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Spectrophotometric determination of rosuvastatin calcium in tablets

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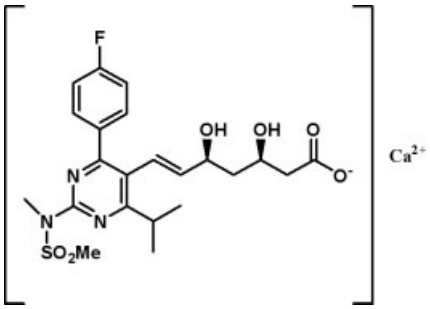
Rosuvastatin calcium is a synthetic lipid lowering agent which is used in hypercholesterolemia. It is a selective and competitive inhibitor of HMG-CoA reductase. In this study a simple, rapid and reliable spectrophotometric method was developed for the determination of rosuvastatin calcium in pharmaceutical preparations. The solutions of standard and pharmaceutical samples were prepared in methanol. 243 nm was chosen for measuring absorbances of rosuvastatin calcium. The developed method was validated with respect to linearity range, limit of detection and quantitation, accuracy, precision, specificity and ruggudness. The linearity range of the method was 1.0–60.0 μg mL^{-1}. The limit of detection was 0.33 μg mL^{-1}. The developed and validated method was applied to the determination of rosuvastatin calcium in pharmaceutical preparations.

1. Introduction

Rosuvastatin calcium (RC) is bis ((*E*)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(methyl(methylsulfonyl)amino) pyrimidin-5yl)(3*R*,5*S*)-3,5-dihydroxyhept-6-enoic acid) calcium salt. The empirical formula of it is $(C_{22}H_{27}FN_3O_6S)_2Ca$.
Rosuvastatin, a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis (Cheng 2004). RC causes an evident reduce on LDL level in comparasion to other statin group drugs (Riesen 2006).



Rosuvastin calcium

In tablet dosage forms, RC has been analysed with HPLC with UV-Vis detector (Mehta et al. 2005) and with MS detector (Hull et al. 2002). High Performance Thin Layer Chromatography (HPTLC) is another reported method for the determination of Rosuvastatin calcium in tablets (Sane et al. 2005). RC has also been determined in plasma by HPLC (Hull et al. 2004; Trivedi et al. 2005). There is no spectrophotometric method for the determination of RC in pharmaceutical dosage forms in the literature.
Chromatographic techniques are time consuming and costly, therefore a simple and accurate validated Ultraviolet-Visible (UV-Vis) spectrophotometric methods can provide a very useful alternative for routine analysis of pharmaceuticals.
In the present study, a simple, sensitive, validated and relatively inexpensive method for the determination of RC in pharmaceutical dosage forms was developted.

2. Investigations, results and discussion

Since RC is sparingly soluable in water at room temperature, an organic solvent, methanol, was choosen to solve RC. The UV-Vis spectra of standard solutions of RC, tablet and synthetic tablet solutions at the same concentrations were identical when recorded from 200 nm to 450 nm in MeOH (Fig).
The spectrum of RC in MeOH had two maximum absorption bands at 205 nm and 243 nm. There was no interference from excipient and MeOH at 243 nm. Therefore 243 nm was selected for quantitative analysis to prevent excipient interference. No difference was observed in 243 nm of all the three spectra.
The developed method for the analysis of RC was validated with respect to stability, linearity, sensitivity, precision, accuracy, specificity, robustness and ruggedness (ICH 1996; Ermer and Ploss 2005; Fabre and Altria 2001; Braggio et al. 1996)
The standard stock solutions of RC were stored under two different conditions (solutions keeped at +4 °C and at ambient temperature) for 1 month and prevented from daylight. During this period, UV spectra of solutions were taken periodically. They were compared with freshly prepared solutions and not any difference was found between them. This indicates that RC is highly stable under the above mentioned conditions.

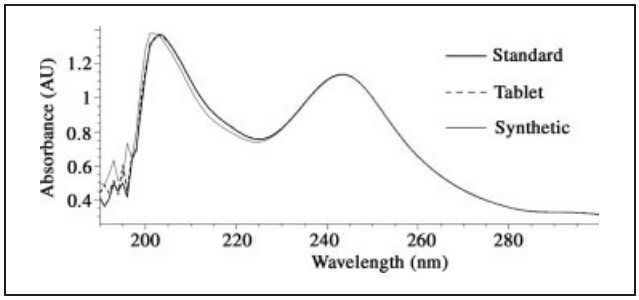


Fig: Spectrum of standard, synthetic and tablet solutions (25 μg mL^{-1})

In quantitative analysis the calibration curve was constructed after analysis of consecutively increased concentrations. The regression equation, standard errors of slope and intercept, correlation coefficient and linearity range are given in Table 1.

