Development and Validation of LC Method for the Simultaneous Estimation of Rosuvastatin Calcium and Olmesartan Medoxomil in Pharmaceutical Dosage Form

TRIPTI SHARMA[1]*, SUDAM C SI[1], DANNANA G SANKAR[2].

[1]Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Siksha'O' Anusandhan University, Bhubaneswar-751003, India.

[2]Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, India.

***Corres.author: triptisharma@soauniversity.ac.in**

Abstract: A simple, fast, and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of Rosuvastatin calcium and Olmesartan medoxomil in bulk drug and pharmaceutical dosage form. The quantification was carried out using Symmetry C18 column (4.6mm×150mm, particle size 5.0μm) and mobile phase comprised of buffer: acetonitrile: tetrahydrofuran in the ratio of (71:25:4 v/v/v). The flow rate was 1.5mL/min and the effluent was monitored at 248nm. The retention time of Rosuvastatin calcium and Olmesartan medoxomil were 11.66±0.85 and 13.67±0.8 min respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of Rosuvastatin calcium and Olmesartan medoxomil were in the range of 5-20μg/mL and 20-80μg/mL respectively. The proposed method is suitable for simultaneous determination of Rosuvastatin calcium and Olmesartan medoxomil in pharmaceutical dosage form and bulk drug.

Keywords: Rosuvastatin calcium, Olmesartan medoxomil, HPLC, method validation.

INTRODUCTION

Hypertension and dyslipidemia are amongst the most important primary risk factors for coronary artery disease and stroke. A majority of hypertension patients have additional cardiovascular risk factors; dyslipidemia is one of the most co-prevalent risk factors. The risk associated with concomitant hypertension and dyslipidemia are generally greater than sum of cardiovascular risks from hypertension and dyslipidemia alone.[1] Fixed dose combination of Rosuvastatin calcium (ROSU) (5mg/10mg/20mg) with Olmesartan medoxomil (OLM) 20mg for hypertension in dyslipidemic patients are in clinical trials.

Rosuvastatin calcium, bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2[methyl(methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5dihydroxyhept-6-enoic acid] calcium salt, 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.

Olmesartan medoxomil, (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate, Olmesartan is a selective AT$_1$ subtype angiotensin II receptor antagonist. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT$_1$ receptor in vascular smooth muscle.

A detailed literature survey for ROSU revealed that several analytical methods are reported for the determination of ROSU single or in combination by LC-MS/MS,[2-6] HPLC[7-12] and spectrophotometric.[13-15] Similarly, a literature survey for OLM revealed that LC-MS/MS,[16-18] HPLC[19-28] and spectrophotometric [29-32] are available for determination of OLM in formulations and biological fluids.The review of the literature revealed that no RP-HPLC method has so far been reported for the combination of ROSU and OLM. So an attempt has been made to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous estimation of ROSU and OLM in combined tablet dosage forms. The method was validated according to the ICH guidelines.[33]

EXPERIMENTAL

Chemicals and Reagents

A reference standard sample of ROSU was obtained from Macleod's Pharmaceuticals Ltd, OLM was provided from Divine SBR International Pvt. Ltd. and commercial dosage form containing the studied drug were purchased Cipla Ltd. Ammonium acetate, glacial acetic acid, tetrahydrofuran, hydrogen peroxide, HPLC grade methanol, acetonitrile and water were purchased from E. Merck, Mumbai, India. All the other chemicals and reagents used were of AR grade and purchased from S.D. Fine Chemicals, Mumbai, India.

Instrumentation and Chromatographic system

The chromatographic system consisted of a JASCO (Japan) chromatograph equipped with an LC – Net II/ADC, an MU – 2010 Plus PDA Detector, a PU – 2089 Plus quaternary pump, an online degasser and a rheodyne model 7725 injector valve with 20µl sample loop. The chromatograph is coupled with "Chrompass" software (version 1.7.403.1). HPLC analysis was performed using Symmetry C18 column (4.6mm×150mm, Particle size 5.0µm). The mobile phase consisted of buffer: acetonitrile: tetrahydrofuran (71:25:4v/v/v) filtered and degassed for 30mins prior to use. The eluent was monitored with PDA detector at 248nm with a flow rate of 1.5mL/min and sample size of 20µL was carried out at room temperature all over the study.

