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Research article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF AMLODIPINE

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ABSTRACT: The present paper describes a simple isocratic RP-HPLC method for the determination of Amlodipine in tablet dosage form. Best symmetric peak shape was obtained with column Zodiac C18 column (250 mm x 4.6 mm, 5µ) at 245nm with retention time of 5.53min. The mobile phase used was Methanol: Water: Acetonitrile 60:20:20 (v/v/v) with flow rate of 1.0 ml/min. The method for estimation of Amlodipine in tablet dosage form was found to be linear, accurate, precise, sensitive and selective. The linearity range was from 60µg/ml to 210µg/ml. Method was found to be highly sensitive as LOD and LOQ were found to 0.4µg/ml and 1.3µg/ml. The repeatability and reproducibility were within the range i.e. less than 2%. The %recovery values were found to be in the range of 98.82-100.93%. The percentage of assay was calculated for market formulation was 99.19%.

Keywords: Amlodipine, HPLC, Linearity, validation

INTRODUCTION

Amlodipine is a calcium blocker of type dihydropyridine which is long acting and efficiently used in the treatment of angina chest cancer and used to lower the blood pressure. The Chemical formula of the drug was $C_{20}H_{25}ClN_2O_5$. The IUPAC name of the drug is (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Fig-1). The drug lowers the blood pressure by relaxing arterial smooth muscles, in turn decreasing the total peripheral resistance and thus reduces blood pressure. The drug is considered to be most important medication needed in basic health system, listed by World health organization's list of essential medicines (WHO, 2014). It is used in the treatment of coronary heart disease (ASHSP, 2011) and in the management of hypertension (Wang, JG, 2009).

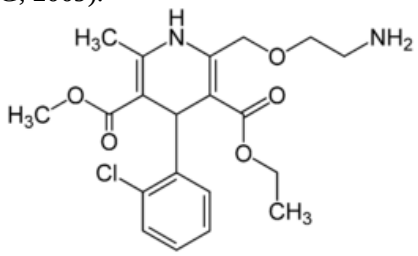

Figure 1: Structure of Amlodipine

There are some adverse effects of intake of drug into the body (Sandoz product information sheet), commonly peripheral edema was observed in 8.3% of drug users, 4.5% of users experience fatigue, 1% of drug users undergo the mild side effects such as dizziness, headache, palpitations; stomach pain, dyspepsia, solmnolence and nausea. Literature survey reveals that few analytical methods have been reports for the estimation of Amlodipine in different pharmaceutical dosage forms in different combinations using different instruments. Among those few were with RP-HPLC(samya M et al,2012, Maste M.M et al, 2011, Nisrin Kaddar et al,2013, Richa Sah et al 2012, Safeer K et al,2010),Spectrophotometric Methods(Praveen Kumar Jampana et al,2014,P. Y. Pawar et al,2013, Laxmileena D. Patil et al,2013, Ramya Gavini et al 2012, Swaroopa Rani K et al,2011, Mehulkumar P et al,2009, Nashwah Gadallah Mohamed et al,2011),UPLC (Reddy, Yarram Ramakoti et al,2012) methods.

Some of these methods have certain draw backs like gradient elution technique, longer run time, lack of sufficient sensitivity, precision and accuracy, and also having expensive mobile phase conditions, contains salt buffers which reduces the life time of the column and may precipitate in the passage route on long stand and also increases the pressure of the pump. Hence an attempt was made to overcome these drawbacks and succeeded in developing a highly specific, accurate, precise and economic method with retention time of 5.53min was best for the routine analysis of the drug in the tablet dosage form.

Materials

Methanol, Acetonitrile and Water were used of HPLC grade. All other chemicals were of Analytical grade. All the reagents used for the analysis were purchased from Merck Specialties Pvt. limited, Mumbai. The standard form of the drug was gifted by Hetero labs, Hyderabad. The pharmaceutical dosage form ie, tablet was purchased from local market.

