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Development and Validation of a Reverse-Phase HPLC Method for the Determination of Rosuvastatin in Pharmaceutical Dosage Forms

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ABSTRACT

A rapid, specific reverse-phase high-performance liquid chromatography method was developed with UV detection for the assaying of rosuvastatin, a new member of a class of cholesterol-lowering drugs, in pharmaceutical dosage forms. The assay was performed isocratically using triethylamine buffer (2.2 mL of triethylamine in 1000 mL of water, P^H adjusted to 4.5 with glacial acetic acid), acetonitrile and methanol (45:25:35) as mobile phase, and a Luna C_{18} column maintained at ambient temperature. The flow rate was 1.0 mL min^{-1} and analyte monitored at 248 nm. The method was found to be linear and has been validated over a concentration range 0.5 to 30 µg $mL^{-1.}$ The developed method was successfully applied for the qualitative and quantitative determination of rosuvastatin in tablet dosage forms without interference from the excipients.

Keywords: Rosuvastatin, Atorvastatin, Chromatography.

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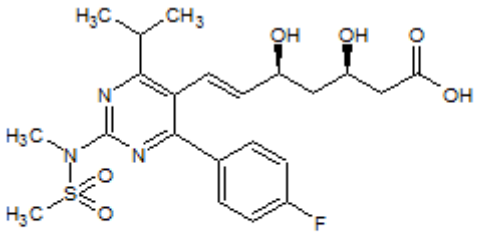
INTRODUCTION

Rosuvastatin (Feg.1) is chemically 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 [3]. Rosuvastatin is an effective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is useful for cholesterol biosynthesis in liver [1-3]. Rosuvastatin is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and apolipoprotein B in the blood [4, 5]. Rosuvastatin also modestly increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease.

Literature survey reveals that till date there are only few analytical methods reported for the estimation of rosuvastatin in pharmaceutical dosage forms. The reported bioanalytical methods were based either on HPLC coupled to UV detector [6] or LC–MS/MS [7, 8, 9] for the quantification of rosuvastatin in plasma. The present investigation has been undertaken to develop a rapid, specific, precise and validated HPLC method for the estimation of rosuvastatin in tablet dosage forms with wide linearity range.



Rosuvastatin

Fig 1: Structural representation of Rosuvastatin.

EXPERIMENTAL

Chemicals and Reagents

Rosuvastatin (99.5% pure), Atorvastatin (99.2% pure, Internal standard) were gift samples from MSN Laboratories Ltd, Hyderabad. Acetonitrile and Methanol (HPLC grade) were obtained from J.T Baker, USA. Triethylamine buffer was obtained from Qualigens fine chemicals, Mumbai, India. All aqueous solutions, including the buffer for the HPLC mobile phase, were prepared with Milli Q (Millipore, USA) grade water.

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Instrumentation

A high-performance liquid chromatography system consisted of a Shimadzu prominence model equipped with two LC-20AD solvent delivery pumps, variable wavelength programmable UV-VISIBLE detector SPD-20AV, auto sampler SIL-20A, CTO-20A column oven, CBM-20A system controller and Shimadzu LC solutions software run on a Compaq Evo computer (operated with windows XP 2000 professional) was used for this method.

Chromatographic Conditions

The chromatographic separation was accomplished with Luna C_{18} column of 250 mm×4.6 mm i.d, 5 µm particle size (Phenomenex, California, USA) protected by a guard column (15×4.6 mm). The column was maintained at ambient temperature. The standard mobile phase consisted triethylamine buffer (2.2 mL of triethylamine in 1000 mL of water, pH adjusted to 4.5 with glacial acetic acid), acetonitrile and methanol (45:25:35) was filtered through 0.45 µm filter before use. The flow rate was maintained at 1.0 mL min^{-1}. Detection was carried out by UV detector at 248 nm and the injection volume was 20 µL.

Preparation of Solutions:

Preparation of Standard Drug Solution

Two independent 1 mg mL^{-1} stock solutions of rosuvastatin (#1 and #2) were prepared by dissolving approximately 10 mg of drug in 10 mL of mobile phase. The stock internal standard (IS) solution was prepared by dissolving 10 mg of atorvastatin in 10 mL of mobile phase. All these solutions were sonicated for complete solubility of the drug. All these solutions were stored at 4°C before use. Working standard solutions of rosuvastatin and internal standard were prepared daily by suitable dilution of the stock solution with mobile phase.

