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Analytical Method Development and Validation of Nimodipine by UV-Spectroscopy

Banothu Bhadru *, Sappidi Harshitha, Tadikonda Rama Rao

CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

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ABSTRACT

A reliable method for quantifying Nimodipine using UV Spectroscopy was developed with a methanol-water solvent mixture (3:2). The absorption spectrum showed a peak at 240 nm, and the method was optimized and validated per ICH guidelines, achieving a correlation coefficient of 0.9989. Intra-precision and intermediate precision yielded %RSD values of 0.511 and 0.347, respectively. An assay of a commercial Nimodipine formulation showed 98.5% purity. This method is suitable for estimating Nimodipine at 50%, 100%, and 150% concentration levels, with statistical validation completed.

KEYWORDS: Nimodipine, UV Spectrophotometer, ICH guidelines, Method development and Method validation, Assay

INTRODUCTION

Nimodipine is antihypertensive, calcium channel blocker drug. Chemically Nimodipine (NM) is isopropyl-2-methoxyethyl-1, 4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5–pyridine dicarboxylate, a dihydropyridine calcium antagonist. It is used mainly in the treatment of cerebrospinal hemorrhage. It is known for its preferential action on cerebral blood vessels and its potential cytoprotective effects by reducing calcium influx into nerve cells [1].It is well absorbed in the gastrointestinal tract after oral administration, but it is subjected to extensive first-pass metabolism that results in very low plasma concentration (ng/ml levels), poor absolute bioavailability and significant inter-individual variations [2].

It is a cerebro vascular disease with rapid onset and frequent recurrence, accompanied by cerebral vasospasm (CVS) and other high-risk complications [3].It is also used in other cerebro vascular disorders, such as ischemic stroke [4], and has been studied in impaired brain function in multi-infarct dementia and senile dementia [5].

A literature survey revealed that several chromatographic and spectroscopic methods have been reported for the estimation of Nimodipine. They include HPLC [6], GC [7], UPLC-MS/MS [8], LC-MS/MS [9], and UV-spectrophotometric techniques [10, 11]. Electro chemical detection [12] or tandem mass spectrometry [13, 14]. Nitrogen–phosphorus detection [15].The structure of Nimodipine was shown in Fig.no.1.

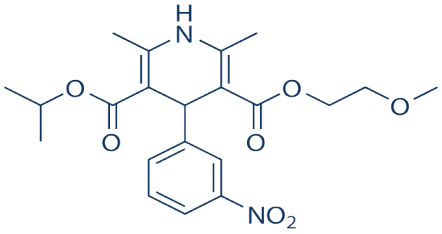

Fig.1. Structure of Nimodipine

MATERIALS AND METHODS

Materials

Nimodipine (API), Nimodipine 40 mg tablets, acetic acid, n-hexane, Ethanol, Cyclo hexane, Methanol, Ether and Water HPLC Grade

Instruments

UV-Visible spectrophotometer with UV Win software, Electronic Balance, Biotech's Ultra Sonicator, pH meter, Water bath shaker, Centrifuge and Refrigerator.

METHOD DEVELOPMENT

Preparation of diluent

To achieve a 3:2 ratio of methanol to water mix 300 ml of methanol with 200 ml of water.

Preparation of a standard stock solution

An exact amount of 10 mg of Nimodipine was accurately weighed and placed into a 10 mL volumetric flask. It was then dissolved in a small volume of diluent until completely dissolved. The volume of the solution was then adjusted to the calibration line with additional diluent, producing a standard stock solution with a concentration of 1000 µg/ml. To prepare a 100 µg/ml concentration from this initial stock, 1 mL was pipetted into a 10 mL volumetric flask, and diluent was added to reach the final volume, resulting in the formation of the second standard stock solution.

Selection of wavelength for analysis of Nimodipine

A 10 ml volumetric flask was accurately filled with 1 ml of standard stock solution-2, which was subsequently diluted to a total volume of 10 ml using diluent, resulting in a concentration of 10µg/ml. This prepared stock solution was utilized for an initial spectral scan within the UV range of 200-400 nm to identify the maximum wavelength.

