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Quantitative determination of pravastatin and its metabolite 3 α -hydroxy pravastatin in plasma and urine of pregnant patients by LC-MS/MS

Xing Zhang, Daria I. Vernikovskaya, Xiaoming Wang, Tatiana N. Nanovskaya, Maged Costantine, Gary D.V. Hankins, and Mahmoud S. Ahmed*

Abstract

This report describes the development and validation of a chromatography/tandem mass spectrometry method for the quantitative determination of pravastatin and its metabolite (3 α -hydroxy pravastatin) in plasma and urine of pregnant patients under treatment with pravastatin, as part of a clinical trial. The method includes a one-step sample preparation by liquid-liquid extraction. The extraction recovery of the analytes ranged between 93.8% and 99.5% in plasma. The lower limits of quantitation (LLOQ) of the analytes in plasma samples were 0.106 ng/mL for pravastatin and 0.105 ng/mL for 3 α -hydroxy pravastatin; while in urine samples they were 19.7 ng/mL for pravastatin and 2.00 ng/mL for 3 α -hydroxy pravastatin. The relative deviation of this method was < 10 % for intra-day and inter-day assays in plasma and urine samples, and the accuracy ranged between 97.2% and 106% in plasma, and 98.2% to 105% in urine. The method described in this report was successfully utilized for determining the pharmacokinetics (PK) of pravastatin in pregnant patients enrolled in a pilot clinical trial for prevention of preeclampsia.

Keywords

Pravastatin; 3 α -hydroxy pravastatin; metabolite; preeclampsia; LC-MS/MS

Introduction

Pravastatin is one of the “statins” which are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the key enzyme in the biosynthesis of cholesterol (Schachter, 2004; Hulskotte *et al.*, 2013). Pravastatin is widely used in the treatment of hyperlipidemia and for the prevention of adult atherosclerotic cardiovascular disease (Costenbader *et al.*, 2007). Currently, an ongoing pilot clinical trial is investigating the efficacy, safety and potential use of pravastatin for prevention of preeclampsia, a serious pregnancy condition with associated maternal and neonatal morbidities and mortality. The PK of pravastatin has been previously determined in men and non-pregnant women, both healthy volunteers and patients (Polagani *et al.*, 2012; Zhu *et al.*, 2003; Kawabata *et al.*, 2005; Sigurbjornsson *et al.*, 1998; Hatanaka 2000), and to the best of our knowledge, there are no reports on its PK in pregnant patients. When compared with other HMG-CoA

*Correspondence to: Mahmoud S. Ahmed, Maternal-Fetal Pharmacology and Bio-development Laboratories, Department of Obstetrics & Gynecology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0587, U.S.A. maahmed@utmb.edu.

reductase inhibitors, pravastatin demonstrates unique PK properties with low absorption and bioavailability, fast absorption rate that limits its elimination, and a relatively low plasma protein binding (Hatanaka 2000). Since pregnancy is associated with physiological changes that alter the PK of medications including clearance and plasma levels (Frederiksen 2001; Unadkat *et al.*, 2007), it became necessary to develop a sensitive and accurate quantitative method for determining the concentration of pravastatin in plasma and urine obtained from pregnant patients in order to determine its PK during pregnancy.

Investigations of the *in vivo* metabolism of pravastatin revealed that 3 α -hydroxy pravastatin was the major metabolite identified in human plasma, urine and feces (Hatanaka 2000) with 23.7% of the oral dose being in plasma and 10% in urine (Everett *et al.*, 1991). In addition, in patients receiving pravastatin for treatment of moderate primary hypercholesterolemia, those with the ratio of the area under the serum concentration-time curve (AUC) of pravastatin to 3 α -hydroxy pravastatin being < 1.6 were associated with an apparent lower reduction of total and low-density lipoprotein cholesterol than the patients with the ratio > 1.6 (Ito 1998).

