Development and Validation of UV Spectrophotometric Method for Estimation of Amlodipine Besylate in Tablet Dosage Form

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ABSTRACT

A new Ultra-violet Spectroscopic method was developed and validated for the determination of amlodipine besylate in the tablet dosage form. The sample was analysed using methanol buffer. The λmax values for amlodipine besylate in the solvent medium were found to be 281 nm. The system obeys Beer's law and shows linearity in the range of 5-30µg/ml.% Recovery studies, Intra-day and interday precision were found to be 98.8%,99.98% and 99.99%. No interference was observed from common tablet adjuvants. The changes in the λmax values (Physical parameter) for amlodipine besylate were evaluated as for stability study. The method was successfully applied to the assay of amlodipine besylate in tablet formulation. The proposed method is fast, accurate and precise for the determination of amlodipine besylate for routine quality control of tablets.

KEYWORDS: *Amlodipine besylate, Methanol buffer, Beer's law, Precision, UV Spectroscopy.*

INTRODUCTION

Amlodipine besylate is a1,2-Dihydropyridine's second-generation calcium channel blocker, which acts on L-type calcium channel antagonist, which is commonly used in the treatment of hypertension, angina and arrhythmias.[4,6] Amlodipine besylate is official in U.S.P. The chemical name of amlodipine besylate is 3-ethyl 5-methyl(4RS)-2- [(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzene sulphonate.[3,5]Amlodipine was found to be slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol, and slightly soluble in 2 propanols.[1,8]

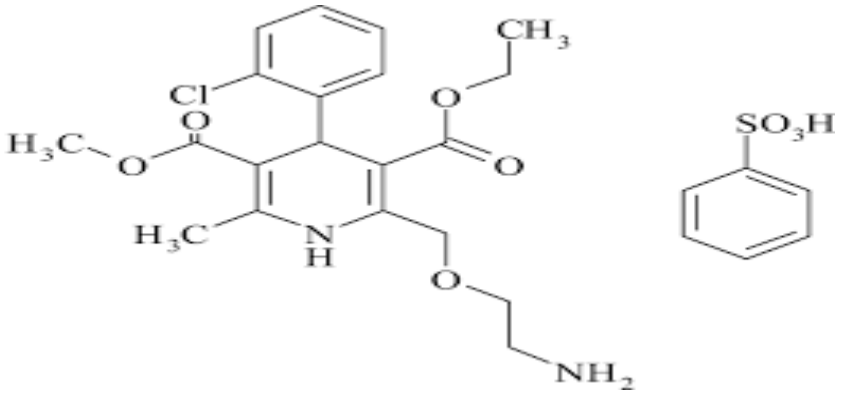

Figure 1 Chemical Structure of Amlodipine Besylate

A literature survey reveals the presence of some analytical methods for the determination of amlodipine besylate by various researchers.[10-19] Ultraviolet spectroscopy is concerned with the study of the measurement of UV radiation with matter in the range of 200 nm to 400 nm.[7,9] Samples for UV absorption can be examined in the form of vapour or solution.

The U.V procedure is simple, rapid and accurate. This is a new method and can be employed for routine analysis in quality control analysis.[1,2]The described method give accurate and precise results for the determination of Amlodipine besylate in marketed formulation.

Materials and Preparation

AmlodipineBesylatepuresamplewasobtainedfromM/SGloryPharmachemIndiaPvt.Ltd, Gajulamandyam,Renigunta, Tirupati-A.P.

Chemicals and Solvents Used:

1. Methanol - Analytical grade
2. Water for analysis
3. Hydrochloric Acid

All the above chemicals and solvents were supplied by our college Sree Vidyanikethan college of pharmacy, A. Rangampet, Tirupati, Andhra Pradesh, India.

Preparation of Standard Stock of Amlodipine Besylate:

50mg of Amlodipine Besylate was taken into 50ml volumetric flask, about 30ml of methanol buffer(0.3ml of HCl was made upto 100ml with methanol) was added and sonicated at room temperature for about 10mins, the volume was made upto 50ml with methanol buffer. The above stock solution is diluted serially to get the concentration range of 5-30 µg/ml. The dilutions thus obtained are analyzed six times and the calibration curve was plotted.

Determination of Absorption Maxima:

The standard solution of Amlodipine besylate (5µg/ml) was scanned at different concentrations in the range of 200- 400nm and the λmax was found to be 281 nm against reagent blank.