Buffer preparation

Accurately weighed and transferred 1.54gm of Ammonium acetate salt in 1000mL of HPLC grade water, sonicated for 2mins to dissolve well and adjusted the pH to 4.0 with glacial acetic acid, mixed well and filtered through 0.45µm membrane filter.

Preparation of stock solution

Accurately weighed and transferred 10mg of ROSU working standard and 40mg of OLM into 100mL volumetric flask. 10mL of methanol was added and sonicated for 5 minutes to dissolve. Volume was made up to the mark with diluents and mixed well.

Calibration standards and quality control sample

To study the linearity range of each component, serial dilutions were made by adding this standard stock solution in the different weights of ROSU in the range of 5–20µg/mL and 20–80µg/mL of OLM. A graph was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. From the standard stock solution, a mixed standard solution was prepared containing 10µg/mL of ROSU and

40µg/mL of OLM. The system suitability test was performed from five replicate injections of mixed standard solution.

Preparation of assay solution

Accurately weighed powder equivalent to 5mg of ROSU and 20mg of OLM was transferred into 10 mL volumetric flask containing 2mLof methanol and sonicated for 30mins. Volume was made up to the mark with diluents methanol to obtain solution of ROSU (500µg/mL) and OLM (2000µg/mL). From this each solution 0.2mL was transferred to 10mL volumetric flask and made up to the mark with diluents methanol to obtain solution of ROSU (10µg/mL) and OLM (40µg/mL). A small portion of sample solution was filtered through 0.45µm nylon filter and used for injection on HPLC.

Method Validation

The proposed method was validated [33] by parameters viz., system suitability, linearity and range, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) and robustness.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

In the present work, an analytical method based on high performance liquid chromatography using photodiode array detection was developed and validated for assay determination of ROSU and OLM in laboratory mixture. The analytical conditions were selected, keeping in mind the different chemical nature, molecular weight and solubility of OLM and ROSU. The column selection was done on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between ROSU and OLM peak. After evaluating all these factors, Symmetry C18 column (4.6mm×150mm, particle size 5.0µm) column was found to be giving satisfactory result. The selection of buffer was based on the chemical structure of both the drugs. For mobile phase selection, preliminary trials using mobile phases of different composition containing water adjusted to acid pH by addition of glacial acetic acid and methanol resulted in poor peak shape. When methanol and water were replaced by buffer: acetonitrile: tetrahydrofuran in the ratio of (71:25:4 v/v/v) better peak shape was obtained. The proportion of the mobile phase components was optimized to reduce retention times and enable good resolution between both molecules. A detection wavelength of 248nm was selected after scanning the standard solution over the range 190-370nm by use of the PDA detector with a flow rate of 1.5mL/min. Detection at 248nm resulted in good response and good linearity. Figure-1 and Figure-2 represent the chromatograms of standard and test preparation respectively.
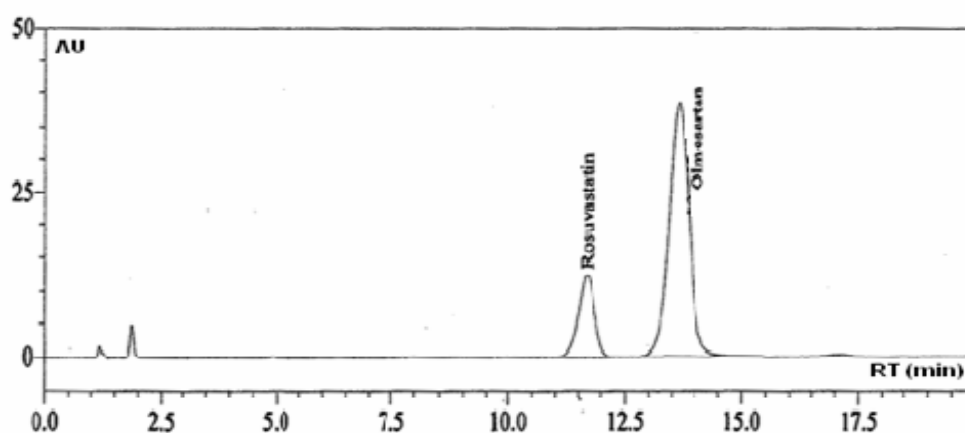

