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A new spectrophotometric method for the estimation of amlodipine besylate and its stress degradation studies

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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 364nm. The calibration was found to be linear in the concentration range of 6-80µg/ml, with regression value of 0.998. Recovery studies were carried out and the average percentage recovery of the sample was found to be 100.4%. 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. 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.

Keywords: Amlodipine besylate, λmax, UV-double beam spectrophotometer, stress degradation studies.

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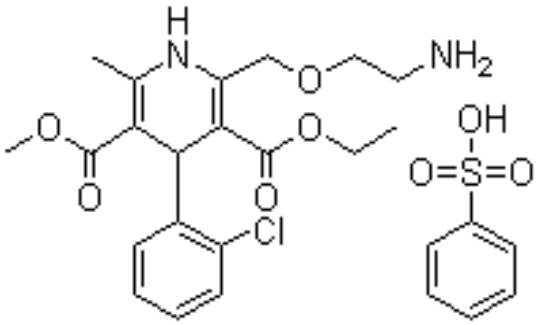
INTRODUCTION

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 [2].



Amlodipine besylate

Literature survey reveals several spectroscopic, HPLC and HPTLC methods for the estimation of amlodipine besylate. Spectrophotometric methods [3], Stability indicating RP-HPLC method [4-7]. However there is no method reported for the detection of Amlodipine besylate in bulk and pharmaceutical formulation by UV spectrophotometry.

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method developed for the detection of RIS in bulk drug and pharmaceutical formulation.

MATERIALS AND METHODS

Instrumentation and chemicals:

Spectral runs were made on a systronics double beam UV- Visible spectrophotometer, model 2202 with spectral band width of 2nm with automatic wavelength correction was employed. The software is PC Link. Amlodipine besylate was kindly provided by Hetero Labs Limited (Hyderabad). Double distilled water was produced from distillation unit.

Preparation of Standard Stock Solution

A stock solution of amlodipine besylate (100µg/ml) was prepared by accurately weighing approximately 10mg of the drug into 100ml A grade volumetric flask and making up to

volume with methanol. Aliquots of the standard stock solution of amlodipine besylate were prepared with double distilled water to give the required final concentration of 10µg/ml.

Absorption maximum: The stock solution of 10µg/ml was subjected to scanning in the UV range and maximum absorption was found at 364nm.

Linearity and calibration: At absorption maxima 364nm, the absorbance were measured for the standard solutions of varying concentrations like 6,7,8,9,10,20,30,40,50,60,70 and 80µg/ml. Linearity of amlodipine besylate was fond to exist between the concentration range of 6 to 80µg/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 0.162%.

Stress Testing Of Amlodipine Besylate

Oxidation studies: Solutions for oxidation studies were prepared in 3% H_2O_2 and the resultant solutions were allowed to stand for one day to facilitate oxidation of the drug.

Acid degradation studies: Solution for acid degradation studies were prepared in 0.1N and 1N HCL and allowed to stand for one day.

Alkali degradation studies: Solutions for alkali degradation studies were prepared in 0.1N NaOH and allowed to stand for one day.

Assay: Twenty tablets were accurately weighed and a quantity of tablet powder equivalent to 5mg of amlodipine besylate was weighed and dissolved in 100ml methanol .The solution was then filled and diluted further to obtain final concentration of 25µg/ml. The sample solution was analyzed and the % drug content was determined from the absorbance using the regression equation obtained in the calibration.

