Research Article

HPTLC METHOD FOR ESTIMATION OF DRONEDARONE HYDROCHLORIDE IN BOTH BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The objective of the current study was to develop a validated, specific HPTLC method for the quantitative determination of Dronedarone and its related substances Simple, accurate and precise high-performance thin-layer chromatography (HPTLC) method for the simultaneous determination of Dronedarone in both, bulk drug and tablet dosage form has been developed and validated. Chromatographic separation was carried out on silica gel 60 GF254 HPTLC plates with Acetone:Methanol (8:2, v/v) using as solvent system. The method validated for accuracy, precision, specificity, Linearity, Range, limit of detection (LOD) and limit of quantification (LOQ). The calibration curve was found to be linear in the concentration range of 200-800 ng/band with Correlation co-efficient is 0.999. Sensitivity of the method was carried out by LOD and LOQ, and was found 34.6 and 75.2 ng/band respectively. Method is specific, selective with potential application in pharmaceutical analysis in tablet dosage form.

Keywords: HPTLC, Dronedarone, Pharmaceutical dosage form, Bulk Drug, Method development, Validation.

INTRODUCTION

Dronedarone hydrochloride, mainly used for the indication of cardiac arrhythmias, which is chemically as N-(2-Butyl-3-(p-(3-(dibutylamino) propoxy)benzoyl)-5-benzofuranyl) methane sulfonamide. Its molecular formula is $C_{31}H_{44}N_2O_5$ HCl, and used as an alternative to amiodarone for the treatment of atrial fibrillation and a trial flutter in people whose hearts have either returned to normal rhythm or who undergo drug therapy or electric shock treatment to maintain normal rhythm[1].

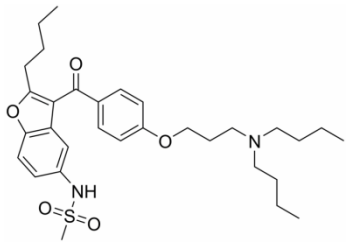

Figure 1: Structure of Dronedarone Hydrochloride

Few HPLC method were available in literature for analysis of Dronedarone includes, Determination of the class III antiarrhythmic drugs dronedarone and amiodarone, and their principal metabolites in plasma and myocardium by high-performance liquid chromatography and UV-detection[2], simultaneous determination of dronedarone and its active metabolite debutyldronedarone in human plasma by liquid chromatography tandem mass spectrometry: Application to a pharmacokinetic study[3], HPLC method development and validation of Dronedarone HCl in its pure and tablet dosage form that informs about the analysis of dronedarone in bulk and pharmaceutical dosage form[4]. Stability indicating method for analysis of dronedarone by HPLC in bulk and dosage form[5]. Literature survey shows that there is no HPTLC method available for quantitative analysis of dronedarone in bulk and pharmaceutical dosage form. Hence here developed a simple, accurate, precise, cost effective method for quantitative analysis of dronedarone in both, bulk and in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals

Dronedarone hydrochloride reference standard was provided by Sanofi-Aventis. Tablets (400 mg) of Dronedarone hydrochloride was produce from a pharmacy. HPLC Grade Methanol and Acetone were obtained from Merck India Limited, Mumbai, India. Membrane filter, 0.45 µm was obtained from Pall Life sciences, Mumbai, India. High purity deionised water was obtained from a Millo-Q (Millipore, Milford, MA, USA) purification system. Nylon syringe filters 0.45 µm were from Millex-Hn (Mumbai, India). TLC plates used were obtained from Merck.

Equipments

The HPTLC system used for quantitative analysis of dronedarone include, Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Developed TLC plate was scanned on Camag TLC scanner III, operated by Wincats Software (V 1.4.2, Camag). The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm.

