Analytical method development and validation of simultaneous estimation of Metolazone and Spironolactone in bulk and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

This paper describes a novel, simple, precise, accurate, sensitive, rapid reversed-phase liquid chromatographic method for simultaneous estimation of Metolazone and Spironolactone in bulk and pharmaceutical dosage form. The chromatographic separation was achieved on WATERS E 2695 HPLC separation module equipped with ALTIMA column (150mm×4.6mm×5µm) and UV-Detector (Waters) using Ortho phosphate buffer and Acetonitrile in the ratio of (35:65v/v) as mobile phase at a flow rate of 1.0ml/min. The detection was carried out at 234nm.The retention time of Metolazone and Spironolactone was found to be 2.127 and 4.103 min respectively. Linearity was observed in the concentration range of 1.25-7.5µg/ml for Metolazone and 12.5-75µg/ml for Spironolactone %Recoveries obtained for Metolazone and Spironolactone were 100.1% & 99.98% respectively .The %RSD below 2.0 shows the high precision of proposed method. The method was validated for precision, Recovery, Specify and Detection and Quantification limits in accordance with ICH guidelines.

KEYWORDS: Metolazone, Spironolactone, RP-HPLC, Simultaneous estimation, Validation.

INTRODUCTION

Metolazone: It is chemically known as 7-chloro-2-methyl-4-oxo-3-o-tolyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide and its empirical formula is $C_{16}H_{16}ClN_3O_3S$ with a molecular weight of 365.835 g/mol. Metolazone is a thiazide-like diuretic marketed under the brand names Zytanix from Zydus Cadila, Zaroxolyn, and Mykrox. It is primarily used to treat congestive heart failure and high blood pressure. Metolazone is sometimes used together with loop diuretics such as furosemide or bumetanide, but these highly effective combinations can lead to dehydration and electrolyte abnormalities

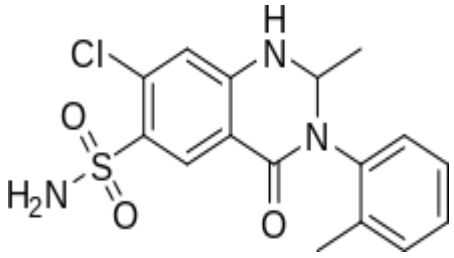


Figure.1. Metolazone

Spironolactone: It is chemically known as 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone or 17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone acetate and its empirical formula is $C_{24}H_{32}O_4S$ with a molecular weight of 416.574 g/mol. Spironolactone is a potassium sparing diuretic that acts by antagonism of aldosterone in the distal renal tubules. It is used mainly in the treatment of refractory edema in patients with congestive heart failure, nephrotic syndrome, or hepatic cirrhosis. Its effects on the endocrine system are utilized in the treatments of hirsutism and acne but they can lead to adverse effects.

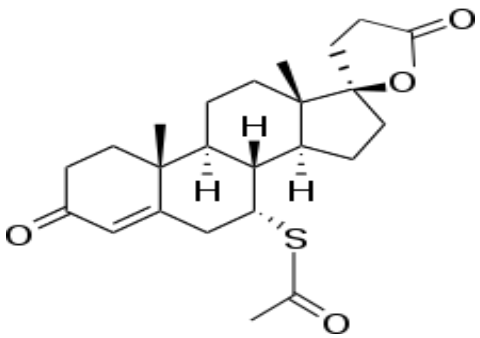


Figure.2. Spironolactone

MATERIALS AND METHODS

All chemicals used of HPLC grade (MERCK.chem.Ltd.,Mumbai) double distilled water was used throughout the study.Fixed dose combination Tablet (METOLACTONE) containing 5mg of Metolazone and 50mg of Spironolactone was procured from local market.

Instruments: HPLC (WATERS E 2695) separation module equipped with ALTIMA column (150×4.6mm×5µm) UV-Detector (Waters), Ultrasonic bath sonicator (SARTORIUS), Analytical balance (ENERTECH) and Vaccum pump (BIOTECHNICS) were used.

Chromatographic mode: The reverse phase HPLC was selected for separation, as it was convenient than any other forms of the liquid Chromatography and was more likely to give good peak shapes at a reasonable retention times.

Preparation of Mobile Phase: Ortho Phosphate Buffer, and Acetonitrile taken in the ratio are 35:65

Selection of Detection wavelength: The detection wavelength was selected as 234 nm where both drugs show significant absorbance and hence this λmax was selected for further studies.

