Sushma Palvai. *et al.* / *International Journal of Pharmacy & Therapeutics*, 4(3), 2013, 182-187.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ROSUVATATIN CALCIUM RELATED SUBSTANCES BY RP-HPLC

Sushma Palvai, Sharath Chandra Seelam, *Dhanalakshmi K, Nagarjuna Reddy

Department of Pharmaceutical Analysis, KLR Pharmacy College,
New Palvoncha, Khammam Dist, Andhra Pradesh – 507115, India.

ABSTRACT

A simple and precise RP-HPLC method was developed for the determination of related substances in Rosuvastatin calcium tablet dosage form. The chromatographic separation was achieved on an kromosil C18 250x4.6mm (5microns) column with mobile phase contain the gradient mixture of the solvent (Water:A:C:N:Methanol:Triethylamine) in the ratio of 450:250:350:1. The eluted compounds were monitored at248nm and the run time was 60 min. the development method separates (two) unknown impurities and rosuvastatin calcium from each other. The calibration curve were linear in the range between 50-200% with correlation coefficient 0.996 and 0.999, %recovery studies were found to be 99.8 and 99.6 for impurity I and II. The development and validated RP-HPLC method is applied for identification of eluted unknown impurities of Rosuvastatin calcium.

Key Words:- Rosuvastatin calcium, RP-HPLC, Method validation, Impurities.

INTRODUCTION

Rosuvastatin (ROSV) is a synthetic lipid-lowering agent, chemically known as (3*R*, 5*S*,6*E*)-7-{4-(4-fluorophenyl) -6- (1- methylethyl) -2- [methyl- [(methyl sulfonyl) amino] pyrimidin-5-yl}-3,5 dihydroxyhept-6-enoic acid calcium salt (2:1). It is used for the treatment of hyperlipidemia and is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis (Harshal KT, Mukesh CP, 2012; Amr MB *et al*., 2011; Alka G *et al*., 2009). ROSV calcium is a salt with pKa of 4.6 and very slightly soluble in aqueous solutions of pH 4.0 and below. It exhibits a high degree of specificity for uptake into the liver and is a potent in vitro and in vivo competitive inhibitor of HMG-CoA reductase (Sakademiai K, 2005; Chirag BP *et al*., 2010; Hasumati AR *et al*., 2009).

The methods for Rosuvastatin calcium related impurities were determined by UPLC but the literature reports do not show any RP-HPLC method for Rosuvastatin calcium related impurities in pharmaceutical formulation, hence there is a need to develop and validate an analytical method for the Rosuvastatin calcium in dosage form (Uyar B *et al*., 2007). Hence the present study aims to develop and validate a method Rosuvastatin calcium tablet dosage form by RP-HPLC respectively (Sandhya D *et al*., 2011; Jamil S *et al*., 2005).

Corresponding Author

Dhanalakshmi K
Email:- dhanadlx@gmail.com

EXPERIMENTAL

Chemicals and reagents

Rosuvastatin calcium standard received as gift sample and rosuvastatin calcium samples (different batches) and potassium hydroxide were purchased from local market, analytical grade solvents and reagents like Methanol(HPLC grade) Acetonitrile(HPLC grade) Glacial

acetic acid(AR grade) Triethyl amine (AR grade), Orthophosphoric acid were received from KLR Pharmacy college. Water (milli pore water) was prepared by using double distillation apparatus.

Instrumentation

An RP-HPLC analysis was performed on Aligent series 1200, a liquid chromatograph is equipped with PDA Detector using chromatographic data software of EZ chrome Elite. The chromatographic separation was achived on kromosil C18 250x4.6mm (5microns) with injection volume of 20microliters using Water: CAN: Methanol: Triethylamine in the ratio of 450:250:350:1 v/v as mobile phase and mix thoroughly, adjust pH to 4.5 with glacial acetic acid. Prior to chromatography the mobile phase was filtered using 0.45 µm membrane filter and degassed by ultrasonic vibrations. Ambient temperature is maintained for Column and sample and the flow rate of the mobile phase was kept at 0.8mL/min with run time of 60 min. All determinations were performed at 248 nm.

VALIDATION

Assay

Preparation of Standard solution

Weigh accurately about 50 mg of Rosuvastatin calcium standard and transfer into a 100mL volumetric flask, add about 50mL of diluents, sonicate to dissolve and make up the volume with diluents, mix well.

Preparation of Sample solution

Weigh accurately about 50 mg of Rosuvastatin calcium test sample and transfer into 100mL of diluents, sonicate to dissolve and make up the volume with diluents, mix well.

System Suitability

Preparation of suitability stock solution

Weigh accurately 25mg of Rosuvastatin calcium Anti Isomer, transferred into 50ml volumetric flask added about 20 ml of diluents and make up the volume, mix well.

