Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Form

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ABSTRACT
A stability indicating, accurate, specific, linear and sensitive reverse phase-HPLC method has been developed and validated for the determination of Rosuvastatin as calcium, (ROS) in pharmaceutical dosage form. The chromatographic separation was performed using end capped (Luna) C18 Column (250 mm x 4.6 mm, 5μm particle size). Mobile phase A was prepared by mixing 3.0g/l Ammonium dihydrogen phosphate in distilled water: Methanol: Acetonitrile: Tetrahydrofuran in the ratios (400:20:100:5v/v). To 1000 ml of the resulting solution 1 ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid. Mobile phase B was prepared by mixing Acetonitrile: Methanol: Tetrahydrofuran in the ratios (500:50:5v/v). Other chromatographic conditions such as flow rate set at 2.0 ml/min and 30°C column temperature with the detection wavelength at 243nm. The retention times of Rosuvastatin was found to be about 16 min. The linearity was performed in the concentration range of 40.0-60.0μg/ml with a squared correlation coefficient of 0.99998. The percentage purity of ROS was found to be >99.8%. The percentage recovery was determined for ROS and was found to be 100.067%. The developed analytical method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness which were within the acceptance limit according to ICH guidelines. All the degradation products obtained by stress conditions were found to be well separated from the principal peak, which means that the ROS peaks were highly pure in all chromatograms obtained. The developed method was successfully employed for routine quality control and stability analysis of ROS in pharmaceutical dosage forms.

Keywords: Rosuvastatin Calcium, Stability-Indicating, RP-HPLC, Validation.

INTRODUCTION
Rosuvastatin (ROS), bis((E)-7[4-(4-Fluorophenyl)-6-isopropyl-2-(Methyl (methylsulfonyl) aminopyrimidin-5yl)-3,5-dihydroxyhept-6-enoic acid) Calcium salt [Fig. 1]. [1] It belongs to the class of drugs called statins which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases. [2] It is highly effective 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. [1, 3,4] In clinical trials, rosuvastatin achieved mark reduction in serum levels of LDL cholesterol, accompanied by modest increases in HDL cholesterol and reduction in triglyceride. [1] The most important related compounds for rosuvastatin are antiisomer and lacton impurity. [6] Literature survey reveals that few Stability-indicating HPLC methods [1,14], spectrophotometric methods [15-17], HPTLC [18-19] methods have been reported for the estimation of ROS as a single or in combined pharmaceutical preparations.

Fig. 1: Chemical Structure for Rosuvastatin Calcium

In the present work we are focused on to develop and validate a stability indicating method with optimum chromatographic conditions for the determination of ROS in pharmaceutical preparations in the presence of its related impurities (Rosuvastatin antiisomer and Lacton impurity) and other unknown degradation products that may be present during stability study. The developed method was validated...
as per ICH guidelines, and can be applied successfully to quality control purposes.

**MATERIALS AND METHODS**

**Reagents and Chemicals**
Rosuvastatin, Rosuvastatin antisomer and Rosuvastatin lacton impurity were purchased from MSN Laboratories Limited, India. All chemicals used were of HPLC grade: Acetonitrile, Tetrahydrofuran, Methanol, Orthophosphoric acid were purchased from J.T. Baker, Triethylamine was purchased from Mallinckrodt Chemicals, and Ammonium dihydrogen phosphate was purchased from Merck. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

**Equipment and Chromatographic Conditions**
A Dionex UltiMate 3000 HPLC system with Chromeleon software “version 6.8”, Photodiode Array Detector and Autosampler was used. It was manufactured by Dionex Corporation Company, USA. An end capped (Luna) C\(_18\) Column (250 mm x 4.6 mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: (3.0 g/l Ammonium dihydrogen phosphate: Methanol, Acetonitrile: Triyrahydrofuran) in the ratios of (400:20:100:5v/v). To 1000 ml of the resulting solution 1ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid. Mobile phase B: (Acetonitrile: Methanol: Triyrahydrofuran) in the ratios (500:50:5v/v) with gradient elution program as presented in Table 1. The flow rate was adjusted to 2.0 ml/min, the injection volume was set at 20µL, the column compartment was operated at 30°C and the UV detection was set at 243nm. The purity analysis was performed over a wavelength range of 200-400nm.

