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Short communication

Determination of nimodipine in human plasma by ultra performance liquid chromatography–tandem mass spectrometry and pharmacokinetic application

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Abstract

A fast and sensitive ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed for the determination and pharmacokinetic study of nimodipine in human plasma. With nitrendipine as the internal standard, sample pretreatment involved one-step extraction with diethyl ether of 0.5 ml plasma. The separation was carried out on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm) with water and acetonitrile (both containing 0.1% formic acid) as the mobile phase under gradient conditions at a flow rate of 0.35 ml/min. The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via electrospray ionization (ESI) source. The standard curves were linear ($r^2 \geq 0.99$) over the concentration range of 0.20–100 ng/ml with a limit of quantification (LLOQ) of 0.20 ng/ml. The intra- and inter-day precision (R.S.D.) values were below 14% and the accuracy (relative error R.E.) was ranged from –2.2% to 7.7% at all three quality control (QC) levels. The method herein described was superior to previous methods and successfully applied to the pharmacokinetic study of nimodipine tablets in healthy male volunteers after oral administration.

Keywords: Nimodipine; UPLC–MS/MS; Human plasma; Pharmacokinetic application

1. Introduction

Nimodipine is a dihydropyridine calcium channel blocker, used in the treatment of senile dementia and in the prophylaxis of the vascular hemierania [1]. It is well absorbed in the gastrointestinal tract after oral administration, but it is subjected to extensive first-pass metabolism that results in very low plasma concentration (ng/ml levels), poor absolute bioavailability and significant inter-individual variations [2]. Sensitive and specific analytical methods are needed for the determination of nimodipine in human plasma.

A number of analytical techniques have been applied to the quantification of nimodipine in biological fluids. Gas chromatography (GC) methods with electron capture [3,4] or nitrogen–phosphorus detection [5] were used to determine nimodipine in plasma which provided a lower limit of quantification (LLOQ) of 5 ng/ml using 1 ml plasma. HPLC with UV detection [6–8] and electrochemical detection [9] had a sensitivity of about 1 ng/ml, but these methods needed long chromatographic run time (longer than 10 min). In the published GC–MS method [10] combined with chiral stationary phase HPLC for the separation and determination of nimodipine enantiomers, although the sensitivity was improved (LLOQ of 0.1 ng/ml), the analytical time was longer than 30 min which was not suitable for the analysis of large number of biological samples. An LC–MS/MS method providing an LLOQ of 0.5 ng/ml using 1 ml plasma sample was used for the quantification of nimodipine enantiomers [11]. Some assays based on liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [12,13] were also described for determining nimodipine in human plasma.

Recently, we published an ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method [14] to determine amlodipine, which had a lower LLOQ (0.15 ng/ml) and a shorter run time (3.0 min per sample) compared with other LC–MS/MS methods. Nimodipine is a structural analogue of amlodipine. The high sensitivity and the fast analysis of UPLC–MS/MS may also benefit the

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pharmacokinetic and clinical studies of nimodipine where the sample throughout and sensitivity is critical.

Here we present a fast, sensitive and selective method for measuring nimodipine in plasma using UPLC–MS/MS carefully optimized based on the previous work [14]. The total run time of the method per sample was 2.5 min which is shorter than those reported for nimodipine [11–13]. The LLOQ of 0.2 ng/ml in plasma corresponded to an on-column sensitivity (the quantity of drug injected on the column per injection) of 2.5 pg nimodipine, which was lower than those reported in the literatures [12,13]. The present method has been proved to be more efficient in analyzing large number of plasma samples for pharmacokinetic study after therapeutic doses of nimodipine.

2. Experimental

2.1. Chemicals and reagents

Reference standards of nimodipine (99.2% of purity), and nitrendipine (internal standard, I.S., 99.4% purity) (Fig. 1) were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile, methanol and formic acid (HPLC grade) were purchased from Dikma (Richmond Hill, NY, USA). Sodium hydroxide and diethyl ether of analytical grade were purchased from Yuwang (Chemical Reagent Plant, Shandong, China). Water was purified by redistillation and filtered through a 0.22 μm membrane filter before use.

