www.ijarp.com ISSN 2456-9992 Page: 01-13

International Journal Advanced Research Publications

Research Article

Volume: 02
Issue: 05

DEVELOPMENT AND EVALUATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF ERYTHROMYCIN AND NIACINAMIDE IN TOPICAL FORMULATION

[*1]Ankit Kumar, [2]Dr. Virendra Kumar Sharma, [3]Dr. Akhilesh Kumar Singhai

[1]Student, Master of Pharmacy School of Pharmacy, LNCT University, J.K Town Sarvadharam C Sector, Kolar Road, Bhopal, Madhya Pradesh, India 462042.

[2]Vice-Principal, School of Pharmacy, LNCT University, J.K Town Sarvadharam C Sector, Kolar Road, Bhopal, Madhya Pradesh, India 462042.

[3]Director, School of Pharmacy, LNCT University, J.K Town Sarvadharam C Sector, Kolar Road, Bhopal, Madhya Pradesh, India 462042.

Article Received: 22 March 2026, Article Revised: 12 April 2026, Published on: 02 May 2026

***Corresponding Author: Ankit Kumar**

Student, Master of Pharmacy School of Pharmacy, LNCT University, J.K Town Sarvadharam C Sector, Kolar Road, Bhopal, Madhya Pradesh, India 462042.

DOI: https://doi-doi.org/101555/ijarp.8091

ABSTRACT

A novel and efficient UV spectrophotometric method has been developed for the simultaneous quantification of Erythromycin and Niacinamide in topical formulations. Characterization revealed Niacinamide as a white crystalline powder and Erythromycin as white to off-white crystals, both confirming purity. Solubility tests indicated Niacinamide's high hydrophilicity, while Erythromycin preferred organic solvents. The method demonstrated linearity in concentrations of 2–10 µg/ml, showing excellent correlation (R^2 values of 0.998 for erythromycin and 0.992 for niacinamide) and precision (RSD values < 2%). The FTIR analysis confirmed the chemical identities of both compounds. Additionally, limits of detection were established at 1.0837 µg/ml for Niacinamide and 2.1165 µg/ml for Erythromycin. This validated method is deemed suitable for routine quality control in pharmaceutical applications due to its accuracy, sensitivity, and reliability.

KEYWORDS: Erythromycin, Niacinamide, UV-spectrophotometry, Topical Gel, Method Validation, FTIR, Drug Formulation.

INTRODUCTION

A common macrolide antibiotic used in dermatological formulations to treat bacterial infections and acne is erythromycin. By attaching itself to the 50S ribosomal subunit, it prevents the synthesis of proteins. Niacinamide, also known as nicotinamide, is a water-soluble form of vitamin B3 that is frequently used in topical therapies and has anti-inflammatory and skin barrier-enhancing qualities. In order to improve therapeutic efficacy, erythromycin and niacinamide are commonly used in acne therapy. For estimation, analytical procedures like HPLC are frequently employed; however, these methods necessitate costly equipment and extended analysis times. For routine analysis, UV spectrophotometry provides an easy, quick, and affordable substitute. In accordance with ICH standards, the current work attempts to create and validate a UV spectrophotometric technique for the simultaneous measurement of niacinamide and erythromycin in topical formulations [1,2].

MATERIAL AND METHODS

Materials

Various analytical grade chemicals and reagents are utilized for development and evalution of quantitative estimation of erythromycin and niacinamide in topical formulation. These include Acetone (Finar), Methanol (Rankem), Petroleum ether (Rankem), DMSO (Rankem), Ethanol (Bio Liqua Pvt. Ltd.). Equipment's are Boiling water bath (Labico), Weighing Machine (Sirtech), UV visible spectrophotometer (Shimadzu-1700) Melting point apparatus (Amtech) FTIR (Perkin Elmer Spectrum BX), Hot air oven (Scientech), Digital pH meter (Lab man), Micropipette (Dragon lab), Ultrasonic bath (Biomall). All chemicals used were of analytical grade & equipment's are calibrated.

Methods

- **Organoleptic Study**

Organoleptic testing looks at a drug's appearance, texture, and smell. To assess the purity of medications, organoleptic testing is crucial. It uses uniform standards for assessment and inspection to guarantee drug quality. Standard medications were inspected for colour, smell, look, and condition [3].

