Development and Validation of a Reversed-Phase HPLC Method for the Analysis of Nimodipine in Pharmaceutical Dosage Forms

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A rapid and sensitive high-performance liquid chromatographic method was developed for the estimation of nimodipine in pharmaceutical dosage forms. Nimodipine was chromatographed on a reverse phase C-18 column in a mobile phase consisting of acetonitrile and water in the ratio of 58 : 42 (v/v). The mobile phase was pumped at a flow rate of 1 mL/min and the eluents were monitored at 241 nm. The calibration curve was linear in the range of 0.1–40 µg/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solution containing 10 µg/mL was 99.95 ± 0.86% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining nimodipine in bulk drug samples and pharmaceutical dosage forms.

Key Words: Nimodipine, Dosage forms, Reversed-phase HPLC.

INTRODUCTION

Nimodipine is a dihydropyridine calcium channel blocker. It is used in the treatment of cerebrovascular disorders, stroke and hypertension[1]. A few analytical methods have been reported for the estimation of nimodipine in pharmaceutical dosage forms[2-5]. Some of the methods utilized gas chromatography with electron capture[4-5] detection and also were complicated by uncontrolled oxidation of nimodipine at high temperatures and the process is considered tedious. Other reported methods such as spectrophotometry and HPLC are not accurate and the process is considered tedious[2-5]. The HPLC methods using the most commonly available columns and detectors like UV are preferred. The present study describes the determination of nimodipine in bulk drug samples and pharmaceutical dosage forms by using RP-C18 column with UV detection. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating nimodipine. The aim of this study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of nimodipine either in bulk drug samples or in pharmaceutical dosage forms.

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EXPERIMENTAL

Nimodipine was a gift sample obtained from M/s Micro Labs, Bangalore, India. Acetonitrile, methanol and water used were of HPLC grade (Qualigens).

A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis Detector SPD-10A VP, CTO-10 AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard^{TM}, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA) and RP C-18 column (150 mm × 4.6 mm I.D., particle size 5 μm; YMC Inc., USA) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

HPLC conditions: The contents of the mobile phase, methanol and water, in the ratio of 52 : 48 v/v, were filtered before use through 0.45 μm membrane filter and degassed with a helium spurge for 15 min. The components of the mobile phase were pumped from the respective solvent reservoirs to the column at a flow rate of 1 mL/min which yielded a column back pressure of 150–160 kg/cm^{2}. The column temperature was maintained at 40°C. The eluents were monitored at 241 nm and detector sensitivity was set at 0.02 a.u.f.s. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems.

Procedure: The solutions were prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. Stock solution of drug was prepared by dissolving 100 mg of nimodipine in 100 mL volumetric flask containing 70 mL of methanol, sonicated for about 15 min and then made up to volume with methanol. Daily working standard solution of nimodipine was prepared by suitable dilution of the stock solution with methanol.

Six sets of the nimodipine solution were prepared in methanol at concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, 4, 10, 20 and 40 μg/mL. Each of these samples (20 μL) was injected six times into the column and the peak area of the drug was recorded.

Assay of nimodipine in tablets: Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 30 mg of nimodipine: was placed in a 100-mL volumetric flask. 70 mL of methanol was added, shaken well and the flasks allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to volume with methanol, thoroughly mixed, and filtered through a 0.45-μm membrane filter. An aliquot of the filtrate 1 mL) was transferred to a volumetric flask and made up to volume with methanol to give an expected concentration of 20 μg/mL of nimodipine. All determinations were conducted in triplicate. The same procedure was used to estimate the amount of nimodipine in two more commercial brands of nimodipine tablets.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area for a set of drug solutions on three different days (n = 5). The intra- and inter-day variation in the peak area of the drug solution (10 or 20 μg/mL) was calculated in terms of coefficient of variation (CV)

and obtained by multiplying the ratio of standard deviation to the mean with 100 [CV = ±SD/mean) × 100].

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount (10 or 20 µg) of the drug to a drug solution of known concentration (10 µg/mL) and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (10 or 20 µg/mL) was added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug. The drug was estimated as per the procedure described above for the estimation of nimodipine in tablet formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery, and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The run time of the method was set at 11 min and nimodipine appeared on the chromatogram at 9.52 min (Fig. 1). When the same drug solution was injected 6 times. The retention time of the drug was same. The ratio of peak areas of nimodipine was calculated and the average values for 6 such determinations were given in Table-1.