Experimental Condition

Chromatography was performed on Merck TLC plates pre-coated with silica gel 60 F254 (10 cm ×10 cm with 250 µm layer thicknesses) from E. Merck, Germany. Before use the plates were prewashed with methanol then dried in

the current of dry air and activated at 110 °C for 5 min. The analysis was carried out by HPTLC using Acetone: Methanol (8:2, v/v) as a solvent system and gel 60GF 254 HPTLC plates (10×10cm) as a stationary phase. Sample were applied on HPTLC plates as 8 mm bands, by camag linomat v automatic sample applicator fitted with 100ul Hamilton syringe with the nitrogen flow. Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) previously saturated with mobile phase vapour for 30 min at room temperature and relative humidity 60 ± 5%. The development distance was approximately 80 mm. After development the plates were dried in current of air by use of an air dryer. Densitometric scanning, at 290 nm, was performed with a scanner, operated by Wincats Software (V 1.4.2, Camag) in absorbance mode. The selection of wavelength was based on maximum absorbance for optimum sensitivity. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm. The slit dimensions were 5 mm × 0.45 mm.

Standard Preparation

25mg of Dronedarone API transfer in 50 mL volumetric flask, dissolve and dilute it up to mark with methanol. Take 5 mL of this dilute solution in 50 mL volumetric flask and dilute up to mark with methanol to a final concentration 50 µg/mL.

Sample Preparation

Amount of powdered tablets equivalent to 25 mg dronedarone was weight and dissolve in methanol in to 50 mL volumetric flask, sonicate for 10 minute, wait for 5 min to room temperature and dilute up to mark with methanol. 5 mL of this sample stock solution was dilute to 50mL to obtained final concentration 50µg/mL. The resultant solution was used for the analysis.

RESULTS AND DISCUSSION

The proposed method describe a HPTLC procedure by using a pre-coated silica gel 60GF 254 HPTLC plates as stationary phase and Acetone:Methanol (8:2, v/v) as a solvent system. Drug solution having concentration of 20 µg/mL were scanned in range of 200-400 nm on a UV visible spectrophotometer for selection of sampling wavelength. After recording the spectra 290nm wavelength was selected as a suitable wavelength for estimation of dronedarone.

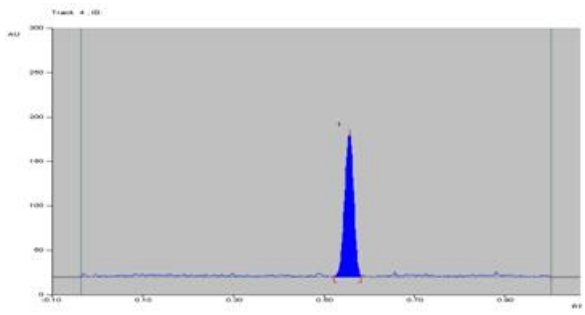

Figure 2: HPTLC chromatogram of dronedarone standard

Method Validation

The proposed HPTLC method was validated as per ICH guideline.

Linearity

The linear response of the dronedarone was determined by analyzing six independent level of calibration curve in the range of 400-800 ng/band of dronedarone and result shown in table 1.

Table 1: Linearity Study of Dronedarone

Concentration ng/band	Peak Area	Parameter	Result
200	1077	Linearity Range (ng/band)	200-800
300	1686		
400	2234	Correlation Coefficient (r^2)	0.999
500	2731		
600	3326	Slope	550.2
700	3847	Intercept	4.46
800	4398		

Precision

Precision study was performed to find out intra-day and inter day variations. The % relative standard deviation (RSD) for precision of Intraday for dronedarone 0.74 (Table-4) and for interday 0.86 which is less than 2% indicating high degree of precision.

Specificity

The specificity of the method was determined by analysis of drug standards and samples. The band for modafinil in the sample was identified by comparing the R_f value and spectrum of the band with those of the band from a standard. The peak purity of modafinil was assessed by comparing spectra acquired at three different positions on the peak, i.e. the peak start (S), peak apex (M), and peak end (E) positions of the peak.

Accuracy

Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking samples of dronedarone (50%, 100% and 150%) with three different excipient. The %RSD for the tablet analysis and recovery studies was less than 2% indicating high degree of accuracy. The results were shown in table 2.

