“ACID- ALKALI DEGRADATION STUDY ON OLMESARTAN MEDOXOMIL AND DEVELOPMENT OF VALIDATED STABILITY-INDICATING CHROMATOGRAPHIC METHODS”

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ABSTRACT

A reversed phase liquid chromatography (RP-HPLC) and thin layer chromatography (HPTLC) densitometry methods as a stability indicating assays of olmesartan medoxomil in presence of its acid or alkaline induced degradation products were developed. Olmesartan medoxomil and its degradation products were analyzed by HPLC equipped with UV-Variable wave length detector at 257 nm where quantitation was achieved by isocratic elution on Agilent, Exclipse XDB- C₁₈ column with mobile phase composed of acetonitrile: methanol: water: glacial acetic acid (40:35:25:0.1 v/v/v/v) at flow rate 1ml min.⁻¹. HPTLC was performed on aluminum packed Nano silica gel 60 F₂₅₄ TLC plates as stationary phase with significant difference in R_f values between olmesartan medoxomil and its degradates using chloroform: methanol: formic acid (8:1.5:0.5 v/v/v) as mobile phase. Densitometric evaluation of intact drug was carried out at 260 nm. The calibration curve of olmesartan medoxomil in bulk form was linear from 0.5- 10 µg ml⁻¹ and 0.05- 1 mg ml⁻¹ with mean percentage accuracy 99.97 ± 1.085 % and 100.35 ± 1.060 % for HPLC and HPTLC methods, respectively. The two proposed methods were successfully applied for the determination of olmesartan medoxomil in drug substance and in drug product. Methods validation was tested for linearity; accuracy; precision; selectivity and robustness, according to USP guidelines.

Keywords: Olmesartan Medoxomil; HPLC; HPTLC; Acid- Alkali Degradation Product; Stability-Indicating.

INTRODUCTION

Olmesartan Medoxomil is an inactive ester prodrug that is completely hydrolyzed during absorption from the GIT to the active form olmesartan which is an angiotensin II receptor antagonist with antihypertensive activity due to mainly selective blockade of AT₁ receptors and the consequent reduced pressor effect of angiotensin II, so it is used in the management of hypertension¹⁻². Olmesartan Medoxomil is non-official in any pharmacopeia up till now.

A limited number of publications were found in the literature concerning the determination of olmesartan medoxomil either in drug substance, drug product or biological fluids. Among those, HPLC procedures have been carried out for the determination of olmesartan in biological fluids using fluorescence detector³ and coupled to tandem mass spectrometry⁴⁻⁵. A RP-HPLC assay was reported for the separation of related substances in olmesartan medoxomil⁶. Celebier et al presented an UV-VIS spectrophotometry and Capillary Zone Electrophoresis methods for the determination of olmesartan medoxomil in tablets⁷⁻⁸. A validated absorption factor spectrophotometric and HPLC methods for analysis of olmesartan medoxomil in combined pharmaceutical formulations were published^{9,10}.

In the present study, a rapid, simple, accurate and reproducible validated chromatographic isocratic RP-HPLC and HPTLC analytical methods were developed for the quantitative estimation of olmesartan medoxomil in drug substance and in drug product without the interference of tablet excipients and its degradates. The proposed methods are suitable for the quality control analysis where the isocratic elution is simpler than gradient elution in routine analysis HPLC; HPTLC method was found to be easy, inexpensive, less time consuming and no need for complicated instruments. So, the two introduced methods are easily applicable in the national quality control laboratories in the developing countries.

EXPERIMENTAL

Chemicals and Reagents

- All the solvents used were of HPLC grade while the other chemicals were of analytical grade.

- Olmesartan Medoxomil was kindly supplied by BIG Pharma (Sabaa Co., Cairo, Egypt) and its purity found to be 99.53± 0.54% according to drug substance in-house specifications¹¹ cited a HPLC separation method using reversed phase column C₁₈, mobile phase acetonitrile: 0.05 M potassium dihydrogen phosphate (50:50 v/v) at flow rate 1.5 ml min⁻¹ and monitoring at the UV range 254 nm.

