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RESEARCH ARTICLE

Development and Validation of Stability Indicating Assay Method for Estimation of Olmesartan Medoximil And Metoprolol Succinate in Combined Dosage Form By UHPLC

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ABSTRACT

A simple, rapid and precise RP-UHPLC method is developed for the simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in bulk drug and pharmaceutical dosage form. The quantification is carried out using Shimadzu Shim-pack XR ODS II (50mm X 1.9mm, 2.0 µm) column with mobile phase-A consist of 50mM phosphate buffer pH-6.8±0.05: Acetonitrile (95:5 %v/v) & mobile phase-B consist of 50mM phosphate buffer pH-6.8±0.05: Acetonitrile (34:66 %v/v) with gradient elution. The flow rate is 0.8 mL/min using 40°C column oven temperature. The eluent is measured at 225 nm. The retention times of Olmesartan Medoxomil and Metoprolol Succinate are about 1.7 min and 0.7 min respectively. The method is validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation. Linearity of Olmesartan Medoxomil and Metoprolol Succinate are in the range of 25-75µg/ml for Olmesartan and 62.5-182.5µg/ml for Metoprolol with regeression co-efficient more than 0.999. The percentage recoveries of both the drugs are between 98 to 102%. The stress testing of dosage form is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation (dry heat and wet heat) conditions and the drugs are well resolved from its degradation products with good resolution. Hence the method is accurate and precise and can be employed for routine analysis of Olmesartan and Metoprolol in different dosage forms.

KEYWORDS

Olmesartan Medoximil, Metoprolol Succinate, Stability Indicating Assay method, Validation, UHPLC

INTRODUCTION

Worldwide, raised blood pressure is estimated to cause 7.5 million deaths, about 12.8% of the total of all deaths. This accounts for 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS. Raised blood pressure is a major risk factor for coronary heart disease and ischemic as well as hemorrhagic stroke. Treating high blood pressure can take a multi-pronged approach including diet changes, medication, and exercise.

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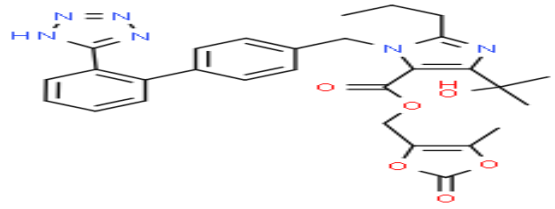

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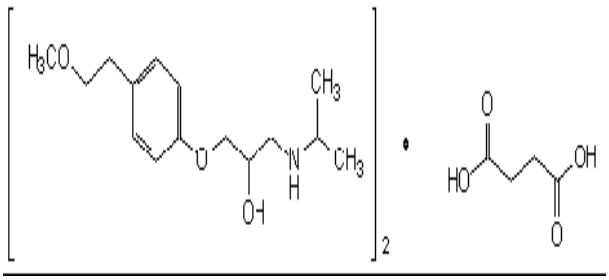

Figure-1: Chemical structures (a) Olmesartan Medoximil; (b) Metoprolol Succinate

Fixed dose combination (FDC) drugs are standard practice in the treatment of infectious diseases, but their role in chronic non-communicable diseases is unclear. FDC anti-hypertensive drugs are an attractive option to improve compliance by reducing the number of pills taken daily, particularly in elderly patients who generally require more than one drug to control blood pressure and often are on multiple other medications. Olmesartan is an angiotensin II receptor blocker (ARB) used in the treatment of Hypertension and it works by relaxing blood vessels so that blood can flow more easily (Fig.1a). Metoprolol succinate is a selective beta-1 blocker and is to treat angina (chest pain) and hypertension (high blood pressure). It is also used to lower your risk of death or needing to be hospitalized for heart failure (Fig.1b).

The combination of Olmesrtan Medoximil (20 mg) and Metoprolol succinate (25 mg) was tentatively approved by US Food and Drug Administration (USFDA) in Oct 2016 for the treatment of hypertension in adults. Pharmaceutical companies are focusing on achieving ever shorter times of drug to market, so it is vital that a tailored, pragmatic approach is adopted when conducting method development for active pharmaceutical ingredients (API) or drug products (DP). Although methods require a high degree of robustness, the overall strategy should encompass full evaluation of the regulatory requirements applicable to the particular phase of the drug lifecycle; this is pivotal to ensure a successful regulatory submission, whereby the applicant must demonstrate suitable validation of all methods used to support the filing. Successfully developed (and validated) analytical methods can reduce overall turnaround times from preclinical to commercial release. Methods should have the desired flexibility built in during early stages to allow easy translation from API to DP, thus potentially reducing costs throughout the product lifecycle.

