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Analytical Review on Chromatographic Based Estimation of Hydrocortisone and Camphor in Topical Dosage Forms

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Abstract: Hydrocortisone and camphor are widely used active pharmaceutical ingredients (APIs) in topical formulations for the treatment of various dermatological conditions such as inflammation, pruritus, and muscular pain. Accurate and reliable estimation of these compounds is crucial for ensuring product quality, efficacy, and patient safety. High-Performance Liquid Chromatography (HPLC) has emerged as a preferred analytical technique for the simultaneous estimation of hydrocortisone and camphor due to its high sensitivity, specificity, and reproducibility. This review explores the current analytical approaches for quantifying hydrocortisone and camphor in topical dosage forms, with a focus on HPLC methodologies. It discusses the principles of HPLC and highlights key parameters including mobile phase composition, detection wavelength, flow rate, column selection, and sample preparation techniques that impact method efficiency and resolution. Furthermore, it evaluates the validation parameters such as linearity, precision, accuracy, robustness, and limits of detection (LOD) and quantification (LOQ) that are essential to comply with regulatory standards. Several studies are analysed to compare their methodological variations and outcomes. The review also emphasizes the significance of method development and optimization for complex matrices like creams, gels, and ointments, where excipients can interfere with accurate detection. Overall, this review provides a comprehensive understanding of HPLC-based estimation methods for hydrocortisone and camphor, which can aid in developing standardized protocols for routine quality control in pharmaceutical manufacturing.

Keywords*: Hplc, Hydrocortisone, Camphor, High-Performance Liquid Chromatography, Topical Formulation.*

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I. INTRODUCTION

A. Importance of Topical Formulations

Topical formulations are an essential component of pharmaceutical and dermatological treatment modalities, offering a versatile, non-invasive approach to drug delivery. These formulations include a variety of dosage forms such as creams, gels, ointments, lotions, pastes, and transdermal patches, each designed for specific therapeutic objectives and skin conditions. The primary objective of a topical formulation is to deliver the active pharmaceutical ingredient (API) directly to a localized site of action on or within the skin, thereby maximizing the therapeutic effect while minimizing systemic exposure and associated side effects [1].

This method of drug delivery is particularly advantageous in the management of dermatological conditions such as eczema, psoriasis, acne, dermatitis, fungal infections, burns, and localized muscle or joint pain. In such cases, systemic administration might be ineffective or produce unnecessary adverse effects. Topical therapy allows clinicians to administer a precise dose to the target area, offering rapid relief and improved patient outcomes [2].

B. Key Benefits of Topical Drug Delivery Include

- *Localized Action:*

Direct application ensures that the drug acts precisely where needed, reducing the amount of drug required and improving efficacy [3].

- *Bypassing First-Pass Metabolism:*

Unlike orally administered drugs, topically applied medications do not undergo first-pass metabolism in the liver. This improves the bioavailability of certain drugs and reduces metabolic degradation.

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- *Reduced Systemic Toxicity:*
  Since the medication is mostly confined to the skin or underlying tissues, the risk of systemic side effects such as gastrointestinal discomfort or hepatic burden is significantly lower [4].

- *Enhanced Patient Compliance:*
  Topical formulations are generally easy to apply, non-invasive, and can be self-administered. This convenience encourages adherence to treatment regimens, especially in chronic skin conditions.

- *Controlled and Sustained Release:*
  Many modern topical delivery systems are designed to release the drug in a controlled manner over time, reducing the need for frequent reapplication and maintaining consistent drug levels in the skin [5].

- *Cosmetic Acceptability:*
  With advancements in formulation science, topical products are now developed with better texture, skin feel, and absorption properties, making them more acceptable to patients for long-term use.

- *Multifunctionality:*
  Topical formulations can be tailored to incorporate multiple agents (e.g., anti-inflammatory, antimicrobial, analgesic), enabling synergistic therapeutic effects in a single application [6].

Moreover, the development of advanced drug delivery technologies, such as liposomes, nanoemulsions, microsponges, and transdermal patches, has significantly enhanced the effectiveness of topical formulations. These innovations allow for deeper skin penetration, targeted drug delivery, and better control over drug release kinetics [7].

