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# Analytical method development and validation of Amlodipine – An antihypertensive and Chlorthalidone - A thiazide diuretic by RP-UPLC method

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**ABSTRACT**

A High sensitive Ultra High Performance Liquid Chromatography method was developed for the estimation of amlodipine and chlorthalidone in combined tablet dosage form.The optimization of UHPLC method was performed using C18 column (4.6 x 50 mm, i.d. 3.5 µm) column, isocratic elution of mobile phase, acetronitrile and potassium dihydrogen ortho phosphate buffer using ortho phosphoric acid pH adjusted to 2.4 (600: 400,v/v)and flow rate of 0.5mL/min at 25$^0$C temperature. The retention time Amlodipine 2.50+ -0.01 and chlorthalidone 1.6+ -0.05 respectively. The developed method was validated according to International Conference on Harmonization (ICH)guidelines. The developed UHPLC method was found to be precise, selective and rapid for the simultaneous determination of Amlodipine, Chlorthalidone.

**Keywords:** Amlodipine, chlorthalidone, validation, UHPLC method.

## 1. INTRODUCTION

The present work propose the analytical method development and validation of amlodipine and chlorthalidone by using UHPLC method. Amlodipine, a long-acting calcium channel blocker (di hydro pyridine class) used as an antihypertensive and in the treatment of angina**.** Like other calcium channel blocker, Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle.[1]

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow channel blocker) that inhibits the trans membrane influx of calcium into vascular smooth muscle. Amlodipine binds to both dihydropyridine and non- dihydropyridine binding sites.[2-6]

Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

Amlodipine is chemically3-ethyl- 5-methyl2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate[7-14]

Chlortalidone prevents reabsorption of sodium and chloride by inhibiting the $Na^+/Cl^-$ symporter in the distal convoluted tubule. Thiazides and related compounds also decrease the glomerular filtration rate, which further reduces the drug's efficacy in patients with kidney impairment (e.g. kidney insufficiency). By increasing the delivery of sodium to the distal renal tubule, Chlortalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism. [15]

Chlorthalidone is chemically2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*isoindol-1-yl)benzene-1- sulfonamide. Chlorthalidone is used in the treatment of High blood pressure, congestive heart failure, symptomatic edema, renal tubular acidosis and prevention of kidney stones.[16-24]

A detailed survey of literature of amlodipine revealed,several methods based on different techniques were reported.[3] A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of anti-hypertensive drugs Amlodipine Besylate, Valsartan, Telmisartan and diuretics Hydrochlorothiazide and Chlorthalidone.[17]

Novel, selective and rapid Gradient Reversed Phase High Performance Liquid Chromatographic (RPHPLC) method for the

analysis of Amlodipine and Losartan in binary mixture has been developed and validated[15,16,17]

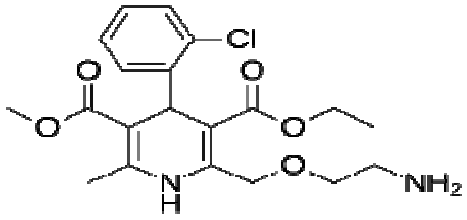


**Figure - 1: Amlodipine**

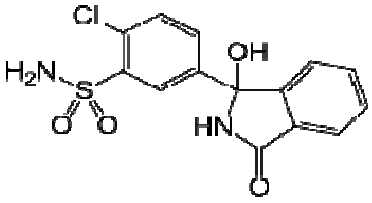


**Figure – 2: Chlorthalidone.**

A reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Aatenolol and Chlorthalidone in marketed formulation.The determinationdeveloped a method for spectrophotometric method for Simultaneous estimation of Amlodipine besylate and Bisoprolal fumarate in pharmaceutical preparation.[18,23]

The literature survey revealed that no UHPLC method for the simultaneous determination of amlodipine,chlorthalidone and in pure drugs in pharmaceutical combined tablet dosage form was reported.Therefore an UHPLC method was developed and found to be simple,rapid,sensitive,selective,accurate, precise and robust for the determination of amlodipine and chlorthalidone in combined tablet dosage form.The Novelty of this method is low limit of quantification(LOQ),Low Limit Of Detection(LOD) highly sensitive and offers good separation with simple mobile phase and shorter analysis time.[23,24]

