Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 808–813
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Determination of lovastatin in human plasma by ultra-performance liquid chromatography–electrospray ionization tandem mass spectrometry and its application in a pharmacokinetic study

Haiyan Yuan, Feng Wang, Jiying Tu, Wenxing Peng, Huande Li *

Clinical Pharmacy & Pharmacology Institute, Second Xiangya Hospital, Central South University, Changsha 410011, PR China

Received 15 September 2007; received in revised form 28 November 2007; accepted 1 December 2007
Available online 8 December 2007

Abstract

A selective, rapid and sensitive ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed for the quantitative determination of lovastatin in human plasma and its application in a pharmacokinetic study. With mycophenolate mofetil as internal standard, sample pretreatment involved a one-step extraction with *tert*-butyl methyl ether of 0.2 ml plasma. The analysis was carried out on an ACQUITY UPLCTM BEH C18 column (50 mm × 2.1 mm, i.d., 1.7 μm) with flow rate of 0.35 ml/min. The mobile phase was 20% water and 80% acetonitrile (v/v). The detection was performed on a triple-quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via electrospray ionization (ESI). Linear calibration curves were obtained in the concentration range of 0.08–24.50 ng/ml, with a lower limit of quantification of 0.08 ng/ml. The intra- and inter-day precision (RSD) values were below 15% and accuracy (RE) was −7.6 to 9.3% at all QC levels. The method was applicable to clinical pharmacokinetic study of lovastatin in healthy volunteers following oral administration.

Keywords: Lovastatin; Determination; UPLC–ESI-MS/MS; Human plasma; Pharmacokinetic

1. Introduction

Lovastatin, a competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, is a highly effective cholesterol-lowering agent, which is widely used in the treatment of hypercholesterolemia [1]. It was reported that lovastatin is also effective in reducing lethality in coronary heart disease [2]. Plasma levels of lovastatin following therapeutic oral doses are reported to be very low. Probably because only 30% of the dosed lovastatin reaches the systemic circulation and is metabolized to many metabolites [3,4]. Therefore, sensitive and selective methods for the determination of lovastatin have been required for therapeutic drug study.

To date, some assays for the determination of lovastatin in human plasma (serum) or urine have been reported, including gas chromatography coupled to mass spectrometry [5,6], high-performance liquid chromatography (HPLC) with tandem mass spectrometry [7,8], reversed-phase HPLC with UV [9,10] and high-performance capillary electrophoresis (HPCE) with UV detection [11]. However, these published methods [5–11] are not ideal for large number of sample determination, because they are time consuming or costly, i.e. derivatization step, arduous sample preparation, long chromatographic run times. Xiao et al. [8] described a liquid chromatography with tandem mass spectrometry (LC–MS/MS) method which achieved better sensitivity. The assay was found to be linear in the range 0.5–30 ng/ml with a LLOQ of 0.5 ng/ml. However, it used 1 ml plasma aliquot to reach the low quantification limit. In addition, it had a relatively longer retention time (about 5.6 min).

Compared with HPLC, UPLC is recently developed technology and provides a higher peak capacity, greater resolution, increased sensitivity and high speed of analysis [12,13]. In this work, a fast new UPLC–MS/MS method was developed for determination of lovastatin in plasma.

* Corresponding author. Tel.: +86 731 5292121; fax: +86 731 4436720.
E-mail address: yuanhy1972@163.com (H. Li).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.12.005

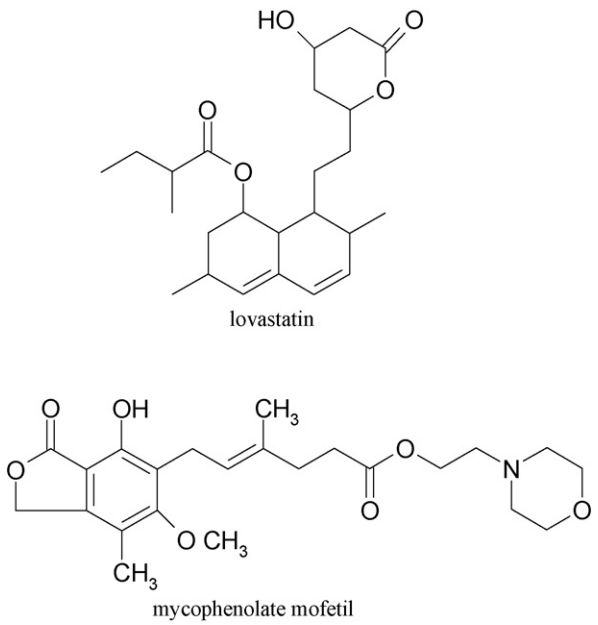

Fig. 1. Chemical structures of lovastatin and mycophenolate mofetil.

