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Utilization of oxidation-reduction reaction of Folin-Ciocalteu's phenol reagent in colorimetric determination of amlodipine in pharmaceutical dosage form

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ABSTRACT

A simple visible spectrophotometric method has been developed for determination of amlodipine in commercial tablets. Amlodipine (AMO) is 1,4-dihydropyridine derivative, this group when reacted with Folin-Ciocaluts phenol reagent in presence of 10% sodium carbonate, intense blue color was formed that exhibits maximum absorbance at wave length 745 nm. The intensity of color was found to be affected by many parameters. Optimization of the method parameters was done. The method was validated over a concentration range of 10 to 110 µg/mL. The values of each validation items were within the acceptable range for precision and accuracy. The lower limit for quantitation and lower of detection were founded to be 3.21 and 9.72 µg/mL, respectively. The proposed method was successfully applied to the quantitative determination of amlodipine in tablets without interference from tablet additives. The simplicity and accuracy of this method will allow many laboratories to determine the concentration of amlodipine in dosage form in easy way.

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1. Introduction

Amlodipine (3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl] -4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate, CAS: 88150-42-9) (Figure 1) is an example calcium channel blocker which is the drug of choice in treatment of mild-to-moderate essential hypertension [1]. Therapeutic action of AMO is due to inhibitions of calcium entry to vessels and heart muscle [2]. There are many methods published for determination of AMO in dosage form and in plasma. Most of these methods utilized high-performance liquid chromatography (HPLC) with UV, fluorescence and mass detections [3-14]. Some of these methods use spectrofluorometry [15,16]. Spectrophotometry has been used for determination of many drugs including AMO in pharmaceuticals preparations [17-22]. Electrochemical techniques also have been used [23-26]. On the other hand oxidation-reduction reactions have been applied to produce colored products could be measured colorimetry. Folin-Ciocalteu's phenol reagent was reported to be used in visible spectrophotometric determination of different drugs. Intense blue color was formed in alkaline medium due to oxidaton-reducion reaction between reagent and drugs [26-29].

Based on our search, there is no reported method used Folin-Ciocalteu's phenol reagent in determination of AMO. The aim of this work is to devolope a simple and senstive colorimetric method could be used in determination of amlodipine in pharmaceutical dosage form without need of sophisticated procedures. This method will be usefull in quick determination of amlodipine in dosage forms.

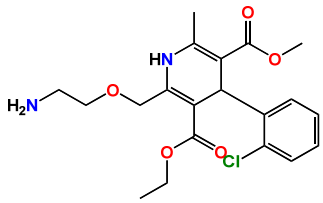

Figure 1. Chemical structure of amlodipine (AMO).

2. Experimental

2.1. Materials

2.1.1. Pure drug

Amlodipine Besylate was given as a gift by Egyptian International Pharmaceutical Industry Co. (EIPICO) (10th of

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Ramadan City, Sharqia, Egypt), with a purity of 99.9% according to the USP method [8].

2.1.2. Dosage from

Windipine 10 tablets labeled to contain 10 mg of amlodipine, produced by Sanofi Aventis, Egypt were purchased from the local market (Batch number 5EG014).

2.1.3. Chemicals and reagents

Mellli-Q water (PureLab Flex) was used throughout the entire procedures. Folin-Ciocalteu's phenol reagent 2.0 N was purchased from Sigma-Aldrich, Steinheim, Germany. Anhydrous sodium carbonate was purchased from El-Nasr Company, Egypt.

2.2. Instrumentation

Jasco V-630 UV-VIS spectrophotometer with Spectra Manager™ II software was used to measure the absorbance of the formed color.

2.3. Preparation of working solutions

Working standard solution of amlodipine was prepared directly by dissolving amlodipine in methanol to yield a concentration of 1 mg/mL and stored at -20 °C. Folin-Ciocalteu's phenol reagent (2.0 N) was used without dilution. Sodium carbonate (10%, *w*:*v*) was prepared by dissolving anhydrous sodium carbonate in water to yield 10% (*w*:*v*) aqueous solution and kept at 4 °C.

