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Spectrophotometric absorption factor method development and validation for estimation of Rosuvastatin Calcium and Telmisartan in solid dosage form

Naman Doshi*, Avani Sheth, Tejas Patel, J B Dave and C N Patel

Shri Sarvajanik Pharmacy College, Department of Quality Assurance, Hemchandracharya North Gujarat University, Mehsana, Gujarat, India

ABSTRACT

A Simple, accurate and precise Spectrophotometric method have been developed for estimation of Rosuvastatin Calcium and Telmisartan by absorption factor method. Rosuvastatin calcium & Telmisartan exhibits λmax at 244 nm and 296 nm respectively. Quantitative estimation of Rosuvastatin Calcium was carried out by subtracting the absorption due to Telmisartan at 244 nm using experimentally calculated absorption factor. Beer's law was obeyed for Rosuvastatin Calcium and Telmisartan 2-7 µg/ml and 4-14 µg/ml respectively.The method was validated using ICH Guidelines.

Key words: Absorption factor 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.

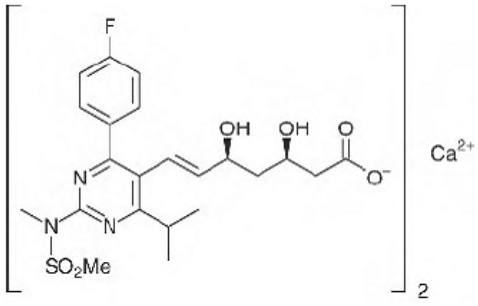


Fig1 Rosuvastatin Calcium

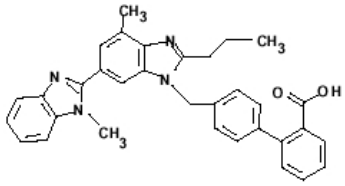


Fig 2 Telmisartan

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.

Experimental Work & Condition:[4-6]

Ultraviolet-visible (UV-Vis) spectrophotometer : Model UV-1700 with Class VP Software.
Materials:

Rosuvastatin Calcium – Zydus CadilaHealthCare
Telmisartan - Hetro Drugs Ltd., India
Water (HPLC Grade) - Milli Q
Diluent: Methanol (HPLC Grade) E. Merck (India) Ltd., Mumbai

Analytical Balance: Model: BP211D, Make: Sartorious Gottingen AG, Germany
Maximum capcacity: 210g
Sonicator: Model: TEC-4, Roop Telesonic Ultrasonix

Marketed Preparation:
Rosatel Tab. (Rosuvastatin Calcium Calcium and Telmisartan Tablets 5 mg + 10 mg, were procured from Zydus CadilaHealthcare Ltd.)

Development of Absorption Factor Method[7-12]:

Selection of solvent for Rosuvastatin Calcium and Telmisartan:
2, 3, 4, 5, 6, and 7 µg/ml solutions of Rosuvastatin Calcium and 4,6,8,10,12, and 14 µg/ml

solutions of Telmisartan were prepared in Diluent and spectrum were recorded between 200-400 nm (Fig 3).

Selection of analytical wavelength for Rosuvastatin Calcium and Telmisartan[14-16]:
For Rosuvastatin Calcium 2, 3, 4, 5, 6 and 7µg/ml solutions of Rosuvastatin Calcium were prepared in Diluent and spectrums were recorded between 200-400 nm. Similarly 4,6,8,10,12, and 14 µg/ml solutions of Telmisartan were prepared in Diluent and spectrums were recorded between 200-400 nm. The overlain derivative spectrums of Rosuvastatin Calcium and Telmisartan at target concentration were recorded (Fig 3).

Preparation of standard stock solution [17-19]:
Rosuvastatin Calcium standard stock solution (200µg/ml):
A 20mg of standard Rosuvastatin Calcium was weighed and transferred to a 100ml volumetric flask and dissolved in 50ml of Diluent. The flask was sonicating for 15min. and volume was made up to the mark with Diluent. From this stock solution working standard solution was prepared by Further 2.5 ml was transferred in 100ml volumetric flask and Diluent was added up to the mark to give a solution containing 5µg/ml Rosuvastatin Calcium.

