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Method development and validation of amlodipine besylate in API and pharmaceutical dosage form by UV spectroscopy

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Abstract
An analytical method was developed for the determination of amlodipine besylate in API and tablet dosage form, and UV-Spectrophotometric was employed to develop a sensitive and accurate method. In order to determine the amount of amlodipine besylate in bulk and its formulation, the current study intended to develop a straight forward, accurate, quick, and affordable UV-Spectrophotometric assay method. The chosen water to use as a solvent while preparing the stock solution. Amlodipine besylate was detected at a λmax of 245nm. Amlodipine used in this approach had a regression coefficient (r^2) of 0.9993 and exhibited linearity at concentrations of 2, 4, 6, 8 and 10µg/ml. With the formulation of amlodipine besylate API, the percent drug content was found to be 100.15. Recovery studies, where recoveries were almost 100% and %RSD is low, demonstrated the accuracy and precision of the method.

Keywords: UV-Spectrophotometer, amlodipine besylate, linearity, precision

Introduction
Amlodipine besylate is used as an anti-hypertensive drug. It is a crystalline white powder; it is soluble in water and sparingly soluble in ethanol. The chemical formula is $C_{20}H_{25}ClN_2O_5$, pKa value is 8.6. IUPAC name of amlodipine is 3-ethyl-5-methyl 2-(2-amino ethoxy-methyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydro pyridine-3,5-dicarboxylate [1, 2, 3].
Amlodipine is a long-acting calcium channel blocker (1,4-dihydro pyridine analogue), that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is indicated for the treatment of hypertension and coronary artery disease when used alone or in combination with another anti-hypertensive agent. Amlodipine is given orally as besylate in general, but doses are calculated in terms of amlodipine base [4, 5].

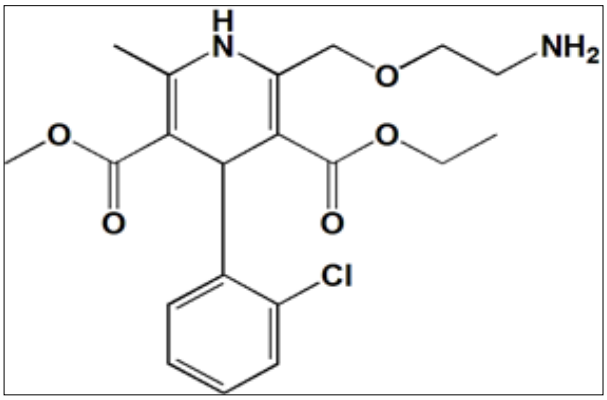

Fig 1: Structure of amlodipine

Literature survey reveals that, only few analytical methods were developed for the estimation of amlodipine besylate with UV spectroscopic methods. The purpose of the study is to develop precise, accurate and validate the method as per ICH guidelines [6, 7].

Materials and methods
Instruments
The present work was carried out with Elico SL 164 UV-Visible spectrophotometer having double beam detector configuration. The absorption spectra of reference and test solution were carried in a 1cm quartz cuvette over the range of 200-800nm.

Chemicals
Amlodipine besylate (API) was procured from Hetero Drugs Limited, Hyderabad, Telangana and amlodipine besylate tablets was purchased from local pharmacy, Hyderabad. All chemicals used are analytical grade.

Preparation of standard [8, 9]
Accurately weigh 100mg of amlodipine besylate (API), transfer it into a clean and dry 100ml volumetric flask, and then sonicate it. Pipette out 10ml of the stock solution from above flask, then transfer it into a 100ml volumetric flask and use the same solvent to make up the volume. A drug's molecular weight, pKa value, solubility, and other characteristics all play a role in the appropriate technique selection. Using a UV-Visible spectrophotometer, the wavelength of maximum absorption (λmax) of a medication in a solvent solution at a concentration of 1mg/ml was scanned between the 200-400nm ranges. The amlodipine UV spectra were acquired using scanning at 200-400 nm. The λ max was observed at 245nm.

Preparation of formulation [10, 11]
Weighed precisely, twenty amlodipine besylate tablets; transferred, crushed, and ground using a clean and dry pestle and mortar. Weigh off the equivalent of 100mg of powder from this, transfer it into a dry 100ml volumetric flask, dissolve it with distil water, sonicate the solution for 15min, filter it, and add water to make up the remaining volume. Then, the solution is run through the UV-spectrophotometer, namely the 200-400nm range. The λmax was observed at 245nm.

