Quantitative Determination of Atorvastatin, Ortho-Hydroxy Atorvastatin, Para-Hydroxy Atorvastatin in Human Plasma Using Rosuvastatin As Internal Standard By LC-MS/MS

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ABSTRACT
High performance liquid chromatographic tandem mass spectrometric method for the estimation of Atorvastatin (ATV), o-Hydroxy Atorvastatin (2-AT), p-Hydroxy Atorvastatin (4-AT) and Hydroxy Atorvastatin in human plasma has been developed and validated using Rosuvastatin (RST) as internal standard. Sample preparation was accomplished by liquid-liquid extraction technique. The processed sample was chromatographed and analyzed on Lichro CRART 55-2, Purospher STAR RP 18e, (3µm) column using mobile phase [0.3% formic acid in water and 0.3% formic acid in Acetonitrile (50:50% v/v ) and diluent as 50% methanol in water. The above analytical method described is validated for the determination for Atorvastatin (over a range of 1.99 ng/mL to 80.52 ng/mL), for 2-AT (over a range of 1.48 ng/mL to 59.84 ng/mL) and for 4-AT (over a range of 0.24 ng/mL to 9.86 ng/mL) using Rosuvastatin as internal standard in human plasma. Atorvastatin, 2-AT, 4-AT were chromatographed and analyzed by MS detector. Signals from the detector were captured in a computer and processed using Analyst software.

Keywords: Atorvastatin, O-Hydroxy Atorvastatin, P-Hydroxy Atorvastatin, Rosuvastatin.

INTRODUCTION
Atorvastatin calcium is ([R-[R*, R*]-2-(4-fluorophenyl)-β, δ- dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]- 1H-pyrrole-1-heptanoicacid, calcium salt (2:1 trihydrate) (Figure1). and rosuvastatin calcium is bis(E)-7-[4-(4 fluoro phenyl)-6- isopropyl-2-[methyl(methylsulfonyl)amino] pyrimidin-5-yl][3R,5S]- 3,5 dihydroxyhept-6-enoic acid] calcium salt (Figure2) which belong to the statin class of drugs used to treat hypercholesterolemia both in patients with established cardiovascular disease as well as those who are at a high risk of developing atherosclerosis. The liver is the primary site of action of atorvastatin, as this is the principal site of both cholesterol synthesis and LDL clearance. It is the dosage of atorvastatin, rather than systemic drug concentration, which correlates with extent of LDL-C reduction. Atorvastatin undergoes rapid absorption when taken orally, with an approximate time to maximum plasma concentration (Tmax) of 1–2 h. Atorvastatin undergoes high intestinal clearance and firstpass metabolism, which is the main cause for the low systemic availability. Administration of atorvastatin with food produces a 25% reduction in Cmax (rate of absorption) and a 9% reduction in AUC (extent of absorption), although food does not affect the plasma LDL-C-lowering efficacy of atorvastatin. Evening dose administration is known to reduce the Cmax and AUC by 30% each. However, time of administration does not affect the plasma LDL-C- lowering efficacy of atorvastatin. The mean volume of distribution of atorvastatin is approximately 381 L. It is highly protein bound (≥98%), and studies have shown it is likely secreted into human breastmilk. Atorvastatin metabolism is primarily through cytochrome P450 3A4 hydroxylation to form active ortho and para hydroxylated metabolites, as well as various beta-oxidation metabolites. The ortho and para hydroxylated metabolites are responsible for 70% of systemic HMG CoA reductase activity. Atorvastatin is primarily eliminated via hepatic biliary excretion, with less than 2% recovered in the urine. Half-life is 14 h and bioavailability is 12%. Statins exert their major effect – reduction of LDL level – through a mevalonic acid – the moiety that competitively inhibits HMG-CoA reductase. By reducing the conversion of HMG – Co A to mevalonate, statins inhibit an early and rate – limiting step in cholesterol biosynthesis. The associated reduction in intracellular cholesterol concentration induces LDL-receptor expression on the hepatocyte cell surface, which results in increased extraction of LDL-C from the blood and decreased.
circulating LDL-C concentrations. In addition statins cause reduction in total cholesterol (TC) and triglycerides (TG) and elevation in high-density lipoprotein-C (HDL-C). Besides lipid lowering effects, statins also have potential roles independent of cholesterol reduction as anti-oxidative, anti-inflammatory, immune-modulator, anti-malarial, and bone forming agents. Thus, due to their so many beneficial effects, there is growing interest in developing analytical methods for statins monitoring. Until the approval of RST in 2003, ATV was the most efficacious drug in the statins class, but recent studies reported RST as a potent inhibitor of HMG-CoA reductase having a higher LDL lowering effects as compared with other statins, which demonstrates that both RST and ATV are the leading drugs in the statins class.

