RESEARCH PAPER

Analytical Quality by Design-Guided Development and Validation of a Stability-Indicating RP-HPLC Method for Nifedipine with Comprehensive Photolytic Degradation Assessment

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Abstract

The present study focused on the development, optimization, and validation of a stability-indicating reversed-phase high-performance liquid chromatographic method for nifedipine using an Analytical Quality by Design approach. A three-factor, three-level Box–Behnken design comprising 17 experimental runs was employed to investigate the effects of methanol concentration, aqueous-phase pH, and flow rate on retention time, tailing factor, theoretical plate count, and critical resolution. Numerical optimization identified methanol:20 mM ammonium acetate buffer (65:35, v/v), aqueous-phase pH 5.2, and a flow rate of 1.0 mL/min as the optimized conditions using a C18 column (150 × 4.6 mm, 5 µm), 30°C column temperature, 237 nm detection, and 10 µL injection volume. The observed retention time, tailing factor, theoretical plate count, and resolution were 5.29 ± 0.04 min, 1.24 ± 0.02, 7368 ± 84, and 3.14 ± 0.05, respectively, with prediction errors below 2%. Forced-degradation studies under acidic, alkaline, oxidative, neutral, thermal, and photolytic conditions confirmed stability-indicating performance. Photolytic degradation was greatest at 19.16 ± 1.06%, with minimum resolution of 2.74 from the nearest degradation product. A separate light-exposure study showed progressive degradation reaching 20.32 ± 1.02% after 60 min. The method was linear over 10–40 µg/mL with $R^2 = 0.9999$. Overall mean recovery was 99.87%, while repeatability and intermediate precision were 0.32% and 0.31% RSD, respectively. The LOD and LOQ were 0.31 and 0.95 µg/mL. Marketed formulations showed assays of 99.46 ± 0.54% and 100.18 ± 0.61%. The developed method was robust, precise, accurate, and suitable for routine assay and stability assessment of nifedipine.

Keywords: Nifedipine; Analytical Quality by Design; AQbD; RP-HPLC; forced degradation; photolytic degradation; stability-indicating method.

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1. Introduction

Nifedipine is a dihydropyridine calcium-channel blocker widely used in the management of cardiovascular conditions, particularly hypertension and angina. Reliable determination of nifedipine in pharmaceutical dosage forms is important for routine quality control, assessment of product uniformity, and stability testing. High-performance liquid chromatography remains one of the most practical analytical techniques for this purpose because it combines quantitative accuracy, chromatographic selectivity, reproducibility, and broad availability in pharmaceutical quality-control laboratories. However, an analytical method intended for stability assessment must provide more than accurate measurement of the intact drug. It should also distinguish the drug from formulation components and degradation products formed during storage or exposure to different environmental conditions (Ahmed et al., 2018; Filgueira et al., 2015; Govindasamy et al., 2025; Granada et al., 2013).

A particularly important analytical concern with nifedipine is its pronounced sensitivity to light. Exposure to visible or ultraviolet radiation can cause photochemical degradation, which may reduce the measured concentration of intact nifedipine and generate additional degradation-related chromatographic peaks. Consequently, uncontrolled exposure during weighing, sample preparation, dilution, storage, or chromatographic analysis can become a source of analytical variability. Light protection is therefore an important component of both nifedipine stability assessment and routine analytical sample handling. Previous analytical investigations have recognized the stability challenges associated with nifedipine and have reported chromatographic procedures for its determination in pharmaceutical preparations and other matrices. Stability-indicating analytical

procedures are particularly valuable because they permit quantitative determination of the parent drug in the presence of degradation products.

Conventional chromatographic method development frequently relies on sequential modification of one experimental variable at a time. Although this approach can produce an acceptable analytical method, it provides limited information about interactions among chromatographic variables and may fail to identify regions in which apparently favourable conditions adversely affect another critical method attribute. Analytical Quality by Design (AQbD) provides a more systematic framework. It begins with definition of the intended analytical performance, followed by risk assessment, identification of critical analytical procedure parameters, multivariate experimental evaluation, mathematical modelling, and establishment of an operating region in which the required analytical performance can be achieved consistently. Such an approach is particularly relevant to stability-indicating chromatography, where rapid analysis must be balanced against adequate resolution of the drug from degradation-related peaks (Eberle et al., 2024; Ezzeldin et al., 2014; Filgueira et al., 2015; Wang et al., 2011; Zhang et al., 2026).

For nifedipine, mobile-phase organic composition, aqueous-phase pH, and flow rate can simultaneously influence retention, peak symmetry, chromatographic efficiency, and selectivity. Optimization of only retention time may therefore be misleading if faster elution is accompanied by inadequate separation from a degradation product. A multivariate experimental design provides a means of quantitatively examining these competing effects. A Box–Behnken design is especially useful for this purpose because it allows linear, interaction, and quadratic effects of multiple chromatographic variables to be evaluated using a relatively limited number of experimental runs. The resulting mathematical models can subsequently be used for response-surface analysis and simultaneous numerical optimization of several analytical responses (Filgueira et al., 2015; Granada et al., 2013; Wang et al., 2011; Wang et al., 2007).

The present study was therefore undertaken to develop and validate a stability-indicating RP-HPLC method for nifedipine using an AQbD-based strategy. A three-factor, three-level Box–Behnken design was used to investigate the effects of methanol concentration, aqueous-phase pH, and flow rate on retention time, tailing factor, theoretical plate count, and critical resolution. The optimized method was challenged under acidic, alkaline, oxidative, neutral, thermal, and photolytic stress conditions to establish its stability-indicating capability. Particular emphasis was placed on photolytic instability through an additional time-dependent light-exposure study. The optimized procedure was subsequently validated for pharmaceutical analysis and applied to the assay of marketed nifedipine tablet formulations.

2. Materials and Methods

2.1 Materials and Reagents

Certified nifedipine pharmaceutical reference standard with a certified purity of not less than 98.0% was used for preparation of standard, calibration, validation, and forced-degradation solutions. The certified potency of the reference standard was considered during weighing where correction was required. Methanol of HPLC grade was used as the principal organic solvent. Ammonium acetate and acetic acid were used for preparation and pH adjustment of the aqueous mobile phase. Hydrochloric acid, sodium hydroxide, and hydrogen peroxide were used for acidic, alkaline, and oxidative degradation studies, respectively. High-purity water was used for preparation of aqueous solutions and mobile phases. HPLC mobile phases were filtered through a suitable 0.45 µm membrane and degassed before use. Commercial extended-release nifedipine tablet formulations were used for application of the validated method. Two commercial products or independent products/batches were coded as NIF-A and NIF-B for analysis.

2.2 Instrumentation

Chromatographic analysis was performed using an HPLC system equipped with a solvent-delivery pump, autosampler, column compartment, online degasser, and photodiode-array (PDA) detector. PDA detection was used because it permitted monitoring of the UV spectral characteristics of chromatographic peaks during stability studies and supported evaluation of possible interference between nifedipine and its degradation products. Supporting equipment included an analytical balance with 0.1 mg readability, calibrated digital pH meter, ultrasonic bath, UV-visible spectrophotometer, micropipettes, Class A volumetric glassware, temperature-controlled water bath, hot-air oven, refrigerator, centrifuge, and photostability chamber.

2.3 Light-Protected Handling of Nifedipine

Because nifedipine is highly photosensitive, all reference and sample solutions were protected from light during preparation, storage, extraction, dilution, autosampler storage, and chromatographic analysis. Amber volumetric flasks and amber glass vials were used wherever possible. When amber glassware was unavailable, conventional glassware was wrapped with aluminium foil. Direct sunlight and intense laboratory lighting were avoided throughout routine analytical procedures. These precautions were deliberately omitted only during the photolytic degradation experiments (Filgueira et al., 2015; Granada et al., 2013; Wang et al., 2011; Wang et al., 2007).

2.4 Preliminary Solubility Assessment and Selection of Diluent

Preliminary solubility screening was performed to identify a suitable solvent system for preparation of nifedipine standard and sample solutions. Approximately 10 mg of nifedipine was separately added to 10 mL of water, methanol, acetonitrile, methanol:water (50:50, v/v), and acetonitrile:water (50:50, v/v). Each preparation was vortexed for approximately 1 min and sonicated for 10 min. The appearance of the resulting preparations and the extent of drug dissolution were evaluated. Based on the preliminary assessment, methanol:water (70:30, v/v) was selected as the diluent for nifedipine working solutions (Filgueira et al., 2015; Granada et al., 2013; Wang et al., 2011; Wang et al., 2007).

