Overview:
The present chapter deals with the determination of rosvastatin related substances in solid oral dosage form using the developed and validated, stability indicating, RP-UPLC method.
Determination of Related Substance in Rosuvastatin Tablets by RP-UPLC Method

4.1 LITERATURE REVIEW

A detailed literature survey for rosuvastatin (ROSV) revealed that few analytical methods are reported for determination of ROSV by HPTLC [1], UV spectroscopy [2-5], assay by RP-HPLC [6-10], capillary electrophoresis [11], Mass spectrometry [12-15], LC-MS/MS [16] and simultaneous determination with atorvastatin by mass spectrometry [17]. Mehta et al. reported a stability-indicating assay method for determination of ROSV in the presence of its degradation products using high performance liquid chromatography [18]. In this assay method total run time is around 35 min to elute all degradation impurities and is applicable for only ROSV estimation but not for its related substances. Gosulu VRR et al. reported a stability-indicating RP-UPLC method for ROSV and its related impurities in pharmaceutical dosage form [19]. In this method, total run time is 12 min to monitor all degradation products in ROSV dosage form. When forced degradation study (acid hydrolysis) of ROSV was performed using this reported method, three major late eluting impurities were observed after 12 min, which is presented in Figure 4.1

Currently, the determination of impurities is one of the most difficult tasks for pharmaceutical analysis during method development, especially if increasing numbers of impurities are required to be determined.

According to our knowledge, none of the currently available analytical methods can separate and quantify all the known related compounds, degradation impurities and unknown degradation compounds (late eluting) of ROSV dosage form in the claimed chromatographic run time. It’s indicated that published RP-HPLC and RP-UPLC methods are not suitable for the related substance determination in ROSV tablets dosage form, as per ICH guidance [20]. It is, therefore, necessary to develop a new stability-indicating method for the determination of ROSV related substances. Hence, we focused on developing a selective, fast, cost-effective, mass compatible
and stability-indicating method using advance technique UPLC for the related substances determination of ROSV in solid pharmaceutical dosage form. Chemical structure of ROSV and related impurities are presented in Figure 4.2.

![Acid degradation chromatogram of reference article](image)

**Figure 4.1** Acid degradation chromatogram of reference article

**A) Rosuvastatin Calcium (ROSV)**

\((3R,5S,6E)-7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoicacid calcium\)
B) Anti-Isomer

\((3R,5R,6E)-7-[4-(4-\text{Fluoro-phenyl})-6-\text{isopropyl}-2-(\text{methanesulfonyl-methyl-amino})-\text{pyrimidin-5-yl}-3,5-\text{dihydroxy-hept-6-enoicacid calcium}\)

![Chemical structure of Anti-Isomer]

C) 5-Oxo impurity

Calcium \(((3R,6E)-7-(4-(4-\text{fluorophenyl})-6-\text{isopropyl}-2-(\text{N-methylmethylsulfonamido})\text{pyrimidin-5-yl})-3-\text{hydroxy-5-oxohept-6-enoate})\)

![Chemical structure of 5-Oxo impurity]
D) Lactone impurity

\[
\text{N-[4-(4-fluoro-phenyl)-5-[2-(4-hydroxy-6-oxo-tetrahydro-pyran-2-yl)-vinyl]-6-isopropyl-pyrimidin-2-yl]-N-methyl-methanesulfonamide}
\]

![Chemical structure of ROSV, Anti-isomer, 5-Oxo and Lactone](image)

Figure 4.2 Chemical structures of ROSV, Anti-isomer, 5-Oxo and Lactone

4.2 THE SCOPE AND OBJECTIVES OF PRESENT STUDY

The main criterion for developing an RP-UPLC method for the determination of related substances in ROSV dosage form in a single run, with emphasis on the method being, a reproducible, mass compatible, stability-indicating, less time-consuming and more selective compared to the present methods. Developed method can separates three known and six major unknown degradation products from each other and from ROSV within short run time. Thereafter, the validation of developed method according to International Conference on Harmonization (ICH) guidelines [20] to show the stability indicating capability of the method.

