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Development of Spectrophotometric Method for Determination of Amlodipine by Picric Acid

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ABSTRACT

Development of UV-visible spectrophotometric technique for determining amlodipine using picric acid was proposed. This method is based on the formation of yellow complex which is obtained by the reaction between the primary amine group of amlodipine and picric acid and also absorbs the UV-visible light at 380 nm. Various parameters affecting on the reaction were carefully determined and optimized. The results suggest that the appropriate concentration of picric acid is 500 μg mL^{-1}. The suitable solvents used for dissolving amlodipine and picric acid are acetonitrile and dichloromethane, respectively. Moreover, the formation of amlodipine-picric complex is independent on the reaction time. The developed method shows linearity concentration of amlodipine in the range of 100 to 400 μg mL^{-1}. The percentage of repeatability and reproducibility are 0.38 and 3.36, respectively. In addition, the lower detection limit of amlodipine is 0.03 μg mL^{-1}. This proposed method can be applied to determination of amlodipine in commercial tablets with percentage recovery of 95 – 100.

Keywords: Amlodipine; Picric Acid; UV-visible Spectrophotometry

1. INTRODUCTION

Amlodipine or commercially named Norvasc, a widely used antihypertensive drug, is classified in the medicinal group namely Calcium Channel Blocker which can treat chest pain caused by decreased blood supply to the heart known as Angina pectoris [1]. Chemical name of amlodipine is 2-[(2-aminoethoxy)methyl]-4-(2-chloro-phenyl)-1,4-dihydro-6-methyl-3,5pyridine dicarboxylic acid, and 3-ethyl-5-methyl ester. The chemical structure is shown in Figure 1. Reactive mechanism of the drug is that the structure of amlodipine is converted at liver and released through urinary system. After taking the drug, amlodipine can retain in the blood for 30-50 hours, thus it can react with other drug and have side effects. Hence, the dosage used for adults is controlled to 5 – 10 mg per day and that for children depends on the discretion of doctor.

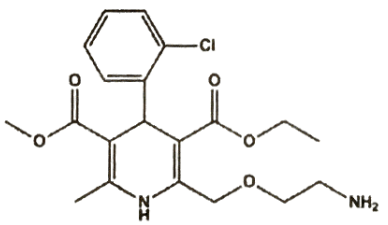

Figure 1 Chemical structure of amlodipine.

Currently, many spectrophotometric method such as HPLC with fluorescence detector [2], UV detection [3-5], voltammetry [6] and LC-MS [7] have been reported for amlodipine analysis but there is not standard method. Additionally, as

known that these instrumentation have requirement of high performance tools, high price, long time of sample preparation and analytical process as well as professional analysts, resulting in high cost. Thus, this present work has focused on method development for analysis of amlodipine using more rapid, simple and high sensitive UV-visible spectrophotometry. It is based on the formation of amlodipine based new complex which exhibits high intensity of absorption band.

There are many reports about the methodology of drug quantitative analysis using picric acid (Figure 2). Sher and coworker has developed the colorimetric visible spectrophotometric technique to determine amount of tranexamic acid and pregabalin by the reaction with picric acid 2,4-dinitrophenol, respectively. The yellow products show maximum absorption at 425 and 418 nm, respectively [8]. In addition, picric acid has been used as a reagent for analysis of gabapentin in a pure drug and capsules [9]. These reports indicate that picric acid is one of potential reagents used for analyzing various types of drug by spectrophotometry but there is no report for amlodipine.

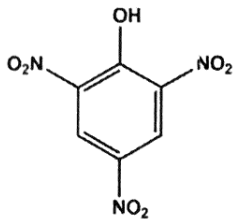

Figure 2 Chemical structure of picric acid.

Therefore, this present work has attempted to develop spectroscopic method to be simple, rapid and high sensitive to quantitate amlodipine based on formation of amlodipine complex with picric acid. Many parameters resulting to the reaction such as type of solvents, concentration of picric acid and suitable wavelength have been investigated. Finally, this improved method has been applied to analysis of amlodipine in commercial tablet drugs.