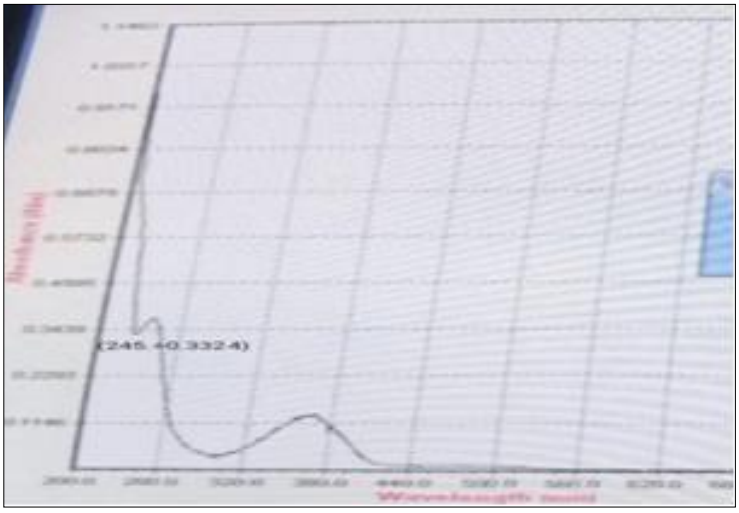

Fig 2: The λmax of amlodipine besylate

Results and discussion

Assay

The maximum absorbance was observed at 245nm in Figure 2, the absorbances was measured for the amlodipine besylate and calculated the assay using following formula:

$$\% \text{ Assay} = \frac{\text{Sample absorbence}}{\text{Standard absorbence}} \times \frac{\text{Wt.of Std}}{\text{Dilution of std}} \times \frac{\text{Wt.of Sample}}{\text{Dilution of Sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Wt.of Tablet}}{\text{Lable claim}} \times 100$$

Table 1: Assay of amlodipine besylate formulation

Amlodipine besylate	Amount of tablet (mg)		% label claim	%RSD
	Labeled	Found		
	10	9.978	99.78	0.90

Linearity

The method's linearity was proven across a concentration range of 2-10µg/ml of the intended concentration. A precisely weighed 100 milligram pure medication was added to a 100-milliliter dry volumetric flask, which was then cleaned, dried, and filled with a little volume of distil water to make the volume reach 100 milliliters. As a result, the drug concentration (Stock solution-I) was 1000µg/ml. From here, 10ml of the solution were pipette out into a 100ml volumetric flask, and distilled water (Stock solution-II, 100µg/ml) was added to bring the volume up to the mark. Concentrations 2, 4, 6, 8, and 10µg/ml were prepared from above prepared Stock solution-II, calibration curve was plotted and the correlation coefficient was calculated. The acquired absorbance readings are plotted against the amlodipine besylate concentration to create the calibration graph. Correlation coefficient of the linearity was found for method and reported in Table.

Table 2: Linearity of amlodipine besylate at 245 nm & statistical data of the regression equation

Concentration (µg/ml)	Absorbance	Amlodipine besylate	
2	0.0763	Parameters	245 nm
4	0.1383	Conc. (µg/ml)	2-10µg/ml
6	0.200	Correlation	0.999
8	0.2547	Slope	0.029
10	0.312	Y- intercept	0.019

