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SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE BESYLATE IN PHARMACEUTICAL DOSAGE FORM BY RP – HPLC

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ABSTRACT

The chromatographic analysis was performed on ODS symmetry C_{18} column (150 × 4.6 mm, 5 µ particle size) with mobile phase consisting of acetonitrile and phosphate buffer (pH 4.0) in the ratio of 60:40 v/v, at a flow rate of 1.2 mL/min and eluents monitored at 237 nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per International Conference on Harmonization (ICH) guidelines. The retention times of amlodipine besylate and telmisartan were 2.633 and 5.600 min, respectively. The calibration curves of peak area versus concentration, which was linear from 2.5-15 µg/mL for amlodipine besylate and 20-120 µg/mL for telmisartan, had regression coefficient (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of amlodipine besylate and telmisartan in tablets. The proposed method is simple, economical, accurate and precise, and could be successfully employed in routine quality control for the simultaneous analysis of amlodipine besylate and telmisartan in tablets.

Keywords: Amlodipine besylate, Telmisartan, RP-HPLC, Simultaneous analysis, Tablets

INTRODUCTION

Telmisartan, 4-[{2-n-propyl-4-methyl-6-(1-methyl benzimidazol-2-yl)-benzimidazol-1-yl} methyl]–bi phenyl-2-carboxylic acid is a new highly selective, nonpeptide angiotensin II type 1 (AT1)-receptor antagonist. Telmisartan lowers blood pressure through blockade of the rennin-angiotensin-aldosterone system (RAAS) and is widely used in the treatment of hypertension[1, 2]. Amlodipine besylate, chemically, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine-dicarboxylic acid 3-ethyl, 5-methyl ester is an antihypertensive and an antianginal agent in the form of the besylate salt, amlodipine besylate[1].

In the literature, quantitation of telmisartan in urine sample has been widely used. Also, determination of telmisartan in human plasma by liquid chromatography-tandem mass spectrometry has been reported. An RP-HPLC method for determination of telmisartan in combination with hydrochlorothiazide has been reported by Wankhede *et al*.[3] and Bhat *et al*.[4] have reported difference spectrophotometric method for determination of telmisartan. Also, Junfeng song *et al*.[5] reported linear sweep polarographic method for determination of telmisartan. Several methods for quantitative estimation of amlodipine besylate in pharmaceutical dosage form and in biological fluids have been reported in the literature. M. Joseffson *et al*.[6] have reported HPLC method for amlodipine besylate in

plasma with amperometric detection and a single step solid phase sample perparation. A. Zarghi *et al*.[7] have also reported HPLC method for amlodipine besylate in plasma. A. Ceccato *et al*.[8] reported LC-MS method for determination of amlodipine besylate in human plasma. Several RP-HPLC methods for determination of amlodipine besylate in combination with atorvastatin calcium have been reported[9-11]. D. Jain and M. R. Khan have also reported spectrophotometric method for estimation of amlodipine besylate in combination with atorvastatin calcium[12]. The aim of this work is to develop an accurate, specific, repeatable, and validated method for simultaneous determination of telmisartan and amlodipine besylate in both bulk and tablet formulations.

EXPERIMENTAL

Materials

Pure telmisartan (TEL) and amlodipine besylate (AMLO) used as working standards, were gifts from Cipla Ltd., India. Tablets containing 5 mg of AMLO and 40 mg of TEL (CRESAR AM) were obtained from Cipla Ltd., India and used within their shelf life period. Acetonitrile and water (HPLC-grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck, India.

Instrumentation

A Shimadzu HPLC system consisting of a LC-2010 CHT binary gradient pump, an inbuilt auto sampler, a column oven and dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data were acquired through the Empower-2 software. The column used was ODS symmetry C_{18} (150×4.6 mm, 5µm). A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds.

