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Research Paper

A spectrophotometric determination of amlodipine drug in pharmaceutical preparations using 2, 6-dihydroxybenzoic acid reagent

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ABSTRACT

Background: Amlodipine is a widely used antihypertensive drug that requires accurate analytical methods for its quantitative estimation in pharmaceutical formulations.
Objective: This study aimed to develop a simple, sensitive, and reliable colorimetric spectrophotometric method for estimating amlodipine in its pure form and pharmaceutical preparations.
Methods: The proposed method is based on the reaction of the secondary amine group of amlodipine with 2,6-dihydroxybenzoic acid in the presence of p-dimethylaminobenzaldehyde, producing a yellow-colored complex. The absorbance was measured at 402 nm.
Results: The method demonstrated linearity over a concentration range of 2–40 µg/mL, with a correlation coefficient of 0.9909 and molar absorptivity of 1.392×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$. The complex remained stable for 50 minutes, with a recovery of 100.596%. The limits of detection and quantification were 0.968 µg/mL and 3.228 µg/mL, respectively. The relative standard deviation was 4.32%.
Conclusion: The developed method offers a practical approach for the quantitative determination of amlodipine in pharmaceutical formulations.

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1. INTRODUCTION

The scientific name of Amlodipine (AM) is (±)-2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine. Its chemical formula is shown in Figure 1, and its molecular formula is The molecular formula of Amlodipine is C20H25ClN2O5, and its molecular weight is 408.879 g/mol. Amlodipine is a white powder that is slightly soluble in water and isopropanol, moderately soluble in ethanol, and freely soluble in methanol [1]. Amlodipine (AM) is an official drug in the United States Pharmacopeia (USP), the Indian Pharmacopoeia (IP), and the British Pharmacopoeia (BP) [2], [3] . Amlodipine (AM) belongs to the dihydropyridine class of calcium channel blockers, which is the most common class of these blockers. Amlodipine is a derivative of the 1,4-dihydropyridine compound, which acts on L-type calcium channels in peripheral arteries and lowers blood pressure by reducing total peripheral resistance. Amlodipine is used to treat hypertension and chronic stable angina (chest pain or discomfort, usually associated with activity or exertion, due to reduced blood flow through the coronary arteries to the heart muscle) [4].

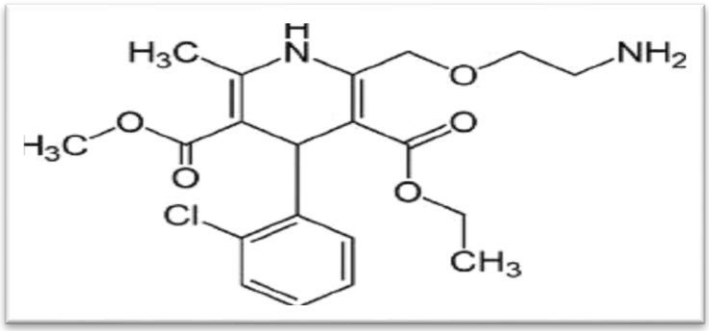


Figure 1. Chemical Formula of Amlodipine

There are several spectroscopic methods for the determination of Amlodipine [5], [6], [7], [8], [9], [10]. In addition to spectroscopic methods, several chromatographic techniques are also employed for the determination of Amlodipine [11], [12], [13], [14], [15].

2. RELATED WORK

The study [16] that used the simultaneous equation method (SEM) with the λmax values of 238 nm indicated the validity and accuracy of the method used, which is consistent with our current studs. Using various [17] analytical techniques in the pharmaceutical field to estimate the quality of active pharmaceutical ingredients and the amount of the drug in biological fluids and formulations. In our current study, we used a number of analytical methods to estimate amlodipine in its pure form and in pharmaceutical preparations. The methods used have proven to be highly accurate and consistent.

3. METHODOLOGY

3.1 Instruments Used

UV-Visible double beam (T92+Spectrophotometer Range (190-800) nm China). Heater HPL-248 China. Sensitive balance (Sartorius BL210 SAG Gottingen – Germany), Computer DEL, Windows 7, Uv probe 2.34 China.

