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Determination of Nimodipine in Human Plasma by HPLC–ESI-MS and Its Application to a Bioequivalence Study

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Abstract

A simple, specific, and precise liquid chromatographic–electrospray ionization mass spectrometric (LC–ESI-MS) method has been developed for determination of nimodipine concentration in human plasma. The method involves the addition of 200 µL of saturated sodium bicarbonate ($NaHCO_3$) solution to plasma to improve the extraction recovery, liquid–liquid extraction of nimodipine from plasma samples with anhydrous diethyl ether, simple reversed-phase chromatography, and ESI mass spectrometric detection in negative ion selected ion monitoring mode (SIM) using target [M-] ions at m/z 417 and m/z 359 for nimodipine and nitrendipine (internal standard, IS), respectively. A complete analytical run was achieved within 3.5 min. The limit of quantification was 0.5 ng/mL. The method was validated within a linear range of 0.5–100 ng/mL. The correlation coefficient for the calibration regression line was 0.9995 or better. Intra- and inter-batch accuracy and precision were acceptable. Analyte was stable in a battery of stability studies. The method has been successfully used in a bioequivalence study.

Introduction

Nimodipine (Figure 1), a dihydropyridine calcium channel blocker, is known for its preferential action on cerebral blood vessels and its potential cytoprotective effects by reducing calcium influx into nerve cells (1). It is so far the only available therapy with proven benefits for reducing the impact of ischemic neurological deficits after subarachnoid hemorrhage (1,2). It is also used in other cerebrovascular disorders, such as ischemic stroke (3), and has been studied in impaired brain function in multi-infarct dementia and senile dementia (4).

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 concentrations and significant inter-individual variations (1). Several methods have been applied for the quantification of nimodipine in plasma. These methods are based on gas chromatography with electron-capture (5), capillary gas chromatography and nitrogen detec-

tion (6), high-performance liquid chromatography with UV detection (7) and electrochemical detection (8), or tandem mass spectrometry (9,10), and ultra-high pressure liquid chromatography with tandem mass spectrometry (11). However, they either have a limited sensitivity, are time-consuming, or require special equipment. In the present article, we report a simple, precise, and sensitive liquid chromatographic–electrospray ionization-mass spectrometry (LC–ESI-MS) method for the quantitative determination of nimodipine in human plasma and its application to a bioequivalence study.

Experimental

Chemicals and reagents

Nimodipine and nitrendipine (used as internal standard, IS) (Figure 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). The purity was 99% for both compounds. Methanol (HPLC-grade) was purchased from Tedia (Fairfield, OH). Sodium bicarbonate ($NaHCO_3$) and anhydrous diethyl ether of analytical-grade were purchased from Nanjing Chemical Reagent Co. (Nanjing, China). All aqueous solutions including the HPLC mobile phase were prepared with purified water (Wahaha, Hangzhou, China). Liquid nitrogen was supplied by the Gas Supplier Center of Nanjing University (Nanjing, China). Blank human plasma was obtained from healthy volunteers in the Central Blood Bank of Nanjing (Nanjing, China).

Figure 1. The chemical structure of nimodipine and nitrendipine (IS).

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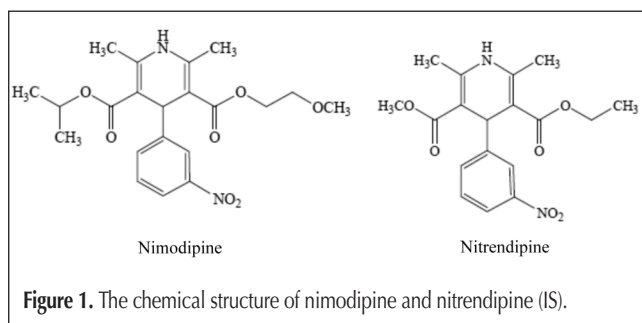
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HPLC–ESI-MS apparatus and conditions

