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A stability indicating assay method development and validation for simultaneous estimation of Olmesartan Medoxamil and Metoprolol Succinate in bulk and pharmaceutical dosage form by RP-HPLC method

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ABSTRACT

A simple, sensitive and reproducible stability indicating RP-HPLC method for the simultaneous determination of Olmesartan medoxomil (OLM) and Metoprolol succinate (METP) in bulk and Pharmaceutical dosage form has been developed and validated. Chromatographic separation was carried out on Thermo Hypersil BDS C_{18} (4.6 x 250 mm, 5µ particle size) column using a mobile phase composed of acetonitrile: phosphate buffer (adjusted to pH 4.8 with 0.1 % OPA) in the ratio of 50:50 % v/v at a flow rate of 1.0 ml/min. The analyte was monitored using UV detector wavelength at 219 nm. The retention time was found to be 2.753 min and 4.112 min for Olmesartan medoxomil and Metoprolol succinate respectively. The proposed method was found to be having linearity in the concentration range of 5-30 µg/ml for Olmesartan medoxomil (r^2 0.99991) and 6.5-37.5 µg/ml for Metoprolol succinate (r^2 0.99994) respectively. The mean % recoveries obtained were found to be 99.86–100.01% for Olmesartan medoxomil and 99.94–100.17% for Metoprolol succinate respectively. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines. The proposed method can be successfully applied for the stability indicating RP-HPLC simultaneous determination of Olmesartan medoxomil (OLM) and Metoprolol succinate (METP) in bulk and combined tablet dosage form and in routine quality control analysis.

Keywords: Olmesartan Medoxomil, Metoprolol Succinate, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Olmesartan Medoxomil is chemically (Fig.1), known as 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. It has a molecular formula of $C_{29}H_{30}N_6O_6$ and molecular weight of 558.59 g/mol. Olmesartan medoxomil belongs to class of angiotensin II receptor antagonists and is a cardio selective drug used to treat hypertension and various cardiovascular disorders. Olmesartan medoxomil is selectively inhibits the binding of angiotensin II to AT1 and this effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure by producing vasodilation, and decreasing peripheral resistance

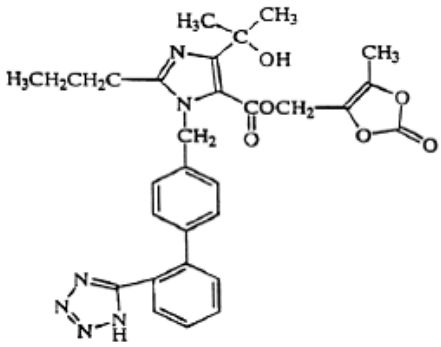

Fig.1 Chemical structure of Olmesartan Medoxomil

Metoprolol succinate is chemically (Fig.2), known as (±) 1(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). It has a molecular formula of $C_{34}H_{56}N_2O_{10}$ and molecular weight of 652.8 g/mol. Metoprolol succinate is an antihypertensive agent (β_1-Adrenergic blocker). Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at β_1-adrenergic receptors in the heart. β_1-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

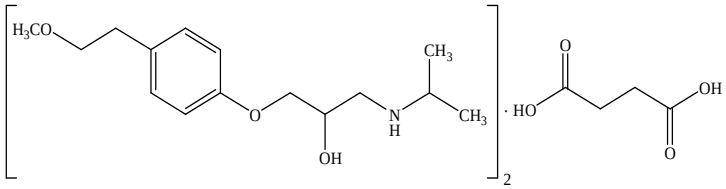

Fig.2 Chemical structure of Metoprolol succinate

Literature survey revealed that few analytical methods were reported so far for both drugs in combination or in alone like Spectrophotometric [1-4], RP-HPLC [5-9], and HPTLC [10] methods. The aim of the present study was to develop a simple, precise, sensitive and selective stability indicating RP-HPLC method with UV detection for the analysis of Olmesartan medoxomil and Metoprolol succinate in bulk and in combined tablet formulation.

