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Development and Validation of Rp-Hplc Method for Estimation of Nicardipine (Anti-Hypertensive) Drug

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ABSTRACT

A simple, accurate ,sensitive and precise Reverse Phase- High Performance Liquid Chromatographic assay method for estimation of Nicardipin in pharmaceutical dosage form was successfully developed.

The chromatographic separation was performed on Waters X Bridge, C18, 5µ, 4.6 × 150mm column. The mobile phase A consists of pH-3.5 phosphate buffer and acetonitrile in the ratio 40:60v/v. mobile phase was delivered isocratic at a flow rate of 1.0ml/min. Samples were injected 20µ L the column temperature was kept at 35ºC and sample temperature 10ºC. The wavelength 250 nm were selected for the evaluation of the chromatogram. The retention time of the drug was found to be 3.3 min. The developed method was found to be linear in a concentration range of 60-180 µg/ml of the drug (r^2=0.9993). The low value of % RSD indicates reproducibility of the method. The method was validated as per ICH guidelines. Thus this method can be used for routine analysis of Nicardipine formulation and to check the stability of bulk samples.

Keywords: Nicardipine , RP-HPLC, Method validation, Assay method

I. INTRODUCTION

Nicardipine is used to treat High blood pressure (Hypertension), it also used for Raynaud's Phenomenon.. Nicardipine belongs to the class of calcium channel Blockers. Nicardipine was Approved by FDA in December 1988. It is more selective for cerebral and coronary blood vessels.

➢ **Objectives:**

The main objectives of the study are as Follows:

- To develop new, simple, sensitive, accurate, and economical analytical method for the determination of assay of Anti-Hypertensive Drug by RP-HPLC.
- To Validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

01. DRUG PROFILE:

Name:Nicardipine

Structure:

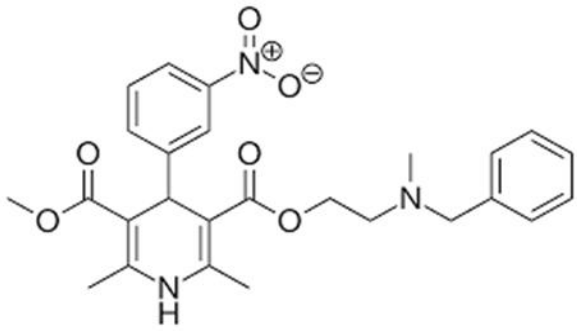

Fig. 1 Structure of Nicardipine

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Table .1 General profile of Nicardipine

Category	**Anti- hypertensive agent**
Chemical Name	3-{2-[benzyl(methyl)amino]ethyl} 5-methyl 2,6-dimethyl- 4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Molecular Formula	C26H29N3O6
Molecular Weight	479.525 g/mole
Odour	Odourless
Description	White crystalline powder
Solubility	Soluble in DMSO (1mg/ml), ethanol:water 25:75-70:30, propylene glycol, or methanol; slightly soluble in acetone, 100% ethanol, chloroform and water and insoluble in 0.1M NaOH
Odour	Odourless
Pka Melting point	8.18 136°c to 138°c

Pharmacological Action

By deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum, nicardipine inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload.

Pharmacodynamics

Nicardipine, a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal's variant angina. Nicardipine is similar to other peripheral vasodilators. Nicardipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes possibly by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload.

II. PLAN OF WORK:

Estimation of Anti-Hypertensive Drug in tablet dosage form will be done by following methods.

2.1 Selection of Drugs and Formulation

- By literature and market survey

2.2. Selection of analytical techniques

- Estimation by UV-Visible spectroscopy.
- Identification by IR Spectroscopy.
- HPLC method.

2.3. Method development by RP-HPLC.

- Selection of preliminary HPLC conditions.
- Selection of mobile phase.
- Selection of column.
- Selection of wavelength.
- Selection of Flow rate**.**
- Selection of Injection Volume.
- Selection of column Temperature.
- Selection of sample Temperature.

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- Analysis of laboratory mixture.

