Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 32 (2003) 387–392
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

High-performance liquid chromatographic analysis of nitrendipine in human plasma using ultraviolet detection and single-step solid-phase sample preparation

Soo Yeon Oh, Kwang Youl Kim, Yoon Gyoon Kim, Hyung-Gun Kim *

Department of Pharmacology, College of Medicine, Dankook University, San 29-1, Anseo-Dong, Cheonan, Choongnam 330-714, South Korea

Received 29 January 2003; received in revised form 6 March 2003; accepted 8 March 2003

Abstract

A high-performance liquid chromatographic (HPLC) method was developed for the determination of nitrendipine in human plasma using solid-phase extraction (SPE) and ultraviolet detection. A 30-μl aliquot of methanol (containing 2 μg/ml of the internal standard, nimodipine) was added to a 1-ml aliquot of biological sample. After vortex-mixing, the mixture was loaded on C_{18} SPE cartridge which was conditioned with methanol and distilled water. After washing with distilled water, the SPE cartridge was eluted with 1-ml aliquot of diethyl ether. The organic phase was collected and evaporated under nitrogen gas. The residue was then reconstituted with a 100-μl aliquot of mobile phase, and a 50-μl aliquot was injected onto the C_{18} reverse-phased column. The mobile phase, 10 mM phosphate buffer (pH 4.5):acetonitrile (50:50, v/v), was run at a flow rate of 1.2 ml/min. The column effluent was monitored using ultraviolet detector at 238 nm. The retention times for nitrendipine and the internal standard were approximately 10.1 and 12.6 min, respectively. The detection limit of nitrendipine in human plasma was 2.0 ng/ml. The coefficients of variation (CV) of the assay were below 16.5% for human plasma, and no interferences from endogenous substances were found. This specific, accurate and precise assay was useful in the study for the pharmacokinetic characteristics of nitrendipine.

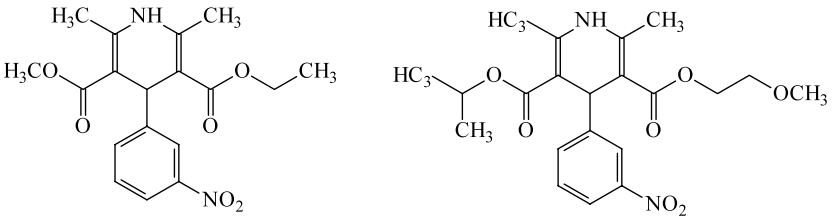
Keywords: Nitrendipine; HPLC; Solid-phase extraction (SPE); Pharmacokinetics

1. Introduction

Nitrendipine (Fig. 1) is one of the dihydropyridine-type calcium antagonist, and widely used for long-term treatment of hypertension. Many of analytical methods for nitrendipine in biological samples were reported [1–5], however, there are some problems about these reported nitrendipine assaying method. Gas chromatography with mass spectrometric detection or electron capture detector showed very high sensitivity and specificity [1–3], but might cause thermal decomposition of nitrendipine. LC–MS method could be best choice considering sensitivity, specificity and stability, but

* Corresponding author. Tel.: +82-41-550-3867; fax: +82-41-550-3866.
E-mail address: hgkimm@dku.edu (H.-G. Kim).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00129-8



Nitrendipine Nimodipine

Fig. 1. Chemical structures of nitrendipine and nimodipine (internal standard).

in many of laboratories LC–MS is not available for economic reason. High-performance liquid chromatographic (HPLC) method using ultraviolet detector is most available but shows low sensitivity and specificity compared with LC–MS method. In our laboratory, HPLC method using electro-chemical detector (amperometric) was firstly tried, however, there were many problems such as stabilizing the detector, interferences by endogenous peak, restriction of mobile phase selection. Then, HPLC method using ultraviolet detector was tried. Janis et al. [4] previously reported HPLC method for nitrendipine in human plasma using liquid extraction that consumed lots of organic solvent. Recently, some HPLC method using ultraviolet detector were reported [6,7], but these method were not sufficient for determining nitrendipine in human plasma for pharmacokinetic study. In this analytical work, HPLC method for nitrendipine in human plasma using solid-phase extraction (SPE) and ultraviolet detection was developed. Also, this method applied to evaluate the pharmacokinetic properties of nitrendipine after oral administration to eight healthy volunteers.

