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Original Article

Unveiling a New Ultra Violet-Spectrophotometric Strategy for Simultaneous Quantification of Adapalene and Erythromycin in Powders and Nanocarriers

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ABSTRACT

Background: This study is aimed to establish a novel and reliable UV-visible spectrophotometric method for simultaneous quantification of Adapalene and Erythromycin. **Purpose:** There is no documented Ultra Violet analysis for these drugs together, especially in nanocarriers. This method is simple, rapid, selective, and precise, suitable for both bulk powders and drug-loaded nanocarriers. **Materials and Methods:** A Shimadzu Ultra Violet/Visible spectrophotometer model 1800, was used. The optimized analysis condition employed Oxolane: Ortho phosphoric acid (OPA) buffer (30:70% v/v) as the solvent. **Results and Discussion:** Erythromycin and Adapalene showed peak absorbance at 214 nm and 235 nm, respectively. The method exhibited a linear relationship between concentration and absorbance. Adapalene showed linearity between 0.1-0.5 µg/mL, and Erythromycin between 10-50 µg/mL. High regression coefficients (0.9985 for Adapalene, 0.9981 for Erythromycin) confirmed excellent quantification accuracy. **Conclusion:** The results confirmed that the excipients present in the nanoformulation did not interfere with the analytical method, indicating its suitability for routine quality control analysis of Erythromycin and Adapalene in both bulk drug substances and formulated products.

Keywords: Adapalene, Erythromycin, UV-visible Spectrophotometry, Simultaneous Estimation.

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INTRODUCTION

Acne vulgaris, a chronic skin condition, involves prolonged inflammation of the pilosebaceous unit, leading to both inflammatory and non-inflammatory lesions (Williams *et al.*, 2012). Long-term treatment, often lasting years, can impact mental health, causing depression and low self-esteem. Acne ranks among the world's ten most prevalent illnesses (Chilicka *et al.*, 2023).

Adapalene (Figure 1A) is known by the chemical name 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid. The FDA has approved the topical retinoid adapalene to treat acne vulgaris. Retinoids are vitamin A derivatives that are usually expressed as generations with higher affinity for the Retinoic Acid Receptor (RAR) with later generations (Arooj *et al.*, 2023). IUPAC name of Erythromycin (Figure 1B) is (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-(((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-14-ethyl-7,12,13-trihydroxy-4(((2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione. Erythromycin, a macrolide antibiotic discovered in 1952, treats both infectious and non-infectious diseases. As a bacteriostatic agent, it inhibits bacterial growth rather than killing bacteria. It works by binding to the 50S ribosomal subunit, blocking protein synthesis in susceptible microorganisms (Sayyafan *et al.*, 2020).

Combination therapy with Adapalene and Erythromycin targets multiple aspects of acne pathogenesis-inflammation and bacterial resistance-unlike monotherapy (Dabade *et al.*, 2011). Adapalene's major side effects include scaling, pruritus, erythema, dryness, and a persistent burning sensation, known as the retinoid reaction (Waller and Sampson, 2018). The major drawback of topical Erythromycin's main drawback is bacterial resistance, which combination therapy helps minimize (Platon *et al.*, 2022). Cubosome-based formulations improve drug bioavailability and provide controlled release, reducing side effects. Cubosomes are non-toxic, inert, non-irritant, aesthetically pleasing, and cosmetically acceptable (Sivadasan *et al.*, 2023).

For accurate standardization and quantification of APIs, excipients, degradation products, and finished formulations, a

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precise analytical method is essential (Sharma *et al*., 2018). Several techniques exist for individual quantification of Erythromycin and Adapalene, including UV spectroscopy (Rattanapoltaveechai *et al*., 2007), HPLC-MS (Roy *et al*., 2015), RP-HPLC (Martins *et al*., 2011; Rathore *et al*., 2010), HPTLC[1] (Thummar *et al*., 2020; Tsuji and Robertson, 1971), GC (Rozet *et al*., 2007). However, no UV spectrophotometric method for simultaneous quantification of both drugs in bulk or combined formulations has been reported. This research aimed to develop and validate a simple, rapid, sensitive, accurate, and precise UV spectrophotometric method suitable for routine quality control analysis of Adapalene and Erythromycin.

