ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

New Ecofriendly Validated Spectrophotometric Method for the Estimation of Amlodipine Besylate in Bulk Drug Using Ninhydrin

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Received:
19th Jan 2013
Received in revised form:
10th Feb 2013
Accepted:
15th Feb 2013
Available online:
25th Feb 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

A new, accurate, precise and economical spectrophotometric method has been developed and validated for the determination of Amlodipine Besylate in bulk drugs. This method is based on the reaction of ninhydrin with primary amine present in the Amlodipine Besylate in the presence of Sodium Bicarbonate. This reaction proceeds quantitatively at 97 ± 1°C in 15 min and produces a purple color product which absorbs maximally at about 566 nm. Beer-Lambert's law is obeyed in the concentration range of 50-250µg/ml and is described by the regression equation Y = 0.700x - 0.20 with a regression coefficient (r^2) = 0.999 (n = 6). The effects of variables such as temperature, heating time, concentration of colour producing reagent and stability of colour were investigated to optimize the procedure. For Amlodipine Besylate, the value of molar absorptivity and Sandell's sensitivity are 3.1077 x 10^3 L/mol/cm and 0.1824µg/cm^2, respectively and of LOD and LOQ are found to be 0.09 and 0.2729µg/ml, respectively. The statistically validated results indicate that the proposed method has good sensitivity, accuracy, precision and stability. The method is eco friendly and economic as it does not involve a any organic solvents.

Keywords: Amlodipine Besylate; Ninhydrin; Sodium Bicarbonate; Spectrophotometry

1. INTRODUCTION

Amlodipine besylate (AM), is a calcium channel blocker. Chemically, it is [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzenesulfonate (Figure 1)[1]. AM is a calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina[2].

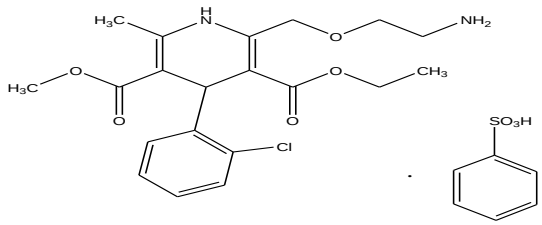

Figure 1: Structure of Amlodipine Besylate (AM)

European Pharmacopoeia describes assay of Amlodipine Besylate by reversed phase high performance liquid chromatography in bulk and pharmaceutical formulations[3]. AM has been studied and determined by relatively other methods such as Spectrophotometric[4-11],Voltametry[12-13], HPLC[14-33], HPTLC[34-36].

Many researchers have dealt with the development of methods that quantify Amlodipine Besylate (AM) in pure form and in tablets. Visible spectrophotometry is the technique of choice even today because of its inherent simplicity, sensitivity, selectivity, accuracy, precision and cost-effectiveness. The scientific references found in the CAS and SCI database, relating to green analytical chemistry or environmentally-friendly analytical methods have been growing significantly in recent years[37-38]. The recent development of new analytical methods with good characteristics such as selectivity and sensitivity are not

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only sufficient but also modern analytical methods need to be green[39-41]. Hence, the purpose of the present study was to improve these methods with respect to two experimental objectives: (i) decrease the heating time, (ii) avoid the use of organic solvent. Both factors are of great significance in reducing time and cost of analysis. In the present investigation, solution of 0.1M HCl has been employed to solubilize a slightly water soluble drug, AM forms a purple colored product with ninhydrin in the presence of saturated solution of $NaHCO_3$ and further spectrophotometric estimation was carried out maximum at about 566 nm without employing any organic solvents. These modifications resulted in increased linear range (50-250µg/ml), molar absorptivity (ε = 3.1077 x 10^3 L/mol/cm) and sensitivity (0.1824µg/cm^2) compared to many existing spectrophotometric methods.

2.0 EXPERIMENTAL

2.1 Apparatus

A Shimadzu 1800 double beam UV-VIS spectrophotometer provided with 1 cm matched quartz cell was used for absorbance measurements.

2.2 Materials and Reagents

Amlodipine Besylate was obtained as gift sample from Wockhardt Pharmaceuticals Pvt. Ltd. (Aurangabad, India). All other reagents used were of analytical grade.

2.3 Solubility Studies

Solubility of Amlodipine Besylate was carried out in 0.1M HCl and further dilution in same solvent.

2.4 Preparation of 0.5% Ninhydrin Solution

500 mg of Ninhydrin was dissolved and made up to 1000 ml with distilled water.

2.5 Preparation of Sodium Bicarbonate (Saturated Solution)

Approximately 25 g of sodium bicarbonate was taken in a beaker containing 100 ml of distilled water and stirred with a magnetic stirrer for twenty minutes. The solution was decanted and filtered using quantitative filter paper.

