International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.14 | Issue 4 | Oct - Dec -2024
www.ijpir.com
DOI :https://doi.org/10.61096/ijpir.v14.iss4.2024.599-608

ISSN: 2231-3656
Print: 2231-3648

Research

Designed Reverse Phase HPLC Method for Validation for determination of API Nifedipine in the Nifedipine 20 mg Tablet Formulation

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Published on: 17 Dec 2024

Published by:
DrSriram Publications

Abstract

A novel, safe and sensitive method of Designed Reverse phase HPLC method for validation for determination of API Nifedipine in the Nifedipine 20 mg tablet formulation. It has been developed for the assay of Nifedipine in its tablet formulation. The method have been developed and validated for the assay of Nifedipine using acetonitrile. Which does not shows any interference in Reverse phase HPLC estimations. All the parameters of the analysis were chosen according to ICH [Q2 (R1)] guideline and validated statistically using RSD and %RSD along with neat Spectrogram. The percentage recoveries for ranged from 99.97-100.0%, respectively. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations. The method gives resolution with a short analysis time. The method parameter was validated and establishes to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of in medical dosage form.

Keywords: Nifedipine, Acetonitrile, Resolution, RP-HPLC, Blood brain barrier, Anti –hypertensive, Dihydropyridine.

INTRODUCTION

Calcium channel blockers (CCB), calcium channel antagonists or calcium antagonists are a group of medications that disrupt the movement of calcium (Ca^{2+}) through calcium channels. Calcium channel blockers are used as antihypertendive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients. Calcium channel blockers are also frequently used to alter heart rate (especially from atrial fibrillation), to prevent peripheral and cerebral vasospam, and to reduce chest pain caused by angina pectorisis. N-type, L-Type, and T-Type voltage – dependent calcium channels are present in the zona glomerulosa of the human adrenal gland, and CCBs can directly influence the biosynthesis of aldosterone in adrenocortical cell, with consequent impact on the clinical treatment of hypertension with these agents. CCBs have been shown

to be slightly more effective than beta blockers at lowering cardiovascular mortality associated with stroke, but they are associated with more side effects. Potential major risks however were mainly found to be associated with short-acting CCBs.

Nifedipine

Nifedipine (1,4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl) - 3, 5-pyridine dicarboxylic acid dimethyl ester) is a dihydropyridine calcium channel blocker used widely in the management of hypertension and angina. It is an important calcium channel blocker with peripheral and coronary vasodilator activity Nifedipine is highly photosensitive and thermally unstable compound.Nifedipine is a dihydropyridine, a methyl ester and a C-nitro compound. It has a role as a calcium channel blocker, a vasodilator agent, a tocolytic agent and a human metabolite.

Molecular Weight :346.3 g/mole,

Molecular Structure : $C_{17}H_{18}N_2O_6$

Aim of the study:The aim of designing a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the validation of Nifedipine in a 20 mg tablet formulation is to establish a precise, accurate, and reliable analytical method for the quantitative determination of Nifedipine, ensuring the quality, efficacy, and safety of the tablet formulation. The method should be robust enough to be used for routine analysis in quality control laboratories.

Objectives:-

MATERIALS AND METHOS

Equipment - Instrument –Glassware-Standard –Solvent-Chemicals Requirement

Sr.No	Requirement		Identification number
1	Analytical Balance	Sartorius	QC/BAL/026
2	pH Meter	Lab India	QC-pH-002
3	Detector	NA	UV, 270nm
4	0.45 nylon membrane filter	Sartorius	membrane filter
5	Glassware	Type A grade	Beaker, volumetric flask, pipette etc.

