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Development and optimization of RP-HPLC method for the estimation of s (-) amlodipine in tablet dosage form

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ABSTRACT

S(-) amlodipine is a potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris. S(-) amlodipine avoids the adverse effect of amlodipine in racemic mixtures. The RP-HPLC method developed for the estimation of s (-) amlodipine in tablet dosage form was done by fixing the parameters as Phenomenex C_8 ODS column (150 x 4.6 mm), 5 μ particle size with mobile phase 20 mM sodium dihydrogen phosphate buffer: acetonitrile (65: 35% v/v) adjusted to pH 8 was used. Mobile phase flow rate was maintained at 1.2ml/min and detected at 239nm. The retention time was 4.20± 0.02 minutes

Keywords: RP-HPLC, S(-) amlodipine.

INTRODUCTION

S(-) amlodipine is a potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris. S(-) amlodipine avoids the adverse effect of amlodipine in racemic mixtures.Literature survey reveals few analytical methods for the determination of amlodipine alone and in combination with other drugs in pharmaceutical preparations and biological fluids including HPLC[1-2], UV spectroscopy[3], LC/MS[4].

MATERIALS AND METHODS

Shimadzu LC-10A(UV detector), Digital electronic balance (BL-220H) (Shimadzu, Japan), S(-) amlodipine (Cipla Pharmaceuticals), HPLC water (Qualigens Fine Chemicals, Mumbai), Sodium Dihydrogen Phosphate (E. Merck (India) Ltd, Mumbai), Acetonitrile HPLC grade (Qualigens fine Chemicals, Mumbai),

Method

Preparation of mobile phase

Several mobile phases at different ratios were used but no favourable results were obtained except for sodium dihydrogen phosphate buffer: acetonitrile in the ratio of 65: 35 % v/v, which gave an acceptable peak. 20 mM was selected as ionic strength for buffer solution at pH 8.0 to get a sharp symmetrical peak with good resolution.

Chromatographic conditions

Phenomenex C_8 ODS column (150 x 4.6 mm), 5 µ particle size with mobile phase 20 mM sodium dihydrogen phosphate buffer: acetonitrile (65: 35% v/v) adjusted to pH 8 was used. Mobile phase flow rate was maintained at 1.2ml/min and detected at 239nm. The retention time was 4.20± 0.02 minutes.

Analysis of formulation

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 10mg of the drug in acetonitrile. It was made up to the volume in a 100 ml standard flask to obtain a concentration of 100µg/ml. From this 1 ml was taken in a 100 ml standard flask and made up to the volume to get a concentration of 1µg/ml. Aliquots of solutions were taken from 1µg/ml to make a concentration of 1-5ng/ml(fig 1).

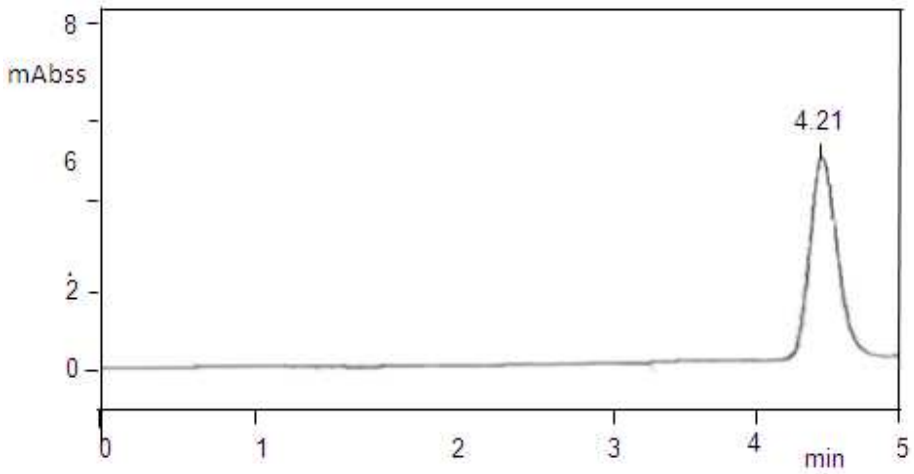

Fig 1:- Chromatogram of standard s (-) amlodipine (5ng/ml)

Preparation of Sample Solution

20 tablets of s (-) amlodipine were weighed and average weight was calculated. The quantity equivalent to 10 mg of s (-) amlodipine was weighed accurately and diluted to get a concentration of 3ng/ml (table 1).