3.2 Chemical Material

The analytical reagents and chemical compounds were used throughout the study are of high purity (Samara IRAQ-SDI, FLUK).

3.3 Solutions, Chemical Materials and Reagents Used

3.3.1 Amlodipine Pharmaceutical Preparation ((1000 µg/ml)

This solution is prepared by dissolving 0.1000 g of pure amlodipine powder (from the General Company for Drug and Medical Supplies, Samarra_ Iraq) in 5ml of ethanol.

3.3.2 The Reagent Solution is 2, 6 di Hydroxy Benzoic Acid (3×10^{-2}M)

0.462 g of the above reagent powder was weighed and dissolved in 5ml of ethanol. The dissolution was complete, and then the volume was made up to the mark in a100ml volumetric flask with distilled water.

3.3.3 Para-dimethylaminobenzene Aldehyde Reagent Solution ($3X10^{-2}$ M)

Prepare this solution by dissolving (0.4470 mg) of the above reagent powder, then add it a little in (10 ml) of ethanol and the dissolution was complete, then complete the volume to one mark in a 100volume bottle with distilled water.

3.3.4 Hydrochloric Acid Solution (1 M)

Prepare by adding 8.47 ml of hydrochloric acid solution (1.8 ml) to a small amount of distilled water in a volumetric bottle (100 ml), then complete the volume related to the distilled water.

3.3.5 Pharmaceutical Preparations Solution (Amlodipine 250 µg/ml solution)

10 tablets of the preparation were ground, and each tablet contains 10 mg of the drug. The weight of 10 tablets was 1.824g, and 0.1 g was taken and dissolved in a quantity of ethanol, and the solution was filtered, and the residue was washed several times with ethanol. Then, 25ml of the prepared solution was taken in a100 ml volumetric flask, and the volume was made up to the mark with distilled water to obtain a solution with a concentration of 250 µg/ml.

4. RESULTS AND DISCUSSION

4.1 General Principle of the Method

Upon addition of a volume of 2,6-dihydroxybenzoic acid (DHBA) solution to an Amlodipine solution, followed by the addition of a para-dimethylaminobenzaldehyde (PDAB) solution, a yellow-colored complex (Mannich complex) is formed. This complex exhibits absorption at a wavelength of 402 nanometers (nm) compared to its blank solution. Relationship between the Drug and Reagent in the Presence of Benzaldehyde.

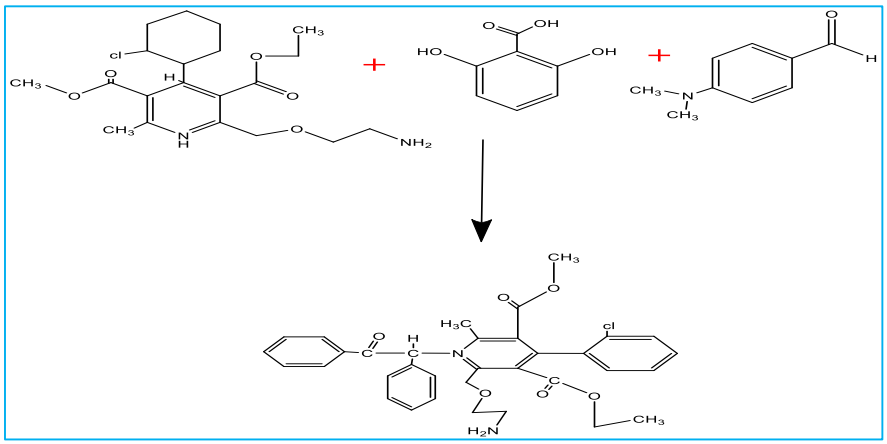


Figure 2. The Proposed Chemical Equation for the Reaction

4.2 Study of Optimal Reaction Conditions

The impact of different variables on retention was concentrated on absorption 3 mL of amlodipine arrangement of focus (250µg/ml) in a last volume of 25 mL (last concentration30µg/ml). The absorbance of the arrangements was estimated at various wavelengths against their blank solution.