Preparation of Working Standard solution

Weigh accurately about 25mg of Rosuvastatin calcium working standard, transferred into 50mL volumetric flask added about 20 ml of diluents and make up the volume, mix well.

Preparation of Impurity 1 stock solution

Weigh accurately 25mg of impurity 1 transfer into 50mL volumetric flask, add 10mL diluent and sonicate to dissolve, make up the volume up to the mark, mix well.

From this transfer 168µL into 100mL volumetric flak containing 20mL of diluents, Make up the volume up to mark, mix well, Such that the resulting solution contains 0.15% IMPURITY 1.

Preparation of Impurity 2 stock solution

Weigh accurately 25mg of impurity 2 transfer into 50mL volumetric flask, add 5mL diluent and sonicate to dissolve, make up the volume up to the mark, mix well.

From this transfer 150µL into 100mL volumetric flak containing 20mL of diluents, Make up the volume up to mark, mix well, Such that the resulting solution contains 0.15% IMPURITY 2.

Limit of Quantification and Limit of Detection

Preparation of LOQ solution

Transfer 12mL of 0.15% IMPURITY 1 solution and 16.7mL of 0.15% IMPURITY 2 solution transferred into 50mL volumetric flask, add 20mL of diluents, and make up the volume up to the mark and mix well.

Preparation of LOD solution

Transfer 3.3ml of LOQ solution into 10mL volumetric flask, make up the volume up to mark with diluents and mix well.

LINEARITY

LOQ solution considered for linearity

Prepare 50%, 100%, 150% and 200% sample solutions each in 3 replicates.

Preparation of 50% Linearity solution

Transfer 42µL of IMPURITY 1 stock solution and 38µL of IMPURITY 2 stock solution into 50 ml volumetric flask, add about 20mL of diluent and make up the volume up to the mark, mix well.

Preparation of 100% Linearity solution

Transfer 84µL of IMPURITY 1 stock solution and 75µL of IMPURITY 2 stock solution into 50 ml volumetric flask, add about 20mL of diluent and make up the volume up to the mark, mix well.

Preparation of 150% Linearity solution

Transfer 126µL of IMPURITY 1 stock solution and 113µL of IMPURITY 2 stock solution into 50 ml volumetric flask, add about 20mL of diluent and make up the volume up to the mark, mix well.

Preparation of 200% Linearity solution

Transfer 168µL of IMPURITY 1 stock solution and 150µL of IMPURITY 2 stock solution into 50 ml volumetric flask, add about 20mL of diluent and make up the volume up to the mark, mix well.

ACCURACY AND PRECISION

Accuracy can be carried out at Limit of Quantification Level.

Preparation of LOQ spiking solution: (prepare in 3 replicates)

Accurately weighed about 12.5mg of test sample and transfer into 25mL volumetric flask, add 10 ml LOQ solution, sonicated to dissolved and made up to the mark, mix well. To this add three different concentrations i.e, 100%, 150%, 200% of working standard stock solution of target concentration level.

Preparation of standard precession solution

Weigh accurately and transfer about 25.00mg of Rosuvastatin Calcium working standard into a 50mL volumetric flask. Add about 5mL of 20mL diluents and sonicate to dissolve. Make up the volume with diluents and mix well.

Preparation of Precision sample solution (Prepare in six replicates)

Transfer carefully different concentrations of tablet content into 6 different 50mL volumetric flask and dissolve in 5mL diluent and make up the volume. Withdraw 10mL aliquot, Filter through 10µm filter and inject into system.

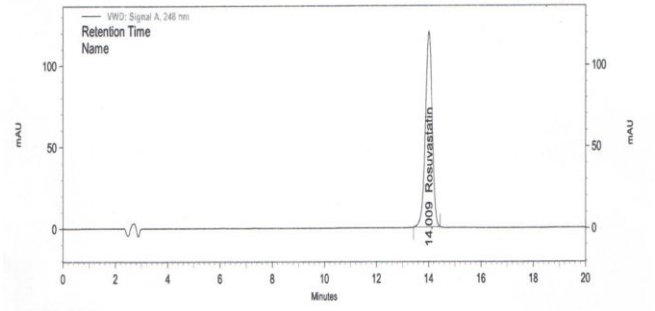
Preparation of precision LOQ solution (Prepare in six replicates)

Transfer 12mL of 0.15% IMPURITY 1 solution and 16.7mL of 0.15% IMPURITY 2 solution transferred into 50mL volumetric flask, add 20mL of diluents, and make up the volume up to the mark and mix well.

