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Research Article

A Rapid RP-HPLC Methodology for the Determination of Clotrimazole Impurities in Topical Dosage Forms

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Abstract

Background: Clotrimazole is an antifungal medication that used to treat skin infection such as athlete's foot, jock itch, ringworm and other fungal skin infection. This medication is also used to treat a skin condition known as pityriasis (tinea versicolor), a fungal infection that causes a lightning or darkening of the skin of the neck, chest, arms, or legs. It is an azole antifungal that works by preventing the growth of fungus.

Aim: To develop a rapid and cost effective RP-HPLC methodology for the determination of Clotrimazole impurities in topical dosage forms.

Method: A rapid and cost effective stability-indicating reverse phase-HPLC method has been developed and validated for the determination of Clotrimazole impurities in topical dosage forms using Clotrimazole as the 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) and gradient elution consisting of Buffer and acetonitrile as the mobile phase. The flow rate was adjusted to 1.0 ml/min. The column oven was set at 40°C and the detection wavelength at 225nm. The retention times of Clotrimazole was found to be 11.8 min with all other known impurities well separated from the placebo, antioxidant and preservative peaks.

Results and Discussion: 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 for routine analysis.

Keywords: Clotrimazole; Methyl Hydroxy Benzoate; Propyl Hydroxy Benzoate; Butylated Hydroxy Toluene Stability-Indicating; RP-HPLC; Validation

Introduction

Clotrimazole (Figure 1) iis a synthetic, imidazole derivative with broad-spectrum, antifungal activity. Its molecular weight is 344.8 g/mol with an empirical formula C22H17ClN2. Clotrimazole is white or pale-yellow crystalline powder that 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 tropical 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

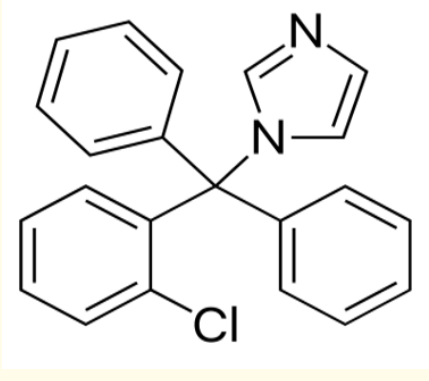

Figure 1: Chemical structure of Clotrimazole.

described as 1-[(2-chlorophenyl) diphenyl methyl]-1H-imidazole. In the present work we have focused to achieve the optimum chromatographic conditions for the determination of Clotrimazole impurities in the presence of preservatives and antioxidants in the topical formulations. The developed method can be applied successfully to quality control and stability testing purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines.

Materials and Methods

- **Chemicals and reagents:** Clotrimazole and all impurities (Clotrimazole impurity A, B, C, D, E and F) and clotrimazole working standards were available in Oman Pharmaceutical Products Co L.L.C. Topical formulation containing Clotrimazole cream 1% 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:** Dissolved 1.0 gm of Potassium dihydrogen orthophosphate and 0.5 gm of tetrabutylammonium hydrogen sulphate into 1000 ml of mili q water.

- **Mobile Phase-A Preparation:** Buffer (100%).

- **Mobile Phase-B Preparation:** Acetonitrile (100%)

- **Diluent:** Methanol was used as diluent.

- **Clotrimazole standard stock solution:** The standard stock solution was prepared by dissolving accurately weighed 25.0 mg of Clotrimazole working standard in 25 mL diluent to obtain 1000µg/mL concentration.

- **Diluted standard solution:** Transferred 1.0 ml of Standard stock solution into 200 ml volumetric flask and dilute to volume with diluent (Concentration: 5µg/mL).

- **Sample preparation for related substance:** Weighed and transferred accurately about 2.5 gm (equivalent to 25 mg of Clotrimazole) of sample into 50 ml centrifuge tube. Added about 20 ml of methanol and kept the centrifuge tube on water bath for 5 min at 50°C, cooled the solution on ice bath for 15 min, centrifuge for 5 min at 5000 rpm, decent the supernant liquid in to 50ml volumetric flask and repeat the extraction with further 20ml of methanol. To the combine extract add methanol to produce 50 ml. Filter the solution with 0.45µ Nylon filter after discarding 5 ml of filtrate. (Clotrimazole 500 µg/ml).