2. Experimental

2.1. Reagents and chemicals

Lovastatin (99.2% of purity) and mycophenolate mofetil (I.S., 99.9% of purity) (Fig. 1) were purchased from National institute for the control of pharmaceutical and biological products (Beijing, China). The primary stock solutions were prepared separately in methanol (56.3 ng/ml for lovastatin and 102.0 μg/ml for mycophenolate mofetil). Methanol and acetonitrile (HPLC grade) were purchased from Caledon Laboratories Ltd. (Georgetown, Canada). Water was purified by redistillation and filtered through 0.22 μm membrane filter before use.

2.2. Apparatus and operation conditions

2.2.1. Liquid chromatography

The chromatography was performed on ACQUITYTM UPLC system (Waters Corp., Milford, MA, USA) with cooling autosampler and column oven enabling temperature control of analytical column. An ACQUITY UPLCTM BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Corp.) was employed. The column temperature was maintained at 40 °C. The mobile phase contained 20% water and 80% acetonitrile (v/v). The flow rate was set at 0.35 ml/min. The auto-sampler was conditioned at 4 °C and the sample volume injected was 5.0 μl. The total run time was 3.5 min.

2.2.2. Mass spectrometry

Triple-quadrupole tandem mass spectrometric detection was carried out on a Micromass® Quattro microTM API mass spectrometer (Waters Corp.) with an electrospray ionization (ESI) interface. The ESI source was set in positive ionization mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 427.00 → 325.00 for lovastatin, *m/z* 434.00 → 113.67 for mycophenolate mofetil (I.S.), respectively, with scan time of 0.10 s per transition. The optimal MS parameters were as follows: capillary, 3.00 kV; cone, 50.00 V; extractor, 4.00 V; RF lens, 0.0 V; source temperature, 120 °C; desolvation temperature, 400 °C; cone gas flow, 46 l/h; desolvation gas flow, 745 l/h; LM 1 resolution, 15.0; HM 1 resolution, 13.0; ion energy 1, 0.5; entrance, 4; collision, 23; exit, 0.3; LM 2 resolution, 15.0; HM 2 resolution, 13.0; ion energy 2, 1.4; multiplier, 650 V. Nitrogen was used as the desolvation and cone gas. Argon was used as the collision gas at a pressure of approximately 2.61×10^{-3} mbar. The optimized collision energy of lovastatin and mycophenolate mofetil were 23.0 eV and 26.0 eV. All data collected in centroid mode were acquired and processed using MassLynxTM NT 4.1 software with QuanLynxTM program (Waters Corp.).

2.3. Preparation of standards and quality control samples

Standard stock solutions of lovastatin and mycophenolate mofetil were both prepared in methanol at the concentration of 56.3 ng/ml and 102.0 μg/ml, respectively. The internal standard working solution was diluted with *tert*-butyl methyl ether to 2.55 ng/ml. And the lovastatin solution was then serially diluted with methanol to provide working standard solutions of desired concentrations. All the solutions were stored at 4 °C.

Calibration standards were prepared by spiking 0.2 ml of blank human plasma with working standard solutions of lovastatin. The effective concentrations in standard plasma samples were 0.08, 0.16, 0.77, 1.50, 3.85, 13.50 and 24.50 ng/ml. One calibration curve was constructed on each analysis day using freshly prepared calibration standards. The quality control samples (QCs) were prepared with blank plasma at LLOQ, low, middle and high concentrations of 0.08, 0.16, 1.50 and 11.80 ng/ml. The standards and quality controls were extracted on each analysis day with the same procedure for plasma samples as described below.

