

## Research Article

# Difference Spectroscopic Method for the Estimation of Amlodipine Besylate in Bulk and in Formulation

SB. Jadhav\*, NA. Patil, AM. Tamboli, SM. More and SA. Shaikh

Sahyadri College of Pharmacy, Methwade, Tal- Sangola, Dist- Solapur. Pin code-413307, Maharashtra, India.

**ABSTRACT**

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of Amlodipine Besylate in bulk drug form by difference spectrophotometric method. Amlodipine Besylate has exhibited maximum absorbance at about 223nm and 241nm in acidic and basic solution respectively. Beer's law was obeyed in the concentration range of 2-10 µg/ml in both the cases. The regression coefficient was found to be  $r^2=0.9972$ . The LOD and LOQ value were found to be 0.75 ppm and 2.28 ppm respectively. The proposed method was successfully applied for the determination of Amlodipine Besylate in bulk drug. As per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory.

**Keywords:** Amlodipine Besylate, NaOH, HCl.

**INTRODUCTION**

Amlodipine Besylate (AMB), 2 - [(2 - amino ethoxy) - methyl] - 4 - (2 - chloro phenyl) - 1, 4 - dihydro - 6 - methyl - 3, 5 - pyridine dicarboxylic acid 3 - ethyl - 5 - methyl ester, benzene sulfonate, is a potent dihydro calcium channel blocker. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium-bound Calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation.

**Objective**

Amlodipine Besylate shows improved absorbing interference by the technique of different spectrophotometry. Thus the objective of the present study was to develop new analytical difference spectrophotometry method and its validation parameters for the proposed method according to ICH guidelines for the estimation of amlodipine besylate bulk drug.

**EXPERIMENTAL METHODS****CHEMICAL AND REAGENTS**

Amlodipine Besylate [bulk drug] used were of analytical reagent grade purchased from research lab fine chem. industries Mumbai, India, NaOH and HCL were purchased from Poona chemical laboratory and double distilled water was used throughout the analysis.

**INSTRUMENTATION**

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements.

**Selection of common solvents**

1N HCL and 1N NaOH were selected as a common solvent for developing spectral characteristics of drug.

**Preparation of solution**

Standard stock solution containing Amlodipine Besylate was prepared by dissolving 10mg in 100ml of methanol and then diluted with 1N NaOH and 1N HCL separately to get series of dilution ranging from 2-10 µg/ml and then absorbance recorded at 223 nm and 241 nm respectively against reagent blank. Calibration curve was prepared by plotting concentration versus difference in absorbance and found to be linear in the concentration range of 2-10 µg/ml.

## VALIDATION

The proposed method was validated according to ICH (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were Selectivity, linearity, precision and accuracy.

### Selectivity

The selectivity of the method was assessed by analyzing standard drug, and pharmaceutical product, comparing the maxima and minima of the standard with that of the sample to determine whether the pharmaceutical product and excipient lead to interfere in the estimation.

### Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$\text{LOD} = 3.3 \sigma / S$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$\text{LOQ} = 10 \sigma / S$$

Where,  $\sigma$  is standard deviation of the response and

S is the slope of the calibration curve.

LOD & LOQ of Amlodipine Besylate was found to be 0.075  $\mu\text{g/ml}$  & 2.283  $\mu\text{g/ml}$  respectively.

### Linearity

Different volumes of stock solutions were suitably diluted with corresponding medium (2,4,6,8, and 10  $\mu\text{g/ml}$ ) to get the desired concentrations. Each solution was analyzed in triplicate. The amplitude values were plotted against the corresponding concentrations to obtain the linear calibration curve.

### Range

2-10  $\mu\text{g/ml}$ .

### Precision

Precision of analytical methods were expressed in relative standard deviation (RSD)

of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively. Precision was calculated as inter-day and intra-day coefficient of variation.

### Accuracy

The accuracy of the method was determined by recovery experiments. A known amount of standard Amlodipine Besylate corresponding to 4, 6 and 8% of the label claim (standard addition method) was added to preanalysed sample of capsule. The recovery studies were carried out in triplicate at each level.

## RESULT AND DISCUSSION

The optical characteristics such as Beer's law limits, percent relative standard deviation and percent range of error were found to be within the limit and satisfactory. All of the analytical validation parameter for the proposed method was determined according to ICH guidelines. The method was found to provide high degree of precision and reproducibility.

