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RESEARCH ARTICLE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF DRONEDARONE HYDROCHLORIDE BULK DRUG AND PHARMACEUTICAL FORMULATION

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Abstract

Three accurate, precise, sensitive and economical procedures for "Analytical method development and validation of UV-VIS Spectrophotometric method for estimation of Dronedarone hydrochloride bulk drug and pharmaceutical formulation" have been developed. The two solutions of standard and samples were prepared in chloroform. The quantitative determination of the drug was measured at 289 nm. Calibration graphs constructed at their wavelength of determination were linear in the concentration range of Dronedarone using 2 – 10 µg/ml (r^2=0.998). All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. This method was successfully applied to Pharmaceutical formulations, because no interferences from tablet excipients were found.

Keywords: Dronedarone hydrochloride, method development, validation, spectrophotometer.

Introduction

Dronedarone is a drug mainly for the indication of cardiac arrhythmias, chemically as N-(2-Butyl-3-(p-(3(dibutylamino) propoxy) benzoyl) -5-benzofuranyl) methane sulfonamide and its structural formula is $C_{31}H_{44}N_2O_5S$ [1]. 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. 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. New investigational drugs like Dronedarone are being used. Dronedarone is the most recent antiarrhythmic 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 repolarization currents, making them less active and hence pro- longing the action potential duration (APD). Dronedarone also exhibits anti adrenergic 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 antiarrhythmic drugs [2-13].

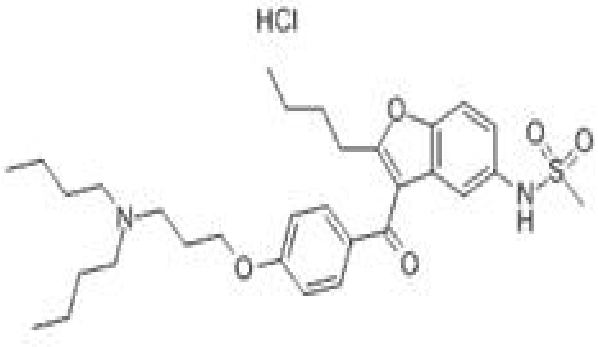

Fig.1 Structure of Dronedarone hydrochloride

Experimental

Instrumentation

A double beam UV-Visible spectrophotometer UV-2080, spectral band width of 1nm, wavelength accuracy ±0.5nm and a pair of 1cm matched quartz cells was used to measure absorbance of the resulting solution.

Materials [7-19]

Standard samples of Dronedarone hydrochloride were taken, manufactured by MSN Pharma Limited (Hyderabad) as a gift sample with 99.59% (w/w) assay value and was used without further purification.

Solvents

Chloroform selected as solvent for developing spectral characteristics of the drug .The selection was made after assessing the solubility of drug in different solvents.

Methods

Solubility of drug

10mg Dronedarone hydrochloride of was weighed and solubility of this sample was checked in water, methanol, chloroform and phosphate buffer. The drug was found to be soluble in chloroform.

Identification of λ_{max} of Dronedarone hydrochloride

10mg of drug was weighed and was dissolved in 10ml of chloroform (1mg/ml). 1ml of this solution was withdrawn and volume was made up to 10ml. Appropriate dilutions were made with chloroform to give concentration of 10µg/ml scanned in UV range from 200-400nm, which could be utilized for analysis and spectrum was recorded (Fig.2).

Preparation of standard stock solution

10 mg of pure Dronedarone hydrochloride was accurately weighed and transferred to 10ml of volumetric flask. Drug was dissolved in chloroform and volume was made up to 10ml. The concentration of drug was 1mg/ml. 1ml of this solution was taken in a 10ml volumetric flask and volume was made up to the mark with chloroform. Thus Dronedarone hydrochloride of strength 100µg/ml was obtained.