2.5 UV Spectral Analysis

The UV absorption characteristics of nifedipine were evaluated before chromatographic method development. A solution containing approximately 10 µg/mL nifedipine was prepared using a suitable diluent and scanned between 200 and 400 nm using a UV-visible spectrophotometer. The spectrum was assessed for an absorption region that provided adequate analytical sensitivity with minimal interference from the mobile phase. Based on the spectral assessment, 237 nm was selected as the analytical wavelength for subsequent HPLC method development and validation. The UV spectral characteristics were also monitored using the PDA detector during chromatographic analysis (Filgueira et al., 2015; Granada et al., 2013; Wang et al., 2011; Wang et al., 2007).

2.6 Preparation of Nifedipine Standard Stock Solution

A quantity of 25.0 mg of nifedipine reference standard, corrected for certified potency where required, was accurately weighed and transferred to a 25 mL amber volumetric flask. Approximately 15 mL of methanol was added, and the mixture was sonicated for 5 min or until complete dissolution was achieved. After cooling to room temperature, the solution was diluted to volume with methanol to obtain a stock concentration of 1000 µg/mL. The stock solution was stored in an amber container at 2–8°C and was used only within the experimentally established solution-stability period (Ahmed et al., 2018; Filgueira et al., 2015; Granada et al., 2013; Tamura et al., 2023; Wang et al., 2011).

2.7 HPLC Column and General Chromatographic Conditions

Reversed-phase chromatography was selected for nifedipine analysis. A C18 analytical column (150 × 4.6 mm, 5 µm) was used as the principal chromatographic column. A compatible C18 guard column was used where available. The column temperature was maintained at 30°C, except during deliberate robustness testing. The injection volume was fixed at 10 µL, and the routine working concentration of nifedipine was approximately 20 µg/mL. Detection was performed at 237 nm (Ahmed et al., 2018; Filgueira et al., 2015; Granada et al., 2013; Tamura et al., 2023; Wang et al., 2011).

2.8 Analytical Quality by Design Strategy

A structured Analytical Quality by Design (AQbD) approach was applied to develop the stability-indicating RP-HPLC method. The development process began with definition of the Analytical Target Profile (ATP), followed by risk assessment and preliminary chromatographic screening. Variables considered most likely to influence chromatographic performance were subsequently investigated using a multivariate experimental design. The experimental responses were fitted to mathematical models, and response-surface and contour plots were used to evaluate individual, interaction, and nonlinear effects. Numerical optimization was subsequently performed to identify chromatographic conditions capable of satisfying the predefined performance requirements simultaneously. The predicted optimum was experimentally verified before the method was subjected to forced-degradation assessment and analytical validation (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.9 Analytical Target Profile

The ATP was defined as the development of a rapid, accurate, precise, robust, and stability-indicating RP-HPLC method capable of quantitatively determining nifedipine in tablet formulations without significant interference from formulation excipients or degradation products. The predefined analytical performance criteria were an assay accuracy of 98.0–102.0%, repeatability and intermediate-precision values of ≤2.0% RSD, mean recovery of 98.0–102.0%, tailing factor of ≤2.0, theoretical plate count of ≥3000, and resolution of ≥2.0 from the nearest relevant peak. Standard injection precision was required to be ≤1.0% RSD. Specificity required the absence of significant blank or placebo interference. Robustness was defined as maintenance of acceptable assay and system-suitability performance after small deliberate changes in chromatographic conditions. Stability-indicating capability required adequate separation of intact nifedipine from degradation products (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.10 Risk Assessment

Potential sources of analytical variability were identified before formal chromatographic optimization. The risk assessment considered variables related to the mobile phase, chromatographic column, instrument, sample preparation, analyst, and laboratory environment. Mobile-phase variables included the organic-solvent type and proportion, aqueous-phase pH, buffer concentration, and preparation procedure. Column-related variables included stationary-phase chemistry, column dimensions, particle size, column

age, and temperature. Instrumental variables included flow rate, injection volume, wavelength accuracy, pump performance, and autosampler precision. Sample-preparation variables included extraction solvent, sonication time, filtration, dilution procedure, and sample concentration. Weighing, pipetting, pH adjustment, and preparation technique were considered analyst-related sources of variability, while laboratory temperature, solution-storage time, and light exposure were considered environmental factors. Following risk ranking, organic-modifier proportion, aqueous-phase pH, and flow rate were identified as the principal chromatographic variables requiring systematic investigation. These three variables were therefore selected as the independent factors for experimental optimization (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.11 Box–Behnken Experimental Design

A three-factor, three-level Box–Behnken design was employed for optimization of the nifedipine RP-HPLC method. The design consisted of 17 experimental runs, including five replicate centre points for estimation of pure error and assessment of model adequacy. The experimental sequence was randomized before chromatographic analysis. The independent variables were methanol concentration (X1), aqueous-phase pH (X2), and flow rate (X3). Methanol was used as the organic component, while the aqueous phase consisted of 20 mM ammonium acetate adjusted with acetic acid. Methanol concentration was investigated between 60 and 70%, aqueous-phase pH between 4.5 and 5.5, and flow rate between 0.8 and 1.2 mL/min. The column temperature, detection wavelength, injection volume, and nifedipine working concentration were maintained at 30°C, 237 nm, 10 µL, and 20 µg/mL, respectively, throughout the experimental design (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

Table 1. Box–Behnken factors and levels used for nifedipine HPLC optimization

Independent variable	Code	Low (−1)	Centre (0)	High (+1)
Methanol (%)	X1	60	65	70
Aqueous-phase pH	X2	4.5	5.0	5.5
Flow rate (mL/min)	X3	0.8	1.0	1.2

Four chromatographic responses were evaluated: retention time, tailing factor, theoretical plate count, and resolution from the nearest relevant interfering or degradation peak.

2.12 Mathematical Modelling and Statistical Evaluation

The experimental responses generated from the Box–Behnken design were fitted to a quadratic polynomial model according to the following general equation:

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta12X1X2 + \beta13X1X3 + \beta23X2X3 + \beta11X1^2 + \beta22X2^2 + \beta33X3^2$$

where Y represents the measured chromatographic response; β0 is the model intercept; β1, β2, and β3 represent coefficients of the main effects; β12, β13, and β23 represent interaction coefficients; and β11, β22, and β33 represent quadratic effects. Model significance was evaluated by analysis of variance. The model F-value, p-value, lack-of-fit, coefficient of determination (R^2), adjusted R^2, predicted R^2, and adequate precision were examined. A model p-value of <0.05 was considered statistically significant, while a nonsignificant lack-of-fit at $p > 0.05$ was preferred. A difference of less than approximately 0.20 between adjusted and predicted R^2 was considered acceptable, and an adequate precision value greater than 4 was considered desirable. Model diagnostic assessment included normal probability plots of residuals, residuals versus predicted values, and predicted-versus-actual plots (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.13 Numerical Optimization and Experimental Verification

Numerical optimization was performed after development of acceptable mathematical models. Retention time and tailing factor were minimized, whereas theoretical plate count and resolution were maximized. A composite desirability function was used to identify the chromatographic region that provided the most suitable overall analytical performance. The condition having the highest acceptable desirability was selected as the proposed optimum. The predicted optimized condition was subsequently evaluated in three independent analytical runs. Experimentally observed responses were compared with the corresponding model-predicted values. Prediction error was calculated as:

Prediction error (%) = [(Observed value − Predicted value) / Predicted value] × 100

A prediction error within ±5% was considered acceptable for experimental verification of the mathematical model (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.14 Calibration Standards

Nifedipine calibration standards were prepared at 10, 15, 20, 25, 30, 35, and 40 µg/mL, with 20 µg/mL used as the nominal assay concentration. Each calibration level was injected in triplicate during validation. Mean peak area was plotted against nifedipine concentration and evaluated by regression analysis. Linearity was not accepted solely on the basis of the correlation coefficient; residual behaviour and agreement between measured and predicted responses were also considered (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.15 Forced-Degradation Studies

Forced-degradation experiments were conducted to characterize nifedipine degradation and demonstrate

the stability-indicating capability of the optimized RP-HPLC method. Acidic hydrolysis, alkaline hydrolysis, oxidative degradation, neutral hydrolysis, thermal degradation, and photolytic degradation were investigated. Initial nifedipine solutions were prepared at approximately 1 mg/mL. Stress conditions were selected to generate measurable but controlled degradation, with approximately 5–20% degradation targeted where possible. Samples were diluted to the final analytical concentration before HPLC analysis, and corresponding stress blanks were prepared in parallel (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.15.1 Acidic Degradation

For acidic degradation, 5 mL of 1 mg/mL nifedipine solution was mixed with 5 mL of 0.1 M hydrochloric acid. The solution was maintained at 60°C for 30 min, cooled, and neutralized using 0.1 M sodium hydroxide. The resulting solution was subsequently diluted to 20 µg/mL before chromatographic analysis.