2.4. Validation of proposed method.

- System suitability parameter
- Linearity and Range
- Accuracy
- Precision

A. System precision.
B. .Method precision.
C .Intermediate precision.

- Specificity
- Robustness

III. EXPERIMENTA.MATERIAL:

3.1 DRUG:

Table 3.1: Drug and drug product samples suppliers and manufacturers

Name of drug and product	Supplier and manufacturer by
Nicardipine	Mylan Laboratories Ltd
Nicardipine Tablet 30 mg	Mylan Laboratories Ltd

3.2 REAGENTS:

Table 3.2: List of Reagent

Sr.No	Chemical	Make
1	Water	Rankem
2	Acetonitrile	Merck life science
3	Phosphoric acid 88%	Merck life science
4	Potassium dihydrogen phosphate	Merck life science
8	0.45 µ PVDF membrane disc filter	Mdi

3.2 INSTRUMENTS:

3.2.1HPLC:

Make	Waters e2695
Detector	Waters 2998 -PDA Detector
Software	Empower PRO4

3.2.2. SPECTROPHOTOMETER: Double beam UV-visible spectrophotometer with 10mm Matched quartz cells

Model	UV1800
Make	shimadzu

3.2.3 ANALYTICAL BALANCE: Digital Analytical balance

Model	XPE26DR
Make	Mettler Toledo

6.2.4 PH METER: Digital pH Meter

Make	Thermo Scientific
Model	Orian Star A211

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3.3 METHOD

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION

The analytical RP-HPLC method developed and method validated. The standard solution of NicardipineAPI and tablet solution was used for method development trials to optimize and validation the method for determination of Nicardipine.

PREPARATION OF SOLUTION

1. Preparation of Diluted Orthophosphoric Acid

Transfer 10 ml of Concentratedorthophosphoric acid in 100 ml volumetric acid added water up to mark and mixed well.

2. Preparation of Buffer pH 3.2:

Dissolve 2.73 g of potassium dihydrogen phosphate in 1000 mL of water and mix well. Adjust to pH 3.5 ± 0.05 using diluted orthophosphoric acid. Filter the solution through a 0.45µm nylon membrane filter.

3. Preparation of Mobile Phase:

Prepare a mixture of Buffer pH 3.5 and acetonitrile in the ratio 40:60 v/v respectively and mix. Sonicate to degas.

4. Preparation of Diluent:

To dissolve the API and tablet prepare a mixture of Water and acetonitrile in the ratio 20:80 v/v respectively and mix. Sonicate to degas.

5. Preparation of Standard solution:

Weighed and transfered accurately about 60 mg of Nicardipinestandard into 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 15 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 5.0 mL of this solution to 25 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 2.0 mL of filtrate and then collected the sample.

(Concentration of Nicardipine standard solution: 120 ppm)

6. Preparation of Sample solution:

Weighed and transferred 10 Nicardipinetablets in to 250 mL clean and dry volumetric flask. Added about 200 mL of diluent, sonicate for 30 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 10.0 mL of this solution to 100 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Sample Solution: 120 ppm)

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION

1) Selection of Stationary phase:

On the basis of literature survey, drug polarity, physical and chemical nature the reversed phase HPLC mode was selected and number of carbon present in molecule (analyte) stationary phase with C18 bonded phase was taken i.ewaters X Bridge, 150 x 4.6 mm, 5µ

2) Selection of Mobile Phase:

The selection of mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey drug polarity and finally mobile phase was selected is Buffer pH 3.5 and acetonitrile in the ratio 40:60 v/v respectively. To give better results.

3) Selection of Detector and Detection wavelength:

The important aspect of chromatography is detection wavelength the UV-visible 2489 and PDA 2998 detector was selected, as it is reliable and easy to set at the correct wavelength and 250 nm wavelengths was selected as detection wavelength for detection of Nicardipine in drug solution.

4) Selection of oven temperature:

The column temperature 35 °C was selected to minimized day to day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

5) Selection of Sample temperature:

The sample temperature 10°C was selected to minimized day to day variation of drug response due to fluctuations in the ambient temperature.

