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Analytical method development and validation of Amlodipine besylate in tablet dosage form

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ABSTRACT

The accurate and precise HPLC analytical method validated for the determination of Amlodipine besylate in pharmaceutical dosage form. The chromatographic separation is carried out on shimadzu HPLC system (LC-2010 CHT) with UV Vissible detector and C18(150mm x3.9 mm) 5 µm Column. The Mobile phase consists of Acetonitrile: Methanol: P^H 3.0 Buffer (15 V: 35 V: 50 V) , at the flow rate of 1.0 ml/min and elutes were monitoring at 237 nm. The observed retention time for Amlodipine besylate was 12.3 min. The % RSD for system precision was 0.41 % and Method precision was 0.58 %. The method was found to linear (R=0.99996) in the Concentration range of 35-105 µg/ml (50 to 150%). The accuracy was in between 99.50-99.91%.

Keywords: HPLC, Correlation coefficient, System suitability, Bias, % RSD and ICH guidelines

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INTRODUCTION

Amlodipine besylate is a dihydropyridine calcium channel blocker indicated in treatment of mild to moderate essential hypertention and for the management of chronic stable angina in pattients. Amlodipine besylate (Figure 1) is chemically name as 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate. Molecular formula for Amlodipine besylate is $C_{26}H_{31}ClN_2O_8S$ and Molecular weight is 567.1

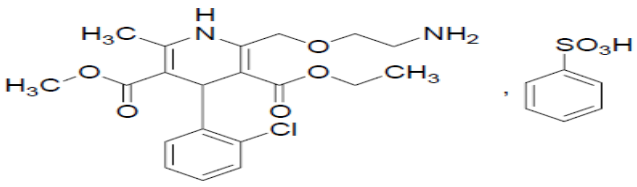

Figure 1: Chemical Structure of Amlodipine besylate

Literature review reveals that there few HPLC method available for the determination of Assay contenets of Amlodipine besylate in Tablet dosage forms.This novel proposed method contribute quick estimation and correct peak shape. The objective of this study was to develop analytical method in order to identify the amount of Amlodipine besylate in Tablet dosage forms.

Factor Calculation:

EXPERIMENTAL

Reagents and Chemicals: Triethylamine and orthophosphoric acid were used of Analytical reagent grade(**Make:** Fisher Scientific) however solvents used for Mobile phase Acetonitrile and Methanol of HPLC grade (**Make :**Fisher Scientific).

Instrumentation: The method development was performed with shimadzu HPLC system(LC-2010 CHT) having UV Vissible detector .

Reference Solution: Weighed accurately 70 mg of Amlodipine besylate (equivalent to 50.47 mg of Amlodipine) transfer into 100 ml of Volumetric flask dissolve it in mobile phase and make the volume upto mark.Transfer 5 ml of this solution to 50 ml of Volumetric flask and make the volume with mobile phase.

Test Solution: Take 20 tablets of Amlodipine besylate and crushed .Weighed to powder accurately equivalent to 70 mg of Amlodipine besylate transfer into 100 ml of Volumetric flask dissolve it in mobile phase and make the volume upto mark.Transfer 5 ml of this solution to 50 ml of Volumetric flask and make the volume with mobile phase.

(Note: use 0.45 µm filter for solution filtration)

$$\text{Factor} = \frac{\text{Molecular weight of Amlodipine}}{\text{Molecular weight of Amlodipine besylate}} = \frac{408.879}{567.09} = 0.721$$

Assay Calculation:

$$\text{(in mg/Tablet)} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{50}{5} \times \frac{P}{100} \times 0.721 \times AW$$

$$\% \text{ Assay} = \frac{\text{mg/tablet}}{LC}$$

Where,

AT = Area of Test Solution

AS = Area of Standard Solution

WS = Weight of Standard

WT = Weight of Test

P = Potency of Standard

AW = Average Weight of 20 Tablets

LC = Label Claim of Amlodipine mentioned on Carton or Strip of tablet

All the acceptance crieteria given in Table 1.

Table 1: Acceptance criteria for System suitability

Sr. No	Parameter	Limit/ Acceptance criteria
1	Theoretical plates	Not less than 2000
2	Tailing Factor	Not more than 1.5
3	% RSD for Area of replicates Injection	Not More than 2.0 %

RESULT AND DISCUSSION

The method was developed with the composition of Acetonitrile: Methanol: PH 3.0 Buffer (15 V: 35 V: 50 V) was showed to good symmetric peaks and less tailing factor. The optimizes parameters are shown in Table 2.

