

**METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BETAMETHASONE, GENTAMICIN & MICONAZOLE BY RP-HPLC METHOD**

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Article Received on 20/05/2020

Article Revised on 10/06/2020

Article Accepted on 30/06/2020

**ABSTRACT**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Betamethasone, Gentamicin and Miconazole in liquid dosage form. Chromatogram was run through Phenomenex C18 150 4.6 mm, 5 $\mu$ . Mobile phase containing Buffer and Acetonitrile in the ratio of 35:65 A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was Water at Temperature was maintained at 30°C. Optimized wavelength for Betamethasone, Gentamicin and Miconazole was 240 nm. Retention time of Betamethasone, Gentamicin and Miconazole were found to be 2.177min, 3.321 min and 2.634 min %RSD of system precision for Betamethasone, Gentamicin and Miconazole. were and found to be 0.8, 0.8 and 0.7 respectively. %RSD of method precision for Betamethasone, Gentamicin and Miconazole. were and found to be 0.4, 0.5 and 0.3 respectively. % recovery was Obtained as 99.56%,99.37%,99.16% for Betamethasone, Gentamicin and Miconazole. Respectively. LOD values are obtained from regression equations of Betamethasone, Gentamicin and Miconazole were 0.09ppm, 0.04ppm, 1.96ppm. LOQ Values 0.28ppm and 0.12ppm, 5.93ppm respectively. Regression equation of Betamethasone was  $y = 3415.x + 485.6$ , Gentamicin was  $y = 5211.x + 876.6$  and of Miconazole was  $y = 4497 .x + 7864$ .

**KEYWORDS:** Betamethasone, Gentamicin and Miconazole, RP-HPLC.**INTRODUCTION**

Chemically Betamethasone Valerate (BTM) acts as a moderately potent glucocorticoid steroid with anti-inflammatory and immunosuppressive properties. Structure of the BTM was shown in figure 1 (A).<sup>[1]</sup>

Chemically Aminoglycosides like gentamicin (GTM) "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically gentamicin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes Structure of the OMB was shown in figure 1 (B).<sup>[2]</sup>

Chemically Miconazole (MCN) interacts with 14- $\alpha$  demethylase, a cytochrome P-450 enzyme necessary to

convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Miconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Structure of the MCN was shown in figure 1 (C).<sup>[3]</sup>

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.<sup>[5-9]</sup> But there is only very few HPLC methods are available for simultaneous estimation of BTM, GTM and MCN, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

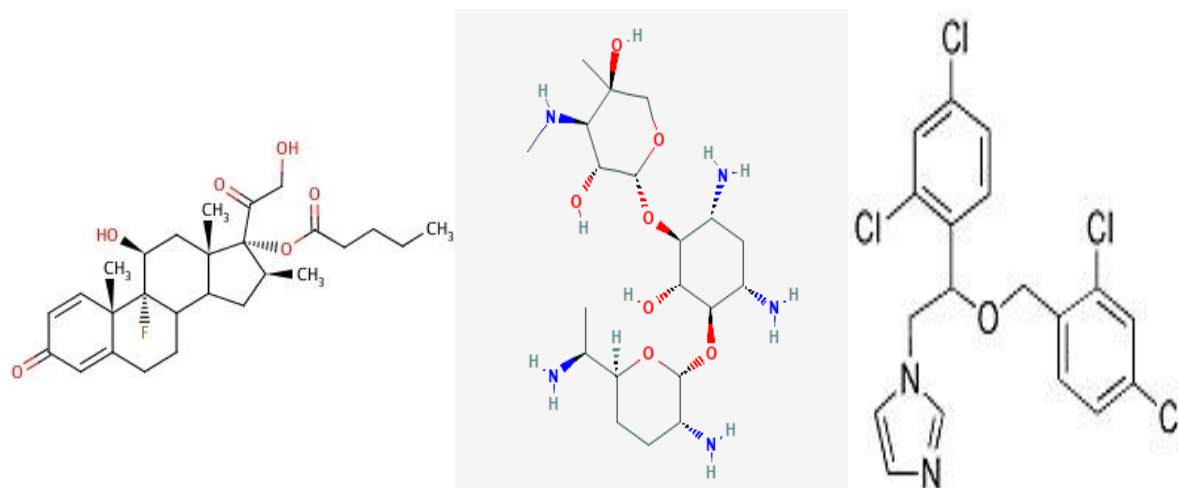


Figure 1: Structure of (A) Betamethasone (B) Gentamicin (C) Miconazole.

