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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF METOLAZONE 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 0.999. 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%. 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% H_2O_2, 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 diuretic chemically 7-chloro-2-methyl-3-(2-methylphenyl) - 4-oxo-1, 2, 3, 4 -tetrahydroquinazoline-6-sulfonamide. Its molecular formula is $C_{16}H_{16}ClN_3O_3S$ and its molecular weight is about 365.835 g/mol g·mol^{-1} **[1]**. Metolazone is used to treat edema (fluid retention) in people with congestive heart failure or kidney disorder. It is also used to treat hyper tension. Metolazone acts primarily to inhibit sodium reabsorption at the cortical diluting site and to a lesser extent in the proximal convoluted tubule. Sodium and chloride ions are excreted in approximately equivalent amounts. The increased delivery of sodium to the distal tubular exchange site results in increased potassium excretion **[6]**. The actions of Metolazone result from interference with the renal tubular mechanism of electrolyte reabsorption. Metolazone is 50-70% bound to erythrocytes, up to 33% bound to plasma proteins, 2-5% of the drug in circulation is unbound. Metolazone metabolizes by Enterohepatic recycling and excretes 70-95% through urine.

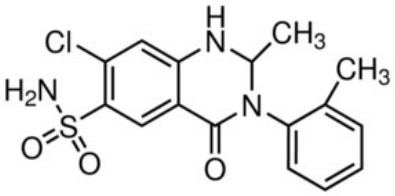

Figure: 1 Metolazone

MATERIALS AND METHODS

Estimation of absorption maxima (λ_{max}) by UV-visible spectroscopy

UV spectroscopic analysis was carried out using UV spectrophotometer. The standard Metolazone (10µg/mL) was placed in cuvettes by using diluent (Acetonitrile: water 50:50% v/v) as reference and the spectra was recorded over a range of 400-200nm.

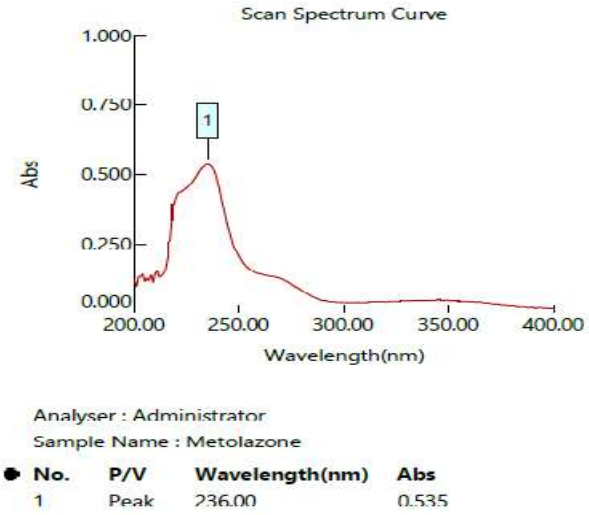

Figure 2: UV spectrum of Metolazone

Chromatographic conditions

Chromatographic conditions were achieved using Hypersil BDS C18 column, (150×4.6mm, particle size 5µ) column for separation. Flow rate of 0.7mL/min. Wavelength at 236nm with an injection volume of 10µL and run time was about 8 minutes at ambient temperature.

Preparation of mobile phase

A mixture of Acetonitrile and HPLC water was prepared in the ratio of 50:50% v/v, sonicated for 10 minutes and filtered with 0.45µ membrane filter.

Assay of Metolazone 2.5mg tablets

Preparation of standard stock solution

Accurately weighed and transferred 100mg of Metolazone into 100mL volumetric flask. 50mL of diluent was added, sonicated for 10 min to dissolve and diluted up to the mark with diluent to obtain 1000µg/mL solution (Solution –A)

Preparation of standard solution

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.

Preparation of sample solution

4 tablets equivalent to 10mg of drug were weighed and transferred into a 50 mL volumetric flask. 30mL of diluent was added, sonicated for 10 min with intermediate shaking, diluted up to the mark with diluent and 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.

