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Simultaneous estimation of Clotrimazole, Betamethasone, antioxidant and preservatives in Topical formulation by RP-HPLC

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ABSTRACT: Background: Clotrimazole and Betamethasone is an antifungal medication that is used to treat athlete's foot, jock itch, ringworm, pityriasis and other fungal skin infection. **Aim:** To develop a simple, rapid, cost-effective methodology for the determination of clotrimazole, betamethasone, antioxidants and preservatives simultaneously by RP-HPLC. **Method:** A stability-indicating reverse phase-HPLC method has been developed and validated for the simultaneous determination of Clotrimazole and Betamethasone in pharmaceutical dosage form. Clotrimazole, betamethasone, butylated hydroxytoluene, methyl hydroxybenzoate and propyl hydroxybenzoate were used as standard. The method was developed using a Thermo Scientific HPLC system (Ultimate 3000) with a Waters Symmetry C8 (4.6 × 150 mm I.D., 5 µm) column and a gradient elution consisting of Buffer and acetonitrile as the mobile phase. The flow rate was adjusted at 1.0 ml/min. The column oven was set at 40°C and the detection wavelength was set at 225 nm. The retention time of Clotrimazole, betamethasone, butylated hydroxytoluene, methyl hydroxybenzoate and propyl hydroxybenzoate was observed to be about 12, 20, 26.5, 7, and 13 min respectively. **Results:** The developed method was validated according to the ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria. **Conclusion:** The proposed method was successfully applied to the topical dosage form consisting of Clotrimazole and Betamethasone for routine analysis.

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INTRODUCTION:

Clotrimazole (Fig 1) is a synthetic, imidazole derivative with broad-spectrum, antifungal activity. Its molecular weight is 344.8 g/mol with an empirical formula $C_{22}H_{17}ClN_2$. Clotrimazole is white or pale-yellow crystalline powder that is soluble in ethanol (96%) and in methylene chloride, practically insoluble in water. Clotrimazole is commonly available without a prescription in various dosage forms, such as topical cream, ointment, or vaginal suppository. It is used for vulvovaginal candidiasis (yeast infection) or yeast

infection of skin. Few HPLC methods were found for the determination of clotrimazole. Clotrimazole (CLOT) is chemically described as 1-[(2-chlorophenyl) diphenyl methyl]-1H-imidazole [1-3].

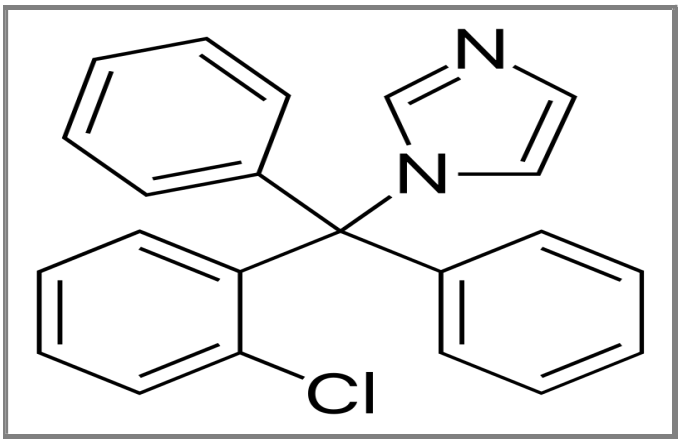

Fig 1. Chemical structure of Clotrimazole.

Methyl paraben (Fig 2) is a 4-hydroxybenzoate ester resulting from the formal condensation of the carboxy group of 4-hydroxybenzoic acid with methanol. It is the most frequently used antimicrobial preservative in cosmetics. It occurs naturally in several fruits, particularly in blueberries. It has a role as a plant metabolite, an antimicrobial food preservative, a neuroprotective agent and an antifungal agent. Its molecular weight is 152.15 g/mol with the chemical formula CH_3 (C_6H_4(OH)COO). It is white crystalline powder, freely soluble in Alcohol and in methanol, slightly soluble in water [4-6].

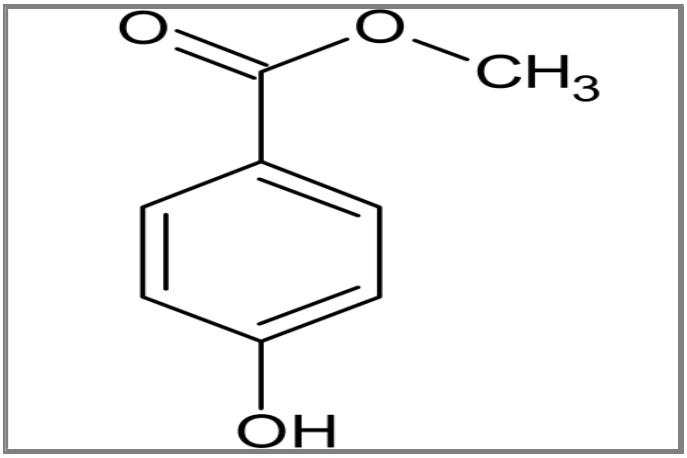

Fig 2. Chemical structure of Methyl Paraben.

Propylparaben (Fig 3), the *n*-propyl ester of p-hydroxybenzoic acid, occurs as a natural substance found in many plants and some insects, although it is manufactured synthetically for use in cosmetics, pharmaceuticals, and foods. It is a member of the class of parabens. It is a preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos, and bath products. As a food additive, it has the E number E216. Its molecular weight is 180.2 g/mol with the chemical formula $C_{10}H_{12}O_3$. It is white crystalline powder, freely soluble in water, sparingly solution in alcohol and practically insoluble in methylene chloride [7-9].