2.6 Preparation of Standard solution of AM

100 mg pure Amlodipine Besylate was transferred to 100 ml volumetric flask and diluted upto the mark with 0.1M HCl to get a concentration of 1000µg/ml solution.

2.7.Optimized Procedure

In a 10 ml volumetric flask, add 1.0 ml standard stock solution (1000µg/ml), 1.0 ml ninhydrin and 1.0 ml saturated sodium bicarbonate solution and diluted upto the mark with 0.1M HCl (100µg/ml). It was heated in boiling water bath at 97 ± 1°C in 15 min and cooled to room temperature. Scan the spectrum and absorbance was measured λmax at 566 nm *versus* the reagent blank (Figure 2).

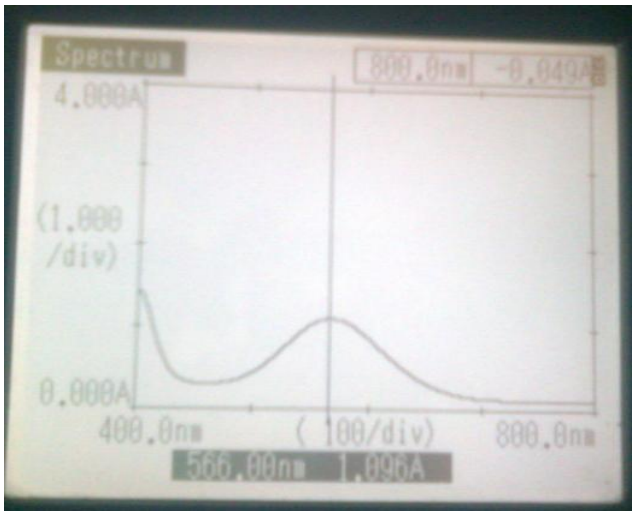


Figure 2: Spectrum of Amlodipine Besylate

3.0. METHOD VALIDATION

3.1. Stability

In order to demonstrate the stability of the Ninhydrin-Amlodipine besylate complex in presence of saturated sodium bicarbonate solution was analyzed over a period of 12 hrs at room temperature. During analysis, the complex was found to be stable over a period of 8 hr at room temperature as shown in Figure 3.

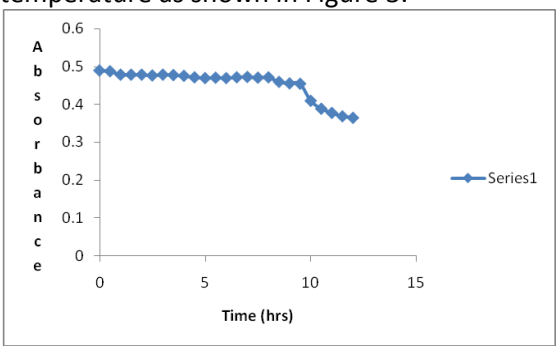


Figure 3: Effect of time (hrs) on absorbance of AM- ninhydrin complex.

3.2. Linearity

From the Standard Stock solution (1000µg/ml), different aliquots (0.5, 1.0, 1.5, 2.0 and 2.5) were taken in a series of 10 ml volumetric flasks and 1.0 ml ninhydrin was added to it followed by 1.0 ml saturated sodium bicarbonate solution and volume made up with 0.1M HCl to get concentration 50-300µg/ml. All flasks were heated for 15 min in boiling water bath and cooled to room temperature and measure the absorbance at 566 nm. Five replicates of analytes were measured and record the absorbance *versus* concentration as shown in Table 1. Plot a graph concentration *versus* absorbance, a linear correlation was found which obeys Beer Lambert's Law in the concentration range of 50-250 µg/ml (Figure 4). Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and the correlation coefficient (r^2).

Concentration (µg/ml)	Absorbance
50	0.136
100	0.490
150	0.867
200	1.210
250	1.528

Table 1: **Observation table for calibration curve of Amlodipine Besylate**

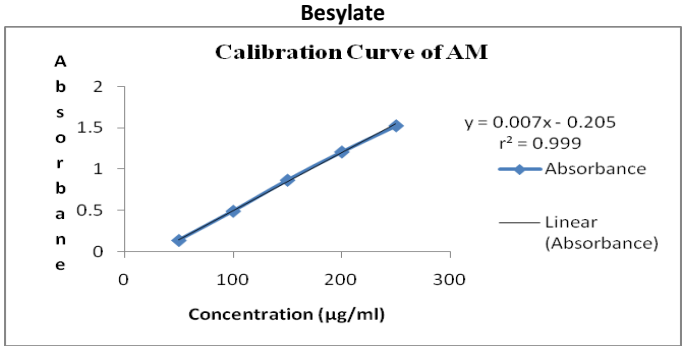


Figure 4: Calibration curve of Amlodipine Besylate (AM)

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

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products. The valve of LOD and LOQ are determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines[42] and given in Table 2.

