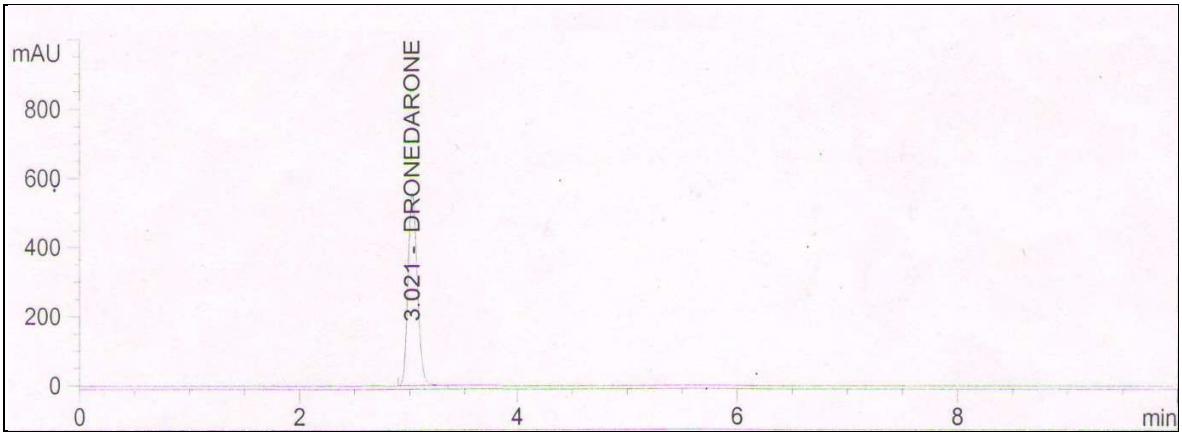

Fig. 1 Chromatogram Of Dronedarone

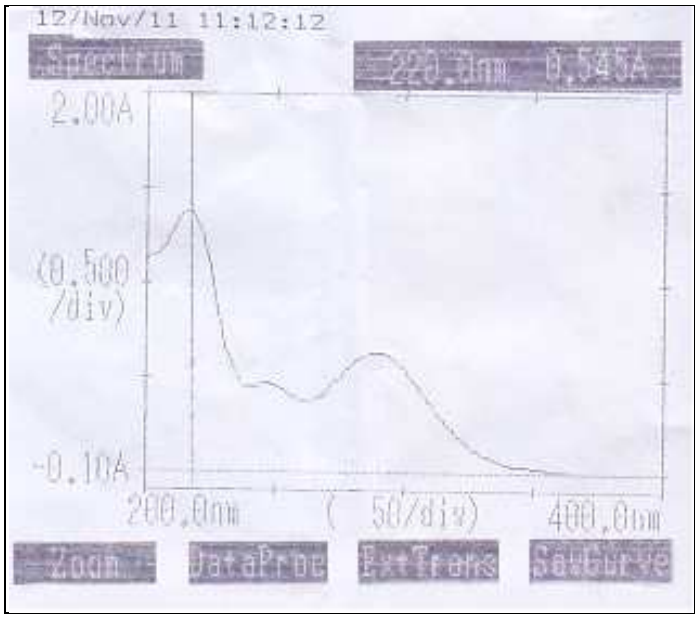

Fig. 2 U.V. spectra of Dronedarone

Table-1 : System Suitability Data

Sr. No.	Parameters	Dronedarone HCl [a]
1	Peak area	4420401
2	No. of theoretical plates	3480.89
3	Retention time (min)	4.2
4	Asymmetry	1.1
5	% RSD	0.4

a : Mean of six determinations

The system suitability results fulfil the acceptance criteria and prove that the results of method are reproducible.