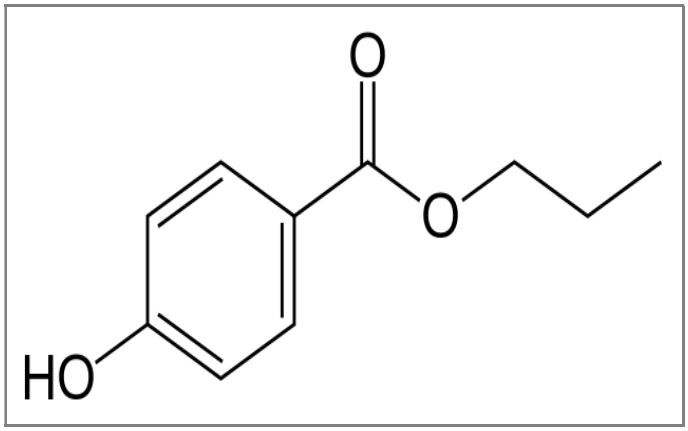

Fig 3. Chemical structure of Propyl Paraben.

Butylated hydroxytoluene (BHT) (Fig 4) also known as dibutyl hydroxytoluene, is a lipophilic organic compound, chemically a derivative of phenol, that is useful for its antioxidant properties. BHT is widely used to prevent free radical-mediated oxidation in fluids and other materials, and the regulation overseen by the U.S. FDA – which consider BHT as 'generally recognized safe' as a small amount to be added in food. At an earlier stage, the National Cancer Institute determined that BHT was noncarcinogenic in an animal model, societal concerns over its broad use have been expressed. Its molecular weight is 220.356 g/mol with empirical chemical formula $C_{15}H_{24}O$. It is white to yellow powder, slightly phenolic odor, soluble in ethanol and insoluble in water and propane-1 2-diol [10,11].

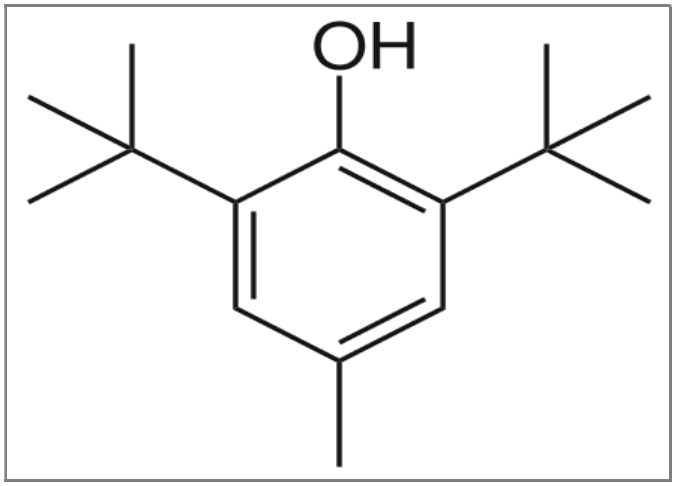

Fig 4. Chemical structure of BHT.

Betamethasone (Fig 5) is a glucocorticoid steroid with anti-inflammatory immunosuppressive abilities. It is applied as a topical cream, ointment, lotion or gel to treat itching and other minor skin conditions such as eczema. It is a synthetic analog of the adrenal corticosteroids. It is white to almost white crystalline powder. Its molecular weight is 504.595 g/mol with empirical chemical formula $C_{28}H_{37}FO_7$ [12,13].

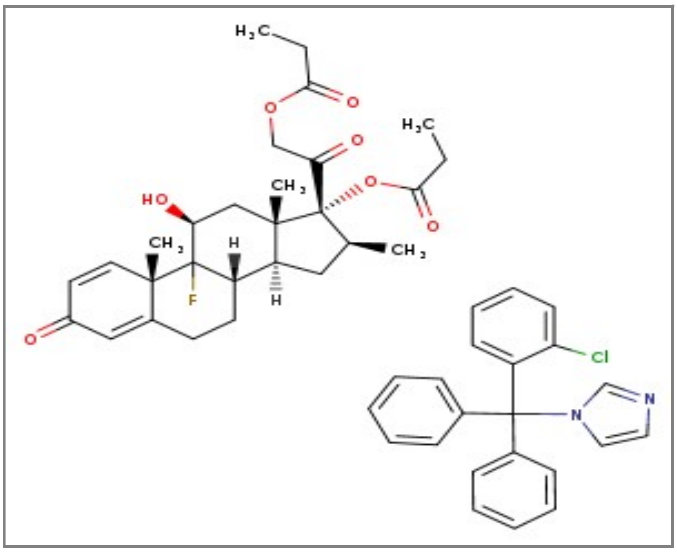

Fig 5. Chemical structure of Betamethasone dipropionate.

Few HPLC methods have been reported for the estimation of Clotrimazole and Betamethasone. The objective of the current study was to develop a simple, rapid and cost effective analytical method for the simultaneous determination of Clotrimazole, Betamethasone and preservative content (Methyl hydroxybenzoate, Propyl hydroxybenzoate), antioxidant (Butylated Hydroxytoluene) in the topical preparation simultaneously using RP-HPLC. The developed method was applied successfully for quality control and stability testing purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [14].

