

## DEVELOPMENT AND VALIDATION OF A REVERSED PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM

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### ABSTRACT

A simple reversed phase HPLC method has been developed for the simultaneous determination of olmesartan medoxomil in combination with hydrochlorothiazide. The method was based on reversed phase liquid chromatography using a xTerra symmetry C18 column (150 × 4.6 mm, 5 $\mu$ ) with UV detection at 230 nm. The mobile phase consisting of acetonitrile and potassium dihydrogen phosphate buffer adjusted to pH 2.5 in a ratio of (45:55, v/v) and at a flow rate of 0.7 ml/min. The method was linear over the concentration range for olmesartan medoxomil 20-60  $\mu$ g/mL and for hydrochlorothiazide 20-60  $\mu$ g/mL. The recoveries of olmesartan medoxomil and hydrochlorothiazide were found to be in the range of 98.0-102.0% and 98.0-102.0% respectively. The method was validated and was successfully employed for the analysis of pharmaceutical formulations containing olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage form.

**Keywords:** Olmesartan, Hydrochlorothiazide, HPLC, Validation.

### INTRODUCTION

Chemically Hydrochlorothiazide (HCT) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide (Fig. 1), one of the oldest and widely used thiazide diuretics<sup>1</sup>. On the other hand Olmesartan medoxomil (OLM) is described chemically as the (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-carboxylic acid (Fig. 2) and is a selective AT1 subtype angiotensin II receptor blocker<sup>2,3</sup>.

The USP describes an RP-HPLC method for the determination of HCT in tablets. Several analytical methods have been reported for the determination of HCT in pharmaceutical formulations including polarography, LC, HPTLC and spectrofluorometry<sup>4-7</sup>. OLM has not yet been officially described in any pharmacopoeia and several analytical methods were reported for its determination

in biological sample such as plasma. A literature survey revealed that very few analytical methods have been reported for the determination of HCT and OLM in a combined tablet formulation. So in this present investigation, an attempt has been made to develop accurate, precise and economically viable reversed phase HPLC method for the simultaneous estimation of hydrochlorothiazide and olmesartan medoxomil in combined tablet dosage form.

### MATERIALS AND METHODS

#### Apparatus and chromatographic condition

The chromatographic separation was performed on a Shimadzu HPLC Prominence series, integrated with Auto Sampler and UV detector. The analytical xTerra symmetry C18 column (15cm x 4.6 mm i.e., 5  $\mu$ m) was used for the separation. The mobile phase consisted of acetonitrile and pot.dihydrogen phosphate

(7.0 grams of  $\text{KH}_2\text{PO}_4$  into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5 with Orthophosphoric acid) in a ratio of 45:55 (v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 0.7 ml/min and the detector wavelength was set at 230 nm. The injection volume was 20  $\mu\text{l}$ .

#### Chemicals and Reagents

The pharmaceutical grade pure samples of Olmesartan medoxomil (99.28%) and Hydrochlorothiazide (99.55%) were received as gift samples from Sumages Pharma Pvt. Ltd., Bhimavaram. HPLC grade Acetonitrile and Analytical grade potassium dihydrogen phosphate and Orthophosphoric acid were obtained from Qualigens Fine Chemicals Ltd., Mumbai.

#### Preparation of Hydrochlorothiazide and Olmesartan standard & sample solution

##### Standard Solution Preparation

Accurately weigh and transfer 10 mg of Hydrochlorothiazide and 10 mg of Olmesartan working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.4 mL of Hydrochlorothiazide and Olmesartan the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

##### Sample Solution Preparation

Accurately weigh and transfer equivalent to 10 mg of Hydrochlorothiazide and Olmesartan sample into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.4 ml of Hydrochlorothiazide and Olmesartan of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

#### Procedure

Inject 20  $\mu\text{L}$  of the standard, sample solution into the chromatographic system and measure the areas for the Hydrochlorothiazide and Olmesartan peaks and calculate the % Assay by using the formulae.

## RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

#### Linearity

The linearity for HPLC method was determined at ten concentration levels ranging from 20-60  $\mu\text{g}/\text{mL}$  for HCT and 20-60  $\mu\text{g}/\text{mL}$  for OLM. The calibration curve was constructed by plotting response factor against respective concentration of OLM and HCT. The plots of peak area Vs respective concentration of OLM and HCT were found to be linear in the range of 20-60  $\mu\text{g}/\text{mL}$  and 20-60  $\mu\text{g}/\text{mL}$  with coefficient of correlation ( $r^2$ ) 0.999 and 0.999 for OLM and HCT respectively.