HPLC-UV methods have also been reported for the determination of ATV alone in human serum, pharmaceutical preparations, along with impurities in pharmaceutical preparations and in combination with amlodipine, nicotinic acid, aspirin, olmesartan, and ezetimibe in dosage forms. Similarly, various analytical methods have been reported for the determination of RST including, spectrophotometric and HPLC-UV. The simultaneous quantification of RST with gemfobozril in human plasma and with other drugs including statins in pharmaceutical preparations has been determined, also the simultaneous analysis of RST and ATV in human serum using HPLC-UV has been studied. Only limited methods have been reported in the HPLC and GC. The objective of the work was to develop and validate LC-MS/MS method for quantification in Human Plasma. The method shows more sensitive limit of detection and Limit of Quantification is very less to the previous reported methods. The method was validated according to standard guidelines and various experimental parameters were optimized with the aim that the reported method could be applied for routine laboratory analysis of these statins and for the determination of pharmacokinetic and drug–drug interaction studies of these drugs in human and animal models.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Atorvastatin Calcium (Purity 93.60 % w/w), o-Hydroxy Atorvastatin Calcium (Purity 98.49 % w/w) p-Hydroxy Atorvastatin Calcium (Purity 95.43 % w/w) and Rosuvastatin Calcium (Purity 91.16 % w/w) working standards were used. HPLC grade Acetonitrile and Methanol were manufactured by Qualigens Fine Chemicals. Formic acid AR grade manufactured by S.D. Fine chemicals, Human Plasma (CPD), Plastic container (Polypropylene), Isopropyl alcohol (HPLC Grade), Ethyl acetate (HPLC Grade) and Water HPLC grade from Milli-Q RO system were used throughout the analysis.
Fig. 3: Atorvastatin metabolized to o-hydroxy and p-hydroxy atorvastatin by CYP3A4

Instrumentation and Chromatographic Conditions
The chromatography was performed on an ACQUITY UPLC system (Waters Corp, USA) with cooling auto sampler and column oven enabling temperature control of the analytical column. LichroCRART 55-2, Purospher STAR RP-18e, (3µm) column was employed. The column temperature was maintained at 45 °C and chromatographic separations were achieved with isocratic elution using a mobile phase composed of [0.3% formic acid in water and 0.3% formic acid in Acetonitrile (50:50% v/v) ]. The flow rate was set at 0.5 ml/min, run time was 4.00 minutes and retention time 2.2 minutes for 2-AT, 1.8 minutes for 4-AT, 2.4 minutes for Atorvastatin, 1.9 minutes for Rosuvastatin. The auto sampler was conditioned at 4°C and the injection volume was 20μl using Auto sampler mode for sample injection.

Compound Setting
Ion source ESI Negative mode, Ion spray voltage at 5.0 kV, temperature at 350 °C, curtain gas at 8 L/min, nebulizer gas at 50 (psi) and Dwell Time 100 (sec) and other parameters are given in table 1.

Table 1: Parameters of compound setting

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2-AT</th>
<th>4-AT</th>
<th>Atorvastatin</th>
<th>Rosuvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent Mass (m/z)</td>
<td>573.3</td>
<td>573.2</td>
<td>557.4</td>
<td>480.2</td>
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<tr>
<td>Product Mass (m/z)</td>
<td>278.1</td>
<td>278.1</td>
<td>278.1</td>
<td>340.2</td>
</tr>
<tr>
<td>Collision energy (eV)</td>
<td>40</td>
<td>45</td>
<td>46</td>
<td>20</td>
</tr>
</tbody>
</table>