RESULTS 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

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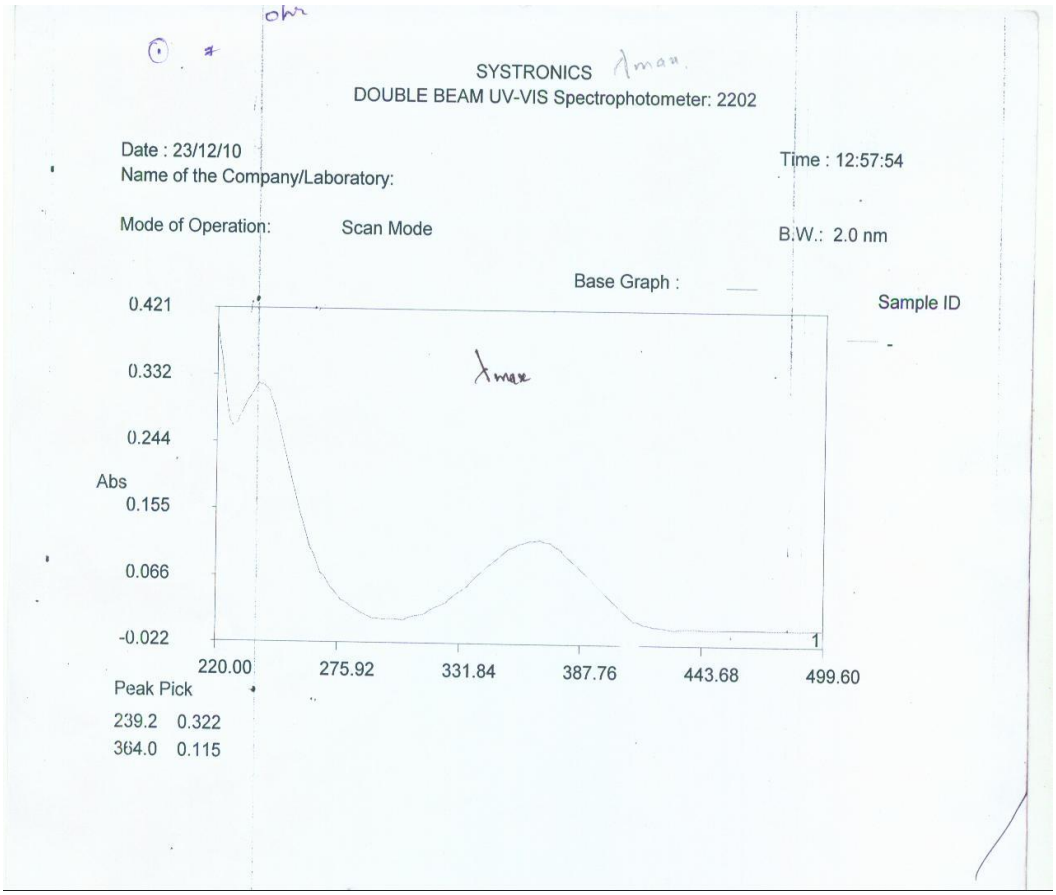
of the drug sample solutions in the entire UV region. It was found to be that only one peak was observed in this method at the wavelength of 364nm and depicted in Figure-1.

Figure-1: Absorption maxima of Amlodipine besylate



Calibration curves

Calibration curve data were constructed in the range of the expected concentrations of 6µg/mL to 80 µg/mL Beer's law was obeyed over this concentration range. The regression equation was found to be Y=0.011x + 0.036. The correlation coefficient (r) of the standard curve was found to be greater than 0.995. 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.

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

Parameters	Optical characteristics
Λmax	364nm
Beer's law limit	6-80µg/ml
Slope	0.011
Intercept	0.036
Correlation coefficient	0.999
Standard error	0.1234
%RSD	0.162
Sandell's sensitivity(µg/)ml	0.064

Table-2.Accuracy of the developed Method

Drug	Amount added (mg)	Amount recovered(mg)	%Recovery
Amlodipine besylate	4	4.008	100.2
	5	5	100
	6	6.06	101

Table-3.Precision

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

Table-4.Assay

Drug	Label claim (mg/tab)	Amount estimated (mg/tab)	%Purity
Amlodipine besylate	5	5.2	104

