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SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND TELMISARTAN IN TABLET DOSAGE FORM BY LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, rapid and accurate reverse phase-high performance liquid chromatographic method for the simultaneous determination of Atorvastatin and Telmisartan in tablet dosage form is developed and validated. The chromatographic analysis was performed on a kromasil C_{18} column (150×4.6 mm, 3.5 µm) in isocratic mode, the mobile phase consisted of acetonitrile and phosphate buffer (adjusted to pH 3.8 with ortho-phosphoric acid) at a ratio of 70:30 v/v, and a flow rate of 1.0 mL/min. The eluents were monitored at 278 nm. The retention time of Atorvastatin and Telmisartan were found to be 2.804 min and 3.875 min, respectively. The linear ranges were found to be 50-90 µg/mL (r^2=0.9992) for Atorvastatin and 12.5-22.5 µg/mL (r^2=0.999) for Telmisartan. The proposed method is also found to be accurate, precise and robust. The method could be applied to routine quality control of pharmaceutical formulations containing Atorvastatin and Telmisartan.

Keywords: Atorvastatin, Telmisartan, RP-HPLC

INTRODUCTION

Telmisartan (TEL), 4-{[2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl] methyl}-biphenyl-2-carboxylic acid is a new highly selective, nonpeptide angiotensin II type 1 (AT1)-receptor antagonist [1]. TEL lowers blood pressure through blockade of the renninangiotensin-aldosterone system (RAAS) and is widely used in the treatment of hypertension [2]. Determination of TEL in human plasma by liquid chromatography-tandem mass spectrometry has been reported [3].

HPTLC [4, 5], Spectrophotometry and RP-HPLC method for determination of TEL in combination with other anti-hypertensive agents has been reported [6-8]. Atorvastatin (ATO) chemically is, (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 (HMGCoA) reductase, the rate limiting enzyme in the biosynthesis of cholesterol [9]. 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 [10-11], aqueous samples [12-13] and tablets [14-17]. Present study involves the efficient RP-HPLC method forthe estimation of ATO and TEL in combined dosage form.

MATERIALS AND METHODS

Materials: ATO and TEL were generous gift samples from Aurobindo Pharma Limited (Hyderabad, India). A commercial Telsartan-AVR containing TEL (40mg) and ATO (10mg) were purchased from local market and used within their shelf-life period. The HPLC grade acetonitrile, methanol and water were purchased from Rankem (New Delhi, India). All other chemicals used were of pharmaceutical or analytical grade from Rankem (New Delhi, India).

Instrumentation: A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Kromasil C_{18} (150×4.6mm, 3.5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Preparation of standard stock solution: The standard stock solutions were prepared by transferring 10 mg of TEL and 10 mg ATO working standards into 10 mL volumetric flasks. To that about 7 mL diluent was added, and the solution was sonicated to dissolve and the volume made up to mark with diluent. The standard solutions were filtered through a 0.45 µm membrane filter. Aliquots of these solutions were transferred using A-grade bulb pipettes into 10 mL volumetric flasks and volume make up to the mark with mobile phase to give the final concentration 100 µg/mL of each analyte.

Method validation

To determine linearity, aliquots of primary standard TEL and ATO stock solutions were taken into 10 mL volumetric flask and diluted up to the mark with the mobile phase such that the final concentrations of TEL and ATO were in the range of 12.5-22.5 µg/mL and 50-90 µg/mL respectively. The solutions (20 µL) were injected three times into the column according to the optimized chromatographic conditions, and the peak areas and retention times were recorded. The calibration curve was constructed by plotting the analyte to internal standard peak area ratio (Response factor) against the concentration (µg/mL).

The accuracy was carried out by recovery studies using standard addition method; known amounts of standard drugs were added to pre-analyzed sample of TEL and ATO in according to 50, 100 and 150% of labeled claim, and then subjected to the proposed HPLC method. The experiment was performed in triplicate. The percentage recovery, RSD (%) and standard error mean (SEM) were calculated for each concentration level.

