High performance liquid chromatographic method validation for determination of rosuvastatin calcium in tablet dosage forms

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Abstract: A precise, sensitive and quick High Performance Liquid Chromatographic (HPLC) method for the determination of rosuvastatin calcium in bulk and tablet dosage forms has been validated. The chromatographic scheme involved: Sil-20A auto sampler, LC-20A pump, SPD-20A UV/visible detector with separation attained by C_{18} column at 40ºC temperature through a mobile phase of acetonitrile and buffer (50:50) at a flow rate of 1.0ml/min. The method is precise (%RSD for intra-day and inter-day extended between 1.06-1.54% and 0.103-1.78%) and linear (r^2=0.9997). Limit of detection and quantification (LOD & LOQ) of the adopted method were 0.78 and 1.56µg/ml. The proposed HPLC method was established to be sensitive, precise and swift that can be proficiently adopted in quality control/quality assurance laboratories for predictable investigation of the bulk and oral solid dosage forms of rosuvastatin calcium.

Keywords: Rosuvastatin calcium, intra-day, inter-day.

INTRODUCTION

Rosuvastatin calcium is chemically bis((E)-7-(4-(4-flurophenyl)-6-1sopropyl-2-methyl(methylsulfonyl) amino) pyrmidin-5yl) (3R, 5S)-3,5-dihydroxyhept-6-enoic acid) calcium salt(Lennernäs and Fager, 1997). Rosuvastatin calcium belongs to the class of lipid lowering drugs that is used in the management of dyslipidemia. It acts by competitively inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase (Nissen *et al*., 2006). The pharmacokinetics parameters of rosuvastatin including AUC and $C_{\max}$ were basically dose related, time to $C_{\max}$ extended from 3-5 hrs, and the elimination half-life ranged from 13 to 21h (Martin *et al*., 2002). It has good solubility in acetone, acetonitrile and N, N-dimethyl formamide, while slight soluble in ethanol and sparingly soluble in water (Alshora *et al*., 2016).

Several analytical techniqueshave been described for the estimation of rosuvastatin calcium in bulk and pharmaceuticals. Saneet *et al*., proposed asimple TLC method for the approximation of rosuvastatin calcium accomplished on HPTLC plates (Sane *et al*., 2005). Another simple and consistent spectrophotometric method was reported for the determination of rosuvastatin calcium (Uyar *et al*., 2007). High Performance Liquid Chromatographic methods were also developed including, Kaila, *et al*. who performed HPLC determination of rosuvastatin calcium by using mobile phase comprising aceonitrile and water (60:40) at pH 3.5 (Kaila *et al*., 2010), Jajam Thriveni, *et al*. developed RP-HPLC method using mobile phase containing acetonitrile: methanol: water (40:40:20) at isocratic mode and showed linearity in the concentration of 1-5µg/ml (Thriveni *et al*., 2013), Najma Sultana, *et al*. developed HPLC method with the mobile phase comprised of ethanol: water: acetonitrile (80: 17.5: 2.5, *V*/*V*) with pH attuned to 3.0 (Najma *et al*., 2011). Previously used methods were time consuming and complex with high proportion of organic solvents (Gajjar and Shah, 2010; Gomes *et al*., 2009; Karunakaran *et al*., 2011; Thriveni *et al*., 2013). Therefore, in the present research a simple and accurate method was adopted and validated for the determination of rosuvastatin calcium in bulk and tablet dosage forms.

MATERIALS AND METHODS

Materials/reagents

Rosuvastatin calcium (reference standard) was provided by Bosch Pharmaceuticals (Pvt.) Ltd. All the reagents and chemicals utilized were of HPLC grade and procured from Merck Chemicals, Germany.