- Olmetec film coated tablets (10 mg per tablet) were purchased from the local market in Egypt (manufactured by Sankyo Pharma, Germany).

Apparatus and Chromatographic Conditions:

For HPLC Method:

An Agilent technologies 1200 series consisted of isocratic pump

(quaternary pump) Model G1310A/ G1311A liquid chromatographic system with 20 µl loop manual injector was used. The analytes were separated on Agilent, Eclipse XDB-C₁₈ column (150 mm X 4.6 i.d., 5 µm particle diameters, made in USA). The mobile phase was programmed to deliver 100% of acetonitrile: methanol: water: glacial acetic acid (40: 35:25: 0.1 v/v/v/v) previously mixed together, filtered through 0.45 µm nylon membrane filter and degassed through Agilent 1200 series vacuum degasser with flow rate 1 ml min.⁻¹ with isocratic elution and the UV- Variable wave length detector Model G1314B/ G13145 (SL) was set at 257 nm. The column was conditioned for ≥ 30 min. All the determinations were performed at ambient temperature 25± 5 °C using thermo stated column compartment Model G1316A/ G1316B and the injection volume was 20 µl.

For HPTLC Method:

High Performance Thin layer chromatographic aluminum plates, precoated with 0.20 mm Nano silica gel 60, 10 X 20 cm cut from 20 X 20 cm plates , with fluorescent indicator at 254 nm (Macherey- NaGel (MN), Germany) were developed in chloroform: methanol: formic acid (8:1.5:0.5 v/v/v) as the mobile phase. For detection and quantification, 10 µl each of each sample solution and standard solutions of different concentrations within the linearity range were applied as separate compact spots 2 cm apart and 1 cm from the top and 2 cm from the bottom of the HPTLC plates using 25 µl Hamilton syringe. The chromatographic glass tank (20 X 20 X 10 cm) was saturated with the mobile phase for one hour prior to development of the plates. The plates were developed up to 7 cm in the usual ascending way, air dried. The spots were detected under a UV- short wave length lamp at 254 nm and scanned at 260 nm with densitometric dual wave length SHIMADZU flying spot Cs-9301 PC (Japan) with spectrodensitometer parameters; photo mode: reflection; scan mode: Zigzag and swing width: 10 mm.

- Unicam UV 300, thermospectronic, vision 32 software equipped with IBM PC computer used for all the absorbance measurements and treatment of data, HP laser jet 1000 series printer and 1- cm path length quartz cuvettes.

- Infra Red Spectroscopy; Jasco FT/ IR 460 plus, Japan.

Stock Solutions:

- Olmesartan Medoxomil stock solution (0.5 mg ml⁻¹) and working solution (0.01 mg ml⁻¹) were prepared in the mobile phase for HPLC.

- Olmesartan Medoxomil stock solution (1 mg ml⁻¹) in methanol for TLC.

- Degradation Product stock solution (1 mg ml⁻¹) and working solutions (20 & 0.2 µg ml⁻¹) in mobile phase for HPLC were prepared.

- Degradation Product stock solution (1 mg ml⁻¹) and working solutions (250, 5 µg ml⁻¹) in methanol for TLC were prepared.

-Preparation of Degradation Product:

One hundred (100) milligrams of Olmesartan Medoxomil was dissolved in 100 mL of 1M hydrochloric acid and refluxed for one (1) hour or in 0.1M NaOH for one (1) hour at room temperature, then stored in refrigerator. Complete degradation of olmesartan Medoxomil was ascertained by neutralizing acid degradation products with 1M NaOH and alkaline degradation product with

0.1M HCL and applying the TLC technique using chloroform: methanol: formic acid (8:1.5: 0.5 v/v/v) as a developing solvent. Prior to each procedure, aliquots of degradation product solution were neutralized with 1M NaOH, filtered and diluted up to the required volume with mobile phase for HPLC and methanol for TLC.

