Development of Colorimetric Method for the Quantitative Analysis of Amlodipine Besylate in Dosage Form Using 4-Dimethyleaminobenzaldehyde as Derivatizing Reagent

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Received 14 November 2019, Revised 02 May 2020, Accepted 20 June 2020

Abstract

The study is based on the determination of amlodipine besylate (AB) after derivatization with 4-dimethyleaminobenzaldehyde (DMAB) using UV/Visible spectrophotometer at pH 5 to yield derivative that is measured at λ max 399 nm. The calibration graph obtained was liner and fulfilled Beer Lambert’s law in the concentration range 0.5-25 µg/mL of (AB) and DMAB having coefficient of determination R² 0.9988 with RSD 0.93% and molar absorptivity 4.04 x 10³ mole⁻¹ cm⁻¹. Quantitative / analytical parameters such as pH, heating time, temperature were optimized. Reagent concentration /volume, interday and intraday studies were also carried out. There was no effect of various solvents and additives observed on the determination of AB in commercially available drugs. The method is stable, accurate, rapid and simple for the study of imine derivative of AB.

Keywords: Colorimetric method, Amlodipine besylate, 4-dimethyleaminobenzaldehyde, Derivatization

Introduction

A calcium channel blocker amlodipine besylate (AB) a family of dihydropyridine drug is highly specific for smooth arterial vascular muscle of heart. It is official drug for the cure of hypertension, abnormal and stable angina. Chemical name (4R, S)-3-ethyl 5-methyl 2-(2amino-ethoxy-methyl)-4-(2-chlorophenyl)-1, 4-dihydro6-methylypyridine-3, 5-dicarboxylate mono benzene sulphonate and is official in Extra Pharmacopoeia of Martindale (EPM) [1]. The impact of that medicine is controlled with marginal and coronary vasodilator properties. Estimation procedure mentioned for AB in European Pharmacopoeia (EP) defines the RP (Reverse Phase) HPLC (High Performance Liquid Chromatography) processes [2] for the study of drug in bulk and dosage formulation. The former methods of analysis is based on HPLC [3–8], RP HPLC [2, 9–11], HPTLC (High Performance Thin Layer Chromatography) [12–15], GC (Gas Chromatography) [16], GCMS (Gas Chromatography Mass Spectrometry) [17], LCTMS (Liquid Chromatography Tandem Mass Spectroscopy) [18] and fluorimetry [19] referred in the previous work. Spectrophotometry is an investigative toll which is commonly used in drug
determination due to its easy handling and economic benefits as a first add analysis. Spectrophotometry still goes to most commonly used analytical technique for qualitative and quantitative analysis of pharmaceutical formulations. It offers significant economic and experimental benefits over other technique. Two derivative spectrophotometric procedures were developed by Prasad and co-workers [20, 21] in mutual tablet preparation. The active content of drug in dosage form was studied by two different spectrophotometric procedures [22]. The primary procedure of determination is depend upon preparation of a complex of an ion pair with active content of medicine by the help of bromothymol sulfone phthalein, the complex was separated in chloroform and measured at $\lambda_{\text{max}}$ of 405 nm. The second procedure comprises the development of an oxidative coupling complex of medicine and hydrazone 3-methyl-2-benzoiazolinone HCl in ammonium ceric sulphate. The amlodipine charge transfer reaction with $\pi$ accepter such as 2,5 p-chloranilic acid and the other was tetrachloro-1,4 benzoquinone (chloranil) [23, 24] was utilized to analyse in pure and dosage form. Jain and Agarwal [25] have developed spectrophotometric procedures for concurrent investigation of lisinopril and amlodipine in dosage form based on diverse mode of assurance over extend 300-190 nm utilizing four diverse examining focuses at 213, 242, 271 and 300 nm. Recently Becker et. al., [26] have been proposed a procedure which is based on NIR attached with integrating sphere with combination of interval partial least squares calibration (iPLS) and synergical siPLS procedure for mutual analysis of three drugs valsartan, hydrochlorothiazide and amlodipine. Hassan et. al., [27] develop HPLC and CE (Capillary Electrophoresis) procedure for concurrent determination of atorvastatin and amlodipine in the existence of their acidic degradation products in dosage form. Therefore, there’s a necessity of a straight forward spectrophotometric procedure for the measure of AB. The process depending upon Schiff’s base reaction of primary amino group of amlodipine with aldehyde group of 4-dimethyleaminobenzaldehyde.

