

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF AMIODARONE HYDROCHLORIDE

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Article Received on
05 Jan. 2018,

Revised on 26 Jan. 2018,
Accepted on 16 Feb. 2018

DOI: 10.20959/wjpr20185-11255

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ABSTRACT

A simple, sensitive and accurate stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the estimation of Amiodarone hydrochloride in bulk and in pharmaceutical dosage form. The separation was performed on pre-coated silica gel 60 GF₂₅₄ aluminum plates using Ethyl acetate: Methanol (9:1 v/v) as mobile phase. The retention factor (R_f) for drug was found to be 0.48 ± 2.05 . The detection of band was carried out at 242 nm. The drug was subjected to different stress conditions like acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH Q2 (R1)

guidelines. The data of linear regression analysis indicated a good linear relationship over the concentration range of 200-1200ng/band with correlation coefficient (R^2) 0.9976. The accuracy of the method was established based on the recovery studies. The developed method was found to be simple, sensitive, selective, accurate and repeatable for analysis of Amiodarone hydrochloride and can be adopted for routine analysis of drug in bulk and in pharmaceutical dosage form.

KEYWORDS: High performance thin layer chromatography (HPTLC), Amiodarone hydrochloride, Stability indicating, Validation.

INTRODUCTION

Amiodarone hydrochloride is a class III anti-arrhythmic agent and one of the most powerful drug used in the treatment of ventricular and supraventricular tachycardia. Amiodarone hydrochloride is a benzofuran derivative, chemically it is 2-butylbenzofuran-3-yl-4-(2-

diethylaminoethoxy)-3,5-diiodophenyl ketone hydrochloride.^[1] Literature survey reveals that several analytical methods have been reported for the estimation of Amiodarone hydrochloride in pharmaceutical dosage form including spectroscopic methods,^[2-3] high performance liquid chromatography (HPLC),^[4-6] stability indicating HPLC methods,^[7-9] FT-Raman spectroscopy^[10] and in biological fluids.^[11] To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of Amiodarone hydrochloride. The present work describes a simple stability indicating HPTLC method for the determination of Amiodarone hydrochloride in bulk and pharmaceutical dosage form (CORDARONE-100 mg) according to the International conference on harmonization (ICH) guidelines.

EXPERIMENTAL

MATERIALS AND METHODS

Reagents and chemicals

Authentic sample of Amiodarone hydrochloride was obtained from Micro Labs Limited, Bangalore. The formulation Cordarone labeled to contain Amiodarone hydrochloride 100 mg was procured from local market. Methanol (AR grade), Ethyl acetate (AR grade) were purchased from S. D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H₂O₂) and sodium hydroxide (NaOH); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Chromatographic conditions

Chromatographic separation of drug was performed on aluminum plates pre-coated with silica gel 60 GF₂₅₄, (10 cm × 10 cm with 250 μm layer thickness). Sample was applied on the plate as a band of 4 mm width using Camag 100 μl sample syringe (Hamilton, Switzerland) with a linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Ethyl acetate: Methanol (9:1 v/v). 10 cm × 10 cm Camag twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run. Migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 200-400 nm, operated by winCATS software, slit dimensions were 3.00×0.45 mm and Deuterium lamp was used as a radiation source.

Selection of detection wavelength

From the standard stock solution (1000 $\mu\text{g/ml}$) further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 242 nm. Representative UV spectrum of Amiodarone hydrochloride is shown in “Fig. 1”.

