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Original Article

A NEW STABILITY INDICATING UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METOLAZONE AND SPIRONOLACTONE IN BULK AND IN ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Objective: The objective of the work is to develop and validate a new, simple, highly sensitive RP-UPLC method for simultaneous estimation of Metolazone and Spironolactone in bulk and in its dosage forms.

Methods: The method was developed on a reversed-phase Hypersil Gold C_{18} (2.1× 100 mm, 2.7 µm) column with isocratic elution. Detection was done by UV-Spectroscopy at a detection wavelength of 235 nm. The analytical procedure was validated by assessing the specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and ruggedness as per ICH guidelines.

Results: The results were obtained as follows- the retention times were found to be around 2.888 min and 3.835 min, the percentage purity was observed to be 99 % w/v and 100 % w/v, the percentage recovery was found to be 99.90% and 99.9% respectively for Metolazone and Spironolactone. Calibration plots were linear ($r^2 > 0.999$) over the concentration range of 12 to 28µg/ml for Metolazolone and 120 to 280µg/ml for Spironolactone. The LOD was 0.0002µg/ml for Metolazone and 0.01µg/ml for Spironolactone. The LOQ was found to be 0.0008µg/ml for Metolazone and 0.003µg/ml for Spironolactone.

Conclusion: The developed analytical method for the simultaneous quantitation of Metolazone and Spironolactone was found to be specific, rapid, reliable, and reproducible. No interference from any component of pharmaceutical dosage form was observed. The method is amenable to the routine analysis of large numbers of samples with good precision and accuracy.

Keywords: RP-UPLC, ICH, Metolazone, Spironolactone, UV-Spectroscopy, LOD, LOQ.

INTRODUCTION

It was the Russian botanist Mikhail Tsvet (Mikhail Semyonovich Tsvet, 1872-1919) [1] who invented the first chromatography technique in 1901 during his research on chlorophyll. He used a liquid-adsorption column containing calcium carbonate to separate plant pigments. The method was described on December 30, 1901 at the XI Congress of Naturalists and Doctors in St. Petersburg. Chromatography is derived from Greek words "chroma" means "color" and "graphing" means "writing" in English [2,3].

UPLC system was designed to provide highest analysis speed and resolution and at the same time keep system pressure at a minimum. UPLC is up to 20 times faster than HPLC with same or better performance, 60% higher resolution, 10% more sensitive, Peak capacity is 1.4 % more, Reduced runtime from 60 to 20 min (related substances) and cut cost by 60%[4] thus this is the fastest, most efficient and cost effective LC system compared to HPLC.

Metolazone (MET) [5], with the chemical name 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo- 1, 2, 3, 4-tetrahydroquinazoline-6-sulfonamide **(Fig.1)** is a quinazoline diuretic, which is widely used in the treatment of hypertension. A proximal action of metolazone has been shown in humans by increased excretion of phosphate and magnesium ions and by a markedly increased fractional excretion of sodium in patients with severely compromised glomerular filtration.

Spironolactone (SPI)[6,7,8], with the chemical name 17-hydroxy-7α - mercapto-3-oxo-17α -pregn-4-ene-21-carboxylic acid γ-lactone acetate **(Fig.2)** is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics. It causes increased amounts of sodium and water to be excreted, while potassium is retained. Spironolactone acts both as a diuretic and as an antihypertensive drug by this mechanism. It may be given alone or with other diuretic agents which act more proximally in the renal tubule. Aldosterone interacts with a cytoplasmic mineralocorticoid receptor to enhance the expression of the Na+, K+-ATPase and the Na+ channel involved in a Na+ K+ transport in the distal tubule. Spironolactone bind to this mineralocorticoid receptor, blocking the actions of aldosterone on gene expression. Aldosterone is a hormone; its primary function is to retain sodium and excrete potassium in the kidneys. Literature survey [9,10,11,12] revealed that no stability indicating UPLC method for simultaneous estimation of Metolazone and Spironolactone was reported. Hence the objective was to develop a stability indicating, simple, sensitive, accurate and precise method for Simultaneous Determination of Metolazone and Spironolactone in bulk and in its Pharmaceutical Dosage form by UPLC.

