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Simultaneous Determination of Luliconazole and Salicylic Acid by Simultaneous Equation Method and Absorbance Ratio Method

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Abstract: *The simultaneous determination of Luliconazole and Salicylic Acid in pharmaceutical formulations holds significant importance for ensuring therapeutic efficacy and patient safety. This research focuses on developing an accurate and efficient method using UV-Visible spectrophotometry, specifically the Simultaneous Equation Method (SEM) and Absorbance Ratio Method (ARM), to quantify these active ingredients in topical creams. The study begins with the preparation of stock solutions and selection of the analytical wavelength, with 298 nm chosen in reference to the isobestic point at 230 nm. Cream samples containing 10 mg Luliconazole and 30 mg Salicylic Acid are prepared, sonicated, and filtered for analysis.*

The UV method is validated in accordance with ICH guidelines, encompassing linearity, precision, LOD, LOQ, specificity, robustness, and accuracy. Calibration curves show excellent linearity within the concentration range of 5-30 µg/ml for both compounds. Intra-day and inter-day precision studies exhibit low % RSD values, indicating the reliability and reproducibility of the method. LOD and LOQ values demonstrate the sensitivity of the method, enabling detection and quantification at trace levels. Specificity is confirmed through overlay spectra and comparisons of analyte responses.

Robustness is assessed by varying temperature and concentration, demonstrating the method's resilience to minor changes. Accuracy is evaluated through the standard addition method, yielding recoveries within acceptable limits. The proposed UV method is successfully applied to the analysis of Luliconazole and Salicylic Acid in commercial cream samples, with assay results confirming its accuracy and suitability for routine quality control.

Keywords: Simultaneous determination, Luliconazole, Salicylic Acid, UV-Visible spectrophotometry, pharmaceutical formulations, combination therapy, analytical validation.

I. INTRODUCTION

The co-formulation of multiple active pharmaceutical ingredients (APIs) has become increasingly common, driven by the desire to improve therapeutic outcomes and patient adherence.[1,2] Within this context, the combination of Luliconazole, a potent antifungal agent, with salicylic acid, a keratolytic compound, holds significant promise. [3,4] These compounds, when combined, offer a multifaceted approach to addressing fungal infections while promoting skin health. [5,6]

Luliconazole demonstrates remarkable antifungal efficacy through its mechanism of action, making it an essential component of topical formulations. [7,8]Salicylic acid, on the other hand, complements this effect by facilitating the removal of dead skin cells, thereby enhancing the penetration and action of Luliconazole. [9,10]This synergistic interaction underscores the importance of accurate quantification of both components, ensuring their optimal concentration and balance within the formulation. [11,12]

Precise and reliable analytical methods are imperative to ensure the potency, safety, and consistency of such combination therapies. [13,14]Yet, the simultaneous determination of Luliconazole and salicylic acid presents a distinct analytical challenge due to their overlapping spectral characteristics. [15,16]Existing methods often involve complex separation techniques or intricate sample preparation, which can be time-consuming and resource-intensive. [17,18]

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To bridge this gap, this research article introduces a novel approach that combines the Simultaneous Equation Method (SEM) and the Absorbance Ratio Method (ARM) utilizing UV-Visible spectrophotometry. SEM leverages the distinctive absorption wavelengths of the individual components to develop mathematical equations that allow their simultaneous quantification. ARM capitalizes on the relative absorbance ratios at specific wavelengths to minimize interference and enhance accuracy.

By focusing on these methodologies, we aim to provide an efficient, cost-effective, and accurate solution for the simultaneous determination of Luliconazole and salicylic acid in complex pharmaceutical formulations. The proposed methods have the potential to streamline quality control processes, accelerate analysis time, and contribute to the broader advancement of combination drug therapies. This study not only addresses a critical research gap but also holds the promise of benefiting both pharmaceutical research and patient care.

