Research Article

Development and Validation of RP-HPLC Method for the Estimation of Rosuvastatin Calcium and Niacin in Combined Tablet Dosage Form

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Rosuvastatin and Niacin individually and combined tablet dosage form. An Inertsil ODS C-18, 5µm column having 15cm x 4.6mm internal diameter in isocratic mode with mobile phase containing potassium (dihydrogen) orthophosphate buffer: acetonitrile (50:50v/v) was used. The flow rate was 1.0 ml/min and effluents were monitored at 254 nm. The retention time for Rosuvastatin and Niacin was 1.58 and 4.84-5.5 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found in ng and recovery of Rosuvastatin and Niacin from tablet formulation was found 98-105 %. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 50%, 100% and 150%.The % recovery was found to be 98-105% for Rosuvastatin and niacin respectively. The low values of % R.S.D are indicative of the accuracy and reproducibility of the method. The % R.S.D value less than 2 indicate that the method is precise. The proposed method was successfully applied for the quantitative determination of Rosuvastatin and Niacin in tablet formulation.

Keywords: Rosuvastatin and Niacin, HPLC, Linearity, Validation, Combined dosage form

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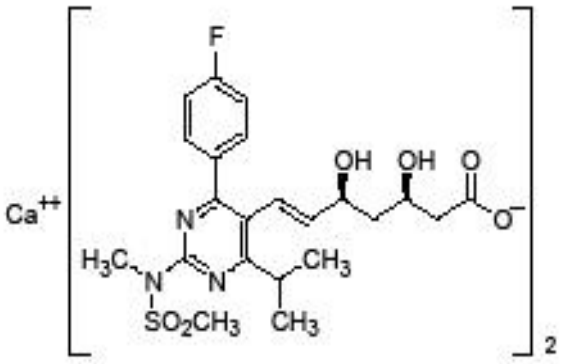
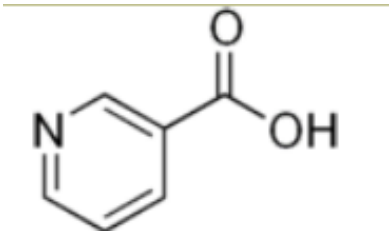
INTRODUCTION

Rosuvastatin HMG-CoA reductase inhibitor (statin) and Niacin (nicotinic acid) are used, along with therapeutic life-style changes, in the primary and secondary prevention of coronary heart disease, carotid artery disease and other atherosclerotic vascular diseases. In US guidelines, the lowering of low-density lipoprotein cholesterol (LDL-C) is the primary goal of lipid-modifying therapy in patients with atherosclerotic disease and those at risk for atherosclerotic disease due to dyslipidemia. However, in patients with atherogenic dyslipidemia (i.e. those with high triglyceride levels, low high-density lipoprotein cholesterol [HDL-C] levels and small dense LDL particles), LDL-C levels may underestimate the cardiovascular risk. Therefore, the US guidelines recommend lowering both LDL-C and non-HDL-C in patients with hypertriglyceridemia. Of the available lipid-modifying drugs, statins are the most effective for lowering plasma LDL-C and are considered the cornerstone of treatment for dyslipidemia. At pharmacologic doses, niacin displays wide-ranging lipid-modifying activity, reducing levels of all atherogenic lipid and lipoprotein subclasses, including total cholesterol, VLDL-C [1],LDL-C, non- HDL-C, triglycerides, apolipoprotein B, and lipoprotein(a), and also significantly increasing levels of HDL-C and apolipoprotein A. Furthermore, the combination of two lipid-lowering agents in one formulation may potentially improve patient compliance [2].

Literature survey revealed that numerous methods have been reported for estimation of Rosuvastatin and Niacin in pharmaceutical formulations individually or

with other drug combination but no HPLC method has been reported for this combination of ROS and NIA. Present study involves development of HPLC method using simple mobile phase which is sensitive and rapid for quantification of Rosuvastatin and Niacin in combined tablet dosage forms as well as subsequent validation of developed method according to ICH guidelines [3,4].

