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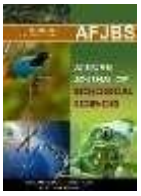

Method development and validation of Nicardipine Hydrochloride injection and its related substances by RP-HPLC

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Abstract

For the estimation and validation of related substances in nicardipine hydrochloride, such as nicardipine monoacids, nicardipine dehydroxy, nicardipine pyridine analog, nicardipine bis analog, and nicardipine dimethyl ester, which exhibits maximum absorbance at 239 nm, an RP- HPLC is a straightforward, accurate, and dependable diode array detector. The purpose of this research is to identify the related impurities present in the nicardipine hydrochloride injection and to identify whether they are complies under unites states food and drug administration guidelines. The calcium channel blocker nicardipine hydrochloride was separated using Inertsil ODS 3V 4.6*250, 5 µm. Four parts methanol and six parts potassium dihydrogen phosphate buffers make up the mobile phase. The isocratic elution mode was used to execute the chromatographic condition at a flow rate of 0.8 ml/min. The method has been validated for LOD and Precision at the LOQ level, accuracy, linearity, method precision, specificity, robustness, and forced degradation tests, in accordance with food and drug administration requirements. For nicardipine hydrochloride, the calibration curve was linear between 4 and 24 µg/ml and correlation coefficient was found to be 0.9997. The forced degradation studies shows that the mass balance was found to be 89.5, respectively. The estimation and validation of nicardipine hydrochloride injection was performed effectively by HPLC using the suggested method.

Keywords: Nicardipine hydrochloride, Nicardipine monoacids, Nicardipine dehydroxy, Nicardipine pyridine analog, Nicardipine bis analog, Nicardipine dimethyl ester.

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Introduction

Dihydropyridine calcium channel blockers, such nicardipine hydrochloride, are widely used to treat hypertension and angina pectoris. It has a therapeutic action that reduces blood pressure and induces vasodilation by specifically blocking calcium ions from entering vascular smooth muscle cells. The High-performance liquid chromatography is a most widely used analytical technique for the quantitative evaluation of pharmaceutical compounds due to its exceptional sensitivity, selectivity, and precision. Reverse phase HPLC (RP-HPLC) is particularly helpful for the analysis of hydrophobic substances like nicardipine hydrochloride and its related compounds. The interaction between a non-polar stationary phase and the non-polar analytes dissolved in a polar mobile phase in RP-HPLC allows for effective separation and quantification [1-10]. The development and validation of a dependable RP-HPLC method for the measurement of nicardipine hydrochloride and its related chemicals is critical to the quality and consistency of pharmaceutical formulations. Such a method should have the highest specificity, accuracy, and precision in separating nicardipine hydrochloride from its impurities and breakdown products. It should also demonstrate that it is suitable for routine stability testing and quality control analysis by being verified in accordance with regulatory standards [11-17]. This work presents the development and preliminary validation of an RP-HPLC method for the measurement of nicardipine hydrochloride and related chemicals. The method optimization, validation parameters are closely studied to provide pharmaceutical quality control laboratories and researchers working on nicardipine hydrochloride injection formulation synthesis with a reliable analytical tool [18-25]. Structure and molecular formula for Nicardipine HCl impurities was shown in table-1.

Materials and Methods

Chemicals and reagents

Water and methanol were utilized in accordance with HPLC grade, whereas potassium hydroxide and dihydrogen phosphate were employed in accordance with analytical research grade purchased from Qualigens, Mumbai, India.