MATERIALS AND METHODS:

Chemicals and reagents:

Clotrimazole, Betamethasone and all preservatives and antioxidants (Methyl hydroxybenzoate, propyl hydroxybenzoate, Butylated hydroxytoluene) working standards were provided by Oman Pharmaceutical Products Co L.L.C. Topical formulation containing Clotrimazole 1 % w/w and Betamethasone 0.05 % w/w was taken from the commercial batch manufactured at Oman Pharmaceutical Products Co L.L.C. HPLC grade Acetonitrile and Methanol was procured from LobaChemie and Merck Ltd. All other chemical reagents were of analytical grade.

Buffer Solution Preparation:

An accurately weighed quantity of Potassium dihydrogen orthophosphate (1.0 g) and tetrabutylammonium hydrogen sulphate (0.5 g) were transferred into a 1000 ml beaker and dissolved in Milli Q water.

The Mobile Phase A and B were buffer (100 %) and Acetonitrile (100 %) (Table 1). The diluent was methanol.

Table 1. Gradient Program.

Time (min)	Mobile Phase A	Mobile Phase B
0	75	25
3	75	25
25	20	80
30	20	80
31	75	25
35	75	25

Clotrimazole standard stock solution (Solution-1):

An accurately weighed quantity of Clotrimazole working standard (25 mg) was taken in a 25 ml volumetric flask. It was dissolved and diluted to volume with diluent to obtain a solution of strength 500 µg/ml.

Betamethasone standard stock solution (Solution-2):

An accurately weighed quantity of Betamethasone dipropionate working standard (31.5 mg) was taken in a 50 ml volumetric flask. It was dissolved and diluted to volume with diluent to obtain a solution of strength 630 µg/ml. Further, 1 ml of the resultant solution was diluted to 20 ml with diluent.

Methyl Hydroxybenzoate standard stock solution (Solution-3):

An accurately weighed quantity of Methyl hydroxybenzoate working standard (25 mg) was taken in a 10 ml volumetric flask. It was dissolved and diluted to volume with diluent to obtain a solution of strength 2500 µg/ml.

Propyl Hydroxybenzoate standard stock solution (Solution-4):

An accurately weighed quantity of Propyl hydroxybenzoate working standard (25 mg) was taken in a 100 ml volumetric flask. It was dissolved and diluted

to volume with diluent to obtain a solution of strength 250 µg/ml.

Butylated hydroxytoluene standard stock solution (Solution-5):

An accurately weighed quantity of Butylated hydroxy toluene working standard (50 mg) in a 20 ml volumetric flask. It was dissolved and diluted to volume with diluent to obtain a solution of strength 1000 µg/ml.

Standard for Assay (Solution-6):

About 5 ml of Solution-1, 5 ml of Solution-2, 2 ml of solution-3, 2 ml of solution-4 and 1 ml of Solution-5 was transferred into a 50 ml volumetric flask and diluted up to the mark with diluent. The resultant solution various strength of drugs in solution that are Clotrimazole (50 µg/ml), Betamethasone (2.5 µg/ml), Methyl hydroxybenzoate (100 µg/ml), Propyl hydroxy benzoate (10 µg/ml), and Butylated hydroxyltoluene (50µg/ml) respectively.

Sample Stock preparation for related substance (Solution-7):

About 2.5 g (equivalent to 25 mg of Clotrimazole, 1.25 mg of Betamethasone dipropionate and 5 mg of Methyl hydroxy benzoate, 0.5 mg of Propyl hydroxybenzoate, and 2.5 mg of Butylated hydroxytoluene) of cream sample was weighed accurately and transferred into a 50 ml centrifuge tube. About 20 ml of methanol was added and the centrifuge tube was kept in a water bath for 5 min at 50°C with occasional swirling. Further, the solution was cooled in an ice bath for 15 min. Next, the solution was centrifuged for 5 min at 5000 RPM and decanted the supernatant liquid into a 50 ml volumetric flask. The extraction procedure was repeated again with 20 ml of methanol. Finally, the combined extract was diluted with methanol to produce 50 ml. The resultant solution was filtered with 0.45 µ Nylon filter after discarding 5 ml of filtrate. The final solution strength was for each moiety as following that are Clotrimazole (500 µg/ml), Betamethasone dipropionate (25µg/ml), Methyl hydroxybenzoate (100 µg/ml), Propyl hydroxybenzoate (10 µg/ml), and Butylated hydroxytoluene (50 µg/ml).

Sample Preparation for Assay (Solution-8): (For Clotrimazole and Betamethasone)

About 5 ml of sample stock solution (Solution-7) filtrate was pipetted into a 50 ml volumetric flask and diluted to volume with diluent which gave a solution of strength for Clotrimazole 50 µg/ml.

Chromatographic study:

Clotrimazole, Betamethasone, Preservatives – Methyl hydroxybenzoate, Propyl hydroxybenzoate, and Antioxidant – Butylated hydroxytoluene content in all solutions were determined by HPLC by using the chromatographic conditions as mentioned in Table 2.

Table 2. Chromatographic condition for analytical study.

