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Rp-HPLC technique for the assay of olmesartan and Rosuvastatin simultaneously: development and validation of method

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Abstract
An isocratic elution mode RP-HPLC method is described for the determination of olmesartan (OST) and Rosuvastatin (RST) simultaneously in pure drug and pharmaceutical formualtions. Chromatography was achieved on BDS C18 column, with a mobile phase consisting of 0.01M sodium dihydrogen orthophosphate (60%) and methanol (40%). Analysis of the analytes was carried out at 265 nm using photodiode array detector. Under optimized chromatographic conditions, the retention times were 3.138 min and 3.827 min for OST and RST, respectively. The peak areas measured was related to OST and RST concentration. Linearity was obtained in concentration ranges of 10-30 µg/ml (RST) and 20-60 µg/ml (OST). The limits of detection (LOD) and quantification (LOQ) were 0.05 and 0.14 µg/ml for OST and 0.002 and 0.005 µg/ml for RST. The relative standard deviations for OST and RST were <1.0%, and the respective percentage recoveries were in the range of 99.31-99.82% (OST) and 100.17-101.48% (RST), implying good precision and accuracy of the method. The proposed method was effectively applied to the estimation of OST and RST in tablet samples with good accuracy and precision. Therefore, the method could be considered as a suitable tool for combined analysis of OST and RST.

Keywords: Olmesartan, Rosuvastatin, analysis, HPLC, formulations

1. Introduction
Olmesartan (OST), an antihypertensive drug belongs to class of medical agents called angiotensin II receptor blockers [1]. OST is prescribed for treating patients with high blood pressure. Angiotensin II causes tightening and narrowing of blood vessels. OST, through inhibiting angiotensin II action, helps to loosen up and broaden the blood vessels and thus lower blood pressure [2, 3]. IUPAC name for OST is (5-methyl-2-oxo-2H-1, 3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl) -2-propyl-1- ({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylate (Fig. 1).
Rosuvastatin (RST), an antilipemic drug belongs to class of medical agents called statins [4]. RST is indicated to decrease plasma levels of triacylglycerol and low density lipoprotein cholesterol, and increase high density lipoprotein cholesterol [5, 6]. RST also prevents blockage of arteries with cholesterol which results in heart stoke and attack. RST competitively hinder the activity of enzyme, Hydroxymethylglutaryl coenzyme A reductase. This enzyme catalyzes the rate limiting step during biosynthesis of cholesterol. IUPAC name for RST is (3R, 5S, 6E)-7-[4-(4-Fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid (Fig. 1).
RST and OST have proven to treat patients suffering from dyslipidemia together with hypertension [7, 8]. A combined fixed dosage (20 mg RST and 40 mg OST) tablet of RST and OST was developed. This dosage form enhanced the dosing convenience. Upon studying the pharmacokinetic properties of combined tablet (RST/OST - 20/40 mg) dosage form developed, it was recommended that RST/OST combination is bioequivalent to co-administration of RST and OST as individual tablets [9].
The RST/OST combination is given in any Pharmacopoeia. Till now three methods using RP-HPLC [10-12] technique was reported for the simultaneous assay of RST and OST in pharmaceutical formulations. In Elijabeth *et al*., method [10], OST and RST was assayed by carrying out chromatography on a Agilent XDB, C18 column. A mixture of potassium dehydrogenate orthophosphate (0.01 M): acetonitrile (55:45 v/v, pH 3.2) was used as the mobile phase in gradient elution mode and detection was done at 240 nm. Using a Symmetry C18 column and phosphate buffer: acetonitrile: Tetrahydrofuran (71:25:4 v/v/v) as mobile

phase at a flow rate 1.5 ml/min and detection at 248 nm, RST and OST was determined by Sharma *et al*.,[11]. Nagavalli and Rao developed RP-HPLC technique to quantify OST and RST combination using Altima C18 column and mobile phase composing of 45% phosphate buffer and 55% acetonitrile with 1.0 ml/min flow rate as mobile phase. Peak area response of OST and RST were measured at 241 nm [12]. The reported methods have limitations like as use of triple solvent system [11], more flow rate [11] and use of gradient elution mode [8].

