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Research Article

Development and validation of a stability – indicating RP –HPLC method for simultaneous estimation of and Sertaconazole Nitrate, Mometasone Fumarate and Preservatives in Tropical cream Formulation including Forced Degradation studies as per ICH guidelines

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ABSTRACT:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Propyl Paraben, Mometasone and Sertaconazole Tropical dosage form. Chromatogram was run through Sunfire C18 (250 x 4.6 mm, 5m). Mobile phase containing 0.01N Kh2po4 and Acetonitrile in the ratio of 65:35 A was pumped through column at a flow rate of 1ml/min.. Temperature was maintained at 30°C. Optimized wavelength for Propyl Paraben, Mometasone and Sertaconazole was 260nm. Retention time of Propyl Paraben, Mometasone and Sertaconazole were found to be 2.222min, 3.308min and 3.991min %RSD of system precision for Propyl Paraben, Mometasone and Sertaconazole were and found to be 0.3, 0.9 and 0.2 respectively. %RSD of method precision for Propyl Paraben, Mometasone and Sertaconazole We're and found to be 0.3, 0.4 and 0.2 respectively. % recovery was obtained as 99.54%, 99.68% and 99.79% for Propyl Paraben, Mometasone and Sertaconazole Respectively. LOD, LOQ values are obtained from regression equations of Propyl Paraben, Mometasone and Sertaconazole were 0.03ppm, 0.05ppm, 0.38ppm, 0.08ppm 0.15ppm, and 1.16ppm respectively. Regression equation of Propyl Paraben was y = 23662x + 242.79, Mometasonee was y = 24014x + 212 and of Sertaconazole was y = 9851.3x + 1592.9

Key Words: Propyl Paraben, Sertaconazole, Mometasone, RP-HPLC.

Introduction:

Superficial fungal infections are frequent skin conditions marked by symptoms including redness, itching, flaking, and discomfort. Treatment may necessitate localized topical antifungal therapy, especially if the infection affects the skin. When a fungal infection presents with a significant inflammatory response, treatments that combine an antifungal agent with a suitable topical corticosteroid can be utilized to tackle both the infectious and inflammatory aspects. [1,2]

Sertaconazole nitrate is a Synthetic imidazole antifungal medication that falls under the azole category. It has wide-ranging effectiveness against dermatophytes and various yeast types that cause superficial mycoses. Its main antifungal effect includes blocking the production of ergosterol, a crucial element of the fungal cell membrane. Blocking ergosterol production disrupts the integrity of fungal membranes and hampers fungal growth and reproduction. At suitable concentrations, sertaconazole may display both fungistatic and fungicidal effects. [2,3] Along with its antifungal properties, sertaconazole has shown anti-inflammatory and antipruritic effects, potentially offering extra advantages in treating inflammatory superficial fungal infections. Experimental research has linked its anti-inflammatory effects to the modulation of the p38–COX-2–PGE_2 pathway. [4]

Mometasone furoate is a synthetic, powerful topical glucocorticoid with anti-inflammatory, antipruritic, and vasoconstrictive effects. It is utilized in treating different inflammatory dermatological conditions that respond to glucocorticoids, such as atopic dermatitis, seborrheic dermatitis, and psoriasis. Its pharmacological effect is

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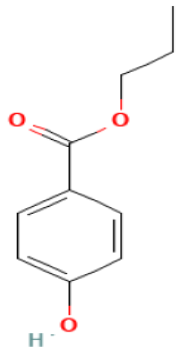
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mediated via glucocorticoid receptors, leading to the regulation of inflammatory processes and inhibition of inflammatory mediators. Mometasone furoate is known to exhibit strong topical anti-inflammatory effects, along with low systemic absorption and a good safety profile for topical use. [5,6]

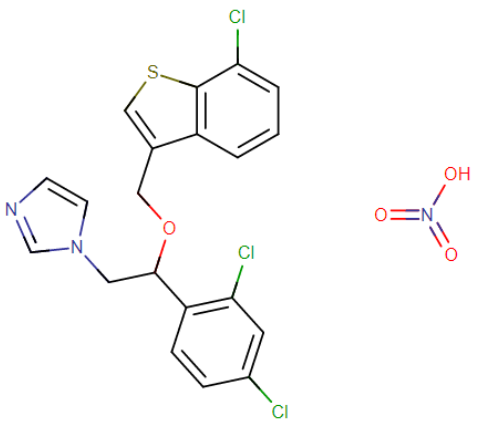
Propyl paraben (propyl 4-hydroxybenzoate; CAS No. 94-13-3) is an ester derived from p-hydroxybenzoic acid and is commonly utilized as an antimicrobial preservative in pharmaceutical, cosmetic, and various other products. Including it in topical formulations aids in safeguarding the mixture from microbial contamination while stored and during application. Propyl paraben is a stable, non-volatile substance that has been utilized as a preservative in cosmetic and pharmaceutical products for many years. [7] Its role as a preservative makes it especially significant for multidose topical formulations that need microbiological protection.