## MATERIALS AND METHODS

**Reagents and Chemicals:** Betamethasone, Gentamicin & Miconazole pure drugs (API), Combination Betamethasone, Gentamicin & Miconazole, Distilled water, Acetonitrile, Tri ethyl amine, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

**Instrumentation:** HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, phenomenex C18 150 x 4.6 mm, 5 $\mu$ . As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

**Chromatographic conditions:** The phenomenex C18 150 x 4.6 mm, 5 $\mu$  column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of Acetonitrile and water was taken in the ratio of (35:65%v/v) mobile phase for the investigation with a flow rate of a 1.0 ml/min. The temperature was maintained at 30 $^{\circ}$ C. The injection volume was 10 $\mu$ l and the UV detection was achieved at 240nm.

**Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0):** Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

### Preparation of mobile phase

**Buffer:** Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water

**Preparation of mixture Standard stock solution:** Accurately weighed 10mg of Betamethasone, 10 mg of Gentamicin and 200mg of Miconazole and transferred to three 50ml volumetric flasks separately. 10ml of methanol was added to flasks and sonicated for 15mins. Flasks were made up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

**Preparation of Sample (Tablet) stock solutions:** 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered.

### Optimized chromatographic conditions

**Column Used:** Phenomenex C18 150 x 4.6 mm, 5 $\mu$

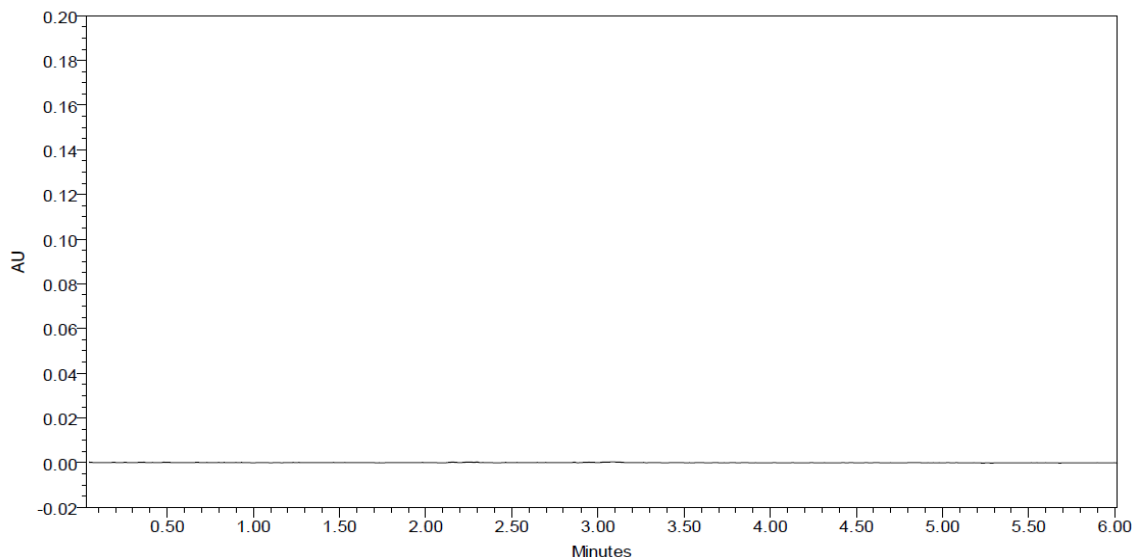
**Mobile phase:** Acetonitrile: Water (35:65v/v)