Column	Waters Symmetry C_8 (150 × 4.6) mm; 5.0µm
Mobile Phase-A	Buffer solution
Mobile Phase-B	Acetonitrile
Flow rate	1 ml/min
Injection volume	10 µl
Wavelength	225 nm
Column Temperature	40 °C
Sampler cooler Temp.	25 °C
Run Time	35 min
Diluent	Methanol
Elution	Gradient

The Chromatographic data were analysed and Specificity, Linearity and range, Robustness, precision, and accuracy were determined.

RESULTS AND DISCUSSION:

The developed method for determination of Clotrimazole, Betamethasone, its preservative and antioxidant were validated by using the following parameters.

System suitability (Assay, Preservative, and Antioxidant):

For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table 3 to 7.

Table 3. System suitability – Clotrimazole (Assay).

Injection #	Area	Tailing Factor	Plate count
1	23095	1.05	55922
2	23077	1.04	54851
3	23145	1.06	53840
4	23085	1.04	53524
5	23204	1.05	53715
6	23395	1.06	55691
Mean	23167	1.05	54591
SD	121.5	-	-
% RSD	0.5	-	-

Table 4. System suitability – Betamethasone dipropionate (Assay).

Injection #	Area	Tailing Factor	Plate count
1	567	0.92	174677
2	588	0.95	168182
3	582	0.93	161236
4	579	0.94	164186
5	572	0.92	166476
6	594	0.96	171939
Mean	580	0.94	167783
SD	10.0	-	-
% RSD	1.7	-	-

Table 5. System suitability – Methyl Hydroxybenzoate (Assay).

Injection #	Area	Tailing Factor	Plate count
1	13875	0.94	14949
2	13822	0.94	14383
3	13924	0.94	13963
4	13890	0.93	13739
5	13978	0.94	13890
6	14122	0.94	14277
Mean	13935	0.94	14200
SD	105.2	-	-
% RSD	0.8	-	-

Table 6. System suitability – Propyl Hydroxybenzoate (Assay).

Injection #	Area	Tailing Factor	Plate count
1	1405	0.93	60416
2	1402	0.94	57553
3	1405	0.94	55786
4	1401	0.93	55178
5	1403	0.94	55718
6	1425	0.93	59060
Mean	1407	0.94	57285
SD	9.0	-	-
% RSD	0.6	-	-

Table 7. System suitability – Butylated Hydroxytoluene (Assay).

Injection #	Area	Tailing Factor	Plate count
1	810.4438	0.97	13759
2	807.9650	0.97	13835
3	829.8211	1.00	15077
4	807.8621	0.99	13808
5	826.3224	1.00	15113
6	807.3674	0.98	13975
Mean	814.9636	0.99	14261
SD	10.27	-	-
% RSD	1.3	-	-

Specificity (Assay, Preservative, and Antioxidant):
There were no interfering peaks at the retention time of Clotrimazole, Betamethasone, preservative – methyl paraben, propylparaben and antioxidant – butylated hydroxy toluene peak in the presence of excipients. Further, to demonstrate the specificity of the method, the sample was subjected to acid, base, oxidation, thermal, and photolytic degradation. This was evaluated by using a Photo Diode Array detector (PDA). The chromatograms are presented in Fig 6 to 11, Table 8 and 9 for the peak purity analysis data.

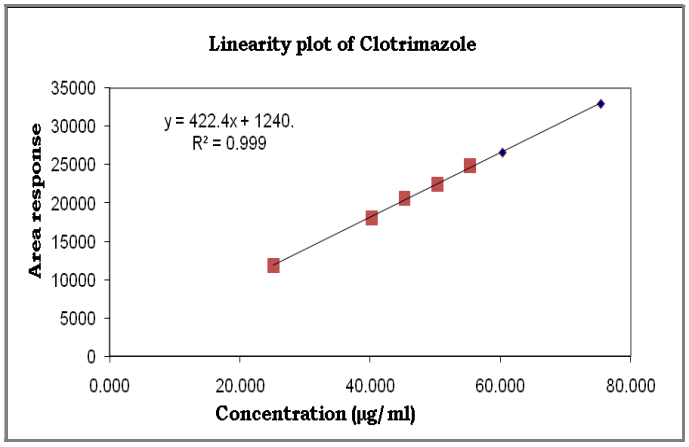

Fig 6. Linearity of Clotrimazole.

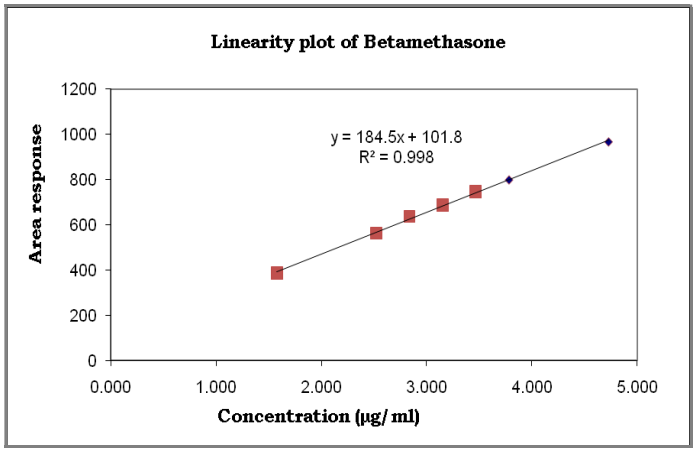

Fig 7. Linearity of Betamethasone.

