

# Simultaneous Determination and Validation of Olmesartan Medoximil and Metoprolol Tartarate by Analytical Technique RP-HPLC

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**Abstract:** A rapid reverse phase high performance liquid chromatography method has been developed and validated for the determination of Olmesartan medoximil and metoprolol tartarate in combined tablet dosage form. Isocratic technique was adopted using C<sub>18</sub> column (150×4.6mm, 5μ XTerra) with a phosphate buffer [pH 2.8] as a mobile phase and the flow rate of 0.5ml/min at UV wavelength 284nm. The R<sub>f</sub> values was found to be 3.624 & 5.178min and the above drugs with a run time of 10min. Various chromatographic parameters including Specificity, Linearity, Accuracy, Precision, LOQ, LOD, Robustness, System suitability have been evaluated. The present investigation was validated as per ICH guidelines for the drugs.

**Keywords:** ICH, Validation, Olmesartan Medoximil, Metoprolol Tartarate, RP-HPLC

## 1. Introduction

Olmesartan Medoximil (Figure 1) is chemically 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1,3-dioxol-4-yl) methyl ester. Olmesartan Medoximil is an angiotensin II receptor antagonist. This has been used for the treatment of hypertension. Olmesartan Medoximil blocking the binding of angiotensin II to the Angiotensin I. Angiotensin II is a powerful vasoconstrictor and increases blood pressure through a variety of mechanisms. Olmesartan reduces vasoconstrictor and the secretion of aldosterone. This lowers blood pressure by producing vasodilation and decreasing peripheral resistance. The structure (fig.1 and 2) of two drugs are shown below.

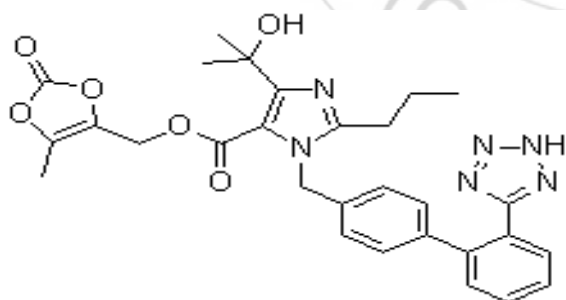


Figure 1: Olmesartan Medoximil

Metoprolol Tartarate (figure-2) is chemically 1-(Isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2- propanol. It is used for the treatment of high B.P. Metoprolol Tartarate may cause changes in blood sugar levels (or) cover signs of low blood sugar, such as rapid pulse rate. Metoprolol Tartarate is used for the treatment of angina, acute myocardial infraction, congestive heart failure and prevention of migraine headaches.

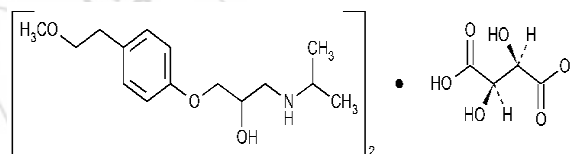


Figure 2 : Metoprolol Tartarate

Literature survey reveals that certain spectroscopic methods were reported for simultaneous determination of Olmesartan Medoximil and Metoprolol Tartarate. The aim of present work was an attempt to develop simple, precise and accurate analytical method for simultaneous estimation of Olmesartan Medoximil and Metoprolol Tartarate in bulk and pharmaceutical dosage forms in accordance with ICH Q2B guidelines.

## 2. Material and Method

### 2.1 Instrumentation

HPLC (WATERS 2690 series (Empower Software), column C18 symmetry (4.6×150nm, make: Xterra) Sonicator (ultrasonic Cleaner Power sonic 420) PH meter, vaccum oven (wadegati), water bath and other glasswares were used.

### 2.2 Chemicals and Solvents:

Marketed formulation OLMEZEST-BETA tablets (20mg of Olmesartan Medoximil and 50mg of Metoprolol Tartarate) was taken for the studies were marketed by Ranbaxy Pvt Ltd. Potassium dihydrogen phosphate, sodium perchlorate, per chloric acid of GR grade was obtained from Merck (India) Ltd, Mumbai, India. HPLC grade Methanol and Acetonitrile were used. (Merck (India) Ltd, Mumbai).

### 2.3. Chromatographic Conditions:

The Column used was C<sub>18</sub> symmetry (4.6×150mm, 5µm, Make: Xterra) for analytical separation. Ortho phosphoric (PH 2.8) and acetonitrile was taken in the ratio of [35:65v/v] for Mobile phase of the investigation with a flow rate 0.5ml/min at ambient temperature (detection wavelength 284nm) The injection volume was 20µl capacity.