However, the success of any topical formulation depends not only on the therapeutic efficacy of the drug but also on the stability, uniformity, and quality of the product. This underscores the critical need for rigorous formulation development protocols, standardization techniques, and robust quality control practices. Accurate quantification of active ingredients, evaluation of penetration and permeation behavior, assessment of pH, viscosity, spreadability, and microbial contamination are some of the essential quality parameters that ensure safety and effectiveness [8].

C. Role of Hydrocortisone and Camphor in Dermatology

Hydrocortisone and camphor are two pharmacologically active agents widely incorporated into topical formulations due to their complementary and synergistic therapeutic effects in managing dermatological conditions. Each has a distinct mechanism of action and set of clinical applications, making them highly valuable in combination for treating inflammation, irritation, and discomfort associated with various skin disorders [9].

D. Hydrocortisone: A Cornerstone Anti-Inflammatory Agent

Hydrocortisone, classified as a low-potency corticosteroid, is one of the most prescribed agents for the treatment of inflammatory skin disorders. It exerts its effects by penetrating the skin barrier and binding to intracellular glucocorticoid receptors. Once bound, the hydrocortisone-receptor complex translocate to the nucleus, where it regulates the transcription of anti-inflammatory genes and suppresses the production of pro-inflammatory cytokines such as prostaglandins, interleukins, and tumor necrosis factor-alpha (TNF-α) [10].

Hydrocortisone, also known as cortisol, is a naturally occurring glucocorticoid hormone produced by the adrenal cortex. Chemically, it belongs to the corticosteroid class and features the cyclopentanoperhydrophenanthrene ring system common to steroid hormones. Its molecular formula is $C_{21}H_{30}O_5$, and it contains several functional groups such as hydroxyl and ketone groups that contribute to its pharmacological activity [11]. In topical formulations, hydrocortisone is often used in esterified forms such as hydrocortisone acetate to improve its solubility and dermal absorption. Although it has limited water solubility, it dissolves well in organic solvents like ethanol and acetone, which are commonly used in pharmaceutical preparations [12].

The mechanism of action of hydrocortisone involves its interaction with intracellular glucocorticoid receptors (GRs) present in the cytoplasm of target cells. Upon binding, the hydrocortisone-receptor complex migrates to the nucleus and modulates gene transcription. This leads to an increased expression of anti-inflammatory proteins such as lipocortin-1, which inhibits phospholipase A2 and subsequently reduces the synthesis of pro-inflammatory mediators like prostaglandins and leukotrienes [13]. Simultaneously, hydrocortisone downregulates the production of pro-inflammatory cytokines such as TNF-alpha, IL-1, and IL-6. This dual action of suppressing inflammation and immune responses makes hydrocortisone highly effective in treating a range of inflammatory skin conditions [14].

Clinically, topical hydrocortisone is used to manage a variety of dermatological disorders including eczema, atopic dermatitis, contact dermatitis, seborrheic dermatitis, psoriasis, and insect bite reactions. Due to its mild potency, hydrocortisone is particularly suitable for use on sensitive skin areas such as the face, neck, and groin [15]. Its ability to relieve redness, itching, swelling, and irritation makes it a preferred choice for both acute flare-ups and maintenance therapy in chronic conditions. Moreover, it is often included in over-the-counter (OTC) formulations for self-treatment of mild inflammatory skin issues, contributing to its widespread availability and use [16].

Despite its therapeutic benefits, the formulation of hydrocortisone into stable and effective topical products presents several challenges. One of the primary concerns is its poor water solubility, which complicates its incorporation into hydrophilic creams and gels. This necessitates the use of solubilizing agents or emulsifiers to enhance drug dispersion.

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Additionally, hydrocortisone is susceptible to photodegradation and oxidation, which can reduce its efficacy over time [17]. Therefore, appropriate packaging in light-resistant containers and the inclusion of antioxidants or stabilizers is essential. The drug's stability is also pH-dependent, with optimal formulation conditions falling within a mildly acidic to neutral range. Furthermore, hydrocortisone can interact unfavorably with certain excipients, particularly oxidizing agents, requiring careful selection of compatible formulation components [18].