RESULTS AND DISCUSSION

Assay

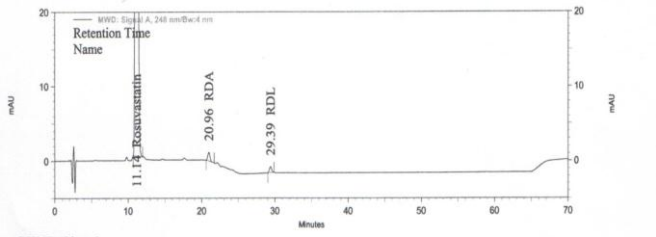
Fig 1. Chromatogram of Rosuvastatin Sample



VWD: Signal A, 248 nm Results

Retention Time	Name	Height	Area	Area %
14.01	Rosuvastatin	2013059	36618370	100.00
Totals		2013059	36618370	100.00

Fig 2. Chromatogram of Rosuvastatin and Related Impurities



MWD: Signal A, 248 nm/Bw:4 nm Results

Retention Time	Name	Height	Area	Area Percent	S/N Ratio
11.14	Rosuvastatin	2956663	43111854	99.80	97546.95
20.96	RDA	2422	51388	0.12	79.91
29.39	RDL	1637	34661	0.08	54.01
Totals		2960722	43197903	100.00	

Fig 3.Impurity 1 Linearity Graph

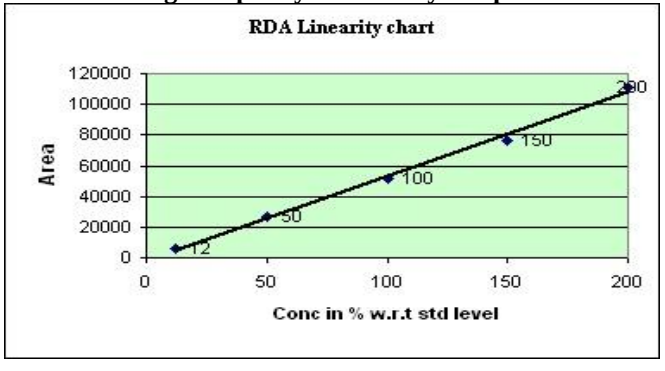


Fig 4. Impurity 2 linearity Graph

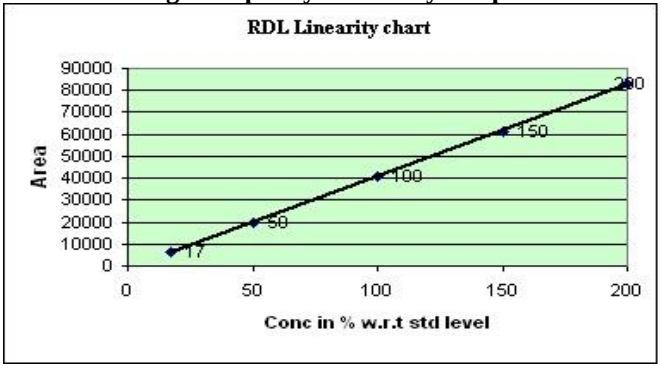


Table 1. System Suitability

Products: Rosuvastatin Calcium						
Parameter : Solution Stability(0.15% Rosuvastatin , Impurity 1 & Impurity 2)						
Hours	Rosuvastatin		IMPURITY 1		IMPURITY 2	
	Area	Area %	Area	Area %	Area	Area %
Initial	70587	43.02	52127	31.77	41355	25.21
44 hrs	71902	43.4	52688	31.8	41077	24.79
Average	**71245**	**43.21**	**52408**	**31.79**	**41216**	**25**
STDV	**930**	**0.27**	**397**	**0.02**	**197**	**0.3**
% RSD	**1.31**	**0.62**	**0.76**	**0.07**	**0.48**	**1.19**

Table 2. System suitability results at different time intervals LOQ

S.No	Compound Name	Area	S/N Ratio	Impurity 1 and 2 conc in % w.r.t to sample conc
1	Impurity 1	6386	10.14	~180 ppm
2	Impurity 2	6142	9.74	~ 250 ppm

LOD

S.No	Compound Name	Area	S/N Ratio	Impurity 1 and 2 conc in % w.r.t to sample conc
1	Impurity 1	1578	2.12	~59 ppm
2	Impurity 2	1586	2.65	~ 83 ppm

Table 3. Precision

S. No	Impurity 1 Area	Impurity 2 Area
1	6015	6182
2	6562	6313
3	6299	6446
4	6303	6131
5	6523	6458
6	6293	6439
AVG	6333	6328
SD	197	144

