Research Article

Stability Indicating Spectrophotometric Method of Amlodipine and Telmisartan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Two simple, rapid, precise and accurate spectrophotometric methods have been developed for determination of Amlodipine Besylate and Telmisartan by simultaneous equation method and stability study method in combined dosage form. The simultaneous equation method is based on measurement of absorbance at 242 nm and 231 nm as two wavelengths selected for quantification of Amlodipine and Telmisartan. Both methods obeyed Beer's law in the concentration range of 5-30 µg/ml for Amlodipine and 2-7 µg/ml for Telmisartan. Amlodipine and Telmisartan were subjected to stress degradation under different conditions recommended by ICH. The samples so generated were used for degradation studies using the developed method. The proposed methods were validated and can be used for analysis of combined dosage tablet formulation containing Amlodipine and Telmisartan.

Keywords: Amlodipine Besylate (AMB), Telmisartan (TEL), Simultaneous equation method, Method development, Stability study, UV-visible spectrophotometer.

INTRODUCTION

Amlodipine besylate (AMB) is a calcium channel blocker, chemically it is [3-ethyl-5-methyl(4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzenesulfonate. fig.1.[1]

Telmisartan (TEM) is an angiotensin receptor blocker, chemically it is 4'-[(1,4'- dimethyl – 2'-propyl [2,6' –bi-1Hbenzimidazol] - 1'-yl) methyl] [1,1'- biphenyl] - 2-carboxylic acid[24]. fig.1.[1]
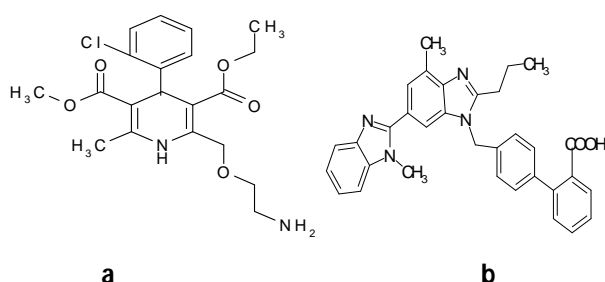
Figure 1: Chemical structure of (a) Amlodipine Besylate & (b) Telmisartan

The literature review reveals that few analytical techniques like RP-HPLC[2], HPTLC[3,4], HPLC[5], Spectroscopic methods[6,7] have been reported for the determination of Telmisartan and its combination with other drugs in pharmaceutical dosage forms. Estimation of Amlodipine in combination with other drugs by spectrophotometric methods[8-11], LC-MS[12], HPLC[13-15], RP-HPLC[16], have been reported. But there are no reported simultaneous estimation methods for Telmisartan and Amlodipine combined dosage form 0.1 N NaOH, as the combined dosage form is not official in any pharmacopoeia.

A combination of Amlodipine and Telmisartan has been reported to show substantial and sustained 24 hour blood pressure (BP) reduction and is well-tolerated in a range of patients with hypertension and at risk of cardiovascular (CV) events. Amlodipine and Telmisartan are available in combined tablet dosage form for the treatment of hypertension. Not a single UV method is reported so far for the simultaneous analysis and stability study of Amlodipine and Telmisartan in their combined dosage form in 0.1N Sodium hydroxide. So a need was felt to develop new methods to analyze these drugs simultaneously. A successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. In this article simple, rapid, accurate, reproducible and economical methods have been described for the simultaneous determination of Amlodipine and Telmisartan in tablet formulations using simultaneous equation method and Stability study method.

MATERIALS AND METHODS

Instrumentation

An UV-visible double beam spectrophotometer (JASCO V-630) with Matched quartz cells corresponding to 1 cm path length. AUX – 220 single pan electronic balance was used for weighing the materials.

Reagent and chemicals

Pure samples of Amlodipine besylate (Micro labs ltd. India), and Telmisartan (Glenmark Pharmaceuticals Ltd., India) were used in the proposed spectrophotometric analysis. TAZLOC-AM (The Madras Pharmaceuticals, Chennai, India) was used as a pharmaceutical dosage form labeled to contain 5 mg AMB and 40 mg of TEL per tablet. Sodium hydroxide (Analytical grade) was procured

from SD Fine Chemicals, Mumbai. All the chemicals used were of analytical grade and were used without further purification. Distilled water was used as a solvent throughout the study. All chemicals and reagents used are of analytical reagent grade.

