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Research Article

Development and Validation of Matrix Interference Method for Estimation of Miconazole Nitrate, Clobetasol Propionate in Combined Pharmaceutical Dosage Form

Abhijeet Welankiwar[1*], Mrunmayee Kulkarni[2], Dehuti Fate[3], Dipti Ruikar[4], Dipti Bonde[5]
[1*]Associate Professor Department of Pharmaceutical chemistry P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
[2]Research Scholar at P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
[3]Assistant Professor Department of Pharmaceutics P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
[4]Professor Department of Quality Assurance P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
[5]Associate Professor Department of Pharmacology P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
Postal Adress Where Research Conducted: P. R. Pote Patil College of Pharmacy, Amravati Pote estate Pote Patil Road Kathora Amravati Maharashtra India 444604
Corresponding Author: Abhijeet Welankiwar
Associate Professor Department of Pharmaceutical chemistry P. R. Pote Patil College of Pharmacy, Amravati Maharashtra 444604.
Email: abhi123welankiwar@gmail.com
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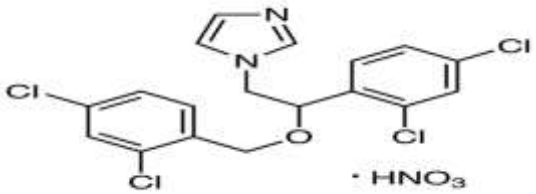
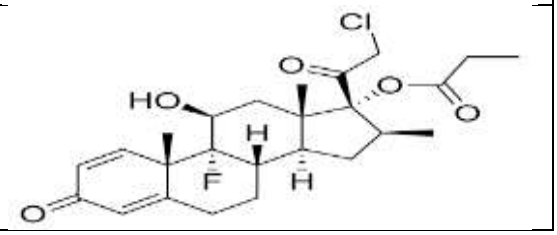
ABSTRACT
Background: Matrix interference caused by excipients and preservatives can affect the accuracy and reliability of analytical methods used for the estimation of active pharmaceutical ingredients (APIs) in combined pharmaceutical dosage forms. Therefore, evaluation of matrix effects is essential for ensuring accurate quantitative analysis.
Aim: The present study aimed to develop and validate a simple, rapid, and economical UV spectrophotometric method for evaluating matrix interference in a topical formulation containing Miconazole Nitrate, Clobetasol Propionate, and Chlorocresol.
Methods: Standard and sample solutions were prepared using methanol and analyzed by UV spectrophotometry at the respective λmax values of Miconazole Nitrate (272 nm), Clobetasol Propionate (239 nm), and Chlorocresol (283 nm). Matrix interference was assessed using the standard addition method and recovery studies at 80%, 100%, and 120% levels. The method was validated according to ICH Q2 (R1) guidelines for linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ).
Results and Discussion: The developed method demonstrated excellent linearity with correlation coefficients (R^2) greater than 0.999 for all analytes. Assay values for Miconazole Nitrate and Clobetasol Propionate were found to be 99.37% and 100.20%, respectively. Recovery studies showed satisfactory results, indicating negligible matrix interference. Precision studies exhibited %RSD values below 2%, confirming good repeatability and reproducibility. Low LOD and LOQ values indicated adequate sensitivity of the method.
Conclusion: The validated UV spectrophotometric method was found to be accurate, precise, sensitive, and suitable for routine quality control analysis of combined pharmaceutical dosage forms with minimal matrix interference.

Keyword: Matrix Interference, UV Spectrophotometry, Method Validation, Multicomponent Analysis, ICH Guidelines, Pharmaceutical Quality Assurance.

INTRODUCTION

Miconazole Nitrate is (RS)-1-(2-(2,4-dichlorobenzyloy)-2-(2,4-dichlorophenyl) ethyl)-1H-imidazole (see Figure 1), a synthetic antifungal agent utilized to treat various fungal infections. Clobetasol propionate, chemically known as [17-(2'-chloroacetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]

propanoate (see Figure 2), is a powerful glucocorticosteroid with minimal mineralocorticosteroid activity. The rationale for this combination is to effectively treat fungal infections while also providing anti-inflammatory effects. Miconazole Nitrate exerts its antifungal effect by converting lanosterol into ergosterol, a process that requires the enzyme 14-α demethylase, which is a cytochrome P-450 enzyme. Miconazole Nitrate inhibits this enzyme, thereby producing its antifungal action. Clobetasol Propionate, on the other hand, is a corticosteroid known for its strong glucocorticoid activity and weak mineralocorticoid activity. It is believed to work by inducing the production of phospholipase A2 inhibitory proteins, collectively referred to as lipocortin. [2]

