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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OFSIMULTANEOUS ESTIMATION OF BETAMETHASONE, GENTAMICIN AND MICONAZOLE IN CREAM FORMULATION

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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 Kromosil 250 x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrie in the ratio of 50:50 A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% OPA buffer at pH 2.5. 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 2.348min, 3.435 min and 4.148 min %RSD of system precision for Betamethasone, Gentamicin and Miconazole. were and found to be 0.3, 0.9 and 0.2 respectively. %RSD of method precision for Chlorthalidone, Gentamicin and Miconazole. were and found to be 0.3, 0.4 and 0.2 respectively. % recovery was Obtained as 99.35%, 98.53%, 99.59% for Betamethasone, Gentamicin and Miconazole. Respectively. LOD, LOQ values are obtained from regression equations of Betamethasone, Gentamicin and Miconazole were 0.06ppm, 0.18ppm, 0.05ppm, 0.15ppm and 0.08ppm, 0.24ppm respectively. Regression equation of Betamethasone. was y =11837.x + 376.1, Gentamicine was y = 12013x +352.8 and of Miconazole was y = 4931.x + 840.9.

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

INTRODUCTION
Betamethasone valerate(1R,2S,10S,11S,13S,14R,15S,17S)-1-fluoro-17-hydroxy-14- (2-hydroxyacetyl)-2,13,15-trimethyl-5-oxotetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}] heptadeca-3,6-dien-14-yl pentanoate glucocorticoid steroid with anti-inflammatory and immunosuppressive properties and used Valerate is used for the treatment, control, prevention of Redness, Itchiness, Hives, Swelling, Inflammation, Itching. Gentamicin (3*R*,4*R*,5*R*)-2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3- {[(2*R*,3*R*,6*S*)-3-amino-6-[(1*R*)-1-(methylamino) ethyl]oxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}- 5-methyl-4-(methylamino)oxane-3,5-diol A complex of three different closely related aminoglycoside sulfates, Gentamicins C1, C2 and C1a, obtained from Micromonospora purpurea and related species and it is used for used to treat minor skin infections (such as impetigo, folliculitis) or minor infections related to some skin conditions (such as eczema, psoriasis, minor burns/cuts/wounds). Gentamicin works by stopping the growth of certain bacteria. Miconazole1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1H-imidazole. An imidazole antifungal agent that is used topically and by intravenous infusion and it is used for treatment of athlete's foot, ringworm, and jock itch. Internal application is used for oral or vaginal thrush (yeast infection). The oral gel may also be used for the lip disorder angular cheilitis.

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for cost effective estimation of Betamethasone, Gentamicin and Miconazole in combination.

The objectives is to develop analytical method
Selecting the HPLC separation mode.
Selecting/ optimizing the mobile phase.
Selecting column for analysis.
Selecting the appropriate detector system.
Selecting appropriate gradient/ isocratic medium.
Selecting appropriate flow rate, temperature and pH

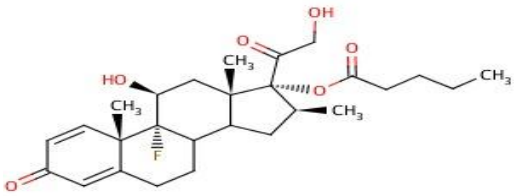

Fig. 1: Structure of Betamethasone valerate.

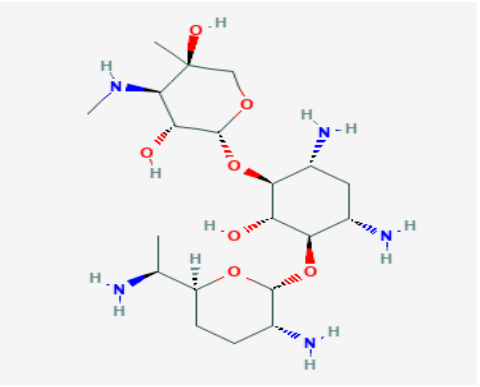

Fig. 1: Structure of Gentamicin.

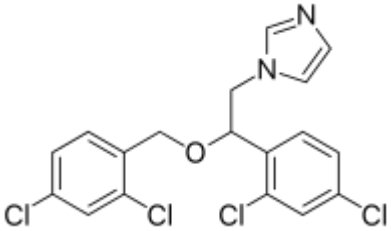

Fig. 3: Structure of Miconazole.

MATERIALS AND METHODS

Materials and Instruments: The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Drug Samples

Were obtained from

- Spectrum pharma research solutions pvt. ltd.

Chemicals and Solvents Used

- Water –HPLC grade
- Acetonitrile - HPLC grade
- Triethyl amine– ARgrade
- Potassium dihydrogen ortho phosphate – AR grade
- Orthophosphoric acid – Argrade
- All the above chemicals and solvents are from Ranchem

Instruments

- Electronics Balance - Denver
- BVK enterprises, India, pH meter
- Waters HPLC 2695 series with quaternary pumps, Photo Diode array detector and auto sampler integrated with empower software
- BVK enterprises, Ultrasonicator.
- Labindia UV double beam spectrophotometer with UVwin5.

