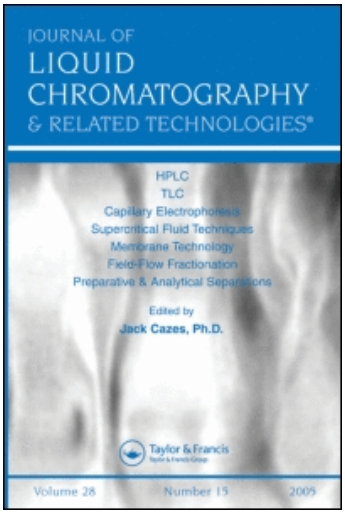

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Stability-Indicating Assay Method for Estimation of Olmesartan Medoxomil and its Metabolite

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Abstract: A novel stability indicating high performance liquid chromatographic assay method was developed and validated for Olmesartan medoxomil and its degradant product. An isocratic HPLC method was developed to separate the drug from the degradation products, using an Inertsil-ODS-3 (C-18) Column (5 µm, 250 mm × 4.60 mm). A mixture of phosphate buffer (pH 4.0) and methanol (30:70) was used as mobile phase. The flow rate was 1.0 mL/min and the detection was carried out at 230 nm. The validation studies were carried out fulfilling the International Conference on Harmonisation (ICH) requirements. The procedure was found to be specific, linear, precise (including intra and inter day precision), accurate, and robust.

Keywords: HPLC, LC-MS/MS, Olmesartan medoxomil, Stability indicating assay method

INTRODUCTION

Olmesartan medoxomil is a prodrug, hydrolyzed to Olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT1 subtype angiotensin II receptor anatagonist. It blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in the vascular smooth muscle.

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Chemically, it is 2, 3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate.[1–3]

The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2)[4] requires that analysis of stability samples should be done through the use of validated stability indicating analytical methods. It also recommends carrying out stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, and oxidizing agents, as well as susceptibility over a wide range of pH values. The objective of the present study was to study degradation of Olmesartan medoxomil under different ICH recommended stress conditions, and to establish a validated stability indicating HPLC method. There is no report yet on the development of a stability indicating assay method for the drug.[5–18]

EXPERIMENTAL

Materials

Olmesartan medoxomil was received from Glenmark Pharmaceuticals Ltd. (Mumbai, India). Sodium hydroxide and hydrogen peroxide were purchased from S.D. Fine-Chem Ltd. Hydrochloric acid and methanol was procured from Merck India Ltd. (Mumbai). All other chemicals were of analytical grade.

Instrumentation

pH of the mobile phase was checked on microprocessor waterproof pH tester (pH testr 20, Eutech Instruments, Oakton, USA). The overall illumination at the point of placement of samples was 6000 lux, which was tested using a calibrated lux meter (Lutron LX-102 digital light meter, Marcucci S.P.A, Vignate, Milan). Thermal stability study was performed in a hot air oven (universal oven with thermotech thermostat TIC-4000 N, S.M. Industries, New Delhi, India). An HPLC system, equipped with a LC- 10ATVP pump and SPD-10ATvp data were acquired and processed using CLASS-VP software (all from Shimadzu, Kyoto, Japan).

Confirmation of the degradation products identities was obtained by using a LC–MS/MS system 410 Prostar Binary LC, with autosampler coupled with ESI–MS Varian 500 IT (Varian, California, USA). The acquisition was performed, in both positive and negative ion mode, recording between 50 and 700 range.

Chromatographic Conditions

HPLC measurements were carried out using a reverse phase Inertsil-ODS-3 (C-18) Column (5 μm, 250 mm × 4.60 mm) operated at ambient temperature isocratically at 1.0 $mLmin^{-1}$ with a mobile phase of phosphate buffer (pH 4.0) and methanol (30:70, v/v). Detection was carried out at 256 nm; injection volume 20 μL. Figure 1 shows the chromatogram of pure olmesartan medoxomil.