The general reaction is shown in scheme 1.

\[
\text{R}^- + \text{R}^\text{NH} \rightleftharpoons \text{R}^- + \text{R}^\text{NH_2} \rightleftharpoons \text{R}^- + \text{R}^\text{NH} + \text{H}_2\text{O}
\]

Scheme 1. A representative diagram for general reaction procedure of Schiff’s base reaction

Materials and Methods

Analytical reagents and chemicals

All the utilized reagents and chemicals were of analytical standard. The acetic acid, ethanol, hydrochloric acid, methanol, potassium chloride, sodium carbonate and sodium bicarbonate were purchased from Merck (Fair Lawn, NJ, USA). Pure AB was voluntarily donated by Bosch pharmaceuticals (PVT) LTD (Karachi, Pakistan), dimethyleaminobenzaldehyde (DMAB) and sodium acetate were purchased from EMD Chemicals (Gibbstown, NJ, USA).

Preparation of stock solutions

Buffer solution

The buffer solutions of pH 1-10 were prepared by utilizing pH 1-10 buffer solutions by using (0.1M) hydrochloric acid, (0.1M) kallium chloride, (0.1M) acetic acid, (0.1M) natrium acetate, natrium carbonate (saturated solution), (0.1M) natrium bicarbonate, (0.1M) ammonium chloride and (0.1M) ammonia solution.

Standard solution

The (1% w/v) stock solution of AB was prepared by dissolving 0.1 g in 10 mL of methanol. Then diluted it up to 0.01% by taking 0.1 mL of standard solution into 10 mL celebrated volumetric flask and the volume was adjusted with methanol. The solution of DMAB 3% was ready by dissolving 0.3 g dimethyleaminobenzaldehyde (DMAB) in 10 mL methanol.

Instrumentation

Perkin Elmer lambda 35 UV/Visible spectrometer (USA) and Thermo Orion 420A pH meter with glass electrode were used.
Procedure (A): analysis of amlodipine besylate standard

The methanolic solution (1-5 mL) containing AB (10-50 μg) was transferred to the 10 mL calibrated flask and then 2 mL DMAB 3% in methanol (w/v) was added followed by 1 mL acetate buffer pH 5. The contents of the flask were warmed at 95°C ± 1°C over water bath for 15 min. The temperature of contents of flask were maintained at room temperature and filled with methanol up to the mark. The λmax was observed at 399 nm compared with blank which was ready by same method without the addition of drug.

Process (B): analysis of amlodipine besylate in dosage form

Twenty tables were weighed precisely and pulverized. The amount of 25 mg of AB was stirred and shaken in 10 mL methanol and the solution was kept for 10 to 15 min. The content was filtered with Whatman filter paper no. 42 and washed with methanol and the volume was adjusted up to mark in 25 mL volumetric flask and finally the procedure for analysis of AB was applied as mentioned above.

Results and Discussion

Scheme 2 shows the reaction mechanism of derivatizing reagent (DMAB) with primary amino group of AB drug during reaction method and new imine derivative AB-DMAB was prepared.

![Scheme 2. Synthetic route for the derivatization of AB with DMAB as derivatizing reagent](image)

AB reacts with DMAB to form an imine derivative AB-DMAB which gives absorbance maximally (λmax) at 399 nm with molar absorptivity of 4.04×10^3 mole^(-1) cm^(-1). The DMAB was confirmed as a derivatizing reagent for the colorimetric analysis of AB. The particular parameters were optimized which impact on the preparation of AB-DMAB derivative likewise effect of pH, effect of reagent concentration, DMAB, temperature and time of heating.

Analytical parameters optimization

Selection of wavelength by using absorption spectra

Wavelength of maximum absorbance play vital role for quantitative analysis. It is very crucial to choose the wavelength where derivative gives optimal absorbance. The absorbance of 10 μg/mL of AB and DMAB derivative was measured within the range of 350-450 nm after heating at 95°C ± 1°C for 15 min using buffer pH 4. It is clear that the λ max was in visible range and at 399 nm against reference and was selected as optimal.

Selection of optimal temperature and heating time for the preparation of derivative

To reach the prime value of absorbance for an analyte the optimization of optimum heating time was measured at 399 nm for 0-30 min with an intermission of 5 min. A prime absorbance value was seen after heating for 15 min at 95°C ± 1°C and was considered as optimum (Fig.1).
Optimization volume and concentration of reagent

The influence of adding different volumes (0.5-3.5 mL) of DMAB (3% in methanol) with intermission of 0.5 mL on absorbance of 10 μg/mL of AB was examined. No change in increase of absorbance was observed after addition 2 mL of 3% reagent. Therefore, the addition of 2 mL 3% DMAB solution was considered as optimal (Fig. 2a and 2b).

![Figure 2](image)

**Figure 2.** Effect of volume of reagent on absorbance of derivative, (a) graphical representation between absorbance and volume and (b) UV-spectra of all readings at \( \lambda_{\text{max}} \) of 399 nm.

**pH effect on derivative**

At the optimum conditions, the effect of addition of 1 mL of 0.1 M buffer solution at pH range 1-10 was studied. A regular increase in absorbance was watched from buffer pH 1-5 and the most extreme absorbance was obtained at pH 5 (Fig. 3a). The addition of buffer pH 8-10 gives precipitation. The buffer pH 5 was taken as optimal. The UV-spectra was obtained at lambda max 397 nm (Fig. 3b).