The blend of sertaconazole nitrate, mometasone furoate, and propyl paraben delivers three synergistic formulation functions: sertaconazole nitrate acts as the antifungal active ingredient, mometasone furoate offers anti-inflammatory and antipruritic effects, while propyl paraben functions as the antimicrobial preservative. Due to significant variations in their chemical structures, physicochemical characteristics, concentrations, and pharmacological functions, the concurrent analysis of these compounds in a combined topical formulation poses a notable analytical difficulty.

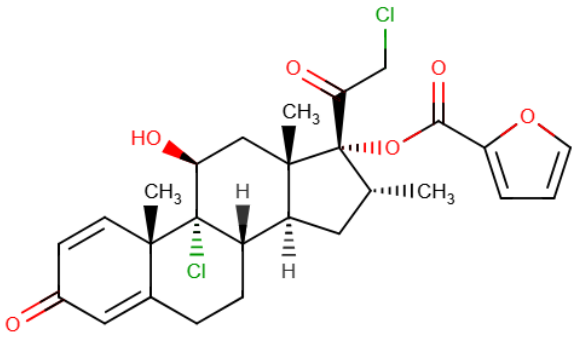
From the viewpoint of pharmaceutical quality control, high-performance liquid chromatography (HPLC), especially reversed-phase HPLC (RP-HPLC), is an appropriate method for the simultaneous assessment of various components in topical pharmaceutical formulations due to its selectivity, reproducibility, sensitivity, and capability to separate compounds with varying physicochemical properties. Existing chromatographic studies have shown that it is possible to determine mometasone furoate and propyl paraben simultaneously, along with other constituents, in topical pharmaceutical formulations through stability-indicating chromatographic techniques. [8] Likewise, RP-HPLC has been utilized for the analysis of propyl paraben along with various active ingredients and preservatives in topical preparations.



- figure 1 Structure of Propyl Paraben



- Figure 2 Structure of Sertaconazole



- Figure 3 Structure of Mometasone

An extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Propyl Paraben, Sertaconazole, and Mometasone, and their medicinal dose form using RP-HPLC.11- 16 must be validated and developed as per ICH guidelines.

Materials and Methods:

Spectrum pharma Research Solution provide with Propyl Paraben, Sertaconazole, and Mometasone pure drugs (API) gift samples and Combination Propyl Paraben, Sertaconazole, and Mometasone Tropical Solution (Onabet SD 30) received from local market. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation:

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Chromatographic Condition:

The primary objective of this study is to provide a highly exact, accurate, sensitive, specific, consistent, and efficient analytical method for the simultaneous quantification of Propyl Paraben, Sertaconazole, and Mometasone in their pure form and throughout Tropical formation.

API Formulation

Accurately Weighed and transferred 5mg of Propyl Paraben and 100mg of Sertaconazole and 5 mg of Mometason working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 15 minutes and make up to the final volume with diluents. (100µg/ml of Propyl Paraben, 2000µg/ml of Sertaconazole 100µg/ml of Mometason)

Sample Formulation

Pipette 1.0 mL of Onabet SD 30 Solution into a 100 mL volumetric flask (equivalent to 20mg of Sertaconazole and 1mg of Mometason) was transferred into a 10mL volumetric flask, 5mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (100µg/ml of Propyl Paraben, 2000µg/ml of Sertaconazole 100µg/ml of Mometason)

Method Validation

As per ICH guidelines, the developed method is validated for validating the analytical procedures. The validation parameters include system suitability, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity and Degradation Studies were performed.

System suitability parameters:

System suitability testing is carried out prior to or during sample analysis to ensure that the HPLC system is functioning properly.

Specificity: Specificity refers to the analytical method's capacity to precisely measure Propyl Paraben, Sertaconazole and Mometason in the presence of other components such as contaminants, degradation products, excipients, solvents, and mobile-phase components.

Precision: Precision indicates the degree of agreement between individual measurements.

It is generally evaluated at 2 levels:

A. Repeatability

Six independent sample preparations at the target concentration are analyzed under the same analytical conditions.

B. Intermediate Precision: Intermediate precision evaluates variations at Different day.

Linearity:

Linearity displays the method's capacity to produce test findings that are directly proportional to the concentration of Propyl Paraben, Sertaconazole and Mometason within the specified range. Of 25%, 50%, 75%, 100%, 125%, 150%.

Accuracy:

Accuracy signifies how closely the experimentally determined value aligns with the true value. It is performed in 3 level from 50% to 150%.

Sensitivity:

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.

Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

Robustness: Robustness indicates the ability of the HPLC method to stay stable despite minor, intentional changes in analytical conditions.

Assay:

The assay and percentage of purity were determined for the brand Onabet SD 30. The measured value was contrasted with the standard value, unaffected by the excipients present in the nasal formulation.