2.4. Preparation of calibration standards

Aliquots of the working solution (0.1-1.1 mL) of amlodipine were transferred to a series of 10 mL volumetric flasks followed by 0.7 mL of Folin-Ciocalteu's phenol reagent, and then 4 mL of 10% sodium carbonate solution was added. The reduction reaction was allowed to proceed for 15 min then the volume was completed to the mark with water then the absorbance was measured at λ = 745 nm in each standard against a reagent blank.

2.5. Application

For the preparations of pharmaceutical dosage form, ten tablets were crushed; powdered and homogenized then extracted with 50 mL methanol, filtered through a dry funnel and filter paper. The filtrate was then filtered through 0.2 µm Millipore filter, transferred to 100 mL volumetric flask and the volume was completed with methanol and stored at 4 °C. An aliquot (0.4 mL) was taken to yield a 40 µg/mL solution of the drug.

3. Results and discussion

3.1. Explanation of color formation

The presence of 1,4-dihyropyridine moiety in amlodipine is responsible for its reducing properties [30]. The reducing action of amlodipine on Folin-Ciocalteu's phenol reagent in alkaline medium generates a blue color that can be measured spectrophotometrically. The principle of this method depends on that the mixed acids in Folin-Ciocalteu's phenol reagent involve the following chemical species ($3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$ and $3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$).

Amlodipine affects reduction of 1, 2 or 3 oxygen atoms from tungstate and or molybdate in the reagent producing one or more of the possible reduced species which have characteristic intense blue color with a maximum absorbance at 745 nm and the 1,4-dihydropyridine moiety is converted to pyridine Figure 2.

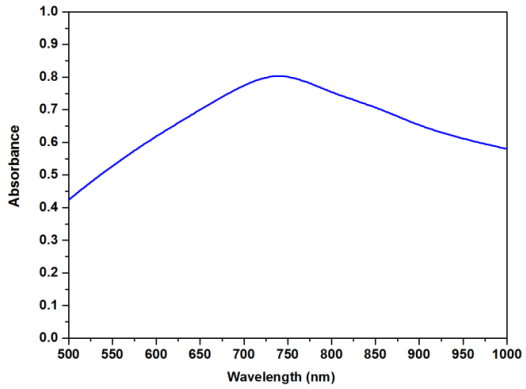

Figure 2. Absorption spectrum of the reduced Folin-Ciocalteu's phenol reagent using 100 ug/mL of amlodipine.

Different factors affecting the reaction were studied. These include volumes of Folin-Ciocalteu's phenol reagent and 10% sodium carbonate solution and reaction time. It was found that 0.7 mL of 2.0 N Folin-Ciocalteu's phenol reagent was sufficient for maximum absorbance 4 mL of 10% sodium carbonate solution was found to be the most suitable volume for maximum absorbance. The intensity of color reaches its maximum at room temperature after 15 minutes so it was selected as the optimal reaction time.

3.2. Method optimization

3.2.1. Effect of the volume of Folin-Ciocalteu's phenol reagent

The effect of volume of the Folin-Ciocalteu's phenol reagent was studied using different volumes of reagent. The results were showed in Figure 3. It showed that maximum absorption was obtained with 0.7 mL reagent.

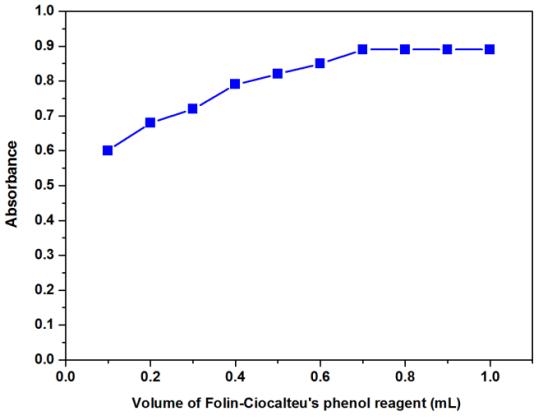

Figure 3. Effect of volume of Folin-Ciocalteu's phenol reagent on the absorbance of reduced species at λ = 745 nm.