2. Experimental

2.1. Chemicals

Nitrendipine for the HPLC assay was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and nimodipine (Fig. 1), the internal standard for the HPLC assay, was used from Nimotop infusion solution (Searle Korea, Seoul, Korea) without further purification. Other chemicals were of reagent grade or HPLC grade.

2.2. Preparation of stock and standard solutions

A stock solution of nitrendipine (1 mg/ml) was prepared in methanol and dilutions of stock solution were made with methanol. Standard solutions of nitrendipine in human plasma were prepared by spiking with an appropriate volume (less than 10 μl/ml of plasma) of the diluted stock solution, giving final concentrations of 2, 5, 10, 20, 50 and 100 ng/ml. The internal standard solution was prepared by diluting Nimotop infusion solution (0.2 mg/ml) with methanol to give final concentration of 2 μg/ml.

2.3. Sample preparation

A 30-μl aliquot of internal standard (nimodipine, 2 μg/ml in methanol) and 50-μl aliquot of 1 N sodium hydroxide were added to 1-ml aliquot of plasma sample. After vigorous mixing, the mixture was loaded onto Sep-Pak C_{18} cartridge (solid phase 500 mg, Waters, Milford, MA, USA) which was conditioned with 2 ml of methanol and 1 ml of water. The cartridge was then washed with 1 ml of water and dried under slight vacuum for 10 min. Finally, the cartridge was eluted with 1 ml of diethyl ether. Flow rates of sample loading and eluting on cartridge were about 0.5 and 1 ml/min, respectively. The effluent was transferred to clean glass tube and then evaporated under a gentle stream of nitrogen. The residue was reconstituted in a 100 μl of mobile phase, and a 50-μl aliquot of the solution was injected onto the HPLC column.

2.4. HPLC apparatus

The HPLC system consisted of a model 7120 injector (Rheodyne, Cotati, CA, USA), a model Series-II basic isocratic pump (LabAlliance, Science Park Road-State College, PA, USA), a reversed-phase (C_{18}) column (YMC-Pack Pro C18; 150 mm, $l \times 4.6$ mm, i.d.; particle size, 5 μm; YMC Co., Ltd., Kyoto, Japan), a model 151 UV/Vis detector (Gilson, Middleton, WI, USA) and a model 1200 recorder (Linear, Reno, NV, USA). The mobile phase, 10 mM phosphate buffer (pH 4.5):acetonitrile (50:50, v/v), was run at a flow rate of 1.2 ml/min at room temperature. The column effluent was monitored using ultraviolet detector at 238 nm.

2.5. Clinical pharmacokinetic study with healthy volunteers

Eight healthy male Korean volunteers completed the study. Volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests. Each volunteer received an oral dose of 20 mg of nitrendipine (Nitren tablet, 20 mg, Boryung Pharm. Co., Ltd., Seoul, Korea). This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice. The protocol of this study was approved by the Ethical Committee of Dankook University Medical Center (Cheonan, Korea).

Subjects were hospitalized (Dankook University Medical Center) at 21:00 h 1 day before this study and fasted 10 h before each drug administration and 4 h after. At 08:00 h, median cubital vein was cannulated (D&B-CATH, Seoul, Korea), and heparinized normal saline injectable solution (20 units/ml), 1 ml, was flushed into the cannula to prevent blood clotting. The doses were taken at 09:00 h of each dosing day with 200 ml of tap water. Approximately 10 ml blood samples were collected via the cannula at the following times; predose, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 h after the administration. The heparinized normal saline injectable solution, 1 ml, was flushed after each blood sampling. The blood sample was centrifuged immediately, and plasma sample was frozen at −70 °C until the HPLC analysis.