RECOMMENDED PROCEDURES

HPLC Method

Aliquots of working solution ($0.01 \mu\text{g ml}^{-1}$) equivalent to 2.5-50 μg olmesartan medoxomil were transferred into a series of 5-ml volumetric flasks and diluted up to the mark with mobile phase acetonitrile: methanol: water: glacial acetic acid (40:35:25:0.1 v/v/v/v). Triplicate 20 μl injections of each solution were made; the procedure was performed as mentioned under HPLC chromatographic conditions. The peak area values were plotted against the corresponding concentrations and the regression equation was computed.

HPTLC Method

Aliquots of stock solution (1 mg ml^{-1}) equivalent to 0.25- 5 mg olmesartan medoxomil were transferred into a series of 5- ml volumetric flasks and diluted up to the mark with methanol. A ten (10) μl of each solution was spotted on HPTLC plates. The procedure was carried out as mentioned under HPTLC chromatographic conditions. The peak area values were plotted against the corresponding concentrations and the regression equation was computed.

Drug Product Preparation

Ten tablets were weighed, decoated and finely powdered. An amount of powder equivalent to 25 mg of olmesartan medoxomil for HPLC and 50 mg for HPTLC was extracted in 40 ml mobile phase for HPLC and 40 ml methanol for TLC, sonicated for 30 min, filtered, transferred quantitatively into 2 separate 50- ml volumetric flasks and completed with appropriate solvents to the mark. The assay was completed as described under recommended procedures. The concentration was calculated using the corresponding regression equation.

RESULTS and DISCUSSION

Elucidation of the degradation products

Stability study was carried out on olmesartan medoxomil (Fig.1), in acidic (1M HCl) and alkaline (0.1M NaOH) media. Olmesartan Medoxomil degradation products were obtained by reflux in 1 M HCl for one hour while degradation in 0.1M NaOH occurred when solution was left on the bench for one hour. The prepared acidic and alkaline degradation products were elucidated by HPLC, TLC, UV and IR. These investigations proved that acidic and alkaline degradates are nearly similar molecules where both degradates have the same RT in HPLC (Fig. 2), R_f on the TLC plates (Fig.3), UV spectra (Fig.4) and IR spectra where the IR spectra of acid and alkali degradates shows the appearance of the characteristic N-H bending at 1595.81 cm^{-1} and O-H stretching at 3435.56 cm^{-1} and the disappearance of the two carbonyl groups present at 1706.69 cm^{-1} and 1831.08 cm^{-1} in the IR spectrum of the intact drug (Fig.5). On the TLC plates, it was observed that acid degradate was separated into two spots near each other (Fig.3) having the same UV and IR spectra and were suggested to be enantiomeric degradates.

Fig.1. Structure of Olmesartan Medoxomil

Fig.2. HPLC chromatogram of olmesartan medoxomil ($10 \mu\text{g ml}^{-1}$) ($RT=3.267 \text{ min.}$) and its acid & alkali degradates ($4 \mu\text{g ml}^{-1}$) ($RT= 1.645, 1.641$ and 2.57 nm.)

Fig.3. TLC Figure of:

- M: Mixture of Olmesartan Medoxomil (I, 1 mg ml^{-1}), acid and alkali degradates (D_1 & D_2 , 0.2 mg ml^{-1}).
- I: Olmesartan Medoxomil intact (1 mg ml^{-1}).
- D_1 : Alkali Degradation product (0.2 mg ml^{-1}).
- D_2 : Acid Degradation product (0.2 mg ml^{-1}).

Fig.4. The absorption spectra of:

- (1) Olmesartan Medoxomil intact ($10 \mu\text{g ml}^{-1}$).
- (2) Alkali & (3) Acid degradates ($10 \mu\text{g ml}^{-1}$) in methanol.