Degradation Studies

These experiments are conducted under different stress conditions to assess the stability of the pure drug substance and assist in establishing appropriate storage conditions. These investigations encompass base, peroxide, acid, neutral hydrolysis, photo, and thermal degradation.

Oxidation:

To 1 ml of Propyl Paraben, Sertaconazole and Mometason stock solution, 1 ml of 20% hydrogen peroxide (H2O2) was added individually. The solutions were stored at 60°C for 30 minutes. In the HPLC analysis, the resulting solution was diluted to achieve a concentration of 10µg/ml, 200 µg/ml, 10 µg/ml, and 10µl was injected into the system, with chromatograms recorded to evaluate the sample's stability.

Acid Degradation Studies:

To 1 ml of stock solution Propyl Paraben, Sertaconazole and Mometason, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. For HPLC study, the resultant solution was diluted to obtain 10µg/ml, 200 µg/ml, 10 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of Propyl Paraben, Sertaconazole and Mometason stock solution, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60°C. For the HPLC analysis, the obtained solution was diluted to create a 10µg/ml, 200 µg/ml, 10 µg/ml concentration, and 10µl was injected into the apparatus, with the chromatograms being documented to evaluate sample stability.

Dry Heat Degradation Studies:

The standard drug solution was heated in an oven at 105°C for 6 hours to investigate dry heat deterioration. For HPLC analysis, the prepared solution was diluted to achieve a concentration of 10µg/ml, 200 µg/ml, 10 µg/ml, and 10µl was injected into the system, with chromatograms recorded to evaluate the sample's stability.

Photo Stability studies:

The drug's photochemical stability was examined by subjecting the 100µg/ml, 2000 µg/ml, 100 µg/ml solution to UV Light, placing the beaker in a UV Chamber for 7 days or 200 Watt hours/m2 in a photostability chamber. In the HPLC analysis, the resulting solution was diluted to achieve a concentration of 10µg/ml, 200 µg/ml, 10 µg/ml, and 10µl was injected into the system while the chromatograms were documented to evaluate the sample's stability

Neutral Degradation Studies:

Stress testing under neutral conditions involved refluxing the drug in water for 6 hours at a temperature of 60º. For the HPLC analysis, the final solution was diluted to create a 10µg/ml, 200 µg/ml, 10 µg/ml concentration, and 10µl were injected into the apparatus while the chromatograms were captured to evaluate the sample's stability.

Result and Discussion

A. Optimization of Chromatographic Conditions:

To develop and establish a suitable RP-HPLC method for estimation of Propyl Paraben, Sertaconazole and Mometason in bulk and Tropical dosage forms, different preliminary tests were performed and different chromatographic conditions were tested. The final analysis was performed by using 60% 0.1N KH2PO4: 40% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 260nm detector wave length and at an injection volume of 10µL using Sunfire C18 4.6 x 250mm, 5µm with run time of 8min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Propyl Paraben, Sertaconazole and Mometason, the optimized chromatogram was obtained as shown in (Figure-4).

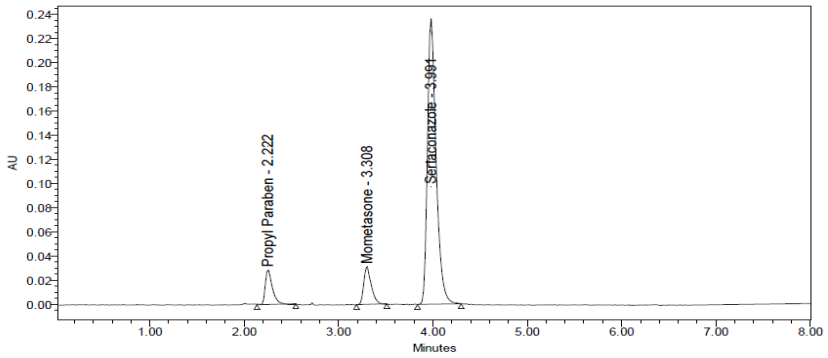


Figure 2: Optimized Chromatogram

B. System Suitability Test:

Table 1: System suitability results

Parameters	Propyl Paraben	Mometason	Sertaconazole
Retention Time (min)	2.222	3.308	3.991
USP Plate count	3646	6286	7234
USP Tailing	1.2	1.2	1.1

