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Determination of Nifedipine in Plasma by High-Performance Liquid Chromatography

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A simple high-performance liquid chromatographic method for the determination of nifedipine, dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate, in plasma is described. The method permits the accurate determination of the drug in plasma at concentrations as low as 2 ng/ml and is suitable for drug monitoring and for investigation of the bioavailabilities of drug preparations.

Keywords——nifedipine; high-performance liquid chromatography; plasma; drug monitoring

Nifedipine (I), dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (Chart 1), is a coronary vasodilator.[1–3] Pharmaceutical preparations containing nifedipine have been widely used clinically. The therapeutic effects of nifedipine should be related to its concentration in plasma. Thus, for comparison of the bioavailabilities of the preparations and for drug monitoring during therapy, a rapid and sensitive method of assay was required.

Several methods have been proposed for the determination of nifedipine in biomedical samples, *i.e.*, fluorometry,[4] gas–liquid chromatography (GC) with electron capture detection,[5] GC–mass spectrometry,[6] and reversed phase high-performance liquid chromatography (HPLC).[7]

Nifedipine is very light-sensitive in solution and special care must be taken in analyzing it. Nifedipine decomposes in daylight to give the 4-(2-nitrosophenyl)-pyridine homolog (II) (Chart 1). Fluorometric determination of nifedipine involves reduction of the nitroso group of II to an amino group and derivation of the amino compound to a fluorescent product. The procedure is rather tedious.

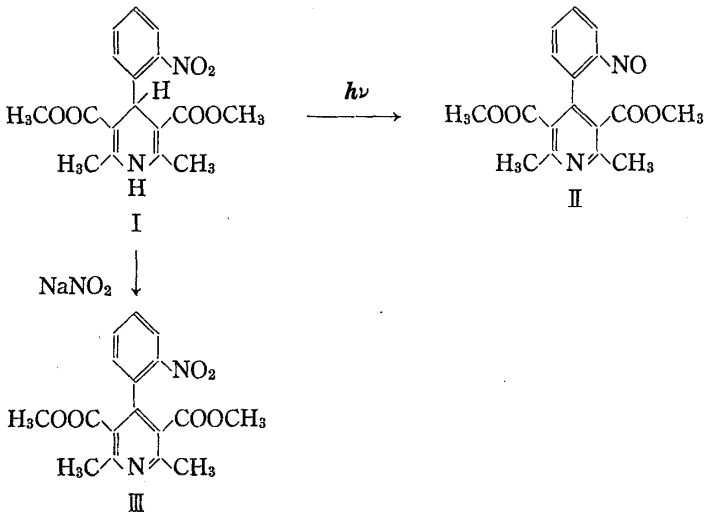

Chart 1

Nifedipine can be oxidized quantitatively with sodium nitrite to give 4-(2-nitrophenyl)-pyridine homolog (III) (Chart 1). Compound III is not a metabolite of nifedipine.[6] We found that III could be separated from plasma substances by normal phase chromatography on silica gel. The present paper describes a high-performance liquid chromatographic procedure for determination of nifedipine in plasma through its conversion to III. The method is simple, sensitive, reliable, and is suitable for comparison of the bioavailabilities of nifedipine preparations and for drug monitoring.

Experimental

Materials——Nifedipine and its preparations were the products of Bayer AG. All solvents and chemicals were obtained from commercial sources and were of reagent grade.

Instrumentation——A Shimadzu LC-3A liquid chromatograph equipped with a Rhodine 7125 syringe loading sample injector valve (100 μl loop) and a Hitachi wavelength-tunable effluent monitor operated at 280 nm was used. The column was LiChrosorb SI-100 (150×4 mm i.d.; Merck Japan, Tokyo, Japan) and the column temperature was maintained at 20°C during the analysis. The pressure was 65 kg/cm², giving a flow rate of 1.5 ml/min.

Standard Procedure——To 3.0 ml of plasma placed in a glass-stoppered centrifuge tube, 1.0 ml of H_2O and 1.0 ml of 2.5 M NaOH were added. The sample was extracted once with 25 ml of $CHCl_3$ with vigorous shaking for 10 min. The phases were subsequently separated by centrifugation (1970 *g*, 15 min). A 20-ml volume of the $CHCl_3$ layer was transferred to a conical flask and was concentrated to about 0.5 ml under a stream of N_2. Then, 1.0 ml of 0.075 M HCl was added and the mixture was evaporated to dryness under N_2. The residue was dissolved in 3.0 ml of 0.075 M HCl and 1.0 ml of 1% aqueous $NaNO_2$ was added. The mixture was allowed to stand for 30 min at room temperature for completion of the oxidation reaction, then 0.05 M borax solution (pH 9.2) was added and the pH of the mixture was adjusted to pH 7.4. Water was added to make the total volume 8.5 ml. The solution was extracted with 8.0 ml of $CHCl_3$ with vigorous shaking for 10 min and the phases were separated by centrifugation (1970 *g*, 5 min). The organic layer (7.0 ml) was transferred to a conical flask and evaporated to dryness under N_2. The procedure described above was carried out under an Na lamp (Toshiba Model NDP-575C, 55W). The residue was dissolved in 110 μl of *n*-hexane containing α-naphthol (1.92 μg/ml) under sonicator mixing (Kokusai Denki Model UO-300FB). A 100 μl aliquot of the *n*-hexane solution was injected into the liquid chromatograph.

