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Journal of Chromatography B, 687 (1996) 413–418

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Liquid chromatographic determination of manidipine in serum with electrochemical detection

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Received 8 August 1995; revised 29 May 1996; accepted 30 May 1996

Abstract

A highly sensitive and selective method for determining a dihydropyridine calcium antagonist, manidipine, by liquid chromatography using column switching with electrochemical detection was developed. Manidipine in serum was extracted by a rapid and simple procedure based on C_8 bonded-phase extraction and then silica extraction. Manidipine and nilvadipine as an internal standard were separated on a C_8 bonded-phase HPLC column and detected by high conversion efficiency amperometric detection at +0.7 V. Manidipine and nilvadipine (I.S.) were separated from an endogenous interference peak in serum and concentrated on a pre-column (C_{18}) by column switching using an isocratic mobile phase, and then the corresponding fractions were introduced to an analytical column with a C_8 stationary phase. Determination of manidipine was possible over the concentration range 0.5–10 ng/ml: the limit of detection was 0.3 ng/ml. The recovery of manidipine added to serum was 93.1–98.4% with coefficients of variation of less than 7.1%. The method is applicable to drug level monitoring in the serum of healthy volunteers treated with manidipine and to the analysis of pharmacokinetics.

Keywords: Manidipine

1. Introduction

Manidipine (Fig. 1), 2-(4-(diphenylmethyl)-1-piperazinyl]ethyl methyl (±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate, is a newly developed potent 1,4-dihydropyridine calcium antagonist having long-lasting activity [1]. The serum concentration of manidipine after oral administration in human is very low [2]. Therefore, a sensitive analytical method for manidipine needed to be developed for pharmacokinetic studies.

A method for the determination of manidipine by HPLC using ultraviolet detection at 230 nm and an ion-pair mobile phase for column separation has been described [3,4]. However, ultraviolet detection at a short wavelength such as 230 nm is not suitable for the sensitive determination of a drug in biological samples because of poor selectivity. The dihydropyridine derivatives may be electrochemically detected with high sensitivity and selectivity. In previous papers, we reported highly selective and sensitive electrochemical detection (ED) methods for the determination of drugs by HPLC [5–7]. HPLC–ED methods with liquid–liquid extraction for the determination of dihydropyridine in plasma has been previously described [8–10]. However, the concentrations of dihydropyridine studied in these methods were 5–400 ng/ml [8], 1–100 ng/ml [9] and 5–500 ng/ml [10] in plasma, respectively. We often encountered the difficulty that the required sensitivity

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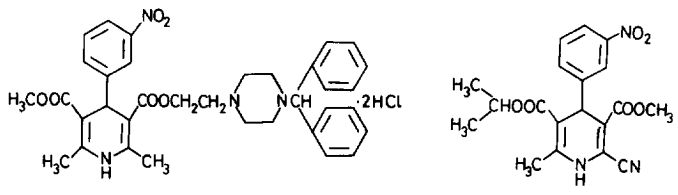
0378-4347/96/$15.00
PII S0378-4347(96)00252-6



(a) (b)

Fig. 1. Chemical structure of (a) manidipine and (b) nilvadipine.

could not be obtained because of interference from endogenous substances.

In previous papers, we described column-switching HPLC methods for the determination of several drugs [5,11,12]. In the present paper, we describe the development of a highly sensitive HPLC–ED method for the determination of manidipine in serum, involving the use of solid-phase extraction and column-switching techniques.

2. Experimental

2.1. Reagents and materials

Manidipine and nilvadipine (Fig. 1) were kindly donated by Takeda Chemical Industries (Osaka, Japan) and Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Bond Elut™ C_8 cartridge and Sep-pak® silica cartridge were purchased from Varian (Harbor City, CA, USA) and Millipore Co. (Bedford, MA, USA). All other solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals of analytical grade were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan).