MATERIALS AND METHODS

Materials

Empree Medicaments INDIA Pvt. Ltd., Belagavi supplied Erythromycin and Adapalene drug. Merk India Limited traded the HPLC grade Acetonitrile. This research work employed only pure chemicals and reagents, including analytical grade triethylamine and orthophosphoric acid. A Shimadzu UV/visible spectrophotometer (UV-Probe, model 1800) with automatic wavelength correction and 10 mm Quartz cuvettes was used. Sample weighing was done using a Systronic electronic analytical balance. All measuring equipment, including cylinders, volumetric flasks, and pipettes, were calibrated before use to ensure accuracy.

Method Development

The UV spectrophotometric method begins with selecting a solvent system and determining its maximum absorption wavelength (λ_{max}). After a literature review, solubility studies for Adapalene and Erythromycin were conducted. Various trials in Methanol, Oxolane, Acetonitrile, and Ortho phosphoric acid led to the final mobile phase: Oxolane and OPA buffer (30:70% v/v). UV spectra of both analytes, individually and combined, were scanned within the 800-200 nm range.

Method Validation

Following ICH guidelines Q2A and Q2B, the analytical method was validated by assessing linearity, accuracy, precision, and ruggedness. These evaluations confirmed the optimized method's suitability by verifying its constraints (Ferenczi-Fodor *et al*., 2001; Mishra and Mundada, 2021).

Preparation of Solvent system

Mobile phase

0.1 mL Orthophosphoric acid and 0.5 mL Triethylamine was dissolved in 100 mL distilled water to prepare OPA buffer. In 70 mL of prepared OPA buffer 30 mL of accurately measured Oxolane was dissolved.

Standard Stock Solution

The preparation of standard stock solutions involved the use of separate 10 mL volumetric flasks containing 10 mg of Erythromycin and Adapalene, which were weighed and mixed with Oxolane to produce 1000 µg/mL stock solution.

Working stock solutions

A 10 mL volumetric flask was used to dilute 0.1 mL of Adapalene and 1 mL of Erythromycin stock solutions, then filled to volume with the mobile phase. Additional concentrations (0.1-0.5 mL Adapalene, 1-5 mL Erythromycin) were pipetted into the same flasks and topped up with the mobile phase.

Selection of wavelength

10 µg/mL of respectively stock solution was examined in the range of 400-200 nm for Erythromycin and Adapalene using OPA buffer as blank solution.

Specificity and selectivity

By scanning both the mobile phase and drug solutions, UV spectra were obtained between 400-200 nm to assess the identity of an analyte among a mixture of similar components in a sample.

Linearity and range

The experiment analyzed absorbance in relation to Adapalene and Erythromycin concentrations. Drug mixtures (0.1-0.5 mL Adapalene, 1-5 mL Erythromycin) created various concentrations (1-5 µg/mL Adapalene, 10-15 µg/mL Erythromycin) and were scanned. This process was repeated three times to ensure consistency. Statistical regression assessed whether absorbance changes correlated with drug concentration (Sanchez, 2018).

Limit of Detection and Limit of Quantification

As per the ICH guidelines: LOD, also known as the Detection Limit (DL), can be computed as LOD=3.3σ/S, whereas the Quantitation Limit (QL), also known as the limit of quantification, is LOQ=10σ/S. S denotes the slope of this calibration curve, and σ represents the standard deviation of that response (Varachhiya *et al*., 2019).

Precision

Reproducibility of the analytical method was evaluated via interday and intraday precision by analyzing three replicates of 0.1, 0.3, and 0.5 mL Adapalene with 1, 3, and 5 mL Erythromycin at their respective λ_{max}. Percentage RSD was calculated for each drug (Pokala *et al*., 2018).

Ruggedness

The method's capability to withstand slight and deliberate modifications is referred to as its robustness. In the current study, triplicate readings were measured on a different UV system for 3

different concentrations at the peak absorbance of Adapalene and Erythromycin (Friedrich *et al*., 2009).

Robustness

The UV spectrophotometry method's robustness was tested by analyzing Adapalene and Erythromycin at 214 nm±2 nm and 235 nm±2 nm. Multiple regression equations and coefficients assessed its performance under varying conditions (Jain *et al*., 2011).

Accuracy (Recovery studies)

To assess the accuracy of the analysis, researchers performed a recovery study. This involved adding standard solutions of the drugs at three different concentration levels (80%, 100%, and 120%) to a sample pre-analysed sample (Uday *et al*., 2021).