Table-2: Preparation of linearity solutions

Linearity level	Stock soln. in µg/ml	Stock soln. to be taken in mL	Dilute to volume (mL) with methanol	Final concentration in µg/ml
10%	1000	2	200	10
25%	1000	5	200	25
50%	1000	5	100	50
100%	1000	5	50	100
120%	1000	6	50	120
150%	1000	7.5	50	150

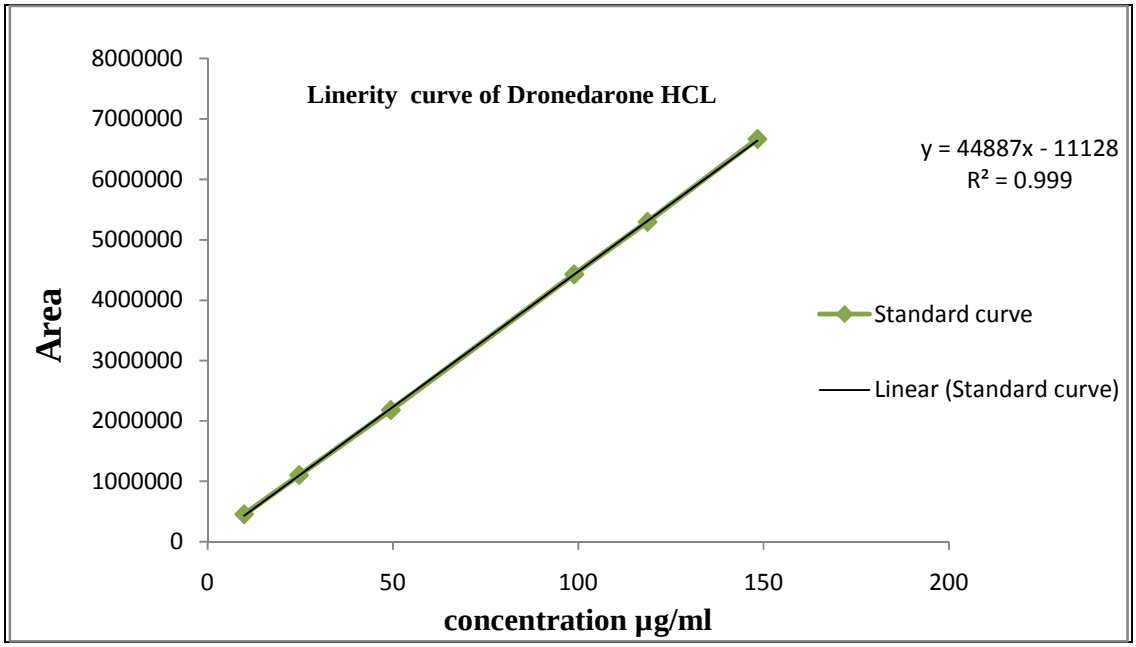

Fig.3: Calibration curve of Dronedarone HCl showing linearity

Table-3: Regression Analysis Data for Calibration Curves

Parameters(Units)	Dronedarone HCl
Linearity range µg/ml	10-150µg/ml
Regression Coefficient r^2	0.9999
Slope	44887.19
Intercept	-11128.79

Table-4: Results of Accuracy Data of Dronedarone HCl

Level of Recovery [a]	Area	Added Amount (mg)	Recovered Amount (mg)	% Recovery	Mean % Recovery	% RSD
50%	1598.889	50.30	49.04	98.8	99.0	0.3
	1591.447	50.00	48.81	98.9		
	1609.350	50.30	49.36	99.4		
100%	3204.018	100.10	98.26	99.5	99.4	0.4
	3194.400	100.30	97.97	99.0		
	3216.822	100.20	98.65	99.7		
150%	4777.469	150.10	146.52	98.9	99.1	0.2
	4791.576	150.39	146.95	99.0		
	4802.626	150.30	147.29	99.3		

a : Accuracy was checked at three levels viz. 50%, 100% and 150

The accuracy results show that the method is accurate to practically achieve added amount at three different levels within 98%-102% with RSD less than 2%. The average % recovery was found to be 99.3%.

Table-5: Results for Method Precision

Set No.	Dronedarone HCl		
	%Assay	Mean %Assay [a]	% RSD
1.	98.8	98.5	0.5
2.	98.5		
3.	98.5		
4.	97.9		
5.	98.2		
6.	99.2		

a: Mean of six determinations

The method precision gave results obtained within 2% RSD suggesting the method is precise.

Table-6: Results for Ruggedness

Set No.	Dronedarone HCl (%Assay)			
	Analyst 1 (Instrument 1)		Analyst 2 (Instrument 2)	
	Column 1	Column 2	Column 1	Column 2
1.	98.5	98.9	98.5	98.3
2.	98.9	98.6	98.6	98.1
3.	98.2	98.2	98.2	98.8
4.	98.6	98.1	98.1	98.6
5.	98.5	98.8	98.6	98.3
6.	98.4	98.4	98.3	98.5
Mean %Assay [a]	98.5	98.4	98.2	98.4
% RSD	0.23	0.38	0.38	0.25

a: Mean of six determinationss
b: Ruggedness studies were carried out using different analysts, instruments and columns

The results for ruggedness evaluation give RSD below 2% suggest the method is rugged to changes.

