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Validated RP-HPLC method for simultaneous estimation of Rosuvastatin Calcium and Telmisartan in pharmaceutical dosage form

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Abstract

A Simple, accurate and precise reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Rosuvastatin Calcium and Telmisartan in marketed formulation is developed. The determination was carried out on a Inertsil ODS 3V C18 (250 x 4.6 mm, 5 µm) column using a mobile phase of Ammonium Dihydrogen Phosphate (pH 3) Buffer solution: Methanol (65:35v/v, pH 3.0). The flow rate was 1.5 ml/min with detection at 298 nm. The retention time for Rosuvastatin was 6.1 min and for Telmisartan 16.2 min. Rosuvasatin Calcium and Telmisartan showed a linear response in the concentration range of 6-18 µg/ml 24-72 µg/ml respectively. The correlation co-efficient ('*r*' value) for Rosuvastatin Calcium and Telmisartan was 0.9986 and 0.9961 respectively. The results of analysis have been validated as per ICH guidelines and by recovery studies. The percentage recoveries obtained for Rosuvastatin Calcium and Telmisartan ranges from 100.04 to 100.57%.

Keywords : RP-HPLC method, Rosuvastatin Calcium, Telmisartan.

Introduction

Rosuvastatin calcium[1] (Fig 1) and Telmisartan [2] (Fig 2) is a fixed dose combination containing Rosuvastatin 5 mg as Lipid Lowering agent and Telmisartan 10 mg as Anti Hypertensive agent. Chemically Rosuvastatin is bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2[

methyl (methylsulfonyl) amino] pyrimidin-5-yl](3R,5S)3,5- dihydroxyhept-6-enoic acid] calcium salt. Chemically Telmisartan is 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. Pharmacologically Rosuvastatin Calcium is is a lipid lowering agent. It is a competitive inhibitor of HMG-Co A reductase. It catalyses the reduction of 3-hydroxyl-3-methylglutaryl coenzymeA to mevalonate, which is a rate limiting step in hepatic cholesterole synthesis. Mevalonate is a small molecule used in the synthesis of cholesterol and other mevalonate derivatives. In this way, it lowers the amount of cholesterol and LDL- cholesterol. Pharmacologically[3] Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Telmisartan does not inhibit the angiotensin converting enzyme, other hormone receptors, or ion channels. This is a new combination is market and so far no analytical methods have been reported for simultaneous analysis of both the drugs together so following experiment was performed.

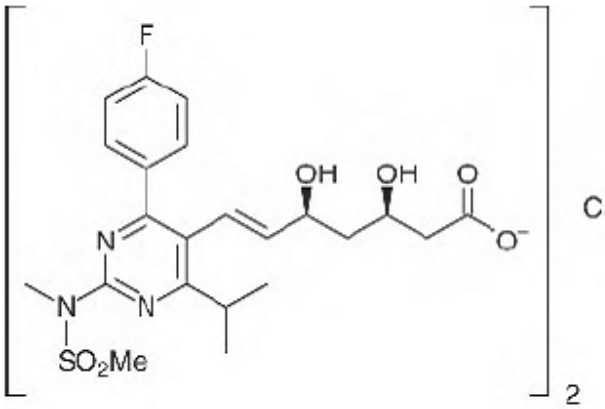


Fig 1 Rosuvastatin Calcium

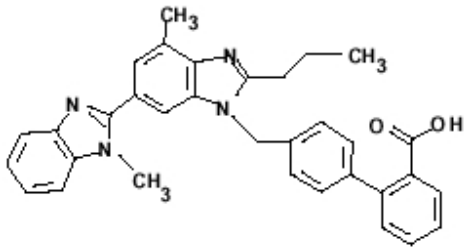


Fig 2 Telmisartan

Experimental Work & Condition: [4-6]-

Materials Used:

Rosuvastatin – Zydus CadilaHealthCare Ankleshwar

Telmisartan - Hetro Drugs Ltd., India.

