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Characterization and method development for estimation and validation of Rosuvastatin Calcium by UV – visible spectrophotometry

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ABSTRACT

Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Recently, approved doses of the Rosuvastatin have been used for the treatment of dyslipidemia. The site of action of Rosuvastatin is the liver, the target organ for cholesterol lowering. Characterization is done by using HPLC Instrument, UV-Visible spectrophotometer and Fourier transmission Infrared spectrum. Estimation is done by Spectral and absorbance measurements made on Perkin Elmer λ 35 UV-Visible spectrophotometer at 242 nm and results are calculated by using standard formulae. The method was validated in terms of specificity, precision, accuracy.

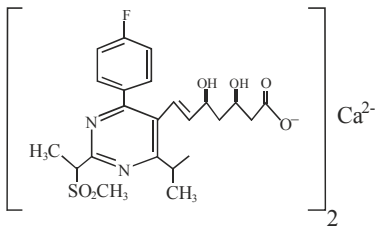
Keywords: Rosuvastatin, cholesterol, HPLC, UV-Visible spectrophotometer, Fourier transmission Infrared spectrum

INTRODUCTION

Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts ***3-hydroxy-3-methylglutaryl coenzyme-A*** to mevalonate, it is early and rate limiting steps in cholesterol biosynthesis[1]. Rosuvastatin calcium was discovered through the synthesis and screening of a series of pyrimidin–substituted 3,5-dihydroxy-6 heptenoates containing a sulphonyl moity introduced to lower lipophilicity and there by improve selectivity for the liver[2]. It is a synthetic enantiomer administered as a calcium salt of the active hydroxy acid active form. Recently approved doses of the Rosuvastatin from 10 to 40 mg/day has been used for the treatment of dyslipidemia [4,5]. The site of action of Rosuvastatin is the liver. Liver is target organ for cholesterol lowering. Rosuvastatin increases the number of hepatic LDL receptor on the cell surface, enhancing uptake and catabolism of LDL and it inhibit the hepatic synthesis of VLDL, thereby reducing the total number of VLDL and LDL particles[6].

Pharmacokinetic study of Rosuvastatin reveals that it has relatively low absolute oral bioavailability. It has been postulated that statins with an absolute bioavailability greater than 50% may show increased risk of muscle and liver toxicity, eg. Cerivastatin with a 60% absolute bioavailability. Peak plasma level of Rosuvastatin accurse at approximately three hours after multiple dosing. Food reduces Rosuvastatin bioavailability by approximately 20%, but extent of absorption is unchanged. Rosuvastatin is strongly and reversibly bound to plasma protein. It has a prolonged effect on hepatic cholesterol synthesis in animal models,

Rosuvastatin Calcium[17] : Chemical name (3R5S,6E) - 7 - [4- (4-flurophenyl)- 6-(1-methylethyl) - 2 - methyl (methysufonyl) amino] - 5-pyrimidinyl]-3,5-dihyroxy-6-heptanoic acid calcium salt (2:1). Trade name: Crestor AstraZeneca, Rosuvas-10 Ranbaxy Ltd and Razel-10 Glenmark pharmaceutical Ltd. Molecular formula: $C_{44}H_{54}CaF_2N_6O_{12}S_2$. Molecular weight**:** 1000.14. Structural formula



Elemental composition (%): **C** 36.36**, H** 44.62, **Ca** 0.82, **F** 1.65, **N** 4.95, **O** 9.91, **S** 1.65.

Rosuvastatin calcium is slightly soluble in water, freely soluble in methanol, acetonitrile & ethanol. The pH of accurately weighed quantity of 100mg Rosuvastatin calcium dissolved in 10ml water was found to be 5.90.