**Preparation of Analytical Solutions**

**Preparation of Ammonium Dihydrogen Phosphate**
It was prepared by dissolving 3.0 g of ammonium dihydrogen phosphate in 1000 ml of distilled water.

**Preparation of Diluent for samples preparation**
It was prepared by mixing 500 ml of Acetonitrile and 500 ml of Distilled Water.

**Preparation of mobile phase A**
It was prepared by mixing 1200 ml of ammonium dihydrogen phosphate, 60 ml of Methanol, 300 ml of acetonitrile and 15 ml of tetrahydrofuran, to 1000 ml of the resulting solution 1.0 ml of triethylamine was added and the pH was adjusted to 6.3 with 5% orthophosphoric acid. Degassed in ultrasonic water bath for 2 minutes and filtered through 0.45µ filter under vacuum filtration.

**Preparation of mobile phase B**
It was prepared by mixing 500 ml Acetonitrile, 50 ml of Methanol and 5 ml of tetrahydrofuran. Degassed in ultrasonic water bath for 2 minutes and filtered through 0.45µ filter under vacuum filtration.

**Preparation of stock system suitability solution**
It was prepared by dissolving 1.0mg of each of Rosuvastatin antisomer and Lacton impurity in 100 ml of diluent, sonicated for 5 minutes, allowed to cool to room temperature and filtered using 0.45µ filter to obtain a solution having a concentration of 0.01mg/ml of each.

**Preparation of stock standard solution for preparation of system suitability solution**
It was prepared by dissolving an accurately weighed quantity of Rosuvastatin calcium equivalent to 25.4 mg of Rosuvastatin (as calcium) in 50.0 ml of diluent, sonicated for 5 minutes, allowed to cool and filtered using 0.45µ filter to obtain a solution having a concentration of 0.508 mg/ml.

**Preparation of system suitability solution**
It was prepared by transferring 5.0 ml of each of stock standard solution and stock system suitability solution to 50 ml volumetric flask and completed to volume with diluent. Mixed and filtered using 0.45µ filter to obtain a solution having a concentration of 0.001 mg/ml of each of Rosuvastatin antisomer and Lacton impurity and 0.0508 mg/ml of Rosuvastatin.

**Preparation of standard solution**
It was prepared by dissolving ROS standard equivalent to 25 mg of Rosuvastatin (as calcium) in 50 ml of diluent, sonicated for 5 minutes, cooled to room temperature then 5.0 ml of the resulting solution was diluted to 50 ml with diluent, mixed well and filtered using 0.45µ filter to obtain a solution having a concentration of 0.05 mg/ml.

**HPLC-Method Development and Validation**
The analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as specificity and peak purity, linearity, precision, accuracy (per cent recovery), and system suitability were tested using the above mentioned chromatographic conditions and instruments.

**Specificity of analytical method and peak purity**
The specificity and peak purity were carried out to determine whether there are any interference due to presence of impurities, degradation products or other components that may be present in retention time of analytical peaks and affect the peak purity and specificity of the analytical method. Forced degradation studies were carried out by using 0.5M HCl (at 90°C for 20 minutes), 2M NaOH (at 90°C for 60 minutes), thermal degradation (at 105°C for 16 Hours), 33% Hydrogen peroxide (at 90°C for 30 minutes) and Photo degradation (for 20 Hours).

**Linearity**
The linearity of the method was established by spiking a series of sample of ROS, the solutions of five different concentrations 40-60µg/ml were injected into the HPLC system. The calibration curve was constructed for the standard solutions by plotting their concentrations against their respective peak areas. Regression curve was obtained and slope-a, intercept-b, and correlation coefficient-R² were determined.

