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Development and Validation of a Stability Indicating RP-HPLC Method for the Estimation of Metalozone in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for estimation of Metolazone in its bulk and formulation. Method development was carried out on Hypersil BDS C18 column, (150×4.6mm, particle size 5µ). The chromatographic separation was achieved using a mobile phase containing acetonitrile and HPLC water in the ratio of 50:50 v/v at flow rate of 0.7 ml/min using detection at 236 nm. Linearity was performed from 1-10 µg/ml with correlation coefficient of 0.9992. The LOD and LOQ for the method were found to be 0.1µg/ml and 0.3µg/ml respectively. The statistical analysis shows that the method was found to be accurate, reliable, simple and reproducible. The %RSD for precision is NMT 2.0%. The chromatographic retention time of proposed method was 3.5 min. The percentage purity of Metolazone was found to be within the limit. For stability studies, the drug was exposed to the stress conditions such as acid, alkaline, oxidation, thermal by using 0.1 M HCl, 0.1 M NaOH, 3.0% H2O2, 80º C. Degradation behavior shows that the major degradation was observed at acidic condition (89.6%) followed by oxidation (90.1%), alkaline (91.9%), and thermal (94.5%). The proposed method was successfully applied for the quantitative determination of Metolazone in bulk and pharmaceutical dosage form.

Keywords: RP-HPLC, Metolazone, Validation, Forced degradation studies.

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. This lowers blood pressure and prevents excess fluid accumulation in heart failure. One of the primary uses of metolazone is for treating edema (fluid retention) associated with congestive heart failure (CHF). In mild heart failure, metolazone or another diuretic may be used alone, or combined with other diuretics for moderate or severe heart failure. In addition to preventing fluid buildup, the use of metolazone may allow the patient to relax the amount of sodium restriction that is required. Although most thiazide diuretics lose their effectiveness in kidney failure, metolazone remains active even when the glomerular filtration rate (GFR) is below 30–40 mL/min (moderate chronic kidney disease).

Metolazone[1-3] functions as a result of contact with the renal tubular process of electrolyte reabsorption. Metolazone is mainly used to prevent sodium reabsorption at the cortical dilution site and to a lesser extent in the proximal convoluted tubule. Sodium and chloride ions are excreted in approximately equivalent amounts. The improved delivery of sodium to the distal tubular exchange site results in increased excretion of potassium.

Metolazone is a cardiovascular agent, specifically a quinazoline diuretic related to the thiazide class. Metolazone works by inhibiting sodium transport across the epithelium of the renal tubules (mostly in the distal tubules), decreasing sodium reabsorption, and increasing sodium, chloride, and water excretion.

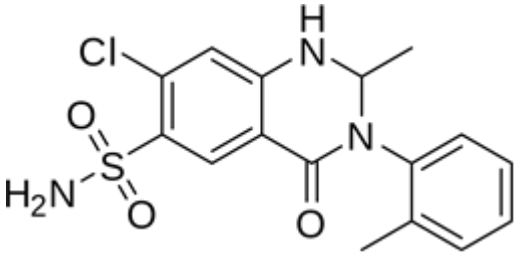

Fig.1: Chemical structure of Metolazone

Extensive literature review was conducted and an attempt was made to develop an unambiguous, valid method for the estimation of Metolazone. Few of spectroscopic, chromatographic, and other analytical methods [4-19] have been reported for the estimation of Metolazone individually and or along with drug combinations in pharmaceutical preparations. The aim of this study is to develop and validate a new simple, accurate and economic stability-indicating RP-HPLC method with less runtime, which would be able to separate and quantify Metolazone in a single run. The developed method was validated as per ICH guidelines [20-21] and can be applied lucratively to quality control purposes.

Materials and Methods:

Equipment:

The Method development and Validation was carried out using Waters Alliance-HPLC system equipped with waters 1525 binary HPLC pump, 2695-separation module connected to 2996-photo diode array detector, and Waters 2707 auto sampler. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents:

Metolazone working standard was kindly given as gift sample by Spectrum Pharma Limited, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions:

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Hypersil BDS C18 column, 150×4.6mm, particle size 5µm as a column with mobile phase of Acetonitrile: HPLC water in the ration of 50:50. The samples were analyzed using 10 µL injection volume, Flow rate was maintained at 0.7 mL/min with runtime of 8 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 236 nm wavelength.