MATERIAL AND METHODS

Rosuvastatin calcium & Ezetimibe received from Dr. Reddy lab Hyderabad & Biocon Ltd. Bangalore respectively were utilized in present study. Chemicals and solvents utilized respectively: Acetonitrile HPLC grade, Qualigence make; Milli-Q grade water; Potassium dihydrogen orthophosphate AR grade, Merck makes; Orthophosperic acid HPLC grade, Merck make; Methanol HPLC grade, Merck make. The instruments, Balance: Metler Toledo AG245 sensitivity –0.001g to 220gm, pH meter: Universal Enterprises with glass electrode, HPLC- separation was carried out on Agilent 1100 series system equipped with Quaternary gradient solvent delivering pump, an automated sample injection device, a Diode array detector with chemstation software, UV-Visible Spectrum- on a Perkin Elmer λ35 UV-Visible spectrophotometer, Fourier transmission Infrared spectrum (FTIR spectrum) on a Perkin Elmer Fourier transmission Infrared spectrophotometer, utilized for the experiment. The Ultraviolet measurement carried out utilizing matched 1 cm quartz cells. The ultraviolet absorption of Rosuvastatin in methanol was scanned from 200 to 400nm. Using methanol as blank, maxima was found at 242.86 nm & minima at 223.65 nm respectively. Percentage transmission at different wave number are given in Table-1 and assignments of the functional group correlated with frequencies are given in Table-2.

Estimation by UV-visible spectrophotometry

Experimental condition

Take the absorbance for standard and sample at 242 nm. The solution of standard and sample were prepared in methanol.

Preparation of solution

Standard solution preparation: Standard solution of Rosuvastatin calcium was prepared by dissolving 20.10 mg of Rosuvastatin calcium in 200ml of methanol to obtain a solution containing 0.1mg/ml. Further diluted 5ml to 50ml with same solvent to obtain a solution containing 10 µg/ml.

Sample solution preparation

Twenty tablets of Rosuvastatin calcium were weighed and powdered. Amount of powdered equivalent to 20 mg taken into a 200ml volumetric flask, 140 ml methanol added to dissolve the drug, cooled the flask up to the room temperature and methanol was added to make up the volume up to the mark, centrifuged at 4000 RPM for 10mins, Further diluted 5ml to 50ml with same solvent to obtain a solution containing 10 µg/ml. Same procedure was used for two other market samples of Rosuvastatin calcium.

Instrumentation

Spectral and absorbance measurements were made on Perkin Elmer λ 35 UV-Visible spectrophotometer with matched 1 cm quartz cells.

Spectral characteristics

In order to ascertain the optimum wavelengths (λ max) of Rosuvastatin calcium, the solution of rosuvastatin in methanol was scanned on a UV-Visible Spectrophotometer in the range of 200-400 nm against the methanol as blank and spectrum for Rosuvastatin calcium was recorded. The λ max was found to be at 242 nm.

Procedure

Absorbance of standard solution and sample solution taken at 242 nm using methanol as a blank and result are recorded in Table-10. Results are calculated by using following formulae.

$$\text{Mg/ tab} = \frac{\text{Abs sample}}{\text{Abs Standard}} \times \frac{\text{Conc. of Std}}{\text{Conc. of Smp}}$$

$$\text{x} \frac{\text{potency}}{100} \text{x Avg wt.}$$

$$\% \text{ Assay as per L.C.} = \frac{\text{Mg/tab}}{\text{Label claim}} \times 100$$

Calculation

For Rosuvas-10 (Ranbaxy Ltd.)

$$\text{Mg/ tab} = \frac{0.480}{0.433} \times \frac{20.10}{311.62} \times \frac{90.20}{100} \times 155.35$$

$$\% \text{ Assay as per L.C.} = \frac{10.02}{10} \times 100 = 100.2\ \%$$

For Razel-10 (Glenmark phar. Ltd)

$$\text{Mg/ tab} = \frac{0.465}{0.433} \times \frac{20.10}{312.20} \times \frac{90.20}{100} \times 158.54$$

$$\% \text{ Assay as per L.C.} = \frac{9.89}{10} \times 100 = 98.9\%$$

Validation of rosuvastatin calcium by UV-visible spectrophotometer

Specificity

Interference of excipients in the determination of Rosuvastatin was studied using above experimental condition. Placebo was prepared in methanol it was found that various excipients present in pharmaceutical preparation do not interfere in the estimation of Rosuvastatin calcium by the above method.

