Development and Validation of a Highly Sensitive and Robust LC-MS/MS with Electrospray Ionization Method for Quantification of Rosuvastatin in Small Volume Human Plasma Samples and its Application to a Clinical Study

Raja Reddy Kallem¹, Arumugam Karthik¹, Lagishetty Chakradhar¹, Ramesh Mullangi¹, Nuggelally R. Srinivas¹.²

¹ Drug Metabolism and Pharmacokinetics, Dr. Reddy’s Laboratories Ltd., Miyapur, Hyderabad (India)
² Drug Development, Discovery Research, Dr. Reddy’s Laboratories Ltd., Miyapur, Hyderabad (India)

Corresponding author: Ramesh Mullangi, Ph. D., Drug Metabolism and Pharmacokinetics, Dr. Reddy’s Laboratories Ltd., Miyapur, Hyderabad 500 049 (India); e-mail address: mullangiramesh@drreddys.com

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- CAS 287714-41-4
- HMG-CoA reductase enzyme inhibitors
- Rosuvastatin, human plasma, LC-MS/MS, method validation, pharmacokinetics

Abstract

A high-throughput, simple, highly sensitive and specific LC-MS/MS method (liquid chromatography coupled with tandem mass spectrometry) has been developed for the estimation of rosuvastatin (CAS 287714-41-4, RST) with 100 µl human plasma using atorvastatin (CAS 134523-00-5) as an internal standard (IS). The API-4000 LC-MS/MS was operated under the multiple reaction-monitoring mode (MRM) using the electro spray ionization technique. The assay procedure involved direct precipitation of RST and IS from plasma with acetonitrile. Sample preparation with this method yielded clean extracts and consistent recoveries: 91.39% for RST and 99.28% for IS. The total chromatographic run time was 3.5 min and the elution of RST and IS occurred at 2.5 and 3.1 min, respectively; this was achieved with a mobile phase consisting of 0.05 mol/L formic acid: acetonitrile (20:80, v/v) at a flow rate of 0.50 ml/min on an Inertsil ODS-3 column (4.6 × 100 mm, 3.0 µm). The developed method was validated in human plasma with a limit of quantitation of 0.05 ng/ml. A linear response function was established for the range of concentrations of 0.05 to 50.0 ng/ml with a correlation coefficient (r) of 0.999. The inter- and intra-day precision in the measurement of RST quality control (QC) samples at 0.05, 0.15, 25 and 40 ng/ml were in the range of 6.55 to 11.40% relative standard deviation (RSD) and 1.76 to 11.17% RSD, respectively. Accuracy in the measurement of QC samples for RST was in the range of 95.02 to 101.37% of the nominal values. RST was stable in the battery of stability studies viz., bench-top, auto-sampler and freeze-thaw cycles. The stability of RST was established for 1 month at –80 °C. The application of the assay to a clinical study confirmed the utility of the assay to derive human pharmacokinetic parameters.
1. Introduction