2.4. Plasma sample preparation

0.2 ml plasma specimens were pipetted into 8.0 ml conical glass tubes and spiked with 1.6 ml internal standard working solution (2.55 ng/ml). After vortex mixed for 3.0 min, the mixture was centrifuged at 5000 × *g* for another 5 min. The upper organic layer was carefully transferred into a vacuum concentration equipment and evaporated. The dry residue was then reconstituted with 150 μl mobile phase and 5 μl solution was injected into the UPLC–MS/MS.

2.5. Method validation

Validation runs were conducted on 3 consecutive days. Each validation run consisted of a minimum of one set of calibration standards and five replicates of LLOQ and QC plasma samples at three concentrations. The results from LLOQ and QC plasma samples in three runs were used to evaluate the precision and accuracy of the method developed.

2.5.1. Selectivity

Selectivity was studied by comparing chromatograms of six different batches of blank plasma obtained from six subjects with those of corresponding standard plasma samples spiked with lovastatin and mycophenolate mofetil and plasma sample after oral doses of lovastatin tablets.

2.5.2. Linearity and lower limit of quantification (LLOQ)

Calibration curves were prepared by assaying standard plasma samples at seven concentrations of lovastatin ranging 0.08–24.50 ng/ml. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of lovastatin to mycophenolate mofetil (I.S.) versus the nominal concentration (x) of lovastatin. The calibration curves were constructed by weighted ($1/x$) least square linear regression. The lower limit of quantification is defined as the concentration which should be at least 5 times the response compared to blank response [14]. It was validated using an LLOQ sample for which an acceptable accuracy (RE) within ±20% and a precision (RSD) below 20% were obtained.

2.5.3. Precision and accuracy

For determining the intra-day accuracy and precision, a replicate analysis of QC plasma samples of lovastatin was performed on the same day. The run consisted of a calibration curve and five replicates of each LLOQ, low, mid, and high concentration quality control samples. The inter-day accuracy and precision were assessed by analysis of three batches on different days. The precision was expressed as the relative standard deviation (RSD) and the accuracy as the relative error (RE).

2.5.4. Extraction recovery and matrix effect

The extraction recovery of lovastatin was determined by calculating the peak areas obtained from blank plasma samples spiked with analyte before extraction with those from blank plasma samples, to which analyte was added after extraction. According to the guidance of USFDA [15], recovery experiments should be performed at three concentrations (low, medium, and high). So this procedure was repeated for five replicates at three concentrations of 0.16, 1.50 and 11.80 ng/ml.

In order to evaluate the matrix effect on the ionization of analyte, i.e. the potential ion suppression or enhancement due to the matrix components. Lovastatin at three concentration levels were added to the extract of 0.2 ml of blank plasma, evaporated and reconstituted with 150 μl of mobile phase, the corresponding peak areas (A) were compared with those of the lovastatin standard solutions evaporated directly and reconstituted with the same mobile phase (B). The ratio ($A/B \times 100$)% was used to evaluate the matrix effect. The matrix effect of internal standard was also evaluated using the same method.

2.5.5. Stability [16]

2.5.5.1. Freeze and thaw stability. The effect of freeze and thaw cycles on the stability of plasma samples containing lovastatin was determined by subjecting five aliquots of QC samples at low, middle and high concentration unextracted quality control samples to four freeze–thaw cycles. After completion of every cycle, the samples were analyzed and the experimental concentrations were compared with the nominal values. The accuracy values of three concentrations in four freeze–thaw cycles were calculated.

2.5.5.2. Long-term stability. Five aliquots of QC samples at low, mid and high concentration unextracted QC samples were stored at −70 °C for 30 days. Then, the samples were processed and analyzed and the concentrations obtained were compared with the nominal values.

2.5.5.3. Short-term stability. Five aliquots of QC samples at low, mid and high concentration unextracted QC samples were kept at ambient temperature (25 °C) for 12 h in order to determine the short-term stability of lovastatin in human plasma. Then, the samples were processed and analyzed and the concentrations obtained were compared with the nominal values.

2.5.5.4. Post-preparation stability. In order to estimate the stability of lovastatin in the prepared sample, five aliquots of QC samples at low, mid and high concentration were kept in an autosampler maintained at 4 °C for about 4 h. Then, the samples were analyzed and the concentrations obtained were compared with the nominal values.