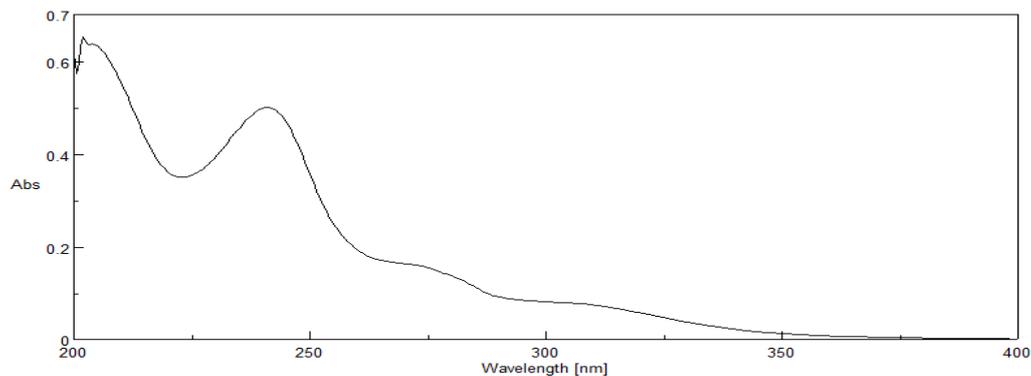


Fig. 1: The UV spectrum of Amiodarone hydrochloride (10 $\mu\text{g/ml}$).

Preparation of standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 $\mu\text{g/ml}$. From the standard stock solution, working standard solution was prepared containing 100 $\mu\text{g/ml}$ of Amiodarone hydrochloride. 4 μl of the resultant solution was applied on TLC plate to get concentration of 400 ng/band. Representative densitogram of Amiodarone hydrochloride (400 ng/band) is shown in “Fig. 2”.

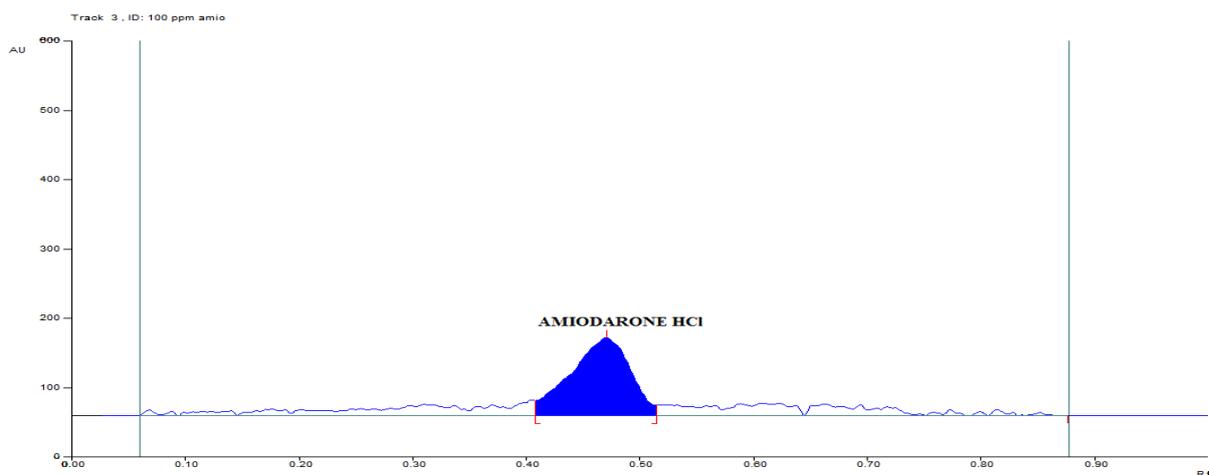


Fig. 2: Densitogram of standard solution of Amiodarone hydrochloride (400ng/band)
Preparation of sample solution.

For determination of the content of amiodarone in amiodarone tablets (label claim: 100 mg Amiodarone HCl per tablet), twenty tablets were weighed; average weight was determined and were finely powdered. A quantity of powder equivalent to 10 mg of amiodarone was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. 1.0 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 100µg/ml.

Stress degradation studies of bulk drug^[12]

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, neutral hydrolysis, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies are carried out at higher concentration level of 2,000ng/band to observe presence of degradation product peak (if any).

Alkaline hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of 1 N NaOH was added. The above solution was kept for 3 hours at room temperature. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 68.97% of Amiodarone hydrochloride was recovered with no peak of degradation. Representative densitogram is shown in “Fig. 3”.

