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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN AND ROSUVASTATIN IN PURE AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A new, simple, rapid, economical, specific, precise and accurate method for simultaneous estimation of Olmesartan and Rosuvastatin in pure and their pharmaceutical combined dosage form has been developed and validated as per ICH Guidelines by using RP-HPLC. The separation was achieved by Develosil (C18) (150mm x 4.6mm) 5µm particle size column and Acetonitrile: Methanol: Phosphate Buffer(25:20:55v/v) used as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 248nm. The retention time of Olmesartan and Rosuvastatin was found to be 1.789 min and 3.488 min, respectively. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, limits of detection, and

quantification for the impurities following the ICH guidelines. Linearity was observed for Olmesartan (10-30µg/ml) and for Rosuvastatin (20-70µg/ml). The mean recovery for Olmesartan and Rosuvastatin is 100.43±0.107 and 100.25±0.211, respectively. The developed method was found to be simple, rapid, accurate, precise, and specific for the simultaneous estimation of Olmesartan and Rosuvastatin in pure and their pharmaceutical combined dosage form.

Keywords: Olmesartan and Rosuvastatin, RP-HPLC, Accuracy, Precision, Robustness

1. INTRODUCTION

Olmesartan is an Antihypertensive agent **[1]**. Olmesartan is a solid-type drug that is insoluble in water and frugally soluble in Methanol **[2-4]**. The Bioavailability of Olmesartan is about 26%. Olmesartan is metabolized by Ester hydrolysis during the absorption from the Gastrointestinal tract. Olmesartan is an Angiotensin Receptor Blocker (ARB) that particularly inhibits the AT1-mediated aldosterone-secreting effects of Angiotensin by inhibiting the binding of Angiotensin -II to AT1 which leads to a decrease in Vascular resistance and blood pressure. Rosuvastatin is an Antihyperlipidemic agent that is used as an adjunct to diet to help in the curing of Hyperlipidemia, Hypertriglyceridemia, and mixed dyslipidemia **[5-7]**. The metabolism of Rosuvastatin is going on in the liver primarily and mainly it acts as a competitive inhibitor of HMG-COA reductase which is helpful in catalyzing the HMG-COA to Mevalonate. Rosuvastatin stimulates the Liver to increase the hepatic production of Low-Density Lipids (LDL) and inhibits Very low-density lipids (VLDL) **[8]**.