2.5.5.5. Stock solution stability. To test the stock solution stability of lovastatin and the I.S., five aliquots of stock standard (56.3 ng/ml for lovastatin) and the I.S. (102.0 μg/ml for mycophenolate mofetil) solution were left at 4 °C for 30 days. Then, the concentrations were analyzed and compared with the fresh stock solution.

2.6. Application to pharmacokinetic study

The method was applied to determine the plasma concentrations of lovastatin from a clinical trial in which 18 healthy male volunteers received a lovastatin tablet 80 mg oral (containing 20 mg lovastatin each). The pharmacokinetic study was approved by the Ethical Committee of XiangYa Second Hospital of Central South University and all volunteers gave their signed informed consent to participate in the study according to the principles of the Declaration of Helsinki. Blood samples were collected before and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10 and 12 h post-dosing. Samples were promptly centrifuged and plasma was separated and stored at −70 °C until analyzed.

The maximum plasma concentration (C_{max}) and their time were noted directly. 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. Optimization of the chromatographic separation and MS/MS working conditions

The separation and ionization of lovastatin and mycoplenolate mofetil were affected by the composition of mobile phase. Therefore, the selection of mobile phase components was critical. In experiment, different ratio (50:50, 40:60, 30:70 and 20:80) of water/acetonitrile was used to mobile phase and 20% water and 80% acetonitrile (v/v) in mobile phase was believed suitable in view of retention time and peak shape of drug. Ammonium acetate was employed to supply the ionic strength. It was found that a mixture of 10–30 mM ammonium acetate buffer–water/acetonitrile could not preferably improve peak shape and was finally not adopted as the mobile phase.

The selection of MRM transitions and associated acquisition parameters (collision energy and cone voltage) were evaluated for best response under positive mode ESI conditions by infusing a standard solution, via a syringe pump, into the mobile phase.

