ejpmr, 2020,7(5), 401-406

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND ATORVASTATIN BY RP-HPLC

Ramakrishna Reddy Voggu*[1], Ravi Teja. Tumburu[2], M. Kishore[3]

[1*]Department of Chemistry, Cleveland State University, Cleveland, OH 44115, USA.
[2]MSN Laboratories Pvt.Limited, Telangana.
[3]Department of Pharmaceutical Analysis, Pratishta Institute of Pharmaceutical Sciences, Suryapet Telangana.

***Corresponding Author: Ramakrishna Reddy Voggu**
Department of Chemistry, Cleveland State University, Cleveland, OH 44115, USA.

Article Received on 29/02/2020 **Article Revised on 19/03/2020** **Article Accepted on 09/04/2020**

ABSTRACT

The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Telmisartan and Atorvastatin. C18 column (Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm) and a mobile phase containing Buffer: ACN, 40:60 v/v mixtures was used for the separation and quantification. The flow rate was0.8 mL/min and the eluents were detected by UV detector at 250 nm. The retention times were found to be 2.766 and 5.383 mins, respectively. The developed method was validated according to ICH guidelines Q2 (R1) and found to be linear within the range of 10-100 μg/mL for both drugs. The developed method was applied successfully for assay of Telmisartan and Atorvastatin in their combined in-house developed dosage forms and *in vitro* dissolution studies.

KEYWORDS: Telmisartan, *in vitro*, Atorvastatin, liquid chromatography (RP-HPLC).

1. INTRODUCTION

Telmisartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Telmisartan does not bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation.

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, this result in a subsequent decrease in hepatic cholesterol levels. Decreased hepatic cholesterol levels stimulates up regulation of hepatic LDL-C receptors which increases hepatic uptake of LDL-C and reduces serum LDL-C concentrations.

2. EXPERIMENTAL DESIGN

2.1. *Chemicals and Reagents:* Telmisartan (94.43%) and Atorvastatin calcium (94.64%) were obtained from the Dr. Reddys laboratories, Hyderabad, India, and Biocon limited, Bangalore, India respectively as gift samples. Water HPLC Grade, acetonitrile HPLC Grade, Ortho phosphoric acid (AR grade) and other reagents of HPLC grade were procured from Merck. Tablets (Telistaplus 40) were purchased from Indian market containing 40mg Telmisartan and 10mg atorvastatin calcium per tablet.

2.2. *HPLC Instrumentation and Conditions:* Quantitative HPLC was performed on an Younglin Acme 9000 High pressure liquid chromatographic instrument for the analysis. The instrument is provided with solvent delivery µmodule with UV-detector and Inertsil extended, ODS Reverse phase column (250mm × 4.6mm and 5µ particle size). Manual injector and window based Autochro 3000 software was used for its recording and analysis. A Sartorius electronic balance was used for weighing the materials. Uv/vis double beam spectrophotometer SL 160 with "Spectra Treats" software.

***2.2.1.* Selection of mobile phase:**

Pure drug of Telmisartan and Atorvastatin calcium of mixed standard stock solution (40µg/ml of telmisartan and 10µg/ml of atorvastatin calcium) were taken and 20µl sample was injected in to RP - HPLC system and run in different solvent systems. Different mobile phases systems like Acetonitrile: Water, Methanol: water, Acetonitrile: Phosphate buffer were tried in order to determine the best conditions for the effective separation of telmisartan and atorvastatin calcium. The mobile phase consisting of acetonitrile and potassium dihydrogen Phosphate buffer, pH is adjusted to 3.5±0.03 in the ratio of (60:40% *v/v*) was selected as it gave high resolution for telmisartan and atorvastatin calcium with minimal tailing.

2.2.2. **Preparation of mobile phase:**[1-3]
The mobile phase consisting of acetonitrile & potassium dihydrogen phosphate buffer, pH is adjusted to 3.5±0.03 in the ratio of (60:40% *v/v*) was prepared and sonicated for 15 minutes. The mobile phase was then filtered through a 0.45µ membrane filter.

