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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ASSAY OF AMLODIPINE IN FORMULATIONS

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ABSTRACT
A simple, sensitive and accurate Isocratic reverse phase high performance liquid chromatography method was developed for determination of amlodipine in formulation. The effective separation was achieved on Phenamex C18, 250 x 4.6 mm, 4.5µm. Mobile was prepared a filtered and degassed mixture of buffer, methanol and acetonitrile in the ratio of 55:35:10. The buffer was prepared by dissolve 7 mL of Triethyl amine in 1000 ml of water, adjust its pH to 3.0 with orthophosphoric acid mix and filter. The flow rate of the mobile phase was 1.2 mL/min and the total elution time was 60 minutes. The UV detection wavelength was carried at 240 nm and experiments were conducted at 30°C. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy and robustness as per the ICH guidelines.

KEYWORDS: Amlodipine, Method development, Validation and RP-HPLC.

INTRODUCTION
Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells.[1] Chemically it is (R.S.)-2 – [(2-aminoethoxy) methyl] 4-ochlorophenyl)-14-dihydro-6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. The chemical structure of amlodipine shown in Figure-1.

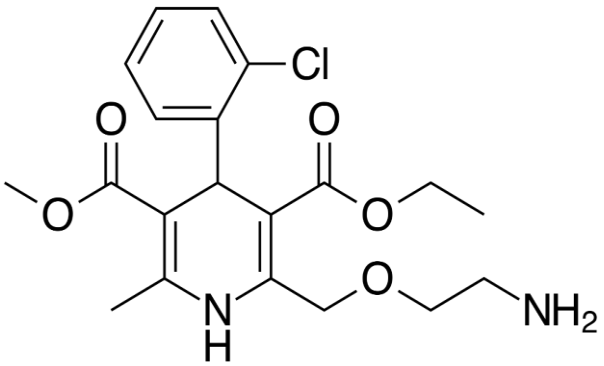

Figure-2: Chemical structure of amlodipine

A literature survey revealed that spectrophotometric, chromatographic methods have been reported for determination of amlodipine[2–7] in single and multi-component pharmaceutical formulations or from biological fluids. However, there were few HPLC methods for simultaneous estimation of HCTZ and AMLO reported. The present work describes a simple, isocratic method for the simultaneous determination of hydrochlorothiazide and amlodipine in tablets as for ICH guidelines.[8-11]

2. EXPERIMENTAL: MATERIALS AND REAGENTS

2.1 INSTRUMENTATION AND SOFTWARE
A high performance liquid chromatography system manufactured by Agilent which consist of VWD detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of Amlodipine. HPLC instrument was controlled by EZ Chrome software. The Phenamex C18, 250 x 4.6 mm, column with particle size of 4.5µm was used as stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment and Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

2.2 CHEMICALS AND REAGENTS
All the reagents were of analytical reagent grade unless stated otherwise. Distilled and de-ionized HPLC-grade

water, HPLC grade acetonitrile, methanol, trimethyl amine and orthophosphoric acid was purchased from Merck, Mumbai.

Buffer preparation
Dissolve 7 mL of Triethyl amine in 1000 ml of water, adjusted its pH to 3.0 with orthophosphoric acid mixed and filtered.

Mobile phase
Mobile was prepared a filtered and degassed mixture of buffer, methanol and acetonitrile in the ratio of 55:35:10.

2.6 PREPARATION OF STANDARD SOLUTIONS
Weigh accurately 50 mg of amlodipine standard into a 100 ml volumetric flask, add 50 ml of mobile phase, sonicated for 5 minutes and make up to the mark with mobile phase.

2.7 PREPARATION OF SAMPLE SOLUTIONS
Transfer a quantity of powder equivalent to about 100 mg of Amlodipine into a 100 ml volumetric flask. Add 70 ml of mobile phase. Shake for 15 minutes, and sonicated for 20 minutes at 25°C. Dilute to volume with mobile phase and mix. Centrifuge a portion at 3500rpm for 15min.

3. METHOD VALIDATION PARAMETERS
The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with Amlodipine in the range of 25 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. The 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. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using Amlodipine and evaluated by making six replicate injections. The accuracy of the method by recoveries of Amlodipine sample solutions at different concentration levels ranging from 25 to 150%. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4. RESULTS AND DISCUSSION
4.1 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS
Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 240 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Amlodipine. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Phenamex C18, 250 x 4.6 mm, 4.5µm column, there was significant improvement in the peak shapes with 1.1 tailing factor and got good number of theoretical plates (5836).

5. METHOD VALIDATION
5.1 SYSTEM SUITABILITY
The RSD from six replicate injections of standard preparation was 1.1%. System suitability data is given in Table-1.

Table-1: System suitability results of Amlodipine

System suitability	Observed value for Amlodipine peak	Acceptance criteria
Tailing factor	1.0	NMT 2.0
Theoretical Plates	5836	NLT 2500
RSD	1.1	NMT 2.0

5.2 SELECTIVITY
Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of Amlodipine. The standard and sample chromatograms shown in the Figures 2-3.