Preparation of Working Standard Solution:

Accurately weighed and transferred 100mg of Metolazone into 100mL volumetric flask. 70mL of diluent was added, mixed well to dissolve and diluted up to the mark with diluent to obtain 1000µg/mL solution (Solution –A). 10mL of the above solution was pipetted out into 100mL volumetric flask and diluted up to the mark with diluent to obtain 100µg/mL solution (Solution-B). 6.0mL from Solution-B was pipetted out into 100mL volumetric flask and diluted op to the mark with the diluent (6µg/mL) concentration and sonicated for about 10 minutes with intermediate shaking.

Preparation of Sample Solution:

10mg of drug i.e., equivalent to 10 tablets were weighed and transferred into a 50 mL volumetric flask, 30mL of diluent was added, sonicated for about 30 min with intermediate shaking and volume diluted up to the mark with diluent. The sample solution was filtered with 0.45µ Millipore Nylon filter. Further 3mL of the above solution was pipetted out, transferred into a 100mL volumetric flask and diluted up to the mark with diluent.

METHOD VALIDATION:

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

System suitability:

System suitability parameters were evaluated to verify system performance. 10 µL of standard solution was injected five times into the chromatograph, and the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined.

Specificity:

The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks.

Precision:

Repeatability/ method precision was performed by injecting six replicates of same concentrations of Metolazone, calculated % assay and %RSD. Reproducibility/ Ruggedness/ Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy:

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (50%, 100% and 150%) of the working standard solutions of Metolazone to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity:

Linearity was evaluated by analyzing different concentrations of the standard solutions of Metolazone. Six working standard solutions ranging between 10µg/mL-80µg/mL were prepared and injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and Limit of quantification:

Limit of detection (LoD) and limit of quantification (LoQ) of Metolazone were determined by calibration curve method. Solutions of Metolazone were prepared in linearity range and injected (n = 3).

Robustness:

To examine the robustness of the developed method, experimental conditions were deliberately changed, resolution, tailing factor, and theoretical plates of Metolazone peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.1mL/minute, organic phase composition in mobile phase was changed ±5% and the detection wavelength was changed ±10nm. In all the above varied conditions, the composition of aqueous component of the mobile phase was held constant.

Forced Degradation Studies:

Stress studies were performed by considering Metolazone working standard solution to provide the stability-indicating property and specificity of the proposed method. Intended degradation was attempted by the stress conditions of exposure to photolytic stress (1.2 million lux hours followed by 200 Watt hours), heat (exposed at 80°C for 24 hours), acid (0.1N HCl for 24 hours at 60°C), base (0.1N NaOH for 24 hours at 60°C), oxidation (3% peroxide for 24 hours at 60°C), water (refluxed for 12 hours at 60°C), and humidity (exposed to 90% RH for 72 hours). The solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION:

System Suitability:

From the results in table 1, the column efficiency for Metolazone peak was identified from the theoretical plate count which is more than 3000, tailing factor less than 2.0, %RSD was found to be less than 2.0%.

Table 1: System suitability data

System suitability parameter	Observed value	Acceptance criteria
Retention time	3.548	--
USP Tailing factor	1.2	NMT 2.0
USP Theoretical Plate Count	5765	NLT 2000
% RSD	0.5	NMT 2.0%

Specificity:

From the obtained chromatograms in figures 2 to 4 it can be inferred that there were no co-eluting peaks at the retention time of Metolazone which shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analyte of interest.