Chemicals and Reagents:

Diluent: Methanol (HPLC Grade) E. Merck (India) Ltd.,Mumbai

Milli-Q Water: In-house production of company.

Ortho-Phosphoric Acid: AR grade, Spectrochem. India

Apparatus and Equipments

Analytical Balance:

Metler Toledo

pH Meter: Thermo Orion, model 420

Roop Telesonic Ultrasonix

HPLC System

Shimadzu LC 2010 HT

Chromatographic condition

Column	: Inertsil ODS 3V (250 X 4.6 mm), 5µ
Detector	: 298 nm
Injection Volume	: 10 µl
Flow Rate	: 1.5 mL min^{-1}
Temperature	: 40º C
Run Time	: 20 minutes
Mobile Phase	: Buffer: Methanol (65:35)
Diluent	: Methanol

Marketed Preparation:

Rosatel Tab. (Rosuvastatin Calcium and Telmisartan Tablets 10 mg + 40 mg, were procured from Zydus CadilaHealthcare Ltd.)

Materials and Methods

Experimental

Material used

Telmisartan, Active Pharmaceutical Ingredient (API) and working standard was supplied by Cadila Healthcare Limited (Ankleshwar, India). Rosuvastatin Calcium, Active Pharmaceutical Ingredient (API) and working standard was supplied by Dr. Reddy Laboratory (Hyderabad, India) Combination drug product of Telmisartan and Rosuvastatin Calcium was provide by Zydus Cadila Healthcare Limited (Ahmedabad, India)

Buffer preparation

Accurately weighed 4 g Ammonium Dihydrogen Phosphate was dissolved in to 2000 mL Milli-Q water and pH was adjusted to 3.0 with ortho-phosphoric acid[1-2].

Preparation of standard solution

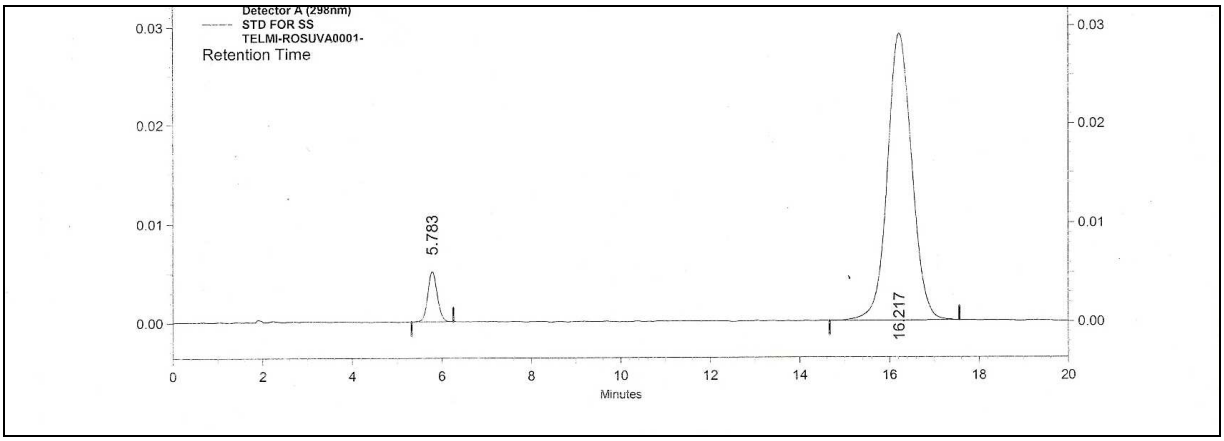
The standard stock solution Rosuvastatin Calcium (200ppm) and Telmisartan (200ppm) respaectively were prepared in 100ml volumetric flask with methanol. Then each 3ml solution were diluted in 50ml volumetric flask to make final standard concentration of Rosuvastatin Calcium and Telmisartan 12 ppm and 48 ppm respectively[3-4].

