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Research Article

DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE IN TABLET DOSAGE FORM

SURESH KUMAR GV [a]*, RAJENDRAPRASAD Y[b]

Department of Medicinal Chemistry, St Johns Pharmacy College, Bangalore-560040, Karnataka, India, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530002, Andhra Pradesh, India. Email: gvsureshkumar@yahoo.com, dryrp@rediffmail.com

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ABSTRACT

A simple, precise and accurate reversed-phase liquid chromatographic method was developed and validated for simultaneous estimation of Telmisartan and Amlodipine in tablet formulations. The chromatographic separation was achieved on (Waters symmetry C18 250mm x 4.6mm 5µm) analytical column with mobile phase consisting mixture of Potassium dihydrogen phosphate (0.02M, pH 3.0 adjusted with ortho-phosphoric acid) and acetonitrile in ratio (60:40 v/v) at flow rate of 1.5ml/min and detector wavelength 237 nm. The retention time of Amlodipine and telmisartan was found to be 3.5 and 8.1 minutes respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both Telmisartan and Amlodipine. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.

Keywords: Telmisartan, Amlodipine, RP-HPLC, validation, assay

INTRODUCTION

Telmisartan (TE) 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, TE shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers and calcium antagonists [1,2].

Amlodipine besylate, 2[(2-aminoethoxy) methyl]-4-(2- chloro-phenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl, 5-methylester besylate is a dihydropyridine derivative with calcium antagonist activity [3,4], used mainly as an antihypertensive and antianginal agent.

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

Literature review reveals various methods for determination of Telmisartan and Amlodipine besylate, individually and in combination with other drugs. A variety of analytical methods for estimation of Amlodipine are previously reported. The majority of methods reported are liquid chromatography coupled to UV [5,6], fluorimetric [7], electrochemical [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 Amlodipine besilate 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 Amlodipine besylate in pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate isocratic reversed-phase HPLC method for simultaneous determination of Telmisartan and Amlodipine besylate in the same tablet dosage form.

EXPERIMENTAL

Chemicals

Telmisartan (94.43%) and Amlodipine (99.31%) was obtained from Dr. Reddy Laboratories, Hyderabad, India, as gift samples. Acetonitrile (HPLC Grade) and Methanol (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. While Potassium di hydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) from (S.D. fine chemicals, Mumbai, India). 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 40mg and Amlodipine 5 mg per tablet.

Equipments

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.

Liquid chromatographic conditions

Chromatographic conditions were obtained using a stainless steel column (Waters symmetry C18 250mm x 4.6mm 5µm), which was maintained at 35^0 C. The analytical wavelength was set at 237 nm and samples of 20µl were injected to HPLC system. The mobile phase was Potassium dihydrogen phosphate (0.02M, pH 3.0 adjusted with ortho-phosphoric acid) and acetonitrile in ratio of 60:40 (v/v) at a flow rate of 1.5ml/min. The mobile phase was filtered through 0.45µm filter (Sartorius, Germany) and degassed for 10 minutes by sonication.

Standard solutions and calibration graphs

Standard stock solution of Telmisartan (400 µg/ml) and amldoipine (75 µg/ml) was prepared in methanol as diluent. To study the linearity range of each component, serial dilutions were made to obtain working standards in the concentration range of Telmisartan (20-80 µg/ml) and Amlodipine (3.75 -15 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 Amlodipine (7.5 µg/ml). The system suitability test was performed from five replicate injection of mixed standard solution.

Sample preparation

Twenty tablets were weighed and finely powdered. The average weight of tablets was determined with the help of weight of 20 tablets. A portion of powder equivalent to weight of five tablets was accurately weighed into 500 ml A-grade volumetric flask and 350 ml diluent was added. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the Telmisartan and Amlodipine, the solutions were then made up to volume with diluent. The solution was filtered through 0.45 µm nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 40 µ/ml of Telmisartan and 7.5 µg/ml of Amlodipine. Twenty microlitres of the test solution was injected and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

Method validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [31]. Assay method precision was determined using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted as described in section 2.5, and were analyzed using the developed HPLC method. Linearity of test solutions were prepared as described in Section 2.4. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions of known concentrations. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 ml/min. The percentage of organic modifier was varied by (±) 5% and pH of mobile phase was varied by (±) 0.1.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In order to develop RP-HPLC method for combination of cardiovascular drugs Telmisartan and Amlodipine 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 (0.02 M-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, 35, 40 and 45 % 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 C18, 250 mm x 4.6 mm, 5 µm column compared to Zorbax C18, 250 mm x 4.6 mm, 5 µm and Inertsil C8, 250 mm x 4.6 mm, 5 µm. The optimum wavelength for detecting both the analytes was ascertained and found to be 237 nm. Peak tailing was observed for Amlodipine when the flow rate was 1.2 ml/min using optimized mobile phase conditions. However, a flow rate of 1.5 ml/min yielded optimum separation and peak asymmetry.

Validation of method

Specificity

The specificity of the HPLC method is illustrated in Fig. 1, where complete separation of Telmisartan and Amlodipine was noticed in presence of tablet excipients. There were no interfering peaks of endogenous compounds observed at the retention time of the analytes. In peak purity analysis with DAD detector (Fig. 2 and 3), purity angle was less than purity threshold for both the analytes. This shows that the peak of analytes was pure and excipients in the formulation doesn't interfere the analytes.