**Precision and ruggedness**
Precision was determined by injecting six independent preparations from a single lot of formulation (50µg/ml) of ROS into HPLC system, while ruggedness was determined by injecting six independent preparations prepared by another analyst into another HPLC system. The retention time and peak area were obtained and the mean and %RSD were found to be within the acceptance criteria.

**Accuracy (per cent recovery)**
The accuracy study was performed on 80%, 100% and 120% of the target concentration of ROS. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level were obtained. The percentage recovery was found to be 0.235. The accuracy study was performed on 80%, 100% and 120% as a mean % recovery of all determinants at the target concentration of ROS. The percentage recovery was found to be 0.99998 for ROS.

RESULTS AND DISCUSSION

The developed analytical method is a new stability indicating RP-HPLC method for the estimation of ROS in pharmaceutical dosage forms. Various mobile phases and columns were used for the development and validation of the analytical method. The final method was optimized with the following conditions: The mobile phase consisted of: mobile phase A: (3.0 g/l Ammonium dihydrogen phosphate: Methanol, Acetonitrile: Tetrahydrofuran), (400:20:100:5 v/v), to 1000 ml of the resulting solution 1 ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid and mobile phase B: Acetonitrile: Methanol: Tetrahydrofuran (500:50:5 v/v) with gradient elution system as presented in Table 1. An end capped (Luna) C18 Column (250 mm × 4.6 mm, 5µm particle size) was used for chromatographic separation. The flow rate was adjusted to 2.0 ml/min and the column oven was operated at 30°C. The injection volume was set to 20µL and the photodiode array detector was set at 243nm. The specificity and peak purity were carried out to determine whether there was any interference due to presence of impurities, degradation products or other components that may be present at the retention time of analytical peak and affect the peak purity and specificity of the analytical method. The purity analysis was performed over a wavelength range of 200-400nm. The linearity was determined as linearity regression of the analyte concentration of the range 40-60μg/ml (ROS). The calibration curve obtained by plotting concentration versus peak area (presented in Table 2) and Fig 2) was linear and the squared correlation coefficient was found to be 0.99998 for ROS.

The precision of the method was determined from the peak areas of six determinants of homogeneous sample preparation. The % Relative Standard Deviation for system precision exhibited in Table 3 was found to be 0.509, and the % Relative Standard Deviation for method precision exhibited in Table 4 was found to be 0.15 and the % Relative Standard Deviation for ruggedness exhibited in Table 5 was found to be 0.235. The accuracy study was performed on 80%, 100% and 120% of the target concentration of ROS. The percentage recovery was determined for ROS and was found to be 100.067% as a mean % recovery of all determinants at the three concentration levels as shown in Table 6.
Chromatogram for photo degradation Figure 6 exhibited three degradation products with retention times at 19 minute, 20.5 minute and 21.6 minute which were found to be well separated from each others and not affecting the ROS peak purity. Chromatogram for 0.5M HCl degradation Figure 7 exhibited two degradation products at retention times 16.7 minute and 25.5 minute which were the same retention times of rosuvastatin antiisomer and lacton impurity respectively. Finally Chromatogram for thermal degradation Figure 8 exhibited a single well separated degradation product with retention time at 25.5 minute. All the degradation products obtained by stress conditions discussed above were found to be well separated from the principal peak, which means that the ROS peaks were highly pure in all chromatograms obtained.

The prescribed analytical method was developed and validated for system suitability, linearity, specificity, accuracy, robustness and ruggedness. All parameters tested were found to be within limits of ICH guideline. The study indicates that the method has significant advantages in term of stability indicating (good resolution between active drugs and Rosuvastatin antiisomer or other degradation products), high purity of active drug, accuracy and precision. The developed analytical method was successfully employed for routine and stability analysis of ROS in pharmaceutical dosage forms.

ACKNOWLEDGMENTS

Authors are thankful to Sama Pharmaceuticals Manufacturing Co. for providing facilities to execute the research work. Authors are thankful to Ala’ Dmaidi for electronic treatment and work.

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