Preparation of Standard Stock Solutions (w/v)
11.12 mg of Atorvastatin Calcium working standard weighted and transferred into 25 ml volumetric flask methanol was added to dissolve working standard. The volume made up to mark with methanol which has concentration 402617.68ng/ml for Atorvastatin. 10.48 mg of o-Hydroxy Atorvastatin Calcium working standard weighted and transferred into 25 ml volumetric flask methanol was added to dissolve working standard. The volume made up to mark with methanol which has concentration 398960.77ng/ml for o-Hydroxy Atorvastatin. 10.69 mg of p-Hydroxy Atorvastatin Calcium working standard weighed and transferred into 100 ml volumetric flask methanol was added to dissolve working standard. The volume made up to mark with methanol which has concentration 402617.68ng/ml for p-Hydroxy Atorvastatin. 11.95 mg of Rosuvastatin working standard weighted and transferred into 10 ml volumetric flask methanol was added to dissolve working standard. The volume made up to mark with methanol which has concentration 1082404.3ng/ml for Rosuvastatin. All calculations calculated on the basis of molecular weight and purity and stored in a refrigerator 2-8°C

Plasma sample Extraction Procedure
Thawed samples were vortexed to ensure complete mixing of contents. 0.400 ml aliquot of sample pipette out in RIA Vials and 0.050 ml of IS (10000 ng/ml) was added and vortexed the content for 30 seconds. 2.5 ml of Ethyl acetate was added. The content was vortexed for 10 mins and centrifuged for 10 mins at 14000 rpm. 2.0 ml of organic layer transferred into another set of RIA Vials and evaporated up to dryness at 35°C and 15 (psi) under a stream of nitrogen gas. The dried residue was
reconstituted with 0.5 ml diluent (methanol 70: water 30) and vortexed for 60 seconds. The Samples were transferred into individual Auto-sampler injector vials for injection.

Validation Parameters
The Method was validated in accordance with FDA Guidelines [48, 49]. Blank screening (Figures 8,9and10), Selectivity, Linearity, Accuracy, Precision, Recovery, Stability (Freeze thaw, bench top, long term, Auto sampler, stock solution at RT and RF) Dilution integrity, Matrix effect and Ruggedness were performed. Each batch of spiked plasma samples includes one complete calibration curve (consisting of one blank plasma, one blank plasma with internal standard and eight different non-zero concentration samples, six replicates of quality control samples LLOQ, LQC, MQC and HQC), except Bench top, freeze thaw stability, long term stability, Auto sampler stability and dilution integrity in which four replicate quality control samples were used. Quality control samples for Atorvastatin prepared by using Citrate Phosphate Dextrose as an anticoagulant and stored at a temperature below -20\(^\circ\)C(deep freezer), protected from light with concentrations taken as in table 2.

<table>
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<tr>
<th>Parameters</th>
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<th>(\alpha)-Hydroxy Atorvastatin</th>
<th>(\beta)-Hydroxy Atorvastatin</th>
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</thead>
<tbody>
<tr>
<td>LLOQ</td>
<td>2.04 ng/ml</td>
<td>1.51 ng/ml</td>
<td>0.252 ng/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>5.54 ng/ml</td>
<td>4.10 ng/ml</td>
<td>0.69 ng/ml</td>
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<tr>
<td>MQC</td>
<td>35.06 ng/ml</td>
<td>25.96 ng/ml</td>
<td>4.34 ng/ml</td>
</tr>
<tr>
<td>HQC</td>
<td>70.12 ng/ml</td>
<td>51.91 ng/ml</td>
<td>8.68 ng/ml</td>
</tr>
</tbody>
</table>

Standardization and calculation
The chromatographic data were acquired and processed using computer based Analyst software. The best-fit lines using weighting factor (1/concentration) linear least square regression analysis were obtained by peak area ratio of Atorvastatin, 2-AT, 4-AT to its internal standard (Rosuvastatin). The concentration of Atorvastatin, 2-AT, 4-AT in plasma samples were calculated using linear regression parameters by corresponding calibration curve.

RESULTS AND DISCUSSION
Chromatography
Representative chromatograms containing solvent reference mix solution, blank plasma, blank plasma with internal standard and quality control samples LLOQ, LQC, MQC and HQC and a calibration curve of representative regression analysis for Atorvastatin.

