

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 C₁₈ 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.

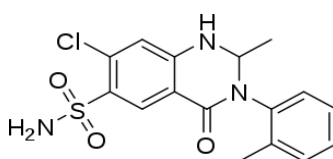
Keywords

Kromosil C₁₈ Column, Metolazone and Spironolactone, RP-HPLC

INTRODUCTION:

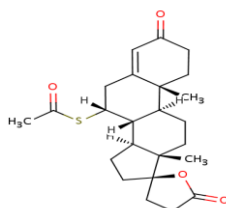
Metolazone is a thiazide-like diuretic marketed under the brand names Zytanix, Metoz, Zaroxolyn, and Mykrox. It is primarily used to treat congestive

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

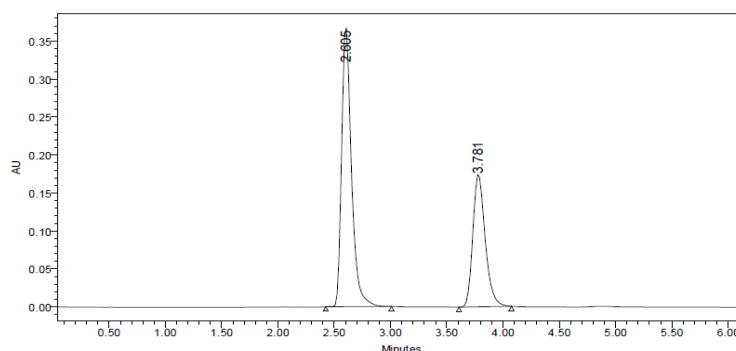


Fig.1 Chromatogram of Trail-6

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 (μ V sec)	Height (μ V)	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 precision 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 precision 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 Precision (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

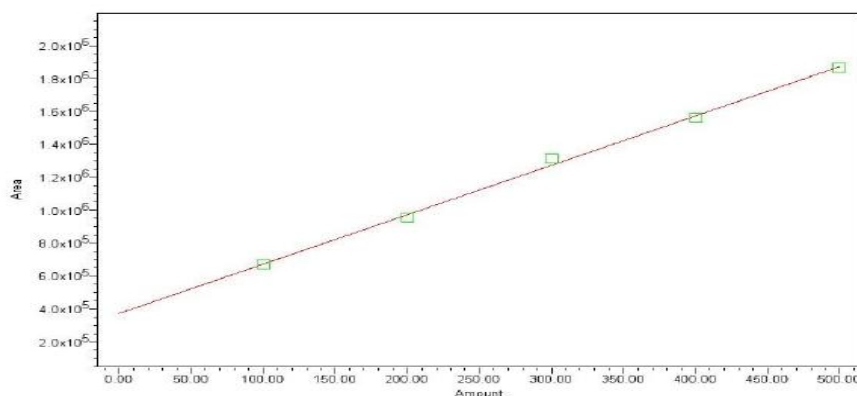


Figure 2 Calibration graph for Metolazone

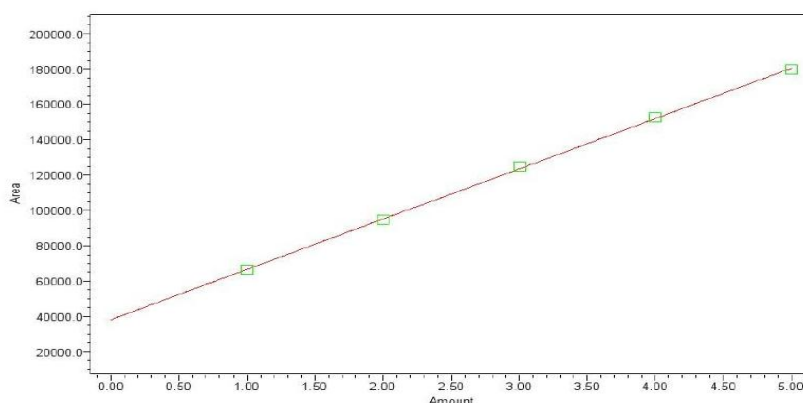


Figure 3 Calibration graph for Spironolactone

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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