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VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METOPROLOL SUCCINATE AND OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM

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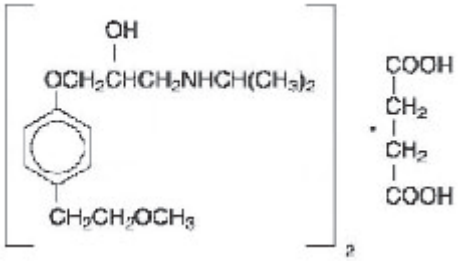
ABSTRACT

The present paper describes simple, rapid, reproducible, accurate and precise stability indicating HPLC method developed for quantitative simultaneous estimation of metoprolol succinate and olmesartan medoxomil in bulk and combined pharmaceutical dosage form. A chromatographic separation of both drugs was achieved with Chromasil 250 x 4.6 mm, i.d 5 ìm C-18 column using methanol:0.05% v/v O-phosphoric acid in water (50:50 v/v) at the flow rate of 1ml/min. The measurements were made at 228.0 nm as detector wavelength. The described method showed excellent linearity over a range of 5-80 ìg/ml for metoprolol succinate and 5-70 ìg/ml for olmesartan medoxomil. The coefficient of correlation for metoprolol succinate and olmesartan medoxomil was found to be 0.9990 and 0.9993 respectively. The retention time for metoprolol succinate and olmesartan medoxomil was found to be 3.485 min and 7.085 min, respectively. The tailing factor for metoprolol succinate and olmesartan medoxomil was found to be 1.02 and 1.13 respectively. Both drugs and their combination drug product were found to be stable in neutral, thermal, oxidative and photolytic stress conditions but mild degradation was observed in acidic and alkaline conditions.

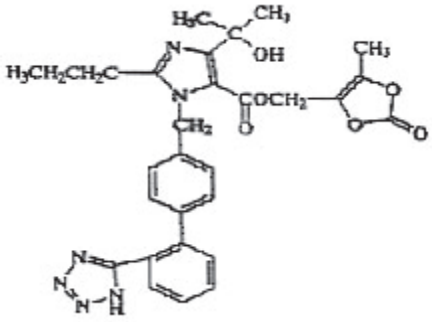
Keywords: *Metoprolol succinate; Olmesartan medoxomil; HPLC method validation; Stability-indicating method; ICH guidelines.*

INTRODUCTION

Chemically, Metoprolol succinate (MET) (Fig.1) is (±)-1-(Isopropylamino-3-[4-(2-methoxyethyl)phenoxy] propan-2-ol.[1] It is used as an antianginal and antihypertensive.[1] The official methods of assay like Potentiometric[2] and HPLC[3] are reported for MET. Chemically, Olmesartan medoxomil (OLM) (Fig. 1) is 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[22 -(1H-tetrazol-5-yl)[1,12 -biphenyl]-4-yl]methyl]-1H-imidazole-5-carboxylic acid (5-methyl -2-oxo-1,-3-dioxol-4-yl)methyl ester[4]. It is selective angiotensin II receptor antagonist and used as an antihypertensive.[1] OLM lowers blood pressure and increases the supply of blood and oxygen to the heart.[1] OLM and MET is a combination of medicines used to treat high blood pressure (hypertension). Literature survey revealed that various methods like UV spectrophotometric[5], HPLC[6-9] and GC-MS[10] for the estimation of metoprolol succinate as single and HPLC in combination with other anti-hypertensive agents[11-13] are reported. The methods such as UV spectrophotometric[14,15], estimation of OLM in plasma, urine and tablet by HPLC [15-19] and LC-MS-MS [20] detection for olmesartan medoxomil as single and HPLC method in combination with other drugs are



Metoprolol succinate (MET)



Olmesartan Medoxomil (OLM)

Fig. 1: *Chemical structure of MET and OLM*

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reported.[21,22] The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommendation of storage conditions, retest periods and shelf-life to be established. There is no report yet of stability indicating RP-HPLC method for these drugs in combination. The aim of the present study accordingly was to establish inherent stability of metoprolol succinate and olmesartan medoxomil in combined tablet dosage form through stress studies under ICH[23] recommended test conditions and to develop and validate stability indicating HPLC method.