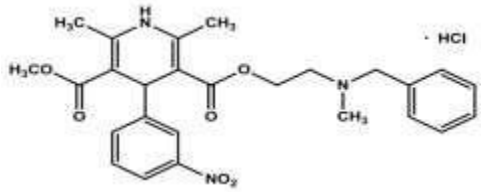
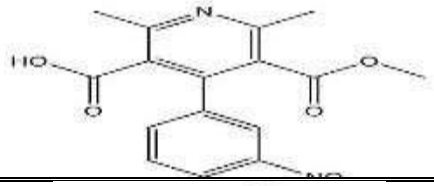
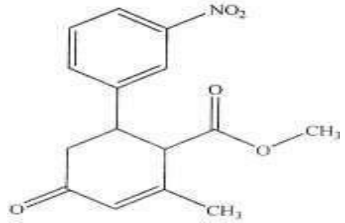
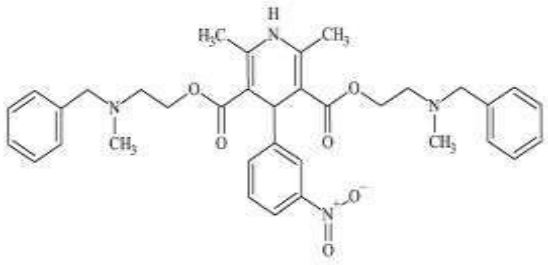
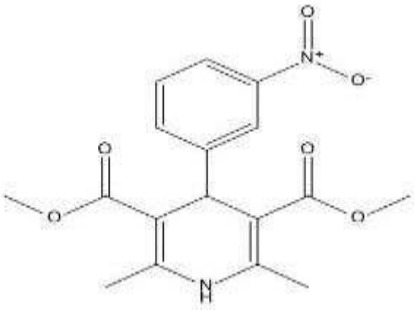
Standards and samples

Nicardipine HCl Injection was used as the sample. Nicardipine Hydrochloride, Nicardipine Monoacid, Nicardipine Dehydroxy, Nicardipine pyridine analog, Nicardipine Bis-Analog, Nicardipine dimethyl ester were used as the standard were obtained from Caplin steriles Pvt, Ltd, Chennai.

. Instruments and chromatographic condition

The following instruments were used for this current study, pH meter, vacuum oven, ultra sonicator, analytical balance, and high-performance liquid chromatography system (Agilent). Chromatographic separation was achieved with an Agilent HPLC system with a DAD detector, Inertsil ODS 3V (250 X 4.6 mm, 5 µm). methanol and potassium dihydrogen phosphate buffer (40:60%v/v) was used as mobile phase. Potassium hydroxide was used as a pH modifying agent to bring the mobile phase pH adjusted to 4.5. then it was degassed, filtered by using a 0.45 µm membrane filter and put onto the column at a flow rate of 0.8 ml/min. The drug solution was loaded to a volume of 100 µl, and at 239 nm, the detection wavelength was measured.

Table 1 Structure and molecular formula for Nicardipine HCl impurities

Chemical Name	Chemical structure	Molecular Formula
Nicardipine HCl		$C_{26}H_{29}N_3O_6 \cdot$
Nicardipine Monoacid		$C_{16}H_{16}N_2O_6$
Nicardipine Dehydroxy		$C_{15}H_{15}NO_5$
Nicardipine Pyridine analog		$C_{26}H_{27}N_3O_6$
Nicardipine Bis analog		$C_{35}H_{40}N_4O_6$
Nicardipine Dimethyl ester		$C_{17}H_{18}N_2O_6$

Preparation of buffer solution

About 5.4 g of potassium dihydrogen phosphate should be weighed and transferred into a beaker. 1000 ml of water should then be added, the mixture should be sonicated, and the mixture should be filtered through a 0.45 µm nylon filter.

Preparation of mobile phase

About 600ml of buffer and 400 ml methanol was measured and transferred into 1000ml standard flask, then sonicate the mixture for approximately five minutes. Mobile phase was used as diluent. Diluent was used as blank.

Preparation of standard stock solution (500 µg/ml)

In a 50 ml volumetric flask, 25 mg of Nicardipine Hydrochloride standard. was weighed and transferred. Next, 30 ml of diluent was added, sonicate to dissolved the sample. Dilute to the volume by using diluent and thoroughly mix.

Preparation of standard solution (0.10 µg/ml)

About 100 ml volumetric flask should be filled with 2.0 ml of the aforementioned standard stock solution. The solution should be well mixed and diluted with diluent. Pipette out 2.0 ml of the aforementioned solution into a 200 ml volumetric flask, dilute with diluent to the volume, and thoroughly mix.

Preparation of sensitivity solution: (0.025 µg/ml)

About 5 ml of the standard solution was transferred into a 20 ml volumetric flask, dilute with diluent to volume, and thoroughly mix.