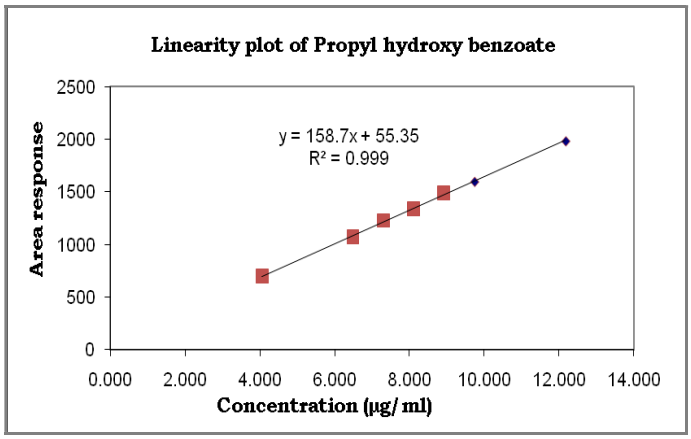

Fig 8. Linearity of Propyl hydroxy benzoate.

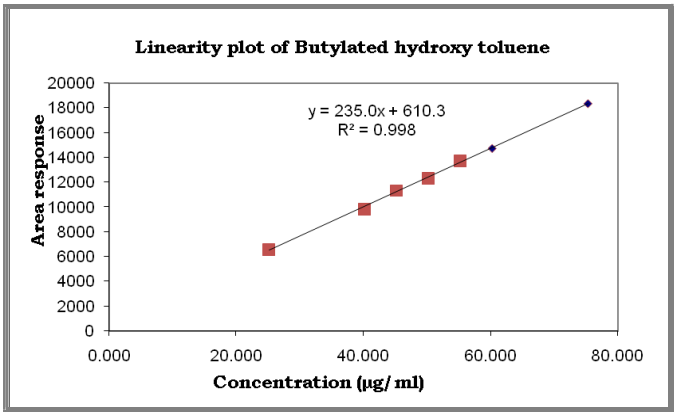

Fig 9. Linearity of Butylated hydroxy toluene.

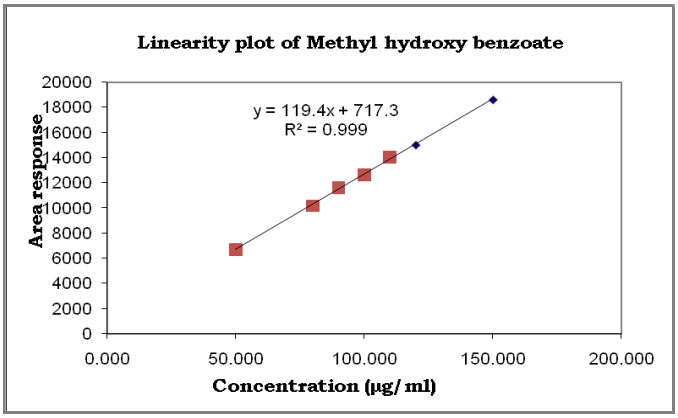

Fig 10. Linearity of Methyl hydroxy benzoate.

Table 8. Force Degradation study summary of Clotrimazole (Assay).

Sample Name	Final DC	PPC	%Dg
AD	5 ml 0.1N HCl @100°C for 15 min	1000	76.4
AA	5 ml 1N NaOH @100°C for 15 min	1000	25.8
OD	5 ml 35% H_2O_2 @100°C for 15 min	1000	28.3
TD	100°C for 24 h	1000	56.2
PD	1.2 million lux h	1000	12.1

Table 9. Force Degradation study summary of Betamethasone (Assay).

Sample Name	Final DC	PPC	%Dg
AD	5 ml 5N HCl @100°C for 15 min	1000	2.2
AA	5 ml 5N NaOH @100°C for 15 min	1000	3.0
OD	5 ml 35% H_2O_2 @100°C for 15 min	1000	5.6
TD	100°C for 24 h	1000	7.7
PD	1.2 million lux h	1000	0.6

AD- Acid Degradation, AA- Alkali Degradation, OD- Oxidative Degradation, TD- Thermal Degradation, PD- Photolytic Degradation, DC- Degradation Condition, PPC- Peak Purity of Clotrimazole and Dg- Degradation.

Linearity and range (Assay, Preservative, and Antioxidant):

Standard solutions containing Clotrimazole, Betamethasone and its preservative (Methyl hydroxybenzoate, Propyl Hydroxybenzoate) with antioxidant (Butylated hydroxytoluene) were prepared. Linearity levels at six different concentrations of 50, 80, 90, 100, 110, 120, and 150 % of the target concentration for Assay. The average peak areas were plotted against concentration. Then, linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

The results obtained are presented in the Table 10 to 14 which demonstrates that the current method was linear for the three analytes in the range specified above with a correlation coefficient better than 0.999. The plots have been represented in Fig 6 to 10.

Table 10. Linearity of Clotrimazole (Assay).

Level	Conc. (µg/ml)	Area
1	25.080	11808
2	40.127	18038
3	45.143	20617
4	50.159	22436
5	55.175	24924
6	60.191	26635
7	75.239	33014
Correlation coefficient (r)		0.9996
Regression coefficient (r^2)		0.9992
Slope		423.7543
Intercept		1240.8454

Table 11. Linearity of Betamethasone (Assay).

Level	Conc. (µg/ml)	Area
1	1.218	385
2	1.949	563
3	2.193	638
4	2.437	687
5	2.680	748
6	2.924	799
7	3.655	967
Correlation coefficient (r)		0.9992
Regression coefficient (r^2)		0.9983
Slope		238.8191
Intercept		101.9574

Table 12. Linearity of Methyl hydroxy benzoate (Assay).