Preparation of test solution

Accurately 20 intact tablets were weighed and average weight of tablet was calculated. Then tablets were finely crushed and 1335.5 mg powder of tablets equivalent to 10 mg Rosuvastatin and 40 mg Telmisartan and transferred into 100 ml volumetric flask. Then add about 50.0 ml diluent was added and sonicate for 40 minutes with intermittent shaking. Filtered it through 0.45µ (PVDF Millipore Filter).This solution was diluted to make final concentration of test solution 12 ppm Rosuvastatin Calcium and 48 ppm Telmisartan[5-6].

Result and discussion

Literature review reveals only individual methods for estimation of Rosuvastatin Calcium and Telmisartan but no methods were reported for simultaneous estimation of Rosuvastatin Calcium and Telmisartan. So method was developed method more superior than previously published methods of individual estimation of both drugs.. The composition of mobile phase is adjusted to maintain highly accurate and specific results. The detection wavelength of 298nm was chosen in order to achieve a good sensitivity for quantitative determination of Rosuvastatin Calcium and Telmisartan in solid dosage form. The chromatographic separation of Rosuvastatin and Telmisartan in the present combination is shown in figure and separation of active ingradients. The compounds eluted in the order of Rosuvastatin Calcium and Telmisartan with retention time of 5.7 min and 16.2 min respectively. The isocratic program throughout HPLC method was adopted to analyze two components in a single run[7-8].



Method Validation

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1).[21] The developed method validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness and solution stability[9].

System suitability and system precision

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented.

Table 1. General Parameters for of RP HPLC Analysis

Compound	Retention Time	N	R	T	K'
Rosuvastatin Calcium	5.7± 0.0022	4335.33	0.0	1.07	153.4
Telmisartan	16.2 ± 0.0029	8660.77	27.3	0.99	33.90

N= Theoretical plates; R= Resolution; T= Assymetry, K'= Capacity Factor.

Linearity

To achieve linearity and range, stock solution containing 0.200 mg mL^{-1} Rosuvastatin Calcium and 0.200 mg mL^{-1} Telmisartan were diluted to yield solution in the concentration range of µg mL^{-1} and 24-72 µg mL^{-1} for Rosuvastatin Calcium and Telmisartan respectively. The solutions were analyzed by using 10µl into HPLC. The results were presented in Table 2.

Table 2: Results of Linearity

Sr. No.	Parameters (n=5)	Rosuvastatin	Telmisartan
1	Retention Time (min)	5.7	16.2
2	Theoritical Plates	7872	8286.41
3	Asymmetry	1.01	1.02
4	Capacity Factor	151.17	75.17
5	% RSD	0.2	0.1
6	Linearity range	0.528 - 18 µg/ml	1.056 – 72 µg ml
7	Linearity equation	y = 5039.3 x + 10150	y = 21659 x + 13603
8	Correlation coefficient	0.9991	0.9986
9	LOD	0.6 µg/ml	2.4 µg/ml
10	LOQ	1.8 µg/ml	7.2 µg/ml

Table 3: Linearity Data of Rosuvastatin

Linearity Range	Stock solution to be taken in ml	Dilute to volume (ml)with diluent	Final concentration in µg/ml Rosuvastatin	Area
50%	1.50	50	6	40044
75%	2.25	50	9	54665
100%	3.00	50	12	71174
125%	3.75	50	15	84516
150%	4.50	50	18	100708

Table 4: Linearity Data of Telmisartan

Linearity Range	Stock solution to be taken in ml	Dilute to volume (ml) with diluent	Final concentration in µg/ml Telmisartan	Area
50%	1.50	50	24	517386
75%	2.25	50	36	808009
100%	3.00	50	48	1056145
125%	3.75	50	60	1327477
150%	4.50	50	72	1557204