4.3 Selection the Best Aldehyde

2 mL of various aldehyde arrangements with shifting focuses (3x10-2M) were taken, alongside 3 mL of amlodipine arrangement with fixation (250µg/ml), and 2 mL of arrangement 2,6-dihydroxybenzoic corrosive with conentration (3x10-2M). The outcomes are kept in the Table 1.

Table 1. Selection the Best Aldehyde

Aldyhyde	Λ_{max}(nm)	Absorbance
P_di methyl amino benzaldyhde	402	0.409
P_chloro benzaldyhyd	400	0.190
P_Bromo benzaldyhde	400	0.218

4.4 Effect of the Volume of Para- Dimethyl amino benzaldehyde Solution

The effect of the volume of para-dimethyl amino benzaldehyde (PDAB) solution was studied by taking different volumes of the aldehyde solution from (0.5-3.5) mL with 3 mL of amlodipine solution and in the presence of 2 mL of 2, 6-dihydroxybenzoic acid reagent solution, in a final volume of 25 mL. The absorbance of the solutions was then measured at a wavelength of 402 nanometers against a blank solution, and the results are tabulated in the Table 2.

Table 2. Effect of the Volume of Para- Dimethylaminobenzaldehyde Solution

Amount(ml)of aldyhyde	Absorbanc
0.5	0.163
1	0.184
1.5	0.321
2	**0.415**
2.5	0.340
3	0.315
3.5	0.213

4.5 Selection of the best Amino Reagent

2 mL of the amino reagent arrangements with a centralization of (3x10-2M), 3 mL of amlodipine arrangement with a grouping of (250µg/ml), and 2 mL of para-dimethylaminobenzaldehyde arrangement with a convergence of (3x10-2M) were taken, and the outcomes are displayed in the Table 3.

Table 3. Selection of the Best Amino Reagent

Regent(3x10^{-2}M)	Λ_{max}	Absorbance
2,6-dihydroxybenzoic acid	402	0.419
Benzidinen	418	0.230
P_Bromo aniline	403	0.127
M_amino benzoic acid	423	0.102
Sulfonilic acid	422	0.252

4.6 Effect of the Volume of the 2, 6 Dihydroxy Benzoic Acid Reagent

Was studied by taking different volumes of the reagent arrangement with a centralization of (3x10-2M) beginning from (0.5-3.5) mL, with 3 mL of the medication amlodipine arrangement and adding 2 mL of p-dimethylaminobenzaldehyde answer for a last volume of 25 mL. The absorbance was then estimated

at a frequency of 402 nanometers against the clear arrangement, and the outcomes are displayed in the Table 4.

Table 4. Impact of the Volume of the 2, 6 Dihydroxy Benzoic Corrosive Reagent

Amount(ml) of Regent $3x10^{-2}M$	Absorbance
0.5	0.298
1	0.361
1.5	0.387
2	0.422
2.5	0.400
3	0.366
3.5	0.260

4.7 Effect of Acid and Base

In this study, acids and bases were used, but their effect on the reaction was negative, so they were excluded.

4.7.1 Effect of Standing Time on the Formation of (Mankh complex)

The time expected for the response to be finished was concentrated on by taking a progression of 25 mL volumetric jars containing 3 mL of amlodipine arrangement with a convergence of (250µg/ml), to which 2 mL of 2, 6 dihydroxy benzoic corrosive arrangement with a grouping of (3x10-2M) and 2 mL of p-dimethylaminobenzaldehyde arrangement with a centralization of (3x10-2M) were added. The arrangements were left for various time spans, then weakened with refined water to the imprint in 25 mL volumetric jars, and the absorbance was estimated at a frequency of 402 nanometers against the clear arrangement. The outcomes are displayed in the Table 5.

