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# Method Development and Validation of Simultaneous Estimation of Metolazone and Spiranolactone Using RP-HPLC Method

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## Abstract

High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Metolazone and Spironolactone was done by RP-HPLC. The Phosphate buffer was $p^H$ 3.0 and the mobile phase was optimized with consists of Phosphate buffer: Methanol PH 4.5(20:80 v/v). Kromosil $C_{18}$ Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Metolazone and Spironolactone were found to be from 100-500 µg/ml of Metolazone and 1-5µg/ml of Spironolactone. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Metolazone and Spironolactone. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise, and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

**Keywords**
Kromosil $C_{18}$ Column, Metolazone and Spironolactone, RP-HPLC

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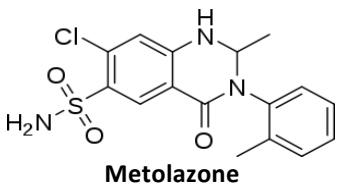
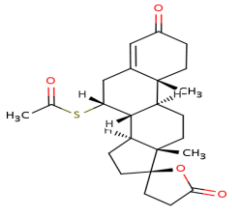
## INTRODUCTION:

Metolazone is a thiazide-like diuretic marketed under the brand names Zytanix, Metoz, Zaroxolyn, and Mykrox. It is primarily used to treat congestive heart failure and high blood pressure. Metolazone indirectly decreases the amount of water reabsorbed into the bloodstream by the kidney, so that blood volume decreases and urine volume increases.




**Metolazone**

Spironolactone, sold under the brand name Aldactone among others, is a medication that is primarily used to treat fluid build-up due to heart failure, liver scarring, or kidney disease.



**Spironolactone**

## MATERIALS AND METHOD:

### Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000$^{+}$pH meter, Weighing machine.

### Chemicals

Metolazone and Spironolactone, Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

**Trial-6 (Optimized)**

**Table 1. Chromatographic condition**

| Parameters | Description |
|---|---|
| Flow rate | 1ml min$^{-1}$ |
| Column | Kromosil $C_{18}$ Column (250mm x 4.6mm)5µg. |
| Mobile Phase | Phosphate buffer:Methanol P$^{H}$ 4.5(20:80 v/v) |
| Buffer | Potassium dihydrogen orthophosphate PH 4.5 adjusted with Orthophosphoric acid |
| Detector | PDA |
| Column temperature | Ambient |
| Type of elution | Isocratic |
| Wavelength | 254 nm |
| Injection volume | 20µl |
| Run time | 10min |

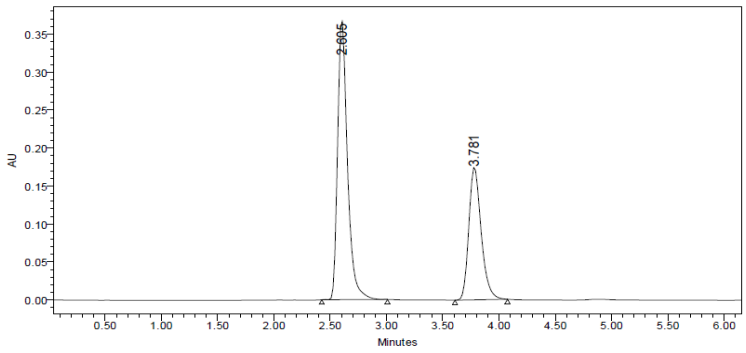



**Fig.1 Chromatogram of Trail-6**

## METHOD VALIDATION

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision

- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

**RESULTS AND DISCUSSION:**

**System suitability**

**Table 2: Results of system suitability parameters for Metolazone and Spironolactone**

| S.No | Name | Retention time (min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | **Metolazone** | 2.5 | 124505 | 213642 | | 1.2 | 4673.4 |
| 2 | **Spironolactone** | 3.9 | 1308495 | 154566 | 6 0 | 1.3 | 6090.3 |

**Precision**

**Table 3: Results of method precession for Metolazone**

| Injection | Area |
|---|---|
| Injection-1 | 1302729 |
| Injection-2 | 1302947 |
| Injection-3 | 1303236 |
| Injection-4 | 1303977 |
| Injection-5 | 1309759 |
| Average | 1304529.8 |
| Standard Deviation | 2961.1 |
| %RSD | 0.2 |

**Table 4: Results of method precession for Spironolactone**

| Injection | Area |
|---|---|
| Injection-1 | 123149 |
| Injection-2 | 123766 |
| Injection-3 | 124271 |
| Injection-4 | 124691 |
| Injection-5 | 124956 |
| Average | 124162.7 |
| Standard Deviation | 725.6 |
| %RSD | 0.6 |

**Intermediate Precession (Ruggedness)**

**Table 5: Results of Intermediate precision for Metolazone**

| Injection | Area |
|---|---|
| Injection-1 | 1300148 |
| Injection-2 | 1304520 |
| Injection-3 | 1305937 |
| Injection-4 | 1306476 |
| Injection-5 | 130871 |
| Average | 1305070.2 |
| Standard Deviation | 3061.8 |
| %RSD | 0.2 |

**Table 6: Results of Intermediate precision for Spironolactone**

| Injection | Area |
|---|---|
| Injection-1 | 122487 |
| Injection-2 | 122626 |
| Injection-3 | 122632 |
| Injection-4 | 122702 |
| Injection-5 | 122962 |
| Average | 122681.8 |
| Standard Deviation | 174.8 |
| %RSD | 0.1 |

