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Development and validation of a liquid chromatography–tandem mass spectrometric assay for pitavastatin and its lactone in human plasma and urine

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ABSTRACT

A rapid, selective and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method with electrospray ionization (ESI) was developed and validated for the simultaneous determination of pitavastatin and its lactone in human plasma and urine. Following a liquid–liquid extraction, both the analytes and internal standard racemic i-prolact were separated on a BDS Hypersil C_8 column, using methanol–0.2% acetic acid in water (70: 30, v/v) as the mobile phase. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode using the transition m/z 422.4 → m/z 290.3 for pitavastatin, m/z 404.3 → m/z 290.3 for pitavastatin lactone and m/z 406.3 → m/z 318.3 for the internal standard, respectively. Linear calibration curves of pitavastatin and its lactone were obtained in the concentration range of 1–200 ng/ml, with a lower limit of quantitation of 1 ng/ml. The intra- and inter-day precision values were less than 4.2%, and accuracies were between −8.1 and 3.5% for both analytes. The proposed method was utilized to support clinical pharmacokinetic studies of pitavastatin in healthy subjects following oral administration.

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1. Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, more commonly known as the statins, are the most commonly prescribed lipid-modifying therapies [1]. Competitive inhibition of HMG-CoA reductase by the statins decreases hepatocyte cholesterol synthesis, which results in increased extraction of LDL-C from the blood and decreased circulating LDL-C concentrations [2].

Pitavastatin (Kowa Company Ltd., Tokyo, Japan), (+)-monocalcium bis(3R,5S,6E)-7-(2-cyclopropyl-4-[4-fluorophenyl]-3-quinolyl-3,5-dihydroxy-6-heptenoate), is a potent synthetic inhibitor of HMG-CoA reductase and was developed for the treatment of hypercholesterolaemia [3]. It can reduce plasma levels of LDL cholesterol by 40% in hypercholesterolaemic patients [4]. In humans, pitavastatin is only minimally metabolized by the cytochrome P450 2C9 isozyme [5]. The major metabolic pathway of pitavastatin involves its initial glucuronidation by uridine diphosphate-glucuronosyltransferase and then spontaneous lactonization by the elimination of the glucuronide moiety. Moreover, the lactone form can be reversibly converted to the parent drug [6,7]. Pitavastatin is excreted predominantly into bile and thereby enters the enterohepatic circulation. Very little parent drug is excreted into the urine [8].

Pitavastatin lactone is hydrolyzed and converted to the open lactone form pitavastatin easily. Pitavastatin is also dehydrated, forming the closed lactone form (Fig. 1). This phenomenon indicates that the mutual conversion may occur through analysis operation, such as extraction and purification. Therefore, it is important to evaluate pitavastatin and its lactone simultaneously, to monitor the stability and conversion of both compounds. Current available data regarding the pharmacokinetics of pitavastatin and its lactone was primarily acquired by a column-switching high-performance liquid chromatography (HPLC) method with ultraviolet detection [9,10]. It needed a long chromatographic run time (>25 min) and time-consuming sample pretreatments. Recently, two LC-MS/MS methods employing electrospray ionization source have been published [11,12]. However, both the methods have not described simultaneous determinations of both the open and closed forms of the drug.

The need to better characterize the clinical pharmacokinetic properties of pitavastatin and its lactone compelled us to set up and validate a simple, specific and sensitive analytical method. In this paper we describe a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the simultaneous determination of pitavastatin and its lactone in human plasma and urine. Following validation, this method was successfully applied to phase I clinical studies of pitavastatin performed in 32 healthy Chinese volunteers after single oral doses from 1 mg to 8 mg, using a 0.2 ml plasma or urine sample.

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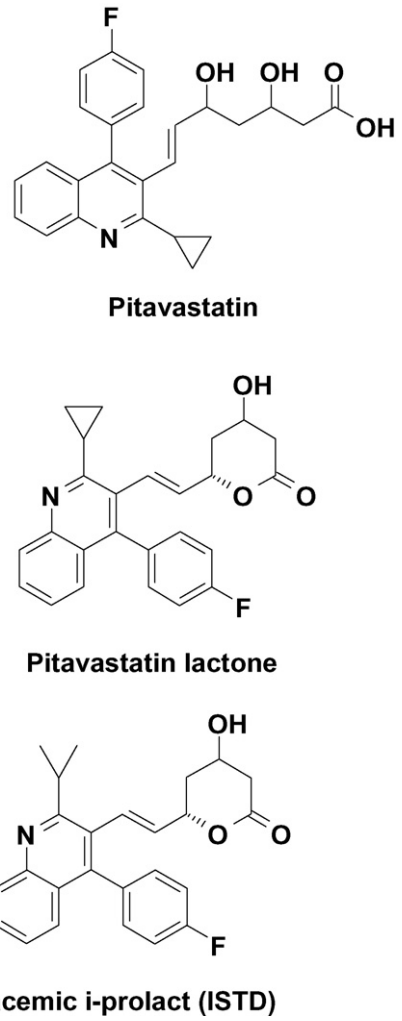

Fig. 1. Chemical structures of pitavastatin, pitavastatin lactone and racemic i-prolact (ISTD).

