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

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ABSTRACT

The aim of the present study was to develop a validated stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of Amiodarone hydrochloride. An isocratic, RP-HPLC method was developed using Hi Q SIL C_{18} (4.6 mm x 250 mm, 5 µm) column, Acetonitrile and 0.5% formic acid (80:20 v/v) as mobile phase at flow rate of 1.0 ml/min. Detection and Quantification was carried at wavelength of 242 nm. The retention time (RT) of drug was 8.329 ± 0.412 min. The method was validated with respect to linearity, precision, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5-30 µg/ml concentrations with a correlation coefficient (R^2) of 0.9993. Amiodarone hydrochloride was subjected to different stress testing conditions. The degradation products were well resolved from the drug under the tested conditions.The developed method was found to be simple, sensitive, selective, accurate and precise for analysis of Amiodarone hydrochloride and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

KEYWORDS: High performance liquid chromatography (HPLC), Amiodarone hydrochloride, Stability indicating method, 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 few 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] FT Raman spectroscopy[7] and bioanalytical HPLC.[8] Although few reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast.[9-12] Hence we have tried to develop stability indicating HPLC method for estimation of Amiodarone hydrochloride. The present work describes a simple stability indicating HPLC method for the determination of Amiodarone hydrochloride in bulk and pharmaceutical dosage form (CORDARONE-100mg) according to the International conference on harmonization (ICH) guidelines.

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.Acetonitrile (HPLC grade) was purchased from S. D. Fine Chemical Laboratories (Mumbai, India), HPLC grade water is collected at college using ELGA water purification system. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH), formic acid 98% (Methanolic acid); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Chromatographic Conditions

HPLC system used was JASCO system equipped with model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), JASCO PDA MD-2010 Plus detector and Borwin chromatography software (version 1.5). A chromatographic column Hi Q SIL C_{18} (4.6 mm x 250mm, 5 µm) was used, for separation at a flow rate of 1.0 ml/min using Acetonitrile:0.5% formic acid (80:20 v/v) as mobile phase and detection at 242nm. The representative chromatogram is shown in"Fig.1".

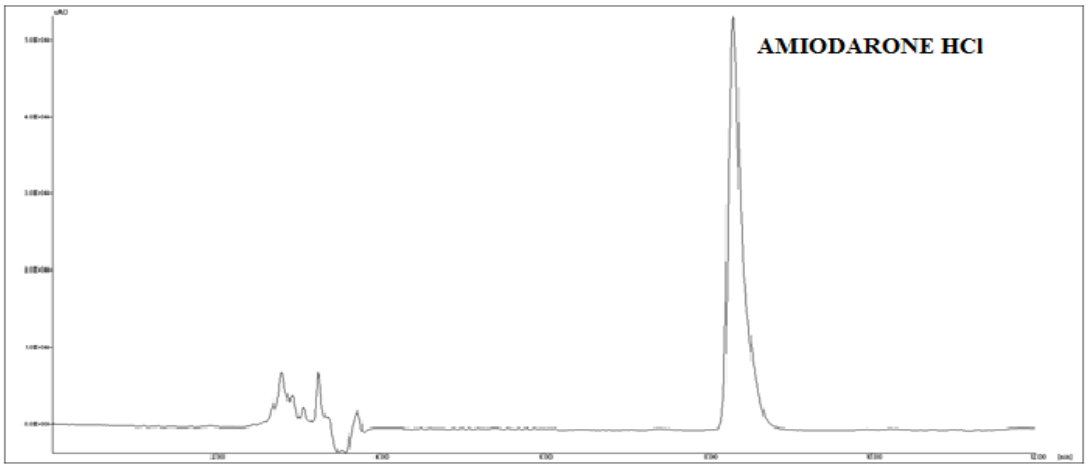

Fig.1: Chromatogram of Amiodarone hydrochloride(10 µg/ml).

Preparation of 0.5% formic acid and mobile phase

0.5% formic acidwas prepared by adding 5 ml of formic acid to 950 ml of HPLC grade water. Mix solution thoroughly. Add HPLC grade water to final volume of 1 L, degas and transfer to container. Mobile phase was prepared by mixing Acetonitrile and 0.5% formic acid in the ratio of 80:20 v/v. It was then filtered and sonicated for 10 min.

Preparation of standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. From this solution further dilution in mobile phase was done to get concentration 10 µg/ml.

Selection of detection wavelength

From the standard stock solution (1000 µg/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 linear, stable and considerable absorbance at 242 nm. Representative UV spectrum of Amiodarone hydrochloride is shown in "Fig. 2".

