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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR AMIODARONE TABLETS IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, sensitive, rapid and precise RP-HPLC method has been developed for the estimation of Amiodarone in pharmaceutical formulations. Hypersil BDS column packed with C_{18} chemically bonded porous silica. (Particle size 5µm) length 4.6mm x 150mm was used for separation. The mobile phase consisting of Acetonitrile: 0.5%Triethylamine Buffer pH to 6.5 with orthophosphoric acid (75:25). The mobile phase was pumped at a flow rate of 2.0 ml/min and the detection was carried out at 240.0 nm. The retention time was found to be 10.92min. This method is validated for Linearity, Specificity, Accuracy, System suitability, Precision, Ruggedness, and Robustness. The proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis in Amiodarone in pharmaceutical formulations.

KEY WORDS

RP-HPLC, Amiodarone and Acetonitrile.

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INTRODUCTION

Amiodarone[1] is a class III antiarrhythmic agent. Chemically it is 2 - butylbenzofuran - 3yl – 4 - (2diethylaminoethoxy) - 3, 5- diiodophenyl ketone hydro chloride. Many methods have been described in the literature for the determination of Amiodarone in human plasma, bioavailability studies[2,3], *in vitro* studies[4-7], capillary electrophoresis, and High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS)[8] has been reported. Officially assay of Amiodarone is not described in

any Pharmacopoeia. The aim of this work was to develop an RP-HPLC[9,10] methods for the estimation of Amiodarone in pharmaceutical formulation.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile HPLC grade and Triethylamine AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a milli-QRO water purification system. Amiodarone was procured from Hetero Pharmaceuticals, Hyderabad.

Chromatographic Conditions

Chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a 10AT SHIMADZU –SPD 10A Detector. Hypersil BDS column packed with C_{18} chemically bonded porous silica. (Particle size 5µm) length 4.6mm x 150mm was used for separation.

Method

A simple, rapid, precise, accurate and specific RP-HPLC method for separation and determination of Amiodarone in pure form and its formulations was developed using UV detector. After several trials with the different combination and ratio of solvents the mobile phase Acetonitrile: 0.5% Triethylamine Buffer pH to 6.5 with orthophosphoric acid (75:25) was selected because it was found ideal to resolve the peaks for the estimation of Amiodarone. Detection wavelength 240.0nm was selected.

Preparation of Standard Solution

Weigh and transfer accurately about 50mg of Amiodarone hydrochloride working standard into a 50 mL clean, dry volumetric flask add 30mL of diluent and sonicate to dissolve. Make up to volume with diluent. Further dilute 5mL of this solution with diluent and mix .Filter the solution through 0.45 µ membrane filter (Millipore or Mdi Nylon).

Preparation of Sample Solution

Weigh and finely powder not less than 20 tablets. Transfer an accurately weighed quantity of the powder or blend, equivalent to about 200 mg Amiodarone Hcl into a 200 mL clean, dry volumetric flask, add 120mL diluent and sonicate at room temperature for about 10 minutes with intermittent shaking. Making up to the volume with diluent and mix. Filter the solution through 0.45µ or finer porosity membrane filter. (Millipore PVDF or Mdi Nylon), further dilute 5 mL of this solution to 50mL with diluent and mix.

RESULTS

Estimation of Amiodarone in pharmaceutical dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The chromatogram of standard and sample solution is given in Figure No.1 and 2. The percentage of individual drugs found in formulations, mean, relative standard deviation, was calculated. The results of analysis show that the amounts of drugs were in good agreement with the label claim of the formulations.

Method Validation

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out Solutions were prepared in duplicate at levels 100% of test concentration using Amiodarone Hcl drug substance and Amiodarone Hcl Tablets placebo as per test method and injected each solution into HPLC as per test methodology.

Precision

The precision of the method was demonstrated by six sample solutions were prepared individually using single batch of Amiodarone Hcl Tablets as per the developed method and injected each solution into HPLC as per methodology.

Stability of sample solution

Standard solution and sample solution were prepared as per methodology and analyzed and at different time intervals by maintaining the solutions at room Temperature.