Estimation of Analysis:

The method was validated according to ICH Q2B guidelines for validation of analytical procedures to determine the linearity, regression, correlation coefficient, stability, per cent recovery studies, slope and intercept for the analyte. [1]

Linearity:

The linearity of Amlodipine Besylate was determined by the analysis of analyte concentration across 5µg/ml to 30µg/ml of Amlodipine Besylate. They were prepared and the absorbance of Amlodipine Besylate was measured at 281nm respectively. Absorbance is plotted graphically as a function of analyte concentration and was showed in Figure2. Percentage curve fitting was calculated.

Table 1 Linearity data of Amlodipine Besylate

CONCENTRATION	ABSORBANCE
5µg/m1	0.08
10µg/ml	0.12
15µg/ml	0.16
20µg/ml	0.2
25µg/ml	0.24
30µg/ml	0.28

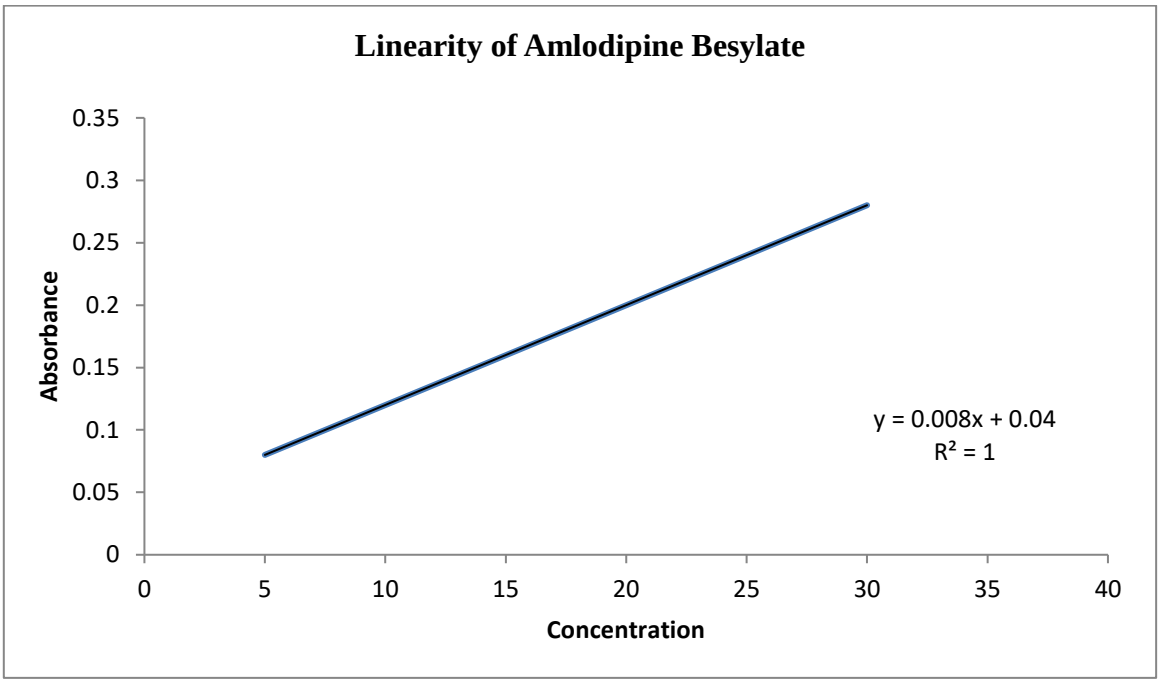

Fig.2:Standard Curve of Amlodipine Besylate (5-30µg/ml)

Recovery studies:

To ensure the suitability and reliability of the proposed method, recovery studies were carried out. Recovery studies by adding known quantities of the drug to previously analyzed pharmaceutical preparations are followed using the proposed procedure. To study per cent recovery, a fixed amount of the sample is taken in a series of volumetric flasks and three different levels of standard solutions are added. Each level of the added drug is repeated six times. The total amount of the drug is then determined by the proposed method, Table.1.

Table2:RecoveryStudies

Level	%Recovery	%RSD
1.	50%	98.2%
2.	49.98%	98.8%
3.	50%	98.6%

Precision:

The precision of an analytical method expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical method is usually expressed as the variance, standard deviation or coefficient of variance of a series of measurements.

The precision of the method was demonstrated by

i) Intraday precision

ii) Inter-day precision

i) Intraday precision

Intraday precision was found by carrying out the analysis of the standard drug at six different concentrations in the linearity range of the drug three times on the same day and %RSD was calculated, Table 3.