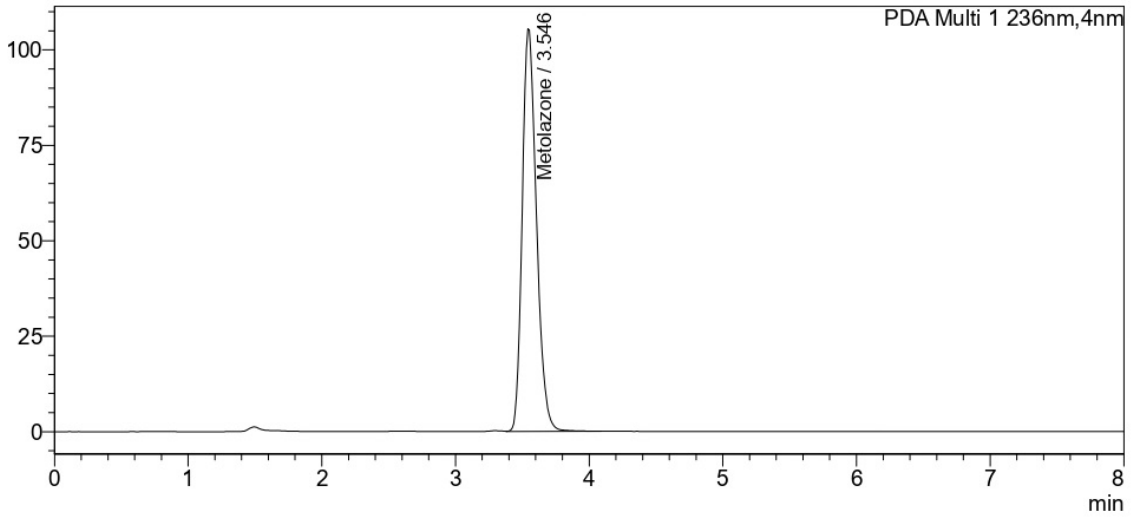

Figure 3: Chromatogram of standard

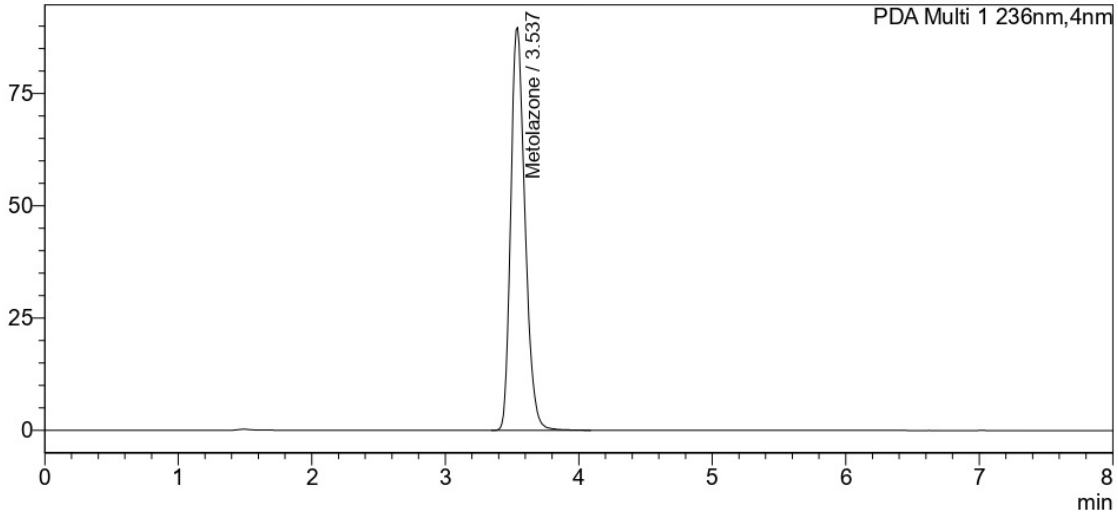

Figure 4: Chromatogram of sample

Drug	Labeled claim	Amount present	% Purity
Metolazone	2.5mg	2.5222mg	100.88

METHOD VALIDATION

SYSTEM SUITABILITY

System suitability tests were performed by increase volumes (10-50µL) of same concentration of Metolazone standard (6µg/mL) and observed for the changes in the parameters such as retention time, number of theoretical plates and tailing factor with increase in injection volume

Acceptance criteria:

The % RSD should be NMT 2.0%, the number of theoretical plates (N) should be NLT 2000 and the Tailing factor (T) should be NMT 2.0.

