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Simple, sensitive and rapid LC–MS/MS method for the quantitation of cerivastatin in human plasma — application to pharmacokinetic studies

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Abstract

A simple and sensitive liquid chromatography–tandem mass spectrometry method was developed and validated for estimation of cerivastatin (I) in human plasma, a potent hydroxy-methylglutaryl-coenzyme A reductase inhibitor. The analyte and internal standard (atorvastatin, II) were extracted by liquid/liquid extraction with diethyl ether/dichloromethane (70/30, v/v). The chromatographic separation was performed on reverse phase Xterra ODS column with a mobile phase of water/acetonitrile (30/70, v/v) with 0.03% formic acid. The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The mass transitions *m*/*z* 460.4 → 356.3 and 559.2 → 440.3 were used to measure I and II, respectively. The lower limit of quantitation was 10 pg/mL with a relative standard deviation of less than 15%. Acceptable precision and accuracy were obtained for concentrations over the calibration curve ranges (0.01–10 ng/mL). Sample analysis time of 2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day. The assay can be used to analyze human plasma samples to support phase I and II clinical studies.

Keywords: Cerivastatin; HMG-CoA reductase inhibitor; LC–MS/MS

1. Introduction

The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (or statins) are potent inhibitors of cholesterol biosynthesis. Several large clinical trials have demonstrated the beneficial effects of statins in the prevention of coronary heart disease [1]. However, the beneficial effects of statins may extend beyond their effects on serum cholesterol levels. Indeed, recent studies emphasize that some non-lipid-related effects of statin present a potential benefit in different diseases, such as atherosclerosis and osteoporosis [2,3]. Denoyelle et al. [4,5] and Bellosta et al. [6] have recently suggested that statins could also potentially play a beneficial role in cancer therapy. The anticancer effect of HMG-CoA reductase inhibitors may be due to blockage of Ras and Rho isoprenylation and induction of p21/p27 by inhibition of the mevalonate pathway [4,7–10].

Cerivastatin may be of particular interest, since it possesses superior lipid-lowering activity at doses equivalent to 1–3% of the doses of other statins [11,12]. It has been reported that cerivastatin can induce leukaemia specific apoptosis and inhibit the proliferation of several solid tumour cell lines [4,12,13]. More importantly, cerivastatin can improve therapeutic index as it is non-toxic to normal human bone marrow progenitors [13]. Cerivastatin also inhibits tumour invasiveness and metastasis in vitro [4]. As with other statins, cerivastatin induced cytotoxicity is mevalonate pathway dependent. Its cytotoxicity can be totally reversed by mevalonate or its derivatives [4,12,13].

Cerivastatin was withdrawn from the market in August 2001, after about 500 cases of rhabdomyolysis (including 100 deaths) were reported in patients taking the drug. In almost half of the cases, cerivastatin had been used in combination with the lipid-lowering fibrate gemfibrozil [14–17]. This

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clinically significant drug interaction probably results from a displacement effect from protein-binding sites [18,19]. For anti-hypercholesterolaemia purposes, cerivastatin requires to be long-term administered which has caused some severe side effects [20]. For anticancer purposes, cerivastatin may be used in combination with standard chemotherapeutic regimen to increase tumour cell killing, rather than being used over prolonged periods as for anti-hypercholesterolaemia treatment [10]. This would minimize the adverse effects related to long-term use of cerivastatin. Even though statins do not differ in their pharmcodynamic properties, the differences in their pharmacokinetic profiles, i.e. affinity for metabolizing enzymes, constitute the rationale for choosing a specific statin especially for combination therapy [20]. Igel et al. [20] stated that cerivastatin, pravastatin and fluvastatin are the least prone to drug interactions.

Thibault et al. [21] conducted a phase I trial of lovastatin in 88 cancer patients with advanced solid tumours. Similarly, Larner et al. [22] conducted a phase I–II trial of lovastatin in anaplastic astrocytoma and glioblastoma multiforme. Kawata et al. [23] conducted a randomized trial of pravastatin in patients with advanced hepatocellular carcinoma. Cerivastatin unlike lovastatin is an open ring, active form drug and belongs to the third generation HMG-CoA reductase inhibitor [12]. Existing data indicate that cerivastatin is the most potent HMG-CoA reductase inhibitor amongst all reported statins [11,12]. Denoyelle et al. [4] and others have recently suggested that cerivastatin could offer a novel approach in the therapy of aggressive forms of cancer in combination with other anti-cancer treatments [10,24]. There are emerging interests to explore the anticancer potentials of cerivastatin in the clinical trials, particularly in tumour sites sensitive to cerivastatin in vitro and in vivo models [4–6,10,12,25,26].

In previously published approaches, radioimmunoassay and enzyme inhibition assay lacked specificity and HPLC assays typically required 10–20 min on-column separation, and both had either a relatively higher quantitation limit (0.025 ng/mL or higher) or a large sample size requirement [27–31]. In order to fully evaluate the pharmacokinetics of cerivastatin in human plasma to support phase I and II clinical studies, it was necessary to develop and validate an assay with appropriate sensitivity, selectivity, accuracy and precision. Potentially large number of samples in clinical studies need a rapid and reliable assay. An ideal method should have simple sample preparation, fast on-column separation, and sensitive and specific detection. Liquid chromatography coupled with mass spectrometry (LC–MS) has become such an analytical tool which meets most of the above needs [32–34]. In many cases, highly specific mass spectrometric detection, especially using tandem mass spectrometry (MS–MS) just requires minimum separation on column. This will greatly shorten the assay time and make it possible to analyze large quantities of samples within a tight time frame. To date only one LC–MS/MS method has been reported for quantitation of cerivastatin in human serum, in which sensitivity of the method was obtained for 25 μL injection volume corresponding to 250 fg on-column with a total run time of 3.5 min [35]. In contrast, we report an LC–MS/MS method with an excellent sensitivity for 10 μL injection volume corresponding to 100 fg on-column with a total run time of 2 min. This paper describes the development and validation of a highly sensitive LC–MS/MS method for the quantitation of cerivastatin in human plasma.

