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Estimation of Nimodipine in Human Plasma by HPLC Method

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A high performance liquid chromatographic (HPLC) assay was developed for the determination of nimodipine in human plasma. Precipitation technique was employed with a RP-HPLC separation for the analysis. Varying amounts of nimodipine (10 to 200 ng), fixed quantity (0.5 μg) of nimesulide (internal standard) and 0.3 mL of acetonitrile were added to blank human plasma (0.5 mL). The mixture was vortexed for 5 min and centrifuged at 3,000 rpm for 10 min and the supernatant liquid was filtered. Twenty microlitres of the resultant filtrate was injected into a reverse phase C-18 column using a mobile phase consisting of methanol and water in the ratio of 68 : 32% v/v at a flow rate of 0.9 mL/min. The eluents were monitored at 237 nm. The method was validated for its linearity, precision and accuracy. The method was found to be simple, precise, sensitive, less time consuming and accurate for the estimation of nimodipine in human plasma.

Key words: Nimodipine, Nimesulide, Human plasma, HPLC, Estimation.

INTRODUCTION

Nimodipine is a dihydropyridine calcium channel blocker which particularly acts on cerebral blood vessels. It is used in the treatment of cerebrovascular disorders, stroke and hypertension[1]. Several analytical methods have been reported for the estimation of nimodipine in plasma[2–5]. These methods utilized gas chromatography (GC) with electron capture[2, 3] and nitrogen[4] detection and also were complicated by uncontrolled oxidation of nimodipine at high temperatures and the process is considered tedious. The HPLC methods are simple, sensitive, precise and highly accurate, and require small quantity of sample. However, the HPLC methods using the most commonly available columns are preferred. In the present study a sensitive, accurate and precise HPLC method has been developed for the estimation of nimodipine in human plasma using RP C-18 column and simple UV detection. It offers alternative to the GC and HPLC procedures available.

EXPERIMENTAL

Nimodipine was a gift sample from M/s Micro Labs, Bangalore, India and nimesulide (used as internal standard) was a gift sample from M/s Dr. Reddy's Labs, Hyderabad, India. Acetonitrile, methanol and water used were of HPLC grade (Qualigens).

Instrumentation: A gradient high pressure liquid chromatography (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable

wavelength programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 5 μm; YMC, Inc., Wilmington, NC 28403, USA) were used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

Chromatographic conditions: The mobile phase components methanol and water were filtered through 0.2 μm membrane filter before use and were pumped from the solvent reservoir in the ratio of 68 : 32% v/v to the column at a flow rate of 0.9 mL/min which yielded a column pressure of 170–180 kg/cm^2. The column temperature was maintained at 40°C. The detector sensitivity was set at 0.0001 a.u.f.s. The volume of each injection was 20 μL.

Methodology: Five sets of plasma samples with varying drug concentrations were prepared by spiking drug-free plasma with an appropriate volume (100 μL) of a known amount of nimodipine at a concentration range of 10 to 200 ng/0.5 mL of plasma along with 100 μL of 0.5 μg/0.5 mL of nimesulide (internal standard) solution.

An aliquot of plasma (0.5 mL) was accurately measured into a 10 mL glass tube with a teflon-lined cap, followed by addition of 100 μL of 0.5 g/0.5 mL of nimesulide (internal standard) solution and 0.3 mL of acetonitrile. The mixture was vortexed to ensure complete mixing of contents for 5 min and centrifuged for 10 minutes at 3,000 rpm. The supernatant liquid was filtered through 0.2 μm membrane filter and twenty microlitres of the filtrate was injected into reverse phase C-18 column and the eluents were monitored at 237 nm. The peak areas of nimodipine and internal standard were recorded. The ratio of peak area of nimodipine to that of internal standard (nimesulide) was calculated, and the regression of the peak area ratio over the plasma concentration of the drug was calculated using the least squares method of analysis.

