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NOVEL UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AMLODIPINE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTACT

A Novel, simple, accurate and precise Zero order derivative spectroscopic method was developed and validated for the estimation of Amlodipine in bulk and pharmaceutical formulation. The stock solution was prepared by weighing 100 mg of standard Amlodipine in 100 ml volumetric flask with 0.1M HCl. The stock solution was made to produce 1000 µg/ml with 0.1M HCl. Further dilutions were prepared as per procedure. The drug solution showed the maximum absorbance at 239 nm. The linearity was found in the concentration range of 3-18 µg/ml. The correlation coefficient was found to be 0.999. The regression equation was found to be Y=0.043x+0.001. The method was validated for linearity, accuracy, precision, ruggedness, robustness, LOD and LOQ. The LOD and LOQ for estimation of Amlodipine were found to be 0.02738 µg/ml and 0.08298 µg/ml respectively. Recovery of Amlodipine was found to be in the range of 99.62-100.62 %. Proposed method was successfully applied for the quantitative determination of Amlodipine in bulk and pharmaceutical formulation.

KEYWORDS: Amlodipine, Zero order UV- Spectroscopy, 0.1M HCl, Accuracy.

INTODUCTION[1-5]

Amlodipine initially approved by the FDA in 1987, is a popular antihypertensive drug belonging to the group of drugs called long acting dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure.

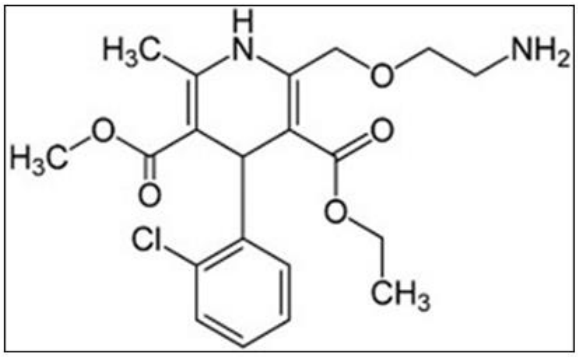

Figure.1: Chemical structure of Amlodipine.

It has a molecular formula of $C_{20}H_{25}ClN_2O_5$ and molecular weight of 408.9 g/mol. It has the structural formula (Fig.1).

The literature survey reveals that various analytical methods have been developed such as UV spectroscopy methods[6-16], HPLC methods[17-20], HPTLC method[21] and UPLC method[22] for the estimation of Amlodipine present in alone or in combination with other drugs or in marketed products. Most of the reported UV Spectroscopic methods are Simultaneous estimation of Amlodipine along with other Antihypertensive drugs, by using several solvents, expensive reagents and often time-consuming. So there is a need to develop novel UV Spectroscopic methods for the estimation of Amlodipine alone in bulk and Pharmaceutical formulation.

A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method, which is simple for the estimation of Amlodipine present in bulk and Pharmaceutical formulation. An effort was made in the present method to develop a novel, simple, sensitive, accurate, reliable and reproducible with minimum Relative Standard Deviation (RSD) values for the estimation of Amlodipine in bulk and Pharmaceutical formulation.

MATERIALS AND METHOD

Instrument

A Shimadzu-1800 UV-Vis double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe software with 1 cm matched quartz cells was used for spectrophotometric measurements in above proposed spectrophotometric methods.

Chemicals

Amlodipine was given as a gift sample by industry.
Tablets of Amlodipine were procured from local market.

Solvent

0.1M HCl

Selection of analytical wavelength

Appropriate dilutions were prepared for drug from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from Zero order method. It shows maximum absorbance at 239 nm were shown in Fig.2 & 2.1.