Rosuvastatin (CAS 287714-41-4), a novel and highly effective inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme, is a recent introduction for the treatment of dyslipidemia [1-3]. Rosuvastatin (RST, Fig. 1) is chemically bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-(methylsulfonyl)amino]pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enocacid calcium salt. In hypercholesterolaemia patients, RST produces marked, dose-dependent decrease in LDL cholesterol [4]. RST was found to be more effective than atorvastatin (CAS 134523-00-5), simvastatin (CAS 79902-63-9) and pravastatin (CAS 81093-37-0), while exerting beneficial effects on triglyceride and high-density lipoprotein (HDL) cholesterol levels [5-8]. To date there are only few bioanalytical methods [9-12] using LC-MS/MS methods (liquid chromatography coupled with tandem mass spectrometry) reported for the estimation of RST in human plasma. The first method, developed by Hull et al. in 2002, utilizes automated solid-phase extraction (SPE), followed by high-performance liquid chromatography with positive ion Turboionspray tandem mass spectrometry with a lower limit of quantitation (LLOQ) of 0.1 ng/ml [9]. However, this method required a relatively large sample volume (1.7 ml) and a complicated and time-consuming sample preparation. Besides, this method required a deuterated internal standard (IS), which is not feasible to synthesize in all analytical laboratories. The second LC-MS/MS method [10] developed by us for simultaneous estimation of RST and fenofibric acid from human plasma (500 µl) involves simple liquid/liquid extraction (LLE) of RST and fenofibric acid along with IS (carbamazepine) into ethyl acetate, wherein the LLOQ of RST was 1 ng/ml. The method developed by us [10] utilizes less sample volume and is easier in sample processing. Of late, Oudhoff et al. [11] developed a sensitive method for estimation of RST using microbore HPLC in combination with tandem mass spectrometry. The most recent publication by Xu et al. [12] also utilized LLE for the extraction of human plasma and the LLOQ of this method was 0.2 ng/ml. Apart from LC-MS/MS methods, we have developed and validated high-performance liquid chromatographic (HPLC) methods for the estimation of RST alone (LLOQ: 0.02 µg/ml) [13] and simultaneously (LLOQ: 0.03 µg/ml) with gemfibrozil [14]. In either HPLC method, we have adopted simple LLE to extract RST from the plasma. In the present report, we summarize the development and validation of RST in small sample volumes (100 µl) after a simple and reproducible precipitation method with commercially available IS and its application to a clinical study. The present method has several benefits over the existing methods viz., high sensitivity, improved LLOQ (0.05 ng/ml), shorter run time (3.5 min), ease of sample preparation of RST from small volume plasma samples (100 µl) and usage of commercially available IS.

2. Materials and methods

2.1. Chemicals and reagents

RST and atorvastatin (IS) were synthesized by the Medicinal Chemistry Group, Discovery Research, Dr. Reddy’s Laboratories Ltd (DRL), Hyderabad and were characterized using chromatographic and spectral techniques by Analytical Research Group, Discovery Research, DRL, Hyderabad. Purity was found to be more than 99% for both the compounds. HPLC grades of acetonitrile, methanol and analytical grade of formic acid and ethylenediaminetetraacetic acid (EDTA) disodium salt were purchased from Qualigens, Mumbai (India). All aqueous solutions including the buffer for the HPLC mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA) grade water. The control human plasma was purchased from Cauvery Diagnostics and Blood Bank, Secunderabad (India).

2.2. Instrumentation and chromatographic conditions

An Agilent (Agilent Technologies, Waldbronn, Germany) 1200 series LC system equipped with a degasser (G1322A) and an isopump (G1311A) along with an auto-sampler (G1367B) was used to inject 25 µl aliquots of the processed samples on an Inertsil ODS-3 column (4.6 x 100 mm, 3.0 µm, GL Sciences Inc., Tokyo, Japan), which was kept at ambient temperature. The isocratic mobile phase, a mixture of 0.05 mol/L formic acid and acetonitrile mixture (20:80, v/v) was delivered at 0.50 ml/min into the mass spectrometer ionization chamber. Quantitation was achieved by MS-MS detection in positive ion modes for the analyte and IS using a MDS Sciex (Foster City, CA, USA) API 4000 mass spectrometer equipped with a Turboionspray™ interface at 475 °C. The ion spray voltage was set at 5500 V. The common parameters viz., nebulizer gas, curtain gas, auxiliary gas and collision gas were set at 35, 20, 40 and 5500 V respectively. Collisions of the compounds parameters viz., de-activating potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) for RST and IS were 70, 49, 10, 11 and 84, 31, 10, 11 respectively. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transitions of the m/z 482.3 precursor ion to the m/z 258.3 product ion for RST and m/z 559.2 precursor ion to the m/z 440.2 product ion for IS. Quadrupoles Q1 and Q3 were set on low resolution. The analytical data were processed by Analyst software (version 1.4.1; Applied Biosystems, Foster City, CA, USA).

