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# Simultaneous Determination of Olmesartan, Amlodipine Besylate and Hydrochlorothiazide in Tablet Dosage Form by Using Stability-Indicating HPLC Method

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## ABSTRACT

A simple, precise and rapid stability-indicating HPLC method was developed for the simultaneous quantitative determination of Olmesartan, Amlodipine and Hydrochlorothiazide from their innovative Pharmaceutical combination drug product, with the presence of degradation products. The separation was achieved on simple gradient method. The detector wavelength was 260 nm. The total runtime was 25 min. and RTof Hydrochlorothiazide, Amlodipine and Olmesartan are 4.2, 11.2 and 16.8 min.respectively.The described method was validated with respect to system suitability, specificity, linearity, precision and accuracy.

**Keywords:** Validation; Olmesartan; Amlodipine; Hydrochlorothiazide and HPLC

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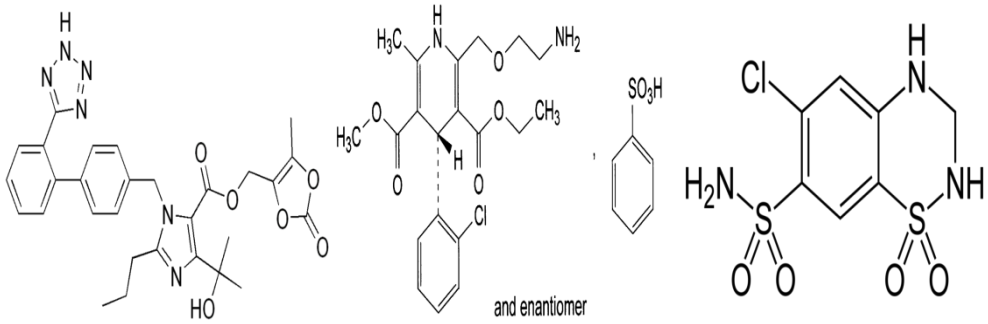
## INTRODUCTION

Cardiovascular diseases (CVDs) are the disorders of heart and blood vessels and primarily include coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. CVDs are the major cause of death in developed countries and also are rapidly emerging as a main cause of death in the developing world. A novel formulation is developed using drugs Olmesartan, Amlodipine and Hydrochlorothiazide for CVDs.OLM is an angiotensin II receptor (type AT1) antagonist used in the management of hypertension. Olmesartan prevents the constriction of blood vessels. Olmesartan is a non- peptide molecule (**Figure 1**).AMD is in a class of drugs called beta-blockers. Beta-blockers affect the heart and circulatory system. Amlodipine Besylate is used to lower blood pressure, lower heart rate, reduce chest pain, and to reduce the risk of re-current heart attacks (**Figure 1**).HYD is a thiazide diuretic. It decreases the amount of fluid in the body by increasing the amount of salt and water lost in the urine. Hydrochlorothiazide is used to lower blood pressure and to decrease edema (swelling). (**Figure 1**).




Olmesartan Amlodipine Hydrochlorothiazide

**Figure 1. Structures of Olmesartan, Amlodipine and Hydrochlorothiazide**

In the pharmaceutical and other fields, there is demand for the development of simultaneous analytical method to minimise the cost and time. Literature survey reveals that a variety of Spectrophotometry and chromatographic methods including UV, colorimetric determination, ratio derivative, and a stability-indicating HPLC methods have been reported for determination OLM, AMD and HYD either single or in double combination with other drugs but no HPLC method has been reported for simultaneous quantitative determination of OLM, AMD and HYD.Hence a rapid simple reproducible HPLC method was developed for simultaneous quantitative determination of OLM, AMD and HYD in pharmaceutical dosage forms in the presence of degradation products.

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## MATERIALS AND METHODS

### Experimental

### Chemicals and Reagents

Standards and tablet (40 mg of Olmesartan, 5 mg of Amlodipine Besylate, 12.5mg of Hydrochlorothiazide).The HPLC grade acetonitrile and methanol,$KH_2PO_4$/Monobasic, OPA.Water for injection was prepared by using Milli Q water purification system.

### Instrumentation

HPLC system of Shimadzu Japan with UV detector and Waters USA with PDA detector used consisting of a quartnary solvent manager. The output signal was monitored and processed using Empower software, water bath equipped with controller (Classic Scientific, India) was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Thermo lab, India). Thermal stability studies were performed in a dry air oven (Newtronic, India).