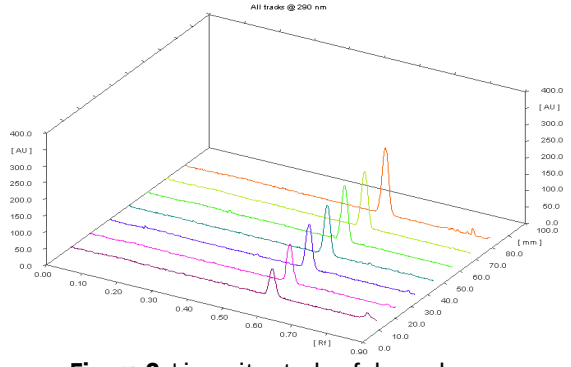

Figure 3: Linearity study of dronedarone

Table 2: Accuracy Study of Dronedarone

Level %	No	Amount of drug added (ng/band)	Amount of drug found (ng/band)	Recovery (%)	Mean Recovery (%)	RSD (%)
50	1	251	251.80	100.32	100.33	0.28
	2	252	253.56	100.62		
	3	252	252.18	100.07		
100	1	502	498.29	99.26	99.47	0.20
	2	501	498.39	99.48		
	3	501	499.30	99.66		
150	1	751	747.55	99.54	99.79	0.25
	2	753	751.34	99.78		
	3	751	751.30	100.04		

Table 3: Assay analysis of Dronedarone Pharmaceutical Tablet

Method	Drug	Label claim [mg/tablet]	Amount found [mg/tablet][a]	Drug Assay [%][a]	% RSD
TLC-Densitometry	Dronedarone	400	397.35	99.33%	0.83%
a= Mean of six determination					

Table 4: Summary of validation parameters

Parameter	Result	%RSD
λ (nm)	290	-
R_F	0.57	-
Accuracy (at 100% level)	100.42	0.86
Intraday precision	99.98	0.74
Interday precision	99.46	0.86
Limit Of Detection (ng/Band)	34.6	-
Limit Of Quantitation (ng/Band)	75.2	-

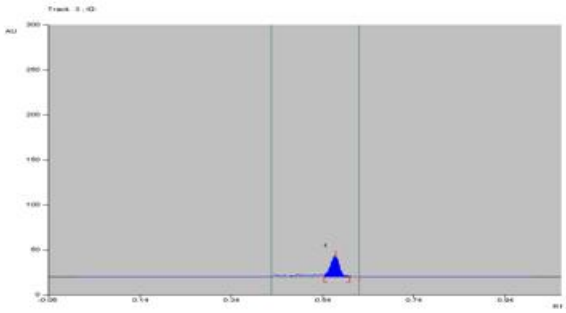

Figure 4: LOD study of dronedarone

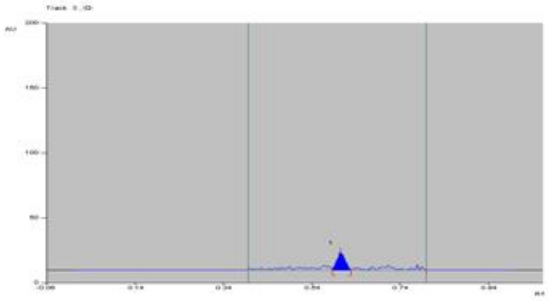

Figure 5: LOQ study of dronedarone

Selectivity

There is no any interference of excipient, and solvent with the drug main peak, so method is selective for Dronedarone analysis.

LOD and LOQ

To determine the limits of detection (LOD) and limit of quantification (LOQ), different dilution of standard solution of drug were applied along with methanol as the blank. The LOD and LOQ were determined on the basis of signal to noise ratio until the average responses of triplicate analysis were obtained approximately 3 and10 times the responses of the blank respectively. LOD and LOQ of dronedarone were found 34.6 and 75.2 ng/band respectively.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ±2 change in wave length ±2 change in mobile phase ratio and chamber saturation time there was no significant impact on the Area.

Ruggedness

The ruggedness study was done by the two analysts. The % RSD for analyst-1 was 1.03 % and for analyst 2 was 0.68 %.

Assay of Pharmaceutical Formulation:

This proposed methods was applied for the determination of dronedarone in commercial tablets. The results shown in Table-4 were satisfactory and with good agreement with the labeled amount.

CONCLUSION

The statistical data have been proven that developed HPTLC method for estimation of dronedarone was found to be more accurate, precise, and sensitive. Therefore the proposed method could be applied for routine analysis in quality control laboratories for both in bulk and pharmaceutical formulation.

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