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Research Article

OPTIMIZED AND VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF NIMODIPINE IN TABLET DOSAGE FORM

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ABSTRACT

The purpose of the investigation was to develop a new RP-HPLC method for the estimation of Nimodipine in pharmaceutical dosage form. Chromatography was carried out on an Altima C-18 column (4.6 x 150mm, 5µ particle size) with a isocratic mobile phase composed of Phosphate buffer (adjusted to pH 3.3 with dilute orthophosphoric acid solution) and acetonitrile (30:70v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 236 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection, limit of quantification, stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention time for Nimodipine was 3.7 min. The percentage recovery of Nimodipine was 100.6%. The relative standard deviation for assay of tablets was found to be less than 2%. The method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of Nimodipine tablets in quality control laboratories and pharmaceutical industries.

Keywords: Nimodipine, RP-HPLC, ICH guidelines.

INTRODUCTION

Nimodipine is a dihydropyridine calcium channel blocker and chemically known as isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate (Figure 1), It is known for its preferential action on cerebral blood vessels and its potential cytoprotective effects by reducing calcium influx into nerve cells and it acts by relaxing the arterial smooth muscle[1]. It is used in the prevention of cerebral vasospasm and resultant ischemia, a complication of subarachnoid hemorrhage (a form of cerebral bleed), specifically from ruptured intracranial berry aneurysms irrespective of the patient's post-ictus neurological condition.

Various UV Spectroscopy[2-7], Spectrofluorometric[8], GC[9], UPLC-MS[10], HPLC with ampherometry[11] and Raman spectroscopic[12] assay methods are reported in the literature for the estimation of Nimodipine. According to literature survey there is no official method for the estimation of Nimodipine by RP-HPLC in tablet dosage forms. Hence, an attempt has been made to develop new method for the estimation and validation of Nimodipine in tablet formulation in accordance with the ICH guidelines[13].

EXPERIMENTAL

Instrumentation

Chromatography was performed with Alliance waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and convenient for LC with class Empower-2 software.

Reagents and chemicals

The reference sample of Nimodipine was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial

tablets (NIMOTOP-30mg) were purchased from the local pharmacy.

Chromatographic condition
The mobile phase consisted of phosphate buffer and acetonitrile was taken in ratio of 30:70 at a flow rate of 1.0 mL/min. Altima, C18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase. 236 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution
Accurately Weighed and transferred 15mg of Nimodipine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of Working Standard Solutions
Aliquot of 0.25, 0.5, 0.75, 1.0, 1.25 & 1.50 mL were pipette out from stock solution into 10 mL volumetric flask and volume was made up to 10 mL with diluent. This gives the solutions of 37.5, 75, 112.5, 10, 150, 187.5 and 225µg/mL for Nimodipine.

Preparation of phosphate Buffer
Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degassed to sonicate and finally make up the volume with water, then pH adjusted to 3.3 with dil. Ortho phosphoric acid solution.

Sample preparation
5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation
Parameters such as systems suitability, Linearity, accuracy, specificity, LOD & LOQ and robustness were performed according to the ICH guidelines.

RESULTS AND DISCUSSION

Method development
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which the drug did not responded properly. The organic content of mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, phosphate buffer: acetonitrile were taken in isocratic ratio: 30: 70 and with flow rate of 1.0 mL/min was employed. Altima C18 column (4.6 x150mm, 5µ particle size) was selected as the stationary phase to reduce the tailing of the peak. 236 nm was selected as the detection wavelength for PDA detector. The retention times was found to about 3.7 min and the results were shown in Table 1 and Figure 2.

Method Validation
System suitability
A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 3. The analytical method validation was carried out as per ICH method validation guidelines.

Linearity
The linearity range was found in the range of 37.5-225 µg/mL. The response for the drug was linear and the regression equation was found to be y=6458x+5816 and correlation coefficient was found to be 0.9999 and the results are given in Table 2 and Figure 3.

Precision
Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intra-day precision and inter-day precision.

Intra-day precision
To study the intra-day precision, six replicate standard solutions of Nimodipine were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.8 which are well within the acceptable criteria of not more than 2.0.