Level	Conc. (µg/ml)	Area
1	42.705	6676
2	68.328	10154
3	76.869	11593
4	85.410	12631
5	93.951	14026
6	102.492	14997
7	128.115	18591
Correlation coefficient (r)		0.9996
Regression coefficient (r^2)		0.9992
Slope		139.9017
Intercept		717.8571

Table 13. Linearity of Propyl Hydroxy benzoate (Assay).

Level	Conc. (µg/ml)	Area
1	5.063	699
2	8.101	1074
3	9.113	1228
4	10.126	1336
5	11.138	1491
6	12.151	1598
7	15.189	1983
Correlation coefficient (r)		0.9996
Regression coefficient (r^2)		0.9992
Slope		127.2485
Intercept		55.6427

Table 14. Linearity of Butylated hydroxy toluene (Assay).

Level	Conc. (µg/ml)	Area
1	24.884	6575
2	39.814	9857
3	44.791	11352
4	49.768	12346
5	54.745	13733
6	59.722	14707
7	74.652	18322
Correlation coefficient (r)		0.9995
Regression coefficient (r^2)		0.999
Slope		237.1522
Intercept		610.5534

Precision (Assay, Preservative, and Antioxidant):

For Assay, precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced. The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 12 and 13.

Ruggedness (Assay, Preservative, and Antioxidant):

Ruggedness of method was demonstrated by preparing the standard and sample as per the methodology by a different analyst on a different day, using a different column lot and using a different HPLC system. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced. The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 15 to 17.

Table 15. Percentage Assay result of Method Precision and Intermediate Precision.

Sample ID #	Opizole-B Cream (B. No. 9ES012A)			
	Clotrimazole		Betamethasone	
	MP	IP	MP	IP
1	91.2	94.8	94.9	96.4
2	91.5	94.8	94.6	96.7
3	93.0	94.9	94.9	96.1
4	92.5	94.7	94.9	96.1
5	94.4	94.6	94.9	95.6
6	100.5	94.8	95.5	96.2
Mean	92.8	94.8	95.0	96.2
SD	1.38	0.10	0.29	037
%RSD	1.48	0.11	0.31	0.38
%Diff	2.0		1.2	

Diff – Difference, MP – Method Precision, IP-Intermediate Precision.

Table 16. Percentage Assay result of Method Precision and Intermediate Precision.

Sample ID #	Opizole-B Cream (B. No. 9ES012A)			
	Methyl hydroxy benzoate		Propyl hydroxy benzoate	
	MP	IP	MP	IP
1	94.3	95.1	88.8	89.0
2	92.6	95.1	87.5	89.2
3	97.1	97.1	89.1	90.6
4	96.1	95.4	89.1	89.5
5	93.3	95.3	89.1	89.3
6	94.3	94.6	89.2	89.2
Mean	94.6	95.4	88.6	89.5
SD	1.70	0.86	0.69	0.58
% RSD	1.79	0.90	0.77	0.65
% Diff	0.8		0.9	

Diff – Difference, MP – Method Precision, IP-Intermediate Precision.

Table 17. Percentage Assay result of Method Precision and Intermediate Precision.

Sample ID #	Opizole-B Cream (B. No. 9ES012A)	
	Butylated Hydroxy Toluene	
	MP	IP
1	86.3	86.1
2	85.0	86.3
3	86.4	87.9
4	86.5	86.8
5	85.7	86.7
6	86.2	86.2
Mean	86.0	86.7
SD	0.57	0.67
%RSD	0.66	0.77
%Diff	0.7	

Diff – Difference, MP – Method Precision, IP- Intermediate Precision.

Accuracy (Assay, Preservative, and Antioxidant):

For Assay, the accuracy of the proposed method had been demonstrated by the recovery study performed by the standard addition method at levels 50, 100, and 150 % of the target concentration. The data obtained had been presented in Table 18 to 22.

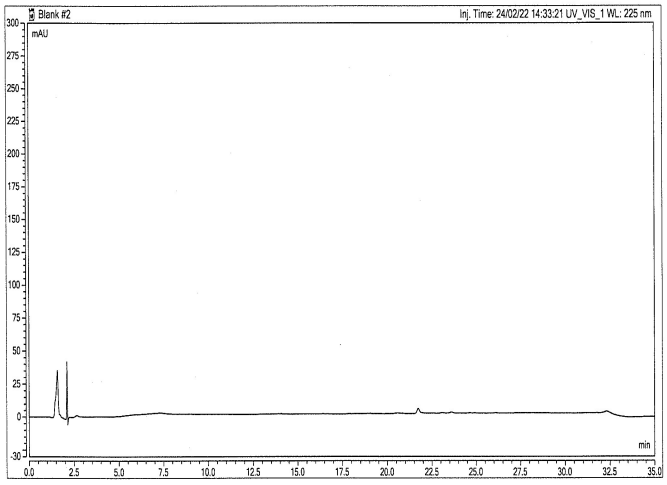

Fig 11. Blank Chromatogram for Assay.