Table 2 : Optimized Chromatographic Analytical method condition

Sr. No.	Parameter	Description /Value
1	Stationary Phase(Column)	C18(150mm x3.9 mm) 5 µm, Preferred to Inertsil ODS-3 .
2	Mobile Phase	15 V Acetonitrile :35 V Methanol:50 V Buffer (Prepared by 7 ml of TEA in 1000 ml HPLC Water. Adjusted PH 3.0 with 25 % Orthophosphoric Acid). Filter through 0.45 µm
3	Flow rate	1 ml/min.
4	Detection wavelength	237 nm
5	Detector	UV Visible
6	Injection Volume	10 µl
7	Column temperature	30 ^{0}C
8	Auto Sampler Temperature	15 ^{0}C

METHOD VALIDATION: The different method validation parameters were performed as per ICH guidelines. All the parameters showed good result and they complies to specified acceptance criteria.

System Suitability: The method for analytical determination of Amlodipine besylate was validated and tested for system suitability according to ICH guidelines.Before start to experiment Stabilize the HPLC system for 30 minutes and Chromatographic system used for analysis must pass system suitability .Inject the Blank , Standard and Test Solution(Each single Injection).The Parameter such as Tailing factor and theoretical plates should meet the acceptance criteria.

Result of System Suitability: All system suitability parameter are found within acceptance criteria. The chromatogram shown in figure 2 and Results are tabulated in Table 3

Table 3: System suitability result of Amlodipine Besylate

Sr. No	Parameter	Results	Acceptance Criteria
1	Theoretical plates	4774	Not less than 2000
2	Tailing Factor	1.0	Not More than 2.0

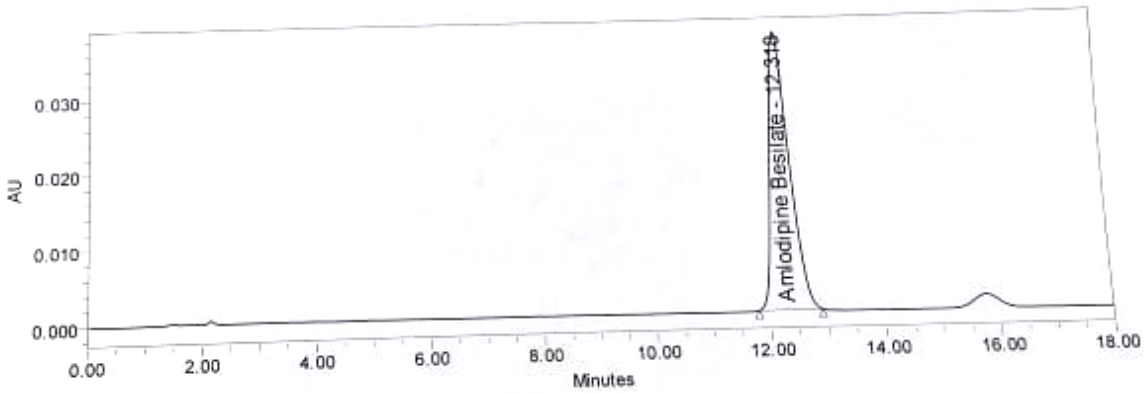

Figure 2 : Typical Chromatogram for Standard Solution

PRECISION: According to the ICH guidelines the precision of an analytical procedure determined the closeness of results obtained by multiple measurement of the same homogeneous sample. Repeatability (System precision and method precision) were done to show the precision of the method. To demonstrate repeatability (System precision) of the test method six replicate injection of Reference solution were analysed and % RSD of assay results was calculated.

RESULT OF PRECISION: The % RSD of six standard Area (For system precision) and for Six Assay result (for method precision) was found within acceptance limit tabulated in Table 4. The acceptance criteria of % RSD for replicate area and Assay content should be not more than 2.0%

Table 4 : Result for Precision study (System and Method)

Sr. No	Peak Area (For System precision)	% Assay(For Method Precision)
1	1441857	98.35
2	1435981	99.32
3	1448956	99.85
4	1436987	99.58
5	1449587	98.95
6	1439589	98.69
Mean	1442160	99.12
SD	5881.97	0.57
% RSD	0.41	0.58

LINEARITY: Linearity was measured by varies the concentration of Amlodipine Besylate 50%, 80%,100% ,120 % and 150% were injected into the HPLC System. A calibration curve was made by plotting the Peak area vs concentration of standard solution. Correlation coefficient (R^2) and linear regression equation were incorporated to statistically evaluate the linearity of the results y = mx+b. Where, y = response, m=slope, x= concentration and b=intercept. Bias is the way of expression to show more imprecision calculated by intercept to area of 100% concentration.