Preparation of stock solution

Amlodipine besylate

Standard stock solution of Amlodipine besylate was prepared by dissolving 10 mg Amlodipine besylate in 50 ml of 0.1N sodium Hydroxide and sonicates for 20 min and thin dilute up to 100 ml to produce a concentration of 100 µg/ml. which is the standard stock solution.
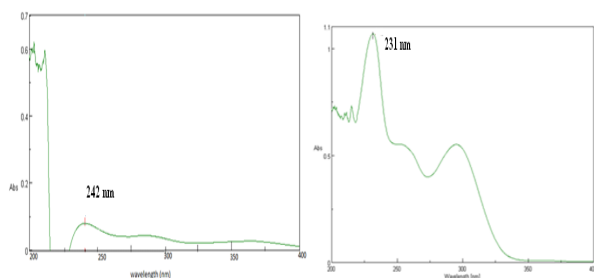
Telmisartan

Standard stock solution of Telmisartan was prepared by dissolving 10 mg of Telmisartan in 100 ml of 0.1N sodium Hydroxide to produce a concentration of 100 µg/ml. which is the standard stock solution.

Mixture (Marketed tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 5 mg and 40 mg of Amlodipine and Telmisartan respectively and transferred into 100 ml volumetric flask containing 50 ml Sodium Hydroxide, sonicate for 15 min., volume was adjusted to mark with same solvent up to 100 ml to produce a concentration 50 µg/ml of AMB and 400 µg/ml of TEL. Then filter with Whatmann filter paper No.41. And these filtrate used for further studies.

Determination of λmax

Standard stock solution containing 100 µg/ml of was prepared of Amlodipine and Telmisartan in 0.1N sodium Hydroxide. Different aliquots were taken from the stock, diluted to 10ml mark with same solvent to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. Amlodipine and Telmisartan showed absorbance maxima at 242 nm and 231 nm respectively. The optical characteristic and linear regression data is summarized in table 1 and fig.2.

Figure 2: λmax of Amlodipine and Telmisartan shows at 242 nm & 231 nm respectively.

Method development

Simultaneous Equation Method[17, 18]

Two wavelengths selected for the 242 nm and 231 nm absorption maxima of AMB and TEL respectively. From stock solution of Amlodipine pipette out 3 ml of solution in each three 10 ml volumetric flask and dilute up to 10 ml with 0.1 N NaOH. The concentration of solution become 30 µg/ml. From stock solution of Telmisartan pipette out 0.7 ml in each three 10 ml of volumetric flask and dilute up to 10 ml with 0.1N NaOH. The concentration of solution becomes 7 µg/ml. From stock solution of mixture pipette out 1 ml each three 10 ml volumetric flask and dilute up to 10 ml with 0.1 N NaOH. The concentration of solution becomes 10 µg/ml. This solution was measured at selected wavelength and absorptivity was determined as a mean of three independent determinations. Concentration of the drug in the samples was obtained using the following equation:

$$Cx = \frac{A1\, ay2 - A2\, ay1}{ax1\, ay2 - ax2\, ay1}$$

$$Cy = \frac{A1\, ax2 - A2\, ax1}{ay1\, ax2 - ay2\, ax1}$$

Where,

A1 and A2 are absorbance of mixture at λ1 and λ2 respectively,

ax1 and ax2 are the absorptivities of AMB at λ1 and λ2 respectively and

ay1, ay2 are the absorptivities of TEL at λ1 and λ2 respectively.