Inter-day precision
To study the inter-day precision, six replicate standard solutions of Nimodipine were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.2 which are well within the acceptable criteria of not more than 2.0.

Specificity
The effect of wide range of excipients and other additives usually present in the formulation of Nimodipine in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Nimodipine.

Limit of detection and limit of quantification
A calibration curve was prepared using concentrations in the linearity range (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Nimodipine were 0.68 and 2.07 µg/mL, respectively (Table 3).

Accuracy
The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD was found to be 0.47. Satisfactory recoveries ranging from 99% to 101.2% were obtained by the proposed method. This indicates that the proposed method was accurate.

Robustness
Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Tablet Analysis
The Content of Nimodipine in the tablets was found by the proposed method. RSD values for Nimodipine are found to be 0.87 and results were shown in table.4 and figure 4.

CONCLUSION
A new precise accurate and simple HPLC method was developed and validated for the estimation of Nimodipine in tablet dosage form. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of nimodipine tablets in QC laboratories and industries.

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Buffer:Acetonitrile (30:70)
2	pH	3.3(+/-0.5)
3	Diluent	Methanol:Water (80:20)
4	Column, make	Altima, C18 (150 x 4.6 mm, 5µ)GL Sci. Inc.
5	Column temperature	30^0C
6	Wave length	236nm
7	Injection volume	10ul
8	Flow rate	1.0ml/min
9	Run time	8mins
10	Retention time	3.72mins

Table 2: Linearity results

S. No.	Concentration in µg/mL	Area
1	37.5	231679
2	75	474462
3	112.5	721795
4	150	961622
5	187.5	1210119
6	225	1445888

Table 3: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (µg/mL)	37.5-225 µg/mL
2	Correlation coefficient	0.999
3	Theoretical plates (N)	8628
4	Tailing factor	1.50
5	LOD (µg/mL)	0.68 µg/mL
6	LOQ (µg/mL)	2.07 µg/mL
7	Regression Equation	Y=6458+5816

