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Determination of nimodipine in human plasma by a sensitive and selective liquid chromatography–tandem mass spectrometry method

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Abstract

A sensitive and highly selective liquid chromatography–tandem mass spectrometry (LC–MS–MS) method was developed to determine nimodipine in human plasma. The analyte and internal standard nitrendipine were extracted from plasma samples by *n*-hexane−dichloromethane−isopropanol (300:150:4, v/v/v), and chromatographed on a C_{18} column. The mobile phase consisted of methanol−water−formic acid (80:20:1, v/v/v). Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via atmospheric pressure chemical ionization (APCI) source. The method has a limit of quantification of 0.24 ng/ml. The linear calibration curves were obtained in the concentration range of 0.24−80 ng/ml. The intra- and inter-day precisions were lower than 4.4% in terms of relative standard deviation (R.S.D.), and the accuracy ranged from 0.0 to 5.8% in terms of relative error (RE). This validated method was successfully applied for the evaluation of pharmacokinetic profiles of nimodipine tablets administered to 18 healthy volunteers.

Keywords: Nimodipine

1. Introduction

Nimodipine (Fig. 1) is a dihydropyridine calcium channel blocker that acts by relaxing the arterial smooth muscle. Able to cross the blood–brain barrier, nimodipine can dilate the cerebral arterioles [1,2]; thus it is used currently to prevent and treat the ischemic damage caused by cerebral arterial spasm in subarachnoid hemorrhage [3]. Nimodipine has also been used in other cerebrovascular disorders, such as ischemic stroke [4] and multi-infarct dementia [5].

Nimodipine is well absorbed in the gastrointestinal tract after oral administration, but it is subject to extensive first-pass metabolism that results in very low plasma concentration (ng/ml levels), poor absolute bioavailability and significant inter-individual variations [6]. Therefore, the determination of nimodipine concentration in plasma requires an analytical method with high sensitivity.

Several analytical techniques have been applied to the quantification of nimodipine in biological fluids. Gas chromatography (GC) methods with electron-capture [7,8] or nitrogen-phosphorus detection [9] were used to determine nimodipine in plasma which can provide a lower limit of quantification (LLOQ) of 5 ng/ml using 1 ml plasma. HPLC with UV detection [10–12] and electrochemical detection [13] have a sensitivity of about 1 ng/ml in 1 ml plasma, but these methods are time-consuming (more than 10 min). Fischer et al. [14] developed a GC−MS method combined with chiral stationary phase HPLC for the separation and determination of nimodipine enantiomers which had an LLOQ of 0.1 ng/ml using 0.5 ml plasma, but the analytical time was more than 30 min which was not suitable for the analyses of large amounts of biological samples. Mück [15] developed an LC−MS−MS method providing an LLOQ of 0.5 ng/ml in 1 ml plasma sample, but the method was also used for the quantification of nimodipine enantiomers. An increase in sample throughput requires a reduced analysis time and simplified sample clean-up procedure. Therefore, the methodological improvements for the rapid and quantitative analyses of nimodipine in biological fluids are still

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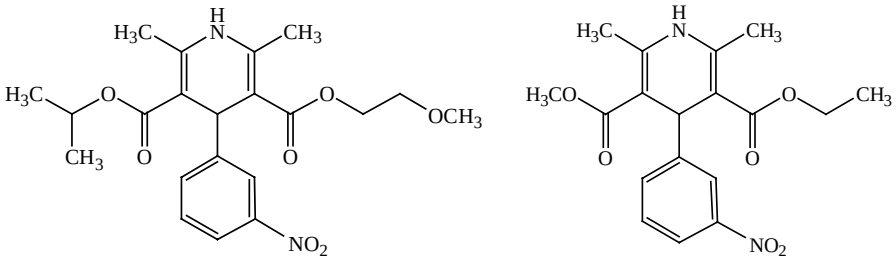

I II

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

needed for clinical trials aimed at improving the quality of preparations, defining single and multi-dose safety, efficacy and pharmacokinetic profiles.

In the present paper, we reported a rapid liquid chromatography–tandem mass spectrometry (LC–MS–MS) method which provided a sensitive and highly selective quantification of nimodipine in human plasma to an LLOQ of 0.24 ng/ml using 0.5 ml of plasma. The method was validated and applied successfully to a pharmacokinetic study after a single dose administration of 60 mg nimodipine to healthy volunteers.