Table 3:IntradayPrecision

Concentration(μg/ml)	Absorbance	%RSD
5 μg/ml	0.1007 0.1022 0.1033	98.4%
10 μg/ml	0.1017 0.1192 0.1023	98.2%
15 μg/ml	0.1057 0.1492 0.1123	98.6%
20μg/ml	0.1087 0.1792 0.1223	98.5%
25 μg/ml	0.1057 0.2092 0.1323	98.4%
30 μg/ml	0.1257 0.2392 0.1423	98.3%

ii) Inter-day precision

Inter-day precision was found by carrying out the analysis of the standard drug at six different concentrations in the linearity range of the drug for three days over one week and %RSD was calculated, Table 4.

Table 4:InterdayPrecision

Concentration(μg/ml)	Day	Absorbance	%RSD
5 μg/ml	1	0.1083	98.32%
	2	0.1103	
	3	0.1123	
10 μg/ml	1	0.1222	98.25%
	2	0.1022	
	3	0.1322	
15 μg/ml	1	0.2037	98.63%
	2	0.1837	
	3	0.2137	
20 μg/ml	1	0.3070	98.54%
	2	0.2870	
	3	0.3170	
25 μg/ml	1	0.3706	98.45%
	2	0.3506	
	3	0.3806	
30 μg/ml	1	0.4906	98.35%
	2	0.4706	
	3	0.4606	

Correlation co-efficient:

The correlation coefficient was calculated from the absorbances obtained which were found to be 0.9996.

Stability:

Stability studies of the drug solutions were carried out at room temperature and the drug was found to be stable for about 24 hours.

Analysis of the Tablet formulation

Preparation of standard solution:

The stock solution of Amlodipine Besylate (1mg/ml) was prepared in methanol buffer, from which different concentrations (5-30μg/ml) were prepared and scanned in UV region, Fig.3.

Preparation of sample solution:

For the estimation of Amlodipine besylate in tablet formulation, 20 tablets of a brand were weighed and triturated to a fine powder. Tablet powder equivalent to 50 mg of amlodipine besylate was weighed and dissolved and further diluted with a quantity sufficient with methanol buffer. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper no. 40 to get the stock solution of 1mg/ml, Table 4. Various dilutions of the tablet solution were prepared and analyzed six times and the concentration was calculated by using the calibration curve for the method,

Table 5:Analysis of formulation

Drug	Amount(mg/Tablet)		%Labelclaim	%RSD
	Labelled	Found		
Amlodipine besylate	50mg	49.988mg	99.76%	99.85%

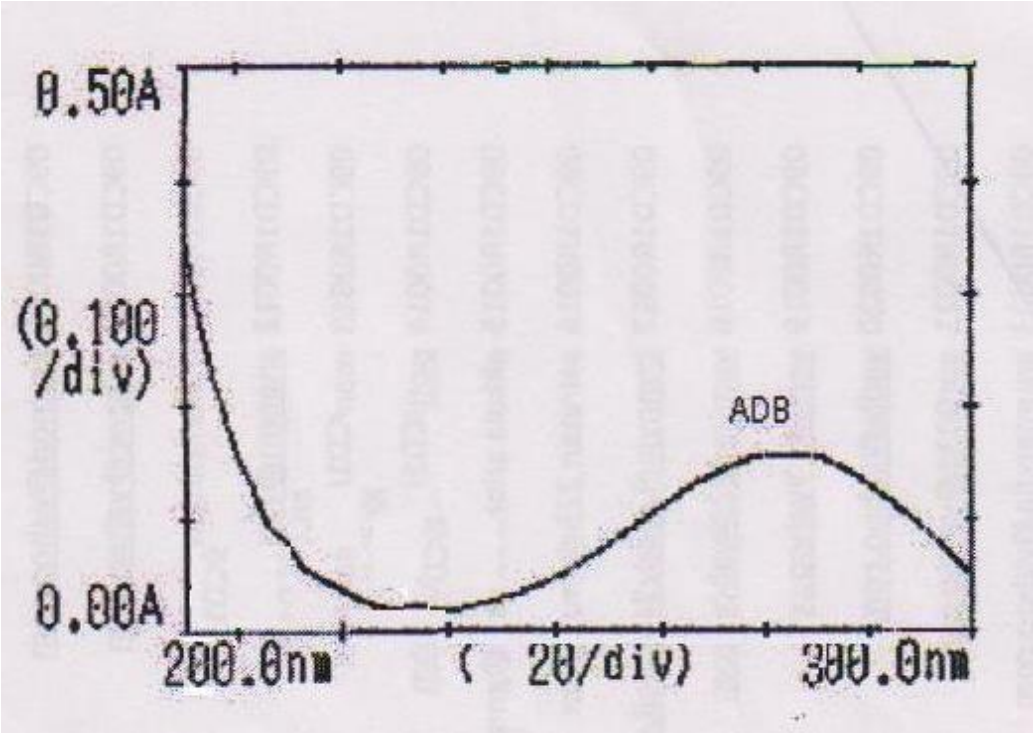

Fig 3:OverlainUVspectraofAmlodipineBesylate (5-30µg/ml)