Chromatographic study

The clotrimazole and its impurities in all solutions were determined by HPLC using the chromatographic conditions as mention in table 2.

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

Time	Mobile Phase A%	Mobile Phase B%
0	75	25
3	75	25
25	20	80
30	20	80
31	75	25
35	75	25

Table 1: Gradient Program.

Instrument	HPLC
Column	Symmetry C8 (150 X 4.6) mm, 5µ Make: Waters
Flow Rate	1.0 mL/min
Detection wavelength	225 nm
Injection volume	10 µL
Column oven	40°C
Run Time	35 minutes
Elution	Gradient

Table 2: Chromatographic condition for analytical study.

Results and Discussion

The developed method for determination of Clotrimazole and its impurities were validated by using the following parameters.

System suitability

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.

Injection #	Area	Tailing Factor	Plate count
1	138.0346	1.55	65428
2	134.236	1.57	65418
3	134.4453	1.54	65192
4	134.2684	1.2	65634
5	134.4393	1.54	65356
6	134.2534	1.51	65417
Mean	135.0274	1.54	65408
SD	1.4829	-	-
%RSD	1.1	-	-

Table 3: System suitability – Clotrimazole (RS).

Sample Name	Final DC	% Impurity	%Dg
AS	As such sample	0.29	-
AD	5 ml 0.1N HCl @100°C for 15 min.	23.48	23.19
AA	5 ml 1N NaOH @100°C for 3 hours.	8.0	7.71
OD	5 ml 35% H2O2 @100°C for 15 min.	5.0	4.71
TD	100°C for 24 hours	2.57	2.28
PD	1.2 million lux hours	3.20	2.91

Table 4: Force Degradation study summary of Clotrimazole (RS). 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.

Solution	Peak Purity (Match Factor)					
Impurity Name	Imp. -A	Imp. -B	Imp. -C	Imp. -D	Imp. -E	Imp. -F
Acid degradation	1000	-	1000	1000	-	1000
Alkali degradation	1000	-	1000	-	-	1000
Oxidative degradation	999	-	1000	990	-	994
Thermal degradation	1000	-	-	1000	-	-
Photolytic degradation	1000	-	1000	-	-	1000

Table 4a: Peak purity date of Force degradation (RS).

Level	Conc. (µg/ml)	Area
1	0.1529	4.7502
2	2.5479	68.2152
3	4.0766	111.8549
4	4.5862	123.2248
5	5.0958	136.8741
6	6.1149	166.7832
7	7.6437	208.6999
Correlation coefficient (r)		0.9998
Regression coefficient (r2)		0.9997
Slope		27.2261
Intercept		-0.3308

Table 5: Linearity of Clotrimazole (RS).

Level	Conc. (µg/ml)	Area
1	0.1497	4.9990
2	2.4956	61.4183
3	3.9929	100.4055
4	4.4920	110.2095
5	4.9911	122.2606
6	5.9893	148.2753
7	7.4867	185.0169
Correlation coefficient (r)		0.9999
Regression coefficient (r2)		0.9997
Slope		24.5413
Intercept		0.8898

Table 6: Linearity of Clotrimazole impurity A (RS).

Specificity

There were no interfering peaks at the retention time of Clotrimazole and impurities peak in the presence of excipients. Further, to demonstrate the specificity of the method, the sample had been subjected to acid, base, oxidation, thermal and photolytic degradation. This was evaluated by using a Photo Diode Array detector (PDA). Refer figure 20 to figure 24 for the chromatograms and table 7 and 7a for the peak purity analysis data.

Injection No.	Clotrimazole (Area)
1	135.8808
2	136.5438
3	136.1872
4	136.8257
5	136.4317
6	136.1641
Mean	136.3389
SD	0.33
%RSD	0.2

Table 7: Area of Standard in Method Precision.

Linearity and range

Standard solutions containing Clotrimazole and clotrimazole impurity A were prepared. Linearity was determined by six different concentrations (LOQ, 50%, 80%, 90%, 100%, 120% and 150% of the target concentration for RS). The average peak areas were plotted against concentrations. 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 5 and 6 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 figure 8 and 9.