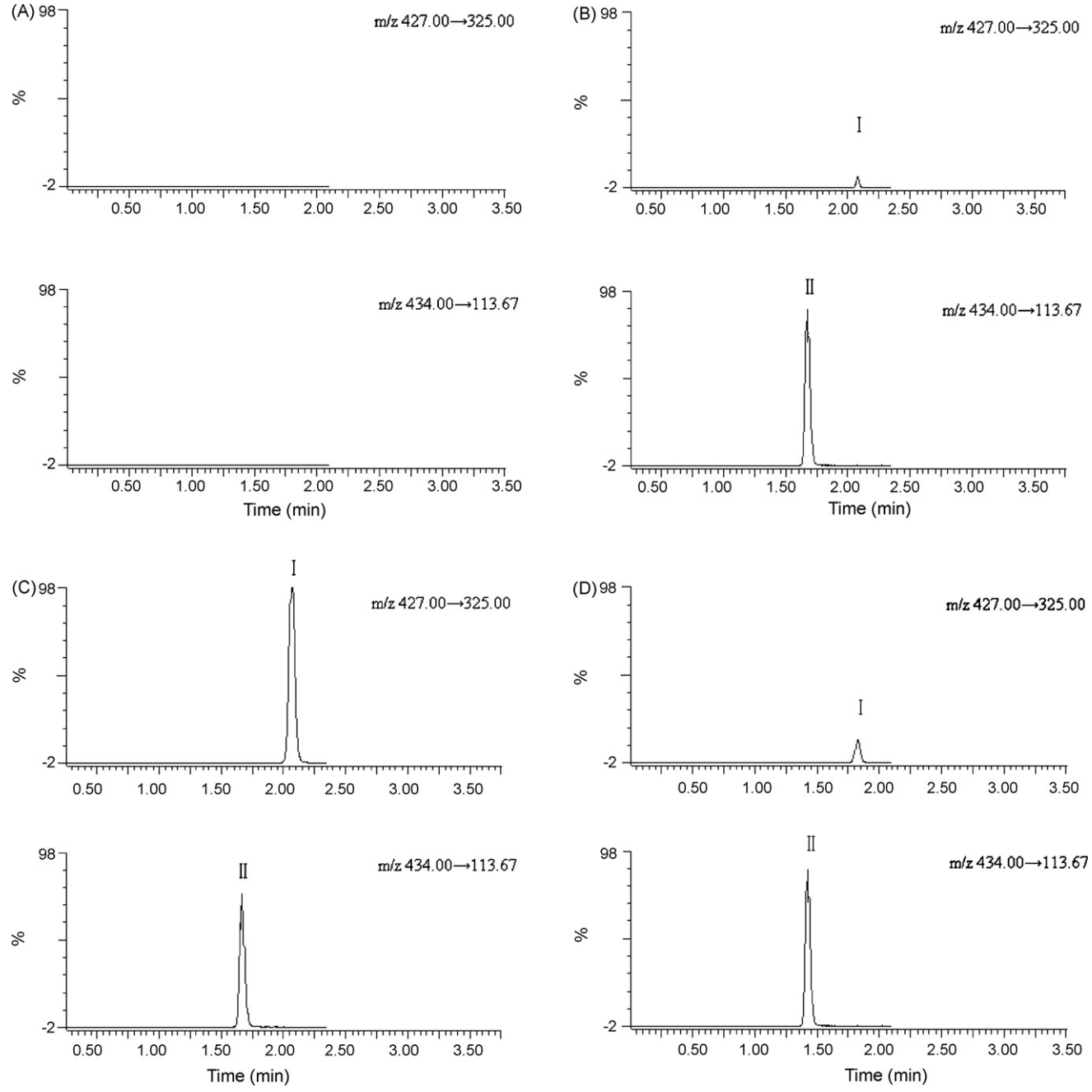

Fig. 2. Representative MRM chromatograms for lovastatin (peak I) and mycophenolate mofetil (peak II, I.S.) from (A) a blank plasma sample; (B) a blank plasma sample spiked with lovastatin at the LLOQ of 0.08 ng/ml and I.S. standards (2.55 ng/ml); (C) a blank plasma sample spiked with lovastatin at 13.5 ng/ml and I.S. standards (2.55 ng/ml); (D) a plasma sample from a volunteer 1.5 h after oral administration of lovastatin (80 mg). The retention times of lovastatin and I.S. were 1.82 and 1.43 min, respectively.

The very narrow chromatographic peaks with a peak width about 5 s, produced by UPLC™ indicated an increase in the chromatographic efficiency which produced a fast separation. Both lovastatin and mycoplenolate mofetil were rapidly eluted with retention times less than 2.0 min (see Fig. 2). The analysis time for lovastatin in the literatures [7–10] which used HPLC–MS and HPLC–MS/MS were about 5 min. The short analysis time may meet the requirement for high sample throughput in bioanalysis.

3.2. Selection of IS

The best internal standard in LC–MS assay is a deuterated form of the analyte. In our laboratory, no deuterated lovastatin was available, therefore, a compound being structurally or chemically similar to the analyte was considered. In LC–MS/MS the I.S. should also have similar chromatographic and mass spectrometric behaviours to the analyte, and mimic the analyte in any sample preparation steps. Firstly, simvastatin was chosen as the internal standard for the assay because of its similarity of structure, retention time and ionization to lovastatin. However, in our laboratory we have not simvastatin control substance. After checking the standards library in our laboratory, mycophenolate mofetil was chosen I.S. Mycophenolate mofetil is a immunosuppressive agent and is applied to resist rejection in organ transplantation. The results showed it was suitable in retention time and ionization of lovastatin.

3.3. Selection of extraction method

As lovastatin is a lipophilic compound, liquid–liquid extraction was applied to extract the analyte. Several extraction solvents such as ethyl acetate, diethyl ether, *N*-hexane, hexane hexamethylene-dichloromethane and *tert*-butyl methyl ether were investigated, and it was found that *tert*-butyl methyl ether extracted the analyte more efficiently. Moreover, the I.S. mycoplenolate mofetil solution which was dissolved in *tert*-butyl methyl ether was used extraction liquid. This extraction method is more convenient than the reported method [7–11].

3.4. Method validation

3.4.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. As shown in Fig. 2, no interference from endogenous substance was observed at the retention time of lovastatin and mycophenolate mofetil.