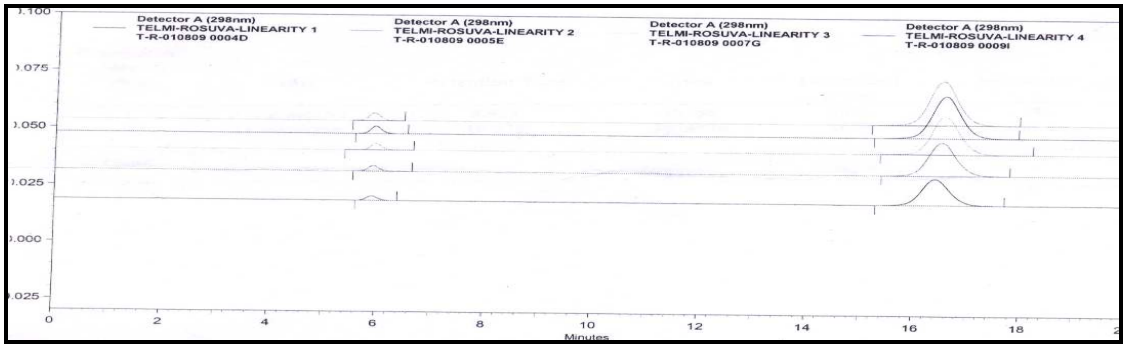
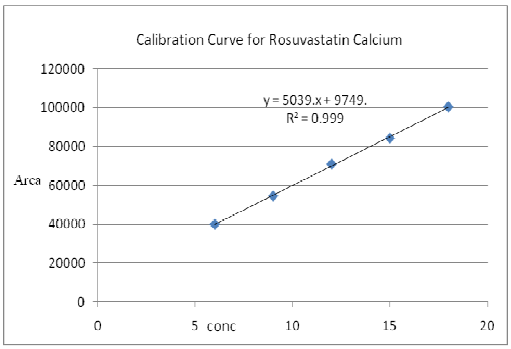
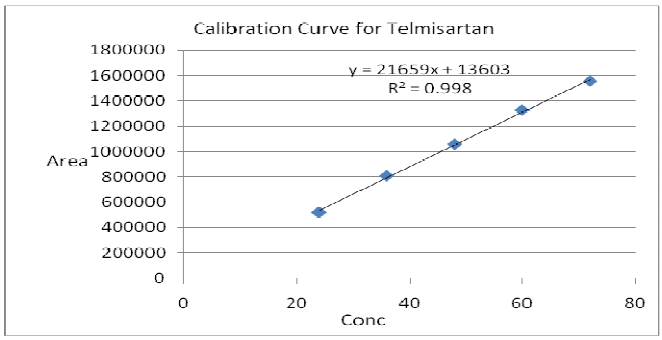


Fig 3 Linearity Chromatograms for Rosuvastatin Calcium and Telmisartan

Fig 4 Linearity and calibration curve.



Rosuvastatin Calcium



Telmisartan

Precision[10]
The method precision was done by preparing six different sample preparations by one analyst under the same condition. The results were presented in Table 3. The results obtained were within 2% RSD.

Ruggedness
Ruggedness test was determined between two different analysts, instruments and Columns. The value of percentage RSD was below 2.0%, showed ruggedness[11] of developed analytical method. The results were presented in Table 5.

Table 5: Results of Method Precision and Ruggedness

Parameters	Telmisartan		Rosuvastatin Calcium	
	%Assay Mean (n=6)	% RSD	%Assay Mean (n=6)	% RSD
Method Precision	99.87	1.2	100.04	0.4
Ruggedness	100.01	0.8	100.03	1.3

Accuracy
The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy[12] was determined at three different level 50%, 100% and 150% of the target concentration in triplicate. The results were presented in Table 4.

