Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 856 (2007) 35–40
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatography/negative ion electrospray tandem mass spectrometry method for the quantification of rosuvastatin in human plasma: Application to a pharmacokinetic study

Jun Gao [a,b], Dafang Zhong [a], Xiaotao Duan [a,b], Xiaoyan Chen [a,*]

[a] *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 646 Songtao Road, Shanghai 201203, PR China*
[b] *Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China*

Received 19 December 2006; accepted 11 May 2007
Available online 26 May 2007

Abstract

A sensitive liquid chromatography/tandem mass spectrometric (LC–MS/MS) method was developed and validated for the determination of rosuvastatin in human plasma. The plasma samples were prepared using liquid–liquid extraction with ethyl ether. Chromatographic separation was accomplished on a Zorbax XDB-C18 (150 mm × 4.6 mm i.d., 5 μm) column. The mobile phase consisted of methanol–water (75:25, v/v, adjusted to pH 6 by aqueous ammonia). Detection of rosuvastatin and the internal standard (IS) hydrochlorothiazide was achieved by ESI MS/MS in the negative ion mode. The lower limit of quantification was 0.020 ng/ml by using 200 μl aliquots of plasma. The linear range of the method was from 0.020 to 60.0 ng/ml. The intra- and inter-day precisions were lower than 8.5% in terms of relative standard deviation (RSD), and the accuracy was within −0.3 to 1.9% in terms of relative error (RE). Compared with the existing methods, the validated method offered increased sensitivity. The method was successfully applied for the evaluation of pharmacokinetics of rosuvastatin after single oral doses of 5, 10 and 20 mg rosuvastatin to 10 healthy volunteers.

Keywords: Rosuvastatin; LC–MS/MS; Pharmacokinetics

1. Introduction

Rosuvastatin is an effective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that has been developed for the treatment of dyslipidemia [1,2]. Compared with several other HMG-CoA inhibitors, rosuvastatin reduces the level of low-density lipoprotein cholesterol more effectively [3,4]. Pharmacokinetic studies in humans receiving oral doses (10–80 mg) showed that the maximum plasma concentrations were maintained at ng/ml levels, the elimination half-life was approximately 20 h and steady-state concentration was reached within 4–5 days after dosing [5,6]. Additionally, the population pharmacokinetic study at 40 mg dose revealed that plasma exposure to rosuvastatin was significantly higher in Asian subjects than in White subjects living in the same environment [7]. As a result, the clinical initial dosage is 10 mg/day in White subjects, but in Chinese subjects, the recommended dosage is lowered to 5 mg/day to achieve the same therapeutic efficacy [8]. To further understand and reveal the pharmacokinetic profile of rosuvastatin at lower dosage in Chinese subjects, a very sensitive analytical method was required.

In recent years, several methods including HPLC-UV [9,10] and LC–MS/MS [11–14] have been reported for the quantification of rosuvastatin in plasma. Most of the published LC–MS/MS methods [11–14] were based on positive electrospray detection, which provided a sensitivity at LLOQ of ≥0.1 ng/ml, using a plasma sample volume of 0.25–1.7 ml. The negative ionization mode was scarcely mentioned except one report in which rosuvastatin was served as internal standard (IS), where the spiked plasma concentration of rosuvastatin was 200 ng/ml [15]. Some methods [11,13] have been successfully applied to characterize the clinical pharmacokinetic profiles of rosuvastatin after 10 mg or higher doses, excepting a more recently described LC–MS/MS method [14], which was applied to determine plasma concentrations of rosuvastatin after an oral administration of 5 mg dose. In the paper [14], the $t_{1/2}$ was

* Corresponding author. Tel.: +86 21 50 800 738; fax: +86 21 50 800 738.
E-mail address: xychen@mail.shcnc.ac.cn (X. Chen).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.05.012

reported as 2–5 h, which was not consistent with other reported data [5,6]. According to the reported $t_{1/2}$ values, the plasma concentrations would be determined only up to 30 h, instead of 72 h as claimed.

