High-Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometric Determination of Nisoldipine in Human Plasma

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Abstract

A selective and sensitive high-performance liquid chromatography–electrospray ionization mass spectrometry (MS) for the determination of nisoldipine in human plasma is first presented. With nimodipine as the internal standard, nisoldipine is extracted from plasma with ethyl acetate. The organic layer is evaporated and the residue is resuspended in the mobile phase of methanol–water (80:20, v/v). An aliquot of 40 µL is chromatographically analyzed on an Agilent ODS C_{18} reversed-phase column (5 µm, 250- × 4.6-mm i.d.) by means of selected-ion monitoring mode MS. The calibration curve of nisoldipine in plasma exhibits a linear range from 0.5 to 20.0 ng/mL with a correlation coefficient of 0.9995. The limit of detection is 0.2 ng/mL. The within- and between-day variations (relative standard deviation) are less than 9.28% and 11.13% (n = 5), respectively. The developed method is validated through successful use for the analysis of nisoldipine contained in biological fluids resulting from a phase-I human pharmacokinetic study.

Introduction

Nisoldipine, a second-generation dihydropyridine calcium antagonist, is a selective arteriolar vasodilator with little effect on other blood vessels and myocardium when used in a therapeutic dosage (1). An analytical method has been reported for the determination of nisoldipine in blood by high-performance liquid chromatography (HPLC) (2). After 40 mg of nisoldipine was taken orally, blood samples were pretreated by liquid–liquid extraction (LLE) with diethyl ethyl absolute, and the supernatant was removed and evaporated, then the residue was dissolved in mobile phase and analyzed by HPLC with UV detection at 237 nm. There were two main shortcomings in the published procedure. First, some endogenous interferences could not be resolved perfectly from nisoldipine. Second, the low quantitative limit (2.0 ng/mL) is not high enough to meet the requirement needed to analyze the compound when only 10 to 20 mg of nisoldipine was given orally in a bioequivalence test. Another paper described the enantioselective assay of the compound in human plasma using HPLC combined with gas chromatography–mass spectrometry (MS) (3). It proved to be suitable for pharmacokinetic studies based on the low quantitation detection (0.05 ng/mL), but a relatively complex sample preparation was needed. The dissertation presented here first describes a simple, selective, and highly sensitive method using HPLC coupled with electrospray ionization (ESI)-MS for the determination of nisoldipine contained in biological fluids resulting from 20 volunteers, to whom 20 mg of nisoldipine was given orally in a single-dose phase-I human pharmacokinetic study.

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Experimental

Chemicals

Nisoldipine and nimodipine were used as the internal standard (IS) and were supplied by Jiangsu Institute for Drug Control (Nanjing, China). Their structures are shown in Figure 1. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

Apparatus

Chromatographic separation was carried out at room temperature with an Agilent ODS C_{18} (5 µm, 250- × 4.6-mm i.d.) reversed-

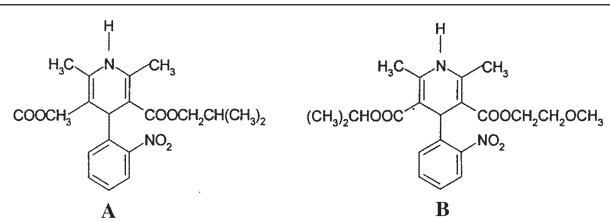

Figure 1. The structures of nisoldipine (A) and nimodipine (B).

phase column on an 1100 series LC system (Agilent Technologies, Palo Alto, CA). The system, equipped with a binary pump, was connected to an autosampler (Hewlett-Packard, Palo Alto, CA). The mobile phase consisted of methanol–water (80:20, v/v) at a flow rate of 1 mL/min, but only 0.4 mL/min eluent volume was introduced into the spectrometer because it was split at the ratio of 4:6 before entering the ESI interface. Detection was performed on an Agilent 1100 series single-quadruple MS equipped with an ESI source at positive single-ion monitoring mode. The [(M+Na)$^+$, *m/z* 411] for nisoldipine and [(M+Na)$^+$, *m/z* 441] for nimodipine were selected as detecting ions, respectively. The MS operating conditions were optimized as follows: temperature, 350°C; drying gas (N_2), 10.0 L/min; nebulizer pressure, 45 psi (N_2); capillary exit voltage, 4000 V; and fragmentor voltage, 110 eV. All data were acquired and processed by Agilent Chemstation Rev. A.08.03 (Agilent) software on a Hewlett-Packard computer.