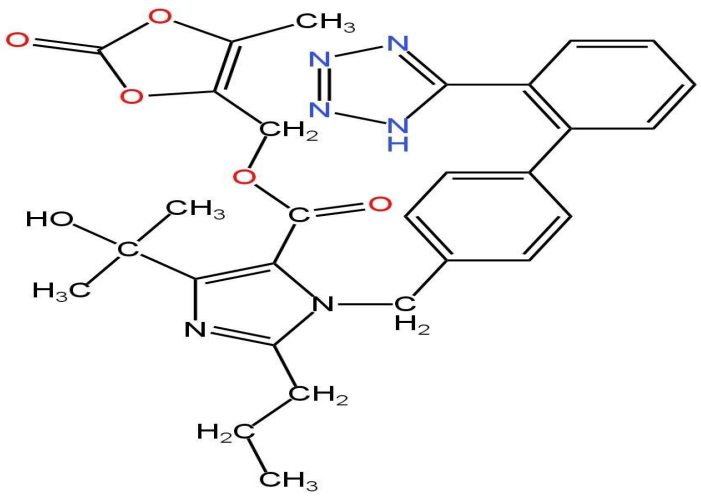

Figure 1: Structure of Olmesartan

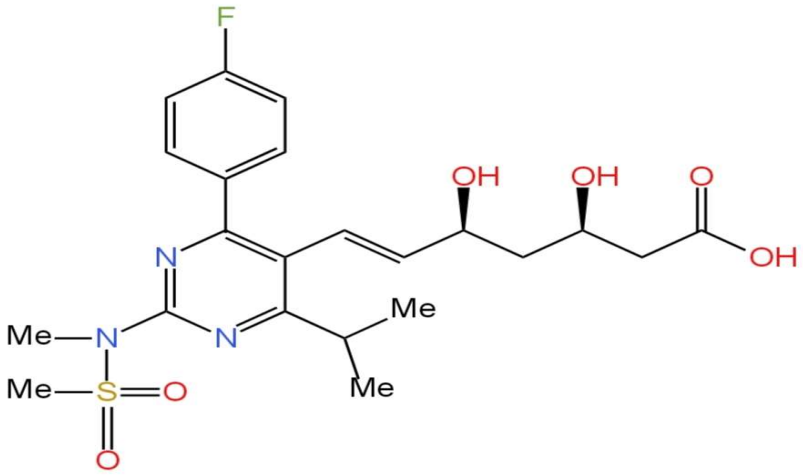

Figure 2: Structure of Rosuvastatin

The literature review has been carried out to estimate the reported analytical methods for our interested drugs individually as well as in the combination with other drugs like HPLC and RP-HPLC have been reported for the Rosuvastatin (RST) and Olmesartan (OST) individually and combinational, few Isocratic RP-HPLC methods for separation and quantification of RST Calcium and OST Medoxomil in bulk drug and Pharmaceutical dosage, few RP-HPLC methods for determination of RST and OST simultaneously in pure drug, and for simultaneous estimation of RST and OST in tablet dosage form, few RP-HPLC methods for assay of RST Calcium in tablet dosage form, and Reproducible HPLC for assay of OST Medoxomil in the tablet dosage form. As we all know, till now there is no RP-HPLC method for simultaneous estimation of Olmesartan and Rosuvastatin have has published **[9]**. The present study is to develop a simple, sensitive, authentic, and economical analytical method for the simultaneous estimation of Olmesartan and Rosuvastatin in bulk form and combined pharmaceutical dosage form **[10]**.

2. MATERIALSANDINSTRUMENTS

2.1. Chemicals and Reagents

OST and RST drugs from Sura labs, Water, Methanol, and Acetonitrile from LICHROSOLV (Merck) for HPLC, etc.

2.2. Instrument Specifications

A WATERS ALLIANCE 2695 High performance liquid chromatogram system which is having Auto sampler and PDA Detector 996 model, PH meter and Lab India, Sartorius Weighing Machine, Volumetric flasks, Pipettes, Burettes and Beakers like Glassware obtained from Borosil and a Digital ultra sonicator from Lab India ; the analysis was performed in the Develosil

column of (C18) (156mm × 4.6mm) with particle size of 5µm, Acetonitrile: Methanol : Phosphate buffer in 25:20:55 v/v with a flow rate of 1ml/1min at a wavelength of 248nm run time for 8 mins.

2.3.Preparation of Solutions

2.3.1. Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer

6.4083gm of Potassium dihydrogen Phosphate buffer is dissolved in 1000 ml of HPLC water, filter and sonicate this solution with the help of Vacuum filtration and Ultra sonicator and PH is maintained at 3.8 by the addition of diluted orthophosphoric acid.

2.3.2. Preparation of Mobile phase

Prepare a mixture of 250 ml of Acetonitrile (25%), 200 ml of Methanol (20%), and 550 ml of Phosphate buffer (55%) and degas in the digital ultra sonicator for 20 mins and filtered under vacuum filtration through 0.45u filter.

2.3.3. Preparation of Diluent

The Mobile phase acts as a Diluent.

2.4. Preparation of Olmesartan and Rosuvastatin Standard Solution

Weighed accurate amounts of 10mg of Olmesartan and 10mg of Rosuvastatin working standard into a clean and dried 10 ml of Volumetric flasks and add about 7 ml of Diluent to it, sonicate to dissolve completely and make the volume up to the mark with the same solvent.