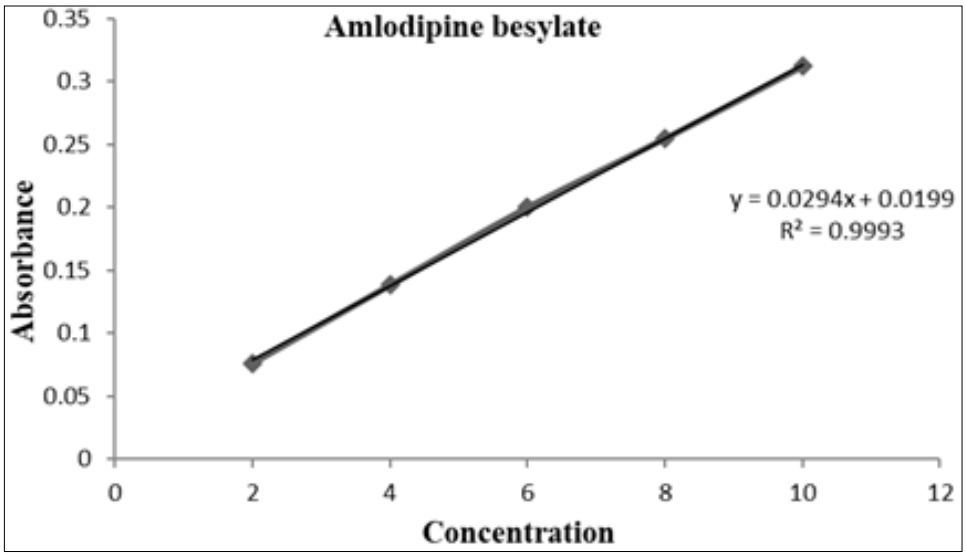

Fig 3: Linearity graph of amlodipine besylate

Limit of detection (LOD)
LOD for amlodipine besylate by the proposed method was determined on the response and slope of the regression coefficient.

LOD = 3.3 × σ/S
Where, σ = standard deviation,
S = linearity curve slope.

Limit of quantization (LOQ)
Limit of quantization for amlodipine besylate by the proposed method was determined on the response and slope of the regression coefficient.
LOQ = 10 × σ/S
Where, σ = standard deviation,
S = linearity curve slope.

Table 3: LOD & LOQ values of amlodipine besylate

Amlodipine besylate	
LOD (µg/ml)	0.13
LOQ (µg/ml)	0.40

Precision
The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation.

Intra and inter-day precision
A variation of results within the same day (intraday), variation of results between days (inter-day) was analyzed. Intra-day precision was determined by analyzing amlodipine besylate for five times in the same day at 245nm. Inter day precision was determined by analyzing drug daily once for five days at 245nm.

Table 4: Precision of amlodipine besylate

Concentration (µg/ml)	Inter-day	Intra-day
	Absorbance Mean + SD	Absorbance Mean + SD
2	0.0745+1.39	0.0763+1.39
4	0.1363+1.35	0.1373+1.40
6	0.2110+1.40	0.2010+1.45
8	0.2550+1.45	0.2547+1.45
10	0.3120+1.50	0.3120+1.40

Accuracy
Accuracy is the closeness of the test results obtained by the method to the true value. The recovery technique was performed to judge the accuracy of the proposed method. For this, known quantities of the amlodipine besylate solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of amlodipine besylate was determined by using the proposed method and the amount of added drug was calculated by the difference.

Table 5: Accuracy of amlodipine besylate

Sample (%level)	Amount taken (µg/ml) (Sample)	Amount added (µg/ml) (API)	Amount recovered (µg/ml)	%Recovery	Mean
80	6.0	4.8	4.76	99.16	**99.50**
80	6.0	4.8	4.81	100.20	
80	6.0	4.8	4.76	99.16	
100	6.0	6.0	6.10	101.66	**101.16**
100	6.0	6.0	6.00	100.00	
100	6.0	6.0	6.11	101.83	
120	6.0	7.2	7.15	99.30	**99.81**
120	6.0	7.2	7.21	100.13	
120	6.0	7.2	7.20	100.00	

Ruggedness and robustness
The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analysts.

Table 6: Ruggedness of amlodipine besylate

S. No.	Assay (% of claim) amlodipine besylate	
	Analyst 1	Analyst 2
1	98.79	99.99
2	101.98	100.98
3	101.59	101.59
4	101.00	101.59
5	101.05	101.13
Mean	**100.88**	**101.05**
SD	**1.397**	**1.316**
RSD	**0.96**	**0.91**

The optimum conditions for UV-spectroscopy method have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of products on the absorbance of the sample and colored species. Beer's law limits, molar absorptivity, sandal's sensitivity, %range of error and %relative standard deviation is summarized in Table. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r^2=0.999) obtained from different concentrations are given in Table 2 and Figure 3. The results showed that the method have reasonable precision. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical dosage forms and the mixtures were analyzed by the proposed methods. The percentage recoveries are given in Table 5. The interference studies veiled that the common excipients and other additives that are usually present in the injection dosage forms did not interfere at their regularly added levels.

Conclusion
From the above results the method described in this paper for the determination of amlodipine besylate from tablet formulation is simple, accurate, sensitive and reproducible. The proposed method could be applied for routine analysis in quality control laboratories. The developed methods were

validated as per ICH guidelines. The result of assay content for pharmaceutical formulation is in good agreement with the label claim of drugs.

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Competing interest statement
All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information
No additional information is available for this paper.

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