Table 5. Effect the Time

Time/mint	2	5	10	15	20
Absorbance	0.391	0.438	0.330	0.334	0.317

4.7.2 Effect of Time on the Stability of the Formed

This study was done by taking 3 mL of amlodipine arrangement addressing a centralization of (30 µg/mL), adding 2 mL of the 2, 6 dihydroxy benzoic corrosive reagent, then, at that point, adding 2 mL of the p-dimethylaminobenzaldehyde arrangement in a 25 mL volumetric flagon, sitting tight for five minutes, and afterward weakening with refined water to the imprint. From the outcomes kept in the Table 6, it is seen that the absorbance esteem stays stable for no less than 50 minutes.

Table 6. Effect of Time on the Stability

Time /mint	Absorbance
5	0.432
10	0.429
15	0.427
20	0.426
25	0.422
30	0.417
35	0.414
40	0.410
45	0.409
50	0.400
55	0.388
60	0.372

4.7.3 Effect of Temperature on Complex Formation

The impact of temperature on the absorbance of the shaded item was concentrated on utilizing different temperature goes from 20 to 50 degrees Celsius. This was finished by adding 3 mL of the medication answer for 2 mL of the reagent arrangement, then, at that point, adding 2 mL of the p-dimethyl amino benzaldehyde arrangement in a 25 mL volumetric flagon. Following five minutes, the volume was left up to the imprint with refined water, and the absorbance of the shaded item was estimated as displayed in the Table 7.

Table 7. Effect of Temperature on Complex Formation

Temperature	Absorbance
20	0.433
25	0.419
30	0.390
35	0.340
40	0.284
45	0.260
50	0.252

4.7.4 Addition Sequence Effect

The grouping of adding the arrangements utilized in some cases affects the power of the subsequent hued complex. Consequently, various examinations were directed with various expansion arrangements, while keeping all volumes and groupings of the materials involved similar in all cases. The results acquired and kept in the Table 8 show that the best expansion succession, which gives the most noteworthy absorbance, is succession number one. This arrangement was utilized in the ensuing advances.

Table 8. Effect of Addition Sequence

NO	Order of Additions	Absorbance
1	D+R+A	0.436
2	A+D+R	0.319
3	R+A+D	0.330

4.8 The Final Absorption Spectrum

Subsequent to arriving at the ideal circumstances which are utilizing 3 ml of amlodipine arrangement with a focus (3x10-2M) and 2 ml of 2,6 dihydroxy benzoic corrosive reagent arrangement with a fixation (3x10-2M) and 2 ml of para-dimethylaminobenzaldehyde arrangement with a fixation (3x10-2M) at room temperature, and passing on the answer for 5 minutes to finish and balance out the response, the volume was finished to the imprint in a 25 ml volumetric flagon with refined water. Then, the last retention of the hued item (Manch complex) was estimated against its clear arrangement, and it was found to give the most elevated retention at a frequency of 402 nanometers, while its clear arrangement didn't give an observable retention around here, as displayed in the Figure 3. The accompanying Table 9 sums up the ideal circumstances for assessing the amlodipine drug.

Table 9. Optimal Conditions

Experimental Conditions	
Λ_{max}	402(nm)
Amount(ml) of 2,6 dihydroxy benzoic $3x10^{-2}M$	2ml
Amount(ml) of para-dimethylaminobenzaldehyde$3x10^{-2}M$	2ml
Solvent	water
Temperature°C	25°C