Sample Processing

Diluents: Based up on the solubility of the drug diluents was selected, firstly dissolved in methanol and diluted with Acetonitrile and Water.

Preparation of Standard stock solutions: Accurately weighed 25mg of Betamethasone, 25 mg of Gentamicin and 100mg of Miconazole and transferred to three 25ml volumetric flasks separately. 10ml of methanol was added to flasks and sonicated for 15mins. Flasks were made up with water and mrthanol (50:50) and labeled as Standard stock solution 1, 2 and 3. **Preparation of Standard working solutions (100% solution):** 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with Water methanol. (20ppm & 20ppm & 400ppm).

Preparation of Sample 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.

Preparation of Sample working solutions (100% solution): From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluents. (20ppm & 20ppm & 400ppm).

Preparation of buffer

0.01N Na_2HPO_4 Buffer: Accurately weighed 1.41gm of sodium dihyrogen 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 with dil. Orthophosphoric acid solution.

0.01N KH_2PO_4 Buffer: Accurately weighed 1.41gm of Potassium dihyrogen 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 5.4 with dil. Orthophosphoric acid solution.

0.1%OPA Buffer: 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

RESULT AND DISCUSSION

System suitability

The system suitability parameters were determined by preparing standard solutions of Betamethasone, Gentamicin and Miconazole and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Table 1: System suitability of Clobetasol, Neomycin, Chlorhexidine and Miconazole.

S no	Betamethasone.			Gentamicin			Miconazole		
Inj	RT(min)	TP	Tailing	RT(min)	TP	Tailing	RT(min)	TP	Tailing
1	2.347	4507	1.59	3.430	7675	1.34	4.133	9359	1.38
2	2.348	4662	1.58	3.435	7763	1.33	4.140	8955	1.39
3	2.350	4206	1.61	3.435	7635	1.37	4.141	8819	1.38
4	2.350	4483	1.59	3.437	7858	1.42	4.147	9267	1.38
5	2.353	4593	1.60	3.438	7994	1.35	4.148	9323	1.37
6	2.355	4519	1.62	3.445	7811	1.34	4.152	9313	1.39

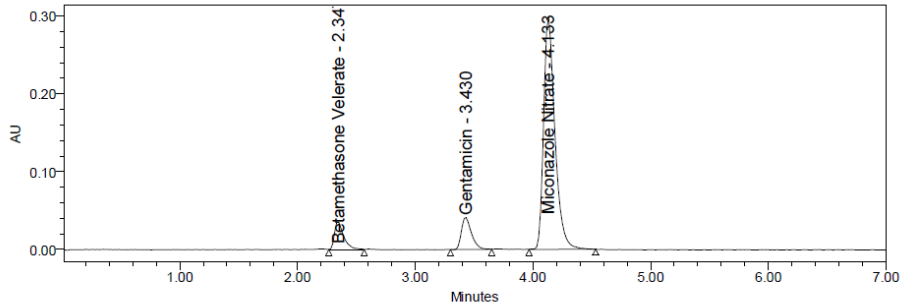

Fig. 1: System suitability chromatogram.

Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

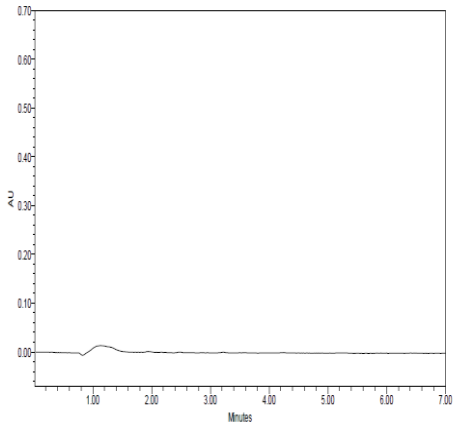

Fig. 2: Blank chromatogram.

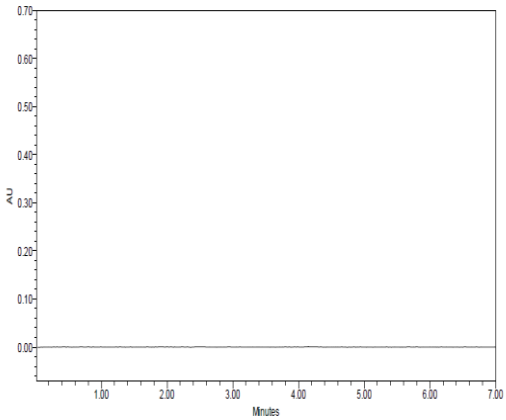

Fig. 3: Placebo chromatogram.