2.2. Apparatus and operating conditions

2.2.1. Liquid chromatographic conditions

The analysis was carried out on an ACQUITY^{TM} UPLC system (Waters Corp., Milford, MA, USA) with cooling autosampler and column oven. An ACQUITY UPLC^{TM} BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm; Waters Corp., Milford, MA, USA) was employed for separation with the column temperature maintained at 40 °C. The chromatographic separation was achieved with gradient elution using a mobile phase composed of water and acetonitrile each containing 0.1% (v/v) formic acid. The gradient elution started at 50% acetonitrile, ramped linearly to 90% acetonitrile in 1.6 min, was maintained at 90% for 0.2 min and then returned to the initial percentage. The flow rate was set at 0.35 ml/min. The autosampler temperature was kept at 4 °C and 5 μl of sample solution was injected with partial loop mode.

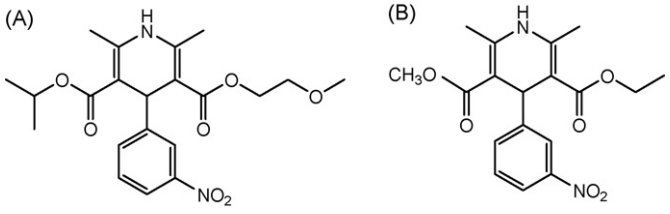

Fig. 1. Structures of nimodipine (A) and nitrendipine (B).

2.2.2. Mass spectrometric conditions

A triple quadrupole tandem mass spectrometer (Micromass® Quattro micro^{TM} API mass spectrometer, Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface was employed for analyte detection. The ESI source was operated in positive ionization mode with optimal operation parameters as follows: capillary voltage 3.0 kV, cone voltage 23 kV, source temperature 110 °C and desolvation temperature 400 °C. Nitrogen was used as the desolvation and cone gas with a flow rate of 400 and 50 l/h, respectively. Argon was used as the collision gas at a pressure of 2.91×10^{-3} mbar. The quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 419 → 343 for nimodipine and m/z 361 → 315 for nitrendipine (I.S.), respectively, with a scan time of 0.05 s per transition. All data were collected in centroid mode and processed using MassLynx^{TM} NT 4.0 software with a QuanLynx^{TM} program (Waters Corp., Milford, MA, USA).

2.3. Preparation of standards and quality control samples

Primary stock solution of nimodipine was prepared by dissolving the accurately weighed reference standard of nimodipine in methanol to yield concentration of 200 μg/ml. Working standard solutions of nimodipine in the concentration range of 1–500 ng/ml were prepared by dilution of the stock solution with water–methanol (50:50, v/v). The stock solution of I.S. was prepared by dissolving appropriate amounts of nitrendipine in methanol to give a concentration of 50 μg/ml. An I.S. working solution of 200 ng/ml was obtained by diluting the stock solution of nitrendipine with water–methanol (50:50, v/v). All the solutions were stored at 4 °C and brought to room temperature before use. Calibration standards were prepared daily by spiking 100 μl of the appropriate working standard solutions to 500 μl of the blank plasma giving concentrations of 0.20, 0.50, 1.00, 5.00, 20.0, 50.0 and 100 ng/ml. QC samples, which were used in the validation and during the pharmacokinetic study, were prepared at the beginning of the experiment by independent dilution at three levels of plasma concentration: 0.50, 20.0 and 80.0 ng/ml and stored at −20 °C after preparation. The standards and quality controls were extracted on each analysis day along with the unknown samples.

2.4. Plasma sample preparation

To prevent the photodegradation of nimodipine, the whole experiment, including standard and QC preparation, plasma collecting, sample preparation and instrumental analysis, was performed under dim light. A 100-μl aliquot of I.S. working solution (200 ng/ml) and 200 μl 1 mol/l sodium hydroxide solution were added to 500 μl of collected plasma sample in 10-ml glass tubes and the mixture was vortexed for 30 s. The sample was extracted with 3 ml of diethyl ether by vortex mixing for 1 min and centrifugation at 3500 × g for 10 min. The upper organic layer was then transferred into another clean glass tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 200 μl of acetonitrile–water (70:30, v/v), followed by vortexing and

centrifugation at 4000 × *g* for 10 min. The supernatant was transferred into 700-μl glass vials, and an aliquot of 5 μl was injected onto the LC–MS/MS system.

2.5. Method validation

The method was validated for selectivity, linearity, accuracy, precision, extract recovery and stability according to the FDA guideline [15] for validation of bioanalytical methods. Validation runs were conducted on three consecutive days. Each validation run consisted of two sets of calibration standards and six replicates of QC plasma samples at three concentrations. The peak area ratios of nimodipine to I.S. of QC samples were interpolated from the calibration curve on the same day to give the concentrations of nimodipine. The results from QC plasma samples in three runs were used to evaluate the precision and accuracy of the method developed.