Analyses were performed using a Shimadzu (Kyoto, Japan) 2010A LC–MS system equipped with a degasser, LC-20AD binary pumps, a model SIL-20AC autosampler, a model CTO-20A thermostat, an electrospray ionization (ESI) interface, and a model CBM-20A system controller. The analysis column was a SepaxGP-C$_{18}$ column (2.1 × 100 mm, 3 μm, Sepax Technologies, Newark, DE) maintained at 40°C. Nimodipine and IS were eluted by mobile phase consisted of methanol and water (75:25, v/v) delivered at a flow rate of 0.3 mL/min. The ESI-MS in negative ion selected ion monitoring (SIM) mode was used for detection of the target ions at m/z 417 and m/z 359 for nimodipine and the IS, respectively. The temperature of desolvation gas and source block was 250 and 200°C, respectively. Nebulizer gas flow was 1.5 L/min. The ESI spray voltage was 1.6 kV. The total run time was 3.5 min. LC–MS Solution (Version 3.4.306) working on Windows 2003 operating system was used for data process.

Preparation of standards samples and calibration curves

Stock solutions of nimodipine (10 mg/mL) and IS (100 ng/mL) were prepared in methanol. All stock solutions were stored away from light at ~ 4°C. Working standard solutions of the analyte were prepared by further dilution of the stock solution with methanol to give a series of concentrations (10, 20, 50, 100, 200, 500, 1000, 2000 ng/mL). The calibration samples were prepared by spiking 50 μL of corresponding working standard solutions of the analyte into 950 μL of blank human plasma to give a calibration range of 0.5–100 ng/mL (0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/mL). The final concentration of the IS in all samples was 5 ng/mL. The samples were then subjected to the sample pretreatment procedures described later. Calibration curves were acquired by plotting the peak area ratios of nimodipine to IS against the nominal concentrations of calibration standard samples. Samples at three levels of concentrations (1, 10, and 50 ng/mL) for method validation and quality control were prepared in the same way.

Table I. Extraction Recovery and Matrix Effect of the Method for Nimodipine in Human Plasma ($n = 5$)

Concentration (ng/mL)	Extraction recovery (Mean ± SD, %)	RSD (%)	Matrix effect (Mean±SD, %)	RSD (%)
1	71.31 ± 6.67	9.36	101.39 ± 5.96	5.88
10	74.15 ± 3.13	4.22	107.59 ± 2.37	2.16
50	77.87 ± 2.08	2.67	105.14 ± 6.27	5.97

Sample pretreatment

Human blood samples were centrifuged at 3,000 rpm for 2 min after collection, and 1 mL of plasma was transferred to a test tube. Plasma samples were stored at –20°C until analysis. To prevent the photodegradation of nimodipine to pyridine derivatives, the experiments including plasma collection, sample preparation, and instrumental analyses were performed under reduced light.

A 50-μL aliquot of the IS stock solution was added into 1 mL of samples and mixed. The samples were basified by mixing with 200 μL of saturated sodium bicarbonate (NaHCO$_3$) solution. After the addition of 5 mL of anhydrous diethyl ether, the samples were mixed for 3 min by vortex and centrifuged at 4000 rpm for 10 min. The organic layer (4 mL) was transferred to a test tube and evaporated to dryness under a stream of air at 40°C. The residue was reconstituted in 0.2 mL of mobile phase. After centrifugation at 12,000 rpm for 5 min, 10 μL of the supernatant was subjected to LC–MS analysis.