	
Rosuvastatin (bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl-sulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxyhept-6-enoicacid] calcium salt	**Niacin** (3-pyridinecarboxylic acid) Chemical formula C6H5NO2 Molecular mass 123.11 g/mol Melting point 236.6 °C Boiling point decomposes

MATERIALS AND METHODS [5-8]

Instrument

The liquid chromatographic system consisted of Cyberlab HPLC –LC-100 model containing binary pump, variable wave length programmable UV- detector and injector with 20µl fixed loop. Chromatographic analysis was performed using Intertsil ODS C-18 column with 15cm x 4.6mm internal diameter and 5µm particle

Reagents and Materials

Methanol and acetonitrile of HPLC grade of research lab-fine ltd. wasused. Water used was double distilled water prepared, filtered and degassed/ultrasonicated. Potassium dihydrogen orthophosphate of analytical grade was used.

Preparation of Standard Stock Solution

A stock solution of Rosuvastatin and Niacin was prepared by accurately weighed50mg of drug, transferred to 50ml of volumetric flask, containing 50ml of mobile phase dissolving it to obtain final standard solution of 1mg/ml of Rosuvastatin and Niacin.

Preparation of Sample Solution

The formulation tablets of Rosuvastatin and Niacin were crushed to give finely powdered material. Powder equivalent to 50mg of Rosuvastatin and Niacin was taken in 50 ml of volumetric flask containing 50ml of solvent and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent and was further diluted to obtain concentration of 100µg/ml.

Chromatographic Conditions

The **mobile phase** consisting of Potassium (dihydrogen) orthophosphate buffer: acetonitrile were filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 50:50 v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 254nm with a run time of 6min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Preparation of buffer solution:

Weigh accurately and dissolve 2.74gm of potassium (dihydrogen) phosphate buffer (monobasic) (KH_2PO_4) In 1000ml volumetric flask and make up the volume with double distilled water. filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter.

Method Validation

The proposed method was validated by studying several parameters such as Specificity, linearity, precision, accuracy and limit of detection (LOD), limit of quantitation (LOQ),and system suitability [1,3,4].

Determination of λmax

The standard solution of Rosuvastatin and Niacin were separately scanned at different concentration inthe range of 200-400 nm and the λmax was determined for each drug.

Specificity

The specificity of the method was checked by a peak purity test of the sample preparation done by UV detector. The peak purity for Rosuvastatin and niacin was found to be 0.988and0.981. The result of the peak purity analysis shows that the peaks of the analytes were pure and also the formation excipients were not interfering with the analyte peaks.

Calibration and linearity

The standard solutions containing 5 - 40 µg/mL of rosuvastatin and niacin in each linearity level were prepared. Linearity solutions were injected. Calibration graphs were found to be linear for both theanalytes in the mentioned concentrations. The coefficient of correlation was found to be 0.988 and 0.981 for rosuvastatin and niacin, respectively.

