

## RP-HPLC Method Development and Validation for the Simultaneous Determination of Telmisartan and Atorvastatin in Bulk and Pharmaceutical Dosage form



### Medical Science

**KEYWORDS :** Telmisartan and Atorvastatin Tablets, RP-HPLC, Validation and Stability Indicating

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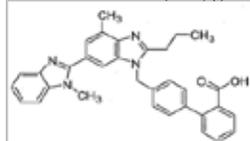
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### ABSTRACT

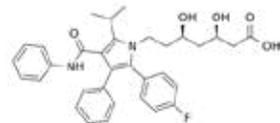
A simple, accurate, precise RP-HPLC method has been developed and validated for the simultaneous estimation of Telmisartan and Atorvastatin in fixed-dosage formulation. The separation was achieved on a C-18 reversed phase column (ACE 5, C-18, 150mm x 4.6mm, 5 $\mu$ ) using Acetonitrile: water: Orthophosphoric acid (95:5:01 v/v) as mobile phase-B & Acetonitrile: water: Orthophosphoric acid (5:95:01 v/v) as mobile phase-A at a flow rate of 1.5mL/min and temperature of 25°C. The UV detection was carried out at 254nm. The retention time of Telmisartan and Atorvastatin was found to be 9.08 and 14.68 min. respectively. The method has been validated for Specificity, Linearity, Accuracy, Precision and Robustness. The calibration curve for Telmisartan and Atorvastatin were linear from the range of 20.01-120.05  $\mu$ g/mL and 2.5-14.97 $\mu$ g/mL respectively. The mean recoveries obtained for Telmisartan and Atorvastatin were 99.9% and 99.2% respectively. The developed method was found to be Specific, Accurate, Precise, Robust and rapid for the simultaneous estimation of Telmisartan and Atorvastatin in Bulk & Tablets 40mg/10mg & 80mg/10mg.

### INTRODUCTION

Telmisartan<sup>1</sup> chemically 4'-[(1, 4'-Dimethyl-2'-propyl-[2, 6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1, 1'-biphenyl]-2-carboxylic acid [Figure 1], is a nonpeptide angiotensin-II receptor antagonist, Atorvastatin calcium chemically<sup>2</sup> [R-(R\*, R\*)]-2-(4-fluorophenyl)- $\beta$ ,  $\delta$ , dihydroxy-5-(1-methyl ethyl)-3-phenyl-4 [(phenyl-amino)-carboxyl]-1 H-pyrrole-1-heptanoic acid calcium salt [Figure 2] is a second generation synthetic 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, Literature survey reveals several methods for determination of Telmisartan and Atorvastatin individually in biological fluids and formulation like HPLC, TLC-densitometric, and derivative spectrophotometer 3-8HPLC and HPTLC methods were reported for determination of Telmisartan and Atorvastatin in combination. However, so far, no method was reported using only polar solvents range of pH2.5 $\pm$ 0.05 with RP-HPLC method for simultaneous determination of Telmisartan and Atorvastatin in bulk and pharmaceutical dosage form. The present developed HPLC method is simple, precise and accurate for simultaneous determination of both drugs in their Pharmaceutical Dosage forms as per International Conference on Harmonization (ICH) guidelines 9



**Figure 1: Structure of Telmisartan (TEL)**



**Figure 2: Structure of Atorvastatin (ATR)**

### METHOD AND MATERIALS

#### Chemicals and Reagents:

Telmisartan and Atorvastatin drug substance and impurities generously sponsored by Vendor, India. Commercially available TEL and ATR tablets were procured from the local market. Orthophosphoric acid (88%) were used of Analytical grade. HPLC grade Methanol, Acetonitrile (Merck) was used. Milli-Q water was used for preparation.

#### Instrumentation:

A Chromatographic system, used for method development and method validation was Waters-Alliance HPLC equipped with separation module consisting of Binary gradient pump, Auto Sampler, thermostatted column compartment, Photo diode array detector (Model 2996), Computer with windows based Empower software.

#### Chromatographic conditions:

##### Table No1- for Chromatographic conditions with Gradient Programme

Column	ACE 5 C18 ,(150 X 4.6)mm ,5 $\mu$ m.		
Flow Rate	1.5 mL/min		
Wavelength	254nm		
Column Temp	25°C		
Sampler Temp	25°C		
Run Time	20 minutes		
Injection Volume	10 $\mu$ L		
Gradient Programme	Time	Mobile Phase-A	Mobile Phase-B
	0.01	95	5
	8.0	65	35
	12.0	50	50
	15.0	5	95
	15.1	95	5
	20.0	95	5

#### Preparation of diluent:

Methanol:Acetonitrile and Water in this ratio50:45:5%v/v.