Extraction recovery and matrix effect

To evaluate extraction recovery, two sets of standards containing nimodipine at 1, 10, and 50 ng/mL were analyzed. One set (B) was prepared in human plasma as described earlier, and the other set was prepared in mobile phase (neat set, A). The absolute extraction recovery (AE) values were calculated as follows: AE (%) = 100% × B/A/4, where B/A means the peak area ratios of the extracted human samples to neat standard solutions of the same concentration.
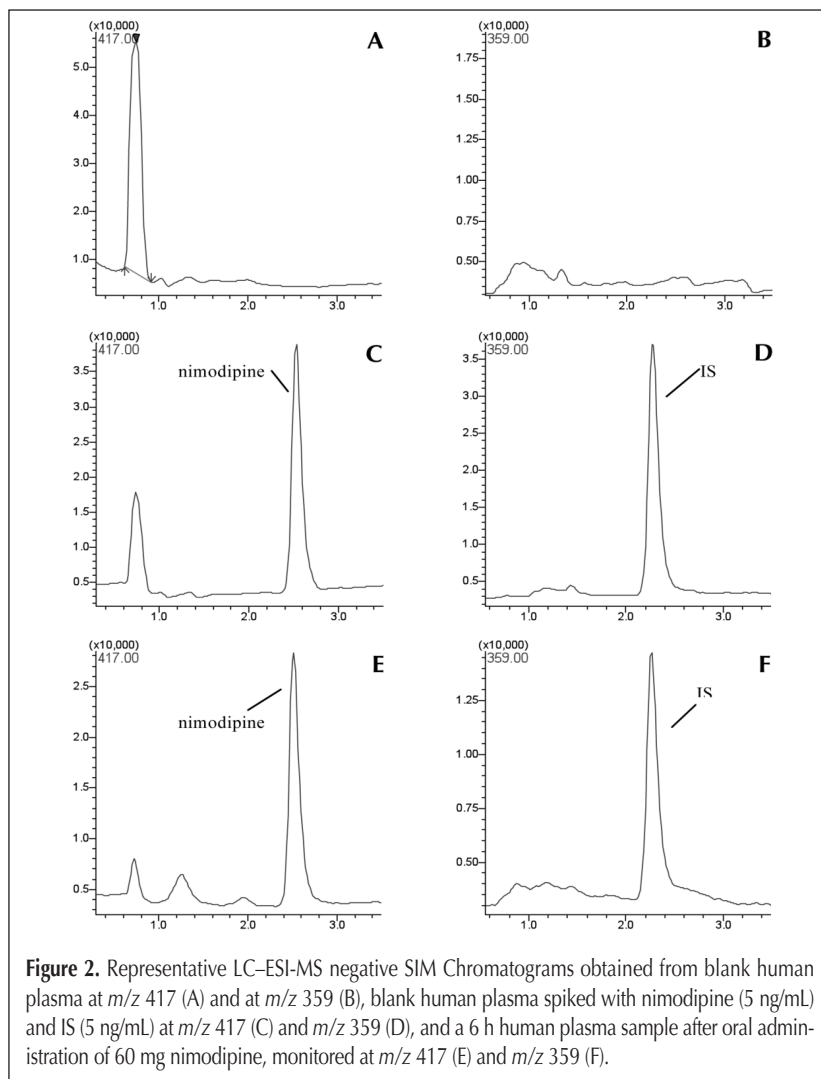

Figure 2. Representative LC–ESI-MS negative SIM Chromatograms obtained from blank human plasma at m/z 417 (A) and at m/z 359 (B), blank human plasma spiked with nimodipine (5 ng/mL) and IS (5 ng/mL) at m/z 417 (C) and m/z 359 (D), and a 6 h human plasma sample after oral administration of 60 mg nimodipine, monitored at m/z 417 (E) and m/z 359 (F).

To evaluate matrix effect, blank plasma is subjected to the sample pretreatment described earlier. The resulting extract was spiked with working standard solutions to prepared solutions (C) containing nimodipine at 1, 10, and 50 ng/mL. Matrix enhancement or suppression of ionization was evaluated by comparing the peak areas of the spiked samples (C) with corresponding neat standard solutions prepared in mobile phase (A).

