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RESEARCH ARTICLE

Analytical Method Development and Validation of Nicardipine by RP-HPLC

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Abstract:
A novel approach was devised to estimate Nicardipine using RP-HPLC with Biorelevant Dissolution Media (FaSSIF). Optimal chromatographic conditions were established utilizing a Kromosil C18 column (4.5×150 mm, 5.0 µm), with a flow rate of 0.8 ml/min and a mobile phase comprising 65% methanol and 35% water. Detection was performed at 265nm using a WATERS HPLC Auto Sampler, Separation module 2695, equipped with a photodiode array detector 996 and Empower software version-2. Retention times were determined to be 2.428 mins, and the Nicardipine purity was assessed at 99.87%. System suitability parameters, including theoretical plates and tailing factor, were found to be 4146 and 1.23, respectively. Method validation was conducted following ICH guidelines (ICH, Q2 (R1)). The linearity study demonstrated Nicardipine's concentration range from 30µg to 150µg, with a correlation coefficient (r2) of 0.997, recovery rate of 100.4%, repeatability %RSD of 0.5, and intermediate precision %RSD of 1.0. Precision evaluation encompassed precision, robustness, and repeatability, with a LOD value of 2.97 and LOQ value of 9.92. Consequently, this RP-HPLC method is recommended for the routine analysis of Nicardipine in both active pharmaceutical ingredients (API) and pharmaceutical dosage forms

Keywords: Kromosil C18; Nicardipine; RP-HPLC; Method development; Validation

1. Introduction

High Performance Liquid Chromatography (HPLC) stands as a cornerstone technique in modern analytical chemistry, offering precise and efficient separation and quantification of compounds within complex mixtures. This methodological approach has gained prominence due to its versatility and applicability across various industries, particularly in pharmaceutical analysis. In the context of pharmaceutical research and development, HPLC plays a vital role in the characterization, quantification, and quality control of pharmaceutical compounds. [1]

One such compound of interest is Nicardipine, chemically identified as 2-(benzyl(methyl)amino)ethyl methyl 1,4–dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate hydrochloride. Nicardipine, classified as a Calcium Channel Blocker, holds therapeutic significance in the treatment of various cardiovascular conditions. [2, 3]

The development of an HPLC method for Nicardipine entails meticulous optimization of chromatographic conditions to ensure accurate and efficient separation of the compound from complex matrices. In the aforementioned abstract, the authors describe the establishment of an RP-HPLC method utilizing Biorelevant Dissolution Media (FaSSIF) for the estimation of Nicardipine. This entails the selection of an appropriate stationary phase, mobile phase composition, flow rate, and detection wavelength to achieve optimal resolution and sensitivity. Moreover, validation of the HPLC method is imperative to ensure its reliability and suitability for intended analytical purposes. [4]

Validation encompasses various parameters such as specificity, linearity, accuracy, precision, robustness, and sensitivity, adhering to regulatory guidelines such as those provided by the International Council for Harmonisation (ICH). The validation process serves to demonstrate the method's ability to accurately and precisely quantify Nicardipine within specified concentration ranges, thus establishing its suitability for routine analysis in both active pharmaceutical ingredients (API) and pharmaceutical dosage forms.

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2. Materials and methods

2.1. Materials

The materials used in this study included HPLC equipment consisting of an auto-sampler and a UV detector, specifically the Separation module 2695 and UV detector 2487, respectively, controlled by Empower software version-2 from Waters. Additionally, a U.V double beam spectrophotometer equipped with M. wave software from Lab India was utilized. Other instruments employed were a pH meter (ADWA Model number AD102U) and a digital weighing machine (Model number ER200A). Chemicals utilized in the study comprised Nicardipine, KH2PO4, water, and methanol for HPLC analysis, as well as acetonitrile for HPLC, ortho phosphoric acid, and K_2HPO_4

2.2. Optimized chromatographic conditions

A Kromosil C18 column measuring 4.5×150 mm with a particle size of 5.0 µm was employed for separation. The column temperature was maintained at ambient levels throughout the analysis. Detection of the target compound was achieved at a wavelength of 265 nanometers. The mobile phase consisted of methanol and water in a ratio of 65:35% v/v, ensuring optimal solubility and elution of the analyte. The flow rate was set at 0.8 milliliters per minute to facilitate efficient elution and separation of components. The auto sampler operated at ambient temperature, maintaining sample integrity during injection. A volume of 20 microliters was injected for analysis, and the entire run was completed within a span of 6 minutes, providing rapid and reliable results. [5] The optimized chromatogram is shown in Figure 1.

