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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF AMLODIPINE BESYLATE FOLLOWING ICH GUIDELINES AND STUDY OF ITS DEGRADATION PROFILE

Priyanka S. Gawarkar*, Pratibha S. Gavarkar, Shrinivas K. Mohite, Rahul S.Adnaik

Rajarambapu College of Pharmacy, Kasegaon, Tal. Walwa, Dist. Sangli, (M S), India

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For Correspondence:

Priyanka S. Gawarkar

Rajarambapu College of Pharmacy, Kasegaon, Tal. Walwa, Dist. Sangli, (M S), India

E-mail:

pgawarkar123@gmail.com

ABSTRACT

A simple spectrophotometric method has been developed for the estimation of Amlodipine besylate using double distilled water as a solvent. Amlodipine besylate is an anti-hypertensive and an antianginal agent. Amlodipine besylate has shown maximum absorption at 358 nm. The calibration was found to be linear in the concentration range of 2-18 µg/ ml, with regression value of 0.9966. Recovery studies were carried out and the average percentage recovery of the sample was found to be 100.07%. Thus the method was found to be accurate. Precesion study was carried and expressed in terms of %RSD, which was found to be less than 2%. So the method was precise. Method is validated according to ICH guidelines and can be adopted for the routine analysis of Amlodipine besylate in pure and tablet dosage form. The drug was subjected to oxidation, hydrolysis, heat and photolysis to apply stress conditions. Degradation products resulting from stress studies did not interfere with the detection of Amlodipine besylate.

INTRODUCTION

Amlodipine besylate 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 benzene sulfonate [1]. Amlodipine besylate is a dihydropyridine calcium channel blocker. Amlodipine besylate is a calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure [3]. Amlodipine Besylate is a peripheral arterial vasodilator that acts directly on the vascular smooth muscle to cause a reduction in peripheral vascular resistance and in blood pressure. Amlodipine Besylate is official in the Indian Pharmacopoeia, British Pharmacopoeia [2].

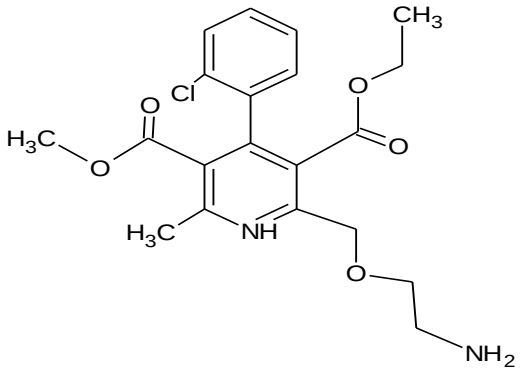


Figure -1: Structure of Amlodipine Besylate

Literature survey reveals the availability of several methods for estimation of Amlodipine besylate includes UV [4-6], HPLC [6-8], HPTLC [9] as alone or in combination with other drugs. Spectrophotometric methods [4-7], Stability indicating RP-HPLC method [10-12]. However there is no method reported for the detection of Amlodipine besylate in bulk and pharmaceutical formulation by UV spectrophotometry.

MATERIALS AND METHODS

Apparatus

Spectral runs were made on a Shimadzu UV-Visible spectrophotometer, model- 1800 was employed with spectral bandwidth of 0.5 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells for all analytical work.

Reagents and chemicals

Amlodipine besylate was obtained from Ipca Laboratories,Mumbai, India as a gift sample and was used as working standards. Methanol AR and double distilled water were used throughout the analysis. All the solutions were protected for light and were analyzed on the day of preparations.

Commercial formulation:

A commercial pharmaceutical preparation of Amlodipine besylate that is AMLOPRES-5 was purchased from the local market.

Selection of common solvent:

Methanol of analytical reagent grade was selected for stock solution as common solvent and further dilutions with double distilled water for developing spectral characteristics of drug. The selection was made after assessing the solubility of the drug in different solvents.

Preparation of Standard Stock Solution:

A stock solution of Amlodipine besylate (100µg/ml) was prepared by accurately weighing approximately 10 mg of the drug into 100 ml volumetric flask and making up to volume with methanol. Aliquots of the standard stock solution of Amlodipine besylate was prepared with double distilled water to give the required final concentration of 10 µg/ml.

Absorption maximum:

The stock solution of 10 µg/ml was subjected to scanning in the UV range and maximum absorption was found at 358 nm.

