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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 metaprolol tartarate in combined tablet dosage form. Isocratic technique was adopted using C_{18} 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_t 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, Metaprolol 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.

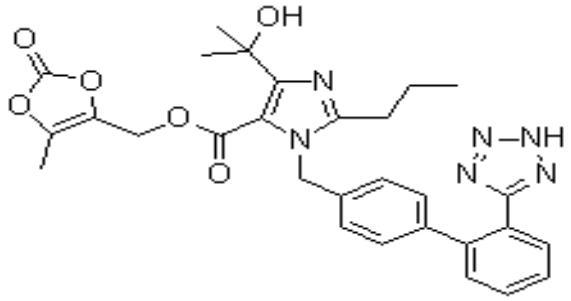


Figure 1: Olmesartan Medoximil

Metaprolol 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. Metaprolol Tartarate may cause changes in blood sugar levels (or) cover signs of low blood sugar, such as rapid pulse rate. Metaprolol Tartarate is used for the treatment of angina, acute myocardial infraction, congestive heart failure and prevention of migraine headaches.

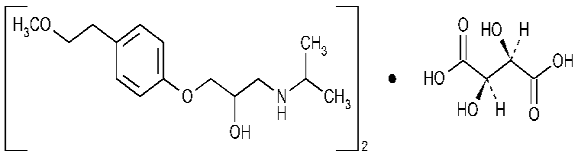


Figure 2 : Metaprolol Tartarate

Literature survey reveals that certain spectroscopic methods were reported for simultaneous determination of Olmesartan Medoximil and Metaprolol Tartarate. The aim of present work was an attempt to develop simple, precise and accurate analytical method for simultaneous estimation of Olmesartan Medoximil and Metaprolol 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 Metaprolol 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_{18} 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 0.45µmembrane 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 0.45µ filter under vaccum filtration.

Preparation of standard stock solution:
10mg of Olmesartan Medoximil and 10mg of Metaprolol 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 Metaprolol Tartarate was pippeted 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 Metaprolol 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 clean solution was transferred to volumetric flask (10ml) and made up to the mark.

Method validation:
The method validation was done as per the ICH Q2Bnorms, 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 directly 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 Metaprolol Tartarate are presented in TableNo1 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 metaprolol tartarate were performed and calculated using S/N ratio method.

Robustness:
It is 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 Metaprolol Tartarate were injected three times in to HPLC system and the values were recorded.

4. Results and Discussion

Olmesartan Medoximil and Metaprolol 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_t of the drugs was 3.624 and 5.178min. The assay limits for Olmesartan Medoximil and Metaprolol Tartarate