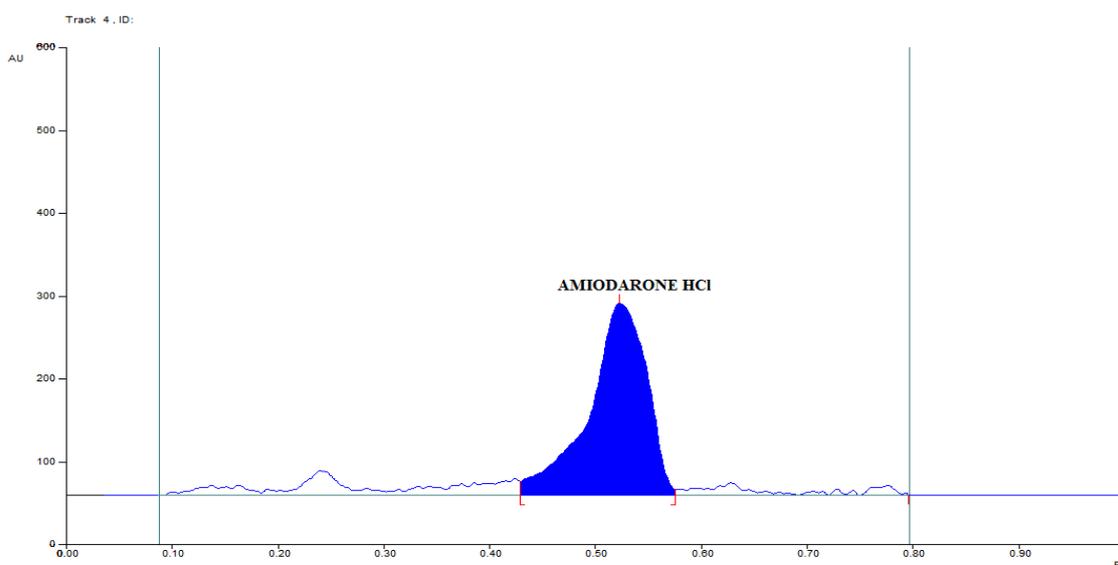


Fig. 3: Representative densitogram of alkali induced degradation of Amiodarone hydrochloride (2000ng/band).

Acid hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 $\mu\text{g/ml}$), 1ml of 0.5 N HCl was added. The above solution was kept for 3 hours at room temperature. 4 μl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 88.63% Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram is shown in “Fig.4”.

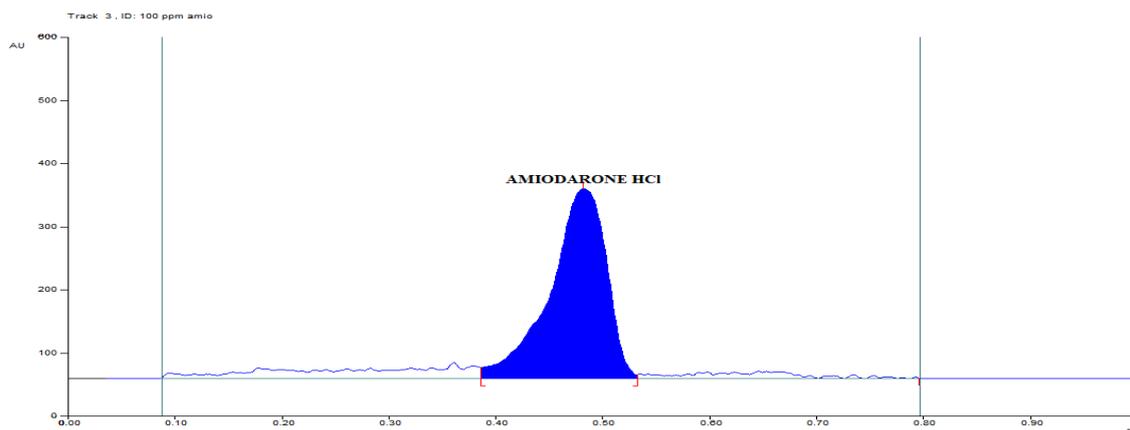


Fig. 4: Representative densitogram of acid induced degradation of Amiodarone hydrochloride (2000ng/band).

