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Method development and validation of amiodarone in bulk and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple rapid sensitive, precise method has been developed for the estimation of amiodarone in pharmaceutical dosage form (Injection) using Hypersil BDS C18 column (150mm×4.6mm) 5µm, used for the separation. The mobile phase consisting of Acetonitrile: Triethylamine buffer (75:25) of pH 6.5 adjusted with ortho-phosphoric acid. The conditions optimized were: flow rate (2 ml/minute), wavelength (240 nm) and run time was 10min. This method is validated for System suitability, Specificity, Accuracy, Linearity, Range and Robustness. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug and met all specifications as per ICH guidelines. The proposed method is good for obtaining reliable results and found to be suitable for the routine analysis in amiodarone in pharmaceutical dosage form.

Keywords: Amiodarone, System Suitability, Precision, Accuracy, ICH Guidelines.

INTRODUCTION [1-11]

HPLC is the most versatile and widely used type of elution chromatography. The technique is used to separate and determine species in a variety of organic, inorganic, and biological materials. HPLC is used either in the liquid-solid adsorption chromatography mode or the liquid-liquid partition chromatography mode, either normal or reversed-phase. Both partition and adsorption chromatography operates on differences in solute polarity since polarity is important in determining both adsorption and solubility. As a general rule, highly polar materials are best separated using partition chromatography, while very non polar are separated using adsorption chromatography. High Performance Liquid Chromatography (HPLC) is fastest growing analytical technique for the analysis of drugs. HPLC has been rapidly developed with the introduction of new pumping methods, more reliable columns and a wide range of detectors. HPLC can also be automated which involve automated sampling, separation, detection, recording, calculation, printing of results due to high selectivity, specificity, sensitivity achieved by HPLC methods. It is the most popular technique today among the different chromatographic procedures. Due to significant evolution of Liquid Chromatography (LC) instruments providing the superior qualitative and quantitative results.

STEPS INVOLVED IN THE METHOD DEVELOPMENT ARE AS UNDER

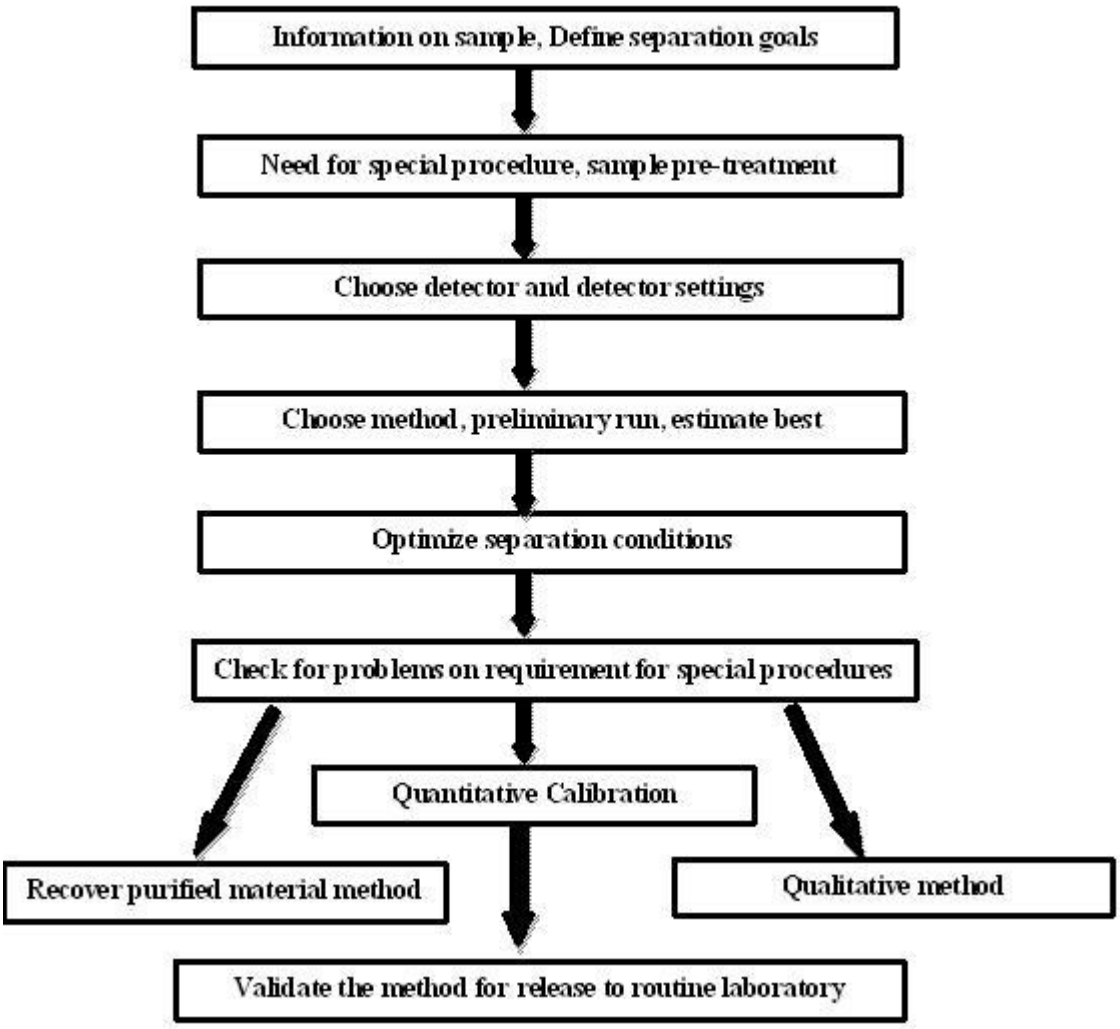

Fig.1: Steps in Method Development

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc. When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.

