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METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF AMLODIPINE USING FOLIN-CIOCALTEU REAGENT BY UV-SPECTROSCOPY

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ABSTRACT

A simple, accurate, precise, and cost-effective UV-Visible spectrophotometric method was developed, validated for the determination of Amlodipine Besylate in pharmaceutical formulations using Folin–Ciocalteu (FC) reagent in accordance with ICH Q2(R1) guidelines. The method is based on the formation of a blue-colored chromogen resulting from the redox reaction between Amlodipine and FC reagent in alkaline medium, with maximum absorbance measured at 760 nm. Standard solutions were prepared in the concentration range of 2–20 µg/mL, exhibiting excellent linearity with a regression coefficient (R^2) of 0.9995. Accuracy of the method was evaluated by recovery studies at 80%, 100%, and 120% levels, with mean recoveries ranging from 99%, 103% and 104% indicating high reliability. Precision studies demonstrated low %RSD values of less than 1% for both intra-day and inter-day analysis, confirming good repeatability and intermediate precision. The method showed good specificity with no interference from common tablet excipients. Robustness testing revealed that small deliberate variations in experimental parameters did not significantly affect the results. The validated method was successfully applied to the assay of Amlodipine Besylate in tablet dosage forms, yielding results within acceptable limits. Therefore, the proposed method can be effectively employed for routine quality control analysis of Amlodipine Besylate in bulk and pharmaceutical formulations.

KEYWORDS: Amlodipine Besylate, UV- Spectroscopy, Folin- Ciocalteau reagent, Method development, validation, ICH Q2 (R1) guidelines.

INTRODUCTION

Amlodipine is long-acting calcium channel blocker widely prescribed for the treatment of hypertension and angina pectoris, Amlodipine besylate is used as an anti-hypertensive drug. It is a crystalline white powder; it is soluble in water and sparingly soluble in ethanol.[1] The chemical formula is C20H25ClN2O5, pKa value is 8.6. IUPAC name of amlodipine is 3-ethyl-5-methyl 2-(2-amino ethoxy-methyl)- 4-(2-chlorophenyl)-6-methyl-1, 4-dihydro pyridine-3,5- dicarboxylate.[2] Hypertension and angina are among the most prevalent cardiovascular disorders world wide, posing a significant burden on health care systems, amlodipine is a third-generation dihydropyridine calcium channel blocker, it is widely used in the management of these conditions due to its efficacy in lowering blood pressure and relieving angina symptoms.[3] It works by inhibiting the influx of calcium into vascular smooth muscle and cardiac myocytes, resulting in vasodilation and reduced cardiac workload.[4]

The drug pharmacokinetics including its long half- life and high bioavailability make it a cornerstone in chronic cardiovascular disease management ensuring quality, safety, efficacy of Amlodipine besylate.[5]

Among various analytical techniques, UV-Visible spectrophotometry is one of the most preferred methods in routine pharmaceutical analysis because of its simplicity, affordability, rapidness, and satisfactory sensitivity while various sophisticated methods such as

HPLC (High-Performance Liquid Chromatography) and LC-MS (Liquid Chromatography- Mass Spectrometry) exist, these techniques are often time consuming and expensive.[6]

Folin–Ciocalteu reagent (FC reagent) is a well-known oxidizing reagent commonly used for the determination of phenolic and reducing compounds. In an alkaline medium, Amlodipine reacts with FC reagent to form a blue-colored chromogen due to the reduction of phosphomolybdic-phosphotungstic acid complexes. The intensity of the developed color is directly proportional to the concentration of the drug and can be measured spectrophotometrically, typically around 760 nm.[7]

Method development involves optimization of experimental parameters such as reagent concentration, reaction time, wavelength selection, pH, and stability of the chromogen to obtain maximum absorbance and reproducibility.[8] Once developed, the method must be validated according to ICH Q2(R1) guidelines to ensure its reliability. Validation parameters include linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness.[9]

IUPAC name: 3-ethyl-5-methyl 2-(2-amino ethoxy-methyl)- 4-(2-chlorophenyl)-6-methyl-1, 4-dihydro pyridine-3,5- dicarboxylate.

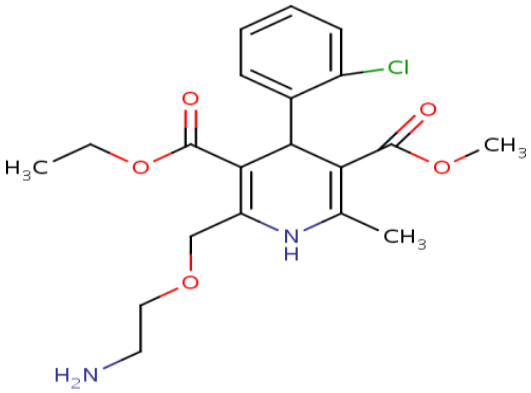


Fig no 1: Chemical structure of amlodipine.

