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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR NIACIN AND ROSUVASTATIN CALCIUM IN SYNTHETIC MIXTURE.

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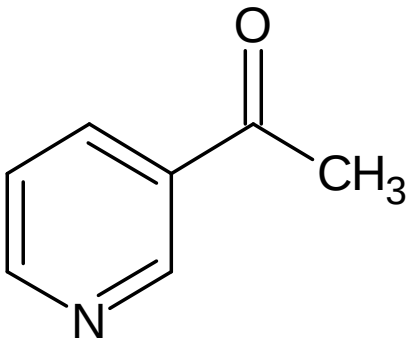
ABSTRACT

A simple, precise and rapid HPLC method has been developed and validated for the Estimation of Rosuvastatin Calcium and Niacin simultaneously in Formulation. Chromatographic Separation of the two drugs was performed on an Eclips XDB C8 column (150mm×4.6 mmid, 5µm particle size).The mobile phase used was a mixture of 0.2% v/v Aq.acetic acid: methanol: acetonitrile (50:25:25% v/v).Detection was performed at 248 nm and sharp peaks were obtained Rosuvastatin calcium and Niacin at retention times of 3.43 min and 2.08 min respectively. The calibration curve was linear in the concentration range 248-752µg/ml for niacin 5.20-15.20µg/ml for Rosuvastatin calcium; the correlation coefficients were 0.990 and 0.998, respectively. The optimized method showed good performance in terms of specificity, linearity, detection and quantitation limits, precision and accuracy in accordance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines.This assay was demonstrated to be applicable for routine quantitation of Rosuvastatin calcium and niacin in Formulation.

KEYWORDS: HPLC, Niacin, Rosuvastatin Calcium, Validation.

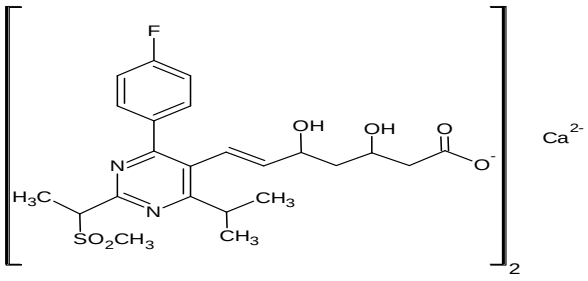
INDRODUCTION

Nia chemically designed as Pyridine 3 carboxylic acid which reduce triglyceride levels is also effective for increasing serum HDL levels it has been demonstrate that this drug lowers the incidence of coronary heart disease in humans. A number of analytical methods have been developed for its determination in pharmaceutical formulation or its in biofluides either alone or in combination with other drugs. Such as determination of Niacin by HPLC, floe injection TLC, HPTLC, Capillary eletrophoric and mass spectrophotometric etc.[7,8]



Rosuvastatin Calcium (Rosu) is chemically (3R,5S)-7-{4-(4-flurophenyl)-6-isopropyl-2-{methyl(methylsulphonylamino)]pyrimidine-5-yl}-3,5-dihydroxyhepten-6-oic acid calcium. Rosu belongs to statin class of drugs used to treat hypercholesterolemia both in patients with established cardiovascular disease as well as those who are at a high risk of developing atherosclerosis. These drugs inhibit the rate limiting key enzyme known as 3-hydroxy-3-methylglutaryl-coenzyme A (*HMG-CoA*) reductase involved in cholesterol biosynthesis. Statins cause reduction in low density lipoproteins-C (LDL-C), total cholesterol (TC) and triglycerides (TG) and elevation in high-density lipoprotein-C (HDL-C) A detailed survey of analytical literature for estimation of Rosu[1-6] revealed several methods based on varied techniques viz, HPLC. Capillary Zone Electrophoresis, Spectrophotometry and High Performance Thin Layer Chromatography (HPTLC) from literature survey reveals that no HPTLC method of both drugs in their combined dosage form. In the present work, an endeavor has been made to estimate both drugs simultaneously by HPTLC method.