2.4.1. Preparation of Olmesartan and Rosuvastatin Standard Stock Solution

Further pipette 0.2ml of above Olmesartan and 0.5ml of Rosuvastatin stock solutions into 10ml volumetric flasks and dilute with the help of diluent.

2.5. Preparation of Olmesartan and Rosuvastatin Sample Solution

Take an average-weighed tablet and crush it in a mortar with the help of a pestle and weigh 10mg of the equivalent weight of Olmesartan and Rosuvastatin in a 10 ml clean volumetric flask and add about 7ml Diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent.

2.5.1. Preparation of Olmesartan and Rosuvastatin Sample Stock solution

Further pipette 0.2mlof above Olmesartan and 0.5mlof Rosuvastatin sample solution into a 10ml volumetric flasks and dilute by the diluent.

2.6. Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay.

3. RESULTS AND DISCUSSION

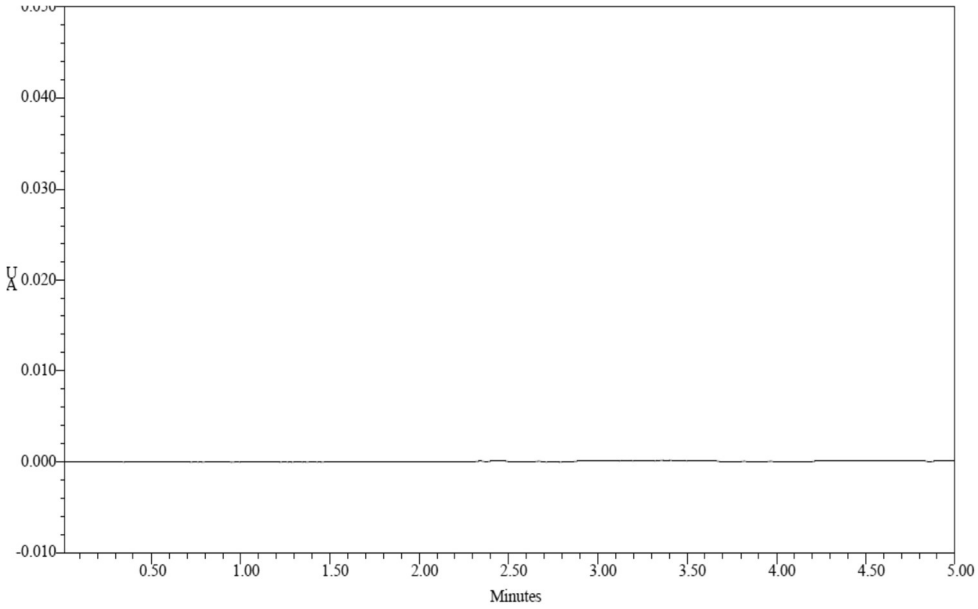

Figure 3: The Chromatogram of Blank Reading

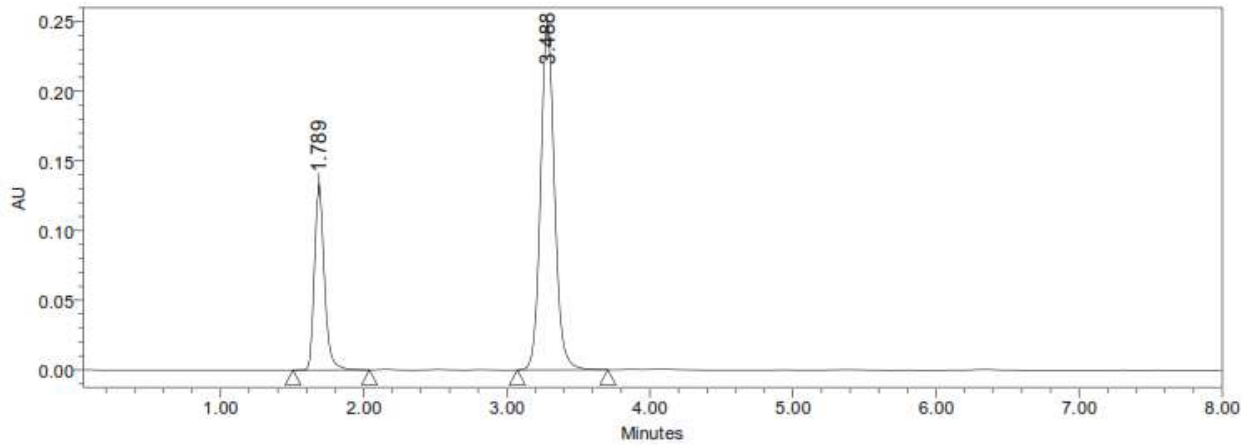

Figure 4: Chromatogram For Assay of Standard

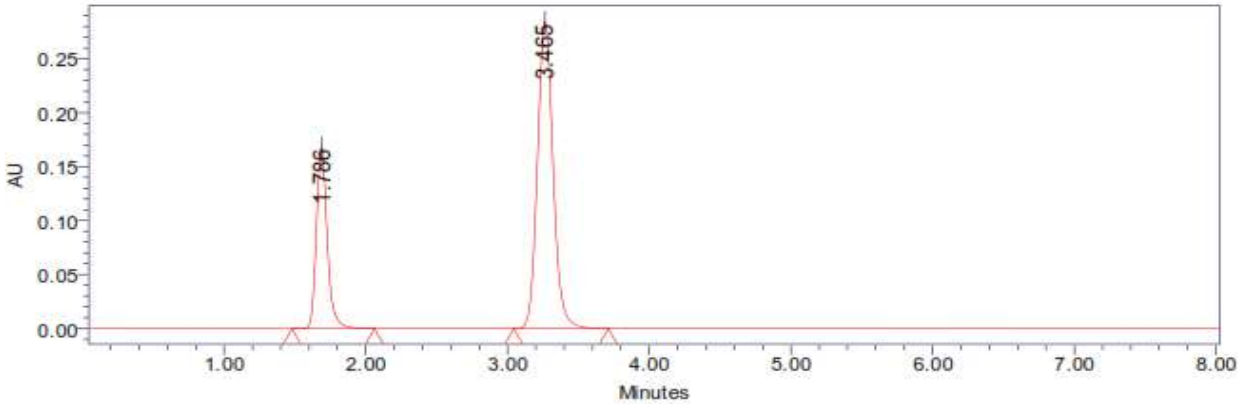

Figure 5: Chromatogram For Assay of Sample

3.1. Validation of the analytical method

As per ICH Standards, the designed chromatographic approach was examined for system suitability, specificity, linearity, accuracy, precision, intermediate precision, and robustness.

3.2. System Suitability