## 3. Preparation of Analytical Solutions

### 3.1 Preparation of 0.01M phosphate buffer (pH: 2.8)

Accurately weighed 7grams of Potassium Dihydrogen Phosphate was dissolved in 100ml of water (HPLC grade) and mixed using ultrasonicator and filter through 0.45µm membrane filter and the resulting solution adjusted to pH 2.8 with the help of dil O- phosphoric acid.

#### Mobile Phase:

Mixture of above buffer solution 350ml (35%) and 650ml of acetonitrile HPLC (65%) were mixed and degassed in ultrasonic water bath for 5min. and filtered through 0.45µm filter under vacuum filtration.

#### Preparation of standard stock solution:

10mg of Olmesartan Medoximil and 10mg of Metoprolol Tartarate working standards were accurately weighed and transferred into volumetric flask (100ml). The diluent (70ml) was added, sonicated for dissolution completely made up to the mark. Further 1.2ml of Olmesartan Medoximil and 3ml of Metoprolol Tartarate was pipetted from the above stock solution into a volumetric flask (10ml), diluted up to the mark.

#### Preparation of sample solution: (Marketed formulation)

10 tablets of Olmesartan Medoximil and Metoprolol Tartarate were weighed and the number of active ingredients present in 10 tablets (156.8 mg) was transferred into a volumetric flask (100ml). 70ml of diluent was added and sonicated for about 30min and was diluted with diluent and filtered. The filtrate 0.6ml of upper clear solution was transferred to volumetric flask (10ml) and made up to the mark.

#### Method validation:

The method validation was done as per the ICH Q2B norms, Accordingly the specificity, Linearity, Accuracy, Precision, LOD, LOQ, Robustness and system suitability studies were evaluated.

#### Specificity:

The Specificity of this method was measured without interference by injecting sample and standard solutions and its retention time was compared.

#### Linearity:

The test results obtained in the investigation are found to be linear. Which is a direct proportionality of the concentration of analyte in samples within given range was studied by analyzing five analyte concentrations of drug ranging from 4-20ppm for Olmesartan Medoximil and 10-

50ppm for Metoprolol Tartarate are presented in Table No 1 and Linearity plot is given in the Figure-7.

#### Accuracy:

Accuracy refers to the nearest of a measured value to a standard (or) known value. The percentage recovery was studied for 50%, 100% and 150%. Each level was injected three times. The accuracy levels are shown in Table-2 and Table-3.

#### Precision:

The precision of this experiment was performed to ascertain the repeatability of the assay results obtained by quantification Methodology. System precision, Method precision and intermediate precision was performed.

#### System precision:

The standard solution (20µl) was injected in HPLC instrument for five times and the peak areas were measured to calculate the %RSD of the areas of five replicate injections.

#### Method Precision:

The sample solution of (20µl) was injected resulting chromatogram for five times and peak areas were calculated and shown in Table

#### Intermediate precision:

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is observed that under different conditions the results are reproducible. Hence the present method was observed to be rugged.

#### LOD and LOQ:

The detection and quantification limits for the olmesartan medoximil and metoprolol tartarate were performed and calculated using S/N ratio method.

#### Robustness:

It is a measure of its capacity to find out unaffected small and deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness measures the lack of internal influences on the test results. As part of the Robustness, deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method.

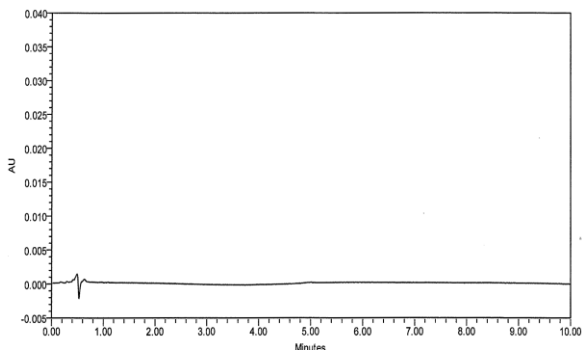
#### System Suitability:

System Suitability tests were carried out on freshly prepared standard stock solutions of Olmesartan Medoximil and Metoprolol Tartarate were injected three times into the HPLC system and the values were recorded.