Linearity and calibration:

At absorption maxima 358 nm, the absorbance was measured for the standard solutions of varying concentrations like 2, 4, 6, 8, 10, 12, 14, 16 and 18 µg/ml. Linearity of Amlodipine besylate was fond to exist between the concentration range of 2 to 18 µg/ml. The linear regression parameters like correlation coefficient, slope and intercept are noted.

Recovery studies:

Recovery studies were carried out by mixing a known quantity of standard drug with preanalysed sample and the contents were reanalyzed by the proposed method. The percentage recovery was calculated.

System precision:

The absorbance of standard solution at working concentration was read six times and the values are noted. The % RSD for these six values is 0.1624%.

Stress Testing of Amlodipine besylate:

Oxidation studies: For oxidative degradation, 2 ml of 50 µg/ml Amlodipine besylate was taken separately in two 10 ml calibrated flask and mixed with 5 ml 3% H_2O_2 (oxidative degradation) and kept on hot water bath set at 80 °C for 2 hr. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10:90) and the absorption spectra of the resulting solutions were recorded.

Acid degradation studies: For acid degradation studies, 2 ml of 50 µg/ml Amlodipine besylate was taken separately in 10 ml calibrated flask and mixed with 5 ml of 0.1 N HCL (acid hydrolysis), and kept on hot water bath set at 80 ℃ for 2 hr. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10:90) and the absorption spectra of the resulting solutions were recorded

Alkali degradation studies: For alkali degradation studies, 2 ml of 50 µg/ml Amlodipine besylate was taken separately in 10 ml calibrated flask and mixed with 5 ml of 0.1N NaOH (alkaline hydrolysis) , and kept on hot water bath set at 80 ℃ for 2 hr. Then, the solution was cooled to room temperature and diluted to the mark with methanol: water (10:90) and the absorption spectra of the resulting solutions were recorded.

Thermal degradation studies: Solid drug was kept in Petri dish in an oven at 80 ℃ for 2 hr and after cooling to room temperature, 5 mg of Amlodipine besylate was weighed and transferred to a 100 ml calibrated flask, dissolved in methanol and diluted up to the mark with distilled water.

The absorption spectrum was recorded from 200-400 nm. The absorbance values obtained in stress studies were compared with the data obtained in calibration curve i.e. in the absence of forced degradation.

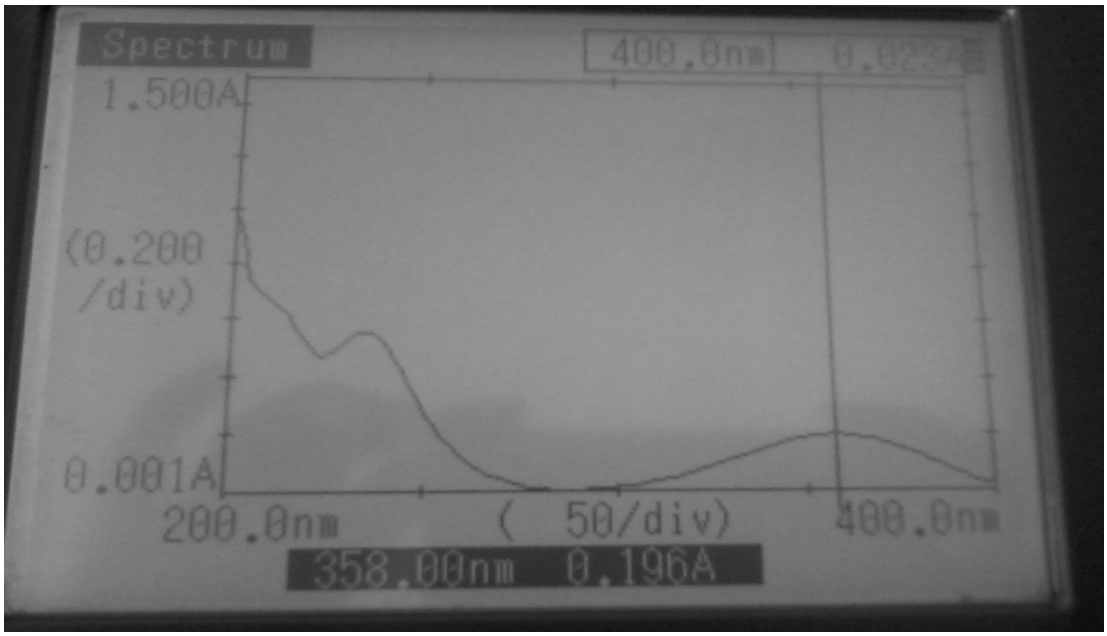
Assay: Marketed tablet formulation containing Amlodipine besylate 5 mg was analyzed using this method. From the 20 tablets , an amount equivalent to 5 mg of Amlodipine besylate was weighed and dissolved in 40 ml of methanol and sonicated for 10 minutes. Then the solution was filtered through whatman filter paper no. 41 and then final volume of the solution was made up to 50 ml with methanol to get a stock solution containing 100 µg/ml of Amlodipine besylate. Appropriate aliquots of Amlodipine besylate within the Beer's law limit was taken. The absorbance of resulting solutions was measured at 358 nm. The concentration of Amlodipine besylate present in the sample solution was calculated. The result of analysis of the tablet formulation is presented in Table No.4.

