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Area under curve method development and validation for estimation of amlodipine besylate in bulk and tablet dosage form

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ABSTRACT

A simple, precise and economical UV - spectrophotometric method has been developed for the estimation of Amlodipine besylate in pharmaceutical dosage form. Method applied was area under curve (AUC) in which area was integrated in the wavelength range of 228.40 nm – 244.80 nm. Calibration curves were plotted for the method by using instrumental response at selected wavelengths and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 3-18 µg/ml for the method. Tablet formulation was analyzed and the percentage of drug determined in the assays was 96.36% – 101.68%. Accuracy and precision studies were carried out and results were satisfactory. The results of the analysis were validated statistically. Limit of detection and limit of quantitation were determined for the method. The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Amlodipine besylate in pharmaceutical formulation.

KEYWORDS

Amlodipine besylate;
UV-spectrophotometry;
Area under curve;
Validation.

INTRODUCTION

Amlodipine Besylate (AB), chemically, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt[1].

A detailed literature survey for Amlodipine besylate revealed that several analytical methods such as Spectrophotometric methods were reported for the quantification of Amlodipine besylate. There are few RP-HPLC methods were reported for the determination of Amlodipine besylate in pharmaceutical dosage form[2-6].

The method was validated according to ICH guidelines[7]. Thus the objective of present study was to develop an applicable method for the routine analysis of Amlodipine besylate in tablet formulations.

EXPERIMENTAL WORK

Material and method

Amlodipine besylate working standard was obtained as gift sample from Lupine Pharma. The drug

was used without further purification. A tablet formulation containing 5 mg of Amlodipine besylate was purchased from local market. Analytical grade solution was used for the experiment.

Instruments used

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ): 10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock and working standard solution

The standard stock solution of Amlodipine besylate was prepared by dissolving accurately weighed 10 mg of the drug in methanol and diluted to 100 mL with same solvent to obtain a final concentration of 100 μg/mL.

Method: Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths 228.40 and 244.80. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The spectrum obtained of zero order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (3-18 μg/mL) versus AUC.

Preparation of sample solution

Ten Amlodipine besylate tablets (5 mg each) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask and 50 mL methanol was added. After ultrasonic vibration for 10 min, the mixture was diluted to volume with methanol and filtered through Whatman filter paper (No. 41). From the filtrate an appropriate aliquot was taken in such a way that the final concentration in 10 mL is 6 μg/mL. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard.

VALIDATION OF METHOD

The proposed method was validated as per ICH-Guidelines Q2 (R1)[7].

Linearity

For the method, calibration curve was prepared on 3 different days. The calibration curve was constructed by plotting the response (*y*) versus the theoretical concentrations of standards (*x*), by using linear regression analysis. Linearity was expressed as a correlation coefficient; the value must be > 0.999.

Precision

The intraday and interday precision of the proposed Spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of Amlodipine besylate for area under curve 6, 9, and 12 μg/mL and the results are reported in terms of percent relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of Amlodipine besylate by the method of standard additions. The study was performed by spiking three known amount concentration of Amlodipine besylate (4.6, 6, and 7.2 μg/mL; ranging from 80% to 120%) into a prequantified sample solution (6 μg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring the response and by fitting these values to the straight-line equation of calibration curve.

Specificity

Results of tablet solution showed that there is no

interference of excipients when compared with the working standard solution. Thus, the method was said to be specific.

Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot (6 µg/mL) in different laboratories by different analysts using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation.

RESULTS AND DISCUSSION

The molecular structure of the Amlodipine besylate is presented in Figure 1. Methanol was selected as the solvent for Amlodipine besylate because provides good solubility and other characteristics for AUC measurements. The absorption spectrum of Amlodipine besylate in methanol is indicated in Figure 2. Optical characteristics of Amlodipine besylate were calculated by the proposed method and are presented in TABLE 1.

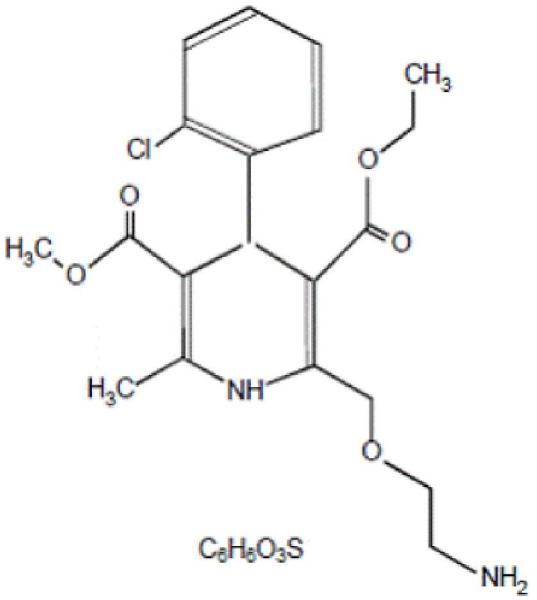

Figure 1 : Chemical structure of Amlodipine besylate.