Preparation of nicardipine bis analog impurity stock solution (10 µg/ml)

About 1.0 mg of the Nicardipine bis analog impurity standard was weighed and transferred into a 100 ml volumetric flask. Next, 5 ml of methanol was added, dissolve and dilute to the volume with methanol. Then the solution was mixed thoroughly.

Preparation of system suitability solution

In a 50 ml volumetric flask, 1 ml of the Nicardipine bis analog impurity stock solution was added, dilute with diluent to the volume and thoroughly mixed. Then 5 ml of the standard stock solution was added.

Preparation of placebo solution

One placebo bag was taken. 20ml of the placebo solution was transferred into clean, dried beaker or test tube. 5.0 ml of the placebo was pipetted out and transferred into a 10 ml volumetric flask, dilute with diluent to the volume and the solution was mixed thoroughly.

Preparation of sample solution (50 µg/ml)

One sample bag was taken. 20 ml of the sample was transferred into a clean, dried beaker or test tube. 5.0 ml of the sample was pipetted out and into a 10 ml volumetric flask, dilute with diluent to the volume and the solution was mixed thoroughly .

Preparation of LOD and LOQ solution

In a 20 ml volumetric flask, pipetted out 400 µl of monoacid stock solution, 400 µl of Dehydroxy stock solution, 145 µl of Pyridine analog stock solution, and 50 µl of standard stock were transferred. Diluted to volume with diluent and thoroughly mixed. This solution was consider as LOQ stock solution. 400 µl of the LOQ Stock solution were pipetted into a 20 ml volumetric flask, diluted with diluent to the appropriate volume, and thoroughly mixed. 10 ml volumetric flask was taken and 3.3 ml of the LOQ solution impurity stock solution via pipette, diluted it to volume with diluent, and thoroughly mixed.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity was checked by comparing the retention time of the analyte against blank, placebo and known impurities

Accuracy

The accuracy of an analytical technique is determined by the extent of concurrence between a measured value and a value acknowledged as either a commonly accepted true value or a recognized reference value. The three concentration of LOQ level, 100.0%, and 300.0% were prepared and used for the accuracy studies. Pipetted out 8.0 ml of the sample into three 10 ml volumetric flask separately, 200, 600, 1800 µ of LOQ stock solution (LOQ level, 100.0%, and 300.0%) were added and diluted to the volume with diluent and mixed well.

Linearity

Linearity refers to the ability of an analytical procedure to generate test results that directly correlate with the concentration (quantity) of the substance being analysed in the sample, within a defined range. Five test concentrations, ranging from 50% to 300% of working concentrations of nicardipine Hcl, nicardipine monoacid, nicardipine dehydroxy, nicardipine pyridine analog, were carried out in accordance with protocol to determine the linearity of the method. In relation to the 100% working concentration, the standard solutions were made at concentrations of 50%, 100%, 150%, 200%, and 300%. Three identical injections were put into the HPLC apparatus for each concentration.

Method Precision

Six homogeneous samples was prepared as per the test method procedure (100 % concentration) Method precision was checked by using this test solution. Pipetted out 8.0 ml of the Sample into six 10 ml volumetric flask, added 600 µL of mixed impurity stock solution and diluted to the volume with diluent and mixed well.Each sample were injected into an HPLC system and the resulting chromatograms were recorded.

Robustness

An analytical procedure robustness, which indicates its dependability under typical operating conditions, is a measure of its ability to withstand slight but intentional changes in technique parameters. In this parameter should adjust flow rate at 0.8 ml/min (±0.1ml) and pH at 4.80 (± 0.05). Pipetted out 8.0 ml of the sample into six 10 ml volumetric flask, added 600 µl of mixed impurity stock solution and diluted to the volume with diluent and mixed well. Finally, this solution was injected into an HPLC system at 20 µl, and the resulting chromatograms were recorded.