➢ **Solubility study:** The greatest quantity of a drug that may dissolve in a solvent at a given temperature to produce a homogenous solution is known as solubility; this is best illustrated by the adage "like dissolves like." Solutions can be classified as unsaturated (having the capacity to dissolve additional solute), saturated (maximised solute quantity), or

supersaturated (unstable with excess solute). Solubility varies from highly soluble molecules like ethanol in water to less soluble ones like silver chloride. Based on the amount of solvent required to dissolve one gram of solute, the United States Pharmacopoeia (USP) divides solubility into seven categories, including extremely soluble, freely soluble, and essentially insoluble. Standard medications are tested for solubility using a variety of solvents, such as acetone and DMSO [4].

- **Melting point determination of standard drug:** The melting point is the temperature at which a solid converts to a liquid, specific to pure crystalline substances. Impurities can lower and broaden the melting range, useful for determining purity. In a melting point apparatus, a capillary tube with the sample is heated gradually, starting from 20°C below the expected range, to maintain thermal equilibrium and ensure accurate temperature readings with the thermometer [5].

- **pH determination of standard drug:** Determining pH is essential in medication analysis as it influences stability and solubility. It affects a medication solution's solubility, chemical stability, and physical stability. The pH meter, which uses an electrometric approach, measures acidity or alkalinity by detecting potential differences between a reference electrode and a pH-sensitive electrode submerged in the sample [6].

- **Identification of pure drug through FTIR:** A Perkin Spectrum BX spectrophotometer was utilized for analysis by creating a 50 mg pellet from dry potassium bromide and 2% powdered medication. A Thermo Nicolet 6700 FTIR spectrometer examined the pellets over a 400–4000 cm^{-1} wavelength range, confirming the structural integrity and authenticity of the pure medication samples through detailed molecular vibration information [7].

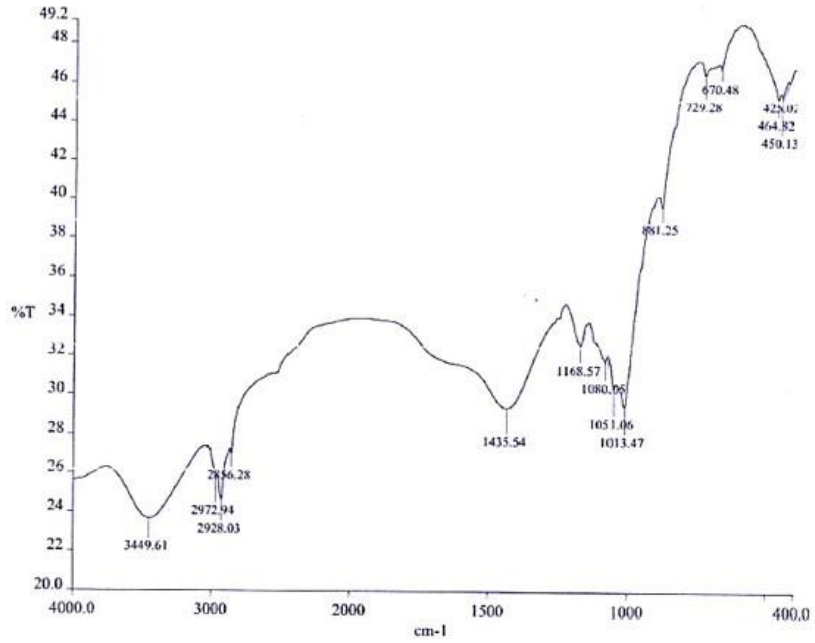


Figure 1: FTIR of pure Erythromycin Drug.

