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HPLC and UV Spectrophotometric Determination of Amrinone Lactate

V. VIJAYA BHASKAR, B.M. GURUPADAYYA*, Y.N. MANOHARA and D.V. PRASAD REDDY
Department of Pharmaceutical Analysis
National College of Pharmacy, Shimoga-577 201, India
E-mail: bm_guru2004@yahoo.co.in

Two assay procedures based on UV spectrophotometry and high performance liquid chromatography (HPLC) have been developed for the determination of amrinone lactate in bulk drug and pharmaceutical formulation (parenteral). UV spectrophotometry involves the determination of amrinone lactate by dissolving it in water followed by measuring absorbance at 259 nm. The HPLC determination was carried out on a reversed phase C-18 column using a mobile phase consisting of water:acetonitril:methanol (40:30:30) at a flow rate of 1.0 mL/min with UV detection at 259 nm. UV Spectrophotometric method is applicable over 2-10 µg/mL range of amrinone lactate with a molar absorptivity of 2.6996×10^4 L mol^{-1} cm^{-1} and a Sandell's sensitivity of 11.11 ng/cm^2. In HPLC method a rectilinear relationship was observed between 0.2-40 µg/mL and analysis time was 7 min. The retention time was found to be 2.710 min. The methods when applied to the determination of amrinone lactate in parenteral gave satisfactory results. The developed methods were found to be precise and accurate from the statistical validation of the analysis data.

Key Words: Amrinone lactate, UV Spectrophotometry, HPLC.

INTRODUCTION

Amrinone lactate[1,2] (AMR) is chemically 5-amino-3,4'-bipyridyl-6(1H)-one (Fig. 1). AMR[3-13] is a cardiovascular drug and used as a non-glycoside cardiotonic. It is a phosphodiesterase inhibitor which prevents enzymatic breakdown of cyc. Amp with the inotropic and vasodilator effects. AMR is used in the short-term management of congestive heart failure unresponsive to digitalis, diuretics or vasodilators. The therapeutic importance of this compound justifies research to establish analytical methods for its determination in bulk drug and pharmaceutical formulation. In literature no analytical methods were reported for the determination of AMR. UV Spectrophotometric method[14] because of its simplicity, sensitivity, speed, reliability and accuracy continues to be a widely used technique in drug quality control. The versatility of HPLC[15] in pharmaceutical analysis

particularly in industrial quality control is well known. In present studies, two methods are proposed for the determination of AMR both in bulk and pharmaceutical formulation. UV Spectrophotometry involves dissolving AMR in water and measuring the change in absorbance at 259 nm with different concentrations. The HPLC analysis was carried out by injecting the drug solution on to a Column C-18, 250 × 4 mm i.d; particle size 5 µm and packing material was eurosphere - 100 with the elution being affected by a mobile phase consisting of water:acetonitrile:methanol (40:30:30) and UV detection at 259 nm.

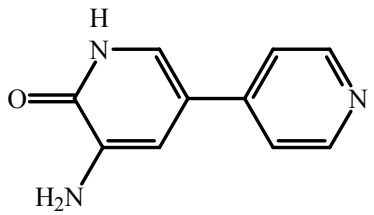

Fig. 1. Chemical structure of amrinone lactate

EXPERIMENTAL

Double distilled water was used in developing UV spectrophotometric method. Acetonitrile, methanol and water (RANKEM, India) of HPLC grade were used in developing RP-HPLC method. The mobile phase is filtered through a 0.45 µM filter. The mobile phase used for chromatography consisted of water:acetonitrile:methanol (40:30:30). Pure sample was received as a gift from Samarth Pharma, Mumbai and was used as such.

Preparation of standard stock solution: A 1 mg/mL drug solution was prepared by dissolving 100 mg of pure AMR in water and diluting to 100 mL in calibrated flask. This solution (1000 µg/mL) was diluted appropriately to get 100 µg/mL with water for use in UV spectrophotometry and HPLC.

