



Research Paper

RP- HPLC Method for Simultaneous Estimation of Telmisartan and Amlodipine besylate in pharmaceutical tablet dosage form

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The chromatographic analysis was performed by Hypersil BDS C18, 250 × 4.6 mm, 5 μ particle size with mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0) in the ratio of 60:40 v/v, at a flow rate of 1.5 ml/min and eluents monitored at 237 nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per ICH guidelines. The retention times of amlodipine besylate and telmisartan were 5.884 and 10.987 min, respectively. The calibration curves of peak area versus concentration, which was linear from 8-48 μg/ml for telmisartan and 1-6 μg/ml for amlodipine besylate, had regression coefficient (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of telmisartan and amlodipine besylate 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 telmisartan and amlodipine besylate in tablets.

Keywords: TLM (Telmisartan), AML (Amlodipine besylate), RP-HPLC (Reverse phase - High performance liquid chromatography), ICH (International Conference on harmonization).

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

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.²

A RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form³, difference spectrophotometric

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determination of telmisartan in tablet dosage form⁴, rapid determination of telmisartan in pharmaceutical preparation and serum by linear sweep polarographic method⁵, several other methods for quantitative estimation of amlodipine besylate in pharmaceutical dosage form and in biological fluids have been reported in the literature. Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation⁶, development and validation of simultaneous estimation of enalapril maleate and amlodipine besylate in combined dosage form⁷, new Spectrophotometric methods for simultaneous determination of amlodipine besylate and lisinoprilin tablet dosage forms⁸, Simultaneous estimation of Amlodipine Besylate and Lisinopril Dihydrate as A.P.I. and in tablet dosage forms by modified form of simultaneous equation method using derivative UV-Spectrophotometry⁹. RP- HPLC Method for Simultaneous Estimation of Losartan potassium and Amlodipine besylate in Tablet Formulation¹⁰ have been reported in literature. A Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies¹¹, enantiomeric

determination of amlodipine in human plasma by liquid chromatography coupled to tandem mass spectrometry¹², RP-HPLC Method for simultaneous determination of atorvastatin and amlodipine in tablet dosage form¹³, simultaneous spectrophotometric determination of amlodipine besylate and atorvastatin calcium in binary mixture¹⁴, simultaneous spectrophotometric determination of atorvastatin calcium and amlodipine besylate in tablet¹⁵ with Validation of analytical procedure, text and methodology¹⁶ were also reported.

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 (TLM) and amlodipine besylate (AML) were used as working standards, gifted from Balaji drugs. Pontasahib(H.P). India. Tablets containing 40 mg of TLM and 5 mg of AML were purchased from market of 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 and Ranbaxy, 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 Hypersil BDS symmetry C18, 250×4.6 mm, 5 μ m. 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 3.0, 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 1.5 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 TLM and AML(500 μ g/ml) were prepared by taking accurately weighed 50 mg of TLM and AML as reference standard in 100 ml volumetric

flask containing 50 ml of methanol and then the volume was made up to the mark with methanol. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of TLM and AML were transferred, using A-grade bulb pipette into 10 ml volumetric flasks and solutions were made up to the mark with the mobile phase to give the final concentrations of 8-48 μ g/ml and 1-6 μ g/ml of TLM and AML respectively.

Estimation of telmisartan and amlodipine besylate from tablets

To determine the content of TLM and AML in tablets (Label claim: 40 mg and 5 mg), 20 tablets were taken and the contents were weighed. 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 methanol and volume was made up to the mark with methanol. The flask was sonicated for 20 minutes to affect complete dissolution. The solution filtered through a 0.45 μ m millipore filter. A suitable aliquot of the filtered solution was transferred into a 100 ml volumetric flask and made up to the volume with the mobile phase to yield the concentration of 40 μ g/ml for TEL and 5 μ g/ml for AML. The experiments were performed six times under the optimized chromatographic conditions described

**Table 1: Linearity data and their analytical performances for TLM and AML**

Drugs	Conc. $\mu\text{g/ml}$	Peak area	Linear Range	Correlation co- efficient	Slope	Intercept
TLM	8	443703	8-48 $\mu\text{g/ml}$	1.0	56031	54
	16	890667				
	24	1343145				
	32	1782414				
	40	2263242				
	48	2689596				
AML	1	81797	1-6 $\mu\text{g/ml}$	0.999	41494	40642
	2	124356				
	3	164193				
	4	207309				
	5	248305				
	6	289265				