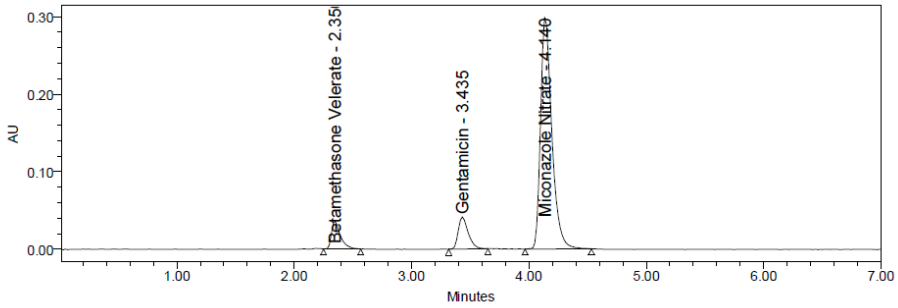

Fig. 4: Optimized chromatogram.

Accuracy

Preparation of Standard stock solutions: Accurately weighed 25mg of Betamethasone, 25 mg of Gentamicin and 100mg of Miconazole and transferred to three 25ml volumetric flasks separately. 10ml of methanol was added to flasks and sonicated for 15mins. Flasks were made up with water and mrthanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluents.

Table 2: Accuracy table of Betamethasone.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	9.943558	99.44	99.35%
	10	9.952091	99.52	
	10	9.921086	99.21	
100%	20	19.81287	99.06	
	20	19.83568	99.18	
	20	19.85899	99.29	
150%	30	29.83027	99.43	
	30	29.8851	99.62	
	30	29.82875	99.43	

Table 3: Accuracy table of Gentamicin.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	9.931175	99.31	99.53%
	10	9.936752	99.37	
	10	9.93567	99.36	
100%	20	20.00584	100.03	
	20	19.87773	99.39	
	20	19.97962	99.90	
150%	30	29.83078	99.44	
	30	29.75478	99.18	
	30	29.92676	99.76	

Table 4: Accuracy table of Miconazole.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	200	9.98	99.40	99.59%
	200	9.91	99.86	
	200	9.97	99.04	
100%	400	19.87	99.46	
	400	20.02	99.84	
	400	19.80	99.88	
150%	600	30.26	99.72	
	600	30.28	99.19	
	600	29.91	99.96	

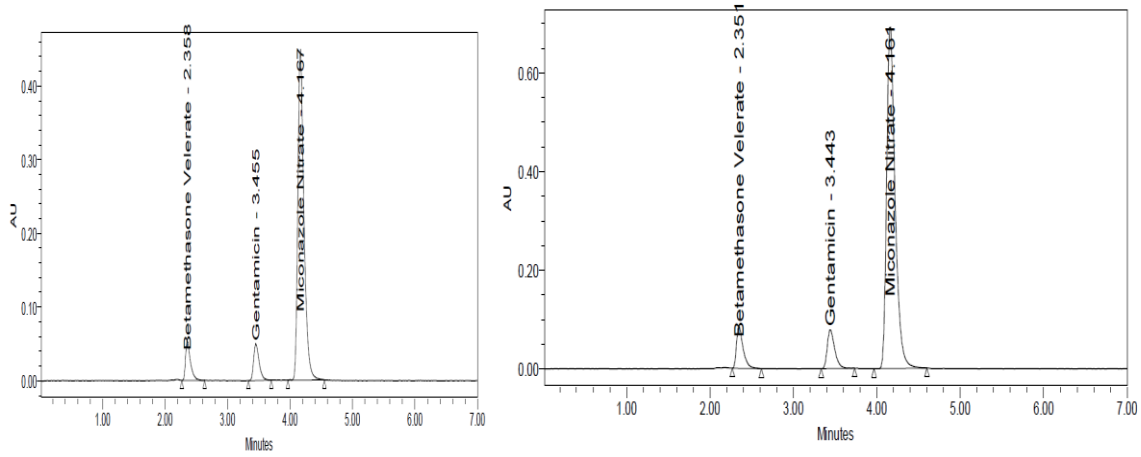

Fig. 5: Accuracy chromatogram.

Precision

Preparation of Standard stock solutions: Accurately weighed 25mg of Betamethasone, 25 mg of Gentamicin and 100mg of Miconazole and transferred to three 25ml volumetric flasks separately. 10ml of methanol was added to flasks and sonicated for 15mins. Flasks were made up with water and mrthanol (50:50) and labeled as Standard stock solution 1, 2 and 3. **Preparation of Standard working solutions (100% solution):** 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask and made up with water and methanol. (20ppm & 20ppm & 400ppm).

Preparation of Sample 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.

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents. (20ppm & 20ppm & 400ppm).

System Precision

Table 5: System precision table of Betamethasone, Gentamicin and Miconazole.