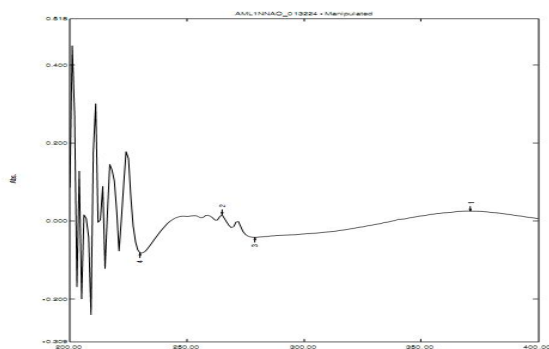
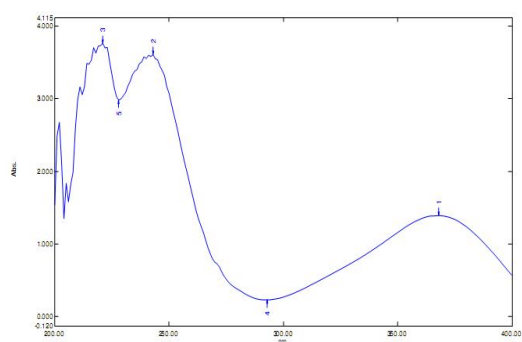
The recovery studies showed that the result were within the limit indicating no interference. The proposed method is simple, sensitive, accurate and precise and can be successfully employed for the routine analysis of the Amlodipine Besylate in bulk drug.

## CONCLUSION

The proposed method is simple, accurate, precise and selective for the estimation of Amlodipine Besylate in bulk drug. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. It can be effectively applied for the routine analysis of Amlodipine Besylate in bulk drug.

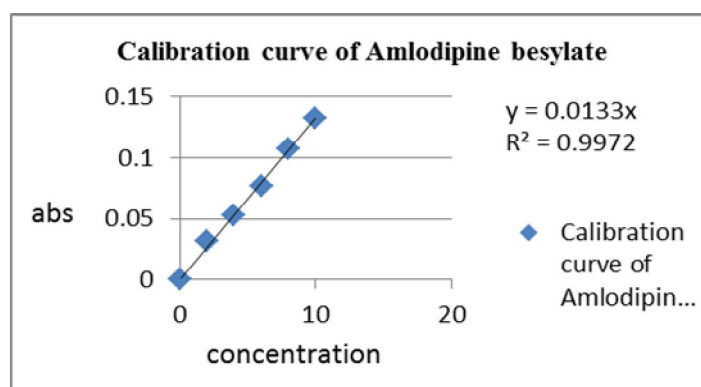
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1N NaOH with  $\lambda_{\text{Max}}$  223nm1N HCL with  $\lambda_{\text{Max}}$  241nm

**Table 1: Linearity of Amlodipine besylate by difference spectrophotometry**

S. No	Concentration Of amlodipine bisulphate ( $\mu\text{g/ml}$ )	Absorbance at 223 nm (1N NaOH)	Absorbance at 241 nm (1N HCl)	Difference in absorbance
1	2	0.0821	0.05	0.0312
2	4	0.142	0.09	0.0528
3	6	0.223	0.147	0.0763
4	8	0.302	0.195	0.1071
5	10	0.37	0.238	0.1329



**Table 3: characteristic and validation parameters of amlodipine besylate**

Parameters	Values	
	NaOH (223nm)	HCl (241nm)
Beers law limit ( $\mu\text{g/ml}$ )	2.5-15	2.5-15
$\lambda_{\text{max}}$	223	241
Regression equation ( $y=a+bc$ )	$Y= 0.0131x + 0.0013$	
Coefficient correlation ( $r^2$ )	0.9975	
Slope (b)	0.0131	
Intercept (a)	0.0013	
linearity	0.9975	
Accuracy range (%)	95.41-114.12	
LOD ( $\mu\text{g/ml}$ )	0.753525	
LOQ ( $\mu\text{g/ml}$ )	2.283408	

$y=mx+c$ , where x is the concentration in ( $\mu\text{g/ml}$ ) and y is absorbance unit

**PRECISION  
1N HCl**

Drug	Conc. [ $\mu\text{g/mL}$ ]	Trial	Trial	Trial	SD	%RSD
Amlodipine	4	0.101	0.105	0.102	0.002082	2.027915
Amlodipine	6	0.127	0.137	0.128	0.005508	4.215295
Amlodipine	8	0.171	0.175	0.170	0.002646	1.538372

**1N NaOH**

Drug	Conc. [ $\mu\text{g/mL}$ ]	Trial	Trial	Trial	SD	%RSD
Amlodipine	4	0.235	0.237	0.207	0.016773	7.410762
Amlodipine	6	0.257	0.242	0.227	0.015000	6.198347
Amlodipine	8	0.292	0.276	0.278	0.008718	3.091489

Standard concentration [ $\mu\text{g/mL}$ ]	Difference in Abs $\times 10^2$	found concentration [ $\mu\text{g/mL}$ ]	Recovery %
2	0.0312	2.28244275	114.122137
4	0.0528	3.93129771	98.2824427
6	0.0763	5.72519084	95.4198473
8	0.1071	8.07633588	100.954198
10	0.1329	10.0458015	100.458015

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