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Development and Validation of analytical method for parenteral drug Nicardipine by using HPLC

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ABSTRACT:

Nicardipine is a second-generation dihydropyridine calcium channel blocker. It is often used in the treatment of hypertension, angina pectoris and associated cardiovascular conditions. The study was conducted to develop and validate a precise, simple and rapid RP-HPLC method for the estimation of Nicardipine in bulk and parenteral formulation. Study was performed with column (Symmetry C18 (4.6 x 250mm, 5µm). A Methanol: Buffer (Pot. Dihydrogen Phosphate (0.1M) (80:20 v/v) was used to create an isocratic mobile elution phase. A UV detector was used for 254 nm detection, which had an 20 µL injection volume, a flow rate of 1.0 mL/min, and an 40°C column temperature. Nicardipine was found to have retention time of 7.118 minute. The approach was validated for accuracy, specificity, selectivity, precision, linearity, sensitivity and robustness. Plots of linear calibration were made for concentrations between 25 and 125 µg/mL. The correlation coefficient (r^2) for the proposed approach was determined to be 0.998. Evaluation of system and method accuracy revealed that the technique is accurate within the acceptable range. In particular, the number of theoretical plates, tailing factor, and RSD were calculated for both solutions. It was evident from the results that the suggested method works well for the quick, precise, and accurate measurement of Nicardipine.

Key words- Nicardipine, RP-HPLC, Parenteral formulation.

INTRODUCTION:

High performance liquid chromatography (HPLC) is becoming the preferred technique for separations and analysis across several disciplines. Virtually every soluble substance may be isolated using a certain kind of HPLC column (1). Reversed phase chromatography has use in both analytical and preparative contexts for biochemical separation and purification. Molecules exhibiting hydrophobic characteristics may be effectively separated by reversed-phase chromatography, achieving high recovery and resolution. The mobile phase consists of water-organic mixtures, whereas the stationary phases may include C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), and cyano columns. It is the primary selection for the majority of samples, particularly neutral or non-ionized chemicals, that solubilize in water-organic mixtures (2). Method development and validation are ongoing procedures that advance concurrently with the progression of medicinal products. Alterations found during pharmaceutical development may need adjustments to current analytical methodologies. Consequently, these alterations to the methodologies may need further evaluation. The emergence of novel techniques and enhanced equipment in analytical fields may lead to the development of more sensitive, precise, and accurate approaches when current methods are inconsistent, unreliable, time-consuming, or excessively costly. Consequently, ongoing development and validation of novel analytical methods are crucial for the expanding drug development initiatives (3).

Nicardipine (Figure 1) is a second-generation dihydropyridine calcium channel blocker that specifically inhibits the contraction of vascular smooth muscle. It is often used in the treatment of angina pectoris, hypertension, and associated cardiovascular conditions (4,5). Nicardipine has a strong affinity for the dihydropyridine binding site and blocks the L-type calcium ion channel, as shown by its capacity to dose-dependently reduce calcium ion-dependent action potentials in ventricular papillary muscle (6) .

There are different HPLC and RPHPLC analytical and bio analytical methods in bulk and formulations reported in the literature for Nicardipine(7–14). Literature also shows the use of UV-Visible method(15–17) and Hyphenated technique (18,19) for nicardipine for the determination of from different dosage form and biological samples. The present study is aiming

to develop and validate a sensitive, simple, rapid, and economic RP-HPLC method for the determination of Nicardipine by using chromatographic method.

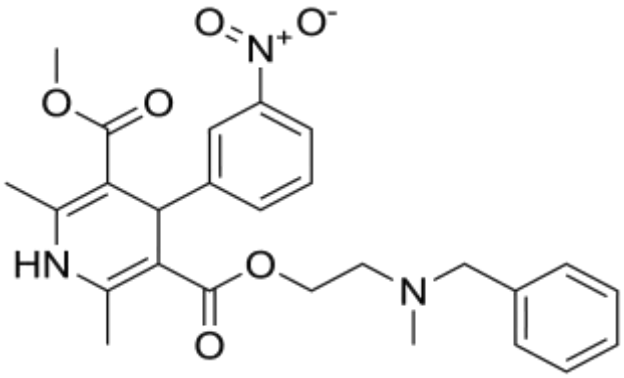

Figure 1: Structure of Nicardipine

METHODS:

Instrumentation:

For the Analytical study of Nicardipine, HPLC system with Empower software was used. The Waters alliance system with 2695 quaternary pump, auto sampler, column (Symmetry C18 (4.6 x 250mm, 5µm) and PDA 2996 were used.