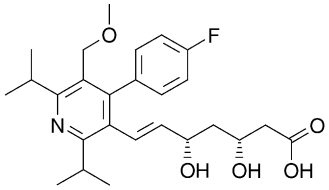
2. Experimental

2.1. Chemicals

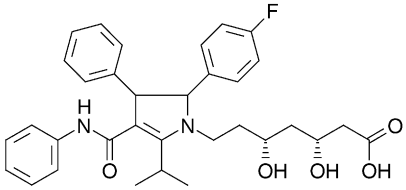
Cerivastatin and atorvastatin (internal standard, I.S.) drug substances were obtained from Cadila Healthcare Ltd. (Ahmedabad, India). Chemical structures are presented in Fig. 1. Stock solutions of cerivastatin (1 mg/mL) and I.S. (1 mg/mL) were separately prepared in 10 mL volumetric flasks with methanol. The stock solutions of cerivastatin and I.S. were stable for at least 3 months at 4 °C. HPLC-grade LiChrosolv methanol, LiChrosolv acetonitrile, diethyl ether and dichloromethane were from Merck (Darmstadt, Germany). Formic acid (100%) was from Merck (Worli, Mumbai, India). HPLC Type I water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

2.2. LC–MS/MS apparatus and conditions

The HPLC Agilent 1100 Series (Agilent Technologies, Waldbronn, Germany) is equipped with G1312A binary pump, G1379A degasser, G1367A autosampler equipped with a G1330B thermostat, G1316A thermostatted column compartment and G1323B control module. The chromatography was on Waters Xterra ODS column (3.5 μm, 100 mm × 3 mm i.d.) at 30 °C temperature. The mobile phase



Cerivastatin (I)



Atorvastatin (II, Internal Standard)

Fig. 1. Chemical structures of cerivastatin and internal standard (atorvastatin).

Table 1
Tandem mass-spectrometer main working parameters

Parameter	Value
Source temperature (°C)	400
Dwell time per transition (ms)	200
Ion source gas (gas 1) (psi)	20
Ion source gas (gas 2) (psi)	40
Curtain gas (psi)	25
Collision gas (psi)	5
Ion spray voltage (V)	5500
Entrance potential (V)	10
Declustering potential (DP) (V)	98 (analyte) and 80 (I.S.)
Collision energy (V)	50 (analyte) and 30 (I.S.)
Collision cell exit potential (V)	30 (analyte) and 13 (I.S.)
Mode of analysis	Positive
Ion transition for cerivastatin (*m*/*z*)	460.4/356.3
Ion transition for atorvastatin (*m*/*z*)	559.2/440.3

composition was a mixture of water/acetonitrile (30/70, v/v) with 0.03% formic acid, which was pumped at a flow-rate of 0.4 mL/min.

Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) using multiple reaction monitoring. A turbo electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized in Table 1. Data processing was performed on Analyst 1.3 software package (SCIEX).

2.3. Sample processing

A 500 μL volume of plasma sample was transferred to a 15 mL glass test tube and then 25 μL of I.S. solution (20 ng/mL) was spiked. After vortexing for 30 s, 5 mL aliquot of extraction solvent, diethyl ether/dichloromethane (70/30) was added using dispensette organic (Brand GmbH, Postfach, Germany). The sample was vortex-mixed for 5 min using a Multi-Pulse Vortexer (Glas-Col, Terre Haute, USA). The sample was then centrifuged using Multifuge 3S-R (Kendro Laboratory Products, Sorvall-Heraeus, Germany) for 5 min at 800 × *g*. The organic layer was quantitatively transferred to a 5 mL glass tube and evaporated to dryness using a TurboVap LV Evaporator (Zymark, Hopkinton, MA, USA) at 40 °C under a stream of nitrogen. Then, the dried extract was reconstituted in 200 μL of water/methanol (50/50, v/v; diluent) and a 10 μL aliquot was injected into chromatographic system.

2.4. Bioanalytical method validation

2.4.1. Calibration and control samples

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution using diluent. The I.S. working solution (20 ng/mL) was prepared by diluting its stock solution with diluent. Twenty five microliter working solutions were added to 475 μL drug-free plasma to obtain the cerivastatin concentration levels of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 ng/mL. The quality control samples were prepared in pool, at concentrations of 0.01 (LLOQ), 0.05 (low), 1.0 (medium) and 8.0 (high) ng/mL, as a single batch at each concentration, and then divided in aliquots that were stored in the freezer at −70 °C until analysis.

2.4.2. Calibration curve

A calibration curve was constructed from a blank sample (a plasma sample processed without an I.S.), a zero sample (a plasma processed with I.S.) and 10 non-zero samples covering the total range (0.01–10 ng/mL), including lower limit of quantification (LLOQ). Such calibration curves were generated on six consecutive days. Linearity was assessed by a weighted ($1/x^2$) least squares regression analysis. The calibration curve had to have a correlation coefficient (r^2) of 0.99 or better. The acceptance criteria for each back-calculated standard concentration was 15% deviation from the nominal value.

2.4.3. Precision and accuracy

The within-batch imprecision and inaccuracy was determined by analyzing six sets of quality control samples in a batch. The between-batch imprecision and inaccuracy was determined by analyzing six sets of quality control samples on three different batches. The quality control samples were randomized daily, processed and analyzed in position either (a) immediately following the calibration curve, (b) in the middle of the batch, or (c) at the end of the batch. The acceptance criteria of within- and between-batch imprecision was <±15% and inaccuracy was <15% across the calibration range [36].