Procedure for plotting calibration curve of pure drug

From the standard stock solution 0.2ml,0.4ml ,0.6ml, 0.8ml, and 1ml dilutions were made in 10ml volumetric flask and volume was made up to the mark with chloroform to obtain concentration in range of 2-10µg/ml. The spectra were recorded, absorbance were measured at 289nm (Table 1) and calibration curve was plotted (Fig. 3).

Analysis of pharmaceutical formulations

20 Tablets were procured from local market and average weight was determined. The powder equivalent to 10mg of Dronedarone hydrochloride was weighed accurately and dissolved in 10ml of chloroform, shaken for ten minutes and filtered. 1ml of this solution was taken in a 10ml volumetric flask and volume was made up to the mark with chloroform. Thus Dronedarone hydrochloride of strength 100 µg/ml was obtained. The solution was diluted in 10 ml volumetric flask with chloroform to get a solution of 10 µg/ml. Absorbance was measured at 289nm. Results are shown in the table 7.

Validation of method

Method validation was performed in terms of linearity, precision and accuracy, Limit of Detection

Fig 2. Identification of λ_{max} of Dronedarone hydrochloride

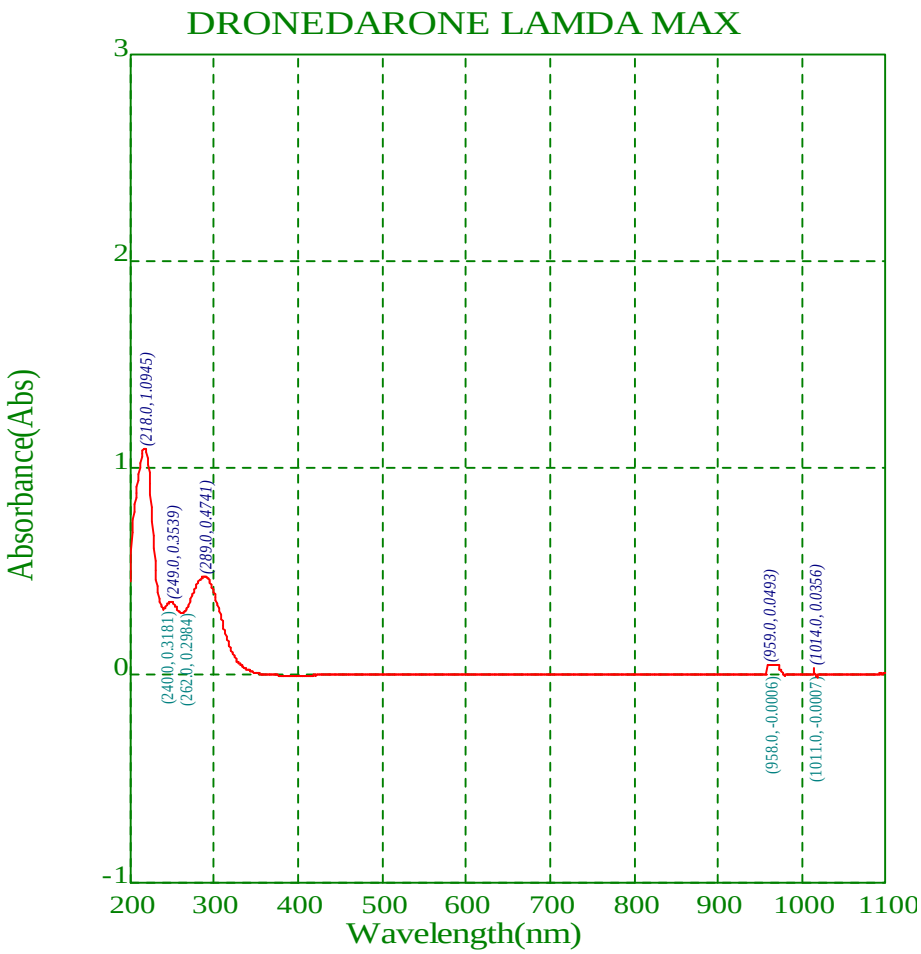

Fig 3. Linearity curve of dronedarone hydrochloride

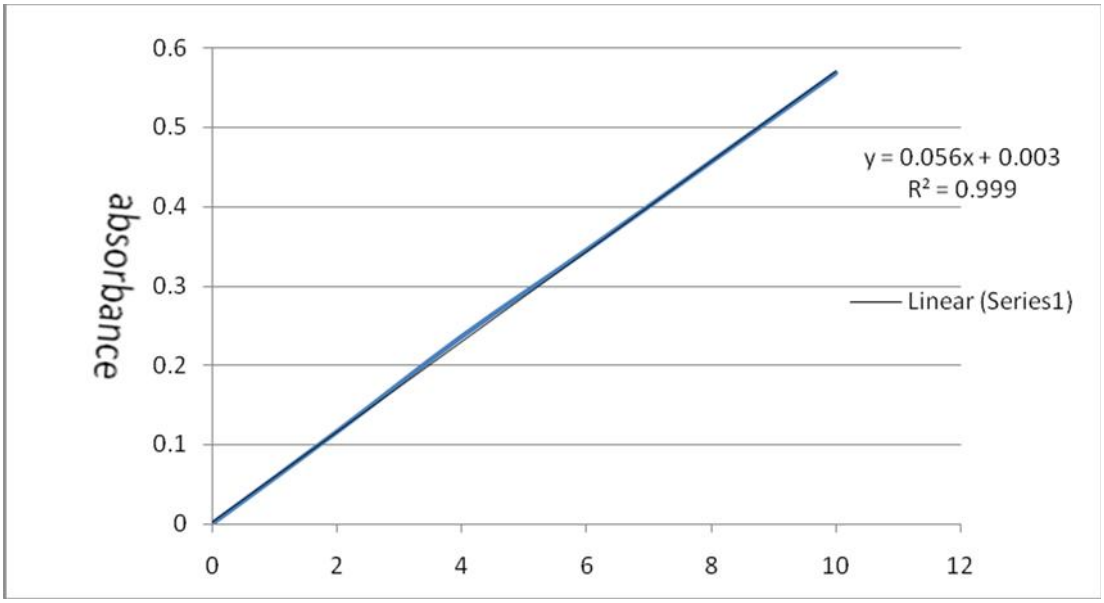

Table 1: Linearity table of Dronedarone hydrochloride

S.No	Concentration	Absorbance
1	0	0
2	2	0.1159
3	4	0.2358
4	6	0.3444
5	8	0.4561
6	10	0.5682

and Limit of Quantification and ruggedness as per **ICH**-Guidelines **Q2B (R1)**.

Linearity

For the method, linearity was repeated 3 times for validation. The calibration curve was constructed by plotting the response y-axis versus the theoretical concentrations of standards x-axis, by using linear regression analysis. Linearity was expressed as a correlation coefficient; r^2 the value must be > 0.999. The results are shown in table 1.

PRECISION

The intraday and inter day precision of the proposed spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of Dronedarone hydrochloride and the results are reported in terms of percent relative standard deviation. Results are shown in the table 4.

Procedure for recovery studies

20 Tablets were procured from local market and average weight was determined. The powder equivalent to 10mg of Dronedarone hydrochloride was weighed accurately and taken in 3 separate 10 ml volumetric flask. To this 8mg, 10mg, 12mg pure drug was added (for 80%, 100% and 120% recovery). 10ml of chloroform was added to make up the volume, shaken for ten minutes and filtered. 2.5ml of this solution was taken in a 25ml volumetric flask and volume was made up to the mark with chloroform. Thus Dronedarone hydrochloride of strength 100 µg /ml was obtained.1ml of this solution was diluted in 10ml volumetric flask up to the mark with chloroform and absorbance was measured at 289nm. This procedure was carried out for 3 times. Results are shown in the table 3.

Limit of detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3×N/S

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

Results are shown in the table 5.