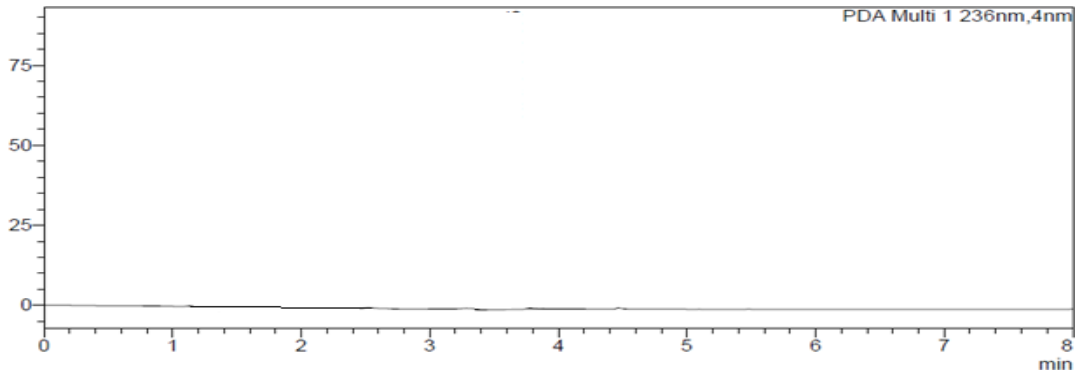

Fig. 2: Blank chromatogram

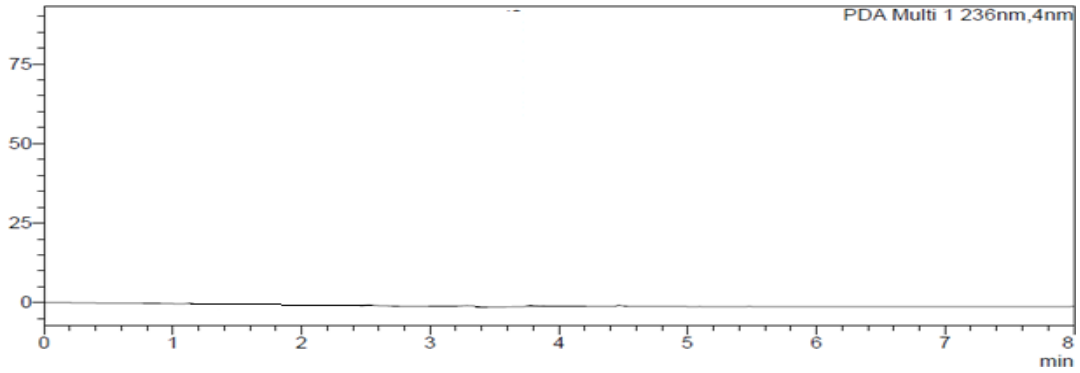

Fig. 3: Placebo chromatogram

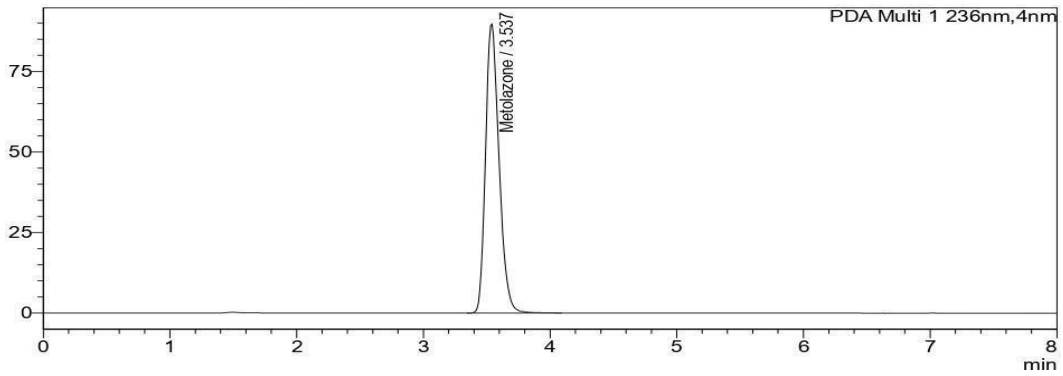

Fig. 4: Chromatogram of Metolazone

Precision:

From the results in table 2, % Assay for Metolazone was found to be in the range of 98 – 102%, and the % RSD for Metolazone to be within 2%. Hence the method is precise, reproducible and rugged for 48 hours' study.

Table 2: Precision data (system precision and method precision)

Number of injections	**Retention time**	**Peak area**	**%Assay**
1	3.540	787571	99.7
2	3.547	799985	99.8
3	3.546	787899	99.3
4	3.545	792132	99.6
5	3.546	772685	99.8
6	3.544	768429	100
Mean		782983	99.7
SD		7369.746	0.2162
%RSD		0.938238	0.9067

Linearity:

Linearity was evaluated by analysing different concentrations of Metolazone. From the results tabulated in table 3, it is inferred that the correlation coefficient was greater than 0.999. The slope and y-intercept values were also provided, which confirmed good linearity between peak areas and concentration.

