**5.4.METHOD VALIDATION**

**1. SYSTEM SUITABILITY:**

Tailing factor for the peaks due to Rosuvastatin and Fenofibrate in standard solution should not be more than 2.0.Theoretical plates for the Rosuvastatin and Fenofibrate peaks in standard solution should not be less than 2000.

**2. SPECIFICITY:**

Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

**Acceptance criteria:**

Chromatogram of standard and sample should be identical with near Retention time.

**Blank interference:**

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

**Acceptance criteria:**

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

**3. LINEARITY:**

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

**Acceptance criteria:**

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

**Statistical Evaluation:**

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

**4. PRECISION:**

**Preparation of sample:**

* Transfer the 200.5mg of sample into a 100ml of volume at flask and add 10ml of water and 10ml of Methanol and sonicate 20min and makeup with water. Transfer the above solution into 5ml into 50ml volume metric flask dilute to the volume with water.
* The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

**Acceptance criteria:** The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times is not more than 2.0.

**5. RECOVERY/ACCURACY**

Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.

**Acceptance criteria:**

The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.

**6. LIMIT OF DETECTION**

The sensitivity of measurement of Rosuvastatin and Fenofibrate by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noice level. The LOD was calculated as three times the noise level.

LOD= 3.3 σ / S

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

**7. LIMIT OF QUANTITATION:**

The sensitivity of measurement of Rosuvastatin and Fenofibrate by the use of proposed method was estimated in terms of limit of quantitation (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

LOQ = 10 σ / S

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

**8. ROBUSTNESS:**

**Effect of variation in flow rate:**

Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

**Effect of variation in wavelength:**

Prepare the system suitability solution as per the test method and injected into the HPLC with ±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.