Limit of quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × N/S

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Dronedarone hydrochloride using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to be reproducible, since there was no significant difference b/w analyst. Thus, the proposed methods could be considered rugged. The results are shown in table 6.

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

Results are shown in the table 5.

Results and Discussion

Standard calibration curve for Dronedarone hydrochloride, covering the range 2-10 µg/ml, prepared by serial dilution with chloroform for pure drug and tablet formulation were developed and validated. The procedure was adopted as per desired protocol, based on ICH Q2B guidelines. The calibration curve was obtained by plotting absorbance Vs analyte concentration. The slope and intercept of the calibration line was determined by linear regression.

Recovery: As shown in Table 3 excellent recovery were made at each added concentration.

Precision: precision evaluated through inter day and intra day of the pure drug from solvent are presented in Table 4.

Limit of detection (LOD) limit of quantification (LOQ): The LOD determined as the amount of drug and LOQ was determined as the lowest concentration for drug shown in Table 5.

Table 2. Linearity regression data for Dronedarone hydrochloride

S.No	Parameters	Results
1	Absorption maxima (nm)	289
2	Linearity range (µg/ml)	2-10
3	Standard regression equation	y = 0.056x + 0.003
4	Correlation coefficient (r^2)	r^2 = 0.999
5	Accuracy (% recovery)	99.71 to 99.92
6	Precision	99.57 - 101.172
7	LOD & LOQ	0.998, 3.025

Table 3 excellent recovery at each added concentration

S.NO	Level of % recovery	Initial amount present µg/ml	Amount of standard added µg/ml	Total amount present µg/ml	Total amount recovered µg/ml	% recovery	Mean	Statistical analysis	
								S.D	% RSD
1	80	10	8	18	17.89	99.38			
	80	10	8	18	17.94	99.66	99.71	0.3682	0.3693
	80	10	8	18	18.02	100.11			
2	100	10	10	20	19.83	99.15			
	100	10	10	20	20.15	100.75	99.58	1.0214	1.0257
	100	10	10	20	19.77	98.85			
3	120	10	12	22	21.98	99.90			
	120	10	12	22	22.08	100.36	99.92	0.4303	0.4306
	120	10	12	22	21.89	99.10			

Table 4: Precision data for proposed method

Sample no	assay of Dronedarone HCL as presence of lebeled amount	
	Assay of intra day precision	Assay of inter day precision
1	100.26	101.132
2	99.78	101.022
3	98.71	100.945
4	99.53	101.590
MEAN	99.57	101.172
STANDARD DEVIATION	0.6484	0.2888
% RSD	0.6512	0.2854

Table 5: LOD and LOQ for drug in solvent

S.No	Conc. (µg/ml)	Absorbance	S.D	Slope	LOD (µg/ml)	LOQ (µg/ml)
1	Dronedarone hydrochloride 10 (µg/ml)	0.5624	0.002439	0.056	0.14	0.43
2		0.5589				
3		0.5629				
4		0.5592				
5		0.5645				

Table 6: Ruggedness data at 10 µg/ml by two analysts at different days

Test concentration µg/ml	Analyst - 1	Analyst - 2
10	0.5624	0.5670
10	0.5589	0.5689
10	0.5629	0.5662
10	0.5592	0.5612
10	0.5645	0.5645
MEAN	0.5616	0.5655
STANDARD DEVIATION	0.002439	0.002906
% RSD	0.004342	0.005138

Table 7: Analysis of pharmaceutical formulations

Formulation	Labeled amount (mg)	Amount recovered (mg)	% drug recovered	Mean	Standard deviation	% RSD
MULTAQ 400	400	393.26	98.315	99.838	1.9398	1.9430
	400	408.09	102.022			
	400	396.71	99.177			

Conclusion

The proposed derivative spectrophotometer method is found to be accurate, precise, economic and rapid for estimation of Dronedarone hydrochloride. It satisfactorily eliminates interference from excipients. Hence it can be employed for routine analysis of drugs in marketed formulations in Quality Control laboratories.