EXPERIMENTAL

Chemicals and reagents

Reference standards of metoprolol succinate and olmesartan medoxomil were procured from Lupin Research Park, Pune and Emcure Pharmaceuticals Ltd., Pune respectively. Methanol (HPLC grade) was obtained from Qualigen Laboratories Pvt. Ltd., Mumbai. Analytical grade of hydrochloric acid (HCl), sodium hydroxide (NaOH), o-phosphoric acid and hydrogen peroxide were obtained from Merck Ltd., Mumbai. The tablets containing metoproplol succinate (25 mg) and olmesartan medoxomil (20 mg) were procured from local market.

Instrument

The chromatographic system used was an Agilent 1120 series, which comprised a degasser, gradient pump and photodiode array detector. The system was controlled through Ezchrome software using Chromasil C18 (4.6 x 250mm, 5 ìm) column maintained at 25°C temperature.

Chromatographic conditions

The separation was achieved using a mobile phase consisting methanol:0.05% v/v o-phosphoric acid in water (50:50 v/v) at a flow rate of 1.0 ml/min and the eluent was monitored using PDA detector at 228.0 nm. The mobile phase was kept in ultrasonicator for 30 min and filtered through a 0.45-ìm nylon membrane filter. The column was maintained at 25°C temperature and injection volume of 20 ìl was used. The peak homogeneity was expressed in terms of peak purity and was obtained directly from software.

Standard stock solutions

The stock solution (100 ìg/ml) of MET and OLM were prepared separately by dissolving accurately 10 mg of each drug in 100 ml methanol HPLC grade in 100 ml volumetric flask.

Calibration curve

Appropriate aliquots of standard stock solutions of MET and OLM were diluted with mobile phase to obtain concentrations in the range of 5, 10, 20, 30, 40, 50, 60, 70 and 80 ìg/ml of MET and 5, 10, 20, 30, 40, 50, 60 and 70 ìg/ml of OLM respectively. The linearity of MET (Fig. 2) and OLM (Fig. 3) was found to be in the concentration ranges of 5-80 ìg/ml and 5-70 ìg/ml, respectively, at their respective maxima. The coefficients of correlation were found to be 0.9990 for MET and 0.9993 for OLM. The mixed standard solution containing 50 ìg/ml of MET and 40 ìg/ml of OLM was prepared from each standard stock solution and injected into HPLC system.

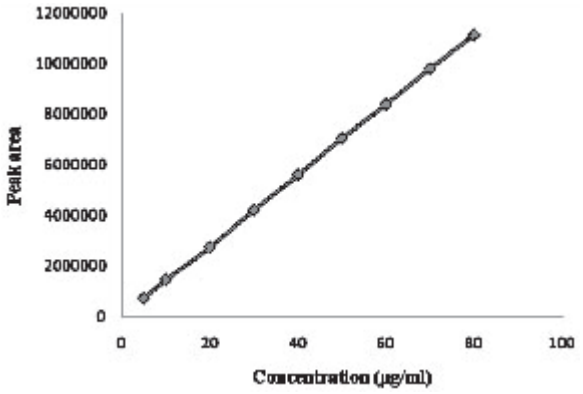

Fig. 2: *Calibration curve of Metoprolol succinate*

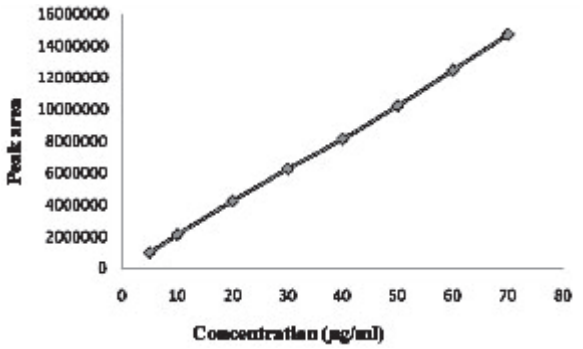

Fig. 3: *Calibration curve of Olmesartan medoxomil*

RESULTS

Optimization of the chromatographic conditions:

