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Validated RP-HPLC method for quantification of metolazone in bulk drug and formulation

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ABSTRACT

A simple, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Metolazone in bulk drug and pharmaceutical formulations was developed and validated in the present study. The mobile phase consists of a mixture of Buffer, Methanol, and Acetonitrile in the proportion 65:28:7. Mix well, adjust the pH Metolazone to 3.0 ± 0.05 with Phosphoric acid. This was found to give a sharp peak of Metolazone at a retention time of 12.568 min. HPLC analysis of Metolazone was carried out at a wavelength of 235nm with a flow rate of 1.1 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50 ppm to 150 ppm. The linear regression equation was y=39.19x75.32. The developed method was employed with a high degree of precision and accuracy for the analysis of Metolazone developed method was validated for accuracy, precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicate that this method is better for the quantification of Metolazone

Keywords: Metalazone, HPLC, Validation.

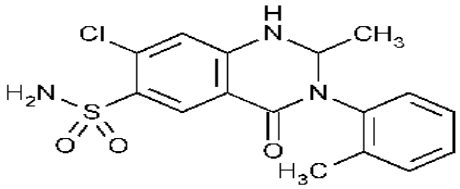
INTRODUCTION

Metolazone is chemically 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2-dihydroquinazoline-6-sulfonamide [3] **(Figure1).** Metolazone is an oral diuretic drug, commonly classified with the thiazide diuretics. 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. This lowers blood pressure and prevents excess fluid accumulation in heart failure.

INTRODUCTION

Metolazone is chemically 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1, 2- hydroquinazoline- 6-sulfonamide [3] (Structure-1). Metolazone is an oral diuretic drug commonly classified with the thiazide diuretics. 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. This lowers blood pressure and prevents excess fluid accumulation in heart failure. The emperical formula for Metolazone is $C_{16}H_{16}ClN_3O_3S$ and the molecular weight is 365.84 grams.

(Structure-1)



Metolazone [$C_{16}H_{16}ClN_3O_3$]

Literature review reveals that methods have been reported for analysis of Metolazone. Ultra violet and Derivative spectrophotometric methods for estimation of Metolazone in pharmaceuticals (2). A Validated UV spectrophotometric method of Metolazone in Bulk and its Tablet Dosage forms (3). Validated RP-HPLC method for simultaneous quantitation of Losartan potassium and Metolazone in bulk drug and formulation(4). Validated HPTLC Method for Simultaneous Estimation of Ramipril and Metolazone in Bulk Drug and Formulation (1). LC-MS-MS development and validation for simultaneous quantitation of Metolazone with other drug in human plasma.

To data there are no published reports on Validation of metolazone by HPLC in bulk drug and in tablet dosage form by the use of mobile phase containing a mixture of Buffer, Methanol, and Acetonitrile in the proportion 65:28:7 respectively. The present study reports there is no significant change in assay level observed up to 48 Hrs for test solution at room temperature. Thus, it can be concluded that the solution is stable up to 48Hrs at room temperature. The proposed method is validated as per ICH Guidelines.

MATERIALS AND METHODS

Instrument:
H.P.L.C- Waters - Alliance 510 with UV- 484 Data Ace software (Instrument I.D: Al-011)
HPLC - Agilent 1100 Series with Chromeleon software (Instrument I.D: AL-013
H PLC Analytical column C18, 150mm x 3.9mm x 5µ (C-048 & C-049)

Chromatographic conditions for Metolazone

Stationary phase	Mobile phase	Flow rate (ml/min)	Run time (min)	Column Temp (0^o)	Volume of injection loop(µl)	Detection wavelength (nm)	Retention time (min)
Hypersil BDS C-18	Buffer, Methanol, and Acetonitrile 65:28:7	1.1	20	25	20	235	12.568

1. Preparation of Mobile phase
For isocratic system, prepare a mixture of Buffer, Methanol, and Acetoitrile in the proportion 65:28:7 respectively. Mix well, adjust the pH to 3.0 ± 0.05 with Phosphoric acid. Filter through 0.5 µm Nylon membrane filter paper and degassed prior to use.

2. Preparation of Buffer
Weigh accurately and transfer 1.38 g of mono basic potassium phosphate monohydrate to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water.

3. Chromatographic conditions
Separation was performed on Chromolith RP- C-18, 150mm x 3.9mm x 5µ column. Methanol used as a Diluent and Mobile phase consists of mixture of Buffer, Methanol, and Acetonitrile in the proportion 65:28:7. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.5 µm Nylon membrane filter paper and degassed with helium purge for 20 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.1 ml/min and wavelength was set to be 235 nm. The column temperature was set at 25^oC.