Drug	Chemical Structure
Miconazole Nitrate	
Clobetasol Propionate	

Based on the literature review, The Matrix Interference Method was performed to evaluate the effect of formulation excipients and other matrix components on the analytical determination of Clobetasol Propionate and Miconazole Nitrate. The study was conducted to demonstrate the specificity of the UV spectrophotometric method by ensuring that the sample matrix does not interfere with the absorbance measurement of the analytes at the selected wavelength. The absence of significant interference confirms that the method is suitable for the accurate and reliable quantification of Clobetasol Propionate and Miconazole Nitrate in the pharmaceutical formulation. In UV analysis, matrix interference refers to absorbance contributed by excipients, solvents, preservatives, or formulation components at or near the λmax of the drug, which can falsely increase or decrease the measured absorbance of the API. Matrix interference in UV-Vis spectroscopy occurs when sample components (e.g., proteins, impurities) other than the analyte alter absorbance, causing inaccuracies. Mitigation methods include matrix matched calibration curves (using blank matrix), standard addition method (adding known analyte amounts), or mathematical corrections like three-point correction to account for background.[4]

MATERIALS AND METHODS:

Materials:

Reference standards of Miconazole Nitrate (MCN), Clobetasol Propionate (CLP), and Chlorocresol (CCL) were obtained as gift samples from a certified manufacturer. Methanol (analytical grade) was used as the solvent throughout the study. All other chemicals and reagents used were of analytical grade.

Instrumentation:

The analysis was carried out using a UV–Visible spectrophotometer equipped with 1 cm quartz cells. An analytical balance, ultrasonicator, and standard laboratory glassware were also used.

Preparation of Working Standard Solution:

Accurately weighed 10 mg each of Miconazole Nitrate, Clobetasol Propionate, and Chlorocresol were separately transferred into 10 mL volumetric flasks and dissolved in methanol. The volume was made up to the mark with methanol to obtain stock solutions of 1000 µg/mL. For Clobetasol Propionate and Chlorocresol, 1 mL of the stock solution was further diluted to 10 mL with methanol to obtain 100 µg/mL working solutions. Appropriate dilutions (10 µg/mL) of each drug were

prepared and scanned in the UV range of 200–400 nm using methanol as blank. The λmax values were found to be 272 nm for Miconazole Nitrate, 239 nm for Clobetasol Propionate, and 283 nm for Chlorocresol, which were selected for further analysis.

Preparation of Sample Solution (Marketed Formulation):

An accurately weighed quantity of cream equivalent to 10 mg Miconazole Nitrate was transferred into 100 mL volumetric flask. About 70 mL methanol was added and sonicated for 20 minutes to ensure complete extraction. The solution was filtered through Whatman filter paper, and volume was made up to the mark with methanol. Suitable dilutions were prepared to bring the concentrations within the linearity range. [6]

Evaluation of Matrix Interference:

Matrix interference was assessed by comparing the absorbance of standard and sample solutions and by performing recovery studies using the standard addition method at 80%, 100%, and 120% levels. The percentage recovery and percentage difference were calculated. The obtained recoveries showed minimal variation, indicating the absence of significant matrix interference from the cream excipients.

Matrix Interference Method:

Matrix interference was evaluated using the spike recovery (standard addition) method. A cream sample (2 g) was accurately weighed, dissolved in 50 mL methanol, and appropriately diluted. Aliquots of the sample solution (1 mL) were transferred into a series of volumetric flasks and spiked with known quantities of standard drug solutions corresponding to their respective linearity ranges.

For Miconazole Nitrate, standard additions equivalent to 100–600 ppm were made, and absorbance was measured at 272 nm.

For Clobetasol Propionate, standard additions equivalent to 2–25 ppm were made, and absorbance was measured at 239 nm.

The absorbance values obtained for the spiked samples were used to assess matrix interference and determine the recovery of each analyte from the cream formulation.

Assay for Cream Formulation:

For the assay, 2 g of cream was accurately weighed, extracted with 70 mL methanol, stirred for 30 min, and filtered through Whatman filter paper. An aliquot (1 mL) of the filtrate was transferred into a series of volumetric flasks. Six flasks were spiked with known concentrations of the respective standard drug solutions within the linearity range, while one flask containing the sample solution was used as the unknown.