Final reversed phase High performance liquid chromatographic condition.

Column	:	Waters X Bridge, 150 x 4.6 mm, 5µ
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL

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Wavelength	:	250 nm
Column Temp	:	35°C
Sample Temp	:	10°C
Run Time	:	8.0 minute
Retention Time	:	About 3.3 minutes

IV. RESULTS AND DISCUSSION

The stability indicating RP-HPLC method was developed on the base of physical and chemical properties of drug molecule and validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity and robustness.

4.1 Selection of Wavelength

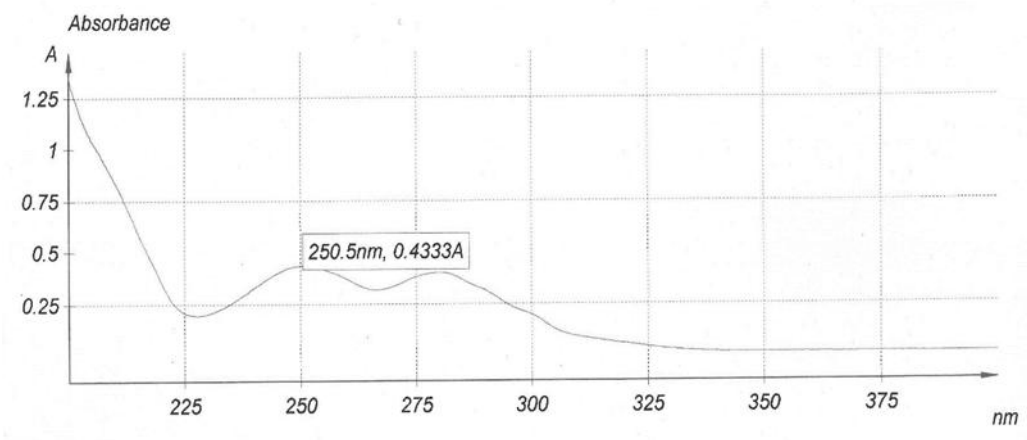

Figure 3.1 Spectra showing λ max of Nicardipine

4.2 Reverse Phase High Performance Liquid Chromatography Method Development

Different trials taken were as follows:

TRIAL: 1
Chromatographic Conditions:

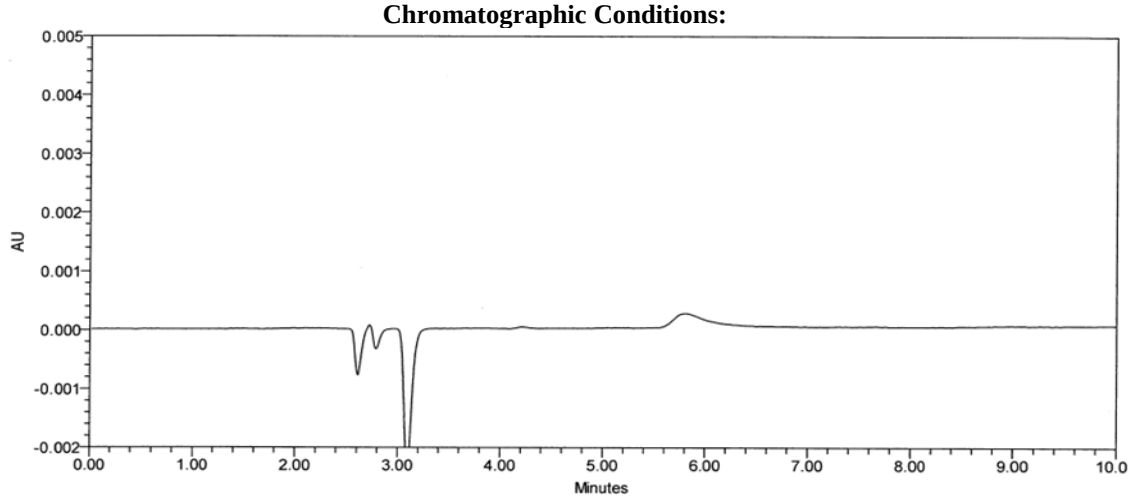

Fig. 4.2 Typical chromatogram for Trial- 1

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TRIAL: 2

Chromatographic Condition:

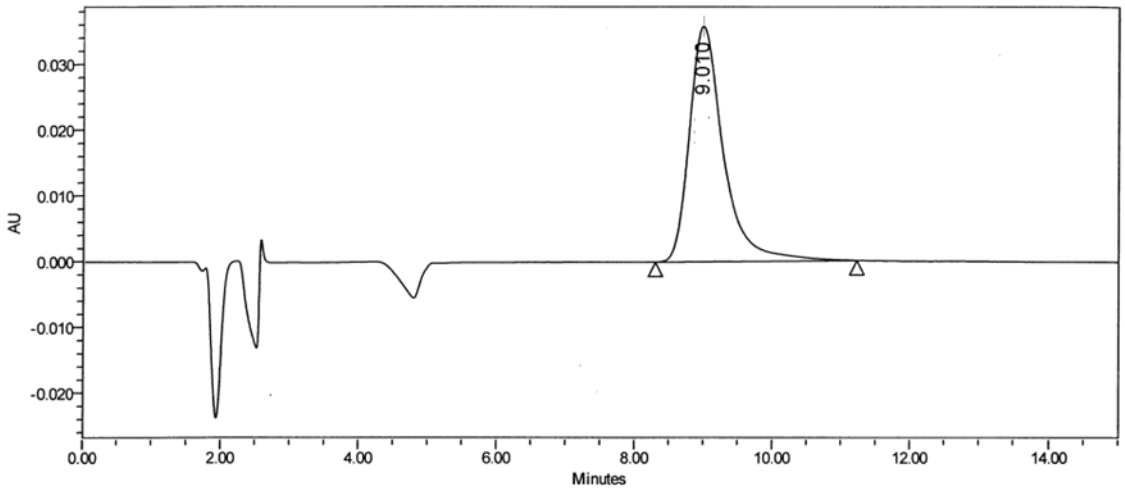

Fig. 4.3 Typical chromatogram for Trial- 2

4.3. METHOD VALIDATION

The following parameters were considered for the analytical method validation of title ingredients.

- System Suitability.
- Specificity.
- Linearity.
- Accuracy.
- Precision.
  a. System Precision.
  b. Method Precision.
  c. ntermediate Precision.
- Robustness.

4.3.1 Specificity: (Identification, Interference & Peak Purity)

The specificity of developed method was determined by Injecting Blank (Diluent), standard solution, placebo solution and sample solution.