The specific objectives of this study are to establish a linear calibration curve within an appropriate concentration range, evaluate the accuracy and precision of the method, and determine validation parameters such as limit of detection (LOD), limit of quantification (LOQ), specificity, robustness, and ruggedness. Additionally, the method is intended to be successfully applied for the assay of Amlodipine in pharmaceutical dosage forms, demonstrating its practical applicability in routine laboratory analysis.[10] The development of a new analytical method using Folin–Ciocalteu reagent for the determination of Amlodipine is necessary to provide a simple, economical, and sensitive alternative to existing analytical techniques.[11] Although chromatographic methods such as HPLC are widely used for the estimation of Amlodipine, they require expensive instrumentation, high maintenance cost, skilled personnel, and longer analysis time.[12] In contrast, a UV–Visible spectrophotometric method based on FC reagent offers a rapid and cost-effective approach that can be easily implemented in routine quality control laboratories, especially where advanced instrumentation is not readily available.[13]

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical reagent grade. Pure Amlodipine reference standard was obtained from (Aurabindo laboratories, Hyderabad) Folin–Ciocalteu reagent, sodium carbonate, and methanol were procured from standard chemical suppliers. Double distilled water was used throughout the study. All reagents were of high purity, ensuring minimal interference in the analysis, methanol is selected as the solvent due to its compatability with UV spectroscopy and its ability to dissolve Amlodipine Besylate efficiently.[14]

Instrumentation

UV–Visible Spectrophotometer (Model No: Analytical TS2080 Plus) equipped with 1 cm matched quartz cells was used for all absorbance measurements, it has a scanning range of 190–1100 nm, allowing detection of both ultraviolet and visible absorbance and enabling accurate determination of the Amlodipine-Folin–Ciocalteu chromogen.[15] Electronic Analytical Balance (Infra Digi-EI) was used for accurate weighing of the drug and reagents. Ultrasonicator (Kshitij 1834) was used for sonication and complete dissolution of the drug solution. Whatman filter paper (No. 41) was used for filtration of tablet sample solutions. All glassware used in the study, including volumetric flasks, pipettes, and beakers, were of Class A grade to ensure accuracy and precision.[16]

Fig no.2: UV-Visible double beam spectrophotometer.

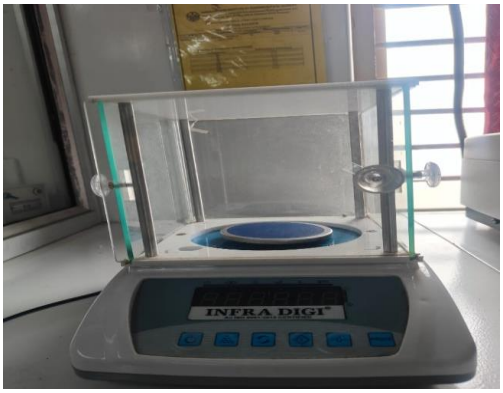


Fig no.3: Electronic balance.

Method Development

Accurately weigh 100mg of Amlodipine besylate transfer it into clean and dry 100ml volumetric flask, add about 50-60ml of methanol and sonicate for 10-15min after complete dissolution, make up the volume to the mark with the same solvent to obtain a stock solution containing 1000 µg/mL (1 mg/mL) of Amlodipine besylate. Pipette out 10ml of the stock solution from above flask, then transfer it into a 100ml volumetric flask, and use the same solvent to make up the volume. A drug's molecular weight, Pka value, solubility, and other characterstics all play a role in the appropriate technique selection. Using a UV- Visible spectrophotometer, the wavelength of maximum absorption (λmax) of a medication in the solvent solution at a concentration of 1mg/ml was scanned between the 200-400nm ranges. The amlodipine UV spectra were acquired using scanning at 200-400 nm. The λ_{max} was observed at 239nm.[17,18,19]

Determination of Absorption maxima of Amlodipine besylate solution

The amlodipine solution equivalent to 10 µg/ml was mixed with 2 ml of sodium hydroxide solution (Sodium hydroxide (2N) is prepared by dissolving 4g of NaoH in 100 ml distilled water) and 2 ml of Folin Ciocalteu reagent in 10 ml volumetric flask. After 15 mins the volume was made upto the mark with sodium hydroxide solution and it is mixed thoroughly. A blank solution was prepared in the same way in absence of amlodipine besylate. The solution is scanned in the range 400-800nm against reagent blank and Maximum absorption was observed at 760nm.[20]