Optimized chromatographic conditions

The chromatography elution was carried out in the isocratic mode using a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.0, pH adjusted with ortho phosphoric acid) in a ratio of 60:40 v/v. The analysis performed at ambient temperature using a flow rate of 1.2 mL/min with a run time of 15 min. The eluent was monitored using DAD at a wavelength of 237 nm. The mobile phase was filtered through whatmann filter paper No.41 prior to use.

Preparation of stock and standard solutions

A stock solution of TEL and AMLO (1000 µg/mL) was prepared by taking accurately weighed 100 mg of TEL and AMLO reference standard in 100 mL volumetric flask containing 50 mL deionized water and then the volume was made up to the mark with deionized water. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of TEL and AMLO were transferred using A-grade bulb pipette into 100 mL volumetric flasks and solutions were made up to the mark with the mobile phase to give the final concentrations of 20-120µg/ml and 2.5 -15.0 µg/ml, respectively.

Estimation of telmisartan and amlodipine besylate from tablets

To determine the content of TEL and AMLO in tablets (Label claim: 40 mg & 5 mg), 20 tablets were taken and the contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask and was dissolved in 25 mL of deionized water and volume was made up to the mark with deionized water. The flask was sonicated for 25min to affect complete dissolution. The solution filtered through a 0.45 µm micro filter. A suitable aliquot of the filtered solution was transferred into a100 mL volumetric flask and made up to the volume with the mobile phase to yield the concentration of 50µg/mL for TEL and 8µg/mL for AMLO. The experiments were performed six times under the optimized chromatographic conditions described above. The peak areas were measured at 237 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity: By appropriate aliquots of the standard TEL and AMLO solution with the mobile phase, five working solutions ranging between 20-120 µg/mL and 2.5-15.0 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of TEL and AMLO to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of TEL and AMLO to which known amounts of standard TEL and AMLO

corresponding to 80,100 and 120% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of TEL and AMLO at a concentration of 40, 60, 80µg/mL and 5, 7.5, 10 µg/mL respectively. Determinations were performed with three replicates on the same day as well as on three consequent days.

Reproducibility: The reproducibility of the method was checked by determining precision on a same instrument, the analysis being performed by another person in the same laboratory. It was analyzing the samples of TEL and AMLO at different concentration 20-120 µg/mL and 2.5-15.0 µg/mL respectively were determined in triplicate and calculate the amount of drug present in the sample.

Limit of detection and the limit of quantification: Limit of detection (LOD) and limit of quantification (LOD) was calculated based on the ICH guidelines.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength and buffer pH were varied by ±2% and 0.2 units, respectively.

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20µL of the working standard solution containing 40µg/mL for TEL and 10µg/mL for AMLO were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time.

RESULTS AND DISCUSSIONS

A RP-HPLC method was proposed as a suitable method for the estimation of TEL and AMLO in the tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate was made on the basis of peak shape, baseline drift, time required for analysis, and the mobile phase consisted of acetonitrile and phosphate buffer (pH 4, adjusted pH with ortho phosphoric acid) in the ratio of 60:40 v/v at a flow rate of 1.2mL/min and analyzed at 237 nm. The retention time observed (2.633 for AMLO and 5.600 for TEL) allows a rapid determination of these drugs. In Figure 1, a typical chromatogram obtained under these conditions is shown.

The calibration plot of peak area against concentration was linear in the range of 20-120 µg/mL and 2.5 -15 µg/mL for TEL and AMLO respectively. The linear regression data for the calibration curves were indicative of a good linear relationship between peak area and concentration over a wide range (Table 1). The correlation coefficient was indicative of high significance. The LOD and LOQ were determined based on analytical responses on 3 and 10 times the background noise, respectively. The LOD and LOQ were found to be 0.027µg/mL and 0.09µg/mL, and 0.13µg/mL and 0.39µg/mL for TEL and AMLO respectively.

