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VALIDATION OF HYDROTROPIC SOLUBILIZATION BASED RP-HPLC METHOD FOR ESTIMATION OF AMIODARONE

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ABSTRACT
A new RP-HPLC method was developed for the estimation of amiodarone in tablets using hydrotropic solubilisation technique and it was validated as per ICH guidelines. The chromatogram for was found to be satisfactory on symmetry C-18 (4.6×150mm, 5µ Thermosil column) using 5% urea solution at a flow rate of 1.0 ml/min. The retention time of amiodarone was found to be 3.715 min. The system suitability parameters proved that the proposed method is suitable for estimation of amiodarone. Tailing factor for the peak was found to be 1.18 and the theoretical plates for separation were found to be 9071. The method was found to be linear in the range of 10-50µg/ml. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD and LOQ were found to be 0.024µg/ml and 0.08 µg/ml respectively. The proposed RP HPLC method was found suitable for the estimation of amiodarone in formulations and is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

KEYWORDS: Amiodarone, hydrotropy, HPLC, estimation, validation, ICH guidelines.

INTRODUCTION
Amiodarone (figure 1) is a benzofuran derivative, anti-arrhythmic drug used commonly in life-threatening ventricular arrhythmias. It blocks potassium currents that cause repolarization of the heart muscle during the third phase of the cardiac action potential.[1] The Indian Pharmacopoeia mentions a chromatographic method comprising of nitrile bonded column, a mixture of 45 volumes of 0.01 M sodium perchlorate and 55 volumes of acetonitrile, the pH of the mixture being adjusted to 3.0 with 2 M phosphoric acid mobile phase and 244nm detection wavelength for estimation of amiodarone in tablet formulations.[2] A few analytical methods have been reported for estimation of amiodarone in dosage forms or biological samples.[3-7] Most of the available methods use organic solvent that possess the potential harm to the nature. Hydrotropy is the ability of a concentrated solution of a chemical compound to increase the aqueous solubility of another compound. Maheshwari has reported the estimation of hydrophobic drugs using hydrotropic agents as mobile phase.[8-10] Hence in the present work, a green method based on hydrotropic solubilization was developed and validated for estimation of amiodarone in tablets by RP-HPLC.

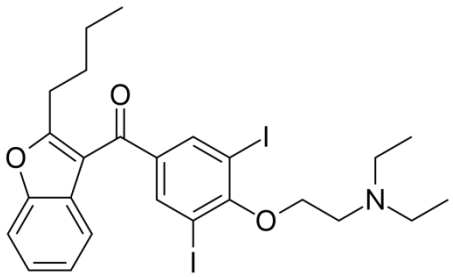

Figure 1: Structure of Amiodarone.

MATERIAL AND METHODS
Amiodarone pure drug was obtained as a gift sample from Medreich Limited, Bangalore. The pure sample was used without further purification. Tablet formulation (Brand Name-Amiodar-100 & 200 manufactured by Micro Labs) was procured from local pharmacy and was used for in the present study. Urea and sodium benzoate were purchased from Rankem while HPLC water was procured from Merck.

Preliminary Solubility analysis[11]
An excess amount of drug was added to screw capped 30ml glass vials containing different aqueous systems viz. distilled water, 5% urea solution and 2M sodium benzoate solution. The vials were shaken mechanically for 12 hours at 28±1°C in a mechanical shaker. These solutions were allowed to equilibrate for next 24hrs and

then centrifuged for 5min at 2000rpm. The supernatant liquid was taken for appropriate dilution after filtration through Whatman filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank.

The drug was found to be more soluble in 5% urea solution as compared to distilled water and 2M sodium benzoate solution and was also found to be stable over the period of 24 hours. Hence 5% urea solution was selected as the hydrotropic agent.

Preparation of mobile phase

A 5% solution of urea was selected as the mobile phase considering the solubility of the drug. The urea solution was filtered through 0.45µ filter under vacuum filtration. The mobile phase was used as diluent in the analysis.

Preparation of Standard Solution

Amiodarone (10 mg) was accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 7 ml of 5% urea solution was added and the flask was sonicated to dissolve the drug completely and the volume was made upto the mark with the distilled water to obtain a concentration of 1mg/mL. (Stock solution I)

Later 5ml of solution was pippetted out from the above stock solution into a 25 ml volumetric flask and the volume was made up to the mark with distilled water (stock solution II)

Further 1.5 ml of solution was pipetted out from stock solution II into a10ml volumetric flask and diluted to the mark with distilled water (stock solution III)

Preparation of Sample Solution

Tablet powder equivalent to 10 mg of Amiodarone was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 70 ml of 5% urea solution was added and sonicated to dissolve the drug completely and the volume was made upto the mark with the distilled water. (Working Stock solution I)

Further 5 ml of solution was pipetted out from the above stock solution into a 25 ml volumetric flask and diluted up to the mark with distilled water (Working Stock solution II)

An accurately measured quantity of 1 ml of above solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with distilled water (Sample solution).

Determination of the Absorption maxima of Amiodarone in 5% urea soluition

Stock solution III of Amiodarone in 5% urea solution was scanned in UV-Visible spectrophotometer over wavelength range of 200-400 nm. The maximum wavelength of Amiodarone was observed (figure 2).