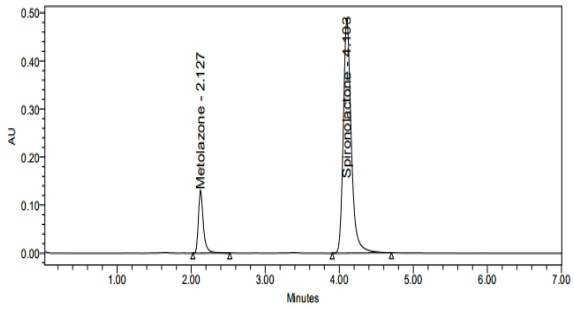
Preparation of Standard Solution: Accurately Weighed quantity of 12.5mg& 5mg of Metolazone & Spiranolactone was transferred in to a 25ml clean dry volumetric flasks, add 3/4th vol of diluent, sonicated for 30 minutes and make up to the final volume with diluent.

Sample preparation:

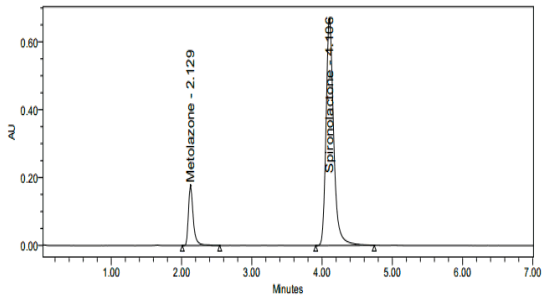
Step 1: 20 tablets were weighed and calculated the average weight of each tablet then the weight equivalent to 20 tablets were transferred into a 100 ml volumetric flask, 70ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

Step 2: From the filtered solution 0.2ml was pippeted out into a 10ml volumetric flask and made upto the mark with diluent.

Procedure for Assay: A steady base line was recorded with the optimized chromatographic conditions and standard and sample solutions of 10µl was separately injected into the HPLC and the chromatogram was recorded from the peak area of Metolazone and Spironolactone the amount of the drug in the sample can be calculated.



Standard Chromatogram for Metolazone and Spironolactone



Formulation Chromatogram for Metolazone and Spironolactone

Method validation: The optimized chromatographic method was completely validated according to the procedures described in ICH Q2 (R1) for the validation of analytical methods.

Linearity: A series of dilutions were prepared from the working standard solution in the concentration range of 1.25-7.5µg/ml for Metolazone and 12.5-75µg/ml for Spironolactone. 10µl of each sample was injected into HPLC. Calibration curve was constructed by plotting the peak area versus the drug concentration (**Figure 3, 4**) (**Table 1, 2**)

Table.1.Linearity of Metolazone

Concentration (µg/ml)	Peak Area
2.5	288687
3.75	429255
5	560030
6.25	702207
7.5	838030

Figure.3. Linearity Graph for Metolazone

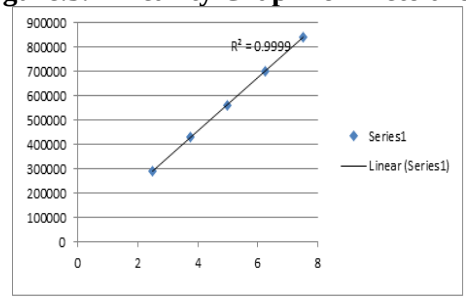
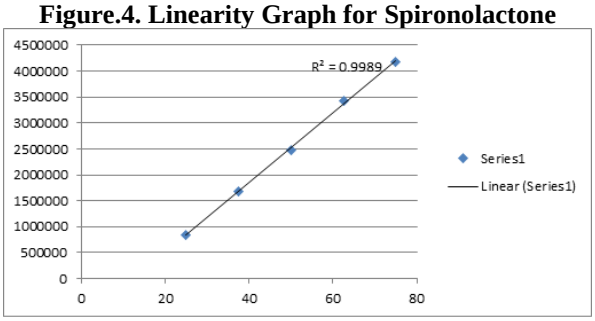


Table.2. Linearity of Spironolactone

Concentration (µg/ml)	Peak Area
25	850686
37.5	1679362
50	2468661
62.5	3425735
75	4168579

Figure.4. Linearity Graph for Spironolactone



Accuracy: The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three levels.50%, 100%, 150% of the target concentrations. The data shows excellent recoveries at all levels (Table 3, 4). The average recoveries for replicate determination at 50%, 100%, 150% levels were within the acceptable criteria.