TABLE 1: Analysis of formulation

Drug	Label claimed	Estimated amount	% Recovery	%RSD*
s(-) amilodipine	5 mg	4.950mg	99.13	0.113

** Mean RSD of three observations*

Validation of the Developed Method

The RP-HPLC method was validated in terms of parameters like linearity and range, accuracy, precision, LOD, LOQ, stability, specificity etc. For all the parameters % RSD values were calculated.

Linearity and range

The standard stock solution was diluted to get a concentration of 1-5ng/ml of s (-) amlodipine. The calibration curves were plotted using peak area Vs concentration for the standard solutions. The correlation coefficient, slope and intercept of s (-) amlodipine was found to be 0.9992, 19227.6, and 4094.3 respectively (fig 2).

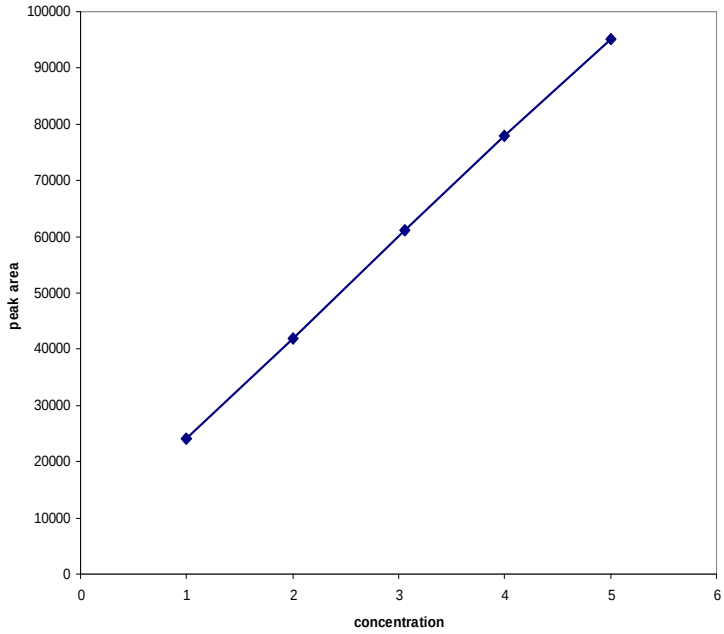

Fig 2:- Calibration curve of s (-) amlodipine (1-5ng/ml)

Accuracy

The accuracy of an analytical method is the looseness of test results obtained by that method to the true value. To study the accuracy of the method, recovery experiments were carried out at 50% and100% levels (table 2). The percentage recovery was calculated using formula given below.

$$\%\ \mathrm{Recovery} = \frac{Amt\ \ calculated\ \ after\ \ addition - Amt\ \ calculated\ \ before\ \ adition}{Amt\ \ standard\ \ added}$$

TABLE 2: ACCURACY

Drug	% Recovery		% RSD*	
	50%	100%	50%	100%
s (-) amlodipine	99.92± 0.019	100.18± 0.012	0.112	0.1478

** Mean RSD of six observations*

Precision

Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample.

a) Intra- day precision.
b) Inter- day precision.
c) Repeatability of injection.

a) Intra-day precision

Intra-day precision was found out by carrying out the analysis of the formulation for three times on the same day and the % RSD was calculated (table 3).

TABLE 3: Intra- day precision

Drug	Concentration	Peak Area	% RSD*
s (-) amlodipine	3 ng/ml	77822 78846 78932	1.21

** Mean RSD of three observations*

b) Inter- day precision

Inter-day precision was found by carrying out the analysis of the formulation for two days. All the solutions were injected into the chromatographic system and % RSD were calculated (table 4).

TABLE 4: Inter-day precision

Drug	Day	Concentration	Peak area	% RSD*
s (-) amlodipine	1st	3ng/ml	77814 77924 78126	1.7
	2nd	3ng/ml	77126 77268 77338	1.26

** Mean RSD of three observations*

c) Repeatability

A sample solution of s (-) amlodipine was injected for 3 times and % RSD were calculated (table 5).

TABLE 5: Repeatability

Drug	Concentration	Peak area	%RSD*
s(-) amlodipine	3ng /ml	77812 77924 77932	1.11

** Mean RSD of three observations*

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under optimized chromatographic conditions. The LOD and LOQ of the s (-) amlodipine was found to be 0.3ng /ml and 1ng/ml respectively (fig 3&4).

