International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

A RAPID LC–MS/MS ASSAY FOR PITAVASTATIN IN HUMAN PLASMA BY USING SOLID PHASE EXTRACTION TECHNIQUE AND ITS APPLICATION TO A PHARMACOKINETIC STUDY

Boligarla Gopi Kalyan Kumar[1], Nageswara Rao Pilli[2], Babu Rao Bhukya[3] and N. Y. Sreedhar[1,*]

[1]Electroanalytical Lab, Department of Chemistry, Sri Venkateswara University, Tirupati–517 502, India
[2]Piramal Clinical Research Laboratories, Ramanthapur, Hyderabad–500 013, India
[3]Pathfinder Institute of Pharmacy Education & Research, Beside Mamnoor Camp, Warangal–506 166, India

***Corresponding author e-mail:** gopi_kalyan@rediffmail.com

ABSTRACT

A rapid and sensitive liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay method has been developed and fully validated for the quantitative determination of pitavastatin in human plasma. A pitavastatin stable labeled isotope (pitavastatin d_4) was used as an internal standard. Analyte and the internal standard were extracted from human plasma *via* solid phase extraction technique. The chromatographic separation was achieved on a C_{18} column by using a mixture of acetonitrile–0.1% formic acid (90:10, v/v) as the mobile phase at a flow rate of 0.85 mL/min. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 0.05–160 ng/mL. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. A run time of 1.5 min for each sample made it possible to analyze more than 450 plasma samples per day. The proposed method was found to be applicable to clinical studies.

Key words: Pitavastatin; Solid–phase extraction (SPE); Liquid chromatography–tandem mass spectrometry (LC–MS/MS); Method validation; Pharmacokinetics

INTRODUCTION

Hypercholesterolemia control is important for the prevention of coronary artery disease (CAD). The statins competitively inhibit the enzyme 3–hydroxy–3–methylglutaryl coenzyme A (HMG–CoA) reductase which is involved in the rate limiting step of cholesterol biosynthesis and are first–choice therapeutic agents for patients with hypercholesterolemia [1–4]. Pitavastatin, a competitive inhibitor of HMG CoA reductase and was developed for the treatment of hypercholesterolaemia [5–7]. It can reduce plasma level of LDL cholesterol by 40% in hypercholesterolaemic patients [8].

For the determination of pitavastatin in biological fluids some high–performance liquid chromatographic methods [9, 10] and liquid chromatography–mass spectrometric methods (LC–MS) [11–15] were reported. Of the LC–MS methods applied to analysis of pitavastatin in human plasma, either the chromatographic run time was long (>4 min) [11–14] and the plasma volume is high (>0.5 mL) [11] or the method was too insensitive for routine application [12–14]. The method proposed by Tian *et al.,* 2008 [12] and Lv *et al.,* 2007 [13] for quantification of pitavastatin in human plasma with a sensitivity of 0.2 ng/mL utilizes a time–cost sample preparation involving liquid–liquid (L–L) extract, evaporation, drying and reconstitution steps. Deng *et al.,* 2007 [14] have determined pravastatin and pitavastatin simultaneously in human plasma by LC–MS/MS using solid phase extraction (SPE) for sample preparation employing 0.30 mL sample for processing (LLOQ of 1.0 ng/mL). However, not

sensitive enough for the determination of pitavastatin concentrations for bioequivalence studies because of its higher LOQ. Another method reported by Shen–Tu *et al.,* 2009 [15] for the determination of pitavastatin in human plasma with plasma concentration range of 0.15–150 ng/mL by using LC–MS/MS. This method employs protein precipitation (PP) technique for the sample preparation. PP is most likely to cause ion suppression, since this method fails to sufficiently remove endogenous compounds such as lipids, phospholipids, fatty acids, etc [16, 17]. A promising method was developed and validated by Di *et al.*, 2008 [11] for the determination of pitavastatin in human plasma using SPE followed by MS detection in the linear dynamic range of 0.08–200 ng/mL and demonstrated suitability for application to phase I clinical pharmacokinetic studies. Although the method is sensitive and has an efficient extraction procedure but the total chromatographic run time is too long (>6 min), which may not be favourable for routine subject analysis. Also, sample processing volume relatively high (> 0.5 mL) and have a very high on–column loading of the analyte at the ULOQ level, which may reduce the efficiency of the column and may affect the column life. The analytical method should gratify the researchers in terms of sensitivity, runtime, sample volume, time consumption and efficient extraction procedure.

The objective of the present study was to develop and validate a rapid and sensitive method for the determination of pitavastatin in human plasma samples based on LC–MS/MS. This method employs a simple solid–phase extraction (SPE) technique for sample preparation and has achieved higher sensitivity (0.05 ng/mL) using a smaller plasma volume (100 µL). We have also employed pitavastatin stable labeled isotope pitavastatin d_4 as an internal standard (IS), this avoid the potential matrix effect related problems and variability in recovery between analyte and IS. Application of this assay method to a clinical pharmacokinetic study in healthy male human volunteers following oral administration of pitavastatin is described. The real time subject data was authenticated by incurred samples reanalysis (ISR).