Filter variability

Standard and Sample solutions of Amiodarone Hcl tablets were prepared. These solutions were filtered by PVDF filter and Nylon filter, Centrifuged (Unfiltered) samples as per methodology.

Specificity

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test

method. As a result no interference due to blank at the retention time of analyte shows that the method was specific. The acceptance limit of the chromatogram (blank) should not show any peak at the retention time of the analyte peak.

DISCUSSION

The objective of the proposed work was to develop some new and sensitive analytical methods for the determination of Amiodarone and to validate the methods according to ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of Amiodarone.

The system with Acetonitrile: 0.5% Triethylamine Buffer pH to 6.5 with orthophosphoric acid (75:25) was selected with 2.0 ml/min flow rate. The optimum wavelength for detection was 240.0 nm at which better detector response for drug was obtained. The retention time for Amiodarone was found to be 11.166 ± 0.03 min.

From the obtained data, the % RSD were within the limits and the developed HPLC method was found to be accurate, precise, linearity and the values obtained demonstrated the suitability of the system for the analysis of this drug (Table No.1-4).

Table No.1: Results - recovery data and study of Accuracy

S.No	Concentration/ Sample ID	Amount Recovered in mg	% Recovered
1	100% Level Sample 1	50.541	100.885
2	100% Level Sample 2	48.744	100.039
3	**Mean**		**100.5**
4	**SD**		**0.6**
5	**% RSD**		**0.6**

Table No.2: Method precision

S.No	Sample I.D	Assay
1	1	99.6
2	2	101.9
3	3	99.5
4	4	101.0
5	5	100.7
6	6	101.1
7	**Mean**	**100.800**
8	**SD**	**1.239**
9	**% RSD**	**1.23**

Table No.3: Filter Variability

For Standard Solution

S.No	Sample ID	Area	% Recovered	% Difference
1	Un Filtered	2894710	-	-
2	PVDF Filtered	2890688	99.86	0.14
3	Nylon Filtered	2899612	100.17	0.17

For Sample Solution

S.No	Sample ID	Area	% Recovered	% Difference
1	Un Filtered	2788679	-	-
2	PVDF Filtered	2814580	100.93	0.93
3	Nylon Filtered	2748948	98.52	1.42

Table No.4: Solution Stability

For Standard Solution

S.No	Time in Hours	Area	% Recovered	% Difference
1	Initial	2894710	-	-
2	1	2900580	100.20	0.20
3	2	2904321	100.33	0.33
4	3	2921129	100.91	0.91
5	4	2899500	100.17	0.17
6	5	2903929	100.32	0.32
7	6	2905872	100.39	0.39
8	7	2892922	99.94	-0.06
9	9	2901414	100.23	0.23
10	11	2928458	100.17	1.17
11	15	2914886	100.70	0.70

For Sample Solution

S.No	Time in Hours	Area	% Recovered	% Difference
1	Initial	2788678.5	-	-
2	1	2782005	99.76	-0.24
3	2	2795044	100.23	0.23
4	4	2843914	101.98	1.98
5	5	2884457	103.43	3.43
6	6	2916535	104.58	4.58
7	7	2928622	105.02	5.02
8	9	2957559	106.06	6.06
9	10	2937355	105.33	5.33
10	11	2935541	105.27	5.27
11	15	2956683	106.02	6.02

