INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Development And Validation of Rp-HPLC Method by Using Amlodipine Besylate

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ARTICLE INFO

Published: 24 May 2025
Keywords:
RP-HPLC, Amlodipine, Method Development, Validation, Anti-hypertensive Drug, Pharmaceutical Analysis
DOI:
10.5281/zenodo.15505690

ABSTRACT

A rapid, precise, and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of the anti-hypertensive drug Amlodipine. The method was optimized using a suitable mobile phase, column, flow rate, and detection wavelength to ensure effective separation and quantification. Various analytical parameters, including system suitability, linearity, accuracy, precision, robustness, and limit of detection (LOD) & limit of quantification (LOQ), were evaluated as per ICH guidelines. The method demonstrated excellent linearity over the specified concentration range, with high precision and accuracy. Robustness studies confirmed the method's reliability under small variations in chromatographic conditions. The developed RP-HPLC method was successfully applied for the routine analysis of Amlodipine in bulk and pharmaceutical dosage forms.

INTRODUCTION

Analytical chemistry is the study of the chemical composition of natural and artificial materials that deals with the separation, identification and determination of components in a sample. In other words, it is the art and science of determining what matter is and how much of it exists, which requires background knowledge of chemical and physical concepts. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. Analytical chemistry has been split into two main types,

Qualitative analysis- identifying functional groups or elements, or information regarding the presence or absence of one or more components of the sample taken.

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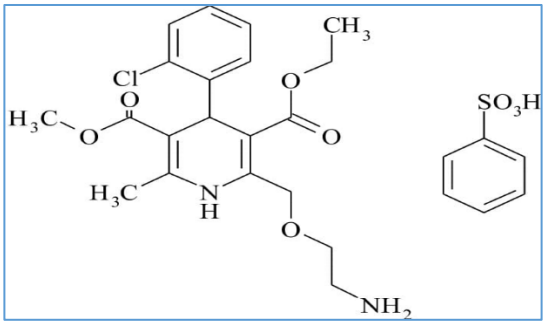
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Quantitative analysis- amount of analyte present in the sample such as mass or volume is determined using appropriate analytical method.

Drug Profile

Amlodipine (AMD), an anti-hypertensive drug belongs to the group of drugs called dihydropyridine calcium channel blockers. It is commonly used in the treatment of high blood pressure and angina. It also has antioxidant properties and ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure.

Amlodipine besylate is a white crystalline powder.



Structure of Amlodipine Besylate

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions primarily peripheral vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. The precise mechanisms for reducing angina is thought to be from: Reduces the total peripheral resistance (afterload) against which the heart works and reduces the rate pressure product, and thus myocardial oxygen demand, at any given level of exercise. Vasospastic angina by inhibiting constriction and restore blood flow in coronary arteries and arterioles in response to calcium, potassium epinephrine, serotonin, and thromboxane A2 analog in experimental animal models and in human coronary vessels in vitro.

MATERIALS AND METHODOLOGY

Materials used:

Table No.01:- List of chemicals and suppliers

Sr. No	Name of chemicals	Suppliers
1.	Amlodipine besylate	Chandra lab Pvt.Ltd.,Hyderabad

Instruments used:

Table No.02:- List of instruments and company

Sr. No	Instrument	Company
1.	UV	Unibloc model;Shimadzu
2.	HPLC	LC-2010;Shimadzu
3.	Analytical balance	LI-2702;Lasany

Chemicals used:

Water- HPLC grade, Methanol- HPLC grade, Acetonitrile- HPLC graded, Potassium dihydrogen phosphate- AR grade, Dipotassium hydrogen phosphate- AR grade, 0.1M sodium hydroxide- AR grade, Hydrochloric acid- AR grade, Ortho phosphoric acid- AR grade.

Active ingredient: Amlodipine besylate.

Methodology:-

1. Initialization of the instrument:

The column was placed on the instrument and switch on the instruments and washed in methanol: water (20:80) for 30 min. Then the system was made to run with the mobile phase for 30 min.

2. Preparation of mobile phase:

Filtered and degassed mixture of water: methanol in the ratio of 30:70 and filtered through 0.45 micron membrane filter.