3. Results and discussion

3.1. Sample preparation

The extraction recovery for nitrendipine from plasma sample was about 65% when using C_{18} cartridge. The extraction recovery was higher when using C_8 or C_2 cartridge, but severe interferences were shown. The addition of sodium hydroxide to plasma samples reduced interferences, but did not affect the extraction recovery significantly. It was reported [8,9] that nitrendipine is unstable in acidic or alkaline medium. However, chemical half-life of nitrendipine in alkaline degradation is ranged from 14.74 to 21.65 days [9]. In this sample preparation, the effect of sodium hydroxide on the stability of nitrendipine seems to be minimal. Diethyl ether showed higher extraction recovery and less interference as an elution solvent compared with other organic solvents such as methanol, acetonitrile and ethyl acetate. Drying step after washing the cartridge with distilled water was very important in this SPE method, because lots of interference peaks were shown when elute the incompletely dried cartridge with diethyl ether.

3.2. Sensitivity and specificity

Fig. 2 shows typical chromatograms of drug-free human plasma, drug standards in human plasma spiked with 10 ng of nitrendipine and internal standard (nimodipine, 60 ng), and plasma collected 3 h after oral administration of 20 mg of nitrendipine to a healthy male volunteer. No interferences from endogenous substances were observed in any of the biological samples. The capacity factor (k′) for nitrendipine and the internal standard were 7.04 and 9.03, respectively. The detection limits for nitrendipine in human plasma was 2.0 ng/ml based on a signal-to-noise ratio of 3.0.