System suitability is to determine whether a particular procedure produces results which are accurate and precise enough. The system convenience is recognized by the completion of method development and its validation. It can be performed by injecting five replicates of the standard solution and measuring the area in HPLC. The %RSD was found to be within the specified limits for the area of all five replicates.

Table 1:Peak results for assay standard of Olmesartan

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Olmesartan	1.788	658458	8649	8269	9454
2	Olmesartan	1.792	658954	8652	8247	9485
3	Olmesartan	1.793	658748	8649	8254	9482
4	Olmesartan	1.788	658965	8675	8235	9486
5	Olmesartan	1.787	658254	8692	8249	9458
Mean			658675.8			
Standard Deviation			312.8885			
%RSD			0.047503			

Table 2: Peak results for assay standard of Rosuvastatin

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosuvastatin	3.438	8476852	63452	9456	1.56
2	Rosuvastatin	3.446	8475825	63526	9486	1.57
3	Rosuvastatin	3.444	8485698	63587	9475	1.56
4	Rosuvastatin	3.465	8478589	63254	9482	1.56
5	Rosuvastatin	3.465	8465875	63526	9472	1.57
Mean			8476568			
Standard Deviation			7113.619			
%RSD			0.083921			

3.3. Linearity

linearity was performed by diluting the standard solution at five different concentrations. 10,15,20,25 and 30 µg/ml of Olmesartan solutions were prepared and injected into the system and the peak area was measured. Similarly, 30,40,50,60, and 70 µg/ml solutions of Rosuvastatin were prepared, and injected into the system and peak area was measured.

