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Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TELMISARTAN AND AMLODIPINE IN COMBINED DOSAGEFORM

SANTOSH KUMAR M*, VENKATESHWAR RAO JUPALLY

Talla Padmavathi College of Pharmacy, Urus, Warangal. Email: mantrisantosh@hotmail.com

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ABSTRACT

Objective: To develop a new stability indicating reversed phase high-performance liquid chromatographic method and to validate for the simultaneous determination of two antihypertensive drugs viz. telmisartan and amlodipine. Methods: Chromatography was carried out on a reversed-phase Hypersil BDS C_{18} Column (100 x 4.6 mm, 5µ.) with mobile phase consisting of a mixture of Buffer (pH was adjusted to 3.6) and Acetonitrile taken in the ratio 60:40, and flow rate of 1 mL/min. The UV detection was performed at 234 nm for telmisartan and amlodipine. The stability-indicating capability of the method was demonstrated through adequate separation of aged and stress degraded telmisartan and amlodipine stability samples. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) and robustness were determined according to International Conference on Harmonization (ICH Q2B) guidelines. Results: The linearity of the calibration curves for each analyte in the desired concentration range is good (r^2 >0.999). The recovery of the method was between 100.46% and 99.91 % for telmisartan and amlodipine respectively. Conclusion: The proposed stability indicating method is rapid, easy, highly sensitive, precise and accurate and it can be successfully applied to estimate the amount of telmisartan and amlodipine in the formulations by easily available low cost materials.

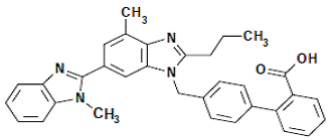
Keywords: RP-HPLC, Telmisartan and Amlodipine, Stability indicating Assay, method development, validation.

INTRODUCTION

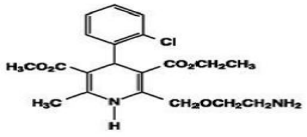
Hypertension, also referred to as high blood pressure, is a condition in which the arteries have persistently elevated blood pressure. Recent study show that treatment with a single-pill combination of telmisartan, an angiotensin receptor blocker(ARB) and amlodipine, a calcium channel blocker (CCB) results in significant reductions in blood pressure (BP) in patients with severe hypertension.

Amlodipine is a calcium channel blocker. Amlodipine relaxes (widens) blood vessels and improves blood flow. It is chemically described as 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate. The structure is given in figure 1.

Telmisartan is an angiotensin II receptor antagonist. Telmisartan keeps blood vessels from narrowing, which lowers blood pressure and improves blood flow. Telmisartan is a non-peptide molecule and chemically described as potassium 4'-[(1,7'-dimethyl-2'-propyl-1H,3'H-2,5'-bibenzimidazol-3'-yl)-methyl]biphenyl-2-arboxylate. The structure is illustrated in figure 1 [1].



Telmisartan



Amlodipine

Fig. 1: Structures of Telmisartan and Amlodipine

Literature survey suggests that a variety of spectrophotometric and chromatographic methods including UV, colorimetric determination, ratio derivative, and a stability indicating HPLC methods have been reported for determination Telmisartan and Amlodipine either single or in combination with other drugs [2-7]. Whereas no stability indicating HPLC method has been reported for simultaneous quantitative determination of Telmisartan and Amlodipine in the combined dosage form.

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH) [8] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

The aim of the present study was to demonstrate inherent stability of Telmisartan and Amlodipine through stress studies under a variety of ICH recommended test conditions [9–10] and to develop and validate a rapid stability-indicating reverse phase assay method [11–13].

MATERIALS AND METHODS

Materials and Reagents: Telmisartan and Amlodipine were supplied by Clearsynth Labs, Mumbai. Other reagents such as Acetonitrile, Orthophosphoric acid Triethylamine and water used were of HPLC and milli-Q grade.

Chromatography conditions: Chromatographic separation was performed on a HPLC (Alliance with PDA detector) at the wavelength of 234 nm. A reverse phase Hypersil BDS C_{18} (100 x 4.6 mm, 5µ.) column was used with mobile phase mixture of Buffer (the pH was adjusted to 3.6) and Acetonitrile taken in the ratio 60:40, and flow rate of 1 mL/min. injection volume was 10µl and the chromatographic runtime of 8 min. was used.