2. Experimental

2.1. Reference compounds and chemicals

Pitavastatin, pitavastatin lactone, and racemic i-prolact (internal standard, ISTD) were all kindly provided by Kowa Company Ltd., with the purity of 99.35, 97.6 and 98.3%, respectively. HPLC grade methanol, acetonitrile and methyl-*tert*-butyl ether were all obtained from Fisher (Fair Lawn, NJ, USA). All other chemicals and reagents were of either HPLC- or analytical-grade and were used without any further purification. Deionized water was generated in-house with a Milli-Q Gradient system (Millipore, Bedford, MA, USA) and was used throughout the study.

2.2. Instrumentation

An Agilent 1100 system (Wilmington, DE, USA) consisting of a vacuum degasser, a binary pump, a column oven and an autosampler was used for solvent and sample delivery. Chromatography was carried out using a BDS Hypersil C_8 column (50 mm × 2.1 mm, 3 μm, Thermo, Waltham, MA), eluting isocratically at 0.2 ml/min with a mobile phase of methanol–0.2% acetic acid in water (70: 30, v/v). The column and autosampler temperature were maintained at 30 and 4 °C, respectively.

An Applied Biosystems MDS Sciex (Concord, Ontario, Canada) API 4000 triple-quadrupole mass spectrometer equipped with a TurboIonSpray ionization (ESI) source was used for mass spectral analysis and the system was operated in positive mode. Optimisation of the MS conditions was carried out using a solution containing 400 ng/ml of pitavastatin, pitavastatin lactone and the internal standard, delivered via a Harvard syringe pump (Harvard Apparatus, SouthNatick, MA, USA) at a constant flow-rate of 10 μl/min. The nebulizer and TurboIonSpray gases (nitrogen) were both set at a value of 30 (instrument units). The optimized TurboIonSpray voltage and temperature were set at 5000 V and 400 °C, respectively. Nitrogen was also used as curtain gas and collision cell gas, which were set at 20 and 4 instrument units, respectively. Quantitation was performed using the multiple reaction monitoring (MRM) transition m/z 422.4 → m/z 290.3 for pitavastatin, m/z 404.3 → m/z 290.3 for pitavastatin lactone and m/z 406.3 → m/z 318.3 for the internal standard, respectively, with a dwell time of 150 ms per transition. The optimized collision energy of 38 eV was used for pitavastatin and its lactone, and 45 eV for the internal standard. The declustering potential (DP) was set at 95, 85 and 110 V for pitavastatin, pitavastatin lactone and the internal standard, respectively. The mass spectrometer was operated at unit mass resolution (peak width at half-height set at 0.7 Da) for both Q1 and Q3.

2.3. Preparation of standard and quality control solutions

Separate stock solutions of pitavastatin and pitavastatin lactone were prepared by dissolving the accurately weighed reference compounds in water for pitavastatin and in acetonitrile for pitavastatin lactone, to yield final concentrations of 100 μg/ml. Successive dilutions from both the stock solutions with acetonitrile gave working standard solutions containing pitavastatin and its lactone at concentrations of 50, 100, 250, 500, 1250, 2500, 5000 and 10,000 ng/ml. The internal standard stock solution of 100 μg/ml was also prepared in acetonitrile. Further dilution of the ISTD stock solution with methyl-*tert*-butyl ether resulted in the extraction solvent containing 2 ng/ml internal standard.

Quality control (QC) stock solutions (100 μg/ml) were prepared from a separate weighing. Dilutions were used to prepare three levels of QC working solutions containing pitavastatin and its lactone at concentrations of 125, 1875 and 8000 ng/ml.

All the solutions were stored at −20 °C and were brought to room temperature prior to use.

The standard and QC working solutions (100 μl) were used to spike blank plasma or urine (4.9 ml, precooling down in an ice bath) either for calibration curves or for QC samples in pre-study validation and during the pharmacokinetic study. The above spiking procedure resulted in final concentrations for the calibration standards of 1, 2, 5, 10, 25, 50, 100 and 200 ng/ml. Similarly, the low, medium and high concentration QC samples were achieved at 2.5, 37.5 and 160 ng/ml, respectively.