To develop a stability-indicating method, different stationary phases like C18, CN, different mobile phases containing buffers like phosphate, ammonium acetate, with different pH (3-7), and organic modifier (acetonitrile) were used. Our objective of chromatographic method development was to achieve peak tailing factor < 2, retention time between 3 to 10 min, along with resolution between MET and OLM > 2. The chromatographic separation was achieved using Chromasil C18 (250 x 4.6 mm i.d., 5 ìm) column, changing the composition of mobile phase and optimized the chromatographic method. To develop a stability-indicating method assessing the effect of change of proportion, MET and OLM were well-resolved from degradation products using mobile phase composition of methanol: 0.05% v/v o-phosphoric acid in water (50:50 v/v) at a flow rate of 1.0 ml/min. with UV detection at 228.0 nm wavelength and injection

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volume 20 ì l. It was found to ideally resolve the peaks of MET (Rt 3.485 min) and OLM (Rt 7.085 min) (Fig.4). Resolution (Rs) between MET and OLM was found to be 10.28. ICH guidelines recommend 10 20 % degradation for establishing stability indicating nature of the assay method.

Resolution (Rs) = $t_2 - t_1 / w_1 + w_2$

t_1, t_2 are retention time of MET and OLM respectively,

w_1, w_2 are width of peaks for MET and OLM respectively,

Rs = 7.085 -3.485 / 0.15+ 0.2

Rs = 3.6 / 0.35 = 10.28

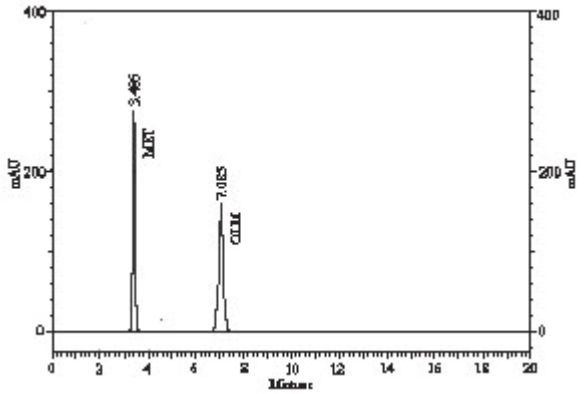

Fig. 4: *Chromatogram of MET and OLM in standard mixture*

Analysis of tablet formulation:

Twenty tablets of Rasotan Beta 25 (Emcure Pharma) each containing 25 mg of metoprolol succinate and 20 mg of olmesartan medoxomil were weighed and crushed in glass mortar to obtain fine powder. The powder sample equivalent to 25 mg of MET and 20 mg of OLM was transferred into a 100 ml volumetric flask and dissolved in 50 ml methanol HPLC grade. The flask was kept in an ultrasonic bath for 20 min. The volume was adjusted to 100 ml with methanol HPLC grade. The solution was filtered through 0.2 ì nylon membrane filter. From this stock solution, 2 ml solution was pipetted out and transferred to 10 ml volumetric flask and made volume up to the mark with mobile phase to get the concentration 50 ìg/ml of MET and 40 ìg/ml of OLM. The solution was injected into HPLC system (Fig. 5). The results of the assay of tablet formulation and its statistical validation data is given in Table 1.