2.4.4. Recovery

Recovery of cerivastatin was evaluated by comparing the mean peak areas of six extracted low and high quality control samples to mean peak areas of six neat reference solutions (unprocessed). Recovery of atorvastatin (I.S.) was evaluated by comparing the mean peak areas of eight extracted samples to mean peak areas of eight neat reference solutions (unprocessed) of the same concentration.

2.4.5. Freeze−thaw stability

The freeze−thaw stability of the analyte was determined at low and high quality control samples (n = 6) over three freeze−thaw cycles within 3 days. In each freeze–thaw cycle, the frozen plasma samples were thawed at room temperature for 2–3 h and refrozen for 12−24 h. After completion of each cycle the samples were analyzed and results were compared with that of zero cycle.

2.4.6. Long-term storage stability

The storage time in long-term stability evaluation brackets the time between the first sample collection and the last sample analysis. Aliquots of human plasma samples (n = 6) spiked with analyte at concentrations of 0.05 and 8.0 ng/mL

were analyzed on day 1. Then the samples from the same pools were analyzed against calibration curves from freshly prepared standards after storage at −70 °C for 15 days.

2.4.7. Processed sample stability

Twelve sets of quality control samples were prepared as described in Section 2.3 and placed into the autosampler to +15 °C. Six sets were analyzed at once (control) and six sets after 24 h. The results were compared with that of control samples.

2.4.8. Short-term stability

Six aliquots each of the low and high quality control samples were kept at room temperature for 24 h. After 24 h the samples were analyzed and the results were compared with that of day 1 samples.

3. Results and discussion

3.1. Mass spectrometry

In order to develop a method with the desired sensitivity (0.01 ng/mL), it was necessary to use MS–MS detection, as the compound did not possess the UV absorbance or fluorescence properties needed to achieve this limit. The inherent selectivity of MS–MS detection was also expected to be beneficial in developing a selective and sensitive method. The positive ion TurboIonspray Q1 mass spectrum and product ion mass spectrum of cerivastatin are shown in Figs. 2 and 3 , respectively. The positive ion TurboIonspray Q1 mass spectrum and product ion mass spectrum of internal standard are shown in Figs. 4 and 5, respectively. $[M + H]^+$ was the predominant ion in the Q1 spectrum, and was used as the precursor ion to obtain product ion spectra. The most sensitive mass transition was from *m*/*z* 460.4 to 356.3 for cerivastatin and *m*/*z* 559.2 to 440.3 for atorvastatin (I.S.).

The ion spray voltage, declusturing potential, entrance potential, collision energy and collision cell exit potential were optimized to deliver effective fragmentation of the $[M + H]^+$ without excessive fragmentation, which would have reduced sensitivity. The parameters presented in Table 1 are the result of this optimization.

3.2. Method development

The HPLC conditions were optimized such that the retention time was kept short at ≈1.3 min in order to assure high throughput. The total runtime for each sample was 2 min. The Waters Xterra C18 HPLC column (3.5 μm, 100 mm × 3 mm i.d.) was chosen based on positive experience in the chromatography of acid compounds and because it demonstrates good stability at the low pH of the mobile phase. The composition of an isocratic mobile phase with water/acetonitrile (30/70, v/v) with 0.03% formic acid was chosen for its compatibility with mass spectrometric detection. The formic acid was found to be necessary in order to lower the pH to protonate the acidic cerivastatin and thus deliver good peak shape.

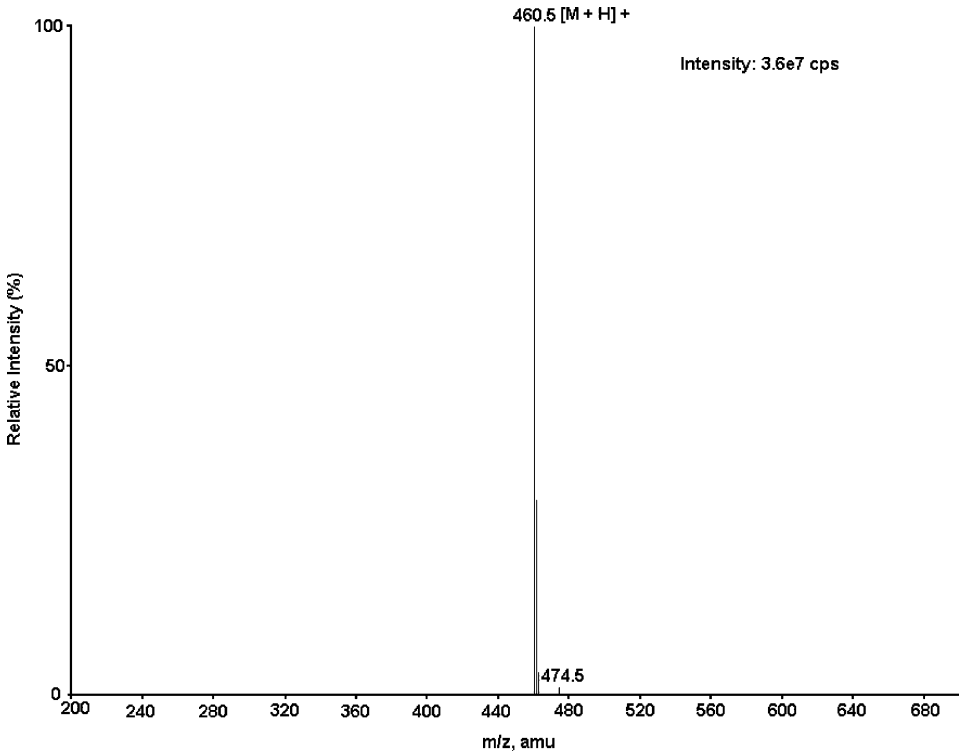


Fig. 2. Full scan positive ion TurboIonspray Q1 mass spectra of cerivastatin.