In the present study, negative electrospray detection has been preferred to determine rosuvastatin in human plasma. High sensitivity and small plasma volume were the main advantage of the technique. The method was validated and applied successfully to a clinical study in Chinese subjects after single oral doses of 5, 10 and 20 mg rosuvastatin.

2. Experimental

2.1. Chemicals and reagents

Rosuvastatin (99.5% pure) and the internal standard hydrochlorothiazide (99.2% pure) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Other chemical reagents (analytical grade) were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China). Heparinized blank (drug free) human plasma was supplied by Shanghai Shuguang Hospital (Shanghai, China). Distilled water, prepared from demineralized water was used throughout the study.

2.2. Instruments

The high-performance liquid chromatography was performed on an Agilent 1100 system equipped with a G1313A autosampler, a G1379A degasser, a G1316A thermostatted column and a G1311A quaternary pump (Agilent, Waldbronn, Germany). The HPLC system was coupled to an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) via a TurboIonspray ionization (ESI) interface for mass analysis and detection. Data acquisition was performed with Analyst 1.4.1 software (Applied Biosystems).

2.3. Standard solutions and quality control (QC) samples

A stock solution of rosuvastatin was prepared by dissolving the accurately weighed reference compound in methanol to give a final concentration of 400 μg/ml. The solution was then serially diluted with methanol/water (50:50, v/v) to obtain the desired concentrations. Calibration curves were prepared by spiking 40 μl of the appropriate standard solution to 200 μl blank plasma. Effective concentrations in plasma samples were 0.020, 0.050, 0.15, 0.50, 2.0, 5.0, 10.0, 20.0 and 60.0 ng/ml for rosuvastatin. A 400 μg/ml stock solution of internal standard hydrochlorothiazide was also prepared in methanol. This was subsequently diluted with water to obtain a 50.0 ng/ml working solution. All the solutions were stored at 4 °C and were brought to room temperature before use.

The quality control samples (QCs) were prepared using a different stock solution of rosuvastatin to obtain the plasma concentrations of 0.020, 0.50 and 50.0 ng/ml, representing low, medium and high concentration levels, respectively. These QCs were stored in plastic tubes at −20 °C until analysis.

The QCs for the recovery and matrix effect evaluation were prepared with blank human plasma at concentrations of 0.050, 0.50 and 50.0 ng/ml, and the QCs for the stability were prepared at concentrations of 0.050 and 50.0 ng/ml.

2.4. Sample preparation

To 200 μl of plasma sample, a 40 μl aliquot of the IS solution (hydrochlorothiazide, 50.0 ng/ml in water) and 40 μl of methanol/water (50:50, v/v) were added, respectively. Then 200 μl of phosphate buffer (pH 5) was added. Samples were mixed for approximately 10 s and then 3 ml of ethyl ether was added. The mixture was vortex-mixed for approximately 1 min and shaked on a mechanical shaker for 10 min. After centrifugation at 2100 × *g* for 5 min, the upper organic layer was separated and evaporated to dryness at 40 °C using a gentle stream of air. The residue was reconstituted in 150 μl of the mobile phase, and then vortex-mixed. A 20 μl aliquot of the resulting solution was injected onto the LC–MS/MS system for analysis.

2.5. LC–MS/MS conditions

Chromatographic separation was performed using a Zorbax XDB-C18 column (150 mm × 4.6 mm i.d., 5 μm; Agilent, Wilmington, DE, USA) with a SecurityGuard C18 guard column (4 mm × 3.0 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA). The column temperature was maintained at 25 °C. A mixture of methanol and water (75:25, v/v, adjusted to pH 6 by aqueous ammonia) was used as the isocratic mobile phase at a flow rate of 0.5 ml/min.