There are analytical methods for determining the concentrations of pravastatin alone, and in combination with its metabolite 3 α -hydroxy pravastatin in human plasma and urine. Briefly, these methods include the following: 1) two HPLC-UV methods for determining pravastatin concentration with an LLOQ that ranged between 1.9 and 200 ng/mL in plasma, and 125 and 2000 ng/mL in urine (Bauer *et al.*, 2005, Campos-Lara *et al.*, 2008). 2) several LC-MS/MS methods with LLOQ of pravastatin in plasma between 0.25 and 0.625 ng/mL (Polagani *et al.*, 2012; Zhu *et al.*, 2003; Mulvana *et al.*, 2000; Jemal *et al.*, 1998; Kawabata *et al.*, 1998). The above mentioned methods had low sensitivity and were not adequate for determining the PK of pravastatin in plasma and urine of pregnant patients. Recently, one LC/MS method had a lower LLOQ of 0.1 ng/mL, but the plasma volume required was 1 mL (Kawabata *et al.*, 2005). Many of the extraction procedures of pravastatin or its metabolite in these reports were carried out by SPE methods (Zhu *et al.*, 2003; Kawabata *et al.*, 2005; Bauer *et al.*, 2005; Mulvana *et al.*, 2000; Kawabata *et al.*, 1998). Although SPE is one of the most commonly used techniques to prepare biological samples, it is more expensive than liquid-liquid extraction. In addition, pravastatin is readily transformed to its isomers and lactonized compounds under aqueous acidic conditions (Hatanaka 2000; Vlckova *et al.*, 2012), a fact that was not addressed in several of the above mentioned methods.

Therefore, the aim of this investigation was to circumvent the above pitfall and, develop and validate a sensitive and rapid LC-MS/MS method for determining the concentrations of pravastatin and its metabolite, 3 α -hydroxy pravastatin in plasma and urine of pregnant patients under treatment with pravastatin. This method is necessary for determining the PK of pravastatin in the ongoing pilot clinical trial conducted by the obstetric-fetal pharmacology research units (OPRU) network sponsored by NICHD and under an IND from the FDA.

Experimental

Chemicals and reagents

Pravastatin sodium, 3 α -hydroxy pravastatin sodium and pravastatin-d₃ sodium were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Acetonitrile, methanol, ammonium acetate, ethyl acetate, formic acid and acetic acid were of LC/MS grade and purchased from Fisher Scientific (Pittsburgh, USA). Blank human plasma was purchased from Innovative Research (Peary Court, USA) and human urine was collected from 6 healthy volunteers.

Preparation of the standards, calibration curves and quality control (QC) samples for plasma analysis

Pravastatin-d₃ was chosen as the internal standard (IS) for the quantification of pravastatin and 3 α -hydroxy pravastatin. The IS stock solution was prepared by dissolving the standard in 30% (v/v) acetonitrile (final conc. 100 ng/mL). A stock solution of the two analytes was prepared in 30% acetonitrile in the following concentrations: pravastatin, 3.71 μ g/mL and 3 α -hydroxy pravastatin, 3.69 μ g/mL. Dilutions of the stock solutions with 30% acetonitrile were used to prepare the working standard solutions. Calibration standards were prepared by spiking 10 μ L of the working standard into 500 μ L of blank human plasma to yield the following ranges of concentrations: pravastatin, 0.106–74.2 ng/mL and 3 α -hydroxy pravastatin, 0.105–73.8 ng/mL. The QC samples for low, medium and high concentrations were prepared as described for calibration curves and were as follows: pravastatin, 0.265, 21.2 and 74.2 ng/mL and 3 α -hydroxy pravastatin, 0.264, 21.1 and 73.8 ng/mL. All standard solutions were stored at 4 °C.

Preparation of the standards, calibration curves and quality control (QC) samples for urine analysis

The IS pravastatin-d₃ stock solution was prepared in 30% acetonitrile (50.0 ng/mL). The stock solutions of the two analytes standards in 30% acetonitrile were prepared in the following concentrations: pravastatin, 19.7 μ g/mL and 3 α -hydroxy pravastatin, 2.00 μ g/mL. The working standard solutions were prepared in 30% acetonitrile by serial dilutions of the stock to the desired concentrations. Calibration standards were prepared by spiking 5 μ L of the working standards solution into 40 μ L of blank human urine to yield the following concentration ranges: pravastatin, 19.7–2.46 $\times 10^3$ ng/mL and 3 α -hydroxy pravastatin, 2.00–250 ng/mL. The QC samples of low, medium and high concentrations were prepared as described above and were as follows: pravastatin, 39.4, 984 and 2.46 $\times 10^3$ ng/mL and 3 α -hydroxy pravastatin, 4.00, 100 and 250 ng/mL. All standard solutions were stored at 4 °C.