Fig.5. The IR Spectrum of (A) Olmesartan Medoxomil Intact
(B) Acid Degradation Product
(C) Alkaline Degradation Product

Method Development

HPLC Method:

In HPLC, the elution order was the degradates at $RT = 1.645$ and 1.641 min. and olmesartan medoxomil at $RT = 3.267$ min. at 257 nm with flow rate 1 ml min.⁻¹ using acetonitrile: methanol: water: glacial acetic acid (40:35:25:0.1 v/v/v/v) as mobile phase (Fig. 2). Linearity was obtained for the concentrations range 0.5 - 10 $\mu\text{g ml}^{-1}$ with mean percentage accuracy 99.97 ± 1.085 %; the parameters of regression equation are shown in (Table 1). The system suitability tests of HPLC method were evaluated (Table 4).

HPTLC Method:

In HPTLC, complete separation was obtained using chloroform: methanol: formic acid (8:1.5:0.5 v/v/v) as a developing mobile phase. The intact and degradation product were satisfactorily resolved with R_f values $\pm$ S.D were 0.70 ± 0.01 , 0.55 ± 0.01 and 0.53 ± 0.30 , respectively (Fig.3). The densitometric evaluation of separated zones was performed at 260 nm (Fig.6). Linearity was obtained in the concentration range 0.05 - 1 mg ml.⁻¹ with mean percentage accuracy 100.35 ± 1.060 %, the parameters of regression equation is shown in (Table 1).

Fig.6. TLC scanning profile of Olmesartan Medoxomil (0.05 - 1 mg ml.⁻¹) at 260 nm

Method Validation

Linearity:

The calibration curves of the two proposed methods were constructed with different concentrations ranging from 0.5 - 10 $\mu\text{g ml}^{-1}$ for HPLC and 0.05 - 1 mg ml.⁻¹ for HPTLC. The linearity was evaluated by linear regression analysis to calculate the slope, intercept and regression coefficient (r^2) (Table1).

Table1. Analytical and Validation parameters for the determination of Olmesartan Medoxomil by HPLC and HPTLC Methods.

Parameters	HPLC Method	TLC Method
Wavelength (nm)	257	260
specificity (mean $\pm$ R.S.D %) ^a	100.08 ± 1.383	100.13 ± 1.389
Linearity range	$0.5 - 10$ $\mu\text{g ml}^{-1}$	$0.05 - 1$ mg ml. ⁻¹
Regression Equation ⁶		
Slope (b)	43.619	804.36
Intercept (a)	6.7437	1619.2
Regression coefficient (r^2)	0.9997	0.9997
Precision ($\pm$ R.S.D %)		
Intraday ^c	± 1.170	± 1.181
Interday ^c	± 1.482	± 1.350
Accuracy (mean $\pm$ R.S.D %) ^d		
Drug substance	99.97 ± 1.085	100.35 ± 1.060
Drug product	102.90 ± 1.818	103.14 ± 1.742
Standard added	100.74 ± 1.469	99.84 ± 1.737

^a Average of $n=6$ for HPLC method.

^b $y = a \pm bc$ where c is the concentration of drug $\mu\text{g}/20\mu\text{l}$ for HPLC, $\mu\text{g}/\text{spot}$ for TLC methods, y the peak area for HPLC and TLC at the specified wave length.

^c Average of $n = 9$

^d Average of $n = 5$

Accuracy:

The accuracy of the two proposed methods in drug substance was determined by the analysis of different concentrations of intact solution, each in triplicate within one day and the results were compared with those of the reported method¹¹ where no significant difference was observed between the calculated student's t test and variance ratio F test values and the theoretical ones (Table 2). The recovery method was studied by analyzing samples of tablets formulation without the interference of tablet excipients; also the

standard addition technique was applied by adding a known amount of standard drug to the drug product and the results were expressed as mean $\pm$ R.S.D %, (Table 1).

Table2. Statistical comparison of results for the determination of Olmesartan Medoxomil in drug substance by the proposed Methods and the reported Method.