RESULT AND DISCUSSION

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. Amlodipine besylate is a UV-absorbing molecule with specific chromophores in the structure that absorbs at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The λmax of the drug for analysis was determined by taking

scans of the drug sample solutions in the entire UV region. It was found to be that only one peak was observed in this method at the wavelength of 358 nm and depicted in Figure-2.

Figure-2: Absorption maxima of Amlodipine besylate



Calibration curves:

Calibration curve data were constructed in the range of the expected concentrations of 2 µg/ml to 18 µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be Y= 0.0385x + 0.0198. The correlation coefficient (r) of the standard curve was found to be greater than 0.9966. Calibration curve of Amlodipine besylate was given in Chart-1. The analytical characteristics and necessary validation parameters for the UV techniques for Amlodipine besylate are presented in Table-1, 2, 3 and 4.

Table-1: Optical characteristics and precision of the developed method

Parameters	Amlodipine besylate
Detection wavelength	356 nm
Beer's Law range	2-18 µg/ml
Regression Equation (Y)	Y= 0.0385x + 0.0198
Slope (m)	0.0385
Intercept (c)	0.0198
Correlation coefficient	0.9966
LOD (µg/ml)	0.4854
LOQ (µg/ml)	1.47

Table-2 : Accuracy of the developed Method

Drug	Amount added (mg)	Amount recovered (mg)	% Recovery
Amlodipine Besylate	4	4.003	100.5
	5	5	100
	6	6.08	101

Table-3 : Precision

Sr. No.	Concentration (µg/ml)	Absorbance	% RSD
1	10	0.156	
2	10	0.155	
3	10	0.157	0.1624
4	10	0.155	
5	10	0.155	
6	10	0.156	

Table-4 : Assay

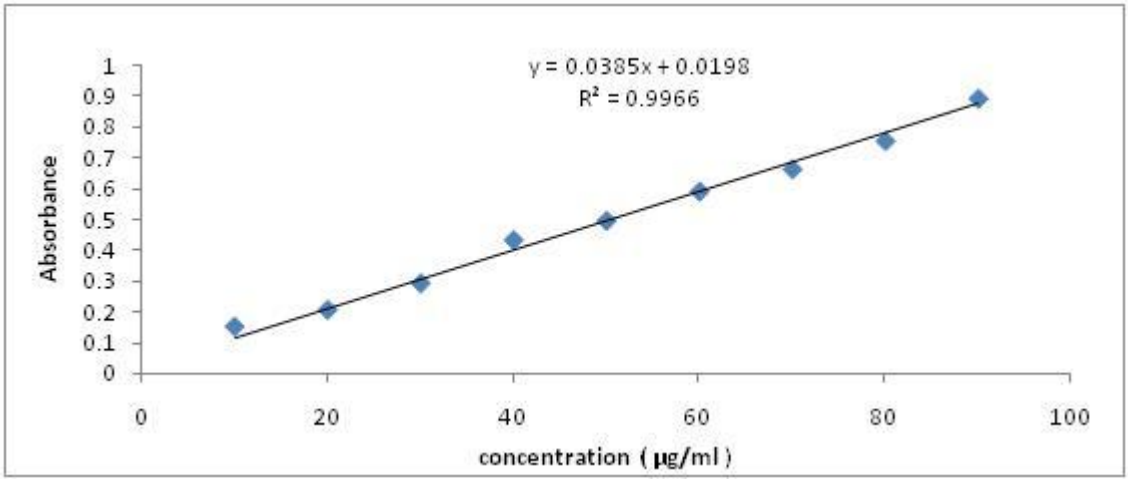
Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)
Amlodipine besylate	5	101.16 ± 0.04725

