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***Short Communication***

# LC-UV Method Development and Validation of Amlodipine in Pure and Tablet Dosage Form

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## Abstract

*The present paper describe a simple, rapid, precise, accurate, economical and less time consuming LC-UV method development for simultaneous determination of in pure and Tablet formulation. Best symmetric peak shape was obtained with column Luna C18 (150mm X 4.6mm, 5µ) at 232 nm using UV-Vis detector, mobile phase used was Acetonitrile: Acetate buffer (pH 5) 50:50 at flow rate 1.0 ml/min. The retention time of Amlodipine besylate was found to be 3.4.*

**Keywords:** LC-UV, Amlodipine besylate, Acetonitrile, Validation.

## Introduction

Amlodipine is a calcium channel blocker which is long acting and efficiently used in the treatment of lower blood pressure. The chemical formula of drug was $C_{20}H_{25}ClN_2O_5$. The IUPAC name of the drug is [3-ethyl, 5-methyl2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.

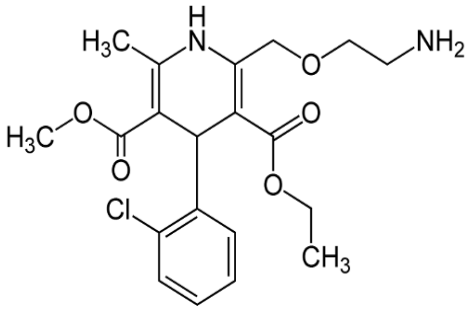

**Figure-1**
**Amlodipine besylate**

## Materials and Methods

Acetonirtile, Methanol and Water were used of HPLC grade. All the reagents for the analysis were purchased from Merck Specialties Pvt. limited, Mumbai. The standard form of the drug was gifted by Emcure Pharmaceutical, Pune.

**Instrumentation:** Chromatographic separation was performed on a YOUNGLIN acme 9000 gradient pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV730 D the output signals was monitored and integrated by chromatographic software version Autochro-3000. Standard and sample drug were weighed by using Shimadzu AY220 electronic analytical balance.

**Preparation of Standard Solution:** In order to prepare the standard solution Amlodipine besylate 5 mg of standard drug was accurately weighed using Shimadzu AY220 electronic balance and dissolve in 100 ml of methanol, 50 µg/ml concentrations. From the stock solution 1 ml was transferred into volumetric flask dilute with 10 ml methanol, thus 5µg/ml concentration respectively. The solution was filtered through nylon memberane of pore size 0.45µm.

**Preparation for Pharmaceutical formulation:** To determine the Amlodipine besylate content of tablet formulation,ten tablets were weighed to determine the average weight of the tablets, and then they were crushed, and mixed using morter and pastle. A sample of the tablet powder equivalent to 10 mg was accurately weighed, and dissolved in methanol make up the volume up to 10ml and the active pharmaceutical ingredient was extracted through given solution by ultrasonication and filtered through a 0.45µm membrane filter. The solution was diluted by additing methanol up to 1000µg/ml concentration.From the tablet solution of 1000µg/ml, 1 ml was pipette out and transferred into volumetric flask of 10 ml dilute with methanol. 100µg/ml solution was used to estimate the formulation assay.

**Chromatographic Conditions:** The mobile phase composition was selected as Acetonitrile: Acetate buffer 50:50 (%v/v). The pH of the mobile phase was set as 5. The Phenominex Luna C18 column was used for analysis. The standard solution of Amlodipine was scanned in the range of 200-400 nm against mobile phase as methanol in Schimatzu spectrophotometer. The maximum absorbance of drug was found and overlaps it and suitable wavelength was found as 232 nm. Ambient temperature was maintained during analysis. Injected volume of 20µl and runtime 10 min was maintained. The drug was eluted with retention time 3.4.

## Results and Discussion

**Method development:** Method d development plays important vital role in estimation of drug in pharmaceutical dosage forms.

Many trials were conducted in order to develop best suitable method for determination of drug in pure and tablet dosage forms. First of all, mobile phase composition was set as 50:50v/v of Methanol: Water Amlodipine was not eluted up to 10 min. The mobile phase was changed Acetonitrile: Acetate buffer 50:50 v/v in this it was found sharp peak, good response with acceptance retention time was found 3.4. pH of the mobile phase was set 5 with Glacial acetic acid. The flow rate of the mobile phase was 1ml/min. The ambient temperature was maintained during the analysis.