The objectives of the present work are as follow:

- Development of rapid, stability indicating RP-UPLC method for determination of ROSV in solid oral pharmaceutical formulation.
- Forced degradation study.
- To separates ROSV from three known and six major unknown degradation products from each other and its placebo compound.
- Perform analytical method validation for the proposed method as per ICH guideline.
4.3 **ROSUVASTATIN (ROSUVASTATIN CALCIUM)**

Rosuvastatin (ROSV) is a synthetic lipid-lowering agent, chemically known as \((3R,5S,6E)-7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl(methylsulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid, calcium salt (2:1).\) It is used for the treatment of hyperlipidemia and is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis [21]. ROSV calcium is a salt with pK\(_a\) of 4.6 and very slightly soluble in aqueous solutions of pH 4.0 and below. It exhibits a high degree of specificity for uptake into the liver and is a potent in vitro and in vivo competitive inhibitor of HMG-CoA reductase.

**Strengths**

Rosuvastatin tablets are available in four strengths equivalent to 5 mg, 10 mg, 20 mg and 40 mg of Rosuvastatin in form of rosuvastatin calcium as an active ingredient for oral administration.

**Innovator**

Crestor tablets, AstraZeneca.

**Pharmacology**

**Pharmacodynamics**

Rosuvastatin is a synthetic, enantiomerically pure antilipemic agent. It is used to lower total cholesterol, low density lipoprotein-cholesterol (LDL-C), apolipoprotein B (apoB), non-high density lipoprotein-cholesterol (non-HDL-C), and triglyceride (TG) plasma concentrations while increasing HDL-C concentrations. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease and high ratios are associated with higher risk of disease. Increased levels of HDL-C are associated with lower
cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, rosuvastatin reduces the risk of cardiovascular morbidity and mortality.

**Indication**

Used as an adjunct to dietary therapy to treat primary hypercholesterolemia (heterozygous familial and nonfamilial), mixed dyslipidemia and hypertriglyceridemia. Also indicated for homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering therapies or when other such therapies are not available.

**Absorption**

Bioavailability is approximately 20%.

**Mechanism of action**

Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL. In vitro and in vivo animal studies also demonstrate that rosuvastatin exerts vasculoprotective effects independent of its lipid-lowering properties. Rosuvastatin exerts an anti-inflammatory effect on rat mesenteric microvascular endothelium by attenuating leukocyte rolling, adherence and transmigration (PMID: 11375257). The drug also modulates nitric oxide synthase (NOS) expression and reduces ischemic-reperfusion injuries in rat hearts (PMID: 15914111). Rosuvastatin increases the bioavailability of nitric oxide (PMID: 11375257, 12031849, 15914111) by upregulating NOS (PMID: 12354446) and by increasing the stability of NOS through post-transcriptional polyadenylation (PMID: 17916773). It is unclear as to how
rosuvastatin brings about these effects though they may be due to decreased concentrations of mevalonic acid.

**Metabolism**

Not extensively metabolized. Only ~10% is excreted as metabolite. Cytochrome P450 (CYP) 2C9 is primarily responsible for the formation of rosuvastatin's major metabolite, N-desmethylrosuvastatin. N-desmethylrosuvastatin has approximately 50% of the pharmacological activity of its parent compound in vitro. Rosuvastatin accounts for greater than 87% of the pharmacologic action. Inhibitors of CYP2C9 increase the AUC by less than 2-fold. This interaction does not appear to be clinically significant.

**Route of elimination**

Rosuvastatin is not extensively metabolized; approximately 10% of a radiolabeled dose is recovered as metabolite. Following oral administration, rosuvastatin and its metabolites are primarily excreted in the feces (90%).