MATERIALS AND METHODS

Chemicals and Solvents:

The pharmaceutical grade pure samples of Olmesartan medoxomil and Metoprolol succinate were received as gift samples from Unichem Laboratories, Mumbai. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem.ltd., Mumbai. All the chemicals used were of analytical reagent grade (Qualigens Fine Chemicals Pvt. Ltd., Mumbai). Fixed dose combination tablet formulation (Olmesar-M) containing 20 mg of Olmesartan Medoxomil and 25 mg of Metoprolol succinate (Manufactured by Macleods Pharmaceuticals Pvt. Ltd., Mumbai) were procured from local market.

Instrumentation:

Quantitative HPLC was performed on Waters technologies 2695 series and UV detector module equipped with auto injector using empower software. An UV-2400PC Series UV/Visible double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements.

Chromatographic condition:

Mobile phase	Acetonitrile: phosphate buffer (adjusted to pH 4.8 with 0.1 % OPA) in the ratio of 50:50 % v/v
Column	Thermo Hypersil BDS, C_{18} column (250 x 4.6mm, particle size 5µ)
UV detector wave length	219 nm
Run time	10 min
Flow rate	1.0 ml/min
Injection volume	20µl
Temperature	30^0C

The analytical Thermo Hypersil BDS C_{18} (250 mm x 4.6 mm, 5µ particle size) column was used at a flow rate of 1.0 ml/min and the UV detector wavelength was set at 219 nm. The injection volume was 20µL and temperature at 30ºc.

Preparation of Phosphate buffer:
Accurately weighed quantity of 1.379 g of sodium dihydrogen orthophosphate was dissolved in 1000 ml of water and then adjusted to pH 4.8 with 0.1% OPA. The buffer was filtered through 0.45 µ filter before use.

Preparation of Mobile Phase:
Sodium dihydrogen orthophosphate buffer and acetonitrile were filtered separately through 0.45 µ membrane filters. The filtered solvents were then mixed in the ratio of 50: 50 (% v/v) and degassed for subjecting mixture to sonication for 10 min and resultant solution used as mobile phase.

Preparation of diluent:
Sodium dihydrogen orthophosphate buffer (adjusted to pH 4.8 with 0.1% OPA) and acetonitrile and (50:50 % v/v) used as diluent.

Preparation of standard solution:
Accurately weighed and transferred 20 mg of Olmesartan medoxomil and 25 mg of Metoprolol succinate working standards into a 100 ml clean and dry volumetric flask, 3/4th volume of diluent was added, sonicated to dissolve for 15 minutes and then made up to the final volume with diluent.

Sample solution preparation:
20 tablets were accurately weighed and determined the average weight of each tablet and then crushed to fine powder in a motor with pestle. Then accurately weighed tablet powder equivalent to 20 mg of Olmesartan medoxomil and 25 mg of Metoprolol Succinate was transferred into a 100 ml volumetric flask, 70 ml of diluent was added and sonicated for 30 min and then made up with diluent and filtered. From the filtered solution 1.0 ml was pipetted out into a 10 ml volumetric flask and made up to volume with diluent to obtain final concentrations of 20µg/ml and 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then Injected 20 µl of filtered portion of the sample and standard preparation into the chromatograph. Recorded the responses for the major peaks. Calculated the content of Olmesartan medoxomil and Metoprolol succinate present in each tablet.

Method validation:
Analytical validation parameters for this proposed method were determined according to ICH guidelines.

System suitability:
System suitability was carried out by injecting 20 µl of the standard solutions five times into the chromatographic system. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms. % RSD for peak area of five replicate injections of standard solutions (% RSD NMT 2) were within the limits. The results for system suitability studies are presented in table 1.

Specificity:
The specificity of the method was performed by injecting standard and sample preparations. Chromatograms were recorded. The effect of wide range of excipients and other additives usually present in the formulations in the determination under optimum conditions was also investigated.

Linearity:
The linearity of an analytical method was determined on six concentration levels ranging from 5-30 µg/ml for Olmesartan medoxomil and 6.5-37.5µg/ml for Metoprolol succinate**.** The calibration curve was constructed by plotting peak area against respective concentrations of Olmesartan medoxomil and Metoprolol succinate respectively. The linearity of proposed method was then evaluated by linear regression analysis. The correlation coefficient, slope and intercept were calculated for both Olmesartan medoxomil and Metoprolol succinate as shown in Fig.3 and Fig. 4.