Table 1: FTIR Interpretation of Erythromycin

S.NO.	Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
1	3500- 3400 (cm^{-1})	3449.61	N-H stretching	primary amine
2	3000-2800 (cm^{-1})	2972.94	N-H stretching	amine salt
3	3000-2840 (cm^{-1})	2856.28	C-H stretching	alkane
4	1440-1395 (cm^{-1})	1435.54	O-H bending	carboxylic acid
5	1170-1155 (cm^{-1})	1168.57	S=O stretching	sulfonamide
6	1085-1050 (cm^{-1})	1080.06	C-O stretching	primary alcohol
7	1070-1030 (cm^{-1})	1051.06	S=O stretching	sulfoxide
8	730-665 (cm^{-1})	729.28	C=C bending	alkene

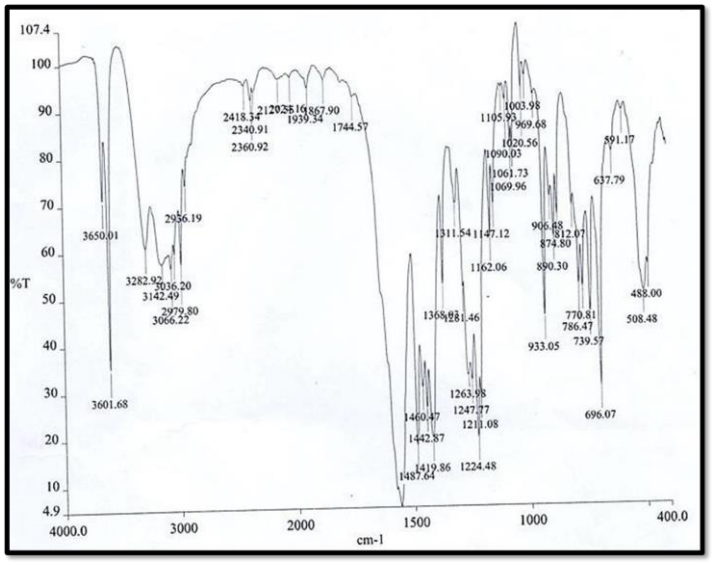


Figure 2: FTIR of pure Niacinamide Drug.

Table 2: FTIR Interpretation of Niacinamide.

S.NO.	Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
1	3700-3584 (cm^{-1})	3650.01	O-H stretching	alcohol
2	3400-3300 (cm^{-1})	3382.92	N-H stretching	aliphatic primary amine
3	3200-2700 (cm^{-1})	3142.49	O-H stretching	alcohol
4	3100-3000 (cm^{-1})	3066.22	C-H stretching	alkene
5	2140-2100 (cm^{-1})	2120.25	CΞC stretching	alkyne
6	1750-1735 (cm^{-1})	1744.57	C=O stretching	esters
7	1440-1395 (cm^{-1})	1419.86	O-H bending	carboxylic acid

- **Preparation of standard stock solution**

➢ **Primary stock solution:** The process to create a concentrated solution of erythromycin and niacinamide involves weighing 10 mg of each standard, transferring them to a 10 ml volumetric flask, and adding methanol to reach the desired volume. The solution is then sonicated for 15 minutes in a sonicator bath.

➢ **Sub stock solution:** The goal is to make a substock solution of erythromycin and niacinamide that can be further diluted into functional solutions. Method: 1 millilitre of basic stock solution to produce a concentration of 100µg/ml, niacinamide and erythromycin standard were pipetted and transferred to a 10 ml volumetric flask. The capacity was then made up with 10 ml of methanol.

➢ **Working standard solutions:** The aim is to create solutions with varying concentrations of niacinamide and erythromycin (2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml) by diluting a substock solution with methanol, facilitating the construction of a calibration curve [8].

- **Determination of wavelength of maximum absorbance (λmax):** UV-visible spectroscopy quantifies light absorption across wavelengths using visible and ultraviolet light (200-400 nm). This technique generates distinct spectra when a sample absorbs light, allowing determination of the drug's maximum absorption wavelength (λ max) through recorded absorbance spectra [9].

- **Method validation by UV Spectrophotometry:** Method validation involves procedures to confirm that an analytical technique is appropriate for a specific test. It ensures the

correctness of measurements and assesses the reliability, consistency, and quality of analytical data through validation outcomes.

- **Linearity and range:**

A. Niacinamide

UV-visible spectrophotometry was used to create the calibration curve for niacinamide across a concentration range of 2–10 µg/ml. Adherence to Beer-Lambert's law within the measured range was confirmed by the ensuing plot, which showed a strong linear association between absorbance and concentration.

Table 3: Calibration data of Niacinamide at 230.0nm.

