ORIGINAL ARTICLE

DEVELOPMENT AND VALIDATION OF AN LC-MS/MS METHOD FOR DETERMINATION OF ATORVASTATIN IN HUMAN PLASMA.

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Abstract
Background: A novel method for the estimation of Atorvastatin in human plasma by using LC-MS/MS and the analyte is Atorvastatin and internal standard is Rosuvastatin have extracted with the tertbutyl methyl ether: n-hexane (70:30, v/v) from human plasma.
Methods: The chromatographic severance was attained of the peak using Agilent Zorbax Eclipse XDB-C_8, (100 mm X 4.6 mm, 3.5 µm) column with a run time is 2.5 min. Atorvastatin and Rosuvastatin were recorded at the total ion current of their relevant multiple reaction monitoring. The LC-MS/MS system composed an Agilent 1100 infinity combined with an AB Sciex Qtrap4000 Thermo Finnigan TSQ quantum discovery triple quadrupole mass spectrometer. All of the parameters must be validated like selectivity, accuracy, precision, linearity, lower limit of quantification, matrix effect, recovery reached the acceptance criteria under the following ICH guidelines.
Results: Atorvastatin has checked the various stability studies like short-term stability at 25 ^oC, long-term stability for 55 days at -70^oC, wet extract stability for 54 hours, autosampler stability for 63 hours, benchtop stability for 14 hours and, freeze-thaw stability at -60 ^oC. Hence, it can be used for routine drug analysis and bioequivalence studies of Atorvastatin in human plasma samples.
Conclusion: The proposed LC-MS/MS method was simple, rapid, precise and accurate for the determination of Atorvastatin in human plasma. The developed LC-MS/MS method can apply for the bioequivalence and pharmacokinetic studies of Atorvastatin in human plasma samples.

Keywords: Atorvastatin, rosuvastatin, estimation, human plasma, LC-MS/MS and validation.

Introduction

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase and it is chemically called as 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Atorvastatin is designated for asthma, nasal polyposis and Crohn's disease[1] and it was used for long-term management of asthma and chronic obstructive pulmonary disease with the help of inhaled corticosteroid therapy[2]. A relevant number of studies for estimation of Atorvastatin have been reported, the methods employed include UV spectroscopy[3,4], HPLC[5-7] and LC-MS[8-10]. Pharmacokinetics and pharmacodynamics of Atorvastatin have been measured in healthy volunteers[11,12] and primary biliary cirrhosis patients[13]. However, no studies regarding the estimation of Atorvastatin in human plasma by using LC-MS/MS have been published so far. The goal of the present study was the estimation of Atorvastatin in human plasma by using LC-MS/MS. Besides, until now, no studies have been reported in the scientific literature regarding the data of full bio-analytical validation of estimation of Atorvastatin in human plasma by using LC-MS/MS. This paper reports the simple, sensitive, rapid, precise and accurate method for the estimation of Atorvastatin in human plasma by using LC-MS/MS. Based on the data obtained the LC-MS/MS method has been applied for analysis of commercial and bioequivalence studies of Atorvastatin samples.

Methodology

Materials

Atorvastatin was procured from Aarti Industries Limited (Mumbai, India). Rosuvastatin was procured from Clearssynth Labs (Mumbai, India). Acetonitrile and methanol, tert butyl methyl ether of HPLC grade were purchased from J.T. Baker (Philipsburg, USA). Formic acid, analytical grade, n-hexane and water, both HPLC grade, were purchased from Merck Limited (Mumbai, India).

Instrument

The LC-MS/MS system composed an Agilent 1100 infinity combined with an AB Sciex Qtrap 4000 Thermo Finnigan TSQ quantum discovery triple quadrupole mass spectrometer and the control was done by the Analyst software version 1.4.2. Ultrapure water (18 MΩ/cm) was produced using the ULTRA-CLEAR system (Richfield, USA). The pH of the buffer solution was measured using a pH/mv-meter Consort P501 (Turnhout-Belgium), provided with a combined pH electrode. UV-vis spectra were recorded on Jasco V-530 spectrophotometer (Tokyo, Japan), in 10 mm quartz cells.

Solutions of LC-MS/MS

A stock solution of Atorvastatin containing 200 µg/ml was prepared in a 10 ml volumetric flask, by dissolving 0.0020 gm of Atorvastatin in methanol (HPLC grade). The flask was filled up to the mark with the same solvent. A stock solution of Rosuvastatin (internal standard) containing 200 µg/ml was prepared in a 10 ml volumetric flask, by dissolving 0.0020 gm Rosuvastatin (internal standard) in acetonitrile and water (50:50, v/v) as diluent. The flask was filled up to the mark with the same solvent. Working solutions containing all of the analytes were prepared in the range 0.100 ng/ml to 3.00 ng/ml using (acetonitrile: water, 50:50, v/v) as diluting the solvent. All the analytes solutions were prepared daily and kept at 2-8°C before and between injections to prevent sample degradation. An ammonium formate buffer solution pH 4.20 was prepared by mixing 0.1578 g of ammonium formate in 500 ml of ultra-pure water. If necessary the pH could be adjusted to pH 4.20 with 0.1% formic acid solution.