Sample preparation

Preparation of plasma samples—Plasma samples were prepared by liquid-liquid extraction method as follows: IS solution (10 μ L, 100 ng/ml) was added to 500 μ L of calibration standards samples or patient plasma samples, vortexed for 1 min followed by the addition of 10 μ L of 10% (v/v) formic acid. The solution was vortexed again for 1 min followed by the addition of 1.0 mL ethyl acetate, vigorous shaking for 3 min and

centrifugation at $12,000 \times g$ for 10 min at 4 °C. The extraction procedure was repeated once more. The organic layers were combined and dried under a stream of nitrogen in an analytical evaporator (ZipVap®, 109A 11-80245, Glas-Col, USA). The residue was dissolved in 120 μ L of aqueous ammonium acetate buffer (10 mM containing 0.05% acetic acid, pH4.5)–acetonitrile solution (68:32, v/v) and vortexed. Aliquots of the samples (70 μ L) were injected into the LC-MS for analysis of pravastatin and 3 α -hydroxy pravastatin.

Preparation of urine samples—Urine samples were prepared by dilution as follows: IS solution (5 μ L, 400 ng/ml) was added to 40 μ L of either calibration standards or patient urine samples. The solution was then diluted with 100 μ L of aqueous ammonium acetate buffer (10 mM containing 0.05% acetic acid, pH4.5)–acetonitrile solution (68:32, v/v) and vortexed. Aliquots of the samples (50 μ L) were injected into the LC-MS for analysis of the two analytes (pravastatin and 3 α -hydroxy pravastatin).

HPLC-MS/MS conditions

Pravastatin and 3 α -hydroxy pravastatin in human plasma and urine samples were quantified using an Agilent HPLC 1200 series system coupled with an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The HPLC system consisted of a degasser, binary pump delivery system, and Hip-ALS auto-sampler controlled by Analyst™ 1.5 Software (MDS Inc. and Applera Corporation, USA). The separation of the analytes was achieved using a Waters Symmetry column (150 mm $\times$ 4.6 mm, 5 μ m) preceded by a Phenomenex C₁₈ guard column (4 mm $\times$ 3.0 mm). The mobile phase consisted of an aqueous ammonium acetate buffer (A, 10 mM containing 0.05% acetic acid v/v, pH4.5) and acetonitrile (B). The flow rate was 1.0 mL/min. The analytes were eluted isocratically by 32% B for 6 min following by increasing phase B to 90% and elution continued for 7 min to remove residual phospholipids from HPLC. Finally, the HPLC column was re-equilibrated with 32% B for 7 min before the following run. The eluent was introduced into ESI source using a post-column splitting ratio of 1:1 (retained/waste).

The MS parameters for all the analytes were set as follows: ion spray voltage, –4500 V; curtain gas, 20 L/h; ion source gas1, 50 L/h; ion source gas2, 50 L/h; source temperature, 500 °C and collision gas, 12 L/h. The multiple reaction monitoring (MRM) transitions were monitored at m/z 423.4→320.9 for pravastatin and 3 α -hydroxy pravastatin, and m/z 426.0→321.0 for IS. The compound-dependent parameters for MRM transitions were: declustering potential, –70 V; entrance potential, –8 V; and collision cell exit potential, –7 V for the analytes and IS. Collision energy was set at –20 V for pravastatin and 3 α -hydroxy pravastatin and –23 V for IS.

Method validation

The method was validated for specificity, matrix effects, linearity, sensitivity, accuracy, precision, recovery, and stability according to the U.S. Food and Drug Administration Guidelines for Industry (US Food and Drug Administration, 2013).

The selectivity was assessed by preparing and analyzing six samples for each of the blank plasma and urine to determine the potential of interfering compounds that could co-elute

with the analytes or IS. The MRM chromatograms of the blank human plasma and urine samples were compared with the corresponding spiked samples at LLOQ levels.

The matrix effect of the analytes and IS was evaluated by comparing the analyte peak areas of the post-extracted samples to those of the standards. The matrix factor of each analyte was determined at three QC concentrations in six individual blank human plasma and urine samples. The efficiency for extraction of the analytes from human plasma and urine was determined by comparing the peak areas of the analytes in QC samples with the spiked samples, following their extraction, at three QC concentrations in six replicates.