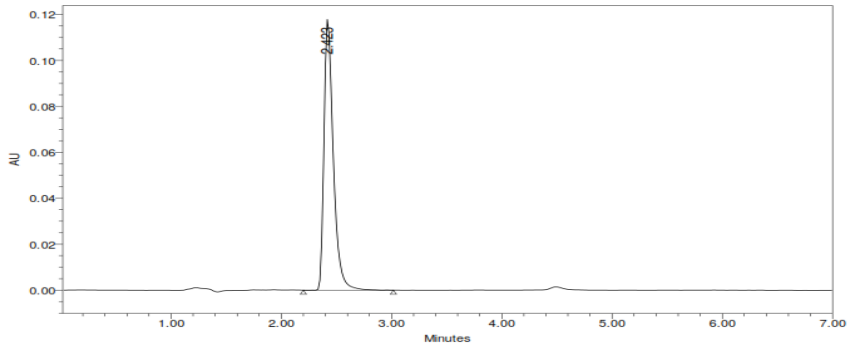

Figure 1. Chromatogram showing optimized injection

2.3. Preparation of Nicardipine sample solution

Initially, 10 milligrams of Nicardipine capsule powder, equivalent to the desired amount, were precisely weighed and placed into a clean, dry 10-milliliter volumetric flask. Approximately 1 milliliter of diluent was added to the flask, followed by sonication to ensure complete dissolution. The flask was then filled up to the mark with the same solvent to create a stock solution. Subsequently, 1 milliliter of the prepared stock solution was transferred into another 10 milliliter volumetric flask and diluted with diluent to reach the desired volume. [6]

2.4. Method validation

2.4.1. Specificity

The specificity of the analytical method was evaluated by analyzing the target compound (Nicardipine) in the presence of potential interfering substances commonly found in pharmaceutical formulations. Any potential interference was assessed by comparing chromatograms of samples containing only Nicardipine with those containing the compound along with potential interfering substances.[7,8]

2.4.2. Linearity

To assess linearity, a series of standard solutions containing Nicardipine at varying concentrations within the specified range were prepared. Each solution was analyzed in triplicate, and a calibration curve was constructed by plotting the peak area against the corresponding concentration of Nicardipine. The linearity of the method was evaluated by assessing the correlation coefficient (r2) of the calibration curve.[7,8]

2.4.3. Range

The range of the method was determined by analyzing Nicardipine standard solutions at concentrations covering the intended range of analysis. This range was established based on the anticipated concentrations of Nicardipine in the samples under investigation.[7,8]

2.4.4. Accuracy

The accuracy of the method was evaluated by conducting a recovery study. Known amounts of Nicardipine standard solution were added to pre-analyzed samples at three different concentration levels. The samples were then analyzed, and the percentage recovery of Nicardipine was calculated. .[7,8]

2.4.5. Precision

Precision was assessed by analyzing multiple replicates of Nicardipine standard solutions at a single concentration level. The intra-day precision was determined by analyzing the solutions within the same day, while the inter-day precision was assessed by analyzing the solutions on different days. The relative standard deviation (RSD) of the peak areas was calculated as a measure of precision. .[7,8]

2.4.6. Repeatability

Repeatability was evaluated by analyzing multiple replicates of a Nicardipine standard solution under the same operating conditions within a short time frame. The RSD of the peak areas was calculated to assess the repeatability of the method. .[9,10]

2.4.7. Intermediate Precision

Intermediate precision, also known as ruggedness, was assessed by analyzing Nicardipine standard solutions on different instruments, by different analysts, and on different days. The RSD of the peak areas obtained under different experimental conditions was calculated. [10,11]

2.4.8. Detection Limit

The detection limit of the method was determined by analyzing Nicardipine standard solutions with progressively lower concentrations until the signal-to-noise ratio reached a predefined value (typically 3:1). The concentration corresponding to this signal-to-noise ratio was considered the detection limit. [10,11]

2.4.9. Quantitation Limit

The quantitation limit was determined by analyzing Nicardipine standard solutions with progressively lower concentrations until the signal-to-noise ratio reached a predefined value (typically 10:1). The concentration corresponding to this signal-to-noise ratio was considered the quantitation limit. [10,11]

2.4.10. Robustness

The robustness of the method was evaluated by introducing deliberate variations in chromatographic conditions, such as flow rate, column temperature, and mobile phase composition. The effect of these variations on the retention time and peak area of Nicardipine was assessed to determine the robustness of the method [12,13]

3. Results and discussion

3.1. Method validation

3.1.1. Linearity

The assessment of linearity encompassed concentrations ranging from 30 ppm to 150 ppm. Every concentration level underwent injection into the chromatographic system, and the resulting area for each level served as the basis for calculating the correlation coefficient. The chromatograms are depicted in Table 1

Table 1. Results of linearity

Concentration (ppm)	**Retention time**	**Area**
30	2.428	1608152
60	2.422	2592905
90	2.430	3778327
120	2.426	5170038
150	2.433	6249400
Co efficient of correlation (R^2)		**0.997**