About 10 tablets were weighed and crushed into fine powder. Powder weighed equivalent to 10 mg of Amlodipine was transferred into a 100 ml volumetric flask and made upto 100 ml with sodium hydroxide and thoroughly agitated for 5 minutes. The content was kept aside for 5 mins, and it is filtered. The filtrate solution was properly diluted with solvent to obtain a required concentration of drug used for the analysis.[21]

Construction of calibration curves

The calibration curve was plotted by taking concentration of the drugs in X-axis and absorbance in Y-axis. The calibration curves were constructed by taking absorbance data in six replicate experiments. The absorbance to concentration called relative response is calculated. Those points falling between 95% to 105% of the average relative response are only considered for construction of calibration.[22]

Validation Parameters

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the accuracy, linearity, precision, robustness of solution.[23]

Linearity

Different aliquots of the working standard of amlodipine solution ranging from 2-20 µg/ml was transferred into a series of volumetric flask and the total volume is made upto 10 ml with solvent. To each flask 2 ml of Folin Ciocalteu reagent solution was added by means of micro burette. The flask contents were mixed and kept at room temperature for 15 mins. The volume is made upto the mark with solvent and absorbance of each solution is measured at 760 nm against the blank.[24] The linearity data is shown in table no 1.

Precision

The reproducibility of the proposed method was determined by evaluating the mid concentration of the standard solution (8 µg/ml) at different time intervals so measure the six replicate absorbance measurements intra and interday precision, %RSD was calculated, the data is shown in table no 3.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out at three different levels (80%, 100%, 120%). The accuracy was performed by spiking 0.8 ml of standard solution(8µg/ml) with 14.4 ml, 16 ml, 17.6 ml of working standard solution. They were made-up to mark with solvent and samples are carried out in triplicates.[25] The accuracy data is shown in table no 7.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the formulae:

LOD = (3.3 × SD) / S LOQ = (10 × SD) / S.

Where, SD represents the standard deviation of the response and S is the slope of the calibration curve. The LOD indicates the smallest amount of Amlodipine Besylate that can be reliably detected, while the LOQ represents the smallest amount that can be quantified with acceptable precision and accuracy. The lower the LOD and LOQ values, the more sensitive the method is for detecting low concentrations of the drug.[26]

Robustness

The robustness of analytical procedure is the measure of its capacity to remain unaffected by small but deliberate

variations in method parameters and provides an indication of its reliability during normal usage.[27] The robustness of data was shown in table no 6.

RESULT AND DISCUSSION

The Folin-Ciocalteu (FC) reagent assay is a redox-based method for determining total phenolic content (TPC) and antioxidant capacity. Under alkaline conditions, phenolic compounds are oxidized to phenolate ions, which reduce the yellow-colored FC reagent a mixture of phosphotungstic and phosphomolybdic acids into a blue-colored molybdenum-tungsten complex.

In alkaline medium, amlodipine besylate reduces the heteropoly acids present in Folin-Ciocalteu reagent to form a blue-colored reduced complex.

Step 1: Ionization in Alkaline Medium

$$\text{Amlodipine–H} + \text{NaOH} \rightarrow \text{Amlodipine}^{-}\,\text{Na}^{+} + \text{H}_2\text{O}$$

Step 2: Redox Reaction

The Folin-Ciocalteu reagent contains phosphomolybdic acid and phosphotungstic acid.

In the original state, these metal ions are in a **high oxidation state (+6)** and the solution is **yellow.**

When a reducing agent (like amlodipine) is added in **alkaline medium (NaOH)**. The drug donates electrons, and the reagent gets **reduced**.

$$\text{Amlodipine (Red)} + 2[PMo_{12}O_{40}]^{3-} \rightarrow \text{Oxidized Amlodipine} + 2[PMo_{12}O_{40}]^{4-}\ \ Mo^{6+} \rightarrow Mo^{5+}$$

The oxidized form of amlodipine itself is colourless (or very pale yellow). After oxidation the oxidized amlodipine have no characteristic colour. The blue colour observed in the reaction is NOT due to oxidized amlodipine. The blue color is due to the reduction of the **Folin–Ciocalteu reagent**, where:

$$Mo^{6+} \rightarrow Mo^{5+}$$

This reduced molybdenum/tungsten complex produces the **intense blue chromogen** measured at 760 nm.

