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COMPARATIVE REVIEW OF RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ADAPALENE, BENZOYL PEROXIDE AND RELATED FORMULATIONS

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ABSTRACT

Analytical determination of adapalene, benzoyl peroxide, and related compounds has gained significant importance due to their widespread use in dermatological formulations and regulatory monitoring in food and pharmaceutical matrices. The reviewed literature demonstrates that reverse-phase high-performance liquid chromatography (RP-HPLC) remains the most widely applied technique for their simultaneous estimation in gels, bulk drug substances, and flour samples. Most reported methods employ C8 or C18 stationary phases with UV or PDA detection and show satisfactory validation characteristics, including good linearity, precision, accuracy, robustness, and specificity in accordance with ICH guidelines. Stability-indicating approaches have also been developed to separate active components from excipients, preservatives, and stress-induced degradation products, particularly in combination therapies containing adapalene, clindamycin, and benzoyl peroxide. Despite these advances, existing methods predominantly rely on conventional solvent-intensive chromatographic systems and lack comprehensive impurity profiling or bio-analytical applications. Future research should therefore focus on the integration of hyphenated detection techniques, chemometric optimization strategies, and green analytical approaches to enhance sensitivity, sustainability, and regulatory applicability. Collectively, the reviewed studies confirm the reliability of RP-HPLC for routine analysis while highlighting the need for more advanced, stability-focused, and formulation-oriented analytical methodologies.

KEYWORDS: Comparative, RP-HPLC, Adapalene, Benzoyl peroxide, Method Development.

INTRODUCTION

Acne vulgaris is one of the most prevalent dermatological disorders worldwide, and its management commonly involves topical combination therapies containing retinoids, antimicrobial agents, and keratolytic compounds. Among these, adapalene, a third-generation synthetic retinoid, and benzoyl peroxide, a widely used antibacterial and keratolytic agent, are frequently employed either individually or in fixed-dose combinations for the treatment of mild to moderate acne. Their complementary mechanisms of action, including regulation of keratinization, anti-inflammatory effects, and reduction of microbial load, make them essential components of modern dermatological therapy. In addition to pharmaceutical applications, benzoyl peroxide is also used in food processing as a flour bleaching agent, necessitating reliable analytical monitoring for safety and regulatory compliance.[1,2]

Accurate quantification of these compounds in pharmaceutical formulations, biological matrices, and food products is therefore critical for quality control, stability assessment, and regulatory evaluation. Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as the most widely applied analytical technique for this purpose due to its high sensitivity, selectivity, reproducibility, and compatibility with complex matrices. Numerous studies have reported validated RP-HPLC methods for the estimation of adapalene and benzoyl peroxide in gels, bulk drug substances, and food samples, often demonstrating satisfactory performance in terms of linearity, precision, accuracy, robustness, and specificity. Recent investigations have also focused on stability-indicating methods capable of separating active pharmaceutical ingredients from degradation products, preservatives, and excipients in multi-component topical formulations.[3]

Despite these advancements, the available literature remains fragmented, with variations in chromatographic conditions, detection approaches, and analytical objectives across different studies. Moreover, emerging requirements for greener analytical methods, impurity profiling, and bio-analytical applications highlight the need for a comprehensive synthesis of existing research. Therefore, the present review aims to critically evaluate the reported analytical methods for adapalene, benzoyl peroxide, and related compounds, comparing chromatographic strategies, validation outcomes, and practical applications while identifying current limitations and future research directions in pharmaceutical and food analysis

Harikishor Barange (2018) et al.[4]

The study conducted by Harikishor Barange et al. (2018) focused on the development and validation of a simple, rapid, and reliable RP-HPLC method for the simultaneous estimation of adapalene and benzoyl peroxide in topical gel formulation. The authors used a C8 column with an isocratic mobile phase consisting of acetonitrile and methanol (90:10), detected at 245 nm. The method showed good chromatographic separation with retention times around 3.7 min for benzoyl peroxide and 5.8 min for adapalene. Validation results demonstrated excellent linearity, precision, accuracy, and robustness, with recovery values close to 100% and %RSD below 2%, indicating high reproducibility. The limits of detection and quantification confirmed adequate sensitivity for routine analysis. The study concluded that the developed method is suitable for quality control testing, assay determination, and simultaneous estimation of both drugs in pharmaceutical formulations, making it useful for industrial and laboratory applications.