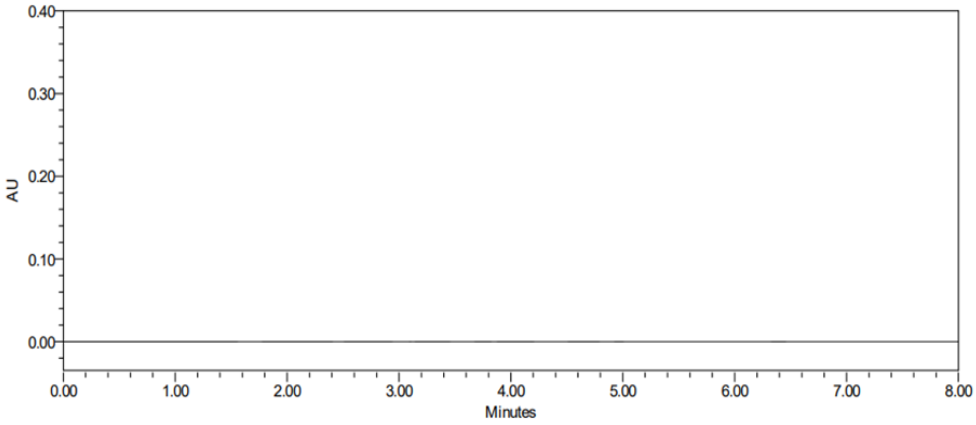

Fig 4.4 Chromatogram of BlankFig4.5 Chromatogram of Standard

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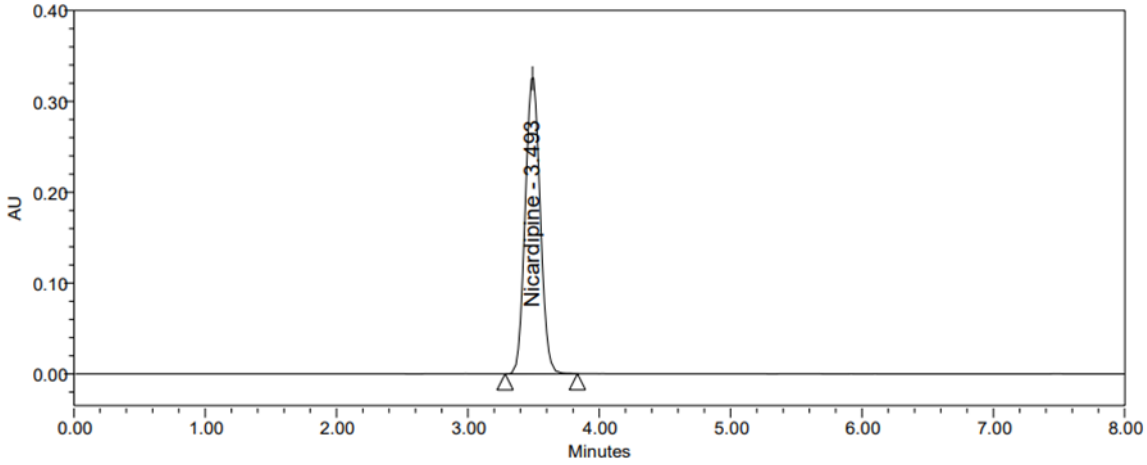

Fig 4.5 Chromatogram of Sample

4.3.2. LINEARITY:

Linearity was evaluated in the range of 50% to 150% of Nicardipine for working concentration. The working concentration of Nicardipine is 120 ppm