## 2. MATERIALS AND METHODS

### 2.1. Materials and reagents

Amlodipine besylate,chlorthalidone standards were purchased from Amoli organics pvt limited&Shanpur pharma chem.HPLC grade Acetonitrile Solvents, Water HPLC-grade from Milli-QRO system, AMLODAC tablets,containing amlodipine 5mg and chlorthalidone 12.5mg manufactured by AEON pharmaceuticals pvt ltd, was purchased from local pharmacy.[22]

### 2.2. Instrumental and Chromatographic conditions

Method development and validation was carried out on UHPLC system,consisting of a LC-pump, degasser and auto sampler. Chromatographic separation was achieved using: C18 column (4.6 x 50 mm, i.d. 3.5 µm) column, with an isocratic elution of the mobile phase system.

Consisting of acetonitrile and potassium dihydrogen orthophosphate buffer pH2.4 using ortho phosphoric acid .in the ratio of 600: 400(v/v)at a constant flow rate of 0.8ul/min and 25degree celcious. the auto sampler was set to inject 10ul/min with chromatographic run time of 10min.[23,24]

### 2.3. Preparation of standard sample solution

Standard stock solution of Amlodipine was prepared by dissolving weighed quantity of the reference standard in 50 ml clean dried volumetric flask, added 30ml of diluents, sonicated for 20 minutes to dissolve the content and made up to volume with diluent. Filtered the final solution through 0.45µm membrane. The solution was diluted toget a concentration of 500µg/mL (stock solution). The stock solution was diluted to obtain a final concentration of 100µg/mL.

Standard stock solution of Chlorthalidone was prepared by dissolving weighed quantity of the reference standard in 50 ml clean dried volumetric flask, added 30ml of diluents, sonicated for 20 minutes to dissolve the content and made up to volume with diluent. Filtered the final solution through 0.45µm membrane. The solution was diluted toget a concentration of 2500µg/mL (stock solution).The stock solution was diluted to obtain a final concentration of 250µg/mL.[20]

### 2.4. Preparation of tablet sample solution

Weighed and finely powdered 20 tablets. An accurately weighed quantity of powder equivalent to Amlodipine and Chlorthalidone transferred into the 50mL volumetric flask; added approximately 35 ml of diluents, sonicated for 20 minutes to dissolve completely; cooled to room temperature and made up the volume with diluent. Filtered the final solution through 0.45µm membrane filter.[20,21,25] The solution was diluted to get a concentration of Amlodipine 100µg/mL and Chlorthalidone 250µg/mL. The final sample solution was filtered through a 0.45 µm membrane filter (Millipore) and 10 µL of the solution was injected into the column and the retention time and peak area obtained was determined. The elute was detected at 240nm and the chromatogram was recorded.[23]

### 2.5. Validation

A method validation was performed as per ICH guidelines for simultaneous estimation of

Amlodipine and Chlorthalidone in combined dosage form. The following validation was addressed: system suitability, specificity, linearity, accuracy, recovery and precision limit of detection and limit of quantification.[16]

### 2.5.1. System Suitability

The system suitability was performed by analyzing five replicate injections of standard sample solution. Results of peak area of Amlodipine and Chlorthalidone were noted and the acceptance crieteria should not be more than 2.0%for the RSD for the peak areas of the both the drugs .[14]

### 2.5.2. Specificity

The diluents solution chromatograms and sample chromatograms were compared for the interference of any extra peak then this indicates that diluents solution used in sample preparation do not interfere in the estimation of Amlodipine and Chlorthalidone.