S. No	Area of Betamethasone.	Area of Gentamicin	Area of Miconazole
1.	231102	241976	1982492
2.	230580	242683	1986256
3.	231635	246898	1982868
4.	232368	241375	1991675
5.	231634	242131	1984267
6.	230582	240833	1981610
Mean	231317	242649	1984861
S.D	698.0	2177.0	3711.7
%RSD	0.3	0.9	0.2

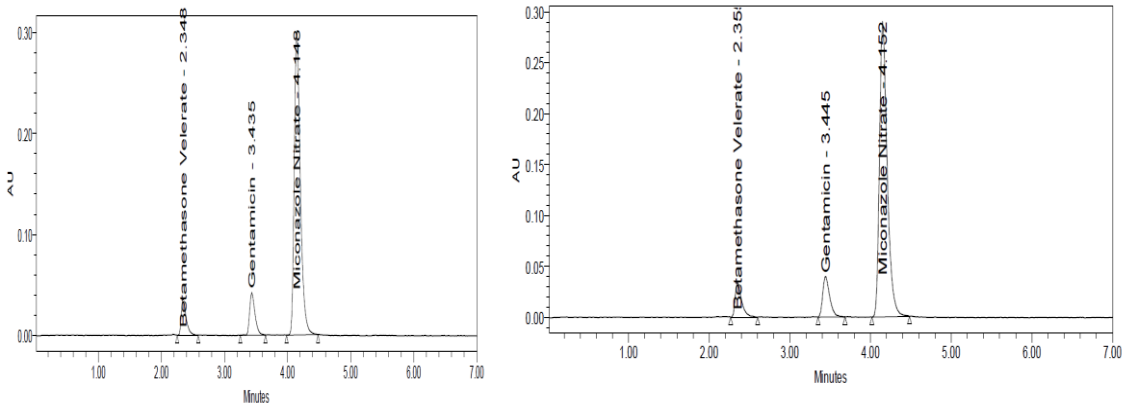

Fig. 6: System precision chromatogram.

Intermediate precision (Day_ Day Precision)

Table 6: Intermediate precision table of Betamethasone, Gentamicin and Miconazole.

S. No	Area of Betamethasone.	Area of Gentamicin	Area of Miconazole
1.	216623	227014	1861948
2.	211895	229939	1857604
3.	214832	226286	1851317
4.	216123	227884	1869448
5.	212412	224047	1869949
6.	213109	228494	1855837
Mean	214166	227277	1861017
S.D	1983.0	2020.9	7540.6
%RSD	0.9	0.9	0.4

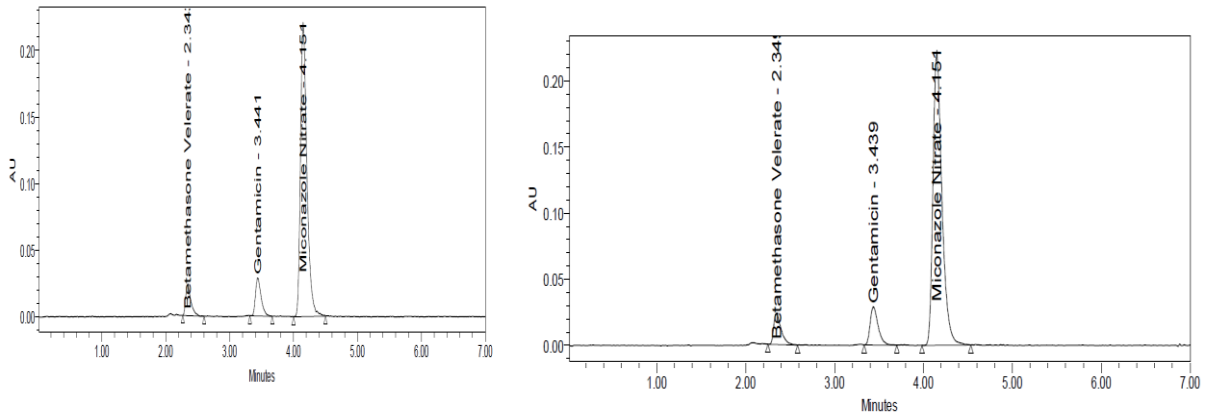

Fig. 7: Intermediate precision chromatogram.

Linearity

Preparation of Standard stock solutions: Accurately weighed 25mg of Betamethasone, 25 mg of Gentamicin and 100mg of Miconazole and transferred to three 25ml volumetric flasks separately. 10ml of methanol was added to flasks and sonicated for 15mins. Flasks were made up with water and mrthanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

25% Standard solution: 0.25ml each from three standard stock solutions was pipette out and made up to 10ml.

50% Standard solution: 0.5ml each from three standard stock solutions was pipette out and made up to 10ml.

75% Standard solution: 0.75ml each from three standard stock solutions was pipette out and made up to 10ml.

100% Standard solution: 1.0ml each from three standard stock solutions was pipette out and made up to 10ml.

125% Standard solution: 1.25ml each from three standard stock solutions was pipette out and made up to 10ml.

150% Standard solution: 1.5ml each from three standard stock solutions was pipette out and made up to 10ml.

Table 7: Linearity table for Betamethasone, Gentamicin and Miconazole.