**Protein binding**

90% bound to plasma proteins (mostly albumin)

**Half life**

19 hours

**Toxicity**

Generally well-tolerated. Side effects may include myalgia, constipation, asthenia, abdominal pain, and nausea. Other possible side effects include myotoxicity (myopathy, myositis, rhabdomyolysis) and hepatotoxicity. To avoid toxicity in Asian patients, lower doses should be considered. Pharmacokinetic studies show an approximately two-fold increase in peak plasma concentration and AUC in Asian patients (Philippino, Chinese, Japanese, Korean, Vietnamese, or Asian-Indian descent) compared to Caucasians patients.
4.4 EXPERIMENTAL

4.4.1 Materials and reagents

Rosuvastatin tablets, impurities, rosuvastatin calcium API and placebo are provided by Cadila Pharmaceutical Ltd. Dholka, Ahmedabad, India, along with the working standard. HPLC grade methanol (MeOH) obtained from J. T. Baker (NJ, USA). Trifluoroacetic acid (TFA) is obtained from Qualigens fine chemicals (Mumbai, India). All solutions are filtered through 0.22 μ nylon filters manufactured by Millipore Pvt. Ltd (Bangalore, India). Nylon membrane filters (0.22 μm) and nylon syringe filters are purchased from Millipore Ltd (India). High purity water is generated with Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

4.4.2 Equipment

Acquity UPLC™ system (Waters, Milford, USA), consisting of a binary solvent manager, sample manager and PDA (photo diode array) detector. System control, data collection and data processing are accomplished using Waters Empower™-2 chromatography data software. Cintex digital water bath is used for specificity study. Photo stability studies are carried out in a photo-stability chamber (SUNTEST XLS+, ATLAS, Germany). Thermal stability studies are performed in a dry air oven (Cintex, Mumbai, India).

4.4.3 Preparation of mobile phase and its gradient program

Table 4.1 Gradients program for elution

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>% MP-A</th>
<th>% MP-B</th>
<th>Gradient curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.3</td>
<td>45</td>
<td>55</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3.5</td>
<td>0.3</td>
<td>40</td>
<td>60</td>
<td>Linear</td>
</tr>
<tr>
<td>6.5</td>
<td>0.3</td>
<td>15</td>
<td>85</td>
<td>Linear</td>
</tr>
<tr>
<td>7.5</td>
<td>0.3</td>
<td>15</td>
<td>85</td>
<td>Isocratic</td>
</tr>
<tr>
<td>7.6</td>
<td>0.3</td>
<td>45</td>
<td>55</td>
<td>Linear</td>
</tr>
<tr>
<td>10</td>
<td>0.3</td>
<td>45</td>
<td>55</td>
<td>Equilibration</td>
</tr>
</tbody>
</table>
Determination of related substance in rosuvastatin tablets by RP-UPLC method

Mobile Phase-A (MP-A): 1 % trifluoroacetic acid in water. MP-A is filtered through 0.22 μm nylon filter and degassed under vacuum prior to use.

Mobile phase-B (MP-B): Methanol

4.4.4 Diluent preparation

Methanol is used as diluents.

4.4.5 Standard solution preparation

The diluted standard solution is prepared by dissolving the rosuvastatin calcium standard and all three impurities in diluent to obtain a solution containing 5 μg/mL.

4.4.6 Sample solution preparation

For the preparation, equivalent 50mg tablets powder is accurately transferred into a 100 mL volumetric flask. Approximately 70 mL of diluent is added to the volumetric flask, which is then sonicated in an ultrasonic bath for 8 min. The resulting solution is then diluted up to the mark with diluent and mixed well. Filter this solution with nylon 0.22μm syringe filter, discard first 3 mL solution.

4.4.7 Placebo solution preparation

In preparing the placebo solution, 750 mg of placebo is accurately transferred into a 100 mL volumetric flask. Approximately 70 mL of diluent is added to the volumetric flask, which is then sonicated in an ultrasonic bath for 8 min. The resulting solution is then diluted up to the mark with diluent and mixed well. Filter this solution with nylon 0.22μm syringe filter, discard first 3 mL solution.