Antiarrhythmic agents are a group of pharmaceuticals that are used to suppress abnormal rhythms of the heart (cardiac arrhythmias), such as atrial fibrillation, atrial flutter, ventricular tachycardia, and ventricular fibrillation. Many attempts have been made to classify antiarrhythmic agents. The problem arises from the fact that many of the antiarrhythmic agents have multiple modes of action, making any classification imprecise. Many attempts have been made to classify antiarrhythmic agents. The problem arises from the fact that many of the antiarrhythmic agents have multiple modes of action, making any classification imprecise. According to at least one source, cardiac anti-arrhythmia drugs have "cost more American lives than the Vietnam War". In the present study amiodarone HCl an antiarrhythmic drug is taken to estimate the quantitative analysis and its validation according to ICH guidelines by RP- HPLC. Drug profile is as under [15-16]

Drug Name : Amiodarone HCl
Chemical Structure :

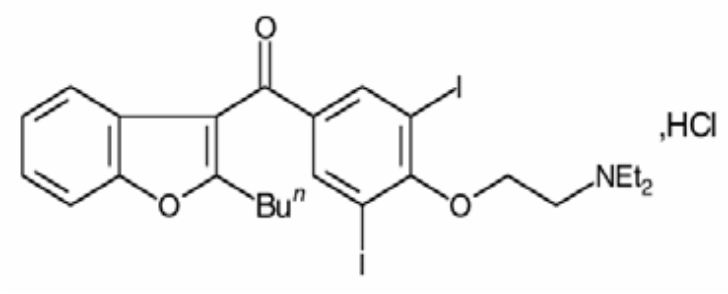

Fig.2: Structure of Amiodarone HCl

IUPAC name : 2-butylbenzofuran-3-yl-4-(2-diethylaminoethoxy)-3,5-diiodophenylketone hydrochloride
Molecular Formula : $C_{25}H_{29}I_2NO_3$.HCl
Molecular Weight : 681.8gm
Melting Point : 158-165°C
Boiling Point : 635.1°C at 760 mmHg
Appearance : A White, or almost white, fine crystalline powder.

SOLUBILITY

Very slightly soluble in water, freely soluble in methylene chloride, soluble in methanol, sparingly soluble in alcohol

AIM

The aim is to develop a method which is accurate and precise and which will be useful in future for further studies related to antiarrhythmic drugs. The primary objective of method development and validation in the analysis of the drug is to design and develop method preferably instrumental one such as UV spectrometric, HPLC that are sensitive and reproducible when applied for analysis of marketed formulation.

OBJECTIVES

In summary, the primary objective of proposed work is to:

Develop new, simple, sensitive, accurate and economical analytical method for the determination of assay of the title ingredient by HPLC. Validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application, i.e. to apply the proposed method for analysis of the drug in its dosage form. Development of HPLC method for determination of Newer Antiarrhythmic Drugs in bulk and pharmaceutical dosage forms. Method validation according to ICH guidelines

MATERIALS AND METHODS [12-14]

INSTRUMENTS AND EQUIPMENTS

Table 1: Instruments and Equipment Use

Name	Details
HPLC System	Waters(2690) , Isocratic LC, Auto injector, PDA (Photo Diode Array Detector) , M-Power2.
UV – VIS Double Beam Spectrophotometer	Schimadzu Model no. 1800 software UV probe Wide wavelength range 1100nm – 190nm Detector – silicon photodiode
Electronic balance	Balance with sensitivity of 0.001gm, Contech India
pH Meter	Elico India

Sonicator	Digital Temperature controller, Bio-Technics, India
Hot Air Oven	Bio-Technics, India
Volumetric Flask	Glass, Class-A
Volumetric Pipettes	Glass, Class-A
Measuring Cylinders	Glass, Class-A
Beakers	Glass, Class-A

CHEMICALS AND REAGENTS

Table 2: Chemicals and Reagents Use

Sr. No.	Chemical Use	Grade	Make
1	Water	HPLC Grade	Research -Lab , Mumbai
2	Methanol	HPLC Grade	Research -Lab, Mumbai
3	Acetonitrile	Analytical Grade	Research -Lab, Mumbai
4	Ortho Phosphoric Acid	Analytical Grade	Research -Lab, Mumbai
5	Triethylamine buffer	Analytical Grade	Research -Lab, Mumbai
6	Hydrochloric Acid	Analytical Grade	Research -Lab, Mumbai
7	Hydrogen Peroxide	Analytical Grade	Research -Lab, Mumbai
8	Sodium Hydroxide	Analytical Grade	Research -Lab, Mumbai

STANDARD / SAMPLE

Table 3: Standard and Sample Used

Sr. No.	Standard / Sample
1	Amiodarone HCl
2	Amiodarone HCl Injection - 50mg/ml

PREPARATION OF SOLUTIONS

PREPARATION OF TRIETHYLAMINE BUFFER

Dissolve 10.0ml of Triethylamine diluted with 1000ml water. Adjust pH 6.5 with orthophosphoric acid (OPA); make up volume up to 2000 mL with water and mix.

PREPARATION OF MOBILE PHASE

250 mL of Triethylamine Buffer (pH 6.5), 750 mL Acetonitrile (HPLC grade) were mixed and filtered through 0.45µm filter paper, sonicated for 15 minutes to degas and used as mobile phase.

STANDARD SOLUTION PREPARATION

Standard stock solution of Amiodarone HCl was prepared by dissolving 50 mg of Amiodarone HCl in 50ml of clean and dry volumetric flask add 30ml of mobile phase and sonicated for few min to dissolve the drug completely. Then it is filtered through 0.45 µm membrane filter and the volume is made up to 50mL with mobile phase to get a 100 µg/ml (Stock solution). From this, Series of working solutions of Amiodarone HCl was prepared by the appropriate dilution of the stock solutions with same solvent to reach the concentration ranges of 10 - 50 µg/ml. solution of concentration 40µg/ml is used throughout the project.

SAMPLE SOLUTION PREPARATION

From this, Series of working solutions of Amiodarone HCl was prepared by the appropriate dilution of the stock solutions with same solvent to reach the concentration ranges of 10 - 50 µg/ml solution of concentration 40µg/ml is used throughout the project.