Table 3: Linearity data

Concentration (µg/mL)	Peak area
1	133264
2	239080
3	399496
4	489978
6	699899
8	952859
10	1191256

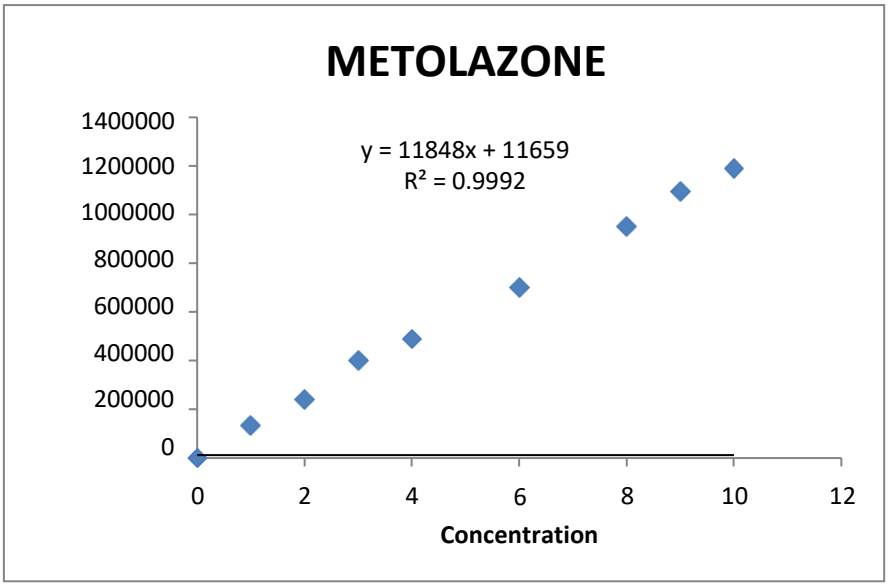

Fig.5: Calibration curve of Metolazone

Accuracy:

From the results in table 4, the % recovery for Metolazone found to be in the range of 98 –102% and the % RSD for Metolazone is less than 2%. Hence the proposed method was accurate.

Table 4: Accuracy data

% Concentration (at specific level)	Area			% recovery	% Mean recovery	Overall %Mean recovery
	Sample area	Average Sample area	Standard area			
50%	392057	391547.3	785488	100.2	100.6%	99.89%
	391065			100.0		
	391520			100.0		
100%	782015	780680.7		99.9	99.76%	
	779812			99.7		
	780215			99.7		
150%	1164589	1171920		99.2	99.86%	
	1172586			99.9		
	1178586			100.5		

LoD and LoQ:

The Limit of Detection and Limit of Quantification of Metolazone were calculated by using following equations (ICH, Q2 (R1)) and the LoD and LoQ values are reported in table 5. These LOD = 3.3 × σ/S and LOQ = 10 × σ/S

Where σ = the standard deviation of the response and S = slope of the calibration curve.

Table 5: LoD and LoQ data

Drug	LOD (µg/mL)	LOQ (µg/mL)
Metolazone	0.1	0.3

Robustness:

From the results in table 6, it is evident that the system suitability parameters such as resolution, RSD, tailing factor, and the theoretical plate count of Metolazone remained unaffected by deliberate changes. The results were presented along with the system suitability parameters of optimized conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

Table 6: Robustness data

S. No	Conditions	Metolazone			
		Retention time (min)	Theoretical plate count	Tailing factor	%RSD
1.	Flow rate (+) 0.8mL	3.110	4042	1.271	0.89%
2.	Flow rate (-) 0.6mL	4.157	5130	1.232	0.77%
3.	Organic phase (-) 45:55 v/v	4.091	5087	1.225	0.9%
4.	Organic phase (+) 55:45 v/v	3.201	4153	1.276	0.94%
5.	Wave length (+) 240nm	3.110	4039	1.271	0.7%
6.	Wave length (-) 232nm	4.136	5039	1.233	0.87%

Forced Degradation Studies:

The samples were analyzed with the above mentioned HPLC conditions using a PDA detector to monitor the homogeneity and purity of the Metolazone. The results which were shown in table 7 indicates that the degradation was not observed in photolytic stress, humidity, acid, base, water hydrolysis, and thermal stress studies. It was interesting to note that all the peaks due to degradation were well resolved from the peaks of Metolazone. Further, the peak purity of Metolazone was found to be homogeneous based on the evaluation parameters such as purity angle and purity threshold. Hence, the method is considered to be "stability-indicating."