3.1.2. Accuracy

The accuracy study for Nicardipine involved assessing concentrations at 50%, 100%, and 150% of the specification level. Each concentration level was injected in triplicate into the chromatographic system, and the resulting area for each level was utilized to calculate the percentage recovery. Chromatograms illustrating the findings are presented in Figure. The accuracy results for Nicardipine are tabulated in Table 2. The table includes the percentage concentration at the specification level, the average area, the amount added (in milligrams), the amount found (in milligrams), and the percentage recovery. The mean recovery values are also provided for each concentration level

Table.2. Showing accuracy results for Nicardipine

%Concentration (at specification level)	**Average area**	**Amount added (mg)**	**Amount found (mg)**	**% Recovery**	**Mean recovery**
50%	1048287	5	5.14	100.2%	100.4%
100%	1378200	10	10.01	98.8%	
150%	1715480	15	15.2	96.5%	

3.1.3. Precision

The precision study involved conducting five injections of Nicardipine standards, with each standard injection introduced into the chromatographic system. The area of each standard injection was then utilized to calculate the percentage Relative Standard Deviation (% RSD). The chromatograms detailing these findings are displayed in Figure, and the corresponding results are presented in Table 3. This table illustrates the retention time (RT) and area for each of the five injections, along with the mean area and standard deviation. Additionally, the % RSD value for precision is provided. Furthermore, the assessment of ruggedness, also known as intermediate precision, was carried out. Table 4 exhibits the results for this analysis, presenting the RT and area for five Nicardipine injections. Similar to Table 3, the mean area, standard deviation, and % RSD value for ruggedness are included. These evaluations contribute to the comprehensive understanding of the method's precision and reliability across different experimental conditions. [14]

Table.3. Results for precision

Sl.No.	Name	RT	Area
1	Nicardipine	2.423	693078
2	Nicardipine	2.424	693338
3	Nicardipine	2.424	695080
4	Nicardipine	2.424	694843
5	Nicardipine	2.423	695336
Mean	694335		
Std.Dev.	1047.5		
%RSD	0.15		

Table.4 Results for intermediate precision

Sl.No.	Name	RT	Area
1	Nicardipine	2.423	693877
2	Nicardipine	2.424	696531
3	Nicardipine	2.424	693977
4	Nicardipine	2.424	695278
5	Nicardipine	2.423	697676
Mean	695468		
Std.Dev.	1642.7		
%RSD	0.24		

3.1.4. Limit of detection and limit of quantification

Limit of Detection (LOD) values were determined based on the standard deviation of the response (σ) and the slope of the calibration curve (s) at levels approximating the LOD, using a specific formula. The standard deviation of the response was established by considering the standard deviation of y-intercepts of regression lines. For Nicardipine, the LOD was calculated to be 2.97 µg. Similarly, the Limit of Quantitation (LOQ) was calculated using the same principles, where the standard deviation of the response and slope of the calibration curve played crucial roles. Again, the standard deviation of the response was determined based on the standard deviation of y-intercepts of regression lines. For Nicardipine, the LOQ was found to be 9.92 µg. These values provide crucial information about the sensitivity and detection capabilities of the analytical method for Nicardipine. The results are shown in Table 5.

Table 5. Results for LOD and LOQ

Parameter	Standard deviation(σ)	Slope
LOD (2.97 µg)	371827.90	563365963
LOQ (9.92 µg)	371827.90	563365963

3.1.5. Robustness

The robustness of the method was assessed by varying the flow rate within the range of 0.8 ml/min to 1.2 ml/min and altering the mobile phase ratio from more organic phase to less organic phase for Nicardipine. The method exhibited robustness under lower flow conditions, and it remained robust even with a ±5% change in the mobile phase composition. Chromatograms illustrating these variations are presented in Figure, while the corresponding results are tabulated in Table 6 and 7.

Table.6. Showing system suitability results (flow rate) for Nicardipine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4352	1.1
2	**1**	**4024**	**1.2**
3	1.2	3730	1.2

Table. 7. Showing system suitability results (mobile phase) for Nicardipine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	10 % less	4331	1.20
2	***Actual**	**4024**	**0.87**
3	10% more	3693	1.26

4. Conclusion

The developed method for the estimation of Nicardipine demonstrates significant attributes of speed, accuracy, precision, and reproducibility. Its linear behavior across a broad concentration range coupled with its cost-effectiveness and the simplicity of its mobile phase preparation enhances its suitability for Nicardipine analysis in both active pharmaceutical ingredients (API) and pharmaceutical dosage forms, particularly when utilizing Biorelevant Dissolution Media (FaSSIF). The method's potential for routine analysis and assay in quality control laboratories underscores its practical utility and value in pharmaceutical research and development.

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