2.2. Apparatus

The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) equipped with a Coulochem Model 5100A electrochemical detector (Environmental Science Assoc., Bedford, MA, USA). The potential of the electrochemical detector was set at +0.7 V vs. a reference electrode. Test samples were introduced using a Rheodyne Model 7120 injector (Rheodyne, Cotati, CA, USA) with an effective volume of 100 μl. Another Rheodyne Model 7120 injector was used as the switching valve. The HPLC precolumn and analytical column contained Develosil ODS-5 and Develosil C_8-5 stationary phase (5 μm) (Nomura Chemical, Seto, Japan). A stainless steel analytical column (150×4.6 mm I.D.) was packed in this laboratory by a conventional high-pressure slurry-packing procedure. The precolumn (50×4.6 mm I.D.) was packed by a low-pressure packing technique. The mobile phase consisted of 0.1 *M* disodium hydrogen-phosphate (pH 4.5, adjusted with 50% phosphoric acid)–acetonitrile (51:49, v/v); the mobile phase was degassed ultrasonically, and the flow-rate was 1.0 ml/min at ambient temperature.

2.3. Sample preparation

Nilvadipine (20 ng) in methanol (10 μl) was added to the serum sample (1 ml) as an internal standard, the serum sample was then diluted with 5 ml water and the solution was briefly mixed. The mixture was applied to a Bond Elut C_8 cartridge that had previously been activated with 5 ml of methanol and water. The cartridge was then washed with 2.0 ml of water and 20% methanol, and then the desired fraction was eluted with 5 ml of 80% methanol. The eluate was evaporated to dryness in vacuum at 60°C. The residue was dissolved in 0.5 ml of chloroform and applied to a Sep-pak Silica cartridge that had previously been activated with 2 ml of chloroform. The cartridge was washed with 3 ml of chloroform, and the desired fraction was eluted with 3.0 ml of chloroform–methanol (20:1). The eluate was evaporated under vacuum and the residue was dissolved in 150 μl of mobile phase. The dissolved extracts (100

μl) were loaded onto the precolumn for the elimination of interfering substances from the serum sample. After thorough washing for 2.8 min, manidipine and nilvadipine were eluted from the precolumn and then led to the analytical column by a column-switching technique using the previously described mobile phase.

2.4. Calibration graphs

Known amounts of manidipine in the range 0.5–10 ng/ml were added to blank serum sample. These serum samples were treated according to the extraction procedure described above. The peak-height ratios of manidipine to nilvadipine were measured and plotted against the respective concentration of analyte.

2.5. Preparation of quality control and calibration samples

Duplicate samples were prepared in 1.0 ml of serum, by adding aliquots of the stock solution of manidipine to drug free serum at three different concentrations – 1.0, 2.5 and 5.0 ng/ml – to determine the accuracy and precision of the method. These samples are designated quality control samples. The quality control samples were stored at −40°C. Calibration samples containing 0.5, 1, 2.5, 5 and 10 ng/ml of manidipine were prepared. The calibration samples were treated in the same manner as the quality control samples. For each validation run quality control samples were thawed and extracted.

2.6. Recovery experiments

Serum was spiked by adding a known amount of manidipine to drug-free serum to obtain a total volume of 1 ml. These samples were extracted by the above described method. Control samples were prepared by adding a known amount of manidipine to 1 ml of methanol. These control samples were not extracted, but directly evaporated to dryness at 60°C, and the residues reconstituted in 150 μl of the mobile phase. An external standard instead of the internal standard was added to all of the samples before the samples were evaporated to dryness. Recoveries were determined by comparison between solid-phase extraction and non-extracted controls.

2.7. Method validation

The accuracy of the method was determined by injection of the manidipine calibration samples and the three different quality control samples after extraction on six separate days. All calibration curves were required to have a correlation value of at least 0.99. The accuracy was calculated as a percentage of the nominal concentration: Accuracy = (Conc.obs/conc.nominal) · 100%. The same data used in the accuracy determinations were used for the calculation of the between-run percentage relative standard deviation [%R.S.D.:R.S.D. = (S.D./mean) · 100%]. The within-run %R.S.D. resulted from analysis of six quality control samples at each concentration with injection on the same day. The detection limit of the HPLC assay after extraction was estimated from the drug quantity in saliva which corresponded to five times the baseline noise. The lower limit of quantitation was defined as the quantity of the sample after preparation and extraction which was quantified with deviation and precision less than 20%.