C. **Specificity data:** No interference was observed

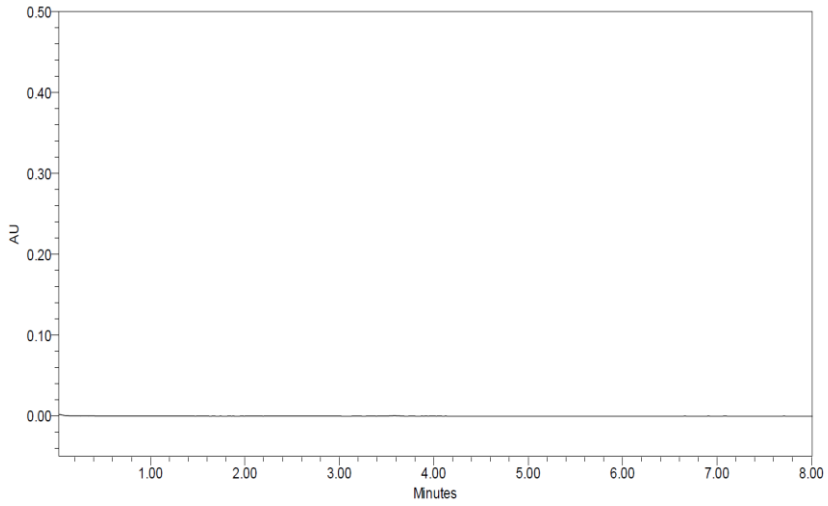


Figure 5: Specificity of Propyl Paraben, Sertaconazole, and Mometasone

D. Linearity:

Calibration data is given in table 4 and regression data in table 4 and calibration curve in figure 6-8

Table 2: Calibration data of Propyl Paraben, Sertaconazole, and Mometasone

Propyl Paraben		Mometasone		Sertaconazole	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
2.5	57062	2.5	58073	50	473537
5	121921	5	123410	100	983883
7.5	174897	7.5	176909	150	1497928
10	238142	10	243527	200	1987388
12.5	301547	12.5	303908	250	2501493
15	350381	15	356401	300	2910812

