IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 11, June 2026
Impact Factor: 8.2

RP-HPLC Method Development and Validation for Estimation of Nimodipine, Amlodipine and Felodipine in Bulk

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Abstract: *A simple, rapid, accurate, and precise Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Nimodipine, Amlodipine, and Felodipine in pharmaceutical formulations. Chromatographic separation was achieved using an ODS Hypersil C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 20 mM phosphate buffer (pH 3.5) and methanol in the ratio of 80:20 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 217 nm using UV detection. The developed method exhibited excellent linearity over the selected concentration ranges with correlation coefficient values above 0.98 for all analytes. Validation studies were performed according to ICH guidelines for parameters including linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Recovery studies showed accuracy in the range of 99.40–103.32%, while precision studies demonstrated %RSD values below 2%, indicating high reproducibility. The developed analytical method was found to be sensitive, robust, and suitable for routine quality control analysis and simultaneous estimation of calcium channel blocker drugs in pharmaceutical dosage forms.*

Keywords: RP-HPLC, Method Development, Method Validation, Nimodipine, Amlodipine, Felodipine, Calcium Channel Blockers, Pharmaceutical Analysis.

I. INTRODUCTION

Cardiovascular diseases (CVDs) remain one of the major global health challenges and are recognized as the leading cause of mortality worldwide. [1,2] Among cardiovascular disorders, hypertension is considered one of the most prevalent chronic conditions affecting millions of individuals and significantly increasing the risk of stroke, myocardial infarction, heart failure, kidney dysfunction, and other vascular complications. According to global health statistics, the increasing incidence of hypertension due to sedentary lifestyle, dietary habits, obesity, diabetes, and aging populations has created a growing demand for effective and long-term pharmacological therapies. Proper management of hypertension is therefore essential for reducing cardiovascular morbidity and improving patient survival outcomes. [3,4]

Among the various classes of antihypertensive agents, calcium channel blockers (CCBs) have emerged as one of the most effective and widely prescribed therapeutic categories because of their ability to reduce peripheral vascular resistance and improve blood circulation. Calcium channel blockers primarily act by inhibiting the influx of calcium ions through L-type calcium channels present in vascular smooth muscle and cardiac muscle cells. This mechanism results in vasodilation, reduction in cardiac workload, and effective lowering of systemic blood pressure. The dihydropyridine class of calcium channel blockers is particularly important due to its selective action on vascular smooth muscle with comparatively fewer effects on cardiac conduction. [5,6]

Amlodipine is a long-acting calcium channel blocker extensively used in the treatment of hypertension, chronic stable angina, and vasospastic angina. It exhibits prolonged duration of action and excellent oral bioavailability, making it one

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of the most commonly prescribed antihypertensive medications worldwide. Nimodipine is another selective calcium antagonist primarily used for the prevention and management of cerebral vasospasm following subarachnoid hemorrhage due to its high affinity for cerebral blood vessels. Felodipine is commonly prescribed for hypertension and angina pectoris because of its potent vasodilatory action and favorable pharmacokinetic profile. Since these drugs belong to the same therapeutic class and are widely utilized in pharmaceutical formulations, accurate analytical estimation becomes essential to ensure dosage accuracy, quality assurance, stability testing, and regulatory compliance. [7,8]

In pharmaceutical quality control laboratories, the development of reliable analytical methods for simultaneous estimation of multiple active pharmaceutical ingredients plays a critical role in ensuring product quality and patient safety. Analytical methods must demonstrate high sensitivity, specificity, reproducibility, and robustness to detect and quantify drug substances accurately even at low concentrations. Among modern analytical techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has become one of the most widely accepted and preferred methods for pharmaceutical analysis because of its high separation efficiency, rapid analysis time, excellent sensitivity, and reproducible performance. RP-HPLC offers several advantages including accurate quantification, minimal sample preparation, high resolution of analytes, and suitability for routine industrial quality control applications. [9,10]

Several analytical methods have previously been reported for the individual estimation of Amlodipine, Nimodipine, and Felodipine in bulk drug substances and dosage forms using spectrophotometric and chromatographic techniques. However, literature reports describing simultaneous estimation of these three calcium channel blockers using a single validated RP-HPLC method remain limited. Simultaneous analytical determination is highly advantageous because it reduces analysis time, decreases solvent consumption, improves laboratory efficiency, and provides cost-effective routine quality control analysis compared with separate analytical procedures for each drug. [11,12]

Method development in RP-HPLC requires careful optimization of multiple chromatographic parameters including mobile phase composition, pH of buffer solution, flow rate, detection wavelength, column selection, solvent system compatibility, and retention characteristics of analytes. Proper optimization is necessary to achieve adequate peak separation, symmetrical peak shape, acceptable retention time, and high analytical reproducibility. Following successful method development, validation studies are required to establish reliability and suitability of the analytical procedure before routine implementation. [13,14]