2.2.3. **Buffer preparation:**[4-5]
1.36gm of potassium dihydrogen phosphate was weighed and dissolved in 100ml of water in a clean dry 1000ml volumetric flask and volume was made up to 1000ml with water. Adjust the pH to 3.5± 0.03 using dilute Orthophosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles.

2.2.4. **Preparation of stock solution:**
For Telmisartan:
10mg telmisartan was weighed and dissolved in 7ml acetonitrile and volume was made up to 10ml with acetonitrile. Sonicated for 10min. (Final concentration is 1000µg/ml).

For Atorvastatin Calcium:
10mg atorvastatin calcium was weighed and dissolved in 7ml acetonitrile and volume was made up to 10ml with acetonitrile. Sonicated for 10min. (Final concentration is 1000µg/ml).

2.2.5. **Preparation of standard solution:**
From the above stock solutions 0.4ml telmisartan and 0.1ml atorvastatin calcium were transferred into 10ml clean dry volumetric and make up to the volume with mobilephase. (Final concentration is 40µg/ml & 10µg/ml).

2.2.6. **Preparation of sample solution:**[6]
Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10mg atorvastatin calcium and 40mg telmisartan was transferred to a 10ml volumetric flask. To the mixture 7ml of acetonitrile was added. The mixture was sonicated for 15 min; the volume was made up to the mark with same solvent and then filtered through a 45 grade filter paper. From the above sample mixture 0.1ml of solution was transferred in to 10ml volumetric flask. The volume was made up to the mark with the mobile phase to get the final concentration of 40µg/ml and 10µg/ml Telmisartan and Atorvastatin calcium respectively.

2.3. Method validation[7-8]
2.3.1. **Specificity:** Specificity is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific.

2.3.2. **Accuracy:** The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. Accuracy was conducted by three replicate measurements at three different concentrations as low, medium, high quality control samples final concentration is 50%, 100%, 150% or 80%, 100%, 120%.

2.3.3. **Precision:** The stock solutions were prepared by dissolving 10mg of telmisartan and 10mg of atorvastatin calcium working standards to get the final concentration of 1000µg/ml and 1000µg/ml of telmisartan and atorvastatin calcium respectively in 10ml volumetric flasks and made up to the volume with acetonitrile. From the stock solution, further dilution was made in a 10ml volumetric flask to get 40µg/ml Telmisartan and 10µg/ml Atorvastatin calcium respectively with diluent.

2.3.4. Linearity: To establish the linearity, a stock solution containing 40ppm telmisartan and 10µg/ml atorvastatin calcium were prepared using mobile phase (mixture containing buffer and acetonitrile in the ratio of 40:60 v/v) and also prepare the solutions in the concentration range of 10-100µg/ml and 2.5-25µg/ml of telmisartan and atorvastatin calcium respectively. Different concentrations of the pure drugs were injected into the chromatographic system and chromatograms were shown in Figure 4.25 to 4.30. Calibration curve of telmisartan and atorvastatin calcium were constructed by plotting peak area vs. applied concentrations and shown in Figures. The obtained results have shown an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area at each level vs. concentration of analyte was calculated and is presented in table.

2.3.5. Limit of Detection (LOD) and Limit of Quantitation(LOQ): The LOD of Telmisartan and Atorvastatin calcium by the proposed method was determined on the basis of response and slope of the regression equation.

LOD value calculated using the formulae:

$$LOD = \frac{3.3 \times \sigma}{s}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve

The LOQ of Telmisartan and Atorvastatin calcium by the proposed method was determined on the basis of response and slope of the regression equation.