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Preparation of Standard Solution

In order to prepare the standard solution of Amlodipine 10mg of standard drug was accurately weighed using Denver electronic balance and dissolved in 10ml of methanol, thus 1000µg/ml standard solution was prepared. The solution was filtered through nylon membrane of pore size 0.45µm. From the standard solution different concentrations were prepared by proper dilutions. The drug solutions of concentration ranging from 60-210µg/ml were prepared, they were considered as working standards.

Procedure for pharmaceutical formulation

To determine the Amlodipine content of tablet formulation, ten tablets of Amlostat were weighed to determine the average weight of the tablets, and then they were crushed, and mixed using a mortar and pestle. A sample of the tablet powder equivalent to 10 mg was accurately weighed, mixed with 10ml methanol and the active pharmaceutical ingredient was extracted into the methanol by vortex mixing followed by ultrasonication and then filtered through a 0.45µm membrane filter. The solution was diluted by adding methanol to obtain a tablet solution of 1000µg/ml. From the tablet solution of 1000µg/ml, 1.2ml was transferred into volumetric flask of 10ml containing methanol. 120µg/ml solution was used to estimate the formulation assay of Amlostall.

Chromatographic Conditions

The mobile phase composition was selected as Methanol: Water: Acetonitrile 60:20:20(%v/v). The pH of the mobile phase was set as 5.9.The Zodiac C18 column was used for the analysis. The standard solutions of Amlodipine were scanned in the range of 200-400 nm against mobile phase as blank in UV-2301 Spectrophotometer. The suitable wavelength showing maximum absorbance was found as 245nm. Ambient temperature was maintained during analysis. Injection volume of 20µl and runtime of 10min was maintained. The drug was eluted with retention time of 5.53min.

Method development

Method development plays vital role in estimation of drug in pharmaceutical dosage forms. Many trails 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 optimized. Methanol and water was taken in different proportions. The mobile phase was varied to get the most suitable composition, so that peak with good resolution and sharpness can be obtained. Mobile phase composition was set as 50:50, 60:40, 70:30, 80:20 v/v of methanol and water. It was found that peak was broad need to have organic phase modifier as mobile phase ingredient. Then acetonitrile was added to the mobile phase. The mobile phase composition was selected in different trails as Methanol: Water and acetonitrile in the ratio 50:30:20, 60:20:20, 70:20:10(%v/v). It was found that mobile phase composition of methanol: water and acetonitrile in the ratio of 60:20:20 (%v/v) gave sharp peak with good response with acceptable retention time of 5.53min. The retention time was suitable for routine analysis of drug in tablet dosage forms.. The pH of the mobile phase was set as 5.9. The C18 column was suitable for analysis, So Zodiac column was used for the analysis. The flow rate of the mobile phase was varied from 0.5-1.5ml/min. The flow rate of 1.0ml/min was selected, as the drug was eluted at 5.53min i.e. suitable retention time for analysis. The ambient temperature was maintained during the analysis.

Method validation

The validation of the analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the indented analytical application.

Validation is also a proof of the repeatability, specificity and suitability of the method. Typical method development and establishment for analytical method includes determination of (1) System suitability, (2) Linearity, (3) precision, (4) accuracy, (5) Ruggedness, (6) Robustness, (7) Sensitivity.
The system suitability parameters such as tailing factor, theoretical plates, retention time all the chromatographic conditions were analyzed for acceptable conditions. Tailing factor must lie below 2, whereas theoretical plates should be more than 2000. The retention time developed in the method should be suitable for the routine analysis of the drug in the pharmaceutical formulation, i.e. elution time should not be too short or too long. The proposed method is said to be linear only when the concentration of the analyte is directly proportional to peak areas of the drug sample. In order to evaluate linearity serial dilution of the drug solution were prepared from stock solution by diluting with the mobile phase to get the concentration range of 60-210µg/ml. The linear regression equation and co-relation coefficient value depicts the linearity of the method. The calibration curve was plotted by taking concentration on x-axis and peak area values on y-axis.