When Mo^{6+} is reduced to Mo^{5+}A **mixed-valence complex** forms. This creates a compound called **molybdenum blue.** It absorbs light in the visible region (~760 nm). Hence, the solution appears **intense blue in colour.** FC reagent produces blue colour because its molybdenum and tungsten ions are reduced from +6 to +5 oxidation state, forming a blue-coloured complex.

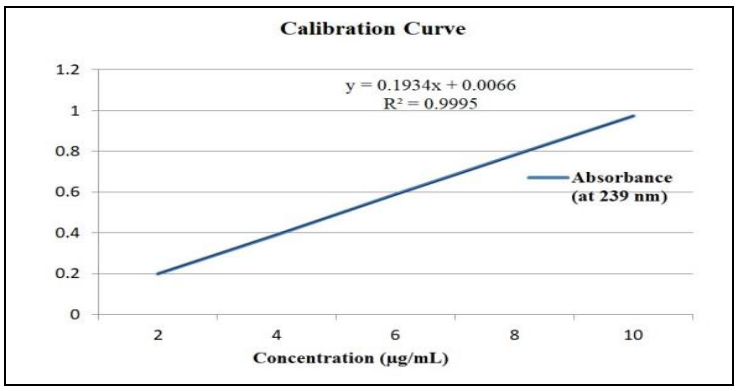


Figure 4: Calibration curve of Amlodipine besylate.

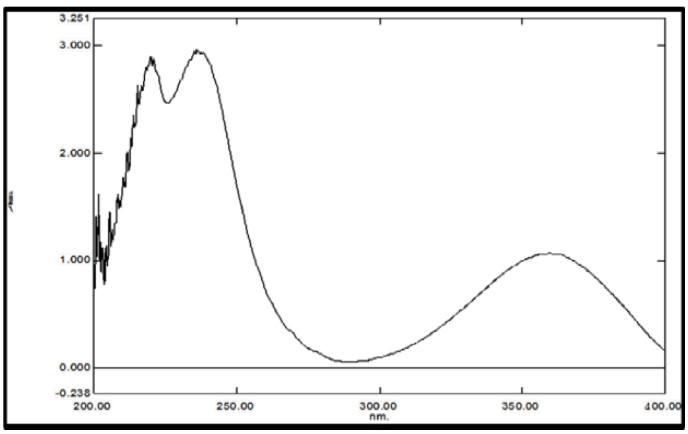
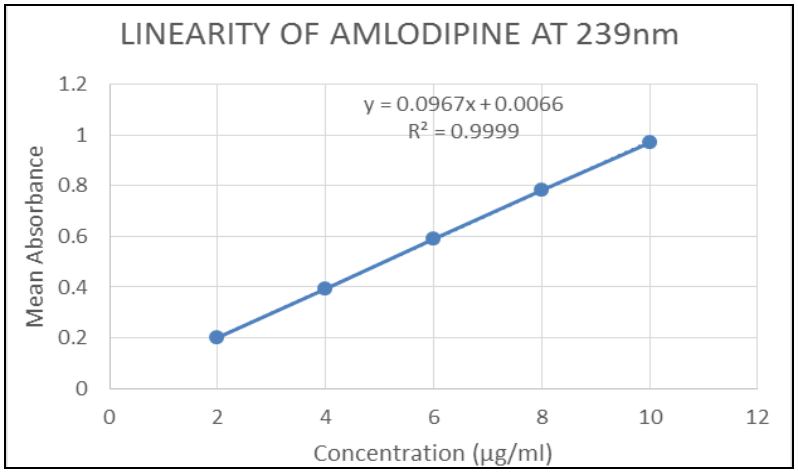


Figure 5: UV spectra of Amlodipine besylate at 200-400nm.

Table 1: Linearity data of Amlodipine besylate.

Sr. No.	Concentration (µg/mL)	Mean Absorbance (at 239 nm)
1	2	0.200
2	4	0.391
3	6	0.590
4	8	0.781
5	10	0.972



