Development and validation of a HPLC analytical assay method for amlodipine besylate tablets: A Potent Ca^{+2} channel blocker

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ABSTRACT

Amlodipine besylate is a potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris. Amlodipine besylate avoids the adverse effect of amlodipine in racemic mixtures. A highly precise and cost effective RP-HPLC method with retention time of 2.60 minutes was developed for the estimation of amlodipine besylate in tablet dosage form, by fixing the parameters as WATERS C18 column 250 mm × 4.6 mm (5μm), with mobile phase as acetonitrile: 70mM potassium dihydrogen orthophosphate buffer: methanol (15:30:55) and pH adjusted to 3.0 using OPA. Mobile phase flow rate was maintained at 1.0 ml/min and detected at 240nm.

Keyword*: RP*-HPLC, amlodipine besylate, diode array detector (DAD).

INTRODUCTION

Amlodipine besylate belongs to the third generation antagonists which retain tissue selectivity and show favourable pharmacokinetic data [1]. The drug does not appear to cause postural hypotension, reflex tachycardia or cardiac conduction disturbances. Thus, amlodipine besylate provides an attractive therapeutic option for the treatment of hypertension, and offers potential for patients with angina pectoris [2]. 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 [3,4], UV spectroscopy [5] and LC/MS [6].

MATERIALS AND METHODS

WATERS (Milford, USA) with DAD detector, S(-) amlodipine (Akums Drugs Ltd., Haridwar, India), Acetonitrile, buffer (70mM potassium dihydrogen orthophosphate) and 85% ortho-phosphoric acid (H_3PO_4), Methanol were purchased from Finar chemical ltd, Ahmadabad and di sodium hydrogen ortho phosphate dehydrate was purchased from CDH, New Delhi.

Preparation of mobile phase

Several mobile phases at different ratios were used but no favourable results were obtained except for acetonitrile: potassium dihydrogen orthophosphate buffer: methanol (15:30:55), which gave an acceptable peak. 70 mM was selected as ionic strength for buffer solution at pH 3.0 to get a sharp symmetrical peak with good resolution.

Chromatographic conditions

WATERS C18 column 250 mm × 4.6 mm (5µm) with mobile phase as acetonitrile: 70mM potassium dihydrogen orthophosphate buffer: methanol (15:30:55) adjusted to pH 3 was used. Mobile phase flow rate was maintained at 1.0ml/min and detected at 240nm. The retention time was 2.60 minutes.

Analysis of formulation

i) **Preparation of buffer**

The freshly prepared buffer was used every day by dissolving 2.381 gm of di-sodium hydrogen ortho phosphate dihydrate in 250 ml of HPLC grade water. Then filtered through a 0.2 µm membrane filter to remove any particulate matter and degassed by sonication before use.

ii) **Sample preparation**

Stock solution of amlodipine besylate (1 mg/ml) was prepared in methanol and stored at 2–8 °C until used. Aliquots were diluted stepwise with the mobile phase to obtain 10 µg/ml. This solution was used for the optimization of the proposed method. Marketed formulation was prepared as similar to pure amlodipine besylate and diluted accordingly after proper separation of excipients (Figure 1).
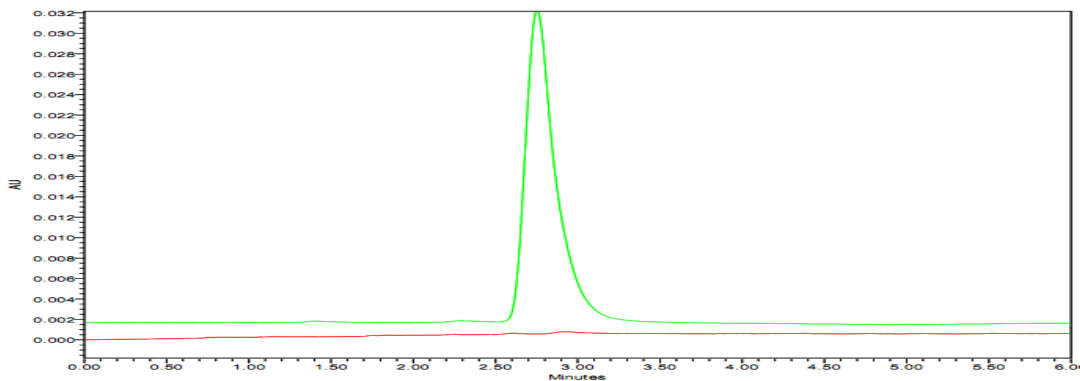
Fig. 1: Chromatographic peak of amlodipine besylate

iii) **Assay of optimized FDT formulation of Amlodipine besylate using HPLC method**

Assay for the tablet dosage form was done with validated chromatographic RP-HPLC method and it was found to be 98.37% w/w S.D. ± 1.27%. Figure 2 explained the selective and specific estimation of amlodipine besylate in presence of excipients of tablet dosage form and percentage purity was found to be 98.37 % w/w. There was no interference of excipients in amlodipine besylate determination from its fast dissolving tablet formulated Table 1.