II. MATERIALS AND METHODS

Preparation of stock solution: Stock solutions of both drugs at a concentration of 100 µg/ml were prepared by dissolving 10 mg of each in a 100 ml volumetric flask, and the volume was made up with Methanol.[19,20]

Selection of analytical wavelength: By appropriately diluting standard solutions of Luliconazole and Salicylic Acid to 10 µg/ml each, they were scanned for the entire wavelength region in spectrum mode, and the peaks were observed. However, the isobestic point was found to be at 230 nm. Thus, the wavelength 298 nm with reference to 230 nm (isobestic point) was selected for the analysis of the sample.[21,22]

Cream Analysis: 100 g of cream contained 1% of Luliconazole and 3% of Salicylic Acid. 1g of cream contained 0.01% of Luliconazole and 0.03% Salicylic Acid, equivalent to 10 mg of Luliconazole and 30 mg of Salicylic Acid. An accurately weighed cream equivalent to 10 mg of Luliconazole and 30 mg of Salicylic Acid cream was taken and transferred into a 100 ml volumetric flask. 50 ml of the mobile phase was added, and the solution was sonicated with occasional shaking for 10 minutes. The solution was then diluted to volume with the mobile phase and filtered through a 0.22 µl syringe filter to obtain concentrations of 100 µg/ml Luliconazole and 300 µg/ml Salicylic Acid.[23-28]

UV method validation according to ICH guidelines:

Linearity: Calibration curves were constructed by measuring absorbances at 232 nm for Salicylic Acid and 298 nm for Luliconazole in the range of 5-30 µg/ml.[29,30]

Precision: Intra-day and inter-day precision of the proposed method were performed by analyzing the corresponding responses three times on the same day (intra-day) and on three different days (inter-day) for three different concentrations of standard solutions of both drugs.[31,32]

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated using the following equations: LOD = 3.3 x Sy/S, LOQ = 10 x Sy/S, where Sy is the standard deviation of the response, and S is the slope of the calibration curve.[33,34]

Specificity: For the spectrophotometric methods, obtaining λ max involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, process impurities, etc.) is compared with the response of the solution containing only the analyte. An assay method was found to be meeting specificity criteria since the response obtained for the analyte i.e. formulation sample was specific and comparable to that with the drug substance at given wavelengths.[35,36]

Robustness: Robustness of the method was determined by analyzing standard solutions under normal operating conditions and by changing some operating analytical conditions, such as change in temperature and concentration.[37,38]

Accuracy: Accuracy was determined by the standard addition method. Three different levels (80%, 100%, and 120%) of standards were spiked to commercial capsules in triplicate. The mean of percentage recoveries and the % RSD was calculated.[39,40]

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III. RESULTS AND DISCUSSION

Table1: Linearity readings for luliconazole

Concentration in µg / ml	Absorbance
5	0.358
10	0.534
15	0.838
20	1.078
25	1.353
30	1.567