3. Preparation of Buffer:

Taken 1.1818gms of potassium dihydrogen phosphate and 0.218gms of Dipotassium hydrogen phosphate was dissolved in 500ml of water and adjusted the pH-3 using Ortho- phosphoric acid.

4. Preparation of standard solution:

Taken 25mg of Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase. Transferred 0.5 ml from stock solution to 10 ml volumetric flask and made up to the volume with mobile phase.

5. Preparation of sample solution:

Weighed 10 tablets, calculated the average weight , powdered, weighed 500mg and transferred to 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase, transferred 0.5 ml to 10 ml volumetric flask and made up to the volume with mobile phase.

VALIDATION:

A. SYSTEM SUITABILITY

Verifies that the entire chromatographic system is functioning correctly and reliably before and during sample analysis.

I. Preparation of stock solution:

Taken 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase.

II. Dilution:

Transferred 0.5 ml from stock solution to 10 ml volumetric flask and made up to the volume with mobile phase.

B. SPECIFICITY

Check for interference from blank, diluents was used as blank.

i.Preparation of standard solution:

Taken 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase. Transferred 0.5 ml from stock solution to 10 ml volumetric flask and made up to the volume with mobile phase.

ii.Preparation of sample solution:

Weighed 500mg of powdered sample and transferred to 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase, transferred 0.5 ml to 10 ml volumetric flask and made up to the volume with mobile phase.

C. LINEARITY

i. Preparation of stock solution:

Taken 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase.

ii. Preparation of linearity solution-I:

Transferred 0.1ml from stock solution to 10 ml Volumetric flask and made up with mobile phase(the solution become 5µg Amlodepine besylate).

iii. Preparation of linearity solution-II:

Transferred 0.2ml from stock solution to 10 ml volumetric flask and made up with mobile phase(the solution become 10µg Amlodepine besylate).

iv. Preparation of linearity solution-III:

Transferred 0.3ml from stock solution to 10 ml volumetric flask and made up with mobile phase(the solution become 15µg Amlodepine besylate).

D. ACCURACY

i. **Preparation of stock solution**:

Taken 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase.

ii. Preparation of standard solution:

Transferred 0.5 ml from stock solution to 10ml volumetric flask made up with mobile phase.

iii. Preparation of Accuracy solution:

Transferred 0.4ml from stock solution to 10 ml volumetric flask and made up with mobile phase(the solution become 25µg Amlodepine besylate) and added 1ml of standard solution.

E. SYSTEM PRECISION

i. Preparation of standard solution:

Taken 15 mg of indapamide and 25 mg of Perindopril erbutmine and 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase. Transferred 0.5 ml from Stock solution to 10 ml volumetric flask and made up to the volume with mobile phase.

ii. Method precision:

Preparation of sample solution: Weighed 500mg of sample and transferred to 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase, transferred 0.5ml to 10 ml volumetric flask and made up to the volume with mobile phase.

iii. Intermediate precision (ruggedness):

The intermediate precision was performed by two analyst on different instrument and different day with the solution used for the method precision.

F. ROBUSTNESS

i. Preparation of stock solution:

Taken 25mg Amlodepine besylate in 50 ml volumetric flask. Dissolved in methanol and diluted to volume with mobile phase.

ii. Dilution:

Transferred 0.5 ml from stock solution to 10ml volumetric flask made up with mobile phase (the solution become 25µg Amlodepine besylate).

G. LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The LOD and LOQ of the drug were derived by visually or calculating the signal- noise ratio. In this method the LOD and LOQ of the drug were calculated by following equation.

LOD = 3.3×Standard deviation / Slope

LOQ = 10.×Standard deviation / Slope

RESULTS AND DISCUSSION

VALIDATION RESULTS

1. System suitability:

In HPLC, injected the solution prepared as per the procedure and recorded the peak response of the chromatogram.

Table No.03:- System suitability result for Amlodipine

S.No	Parameter	Amlodipine besylate
1	RT(min)	3.62
2	Tailing Factor	1.250
3	No.of theoretical plates	7394

Result :

On the evaluation of above results it was found that system suitability parameters were within the satisfactory limit.