Betamethasone.		Gentamicin		Miconazole	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
5	57649	5	58336	100	473800
10	122242	10	123673	200	984146
15	174087	15	177172	300	1498191
20	239104	20	243790	400	1987651
25	301686	25	304171	500	2501756
30	350747	30	356664	600	2916075

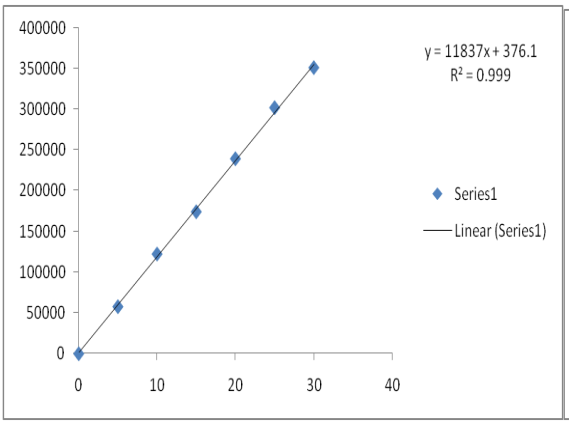

Fig. 8: Calibration curve of Betamethasone.

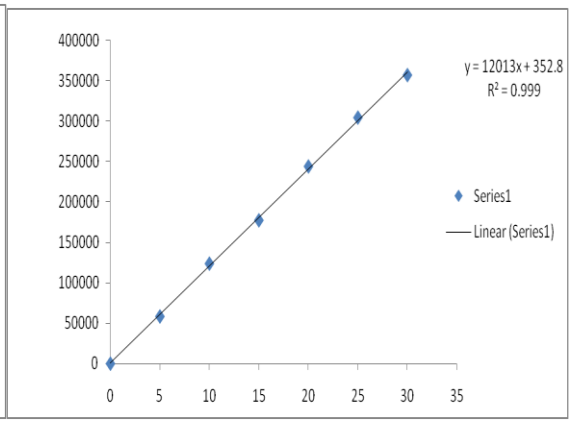

Fig. 9: Calibration curve of Gentamicin.

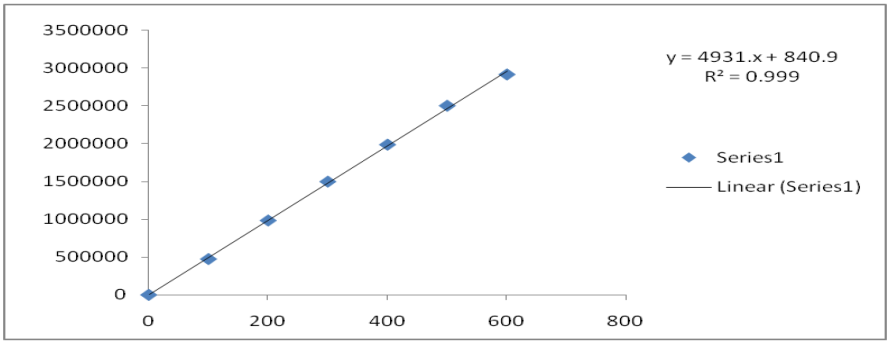

Fig. 10: Calibration curve of Miconazole.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

Table 8: Robustness data for Betamethasone, Gentamicin and Miconazole.

S.no	Condition	%RSD of Betamethasone.	%RSD of Gentamicin	%RSD of Miconazole
1	Flow rate (-) 0.9ml/min	0.6	0.6	0.9
2	Flow rate (+) 1.1ml/min	0.9	0.4	0.6
3	Mobile phase (-) 60B:40A	1.1	1.1	0.4
4	Mobile phase (+) 50B:50A	0.6	1.2	1.0
5	Temperature (-) 25°C	0.9	1.0	0.9
6	Temperature (+) 35°C	1.4	0.4	0.9

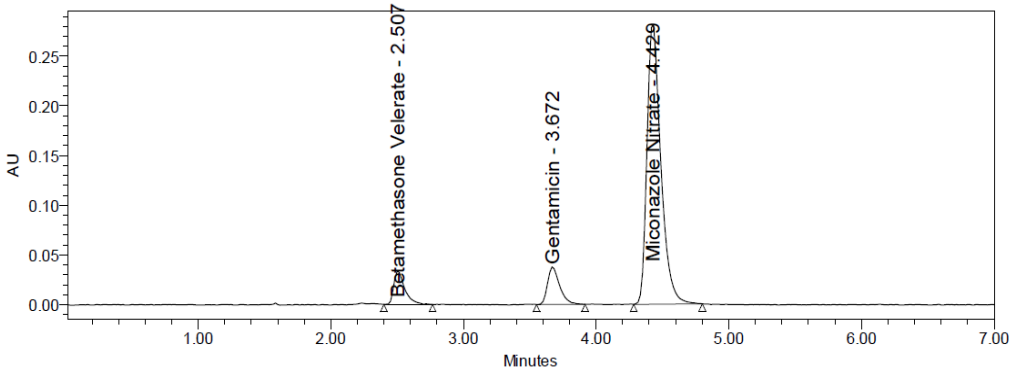

Fig. 11: Flow minus chromatogram injection.