The absorbance of the prepared solutions was measured at 272 nm for Miconazole Nitrate and 239 nm for Clobetasol Propionate. The drug content in the cream formulation was determined using the standard addition method.

Method Validation:

Method validation was performed according to ICH Q2 (R1) guidelines.

1) Linearity:

Calibration curves were conducted by plotting absorbance versus concentration at respective λmax values. Correlation coefficient (R^2), slope, and intercept were calculated.

1. Miconazole Nitrate:

Accurately weigh 10 mg of Miconazole Nitrate and transfer it into a 10 mL volumetric flask. Dissolve and make up the volume with methanol to obtain a 1000 µg/mL stock solution. Aliquots of 1, 2, 3, 4, 5, and 6 mL of the stock solution were transferred into separate 10 mL volumetric flasks and diluted to volume with methanol to obtain concentrations of 100, 200, 300, 400, 500, and 600 µg/mL, respectively. The absorbance of each solution was measured at 272 nm, and a calibration curve of absorbance versus concentration was constructed. [9]

2. Clobetasol Propionate:

Accurately weigh 10 mg of Clobetasol Propionate and transfer it into a 10 mL volumetric flask. Dissolve and make up the volume with methanol to obtain a 1000 µg/mL stock solution. Further, dilute 1 mL of this stock solution to 10 mL with methanol to obtain a 100 µg/mL working standard solution. Aliquots of 0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the working solution were transferred into separate 10 mL volumetric flasks and diluted to volume with methanol to obtain concentrations of 2, 5, 10, 15, 20, and 25 µg/mL, respectively. The absorbance of each solution was measured at 239 nm, and a calibration curve of absorbance versus concentration was constructed.[9]

3. Chlorocresol:

Accurately weigh 10 mg of Chlorocresol and transfer it into a 10 mL volumetric flask. Dissolve and make up the volume with methanol to obtain a 1000 µg/mL stock solution. Further, dilute 1 mL of the stock

solution to 10 mL with methanol to obtain a 100 µg/mL working standard solution**.** Aliquots of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL of the working solution were transferred into separate 10 mL volumetric flasks and diluted to volume with methanol to obtain concentrations of 5, 10, 20, 30, 40, and 50 µg/mL**,** respectively. The absorbance of each solution was measured at 283 nm**,** and a calibration curve of absorbance versus concentration was constructed. [9]

2) Accuracy:

For Miconazole Nitrate:

Accuracy of the proposed method was evaluated by the standard addition method at 80%, 100%, and 120% levels. Cream samples equivalent to 1.6 g, 2.0 g, and 2.4 g of Tenovate M cream, respectively, were extracted with 70 mL methanol, stirred for 30 min, and filtered through Whatman filter paper. An aliquot (1 mL) of the filtrate was transferred into a series of volumetric flasks and spiked with known concentrations of Miconazole Nitrate standard solution within the linearity range. The absorbance of the resulting solutions was measured at 272 nm, and the percentage recovery was calculated to assess the accuracy of the method. [10]

For Clobetasol Propionate:

Accuracy of the proposed method was evaluated by the standard addition method at 80%, 100%, and 120% levels. Cream samples equivalent to 1.6 g, 2.0 g, and 2.4 g of Tenovate M cream, respectively, were extracted with 70 mL methanol, stirred for 30 min, and filtered through Whatman filter paper. An aliquot (1 mL) of the filtrate was transferred into a series of volumetric flasks and spiked with known concentrations of Clobetasol Propionate standard solution within the linearity range. The absorbance of the resulting solutions was measured at 239 nm, and the percentage recovery was calculated to evaluate the accuracy of the method. [10]

3) Precision:

Interday precision for Miconazole Nitrate:

Interday precision was determined by preparing six replicate solutions of the mixed standard solution. The absorbance was measured at 272 nm over three consecutive days, and the %RSD was calculated to evaluate method precision.

Interday precision for Clobetasol Propionate:

Interday precision was evaluated by preparing six replicate solutions of the mixed standard solution. The absorbance was measured at 239 nm on three consecutive days, and the %RSD was calculated to assess the precision of the method. [12]

Intra-day precision for Miconazole Nitrate:

Intraday precision was evaluated by preparing six replicate solutions of the mixed standard solution. The absorbance was measured at 272 nm on the same day at different time intervals (1–2 h), and the %RSD was calculated to assess the precision of the method.