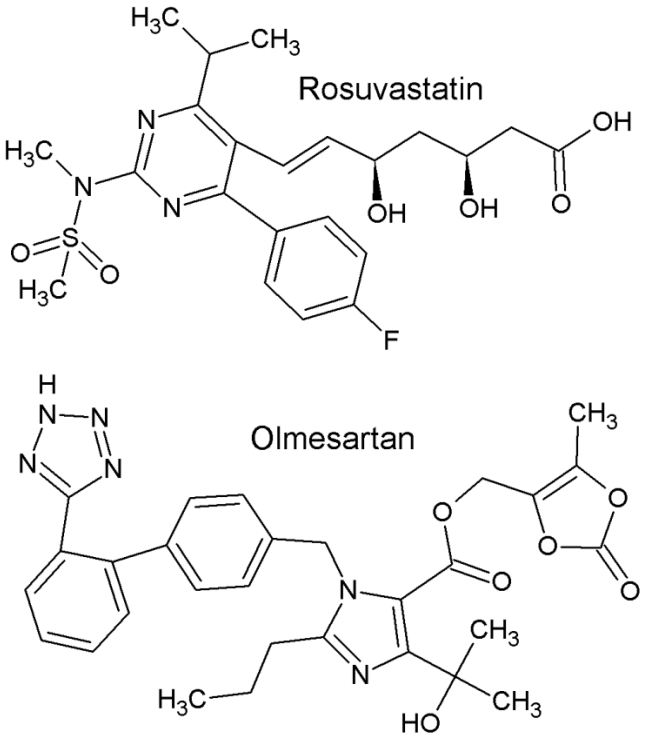

Fig 1: RST and OST chemical structures

The present investigation was aimed to establish an RP-HPLC method appropriate for the simultaneous quantitation of OST and RST in bulk and tablet dosage form.

2. Materials and methods

2.1. Instrumentation

The Waters HPLC system with binary HPLC pump model 2965, PDA detector model 2998 with 10 µl injection loop was used. The chromatographic data was processed by Waters Empower2 software.

2.2. Materials

OST and RST reference substances were kindly provided by Lara Drugs Private Limited (Telangana, India). Olbet 40 MG tablets (Zuventus Healthcare Pharmaceuticals Ltd, Mumbai, India) containing 40 mg of OST and Rosuvas 20 MG tablets (Sun Pharma Laboratories Ltd, East Sikkim, India) containing 20 mg of RST were obtained from commercial sources. Acetonitrile (HPLC grade) was purchased from Merck Pvt Ltd., Mumbai, India. Sodium dihydrogen orthophosphate of analytical reagent grade were obtained from Sd Fine Chemicals Ltd., Mumbai, India. Purified water was obtained from Milli-Q system was used in the study.

2.3. Chromatographic conditions

The chromatographic separation and analysis of selected drug combination were carried out on a BDS C18 (250 ×4.5 mm i.d., particle size 5 µm). The mobile phase was a mixture of 0.01M Sodium dihydrogen orthophosphate and methanol (60:40, *v/v*; pH 3.5) delivered at a flow rate of 1 ml/min. The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 10 min. Analysis was performed at 30ºC temperature. The elution of the analytes was monitored by photodiode array detector set at 265 nm. The injection volume was 10 µl.

2.4. Stock standard and tablet sample solutions

The stock solution was prepared by weighing 20 mg RST and 40 mg OST, and dissolving them in 30 ml of mobile phase in a 100 ml volumetric flask. The final volume was achieved using the same solvent system to obtain a final concentration of 200 µg/ml and 400 µg/ml of RST and OST, respectively. The stock solution was further diluted with the mobile phase to obtain working standards with concentration 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, & 30 µg/ml for RST and 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml & 60 µg/ml for OST.

RST and OST combination is still in clinical trial, therefore marketed combination is not available. Hence laboratory tablet sample mixture was prepared by taking two commercially available brands of RST and OST. The brands used in the investigation are Olbet 40 MG and Rosuvas 20 MG tablets labeled to contain 40 mg and 20 mg of OST and RST, respectively. Ten tablets of each brand were powdered. Tablet powder equivalent to 40 mg of OST and 20 mg of RST was accurately weighed into a 100 ml volumetric flask and mixed with 30 ml of mobile phase. The solution was sonicated for 20 min and filled with mobile phase to obtain a final concentration of 400 µg/ml of OST and 200 µg/ml of RST. The solution was filtered through a 0.45 µm membrane filter. For analysis, the above prepared tablet sample solution was diluted further to get a concentration of 40.0 µg/ml and 20 µg/ml OST and RST, respectively with mobile phase.