Telmisartan standard stock solution: (200µg/ml)
A 20mg of standard Telmisartan was weighed and transferred to a 100ml volumetric flask and dissolved in 50ml of Diluent. The flask was sonicating for 15min. and volume was made up to the mark with Diluent. From this stock solution working standard solution was prepared by Further 5.0ml was transferred in 100ml volumetric flask and Diluent was added up to the mark to give a solution containing 10µg/ml Telmisartan.

Construction of Calibration Curve
Calibration Curve Rosuvastatin Calcium

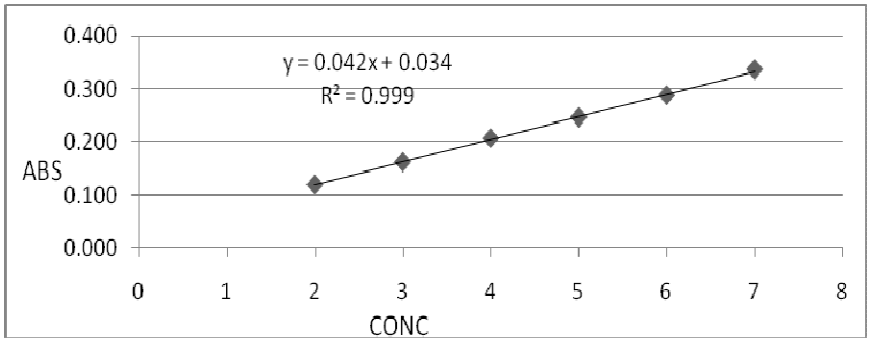
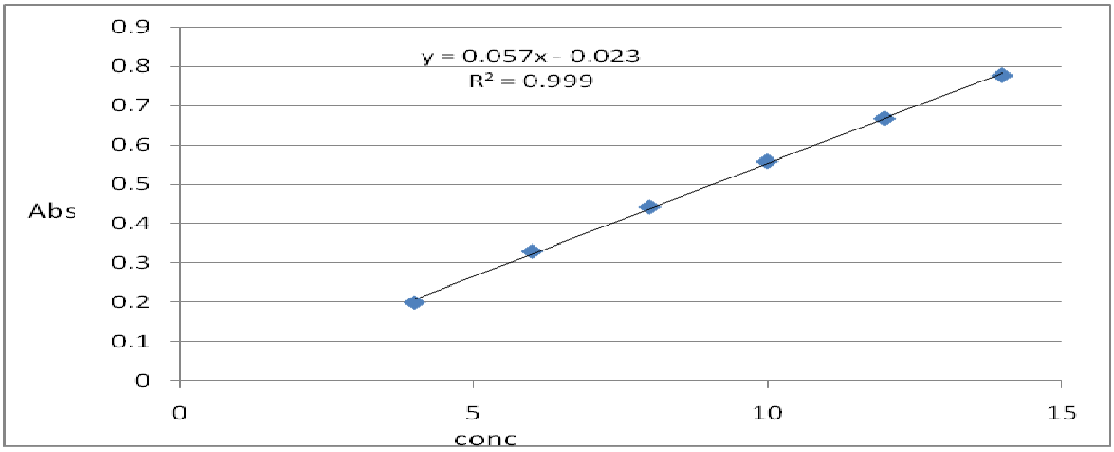


Fig 3 Calibration curve for Rosuvastatin Calcium (2 - 7µg/ml)

Appropriate volume of aliquots from standard Rosuvastatin Calcium stock solution was transferred to different volumetric flasks of 200ml capacity .The volume was adjusted to the mark with the Diluent to obtain the concentration of 2, 3, 4, 5, 6 and 7µg/ml. Calibration curve of each solution against the Diluent was recorded at 244nm was measured and the plot of absorbance v/s concentration was plotted. The straight-line equation was determined. (Figure 3)

Calibration Curve Telmisartan



(Fig 4). Calibration curve for Telmisartan (4 - 14µg/ml)

Appropriate volume of aliquots from standard Telmisartan stock solution was transferred to different volumetric flasks of 200ml capacity .The volume was adjusted to the mark with the Diluent to obtain the concentration of 4, 6, 8, 10, 12 and 14µg/ml. Calibration curve of each solution against the Diluent was recorded at 296nm was measured and the plot of absorbance v/s concentration was plotted. The straight-line equation was determined (Figure 4).