METHOD DEVELOPMENT

FINAL OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Column	: Hypersil BDS C18 (150x4.6mm), 5µm
Flow rate	: 2.0 ml/min
Wavelength	: 240nm
Injection volume	: 20µl
Run time	: 10 min
Column Temp	**:** Ambient
Mobile phase	: Acetonitrile : Triethylamine buffer (75:25)

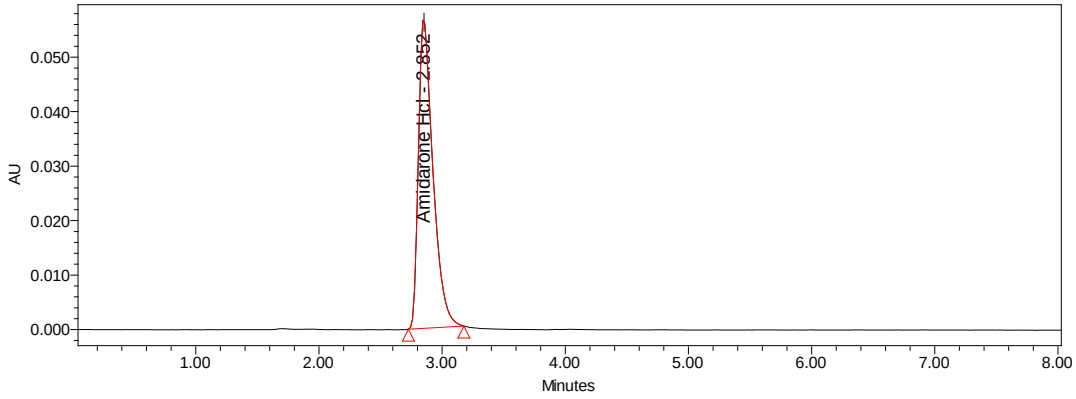

Fig.3: Chromatogram of Standard

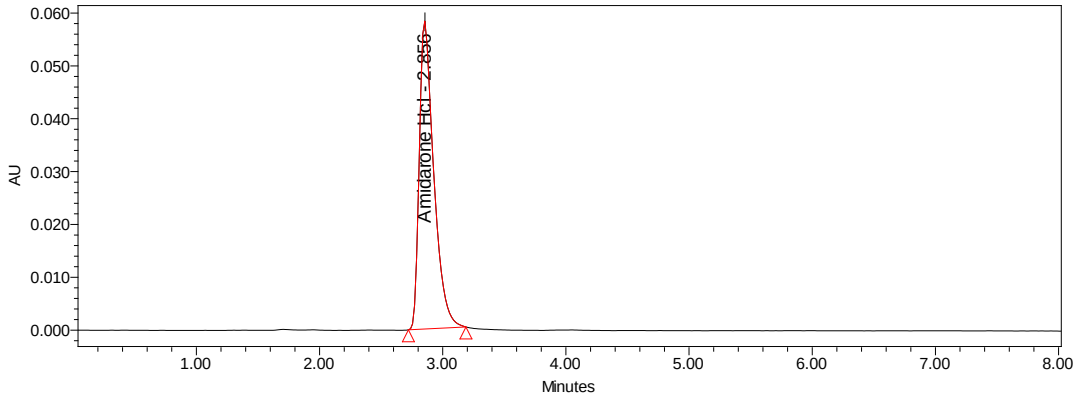

Fig.4: Chromatogram of Sample

OBSERVATION

Amiodarone HCl peak is good and retention time is2.856. The method was further considered for validation and routine analysis. The following parameter was considered for the validation of analytical method of Assay of Amiodarone HCl.

SYSTEM SUITABILITY TEST

To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. The standard solution which is prepared as per the procedure is injected 3 times to check the instrument is giving consistent results. Inject separately blank (1 inj.) and standard solutions (3 inj.) into the HPLC and record the chromatograms and check the system suitability.

PRECISION

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was performed as intraday precision, Inter day precision. The precision of the method was measured by the percentage relative standard deviation (% RSD) over the concentration range of high, middle and low QC samples respectively of drug during course of validation.

There are two types of precisions Intraday and Inter-days Precision.

a) SYSTEM PRECISION

To study the intraday precision, three replicate standard solutions (10, 30, 50µg/ml) of Amiodarone HCl was injected. The percent relative standard deviation (% RSD) was calculated, which are well within the acceptable criteria of not more than 2.0.

b) METHOD PRECISION

To study the inter days precision, three replicate standard solutions (10, 30, 50µg/ml) of Amiodarone HCl was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated which are well within the acceptable criteria of not more than 2.0.

LINEARITY

It is the ability of the method to elicit test results directly proportional to analyate concentration within a given range. Linearity was performed by preparing standard solutions ofAmiodarone HClat different concentration levels, twenty micro liters of each concentration was injected into the HPLC system. The peak responses were read at 240nm and the corresponding chromatograms were recorded. Linearity plots of concentration over areas were constructed individually. The Amiodarone HCl obeyed linearity in the range of 10-50µg/ml.

ACCURACY

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed Standard solution. The standard addition method was performed at 50%, 100% and 150% level of 20µg/ml. The solutions were analyzed in triplicate at each level as per the proposed method. Satisfactory recoveries ranging from 99% to 101% were obtained by the proposed method. This indicates that the proposed method was accurate. The percent recovery and % RSD was calculated.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

A Calibration curve was prepared using concentrations in the range of 10-50 µg/ml. The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit. The LOD and LOQ were calculated according to the formula given by the ICH guidelines as described below,

LOD is calculated from the formula: -

$$\mathbf{LOD} = \frac{\mathbf{3.3\sigma}}{\mathbf{S}}$$

LOQ is calculated from the formula: -

$$\mathbf{LOQ} = \frac{\mathbf{10\sigma}}{\mathbf{S}}$$

Where,

σ = Standard deviation of the response of calibration curve.

S = Slope of the calibration curve.

ROBUSTNESS

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution which is prepared as per the procedure is injected 3 times (diluent-1 inj., Standard-3 inj.). Record the chromatograms and check the system suitability parameter for all conditions.