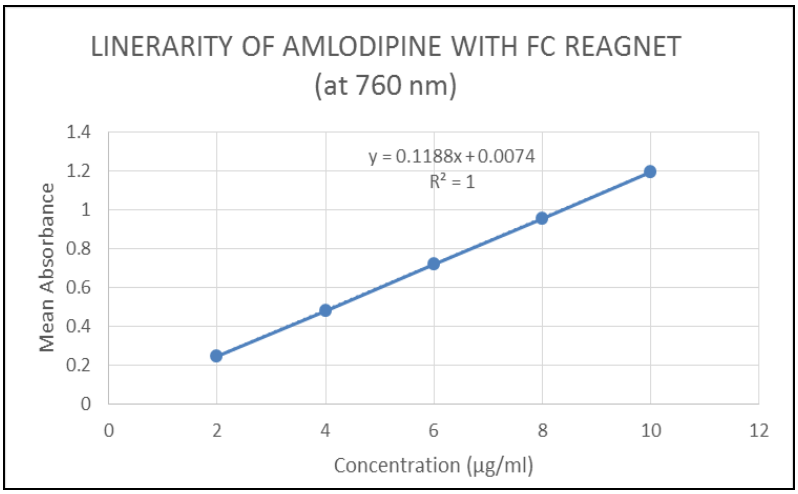


Table 1.1: Linearity data of Amlodipine besylate with FC reagent.

Sr. No.	Concentration (µg/mL)	Mean Absorbance (at 760 nm)
1	2	0.245
2	4	0.482
3	6	0.721
4	8	0.958
5	10	1.195

Table-2: Analytical Parameters for determination of drugs By Red-Ox reaction with Folin-Ciocalteu reagent.

S.no.	Optical characters	Results
1	λmax	760 nm
2	Beers law limit (µg/ml)	2-20 µg/ml
3	Slope	0.0453
4	Correlation Coefficient	0.9995

Table 3: Intraday Precision of Amlodipine.

Concentration (µg/ml)	Absorbance
8	0.364
8	0.365
8	0.366
8	0.366
8	0.367
8	0.368
Mean	0.366
Standard deviation	0.0016
%RSD	0.436

Table 4: Interday precision of Amlodipine.

8	0.366	0.367
8	0.367	0.368
8	0.366	0.369
Mean	0.366	0.368
Standard deviation	0.0006	0.0010
%RSD	0.164	0.272

Table 5: LOD and LOQ of Amlodipine.

Parameters	Amlodipine (µg/ml)
LOD	0.42
LOQ	1.26

Table 6: Robustness of Amlodipine.

S.no.	Wavelength	Absorbance
1	748 nm	0.347
2	750 nm	0.395
3	760 nm	0.424

Table 7: Accuracy of Amlodipine.

S.no.	Sample level (%)	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Mean	% Recovery
1	80 80 80	8 8 8	14.4 14.4 14.4	7.9 8.0 8.0	7.9	99%
2	100 100 100	8 8 8	16 16 16	8.2 8.3 8.4	8.3	103%
3	120 120 120	8 8 8	17.6 17.6 17.6	8 8 9	8.3	104%

Procedure involved in the Assay of Amlodipine

Weigh powder equivalent to 5mg of Amlodipine besylate and transfer to a 25ml pf volumetric flask add 20ml of methanol and sonicated for 15min. Make up the volume upto 25ml and mix, the transfer 5ml of above solution to a 25ml volumetric flask add F.C reagent, makeup the volume 25ml with 0.1N NaoH and mix, prepare blank in the same manner filter both the blank and drug solution using Whatmann filter paper and discard first few ml of filtrate. Measure the absorbance at 760nm.

Table 8: Assay of Amlodipine.

Label claim	Amount found	%Assay
Amlodipine besylate (5 mg)	4.98mg	99.60

% Assay is calculated using the formula

% Assay = Amount Found / label claim ×100

% Assay = 4.98/5.00 × 100

% Assay of Amlodipine= 0.996 × 100 = 99.60%

Values indicate that the method is accurate and precise, suitable for routine quality control of Amlodipine besylate.

The results of the method development and validation show that the amlodipine assay using Folin-Ciocalteu reagent is accurate, precise, and robust. The standard curve is linear over the range of concentrations tested, and the accuracy is within acceptable limits that is determined by %Recovery. The precision of the method is also within acceptable limits as determined by the %RSD that is less than 2. The LOD and LOQ values indicate that the method was sensitive.

CONCLUSION

A simple, selective, rapid and sensitive method has been proposed for the assay of drugs and in pharmaceutical formulations. The main objective of this study was to develop a method and validation using UV Spectroscopy for the drug Amlodipine shows absorbance at about 760nm, Assay formulations are conducted as per as ICH guidelines. The method is focused on the well-characterized and proven red-ox reaction and uses very simple, cheaper chemicals and easily accessible instruments. Other features like short performance time, ease of handling and the non-use of organic solvents also suggest this procedure as a routine laboratory method. The method is successfully applied to quantifying drugs in tablets and injecting them without intervention from common excipients. The current method is ideal for evaluation of Amlodipine besylate in bulk drugs and pharmaceuticals; therefore, this method can be used in laboratories for quality control.

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