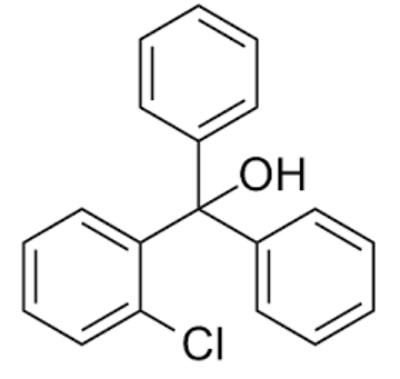

Figure 2: Chemical structure of Clotrimazole Impurity A.

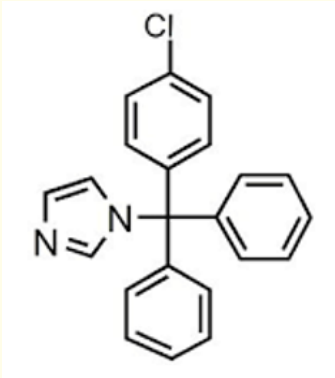

Figure 3: Chemical structure of Clotrimazole Impurity B.

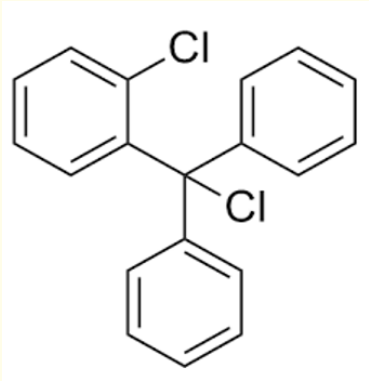

Figure 4: Chemical structure of Clotrimazole Impurity C.

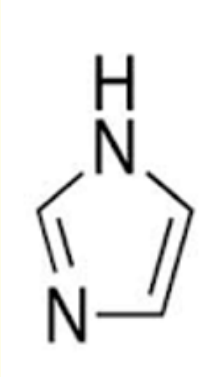

Figure 5: Chemical structure of Clotrimazole Impurity D.

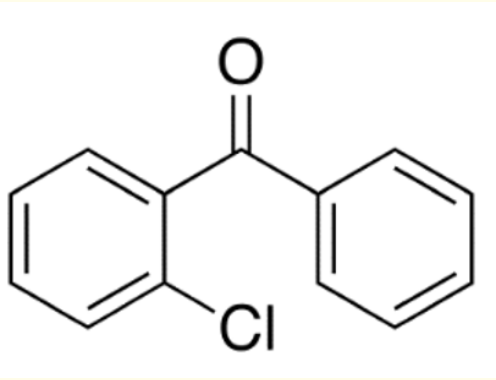

Figure 6: Chemical structure of Clotrimazole Impurity E.

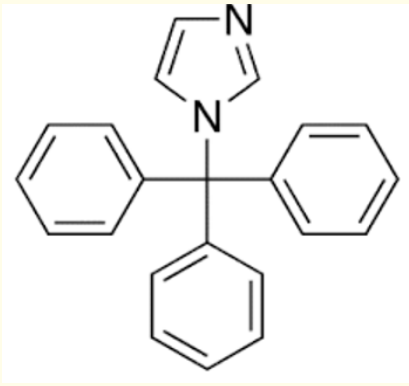

Figure 7: Chemical structure of Clotrimazole Impurity F.

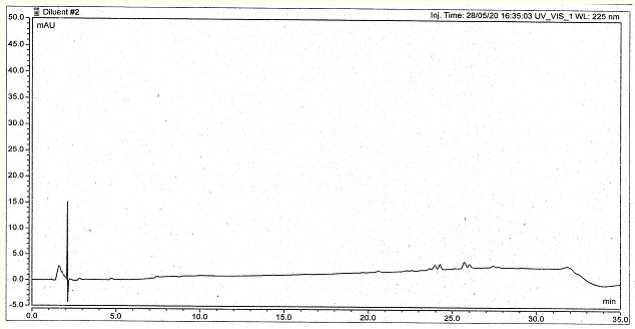

Figure 10: Reference chromatogram of Blank (@225nm).