Chromatographic separation for LC–MS measurements were performed using a reversed hase Polaris C-18-A 250 mm × 2.0 mm, 5 μm particle size column. Flow rate was 1 $mLmin^{-1}$ with a mobile phase trifluroaceticacid methanol (30:70).

Generation of Stress Samples

The reactions were carried out at a drug concentration of 1 $mgmL^{-1}$. The stress conditions were as follows:

Hydrolytic Conditions

On heating the drug in 0.01 N HCl for 12 h, around 20% degradation was seen with a corresponding rise in degradation product peak, as shown in Figure 2. Olmesartan medoxomil was found to be very labile in alkali. Fifty percent degradation of the drug was observed in 0.01 M NaOH at

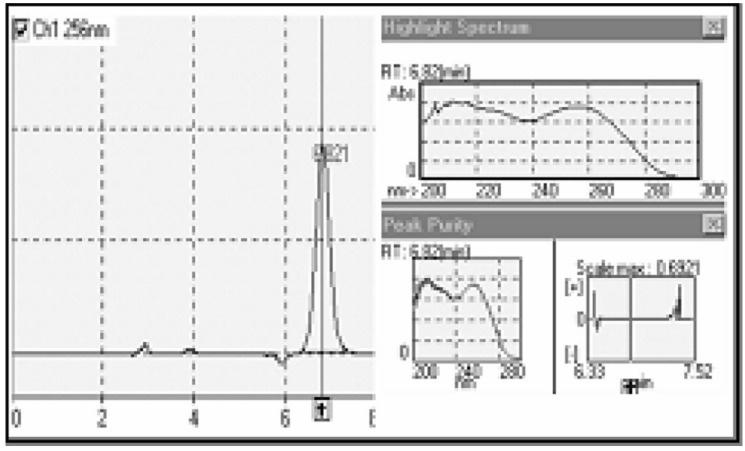

Figure 1. Chromatogram of pure Olmesartan medoxomil (t_R6.8).

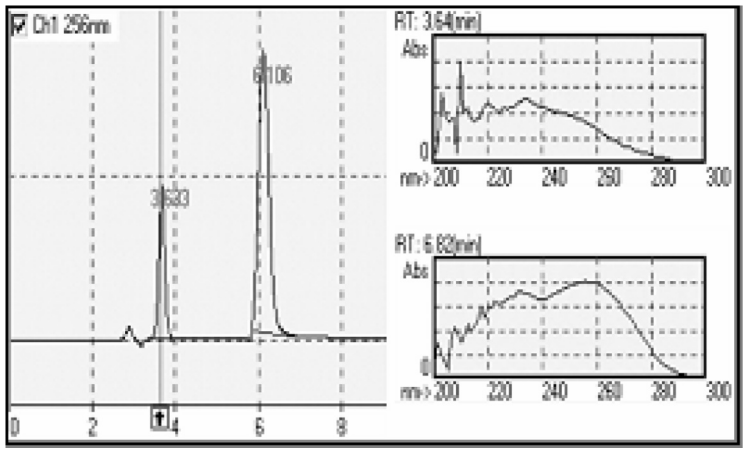

Figure 2. Chromatogram of Olmesartan medoxomil (t_R6.1) and degradation product (t_R3.6) in 0.01 N HCl.

room temperature within 5–10 min (Figure 3). An LC–MS investigation indicates formation of only one major degradation product apart from the drug. No other additional peaks showed up in the mass chromatogram. The study of mass values indicates one degradation product to have molecular weights of m/z 429. The peak with m/z 429 was characterized to be due to absence of cyclic 2, 3-carbonate.

