Muraka Sirisha. *et al.* / *International Journal of Pharmacy & Therapeutics*, 4(1), 2013, 1-6.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND TELMISARTAN IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

A simple and reliable reverse phase high-performance liquid chromatography method was developed and validated for analysis of Atorvastatin and Telmisartan in pure and pharmaceutical dosage form. The method was developed on symmetric C18 (4.6 x 150mm, 3.5 µm, Make: Hypercil), with a mobile phase of phosphate buffer (P_H 3.0): Acetonitrile (40:60) %v/v. The effluent was monitored by Waters HPLC model containing Alliance 2695 with 2487 detector, variable wavelength prominence UV/ VIS detector SPD-20A (VP series). Calibration curve was linear over the concentration range of 50–90µg/ml for Atorvastatin and 12.5–22.5 µg/ml for Telmisartan. Recovery of Atorvastatin and Telmisartan was found to be in the range of 100.2 -99.80%. The limit of detection (LOD) and quantification (LOQ) were 2.97 and 9.79 for Atorvastatin and 2.93 and 9.95 for Telmisartan, respectively. The retention time and run time was very short; hence it is cost effective, making it more economical and rapid. Hence, this method can be used for the analysis of large number of samples.

Key Words:- Atorvastatin, Telmisartan, RP-HPLC, Validation.

INTRODUCTION

Atorvastatin calcium (Fig 1) is the calcium salt (2:1) trihydrate of [R-(R*, R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenylamino) carbonyl]-lHpyrrole1 heptanoic acid. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzymeA (HMGCoA) Reductase. Atorvastatin is the most efficacious of the currently available HMG-CoA Reductase inhibitors in terms of lowering plasma cholesterol levels by suppressing the hepatic production of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol.

Telmisartan (TELM) (Fig 2) chemically described as 4[(1,4-dimethyl-2-propyl(2,6-bi- 1H-benzi midazol]-1-yl)methyl][1,1-biphenyl]-2-carboxylic acid is a potent, long-lasting nonpeptide antagonist of the angiotensin II(AT1) receptor that is indicated for the treatment of essential hypertension. It selectively and insurmountably inhibits stimulation of the AT1receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. In clinical studies,

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Telmisartan shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin converting enzyme (ACE) inhibitors, beta-blockers and calcium antagonists.Literature review revealed that there are various methods for determination of Telmisartan and Atorvastatin calcium, individually and in combination with other drugs. The majority of methods reported are liquid chromatography coupled to UV (Shah D.A *et al.*2007,) electrochemical (Sahu R *et al*., 2007) or mass spectrometry (Chaudhari B.G *et al*., 2006) detection but some determinations were also performed by thin layer (Wang Y *et al*., 2005) and gas

Chromatography (Chen B *et al.*, 2005) or Spectrophotometry (Wankhede S.B *et al*., 2007) .A LC method for the assay and related substances of Atorvastatin is also reported in the European Pharmacopoeia. Due to their high sensitivity and selectivity, analytical methods such as liquid or capillary gas chromatography were previously reported. Telmisartan in pharmaceutical dosage forms is determined by various techniques such as linear sweep polarography, parallel catalytic hydrogen wave method and HPLC (Murthy M., 2009) however few references have been found for simultaneous determination of Telmisartan and Atorvastatin in combined pharmaceutical preparations. The present study describes a simple, rapid, precise and accurate isocratic Reversed-phase HPLC method for simultaneous determination of Telmisartan and Atorvastatin in the same tablet dosage form.

MATERIALS AND METHODS

Chemicals and solvents

The reference sample of Atorvastatin and Telmisartan was supplied by Pharma train Labs Pvt Ltd., Hyderabad. HPLC grade water and Methanol were purchased from E. Merck (India) Ltd., Ahmedabad. Sodium Dihydrogen phosphate and glacial acetic acid of AR Grade was obtained from S.D. Fine Chemicals Ltd., Mumbai. Tablet formulations ARBITEL-AV were procured from a local pharmacy with labelled amount of 10 mg of Atorvastatin and 40mg of Telmisartan per tablet.

Instrument

The HPLC system is of Waters, Alliance 2695 with 2487 detector UV-VIS detector, HPLC Pump LC-20AT pump and Injector Loop rheodyne, model No. 2767, Made in USA 20 µl volume loop. Data acquisition was performed by the Empower software.

Chromatographic Condition

Chromatographic analysis was performed on a Hypercil reversed phase C-18 column with 150 x 4.6mm internal diameter and 3.5µm particle size. The mobile phase consisted of Phosphate buffer (pH 3.0): acetonitrile (40: 60 v/v) and that was set at a flow rate of 1.0 ml/min. The mobile phase was degassed and filtered through 0.45 µ filter under vacuum before pumping into HPLC system. The effluent was monitored by UV detection at 276 nm.

Preparation of Phosphate buffer (0.02M)

Weighed 2.72 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust the pH to 3.0 with Glacial acetic acid.

Preparation of mobile phase

Mix a mixture of above buffer 40 mL (40%) and 600 mL of Acetonitrile HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Use the Mobile phase as Diluent.

