IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 February, 2012; received in revised form 22 March, 2012; accepted 28 May, 2012

HPLC METHOD DEVELOPMENT OF AMLODIPINE BY RP-HPLC IN ITS BULK DOSAGE FORMS

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Keywords:
Amlodipine,
validation,
C-18 column,
Reverse phase

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ABSTRACT

A new, simple, precise, sensitive, accurate and reproducible Reverse phase HPLC method was developed and validated for the analysis of amlodipine in bulk dosage forms. The separation was conducted by using c-18 RP-HPLC coloumn, which was maintained at ambient temperature. The mobile phase consisting of Phosphate buffer and Acetonitrile (90:10v/v) was delivered at a rate of 1.5 ml/min. The analysis was detected by using UV detector at the wave length of 225nm.The method is validated for its specificity, precision, accuracy, linearity and robustness. The method was found to be linear over the concentration range 10-100 μg/ml (r^2 =0.999). The retention time for amlodipine was found to be 3.34min. Limit of quantification of the method is 0.179μg/ml and limit of detection is 0.054 μg/ml.

INTRODUCTION: Amlodipine chemically known as 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate [1]. Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. That inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels.

Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Serum calcium concentration is not affected by amlodipine. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

This paper describes a fast, sensitive, rapid and accurate method for developed and validated [2, 3, 4]. The analysis of amlodipine in bulk dosage forms by using Reverse Phase- High Performance Liquid Chromatography(RP-HPLC) [3]. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [2].

MATERIALS AND METHODS:

Instrumentation: Quaternary isocratic HPLC (Younglin HPLC YL9000 series) with YL 9110 Pump and with autochro 3000 software and UV-Vis detector YL9120, electronic balance (Shimadzu) was used for weighing the sample.

Reagents and Chemicals: Amlodipine was obtained from FDC limited Goa and acetonitrile and water employed for the preparation of mobile phases were of HPLC grade (qualigens fine chemicals, Mumbai). All the other chemicals and solvents viz phosphate buffer and acetonitrile are of ambient grade.

Selection of Chromatographic Parameters:

a) Column : Inertsil, C-18, 250 x 4.6mm.5µ.

b) Flow rate : 1.5 ml/min

c) Temperature : Room Temp

d) Detection wavelength : 225nm

e) Injection volume : 20µl

f) Run time : 5 min

Selection of Mobile Phase: The solution of Amlodipine was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally Phosphate buffer and Acetonitrile (90:10v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Amlodipine.

Preparation of Mobile Phase: Mobile phase comprised of 10 mM Potassium di- hydrogen ortho phosphate. (Adjusted to P^H 3.5±0.05 with Ortho phosphoric acid), and acetonitrile (90:10 v/v), diluent (pH 7) used was water. Mobile phase was filtered through a 0.45-µ m membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.5 ml/min), which yields a column back pressure of 538-543 psi .Run time was set as 5 min, column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 225 nm and data were acquired, stored and analyzed with the software"Autochro-3000" (Young Lin).

Preparation of Standard Stock Solution: A stock solution of Amlodipine was prepared by dissolving Amlodipine (100 mg) in a volumetric flask (100 ml) containing 25 ml of diluent, sonicated for 20 min and then made up to the volume with diluent. Working standard solution of Amlodipine (300µg/ml) was prepared by suitable dilution of stock solution with diluent. Linearity solutions were prepared in diluents containing RS (10-100µg/ml). Each of these drug solutions (20µl) was injected into the column and the peak area and retention times were recorded.

Selection of Analytical Wavelength: From the standard stock solution, further dilutions were prepared using mobile phase and scanned over the range of 200 – 400 nm and the spectrum was overlain. It was observed that 225 nm is the λ_{max} for Amlodipine and the wavelength suitable for Amlodipine was preferred.

RESULTS AND DISCUSSION: The retention time for Amlodipine was 3.34 minutes for a run period of 5 minutes. Each sample was injected five times and the similar retention times were observed in all cases. The peak areas of different concentrations set up as above were calculated and average value for 5 such determinations are shown in Tables given below. The peak area for drug solution was reproducible as indicated by low coefficient of variation. A good linear relationship (r= 0.999) was observed between the concentration of Amlodipine and the respective peak areas.

The calibration graph was found to be linear in the range of 10-100 µg/mL, when the Amlodipine solution was analyzed by the proposed RP-HPLC method. In intra and inter day variation studies, Inter day and intraday precision was determined by analyzing the drug sample at three different concentration levels. The results are presented in the form of %RSD which is below 1.00. which shows that the proposed HPLC method was highly precise.. The drug content in the Capsules was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

Evaluation of Analytical Methods:

1. **Linearity:** A liquots ranging from 10-100µg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Amlodipine, the higher concentration range was used to improve signal to noise ratio. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100µg/ml for Amlodipine.

2. **Precision:** Five sets of aliquots with same concentration (50 µg/ml) were prepared and these solutions were analyzed to record any intra and inter day variations in the results. The results obtained for Intra and interday variations are shown in Tables respectively.