Chemicals and Reagents:

The Pure API of Nicardipine was obtained as a gift sample from Wockhardt Research Centre, Chhatrapati Sambajinagar. The Formulation was procured from the local market. Acetonitrile, Methanol, potassium dihydrogen phosphate used were of HPLC grade.

Method Development by RP-HPLC

Preparation of Buffer (Mobile Phase A):

Accurately weigh 1.36 gm of potassium dihydrogen phosphate in 1000 ml of HPLC-grade water and mix thoroughly with agitation. Sonicated for 15 minutes and filtered through a 45 µm membrane filter.

Preparation of Mobile Phase:

Mobile phase A and Methanol in the ratio of 20:80 v/v were mixed and degas by sonication for 15 minutes.

Standard stock Preparation:

Accurately weighed 25 mg of nicardipine API and transferred into 25 mL volumetric flasks. 15 ml of Mobile phase A and Acetonitrile in the ratio of 50:50 v/v is added to flask, sonicated and volume upto 25 mL was maintained with diluent to get 1000 µg/ml concentration of Nicardipine.

1 ml of above solution was taken and diluted upto 10 ml with diluent. Further dilutions were prepared for the study as per the requirement of the study from the stock solution of 1000 ppm or 100 ppm.

Method Development and Chromatographic Conditions Optimization:

A robust RP-HPLC technique for Nicardipine measurement was developed using several mobile phases to optimize separation and resolution. The technique development started using a C18 column, with a mobile phase composed of water and methanol or acetonitrile at varying concentrations as organic modifiers. The peaks were inadequately resolved during this mobile phase. Phosphate buffer was used instead of water to enhance the peak forms. It culminated in pronounced peaks. The optimal findings were obtained using a Symmetry C18 column (4.6 x 250 mm, 5 µm), with a mobile phase comprising Methanol and Buffer (Potassium Dihydrogen Phosphate (0.1M) at a ratio of 80:20 v/v) at a flow rate of 1.0 ml/min. The aforementioned chromatographic settings produced crisp peaks with little tailing, excellent resolution, and reduced runtime for Nicardipine.

RESULT AND DISCUSSION:

Chromatographic Conditions:

Utilising the physiochemical features of Nicardipine and existing literature on analytical techniques for similar drugs, several solvent systems were used to achieve optimal separation, specifically in terms of resolution and selectivity (Figure 2). The various mobile phases used to attain optimised chromatographic conditions for the separation and quantification of Nicardipine. Inadequate resolution, suboptimal peak forms, and baseline disturbances were among the primary reasons for the rejection of the trials. In Figure 2 optimized trial obtained for estimation of nicardipine were depicted.

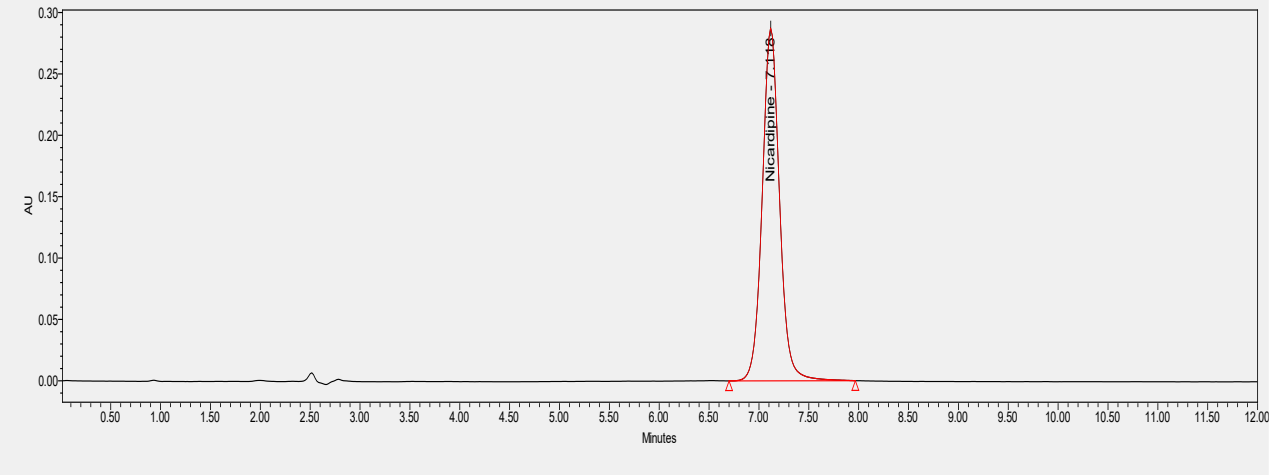

Figure 2: Optimized trial for Method development of Nicardipine

Chromatographic Conditions

Based on system suitability factors, the finalized chromatographic conditions were as follows:

Column : Symmetry C18 (4.6 x 250mm, 5µm)

Column Temp : 40°C

Wave length : 254 nm

Run Time : 12 min

Pump Mode : Isocratic

Retention time : About 7.118 minutes

Flow Rate : 1.0 ml/min

Injection Volume: 20 µl

Method Validation by RP-HPLC

a. System Suitability Study;

An HPLC technique has been established for quantifying the percentage assay of Nicardipine. The retention time (RT) for Nicardipine was determined to be 7.118 minutes, and other characteristics such as tailing factor, resolution, and theoretical plates (see Table 1) were within acceptable limits.

Table 1: System Suitability Parameters for Nicardipine

Sr. No.	Name	RT (min)	Area	Tailing Factor	Theoretical PlateCount	Selectivity	Resolution
1	Nicardipine	7.118	3460964	1.059	8577.827	1.128	17.628

b. Specificity

The lack of supplementary peaks in the chromatogram indicates the absence of excipient interference. The blank exhibited no interference during the retention period of the analyte peaks.

c. Linearity and Range

Linearity was established by creating standard solutions of Nicardipine within the range of 25-125 µg/ml. Twenty microlitres of each concentration were put into the HPLC. The mean peak areas were derived from the chromatograms, and individual linearity plots of concentration against mean peak areas were generated. The regressions of the plots were calculated using the least squares regression technique (Table 2 and Figure 3).

Table 2: Linearity and Range for Nicardipine

Level	Concentration(µg/ml)	PeakArea
Level 1	25	691881
Level 2	50	1549027
Level 3	75	2548607
Level 4	100	3296552
Level 5	125	4248623
Slope		**35444**
Intercept		**-191365**
Correlation coefficient		**0.9986**

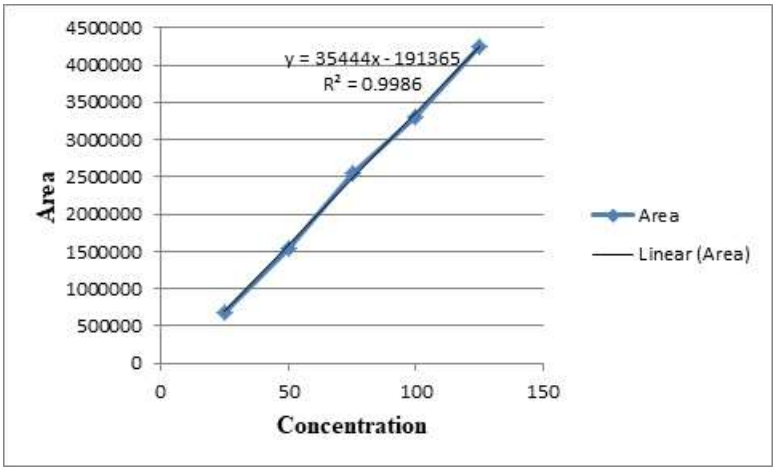

Figure 3: Standard Calibration (Linearity) Curve for Nicardipine

d. Precision

i. System Precision

It was performed by taking of peak area for standard drugs solutions in five replicates. % RSD for Nicardipine was found to be 0.725% (Table 3).

Table 3: System Precision Data of Nicardipine

Sr. No.	Peak areas of Nicardipine
1.	3360964
2.	3363257
3.	3353039
4.	3359295
5.	3305522
Mean	3348415.4
SD (±)	24276.16
RSD (%)	0.725

ii. Method Precision

It was performed by taking the peak area for sample solutions in five replicates. The % assays for nicardipine with five samples were calculated (Table 4).

Table 4: Method Precision Data of Nicardipine

Sample No.	% Assay of Nicardipine (w/w)
1.	100.22
2.	100.29
3.	100.56
4.	100.18
5.	98.66
Mean	99.98
SD (±)	0.755
RSD (%)	0.755

iii. Intraday and Inter-day Precision

The % RSD in intraday precision for Nicardipine (25, 75, 125 µg/ml) was found to be 0.475, 0.072, 0.524 %. In inter-day precision % RSD for Nicardipine (25, 75, 125 µg/ml) was found to be 0.090, 1.101, 0.892 % respectively. % RSD in intraday and inter-day studies was found well within the acceptable limits (Table 5 and 6).