The mass spectrometer was operated in the negative ion mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 480 → 418 for rosuvastatin and *m/z* 296 → 269 for IS, respectively, with a dwell time of 200 ms per transition. The product ion spectra of $[M-H]^-$ ions of rosuvastatin and hydrochlorothiazide (IS) are shown in Fig. 1. The tuning parameters were optimized by infusion of a standard solution (10.0 ng/ml) of the analyte and IS at a flow rate of 10 μl/min, using a syringe pump integrated into the API 4000 mass spectrometer. The optimized TurboIon Spray voltage and temperature were set at −3800 V and 450 °C, respectively. The declustering potential (DP) voltage was set at −55 V for each analyte. Ion source gas 1 (N_2), gas 2 (N_2) and curtain gas (N_2) pressure were set at 40, 55 and 10 psi, respectively. The optimized collision energies of −22 and −27 eV were used for rosuvastatin and IS, respectively.

2.6. Method validation

This method was validated for selectivity, accuracy, precision, calibration curve range, recovery, stability and matrix effect.

Selectivity was performed by analyzing the blank plasma from six different sources to test interference at the retention times of analyte and internal standard.

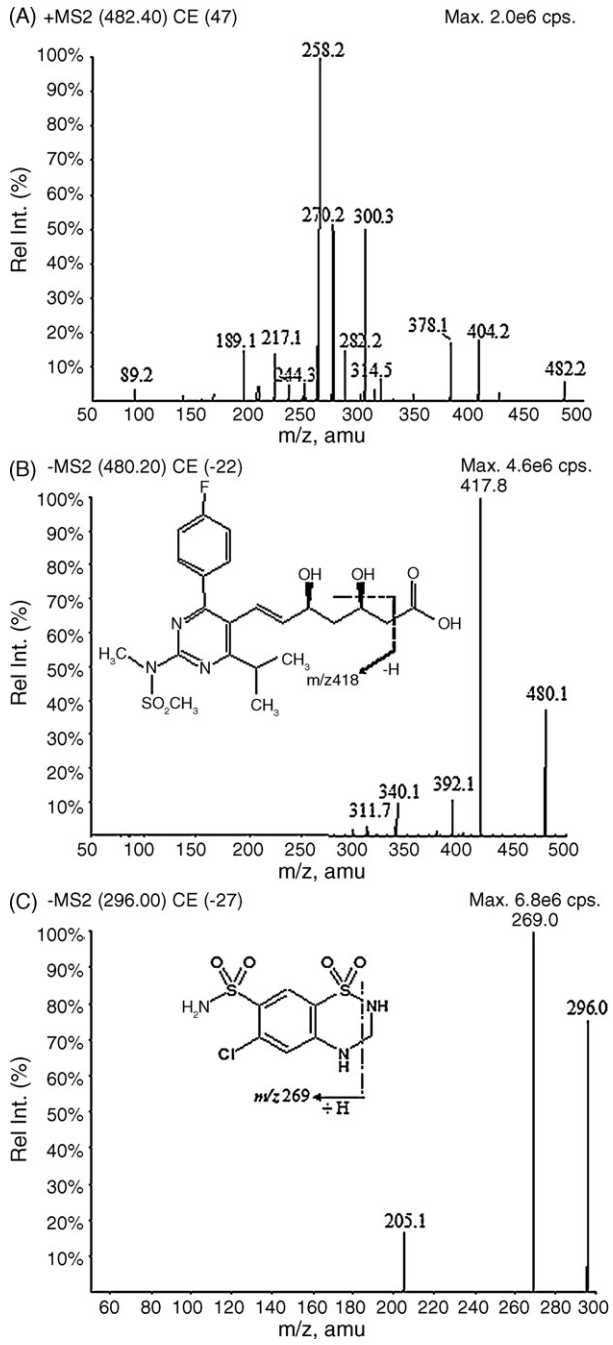

Fig. 1. Full-scan product ion spectra of $[M+H]^-$ of rosuvastatin (A) in positive ion mode, $[M-H]^-$ of rosuvastatin (B) and hydrochlorothiazide (C) in negative ion mode.