Analytical method development, validation, and transfer are key elements of any pharmaceutical development program. The need to develop new analytical methods with extremely high sensitivity along with precision and accuracy thus became mandatory. These methods are needed for assurance of quality, safety and efficacy of medicine and pharmaceuticals. There are five analytical tests that are considered universal by the FDA for formulated products: description, identification (ID), assay, dissolution and impurities. Description is a physical characteristic of the finished product but for identification, assay, dissolution and impurities analytical methods required. Fixed dose combination of Olmesartan medoximil and Metoprolol succinate is not official in Indian pharmacopeia.

Assay by UHPLC method for the estimation of Olmesartan Medoximil and Metoprolol Succinate is not available in any pharmacopeia and not reported in any scientific journal. This present work describes the development of simple, selective, accurate and precise RP-HPLC method for the determination of Assay by UHPLC of Olmesartan Medoximil and Metoprolol Succinate in Tablet formulation.

Materials and Methods

Chemicals and Reagents:

Potassium bromide, Sodium dihydrogen phosphate, hydrochloric acid (35%), sodium hydroxide, Hydrogen peroxide and HPLC grade acetonitrile were purchased from Merck. Analytical standards were provided as gift samples by Piramal Enterprises Ltd, Pharmaceutical Development Service Ltd. Tablet Olmetor-M tablet formulation purchased from market.

Instrument used:

The UHPLC used for method development and validation was Shimadzu N-Series and Nexera X2 UHPLC.

API Evaluation

Identification by UV test was performed for evaluation of Olmesartan Medoxilmil and Metoprolol Succinate. Solution of 10ppm of Olmesartan Medoxilmil and Metoprolol Succinate individually was prepared in a

mixture of 50 volumes of water and 50 volumes of acetonitrile and scanned from 200-400 nm in UV spectrophotometer. Identification by IR was carried out for all APIs. Accurately weighed 2 mg of individual API was mixed with 200 mg of previously dried potassium bromide at 105°C for 1 hr. and triturated to get homogenous mixture. This sample was scanned in the range of 400-4000cm-1 in FTIR. Melting point determination was carried out for all APIs. Melting point was determined by capillary method using Lab India melting point apparatus.

Method Development

The step wise logical scientific method development has been described along with the reasoning. Method development was initiated by converting a HPLC method from literature to UHPLC method. Initial trial were taken using 0.1% OPA in water and Acetonitrile on a C18 column. But it was observed that the peak shape was not proper for Olmesartan. Later the ratio was changed, but it always led to more retention of Olmesartan peak and run time was increasing. Then we shifted from 0.1% OPA in water to phosphate buffer and found improvement in peak shapes. Later trials with various gradient composition were taken during forced degradation development to assure specificity. Final optimized method parameters are as follows: 50mM phosphate buffer pH-6.8±0.05: Acetonitrile (95:5 %v/v) as a mobile phase-A and 50mM phosphate buffer pH-6.8±0.05: Acetonitrile (34:66 %v/v) as a mobile phase-B. Samples were injected in C18 column Shimadzu Shimpack XR ODS II (50mm X 1.9mm, 2.0 µm) which was eluted at 0.8mL/min. Injection volume kept 10µL. UHPLC column temperature was set to 40°C and auto sampler temperature kept ambient. Selected gradient was as follows: 0.0-1.5 min, linear gradient 40-70% B 1.5-2.5 min, isocratic 70% B; 2.5-3.5 min, linear gradient 70-40% B; 3.5-40 min, isocratic 40% B. Forced degradation study was performed on tablet formulation to check stability indicating nature of method. In forced degradation study acid-base hydrolysis, oxidation using hydrogen peroxide, thermal stress and photo stability stress were carried out. Acid, base and peroxide hydrolysis was performed at 100°C. For thermal stress tablet formulation was kept at 105°C for 24 hours and photo stress carried out at 1 ICH Cycle.

Method Validation

The performance characteristics considered for validation of the optimized method were: system suitability, specificity, filter study, linearity, accuracy, precision and robustness.