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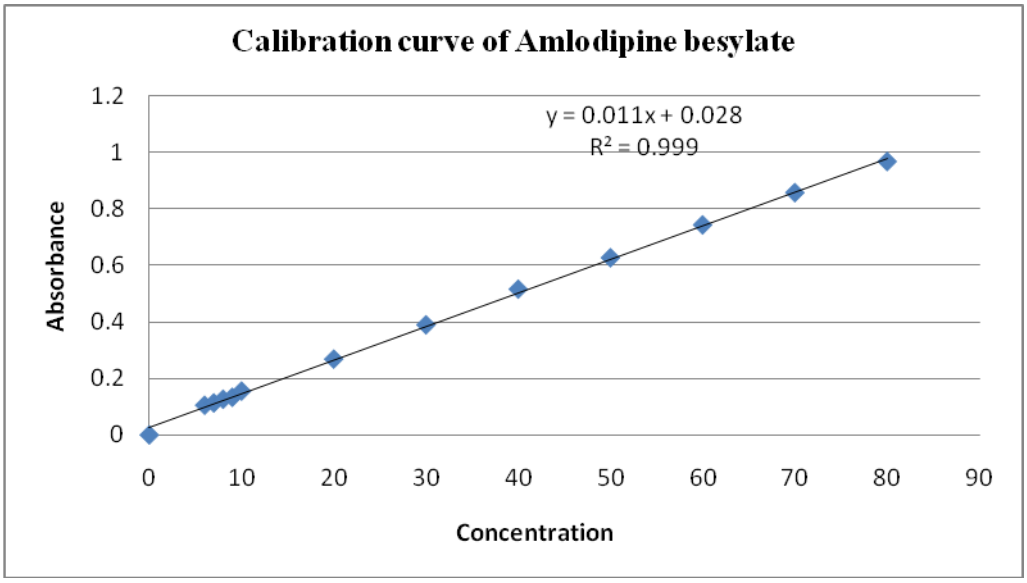
Chart.1. Calibration curve of Amlodipine besylate



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, 1N HCL, and 0.1N NaOH and 3%H_2O_2. 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 5mg. 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 application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets. These results reveal that the developed method have an adequate precision and accuracy and consequently can be applied the determination of Amlodipine besylate tablet in pharmaceuticals without any interference from the excipients. The stability of Amlodipine besylate in 0.1N HCL, 0.1N HCL, 1N NaOH and 3%H_2O_2 solution was evaluated to verify whether any spontaneous degradation occurs when the samples were prepared. The stability profile for 0, 3, 24 and 48hrs are

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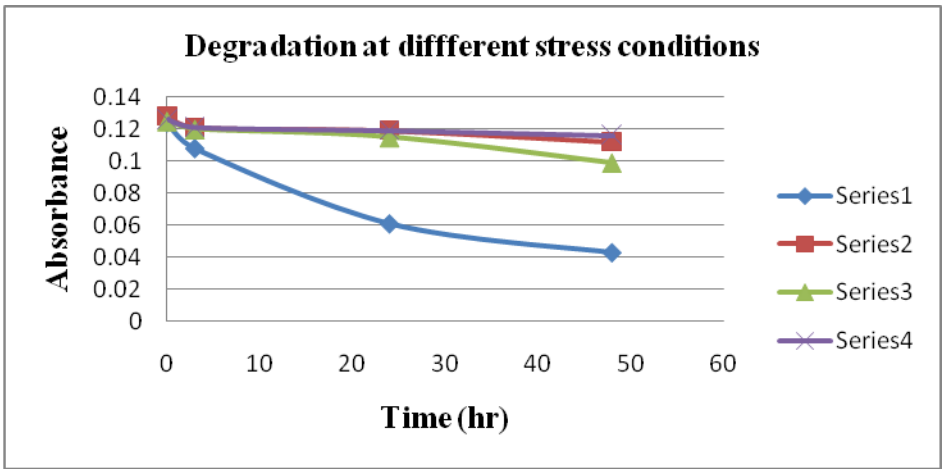
mentioned in Table-5 and chart-2. Amlodipine besylate degradation in 1N HCL, 0.1N NaOH and 3%H_2O_2 at 24hrs were mentioned in figures-2, 3 and 4.