**Fig. 4:** Chromatogram of an aqueous sample of Atorvastatin

**Fig. 5:** Chromatogram of an aqueous sample of 2-AT
Fig. 6: Chromatogram of an aqueous sample of 4-AT

Fig. 7: Chromatogram of an internal standard (Rosuvastatin)

Fig. 8: Chromatogram of Blank plasma sample for Atorvastatin

Fig. 9: Chromatogram of Blank plasma sample for 2-AT

Fig. 10: Chromatogram of Blank plasma sample for 4-AT
Selectivity
Six lots of plasma with CPD anticoagulant were evaluated and none showed significant interfering peaks at the retention time of Atorvastatin (Figure 4), 2-AT (Figure 5), 4-AT (Figure 6) and Rosuvastatin (IS) (Figure 7).

Linearity
The linearity Atorvastatin of was determined by weighted least square regression analysis of standard plot associated with eight point standard curve (Figure11, 12 and13). The calibration was shown to be linear from 1.99 ng/ml to 80.52 ng/ml for Atorvastatin,1.48 ng/ml to 59.84 ng/ml for 2-AT and 0.24 ng/ml to 9.86 ng/ml for 4-AT. Best –fit calibration lines of chromatographic response versus concentration were determined by weighted least square regression analysis with weighting factor of 1/concentration. The coefficient of correlation ($r^2$) was consistently greater than or equal to 0.99 during the course of validation.

Sensitivity
The limit of Quantitation was 2.04 ng/ml for Atorvastatin, 1.51 ng/ml for 2-AT and 0.252 ng/ml for 4-AT. The between batch precision and accuracy at LLOQ concentration for Atorvastatin, 2-AT and 4-AT using internal standard ratio method were 8.6% - 96.9%, 9.5% - 100.6% and 6.6% - 101.0% respectively.

Accuracy
The accuracy of the assay was defined as the absolute value of calculated mean values of the quality control samples to their respective nominal values, expressed as percentage.
Within batch accuracy
The within batch accuracy using internal standard area ratio method ranged from 87.3% to 110.0% for Atorvastatin 86.5% to 106.2% for 2-AT and 86.8% to 102.6% for 4-AT, which are within limits (Refer Table 3).

Between batch accuracy
The between batch accuracy using internal standard area ratio method ranged from 95.6% to 103.3% for Atorvastatin 89.8% to 106.2% for 2-AT and 86.8% to 102.6% for 4-AT, which are within limits (Refer Table 3).

Precision
The precision of the assay was measured by the percent coefficient of variation over the concentration range of LLOQ (Figure14, 15 and 16), LQC (Figure17, 18 and19), MQC (Figure20, 21 and 22) and HQC (Figure 23, 24 and 25) quality control samples of Atorvastatin.

Within Batch Precision
The within batch precision using internal standard area ratio method ranged from 1.6% to 10.7% for Atorvastatin 1.1% to 9.3% for 2-AT and 0.7% to 8.0% for 4-AT, which are within limits (Refer Table 3).

Between Batch Precision
Between Batch Precision using internal standard area ratio method ranged from 7.% to 9.2% for Atorvastatin 4.9% to 9.5% for 2-AT and 5.4% to 9.1% which are within limits (Refer Table 3).

Table 3: Results of Accuracy, Precision and Recovery

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Acceptance Criteria</th>
<th>Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nominal concentration: 85-115%</td>
<td>Atorvastatin</td>
</tr>
<tr>
<td>1</td>
<td>Within Batch Accuracy</td>
<td>87.3-110.0</td>
<td>86.5-106.2</td>
</tr>
<tr>
<td>2</td>
<td>Between Batch Accuracy</td>
<td>95.6-103.3</td>
<td>89.8-106.2</td>
</tr>
<tr>
<td>3</td>
<td>Within Batch Precision</td>
<td>% CV : 15%</td>
<td>1.6-10.7</td>
</tr>
<tr>
<td>4</td>
<td>Between Batch Precision</td>
<td>% CV : 15%</td>
<td>7.0-9.2</td>
</tr>
<tr>
<td>5</td>
<td>Recovery of Analyte</td>
<td>% CV : 20%</td>
<td>13.5</td>
</tr>
<tr>
<td>6</td>
<td>Recovery of internal standard</td>
<td>% CV : 20%</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Recovery of Analyte
The percentage recovery of Atorvastatin was determined by measuring the peak area response of spiked (extracted) quality control samples (LQC, MQC and HQC) against the peak area response of aqueous (Unextracted) quality control samples (LQC, MQC and HQC) of equivalent concentrations. The percent recovery at LQC, MQC and HQC quality control samples were 86.26%, 90.03% and 72.32% for Atorvastatin, 82.98%, 84.11% and 71.81% for 2-AT and 85.70%, 86.78%, 72.35% for 4-AT. The % CV for recovery of inter-quality control samples for Atorvastatin 13.5%, for 2-AT was 8.5% and for 4-AT was 9.8%, which are within limits. The % mean of recovery were 84.54% for Atorvastatin 79.63%, for 2-AT was 81.61% and for 4-AT, which are within limits (Refer Table 3).