SPECIFICITY

Specificity is determined by comparing the chromatograms of blank, the Metolazone standard and sample and observed for any interference at the retention time of Metolazone standard **[1]**. Solutions of standard, sample and diluent were prepared as per the test method and injected into the chromatographic system and the chromatograms were recorded.

Acceptance criteria:

The chromatogram of blank should not show any peak at the retention time of the main analyte.

The retention time should be identical for both the standard and sample chromatograms.

LINEARITY

The linearity of the method was demonstrated over the concentration range of 13% - 175% of the target concentration. Aliquots of 13%, 25%, 50%, 75%, 100%, 125%, 150%, 175% were prepared from standard stock solution and injected into the chromatogram and the obtained peak areas were used for the construction of calibration plot. From the resultant data, parameters such as slope, intercept and regression equation were calculated.

Acceptance criteria

The plot of concentration verses peak area of Metolazone at 13% to 175% level should be linear with a correlation coefficient (r) value is not less than 0.999.

PRECISION

The precision of the method was determined by system precision and method precision using 100% standard and sample solutions.

System precision

System precision was evaluated by injecting the Metolazone standard (6µg/mL) was taken from the same aliquot for 6 times and percentage relative standards (%RSD) for peak area were calculated.

Method precision

Method precision was evaluated by injecting six assay samples of drug product of the sample concentration (6µg/mL) were prepared and injected into the chromatographic system and the chromatograms were recorded.

Acceptance criteria:

The % RSD should be NMT 2.0%, the number of theoretical plates (N) should be NLT 2000 and the Tailing factor (T) should be NMT 2.0

ACCURACY

In the present research work, accuracy is performed by standard addition method **[2]**. This method involves the addition of known concentration of standard at three levels (80, 100, and 120%) to the known concentration of sample solution. From the data obtained %

RSD and percentage recovery were calculated for each level.

Acceptance criteria: The mean % recovery for each spiked level should be not less than 98% and not more than 102%.

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations (±2%) in method parameters and provides an indication of its reliability during normal usage **[2]**.

Conditions:

- Flow rate changed to 0.6mL/min and 0.8mL/min
- Organic phase ratio is changed to 55:45% v/v and 45:55%v/v
- Wavelength is changed to 246nm and 226nm

The Metolazone standard solution (6µg/mL) was injected for each minor change and the system suitability parameters were noted at each change. The parameters should be within the limits for the method to be robust.

LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ)

The LOD and LOQ were calculated from the data obtained from the standard calibration curve using the following equation:

$$\mathbf{LOD} = \frac{3.3\,x\,\sigma}{m}\mathbf{;\ LOQ} = \frac{10\,x\,\sigma}{m}$$

FORCED DEGRADATION STUDIES

Degradation studies were carried out as per ICH guidelines. The Metolazone API and formulation were subjected to acidic (0.1N HCl), alkali (0.1N NaOH), peroxide (3% H_2O_2) conditions for 24 hours at room temperature and thermal degradation by placing in hot air oven at a temperature of 80ºC for 24 hours.