Preparation of buffer solution: Accurately weighed and transferred 1ml of Concentrated Orthophosphoric acid in 1000ml of Volumetric flask and about 900ml of milli-Q water added and subjected to sonication for degassing and finally made the volume with water then added 0.5ml of Triethylamine then pH adjusted to 3.6 with dil. Orthophosphoric acid solution.

Preparation of mobile phase: 1000 mL of mobile phase was prepared by mixing 600ml of buffer and 400ml of Acetonitrile.

Preparation of Standard solution: Accurately Weighed and transferred 10mg of Amlodipine and 10mg of Telmisartan working Standards into a 10 ml clean, dry volumetric flask, added 7ml of diluent (methanol : water:: 80:20) , sonicated for 5 minutes and made up to the final volume with diluent.

Preparation of Sample solution: five tablets were weighed and calculated the average weight then the weight equivalent to one tablet was transferred into a 100 mL volumetric flask, 80mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation: Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy, Limit of detection and Limit of quantification.

Stability studies: The stability studies were carried out by attempting deliberate degradation of the sample with exposure to stress conditions like acidic (2N HCl), alkaline (2N NaOH), 105^{0}C dry heat, oxidizing agents (H_2O_2) and Water.

Oxidation: To 1 ml of stock solution of Amlodipine and Telmisartan, 1 ml of 20% hydrogen peroxide (H_2O_2) was added. The solution was kept for 30 min at 60^{0}C. For HPLC study, the resultant solution was diluted to obtain 100µg/ml & 12.5µg/ml concentration and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock solution Amlodipine and Telmisartan, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0}C .The resultant solution was diluted to obtain 100µg/ml & 12.5µg/ml concentration and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Amlodipine and Telmisartan, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^{0}C. The resultant solution was diluted to obtain 100µg/ml & 12.5µg/ml concentration and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105^{0}C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml & 12.5µg/ml concentration and10µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the drug stock solution to UV Light by keeping the beaker in UV Chamber for 6h. For HPLC study, the resultant solution was diluted to obtain 100µg/ml & 12.5µg/ml concentration and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Precision: The precision of the method was evaluated by carrying out six independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

Accuracy: Accuracy for the assay of Telmisartan and Amlodipine determined by applying the method in triplicate samples to which known amount of Telmisartan and Amlodipine standard was added at different levels (50%, 100%, and 150%). Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

Linearity: The Linearity of detector response was established by plotting a graph of concentration versus area of Telmisartan and Amlodipine standard and determining the correlation coefficient. A series of solution of Telmisartan and Amlodipine standard solution in the concentration ranging from about 10–150 ppm of Telmisartan and 1–20 ppm of Amlodipine respective levels of the target concentration were prepared and injected into the HPLC system. (Correlation coefficient should be not less than 0.999.)

Limit of Detection (LOD) Limit of Quantification (LOQ): LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

RESULTS AND DISCUSSION

Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, and pH of mobile phase were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, peak tailing, theoretical plates, and run time were the major tasks while developing the method. Buffers like sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate did not yield the desired results. The composition of mobile phase Acetonitrile: Buffer (80:20v/v) of pH 3.6 with flow rate of 0.8ml/min and at runtime of 6min shown merging of two drugs. The composition of mobile phase Acetonitrile: Buffer (70:30v/v) of pH 3.6 with flow rate of 1ml/min and runtime of 15 min yielded peaks with non-sink in the base line with unstable retention times.

At Acetonitrile: Buffer (40:60v/v) of pH 3.6 with flow rate of 1ml/min and detection at 234nm of runtime of 8min, a perfect chromatogram was eluted. The typical chromatogram obtained from final HPLC conditions are depicted in Figure 2.

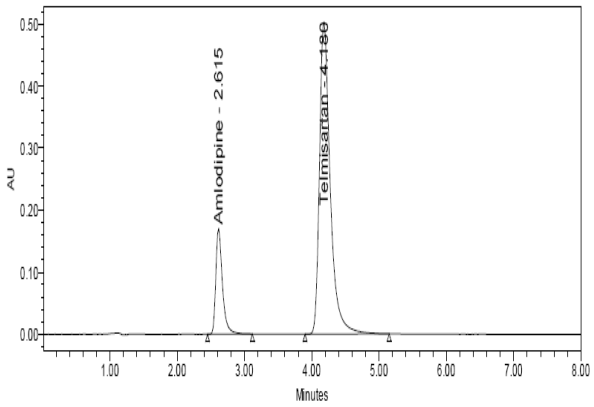


Figure 2: Typical chromatogram of Amlodipine and Telmisartan by proposed method

Method validation: Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to specificity, system suitability, linearity, accuracy, precision, LOD and LOQ as follows.