Accuracy:
The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 80%, 100% and 120 % levels using standard addition method, where sample preparations were spiked with known amount of standard preparations and then each concentration was injected triplicate into the chromatographic system.

Precision
System precision
System precision was established by six replicate injections of the standard solution into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Method precision
The method precision study was performed by injecting six sample preparations of marketed formulations into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Intermediate precision
A study was carried out by injecting six standard preparations on different days into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Robustness
Robustness of the method was determined by small deliberate changes in flow rate, mobile organic phase temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Forced degradation studies:
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results showed that for both the solutions, the retention time and peak area of Olmesartan medoxomil and Metoprolol succinate are remained almost similar (%RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr., which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented.

Acid degradation studies:
To 1.0 ml of stock solution of Olmesartan medoxomil and Metoprolol succinate, 1.0 ml of 2N hydrochloric acid was added and refluxed for 30 min at 60 °C. The resultant solution was suitably diluted to obtain 20µg/ml & 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then 20µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies:
To 1.0 ml of stock solution of Olmesartan medoxomil and Metoprolol succinate, 1.0 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60°C. The resultant solution was suitably diluted to obtain 20µg/ml & 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then 20µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Peroxide degradation studies:
To 1.0 ml of stock solution of Olmesartan medoxomil and Metoprolol succinate, 1.0 ml of 20 % hydrogen peroxide (H_2O_2) solution was added and the resultant solution was kept for 30 min at 60 °C. For HPLC study, the resultant solution was suitably diluted to obtain 20µg/ml & 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then 20µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies:
1.0 ml of stock solution of Olmesartan medoxomil and Metoprolol succinate was placed in oven at 105 °C for 6 hr to study dry heat degradation. The resultant solution was diluted to 20µg/ml & 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then 20µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the drug solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was suitably diluted to obtain 20µg/ml & 25µg/ml of Olmesartan medoxomil and Metoprolol succinate respectively. Then 20µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the estimation of Olmesartan medoxomil and Metoprolol succinate in bulk and pharmaceutical dosage form. Separation was done by using mobile phase composed of acetonitrile: phosphate buffer (adjusted to pH 4.8 with 0.1% OPA) in the ratio of 50:50 % v/v on Thermo Hypersil BDS

C_{18} (4.6 x 250mm, 5µ particle size) column at a flow rate 1.0 ml/min using UV detection at 219 nm. The retention times were found to be 2.753 min and 4.112 min for Olmesartan medoxomil and Metoprolol succinate respectively. The Isobestic point of Olmesartan medoxomil and Metoprolol succinate was found to be 219 nm (as shown in figure 3) after scanning 10µg/ml standard solutions of both Olmesartan medoxomil and Metoprolol succinate in the UV region of 200-400 nm against reagent blank methanol and was utilized for HPLC method development.

Linearity was evaluated in the concentration range of 5-30 µg/ml for Olmesartan Medoxomil and 6.5-37.5 µg/ml for Metoprolol succinate. The calibration curves of Olmesartan Medoxomil and Metoprolol succinate were described by the equation y = 66826.9x +2163.5 and y = 69826.9x +2010.3 with correlation coefficient of 0.9999 as shown in figure 4 and figure 5 respectively. The standard and sample chromatograms in the specifity studies are shown in figure 6 and figure 7. System suitability results are shown in table 1. The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the method was precise, accurate and robust. Accuracy data as shown in table 2. The validation summary parameters and assay results obtained from the marketed formulations are shown in table 3 and table 4. The results of robustness studies are shown in table 5 and table 6. The stress testing chromatograms for both Olmesartan Medoxomil and Metoprolol succinate are shown in figure 8 to figure 12 and results are shown in table 7 and table 8.