Precision of the method

The precision of the method in the determination of Rosuvastatin calcium was tested by absorbance of the six samples containing 10 µg / ml of Rosuvastatin calcium. The relative standard deviation and the mean percentage are recorded in Table 3.

Accuracy of method (recovery of method)

The accuracy of Rosuvastatin was determined by spiking known amounts of Rosuvastatin calcium using above experimental condition and result are recorded in Table 4 .

RESULTS AND DISCUSSION

Spectrophotometric estimation was carried out by using 10 mm cuvette 242nm. The method was applied to estimate the Rosuvastatin content in two different market samples under the brand name Rosuvas-10 Ranbaxy Ltd, Razel-10 Glenmark pharmaceutical Ltd, the assay found 100.2% and 98.9% as per label claim respectively. The method was validated in term of specificity, precision, accuracy. During spectrophotometric estimation of Rosuvastatin found no interference of exipient, hence, method is specific. The precision was determined by analyzing 6 preparation of homogeneous sample, the mean assay and %RSD was found 99.9% and 0.4 respectively, hence, method is precise. The accuracy of the method was evaluated by carrying recovery study. The recovery was found 100%, 99.9% and 100.8% at a recovery level 50%, 100% and 150% of the optimum concentration respectively; hence, method is capable to recover the Rosuvastatin content at 50% to 150% of optimum concentration.

REFERENCES

[1] Tobert, J.A.Lovastatin and beyond: the history of HMG-CoA reductase inhibitors. *Nature Rev. Drug Discov.* **2**: 517-526 (2003).

[2] Watanabe, M.etal, synthesis and biological activity of methansulfonamide pyrimidine and N-methansulphonyl pyrrole-substituted 3,5-dihydroxy-6-heptanoate, a novel series of HMG-CoA reductase inhibitors. *Bioorg.Med. Chem.* **5**: 437-444 (1997).

[3] Davidson MH Rosuvastatin: a highly efficacious statin for the treatment of

dyslipidamia. *Expert opin Invest Drugs,* **11** (1): 125-141(2002).

[4] Schuster H. Rosuvastatin –a highly effective new 3 hydroxy-3- methylglutaryl coenzyme A reductase inhibitor. A review of clinical trial data at 10-40 mg doses in dyslipidemic patient. Cardiology, **99**(3): 126-139(2003).

[5] Brewer Jr. HB Benefit-risk assessment of Rosuvastatin 10-40 mg. *Am J Cardiol*., **92** (4B): 23K- 29 K.

[6] Physician desk reference, Crestor (AstraZeneca).

[7] White CM. A review of the pharmacologic and pharmacokinetics aspects of Rosuvastatin. *J Clin pharmacol*., **42**: 963-970. 2002.

[8] Crestor (Rosuvastatin calcium) prescribing information.Wilminton, DE. AstraZeneca pharmaceutical LP, August 2003.

[9] Mc Taggart F, Buckett L, Davidson R, Holdgate G,Mccomick A. Schneck D, etal. Preclinical & clinical pharmacology of Rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Am J Cardiol 87 (suppl) : 28B-32B, 2001.

[10] FDA Drugs approval list (cited 26 aug 2003).

[11] AstraZeneca pty ltd. Crestor product information. 11 april 2006.

[12] Wolfe SM. Dangers of Rosuvastatin identified before and after FDA approval. Lancet, **363**: 2189-2190, 2004.

[13] Glason SK, Re: Docket No 200 4p -0113/ CPI. Rockville: Department of health and human service, food and drug administration, 2005.