Forced degradation study

Forced degradation studies are carried out to establish degradation pathways of drug substances and drug products. They are processed by Acid, Base, Peroxide, Thermal and photolytic degradation process. For acid stress, 10 ml sample solution was transferred into a 10 ml volumetric flask and added 0.25 ml of 5 N HCl solution, mixed and kept in water bath for 2 hours at 80°C, removed flask and allowed to attain the room temperature, then neutralized with 0.25 ml of 5 N NaOH solution and mixed well. Diluted 4 ml of this solution to 5 ml with diluent and mixed well. For base stress, 10 ml sample solution was transferred into a 10 ml volumetric flask and added 0.25 ml of 5 N NaoH solution, mixed and kept in water bath for 1 hour at 80°C, removed flask and allowed to attain the room temperature, then neutralized with 0.25 ml of 5 N HCl solution and mixed well. Diluted 4 ml of this solution to 5 ml with diluent and mixed well. For peroxide stress, 20 mL sample was transferred into a 20 ml volumetric flask and added 0.5 ml of 10% hydrogen Peroxide, mixed and kept at room temperature in dark area for 5 hours and mixed well. Diluted 8 ml of this solution to 10 ml with diluent and mixed well. For photolytic stress, 8 ml of sample was Pipetted out and transferred into 10 ml volumetric flask, diluted to volume with diluent and mixed well. Then it was

exposed to 1.2 million LUX hour in normal light and 200 Watt hour/Sq.meter in UV light. For thermal stress, 8 ml of sample was pipetted out and transferred in to 10 ml volumetric flask, diluted to volume with diluent and mixed well.Then the solution were were exposed to 80 °C temperature for 14 days in a vacuum oven and then cooled to room temperature. Each stress solution were injected into the chromatographic system and the chromatograms were recorded.

Results and Discussion

The current FDA recommendations were followed during the optimization and validation of the analytical technique in order to achieve the desired levels of precision and LOD at the LOQ level, accuracy, linearity, robustness, specificity, and forced degradation research.

LOD and precision at LOQ level

LOD is computed using test replicates of a sample that is known to contain modest amounts of analyte in addition to the reported LOD. LOQ is the lowest concentration at which the analyte can be reliably identified while still satisfying bias and imprecision targets. For LOQ precision, %RSD value was found to be 3.2, 1.3,2.5, and 3.1% for all impurities. LOQ precision, %RSD value was found to be 2.2, 3.2, 4.4 and 12.2% for all impurities. The %RSD Values were within the acceptance criteria. The concentration of LOD and LOQ for all impurities were found to be 0.014µg/ml and 0.04µg/ml. Less concentration of all impurities results showed , the sensitive of the method was very high. The reports was shown in table -2 and 3.

Table 2: Results for LOQ precision

Parameters	Nicardipine Hcl	Monoacid	Dehydroxy	Pyridine analog
Mean Area	10371	11167	9003	5033
SD	334.0	149.5	227.1	158.2
% RSD	3.2	1.3	2.5	3.1
Conc. (µg/ml)	0.04	0.04	0.04	0.04
USP s/n Ratio	41	226	91	22

Table 3: Results for LOD precision

Parameters	Nicardipine Hcl	Monoacid	Dehydroxy	Pyridine analog
Mean Area	3835	3587	2799	1368
SD	84.1	113.1	122.3	166.2
% RSD	2.2	3.2	4.4	12.2
Conc. (µg/ml)	0.014	0.014	0.014	0.014
USP s/n Ratio	15	75	30	6

Specificity

The specificity of an analytical method refers to its capacity to differentiate between the analyte(s) and the additional constituents present within the sample matrix. The retention time, relative retention time, peak purity and purity threshold studies were calculated. The reports were shown in table-4. From the results it was clearly showed no additional constituents present in the standard,

impurities amd samples. This indicated the method was specific. The specific chromatograms were shown in figure1-9.