S. No.	Concentration (µg/ml)	Absorbance 1 at 230.0nm	Absorbance 2 at 230.0nm	Absorbance 3 at 230.0nm	Mean Absorbance 230.0 nm
1	2	0.463	0.466	0.468	0.465
2	4	0.696	0.691	0.698	0.695
3	6	0.989	1.146	1.161	1.090
4	8	1.271	1.341	1.445	1.352
5	10	1.662	1.791	1.831	1.761
Mean					**1.0746**
SD					**0.053293152**
%RSD					**4.934**

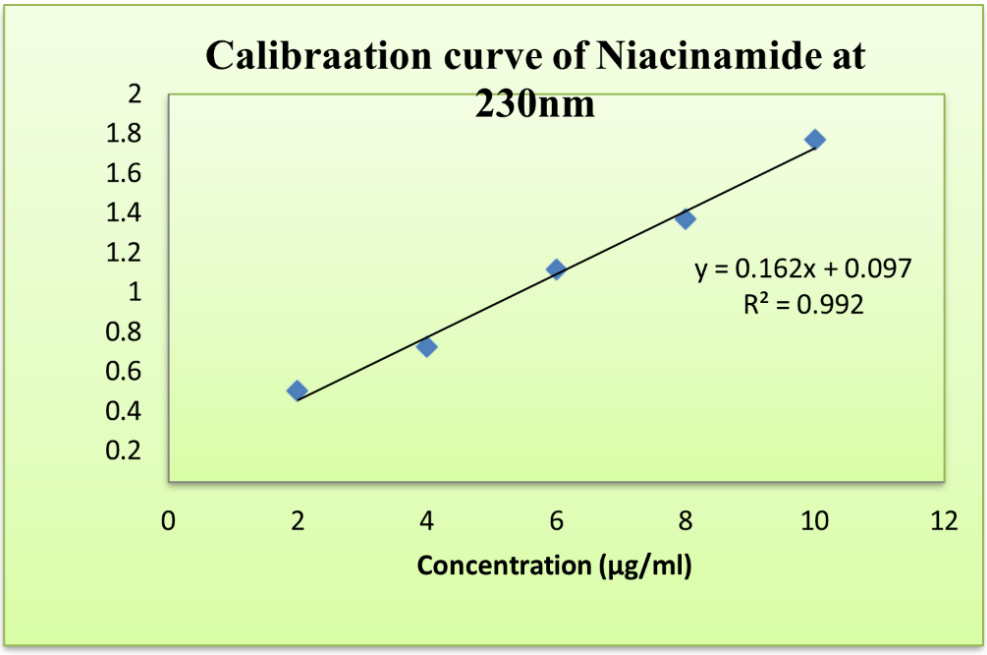


Figure 3: Calibration curve of Niacinamide at 230.0nm

B. Erythormycin

Table 4: Calibration data of Erythromycin at 218.0nm.

S. No.	Concentration (µg/ml)	Absorbance 1 at 218.0nm	Absorbance 2 at 218.0nm	Absorbance 3 at 218.0nm	Mean Absorbance 218.0 nm
1	2	0.08	0.098	0.095	0.090
2	4	0.121	0.145	0.165	0.143
3	6	0.185	0.198	0.214	0.199
4	8	0.234	0.268	0.295	0.265
5	10	0.292	0.371	0.298	0.320
Mean					**0.203933333**
SD					**0.018693671**
%RSD					**9.122**

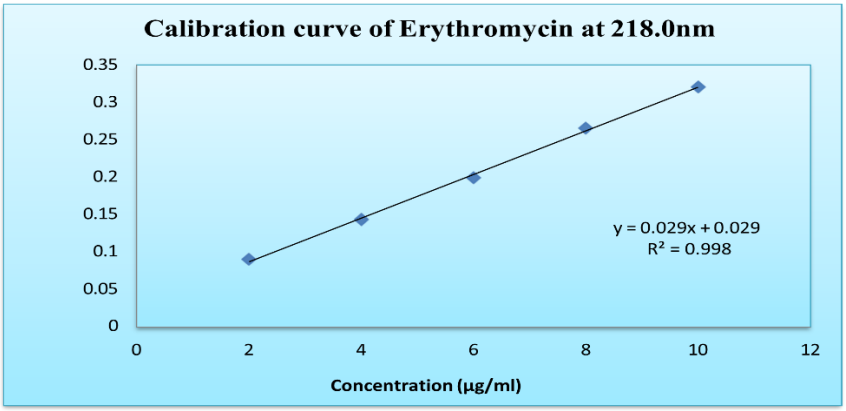


Figure 4: Calibration curve of Erythromycin at 218.0nm.