Another important consideration is skin permeation. While hydrocortisone is intended for localized action, ensuring adequate penetration through the stratum corneum is critical for its efficacy. To address this, pharmaceutical scientists have explored various delivery strategies, including the use of liposomes, nano-carriers, and ester derivatives to enhance skin absorption. Lastly, as topical products are often stored for extended periods and exposed to environmental factors, preservatives must be added to prevent microbial contamination, particularly in water-based formulations [19].

➢ *Clinically, Hydrocortisone Is Effective In Managing Conditions Like*

- Eczema and dermatitis
- Psoriasis (mild forms)
- Seborrheic dermatitis
- Insect bite reactions
- Contact allergies and rashes [20]
- Its primary actions include:
- Anti-inflammatory: Reduces swelling, erythema, and pain by suppressing the local immune response [21].
- Antipruritic: Provides relief from itching by modulating inflammatory signals and histamine activity.
- Vasoconstrictive: Decreases capillary permeability and local blood flow, reducing skin redness and heat [22].
- Hydrocortisone is preferred in sensitive areas such as the face, groin, and underarms, where more potent steroids may cause thinning of the skin or systemic absorption.

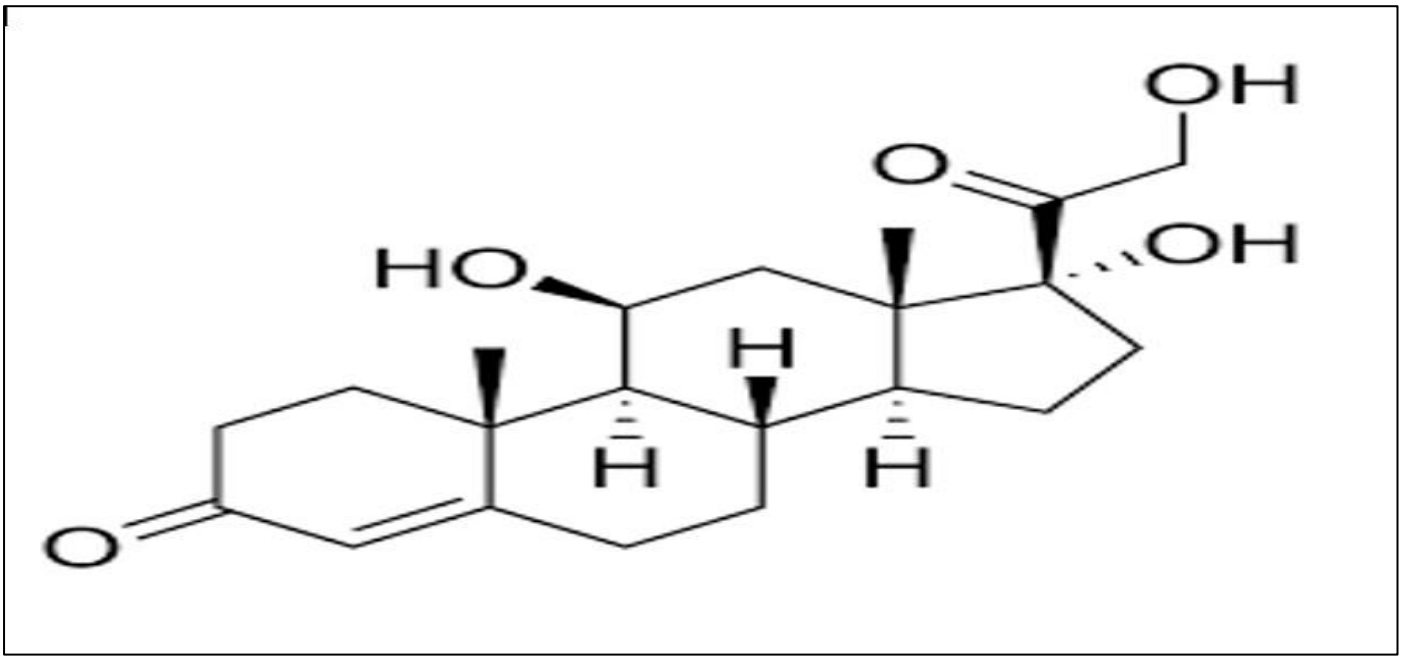

Fig 1 Hydrocortisone Molecular Structure [23]