The linearity of the assay method was determined by plotting the peak area ratios of rosuvastatin to IS against the concentrations of rosuvastatin in plasma in duplicate on three consecutive days. Least-squares linear regression was used for curve fitting with $1/x^2$ as the weighting factor.

Inter- and intra-day accuracy and precision for the assay were determined at the three concentration levels (see Table 1) on three separate days, and on each day six replicates were analyzed together with an independently prepared calibration curve. Accuracy was expressed by relative error (RE), and precision by relative standard deviation (RSD) [16]. The inter- and intra-day precision should not exceed 15% and the accuracy should be within ±15%, except at the lower limit of quantification (LLOQ), where precision should be below 20% and accuracy within ±20%.

Table 1
Precision and accuracy of the LC–MS/MS method to determine rosuvastatin in human plasma (on 3 consecutive days, six replicates for each day)

Nominal concentration (ng/ml)	Calculated concentration (ng/ml, mean ± SD)	Intra-day RSD (%)	Inter-day RSD (%)	Relative error (%)
0.020	0.020 ± 0.002	8.1	8.5	1.9
0.50	0.50 ± 0.032	6.4	5.6	0.9
50.0	49.8 ± 2.58	4.9	6.8	−0.3

Recovery of rosuvastatin was evaluated by comparing the mean peak areas of the regularly prepared samples ($n=6$) at 0.050, 0.50 and 50.0 ng/ml with the mean peak areas of spiked-after-extraction samples, which represented the 100%. To prepare the spiked-after-extraction samples, blank human plasma was processed according to the sample preparation procedure as described above. The supernatant was evaporated to dryness, and were reconstituted with the mobile phase by addition of appropriate standards at concentrations corresponding to the final concentration of the pretreated plasma samples.

Rosuvastatin stability in plasma was assessed by analyzing replicates ($n=3$) of stability samples at concentrations of 0.050 and 50.0 ng/ml, respectively, exposed to different time and temperature conditions. The results were compared with those for samples freshly prepared, and the percentage concentration deviation was calculated. The short-term stability was evaluated after exposure of the plasma samples to room temperature for 2 h. The bench-top storage stability was assessed by placing extracted plasma samples in the HPLC autosampler at 25 °C for a fixed period of time. The stability of stock solution in methanol was also evaluated at 4 °C for 7 days.

Matrix effects were determined by comparing the mean chromatographic peak areas of spiked-after-extraction samples with those from corresponding neat standard solution at the same concentrations. If the peak area ratios for the plasma extracts versus neat standard solutions were <90 or >110%, a matrix effect was implied.

2.7. Pharmacokinetic study

A pharmacokinetic study was performed in healthy Chinese volunteers approved by the Ethical Committee of Xijing Hospital, Xi'an, China. Single oral doses of 5, 10 and 20 mg rosuvastatin were administered to 10 healthy volunteers, respectively. The blood samples were collected into sodium–heparin-containing tubes predosing and 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 14, 24, 36, 48 and 72 h postdosing. Plasma was harvested by centrifuging the blood at 2100 × *g* for 10 min and stored frozen at −20 °C until analysis.

Pharmacokinetic parameters of rosuvastatin were calculated by non-compartmental analysis using the WinNonlin 5.0.1 (Pharsight Corp., Mountain View, CA, USA). The peak plasma

concentration (C_{max}) and the corresponding time (t_{max}) were directly obtained from the raw data. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method. The terminal elimination rate constant (k_e) was estimated by linear least-squares regression of the terminal portion of the plasma concentration–time curve, and the corresponding elimination half-life ($t_{1/2}$) was then calculated as $0.693/k_e$.

3. Results and discussion

3.1. Mass spectrometry

Rosuvastatin has a pyrimidine ring and a carboxylic group in its structure, hence it could be detected either in the positive or negative ionization. However, the reported LC–MS/MS methods [11–14] for the quantification of rosuvastatin were all performed in positive ionization mode. During the initial method development, we also attempted to optimize ESI conditions under positive ionization mode. However, the observed signal intensity was not sensitive enough for determination of expected rosuvastatin concentrations, especially for low dosage administration, which might be attributed to more fragment ions produced in the product ion spectrum of $[M+H]^+$ (see Fig. 1A). To improve the sensitivity, the negative ESI detection was taken into consideration in this experiment.