4.4.8 Chromatographic conditions

The chromatographic condition is optimized using 100 x 2.1 mm, 1.7 μm Waters Acquity UPLC BEH C-18 column (Milford, USA). The optimized conditions are as follows: injection volume:
7.0 μL, flow rate: 0.3 mL/min at a column temperature of 40 °C, sample cooler temperature: 10 °C and detection wavelength: 240 nm, gradient elution [Table 4.1]. Under these conditions the system back pressure is about 7200 psi. The stress degraded samples are analyzed using a PDA detector over a range of 200 – 400 nm.

4.5 METHOD VALIDATION

After development, this method is subjected to validation according to ICH guidelines. The method is validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (specificity, system suitability, precision, accuracy, linearity, robustness, solution stability and filter compatibility).

4.5.1 Specificity

Forced degradation studies are performed to demonstrate selectivity and stability-indicating capability of the proposed method. The sample are exposed to acid hydrolysis [0.1 N HCl, 80°C, 2h], base hydrolysis [0.5 N NaOH, 80°C, 6h], oxidative [3 % H₂O₂, 80°C, 6h], thermal [100 °C, 8h], and photolytic degradation [1.2 million Lux hours]. All exposed samples are than analysed by the proposed method.

4.5.2 System suitability

System suitability parameters are measured so as to verify the system, method and column performance. System precision is determined on six replicate injections of standard preparation. All important characteristics including % RSD, tailing factor and theoretical plate number for ROSV are measured.

4.5.3 Precision

The precision of the system is determined using the sample preparation procedure described above for six real samples of solid oral (tablets) formulation and analysis using the same
proposed method. Intermediate precision is studied by other scientist, using different columns, different UPLC, and is performed on different days.

4.5.4 Accuracy

To confirm the accuracy of the proposed method, recovery experiments are carried out by the standard addition technique. Four levels (LOQ, 50 %, 100 % and 200 %) of standards are added to pre-analyzed placebo samples in triplicate. The percentage recoveries of all known impurities and unknown impurity at each level and each replicate are determined. The mean of percentage recoveries (n=12) and the relative standard deviation are also calculated.

4.5.5 Linearity

Linearity is demonstrated from LOQ to 150 % of standard concentration using a minimum of six calibration levels (LOQ, 50 %, 75 %, 100 %, 125 % and 150 %) for ROSV and its impurities. The method of linear regression is used for data evaluation. The peak areas of the compounds are plotted against the respective concentration of individual compound. Linearity is described by the linearity equation, correlation coefficient and Y-intercept bias is also determined.

4.5.6 Limit of quantification (LOQ)

The LOQ of ROSV and Impurities are determined by using signal to noise ratio method as defined in International Conference on Harmonization (ICH) guideline [20]. Increasingly dilute solution of ROSV and impurities are injected into the chromatograph and signal to noise (S/N) ratio are calculated at each concentration for each components.

4.5.7 Robustness

The robustness is a measure of the capacity of a method to remain unaffected by small but deliberate changes in flow rate (± 0.03 mL/min), change in column oven temperature (± 5°C) and change in wavelength (± 2 nm).
4.5.8 Solution stability

The stability of the sample solution is established by storage of the sample solution at ambient temperature for 24h. The sample solution is re-analyzed after 6, 12 and 24h, and the results of the analysis are compared with the results of the fresh sample. The stability of standard solution is established by the storage of the standard solution at ambient temperature for 24h. The standard solution is re-injected after 6, 12 and 24h, and % RSD are calculated.

4.5.9 Filter compatibility

Filter compatibility is performed for nylon 0.22 μ syringe filter and PVDF 0.22 μ syringe filter (Millipore). To confirm the filter compatibility in proposed analytical method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filters and percentage impurities are determined and compared against centrifuged sample.