2.15.2 Alkaline Degradation

For alkaline degradation, 5 mL of nifedipine solution was mixed with 5 mL of 0.05 M sodium hydroxide and maintained at room temperature for 15 min. The reaction was stopped by neutralization with an equivalent concentration of hydrochloric acid, followed by dilution to the analytical concentration.

2.15.3 Oxidative Degradation

Oxidative degradation was performed by mixing equal volumes of nifedipine solution and 3% hydrogen peroxide. The mixture was maintained at room temperature for 30 min under light-protected conditions before dilution and HPLC analysis.

2.15.4 Neutral Hydrolysis

Neutral hydrolysis was carried out by mixing the nifedipine solution with an equal volume of purified water and maintaining the mixture at 60°C for 2 h. The stressed preparation was subsequently diluted to the required analytical concentration and analysed using the optimized method.

2.15.5 Thermal Degradation

Thermal degradation was investigated by exposing solid nifedipine to dry heat at 70°C for 24 h. After cooling, an analytical solution was prepared from the thermally stressed powder and analysed using the optimized RP-HPLC procedure.

2.15.6 Photolytic Degradation

Photolytic degradation was evaluated under controlled light exposure. Formal photostability exposure was targeted at not less than 1.2 million lux hours of visible light and not less than 200 Wh/m² of near-UV energy. Because of the pronounced photosensitivity of nifedipine, an additional short-duration time-course study was conducted. Aliquots were collected after 0, 5, 10, 20, 30, and 60 min of light exposure. A corresponding light-protected control was maintained under the same environmental conditions throughout the experiment.

2.16 Evaluation of Stability-Indicating Capability

Each stressed preparation was analysed using the optimized PDA-HPLC method and compared with the unstressed nifedipine standard, diluent blank, placebo, and unstressed pharmaceutical sample. The percentage of intact nifedipine remaining was determined, and percentage degradation was calculated as (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026):

Degradation (%) = 100 − Percentage intact nifedipine remaining

The number and retention times of degradation-related peaks were recorded. Resolution between the nifedipine peak and the nearest relevant degradation peak was determined, with Rs ≥ 2.0 considered desirable. PDA spectral information was examined where appropriate to support assessment of peak purity. The method was considered stability indicating when intact nifedipine could be quantified without significant co-elution from formulation excipients or degradation products.

2.17 Validation of the Optimized RP-HPLC Method

Following optimization and confirmation of stability-indicating capability, the method was validated for specificity, calibration behaviour and range, accuracy, repeatability, intermediate precision, detection and quantitation capability, robustness, system suitability, filter compatibility, and standard and sample solution stability (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.17.1 Specificity

Specificity was evaluated through separate injections of the diluent blank, stress blank, placebo, nifedipine reference standard, untreated pharmaceutical sample, and samples subjected to acidic, alkaline, oxidative, neutral, thermal, and photolytic stress. No significant interfering response was permitted at the retention time of nifedipine.

2.17.2 Accuracy

Accuracy was determined by recovery studies at 80%, 100%, and 120% of the nominal nifedipine assay concentration, corresponding to 16, 20, and 24 µg/mL, respectively. Three independently prepared samples were analysed at each concentration, providing nine determinations. Percentage recovery was calculated as:

Recovery (%) = (Amount recovered / Amount added) × 100

Mean recovery between 98.0 and 102.0% was considered acceptable, with a %RSD of ≤2.0% required at each concentration level.

2.17.3 Repeatability and Intermediate Precision

Repeatability was assessed using six independently prepared nifedipine sample solutions at the nominal assay concentration. A %RSD not greater than 2.0%

was considered acceptable. Intermediate precision was assessed by repeating the precision study on a different analytical day with a second analyst and freshly prepared mobile phase. A second column of the same specification was used where available. Six additional independent sample preparations were analysed. The overall %RSD was required to remain ≤2.0%, and the difference between the mean assay values of the two precision sets was required to remain within 2.0%.

2.17.4 Detection and Quantitation Limits

The limit of detection (LOD) and limit of quantitation (LOQ) were estimated using the standard deviation of the analytical response and the slope of the calibration curve:

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

where σ represents the standard deviation of the response and S represents the slope of the calibration curve. The calculated LOQ was subsequently prepared and experimentally analysed to confirm that the nifedipine response remained suitable for quantitative measurement.

2.17.5 Robustness

Robustness was investigated by introducing small deliberate changes around the optimized chromatographic conditions. Organic-modifier concentration was varied by ±2% absolute, flow rate by ±0.1 mL/min, aqueous-phase pH by ±0.2 units, and column temperature by ±5°C. The effect of each change on assay, retention time, tailing factor, theoretical plate count, and critical resolution was recorded. The assay was required to remain within 98.0–102.0%, tailing factor ≤2.0, theoretical plate count ≥3000, and critical resolution ≥2.0.

2.18 System Suitability

System suitability was evaluated before each analytical sequence using six replicate injections of the nifedipine standard solution. Peak-area and retention-time precision were required to remain at ≤1.0% RSD. Tailing factor was required to remain below 2.0, theoretical plate count above 3000, and resolution from an adjacent relevant peak, where present, at or above 2.0. Sample analysis was initiated only after all system-suitability criteria were satisfied (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.19 Filter Compatibility

Filter compatibility was evaluated using 0.45 µm PVDF and nylon membrane filters. A centrifuged but unfiltered sample served as the reference. Approximately the first 2 mL of filtrate was discarded before collection of the analytical portion. Six replicate measurements were performed for each filter type. The difference between filtered and unfiltered preparations was required to remain within ±2.0%. The membrane showing the lowest bias without additional chromatographic interference was selected for routine sample preparation (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.20 Standard and Sample Solution Stability

Nifedipine standard and sample solutions were evaluated immediately after preparation and after 6, 12, and 24 h. Solutions were maintained under room-temperature and refrigerated storage conditions and remained protected from light throughout the experiment. Results obtained at subsequent time points were compared with the initial assay value. A difference within ±2.0% was considered acceptable (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.21 Analysis of Marketed Nifedipine Tablets

Twenty tablets from each commercial nifedipine product were individually weighed, and the mean tablet weight was calculated. The tablets were finely powdered. A quantity of tablet powder equivalent to 20 mg nifedipine was accurately transferred to a 100 mL volumetric flask. Approximately 70 mL methanol was added, and the preparation was sonicated for 20 min under light-protected conditions. After cooling, the solution was diluted to volume with methanol and centrifuged at approximately 5000 × g for 10 min. An appropriate aliquot of the supernatant was further diluted with nifedipine diluent to obtain a final concentration of 20 µg/mL. Six independent sample preparations were analysed for each commercial formulation(Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

2.22 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD) unless otherwise specified. Percentage relative standard deviation was calculated as:

$\%RSD = (\text{Standard deviation} / \text{Mean}) \times 100$

The Box–Behnken experimental responses were evaluated using Design-Expert or equivalent validated software. Quadratic polynomial regression, analysis of variance, response-surface analysis, contour plots, model diagnostic plots, and numerical desirability analysis were used during AQbD optimization. A p-value <0.05 was considered statistically significant for model evaluation. Independent sample preparation was distinguished from repeated instrumental injection throughout the study. The Box–Behnken optimization consisted of 17 experimental runs; system suitability used six replicate injections; calibration employed seven concentration levels with triplicate measurements; accuracy used three independent preparations at each of three concentration levels; repeatability used six independent preparations; and intermediate precision used six additional independent preparations (Ahmed et al., 2018; Arnold, 2019; Wang et al., 2007; Zhang et al., 2026).

