Journal of Chromatography, 341 (1985) 341—347
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2583

DETERMINATION OF NIFEDIPINE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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(First received November 14th, 1984; revised manuscript received February 6th, 1985)

SUMMARY

A rapid, accurate and sensitive high-performance liquid chromatographic assay was developed for the determination of nifedipine in human plasma. A toluene extract of an alkalinized plasma sample was chromatographed on a reversed-phase column with electrochemical detection at +0.95 V. The recovery of nifedipine from plasma was about 100%. The detection limit for nifedipine in plasma was 2 ng/ml using 0.5 ml of sample. The assay gave a linear response over the concentration range 5—400 ng/ml in plasma. The coefficients of variation from 9.6 to 191.0 ng/ml varied between 5.2 to 1.0% and the accuracy did not exceed 3.0%. Photodegradation products and metabolites of nifedipine did not interfere in the analysis. This method allowed the behaviour of nifedipine in humans to be studied.

INTRODUCTION

Nifedipine belongs to a group of calcium channel antagonists widely used as coronary vasodilators. The major therapeutic application is for angina pectoris [1] and hypertension [2]. Nifedipine is more than 90% absorbed from oral doses and almost completely metabolized before it is excreted [3—5]. As shown in Fig. 1, dimethylpyridinecarboxylic acid (M-I), hydroxymethylpyridinecarboxylic acid (M-II) and the corresponding lactone (M-III) are reported as metabolites [3—6]. Also, the pyridine derivative (P-I) is postulated as a precursor of known metabolites of nifedipine [7—10]. Nifedipine is very sensitive to light and the 2-nitroso derivative (P-II) and pyridine derivative (P-I) have been detected as its light degradation products [11]. These metabolites and photodegradation products are pharmacologically inactive [3, 5].

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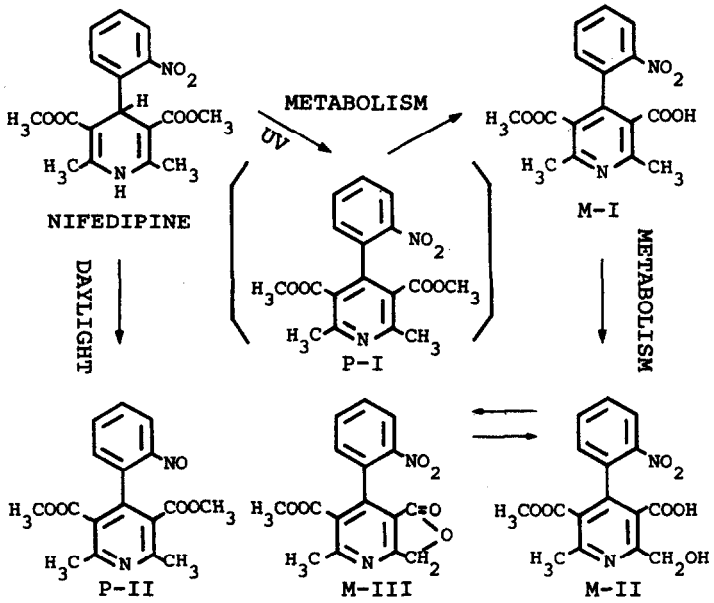


Fig. 1. Structures of nifedipine, its metabolites and photodegradation products.

Many methods for the determination of nifedipine in plasma have been described. Recently, novel gas chromatographic (GC) [6—14], high-performance liquid chromatographic (HPLC) [15—18] and radio-receptor assays [19] have been developed. However, the GC determination of nifedipine suffers from a serious problem as high temperatures are employed and the non-reproducible degradation of nifedipine to P-I during chromatography cannot be avoided. Therefore, nifedipine is oxidized to its more stable pyridine derivative (P-I) prior to analysis [6, 12]. The specificity of the method, however, may be reduced when considerable amounts of P-I are present in the original sample. The HPLC methods are highly accurate and selective, but the sensitivity of most of them is low owing to the use of a UV detector, and they therefore require a large amount of plasma [16, 17] and/or complex sample preparation [15].

The purpose of this study was to develop a simple, sensitive and selective HPLC method using an electrochemical detector for the determination of nifedipine in human plasma.

EXPERIMENTAL

Materials

The nifedipine supplied (Kanebo, Osaka, Japan) was used without further purification. Diethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate, used as an internal standard, was synthesized according to the reported procedure [6]. HPLC-grade methanol, tetrahydrofuran and toluene were purchased from Wako (Osaka, Japan). Other reagents and chemicals were of analytical-reagent grade.