Precision was determined as repeatability, intermediate precision and reproducibility in accordance with ICH recommendations [18]. Repeatability was determined as intra- day variation and intermediate precision was determined by measurement of inter day variation. The reproducibility was checked by measuring the precision of the method in same laboratory on a same instrumentation with analysis being performed by another person. For both intra-day and inter-day variation, standard solutions of TEL and ATO at single concentration were determined in five times.

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated based on the ICH guidelines [18]. Robustness was done by deliberately changing the chromatographic conditions like ± 0.2 in pH of the buffer and ±0.1mL in flow rate. To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like asymmetry factor, theoretical plate number (N), resolution (Rs) and retention time (t_R) were analyzed by using 20 µL of the working standard solution containing TEL (15 µg/mL) and ATO (60 µg/mL) injecting five times into HPLC system.

For analysis of marketed samples, twenty tablets of Telsartan-AVR each containing TEL (40 mg) and ATO (10mg) were weighed and finely powdered. A quantity of the powder equivalent to one tablet content was accurately weighed, transferred into 100 mL volumetric flask containing 70 mL of diluent, sonicated for about 15 min and the volume make up to the mark with methanol. This solution was filtered through a 0.45 µm membrane filter paper and filtrate was again diluted with mobile phase. The standard and sample solutions (20 µL) were separately injected into HPLC system. The possibility of interference from the excipients in the analysis was studied.

RESULTS AND DISCUSSION

In order to achieve simultaneous elution of the two components, initial trails were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions, such as methanol-water (80:20 v/v), acetonitrile-water (80:20 v/v), methanol-0.05M phosphate buffer (80:20 v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid), methanol-acetonitrile-0.05M phosphate buffer (80:10:10 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid) and acetonitrile-0.05M phosphate buffer (80:20 v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid). Finally, acetonitrile and phosphate buffer (adjusted to pH 3.8 with ortho-phosphoric acid) at a ratio of 70:30 v/v was selected

as the optimum mobile phase and a flow rate of 1.0 mL/min. Under these conditions, the analyte peaks were well resolved and were free from tailing. The tailing factor was <1.5 for both the analytes. The retention times of ATO and TEL were found to be 2.804 min and 3.875 min, respectively. The resolution (Rs) between ATO and TEL was found to be 2.35, indicating good separation of both analytes from each other. The theoretical plate number for TEL and ATO were found to be 4140 and 2665, respectively, thus indicating good column efficiency. A typical chromatogram was recorded at 278 nm, shown in Figure 1. The calibration plot was constructed by plotting peak area versus concentration (µg/mL) of TEL and ATO which were found to be linear in the range of 12.5-22.5 µg/mL (r^2=0.999) and 50-90 µg/mL (r^2=0.999), respectively (Table 1). Limit of detection (LOD) values of TEL and ATO were experimentally verified to be 0.042µg/mL and 0.063µg/mL, respectively. Limit of quantitation (LOQ) values of TEL and ATO were found to be 0.14µg/mL and 0.21µg/mL, respectively, which indicated that the method can be used for analysis of TEL and ATO over a very wide range of concentrations.

The percentage recoveries of TEL and ATO were found to be in the range of 99.81-100.62% and 99.65-100.68%, respectively. The results were shown in Table 2, which indicates that the method is accurate. The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample. Results from determination of repeatability and intermediate precision, expressed as RSD (%). The low values of %RSD indicated that the method is precise. The reproducibility results were shown that, there were no significant differences between %RSD values for intra-day and inter-day precision, which indicated that the method, is reproducible. Robustness was done by small deliberate changes in the chromatographic conditions. There were no significant changes in the peak areas and retention times of TEL and ATO when the pH and flow rate of the mobile phase were changed. The results were indicating that the proposed method is robust.

The proposed method was applied to the simultaneous estimation of TEL and ATO in tablets. The assay results show that the proposed method was selective for the simultaneous determination of TEL and ATO without interference from the excipients used in the tablet dosage form. The values were shown in Table 3. The assay results and low %RSD values indicated that the developed method can be used for routine analysis of TEL and ATO in pharmaceutical dosage forms.