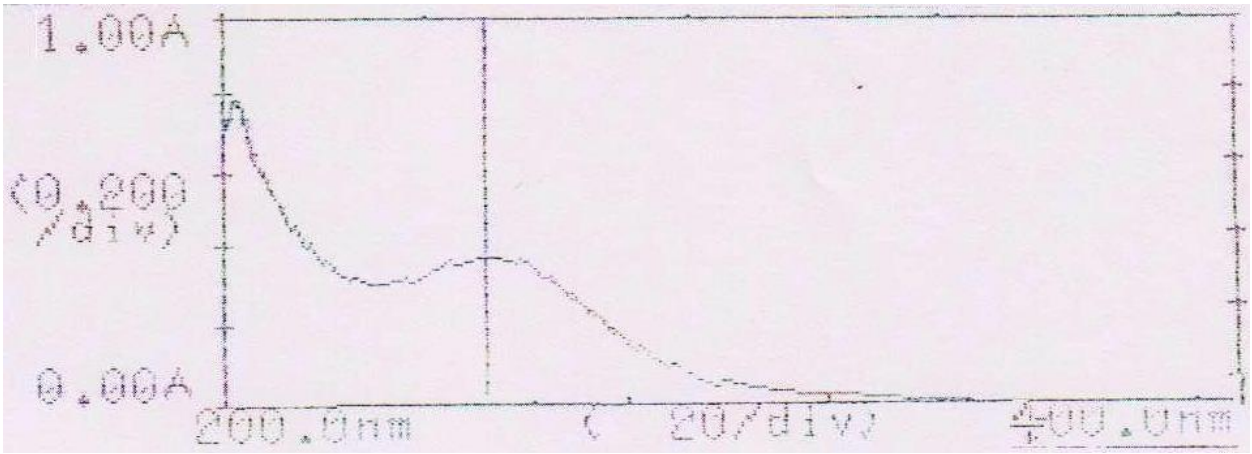

Fig 4:UVSpectrumofFormulation(10µg/ml)

RESULTS

The object of the study was to develop an analytical method for the Estimation of Amlodipine besylate. Hence an attempt was made to develop a new method based on U.V and HPLC methods.

The U.V. method for the estimation of the drug was found to be sensitive with accuracy and precision. The method of the U.V. developed has been validated as per ICH guidelines. The normal U.V spectra of Amlodipine Besylate were recorded in a methanol buffer. From the recorded U.V spectra, it was found that as the concentration increases absorbance also increases. Hence, Beer's law is verified. Linearity was checked in different concentrations. The calibration curve was obtained for Amlodipine Besylate in the range of 5-30 ug/ml respectively. The slope and intercept values of Amlodipine Besylate were found at 281nm respectively.

The recovery studies were carried out to ensure the suitability and reliability of the method by adding a known amount of standard drug solution and analysis was carried out as per the developed procedure. The precision of an analytical method is usually expressed as the variance, standard deviation or coefficient of variance of a series of measurements. The precision of the method was demonstrated by intraday precision and inter-day precision which was found to be abrupt.

The correlation coefficient was calculated from the absorbances obtained which were found to be 0.9996. Stability studies of the drug solutions were carried out at room temperature and the drug was found to be stable for about 24 hours.

DISCUSSION

An attempt was made to develop and validate a new U.VSpectrophotometric method. The so proposed method obeyed beer's law and results were also statistically validated as per ICH guidelines.

CONCLUSION

This is a new and novel method and can be employed for routine analysis in quality control studies of amlodipine besylate. The described method gave accurate and precise results. The Correlation coefficient was found to be 0.9996 and the drug solution was found to be stable for 24 hrs. The method relies on the use of simple and cheap chemicals and techniques but provides sensitivity comparable to that achieved by a sophisticated and expensive technique like HPLC, or HPTLC. Thus these can be used as alternatives for rapid and routine determination of bulk samples and tablets.

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Conflict of Interest; The authors declared no conflict of interest.

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