Stability studies

Oxidation: For hydrogen peroxide-induced degradation, The Fig.3. Shows the major degradation found at RT 1.084. All the major and minor degradation products were well separated from Telmisartan and Amlodipine peaks. The peak purity is checked for Telmisartan and Amlodipine and the results are summarized in Table 1.

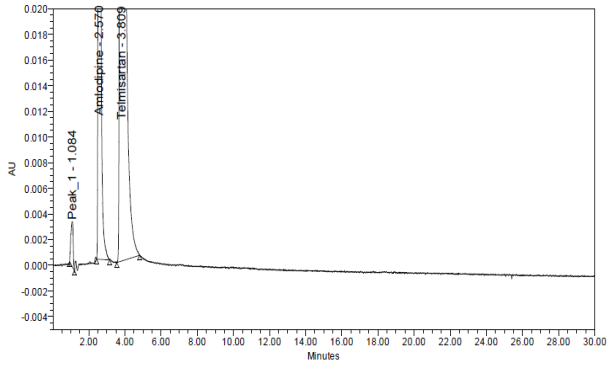


Figure 3: chromatogram for peroxide induced degradation

Acid Degradation Studies: For acid induced degradation, The Fig.4. Shows the major degradation found at RT 1. 934. All the major and minor degradation products were well separated from Telmisartan and Amlodipine peaks. The peak purity is checked for Telmisartan and Amlodipine and the results are summarized in Table 1.

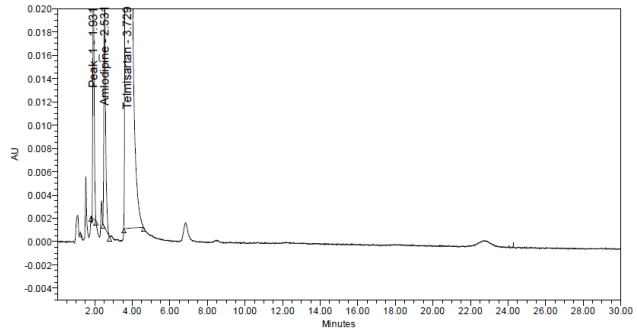


Figure 4: chromatogram for acid induced degradation

Alkali Degradation Studies: The study in basic solution was depicted in figure 5, which shows the major degradation found at RT 1. 434. All the major and minor degradation products were well separated from Telmisartan and Amlodipine peaks. The peak purity is checked for Telmisartan and Amlodipine and the results are summarized in Table 1

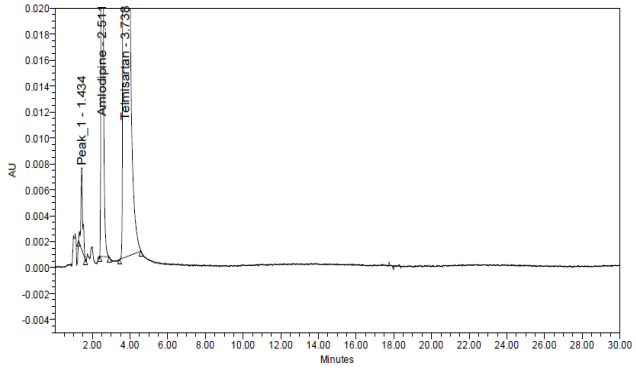


Figure 5: chromatogram for Alkali induced degradation

Dry Heat Degradation Studies:

For heat induced degradation, the figure 6 shows the major degradation found at RT 1. 096. All the major and minor degradation products were well separated from Telmisartan and Amlodipine peaks. The peak purity is checked for Telmisartan and Amlodipine and the results are summarized in Table 1