2. Experimental

2.1. Materials

Nimodipine (99.3% purity) and nitrendipine (internal standard, 99.1% purity) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol (Yuwang Chemical, Shandong, China) were of HPLC grade. Other chemicals were of analytical grade. Blank (drug-free) human plasma was obtained from Shenyang Blood Donor Service (Liaoning, China). Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Instrumentation

A Finnigan TSQ API II tandem mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (San Jose, CA, USA), an Agilent 1100 autosampler (Agilent, Wilminton, DE, USA) and a Shimadzu LC-10AD pump (Kyoto, Japan) were used for LC–MS–MS analyses. Analytical data were acquired using Xcalibur 1.1 software, and quantitative processing was performed using LCQuan software (Finnigan).

2.3. LC–MS–MS conditions

The LC separation was performed using a Zorbax Extend C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Wilminton, DE, USA) and a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d., Phenomenex, Torrance, CA, USA). The isocratic mobile phase consisted of methanol–water–formic acid (80:20:1, v/v/v) at a flow rate of 0.75 ml/min. The column temperature was maintained at room temperature (20 °C).

The mass spectrometer was operated in the positive ion detection mode with the corona discharge current set at 4.00 μA. Nitrogen was used as the sheath gas (0.6 MPa) and auxiliary gas (3 l/min) for nebulization. The heated capillary and vaporizer temperatures were set at 250 and 450 °C, respectively. For collision-induced dissociation (CID), argon was used as the collision gas at a pressure of 0.19 Pa. Quantification was performed using selected reaction monitoring (SRM) of the transitions *m/z* 419 → 343 for nimodipine and *m/z* 361 → 315 for nitrendipine, respectively, with a scan time of 0.3 s per transition. The optimized collision energies of 20 and 25 eV were chosen for nimodipine and nitrendipine, respectively.

2.4. Sample preparation

To prevent the photodegradation of nimodipine to pyridine derivatives, the whole experiments including plasma collecting, sample preparation and instrumental analyses were performed under feeble yellow light. A 100 μl aliquot of internal standard (nitrendipine, 200 ng/ml in methanol) and 100 μl of the mobile phase were added to 500 μl of human plasma samples, respectively. Then 500 μl of 0.01 mol/l NaH_2PO_4 buffer (pH 12) was added. The mixture was vortexed and extracted with 3 ml of *n*-hexane–dichloromethane–isopropanol (300:150:4, v/v/v) by shaking for 10 min. After centrifugation at 2000 × *g* for 10 min, the organic phase was transferred into another tube and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was reconstituted in 200 μl of the mobile phase and vortexed for 1 min. A 20 μl aliquot of the solution was injected onto the LC–MS–MS system.

To prepare the standard calibration samples and QC samples, 100 μl internal standard (nitrendipine, 200 ng/ml in methanol) and 100 μl of the standard working solutions

were added to 500 μl blank human plasma. The following procedures were the same as described above. Calibration standards were prepared to achieve the final standard plasma concentrations of 0.24, 0.48, 1.6, 4.8, 16.0, 40.0 and 80.0 ng/ml for nimodipine.

2.5. Method validation

Plasma samples were quantified using the ratio of the peak area of nimodipine to that of nitrendipine as the assay response. Linear calibration curves were obtained with correlation coefficients of greater than 0.998 using a $1/x^2$ weighted linear regression model.

To evaluate linearity, plasma calibration curves were prepared and assayed in triplicate on three consecutive days. Accuracy and precision were also assessed by determining QC samples at three concentration levels on the three different validation days. The accuracy was expressed by relative error (RE) and the precision by relative standard deviation (R.S.D.).

The extraction recoveries of nimodipine at three QC levels were determined by comparing the peak area ratios of analyte to internal standard in sample that had been spiked with analyte prior to extraction with samples to which the analyte had been added post-extraction. The internal standard was added to both sets of samples post-extraction.

The stability of nimodipine in the reconstituted solution under feeble yellow light was assessed by placing QC samples under ambient conditions for 24 h. The freeze–thaw stability of nimodipine was also assessed by analyzing QC samples undergoing three freeze (−20 °C)−thaw (room temperature) cycles.