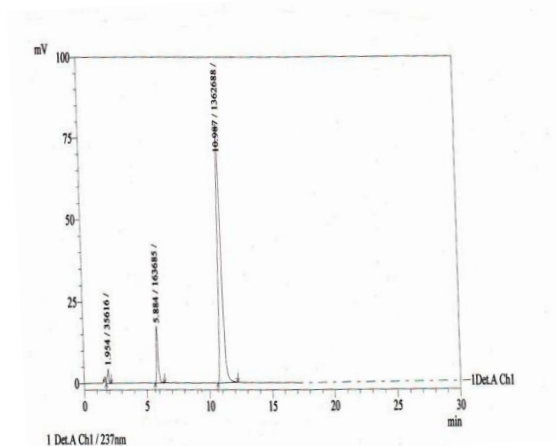
prior. The peak areas were measured at 237 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity: By appropriate aliquots of the standard TLM and AML solution with the mobile phase, six working solutions ranging between 8-48 $\mu\text{g/ml}$ and 1-6 $\mu\text{g/ml}$ 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 TLM and AML 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 TLM and AML to which

known amounts of standard TLM and AML, corresponding to 80,100 and 120%

**Fig.1: Chromatogram of TLM and AML**

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



TLM and AML at a concentration of 16, 24, 48 $\mu\text{g/ml}$ and 2, 3, 4 $\mu\text{g/ml}$ respectively. Determinations were performed with three replicates on the same day as well as on three consequent days.

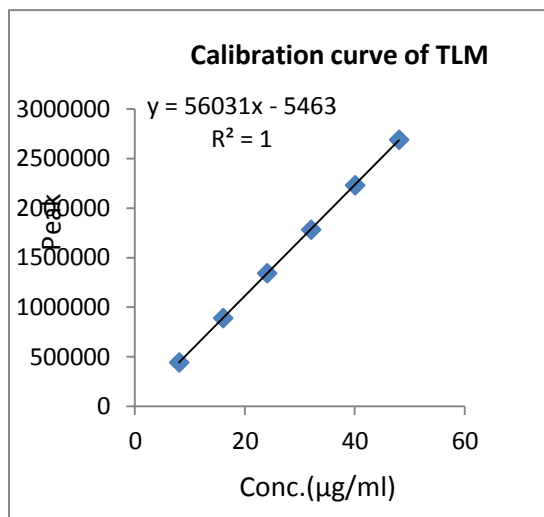


Fig.2: Linearity curve of TLM

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 TLM and AML at different concentration in between 8-48 $\mu\text{g/ml}$ and 1-6 $\mu\text{g/ml}$ in triplicate respectively and calculates the amount of drug present in the sample.

Limit of detection and the limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) 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 $\pm 2\%$ and 0.2 units, respectively.

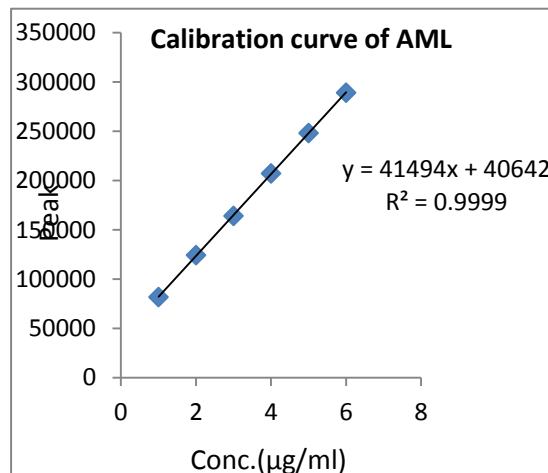


Fig.3: Linearity curve of AML

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20 μl of the working standard solution containing 40 $\mu\text{g/ml}$ for TLM and 5 $\mu\text{g/ml}$ for AML were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time.

Table 2: System suitability parameters

Parameter	TLM	AML
Retention time (min.)	10.987	5.884
Resolution	14.885	17.583
No. of Theoretical plates	10112	8966
Tailing factor	1.592	1.432



RESULTS AND DISCUSSION

A RP-HPLC method was proposed as a suitable method for the estimation of TLM and AML 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 3, adjusted pH with ortho phosphoric acid) in the ratio of 60:40 v/v at a flow rate of 1.5 ml/min and analyzed at 237 nm. The retention time observed (5.884 for AML and 10.987 for TLM) 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 8-48 µg/ml and 1-6 µg/ml for TLM and AML 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 0.0069 µg/ml and 0.0232 µg/ml, and 0.0023 µg/ml and 0.0079 µg/ml for TLM and AML 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 TLM and AML solution, and the recovery values were found to be in the range of 99.95-100.21% and 99.83-100.74% respectively. The values of % recovery and %COV were indicated that the method is accurate. The precision of the method was assessed in accordance with ICH guidelines. The low %COV (<2) values indicate that the method is precise.

Table 3: Estimation of amount present in tablet dosage form.

Brand name	Tablet Formulation	Label Claim per Tablet (mg)	% Label claim estimated (Mean) N=5	SD	%COV	% Drug estimated
CRESAR AM	TEL	40	40.06	0.312	0.3115	100.5
	AML	5	5.02	0.761	0.7684	100.4



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 %COV (<2) values indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered being variations in the pH (0.2 units) and strength of acetonitrile ($\pm 2\%$). 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 TLM and AML 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 TLM and AML 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 TLM and AML in tablet dosage forms. The method can be successfully used for routine analysis of TLM and AML in bulk drugs and pharmaceutical dosage forms without interference.

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