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# Development and validation of reversed-phase HPLC method for Simultaneous Estimation of Atorvastatin calcium and Telmisartan in Tablet dosage form

**Suresh Kumar GV [1]*, Rajendraprasad Y[2], Chandrashekar SM[1]**

**[1]St Johns Pharmacy College,Department of Medicinal Chemistry,6, 2nd stage Vijaynagar, R.P.C.Layout,Bangalore-560040,Karnataka,India**

**[2]Department of Pharmaceutical Sciences,Andhra University,Visakhapatnam-530002Andra Pradesh India**

***Corres. Author:gvsureshkumar@yahoo.com***
***Telephone: +91-80-23190191***
***Mobile Phone: +91-9886104039***
***Fax: +91-80-23350035***

**Abstract:** A simple, precise and accurate reversed-phase liquid chromatographic method has been developed for the simultaneous estimation of atorvastatin calcium and telmisartan in tablet formulations. The chromatographic separation was achieved on (Waters symmetry C18, 250mm x 4.6mm, 5µ) analytical column. A mixture of ammonium acetate (0.02M, pH 4.0 adjusted with glacial acetic acid) and acetonitrile in ratio (40:60 v/v) at flow rate of 1.0ml/min and detector wavelength 254 nm. The retention time of atorvastatin calcium and telmisartan was found to be 4.6 and 6.1 minutes respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both atorvastatin calcium and telmisartan. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.
**Key words:** Telmisartan, Atorvastatin calcium, RP-HPLC, validation, assay

## 1. Introduction

Atorvastatin calcium (βR,αR)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole- 1-heptanoic acid as the calcium salt belongs to the group of statins. All statins, including atorvastatin reduce the production of cholesterol in the liver by the competitive inhibition of 3-hydroxy- 3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in the biosynthesis of cholesterol [1].

Telmisartan is chemically described as 4[(1,4-dimethyl-2-propyl(2,6-bi-1H-benzimidazol]-1-yl)methyl] [1,1-biphenyl]-2-carboxylic acid [2]. Telmisartan is recently introduced as angiotensin II receptor antagonist for the treatment of essential hypertension [3, 4]. It is useful in treatment of mild to moderate hypertension and well tolerated with lower incidence of cough than ACE inhibitors [5].

A novel formulation commercially available in combination of telmisartan and atorvastatin calcium is intended for prophylaxis or in the treatment of cardiovascular, cardiopulmonary, pulmonary renal diseases by improving endothelial function of organs, tissues and vessels when indication requires in blood pressure and lipid level control [6].

Literature review reveals various methods for determination of atorvastatin calcium and telmisartan individually and in combination with other drugs.

Analytical methods for atorvastatin calcium usually combine reversed phase chromatographic methods and UV detection at characteristic absorption maxima or different modes of MS detection. Several methods have been reported for quantitative determination of atorvastatin in biological samples [7-9], aqueous samples [10, 11] and tablets [12-14]. Telmisartan in pharmaceutical dosage forms is determined by various techniques such as linear sweep polarography [15], parallel catalytic hydrogen wave method [16] and HPLC [17-19]. However no references have been found for simultaneous determination of telmisartan and atorvastatin calcium in pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate isocratic reversed-phase HPLC method for simultaneous determination of atorvastatin calcium and telmisartan in the same tablet dosage form.