Neutral hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 $\mu\text{g/ml}$), 1 ml of distilled water was added. The above solution was kept for 3 hours at room temperature. 4 μl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 81.35% of Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram is shown in “Fig. 5”.

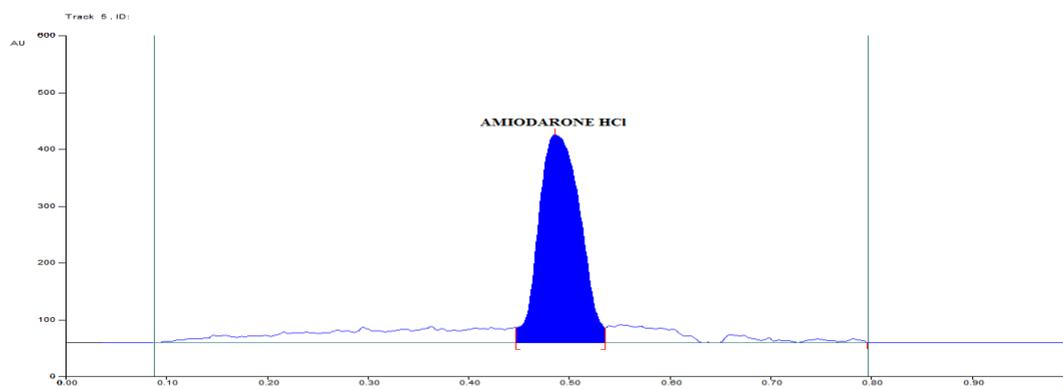


Fig. 5: Representative densitogram of neutral degradation of Amiodarone hydrochloride (2000 ng/ band).

Degradation under oxidative conditions

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of 30% H₂O₂ was added. The above solution was kept for 3 hours at room temperature. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 88.03% of Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram is shown in “Fig. 6”.

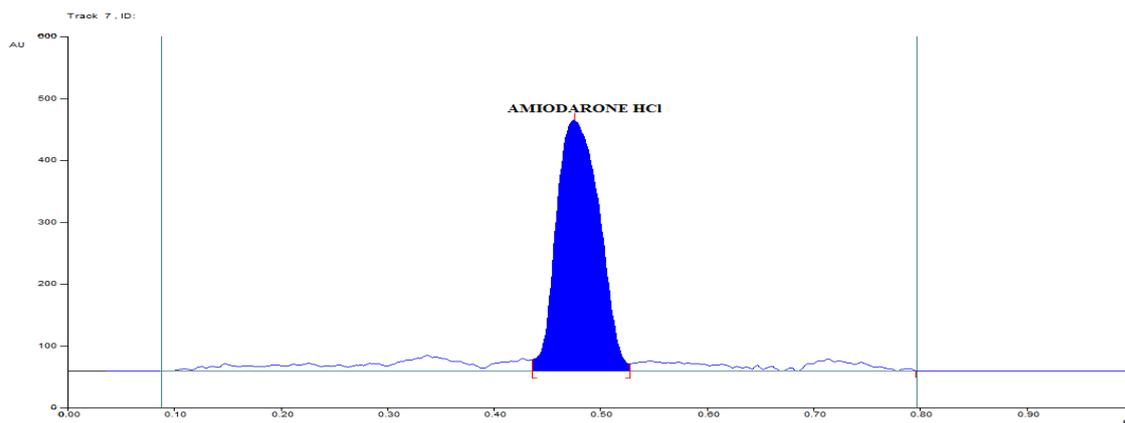


Fig. 6: Representative densitogram of peroxide induced degradation of Amiodarone hydrochloride (2000ng/band).

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (50⁰ C) for a period of 3 hours. Sample was withdrawn, dissolved in methanol and diluted to get 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 93.63% Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram is shown in “Fig. 7”.