C. Combine Calibration curve of Niacinamide and Erythromycin

Table 5: Calibration data of Niacinamide and Erythromycin.

S. No.	Concentration (µg/ml)	Absorbance 218.0 nm
1	2	0.192
2	4	0.268
3	6	0.361
4	8	0.462
5	10	0.559
Mean		**0.203933333**
SD		**0.018693671**
%RSD		**9.122**

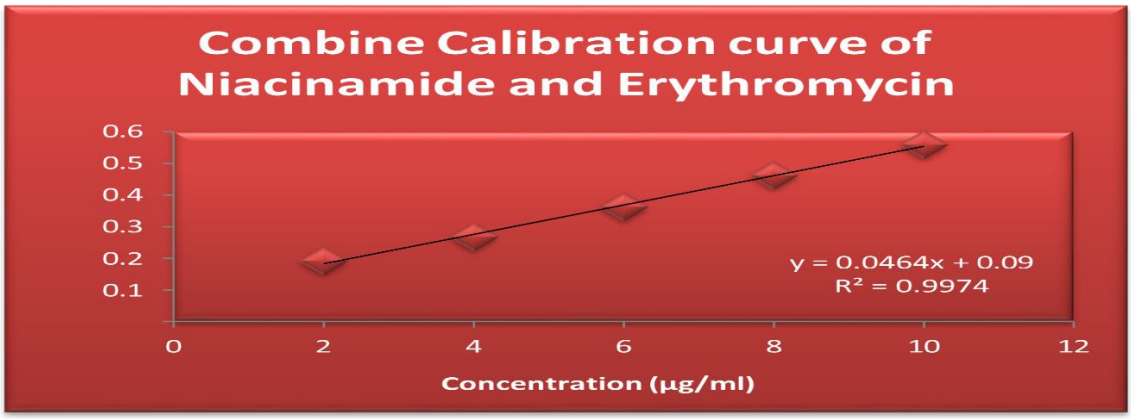


Figure 5: Combine Calibration curve of Erythromycin and Niacinamide at 227.0nm

- **Preparation of Drug loaded Topical gel:** In the preparation of a niacinamide and erythromycin-loaded topical gel, 1.0 g of Carbopol 940 and 1.10 g of carboxymethyl cellulose (CMC) were dispersed in distilled water (up to 100 mL) with stirring for hydration. To improve texture, 1.0 mL of propylene glycol was incorporated as a plasticiser and humectant, while 0.3 g of methylparaben was added as a preservative to prevent contamination. Subsequently, 100 mg of the drug-loaded gel was mixed into the gel base, followed by the gradual addition of triethanolamine to neutralise Carbopol and adjust the pH for safe topical application. The final gel was stored at 4 °C for further characterization and evaluation [10].

➢ **Composition of Topical gel formulation**

Sr. No.	Ingredients	Amount of gel formulation
1	Carbopol 940	1.0 g
2	Carboxymethyl Cellulose (CMC)	1.10 g
3	Propylene glycol	1.0 mL
4	Methyl Paraben	0.3g
5	Triethanolamine	q.s
6	Distilled Water	100 mL

➢ **Characterization parameter of Drug loaded topical gel**

Physical Appearance: The topical gel was assessed and found to be consistent, smooth, and visually appealing with a clear to slightly transparent appearance. It exhibited no lumps, air bubbles, or debris, indicating a homogeneous formulation. Its consistency was optimal for application, with no signs of phase separation or syneresis, and the color remained constant. Additionally, it had a neutral or pleasant smell, enhancing consumer acceptance. Overall, these qualities suggest that the formulation is stable and suitable for topical use [11].

Homogeneity of topical gel: All developed gels were visually inspected for homogeneity after setting in the container, focusing on their appearance and any aggregates present [12].