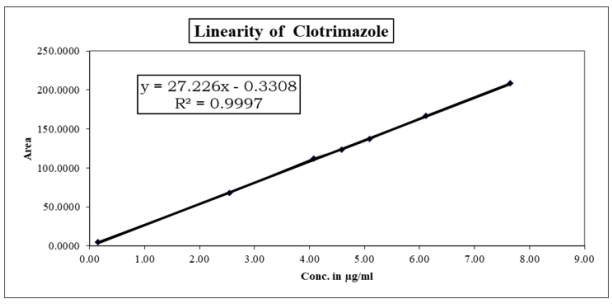

Figure 8: Linearity Plot of Clotrimazole – Related Substances.

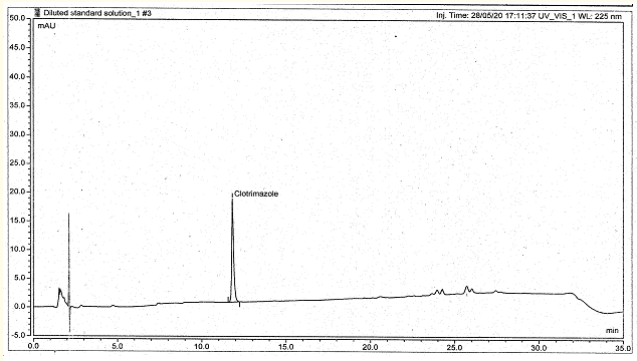

Figure 11: Reference chromatogram of Clotrimazole Standard (@ 225 nm).

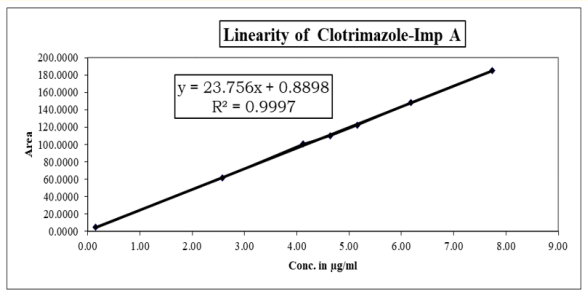

Figure 9: Linearity Plot of Clotrimazole impurity A – Related Substances.

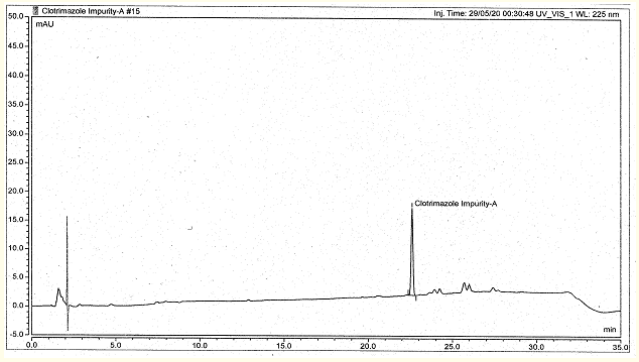

Figure 12: Reference chromatogram of Clotrimazole Impurity A (@ 225 nm).

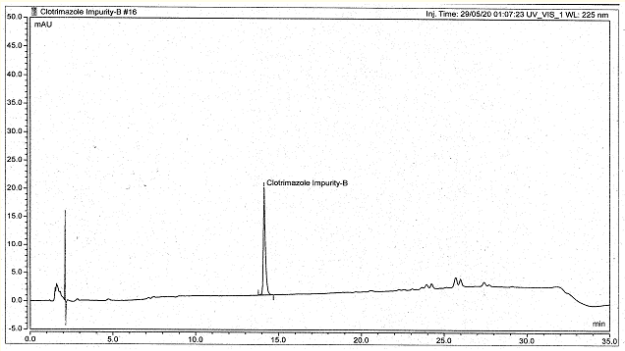

Figure 13: Reference Chromatogram of Clotrimazole impurity B (@ 225nm).