Recovery of Internal Standard
The percentage recovery of Rosuvastatin (IS) was determined by measuring the peak area response of spiked (extracted) quality control samples (MQC) against the peak area response of aqueous (unextracted) IS dilution of same concentration. The mean percentage recovery and % CV for recovery were 90.36% and 1.4% which is within limits (Refer Table 3).
Fig. 14: Chromatogram of LOQQ sample for Atorvastatin

Fig. 15: Chromatogram of LOQQ sample for 2-AT

Fig. 16: Chromatogram of LOQQ sample for 4-AT

Fig. 17: Chromatogram of LQC sample for Atorvastatin

Fig. 18: Chromatogram of LQC sample for 2-AT

Fig. 19: Chromatogram of LQC sample for 4-AT
Fig. 20: Chromatogram of MQC sample for Atorvastatin

Fig. 21: Chromatogram of MQC sample for 2-AT

Fig. 22: Chromatogram of MQC sample for 4-AT

Fig. 23: Chromatogram of HQC sample for Atorvastatin

Fig. 24: Chromatogram of HQC sample for 2-AT
Freeze thaw stability
The stability of the spiked plasma samples was determined during three freeze-thaw cycles. Four replicates numbers of LQC and MQC samples (Stability samples) kept at -80°C and were analysed after third freeze thaw cycle against freshly spiked calibration curve standards and freshly spiked QC samples (comparison samples). The comparative stability ranged from 99.8% to 100.8% for Atorvastatin, 97.5% to 99.9% for 2-AT and 96.4% to 97.9% for 4-AT, which are within limits (Refer Table 4).

Bench top stability
The bench top stability (Short term stability in matrix) determined by analyzing four replicates of low and high QC stability samples, which had been kept at room temperature for a designed time against the freshly spiked QC samples (comparison samples). The comparative stability ranged from 99.8% to 102.8% for Atorvastatin, 101.5% to 102.7% for 2-AT and 99.3% to 99.4% for 4-AT, which are within limits (Refer Table 4).

Auto sampler stability
The auto sampler stability (Post-processing stability) determined by analyzing four replicates of low and high QC stability samples, which had been processed and kept in Auto sampler and were analyzed after against freshly spiked calibration curve standards and freshly spiked QC samples (comparison samples). The comparative stability ranged from 97.6% to 103.5% for Atorvastatin, 99.2% to 103.5% for 2-AT and 99.4% to 102.5% for 4-AT, which are within limits (Refer Table 4).

Long term stability
For long term stability (Long term stability in matrix) determined by analyzing four replicates of low and high QC stability samples were analyzed against freshly spiked calibration curve. The low and high QC samples were stored for 07 days in deep freezer (at below -20°C) with CPD as an anticoagulant in polypropylene tubes. The stability ranged from 101.4% to 104.5% for Atorvastatin, 101.2% to 106.6% for 2-AT and 97.4% to 100.1% for 4-AT, which are within limits (Refer Table 4).

Stock solution stability at room temperature
Stock solution stability at room temperature was performed by storing for Atorvastatin, 2-AT, 4-AT and Rosuvastatin (IS) stock solutions at room temperature. The evaluation of stability was done by assaying six replicate injections of appropriately prepared dilutions of stored stock aliquot solutions of Atorvastatin, 2-AT, 4-AT and Rosuvastatin (IS) against six replicate injection of appropriately prepared dilution from fresh stock solutions of Atorvastatin, 2-AT, 4-AT and Rosuvastatin (IS). The stock solution Atorvastatin, 2-AT, 4-AT and Rosuvastatin (IS) was found to be both Analyte and IS, when stored at room temperature. The percent stability of the stock solutions of Atorvastatin, 2-AT, 4-AT and Rosuvastatin (IS) were 97.9% and 101.5% respectively, which are within limits (Refer Table 4).