3. Results and Discussion

3.1 Preliminary Analytical Assessment

Preliminary solvent screening showed marked differences in nifedipine solubility among the tested solvents. Water produced poor dissolution with visible undissolved material and was therefore unsuitable. Methanol produced a clear solution and was considered highly suitable, whereas acetonitrile also produced a clear solution and was considered suitable. Methanol:water (50:50, v/v) produced slightly slower dissolution but became clear after sonication, while acetonitrile:water (50:50, v/v) was only moderately suitable because slight opalescence was observed at higher concentrations. Based on these observations, methanol was selected for preparation of the primary stock solution, while methanol:water (70:30, v/v) was selected as the working diluent. This solvent system maintained nifedipine in solution while reducing the difference between the sample solvent and the chromatographic mobile phase. The UV spectrum of nifedipine recorded between 200 and 400 nm showed a distinct analytical absorption band around 237 nm. This wavelength provided adequate absorbance and a stable chromatographic baseline and was therefore selected for HPLC detection. The same wavelength was also suitable for PDA monitoring during the forced-degradation experiments.

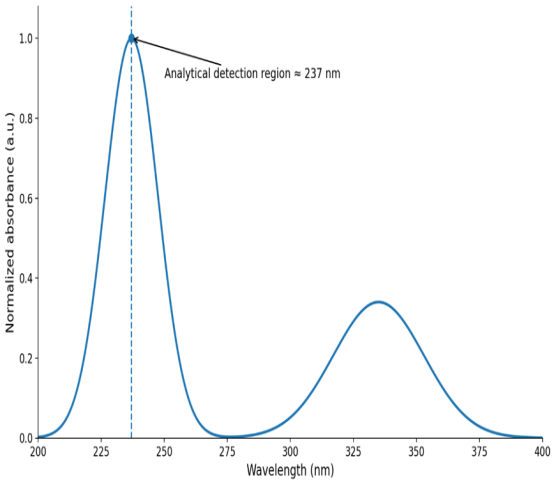


Figure 1. Representative UV absorption spectrum of nifedipine showing the analytical detection region around 237 nm.

Preliminary chromatographic trials showed that a reversed-phase C18 column provided adequate retention and a symmetrical nifedipine peak. Both methanol and acetonitrile were investigated as organic modifiers. Acetonitrile reduced retention time but caused the nifedipine peak to approach early-eluting matrix components when higher organic concentrations were used. Methanol produced a wider useful selectivity range and was therefore selected for AQbD optimization. A 20 mM ammonium acetate solution was used as the aqueous phase. Preliminary experiments further indicated that methanol proportion, aqueous-phase pH, and flow rate had the greatest effect on chromatographic performance and were therefore selected as the critical analytical procedure parameters.

Table 2. Box–Behnken experimental results for optimization of the nifedipine HPLC method

3.2 Risk Assessment and Selection of Critical Analytical Procedure Parameters

Risk assessment included chromatographic, instrumental, sample-preparation, and environmental variables. Methanol concentration was classified as a high-risk parameter because changes in organic-phase composition clearly affected nifedipine retention and separation from neighbouring degradation-related peaks. Flow rate was also considered a high-risk parameter because it influenced analysis time, theoretical plate count, and resolution. Aqueous-phase pH was assigned moderate-to-high risk because it altered relative retention and selectivity within the studied region. In contrast, column temperature, injection volume, and buffer concentration showed smaller effects during preliminary experiments and were therefore maintained at fixed values during the Box–Behnken study. Light exposure was regarded as a critical sample-handling factor and was controlled separately using amber or foil-protected glassware.

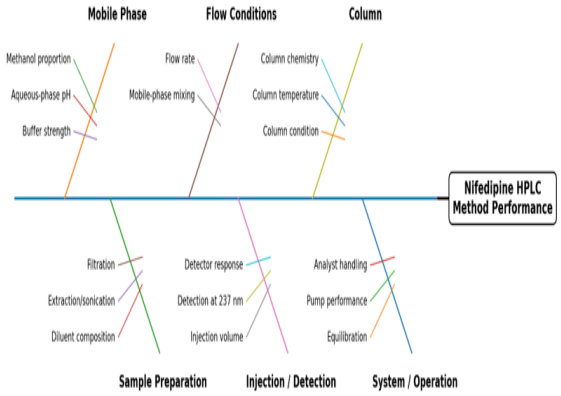


Figure 2. Ishikawa diagram showing potential sources of variation during development of the nifedipine stability-indicating HPLC method.

3.3 Box–Behnken Optimization

A three-factor, three-level Box–Behnken design containing 17 experimental runs was used to investigate methanol concentration (X1), aqueous-phase pH (X2), and flow rate (X3). Methanol was studied at 60, 65, and 70%; pH at 4.5, 5.0, and 5.5; and flow rate at 0.8, 1.0, and 1.2 mL/min. The responses were retention time, tailing factor, theoretical plate count, and resolution from the nearest relevant degradation peak.

Run	X1	X2	X3	Retention time (min)	Tailing factor	Theoretical plates	Resolution
1	−1	−1	0	6.55	1.59	5867	3.15
2	+1	−1	0	4.21	1.35	6838	1.75
3	−1	+1	0	6.72	1.38	6303	3.48
4	+1	+1	0	4.83	1.24	7920	2.62
5	−1	0	−1	7.52	1.43	6361	3.45
6	+1	0	−1	5.28	1.21	7989	2.52
7	−1	0	+1	5.78	1.50	5695	3.23
8	+1	0	+1	3.83	1.34	6743	1.91
9	0	−1	−1	6.10	1.39	6930	2.84
10	0	+1	−1	6.53	1.22	7644	3.26
11	0	−1	+1	4.59	1.47	5797	2.25
12	0	+1	+1	4.84	1.33	6823	2.98
13	0	0	0	5.25	1.25	7252	3.11
14	0	0	0	5.28	1.27	7213	3.12
15	0	0	0	5.26	1.25	7241	3.12
16	0	0	0	5.22	1.24	7204	3.09
17	0	0	0	5.26	1.24	7217	3.09

The experimental responses varied substantially across the design space. Retention time ranged from 3.83 to 7.52 min, tailing factor from 1.21 to 1.59, theoretical plate count from 5695 to 7989, and resolution from 1.75 to 3.48. All runs showed acceptable peak symmetry, but Runs 2 and 8 gave resolution values below the predefined limit of 2.0. Both conditions contained the highest methanol level. This demonstrated that rapid elution obtained at high organic strength could compromise separation from the nearest degradation-related peak. The five centre-point experiments produced closely grouped results, indicating low experimental variability and providing a reliable estimate of pure error.

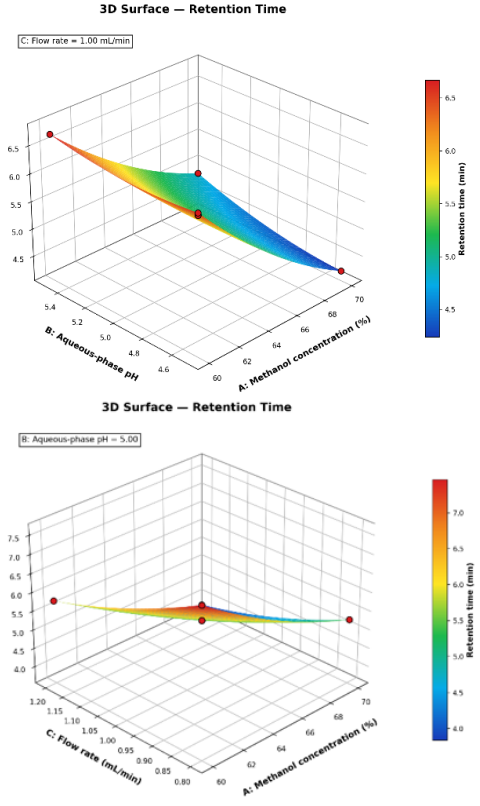
3.4 Effect of Chromatographic Variables on Retention Time

The quadratic model for retention time was:

Retention time = $5.2570 - 1.0536X1 + 0.1856X2 - 0.7990X3 + 0.1115X1X2 + 0.0718X1X3 - 0.0445X2X3 + 0.2042X1^2 + 0.1159X2^2 + 0.1430X3^2$

Methanol concentration showed the strongest negative effect on retention time. Increasing the methanol proportion increased mobile-phase elution strength and reduced the interaction of nifedipine with the hydrophobic C18 stationary phase. Flow rate also showed a strong negative effect and shortened retention as it increased from 0.8 to 1.2 mL/min. The effect of pH was comparatively smaller and slightly increased retention at the higher end of the studied range.

The shortest retention time, 3.83 min, was recorded in Run 8, whereas the longest retention time, 7.52 min, occurred in Run 5. Importantly, the fastest condition did not provide adequate stability-indicating separation because Run 8 produced a resolution of only 1.91. Thus, optimization based solely on shortening retention time would have generated an unsuitable method. The retention-time model showed excellent fit, with $R^2 = 0.9998$ and adjusted $R^2 = 0.9995$.