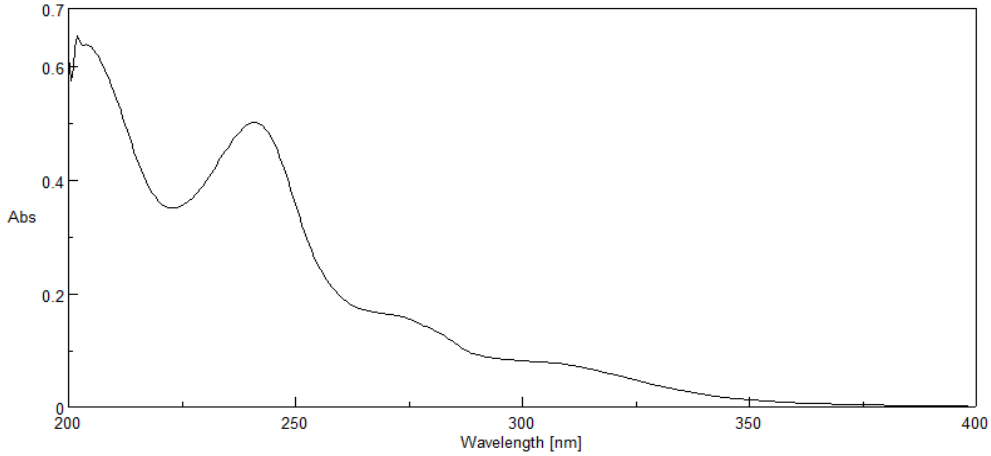

Fig. 2: The UV spectrum of Amiodarone hydrochloride (10 µg/ml).

Preparation of sample solution

20 tablets each containing 100 mg of Amiodarone hydrochloride (Cordarone 100 mg) was weighed and powdered. A quantity of powder equivalent to 10 mg of Amiodarone hydrochloride was transferred to a 10 ml volumetric flask containing 5ml 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 to get concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. Further dilution in mobile phase was done to get concentration 10 µg/ml.

STRESS DEGRADATION STUDIES OF BULK DRUG[13]

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 concentration level of 20 µg/ml.

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. After exposure the volume was made up to 10 ml with methanolto get the concentration of 100µg/ml.From this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Alkali degradation blank is prepared in the same way without using analyte. Under alkaline hydrolysis, percent recovery obtained for Amiodarone hydrochloride was 87.10% with no peak of degradant. The representative chromatogram is shown in"Fig. 3".

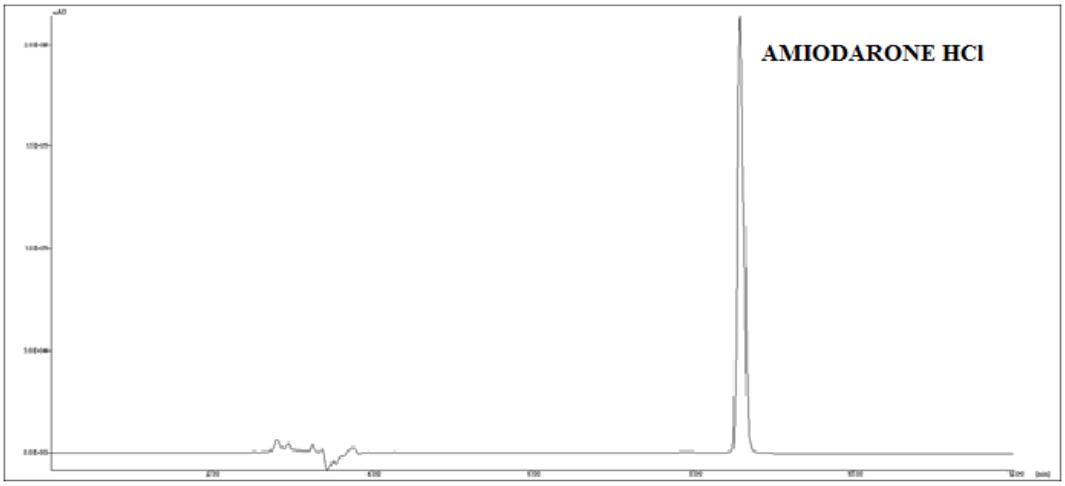

Fig. 3: Chromatogram of Amiodarone hydrochloride after alkaline degradation.