Sample Preparation

Eight tablets were weighed to get the average tablet weight and pulverized. Amount equivalent to 100 mg of rosuvastatin from powdered formulation was dissolved in 100 mL of mobile phase and filtered through a 0.45 µm membrane filter, to get the concentration of 1 mg mL^{-1}. Ten sets of the sample solution were prepared in mobile phase containing rosuvastatin at a concentration range of 0.5 to 30 µg mL^{-1} along with a fixed concentration 2 µg mL^{-1} of internal standard.

Method Validation

System Suitability

The system suitability was assessed by six replicate analysis of the drug from stock solutions (#1), (#2) at 10 µg mL^{-1} level. The acceptance criteria were tailing factor and %

relative standard deviation (%R.S.D) for peak area of rosuvastatin from stock (#1) not more then 2.0. Similarity factor between two stocks was in the range of 0.985 to 1.015.

Limit of Quantitation and Limit of Detection

The quantitation limit (LOQ) was defined as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than 10. The detection limit (LOD) was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The LOD and LOQ were determined using six replicate injections at different lowest concentrations which were prepared from standard stock solutions and analysed.

Accuracy and Precision

The accuracy and precision of the proposed method was assessed by six replicate analysis of three different concentrations at 0.5, 10, 30 µg mL^{-1}, prepared and analyzed on the same day (intra-day) and three different days (inter-day) over a period of two weeks. The acceptance criteria for intra-day and inter-day % R.S.D should be not more than 2.0 at each concentration level. The intra-day and inter-day average nominal concentration should be 100±2% at each concentration level.

Linearity

The calibration curve was constructed with nine concentrations ranging from 0.5 to 30 µg mL^{-1}. The peak area ratio of drug to the IS was considered for plotting the linearity graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Recovery

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed method. A known amount of pure drug was added to each of the previously analyzed formulation and the total amount of the drug was once again determined by the proposed method. The acceptance criteria was mean recovery of rosuvastatin should be 100±2% at each spike level.

Ruggedness

The ruggedness of assay method was assessed by conducting the system to system /analyst to analyst/column to column variability study on different HPLC system, different column and different analyst under similar conditions at different times. Six samples were prepared and each was analysed as per test method. The acceptance criteria was % R.S.D should be not more than 2.0 on the columns, systems and analysts.

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Robustness

Robustness of the method was investigated by varying the instrumental conditions such as flow rate (±10%), column oven temperature (35°C) and organic content in mobile phase (±2%). Sample and standard solutions were injected under each condition and analyzed. The acceptance criteria were tailing factor and % R.S.D for peak area of rosuvastatin at each condition should be not more then 2.0.

Estimation of Rosuvastatin in Tablet Dosage Forms

Two commercial brands of tablets (RAZEL, Glen mark Pharmaceutical Ltd., Mumbai, India; ROSUVAS, Ranbaxy laboratories Ltd., Gurgaon) were chosen for testing suitability of the proposed method to estimate rosuvastatin in tablet dosage forms.

RESULTS AND DISCUSSION

Method Validation

System Suitability

The % R.S.D for peak area and tailing factor of rosuvastatin peak were within 2.0 indicating the suitability of the system (Table 1). Similarity factor between two rosuvastatin standard preparations was 0.996.

Table 1: Results of system suitability study

	Rosuvastatin (#1)		Rosuvastatin (#2)	
	Peak area	***Tailing factor***	***Peak area***	***Tailing factor***
Mean (n=6)	2373977	1.285	2366668	1.289
S.D	1479.1	0.01	2723.9	0.01
% R.S.D	0.06	0.72	0.12	0.85

Limit of Quantitation and Limit of Detection

At 0.5 μg mL^{-1} concentration level, peak area to signal-noise ratio was higher than 10. So this concentration level was considered as the quantitation of limit (LOQ). The method was found to be sensitive as determined from the six replicate injections of the LOQ where the % R.S.D was 0.55 (Table 2, Intra-day). At 0.2 μg mL^{-1} concentration level, peak area to signal-noise ratio was higher than 3. So this concentration level was considered as the limit of detection (LOD). There are no significant interferences at the retention time of analyte with this method (Fig. 2).