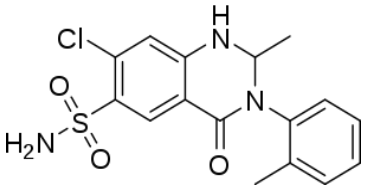

Fig. 1: Chemical Structure of Metolazone.

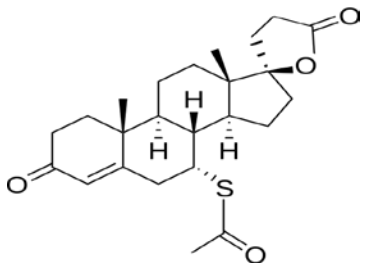

Fig. 2: Chemical Structure of Spironolactone.

MATERIALS AND METHODS

Chemicals and reagents

Pure samples of Metolazone and Spironolactone were obtained from The Madras Pharmaceuticals, Chennai. The commercial samples of the tablets Metolactone containing Metolazone-5mg and Spironolactone-50mg were provided by CENTAUR Pharmaceuticals Pvt. .Ltd. HPLC grade Acetonitrile and Methanol were procured from Merck Ltd. (Mumbai, India.). Water (HPLC) was obtained from a Milli-QRO water purification system. All the other used reagents were of analytical grade.

Chromatographic Conditions

Chromatographic separation was achieved by using a Waters e2695 Separation Module UPLC system and a UV-visible detector. The chromatographic column utilized in the study was Hypersil Gold C_{18}(2.1× 100 mm, 2.7 µm). Different mobile phases were tried, and the one containing Methanol, Acetonitrile and Phosphate Buffer (pH 3.5 adjusted with Orthophosphoric acid) in the ratio of 50:32:18 v/v was considered to be appropriate.

The mobile phase was filtered through 0.45µm membrane filter and ultra sonicated for 10 minutes. The flow rate selected was 1.0 ml/min with wave length of 235 nm. All the determinations were performed at constant column temperature (Ambient) and an injector volume is 10µl. The spectra were obtained from the UV detector.

Preparation of standard solution metolazone & spironolactone

Accurately 5 mg Metolazone and 50 mg spironolactone were weighed and transferred into 50 ml volumetric flask, about 15 ml of diluent was added and sonicated for 30 minutes to dissolve it. The volume was made up with diluent.

From this 5 ml of solution was pipetted out and transferred into 25 ml of volumetric flask and the volume was made up with diluents and it gives the concentration of 20 µg/ml Metolazone and 200 µg/ml spironolactone.

Preparation of sample solution metolazone & spironolactone

Metalactone tablets containing 5mg metolazone and 50mg spironolactone were weighed and the average weight was calculated. The tablets were powdered in a mortar and a sample of powder equivalent to 5mg metolazone and 50mg spiranolactone was transferred into a 50 ml volumetric flask and 15 ml of diluent was added, sonicated for 30 min and made up to the mark with diluents. Pipette out 5 ml of above solution into 25 ml volumetric flask and made up with diluents to obtain a concentration of 20 µg/ml, 200 µg/ml of Metolazone and spironolactone respectively.

Assay

10 µl of standard and sample solutions were injected into the injector of UPLC, and the peak dareas of the drugs in standard and sample were compared and assay was performed. Metolazone and Spironolactone shows the percentage purity values of 99 % w/v and 100 % w/v respectively.

RESULTS AND DISCUSSION

Specificity

The specificity of the method was confirmed by injecting the placebo and placebo spiked standard and observed that there was no shift in wavelength interference due to placebo. This confirms the specificity of the proposed method. There is no peak in the blank and Placebo solution run at the retention time corresponding to Metolazone and Spironolactone as in standard run as shown in **(Fig.3).**

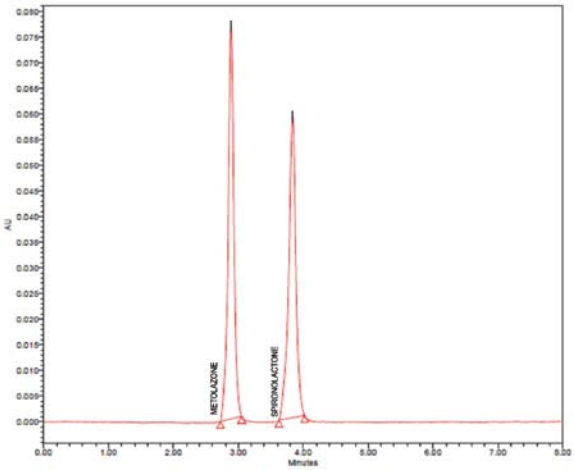

Fig. 3: chromatogram showing specificity of metolazone and spironolactone.