Values	HPLC Method	TLC Method	Reported ^c Method ¹¹
Mean ^a $\pm$ R.S.D %	99.97 $\pm$ 1.085	100.35 $\pm$ 1.060	99.53 $\pm$ 0.54
S.D	1.084	1.063	0.54
S.E	0.485	0.476	0.24
Variance	1.176	1.131	0.29
<i>t</i> (2.31) ^b	0.808	1.524	
<i>F</i> (6.39) ^b	3.995	3.841	

^a Average of n=5 for HPLC, TLC and reported method.

^b Theoretical values of *t* and *F* at 95% confidence level.

^c HPLC procedure using C_{18} column, Acetonitrile: 0.05 Potassium Dihydrogen Phosphate (50:50 V/V) as mobile phase and UV detector at 254 nm.

Precision:

The intra-day and inter-day variations were expressed by $\pm$ R.S.D %, (Table 1), were determined by using triplicate analysis of three different concentrations (2, 6, 10 μ g ml⁻¹ for HPLC); (0.2, 0.4, 0.6 mg ml⁻¹ for HPTLC) over a period of three days (Table 1).

Specificity:

The specificity of the two proposed methods was determined by preparing laboratory mixtures of intact and its degradation product containing 0.1- 100 % w/w of the degradation product (Table 3). The assay results are unaffected by the presence of extraneous materials (degradates, excipients such as micro crystalline cellulose, lactose monohydrate, hydroxyl propyl cellulose, low substituted hydroxyl propyl cellulose, magnesium stearate, titanium dioxide (E171), talc, hypromellose) ¹². The good results of laboratory- prepared mixtures prove the specificity of proposed methods (Table 3).

Table3. Determination of Olmesartan Medoxomil by the proposed methods in presence of its degradation products

Sample Number	% of degradation ^a products	HPLC Method ^b found %	HPTLC Method ^b found %
1	0.1	101.59	101.68
2	0.5	100.45	100.75
3	5	98.65	98.36
4	10	101.23	98.66
5	50	100.37	101.37
6	100	98.15	99.98
Mean ^c $\pm$ R.S.D%		100.08 $\pm$ 1.383	100.13 $\pm$ 1.389

^a Calculated with respect to intact weight (degradation products -drug mixture wt/wt%).

^b Found (%) of intact sample.

^c Average of n=6 for HPLC and TLC method.

System Suitability of HPLC Method:

The % R.S.D of injection repeatability and retention time for drug substance are within 2% indicating the suitability of the system (Table 4). The efficiency of the column as expressed by the number of theoretical plates was 6955 and the tailing factor was 0.6 (Table 4).

Table 4. Results of System Suitability Tests of HPLC Method

Parameters	Values	Comments
Retention Time	3.261 $\pm$ 0.040	$\pm$ Standard deviation
Injection Repeatability	1.212	RSD for five injections
K	2.16	Capacity factor
Selectivity Factor (α)	3.66	Separation factor calculated as K_2/K_1
Tailing Factor	0.63	calculated at 5% of peak height
Theoretical Plates (N)	6955	Column efficiency theoretical Plate/ column
HETP	0.02	Height equivalent theoretical plate
Resolution	3.244	Calculated by $2(t_2-t_1)/w_2+w_1$

Robustness:

Two sets of experiments were performed on Agilent 1200 series HPLC and HPTLC densitometry by two different analysts in different days with respect to the peak area as a response factor calculated and expressed as $\pm$ R.S.D% and no significant difference was obtained between the results in this study. Also, small changes in the composition of the mobile phase of both methods did not affect on the response factor.

CONCLUSION

This paper described RP-HPLC and HPTLC methods which can be used as stability indicating assay for the determination of olmesartan medoxomil in presence of its degradates and in drug product without interference from tablet excipients. The advantages of the suggested methods were the ease of performance, the reproducibility and the lack of complicated pretreatments before analysis. In addition these methods have a potential application in quality control laboratories.

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