3. Results and discussion

3.1. Method optimization

Chromatographic parameters like retention time, tailing factor, number of theoretical plates, and resolution were determined to optimize the method. For that, series of trials were carried out, such as different ratios of mobile phase and different types of stationary phase, with different temperature, pH values and flow rate. On the basis that the method will be employed for separation of OST and RST from each other, the BDS C18 (250 × 4.6 mm, 5 µm) analytical column with temperature 30 ºC was selected which gave good symmetric and sharp peaks. Based on less analysis time, peak response, peak symmetry and column efficiency, a mixture of 0.01M sodium dihydrogen orthophosphate and methanol (60:40 *v/v*) was selected as the mobile phase, adjusted to pH 3.5 and a flow rate of 1.0 ml/min. Using the photodiode array detector, a wavelength of 265 nm was selected as detection wavelength. The chromatographic parameters optimized exhibiting a good peak shape, resolution and good number of theoretical plates. The typical chromatogram of OST and RST by the developed method is presented in Fig. 2.

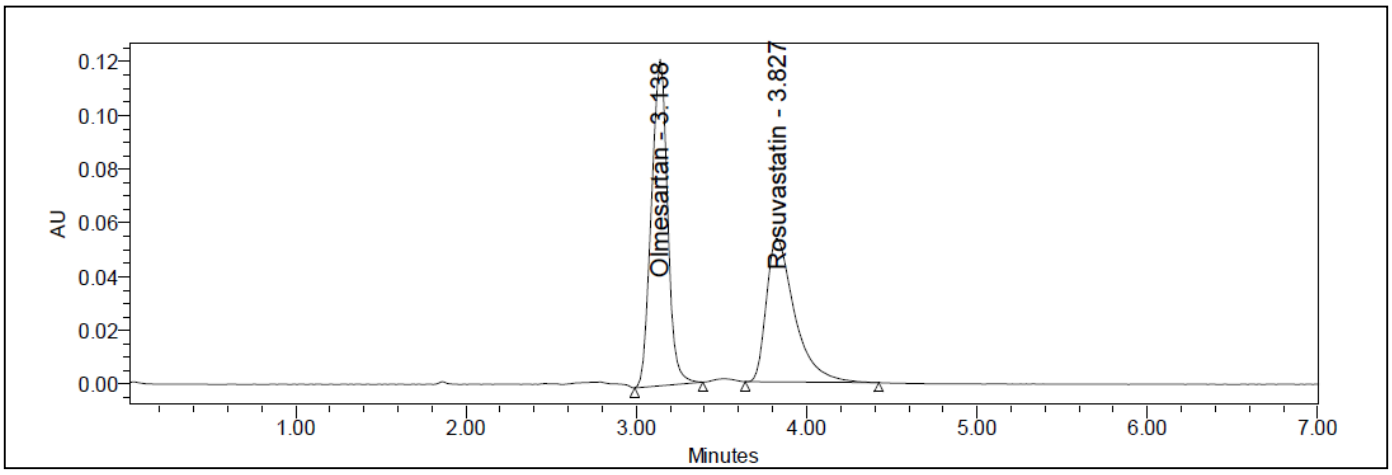

Fig 2: Typical chromatogram of OST and RST by the developed method

3.2. Method validation

Validation was performed according to ICH guidelines [13]. System suitability, selectivity, linearity, sensitivity, accuracy, precision, specificity and robustness were determined.

3.2.1. System suitability study

The system suitability was established by six consecutive injections of the same working standard solution. The parameters considered were: plate count, peak tailing, resolution, and relative standard deviation of the peak areas and retention times of OST and RST. The values for the system suitability parameters of the method, as presented in Table 1, are within acceptance limits.