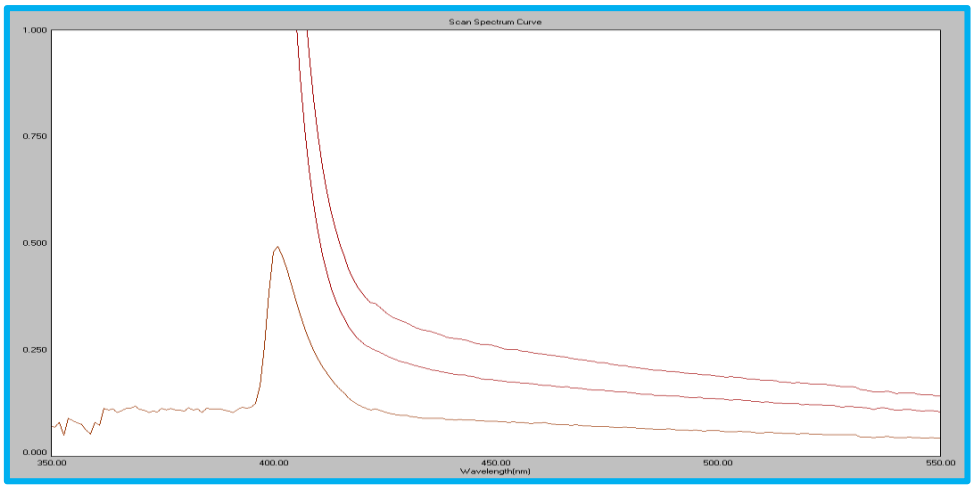


Figure 3. Final Absorption Spectrum from AMLO

4.9 Working Method and Calibration Curve

In a progression of volumetric carafes with a limit of 25 mL, different volumes (0.2 - 4 mL) of an amlodipine solution with a concentration of 250 µg/ml were taken. Then, 2 mL of a $3x10^{-2}$ M 2,6-dihydroxybenzoic acid solution and 2 mL of a $3x10^{-2}$ M p-dimethylaminobenzaldehyde solution were added. The solution was left for five minutes to allow the reaction to complete and stabilize. The volume was then made up to the mark with distilled water. The absorbance of the solutions was measured at a wavelength of 402 nm against a blank solution. The Figure 4 illustrates this process.

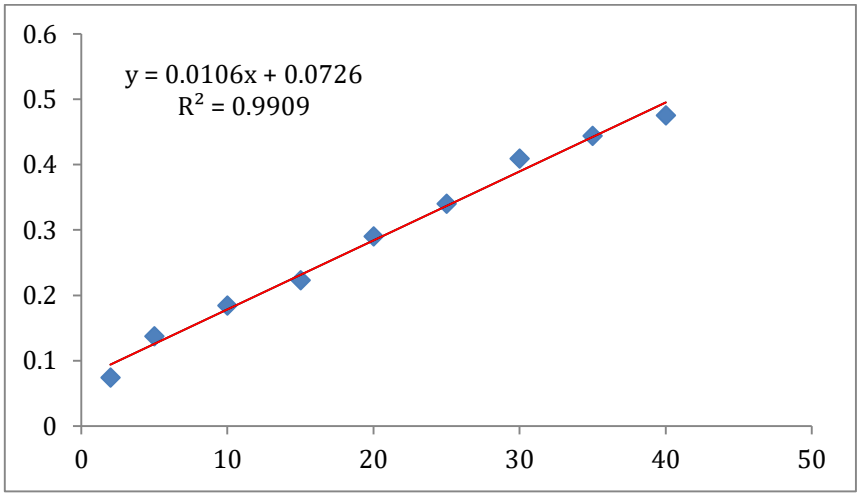


Figure 4. (AML- complex) Calibration Curve

4.10 The Nature of the Formed Product

The determination of the binding ratio between amlodipine and the 2, 6-dihydroxybenzoic acid reagent using two methods:

4.10.1 Continuous Variation Method (Job's Method)

In this method, different volumes of the drug solution ranging from 0.5 to 4.5 mL were placed in 25 mL volumetric flasks. To each of these volumes, 5 mL of the reagent solution was added, and the total volume was made up to the mark with distilled water. The absorbance of the solutions was measured at 402 nm. The plot Figure 5 shows that the binding ratio between the drug and the reagent is 1:1.