Dr Abhilash Babu G(2023) et al.[3]

In another study, Babu et al. (2023) developed an isocratic HPLC method for the determination of benzoyl peroxide in flour products to monitor food safety and regulatory compliance. The method employed a C18 column with a water–

acetonitrile mobile phase and UV detection at 235 nm. The method showed excellent linearity across the tested concentration range, along with good accuracy, precision, and selectivity. Recovery experiments and stability testing demonstrated that the method was suitable for routine laboratory monitoring of benzoyl peroxide residues in food matrices. This study highlights the broader application of HPLC techniques not only in pharmaceuticals but also in food quality assessment and regulatory monitoring**.**

N. Shravya[1], P. Mary (2021) et al.[5]

The study by N Shravya et al. (2021) focused on the development and validation of a simple, accurate, and economical RP-HPLC method for the simultaneous estimation of adapalene and benzoyl peroxide in bulk drug and gel formulation. Chromatographic separation was achieved on a Symmetry C18 column using a mobile phase consisting of phosphate buffer (pH 3) and methanol (30:70 v/v) with a flow rate of 1 mL/min and PDA detection at 254 nm, as described in the chromatographic conditions on page 2. The optimized method produced sharp and well-resolved peaks with retention times around 2.97 min for adapalene and 3.55 min for benzoyl peroxide. Validation studies performed according to ICH guidelines demonstrated excellent linearity within the studied range, high precision (%RSD < 2%), and recovery values close to 100%, indicating good accuracy. Sensitivity results presented in Table 9 on page 7 showed LOD values of 0.3 µg/mL for adapalene and 1.2 µg/mL for benzoyl peroxide, confirming the method's suitability for low-level detection.

Robustness testing under varied flow rate, temperature, and mobile-phase composition showed no significant changes in chromatographic performance, proving the reliability of the method. The authors concluded that the developed RP-HPLC method is suitable for routine quality-control analysis, assay determination, and simultaneous estimation of adapalene and benzoyl peroxide in pharmaceutical dosage forms.

Ranjith et al. (2024) et al.[2]

The study by Ranjith et al. (2024) focused on the development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of clindamycin phosphate, adapalene, and preservatives in topical gel formulation. The chromatographic separation was achieved using a C18 column with a mobile phase consisting of acetonitrile and buffer at acidic pH, with detection at 210 nm for clindamycin and preservatives and 321 nm for adapalene. The optimized method produced well-resolved peaks with acceptable retention time and system suitability parameters, as shown in the chromatographic conditions table on page 5. Validation studies demonstrated good linearity ($r^2 \approx 0.999$), high accuracy with recovery values around 98–102%, and excellent precision with %RSD values below 2%, confirming the reliability of the method. Forced degradation studies under acid, base, peroxide, thermal, humidity, and photolytic conditions proved that the method is stability-indicating and capable of separating degradation products from the drug peak. The authors concluded that the developed RP-HPLC method is simple, accurate, precise, and suitable for routine quality-control testing, stability studies, and pharmaceutical analysis of clindamycin-containing gel formulations.

Yi-Cheng Chen2015 et al.[6]

The study by **Chen et al. (2015)** aimed to develop and optimize a reliable RP-HPLC method for the simultaneous determination of adapalene and benzoyl peroxide in pharmaceutical formulations using response surface methodology (RSM). The chromatographic separation was performed on a C18 column with detection at 270 nm and a flow rate of 1 mL/min, while the mobile phase composition was statistically optimized using tetrahydrofuran, acetonitrile, and aqueous acetic acid. The optimized mobile phase (25:50:25) produced well-resolved peaks with acceptable tailing

factors and retention times, as shown in the chromatographic optimization results on pages 4–5. Method validation demonstrated excellent specificity with no interference from excipients, strong linearity with correlation coefficients above 0.999, and good precision with %RSD values below 1%. Accuracy and recovery studies reported recoveries above 97%, confirming the reliability of the method. Robustness testing indicated that small variations in wavelength, flow rate, and solvent composition did not significantly affect chromatographic performance. The method was successfully applied to the analysis of a commercial gel formulation, confirming its suitability for routine quality-control testing and pharmaceutical analysis of combination acne therapy products.

Comparative Analysis of Chromatographic Conditions and parameters

Parameter	Harikishor Barange et al (2018)	Dr Abhilash Babu et al G (2023)	N. Shravya[1], P. Mary et al(2021)	K. Ranjith1, et al (2024)	Yi-Cheng Chen et al (2015)
Column	C8 (250×4.6 mm, 5µm)	C18 (250×4.6 mm, 5µm)	C18 (250×4.6 mm, 5µm)	LiChroCart–Lichrospher B250 mm × 4.6mm(Particle size: 5.0 µm)	LiChrosorb C18 (250 × 4.6 mm)
Mobile Phase	ACN:Methanol (90:10)	Water:ACN (45:55)	Buffer:Methanol (30:70)	Acetonitrile: Buffer 225 :775 (v/v) equivalent to 25 : 75	Acetonitrile : Tetrahydrofuran Water (0.1% acetic acid) 25 : 50 : 25 (v/v/v)
Flow Rate	1.0 mL/min	1.5 mL/min	1.0 mL/min	1.0 mL/min	1.0 mL/min
Wavelength	245 nm	235 nm	254 nm	210 nm	270 nm
Injection	20 µL	20 µL	20 µL	20 µL	20 µL
RT (BP)	3.7 min	13.8 min	3.5 min	7.min	15 min
RT (ADA)	5.8 min	--	2.9 min	--	4.2 min
Run Time	8-10 min	20 min	25 min	25 min	15min

