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

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Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for estimation of Amlodipine Besylate in tablet dosage form have been developed. First method employs zero order method using 237.5 nm as analytical wavelengths in methanol. The second method is area under curve based on estimation of drug in the range of 232- 242 nm. Recovery studies range from 99.33% for Amlodipine Besylate in case of zero order method and 102.93% in case of area under curve method confirming the accuracy of the proposed methods. The proposed methods are recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

Keywords: Amlodipine Besylate, Area under curve method, Zero order method.

INTRODUCTION

Amlodipine, 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, Amlodipine besylate. It is not official in any Pharmacopoeia. Various analytical methods have been reported for the assay of Amlodipine besylate[1] in pure form as well as in pharmaceutical formulations. They include high performance liquid chromatography,[2-7] reversed phase high performance liquid chromatography,[8-11] high performance thin layer chromatography,[12-15] gas chromatography,[16] gas chromatography–mass spectrometry,[17] liquid chromatography with tandem mass spectrometry[18] and fluorimetry,[19] derivative spectroscopy,[20,21] simultaneous multi-component mode of analysis and difference spectrophotometry[22-24].

By these two methods no UV spectrophotometric study on Amlodipine in tablet dosage form in pharmaceutical preparations has been found in literature survey. The objective of the present work is to develop and validate new analytical methods for determination of Amlodipine Besylate in tablet dosage form. This communication forms the first report of two simple, sensitive and reproducible methods for the estimation of Amlodipine Besylate from tablet dosage form.

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MATERIALS AND METHODS

Materials:

Spectral runs were made on a Shimadzu UV-Visible spectrophotometer, model- 1700 (Japan) 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. Glassware's used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Amlodipine besylate reference standard was kindly provided by Shreya Life Sciences Pvt. Ltd. Aurangabad (M. S.). The pharmaceutical preparations of combination of Amlodipine that is Amlodac tablet (ARISTO, Mumbai). Methanol of analytical reagent grade was purchased by Loba Chemie Pvt. Ltd. (India). All the solutions were protected for light and were analyzed on the day of preparations.

Selection of common solvent:

Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of the drug in different solvents.

Preparation of Standard Drug Solution:

An accurately weighed quantity of about 2.5 mg of Amlodipine besylate was taken in 50 mL volumetric flask dissolved in sufficient

Table 1: Optical Characteristics and Precision

Parameters	Method I	Method II
Absorption maxima (nm)	237.5	232-242
Beer's law limit (µg/mL)	2-20	2-20
Correlation coefficient	0.9991	0.9996
Slope (m)	0.0476	0.782897
Intercept	-0.0065	-0.17447
% COV	-	0.0009
LOD (µg/mL)	1.24	1.19
LOQ (µg/mL)	3.72	3.57

quantity of methanol, sonicated and diluted to 50 mL with the same so as to get the concentration of 50µg/ mL. Further dilutions were made from this stock solution to get required concentration.

Determination of Absorption Maxima:

The standard solution of Amlodipine besylate (20µg/ mL) was scanned at different concentrations in the range of 200-400nm and the λ max was found to be 237.5 nm against reagent blank (Fig. 1).

I. Zero Order Method

The solutions were scanned in the range from 400-200 nm, and the peaks were observed at 237.5 nm and 360 nm. The wavelength selected for the analysis of the drug was 237.5 nm (figure 2). The drug followed the Beer's- Lamberts law in the range of 2-20 µg/ml. By using the calibration curve the concentration of the sample solution can be determined.

II. Area Under Curve Method (AUC)

The AUC (Area Under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 232 nm and 242 nm (Fig. 2). 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 the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (50µg/ mL) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm (fig. 2) and the calibration curve was plotted.

All the two method were checked by analyzing the samples with known concentration. As the result obtained were satisfactory, the method was applied for the pharmaceutical formulations.

Analysis of the Tablet formulation:

For the estimation of Amlodipine besylate in tablet formulation by two methods, 10 tablets of brand were weighed and triturate to fine powder. Tablet powder equivalent to 5 mg of AB was weighed and the dissolved and further diluted with quantity sufficient with methanol. It was kept for ultra- sonication for 30 min; this was filtered through Whatmann filter paper no. 41 to get the stock solution of 50 µg/ mL. Various dilutions of the tablet solution were prepared and analyzed for six times and the concentration was calculated by using the calibration curve for three methods.