Acid hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of 0.5 N HCl was added. The above solution was kept for 15 min. at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of 100 µg/ml. From this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Acid degradation blank is prepared in the same way without using analyte. Under acid hydrolysis, percent recovery obtained for Amiodarone hydrochloride was 12.03% with peak of degradant (D_1) at 5.320 min. The representative chromatogram is shown in"Fig. 4".

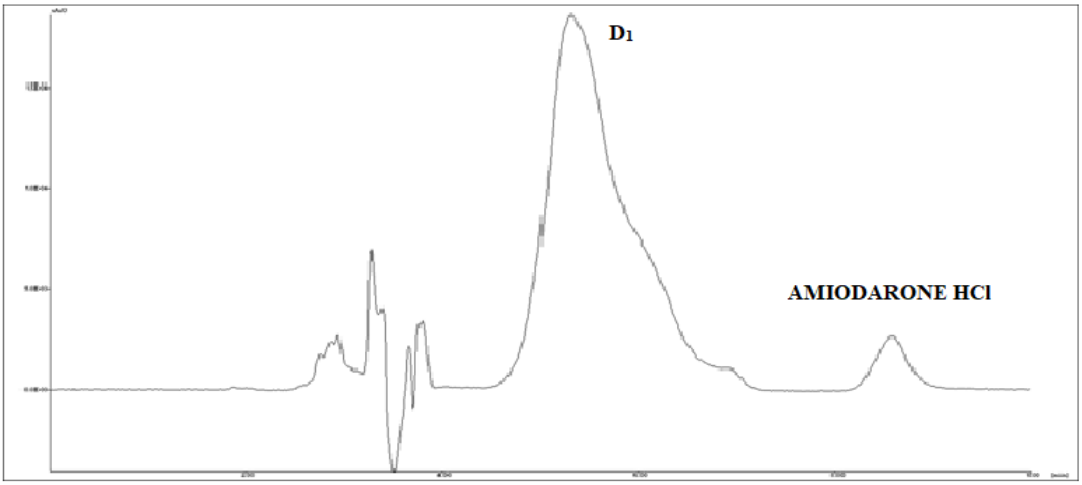

Fig. 4: Chromatogram of Amiodarone hydrochloride after acidic degradation.

Neutral hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of distilled water was added. The above solution was kept for 3 hours at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of 100 µg/ml. From

this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Neutral degradation blank is prepared in the same way without using analyte. Under neutral hydrolysis, percent recovery obtained for Amiodarone hydrochloride was 96.21% with no peak of degradant. The representative chromatogram is shown in"Fig. 5".

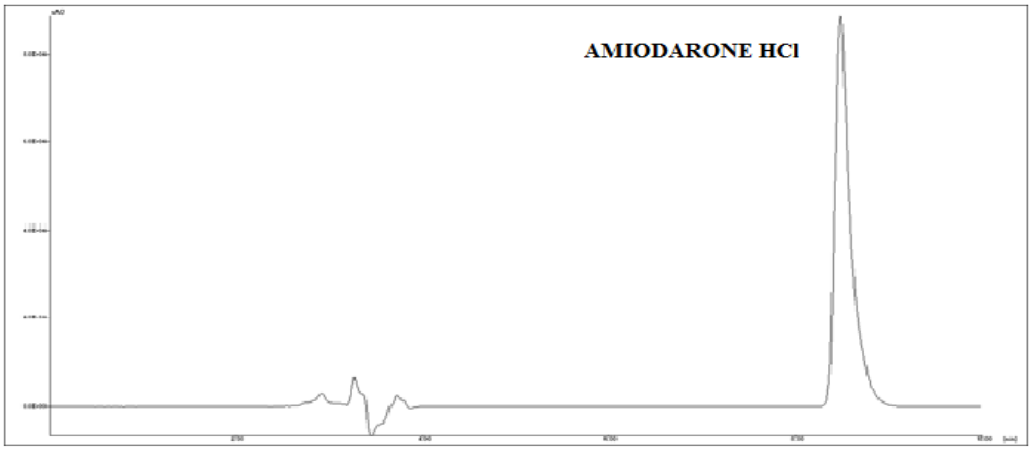

Fig. 5: Chromatogram of Amiodarone hydrochloride after neutral degradation.

Degradation under oxidative condition

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of 30% H_2O_2 was added. The above solution was kept for 3 hours at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of 100 µg/ml. From this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Oxidative degradation blank is prepared in the same way without using analyte. Under oxidative degradation, percent recovery obtained for Amiodarone hydrochloride was 95.20% with no peak of degradant. The representative chromatogram is shown in"Fig. 6".