2.3. Calibration standard solutions

Primary stock solutions of RST for preparation of standard and quality control (QC) samples were prepared from separate
weighing. The primary stock solutions of the analytes and IS were prepared in methanol (1.0 mg/ml) and stored at –20 °C, which were found to be stable for one month (data not shown). Appropriate dilutions were made in methanol for RST to produce working stock solutions of 500, 200, 100, 50, 10, 2, 1 and 0.5 ng/ml on the day of analysis and these stocks were used to prepare the calibration curve (CC). Another set of working stock solutions of RST were made in methanol (from the primary stock) at 400, 250, 150 and 0.5 ng/ml for preparation of QC samples. Working stock solutions were stored at approximately 5 °C for a week (data not shown). Individual QC and CC working stock solutions of RST were made before spiking into QC and CC samples accordingly. A working IS solution (1.00 µg/ml) was prepared in methanol. Calibration samples were prepared by spiking 50 µl of control human plasma with the appropriate amount of RST (10 µl) and IS (10 µl) on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control human plasma in bulk at appropriate concentrations (0.05 ng/ml (LLOQ), 0.15 ng/ml (QC low), 25.0 ng/ml (QC medium) and 40.0 ng/ml (QC high)) and 100 µl volumes were aliquoted into different tubes and, depending on the nature of experiment, samples were stored at –80 °C until analysis.

2.4. Sample preparation
To 100 µl of plasma sample, IS solution (10 µl) equivalent to 100 ng was added and mixed for 15 s on a cyclomixer (Remi Instruments, Mumbai, India), followed by precipitation with 300 µl of acetonitrile. The mixture was vortexed for 1 min, followed by centrifugation for 2 min at 1,680 g on Biofuge (Heraeus, Germany) and 25 µl of supernatant was injected onto the LC-MS/MS system.

2.5. Specificity and selectivity
The lack of chromatographic interference from endogenous plasma components was investigated using pooled blank samples as well as samples obtained from individual volunteer.

2.6. Calibration curve
Calibration curves were acquired by plotting the peak area ratio of RST:IS against the nominal concentration of calibration standards. The concentrations used were 0.05, 0.10, 0.20, 1.00, 5.00, 10.0, 20.0 and 50.0 ng/ml. The results were fitted to linear regression analysis using 1/X² as weighting factor. The calibration curve had to have a correlation coefficient (r) of 0.99 or better. The acceptance criterion for each back-calculated standard concentration was a 15% deviation from the nominal value except for the LLOQ, which was set at 20% [15, 16].

2.7. Precision and accuracy
The intra-day assay precision and accuracy were estimated by analyzing four replicates at four different QC levels i.e., 0.05, 0.15, 25.0 and 40.0 ng/ml. The inter-assay precision was determined by analyzing the four levels QC samples on four different runs. The criteria for acceptability of the data included accuracy within ±15% deviation (DEV) from the nominal values and a precision of ±15% relative standard deviation (RSD), except for the LLOQ, where it should not exceed ±20% of the coefficient of variation (CV) [16, 17].

2.8. Recovery
The recovery of RST and IS through the direct precipitation procedure was determined by comparing the responses of the analytes extracted from replicate QC samples (n = 4) with the response of analytes from non-precipitated standard solutions at equivalent concentrations [18]. Recoveries of RST was determined at low, middle and high concentrations viz., 0.15, 25.0 and 40.0 ng/ml, whereas the recovery of the IS was determined at a single concentration of 100 ng/ml.

2.9. Stability experiments
The stability of RST and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples up to 12 h (in an auto-sampler) after the initial injection. The peak areas of RST and IS obtained at the initial cycle were used as the reference to determine the relative stability of the analytes at subsequent points. The stability of RST in the biomatrix during 8 h (bench-top) was determined at ambient temperature (25 ± 3°C). The freezer stability of RST in human plasma was assessed by analyzing the QC samples stored at –80 °C for at least one month. The stability of RST in human plasma following repeated freeze-thaw cycles was assessed using QC samples spiked with RST. The samples were stored at –80°C between freeze-thaw cycles. The stability of RST was assessed after three freeze-thaw cycles. All the stability experiments were carried out at low and high QC concentrations in four replicates. The samples were processed using the same procedure as described for the sample preparation (section 2.4.). Samples were considered to be stable if assay values were within the acceptable limits of accuracy (i.e. ±15% DEV) and precision (i.e. ±15% RSD).

