Method Development and Validation For The Estimation of Amlodipine in Api And Its Pharmaceutical Formulation

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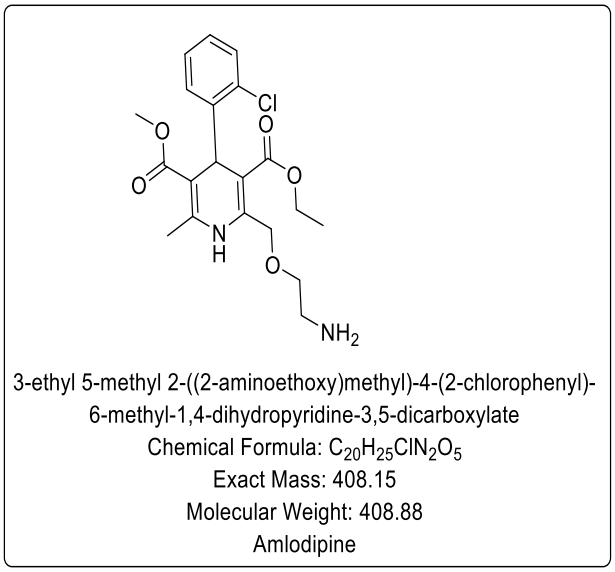
Abstract- *A simple, rapid, economical, and highly sensitive Assay HPLC method was developed and fully validated for determination of Amlodipine in bulk and its pharmaceutical dosage form namely Tamiflu. Chromatographic separation was achieved on ZORBAX Eclipse XDB C8 EPS column (250 mm X 4.6 mm, 5 µm). Chromatographic separation was carried out at ambient temperature. All analytes were separated isocratically with a mobile phase consisting of Acetonitrile: Methanol and Water (35:35:30 v/v/v), at a flow rate 1.0 ml/min with injection volume 20 µL. The wavelength was monitored at 210 nm. The proposed method was validated according to ICH guidelines with total run time less than 13 min. The correlation coefficient (r2) was noted as 0.9992 which states that the method was good linear to the concentration versus peak area responses. The average percentage recovery were in the range of 95.0-105.0 %.A rapid, economical, simple and sensitive HPLC method was developed and validated for the determination of Amlodipine in tablet. The developed, validated method was successfully applied for the determination of Amlodipine in formulation.*

Keywords- Amlodipine, Assay, RP-HPLC, Validation, ICH guidelines.

I. INTRODUCTION

Amlodipine is chemically ethyl 3-Ethyl 5-methyl -2-[(2-aminoethoxy) methyl] – 4 - (2-chlorophenyl) - 6- methyl - 1,4- dihydropyridine-3, 5- dicarboxylate benzenesulfonate. Amlodipine is a calcium channel blocker medication used to treat high blood pressure and coronary artery disease. While not typically recommended in heart failure, amlodipine may be used if other medications are not sufficient for treating high blood pressure or heart-related chest pain. It is taken by mouth and has an effect that lasts for at least a day. Amlodipine is considered a peripheral arterial vasodilator that exerts its action directly on vascular smooth muscle to lead to a reduction in peripheral vascular resistance, causing a decrease in blood pressure.



3-ethyl 5-methyl 2-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate
Chemical Formula: $C_{20}H_{25}ClN_2O_5$
Exact Mass: 408.15
Molecular Weight: 408.88
Amlodipine

Figure 1. Structure of Amlodipine

II. MATERIALS AND METHOD

2.1 Materials:

Amlodipine (API) - White powder, used to treat high blood pressure and coronary artery disease.

Amidine 5 mg drug contain each Tablet

Acetonitrile, Methanol, Acetate Buffer (HPLC grade) by Merck Life Science, Distilled Water.

Instruments used in Experimental Work:

Table 1. List of Instruments

Sr. No.	Name of Equipment's/ Instruments	Model / Specification	Manufacturer
1	HPLC	Series LC2030	Shimadzu
	Pump	PU2030	
	Injection Port	Autosampler	
	UV/Vis Detector	UV 2075 plus	
	Software	LabSolutions	
2	pH Meter	101	Chemiline
3	Balance	AY-120	Shimadzu
4	Sonicator	UCB-40	Rolex

2.2 Method:

2.2.1 Optimized chromatographic conditions: HPLC system equipped with Shimadzu LC2030, ZORBAX Eclipse XDB C8 EPS column (250 mm X 4.6 mm, 5 µm). Chromatographic separation was carried out at ambient temperature (25 °C ±0.5 °C). All analytes were separated isocratically with a mobile phase consisting of ACN : MeOH : Water(35:35:30, v/v/v), at a flow rate 1.0 ml/min with injection volume 20 µL. The wavelength was monitoredat 210 nm.