Under negative ESI mode, rosuvastatin produced abundant deprotonated molecule $[M-H]^-$ at m/z 480. In the product ion mass spectrum of $[M-H]^-$, fewer fragment ions were formed compared with that of $[M+H]^+$. A dominant fragment ion at m/z 418 (Fig. 1B) was derived from neutral loss of H_2O and CO_2. The intensities of other fragments were less than the 10% of base peak intensity. Therefore, the MRM transition m/z 480 → 418 was selected. In addition, it was observed that (−) ESI mode produced lower chemical background noise than positive ion mode. As a result, (−) ESI was chosen to obtain high sensitivity for rosuvastatin in this experiment. The LLOQ of 0.020 ng/ml was achieved under (−) ESI ionization mode,

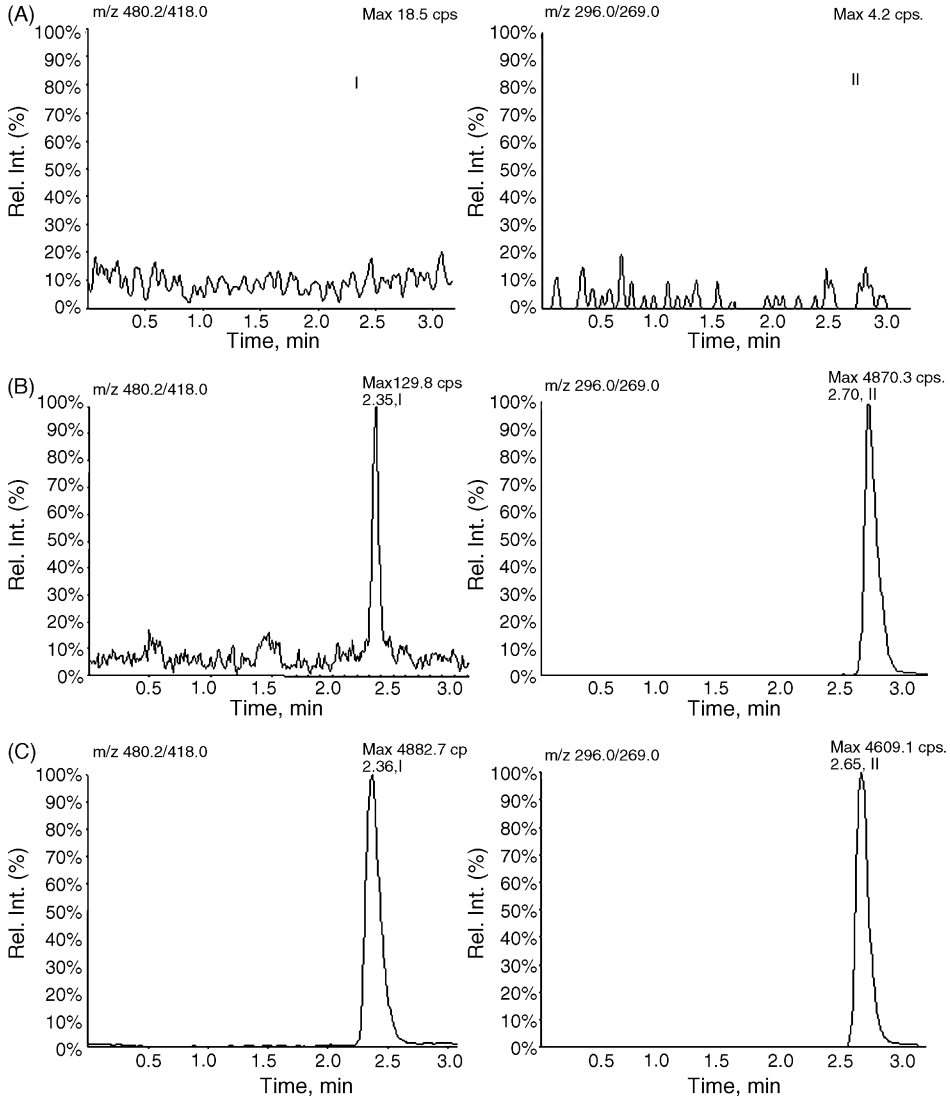

Fig. 2. Typical MRM chromatograms of rosuvastatin (I) and hydrochlorothiazide (IS, II) in human plasma samples, (A) blank plasma sample; (B) plasma sample spiked with rosuvastatin (0.020 ng/ml) and I.S. (10.0 ng/ml); (C) plasma sample 4 h after single oral dose of 10 mg rosuvastatin to a subject.

at least five times more sensitive than the reported methods [11–14].

3.2. Chromatography

Chromatographic conditions, the composition of mobile phase in particular, were optimized to achieve good sensitivity and peak shapes for the compounds, as well as a short run time. Methanol revealed higher mass spectrometric response and lower background noise than acetonitrile and was chosen as the organic phase. It was found that the ionization efficiency of rosuvastatin was dependent on the pH value of the mobile phase. The highest mass response was observed when the pH values of the mobile phase were maintained at 6–9. In view of the tolerance of the chromatographic column, the pH value of the mobile phase was adjusted to 6 with aqueous ammonia in this experiment.

3.3. Method validation

3.3.1. Selectivity