Equipment

High Performance Liquid Chromatography (Shimadzu Corporation, Japan) was used, equipped with Sil-20A HT auto sampler, LC-20 AT pump and SPD-M20A UV-VIS detector. Chromatographic separation was accomplished on a system that was integrated via Shimadzu model CBM-20Awith LC solution software. C_{18} column (5µm, 150mm×4.6mm) (Phenomenex, USA) and auto sampler was used to carried out presented chromatographic study. Furthermore, for standard and sample solution

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**Corresponding author:* e-mail: tariq.ali@duhs.edu.pk preparation, analytical balance, micropipette (Gilson Pipetman), micropore filtration assembly, vortex mixer, sonicator (Power Sonic410) were also used.

Chromatographic condition

The mobile phase used to carry out chromatographic studies consisted of acetonitrile: Phosphate buffer in a ratio of 50:50 (pH 4.5 adjusted with ortho-phosphoric acid). Mobile phase was filtered using micropore filtration assembly. The process was preceded at a flow rate of 1.0ml/min at 40ºC using C_{18} column. 10ul of injection volume was used for sample and standard injections to HPLC system under isocratic condition. Chromatogram was detected using SPD- 10A VP Shimadzu UV-VIS detector at a wavelength 0f 254nm.

Preparation of rosuvastatin calcium reference standard solution

Stock solution for the standard of rosuvastatin calcium was prepared by transferring accurately weighed 10mg of reference standard in 10ml volumetric flask and adding 5ml of methanol. After sonication (10 minutes), volume was makeup with methanol up to 10ml. The resulting concentration was 1mg/ml or 1000µg/ml. This stock solution was further diluted in mobile phase for the preparation of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78ug/ml dilutions as required for calibration and linearity.

Preparation of sample solutions from tablets

A total of 20 tablets of 10mg rosuvastatin calcium were weighed accurately and then triturated in mortar and pestle in order to obtain a fine powder of weighed tablets. The amount of powder equal to 10mg of rosuvastatin calcium was shifted to a 10ml volumetric flask and adds 5 ml of methanol. The mixture was sonicated for 5 min in order to dissolve sample in methanol and makeup the volume with methanol. The final concentration of sample solution was kept equivalent to 1mg/ml or 1000µg/ml. Stock solution of sample was then filtered with Whatman filter paper. This solution was further diluted in mobile phase to prepare 100ug/ml sample solution. Sample solution was filtered through 0.45µm HV Millipore membrane filter and injected to the HPLC system.

Validation procedure

HPLC method was developed to obtain speedy and precise method for the quantitative determination of rosuvastatin calcium. Developed HPLC technique was validated according to International Conference on Harmonization and United State Pharmacopeias (ICH, 1997; USP, 2008). This method was validated for suitability, specificity, precision, linearity, accuracy, LOD, LOQ and robustness.

System suitability and specificity

Before each stage of validation parameters, the system suitability was tested for chromatographic system. The suitability of system was checked by injecting three replicates of standard preparation to authenticate that the resolution/reproducibility of the chromatographic system satisfactory for the investigation. This was assessed by analyzing tailing factor, retention time, peak height, peak area and theoretical plates. Specificity was assessed by paralleling the chromatograms of blank, reference standard and sample prepared from tablets dosage forms.

