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# METHOD DEVELOPMENT AND VALIDATION FORTHE SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND METOPROLOL TARTRATEIN BULK AND SOLID DOSAGE FORM BY RP-HPLC.

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**ABSTRACT**

A simple, Accurate, precise technique was developed for the simultaneous estimation of Olmesartam Medoxomil and Metoprolol Tartrate in bulk and solid dosage form. Chromatogram was run through Symmetry Xterra- $C_{18}$, BDS column (150 x 4.6mm, 5µ) column. Mobile phase containing Phosphate Buffer (pH-2.8)and Acetonitrile taken in the proportions 35:65 v/v was pumped through column at flow rate of 0.5 ml/min. Temperature was maintained Ambient. Optimised wavelength selected was 284nm. Retention time of Olmesartam Medoxomil and Metoprolol Tartratewere observed to be 3.624min and 5.178min. %RSD of the Olmesartam Medoxomil and Metoprolol Tartratewere and observed to be 0.39 and 0.86 respectively. %Recovery was obtained as99.69% for Olmesartam Medoxomil and99.45% for Metoprolol Tartrate respectively. LOD, LOQ values obtained from regression equations of Olmesartam Medoxomil and Metoprolol Tartrate were 0.015, 0.13 and 0.046, 0.40 respectively. Regression equation of Olmesartam Medoxomilis y = 251942x + 110535, and y = 9709x + 11274 of Metoprolol Tartrate. Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular quality control test in industries.

## INTRODUCTION

Olmesartan medoxomil is a potent, orally active, selective angiotensin II receptor (type $AT_1$) antagonist. Used to treat high blood pressure (hypertension). It is used in lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. *Metoprololtartrate* is a cardio-selective beta-adrenergic blocking agent. It is used to treat and prevent heart attacks, lower high blood pressure and reduce chest pain (angina). Olmesartan Medoxomil + Metoprolol Succinate is used in the treatment of Hypertension (high blood pressure). [(1-2)]

## MATERIALS and METHODS:

**Preparation of phosphate buffer:** Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate was taken into a 1000ml of volumetric flask, add about 900ml of distilled water. The flask was shaken until the particles get dissolved, made up to the mark with water and then ad 1ml of triethylamine. The pH was

adjusted to 3.0 with dilute ortho phosphoric acid solution.

**Preparation of Mobile phase:** Accurately measured500ml of (50%) of HPLC acetonitrile and 500ml of phosphate buffer (50%) were mixed and degassed in a digital ultra sonicator for 25 minutes and then filtered through 0.45 microns filter under vacuum filtration.[(3)]

**Preparation of sample solution**: Accurately weighed 10 tablets 10 mg of powder equivalent Olmesartan Medoxomil and 10 mg of Metoprolol Tartrate API standards were accurately weighed and is transferred into a neat and dry volumetric flask of 100ml. About 70ml of diluent was added and allow for sonicate to remove the complete air bubbles formed in it, which is again make up to mark with same diluent. From the stock solution 1.2ml of Olmesartan Medoxomil and 3ml of Metoprolol Tartrate stock solutions was pipetted out and transferred in to 10ml volumetric flask which is again diluted with diluent up to the mark to get 12µg/ml Olmesartan Medoxomil and 30µg/ml Metoprolol Tartrate.

**Preparation of Standard solution:** 10 mg of Olmesartan Medoxomil and 10 mg of Metoprolol Tartrate API standards were accurately weighed and is transferred into a neat and dry volumetric flask of 100ml. About 70ml of diluent was added and allow for sonicate to remove the complete air bubbles formed in it, which is again make up to mark with same diluent. From the stock solution 1.2ml of Olmesartan Medoxomil and 3ml of Metoprolol Tartrate stock solutions was pipetted out and transferred in to 10ml volumetric flask which is again diluted with diluent up to the mark to get 12µg/ml Olmesartan Medoxomil and 30µg/ml Metoprolol Tartrate.[(4)]

**Procedure:** Samples were injected by changing chromatographic conditions and the chromatograms were recorded.

## RESULTS AND DISCUSSION:

**Method validation:** Specificity, linearity, range, Accuracy, precision, Repeatability,Intermediate precision,limit of detection,limit of Quantification,Robustness.

**SPECIFICITY:** The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.[(5)]

**LINEARITY:** The regular stock solution consists of six linear concentrations (4µg/ml-20µg/ml Olmesartan Medoxomil and 10µg/ml-50µg/ml-Metoprolol tartrate). They were tabulated in table num-1 and 2.