Linearity and range

Linearity was evaluated by visual inspection of plot of peak are as a function of analyte concentrations for Metolazone and Spironolactone. From the linearity studies the specified range was determined for Metolazone and Spironolactone given as 12 - 28 µg/ml and 120-280 respectively. The results are reported in **Table-1**. The calibration curves are shown in **(Fig.4 & 5)**.

Table 1: Linearity Results for Metolazone and Spironolactone

Parameters	Metolazone	Spironolactone
Linear Dynamic Range	12-28µg/ml	120-280µg/ml
Correlation Coefficient	0.999	0.999
Slope (m)	23957	2335

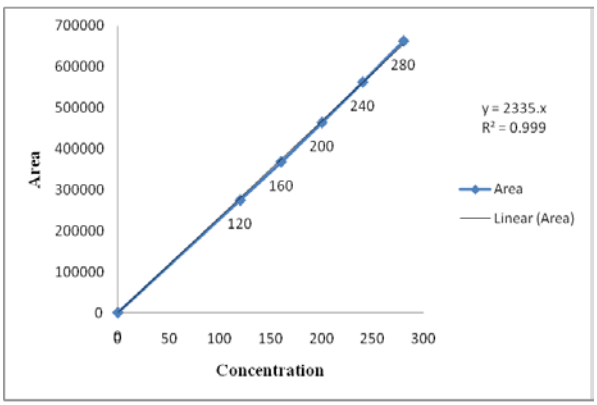

Fig. 4: Linearity of spironolactone

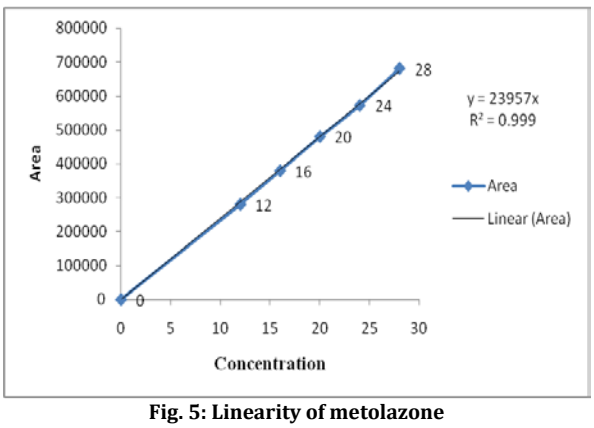

Fig. 5: Linearity of metolazone

Table 2: system precision data for Metolazone

	Sample Name	Name	R_T	Area	Height	Tailing	Plate Count
1	Sys pre	MET	2.880	462743	76652	1.0	5874
2	Sys pre	MET	2.899	466576	76985	1.0	5833
3	Sys pre	MET	2.888	467653	77300	1.0	5967
4	Sys pre	MET	2.889	465737	76621	0.9	5834
5	Sys pre	MET	2.890	463971	76647	0.9	5870
6	Sys pre	MET	2.890	465863	76654	0.9	5798
Mean				465960	76841		
Std. .Dev				1347	297		
%RSD				0.3	0.4		

Table 3: system precision data for Spironolactone

	Sample Name	Name	R_T	Area	Height	Tailing	Plate Count
1	Sys pre	SPI	3.835	443864	59742	0.9	6716
2	Sys pre	SPI	3.837	444847	58975	0.9	6713
3	Sys pre	SPI	3.836	444064	59143	0.9	6742
4	Sys pre	SPI	3.837	447868	58651	0.9	6583
5	Sys pre	SPI	3.838	445701	58791	0.9	6763
6	Sys pre	SPI	3.838	449686	58839	0.9	6673
Mean				446433	58882		
Std. .Dev				2308	185		
%RSD				0.5	0.3		