All the calibration standards and QC samples were stored at −70 °C until required for the assay.

2.4. Sample preparation

An aliquot of plasma or urine (0.2 ml) was mixed with 0.2 ml of 0.02 M phosphate buffer (pH 3) in an ice bath, then extracted with 3 ml methyl-*tert*-butyl ether (containing 2 ng/ml ISTD) by vortexing for 3 min. The organic and aqueous phases were separated by centrifugation at 3000 × *g* for 10 min at 4 °C. The upper organic phase was transferred to another glass tube and was evaporated to dryness at room temperature under a gentle stream of nitrogen. The residue was dissolved in 400 μl of the mobile phase, and a 10 μl aliquot was injected onto the LC–MS/MS system for analysis.

2.5. Data acquisition and analysis

Data were collected and analyzed by Analyst 1.3.1 software (Applied Biosystems MDS Sciex). Calibration of analyte was performed by establishing a linear regression function after $1/x$ weighing of the analyte/ISTD peak area ratio versus analyte concentration relationship. Drug concentrations in the unknown and quality control samples were calculated by interpolation from the calibration curves prepared in the same analysis run.

2.6. Method validation

The specificity of the method was measured by analysis of six blank plasma and urine samples of different origin for interference at the retention times of the analytes and ISTD. The specific determination of pitavastatin and its lactone was illustrated by analysis of three MRM transitions characteristic of the analytes and ISTD.

In order to assess the intra- and inter-day precision and accuracy, complete analytical runs were performed on the same day and on four consecutive days. Each analytical run consisted of a matrix blank (matrix sample processed without ISTD), a zero sample (matrix sample processed with ISTD), eight nonzero standards as mentioned above, six replicate LLOQ samples, and a set of low, medium and high concentration QC samples. Concentrations for the QC samples were calculated by reference to the calibration curve generated from the calibration standards. The LLOQ was defined as the concentration of the lowest concentration standard in the calibration curve that was analyzed with accuracy within ±15% and a precision ≤15%. During routine analysis each analytical run included a matrix blank, a set of calibration samples, a set of QC samples in duplicate and unknowns.

The extraction recoveries of pitavastatin and its lactone were determined at low and high QC levels by comparing the analyte/ISTD peak area ratios in spiked samples with the peak area ratios of samples that had the analyte-spiked post-extraction. The internal standards were added to both sets of samples post-extraction.

Stability tests were performed for analyte-spiked plasma and urine samples under various storage and process conditions by analyzing 6 replicates at low and high QC concentrations.

3. Results and discussion

3.1. LC–MS/MS

Due to the alkaline nitrogen atom on the pyridine cycle in the molecular structure, the positive ionization mode should be more appropriate for pitavastatin and its lactone than the negative mode. All of the positive ion electrospray mass spectra of pitavastatin, pitavastatin lactone and the internal standard in the full scan Q1 mode showed the protonated molecular ion $[M+H]^+$ as the base peak, m/z 422.4 for pitavastatin, m/z 404.3 for pitavastatin lactone and m/z 406.3 for the internal standard. By increasing the collision energy, the fragmentation patterns of the protonated molecular ions were observed. The product ion mass spectra of both analytes and the internal standard were shown in Fig. 2, where the most intense product ions were observed at m/z 290.3 for pitavastatin and its lactone, and m/z 318.3 for the internal standard. These fragmentation schemes are also shown in Fig. 2. Additional tuning of the ESI source parameters for the transitions m/z 422.4 → m/z 290.3, m/z 404.3 → m/z 290.3 and m/z 406.3 → m/z 318.3 further improved the sensitivity.

Using acetonitrile or methanol as an organic additive in the mobile phase yielded similar responses, but as a protonic solvent methanol was more compatible for the positive mode, and provided a more stable signal. The high organic solvent content shortened the chromatographic cycle time and the acid modifier, acetic acid, improved the signal intensity greatly. Therefore, the mobile phase consisting of methanol–0.2% acetic acid in water (70: 30, v/v) was chosen to obtain a good chromatographic peak shape and to keep the analytes and the internal standard at a suitable retention time (~2.5 min for all of the three compounds). The fast analysis had a total run time of 4.5 min per sample, showing a much higher throughput than previous reports [9,12].