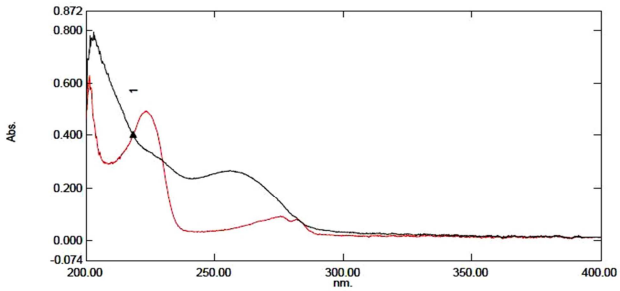

Fig. 3 Isobestic point of Olmesartan medoxomil and Metoprolol succinate (1=219 nm)

Table 1: System Suitability Results

S.No	System suitability parameters	Olmesartan medoxomil	Metoprolol succinate
1	USP Tailing	1.11	1.12
2	Resolution	6.14	
3	Retention time (min)	2.753	4.112
4	USP Plate count	3603	4265

Table 2: Accuracy data

Sample	Concentration Level	Peak area*	Amount added (mg)	Amount recovered (mg)	Mean % Recovery *± SD
Olmesartan Medoxomil	80%	1082628	16.00	15.98	99.86 ±0.17
	100%	1354047	20.00	19.98	99.91±0.55
	120%	1626363	24.00	24.00	100.01± 0.62
Metoprolol Succinate	80%	1413256	20.00	20.01	100.07±0.27
	100%	1768304	25.00	25.04	100.17±0.43
	120%	2117093	30.00	29.98	99.94±0.71

**Mean of three determinations*

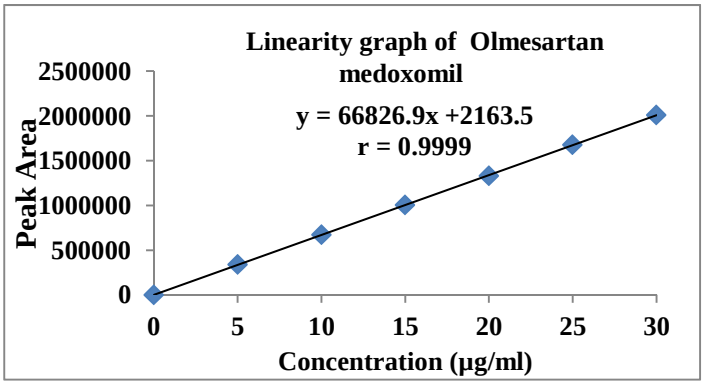

Fig.4 Linearity Graph of Olmesartan medoxomil (5-30 µg/ml)

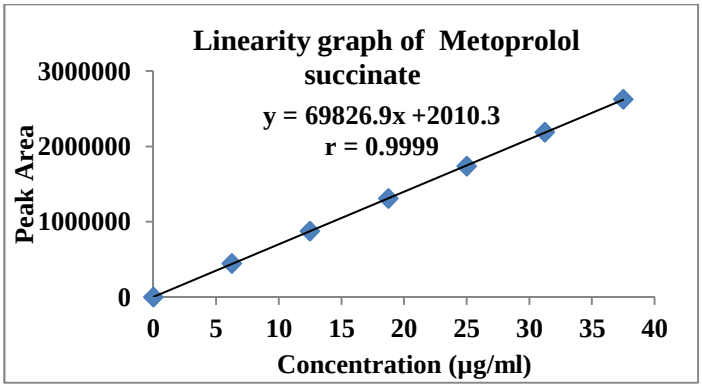

Fig.5 Linearity Graph of Metoprolol succinate (6.25-37.5 µg/ml)

Linearity:

The calibration curve was found to be linear over the concentration range of 5-30 µg/ml for Olmesartan Medoxomil and 6.25-37.5µg/ml for Metoprolol succinate. The correlation coefficient was found to be 0.9999 for both Olmesartan Medoxomil and Metoprolol succinate respectively.