TABLE-1
CALIBRATION OF THE HPLC METHOD

Concentration of nimodipine (µg/mL)	Peak area	C.V. (%)
0.00	0	0
0.05	4032	2.12
0.10	8189	1.42
0.20	16503	2.42
0.50	41779	0.47
1.00	84532	1.21
2.00	173029	0.27
4.00	344763	0.88
10.00	864232	1.52
20.00	1715374	0.92
40.00	3451023⁻	0.85

* Mean of six determinations
Regression Equation: $Y = -747.54 + 86212.96X$ (r = 0.99999)

When the concentration of nimodipine and its respective peak area were subjected to regression analysis by least squares method, a high correlation coefficient was observed (r = 0.99999) in the range of 0.05 to 40 µg/mL only. The regression of nimodipine concentration over its peak area ratio was found to be $Y = -747.54 + 86212.96X$ where 'Y' is the peak area and 'X' is the concentration of nimodipine. This regression equation was used to estimate the amount of nimodipine either in tablet formulations or in validation study.
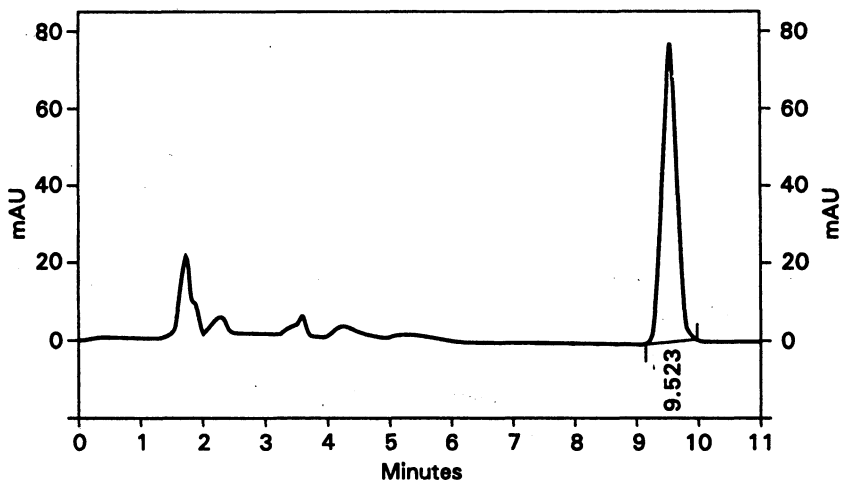
Fig. 1.   Typical chromatogram for nimodipine

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 10 or 20 µg/mL of nimodipine were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area of the drug for five replicate injections was found to be less than 1.5%. Also, the inter-day variation (3 days and five injections) was found to be less than 2.5% (Table-2). Thus, the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (10 or 20 µg) was added to a known concentration of drug solution (10 µg/mL), there was a high recovery (99.95 ± 0.86%) of nimodipine (Table-3) indicating that the proposed method is highly accurate.

TABLE-2

INTER- AND INTRA-DAY PRECISION FOR NIMODIPINE
ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY
THE PROPOSED HPLC METHOD

| Nimodipine concentration (µg/mL) | Concentration of nimodipine (µg/mL) found on | | | |
| | Intra-day | | Inter-day | |
	Mean (n = 5)	C.V. (%)	Mean (n = 5)	C.V. (%)
10	10.02	0.99	10.05	1.12
20	20.09	1.29	20.03	2.16

* Mean of 5 determinations

TABLE-3
RECOVERY OF NIMODIPINE USING THE PROPOSED HPLC METHOD

Amount of drug added to pre-analyzed drug sotution (10 µg/mL)	Recovery of nimodipine	
	Mean (±s.d.) amount (µg) found (n = 5)	Mean (±s.d.) % recovery (n = 5)
10	19.95 ± 0.18	99.75 ± 1.20
20	20.98 ± 0.32	99.95 ± 0.86

The HPLC method, developed in the present study, has also been used to quantify nimodipine in tablet dosage forms. Nimodipine tablets (containing 30 mg of the drug) were analyzed as per the procedure described above. The average drug content was found to be 99% of the labeled amount (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method.

TABLE-4
MEAN (±s.d.) AMOUNT OF NIMODIPINE IN TABLET
DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the capsule	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AA	30	29.94 ± 0.07	99.80 ± 2.33
BB	30	29.89 ± 0.03	99.63 ± 1.00
CC	30	29.68 ± 0.03	98.93 ± 1.00

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