Calibration curves were plotted between peak area (on y-axis) and concentration (on x-axis) and correlation coefficient was calculated.

The results of linearity were given below in **Table 3 and 4** and calibration curves are shown in **Figure 6, 7**.

Table 3: Linearity data for Olmesartan

Concentration (µg/ml)	Averagepeak area
10	364859
15	538493
20	698898
25	869582
30	1036878

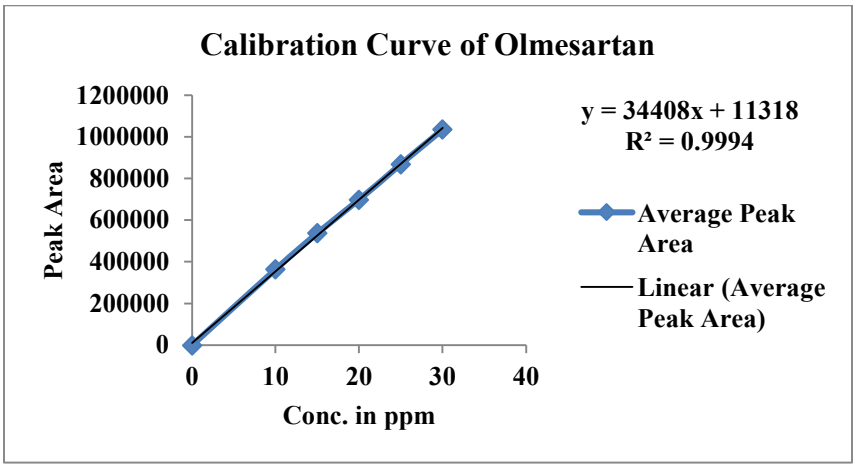

Figure 6: Calibration Curve of Olmesartan

Table 4: Linearity data for Rosuvastatin

Concentration (µg/ml)	Average peak area
30	4898784
40	6586458
50	8256847
60	9854752
70	11456982

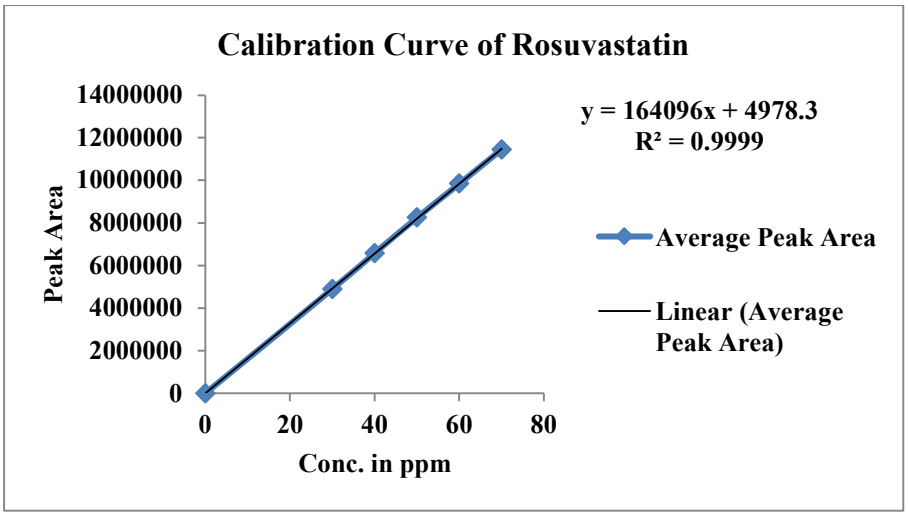

Figure 7: Calibration Curve of Rosuvastatin

3.4.Precision

3.4.1. *Repeatability*

The standard solution was injected into the system five times and the area was measured for all five injections. The % RSD for both Olmesartan and Rosuvastatin was found to be within specified limits. The results of repeatability of Olmesartan ad Rosuvastatin are mentioned in **Table 5 and 6** Respectively.