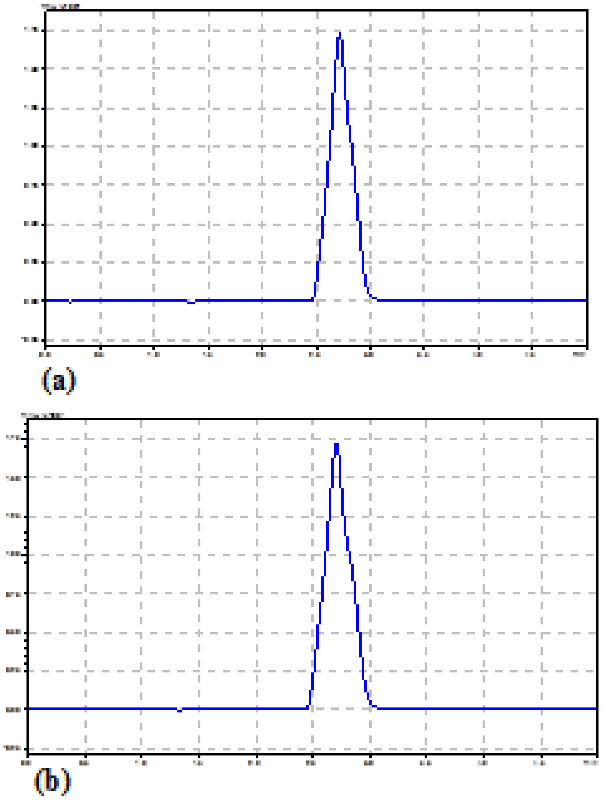

Fig. 1: HPLC Chromatograms of rosuvastatin calcium, reference standard (a) and tablet (b).

Linearity

From the standard stock solution of rosuvastatin calcium (1000µg/ ml), serial dilutions of standard were prepared in mobile phase in a range of 0.78-100ug/ml (n=8). The Standard solutions were injected in HPLC system using a 10µl fixed loop system and chromatograms were recorded. Calibration curve were plotted against prepared concentration and peak area and regression equation were computed to obtain value of coefficient of determination (r^2). Limit of detection and limit of quantification was also assessed.

Recovery

Percentage recovery was calculated for additional validation by making drug solutions at three concentration levels (n=3) i.e. 70%, 100% and 130% levels of the target rosuvastatin calcium amount and injecting to HPLC. The percentage recoveries at each of three levels, average recoveries and relative standard deviation were then determined.

Table 1: System suitability of HPLC method for determination of rosuvastatin calcium

S. no.	Retention time (min)	Peak area	Peak height	USP tailing factor	Theoretical plates	Resolution	k factor
1	2.757	1689111	175953	0.941	1518.39	1.212	0.835
2	2.742	1685728	169894	0.959	1399.29	1.082	0.852
3	2.747	1687940	163811	0.95	1374.93	1.171	0.841
4	2.756	1688392	176242	0.909	1489.8	1.099	0.892
5	2.749	1692443	168233	0.929	1387.67	1.124	0.873
6	2.737	1680579	173487	0.968	1507.57	1.125	0.849
Mean	2.748	1687365.5	171270	0.943	1446.276	1.136	0.857
SD	0.0078	3973.65	4864.56	0.0195	65.71	0.048	0.022
% COV	0.284	0.235	2.84	2.07	4.5	4.22	2.45

Table 2: Linearity of the proposed HPLC method

Drug Conc (ug/ml)	Area	Mean Area
100	1689111	1687593
	1685728	
	1687940	
50	856028	866220
	872924	
	869708	
25	434434	443420
	448010	
	447817	
12.5	221070	223382
	222453	
	226624	
6.25	103323	103880
	103359	
	104958	
3.15	53555	53309
	53511	
	52860	
1.56	28088	28480
	27938	
	29413	
0.78	15856	15702
	15339	
	15911	
Pearson correlation (R^2)		0.9997
Slope		16915
y-Intercept		6484.8

Precision/accuracy

Precision/accuracy of the developed method was judged in terms of repeatability. 12.5, 25, 50 and 100ug/ml standard solution of rosuvastatin calcium were prepared. These concentrations were injected three times of same day for intraday precision and for interday precision concentrations were injected three times on 2 more consecutive days.

Robustness/ruggedness

Robustness/ruggedness of the method was determined by altering of flow rate, wavelength and changing proportion of mobile phase like, flow rate (±0.1mL min-1), percentage of buffer and methanol (45:55 and 55:45, v/v), and analyzing wavelength (±4.0). The percentage recovery and %RSD was noted for rosuvastatin calcium.

Assay

Assay of rosuvastatin calcium 10mg marketed formulation was performed using proposed HPLC method. Label claim and percentage were calculated.