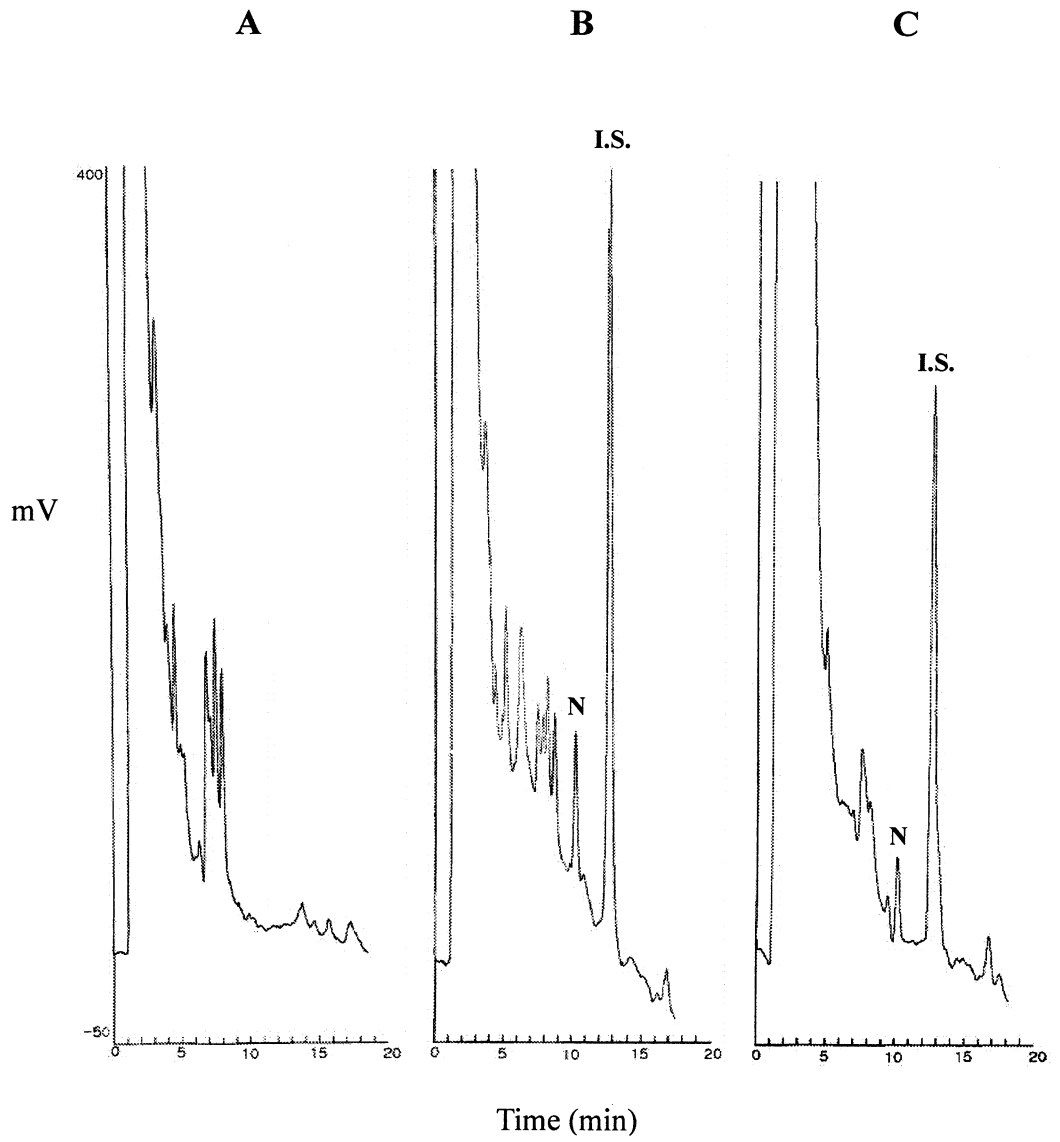


Fig. 2. Chromatograms after extraction of drug-free human plasma (A), human plasma spiked with 10 ng/ml of nitrendipine and 60 ng of internal standard (B), plasma collected from a volunteer at 3 h after oral administration of 20 mg of nitrendipine (C), peak: nitrendipine (10.1 min); internal standard (12.6 min). The detector's sensitivity was set at 0.01 AUFS. N, nitrendipine; I.S., internal standard.

3.3. Linearity

Six point standard curves were linear over the concentration range of 2–100 ng/ml for nitrendipine ($y = 0.01913 \pm 0.00064x - 0.00773 \pm 0.00328$, $n = 4$; y: nitrendipine peak height divided by I.S. peak height, x: theoretical concentration of nitrendipine). The correlation coefficients (r^2) of the standard curves generated during the validation ranged from 0.9976 to 0.9995. The standard curves obtained as described above were used for all the calculations.

Table 1
Intra-day and inter-day response factors and accuracies of nitrendipine at various concentrations in human plasma using SPE method

	Theoretical concentration (ng/ml)	Response factor[a]	Accuracy (%)[b]
Intra-day (n = 4)	2	0.0175 (16.5)	89.4
	5	0.0209 (11.0)	106.5
	10	0.0205 (2.9)	104.3
	20	0.0201 (4.3)	102.3
	50	0.0197 (13.2)	100.4
	100	0.0190 (2.1)	97.0
Inter-day (n = 4)	2	0.0196 (10.3)	100.1
	5	0.0189 (10.7)	96.6
	10	0.0182 (2.7)	92.9
	20	0.0190 (4.8)	96.9
	50	0.0199 (10.5)	101.6
	100	0.0190 (6.6)	96.9

Values in parentheses are CV (%).
[a] (Drug peak height divided by its concentration (ng/ml))/(internal standard peak height); mean.
[b] (Mean observed concentration/theoretical concentration) × 100; mean.

3.4. Precision and accuracy

The intra-day and inter-day coefficients of variations (CVs) for response factor were both lower than 16.5 and 10.7%, respectively (Table 1). The ranges of intra-day and inter-day CVs of nitrendipine in human plasma were 2.1–16.5 and 2.7–10.7%, respectively, within the concentration ranges of 2.0–100 ng/ml (Table 1). The intra-day and inter-day accuracies [(mean observed concentration/theoretical concentration) × 100] of nitrendipine were 89.4–106.5 and 92.9–101.6%, respectively, within the concentration ranges of 2.0–100 ng/ml (Table 1).