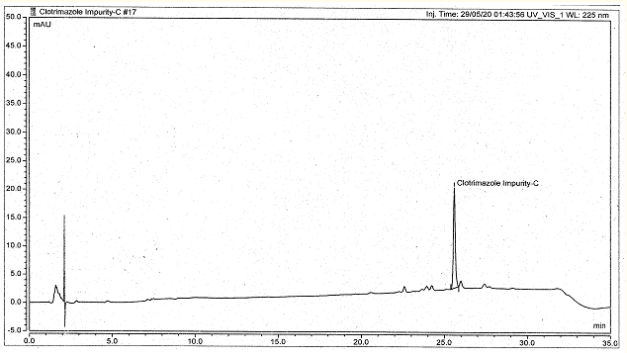

Figure 14: Reference Chromatogram of Clotrimazole impurity C (@ 225nm).

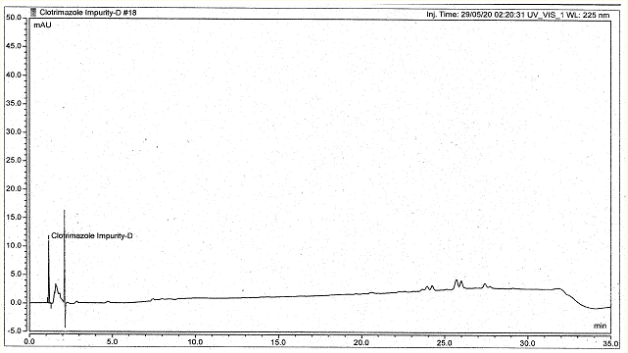

Figure 15: Reference Chromatogram of Clotrimazole impurity D (@ 225nm).

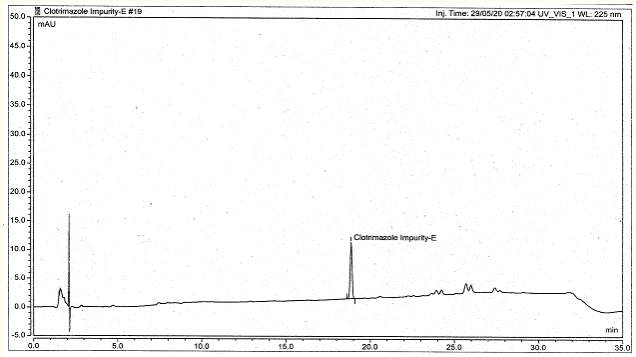

Figure 16: Reference Chromatogram of Clotrimazole impurity E (@ 225nm).

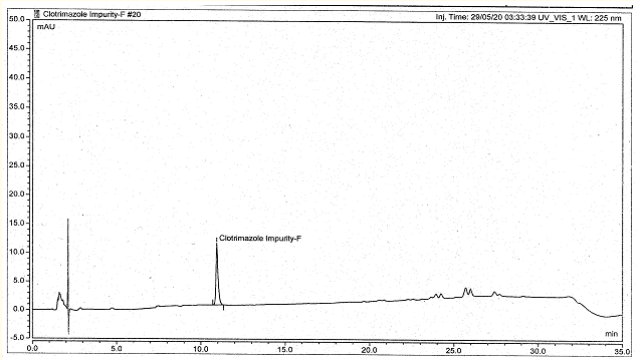

Figure 17: Reference Chromatogram of Clotrimazole impurity F (@ 225nm).

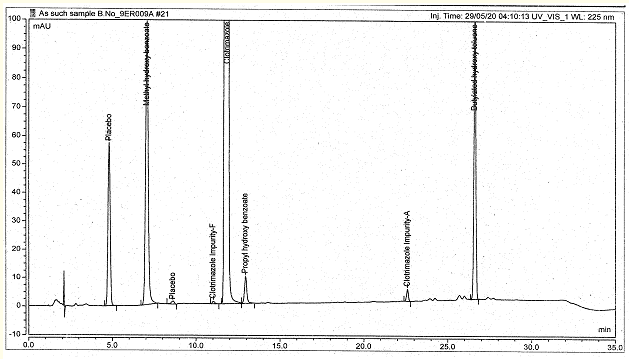

Figure 18: Reference chromatogram of Clotrimazole as such sample (@225 nm).