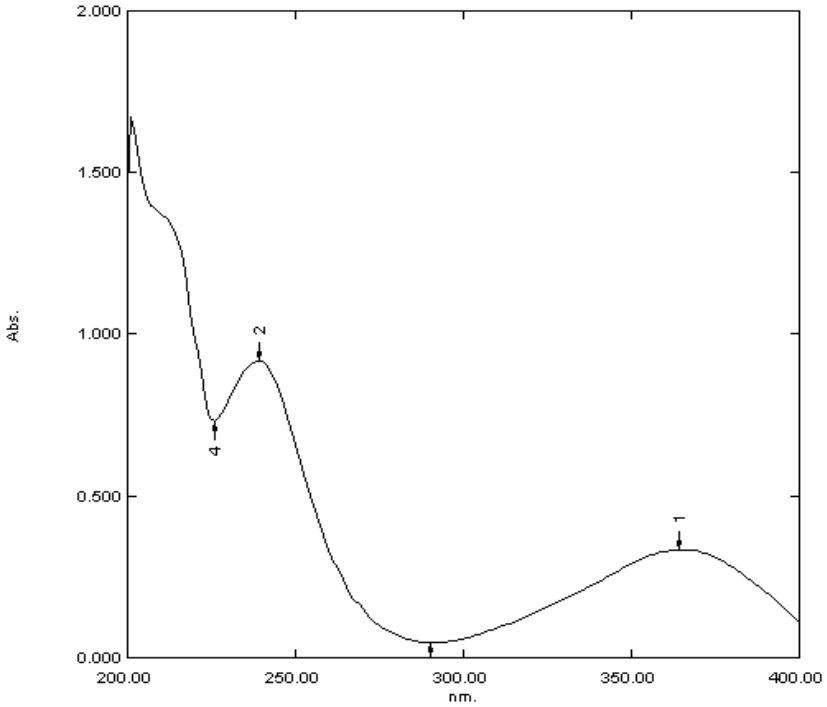

Fig.2: Zero order spectra of Amlodipine (Bulk drug) showing absorbance at 239 nm

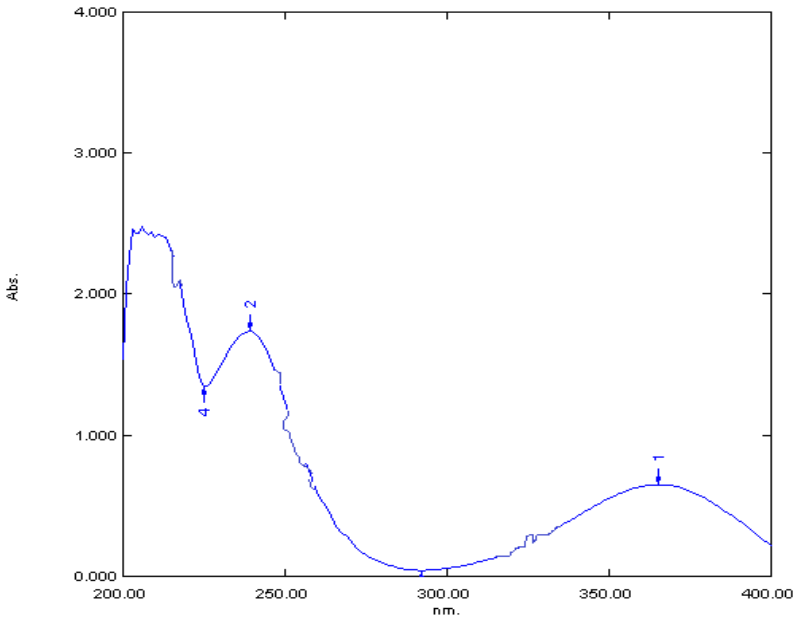

Fig.2.1: Zero order spectra of Amlodipine (Tablet) showing absorbance at 239 nm.