Structure



Rosuvastatin calcium

MATERIAL AND METHODS

Chemical and Reagents

Standard gift samples of Rosuvastatin calcium and Niacin were provided by Glenmark Generic Limited, Ankleshwar, Gujarat. Rosuvastatin Calcium (10 mg) and Niacin (500mg) in synthetic mixture all chemicals and reagents used were of AR grade.

Instrumentation and Chromatographic Conditions

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

Optimized chromatographic conditions
HPLC Column: XDB Eclips C_8 column (5 µm, 150 × 4.6 mm ID)
Column temperature: Ambient temperature
Mobile Phase: Aq.aceticacid (0.2%)
Acetonitrile: methanol (50:25:25v/v)
Flow rate: 0.8 ml/min.
UV detection: 248 nm
Injection volume : 20 µL
Run time: 10 mins

Preparation of standard solutions

About 10 mg each of Rosu and Nia were accurately weighed accurately and transferred to separate 100 ml volumetric flasks respectively. It was dissolved in the mobile phase consisting of 0.2%Aq.acetic acid: methanol: acetonitriel (50:25:25*v/v*) and the solutions were made up to volume with same solvent to obtain stock solutions of concentration 100 µg mL^{-1} each of the drugs, respectively.

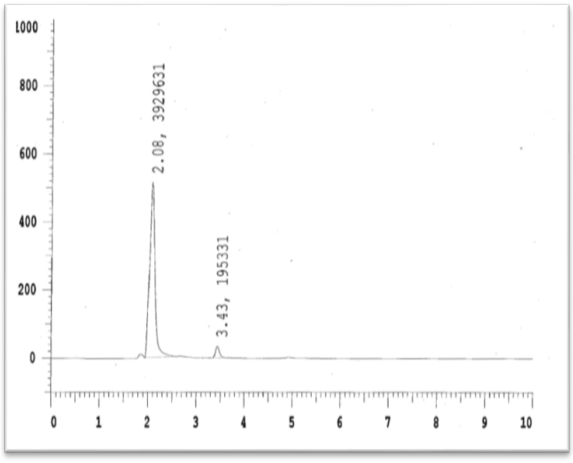


Fig. 1: Chromatogram of standard Rosu (R_t=3.43) and Nia (R_t=2.08.

Preparation of the sample solutions

API powder equivalent to 10.0 mg Rosuvastatin Calcium and 500.0 mg of Niacin was taken and dissolved in mobile phase and sonicated for 20 min. and then volume was made up to the mark with mobile phase. It was dissolved in the mobile phase consisting of 0.2%Aq.acetic acid: methanol: acetonitriel (50:25:25*v/v*) and the solutions were made up to volume with same solvent to obtain sample solutions of concentration of each of the drugs, respectively.

RESULT AND DISCUSSION

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

A. Linearity and Range

Suitable dilutions using mobile phase were made from the standard stock solution containing 500 µg/ml of Niacin and 10 µg/ml of Rosuvastatin Calcium to prepare range of standard solutions containing six different concentrations of analytes. Three replicates per concentration were injected. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 248-752 µg/ml of Niacin and 5-15 µg/ml for Rosuvastatin Calcium.The result obtained are shown in table. No 1 and 2.

Table 1: Standard calibration data of Niacin.

Sr.No.	Concentration of Niacin	Peak area of Niacin
1	248	2040204
2	376	3213361
3	504	3939018
4	624	4875059
5	752	5816777

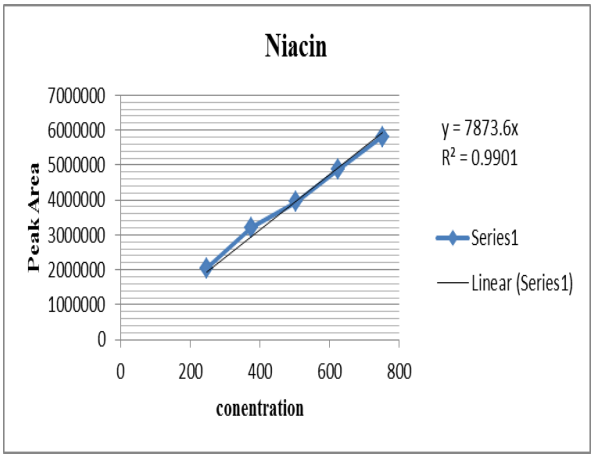


Fig. 2: Calibration curve of Niacin.