4.6 RESULTS AND DISCUSSION

4.6.1 Method Development and Optimization

The main criterion for developing an RP-UPLC method for the determination of related substances in ROSV dosage form in a single run, with emphasis on the method being accurate, reproducible, robust, stability indicating, linear, free of interference from other formulation excipients and convenient enough for routine use in quality control laboratories.

Table 4.2 Working concentration of rosuvastatin and its impurities

<table>
<thead>
<tr>
<th>Compound</th>
<th>Working concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/mL)</td>
</tr>
<tr>
<td>ROSV</td>
<td>0.5</td>
</tr>
<tr>
<td>ROSV impurities</td>
<td>0.005</td>
</tr>
</tbody>
</table>
A spiked solution of impurities (5 µg/mL), ROSV (500 µg/mL) and placebo peaks are subjected to separation by RP-UPLC. Initially, the separation of all peaks is studied using 0.1% trifluoroacetic acid (TFA) as mobile phase A and methanol as mobile phase B on an Acquity BEH C18 (100 × 2.1 mm, 1.7µ) column and Waters (UPLC) system with an isocratic program. The 0.3 mL/min flow rate is selected to achieve the separation of peaks. The column oven temperature is maintained at 40°C. These conditions resulted in separation of the ROSV peak with the placebo peaks and impurities peaks, represented in Figure 4.1. But during force degradation study some late elute peaks are observed. It is not incorporate with reference method. Based on obtained results, isocratic program is replaced with gradient program in an effort to achieve high resolution between the, known impurities and all degradants peaks. With the Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 µ) column, different combinations of mobile phase A and B are studied to optimize the method, and the results of the optimization study are summarized in Table 4.3.

### Table 4.3 Summary of method optimization

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture of methanol and 0.1% TFA in the ratio of 50:50 employing isocratic elution; acquity UPLC BEH C18 column (100 x 2.1 mm, 1.7µ); 40°C</td>
<td>During acid degradation study some late elute peaks are observed</td>
</tr>
<tr>
<td>0.1% TFA (MP-A) and methanol (MP-B), linear gradient; acquity UPLC BEH C18 column (100 x 2.1 mm, 1.7µ); 40°C</td>
<td>Satisfactory peak separation and peak shape observed within 10 minutes</td>
</tr>
</tbody>
</table>

From the mobile phase selection study, the optimized UPLC parameters are as follows: flow rate, 0.3 mL/min; column oven temperature, 40°C; sample cooler temperature, 10°C; injection volume, 7 µL; and an gradient program with mobile phase A and B. Based on the UV spectrum of the compound, 240 nm is found to be appropriate for the determination of ROSV impurities in pharmaceutical formulations. ROSV and all impurities are well resolved with respect to each
other in a reasonable time of 10 minutes [Figure 4.3]. No chromatographic interference due to the blank (diluent) and other excipients (placebo) at the retention time of ROSV and all impurities are observed, as shown in Figure 4.3.

![Overlaid chromatograms of diluent, placebo and standard (for identification)](image)

**Figure 4.3** Overlaid chromatograms of diluent, placebo and standard (for identification)

![Acid degradation chromatogram of ROSV](image)

**Figure 4.4** Acid degradation chromatogram of ROSV

### 4.6.2 Analytical Parameters and Validation

After satisfactory development of method it is subjected to method validation as per ICH guideline [20]. The method is validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (specificity, system suitability, precision, accuracy, linearity, robustness, LOQ, solution stability and filter compatibility).
4.6.2.1 Specificity

Forced degradation studies are performed to demonstrate the selectivity and stability-indicating capability of the proposed RP-UPLC method. Figure 4.3 shows that there is no interference at the RT (retention time) of ROSV and all known impurities from the blank and other excipients. Significant degradation is not observed when ROSV is subjected to oxidation, base, photolytic and thermal, whereas significant degradation is observed when the ROSV is subjected to acid hydrolysis (0.1N HCl, 80°C, 2h) condition, leading to the formation of rosuvastatin anti isomer and unknown impurities. The acid hydrolysis product (rosuvastatin anti isomer) and ROSV are well separated from each other, as seen in Figure 4.4. The peak attributed to ROSV is investigated for spectral purity in the chromatogram of all exposed samples and it’s found to be spectrally pure. The purity and related substances of ROSV is unaffected by the presence of other excipients and thus confirms the stability-indicating power of this method. The results of the forced degradation study are summarized in Table 4.4. Specificity study specimen chromatograms are presented in Figure 4.5 to 4.9.

