Extractive Spectrophotometric Methods for the Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations by Using Safranin O and Methylene blue

MAROTHU VAMSI KRISHNA and DANNANA GOWRI SANKAR*

Pharmaceutical Analysis and Quality Assurance Division,
Department of Pharmaceutical Sciences,
Andhra University, Visakhapatnam, India.
E-mail:marothu_vamsi@rediffmail.com

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Abstract: Two simple extractive Spectrophotometric methods are described for the determination of rosuvastatin calcium (RST) in pure form and in pharmaceutical formulations. These methods are based on the formation of ion association complexes of the RST with basic dyes safranin O (Method A) and methylene blue (Method B) in basic buffer of pH 9.8 followed by their extraction in chloroform. The absorbance of the chloroform layer for each method was measured at its appropriate λ_{max} against the reagent blank. These methods have been statistically evaluated and are found to be precise and accurate.

Keywords: Rosuvastatin calcium, Extractive Spectrophotometry, Determination.

Introduction

Rosuvastatin calcium (RST) is an anti hyperlipidemic agent and is chemically known as 6-heptenoic acid, 7-[4-((4-fluorophenyl)-6-(1-methylethyl)-2-[methyl (methylsulphonyl) amino]-5-pyrimidinyl]-3, 5-dihydroxy-, calcium salt (2:1), (3R, 5S, 6E). It acts by inhibiting the enzyme 3-hydroxy-3-methyl glutaryl- coenzyme A (HMG-coA) reductase. To the best of our knowledge, there is no work in the literature reported about the visible spectrophotometric methods for the quantification of RST in pure drug and in pharmaceutical formulations, however one HPTLC¹ and few LC-MS²⁻⁴ methods have been reported for the estimation of RST in pharmaceutical preparations and in biological fluids. The proposed methods are based on the formation of ion association complexes of the RST

with basic dyes safranin O (SFN O) and methylene blue (MTB) in basic buffer of pH 9.8 followed by their extraction in chloroform.

Experimental

Apparatus

All spectral and absorbance measurements were made on a systronic model 106 digital spectrophotometer with 10mm matched quartz cells.

Materials and reagents

All chemicals used were of analytical reagent grade. RST was obtained from Dr.Reddy's labs Hyderabad. Fortius, Rostar and Novostat are the commercial tablet formulations labeled to contain 5, 10 and 20mg of RST per tablet respectively. SFN O (0.2% w/v) solution was prepared by dissolving 200 mg of Safranin O in 100 ml of distilled water. MTB (0.2% w/v) solution was prepared by dissolving 200 mg of Methylene blue in 100 ml of distilled water. Ammonia- ammonium chloride buffer solution (pH 9.8) was prepared by mixing 7g of ammonium chloride with 56.8 ml of liquor ammonia solution and diluted to 100 ml with distilled water and pH was adjusted to 9.8.

Standard solution of RST (1mg/ml) was prepared by dissolving 100 mg of drug in 100 ml of methanol. It was further diluted with methanol to get the working standard solution of 50 μ g /ml.

General procedure and calibration

Different aliquots of standard solution (50 μ g/ ml) from 1.0 -5.0 ml (for method A) or 0.5 - 2.5 ml (for method B) and 1.0 ml of pH 9.8 buffer solutions were placed separately in a series of 125 ml separating funnels. A volume of 1.5 ml of SFN O for method A or 0.5 ml of MTB for method B was added respectively. The total volume in each funnel was adjusted to 10 ml with distilled water. Then 10 ml of chloroform was added to each separating funnel and the contents were shaken for 2 minutes and allowed to separate. The organic layer was collected through cotton plug and the absorbance was measured immediately at 530 nm for method A and at 655 nm for method B against the reagent blank. Both the colored species were stable for 2 hours. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Procedure for tablets

Twenty tablets were weighed accurately and ground in to a fine powder. An amount of powder equivalent to 100 mg of RST was weighed into a 100ml volumetric flask, 50 ml of the methanol was added and shaken thoroughly for about 10 min, then the volume was made up to the mark with the methanol, mixed well and filtered using a quantitative filter paper. The assay of the tablets was completed according to the general procedure.

Results and Discussion

Optical characteristics such as Sandell's sensitivity, molar absorptivity, precision and accuracy were found by performing eight replicate determinations containing 3/4th of the amount of the upper beer's law limits. The measured standard deviation (S.D.), Relative standard deviation (RSD), and confidence limits can be considered satisfactory for both the methods and are summarizes in Table 1. Pharmaceutical formulations of RST were successfully analyzed by the proposed and the reference methods. The results obtained by

proposed and reference methods are presented in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed samples and the mixtures were analyzed by the proposed methods. The ingredients present in the formulations of RST did not interfere with the proposed analytical methods. In conclusion the proposed extractive spectrophotometric methods for the estimation of RST are simple, sensitive, accurate and useful for routine quality control analysis.

Table1. Optical and regression characteristics, precision and accuracy of the proposed methods for RST

Parameter	Method A	Method B
$\lambda_{\max}$ (nm)	530	655
Beer's law limits ($\mu\text{g ml}^{-1}$)	5.0– 25.0	2.5 – 12.5
Detection limits ($\mu\text{g ml}^{-1}$)	0.099	0.076
Molar absorptivity ($\text{L mole}^{-1} \text{cm}^{-1}$)	3.60×10^4	6.54×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$ / 0.001 absorbance unit)	0.027	0.015
Optimum photometric range ($\mu\text{g ml}^{-1}$)	6.0 – 23.0	3.5 – 10.5
Regression equation ($Y = a + bC$)		
Slope (b)	3.61×10^{-2}	6.52×10^{-2}
Standard deviation of slope (S_b)	0.70×10^{-4}	2.00×10^{-4}
Intercept (a)	-3.00×10^{-4}	1.40×10^{-3}
Standard deviation of intercept (S_a)	1.20×10^{-3}	1.67×10^{-3}
Standard error of estimation (S_e)	1.14×10^{-3}	1.59×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)*	0.172	0.108
% Range of error (Confidence limits)*		
0.05 level	0.144	0.090
0.01 level	0.213	0.134
% Error in bulk samples**	-0.260	0.038

*Average of eight determinations

** Average of three determinations

In $Y = a + bC$, Y is absorbance and C is concentration.

Table2. Results of analysis of tablet formulations containing RST

Method	Formulations	Labeled amount (mg)	% Recovery ^a	% recovery of reference method ^b
A	Fortius	5	99.95	99.98
	Rostar	10	100.00	100.35
	Novostat	20	100.08	100.10
B	Fortius	5	100.05	99.98
	Rostar	10	100.15	100.35
	Novostat	20	100.02	100.10

^a Average of six determinations

^b UV method developed in our laboratory.

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