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# Simultaneous Estimation of Chlorthalidone, Amlodipine and Telmisartan by RP-HPLC Method in Pharmaceutical Dosage Form

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**ABSTRACT**

A novel, simple, accurate reverse phase – High Performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine in pharmaceutical dosage form was developed and validated. This method was performed on C18 column (250x4.6mm) using mobile phase Buffer: Acetonitrile in ratio 60:40. Buffer solution was mixing 1ml of o- phosphoric acid in 1000ml of water. The flow rate was 0.8 ml /min and detection was carried out at 221nm. The retention times were 3.1, 7.4 and 10.5 min for Chlorthalidone, Telmisartan and Amlodipine respectively. The method was linear over the concentration range of 4-20µg/ml for chlorthalidone and 10-50 µg/ml for telmisartan and 3-7µg/ml for amlodipine with correlation coefficient of 0.998, 0.997 and 0.987 respectively. The developed method was validated as per ICH guidelines for linearity, accuracy, precision, robustness, specificity, limit of detection, limit of quantitation.

## INTRODUCTION

Chlorthalidone (CHL) may be a long acting thiazide-like diuretic of the sulfamoylbenzamide class that's unproductive of the benzothiadiazine structure. Chlorthalidone is employed within the treatment of high vital sign, edema and congestive coronary failure. The elimination half-life of Chlorthalidone is about 40 to 50 hours. IUPAC name of Chlorthalidone is 2-chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl)benzenesulfonamide. It is a racemic mixture of 2-chloro-5(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide, with the structure of Chlorthalidone was shown in figure 1.

Telmisartan (TEL) is an angiotensin II receptor antagonist (ARB) utilized in the management of hypertension. IUPAC name of Telmisartan is 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid. It is used alone or in combination with other classes of antihypertensives for the treatment of hypertension. Structure of Telmisartan was shown in figure 2.

Amlodipine (AML), initially approved by the FDA in 1987, maybe a popular antihypertensive belonging to the group of medicine called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are related to a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. IUPAC name of Amlodipine is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Structure of Amlodipine was shown in figure 3.

There is an HPLC method that described about simultaneous determination of Amlodipine Besylate, Valsartan, Telmisartan, Hydrochlorothiazide and Chlorthalidone (R. A. Mhaske *et al).* There is also a paper which describe about simultaneous estimation of Chlorthalidone, Telmisartan and Amlodipine by UPLC method (G Sivakamasundari *et al*) and there are many more method are available which are individual or in combination with other drugs. However, no method was reported for simultaneous determination of Chlorthalidone, Telmisartan and Amlodipine by RP-HPLC though there is method available which is on UPLC.

Therefore, the aim of this study was to develop and validate an efficient and new RP-HPLC method for simultaneous determination, and this new method could also be used for routine analysis of CHL, TEL and AML in pharmaceutical dosage form.