EXPERIMENTAL

Standards and chemicals

Pitavastatin calcium reference standard (97.80% pure) was obtained from Hetero Drugs Limited (Hyderabad, India). Pitavastatin d_4 calcium salt (98.97% pure) was employed as an internal standard and was obtained from Clearsynth Labs Limited (Mumbai, India). Their structures are shown in Fig. 1. HPLC grade acetonitrile was purchased from J.T. Baker (Phillipsburg, USA). Analytical grade formic acid was purchased from Merck Ltd (Mumbai, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). The control human plasma sample was procured from Deccan's Pathological Lab's (Hyderabad, India).

LC–MS/MS instrument and conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a Zorbax SB C_{18} column (50 mm x 2.1 mm, 5 µm), a binary LC–20AD prominence pump, an auto sampler (SIL–HTc) and a solvent degasser (DGU–$20A_3$) was used for the study. Aliquot of 20 µL of the processed samples were injected into the column, which was kept at 40 °C. An isocratic mobile phase consisting of a mixture of acetonitrile–0.1% formic acid (90:10, v/v) was used to separate the analyte from the endogenous components and delivered at a flow rate of 0.85 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantification was achieved with MS–MS detection in positive ion mode for the analyte and the IS using an MDS Sciex API–4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ interface at 500 °C. The ion spray voltage was set at 5500 V. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 36, 30, 20, and 8 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 45, 42, 10, 12 V for pitavastatin and 45, 42, 10, 12 V for the IS. Detection of the ions was carried out in the multiple–reaction monitoring mode (MRM), by monitoring the transition pairs of *m/z* 442.10 precursor ion to the *m/z* 290.20 for pitavastatin and *m/z* 426.10 precursor ion to the *m/z* 294.30 product ion for the IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst Software™ (version 1.4.2).

Preparation of stock and working solutions

Two standard stock solutions of pitavastatin were prepared separately in HPLC grade methanol (1 mg/mL). Their concentrations were corrected according to the actual amount weighted accounting for its potency. Working standard solutions necessary for plotting the calibration curve (CC samples) were prepared by appropriate dilution of the one of the above stock solution of the pitavastatin using a mixture of acetonitrile and water (60:40, v/v; diluent). Similarly, Quality controls samples (QC samples) for determination of accuracy and precision

were prepared by appropriate dilution of the second standard stock solution prepared above using the same diluent. The concentrations of the QC samples are selected form the five different levels of the calibration curve range.

A 1 mg/mL of pitavastatin d_4 stock solution was prepared by dissolving the compound in HPLC grade methanol. The working concentration of pitavastatin d_4 (2000 ng/mL) was prepared form the above stock solution using the diluent.

Preparation of calibration curve standards and quality control samples in human plasma

Six lots of K2–EDTA human plasma were screened and used to prepare calibration standards, quality control samples and dilution integrity (DIQC) samples. After bulk spiking, aliquots of 200 µL for CCs and 200 µL for QCs of spiked plasma samples were pipetted out into a prelabelled micro centrifuge tubes (2 mL) and then all the bulk spiked samples were stored in deep freezer at –70±10 °C.

Calibration samples were prepared by spiking 950 µL of control K_2 EDTA human plasma with the 50 µL working standard solution of the analyte as a bulk, to obtain pitavastatin concentration levels of 0.05, 0.10, 1.01, 10.1, 20.1, 40.2, 80.4, 120 and 160 ng/mL as a single batch at each concentration. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations of 0.052 (lower limit of quantitation, LLOQ), 0.157 (low quality control, LQC), 17.5 (medium quality control, MQC1), 87.3 (MQC2) and 139 ng/mL (high quality control, HQC) as a single batch at each concentration.

Sample processing protocol

All frozen subject samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature prior to analysis. A 100 µL aliquot of human plasma sample was mixed with 10 µL of the internal standard working solution (2000 ng/mL of pitavastatin d_4). To this, 100 µL of 0.1% formic acid solution was added after vortex mixing for 10 s. The sample mixture was loaded onto an Orpheus C_{18} cartridge (30 mg/1 mL) that was pre–conditioned with 1.0 mL of methanol followed by 1.0 mL of water and 1.0 mL of 0.1% formic acid solution. The extraction cartridge was washed with 1.0 mL of 0.1% formic acid solution followed by 1.0 mL of 5% methanol. Analyte and IS were eluted with 0.5 mL of mobile phase. Aliquot of 20 µL of the extract was injected into the chromatographic system.

