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Der Pharmacia Sinica, 2013, 4(2):145-152



# Development and validation of stability indicating HPLC assay method for simultaneous determination of amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide in tablet formulation

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## ABSTRACT

The purpose of the research described herein was to develop simple, precise and accurate isocratic stability indicating reversed phase HPLC assay method for determination of simultaneous determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide solid dosage forms. Isocratic RP-HPLC method was developed on Phenomenex Gemini C18  $250 \times 4.6$ mm,  $5\mu$ m column using mobile phase as 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/min and the detection was carried out at 241 nm using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The validation element investigated showed that the method has acceptable specificity, accuracy, linearity, solution stability, precision and robustness.

Keywords: Amlodipine besylate, Olmesartan medoxomil, Hydrochlorothiazide Stability indicating assay, Method development, Method validation

## INTRODUCTION

Stress testing is a part of developmental strategy under International Conference on Harmonization (ICH) requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on a drug's inherent stability and assist in the validation of analytical methods to be used in stability studies (1-3). It is suggested that stress testing should include the effects of temperature, light, oxidizing agents and susceptibility across a wide range of pH values. It is also recommended that analysis of stability samples should be accomplished through the use of a validated stability-testing method.

Amlodipine besylate is chemically described as 3-Ethyl-5-methyl ( $\pm$ )-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. Its empirical formula is C<sub>20</sub>H<sub>25</sub>CIN<sub>2</sub>O<sub>5</sub>•C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>S, and its structural formula is shown in figure 1. Amlodipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. Similar to other DHP CCBs, amlodipine binds directly to inactive L-type calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. Amlodipine is a long-acting CCB that may be used to treat mild to moderate essential hypertension and exertion-related angina (chronic stable angina).



Figure-1 Structure of Amlodipine Besylate

Olmesartan medoxomil is chemically (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-( $\{4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl\}phenyl\}phenyl\}methyl)-1$ *H*-imidazole-5-carboxylate [figure 2]. Its molecular formula is C<sub>29</sub>H<sub>30</sub>N<sub>6</sub>O<sub>6</sub> having molecular mass 558.58 gm/mole. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT<sub>1</sub> receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis.



Figure-2 Structure of Olmesartan medoxomil

Hydrochlorothiazide is chemically6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4,benzothiadiazine-7-sulfonamide. Its molecular formula is  $C_7H_8N_3O_4S_2Cl$  having molecular mass 297.74 gm/mole. Hydrochlorothiazide belongs to the thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na+-Cl- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport.



Many methods, either individual or in a combination of Amlodipine, Olmesartan and Hydrochlorothiazide have been reported, including simultaneous determination of Amlodipine with Olmesartan, Olmesartan with Hydrochlorothiazide and Amlodipine with Olmesartan. Few methods are also reported for simultaneous determination of Amlodipine with Olmesartan and Hydrochlorothiazide in combination formulations by ultraviolet (UV) absorption and UV derivative spectrophotometry, spectrophotometric determination with artificial neural network, high performance liquid chromatography (10–12), ultra high performance liquid chromatography and thin-

layer chromatographic determination (13). Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method in such a way which includes shortest runtime with maximum selectivity. Hence, it can be maximum utilize for the analysis of formulation development and stability testing as well as at quality control laboratory for routine use.

This paper deals with forced degradation of Amlodipine, Olmesartan and Hydrochlorothiazide under acidic hydrolysis, alkali hydrolysis and oxidation, thermal and photolytic stress condition and the validation of developed method for assay of Amlodipine with Olmesartan and Hydrochlorothiazide from its dosage form (tablets).

#### MATERIALS AND METHODS

#### 2.1 Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

#### 2.2 Reagents and Reference substance

Olmesartan medoxomil and Amlodipine besylate standards were provided by Cadila pharmaceuticals Ltd., Ahmadabad (India) and Hydrochlorothiazide standard was provided by Alembic pharmaceuticals Ltd., Baroda (India). Olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide tablets containing 40mg Olmesartan medoxomil, 10mg amlodipine besylate and 25mg hydrochlorothiazide and the inactive ingredient used in drug matrix were obtained from market. HPLC grade acetonitrile was obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade ammonium acetate, hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

#### 2.3 Chromatographic conditions

Chromatographic analysis was performed on Phenomenex Gemini C18 (250mm  $\times$  4.6mm i.d., 5µm particle size) column applying an isocratic elution using a mixture of 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v) as a mobile phase. The mobile phase was filtered through 0.45µm membrane filter and degassed for 30 minute in an ultrasonic bath prior to its use. Flow rate of mobile phase was adjusted to 1.00 ml/min and injection volume was 20 µL. Detection was performed at 241 nm. Normal run time was chosen as 20 minutes but for degradation study chromatographic run was carried out up to 60 minutes to confirm that any degradation peak is eluted after 20 minutes or not.