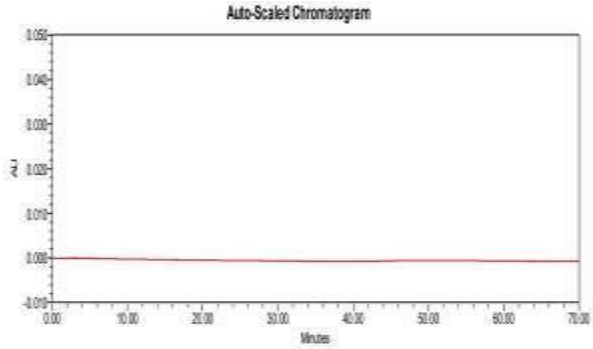


Fig. 1: chromatogram for Blank

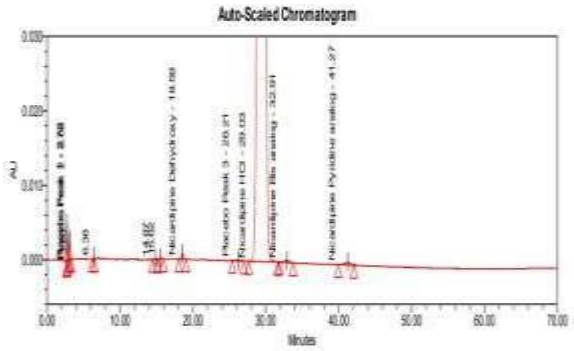


Fig. 2: Chromatogram for control Sample.

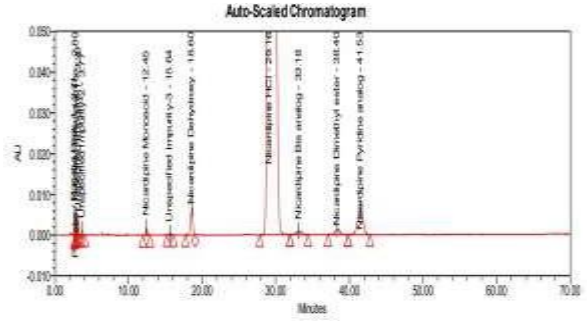


Fig.3:Chromatogram for Impurity spiked sample

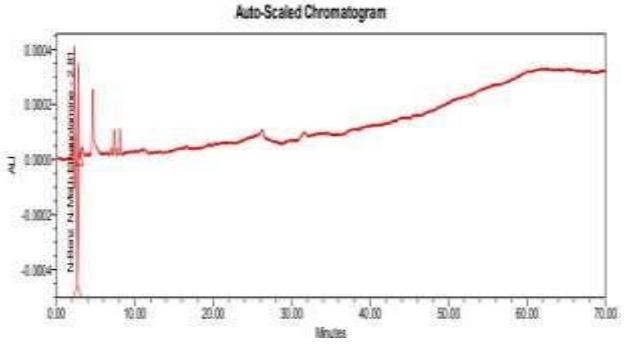


Fig.4 Chromatogram for N-M N-B ethanolamine .

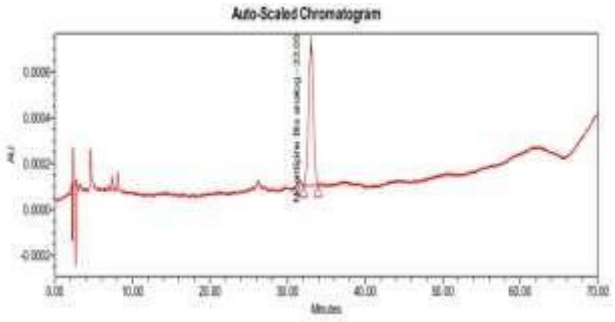


Fig. 5: Chromatogram for Nicardipine bis analog.