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References

1. The Merck Index, 14th ed. 2007. P-3449.
2. Gerald V. Naccarelli, *et al*. Safety and efficacy of dronedarone in the treatment of atrial fibrillation/flutter. Clin Med Insight Cardiol 2011; 5: 103-19.
3. V.K. Ahirao, *et al*. Stress degradation studies of dronedarone in pharmaceutical dosage form by a validated stability indicating method. J Chil Chem Soc 2012; 57(3): 1272-6.
4. Disha Patel, Avijit Chowdhury. Development and validation of Dronedarone HCl in plasma by RP-HPLC method coupled with UV detector. Iventi Impact: Biomedical analysis 2012; Vol 2012.

5. Tondepu Naresh, Sait, Shakil S, Surendranath KV, Kiran Kaja, Ravi Kumar, Suresh A. Stability indicating HPLC method for Dronedarone in bulk drugs and pharmaceutical dosage forms. Amer J Anal Chem 2012; 3(8): 544-7.
6. Arpan Patel, Jawed Akhtar, Chirag Sharma. Spectrophotometric estimation of Dronedarone in pure drug and pharmaceutical formulation. Asian J Biomed Pharm Res 2012; 2(1): 266-71.
7. Batuk Dabhi, Yashwantsinh Jadeja, Madhavi Patel, Hetal Jehaliya, Dinish Karia, Anamik Shah. Method development and validation of a stability indicating RP-HPLC method for the quantitative analysis of Dronedarone hydrochloride in pharmaceutical tablets. Scipharm 2013; 81: 115-122.
8. Rajyalakshmi Ch, Benjamin T. Forced degradation study on dronedarone and application of validated stability indicating HPLC-UV method in stability testing of dronedarone tablets. Der Pharm Chem 2013; 5(1): 189-195.
9. Arpan Patel, Javed Akhtar. RP-HPLC Method development and validation of Dronedarone HCL in its pure form and tablet dosage form. J Chem Pharm Res 2012; 4(4): 2173-2179.
10. Batuk Dobhi, Hetal Jehaliya, Madhavi Patel, Yashwantsinh Jadeja, Denish Karia, Anamik Shah. 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.
11. Boldeman R. W, Hermans J. J, Maessen JG. Determination of the class iii antiarrhythmic drugs Dronedarone and Amiodarone, and their principal metabolite in plasma and myocardium by high performance liquid chromatography and UV detection. J Chromatogr B; Anal Tech Biomed Life Sci 2009; 877: 1727-31.
12. Dinesh Sahu, Ghazal khan. RP-HPLC method development and validation of Dronedarone HCl in its pure form and tablet dosage form. Asian J Biomed Pharm Res 2011, 1(2), 352**.**
13. Cen xie, Shilei Yang, Dafang Zhong. Simultaneous determination of dronedarone and its active metabolite debutyldronedarone in active plasma by liquid chromatography- tandem mass chromatography: application to a pharmacokinetic study. J Chromatogr B; Analyt Technol Biomed Life Sci 2011; 879(28): 3071-5.
14. M. Saeed Arayne, Najma Sultana, Saima Sher Bahadur. Multivariate calibration in UV Spectrophotometric analysis. Pak J Pharm Sci 2007; 20(2): 163-74.
15. International Conference on Harmonization [ICH] of technical requirements for registration of pharmaceuticals for human use guideline on validation of analytical procedure – Methodology ICH, Geneva, Switzerland 1996.
16. US FDA. Guideline for industry: text on validation of analytical procedures: ICH Q2A. Rockville, MD: Mar 1995.
17. GLP-The United Kingdom Compliance Programme (Department of Health) 1989.Beckett, A. H., Stenlake, J. B., Practical Pharmaceutical Chemistry, 4th edition, Part IICBS Publishers and Distributors, New Delhi (1997), 275-295.
18. Instruction Manual Users system Guide UV-2080 UV- Visible Spectrophotometer (Double beam), Analytical technologies.