The intra-day and inter-day precision values (%RSD) were calculated for Amlodipine besylate (results shown in TABLE 3) and the values obtained (≤2%) comply with the stated limits of the guidelines. The accuracy of Amlodipine besylate was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% and the values obtained were found to

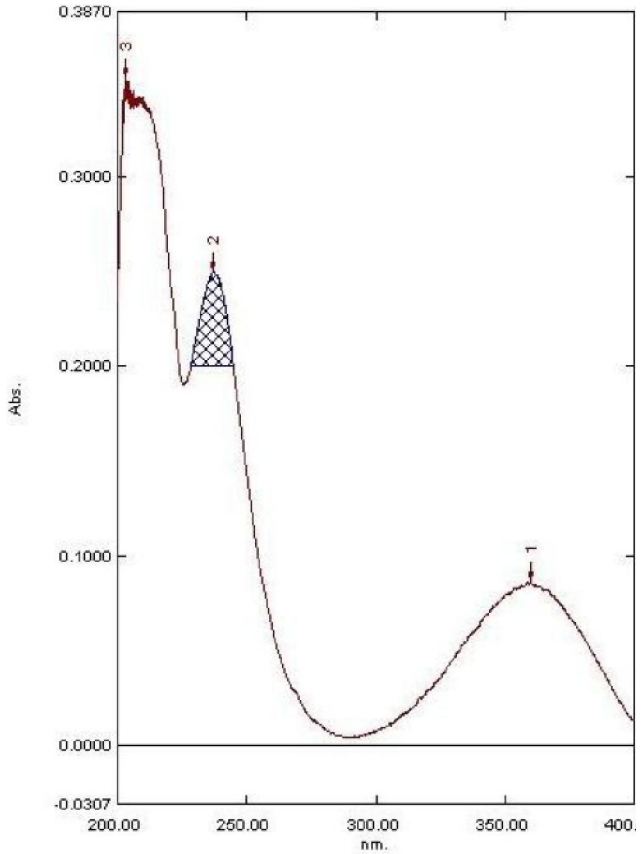

Figure 2 : Area under curve spectrum of Amlodipine besylate in methanol

TABLE 1 : Optical characteristics of Amlodipine besylate

Parameters	Amlodipine besylate
Beer-Lambert's range(µg/mL)	3-18 ug/ml
λ max(nm)/ wave length range (nm)	239
Slope	0.076
Intercept	0.036
Correlation coefficient	0.999
Limit of detection (µg/mL)	0.30
Limit of quantitation (µg/mL)	0.92

TABLE 2 : Assay results of commercial Amlodipine besylate tablet.

Amlodipine besylate marketed formulation	Label claim/Tablet (Amlodac 5)	% Recover*	% RSD
Tablet	5 mg	100.5%	0.92

***Average of three determinations**

be in the acceptable limits (≤2%) (results presented in TABLE 4). This indicates that there was no interference from the excipients present in the dosage form. Ruggedness of proposed method was determined with the help of two different analysts. and results were evalu-

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ated by calculating the %RSD value and lying within the range (results shown in TABLE 5).

TABLE 3 : Precision

Conc. µg/mL	Intraday		Interday	
	% Recovery	% RSD	% Recovery	% RSD
6	99.89	1.63	99.45	1.39
9	99.69	1.09	99.53	1.02
12	100.0	1.37	99.67	1.66

TABLE 4 : Accuracy

Nominal Value %	Initial amt.	Added amt.	% Recovery	%RSD
80	6	4.8	99.07	0.55
100	6	6	99.16	0.67
120	6	7.2	99.24	0.93

TABLE 5 : Ruggedness

Analyst	Amount found of Amlodipine Besylate [%]	%RSD [n=3]
I	99.96	0.78
II	99.89	0.75

n= no. of estimations

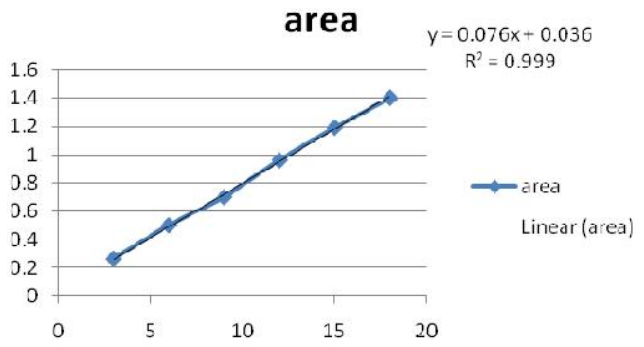

Figure 3 : Calibration curve of Amlodipine besylate at 239nm

CONCLUSION

The UV spectrophotometric AUC method developed for determination of Amlodipine besylate was based on different analytical techniques, UV-Spectrophotometric, AUC method. The method was validated and found to be simple, sensitive, accurate, and precise in compliance to the limits stated in the ICH guidelines. Hence, we conclude that the method can be used successfully for routine analysis of pharmaceutical dosage forms containing Amlodipine besylate. The proposed spectrophotometric method will not replace the presently known methods available for the analysis of Amlodipine besylate. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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