Experiment Requirements

Requirement: Chemical, Reagent, Placebo and Standards

Sr.No	Requirement		Mechanism Use
1.	Water	Reagent	Solvent
2.	Potassium Di hydrogen phosphate	Chemical	buffering agent- Lower the pH
	Trtrahydrofuran	Chemical	a competing base for the retention control and peak shape improvement
3.	Ortho Phosphoric acid	Chemical	If the pH of the mobile phase needs to be increased to enhance LC separations then ammonium hydroxide (ammonia solution) is suitable.
4.	Methanol	Reagent	Lower boiling point, high solubility and low toxicity.
5.	Acetonitrile	Reagent	The choice-solvent for almost all chromatographic separations, due to its low chemical reactivity, high miscibility with water mixtures, low viscosity and low ultraviolet cut-off.
6.	Sodium Hydro xide	Merck	Ph adjustment
7.	Hydrochloric acid	Rankem	Of the mobile phase needs to be increased to enhance LC separations then ammonium hydroxide (ammonia solution) is suitable.
8.	Hydrogen Peroxide	Ranken	Limits of detection are very low for both peroxides, and the linear ranges for determination can be adapted easily to varying sampling conditions by dilution of either the sample or the reagents.
9.	Nifedipine	Reference standard	Active material with potency

Sr.No	Requirement		Mechanism Use
10.	Placebo	Product Placebo	Mixture of excipient in product
11.	tablets IP 80 mg	Tablets	Finished product

Design of Experiment –DOE by different trails by Reverse Phase -HPLC Method
Requirement: Chemical, Reagent, Placebo and Standards

Sr.No	Requirement		Mechanism Use
1.	Water	Reagent	**Solvent**
2.	Sodium Di hydrogen phosphate	Chemical	buffering agent- Lower the pH
3.	Ortho Phosphoric acid	Chemical	If the pH of the mobile phase needs to be increased to enhance LC separations then ammonium hydroxide (ammonia solution) is suitable.
4.	Acetonitrile	Reagent	The choice-solvent for almost all chromatographic separations, due to its low chemical reactivity, high miscibility with water mixtures, low viscosity and low ultraviolet cut-off.

Selection of Chromatographic System

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1223A), binary pump (G1412A), auto injector (G1927C), column oven (G1240B), DAD detector (G1815C) and Empower (software).

The published methods of analysis for determination and separation of Nifedipine in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Nifedipine

Selection of Buffer in Mobile Phase

Dilute orthophosphoric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (60:40) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of Mobile Phase

Different ratios of Acetonitrile and Buffer was used to optimize the retention time from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Acetonitrile) (60:40) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of HPLC Column

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Xterra RP, 155 mm X 4.5, µm or equivalent column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

Selection of Diluent / Solvent for extraction

Different solvents were tried including single solvent and combination of solvents like ACN: Water, Buffer in different concentrations, But Nifedipine Tablet gets dissolved in Acetonitrile. Hence first stock was prepared in methanol and followed by second dilution done in diluents as (Buffer: Acetonitrile) (60:40) %v/v same as that of mobile phase to reduce the peak shape related problems.

The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria.

Methodology

Chemical and Reagent

Sr.No	Chemical	Source	Grade	Batch No	Purity
1.	**Water**	**Merck**	**HPLC grade**	**PE2985**	**NA**
2.	**Sodium Dihydrogen phosphate dehydrate**	**Merck**	**GR Grade**	**HRU235694**	**NA**
3.	**Orthophosphoric acid**	**Merck**	**GR Grade**	**QD36954**	**NA**
4.	**Acetonitrile**	**Rankem**	**HPLC grade**	**TS232223**	**NA**
5.	**Nifedipine standard**	**USP**	**Pharmocopiea**	**RS/24/008**	**99.6%**
6.	**Nifedipine placebo**	**In-house**	**In-house**	**----**	**NA**
7.	**Nifedipine 10 mg**	**In-house**	**In-house**	**NELM56984**	

Instruments, Equipment's and Apparatus

Sr.No	Requirement		Identification number
1.	HPLC	Agilent	QC-HPLC-005
2.	Dissolution Apparatus	Lab India	QC-Disso-003 USP type ll paddle
3.	Analytical Balance	Sartorius	QC-BAL-006
4.	pH Meter	Lab India	QC-pH-006
5.	Column	Inertsil	Xterra RP, 155 mm X 4.5, µm or equivalent column
6.	Detector	NA	UV, 275± 5 nm
7.	0.45 nylon membrane filter	Sartorius	0.45 membrane filter
8.	Glassware	Type A grade	Beaker, volumetric flask, pipette etc.

Preparation of dilute orthophosphoric acid: Dilute2 ml of orthophosphoric acid to 200 ml with water and mix.
Preparation of Buffer solution: Weight and transfer about 2.36 of Sodium Di hydrogen phosphate dehydrate into a beaker containing 1000ml of water. Adjust pH of the solution to 5.0± 0.05 with Orthophosphoric acid and filter through 0.45µ nylon membrane filter.