2.10. Human pharmacokinetic study
A pharmacokinetic study was performed in healthy male subjects. The ethics committee (Lotus Labs, Bangalore, India) approved the protocol and the volunteers provided written informed consent. Blood samples were obtained following administration of 10 mg RST into polypropylene tubes containing EDTA solution as an anti-coagulant at pre-dose, 0.5, 1, 2, 3, 5, 8, 10, 12, 24, 36, 48 and 72 h. Plasma was harvested by centrifuging the blood using Biofuge at 1,760 g for 5 min and stored frozen at –80°C until analysis. Plasma (100 µl) samples were spiked with IS and processed as described above. Along with clinical samples, QC samples at low, medium and high concentration were assayed in duplicate and were distributed among calibrators and unknown samples in the analytical run; no more than 33% of the QC samples were greater than ±15% of the nominal concentration. Plasma concentration-time data of RST was analyzed by a non-compartmental method using WinNonlin Version 5.1 (Pharsight Corporation, Mountain View, CA, USA).

3. Results
3.1. Matrix effect, specificity and selectivity
The matrix effect was evaluated by analyzing three batches of QC samples for the analyte and at 100 ng/ml concentration for the IS. The average matrix effect values obtained were 84.19, 82.66 and 80.78% for QCs at concentration levels of 0.15, 25.0 and 40.0 ng/ml for RST and 94.64% for the IS at 100 ng/ml. No significant peak area differences were observed.

Fig. 2 shows a typical overlaid chromatogram for the control human plasma (free of analyte and IS), human plasma spiked with RST at LLOQ and IS and an in vivo
plasma sample obtained at 2 h after oral administration of the RST tablet. No interfering peaks from endogenous compounds were observed at the retention times of the analyte and IS. The retention time of RST and IS were 2.5 and 3.1 min, respectively. The total chromatographic run time was 3.5 min, which was shorter than those reported in literature [9–12].

3.2. Calibration curve
The plasma calibration curve was constructed using eight calibrators in duplicate (viz. 0.05–50.0 ng/ml). The standard curve had a reliable reproducibility over the standard concentrations across the calibration range. The calibration curve was prepared by determining the best fit of peak-area ratios (peak area analyte/peak area IS) versus concentration, and fitted to $y = mx + c$ using weighing factor $(1/X^2)$. The average regression ($n = 4$) was found to be $r > 0.999$. The lowest concentration with the RSD < 20% was taken as LLOQ [14] and was found to be 0.05 ng/ml. The % accuracy observed for the mean of back-calculated concentration for four linearities was within 93.60–112.17, while the precision (% CV) values were in the range of 4.30–12.17.

3.3. Accuracy and precision
Accuracy and precision data for intra- and inter-day plasma test samples were presented in Table 1. The in-

Fig. 2: Typical MRM chromatograms of RST (left panel) and IS (right panel) in (a) human blank plasma, (b) human plasma spiked with RST at LLOQ (0.05 ng/ml) and IS, (c) a 2.0 h in vitro plasma sample showing RST peak obtained following oral dose of RST along with IS.
tra-day accuracy (%) for RST ranged from 96.10–102.56 at 0.05 ng/ml, 98.44–105.60 at 0.15 ng/ml, 86.16 to 106.87 at 25.0 ng/ml and 87.10–106.80 at 40.0 ng/ml. The inter-day accuracy (%) for RST was 99.95, 101.37, 98.10 and 95.02 at 0.05, 0.15, 25.0 and 40.0 ng/ml. The intra-day precision (%) for RST ranged from 6.93–11.17 at 0.05 ng/ml, 5.60–6.90 at 0.15 ng/ml, 1.76–9.61 at 25.0 ng/ml and 2.12–8.56 at 40.0 ng/ml. The inter-day precision (%) for RST was 9.04, 6.55, 11.40 and 10.40 at 0.05, 0.15, 25.0 and 40.0 ng/ml.

### 3.4. Recovery

The results of the comparison of neat standards versus plasma-extracted standards were estimated for RST at 0.15, 25.0 and 40.0 ng/ml and the absolute mean recoveries were 84.31, 97.68 and 92.19%, respectively, across the concentrations. The absolute recovery of the IS at 100 ng/ml was 99.28%.