[14] Crestor: Rosuvastatin calcium-package insert. AstraZeneca 2004.

[15] Davidson MH. Controversy surrounding the safety of cerivastatin. *Exper opin Drug Saf.* 1: 207-212, 2002.

[16] Public Citizens Petition to the FDA.

[17] The Merck index 13th editions publish by Merck research laboratories division of Merck and co., inc. white house statin page no. 3949.

[18] Akmar SK, Kothapalli Lata, Thomas Asha, Jangam Sumitra, Deshpande AD, "Reverse phase high performance liquid chromatography method for estimation of Ezetimibe in bulk and pharmaceutical formulations" **69**: 5, 695-697, 2007.

Table 1. Fourier transmission Infrared % Transmission for Rosuvastatin calcium

Wavenumber	% Transmission	Wavenumber	% Transmission
3401.01	61.20	1605.27	54.57
1437.55	52.53	1336.50	55.60
1068.22	63.66	900.64	66.98
775.84	61.46	518.96	60.81
2929.96	64.51	1547.86	44.86
1381.40	45.88	1228.85	58.55
964.46	54.96	844.11	61.89
575.97	61.92	1509.56	51.58
1154.69	52.07	810.26	66.68

Table 2. Wave number with possible functional group for Rosuvastatin calcium

Wave number (1/λ) in cm^{-1}	Possible functional group
2990-2855 1485-1415	General alkyl group C-H
3450-3300 3000-2850 1470-1365 1200-0950	Alkyl group –hydroxyl or possible amino substituent
1620-1585 1525-1475 1340-1210 880-690	Aromatic compound possibly phenoxy or amino sustituent
3540-3200 1205-0885	Hydroxyl or amino group general R-OH
3650-3175 1635-1565 1525-1460 1390-1330 1280-1150 870-715	Phenyl –general or aryl ether Hydroxyl or amino sustituent

Table 3. Results for estimation of Rosuvastatin by UV-Visible spectrophotometer

S.No.	Standard Absorbance	Sample Absorbance	Standard Weight (mg)	Sample weight (mg)	Average weight (mg)	Result (%)
Rosuvas-10	0.433	0.480	20.10	311.62	155.35	**100.2**
Razel-10	0.433	0.465	20.10	312.20	158.54	**98.9**

Table 4. Precision results for Rosuvastatin by UV-Visible spectrophotometer

S.No.	Standard Absorbance	Sample Absorbance	Standard weight (mg)	Sample weight (mg)	Average weight (mg)	Result (%)
1	0.434	0.481	50.20	778.12	155.35	100.2
2	0.434	0.478	50.20	777.20	155.35	99.7
3	0.434	0.479	50.20	778.50	155.35	99.7
4	0.434	0.48	50.20	776.80	155.35	100.2
5	0.434	0.477	50.20	778.20	155.35	99.3
6	0.434	0.479	50.20	776.50	155.35	100.0
Mean percentage assay						**99.9**
% of RSD						**0.4**

Table 5. Accuracy results for Rosuvastatin by UV-Visible spectrophotometer

S. No.	Level %	Std Absorbance.	Smp Absorbance	Std weight (mg)	Amount spiked (mg)	Amount Found (mg)	Recovery (%)
1	50	0.434	0.216	20.2	10.05	10.05	100.0
2	50	0.434	0.211	20.2	10.01	9.82	98.1
3	50	0.434	0.219	20.2	9.94	10.19	102.5
	Mean						**100.2**
1	100	0.434	0.429	20.2	20.09	19.97	99.4
2	100	0.434	0.423	20.2	20.03	19.69	98.3
3	100	0.434	0.432	20.2	20.11	20.11	100.0
	Mean						**99.2**
1	150	0.434	0.658	20.2	30.12	30.63	101.7
2	150	0.434	0.645	20.2	20.22	30.02	99.3
3	150	0.434	0.652	20.2	30.20	30.05	100.5
	Mean						**100.5**