A comparative evaluation of the reviewed RP-HPLC methods indicates that chromatographic optimization across studies consistently aimed to achieve rapid separation, symmetrical peaks, and acceptable system suitability parameters while maintaining robustness and reproducibility. Most methods employed reversed-phase columns, particularly C8 or C18 silica-based stationary phases, reflecting their suitability for moderately lipophilic dermatological drugs such as adapalene, benzoyl peroxide, and clindamycin.[2,4] The selection of stationary phase was primarily guided by analyte hydrophobicity and required resolution, with C18 columns offering stronger retention for non-polar compounds, while C8 phases allowed faster elution and shorter analysis times.[4]

Mobile phase composition showed strong similarity across studies, with acetonitrile emerging as the dominant organic modifier due to its low viscosity, high elution strength, and compatibility with UV detection. It was frequently combined with methanol or aqueous buffers to fine-tune selectivity and retention.[3,4] Buffered systems with acidic pH values around 2.5 were commonly adopted, particularly in stability-indicating assays, as they suppressed ionization, improved peak symmetry, and enhanced reproducibility.[2] Diluents were usually matched to the mobile phase composition to ensure analyte solubility and minimize chromatographic distortion.[4]

Flow rates in most methods were maintained near 1.0 mL min^{-1}, although some studies used slightly higher rates to shorten run time while preserving resolution.[3,4] Column temperatures were either ambient or moderately controlled (approximately 25–40 °C), indicating that thermal control was used mainly to maintain reproducibility rather than to

influence selectivity.[3] Injection volumes of 20 µL were consistently reported, suggesting this volume provides optimal balance between sensitivity and peak shape without causing column overload.[2,4]

Detection was predominantly carried out using UV or PDA detectors due to the aromatic chromophores present in the analytes. Wavelength selection depended on the absorbance maxima of the individual drugs, with values around 210–245 nm commonly used to achieve sufficient sensitivity[2,4] Efficient chromatographic performance was reflected in relatively short retention times, typically between 3 and 7 minutes for active ingredients, although total run times ranged from about 20 to 25 minutes to allow separation of impurities, excipients, and degradation products[2,4] Earlier elution of benzoyl peroxide compared with adapalene in several studies highlighted the influence of molecular polarity on retention behaviour.[4]

Overall, the comparative analysis demonstrates that successful RP-HPLC method development for dermatological formulations relies on a consistent strategy: use of reversed-phase silica columns, organic-rich mobile phases with controlled acidic pH, moderate flow rates, standardized injection volumes, and UV detection at optimized wavelengths. These shared methodological principles collectively ensure reliable retention, efficient separation, and regulatory-compliant analytical performance across different formulations and laboratories.[2–4]

CONCLUSION

The collective evidence from the reviewed studies confirms that RP-HPLC remains a reliable and widely applicable analytical technique for the determination of adapalene, benzoyl peroxide, and related compounds in pharmaceutical and food matrices. The reported methods consistently demonstrate acceptable validation parameters, including good linearity, precision, accuracy, specificity, and robustness, indicating their suitability for routine quality control and regulatory analysis. For instance, the simultaneous estimation method developed for gel formulations showed accurate assay results and robust performance, confirming its applicability for pharmaceutical testing and content uniformity assessment. Similarly, HPLC methods developed for bulk drugs and food samples highlight the versatility of chromatographic techniques across different analytical contexts.

However, despite these strengths, the reviewed literature reveals that most methods rely on conventional UV detection and solvent-intensive chromatographic conditions, limiting their sensitivity for impurity profiling and degradation pathway characterization. In addition, many studies focus on individual or dual-drug systems, whereas modern dermatological therapies increasingly involve complex multi-component formulations. Future analytical research should therefore prioritize the development of high-sensitivity stability-indicating methods, incorporation of hyphenated techniques, chemometric optimization, and greener chromatographic approaches. Expanding these methods to advanced drug delivery systems and bio-analytical applications would further enhance their clinical and industrial relevance.

Overall, the reviewed work establishes a strong analytical foundation for the estimation of adapalene and benzoyl peroxide while clearly indicating the need for more comprehensive, sensitive, and formulation-oriented analytical strategies to meet evolving pharmaceutical and regulatory requirements.

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