4. Preparation of Placebo solution:
Weighed and transferred 974 mg of placebo (Excipient mixture) into a 25 ml volumetric flask. Add about 10 ml of diluent and shaken for 20 minutes by manually and further sonicate for 30 minutes. Dilute up to mark with diluent. Centrifuge this solution at 8000 rpm for 10 minutes. Decant the supernatant solution into another test tube and transfer 1.0 ml of supernatant solution into another 10 ml volumetric flask and make up the volume with diluent. Filter the solution through 0.5µm nylon membrane filter.

(Dilution scheme: 974mg →25ml / 1 ml→10.0 ml)

5. Preparation of Metolazone Standard Solution:
Weigh accurately about 25 mg of Metolazone working standard and transfer in to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

(Dilution scheme: 25mg → 25.0 ml → 1 ml /10.0 ml)

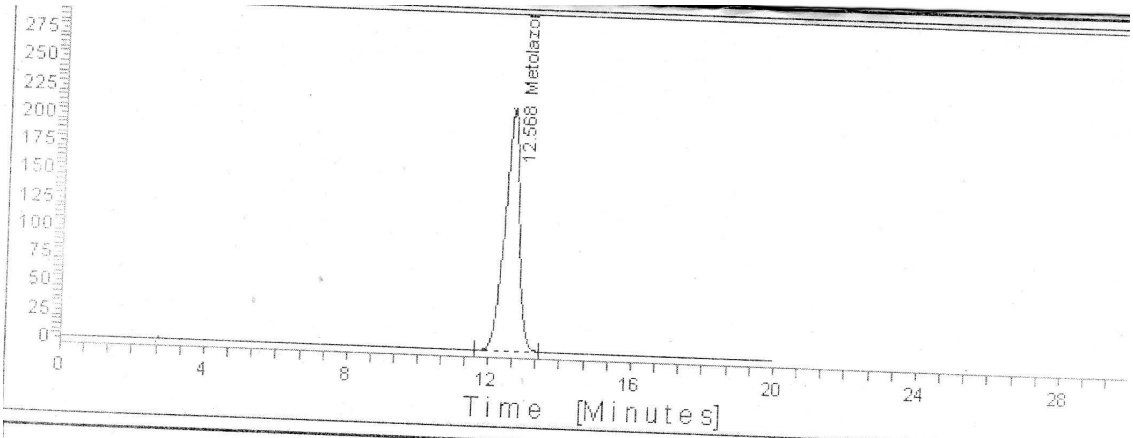
6. Preparation of Test Solution:
Weigh and transfer 999mg of sample powder into a 25 ml volumetric flask. Add about 10 ml of diluent and shake for 20 minutes by manually and further sonicate for 30 minutes. Dilute up to mark with diluent. Centrifuge this solution at 8000 rpm for 10 minutes. Decant the supernatant solution into another test tube and transfer 1.0 ml of supernatant solution into another 10 ml volumetric flask and make up the volume with diluent. Filter the solution through 0.5µm nylon membrane filter.

(Dilution scheme: 999mg →25ml / 1 ml→10.0 ml)

7. System Suitability Solution:
Use Metolazone standard working solution as system suitability solution.

8. Procedure:
Separately inject equal volumes of blank, five replicate injections of system suitability solution (Metolazone standard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Metolazone standard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Metolazone standard working solution).

Figure1: Chromatogram of Metolazone



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	12.568	5962.803	215.31	100	100	0.449
Total		**5962.803**	**215.31**	**100**	**100**	

Figure 2: Linearity of Metolazone Standard

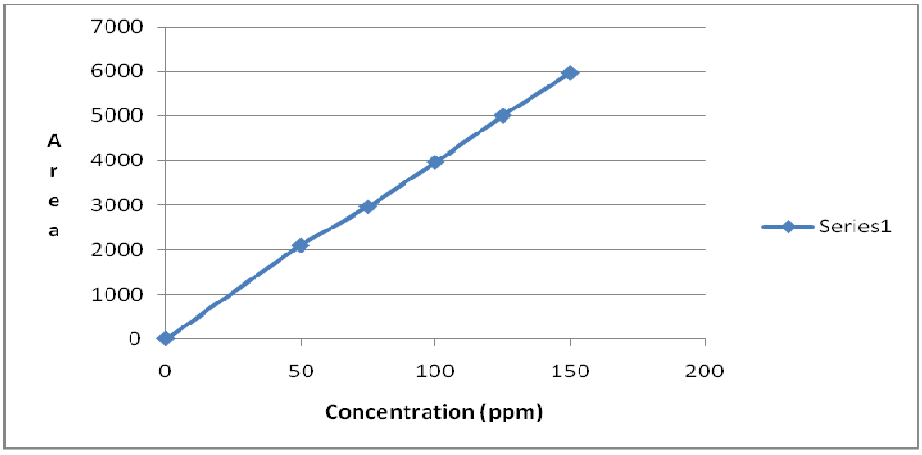


Table -1: Performance calculations, detection characteristics precision and accuracy of the proposed method for Metolazone