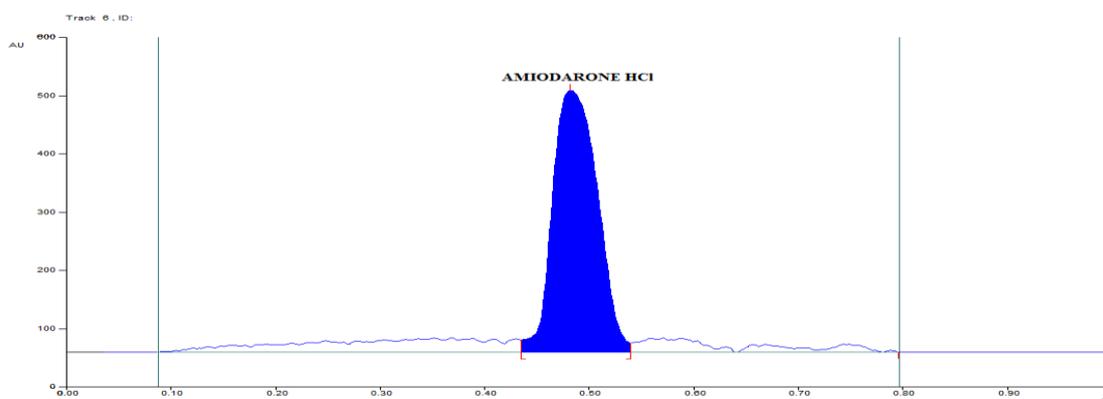


Fig. 7: Representative densitogram of dry heat degradation of Amiodarone hydrochloride (2000ng/band).

Photodegradation studies

1. UV illumination

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m². After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain 1000 µg/ml. 5 ml of the resultant solution was then diluted with methanol to get the concentration of 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 95.15% of Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to UV Illumination is shown in “Fig. 8”.

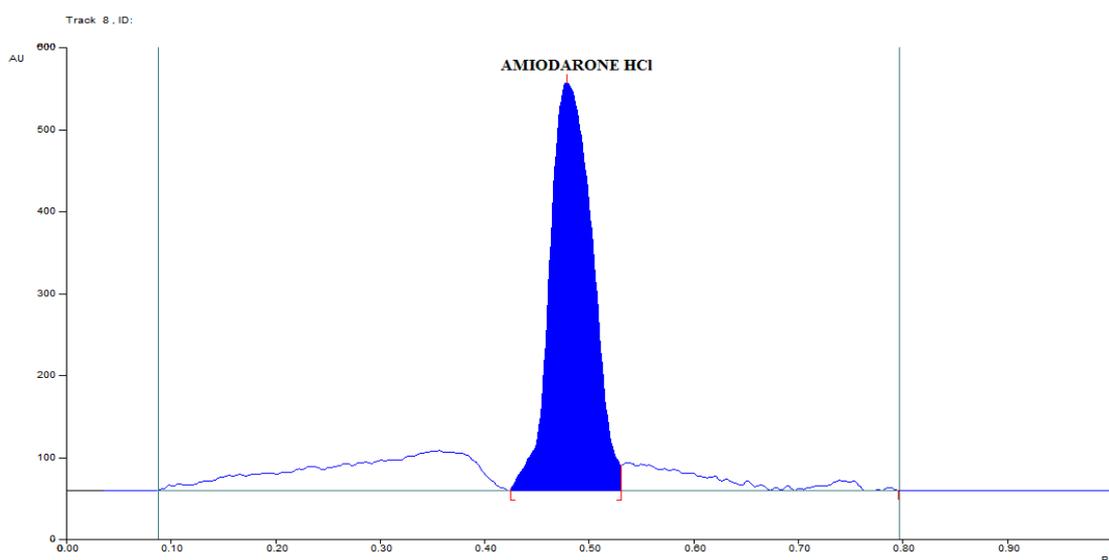


Fig. 8: Representative densitogram of Photolytic UV degradation of Amiodarone hydrochloride (2000ng/band).