Precision and accuracy

The precision and the accuracy of the method were confirmed by the five replicate determinations of plasma containing nimodipine at three concentrations (1, 10, and 50 ng/mL) in a single batch and three different batches. The determined concentrations, which were obtained from a calibration curve prepared concurrently, were used to evaluate the precision and accuracy of the method. The precision was evaluated by the coefficient of variation (CV), and the accuracy was expressed as the relative error (RE) according to the equation:

RE (%) = 100% × (measured concentration –
nominal concentration) / nominal concentration.

The criteria for acceptability of the data included precision (CV) within 15% and accuracy (RE) within ± 15% of the nominal values (12).

Stability experiments

Spiked samples of three concentrations (1, 10, and 50 ng/mL) were assessed after the storage under reduced light at room temperature for up to 4 h, after three freeze-thaw cycles, and at –20°C for 1 month. The stability of nimodipine in the injection solvent was determined periodically by injecting processed samples for up to 12 h (in autosampler at 5°C) after the initial injection. Samples were considered to be stable if the bias between the concentrations determined from 0 h and under different stability conditions was within the acceptance criterion recommended by the Food and Drug Administration (U.S. FDA) (http://www.fda.gov/cder/guidance/index.htm, 2001). The bias was calculated as follows:

% bias = 100 × (C − C_{0 h}) / C_{0 h}

Application of the assay to a bioequivalence study in human

The present HPLC–ESI-MS method was employed to a crossover bioequivalence study approved by the Human Ethics Committee of Institute of Dermatonosis, Chinese Academy of Medical Sciences (Nanjing, China). Informed consent was obtained from the subjects. Twenty healthy male volunteers (mean age 23.7 years, mean body mass index 21.5) were enrolled. Each participant received a single oral dose (60 mg) of test nimodipine tablets (20 mg each, Tianjin, China) and reference tablets (Nimotop, 30 mg each, Bayer AG, Germany) in a balance 2×2 Latin square design experiment, separated by one week washout period. The volunteers fasted overnight prior to the dose administration and 4 h post-dosing. Any medication, cigarette, and drinks containing caffeine or alcohol were not allowed at least two weeks prior to and during the periods of the test. Blood was obtained before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after dose administration.

Pharmacokinetics and statistical analysis

The pharmacokinetic parameters, namely, the maximum plasma concentration (C_{max}) and time point of maximum plasma concentration (T_{max}) were obtained directly from the measured data; half-life of drug elimination during the terminal phase

Table III. Determination of the Stability of Nimodipine ($n = 4$)*

Spiked conc. (ng/mL)	Conditions	Mean measured conc. ± SD (ng/mL)	Bias (%)a	CV (%)
0.5	0 h (for all)	0.86 ± 0.047		5.41
	4 h at room temperature	0.85 ± 0.020	–0.99	2.35
	3 F/T	0.83 ± 0.028	–3.32	3.33
	1 mouth at –20°C	0.90 ± 0.087	4.25	9.66
	12 h (in autosampler)	0.83 ± 0.030	–3.16	3.63
10	0 h (for all)	9.48 ± 0.108		1.14
	4 h at room temperature	9.66 ± 0.539	1.85	5.58
	3 F/T	10.16 ± 0.274	7.17	2.70
	1 mouth at –20°C	10.37 ± 0.408	9.32	3.86
	12 h (in autosampler)	9.99 ± 0.754	5.30	7.55
50	0 h (for all)	44.64 ± 0.186		0.42
	4 h at room temperature	43.94 ± 2.161	–1.58	4.92
	3 F/T	47.40 ± 3.041	6.18	5.92
	1 mouth at –20°C	49.19 ± 2.319	9.72	4.51
	12 h (in autosampler)	47.96 ± 4.381	7.44	9.13

* CV = coefficient of variation; F/T = freeze-thaw; and Bias was calculated as follows: % bias = 100 × (C − C_{0 h}) / C_{0 h}.