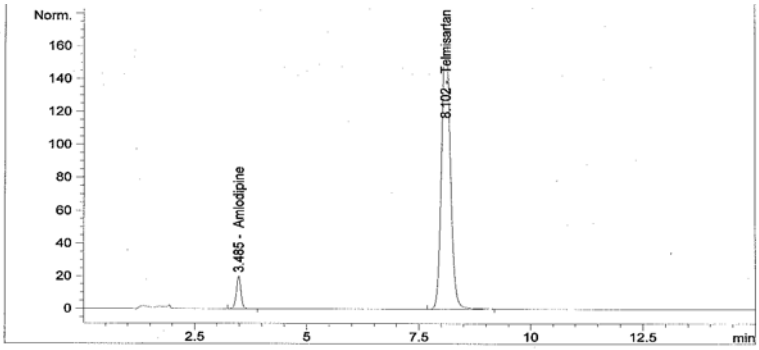

Fig 1: A typical chromatogram of test solution containing 7.5 µg/ml of amlodipine calcium and 40 µg/ml of telmisartan.

Table 1: Results of the recovery analysis of Telmisartan and Amlodipine

Compound	Wt spiked (mg)	Wt recovered (mg)	Recovery (%)	RSD (%) N=3
Amlodipine	7.52	7.50	99.73	0.52
	15.08	15.03	99.67	0.29
	22..54	22.52	99.91	0.41
Telmisartan	20.12	20.09	99.85	0.25
	80.06	80.03	99.96	0.51
	160.01	159.8	99.87	0.34

R.S.D.: relative standard deviation Wt: weight.

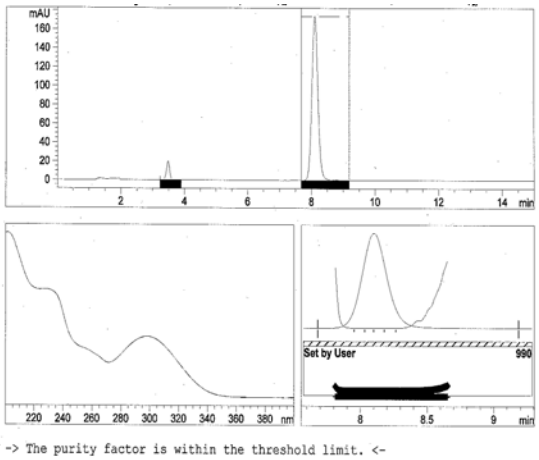

Fig 2: Purity factor of Amlodipine

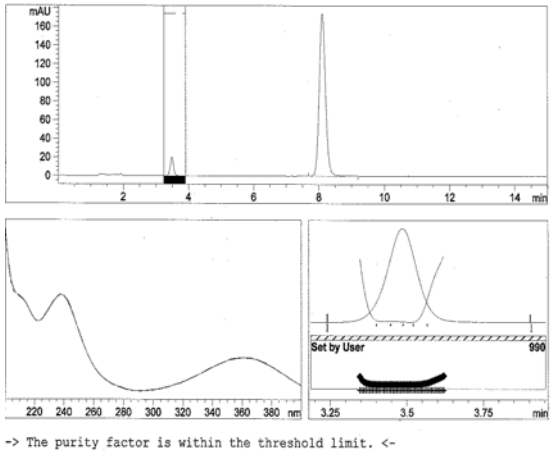

Fig 3: Purity factor of Telmisartan

Accuracy

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 Amlodipine were 99.89 and 99.77 respectively.

Precision

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 five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for obtained Telmisartan and Amlodipine was 0.37 and 0.41% respectively (Table 2).

The intra- and inter-day variability or precision data are summarized in Table 3. The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. 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 for consecutive 3 days. The results indicated the good precision of the developed method (Table 3).

Robustness

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).

Table 2: System suitability parameters

Parameters	Telmisartan	Amlodipine
Theoretical plates[a]	8602	5857
USP resolution[a]	17.37	--
peak symmetry[a]	1.1	0.96
% RSD	0.37	0.41

[a] USP-NF 29 section 621, pp.2135

Table 3: Intra and Inter-day assay precision data (n=9)

Actual concentration	Measured concentration (µg/ml), RSD. (%)	
	Intra -day	Inter-day
Amlodipine (µg/ml)		
3.27	3.26 (0.42)	3.14 (0.75)
7.53	7.57 (0.38)	7.44 (0.89)
15.11	15.07 (0.54)	15.81 (0.42)
Telmisartan (µg/ml)		
200.69	200.62 (0.43)	200.12 (0.37)
800.04	799.61 (0.58)	800.49 (0.49)
1600.11	1599.67 (0.64)	1599.37 (0.74)

Data expressed as mean for "measured concentration" values.

Table 4: Results of robustness study

Factor	Level	Amlodipine	Telmisartan
		Mean % assay (n=3) (% R.S.D.)	Mean % assay (n=3) (% R.S.D.)
pH of mobile phase	2.9	99.7 (0.38)	100.1(0.59)
	3.1	99.3(0.43)	99.9 (0.71)
Flow rate (ml/min)	1.4	99.7(1.15)	99.4(1.26)
	1.6	99.1(0.92)	99.1(0.44)
% of acetonitrile	35	99.0 (0.80)	98.6(1.25)
	45	100.9 (1.73)	99.0(1.09)

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Amlodipine 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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