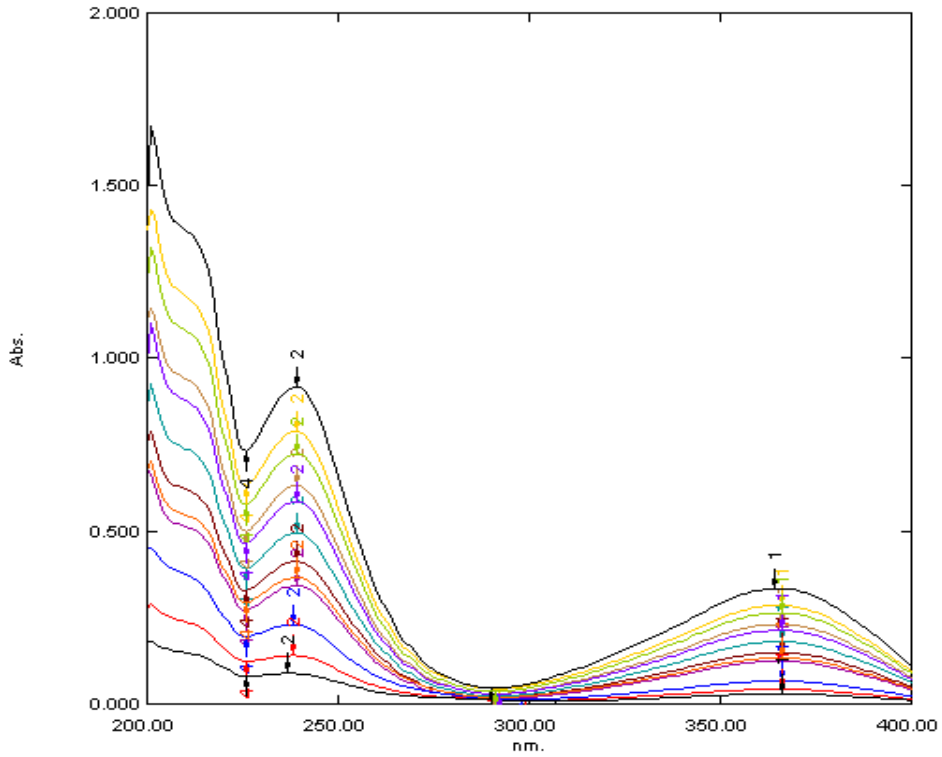

Fig. 3: Zero order overlain spectra of Amlodipine at 239 nm.

Preparation of Standard stock solution

Accurately weigh 100mg of Amlodipine was transferred into 100ml volumetric flask and diluted with up to the mark. From this pipette out 10ml into 100ml volumetric flask and diluted with 0.1M HCl up to the mark, from this solution pipette out 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 ml in 10ml individual volumetric flask and add 0.1M HCl up to the mark , this gives 3, 6, 9, 12, 15 and 18µg/ml concentrations.

Preparation of Sample solution

The commercially available Amlong contains 10 mg of Amlodipine. From this twenty Tablets were weighed and powdered. The Tablet powder equivalent to 100 mg of Amlodipine was transferred into 100 ml volumetric flask then it was diluted with the 0.1M HCl and made up to the mark and the solution was filtered through whatman filter paper NO. 41. From the above solution 10 ml was pipetted out into 100 ml volumetric flask and the volume was made up to the mark with 0.1M HCl. The final concentration of Amlodipine was brought to 6 µg/ml.

Method validation

The method is validated according to the ICH guidelines.[23,24,25]

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

The working standard solution were diluted serially with 0.1M HCl to obtain the range of 3-18µg/ml. a calibration curve for Amlodipine was obtained by measuring the absorbance at the λmax of 239 nm and absorbance values are shown in Table.1 and Calibration graph were presented in Fig.3. Statistical parameters like slope, intercept, coefficient of correlation, and Sandel's sensitivity were determined and presented in Table.2.

Table 1: Results of calibration curve for Amlodipine at 239 nm by zero order Spectroscopy.

SL. NO	Concentration in µg/ml.	Mean Absorbance±Standard deviation
1.	3	0.128±0.0015
2.	6	0.261±0.0010
3.	9	0.390±0.0025
4	12	0.517±0.0020
5.	15	0.648±0.0052
6.	18	0.772±0.0051