The parameters which are changed are as follows,

i) Change in flow rate ± 2 nm

ii) Change in pH ± 0.2

SYSTEM SUITABILITY TEST

Table 4: Results of System Suitability Test

Sr. NO.	Sample	Retention Time(Min.)	Area Response
1	Std 01	2.854	7037.5151
2	Std 02	2.854	7039.6279
3	Std 03	2.854	7037.5151

4	Std 04	2.853	7041.1612
5	Std 05	2.853	7041.5928
Average		2.85351	7093.482
SD		0.0010	1.93867
% RSD		0.024156	0.02754
Tailing Factor		1.123	1.112

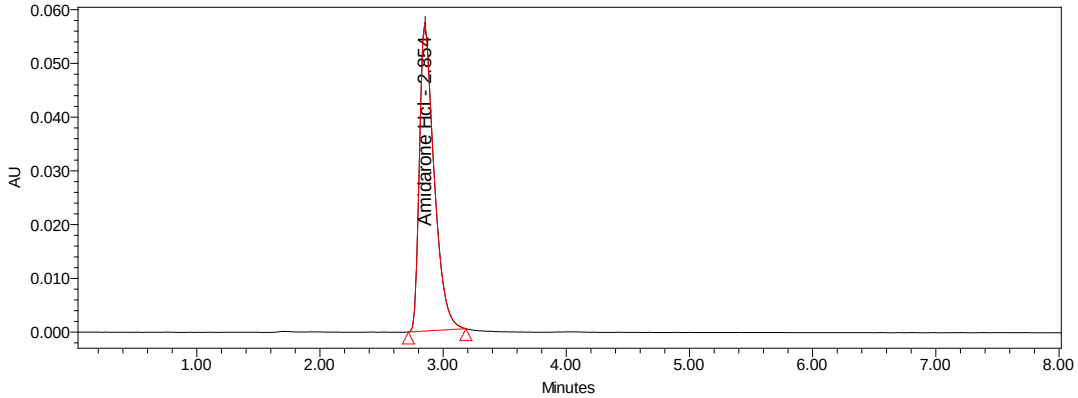

Fig.5: Chromatogram of system suitability

DATA INTERPRETATION

From the above results, it can be concluded that the system is suitable for analytical method validation.

SPECIFICITY

Table 5: Results of Specificity

Sr. No.	Solution	Retention time
1	Standard	2.854
2	Sample	2.854

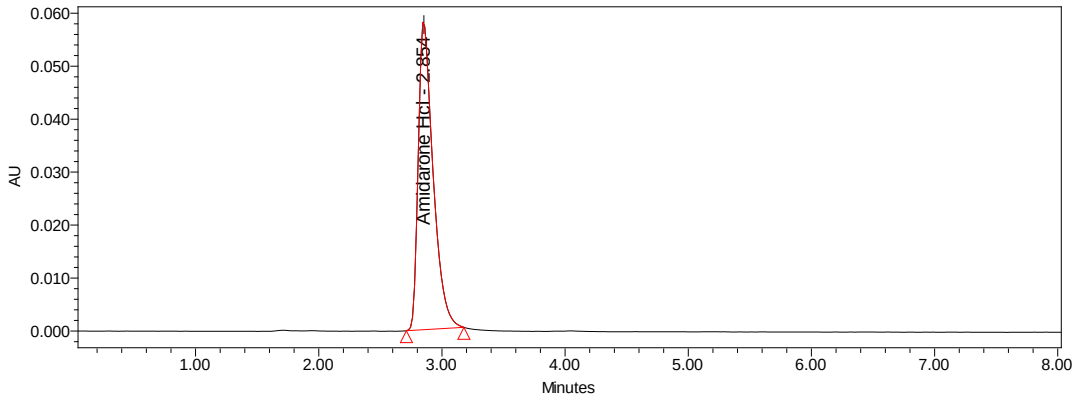

Fig.6: Chromatogram of Standard

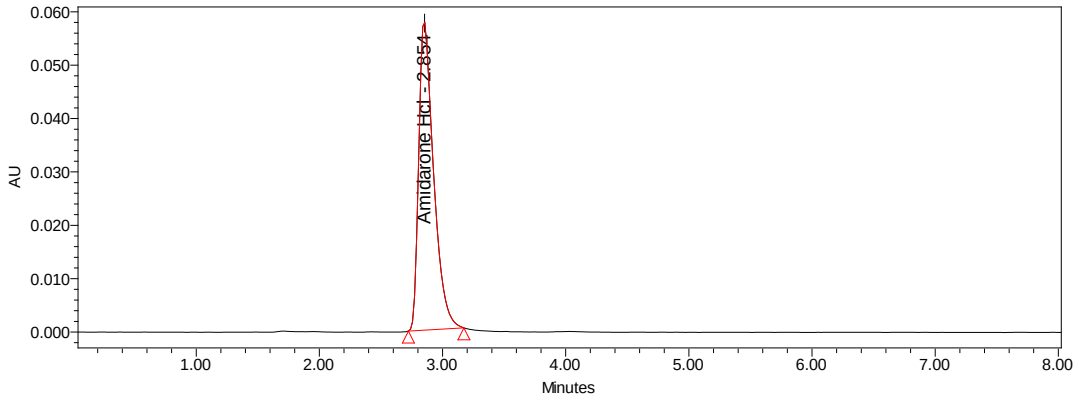

Fig.7: Chromatogram of Sample

DATA INTERPRETATION

The Amiodarone Hydrochloride peak is pure. Hence, the Assay method is considered specific & stability indicating.