2. Fluorescent light

The photo degradation study of the drug was studied by exposing the drug to fluorescent light providing illumination of NLT 1.2×10⁶ Lux hr of fluorescent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain 1000µg/ml. 5 ml of the resultant solution was then diluted with methanol to get the concentration of 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 95.37% of Amiodarone hydrochloride was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to Fluorescent light is shown in “Fig. 9”.

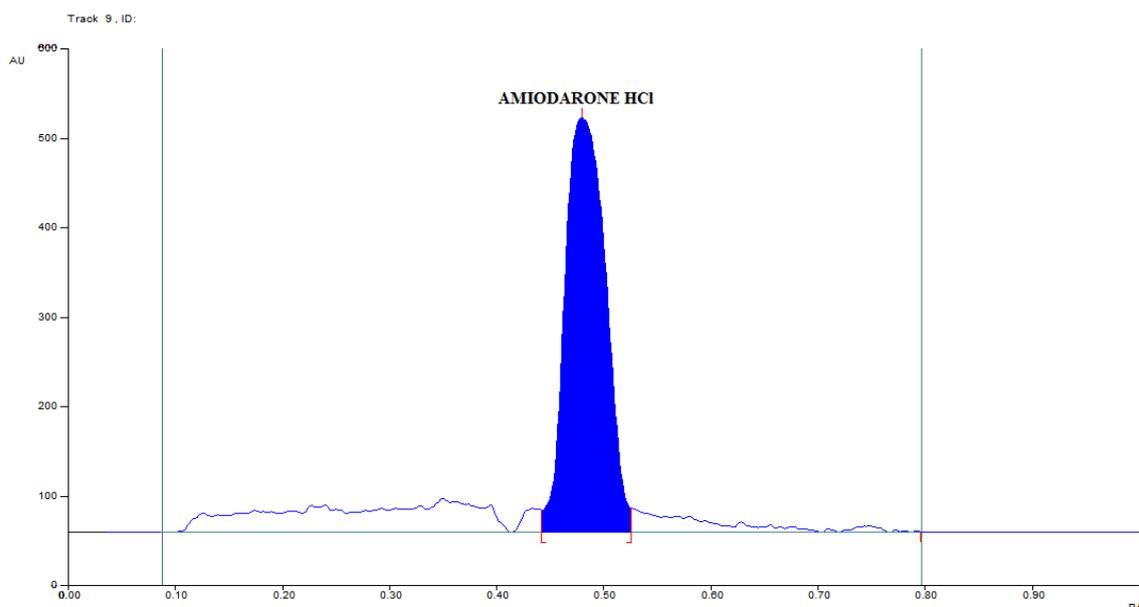


Fig. 9: Representative densitogram of Photolytic fluorescent light degradation of Amiodarone hydrochloride (2000 ng/band).

Validation of analytical method^[13]

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity and range

From the standard stock solution (1000 µg/ml) of Amiodarone hydrochloride, solution was prepared containing 100 µg/ml of Amiodarone hydrochloride. The solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analysing six concentrations over the concentration range of 200-1200 ng/band for Amiodarone hydrochloride. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve. The results found to be linear with regression equation of $y = 8.6235x - 115.58$ with $R^2 = 0.9976$. The calibration curve is shown in “Fig. 10”.