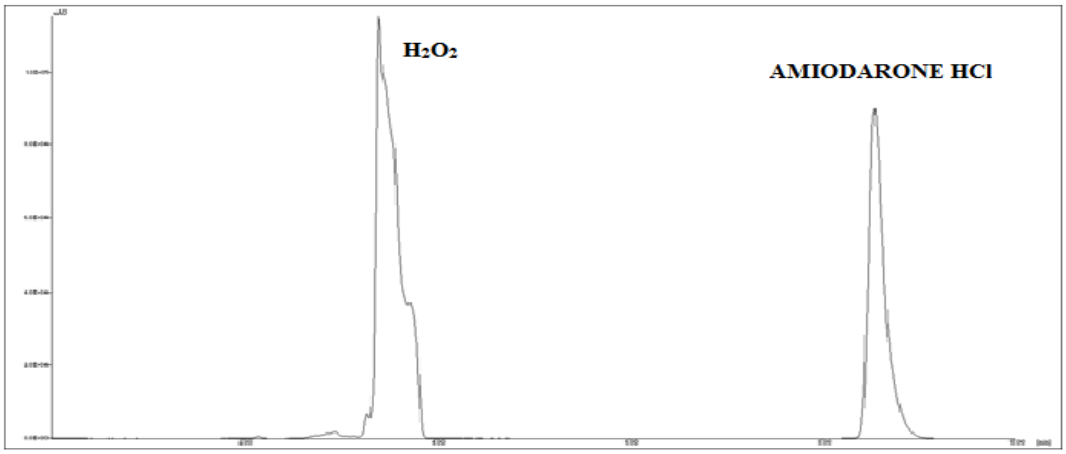

Fig. 6: Chromatogram of Amiodarone hydrochloride after oxidative degradation.

Degradation under dry heat:

Dry heat studies were performed by keeping drug sample in oven (50^0 C) for a period of 3 hours. A sample was withdrawn, and transferred to 10 ml volumetric flask then dissolved in methanol to get concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. And then again from this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Under dry heat degradation condition, percent recovery obtained for Amiodarone hydrochloride was 61.70% with no peak of degradant. The representative chromatogram is shown in"Fig. 7".

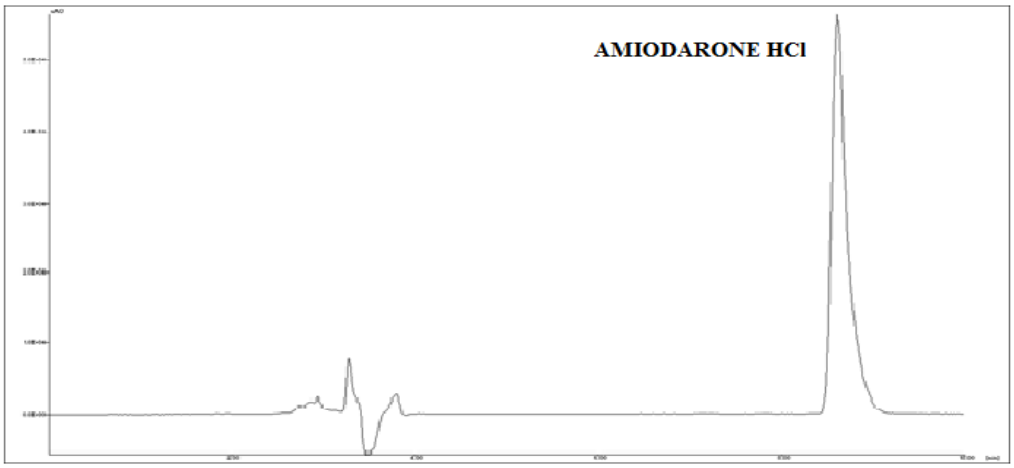

Fig. 7: Chromatogram of Amiodarone hydrochloride after dry heat degradation.

Photo-degradation 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/m^2. After exposure accurately weighed 10 mg of drug was transferred to 10ml volumetric flask; the volume was made up with methanol to obtain concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. And then again from this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Under photo degradation study by UV light, percent recovery obtained for Amiodarone hydrochloride was 42.02% with no peak of degradant. The representative chromatogram is shown in"Fig. 8".