Table 3: Validation Parameters of the proposed RP-HPLC Method

Parameter	Olmesartan medoxomil	Metoprolol succinate
Regression equation	y = 66826.9x +2163.5	y = 69826.9x +2010.3
Correlation coefficient	0.99991	0.99994
LOD (µg/ml)	0.76	0.24
LOQ (µg/ml)	2.48	0.84
System precision (% RSD)	0.31	0.27
Method precision (% RSD)	0.53	0.12
Intermediate precision (% RSD)	0.22	0.34

Table 4: Results of assay in Marketed formulation

Brand	Drug	Standard peak area	Sample peak area	Labeled amount (mg)	Amount found (mg)	% Assay	% RSD*
Olmesar-M	Olmesartan Medoxomil	1354144	1353279	20.0	19.89	99.54%	0.27
	Metoprolol succinate	1767883	1763133	25.0	24.96	99.53%	0.34

**Mean of two determinations*

Specificity studies:

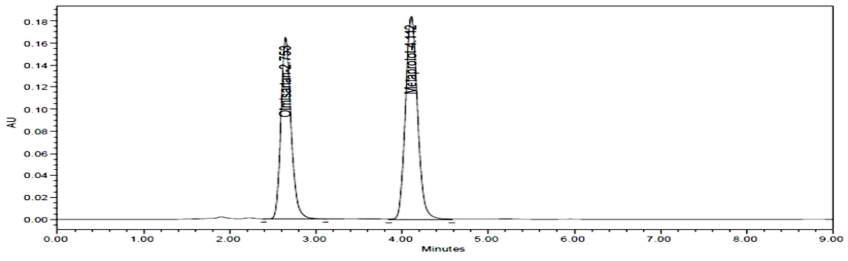

Fig.6 Typical chromatogram of standard

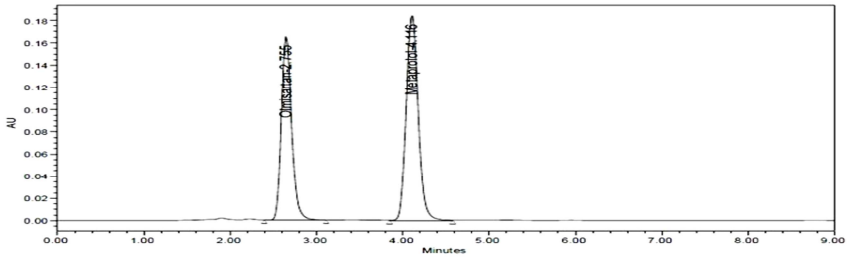

Fig.7 Typical chromatogram of sample

Robustness:

The developed method is robust with deliberate changes in variation of mobile organic phase composition, flow rate and temperature for both Olmesartan Medoxomil and Metoprolol succinate respectively.

Table 5: Results of robustness study of Olmesartan Medoxomil

S.No.	Parameter	Change Level	Olmesartan Medoxomil			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.8	2.834	1454193	1.22	3952
		1.2	2.489	1277468	1.12	3253
2.	Mobile organic phase composition (±10%v/v/v)	40:60	2.512	1382544	1.14	4922
		60:40	2.234	1409558	1.18	3014
3.	Temperature(±5°C)	25 ^{0}C	2.637	1359952	1.17	3846
		35 ^{0}C	2.664	1371334	1.09	4025

Table 6: Results of robustness study of Metoprolol succinate

S.No.	Parameter	Change Level	Metoprolol succinate			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.8	4.393	1898735	1.18	5122
		1.2	3.856	1666770	1.12	3847
2.	Mobile organic phase composition(±10%v/v/v)	40:60	4.242	1382544	1.21	5465
		60:40	3.546	1409558	1.17	3781
3.	Temperature(±5°C)	25 ^{0}C	4.381	1774045	1.06	3964
		35 ^{0}C	3.799	1786499	1.14	4728

Forced degradation studies:

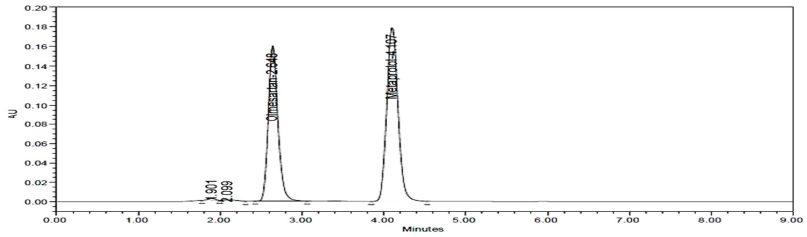

Fig.8 Chromatogram of Acid hydrolysis

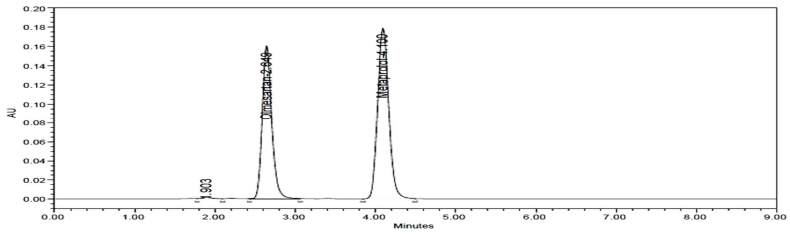

Fig.9 Chromatogram of Base hydrolysis

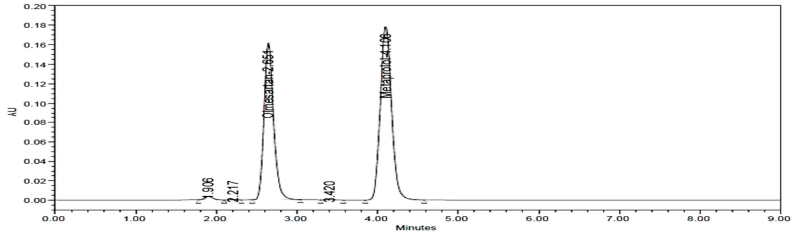

Fig.10 Chromatogram of Oxidation (peroxide)

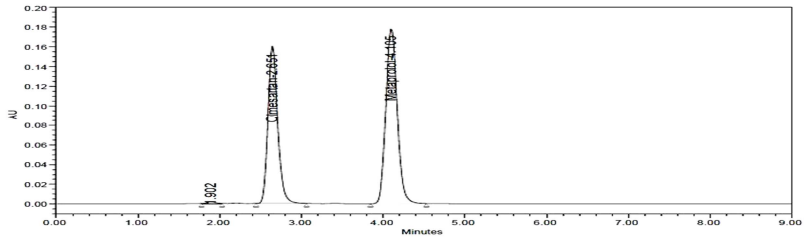

Fig.11 Chromatogram of Heat Exposure

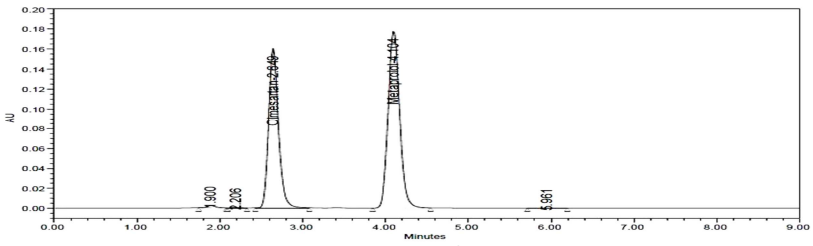

Fig.12 Chromatogram of UV Exposure

Table 7: Degradation Study of Olmesartan medoxomil

S.No.	Stress Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation Hydrolysis	1306186	96.00	3.54
2	Base Hydrolysis	1314194	96.59	2.95
3	Heat Exposure	1316691	96.78	2.76
4	Oxidation degradation	1291402	94.92	4.62
5	UV Exposure	1311905	96.42	3.12

Table 8: Degradation Study of Metoprolol succinate

S.No.	Stress Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation Hydrolysis	1703500	95.68	3.85
2	Base Hydrolysis	1708644	95.97	3.56
3	Heat Exposure	1714147	96.28	3.25
4	Oxidation degradation	1699110	95.43	4.10
5	UV Exposure	1707607	95.91	3.62

CONCLUSION

From this study, it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Olmesartan medoxomil and Metoprolol succinate in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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