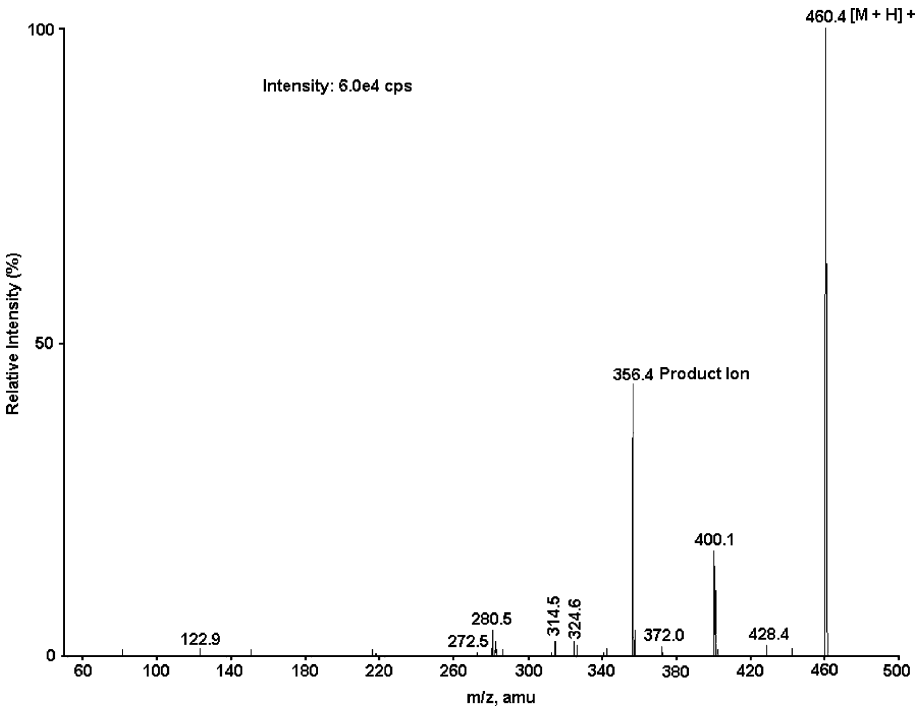


Fig. 3. Full scan positive ion TurboIonspray product ion mass spectra of cerivastatin.

The percentage of formic acid was optimized to maintain this peak shape whilst being consistent with good ionization and fragmentation in the mass spectrometer.

A stable isotope labeled analyte has to be used as an internal standard to deal with sample matrix effects. Since such internal standard is not commercially available, an alternative approach has been used. Internal standard substance should match the chromatographic retention, recovery and ionization properties with the matrix of cerivastatin. Atorvastatin (Fig. 1) was found to fulfill these criteria sufficiently. The

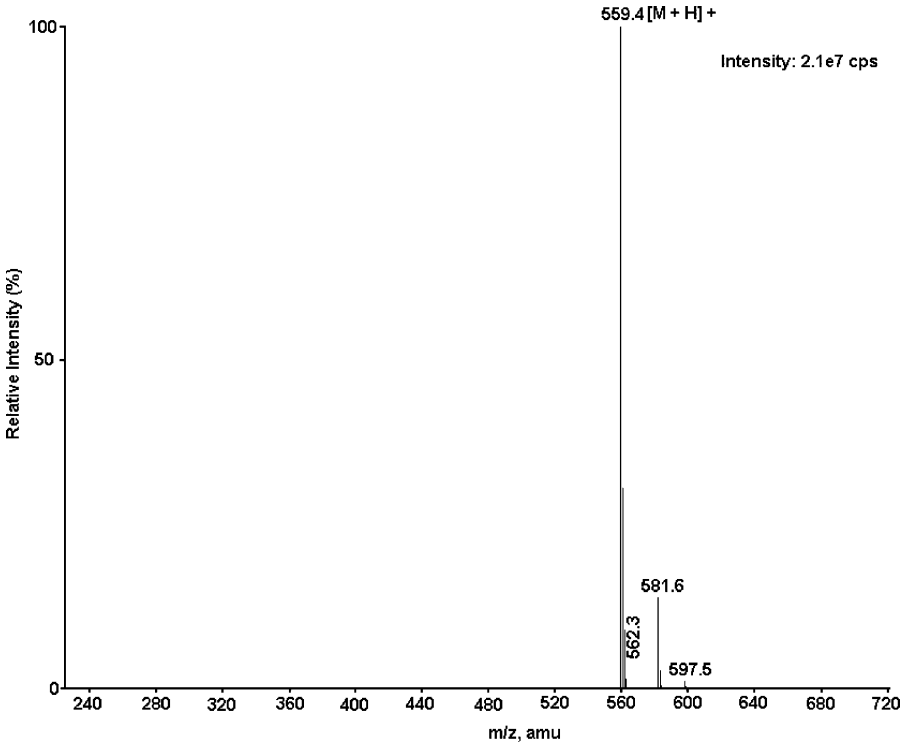


Fig. 4. Full scan positive ion TurboIonspray Q1 mass spectra of internal standard, atorvastatin.