Table 1: System suitability studies of OST and RST

Property	OST (%RSD)	RST (%RSD)	Accepted limits
Retention time (t_R)	3.138 (0.315)	3.825 (0.348)	RSD ≤ 2
Peak area (mAU)	522524 (0.815)	521400 (0.426)	RSD ≤ 2
Theoretical plates (N)	6977 (0.559)	7482 (0.624)	> 2000
Tailing factor (T)	1.22 (0.862)	1.04 (0.561)	≤ 2
Resolution (R)	-	4.56 (0.418)	> 1.5

3.2.2. Selectivity

The selectivity of the optimized method was examined with working standard solution of OST (40 µg/ml) and RST (20 µg/ml) relative to the blank mobile phase and tablet sample solution (OST -40 µg/ml and RST-20 µg/ml) (Fig. 3). No interference was observed by coelution of components of mobile phase at the same retention time of OST and RST at 265 nm, demonstrating the selectivity of this method. The retention times of OST and RST in working standard and tablet sample solutions are same.

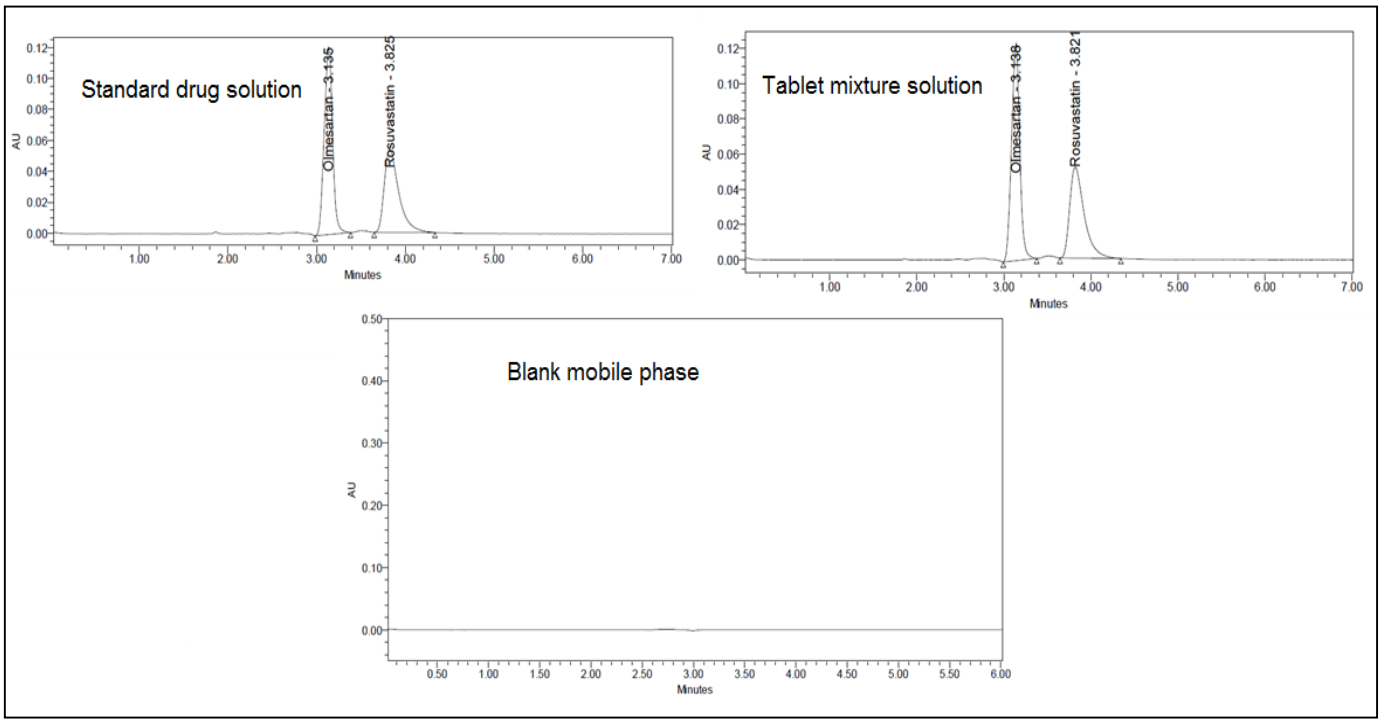

Fig 3: Chromatograms of selectivity study

3.2.3. Linearity and sensitivity

Linearity was done by preparing standard solutions of OST and RST at five concentration levels. The linearity of detector response for OST and RST was verified by prepared solutions of over the concentration range of 20-60 µg/ml and 10-30 µg/ml, respectively. The peak area of each sample against respective concentration of analytes was found to be linear. The correlation coefficient for both analytes was greater than ≥0.9990. Linearity results were presented in Table 2.