Table II. The Intra- and Inter-day Precision and Accuracy of the Method for Nimodipine in Human Plasma ($n = 5$)*

	Spiked conc. (ng/mL)	Measured conc. (mean ± SD, ng/mL)	Precision (RSD, %)*	Accuracy (relative error, %)
Intra-day	1	1.00 ± 0.06	6.22	–0.22
	10	9.64 ± 0.35	3.66	–3.56
	50	47.16 ± 3.98	8.43	–5.67
Inter-day	1	0.91 ±0.08	8.67	–4.88
	10	9.70 ±0.69	7.16	–3.03
	50	49.56 ±3.59	7.25	–0.88

* RSD = 100% × (SD/mean).

Relative error r = 100% × (measured concentrateion – spiked concentrateon) / spiked concentration

Table IV. Pharmacokinetic Parameters of Nimodipine in 20 Healthy Male Volunteers Receiving a Single 60-mg Oral Dose of Test and Reference Nimodipine Tablets

Parameters	Reference (Nimotop)	Test
C_{max} (ng/mL)	48.43 ± 12.81	44.27 ± 16.10
T_{max} (h)	0.81 ± 0.38	0.87 ± 0.33
$t_{1/2}$ (h)	2.01 ± 0.20	1.85 ± 0.42
MRT (h)	2.93 ± 0.60	2.70 ± 0.50
$AUC_{0-12 h}$ (ng × h /mL)	100.75 ± 36.26	93.84 ± 25.14
$AUC_{0-\infty}$ (ng· h /mL)	104.33 ± 38.55	96.89 ± 25.85
F (%)		93.14 ± 16.70

($t_{1/2\beta}$), area under the plasma concentration-time curve from 0 to the last measurable concentration (AUC_{0-12h}), area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$), and mean residence time (MRT) were computed using WinNolin 5.0.1 (Pharsight, Mountain View, CA)

Statistical analysis of bioequivalence parameters was carried out using SPSS Software-Version 10.0. A Wilcoxon test was established for T_{max}. For $\ln C_{max}$, $\ln AUC_{0-12h}$, and $\ln AUC_{0-\infty}$, variance analysis was used to assess period, person and product effects. Two/one-side student t-tests for pharmacokinetic parameters ($\ln C_{max}$, $\ln AUC_{0-12h}$ and $\ln AUC_{0-\infty}$) of nimodipine were carried out. Bioequivalence was assessed using a 90% confidence interval (CI) for the ln-transformed bioequivalence parameters, within an acceptable range of 0.80–1.25 (13).

Results

Method validation
Specificity

No interfering endogenous compound peaks eluting at the retention time of nimodipine or IS were detected using negative ion SIM at the given *m/z* (417, 359) for blank human plasma from six different sources. Representative LC–ESI-MS chromatograms obtained from blank human plasma and nimodipine-spiked plasma are shown in Figure 2. The retention time of nimodipine and IS were ~ 2.56 and 2.29 min, respectively. The overall chromatographic run time was within 3.5 min.

Linearity and limits of detection and quantitation

Least squares regression calibration curves were constructed by plotting the peak area ratios of analyte to IS versus nominal concentrations. The correlation coefficient (r^2) was typically better than 0.9995 over the concentration range of 0.5–100 ng/mL. The deviation between the nominal concentrations and measured concentrations was generally within 10% (the lowest concentration within 20%) with the use of the least squares regression ($n = 5$).

The lower limit of quantification (LLOQ) for nimodipine in human plasma, which was defined as the concentration of analyte that gave a signal-to-noise ratio of 10 and both precision and accuracy less than or equal to 20%, was 0.5 ng/mL, the lowest concentration on the calibration curves.

Extraction recovery and matrix effect

The absolute extraction recoveries of nimodipine from human plasma are shown in Table I.

No significant matrix effect was observed. The negligible matrix effect was also confirmed by the low coefficient of variation (CV < 1.3%) between the slopes of the five standard curves.