Sample preparation

Twenty healthy male volunteers were given a single oral dose of 20 mg nisoldipine in a random two-way cross-over design. Blood samples were collected at the time of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 24.0 h after oral administration of the capsules. The blood samples were immediately centrifuged at 3000 g for 10 min. The plasma was removed and stored at –20°C until the analysis was completed.

A 1.0-mL volume of plasma, 8 ng nimodipine (IS), and 0.1 mL of 1.0M NaOH were mixed with 5.0 mL of ethyl acetate in a 10-mL centrifuge tube and vortexed for 3 min. The mixture was centrifuged at 4000 rpm for 15 min, and the supernatant was transferred to another tube for evaporation to dryness under a gentle stream of nitrogen in a water bath at 35°C. The residue was redissolved in 80 µL of mobile phase. An aliquot of 40 µL was injected into the LC–MS system. All of the operations were accomplished in darkroom because of the photochemical decomposition of nisoldipine.

Table I. Recovery of Nisoldipine from Plasma (*n* = 5)

Added (ng/mL)	Recovery (%)	RSD (%)
0.5	89.31	6.33
6.0	98.36	3.52
20.0	94.92	1.65

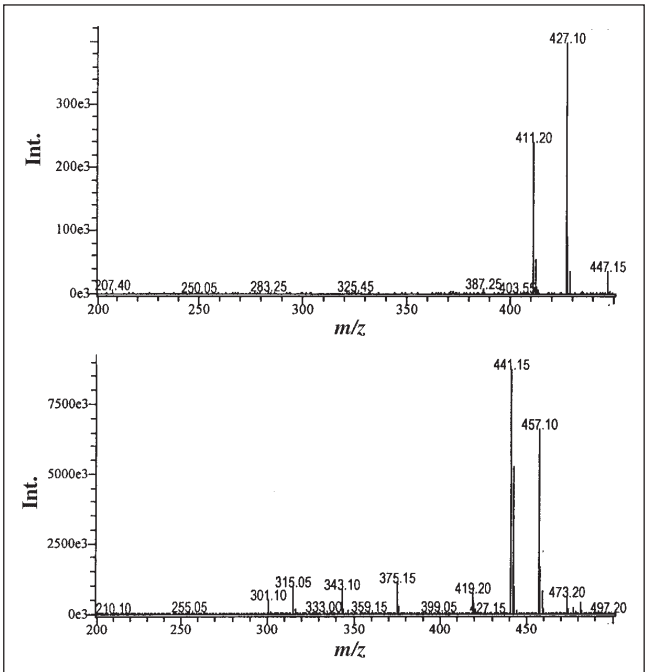

Figure 2. ESI-MS of nisoldipine and nimodipine.

Calibration and quality control samples

Stock nisoldipine solution (1 mg/mL) was prepared by dissolving 10 mg nisoldipine in 10 mL of methanol and stored in brown quantitative bottling at –4°C because nisoldipine decomposes under light. The solution was stable for 2 weeks under these conditions (4).