Table-7: LOD & LOQ

S. No.	Conc. µg/ml	Peak Area	S. D.	Slope	LOD µg/ml	LOQ µg/ml
1	Dronedarone HCl 100 µg/ml	4420101	11902.7	44887.19	0.87	2.65
2		4453781				
3		4428694				
4		4427364				
5		4424281				
6		4426891				

The LOD for Dronedarone HCl was found to be 0.6 µg /ml while LOQ was 1.83 µg /ml.

Table-8: Results of Robustness Study

Sr. No.	System Suitability	Temp. -5°C	Temp. +5°C	Flow -10%	Flow +10%
1	427578.5	427347.9	427156.3	518314.2	364188.7
2	426461.5	425755.8	427181.1	517152.3	364375.3
3	426073.6	426106.0	427359.7	517872.9	364375.3
4	425727.1	427456.0	427726.7	519110.7	364441.7
5	426087.8	426774.7	427156.3	518354.6	364072.3
6	430062.1	427347.9	427156.3	517825.6	364859.5
% RSD	0.4	0.2	0.1	0.1	0.1

Sr. No.	System Suitability	Org. Ph +2%	Org. Ph -2%	pH 3.2	pH 2.8
1	427578.5	427521.7	434170.4	427595.5	426635.8
2	426461.5	428645.2	438458.2	427773.4	426676.5
3	426073.6	428896.1	440732.9	426453.3	426144.7
4	425727.1	429748.3	438955.8	427487.2	427357.9
5	426087.8	430009.5	437276.3	427387.6	426635.8
6	430062.1	429856.4	737276.3	427595.5	427589.2
% RSD	0.4	0.2	0.5	0.1	0.1

Concentration level used for robustness evaluation was 100 µg /ml. Three factors were slightly changed at three levels (-1, 0, 1). The results show area obtained.

Results for robustness evaluation for drug are presented in Table-8. Insignificant differences in peak areas and less variability in retention times were observed. The results mentioned are of area obtained.

Table-9: Results for Solution Stability for Sample Preparation

Time (hrs)	Dronedarone HCl	
	Area	% Difference
Initial	4442292	-
4	4438694	-0.1
8	4475163	0.7
12	4487724	1.0
16	4460564	0.4
20	4452964	0.2
24	4449764	0.1

The standard and sample solutions were found to be stable up to 24 hrs.

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination Dronedarone in tablet formulation. The method is very simple and specific with runtime of 7 min, makes the developed method it's suitable for routine quality control analysis work.

Acknowledgment
Sincere thanks to Jaipur National University for providing the research facilities and Cadila Healthcare Ltd., India for providing the Dronedarone drug samples.

REFERENCES

[1] Swarbrick, J. and Boylan, J.C., In; Encyclopedia of Pharmaceutical Technology, New York, Marcel Dekker Inc., Vol. 15, **1997**, 329-331.
[2] Connors, K.A., In; A Textbook of Pharmaceutical Analysis, India, Jhon Wiley and Sons, 3th Edn. **1999**, 196-198
[3] Harris, D.C., In; Quantitative Chemical Analysis, New York, W. H. Freeman and Company, 6th Edn. **2003**, 7-3.
[4] Ghazala Khan, Dinesh Sahu, *Asian Journal of Biochemical and Pharmaceutical Research*, **2011**, 1(2), 352
[5] Gunjan Modi, *Journal of Chemical and Pharmaceutical Research*, **2011**, 3(3), 670-675.
[6] Jawad Akthar, Parth Bhatt, *Research Journal of Pharmacy and Technology*, **2012**, 5(1**)**, 60.
[7] Patel Nirav M, *Asian Journal of Biochemical and Pharmaceutical Research*, **2011**, 1(2), 379
[8] ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October **1994**, 1-5.
[9] ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November **1996**, 1-8.
[10]http://www.docstoc.com/docs/81444469/RP-HPLC-method-for-amiodarone-in-tablets.