2.6. Application of the method to pharmacokinetic study in healthy volunteers

To demonstrate the reliability of this method for the study of pharmacokinetics of nimodipine, it was used to determine nimodipine concentrations in plasma samples 0–10 h after administration of 60 mg nimodipine to 18 healthy volunteers in a pharmacokinetic study approved by the Ethic Committee. The age of 18 male healthy volunteers ranged from 18 to 27 years (23 ± 5 years), and the weight ranged from 57 to 74 kg (66 ± 8 kg). All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Serial blood samples (3 ml) were collected from antecubital vein at 0, 0.17, 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 10 h post-dose. Plasma was separated by centrifugation at 2000 × g for 10 min and stored frozen at −20 °C until analysis.

Pharmacokinetic parameters were determined from the plasma concentration–time data. The elimination half-life ($t_{1/2}$) was calculated with non-compartmental model of TOPFIT program on a personal computer. The area under the plasma concentration–time curve from time zero to the last measurable plasma concentration point (t = 10 h) (AUC_{0-t}) was calculated by the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$, where C_t is the last measurable plasma concentration and k_e is the elimination rate constant.

3. Results and discussion

3.1. LC−MS−MS optimization

The LC−MS−MS method for the determination of nimodipine in human plasma was investigated on the basis of literature [16] which determined nitrendipine and its metabolite in human plasma using LC−MS−MS method. Firstly nimodipine and nitrendipine were introduced directly to mass spectrometer using APCI ionization. Parameters such as corona discharge, orifice voltage, ring voltage, flow of sheath and auxiliary gas (N_2) were optimized in order to obtain more abundant protonated molecular ions of analytes. Fig. 2 shows product ion spectra of $[M + H]^+$ ions of nimodipine and nitrendipine. By positive APCI mode, the analyte and internal standard formed predominately protonated molecules $[M + H]^+$ in full scan mass spectra. The major fragment ions at m/z 343 and 315 were chosen in the SRM acquisition for nimodipine and nitrendipine, respectively. The most suitable collision energy was determined by observing the maximum response obtained for the fragment ion peak m/z.

The possibility of using electrospray ionization (ESI) or atmospheric pressure chemical ionization sources under positive ion detection mode was evaluated during the early stage of method development. It was found that APCI could offer higher sensitivity and better linearity for the analyte than ESI. Higher flow rate of 0.75 ml/min could be used in APCI mode than that in ESI mode (usually less than 0.5 ml/min) that reduced the chromatographic separating time. And the stable response of APCI source provided reproducibility of the measurement.

The chromatographic conditions were investigated to optimize for sensitivity, speed and peak shape. The compositions of mobile phase were optimized with varying percentages of organic solvent. It was found that high organic solvent contents (about 80%) in HPLC system decreased the background noise and provided rapid separation and stable MS signal throughout an analytical run, allowing the enhancement of sensitivity. Methanol was chosen as the organic solvent because it was less toxic and cheaper than acetonitrile while providing same sensitivity as acetonitrile. It was also found that the addition of acidic modifiers (formic acid) to the mobile phase could improve the sensitivity by promoting the ionization of the analytes, but the contents of formic acid in mobile phase had no significant effect on the sensitivity of the analytes under APCI conditions. To achieve symmetrical peak shapes, a short chromatographic run time, and to eliminate the matrix effect, a mobile phase consisting