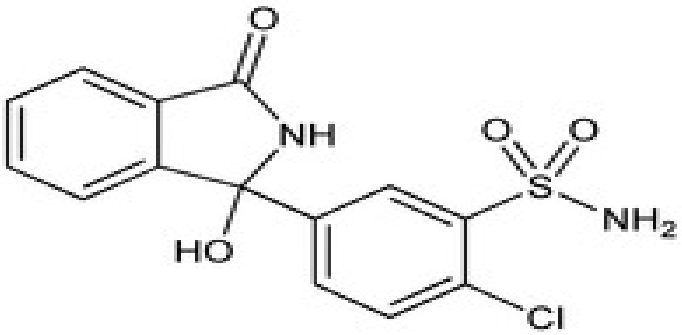


**Figure No. 1: Structure of Chlorthalidone**

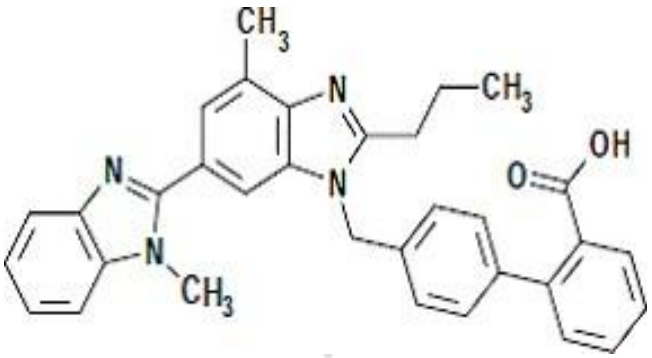


**Figure No. 2: Structure of Telmisartan**

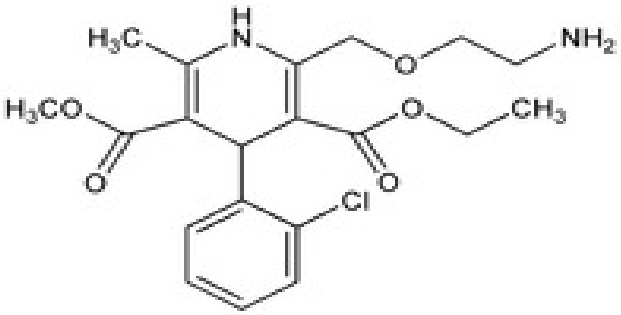


**Figure No. 3: Structure of Amlodipine**

## MATERIALS AND METHODS

### MATERIALS

Chlorthalidone, Telmisartan, Amlodipine, Methanol (HPLC grade), Water (HPLC grade), Acetonitrile (PLC grade)

Marketed formulation-

Eritel Trio CH-40 tablet

Instruments used: Digital balance-scale etc,

HPLC Agilent – EZ- Chrome elite

UV-Visible Spectrophotometer- UV-1900 Shimadzu, Japan

Ultra sonicator- Lifecare, Mumbai

**Instrumentation and chromatographic condition**

Chromatography was performed with Agilent 1120 compact LC system using software EZ – Chrome elite performed data acquisition, data handling and instrumentation control. c18 column (250x4.6mm) was used for chromatographic separation under the suitable condition. mobile phase was Buffer: Acetonitrile in ratio 60:40. Buffer solution was mixing 1 ml of o-phosphoric acid in 1000ml of water. The mobile phase was sonicated and degassed before use. The diluents were methanol. It was pumped through the column at a flow rate of 0.8ml/min. The detection was carried out at 221nm. The retention time obtained for the drugs were 3.1 min for Chlorthalidone, 7.4 min for Telmisartan and 10.5 min for Amlodipine. A typical chromatogram is shown in fig 4.

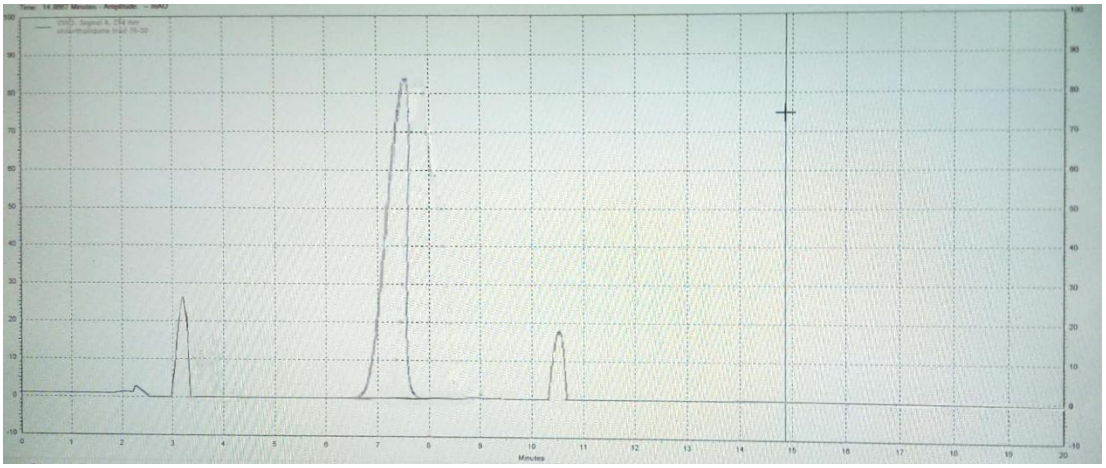

**Figure No. 4: Typical chromatogram of Chlorthalidone, Telmisartan and Amlodipine**

**Standard Preparation**

20 mg of Telmisartan(TEL), 6.25 mg of Chlorthalidone(CHL) and 2.5 mg of Amlodipine (AML) working standards was accurately weighed and separately transferred in to a 50 ml volumetric flask and diluted with 20 ml of diluent and sonicated to dissolve TEL, CHL and AML, volume was made up to the mark with diluents from this 1 ml each solution was pipetted out and taken in to a 10 ml volumetric flask and volume made up with diluents to get final concentration 40 µg/ml of TEL 12.5 µg/ml CHL and 5 µg/ml of AML.