### 3.5. Stability

#### 3.5.1. Autosampler and bench-top stability

Over a 12 h injection time period in the auto-sampler at ambient temperature and over the bench-top for an 8 h period, the predicted concentrations for RST at 0.15 and 40.0 ng/ml, samples deviated within the nominal concentrations. The results were found to be within the assay variability limits (Table 2).

#### 3.5.2. Freeze-thaw stability

Table 2 shows the results of the analyses of the QC samples following 3 freeze-thaw cycles. RST was shown to be stable in the frozen plasma at −80°C for at least 3 freeze-thaw cycles.

#### 3.5.3. Freezer stability

RST was found to be stable when stored at −80°C for at least one month. Both accuracy and precision of QC samples in this evaluation were within the assay variability limits (Table 2).

### 3.6. Application of the method

The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the plasma pharmacokinetics of RST in healthy volunteers. Profiles of the mean plasma concentration versus time are shown in Fig. 3. Maximum concentration in plasma (C_{max} = 11.37 ± 0.81 ng/ml) was achieved at 2.00 ± 0.00 h (T_{max}). The half-life (t_{1/2}) of RST was 20.36 ± 1.74 h, while the AUC(0–∞) was 85.68 ± 7.32 ng · h/ml. The current method could quantify the plasma concentrations of RST at the dose of 10 mg up to 72 h post dose. The fact that only 100 µl of plasma was used indicates its higher sensitivity compared to the currently existing methods in literature.

### Table 1: Intra- and inter-day precision data of RST quality controls in human plasma.

<table>
<thead>
<tr>
<th>Theoretical concentration (ng/ml)</th>
<th>Run</th>
<th>Measured concentration (ng/ml)</th>
<th>Mean</th>
<th>SD</th>
<th>% CV</th>
<th>Accuracy (%)</th>
</tr>
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<tbody>
<tr>
<td>0.05</td>
<td>1</td>
<td>0.05</td>
<td>0.01</td>
<td>10.01</td>
<td>102.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.05</td>
<td>0.01</td>
<td>11.17</td>
<td>100.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.05</td>
<td>0.00</td>
<td>6.93</td>
<td>96.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.05</td>
<td>0.00</td>
<td>9.66</td>
<td>101.27</td>
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<tr>
<td>0.15</td>
<td>1</td>
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<td>0.01</td>
<td>6.90</td>
<td>101.50</td>
<td></td>
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<tr>
<td></td>
<td>2</td>
<td>0.16</td>
<td>0.01</td>
<td>5.60</td>
<td>105.60</td>
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<tr>
<td></td>
<td>3</td>
<td>0.15</td>
<td>0.01</td>
<td>6.90</td>
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<tr>
<td></td>
<td>4</td>
<td>0.15</td>
<td>0.01</td>
<td>6.90</td>
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<tr>
<td>25.0</td>
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<td>26.22</td>
<td>2.52</td>
<td>9.61</td>
<td>104.87</td>
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</tr>
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<td></td>
<td>2</td>
<td>26.72</td>
<td>1.60</td>
<td>6.00</td>
<td>106.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21.54</td>
<td>0.38</td>
<td>1.76</td>
<td>86.16</td>
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<tr>
<td></td>
<td>4</td>
<td>22.43</td>
<td>0.98</td>
<td>4.39</td>
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<tr>
<td>40.0</td>
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<td>42.72</td>
<td>2.50</td>
<td>5.86</td>
<td>106.80</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>39.96</td>
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<td>8.56</td>
<td>99.90</td>
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<tr>
<td></td>
<td>3</td>
<td>35.10</td>
<td>1.23</td>
<td>3.49</td>
<td>87.75</td>
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<tr>
<td></td>
<td>4</td>
<td>34.84</td>
<td>0.74</td>
<td>2.12</td>
<td>87.10</td>
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</tr>
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</table>

