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Determination of Nifedipine by Validated RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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Abstract

The present paper deals with the development and validation of reverse phase HPLC method for the determination of Nifedipine on Nucleosil 100, 5 µm, C_8, 250 x 4.0 mm column. A mobile phase consisting of 40 ml 2-propanol: 60 ml phosphoric acid 0.85% was employed in this study. The flow rate was kept at 0.8 ml/min and the injection volume was 10 µl. The separation was performed at 40°C. Eluents were monitored by UV detector set at 237 nm. The developed method was statistically validated for the linearity, precision, robustness, specificity and solution stability. The specificity of the method was ascertained by force degradation studies by acid and alkali hydrolysis, oxidation, heat and photo degradation. The degraded products were well resolved from the analyte peak with significant differences in their retention time values.

Keywords: *Reverse phase HPLC, Nifedipine and Stability.*

1. Introduction

Nifedipine (1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl) - 3, 5-pyridine dicarboxylic acid dimethyl ester) (Fig. 1) is a dihydropyridine calcium channel blocker used widely in the management of hypertension and angina (1, 2). It is an important calcium channel blocker with peripheral and coronary vasodilator activity (3-5). Nifedipine is highly photosensitive and thermally unstable compound. These unfavorable pharmacokinetics and physic characteristics make determination of nifedipine in formulation difficult. The drug and its formulations are official in USP (6) and BP (7), which recommended HPLC and nonaqueous titration for its assay, respectively. Literature survey reveals that the drug has been determined by a variety of analytical techniques, such as HPLC (8-14), reversed-phase HPLC (15-18), HPTLC (19), gas chromatography (20-26), micellar electrokinetic chromatrography (27), electroanalytical methods (28-31), flow-injection analysis (32), mass spectrometry (33) and UV spectrophotometry (34-38). Keeping this survey into consideration, the present paper describes a simple, rapid, precise, specific and stability indicating RP- HPLC method for quantification of Nifedipine, a light sensitive drug, as bulk drug and solid oral dosage forms.

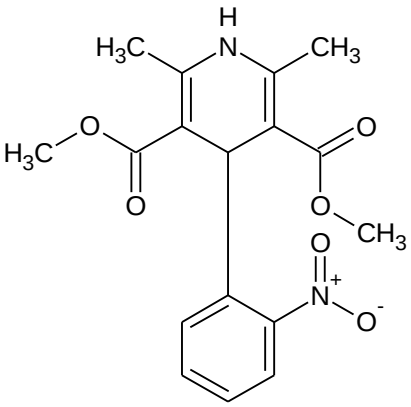

Fig. 1: Structure of Nifedipine.

2. Materials And Methods

Instruments used

Shimadzu LC-2010 HT with liquid chromatograph, Metller Toledo electronic analytical balance, Transsonic Digital S (Sonicator), Chromatographic software – CLASS-VP and Nucleosil 100, 5 µm, C_8, 250 x 4.0 mm column was used as a stationary phase.

Reagents and chemicals used

HPLC grades Methanol, AR grade ortho phosphoric acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide was procured from E. Merck.

Note: Nifedipine is extremely light sensitive, especially in solution. Hence, all labor must be done protected from light and as fast as possible.

Diluent: 2-Propanol: Ortho phosphoric acid 85% (65: 35)

Preparation of standard stock solution

Accurately, about 20 mg of standard Nifedipine was weighed and transferred to a 100 ml volumetric flask with 65 ml 2-Propanol and sonicated for 10 minutes cooling with ice. Equilibrated to room temperature and made up to graduation with ortho phosphoric acid 85%. The final concentration for the standard solution is 200 µg/ml.

Selection and preparation of mobile phase

Pure drug of Nifedipine was injected into the HPLC system and run in different solvent systems. It was found that 2-propanol and phosphoric acid 0.85% gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase employed was 40 ml 2-propanol: 60 ml phosphoric acid 0.85%. The prepared mobile phase was ultrasonicated for 20 mins.