Table 4.4 Summary of forced degradation results

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>Peak Purity (ROSV)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>Pass</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Acid hydrolysis (0.1 N HCl, 80°C, 2h)</td>
<td>Pass</td>
<td>Significant degradation</td>
</tr>
<tr>
<td>Alkaline hydrolysis (0.5 N NaOH, 80°C, 6h)</td>
<td>Pass</td>
<td>No significant degradation</td>
</tr>
<tr>
<td>Oxidation (3 % H₂O₂, 80°C, 6h)</td>
<td>Pass</td>
<td>No significant degradation</td>
</tr>
<tr>
<td>Thermal (100 °C, 8h)</td>
<td>Pass</td>
<td>No significant degradation</td>
</tr>
<tr>
<td>Photolytic (1.2 million Lux hours)</td>
<td>Pass</td>
<td>No significant degradation</td>
</tr>
</tbody>
</table>
Figure 4.5 Specimen chromatogram of diluent

Figure 4.6 Specimen chromatogram of placebo solution

Figure 4.7 Specimen chromatogram of lactone impurity
4.6.2.2 System suitability

The percentage relative standard deviation (RSD) of area from six replicate injections is below 5.0 % (diluted standard solution, 5µg/mL). Low values of RSD for replicate injections indicate that the system is precise. The results of other system suitability parameters such as peak tailing and theoretical plates are presented in Table 4.5. As seen from this data, the acceptable system suitability parameters would be as follows: the relative standard deviation of six replicate injections is not more than 5.0 %, the tailing factor ROSV is not more than 1.5 and the theoretical plates are not less than 10000
Table 4.5  System suitability results (precision, intermediate precision and robustness)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Theoretical plates*</th>
<th>Tailing factor*</th>
<th>% RSD* of standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>13644</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>13222</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>At 0.33 mL/min flow rate</td>
<td>15573</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>At 0.27 mL/min flow rate</td>
<td>12565</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>At 35°C column temp</td>
<td>13980</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>At 45°C column temp</td>
<td>13973</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>At 238 nm</td>
<td>13542</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>At 242 nm</td>
<td>12910</td>
<td>1.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*… Determined on six values

4.6.2.3  Precision

The purpose of this study is to demonstrate the reliability of the test results with variations. The results are shown in Table 4.6, along with intermediate precision data. Low RSD values indicate that this method is precise. Overlaid chromatograms of method precision study are presented in Figure 4.10.

Table 4.6  Precision and Intermediate precision results for impurities

<table>
<thead>
<tr>
<th>Substance</th>
<th>Precision</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% impurity #</td>
<td>% RSD*</td>
</tr>
<tr>
<td>Anti-Isomer</td>
<td>1.074</td>
<td>0.5</td>
</tr>
<tr>
<td>Lactone</td>
<td>0.989</td>
<td>3.3</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>1.087</td>
<td>0.4</td>
</tr>
</tbody>
</table>

#... Average of six determinations; *… Determined on six values
Determination of related substance in rosuvastatin tablets by RP-UPLC method

4.6.2.4 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. The amount recovered (for 50, 100 and 200 % level) is within ± 10 % of amount added; for the LOQ level, the amount recovered is within ± 20 % of the amount added, indicating that the method is accurate and that there is no interference due to other excipients presents in the injection. The results of the recovery assay are shown in Table 4.7. Overlaid specimen chromatograms are presented in Figure 4.11.