Parameter	HPLC Method
Wavelength (nm)	235
Retention time (t) min	12.568
Linearity range (ppm)	50 – 150
LOD (ppm)	1.4575
LOQ (ppm)	4.858
Regression equation (y=bc+a)	
Slope (b)	39.19
Intercept (a)	75.32
Standard deviation (SD)	19.04
Correlation coefficient(r^2)	0.999
Relative Standard deviation (%RSD)	0.475
Intermediate Precision (%RSD)	0.77
Range of errors	
Confidence limits with 0.05 level	16.688
Confidence limits with 0.01 level	21.933

RSD of 5 independent determinations
RSD of 15 independent determinations (5 independent samples per day for 3 days)

Table -2: System suitability - Linearity of standard

Sr. No.	Area of Metolazone	Tailing factor	Theoretical plates
1	3902.45	1.16	4352
2	4012.04		
3	3905.81		
4	3969.63		
5	3920.91		
Mean	3942.17		
Standard Deviation (±)	47.40		
(%) Relative Standard Deviation	1.20		

Table -3: Results of linearity of standard

Linearity Level	Sample Concentration (In %)	Sample Concentration (in ppm)	Average Area (n = 3)	Correlation Coefficient
Level – 1	50	50	2088.66	0.999
Level – 2	75	75	2954.83	
Level – 3	100	100	3961.28	
Level – 4	125	125	5004.07	
Level – 5	150	150	5962.80	

The linearity plot of peak area of Metolazone Vs. standard concentration in percentage

Table -4: Results of linearity of sample

Linearity Level	Sample Concentration (Wt in %)	Sample Concentration (in ppm)	Average Area (n = 3)	Correlation Coefficient
Level – 1	50	50	2064.53	0.999
Level – 2	75	75	2948.20	
Level – 3	100	100	3933.73	
Level – 4	125	125	5014.10	
Level – 5	150	150	5931.11	

The linearity plot of peak area of Metolazone Vs. sample concentration in percentage

Table -5: Results of linearity of standard in presence of placebo

Linearity Level	Standard Concentration (in %)	standard Concentration (in ppm)	Placebo added to the standard solution	Average Area (n = 1)	Correlation Coefficient
Level – 1	50	50	974mg	2061.90	0.999
Level – 2	75	75	974mg	2943.52	
Level – 3	100	100	974mg	3928.74	
Level – 4	125	125	974mg	4971.95	
Level – 5	150	150	974mg	5960.84	

The linearity plot of peak area ofMetolazone Vs. Standard concentration in presence of placebo in percentage.

Table -6: Results of linearity of standard in presence of placebo

Linearity Level	standard Concentration (in %)	standard Concentration (in ppm)	Placebo added to the standard solution	Average Area (n = 1)	Correlation Coefficient
Level – 1	50	50	974mg	2061.90	0.999
Level – 2	75	75	974mg	2943.52	
Level – 3	100	100	974mg	3928.74	
Level – 4	125	125	974mg	4971.95	
Level – 5	150	150	974mg	5960.84	

Five test solutions ofMetolazone were prepared as per the analytical method. The % of RSD of Five test solutions was calculated

Table -7: Results of intermediate precision

Test Solution	% Assay of Metolazone
1	98.53
2	99.74
3	99.85
4	99.62
5	100.80
6	100.34
Mean	99.81
Standard Deviation (±)	0.77
(%) Relative Standard Deviation	0.77

Five test solutions of Metolazone were prepared as per the analytical method. The % of RSD of Five test solutions was calculated

Table – 8: Results of Accuracy (%Recovery)

