SIMULTANEOUS DETERMINATION OF ATORVASTATIN CALCIUM AND TELMISARTAN IN PHARMACEUTICAL FORMULATIONS BY REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT:
A simple, sensitive and reproducible reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed for simultaneous quantitative estimation of Atorvastatin calcium (ATOR) and Telmisartan (TELM) in the pharmaceutical formulations. Chromatographic separation was achieved on a 250 × 4.6 mm, 5µ, Waters symmetry column. The flow rate was 1ml/min and eluent was monitored by absorbance at 267 nm using a mixture of Methanol and Acetonitrile (pH 3.0±0.01) in the ratio of 25:75 (v/v). The retention time of Atorvastatin calcium and Telmisartan was found to be 5.6 and 8.2 min respectively. Calibration plots were linear in the concentration range of 5-25 µg/ml for Atorvastatin calcium and 10-50 µg/ml for Telmisartan with correlation coefficient (R^2) 0.999 respectively. The proposed method was validated by testing its linearity, recovery, specificity, system suitability, precision (Interday, intraday, analyst and instrument precision), robustness and LOD/LOQ values and it was successfully employed for the determination of Atorvastatin calcium and Telmisartan in pharmaceutical tablet formulations.

Keyword: Atorvastatin calcium, Telmisartan, Validation

1. 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]-lH-pyrrole 1-heptanoic acid. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (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[1].

Telmisartan (TELM) (Fig. 2) chemically described as 4[(1,4-dimethyl-2-propyl(2,6-bi-1H-benzimidazol]-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 AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. In clinical studies, TELM shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin converting enzyme (ACE) inhibitors, beta-blockers and calcium antagonists[2].

A novel formulation commercially available in combination of Telmisartan and Atorvastatin calcium, benefits from the complementary modes of action of long-lasting angiotensin receptor and antihyperlipidemic action. This provides powerful efficacy for day long control of BP and has proven evidence in cardiovascular (CV) outcomes of both Telmisartan and Atorvastatin calcium.

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[5,6], fluorimetric[7] electro-chemical[8,9], or mass spectrometry detection[10-13] but some determinations were also performed by thin layer[14, 15], micellar electrokinetic[16] and gas chromatography [17, 18] or spectrophotometry [19, 20].

A LC method for the assay and related

substances of Atorvastatin is also reported in the European Pharmacopoeia[21]. Due to their high sensitivity and selectivity, analytical methods such as liquid[22-26] 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 [27] and HPLC[28-30].

However no references have been found for simultaneous determination of Telmisartan and Atorvastatin calcium in combined pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate isocratic Reversed-phase HPLC method for simultaneous determination of Telmisartan and Atorvastatin calcium in the same tablet dosage form.

2. EXPERIMENTAL:

a. Chemicals: Telmisartan (99.4%) and Atorvastatin (101.5%) were obtained from Cipla Pharmaceutical Ltd, Mumbai, India, as gift samples. Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Potassium dihydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) were purchased from E. Merck (India) Ltd. 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 of Telmisartan 40 mg and Atorvastatin 10 mg per tablet.

b. Instruments: Analysis was performed on a chromatographic system Agilent 1200 series separation module (Japan) equipped with an auto injector (G1329A), Diode array detector SL (G1315C), Quaternary pump (G1311A) and column thermostat (G1316A). Data acquisition was made with Chemstation software. The peak purity was evaluated with DAD detector.

c. Liquid chromatographic conditions: Chromatographic conditions were obtained using a stainless steel column (Waters symmetry $C_{18,}$ 250mm x 4.6mm 5µm), which was maintained at 40^0C. The analytical wavelength was set at 267 nm and samples of 20µl were injected to HPLC system. The mobile phase was Potassium dihydrogen phosphate (10mM, pH 3.0 adjusted with ortho-phosphoric acid) and acetonitrile in ratio of 60:40 (v/v) at a flow rate of 1ml/min. The mobile phase was filtered through 0.45µm filter and degassed for 10 minutes by sonication.

d. Standard solutions:

1. Stock standard solutions: An accurately weighed quantity of 40 mg of Telmisartan and 10 mg of Atorvastatin was transferred into a 100 ml volumetric flask separately. Dissolved with 25 ml of methanol and diluted to required volume with mobile phase, having the concentration of 400 µg/ml of Telmisartan and 100 µg/ml of Atorvastatin.

2. Preparation of working standard: From the standard stock solution 10 ml is pipette out into 100 ml volumetric flask and made up the volume with mobile phase, having the concentration of 40 µg/ml of Telmisartan and 10 µg/ml of Atorvastatin.

3. Preparation of laboratory mixture: Accurately weighed quantities of TELM and ATOR (≈ 40 mg and ≈10 mg respectively) were mixed and transferred into a 100 ml volumetric flask, than dissolved with 25 ml of methanol and diluted to required volume with mobile phase, having the concentration of 400 µg/ml of TELM and 100 µg/ml of ATOR. An accurately measured 1.0 ml portion of the resultant solution was diluted to 10.0 ml with diluent to obtain a laboratory mixture having concentration similar to marketed formulation.