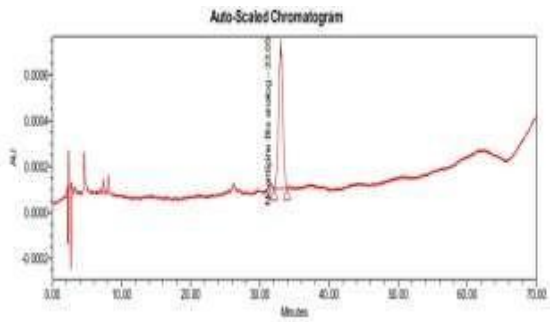


Fig. 6: Chromatogram for Nicardipine dehydroxy

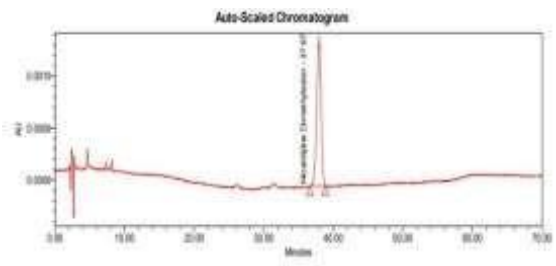


Fig. 7: Chromatogram for Nicardipine dimethyl ester

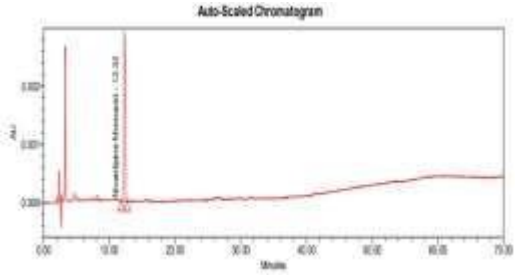


Fig. 8:Chromatogram for Nicardipine monoacid

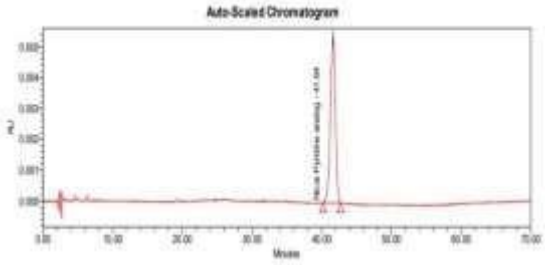


Fig. 9: Chromatogram for Nicardipine pyridine analog solution.

Table 4: Specificity reports

Standard preparation					
Impurity name	RT (min)	RRT	Purity angle	Purity threshold	Purity flag
Nicardipine Hcl	29.781	NA	0.655	0.984	No
Individual impurity					
Monoacid	12.323	NA	0.328	0.661	No
Dehydroxy	18.395	NA	0.145	0.474	No
Bis analog	33.049	NA	1.176	2.052	No
Dimethyl ester	37.967	NA	0.596	0.931	No
Pyridine analog	41.639	NA	0.096	0.336	No
Control sample					
Nicardipine Hcl	29.030	1.00	0.004	0.228	No
Monoacid	Not detected				
Dehydroxy	18.587	0.64	0.946	1.477	No
Bis analog	32.914	1.13	2.695	3.723	No
Dimethyl ester	Not detected				
Pyridine analog	41.271	1.42	1.217	1.891	No

Accuracy

The three concentration (LOQ level, 100.0%, and 300.0%) was used to evaluate accuracy for all impurities. The procedure was followed in order to create standard and spiked sample solutions at 100%, 300%, and LOQ levels of concentration. The recovery percentage was computed using the area designated for each concentration for monoacid, dehydroxy and pyridine analog impurities. The %RSD value was found to be 5.3,4.1 and 9.5 % for monoacid, dehydroxy and pyridine analog impurities. The results showed at each level meets the within the acceptance criteria. Hence, the method was found to be accurate in the range of LOQ to 300 % of specification level.The results were shown in table 5

Table-5: Reports for accuracy

	Recovery

% Level	Monoacid	Dehydroxy	Pyridine analog
LOQ (0.05%)	97.4	110.5	80.9
100%	96.9	103.3	98.6
300%	89.4	102.1	96.3
Overall mean	94.6	105.3	91.9
%RSD	5.3	4.1	9.5

Linearity

The linearity range of 50 % to 300 % for nicardipine Hcl, nicardipine monoacid, nicardipine dehydroxy and nicardipine pyridine analog The correlation Coefficient value for all impurities were found to be within the limit of not less than 0.990. So the method was linear from the above said range. The results were shown in table: 6.

Table 6: Results for linearity

Impurity	Nicardipine HCl	Monoacid	Dehydroxy	Pyridine analog
Slope	235362.298	343429.979	233290.499	136862.496
Y Intercept	1069.2	178.49	222.22	-243.99
% y-Intercept	2.72	0.32	0.20	-0.09
CC (r)	0.9997	0.9998	0.9998	0.9998
RF	NA	0.69	1.01	1.72

Method Precision

Method precision was done for the six replicate analysis of Mono acid, Dehydroxy and Pyridine analog. The %RSD was found to be 3.9, 2.3 and 2.7% for impurities. Therefore, the results showed % RSD value was found to be within the limit of the acceptance criteria. Hence, it concluded and the method was précise for the determination of Nicardipine HCl. The report was shown table 7.