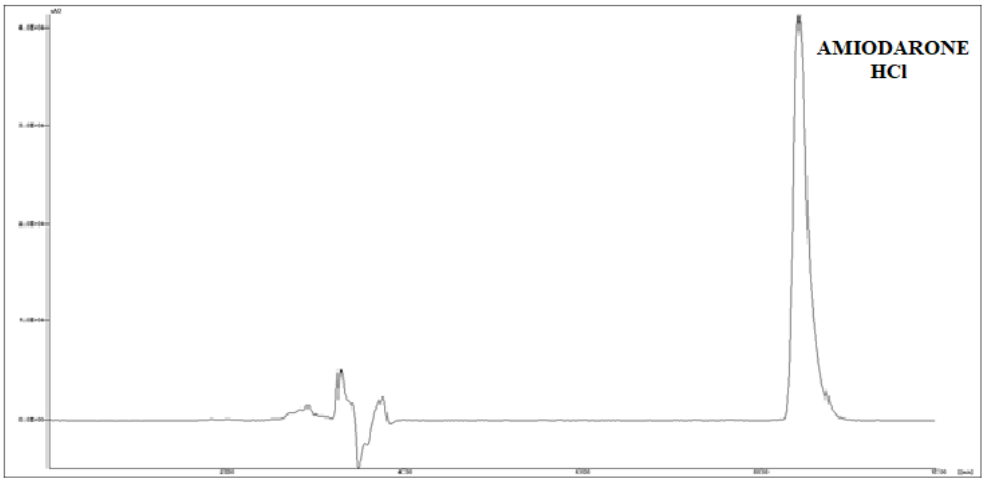

Fig. 8: Chromatogram of Amiodarone hydrochloride after UV light exposure.

2.Fluroscent light

The photo degradation study of the drug was studied by exposing the drug to fluroscent light providing illumination of NLT 1.2×10^6 Lux hr of fluroscent 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 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. And then again from this solution 2 ml was taken in 10 ml volumetric flask and volume was made up with mobile phase to get concentration of solution 20 µg/ml and then this solution was injected. Under photo degradation study by Fluroscent light, percent recovery obtained for Amiodarone hydrochloride was 42.50% with no peak of degradant. The representative chromatogram is shown in"Fig. 9".

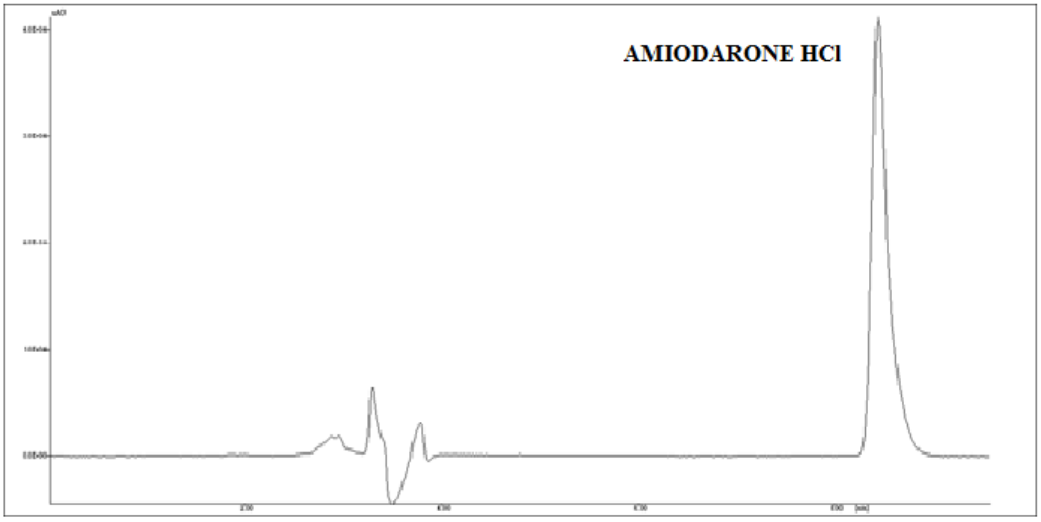

Fig. 9: Chromatogram of Amiodarone hydrochloride after fluroscent light exposure.

VALIDATION OF ANALYTICAL METHOD[14]

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.997, indicating no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µg/ml) of Amiodarone hydrochloride, solution was prepared containing 100 µg/ml of Amiodarone hydrochloride with methanol.This solution was further diluted with mobile phase to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml,the equation of calibration curve was found to be y = 53476x + 28460.The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in "Fig. 10".