Preparation of standard and sample Solutions

Preparation of Standard Solution

Accurately weigh and transfer 10 mg each of Atorvastatin & Telmisartan working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.7ml of Atorvastatin & Telmisartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution

Accurately weigh and transfer equivalent to 10 mg of Atorvastatin / Telmisartan sample into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.7ml of Atorvastatin & Telmisartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD DEVELOPMENT AND OPTIMIZATION

A mixture of Phosphate buffer (pH 3.0) and acetonitrile in the ratio of 40:60v/v was found to be the most suitable mobile phase for ideal separation of Atorvastatin and Telmisartan. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The

detection of the drug was monitored at 276 nm. The run time was set at 7 min. Under these optimized chromatographic conditions the retention time obtained for Atorvastatin was 2.813min and for Telmisartan was 3.886min. A typical chromatogram showing the separation of the drug is given in Fig 3.

VALIDATION PROCEDURES

The method validation was carried out according to the recommendations for analytical method validation.

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (tR), number of theoretical plates (N) and tailing factor (T) were evaluated for five replicate injections of the drug at a concentration of 70 µg/ml. The results which are given in Table 2 were within acceptable limits

Linearity

The linearity of the method was determined at concentration levels ranging from 50 to 90ppm for Atorvastatin and 12.5 to 22.5ppm for Telmisartan. The calibration curve was constructed by plotting response factor against concentration of drugs which is shown in Fig 4 and 5.

Lod and Loq

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. Determination of the signal-to-noise ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out three times and the percentage recovery were calculated and presented in Table 3.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day precision

In the intra- day studies, five injections of standard solution (70µg/ml) were injected into the chromatographic system in different time interval within a day. %RSD was calculated and shown in Table 4.

Inter-day precision

In the inter-day variation studies, five injections of standard solution (70µg/ml) were injected at different days. % RSD was calculated and shown in Table 5.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase composition and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Ruggedness

It was checked by determining precision on the same instrument, but by a different analyst.

RESULT AND DISCUSSION

A simple, accurate and precise RP HPLC method was developed for the simultaneous estimation of Atorvastatin and Telmisartan in Pharmaceutical dosage forms. All the results were summarized in Table 1. The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. The results of the system suitability test were summarized in Table 2. Linearity of the method was evaluated at 5 different concentration levels 50-90 µg/ml and 12.5-22.5 µg/ml for Atorvastatin and Telmisartan respectively. Both the drugs were found to give linear detector response in the concentration under study with correlation coefficient of 0.999 and 0.999 for Atorvastatin and Telmisartan respectively. Accuracy of the method was determined by recovery test. The percentage recovery was found to be 100.2% for Atorvastatin and 99.80% for Telmisartan (Table 3). All results indicate that the method is highly accurate. This method was validated for its inter-day and intra-day precision. The results obtained were within the acceptable limit (Table 4&5). The ruggedness and robustness of the method were determined, which demonstrate that the developed method is rugged and robust. Detection limit for Atorvastatin and Telmisartan was 2.97µv and 2.93µv and quantification limit was 9.79µv and 9.95µv. All the results of validation parameters are summarized in the Table1. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures.