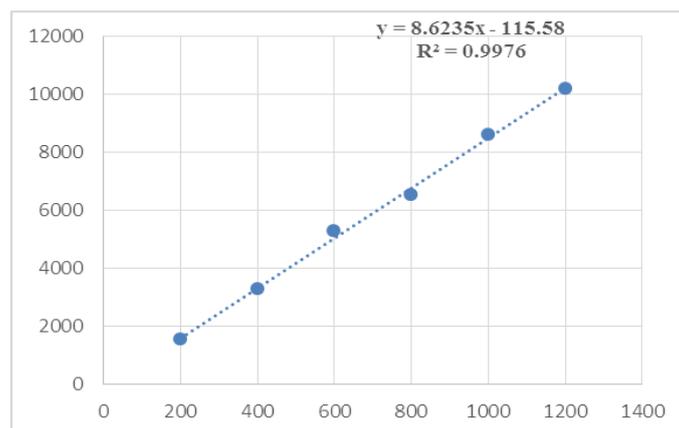


Fig. 10: Calibration curve of Amiodarone hydrochloride (200-1200 ng/band) reference standard.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analysed on the same day and percentage RSD was calculated. For the inter-day variation studies, 3 replicates of 3 concentrations were analysed on 3 consecutive days and percentage RSD was calculated. For intra-day precision and inter-day precision results obtained are shown in Table 1.

Table 1: Intra-day and inter-day variation studies data for Amiodarone hydrochloride.

Concentration (ng/band)	Intra-day precision			Inter-day precision		
	Area	% recovery	% RSD	Area	% recovery	% RSD
400	3339.7	100.170	0.681	3329.8	99.883	0.608
	3341.3	100.217		3335.6	100.052	
	3299.9	99.017		3368.8	101.014	
600	5104.8	100.894	0.362	5108.5	100.966	0.239
	5135.2	101.482		5099.9	100.800	
	5100.5	100.811		5124.5	101.275	
800	6792.4	100.133	0.809	6788.9	100.082	0.408
	6799.8	100.240		6775.6	99.890	
	6699.9	98.792		6829.7	100.674	

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula $LOD = 3.3 \sigma / S$ and $LOQ = 10 \sigma / S$ where σ is standard deviation of the y intercept of linearity equation and S is slope of the calibration curve of the analyte. The LOD and LOQ were found to be 34.04 ng/ band and 103.16ng/band, respectively.

Assay

CORDARONE-100 mg tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Basic concentration of sample chosen was 400 ng/band from tablet solution. Concentration and % recovery was determined from linearity equation. Assay results obtained are shown in Table 2.

Table 2: Assay of marketed formulation.

S. N.	Peak area	Amount recovered (ng/band)	% recovery	% RSD
1	3381.3	405.506	101.376	0.541
2	3345.4	401.343	100.336	
3	3342.3	400.983	100.246	
4	3339.2	400.624	100.156	
5	3329.9	399.545	99.886	
6	3332.1	399.801	99.950	

Accuracy

To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 400ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Table 3: Accuracy studies of Amiodarone hydrochloride.

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	% recovery	% RSD
50 %	400	200	5101.2	100.825	0.395
			5110.2	100.999	
			5140.6	101.586	
100 %	400	400	6873.2	101.304	0.657
			6799.6	100.237	
			6789.9	100.097	
150 %	400	600	8610.2	101.186	0.174
			8580.2	100.838	
			8591.6	100.970	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, Time was changed from spotting to development and development to scanning and the effect on the area was noted. It was found that method is robust.

RESULT AND DISCUSSION

Amiodarone hydrochloride has absorbance maxima at 242 nm. The calibration curve data show good linear relationship in concentration range 200-1200 ng/band. Recovery study is carried out at three different level 50%, 100% and 150% by adding pure drug to the previously analysed test sample. Percentage recovery for drug was determined by linearity equation method and found to be within acceptance criteria. The precision and accuracy was found to be good, which is evident by low standard deviation values.

Sr. No.	Validation parameters	Amiodarone hydrochloride
1.	Linearity equation	$y = 8.6235x - 115.58$
	R ²	R ² = 0.9976
	Range	200-1200ng/band
2.	Precision	(%RSD)
	Intra-day	0.62
	Inter-day	0.42
3.	Assay	99.87% - 101.38%
4.	Accuracy	
	50	100.82 % - 101.57 %
	100	100.10 % - 101.30 %
	150	100.84 % - 101.19 %
5.	Limit of detection	34.04 ng/band
6.	Limit of quantitation	103.16 ng/band
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

A simple, precise, accurate, reproducible, and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of Amiodarone hydrochloride as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of Amiodarone hydrochloride in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

ACKNOWLEDGEMENT

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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