**Sample Preparation**

Twenty tablets were accurately weighed and average weight was determined and transferred to clean and dry glass mortar and ground into a fine powder. The quantity of powder equivalent to 40 mg of Telmisartan, 12.5 mg of Chlorthalidone and 5 mg of Amlodipine was weighed accurately and transferred to 50 ml volumetric flask and 10 ml of methanol (HPLC grade) was added. The contents were sonicated for 20 min and made up the volume up to the mark 50 ml by Water (HPLC grade). This solution was filtered through 0.45 µm pore size nylon 66 membrane. 1ml of stock solution was transferred into 25ml volumetric flask and diluted to the volume with mobile phase.

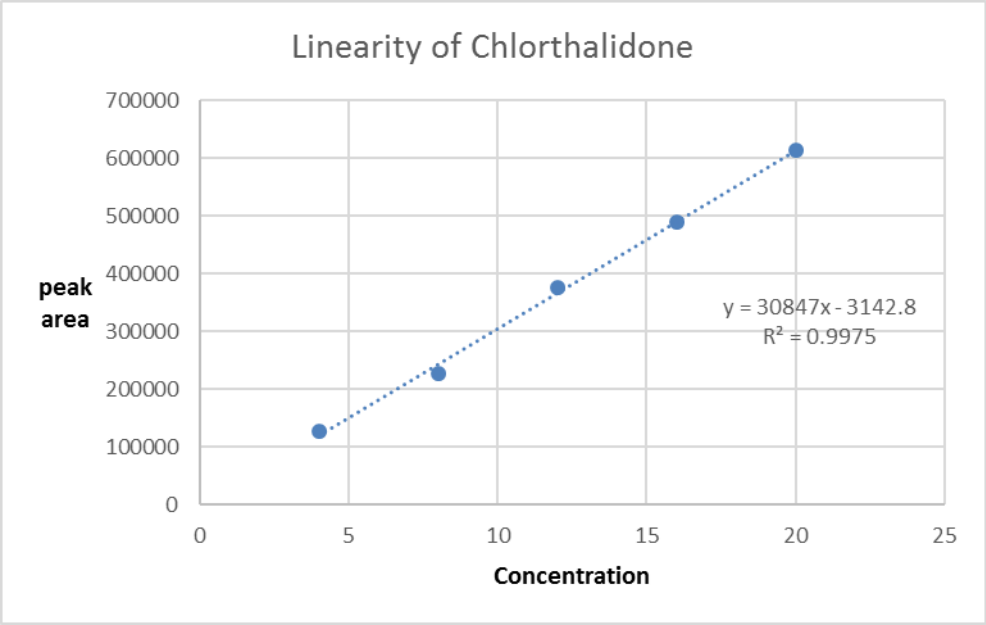


**Figure No. 5: Calibration curve of Chlorthalidone**

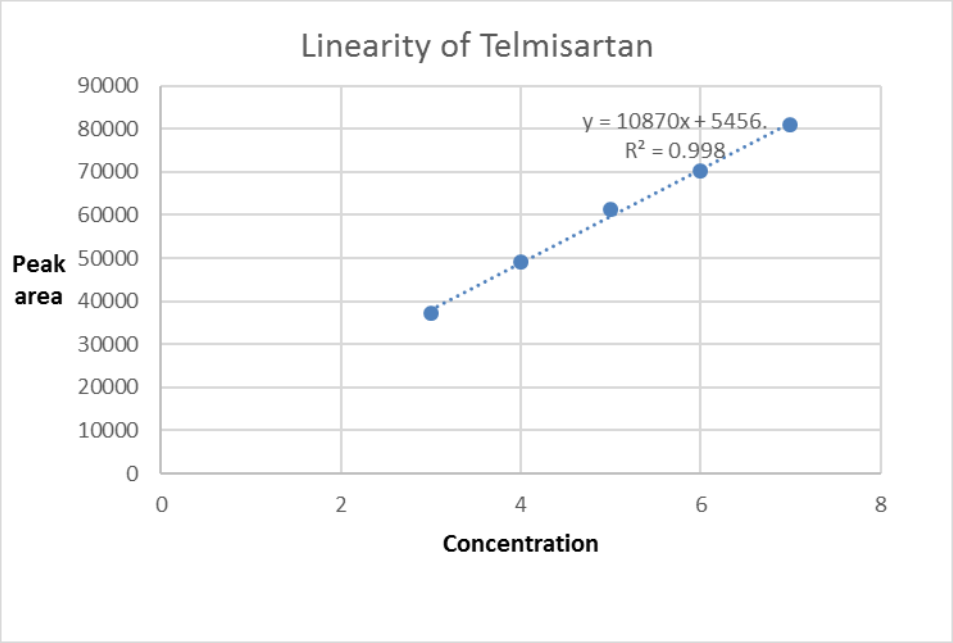


**Figure No. 6: Calibration curve of Telmisartan**

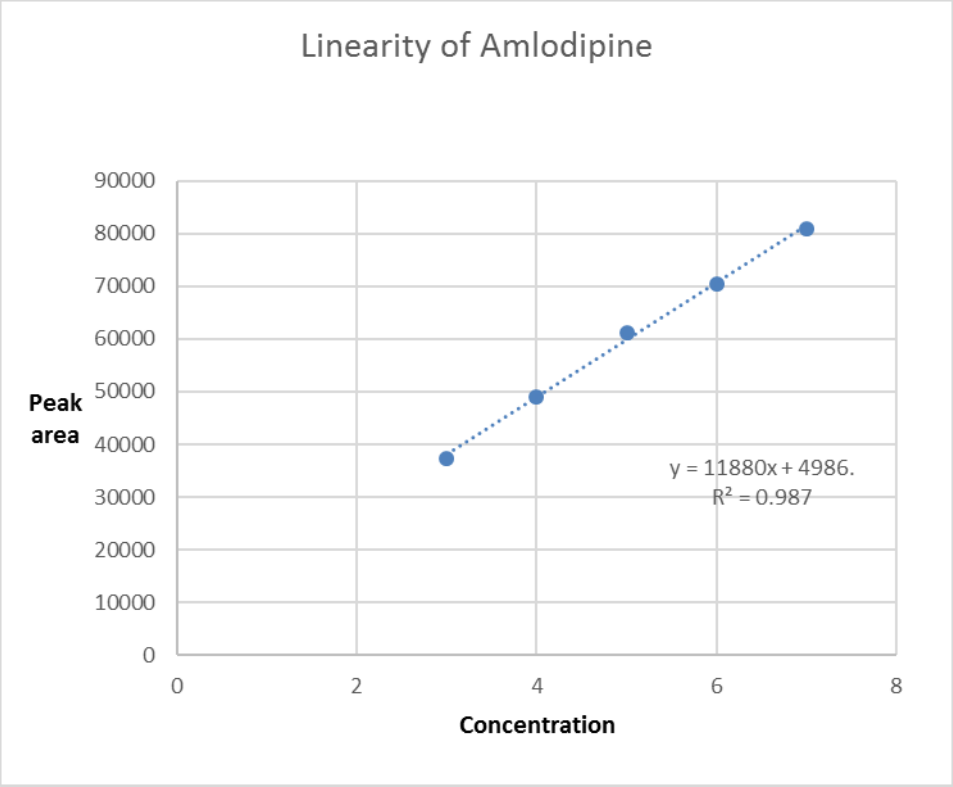


**Figure No. 7: Calibration curve of Amlodipine**

## RESULTS AND DISCUSSION

### Method development

The RP-HPLC method aimed to estimate the Chlorthalidone, Telmisartan, and Amlodipine in the pharmaceutical dosage form and validate the method as per ICH guidelines. Preliminary tests were performed to select optimum HPLC condition. Parameters such as choice of column mobile phase composition, detection wavelength and other factors were studied. After several trials the mobile phase selected as 60:40 (v/v) mixture of freshly prepared buffer (1 ml of O-phosphoric acid in 1000 ml of water) and acetonitrile. Column C18, (250x4.6mm) was found to be suitable for chromatographic separation. The wavelength was Selected at 221 nm based on the UV –overlain spectra of three drugs using optimised mobile phase. The flow rate was 0.8 ml and retention time obtained for the drugs were 3.1 minutes for Chlorthalidone, 7.4 minutes for Telmisartan, 10.5 minutes for Amlodipine respectively.