Method validation procedures

The validation of the above method was carried out as per US FDA guidelines (US DHHS, *et al*., 2001) [18]. System suitability test was performed by injecting six repeated injections of aqueous mixture of the analyte and the IS. Injector carryover experiment was performed to verify any carryover of analyte and the IS, which may reflect in subsequent runs. The design of the carryover test comprised of the following sequence of injections i.e. blank plasma sample → six samples of LLOQ → blank plasma sample → ULOQ sample → blank plasma samples to check for any interference due to carry over. The selectivity of the method was assessed in six different sources of plasma, of which, four were normal K_2–EDTA plasma and one each of Lipemic and helolyzed plasma. Sensitivity of the method was assessed by analyzing six sets of spiked plasma samples at lowest level of the calibration curve concentrations (LLOQ). Matrix effect was checked with six different lots of K_2 EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total).

The linearity of the method was determined by analysis of three standard calibration curves (CC) containing nine non–zero concentrations. In addition, each curve contains one blank plasma sample and one blank plasma sample with internal standard (zero standard). Each CC was analyzed individually by least square weighted ($1/x^2$) linear regression. Intra–day accuracy and precision was determined using six replicates of LLOQ QC, LQC, MQC–1, MQC–2, and HQC samples were analyzed along with a calibration curve in a single day. Inter–day accuracy and precision were assessed by analyzing three batches of samples on two consecutive days. The precision (% CV) at each concentration level from the nominal concentration should not be greater than 15%, except for LLOQ QC where it should be 20%. The accuracy (%) must be within ±15% of their nominal value at each QC level except LLOQ QC where it must be within ±20%.

Recovery for the analyte and the IS was calculated by comparing the mean peak response of pre–extraction spiked samples (spiked before extraction) to that of post–extraction spiked samples (spiked after extraction) at each QC level. Recoveries of analyte and the IS were determined by comparing the peak area of extracted analyte standard with the peak area of non–extracted standard. Recovery of pitavastatin was determined at a concentration of 0.157 (LQC), 87.3 (MQC-2) and 139 (HQC) ng/mL, whereas for the IS was determined at concentration of 2000 ng/mL.

Stock solution stability of the analyte and the IS was tested at room temperature for 15 h and at 2–8 °C in refrigerator for 21 days. The stock solution stability was performed by comparing the area response

stability samples with the response of the sample prepared from fresh stock solution. The solutions were considered stable if the deviation within ±10% form nominal value. Bench top stability at room temperature (13 h), processed samples stability (autosampler stability for 44 h, wet extract stability for 39 h and reinjection stability for 26 h), freeze–thaw stability (4 cycles) were performed at LQC and HQC levels using six replicates at each level. Similarly, the long term stability of spiked plasma samples stored at −70±10°C for 60 days was also studied at both the QC levels. The stability samples were processed and quantified against freshly spiked calibration curve standards along with freshly spiked QC samples. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD). Dilution reliability was performed to extend the upper concentration limit with acceptable precision and accuracy. Six replicates each at a concentration of about 1.60 times of the uppermost calibration standard were diluted two– and four–fold with screened blank plasma. The diluted samples were processed and analyzed with un–diluted calibration curve samples.

Pharmacokinetic study design

The proposed method was applied to determine pitavastatin plasma concentration for a pharmacokinetic study conducted in 6 healthy Indian subjects. All the volunteers provided with written informed consent and were fasted for 12 h before the drug formulation administration. The subjects were orally administered a single dose of pitavastatin (4 mg tablet) with 200 mL of water. Blood samples were collected following oral administration of 4 mg pitavastatin tablet at pre–dose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 20, 24, 36, 48 and 72 h, in K_2-EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged at 3200 rpm for 10 min and the plasma was collected. The collected plasma samples were stored at −70 ± 10 °C till their use. Plasma samples were spiked with the IS and processed as per the extraction procedure described earlier. The QC samples at low, middle 1, middle 2 and high concentration levels were also assayed in triplicate along with the clinical samples. WinNonlin software (Version 5.1) was used to analyze the plasma concentration–time profile of pitavastatin. An ISR was also performed by selecting the 12 subject samples (2 samples from each subject) near C_{max} and the elimination phase (3 x LLOQ) in the pharmacokinetic profile of the drug. The ISR values were compared with the initial values. The percent change in the value should not be more than ±20%.

RESULTS AND DISCUSSION

Mass spectrometry

Mass parameters were tuned in both positive and negative ionization modes using electrospray ionization source. Highest intensity was obtained in positive ion mode than the negative ion mode the analyte and IS having the ability to accept protons. Protonated form of analyte and the IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 442.10 to 290.20 for pitavastatin and from m/z 426.10 to 294.30 for the IS. The dwell time for each transition was 200 ms. As earlier publications have discussed the details of fragmentation pattern pitavastatin [11], we are not presenting the data pertaining to this. LC–MRM is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. Thus, the MRM technique was chosen for the assay development.

Method development

The aim of the present work was to develop and validate an LC–MS/MS method for the quantitative determination of pitavastatin in human plasma to monitor the pitavastatin concentration for pharmacokinetic/bioequivalence studies. Analytical method development includes the judicious selection of mobile phase composition, flow rate, chromatographic column and injection volume. These parameters should be copiously monitored to obtain better resolution form the endogenous components which in turn affect sensitivity and reproducibility of the method.