According to International Council for Harmonisation guidelines, analytical method validation must demonstrate acceptable performance with respect to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and system suitability parameters. Compliance with these internationally accepted guidelines ensures regulatory acceptance and scientific reliability of the developed analytical method.[15]

Therefore, the objective of the present investigation was to develop and validate a simple, rapid, precise, accurate, economical, and reproducible Reverse Phase High Performance Liquid Chromatography method for the simultaneous estimation of Nimodipine, Amlodipine, and Felodipine. The developed method was optimized under suitable chromatographic conditions and validated according to ICH analytical validation guidelines to establish its applicability for routine pharmaceutical quality control analysis and simultaneous estimation in pharmaceutical dosage formulations.

MATERIALS AND METHODS

The analytical study was carried out for the simultaneous estimation of Nimodipine, Amlodipine, and Felodipine using Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Reference standard samples of the three drugs were procured from certified pharmaceutical suppliers and analytical grade methanol, phosphate buffer reagents, and distilled water were used throughout the study. Chromatographic separation was achieved on an ODS Hypersil C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at ambient temperature. The mobile phase consisted of 20 mM phosphate buffer adjusted to pH 3.5 and methanol in the ratio of 80:20 (v/v), filtered through a 0.45 µm membrane filter and degassed prior to use. The mobile phase was delivered at a flow rate of 1.0 mL/min and detection was performed at 217 nm using a UV detector. Standard stock solutions of each drug were prepared in methanol and

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diluted to obtain working concentrations within the Beer's law range for calibration studies. Method development was performed by optimizing parameters including solvent system, mobile phase composition, pH, buffer concentration, column selection, and detection wavelength to obtain satisfactory peak resolution and symmetrical peak shape. The developed method was validated according to International Council for Harmonisation guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), system suitability, and solution stability to establish reliability and reproducibility of the analytical procedure.[16-18]

RESULTS AND DISCUSSION

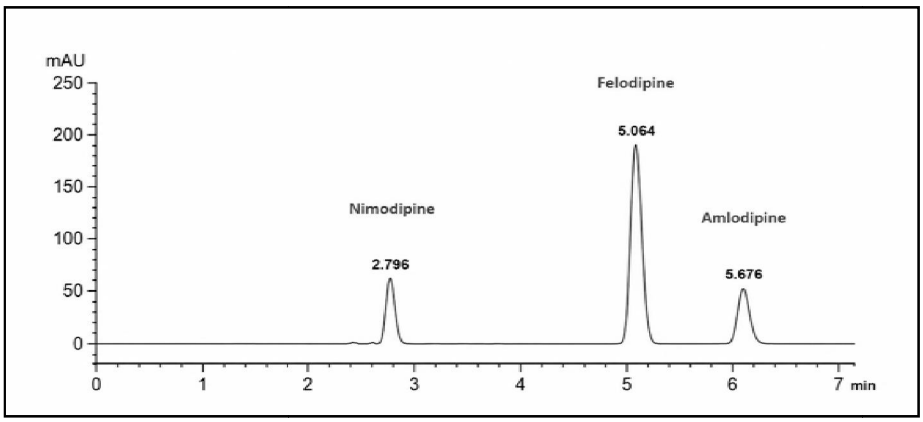

Fig 1: Optimized Chromatogram of standard of Nimodipine, Felodipine and Amlodipine

Method Development and Optimization

A robust Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was successfully developed for the simultaneous estimation of Nimodipine, Amlodipine, and Felodipine. Chromatographic separation was achieved using an ODS Hypersil C18 column (250 mm × 4.6 mm, 5 µm particle size) maintained at ambient temperature. The optimized mobile phase consisted of 20 mM phosphate buffer (pH 3.5) and methanol in the ratio of 80:20 v/v, delivered at a flow rate of 1.0 mL/min, while detection was carried out at 217 nm.

Several experimental trials were conducted during method development by varying solvent composition, buffer concentration, pH, column type, and flow rate to achieve optimal chromatographic performance. The finalized chromatographic conditions produced sharp, symmetrical peaks with satisfactory resolution among all three analytes. Blank chromatogram analysis confirmed absence of interference from mobile phase components, demonstrating good method specificity.

System Suitability Studies

System suitability testing was performed to evaluate chromatographic performance and reliability of the developed analytical method. The results confirmed satisfactory performance with acceptable values for linearity, accuracy, precision, and sensitivity.