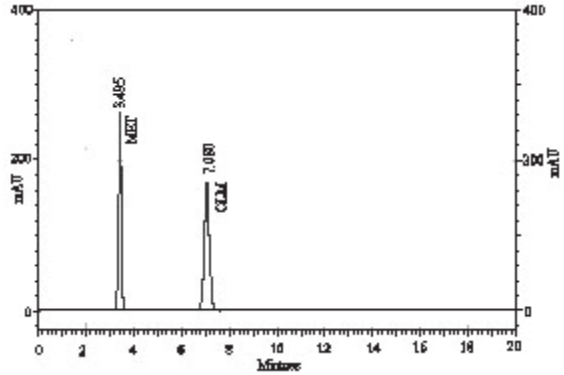

Fig. 5: *Chromatogram of MET and OLM in Tablet mixture*

Table 1: *Analysis of Tablet Formulation*

Tablet sample	Label claim (mg/tablet)	Amount found (mg/tablet)	% Label claim found*	± Standard deviation	Standard error
MET	25	24.96	99.84	0.4134	0.1688
OLM	20	19.96	99.80	0.5030	0.2053

**Average of six readings*

MET and OLM denotes metoprolol succinate and olmesartan medoxomil respectively

Procedure for forced degradation study of drug substances

Forced degradation of each drug substances and the drug product was carried out under acid, base, neutral, oxidative, thermolytic and photolytic stress conditions.

Acidic degradation

The accurate quantity of 2.5 mg MET and 2.5 mg OLM was weighed and transferred to 25 ml volumetric flask separately; 10 ml of 0.1 N HCl was added into each flask separately and the flask was kept for 2 hrs at room temperature. Then it was neutralized with 0.1 N NaOH and solution was sonicated for 30 min with intermittent shaking in ultrasonicator. Then the volume was made up with methanol HPLC grade and each solution was filtered through 0.2 µm membrane filter. From the filtered stock solutions, 5 ml of MET and 4 ml of OLM was pipetted out separately and transferred into a 10 ml volumetric flask and diluted to volume with mobile phase to obtain final concentration of 50 µg/ml of MET and 40 µg/ml OLM. Then the solution was injected into system (Fig. 6).

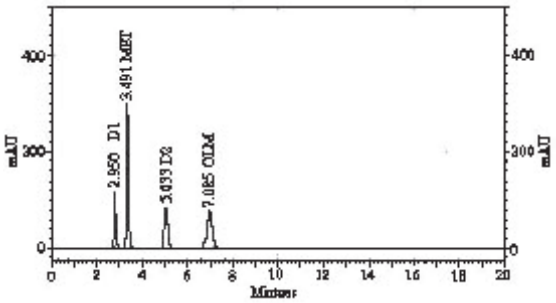

Fig. 6: *Acidic degradation of MET and OLM in standard mixture*

Alkali degradation

Alkali degradation was carried out by adding 10 ml of 1 N NaOH and the mixture was refluxed for 30 min at 60°C. Then it was neutralized with 1 N HCl and the solution was sonicated for 30 min with intermittent shaking in ultrasonicator (Fig. 7).

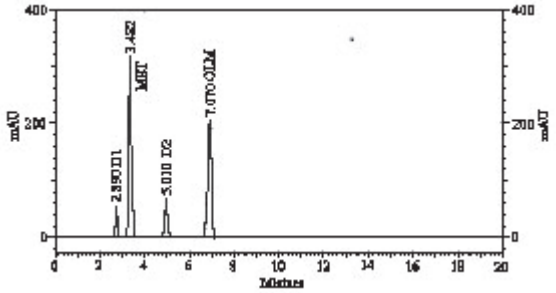

Fig. 7: *Alkali degradation of MET and OLM in standard mixture*

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Neutral degradation

10.0 ml distilled water was added to each stock solution of MET and OLM separately. Both solutions were refluxed at 60°C for 2 hrs and cooled at room temperature (Fig. 8).

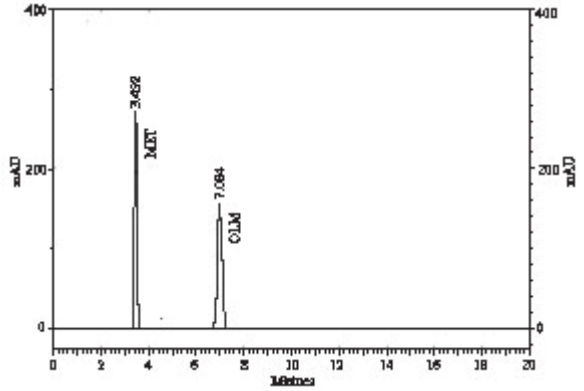

Fig. 8: *Neutral degradation of MET and OLM in standard mixture*

Oxidative degradation

Oxidative stress degradation of MET and OLM was conducted with 30% H_2O_2 for 2 hrs at 60°C in a water bath (Fig. 9).