Initially, organic solvents like acetonitrile and methanol were tried in different volume ratio in combination with acidic buffers such as ammonium formate, ammonium acetate, as well as acid additives like acetic acid and formic acid in varying strength. A mobile phase composition with a small amount of formic acid helped in achieving the symmetric peak shape and improved the analyte detection. It was observed that acetonitrile and 0.1% formic acid (90:10, v/v) as the mobile phase was most appropriate to give best sensitivity, efficiency and peak shape. Separation was attempted on different columns like C_8 and C_{18} of different makes (Hypurity advance, Zorbax, Inertsil, Ace, Grace, Kromasil, Hypersil etc). Zorbax SB C_{18} (50 mm x 2.1 mm, 5 µm) column gave good peak shape and response even at lowest concentration level for the analyte. The isocratic mobile phase was operated at a flow rate of 0.85 mL/min. The retention time of analyte and the IS were low enough (0.85 and 0.85 min) allowing a short run time of 1.5 min.

The reported procedures have employed LLE [12, 13], SPE with evaporation and reconstitutions steps [14] and PP [15] to extract pitavastatin from human plasma. But the as a purpose to develop a simple, effective and sensitive method with minimum or no matrix effect, SPE was tested. SPE was checked with Oasis HLB and Orpheus C_{18} cartridges in presence of formic acid and ammonium acetate in different strengths. Consistent and reproducible recovery (~96%) was obtained with Orpheus C_{18} cartridges at all QC levels for the analyte. Thus, the simple SPE procedure was employed for the sample preparation in this work and provided high recovery for the analyte. Addition of 0.1% formic acid to the plasma samples helped in achieving consistent and reproducible recovery. Initially, samples were eluted with methanol, evaporated and reconstituted with mobile phase. But the peak shape of the analyte was not acceptable at lower concentration levels and hence samples were eluted with mobile phase and injected. The mean recovery for analyte and the IS was good and reproducible. Also, the validation results and real sample analysis study encourages this extraction methodology and hence was accepted in the present study.

An ideal internal standard should mimic the analyte in as many ways as possible. It should have a similar structure, same physicochemical properties or can be a labeled analogue. For LC–MS/MS analysis, use of stable isotope–labeled drugs as internal standards proves to be helpful when a significant matrix effect is possible and to increase assay precision and limit variable recovery between analyte and the IS [19, 20]. At the initial stages of this work, several compounds were investigated to find a suitable IS and finally pitavastatin stable labeled isotope pitavastatin d_4 was found to be best for the present purpose.

System suitability and carryover effect

During method validation, the precision (% CV) of a system suitability test was found to be in the range 0.00–0.02% for retention time and 0.24–1.02% for the area response of pitavastatin and the IS.

Carryover evaluation was performed to ensure that it does not affect the accuracy and precision of the proposed method. No significant carryover was observed in blank sample after injection highest concentration of analyte (ULOQ; upper limit of quantitation) which indicates no carry–over of the analyte in subsequent samples (data not shown).

Chromatography and selectivity

The selectivity of the method was examined by analyzing blank human plasma extract (Fig. 2A) and an extract spiked only with the IS (Fig. 2B). As shown in Fig. 2A, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug–free human plasma at the retention time of the analyte and the IS. Similarly, Fig. 2B shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 2C depicts a representative ion–chromatogram for the LLOQ (CS–1) sample (0.05 ng/mL). A representative chromatograms resulting from the analysis of subject blank plasma sample and 1.00 h subject plasma sample after the administration of a 4 mg oral single dose of pitavastatin is shown in the Fig. 3.

Matrix effect and sensitivity

Matrix effect assessment was done with the aim to check the effect of different plasma lots (n=6) on the back calculated value of QC's nominal concentration. The results found were well within the acceptable limits as shown in Table 1. No considerable matrix effect was observed in all the six batches of human plasma for the analyte at low and high quality control concentrations.

The lowest limit of reliable quantification for the analyte was set at the concentration of the LLOQ (0.05 ng/mL). At this concentration, the precision and accuracy of analyte was found to be 4.72 and 103%, respectively.

Linearity, precision and accuracy

The nine–point calibration curve was found to be linear over the concentration range of 0.05–160 ng/mL for pitavastatin. After comparing the two weighting models (1/x and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration was found to produce the best fit for the concentration–detector response relationship. The mean correlation coefficient of the weighted calibration curves generated during the validation was ≥ 0.99.

The results for intra–day and inter–day precision and accuracy in plasma quality control samples are summarized in Table 2. The intra–day and inter day precision deviation values were all within 15% of the relative standard deviation (RSD) at low, middle and high quality control level, whereas within 20% at LLOQ QCs level. The intra–day and inter–day accuracy deviation values were all within 100 ± 15% of the actual values at low, middle, and high quality control level, whereas within 100± 20% at LLOQ QCs level. The results revealed good precision and accuracy.