Estimation of Rosuvastatin Calcium and Telmisartan by Absorption Factor

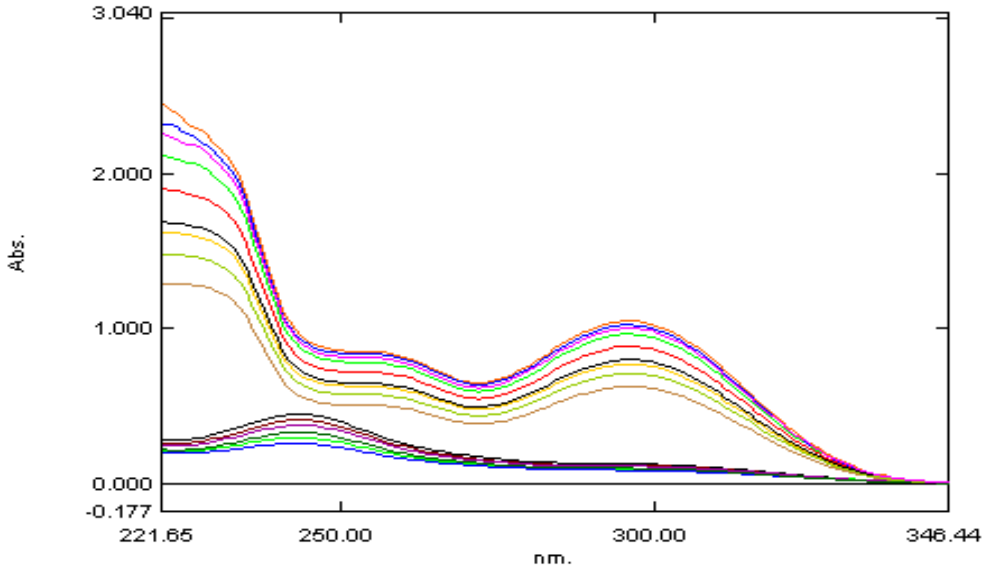


Fig 3 Overlain Spectras of Telmisartan and Rosuvastatin Calcium

Sample preparation

An accurately weighed five intact tablets equivalent to 5 mg of Rosuvastatin Calcium and 10 mg of Telmisartan in to a 100-ml volumetric flask. Add 50 ml of Diluent and sonicate it for 20 min. filter it through 0.45 µm HVLP filter. Transfer 10.0ml of filtrate into 100ml volumetric flask and

add Diluent upto mark to get final concentration of Rosuvastatin Calcium 5µg/ml and Telmisartan 10µg/ml.

Methods:

Rosuvastatin Calcium and Telmisartan solution in diluent of know concentrations were scanned against blank on spectrophotometer. The value of Absorption factor was found to be 1.7456. Quantitative estimation of the Rosuvastatin Calcium and Telmisartan was carried out using following equation.

Absorption of Rosuvastatin Calcium at 244 nm=

$$\text{Abs 244 (Ros+Tel)} - \frac{\text{Abs 244(Tel)}}{\text{Abs 296 (Tel)}} * \text{Abs296 (Ros+Tel)}$$

Abs: Absorption Value.
Ros: Rosuvastatin Calium.
Tel: Telmisartan.

Validation of Absorption Factor method:

Accuracy:

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy is performed at three levels 50, 100 and 150%. Percentage recovery and low relative standard deviation value show's accuracy of the spectrophotometric method (Table: 1-2).