The precision of a method is a measure of random error and is defined as the Agreement between replicate measurements of the same sample. It is expressed as the relative standard deviation (R.S.D.) of the replicate measurements. In this study the %RSD value illustrates the precision of the method. The precision was determined by intraday and interday precision studies. The recovery of the method is determined by spiking of standard drug solution to pre analyzed sample at three different levels i.e., at 50, 100, and 150%. The %recovery values were found to be in the range of 98.82-100.93%. The ruggedness of the method is described by measure of reproducibility of test results under normal expected conditions from instrument to instrument and from analyst to analyst. It is generally evaluated by carrying out the analysis by different analysts in the laboratory. The robustness of the method is to determine that the method is suitable and slight variations to the method conditions whether alter the system suitability conditions. A method is said to be robust only if the results of the analysis were unaffected by small deliberate changes to the operational conditions. Many of the chromatographic conditions were slightly varied in order to check the robustness of the method, but the most common conditions that were generally altered were detection wavelength of UV detector, composition of mobile phase and pH of the system.
The sensitivity of the method was determined by estimation of Limit of detection and Limit of quantification. They were determined by using the formula associated with the signal to noise ratio (S/N). To determine the value of LOD S/N (signal/noise ratio), acceptable ratio of LOD was 2:1 or 3:1, while S/N of 10:1 is often considered to be necessary for determination of LOQ. A typical signal is measured from the base line to apex and divided by the peak to peak noise, which is determined from the blank chromatogram.

RESULTS AND DISCUSSIONS

Method development

During this phase of analysis most suitable chromatographic conditions were developed for the estimation of the drug. It was stated that the mobile phase composition of methanol: water: acetonitrile in the ratio of 60:20:20(%v/v) was best suited, as it gave good resolute and sharp peak and retention time of 5.53min was best for the routine analysis of the drug in the tablet dosage form. The flow rate was maintained as 1.0ml/min. The pH was maintained as 5.9. All the system suitability parameters were satisfied as tailing factor was obtained as 1.23, theoretical plates were 10318 and retention time was found to be 5.53min. The response was good and peak shape was sharp.