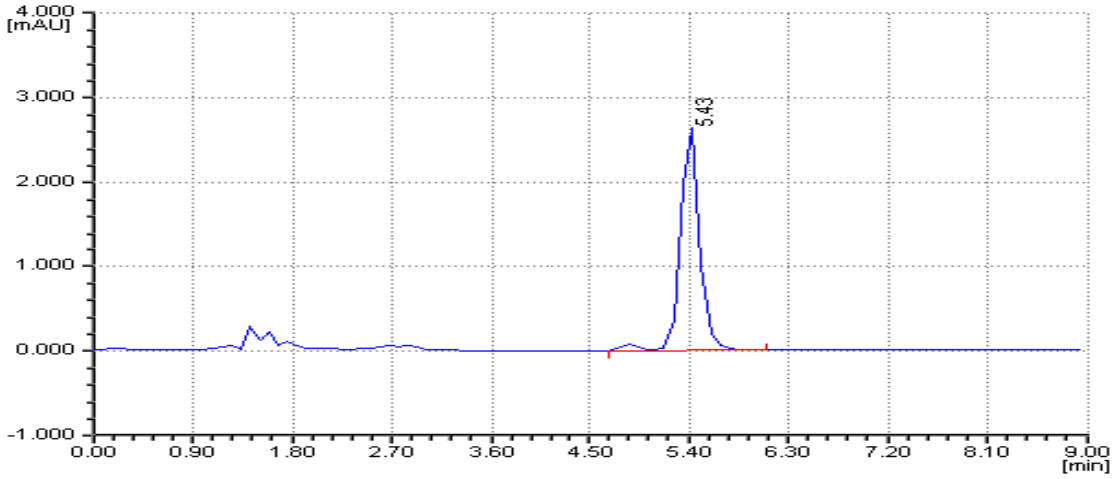


Figure 1: Typical chromatogram of ROS

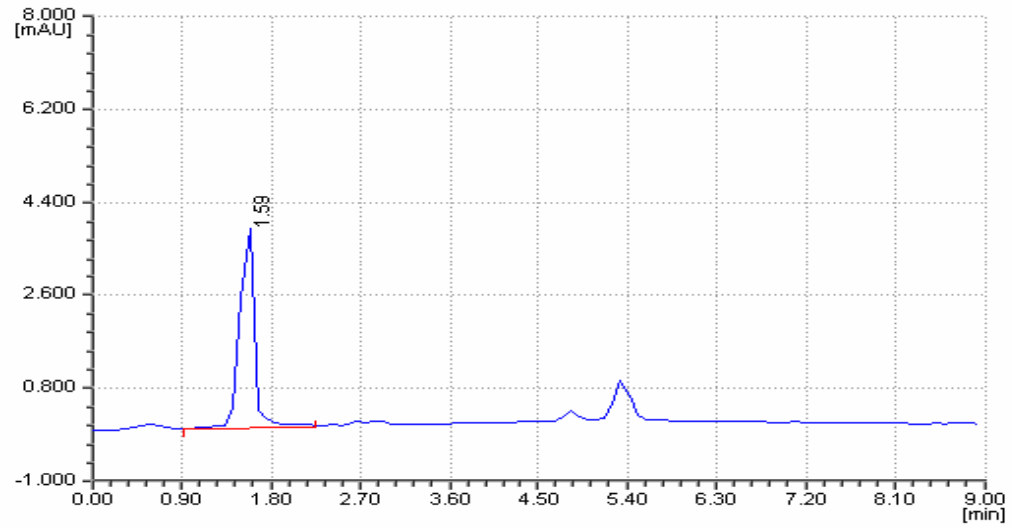


Figure 2: Typical chromatogram of NIA

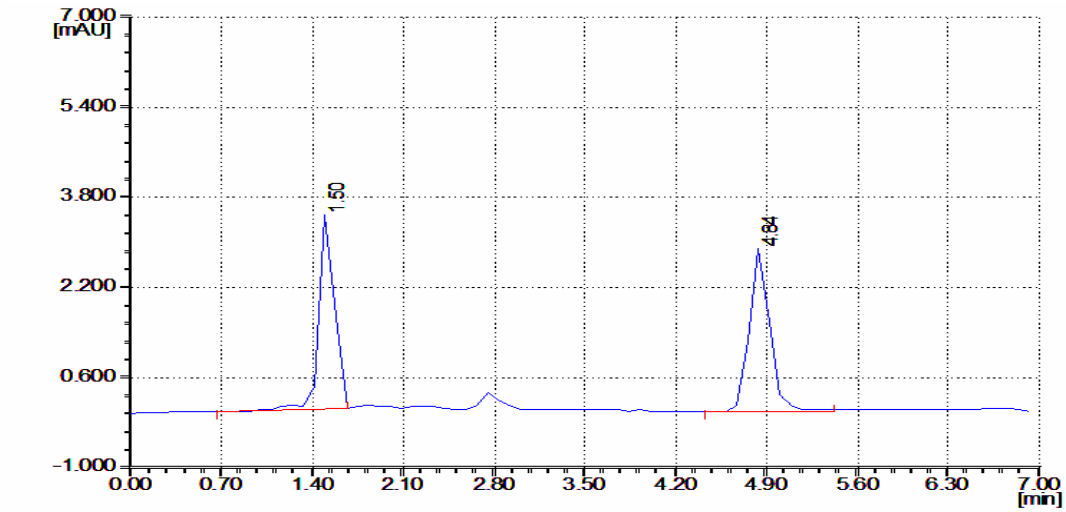


Figure 3: Typical chromatogram of mixture

CALIBRATION CURVE:

Appropriate aliquots of Rosuvastatin and Niacin standard stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 5, 10, 15, 20, 25, 30, 35, 40 µg/ml of Rosuvastatin and Niacin. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Rosuvastatin and Niacin was constructed by plotting peak area ratio versus applied concentration of Rosuvastatin and Niacin and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Rosuvastatin and Niacin in tablet sample was found out using regression equation.

Table 1: Calibration curve data of Rosuvastatin

Component	Concentration in µg/ml	Concentration in gm/lit.	Area at 254 nm
Rosuvastatin	5	0.005	1480.2
	10	0.010	2715
	15	0.015	3991.9
	20	0.020	5141
	25	0.025	6632
	30	0.030	7563
	35	0.035	8544
	40	0.040	9965

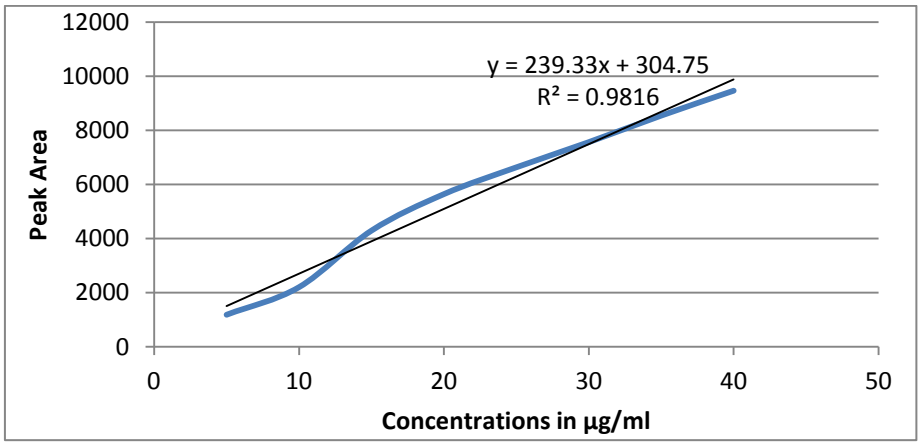


Figure 4: Calibration curve for Rosuvastatin

Table 2: Calibration curve data of Niacin

Component	Concentration in µg/ml	Concentration in gm/lit.	Area at 254 nm
Niacin	5	0.005	1083.5
	10	0.010	2223
	15	0.015	3246
	20	0.020	4499
	25	0.025	5715
	30	0.030	7010
	35	0.035	8354
	40	0.040	9500

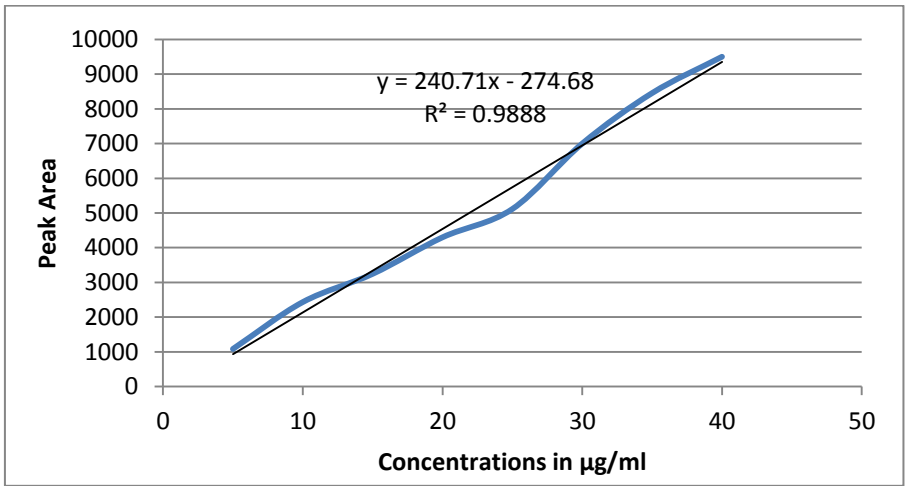


Figure 5: Calibration curve for Niacin

Table 3: Linear regression data for rosuvastatin and niacin

Parameters	Rosuvastatin	Niacin
Linearity range	5-40 µg/ml	5-40 µg/ml
R^2 (Regressioncoefficient)	0.981	0.988
Slope	239.33	240.71
intercept	304.75	274.68