2. Specificity:

Diluents ,standard preparation and assay were prepared as per the method and the solutions were injected into the HPLC and the chromatograms recorded. The retention time were given in the following table,

Table No.04:- Specificity result for Amlodipine

S. No	Solution	Retention Time(min)
1	Amlodepine besylate standard preparation	3.63
2	Amlodepine besylate assay preparation	3.62

Result :

No peaks should be detected at the retention time of Amlodepine besylate in the chromatograms of diluents preparation.

In HPLC,injected the each concentration of the solution prepared as per the method and the results were given in the following tables,

3.Linearity

Table No.05:- Linearity result for Amlodipine

S. No	Linearity Level	Concentration (µg)	Area (mV.s)
1	I	5	157.532
2	II	10	301.879
3	III	15	436.291
4	IV	20	552.655
5	V	25	666.779
6	VI	30	790.165
Correlation Coefficient			**0.998**

Results :

On evaluation of above results % RSD values are within the limit hence the curve shows linearity at concentration range 5µg/ml-30µg/ml for Amlodepine besylate.

3. Accuracy:

In HPLC, injected each concentration of the solution prepared as per the method and the results were given in the following tables,

Table No.06:- Accuracy result for Amlodipine

%Concentration (at specification Level)	Area	Amount Added (µg)	Amount Found (µg)	% Recovery	Mean Recovery
80%	637.1297	22.5	22.31	99.16%	
100%	777.3993	27.5	27.22	98.98%	99.21%
120%	883.5633	35.5	32.34	99.50%	

Results:

From the results shown in accuracy, it was found that the percentage recovery values pure drug from the pre analyzed solutions of formulations were in between 99.48% for Amlodepine besylate which indicates that the method was accurate.

4. Precision:

In HPLC,injected six replicate injection of the solution prepared as per the method and the results were given in the following table,

i.System Precision:

Table No.07:- System Precision result for Amlodipine

Injections	Area[mV.s]
1	685.131
2	680.701
3	676.947
4	679.709
5	680.747
6	679.988
Avg	680.5372
Std dev	2.647675
% RSD	0.40

iii. Method precision:

Table No.08:- Method Precision result for Amlodipine

Injections	Area[mV.s]
1	682.656
2	684.543
3	684.116
4	683.652
5	690.639
6	683.454
Avg	684.8433
Std dev	2.910083
%RSD	0.5

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

Result : From the results shown in precision, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility.

The robustness of the method established by making minor variations in the method parameters like, change in flow rate by ± 10 % of actual flow rate .

5. Robustness:

Table No.09:- Robustness result for Amlodipine

S. No	Flow Rate (ml/min)	10.9 USP Plate Count	USP Tailing
1	0.9	2804	1.6
2	1.0	7394	1.2

Result

The flow rate was varied at 1.0 ml/min to 1.2ml/minThe results are summarized On evaluation of the above results, it can be concluded that the variation in flow rate affected he method significantly. Hence it indicates that the method is not robust even by change in the flow rate ±10%.

6.Limit of detection:

Table no.10:- Limit of detection result

Drug Name	Standard Deviation	Slope	Result (µg/ml)
Amlodepine besylate	2.647675	24.996	0.95

7.Limit Of Quantification:

Table No.11:- Limit of quantification result

Drug Name	Standard Deviation	Slope	Result (µg/ml)
Amlodepine besylate	2.647675	24.996	1.05

CONCLUSION:

The development and validation of a robust RP-HPLC method for Amlodipine ensure accurate, precise, and reproducible quantification of the drug in pharmaceutical formulations. The optimized chromatographic conditions, including the choice of mobile phase, column, flow rate, and detection wavelength, contribute to the method's efficiency and reliability. Validation parameters such as linearity, accuracy, precision, specificity, and robustness confirm its suitability for routine quality control analysis. This method provides a cost-effective and time-efficient approach for the determination of Amlodipine, meeting regulatory requirements and industry standards. Further studies may explore its application in biological matrices for pharmacokinetic and bioavailability assessments.