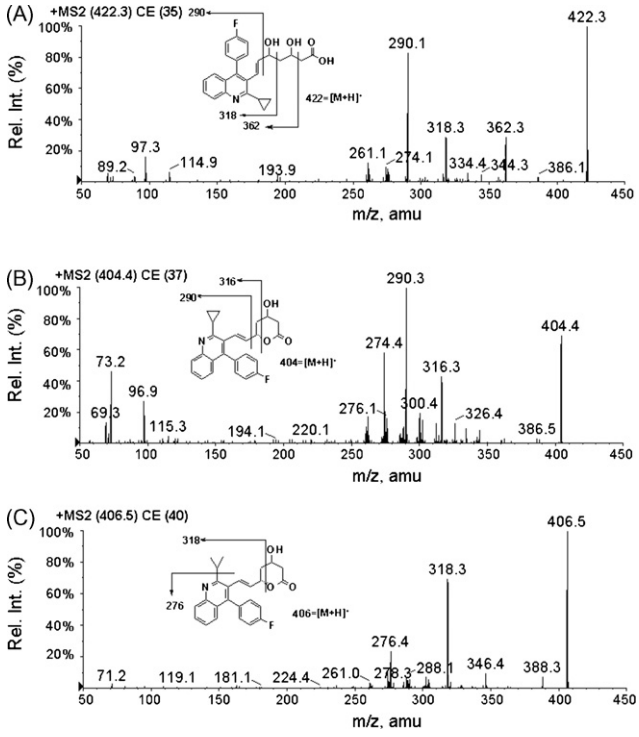

Fig. 2. Positive ion ESI mass spectra of (A) pitavastatin; (B) pitavastatin lactone and (C) racemic i-prolact (ISTD) with each protonated molecule $[M+H]^+$ as precursor ion.

3.2. Method validation

3.2.1. Assay specificity

The coupling of LC with MS/MS detection in the MRM mode has high specificity because only ions derived from the analytes of interest are monitored. Assay specificity was confirmed by the absence of interfering peaks at the retention times of the analytes and internal standard (Figs. 3 and 4).

Blank matrices were spiked at the concentration of the low and high QC sample and evaluated to determine whether the source of the matrix has any effect on the quantification of the analytes. Matrices from six different donors were spiked at each concentration and analyzed against calibration standards and QCs. In human plasma, the relative errors for pitavastatin ranged from −8.6 to 10.0%, while for pitavastatin lactone the corresponding values ranged from −5.4 to 2.0%. In human urine, the corresponding relative errors for pitavastatin were between −9.5 and 9.2%, while for pitavastatin lactone the corresponding values were between −11.7 and 2.3%. These data were all within ±15% of the theoretical, indicating that no significant matrix effect was observed in any of the blank plasma or urine samples.

3.2.2. Linearity of calibration curve and lower limit of quantitation

Linear calibration curves with correlation coefficients greater than 0.998 were obtained over the concentration range of