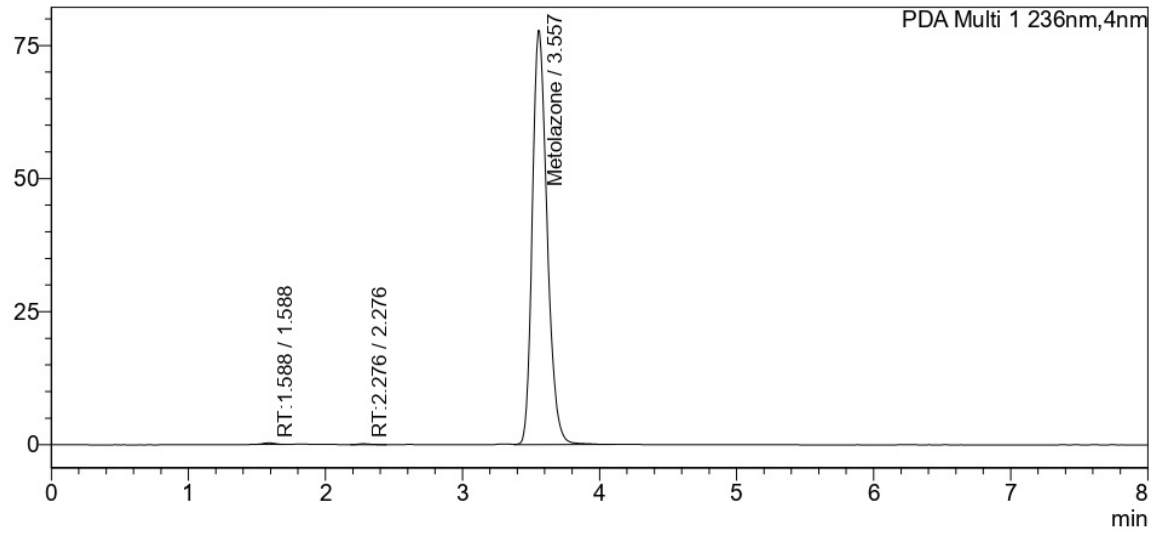

Figure 6: Chromatogram of acid degradation in API

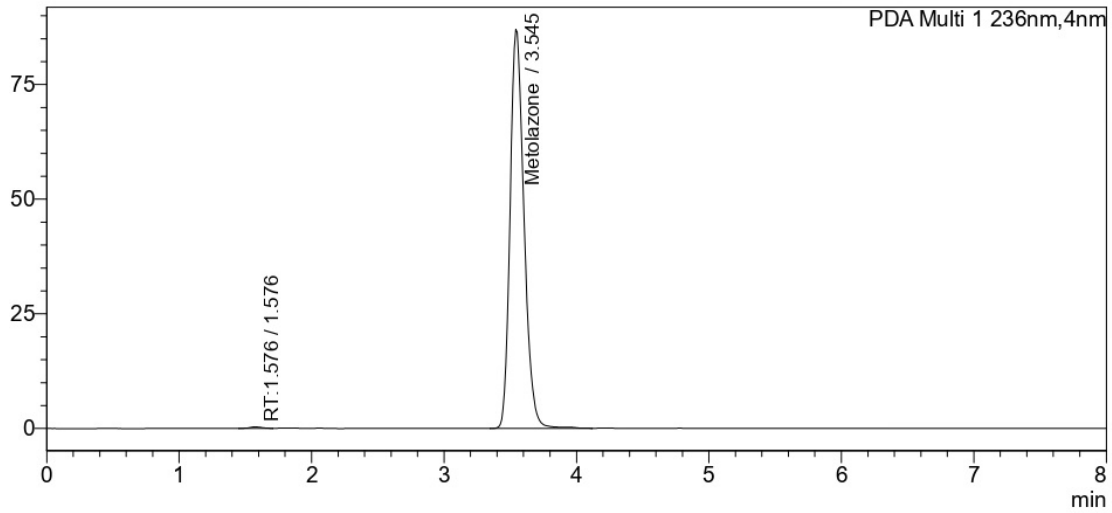

Figure 7: Chromatogram of acid degradation in formulation

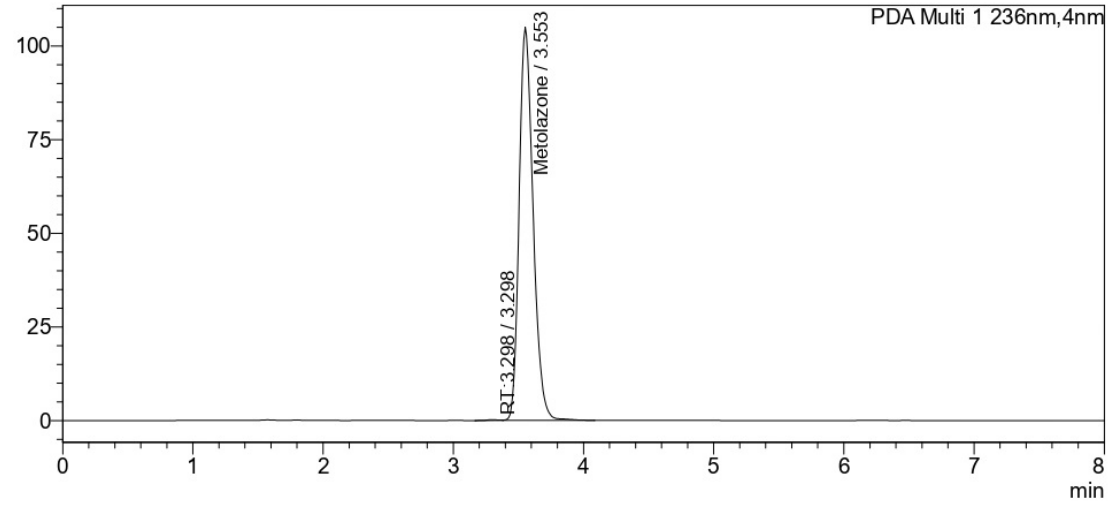

Figure 8: Chromatogram of alkali degradation in API

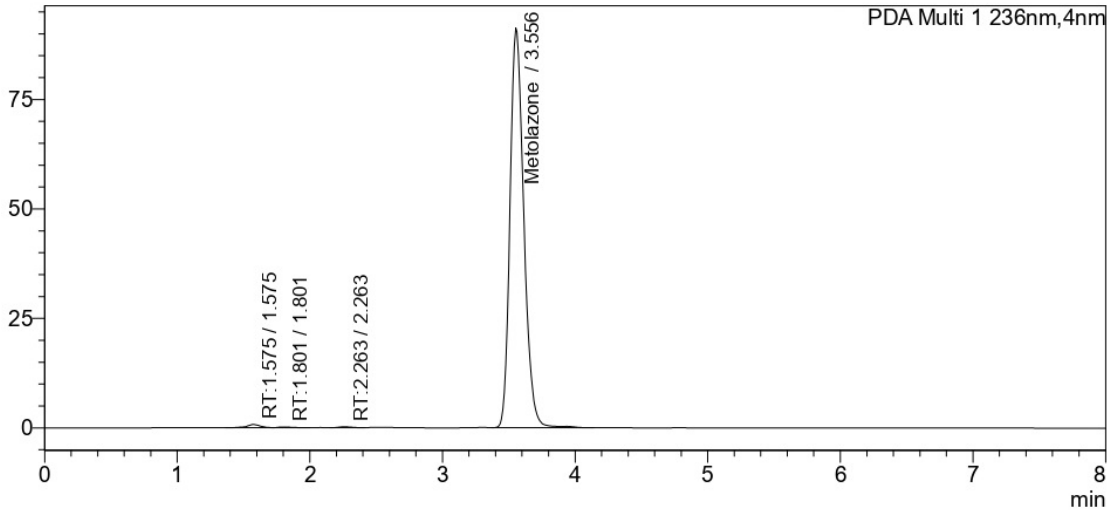