A Shimadzu UV/Visible double beam spectrophotometer (model 1601) with 1 cm matched quartz cells were used for all spectral measurements. An isocratic high performance liquid chromatography (Knauer HPLC) with Wellchrom HPLC-Pump K 501 and UV/Vis detector K 2501 (Knauer). Column C-18, 250 × 4 mm i.dl; particle size 5 µm and packing material was eurosphere-100.

UV spectrophotometric method (Method A): Different aliquots (0.2-1.0 mL) of 100 µg/mL AMR were accurately transferred into a series of 10 mL calibrated flasks and made up to the volume with water. The contents were mixed well and absorbance maximum is found at 259 nm (Fig. 2).

The obtained absorbance values when plotted against the concentration of AMR gives the calibration graph (Fig. 3). The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from Beer's law data.

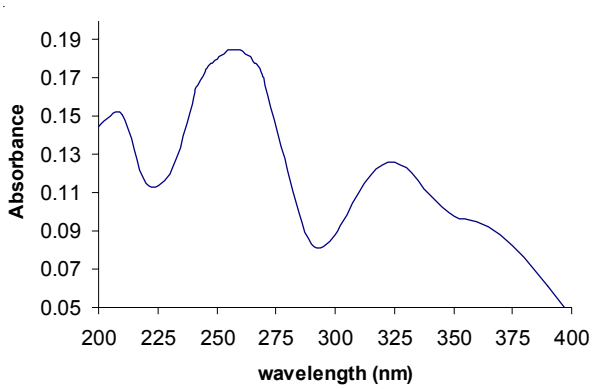

Fig. 2. UV absorption spectrum of amrinone lactate in water

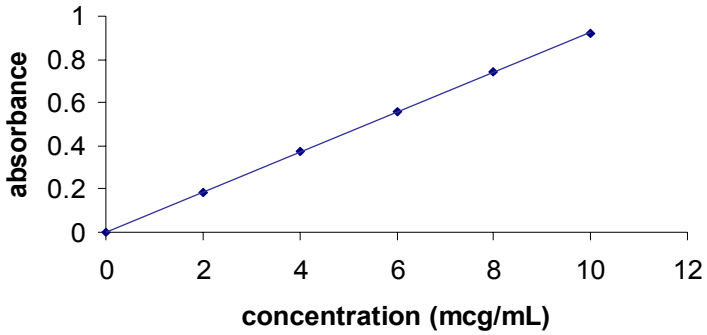

Fig. 3. Beer's law plot or calibration curve of amrinone lactate in UV spectrophotometry

Chromatographic assay (Method B)

Chromatographic conditions: Chromatographic separation was achieved at ambient temperature on a reversed phase isocratic high performance liquid chromatography (Knauer HPLC) with Wellchrom HPLC-Pump K 501 and with soft ware C2000 version 1.7 and UV/Vis detector K 2501(Knauer). Column C-18, 250 × 4 mm i.dl; particle size 5 µm and packing material was eurosphere-100, using a mobile phase consisting of water:acetonitrile:methanol (40:30:30) at a flow rate of 1.0 mL/min. The

detector wavelength was set at 259 nm and retention time at which sample eluted was 2.710 min.

Calibration graph: Working standard solutions containing 0.2-40 µg/mL AMR were prepared by appropriate dilution of the stock solution with the diluent solution (mobile phase). 20 µL aliquot of each solution was injected manually onto the column and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area as a function of AMR concentration (Fig. 4). The amount of drug present in formulation was calculated making use of standard calibration curve.

Analysis of formulations: The contents of ten formulations were emptied into a beaker and mixed.