Cx and Cy are the concentrations of AMB and TEL in µg/ml respectively.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Ruggedness, Limit Of Detection (LOD) and Limit Of Quantification (LOQ) as per the ICH (International Conference on Harmonization).[19,20]

Linearity

Various aliquots were prepared form the stock solution (100µg/ml) ranging from 1- 30 µg/ml. The samples were scanned in UV-VIS Spectrophotometer using Sodium hydroxide as blank. It was found that the selected drug Amlodipine shows linearity between the 5-30 µg/ml and Telmisartan shows between the 2-7 µg/ml. The optical

characteristic and linear regression data is summarized in table 1, calibration curve shown in fig.3.

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 10µg/ml were prepared and analyzed three times in a day i.e. morning, afternoon and evening and the absorbance were noted. The result was indicated by % RSD. In the interday variation study, solutions of same concentration 10µg/ml were prepared and analyzed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD in table 1.

Accuracy

The accuracy of an analytical procedure express the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant i.e. 100% and the amount of pure drug of Amlodipine and Telmisartan was added for 80%, 100% and 120% in marketed formulation respectively. The solutions were prepared and the accuracy was indicated by % recovery. The recovery values for Amlodipine and Telmisartan ranged from 97% to 103% shown in table 1.
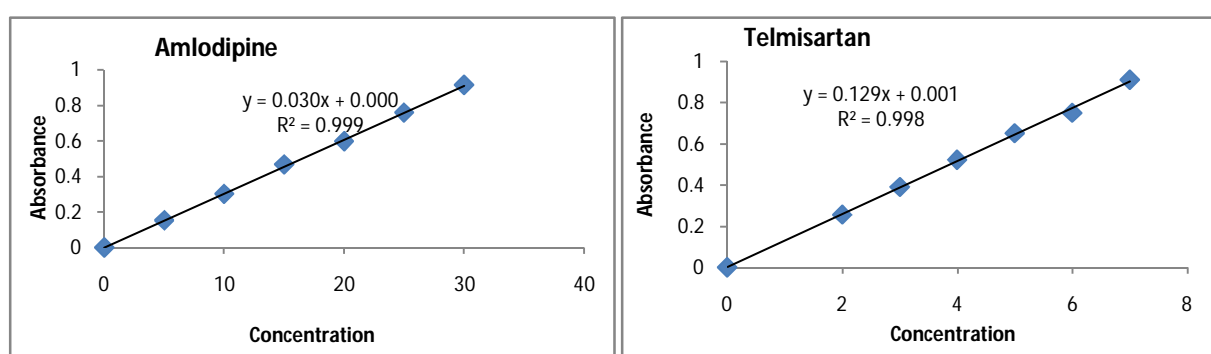

Figure 3: Callibration curve for Amlodipine and Telmisartan in 0.1 N NaOH

Table 1: Summary of optical characteristic and method validation result of Amlodipine and Temisartan

Parameter	UV-Spectrophotometric					
	AMB			**TEL**		
λ max (nm)	242			231		
Beer's Law limits (µg/ml)	5-30 (µg/ml)			2-7 (µg/ml)		
Regression equation (*Y)	0.030x+0.000			0.129x+0.001		
Slope (m)	0.030			0.129		
Intercept (c)	0.000			0.001		
Standard deviation	0.2978			0.2433		
Correlation co-efficient (r)	0.999			0.998		
	% Mean	S.D	%R.S.D	% Mean	S.D	%R.S.D
	99.74	0.1630	0.16	99.78	0.03	0.030
	99.41	0.3569	0.35	99.64	0.04	0.040
Accuracy						
Level of % recovery	% recovery of dugs		Standard Deviation		%R.S.D	
	AMB	TEL	AMB	TEL	AMB	TEL
80	97.21	99.90	0.01	0.02	0.010	0.020
100	101.79	102.38	0.005	0.10	0.0049	0.0976
120	101.65	102.84	0.01	0.04	0.0097	0.0388
	AMB			TEL		
LOD	1.82 mg/ml			1.37 mg/ml		
LOQ	5.52 mg/ml			4.17 mg/ml		

Table 2: Analysis data of marketed formulation

Label claim (mg/tablet)		Amount found (mg/ml)		% of label claim estimated	
Amlodipine	Telmisartan	Amlodipine	Telmisartan	Amlodipine	Telmisartan
5	40	4.96	39.64	98.8	99.1

Limit of detection (LOD)

The limit of detection (LOD) was determined based on standard deviation of response of the calibration curve. The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOD. The result was shown in table 1.