4. Sample preparation: Twenty tablets (CRESLIP, Cipla Pharmaceutical Ltd.) were weighed and ground to a fine powder. An amount of powder equivalent to 40mg of Telmisartan and 10mg of Atorvastatin was weighed accurately and transferred into a 100 ml A-grade volumetric flask containing 25 ml of methanol and sonicated for 30 min to effect complete dissolution of the Telmisartan and Atorvastatin and diluted upto 100 ml with diluent, then the solution was filtered through 0.45 µm membrane filter and 10 ml of filtrate taken into 100 ml volumetric flask. The aliquot portion of the filtrate was further diluted to get final concentration of 40 µg/ml of Telmisartan and 10 µg/ml of Atorvastatin.

e. Selection of wavelength of HPLC detection: The aliquots portions of stock standard solution of Telmisartan and Atorvastatin were diluted appropriately with mobile phase to obtain a concentration 10µg/ml of each drug. The solutions were scanned in the range of 400-200 nm in 1.0 cm cell against blank.

f. Linearity study and Calibration curve: To study the linearity range of each component, serial dilutions were made to obtain working standards in the concentration range of Telmisartan (10-50 µg/ml) and Atorvastatin (5-25 µg/ml). A graph was plotted as concentration of drugs versus peak area response

and results found linear for both analytes. From the standard stock solution, a mixed standard of working concentration was prepared containing Telmisartan (40 µg/ml) and Atorvastatin (10 µg/ml). The system suitability test was performed from five replicate injection of mixed standard solution.

g. Analysis of Laboratory Mixture: In order to establish suitability of the proposed method for quantitative simultaneous estimation of Telmisartan and Atorvastatin in the pharmaceutical formulations, the method was first tried for the estimation of the components in a standard laboratory mixture of two drugs by using eq. 1and 2.

h. Analysis of Marketed Formulation: 20 µl of the standard and sample are injected separately and chromatograms are generated. With peak area obtained for standard and sample, the content of ATOR and TELM in each tablet was calculated using the following equation:

$$\textbf{Amount of drug present in each tablet} = \frac{\text{Sample area x Std.Conc. x Std. Purityx Avg. weight}}{\text{Std. area x Sample conc.}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\textbf{Percentage label claim} = \frac{\text{Amount present}}{\text{Label claim}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

i. Recovery study: Recovery studies were performed to validate the accuracy of developed method. For recovery study different concentrations (50%, 100% and 150%) of standard drug was prepared and then its recovery was analyzed.

j. Method validation: The HPLC method was validated in terms of precision, accuracy, specificity and linearity according to ICH guidelines (30).

Accuracy: The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high i.e. 50%, 100% and 150%) of the authentic standards were added to the placebo. The mixtures were extracted as described in section 2d, and were analyzed using the developed HPLC method.

Precision: Assay method precision was determined using nine-independent test solutions (3 concentration/3 replicates). To study precision 80%, 100% and 120% concentration was prepared and three replicate of each concentration was injected. The intermediate precision of the assay method was also evaluated using different analyst different days.

Specificity: Accurately weighed quantities of the tablets powder equivalent to about 40 mg of TELM and 10 mg of ATOR were taken in a dry 50.0 ml volumetric flask. Each sample solution was stored under following different relevant small stress conditions (light, heat, acid/base hydrolysis and oxidation) for sufficient time (24 hrs) to achieve 10 to 30% degradation of the initial sample.

1. Addition of small amount of alkali solution (0.1 N NaOH).
2. Addition of small amount of acid solution (0.1 N HCl).
3. Addition of small amount of oxidative agent (3% H_2O_2).
4. Sample solution was heated 50 ^{0}C on water bath for a sufficient time
5. Sample solution was exposed 600 foot-candle of UV light for a sufficient time.

After 24 hr each treated sample was analyzed and percent labeled claims were calculated by the method using formula under estimation of TELM and ATOR by proposed method.

Linearity: Solutions for linearity study were prepared as described in Section 2f. Six replicates of each concentration were injected and results are examined and it was found that calibration curve was linear in the concentration range of 5-25 µg/ml for ATOR and 10-50 µg/ml for TELM with correlation coefficient (R^2) 0.999.

LOD and LOQ: The LOD and LOQ for analytes were estimated by SD of injecting a series of dilute solutions of known concentrations.

Ruggedness: Ruggedness was ascertained by getting the sample analyzed from different analysts and carrying out analysis on different days by proposed method.

Robustness: To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The ratio of mobile phase was varied.

3. RESULTS AND DISCUSSION:

The attempt was made to develop an alternative and economical method for simultaneous estimation of Telmisartan and Atorvastatin by high performance liquid chromatography.

Optimization of the chromatographic conditions: In order to develop RP-HPLC method for combination of cardiovascular drugs

Telmisartan and Atorvastatin 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 Phosphate buffer (10mM Phosphate buffer pH at 3.0) gave better peak shapes than their acetate 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 30 and 40% of acetonitrile in mobile phase. The best peak shape and maximum separation was achieved with mobile phase composition consisting acetate buffer-acetonitrile (60:40 v/v). The best separation, peak symmetry and reproducibility were obtained on Waters symmetry C_{18}, 250 mm x 4.6 mm, 5 µm column compared to Hypersil ODS C_{18}, 250 mm x 4.6 mm, 5 µm. The optimum wavelength for detecting both the analytes was ascertained and found to be 267 nm.