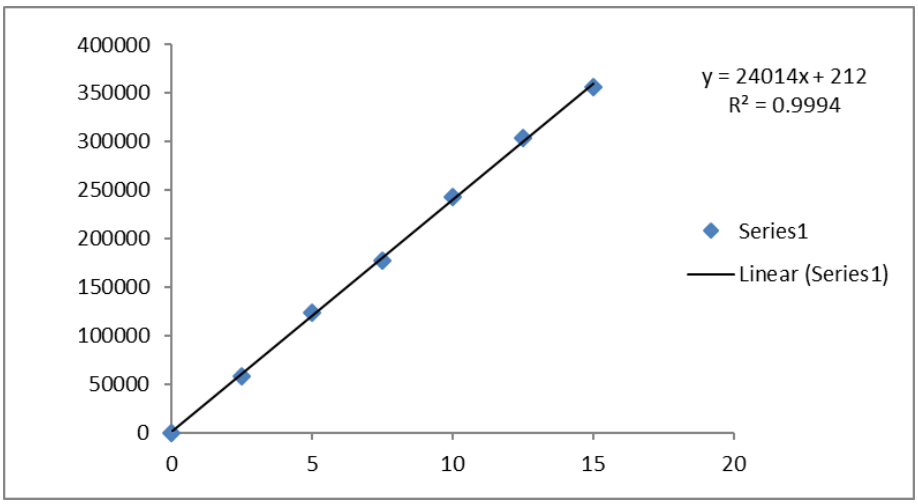


Figure 6 Calibration curve of Sertaconazole

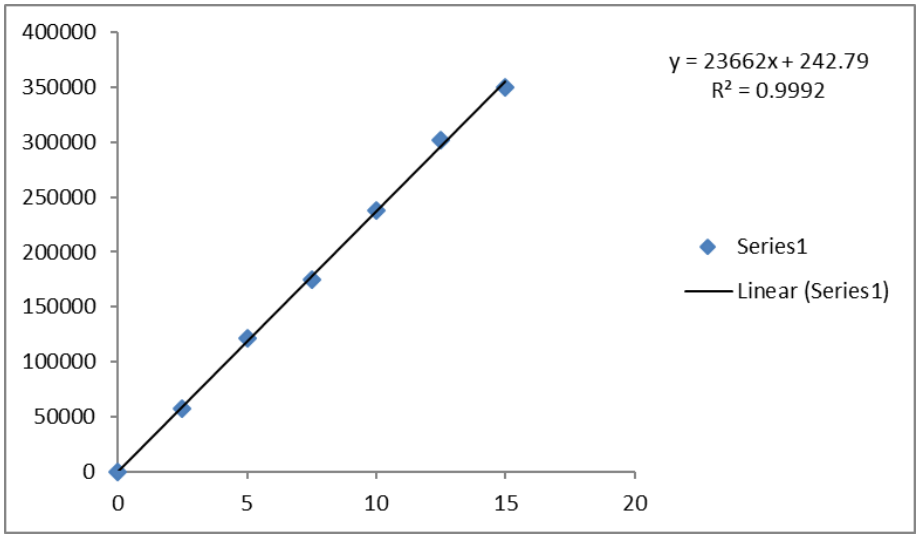


Figure 7 Calibration curve of Mometasone

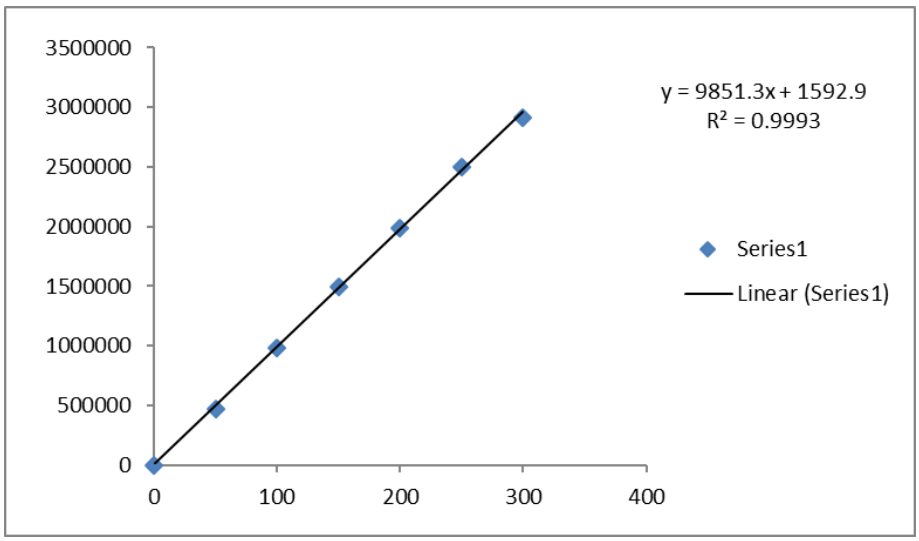


Figure 8: Calibration curve of Propyl Paraben

Table 3: regression data

Parameter	**Propyl Paraben**	Sertaconazole	Mometasone
Conc range (µg/mL)	2.5-15µg/ml	50-300µg/ml	2.5-15µg/ml
Regression Equation	y = 5641.2x + 8389.3	y = 6070.3x + 132	y = 5843.6x + 75.1
Co-relation	0.999	0.999	0.999

E. Accuracy: Recovery data shown in table 4

Table 4: recovery data of Propyl Paraben, Sertaconazole, and Mometasone

Drug	level	Amount added	Amount Recovered	% Recovery	Total Mean
Propyl Paraben	50%	5	4.98	99.65	99.54
	100%	10	9.93	99.34	
	150%	15	14.9	99.62	
Mometasone	50%	5	4.98	99.57	99.68
	100%	10	9.99	99.91	
	150%	15	14.93	99.56	
Sertaconazole	50%	100	99.68	99.68	99.79
	100%	200	199.8	99.90	
	150%	300	299.35	99.78	

F. Precision : System precision was performed and the data was shown in table 5

Table 5: Method precision

S. No	Area of Propyl Paraben	Area of Sertaconazole	Area of Mometasone
1.	230593	1976556	239941
2.	230997	1978960	240041
3.	230486	1975449	241245
4.	230495	1976358	241116
5.	231402	1975365	242099
6.	232284	1986247	241977
Mean	231043	1978156	241070
S.D	704.9	4171.8	921.7
%RSD	0.3	0.2	0.4

The % RSD for the peak areas of Propyl Paraben, Sertaconazole, and Mometasone obtained from six replicate injections of standard solution was within the limit.

Intermediate Precision: The precision of the method was determined by analyzing a sample of Propyl Paraben, Sertaconazole, and Mometasone and shown in table 6

Table 6: Intermediate Precision

S. No	Area of Propyl Paraben	Area of Sertaconazole	Area of Mometasone
1.	215834	1861685	226751
2.	211106	1857341	229676
3.	214043	1851054	226023
4.	215334	1869185	227621
5.	211623	1869686	223784
6.	212320	1855574	228231
Mean	213377	1860754	227014
S.D	1983.0	7540.6	2020.9
%RSD	0.9	0.4	0.9

From the above results, the % RSD of method precision study was within the limit for Propyl Paraben, Sertaconazole, and Mometasone.

G. Robustness:

Robustness conditions like Flow rate, mobile phase, and temperature and was altered and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table7: Robustness data for Propyl Paraben, Sertaconazole, and Mometasone.

Condition	%RSD of Propyl Paraben.	%RSD of Mometasone	%RSD of Sertaconazole
Flow rate (-) 0.8ml/min	0.5	0.5	0.6
Flow rate (+) 1.0ml/min	0.5	1.5	0.2
Mobile phase (-) 65B:35A	0.3	0.8	0.4
Mobile phase (+) 55B:45A	0.5	0.3	0.3
Temperature (-) 25°C	0.5	0.6	0.5
Temperature (+) 35°C	0.6	0.6	0.2

H. Sensitivity:

Table 8 sensitivity of Propyl Paraben, Sertaconazole, and Mometasone.