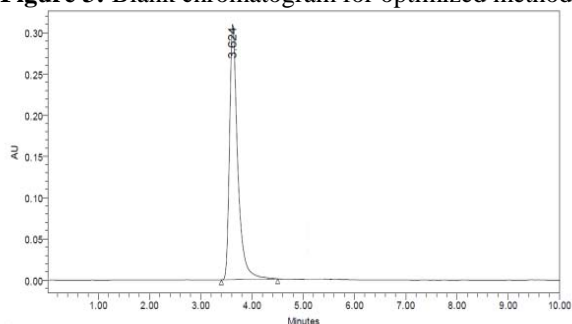
## 4. Results and Discussion

Olmesartan Medoximil and Metoprolol Tartarate can be effectively analyzed by the RP-HPLC method with phosphate buffer (pH: 2.8) Acetonitrile: water (35:65, v/v) a flow rate of 0.5ml/min and detection wavelength of 284nm. The R<sub>t</sub> of the drugs was 3.624 and 5.178min. The assay limits for Olmesartan Medoximil and Metoprolol Tartarate

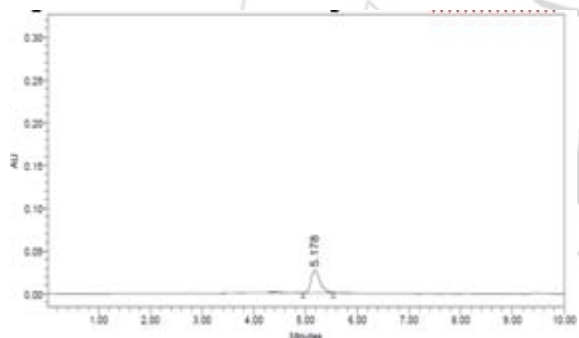
was 90-100% and the results were obtained for Olmesartan Medoximil and Metoprolol Tartarate was found to be 99.2% and 100%. Hence the results were within the limit.



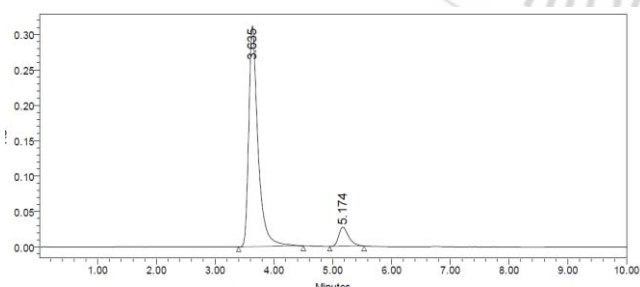
**Figure 3:** Blank chromatogram for optimized method



**Figure 4:** Standard chromatogram for Olmesartan Medoximil



**Figure 5:** Standard chromatogram for Metoprolol Tartarate

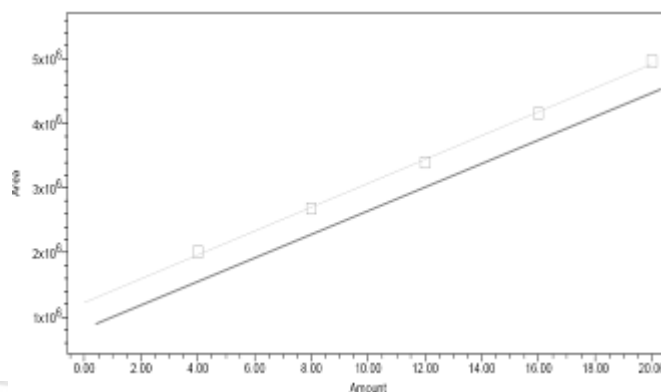


Name	Retention time	Area	USP Resolution	USP Tailing	USP Plate count
OM	3.642	3410176		1.5	4874
MT	5.124	319570	5.1	1.4	3579

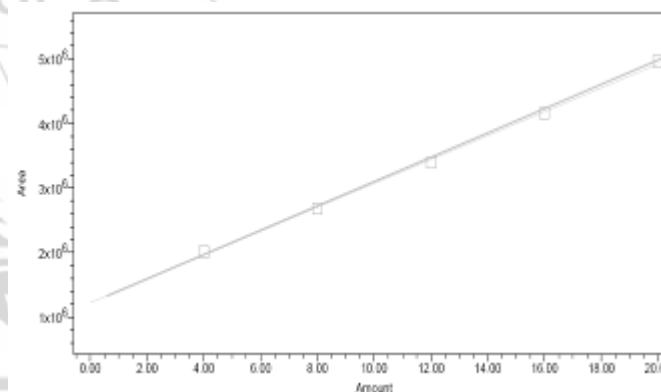
**Figure 6:** Sample Chromatogram for Olmesartan Medoximil and Metoprolol tartarate

This method was specific. Since there was no interference due to placebo and sample at the retention time of analyte

peak. The linearity range was observed to be 4-20ppm for Olmesartan Medoximil and 10-50ppm for Metoprolol Tartarate. Calibration curve was plotted and correlation coefficient for both the drugs Olmesartan Medoximil and Metoprolol Tartarate found to be 0.995 and 0.997. Hence the results were obtained within the limit.