Recovery and dilution integrity

Six replicates at low, medium and high quality control concentration for pitavastatin was prepared for recovery determination. SPE with C_{18} cartridges

provided the cleanest samples and proved to be robust. The mean overall recovery (with precision range) of pitavastatin was 96.0±1.78% (2.21–3.64%) and the recovery of IS was 94.8% with the precision range of 1.36–3.11%.

The upper concentration limit of pitavastatin can be extended to 277 ng/mL for by 1/2 and 1/4 dilutions with screened human blank plasma. The precision (%CV) for dilution integrity of 1/2 and 1/10th dilution was found to be 5.47% to 1.95%, while the accuracy results were found to be 105% and 101%, respectively.

Stability studies

Analyte stability at various conditions was evaluated. In the different stability experiments carried out viz. bench top stability (13 h), autosampler stability (44 h), wet extract stability (39 h), repeated freeze–thaw cycles (4 cycles), reinjection stability (26 h) and long term stability at −70 °C for 60 days the mean % nominal values of the analyte were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 3). Therefore, the results were found to be within the acceptable limits during the entire validation.

Stock solutions of pitavastatin and IS were found to be stable for 21 days at 2–8 °C. The percentage stability (with the precision range) of pitavastatin and the IS was 96.8% (1.48–2.73%) and 96.6% (2.37–3.64%), respectively.

Pharmacokinetic study and incurred samples reanalysis

The proposed method was successfully used to quantify pitavastatin plasma concentration for a pharmacokinetic study in healthy South Indian adult male subjects (*n*=6). Fig. 4 depicts (presented upto 24 h in order to depict the plot with better clarity) the mean plasma concentration *vs* time profile of pitavastatin after administration of a single 4 mg oral dose of pitavastatin under fasting condition. Table 4 summarizes the mean pharmacokinetic parameters of pitavastatin.

The reproducibility of the present method was established by reanalysis of incurred samples (ISR). For incurred samples analysis two plasma samples from each subject were selected and re–assayed in a single bioanalytical run. The differences in concentrations between the ISR and the initial values for all the tested samples were less than 20% (Table 5), indicating good reproducibility of the present method.

CONCLUSIONS

This paper presents the successful development and validation of a simple, sensitive and rapid LC–MS/MS method for the determination of pitavastatin in human plasma samples according to commonly acceptable FDA guidelines. The method presented has the highest sensitivity (0.05 ng/mL) and employs low plasma volume (100 µL) for processing compared to other procedures, therefore volume blood sample collected from an individual during the study is reduced significantly—this allows inclusion of additional points. Moreover, the total analysis time (chromatography and extraction) is the shortest. Thus, the advantage of this method is that a relatively more number of samples can be analyzed in short time, thus increasing the output. A simple SPE technique with direct injection (avoids drying, evaporation and reconstitution steps) for sample preparation, thereby significantly reduces the sample processing time. The method showed suitability for pharmacokinetic studies in humans. From the results of all the validation parameters, we can conclude that the developed method can be useful for bioavailability and bioequivalence (BA/BE) studies and routine therapeutic drug monitoring with the desired precision and accuracy.

Table 1: Matrix effect of pitavastatin in human plasma (*n* = 3).

Plasma lot	LQC (0.157 ng/mL)		HQC (139 ng/mL)	
	Concentration found (mean ± SD; ng/mL)	% Accuracy	Concentration found (mean ± SD; ng/mL)	% Accuracy
Lot 1	0.152 ± 0.001	96.6	143 ± 3.22	103
Lot 2	0.154 ± 0.004	97.9	144 ± 1.27	104
Lot 3	0.154 ± 0.004	98.1	142 ± 0.65	102
Lot 4	0.153 ± 0.004	97.7	143 ± 0.59	103
Lot 5	0.154 ± 0.002	98.1	146 ± 1.78	105
Lot 6	0.156 ± 0.005	99.6	145 ± 1.75	104

Table 2: Precision and accuracy data for pitavastatin

Quality control	Run	Concentration found (mean ± SD; ng/mL)	Precision (%)	Accuracy (%)
Intra–day variations (Six replicates at each concentration)				
	1	0.056 ± 0.002	2.85	108
LLOQ	2	0.055 ± 0.005	8.75	106
	3	0.057 ± 0.001	2.06	109
	1	0.161 ± 0.003	1.69	103
LQC	2	0.166 ± 0.007	4.38	106
	3	0.158 ± 0.005	3.12	100
	1	16.9 ± 0.31	1.81	96.6
MQC1	2	17.6 ± 0.66	3.75	101
	3	16.7 ± 0.25	1.47	95.7
	1	90.5 ± 0.62	0.68	104
MQC2	2	93.2 ± 3.76	4.03	107
	3	89.3 ± 1.46	1.63	102
	1	138 ± 2.24	1.62	99.5
HQC	2	141 ± 6.83	4.83	102
	3	136 ± 2.21	1.63	98.1
Inter–day variations (Eighteen replicates at each concentration)				
LLOQ		0.056 ± 0.003	5.18	108
LQC		0.162 ± 0.006	3.72	103
MQC1		17.0 ± 0.58	3.38	97.7
MQC2		91.0 ± 2.77	3.05	104
HQC		138 ± 4.69	3.39	99.9

Spiked concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 0.052, 0.157, 17.5, 87.3 and 139 ng/mL, respectively.