**Preparation of Standard Solution**

Accurately weigh and transfer about 80 mg of TEL and about 10 mg of ATR standards into a 100 mL volumetric flask, add 50 mL of methanol and sonicate to dissolve and dilute with methanol to volume, and mix well. Further 5 mL to 50 mL volumetric flask dilute with diluent.

**Preparation of sample solution for 40/10mg & 80/10 mg Strengths**

Weigh transfer tablet powder equivalent to one dose into a 100 mL volumetric flask. Add 50 mL of methanol and sonicate for 45 minutes with intermediate shaking. And add 25 mL of diluent and sonicate for 15 minutes with intermediate shaking and dilute to volume with diluent and mix well. Centrifuge a portion of the solution with lid at 5000 RPM about 10 minutes. Pipette out 5 mL of above clear centrifuged solution into a 50 mL volumetric flask, dilute to volume with diluent and mix well.

**RESULTS AND DISCUSSION**

**Method Development:**

The HPLC procedure was optimized with a view to develop an assay method, Pure drug along with its available pharmaceutical dosage form were injected and run in different solvent systems. Initially run by isocratic than gradient was chosen for better resolution between two actives, finally several logical trails was made than achieved good peak symmetry, resolution and theoretical plates for actives with acceptable retention time and method has been finalized.

**Method validation:**

Telmisartan and Atrovastatin tablets are available in different strengths such as 40/10mg & 80/10mg tablets. However 80/10mg strength was considered for entire validation experimentation. The developed method was validated for Specificity, Precision, Linearity, Accuracy, Solution Stability and Robustness as per ICH recommendation.

**Specificity-Blank and Placebo interference**

To establish the interference of placebo, study was conducted. Assay was performed on placebo in duplicate equivalent to test concentration. Blank and Placebo chromatograms solutions showed no peaks at the retention time of TEL and ATR peak. This indicates that method is specific. The chromatogram of placebo, and standard shown in Fig 3, 4 and 5.

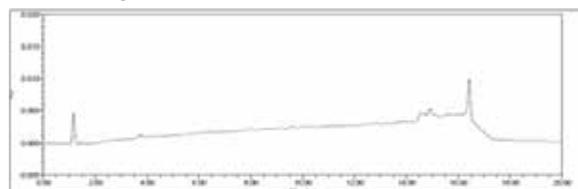


Figure 3: Chromatogram of placebo

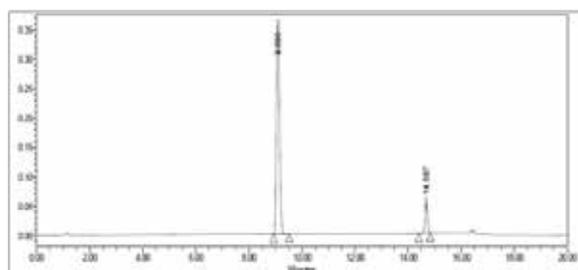


Figure 4: Chromatogram of Standard

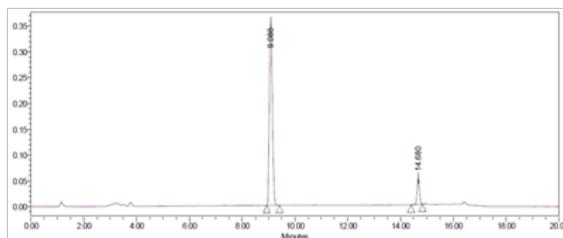


Figure 5: Chromatogram of sample

**Linearity:**

Linearity was studied by plotting a graph of concentration versus response and determining the correlation coefficient, slope and Y-intercept. A series of solutions were prepared using TEL and ATR standard at concentration levels from 25% to 150% of test concentration (80/10mg). Linearity results obtained are presented in Table 2. The linearity was found to be linear with a correlation coefficient of 0.999 & 0.999 respectively, linearity graph of TEL and ATR shown in Fig 6 & 7.