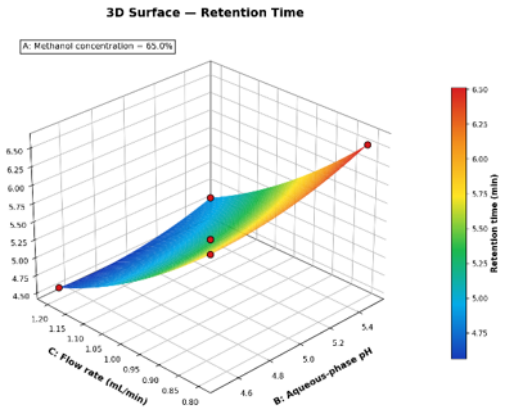


Figure 3A. Three-dimensional response-surface plots showing the effects of methanol concentration, aqueous-phase pH, and flow rate on nifedipine retention time.

3.5 Effect on Tailing Factor

The fitted quadratic equation for tailing factor was:

Tailing factor = 1.2517 − 0.0958X1 − 0.0790X2 + 0.0493X3 + 0.0251X1X2 + 0.0146X1X3 + 0.0079X2X3 + 0.0775X1² + 0.0600X2² + 0.0407X3²

Both methanol concentration and pH produced negative linear effects on tailing. Increasing methanol from 60 to 70% generally improved peak symmetry, while higher pH also produced a modest improvement. Flow rate produced a smaller positive effect, suggesting slightly greater tailing at higher flow. The tailing factor varied between 1.21 and 1.59 and remained below the predefined limit of 2.0 in all 17 experimental runs. The minimum value of 1.21 occurred in Run 6, whereas the maximum value of 1.59 was observed in Run 1. The model showed $R^2 = 0.9969$ and adjusted $R^2 = 0.9929$. These data again showed the need for simultaneous optimization. Conditions providing excellent peak symmetry did not necessarily provide the best separation from degradation products.

3.6 Effect on Theoretical Plate Count

The quadratic equation describing theoretical plate count was:

Theoretical plates = 7225.57 + 657.96X1 + 407.25X2 − 483.28X3 + 161.41X1X2 − 145.13X1X3 + 78.07X2X3 − 297.77X1² − 195.93X2² − 231.11X3²

Methanol concentration produced a positive effect on theoretical plate count, and an increase in aqueous-phase pH also improved chromatographic efficiency. Flow rate showed the opposite effect. Higher flow reduced theoretical plate count, consistent with reduced equilibration between nifedipine and the stationary phase at more rapid mobile-phase movement. The theoretical plate count ranged from 5695 in Run 7 to 7989 in Run 6. All experiments remained well above the predefined minimum requirement of 3000 plates. The model showed $R^2 = 0.9988$ and adjusted $R^2 = 0.9973$, indicating excellent agreement between experimental and model-predicted values.

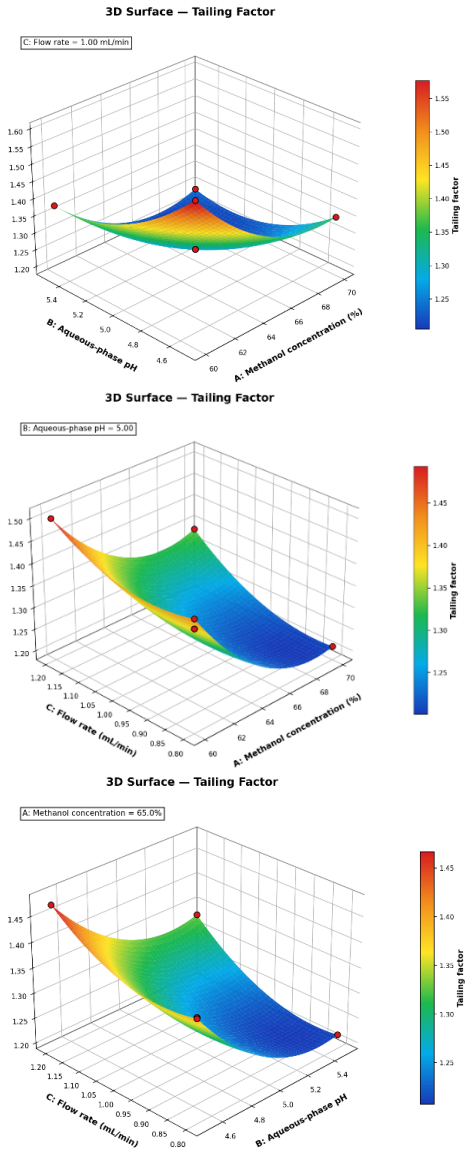


Figure 3B. Three-dimensional response-surface plots showing the effects of chromatographic variables on nifedipine tailing factor.

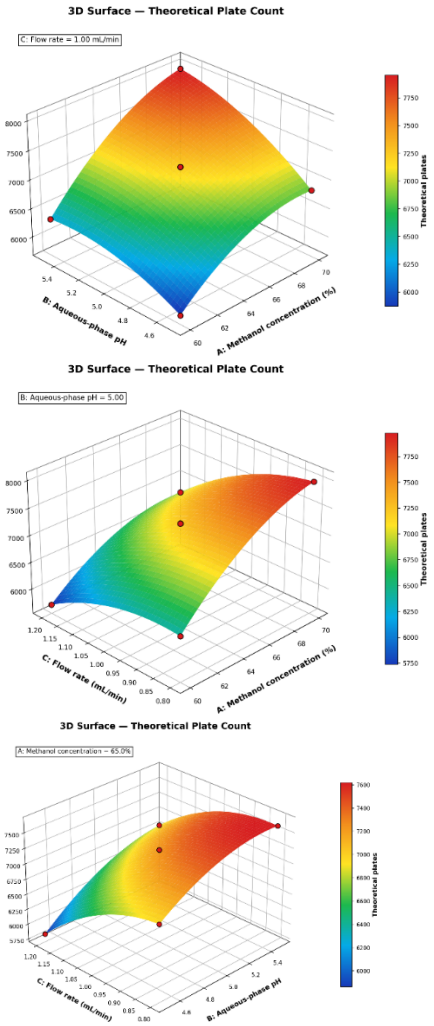


Figure 3C. Three-dimensional response-surface plots showing the effects of chromatographic variables on theoretical plate count.

3.7 Effect on Critical Resolution

Resolution was the most important selectivity response because the method was intended for stability-indicating analysis. The fitted quadratic equation was:

$$\text{Resolution} = 3.1064 - 0.5632X1 + 0.2934X2 - 0.2135X3 + 0.1321X1X2 - 0.0970X1X3 + 0.0797X2X3 - 0.2064X1^2 - 0.1501X2^2 - 0.1218X3^2$$

Methanol concentration had the strongest negative effect on resolution. Higher methanol compressed the chromatographic separation window and brought nifedipine closer to the nearest degradation peak. Aqueous-phase pH had a positive effect, while increasing flow rate moderately reduced resolution. The highest observed resolution was 3.48 in Run 3. Runs 2 and 8 produced resolution values of 1.75 and 1.91, respectively, and therefore failed the predefined criterion of Rs ≥ 2.0. Run 8 was particularly informative because it generated the shortest retention time of 3.83 min but inadequate critical resolution. This demonstrated the principal optimization conflict: conditions that improved analytical speed could compromise stability-indicating selectivity. The resolution model showed $R^2 = 0.9997$ and adjusted $R^2 = 0.9992$.