#### Recovery

Five different samples of known concentration ranging from 20-60  $\mu\text{g}/\text{mL}$  for OLM and 20-60  $\mu\text{g}/\text{mL}$  for HCT were prepared and these are analyzed against standard solution. The result of recovery analysis of olmesartan medoxomil and hydrochlorothiazide were found to be in the range of 98.0-102.0% and 98.0-102.0% respectively.

#### Sensitivity

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02  $\mu\text{g}/\text{ml}$  and 0.07  $\mu\text{g}/\text{ml}$  for OML and 0.07  $\mu\text{g}/\text{ml}$  and 0.025  $\mu\text{g}/\text{ml}$  for HCT. The LOD and LOQ showed that the method is sensitive for OLM and HCT.

#### System suitability test

The specificity of this method was determined by complete separation of OLM and HCT as shown in Fig. 3 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of OLM and HCT was less than 2% and resolution was satisfactory. The average retention time  $\pm$  standard deviation for OLM and HCT were found to be 0.26  $\pm$ 0.004 and 1.00  $\pm$ 0.005 respectively, for five

replicates. The peaks obtained for OLM and HCT were sharp and have clear baseline separation. Analysis were also performed for active OLM and HCT, placebo (All the ingredients except active OLM & HCT) and sample both stressed (at 105°C for 24hr) and unstressed condition. After analysis it was found that there is no interference of peak in the Olmesartan medoxomil and Hydrochlorothiazide region for the stressed sample, Placebo & Active. Hence the developed method was specific for the analysis of this product.

#### Ruggedness and Robustness

Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

#### Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of HCT and OLM remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process.

#### CONCLUSION

The developed HPLC method is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of olmesartan medoxomil and hydrochlorothiazide in a combined tablet dosage form.

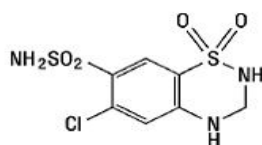


Fig. 1: Structure of Hydrochlorothiazide

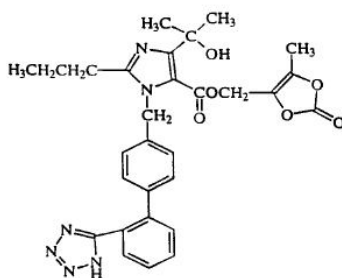
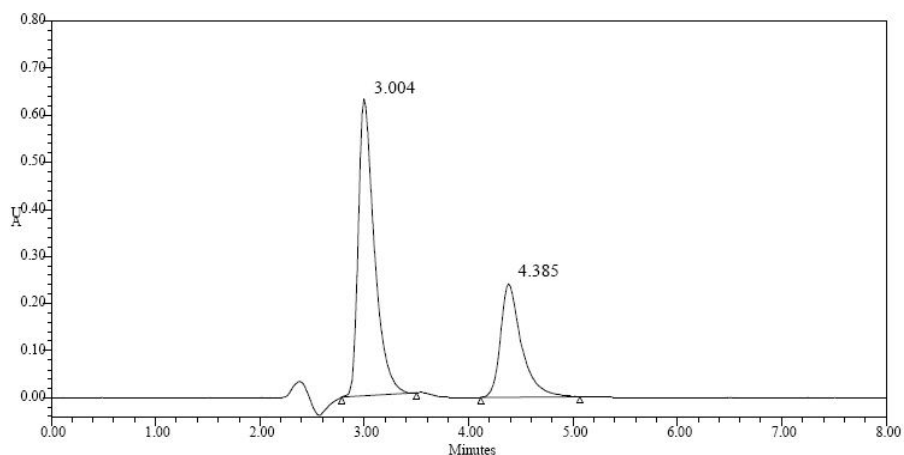


Fig. 2: Structure of Olmesartan Medoxomil



**Fig. 3: Typical chromatogram of Olmesartan medoxomil and Hydrochlorothiazide**

**Table 1: Analytical Validation Parameters**

Parameter	OLM	HCT
Linearity	20-60 µg/mL	20-60 µg/mL
Correlation Coefficient	0.999	0.999
Theoretical Plates	2537	2651
Tailing Factor	1.5	1.6
Limit of Detection (LOD)	0.02 µg/ml	0.007µg/ml
Limit of Quantitation (LOQ)	0.07 µg/ml	0.025 µg/ml
Accuracy (%)	98.0-102.0	98.0-102.0
Retention Time (min)	4.382	3.006

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