Table 3: Stability data for pitavastatin in plasma (*n*=6)

Stability test	QC (spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/ Stability (%)
Process[a]	0.157	0.151 ± 0.003	1.76	96.4
	139	135 ± 1.75	1.30	97.3
Process[b]	0.157	0.150 ± 0.003	2.11	95.5
	139	134 ± 2.05	1.53	96.8
Bench top[c]	0.157	0.156 ± 0.003	1.76	99.2
	139	134 ± 1.36	1.01	96.7
FT[d]	0.157	0.152 ± 0.002	1.30	96.6
	139	135 ± 1.23	0.91	97.4
Reinjection[e]	0.157	0.156 ± 0.004	2.58	99.6
	139	142 ± 3.16	2.22	103
Long–term[f]	0.157	0.152 ± 0.003	1.92	96.9
	139	135 ± 0.87	0.64	97.6

[a] after 44 h in autosampler at 10°C; [b] after 39 h at 2–8°C; [c] after 13 h at room temperature; [d] after 4 freeze and thaw cycles; [e] after 26 h of Reinjection;
[f] at –70°C for 60 days

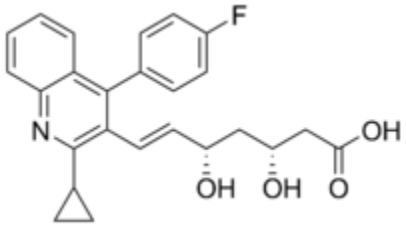
Table 4: Pharmacokinetic parameters of pitavastatin after single oral administration of 4 mg tablet to healthy South Indian male subjects (*n*=6, Mean ± SD).

Parameter	Estimated value
C_{max} (ng/mL)	115 ± 7.31
t_{max} (h)	0.63 ± 0.14
AUC_{0-t} (ng h/mL)	281 ± 24.7
AUC_{0-inf} (ng h/mL)	286 ± 27.4
$t_{1/2}$ (h)	14.2 ± 1.94

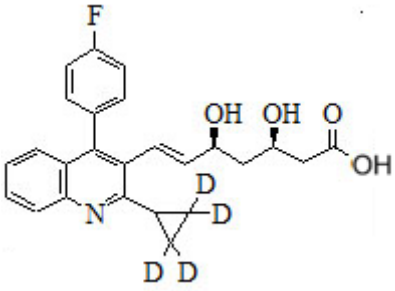
Table 5 : Incurred samples re–analysis data of pitavastatin.

Sample	Initial conc. (ng/mL)	Re–assay conc. (ng/mL)	Difference [a] (%)
1	115	116	–0.96
2	0.23	0.19	16.4
3	113	121	–6.78
4	0.41	0.46	–10.6
5	96.4	92.7	3.95
6	0.70	0.69	2.30
7	99.4	109	–9.22
8	0.31	0.29	7.26
9	108	94.3	13.3
10	0.76	0.83	–9.45
11	108	101	6.51
12	0.22	0.23	–3.12

[a] Expressed as [(initial conc.−re–assay conc.)/average]×100%.



pitavastatin



pitavastatin d_4 (IS).

Figure 1: Chemical structures of pitavastatin and pitavastatin d_4 (IS).