**ACCURACY:** Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no.3 and 4.

**PRECISION:** The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Olmesartan Medoxomil and Metoprolol Tartratewere injected atintermediate precision and repeatability. The %RSD was calculated and results were reported at table no. 5, 6, 7 and 8.[(6)]

**LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):** The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 9 .[(7)]

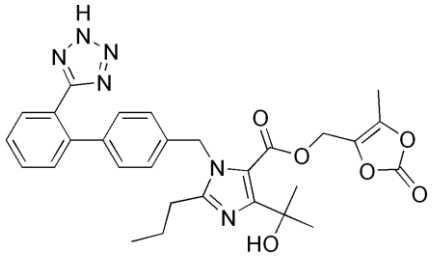

**Fig-1: structure of Olmesartan**

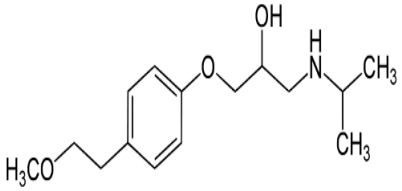

**Fig-2: structure of Metoprolol Tartrate**

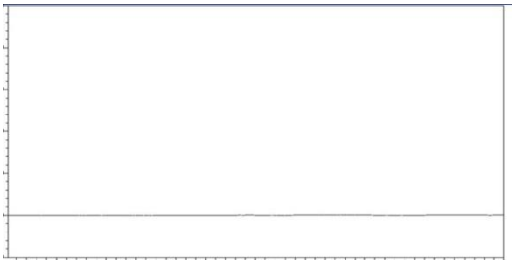

**Fig-3: Chromatogram showing blank**

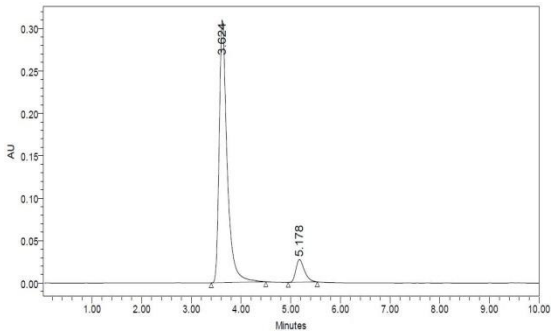


**Fig-4: Chromatogram showing optimised condition**

**Table 1: Linearity results for Olmesartan Medoxomil**

| Linearity Level | Concentration (µg/ml) | Peak Area |
|---|---|---|
| Level 0 | 0 | 0 |
| Level 1 | 4 | 1181514 |
| Level 2 | 8 | 2181557 |
| Level 3 | 12 | 3190741 |
| Level 4 | 16 | 4161134 |
| Level 5 | 20 | 5064755 |

Table 2: Linearity results for Metoprolol Tartrate

| Linearity Level | Concentration (µg/ml) | Peak Area |
|---|---|---|
| Level 0 | 0 | 0 |
| Level 1 | 10 | 109398 |
| Level 2 | 20 | 218339 |
| Level 3 | 30 | 311805 |
| Level 4 | 40 | 394694 |
| Level 5 | 50 | 489759 |