Figure-1: Typical chromatogram of Rosuvastatin and Olmesartan medoxomil in standard solution
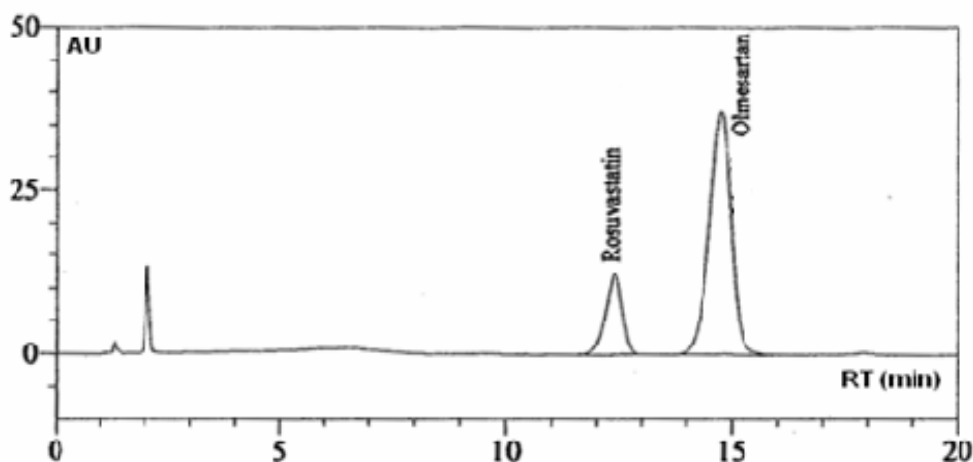
Figure-2: Typical chromatogram of Rosuvastatin calcium and Olmesartan medoxomil test solution

Method Validation

System suitability

System suitability test is used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests was carried out on freshly prepared standard solutions. The method complies with the system suitability parameters. Retention time, number of theoretical plates, asymmetrical factor, and peak area were evaluated for five replicate injections of the drugs. The results given in Table-1 were within acceptable limits.

Linearity

Linearity was determined separately for ROSU and OLM by plotting peak area against concentration. From these calibration plots it was clear that response was a linear function of concentration over the ranges 5-20µg/mL for ROSU and 20-80µg/mL for OLM. The linear regression equations for ROSU and OLM were: $y = 29708.67\ x + 5489$ and $y = 28042.33x + 16685$ where y is response (peak area) and x the concentration. The results are shown in Table -2.

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 50, 100, and 150% of those expected, in accordance with ICH guidelines, by replicate analysis ($n = 6$). Standard drug solutions were added to a pre-analyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in Table -3. From these results it was clear that the method enables very accurate quantitative estimation of ROSU and OLM in tablet dosage form, because all the results were within acceptable limits.

Precision

The intra-day and inter-day precision were determined by assaying the tablets in six times in a day for consecutive six days and expressed as standard deviation. The standard deviations were below 2%, which signifies the precision of both the methods (Table - 4).

Table -1: Results from system suitability studies for ROSU and OLM

Parameters	ROSU	OLM
Theoretical plates (N)	6435±0.46	6024±0.26
Peak area	303493 ± 0.28	1137031±0.14
Retention Time (R_T)	11.66 ± 0.85	13.67 ± 0.80
Tailing factor (T)	0.95±0.04	1.01±0.02

*Mean of six determinations

Table -2: Linear regression data for calibration curve of ROSU and OLM

Parameters	ROSU	OLM
Linearity range(μg/mL)	5-20	20-80
r * ± S.D.	0.9997±0.04	0.9999±0.05
Slope* ± S.D.	29708.67±0.03	28042.33±0.50
Intercept* ± S.D.	5489±0.08	16685±0.45

*Mean of three determinations

Table -3: Results from recovery studies

Name of the drug	Amount taken (μg/mL)	Amount (%)	Amount added (μg)	Recovery (%)	% RSD
ROSU	5	50	2.5	100.46	0.82
		100	5	100.62	0.79
		150	7.5	100.12	0.63
OLM	20	50	10	100.08	0.47
		100	20	99.52	0.95
		150	30	99. 94	0.22

*Mean of six determinations

Table -4: Precision of ROSU and OLM by HPLC method

Name of the drug	Intra-day precision	Inter-day precision
	Mean* (%)± S.D.	Mean* (%) ± S.D.
ROSU	100.02±0.26	99.82± 0.67
OLM	99.91±0.49	100.05±0.59

*Mean of three determinations injected six times at each concentration level

Limits of Detection (LOD) and Quantification (LOQ)

Limit of detection was calculated by using the formula:

LOD = 3.3 SD/*S*

SD = Standard deviation of the response,

S =Slope of calibration curve of the analyte.