Figure 9: Chromatogram of alkali degradation in formulation

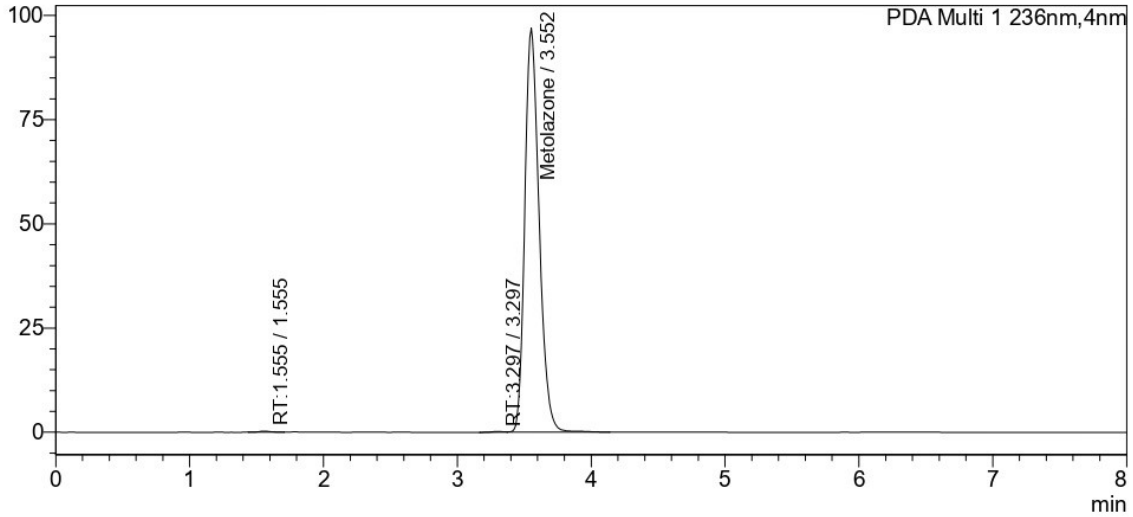

Figure 10: Chromatogram of oxidative degradation in API

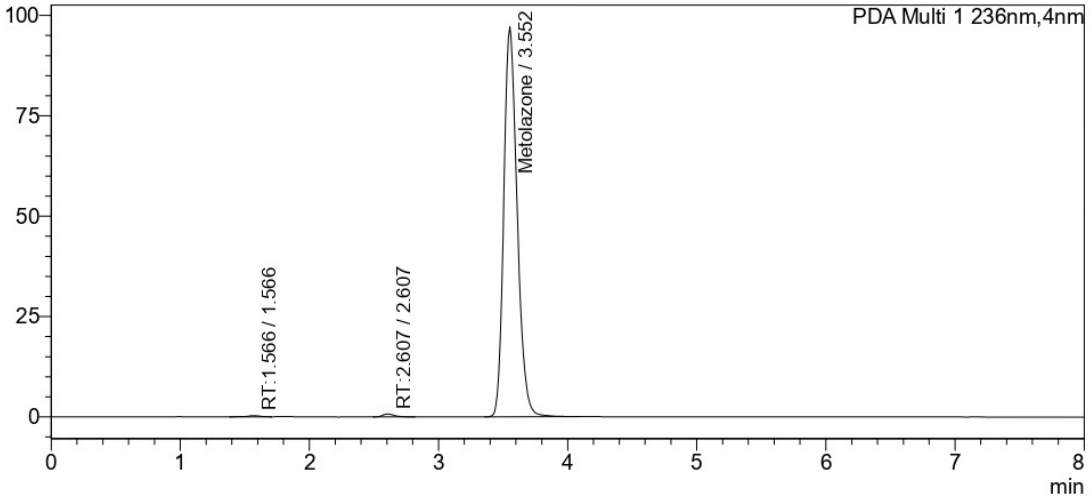

Figure 11: Chromatogram of oxidative degradation in formulation

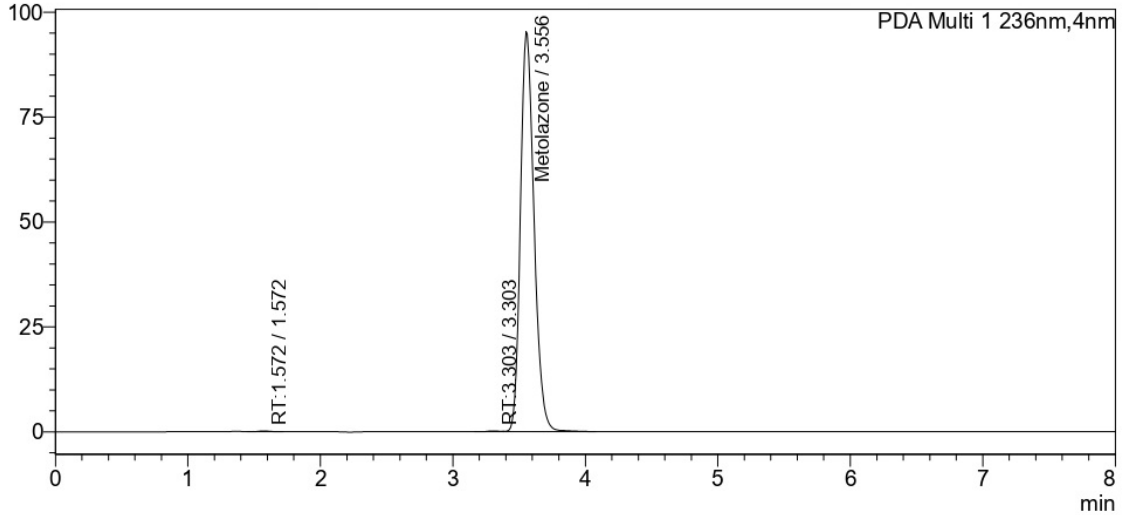

Figure 12: Chromatogram of thermal degradation in API

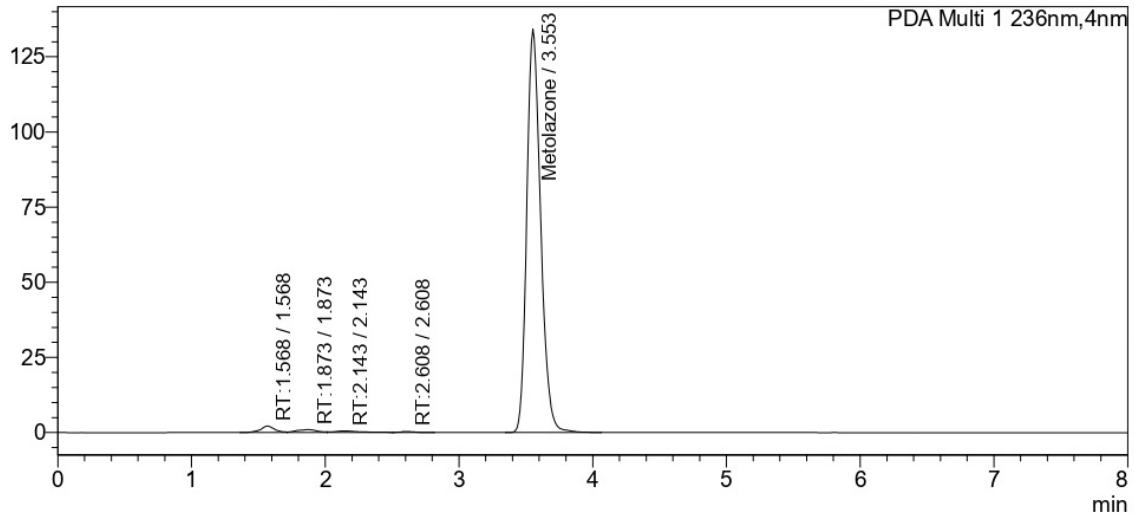

Figure 13: Chromatogram of thermal degradation in API

RESULTS

System suitability

Table No1: System suitability of Standard

Number of injections	Retention time	Peak area
1	3.548	785371
2	3.548	798989
3	3.546	787299
4	3.546	782832
5	3.543	779615
6	3.542	778821
Mean		785488
SD		7369.746
%RSD		0.938238

Table No: 2 System suitability of Sample

Number of injections	Retention time	Peak area
1	3.537	781058
2	3.540	782058
Mean		781558
SD		707.1068
%RSD		0.90474

LINEARITY

Table 3: Linearity of Metolazone

Concentration (µg/mL)	Retention time	Peak area
0	0	0
1	3.553	133264
2	3.545	239080
3	3.543	399496
4	3.540	489978
6	3.540	699899
8	3.542	952859
9	3.535	1093919
10	3.535	1191256
R^2		0.999