![Figure 3a-b](image)

**Figure 3a-b.** Effect of pH on absorbance of derivative

**Effect of solvent**

The addition of various solvents such as propanol, isopropanol, butanol, n-hexane, acetonitrile, ethyleacetate, tetrahydrofuraran, water and acetone has no effect on absorbance. The study of solvent effects was checked by addition 1 mL and 2 mL of mentioned solvents and 2 mL of 3% methanolic solution of DMAB and 1 mL acetate buffer pH 5 then heated for 15 min at 95°C ± 1°C as shown in Table 1.

**Table 1.** Effect of different solvents on absorbance of derivative.

| Solvents     | Volume (mL) Added | Absorbance with methanol | Absorbance with other solvents |
|--------------|-------------------|--------------------------|-------------------------------|
| 1-propanol   | 2                 | 0.3021                   | 0.3020                        |
| Iso-propanol | 2                 | 0.3021                   | 0.3019                        |
| Butanol      | 2                 | 0.3021                   | 0.3017                        |
| n-hexane     | 2                 | 0.3021                   | 0.3020                        |
| Acetonitrile | 2                 | 0.3021                   | 0.3018                        |
| Ethyl acetate| 2                 | 0.3021                   | 0.3017                        |
| Tetrahydrofuran | 2           | 0.3021                   | 0.3021                        |
| Water        | 2                 | 0.3021                   | 0.1603                        |
| Acetone      | 2                 | 0.3021                   | 0.3016                        |
Effect of mixing order of reagents

The mixing sequence of reagent for the derivatization procedure plays an imperative part in improvement of absorbance and precision of result. Different orders of mixing were applied in the current work. It was noticed that the addition of 1 mL buffer pH 5 in the 25 μg/mL of AB followed by addition of 2 mL 3% DMAB results in decreasing in the absorbance value. Changing the sequence of mixing by taking DMAB first then add buffer followed by AB solution also has shown low rate of absorbance. The maximum absorbance resulted when 2 mL of reagent DMAB was mixed to the pure AB solution followed by addition of 1 mL buffer solution pH 5.

Excipient study

The influence of related additives likewise, glucose, PVP (polyvinylpyrrolidone) k-30, galactose, eurorget fructose, sucrose, polyethylene glycol (PEG-6000) and M-paraben P-paraben was investigated in same and ten times the amount of AB. It was seen that no one of the additive affected with any variation in absorbance of more than ±5% error (Table 2).

Percent recovery from dosage form

Table 3 shows the percent recovery of AB-DMAB derivative from five different pharmaceutical formulations by following mentioned procedure (B). The percent recovery was found more than 98 % in all selected formulations.

Stability of derivative

The stability of AB-DMAB derivative was verified in the terms of absorbance at the concentration of 10 μg/mL AB but no remarkable variation in the absorbance of more than 3% was detected within 48 h.

Calibration graph (Beer’s Law)

The impact of change in the concentration of AB on its absorbance was studied. A linear calibration curve was obtained which fulfilled the Beer’s law within the concentration range 5-25 μg/mL of AB with coefficient of determination R² 0.9988 (Fig. 4).
Day to day reproducibility /repeatability

For the stability of derivative, the assessment of interday and intraday repeatability of the procedure is an important parameter. The methanolic solution of AB 10 μg/mL was taken in three separate (10 mL) calibrated flasks and the method was applied as mentioned procedure (A). The mentioned method was repeated for three days (n=3). The average mean absorbance of intraday and interday reproducibility for imine derivative was seen as 0.3021 and 0.2790 with (RSD) values 0.93% and 1.16%, respectively (Table 4).

Table 4. Sensitivity comparison of proposed method for imine derivative with AB.

| Parameters                  | Imine derivative | Amlodipine drug |
|-----------------------------|------------------|-----------------|
| Precision (n=3)             | Inter-day        | 0.2790          | --              |
|                             | Intra-day        | 0.3021          | --              |
| Sensitivity (μg/mL⁻¹)       | Limit of detection (LOD) | 0.992              | 1.67          |
|                             | Limit of quantification (LOQ)| 3.31              | 3.67          |

Conclusion

The new developed method is modest, easy, linear, straightforward, repeatable and fast using low cost common laboratory chemical reagents. The developed procedure validated for the quantitative determination of amlodipine besylate drug. The amlodipine besylate drug was derivatized by 4-dimethylaminobenzaldehyde reagent with buffer (pH 5) on heating at 95°C for 15 min. The content of derivative absorbs in visible region at \( \lambda_{\text{max}} \) of 399 nm with bathochromic shift. The developed procedure avoids any interference with binding material which might absorb in UV region. The process was used for the assay of amlodipine besylate on commercially available products.

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