Quality control samples were prepared by spiking control

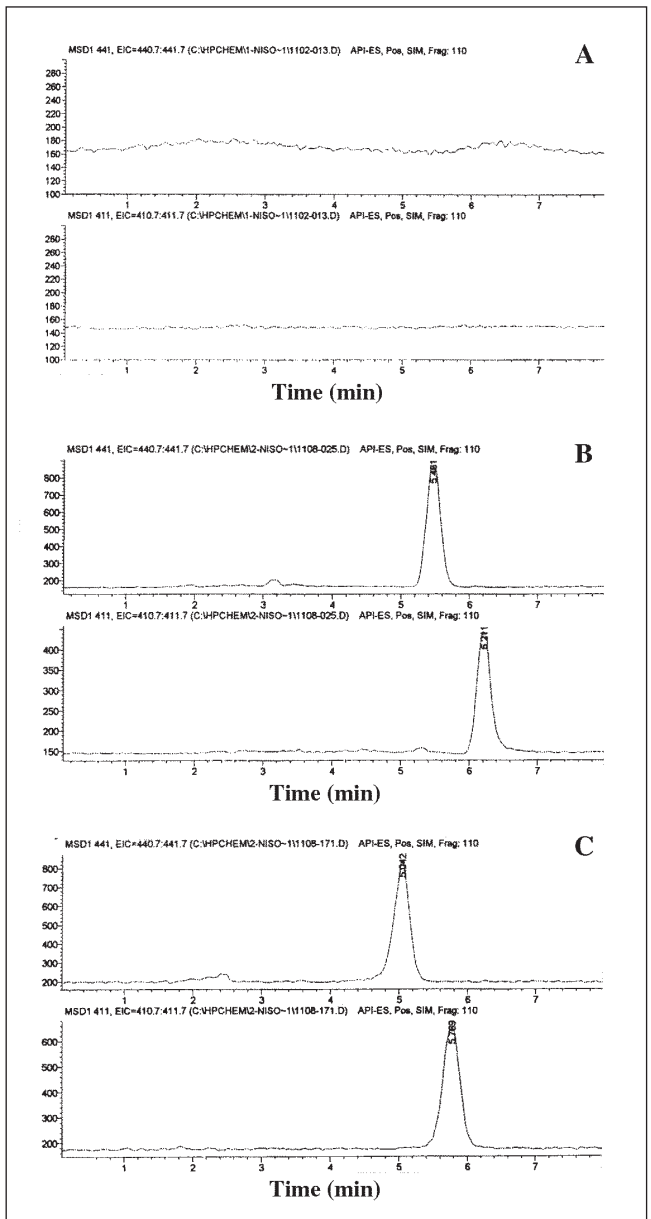

Figure 3. Chromatograms of the (A) blank plasma, (B) blank plasma spiked with nisoldipine and nimodipine, and (C) volunteer's plasma.

plasma samples of 1 mL with nisoldipine at three concentration levels (0.5, 6.0, and 20.0 ng/mL) on the day of blood sampling. The samples were stored at –20°C together with the unknown samples and were analyzed at each concentration level in each sample sequence.

Calibration samples were prepared by spiking 1 mL of control plasma with the proper amount of stock nisoldipine solution at 0.5, 1.0, 2.0, 4.0, 6.0, 10.0, 20.0 ng/mL, and 8 ng nimodipine (IS) and then treated and analyzed in the same way as mentioned in Sample preparation section. The ratio (Ai/As) of the peak areas of the analyte (Ai) and IS (As) in each fraction was calculated. The calibration curves were constructed by plotting the peak area ratio versus concentration of nisoldipine, and the plot was then subjected to linear regression analysis.

Nine spiking control plasma samples at three concentration levels of nisoldipine (0.5, 6.0, 20.0 ng/mL × 3) were prepared by adding 0.1, 0.2, and 0.5 mL of 1.0M NaOH to them, respectively, and then treating and determining them (in the same way as mentioned in Sample preparation section) to demonstrate the effect of varying levels of sodium on the results.

Validation

The method was validated by analysis of human plasma quality control samples with satisfactory results. Quality control samples were analyzed on 6 days. On the first day, 15 quality control samples (0.5, 6.0, and 20.0 ng/mL × 5) were analyzed. Over the following 5 days, the samples (0.5, 6.0, and 20.0 ng/mL × 1) were detected per day. The precision of the assay was studied by calculating the relative standard deviation (RSD) of replicate measurements at each concentration level of nisoldipine in plasma. Accuracy of the method was also investigated by calculating the difference from the theoretical value. In order to be acceptable, the calculated value of precision or accuracy should be less than 15% at all concentration levels.

The absolute recovery of extraction of nisoldipine was determined by comparing the peak area ratio resulting from the sample after extracted with that obtained from the sample that contained the same amount of nisoldipine in the extracted plasma but that was not extracted again after the addition of the drug.

The limit of detection (LOD) was defined as the sample concentration resulting in a peak area of three times the noise level. The limit of quantitation (LOQ) was defined as the lowest drug concentration that can be determined with acceptable accuracy and precision.

A freezing test was essential for this assay because nisoldipine is an unstable chemical. The freezing test samples were prepared by spiking 5 mL of the control plasma samples with nisoldipine at three concentration levels (0.5, 6.0, and 20.0 ng/mL) of blood sample. The samples were divided into 1-mL aliquots in each bottle and stored at –20°C and were detected every 5 days. The MS signal of the samples was similar until the fifth detection. This meant that the samples were stable within at least 20 days.