LOD sample Preparation: 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 0.1ml, 0.1ml and 0.1ml of Betamethasone, Gentamicin and Miconazole solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from three standard stock solutions was pipette out and transferred to 3 separate 10ml volumetric flask and made up with diluents from the above solutions 0.3ml, 0.3ml and 0.3ml of Betamethasone, Gentamicin and Miconazole solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

Table 9: Sensitivity table of Betamethasone, Gentamicin and Miconazole.

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Betamethasone.	0.06 µg/ml	0.18 µg/ml
Gentamicin	0.05 µg/ml	0.15µg/ml
Miconazole	0.08 µg/ml	0.24 µg/ml

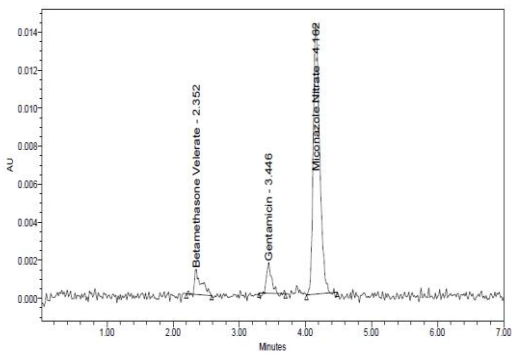

Fig. 12: LOD chromatogram of standard.

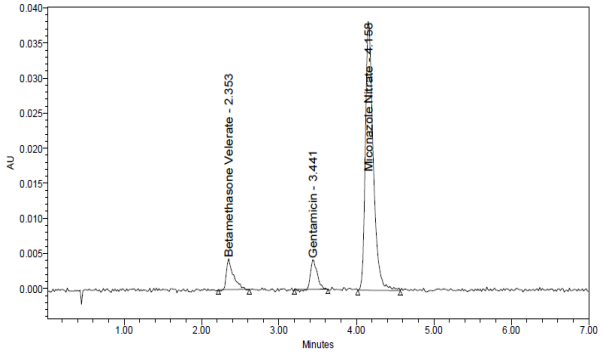

Fig. 13: LOQ chromatogram of standard.

Assay

Betnovate-GM (0.1% w/w+0.1% w/w+2.0%), baring the label claim Gentamicin 20mg Betamethasone, 20mg Miconazole 400mg per unit formulation Assay was performed with the above formulation. Average % Assay for Betamethasone, Gentamicin and Miconazole. Obtained was 99.03%, 99.56% and 99.49% respectively.

Table 10: Assay Data of Betamethasone.

S.no	Standard Area	Sample area	% Assay
1	231102	229082	98.84
2	230580	229486	99.01
3	231635	228975	98.79
4	232368	228984	98.79
5	231634	229891	99.18
6	230582	230773	99.57
Avg	231317	229532	99.03
Stdev	698.0	704.9	0.304
%RSD	0.3	0.3	0.31

Table 11: Assay Data of Gentamicin.

S.no	Standard Area	Sample area	% Assay
1	241976	240937	99.10
2	242683	241037	99.14
3	246898	242241	99.63
4	241375	242112	99.58
5	242131	243095	99.98
6	240833	242973	99.93
Avg	242649	242066	99.56
Stdev	2177.0	921.7	0.38
%RSD	0.9	0.4	0.38

Table 12: Assay Data of Miconazole.

S.no	Standard Area	Sample area	% Assay
1	1982492	1977082	99.41
2	1986256	1979486	99.53
3	1982868	1975975	99.35
4	1991675	1976884	99.40
5	1984267	1975891	99.35
6	1981610	1986773	99.90
Avg	1984861	1978682	99.49
Stdev	3711.7	4171.8	0.210
%RSD	0.2	0.2	0.21