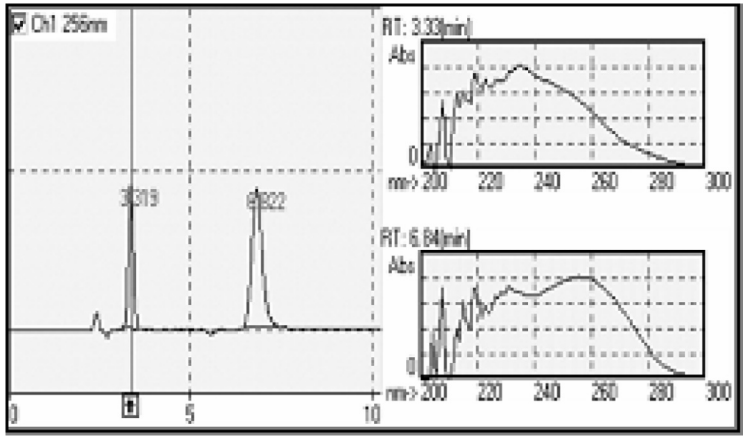

Figure 3. Chromatogram of Olmesartan medoxomil (t_R6.8) and degradation product (t_R3.3) in 0.01 N NaOH.

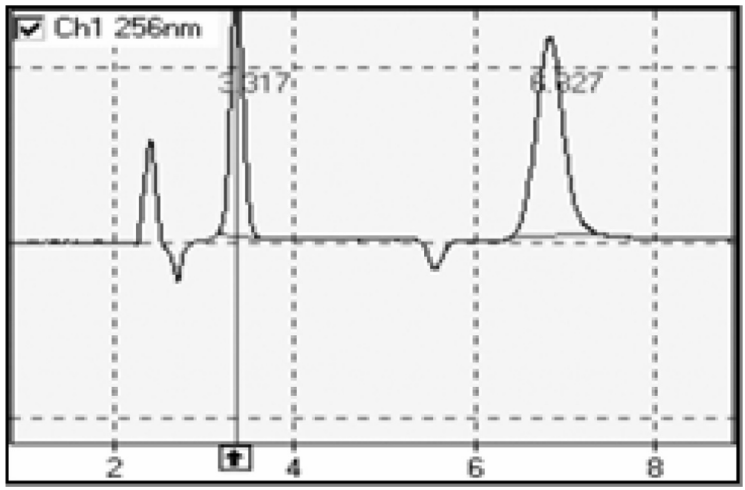

Figure 4. Chromatogram of Olmesartan Medoxomil (t_R6.8) and degradation product (t_R3.3) in 6% H_2O_2.

Oxidative Condition

Oxidative decomposition was carried out using 3% and 6% H_2O_2 resulted in one degradation product at RT 3.3 as shown in Figure 4.

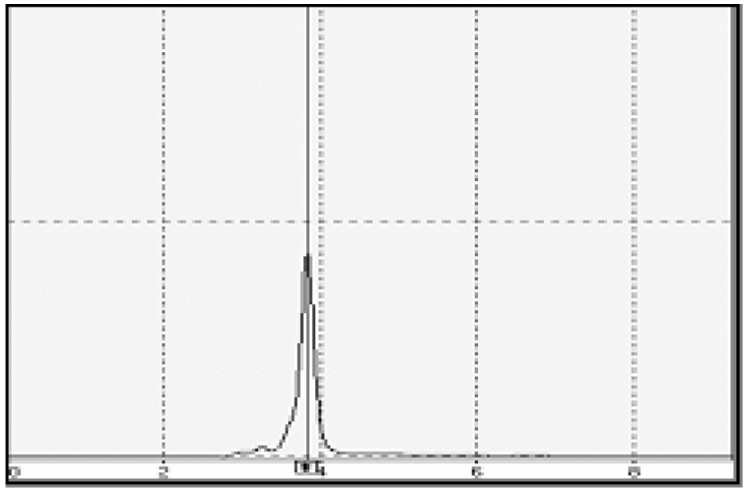

Figure 5. Chromatogram of Olmesartan medoxomil (t_R6.8) after two days exposure to direct sunlight.