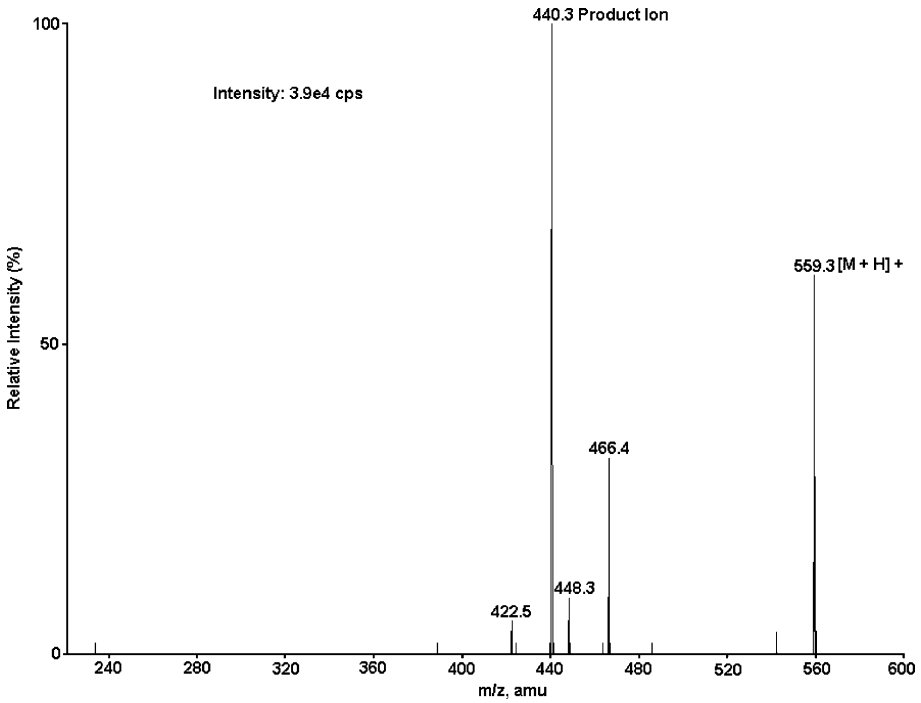


Fig. 5. Full scan positive ion TurboIonspray product ion mass spectra of internal standard, atorvastatin.

matrix effects were similar to the matrix effects of cerivastatin. Hence atorvastatin has been chosen as internal standard in the quantitative assay for cerivastatin from plasma.

3.3. Extraction recovery

The extraction recovery of cerivastatin was 105.15% on average, and the dependence on concentration is negligible. The recovery of the internal standard was 76.92% at the concentration used in the assay (20 ng/mL). With the high extraction recovery of cerivastatin and internal standard (to compensate for extraction variability and MS sensitivity), the assay has proved to be robust in high throughput bioanalysis.

3.4. Specificity and selectivity

The specificity/selectivity of the method was investigated by analyzing blank human plasma extract (Fig. 6) and an extract spiked only with the internal standard (Fig. 7) (n = 6). As shown in Fig. 6, no significant interference in the blank plasma traces was seen from endogenous substances in drug-free human plasma at the retention time of the analyte. Fig. 7 shows the absence of interference from the internal standard to the MRM channels of the analyte. Fig. 8 depicts a representative ion-chromatogram for the LLOQ (0.01 ng/mL) of the calibration curve. The mean response for the analyte peak at the assay sensitivity limit (0.01 ng/mL) was ≈12.78-folds greater than the mean response for the peak in six blank human plasma samples at the retention time of the analyte. Excellent sensitivity was observed for 10 μL injection volume corresponding to 100 fg on-column. The product ion-chromatogram obtained from an extracted plasma sample of a subject was depicted in Fig. 9.

3.5. Bioanalytical method validation

3.5.1. Calibration curves

Calibration curve was linear over the concentration range of 0.01–10 ng/mL for the analyte. The 10-point calibration curve gave acceptable results for the analyte and was used for all the calculations. The calibration curve was fitted to a $1/x^2$ weighted linear regression (where x was the concentration of the analyte) as this was judged to be the weighting, which made the assay most robust. The mean linear regression equation of calibration curve for the analyte was y= 0.0312(±0.0286) + 1.8250(±0.2668)x, where y was the peak area ratio of the analyte to I.S. and x was the concentration of the analyte. The mean correlation coefficient of the weighted calibration curves generated during the validation was 0.9983 for the analyte. Table 2 summarizes the calibration curve results for the analyte. The calibration curves obtained as described above were suitable for generation of acceptable data for the concentrations of the analyte in the samples during the between-batch and within-batch validations.

3.5.2. Lowest concentration

The lower limit of quantitation (LLOQ) of cerivastatin in human plasma assay was 0.01 ng/mL. The between-batch imprecision at the LLOQ was 2.96% and inaccuracy was −2.27% (Table 3). The within-batch imprecision was 11.16% and inaccuracy was −6.29% for cerivastatin.