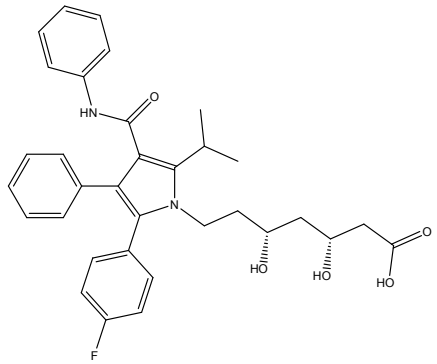



**Fig 1. Structure of Atorvastatin**

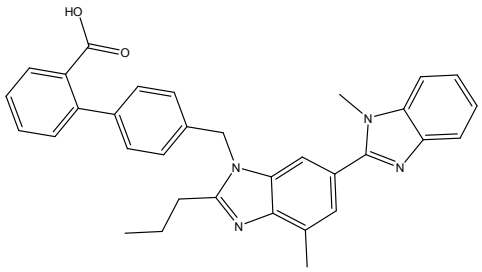



**Fig 2. Structure of Telmisartan**

## 2. Experimental

### *2.1. Chemicals*

Telmisartan (94.43%) and Atorvastatin calcium (94.64%) were obtained from Dr. Reddys Laboratories, Hyderabad, India, and Biocon Limited, Bangalore, India respectively as gift samples. Acetonitrile (HPLC Grade) and Methanol (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. While Ammonium acetate (AR Grade) and glacial acetic acid (AR Grade) from S.D. fine chemicals, Mumbai, India. The 0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. Mili-Q water was used throughout the experiment. Tablets were purchased from Indian market containing telmisartan 40mg and atorvastatin calcium 10mg per tablet.

### *2.2. Equipments*

Analysis was performed on a chromatographic system Agilent 1200 series separation module (Japan) equipped with an auto injector (G1329A), Diode array detector (DAD) SL (G1315C), Quaternary pump (G1311A) and column thermostat (G1316A). A chromatographic separation was achieved by Symmetry C-18, 250 x 4.6mm, 5µ analytical column. Data acquisition was made with Chemstation software. The peak purity was checked with the DAD detector.

### *2.3 Liquid chromatographic conditions*

Chromatographic conditions were obtained using a stainless steel column (Waters symmetry C18 250mm x 4.6mm 5µm), which was maintained at 25$^{o}$ C. The analytical wavelength was set at 254 nm and samples of 10µl were injected to HPLC system. The mobile phase was a mixture of ammonium acetate (0.02M, pH 4.0 adjusted with glacial acetic acid) and acetonitrile in ratio of 40:60 (v/v) at a flow rate of 1.0ml/min. The mobile phase was filtered through 0.45µm filter (Sartorius, Germany) and degassed for 10 minutes by sonication.

### *2.4. Standard solutions and calibration graphs*

Standard stock solution of Telmisartan (4.0 mg/ml) (Figure-2) and atorvastatin calcium (1.0 mg/ml) (Figure-1) was prepared in methanol as diluent. To study the linearity range of each component, serial dilutions were made to obtain working standards in the concentration range of telmisartan (200-600 µg/ml) and atorvastatin calcium (50 -150 µg/ml). A graph was plotted as concentration of drugs versus peak area response and results were found linear for both analytes. From the standard stock solution, a mixed standard solution was prepared containing telmisartan (400 µg/ml) and atorvastatin calcium (100 µg/ml). The system suitability test was performed from five replicate injections of mixed standard solution.

### *2.5. Sample preparation*

Twenty tablets were weighed and finely powdered. The average weight of tablets was determined with weight of 20 tablets. A portion of powder equivalent to the weight of one tablet was accurately weighed into 100 ml A-grade volumetric flask and 70 ml diluent was added. The volumetric flasks were sonicated for about 20min to effect complete dissolution of the telmisartan and atorvastatin calcium, the solutions were then made up to volume with diluent. The solution was filtered through 0.45 µ m nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 400 µg/ml of telmisartan and

100 µg/ml of atorvastatin calcium. Ten microlitres of the test solution was injected and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

### *2.6. Method validation*

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [20]. Assay method precision was determined by using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted as described in Section 2.5 and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Section 2.4. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 ml/min. The percentage of organic modifier was varied by (±) 5% and pH of mobile phase was varied by (±) 0.1.

## 3. Results and discussion

### *3.1. Optimization of the chromatographic conditions*

In order to develop RP-HPLC method for combination of cardiovascular drugs telmisartan and atorvastatin calcium in single formulation. The chromatographic conditions were optimized for better resolution by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that acetate buffer (0.02 M-ammonium acetate buffer pH at 4.0) gave better peak shapes than their phosphate and citrate counterparts. With methanol as solvent both the peaks shows less theoretical plates and bad peak shapes, on changing to acetonitrile the peak shape improved along with theoretical plates. Further optimization experiments were carried out with 50, 60 and 70 % of acetonitrile in mobile phase. The best peak shape and maximum separation was achieved with mobile phase composition comprising mixture of acetate buffer-acetonitirle (40:60 v/v).

The best separation, peak symmetry and reproducibility were obtained with Waters symmetry C18, 250mm x 4.6mm, 5µm column compared to Zorbax C18, 250mm x 4.6mm, 5µm and Inertsil C8, 250mm x 4.6mm, 5µm. The optimum wavelength for detecting both the analytes was ascertained and found to be 254 nm. Peak tailing was observed for atorvastatin calcium when the flow rate was 0.8ml/min using optimized mobile phase conditions. However, a flow rate of 1.0ml/min yielded optimum separation and peak asymmetry.