PRECISION

SYSTEM PRECISION

Table 5: Results for System Precision

	Injection	Peak Area	% Assay
Concentration 40 µg/ml	1	7035.56	100.56
	2	7037.58	100.88
	3	7035.56	100.78
	4	7045.13	100.06
	5	7038.796	101.02
Statistical Analysis	Mean	**7038.796**	**100.06**
	SD	**4.011699**	**52.3**
	%RSD	**0.056994**	**0.09**

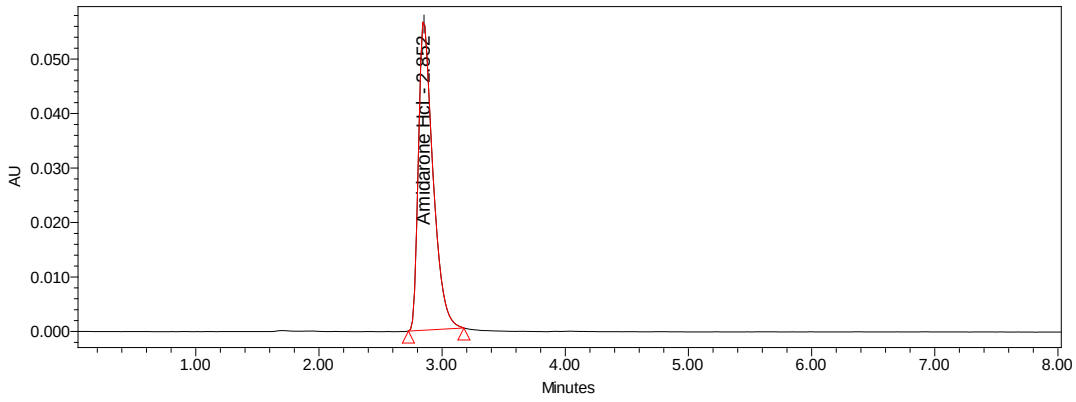

Fig.8: Chromatogram for system precision

DATA INTERPRETATION

From the above results, it can be concluded that Retention time & Area response are consistent as evidenced by Relative standard deviation. Hence, it is concluded that the system precision parameters meets the requirement of Method validation.

METHOD PRECISION

Table 6: Results for Method Precision

	Injection	Peak Area	% Assay
Concentration 40 µg/ml	1	7037.5151	100.86
	2	7039.6279	100.91
	3	7037.5151	100.86
	4	7040.1612	100.92
	5	7046.1712	100.06
Statistical Analysis	Mean	**7039.482**	**100.77**
	SD	**1.734005**	**44.5**
	%RSD	**0.024633**	**0.08**

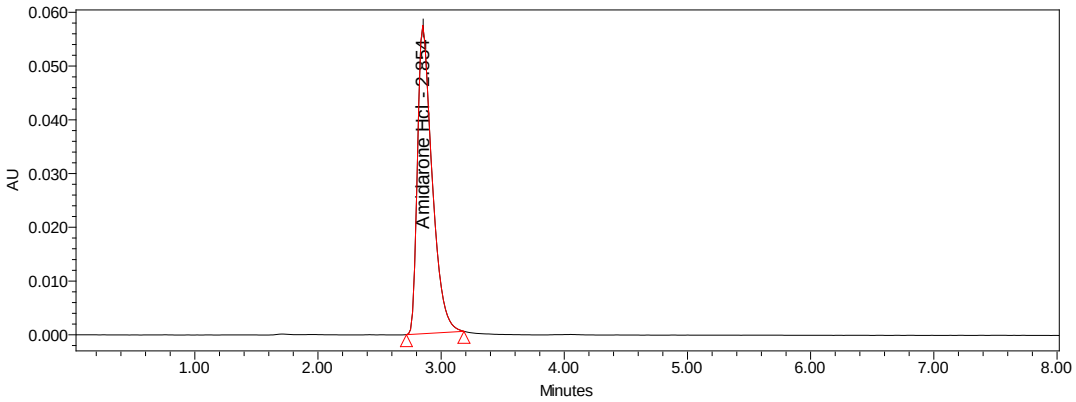

Fig.9: Chromatogram for Method precision

DATA INTERPRETATION

From the above results, it can be concluded that the method is precise.

INTERMEDIATE PRECISION

Table 7: Results for Intermediate Precision by comparing with Method precision (Table 13)

	Injection	Peak Area	% Assay
Concentration 40 µg/ml	1	7034.01	100.54
	2	7036.79	100.86
	3	7039.451	100.12
	4	7042.512	100.56
	5	7048.98	100.04
Statistical Analysis	Mean	**7048.98**	**100.88**
	SD	**1.85649**	**67.0**
	%RSD	**0.4525**	**0.8**

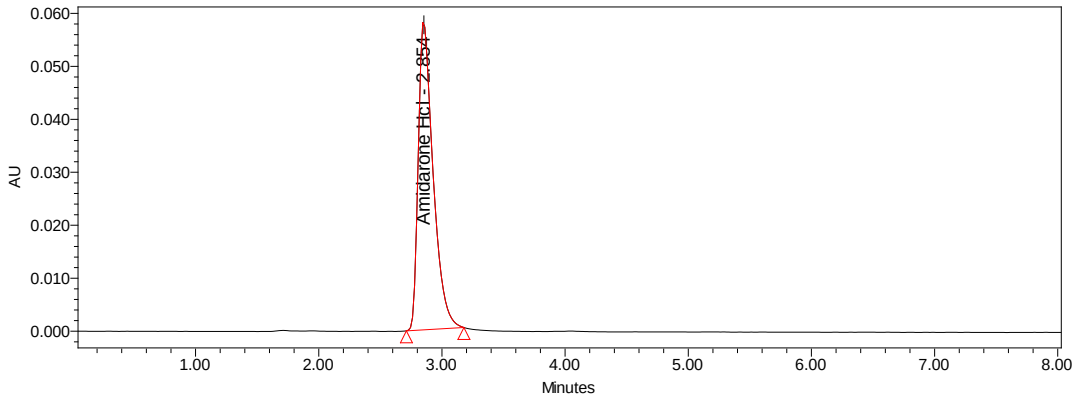

Fig.10: Chromatogram for Intermediate precision

DATA INTERPRETATION

From the above results, it can be concluded that the method is rugged.