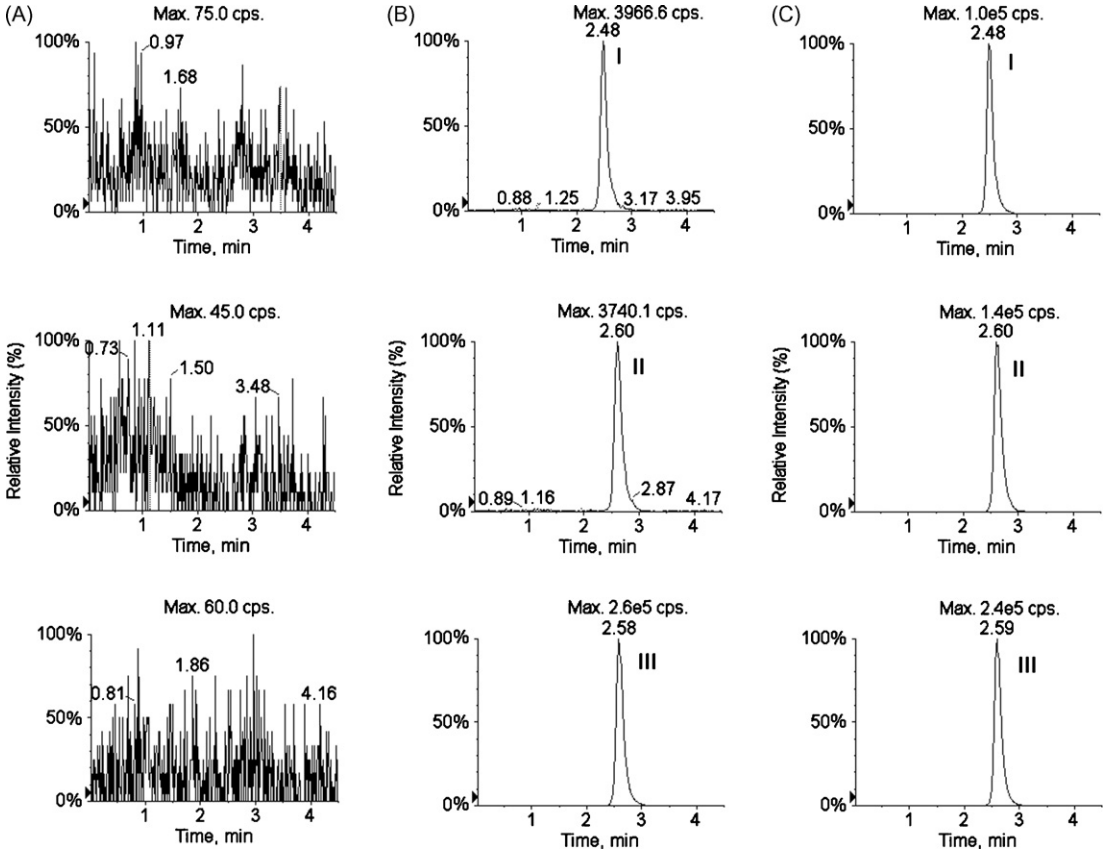

Fig. 3. Representative MRM chromatograms of (A) blank plasma sample; (B) blank plasma sample spiked with pitavastatin (1 ng/ml), pitavastatin lactone (1 ng/ml) and internal standard (30 ng/ml); and (C) a plasma sample from a volunteer 1.0 h after oral administration of 4 mg pitavastatin (25.8 ng/ml for pitavastatin and 43.0 ng/ml for pitavastatin lactone). Peaks I, II and III refer to pitavastatin, pitavastatin lactone and the internal standard, respectively.

1–200 ng/ml for both pitavastatin and its lactone. The current assay had a lower limit of quantitation of 1 ng/ml with a signal-to-noise ratio above 20. In fact, a much lower LLOQ (less than 0.1 ng/ml) could be obtained by decreasing the solvent volume for dissolving the residue and increasing the volume injected onto the LC–MS/MS system.

3.2.3. *Precision, accuracy and extraction recovery*

Data for intra- and inter-day precision and accuracy of the assay are summarized in Table 1. In human plasma and urine, the intra- and inter-day precision were less than 4.2%, and the accuracies, expressed in the relative error (R.E.), were between −8.1 and 3.5% for both analytes.

In human plasma, the mean extraction recoveries for pitavastatin were 74.9 ± 9.8 and 70.7 ± 8.7% at concentrations of 2.5 and 160 ng/ml, respectively, while for pitavastatin lactone the corresponding values were 78.7 ± 2.7 and 80.5 ± 3.3%. In human urine, the corresponding extraction recoveries for pitavastatin were 82.1 ± 3.1 and 74.5 ± 1.1%, respectively, while for pitavastatin lactone the corresponding values were 75.7 ± 3.2 and 69.9 ± 1.9%.

Table 1
Precision, accuracy and LLOQ results for pitavastatin and its lactone in human plasma and urine extracts (n = 4 day, six replicates per day)

Matrix	Compound	Added C (ng/ml)	Intra-day			Inter-day		
			Found C (ng/ml)	R.S.D. (%)	R.E. (%)	Found C (ng/ml)	R.S.D. (%)	R.E. (%)
Plasma	Pitavastatin	2.5	2.44	3.3	−2.5	2.45	2.5	−1.7
		37.5	37.3	2.7	−0.5	36.2	2.4	−3.5
		160	161	2.5	0.7	153	2.5	−4.2
		1	0.92	2.8	−8.1	0.97	1.9	−3.5
	Pitavastatin lactone	2.5	2.40	2.0	−3.9	2.45	3.5	−1.9
		37.5	35.9	1.1	−4.2	36.3	1.6	−3.2
		160	153	2.5	−4.2	157	3.0	−2.2
		1	0.97	3.2	−3.2	0.99	3.0	−0.7
Urine	Pitavastatin	2.5	2.38	2.9	−5.0	2.35	3.1	−5.7
		37.5	35.6	3.2	−5.1	37.8	2.7	0.7
		160	164	3.1	2.3	164	4.1	2.2
		1	1.03	4.2	3.2	1.03	3.9	2.9
	Pitavastatin lactone	2.5	2.54	3.6	1.5	2.43	3.0	−2.6
		37.5	36.0	3.6	−4.1	36.7	3.7	−2.2
		160	159	4.0	−0.6	152	3.0	−5.4
		1	1.04	2.5	3.5	0.96	4.1	−4.0