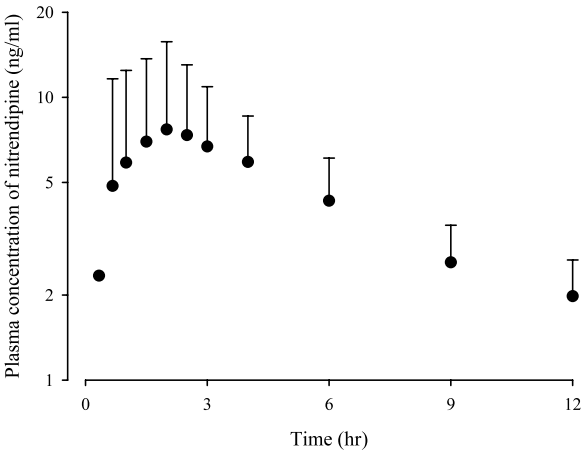


Fig. 3. Mean plasma concentration–time profile of nitrendipine after oral administration of nitrendipine, 20 mg, to eight healthy volunteers. Vertical bar represent standard deviation.

3.5. Clinical application

This HPLC method was also successful for the pharmacokinetic study in human. After oral administration of nitrendipine (20 mg) to eight healthy volunteers, the plasma concentration–time profile of nitrendipine is shown in Fig. 3, and

Table 2
Pharmacokinetic parameters of nitrendipine after oral administration of nitrendipine tablet (20 mg) to eight healthy volunteers

	Mean ± S.D.
AUC (ng h/ml)	67.2 ± 28.5
C_{max} (ng/ml)	11.4 ± 7.4
T_{max} (h)	3.2 ± 1.9
$t_{1/2}$ (h)	6.2 ± 3.1

relevant pharmacokinetic parameters are listed in Table 2.

4. Conclusions

The method described in this report is able to quantitate nitrendipine in human plasma. The SPE clean-up procedure and ultraviolet detection used on the plasma samples gives a very clean chromatogram in which nitrendipine and internal standard, nimodipine, peaks are well enough resolved. The detection limit of this method for nitrendipine is 2 ng/ml, which is enough to detect terminal phase concentrations of nitrendipine after oral administration of 20 mg dose of nitrendipine to eight healthy volunteers. This method could be very useful for bioequivalence test and pharmacokinetic studies for nitrendipine.

Acknowledgements

This study was supported by the research fund of Korea Food and Drug Administration (2002) and of Dankook University in 2002.

References

[1] O. Beck, T. Ryman, J. Chromatogr. 337 (1985) 402–407.
[2] C. Fischer, B. Heuer, K. Heuck, M. Eichelbaum, Biomed. Environ. Mass Spectrom. 13 (1986) 645–650.
[3] P.A. Soons, D.D. Breimer, J. Chromatogr. Biomed. Appl. 13 (1988) 362–368.
[4] R.A. Janis, G.J. Krol, A.J. Noe, M. Pan, J. Clin. Pharmacol. 23 (1983) 266–273.
[5] K. Heinig, F. Bucheli, J. Chromatogr. B 769 (2002) 9–26.
[6] Y.P. Patel, S. Patil, I.C. Bhoir, M. Sundaresan, J. Chromatogr. A 828 (1998) 283–286.
[7] V. Marinkovic, D. Agbaba, S. Vladimirov, S. Stankovic, J. Pharm. Biomed. Anal. 24 (2001) 993–998.
[8] A. Alvarez-Lueje, J. Sturm, J.A. Squella, L.J. Nunez-Vergara, J. Pharm. Biomed. Anal. 25 (2002) 887–895.
[9] D.N. Tipre, P.R. Vavia, J. Pharm. Biomed. Anal. 24 (2001) 705–714.