The sensitivity of the method was represented as limits of detection (LOD) and quantitation (LOQ). The detection and quantitation limits were determined based on the signal-to-noise ratio of 3:1 and 10:1, respectively. The determined LOD and LOQ for OST and RST were shown in Table 2 and chromatograms are presented in Fig. 4.

Table 2: Regression analysis and sensitivity of OST and RST

Parameter	OST	RST
Linearity (µg/ml)	20-60	10-30
Regression equation (y= mx + c)	y = 3442 x + 879.9	y = 6855 x + 660.9
Slope (m)	3442	6855
Intercept on Y-axis (c)	879.9	660.9
Regression coefficient (R^2)	R^2 = 0.9995	R^2 = 0.9994
LOD (µg/ml)	0.05	0.002
LOQ (µg/ml)	0.14	0.005

y = Peak area of OST/RST; x = concentration of OST/RST (µg/ml)

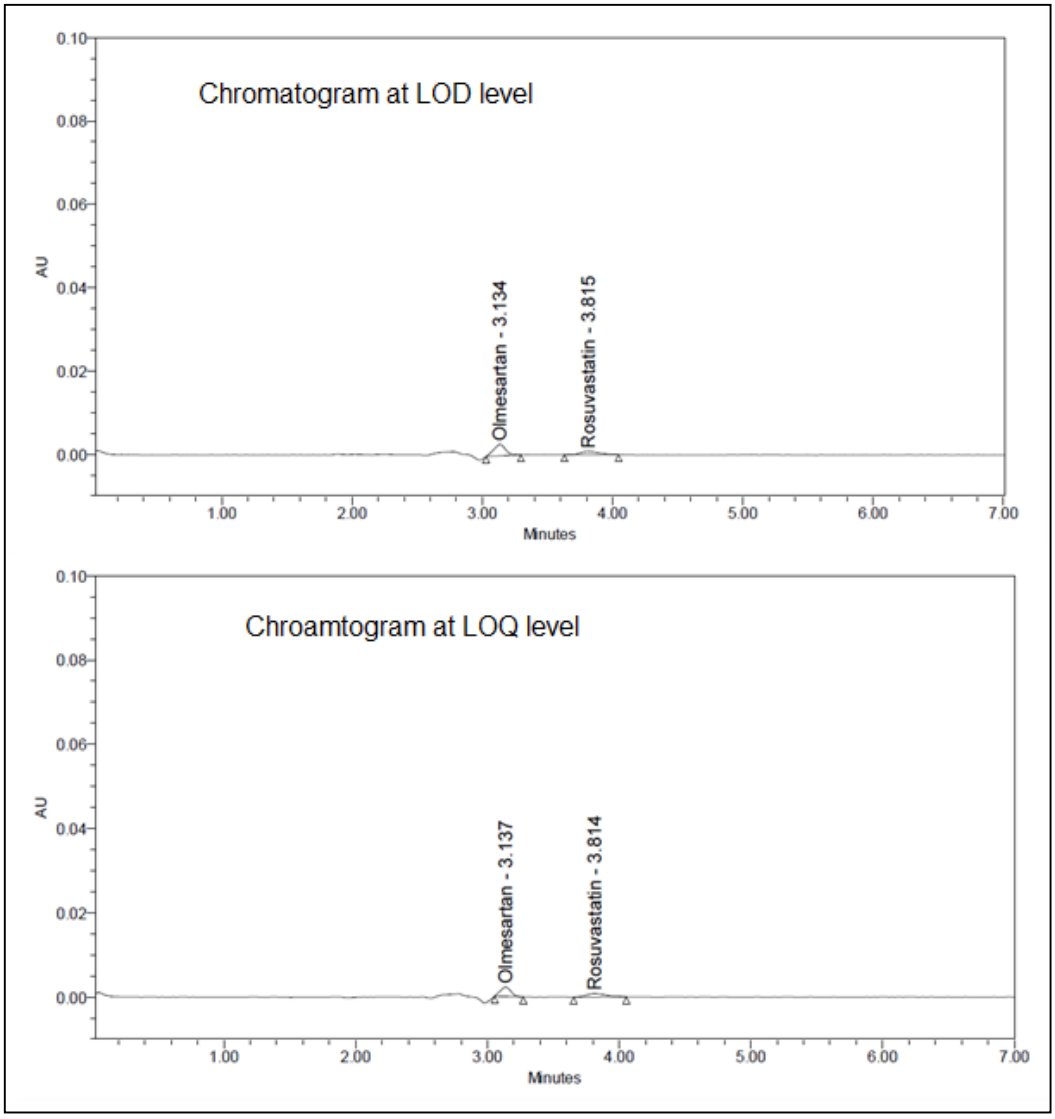

Fig 4: OST and RST chromatograms at LOD and LOQ levels

3.2.4. Precision and accuracy

These parameters were studied as intra- and inter-day. For precision studies, the same standard solutions of RST and OST were injected 6 times into the HPLC system on the same day (intra-day analysis) and on three consecutive days (inter-day analysis). The percentage RSD values calculated for peak areas of RST and OST were less than 0.1% (Table 3) indicating the precise assay with the developed HPLC method. For accuracy, the percentage recovery was calculated for OST and RST on the same day (intra-day analysis) and on three consecutive days (inter-day analysis). The results are acceptable with good percent recovery (Table 3).