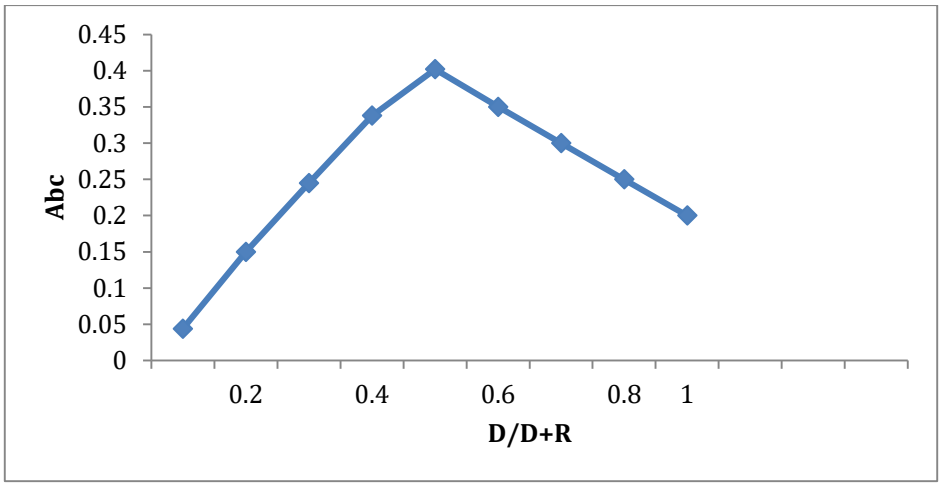


Figure 5. Job's Method of Continuous Variation of AML_2, 6 Di Hydroxy Benzoic Acid

4.10.2 Molar Ratio Method

This method was used to ensure that the molar ratio between amlodipine and 2,6 dihydroxy benzoic acid is 1:1. 3 mL of the medication arrangement was put in a progression of volumetric flagons of 25 mL limit, and various volumes of the reagent from (0.5-4.5) mL were added to it. The excess increments were made with the ideal volumes and weakened with refined water to the imprint, and the absorbance of these arrangements was estimated at a frequency of 402 nanometers against their clear arrangement. It was found that the molar proportion concurs with the constant variety technique, and the Figure 6 affirms that the proportion is 1:1 between the medication and the reagent

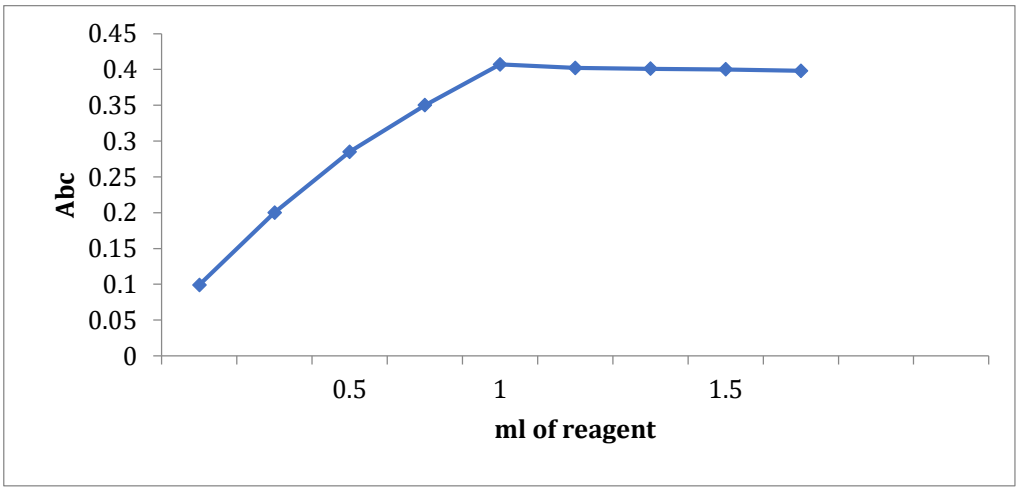


Figure 6. Mole_ Ratio Method of AML_2, 6 Di Hydroxy Benzoic Acid

4.11 The Precision and Accuracy of the Method

The precision and accuracy of the method are assessed by analyzing three different concentrations (2, 25, 40 µg/ml) with six replicates for each concentration. The relative standard deviation (RSD %) and relative error (RE %) are calculated, and the results are presented in Table 10.

Table 10. Accuracy and Precision

Amount (µg.ml-1)		RE%	Recovery%	Average	RSD %
Taken	measured				
2	2.0260	1.302	101.302	100.59	2.791
25	25.453	1.812	101.812		4.789
40	40.2632	0.6580	100.65		5.408

4.12 The detection limit (LOD) and quantification limit (LOQ)

It was possible to calculate the detection limit and quantitative limit for the estimation of the drug amlodipine at the wavelength of 402 nanometers by measuring the absorbance of the sample solution against distilled water, and 6 readings were taken under the same conditions. The results are tabulated in the Table 11.