Preparation of mobile phase: Thoroughly mix buffer and acetonitrile solution in the ratio of 65: 35 % v/v
Dissolution

Medium	:	Purified Water
Volume	:	900ml
Apparatus	:	USP type ll
Speed	:	50rpm
Temperature	:	37±0.5ºC
Sampling point	:	30 minutes

Chromatographic conditions: Column : Xterra RP, 155 mm X 4.5, µm or equivalent column or Equivalent,Wavelength: UV 275± 5, Flow rate Injection : 1.2ml/minute Volume: 20µLColumn oven Temperature: 35°C Run time: 10 minute.

Preparation of the standard solution: Accurately weigh and transfer about 58 mg of Nifedipine standard into a 200 ml volumetric flask add about 120.0ml of dissolution media t and sonicate to dissolve and dilute to volume with diluent. Transfer 2.0 ml of this solution to 100 ml volumetric flask and dilute the volume with dissolution media and mix.

Preparation of Test Sample solution: Set the parameters of dissolution apparatus as mentioned above. Place one tablet into each of the dissolution jar. At the end of the specified time point withdraw 10ml of the sample solution through 10 µm full flow filters from each dissolution vessel .Filter the solution through 0.45 membrane filter by discarding first 5ml of filtrate

Evaluation of System Suitability parameters: The column efficiency as determined for the Nifedipine from standard solution is not less than 2000 theoretical plates and tailing factor for the same peak is not more than 2.0, The relative standard deviation for Nifedipine peak area obtained from five replicate injections of standard solution is not more 2.0, The rention time of peak is Nifedipine about 5 minutes

Specificity and System suitability

- **Specificity** is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix [13]. In case of an HPLC method, it is assured by complete separation of peak(s) of analyte(s) from other peaks originated from the sample matrix
- **The System Suitability Testing (SST)** is used to verify that an analytical method was suitable for its intended purpose the day the analysis was done. It is an essential parameter to ensure the quality of the method for correct measurements.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	**Identification**	Prepared standard and sample solution as per the test method and inject into the chromatographic system	
2.	**Blank and Placebo Interference**	Prepared blank and placebo solution as per the test method and inject into the chromatographic system	
3.	**System Suitability**	Prepared standard as per the test method and inject five times into the chromatographic system	
4.	**System Suitability Acceptance Criteria**		
5.	**Identification and RT Confirmation:** • The retention time of standard solution and sample solution should be comparable with respect to retention time • The retention time of analyte peak obtained from sample solution should be within ±0.5 minutes of the retention time of analytic peak obtained from the standard solution **Blank and Placebo Interference:** There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard **System Suitability** • The column efficiency as determined for the Nifedipine from standard solution is not less than 32000 theoretical plates. • Tailing factor for the same peak is not more than 2 • The relative standard deviation for Nifedipine peak area obtained from five replicate injections of standard solution is not more 2.		
Observed Value			
6.	Identification and RT Confirmation	Name: Standard; Sample 5 mg Retention time in minute: 4.153; 4.235	The retention time of standard solution and sample solution should be comparable with respect to retention time The retention time of analyte peak obtained from sample solution should be within ±0.5 minutes of the retention time of analytic peak obtained from the standard solution
Conclusion: RT of Nifedipine obtained with standard and test sample are comparable. Hence method is specific			
7.	**Blank and Placebo Interference**	There are no interference peak observed due to place to at the retention time of Nifedipine peak. Hence method is specific	There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard

Sr.No	Validation Parameter		Results	Acceptance Criteria
8.	System Suitability	Theoretical plate	7231	The column efficiency as determined for the Nifedipine from standard solution is not less than 3000 theoretical plates
		Tailing Factor	1.02	Tailing factor for the same peak is not more than 2.
		Peak Area	0.08	The relative standard deviation for Nifedipine peak area obtained from five replicate injections of standard solution is not more 2.0