Table 5: Results of repeatability for Olmesartan

S. No.	Peak Name	Retention Time	Area (µV* sec)	Height (µV)	USP Plate count	USP Tailing
1	Olmesartan	1.792	658795	8659	8269	1.38
2	Olmesartan	1.791	658469	8675	8275	1.39
3	Olmesartan	1.790	658742	8629	8294	1.38
4	Olmesartan	1.790	658695	8675	8275	1.38
5	Olmesartan	1.789	658492	8629	8295	1.39
Mean			658638.6			
Std.Dev			148.81969			
%RSD			0.022595			

Table 6: Results of repeatability for Rosuvastatin

S. No.	Peak Name	Retention time	Area (µV* sec)	Height (µV)	USP plate count	USP tailing
1	Rosuvastatin	3.435	8475886	63598	9485	1.56
2	Rosuvastatin	3.428	8475898	63785	9468	1.57
3	Rosuvastatin	3.419	8475898	63985	9478	1.56
4	Rosuvastatin	3.414	8475986	63598	9498	1.56
5	Rosuvastatin	3.408	8478598	63785	9482	1.57
Mean			8476453			
Standard deviation			1199.651			
%RSD			0.014153			

3.4.2. *Intermediate precision:*

It is also called Ruggedness.

Here precision was performed on different days under the same conditions. It was performed by injecting the standard solution three times and the area was measured. The % RSD was calculated and was found to be within limits. The results of intermediate precision on day 1 and day 2 of both drugs are given in **Tables 7, 8, 9 and 10**.

Table 7: Results of Intermediate precision day 1 for Olmesartan

S. No.	Peak Name	RT	Area (µV* sec)	Height (µV)	USP plate count	USP Tailing
1	Olmesartan	1.787	665985	8756	8365	1.39
2	Olmesartan	1.789	662598	8725	8495	1.40
3	Olmesartan	1.789	664875	8795	8367	1.39
Mean			664486			
Std. dev			1726.683			
%RSD			0.259852			

Table 8: Results of intermediate precision day 1 for Rosuvastatin

S. No.	Peak Name	RT	Area (µV* sec)	Height (µV)	USP plate count	USP Tailing
1	Rosuvastatin	3.482	8596852	64589	9568	1.58
2	Rosuvastatin	3.477	8578458	64856	9548	1.59
3	Rosuvastatin	3.477	8596854	65823	9653	1.58
Mean			8590721			
Std. dev			10620.36			
%RSD			0.123626			

Table 9: Results of intermediate precision day 2 for Olmesartan

S. No.	Peak Name	RT	Area (µV* sec)	Height (µV)	USP plate count	USP Tailing
1	Olmesartan	1.790	648958	8569	8156	9365
2	Olmesartan	1.789	648965	8647	8125	9386
3	Olmesartan	1.793	648537	8652	8248	9347
Mean			648820			
Std. dev			245.1102			
%RSD			0.037778			

Table 10: Results of intermediate precision day 2 for Rosuvastatin

S. No.	Peak Name	RT	Area (µV* sec)	Height (µV)	USP plate count	USP Tailing
1	Rosuvastatin	3.474	8365985	62589	9365	1.54
2	Rosuvastatin	3.473	8345876	62847	9384	1.55
3	Rosuvastatin	3.478	8352884	62598	9368	1.54
Mean			8354915			
Std. dev			10207.19			
%RSD			0.12217			

3.5. Accuracy

Accuracy was performed by preparing 50%,100%, and 150% standard stock solutions from the standard solution. Inject the individual concentrations (50%,100%, and 150%) in three replicates, calculate the amount added and the amount found, then individual recovery and mean recovery. Record the chromatograms and measure the peak responses as well. The accuracy results of Olmesartan and rosuvastatin were given in **Tables 11 and 12** respectively.