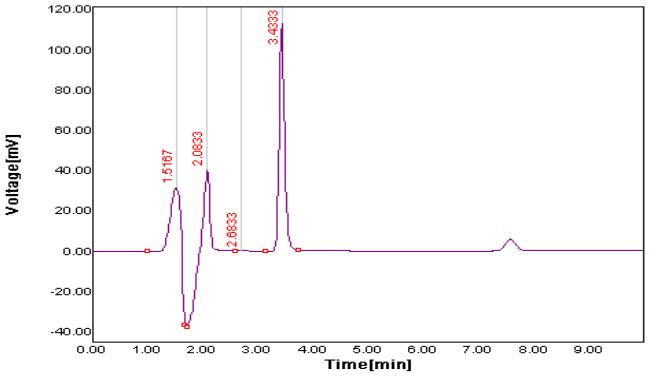


**Figure-2**
**Standard chromatogram of Amlodipine besylate**

**Method validation:** Method validation, according to ICH guidelines is performed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specific range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations.

**Table-1**
**System suitability of Amlodipine besylate**

| System suitability parameters | Amlodipine besylate |
|---|---|
| RSD | 0.38 |
| Tailing factor | 1.1 |
| No. of theoretical plates | 6096 |
| Resolution | 8.07 |
| LOD (µg/ml) | 0.04 |
| LOQ (µg/ml) | 0.14 |
| Retention Time | 4.4 |

**Linearity:** Linearity was checked in the concentration range 3-7 µg/ml AMLO using for pure drug and result were tabular form.

**Table-2**
**Linearity results of Amlodipine besylate**

| Concentration (µg/ml) | Area |
|---|---|
| 7 | 220.82 |
| 6 | 190.13 |
| 5 | 160.12 |
| 4 | 125.12 |
| 3 | 96.46 |

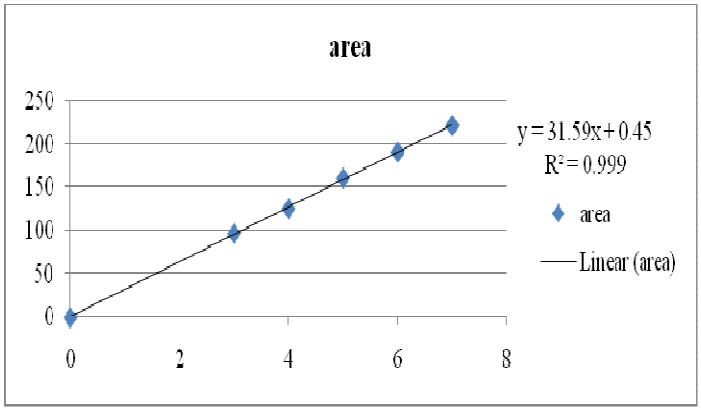


**Figure-3**
**Calibration curve of Amlodipine besylate**

**Precision:** Precision of the method was analyzed in two ways: intraday and interday precision. Intraday precision was estimated by injection concentration of 100µg/ml on the same day of preparation of drug solution.

**Table-3**
**Intraday Precision**

| Sample (µg/ml) | Amlodipine besylate |
|---|---|
| 1 | 161.04 |
| 2 | 160.25 |
| 3 | 160.3 |
| 4 | 159.8 |
| 5 | 161.2 |
| 6 | 160.5 |
| %RSD | 0.32 |

**Table-4**
**Intraday Precision**

| Sample (µg/ml) | Amlodipine besylate |
|---|---|
| 1 | 158.02 |
| 2 | 154.07 |
| 3 | 156.3 |
| 4 | 153.4 |
| 5 | 159.2 |
| 6 | 158.5 |
| % RSD | 0.38 |

**Recovery:** The % recovery values were found to be in the range of 96.2 – 102% three different spiked concentrations used in the recovery studies.

**Table-5**
**Recovery**

| Drug | Concentration | Amount recover | % Recovery |
|---|---|---|---|
| Amlodipine | 80 | 2.05 | 102 |
| | 100 | 2.5 | 100 |
| | 120 | 2.9 | 96.6 |

**Robustness:** Robustness of the development chromatographic condition was analyzed by making the small variation in the chromatographic condition. In the present study two important chromatographic conditions wavelength and flow rate were varied.

**Table-6**
**Robustness**

| Criteria | | Amlodipine besylaye |
|---|---|---|
| Flow rate | 0.9 | 4.6 |
| | 1.1 | 4.3 |
| Wavelength | 230 | 4.5 |
| | 234 | 4.5 |

**Sensitivity:** The sensitivity of the method reflects the capability of the method to detect and quantify very low concentration of the drug in the sample solution. The sensitivity of the developed method influences many important factor such as if the method was very sensitive it can detect and quantify drug in wide range.

**Table-7**
**Sensitivity**

| Parameter | Drug | µg/ml |
|---|---|---|
| LOD | Amlodipine besylate | 0.04 |
| LOQ | | 0.14 |

**Table-8**
**Formulation assay**

| Formulation | Drug | Concentration (µg/ml) | Amount found |
|---|---|---|---|
| | Amlodipine besylate | 5 | 5.1 |

## Conclusion

The development LC-UV method was simple, specific, accurate, rugged, robust and sensitive validation of the proposed procedure was carried out according to the ICH guidelines from the result it can be concluded the method can be used for routine analysis of Amlodipine in Pure and Tablet Dosage Form.

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