LOQ value calculated using the formulae:

$$LOQ = \frac{10 \times \sigma}{s}$$

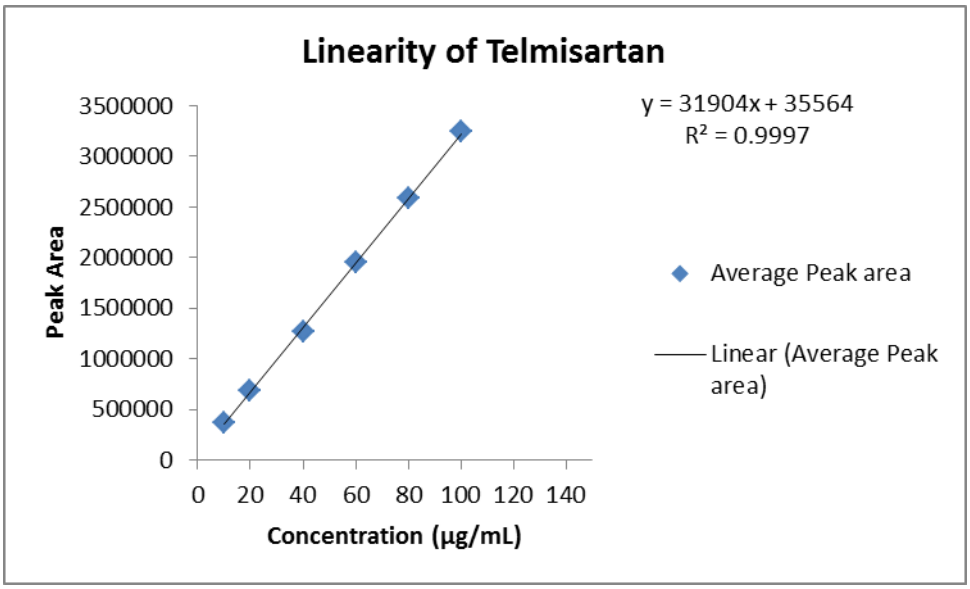
Where, σ = the standard deviation of the response
S = the slope of the calibration curve

2.3.6. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the flow rate, mobile phase composition and temperature variation was made to evaluate the impact on the method.The sample was analyzed separately by slightly changes in the analytical method.

2.3.7. System Suitability: For system suitability, six replicates of the working standard sample were injected and the parameters like retention time, plate number (N), resolution and peak asymmetry of samples were calculated and the results were listed.

Table 1: Linearity results for Telmisartan and Atorvastatin calcium.

Telmisartan Conc. (µg/ml)	Level	Peak Area	Atorvastatin Calcium Conc. (µg/ml)	Peak Area
10	I	372215	**2.5**	105315
20	II	684365	**5**	214365
40	III	1273021	**10**	402858
60	IV	1947635	**15**	617635
80	V	2586107	**20**	822107
100	VI	3240216	**25**	1041216