Accuracy (recovery test):

The accuracy of the method was done by recovery study. The recovery experiments were performed by adding known amounts of the pure drug to the preanalyzed sample. The recovery was done at three levels: 50%, 100%, and 150% of the label claim. Three samples were prepared for each recovery level.

Table 4: Statistical validation of Accuracy (recovery test)

Component	Percentage	Mean*	Standard Deviation	Percentage Relative Standard Deviation	Standard Error
Rosuvastatin	50%	101	1.217908	1.20	0.7
	100%	101	0.450925	0.44	0.26
	150%	100.6	0.677348	0.67	0.39
Component	**Percentage**	**Mean***	**Standard Deviation**	**Percentage Relative Standard Deviation**	**Standard Error**
Niacin	50%	100	1.101514	1.10	0.63
	100%	100.8	1.451149	1.43	0.83
	150%	100.8	1.442221	1.43	0.83

*Average recovery = average of three levels, nine determinations

Precision:
The intra-day precision study of Rosuvastatin and niacin was carried out by estimating the correspondence responses six times on the same day with 10µg/ml concentration and inter-day precision study of Rosuvastatin and niacin was carried out by estimating the correspondence responses six times next day with 10µg/ml concentration.

Table 5: Results for precision study of tablet dosage form

Component	Concentration (µg/ml)	Mean*	Standard Deviation	Percentage Relative Standard Deviation	Standard Error
Rosuvastatin	10	100.3	1.451551	1.447	0.649
Niacin	10	103.6	0.691375	0.667	0.309

Stability
In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 6 hours at room temperature.

Robustness
Robustness of the method was studied by changing the composition of mobile phase by ±4% and the PH by ± 0.1, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

Determination of the limits of detection and Quantitation(LOD and LOQ)
For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the RSD of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples in the range of the detection and quantitation limits.
The LOD and LOQ ofRos and Nic were calculated by mathematical equation:
LOD = 3.3 × standard deviation / slope
LOQ = 10× standard deviation / slope

System suitability
For system suitability studies, five replicate injections of mixed standard solutions were injected, and the parameters like RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are reported.

Table 6: System Suitability Parameters

Parameters	Results	
	Rosuvastatin	Niacin
Theoretical plates (N)	4163	516.93
Retention time (min)	4.84	1.58
LOD (ng)	6.21	19.89
LOQ (ng)	18.84	60.28
Accuracy (%)	98-105(101)	98-105(100.8)
R.S.D. (%)	0.44	1.43
Tailing factor	0.25	1.11

RESULTS AND DISCUSSION
The proposed method was found to be linear in the concentration range of 5-40µg/ml for Rosuvastatin Calcium and niacin. The method was specific since excipients in the formulation did not interfere in the estimation of Rosuvastatin Calcium and Niacin. Regression coefficient was found 0.98for both drugs. Accuracy of the method was indicated by the recovery values 98-105% for Rosuvastatin Calcium and niacin. Precision is reflected by %RSD as 1.45 for ROS and 0.69 for Niacin which was less than 2. The LOD and LOQ values were 6.21ng/ml and 19.89 ng/ml for ROS and 18.84ng/ml and 60.28ng/ml for Niacin.

CONCLUSION
The proposed method is found to be simple, sensitive and reproducible and hence it can be used in routine analysis for simultaneous

determination of Rosuvastatin and Niacin in bulk as well as in pharmaceutical preparation.Statistical analysis of the results has been carried out revealing high accuracy and good precision. The results of linearity, precision, accuracy and specificity, proved to be within the limits.

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