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

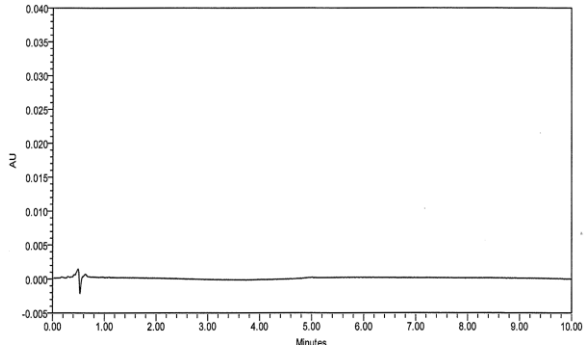


Figure 3: Blank chromatogram for optimized method

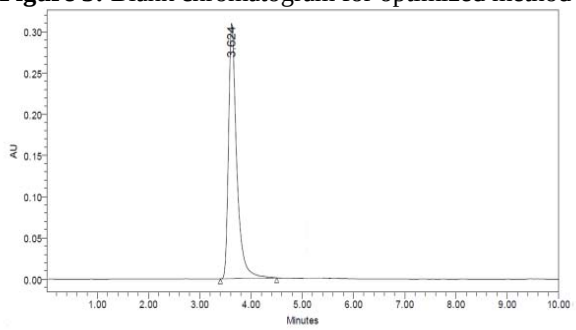


Figure 4: Standard chromatogram for Olmesartan Medoximil

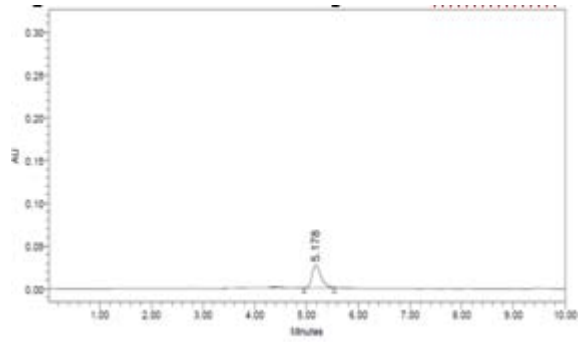
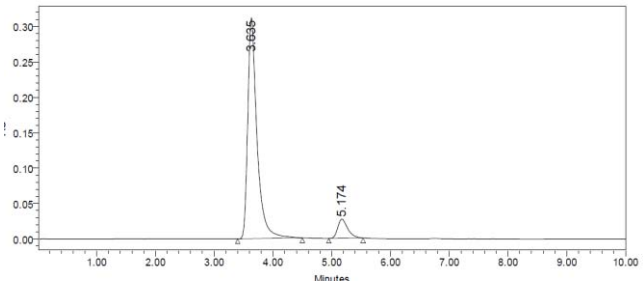


Figure 5: Standard chromatogram for Metaprolol Tartarate



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 Metaprolol 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 Metaprolol Tartarate. Calibration curve was plotted and correlation coefficient for both the drugs Olmesartan Medoximil and Metaprolol Tartarate found to be 0.995 and 0.997. Hence the results were obtained within the limit.

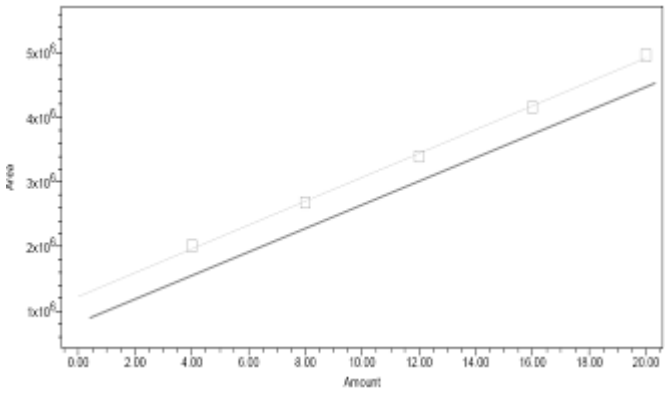


Figure 7 (A): Linearity plot for Olmesartan Medoximil

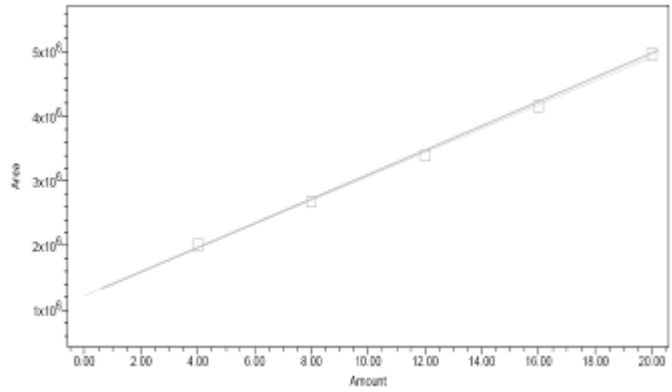


Figure 7 (B): Linearity plot for Metaprolol Tartarate

Table 1: Linearity Studies of Olmesartan Medoximil and Metaprolol Tartarate

S. No	*Olmesartan Medoximil*		*Metaprolol 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 Metaprolol Tartarate at 50%, 100% and 150%. The % recovery of the Olmesartan Medoximil and Metaprolol 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 Metaprolol 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 Metaprolol 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 Metaprolol Tartarate

Injections	*Olmesartan Medoximil*		*Metaprolol 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 Metaprolol Tartarate

Drug Name	*LOD*	*LOQ*
Olmesartan Medoximil	2.96	10
Metaprolol Tartarate	2.98	9.98

Robustness studies for the changed flow rates (±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*		*Metaprolol 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 Metaprolol 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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