### 3.2. Validation of method

#### *3.2.1. Specificity*

The specificity of the HPLC method is illustrated in Figures. 3,4 and Figure 5 which depicts complete separation of telmisartan and atorvastatin calcium in presence of tablet excipients. And no interfering peaks of endogenous compounds observed at the retention time of the analytes. In peak purity analysis with DAD detector, purity angle was less than purity threshold for both the analytes, which implies that both analytes are pure and excipients in the formulation doesn't interfere the analytes.

#### *3.2.2. Accuracy*

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage of recoveries obtained for telmisartan and atorvastatin calcium was found to be 99.28 and 99.61% respectively.

#### *3.2.3. Precision*

Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analysis of the same working solution. The relative standard deviation (R.S.D.) obtained for telmisartan and atorvastatin calcium are 0.57 and 0.15% respectively (Table 2).

The intra- and inter-day variability or precision data are summarized in Table 3. The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The R.S.D. of the assay results, expressed as percentage of label claim, was used to evaluate the method precision. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method (Table 3).

#### *3.2.4. Linearity*

Linearity was determined for telmisartan in the range of telmisartan 100–800µg/ml and for atorvastatin 50–150µg/ml. The correlation coefficient ('*r*') values for both the drugs were >0.999.

#### *3.2.5. Limit of detection (LOD) and limit of quantitation (LOQ)*

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels [20]. The limit of quantification (LOQ) is defined as the

lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as

$$LOD = \frac{3.3 \times Syx}{b}$$

$$LOQ = \frac{10.0 \times Syx}{b}$$

where Syx is residual variance due to regression; *b* is slope.

LOD and LOQ for telmisartan were 0.7 and 2.1 µg/ml respectively and for atorvastatin calcium were 0.008 and 0.02 µg/ml, respectively.

***3.2.6. Robustness***

The robustness of an analytical procedure is measure of its ability to remain unaffected by small, but deliberate variations in method parameters. Robustness of the method was investigated by varying experimental conditions such as changes in wavelength, flow rate, pH and composition of mobile phase. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained implies method is robust for routine quality analysis (Table 4).

## 4. Conclusion

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of atorvastatin calcium and telmisartan in new tablet formulation. The method is very simple and specific as both peaks are well separated from its excipient peaks and with total runtime 12 min, makes the developed method suitable for routine quality control analysis.

**Table 1: Results of the recovery analysis of Atorvastatin calcium and Telmisartan**

| Compound | Wt spiked (mg) | Wt recovered (mg) | Recovery (%) | RSD (%) N=3 |
|---|---|---|---|---|
| Atorvastatin | 5.01 | 4.99 | 98.60 | 0.57 |
| | 10.01 | 9.94 | 99.30 | 0.23 |
| | 15.03 | 15.02 | 99.93 | 0.26 |
| Telmisartan | 20.04 | 20.03 | 99.95 | 0.4 |
| | 40.06 | 39.83 | 99.43 | 0.28 |
| | 60.12 | 59.8 | 99.47 | 0.42 |

R.S.D.: relative standard deviation Wt: weight.

**Table 2: System suitability parameters.**

| Parameters | Telmisartan | Atorvastatin |
|---|---|---|
| Theoretical plates[a] | 9891 | 9886 |
| USP resolution[a] | 6.87 | -- |
| peak symmetry[a] | 1.1 | 1.14 |
| % RSD | 0.57 | 0.15 |