Table 4: Assay results

S. No.	Formulation	Label claim	Amount found	%Assay
1	NEMOTIDE	30 mg	30.14mg	100.6%

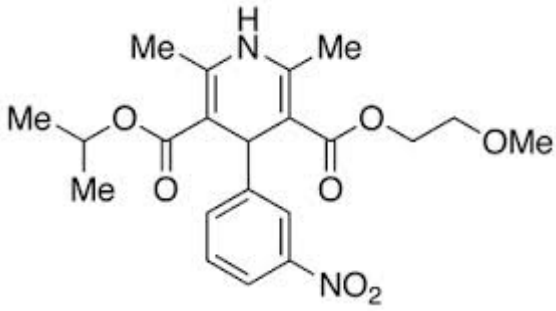

Fig. 1: Structure of Nimodipine

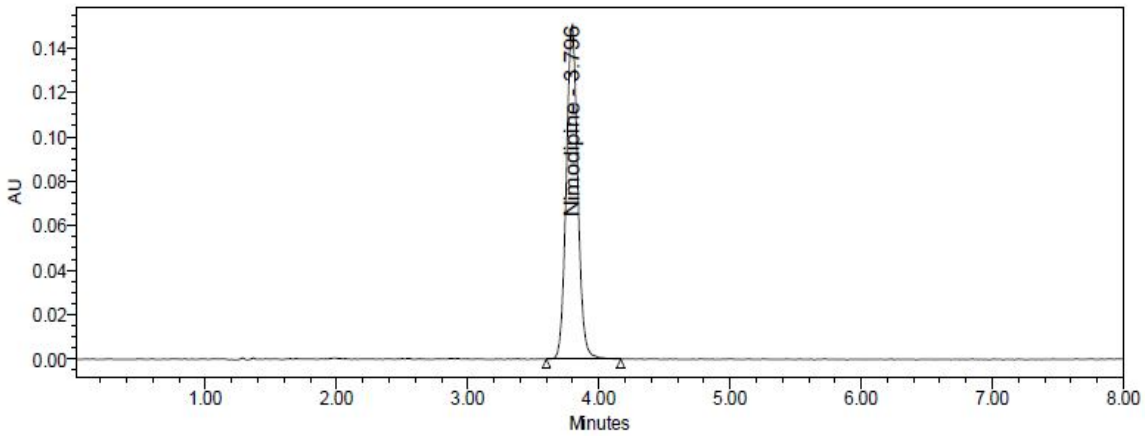

Fig. 2: Chromatogram of Nimodipine Standard

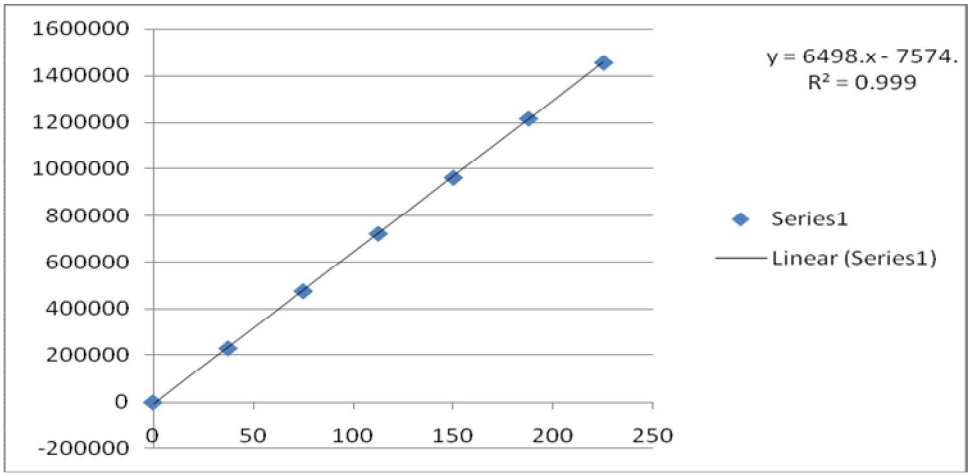

Fig. 3: Linearity curve

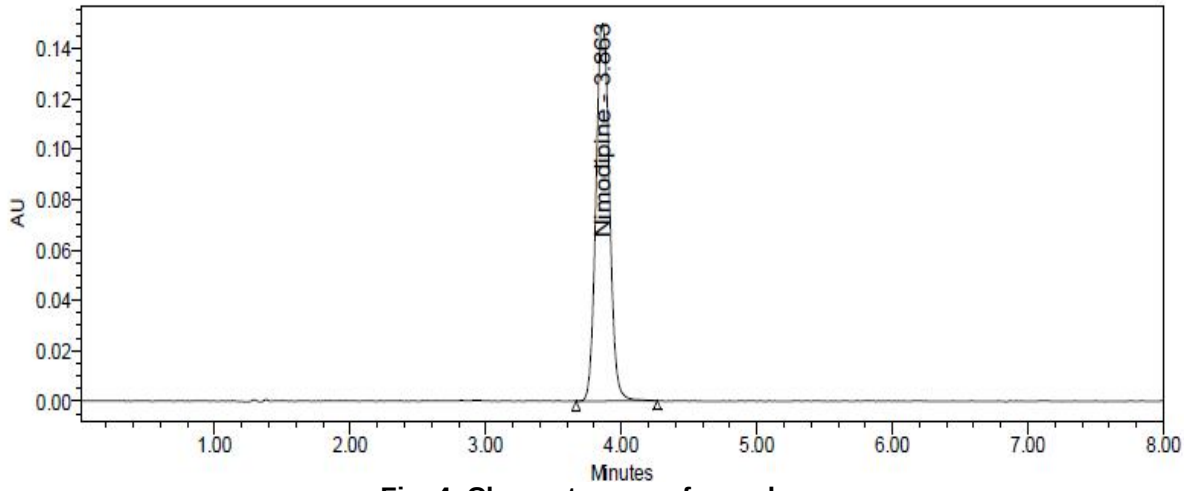

Fig. 4: Chromatogram of sample

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