Intra-day precision for Clobetasol Propionate:

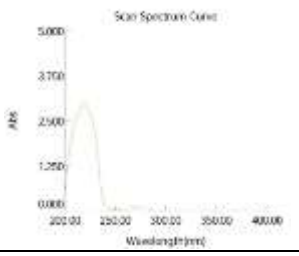
Intraday precision was evaluated by preparing six replicate solutions of the mixed standard solution. The absorbance was measured at 239 nm on the same day at different time intervals (1–2 h), and the %RSD was calculated to assess the precision of the method. [13]

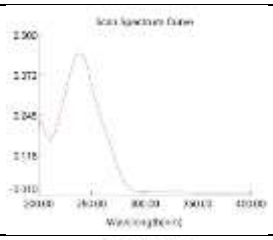
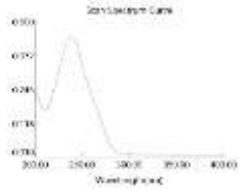
4) Limit of Detection and Limit of Quantification:

LOD and LOQ were calculated from the equation. [14]

$LOD = S3.3 \times \sigma$ $LOQ = S10 \times \sigma$

RESULT AND DISCUSSION:

Sr. No.	Drug	Calibration Curve
1.	Miconazole Nitrate	

2.	Clobetasol Propionate	
3.	Chlorocresol	

Solubility Study for Miconazole Nitrate and Clobetasol Propionate:

The solubility study revealed that methanol effectively dissolved both Miconazole Nitrate and Clobetasol Propionate, whereas both drugs exhibited negligible solubility in distilled water. Based on these findings, methanol was selected as the extraction solvent to ensure complete dissolution of the analytes from the cream formulation. The use of methanol minimized the risk of incomplete extraction and contributed to consistent and reproducible absorbance measurements during UV spectrophotometric analysis. Appropriate solvent selection is a crucial aspect of analytical method development, as complete dissolution of the analytes directly influences the accuracy, precision, and reliability of the analytical results.

Assay for cream formulation:

Table No 01: Assay Reading of Miconazole and Clobetasol

Sr. No.	Concentration for Miconazole	Absorbances	Concentration for Clobetasol	Absorbances
1	100 ppm	0.396	2 ppm	0.279
2	200 ppm	0.590	5 ppm	0.352
3	300 ppm	0.800	10 ppm	0.466
4	400 ppm	1.032	15 ppm	0.610
5	500 ppm	1.211	20 ppm	0.716
6	600 ppm	1.410	25 ppm	0.829
Unknown		0.349	Unknown	0.183

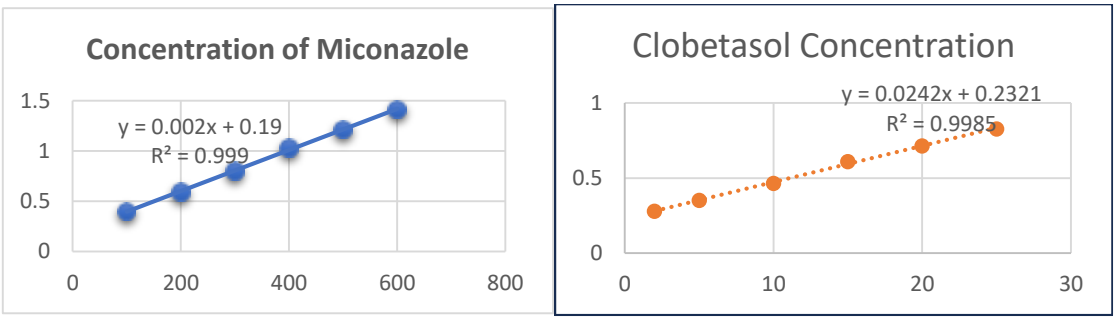


Figure No 01: Calibration Curve of Miconazole and Clobetasol (Assay)