Precision and accuracy

The data proved good precision and accuracy of the method. As shown in Table II, the intra- and inter-day precision of the assay was lower than 10%. The accuracy (RE) was also lower than 10%. The results indicated that the method was reproducible.

Stability

Assessment of the stability of nimodipine in human plasma at room temperature, at –20°C for 1 month, and after three freeze-thaw cycles was performed. The results indicated that nimodipine was stable in plasma under those conditions (Table III). Stability of processed samples in the autosampler up to 12 h was also evaluated. The bias between the concentrations determined from the initial injection and the re-injection was within 10%.

Application to bioequivalence study

The plasma concentration-time profiles of nimodipine are shown in Figure 3. The main pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-12h}, $AUC_{0-\infty}$, $t_{1/2\beta}$) of nimodipine are shown in Table IV. The relative bioavailability of the test tablets was 93.14 ± 16.70%. A Wilcoxon test was established for T_{max} and no statistical difference was shown between the two products ($p > 0.05$). The two/one-side student t-tests and variance analysis for pharmacokinetic parameters ($\ln C_{max}$, $\ln AUC_{0-12h}$ and $\ln AUC_{0-\infty}$) of nimodipine were calculated using SPSS 10.0. The results indicated that there were no significant differences in the periods and products, and the two products of nimodipine were bioequivalent in 90% confidential limit.

Conclusion

Liquid–liquid extraction methods using a mixture of organic solvents have been reported for the extraction of nimodipine from plasma samples (9,10,14). Qin et al. used diethyl ether to extract nomidipine from plasma (11). However, sodium hydroxide solution, which is a strong base and may hydrolyze nimopidine, was used to basify plasma samples (11). In the present study, we developed a liquid–liquid extraction method using diethyl ether. This method yielded clean plasma preparations. In addition, sodium bicarbonate solution was used to adjust the plasma pH. The mild alkaline solution of sodium bicarbonate is not expected to cause any hydrolysis of nimopidine. Under alkaline conditions, the free base form of the amino group in the dihydropyridine ring of nimodipine molecule is favored. Therefore, extraction recovery is enhanced with the addition of sodium bicarbonate solution. Our liquid–liquid extraction procedures are easy to operate and control.

Several methods have been reported for the quantification of nimodipine in biological fluids. These methods, which utilized
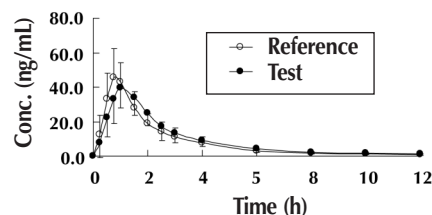

Figure 3. The mean plasma concentration-time curves after oral administration of 60 mg nimodipine of test and reference tablets ($n = 20$).

gas chromatography with electron-capture (5), capillary gas chromatography and nitrogen detection (6), HPLC with UV detection (7), and electrochemical detection (8), provide LLOQ of 1–10 ng/mL. But these methods were time-consuming with a run time of more than 10 min. Qiu et al. developed an LC–MS–MS method (10) and an ultra-high pressure liquid chromatography–MS–MS method (11) that provided LLOQ of 0.24 ng/mL and 0.20 ng/mL, respectively. However, these methods required a sophisticated tandem mass spectrometer. Our method in the present study provided a comparable LLOQ value with a short rune time of 3.5 min. Moreover, sensitive and specific detection of nimopidine was achieved with a less sophisticated LC–MS method.

Our rapid, simple, and sensitive LC–MS method for nimodipine determination was fully validated for specificity, sensitivity, precision, and accuracy. The concentrations of nimodipine used in the validation study covered the need of our pharmacokinetic study. The pharmacokinetic application of this LC–MS method further proved that it was sensitive and reproducible for the pharmacokinetic study of nimodipine. Our study demonstrated that test and reference (Nimotop) tablets were bioequivalent.

