ORIGINAL ARTICLE

RB SCIENCE
ENTERPRISING BIOSCIENCE RESEARCH

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

Published by RB Science Home Page: www.rbscience.co.in

Development of stability indicating assay for estimation of amiodarone

Narsingh Goyal*, Ankit Diwan, Kuldeep Ganju

Sagar Institute of Pharmacy and Technology, Bhopal, Madhya Pradesh, India

Article History

Received on: 26/05/2022

Revised on: 13/06/2022

Accepted on: 18/06/2022

Published on: 02/08/2022

Keywords

Amiodarone,

RP-HPLC,

Stability,

Buffer,

Stress Degradation,

Validation

ABSTRACT

A new RP-HPLC method was developed for the estimation of amiodarone in tablets 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 mobile phase composed of 70:30%v/v acetate buffer (pH 4.5) : methanol-isopropyl alcohol (30-70) at a flow rate of 0.8ml/min. The retention time of amiodarone was found to be 5.317 min at detection wavelength of 310.4 nm. The method was found to be linear in the range of 10-50µg/ml. 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. The method was used for analyzing the stability of amiodarone using forced degradation studies in basic, acidic and oxidative stress conditions induced by 0.1N NaOH, 0.1M HCl and 5% H_2O_2 solution respectively. The method was found to be highly effective in the analysis of amiodarone in presence of degradation products and could easily differentiate between the peaks of the degradation product as well as the parent molecule.

*Corresponding Author
Narsingh Goyal
Email: narsinghgoyal4@gmail.com

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

ISSN No. 2456-8244

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Introduction

Amiodarone is a benzofuran derivative, anti-arrhythmic drug used commonly in a variety of settings. The FDA approved indications for amiodarone are recurrent ventricular fibrillation (VF) and recurrent hemodynamically unstable ventricular tachycardia (VT). It is a class III anti-arrhythmic drug. It blocks potassium currents that cause repolarization of the heart muscle during the third phase of the cardiac action potential (drugbank, 2022). As a result it increases the duration of the action potential as well as the effective refractory period for cardiac cells (myocytes). A few methods have been reported for estimation of Amiodarone. These methods make use of complex or costly methodology like LC-MS, LC-MS/MS and GC-MS or less sensitive methods like UV (Coelho et al., 2020; Rajendran et al., 2006; Khan et al., 2005) . Some reported method did not make use of internal standards while some used internal standards but they are not easily available in market. Thus, the above observations warrant the need for development and validation of new method for estimation of Amiodarone with high sensitivity, accuracy, precision, rapid and economical the using of suitable internal standard as per ICH, USFDA and other guidelines. It was therefore envisioned to develop and validate a suitable stability indicating method for the estimation of Amiodarone in dosage form.

Material and Methods

Amiodarone was obtained as a gift sample from Medreich Pharmaceuticals, Bangaluru; all other chemicals, and reagents were of analytical grade and procured from various chemical suppliers.

Preparation of mobile phase

The mobile phase consisted of acetate buffer pH 4.5 (35 ml) and a mixture of methanol and isopropyl alcohol (30:70, 65 ml), filtered through 0.45µ filter under vacuum filtration. The mobile phase was also 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 7ml of diluent was added and sonicated to dissolve the drug completely and the volume was made up to the mark with the same solvent. This solution was appropriately diluted using the diluent to obtain the working standard solution.

Preparation of Sample Solution

Tablet powder equivalent to 50 mg of amiodarone was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve the drug completely and the volume was made upto the mark with the same solvent. This solution was appropriately diluted to obtain the sample solution.

Analysis of tablet formulation

20 µl 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.

Optimization of elution conditions

Several trial runs were carried out and the following conditions were found to be most suitable in eluting amiodarone from the stan-

dard as well as sample solutions.

Mobile phase: Acetate buffer (pH 4.5) and [methanol: isopropyl alcohol (30:70)] in the ratio of 35:65 v/v

Column: Octadecylsilane (ODS) (4.6 x 150mm, 5µm, Thermosil)

Flow rate: 0.8 ml per min

Wavelength: 310.4 nm

Injection volume: 20 µl

Column oven temperature: Ambient

Validation of the method

The developed method was validated according to ICH guidelines for system suitability, linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) (Sahu and Singh, 2017; Biswas et al., 2018).

Application of method in forced degradation conditions (Kumar et al., 2020)

Basic degradation

Amiodarone (100 mg) was accurately weighed and transferred into a 10 ml clean, dry volumetric flask and 10 ml of diluent was added and sonicated to dissolve the drugs completely. It was then diluted with 10 mL of 0.1N NaOH (basic hydrolysis) and kept for 30 minutes, further diluted with the diluents up to the mark. 1 ml of this solution was filtered through 0.45 µm filter paper, transferred into 10 mL volumetric flask to obtain a concentration of 10 µg/ml and volume was made up to the mark with mobile phase. The sample was withdrawn at 1, 24, 48 and 72 hrs intervals and periodically analyzed by developed HPLC method.