System suitability

System suitability of analytical method was checked throughout whole analysis by measuring the %RSD for known standard, Tailing factor, resolution and plate count.

Specificity

Specificity was performed by checking interference from blank, placebo (excipients of formulation) at the retention time of both these active peaks. Peak purity of Olmesartan and Metoprolol were checked for specificity Forced degradation study was also performed.

Filter study

Filter study was performed to select suitable filter to get clear solution. Filters were evaluated against centrifuged sample solution and absolute difference between filter and centrifuged sample was calculated.

Linearity

Linearity was assessed visually and by means of a lack-of-fit test. The working range was defined as the interval between the upper and the lower levels of the analytes within the calibration curve. Linearity was evaluated from 50% level to 150% level.

Accuracy

Accuracy of analytical method was evaluated by recovery study. Known amount of API spiked in placebo mixture preparation at 50%, 100% and 150% level.

System precision

The five replicate injections of standard preparation were injected to determine the reproducibility of the instrument.

Method precision

The six different sample sets were prepared and injected to determine the repeatability of method.

Standard solution preparation

Accurately weighed and transferred about 20 mg OLM and 25mg of MET into 100 mL of clean, dry volumetric flask. 50 mL of diluent (Water: Acetonitrile (1:1)) added and sonicated to dissolve and volume made up to the mark with diluent. 5mL of standard stock transferred into 50mL of volumetric flask and volume made up to the mark with diluent.

Sample preparation

10 tablets were weighed and crushed to fine powder. Powder equivalent to 20 mg OLM and 25mg of MET was transferred into 100 mL of clean, dry volumetric flask. 50 mL of diluent (Water: Acetonitrile (1:1)) added and sonicated for 15 min and volume made up to the mark with diluent. 5mL of standard stock transferred into 50mL of volumetric flask and volume made up to the mark with diluent. It was filtered using 0.45µ Filter discarding 3mL of filtrate.

Forced degradation study

Forced degradation study on formulation was carried out in solution state. For acid stress, 5 mL of sample stock solutions were transferred into 50 mL of volumetric flask and 2 mL of 0.1N HCl added. Sample solution was kept at 100°C for 1 hour. After 1 hour sample was neutralized with 2 mL of 0.1N NaOH and volume made up to the mark with diluent. Similarly solution for base stress was prepared. For oxidation stress, 0.1% hydrogen peroxide was used and sample was kept at 100°C for 1 hour. Thermal and photo stress were carried out on solid state. For thermal stress, tablet formulation was kept at 105°C for 24 hours, for photo stress tablets were exposed to 1 ICH cycle.

Robustness

Robustness study was performed with deliberate changes in method parameters with respect to flowrate, column oven temperature, detection wavelength and pH of buffer.

Results and Discussion

The method has been employed successfully for quantitative determination of OLM and MET by Reverse Phase High Performance Liquid Chromatographic method and validated according to ICH Q2 (R1) guidelines.

Identification by UV

A 10ppm solution of Olmesartan Medoximil and Metoprolol Succinate individually was scanned in the range of 200 to 400 nm and maximum absorbance observed at 257 nm for Olmesartan Medoximil and 222 nm for Metoprolol Succinate. Result of identification by UV is given in Fig-2a and 2b.

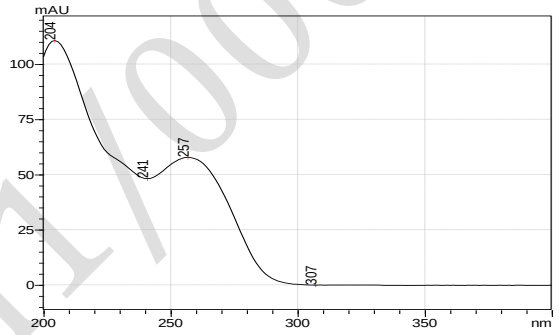

Figure-2a: Identification of Olmesartan Medoximil by UV

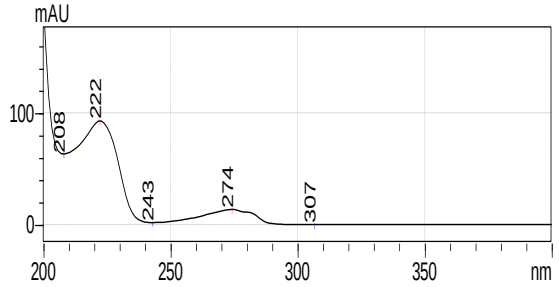

Figure-2b: Identification of Metoprolol Succinate by UV

Identification by IR

Identification by IR was carried out for all APIs. Samples were scanned in the range of 400-4000cm-1. IR peaks observed in sample preparation were matched with the reference spectra available in pharmacopeia. Result of identification by IR is given in Fig-3a and 3b