Chart.2.Degradation of Amlodipine besylate at different stress conditions



Series1-0.1N NaOH Series2-0.1N HCL

Series3-1N HCL Series4-3% H_2O_2

Table-5.Stress degradation study using the proposed method

S.No.	Duration (hrs)	0.1N NaOH	0.1NHCL	1NHCL	3%H_2O_2
1	0	0.124	0.128	0.125	0.126
2	3	0.108	0.121	0.120	0.121
3	24	0.061	0.119	0.115	0.119
4	48	0.043	0.112	0.099	0.116

Figure -2: Degradation of Amlodipine besylate by 1N HCL at 24hrs

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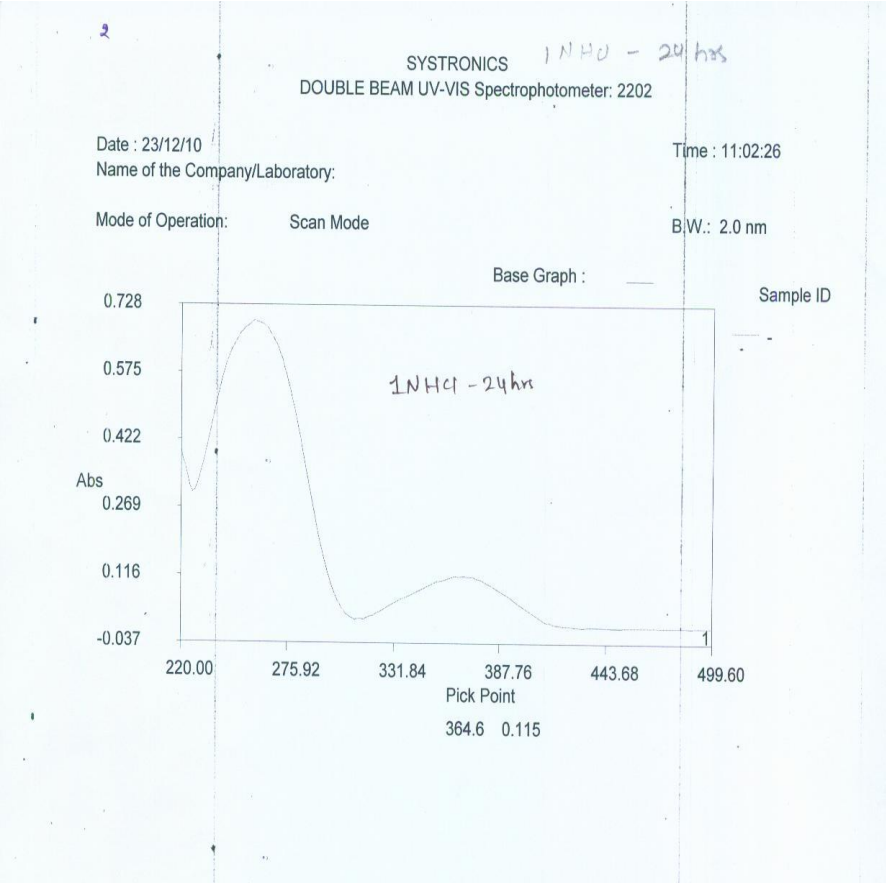