LINEARITY

Table 8: Results of Linearity Study of Amiodarone HCl

Sr. No.	Concentration (µg/ml)	Area
1	20	3385.24
2	30	5478.25
3	40	7039.48
4	50	8998.56
5	60	10886.33
Y-Intercept		**295.1**
Slope		**186.5**
Correlation Coefficient		**0.999**

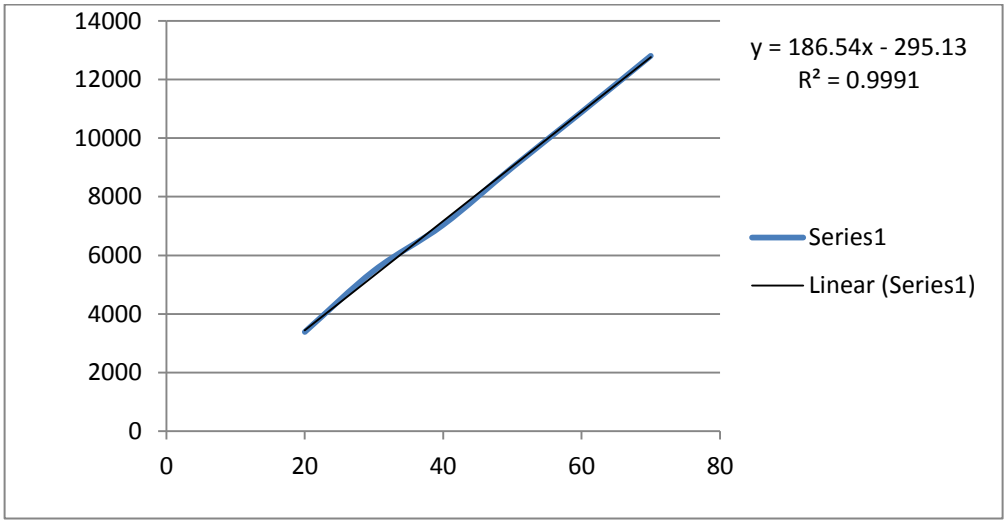

Fig. 11: Linearity of Amiodarone HCl

DATA INTERPRETATION

From the statistical treatment of linearity data of Amiodarone Hydrochloride, it is clear that the response of Amiodarone Hydrochloride is linear between 20 to 40 % level of specification limit. The correlation & regression coefficient is 0.999.

ACCURACY

Table 9: Results of % Recovery of Amiodarone HCl

Spike Level In %	Sample	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean	%RSD
50%	1	19.68	20	**99.82**		
	2	19.74	20	**98.89**	**99.82**	**0.82**
	3	19.85	20	**98.98**		
100%	1	39.96	40	**100.92**		
	2	38.92	40	**100.83**	**100.3**	**1.62**
	3	38.84	40	**100.56**		
150%	1	59.84	60	**101.38**		
	2	59.52	60	**101.50**	**101.5**	**0.62**
	3	59.79	60	**101.42**		