### Chromatographic Conditions

The chromatographic column used was Inertsil ODS 3V, 150 mm × 4.6 mm, 5.0 µm particles. The Mobile phase A is 0.025 M$KH_2PO_4$/Monobasic& adjust pH 4.4 (± 0.05) with dilute OPA (1ml to 100ml) and mobile B isAcetonitrile. The flow rate was 1.1 mL·min–1.The gradient program (T/%B) was set as 0/22, 5.2/28, 7/35, 19/46, 20/22 and 25/22. The detector wavelength was 260 nm. The diluent contains a mixture of Buffer: Acetonitrile: Methanol (40:40:20) adjust pH 3.0 (± 0.1) with dilute Orthophosphoric acid (1 in 10).

### Preparation of Standard and sample Solutions

### Standard preparation (solution-a)

80 mg Olmesartan Medoxomil and 25.0 mg of Hydrochlorothiazide were prepared by using diluent and kept in ultrasonic bath to dissolve the drug completely.

### Amlodipine standard preparation (solution-b)

35mg Amlodipine besilate was prepared in diluent and kept in ultrasonic bath to dissolve the drug completely.

**Mix standard preparation:** 5 ml of **solution-a** and 2 ml of **solution-b**were mixed and diluted to 50 ml with diluent

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**Preparation of Sample Solution**

200 mg of Olmesartan Medoxomil, 62.5 mg of Hydrochlorothiazide & 25 mg of Amlodipine were diluted with 500 ml of diluent and sonicated. 5 ml of above solution was diluted with 25 ml diluent.

## RESULTS AND DISCUSSION

**Chromatographic Conditions**

The mobile phase conditions were optimized so that the three drugs would be separated in short run time.

The UV spectra of the solutions showed the absorptions 257.5nm for Olmesartan, 225 and 271 nm for Hydrochlorothiazide and 257 nm for Amlodipine for UV max. (**Figure 3**).Under the optimum chromatographic conditions, the retention times of Hydrochlorothiazide, Amlodipine and Olmesartan are 4.2, 11.2 and 16.8 min. respectively (**Figure 2**). The retention times of individual analyte are confirmed by injecting individual working solutions (**Figure 4**).

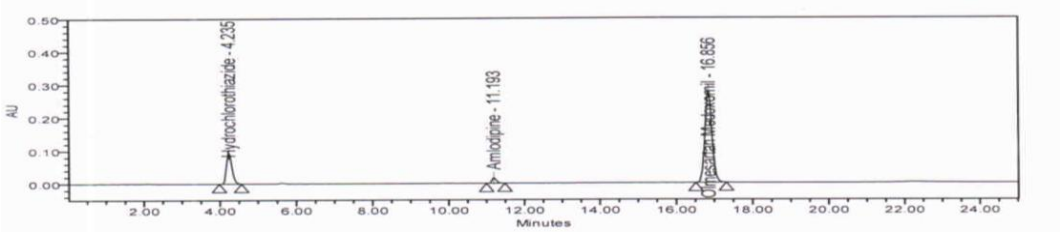


**Figure-2. Chromatogram of Hhydrochlorothiazide, Amlodipine and Olmesartan Medoxomil in pharmaceutical sample solution and their retention time.**

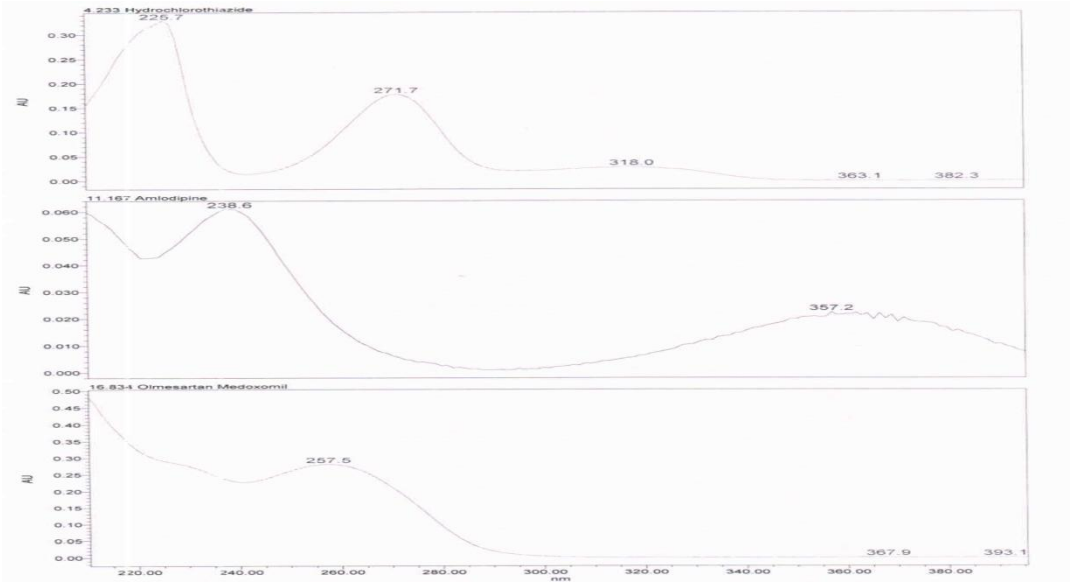

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**Figure-3. PDA spectra of Hydrochlorothiazide, Amlodipine and Olmesartan Medoxomil**