**Figure 7 (A):** Linearity plot for Olmesartan Medoximil



**Figure 7 (B):** Linearity plot for Metoprolol Tartarate

**Table 1:** Linearity Studies of Olmesartan Medoximil and Metoprolol Tartarate

S. No	Olmesartan Medoximil		Metoprolol Tartarate	
	Concentration	Area	Concentration	Area
1	4ppm	2011514	10ppm	189398
2	8ppm	2681557	20ppm	258331
3	12ppm	3390741	30ppm	321805
4	16ppm	4161134	40ppm	394694
5	20ppm	4964755	50ppm	459759
Correlation Coefficient		0.995		0.997

The Accuracy Studies were shown as % recovery for Olmesartan Medoximil and Metoprolol Tartarate at 50%, 100% and 150%. The % recovery of the Olmesartan Medoximil and Metoprolol Tartarate was observed to be within the range of 99.3-100.3% and 99.13-100%. The accuracy results are shown in Table-2 and Table-3.

**Table 2:** % Recovery results for Olmesartan Medoximil

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.96	99.2%	100.3%
		5	4.99	99.8%	
		5	5.1	102%	
2	100 %	10	9.92	99.2%	99.4%
		10	9.94	99.4%	
		10	9.98	99.8%	
3	150 %	15.3	15.1	98.6%	99.3%
		15.3	15.2	99.3%	
		15.3	15.3	100%	

**Table 3:** % Recovery results for Metoprolol Tartarate

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	14.8	14.72	99.4%	99.69%
		14.8	14.79	99.9%	
		14.8	14.77	99.79%	

The precision study %RSD was found to be less than 1%. For Olmesartan Medoximil 0.4% and Metoprolol Tartarate 0.36%. System precision indicates that the system has good reproducibility. The results obtained for precision values are shown in Table-4.

**Table 4:** Method Precision values for Olmesartan Medoximil and Metoprolol Tartarate

Injections	Olmesartan Medoximil		Metoprolol Tartarate	
	Retention Time	Area	Retention Time	Area
1	3.623	3480636	5.175	323863
2	3.624	3463594	5.17	325248
3	3.629	3498779	5.174	322052
4	3.629	3497870	5.174	328133
5	3.629	3490276	5.174	328655
Avg		3486743		3281662
SD		14601.3		2802.3
%RSD		0.42		0.36

The results of LOD and LOQ are shown in Table- 5.

**Table 5:** LOD and LOQ of Olmesartan Medoximil and Metoprolol Tartarate

Drug Name	LOD	LOQ
Olmesartan Medoximil	2.96	10
Metoprolol Tartarate	2.98	9.98

Robustness studies for the changed flow rates ( $\pm 0.1$ ) and changing Mobile Phase composition by changing the organic ratio by 10% assay carried out the variation in 10% organic composition both the studies were within the acceptance limits.

**Table 6:** Robustness studies for change in flow rate

Synod	Flow rate (ml/min)	Olmesartan Medoximil		Metoprolol Tartarate	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.4	4859	1.62	3330.4	1.52
2	0.5	4890	1.58	3437.6	1.47
3	0.6	4895	1.58	3228.7	1.47

The system suitability parameters like Theoretical plates (N), Tailing Factor (T) were calculated the proposed RP-HPLC method was accurate and precise as presented in the **Table No.7.**

**Table 7:** System suitability parameters for Olmesartan Medoximil and Metoprolol tartarate

Parameters	Olmesartan Medoximil	Metoprolol Tartrate
Retention Time	3.654	5.181
Theoretical Plates	6899	4380
Tailing Factor Area	1.6	1.4

## 5. Summary and Conclusion

The present method was specific, precise, accurate, rapid and economical for simultaneous estimation of Olmesartan Medoximil and Metoprolol Tartrate in pharmaceutical dosage forms. These methods were validated as per ICH guidelines. The sample recoveries in all formulations were in good agreement with their respective label claims.

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