### 2.5.3. Linearity

The calibration curve was established by plotting a graph of concentration versus area of Amlodipine and Chlorthalidonestandards and determining the correlation coefficient (R2).A series of concentration s ranging from 2.5ng/ml to 7.5ng/ml of amlodipine and 8.75ng/ml to 18.75ng/ml of chlorthalidone were prepared and analysed in triplicates(correlation coefficient should be not less than 0.99)[16]

### 2.5.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.The percent recoveries were carried out using standards and the tablet samples at 80% ,100% and 120%level,in triplicate in each level and analysed by UHPLC .The calculated values of percentage of recovery should be between 98.00% -102.00%.[19]

### 2.5.5. Precision

The precision of the method was evaluated as intra –day and inter –day by carrying out six independent assays of test samples against a qualified reference standard and the %RSD of assay was calculated (%RSD should not be more than 2%)

### 2.5.6. Robustness

The robustness of a method is the ability of the method to remain unaffected by making slight deliberate changes in the chromatographic conditions. The robustness of the method was studied by making slight changes in ratio of the mobile phase, flow rate and %RSD was calculated by 3 replicate injections.[6]

### 2.5.7. Limit of Detection and Limit of Quantification

Limit of Detection and limit of quantification were calculated using formula 3.3sigma/S and 10sigma /S respectively, where sigma is the standard deviation of the response (y-intercept) and Sis the slope of the calibration plot.[2]

## 3. RESULTS AND DISCUSSION

### 3.1. METHOD DEVELOPMENT

A UHPLC method was developed for the simultaneous estimation of amlodipine and chlorthalidone in tablet dosage form, which can be conveniently employed for routine analysis. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for drugs was selected based on their polarity. Different trials were taken finally the optimized mobile phase was Acetonitrile :potassium dihydrogen ortho phosphate buffer pH2.4 using ortho phosphoric acid (600:400 V/V) with a flow rate of 0.8ml/min .The retension time of Chlorthalidone is 1.6+ -0.05,and Amlodipine is 2.50+ -0.01 respectively.[13]

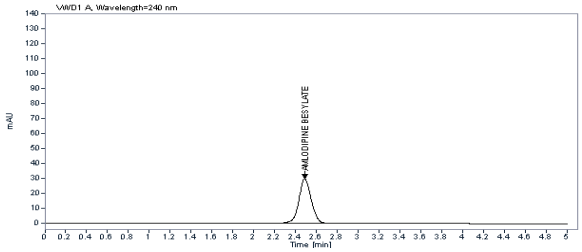


**Figure – 3: Chromatogram (Amlodipine Besylate)**

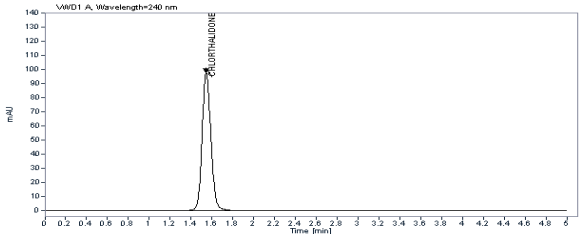


**Figure – 4: Chromatogram (Chlorthalidone)**

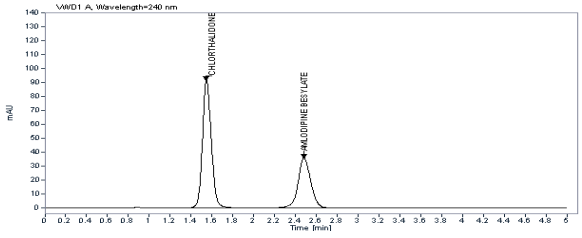


**Figure – 5: Chromatogram (Amlodipine and Chlorthalidone)**

The method was validated as per ICH (Q2B) regulatory guidelines .summary of validation parameters of UHPLC method for simultaneous estimation of Amlodipine and chlorthalidone is given in table 1.[9,10]

### 3.2 Validation

#### 3.2.1 System Suitability

The system suitability was performed by analyzing five replicate injections and the %RSD for Amlodipine and Chlorthalidone wasfound to be 0.3 and 0.2 respectively, which is less than 2.[26]