Table 2: Standard calibration data of Rosuvastatin Calcium.

Sr. No.	Concentration of Rosuvastatin Calcium	Peak area of Rosuvastatin Calcium
1	5.20	96998
2	7.60	151439
3	10.00	195782.3
4	12.40	265391.0
5	15.20	300175.7

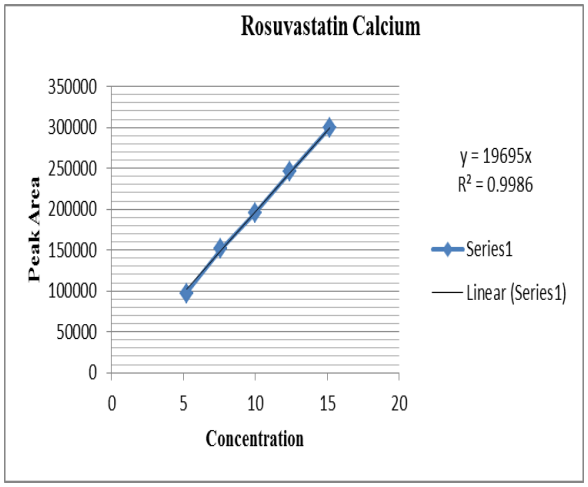


Fig. 3: Calibration curve of Rosuvastatin Calcium.

Precision

B. Repeatability

To check the degree of repeatability of the developed HPLC method, six samples of the formulation were analyzed as per the procedure given under Analysis of formulation. The standard deviation and % Relative Standard Deviation (% R.S.D.) were calculated.

(Table No. 3)

Table 3: Repeatability precision.

Sr.No	Amount present(µg ml^{-})		Peak area ratio		Amount found in mg/ml		% of drug found*	
	Nia	Rosu	Nia	Rosu.	Nia	Rosu	Nia	Rosu
1	500	10	394066	195678	500.20	9.99	100.04	99.94
2	500	10	3940660	195668	500.20	9.99	100.04	99.94
3	500	10	3941415	196071	500.30	10.01	100.06	100.14
4	500	10	3940660	194828	500.20	9.95	100.04	99.51
5	500	10	3941405	195060	500.30	9.96	100.06	99.63
6	500	10	3940960	195407	500.24	9.98	100.04	99.80
						Mean	100.04	99.82
						S.D	0.0109	0.2562
						RSD	0.01	0.2566

*Average of six determinations

C. Intermediate precision (Intra-day and Inter-day precision)

The Intra and Inter-day precision were determined by assay of the sample solutions on the same day at different time intervals and on different days respectively. The S.D. and % R.S.D. were calculate (Table No 4 & 5).

Table 4: Intraday Precision.

Sr. No.	Wt. of sample taken (mg)		Mean peak area		Percent label claim	
	Nia	Rosu	Nia	Rosu	Nia	Rosu
01.	24.8	25.0	3940019	195579	100.04	100.05
02.	25.0	24.9	3941495	196140	100.07	100.34
03.	25.1	24.9	3938061	196386	99.99	100.47
04.	25.2	25.1	3939642	196455	100.03	100.50
05.	25.1	25.0	3938377	196050	99.99	100.29
06.	25.2	25.1	3938997	196471	100.01	100.51
				Mean	100.02	100.36
				SD	0.031	0.1764
				RSD	0.030	0.175

Table 5: Interday precision.

Sr. No.	Wt. of sample taken (mg)		Mean peak area		Percent label claim	
	Nia	Rosu	Nia	Rosu	Nia	Rosu
01.	24.7	25.1	3936130	196388	100.01	100.27
02.	24.9	25.0	3940019	196290	100.11	100.22
03.	25	25.2	3941495	195650	100.15	99.89
04.	24.6	24.9	3934469	195864	99.97	100.00
05.	25.0	25.1	3939801	196589	100.11	100.37
06.	25.1	25.0	3941873	196493	100.16	100.32
				Mean	100.08	100.17
				SD	0.07739	0.19072
				RSD	0.077	0.190

D. Limit of detection (LOD) and Limit of quantitation (LOQ)

The detection of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an extract value.The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ. (Table No 6).