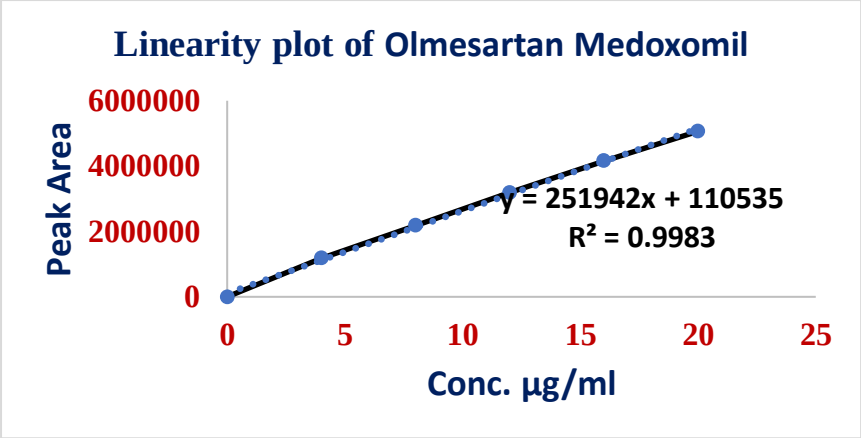


**Fig-5: showing calibration curve ofOlmesartan Medoxomil**

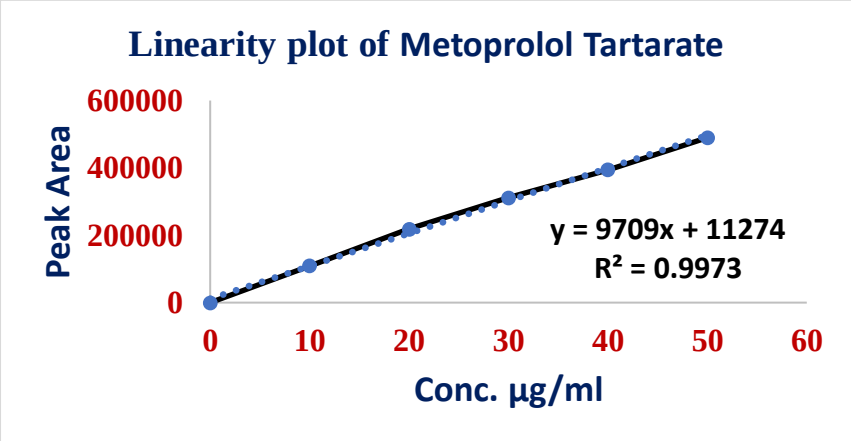


**Fig-6: showingcalibration curve of Metoprolol Tartrate**

Table 3 : Accuracy data for Olmesartan Medoxomil

| Concentration of sample taken (µg/ml) | % of spiked level | Amount added (µg) | Amount found (µg) | % Recovery | Statistical Analysis of % Recovery |
|---|---|---|---|---|---|
| 10µg/ml | 50% Injection 1 | 6 | 5.96 | 99.2 | MEAN = 100.33 %RSD = 1.47 |
| | 50% Injection 2 | | 5.99 | 99.8 | |
| | 50% Injection 3 | | 6.1 | 102 | |
| | 100% Injection 1 | 12 | 11.92 | 99.2 | MEAN = 99.46 %RSD = 0.31 |
| | 100% Injection 2 | | 11.94 | 99.4 | |
| | 100% Injection 3 | | 11.98 | 99.8 | |
| | 150% Injection 1 | 18 | 17.8 | 98.7 | MEAN = 99.30 %RSD = 0.65 |
| | 150% Injection 2 | | 17.9 | 99.4 | |
| | 150% Injection 3 | | 18 | 100 | |

Table 4 : Accuracy data for Metoprolol Tartrate

| Concentration of sample taken (µg/ml) | % of spiked level | Amount added (µg) | Amount found (µg) | % Recovery | Statistical Analysis of % Recovery |
|---|---|---|---|---|---|
| 150µg/ml | 50% Injection 1 | 15 | 14.95 | 99.6 | MEAN = 99.73 %RSD = 0.51 |
| | 50% Injection 2 | | 14.91 | 99.3 | |
| | 50% Injection 3 | | 15.06 | 100.29 | |
| | 100% Injection 1 | 30 | 29.88 | 98.5 | MEAN = 99.27 %RSD = 0.68 |
| | 100% Injection 2 | | 29.91 | 99.6 | |
| | 100% Injection 3 | | 29.95 | 99.73 | |
| | 150% Injection 1 | 45 | 44.72 | 99.27 | MEAN = 99.36 %RSD = 0.08 |
| | 150% Injection 2 | | 44.79 | 99.43 | |
| | 150% Injection 3 | | 44.77 | 99.38 | |

Table-5: Intermediate precision data forOlmesartan Medoxomil

| Injection | Retention Time | Area of the peak | Height of the Peak | Peak plate count | Peak tailing |
|---|---|---|---|---|---|
| 1 | 3.623 | 3460837 | 32985 | 4761 | 1.4 |
| 2 | 3.629 | 3471289 | 32662 | 4782 | 1.5 |
| 3 | 3.629 | 3488123 | 32945 | 4639 | 1.4 |
| 4 | 3.623 | 3487128 | 33312 | 4614 | 1.5 |
| 5 | 3.633 | 3484139 | 32544 | 4638 | 1.4 |
| 6 | 3.635 | 3482645 | 33985 | 4715 | 1.5 |
| Mean | | 3479026 | | | |
| SD | | 10754 | | | |
| % RSD | | 0.31 | | | |