RESULTS

Chromatographic parameters

For the quantification of rosuvastatin calcium in tablets, a HPLC method was proposed as an appropriate method for QA/QC analysis. Method was developed on C_{18} column (5µm, 150mm×4.6mm) (Phenomenex, USA) which deliver efficient separation at 40ºC temperature. Different composition of acetonitrile: buffer and methanol: buffer was tried. It was observed that with methanol rosuvastatin calcium was damaged and peak splitting was observed.

With acetonitrile this problem was resolved but when the composition of acetonitrile was reduced below 40% the peaks were again fragmented. Symmetry of peaks was not as of required standard. Different pH was also used to observe the HPLC chromatograms and finally pH 4.5 was set for the proposed method. Peak responses were also noted at different wave length and the wavelength of 254nm was selected because the maximum peak response was observed at this wavelength. The mobile phase was comprised of acetonitrile: Phosphate buffer (50:50) generated baseline resolved peaks with a brilliant resolution.

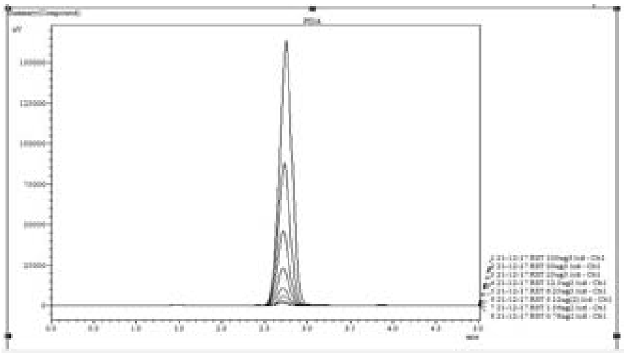

Fig. 2: HPLC Chromatograms of linearity solutions.

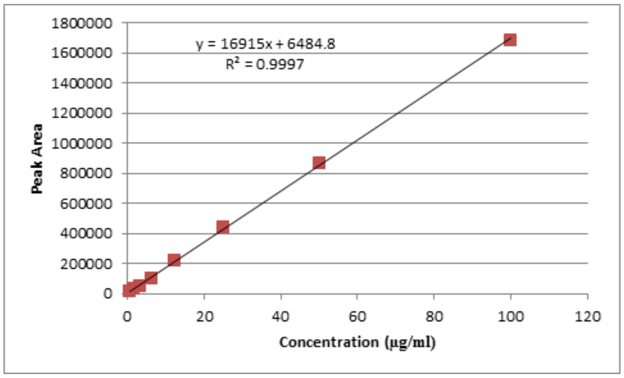

Fig. 3: Calibration curve showing linearity

Validation protocol

Method validation was executed according to guidelines given by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH, 1997). Validation parameters included the linearity, specificity, system suitability, accuracy, precision, LOD, LOQ and ruggedness/robustness, as follows:

System suitability and specificity

System suitability was found to be in the FDA recommended limits (table 1). The proposed method was specific and selective for the quantification of rosuvastatin calcium in oral solid dosage forms without any peak interference. Method exhibited good sensitivity and selectivity. The chromatogram demonstrated that there is no interference even in the presence of excipients in the formulations (fig. 1).

Linearity, LOD and LOQ

The linearity was in the range of 0.78-100ug/ml with the least square regression of 0.9997 (y=16915x+6484.8) (table 2, fig. 2). LOD and LOQ were found to be 0.78 and 1.56ug/ml.

Precision and accuracy

The reported method was accurately quantifying rosuvastatin calcium with reproducibility. Satisfactory mean recoveries of rosuvastatin calcium were in the range of 98.89% -100.66% when sample was spiked in the level of 70%, 100% and 130% (table 3). Inter and intra-day precision was found in the recommended range (%RSD for intra-day and inter-day extended between 1.06-1.54% and 0.103-1.78%)(table 4).

Robustness/ruggedness

The method established to be judiciously precise and robust as there was no momentous changes in the % RSD as showed in table 5.