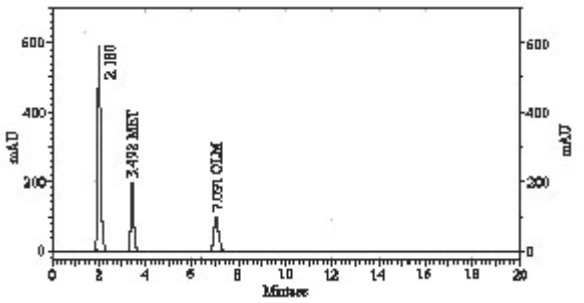

Fig. 9: *Oxidative degradation of MET and OLM in standard mixture*

Thermal degradation

About 100 mg of drug substances were placed in a controlled temperature oven at 80°C for 48 hrs. (Fig.10)

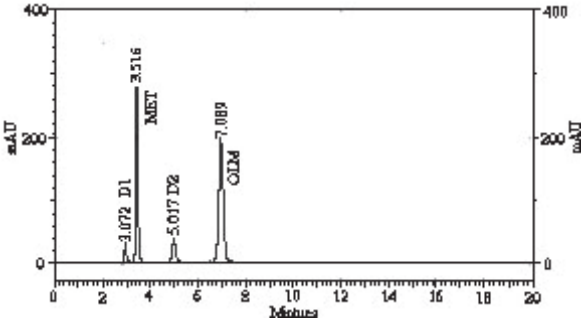

Fig. 10: *Thermal degradation of MET and OLM in standard mixture*

Photodegradation (UV light)

Photodegradation was performed by spreading the drug substance in petri dish as thin film and kept in photostability chamber equipped with ultraviolet light with energy of not less than 200 watt hours/square meter (Fig. 11).

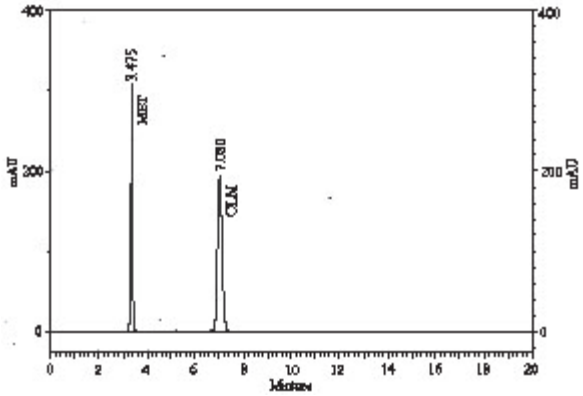

Fig. 11: *Photolytic degradation under UV light of MET and OLM in standard mixture*

Photodegradation (fluroscence light)

Photodegradation was performed by exposing the drug substance in photostability chamber equipped with fluroscence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved and diluted to obtain final concentration and injected into system. (Fig. 12)

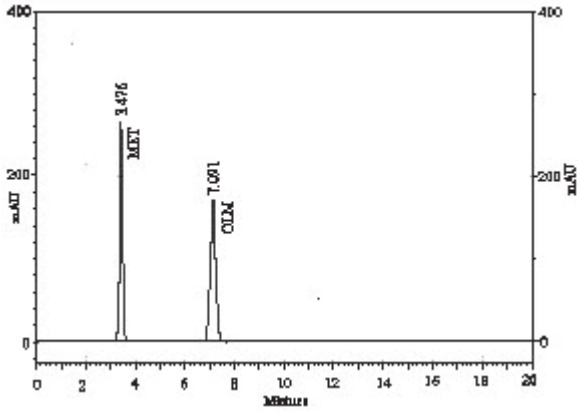

Fig. 12: *Photolytic degradation under fluorescence light of MET and OLM in standard mixture*

The results of forced degradation study of standard the proposed method are shown in Table 2.