Table 1 Data derived from the Accuracy of Experiment for Rosuvastatin Calcium

Levels	Sets	Absorbance	Mg Added	Mg Added (Actual)	Mg Recovered	% Recovery	Mean Recovery	% RSD
50%	1	0.140	2.50	2.48	2.48	100	99.8	0.4
50%	2	0.140	2.45	2.46	2.46	100		
50%	3	0.141	2.60	2.55	2.50	99.5		
100%	1	0.246	5.10	5.08	5.00	99.3	99.5	0.3
100%	2	0.246	5.20	5.15	5.10	99.5		
100%	3	0.246	5.10	5.08	5.05	99.8		
150%	1	0.358	10.20	10.18	10.18	100	100.5	0.6
150%	2	0.358	10.10	10.08	10.18	101		
150%	3	0.358	10.20	10.18	10.18	100		

Table 2 Data derived from the Accuracy of Experiment for Telmisartan

Levels	Sets	Absorbance	Mg Added	Mg Added (Actual)	Mg Recovered	% Recovery	Mean Recovery	% RSD
50%	1	0.255	5.10	5.05	5.00	99.5	99.5	0.6
50%	2	0.256	5.10	5.00	4.96	99.3		
50%	3	0.255	5.20	5.10	5.08	99.8		
100%	1	0.558	10.2	10	10.00	100	100.1	0.4
100%	2	0.558	10.1	10	10.00	100		
100%	3	0.557	10.1	10	10.10	100.5		
150%	1	0.846	15.1	15.05	15.00	100	100	0.1
150%	2	0.845	15	15	15.00	100		
150%	3	0.845	15.1	15	15.02	100.1		

Precision:

The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectrophotometric (Table: 3-4).

Table 3 Data derived from the Precision of the Experiment for Rosuvastatin calcium

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay	Mean % Assay	% RSD
1	620.40	0.250	5.05	100.05	100.2	0.5
2	618.80	0.245	5.00	100.00		
3	622.50	0.255	5.10	100.10		
4	621.20	0.260	5.15	100.15		
5	626.50	0.270	5.25	100.50		
6	619.90	0.265	5.20	100.40		

Table 4 Data Derived from the Precision of the Experiment for Telmisartan

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay	Mean % Assay	% RSD
1	620.40	0.560	10.52	101	101.6	1.2
2	618.80	0.570	10.63	101.7		
3	622.50	0.575	10.72	101.7		
4	621.20	0.580	10.84	102		
5	626.50	0.590	10.92	102.1		
6	619.90	0.558	10.10	100		

Method ruggedness:

Ruggedness test was determined between two different days, analysts and instruments. The value of RSD was to be found below 2.0% showed ruggedness of developed spectrophotometric method (Table: 5-6).

Table 5 Data Derived from the Intermediate Precision of the Experiment for Rosuvastatin calcium

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay	Mean % Assay	% RSD
1	625.48	0.240	4.95	99.50	99.10	1.3
2	618.53	0.235	4.90	99.10		
3	623.70	0.245	5.00	100.00		
4	622.20	0.230	4.85	98.35		
5	623.50	0.238	4.92	98.65		
6	629.90	0.245	5.00	100.00		

Table 6 Data Derived from the Intermediate Precision of the Experiment for Telmisartan

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay	Mean % Assay	% RSD
1	625.48	0.560	10.52	101	101.6	1.7
2	618.53	0.570	10.63	101.7		
3	623.70	0.575	10.72	101.7		

4	622.20	0.580	10.84	102		
5	623.50	0.590	10.92	102.1		
6	629.90	0.558	10.10	100		

Linearity and Range:

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within the given range. The range of the analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of accuracy and linearity (Table:7-8).

Table 7 Data Derived from the Linearity of the Experiment for Rosuvastatin Calcium

ML Added	Diluted to (ml)	Concentration (ppm)	Absorbance
1.0	100	2	0.120
1.5	100	3	0.162
2.0	100	4	0.207
2.5	100	5	0.246
3.0	100	6	0.288
3.5	100	7	0.336

Table 8 Data Derived from the Linearity of the Experiment for Telmisartan

ML Added	Diluted to	Concentration (ppm)	Absorbance
0.20	100	4	0.198
0.30	100	6	0.329
0.40	100	8	0.442
0.50	100	10	0.558
0.60	100	12	0.668
0.70	100	14	0.758

Specificity and Selectivity:

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a preweighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs (Table:9-10).