Solvent and standard stock solution stability

The experiment assessed the stability of the solutions by storing them under unstable conditions for 72 hr (3 days). After this period, they evaluated whether the solutions remained unchanged (Nagao *et al*., 2023).

Formulation of Cubosomal Dispersion

Cubosomes were prepared using the Top-Down approach, where Glyceryl Monooleate (GMO) and Pluronic F-127/Poloxamer 407 were melted at 40ºC. Erythromycin and Adapalene were then incorporated, followed by mixing with melted Pluronic F-127. Warm water was added dropwise under constant stirring, and sonication ensured uniform dispersion. The entrapped drug was then estimated.

Characterization of Cubosomal Dispersion

The drug-loaded cubosomal dispersion was diluted with distilled water to obtain suitable concentrations for analysis. Dynamic Light Scattering (DLS) was performed using a Malvern Zetasizer to determine the Polydispersity Index (PDI) and zeta potential of the formulation. All measurements were conducted in triplicate to ensure reproducibility and reliability of the data.

Separation of Unentrapped Drug from Cubosomes

A 2.5 mL aliquot of drug-loaded cubosomal dispersion was centrifuged at 15,000 rpm for 30 min at 4ºC using a KUBOTA-7000 refrigerated centrifuge. After separation, 0.1 mL of the supernatant, containing free drug, was diluted to 10 mL with Milli-Q water. To remove residual cubosomal particles, the diluted supernatant was filtered through a Millex® PTFE syringe filter.

Quantification of Encapsulated Drug UV Spectrophotometrically

The filtered solution was analyzed spectrophotometrically at 214 nm for Adapalene and 235 nm for Erythromycin. A blank cubosomal dispersion served as the reference for background subtraction. Standard calibration curves determined the concentration of encapsulated drugs. Entrapment efficiency was calculated using the given formula:

$$\%EE = \frac{Total\ amount\ of\ drug\ added - Amount\ of\ drug\ in\ the\ supernatant}{Total\ amount\ of\ drug\ added} \times 100$$

RESULTS

Method Validation

Adapalene and Erythromycin showed maximum absorption at 214 nm and 235 nm, and is represented in Figure 2 respectively.

Linearity

The experiment confirmed a linear relationship between drug concentration and absorbance for both Adapalene (ADP) and Erythromycin (ERY) with the of 0.1-0.5 µg/mL for ADP (Figure 3) and 10-50 µg/mL for ERY (Figure 3). This held true at both analyzed wavelengths (214 nm and 235 nm). Interestingly, the conclusion is further supported by the high correlation coefficients (R^2) obtained: 0.9985 for ADP and 0.9981 for ERY.

LOD and LOQ

This research evaluated the analytical method's sensitivity using Limit of Detection (LOD) and Limit of Quantitation (LOQ).

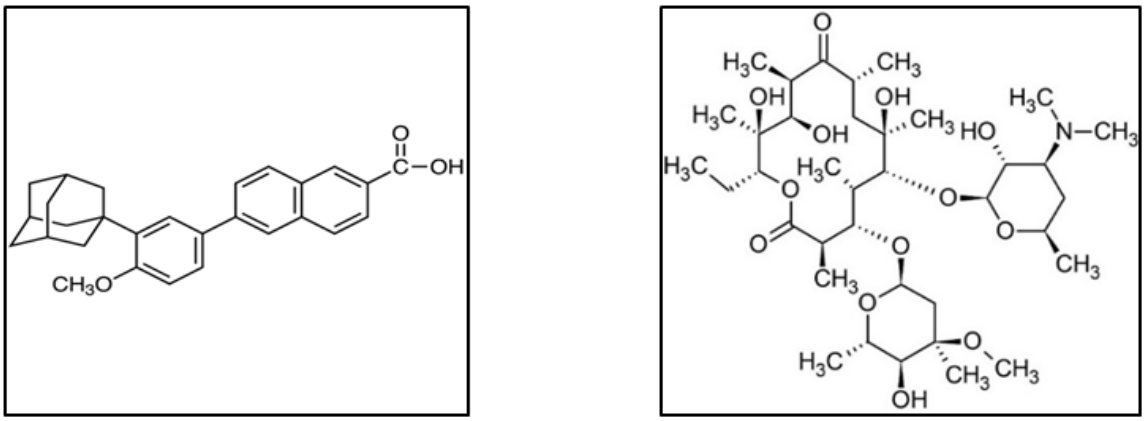

Figure 1: Chemical structure of A) Adapalene B) Erthromycin.