Table 3: Valuation of precision and accuracy for OST and RST

Injection No.	OST (40 µg/ml)		RST (20 µg/ml)	
	Peak area	Recovery (%)	Peak area	Recovery (%)
Intra-day analysis				
1	523411	40.07	523147	19.93
2	527909	40.41	523733	19.91
3	526013	40.27	521467	20.00
4	524636	40.16	526760	19.80
5	523955	40.11	526392	19.81
6	524043	40.12	529907	19.68
Mean	525311	40.213	525651	19.839
RSD	0.318	0.318	0.610	0.610
Inter-day analysis				
Day 1	525215	40.21	523347	20.07
Day 2	522929	40.03	522382	20.04
Day 3	520375	39.84	522444	20.04
Mean	522840	40.02	522724	20.05
RSD	0.463	0.463	0.103	0.103

3.2.5. Recovery test

The accuracy of the developed method was further evaluated through recovery test. The recovery test was determined by means of standard addition method. The recovery experiments were performed by adding OST and RST standards to the preanalyzed tablet mixture sample for three times. The recovery test results are summarized in Table 4 and corresponding chromatograms are shown in Fig. 5. Hence, the obtained results indicated that the developed HPLC method was accurate enough for simultaneous quantitative evaluation of OST and RST. There was no interference noticed from the excipients of the tablet.

Table 4: Recovery of OST and RST

Sample	Labeled claim (mg)	Spiked (mg)	Total found (mg)	Recovered (%)	RSD (%)
OST	40	20	59.84	99.73	0.531
	40	40	79.45	99.31	0.205
	40	60	99.82	99.82	0.364
RST	20	10	30.18	100.61	1.201
	20	20	40.07	100.17	1.014
	20	30	50.74	101.48	0.178