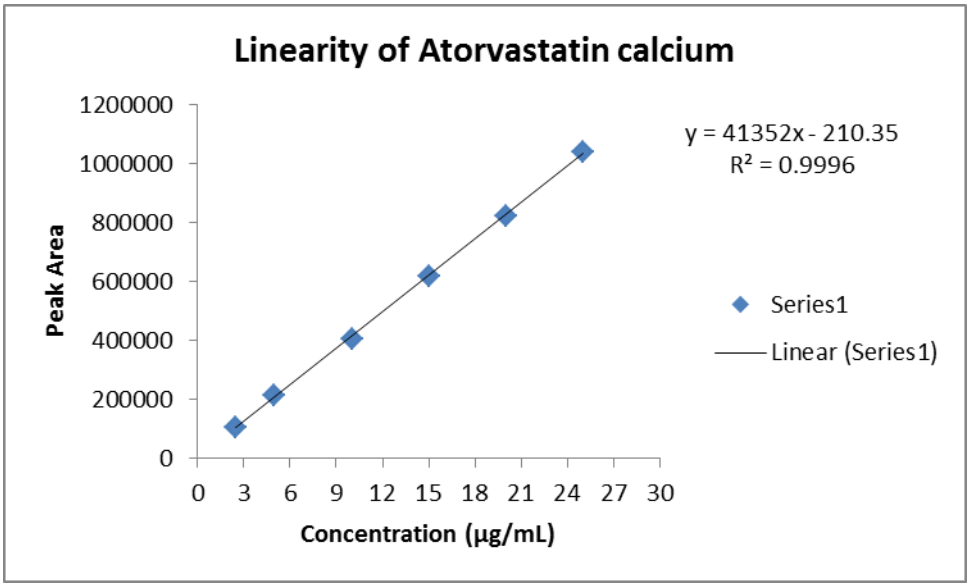


Table 2: Summary results of Accuracy parameter for Telmisartan and Atorvastatin Calcium.

Drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery
Telmisartan	50	20	19.72	98.59	99.20
	100	40	39.96	99.95	
	150	60	59.41	99.06	
Atorvastatin calcium	50	5	4.97	99.57	99.45
	100	10	9.99	99.98	
	150	15	14.82	98.80	

Table 3: Injection precision results for Telmisartan and Atorvastatin calcium.

Injection	Area of Telmisartan	Area of Atorvastatin calcium
Injection-1	1273926	403926
Injection-2	1267798	402798
Injection-3	1269245	404245
Injection-4	1273846	403846
Injection-5	1274947	401947
Injection-6	1273542	403542
Average	1272217	403384
Standard Deviation	2937.454	858.121
%RSD	0.230892	0.212731

Table 4: Intraday Precision results for Telmisartan and Atorvastatin calcium

Injection	Area of Telmisartan	Area of Atorvastatin calcium
Injection-1	1268459	406942
Injection-2	1278203	402503
Injection-3	1270250.5	405252
Injection-4	1270870.5	406850.5
Injection-5	1273362.5	404352.5
Injection-6	1277362	405362
Average	1273084.6	405210.33
Standard deviation	3976.1159	2874.1092
%RSD	0.31232142	0.70928824

Table 5: LOD results for Telmisartan and Atorvastatin calcium.

	Telmisartan	Atorvastatin Calcium
Slope (S)	31904.78	41459.72
Standard deviation (σ)	3976.1159	2874.1092
LOD (µg)	0.41	0.23

Table 6: LOQ results for Telmisartan and Atorvastatin calcium.

	Telmisartan	Atorvastatin Calcium
Slope (S)	31904.78	41459.72
Standard deviation (σ)	3976.1159	2874.1092
LOQ (µg)	1.246	0.695

Table 7: Robustness results for Telmisartan and Atorvastatin calcium.

Telmisartan					
Parameters	**Adjusted to**	**Avg. Area**	**RT**	**SD**	**% RSD**
Flow rate as per method 1.5ml/min	**1.4 ml/min**	1635126.7	3.17	9505.82	0.58
	1.5 ml/min	1276041.5	2.75	1998.77	0.16
	1.6ml/min	862340.33	2.38	4341.03	0.50
Mobilephase composition(Buffer:Acetonitrile, 40:60)	**Buffer:Acetonitrile (35:65)**	1419049.83	3.26	3096.83	0.22
	Buffer:Acetonitrile (40:60)	1276041.5	2.75	1998.77	0.16
	Buffer:Acetonitrile (45:55)	1083250.00	2.25	3340.39	0.31
Atorvastatin Calcium					
Flow rate as per method 1.5ml/min	**1.4 ml/min**	457168.33	5.83	1815.64	0.40
	1.5 ml/min	404118.17	5.37	805.46	0.20

	1.6ml/min	344486.33	4.96	1226.28	0.36
Mobilephase composition(Buffer:Acetonitrile, 40:60)	**Buffer:Acetonitrile (35:65)**	437538.83	5.96	957.21	0.22
	Buffer:Acetonitrile (40:60)	404118.17	5.38	805.46	0.20
	Buffer:Acetonitrile (45:55)	368006.83	4.85	3193.25	0.87

Table 8: System suitability parameters.