**Flow rate:** 1.0ml/min

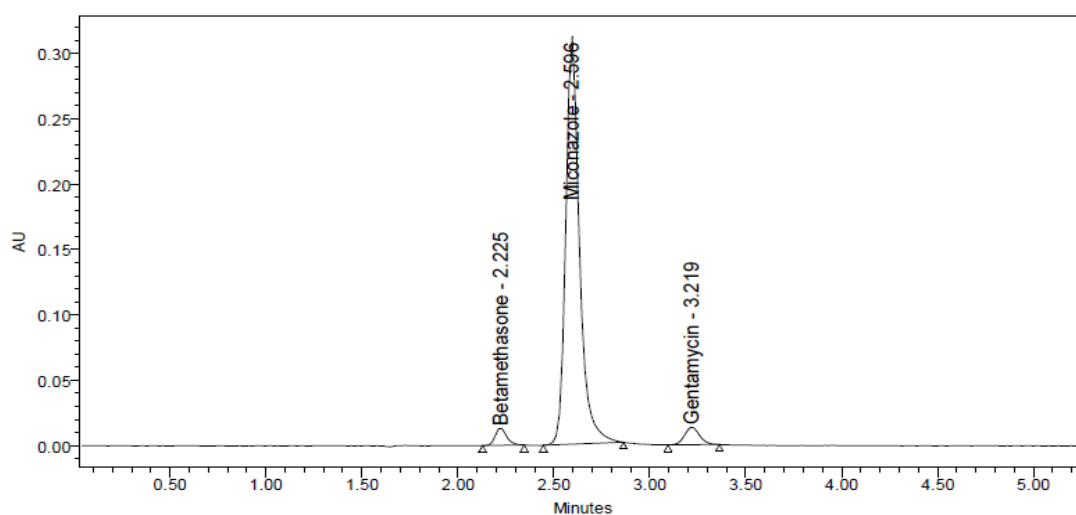
**Wavelength:** 240.0 nm

**Temperature:** 30 $^{\circ}$ C

**Injection Volume:** 10.0 $\mu$ l

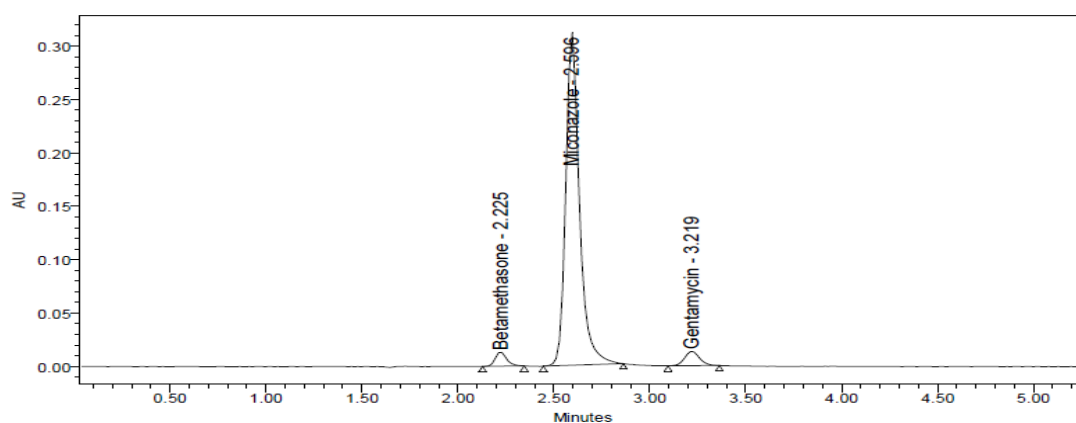


**Figure 2: Blank chromatogram.**



**Figure 3: Chromatogram of standard mixture of BTM, GTM & MCN.**

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Betamethasone	2.225	1394994	1.29	5	7038
2	Gentamicin	2.219	107049	1.11	4.9	7838
3	Miconazole	2.596	2886583	1.30	4.6	6818



**Figure 4: Chromatogram of sample mixture of BTM, GTM & MCN.**

Table 1: Linearity table for BTM, GTM &amp; MCN.

Betamethasone.		Gentamicin		Miconazole	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Conc (µg/mL)	Peak area	Conc (µg/mL)
5	17517	5	5	17517	5
10	35489	10	10	35489	10
15	51907	15	15	51907	15
20	68513	20	20	68513	20
25	85626	25	25	85626	25
30	102977	30	30	102977	30

Table 2: System precision table of BTM, GTM &amp; MCN.