Preparation of a sample solution

Ten tablets were accurately weighed, and their average weight was calculated before being pulverized into a fine powder. A quantity of powder equivalent to 10 mg of Nimodipine was subsequently dissolved in a diluent through sonication. The solution was then brought to a final volume of 10 ml in a volumetric flask with additional diluent, achieving a concentration of 1000 µg/ml. The content of Nimodipine in the commercially available formulation was evaluated using a pre-validated analytical method at 240 nm.

Assay

$$\%\text{Assay} = \frac{\text{Sample absorbence}}{\text{Stad.absorbence}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tab.}}{\text{Label claim}} \times 100$$

Table 1: Assay of Nimodipine formulation

Nimodipine	Labelled claimed(mg)	Amount found(mg)	% assay	% RSD
	40	39.95	99.5	0.04

Method validation

The proposed method has been validated based on ICH Q2 (R1) and USP standards, covering various parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness, and stability assessments.

Linearity

Serial dilutions were carried out starting from the standard stock-2 solution to achieve concentrations of 5µg/mL, 10µg/mL, 15µg/mL, 20µg/mL, and 25µg/mL. The absorbance for each concentration was recorded at a wavelength of 240 nm. A calibration

curve was subsequently established by graphing the absorbance values on the y-axis in relation to the corresponding concentrations on the x-axis.

Precision

The precision of the method was determined by repeatability (intraday precision) and intermediate precision (inter-day precision) for the standard solution. Six replicate absorbance measurements were taken from the homogeneous solution, and the %RSD was calculated. The results were expressed as %RSD of the measurement.

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. Accuracy of the method is demonstrated at three different concentration levels: 50%, 100%, and 150%. This is achieved by spiking a known quantity of standard drug into the analyzed sample in triplicate.

LOD and LOQ

The detection limit (LOD) of an individual's analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

$$LOD = 3.3S/b$$

$$LOQ = 10S/b$$

Where

S = the standard deviation of the intercept

b = slope of the calibration curve

Ruggedness

Ruggedness of the method was determined by carrying out the analysis on different days, with different analysts using different makes of reagents and instruments.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. It was performed by varying λmax value of ±2 nm for 5µg/ml in triplicate.

RESULTS AND DISCUSSION

Linearity

Five linearity solutions were prepared using Nimodipine API at concentration levels ranging from 5-25% of the target concentration of tablet diluent. The absorbance of the solutions was measured in a 1 square cm cell on a suitable UV Spectrophotometer at 240 nm, with the diluent used as a blank. The Linearity data was shown in table no. 1. And the calibration curve was shown in **Fig. no. 2.**

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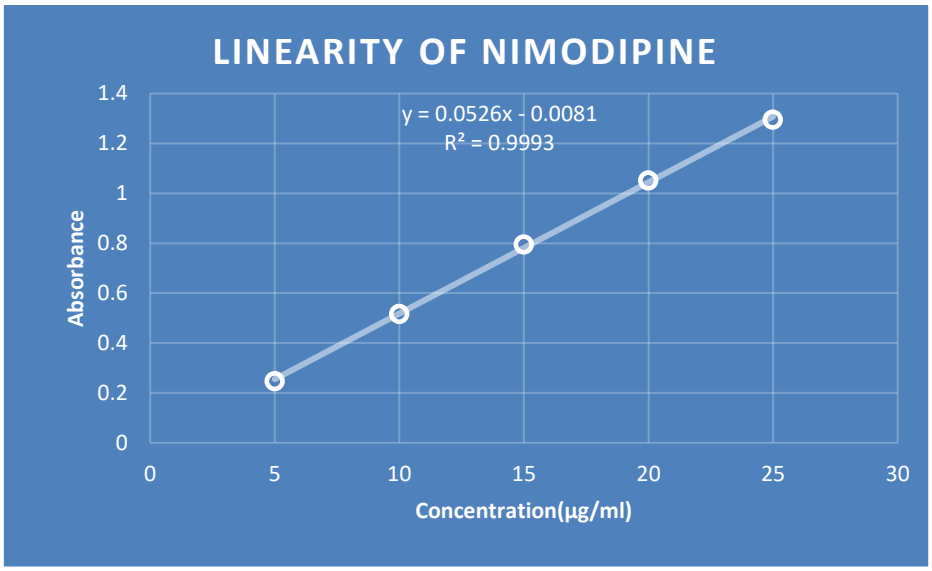