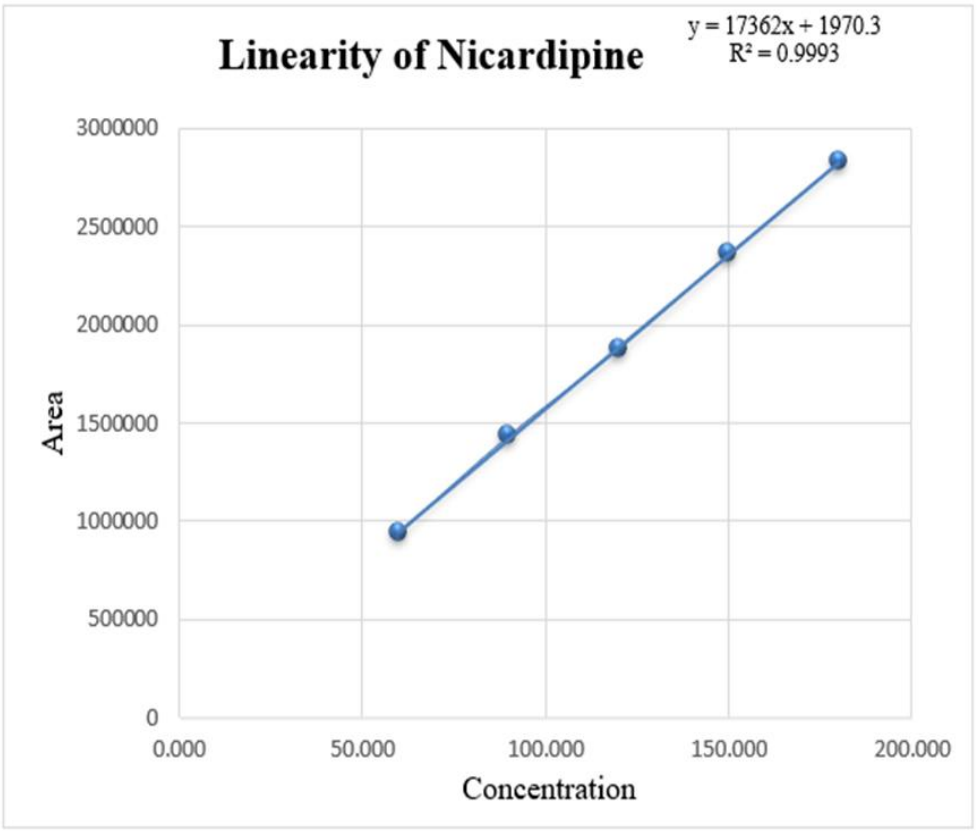

Fig 4.6 Linearity plot of Nicardipine

4.3.3 Accuracy (Recovery):

Evaluated accuracy at three levels 50%, 100% and 150% of the working concentration for Nicardipine. The working concentration level of Nicardipine is 120 ppm. Each level prepared in triplicates

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Table : 4.1% Recovery for Nicardipine

Level %	Conc added (µg/mL)	Injection-1	Injection-1	Peak Area	% Recovery	% Mean recovery
50%	60	938745	936658	937702	100.7	**100.6**
	60	940065	939349	939349	100.7	
	60	935127	933870	934499	100.2	
100%	120	1866847	1876345	1871596	100.4	**100.4**
	120	1855587	1861058	1858323	99.7	
	120	1851439	1849654	1850547	99.2	
150%	180	2806947	2824852	2815900	100.7	**100.7**
	180	2816308	2810023	2813166	100.6	
	180	2821280	2826647	2823964	100.1	
% Overall Mean recovery						**100.6**

4.3.4 Precision:

4.3.4.1 System Precision:

Single injection of Blank (Diluent) and six replicate injections of Standard solution were injected into the chromatographic system. The data obtained is summarized in Table 7.7

Table 4.2 System precision

Component	**Nicardipine**
USP Tailing	1.1
Theoretical Plates	13908
S. No.	**Peak Area**
1	1880634
2	1876318
3	1891453
4	1879548
5	1883640
6	1890164
Mean Area	**1883626**
%RSD	**0.3**

4.3.5 Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 4.3 Robustness for Nicardipine

Changes in parameters	**Values**	**Retention time**	**% Assay**	**% Difference**
Control	**As Such**	3.2586.	99.7	NA
Change in Flow rate (± 0.1 mL/min)	**+ 0.1 mL/min**	3.496	99.5	-0.2
	- 0.1 mL/min	3.0902	99.7	0
Change in wavelength (± 5 nm)	**+ 5 nm**	3.302	99.4	-0.3
	- 5 nm	3.023	100.0	0.3
Change in Column temperature	**+ 5°C**	3.210	99.5	-0.2
	- 5°C	3.435	99.7	0

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(± 5°C)				

V. CONCLUSION

The stability indicating RP-HPLC method was found to be simple, rapid, sensitive, specific, precise, accurate and cost effective for estimation of Nicardipine in tablet dosage form and bulk drugs substance. This technique was employed in the present investigation for estimation of Nicardipine using HPLC with Waters X Bridge, 150 x 4.6 mm, 5µ column, UV/PDA detector at 250 nm wavelength, flow rate was 1 ml/min, injection volume with empower Software was used for the study. The standard and sample solution of Nicardipine were prepared in diluent. the column make injection volume and different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was Buffer pH 3.5 and acetonitrile, the wavelength 250 nm were selected for the evaluation of the chromatogram of Nicardipine respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: acetonitrile. This selected chromatographic condition gave good resolution and optimum retention time with appropriate theoretical plates, tailing factor.

The practically taken development trails results and validation of developed RP-HPLC results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of Nicardipine in tablet dosage form and bulk drugs substance.

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