S. No	Area of Betamethasone.	Area of Gentamicin	Area of Miconazole
1.	65726	82122	1822868
2.	64495	82971	1834410
3.	64852	82876	1822207
4.	64304	83217	1843493
5.	65007	82535	1838788
6.	65220	81376	1810744
Mean	64934	82516	1828752
S.D	512.0	675.8	12262.0
%RSD	0.8	0.8	0.7

Table 3: degradation data of BTM.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.55	0.695	0.722
2	Alkali	5.55	0.668	0.792
3	Oxidation	7.04	0.668	0.792
4	Thermal	1.90	0.850	0.905
5	UV	2.42	0.241	0.312
6	Water	0.38	0.283	0.312

Table 4: degradation data of GTM.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.37	0.712	1.016
2	Alkali	3.71	1.009	1.397
3	Oxidation	5.04	1.009	1.397
4	Thermal	1.60	0.937	1.306
5	UV	1.44	0.990	1.557
6	Water	1.96	0.688	1.020

Table 5: degradation data of MCN.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	2.59	0.110	0.298
2	Alkali	3.15	0.150	0.311
3	Oxidation	4.50	0.150	0.311
4	Thermal	1.54	0.113	0.308
5	UV	1.17	0.141	0.332
6	Water	0.53	0.153	0.302

Table 6: Summary of validation data of BTM, GTM &amp; MCN.

Parameters	Betamethasone.	Gentamicin	Miconazole	LIMIT	
<b>Linearity</b>					
Range (µg/ml)	5-30 µg/ml	5-30 µg/ml	100-600 µg/ml	R < 1	
Regression coefficient	0.999	0.999	0.999		
Slope(m)	3415	5211	4497		
Intercept(c)	485.6	876.6	7864		
Regression equation (Y=mx+c)	y = 3415x + 485.6	y = 5211x + 876.6	y = 4497x + 7864		
<b>Assay (% mean assay)</b>	99.56%	99.37%	99.16%	90-110%	
<b>Specificity</b>	Specific	Specific	Specific	No interference of any peak	
<b>System precision %RSD</b>	0.8	0.8	0.7	NMT 2.0%	
<b>Method precision %RSD</b>	0.4	0.5	0.3	NMT 2.0%	
<b>Accuracy % recovery</b>	98.83%	98.65%	99.21%	98-102%	
<b>LOD</b>	0.09µg/ml	0.04 µg/ml	1.96µg/ml	NMT 3 µg/ml	
<b>LOQ</b>	0.28 µg/ml	5.93 µg/ml	0.12 µg/ml	NMT 10µg/ml	
<b>Robustness</b>	<b>FM</b>	1.8	1.1	0.3	%RSD NMT 2.0
	<b>FP</b>	0.6	0.5	0.3	
	<b>MM</b>	0.2	0.5	0.9	
	<b>MP</b>	1.5	0.9	0.1	
	<b>TM</b>	1.8	1.2	0.5	
	<b>TP</b>	0.5	0.5	0.5	

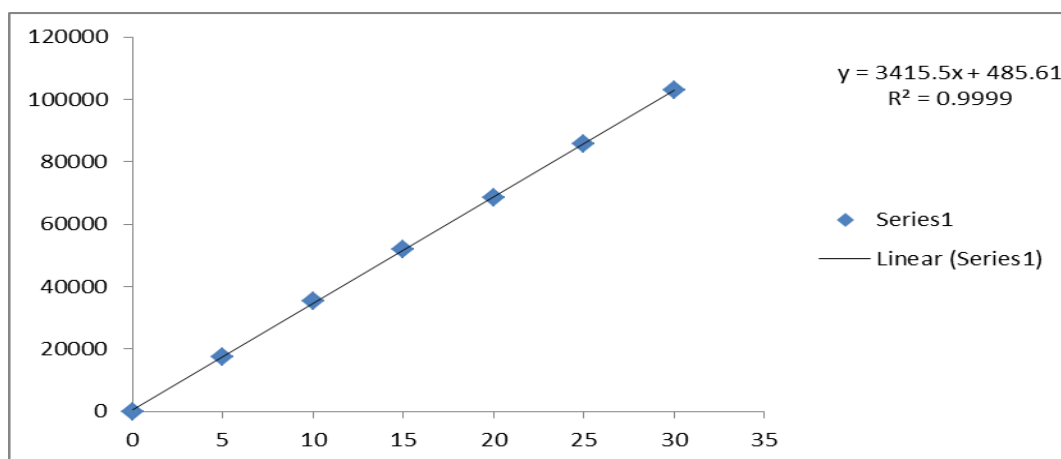


Fig. 7: Linearity curve of Betamethasone.