Table 1: Assay of the prepared formulation

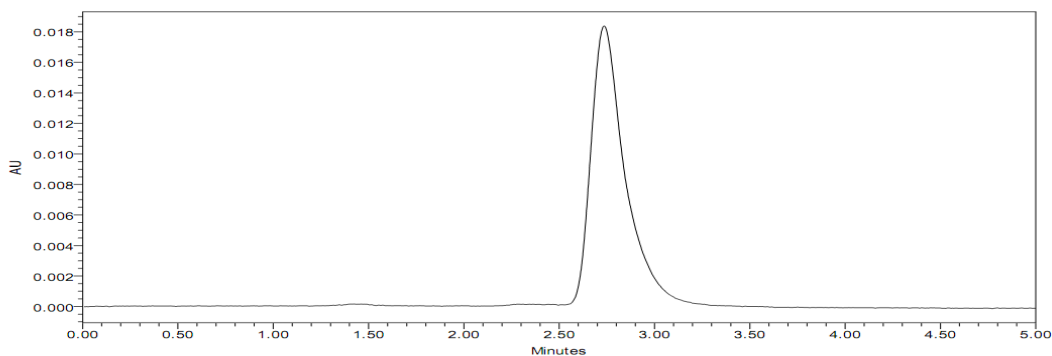| Sample amount added (µg/ml)	Amount estimated (µg/ml)	Assay
8	7.83	98.37 %

Fig. 2: Chromatographic peak of amlodipine besylate in presence of excipients in tablets dosage form

Validation of the Developed Method

After chromatographic method development and optimization it was validated. The validation of an analytical method verifies that characteristics of the method satisfy the requirements of application domain. Proposed method was validated according to ICH guidelines for linearity, precision, accuracy sensitivity, and recovery (ICH Q2A, 2005).

a) Calibration curve (linearity)

The method was found to be linear in the range of 0.5-8 µg/ml (Figure 3) with correlation coefficient r^2=0.990. The overlay of peaks is shown in figure 4. Validation parameters are given in table 2.
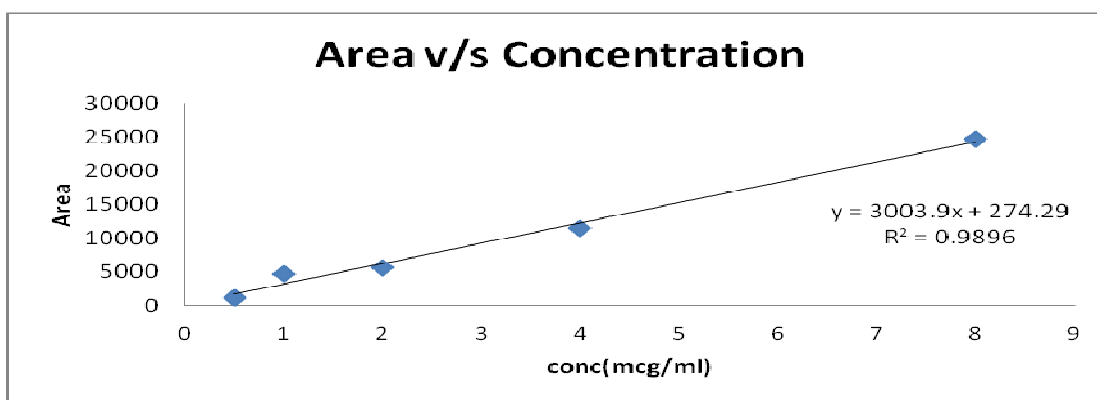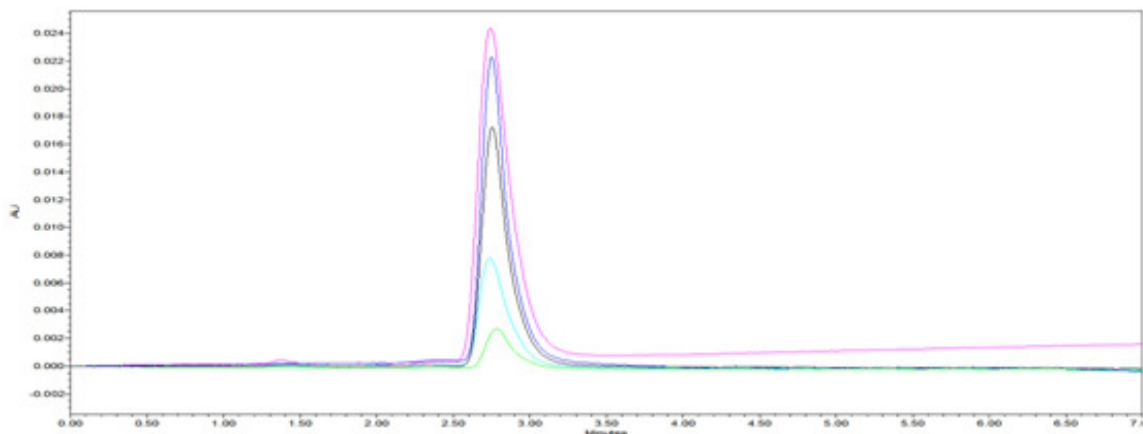

Fig. 3: Calibration curve of amlodipine besylate

Fig. 4: Overlay peaks of amlodipine besylate found in RP-HPLC method

b) Precision

The intra-day and inter-day variations of the method were determined using three replicate injections of four concentrations and analyzed on the same day at three different time

intervals and at three different days. The result revealed the precision with % RSD (0.025 % and 0.022 %) respectively for intra-day and inter-day.