The selectivity of this method was investigated by preparing and analyzing six individual human blank plasma samples with corresponding plasma samples spiked with nimodipine and nitrendipine and plasma samples after oral administration of nimodipine tablet.

The linearity was determined in the range of 0.20–100 ng/ml by plotting the peak area ratio of nimodipine to I.S versus the nominal concentration of nimodipine in plasma. The calibration curves were constructed by weighted ($1/x^2$) least squares linear regression. The precision and accuracy were determined by repeated analysis of six replicates at each QC level (LLOQ, low, mid and high levels) on three different days. The precision was defined as relative standard deviation (R.S.D.) and the accuracy was expressed as relative error (R.E.). The extraction recovery of nimodipine at three QC concentrations was determined by comparing the peak area of extracted samples spiked with known amount of the analytes with that of spiked post-extraction at corresponding concentrations.

Stability experiments were performed to evaluate the stability of nimodipine in plasma samples under different temperature and timing conditions. Five aliquots of QC plasma samples of low, mid and high concentrations were subjected to the following conditions: three freeze (−20 °C)–thaw (room temperature) cycles, storage at −20 °C for 30 days. Short-term stability was assessed by analyzing QC plasma samples kept at room temperature for 10 h that exceeded the routine preparation time of samples. Post-preparative stability was assessed by analyzing the extracted QC samples kept in the autosampler at 4 °C for 24 h.

2.6. Pharmacokinetic study

The pharmacokinetic study was approved by the local Ethics Committee and carried out in the hospital. All volunteers gave their signed informed consent to participate in the study according to the principles of the Declaration of Helsinki. One nimodipine tablet (containing 60 mg nimodipine each) was administered to each healthy male volunteer after 12 h fasting. Blood samples were collected before and at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 h post-dosing. Plasma was separated by centrifugation and stored at −20 °C until analyzed.

The maximum plasma concentrations (C_{max}) and their times (T_{max}) were noted directly from the measured data. The elimination rate constant (k_e) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_e$. The area under the plasma concentration–time curve (AUC_{0-t}) to the last measurable plasma concentration (C_t) was calculated by the linear trapezoidal rule. The area under the plasma concentration–time curve to time infinity ($AUC_{0-\infty}$) was calculated as: $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$.

3. Results and discussion

3.1. UPLC–MS/MS optimization

In our previous study [14] where nimodipine was employed as the I.S. the UPLC–MS/MS conditions were established for the determination of amlodipine. In this work, UPLC–MS/MS operation parameters were carefully optimized for the determination of nimodipine based on the previous study. A standard solution of nimodipine and nitrendipine was directly infused along with the mobile phase into the mass spectrometer with ESI as the ionization source. And the mass spectrometer was tuned in both positive and negative ionization modes for optimum response of nimodipine. It was found that the signal intensity of positive ion was higher than negative ion. In the precursor ion full-scan spectra, the most abundant ions were protonated molecules $[M+H]^+$ *m/z* 419 and 361 for nimodipine and nitrendipine, respectively. Parameters such as desolvation temperature, ESI

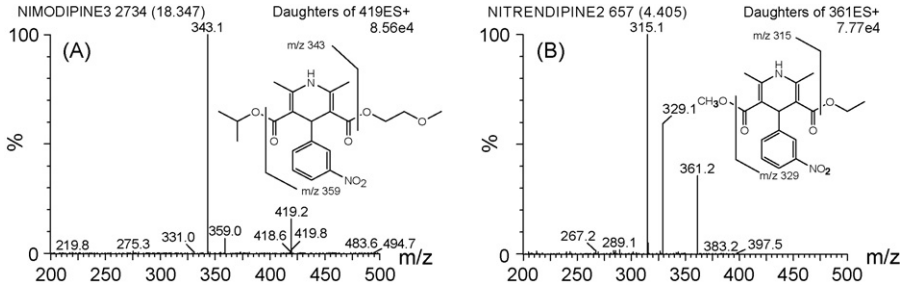

Fig. 2. Full-scan product-ion mass spectra of $[M+H]^+$ of nimodipine (A) and nitrendipine (B).

source temperature, capillary and cone voltage, flow rate of desolvation gas and cone gas were optimized to obtained highest intensity of protonated molecule of nimodipine. The product-ion scan spectra showed high abundance fragment ions at *m/z* 343 and 315 for nimodipine and nitrendipine, respectively. The product-ion spectra of these compounds are shown in Fig. 2. The collision gas pressure and collision energy of collision-induced decomposition (CID) were optimized for maximum response of the fragmentation of *m/z* 343 for nimodipine. The ion transitions of *m/z* 419 → 343 for nimodipine and *m/z* 361 → 315 for nitrendipine were chosen for MRM.