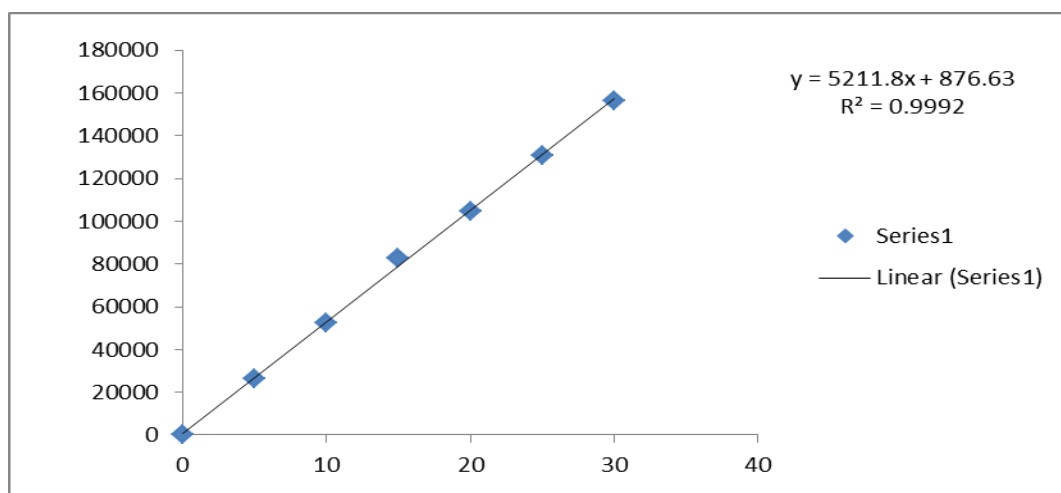


Fig. 8: Linearity curve of Gentamicin.

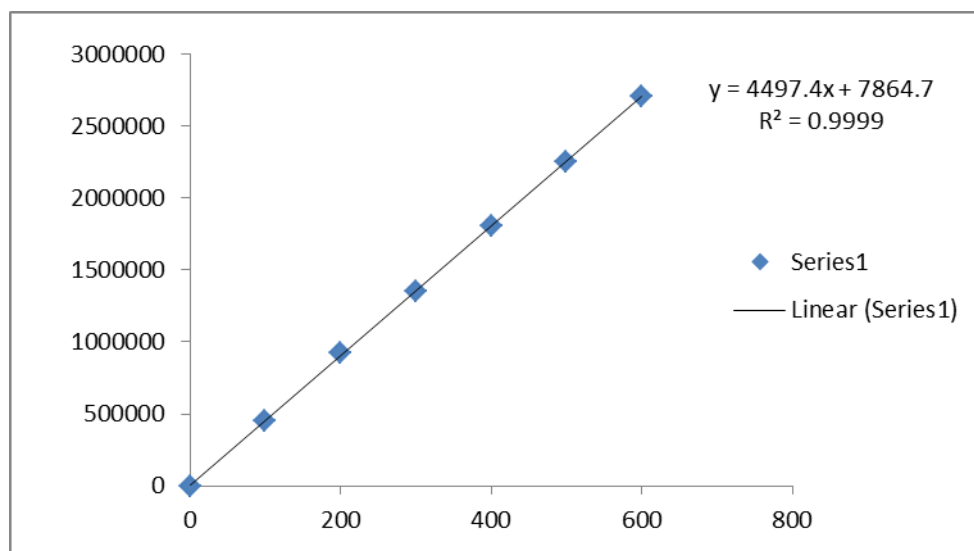


Fig. 9: Linearity curve of Miconazole.

### Validation

The above optimized chromatographic method has been validated for the assay of BTM, GTM & MCN using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Betamethasone, Gentamicin and Miconazole (BTM, GTM & MCN) drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug ( $\mu\text{g/ml}$ ) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Betamethasone (10mg)+ Gentamicin (10mg)+ Miconazole (200mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Betamethasone (10mg)+ Gentamicin (10mg)+ Miconazole (200mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80, 100 and 120% of Betamethasone, Gentamicin and Miconazole. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae  $\text{LOD} = 3.3 \times \text{standard deviation} / \text{slope}$ ;  $\text{LOQ} = 10 \times \text{standard deviation} / \text{slope}$ . Robustness was performed by following the same method with different flow rate.