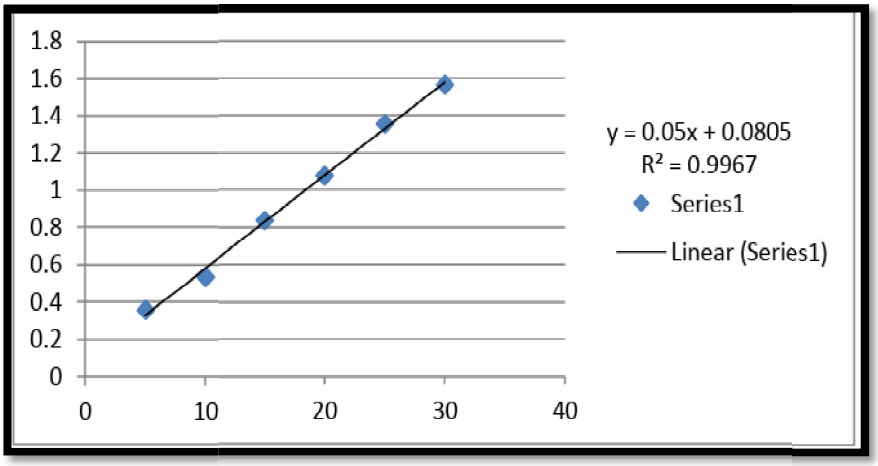

Fig 1: Linarity graph for luliconazole

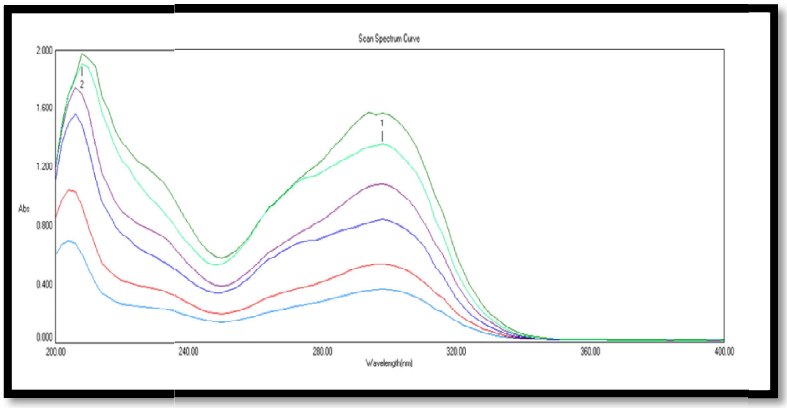

Fig 2: Overlay spectra for luliconazole

Table 2: Intra – Day Precision

No.	Concentration in µg / ml	Absorbance
Set I (10.30AM)	7	0.503
	17	0.993
	27	1.380
Set II (1.30PM)	7	0.494
	17	0.996
	27	1.375
Set III	7	0.500

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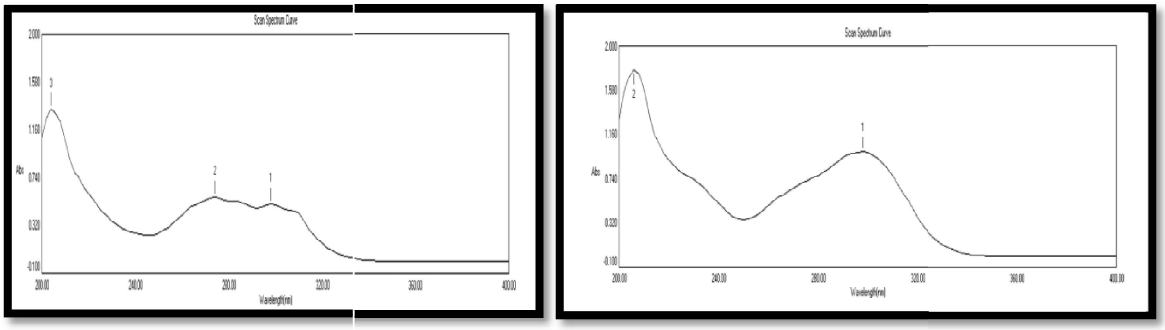
(4.30PM)	17	0.993
	27	1.376

Observations- Intra – Day precision parameter with% RSD

Table 3: Inter –Day Precision

No.	Concentration in µg / ml	Absorbance
Day 1	7	0.503
	17	0.993
	27	1.380
Day 2	7	0.501
	17	0.996
	27	1.379
Day 3	7	0.509
	17	0.994
	27	1.383



a b

Fig 3: Intra-day Precision spectra of a) 7 µg/ml b)17 µg/ml (Set I)

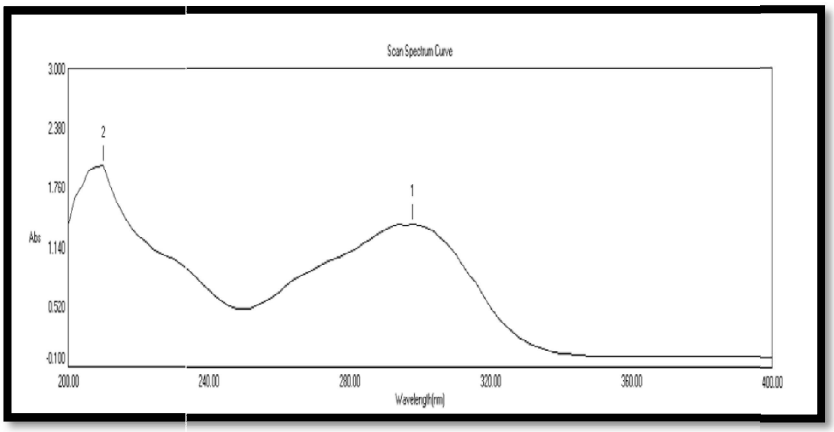


Fig 4: Intra-day Precision spectra of 27 µg/ml (Set I)