In the literature [12] atmospheric pressure chemical ionization (APCI) could offer higher sensitivity and better linearity for nimodipine than ESI. In our study, however, ESI provided better sensitivity and a wider linear range for the analyte. This could be due to a low flow rate (0.35 ml/min) used with the UPLC column of small particles and narrow internal diameter, which suits the ESI source better.

The chromatographic conditions were modified to obtain high sensitivity and sample throughput. The separation and ionization of nimodipine and nitrendipine were affected by the composition of mobile phase. The mobile phase system of acetonitrile–water same as in the determination of amlodipine in human plasma was used. In the previous study 0.3% formic acid was employed to enhance detection sensitivity. In the nimodipine case it was found that 0.1% formic acid in the mobile phase was optimum for the ionization and detection of analyte. Again, gradient elution was performed on UPLC column to push the speed of analysis, provide a better peak shape and extend the column life.

In the analysis of biological sample the MS system was easily contaminated. In order to avoid the contamination of earlier eluted endogenous components of the sample matrix onto the MS system a switch technique was developed. The first 0.7-min eluate was switched away from the MS detector, and the eluate from 0.7 to 1.6 min was allowed to enter into the MS system and be recorded. Two channels were used for recording the response, channel 1 for nimodipine with a retention time of 1.31 min, and channel 2 for the I.S. with a retention time of 1.20 min. Under the current chromatographic conditions, both nimodipine and I.S. were rapidly eluted and the total run time was just 2.5 min per sample, which was less than the values in the literatures [12,13]. As shown in Fig. 3, the very narrow chromatographic peaks with a peak width about 5 s, produced by UPLC resulted in an increase in the chromatographic efficiency and sensitivity. Both high sample throughput and high sensitivity met the requirement for pharmacokinetic study.

The ideal I.S. in LC–MS analysis is the compound that has structure, chromatographic and mass spectrometric behavior similar to the analyte. Based on our previous work [14], amlodipine would be an internal standard of choice since a very good chromatographic separation between nimodipine and amlodipine was obtained. However, it was found that nitrendipine is more stable than amlodipine under the experimental conditions. Although the resolution between nimodipine and nitrendipine was smaller than that with amlodipine, the high selectivity of MRM in which only required ion reactions were monitored guaranteed the reliability of the detection. Nitrendipine was chosen as the I.S. not only by virtue of its similarity in chromatographic behavior, mass spectrometric response and extraction recovery to nimodipine but also for its relative stability.