Figure-3: Degradation of Amlodipine besylate by 0.1N NaOH at 24hrs

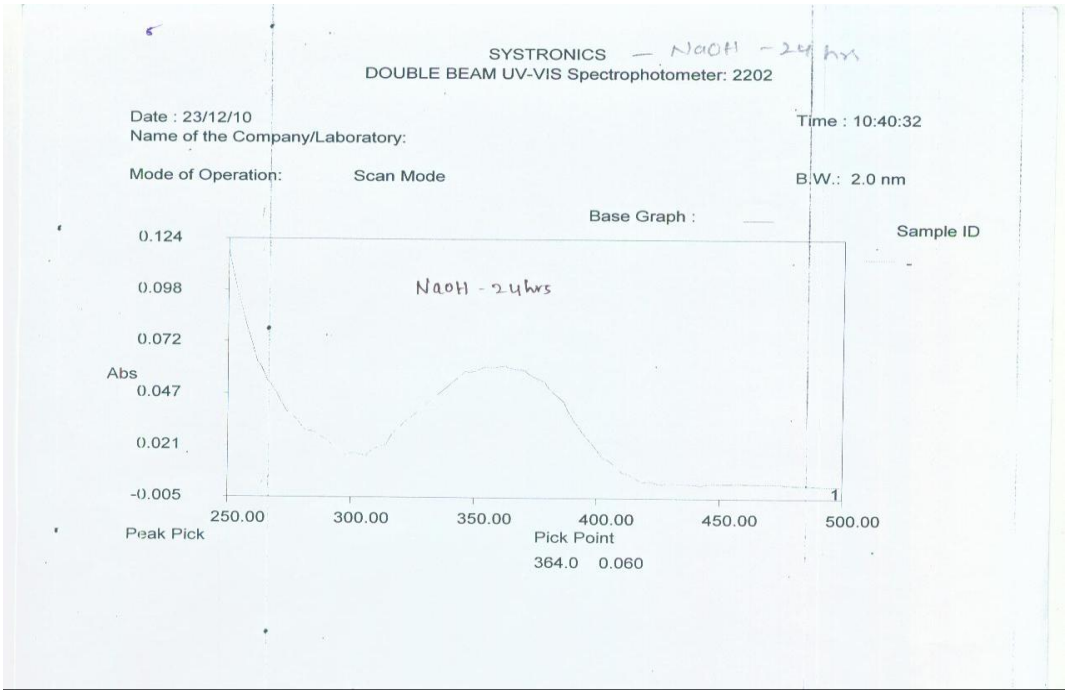

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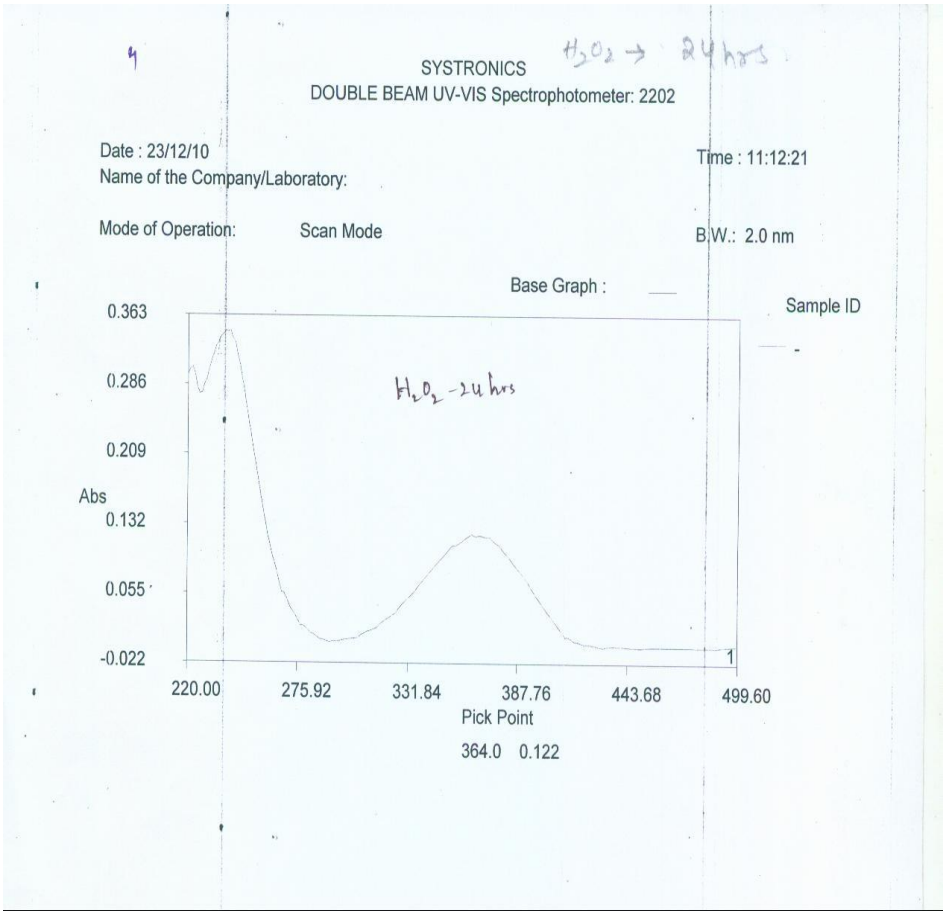
Figure-4: Degradation of Amlodipine besylate by 3% H_2O_2 at 24hrs



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 degraded more in 0.1N NaOH, compared to 0.1N HCL, 1N HCL and 3%H_2O_2. Degradation in 1N HCL was more compared to 0.1NHCL.

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