Fig.2.Calibration curve of Nimodipine

Table.2. Linearity of Nimodipine at 240 nm & Statistical Data

S.No	Concentration(µg/ml)	Absorbance	Nimodipine	
1.	5	0.247	λmax	240 nm
2.	10	0.517	Con. (µg/ml)	5-25 µg/m
3.	15	0.795	Correlation	0.999
4.	20	1.052	Slope	0.052
5.	25	1.295	Y- intercept	0.008

Precision:

The precision study indicated that there were no notable differences in precision values, thereby validating the method's effectiveness for the analysis of Nimodipine in tablet formulations. Additionally, there was no indication of interference from excipients. The results varied between 98.30% and 100.56%, with an acceptable deviation of 2%.

Table.3. Precision data of Nimodipine

S.No	Concentration(µg/mL)	Absorbance
1	6.0	0.578
2	6.0	0.584
3	6.0	0.587
4	6.0	0.592
5	6.0	0.598
6	6.0	0.602
	Mean	0.5922
	Stdev	0.0072
	%RSD	1.44

LOD and LOQ

The LOD (Limit of Detection) was estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as

LOD = 3.3 × (S.D./Slope) Where,

SD = Standard deviation of the Y- intercepts of the 6 calibration curves

Slope = Mean slope of the 6 calibration curves

The LOQ (Limit of Quantization) was estimated from the set of 6 calibration curves used to determine method linearity.

The LOQ may be The LOQ may be calculated as

$$LOQ = 10 \times (S.D./Slope)$$

Where SD = Standard deviation of the Y- intercepts of the 6 calibration curves

Slope = Mean slope of the 6 calibration curves

Table: 4. LOD and LOQ results of Nimodipine

Parameters	Nimodipine (µg/mL)
LOD	0.18
LOQ	0.62

Accuracy (Recovery studies)

Recovery study for carbamazepine was carried out at 50%, 100%, and 150% concentration. The results were shown in the table using the data below. Drug-drug interaction, drug excipients interaction, and drug solvent interaction have not been noticed or identified. Hence, it has been proved that there is no interference of any component with the drug.

Table.5.Recovery studies of Nimodipine

Sample (%level)	Amount Taken(µg/mL)	Amount Added(µg/mL)	Amount Recovered(µg/mL)	% Recovery	Average
50	6	3	8.76	99.6	
50	6	3	8.80	99.8	99.6
50	6	3	8.82	99.4	
100	6	6	12.18	101.2	
100	6	6	12.16	101.3	101.2
100	6	6	12.14	101.1	
150	6	8	15.16	100.9	
150	6	8	15.14	100.7	100.4
150	6	8	15.12	100.4	

Robustness

The robustness of this procedure was evaluated by measuring the absorbance of a 6µg/mL Nimodipine standard solution at different maximum wavelengths (specifically ±1nm) around the actual maximum.

Table.6. Robustness data of Nimodipine

S.No	Wavelength	Absorbance
1	239	0.572
2	240	0.593
3	241	0.598

Ruggedness

The assessment of ruggedness was conducted by analyzing data collected from various analysts, each utilizing different reagents and instruments. Each analyst prepared six samples from the same batch, and the resulting data is presented in the table.

Table.7.Ruggedness of Nimodipine

S.No	Assay (% of claim)Nimodipine	
	Analyst 1	**Analyst 2**
1	99.82	99.62
2	99.56	98.32
3	100.14	100.7
4	98.48	99.83
5	99.78	98.91
Mean	99.56	98.32
SD	1.389	1.311
RSD	1.38	1.37

Stability Studies

To check the stability of the solution, a sample solution of carbamazepine with a concentration of 6µg/mL was taken. In table 9, stability studies information was displayed.

Table.8. Stability Studies data of Nimodipine

Time	% Assay
Initial	99.6
24hours	98.9

CONCLUSION

A new method was developed and validated for the determination of Nimodipine using UV spectroscopy. The proposed method was found to be simple, accurate, precise, reproducible and robust. The developed method can be applied for the assay of commercial tablets containing Nimodipine in routine quality control analysis.

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Conflict of Interest Statement: All authors have nothing else to disclose.