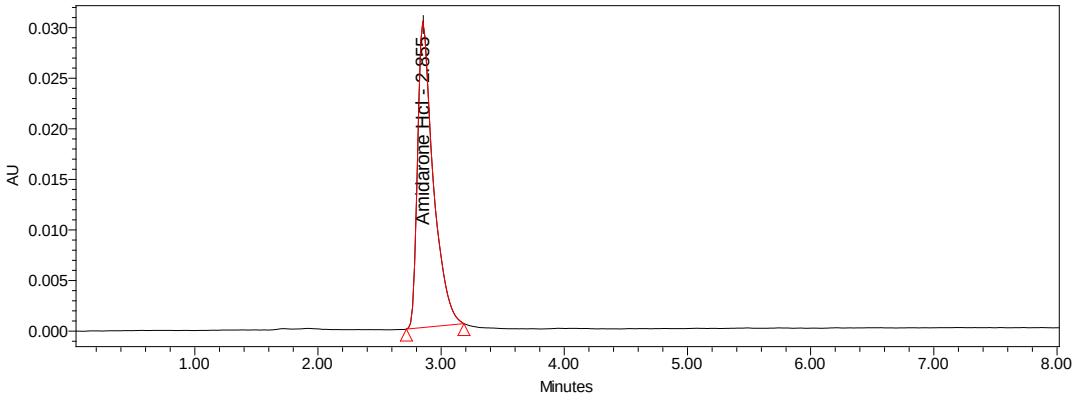

Fig.12: Chromatogram for Accuracy 50%

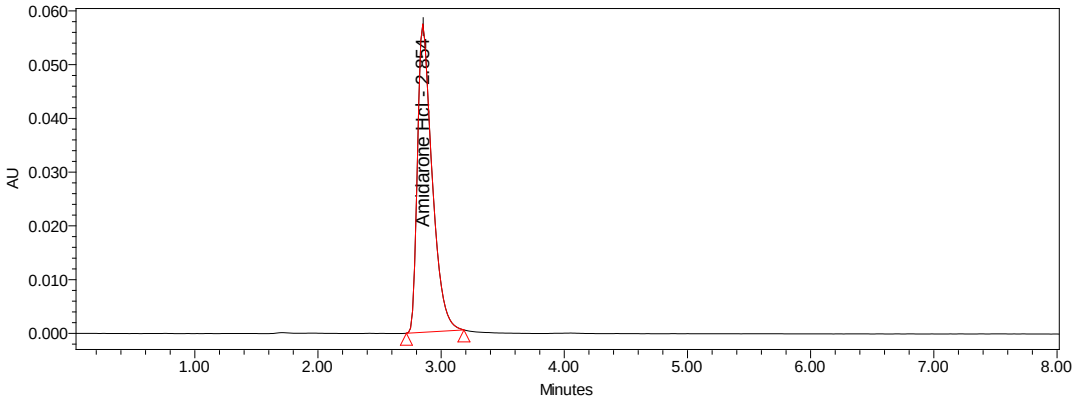

Fig.13: Chromatogram for Accuracy 100%

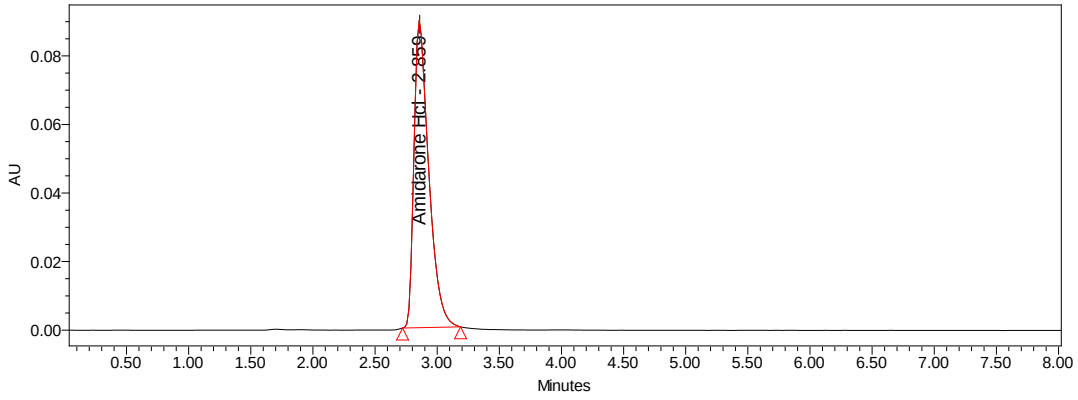

Fig.14: Chromatogram for Accuracy 150%

DATA INTERPRETATION

From the above results, it can be concluded that the recovery is well within the limit. Hence, the Method is accurate.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

Table 11: LOD and LOQ of Amiodarone HCl

Parameter	Measured values
Limit of Detection	**0.03**
Limit of Quantitation	**0.09**

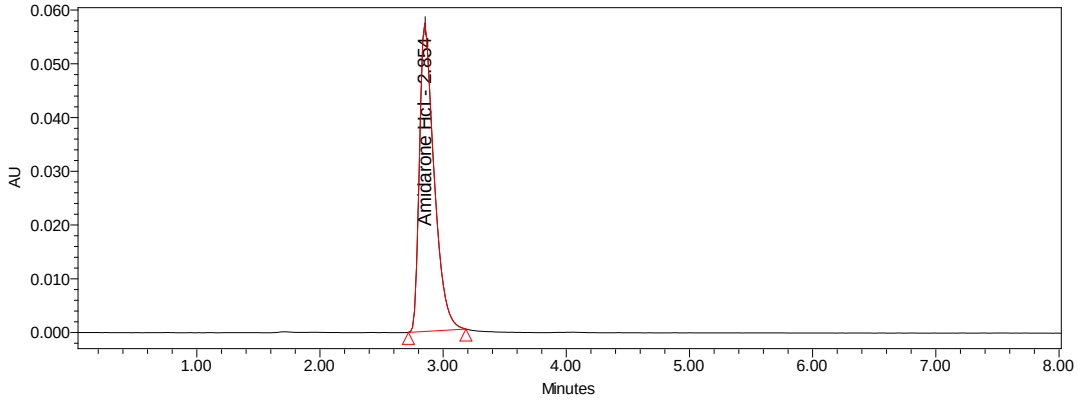

Fig.15: Chromatogram for LOD and LOQ

ROBUSTNESS

CHANGE IN FLOW RATE

Table12: Results of Robustness-Change in Flow Rate

Sample	0.8ml/min	1ml/min	1.2ml/min
Std 01	6079.40	7037.51	7035.56
Std 02	5859.63	7039.62	7037.58

Std 03	5935.37	7037.51	7035.56
Std 04	6056.36	7041.16	7040.15
Std 05	6059.63	7041.59	7045.13
Average	**6005.278**	**7039.48**	**7038.79**
% RSD	**1.39**	**0.024633**	**0.0569**
Tailing Factor	**1.112**	**1.111**	**1.1238**