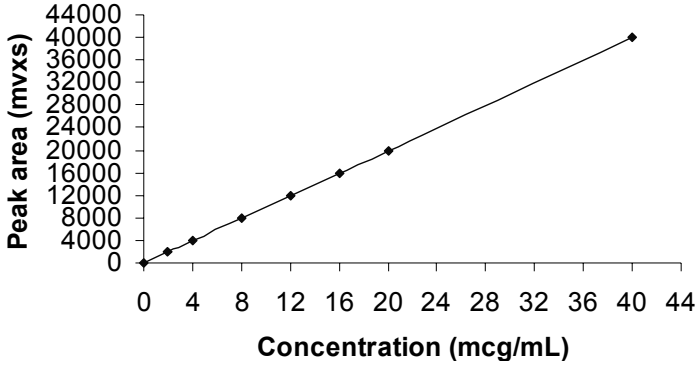

Fig. 4. Beer's law plot or calibration curve of amrinone lactate in RP-HPLC

Methods A and B: 20 µL of injection solution equivalent to 100 mg of AMR was quantitatively transfered in to a 100 mL calibrated flask and diluted to the mark with distilled water. The solution was then analyzed after dilution by UV spectrophotometric and HPLC methods. The stock solution was diluted in UV spectrophotometry in such a way that various aliquots are in the range of 2-10 µg/mL. In HPLC the stock solution was filtered through a 0.45 µm filter and diluted suitably with mobile phase to get various concentrations in the range of 0.2-40 µg/mL.

Optimized chromatographic conditions: The optimized chromatographic conditions are as follows

Parameters	Method
Stationary phase (column)	((Knauer HPLC C-18 (250 × 4 mm i.dl; Particle size 5 µm and packing material was eurosphere-100)
Mobile phase	Water:acetonitrile: methanol
Flow rate (mL/min)	1.0 mL/min

Column back pressure (1500 psi)	1500
Run time (min)	7
Column temperature (ºC)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	259
Retention time (min)	2.710

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and sandell's sensitivity are presented in Table-1. The regression analysis using the method of least squares was made for the slope (b), intercept (a), correlation (r) obtained from different concentrations and results are summarized in Table-1. The limit of detection and limit of quantification were 0.725 and 1.5 µg/mL, respectively

TABLE-1
OPTICAL CHARACTERISTICS FOR METHOD A

λ_{max} (nm)	259
Beer's law limits (µg/mL) (C)	2-10
Molar absorptivity (L mol^{-1} cm^{-1})	2.6996×10^4
Sandell's sensitivity (µg/cm^2-0.001 absorbance unit)	0.011
Regression equation (y*) Slope (b)	0.0369
Intercept (a)	0.0690
Correlation coefficient (r^2)	0.9997
Range of errors**	
Confidence limits with 0.05 level	0.0056
Confidence limits with 0.01 level	0.0082

*y = a + bc where c is the concentration of AMR in µg/mL and y is the absorbance at the respective λ_{max}; **For average of eight measurements.

The graphs showed negligible intercept as described by the regression equation:

$$y = a + bc$$

(where y is the absorbance unit and c is concentration in µg/mL) obtained by the method of least squares. The per cent range of error (0.05 and 0.01 level of confidence limits) calculated from the eight measurements, ¾th of the amount of upper Beer's law limits in each method are summarized in Table-1. The results showed that these methods have reasonable precision.

The other active ingredients and excipients usually present in the pharmaceutical dosage forms are not present in this formulation, so they doesn't interfere in the estimation when formulation was analyzed by this method.

The optimum concentration for the estimation of AMR was established by varying drug concentration. To evaluate the accuracy of the method, a known amount of pure drug was added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The per cent recoveries are given in Table-4. Inter-day and intra-day precision of the methods was determined by repeat analysis (n = 6) of the standard solution containing AMR at two different levels. The results of method are presented in Table-2. The % RSD value for the peak area based and retention time based values did not exceed 1.2 %, which shows the method is more precise. The method reported here is found to be simple, sensitive, accurate, precise, reproducible and economical and can be used in the determination of AMR in bulk drug and its pharmaceutical dosage forms (injection) in a routine manner.