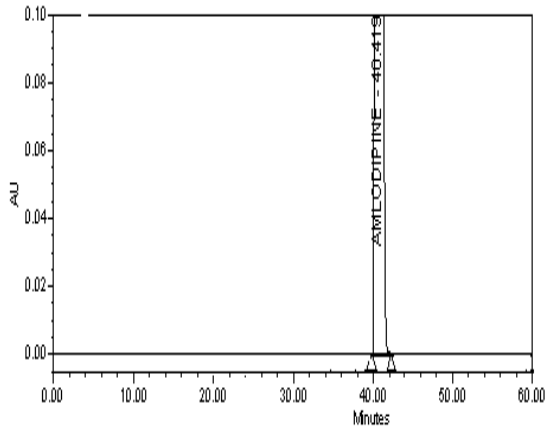

Fig-3: Chromatogram of Standard

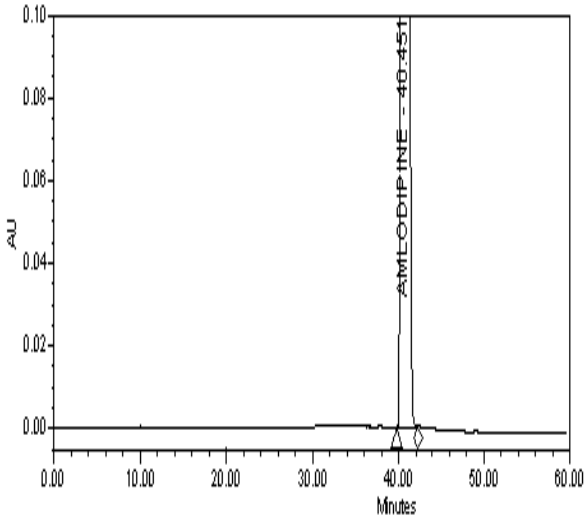

Fig-3: Chromatogram of sample

5.3 LINEARITY

To demonstrate the linearity with Amlodipine standard in the range of 25 to 150% of specification limit**.** Correlation coefficient of Amlodipine was 0.9996.The linearity results shown in the below Table -2. Linearity curve of Amlodipine shown in the Figure-3.

Table-2: Linearity results of Amlodipine

Standard concentration in %	Amlodipine Area
0	0
25%	364778
50%	801716
75%	1200382
100%	1569219
125%	1990519
150%	2312938
Coefficient of correlation(r)	0.9996
Slope (m)	15496.85
Intercept (b)	7671.821429

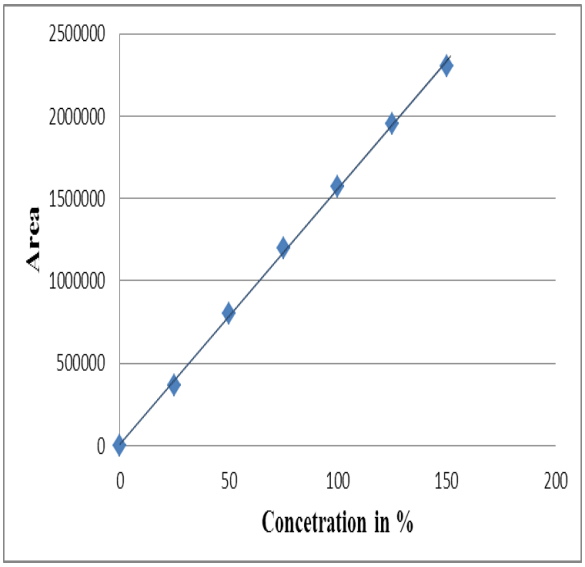

Figure-5 Linearity curve of Amlodipine

5.4 PRECISION

The precision of test method was validated by assaying six samples prepared on Amlodipine and calculate relative standard deviation of area results. The precision results are given Table-3.

Table-3: Precision results of Amlodipine

Sample No.	% Assay of Amlodipine
01	99.3
02	99.8
03	99.1
04	98.9
05	99.9
06	99.8
Average	99.5
SD	0.39
%RSD	0.4

5.5 ACCURACY

Accuracy study found that the mean % of recovery was more than 98.0% and less than 102.0% at each level 25 to 150% of concentration levels, hence method is accurate. The accuracy results are given Table-4.

Table-4: Accuracy results

Sample No.	Spike level	% Recovery of Amlodipine
01	25	101.2
02	50	100.8
03	75	100.1
04	100	99.8
05	125	101.1
06	150	101.3

5.6 ROBUSTNESS

The method robustness was studied by injecting the system suitability solution at change in the pH of buffer solution, flow rate and column temperature. The results were obtained as shown in the below Table-5.

Table-5: Robustness results

Condition	% RSD (NMT: 2.0)	Theoretical Plates (NLT: 2000)	Tailing factor (NMT: 2.0)
Normal Condition	1.1	5836	1.0
Change in buffer pH 2.8	1.2	6246	1.2
Change in buffer pH 3.2	1.1	5937	1.0
Column temperature 25°C	1.0	5905	1.1
Column temperature 35°C	1.2	6346	1.0
Flow rate 1.0 mL/min	1.1	5505	1.0
Flow rate 1.4 mL/min	1.2	5289	1.2

6. CONCLUSION

A simple Isocratic HPLC method has been developed and validated for the determination of Amlodipine. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of Amlodipine and also for the analysis of stability samples.

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