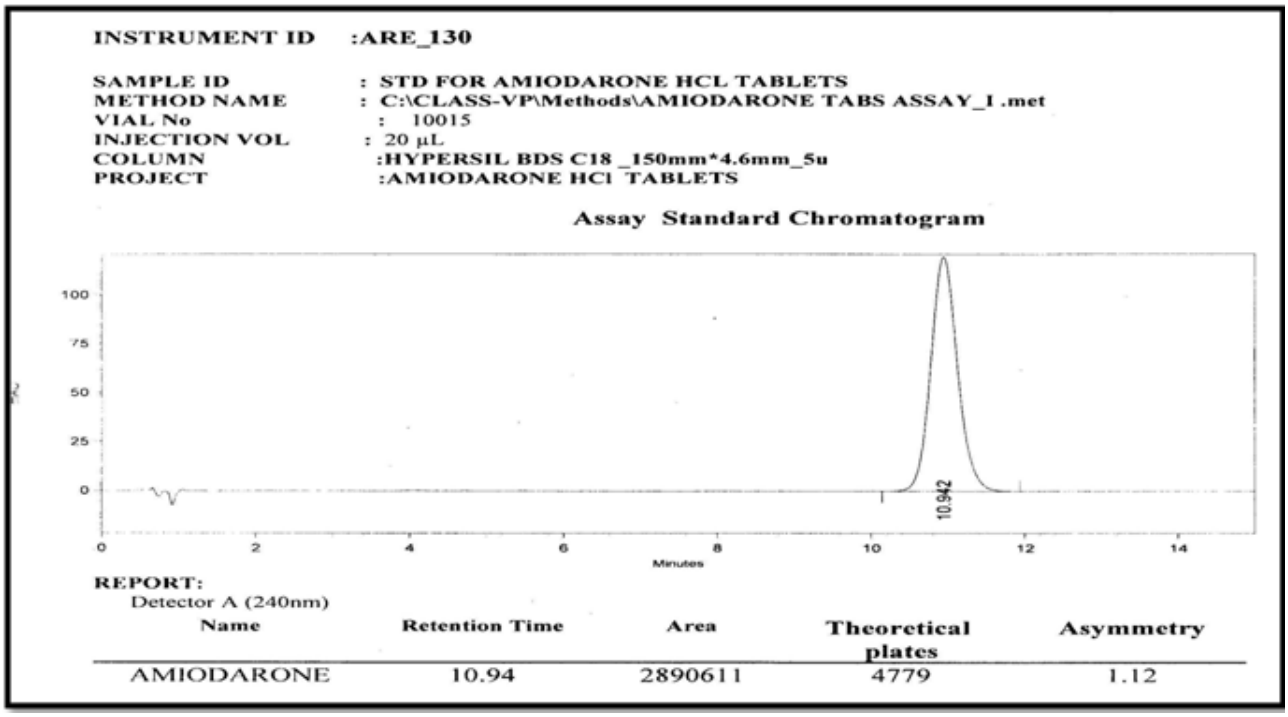

Figure No.1: Chromatogram of standard solution

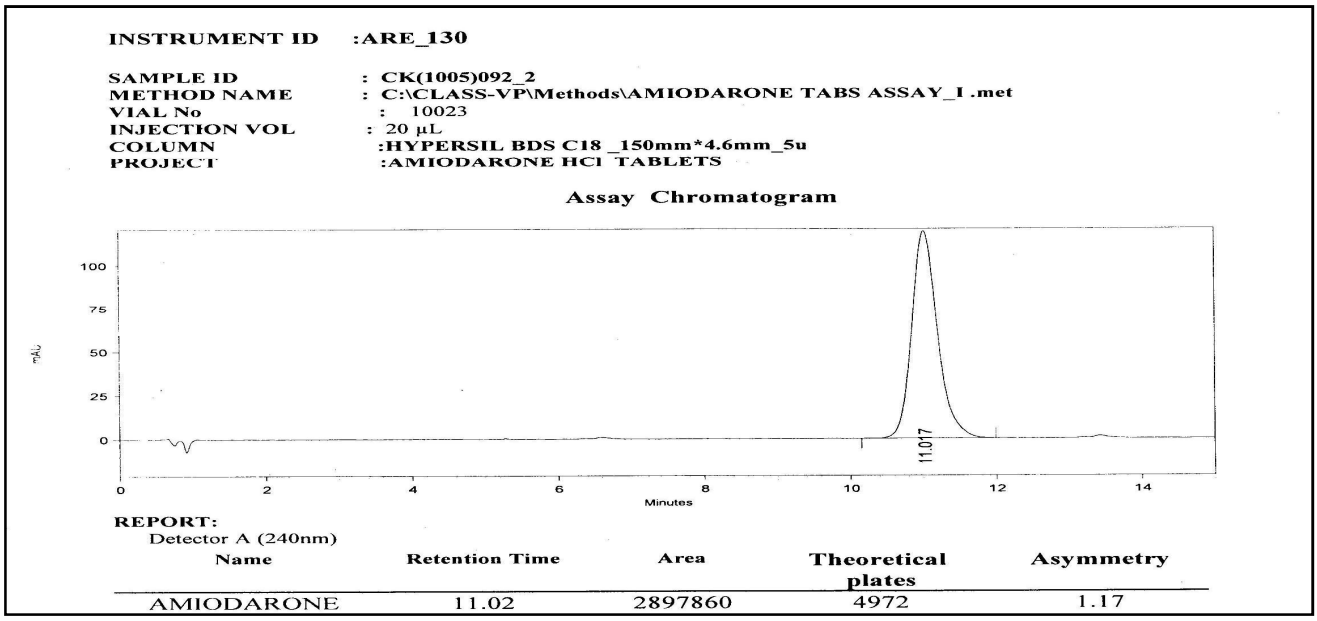

Figure No.2: Chromatogram of sample solution

CONCLUSION

The establish method capable of analyzing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool. The run time of the HPLC procedure is only 11.02 minutes. Good agreement was seen in the assay results of pharmaceutical formulation as well as in laboratory prepared mixtures by developed methods. We concluded that all the proposed methods are a good approach for obtaining reliable results and were found to be suitable for the routine estimation of Amiodarone in pharmaceutical formulation.

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BIBLIOGRAPHY

1. Amiodarone; The free encyclopedia. Available from: URL: http:// www.wikipedia.com.
2. Matthew J. Christopherson, Kenton J. Yoder, Brent Miller R. Validation of a Stability-Indicating HPLC Method for the Determination of Amiodarone HCl and Its Related Substances in Amiodarone HCl Injection, *Journal of Liquid Chromatography and Related Technologies*, 27(1), 2004, 95-111.
3. Thyagarajapuram N and Alexander K S. A Simplified Method for the Estimation of Amiodarone Hydrochloride by Reverse-Phase High Performance Liquid Chromatography, *Journal of Liquid Chromatography and Related Technologies*, 26(8), 2003, 1315-1326.