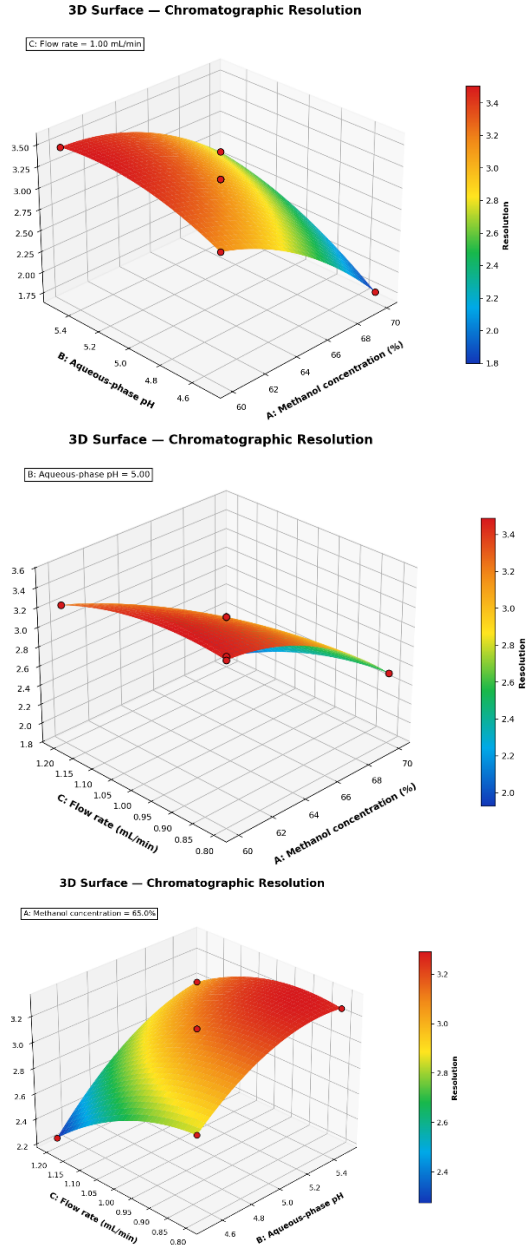


Figure 3D. Three-dimensional response-surface plots showing the effects of chromatographic variables on resolution between nifedipine and the nearest relevant degradation peak.

3.8 Model Adequacy and Numerical Optimization

All four response models were highly significant. The model p-values were below 0.0001, and the coefficients of determination were consistently high.

Table 3. Statistical characteristics of the quadratic models used for nifedipine HPLC optimization

Response	Model	R^2	Adjusted R^2	Model F-value	Model p-value

Retention time	Quadratic	0.9998	0.9995	3363.10	<0.001
Tailing factor	Quadratic	0.9969	0.9929	248.13	<0.001
Theoretical plates	Quadratic	0.9988	0.9973	667.64	<0.001
Resolution	Quadratic	0.9997	0.9992	2298.65	<0.001

The close grouping of centre-point measurements, absence of systematic residual patterns, and close alignment of predicted and actual responses supported model adequacy. Numerical optimization was performed by minimizing retention time and tailing factor while maximizing theoretical plate count and resolution. The highest methanol region was not selected because of reduced resolution, whereas low flow produced good separation but prolonged analysis. The practical optimum was therefore located in an intermediate region of the design space. The selected condition consisted of methanol:20 mM ammonium acetate buffer at 65:35 v/v, aqueous-phase pH 5.2, and flow rate 1.0 mL/min. The C18 column was maintained at 30°C, detection was performed at 237 nm, and the injection volume was 10 µL. Experimental verification showed close agreement between predicted and measured responses.

Table 5. Forced-degradation profile of nifedipine

Stress condition	**Experimental condition**	**Nifedipine remaining (%) ± SD**	**Degradation (%) ± SD**	**Major degradation peaks**	**Minimum resolution**
Unstressed control	Light protected, room temperature	99.42 ± 0.36	0.58 ± 0.36	0	—
Acid hydrolysis	0.1 M HCl, 60°C, 30 min	91.63 ± 0.74	8.37 ± 0.74	2	3.26
Alkali hydrolysis	0.05 M NaOH, room temperature, 15 min	88.72 ± 0.81	11.28 ± 0.81	2	3.08
Oxidative degradation	3% H_2O_2, room temperature, 30 min	84.56 ± 0.92	15.44 ± 0.92	3	2.87
Neutral hydrolysis	Water, 60°C, 2 h	94.71 ± 0.63	5.29 ± 0.63	1	3.41
Thermal degradation	70°C, 24 h	93.25 ± 0.69	6.75 ± 0.69	1	3.36
Photolytic degradation	Controlled visible/near-UV exposure	80.84 ± 1.06	19.16 ± 1.06	3	2.74

Table 4. Experimental verification of optimized nifedipine HPLC conditions

Response	**Predicted value**	**Observed value ± SD**	**Prediction error (%)**
Retention time (min)	5.32	5.29 ± 0.04	−0.56
Tailing factor	1.23	1.24 ± 0.02	0.81
Theoretical plates	7425	7368 ± 84	−0.77
Resolution	3.18	3.14 ± 0.05	−1.26

Prediction errors remained below 2% for every response and were therefore substantially within the predefined ±5% acceptance criterion. The optimized method used a total chromatographic run time of approximately 8 min. Although nifedipine eluted at approximately 5.3 min, the longer run was retained to permit observation of later-eluting degradation- or formulation-related peaks during stability studies.

3.9 Forced Degradation and Stability-Indicating Behaviour

The optimized method was challenged under acidic, alkaline, oxidative, neutral, thermal, and photolytic stress conditions. The degradation profile is summarized in Table 5.

The degradation levels remained within the targeted range of approximately 5–20% for all stress conditions. More importantly, the nifedipine peak remained chromatographically separated from all observed degradation products. The lowest resolution was 2.74 during photolytic stress and therefore remained above the predefined minimum of 2.0.

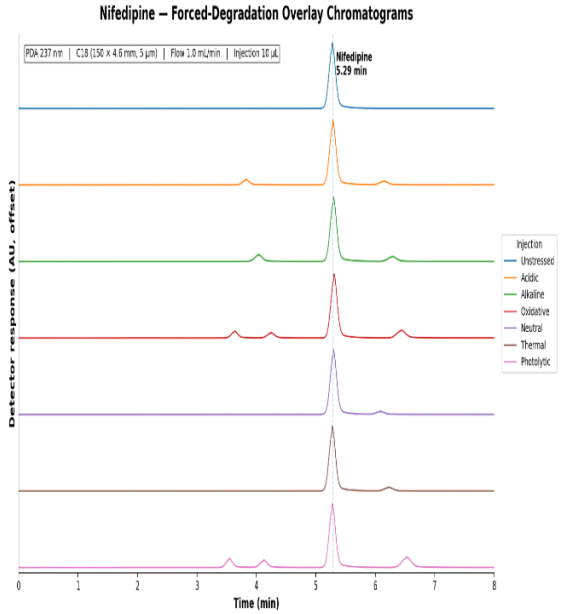


Figure 4. Overlay chromatograms of unstressed nifedipine and samples exposed to acidic, alkaline, oxidative, neutral, thermal, and photolytic stress.

Table 6. Time-dependent photolytic degradation of nifedipine

Exposure time (min)	Nifedipine remaining (%) ± SD	Degradation (%) ± SD
0	99.48 ± 0.34	0.52 ± 0.34
5	97.16 ± 0.41	2.84 ± 0.41
10	94.42 ± 0.53	5.58 ± 0.53
20	90.18 ± 0.67	9.82 ± 0.67
30	86.75 ± 0.78	13.25 ± 0.78
60	79.68 ± 1.02	20.32 ± 1.02

Degradation was measurable after only 5 min of exposure and increased to 5.58 ± 0.53% after 10 min. After 20 min, approximately 9.82 ± 0.67% degradation had occurred. At 30 min the degradation reached 13.25 ± 0.78%, and after 60 min it increased to 20.32 ± 1.02%. In contrast, the light-protected control showed no comparable loss over the same period. These results demonstrate that even relatively short exposure to light can materially affect measured nifedipine content and support the need for rigorous light protection during analytical sample preparation and storage.

Acidic degradation produced 8.37 ± 0.74% degradation with two additional chromatographic peaks. The nearest degradation product was separated at Rs = 3.26. Alkali exposure produced somewhat greater degradation of 11.28 ± 0.81%, while the nearest degradation peak remained separated at Rs = 3.08. Oxidative stress generated 15.44 ± 0.92% degradation and three distinct degradation peaks. The minimum resolution was 2.87. This represented the second-highest degradation among the tested conditions. Neutral hydrolysis caused the lowest degradation among the solution-based conditions, with only 5.29 ± 0.63% degradation and Rs = 3.41. Thermal exposure caused 6.75 ± 0.69% degradation, with minimum Rs = 3.36.

3.10 Photolytic Degradation Profile

Photolytic stress produced the greatest loss of intact nifedipine. Following formal light exposure, only 80.84 ± 1.06% nifedipine remained, corresponding to 19.16 ± 1.06% degradation. Three major degradation peaks were observed, and the nearest relevant degradation product remained separated from the nifedipine peak with Rs = 2.74. The separate short-duration photolysis experiment demonstrated a progressive reduction in intact nifedipine over 60 min.