2. MATERIALS AND METHODS

2.1 Instrumentation

Detection of the UV-visible signals for all studied parameters were performed on a double beam JASCO V-650 UV-vis spectrophotometer with a high sensitive photomultiplier tube detector.

2.2 Chemicals reagents

All reagents were obtained from commercial sources. Amlodipine was purchased from Pharma. Picric Acid and solvents were obtained from Ajax Chemicals. D-glucose and vitamin C were purchased from APS Chemicals. Sucrose was obtained from Promaster. Starch and sodium chloride were purchased from Ajax Chemicals. The chemical reagents were analytical grade and used without further purification.

2.3 Standard solutions

2.3.1 Stock solution of amlodipine

Amlodipine (0.1000 g) was dissolved in methanol (1 mL), then adjusted the total volume to 100 mL by acetonitrile.

2.3.2 Solution of picric acid

Picric acid (0.0625 g) was dissolved by solvent (dichloromethane, chloroform, acetonitrile, acetone or methanol) and adjusted the total volume to 25 mL in volumetric flask.

2.3.3 Solutions of interference

The solutions of interference were prepared by dissolving starch, D-glucose,

sucrose, vitamin C or sodium chloride (0.0250 g) in acetonitrile. The total volume was adjusted to 25 mL by adding the same solvent into the volumetric flask.

All standard solutions were utilized to prepare sample solutions for studying the optimal conditions of amlodipine analysis.

2.4 Procedure

2.4.1 Method optimization

The suitable wavelength was investigated as described. Picric acid (2.00 mL) was added into the 10 mL volumetric flasks containing amlodipine solution (0.50–2.00 mL). The mixtures were diluted by acetonitrile to the total volume of 10 mL. Absorption band of each sample mixture was recorded on UV-visible spectrophotometer from 375 to 475 nm with acetonitrile used as a blank.

In addition, the effect of picric acid concentration was also determined by adding picric acid (2.0 mL) into 10 mL volumetric flasks containing amlodipine (0.50, 1.00, 1.50 and 2.00 mL). The total volume of mixture solutions were adjusted by acetonitrile to 10 mL and measured the absorbance at 380 nm. The experiments were repeated by changing the volume of picric acid solution to 0.8, 1.2, 1.6 and 2.4 mL.

Effect of solvents was further studied. Amlodipine or picric acid solutions (0.50, 1.00, 1.50 and 2.00 mL) were added into 10 mL volumetric flasks. The total volume was adjusted by addition of dichloromethane, acetone, acetonitrile, chloroform or methanol and measured the absorbance at 380 nm.

2.4.2 Determination of amlodipine in commercial drug samples

The calibration curve was initially constructed from the standard solution of amlodipine. Picric acid (2.00 mL) was added into each 10 mL volumetric flask containing standard amlodipine solution of 1.00, 2.00, 3.00 and 4.00 mL. The mixtures were diluted by adding acetonitrile to adjust the total volume to 10 mL. The absorbance was measured at 380 nm.

Twenty amlodipine tablets were finely powdered and weighed. An accurately weighed quantity of the mixed power containing an equivalent to 100 mg of amlodipine was dissolved in methanol (1 mL). The final volume was adjusted by adding acetonitrile to 100 mL. Drug solution (3.00 mL) was then added into 10 mL volumetric flask containing picric acid (2.00 mL) and diluted by acetonitrile to obtain the total volume of 10 mL. The absorption bands of all samples were recorded at 380 nm.

3. RESULTS AND DISCUSSION

3.1 Method optimization

For method optimization, a set of four standard amlodipine solutions was measured and the slope of standard curve was determined at different studied parameters.