Parameter	Analytical data
Linearity Range (µg/ml)	50-250
λ max (nm)	566
Molar extinction coefficient	$3.1077x10^3$
Sandell's sensitivity	0.1824
Slope	0.7×10^{-2}
Intercept	-205
Standard deviation about regression (Sy)	±0.2637
Standard deviation of Slope (Sb)	±0.0970
Standard deviation of intercept (Sa)	±0.1635
Correlation co-efficient (r)	0.999
Limit of detection (LOD, µg/ml)	0.09
Limit of quantification (LOQ, µg/ml)	0.2729

Table 2: Optical characteristics data and validation parameters of Amlodipine Besylate

3.4. Precision

To determine precision, 7 days measurement (intra-days and interday) were computed with relative standard deviation (RSD%) for replicate samples (n = 5) using concentration 100, 150 and 200µg/ml Both the intra-day and interday samples were calibrated with standard curve concurrently prepared in the same day of analysis.

3.4.1. Intraday Precision

Intraday precision of test method is demonstrated by three samples of the same batch (same concentration) at initial, 24 and 48 hrs (Table 3).

3.4.2. Interday Precision

Interday precision of test method is demonstrated by three samples of the same batch (same concentration) on three successive days (Table 3).

AM taken (µg/ml)	Intraday Accuracy and precision			Interday Accuracy and precision		
	AM found (µg/ml)	RE %	RSD %	AM found (µg/ml)	RE %	RSD %
100	100.35	0.2045	0.4992	99.81	0.2197	0.5391
150	150.11	0.3239	0.5259	149.68	0.4437	0.7262
200	200.15	0.4667	0.5711	199.85	0.5863	0.7100

Table 3: Evaluation of intra-day and inter-day accuracy and precision

3.5. Accuracy

To determine the accuracy of the proposed method, recovery study was carried out by adding different amount (80%, 100%. 120%) of bulk sample of Amlodipine Besylate within the linearity range and results obtained are compiled in Table 4 and show good accuracy for the method.

Level	Amount of AM added (µg)	Amount of AM found (µg)	% Recovery	% RSD
80 %	80	81.71	100.95	1.43
100 %	100	99.07	99.07	1.32
120 %	120	119.60	99.66	1.75

*An average value ± relative standard deviation of 5 observations

3.6 Assay

Assay of tablet dosage form was carried by same procedure as mentioned in methodology to equivalent weight of Amlodipine Besylate by proposed spectrophotometric method. The percent purity was found out using regression analysis(Table 5) .

Formulation	Actual amount (µg)	Amount Found (µg)	% of Drug Found
Tablet	50	51.33	102.66

Table 5: Assay Results of Tablet Dosage Form

4.0. RESULTS AND DISCUSSION

Preliminary studies were carried out to establish the optimum conditions for assay of the Amlodipine Besylate.

4.1. Effect of temperature

The effect of temperature on the complexation reaction at 80^0, 90^0 97 ± 1°C were examined (Table 6). It was observed that ninhydrin–AM complex in saturated sodium bicarbonate required 97 ± 1°C for obtaining maximum and stable absorbance and remained constant for about a further 8 hrs.

Temperature (^{0} C)	Absorbance
80 ± 1	0.455
90 ± 1	0.585
97 ± 1	0.765

Table 6: Effect of temperature on absorbance

4.2. Effect of the reaction heating time

The effect of time on the complexation reactions at room temperature was examined. It was observed that ninhydrin–AM charge transfer complex in saturated sodium bicarbonate required 15 min for obtaining maximum and stable absorbance as shown in Figure 5.