No interference peaks were detected for the analyte or IS from the six different sources of plasma. The typical chromatograms of a blank, a spiked plasma sample with rosuvastatin at LLOQ and IS, and a plasma sample from a healthy volunteer 4 h after an oral administration are shown in Fig. 2. The retention times for rosuvastatin and IS were 2.35 and 2.70 min, respectively. The total chromatographic run time was 3.2 min.

3.3.2. Linearity of calibration curves and lower limits of quantification

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range of 0.020–60.0 ng/ml for rosuvastatin in human plasma. The mean linear regression equation of the calibration curves generated during the validation was $y = (0.112 \pm 0.015)x + (0.00981 \pm 0.00374)$, where y represents the ratios of rosuvastatin peak area to that of IS and x represents the plasma concentrations of rosuvastatin. The correlation coefficients of the weighted calibration curves ranged from 0.9961 to 0.9992. Good linearity was seen in the concentration ranges.

The lower limit of quantification was established at 0.020 ng/ml for rosuvastatin. The precision and accuracy values corresponding to LLOQ are shown in Table 1. Under the present LLOQ of 0.020 ng/ml, the plasma concentration of rosuvastatin could be determined up to 72 h (at least five half-lives) after a single oral dose of 5 mg rosuvastatin, which was sensitive enough to characterize the pharmacokinetic profiles.

3.3.3. Precision and accuracy

Table 1 summarizes the inter- and intra-day precision and accuracy values for QCs. In this assay, the intra-day precision was 8.1% or less, and the inter-day precision was 8.5% or less for each QC level of rosuvastatin. The accuracy was within −0.3 to 1.9%. The above values were within the acceptable range, and the method was thus judged to be suitably accurate and precise.

3.3.4. Recovery

The recoveries of rosuvastatin extracted from plasma were 71.7 ± 5.2%, 69.4 ± 3.8% and 70.5 ± 6.3% at concentrations of 0.050, 0.50 and 50.0 ng/ml, respectively. Mean recovery for the IS was 76.2 ± 4.7%. The RSDs for all recoveries were less than 6.3% throughout the entire concentration ranges, indicating solid assay consistency.