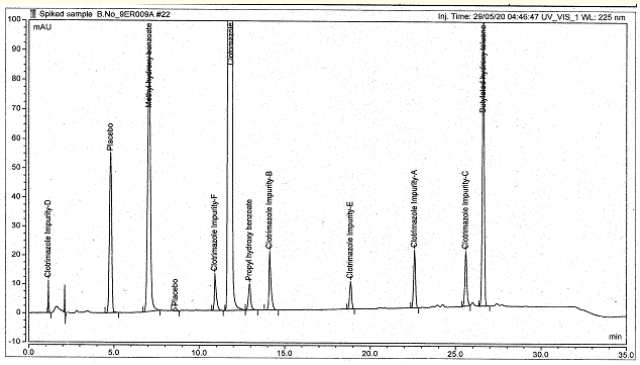

Figure 19: Reference chromatogram of Clotrimazole spiked sample (@225 nm).

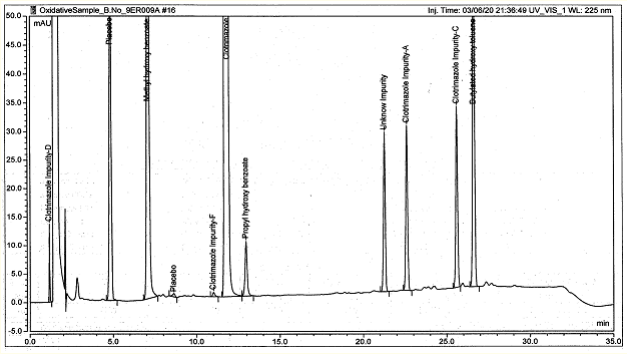

Figure 22: Reference chromatogram of Oxidative Degradation (@225 nm).

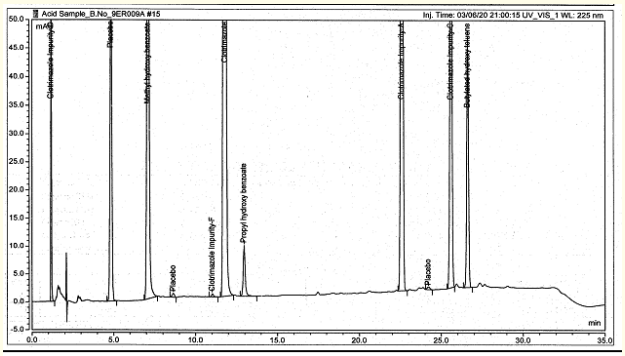

Figure 20: Reference chromatogram of Acid Degradation (@225 nm).

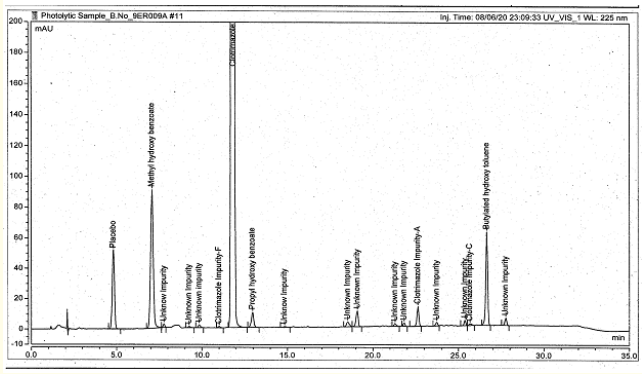

Figure 23: Reference chromatogram of Photolytic Degradation (@225 nm).

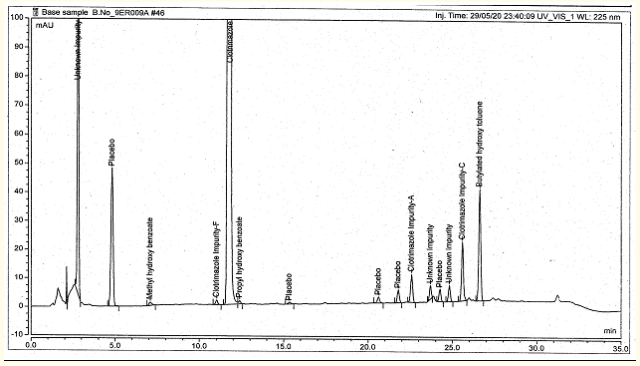

Figure 21: Reference chromatogram of Base Degradation (@225 nm).