RESULTS OF LINEARITY: The observed value for correlation coefficient 0.99996 (limit:not less than 0.9999) and Bias 0.74 (Limit ±3) found within acceptance criteria in the Concentration range of 35-105 µg/ml. The linearity data shown in the Table 5 and Calibration plots are given in the Figure 3 .

Table 5: Results of Linearity study

Sr. No	Concentration(in %)	Peak area
1	50%	438598
2	80%	701442
3	100%	876758
4	120%	1052464
5	150%	1305698
	Correlation: 0.99996 **Slope** : 8685.42 **Intercept** : 6449.93 **Bias** : 0.74	

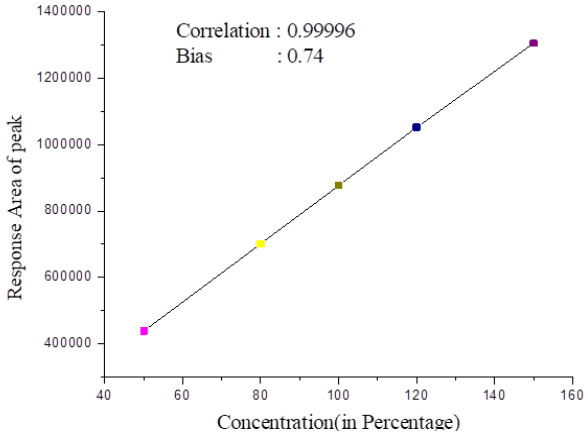

Figure 3 : Calibration curve between concentration and Response area

RECOVERY: The accuracy of an Analytical procedure shows to closeness of result with the conventional value. The accuracy of the method was planned by standard addition process.A known amount of standard weight added at different level 50%,100% and 150% to get final concetration of 35µg/ml, 70 µg/ml and 105 µg/ml than there solution were analysed for Assay content.Triplicate set of each levels were prepered for the experiment.

RESULTS OF RECOVERY : The concentration of 50% solution showed % Mean recovery 99.50 % and % RSD

0.34%. The concentration of 100% solution showed % Mean recovery 99.58 % and % RSD 0.34%. The concentration of 150% solution showed % Mean recovery 99.91 % and % RSD 0.03%. All the result were tabulated in Table 6.The recovery Acceptance limit of Assay content should be 98 to 102 % and % RSD not more than 2.0%.

Table 6: Results of Accuracy study

Spiked Concentration	Amount added (in mg)	Amount recovered (in mg)	% Recovery
50% -01	35.25	35.13	99.66 %
50% -02	34.98	34.67	99.11%
50% -03	35.68	35.58	99.72%
100%-01	70.35	70.14	99.70 %
100%-02	69.85	69.74	99.84 %
100%-03	71.25	70.68	99.20%
150%-01	105.68	105.58	99.91 %
150%-02	104.85	104.79	99.94%
150%-03	104.35	104.23	99.89%

Mean for 50% : 99.50
Mean for 100% : 99.58
Mean for 150% : 99.91

RSD for 50% : 0.34
RSD for 100% : 0.34
RSD for 150% :0.03

Table 7: Summary of all results

Validation parameter	Results
Linearity	
Coefficient of correlation	0.99996
Bias	0.74
Precision	
System Precision	0.41 %
Method Precision	0.58%
Accuracy	
50%	99.50%
100%	99.58%
150%	99.91%

CONCLUSION

The developed and validated HPLC method was showed good symmetric peak and less tailing factor. The method was exposed good precision, Accuracy and the observed value during experiment meet with specified acceptance limit. The linearity graph showed good correlation between different concentration solution and area, the R^2 value were found to be 0.99996. The proposed method can help to research scholar and Quality control in routine analysis. The result of assay content for pharmaceutical formulation is in good agreement with the label claim of drugs.

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