### RESULTS AND DISCUSSION

The regression equation for BTM was found to be  $y = 3415x + 485.6$  (slope, intercept and correlation coefficient were found to be 3415, 485.6 and 0.999 respectively) and linear over beer's range of 5-30  $\mu\text{g/ml}$ . The regression equation for GTM was found to be  $y =$

$5211x + 876.6$  (slope, intercept and correlation coefficient were found to be 5211, 876.6 and 0.999 respectively) and linear over beer's range of 5-30  $\mu\text{g/ml}$ . The regression equation for MCN was found to be  $y = 4497x + 7864$  (slope, intercept and correlation coefficient were found to be 4497, 7864 and 0.999 respectively) and linear over beer's range of 100-600  $\mu\text{g/ml}$ . Linearity graph of BTM, GTM & MCN were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Betamethasone, Gentamicin and Miconazole were and found to be 0.8, 0.8 and 0.7 respectively. %RSD of method precision for Betamethasone, Gentamicin and Miconazole were and found to be 0.4, 0.5 and 0.3 respectively. % recovery was obtained as 98.83%, 98.65% and 99.21% for Betamethasone, Gentamicin and Miconazole respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug BTM, GTM & MCN and along with 5  $\mu\text{g/mL}$  of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.56%, 99.37% and 99.16% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for BTM, GTM & MCN was found to be 0.09  $\mu\text{g/ml}$ , 0.04  $\mu\text{g/ml}$  and 1.96  $\mu\text{g/ml}$  respectively. LOQ for BTM, GTM & MCN was found to be 0.28  $\mu\text{g/ml}$ , 5.93  $\mu\text{g/ml}$  and 0.12  $\mu\text{g/ml}$  respectively. Summary of all the validation parameter shown in table 6.

### Degradation

Degradation studies were performed with the formulation and the degraded samples were injected.

Assay of the injected samples was calculated and all the samples passed the limits of degradation.

## CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Betamethasone, Gentamicin and Miconazole in Tablet dosage form was developed and the proposed method as suitable for routine analysis of BTM, GTM & MCN.

## REFERENCES A) BETAMETHASONE (B) GENTAMICIN (C) MICONAZOLE

1. Betamethasone monograph (Online) available on URL: <https://www.drugbank.ca/drugs/DB00443>.
2. Gentamicin monograph (Online) available on URL. <https://www.drugbank.ca/drugs/DB00798>.
3. Miconazole monograph (Online) available on URL. <https://www.drugbank.ca/drugs/DB01110>.
4. R. G Chatwal, Anand K.S. High performance liquid chromatography. Instrumental methods of chemical analysis, 5<sup>th</sup> ed; Himalaya publishers: Mumbai, 2010; 2.570-2.629.
5. B. K Sharma, High performance liquid chromatography. Instrumental methods of chemical analysis, 24<sup>th</sup> ed; Goel publishers: Meerut, 2005; 295-300.
6. W.M. Dong HPLC Instrumentation and trends. Modern HPLC for practicing scientists. USA, 2006; 5-10, 78-110.
7. A. Skoog, DM West, FJ Holler, Fundamentals of Analytical Chemistry, 7th edition, Saunders College Publishing, Philadelphia, 1992; 1-3.
8. K. A Corners. Textbook of Pharmaceutical Analysis, A Wiley- inter science Publication, 1st edition, 1967; 475-478.
9. A.V Kasture., Wadodkar S.G., Mahadik K.R., More H.N. Textbook of Pharmaceutical Analysis – II, Published by Nirali Prakashan, 13th edition, 2005.
10. A.H. Beckett and Stanlake J.B. Practical Pharmaceutical Chemistry, Part 2, CBS Publishers and Distributors; 4th edition, 2002; 157-174.
11. R.L Snyder, Kirkland J.J, Glajch L.J. Practical HPLC method development, 2<sup>nd</sup> ed; new York, 1997; 30-100.
12. A. Satinder, Dong M.W, Method development and validation .Pharmaceutical analysis by HPLC, 15<sup>th</sup> ed; Newyork, 2005; 16-70.