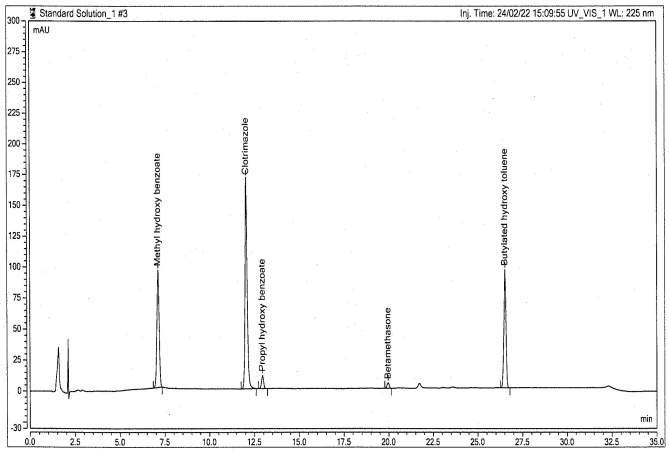

Fig 12. Standard Chromatogram for Assay.

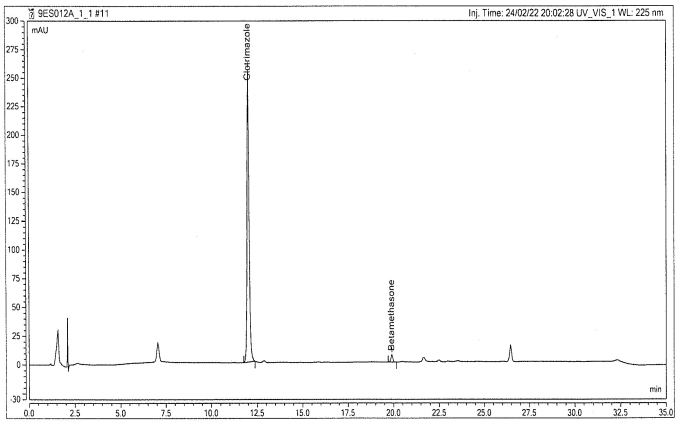

Fig 13. Sample Chromatogram for Assay.

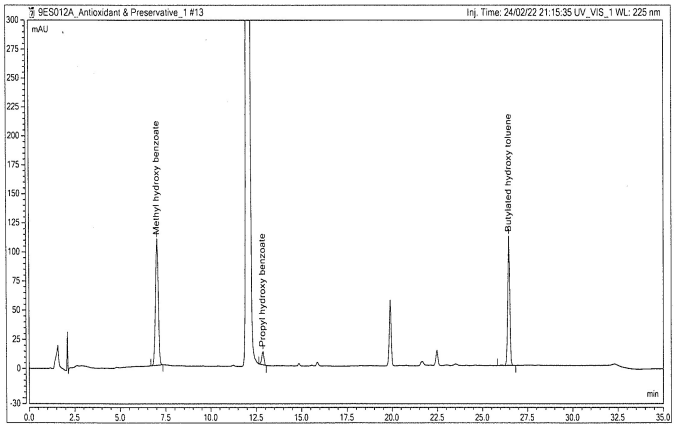

Fig 14. Sample Chromatogram for Assay (Preservatives and Anti-oxidant).

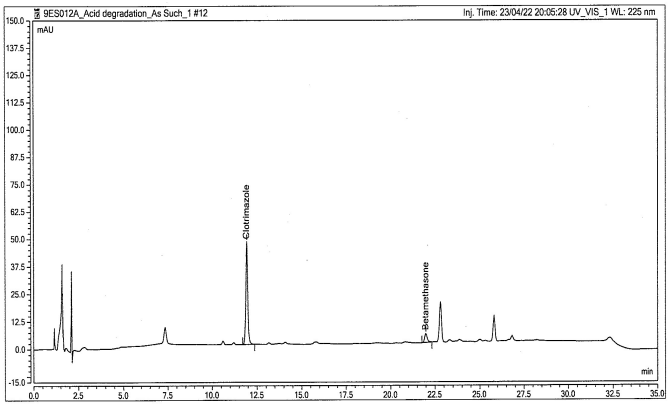

Fig 15. Acid Degradation Chromatogram.

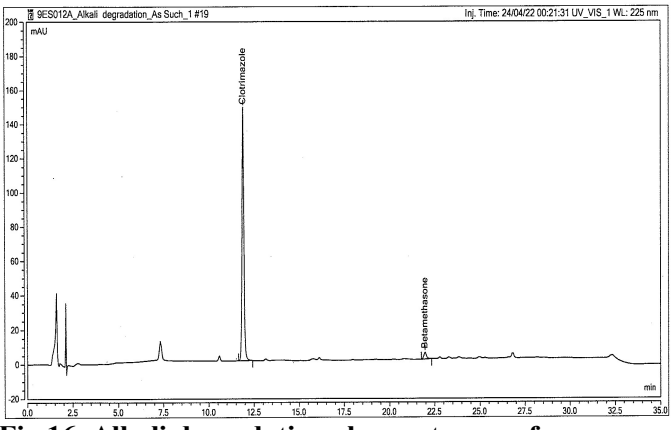

Fig 16. Alkali degradation chromatogram for assay.