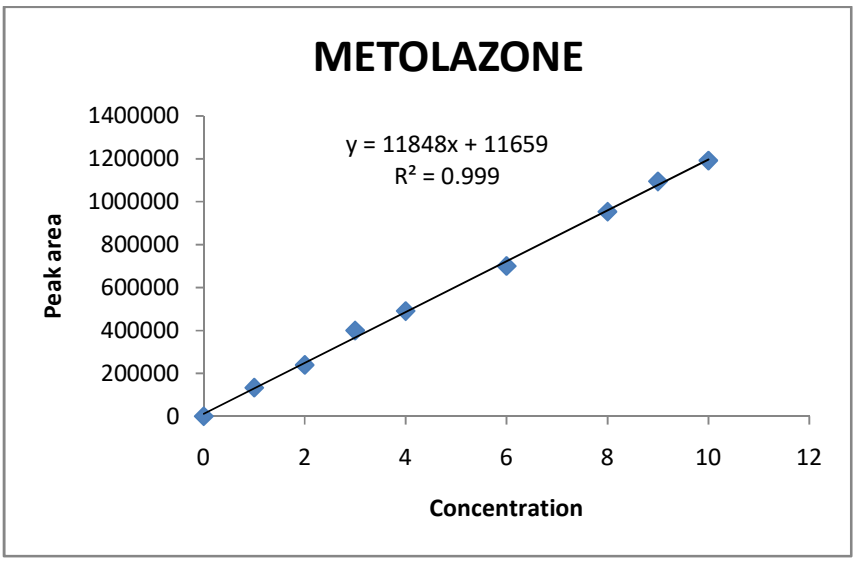

Figure 14: Calibration curve of Metolazone

PRECISION

System precision

Table 4: System precision of Metolazone

Number of injections	Retention time	Peak area	%Assay
1	3.540	785371	99.7
2	3.547	798989	99.8
3	3.546	787299	99.3
4	3.545	782832	99.6
5	3.546	779615	99.8
6	3.544	778821	100
Mean		785488	99.7
SD		7369.746	0.2162
%RSD		0.938238	0.9067

Method precision

Table 5: Method precision of Metolazone

Number of injections	Retention time	Peak area	%Assay
1	3.544	777192	99.3
2	3.546	779021	99.6
3	3.545	780932	99.8
4	3.546	780999	99.7
5	3.547	781058	99.8
6	3.540	782058	100
Mean		780210	99.7
SD		1622.758	0.216025
%RSD		0.90799	0.916675

ACCURACY

Table 6: Accuracy of Metolazone

% 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

Table 7: LOD and LOQ of Metolazone

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

ROBUSTNESS

Table 8: Robustness of Metolazone

S.No	Conditions	Metolazone			
		Retention time (min)	Theoretical plate count (USP)	Tailing factor (USP)	%RSD
1.	Flow rate (+) 0.8mL	3.110	4042	1.271	0.89%
		3.110	4047	1.271	
2.	Flow rate (-) 0.6mL	4.157	5130	1.232	0.77%
		4.136	5034	1.233	
3.	Organic phase (+) 55:45%v/v	4.091	5087	1.225	0.9%
		4.092	5091	1.220	
4.	Organic phase (-) 45:55%v/v	3.201	4153	1.276	0.94%
		3.199	4126	1.276	
5.	Wave length (+) 246nm	3.110	4039	1.271	0.7%
		3.110	4045	1.271	
6.	Wave length (-) 226nm	4.136	5039	1.233	0.87%
		4.157	5137	1.232	

FORCED DEGRADATION STUDIES

Table 9: Forced degradation studies of Metolazone

S. No	Degradation	Conditions	Metolazone Peak area		%Assay	
			API degradation	Formulation degradation	API	Formulation
1.	Acid degradation	0.1 N HCl for 24 hours	712452	697168	89.8%	89.6%
2.	Alkali degradation	0.1 N NaOH for 24 hours	725485	715285	91.9%	91.9%
3.	Oxidative degradation	3% H_2O_2 for 24 hours	722477	700531	90.6%	90.1%
4.	Thermal degradation	80°C for 24 hours	728956	735269	93.3%	94.5%

DISCUSSION

A simple, sensitive, precise and specific validated stability indicating RP-HPLC method for estimation of Metolazone in bulk and pharmaceutical dosage form was developed and validated. The separation was performed on Hypersil BDS C18 column, (150×4.6mm, particle size 5µ) chromatographic column. The mobile phase was mixture of Acetonitrile and Water (50:50% v/v). The flow rate was 0.7 mL/ min and was detected at 236 nm. According to guidelines, system suitability parameters constitute integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The developed method was validated according to ICH guidelines. The linear response was observed in the range of 1-10µg /ml for Metolazone. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98 – 102 %. System precision and method precision were found to be within limits and method was found to be robust. Summary of validation parameters is shown in below table. The method was validated statistically and was applied successfully for estimation of Metolazone.

CONCLUSION

The results obtained in this study demonstrates that the Metolazone stability indicating RP-HPLC method described in the protocol is selective, linear, precise, accurate and robust for the determination of stability of Metolazone drug. Therefore, the proposed method can be used for routine analysis for the estimation of Metolazone in its tablets formulation.

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