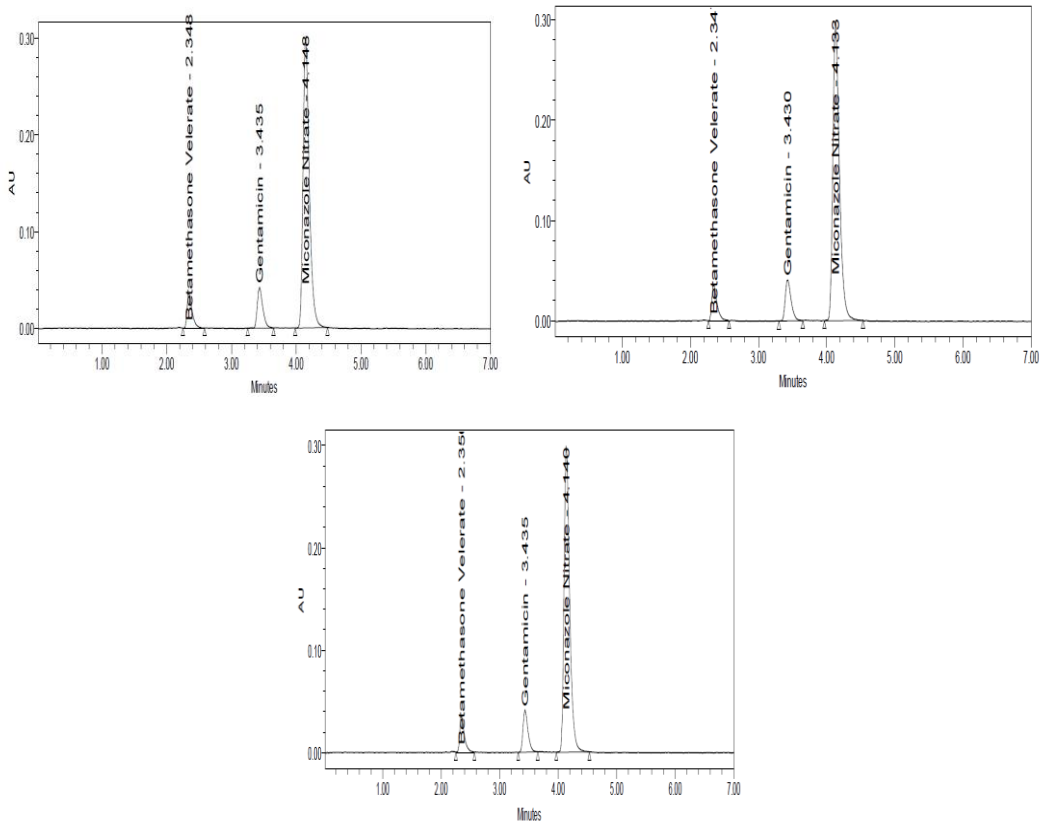

Fig. 14: Chromatogram of working standard solution.

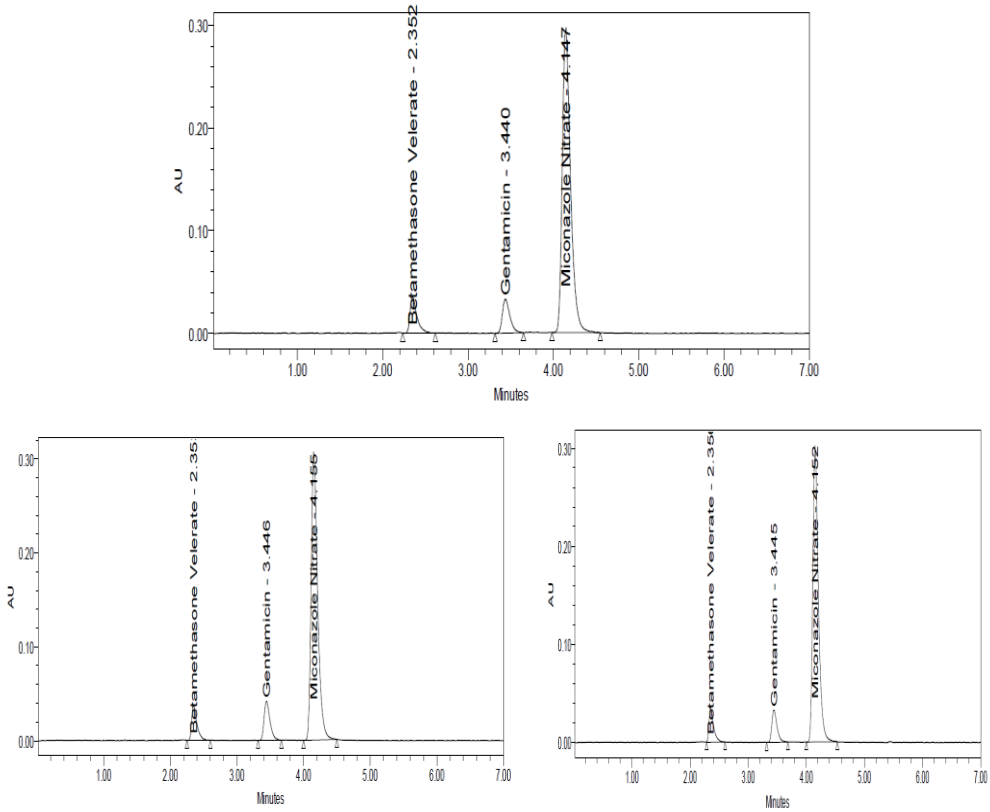

Fig. 15: Chromatogram of working sample solution.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Betamethasone, Gentamicin and Miconazole in Tablet dosage form. Retention time of Betamethasone, Gentamicin and Miconazole. Were found to be 2.348min, 3.435 min and 4.148 min %RSD of system precision for Betamethasone, Gentamicin and Miconazole were and found to be 0.3, 0.9 and 0.2 respectively. %RSD of method precision for Chlorthalidone, Gentamicin and Miconazole were and found to be 0.3, 0.4 and 0.2respectively. % recovery was obtained as 99.35%, 98.53%, and 99.59% for Betamethasone, Gentamicin and Miconazole respectively. LOD, LOQ values are obtained from regression equations of Betamethasone, Gentamicin and Miconazole were 0.06ppm, 0.18ppm, 0.05ppm, 0.15ppm and 0.08ppm, 0.24ppm respectively. Regression equation of Betamethasone was y =11837.x + 376.1, Gentamicine was y = 12013x +352.8 and of Miconazole was y = 4931.x + 840.9. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