Table 6: Results of accuracy

Results of Accuracy Data of Rosuvastatin

For Rosuvastatin Calcium					
Level	**Amount of Drug added (mg)**	**Amount of Drug recovered (mg)**	**Recovery (%)**	**Mean**	**% RSD**
50 %	52.30	53.44	102.2	100.3	0.2
	52.50	52.97	100.3		
	52.40	52.64	100.5		
100 %	104.80	105.54	100.7	100.7	0.1
	104.20	105.12	100.9		
	104.80	105.53	100.7		
150 %	156.30	156.70	100.3	100.1	0.2
	156.10	156.19	100.1		
	156.30	156.18	99.9		

Table 7: Results of Accuracy Data of Telmisartan

For Telmisartan					
Level	**Amount of Drug added (mg)**	**Amount of Drug recovered (mg)**	**Recovery (%)**	**Mean**	**% RSD**
50 %	75.50	76.53	101.4	100.3	0.3
	74.50	74.22	99.6		
	75.20	75.69	100.6		
100 %	150.40	151.68	100.9	100.8	0.2
	149.50	150.35	100.6		
	150.0	151.04	100.7		
150 %	224.80	223.64	99.5	100.2	0.1
	225.50	225.74	100.1		
	216.00	219.22	101.5		

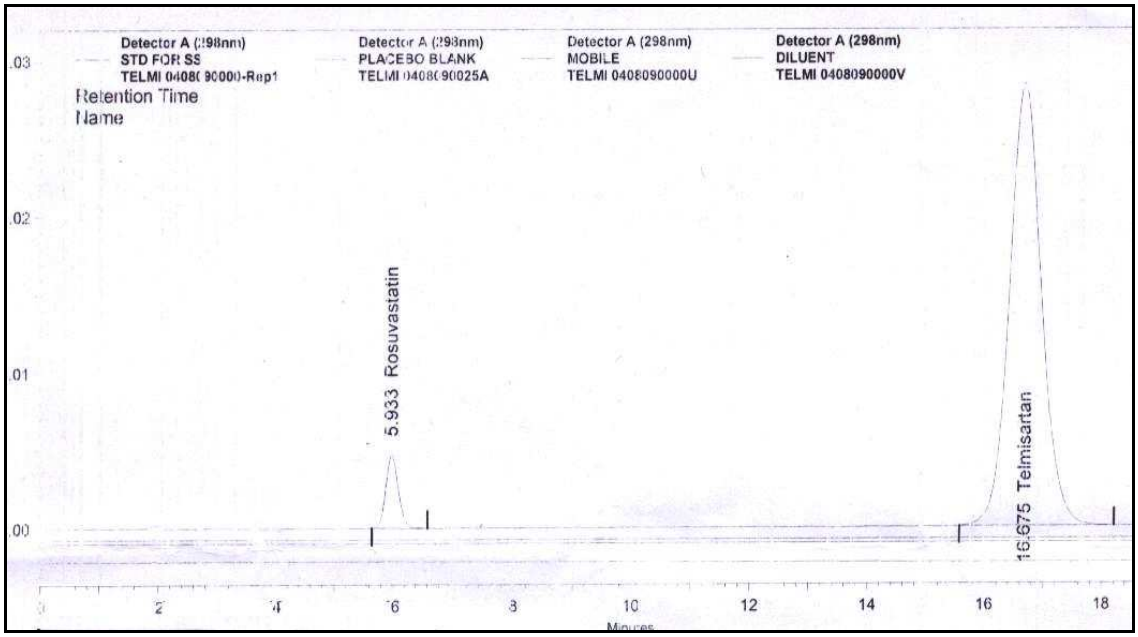


Fig. 5 Overlay chromatogram of Placebo, standard, Mobile and Diluent in specificity study.

Solution stability[13-14]**:**
The standard and sample solutions were found stable up to 24 hours at room temperature. After 4, 8, 12, 16, 20, 24 hours the solutions were analysed. No significant changes (<2%) were observed for the chromatographic responses for the solution analysed, relative to freshly prepared standard. Results related to solution stability are summarized in Table 8.