Sample collection and preparation

Blood samples were collected into blank tubes and centrifuged for 5 minutes at approximately 4000 rpm at 4°C. The plasma samples (supernatant) were separated and stored at -20°C until analysis. Atorvastatin was extracted from the plasma samples where with protein precipitation. Concisely, 200 µl of plasma sample was extracted in 4 ml of extraction solvent (tert butyl methyl ether:n-hexane, 70:30, v/v) including the internal standard (Rosuvastatin at 200 ng/ml). The mixture was vortexed for 20 min and centrifuged at 4000 rpm, for 5 min at 4°C. 3 ml supernatant layer was separated and evaporated under a gentle stream of nitrogen gas at 45°C up to dryness. Then the residue was reconstituted with 500 µl mobile phase (acetonitrile:5Mm ammonium formate buffer in 0.1% formic acid, 60:40, v/v) and loaded into a pre-labeled autosampler vial.

Chromatographic and mass spectrometric conditions

Chromatographic studies were performed using an Agilent Zorabax Eclipase XDB-C_8 purchsed from Thermo Electron corporation, USA, with the dimentions 150 mm X 4.6 mm and 3.5 µm particle size and the injection volume was 5 µl. The mass spectrometer was operated under positive ionization mode and the detection of the analytes was based on multiple reaction monitoring of m/z 431.3/323.1 and 313.40/245.30 for Atorvastatin and Rosuvastatin, respectively. The ion spray voltage, the source temperature, declustering potential, collision cell exit potential, entrance potential and dwell time sets at 5000V, 500°C, 90V, 11V, 10V and 200ms, respectively.

Validation of the method

The method was validated according to the ICH guidelines[14].

Software

Calculating the molecular mass of analyte and internal standard by using version 1.4.2 for Analystsoftware.

Results

Method development

Atorvastatin and all of the compounds enclosed in this study ionize according to the pH mobile phase. The development of the new LC-MS/MS method is based on the behavior of ionizable compounds that have retention closely related to the pH of the mobile phase. The results were shown in Figure 1 and Figure 2. As shown in Figure 1, Atorvastatin exists molecular mass: parent peak molecular massis 413.5 m/z and daughtier peak molecular mass is 323.1 m/z, respectively.

Method validation

The final developed method (as described in the Experimental section) was validated, checking for linearity, precision, accuracy, matrix effect and stability studies.

Linearity

Linearity has been checked directly for each compound studied in this paper by dilution of a standard stock solution. The slope and y-intercept were provided as equations to there with the correlation coefficient to demonstrate the linearity of the method. Linearity was established for a working solution containing a concentration between 0.103-3.010 ng/ml for Atorvastatin. The equation was obtained y= 0.101+0.00158 (r^2=0.9989) shown in Figure 2.

Precision and accuracy

The precision was estimated by both with-in batch precision and between batch precision. According to the international rules of validation of with-in batch, precision was checked on four different concentrations like HQC, MQC, LQC and, LLOQ QC. Between batch, precision was established on another day, by a different investigator using the percent coefficient of

variation. The obtained values for %CV are below 15% (of HQC, MQC and LQC) and below 20% (LLOQ QC) showing a good precision of the method. The results are present in Table 1.

Accuracy was determined by calculating the recovery of each analyte at three different concentration levels like HQC, MQC and LQC. The obtained values for the recoveries were in the range of 72.48-81.48%. The results are present in Table 2.

Matrix effect

It is a combined effect of all components of the sample other than the analyte. The matrix effect was checked by three different concentration levels, these are HQC, MQC and LQC. The %CV values below 15% showing a good matrix effect of the method. The results are presented in Table 3.

Stability studies

Stabilities were estimated at low and high concentrations of the sample of Atorvastatin in plasma. Checking the stabilities like short term stability at -20^{0}C, long term stability for 55 days at -70^{0}C, wet extract stability for 54 hours, autosampler stability for 63 hours, benchtop stability for 14 hours and freeze and thaw stability (3) cycles at -70^{0}C, respectively. The %CV values below 15% showing a good stability effect of the method. The results are shown in Table 4.