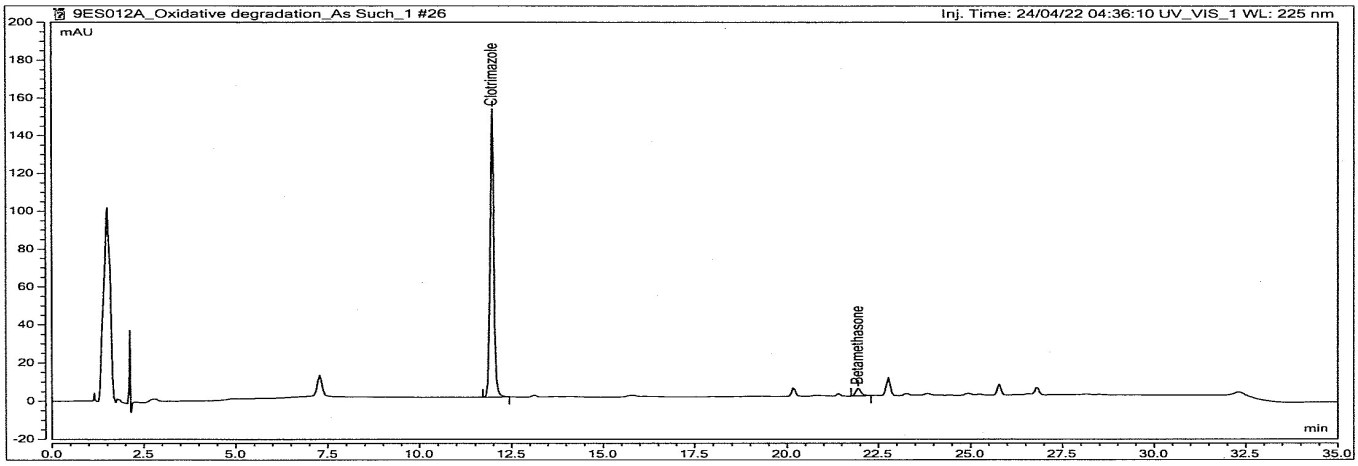

Fig 17. Oxidative Degradation Chromatogram for Assay.

Table 18. Accuracy of Clotrimazole (Assay).

Level	Mean Area	Added value (µg/ml)	Found value (µg /ml)	% Recovery	% Mean Recovery	% RSD
50 %	12241	25.219	25.323	100.4	99.7	0.6
	12130	25.219	25.093	99.5		
	12089	25.219	25.008	99.2		
100 %	25296	50.438	50.677	100.5	101.4	0.8
	25679	50.438	51.444	102.0		
	25580	50.438	51.246	101.6		
150 %	36474	75.657	73.070	96.6	98.6	1.8
	37660	75.657	75.446	99.7		
	37579	75.657	75.284	99.5		

Table 19. Accuracy of Betamethasone (Assay).

Level	Mean Area	Added value (µg/ml)	Found value (µg /ml)	% Recovery	% Mean Recovery	% RSD
50 %	421	1.314	1.306	99.4	98.8	0.5
	417	1.314	1.293	98.4		
	418	1.314	1.297	98.7		
100 %	823	2.628	2.553	97.1	98.0	0.8
	834	2.628	2.587	98.4		
	834	2.628	2.587	98.4		
150 %	1176	3.942	3.819	96.9	98.5	1.5
	1211	3.942	3.933	99.8		
	1199	3.942	3.894	98.8		

Table 20. Accuracy of Methyl hydroxy benzoate.

Level	Mean Area	Added value (µg/ml)	Found value (µg /ml)	% Recovery	%Mean Recovery	% RSD
50%	6138	42.812	42.522	99.3	100.0	0.6
	6197	42.812	42.931	100.3		
	6199	42.812	42.945	100.3		
100%	12372	85.624	85.709	100.1	99.6	0.6
	12351	85.624	85.564	99.9		
	12223	85.624	84.677	98.9		
150%	18316	128.436	126.887	98.8	99.3	0.5
	18427	128.436	127.656	99.4		
	18492	128.436	128.107	99.7		

Table 21. Accuracy of Propyl Hydroxy benzoate.

Level	Mean Area	Added value (µg/ml)	Found value (µg /ml)	% Recovery	%Mean Recovery	% RSD
50 %	743	5.032	5.055	100.5	101.1	0.5
	751	5.032	5.109	101.5		
	750	5.032	5.102	101.4		
100 %	1423	10.064	9.952	98.9	99.0	0.4
	1430	10.064	10.001	99.4		
	1421	10.064	9.938	98.7		
150 %	2175	15.096	15.212	100.8	100.6	0.3
	2171	15.087	15.132	100.3		
	2165	15.087	15.133	100.3		

Table 22. Accuracy of Butylated hydroxy toluene.

Level	Mean Area	Added value (µg/ml)	Found value (µg /ml)	% Recovery	% Mean Recovery	% RSD
50 %	7008	25.280	24.959	98.7	99.3	0.5
	7053	25.280	25.120	99.4		
	7074	25.280	25.195	99.7		
100%	14396	50.561	51.272	101.4	100.5	0.9
	14243	50.561	50.727	100.3		
	14151	50.561	50.400	99.7		
150%	21183	75.841	75.445	99.5	99.1	0.4
	21097	75.841	75.138	99.1		
	21044	75.841	74.950	98.8		

CONCLUSION:

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. Also, it is found to be accurate, precise, specific, stability indicating and rugged. Hence, it can be employed for the routine estimation of Clotrimazole, Betamethasone, preservatives (Methyl hydroxybenzoate and propyl hydroxybenzoate) and antioxidant (Butylated hydroxytoluene) in Clotrimazole (0.1 % w/w) and Betamethasone(0.05 % w/w) cream topical dosage form.

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