Robustness:

Observations: Robustness Parameters with Absorbance

Table 4: Parameters for robustness

Parameters	Absorbance
Temperature	
Room Temperature	0.546

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8 ° C	0.565
Concentration	
55:45 v / v (MeOH : 0.1 % Ac . acid)	0.524
90:10 v / v (MeOH : 0.1 % Ac . acid)	0.511

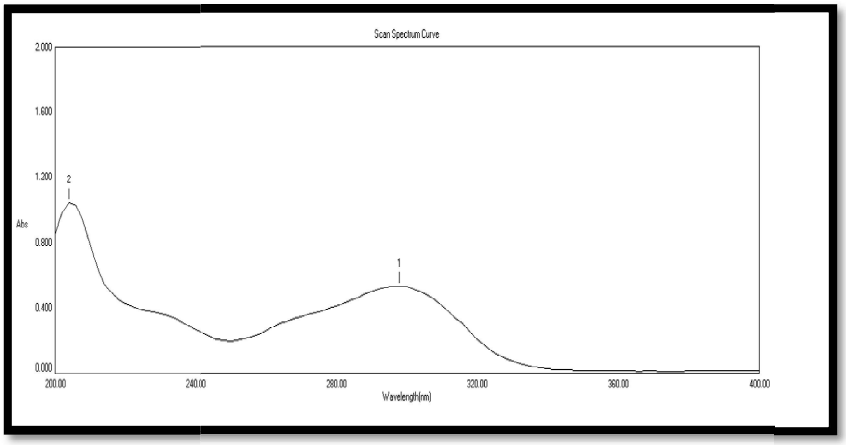

Fig 5: Luliconazole spectra at Room Temperature

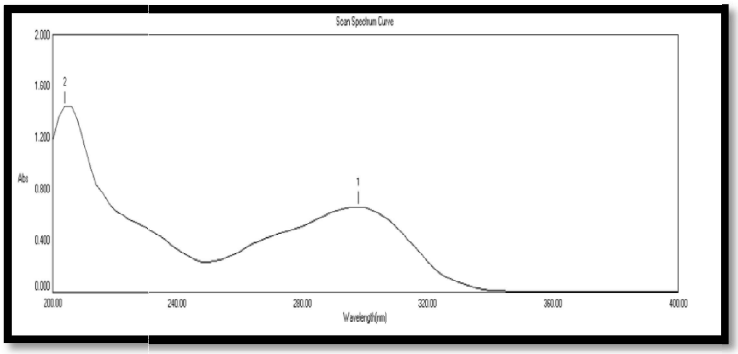

Fig 6: Luliconazole spectra at 8°C

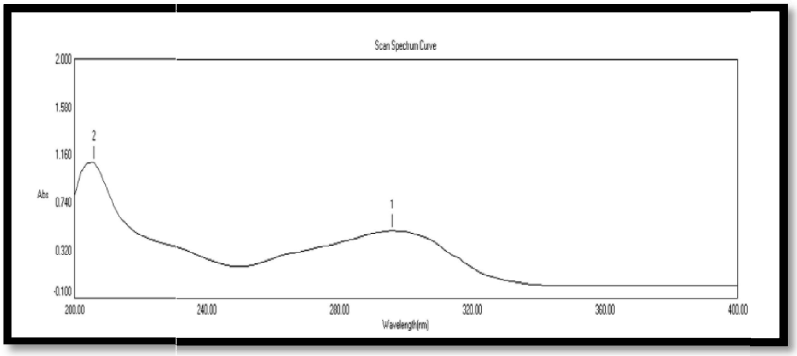

Fig 7: Luliconazole spectra 90:10v/v concentration

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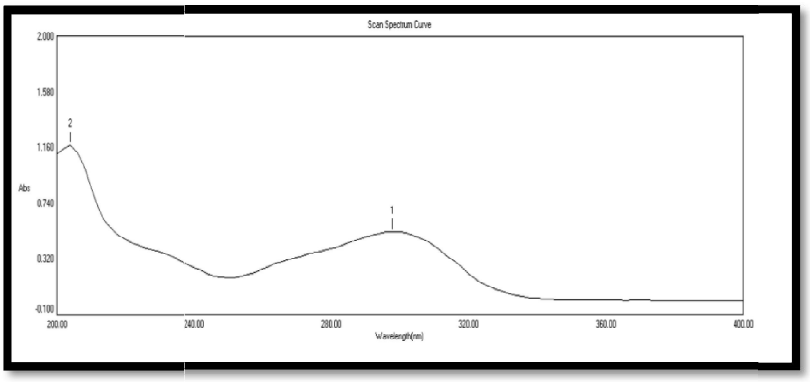

Fig 8: Luliconazole spectra 55:45 v/v concentration

Salicylic acid