REFERENCES

1. R. G Chatwal, Anand K.S. High performance liquid chromatography. Instrumental methods of chemical analysis, 5th ed; Himalaya publishers: Mumbai, 2010; 2.570-2.629.
2. B. K Sharma, High performance liquid chromatography. Instrumental methods of chemical analysis, 24th ed; Goel publishers: Meerut, 2005; 295-300.
3. W.M. Dong HPLC Instrumentation and trends. Modern HPLC for practicing scientists. USA., 2006; 5-10, 78-110.

a. Skoog, DM *West*, FJ Holler, Fundamentals of Analytical Chemistry, 7th edition, Saunders College Publishing, Philadelphia, 1992; P.1-3.

4. K. A Corners. Textbook of Pharmaceutical Analysis, A Wiley- inter science Publication, 1st edition, 1967; P.475-478.
5. A.V Kasture., Wadodkar S.G., Mahadik K.R., More H.N. Textbook of Pharmaceutical Analysis – II, Published by Nirali Prakashan, 13th edition, 2005.
6. A.H. Beckett and Stanlake J.B. Practical Pharmaceutical Chemistry, Part 2, CBS Publishers and Distributors; 4th edition., 2002; 157-174.
7. R.L Snyder, kirklannd J.J, Glaijch L.J. Practical HPLC method development, 2nd ed; new York, 1997; 30-100.

a. Satinder, Dong M.W, Method development and validation. Pharmaceutical analysis by HPLC, 15th ed; Newyork, 2005; 16-70.

8. M.E Swartz, Ira krull, Analytical method development and validation, 1st ed; Marcel Dekker, New York, 2009; 17-80.
9. Chandra nath saha1 and sanjib bhattacharya2* a validated simultaneous rp-hplc method for determination of betamethasone dipropionate and tolnaftate in combined semisolid formulation international journal of chemtech research, July-Sept. 2009.

10. R,* k. mukkanti,[1] s. durgaprasad,[2] and m. khan simultaneous hplc determination of butenafine hydrochloride and betamethasone in a cream formulation indian j pharm sci., 2009 Sep-Oct; 71(5): 547–551.
11. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2010; 2: 1657-1658.
12. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 2011; 2: 1408-1409.
13. "https://www.drugbank.ca/drugs/DB00443
14. https://www.drugbank.ca/drugs/DB00798
15. https://www.drugbank.ca/drugs/DB01110
16. Aaburustum development and validation of a rp-hplc method for the determination of gentamicin sulfate and its related substances in a pharmaceutical cream using a short pentafluorophenyl column and a charged aerosol detector journal of pharmaceutical and biomedical analysis, 5 February 2010; 51(3).
17. Neha sharad sinnarkar and manisha s. phoujdar* development and validation of hptlc method for determination of betamethasone valerate in api and pharmaceutical dosage form world journal of pharmaceutical sciences received: 19-03-2017 / revised: 24-04-2017 / accepted: 26-04-2017 / published: 27-04-2017.
18. Mmaría s.cámara[a] juan c.robles[a] silvina v.kergaravat[a] héctor c.goicoechea[b] elsever, 15 August 2009; 79(3).
19. Jonathan byrne trinidad velasco-torrijos robert reinhardt an rp-hplc method for the stability-indicating analysis of impurities of both fusidic acid and betamethasone-17-valerate in a semi-solid pharmaceutical dosage form *journal of chromatographic science*, 1 October 2015; 53(9): 1498–1503.
20. philip j kuehl1*, samiran de3, barbel eppler2, jane marsters1, laura matthews2, matthew d reed1 and james d talton2 development and validation of an hplc assay for dual detection of gentamicin sulfate and leucine from a novel dry powder for inhalation journal of uoj analytical & bioanalytical techniques kuehl et al., j anal bioanal techniques, 2012; 3: 6.
21. m. b. ali*, m. attia[1], n. bellili[1] and sami fattouch development and validation of a rp-hplc method for simultaneous determination of betamethasone and sodium benzoate in oral liquid pharmaceutical formulation indian journal of pharmaceutical sciences, 19 Jan 2016; revised date: 16 jun 2016; accepted date: 20 jun 2016.
22. chinmoy roy*1,2, jitamanyu chakrabarty2 and rammohan rao ratti1 development and validation of a stability-indicating np-hplc method for simultaneous determination of betamethasone dipropionate and calcipotriene in topical dosage form scholars research library archives of applied science research, 2013; 5(2): 15-24.
23. venkata raj kumar prava1* and ganapaty seru2 rp-hplc method development and validation for the simultaneous determination of clindamycin and miconazole in pharmaceutical dosage forms pharmaceutical methods, Jul-Dec 2014; 5(2).