A signal to noise ratio (S/N) of approximately 3 : 1 is generally considered to be acceptable for estimating the limit of detection (LOD), which is the lowest concentration that can be detected. The LOD obtained was 0.33 μg mL^{-1} for RC.

Limit of quantification (LOQ) is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision (Fabre and Altria 2001; Braggio et al. 1996). By this way, for RC, the LOQ value was found 1.00 μg mL^{-1} (Table 1).

After three different concentrations of RC (10, 25, 50 μg · mL^{-1}) in the linear range had been selected, this independent series was prepared and analyzed in one day (intra-day) and six consecutive days (inter-day). The RSD % values varied from 0.38 to 1.41 for intra-day precision (repeatability) and from 0.26 to 0.29 for inter-day precision (reproducibility) (Table 2). These RSD% values showed that the method had high precision.

Table 1: Data of the calibration curve for RC determined by the proposed method (n = 6)

Regression equation	* y = 0.0447x + 0.0048
r	0.9998
S_1	0.0004
S_2	0.0010
Linearity range (μg mL^{-1})	1.00–60.00
LOQ (μg mL^{-1})	1.00
LOD (μg mL^{-1})	0.33

* y = aX + b where X is the concentration of rizatriptan (μg/mL); y is the absorbance for UV-Vis spectrophotometric method; (a is the slope and b is the intercept)
r: The coefficient of correlation
S1: Standard error of intercept at regression line
S2: Standard error of slope at regression line
LOQ: The limit of quantitation
LOD: The limit of detection

The accuracy of the proposed method was also tested by recovery experiments. Therefore the proposed method was reapplied six times (n = 6) for the determination of RC (25 μg mL^{-1}) in synthetic tablet solutions (Table 3).

Another recovery study of the method was performed using the standard addition technique and its calculation results, namely the mean ± SE, relative standard deviation (RSD) and recovery % are presented in Table 4.

For both these recovery experiments, the high recovery rate indicated that the method had a good accuracy.

The spectrum obtained from both tablet and synthetic tablet solutions were identical to the spectrum obtainded from standard solution containing an equivalent concentration of RC (Fig.).

In addition the standard addition technique was applied to the same preparations which were analysed by calibration curve methods. The regression equations of standard addition curves of the method for tablet analysis were found to be y = 0.0446x + 1.1423 (r = 0.9996) (x is the concentration of RC (μg mL^{-1}); y is the absorbance for UV-Vis spectrophotometric method; a is the slope and b is the intercept).

Since the slopes of the standard and standard addition curves were identical, it was concluded that there was no spectral interaction in the analysis of RC in pharmaceutical formulatios.

Ruggedness test for the UV-Vis Spectrophotometric analysis of RC was performed by the application of developed method to the standard solution of RC (25 μg mL^{-1}) by different analysts on different days. Obtained results were compared statistically (Mann Whitney-U Test). The obtained results for the RC (25 μg mL^{-1}) from different analyst are statistically acceptable (Uc = 24 < Ut = 29 Uc = Calculated Ut = Table). Therefore it could be said that the method is rugged.

Robustness of the proposed method was tested by minor changes on the selected wavelength. Since the absorbance was not significantly effected, the proposed method could be considered as robust.

The proposed method was succesfully applied to the determination of RC from pharmaceutical tablet formulation (Crestor®). RC in six different tablet solutions containing one dosage form (10 mg) was analyzed by the calibration curve and standard addition methods. Both of these methods were performed at 243 nm. With the calibration curve method, the tablet solutions of RC, prepared as six indipendent series, were analyzed three times.

With the standard addition method, tablet analysis was performed six times by the addition of known amounts of RC standard solutions (10 μg mL^{-1}, 20 μg mL^{-1} and 30 μg mL^{-1}) to the tablet solution (10 μg mL^{-1}). After the measurement of absorbances, the concentration of tablet solutions was determined according to the standard and calibration curve regression equation. The results are given in Table 5.