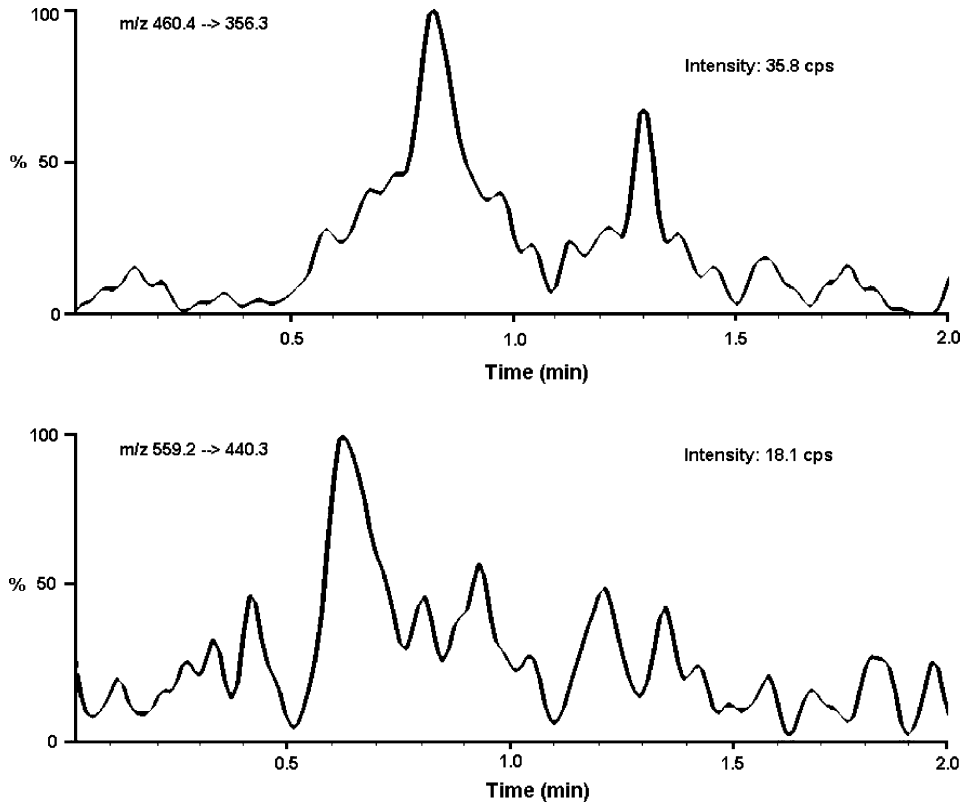


Fig. 6. MRM ion-chromatograms resulting from the analysis of blank (drug and internal standard free) human plasma for cerivastatin and internal standard.

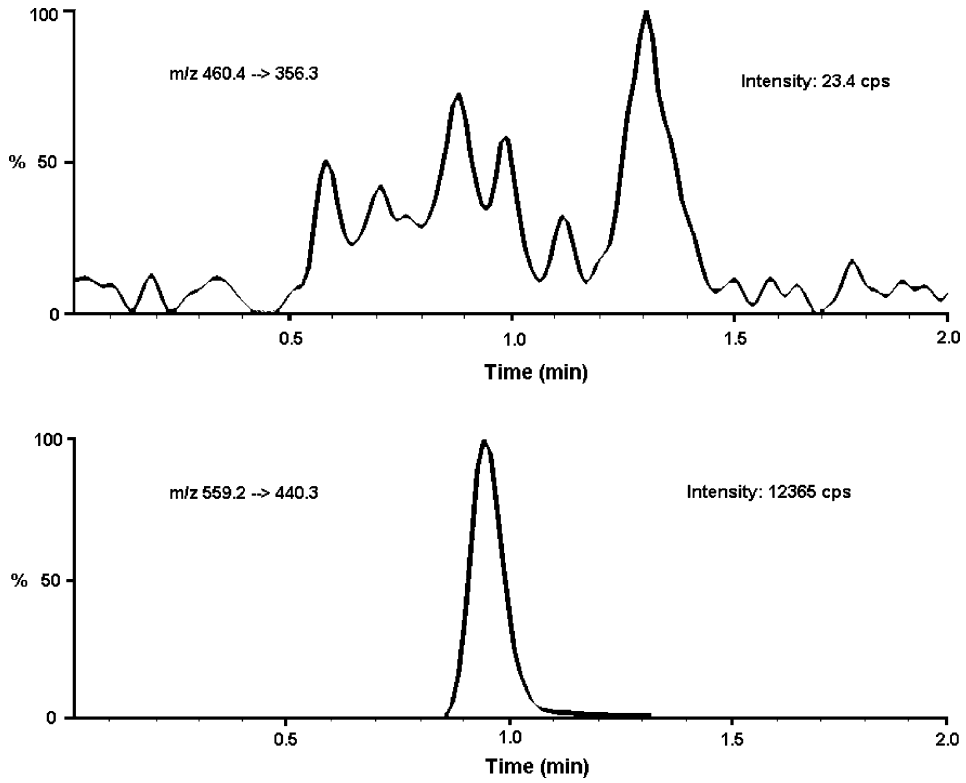


Fig. 7. MRM ion-chromatograms resulting from the analysis of blank (drug-free spiked with I.S.) human plasma for cerivastatin and internal standard.

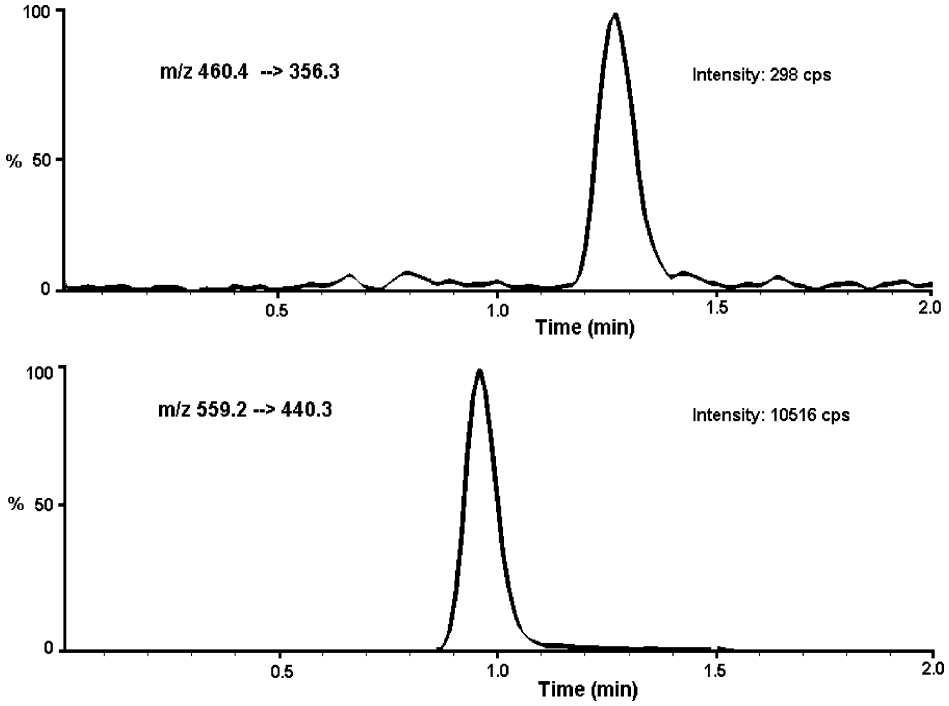


Fig. 8. Representative MRM ion-chromatograms resulting from the analysis of 0.01 ng/mL (LLOQ) of cerivastatin spiked with the internal standard.