3.2.2. Effect of volume of 10% sodium carbonate solution

Color formation was enhanced in alkaline pH, however, alkali hydroxide was found to precipitate reagent in the form of metal hydroxide. It was found that sodium carbonate solution provided the required alkalinity without precipitation of reagent. Different volumes of 10% sodium carbonate were used the result was illustrated in Figure 4. It was found that 4 mL 10% sodium carbonate produced maximum absorption.

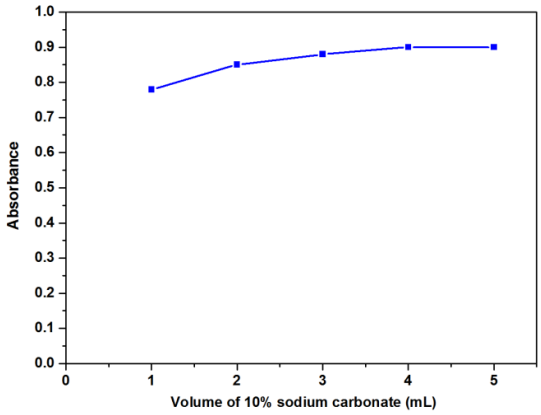

Figure 4. Effect of volume of 10% sodium carbonate on the absorbance of reduced Folin-Ciocalteu's phenol reagent on the absorbance of reduced species at λ = 745 nm.

3.2.3. Effect of time for reaction

In order to determine the time required to obtain full color formation, different waiting times after addition of reagent and 10% sodium carbonate to the drug were used. The result was illustrated in Figure 5. It was found that blue colored product exhibited maximum absorption after 15 min at λ = 745 nm.

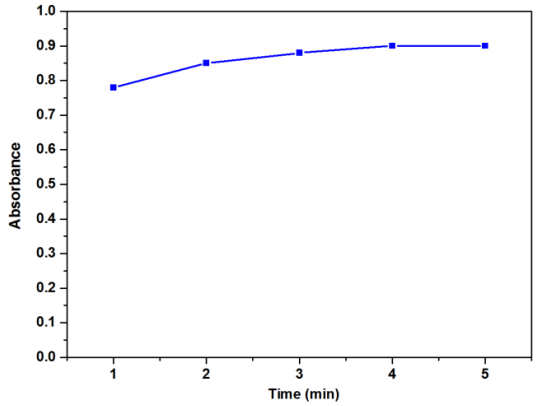

Figure 5. Effect of time on the reduction of Folin-Ciocalteu's phenol reagent on the by amlodipine.

3.3. Method validation

3.3.1. Linearity and range

Under the optimized experimental conditions, the method has been validated for linearity, limits of detection and quantization, accuracy and precision according to ICH guidelines [31]. From the resulting peaks of calibration, the absorbance values were plotted versus the corresponding concentrations (µg/mL) and the calibration graph was obtained Figure 6. Calibration graph was linear over the range of 10-110 µg/mL. The coefficient of determination (r^2) was 0.999. The calibration graph had a reliable reproducibility across the calibration range. The following regression equation (1) was computed.

$$y = 0.00975x - 0.0609 \qquad r = 0.999 \qquad (1)$$

where x is the concentration in µg/mL, y is the absorbance and r is the regression coefficient. Based on residual standard deviation of the regression line, the limit of detection (LOD) and limit for quantitation (LOQ) were calculated and found to be 3.21 and 9.72 µg/mL, respectively. Summary of these results was in Table 1.

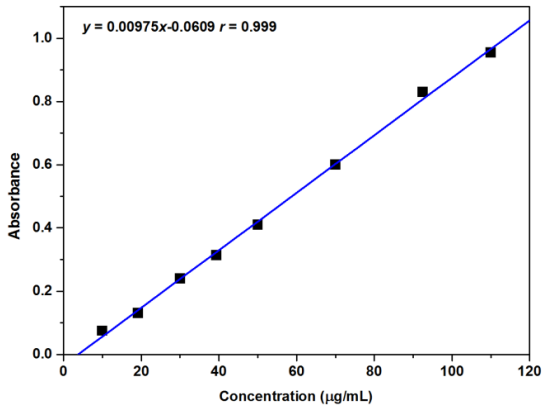

Figure 6. Linearity of the reaction between Folin-Ciocalteu's phenol reagent and amlodipine.