For the calibration curves, the ratios of peak area of each analyte to that of IS in plasma and urine were plotted against eight corresponding concentrations using a weighted linear least-squares regression model. The limit of quantification was determined by measuring the signal-to-noise ratio (S/N) for each analyte and was 10:1 for LLOQ. In addition, the analyte peak (response) at LLOQ concentration was identifiable, discrete, and reproducible with an accuracy within $\pm 20\%$ and a precision variation below 20%.

Precision and accuracy were determined by analyzing five sets of spiked plasma and urine QC samples at LLOQ, low, medium, and high concentrations on the same day and over three days according to the following criteria: the precision determined at each concentration did not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it did not exceed 20% of the CV. Accuracy of the method was evaluated by comparing the calculated concentration using calibration curves, with the added concentration. The mean value of five determinations per concentration was within 15% of the actual value except for LLOQ which did not deviate by more than 20%.

The stability of the analytes in plasma and urine was investigated at low and high concentrations as follows: 1) Short-term stability (4 hours); three aliquots of each of the two concentrations were thawed at room temperature (22–25 °C), kept for 4 h then analyzed. 2) Freeze-thaw stability was determined following three freeze-thaw cycles (–70 °C to room temperature is defined as one cycle). 3) The long-term stability (30 days) was determined after three aliquots of each of the two concentrations were kept at –70 °C for 30 days. The analytes were considered stable if the RSD for each concentration did not exceed 15%, and the accuracy did not deviate by more than 15% of the nominal concentration (US Food and Drug Administration, 2013).

Results and discussion

Optimization of mass spectrometric conditions

The mass spectrometric parameters of pravastatin and 3 α -hydroxy pravastatin were optimized by direct infusion of their neat solutions in the initial mobile phase under both positive and negative ion modes of ESI. In the full scan mass spectra, the predominant ions of the two analytes observed were $[M-H]^-$ in negative mode and $[M + Na]^+$ in positive mode. The sodium adduct ion in positive mode provided a slightly better response than the deprotonated ion in negative mode. However, $[M-H]^-$ ion provided more reproducible results during the experiment using MRM quantification. Therefore, the $[M-H]^-$ ions of

pravastatin and 3 α -hydroxy pravastatin at m/z 423 were further used as precursor ion (Polagani *et al.*, 2012; Jemal *et al.*, 1998). The spectra of the product ions collected in the MS² experiment for the [M-H]⁻ at m/z 423 yielded an ion at m/z 321 from the two analytes and was the prominent peak of the product ion mass spectra (Fig. 1). Similar to the two analytes, the MRM transitions of pravastatin-d₃ (IS) was chosen as m/z 426→321 (Fig. 1).

Optimization of chromatographic conditions

The factors that could influence the chromatographic separation of pravastatin and 3 α -hydroxy pravastatin were investigated and included the type of HPLC column and composition of the mobile phase. The following HPLC columns were tested: Waters Symmetry C18, Phenomenex Luna C18 and Agilent Eclipse XDB C18. Waters Symmetry C18 column gave a better peak shape and response. The composition of the mobile phase was optimized on basis of the physico-chemical properties of the analytes and their response in LC/MS analysis. Accordingly, the addition of ammonium acetate (5 mM and 10 mM), ammonium hydrogen carbonate (5 mM and 10 mM), and ammonium hydroxide (0.1%) were tested. The results revealed that the type of buffer did not have a significant effect on the resolution of the analytes, but affected their ESI response. The best ESI response for the two analytes was achieved by using ammonium hydroxide, followed by ammonium acetate. However, ammonium hydroxide was not used because of the low reproducibility of the peak area of the analytes and the lack of linearity. For the organic solvent, acetonitrile was used instead of methanol because it resulted in better retention time and resolution of the two analytes. Reports on the lack of pravastatin stability under aqueous acidic conditions (pH<3.0) and its transformation to isomers and lactonized compounds (Hatanaka 2000; Vlckova *et al.*, 2012) led us to test the most favorable pH value of the aqueous buffer. Our data revealed that adjusting the pH of the aqueous phase to approximately 4.5, using 0.05% acetic acid, resulted in a stable pravastatin and its metabolite in the reconstituted samples at room temperature in the duration of analytical run (approx. 12 h).