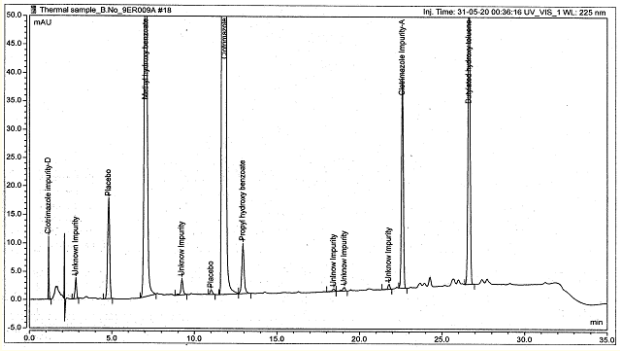

Figure 24: Reference chromatogram of Thermal Degradation (@225 nm).

Precision

For RS, 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 data obtained for six replicate standard injections and the six sample preparations have been presented in table.

Sample	% Clotrimazole Impurity- A RRT-0.20	%Total Impurities
1	0.25	0.31
2	0.25	0.30
3	0.25	0.30
4	0.24	0.30
5	0.24	0.30
6	0.25	0.30
Mean	0.25	0.30
SD	0.00	0.00
% RSD	1.3	1.4

Table 8: % impurity result for as such sample in Method Precision.

Sample	% Clotrimazole Impurity-A RRT-0.20	%Total Impurities
1	1.22	1.35
2	1.22	1.39
3	1.21	1.34
4	1.23	1.36
5	1.23	1.37
6	1.21	1.37
Mean	1.22	1.36
SD	0.01	0.02

Table 9: % impurity result for as such sample in Method Precision.

Sample ID #	Opizole Cream (B. No. 9ER09A) As such sample	
	Clotrimazole impurity A	
	MP	IP
1	0.252	0.230
2	0.246	0.240
3	0.245	0.237
4	0.244	0.248
5	0.243	0.242
6	0.245	0.236
Mean	0.246	0.239
SD	0.00	0.01
%RSD	1.3	2.5
%Diff	2.85	

Table 10: % Impurity result of Method Precision and Intermediate Precision. MP: Method Precision; IP: Intermediate Precision; Diff: Difference; MP: Method Precision; IP: Intermediate Precision.

Sample ID #	Opizole Cream (B. No. 9ER09A) Spiked sample	
	Clotrimazole impurity A	
	MP	IP
1	1.216	1.191
2	1.216	1.170
3	1.211	1.179
4	1.225	1.165
5	1.225	1.152
6	1.211	1.143
Mean	1.217	1.167
SD	0.01	0.02
%RSD	0.5	1.5
%Diff	4.11	

Table 11: % Impurity result of Method Precision and Intermediate Precision. MP: Method Precision; IP: Intermediate Precision; Diff: Difference; MP: Method Precision; IP: Intermediate Precision.

Accuracy of Clotrimazole related compound-A							
Level	Sample	Mean Area	Added value (µg/ml)	Found value (µg/ml)	Recovery	%Mean Recovery	% RSD
50%	1	31.7292	0.0075	0.0069	92.0	91.6	2.2
	2	31.8201	0.0075	0.0070	93.3		
	3	31.7891	0.0075	0.0067	89.3		
100%	1	146.5340	0.2513	0.2408	95.8	94.9	0.9
	2	147.0266	0.2513	0.2377	94.6		
	3	148.0014	0.2513	0.2368	94.2		
150%	1	206.5162	0.3769	0.3578	94.9	95.9	1.0
	2	205.3567	0.3769	0.3611	95.8		
	3	205.9394	0.3769	0.3653	96.9		

Table 12: Accuracy of Clotrimazole Impurity A(RS).

Ruggedness

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.

Accuracy's

For Related substances, the accuracy of the proposed method had been demonstrated by the recovery study performed by the standard addition method at levels LOQ, 100% and 150% of the target concentration. The data obtained had been presented in table 9 [1-13].

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 and its related impurities in Clotrimazole Cream topical dosage form.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

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