Dilution Integrity
Dilution Integrity was determined by assaying four replicates QC spiked approximately two times the concentration of ULOQ. Samples were diluted by an appropriate factor (two) prior to extraction, against freshly spiked calibration standard samples. The accuracy for two times diluted concentration were 100.1%, 90.3% and 101.9% and four times diluted concentration were 100.2%, 86.9% and 102.3% for Atorvastatin, 2-AT, 4-AT respectively. The precision two times diluted concentration were 7.0%, 2.4%, 1.9% and four times diluted concentration were 3.6%, 1.5% and 12.1% for Atorvastatin, 2-AT, 4-AT respectively which are within limits (Refer Table 4).
Matrix effect
Matrix effect was calculated by spiking Analyte and IS at LQC and HQC levels into each of blank plasma extracts from six different batches of matrix respectively and analyzed in duplicate against six replicate injections of aqueous samples at low and high QCs samples. At low and high concentration QCs samples, the % coefficient of variation for matrix factor was found to be 9.44% to 5.45%, 9.24% to 6.80% and 11.11% to 5.76%, respectively for Atorvastatin, 2-AT, 4-AT which are within limits (Refer Table 4).

Table 4: Result of stabilities Dilution integrity, Matrix effect and Ruggedness.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Acceptance Criteria</th>
<th>Results (%)</th>
<th>Atorvastatin</th>
<th>2-AT</th>
<th>4-AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Freeze and thaw cycle at -80°C</td>
<td>Mean % change after 3 cycles: ± 15%</td>
<td>99.8-100.8</td>
<td>97.5-99.9</td>
<td>96.4-97.9</td>
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<tr>
<td>2</td>
<td>Bench top stability</td>
<td>Mean % change after 10.14 h: ± 15%</td>
<td>99.8-102.8</td>
<td>101.5-102.7</td>
<td>99.3-99.4</td>
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<tr>
<td>3</td>
<td>Auto sampler stability</td>
<td>Mean % change after 50.04 h : 85-115 %</td>
<td>97.6-98.0</td>
<td>97.6-98.0</td>
<td>99.4-102.5</td>
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<tr>
<td>4</td>
<td>Long term Stability</td>
<td>Mean % change after 7 Days: ± 15%</td>
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<td>97.4-100.1</td>
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<td>5</td>
<td>Stock solution stability at room temp.</td>
<td>% Nominal Concentration: ± 15%</td>
<td>97.9</td>
<td>101.5</td>
<td>100.6</td>
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<tr>
<td>6</td>
<td>Dilution integrity</td>
<td>Accuracy (% Nominal): ± 15%</td>
<td>100.1</td>
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<td>101.9</td>
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<td></td>
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<td>7</td>
<td>Matrix effect</td>
<td>% CV: ≤15%</td>
<td>9.44</td>
<td>9.24</td>
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<tr>
<td></td>
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<td>8</td>
<td>Ruggedness</td>
<td>% Nominal : ± 15%</td>
<td>87.1-107.8</td>
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<td>92.8-105.1</td>
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<td>% CV : ≤15%</td>
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<td>1.5-12.2</td>
<td>3.0-7.1</td>
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Ruggedness
The ruggedness of the extraction procedure and chromatographic method was evaluated by analysis of a batch of six sets of quality control samples and a set of calibration standards using different column (same type) by another analyst. The within batch accuracy ranged from 105.0% to 109.6% for Lornoxicam. The within batch precision ranged from 87.1% to 107.8%, 87.4% to 94.2% and 92.8% to 105.1%, respectively for Atorvastatin, 2-AT, 4-AT. The results indicated that the batch met the acceptance criteria of linearity, precision and accuracy data of the quality control samples, which are within limits (Refer Table 4).

CONCLUSION
The above analytical method described is valid for the determination for Atorvastatin (over a range of 1.99 ng/mL to 80.52 ng/ml), for 2-AT (over a range of 1.48 ng/ml to 59.84 ng/ml) and for 4-AT (over a range of 0.24 ng/ml to 9.86 ng/ml) using Rosuvastatin as internal standard in human plasma and by using Lichro CRART 55-2, Purospher STAR RP-18e, (3µm) as column. This method is for quantification of Atorvastatin 2-AT and 4-AT in human plasma which is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma.

Declaration of interest
The authors are thankful to Management, for providing the research grant. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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REFERENCES
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