[a] USP-NF 29 section 621, pp.2135

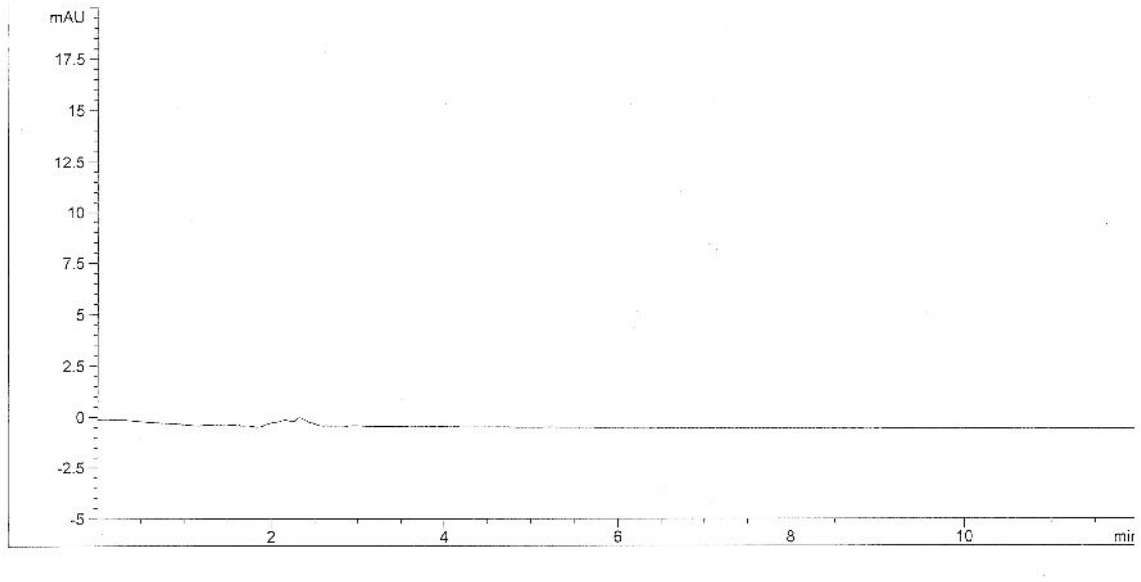
**Table 3: Intra and Inter-day assay precision data (n=9)**

| Actual Concentration | Measured concentration (µg/ml), RSD. (%) | |
|---|---|---|
| | Intra -day | Inter-day |
| Atorvastatin (µg/ml) | | |
| 100 | 99.42 (0.19) | 99.14 (0.75) |
| 150 | 151.17(0.65) | 151.37 (0.89) |
| 300 | 298.31 (0.56) | 298.81 (0.45) |
| Telmisartan (µg/ml) | | |
| 400 | 400.68 (0.45) | 400.12 (0.57) |
| 600 | 599.3 (0.28) | 600.45 (0.49) |
| 1200 | 1199.57 (0.24) | 1199.37 (0.81) |

Data expressed as mean for "measured concentration" values.

**Table 4: Results of robustness study**

| **Factor** | **Level** | **Atorvastatin calcium** | **Telmisartan** |
|---|---|---|---|
| | | **Mean % assay (n=3) (% R.S.D.)** | **Mean % assay (n=3) (% R.S.D.)** |
| pH of mobile phase | 3.9 | 99.3 (0.4) | 99.6(0.32) |
| | 4.1 | 100.8(0.5) | 99.5(0.35) |
| Flow rate (ml/min) | 0.9 | 99.4(0.78) | 99.8(0.87) |
| | 1.1 | 100.3(0.75) | 99.8(0.42) |
| % of acetonitrile | 55 | 100.9(0.46) | 99.7(0.25) |
| | 65 | 100.7(0.39) | 99.5(0.30) |
| Wavelength (nm) | 252 | 99.7 (0.81) | 99.4 (0.38) |
| | 256 | 99.3 (0.78) | 99.0 (0.83) |