Therefore, the Waters Symmetry C18 column was used to separate pravastatin and 3 α -hydroxy pravastatin with an acceptable resolution and ESI response. Sample stability was achieved by using a mobile phase composed of an aqueous ammonium acetate buffer (10 mM, 0.05% acetic acid) and acetonitrile (68:32, v/v).

Sample preparation

In previous reports, the extraction of pravastatin and 3 α -hydroxy pravastatin from biological matrices was frequently achieved by either SPE methods (Zhu *et al.*, 2003; Kawabata *et al.*, 2005; Bauer *et al.*, 2005; Mulvana *et al.*, 2000; Kawabata *et al.*, 1998) or liquid-liquid extraction (Polagani *et al.*, 2012). In this investigation, liquid-liquid extraction (LLE) and protein precipitation (PP) were tested because they are simpler and more cost effective. Moreover, LLE increased the extraction efficiency because pravastatin is an acidic compound (pK_a4.7) and the acidic extraction promotes protonation of the analytes. However, the stability of pravastatin and its metabolite is an important factor and should be considered for both the LLE and PP methods. Accordingly, the following types of extraction solvents and deproteinization agents were tested: Chloroform (0.1% formic acid), ethyl acetate (0.1% formic acid), and chloroform-isopropanol (5:0.2, v/v, 0.1% formic acid) for

LLE; and acetone, acetonitrile, acetone (0.1% formic acid) and acetonitrile (0.1% formic acid) for PP. Good extraction efficiency and sample stability were achieved by using LLE with ethyl acetate (0.1% formic acid) and PP with acetonitrile. However, the PP method resulted in a relatively high background in mass detection. Therefore, ethyl acetate (0.1% formic acid) was chosen for prepare plasma samples because it provided a clean extract and minimized matrix effect. In addition, one time extraction recovery of pravastatin and 3 α -hydroxy pravastatin with ethyl acetate (0.1% formic acid) were 78.0% and 88.7%, while the extraction recovery can be increased to 93.8% and 99.5% after second time extraction. Therefore, two times extraction procedure was adopted for plasma sample preparation. Urine is a relatively simpler matrix than plasma, and direct dilution with initial mobile phase was applied before LC-MS/MS detection.

Method validation

Selectivity

The typical MRM chromatograms of plasma blank samples and urine as well as those spiked with the two analytes at low concentrations are shown in Fig. 2 and Fig. 3. No significant interference was detected in blank samples from endogenous substances with the analytes and IS.

Matrix effects and recovery

The matrix factor of analytes and IS were evaluated in six blank/control human plasma and urine samples at low, medium, and high concentrations.

The matrix factor for pravastatin and 3 α -hydroxy pravastatin ranged between 84% and 104%, with < 13% relative standard deviation (Table 1). These results indicated that the ion suppression or enhancement of the analytes in the plasma matrix was negligible. The matrix factor for urine samples was also acceptable and in agreement with the FDA guidelines.

The extraction recovery of pravastatin and 3 α -hydroxy pravastatin from plasma ranged between 94% and 99%, with a variation of < 14%, at low, medium, and high concentrations (Table 1). The extraction recovery was consistent and reproducible. Therefore, the liquid-liquid extraction procedure of the analytes used in this analytical method was simple and efficient.

Linearity and lower limit of quantification (LLOQ)

Calibration curves for the plasma and urine samples were constructed by the internal standard method and fit by weighted least-squares linear regression analysis (1/x for plasma, and 1/y for urine) of the peak area ratio of each analyte to IS. The calibration curve in the tested range exhibited good linear regressions ($r^2 > 0.99$). The LLOQ for pravastatin in plasma was 0.106 ng/mL, and for 3 α -hydroxy pravastatin 0.105 ng/mL, while in urine, they were 19.7 ng/mL for pravastatin, and 2.0 μ g/mL for 3 α -hydroxy pravastatin. At LLOQ concentrations of pravastatin and 3 α -hydroxy pravastatin in plasma and urine, the relative deviation was less than 17% and 12% for intra-day and inter-day, and the accuracy ranged between 91 and 101% for plasma, and 93% to 98% for urine (Table 2)

Precision and accuracy

Precision and accuracy were validated at LLOQ, low, medium, and high concentrations of the analytes (Table 2). The intra-day accuracy for pravastatin and 3 α -hydroxy pravastatin in samples of plasma ranged between 97.2% and 104% with precision < 10%, and the inter-day accuracy ranged between 98.8% and 106% with precision < 7%, while in urine, the intra-day accuracy for the analytes ranged between 98.2% and 105% with precision < 6%, and the inter-day accuracy ranged between 99.2% and 104% with precision < 2%. All of the values were within acceptable range, indicating that the method was accurate, precise, and reproducible.