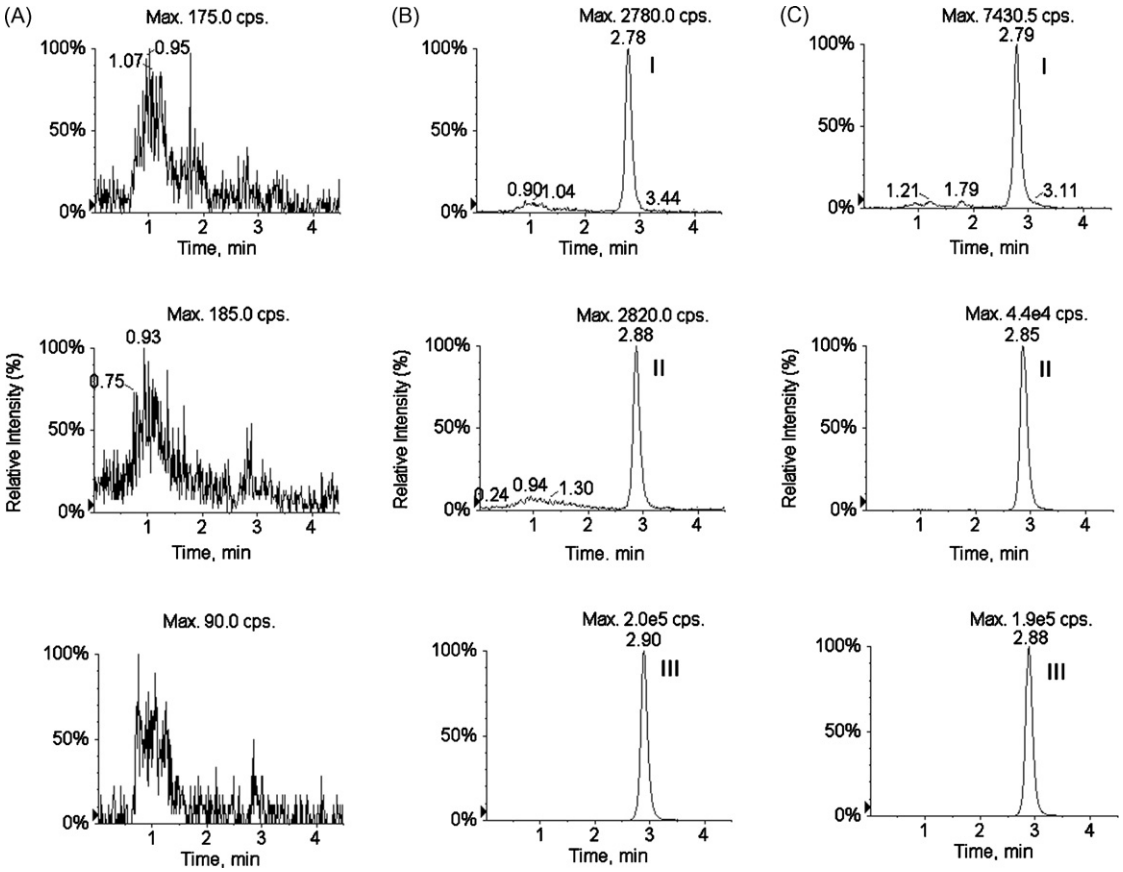

Fig. 4. Representative MRM chromatograms of (A) blank urine sample; (B) blank urine sample spiked with pitavastatin (1 ng/ml), pitavastatin lactone (1 ng/ml) and internal standard (30 ng/ml); and (C) a urine sample from a volunteer after oral administration of 2 mg pitavastatin (2.3 ng/ml for pitavastatin and 19.5 ng/ml for pitavastatin lactone). Peaks I, II and III refer to pitavastatin, pitavastatin lactone and the internal standard, respectively.

The mean extraction recoveries of the internal standard were 84.9 ± 8.0% for plasma and 58.1 ± 10.0% for urine.

3.2.4. Stability

As a part of the method validation, data were also generated to ensure whether pitavastatin and its lactone were stable at distinct timing and temperature conditions. The stability test results are summarized in Table 2.

Pitavastatin and its lactone were stable in human plasma and urine when stored frozen at −70 °C for at least two months. The analytes were also shown to be stable after four freeze (−70 °C)–thaw (ice bath) cycles and after 24 h of storage in reconstitution solutions at 4 °C. The REs for the both analytes were all less than 10% in these biological samples.