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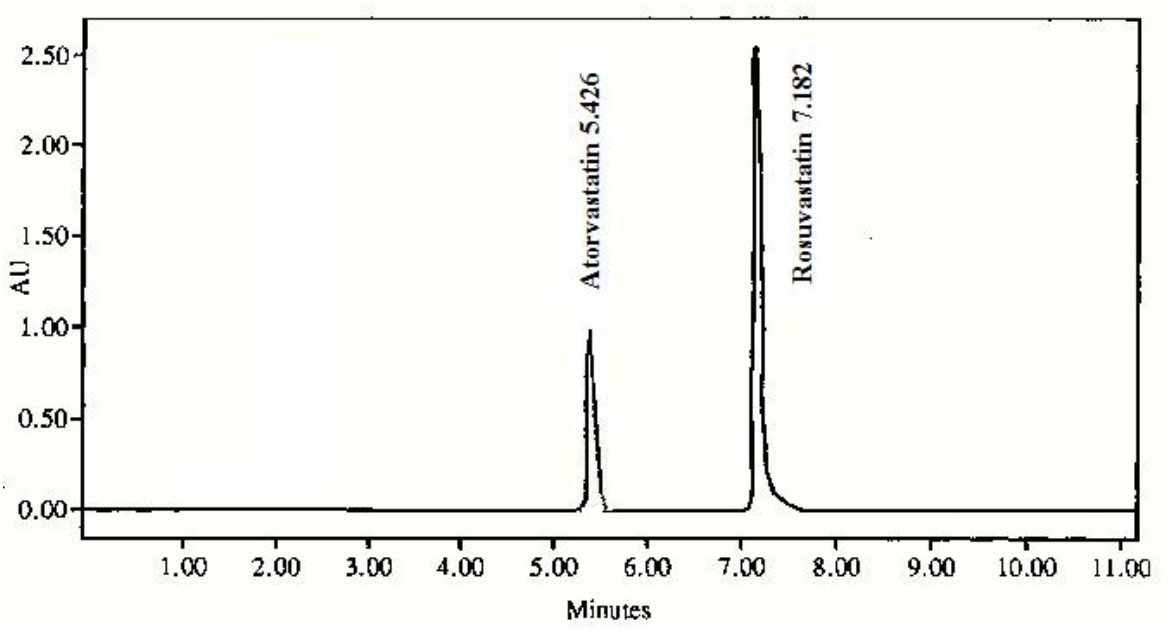

Fig 2: Model Chromatogram for Rosuvastatin.

Accuracy and precision:

The intra-day percentages of nominal concentrations were ranged 99.59 to 100.67. The intra-day % R.S.D was 0.25 to 1.89. The inter-day percentages of nominal concentrations were ranged 99.74 to 100.18. The inter-day % R.S.D was 0.24 to 0.87. Results are summarized in Table 2.

Table 2: Accuracy and precision of the proposed method

Concentration of rosuvastatin (µg mL^{-1})	Intra-day			Inter-day		
	Mean(n=6) Con. found	*Mean(n=6) % Nominal*	*% R.S.D*	*Mean(n=18) Con. found*	*Mean(n=18) % Nominal*	*% R.S.D*
0.5	0.498	99.59	0.55	0.501	100.18	0.71
10	10.07	100.67	1.89	9.98	99.74	0.87
30	30.04	100.1	0.25	29.98	99.91	0.24

Linearity

A good linear relationship (r = 0.9999) was observed (Fig.3) between the concentration of the rosuvastatin and the respective ratio of peak areas (Table 3). The calibration equation was found to be Y = 0.007319 + 0.40089 X (where Y is the ratio of peak area of drug to that of internal standard and X = concentration of rosuvastatin).The peak area ratio of the drug to IS was linear in the range of 0.5–30 µg mL^{-1}.