VALIDATION:

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

RESULTS AND DISCUSSION

All the methods -Zero order and Area Under Curve (AUC) method for the estimation of Amlodipine besylate in tablet dosage were found to be simple, accurate and reproducible. Beer- Lambert's law was obeyed in the concentration range of 02-20 µg/ml in all these methods. The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The %RSD value is less than 2 indicative of accuracy of the method. Results found are satisfactory.

Table 2: Results of analysis of tablet and recovery

Method	Drug	Label Claim	% Label Claim	% Recovery* (Mean ± R. S. D)
I	AB	5	99.74	99.33 ± 0.150
II		5	100.00	99.02 ± 0.67

Average of ten determinations;
R.S.D: Relative Standard Deviation

Table 3: Statistical analysis of results

Method	SD*	COV (%)*	SE*
I	0.15	0.0002	0.0474
II	0.67	0.000006	0.2118

* Mean of ten readings
SD- Relative Standard deviation,
COV-Coefficient of Variation,
SE- Standard Error

Method validation:

Linearity- Linearity was observed in the range of 02-20 µg/ mL for zero order, first

Table 4: Results of intermediate precisions.

Day	Method I	Method II
	% Label claim estimated* (Mean ± % R.S.D.)	% Label claim estimated* (Mean ± % R.S.D.)
Intra day	99.04 ± 0.43	102.5 ± 1.58
Inter day	104.05 ± 1.74	98.75 ± 1.46

*Average of three determinations;
R.S.D.; Relative Standard Deviation.

order derivative and AUC method. The calibration curve yielded coefficient of correlation (r) 0.9991, 0.9996 for zero order and AUC method respectively, given in table no. 9 and 12.

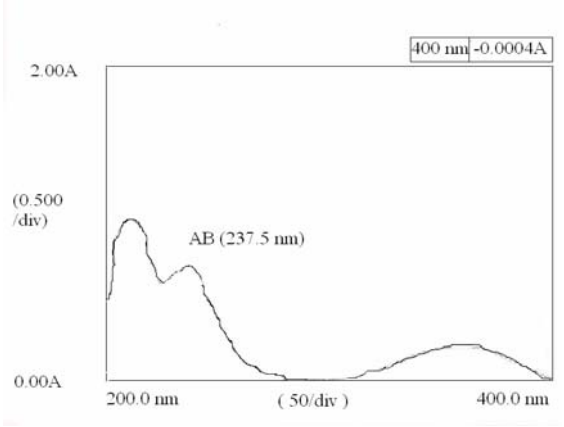

Fig.1 Wavelength selected for Zero order method.

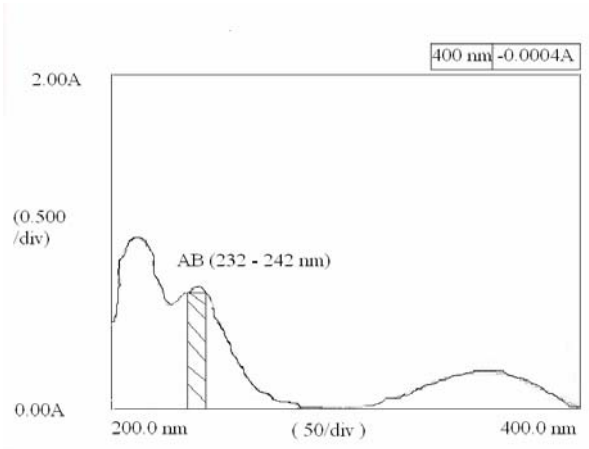

Fig. 2 Wavelength range selected for AUC method.

Sensitivity- High Molar absorptivity and low Sandell's sensitivity for the respective method reveals that the method is highly sensitive.

System precision- % COV calculated from 3 replicate readings (absorbance values) at concentration (2µg/ mL) confirm the precision of the method given in table no. 11 and 14.

Assay results- Amlodipine besylate tablet was analyzed by proposed methods, the percentage in tablet were determined and presented in the table. Assay results obtained are within limit given in table no. 10 and 13.

Accuracy and precision –The low values of S.D, % COV, and 95% confidence interval indicate that method is precise. % recovery was found to be within limit indicate the non-interference from the formulation excipients and confirm the accuracy and precision of the method.

CONCLUSION

The most striking feature of these methods are its simplicity and rapidity, non- requiring-consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. All the above result indicates that, the methods employed here are very simple, accurate, simple, economical and rapid for routine analyses of the drug, Amlodipine besylate. The recovery was 99.33% and 99.02% for two methods, which is close to 100% indicating reproducibility and accuracy of the method.

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