Acidic degradation

Amiodarone (100 mg) was accurately weighed and transferred into a 10 ml clean, dry volumetric flask and 10 ml of diluent was added and sonicated to dissolve the drugs completely. It was then diluted with 10 mL of 0.1N HCl (acidic hydrolysis) and kept for 30 minutes, further diluted with the diluents up to the mark. 1 ml of this solution was filtered through 0.45 µm filter paper, transferred into 10 mL volumetric flask to obtain a concentration of 10 µg/ml and volume was made up to the mark with mobile phase. The sample was withdrawn at 1, 24, 48 and 72 hrs intervals and periodically analyzed by developed HPLC method.

Oxidative degradation

Amiodarone (100 mg) was accurately weighed and transferred into a 10 ml clean, dry volumetric flask and 10 ml of diluent was added and sonicated to dissolve the drugs completely. It was then diluted with 10 mL of 5% H_2O_2 solution and kept for 30 minutes, further diluted with the diluents up to the mark. 1 ml of this solution was filtered through 0.45 µm filter paper, transferred into 10 mL volumetric flask to obtain a concentration of 10 µg/ml and volume was made up to the mark with mobile phase. The sample was withdrawn at 1, 24, 48 and 72 hrs intervals and periodically analyzed by developed HPLC method.

The % degradation of drugs to be remained, retention time and % RSD was calculated from the standard concentration of drug.

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 was obtained at 310.4 nm (Figure 1).

The chromatogram obtained using the optimized elution conditions revealed the reten-

tion time of amiodarone to be 5.317 min (Figure 2).

Validation of the method

System Suitability

From the system suitability studies it was observed that all the parameters (theoretical plates, tailing factor, retention time) were within prescribed limits (Table 1). The average retention time of six replicate analyses was 5.317 min with a relative standard deviation (RSD) of 0.225; the number of theoretical plates for separation was 8884 and the tailing factor was 1.26. Hence it was concluded that the instrument, reagents and column were suitable to perform the assay.

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 3.

Accuracy

The accuracy of a method is the closeness of test results obtained by the analytical method to the true value. Accuracy was studied using recovery method whereina known quantity of standard drug is spiked into a pre-analyzed sample and the concentration of the same is determined using the method under consideration. The method passed the test for accuracy, as the percentage recovery was found to be 100.30 % with a RSD of 0.744 % (Table 1).

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 precision was evaluated in terms of repeatability as well as intermediate precision and the method exhibited a RSD of less than 2.0% suggest good precision of the method.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase and temperature (Table 2-4).

LOD and LOQ were calculated using signal to noise ratio method and the LOD was found to be 0.08µg/mL while the LOQ was found to be 0.27 µg/mL.

Stability Indicating Studies

In order to ascertain whether the developed method was stability indicating, the pure samples of amiodarone were subjected to stress under different conditions to promote degradation as per the ICH guidelines. Stability indicating studies were performed by base stress using 0.1N NaOH, acid stress using 0.1 M HCl and oxidative stress using 5% solution of H_2O_2.

The degradation products in each condition were completely distinguishable from the parent compound.

The % RSD of the peak areas obtained in each of stressed degradation study was less than 1.1% revealing that the method is a good stability indicating assay method for amiodarone hydrochloride in formulations (Table 5-7).

Conclusion

The investigation resulted in the development of a new RP – HPLC method for the estimation of Amiodarone hydrochloride in bulk and in formulations. 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 highly effective in the analysis of amiodarone in presence of degradation products and could easily differentiate between the peaks of the degradation product as well as the parent molecule.

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Table 1. Recovery Study

Conc. of drug in tablet sample (µg/ml)	Conc. of drug added to final (µg/ml)	% Amount Recovered						
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	% Recovered (mean)
25	5	100.40	101.20	99.61	101.19	97.85	97.21	99.57
25	10	99.40	101.41	100.50	99.41	99.40	101.49	100.27
25	15	100.87	98.28	101.41	99.20	99.67	100.54	99.99
Mean Recovery								99.91
Standard Deviation								0.350
RSD								0.350

Table 2. Effect of variation in flowrate (-0.1ml/min)

Concentration (µg/ml)	Retention time (min)*	Peak Area*	Standard deviation	% RSD
20	5.335	2900.16	20.177	0.695
30	5.321	4198.45	19.995	0.476
50	5.318	6913.28	11.98	0.173

* Average of six replicate values

Table 3. Effect of variation in flow rate (+0.1 ml/min)