Table5: Linearity readings for Salicylic acid

Concentration in µg / ml	Absorbance
5	0.330
10	0.513
15	0.756
20	1.046
25	1.336
30	1.551

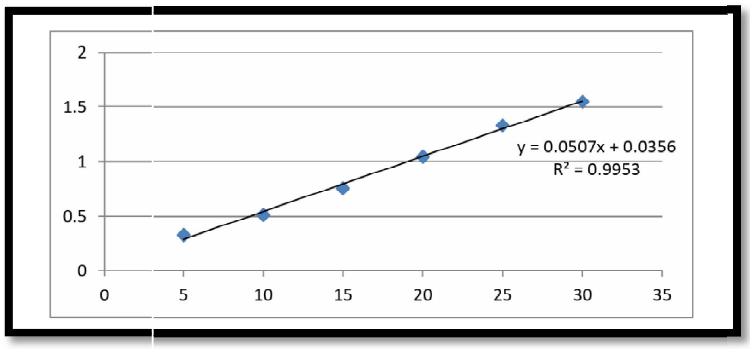

Fig 9: Linearity for Salicylic acid

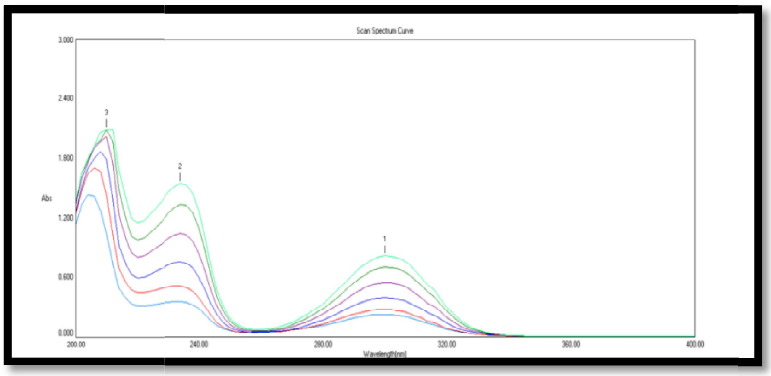

Fig10: Overlay Spectra of Linearity

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Table.6: Intra – Day Precision

No.	Concentration in µg / ml	Absorbance
Set I (11.30AM)	7	0.409
	17	0.903
	27	1.381
Set II (2.30PM)	7	0.407
	17	0.903
	27	1.359
Set III (5.30PM)	7	0.406
	17	0.904
	27	1.360

+Observations- Intra – Day precision parameter with% RSD\

Table7: Inter –Day Precision

No.	Concentration in µg / ml	Absorbance
Day 1	7	0.409
	17	0.903
	27	1.381
Day 2	7	0.406
	17	0.905
	27	1.368
Day 3	7	0.404
	17	0.906
	27	1.383