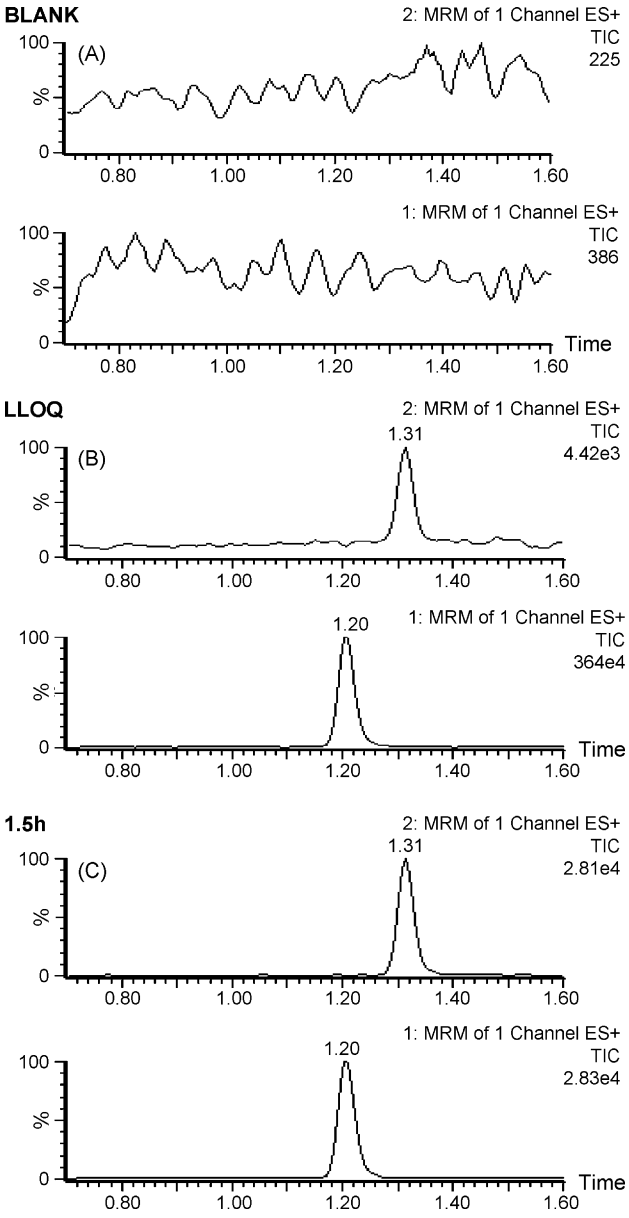

Fig. 3. Representative MRM chromatograms of nimodipine (peak 2, channel 2) and nitrendipine (peak 1, channel 1) in human plasma samples. (A) A blank plasma sample; (B) a blank plasma sample spiked with nimodipine at the LLOQ of 0.20 ng/ml and I.S. (200 ng/ml); (C) plasma sample from a volunteer 1.5 h after oral administration of nimodipine. The retention times for nimodipine and I.S. were 1.31 and 1.20 min, respectively.

3.2. Method validation

Comparing the chromatograms of six batches of blank plasma with the spiked plasma demonstrated a good selectivity of the method. The representative chromatograms are shown in Fig. 3A–C. All plasma lots were found to be free of interferences with the compounds of interest.

Table 1
Precision and accuracy for the determination of nimodipine in human plasma (intra-day: $n = 6$; inter-day: $n = 6$ series per day, 3 days)

Concentrations (ng/ml)		R.S.D. (%)		R.E. (%)
Added	Found (mean ± S.D.)	Intra-day	Inter-day	
0.20	0.21 ± 0.02	7.0	12	4.8
0.50	0.49 ± 0.04	7.2	10	−2.2
20.0	20.4 ± 1.5	5.6	14	1.8
80.0	86.1 ± 4.2	3.1	11	7.7

The evaluation of matrix effect from the influence of coeluting components on analyte ionization is needed for an LC–MS/MS method. The effect of the plasma matrix on ionization efficiency was expressed as the ratio of the mean peak area of analytes spiked after extraction to that of the neat standard solutions dried directly and reconstituted with mobile phase. All the ratios were between 85% and 115% indicating no significant matrix effect for nimodipine and nitrendipine in this method.

The linear regression of the peak area ratios versus concentrations was fitted over the concentration range of 0.20–100 ng/ml for nimodipine in human plasma. A typical regression equation for the calibration curve was: $y = 9.500 \times 10^{-2}x + 8.117 \times 10^{-3}$, $r = 0.9948$, where y is the peak area ratio of nimodipine to I.S., and x is the concentration of nimodipine in plasma.

The lower limit of quantification for nimodipine was 0.20 ng/ml corresponded to an on-column sensitivity of 2.5 pg, which was lower than those reported in literatures [12,13]. The high sensitivity could be attributed to the extra resolution and peak sharpness produced by the UPLC chromatographic system and the improved ionization efficiency of the MS system.

The intra-day precision (R.S.D.) for QC samples of 0.50, 20.0 and 80.0 ng/ml was 7.2%, 5.6% and 3.1% and that of inter-day analysis was 10%, 14% and 11%, respectively with accuracy (R.E.) within −2.2% to 7.7% (Table 1). All these data were within acceptable range and the precision and accuracy of the present method were adequate for the determination of nimodipine in human plasma from pharmacokinetic study.

Liquid–liquid extraction was applied to extract the analytes. The extraction recoveries of nimodipine from human plasma QC samples were 89.2 ± 3.9%, 90.3 ± 2.2% and 91.3 ± 3.1% at concentration levels of 0.50, 20.0 and 80.0 ng/ml, respectively, and the mean extraction recovery of nitrendipine was 87.4 ± 2.5%. Several extraction solvents such as diethyl ether–dichloromethane, *n*-hexane-diethyl ether and diethyl ether were investigated, and it was found that diethyl ether extracted the analyte more efficiently. Moreover, the boiling point of diethyl ether is lower, it was evaporated to dryness more quickly. This extraction procedure was more convenient and offered higher recovery for nimodipine compared with those reported in literatures [12,13].