Table 7: Results for method precision

Parameters	Mono acid	Dehydroxy	Pyridine analog
Average area	0.193	0.678	2.493
SD	0.00756	0.01555	0.06714
% RSD	3.9	2.3	2.7

Robustness

An analytical procedure robustness, which indicates its dependability under typical operating conditions, is a measure of its ability to withstand slight but intentional changes in technique parameters. The robustness conditions were shown in table 8. System Suitability Criteria in Robustness condition (High and Low pH of Mobile phase) was met the acceptance criteria. No intereference was observed in main peak and known impurities by diluent (Blank) and Placebo. Bis analog Impurity was co-eluted with the main peak (Nicardipine HCl) in sample by changing the Lower Buffer pH 4.80 (Lower in robustness condition $\pm$0.05). Thus the method was sensitive to the buffer pH. So the pH range 4.85$\pm$0.03 is the optimum pH for buffer and suggested to this method.

The system suitability reports and retention time, relative retention time reports were shown in table-9 and 10.

Table 8: Condition for Robustness

Robustness	Test conditions	High	Low
Flow rate (ml/min)	0.8 ml/min	0.9 ml/min	0.7 ml/min
Buffer pH	4.80	4.85	4.75

Table 9: System suitability reports for Robustness

System suitability evaluation (test condition)				
Parameter	Flow rate		pH	
	Initial	Bracketing	Initial	Bracketing
S/N ratio (sensitivity solution)	34	–	34	–
USP resolution (SST solution)	3.21	3.22	3.21	3.22
USP tailing (std solution)	0.94	0.93	0.94	0.93
%RSD (standard)	1.4	1.4	1.4	1.4
System suitability evaluation (low)				
Parameter	Before	After	Before	After
S/N ratio (sensitivity solution)	43	–	28	–
USP resolution (SST solution)	3.05	3.51	NA	NA
USP tailing (std solution)	0.94	0.92	0.95	0.95
%RSD (standard)	1.1	1.3	1.3	1.3
System suitability evaluation (high)				
Parameter	Before	After	Before	After
S/N ratio (sensitivity solution)	18	–	22	–
USP resolution (SST solution)	3.56	3.27	5.14	5.14
USP tailing (std solution)	0.89	0.96	0.93	0.92
%RSD (standard)	1.0	1.5	1.6	1.8

Table 10: RT and RRT evaluation for Robustness study

Standard preparation

Component name	Test condition		Low flow		High flow		Low pH		High pH	
	RT (min)	RRT	RT (min)	RRT	RT (min)	RRT	RT (min)	RRT	RT (min)	RRT
Nicardipine Hcl	29.45	NA	34.959	NA	29.691	NA	29.244	NA	29.897	NA
Control sample										
Nicardipine Hcl	28.71	1.00	33.969	1.0	28.840	1.00	28.518	1.00	29.277	1.00
Nicardipine monoacid	12.13	0.42	ND	NA	ND	NA	ND	NA	ND	NA
Nicardipine dihydroxy	18.52	0.65	21.380	0.63	18.082	0.63	18.792	0.66	18.237	0.62
Nicardipine bis analog	32.21	1.12	38.322	1.13	32.567	1.13	30.282	1.06	35.023	1.20
Nicardipine pyridine analog	40.74	1.42	47.902	1.41	40.582	1.41	39.294	1.38	42.396	1.45
Spiked sample										
Nicardipine Hcl	28.75	1.00	33.964	1.00	28.832	1.00	28.068	1.00	29.885	1.00
Nicardipine monoacid	12.42	0.43	14.329	0.42	12.163	0.42	12.707	0.45	12.148	0.42
Nicardipine dihydroxy	18.52	0.64	21.289	0.63	18.085	0.63	18.798	0.67	18.258	0.63
Nicardipine bis analog	32.33	1.12	38.579	1.14	32.506	1.13	30.194	1.08	34.98	21.21
Nicardipine pyridine analog	40.85	1.42	48.130	1.42	40.530	1.41	39.367	1.40	42.43	1.47

Forced Degradation study:

Drug substances and products undergo forced degradation, which breaks them down under worse circumstances than stress. This leads to the formation of breakdown components, which can be scrutinized to assess the stability of the molecule, identify the pathways of degradation, and confirm the effectiveness of the stability-testing methods employed. The % degradation acceptance criteria is 5-20%. Based on above Degradation study, observed that Photolylic degradation was observed 20.662 %. Mass balance of all stressed conditions were met the acceptance criteria except Photolytic degradation. In Photolytic degradation condition, 82.4% mass balance was observed, The degradation study chromatogram was shown in figure 10 and the results were shown in table 11.