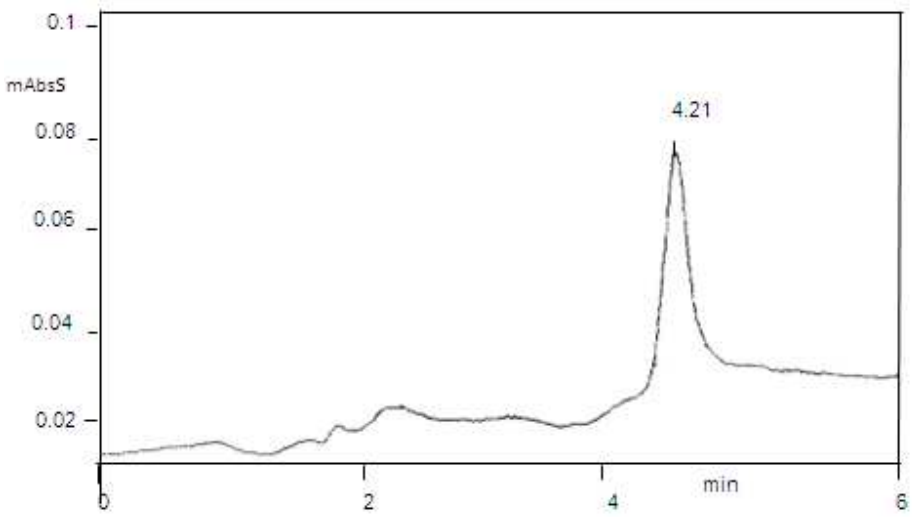

Fig 3:- Limit of Detection (LOD) of s (-) amlodipine

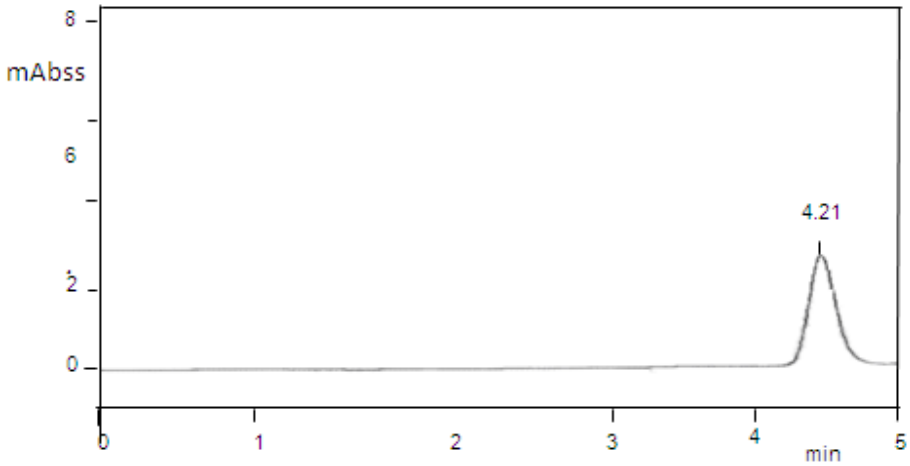

Fig 4:- Limit of quantification (LOQ) of s (-) amlodipine

Stability

The sample solution was subjected to stability studies under room temperature. Stability was studied by comparing the peak areas of freshly prepared solution with the sample solution. The solution stored under room temperature was stable up to 3 hours (table 6).

TABLE 6: Stability of solution

Time (hrs)	Peak area
0	77822
½	77022
1	76821
1½	76019
2½	75818
3	74817

RESULTS AND DISCUSSION

The RP-HPLC method developed for the estimation of s (-) amlodipine in tablet dosage form was done by fixing the parameters as Phenomenex C_8 ODS column (150 x 4.6 mm), 5 µ particle size with mobile phase 20 mM sodium dihydrogen phosphate buffer: acetonitrile (65: 35% v/v) adjusted to pH 8 was used. Mobile phase flow rate was maintained at 1.2ml/min and detected at 239nm. The retention time was 4.20± 0.02 minutes.

Validation parameters like accuracy, precision, linearity and stability studies showed low %RSD values which indicates that the method is precise and sensitive. The linearity for S(-) amlodipine was found to be 0.9992. The LOD and LOQ of the s (-) amlodipine was found to be 0.3ng /ml and 1ng/ml respectively. The accuracy for S(-) amlodipine was found to be 99.92%. Intra day precision and inter day precision were found to be 0.64% RSD and 1.26% RSD respectively. The solution stored under room temperature was stable up to 3 hours.

CONCLUSION

RP-HPLC method for the estimation of S(-) amlodipine in tablet dosage form was found to be within the limits. Validation parameters like accuracy, precision and linearity showed low %RSD values which indicates that the method is precise and sensitive. Hence this method can be employed for laboratory works.

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