Chromatography

The chromatographic system consisted of a Model 510 solvent delivery

system, a U6K universal injector (Waters Assoc., Milford, MA, U.S.A.) and a Model VMD-501 electrochemical detector (Yanagimoto Seisakusho, Kyoto, Japan). The potential of the detector was set at +0.95 V versus an Ag/AgCl reference electrode. Chromatographic experiments were performed on a Unisil Pack 5C18-150A column (octadecylsilica, 15 cm × 4.6 mm I.D., particle size 5 μm) (Gasukuro Kogyo, Tokyo, Japan). Methanol—tetrahydrofuran—0.05 *M* phosphate buffer (pH 3.0) (660:10:330) was employed as the mobile phase at a flow-rate of 0.8 ml/min. The mobile phase was degassed by vacuum plus sonication prior to use. The chromatography was carried out at 20°C.

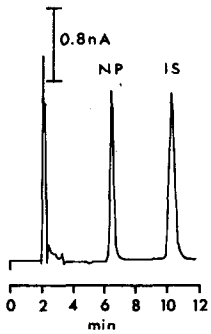
Sample preparation

To 0.5 ml of plasma in a 10-ml conical extraction tube fitted with a glass cap were added 50 μl of the internal standard solution (1 μg/ml in methanol). Then 0.5 ml of 0.1 *M* borate buffer (pH 9, adjusted with sodium hydroxide solution) was added and the mixture was vortex-mixed for 10 sec. The extraction was carried out on a shaking board for 15 min with 6 ml of toluene followed by centrifugation at 2000 *g* for 10 min. A 5-ml volume of the organic layer was transferred into a second tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase and 10-μl aliquots were injected into the chromatograph. All steps were carried out in a dark room and samples were shielded from exposure to direct lighting to prevent light degradation of nifedipine [11].

RESULTS AND DISCUSSION

Electrochemistry

The electrochemical detector used was equipped with dual electrodes, but we used only a single electrode. The chromatogram shown in Fig. 2 was obtained from a standard solution of nifedipine and the internal standard at a detector potential of +0.95 V vs. Ag/AgCl. Hydrodynamic voltammograms observed for the oxidation of nifedipine and the internal standard under the HPLC conditions used are illustrated in Fig. 3. Based on these curves, +1.15 V



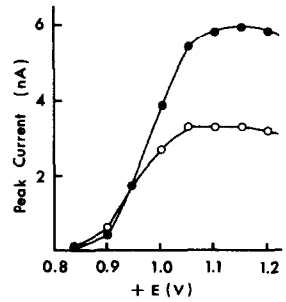


Fig. 2. Chromatogram of a standard mixture of nifedipine (NP) and the internal standard (IS). All amounts injected were 7.5 ng.

Fig. 3. Hydrodynamic voltammograms of nifedipine (•) and the internal standard (○). All amounts injected were 7.5 ng.

was the most sensitive potential. However, at high potentials (> +1.0 V) the background current becomes high, the baseline drifts owing to the oxidation of water, oxygen and other mobile-phase components and the performance of the electrode decreases rapidly [20]. Therefore, an applied electrode potential of +0.95 V was chosen. The detection limit (signal-to-noise ratio = 2) for a standard solution was approximately 40 pg injected at +0.95 V. This detection limit could be lowered by increasing the detector potential.

Calibration

Calibration samples were prepared by using drug-free plasma. Plasma samples (0.5 ml) spiked with nifedipine at known concentrations (0, 5, 10, 30, 60, 100, 200 and 400 ng/ml) were assayed as described above. A least-squares linear regression evaluation of the peak height ratio (y) versus concentration (x) relationship gave $y = 0.0114x - 0.0241$, with a correlation coefficient of 0.9998.

Reproducibility and accuracy

Reproducibility and accuracy were determined for five or six spiked plasma samples with respect to a calibration graph (Table I). The within-day coefficients of variation were 1.0—5.2%. The day-to-day coefficients of variation for analyses of the same plasma samples on three days over a period of one week were 3.2% at 41.8 ng/ml ($n = 6$) and 2.9% at 83.1 ng/ml ($n = 5$). The accuracy of the method expressed as the mean deviation of all concentrations from the theoretical value ranged from —2.9% to 2.3%.

TABLE I

REPRODUCIBILITY AND ACCURACY FOR NIFEDIPINE

Spiked value (ng/ml)	Number of samples	Assay value (ng/ml) (mean ± S.D.)	Coefficient of variation (%)	Accuracy (%)
Within-day				
9.6	6	9.5 ± 0.5	5.2	—0.2
47.8	6	46.4 ± 1.3	2.8	—2.9
95.5	6	96.8 ± 1.0	1.0	1.4
191.0	6	195.1 ± 4.4	2.2	2.2
Day-to-day				
41.8	6	41.7 ± 1.3	3.2	—0.3
83.1	5	81.6 ± 2.3	2.9	2.3

Recovery

The recovery of nifedipine was estimated as follows. Control plasma samples spiked with 10, 50 and 100 ng/ml nifedipine were extracted as described above without addition of the internal standard solution. Before evaporation the internal standard solution was added to each extract and the subsequent procedure was carried out as described above. The recovery was calculated by comparing the peak height ratios of control plasma samples with those of non-extraction standards.