Limit of quantification was calculated by using the formula:

LOQ = 10SD/*S*

SD = Standard deviation of the response,

S =Slope of calibration curve of the analyte.

The LOD for ROSU and OLM were found to be 0.05μg/mL and 0.89μg/mL, respectively. The LOQ was 0.15μg/mL and 2.6μg/mL for ROSU and OLM respectively.

Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.35mL/min and 1.65mL/min. Mobile phase composition was changed buffer: acetonitrile: tetrahydrofuran (70:25:5, (v/v/v)) and (72:25:3, (v/v/v)). Detection wave length was changed ±5nm, i.e. 243nm & 253nm. The standard solution and three different sample preparations were injected in each varied conditions and the assay was checked under all deliberately varied conditions, the %RSD for the assay values (n=3) for ROSU and OLM were found to be well within the acceptance limit of 2%. The results are reported in Table 5.

Table -5: Results from testing the robustness of the method

Conditions	Modific ation	ROSU			OLM		
		Mean area ± SD	RSD (%)	Mean *R*T (min) ± SD	Mean area ± SD	RSD (%)	Mean *R*T (min) ± SD
Mobile phase composition (v/v/v)	72:25:3	303822 ± 789	0.25	11.57 ± 0.11	1141960 ± 2696	0.23	13.55 ± 0.10
	71:25:4	310224 ± 4996	1.61	11.67 ± 0.12	1137608 ± 7069	0.62	13.65 ± 0.12
	70:25:5	304122 ± 4715	1.55	11.83 ± 0.05	1133048 ± 6755	0.59	13.84 ± 0.05
Mobile phase flow rate (mL/min)	1.35	315653 ± 1938	0.61	12.15 ± 0.43	1152118 ± 5610	0.48	14.27 ± 0.63
	1.50	305178 ± 1669	0.54	11.66 ± 0.10	1136971 ± 5840	0.51	13.67 ± 0.11
	1.65	313571 ± 1109	0.35	11.32 ± 0.07	1147041 ± 10107	0.88	13.31 ± 0.09
Detector wavelength (nm)	243	322989 ± 3517	1.08	11.50 ± 0.17	1154045 ±7391	0.64	13.49 ± 0.15
	248	304959 ± 3205	1.05	11.65 ± 0.07	1136236 ± 3426	0.3	13.67 ± 0.06
	253	313567 ± 948	0.3	11.74 ± 0.13	1157143 ± 11544	0.99	13.76 ± 0.12

*Mean of three determinations

Specificity

The specificity of the HPLC method was found in complete separation of ROSU and OLM in tablets in presence of excipients. The average retention time ± standard deviation for ROSU and OLM were found to be 11.66 ± 0.10and 13.67± 0.11min respectively, for six replicates. The peaks are sharp and have clear baseline separation.

Application of the Method for Analysis of Marketed Formulations

As the combination of ROSU and OLM is in clinical trial marketed combination is unavailable, so laboratory mixture was made by taking two marketed brands of ROSU and OLM. Olmezest and Olmy -20 containing OLM 20mg; Rozavel and Zyrova containing ROSU 5mg of Sun pharmaceutical Ltd and Zydus Cadila Healthcare Ltd respectively and mixing equivalent amount. The results were shown in Table-6.

Table -6: Results from assay of OLM and ROSU in marketed formulations

Formulation	Mean concentration ± SD	RSD (%)	Recovery (%)
Formulation I			
ROS	4.92±0.08	1.68	98.4
OLM	19.94±0.13	0.65	99.72
Formulation II			
ROS	4.94±0.05	1.05	98.9
OLM	19.90±0.16	0.8	99.53

*Mean of five determinations

CONCLUSION

The developed and validated LC method enables specific, accurate, robust and precise simultaneous analysis of ROSU and OLM in tablet formulations. The method is sensitive enough for quantitative detection of the analytes in pharmaceutical preparations. The proposed method can thus be used for routine analysis, quality control and for studies of the stability of pharmaceutical tablets containing these drugs.

ACKNOWLEDGEMENTS

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Tripti Sharma *et al* /Int.J. ChemTech Res.2014,6(2),pp 1115-1123.

1122

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