3.1.1 The appropriate wavelength

Amlodipine analysis is based on the complexation with picric acid. Fitting wavelength is firstly required to reduce the effect of interference and obtain high absorption intensity of the complex. When the solution of picric acid was mixed with the solution of amlodipine in acetonitrile, a yellow product was formed. Absorption spectra of product were scanned from 375 to 475 nm. Upon increasing amlodipine concentration from 50 to 200 $\mu g\ mL^{-1}$, an enhancement of absorption intensity was observed with the maximum absorption at 380 nm (Figure 3). It corresponds to charge transfer electron transitions (CT) which have absorption in near visible region [10].

The possible reaction mechanism of picric acid and amlodipine is shown in Figure 4. It is based on the proton transfer from the hydroxyl group of Lewis acid picric acid to the primary amino group of the Lewis base, amlodipine, resulting in the intensely yellow colored complexes which much more deeply colored than free picric acid when solvated by suitable organic solvents [11].

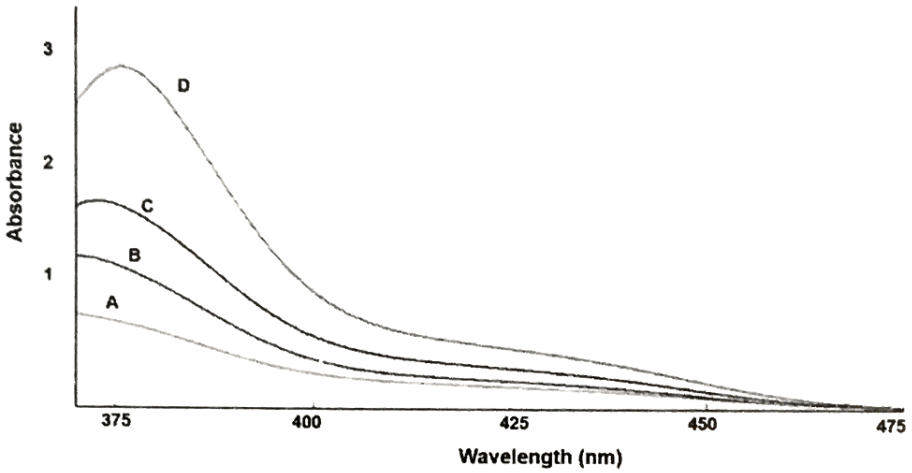

Figure 3 Absorption spectra of yellow product from reaction of amlodipine (50 (A), 100 (B), 150 (C) and 200 μg mL^{-1} (D) and picric acid (500 μg mL^{-1}).

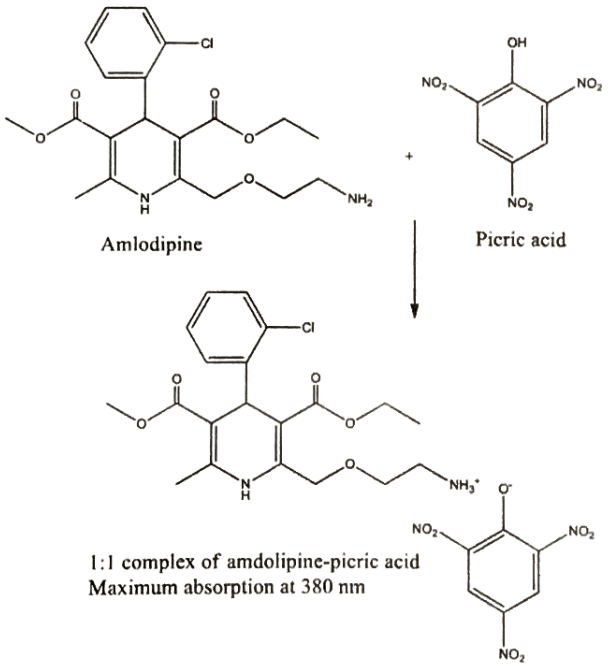

Figure 4 The possible reaction mechanism of amlodipine and picric acid.

Additionally, there is further decision from sensitivity and linear regressive values (R^2). Figure 5 shows the highest sensitivity at 380 nm, confirming that the wavelength of 380 nm is suitable for further studies.