Table 2. Linearity Study for TEL and ATR

Level	TEL		ATR	
	Concentration (µg/mL)	Response	Concentration (µg/mL)	Response
25%	20.01	711408	2.50	81237
50%	40.02	1408461	4.99	162474
80%	64.03	2253955	7.98	259959
90%	72.03	2568366	8.98	294454
100%	80.03	2822537	9.98	324949
120%	96.04	3395018	11.98	389938
150%	120.05	4218877	14.97	487423
Slope	35169		32569	
Intercept	9746		193	
%Y-Intercept	0.3		0.1	
Correlation Coefficient	0.99994		0.99992	

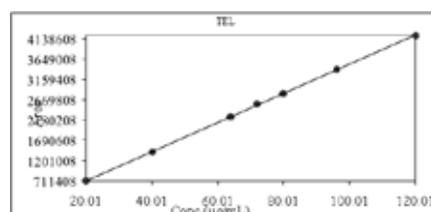


Figure 6: Linearity Graph of Telmisartan

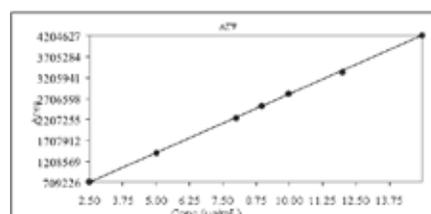


Figure 7: Linearity Graph of Atrovastatin

**Method Precision and Intermediate Precision:**

The Method precision was established by conducting assay in six samples of TEL and ATR tablets. The average % assays & %RSD of TEL and ATR tablets were found to be 99.9 & 99.6 and 0.72 & 0.61 respectively. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument. The results were given in Table 3.

**Table 3. Precision data for TEL and ATR**

Sample No.	TEL		ATR	
	% Assay in Method precision	% Assay in Intermediate precision	% Assay in Method precision	% Assay in Intermediate precision
1	99.7	100.9	100.6	101.5
2	100.6	98.9	99.5	98.4
3	99.0	100.1	99.1	99.2
4	99.5	99.6	99.1	98.7
5	100.9	99.2	100.1	99.3
6	99.6	99.7	99.3	99.0
Average(n=6)	99.9	99.7	99.6	99.3
%RSD (n=6)	0.72	0.71	0.61	1.11
%RSD(n=12)	0.65		0.91	

**Accuracy:**

A known amount of TEL and ATR drug is added to the placebo at 50% (respect to lower strength), 100% and 150% of the analyte concentration. Sample solutions were prepared in triplicate for each spike level and assay was determined as per proposed method. The observed recovery results were found in the range between 98 to 102% for TEL and 98 to 102% for ATR, demonstrating that the method is accurate within the desired range. The results were given in Table 4 & 5.

**Table 4 Accuracy data of Telmisartan**

S No.	% Level	Amount added(mg)	Amount recovered(mg)	% Recovery	% Mean recovery	% RSD
1.	25	20.125	20.246	100.6	100.2	0.4
2.		20.145	20.125	99.9		
3.		20.042	20.082	100.2		
1.	100	80.016	79.456	99.3	99.6	0.3
2.		80.115	79.795	99.6		
3.		80.017	79.937	99.9		
1.	150	120.03	120.15	100.1	99.8	0.4
2.		120.19	119.47	99.4		
3.		120.10	119.86	99.80		

**Table 5 Accuracy data of Atorvastatin**

S. No.	% Level	Amount added(mg)	Amount recovered(mg)	% Recovery	% Mean recovery	% RSD
1.	50	5.1254	5.1049	99.6	99.0	0.6
2.		5.1453	5.0630	98.4		
3.		5.0421	4.9917	99.0		
1.	100	10.016	9.826	98.1	99.1	1.0
2.		10.115	10.034	99.2		
3.		10.017	10.017	100.0		
1.	150	15.036	15.05	100.1	99.6	0.6
2.		15.198	15.03	98.9		
3.		15.142	15.11	99.8		

**Solution Stability:**

Standard and Sample Solutions were prepared as per proposed method and analyzed initially and at different time intervals by keeping the solutions at Room Temperature (~ 25°C) for 96 hours. % Difference between the assays obtained for TEL and ATR, initial and different time interval should not be more than 2.0. From the results it can be concluded that the Standard and Sample Solutions are stable up to 48 hours at room temperature (~ 25°C).

**Robustness:**

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.1 mL min<sup>-1</sup>), wavelength (altered by ±5 nm), variation in mobile phase composition (±0.2% absolute), Column Oven temperature (±5°C), standard solution was prepared and injected into HPLC system. The system suitability parameters were evaluated. In all the cases, the %RSD obtained was less than 1. From the above study the proposed method was found to be robust.

**CONCLUSION**

The HPLC method developed is accurate, precise, reproducible, and specific there is allowable variation in flow rate, temperature, pH, and mobile phase composition which indicate that method is robust enough. The low RSD value for percent assay of test preparation revealed that the proposed method is rugged and can be applied for the routine and less cost effective method for Bulk and dosage form.

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