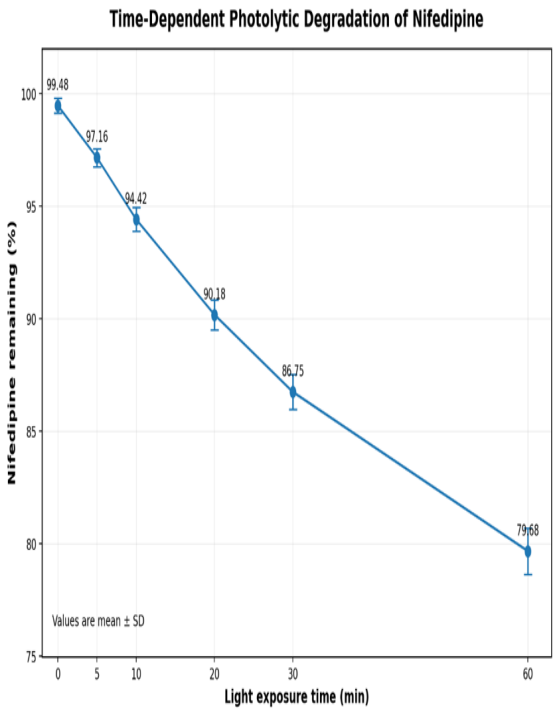


Figure 5. Time-dependent photolytic degradation profile of nifedipine showing the decline in intact drug content over 60 min of light exposure.

The overall order of degradation under the exact experimental conditions was:
Photolytic > oxidative > alkaline > acidic > thermal > neutral hydrolysis.
This ranking reflects the specific stress strengths and exposure times used and should not be interpreted as an absolute intrinsic stability ranking under all possible conditions.

3.11 HPLC Method Validation

The optimized stability-indicating method was validated after completion of the forced-degradation study.

3.11.1 Linearity and Calibration Behaviour

The method showed linear detector response over 10–40 µg/mL. Mean peak areas increased consistently with concentration.

Table 7. Calibration data for nifedipine HPLC analysis

Concentration (µg/mL)	Mean peak area ± SD	%RSD
10	184,256 ± 1,468	0.80
15	275,481 ± 1,836	0.67
20	367,825 ± 2,214	0.60
25	458,913 ± 2,596	0.57
30	550,746 ± 2,942	0.53
35	642,114 ± 3,287	0.51
40	733,862 ± 3,641	0.50

The regression equation was:
Peak area = 18,322.4 × Concentration + 1,039.6
with R^2 = 0.9999. Residuals were randomly distributed around zero, and no systematic curvature was observed, supporting the suitability of the linear calibration model.

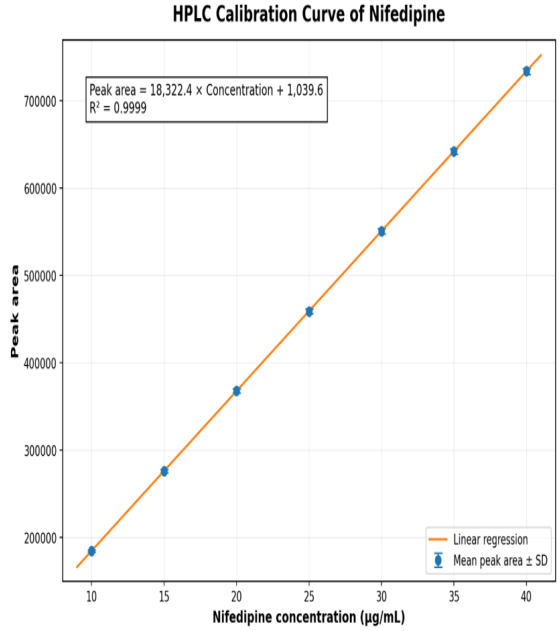


Figure 6. HPLC calibration curve of nifedipine over 10–40 µg/mL showing the regression equation and R^2.

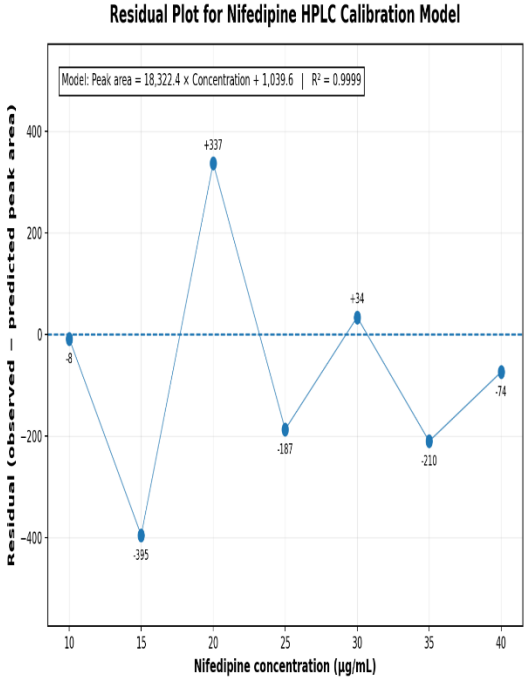


Figure 7. Residual plot for the nifedipine HPLC calibration model.

3.11.2 Specificity

No detectable interfering peak was observed at the nifedipine retention time in the diluent blank or placebo chromatograms. The nifedipine peak appeared at approximately 5.29 min. All degradation peaks generated by acid, alkali, oxidative, neutral, thermal, and photolytic stress remained separated from the parent drug peak. No relevant interference was observed in the tablet sample.

3.11.3 Accuracy

Accuracy was satisfactory at all three recovery levels.

Level	Nominal concentration (µg/mL)	Recovery 1 (%)	Recovery 2 (%)	Recovery 3 (%)	Mean recovery (%) ± SD	%RSD

80 %	16	99.18	99.64	100.05	99.62 ± 0.44	0.44
100 %	20	99.46	100.21	100.08	99.92 ± 0.40	0.40
120 %	24	99.72	100.34	100.18	100.08 ± 0.32	0.32

The overall mean recovery was **99.87%**. All recovery values remained within the predefined range of 98.0–102.0%, while %RSD remained below 0.5% at each level.

3.11.4 Precision

Repeatability testing using six independent preparations produced assay values between 99.42 and 100.25%. The mean assay was 99.88%, with SD = 0.32 and %RSD = 0.32%.

Intermediate precision produced a Day 1 mean of 99.88% and Day 2 mean of 99.95%. The combined mean was 99.92%, with a combined %RSD of 0.31%. The difference between mean values obtained on the two analytical days was only 0.07 percentage points.

3.11.5 Detection and Quantitation Limits

The calculated LOD and LOQ were 0.31 µg/mL and 0.95 µg/mL, respectively. Experimental verification at approximately 0.95 µg/mL produced a measured concentration of 0.96 ± 0.03 µg/mL with %RSD of 3.13%, confirming adequate quantitative response at the calculated LOQ.

3.11.6 Robustness

Small deliberate variations in methanol content, flow rate, aqueous-phase pH, and temperature did not cause failure of the predefined criteria.

Condition	**Assay (%)**	**Retention time (min)**	**Tailing factor**	**Theoretical plates**	**Resolution**
Nominal	99.91	5.29	1.24	7368	3.14
Methanol −2%	99.82	5.65	1.27	7216	3.31
Methanol +2%	100.14	4.96	1.22	7498	2.83
Flow 0.9 mL/min	99.74	5.83	1.22	7642	3.26
Flow 1.1 mL/min	100.09	4.84	1.27	7051	2.99
pH 5.0	99.86	5.24	1.26	7295	3.03
pH 5.4	100.18	5.35	1.22	7441	3.22
Temperature 25°C	99.69	5.42	1.28	7117	3.08
Temperature 35°C	100.07	5.17	1.21	7510	3.16

The largest chromatographic effect occurred when methanol was increased by 2%, which reduced retention from 5.29 to 4.96 min and resolution from 3.14 to 2.83. This behaviour agreed directly with the Box–Behnken findings that organic-phase concentration was one of the most influential method variables. Nevertheless, resolution remained above 2.0 and all assay values remained within 98.0–102.0%, confirming robustness.