2.8. Drug administration and sampling

Manidipine tablets (10 and 20 mg of Calslot® brand of manidipine, Takeda) were orally administered to seven healthy volunteers (10 mg to two and 20 mg to five). Blood samples (5 ml) were collected by venepuncture at 1, 2, 4, 6, 12 and 24 h after administration. Serum samples were separated by centrifugation at 1900 *g* for 15 min and stored at −40°C until analysis.

3. Results

The electrochemical detector used was equipped with coulometric dual electrodes, but only the first electrode was used. Hydrodynamic voltammograms of manidipine and nilvadipine were obtained in the HPLC chromatogram. Based on these curves, +0.8 V was the most sensitive potential and half-wave potentials ($E_{1/2}$) of manidipine and nilvadipine were

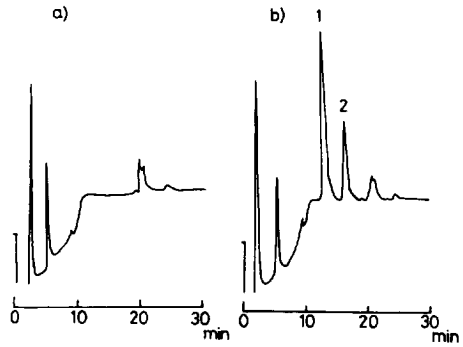


Fig. 2. Chromatograms of (a) plasma blank and (b) added standards in serum. Peaks: 1 = manidipine, 2 = internal standard (nilvadipine).

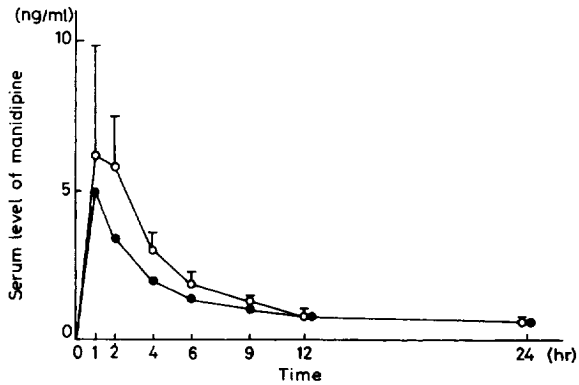


Fig. 3. Serum concentration–time profile of manidipine after single 10-mg and 20-mg oral dose of manidipine to two (10 mg) and five (20 mg) healthy volunteers. Dose: (●) 10 mg, (○) 20 mg.

observed at +0.62 and +0.65 V, respectively. A stable and high electrochemical response was obtained at +0.7 V. Therefore, the applied potential of this HPLC–ED system was set at +0.7 V. A typical chromatogram of a standard mixture of manidipine and nilvadipine is shown in Fig. 2. Low interference from endogenous components of serum was obtained by this extraction method employing a column-switching technique. Calibration graphs for manidipine in human serum were linear in the range 0.5–10 ng/ml. The limit of detection for manidipine was 0.3 ng/ml (signal-to-noise ratio = 5). The results of recovery studies are shown in Table 1. The recovery of manidipine was determined by adding the three known levels of 1.0, 2.5 and 5.0 ng/ml to blank serum. The recovery values for manidipine were 93.1–98.4% in serum. Coefficients of variation were less than 7.1%. The time course of concentrations of manidipine in serum samples from seven healthy volunteers receiving 10 mg (two volunteers) and 20 mg (five volunteers) manidipine orally were determined using the proposed method (Fig. 3). Pharmacokinetic parameters of seven healthy volunteers were calculated and are shown in Table 2.