Table 5: Intraday Precision data of Nicardipine

Sr. no.	Conc. (µg/ml)	Area	Mean Peak Area	SD(±)	%RSD
1	25	697572	694663	3301.190	0.475
		695341			
		691075			
2	75	2575341	2574275.67	1845.211	0.072
		2572145			
		2575341			
3	125	4269292	4243949.33	22250.311	0.524
		4227619			
		4234937			

Table 6: Inter-day Precision data of Nicardipine

Sr. no.	Day	Conc. (µg/ml)	Peak Area	Mean Peak Area	SD(±)	%RSD
1	1	25	696461	697117	629.01	0.090
	2		697715			
	3		697175			
2	1	75	2587058	2575045.33	28360.02	1.101
	2		2595422			
	3		2542656			
3	1	125	4233377	4250304	37916.86	0.892
	2		4223799			
	3		4293736			

e. Accuracy (Recovery Study)

The standard addition procedure was conducted at 80%, 100%, and 120% levels. The solutions were examined in triplicate at each level according to the specified methodology. The percentage recovery and % RSD were determined, and the findings are reported in Table 7. The suggested approach yielded satisfactory recoveries between 96.44 and 98.93. This signifies that the recommended procedure was accurate.

Table 7: Recovery study for Nicardipine

Level	Set	Amount added(µg/ml)	Area	Amount found(µg/ml)	Mean Conc.	SD	%RSD	%Recovery
80%	1	90	2953100	88.72	89.04	0.3412	0.3832	98.93
	2	90	2977213	89.40				
	3	90	2963545	89.01				
100%	1	100	3256034	97.26	96.44	0.7449	0.7724	96.44
	2	100	3204701	95.81				
	3	100	3219656	96.23				
120%	1	110	3614614	107.38	107.95	0.5564	0.5155	98.13
	2	110	3635449	107.97				
	3	110	3654036	108.49				

f. Sensitivity:

The LOD and LOQ for Nicardipine were determined to be 0.8248 and 2.499 µg/ml, respectively. These figures demonstrate that the approach is appropriate for ascertaining lower concentrations and affirm that the suggested method is sensitive for the estimation of nicardipine.

g. Robustness

The robustness investigation was conducted by making minor adjustments to the flow rate of the mobile phase. No significant alterations were noticed in the chromatograms, indicating that the new approach exhibited robustness. The findings of the robustness study are shown in Table 8.

Table 8: Robustness data of Nicardipine

Flow Rate	**Parameters**			
0.8 ml/min				
	RT	**Area**	**Theoretical Plates**	**Tailingfactor**
Average	8.6933	4320016	7089.18	1.08
S. D	0.0200	6377.41	35.384	0.0100

% RSD	0.2304	0.1476	0.499	0.925
1.2 ml/min				
Average	5.696667	2837365	5549.93	1.11
S. D	0.0080	24842.19	57.735	0.0058
% RSD	0.1408	0.8755	1.040	0.519

h. Analysis of Marketed Formulation

The concentrations in the samples were determined using a multilevel calibration method that was established on the same HPLC equipment and under the same circumstances. This calibration method used a linear regression equation. The findings are shown in Table 9.

Table 9: Analysis of marketed formulations for Nicardipine

Injection	**Formulation 1**		**Formulation 2**	
	Peak Areas	**%Assay**	**Peak Areas**	**%Assay**
1	3262415	97.443	3277681	97.874
2	3228665	96.491	3323510	99.167
3	3225265	96.395	3294357	98.344
4	3244713	96.944	3390263	101.050
5	3210837	95.988	3270929	97.684
Mean	3234379	96.652	3311348	98.824
SD	19765.813	0.558	48557.564	1.370
% RSD	0.611	0.577	1.466	1.386

Conclusion:

The proposed method has been validated to evaluate its robustness, ruggedness, linearity, LOD, and LOQ. The validation findings indicate that these parameters conform to the permissible limits set by ICH standards. The proposed HPLC method for quantifying Nicardipine in pharmaceutical formulations was found to be user-friendly, accurate, reliable, and sensitive. This method may efficiently conduct regular quality control assessments of both pure and therapeutic dosage forms of Nicardipine. The newly developed and validated method for Nicardipine, in both parenteral and bulk forms, may be advantageous for researchers engaged in the development of advanced drug delivery systems and subsequent investigations.

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