The short-term stability results showed that pitavastatin and its lactone were stable in human plasma for at least 4 h at 4 °C, and in

Table 2
Stability results for pitavastatin and its lactone in human plasma and urine (n = 6, values expressed as mean (R.E.%))

Stability test condition	Nominal concentration added (ng/ml)							
	Pitavastatin in plasma		Pitavastatin lactone in plasma		Pitavastatin in urine		Pitavastatin lactone in urine	
	2.5	160	2.5	160	2.5	160	2.5	160
Long-term stability								
2 weeks at −70 °C	2.57 (2.7)	160 (0.1)	2.51 (0.2)	163 (1.6)	2.34 (−6.4)	166 (3.7)	2.37 (−5.1)	159 (−0.4)
1 months at −70 °C	2.43 (−2.6)	165 (2.9)	2.46 (−1.7)	161 (0.9)	2.42 (−3.1)	164 (2.3)	2.44 (−2.6)	158 (−1.1)
2 months at −70 °C	2.49 (−0.3)	170 (6.5)	2.39 (−4.6)	159 (−0.5)	2.44 (−2.2)	169 (5.5)	2.31 (−7.7)	159 (−0.6)
Short-term stability								
2 h at 4 °C	2.62 (4.9)	163 (1.6)	2.36 (−5.5)	152 (−4.7)	2.51 (0.5)	157 (−2.0)	2.36 (−5.6)	156 (−2.6)
4 h at 4 °C	2.62 (4.9)	164 (2.6)	2.20 (−11.8)	145 (−9.2)	2.60 (4.0)	164 (2.3)	2.32 (−7.2)	152 (−5.2)
12 h at 4 °C	3.73 (49.2)	234 (46.2)	1.43 (−42.7)	106 (−33.5)	2.52 (0.7)	162 (1.4)	2.37 (−5.3)	155 (−3.4)
24 h at 4 °C	NA	NA	NA	NA	3.26 (30.4)	203 (26.6)	1.94 (−22.3)	119 (−25.6)
2 h at room temperature	3.60 (43.8)	237 (48.0)	1.70 (−32.1)	124 (−22.5)	2.60 (3.9)	168 (4.9)	2.40 (−4.1)	150 (−6.5)
24 h at room temperature	NA	NA	NA	NA	5.39 (115.7)	316 (97.2)	ND	24.6 (−84.6)
Freeze and thaw stability								
4 cycles at −70 °C	2.57 (2.8)	162 (1.2)	2.42 (−3.1)	157 (−1.6)	2.33 (−6.9)	156 (−2.3)	2.46 (−1.8)	156 (−2.8)
Post-preparative stability								
24 h at 4 °C	2.61 (4.4)	159 (−0.3)	2.55 (2.0)	159 (−0.9)	2.30 (−8.0)	156 (−2.4)	2.47 (−1.2)	162 (1.0)

NA: not available; ND: not detectable.

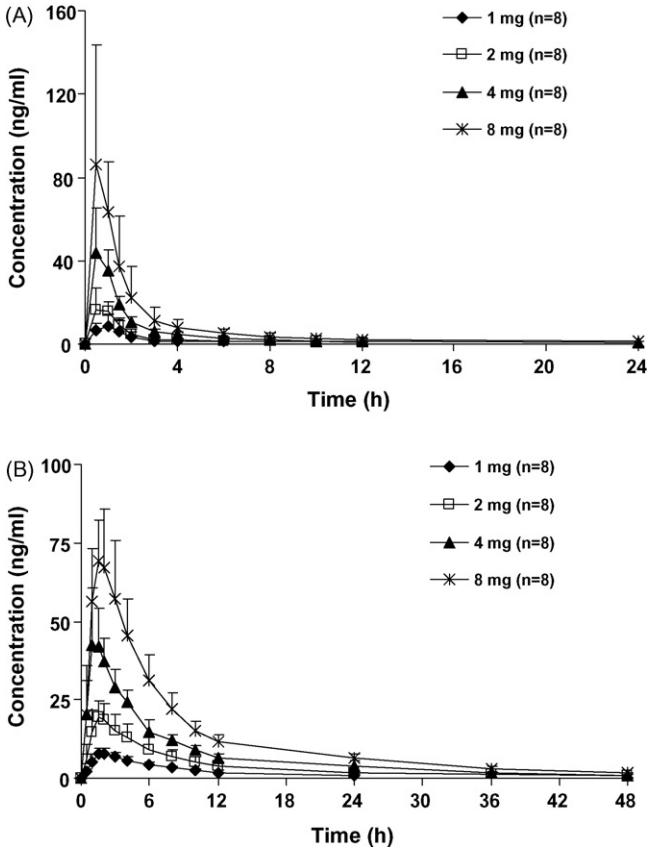

Fig. 5. Mean plasma concentration–time profiles of (A) pitavastatin and (B) pitavastatin lactone after a single oral dose of 1, 2, 4 or 8 mg of pitavastatin to healthy subjects.