Table 8: Results of standard and solution stability

Time (hour)	Area		% Difference	
	Rosuvastatin	Telmisartan	Rosuvastatin	Telmisartan
0 (Initial)	70337	1057672	==	==
4	70084	1057215	-0.4	0.1
8	70298	1057197	-0.3	0.1
12	70099	1058281	-0.6	0.1
16	70467	1055380	0.2	-0.2
20	71010	1058984	0.7	0.2
24	71064	1059635	1.1	0.4
% Mean RSD			0.17	0.18

Table 9: Results of Sample Solution Stability

Time (hour)	Area		% Difference	
	Rosuvastatin	Telmisartan	Rosuvastatin	Telmisartan
0 (initial)	70893	1058772	==	==
4	70356	1067215	-0.2	0.4
8	70265	1077187	-0.3	0.6
12	71230	1078282	0.5	0.9
16	71567	1075481	0.7	0.8
20	71310	1078984	0.6	1.2
24	71069	1079635	0.7	1.3
% Mean RSD			0.33	0.86

Robustness

Robustness[15-16] of the method was carried out by deliberately made small change in the flow rate, pH, organic phase ratio and column oven temperature[17-19]. Results were presented in Table 9.

Table 10: Results of Robustness study

Rosuvastatin Calcium Robustness Study

Sr. No	Sys. Suit.	Temp. -5°C	Temp. +5°C	Flow -10%	Flow +10%	Org. -2%	Org. +2%	pH = 3.2	pH = 2.8
1	69342	69784	69847	69652	70289	69265	69265	70693	70754
2	69469	68497	68702	69525	70382	68700	69700	70599	70655
3	69181	69942	69087	69039	70635	69710	68721	70318	70256
4	69219	69191	68582	69869	70174	69110	69110	70289	70231
5	69281	69967	70384	69857	70085	70467	68467	70618	70523
%RSD	0.3	0.7	1.0	0.3	0.5	1.1	0.4	0.4	0.6

Table 11: Results of Robustness Study

Telmisartan Robustness Study

Sr. No	Sys. Suit.	Temp. -5°C	Temp. +5°C	Flow -10%	Flow +10%	Org. -2%	Org. +2%	pH = 3.2	pH = 2.8
1	1057672	1053621	1036265	1036352	1045623	1035264	1032643	1032654	1031526
2	1057215	1056234	1031564	1032563	1032652	1032652	1032652	1032646	1056234
3	1057197	1035621	1032162	1032562	1032642	1032645	1065231	1065895	1032654
4	1058281	1034536	1035623	1032546	1032654	1032654	1032156	1032263	1054652
5	1055380	1023562	1032561	1032564	1032655	1035624	1032642	1031361	1053552
%RSD	0.2	0.3	0.5	0.4	0.6	0.7	0.9	1.1	0.9

Table 12: Summary of Validation Parameters of RP HPLC Method for Simultaneous estimation of Rosuvastatin Calcium and Telmisartan

Parameter	Acceptance Crieteria	Rosuvasatin Calcium	Telmisartan
Range of Linearity Correlation Coefficient	Correlation coefficient $r^2 > 0.999$ or 0.995	0.528 - 18 µg/ml 0.9991	1.056 – 72 µg ml 0.9986
LOD	S/N > 2 or 3	0.6 µg/ml	2.4 µg/ml
LOQ	S/N > 10	1.8 µg/ml	7.2 µg/ml
Precision	RSD < 2%	%RSD = 0.4	%RSD = 1.2
Intermediate Precision	RSD < 2%	%RSD = 1.3	% RSD =0.8
Accuracy	Recovery 98- 102% (individual)	% recovery= 100.5	% recovery = 100.6
Specificity	1) No intereference from blank, placebo and other degradation products with the main peak. 2) The peak purity index > 0.999	No intereference. **Peak purity** 1)Test sample = 0.9992 2)Spiked sample = 0.9998	No intereference. **Peak purity** 1)Test sample = 0.9998 2) Spiked sample = 0.9998
Solution Stability	> 12 hour	Stable up to 24 hour %RSD = 0.3	Stable up to 24 hour %RSD = 0.8
Robustness	RSD NMT 2% in modified condition	Complies	Complies

Conclusion

Thus proposed method was found to be simple, accurate, precise selective and economical for simultaneous routine analysis of Rosuvastatin Calcium and Telmisartan in tablet dosage form

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