Selection of analytical wavelength

By appropriate dilution of standard stock solution with mobile phase, various concentrations of Nifedipine were prepared separately. The solutions were scanned using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. The λ_{max} of Nifedipine was found to be 237 nm which was selected as the analytical wavelength for further analysis.

Optimization of Chromatographic conditions

Column	Nucleosil 100, 5µm, C_8, 250 x 4.0 mm column
Detector	237 nm
Injection volume	10 µl
Flow rate	0.8 ml/min
Temperature	40˚ C
Run time	20 min
Mobile phase	40 ml 2-propanol: 60 ml phosphoric acid 0.85% (40: 60) v/v

Method validation

System suitability

System suitability parameters were calculated at the start of study of each validation parameter. The values of system suitability results obtained during the entire study are recorded in Table 1.

Table 1. System Suitability and System Precision

Parameter	% RSD	Tailing Factor	%Recovery
1) Specificity	1.01	1.11	100.21
2)Linearity	0.32	1.20	101.19
3)Method Precision	0.20	1.12	101.22
4)Intermediate Precision	0.18	1.21	101.12
5)Accuracy	0.12	1.03	99.68
6)Solution Stability			
Initial	0.12	1.08	100.21
After 4hrs	0.09	1.08	100.80
After 8hrs	0.15	1.09	100.62
After 12hrs	0.05	1.08	101.31
After 24hrs	0.11	1.09	99.12

Linearity

Linearity was determined at five levels over the range of 70% to 130% of test concentration. A standard Linearity solution was prepared to attain concentration of 70%, 80%, 100%, 120%, and 130% of the test concentration. Each linearity solution was injected in triplicate. The mean area at each level is calculated and a graph of mean area versus concentration is plotted. The correlation co-efficient (r), Y-intercept, slope of regression line, residual sum of squares is calculated and recorded in Table 2. The plot of peak area response against concentration is presented in Fig 2. The Beer Lambert's law was obeyed in the concentration range of 70% to 130% for Nifedipine. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient. (r^2 = 0.9989).

Table 2. Characteristics Of The Analytical Method Derived From The Standard Calibration Curve

Parameters	Characteristics
Linear dynamic range µg/ml	140-260 µg/ml
Correlation coefficient	0.9989
Y- intercept	53557
Slop of regression line	51701x

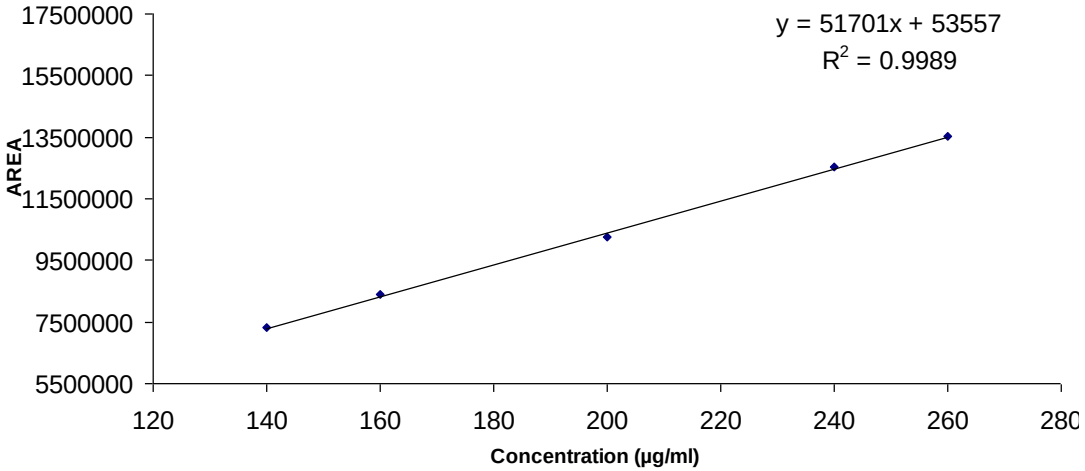

Fig. 2: Linear calibration curve for Nifedipine.