Table.3. Accuracy-%Recovery of Metolazone

% Conc	Area	Amount added(mg)	%Recovery	Avg %Recovery	Mean recovery
50	273511	12.5	100.3	99.85	100.1
	271007		99.38		
	272310		99.85		
100	547719	12.5	100.4	100.2	
	543183		99.59		
	549023		100.6		
150	815571	12.5	99.69	100.3	

Table.4. Accuracy-%Recovery of Spironolactone

% Conc	Area	Amount added(mg)	%Recovery	Avg %Recovery	Mean recovery
50	1747632	5	99.97	99.9	99.98
	1758904		100.6		
	1733419		99.16		
100	3503257	5	100.2	99.98	
	3482512		99.6		
	3495513		99.9		
150	5237753	5	99.88	100.1	

Precision:

Method precision: Six assay samples of drug product at 100% of working sample concentration were prepared and injected into the chromatographic system. The percent assay and % RSD are calculated. (**Table 5**)

System precision: Six injections of the standard solution were injected into the chromatographic system. The percent assay and %RSD are calculated. (Table 6)

Table.5.Method Precision

S.No	Metolazone		Spironolactone	
	Retention time	Area	Retention time	Area
1	2.121	542303	4.072	3520746
2	2.122	543577	4.074	3487769
3	2.123	546168	4.083	3470810
4	2.125	546084	4.090	3505854
5	2.125	542618	4.105	3466596
6	2.126	549121	4.095	3514816
AVG		544949		3494432
SD		2626.4		22872
%RSD		0.5		0.65

Table.6.System Precision

S.No	Metolazone		Spironolactone	
	Retention time	Area	Retention time	Area
1	2.117	546038	4.064	3500396
2	2.121	545280	4.073	3490618
3	2.121	546040	4.085	3494764
4	2.122	543895	4.086	3504638
5	2.125	546457	4.089	3491068
6	2.125	541325	4.093	3473533
AVG		544839		3492503
SD		1945.9		10771.7
%RSD		0.4		0.3

Robustness: Robustness was evaluated by small deliberate variation in the chromatographic conditions at three different levels -1, 0, +1. The factors selected were flow rate (±0.1ml/mm) and the organic modifier in the mobile phase ±1%. The results obtained were unaffected by small variations in these parameters.

Specificity: Specificity was performed to exclude the possibilities of interference with excipients in the region of elution of Metolazone and Spironolactone. The retention times of the drug standards and the drug from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablet.

Sensitivity: Sensitivity of the proposed method was estimated in terms of limit of Detection (LOD) and limit of Quantitation(LOQ). LOD=3.3 SD/s LOQ=10SD/s Where SD is the residual standard deviation and‘s’ is the slope of the line.

Table.7. Validation parameters

Parameters		Results					
		Metolazone			Spironolactone		
Linearity		Correlation coefficient: 0.999			Correlation coefficient: 0.998		
Method Precision		%RSD 0.5			%RSD 0.65		
Accuracy	50%	99.85			99.9		
	100%	100.2			99.98		
	150%	100.3			100.1		
LOD		0.54			0.09		
LOQ		1.63			0.27		
Robustness							
		RT	Area	Tailing	RT	Area	Tailing Factor
Flow +10%		2.130	522510	1.39	4.111	3448059	1.28
Flow -10%		2.349	579874	1.30	4.514	3874102	1.31
Temp +5°C		2.127	594725	1.37	4.107	3620764	1.28
Temp -5°C		2.121	526474	1.28	4.072	3852136	1.32

RESULTS AND DISSCUSSIONS

A satisfactory separation and good peak symmetry was obtained with stationary phase ALTIMA column [150×4.6mm×5µm] mobile phase comprising of Ortho phosphate buffer : Acetonitrile in ratio of (35:65v/v) at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 234nm, based on peak area. The retention time was found to be 2.127 and 4.103 min for Metolazone and Spironolactone respectively (Figure 5).

Linearity: Linear regression equation was found to be Y=11200x+1825 for Metolazone and y=67107x+1782 for Spironolactone. R2 was found to be 0.999 for both the drugs. The results are expressed in the table. (Figure 3, 4) (Table 1, 2)

Accuracy: The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. %Recovery was found to be 100.1% for Metolazone and 99.98% for Spironolactone and the results were expressed in table. (Table 3,4)

Precision: The %RSD of method precision for Metolazone and Spironolactone was found to be 0.5 and 0.65 respectively. %RSD of system precision for Metolazone and Spironolactone was found to be 0.4 and 0.3 respectively. The %RSD below 2.0 shows high precision of proposed method. (Table 5, 6)

Robustness: The Robustness was found that the system suitability parameters were within the limits at all variable conditions. From the results obtained it can be concluded that, this method is robust towards small variations in method parameters.

Specificity: There was no other interfering peak around the retention time of Metolazone and Spironolactone and hence the method was found to be specific**.**

Sensitivity: The LOD for this method was found to be 0.54 µg/ml and 0.09 µg/ml for Metolazone and Spironolactone

The LOQ for this method was found to be 1.63µg/ml and 0.27 µg/ml for Metolazone and Spironolactone.

CONCLUSION

The proposed HPLC method was found to be simple,precise, accurate and sensitive for the simultaneous estimation of Metolazone and Spironolactone in pharmaceutical dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Metolazone and Spironolactone in pure and its pharmaceutical dosage forms.

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