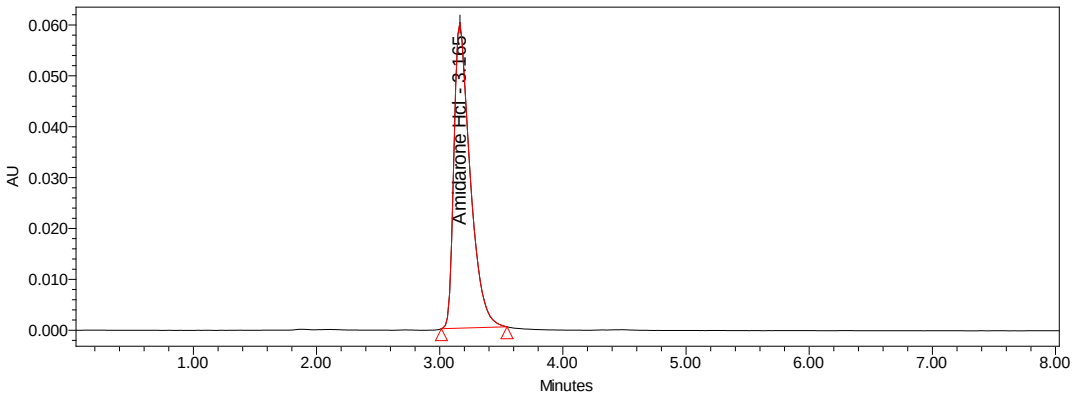

Fig.16: Chromatogram of Flow Rate-0.8 ml/min

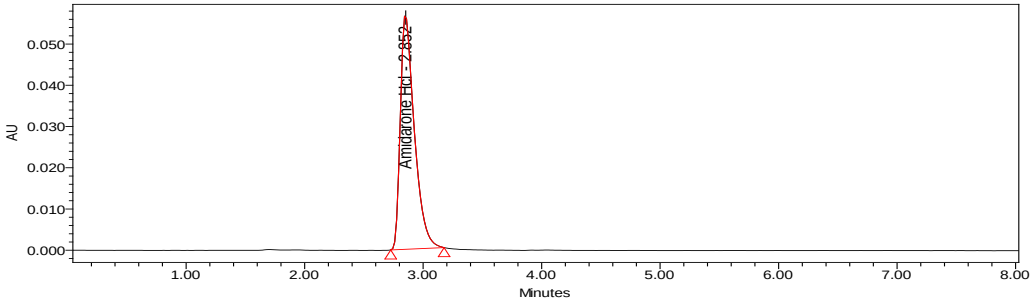

Fig.17: Chromatogram of Flow Rate-1 ml/min

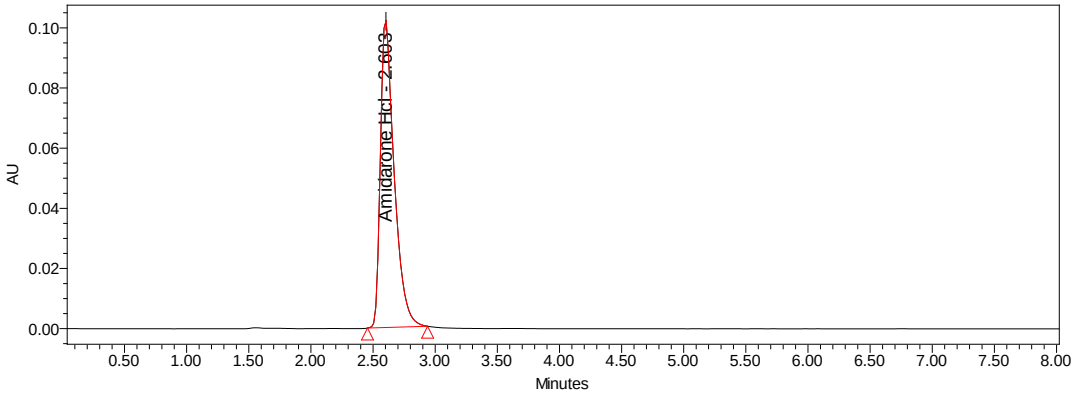

Fig.18: Chromatogram of Flow Rate-1.2 ml/min

DATA INTERPRETATION

From the above results, it can be concluded that the Method is robust.

RUGGEDNESS

Table 13: Results of Ruggedness

Sr. No.	Peak area	% Assay
1	7039.6128	100.90
2	7040.9618	100.93
3	7046.6418	101.06
4	7038.2192	100.87
5	7047.2916	101.07
6	7046.1216	101.05
Mean	7043.141	100.98
%RSD	1.020	0.08

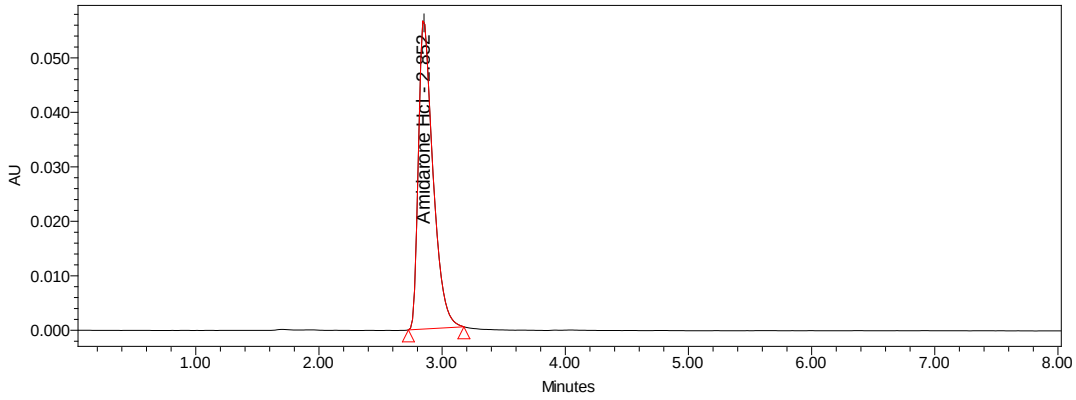

Fig.19: Chromatogram of Ruggedness

DATA INTERPRETATION

From the above results, it can be concluded that the Method is rugged.

DISCUSSION [17-34]

The Amiodarone HCl drug was analyzed by using RP-HPLC method in bulk and pharmaceutical dosage form. The aim is to develop accurate and precise method for the estimation of Amiodarone HCl in bulk and pharmaceutical dosage form.

An isocratic reversed phase HPLC assay with UV detection used for the proposed method. The separation was performed on Hypersil BDS C18 column (150mm×4.6mm) at ambient temperature. The mobile phase was triethylamine buffer (pH 6.5) and acetonitrile (25:75), was pumped at a flow rate 2.0 mL/min. The run time was 10 min. detection was performed with variable wavelength detector at 240nm. System suitability tests were performed and chromatographic parameter such as asymmetry factor, resolution, retention time, no. of theoretical plates, and area were calculated. The validity of the Amiodarone HCl was established through a study of system suitability, linearity, precision, intraday and inter days precision, accuracy, robustness, ruggedness. In system suitability study, there was no significant impact on the asymmetry factor, retention time, and no. of theoretical plates. The linearity was established with a series of working standard solution prepared by diluting the stock solution with dilution to the final concentration of 10-50µg/ml. Each concentration was injected in to liquid chromatography and the value of peak area was taken for the calibration curve. The calibration curve was plotted using concentration against peak area. The correlation co-efficient values were found to be 0.999 indicates that the concentration of Amiodarone HCl had good linearity. In precision, intraday and interdays precision study was carried, in intraday precision study,

the %RSD for Amiodarone hydrochloride was found to be 0.08.In interdays precision study, the % RSD was found to be 0.09. The result indicates that the method is validated, there is no significant difference by different time intervals and on different day, therefore the method can be consider to be acceptable. In accuracy or recovery studies, the overall % of recovery and % RSD for Amiodarone HCl had no significant difference. Therefore, accuracy of the method considered acceptable as it was well within 98-102%. The result of robustness study also indicates that the method is robust and is unaffected by small variation in chromatographic conditions (change in flow rate).

CONCLUSION

The proposed method of Amiodarone HCl injection (50mg/ml) formulation was carried out. This was found to be specific, precise and accurate for the quantitive analysis of Amiodarone HCl in both bulk and formulation. The method was found to be linear in the specified range for Amiodarone HCl injection. Accuracy of the method is established for the drug product. The method was found to be precise, robust. A system suitability test was established and recorded. Hence, this method stands validated and can be used for routine analysis.

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