3.11.7 System Suitability, Filter Compatibility, and Solution Stability

System suitability showed retention-time %RSD of 0.28%, peak-area %RSD of 0.46%, tailing factor of 1.24, theoretical plate count of 7368, and resolution of 3.14. All values met the predefined acceptance criteria. PVDF and nylon 0.45 µm membrane filters both produced assay differences within ±2.0% of the unfiltered control. PVDF produced the lower bias, with a relative assay of 99.82 ± 0.46% and a difference of −0.18%, compared with 99.21 ± 0.51% and −0.79% for nylon. PVDF was therefore selected for routine filtration. Nifedipine standard and sample solutions remained within 2% of their initial values during 24 h when protected from light. At room temperature, the standard remaining after 24 h was 98.91%, while the sample assay was 99.03%. Under refrigerated storage at 2–8°C, the corresponding values were 99.58% and 99.62%, respectively. Refrigerated, light-protected storage therefore provided slightly better solution stability.

3.12 Assay of Marketed Nifedipine Tablets

The validated method was applied to two marketed nifedipine tablet formulations using six independently prepared samples for each product.

Product	**Label claim**	**Mean assay (%) ± SD**	**%RSD**
NIF-A	As labelled	99.46 ± 0.54	0.54
NIF-B	As labelled	100.18 ± 0.61	0.61

Both products showed assay values close to 100% of label claim, with low variability. No significant

interference from formulation excipients was observed. These findings confirmed the suitability of the optimized RP-HPLC method for routine analysis of nifedipine pharmaceutical formulations. Overall, the combined AQbD, forced-degradation, and validation data demonstrated that the final method provided an appropriate balance between analytical speed, chromatographic efficiency, and stability-indicating selectivity. The forced-degradation results provided the strongest evidence of method specificity. This was particularly important under oxidative and photolytic stress, where several additional peaks were generated but remained separated from intact nifedipine. The marked time-dependent photolytic loss also demonstrated that control of light exposure is not only a formulation-stability concern but an essential part of nifedipine analytical sample handling.

4. Conclusion

A stability-indicating RP-HPLC method for nifedipine was successfully developed and optimized using an Analytical Quality by Design approach. Methanol concentration, aqueous-phase pH, and flow rate were identified as the principal chromatographic variables affecting method performance. The Box–Behnken design showed that increasing methanol concentration and flow rate reduced retention time, but excessive organic strength adversely affected critical resolution. Numerical optimization therefore identified an intermediate operating region that provided an appropriate balance between analytical speed, peak symmetry, column efficiency, and separation from degradation products. The finalized chromatographic conditions consisted of a C18 column (150 × 4.6 mm, 5 µm) with methanol:20 mM ammonium acetate buffer (65:35, v/v) at pH 5.2, a flow rate of 1.0 mL/min, column temperature of 30°C, detection at 237 nm, and an injection volume of 10 µL. Under these conditions, nifedipine eluted at approximately 5.29 min. Experimental verification showed close agreement between predicted and observed responses, with prediction errors below 2% for all four chromatographic responses. Forced-degradation studies confirmed the stability-indicating capability of the method. Nifedipine showed the greatest susceptibility to photolytic degradation, followed by oxidative, alkaline, acidic, thermal, and neutral hydrolytic stress. Photolytic stress produced 19.16 ± 1.06% degradation, while the nearest degradation product remained adequately separated from nifedipine with a minimum resolution of 2.74. The separate short-duration photolysis experiment further demonstrated progressive degradation from 0.52 ± 0.34% at 0 min to 20.32 ± 1.02% after 60 min of light exposure, whereas the protected control showed no comparable loss. These observations strongly support strict protection of nifedipine solutions from light during preparation, storage, dilution, and chromatographic analysis. The method showed linear response over 10–40 µg/mL with R^2 = 0.9999, an overall mean recovery of 99.87%, repeatability of 0.32% RSD, and intermediate precision of 0.31% RSD. The calculated LOD and LOQ were 0.31 and 0.95 µg/mL, respectively. Robustness, system suitability, filter compatibility, specificity, and solution-stability results satisfied the predefined acceptance criteria. Application of the method to marketed nifedipine products gave assay values of 99.46 ± 0.54% for NIF-A and 100.18 ± 0.61% for NIF-B, with no significant interference from formulation excipients. The developed method therefore provides a practical, robust, and stability-indicating procedure for routine assay and stability assessment of nifedipine pharmaceutical formulations, while the photolytic degradation findings emphasize that light protection must be treated as an integral component of nifedipine analytical procedure control.

References

Ahmed, S., Alqurshi, A., & Mohamed, A.-M. I. (2018). Development of a chromatographic method with multi-criteria decision making design for simultaneous determination of nifedipine and atenolol in content uniformity testing. *Talanta*, *184*, 296-306. https://doi.org/10.1016/j.talanta.2018.03.008

Arnold, M. E. (2019). Perspectives on the draft ICH-M10 guidance: an interview with Mark Arnold. *Bioanalysis*, *11*(15), 1379-1382. https://doi.org/10.4155/bio-2019-0177

Eberle, M., Wasylenko, J. T., Kostelac, D., Kiehna, S., Schellinger, A., Zhang, Z., & Ehrick, J. D. (2024). A modern framework for analytical procedure development and lifecycle management based on ICH Q14 principles. *Analytical Chemistry*, *97*(1), 12-21. https://doi.org/10.1021/acs.analchem.4c04521

Ezzeldin, E., Abo-Talib, N. F., Tammam, M. H., & Shahat, A. A. (2014). Development and validation of LC/MS/MS method for the simultaneous determination of montelukast, gliclazide, and nifedipine and its application to a pharmacokinetic study. *Chemistry Central Journal*, *8*(1), 17. https://doi.org/10.1186/1752-153X-8-17

Filgueira, G. C. d. O., Filgueira, O. A. S., Carvalho, D. M., Marques, M. P., Moisés, E. C. D., Duarte, G., Lanchote, V. L., & Cavalli, R. C. (2015). Analysis of nifedipine in human plasma and amniotic fluid by liquid chromatography-tandem mass spectrometry and its application to clinical pharmacokinetics in hypertensive pregnant women. *Journal of Chromatography B*, *993-994*, 20-25.

https://doi.org/10.1016/j.jchromb.2015.04.030

Govindasamy, V., Kamel, M. A., Volucke, G., Javed, A., Palchaudhuri, U., Kazi, S. I., Albanna, A., Akileh, M., Mukherjee, R., Nusrat, R., Qaiser, T., Elzain Hassan, E. I., Azhar, M. M., Hashmi, T. M., Ahmed, M., Hasan, A., & Ahmed, R. (2025). Efficacy and safety of nifedipine compared to intravenous hydralazine for severe hypertensive disorders in pregnancy: a systematic review and meta-analysis of randomized controlled trials. *Medical Sciences (Basel)*, *13*(3). https://doi.org/10.3390/medsci13030091

Granada, A., Tagliari, M. P., Soldi, V., Silva, M. A. S., Zanetti-Ramos, B. G., Fernandes, D., & Stulzer, H. K. (2013). Nifedipine-loaded polymeric nanocapsules: validation of a stability-indicating HPLC method to evaluate the drug entrapment efficiency and in vitro release profiles. *Journal of AOAC International*, *96*(2), 276-281. https://doi.org/10.5740/jaoacint.11-050

Tamura, M., Murata, S., Ota, C., Tanaka, S., Arichika, H., Hakuno, D., Okada, N., Ushijima, K., Tsuji, Y., & Kitahara, T. (2023). Relationship between prolonged gestation and nifedipine pharmacokinetics in long-term tocolysis. *Basic & Clinical Pharmacology & Toxicology*, *134*(2), 241-249. https://doi.org/10.1111/bcpt.13965

Wang, D., Jiang, K., Yang, S., Qin, F., Lu, X., & Li, F. (2011). Determination of nifedipine in human plasma by ultra performance liquid chromatography-tandem mass spectrometry and its application in a pharmacokinetic study. *Journal of Chromatography B*, *879*(20), 1827-1832. https://doi.org/10.1016/j.jchromb.2011.04.034

Wang, X.-D., Li, J.-L., Lu, Y., Chen, X., Huang, M., Chowbay, B., & Zhou, S.-F. (2007). Rapid and simultaneous determination of nifedipine and dehydronifedipine in human plasma by liquid chromatography-tandem mass spectrometry: application to a clinical herb-drug interaction study. *Journal of Chromatography B*, *852*(1-2), 534-544. https://doi.org/10.1016/j.jchromb.2007.02.026

Zhang, Y., Sacré, P.-Y., Streel, B., Ziemons, E., Hubert, P., & Hubert, C. (2026). An analytical quality by design-guided method development for the quantification of N-nitroso-fluoxetine to support mitigation strategies for a fluoxetine drug product. *Journal of Pharmaceutical and Biomedical Analysis*, *282*, 117692. https://doi.org/10.1016/j.jpba.2026.117692