Table 4: Method precision data for Metolazone

S. No.	Sample Name	Name	RT	Area	Height
1	PRECISION	MET	2.889	464597	76296
2	PRECISION	MET	2.890	463482	76027
3	PRECISION	MET	2.891	464533	75700
4	PRECISION	MET	2.890	466393	75544
5	PRECISION	MET	2.891	461733	75126
6	PRECISION	MET	2.889	466272	75181
Mean				464502	75646
Std. Dev.				1756	462
% RSD				0.4	0.6

Table 5: Method precision data for Spironolactone

S. No.	Sample Name	Name	RT	Area	Height
1	PRECISION	SPI	3.838	449484	58662
2	PRECISION	SPI	3.839	448445	58434
3	PRECISION	SPI	3.839	449133	58204
4	PRECISION	SPI	3.839	446001	57950
5	PRECISION	SPI	3.839	446901	57793
6	PRECISION	SPI	3.838	447322	57659
Mean				447881	58117
Std. Dev.				1361	387
% RSD				0.3	0.7

Table 6: Results of Precision for Metolazone & Spironolactone:

S. No.	Sample weight	Sample area-1	Sample area-2	% Assay-1	% Assay-2
1	242	464597	449484	99	101
2	242	463482	448445	98	100
3	242	464533	449133	99	101
4	242	466393	446001	99	100
5	242	461733	446901	98	100
6	242	466272	447322	99	100
Avg. assay				99	100
STD				0.37	0.30
% RSD				0.38	0.30

Precision

1) System precision

The precision of the system was determined by six replicate injections of mixed standard solution. The % R. .S. .D of Area, retention time are present within the Acceptance criteria of 2 %. The results are reported in **Table-2 & 3**. The chromatograms are shown in **(fig.6)**.

2) Method Precision

The precision of the method was determined by six replicate injections of sample solution. The % R. S. D of Area and retention time and assay are present within the acceptance criteria of 2 %.

The results are reported in **Table- 4, 5& 6.** The chromatograms are shown in **(fig.7).**

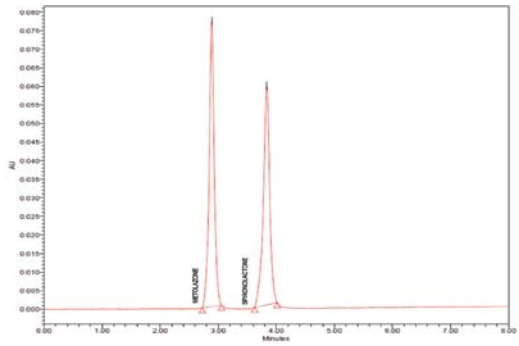

Fig. 6: Chromatogram showing system precision

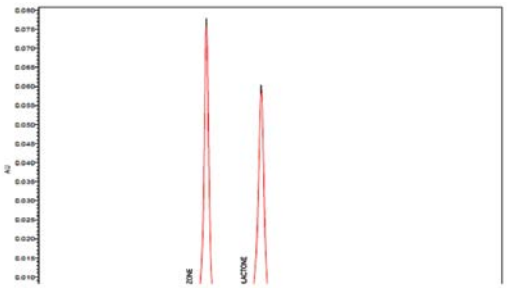

Fig. 7: Chromatogram showing method precision

Thus the proposed method was found to be high degree of precision and reproducibility.

D. System suitability

System suitability parameters such as resolution, tailing factor, no. of theoretical plates were calculated and the results are presented in **Table- 7**. The chromatograms are shown in **(fig-8**). The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicates the complete separation of the drugs.

The tailing factor values for Metolazone and Spironolactone indicating the symmetrical nature of the peak.

The no. of theoretical plates was high indicating the efficient performance of the column.

Table 7: System suitability parameters of Metolazone & Spironolactone

Parameters	Metolazone	Spironolactone
Tailing factor	1.0	0.9
Retention time	2.888	3.835
Theoretical plates per unit	5907	6717

E. Accuracy

The validation of the proposed method was further verified by recovery studies. Acceptance criteria are 98 - 102 % w/v. The results are reported in Table - 8.