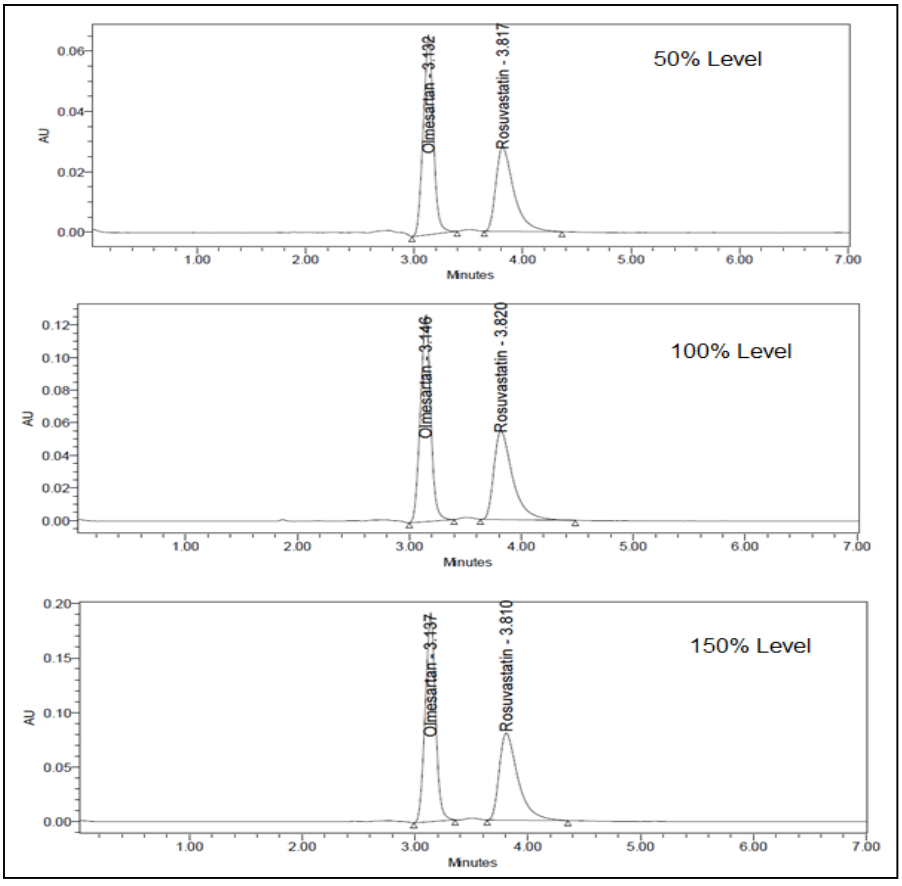

Fig 5: OST and RST chromatograms at different concentration levels

3.2.6. Robustness

Under the slightly varied chromatographic conditions (flow rate of mobile phase-±0.1 ml/min; methanol ratio in mobile phase - ±5% and column temperature - ±2 ºC), the OST and RST peaks were well separated and there was no significant change peak area response as shown by low percent relative standard deviation values (Table 5), which illustrated the robustness of the method.

Table 5: Robustness data of OST and RST

Robustness condition	OST %RSD of peak area	RST %RSD of peak area
Flow minus (0.9 ml/min)	0.518	0.339
Flow Plus (1.1 ml/min)	0.625	0.585
Methanol ratio minus (35%)	0.259	0.227
Methanol ratio Plus (45%)	0.716	0.946
Temperature minus (28 ºC)	0.617	0.718
Temperature Plus (32 ºC)	0.925	0.953

3.3. Application of developed method to tablet dosage form

To demonstrate the applicability of the proposed method, OST and RST were assayed in tablets using the proposed method. Working standard solutions and tablet mixture sample solution was from prepared from the formulation. Sample and standards are injected six homogeneous samples. OST and RST content in the formulation was quantified by taking the standard as the reference. The average percent assay and percent relative standard deviation were calculated for OST and RST (Table 6). The values indicated the accuracy and precision of the method for the assay of OST and RST by the proposed method. Interference from the tablet excipients was not observed.

Table 6: Assay of OST and RST in formulation using proposed method

OST			RST		
Labeled claim (mg)	**Found (mg)**	**Recovered (%)**	**Labeled claim (mg)**	**Found (mg)**	**Recovered (%)**
40	40.07	100.19	20	19.94	99.70
40	39.75	99.37	20	19.96	99.81
40	40.23	100.57	20	20.27	101.34
40	40.15	100.37	20	20.08	100.41
40	40.11	100.27	20	20.07	100.34
40	40.11	100.28	20	19.81	99.05
Mean	40.068	100.17	Mean	20.038	100.19
RSD	0.465	0.465	RSD	0.481	0.481

4. Conclusion

An isocratic elution mode RP-HPLC method has been developed and validated for simultaneous estimation of OST and RST in bulk and in dosage forms. The proposed method was proved to be simple, economical, selective, accurate, precise and rapid. Good recoveries, excipients interference free and reproducible chromatograms were obtained. Therefore, the developed method can be used for the regular analysis of OST and RST in routine quality control laboratories.

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