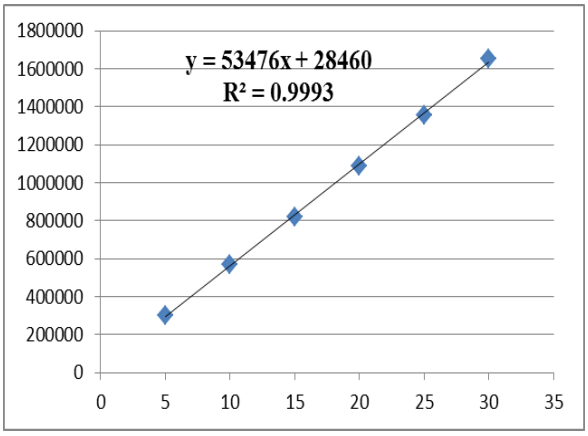

Fig. 10: Linearity curve of Amiodarone hydrochloride (5-30 µg/ml).

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 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1 and Table 2.

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

Replicates	Conc. (µg/ml)		
	5	10	15
1	302514.914	570865.903	830488.902
2	299152.601	567589.466	832403.485
3	301012.105	564655.21	829874.033
Mean	300893.207	567703.526	830922.140
SD	1684.307	3106.917	1319.206
%RSD	0.560	0.547	0.159

Table 2: Inter-day variation studies data for Amiodarone hydrochloride.

Replicates	Conc. (µg/ml)		
	5	10	15
1	297109.604	568589.476	837512.843
2	301742.605	572707.084	828784.753
3	300597.057	565655.356	834894.945
Mean	299816.422	568983.972	833730.847
SD	2413.134	3542.377	4478.977
%RSD	0.805	0.623	0.537

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

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ was found to be 0.166 µg/ml and 0.504 µg/ml, respectively.

CORDARONE-100mg tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded. Basic concentration of sample chosen was 10 µg/ml from tablet solution. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 3.

Assay

Table 3: Assay of marketed formulation.

Sr. No.	Amiodarone hydrochloride		
	Peak area	Amount Recovered (µg/ml)	% Recovery
1	564268.359	10.020	100.196
2	565994.809	10.052	100.519
3	559990.131	9.940	99.396
4	565236.615	10.038	100.377
5	570105.577	10.129	101.288
6	566427.868	10.060	100.600
Mean	565337.227	10.040	100.396
SD	3289.471	0.062	0.615
%RSD	0.582	0.613	0.613

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the CORDARONE-100mg tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 10 µg/ml of Amiodarone hydrochloride. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Table 4: Accuracy of Amiodarone hydrochloride.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Area	Amount recovered (µg/ml)	% recovery
50%	10	5	829531.213	14.980	100.132
			832186.102	15.030	
			833261.933	15.050	
100%	10	10	1110007.762	20.225	100.528
			1101316.311	20.062	
			1099542.441	20.029	
150%	10	15	1369116.248	25.070	100.846
			1391734.322	25.493	
			1369147.791	25.071	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition (± 2% Composition), detection wavelength (± 1 nm), flow rate (± 0.1 ml/min) were altered and the effects on the area were noted. The method was found to be robust.The results obtained are shown in Table 5.

Table 5: Robustness study.

% RSD Found For Robustness Study(peak area)								
MP COMPOSITION (± 2% Composition)			DETECTION WAVELENGTH (± 1 nm)			FLOW RATE (± 0.1 ml/min)		
78:22	**80:20**	**82:18**	**241**	**242**	**243**	**0.9**	**1**	**1.1**
0.968	0.646	0.513	0.912	0.551	0.389	0.681	0.262	0.291

RESULTS AND DISCUSSION

The developed method was found to be simple, sensitive, specific, accurate and repeatable for analysis ofAmiodarone hydrochloridein bulk and pharmaceutical dosage form without any interference from the excipients. The results indicated the suitability of the method to study stability of Amiodarone hydrochloride under various forced degradation conditions.

Table 6: Summary of Validation Parameters.

Sr. No.	Validation parameters	Amiodarone hydrochloride
1.	Linearity equation R^2 Range	y = 53476x + 28460 R^2= 0.9993 5-30 µg/ml
2.	Precision	(%RSD)
	Intra-day	0.453
	Inter-day	0.700
3.	% Assay (Mean ± %RSD)	100.396± 0.613
4.	Accuracy	Mean ± %RSD
	50	100.132% ± 0.239
	100	100.528% ± 0.521
	150	100.846% ± 0.968
5.	Limit of detection	0.166µg/ml
6.	Limit of quantitation	0.504µg/ml
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

A simple, precise, accurate, reproducible and stability indicating HPLC 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.

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