2.2.2Preparation of mobile phase: 70 mL of HPLC grade Methanol and Acetonitrile were taken as 30 mL of Water.

2.2.3Preparation of standard solution: Stock solution was prepared by dissolving 10 mg Amlodipine Besylate in water and then diluted with Water in 10 ml of volumetric flask to get concentration of 1000 µg/ml. From the resulting solution 0.1 ml was diluted to 10 ml with water to obtain concentration of 10 µg/ml of Amlodipine Besylate and labelled as standard stock Amlodipine Besylate.

2.2.4 Selection of detection wavelength: From the standard stock solution further dilutions were done using water and scanned over the range of 200-400 nm and the spectra were overlain. It was observed that drug showed considerable absorbance at 210 nm.

III. RESULT AND DISCUSSION

3.1 Linearity and range:

In above method linearity was determined by constructing the calibration curves. For this purpose different standard solution of Amlodipine besylate of different concentration level (2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml,10 µg/ml and 12 µg/ml) were used. Measurement of each concentration was carried out & the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves & correlation coefficients (figure 2). A table 2 represents the results were directly proportional to the concentration of analyte in the given sample.

Table 2. Linearity Result of Amlodipine

Linearity		
Sr. No	Concentration (µg/mL)	Peak Area
1	2	52451
2	4	104902
3	6	150353
4	8	209804
5	10	262255
6	12	314706

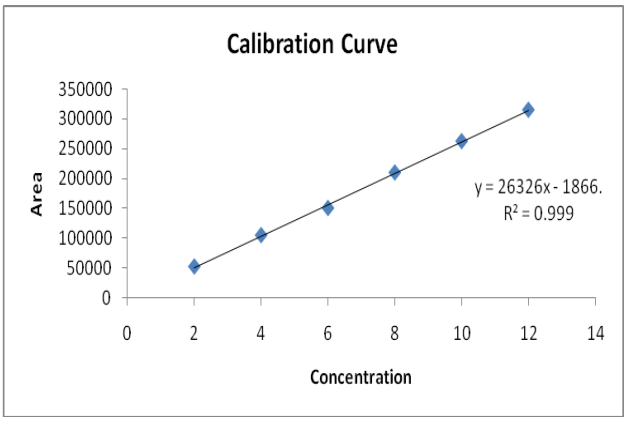


Figure 2. Calibration Curve of Amlodipine

Table 3. Characteristic parameters of Amlodipine for the proposed HPLC method.

Parameter	Result
	Amlodipine
Calibration range (µg/ml)	2-12
Detection wavelength (nm)	210
Solvent (Acetonitrile + Methanol: Water)	70:30
Regression equation (y*)	y = 26326.2x + 1866.7
Slope (b)	26326.2
Intercept (a)	1866.7
Correlation coefficient(r2)	0.9992
Limit of Detection (µg/ml)	2.7715
Limit of Quantitation (µg/ml)	8.3985

3.2 System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 8 µg/ml. The results which are given in Table 4.Were within acceptable limits.

Table 4. System suitability studies of Amlodipine by HPLC method.

Sr. No.	Properties	Values
1.	Retention time	7.8175
2.	Area	215028
3.	Asymmetry	1.12

3.3 Specificity:

Chromatogram of blank was taken as shown in Figure 3. Chromatogram of Amlodipine showed peak at a retention time of 7.175 min (fig 4). The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Amlodipine sample was 7.175 min (fig 5). The wavelength 210 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Amlodipine from the tablet formulation was Amlodipine. Specificity of Amlodipine represent in table 5.

Table 5. Specificity of Amlodipine by HPLC method

Specificity				
Sample	Label Claim (mg)	Amount Found	Recovery	Retention Time
Tablet	5	4.91	98.2	7.175