#### 3.2.2 Specificity

The optimized UHPLC method was used for the analysis and identification of Amlodipine and Chlorthalidone were 2.50+ -0.01 and 1.6+ -0.05 minutes respectively. No interfereing peaks were observed with the same retension time of the analytes.[10,11]

#### 3.2.3 Linearity

A linear response was observed for the intensity of the peak area versus concentration over a working range of 2.5ng/ml to 7.5ng/ml of amlodipine and 8.75ng/ml to 18.75ng/ml of chlorthalidone were prepared and analysed in triplicates(correlation coefficient of 0.9991 (figure III) and 0.9990 (figure iv)

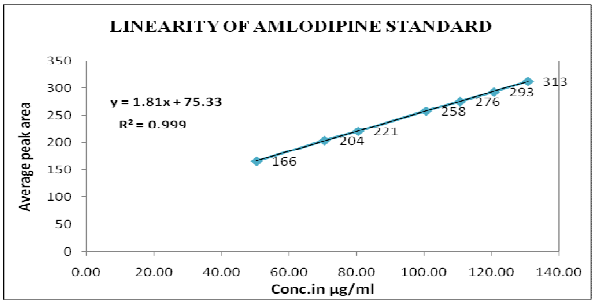


**Figure - 6: Linearity Results of Amlodipine.**

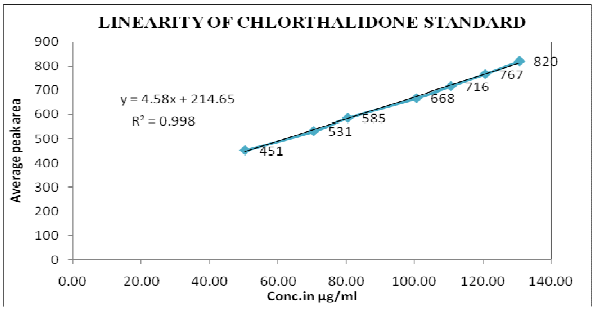


**Figure – 7: Linearity Results of Chlorthalidone.**

#### 3.2.4. Accuracy

Percent recovery of amlodipine ranged from 100.80% to 101.20%) and the chlorthalidone 99.30%-100.60%) showing the good accuracy of the method .[5]

**Table - 1: Analytical method validation report for Amlodipine and Chlorthalidone tablets**

| Parameters | Limit | Observations | Passes/Fails |
|---|---|---|---|
| Specificity | No interference at retension time of the analyte peak | No interference at retension time of the analyte peak | Passes |
| System precision | RSD 1.0% | Amlodipine 0.3%<br>Chlorthalidone 0.2% | Passes |
| Method precision | RSD 1.0% | Amlodipine 0.3%<br>Chlorthalidone 0.4% | Passes |
| Linearity of detector response | Correlation co-efficient NLT 0.99 | Amlodipine - 0.9999<br>Chlorthalidone -0.9991 | Passes |
| Accuracy | % Recovery range (98.0%-102.0%) | Amlodipine 100.80 % to 101.20%)<br>Chlorthalidone 99.30%-100.60%) | Passes |
| Ruggedness | RSD NMT 2.0% | Amlodipine besylate –within the limits<br>Chlorthalidone –within the limits | Passes |
| Robustness | RSD NMT 2.0% | Amlodipine –within the limits<br>Chlorthalidone –within the limits | Passes |

#### 3.2.5. Precision

The precision of the analytical method was studied byn determining the concentration of each drug in the tablet in six replicates .The results of the precision study indicate that the method is reliable and the %RSD for the precision study was 1.4 % and 1.2% (inter-day precision ),1.0% and 1.7% (intra-day precision) for amlodipine and chlorthalidone.[4,25]

#### 3.2.6. Robustness

The result of the robustness study indicates that the study indicates that the method

is robust and is unaffected by small variations in the chromatographic conditions .The %RSD was found to be less than 2.[25]

## 4. CONCLUSION

Amlodipine and chlorthalidone in tablet dosage form was validated and found to be accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of amlodipine and chlorthalidone in combined tablet formulation.

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