LOD is calculated from the formula:
LOD=3.3σ/S
σ= standard deviation of the response
S=slope of the calibration curve

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte sample that can be quantitatively determined with suitable precision and accuracy.

LOQ is calculated from the formula:
LOQ=10σ/S
LOD and LOQ of Nia and Rosu:

Table 6: LOD and LOQ of Niacin and Rosuvastatin Calcium.

Drugs	LOD*(µg/ml)	LOQ*(µg/ml)
Rosuvastatin Calcium	0.077	0.23
Niacin	1.60	4.85

E. Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 50, 100 and 150 % of the test concentration as per ICH guidelines.

To perform recovery studies at 80 % of the test concentration, a preanalyzed synthetic mixture containing 10 mg of Rosu and 500 mg of Nia was weighed. Sample powder containing equivalent to 10mg of Rosu and 500mg of Nia was weighed and transferred to a 100 ml volumetric flask. To it, mobile phase was added and the contents were shaken in a sonicator for 30 minutes. Finally the volume was made up to the mark with the same solvent. The solution was filtered through Whatmann filter paper No. 42 and like this, nine recovery solution were prepared are prepared as follows,
Flask 1, 2, 3- 30 ml sample solution + 15 ml Standard solution
Flask 3, 4, 6- 30 ml sample solution + 30 ml Standard solution
Flask 7,8, 9- 30 ml sample solution + 45 ml Standard solution
The results of the recovery studies and its statistical validation data are given in.

(Table No. 7)

Table 7: Results of Recovery Studies.

Sr. No.	Wt. of Powder		Amount of pure drug added (mg)		Amount of drug recovered		Percent Recovery	
	NIA	ROSU	NIA	ROSU	NIA	ROSU	NIA	ROSU
1.	300	30	150	15	154.18	14.92	102.78	99.46
2.	300	30	150	15	153.88	14.88	102.78	99.2
3.	300	30	300	30	299.52	29.95	99.84	99.83
4.	300	30	300	30	299.65	29.84	99.88	99.46
5.	300	30	450	45	442.93	45.81	98.42	101.8
6.	300	30	450	45	443.37	45.87	98.44	101.93
						Mean	100.35	100.28
						SD	1.983	1.24
						RSD	1.976	1.236

F. Specificity: A blend of commonly used synthetic mixture of API and the chromatogram showed no interfering peaks at retention time of the two drugs. Shown in below.

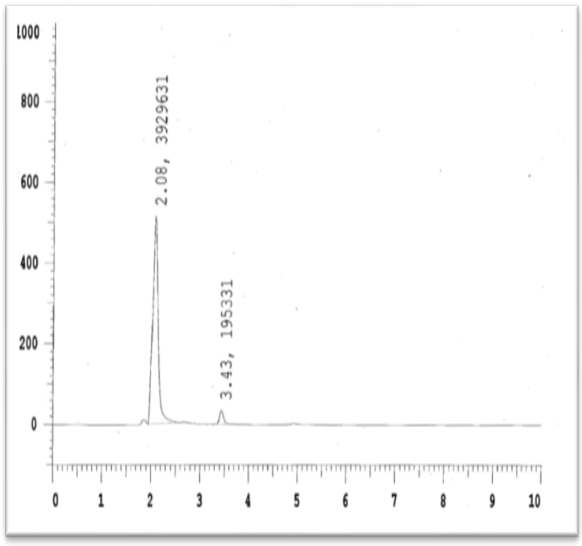


Fig. 4: Chromatogram of standard Rosu ($R_{t=}$3.43) and Nia ($R_{t=}$2.08).

CONCLUSION

The proposed RP- HPLC method was found to be highly accurate, sensitive and precise, Selective and economic as per ICH Guidelines. Therefore, this method can be applied for the routine quality control analysis of Niacin and Rosuvastatin Calcium in pure tablet.

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