Viscosity of Topical gel: The rheological properties of topical gels containing niacinamide and erythromycin were evaluated by measuring their viscosity using a Brookfield viscometer. The process involved filling a sample cup with approximately 10g of gel and using spindle No. 4 at 60 rpm and 25 ± 1 °C. After stabilization, viscosity readings in centipoise (cP) were recorded three times for each formulation to ensure accuracy. The flow behavior, including shear thinning and gel consistency, was also observed, providing insights into the gels' application performance and spreadability [13].

pH determination of topical gel: To ensure skin compatibility and formulation stability, the pH of topical gels containing niacinamide and erythromycin was evaluated. Approximately 1g of gel was mixed with 10 mL of distilled water in a clean beaker to form a uniform dispersion. A calibrated digital pH meter was then used to measure the pH of the mixture, recording the reading after the electrode stabilized, while avoiding contact with the beaker's walls [14].

Spreadability of topical gel: The study evaluated the spreadability of a topical gel containing niacinamide and erythromycin using the slip and drag method. In this test, 1.0g of gel was placed between two glass slides, with a 50g weight applied for one minute. The spread diameter was then measured in centimeters, averaged over three trials for each formulation. A larger diameter indicates better spreadability, essential for comfort and effective drug delivery. Observations on stickiness and gel consistency were also recorded during testing [15].

RESULT AND DISCUSSION

The study focused on developing a topical gel formulation with erythromycin and niacinamide, including extensive analytical and physicochemical characterization. Organoleptic analysis showed erythromycin as a white to off-white and odorless solid, while niacinamide was identified as a white, odorless crystalline substance. Solubility tests revealed erythromycin's minimal solubility in water but good solubility in methanol and ethanol, and niacinamide's high solubility in methanol, water and PBS. Drug purity was confirmed through melting temperature measurements of erythromycin (139°C) and niacinamide (130°C) (Table 6), aligning with standard values, and FTIR analysis verified the presence of

distinctive functional groups in both compounds. Niacinamide and erythromycin had λmax values of 230 and 218 nm, respectively, with an isobestic point at 227 nm, according to UV spectrophotometric study (Figure 6). With correlation values of 0.992 for niacinamide and 0.998 for erythromycin, the calibration curves demonstrated outstanding linearity in the range of 2–10 µg/ml. Method repeatability was confirmed by precision experiments that showed low %RSD values (intraday: 0.159% and 0.378%; interday: 0.143% and 0.376%). Niacinamide's LOD and LOQ were determined to be 1.0837µg/ml and 3.2839µg/ml, whereas erythromycin's were 2.1165µg/ml and 6.4137µg/ml (Table 7). With a viscosity of 7526 ± 0.32 cps, a pH of 5.78, a spreadability of 6.0 g cm/sec, (Table 8) and no indications of skin irritation, the topical gel's formulation demonstrated satisfactory homogeneity.

Table 6: Melting point Niacinamide and Erythromycin.

S.No.	Drug	Reference Range	Observation
1	Niacinamide	128-131°C	130ºC
2	Erythromycin	135-145°C	139ºC

Table 7: Optical Characteristics and Validation Study of Drugs.

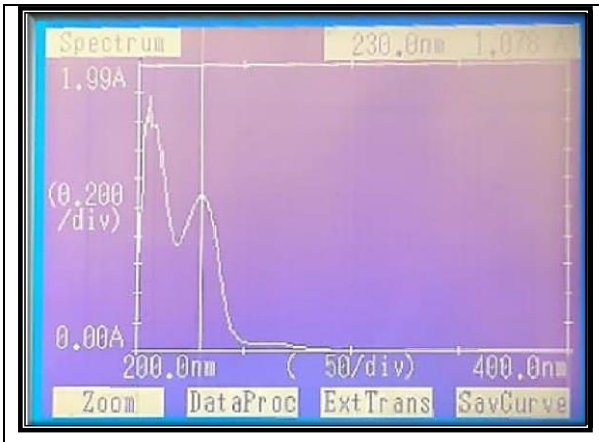
S.No	Parameters	Niacinamide	Erythromycin
1	Wavelength λ max nm	230.0nm	218.0nm
2	Beer's law limit µg/ml	2-10	2-10
3	Correlation coefficient (R^2)	R^2 = 0.992	R^2= 0.998
4	Slope	0.162	0.029
5	Intercept	0.097	0.029
6	SD	0.053293	0.018693
7	% RSD	4.934	9.122
8	Precision Intraday (% RSD) Interday (% RSD)	0.159 0.143	0.378 0.376
9	Repeatability (% RSD)	0.144	0.377
10	Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	0.143 0.144	0.378 0.760
11	Robustness Temp.25ºC (% RSD) Temp.45ºC (% RSD)	0.143 0.143	0.760 0.375
12	LOD (µg/ml)	1.0837	2.1165
13	LOQ (µg/ml)	3.2839	6.4137

Table 8: Characteristic of Topical Gel.