Table 1. Summary of System Suitability Parameters

Parameter	Nimodipine	Amlodipine	Felodipine
Beer's Law Range (µg/mL)	2–10	100–500	20–100
LOD (µg/mL)	0.5862	1.4621	2.3145
LOQ (µg/mL)	1.7764	4.4306	7.0132
Regression Equation	y = 134.2x + 705.4	y = 260.18x + 29765	y = 339.84x + 19122
Correlation Coefficient (R^2)	0.9824	0.9952	0.9921

DOI: 10.48175/568

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Accuracy (% Recovery)	99.618	101.984	102.874
Intraday Precision (%RSD)	1.3928	0.1126	0.5632
Interday Precision (%RSD)	1.4573	0.1024	0.1865

The system suitability study demonstrated excellent chromatographic reproducibility. Correlation coefficient values greater than **0.98** indicated strong linearity for all analytes. Low %RSD values (<2%) confirmed high precision of the developed RP-HPLC method.

Linearity Studies

Linearity studies were performed using standard solutions prepared at different concentration levels within Beer-Lambert's law range. Calibration curves showed a direct proportional relationship between concentration and detector response.

Table 2. Calibration Curve Data

Concentration (µg/mL)	Nimodipine	Amlodipine	Felodipine
0	0	0	0
4	0.129	0.109	0.417
8	0.245	0.259	0.662
12	0.372	0.378	1.146
16	0.468	0.493	1.332
20	0.558	0.602	1.734

All three drugs exhibited excellent linear response over the tested concentration range. Regression analysis demonstrated strong linear correlation with **R^2 values ranging from 0.9817 to 0.9952**, indicating suitability of the method for quantitative analysis.

Accuracy Studies (% Recovery)

Accuracy was evaluated by standard addition method at **50%, 100%, and 150% concentration levels** in triplicate.

Table 3. Accuracy Study Summary

Drug	Mean Recovery (%)	%RSD
Nimodipine	99.403	5.38
Amlodipine	103.32	1.22
Felodipine	102.403	1.845

Recovery values ranged between **99.40% and 103.32%**, confirming excellent accuracy of the method. The obtained values were within acceptable limits recommended by **ICH analytical validation guidelines**, indicating minimal analytical error.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Sensitivity of the developed method was assessed through determination of detection and quantification limits.

Table 4. LOD and LOQ Results

Parameter	Nimodipine	Amlodipine	Felodipine
Slope	132.8	258.55	341.07
LOD (µg/mL)	0.5714	1.475	2.279
LOQ (µg/mL)	1.7316	4.4698	6.906

Low LOD and LOQ values indicate high sensitivity of the analytical method. The method was capable of detecting and quantifying very small concentrations of analytes, making it suitable for routine pharmaceutical analysis.

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Precision Studies

Precision was evaluated by analyzing replicate injections under intraday and interday conditions.

Table 5. Precision Study Summary

Parameter	Nimodipine	Amlodipine	Felodipine
Intraday %RSD	1.4166	0.1048	0.5891
Interday %RSD	1.4824	0.0983	0.1797

The obtained %RSD values were well below the acceptance criterion of **2%**, confirming excellent repeatability and intermediate precision. These results demonstrate reproducibility of the developed chromatographic method during routine laboratory analysis.

Robustness Study

Robustness was investigated by deliberately introducing small variations in chromatographic parameters such as mobile phase composition. A **±5 mL variation in mobile phase ratio** was applied to examine method stability.

No significant changes were observed in retention time, peak symmetry, or chromatographic resolution. The analytes remained well separated under altered experimental conditions, demonstrating that the developed RP-HPLC method is sufficiently robust and reliable for routine quality control analysis.

Discussion

The present study successfully developed and validated a simple, precise, accurate, and sensitive RP-HPLC method for simultaneous determination of Nimodipine, Amlodipine, and Felodipine. Validation parameters including specificity, linearity, accuracy, precision, sensitivity, and robustness were evaluated according to International Council for Harmonisation guidelines.

High correlation coefficients (>0.98), recovery values close to 100%, and precision values below 2% RSD confirmed reliability of the analytical procedure. The low detection and quantification limits further indicated excellent sensitivity of the method. Overall, the developed RP-HPLC method can be effectively employed for routine quality control analysis, simultaneous estimation, and pharmaceutical formulation analysis of calcium channel blocker drugs.

II. CONCLUSION

The present study successfully developed and validated a simple, rapid, accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Nimodipine, Amlodipine, and Felodipine. The optimized chromatographic conditions provided excellent peak resolution, satisfactory retention time, and reliable separation of all analytes without interference. Validation studies confirmed that the method exhibited excellent linearity, high accuracy with recovery values close to 100%, good precision with %RSD values below 2%, and high sensitivity as demonstrated by low LOD and LOQ values. Robustness testing further confirmed the stability and reproducibility of the analytical procedure under small deliberate variations in chromatographic conditions. Based on the obtained results, the developed RP-HPLC method can be effectively applied for routine quality control testing, simultaneous drug estimation, and pharmaceutical formulation analysis, making it highly suitable for industrial and research applications.

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