**Accuracy**

**Table-7 Accuracy (recovery) data for Metolazone**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 656659.5 | 5.0 | 5.036 | 100.7% | |
| 100% | 1304258 | 10.0 | 10.003 | 100.0% | 99.84% |
| 150% | 1854608 | 14.4 | 14.224 | 98.780% | |

**Table-8 Accuracy (recovery) data for Spironolactone**

| % Concentration (At specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 65800 | 5.3 | 5.34 | 100.8% | |
| 100% | 124353 | 10 | 10.10 | 100.01% | 100.51% |
| 150% | 177940 | 14.2 | 14.45 | 99.68% | |

**Linearity**

**Table-9 Area of different concentration of Metolazone**

| S.No. | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 100ppm | 668934 |
| 2 | II | 200ppm | 956781 |
| 3 | III | 300ppm | 1313873 |
| 4 | IV | 400ppm | 1563458 |
| 5 | V | 500ppm | 1867084 |
| **Correlation Coefficient** | | | **0.999** |

**Table-10 Area of different concentration of Spironolactone**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 1ppm | 66510 |
| 2 | II | 2ppm | 94701 |
| 3 | III | 3ppm | 124802 |
| 4 | IV | 4ppm | 152731 |
| 5 | V | 5ppm | 179732 |
| **Correlation Coefficient** | | | **0.999** |

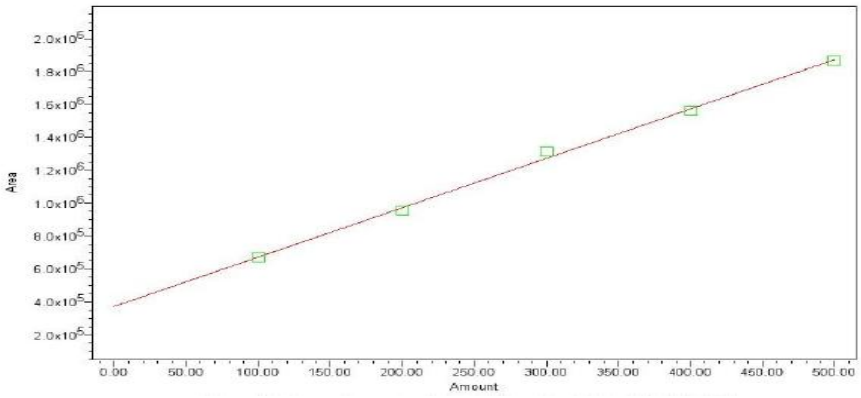



**Figure 2 Calibration graph for Metolazone**

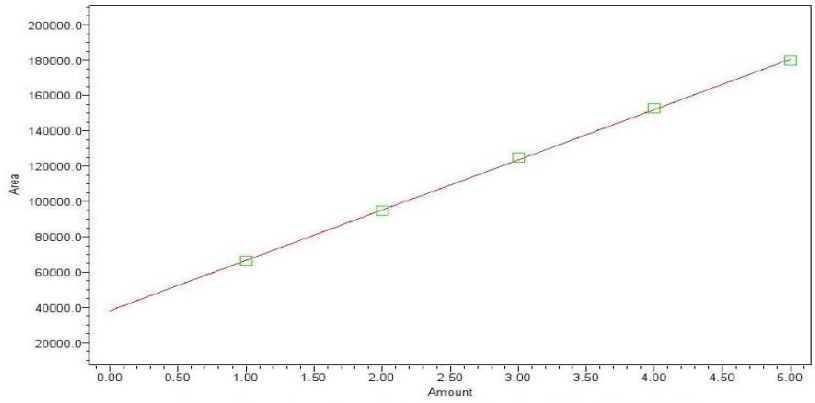



**Figure 3 Calibration graph for Spironolactone**

**Table-11 Analytical performance parameters of Metolazone and Spironolactone**

| Parameters | Metolazone | Spironolactone |
|---|---|---|
| Slope (m) | 66574 | 12529 |
| Intercept (c) | 53592 | 50245 |
| Correlation coefficient ($R^2$) | 0.999 | 0.999 |

**Limit of Detection**

**Table-12 Results of LOD**

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| Metolazone | 52 | 152 | 2.9 |
| Spironolactone | 52 | 156 | 3 |

**Table no-13 Results of LOQ**

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| Metolazone | 52 | 522 | 10.03 |
| Spironolactone | 52 | 524 | 10.1 |

**Robustness**

**Table-14 Flow Rate (ml/min) data for Metolazone**

| S. No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.6 | 5339.9 | 1.4 |
| 2 | 0.8 | 4673.4 | 1.3 |
| 3 | 1.0 | 5216.0 | 1.4 |

**Table-15 flow rate (ml/min) data for Spironolactone.**

| S. No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.8 | 7063.3 | 1.3 |
| 2 | 1.0 | 6090.3 | 1.2 |
| 3 | 1.2 | 6998.0 | 1.3 |

**Table -16 Change in Organic Composition in the Mobile Phase for Metolazone**

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 4508.4 | 1.3 |
| 2 | *Actual | 4673.4 | 1.4 |
| 3 | 10% more | 4318.1 | 1.3 |

**Table -17 Change in Organic Composition in the Mobile Phase for Spironolactone**

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 6387.7 | 1.2 |
| 2 | *Actual | 6090.3 | 1.2 |
| 3 | 10% more | 6232.5 | 1.2 |

**SUMMARY AND CONCLUSION:**

The study is focused to develop and validate HPLC methods for estimation of Metolazone and Spironolactone in tablet dosage form.

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