TABLE -2
INTER-AND INTRA-DAY PRECISION FOR AMRINONE LACTATE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED UV METHOD

Conc. of amrinone lactate (µg/mL)	Concentration of amrinone lactate found on			
	Intra-day		Inter-day	
	Mean (n = 6)	CV (%)	Mean (n = 6)	CV (%)
4	3.98	0.426	4.02	0.587
6	6.02	0.518	6.07	0.787

TABLE 3
INTER-AND INTRA-DAY PRECISION FOR AMRINONE LACTATE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Conc. of amrinone lactate (µg/mL)	Concentration of amrinone lactate found on			
	Intra-day		Inter-day	
	Mean (n = 6)	CV (%)	Mean (n = 6)	CV (%)
4	4.01	0.0562	4.04	0.218
8	8.08	0.321	8.02	0.398

TABLE -4
RESULTS OF ASSAY OF FORMULATION BY THE PROPOSED METHODS

Formulation and brand name*	Label claim (mg/mL)	Found† (% recovery of nominal amount ± SD)	
		Method A	Method B
Amicor injection	5	99.68 ± 0.621	99.99 ± 0.582
	1	99.72 ± 0.457	99.99 ± 0.259

*Marketed by Samarth Pharma Pvt Ltd, Mumbai.
†Mean value of three determinations.

The objective of present study was to develop a rapid and sensitive HPLC method for the analysis of AMR in bulk drug and its formulations using the most commonly employed RPC-18 column with UV-detection at 259 nm. The composition and pH of the mobile phase were varied to optimize the chromatographic conditions. Mobile phase consisting of water: acetonitrile: methanol (40:30:30) had given immacculate results when compared to other mobile phases. At a flow rate of 1.0 mL/min, the retention time for AMR was 2.710 min. The run time was set at 7 min. Each sample was injected 6 times and the retention times were same. The peak area of the drug was reproducible as indicated by low coefficient of variance (0.435 %). The calibration curve points of the proposed method were shown in Table-5. Under the described experimental conditions, the analyte peak was well defined and free from tailing. AMR was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r^2 = 0.9999) over the concentration range 0.2-40 µg/mL. Using regression analysis the linear equation,

$$Y = 999.98X + 3.7262$$

where y is the mean peak area and X is concentration in µg/mL. The limit of detection and limit of quantification were 0.1 and 0.15 µg/mL respectively. The regression equation was used to estimate the amount of AMR either in formulations or in validation study (precision and accuracy). To evaluate the accuracy of the method, a known amount of pure drug was added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The per cent recoveries are given in Table-4. It is more sensitive method as signal/concentration of drug value varies more from one concentration to other.

TABLE-5
CALIBRATION CURVE POINTS OF THE PROPOSED METHOD (HPLC) FOR ESTIMATION OF AMR

Concentration of amrinone lactate (µg/mL)	Peak area* (mvxs)	CV (%)
0	0	0
2	2010.7489	0.01541
4	4005.5879	0.05370
8	8009.2547	0.05230
12	12010.5489	0.0137
16	16005.9874	0.0239
20	20015.3456	0.4350
40	40008.4897	0.2820

*Mean of six determinations.

Inter-day and intra-day precision of the methods was determined by repeated analysis (n = 6) of the standard solution containing AMR at two

different levels. The results of method are presented in Table-3. The %RSD value for the peak area based and retention time based values did not exceed 0.5 %, which shows the method is more precise.

Application: Results obtained with proposed methods confirm the suitability of these methods for pharmaceutical dosage forms. The results obtained by the proposed methods agreed well with the label claim in all instances.

Conclusion

Amrinone lactate has been determined in bulk drug and in pharmaceutical products by employing two different techniques. The methods offer the advantages of speed and convenience since they do not require special working conditions. In comparison of the two methods, HPLC is more sensitive, precise, selective and accurate method. When it comes for simplicity, UV spectrophotometric method is superior to HPLC method.

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