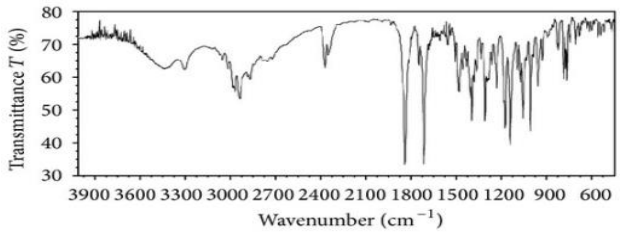

Figure-3a: Identification of Olmesartan Medoximil by IR

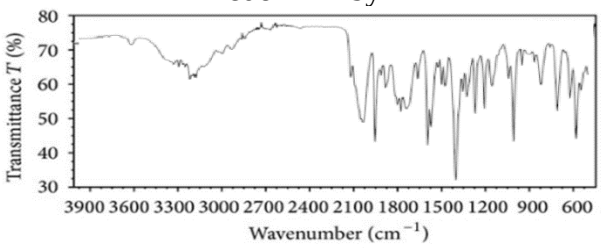

Figure-3b: Identification of Metoprolol Succinate by IR

Melting point determination

Melting point was determined by using capillary method. Test results were compared to reference results available in COA and met acceptance criteria. Result of identification by melting point is given in Table-1.

API Name	Observation			Specification
	Start temperature	End temperature	Melting point	
Olmesartan Medoximil	176.1°C	178.7°C	177.3°C	175°C-180°C
Metoprolol Succinate	118.1°C	119.3°C	118.8°C	118°C-120°C

Table-1: Melting Point Determination

Chromatographic conditions

The optimized UHPLC conditions are given in Table-2.

Column	Shimadzu Shimpack XR ODS II (50mm X 1.9mm, 2µm)		
Mobile Phase-A	50mM pH-6.8 phosphate buffer: Acetonitrile (95:5)		
Mobile Phase-B	50mM pH-6.8 phosphate buffer: Acetonitrile (34:66)		
Mobile Phase program	Gradient		
Column temperature	40°C		
Injection volume	10 µL		
Flow rate	0.8 mL/minute		
Detection	225 nm, UV		
Run time	4 minutes		
Gradient	**Time (min)**	**Mobile Phase-A**	**Mobile Phase-B**
	0.00	60	40
	1.50	30	70
	2.50	30	70
	3.50	60	40
	4.00	60	40

Table-2: Optimized Chromatographic Condition

Method Validation

The results for various validation parameters viz. system suitability, specificity, linearity, accuracy, precision, forced degradation are depicted below.

System suitability

Results for various system suitability parameters for analytical method was checked throughout and reported in Table-3.

Parameters	Specification	Observation	
		Olmesartan Medoximil	**Metoprolol Succinate**
%RSD	NMT 2.0%	0.1%	0.1%
Tailing Factor (T)	≤ 2.0	1.0	1.1
USP Plate count	NLT 2000	19	36
USP Resolution	NLT 2.0	5.3	

Table 3: Result of System Suitability Test

Specificity

Sr. No	Condition	% Assay	
		OLM	MET
1	Control Sample	99.8	99.6
2	0.1N HCl at 100°C for 1 hour	98.3	93.1
3	0.1N NaOH at 100°C for 1 hour	98,5	93.9
4	0.1% H2O2 at 100°C for 1 hour	97.2	95.1
5	105°C for 24 hours	99.3	98.6
6	1 ICH Cycle	99.7	99.1

Table-4: Results of Forced Degradation Study

Interference from blank, placebo (excipients of formulation) at the retention time of both these active peaks was checked. No interference was observed at the retention time of Olmesartan and Metoprolol. (Fig. 4). Peak purity of Olmesartan and Metoprolol were passing by total point method for specificity as well as forced degradation study (Table-4).