Parameter	Telmisartan	Atorvastatin calcium
Retention time(min.)	2.766	5.383
Resolution	6.5897	8.7095
N	6387.6	3423.2
TF	1.000	1.06

3. RESULTS AND DISCUSSION

3.1. Optimization of Chromatographic Conditions. To develop suitable RP-HPLC method for simultaneous estimation of Telmisartan and Atorvastatin, different chromatographic conditions were applied and optimized chromatographic conditions were developed.

Optimized chromatographic conditions are as follows:
Mobile phase: Buffer: Acetonitrile (40:60)
Column: C_{18} column (Inertsil 250 × 4.6 mm, packed with 5 µm)
Injection volume: 20 μL,
Flow rate: 0.8 mL/min,
Detection wavelength: 250 nm,
Run time: 8min,
Temperature: Ambient (25^0C).

Validation

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. LOD and LOQ of Telmisartan and Atorvastatin were determined by calibration curve method. Solutions of telmisartan and Atorvastatin were prepared in the range of 10-100 μg/mL and injected in triplicate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of Telmisartan and Atorvastatin were determined by calibration curve method. Solutions of Telmisartan and Atorvastatin were prepared in the range of 0.41 and 0.23.

Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for Telmisartan and Atorvastatin were 99.20% and 99.45%, respectively

Robustness: The method for the development of RP-HPLC method for the simultaneous estimation of Telmisartan and Atorvastatin was found to be robust as the % RSD was found to be less than 2.

System Suitability: The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 10-100μg/mL was injected and repeated five times and the chromatograms were recorded. The retention time, resolution, number of theoretical plates, and peak asymmetry were calculated as 2.766, 5.383, 6.5897, 8.7095 6387.6, 3423.2, 1.000 and 1.06. The result complies with the recommended limits.

CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of Telmisartan and Atorvastatin was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, *in vitro* dissolution study of combinational dosage formulations containing Telmisartan and Atorvastatin.

REFERENCES

1. J. M. Zuckerman, "Macrolides and ketolides: azithromycin, clarithromycin, telmisartan," *Infectious Disease Clinics of North America*, 2004; 18(3): 621–649.
2. Ravi Teja.Tumburu Ramakrishna Reddy Voggu, M. Kishore. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in Combined Formulation. 2020; 10(1): 19-27.
3. D. Adam, E. Glaser-Caldow, J. Wachter et al., "Comparative efficacy of clarithromycin modified-release and telmisartan immediate-release formulations in the treatment of lower respiratory tract infection," *Clinical Therapeutics*, 2001; 23(4): 585–595.
4. A. Giannopoulos, G. Koratzanis, E. J. Giamarellos-Bourboulis, C. Panou, I. Adamakis, andH. Giamarellou, "Pharmacokinetics of clarithromycin in the prostate: implications for the treatment of chronic abacterial prostatitis," *Journal of Urology*, 2001; 165(1): 97–99.
5. P. G. Davey, "The pharmacokinetics of telmisartan and its 14-OH metabolite," *Journal of Hospital Infection*, 1991; 19: 29–37.
6. M. E. Bosch, A. J. R. S´anchez, F. S. Rojas, and C. B. Ojeda, "Determination of paracetamol: historical evolution," *Journal of Pharmaceutical and Biomedical Analysis*, 2006; 42(3): 291–321.
7. M. Khanmohammadi, M. Soleimani, F. Morovvat, A. B. Garmarudi, M. Khalafbeigi, and K. Ghasemi, "Simultaneous determination of Atorvastatin and codeine phosphate in tablets by TGA and

chemometrics," *Thermochimica Acta*, 2012; 530: 128–132.
8. Y. Ozkan, A. Savaser, Y. Ozalp, and A. Isimer, "Dissolution properties of different designed and formulated salbutamol tablet dosage forms," *Acta Poloniae Pharmaceutica*, 2000; 57(4): 33–41.