Table 9 Data Derived from the Specificity of Experiment for Rosuvastatin Calcium

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay
Set 1	618.60	0.250	5.05	100.5%
Placebo Spiked	605.10	0.240	4.98	99.5%

Table 10 Data Derived from the Specificity of Experiment for Telmisartan

Set	Test Wt. (Mg)	Test Reading	Mg/Tab	% Assay
Set 1	618.60	0.558	10.10	101%
Placebo Spiked	605.10	0.561	10.20	101.5%

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 48 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 48 h at room temperature **(Table:11)**

Table 11 Data Derived from Solution Stability of the Rosuvastatin & Telmisartan

ROSUVASTATIN CALCIUM						TELMISARTAN					
STANDARD SOLUTION			SAMPLE SOLUTION			STANDARD SOLUTION			SAMPLE SOLUTION		
Time Hrs.	Abs	% Diff	Time Hrs.	Abs	% Diff	Time Hrs.	Abs	% Diff	Time Hrs.	Abs	% Diff
Initial	0.245	-------	Initial	0.246		Initial	0.558	0.0	Initial	0.558	0.0
2	0.245	0.0	2	0.245	-0.70	2	0.558	0.0	2	0.558	0.0
4	0.245	0.0	4	0.245	-0.70	4	0.558	0.0	4	0.558	0.0
6	0.245	0.0	6	0.245	-0.7	6	0.558	0.0	6	0.558	0.0
8	0.245	0.0	8	0.245	-0.7	8	0.558	0.0	8	0.558	0.0
10	0.246	0.7	10	0.246	0.0	10	0.558	0.0	10	0.558	0.0
12	0.246	0.7	12	0.246	0.0	12	0.558	0.0	12	0.558	0.0
14	0.246	0.7	14	0.246	0.0	14	0.558	0.0	14	0.561	1.3
16	0.248	1.2	16	0.248	1.2	16	0.557	-0.5	16	0.562	1.7
18	0.248	1.2	18	0.248	1.2	18	0.559	0.5	18	0.562	1.7
20	0.248	1.2	20	0.248	1.2	20	0.559	0.5	20	0.562	1.7
22	0.249	1.6	22	0.249	1.7	22	0.560	1.2	22	0.562	1.7
24	0.249	1.6	24	0.249	1.7	24	0.560	1.2	24	0.562	1.7

Table 12: Summary of Validation Parameters of Rosuvastatin Calcium and Telmisartan by Absorption Factor Method

Parameter	**Acceptance Crieteria**	**Rosuvstatin Calcium**	**Telmisartan**
Linearity Range Correlation Coefficient	Corelation coefficient r^2 > 0.999 or 0.995	2-7 µg/ml r^2 = 0.9990	4-14 µg/ml r^2 = 0.9997
Precision	RSD < 2%	%RSD = 0.5	%RSD = 1.2
Intermediate Precision	RSD < 2%	%RSD = 1.3	%RSD = 1.7
Accuracy	Recovery 98- 102% (individual)	% Recovery = 99.5-100.5	% Recovery = 99.5 - 100.1
Specificity	1) No intereference from blank, placebo.	No interference **% Assay** 1) Test sample = 100.5 2) Spiked sample= 99.5	No interference **% Assay** 1) Test sample = 100 2) Spiked sample= 101.5
Solution Stability	> 12 hour	Stable up to 24 hour %RSD = 1.7	Stable up to 24 hour %RSD = 1.7

RESULTS AND DISCUSSION

The proposed analytical method is simple, accurate and reproducible. Rosuvastatin Calcium and Telmisartan showed λ_{max} at 244 nm and 296nm respectively. As their λmax differ more then 20nm, absorption factor method was tried for their simultaneous estimation in formulation. Telmisartan also showed absorbance at 244nm and give interference in determination of Rosuvastatin Calcium. Quantitative estimation of Rosuvastatin Calcium was carried out by subtracting interference of Telmisartan using experimentally calculated absorption factor.

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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