System Suitability				
Sr.No	**Retention Time**	**Peak Area**	**Theoretical Plates**	**Asymmetry**
1.	4.812	217760	5511	1.02
2.	4.835	218807	5675	1.02
3.	4.836	219933	5603	1.03
4.	4.835	214178	5640	1.04
5.	4.853	213104	5622	1.02
Mean	4.832	214956	622	1.03
SD	0.21	179.01		
% RSD	0.15	0.05		

Conclusion :

- The retention time of standard solution and sample solution is comparable with respect to retention time
- There is no any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard
- The column efficiency as determined for the Nifedipine from standard solution is not less than 2000 theoretical plates
- Tailing factor for the same peak is not more than 2.
- The relative standard deviation for Nifedipine peak area obtained from five replicate injections of standard solution is not more 2

Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Sr.No	**Validation Parameter**	**Results**		**Acceptance Criteria**
Method and Procedure				
1.	**Method**	Five linearity solutions were prepared by using Nifedipine standard at concentration levels ranging from 50% to 150 % of target concentration of Nifedipine. Measured the peak area response of solution at Level 1 and Level 5 six times and other levels		
2.	**Acceptance criteria**	**Linearity:** • The co-relation is not less than 0.999 • The % Y intercept is between +5 % • % RSD of peak Reponses of 02 % level and 120% level should be NMT 2.0		
3.	Observed results	Correlation Coefficient	0.99998	Correlation coefficient should be not less than 0.999
		%y-intercept	-0.35	%y-intercept should be ±2.0

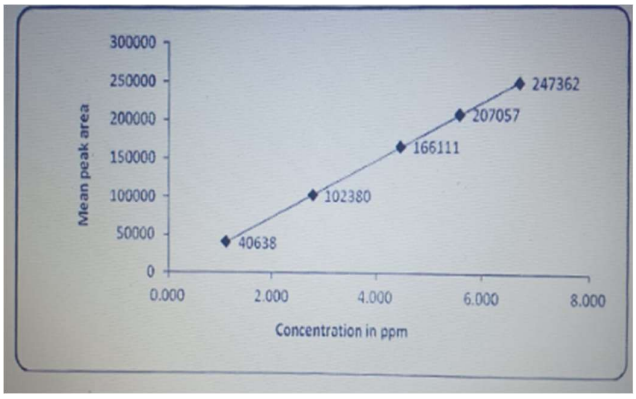
Sr.No	Validation Parameter	Results		Acceptance Criteria	
		Residual were within ±5 % of the 100% concentration response		Residual should be within ±5 % of the 100% concentration response	
		% RSD at lower level	0.12	% RSD of peak area response of 6 replicates at lower and higher levels should be more than 2.0	
		% RSD at higher level	0.03		
Linearity level	**Concentration in ppm**	**Area-Average**	**% of RSD**	**Statistic Analysis**	
Level -1	1.123	42651	0.12	R^2	0.99996
Level -2	2.7878	112329	---	Slope	37215.08
Level -3	4.467	151171	---	Y Intercept	-743.08
Level -4	5.5765	227078	--	% Y Intercept	-0.36
Level 5	6.687	237398	0.04	Correlation coefficient	0.999999

Conclusion :
Response of Nifedipine is linear overt the concentration range 20% to 120% target concentration

Linerity level	Concentraion in ppm	Y –Practical responses i.e mean peak areas are obtaines	Theroticalrepbse	Residaul	Residual Squares
Level-1	1.113	406381	456943	761	692915
Level-2	2.628	1023802	114562	615	411392
Level-3	4.321	1661112	165287	895	800621
Level-4	5.265	2070572	225634	589	181841
Level5	6.862	2473624	222548	712	565512
		Reqsidual sum of square			1875242

- Trend line equation
- Y=MX + (C)
- Y= Response
- X= Concentration
- M(Slope)=37215.08
- C (Y- Intercept) = -743.08
- Response of 1000% Concentration = 20705
- 5 % of 100% Concentration= 10353

Linearity Graph



Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Sr.No	Validation Parameter	Results		Acceptance Criteria
A. System Suitability : Method and Procedure				
1.	**System Suitability**	Prepared standard solution as per the test methods and inject six times into the chromatographic system		
	Acceptance criteria	• The column efficiency as determined for the Nifedipine from standard solution is not less than 3200 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for Nifedipine peak area obtained from five replicate injections of standard solution is not more 2.0		
2. Observed Values				
System Precision		Theoretical Plates	6789	The column efficiency as determined for the Nifedipine from standard solution is not less than 2000 theoretical plates.
		Tailing Factors	1.02	Tailing factor for the same peak is not more than 2.0
		% RSD	0.329	The % RSD of % assay from Five samples should be more than 2.0
3. **Results :**				