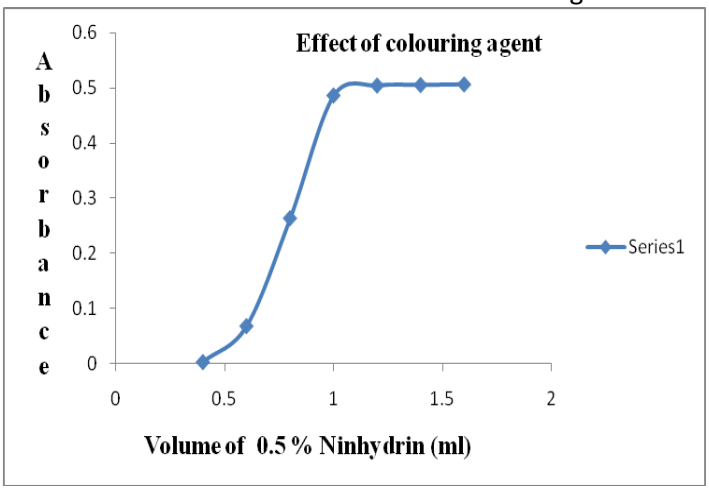
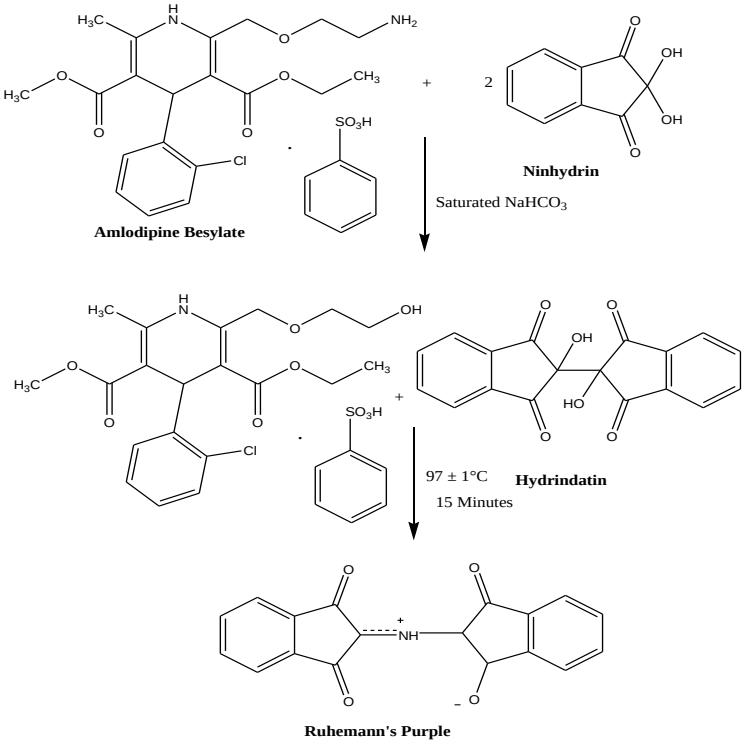


Figure 6: Effect of volume of 0.5% ninhydrin on the absorbance of reaction product

It was reported that in alkaline medium, ninhydrin is converted to ocarboxyphenyl glyoxal which would reduce ninhydrin to 2-hydroxyindan-1,3-dione. The primary amino group of Amlodipine besylate reacted with ninhydrin in presence of saturated $NaHCO_3$ solution (alkaline medium) to give diketohydrindylidene diketohydrindamine. Amlodipine besylate reacts with ninhydrin via oxidation deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored Ruhemann's purple without employing any organic solvent., which absorbs a maximum at 566 nm as shown in Figure 2. The proposed reaction between Amlodipine besylate and ninhydrin is shown in the Scheme I.



Scheme I: Formation of Ruhemann's purple complex between AM and ninhydrin.

To optimize the reaction conditions, different parameters have been investigated such as temperature, heating time, reagent concentration, and color stability. Reaction between ninhydrin and Amlodipine Besylate did not give any colored product in the absence of $NaHCO_3$, not even after prolonged heating. It was observed that complete colour development was attained at 97± 1°C. The optimum reaction time was determined by heating the reaction mixture on a water bath at 97± 1°C. It was noted that complete colour development was attained in five minutes. The effect of ninhydrin concentration on the colour development was investigated. 1 ml of 0.5% ninhydrin reagent produced maximum colour intensity.Interestingly, reaction was found to be specific in $NaHCO_3$ medium.

5.0. CONCLUSIONS

From the result and discussion it can be concluded that spectrophotometric method has found to be new, accurate, precise and economic for the determination of Amlodipine besylate. The proposed method shows better sensitivity. In comparison with the existing visible spectrophotometric methods for the quantification of Amlodipine besylate, the present modified method can be considered green as it demonstrates that visible spectrophotometry can be utilized without the usage of organic solvent. In addition, the proposed method employs an inexpensive instrument. Overall the proposed new and ecofriendly spectrophotometric method is

economical and suitable for quality control of Amlodipine besylate in bulk, fixed-dose combination tablets

6.0.ACKNOWLEDGEMENT

The authors are gratefully acknowledging the receipt of pure Amlodipine besylate as gift sample from Wockhardt Pharmaceuticals Pvt. Ltd.(Aurangabad, India). Authors express their gratitude to Yash Institute of Pharmacy, Aurangabad for providing the instrumental and chemicals facility.

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Conflict of Interest: None Declared