Specificity

The specificity of the HPLC method was determined by complete separation of Nifedipine in the presence of its degradation products. There was no interference from sample and its degraded products

the peak purity of Nifedipine is 0.9991. It shows that developed analytical method is specific for the analysis of Nifedipine.

Precision

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The chromatogram for standard was given in Fig. 3. The value of standard deviation shows that the method is precise. The results obtained are presented in Table 3.

Table 3. Method Precision

Sample Preparation No.	Assay (%)
1	100.49
2	100.12
3	99.93
4	98.43
5	100.03
6	101.66
Mean	**100.11**
SD	**1.04**
RSD (%)	**1.04**
95% Confidence Interval	**1.09**

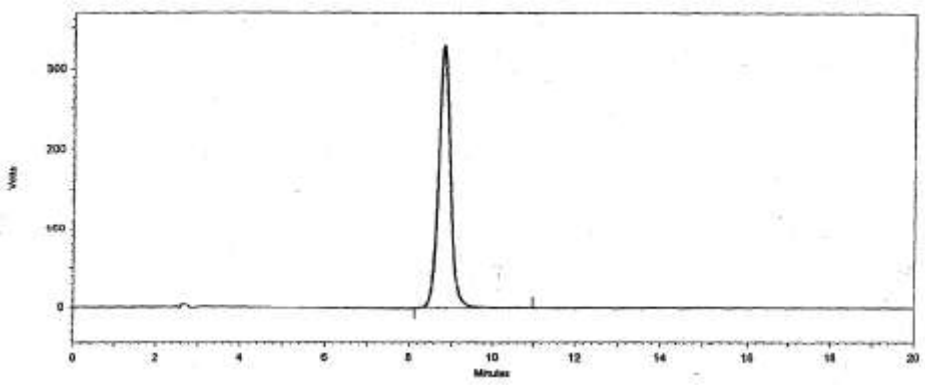

Fig. 3: Chromatogram for standard solution.

Recovery studies

To check the accuracy of the proposed method, recovery studies were carried out at 70, 100 and 130 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 4.

Table 4. Recovery Studies

Level	Amount found (mg/ml)	Amount added (mg/ml)	Recovery (%)	Mean (%)	% RSD
	0.14184	0.14256	99.50	99.54	0.24
Level-1 (70%)	0.14179	0.14276	99.32		
	0.14257	0.14286	99.80		
	0.20575	0.20685	99.47	100.23	1.04
Level-2 (100%)	0.20715	0.20425	101.42		
	0.20486	0.20525	99.81		
	0.25711	0.26056	98.68		
Level-3 (130%)	0.25817	0.26205	98.52	98.80	0.37
	0.26017	0.26225	99.21		
Mean			**99.52**		
% RSD			**0.84**		

Robustness of method

The robustness of the developed method was studied by making small deliberate variations in the method parameters such as the small components in the mobile phase, flow rate, wave length and the

column temperature. The solution containing 200 µg/mL of Nifedipine was injected into sample injector of HPLC under the different conditions. The results of the robustness study are given in Table 5.

Table 5. Method Robustness

Condition	% RSD	Tailing Factor	%Recovery of reference 2
1) Change in Flow rate			
Normal Condition (0.8 ml per minute)	0.05	1.09	99.48
Flow rate (0.7ml per minute)	0.06	1.08	99.17
Flow rate (0.9 ml per minute)	0.09	1.08	98.38
2) Change in minor component in the mobile phase			
Normal Condition (Buffer: Propanol) (60: 40))	0.05	1.08	99.59
(Buffer: Propanol) (62: 38)	0.18	1.08	99.43
(Buffer: Propanol) (58: 42))	0.09	1.06	101.57
3) Change in column oven temperature			
Normal Condition (40°C)	0.04	1.08	99.39
Oven temperature (45°C)	0.05	1.08	98.73
Oven temperature (35°C)	0.07	1.09	98.16
4) Change in Wave Length			
Normal: Wave Length 237nm	0.04	1.09	99.89
Wave Length 242nm	0.09	1.05	98.97
Wave Length 232nm	0.08	1.07	98.83

Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, exhibits the ruggedness of developed analytical method and results are presented in Table 6.