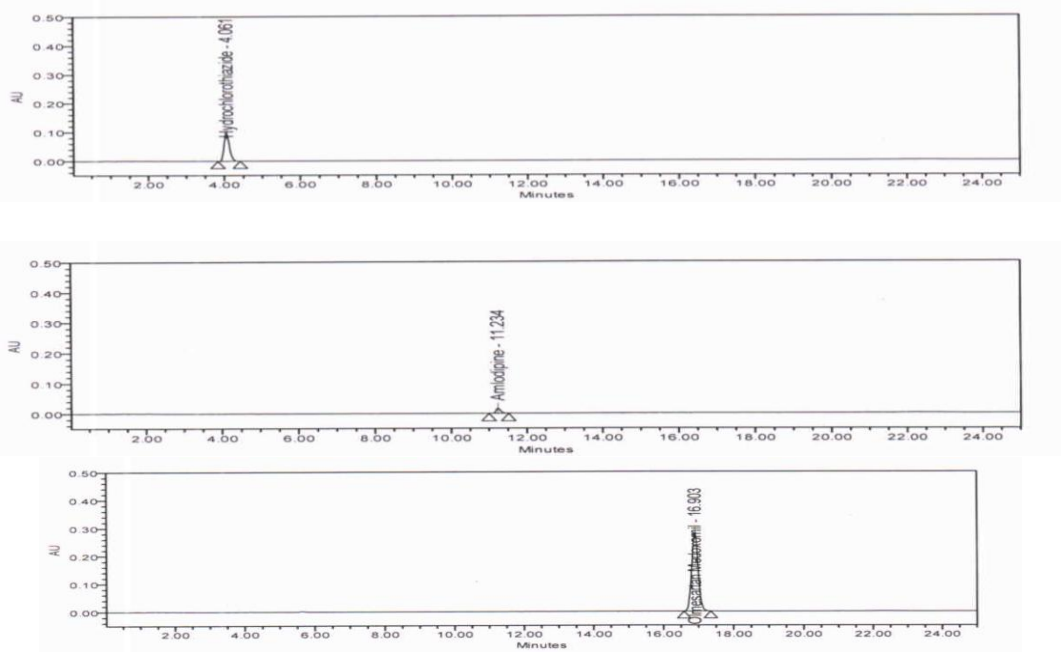


**Figure-4.Chromatograms of individual standard and their retention times.**

## Validation

### System suitability

In order to find the adequate peak separation (resolution) and repeatability of the proposed method, suitability parameters including retention factor, selectivity and asymmetry factor were investigated & the results were summarised in Table 1.

**Table -1-System suitability test parameters**

| System suitability test parameters | HYD | AML | OLM |
|---|---|---|---|
| Retention time (min)(mean ± S.D. n=5) | 4.371 ± 0.022 | 11.54±0.037 | 17.00 ± 0.057 |
| Repeatability of Retention time RSD % (n=5) | 0.510 | 0.324 | 0.332 |
| Repeatability of Peak area,RSD % (n=5) | 0.032 | 0.105 | 0.208 |
| Resolution | --- | 33.066 | 20.777 |
| Tailing factor (asymmetry factor) | 1.135 | 1.162 | 0.986 |
| USP plate count | 5452 | 54972 | 42791 |

### Specificity

Retention time of Olmesartan Medoxomil, Hydrochlorothiazide, and Amlodipine peaks in sample preparation are comparable with standard preparation. Therefore, the HPLC method

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for the determination of assay for Olmesartan Medoxomil, Hydrochlorothiazide, and Amlodipine in Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Tablets is specific.

**Forced degradation studies**

Stress testing of a drug substance can help to identify the likely degradation products, which can help to establish the degradation pathways and the intrinsic stability of the molecule. Acid induced degradation, Base induced degradation, and Hydrogen peroxide induced degradation, Photo-degradation, Humidity Degradation (25 ° C/92% for 24 hrs.)Was carried out with respective condition and details mentioned in Table-2.