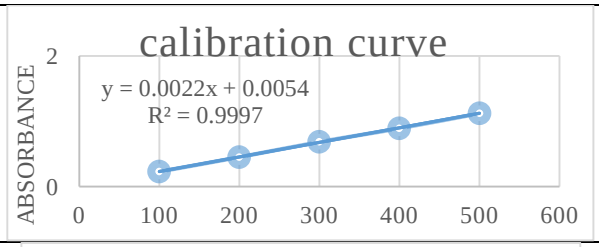
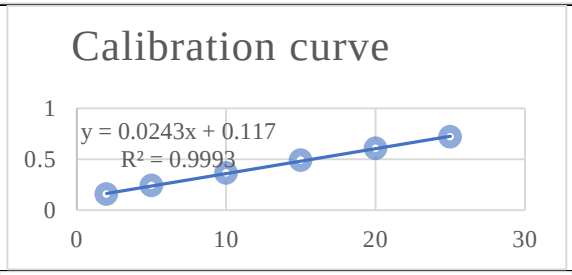
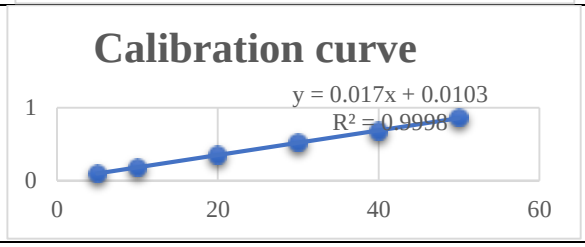
The assay results demonstrated that the developed UV spectrophotometric method accurately quantified both Miconazole Nitrate and Clobetasol Propionate in the marketed cream formulation. The percentage assay values obtained for Miconazole Nitrate (99.37%) and Clobetasol Propionate (100.20%) were found to be in close

agreement with their respective labelled claims, indicating excellent analytical accuracy. Furthermore, the absence of significant interference from formulation excipients confirmed the specificity of the method and highlighted the effectiveness of the standard addition technique for the quantitative estimation of both drugs in a complex semisolid dosage form. These findings establish the reliability and suitability of the proposed method for routine quality control analysis. (Table no. 01 and Figure no. 01)

1) Linearity and Range:

Table No 02: Linearity of Miconazole, Clobetasol and Chlorocresol

Sr. No.	Conc. of Miconazole	Absorbances	Conc. of Clobetasol	Absorbances	Conc. of Chlorocresol	Absorbances
1	100 ppm	0.227	2 ppm	0.158	5 ppm	0.097
2	200 ppm	0.450	5 ppm	0.240	10 ppm	0.178
3	300 ppm	0.684	10 ppm	0.365	20 ppm	0.348
4	400 ppm	0.892	15 ppm	0.488	30 ppm	0.524
5	500 ppm	1.122	20 ppm	0.605	40 ppm	0.683
6	600 ppm	1.380	25 ppm	0.718	50 ppm	0.861

Sr. No.	Drug	Calibration curve
1.	Miconazole Nitrate	
2.	Clobetasol Propionate	
3.	Chlorocresol	

The calibration curves demonstrated excellent linearity across the selected concentration ranges, with correlation coefficient (R^2) values exceeding 0.999 for all analytes. This strong linear relationship between absorbance and concentration confirms adherence to Beer–Lambert's law within the studied range. The high degree of linearity ensures reliable quantification by minimizing analytical errors during routine measurements and validates the suitability of the selected concentration ranges for pharmaceutical analysis. These findings indicate that the proposed method is capable of providing accurate and reproducible results over the investigated concentration range, consistent with previously reported UV spectrophotometric methods developed for the

analysis of multicomponent pharmaceutical formulations. (Table no. 02)

2) Accuracy

1. Miconazole Nitrate

Table No 03: Accuracy for Miconazole Nitrate

Sr. No.	Conc. for 80%	Spiked Absorbances	Conc. for 100%	Spiked Absorbances	Conc. for 120%	Spiked Absorbances
1.	100 ppm	0.316	100 ppm	0.396	100 ppm	0.475
2.	200 ppm	0.472	200 ppm	0.590	200 ppm	0.708
3.	300 ppm	0.640	300 ppm	0.800	300 ppm	0.960
4.	400 ppm	0.825	400 ppm	1.032	400 ppm	1.238
5.	500 ppm	0.968	500 ppm	1.211	500 ppm	1.453
6.	600 ppm	1.128	600 ppm	1.410	600 ppm	1.692
7.	Unknown	0.256	Unknown	0.349	Unknown	0.466