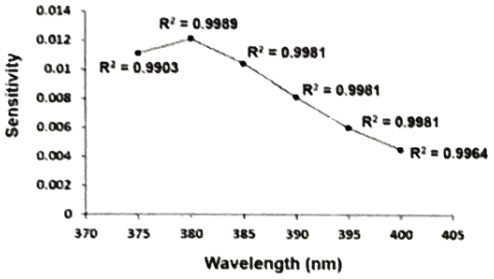

Figure 5 Effect of varying wavelengths on sensitivity for amlodipine determination.

3.1.2 Effect of picric acid concentration

Effect of various concentrations of picric acid were examined to obtain the suitable concentration for amlodipine analysis.

When the standard solutions of amlodipine were mixed by different concentration of picric acid (200, 300, 400, 500 and 600 μg mL^{-1}) and measured absorbance at 380 nm, the amount of picric acid affected on absorption of the complexes. The data was analyzed by linear relation of absorbance and concentration of amlodipine. The correlation of slope reflecting to sensitivity and concentrations of picric acid were plotted and inserted with linear regressive values (R^2) (Figure 6). It was observed increasing in tendency of sensitivity from 200 to 400 μg mL^{-1} and decreasing to 600 μg mL^{-1}. This evidence indicates effect of picric acid concentration in which at too low concentration, the absorptivity of amlodipine-picric acid complex is low, but at too high concentrations, detection of the complexes would be interfered and lose sensitivity. Moreover, the sensitivity and R^2 values of 400 μg mL^{-1} and 500 μg mL^{-1} were further compared. At 400 μg mL^{-1}, it appears higher sensitivity but lower R^2 value than those of 500 μg mL^{-1}. Hence, picric acid concentration of 500 μg mL^{-1} would be appropriate concentration to apply for the next experiment.

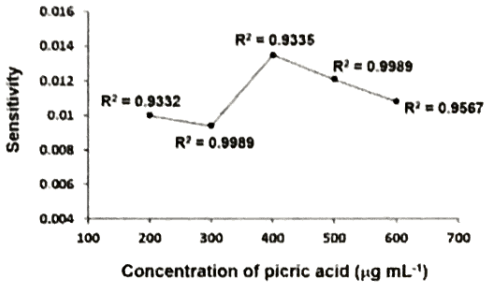

Figure 6 Effect of varying picric acid concentrations on sensitivity for amlodipine determination.

3.1.3 Effect of solvent

Several organic solvents including chloroform, acetonitrile, dichloromethane, acetone and methanol were investigated in order to choose an appropriate solvent for preparation of amlodipine and picric acid solutions, resulting to a good absorption signal of the complexes. For picric acid, it was found that dichloromethane and chloroform provided the slightly different sensitivity. Consideration from R^2 values, it revealed that dichloromethane (0.9989) was more suitable than chloroform (0.9970) to prepare picric acid solutions. In the case of amlodipine, dichloromethane presented the greatest sensitivity of 0.0137 but low R^2 value of 0.9606 which was too low for quantitative analysis. While acetonitrile gave lower sensitivity than dichloromethane (0.0103), its R^2 value was better (0.9818). As a result, acetonitrile was selected to use as a solvents for amlodipine.

3.1.3 Stability of amlodipine-picric acid complexes

The stability of amlodipine-picric acid complexes was examined by

quantifying absorption intensity upon time increase from 1 to 24 min after the reaction taken place. Figure 7 reveals that the absorbance of yellow complexes are most likely similar while time changes, meaning that the time does not affect on the stability of amlodipine-picric acid complex under this experimental conditions. However, in order to control kinetic effect, the absorbance of all samples were detected after reached the reaction for 1 min.

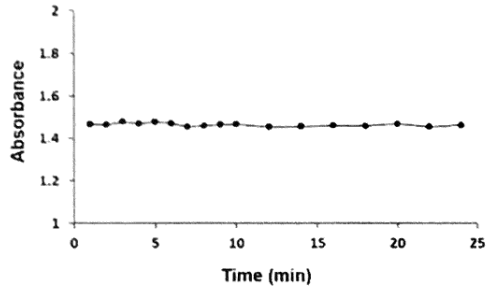

Figure 7 Effect of time on the stability of amlodipine-picric acid complex.