Table II. Regression Parameters for the Calibration Curves

Batch	Slope (A)	Intercept (B)	R
1	0.1250	0.0419	0.9986
2	0.1279	0.0345	0.9987
3	0.1213	0.0088	0.9991
4	0.1194	0.0099	0.9997
5	0.1156	0.0129	0.9997

Table III. Accuracy for the Method for Determining Nisoldipine in Human Plasma (*n* = 5)

Conc. added (ng/mL)	Conc. found (mean ± SD, ng/mL)	Accuracy (%)
0.5	0.46 ± 0.09	8.06
6.0	5.84 ± 0.19	2.67
20.0	20.46 ± 0.69	2.31

Results and Discussion

At the start of this investigation, the authors tried to use the published method (2) to analyze nisoldipine in blood by HPLC coupled with UV detection. However, some endogenous interferences gave a very similar absorbance and could not be resolved perfectly from the analyte. More critically, the LOD reported in

Table IV. Between- and Within-Day Variability of the HPLC–MS Method (*n* = 5)

Conc. added (ng/mL)	Between-day variability		Within-day variability	
	Mean (ng/mL)	RSD (%)	Mean (ng/mL)	RSD (%)
0.5	0.47	11.13	0.49	9.28
6.0	5.65	6.17	5.86	3.23
20.0	19.97	4.06	20.46	3.38

Table V. Results of the Freezing Test for the Determination of Nisoldipine (*n* = 5)

Conc. (ng/mL)	Mean (ng/mL)	RSD (%)
0.5	0.48	6.11
6.0	5.88	4.08
20.0	19.85	4.44

Table VI. Effect of Variability in Background Sodium Levels on the Assay Results

Conc. (ng/mL)	1.0M NaOH added		
	0.1 mL	0.2 mL	0.5 mL
0.5	0.49	0.48	0.48
6.0	5.94	5.88	5.86
20.0	19.92	19.85	19.79

the reference was 2.0 ng/mL of nisoldipine, which was not sensitive enough to meet the requirement of detection in this investigation. The HPLC–ESI-MS technology described here provides a simple, rapid, selective, and highly sensitive alternate for the analysis of nisoldipine contained in biological fluids, especially for a large number of samples at low concentrations resulting from animal or human (or both) pharmacokinetic study.

Sample preparation

LLE was necessary and important because this technique can not only purify but also concentrate the sample. Diethyl ethyl absolute and ethyl acetate were all tested for the extraction, and ethyl acetate was finally adopted because of its high extraction efficiency. An amount of 0.1 mL NaOH (1.0M) was added to the mixture of plasma and ethyl acetate in order to reduce interference because the most endogenous consist of acidic compounds. The results of the absolute recovery of extraction test of nisoldipine are shown in Table I.

Selection of IS

It is necessary to use an IS to get high accuracy when MS is equipped with an HPLC detector. Nimodipine, nitrendipine, and nicardipine hydrochloride were investigated to find out which was the more suitable one. Nimodipine was adopted in the end because of its similarity of retention action, ionization, and extraction efficiency, as well as less endogenous interference at *m/z* 441.

Analytical conditions and optimization

The sensitivity and selectivity in the HPLC–MS mainly depended on the analyte properties (i.e., how easily it is ionized) and the constituent of the mobile phase used. Increasing the percentage of organic solvent in mobile phase will strengthen the MS signal, and methanol generally gives a stronger signal than acetonitrile. Therefore, a mobile phase of methanol–water (80:20, v/v) was adopted at the end. Under these conditions, the peak shape was good and no significant endogenous interference appeared near the retention of nisoldipine and IS in the chromatogram; the retention time of nisoldipine and nimodipine were 5.0 and 5.8 min, respectively. This was also very important to ensure the analytical speed of a large number of samples from clinical trials.

Positive and negative ESI modes were studied, and the results showed that the first one was more suitable for nisoldipine and IS. Different values from 70 to 120 eV of fragment voltage were tested in order to increase the sensitivity of detection, and the strongest response was given by 110 eV. There were two main positive fragment peaks of $[M+Na]^+$ and $[M+K]^+$ in the extracted MS spectra. Therefore, *m/z* 411 produced by the quasimolecule ion $[M+Na]^+$ of nisoldipine and *m/z* 441 by the quasimolecule ion $[M+Na]^+$ of nimodipine were selected for monitoring.