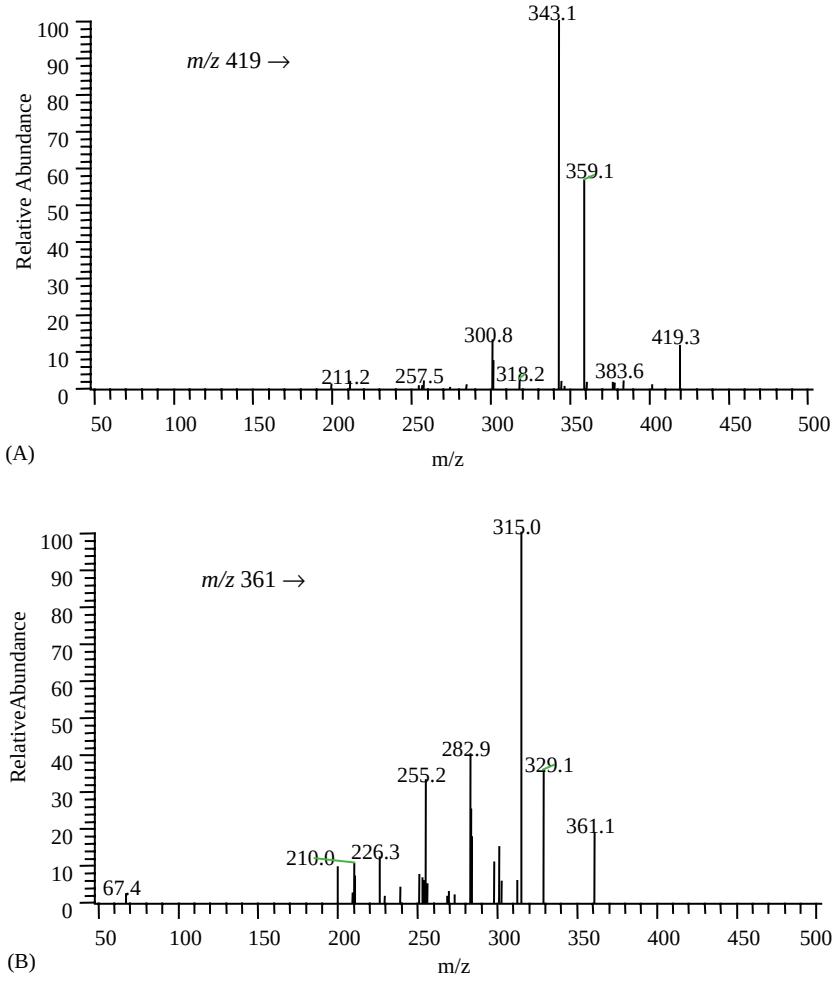

Fig. 2. Full-scan product ion spectra of $[M + H]^+$ of (A) nimodipine and (B) nitrendipine.

of methanol−water−formic acid (80:20:1, v/v/v) was used in the experiment.

Some kinds of liquid–liquid extraction and solid phase extraction methods had been published for the extraction of nimodipine from plasma samples. In the present experiment, different liquid–liquid extraction conditions were evaluated including different extraction solvents and aqueous pH buffers. Three organic extraction solvents (diethyl ether, diethyl ether−dichloromethane and *n*-hexane−dichloromethane−isopropanol) were evaluated. Among them, the last one yielded a good clean-up of the plasma samples and adequate recovery values. It was found that 0.01 mol/l NaH_2PO_4 buffer (pH 12) could significantly increase the extraction of nimodipine.

3.2. Method validation

3.2.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Fig. 3 shows the typical chromatograms of a blank plasma sample, a blank plasma sample spiked with nimodipine at the LLOQ and nitrendipine, and a plasma sample from a healthy volunteer 3 h after an oral administration. No significant interferences from endogenous substances with analyte or nitrendipine were detected. Typical retention times for nimodipine and nitrendipine were 3.6 and 3.4 min, respectively.

3.2.2. Linearity and lower limit of quantification

The linear regression of the peak area ratios versus concentrations was fitted over the concentration range of 0.24−80 ng/ml in human plasma. A typical equation of the calibration curve was as follows: $Y = 8.337 \times 10^{-3} + 1.689 \times 10^{-2}X$ ($r = 0.9978$), where Y is the peak area ratio of nimodipine to nitrendipine, and X is the concentration of nimodipine.

The present LC−MS−MS method offered an LLOQ of 0.24 ng/ml with an accuracy of 11.8% in terms of RE and a precision of 13.1% in terms of R.S.D. ($n = 5$), which is more sensitive than or comparable to the reported methods [7–13]. Under present LLOQ of 0.24 ng/ml, the nimodipine concentration can be determined in plasma samples until 10 h after a single oral dose of 60 mg ni-