3.3.5. Stability

Rosuvastatin in plasma was reported to be stable at −20 °C for 6 months and after three freeze/thaw cycles [11,14]. Due to the need for delayed injection for extracted samples, stability of rosuvastatin in the final reconstituted extraction fluid was assessed, and the extracted plasma samples showed no significant degradation in the HPLC autosampler (25 °C) for at least 24 h (RE from −0.5 to 7.0%). Rosuvastatin was also found to be stable (RE from 1.7 to 4.7%) in human plasma at room temperature for 2 h. In addition, stock solution of rosuvastatin in methanol was shown to be stable for 7 days at 4 °C (RE from −3.6 to 5.2%).

3.3.6. Matrix effect

In our study, the estimation of matrix effect was conducted following the procedures described in Section 2.6. By the analysis of six batches of samples, matrix effect values were calculated. Average matrix effect values obtained were 92.6, 90.1 and 94.3% for QC samples at concentrations of 0.050, 0.50 and 50.0 ng/ml. No significant matrix effect was observed.

3.4. Application of the method to pharmacokinetic study

The validated LC–MS/MS method was applied to investigate the pharmacokinetic profiles of rosuvastatin after single oral doses of 5, 10 and 20 mg rosuvastatin. Profiles of the mean plasma concentration versus time are shown in Fig. 3.

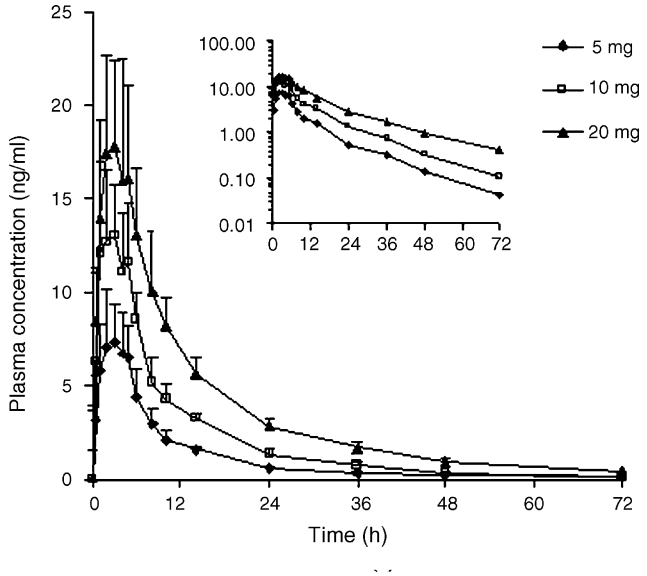

Fig. 3. Mean plasma concentration–time curve of rosuvastatin after single oral doses of 5, 10 and 20 mg to 10 healthy subjects, respectively.

Table 2
Major pharmacokinetic parameters of rosuvastatin after single oral doses of 5, 10 and 20 mg to 10 healthy subjects, respectively

Parameters	Dose groups		
	5 mg ($n = 10$)	10 mg ($n = 10$)	20 mg ($n = 10$)
C_{max} (ng/ml)	8.32 ± 2.44	14.8 ± 3.97	20.1 ± 5.02
t_{max} (h)	3.1 ± 0.99	2.0 ± 0.81	3.4 ± 1.6
$AUC_{0\text{-}t}$ (ng h/ml)	75.8 ± 22.0	151 ± 36.8	258 ± 61.6
$AUC_{0-\infty}$ (ng h/ml)	77.0 ± 22.6	153 ± 38.3	270 ± 60.6
$t_{1/2}$ (h)	12.0 ± 3.9	12.5 ± 3.8	18.5 ± 4.9
CL/F (l/h)	70.8 ± 20.7	69.0 ± 17.8	77.2 ± 16.7

The corresponding pharmacokinetic parameters (C_{max}, t_{max}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$) are presented in Table 2. At 5–20 mg dose levels, plasma exposure to rosuvastatin appeared increasing dose-proportionally, and the plasma elimination half-lives were prolonged with increased doses. When the same doses (10 and 20 mg) of rosuvastatin were administered, the higher plasma concentrations were observed in Chinese subjects than the reported in white subjects [5,17].