No fragment ions were present in the complete ESI-MS spectrum (Figure 2). This meant that nisoldipine was thermally stable under the ESI conditions used.

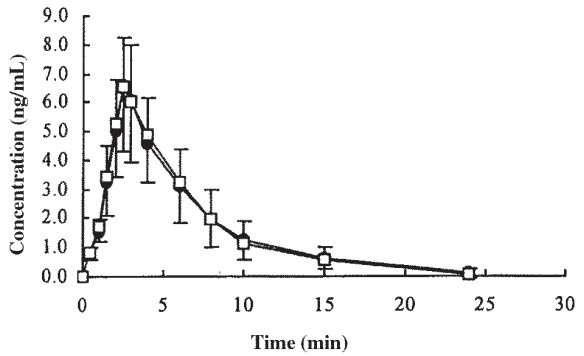

Figure 4. Mean drug plasma concentration-time curve of nisoldipine in 20 volunteers after oral administration.

Table VII. Pharmacokinetic Parameters of 20 Healthy Male Volunteers after Oral Administration of 20 mg Nisoldipine

Parameters	Test	Reference
$T_{1/2}$/h	3.80 ± 0.90	3.89 ± 1.21
T_{max}/h	2.5 ± 0.4	2.4 ± 0.3
C_{max}/ng/mL	7.13 ± 2.17	7.02 ± 1.95
$AUC_{0\text{-}24}$/ng.h.mL^{-1}	37.92 ± 14.73	38.31 ± 15.43
$AUC_{0\text{-}\infty}$/ng.h.mL^{-1}	39.97 ± 14.61	40.27 ± 15.82

Specificity

Comparing the HPLC–MS chromatograms of blank and validation samples obtained from validation experiments with those of control human plasma from individual volunteers showed that the procedure has a very good specificity for nisoldipine and IS. The typical chromatograms of blank plasma, blank plasma spiked with nisoldipine, and IS, as well as the volunteer's plasma, are shown in Figure 3.

Assay performance

Assay performance of the present method was assessed by the following criteria: linearity, accuracy, precision, stability, recovery, LOD, and LOQ. The assays exhibited linearity between the response (Ai/As) and corresponding concentration of nisoldipine (Ci) over the range of 0.5–20.0 ng/mL in the sample. Calibration curve intercepts were not significantly different from zero. The results are presented in Table II.

Some ESI interface designs were susceptible to performance if large numbers of samples were introduced at high flow rates. Data presented in Table II shows an approximately 10% drop in slope taken over the five batch trials. It was acceptable as thought of these was obtained during five days. Further studies showed that the drop in performance did not increase with larger number of samples.

Accuracy values were within acceptable limits (Table III). The results of within- and between-day precision for control samples ranged from 3.2% to 11.13%, respectively (Table IV). Data of the freezing test were listed in Table V, which shows that nisoldipine and IS were stable within at least 20 days under the condition. The effect of variability in background sodium levels on the assay results could be ignored (Table VI). The LOD and LOQ in this investigation were 0.2 and 0.4 ng/mL, respectively.

Results of pharmacokinetic study

The applicability of this method has been demonstrated by the determination of nisoldipine in plasma from 20 subjects that each received an oral dose of 20 mg nisoldipine in a bioequivalent study. The developed procedure was sensitive enough to assure the quantitative analysis of nisoldipine in plasma with acceptable accuracy and precision over a period of 15 h after a single oral administration. The mean plasma concentration-time curve of nisoldipine of the 20 volunteers is shown in Figure 4. The kinetic parameters of the test and reference capsules are listed in Table VII. The test capsules were found to be bioequivalent to the reference.

Conclusion

A simple, rapid, specific, and highly sensitive method for the determination of nisoldipine in plasma by HPLC–ESI-MS has been established and validated through its successful use for the analysis of nisoldipine in plasma samples resulting from a phase-I human pharmacokinetic study. The method has solved the existing problems of low specificity and sensitivity of the previously reported procedure (2). It serves as a suitable alternative for large numbers of biological samples because of its simple LLE processing and short analytical time consumption.

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