FUTURE PROSPECTS:-

The future prospects of Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method are promising, driven by advancements in technology, increasing demands in various industries, and the need for more efficient, accurate, and environmentally friendly analytical techniques. Below are some key trends and prospects:

1. Technological Advancements

- **Ultra-High-Performance Liquid Chromatography (UHPLC):**

RP-HPLC is evolving toward UHPLC systems, which use smaller particle sizes (sub-2 µm) in columns and higher pressures. This leads to faster separations, improved resolution, and greater sensitivity, making it ideal for complex samples in pharmaceuticals, biotechnology, and food analysis.

- **Column Chemistry Innovations:** Development of novel stationary phases (e.g., hybrid silica, core-shell particles) will enhance selectivity, stability, and reproducibility, allowing RP-HPLC to tackle a broader range of analytes, including polar and biomolecules.

- **Automation and Robotics:** Automated method development systems integrated with artificial intelligence (AI) and machine learning (ML) will streamline optimization processes, reducing human error and time while predicting optimal conditions for separation.

2. Pharmaceutical and Biopharmaceutical Applications

- **Biologics and Biosimilars:** As the biopharmaceutical industry grows, RP-HPLC will play a critical role in characterizing large molecules like monoclonal antibodies, peptides, and oligonucleotides. Method validation will focus on ensuring robustness for these complex entities.

- **Quality-by-Design (QbD):** Regulatory agencies like the FDA and ICH emphasize QbD approaches. Future RP-HPLC methods will integrate QbD principles, enhancing method robustness and ensuring compliance with stringent guidelines (e.g., ICH Q2(R1) for validation).

- **Impurity Profiling:** With increasing focus on drug safety, RP-HPLC will see advancements in detecting trace-level impurities and degradation products, supported by highly sensitive detectors like mass spectrometry (MS).

3. Green Analytical Chemistry

- **Sustainable Practices:** The push for environmentally friendly methods will drive the adoption of greener solvents (e.g., ethanol instead of acetonitrile), miniaturization of systems (e.g., micro-HPLC), and reduced waste generation in RP-HPLC processes.

- **Energy Efficiency:** Future developments may focus on low-energy systems and recyclable materials for columns and accessories.

4. Integration with Advanced Detection Systems

- **Hyphenated Techniques:** Coupling RP-HPLC with advanced detectors like tandem mass spectrometry (LC-MS/MS), nuclear magnetic resonance (NMR), or infrared spectroscopy will expand its capabilities for structural elucidation and trace analysis.

- **Multi-Dimensional Chromatography:** Combining RP-HPLC with other modes (e.g., ion-exchange or size-exclusion) in 2D-LC systems will improve separation of complex mixtures, particularly in proteomics and metabolomics.

5. Personalized Medicine and Clinical Research

- **Biomarker Analysis:** RP-HPLC will be increasingly used for quantifying biomarkers in personalized medicine, requiring validated methods with high throughput and sensitivity for clinical samples.

- **Point-of-Care Applications:** Miniaturized RP-HPLC systems could emerge for rapid diagnostics, supported by portable detectors and simplified validation protocols.

6. Regulatory and Industry Demands

- **Global Harmonization:** As regulatory standards evolve, RP-HPLC method validation will align with international guidelines, ensuring consistency across regions and industries.

- **Speed to Market:** In drug development, faster method development and validation cycles will be critical to accelerate product release, especially in competitive sectors like generics and biosimilars.

7. Artificial Intelligence and Data Analytics

- **Predictive Modeling:** AI-driven tools will predict chromatographic behavior, optimize mobile phases, and validate methods faster by analyzing large datasets from previous experiments.

- **Real-Time Monitoring:** Integration of smart sensors and data analytics will enable real-time validation and adjustment of RP-HPLC methods during runs.

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HOW TO CITE: Ritika Todkar*, Mahesh Kshirsagar, Gaffar Sayyed, Sanjay Garje, Development And Validation of Rp-HPLC Method by Using Amlodipine Besylate, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 5, 4043-4054. https://doi.org/10.5281/zenodo.15505690