### Method Validation

The developed HPLC method was validated to confirm that it was suitable for its intended purposes as described in an international conference on harmonization (Q2B) guidelines. The validation parameters include system suitability, linearity, accuracy, precision (system precision, method precision), limit of detection (LOD), limit of quantitation (LOQ), sensitivity, robustness (flow rate, wavelength).

**Table No. 1: Result of method validation**

| Parameter | Chlorthalidone | Telmisartan | Amlodipine |
|---|---|---|---|
| Linearity | 4-20µg/ml | 10-50µg/ml | 3-7 µg/ml |
| Regression equation | y = 30847x - 3142 | y = 10870x + 5456 | y = 11880x + 4986 |
| Correlation coefficient ($R^2$) | 0.998 | 0.997 | 0.987 |
| Accuracy (%) | | | |
| 80% | 99.1% | 100.93% | 101.84% |
| 100 % | 100.63% | 99.36% | 99.56% |
| 120% | 99.93% | 98.93% | 98.43% |
| precision | 99.43% | 99.36% | 99.29% |
| LOD(µg/ml) | 0.59 | 0.71 | 0.45 |
| LOQ(µg/ml) | 1.54 | 1.69 | 1.37 |
| Assay (%) | 98.4% | 97.75% | 98% |
| Retention time | 3.1min | 7.4min | 10.5min |

**Linearity**

For establishing the linearity for Telmisartan(TEL), Chlorthalidone(CHL) and Amlodipine (AML), a series of standard preparation of Telmisartan(TEL), Chlorthalidone(CHL) and Amlodipine (AML) were prepared and the method was found linear between 4-20µg/ml for chlorthalidone and 10-50 µg/ml for telmisartan and 3-7µg/ml for amlodipine, and calibration curve was constructed by plotting concentration on X-axis and peak area on Y-axis. The linearity graph for individual drug is shown in figure 5, 6 and 7.