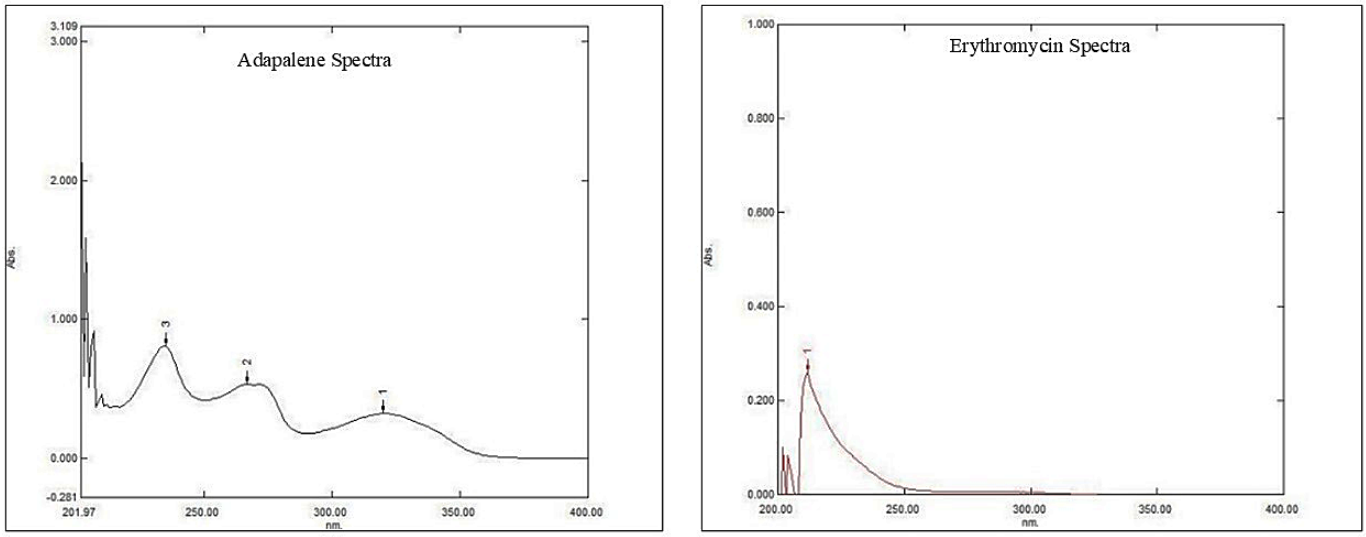

Figure 2: UV-Spectrum of Adapalene and Erythromycin.

Table 1: System Precision and Intraday Precision.

Concentration (µg/mL)	Erythromycin Absorbance (Avg.)	Erythromycin % RSD	Adapalene Absorbance (Avg.)	Adapalene % RSD
System Precision				
10	0.193-0.194	0.298-0.299	0.122-0.123	0.471-0.472
30	0.347-0.348	0.166	0.205-0.206	0.280-0.281
50	0.518	0.223	0.291-0.292	0.198
Intraday Precision (Morning, Afternoon, Evening)				
10	0.193-0.194	0.298-0.299	0.122-0.123	0.471-0.472
30	0.347-0.348	0.166	0.205-0.206	0.280-0.281
50	0.518	0.223	0.291-0.292	0.198

LOD is the lowest detectable amount of a drug, while LOQ is the minimum concentration measurable with accuracy. The LOD for Erythromycin was 1.11 µg/mL and for Adapalene 0.02 µg/mL, while LOQ was 3.37 µg/mL for Erythromycin and 0.06 µg/mL for Adapalene.

Precision

The analysis method demonstrated good precision, as the percent Relative Standard Deviation (%RSD), calculated for three repeated measurements (replicates) at three different concentrations for both Adapalene and Erythromycin, consistently fell below 2%. Detailed results are presented in Tables 1 and 2.

Ruggedness

Method's Ruggedness was assessed by measuring the %RSD for Adapalene and Erythromycin under different analysts and instruments. The %RSD values remained below 2% for all replicates, indicating the method's robustness and reproducibility. These findings are detailed in Table 3.