Table 8: Accuracy data for Metolazone and Spironolactone

Parameters	Metolazone	Spironolactone
% recovery		
50 %	99.6	99.7
100 %	99.8	99.7
150 %	99.8	99.9

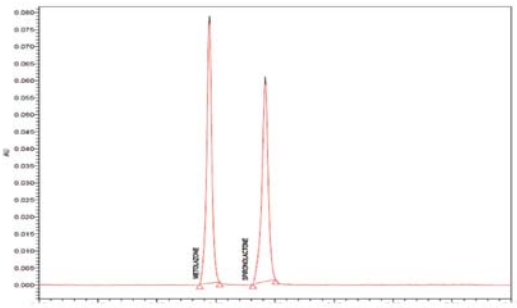

Fig. 8: chromatogram showing system suitability of metolazone and spironolactone.

This serves as a good index of the accuracy and reproducibility of the proposed method.

F. Robustness

Robustness was determined by carrying out the assay during which flow rate and temperature were altered slightly. The results are reported in **Table- 9**.

Table 9: Robustness data for Metolazone and Spironolactone

Parameters	% RSD Metolazone	% RSD Spironolactone
Flow rate	0.2	0.3
Temperature	0.4	0.3

% RSD values for robustness indicated that the method is robust and does not show variations in the results on slight variations in flow rate and temperature.

G. Ruggedness

Ruggedness was determined by carrying out the assay during under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. There was reproducibility of test results under normal, expected operational conditions from laboratory and from analyst to analyst. The results are reported in **Table - 10.**

The method is rugged and does not show variations in the results on slight variations of parameters.

Table 10: Ruggedness data for Metolazone and Spironolactone

Parameters	% RSD Metolazone	% RSD Spironolactone
Analyst 1	0.004	0.0003
Analyst 2	0.002	0.001

% RSD values for ruggedness indicated that the method is rugged and does not show variations in the results when performed by different analysts.

Limit of detection

The limit of detection of Metolazone and Spironolactone were calculated and found to be 0.0002 µg/ml and 0.011 µg/ml respectively.

Limit of quantification

The limit of quantification of Metolazone and Spironolactone were calculated and found to be 0.0008 µg/ml and 0.003 µg/ml respectively.

Degradation studies degradation studies

Degradation studies were carried out as per ICH guidelines. The sample solutions were subjected to acidic, basic, peroxide, water and light. Where as in acidic, basic the % degradations were found to be -7 %, -8 % and -9 %, -9 % for Metolazone and spironolactone respectively. The % degradation by peroxide was found to be -6 %

and -5 %. The % degradation by water was found to be -8 % and -7 %. The solid sample was subjected to light for 7 days and then the % degradation were found to be -5 % and -6 %. The results are reported in **Table-11.**

Table 11: Results of degradation studies for metolazone and spiranolactone

S. No.	Name	Sample weight	Sample area-1	Sample area-2	% Assay-1	% Assay-2	% DEG-1	% DEG-2
1	Acid	242	464433	449828	92	92	-7	-8
2	Base	242	463248	444682	90	91	-9	-9
3	Peroxide	242	464134	445453	93	95	-6	-5
4	Water	242	463892	444004	91	93	-8	-7
5	Light	242	462924	446801	94	94	-5	-6

CONCLUSION

A new stability indicating analytical method is developed and validated for simultaneous estimation of Metolazone and Spironolactone by RP-UPLC technique. The sample preparation is simple, consumes less amount of mobile phase and the required time for analysis is very short, the information presented in the study could be very useful for the quality monitoring of metolazone and spironolactone in combined pharmaceutical dosage forms and can be used to check drug quality during stability testing. The analytical procedure is validated as per ICH guidelines and shown to be accurate, precise and specific. This method represents a fast analytical procedure for the simultaneous quantitation of Metolazone and Spironolactone. The method is amenable for the routine analysis of large numbers of samples with good precision and accuracy.

CONFLICT OF INTERESTS

Declared None

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