Concentration (µg/ml)	Retention time (min)*	Peak Area*	Standard deviation	% RSD
20	5.31	2903.49	27.003	0.93
30	5.298	4204.28	20.389	0.484
50	5.305	6911.61	12.175	0.176

* Average of six replicate values

Table 4. Effect of variation in composition of mobile phase ratio

Mobile Phase ratios	Retention time (min)*	Peak Area*	Standard deviation	% RSD
20-80	5.319	1546.55	28.515	1.84
25-75	5.317	1544.66	29.373	1.901
35-65	5.319	1546.81	28.456	1.839

* Average of six replicate values

Table 5. Result of Basic stress study

Time (hrs)	Mean Peak Area*	Standard deviation	% RSD
1	627.82	4.033	0.642
24	624.54	3.871	0.619
48	597.25	4.614	0.772
72	518.63	4.215	0.812

*Average of six replicate analyses

Table 6. Result of Acidic stress study

Time (hrs)	Mean Peak Area*	Standard deviation	% RSD
1	800.91	6.216	0.776
24	756.34	6.308	0.834
48	741.96	5.166	0.696
72	710.33	4.76	0.670

*Average of six replicate analyses

Table 7. Result of Oxidative stress study

Time (hrs)	Mean Peak Area*	Standard deviation	% RSD
1	698.61	7.315	1.047
6	731.25	3.684	0.503
24	712.17	4.027	0.565
48	687.55	2.849	0.414

*Average of six replicate analyses

Figure 1. Absorption maxima of Amiodarone

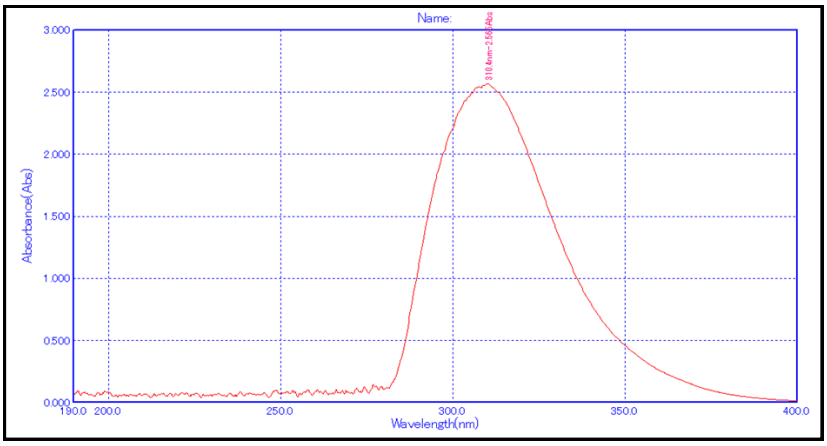
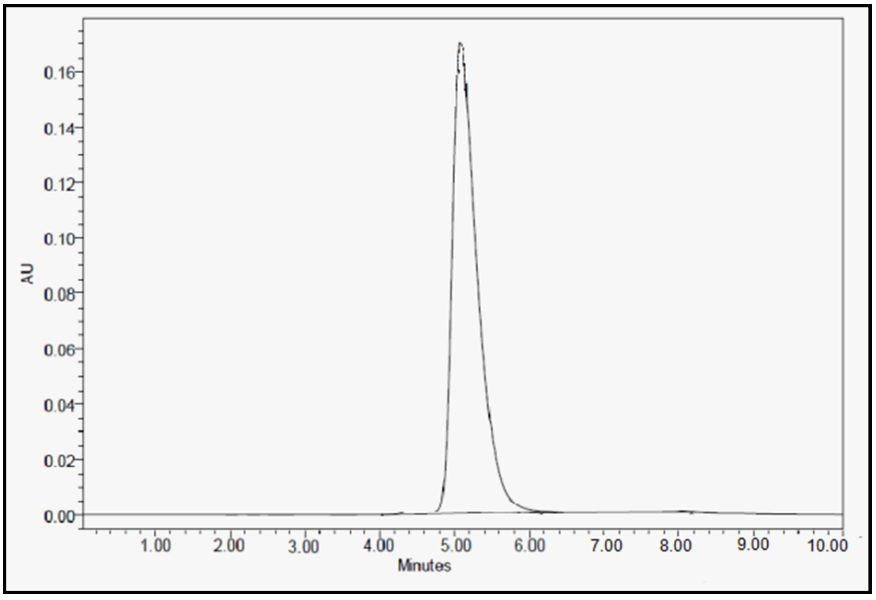


Figure 2. Chromatogram of amiodarone in optimized experimental conditions



Cite this article as

Goyal N, Diwan A, Ganju K. Development of stability indicating assay for estimation of amiodarone. J Pharmacol Biomed. 2022; 6(3): 514-521