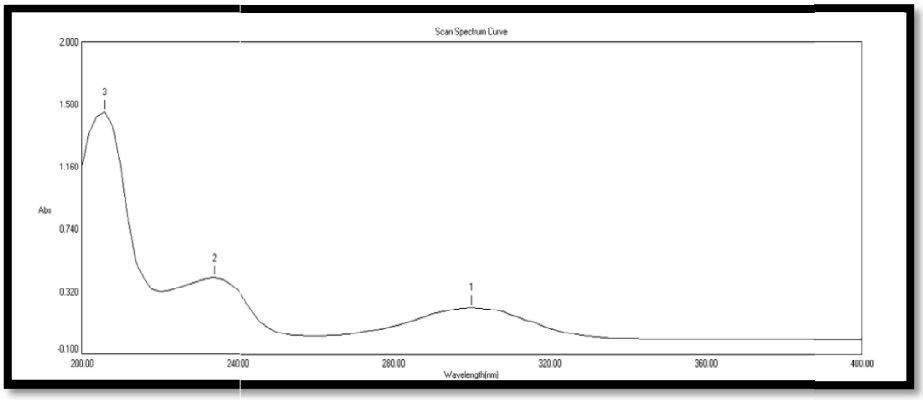

Fig 11: Intra-day Precision spectra of 7 µg/ml (Set I)

Table 8: parameters for robustness

Parameters	Absorbance
Temperature	
At Room Temperature	0.517
At 8 ° C	0.557
Concentration	
90:10 v / v (Methanol : 0.1 % Acetic acid)	0.522
55:45 v / v (Methanol : 0.1 % Acetic acid)	0.503

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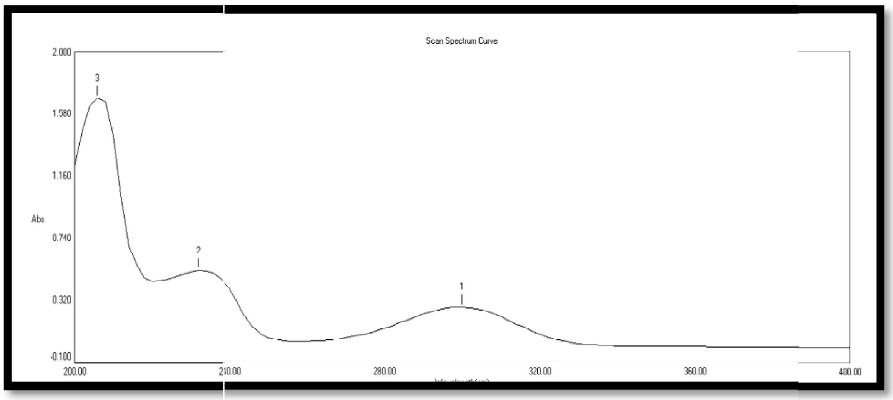