human urine for at least 12 h at 4 °C. With the lapse of time, some of the pitavastatin lactone changed gradually to pitavastatin. The relative errors for plasma pitavastain concentration ranged from 46.2 to 49.2% when stored at 4 °C for 12 h, while the corresponding values for pitavastatin lactone ranged from −42.7 to −33.5%. Similar results were obtained when the spiked plasma samples were kept at room temperature for 2 h. Similarly, the relative errors for urine pitavastatin concentrations ranged from 26.6 to 30.4% when stored at 4 °C for 24 h, while the corresponding values for pitavastatin lactone ranged from −25.6 to −22.3%. Over 90% of pitavastatin lactone was hydrolyzed and converted to pitavastatin when stored at room temperature for 24 h. These results indicated that pitavastatin lactone was somewhat unstable in plasma and urine samples. The collected biological samples should be immediately kept in an ice bath and then stored at −70 °C to minimize the hydrolysis of the lactone. Also the quantification operations, such as preparation of standard solutions and sample extraction, should be performed at low temperature (≤4 °C).

3.3. Application of the analytical method in pharmacokinetic studies

The present method was utilized for the analysis of biological samples obtained from 32 healthy subjects after single oral administration of pitavastatin from 1 to 8 mg, as part of a phase I study. The mean plasma concentration–time curves of pitavastatin and its lactone are represented in Fig. 5. At all single dose levels of pitavastatin, absorption of pitavastatin was rapid, with median T_{max} ranging between 0.5 and 1.0 h. The metabolite pitavastatin lactone also appeared rapidly in plasma, with T_{max} occurring between approximately 0.5 and 1.5 h later than that for the parent drug. There was no detectable difference in dose-normalized C_{max} and $AUC_{0-\infty}$, which indicated dose proportionality of pitavastatin in the dosage levels of 1–8 mg. The total amount of pitavastatin excreted as unchanged drug in the urine was less than 1% of the administered dose, indicating that pitavastatin is subject to non-renal elimination in healthy subjects.

4. Conclusion

The optimized method was validated to guarantee a reliable determination of pitavastatin and its lactone in human plasma and urine. The method has a lower limit of quantitation of 1 ng/ml for both analytes and has been shown to be sensitive, selective and reproducible. The short chromatographic cycle time (4.5 min) allowed high-throughput analysis with minimal matrix interference. The method described has been shown to be successfully applied to phase I pharmacokinetic studies in healthy subjects.

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References

[1] R.J. Havel, E. Rapaport, N. Engl. J. Med. 332 (1995) 1491.
[2] D.J. Maron, S. Fazio, M.F. Linton, Circulation 101 (2000) 207.
[3] T. Aoki, H. Nishimura, S. Nakagawa, J. Kojima, H. Suzuk, T. Tamaki, Y. Wada, N. Yokoo, F. Sato, H. Kimata, M. Kitahara, K. Toyoda, M. Sakashita, Y. Saito, Arzneimittelforschung 47 (1997) 904.
[4] Y. Saito, N. Yamada, T. Teramoto, H. Itakura, Y. Hata, N. Nakaya, H. Mabuchi, M. Tushima, J. Sasaki, N. Ogawa, Y. Goto, Atherosclerosis 162 (2002) 373.
[5] H. Fujino, I. Yamada, S. Shimada, T. Nagao, M. Yoneda, Arzneimittelforschung 52 (2002) 745.
[6] H. Fujino, I. Yamada, S. Shimada, M. Yoneda, J. Kojima, Xenobiotica 33 (2003) 27.
[7] I. Yamada, H. Fujino, S. Shimada, J. Kojima, Xenobiotica 33 (2003) 789.
[8] H. Fujino, I. Yamada, J. Kojima, M. Hirano, H. Katsumoto, M. Yoneda, Xenobio. Metabol. Dispos. 14 (1999) 415.
[9] J. Kojima, H. Fujino, M. Yosimura, H. Morikawa, H. Kimata, J. Chromatogr. B. Biomed. Sci. Appl. 724 (1999) 173.
[10] C.K. Hui, B.M.Y. Cheung, G.K.K. Lau, Br. J. Clin. Pharmacol. 59 (2004) 291.
[11] J.W. Deng, K.B. Kim, I.S. Song, J.H. Shon, H.H. Zhou, K.H. Liu, J.G. Shin, Biomed. Chromatogr. 22 (2008) 131.
[12] H. Lv, J.G. Sun, G.J. Wang, X.Y. Zhu, Y. Zhang, S.H. Gu, Y. Liang, J. Sun, Clin. Chim. Acta 386 (2007) 25.