*Average of 6 determination, S.D. = Standard Deviation

Table-5 : Accuracy assessment by recovery experiments

Amount of sample	Amount of drug added	Percentage recovery
Amlodipine besylate (µg/ml)	Amlodipine besylate (µg/ml)	Amlodipine besylate
5.0	4.0	100.28
5.0	5.0	99.95
5.0	6.0	99.99

Chart.1: Calibration curve of Amlodipine besylate



Performing replicate analysis of the standard solutions was used to assess the accuracy, precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in 0.1N HCL, 0.1N NaOH, 3%H_2O_2 and Thermal degradation.These samples were analyzed with the relevant calibration curves to determine

the intra and inter day variability. The Intra and Interday precision were determined as the %RSD. The precision, accuracy and reproducibility of the results are given in Tables-2, 3 and 4, which demonstrate a good precision, accuracy and reproducibility.

The proposed methods can be successfully applied for Amlodipine besylate assay in tablet dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim 5 mg. The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method. In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets.

The stability of Amlodipine besylate in 0.1N HCL, 0.1N NaOH, 3%H_2O_2 and Thermal degradation. Solution was evaluated to verify whether any spontaneous degradation occurs when the samples were prepared. Amlodipine besylate degradation in 0.1N HCL, 0.1N NaOH ,3%H_2O_2 and Thermal degradation at were mentioned in figures-3, 4, 5 and 6.

Table-6 : Stress degradation study using the proposed method

Duration(2 hrs at 80°C)	0.1 N NaOH		0.1 N HCl		3%H_2O_2		Thermal degradation	
	Before degrad-ation	After degrad-ation	Before degrad-ation	After degrad-ation	Before degrad-ation	After degrad-ation	Before degrad-ation	After degrad-ation
Absorbance	0.522	0.526	0.940	0.945	0.580	0.588	0.990	0.996