TABLE II

EXTRACTION YIELD OF NIFEDIPINE FROM SPIKED PLASMA SAMPLES (n = 3)

Spiked value (ng/ml)	Extraction yield (%)	Coefficient of variation (%)
10	101.2	5.1
50	101.7	2.0
100	102.6	1.4

As shown in Table II, nifedipine was recovered quantitatively, with a range of 101.2—102.6%.

Chromatography

In reversed-phase HPLC, the mobile phase is commonly a binary mixture of solvents. In this study, various combinations of methanol and buffer solution were examined, but a small peak in the blank plasma was not well separated from the internal standard. A ternary solvent system was then investigated using combinations of methanol, acetonitrile and tetrahydrofuran [21]. As a result, methanol—tetrahydrofuran—0.05 *M* phosphate buffer (660:10:330) was selected for their assay.

Typical chromatograms of a blank plasma and a plasma sample taken 20 min after oral administration of 10 mg of nifedipine to a healthy volunteer are shown in Fig. 4. The retention times of nifedipine and the internal standard were approximately 6.5 and 10.6 min, respectively. No interfering peaks were found in several blank plasma samples examined. The concentration of nifedipine in this sample was about 120 ng/ml. Based on a signal-to-noise ratio of 2, the detection limit of the assay for a plasma sample (0.5 ml) was ca. 2 ng/ml, which is well below the drug concentration expected in biological specimens from patients given therapeutic doses of nifedipine.

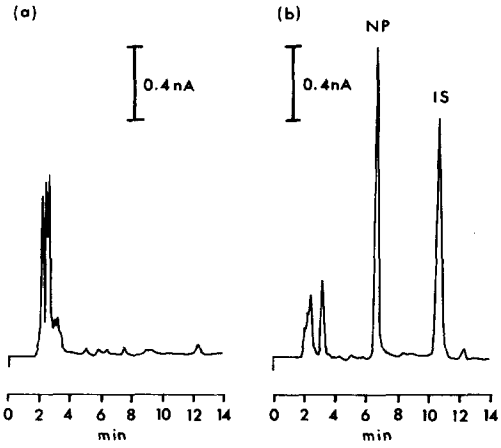


Fig. 4. Chromatograms of extracts from (a) human blank plasma and (b) human plasma collected 20 min after oral administration of 10 mg of nifedipine preparation. Peaks: NP = nifedipine; IS = internal standard.

Selectivity

The metabolites and photodegradation products of nifedipine did not interfere as they did not have electrochemical activity. Nifedipine may be administered in combination with other drugs such as pindolol, carteolol, acebutolol, trichlormethiazide, furosemide, methyldopa, reserpine, diazepam, oxazepam, aspirin, warfarin and trapidil. Among the drugs tested, pindolol, methyldopa and resperine had electrochemical activity, but they did not interfere in the assay because they were well resolved from nifedipine and the internal standard.

Application to biological samples

The proposed method was applied to the determination of nifedipine in plasma samples obtained from three healthy, fasting volunteers who received orally fine granules containing 10 mg of nifedipine (Sepamit; Kanebo, Japan). Blood samples were drawn 0, 20, 40, 60, 120, 240 and 420 min after administration. After immediate centrifugation the plasma was stored at −20°C until taken for assay.

Fig. 5 shows the mean plasma concentration curve of the three subjects and Table III gives the parameters calculated from the data in Fig. 5. Nifedipine was rapidly absorbed from this preparation with maximum concentrations of ca. 150 ng/ml 20 min after administration. These values are in agreement with a previous report [5].

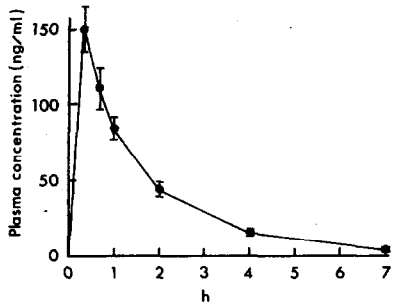


Fig. 5. Mean plasma concentrations of nifedipine in three healthy volunteers following a single oral administration of fine granules containing 10 mg of nifedipine (Sepamit). Each point represents the mean ± standard error for three subjects.

TABLE III

INDIVIDUAL PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF 10 mg NIFEDIPINE (SEPAMIT)

Subject	T_{max} (min)	C_{max} (ng/ml)	$AUC_{0-\infty}$* (ng · h/ml)	$t_{1/2}$ (h)
A	20	174.3	316.3	1.6
B	20	119.4	212.7	1.3
C	20	157.9	287.2	1.9
Mean	20	150.5	272.1	1.6

*AUC = Area under the curve.

CONCLUSION

The method described is sufficiently simple, sensitive, specific and rapid for the determination of nifedipine at therapeutic concentrations in human plasma. It can be used for routine clinical monitoring and in pharmacokinetic studies of small animals.

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