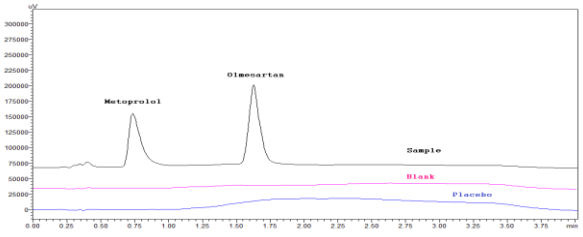

Figure-4: Overlay chromatograph of blank, placebo & sample preparation

Filter study

Results of Filter study performed to select suitable filter against centrifuged sample solution are shown in Table-5.

Drug Name	% Assay				
	Centrifuged	0.45 µm PVDF filter	Absolute difference	0.45 µm Nylon filter	Absolute difference
Olmesartan Medoximil	99.9	99.5	0.4	98.9	1.0
Metoprolol Succinate	99.3	99.1	0.2	98.9	0.4

Table 5: Result of Filter Study

Linearity

Name of Active/ Impurity	Linearity Level	Conc. (µg/mL)	Area	Correlation co-efficient (r)	Slope	y-Intercept
Olmesartan Medoximil	**50 %**	25.445	508976	1.000	20337	10224
	80 %	40.712	799567			
	100 %	50.890	1006540			
	120 %	61.068	1298671			
	150 %	76.335	1509876			
Metoprolol	**50 %**	62.540	588756	1.000	9324	9944

Succinate	**80 %**	100.064	932987			
	100 %	125.0800	1198730			
	120 %	150.0966	1409567			
	150 %	187.6200	1750987			

Results of Linearity for both the analytes evaluated from 50% level to 150% level are depicted in Table-6. The correlation co-efficient value was greater than 0.999.

Accuracy

Accuracy of analytical method was evaluated by recovery study.

Known amount of API was spiked in placebo mixture preparation at 50%, 100% and 150% level. The % recovery obtained for the analytes is shown in Table-7.

Name of Impurity	**Recovery at 50%**				**Recovery at 100%**			**Recovery at 150%**		
	No of sets	**% Recovery**	**Mean**	**%RSD**	**% Recovery**	**Mean**	**%RSD**	**% Recovery**	**Mean**	**%RSD**
Olmesartan Medoximil	Set-1	101.45	100.8	0.6	100.43	100.4	1.0	99.08	99.2	0.4
	Set-2	100.67			100.99			99.26		
	Set-3	100.16			99.06			99.87		
Metoprolol Succinate	Set-1	99.98	100.8	0.8	101.98	100.4	1.5	99.56	99.2	0.6
	Set-2	101.67			100.76			100.78		
	Set-3	100.67			99.03			99.98		

Table-7: Recovery Results

System precision

The five replicate injections of standard preparation were injected to determine the reproducibility of the instrument and %RSD was reported in Table-8.

Parameter	**Specification**	**Observation**	
		Olmesartan Medoximil	**Metoprolol Succinate**
% RSD of standard preparation	NMT 2.0%	0.5%	0.7%

Method precision

The six different sample sets were prepared and injected. Results obtained for %Assay of 6 different sample preparations is depicted in Table-9.

Sample preparation	**% Assay**	
	Olmesartan Medoximil	**Metoprolol Succinate**
1	99.78	100.34
2	100.67	99.78
3	98.99	99.56
4	100.04	98.95
5	99.67	100.67
6	100.98	99.56
Mean	100.0	99.8
% RSD	0.7	0.6

Table-9: Results of Method Precision

Robustness

Robustness results obtained with deliberate change as shown in below Table-10.

Parameters	**Condition**	**% RSD (OLM)**	**% RSD (MET)**
Change in Flow rate (0.8mL/min°C ± 0.1mL/min)	0.7mL/min	0.4%	0.7%
	0.9mL/min	0.6%	0.3%
Change in Column oven (40°C ± 5°C)	35°C	0.6%	0.5%
	45°C	0.7%	0.4%
Change in Wavelength (225nm ± 2 nm)	223	0.4%	0.6%
	227	0.5%	0.4%
Change of pH in buffer (6.8 ± 0.2)	6.6	0.3%	0.5%
	7.0	0.6%	0.3%

Table-10: Results of Robustness

Conclusion

The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method shows no interference by the excipients.

The statistical parameters and recovery data reveals the good accuracy and precision. This method can be useful and suitable for the estimation of the OLM & MET in bulk and pharmaceutical formulations.

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