Table-6: Intermediate precision data for Metoprolol Tartrate

| Injection | Retention Time | Area of the peak | Height of the Peak | Peak plate count | Peak tailing |
|---|---|---|---|---|---|
| 1 | 5.175 | 336517 | 4261 | 3519 | 1.4 |
| 2 | 5.174 | 336824 | 4316 | 3494 | 1.3 |
| 3 | 5.202 | 339674 | 4317 | 3439 | 1.4 |
| 4 | 5.174 | 331423 | 4298 | 3425 | 1.3 |
| 5 | 5.193 | 338354 | 4279 | 3525 | 1.4 |
| 6 | 5.174 | 339712 | 4312 | 3493 | 1.3 |
| Mean | | 337084 | | | |
| SD | | 3087 | | | |
| % RSD | | 0.92 | | | |

Table-7: Repeatability results for Olmesartan Medoxomil

| Injection | Retention Time | Area of the peak | Height of the Peak | Peak plate count | Peak tailing |
|---|---|---|---|---|---|
| 1 | 3.623 | 3480636 | 33168 | 4874 | 1.5 |
| 2 | 3.624 | 3463599 | 33210 | 4873 | 1.5 |
| 3 | 3.629 | 3498779 | 33153 | 4574 | 1.5 |
| 4 | 3.802 | 3497870 | 33421 | 4567 | 1.5 |
| 5 | 3.624 | 3490276 | 33521 | 4500 | 1.5 |
| 6 | 3.822 | 3496298 | 34621 | 4618 | 1.5 |
| Mean | | 3487909 | | | |
| SD | | 13691 | | | |
| % RSD | | 0.39 | | | |

Table-8: Repeatability results for Metoprolol Tartrate

| Injection | Retention Time | Area of the peak | Height of the Peak | Peak plate count | Peak tailing |
|---|---|---|---|---|---|
| 1 | 5.175 | 323863 | 4361 | 3579 | 1.4 |
| 2 | 5.170 | 325248 | 4298 | 3574 | 1.4 |
| 3 | 5.174 | 322052 | 4317 | 3339 | 1.4 |
| 4 | 5.408 | 328133 | 4328 | 3325 | 1.4 |
| 5 | 5.170 | 328655 | 4289 | 3325 | 1.4 |
| 6 | 5.407 | 328712 | 4302 | 3463 | 1.4 |
| Mean | | 326110 | | | |
| SD | | 2814 | | | |
| % RSD | | 0.86 | | | |

Table-9: LOD and LOQ data for Olmesartan Medoxomil and Metoprolol Tartrate

| Drug | Average slope | Average intercept | Standard deviation of the intercept | Regression coefficient ($R^2$) | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|---|---|---|---|
| Olmesartan medoxomil | 251942 | 110535 | 1168 | 0.998 | 0.015 | 0.046 |
| Metoprolol Tartrate | 9709 | 11274 | 398 | 0.997 | 0.13 | 0.40 |

Table-10: Robustness data for Olmesartan Medoxomil

| Flow 0.4 ml | Std Area | Tailing factor | Flow 0.5ml | Std Area | Tailing factor | Flow 0.6 ml | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| | 4051994 | 1.62 | | 4532189 | 1.58 | | 4964755 | 1.58 |

Table-11: Robustness data for Metoprolol Tartrate

| Flow 0.4 ml | Std Area | Tailing factor | Flow 0.5 ml | Std Area | Tailing factor | Flow 0.6 ml | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| | 395859 | 1.52 | | 432687 | 1.47 | | 459759 | 1.47 |

**ROBUSTNESS:** The small deliberate changes in method like flow ratewas made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.4ml/min), flow plus (0.6ml/min), temperature ambient was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.10 and 11.[8]

## CONCLUSION:

A simple, fast, accurate and specific RP-HPLC method has been developed for the simultaneous quantification of Olmesartan and Metoprolol in bulk and tablet dosage form by studying different parameters. The maximum absorbance was found to be at 284nm for Olmesartan and 284nm for Metoprolol. The common wavelength observed was 284nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry Xterra – C18, BDS column and resulted good peak shape. 30℃ temperature was found to be suitable for the nature of drug solution.The flow rate was fixed at 0.5ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 65:35(Acetonitrile:Phosphate buffer) was fixed due to good symmetrical peaks and good resolution. So, this mobile phase was used for the proposed study.

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