Table 11. Detection Limit

Slope	T	SD	LOD µg/ml	LOQ µg/ml
0.0106	0.2483	0.003422	0.96849	3.22830

4.13 Discussions

The proposed method was applied to the pharmaceutical preparation containing amlodipine in the form of tablets as follows:

4.13.1 Direct Assessment of Amlodipine

The immediate strategy was concentrated on by taking three unique groupings of the drug planning arrangement (tablets) (2, 25, 40) µg/mL of 10 µg/mL focus and these arrangements were treated with similar advances continued in the alignment bend as per the ideal circumstances. The absorbance was estimated against the clear arrangement at a frequency of 402 nm. The normal of six readings for every focus was determined, and the relative blunder, recuperation, and results are displayed in Table 12.

Table 12. Standard Addition Method

Drug of Amlo taken	Con of Amlo found	%RE	%REC	Average
2	2.032	1.642	101.64	100.67
25	24.997	-0.001	99.9	
40	40.151	0.379	100.37	

4.13.2 The Standard Addition Method

For determining amlodipine in pharmaceutical preparations, to ensure that the proposed method is free from interferences, the standard additions were applied in the estimation of amlodipine in its pharmaceutical preparations. The method involves the addition of a fixed quantity (0.5 mL) of the prepared pharmaceutical preparation solution (concentration 250 µg/mL) to a series of 25 mL volumetric flasks to give a final concentration of 2 µg/mL, followed by the addition of increasing volumes (1, 2, 3, 4 mL) of the pure amlodipine standard solution (concentration 250 µg/mL). Flask number 5 was left without any addition and all the above arrangements were treated in a similar way as the alignment bend. The absorbance of six readings for every arrangement was estimated against the clear arrangement at a frequency of 402 nm, and the outcomes are displayed in the Figure 7.

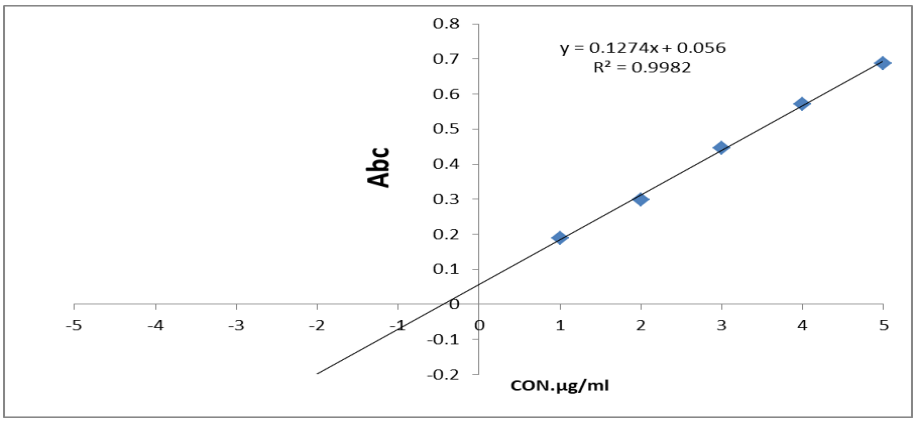


Figure 7. The Standard Addition Method

5. CONCLUSION

The proposed method is simple, accurate, precise and rapid. Therefore, this approach could be considered for the analysis of Amlodipine in the quality control laboratories. Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. Method is sufficiently sensitive to permit determination even down to 0.071 µg.ml-1. The sensitivity in terms of molar absorptivity and the precision in terms of RSD% of the method are very suitable for the determination of Amlodipine fumarate in pure and dosage forms. The commonly used additives such as starch, lactose, titanium dioxide and magnesium stearate do not interfere with the assay procedure.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Muhammad Fadil Hamad	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Shahla Jamal Shakur	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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