### Table 2: Stability data of RST quality controls in human plasma.

<table>
<thead>
<tr>
<th>QC (spiked) concentration (ng/ml)</th>
<th>Stability</th>
<th>Mean ± SD(^a) (ng/ml)</th>
<th>Accuracy (%)(^b)</th>
<th>Precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>0 h (for all)</td>
<td>0.151 ± 0.01</td>
<td>111.26</td>
<td>6.88</td>
</tr>
<tr>
<td></td>
<td>3 F/T</td>
<td>0.170 ± 0.01</td>
<td>93.64</td>
<td>7.22</td>
</tr>
<tr>
<td></td>
<td>8 h (BT)</td>
<td>0.141 ± 0.01</td>
<td>95.03</td>
<td>8.69</td>
</tr>
<tr>
<td></td>
<td>12 h (in injector)</td>
<td>0.136 ± 0.08</td>
<td>90.07</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>30 day at −80 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.0</td>
<td>0 h (for all)</td>
<td>42.72 ± 2.50</td>
<td>106.68</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>3 F/T</td>
<td>45.58 ± 0.22</td>
<td>85.25</td>
<td>4.16</td>
</tr>
<tr>
<td></td>
<td>8 h (BT)</td>
<td>36.42 ± 1.52</td>
<td>95.08</td>
<td>7.72</td>
</tr>
<tr>
<td></td>
<td>12 h (in injector)</td>
<td>40.62 ± 3.14</td>
<td>88.18</td>
<td>3.77</td>
</tr>
<tr>
<td></td>
<td>30 day at −80 °C</td>
<td>37.67 ± 1.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QC: quality control; % CV: coefficient of variation; F/T: freeze/thaw; BT: bench top.

\(^a\)Back-calculated plasma concentrations.

\(^b\)(Mean assayed concentration – nominal concentration)/(nominal concentration) x 100.
4. Discussion

Hitherto, only few LC-MS/MS methods for the quantitation of rosuvastatin from various biological matrices have been reported in literature. The first method reported by Hull et al. [9] involved a very complicated sample preparation step with higher plasma volume and further required the use of deuterated IS for quantitation of RST with an LLOQ of 0.1 ng/ml. The second method developed [10] in our laboratories overcame the limitations of the first method as it simplified the sample processing and usage of commercial IS, although it had a drawback in terms of assay sensitivity because of higher LLOQ (1 ng/ml). Subsequently, Oudhoff et al. (2006) reported an LC-MS/MS method using microbore HPLC column. In this method, the sample preparation was achieved using SPE, and the LC was done under gradient conditions to avoid the background noise on mass spectrometry [11]. The most recent method developed by Xu et al. [12] achieved an LLOQ of 0.2 ng/ml, using 250 µl of plasma, and it involved LLE.

The objectives of our work were two-fold: (a) to develop and validate a highly sensitive and specific assay with simple sample preparation process; and (b) to increase the assay throughput via shortening the run time and using small plasma volume. In order to achieve our objectives, multiple parameters such as the sample processing/extraction procedure, chromatographic conditions and fine tuning of mass spectrometry parameters were evaluated. While optimizing the extraction scheme of RST from human plasma, we found that we could achieve the required S/N ratio (as per the guidelines) [15] to set the LLOQ of 0.05 ng/ml by simple protein precipitation with acetonitrile (S/N ratio 7:1). These methods resulted in cleaner extracts with a recovery of > 91 % (analyte and IS), and the background noise interference from plasma was drastically reduced. Hence the present method can be used for routine therapeutic drug monitoring. In order to increase the throughput, we have further optimized the mobile phase composition and column selection. Use of 0.05 mol/L formic acid buffer with acetonitrile combination (20:80, v/v) on an Inertsil ODS 3 column (4.6 × 100 mm, 3.0 µm) provided the maximum throughput advantage without compromising on the selectivity and desired sensitivity required for the RST determination. Overall, the simplified extraction process, usage of commercial IS and shorter analytical run along with improved LLOQ renders the present method suitable for routine clinical samples analysis.

5. Conclusion

In summary, we have developed and validated a highly sensitive, specific, reproducible and high-throughput LC-MS/MS assay to quantify RST using commercially available IS from small volumes of human plasma. To the best of our knowledge, the present method offers the highest sensitivity (0.05 ng/ml) than any other methods described in the literature. In addition, the present method utilizes direct precipitation of plasma and offers high-throughput because of optimization of chromatographic conditions. The bioanalytical validation data support the utility of the method for routine therapeutic drug monitoring and also for routine clinical samples analysis with desired precision and accuracy along with improved high throughput.

References

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