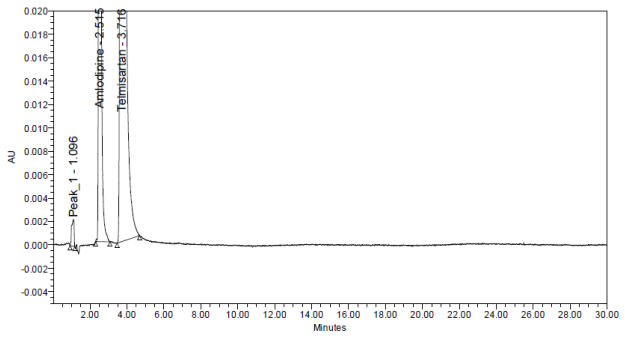


Figure 6: chromatogram for dry heat induced degradation

Photo Stability studies: The drugs Telmisartan and Amlodipine are stable under photolytic conditions and the corresponding chromatogram depicted in fig.7. The peak purity is checked for Telmisartan and Amlodipine and the results are summarized in Table 1.

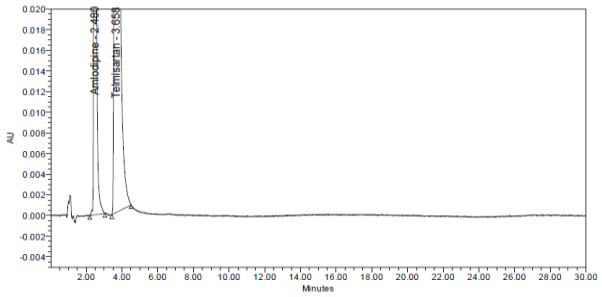


Figure 7: chromatogram for photolytic degradation

Table 1: Peak purity results of Telmisartan and Amlodipine

Study	Purity Angle		Purity Threshold	
	Telmisartan	Amlodipine	Telmisartan	Amlodipine
Oxidation	0.051	0.143	0.289	0.356
Acid Degradation	0.053	2.969	0.289	0.933
Alkali Degradation	0.066	0.343	0.297	0.522
Dry Heat Degradation	0.062	0.191	0.299	0.415
Photo Stability	0.051	0.179	0.286	0.377

Precision: The % R.S.D of Telmisartan and Amlodipine assay during the method precision was found to be 0.42% and 0.40% respectively, indicating excellent precision of the method. The results are summarized in table 2.

Table 2: Results of precision

S.No	Telmisartan Peak area	Amlodipine Peak area
1	5578884	1164330
2	5584869	1166733
3	5588253	1165339
4	5643296	1177530
5	5600428	1170074
6	5589537	1166791
AVG	5597544	1168466
SD	23498.1	4846.5
%RSD	0.42	0.40

Accuracy: Percent recovery of Amlodipine samples ranged from 99.51% to 100.12%, and the Percent recovery of Telmisartan samples ranged from 99.64% to 101.09% showing the good accuracy of the method. The result is shown in Table 3.

Table 3- Summary results of Accuracy for Telmisartan and Amlodipine

AMLODIPINE						
Recovery Level	Resultant solution (ppm)	Standard Injections inj-1	inj-2	inj-3	% Recovery	%RSD
50%	6.25	198919	198819	199324	99.5165	0.77877
100%	12.5	265327	263596	264886	100.1282	0.78835
150%	18.75	328571	328226	332412	100.1025	0.76921

TELMISARTAN						
Recovery Level	Resultant solution (ppm)	Standard Injections inj-1	inj-2	inj-3	% Recovery	%RSD
50%	50	7994720	7969916	8013199	101.0993	0.78213
100%	100	10634142	10667001	10661007	100.6596	0.79654
150%	150	13231357	13248359	13246609	99.6416	0.77254

Limit of Detection (LOD) Limit of Quantification (LOQ): The LOD of Telmisartan and Amlodipine were found to be 0.10µg/ml and 0.19µg/ml respectively. The LOQ was 0.31 µg/ml and 0.58µg/ml for Telmisartan and Amlodipine respectively. Since the LOQ and LOD values of Telmisartan and Amlodipine achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 10-150 ppm for Telmisartan and 1-20 ppm for Amlodipine three times, and the correlation coefficient obtained was 0.9999 for both the drugs, thus indicating excellent correlation between peak areas and concentrations of the analytes.

CONCLUSION

The developed RP-HPLC method proves to be simple, linear, precise, accurate and specific. The total runtime was 8 minutes within which two drugs and their degradation products were separated. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs Telmisartan and Amlodipine in presence of degradation products by the industry. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with other available HPLC methods. The shorter run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control method of Telmisartan and Amlodipine in combined dosage forms and as well as for single drug analysis.

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