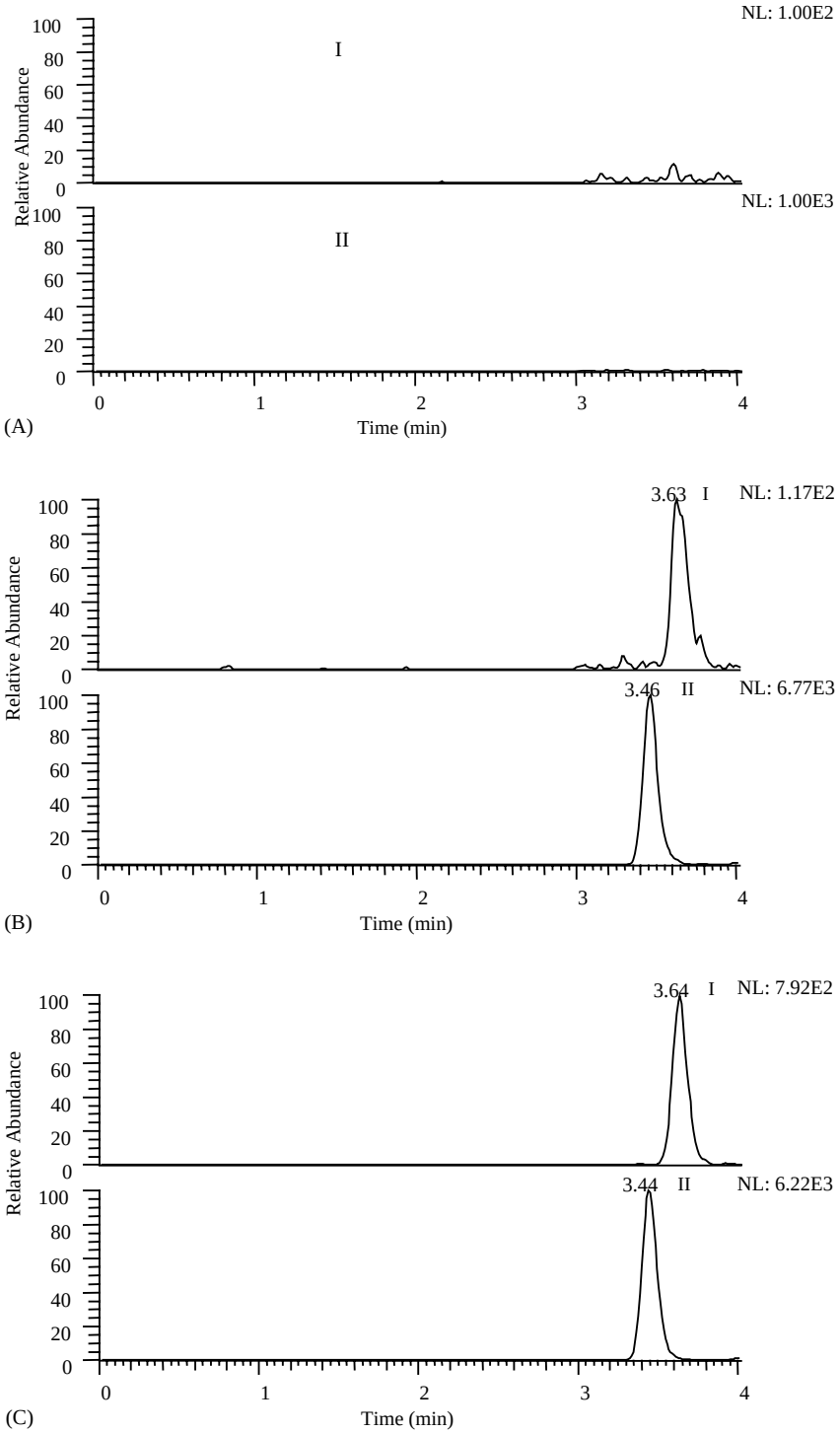

Fig. 3. Representative SRM chromatograms of nimodipine plasma samples determined by LC−MS−MS method: (A) a blank plasma sample; (B) a blank plasma sample spiked with nimodipine at the LLOQ of 0.24 ng/ml and nitrendipine (IS, 40 ng/ml); (C) a human plasma sample collected at 3 h after oral administration of nimodipine (60 mg) to a healthy volunteer (concentration determined was 3.4 ng/ml). Peak I, nimodipine; peak II, nitrendipine.

modipine, which is sensitive enough to investigate the pharmacokinetic behaviors of nimodipine, to establish the relationship between dose and pharmacological effect and identify doses that produce toxic responses in humans.