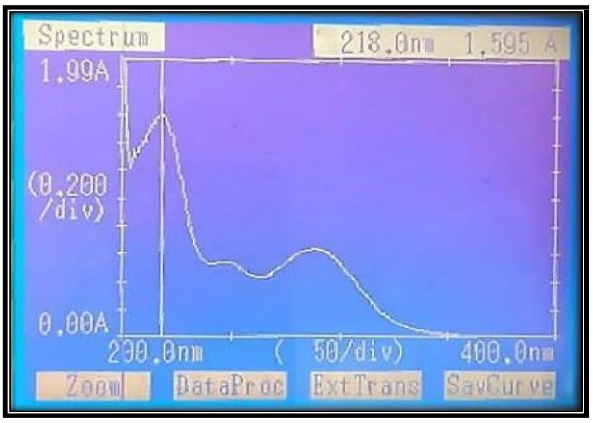
S .No.	Formulation	Parameters
Viscosity		
1	Topical gel	7526 ±0.32 cps
pH		
2	Topical gel	5.78pH
Spreadability		
3	Topical gel	6.0g-cm/sec

Figure 6: Determination of wavelength of maximum absorbance (λmax)

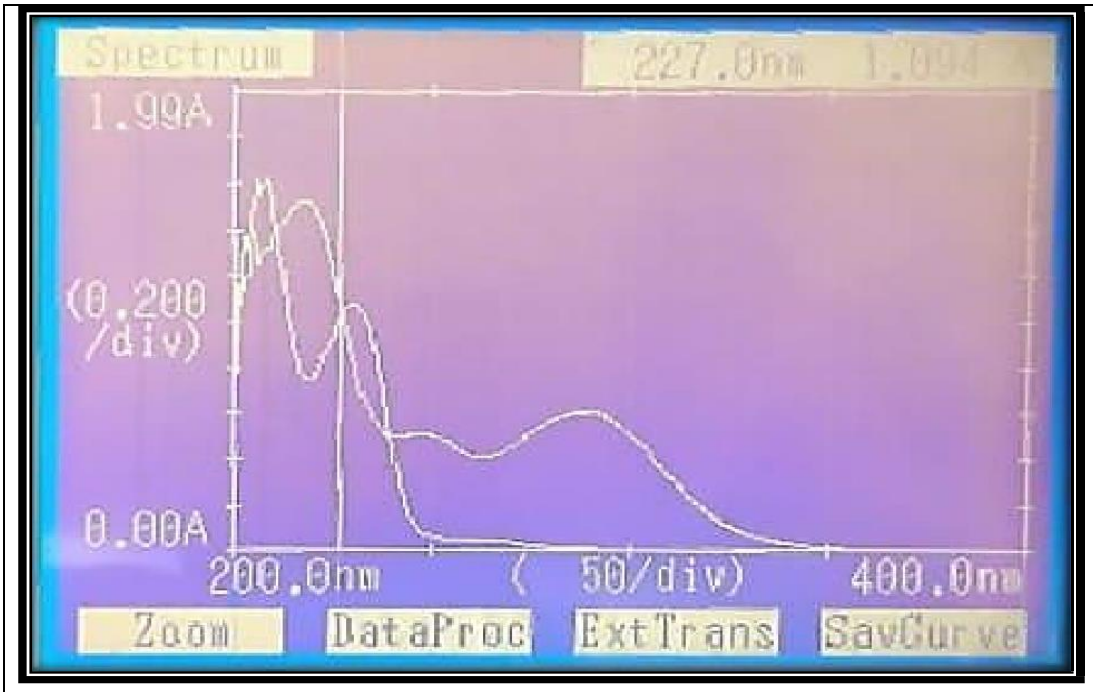
Spectrum scan on UV-Vis spectrophotometer (Shimatzu 1700) was done between the range of 200 .0nm-400 .0nm.