Table 2: Validation Parameters of RP-HPLC Method for amlodipine besylate

S. No.	Regression Analysis Parameters	Results
1	Linearity range (µg/ml)	0.5-8 µg/ml
2	Coefficient of determination (r^2)	0.99
4	Regression equation (Y^a)	y = 3003.x + 274.2
4	Slope (b)	3003
5	Intercept (a)	274.2

c) Accuracy

To examine the accuracy of the method, recovery studies were carried out by standard addition method. Accuracy was found to be 104.7 ±1.82. The recovery results are described in table 3.

Table 3: Accuracy data of RP-HPLC method for amlodipine besylate

Sample amount (µg/ml)	Spiked drug amount (µg/ml)	Amount recovered (µg/ml)	% Recovery
8	6.4 (80%)	14.22	97.21
8	8.0 (100%)	16.1	101.36
8	9.6 (120%)	17.53	99.27
Mean Recovery (%)			99.28

d) Detection and quantitation limits (LOD & LOQ)

The LOD and LOQ were calculated and were found to be 0.0204496µg/ml and 0.0674µg/ml respectively.

The validation data so obtained indicated that the developed method is simple, sensitive, specific, and accurate. So far, reported methods have been time (retention time more than 10 min) consuming for they use higher proportion of buffer in mobile phase, pre column detivatization and fluorimetric detection making them costly in routine determination

(Shriram M. Pathak et al., 2008, Tsukasa Uno et al., 2004, M. Saeed Arayne et al., 2011). However the developed and validated method is not only more precise, accurate but also less time consuming making its useful for routine determination of amlodipine besylate in bulk drug and pharmaceutical formulation.

System suitability parameters

To know reproducibility of the method system suitability tests were performed such as asymmetric factor, tailing factor, theoretical plates and plate numbers. The values found for these parameters are described in table 4. All the system suitability parameters found to be according to the acceptable limits of the analytical methods.

Table 4: System Suitability Parameters of amlodipine besylate for RP-HPLC Method

System suitability parameters	Results	Acceptable limits
Asymmetric factor	0.8	< 1.5
Tailing factor	1.57	< 2
Plate No.	3024.2	> 2000
Capacity Factor	2.67	
Resolution	12.21	

Peak purity

The result of peak purity revealed that the chromatogram obtained of the drug was found to be pure. As peak purity was checked at different point of chromatogram and the spectrum of the sample corresponding to these points of the chromatogram (Figure 5) showed that the spectra were same and overlaid at same wavelength, this showed the purity of the chromatogram.
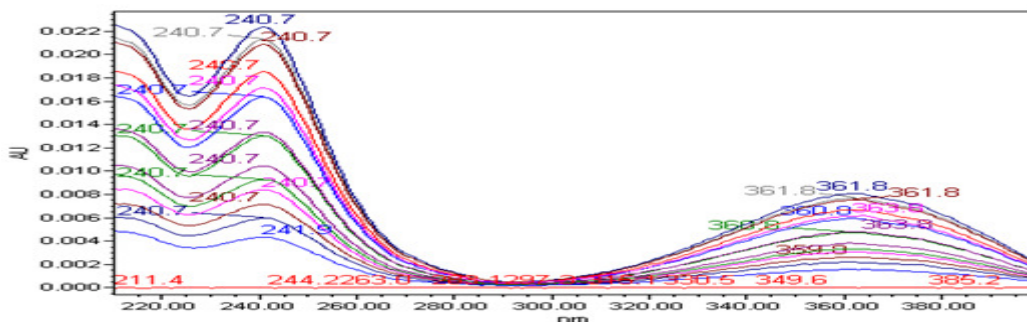

Fig. 5: Overlay spectra for peak purity

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 WATERS C18 column 250 mm × 4.6 mm (5µm), with mobile phase as acetonitrile: 70mM potassium dihydrogen orthophosphate buffer: methanol (15:30:55) adjusted to pH 3 using OPA. Mobile phase flow rate was maintained at 1.0 ml/min and detected at 240nm. The retention time was 2.60 minutes.

Validation parameters like accuracy, precision, linearity showed low %RSD values which indicates that the method is precise and sensitive. The linearity range for S(-) amlodipine was found to be 0.5-8 µg/ml with r^2 value of 0.99. The LOD and LOQ of the *s* (-) amlodipine was found to be 0.0674µg/ml and 0.0204496µg/ml respectively. The accuracy for Amlodipine besylate was found to be 104.7 ±1.82%. Intra-day precision and inter-day precision were found to be 0.025 % and 0.022 % RSD respectively.

CONCLUSION

RP-HPLC method for the estimation of Amlodipine besylate 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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