**Minutes**

**Fig 3. A typical chromatogram of placebo solution,**

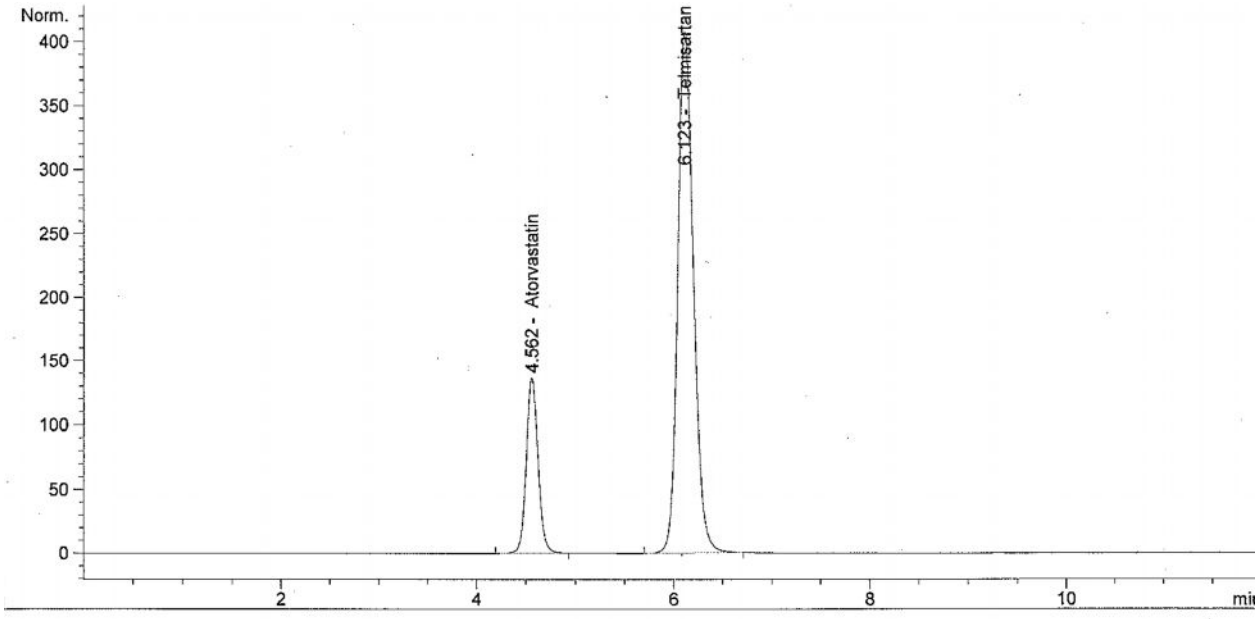



**Fig 4. A typical chromatogram of standard solution containing 100 µg/ml of atorvastatin calcium and 400 µg/ml of telmisartan.**

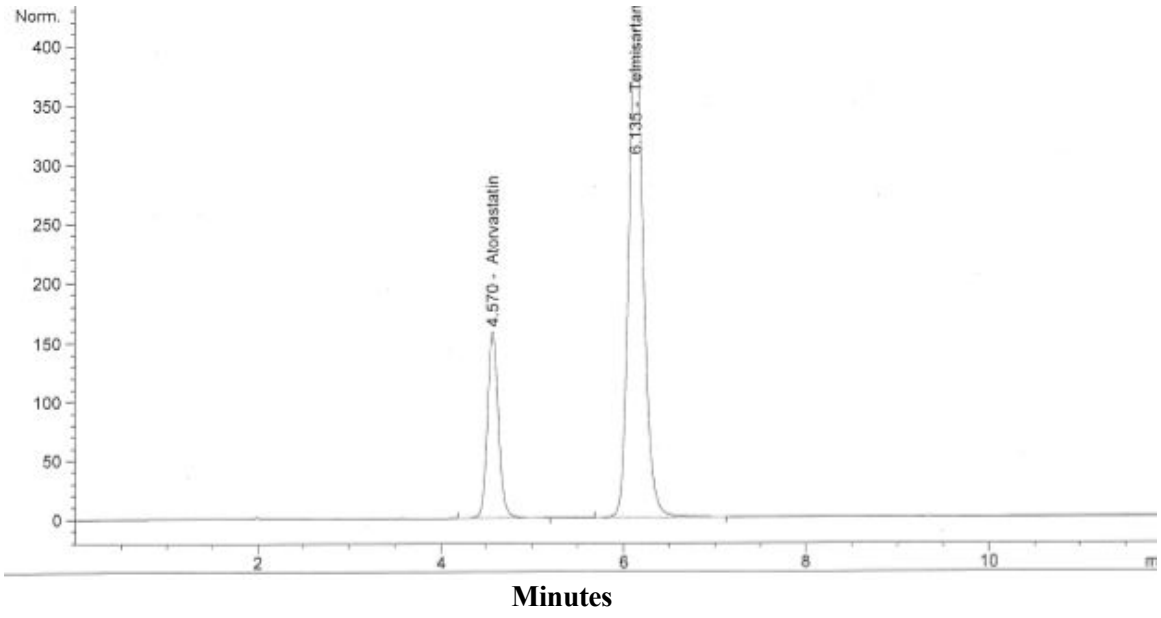



**Fig 5. A typical chromatogram of sample solution containing 100 µg/ml of atorvastatin calcium and 400 µg/ml of telmisartan.**

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