LOD=3.3 X S.D/S

Where;   S.D=standard deviation

            S=slope of absorbance of calibration curve

Limit of quantification (LOQ)

The limit of quantification (LOQ) was determined based on standard deviation of response of the calibration curve. The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOQ. The result was shown in table 1.

LOQ=10 X S.D/S

Where;   S.D=standard deviation

            S=slope of absorbance of calibration curve

Assay of marketed formulation (Tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 5 mg and 40 mg of Amlodipine and Telmisartan respectively and transferred into 100 ml volumetric flask containing 50 ml Sodium Hydroxide, sonicate for 15 min., volume was adjusted to mark with same solvent up to 100 ml to produce a concentration 50 µg/ml of AMB and 400 µg/ml of TEL. Then filter with Whatmann filter paper No.41. And these filtrate used for further dilutions using 0.1 N NaOH. Calculate amount found (mg/ml) and % of label claim estimated of AMB and TEL in marketed formulation. The result was shown in table 2.

Degradation studies

The International Conference on Harmonization (ICH)[19,20] guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Amlodipine and Telmisartan (Mixture) using the method developed.

### Stress degradation by hydrolysis under acidic condition

To 1ml of stock solution of Amlodipine and Telmisartan (tablet), 5ml of 1N HCL was added in 10ml of volumetric flask and kept for 3hours. After 3 hours solution was diluted with 0.1N sodium hydroxide up to the mark and the solution was taken in cuvette for the UV-VIS Analysis.[21] the result was shown in table 6. Acidic degradation of Amlodipine and Telmisartan (tablet) after 3 hours shown in fig.4(A).

Stress degradation by hydrolysis under basic condition

To 1ml of stock solution of Amlodipine and Telmisartan (tablet), 5ml of 1N NaoH was added in 10ml of volumetric flask and kept for 3 hours. After 3 hours solution was diluted with 0.1N sodium hydroxide up to the mark and the solution was taken in cuvette for the UV-VIS Analysis.[21] the result was shown in table 6. Basic degradation of Amlodipine and Telmisartan (tablet) after 3 hours shown in fig.4(B).

Oxidative degradation

To 1ml of stock solution of Amlodipine and Telmisartan (tablet), 5ml of 6% Hydrogen Peroxide was added in 10ml of volumetric flask and kept for 3 hours. After 3 hours solution was diluted with 0.1N sodium hydroxide up to the mark and the solution was taken in cuvette for the UV-VIS Analysis.[21] the result was shown in table 6. Oxidative degradation of Amlodipine and Telmisartan (tablet) shown in fig.4(C).
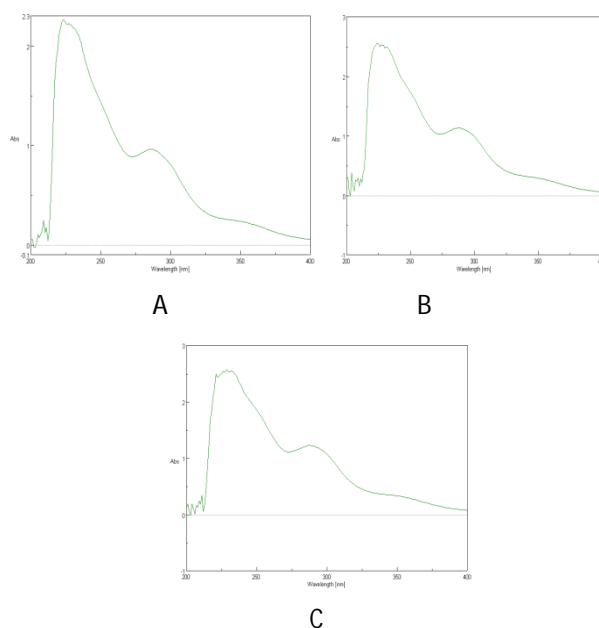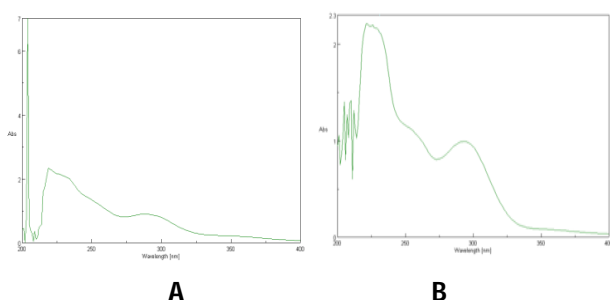