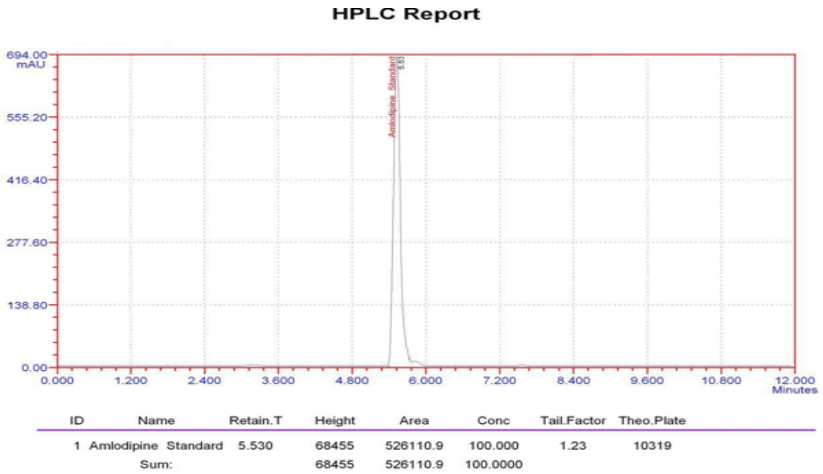

Figure 2: Standard chromatogram of Amlodipine

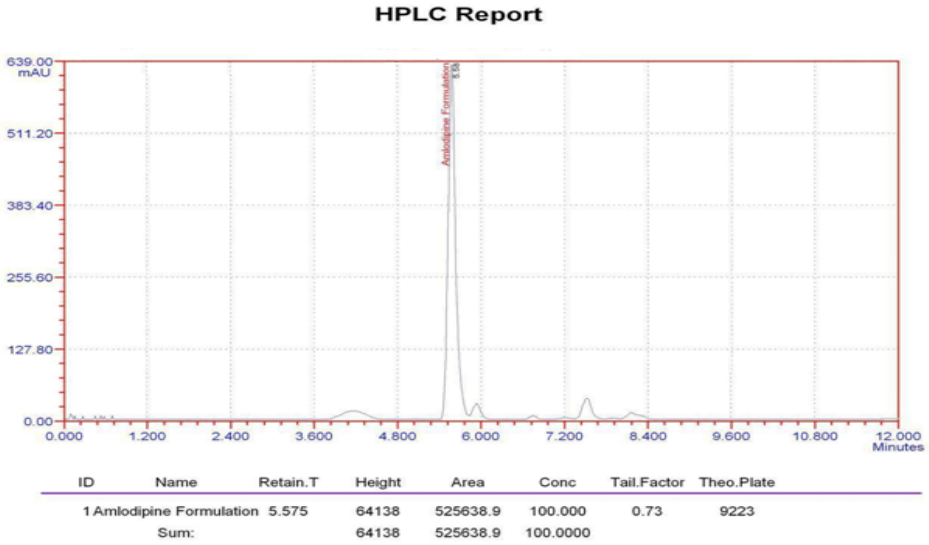

Figure 3: Formulation chromotogram of Amlodipine

Method validation

The developed method was validated according to ICH guidelines. The different validation parameters such as linearity, precision, recovery, robustness, robustness, sensitivity and formulation assay were estimated.

Linearity

The linearity of this method was found to be in the range of 60-210µg/ml for Amlodipine (API) with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of y =4221x+13952. The wide range of concentration shows that the drug can be estimated accurately and can be used for routine analysis. Results are given in Table 1, Fig-4

Table 1: Linearity results of Amlodipine

S.No	Concentration (µg/ml)	Area
1	60	279895
2	90	398673
3	120	526110
4	150	643129
5	180	779681
6	210	889181
	Slope Intercept CC	4221 13952 0.9995

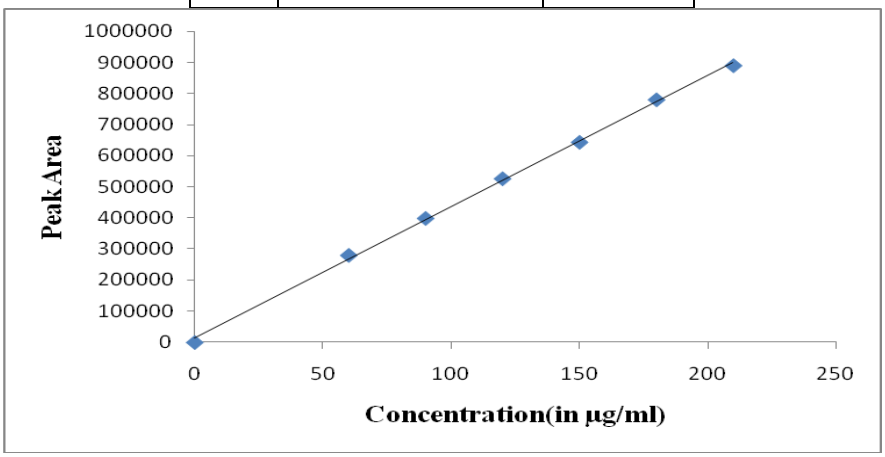

Figure 4: Calibration curve of Amlodipine

Precision

Precision of the method was analyzed in two ways: intraday and interday precision. Intraday precision was estimated by injecting six replicate concentration of 120µg/ml on the same day of preparation of drug solution. The %RSD of the six replicate injections of drug sample was found to be 0.44. In case of intraday precision, six replicate injections were injected into the chromatographic column on consecutive days within one week of sample preparation. It was observed that %RSD was found to be 0.56; the %RSD values depict the repeatability of the method. The results are given in table 2 and 3.