Molecule	LOD	LOQ
Propyl Paraben.	0.03 µg/ml	0.08 µg/ml
Sertaconazole	0.38 µg/ml	1.16 µg/ml
Mometasone	0.05 µg/ml	0.15µg/ml

I. Force Degradation Studies:

Table 9 shows degradation conditions and table 9 shows the obtained degraded data and purity plot chromatogram in figure 9-14

Table 9: degradation data

Type of degradation	Propyl Paraben		Sertaconazole		Mometasone	
	% Rccovered	% Degraded	% Rccovered	% Degraded	% Rccovered	% Degraded
Acid	95.28	4.72	95.36	4.64	94.90	5.10
Base	97.18	2.82	97.31	2.69	97.39	2.61
Peroxide	98.52	1.48	98.38	1.62	97.92	2.08
Thermal	99.31	0.69	99.84	0.16	99.00	1.00
UV	99.53	0.47	99.51	0.49	99.40	0.60
Water	99.44	0.56	99.80	0.20	98.94	1.06

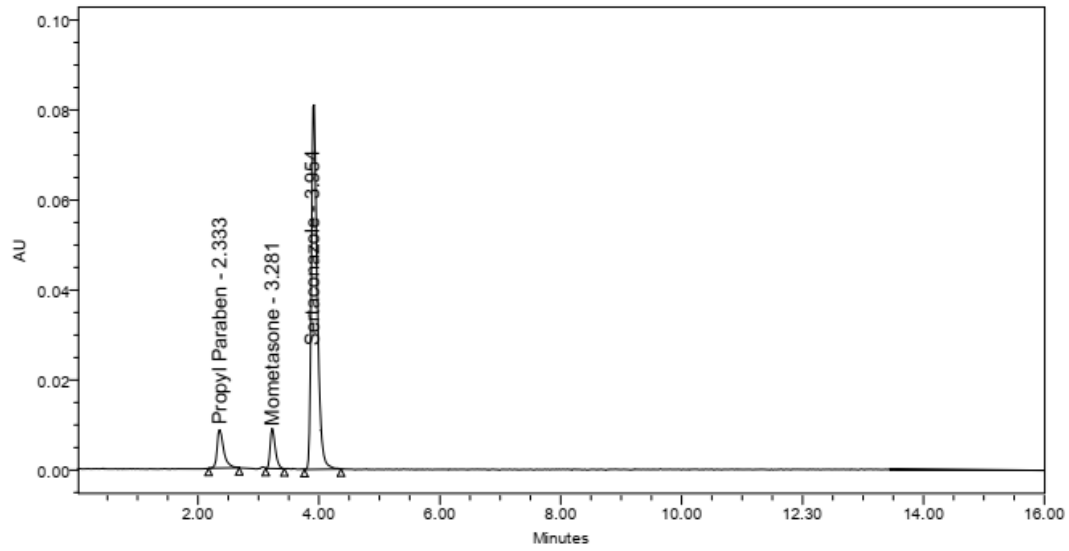


Figure 9: Acid degradation chromatogram

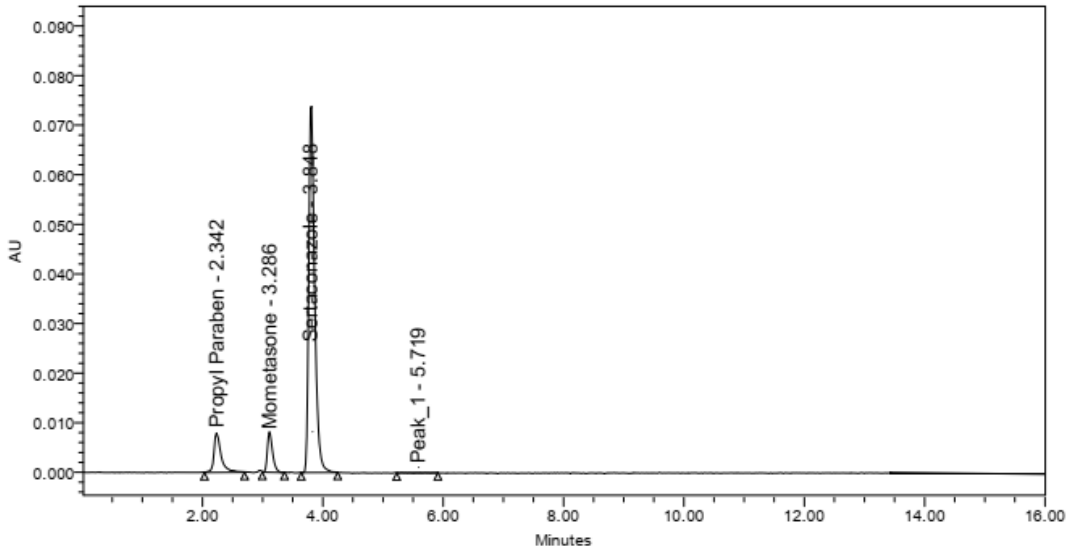


Figure 10: Base degradation chromatogram

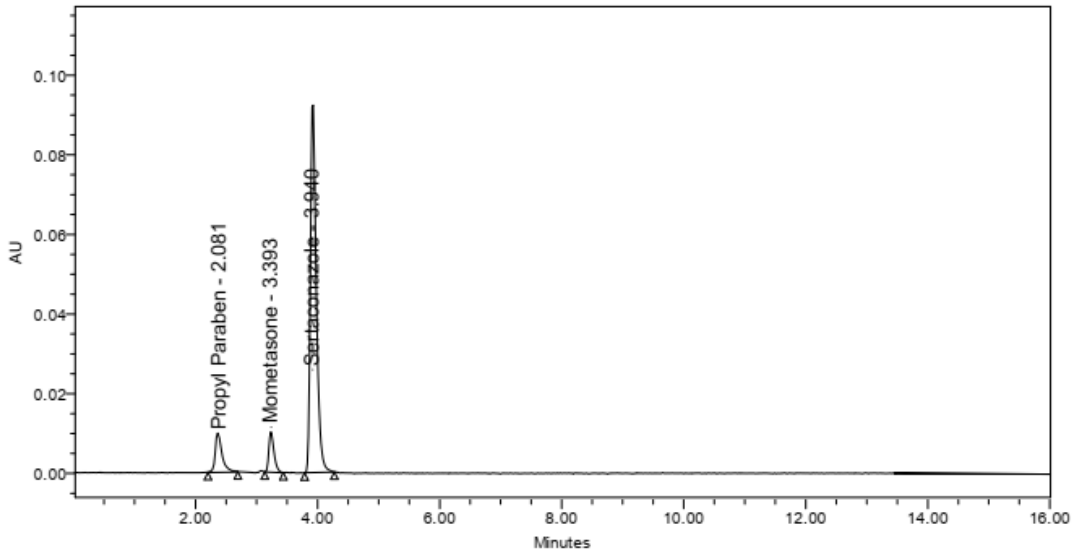


Figure 11: Peroxide degradation chromatogram

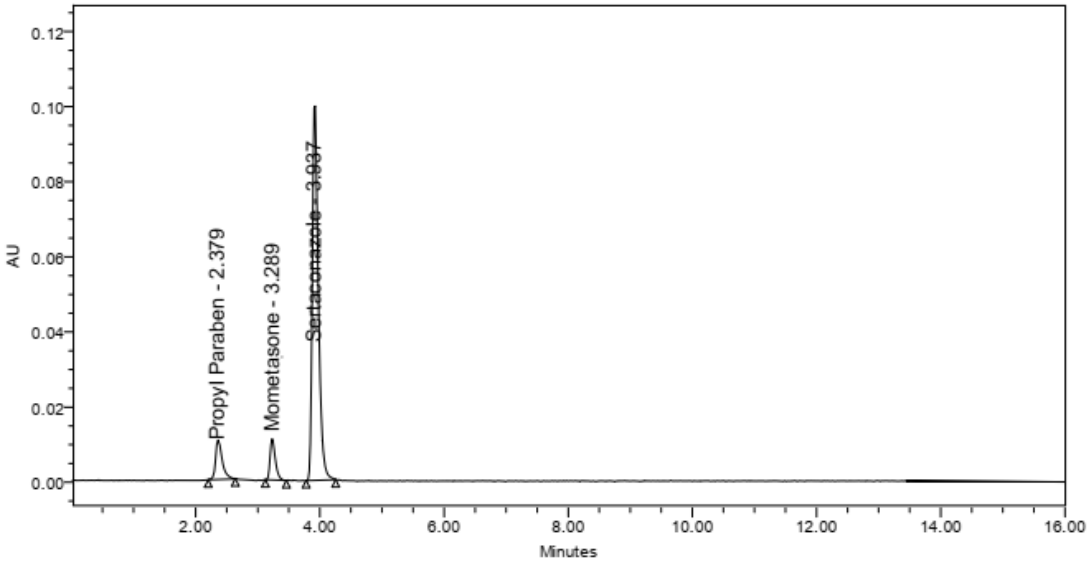