**Table-2- Peak purity results of OLM, AML & HYD in forced degradation study**

| Name | Purity Angle | | | Purity Threshold | | | Purity Criteria | | |
|---|---|---|---|---|---|---|---|---|---|
| | OLM | AML | HYD | OLM | AML | HYD | OLM | AML | HYD |
| Acid degradation3 | 0.226 | 0.579 | 0.133 | **0.405** | **0.737** | **0.338** | **Pass** | **Pass** | **Pass** |
| Base degradation | 0.197 | 0.614 | **0.132** | **0.391** | **0.77** | **0.344** | **Pass** | **Pass** | **Pass** |
| Peroxide degradation | 0.200 | 0.554 | **0.139** | **0.406** | **0.769** | **0.363** | **Pass** | **Pass** | **Pass** |
| Thermal degradation | 0.204 | 0.686 | **0.138** | **0.372** | **0.787** | **0.331** | **Pass** | **Pass** | **Pass** |
| Photolytic degradation | 0.218 | 0.864 | **0.127** | **0.442** | **0.921** | **0.356** | **Pass** | **Pass** | **Pass** |
| Humidity degradation | 0.134 | 0.679 | **0.134** | **0.403** | **0.906** | **0.336** | **Pass** | **Pass** | **Pass** |

**Linearity**

Linearity solutions were prepared from stock solution at six concentration levels from 50 to150% of analyte concentrations .The slope, Y-intercept and correlation coefficient were calculated and summarized in Table 3.

**Table-3-Linearity for Olmesartan Medoxomil, Hydrochlorothiazide, and Amlodipine**

| Analyte | Concentration range | Correlation Coefficient | Slope | Intercept |
|---|---|---|---|---|
| HYD | 12.43-37.28 µg/mL | 0.99990 | 33367 | 21864 |
| ALM | 4.94-14.83 µg/mL | 0.99998 | 17937 | 605 |
| OLM | 40.06-120.18 µg/mL | 0.99998 | 42345 | 19401 |

**Precision**

The precision of the assay method was evaluated by carrying out six independent assays of OLM, AML, and HYD test samples against qualified standard.

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**Table-4. Intraday and inter day precision results for OLM, AML & HYD**

| Day | Active name | Pre-1 %Assay | Pre-2 %Assay | Pre-3 %Assay | Pre-4 %Assay | Pre-5 %Assay | Pre-6 %Assay | Mean | % RSD |
|---|---|---|---|---|---|---|---|---|---|
| Intra day | OLM | 99.7 | 99.5 | 99.4 | 9.4 | 99.2 | 99.4 | 99.4 | 0.164 |
| | AML | 100.2 | 101.6 | 99.9 | 99.9 | 99.4 | 99.7 | 100.1 | 0.772 |
| | HYD | 99.9 | 99.8 | 99.9 | 100.1 | 100 | 100.5 | 100 | 0.25 |
| Inter day | OLM | 98.8 | 98.4 | 100.9 | 98.6 | 98.7 | 99 | 0.97 | 0.97 |
| | AML | 99.6 | 99.2 | 99.4 | 101.9 | 99.7 | 10 | 100 | 0.985 |
| | HYD | 99.9 | 99.6 | 99.6 | 101.9 | 100 | 100.2 | 100.2 | 0.863 |

## Accuracy

The accuracy of an analytical method expresses the nearness between the reference value and found value. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 80%, 100% and 120% of target test concentration (159.80 to 238.94 µg/mL for OLM, 20.30 to 30.15 µg/mL for AML and 50.15 to 75.07 µg/mL for HYD) in tablets. The results obtained are shown in Table 5.

**Table 5**

| Analyte . | Recovery level | Amount Added (mg) | Amount Recovered (mg) | % RSD |
|---|---|---|---|---|
| | 80 % | 50.36 | 49.85 | 0.12 |
| Hydrochlorothiazide | 100% | 62.70 | 62.20 | 0.07 |
| | 120 % | 75.22 | 74.17 | 0.08 |
| | 80 % | 20.34 | 20.11 | 0.21 |
| Amlodipine | 100% | 25.44 | 25.17 | 0.09 |
| | 120% | 30.32 | 30.04 | 0.18 |
| | 80 % | 159.48 | 159.25 | 0.19 |
| Olmesartan | 100% | 199.62 | 198.80 | 0.09 |
| | 120% | 239.61 | 237.08 | 0.18 |

## Solution Stability and Mobile Phase Stability

The solution stability of OLM, AML, and HYD was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 48 hrs. The same sample solution was assayed for a 24 hours interval against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 24 hours interval up to 48 hours The % RSD of the assay of OLM, AML, and HYD during solution stability and mobile phase experiments were within 1% and it indicates that both standard and test preparation and mobile phase were stable for 2 days on bench top at room temperature.

## CONCLUSIONS

The established HPLC method proves to be simple, linear, precise, accurate and specific. The method was validated and shows satisfactory data for all the method validation parameters

tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs OLM, AML, and HYD in presence of degradation products in stability by the industry.

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