#### 2.4. Standard preparation

Olmesartan medoxomil standard stock solution containing  $400\mu$ g/ml was prepared in a 100 ml volumetric flask by dissolving 40.00 mg of Olmesartan medoxomil and then diluted to volume with diluent. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of  $200\mu$ g/ml). Amlodipine besylate standard stock solution containing  $100\mu$ g/ml was prepared in a 100 ml volumetric flask by dissolving 10.00 mg of Amlodipine besylate and then diluted to volume with diluent. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of  $50\mu$ g/ml). Hydrochlorothiazide standard stock solution containing  $250\mu$ g/ml was prepared in a 100 ml volumetric flask by dissolving 25.00 mg of Hydrochlorothiazide and then diluted to volume with diluent.Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent.Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of  $50\mu$ g/ml).

#### 2.5. Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml of diluent was added and sonicated for a minimum 30 minute with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45 $\mu$ m nylon syringe filter. Further take 25 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent.The concentration obtained was 200  $\mu$ g/ml of Olmesartanmedoxomil, 50 $\mu$ g/ml of Amlodipine besylateand 125  $\mu$ g/ml of Hydrochlorothiazide.



#### 2.6 Degradation Study

2.6.1. Acidic degradation condition

Acidic degradation study was performed by taking the drug content in 0.1 N HCl at room temperature for 2.0 hours and mixture was neutralized.

### 2.6.2. Alkali degradation condition

Alkaline degradation study was performed by taking the drug content in 0.05 N NaOH at room temperature for 2.0 hours and mixture was neutralized.

## 2.6.3. Oxidative degradation condition

Oxidative degradation study was performed by taking the drug content in  $30\% \text{ v/v} \text{ H}_2\text{O}_2$  at room temperature for 2 hours.

2.6.4. Thermal degradation condition

Thermal degradation was performed by exposing solid drug at 80°C for 72 hours.

## 2.6.5. Photolytic degradation condition

Photolytic degradation study was performed by exposing the drug content in UV-light for 72 hours.

### 2.7. Method validation

2.7.1 Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

## 2.7.2 Linearity

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration. The peak areas versus concentration data were evaluated by linear regression analysis.

## 2.7.3 Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

## 2.7.4 Accuracy

An accuracy study was performed by adding known amounts of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide to the placebo preparation. The actual and measured concentrations were compared. Recovery

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of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in triplicate.

#### 2.7.5. Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate ( $\pm 0.1$  ml/min), mobile phase composition 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (62: 38 and 58: 42 v/v) and using different lot of LC column.

## 2.7.6. Solution stability

The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and  $2-5^{\circ}$ C and tested at interval of 12, 24, 36 and 48 hours. The responses for the aged solution were evaluated against a freshly prepared standard solution.





Figure-7 Chromatogram of Oxidative forced degradation study

	Amlodipine besylate (% Assay)		Olmesartanmedoxomil (% Assay)		Hydrochlorothiazide (% Assay)	
	Intraday $(n = 6)$	Interday $(n = 6)$	Intraday $(n = 6)$	Interday $(n = 6)$	Intraday $(n = 6)$	Interday $(n = 6)$
Set	Intraday $(n = 6)$	Interday $(n = 6)$	Intraday $(n = 6)$	Interday $(n = 6)$	Intraday $(n = 6)$	Interday $(n = 6)$
1	100.9	100.2	100.8	100.9	100.2	100.4
2	100	99.51	99.47	99.9	99.86	99.73
3	100	100.1	101.8	99.74	100.2	100
4	99.21	101.1	100.2	98.95	98.85	100.5
5	99.43	98.94	101.1	100.5	101	101.3
6	99.67	100.1	100	99.21	100.9	101.1
Mean	99.9	100	100.6	99.9	100.2	100.5
Standard deviation	0.596	0.734	0.832	0.736	0.773	0.625
% RSD	0.597	0.734	0.827	0.737	0.771	0.622