The mean relative errors of nimodipine concentration measured in QC samples being subjected to certain conditions are shown in Table 2. The results indicated the stability of nimodipine in plasma stored at room temperature for 10 h, at −20 °C for 30 days and during the three freeze–thaw cycles, and in the prepared samples at 4 °C for 24 h.

Table 2
Stability of nimodipine in plasma samples ($n = 5$)

Nominal concentration (ng/ml; $n = 5$)	Concentration found (ng/ml; mean ± S.D.)	R.S.D. (%)	R.E. (%)
Three freeze–thaw cycles			
0.50	0.49 ± 0.04	8.2	−1.9
20.0	20.6 ± 1.4	6.6	3.2
80.0	83.1 ± 4.5	5.4	3.9
Long term (−20 °C for 30 days)			
0.50	0.47 ± 0.02	4.9	−6.0
20.0	22.2 ± 0.3	1.3	11
80.0	85.9 ± 2.8	3.3	7.3
Short term (room temperature for 10 h)			
0.50	0.49 ± 0.03	6.4	2.0
20.0	19.9 ± 0.7	3.5	−0.7
80.0	80.7 ± 2.3	2.9	0.9
Post-preparative (4 °C for 24 h)			
0.50	0.51 ± 0.04	7.8	2.1
20.0	21.9 ± 1.0	4.4	9.6
80.0	83.6 ± 7.9	9.4	4.6

3.3. Application to a pharmacokinetic study

This validated UPLC–MS/MS method was applied to a pharmacokinetic study of nimodipine in healthy volunteers following a single oral administration. The profile of the mean plasma concentration–time is shown in Fig. 4. The maximum plasma concentration (C_{max}) was 12.7 ± 9.0 ng/ml, the time of maximum plasma concentration (T_{max}) was 1.59 ± 0.63 h, the area under the plasma concentration–time curve from 0 h to the time of last measurable concentration (AUC_{0-t}) was 50.5 ± 19.8 ng h/ml, area under the plasma concentration–time curve from 0 h to infinity ($AUC_{0-\infty}$) was 53.7 ± 20.2 ng h/ml, the half-life of drug elimination at the terminal phase ($t_{1/2}$) was 2.45 ± 0.47 h. These parameters were in accordance with those reported in the literature [12].

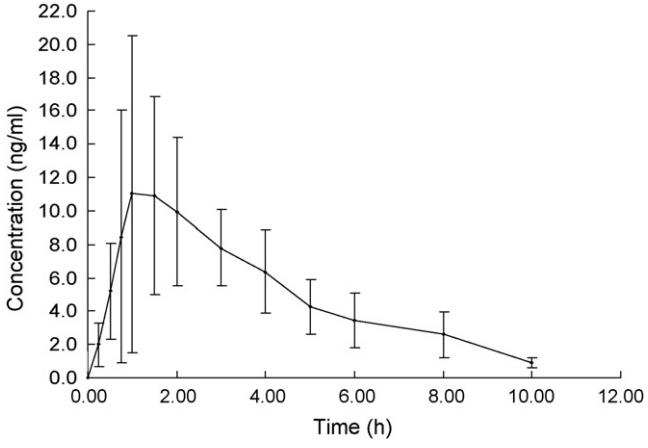

Fig. 4. Mean plasma concentration–time profile of nimodipine after oral administration of nimodipine tablet (containing 60 mg nimodipine) to 18 healthy volunteers (each point represents mean ± S.D.).

4. Conclusion

A fast and sensitive UPLC–MS/MS method for quantification of nimodipine in human plasma was developed and validated. Compared with the published methods, the sharp peaks (high efficiency), short run time and reduced ion suppression produced by using special UPLC column are of particular advantage when coupled to electrospray mass spectrometer. An LLOQ of 0.2 ng/ml in plasma corresponding to an on-column sensitivity of 2.5 pg nimodipine was achieved. The time for instrumental determination of one sample was only 2.5 min and more than 200 plasma samples could be assayed daily, which make the method attractive particularly in high-throughput bioanalysis of nimodipine. The method was proved superior in sensitivity and speed to previously reported methods and was successfully applied to the pharmacokinetic study of nimodipine tablets in healthy volunteers.

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