Stability

The stability of the analytes under the following conditions of storage and processing was determined namely: short- and long-term storage as well as freeze-thaw cycles. Short-term stability was determined by “storing” for 4 h at room temperature (22–25 °C). The accuracy for the concentrations of the analytes in plasma ranged between 93.1% and 96.9% with precision of < 6% at the two QC levels (low and high). In urine, the accuracy ranged between 98.0% and 100% with precision of < 6%. These results indicate that the analytes in plasma and urine samples were stable for at least 4 h at room temperature (Table 3). The effect of three freeze-thaw cycles of the analytes in plasma and urine samples was determined and revealed the following: The stability of the analytes in plasma ranged between 93.3% and 97.6% with precision of < 4%, while for urine samples, they ranged between 94.3% and 105% with precision of < 10%. These data indicated that the analytes were stable under these conditions (Table 3). Moreover, the analytes in human plasma and urine samples were stable following their storage at –70°C for 30 days (Table 3).

Method application

The methods cited in this report were validated and used to determine the concentrations of pravastatin and 3 α -hydroxy pravastatin in pregnant patients in an ongoing double blind, placebo controlled pilot clinical trial. Plasma and urine samples were collected from patients to determine the PK of the medication in this patient population. QC samples at low, medium, and high concentrations were prepared daily and analyzed together with the patient samples. At the time of preparing this manuscript, 578 samples from 13 pregnant patients enrolled in the clinical trial had been analyzed while blinded to whether the patient is in the control or treated group. Among these samples, the concentrations of the two analytes in biological samples from 6 pregnant patients were quantified. According to the concentration profiles, we deduced these patients were under treatment with pravastatin. The data obtained were as follows: the concentration of pravastatin in plasma samples ranged between 0.106 ng/mL and 32.6 ng/mL while in urine samples it ranged between 24.0 ng/mL and 2.23×10^3 ng/mL. The concentrations of 3 α -hydroxy pravastatin in plasma ranged between 0.127 ng/mL and 10.7 ng/mL while in urine it ranged between 2.16 ng/mL and 218 ng/mL. The sensitivity and calibration range of this method were adequate for profiling during the 24 h sampling period.

Conclusions

The development and validation of a chromatography/tandem mass spectrometry method for the quantification of pravastatin and its major metabolite, 3 α -hydroxy pravastatin in plasma and urine of pregnant patients is described in this report. The challenge of pravastatin stability was overcome by optimizing the procedure for sample preparation and chromatographic conditions. The method reported here provides better sensitivity than those previously reported with an LLOQ of 0.106 ng/ml for pravastatin, and 0.105 ng/ml for 3 α -hydroxy pravastatin in plasma samples. The low detection limit, achieved in our method, is crucial for determining the PK of pravastatin in pregnant patients since it will be the basis for recommendations on the dose for treatment of this patient population.

Moreover, the method reported here is cost-effective and the use of liquid-liquid extraction allowed a simple protocol for preparation of the biological fluids samples obtained from the patients. Finally, the method has been successfully applied for the analysis of approximately 578 samples from patients under treatment with pravastatin or placebo (double blind) during pregnancy.

Acknowledgments

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Abbreviations used

PK	pharmacokinetic
MRM	multiple reactions monitoring
LLE	liquid-liquid extraction
PP	protein precipitation
SPE	solid phase extraction

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Figure 1.
MS² product ion spectra of pravastatin (A), 3α-hydroxy pravastatin (B) and pravastatin-d₃ (C).

Figure 2.

Typical MRM chromatograms of pravastatin (**1**) and 3 α -hydroxy pravastatin (**2**) (left panel), and the IS (**3**) (right panel) in (A) human blank plasma, (B) blank human plasma spiked with pravastatin (0.106 ng/mL) and 3 α -hydroxy pravastatin (0.105 ng/mL) (C) human plasma of a pregnant patient under treatment with pravastatin.

Figure 3.