Figure 4: Acidic degradation (A), Basic degradation (b) & Oxidative degradation of marketed formulation (tablet) after 3 hr

Photolytic degradation

Photolytic degradation Sample of Amlodipine and Telmisartan (tablet) was exposed to near ultraviolet lamp in photostablity chamber at 190-320 nm for 3 hour. After that 387 mg of sample was dissolved 0.1N NaOH and volume made up to 100 ml. From this solution appropriate dilution was made using 0.1N NaOH and the solution was taken in cuvette for the UV analysis. [21] The result was shown in table 6.Photolytic degradation shown in fig.5(A).

Dry heat induced degradation

Amlodipine and Telmisartan (tablet) sample was taken in a Petri plate and exposed to a temperature of 50°c for 48 hours in an oven. After 48 hours, 387 mg of the sample was diluted with 0.1N NaOH in order to make the volume up to 100 ml. From this solution, dilutions were carried out and the solution was taken in cuvette for the UV-VIS Analysis.[21]The result was shown in table 6. Dry heat induced degradation of Amlodipine and Telmisartan (tablet) shown in fig.5(B).

A   **B**

Figure 5: Photolytic degradation (A) of marketed formulation (tablet) after 3 hr & Dry heat induced degradation (B) of marketed formulation (tablet) after 48 hr

Table 3: Result of Degradation study Amlodipine of Telmisartan

Stress condition	Time	% Assay of active substance of drugs	
		Amlodipine	**Telmisartan**
Acidic degradation	3 h	97.92%	85.28%
Basic degradation	3 h	85.92%	94.51%
Oxidative degradation	3 h	89.87%	98.92%
Photolytic degradation	3 h	95.13%	85.11%
Dry heat induced degradation	48 h	95.13%	88.91%

RESULTS AND DISCUSSION

The proposed methods of analysis for AMB and TEL in combination were validated as per the recommendations of ICH guidelines for parameter like accuracy, precision, linearity, and range, limit of detection and limit of quantification. The drugs obeys Beer's law in concentration range of 5-30 µg/ml for AMB and 2-7 µg/ml for TEL with correlation coefficient greater than 0.999. Precision of both the methods were calculated by

intraday and inter day variation study and %RSD of observation were found to be less than 2, shown in table 1. Results of recovery studies were within 97-103%, shown in table 1. The stress degradation studies showed that Amlodipine and Telmisartan (Marketed formulation) i.e. tablet undergoes degradation in acidic, alkaline, oxidative, photolytic conditions whereas it is relatively stable when exposed to dry heat, conditions. Summary of the results of stress degradation studies of Amlodipine and Telmisartan are shown in the Table 3.

CONCLUSION

A simple, accurate, precise, robust and rapid UV visible spectrophotometric method has been developed for simultaneous estimation of AMB and TEL in pharmaceutical dosage form. The method has been validating for AMB and TEL in pharmaceutical dosage form. The present study was concluded to understand the degradation behavior of AMB and TEL under ICH recommended stress conditions. This study is an example of the development of a stability indicating assay method for AMB and TEL where forced degradation was carried out under all stress conditions. These drugs showed degradation in all of the studied conditions. However the extent of degradation was different. The method developed for quantitative determination of AMB and TEL is rapid, precise, accurate and selective. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of AMB and TEL in bulk and pharmaceutical formulation. The developed method can be conveniently used for the assay determination of in bulk AMB and TEL drugs and pharmaceutical dosage form.

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