3.4.2. Linearity and LLOQ

The standard calibration curves for lovastatin were linear over the concentration range of 0.08–24.50 ng/ml ($r^2 > 0.99$) by using weighted least square linear regression analysis with a weigh factor of $1/x$. Atypical equation for the calibration curves was: $y = 1.68 \times 10^{-1}x - 4.16 \times 10^{-3}$, $r = 0.996$.

The lower limit of quantification (LLOQ) for lovastatin was 0.08 ng/ml (S/N ≥ 5) with 5 μl injected into the UPLC column with precision and accuracy presented in Table 1 with RE within ±20% and RSD lower than 20%. Compared with the previous method regarding the determination of lovastatin in human plasma, the present method gave a higher sensitivity with an LLOQ of 0.08 ng/ml. The high sensitivity could be attributed to the extra resolution and peak sharpness produced by the UPLC chromatographic system and the improved ionization efficiency under the mass spectrometric conditions.

Table 1
Precision and accuracy for the determination of lovastatin in human plasma (intra-day: $n = 5$; inter-day: $n = 5$ series per day, 3 days)

Added *C* (ng/ml)	Found *C* (ng/ml)	Intra-run RSD (%)	Inter-run RSD (%)	Accuracy RE (%)
0.08 (LLOQ)	0.08 ± 0.05	5.4	4.3	9.3
0.16 (Low)	0.17 ± 0.01	5.2	13.5	8.4
1.50 (Middle)	1.38 ± 0.08	5.9	12.6	−7.6
11.80 (High)	10.95 ± 0.80	7.3	11.6	−7.2

3.4.3. Precision and accuracy

The data of intra-day and inter-day precision and accuracy for the method are listed in Table 1. The intra-day precision for low, mid and high QC levels of lovastatin were 5.2, 5.9 and 7.3%, respectively, and that of inter-day analysis were 13.5, 12.6 and 11.6%, respectively, with an accuracy (RE) within −7.6 to 8.4%. The precision and accuracy of the present method conform to the criteria for the analysis of biological samples according to the guidance of USFDA [15] where the precision (RSD) determined at each concentration level is required not exceeding 15%.

3.4.4. Extraction recovery and matrix effect

The extraction recoveries of lovastatin from human plasma were 85.8 ± 4.9, 86.0 ± 2.9, and 87.5 ± 4.8% at concentration levels of 0.16, 1.50 and 11.80 ng/ml, respectively, and the mean extraction recovery of mycophenolate mofetil was 91.5 ± 2.6%.

In terms of matrix effect, all the ratios $(A/B \times 100)$% defined as in Section 2 were between 85 and 115%, which means no matrix effect for lovastatin and mycophenolate mofetil in this method.

3.4.5. Stability

The stock solution of lovastatin in plasma were found to be stable at room temperature for 12 h, at 4 °C for 4 h, at the −70 °C for 30 days, at freeze and thaw stability (Table 2). The stock solutions were stable for at least 1 month. The difference values between the fresh samples and the test solution in stock solution

Table 2
Stability of lovastatin in human plasma at three QC levels ($n = 5$)

Stability	Accuracy (mean ± RSD) (%)		
	0.16 (ng/ml)	1.50 (ng/ml)	11.80 (ng/ml)
Short-term stability	98.2 ± 5.3	100.2 ± 8.8	100.3 ± 3.0
Long-term stability	100.1 ± 7.0	98.6 ± 4.3	99.6 ± 7.9
Freeze–thaw stability	99.9 ± 9.1	99.9 ± 5.6	101.2 ± 4.8
Post-preparation stability	98.4 ± 8.4	99.0 ± 7.3	99.4 ± 6.2

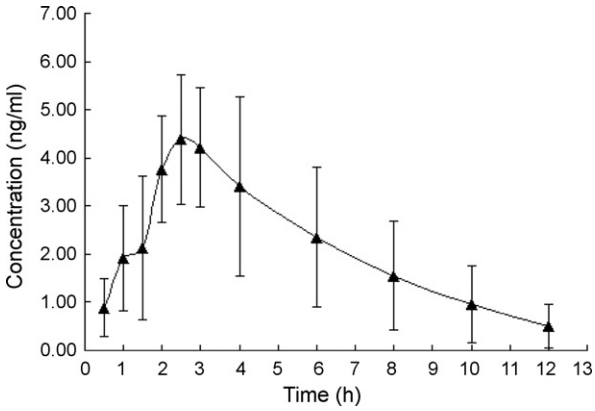

Fig. 3. Mean plasma concentration–time curve of lovastatin in 18 volunteers after a single oral dose (80 mg) of lovastatin.

stability were <5% for lovastatin and mycophenolate mofetil, respectively.