Robustness

To assess robustness, the analysis method underwent slight variations. The %RSD values calculated from the resulting data (regression coefficients) were all below 2%. These results, detailed in Table 4, demonstrate that the method remains unaffected by minor changes.

Accuracy (recovery studies)

Accuracy was tested by spiking samples and measuring recovery. Recoveries for Adapalene (98.98-100.23%) and Erythromycin (98.77-100.14%) were excellent.

Solvent and standard stock solution stability

To confirm that the working solutions remained stable and were not degraded in the diluent medium during the analysis, solution stability studies were performed. The %RSD values, derived from regression coefficients, were all found to be below 2%.

Characterization of Cubosomal Dispersion

The particle size distribution, centered at 113.6 nm, is ideal for cubosomal formulations. A PDI of 0.489 indicates uniformity,

while a zeta potential of -28.08 mV ensures colloidal stability by preventing aggregation. TEM imaging (Figure 4) confirms smooth, oval-shaped particles.

Spectrophotometric Analysis of Cubosomes

Analysis of the final cubosome dispersion revealed high Entrapment Efficiencies (EE) of 85.2% and 86.2% for Erythromycin (ERY) and Adapalene (ADP), respectively. These values indicate successful encapsulation of a significant portion of both drugs within the cubosomes.

DISCUSSION

The drugs' absorptivity remained stable within 0.1-0.5 µg/mL for Adapalene and 10-50 µg/mL for Erythromycin, confirming Beer-Lambert's Law. High R^2 values (0.9985 for Adapalene, 0.9981 for Erythromycin) indicate strong linearity. LOQ exceeded LOD, highlighting the need for sensitivity and precision. Results were consistent across concentrations, demonstrating precision. The method showed ruggedness under slight lab variations. %RSD remained below 2%, confirming robustness and repeatability. The validation characteristics and results you described-such as linearity, precision, accuracy, robustness, ruggedness, LOD/LOQ, and compliance with Beer-Lambert's Law-are directly aligned with internationally recognized regulatory guidelines for analytical method validation (ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1) Retrieved from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2014). Recovery studies demonstrated the method's accuracy, with high recovery rates for Adapalene (98.98-100.23%) and Erythromycin (98.77-100.14%). Solution stability confirmed no degradation of working solutions, ensuring reliable results. Cubosomes showed high entrapment efficiency-85.2% for Erythromycin and 86.2% for Adapalene-demonstrating their potential for targeted drug delivery and efficient transport to specific sites (Boyd *et al.*, 2009).

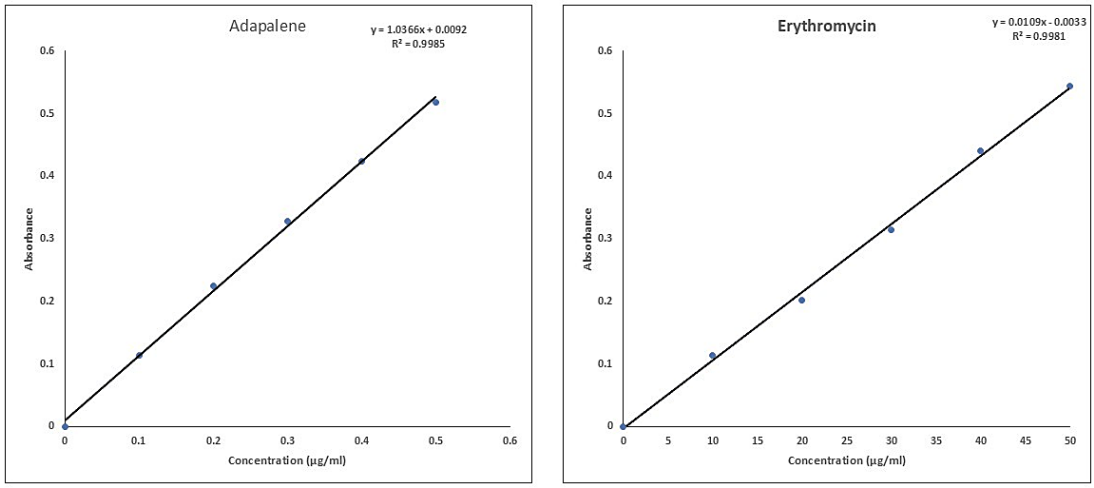

Figure 3: Standard Calibration Curve for Adapalene and Erythromycin.