Table 2: Precision and accuracy of the developed spectrophotometric method for the analysis of RC (n = 6)

Added (μg mL^{-1})	Intra-Day			Inter-day		
	Found $\bar{x}$ (μg mL^{-1})	Precision RSD %	Accuracy Bias %	Found $\bar{x}$ (μg mL^{-1})	Precision RSD %	Accuracy* Bias %
10	9.98 ± 0.06	1.41	0.18	9.96 ± 0.01	0.26	0.41
25	25.1 ± 0.11	1.09	−1.02	25.03 ± 0.01	0.12	−0.33
50	49.95 ± 0.08	0.38	0.53	50.03 ± 0.06	0.29	−0.28

x: Mean ± SE
RSD %: Relative standard deviation %
* Accuracy: ((found − added)/added) × 100

Table 3: Recovery results obtained from the synthetic mixture by applying the proposed analytical methods to the synthetic mixture (n = 6)

Synthetic tablet	$\bar{x} \pm SE$	RSD (%)	Recovery %
10 mg	10.016 ± 0.01	0.29	100.16

Table 4: Recovery results obtained with the standard addition technique (n = 6)

Crestor® tablet (10 mg RC)

Added ((g mL^{-1})	$\bar{x} \pm SE$	RSD (%)	Recovery %
10	10.10 ± 0.08	1.89	101.03
20	20.04 ± 0.04	0.53	100.22
30	30.01 ± 0.03	0.21	100.03

x: Mean ± SE
SE = Standard Error

Table 5: Analysis of the pharmaceutical preparation (tablet) by the developed method (n = 6)

Crestor® tablet (10 mg rosuvastatin calcium)

Calibration curve method	Standard addition method
9.71 ± 0.08	9.95 ± 0.08
9.74 ± 0.08	10.25 ± 0.08
9.99 ± 0.08	10.00 ± 0.08
9.77 ± 0.07	10.00 ± 0.07
9.80 ± 0.07	$10{,}26 \pm 0.07$
10.03 ± 0.07	10.02 ± 0.07
$\bar{x}$: 9.94	$\bar{x}$: 9.98
SD: 0.2	SD: 0.18
SE: 0.08	SE: 0.07
RSD %: 1.99	RSD %: 1.79
$p = 0.249 > p = 0.05$	

x: Mean ± SE
SE = Standard error
RSD %: Relative standard deviation %

The obtained results and statistical data are given in Table 5. The results obtained from calibration curve and standard addition method were compared by the Mann-Whitney U test and no difference was found statistically.

The presented method was found to be simple, accurate, precise, rugged and robust. It can be directly and easily applied to the analysis of the pharmaceutical tablet formulation of RC (Crestor®).

Moreover, the present method is fast and inexpensive in comparison to chromatographic techniques. Therefore, it can be concluded that the proposed method provides an alternative procedure for the quality control of RC in pharmaceutical formulations.

3. Experimental

3.1. Apparatus

The spectrophotometric measurements were carried out using an Aglient 8453 model UV-VIS spectrophotometer with a diode array detector (DAD) (190–1100 nm). UV spectra of reference and sample solutions were recorded in 1 cm quartz cells.

3.2. Chemicals and reagents

RC was kindly supplied by Dr. Reddy's Laboratories. Pharmaceutical preparations of RC was obtained from local pharmacies. Methanol (MeOH) was purchased from Merck. RC was tested for purity by controling its melting point, UV and IR spectra. No impurities was found.

3.3. Standard solutions

Stock solution of RC was prepared at a concentration of 1000 μg mL^{-1} in MeOH. 50.00 mg RC was accurately weighed and transfered to a 50 mL volumetric flask and 30 mL MeOH was added. It was treated in ultrasonic bath for 15 min at 25 °C and then the volume completed with MeOH. This solution was kept at +4 °C maximum for 1 month and the stock solution was stable during this period. Working standard solutions were daily prepared in 10 mL volumetric flask from diluting the stock solution with MeOH.

3.4. Tablet solutions and procedure

Ten tablets of RC (Crestor® 10 mg) were accurately weighed and powdered. The amount equivalent to one tablet was weighed and transfered to a 50 mL volumetric flask and 30 mL MeOH was added. It was treated in an ultrasonic bath for 15 min at 25 °C and then the volume was completed with MeOH. After shaking, part of the flask content was centrifuged at 3500 rpm for 15 min. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with MeOH to give a final concentration of 10 μg mL^{-1}. UV spectra were recorded against MeOH which is used as a blank solution. From calibration curve the amount of one tablet was calculated.

3.5. Synthetic tablet preparations

Pharmaceutical preparations of RC contain 10 mg of standard RC and the following inactive ingredients: microcrystalline cellulose, lactose monohydrate, tribasic calcium phosphate, crospovidone, magnesium stearate, hypromellose, triacetin, titanium dioxide, yellow ferric oxide, and red ferric oxide. For preparing the synthetic tablet these inactive ingredients and standard RC equivalent amount to one tablet were weighed and transfered to a 50 mL volumetric flask and completed to 50 mL as descriped in section 3.4.

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