**Accuracy**

Accuracy study were carried out, by applying the method drug content in the tablet formulation, to which known amount of drugs was added 80%, 100%, 120% levels. The technique includes addition of standard drug solution to pre-analyzed sample solution. Recovery test was performed at 3 times each level.

**Precision**

To study precision, six replicate standard solutions of Chlorthalidone, Telmisartan and Amlodipine were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be within the acceptance criteria of not more than 2.0 %.

**Robustness**

Robustness is a measure of the capacity of the analytical method to remain unaffected by small but deliberate variation of the operating condition. This was tested by studying the effect of changing the flow rate and wavelength.

**Limit of detection (LOD)**

To determine the Limit of Detection (LOD) sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliable detected.

**Limit of quantitation (LOQ)**

Limit of quantitation was determined by the analysis of sample with known concentration of analyte and by establishing the minimum analyte can be reliably quantities.

## CONCLUSION

From all statistical data it is concluded that the developed RP-HPLC method is validated as per ICH guideline it is more specific, linear, precise, accurate and robust for assay of Chlorthalidone (CHL), Telmisartan (TEL) and Amlodipine (AML) in tablets dosage form. Hence this method could be introduced in to routine use for the assay study and related substances of Chlorthalidone (CHL), Telmisartan (TEL) and Amlodipine (AML) tablets. It can be concluded that developed RP-HPLC method have been accepted due to more specific, precise, linear, robust and sensitive. Thus, it represent another good alternative for the already existing methods.

## ACKNOWLEDGEMENT

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