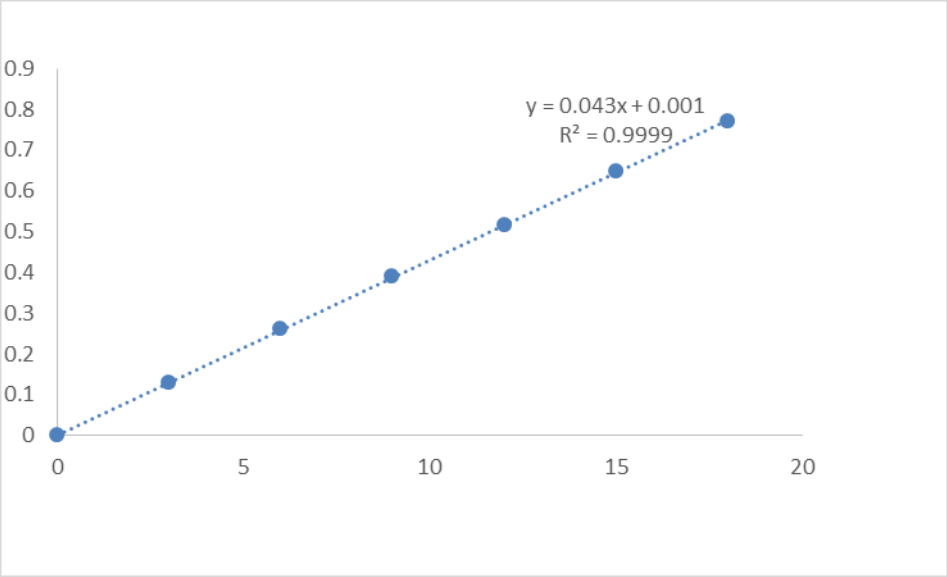

Fig.3: Calibration curve for Amlodipine at 239 nm by Zero order Spectroscopy

Table no. 2: Regression parameters for Amlodipine by zero order spectroscopy.

Optimum condition	UV Method
λmax (nm)	239nm
Beer's law limits (µg/ml)	3-18
Molar extinction coefficient (L.mol^{-1} cm^{-1})	0.0435
Sandell's sensitivity(mcg / cm^2-0.001 absorbance units)	0.02343
Regression equation (Y*)	Y=0.043x+0.001
Slope (b)	0.043
Intercept (a)	0.001
Correlation coefficient(r2)	0.999

Precision

Precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 3, 6, 9, 12, 15 and 18µg/ml concentration for three times in same day. Inter-day precision was determined by analyzing the same concentration of solution daily for three days. Precision results are shown in Table.3.

Table 3: Determination of precision results for Amlodipine at 239 nm by Zero order derivative spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance±SD**	%RSD	Inter-day Absorbance±SD**	%RSD
3	0.128±0.0015	1.19	0.130±0.0025	1.92
6	0.261±0.0010	0.38	0.260±0.0036	1.38
9	0.390±0.0025	0.64	0.390±0.0070	1.79
12	0.517±0.0020	0.40	0.521±0.0085	1.63
15	0.648±0.0052	0.81	0.651±0.0110	1.68
18	0.772±0.0051	0.65	0.780±0.013	1.66

Accuracy

To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i. e, 50%, 100% and 150%. In which the formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in Table.4.

Table 4: Determination of accuracy results for Amlodipine by Zero order derivative spectroscopy.

Tablet	Spiked levels	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Amount recovered	% Recovery±SD**	%RSD
Amlong 10 mg	50	6	3	9.05	100.62±1.3903	1.38
	100	6	6	12.03	100.27±0.5021	0.50
	150	6	9	14.94	99.62±0.4714	0.47

Robustness

Robustness of the method was determined by carrying out the analysis at different wavelengths (238 & 240 nm i.e ±1nm) by using 12 µg/ml concentrations. The value of %RSD was found to be less than 2 were shown in Table.5.

Table 5: Determination of Robustness results for Amlodipine at 238 and 240 nm by Zero order Spectroscopy.

Parameter	At 238 nm	At 240 nm
Mean absorbance	0.395	0.390
Standard deviation	0.001	0.0015
%RSD	0.253	0.393

Ruggedness

Ruggedness was determined between different analysts. The value of % RSD was found to be less than 2 were shown in Table.6.

Table 6: Determination of Ruggedness results for Amlodipine at 239 nm by Zero order Spectroscopy.

Analysts	Analyst-1	Analyst-2
Mean absorbance	0.517	0.521
Standard deviation	0.0020	0.0085
%RSD	0.40	1.63

Limit of detection and Limit of Quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the Response and slope of linearity curve. LOD and LOQ values of Amlodipine were found to be 0.02738 µg/ml and 0.08298 µg/ml. Shown in Table no. 7.

Table 7: Determination of LOD and LOQ results for Amlodipine at 239nm by Zero order spectroscopy.

SL.NO	Parameters	Values
1	SD of Intercepts*	0.000361
2	Average of Slopes*	0.0435
3	LOD(3.3×SD of intercepts/average of slopes)	0.02738
4	LOQ(10×SD of intercepts/average of slopes)	0.08298

*Mean value obtained from 6 calibration curves.

CONCLUSION

In the present investigation, we have developed Novel, simple, accurate and Precise UV- spectrophotometric method like Zero order derivative spectroscopy for the routine estimation of Amlodipine in bulk and pharmaceutical formulation and the method were validated in terms of linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ.

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