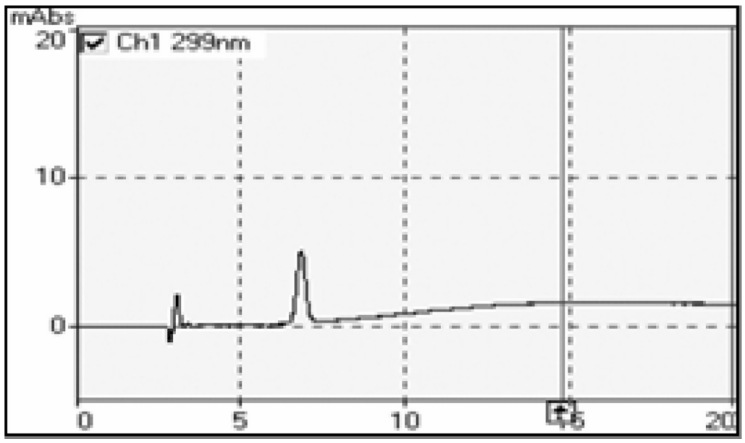

Figure 6. Chromatogram of Olmesartan medoxomil (t_R6.8) along with degradation product (t_R15.8) formed under thermal condition.

Photolytic Condition

Drug remains stable after exposure to direct sunlight for 2 days ~70,000–80,000 lux sun exposure (Figure 5).

Thermal Stress

As shown in Figure 6, a negligible degradation was seen on subjecting the drug to dry heat at 50°C for 3 months.

Separation Studies

The initial analyses of different stressed samples were performed on an HPLC system using a C-18 column and a mobile phase composed of acetate buffer (pH 3.7) and methanol (30:70). It was filtered through 0.45 μm nylon membrane filters and sonicated before use. The injection volume was 20 μL, and the mobile phase flow rate was 1 mL min^{-1}. The detections were carried out at 256 nm.

RESULTS AND DISCUSSION

Optimization Studies

The method was optimized to separate major degradation products formed under various conditions. Resolution was also checked on

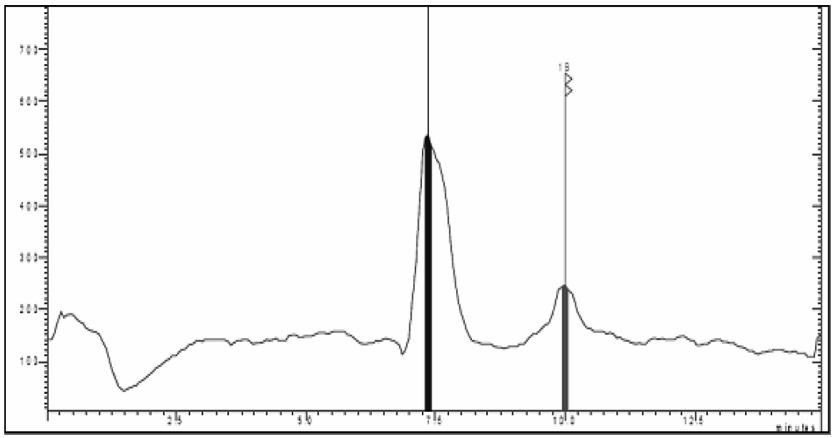

Figure 7. LC-MS/MS chromatogram of Olmesartan medoxomil (t_R 7.6) and degradation product of thermal condition (t_R 10.0).

mixtures of the degradation solutions to confirm the separation behavior. During thermal degradation studies non-chromophoric degradation products were formed, which were not detected by a normal UV detector. The further study of degradation products was carried out using LC-MS/MS, the resulting chromatogram was shown in Figure 7.