Table 1. Linearity data of amlodipine by the proposed redox spectrophotometric method.

Parameters	Value
Linearity range (µg/mL)	10-110
Slope	0.010
Intercept	-0.061
Correlation coefficient	0.999
r^2	0.999
S.E. of slope	1.025×10^{-4}
Confidence limit of slope	$0.010\pm2.51\times10^{-4}$
S.E. of intercept	6.339×10^{-3}
Confidence limit of intercept	-0.061 ± 0.016
Residual S.D. ($S_{y/x}$)	9.477×10^{-3}

3.3.2. LOD and LOQ

Limit of detection is the lowest concentration of analyte that can be readily detected, while limit for quantitation is the lowest concentration of analyte that can be quantified by the method [32,33]. Based on the residual standard deviation of the regression line, the LOD and LOQ were calculated and found to be 3.21 and 9.72 µg/mL, respectively.

3.3.3. Accuracy and precision

Evaluation of the accuracy was made by the analysis of five concentrations of the working standard solution within the linearity range of the drug, each three times. The results of the proposed method were compared with those obtained from the official method [8].

The method was found to have acceptable accuracy (Table 2). Statistical comparison of the performance of the proposed method with that of the official method showed that there was no significant difference in their accuracy and precision as shown by the results of student's *t*-test and variance ratio F-test, respectively (Table 3). Intra-day and inter-day precision testing indicate that the repeatability of the proposed method is good as indicated by small values of %RSD (Table 4).

Table 1. Accuracy results for amlodipine by the proposed redox spectrophotometric method.

Concentration taken (µg/mL)	Recovery percentage (%) *	Mean±SD
15	99.08	100.36±1.40
35	101.95	
55	101.24	
75	98.72	
95	100.83	

* Average of three different determinations.

Table 3. Statistical comparison of the performance of the proposed method with that of the official method for the analysis of amlodipine in pure form *.

Item	Proposed	Official
Mean accuracy±SD	100.36±1.40	99.48±0.68
% RSD	1.39	0.68
% RSE	0.63	0.31
n	5	5
Variance	1.96	0.46
t-test (2.31)	1.27	
F-test (6.39)	4.19	

* Values in parenthesis represent the tabulated values of *t*- and F-test at $p = 0.05$.

Table 4. Results of testing the precision of the proposed redox spectro-photometric method for the analysis of amlodipine in pure form.

Intra-day precision (% RSD)	1.15
Inter-day precision (% RSD)	1.45

Table 5. Assay of amlodipine tablets by the proposed redox spectrophotometric method using standard addition technique.

Item	Taken concentration (µg/mL)	Added concentration (µg/mL)	Recovery percentage (%) *
	40	15	100.44
	40	25	100.47
	40	35	101.36
	40	45	97.98
	40	55	99.19
	40	65	101.60
Mean ± SD	96.13±0.51		100.17±1.37
% RSD	0.53		1.37
% RSE	0.30		0.56
Variance	0.26		1.88

* Average of three different determinations.

3.4. Application of the proposed method to pharmaceutical dosage form

The proposed redox spectrophotometric method was applied to the assay of amlodipine in Windipine 10 mg tablets to demonstrate its efficiency in the analysis amlodipine in pharmaceutical dosage form for quality control testing. The concentration of amlodipine was calculated using the regression equation that was obtained from the proposed method. To validate the application on pharmaceutical dosage form; standard addition technique was applied. The results were illustrated in Table 5.

4. Conclusion

As a conclusion we developed a simple and sensitive colorimetric method for determination AMO in pharmaceutical dosage form. This method could be used in routine analysis of AMO in bulk powder and pharmaceutical preparations and economically of the method are the main advantages of this method. The developed method can be use in routine analysis of ASN in bulk powder as well as applied in quality control laboratories for the routine analysis of the investigated drugs in raw materials, in pharmaceutical formulations.

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