Table 11: Accuracy results for Olmesartan

% Concentration (at specification Level)	Area	Amount Added(ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	357480.6667	10	10.060	100.600%	100.43%
100%	703252.7	20	20.109	100.545%	
150%	1044939	30	30.040	100.133%	

Table 12: Accuracy results for Rosuvastatin

%Concentration (at specific Level)	Area	Amount Added(ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	417522	25	25.141	100.564%	100.25%
100%	826823.3	50	50.085	100.170%	
150%	1236008	75	75.021	100.028%	

3.6.LOD AND LOQ:

LOD is the smallest concentration of analyte that gives the measurable response. whereas, LOQ is the smallest concentration of analyte, which gives a response that can be accurately quantified. the results of LOD&LOQ are given in **Table 13**.

Table 13: LOD and LOQ of Olmesartan and rosuvastatin

DRUG	LOD	LOQ
Olmesartan	1.13µg/ml	3.39µg/ml
Rosuvastatin	2.35µg/ml	9.024µg/ml

3.7. Robustness

The robustness was performed by altering the flow rate (±0.1) and mobile phase ratio (±5%). There was no significant change in the parameters like tailing factor, asymmetric factor, resolution, and plate count. The results of the robustness of Olmesartan and rosuvastatin were given in **Tables 14 and 15** respectively.

Table 14: Results for the robustness of Olmesartan

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 1.0mL/min	658798	1.789	8257	1.38
Less flow rate of 0.9mL/min	706589	1.867	8846	1.35
More flow rate of 1.1mL/min	635284	1.744	8458	1.36
Less organic phase (about 5% decrease in organic phase)	625879	1.831	8652	1.34
More organic phase (about 5% increase in the Organic phase)	618547	1.874	8745	1.32

Table 15: Results for the robustness of Rosuvastatin

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 1.0ml/min	8475847	3.488	9475	1.56
Less flow rate of 0.9mL/min	9154885	3.721	9365	1.53
More flow rate of 1.1mL/min	8365984	3.097	9585	1.54
Less organic phase (about 5% decrease in organic phase)	8265945	6.242	9582	1.55
More organic phase (about 5% increase in organic phase)	8569854	2.402	9658	1.52

CONCLUSION

A new method was developed for the simultaneous estimation of Olmesartan and Rosuvastatin by the RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Olmesartan and Rosuvastatin by using Develosil C18 column which measures 150mm* 4.6mm, and 5µm particle size. The flow rate was maintained at 1ml/min, the mobile phase ratio was Acetonitrile:

methanol: phosphate buffer (25:20:55v/v) and the detection wavelength was 248nm.

The WATERS HPLC autosampler instrument, separation module 2695, photodiode array detector 996, empower-software version-2 was used. The retention times of Olmesartan and Rosuvastatin were found to be 1.789mins and 3.488mins respectively. The system suitability parameters of both the drugs such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)).

The mean recovery of Olmesartan and Rosuvastatin was found to be 100.43% and 100.25%, the %RSD for repeatability was 0.022 and 0.014 the precision study was precise, robust, and repeatable. The linearity study in the concentration range of 10µg-30µg (Olmesartan) and 30µg-70µg (Rosuvastatin) and correlation coefficient (r^2) was found to be 0.999 and 0.999.LOD value was 1.13 and 3.39, and LOQ value was 2.35 and 9.024 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Olmesartan and Rosuvastatin in bulk and combined pharmaceutical dosage form.

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