Table 4.7 Accuracy results (n=3)

<table>
<thead>
<tr>
<th>Substance</th>
<th>At LOQ 0.075 µg/mL</th>
<th>At 50 % 2.5 µg/mL</th>
<th>At 100 % 5 µg/mL</th>
<th>At 200 % 10 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-isomer</td>
<td>Mean Accuracy</td>
<td>111.6</td>
<td>104.3</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>%RSD*</td>
<td>6.6</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>Mean Accuracy</td>
<td>100.4</td>
<td>96.7</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>%RSD*</td>
<td>11.5</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Lactone</td>
<td>Mean Accuracy</td>
<td>114.3</td>
<td>93.4</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td>%RSD*</td>
<td>10.1</td>
<td>0.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*… Determined on three values; #... Mean of three determinations
4.6.2.5 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in that sample within a given range [22]. The response is found to be linear in the range of 0.075 to 10 µg/mL. The regression statistics are shown in Table 4.8, with the linearity curve for ROSV, Anti isomer, 5-Oxo and Lactone represented in Figure 4.12-4.15.

Table 4.8 Regression statistics

<table>
<thead>
<tr>
<th>Substance</th>
<th>Linearity range (µg/mL)</th>
<th>Correlation Coefficient (R²)</th>
<th>Y-intercept bias in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROSV</td>
<td>0.075 to 11.84</td>
<td>0.999</td>
<td>1.4</td>
</tr>
<tr>
<td>Lactone</td>
<td>0.075 to 14.45</td>
<td>0.999</td>
<td>0.5</td>
</tr>
<tr>
<td>Anti isomer</td>
<td>0.075 to 11.25</td>
<td>0.999</td>
<td>0.9</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>0.075 to 8.12</td>
<td>0.999</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Determination of related substance in rosuvastatin tablets by RP-UPLC method

**Figure 4.12** Linearity of rosuvastatin

**Figure 4.13** Linearity of rosuvastatin anti-isomer

**Figure 4.14** Linearity of lactone
The concentration (in μg/mL) with a signal to noise ratio (S/N) of at least 10 is taken as the LOQ, which meets the criteria defined by ICH guidelines. The LOQ for the ROSV, rosuvastatin anti isomer, 5-oxo and lactone peaks are found to be 0.075 μg/mL. The precision is also established at the quantification level. The % RSD of the peak area is well within the acceptance limit of <10.0 %. The determined limit of qualification and precision at LOQ values for ROSV, rosuvastatin anti isomer, 5-Oxo and lactone are presented in Table 4.9. Specimen chromatogram of LOQ study is presented in Figure 4.16.

### Table 4.9  LOQ and its precision results

<table>
<thead>
<tr>
<th>Substance</th>
<th>LOQ (µg/mL)</th>
<th>S/N</th>
<th>Precision (% RSD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROSV</td>
<td>0.075</td>
<td>165.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Anti isomer</td>
<td>0.075</td>
<td>77.0</td>
<td>6.1</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>0.075</td>
<td>26.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Lactone</td>
<td>0.075</td>
<td>60.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

*… Determined on six values
4.6.2.7 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. No significant effect is observed on system suitability parameters such as RSD, tailing factor, or the theoretical plates of ROSV when small but deliberate changes are made to chromatographic conditions. The results are presented in Table 4.5, along with the system suitability parameters of normal conditions. Thus, the method is found to be robust with respect to variability in applied conditions.

4.6.2.8 Solution stability

Drug stability in pharmaceutical formulations is a function of storage conditions and chemical properties of the drug, preservative and its impurities. Condition used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data is required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling [22 - 25]. Stability of sample solution is established by storage of sample solution at ambient temperature for 24h. Precision solution is re-injected after 6, 12 and 24h time intervals and observed % impurity difference from initial. Sample solution did not show any appreciable
change in % impurity value when stored at ambient temperature up to 24h [Table 4.10]. Standard solution is also found to be stable up to 24h at ambient temperature. No significant area change (% RSD less than 0.5) is observed in re-injected standard preparation up to 24h.