The specificity of the HPLC method is illustrated in Fig. 3 and Fig. 4, where complete separation of Atorvastatin was noticed in presence of tablet excipients and its impurities produced by alkali and thermal degradation. There were no interfering peaks of endogenous compounds observed at the retention time of the analytes.

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage recoveries obtained for Telmisartan and Atorvastatin were 99.90 and 99.66 respectively.

Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing 80%, 100% and 120% analyses of the working solution.

The intra-day, inter-day, analyst and instruments variability or precision data are summarized in Table 3. The R.S.D of the assay results, expressed as percentage of the label claim, was used to evaluate the method precision. The inter-day precision was also determined by assaying the tablets in triplicate per day. The results indicated the good precision of the developed method.

The developed method was applied to the analysis of Telmisartan and Atorvastatin in tablet dosage from marketed as Creslip (Label claim 40 mg Telmisartan and 10 mg Atorvastatin strength, Cipla Pharmaceutical Ltd.). The results of analysis are given in Table 5. The contents of marketed tablet dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Telmisartan and Atorvastatin in tablet dosage form.

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. Robustness of the method was investigated under a variety of conditions including changes of pH of the mobile phase, flow rate, percentage of acetonitrile in the 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 as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Atorvastatin 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 of 12 min, makes the developed method it's suitable for routine quality control analysis work.

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Fig. 1 Structure of Atorvastatin

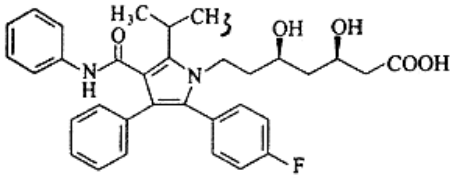

Fig. 2 Structure of Telmisartan

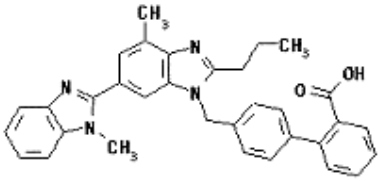

Fig. 3 Alkali degradation test solution for specificity

Fig. 4 Thermal degradation test solution for specificity

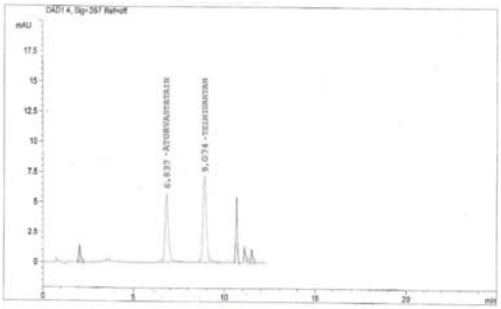

Table 1 Results of recovery analysis of Atorvastatin and Telmisartan

Compound	Wt. Spiked (%)	Wt. recovered (%)	Recovery (%)	RSD (%) n=3
Atorvastatin	50	49.89	99.78	0.023
	100	99.72	99.72	0.020
	150	149.26	99.50	0.010
Telmisartan	50	49.94	99.88	0.023
	100	99.95	99.95	0.015
	150	149.82	99.88	0.017

Table 2 System suitability parameter of Atorvastatin and Telmisartan

Parameters	Atorvastatin	Telmisartan
Theoretical plates	7420	8521
Peak Height	5.56	7.56
Peak Symmetry	0.935	0.981
USP tailing	1.028	1.027
Width at half height	0.283	0.368

Table 3 Results of precision of Atorvastatin and Telmisartan

Compound	Precision	Mean	RSD (%)
Atorvastatin	Intra day	99.66	0.007
	Inter day	99.64	0.021
	Analyst	99.67	0.014
	Instrument	99.68	0.028
Telmisartan	Intra day	100.57	0.007
	Inter day	100.56	0.014
	Analyst	100.58	0.021
	Instrument	100.58	0.014

Table 4 Results of robustness study of Atorvastatin and Telmisartan

Factor	Level	Atorvastatin Mean % assay (n=3) RSD (%)	Telmisartan Mean % assay (n=3) RSD (%)
pH of mobile phase	3	99.2 (0.209)	99.1 (0.307)
	3.2	98.8 (0.210)	98.6 (0.058)
Flow rate (ml/min)	1	99.6 (0.209)	99.55 (0.020)
	1.3	99.1 (0.308)	99.0 (0.210)
% of Acetonitrile	30	99.5 (0.058)	99.2 (0.058)
	40	100.7 (0.151)	100.3 (0.149)

Table 5 Quantitative analysis of marketed formulation of Atorvastatin and Telmisartan

Tablet Sample	Label Claim (mg)	Amount present (mg/tablet)	% Label Claim	% Deviation
ATOR	10	10.04	100.42	+0.42
TELM	40	39.65	99.12	-0.88