3.2.3. *Precision and accuracy*

Table 1 summarizes the intra- and inter-day precision and accuracy for nimodipine from QC samples. In this assay, the intra- and inter-day precisions ranged from 1.9 to 4.2% and from 2.9 to 4.4% for each QC level, respectively. The

Table 1
Precision and accuracy of the LC−MS−MS method to determine nimodipine in human plasma ($n = 3$ days, six replicates per day)

Concentration (ng/ml)		R.S.D. (%)		RE (%)
Added	Found	Intra-day	Inter-day	
0.48	0.48	4.2	4.4	0.0
16.0	16.9	1.9	2.9	5.6
72.0	73.0	4.0	3.0	1.4

accuracy was within 5.8%. The results, calculated using one-way ANOVA, indicated that the values were within the acceptable range and the method was accurate and precise [17].

3.2.4. Extraction recovery and stability

The mean extraction recovery of nimodipine, determined at three concentrations (0.48, 16.0, 72.0 ng/ml), were 84.7, 85.1 and 87.5% ($n = 6$), respectively. The mean extraction recovery of nitrendipine was investigated as 81.9% ($n = 6$).

It is known that most of the dihydropyridine drugs are sensitive to the light, undergoing oxidation to pyridine analogues. But during the experiment we found that nimodipine was stable in the reconstituted solution of methanol−water−formic acid (80:20:1, v/v/v) for at least 24 h at room temperature under experimental conditions. The mean relative error of nimodipine between the initial concentrations and the concentrations of the following three freeze–thaw cycles ranged from −5.9 to 4.3%, which indicated the stability of nimodipine during the three freeze–thaw cycles. Nevertheless, in order to prevent the probable photodegradation of nimodipine, the whole procedures including plasma collecting, sample preparation and instrumental analyses were performed under feeble yellow light.

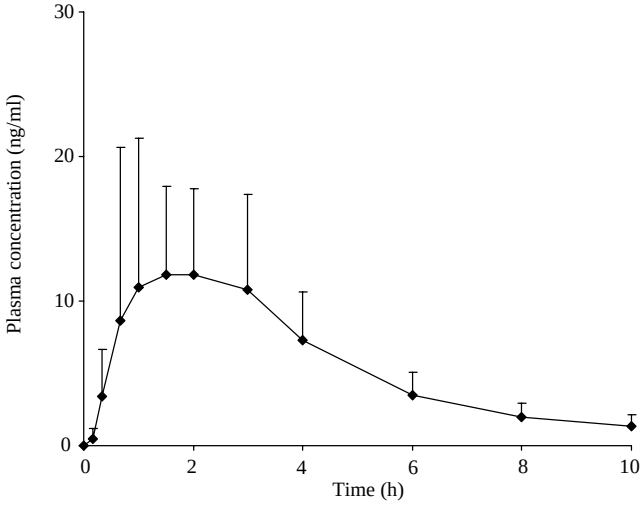

Fig. 4. Mean plasma concentration–time profile of nimodipine after an oral administration of 60 mg nimodipine to 18 healthy volunteers. Each point represents the mean + S.D. ($n = 18$).

3.3. Pharmacokinetic study

The method was applied to determine the plasma concentration of nimodipine following a single oral administration (60 mg) to eighteen healthy volunteers. Mean plasma concentration–time profiles of nimodipine are presented in Fig. 4. The main pharmacokinetic parameters of nimodipine in 18 volunteers were calculated. After oral administration of 60 mg nimodipine, T_{max} and C_{max} values were found to be 2.1 ± 0.9 h and 16.3 ± 11.9 ng/ml, respectively. Plasma concentrations declined with $t_{1/2}$ of 2.7 ± 1.2 h. The elimination rate constant (k_e) was calculated as $0.29 \pm 0.10\ h^{-1}$. The AUC_{0-t} and $AUC_{0-\infty}$ values obtained were 57.0 ± 23.6 and 62.9 ± 25.3 ng h/ml, respectively.

4. Conclusions

An LC−MS−MS method was developed and validated for the determination of nimodipine in human plasma. The method is rapid, sensitive and highly selective with an LLOQ of 0.24 ng/ml using 0.5 ml human plasma. The determination of one plasma sample only needed 4 min and more than 120 samples could be assayed daily, including sample preparation, data acquisition and processing. It was proved superior in sensitivity and speed than the reported methods. The method was successfully applied to evaluate the pharmacokinetics of nimodipine after an oral dose of 60 mg to healthy volunteers. The present method provided an example for biological samples analysis of dihydropyridine drugs.

Acknowledgements

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