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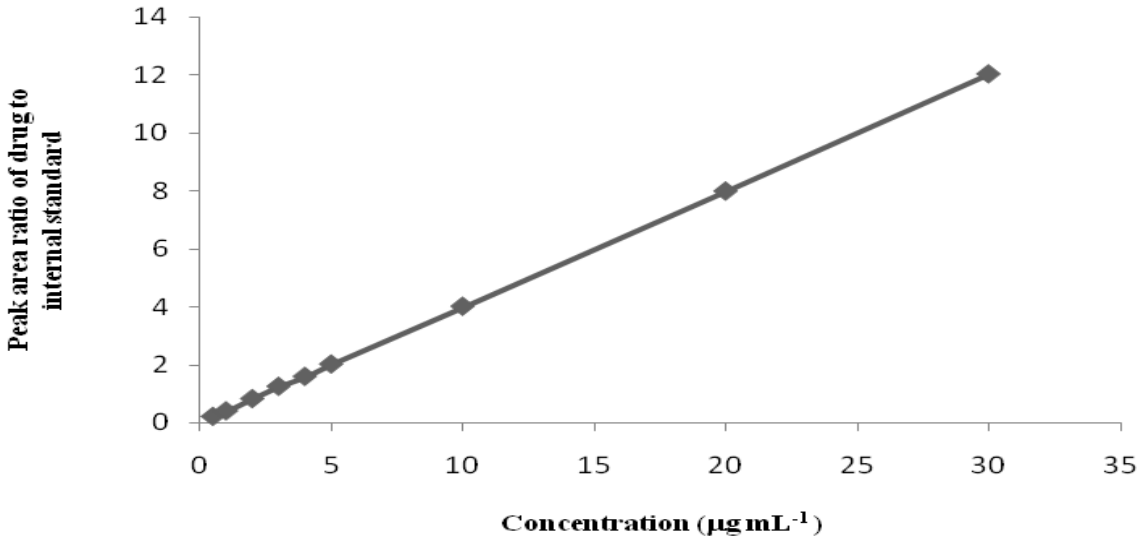

Fig 3: Standard calibration graph of Rosuvastatin.

Table 3: Linearity of the proposed method

Concentration of rosuvastatin (µg mL^{-1})	Mean peak area ratio (n=6)	Coefficient of variation %CV
0.5	0.207	0.05
1	0.398	0.25
2	0.821	0.61
3	1.245	1.12
4	1.589	0.73
5	2.015	0.12
10	4.011	0.83
20	7.993	0.44
30	12.04	0.87

Recovery

The mean % recovery of rosuvastatin from the preanalyzed samples ranged 99.7 to 100.15, indicating (Table 4) high accuracy of the proposed method.

Table 4: Results of recovery study

Amount of drug added (µg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount Found (n=6)	*Mean % recovery*	*Mean amount Found (n=6)*	*Mean % recovery*
10.0	9.99	99.9	9.97	99.7
15.0	14.99	99.93	15.01	100.06
20.0	20.03	100.15	19.98	99.9

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Ruggedness

The % R.S.D was within the range of 0.05 to 0.11 at different variants. The results (Table 5) indicated that the proposed method was rugged and reproducible by using different system, different analyst, and different columns.

Table 5: Ruggedness of the proposed method

Variants	Rosuvastatin	
	% Nominal (mean n=6)	%R.S.D
Control	99.9	0.05
Different System	99.3	0.10
Different Analyst	99.7	0.11
Different Column	99.8	0.07

Robustness

The % R.S.D for peak area and tailing factor of rosuvastatin peak at different variants were within 2.0 indicating the Robustness of the proposed method (Table 6).

Table 6: Robustness of the proposed method

Variants	Peak area		Tailing factor	
	Mean (n=6)	% R.S.D	Mean (n=6)	% R.S.D
SET 1[a]	2337322	0.06	1.298	0.53
SET 2[b]	2359839	0.28	1.285	0.59
SET 3[c]	2325915	0.07	1.319	0.93
SET 4[d]	2380750	0.07	1.309	0.83
SET 5[e]	1943107	0.08	1.277	0.61
SET 6[f]	2905375	0.04	1.313	0.58

[a] Set 1: Control (Proposed method), [b] Set 2: Variation in flow rate (-10%), [c] Set 3: Variation in flow rate (+10%), [d] Set 4: Column oven temperature (35°C), [e] Set 5: Variation in organic content in mobile phase (-2%), [f] Set 6: Variation in organic content in mobile phase (+2%).

Estimation of Rosuvastatin in Tablet Dosage Forms

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of rosuvastatin in two different brands of tablets dosage forms was shown in Table 7.

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Table 7: Assay of rosuvastatin in tablet dosage forms

Brand name	Labeled amount (mg)	Amount found (mg) (Mean n=6)	% S.D
RAZEL	20	19.87	0.16
ROSUVAS	10	9.92	0.15

CONCLUSION

A sensitive and selective HPLC method has been developed for the quantification of rosuvastatin in pharmaceutical dosage forms using a UV detector. The method was validated for accuracy, precision, recovery and linearity. The method was found to be linear and has been validated over a concentration range of 0.5 to 30 µg mL^{-1}. Hence, this HPLC-UV method can be used for the routine drug analysis.

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