System Suitability and System Precision	**Sr.No**	**Peak Area**	**Theoretical factor**	**Tailing Factor**
	1	248989	5895	1.05
	2	283805	5982	1.05
	3	208175	5573	1.03
	4	286942	5779	1.02
	5	286223	5746	1.02
	6	287222	5824	1.02
	Mean	282789		
	SD	639.28		
	% RSD	0.21		

Observed Results:

- The observed theoretical plates obtained for the Nifedipine from standard solution is more than 3000 theoretical plates.
- The Observed Tailing factor obtained for the Nifedipine peak from the standard solution is less than 2.0.
- The % RSD of the peak area of Nifedipine obtained from five replica injections of the standard solution is 0.73

Conclusion :

The above data shows that the system is precise.

B. **Method Precision : Method and Procedure**				
1.	**Methods Precision**	Prepared six sample solution of Nifedipine tablets 5 mg as per the test methods and inject into the chromatographic system		
	Acceptance criteria	The % RSD of % assay from six samples should be more than 2.0		
2. Observed Value				
Method Precision		% RSD	1.785	The % RSD of % assay from six samples should be more than 2.0
3. **Results:**				

Sr.No	**Dissolution % labeled amount**
Injection-1	100.0
Injection-2	101.3
Injection-3	1014
Injection-4	100.9
Injection-5	100.9
Injection-6	100.1
Mean	**100.1**

Sr.No	Validation Parameter	Results	Acceptance Criteria
SD		**1.79**	
% RSD		**1.69**	
95% confidence interval of mean		**99.8 to 102.5**	
Conclusion : The above results show that the methods is precise			

Accuracy:

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	Accuracy was performed by spiking the Nifedipine drugs substance to the placebo at 20% to 120 % of target concentration of Nifedipine in triplicate at each level and analyzed as per the test method			
	Acceptance Criteria	The % recovery of accuracy levels should be not less than 95.0 and not more than 105% Report the 95 % confidence interval of mean		
2.	**Observed Values**			
Accuracy		Mean recovery	% 99.3	The % recovery of accuracy levels should be not less than 95.0 and not more than 105.0

3. **Results**

Accuracy level		Amount added in mg	Amount found in mg	% Recovery	Statistical Analysis		
					Mean	SD	%RSD
Level 1	Sample -1	1.001	0.994	99.5	99.3	0.13	0.13
	Sample -2	1.003	0.996	99.2			
	Sample -3	1.001	0.998	99.3			
Level 2	Sample -1	2.502	2.482	99.1	98.6	0.22	0.22
	Sample -2	2.503	2.463	98.3			
	Sample -3	2.501	2.465	98.5			
Level 3	Sample -1	5.011	4.953	98.3	98.7	0.14	`0.14
	Sample -2	5.011	4.942	98.5			
	Sample -3	5.012	4.941	98.8			
Level 4	Sample -1	6.021	6.036	99.8	99.8	0.14	0.14
	Sample -2	6..021	5.957	99.7			
	Sample -3	6.021	6.070	99.6			

Overall Statistical Analysis

Mean	99.2	**SD**	0.51	**% RSD**	0.51

Conclusion : The Form the above results , it is concluded that the test method is accurate from 20 % to 120% of test stock concentration

Range

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	Range of analytical method can be obtained from linearity, Precision and accuracy data. Report range in % with respect to sample concentration.		
Observed Values			

Sr.No	Validation Parameter	Results	Acceptance Criteria
2.	Range	The analytical method is linear , Precise and accurate from 20% 120% of target concentration	----

Conclusion :
It was concluded from the linearity, Precision and accuracy data that the analytical method is islinear , Precise and accurate from 20% to 120% of target concentration.

CONCLUSION

In the current study the effort has been undertaken to improve most simple, economical, sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives resolution with a short analysis time (< 10 min). The method parameter was validated and establish to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of Nifedipine in medical dosage form.

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