CONCLUSION

The developed RP-HPLC method was accurate, precise, reproducible and robust. The developed method has been found to be better, because of its wide range of linearity, use of a readily available mobile phase, lack of extraction procedure and low retention times. All these factors make the proposed method suitable for the quantification of TEL and ATO in bulk drugs and in table dosage form. The method can be successfully used for the routine analysis of TEL and ATO in pharmaceutical dosage forms without interference.

Table 1: Linearity data of TEL and ATO

Analyte	Conc. (µg/mL)	Mean peak area ± SD (n=3)	RSD (%)	Linear regression
TEL	12.5	1616125±4865	0.301	R^2=0.999
	15	1928367±8942	0.4786	
	17.5	2234843±10245	0.4584	
	20	2571642±13586	0.5283	
	22.5	2885708±22384	0.7757	
ATO	50	228407±548	0.2398	R^2=0.999
	60	276978±1710	0.6175	
	70	339892±5215	1.5342	
	80	393459±3790	0.9632	
	90	451862±680	0.1505	

` Table 2: Results of recovery studies by standard addition method

Analyte	%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	% Recovery
ATO	50	15	15.02	100.13
	100	20	19.93	99.65
	150	25	25.17	100.68
TEL	50	60	60.21	100.35
	100	80	79.85	99.81
	150	100	100.62	100.62

Table 3: Estimation of amount present in tablet dosage form

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)
ATO	10	99.82±0.604	0.605
TEL	40	101.36±0.721	0.711

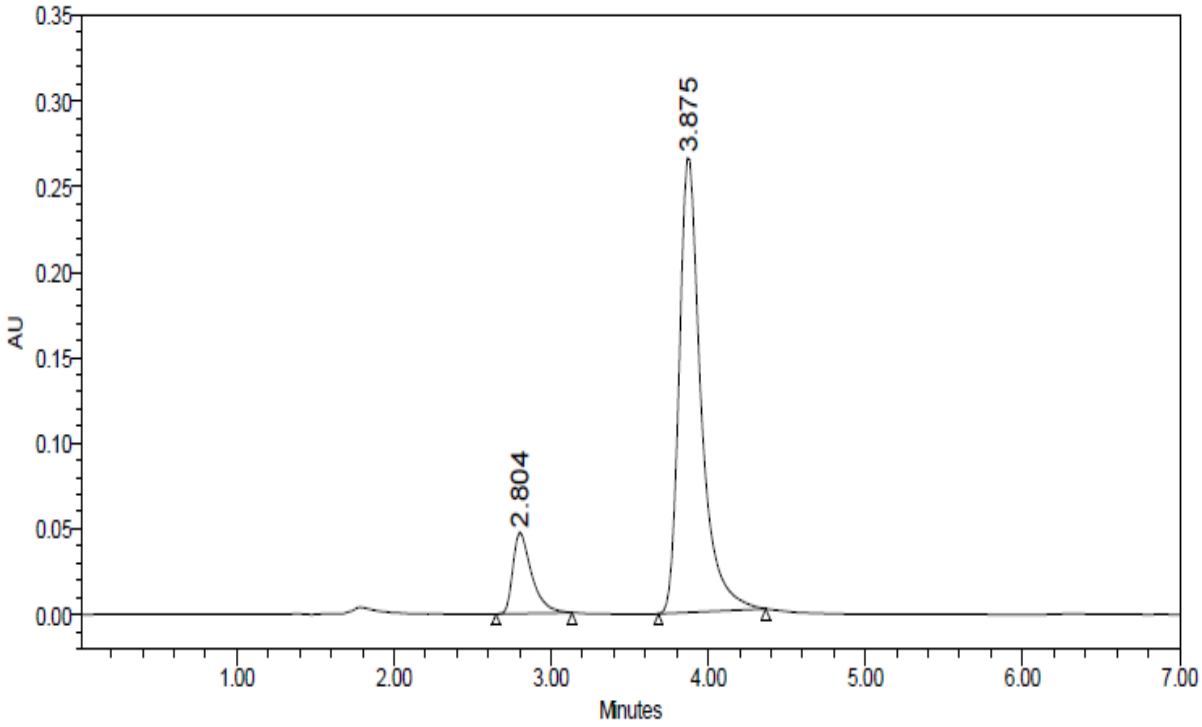

Figure 1: A typical chromatogram of ATO and TEL

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