Figure -3: Degradation of Amlodipine besylate by 0.1 N HCL at 2 hrs

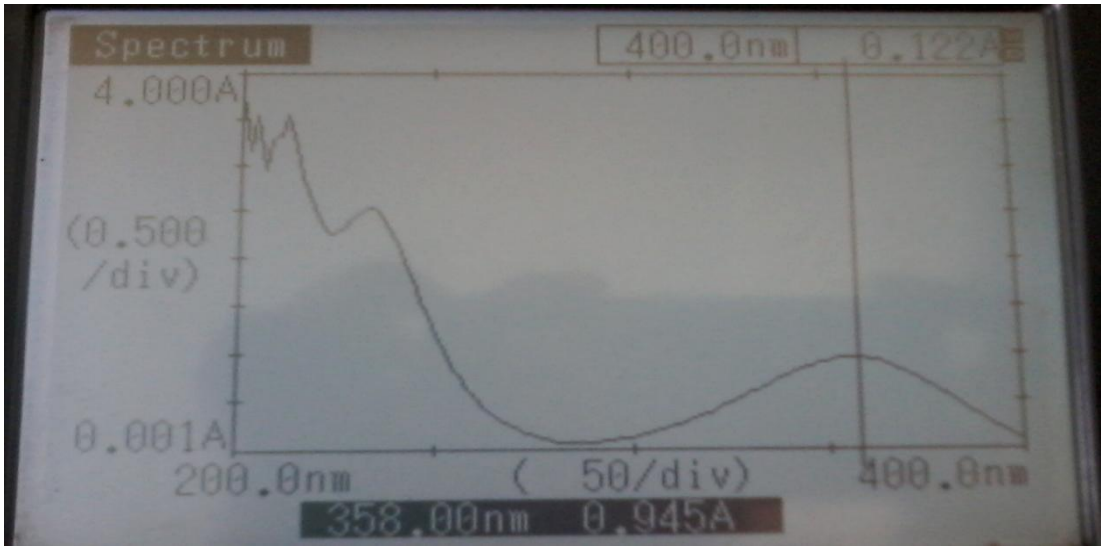


Figure-4: Degradation of Amlodipine besylate by 0.1N NaOH at 2hrs

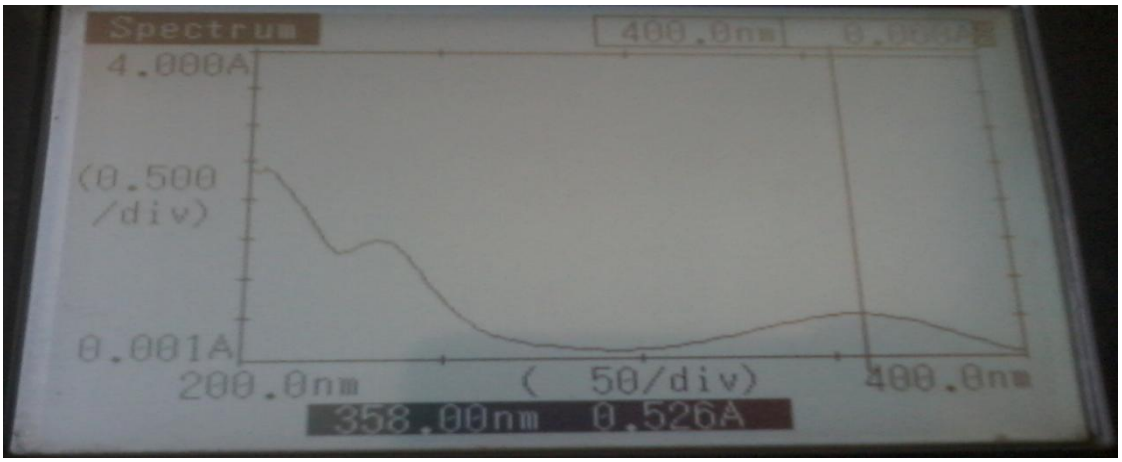
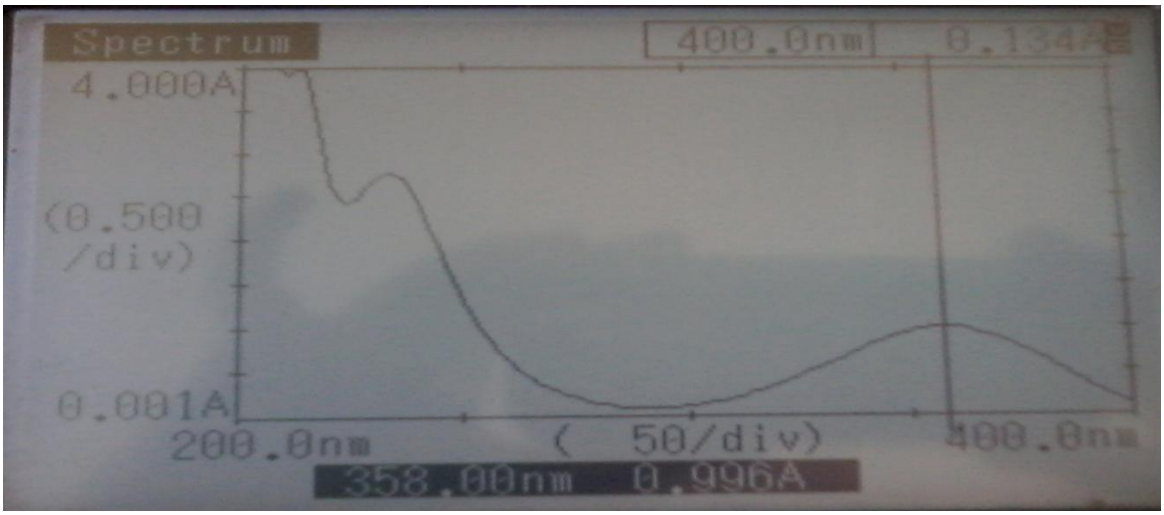


Figure-5: Degradation of Amlodipine besylate by 3% H_2O_2 at 2hrs

Figure-6: Degradation of Amlodipine besylate by Thermal degradation at 2hrs



CONCLUSION

This developed UV method for estimation of Amlodipine besylate is accurate, precise and stability indicating from the above results, it was concluded that the drug did not undergo degradation under these above conditions. Since the absorbance values obtained under these stressed conditions were similar to those of standard Amlodipine besylate sample. Degradation study showed that Amlodipine besylate is stable under conditions as acid, alkali, thermal and oxidation. The developed method may be employed for analysis of stability samples of Amlodipine besylate. This method is found to be useful for simultaneous qualitative and quantitative analysis of Amlodipine besylate in tablet formulations.

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