3. **Limit of Detection (LOD):** The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula;

$$LOD = \frac{3.3\sigma}{S}$$

LOD = 0.054µg/ml

Where, σ = standard deviation of the response, S = slope of the calibration curve

4. **Limit of Quantitation (LOQ):** The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula;

$$LOQ = \frac{10\sigma}{S}$$

LOQ= 0.179µg/ml

Where, σ = standard deviation of the response, S = slope of calibration curve

5. **Range:** Amlodipine : 10-100 µg/ml

TABLES:

1) Resolution of drug

Drug	RT (min)	Peak Area	Height	Plates	HETP
Amlodipine	3.34	3883448	43314	3441	0.0435

2) Accuracy

Concentration(µg/ml)	Retention Time(min)	Peak Area
10	3.47	86542
25	3.46	2365421
50	3.46	3965423
75	3.348	5652139
100	3.346	7354629

3) **Precision**

Intra-day Precision for Amlodipine

Concentration(µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3965423			
50	3883621			
50	3883425	3783425.5	53289	0.35
50	3923748			
50	3843218			

Inter day precision of Amlodipine

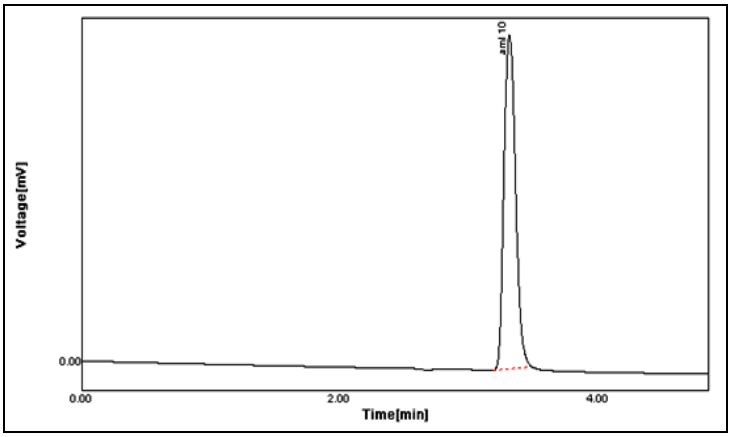
Concentration(µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3873448			
50	3883621			
50	3783425	3833523	54289	0.23
50	3923748			
50	3924568			

4) Limit of Detection and Quantification (LOQ & LOD):

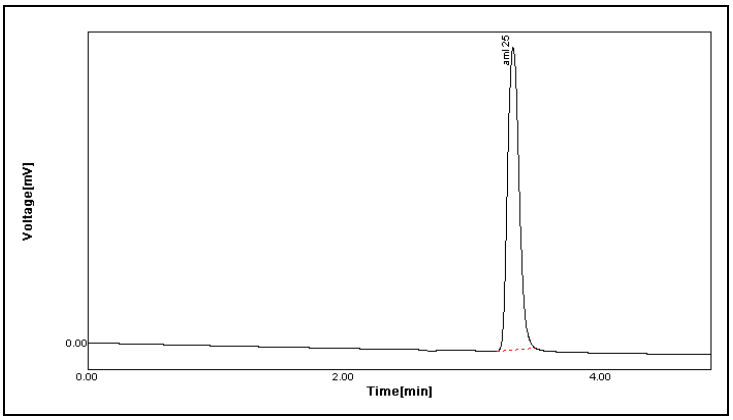
Limit of Detection	Limit of Quantiation
0.075µg/ml	0.179µg/ml

CHROMATOGRAMS:

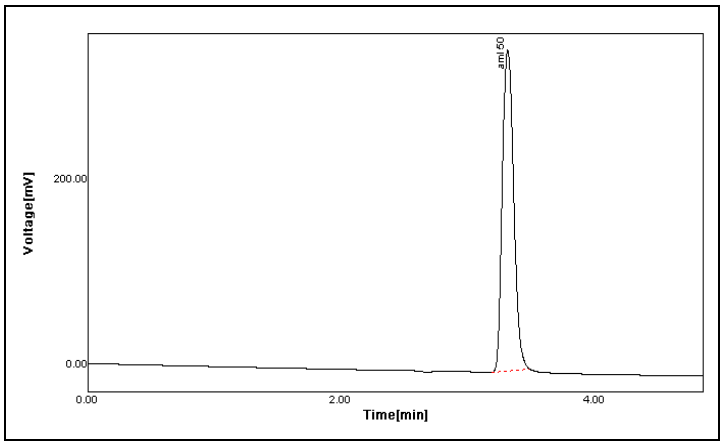
1) Linearity:



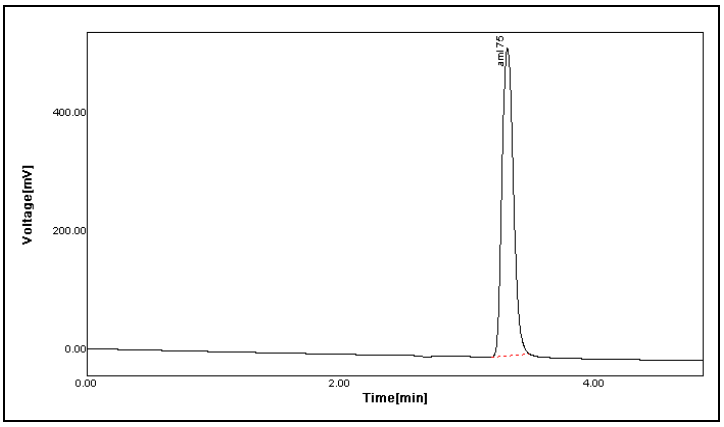
A TYPICAL CHROMATOGRAM FOR AMLODIPINE (10µg/ml)



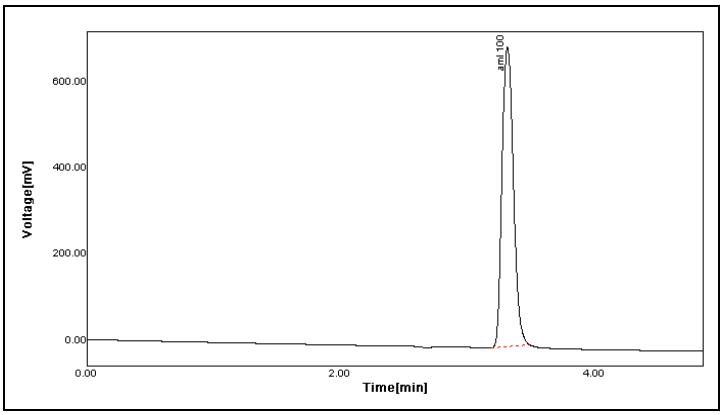
A TYPICAL CHROMATOGRAM FOR AMLODIPINE (25µg/ml)



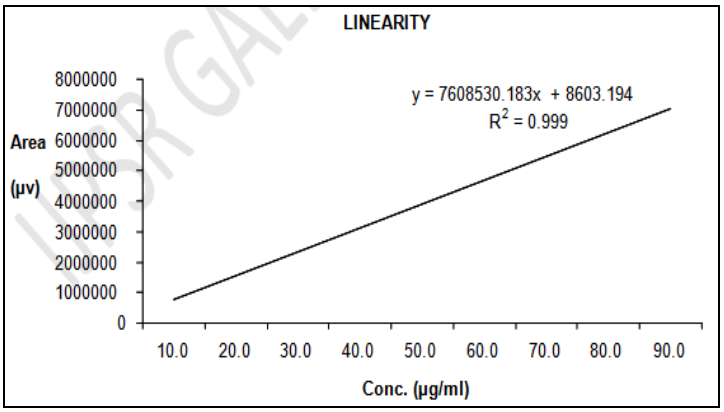
A TYPICAL CHROMATOGRAM FOR AMLODIPINE (50µg/ml)



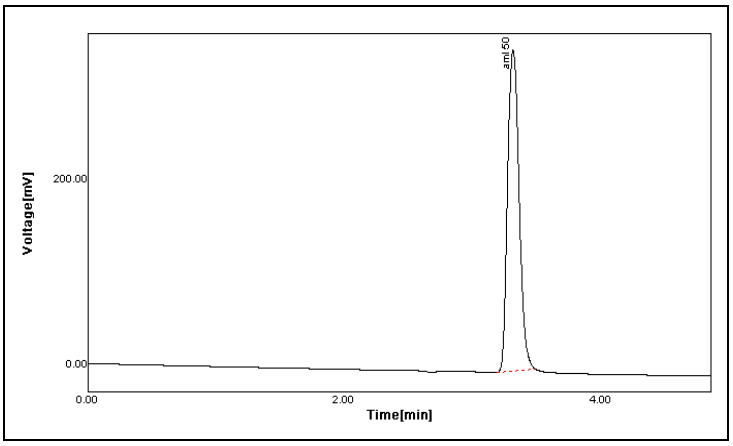
A TYPICAL CHROMATOGRAM FOR AMLODIPINE (75µg/ml)



A TYPICAL CHROMATOGRAM FOR AMLODIPINE (100µg/ml)



CALIBRATION CURVE OF AMLODIPINE:



A TYPICAL CHROMATOGRAM FOR AMLODIPINE STANDARD SOLUTION (50µg/ml)

CONCLUSION: The developed method was validated in terms of precision, linearity, limit of detection & and limit of quantification. A good linear relationship was observed for amlodipine in the concentration range of 10-100µg/ml.

The co-relation co-efficient for amlodipine was found to be as 0.999.The intra and inter day precision was good enough to indicate that the developed method is precise and reproducible.

This demonstrated that the current developed RP-HPLC method is simple, linear, pricise.

ACKNOWLEDGEMENT: The authors are thankfull to the Management of Brown's College of Pharmacy for granting permission for the publication of this work.

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