Precision: Blank human plasma was spiked with 100 μL of internal standard solution (containing 0.5 μg/0.5 mL) and of nimodipine solutions (100 μL) so as to yield concentrations of 10, 50 and 100 ng/0.5 mL of plasma. Each sample was treated, as described above, and the filtrate was injected in to the HPLC column (n = 5). Each sample was prepared in triplicate on three consecutive days, and were injected in to the HPLC column (n = 5) to observe the precision of the method.

Accuracy: The preanalyzed plasma samples containing 50 ng/ 0.5 mL were added with known quantity of nimodipine (10, 20 or 100 ng/0.5 mL) and was subjected to the proposed HPLC method, in triplicate. The differences in the measured concentration and that of the added quantity (10, 20 or 100 ng/0.5 mL) were expressed as per cent recovery.

RESULTS AND DISCUSSION

The run time of the method was set at 10 min. The retention times of nimodipine and nimesulide (internal standard) were 7.5 min and 4.6 min respectively (Fig. 1). When the same drug solution was injected 5 times, the retention time of the drug and internal standard was same. Table-1 shows the

mean peak area ratios of nimodipine to that of internal standard solutions for 5 such determinations. When the concentration of nimodipine and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed (r = 0.9995) in the range of 10 to 200 ng/0.5 mL. The regression of nimodipine concentration over its peak area ratio was found to be Y = 0.0136 + 0.0102X where 'Y' is the peak area ratio and 'X' is the concentration of nimodipine. This regression equation was to estimate the amount of nimodipine in plasma or in validation study (precision and accuracy).

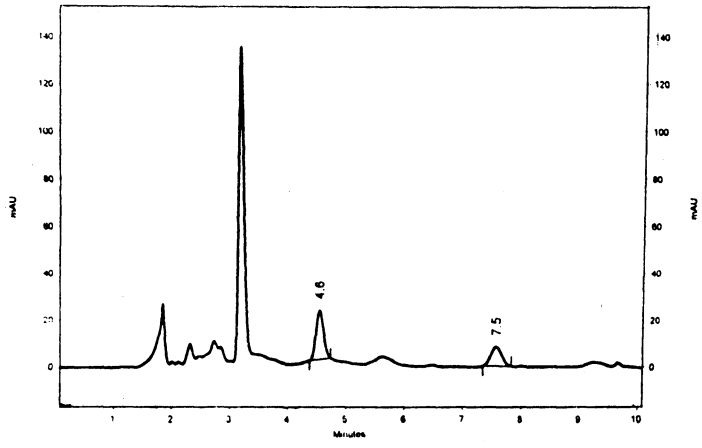

Fig. 1. Typical HPLC chromatogram of nimodipine in human plasma

TABLE-1
CALIBRATION CURVE FOR THE ESTIMATION OF NIMODIPINE IN HUMAN PLASMA BY HPLC METHOD

Amount of nimodipine added to 0.5 mL human plasma (ng)	Peak area ratio*	C.V. (%)
0	0	0.00
10	0.0788	0.21
20	0.1867	0.43
50	0.4605	0.56
100	1.0402	0.71
200	1.9993	0.59

*Mean of five determinations
Regression equation: Y= 0.0136 + 0.0102X (r = 0.9995)

The present HPLC method was also validated for intra- and inter-day variation. To assess the assay recovery from plasma by the present HPLC method, the drug (10, 50 and 100 ng/mL) was added to drug-free plasma along with internal

standard solution. The plasma was extracted, as per the procedure described above, and the resultant filtrate was repeatedly injected on the same day and on three different days. The coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 1.8%. Also, the inter-day variation (3 days and five injections) was found to be less than 2%. Thus, the results show that this HPLC method is highly reproducible. When a known amount of drug solution (50 ng/mL) was added to preanalyzed plasma samples (10, 20 or 100 ng/mL), there was a high recovery (97.83%) of nimodipine indicating that this HPLC method is highly accurate. Hence the proposed HPLC method was found to be simple, precise, highly accurate, specific and less time consuming than the reported methods.

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