For the preparation of the calibration curve, a series of nifedipine standard solutions was made. To 3.0 ml of drug-free plasma, 1.0 ml of the standard solution and 1.0 ml of 2.5 M NaOH were added, and the mixture was extracted with 25 ml of $CHCl_3$. These standards were carried through the procedure described above.

Bioavailability——Capsules containing a known amount of nifedipine were administered orally with 60 ml of water to a group of drugs (three) weighing 14±3.0 kg. Blood samples were drawn at predetermined intervals through the vein of the foreleg and plasma nifedipine levels were determined by the method described above. The administration, blood sampling, and the determination of nifedipine were carried out under the Na lamp.

Results and Discussion

A chromatogram of a plasma sample containing nifedipine is shown in Fig. 1. The mobile phase used was *n*-hexane containing 10% dichloromethane, 0.125% methanol, and 0.008% aqueous ammonia. The retention times were 11.8 min for nifedipine and 13.5 min for α-naphthol, the reference standard. No interfering peak arose from endogenous plasma conponents.

The mobile phase used was found to be the most suitable for the present purpose. Decreasing the concentration of dichloromethane made the retention times longer. A solvent of higher dichloromethane content caused the peaks to overlap partially. Increased methanol concentration resulted in an interfering peak from plasma components. When the content of aqueous ammonia was less than 0.008%, the peaks of nifedipine and the reference standard became closer and overlapped.

The ratio of the area of the peak of nifedipine to that of the reference standard was plotted against the amount of nifedipine in the standard solution. The calibration curve thus obtained was linear up to at least 400 ng/ml and passed through the origin.

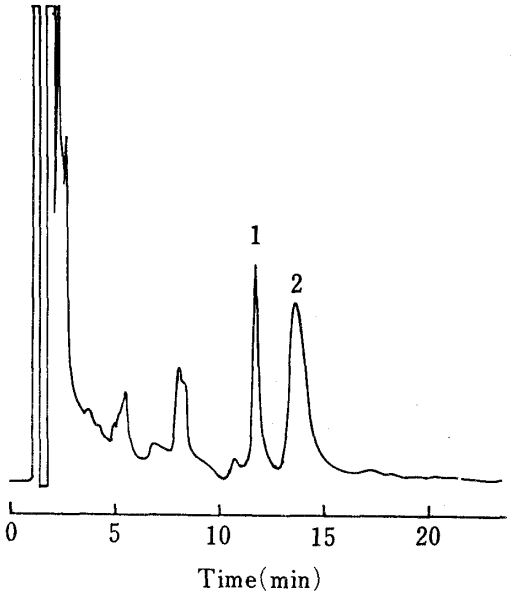

Fig. 1. Chromatogram of Dog Plasma containing Nifedipine

1, nifedipine; 2, α-naphthol (reference standard).

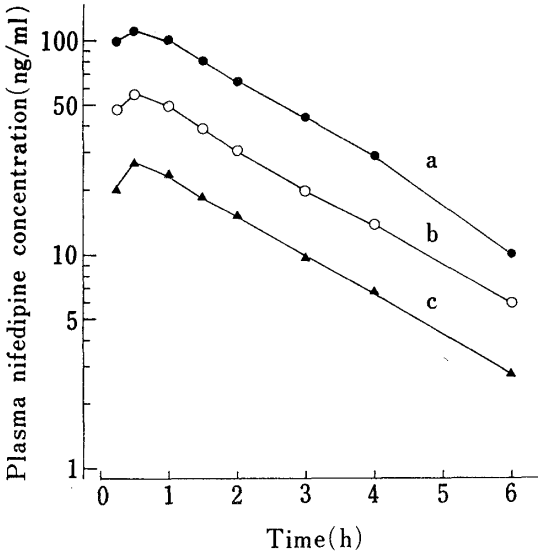

Fig. 2. Plasma Concentration of Nifedipine following Its Oral Administration to Dogs

Doses of nifedipine were 20 mg (a), 10 mg (b), and 5 mg (c) per animal. Analytical data are averages of three animals.

The recovery of nifedipine added to plasma (102 and 205 ng/ml) was complete (100±1.8% (mean±SD), $n=10$ each). When the reference standard was added to plasma just before the extraction, its recovery was sometimes erratic.

In the determination of nifedipine in plasma containing 28 ng/ml, the coefficient of variation was 2% ($n=20$). The present method permits the accurate determination of nifedipine in plasma at concentrations as low as 2 ng/ml plasma.

The plasmas of drugs given nifedipine orally were analysed by the present method. The results are shown in Fig. 2. The plasma nifedipine level increased immediately after the administration, reached a maximum in 30 min, and then decreased at a first order rate.

It was demonstrated by the present method that nifedipine-containing capsules (Adalat) of different lots were bioequivalent in dogs.

References and Notes

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