Optimization of experimental conditions

Various trials were performed by varying the concentration of urea solution for determining the most efficient concentration using fixed flow rate of 1.0 ml/min; detection wavelength of 243 nm; injection volume 20 µl; and C18 column (4.6 x 150mm, i.d, 5µm, Thermosil). Stock solution III of the standard solution was injected into the HPLC system and the retention time and peak shape was observed (figure 3).

Analysis of tablet formulation

20µl each of the standard and sample solutions of Amiodarone were injected into the chromatographic system using the optimized conditions and the area for the Amiodarone peak was measured and the drug content of the tablets was calculated by comparing the areas of standard and sample solutions.

Validation of the method[12-13]

System Suitability

Stock solution-III of Amiodarone standard was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system. A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Linearity

Dilutions of the standard stock solution II were prepared at five different levels (10-50 ppm) by appropriate dilution using the mobile phase. Each level was injected into the chromatographic system in six replicates and the peak area was measured. A calibration curve was plotted taking concentration on X-axis v/s peak area on Y-axis and is presented in figure 4. The results are tabulated in Table 2.

Accuracy

Accurately weighed quantity of Amiodar-200 tablet powder equivalent to 10 mg was transferred into a 25 ml clean dry volumetric flask and about 20mL of diluent was added and sonicated to dissolve the drug completely. The volume was made up to the mark with the distilled water. 5 ml of this solution was further diluted to 25 ml in a volumetric flask with the distilled water. Accurately pipetted out 1.5 ml of the above solution was diluted to10 ml with the distilled water. The samples were spiked with 80, 100 and 120% solutions and recovery was studied. The prepared concentration (80%, 100% and 120%) solutions were injected into the HPLC system. The amounts added, amounts estimated and the individual recovery and mean recovery values were calculated and the results were summarized in table 3.

Precision

The precision of the method was validated in terms of repeatability and inter-day repeatability (intermediate precision). The stock solution III of the standard solution was injected in six replicates in the HPLC system and the area for all six injections in HPLC was measured. Similar procedure was repeated for all the concentrations of the linearity range. The % RSD for the area was calculated.

Robustness

In order to evaluate the robustness of the method, deliberate changes in the flow rate were made and the drug was assayed using the proposed conditions.

Limit of Detection

Amiodarone (10 mg) was accurately weighed and transferred into a 10 mL clean dry volumetric flask and about 7 mL of diluent was added and sonicated to dissolve it completely The volume was made up to the mark with distilled water. 0.3 ml of the above solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with distilled water (30 µg/ml solution). 1 ml of the above solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with distilled water. 0.08 mL of this was pipetted into a 10 ml of volumetric flask and diluted up to the mark with distilled water. This solution was injected in the system and studied for peak area.

Limit of Quantification

A solution similar to LOD was prepared and 0.27 mL of this was pipetted into a 10 ml of volumetric flask and diluted up to the mark with distilled water. This solution was injected in the system and studied for peak area.

RESULTS AND DISCUSSION

The wavelength for detection of Amiodarone by HPLC was selected on the basis of the absorption maxima obtained from UV spectrum scan of the drug. The maximum absorption in 5% urea solution was obtained at 243 nm.

Figure 2: Absorption maxima of Amiodarone.

For optimizing the mobile phase and the experimental conditions, different compositions of the mobile phase were tried out and the peak was observed for its proper formation and the retention time was also observed (Figure 3). The optimized mobile phase included 5% urea solution.

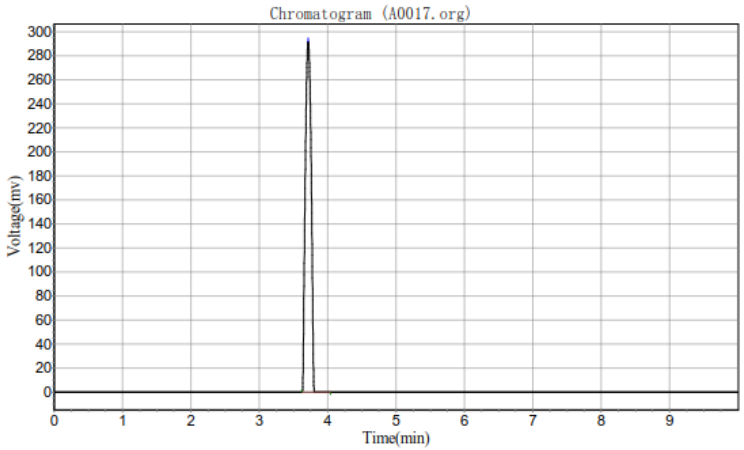

Figure 3: Chromatogram of Amiodarone in optimized experimental conditions.

Validation of the method

The developed method was validated as per ICH, US FDA guidelines and USP.

System Suitability

From the system suitability studies it was observed that all the parameters were within limit (Table 1). Hence it was concluded that the instrument, reagents and column were suitable to perform the assay.

Table 1: System suitability parameters.