Fig 1. Structure of Atorvastatin

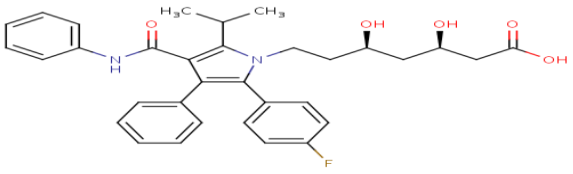

Fig 2. Structure of Telmisartan

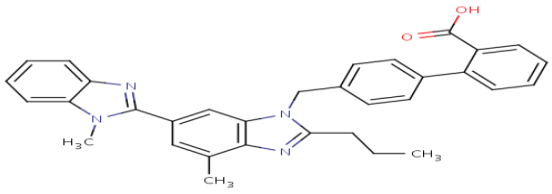

Fig 3. Typical HPLC Chromatogram of Atorvastatin and Telmisartan

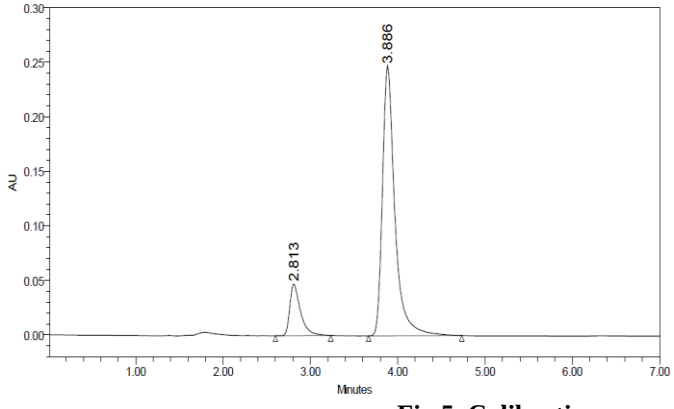

Fig 4. Calibration curve of Atorvastatin by HPLC

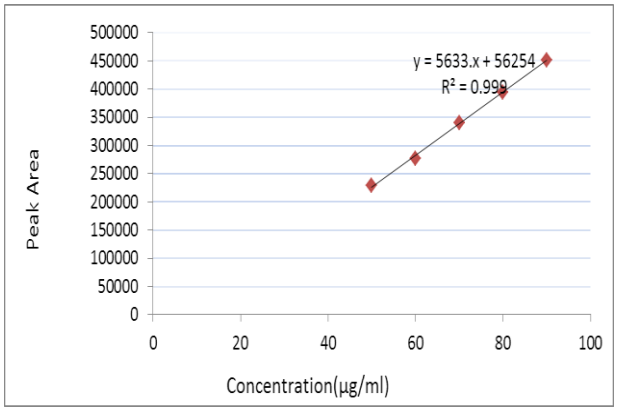

Fig 5. Calibration curve of Telmisartan by HPLC

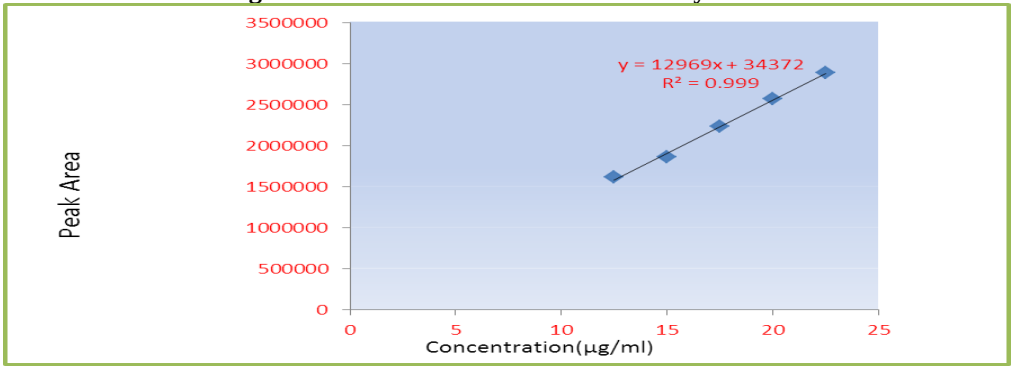

Table 1. Results of Validation of the developed HPLC Method

S.No	Parameters*	Atorvastatin	Telmisartan
1	Linearity (µg/ml)	50-90	12.5-22.5
2	Correl. coefficient	0.999	0.999
3	% Recovery	100.2%	99.80%
4	Inter- day precision (% RSD)	0.68	0.53
5	Intra- day precision (% RSD)	0.004	0.009
6	LOD(µv)	2.97	9.79
7	LOQ(µv)	2.93	9.95
8	Ruggedness (%RSD)	0.004	0.009

*Mean of five determinations ATV- Atorvastatin, TEL- Telmisartan

Table 2. Results of System Suitability Parameters for the analysis of Atorvastatin and Telmisartan

S.No	Parameters*	Atorvastatin	Telmisartan
1.	Peak area	308415	2398637
2.	Theoretical Plates	2664.8	4144.2
3.	Tailing Factor	1.66	1.55
4.	Retention time (minutes)	2.80	3.88
6.	% RSD of Peak area	0.006	0.009

*Mean of five determinations Atorvastatin, Telmisartan

Table 3. Results of Recovery Studies of Marketed Formulation

S.No	Level of Recovery	Drug	Amount of drug added in µg/ml	% Recovery*
1	50%	ATV	15.0	100.15
		TEL	60.0	100.33
2	100%	ATV	20.0	99.66
		TEL	80.0	99.79
3	150%	ATV	25.0	100.69
		TEL	100.5	100.59

*Mean of three determinations ATV- Atorvastatin, TEL- Telmisartan

Table 4. Intraday Precision

S.No	Injection	Atorvastatin	Telmisartan
1	Injection-1	294627	2468343
2	Injection-2	290243	2461738
3	Injection-3	289829	2481354
4	Injection-4	290561	2453947
5	Injection-5	292263	2447184
AVG		291504	2462513
STD		1975.74	13209.16
%RSD		0.68	0.53

*Mean of five determinations ATV- Atorvastatin, TEL- Telmisartan

Table 5. Interday Precision

S.No	Injection	Atorvastatin	Telmisartan
1	Injection-1	308415	2398627
2	Injection-2	306345	2362485
3	Injection-3	305677	2341659
4	Injection-4	306397	2376247
5	Injection-5	308685	2357893
AVG		307103.8	2367382
STD		1353.74	21392.36
%RSD		0.004	0.009

*Mean of five determinations ATV- Atorvastatin, TEL- Telmisartan

CONCLUSION

The developed RP HPLC method for the simultaneous estimation of Atorvastatin and Telmisartan offers simplicity, selectivity, precision and accuracy. All the results of validation parameters are summarized in the Table 1. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures. The method gives good resolution between the compounds with a short analysis time. So the developed method can be used for the routine analysis of Atorvastatin and Telmisartan in Pharmaceutical formulations.

ACKNOWLEDGEMENT

The authors whole heartedly wish to thank Pharma Train Ltd, Hyderabad for providing the necessary facilities to carry out my work.

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