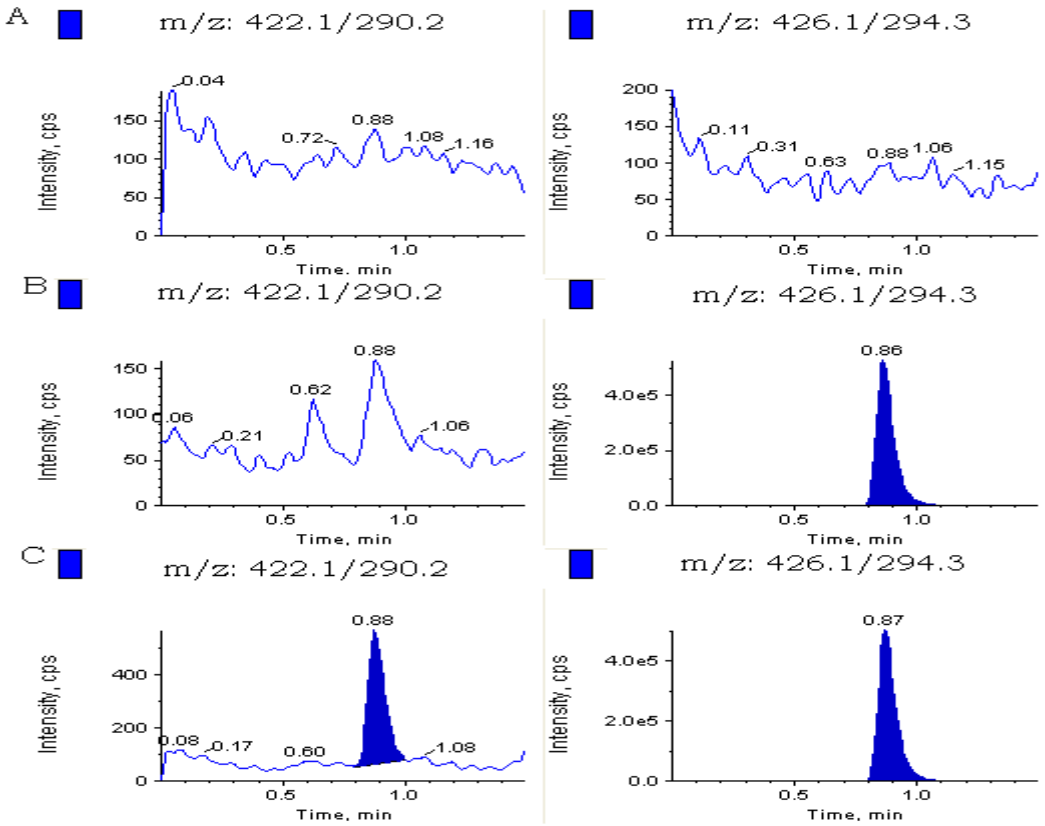


Figure 2: Typical MRM chromatograms of pitavastatin (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C).

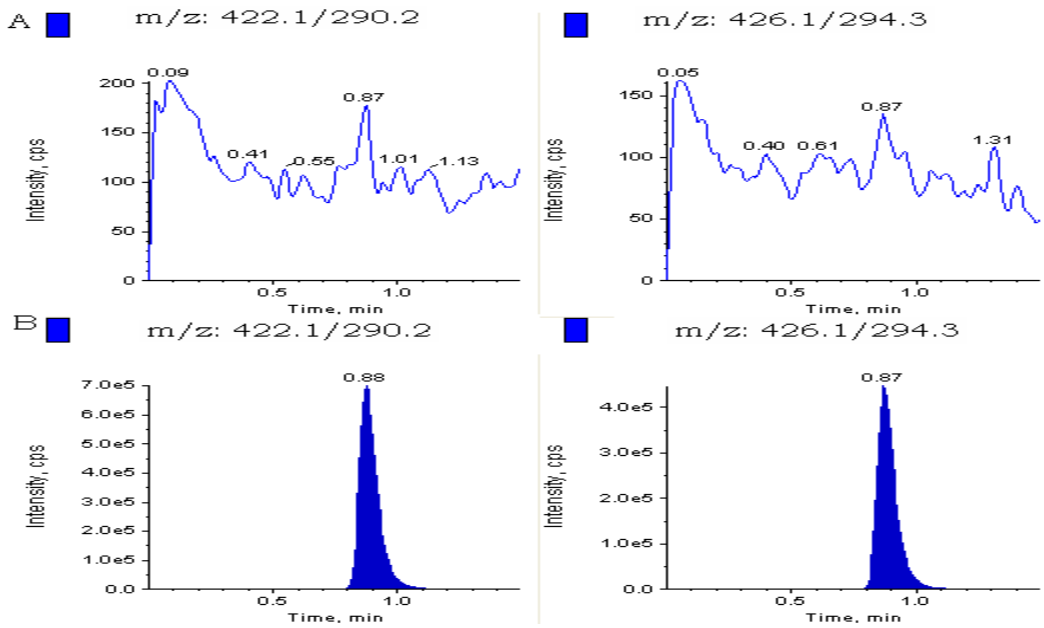


Figure 3: MRM chromatograms resulting from the analysis of subject blank plasma sample (A) and 1.00 h subject plasma sample (B), after the administration of a 4 mg oral single dose of pitavastatin tablet. The sample concentration was determined to be 98.0 ng/mL.

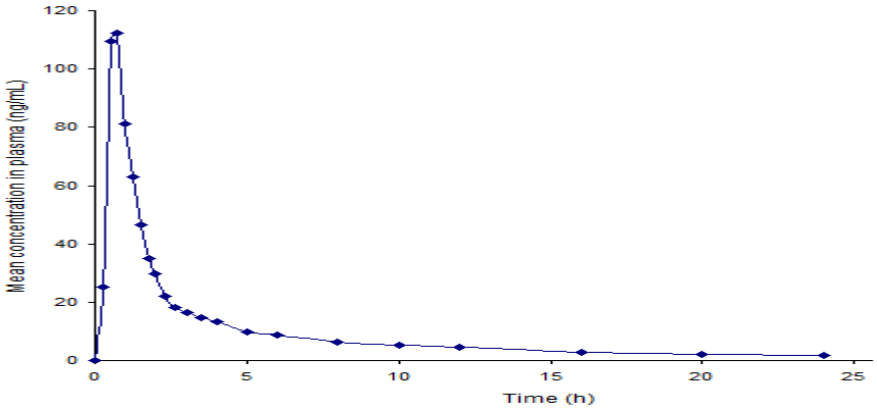


Figure 4: Mean plasma concentration–time profile of pitavastatin in human plasma following oral administration of pitavastatin (4 mg tablet) to healthy volunteers (*n*=6).