Validation of the Developed Methods

Linearity

Table 1 lists the mean HPLC area response for Olmesartan medoxomil. The calibration curves (n = 3) constructed for Olmesartan medoxomil were linear over the concentration range of 0.5–200 μg/mL. Peak areas of olmesartan medoxomil were plotted verses concentration and regression analysis performed on the resultant curve. Typically, the regression equation for the calibration curve was:

$$y = 48804x + 6039.4, (r^2 = 0.9996)$$

Precision

The injection (system) precision was determined by performing six replicate injections of the standard solution (30 μg/mL. The intra-day and inter-day precisions were determined by performing five replicate assays of independently prepared samples of olmesartan medoxomil. The

Table 1. Linearity and calibration data of Olmesartan medoxomil (^{c}n = 3)

Concentration (μgmL^{-1})	Mean peak area ± ^{a}S.D., ^{b}R.S.D. (%)
0.5	43109 ± 800.79, 0.0186
01	130025 ± 4462.28, 0.0334
05	212085 ± 2817.0, 0.0133
10	389222 ± 872.64, 0.0022
20	947505 ± 9231.0, 0.0098
30	1519340 ± 12325.5, 0.0081
40	2025832 ± 17240.7, 0.0085
50	2413302 ± 11657.6, 0.0048
100	4833342 ± 137102.1, 0.0284
200	9648733 ± 218759.0, 0.0227

^{a}S.D. = Standard deviation.
^{b}R.S.D. = Relative standard deviation.
^{c}n = Number of determination.

Table 2. Recovery study (^{c}n = 3)

Spiked concentration (μgmL^{-1})	Measured concentration ± ^{a}S.D., ^{b}R.S.D. (%)	Recovery (%)
05	5.0133 ± 0.090185, 1.798	100.27
10	10.0566 ± 0.15695, 1.560	100.57
15	14.87 ± 0.72111, 4.84	099.13
20	19.7466 ± 0.351188, 1.778	098.73
25	24.6066 ± 0.536315, 2.179	098.43

^{a}S.D. = Standard deviation.
^{b}R.S.D. = Relative standard deviation.
^{c}n = Number of determination.

Table 3. Intra day precision studies (^{c}n = 3)

Spiked concentration (μgmL^{-1})	Measured concentration ± ^{a}S.D., ^{b}R.S.D. (%)
10	9.85 ± 0.0153, 0.157
20	19.68 ± 0.2183, 1.103
30	31.0 ± 0.2570, 0.821
40	41.38 ± 0.3503, 0.848
50	49.32 ± 0.2425, 0.495

^{a}S.D. = Standard deviation.
^{b}R.S.D. = Relative standard deviation.
^{c}n = Number of determination.

Table 4. Inter day precision studies ($n^c = 3$)

Spiked concentration (μgmL^{-1})	Measured concentration $\pm$ [a]S.D., [b]R.S.D. (%)
10	9.91 ± 0.1044, 1.051
20	19.76 ± 0.360, 1.82
30	31.21 ± 0.4877, 1.564
40	41.31 ± 0.326, 1.18
50	50.17 ± 1.235, 2.468

[a]S.D. = Standard deviation.
[b]R.S.D. = Relative standard deviation.

R.S.D. values were 2.13 and 2.38%, respectively. Tables 2 and 3 give the intra-day and inter-day precision values.

Accuracy

The accuracy was evaluated by the recovery studies, which were carried out by spiking five known amounts of olmesartan medoxomil standards in preanalyzed mixtures of degradation solutions. The average recovery at each level was within $100 \pm 2.25\%$ and the R.S.D. values at each level were 0.95%, as given in Table 4.

Specificity

The purity curve of the drug was ≥ 0.999 pure when checked with the upslope and down slope method. The resolution factor for the drug peaks in the mixture of degradation solution was ~ 2 from the nearest resolving peak.

CONCLUSION

In this study, olmesartan medoxomil was subjected to stress studies under various ICH recommended conditions. The drug was found to degrade in acidic, alkaline, oxidative, and thermal conditions. The drug undergoes extensive degradation under acidic and alkaline stress, degrades to a mild extent in oxidative and thermal conditions, and is stable to photolytic stress. The drug can be analyzed specifically in the presence of different chromophoric degradation products by using isocratic conditions and a simple mobile phase containing phosphate buffer (pH 4.0) and methanol in the ratio of 30:70.

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