Typical MRM chromatograms of pravastatin (**1**) and 3 α -hydroxy pravastatin (**2**) (left panel), and the IS (**3**) (right panel) in (A) human blank urine, (B) blank human urine spiked with pravastatin (19.7 ng/mL) and 3 α -hydroxy pravastatin (2.00 ng/mL) (C) human urine of pregnant patient prescribed of pravastatin.

Table 1Matrix effects and recovery of pravastatin and 3 α -hydroxy pravastatin in human plasma and urine (n = 6)

Analytes	Matrix	Conc. (ng/mL)	Matrix effects (n = 6)		Extraction recovery (n = 6)	
			Matrix factor (%)	CV (%)	Mean recovery (%)	CV (%)
Pravastatin	plasma	0.265	95.7	12	99.5	8.3
		21.2	91.1	7.3	93.8	14
		74.2	84.4	13	93.9	8.7
	urine	39.4	85.7	5.4	–	–
		984	86.7	2.6	–	–
		2.46 $\times 10^3$	81.4	3.2	–	–
3 α -hydroxy pravastatin	plasma	0.264	104	11	99.2	3.7
		21.1	92.4	8.6	94.2	12
		73.8	87.5	10	94.6	10
	urine	4.00	93.6	7.9	–	–
		100	96.9	3.6	–	–
		250	99.2	2.7	–	–

Table 2

Intra-day and inter-day precision and accuracy of the method for human plasma and urine

Analytes	Bio-matrix	Concentration (ng/mL)	Intra-day (n = 5)			Inter-day (n = 3)		
			Mean (ng/mL)	Accuracy ^a (%)	CV (%)	Mean (ng/mL)	Accuracy ^a (%)	CV (%)
Pravastatin	plasma	0.106	0.0970	91.5	8.4	0.0981	92.5	12
		0.265	0.269	101	1.4	0.266	100	3.6
		21.2	22.1	104	3.1	22.5	106	2.4
		74.2	74.6	101	2.5	73.3	98.8	2.3
	urine	19.7	18.7	94.8	2.2	18.4	93.6	1.2
		39.4	40.0	102	2.3	40.1	102	0.9
		984	1.03×10 ³	105	2.1	1.02×10 ³	104	0.8
		2.46×10 ³	2.42×10 ³	98.2	1.3	2.44×10 ³	99.2	1.9
3α-hydroxy pravastatin	plasma	0.105	0.107	101	17	0.0984	92.8	12
		0.264	0.268	101	6.3	0.269	102	4.5
		21.1	20.6	97.2	9.6	21.9	103	6.7
		73.8	77.4	104	6.7	74.1	99.9	5.3
	urine	2.00	1.93	96.3	5.9	1.96	97.8	1.5
		4.00	4.10	102	3.3	4.03	101	1.7
		100	103	103	1.1	102	102	1.3
		250	248	99.0	1.9	250	100	1.6

^a Accuracy = (obtained concentration/added concentration) × 100%.

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Table 3

Stability of pravastatin and 3α-hydroxy pravastatin in human plasma and urine

Analytes	Bio-matrix	Concentration (ng/mL)	Short-term stability (4 h, 22–25 °C)			Freeze and thaw stability (3 cycles, –70 °C)			Long-term stability (30 days, –70 °C)		
			Mean (ng/mL)	Mean accuracy ^a (%)	CV (%)	Mean (ng/mL)	Mean accuracy ^a (%)	CV (%)	Mean (ng/mL)	Mean accuracy ^a (%)	CV (%)
Pravastatin	plasma	0.265	0.257	96.9	4.3	0.250	94.5	3.9	0.288	109	3.1
		74.2	70.6	95.2	1.1	69.5	93.7	1.4	75.9	102	0.8
	urine	39.4	39.5	100	3.3	41.1	105	8.2	39.3	99.7	1.6
3α-hydroxy pravastatin		2.46×10 ³	2.41×10 ³	98.0	4.9	2.43×10 ³	98.7	7.7	2.42×10 ³	98.2	1.8
		0.264	0.256	96.8	5.3	0.258	97.6	2.6	0.239	90.6	1.7
	plasma	73.8	68.7	93.1	0.9	68.9	93.3	2.0	73.9	100	3.6
	urine	4.00	4.00	98.8	2.7	3.80	94.3	9.2	3.85	96.1	3.1
		250	248	99.0	5.2	242	96.8	6.7	242	96.8	0.8

^aAccuracy = (obtained concentration/added concentration) × 100%.