Figure 12: Thermal degradation chromatogram

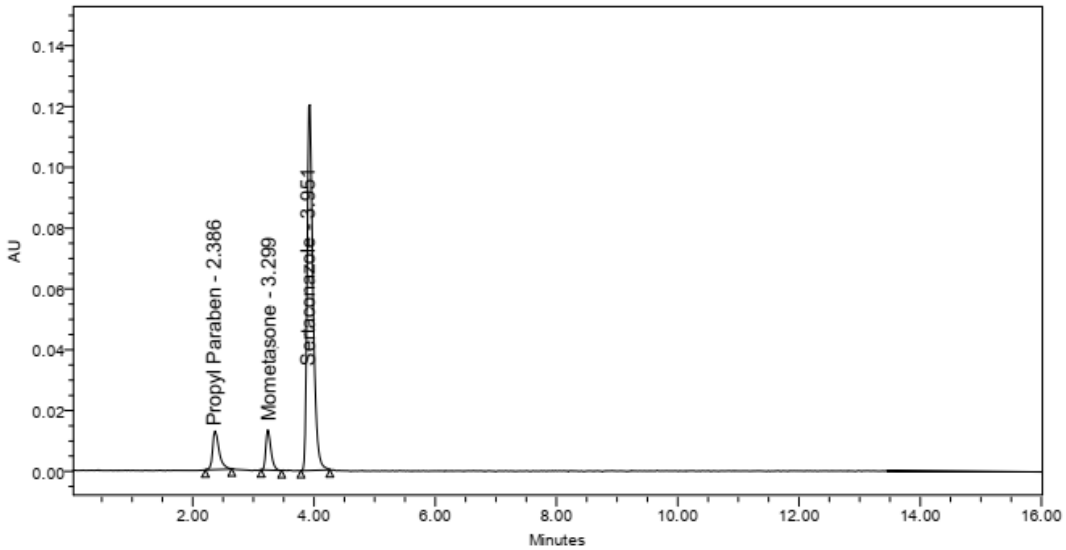


Figure 13: UV degradation chromatogram

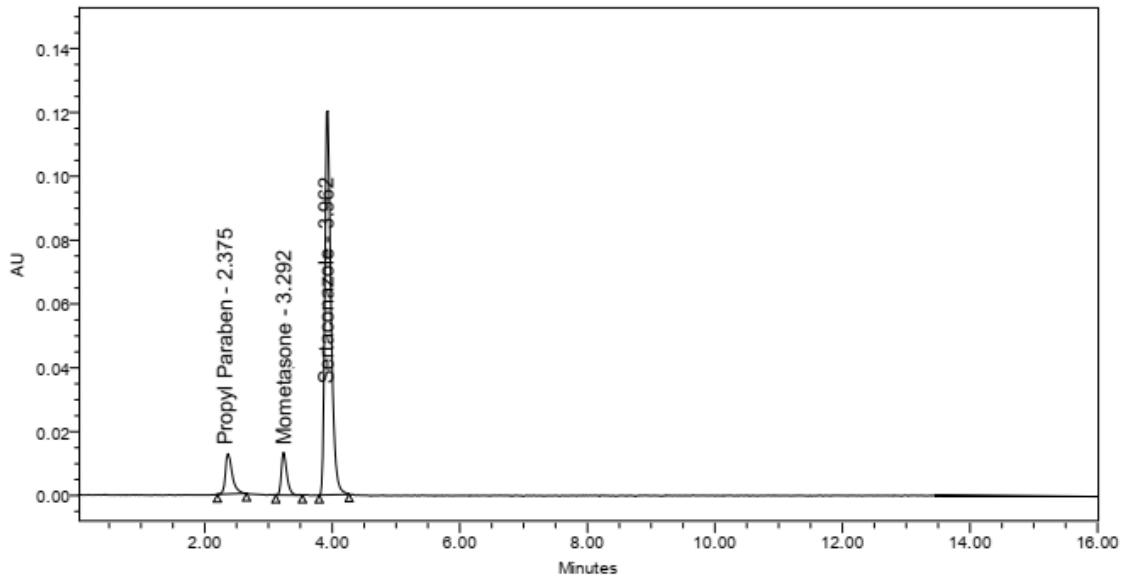


Figure 14: Neutral degradation chromatogram

J. Assay:

Average % Assay for Propyl Paraben, Sertaconazole and Mometasone. Obtained was:

Table 10: assay data

Formulation	Label claim(mg)	% Assay*
Onabet SD 30	Sertaconazole 2%(w/v)	99.47 %
	Mometasone 0.1%(w/v)	99.30 %

Conclusion:

An uncomplicated, quick, precise, accurate, specific, and reliable RP-HPLC technique can be effectively established for the concurrent assessment of Sertaconazole nitrate, Mometasone furoate, and Propyl paraben in a combined topical medicinal preparation. The established chromatographic technique ensures efficient separation and accurate quantification of the two active pharmaceutical components and the preservative, confirming its appropriateness for quality-control purposes in pharmaceuticals.

The addition of Propyl paraben as a preservative is crucial for preserving the microbiological integrity of the topical formulation, whereas Sertaconazole nitrate offers antifungal properties and Mometasone furoate delivers anti-inflammatory and antipruritic benefits. The simultaneous analysis of all three components using one chromatographic method can streamline routine testing and decrease the necessity for distinct analytical techniques.

The established RP-HPLC method can thus be regarded as an effective analytical technique for formulation development, regular quality assessment, and stability analysis of topical formulations with Sertaconazole nitrate, Mometasone furoate, and Propyl paraben. When backed by adequate validation results, the method can offer a consistent and repeatable approach for the concurrent estimation of these components in pharmaceutical formulations.

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