2. Clobetasol Propionate:

Table No 04: Accuracy for Clobetasol Propionate

Sr. No.	Conc. for 80%	Spiked Absorbances	Conc. for 100%	Spiked Absorbances	Conc. for 120%	Spiked Absorbances
1.	2 ppm	0.223	2 ppm	0.279	2 ppm	0.334
2.	5 ppm	0.281	5 ppm	0.352	5 ppm	0.422
3.	10 ppm	0.372	10 ppm	0.466	10 ppm	0.559
4.	15 ppm	0.482	15 ppm	0.610	15 ppm	0.722
5.	20 ppm	0.572	20 ppm	0.716	20 ppm	0.859
6.	25 ppm	0.663	25 ppm	0.829	25 ppm	0.994
7.	Unknown	0.154	Unknown	0.183	Unknown	0.348

The accuracy of the proposed UV spectrophotometric method was evaluated using the standard addition technique, and the recovery results confirmed its satisfactory performance. The percentage recoveries obtained for both Miconazole Nitrate and Clobetasol Propionate were found to be within the acceptable range, demonstrating the accuracy of the method. Furthermore, the results indicated that the excipients and other components present in the cream formulation did not produce any significant interference during analysis, suggesting negligible matrix effects. These findings establish the reliability of the proposed method and support its suitability for the routine quantitative analysis and quality control of topical pharmaceutical formulations. (Table no. 03 and Table no. 04)

3) Precision (Interday):

Table No 05: Precision for Miconazole Nitrate and Clobetasol Propionate

Day	Absorbance 1	Absorbance 2	Absorbance 3	Mean	SD	%RSD
1. Miconazole Nitrate						
1	0.512	0.515	0.510	0.512	0.0025	0.49%
2	0.508	0.511	0.509	0.509	0.0015	0.29 %

3	0.514	0.516	0.513	0.514	0.0015	0.20 %
2. Clobetasol Propionate						
1	0.428	0.431	0.429	0.429	0.0015	0.35 %
2	0.432	0.430	0.433	0.431	0.0015	0.35 %
3	0.427	0.429	0.428	0.428	0.0010	0.23 %

4) Precision (Intra-day)

Table No. 06: Precision for Miconazole Nitrate and Clobetasol Propionate

Replicate	Absorbances	Replicate	Absorbances
Miconazole Nitrate		Clobetasol Propionate	
1	0.506	1	0.423
2	0.509	2	0.426
3	0.507	3	0.424
4	0.510	4	0.425
5	0.508	5	0.427
6	0.509	6	0.424
Mean	0.508	Mean	0.424
SD	0.0015	SD	0.0015
%RSD	0.30 %	%RSD	0.35%

The low %RSD values obtained from both intra-day and inter-day precision studies demonstrated the excellent precision of the proposed method. The minimal variation among replicate measurements confirmed its good repeatability and reproducibility, indicating that the method is reliable for routine pharmaceutical analysis. (Table no. 05 and Table no. 06)

5) LOD and LOQ:

Table No. 07: LOD and LOQ for clobetasol and miconazole

Drug	LOD	LOQ
Miconazole Nitrate	0.35 µg/mL^{-1}	1.05 µg/mL^{-1}
Clobetasol Propionate	0.28 µg/mL^{-1}	0.85 µg/mL^{-1}

The low LOD and LOQ values obtained for both analytes demonstrate the good sensitivity of the proposed UV spectrophotometric method. These results indicate that the method is capable of detecting and accurately quantifying low concentrations of the drugs, making it suitable for routine quality control of pharmaceutical formulations. (Table no. 07)

CONCLUSION:

The present study successfully developed and validated a simple, rapid, and cost-effective UV spectrophotometric method for the evaluation of matrix interference in a combined topical pharmaceutical formulation containing Miconazole Nitrate, Clobetasol Propionate, and Chlorocresol. The method demonstrated excellent linearity over the selected concentration ranges with correlation coefficients (R^2) greater than 0.999 for all analytes. Validation parameters, including accuracy, precision, specificity, LOD, and LOQ, were found to be within acceptable limits as per ICH Q2(R1) guidelines. Recovery studies showed results within the acceptable range, confirming the absence of significant interference from excipients and preservatives present in the formulation. The %RSD values for both intra-day and inter-day precision were below 2%, indicating good reproducibility of the

method. The assay results for Miconazole Nitrate and Clobetasol Propionate were found to be within acceptable limits, confirming the reliability of the method for quantitative estimation. Additionally, low LOD and LOQ values indicated high sensitivity of the developed method. Overall, the proposed method is accurate, precise, sensitive, and robust, and can be effectively applied for routine quality control analysis of combined pharmaceutical dosage forms. The study confirms that matrix interference does not significantly affect the analytical performance under the optimized conditions.

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