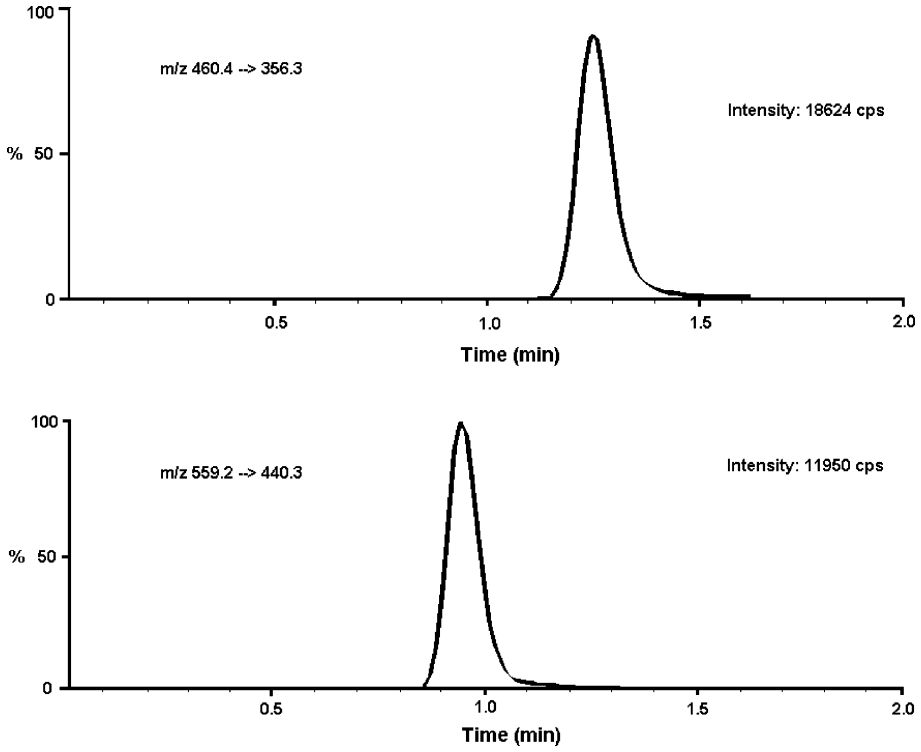


Fig. 9. MRM ion-chromatograms resulting from the analysis of subject plasma sample after the administration of 0.3 mg oral single dose of cerivastatin. The sample concentration was 640 pg/mL.

Table 2
Precision and accuracy data of back-calculated concentrations of calibration samples for cerivastatin in human plasma

Concentration added (ng/mL)	Concentration found (mean ± S.D. $n = 6$) (ng/mL)	Imprecision (%)	Inaccuracy (%)
0.01	0.009 ± 0.001	9.94	−7.41
0.02	0.020 ± 0.002	8.86	2.03
0.05	0.052 ± 0.004	8.09	4.73
0.1	0.099 ± 0.006	5.73	−0.66
0.2	0.208 ± 0.010	4.63	3.88
0.5	0.512 ± 0.020	3.87	2.40
1.0	0.998 ± 0.076	7.57	−0.17
2.0	1.980 ± 0.080	4.02	−1.02
5.0	5.034 ± 0.306	6.08	0.68
10.0	9.786 ± 0.451	4.61	−2.14

Table 3
Precision and accuracy of the method for determining cerivastatin concentrations in plasma samples

Concentration added (ng/mL)	Within-batch precision ($n = 6$)			Between-batch precision ($n = 3$)		
	Concentration found (mean ± S.D.) (ng/mL)	Imprecision (%)	Inaccuracy (%)	Concentration found (mean ± S.D.) (ng/mL)	Imprecision (%)	Inaccuracy (%)
0.01	0.009 ± 0.001	11.16	−6.29	0.010 ± 0.000	2.96	−2.27
0.05	0.053 ± 0.004	7.95	5.42	0.047 ± 0.005	10.18	−6.92
1.0	0.975 ± 0.069	7.11	−2.46	1.023 ± 0.104	10.29	2.32
8.0	7.617 ± 0.441	5.79	−4.79	8.031 ± 0.829	10.39	0.39
100[a]	99.254 ± 0.376	7.59	−0.75	99.555 ± 0.269	0.27	−0.45

[a] The sample was processed with 20-fold dilution.

3.5.3. Middle and upper concentrations

The middle and upper quantitation levels of cerivastatin ranged from 0.05 to 8.0 ng/mL in human plasma. For the between-batch experiment, the imprecision ranged from 10.18% to 10.39% and inaccuracy ranged from −6.92% to 2.32% (Table 3). For the within-batch experiment, impression was <±12% and inaccuracy was <7% and were in keeping with the recommendations of Shah et al. [36].

3.5.4. Freeze−thaw stability

The results showed that the analyte was stable in human plasma through three freeze−thaw cycles (Table 4). The imprecision was <±10% and inaccuracy was <14%. The results demonstrated that human plasma samples could be thawed and refrozen without compromising the integrity of the samples. The stability data were used to support repeat analysis.

