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Research Article

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Investigation of Reverse Phase HPLC Method for Determination of Nimodipinein Pharmaceutical Dosage Form using Nanoparticles Modified with Cetyltrimethylammonium Bromide

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Abstract A rapid and sensitive high-performance liquid chromatogaphic 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. A pre-concentration of nimodipine was applied using Nanoparticles Modified with Cetyltrimethylammonium Bromide. 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 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 the proposed HPLC method may be used for determining nimodipine in bulk drug samples and pharmaceutical dosage forms.

Keywords Nimodipine; Reversed-phase HPLC; Nanoparticle; Cetyltrimethylammonium Bromide

1. Introduction

Nimodipine is a dihydropyridine calcium channel blocker. It is used in the treatment of cerebrovascular disorders, stroke and hypertension. A few analytical methods have been reported for the estimation of nimodipine in pharmaceutical dosage forms. Some of the methods utilized gas chromatography with electron capture 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. 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-C 18 column with UV detection. Owing to the widespread use of HPLC in routine analysis, it is important that well validated 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.

Magnetic nanoparticles offer many advantages over the traditional sorbents. They have very large surface area, highly active surface sites, and a short diffusion route. These particles tagged to the target can be removed from a matrix quickly by applying a magnetic field and do not agglomerate after removal of the field and can be reused or recycled easily; however, these nanometer sized metal oxides are not target-selective; therefore, overcoming this limitation modification of these magnetic nanoparticles is necessary [6-8]. Hemimicelles and admicelles are formed by the adsorption of ionic surfactants on surface of mineral oxides such as alumina, silica, titanium dioxide, and iron

oxides [9-10] and have recently been employed as useful sorbent for the SPE of some organic compounds [11]. Few SPE methods based on surfactant-coated Fe_3O_4NPS have been reported [12].

2. Materials and Methods

2.1 Materials

Nimodipinewas purchased from Bayer Schering Pharma, Germany in tablet 30 mg. Acetonitrile, methanol and water used were of HPLC grade from Merck, Germany. A gradient (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 x 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 8.03 (Shimadzu)".

2.2 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 pm 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 backpressure 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.

2.3 Sample Preparation

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, l, 2, 4, 10, 20 and 40 µg/ml. Each of these samples (20 PI,) was injected six times into the column and the peak area of the drug was recorded.

2.4 Preconcentration and Assay Procedure

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.

By the addition of appropriate volume of the drug's stock solution in 20mL of distilled water, the aqueous solution of each drug (100 ng/mL) was prepared and then 0.75mL of the MNP suspension (containing 10mg of Fe_3O_4NPs) was added to the drug's solution and the pH was adjusted to 8.5. Then, 0.5mL of the 10 mg/mL−1 CTAB was added and the mixture was shaken for 5 min to enhance the drug's adsorption efficiency and then by use of a strong magnet Fe_3O_4NPs placed at the bottom of the beaker was separated quickly from sample solution. The magnet was removed and the supernatant water was decanted. Finally the drugs were desorbed with 500 μL methanol from MNPs. Calculation of ER% showed that desorption of drugs was completed during 30 s in ultrasonic bath and 30 s in vortex. The magnet was used again to settle the MNPs and the eluent was decanted into a microtube. Then, 20 μL of the solution was injected into the HPLC instrument for analysis. All the experiments were carried out at the room temperature. The same procedure was used to estimate the amount of nimodipine in two more commercial brands of nimodipine tablets.

3. Results and Discussion

The run time of the method was set at 1-1 min and nimodipine appeared on the chromatogram at 9.52 min (Figure 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)	Pleak Area	CV (%)
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 = 0.9999)

When the concentration of nimodipine 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.

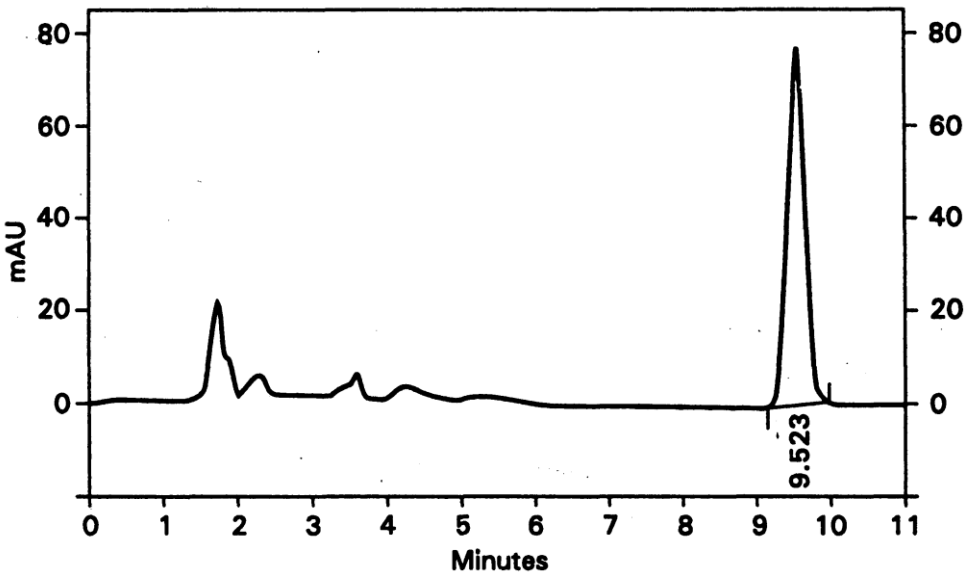

Figure 1: Typical chromatogram for nimodipine

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 10 or 20 µg/mL of nimodipinewere 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

The 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 IOLC method.

Table 3: Recovery of nimodipine using the proposed HPLC method

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

The 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 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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