Table 2: *Results of forced degradation study*

Stress condition	MET			OLM		
	Degradation (%)	Peak purity		Degradation (%)	Peak purity	
		Front	Tail		Front	Tail
Acidic (0.1 N HCl) 2 hr	19.76	994	991	19.53	993	992
Alkaline (1 N NaOH) reflux 60°C 2hr	7.12	995	992	9.25	995	991
Neutral Hydrolysis 60°C 2 hr	0.58	997	993	0.15	996	992
Oxidative (30 % H_2O_2) 2 hr	9.60	994	992	8.20	995	993
Dry heat 60°C (7 days	3.60	992	990	3.00	996	992
Photolysis (UV 20 Watt hours/square meter	0.16	996	990	0.30	995	992
Fluorescence (1.2 million Lux hrs	0.08	994	992	0.10	997	993

MET and OLM denotes metoprolol succinate and olmesartan medoxomil respectively.

Metoprolol and Olmesartan determination
Method Validation
Linearity
Linearity for MET and OLM was selected at 5-80 ìg/ml and 5-70 ìg/ml. The correlation coefficients were selected at 0.9990 and 0.9993 for MET and OLM, respectively. The results are shown in Table 3.

Table 3: *System Suitability Parameters*

Parameter	MET	OLM
Linearity range* (µg/ml)	5-80	5-70
Correlation coefficient*	0.9990	0.9993
Slope*	13949	20783
Limit of detection (µg/ml)	0.020	0.024
Limit of quantitation (µg/ml)	0.060	0.073
Retention time* (min)	3.485	7.085
Tailing factor*	1.02	1.13
Theoretical plates*	8245	6432

**Average of six readings*
MET and OLM denotes metoprolol succinate and olmesartan medoxomil respectively.

Specificity
Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak. The peak purity values for analyte peaks, MET and OLM, were in the range of 999–1000 for drug substance and in the range of 998–1000 for tablets, indicating homogeneous peaks and thus establishing the specificity of assay method.

Determination of Limits of Quantification and Detection
The limit of detection (LOD) and limit of quantitation (LOQ) for MET and OLM were determined at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The LODs for MET and OLM were 0.020 ìg/ml and 0.024 ìg/ml, respectively and the LOQs were 0.060 and 0.073 ìg/ml, respectively (Table 3).

Precision (repeatability)
The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The results of the precision study indicate that the method is reliable (%RSD<2).

Accuracy (recovery test)
Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels, 80 %, 100 %, and 120 % of the label claim of the tablet (25 mg of MET and 20 mg of OLM). The results are shown in Table 4.

Robustness
The robustness of a method is the ability of method to remain unaffected by small changes in parameters like mobile phase composition, flow rate, pH of mobile phase and temperature etc.

Table 4: *Recovery studies*

Drug	Level of % recovery	%Mean*	Standard deviation	%RSD	Standard error
MET	80	99.55	0.1213	0.1207	0.0495
OLM	80	99.72	0.2317	0.2310	0.0946
MET	100	99.48	0.1497	0.1489	0.0611
OLM	100	99.85	0.4527	0.4520	0.1848
MET	120	99.84	0.3240	0.3234	0.1323
OLM	120	99.75	0.1432	0.1428	0.0584

**Average of six readings*
MET and OLM denotes metoprolol succinate and olmesartan medoxomil respectively.

Both drugs and their combination drug product were found to be stable in neutral, thermal, oxidative and photolytic stress conditions but mild degradation was observed in acidic and alkaline conditions. The results obtained by the stress degradation conditions of the drugs show that the method is specific and stability-indicating.

DISCUSSION
The results obtained by the stress degradation conditions of both drugs showed that validated stability-indicating RP-HPLC method is specific, simple, rapid, reproducible, accurate and precise method developed for the quantitative simultaneous estimation of metoprolol succinate and olmesartan medoxomil in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in API, formulations, dissolution studies, bioavailability and pharmacokinetic studies.

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