Fig 11:Salicylic acid spectra at Room Temperature

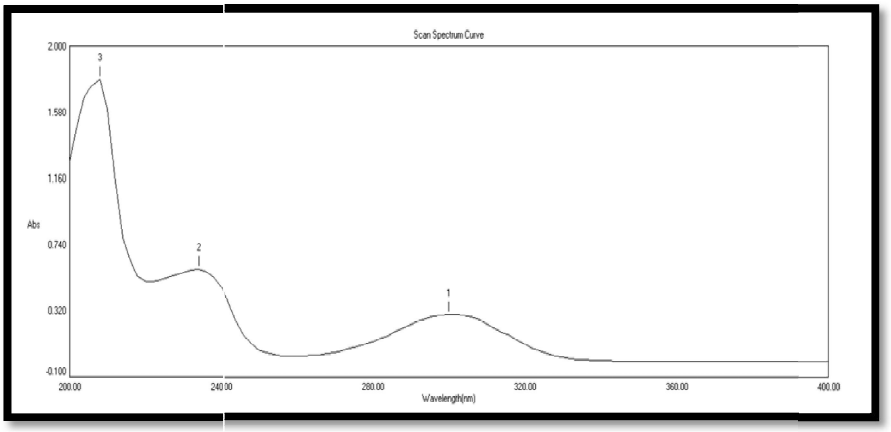

Fig 12:Salicylic acid spectra at 8°C

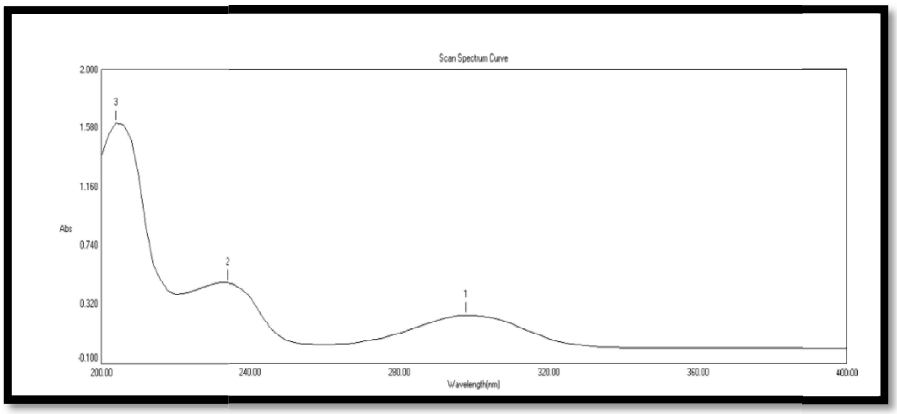

Fig 13: Salicylic acid spectra 55:45v/v concentration

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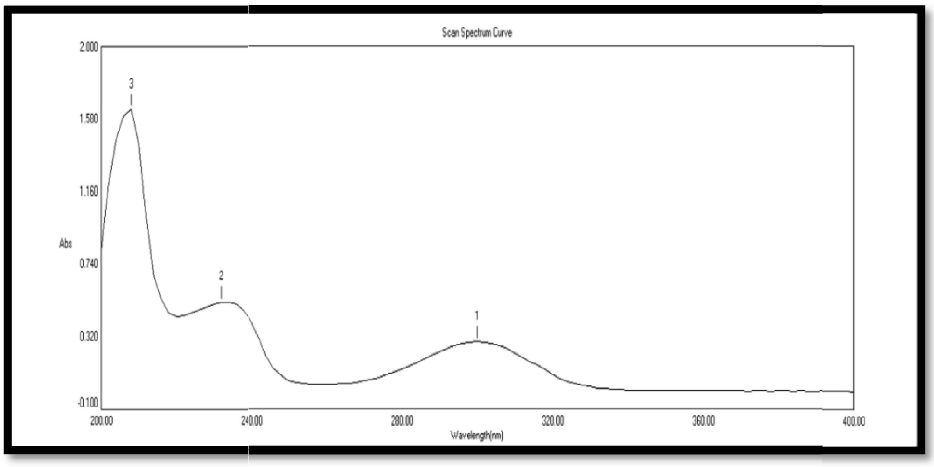