4. Di Pietra A M, Cavrini V, Gatti R et al. Determination of Amiodarone hydrochloride in pharmaceutical formulations by derivative UV Spectrophotometry and high-performance liquid chromatography (HPLC), *Pharm Res*, 5(11), 1988, 709-12.
5. Khan M A, Kumar S, Jayachandran J, Vartak S V, Bhartiya A, Sinha S. Validation of a Stability Indicating LC Method for Amiodarone HCL and

Related Substances, *Chromatographia*, 61(11), 2005, 599-607.

6. Pérez-Ruiz T, Martínez-Lozano C, Sanz A, Bravo E. Development and validation of a capillary electrophoretic method for the determination of amiodarone and desethylamiodarone, *Chromatographia,* 56(1-2), 2002, 63-67.
7. Hanioka N, Saito Y, Soyama A, Ando M, Ozawa S, Sawada J. High-performance liquid chromatographic assay for amiodarone N-deethylation activity in human liver microsomes using solid-phase extraction, *J Chromatogr B Analyt Technol Biomed Life Sci,* 774(1), 2002, 105-13.
8. Bolderman R W, Hermans J J, Maessen J G. 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, *J Chromatogr B Analyt Technol Biomed Life Sci*, 877(18-19), 2009, 1727-31.
9. Maes A, Baert K, Croubels S, De Clercq D, van Loon G, Deprez P, De Backer P. Determination of amiodarone and desethylamiodarone in horse plasma and urine by high-performance liquid chromatography combined with UV detection and electrospray ionization mass spectrometry, *J Chromatogr B Analyt Technol Biomed Life Sci*, 836(1-2), 2006, 47-56.