4. Conclusion

A sensitive and selective LC–MS/MS method for the quantification of rosuvastatin in human plasma was developed and validated. In our study, the desired sensitivity with an LLOQ of 0.020 ng/ml was achieved by using negative ESI/MS/MS instead of positive ESI detection, which proved to be superior in sensitivity in comparison to the methods reported previously. It allows the determination of rosuvastatin up to 72 h after single oral doses of 5, 10 and 20 mg.

Acknowledgements

The authors express great appreciation to Dr. A.D. Wen of Xijing Hospital (Xi'an, China) for his efforts in the PK studies of rosuvastatin. Thanks for Dr. Y.F. Zhang for the PK data analysis.

References

[1] M.J. Chapman, F. McTaggart, Atheroscler. Suppl. 2 (2002) 33.
[2] K. Nezasa, K. Higaki, T. Matsumura, K. Inazawa, H. Hasegawa, M. Nakano, M. Koike, Drug Metab. Dispos. 30 (2002) 1158.
[3] A.G. Olsson, J. Pears, J. McKellar, J. Mizan, A. Raza, Am. J. Cardiol. 88 (2001) 504.
[4] M.F. Bullano, D.A. Wertz, G.W. Yang, S. Kamat, G.M. Borok, S. Gandhi, K.L. McDonough, V.J. Willey, Pharmacotherapy 26 (2006) 469.
[5] P.D. Martin, M.J. Warwick, A.L. Dane, M.V. Cantarini, Clin. Ther. 25 (2003) 2215.
[6] P.D. Martin, A.L. Dane, O.M. Nwose, D.W. Schneck, M.J. Warwick, J. Clin. Pharmacol. 42 (2002) 1116.
[7] E. Lee, E. Ryan, B. Birmingham, J. Zalikowski, R. March, H. Ambrose, R. Moore, C. Lee, Y. Chen, D. Schneck, Clin. Pharmacol. Ther. 78 (2005) 330.
[8] A.G. Olsson, H. Istad, O. Luurila, L. Ose, S. Stender, J. Tuomilehto, O. Wiklund, H. Southworth, J. Pears, J.W. Wilpshaar, Am. Heart J. 144 (2002) 1044.
[9] T.R. Kumar, N.R. Shitut, P.K. Kumar, M.C. Vinu, V.V. Kumar, R. Mullangi, N.R. Srinivas, Biomed. Chromatogr. 20 (2006) 881.
[10] S. Vittal, N.R. Shitut, T.R. Kumar, M.C. Vinu, R. Mullangi, N.R. Srinivas, Biomed. Chromatogr. 20 (2006) 1252.
[11] C.K. Hull, A.D. Penman, C.K. Smith, P.D. Martin, J. Chromatogr. B 772 (2002) 219.
[12] K.A. Oudhoff, T. Sangster, E. Thomas, I.D. Wilson, J. Chromatogr. B 832 (2006) 191.
[13] R.K. Trivedi, R.R. Kallem, R. Mullangi, N.R. Srinivas, J. Pharm. Biomed. Anal. 39 (2005) 661.
[14] D.H. Xu, Z.H. Ruan, Q. Zhou, H. Yuan, B. Jiang, Rapid Commun. Mass Spectrom. 20 (2006) 2369.
[15] R.V.S. Nirogi, V.N. Kandikere, W. Shrivastava, K. Mudigonda, P.V. Datla, Rapid Commun. Mass Spectrom. 20 (2006) 1225.
[16] V.P. Shah, K.K. Midha, J.W. Findlay, J.D. Hulse, I.J. Mcgliveray, G. McKay, K.J. Miller, R.N. Patnaik, M.I. Powell, A. Tonelli, C.T. Viswanathan, J. Pharm. Sci. 17 (2000) 1511.
[17] P.D. Martin, P.D. Mitchell, D.W. Schneck, Br. J. Clin. Pharmacol. 54 (2002) 472.