Table 4.10 Solution stability results

<table>
<thead>
<tr>
<th>Substance</th>
<th>% impurity #Initial</th>
<th>% impurity #After 6h</th>
<th>% impurity #After 12h</th>
<th>% impurity #After 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Isomer</td>
<td>1.074</td>
<td>1.073</td>
<td>1.073</td>
<td>1.074</td>
</tr>
<tr>
<td>Lactone</td>
<td>0.989</td>
<td>0.991</td>
<td>0.992</td>
<td>0.992</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>1.087</td>
<td>1.088</td>
<td>1.089</td>
<td>1.088</td>
</tr>
</tbody>
</table>

4.6.2.9 Filter compatibility

Filter compatibility is performed for nylon 0.22 µ syringe filter and PVDF 0.22 µ syringe filter. To confirm the filter compatibility in proposed method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filter and percentage impurities are determined and compared against centrifuged sample. Sample solution does not show any significant changes in percentage impurities with respect to centrifuged sample. Percentage impurities results are presented in Table 4.11. In displayed result difference in % impurities are not observed more than ±0.05, which indicates that both syringe filters having a good compatibility with sample solution.

Table 4.11 Filter compatibility study results

<table>
<thead>
<tr>
<th>Substance</th>
<th>Centrifuged sample solution</th>
<th>PVDF Syringe filter 0.2µm</th>
<th>% Difference in impurities from centrifuged</th>
<th>Nylon Syringe filter 0.2µm</th>
<th>% Difference in impurities from centrifuged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Isomer</td>
<td>1.071</td>
<td>1.074</td>
<td>0.003</td>
<td>1.076</td>
<td>0.005</td>
</tr>
<tr>
<td>Lactone</td>
<td>0.982</td>
<td>0.989</td>
<td>0.007</td>
<td>0.976</td>
<td>0.006</td>
</tr>
<tr>
<td>5-Oxo</td>
<td>1.081</td>
<td>1.087</td>
<td>0.006</td>
<td>1.075</td>
<td>0.006</td>
</tr>
</tbody>
</table>
4.7 CALCULATION FORMULA

4.7.1 Related Substances (% w/w)

Calculated the quantity, in %, of impurities in the portion of solid oral pharmaceutical formulation using the following formula:

\[
\text{Impurity (\% w/w)} = \frac{C_{\text{std}} \times R_s \times 10000}{C_s \times R_{\text{std}}}
\]

Where,

- \(C_{\text{std}}\) = Concentration of standard solution in mg/mL
- \(C_s\) = Concentration of sample solution in mg/mL
- \(R_s\) = Compound peak response obtained from the sample preparation
- \(R_{\text{std}}\) = Compound peak response (mean peak area) obtained from the standard preparation

4.7.2 Relative standard deviation (% RSD)

It is expressed by the following formula and calculated using Microsoft excel program in a computer.

\[
\text{Related Standard Deviation (\%)} = \frac{SD \times 100}{\bar{X}}
\]

Where,

- SD = Standard deviation of measurements;
- \(\bar{X}\) = Mean value of measurements

4.7.3 Accuracy (% Recovery)

It is calculated using the following equation:

\[
\% \text{ Recovery} = \frac{\text{Amount of substance found (mg) \times 100}}{\text{Amount of substance added (mg)}}
\]
4.8 CONCLUSION

A new RP-UPLC method is successfully developed for the estimation of related substances in rosuvastatin tablets. The method validation results have verified that the method is selective, precise, accurate, linear, robust and stability-indicating. The run time (10.0 min) enables rapid determination of impurities. This stability-indicating method can be applied for the determination of related substances in bulk drugs, pharmaceutical formulations and chemical processing. The developed method can also be applied for identification of unknown impurities.
4.9 REFERENCES


Determination of related substance in rosuvastatin tablets by RP-UPLC method