The results from all stability tests presented in demonstrated a good stability of lovastatin over all steps of the determination.

3.5. Pharmacokinetic application

The present method was successfully applied to the pharmacokinetic study of lovastatin after oral administration in healthy male volunteers. Mean plasma concentration–time curve of lovastatin in single dose study is shown in Fig. 3.

After administration of a single dose of 80 mg lovastatin, the C_{max} and T_{max} were 5.8 ± 5.0 ng/ml and 2.9 ± 1.0 h, respectively. Plasma concentration declined with the $t_{1/2}$ of 2.7 ± 1.2 h. The AUC_{0-12} and $AUC_{0-\infty}$ values obtained were 25.0 ± 19.5 and 25.8 ± 20.0 ng h/ml, respectively. In this experiment, the evident individual differences of pharmacokinetics were observed. Therefore, lovastatin treatment must be individuation in clinical application.

4. Conclusion

A sensitive, selective and rapid UPLC–ESI-MS/MS method for the determination of lovastatin in human plasma is described. Compared with the published methods, the sharp peaks produced by UPLC are particular advantage when coupled to electrospray mass spectrometry, reducing ion suppression and offering superior sensitivity with an LLOQ of 0.08 ng/ml, satisfactory selectivity and short run time of 2.0 min. The method has been successfully applied to a pharmacokinetic study of lovastatin given in tablet form to healthy volunteers.

References

[1] R.H. Bradford, C.L. Shear, A.N. Chremos, Arch. Intern. Med. 151 (1991) 43–49.
[2] J.A. Tobert, Am. J. Cardiol. 62 (1988) 28J–34J.
[3] B.K. Tang, W. Kalow, Eur. J. Clin. Pharmacol. 7 (1995) 449–451.
[4] H. Lennernas, G. Fager, Clin. Pharmacokinet. 32 (1997) 403–425.
[5] M.J. Morris, J.D. Gilbert, J.Y. Hsieh, B.K. Matuszewski, H.G. Ramjit, W.F. Bayne, Biol. Mass Spectrom. 22 (1993) 1–8.
[6] W.H. Zeng, J.H. Cai, Y.L. Wu, Fenxi Ceshi Xuebao 19 (2000) 69–73.
[7] Y. Wu, J. Zhao, J. Henion, W.A. Korfmacher, A.P. Lapiguera, C.C. Lin, J. Mass Spectrom. 32 (1997) 379–387.
[8] H. Xiao, H. Shen, J.F. Chen, D.W. Xiao, J. Chin. Pharm. Sci. 15 (2006) 233–236.
[9] J.T. Strode, L.T. Taylor, A.L. Howard, D. Ip, J. Pharm. Biomed. Anal. 20 (1999) 137–143.
[10] L.Y. Ye, P.S. Firby, M.J. Moore, Ther. Drug Monit. 22 (2000) 737–741.
[11] M. Li, L.Y. Fan, W. Zhang, C.X. Cao, Anal. Bioanal. Chem. 387 (2007) 2719–2725.
[12] C.C. Leandro, P. Hancock, R.J. Fussell, B.J. Keely, J. Chromatogr. A 1103 (2006) 94–101.
[13] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, J. Chromatogr. B 825 (2005) 134–142.
[14] International Conference on Harmonization, Draft Guideline on Validation Procedures Definitions and Terminology, Federal Register, vol. 60, IFPMA, Switzerland, 1995, pp. 11260−11266.
[15] USFDA, 2001, http://www.fda.gov/cder/guidance/4252fnl.htm.
[16] SFDA, 2005, http://www.sda.gov.cn/gsz05106/08.pdf.