Table 4
Stability of the samples

Sample concentration (ng/mL) ($n = 6$)	Concentration found (mean ± S.D.) (ng/mL)	Imprecision (%)	Inaccuracy (%)
Short-term stability – 24 h in plasma			
0.05	0.057 ± 0.005	8.67	6.67
8.0	8.353 ± 0.782	9.36	9.67
Freeze and thaw stability			
Cycle 1			
0.05	0.049 ± 0.002	3.77	−8.67
8.0	7.715 ± 0.743	9.63	−13.12
Cycle 2			
0.05	0.059 ± 0.003	5.73	11.42
8.0	9.321 ± 0.393	4.22	4.96
Cycle 3			
0.05	0.058 ± 0.006	9.53	8.64
8.0	9.385 ± 0.307	3.27	5.68
Autosampler stability – 24 h (after extracting and reconstitution)			
0.05	0.046 ± 0.003	5.48	−8.59
8.0	7.440 ± 0.318	4.28	−6.85
15-days stability at −70 °C			
0.05	0.053 ± 0.004	6.99	−1.91
8.0	7.020 ± 0.594	8.46	−7.83

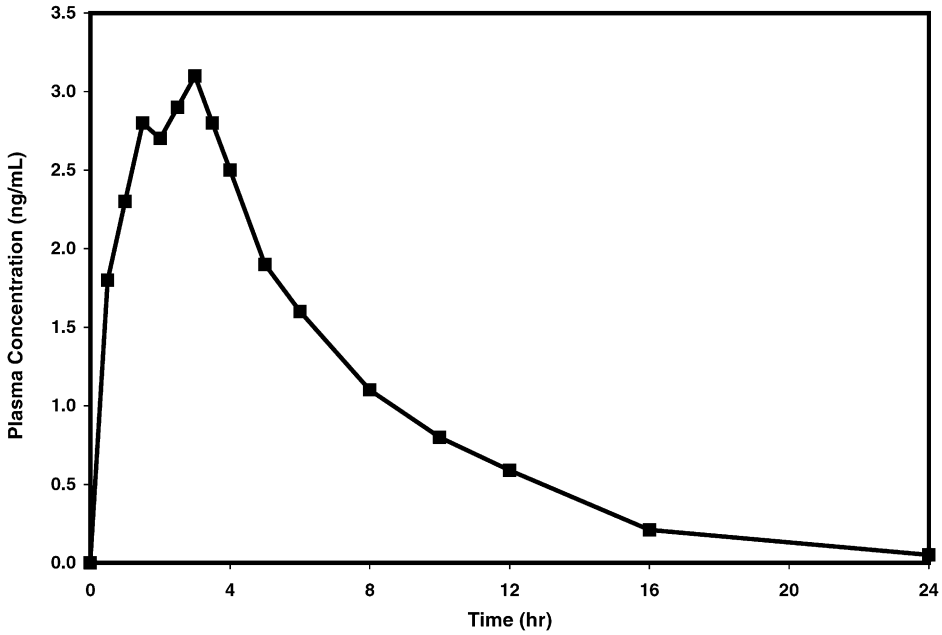


Fig. 10. Concentration vs. time profile over 24 h of cerivastatin in human plasma from a subject receiving a single 0.3 mg oral dose of cerivastatin.

3.5.5. Long-term storage stability

The imprecision and inaccuracy for the analyte on day 15 was <±7% and <8%, respectively (Table 4). The sample long-term storage stability at −70 °C was acceptable for storage for subject samples.

3.5.6. Processed sample stability

The imprecision and inaccuracy for the analyte was <±6% and <9%, respectively (Table 4). The results demonstrated that extracted samples could be analyzed after keeping in the autosampler for at least 24 h with an acceptable precision and accuracy.

3.5.7. Short-term stability

The imprecision and inaccuracy for the analyte was <±10% and <10%, respectively (Table 4). The results indicate that the analyte was stable in neat plasma for up to 24 h at room temperature.

3.6. Dilution

The upper concentration limits can be extended with acceptable precision and accuracy to 100 ng/mL by a 20-fold dilution with control human plasma. The results suggested that samples whose concentrations are greater than the upper limit of the calibration curve can be assayed to obtain acceptable data (Table 3).

3.7. Matrix effect

The matrix effects in the LC–MS/MS method were evaluated by spiking blank plasma extracts with low and high QC samples. Six independent plasma lots were used with six samples from each lot. Percent nominal concentrations estimated were well within the acceptable limits. Hence the effect of matrix on estimation of drug is negligible.

3.8. Application

The validated method has been successfully used to quantify the cerivastatin concentration in the human plasma samples after the administration of a single 0.3 mg oral dose of cerivastatin. The concentration versus time profile of a subject receiving a single dose of cerivastatin is presented in Fig. 10.

4. Conclusions

A high throughput and sensitive LC–MS/MS method for the quantitation of cerivastatin in human plasma was developed and validated to support cerivastatin analysis in clinical studies. Bioanalytical method demonstrated in this publication has broader calibration range with acceptable accuracy and precision. With dilution integrity up to 20-folds, we have established that, upper limit of quantitation is extendable up to 100 ng/mL. Hence, this method is useful for single and multiple ascending dose studies in human subjects. Acceptable data were generated for cerivastatin using weighted linear regression ($1/\text{concentration}^2$) and full calibration curves for human plasma samples. The desired sensitivity for cerivastatin was achieved with an LLOQ of 0.01 ng/mL. Acceptable precision and accuracy were obtained for concentrations above the sensitivity limit and within the calibration curve range of 0.01–10 ng/mL. Cerivastatin was shown to be stable in routine analysis conditions and in human plasma for up to

15 days when stored at −70 °C. The method is fast, rugged with a throughput more than 400 sample per day. The method described is simple, rapid, sensitive, specific, accurate, reproducible and validated as per FDA guidelines [37].

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