Level of Addition	Amount of Metolazone added in (mg)	Amount of Metolazone found in (mg)	Recovery (%)
First Level (Rec-50 %)	13.4	13.29	99.18
Second Level (Rec-75 %)	19.1	18.97	99.32
Third Level (Rec-100 %)	25.4	25.31	99.65
Fourth Level (Rec-125 %)	32.3	32.04	99.2
Fifth Level (Rec-150 %)	38.6	38.41	99.51
Mean			99.37
Standard Deviation (±)			0.20
(%) Relative Standard Deviation			0.20

These test solutions were prepared by adding a quantity of Metolazone API to excipient blend to produce three different concentration solution equivalent to 50 %, 75 %, 100 %, 125 % and 150 % of test concentration

Table – 9.1: Results for Change in Column Lot

Flow rate →	Same column	Diff column
Sample	% Assay	
Test solution	100.01	101.01
Average assay result from method precision	99.86	99.86
Mean	99.93	100.43
Standard Deviation (±)	0.10	0.81
(%) Relative Standard Deviation	0.10	0.81

Table – 9.2: Results for change in flow rate

Flow rate →	0.9ml/minute	1.3 ml/minute
Sample	% Assay	
Test solution	100.38	102.21
Average assay result from method precision	99.86	99.86
Mean	100.12	101.04
Standard Deviation (±)	0.37	1.66
(%) Relative Standard Deviation	0.37	1.64

Table – 9.3: Results for change in wavelength

Wavelength →	233 nm	237 nm
Sample	% Assay	
Test solution	100.19	101.61
Average assay result from method precision	99.86	99.86
Mean	100.03	100.73
Standard Deviation (±)	0.23	1.24
(%) Relative Standard Deviation	0.23	1.23

Table -9.4: Results for change in pH of mobile phase

pH	2.8	3.2
Sample	% Assay	
Test solution	99.37	99.51
Average assay result from method precision	99.86	99.86
Mean	99.62	99.69
Standard Deviation (±)	0.35	0.25
(%) Relative Standard Deviation	0.35	0.25

Table -10: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	% Assay of Metolazone
0^{th} hr	100.98
12^{th} hr	100.94
24 hr	100.87
36 hr	101.81
48 hr	99.80
Mean	100.88
Standard Deviation (±)	0.72
(%) Relative Standard Deviation	0.71

RESULTS AND DISCUSSION

The appropriate wavelength in UV region has been selected for the measuring of active ingredient in the proposed method. This method was validated by linear fit curve and all the parameters were calculated.

Parameters Fixation

In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

A) Mobile phase characteristics
In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile phase, total pH of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

B) Detection Characteristics
To test whether Metolazone had been linearly eluted from the column, different amounts of Metolazone were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig - 2, the linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, Intercepts and correlation coefficient. The results are presented in Table – 1, 2.

C) Performance Calculations
To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results are recorded in Table-1.

D) Method validations
The UV absorption maximum for Metolazone was fixed at 235 nm respectively. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

E) Precision
The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of Drug. The percent of Relative Standard deviation calculated for Metolazone and are presented in Table-6.The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of Drug solution was calculated in terms of coefficient of variation (CV) and the results are presented in Table-7.

F)Accuracy
To determine the accuracy of the proposed methods, different of technical grade samples of Metolazone within the linearity limits were taken and analyzed by the proposed methods. The results are recorded in Table-8.

F)Interference Studies
The effect of wide range of excipients and other additives usually present in the formulations of Metolazone in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of Drug. In fact many have no absorption at this UV maximum.

G) Analysis of Formulation
To find out the stability of the proposed methods for the assay of formulations containing Metolazone was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision and accuracy from reference method. The results are recorded in Table-8.

H) Ruggedness and Robustness
Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, Change in Flow rate, Change in wavelength and Change in p^H of the Mobile phase . The results were indicated by % CV in Table -9.1, 9.2 & 9.4.

Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 249 nm and 253 nm and the results were indicated by % CV in Table -9.3.

I) Recovery Studies
Recovery studies were conducted by analyzing each formulation in the first instance for the active ingredient by the proposed methods known amounts of pure Drug was then added to each of the previously analyzed formulations and

the total amount of the Drug was once again determined by the proposed methods after bringing the active ingredient concentration within the linearity limits. The results are recorded in Table -8.

J) Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table -10.

CONCLUSION

The method was found to be accurate and precise, as indicated by recovery studies close to 100 and % RSD is not more than 2. The summery of validation parameters of proposed HPLC method is given in tables.

The simple, accurate and precise HPLC method for the determination of Metolazone as bulk and in tablet dosage form has been developed. The method may be recommended for routine and quality control analysis of the investigated drug in bulk and pharmaceutical formulations. The analytical solution is found to be stable up to 48 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

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