DISCUSSION

A selective, accurate and quick HPLC method for the quantification of rosuvastatin calcium in bulk and oral solid dosage forms has been adopted and validated. Previously used methods were time consuming and complex with high proportion of organic solvents. Therefore, a simple and accurate method was proposed. Method was quick as the run time was less than 4 min (2.748±0.0078) compared to the methods where the run time was around 10 minutes (Gomes *et al.*, 2009; Hasumati A. Raj *et al.*, 2009; Kaila *et al.*, 2010; Najma *et al.*, 2011; Raj *et al.*, 2009; Thriveni *et al.*, 2013). Method developed with the less proportion of organic solvent to keep the method less expensive. Other reported methods used the proportion of organic solvent higher than the proposed method (Gajjar and Shah, 2010; Karunakaran *et al.*, 2011; Najma *et al.*, 2011). The pH of mobile phase was maintained at 4.5 compared to others studies where pH was approximately 3 (Gomes *et al.*, 2009; Najma *et al.*, 2011) to decrease the retention time as of the acidic nature of rosuvastatin calcium. Recognized on the peak purity conclusions acquired from the investigation of rosuvastatin calcium sample solutions by the projected method, it can be obvious that there is no co-eluting peak with the principal peaks demonstrating the specificity of method for the estimation of rosuvastatin calcium in tablet dosage forms. The suggested method is found reproducible, repeatable (%RSD for intra-day and inter-day were in the suggested range of <5%) and linear (R^2=0.9997). The LOD and LOQ of the suggested method were 0.78 and 1.56µg/ml, comparable to previous reports (Najma *et al.*, 2011; Thriveni *et al.*, 2013). Linearity was found over a wide concentration range which is better than some previous reported methods (Gomes *et al.*, 2009; Mostafa *et al.*, 2014). The suggested method for

Table 3: Accuracy of proposed method

S#	Concentration Added	Concentration Found	Mean	% Recovery	% Precision	% Accuracy
1	100	98.892	99.164	98.89	0.3099	0.8910
		99.326		99.33		
		99.274		99.28		
2	70	70.448	70.447	100.641	0.0197	0.6390
		70.432		100.617		
		70.460		100.656		
3	130	130.553	130.447	100.425	0.1083	0.3440
		130.502		100.386		
		130.287		100.220		

Table 4: Precision of proposed method

Reproducibility	Concentration (µg/ml)	Concentration determined	Accuracy[b]	RSD[c]
Precision Interday[a]	100	99.386	99.39	0.103
	50	50.830	101.7	1.043
	25	25.836	103.3	1.78
Precision Intraday[a]	100	98.74	98.7	1.54
	25	24.09	96.36	1.06
	12.5	12.828	102.6265	1.33

[a]Determined on three replicate of standard injections. [b]Accuracy is error * 100 / actual conc. [c]RSD= Relative Standard Deviation

Table 5: Method robustness

Changes in chromatographic conditions		Peak area	Peak height	Tailing
Flow rate (ml/min)	0.9	1.32*	0.89	0.05
	1.1	1.5	1.28	0.14
Proportion of buffer (%)	45	0.67	0.43	1.06
	55	1.18	1.43	1.26
Detection wavelength (nm)	250	1.09	0.78	0.37
	258	0.49	1.3	0.99

* Results are expressed as % RSD

assay of rosuvastatin calcium was, thus, found validated conferring to the ICH guidelines in expressions of selectivity, linearity, accuracy and reproducibility. The method is competent to determine drug in a solo run in addition, also accurate, specific, robust, lack of interference due to additives/excipients and simple to adopt in the quality control and quality assurance laboratories.

CONCLUSION

The proposed HPLC method was established to be sensitive, precise and swift that can be proficiently adopted in quality control/quality assurance laboratories for predictable investigation of the bulk and oral solid dosage forms of rosuvastatin calcium.

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