Table-2 Evaluation Data of Accuracy study

Drug	Level (%)	Amount added concentration <sup>a</sup> (mg/ml)	Amount found concentration <sup>a</sup> (mg/ml)	% Recovery	% RSD
Amlodipine	50	0.0248	0.0248	99.72	0.619
	100	0.0500	0.0498	99.67	0.414
	150	0.0750	0.0740	98.70	0.686
Olmesartan	50	0.1002	0.0995	99.35	0.426
	100	0.1997	0.1979	99.09	0.594
	150	0.3002	0.2986	99.47	0.832
Hydrochlorothiazide	50	0.0625	0.0620	99.23	0.990
	100	0.1255	0.1251	99.69	0.546
	150	0.1877	0.1870	99.67	0.564

<sup>a</sup> Each value corresponds to the mean of three determinations

Table-3 Evaluation Data of Solution Stability study

Intervals	%Assay for test solution stored at 2-8° C			% Assay for test solution stored at ambient temperature		
	Amlodipine	Olmesartan	HCTZ	Amlodipine	Olmesartan	HCTZ
Initial	100.96	99.98	100.09	100.99	99.94	100.13
12 h	100.92	99.76	99.99	100.90	99.67	99.67
24 h	100.82	99.67	99.66	100.58	99.32	99.62
36 h	100.53	99.56	99.62	100.07	99.06	99.31
48 h	100.16	99.37	99.42	99.64	98.78	99.08

### **RESULTS AND DISCUSSION**

To develop a rugged and suitable HPLC method for the quantitative determination of amlodipine, olmesartan and hydrochlorothiazide, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other

chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By keeping mobile phase composition as 0.02M ammonium acetate buffer pH 4.5 - Acetonitrile (60:40, v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, Acetonitrile was chosen to reduce run time and to attain good peak shape. Chromatogram of standard preparation is represented in (Fig. 2). A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less than 2.0 and % RSD of peak area was found less than 2.0. The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of all drug peaks during the force degradation study. There was no interference of any peak of degradation product with drug peak. Major degradation was found in oxidative condition in which drug products were degraded up to 24%. (Fig. 3). In oxidative degradation, it was found that around 15 % of the drugs contents were degraded (fig. 5) and in alkaline condition around 8 % of the drugs contents were degraded and impurity peak was found at 5.602 min (Fig. 5). Drug contents were found to be degraded around 2% in thermal degradation while slightly degradation was observed under the photolytic condition. For linearity seven points calibration curve were obtained in a concentration range from 50.0-200.0 µg/ml for Amlodipine, from 80-320 µg/ml for Olmesartan and from 50-200 µg/ml for Hydrochlorothiazide. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 2E+07x + 3802 with correlation coefficient 0.999 for Amlodipine, y = 7E+07x + 15600 with correlation coefficient 0.999 for Olmesartan and was y = 1E+08x + 53918 with correlation coefficient 0.999 for Hydrochlorothiazide. The result of repeatability and intermediate precision study is shown in Table 1. The % RSD values for intraday precision study and interday precision study was < 2.0 % for all drugs which confirm that the method is precise. The HPLC area responses for accuracy determination are depicted in Table 2. The results show that best recoveries (99.72-98.70% for Amlodipine, 99.09-99.47% for olmesartan and 99.23-99.67% for Hydrochlorothiazide) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Table 3 shows the results obtained in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hours at  $2 - 5^{\circ}$  C and ambient temperature as during this time the result was not decreased below the minimum percentage. The result of robustness study showed that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

#### CONCLUSION

A new analytical method has been developed to be routinely applied to simultaneous determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in pharmaceutical dosage form. In this study, stability of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

#### Acknowledgements

The authors are thankful for facilities & grants given under UGC- Special Assistance Programme Department Research Support (DRS) and Department of Science & Technology (DST) New Delhi, Fund For Improvement of Science & Technology (FIST), National Facility for Drug Discovery (NFDD) and Department of Chemistry, Saurashtra University, Rajkot – 360 005 (INDIA) for providing analytical facilities.

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