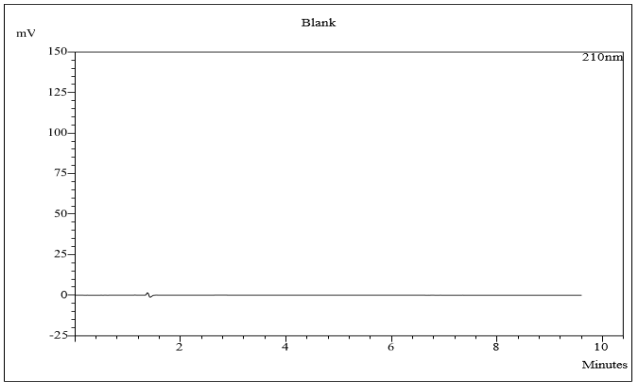


Figure 3. A typical chromatogram of Blank

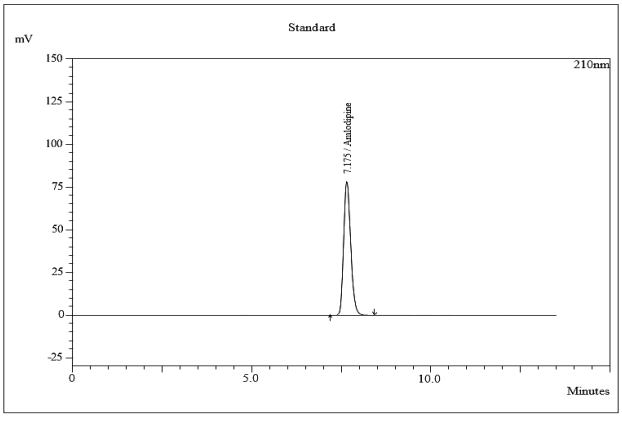


Figure4. A typical chromatogram of Amlodipine Standard [Concentration 8 µg/ml]

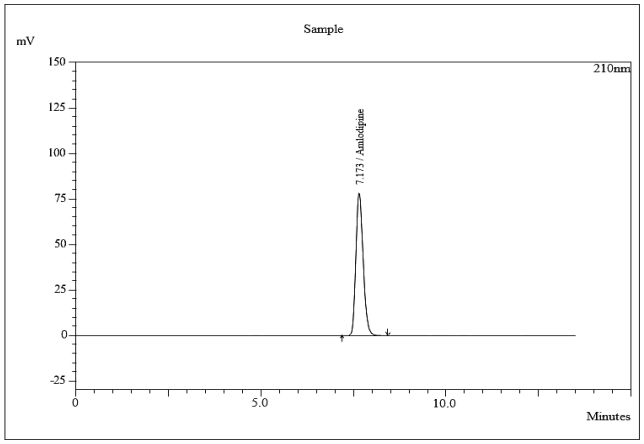


Figure 5. A typical chromatogram of Amlodipine Sample [Concentration 8 µg/ml]

3.4 Precision:

Demonstration of precision was done under two categories. The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Amlodipine and the % RSD of the replicate injections was calculated. In addition, to demonstrate the precision of method (Method Precision), six samples from the same batch of formulation were analysed individually and the assay content of each sample was estimated. The average for the six determinations was calculated along with the % RSD for the replicate determinations. Both the system precision and method precision were subjected for repeatability variations as reported in Table No 6.

Table 6.Precision Repeatability

Precision		
Repeatability		
Sr. No	Concentration (µg/mL)	Peak Area
1	8	215022
2	8	210022
3	8	211035
4	8	208148
5	8	209022
6	8	212810
Average		211009.83
Standard Deviation		2547.79
RSD%		1.21

3.5 Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Amlodipine (8 µg/ml) were spiked with 80, 100, and 120 % extra Amlodipine standard and the mixtures were analysed by the proposed method.

Standard deviation of the % recovery and % RSD were calculated and reported in Table 7.

Table 7. Accuracy of Amlodipine

Accuracy				
Sr. No	Concentration (µg/mL)	Peak area	Found Concentration (µg/mL)	% Recovery
1	6.4	155724.8	6.39	99.81
2	8	194656	7.99	99.88
3	9.6	583968	9.69	100.91

3.6 Robustness:

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the Optimized method parameters were done. The effect of change in flow rate, wavelength, and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes in flow rate and changes in wavelength. The results are shown in Table 8.

Table 8. Robustness of Amlodipine

Robustness							
Sr. No	Parameter		Response	Parameter	Response	Parameter	Response
ACN+MeOH : Water (V/V)			Retention Time (min)	Detection Wavelength (nm)	Peak Area	Flow Rate (mL/min)	Retention Time (min)
1	69	31	7.078	208	199677	0.9	7.306
2	70	30	7.175	210	209992	1	7.175
3	71	29	7.274	212	203256	1.1	7.1709
Average			7.176	Average	201642	Average	7.217
Standard Deviation			0.080	Standard Deviation	0.369	Standard Deviation	0.0627
RSD%			1.115	RSD%	1.071	RSD%	0.869

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