Fig 14: Salicylic acid spectra 90:10 v/v concentration

Assay –

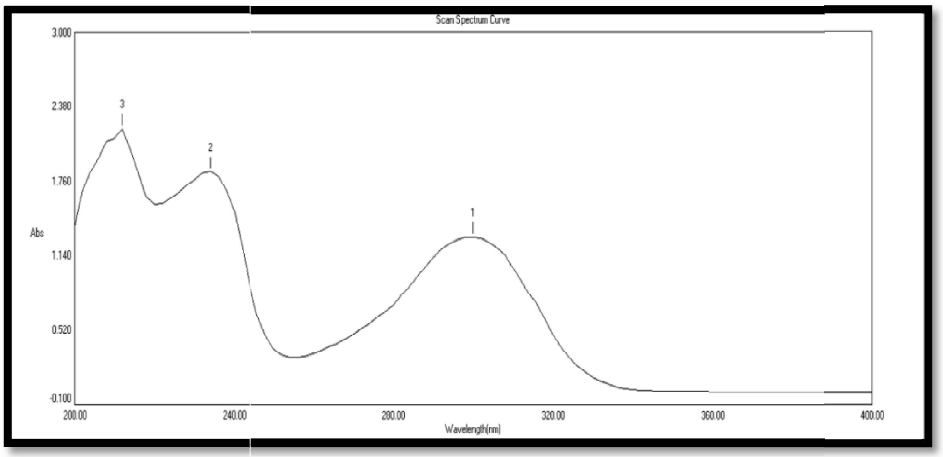

Fig 15: Standard-Luliconazole Salicylic acid spectra

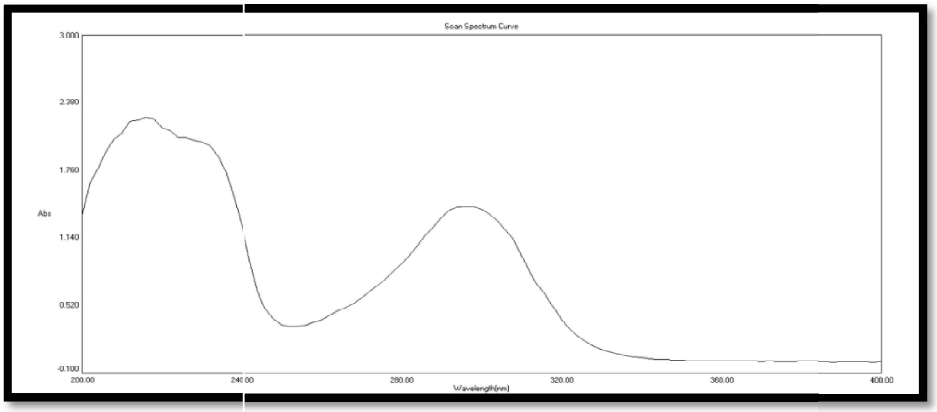

Fig 16: Sample-Lulian cream spectra

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Table 9: Salicylic acid content

No.	P / V	Wavelength (nm)	Absorbance
1	Peak	298.00	1.246
2 .	Peak	232.00	1.844

No.	ID	Mode	A	Assay
			232.00nm	(%)
1	Sample	Abs	1.844	100.3 %
2	Standard	Abs	1.838	

Table 10: Luliconazole content

No.	ID	Mode	A	Assay
			298.00nm	(%)
1	Sample	Abs	1.246	98.9 %
2	Standard	Abs	1.259	

Calculations- % Luliconazole content-

$$\frac{\text{Abs. of sample} \times \text{Standard Conc.} \times \text{Potency} \times 100}{\text{Abs. of standard} \times \text{Sample Conc.} \times 100}$$

$$= \frac{1.246 \times 10 \times 100 \times 100}{1.259 \times 10 \times 100}$$

$$= \mathbf{98.9\%}$$

% Salicylic acid content-

$$\frac{\text{Abs. of sample} \times \text{Standard Conc.} \times \text{Potency} \times 100}{\text{Abs. of standard} \times \text{Sample Conc.} \times 100}$$

$$= \frac{1.844 \times 30 \times 100 \times 100}{1.838 \times 30 \times 100}$$

$$= \mathbf{100.3\%}$$

Discussion

% Assay for Luliconazole and Salicylic Acid was found to be 98.9% and 100.3% indicates that method is accurate. Limit of detection (LOD) – Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels.

Luliconazole-

$$LOD = 3.3(Sy/S) = \frac{3.3 \times 0.0302}{0.05} = 1.99\mu g/ml$$

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Salicylic acid-

$$LOD = 3.3(Sy/S) = \frac{3.3 \times 0.0363}{0.0507} = 2.36\ \mu g/ml$$

Limit of quantitation (LOQ) -Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels.

Luliconazole-

$$LOQ = 10(Sy/S) = \frac{10 \times 0.0302}{0.0507} = 6.04\ \mu g/ml$$

Salicylic acid-

$$LOQ = 10(Sy/S) = \frac{10 \times 0.0363}{0.05} = 5.96\ \mu g/ml$$

IV. CONCLUSION

In conclusion, the developed UV-Visible spectrophotometric method employing the Simultaneous Equation Method and Absorbance Ratio Method proves to be a robust, sensitive, and accurate approach for the simultaneous determination of Luliconazole and Salicylic Acid in topical pharmaceutical creams. The method's validation adheres to ICH guidelines, encompassing linearity, precision, specificity, robustness, and accuracy assessments. The method's successful application to commercial cream samples confirms its practical utility in routine quality control analysis. This research addresses the research gap in simultaneous determination techniques for these compounds and provides a valuable contribution to pharmaceutical analysis, facilitating the assessment of combination drug formulations with enhanced precision and efficiency.

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