Table 7: Forced degradation studies at different stress conditions

S. No	Degradation	% Degradation Assay		% Assay
		API	Formulation	
1.	Acid degradation	89.8%	89.6%	
2.	Alkali degradation	91.9%	91.9%	
3.	Oxidative degradation	90.6%	90.1%	
4.	Thermal degradation	93.3%	94.5%	100.8%

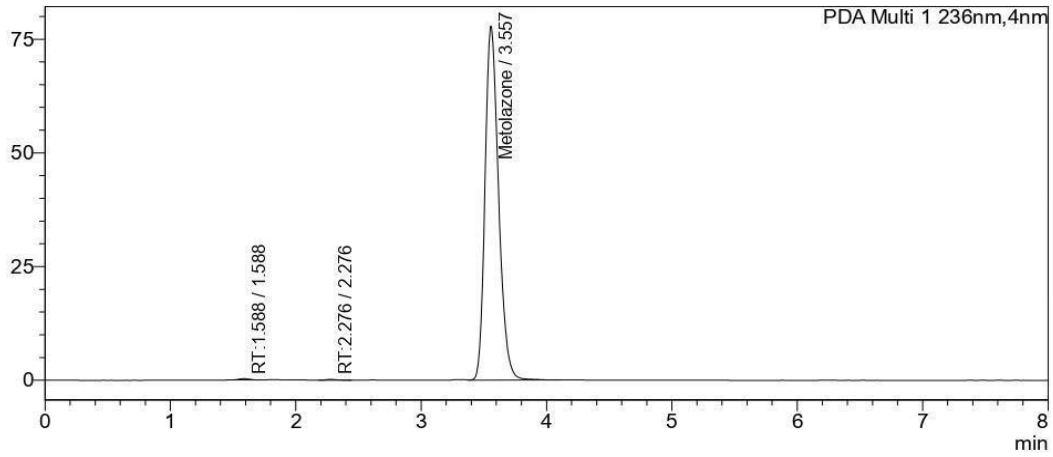

Fig. 6: Chromatogram of acid degradation

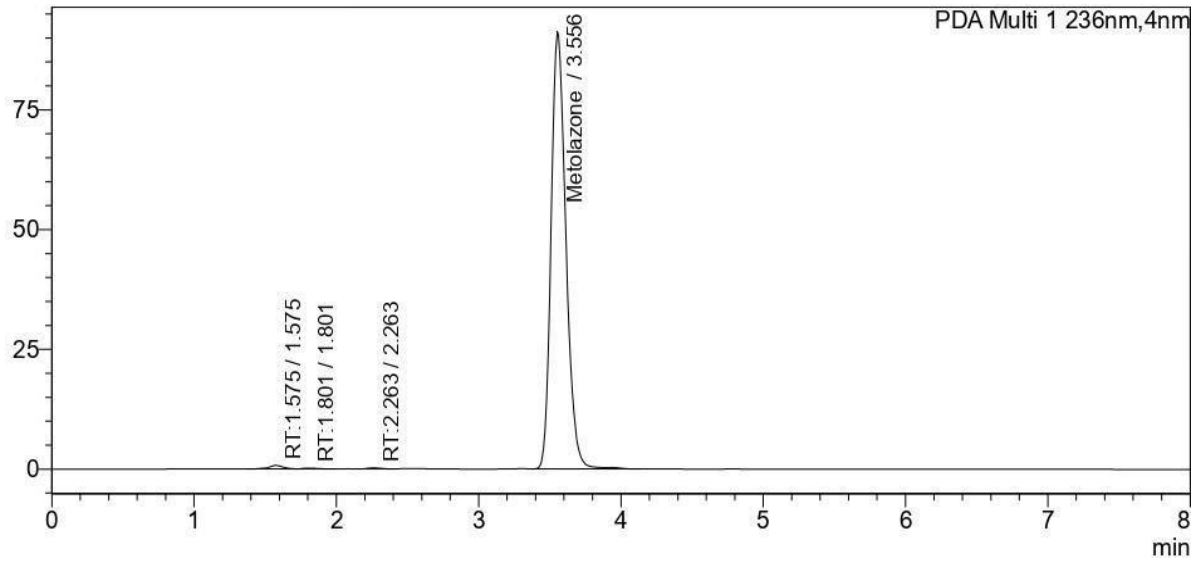

Fig. 7: Chromatogram of alkali degradation

Conclusion:

A simple, rapid, cost less effective and accurate RP-HPLC method was developed for the determination of Metolazone in tablets by isocratic mode elution. The analytical conditions and the solvent system developed provided good separation within a short run time. The RP-HPLC method was validated and demonstrated good linearity, precision, accuracy and specificity. Thus, the developed RP-HPLC method can be utilized for routine analysis during the analysis of Metolazone. All the parameters for the drug Metolazone had met the criteria of ICH guidelines for method validation. However, RP-HPLC method is considered more specific and sensitive. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. Good agreement was seen in the assay results of capsules as well as in laboratory prepared mixtures by developed methods. We concluded that the proposed method us a good approach for obtaining reliable results and was found to be suitable for the routine estimation of Metolazone in tablets.

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