Previous pharmacokinetic data on nimodipine were limited and controversial after oral administration of 60 mg nimodipine tablets. The mean C_{max} described in different publications ranged from 12.1 to 81.7 ng/mL while the mean AUC values ranged from 53.7 to 256 ng·h/mL (9,10,11,14). Those differences could be caused by different manufacturers of the tablets and ethnic differences. In a Nimotop pharmacokinetic study using LC–MS–MS, He et al. (9) reported that the C_{max}, AUC_{0-12h}, and $AUC_{0-\infty}$ values were 50.11 ± 17.73 ng/mL, 92.76 ± 35.18 ng·h/mL, and 96.57 ± 36.73 ng·h/mL, respectively, which were similar to the results in the present study.

In conclusion, a rapid, simple and sensitive LC–MS assay was developed, validated, and successfully applied to a bioequivalence study for nimodipine.

References

1. M. Langley and E. Sorkin. Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease. *Drugs.* **37:** 669–699 (1989).
2. S. Keyrouz and M. Diringer. Clinical review: Prevention and therapy of vasospasm in subarachnoid hemorrhage. *Crit Care.* **11:** 220 (In press) (2007)
3. M. Sobrado, M. López, F. Carceller, A. García, and J. Roda. Combined nimodipine and citicoline reduce infarct size, attenuate apoptosis and increase bcl-2 expression after focal cerebral ischemia. *Neuroscience.* **118:** 107–113 (2003).
4. J. López and J. Birks. Nimodipine for primary degenerative, mixed and vascular dementia. *Cochrane Database Syst Rev.* **3:** CD000147(2002);.
5. P. Jakobsen, E. Mikkelsen, J. Laursen and F. Jensen. Determination of nimodipine by gas chromatography using electron-capture detection; external factors influencing nimodipine concentrations during intravenous administration. *J. Chromatogr.* **374:** 383–387 (1986).
6. M. Rosseel, M. Bogaert and L. Huyghens. Determination of the calcium antagonist nimodipine in plasma by capillary gas chromatography and nitrogen detection. *J. Chromatogr.* **533:** 224–228 (1990).
7. M. Qian and J. Gallo. High-performance liquid chromatographic determination of the calcium channel blocker nimodipine in monkey plasma. *J Chromatogr.* **578:** 316–320 (1992).
8. J. López, V. Martínez, R. Alonso, and R. Jiménez. High-performance liquid chromatography with amperometric detection applied to the screening of 1,4-dihydropyridines in human plasma. *J. Chromatogr A* **870:** 105–114 (2000).
9. Z. He, D. Zhong, X. Chen, X. Liu, X. Tang, and L. Zhao. Development of a dissolution medium for nimodipine tablets based on bioavailability evaluation. *Eur J. Pharm. Sci.* **21:** 487–491 (2004).
10. F. Qiu, X. Chen, X. Li, and D. Zhong. Determination of nimodipine in human plasma by a sensitive and selective liquid chromatography-tandem mass spectrometry method. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **802:** 291–297 (2004).
11. F. Qin, Y. Ma, Y. Wang, L. Chen, D. Wang, and F. Li. Determination of nimodipine in human plasma by ultra performance liquid chromatography-tandem mass spectrometry and pharmacokinetic application. *J. Pharm. Biomed. Anal.* **46:** 557–562 (2008)
12. FDA Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), http://www.fda.gov/cder/guidance/index.htm (2001).
13. FDA Guidance for Industry, Statistical Approaches to Establishing Bioequivalence, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), http://www.fda.gov/cder/guidance/index.htm (2001).
14. Blardi P, Urso R, De Lalla A, Volpi L, Perri TD, and Auteri A. Nimodipine: drug pharmacokinetics and plasma adenosine levels in patients affected by cerebral ischemia. *Clin. Pharmacol. Ther.* **72:** 556–561 (2002).