Conclusion

The proposed LC-MS/MS method was simple, rapid, precise and accurate for the determination of Atorvastatin in human plasma. Sample preparation showed high recovery for the quantitative estimation of Atorvastatin in human plasma and this method allows higher sample throughout put due to the short chromatography time (3.5 min) and simple sample preparation. Thus, the developed LC-MS/MS method can apply for the bioequivalence and pharmacokinetic studies of Atorvastatin in human plasma samples.

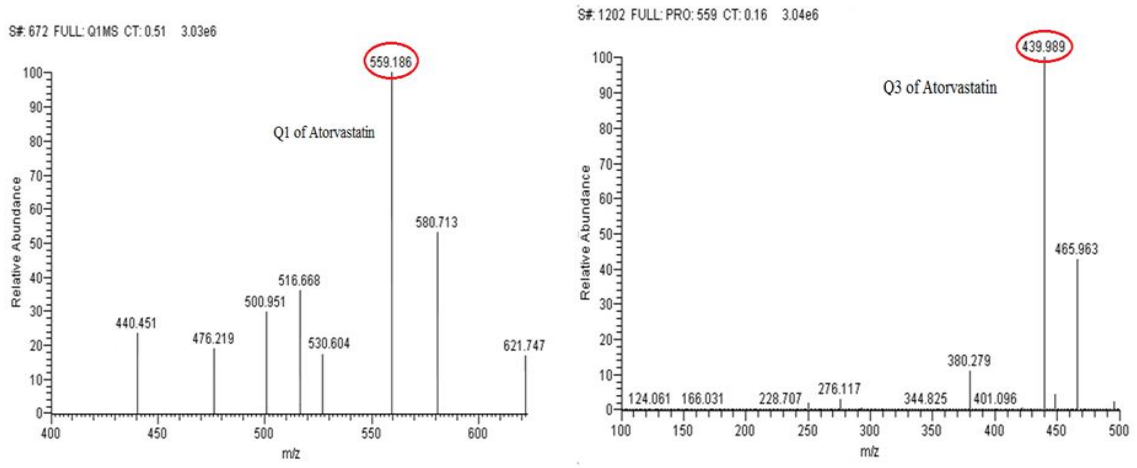

Figure 1. Product ion spectra of Atorvastatin

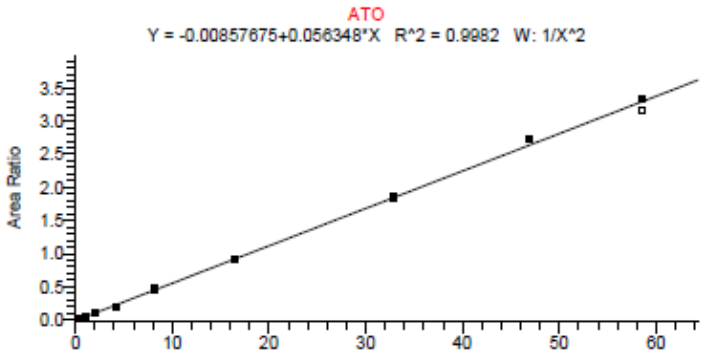

Figure 2. Linearity plot of Atorvastatin

Table 1. Precision data of Atorvastatin

Within-batch precision (n=6) for Atorvastatin			
Sample ID	**Concentration found (mean±SD, n=6; µg/ml)**	**Precision (%)**	**Accuracy (%)**
HQC	2.633± 0.155	5.26	105.58
MQC	1.570±0.073	4.65	99.61
LQC	0.268±0.010	3.86	103.40
LLOQ QC	0.114±0.010	9.19	107.17
Between batch precision for Atorvastatin			
HQC	2.843± 1.94	6.85	107.98
MQC	0.543±0.033	6.26	104.47
LQC	0.266±0.017	6.73	102.33
LLOQ QC	0.113±0.008	7.81	105.86

Note: S.D. = standard deviation; %CV = percent coefficient of variation

Table 2. Accuracy data of Atorvastatin

Parameter	**LQC**	**MQC**	**HQC**
% Recovery	76.43	72.48	81.48
% CV	2.92	6.71	4.54

Table 3. Matrix effect data of Atorvastatin

Parameter	**HQC**	**MQC**
%Accuracy	100.12	99.89
S.D(+/-)	0.002	0.065
%CV	0.95	2.50

Table 4. Stability studies data of Atorvastatin

Parameter	Results	
	HQC	**LQC**
Short term stability(-20^{0}C)	105.16%	103.27%
Long term stability(-70^{0}C) 55 days	105.55%	105.38%
Wet extract stability(54 hours)	104.51%	99.87%
Autosampler stability(63 hours)	99.00%	98.21%
Bench-top stability(14 hours)	93.56%	95.71%
Freeze & thaw stability(3)cycles at -60^{0}C	107.80%	100.32%

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