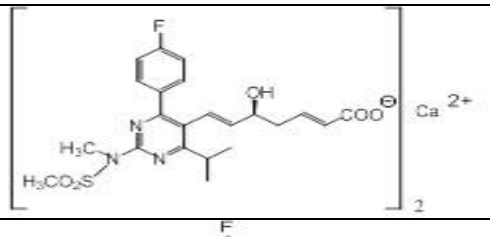
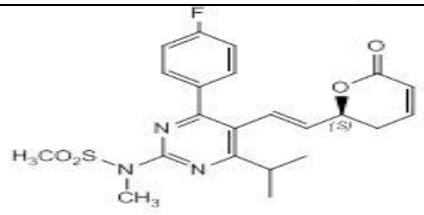
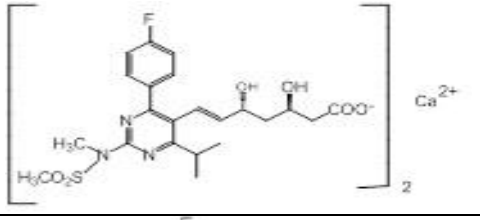
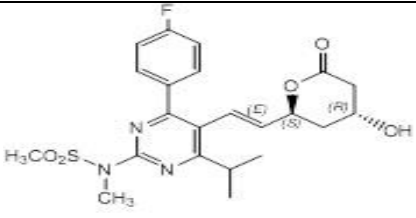
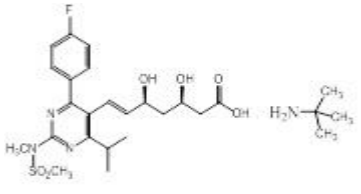
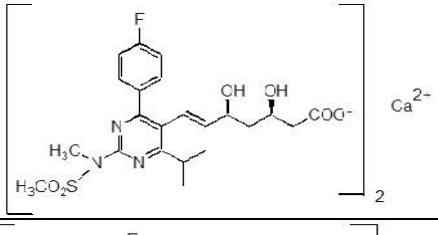
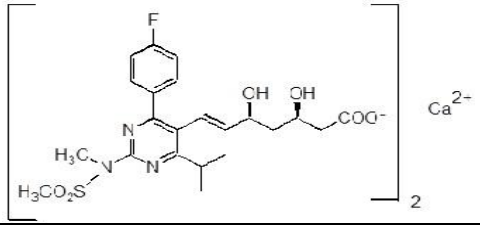
Table 4. Accuracy

S. No	Impurity 1 Area		Impurity 2 Area
1	**LOQ std average**	6292	6314
2	**Sample + LOQ std average**	6282	6117
3	**Sample average**	0	0
4	**Net Area**	6282	6117
5	**Recovery%**	99.8	96.9

Table 5. Linearity

Impurity 1 conc (%) w.r.t standard level	Impurity 2 conc (%) w.r.t standard level	Average area	
		Impurity 1	Impurity 2
12	17	6373	6343
50	50	26491	19464
100	100	51913	40912
150	150	76287	61319
200	200	111188	82971
Correlation	Coefficient	0.9976	0.9999

Table. 6 Structures

Name	R.T	Chemical Name	Structure
Rosuvastatin dehydro acid	20.91	Bis [(S,2E,6E)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl methylsulfonamido)pyrimidin-5-yl)-5-hydroxyhepta-2,6-dienoic acid] calcium salt	
Rosuvastatin dehydro lactone	29.4	(S,E)-N-(4-(4-fluorophenyl)-6-isopropyl-5-(2-(6-oxo-3,6-Dihydro -2H-pyran-2-yl) vinyl) pyrimidin-2-yl)-N-methyl Methane sulfonamide.	
Anti isomer	13.29	((+)-(3R, 5S)-7[4-(4-Fluorophenyl)-6-isopropyl-2-(N-Methyl-N-Methane sulfonyl amino) Pyrimidine- 5-yl]-3,5-dihydroxy-6 (E)-heptenoicacid Calcium salt (2:1)	
Lactone impurity	6.01	[(E)-7-[-[4-(4-fluorophenyl)-6-isopropyl-2-(methyl (methylsulfonyl) amino] pyrimidin-5-yl]-(3R, 5S) 3, 5-di-hydroxyhept-6-enoic acid-(3, 6) lactone.	
5-Ketoacid	4.76	tert. butyl amine salt of (+)-(3R,5S)-7[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl]-3,5-dihydroxy-6(E)- heptenoic acid	
Rosuvastatin dehydro triene	13.9	Bis [(2, 4, 6E)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methylmethylsulfonamido) pyrimidin-5-yl)hepta-2, 4, 6-trienoic acid] calcium salt.	
Rosuvastatin		(3R, 5S)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl]-3, 5- dihydroxy-6(E)-heptenoic acid calcium salt (2:1).	

CONCLUSION

An RP-HPLC technique developed for the analysis of Rosuvastatin calcium and related substances in tablet dosage as per USP, it is simple, accurate, and precise. The assay method is within the limits and fit with system suitability. The method is repeatable and can be used for routine analysis of Rosuvastatin calcium in bulk and in pharmaceutical formulation. Hence it is more effective, sensitive and the IMPURITES profile can be analysed easily through this method.

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