Table 2: Interday Precision data for Adapalene and Erythromycin.

Concentration (µg/mL)	Erythromycin Absorbance (Avg.)	Erythromycin % RSD	Adapalene Absorbance (Avg.)	Adapalene % RSD
Day 1				
10	0.193	0.299	0.122	0.472
30	0.348	0.166	0.206	0.281
50	0.519	0.111	0.292	0.198
Day 2				
10	0.194	0.298	0.123	0.471
30	0.347	0.166	0.206	0.281
50	0.518	0.111	0.291	0.198
Day 3				
10	0.194	0.298	0.122	0.472
30	0.348	0.166	0.206	0.281
50	0.519	0.111	0.292	0.198

Table 3: Ruggedness data of Erythromycin and Adapalene Change in Instrument and Analyst.

Concentration (µg/mL)	Erythromycin Absorbance (Avg.)	Erythromycin % RSD	Adapalene Absorbance (Avg.)	Adapalene % RSD
Instrument Change				
10	0.193	0.518	0.124	0.806
30	0.346	0.289	0.206	0.743
50	0.514	0.297	0.293	0.522
Analyst Change				
10	0.193	0.299	0.123	0.471
30	0.348	0.166	0.205	0.281
50	0.519	0.111	0.291	0.198

Table 4: Robustness data of Erythromycin and Adapalene.

Concentration (µg/mL)	Erythromycin Absorbance (nm)			Concentration (µg/mL)	Adapalene Absorbance (nm)		
	212	214	216		235	237	233
30	0.345	0.348	0.349	0.3	0.205	0.206	0.208
30	0.344	0.348	0.349	0.3	0.205	0.206	0.209
30	0.345	0.347	0.348	0.3	0.204	0.205	0.209
Average	0.345	0.348	0.349	Average	0.205	0.206	0.209
%RSD	0.168	0.166	0.166	%RSD	0.282	0.280	0.277

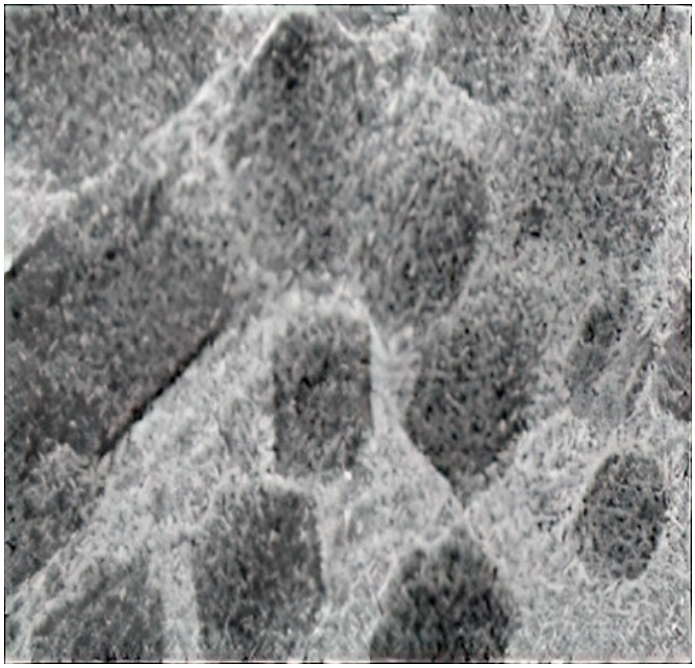

Figure 4: TEM Analysis of Drug Loaded Cubosomal Dispersion.

CONCLUSION

The UV-Spectrophotometry method was designed and validated for detecting Erythromycin and Adapalene in bulk drugs and cubosomal dispersion. Detection wavelengths were set at 214 nm for Adapalene and 235 nm for Erythromycin, using Oxolane: OPA buffer (30:70% v/v) as the solvent. All validation parameters met ICH standards, confirming the method's suitability for routine quality control (Skoog *et al.*, 2008).

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ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **UV:** Ultra-violet; **HPLC-MS:** High Performance Liquid Chromatography-Mass Spectroscopy; **RP-HPLC:** Reverse phase-High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **GC:** Gas Chromatography; **ICH:** International Council on Harmonisation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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