Table 2: Intraday Precision results of Amlodipine

Sample (µg/ml)	Area
1	526110
2	528753
3	522545
4	523176
5	526454
6	524272
% of RSD	**0.443**

Table 3: Interday Precision results of Amlodipine

Sample (µg/ml)	Area
1	524965
2	518622
3	517249
4	520593
5	522965
6	522954
% of RSD	**0.56**

Recovery

The %recovery values were found to be in the range of 98.82-100.93%. Three different spiked concentrations used in the recovery studies were 60, 120, and 180µg/ml. The recovery was carried out by using standard addition method in the range of 50, 100 and 150%. The results of recovery studies were illustrated in Table 4.

Table 4: Recovery results of Amlodipine

S.No	Recovery	Concentration	% Estimation Conc Found	% of Recovery
1	50 %	60 µg/ml	60.30	100.50
		60µg/ml	59.89	99..82
		60µg/ml	59.49	99.16
2	100%	120 µg/ml	119.0	99.17
		120µg/ml	120.67	100.57
		120µg/ml	120.13	100.11
3	150 %	180 µg/ml	179.86	99.92
		180 µg/ml	181.67	100.93
		180 µg/ml	177.8	98.82

Ruggedness

The %RSD value 0.81 illustrate that method was rugged, in spite of changing the analyst no significant changes were observed in the estimation of the drug in the pure and tablet dosage forms. Thus, the developed chromatographic conditions were suitable for the routine analysis of the drug in pharmaceutical formulation.Table-5.

Table 5: Ruggedness results of Amlodipine

Sample (µg/ml)	Area
1	519437
2	517687
3	519491
4	526725
5	525325
6	527192
% of RSD	**0.81**

Robustness

Robustness of the developed chromatographic condition was analyzed by making the small variations in the chromatographic conditions. In the present study, three of the important chromatographic conditions mobile phase, wavelength and flow rate were varied. Mobile phase composition was varied as Methanol: Water: Acetonitrile in the ratio 55:22.5:22.5(%v/v) and 60:17.5:17.5(%v/v). The wavelength of UV detector was varied from 245nm to 242 and 248nm. The flow rate was maintained as 0.8 and 1.2ml/min. It was observed that there were no considerable changes in the peak shape and retention time of Amlodipine, Table-6.

Table 6: Ruggedness results of Amlodipine

S.No	Parameter	Change	Area	% of Change
1	Standard	……	526110	……
2	Mobile Phase	55:22.5:22.5	529512	0.65
		60:17.5:17.5	521860	0.81
3	Wavelength	242nm	523625	0.47
		248nm	525603	0.1
4	Flow rate	0.8ml/min	520964	0.98
		1.2ml/min	527887	0.34

Sensitivity

The sensitivity of the method reflects the capability of the method to detect and quantify very low concentrations of the drug in the sample solution. The sensitivity of the developed method influences many important factors such as if the method was very sensitive it can detect and quantify drugs in wide range of sample solution. The Limit of Detection and Limit of quantification depicts the sensitivity of the method, as the LOD value was calculated to be 0.4µg/ml and LOQ was 1.3µg/ml. Table-7.

Table 7: Sensitivity results of Amlodipine

LOD	0.4µg/ml
LOQ	1.3µg/ml

Formulation assay

In order to find the %assay of the drug sample in the pharmaceutical tablet dosage form AMLOSTAT, the formulation assay was carried out. The concentration of 120µg/ml of tablet solution was prepared in methanol and injected into the chromatographic column corresponding peak area of tablet and pure drug form was noted. By using the formulae, %assay of the drug in tablet dosage form was calculated and it was found to be 99.91%. Table-8.

Table 8: Sensitivity results of Amlodipine

Formulation	Dosage	Concentration	Amount found	% Assay
Amlostall	5 mg	120 µg/ml	119.89	99.91

CONCLUSION

The developed RP-HPLC method was simple, specific, precise, accurate, rugged, robust and sensitive. Validation of the proposed procedures was carried out according to the ICH and USP guidelines. From the results it can be concluded the method can be used for routine analysis of Amlodipine in pure and tablet dosage form.

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