UV-Vis spectrum of Niacinamide



UV-Vis spectrum of Erythromycin



Overlay of Niacinamide and Erythromycin showing Isobestic point at 227.0nm

CONCLUSION:

To sum up, the new topical gel formulation with erythromycin and niacinamide showed good analytical and physicochemical qualities. With strong linearity (R^2 = 0.992 and 0.998), low %RSD values (<1%), and appropriate sensitivity (LOD: 1.0837 µg/ml and 2.1165 µg/ml; LOQ: 3.2839 µg/ml and 6.4137 µg/ml), the UV spectrophotometric technique was effectively validated. Effective and safe topical use was ensured by the formulation's ideal properties, which included appropriate viscosity (7526 cps), skin-friendly pH (5.78), good spreadability (6.0 g·cm/sec), and lack of irritation. As a result, the created formulation has strong therapeutic potential and patient compliance, and it is stable, dependable, and appropriate for dermatological usage.

REFERENCES

1. Penndorf R. Luminous and Spectral Reflectance as Well as Colors of Natural Objects:(Albedo and Color of Terrain Features). Geophysics Research Directorate, Air Force Cambridge Research Center; 1956.
2. Goetz AF, Davis CO. High resolution imaging spectrometer (HIRIS): science and instrument. International Journal of Imaging Systems and Technology. 1991 Jun;3(2):131 43.
3. Stéphane Guilbert, Nathalie Gontard. Agro-polymers for edible and biodegradable films: Review of agricultural polymeric materials, physical and mechanical characteristics. Food Science and Technology. 2005; 263-276
4. Stéphane Guilbert, Nathalie Gontard. Agro-polymers for edible and biodegradable films: Review of agricultural polymeric materials, physical and mechanical characteristics. Food Science and Technology. 2005; 263-276.
5. Yogesh S. Thorat*, Indrajeet D. Gonjari and Avinash H. Hosmani. Solubility Enhancement Techniques: A Review on Conventional and Novel Approaches. Ijpsr. 2011; Vol. 2(10): 2501-2513.
6. O'C. Young JC. True melting point determination. The Chemical Educator. 2013 Feb;18(1):203-8.
7. Amir M, Alam MA, Aftab MF, Nabi MG, Alam MS, Rathi J, Thakur SS. Effect of pH on Pharmaceutical Ingredients / Drugs / Chemicals, Asian Journal of Dental and Health Sciences. 2022; 2(3):9-11
8. Parmeshwar B. Karhale, Pratiksha R. Meshram et al. European Journal of Pharmaceutical and Medical Research. Ejpmr. 2025;12(7).

9. Khokrale, S., Lulla, J., & Borse, L. (). A review of UV-visible spectroscopy's method development and validation. International Journal of Pharmaceutical Sciences. 2024; 2(6).
10. Wasankar SR, Faizi SM, Deshmuk AD. Formulation and development of liposomal gel for topical drug delivery system. International journal of pharmaceutical sciences and research. 2012 Nov 1;3(11):4461
11. Hetrick EM, Vannoy J, Montgomery LL, Pack BW. Integrating tristimulus colorimetry into pharmaceutical development for color selection and physical appearance control: A quality-by-design approach. Journal of pharmaceutical sciences. 2013 Aug 1;102(8):2608-21.
12. Gupta A, Mishra AK, Singh AK, Gupta V, Bansal P. Formulation and evaluation of topical gel of diclofenac sodium using different polymers. Drug invention today. 2010 May 1;2(5):250-3.
13. Ji JA, Ingham E, Wang JY. Effect of EDTA and methionine on preventing loss of viscosity of cellulose-based topical gel. AAPS PharmSciTech. 2009 Jun;10(2):678-83.
14. Priya Pa H, Murthy PN, S G P, Matadh AV, Murthy SN, H N S, Maibach H, Murthy SN. Skin can modulate the pH of topical creams and gels. AAPS PharmSciTech. 2025 Jun;26(5):1-9. 7
15. Al-Barghouthy EY, Hamed S, Mehyar GF, AlKhatib HS. Comparative Evaluation of Spreadability Measurement Methods for Topical Semisolid Formulations/A Scoping Review. Gels. 2025 Dec 12;11(12):1006.