4. Discussion

HPLC determination methods for 1,4-dihydropyridine derivatives have been described in previous papers [13–16]. Nimodipine [13], nicardipine [14], nitrendipine [15] and nifedipine [16] were determined by HPLC with ultraviolet detection. The sensitivity of the ultraviolet detector was not sufficient for the low concentration of 1,4-dihydropyridine derivatives (1–2 ng/ml). Two previous papers reported determination of manidipine in plasma by HPLC–UV [3,4]. The sensitivity of this method was 0.2 ng/ml of manidipine. However, it is considered that the confidence range of the detection limit is greater than 1 ng/ml in plasma or serum for several compounds as measured by HPLC–UV. A sensitive

Table 1
Accuracy and precision of determination of manidipine in human serum

Added (ng/ml)	Found (mean±S.D.) (ng/ml)	Accuracy (%)	Between-run precision (%)	Within-run precision (%)
1.0	0.99±0.07	98.4	7.1	3.9
2.5	2.40±0.11	95.7	4.6	4.4
5.0	4.66±0.19	93.1	4.1	2.3

(n = 6).

Table 2
Pharmacokinetic parameters of manidipine in healthy volunteers

Manidipine (mg)	C_{max} (ng/ml)	T_{max} (h)	K_e (h^{-1})	$T_{\frac{1}{2}}$ (h)	AUC (ng·h/ml)
10 ($n=2$)	5.0	1.0	0.11	6.80	29.5
20 ($n=5$)	7.6±1.9	1.4±0.5	0.11±0.02	6.4±1.1	40.4±7.7

and selective HPLC–ED method has been reported in previous papers [8–10]. An amperometric electrochemical detector was used for these HPLC detection systems. It is recognized that the efficiency of the electrochemical reaction of the amperometric electrochemical detection is about 3–10%. On the other hand, a high conversion efficiency amperometric detector could obtain a high efficiency (about 70–100%), which is the expected high sensitivity detection limit. We have reported highly sensitive HPLC methods with high conversion efficiency electrochemical detection for several drugs [5–7]. Initially, our efforts were directed toward developing a highly sensitive high conversion efficiency electrochemical detection of manidipine by HPLC. The electrochemical response of manidipine and nilvadipine was examined on hydrodynamic voltammograms. Based on these curves, manidipine and nilvadipine were detected with greatest sensitivity at a potential above +0.8 V. However, the background current and baseline drifts became high owing to the oxidation of water, oxygen and mobile-phase components. Also, the interference peak from serum samples was higher at a potential of above +0.8 V than below +0.8 V. Therefore, an applied electrode potential of +0.7 V was chosen. The detection limit (signal-to-noise ratio = 5) was approximately 50 pg for the standard solution. We then directed our project towards establishing an extraction method for manidipine and nilvadipine, and elimination methods of endogenous substances from plasma. Several extraction methods with liquid–liquid extraction have been described [8–10,13,14,16], but such methods are tedious. Bocker et al. [15] reported a simple extraction method for nitrendipine and its metabolites in an incubation mixture of liver microsomes using a Bond Elut C_{18} extraction column.

However, in our present study, the high interference peak of endogenous components in serum was not removed. Next, we attempted further purification of the eluate from Bond Elut C_8 by the use of a Sep-pak silica cartridge coupled with column-switching technique. No interference was observed in the chromatograms at the retention time of the analyte (Fig. 2). Calibration graphs for manidipine in human serum were linear in the range 0.5–10 ng/ml. The sensitivity and the calibration range of the present method is appropriate for therapeutic drug monitoring of manidipine in patients. The results of recovery studies (Table 1) show that the proposed method is satisfactory with respect to accuracy and precision. The method described was used to study the pharmacokinetics of manidipine at oral doses of 10 and 20 mg in healthy volunteers (Fig. 3). In a previous paper, Miyabayashi et al. [3] described the pharmacokinetics of manidipine. The pharmacokinetic parameters obtained in the present study (Table 2), are roughly similar to those of the previous paper [3].

From these results, it was shown that the proposed method for the determination of manidipine could be applied to pharmacokinetic studies in patients receiving manidipine treatment. Further pharmacokinetic studies of drug interaction are being carried out in these laboratories, and the details will be reported elsewhere.

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