As results, it can be concluded the optimal condition for determination of amlodipine in Table 1.

Table 1 Summarization of the suitable conditions for the analysis of amlodipine by picric acid.

Parameters	Results
λ_{max}	380 nm
Picric acid Concentration	500 ppm
Solvent for picric acid	Dichloromethane
Solvent for amlodipine	Acetonitrile
Reaction time	1 min

3.2 Method validation

3.2.1 Linear range

According to the optimization of various parameters including wavelength, solvents, time and concentration of picric acid, the linear range of standard solution of amlodipine was further investigated with triplicate measurements. Calibration curve can be plotted by linear correlation of absorbance and the concentration of amlodipine (Figure 8). The results shows that the concentration of amlodipine which corresponds to Beer's law and in linear range from 100 to 400 μg mL^{-1} can be used for quantitative analysis of amlodipine.

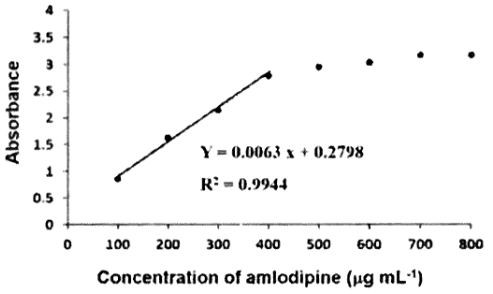

Figure 8 Linear range and calibration curve for amlodipine analysis.

3.2.2 Spectrophotometric parameters

After obtained the calibration curve, the spectrophotometric parameters were also studied and summarized in Table 2.

Reproducibility and repeatability shown by average absorbance value with standard deviation (SD) and percentage of relative standard deviation (%RSD) indicate that the improved analytical method has high precision with LOD of 0.03 μg mL^{-1} and can repeat sample analysis obtaining good results and low deviation.

3.2.3 Effect of interferences

Effect of many interferences was investigated by measuring absorption intensity of the complex (100 μg mL^{-1}) in the presence of selected interferences including starch, glucose, sucrose, vitamin C and sodium chloride (50-300 μg mL^{-1}). It is found that at lower concentration than 200 ppm gives percentage of deviation less than 5%, meaning that the interferences do

not affect to the analyze. In a contrast, at higher concentration than 300 ppm shows more than 5% deviation caused by the interferences.

Table 2 Parameters of spectrophotometric analysis

Parameters	Results
Beer's law limit	100-400 ppm
Linear equation	$y = 0.0063x + 0.2798$
R^2	0.9944
Reproducibility ($\overline{X} \pm$ SD)	1.4498 ± 0.0488 %RSD 3.36
Repeatability ($\overline{X} \pm$ SD)	1.5141± 0.0057 %RSD 0.3783
Limit of detection (LOD)	0.03 ppm

3.4 Determination of amlodipine in drug samples

The improved method was applied to quantitate amlodipine in drug samples A, B, and C by using the calibration curve constructed from the standard solutions of amlodipine as shown in Figure 7. As a result, amlodipine was found in drug samples A, B and C of 8.63, 4.45 and 8.73 mg/table, respectively. When compared with the labeled amounts of 10, 5 and 10 mg/table, respectively, by using T-test and F-test statistics, it shows 95% confidence level which can be acceptable and percent recovery of 95.1-99.9% indicates the efficiency of the developed method of amlodipine analysis.

4. CONCLUSION

The UV-visible spectrophotometric method has been developed for amlodipine analysis in drug samples by formation of yellow amlodipine-picric acid complex. According to the results of method optimization and method validation, it reveals that this method shows high sensitivity, good accuracy and precision and high percent recovery. Furthermore, it also is simple, rapid and efficient for quantitative determination and would be applied for analysis of drugs in the pharmaceutical laboratories.

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