Table 6. Method Ruggedness

Sample Preparation No.	Assay (%)
1.	99.47
2.	99.06
3.	98.92
4.	99.10
5.	99.47
6.	97.48
Mean	**98.92**
SD	**0.88**
RSD (%)	**0.89**
95% Confidence Interval	**0.92**
Difference between method precision and intermediate precision assay	**1.19**

Solution stability studies

The sample solution was prepared at test concentration and initial assay was determined. Solution was stored up to 24 hours at room temperature and about 4°C and assay was determined at 4 hours, 8 hours, 12 hours and 24 hours against freshly prepared standard and also analyzed about 4 °C at 24 hours. The assay obtained at different time intervals was compared with the initial assay value and recorded. The relative standard deviation was found below 2.0%. It proves that both standard and sample solutions are stable up to 24 hours at room temperature and at 4 °C.

Analysis of the marketed formulation

To determine the content of Nifedipine in conventional tablets [CALCIGARD RTD- tab (Torrent Pharmaceuticals Ltd.) label claim: 20 mg/tab], twenty tablets of Nifedipine were weighed; their average weight was determined and crushed to fine powder. The tablet powder equivalent to 20 mg of Nifedipine was weighed and transferred to a 100 ml volumetric flask with 65 ml 2-Propanol and sonicated for 10 minutes cooling with ice. Equilibrated to room temperature and made up to graduation with ortho phosphoric acid 85%. The solution was filtered through a 0.22 µ membrane filter. The final

concentration for the standard solution is 200 µg/ml. A 10 µl volume of sample solution was injected into the sample injector of HPLC under the optimized chromatographic conditions. Area of peak was measured at 237 nm. The amount of drug present in the sample was determined using the prepared calibration curve of standard Nifedipine.

3. Results and Discussion

Nifedipine is an important calcium channel blocker with peripheral and coronary vasodilator activity. Literature survey reveals that there is no stability indicating HPLC method reported so far for the determination of Nifedipine. Keeping this point into consideration an attempt was made to develop a simple and accurate RP-HPLC method to determine Nifedipine in presence of its degradation products.

The mobile phase consisting of 40 ml 2-propanol: 60 ml phosphoric acid 0.85% was employed in this study. The flow rate was kept at 0.8 ml/min and the injection volume was 10 µl. The separation was performed at 40°C. Eluents were monitored by UV detector set at 237 nm. The separation was carried on on Nucleosil 100, 5 µm, C_8, 250 x 4.0 mm column. The run time was set at 20 min. The retention times of Nifedipine was found to 9.35 mins (Fig No. 2). The peak areas of the drug were reproducible as indicated by the low coefficient of variation. The amount of drug found was between 98 to 102%. The sample recoveries in formulation were in good agreement with their respective label claim which suggested non-interference of formulation excipients in the estimation. Also, the % RSD for both the tablet analysis and recovery studies was less than 2 % indicating high degree of precision and accuracy of the proposed method. The mean % assay of intra-day and inter-day precision was found to be 100.11and 98.92 respectively. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions.

4. Conclusion

Also, the developed method was capable of determining Nifedipine in presence of its degradation products. The solutions of both standard and sample solutions are stable up to 24 hours at room temperature and at 4 °C. Hence, it can be concluded that the developed RPHPLC method is a stability indicating simple, accurate, precise and robust method and can be employed successfully for the estimation of Nifedipine in bulk and formulation.

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