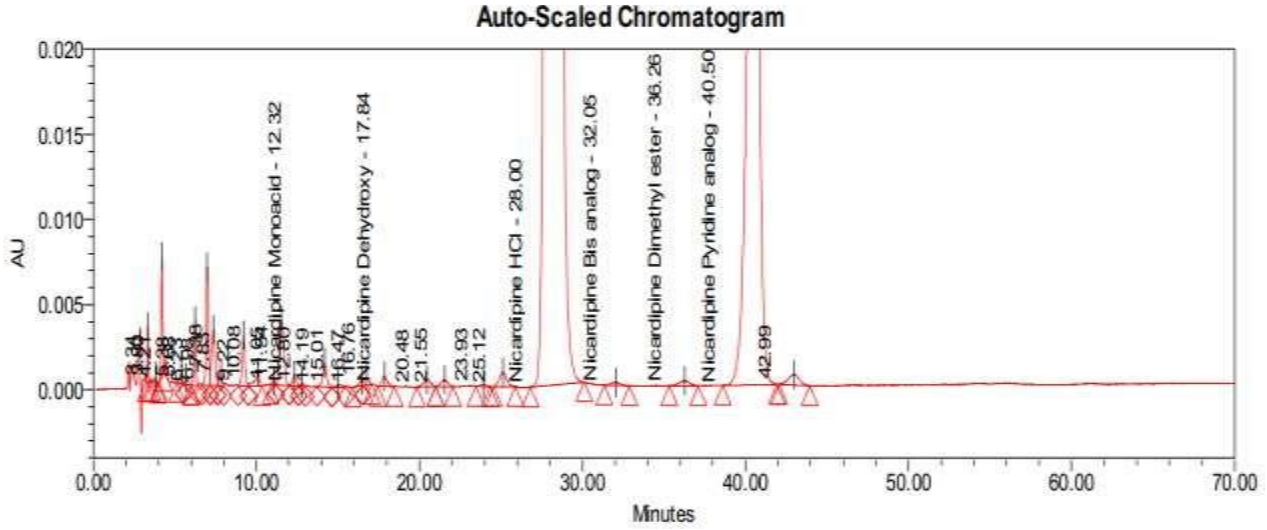


Fig 10. Chromatogram for Degradation study

Table 11: Results for Forced Degradation

Impurity@RRT	Control sample	Mode of degradation				
		Acid	Base	Peroxide	Light	Thermal
		0.1N HCl-0.5mL-80°C-65hr (0.1 mg/ml)	0.1N NaOH_0.5mL-80°C -7 hr	30% H_2O_2-0.5ml-RT-22 hr	1° Pack	Thermal 80°C-14 days
Tot imp (RS-1)	0.351	1.704	3.012	2.581	19.96	2.731
Tot imp (RS-2)	0.171	0.682	1.382	0.675	0.694	1.102
Total impurity	0.522	2.386	4.394	3.256	20.66	3.833
%Assay	96.9	93.0	90.7	89.6	59.6	93.3
Mass balance	-	97.9	97.6	95.4	82.4	99.7

Conclusion

A highly effective isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) technique was developed, fine-tuned, and validated for investigating the associated impurities of nicardipine hydrochloride. Pre-validation has been completed on the suggested HPLC method for identifying organic contaminants in "Nicardipine HCl Injection." A test for system appropriateness is developed. It is discovered that the procedure is accurate, linear, precise, and specific. By determining the LOD and Precision at the LOQ Level, the method sensitivity was assessed. After robustness metrics were run, it was discovered that the approach is pH-sensitive to the buffer. To identify the degradation pathways and demonstrate that the approach indicates stability, a forced degradation research was carried out. Thus, this method has been validated and is appropriate for both regular and stable samples.

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