System suitability parameters	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Mean	R.S.D
AUC X 10^6	1.74	1.73	1.74	1.74	1.73	1.74	1.74	-
Retention Time	3.715	3.715	3.718	3.712	3.714	3.715	3.715	0.002
Tailing Factor	1.01	1.26	1.26	1.21	1.15	1.18	1.18	-
No. of Theoretical plates	9173	9002	9118	9204	9041	8887	9071	-

Specificity

The observation of the chromatograms obtained from injecting the standard solution, sample solution as well as the blank (mobile phase) did not exhibit any other significant peaks other than the peak of Amiodarone. Hence it was concluded that the developed method is specific in nature.

Linearity and Range

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The linearity of the method was determined by analysis of standard plots associated with five point standard calibration curve. The peak area obtained from each preparation level of the Amiodarone standard solution was recorded. A correlation coefficient of not less than 0.9990 was considered as significant to ascertain the linearity and range of the method. The standard calibration curve is represented in the figure 4.

Table 2: Linearity of Amiodarone.

Concentration (µg/ml)	Area under curve						Mean
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
0	0	0	0	0	0	0	0
10	1507.36	1502.74	1520.17	1518.92	1497.33	1503.79	1508.39
20	3074.22	3014.72	3005.48	3040.34	3037.84	2994.66	3027.88
30	4397.18	4406.29	4548.63	4579.08	4488.26	4389.18	4468.1
40	6048.44	6010.96	6080.68	6075.55	6029.17	6075.24	6053.34
50	7318.18	7610.21	7298.58	7069.6	7425.27	7369.56	7348.57
Correl Coeff (r2)	0.999						
Slope (m)	148						
Intercept (c)	33.09						

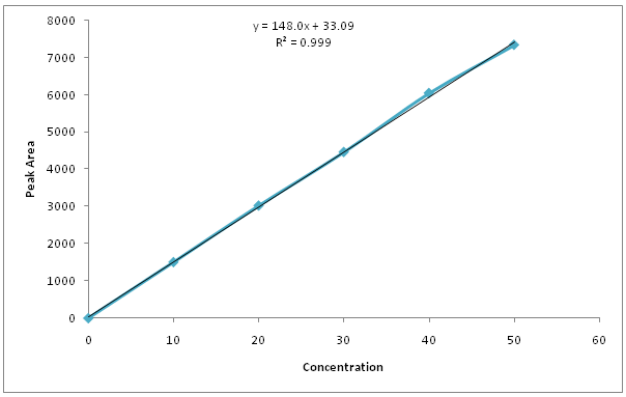

Figure 4: Calibration curve of Amiodarone.

Accuracy

The accuracy of a method is the closeness of test results obtained by the analytical method to the true value. The method passed the test for accuracy, as the percentage recovery was found to be 100.30% with a RSD of 0.288%. The result is presented in table 3.

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Precision

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample. The method passed the test for repeatability as the % RSD was less than 2%.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase and temperature. Effects of variation in the flow rate (±1 ml/min) were studied at three different concentrations and temperatures (±1°C to ±5°C) were studied. The method was able to maintain steady retention time of amiodarone on these deliberate changes in flow and temperature.

Table 3: Recovery of Amiodarone at different spiking levels.

Conc. of drug in tablet sample □g/ml)	Conc. of drug added to final (□g/ml)	Amount Recovered (µg/ml)						% Amount Recovered						% Recovered (mean)
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
100	5	105.048	104.991	105.047	105.063	104.971	105.198	100.046	99.991	100.045	100.060	99.972	100.189	100.050
100	10	110.021	110.126	109.798	110.022	109.988	110.087	100.019	100.115	99.816	100.020	99.989	100.079	100.006
100	15	115.158	115.121	114.875	114.797	115.103	115.156	100.137	100.105	99.891	99.823	100.090	100.136	100.030
Mean														100.029
SD														0.022
%RSD														0.022

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the signal to noise ratio method. The average base line noise obtained from the blank run (mobile phase injection) was found to be 58 µV whereas the signal obtained from LOD solution (0.08% of target assay concentration) was found to be 171 µV.

S/N = 171/58 = 2.94

The S/N Ratio value shall be 3 for LOD solution.

The signal obtained from the LOQ solution (0.27% of target assay concentration) was found to be 571 µV.

S/N = 571/58 = 9.84

The S/N Ratio value shall be 10 for LOQ solution.

Application of the method to marketed formulation

The developed and validated method was applied for the analysis of the marketed formulation of Amiodarone and the results obtained are presented in tables 4. The percentage recovery of Amiodarone was found to be 99.86% and 99.84% respectively.

Table 4: Results of assay of marketed formulation.

Brand name & label content	Amount found (mg)*	Standard deviation	% RSD	Percentage recovery
Rizora, 100 mg	100.21	0.469	4.593	102.1
Rizora, 200 mg	199.918	0.317	6.445	98.36
* Average of six replicate values				

CONCLUSION

The investigation resulted in the development of a new RP – HPLC method for the estimation of amiodarone in bulk and in formulations using hydrotropic agent. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis. The method was found to be an efficient alternative to the harmful organic solvents usually utilized for analysis of drug and pharmaceuticals.

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