REFERENCES

1. Matsubara K, Yamamoto Y, Sonoyama K, Igawa G, Furuse M, Yatsuhashi T, Hamada T, Ogino K, Igawa O, Hisatome I and Shigemassa C. Current status of lipid management of hypertensive patients. Hypertens Res, 2003; 26(9): 699–707.
2. Shepherd J. Preventing coronary artery disease in the West Scotland: implications for primary prevention. Am J Cardiol, 1998; 82(10B): 57T–59T.
3. Williams D and Feely J. Pharmacokinetic– pharmacodynamic drug interactions with HMG –CoA reductase inhibitors. Clini Pharmacokinet, 2002; 41(5): 343–70.
4. Davidson MH and Toth PP. Comparative effects of lipid–lowering therapies. Prog Cardiovasc Dis, 2004; 7(2): 73–104.
5. Aoki T, Nishimura H, Nakagawa S, Kojima J, Suzuki H, Tamaki T, Wada Y, Yokoo N, Sato F, Kimata H, Kitahara M, Toyoda K, Sakashita M, Saito Y. Pharmacological profile of a novel synthetic inhibitor of 3–hydroxy–3–methylglutaryl–coenzyme A reductase. Arzneimittelforschung, 1997; 47(8): 904–9.
6. Wensel TM, Waldrop BA, Wensel B. Pitavastatin: a new HMG–CoA reductase inhibitor. Ann Pharmacother, 2010; 44(3): 507–14.
7. Mukhtar RY, Reid J and Reckless JP. Pitavastatin. Int J Clin Pract, 2005; 59(2): 239–52.
8. Saito Y, Yamada N, Teramoto T, Itakura H, Hata Y, Nakaya N, et al. A randomized, double–blind trial comparing the efficacy and safety of pitavastatin versus pravastatin in patients with primary hypercholesterolaemia. Atherosclerosis, 2002; 162(2): 373–9.
9. Ojha A, Guttikar S, Vayeda C, Padh H. Determination of pitavastatin from human plasma using high performance liquid chromatography with fluorescence detection. Se Pu, 2007, 25(5): 715–8.
10. Kojima J, Fujino H, Yosimura M, Morikawa H, Kimata H. Simultaneous determination of NK–104 and its lactone in biological samples by column–switching high–performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl, 1999; 724(1): 173–80.
11. Di B, Su MX, Yu F, Qu LJ, Zhao LP, Cheng MC, He LP. Solid–phase extraction and liquid chromatography/tandem mass spectrometry assay for the determination of pitavastatin in human plasma and urine for application to Phase I clinical pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci, 2008; 868(1–2): 95–101.
12. Tian L, Huang Y, Jia Y, Hua L, Li Y. Development and validation of a liquid chromatography–tandem mass spectrometric assay for pitavastatin and its lactone in human plasma and urine. J Chromatogr B Analyt Technol Biomed Life Sci, 2008; 865(1–2): 127–32.
13. Lv H, Sun JG, Wang GJ, Zhu XY, Zhang Y, Gu SH, Liang Y, Sun J. Determination of pitavastatin in human plasma via HPLC–ESI–MS/MS and subsequent application to a clinical study in healthy Chinese volunteers. Clin Chim Acta, 2007; 386(1–2): 25–30.
14. Deng JW, Kim KB, Song IS, Shon JH, Zhou HH, Liu KH, Shin JG. Determination of two HMG–CoA reductase inhibitors, pravastatin and pitavastatin, in plasma samples using liquid chromatography–tandem mass spectrometry for pharmaceutical study. Biomed Chromatogr, 2008; 22(2): 131–5.

15. Shen–Tu J, Xu X, Liu J, Hu X, Chen J, Wu L, Huang M and Zhou H. Determination of Pitavastatin in Human Plasma by LC–MS–MS. Chromatogr, 2009; 69(9–10): 1041–1047.
16. Kole PL, Venkatesh G, Kotecha J, Sheshala R. Recent advances in sample preparation techniques for effective bioanalytical methods. Biomed Chromatogr, 2011; 25(1–2): 199–217.
17. Van Eeckhaut A, Lanckmans K, Sarre S, Smolders I, Michotte Y. Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects. J Chromatogr B Analyt Technol Biomed Life Sci, 2009; 877(23): 2198–2207.
18. US DHHS, FDA and CDER. Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, 2001. Available at: http://www/ fda.gov/cder/guidance/index.htm.
19. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG and Weiner R. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007; 24(10): 1962–1973.
20. Gajula R, Maddela R, Ravi VB, Inamadugu JK, and Pilli NR. A rapid and sensitive liquid chromatography–tandem mass spectrometric assay for duloxetine in human plasma: Its pharmacokinetic application. J Pharm Anal, 2013; 3(1): 36–44.