The accuracy was assessed from three replicates containing a concentration range of 80, 100 and 120%. The recovery of the method determined by spiking a previously analyzed test solution with standard TEL and AMLO solution, and the recovery values were found to be in the range of 99.70-99.90% and 99.70-99.76% respectively. The values of % recovery and %RSD were indicates that the method is accurate.

The precision of the method was assessed in accordance with ICH guidelines. The low %RSD (<2) values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %RSD (<2) values indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider being variations in the pH (0.2 units) and strength of acetonitrile (±2%).The results and the experimental range of the selected variables, together with the optimized conditions. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 2, showing that the parameters are within the suitable range.

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 3. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference

was observed from the tablet excipients. The TEL and AMLO content was found to be 100.21% and 99.77% respectively.

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of TEL and AMLO from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures. All these factors make this method suitable for quantification of TEL and AMLO in tablet dosage forms. The method can be successfully used for routine analysis of TEL and AMLO in bulk drugs and pharmaceutical dosage forms without interference.

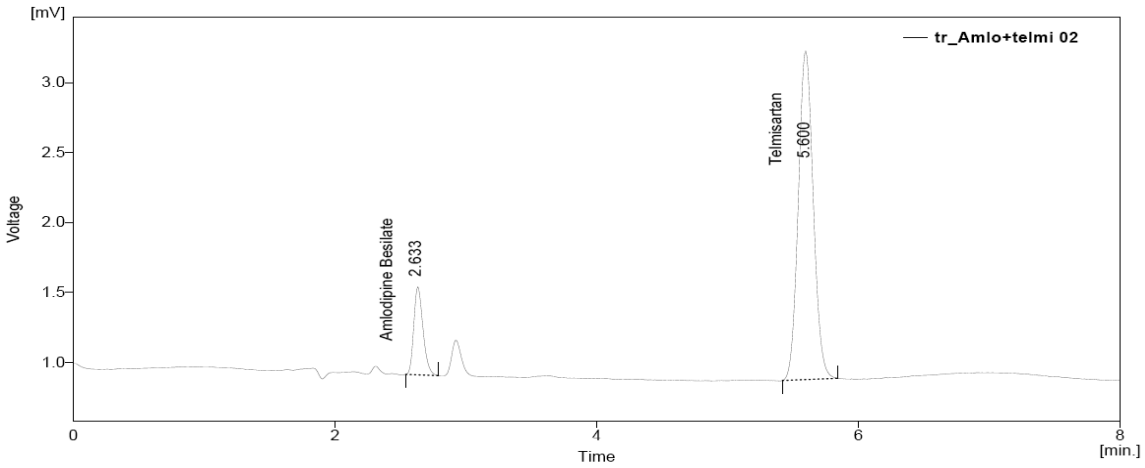

Figure 1: A typical chromatogram of amlodipine and telmisartan

Table 1: Linearity data and their analytical performances for telmisartan and amlodipine

Drug	Conc. µg/ml	Peak area	Linear Range	Correlation co-efficient	Slope(m)	Intercept
TEL	20	987.511	20-120 µg/ml	0.999	45.40	153.2
	40	2031.509				
	60	2899.555				
	80	3800.012				
	100	4717.711				
	120	5552.031				
AMLO	2.5	169.254	2.5-15 µg/ml	0.999	65.10	19.30
	5.0	347.523				
	7.5	518.159				
	10.0	680.211				
	12.5	832.696				
	15.0	985.623				

Table 2: System Suitability Parameters

Parameter	**TEL**	**AMLO**
% RSD of Retention time	0.32	0.79
% RSD of Peak area	0.14	0.21
Resolution	3.85	-----
No. of Theoretical plates	3745	3238
Tailing factor	1.71	1.06

Table 3: Estimation of amount present in tablet dosage form

Brand Name	Tablet Formulation	Label Claim per Tablet (mg)	% Label claim estimated (Mean) N=3	RSD (%)	% Drug estimated
CRESARAM	TEL	40	39.95	0.529	99.86
	AMLO	5	5.02	0.918	100.40

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