E. Camphor: A Versatile Natural Therapeutic

Camphor is a naturally occurring terpenoid obtained from the wood of the camphor tree (*Cinnamomum camphora*) or synthesized chemically. It has been used for centuries in traditional medicine for its diverse pharmacological properties. In dermatology, it serves multiple roles owing to its ability to produce a cooling and soothing effect upon topical application [24].

Mechanistically, camphor acts as a counter-irritant. It activates transient receptor potential (TRP) channels, particularly TRPV1 and TRPM8, which are responsible for temperature and pain sensation. This interaction causes a paradoxical effect — a cooling sensation followed by a warming feeling — that distracts the brain from deeper pain or itch stimuli [25].

Camphor is a naturally occurring terpenoid compound that has been used for centuries in traditional and modern medicine, particularly in topical preparations. It is primarily obtained from the wood of the camphor tree (*Cinnamomum camphora*), though it can also be synthesized chemically from turpentine oil. Camphor is a white, crystalline substance with a strong, penetrating aroma and a waxy texture [26]. Chemically, it belongs to the class of bicyclic monoterpenes, with the molecular formula $C_{10}H_{16}O$. Its lipophilic structure allows it to readily penetrate the skin barrier, making it ideal for topical applications. It is highly volatile, subliming at room temperature, which contributes to its characteristic cooling effect and rapid onset of action [27].

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Camphor exhibits a wide range of therapeutic properties that make it useful in treating various dermatological and musculoskeletal conditions. It functions as an analgesic, antipruritic, rubefacient, and mild antimicrobial agent. When applied topically, camphor activates transient receptor potential (TRP) channels on sensory neurons, especially TRPV1 and TRPM8, which are involved in heat and cold sensation [28]. This action induces a cooling effect that temporarily masks pain and itching. Additionally, camphor enhances local blood flow by causing mild vasodilation, which explains its warming and counter-irritant effects. It is commonly used in formulations for muscle and joint pain relief, insect bites, minor burns, cough and cold balms, and skin irritation. The dual action of cooling and heating sensations provides symptomatic relief and soothes discomfort effectively [29].

However, camphor's volatility and lipophilicity pose specific formulation challenges. Its strong odor and tendency to evaporate can affect product stability and consistency, especially in open or semi-solid preparations like creams and ointments. This necessitates the use of airtight, well-sealed packaging to prevent sublimation and maintain the therapeutic concentration of the active compound [30]. Additionally, its solubility in water is very limited, so camphor must be incorporated into oil phases or alcohol-based solutions, or dispersed using emulsifying agents. In high concentrations, camphor can be toxic and irritant, so its dosage must be carefully regulated—usually restricted to 0.1–3% in over-the-counter topical formulations, as per safety guidelines [31].

From a formulation perspective, compatibility with other active and inactive ingredients is essential. Camphor can interact with certain compounds, such as strong oxidizers, and may destabilize emulsions if not properly balanced. Despite these challenges, camphor remains a highly valued compound in dermatology and pharmaceutical sciences, especially when used in combination with other agents like hydrocortisone. The synergistic effects of such combinations can enhance therapeutic outcomes by combining anti-inflammatory action with symptomatic relief of pain and itching [32].

In conclusion, camphor is a potent, versatile agent in topical therapy. Its unique pharmacological properties—ranging from sensory modulation to vasodilation—make it an essential component of many skin-care and pain-relief formulations. With careful formulation design and quality control, camphor continues to provide effective and safe relief for a wide range of skin and musculoskeletal conditions [33].

➢ *Camphor Is Beneficial For*:

- Muscle and joint pain (topical analgesia)
- Itching and irritation from insect bites or minor rashes
- Burns and skin irritation
- Cough and cold when applied as a vapor rub (non-dermatologic use) [34]
- Its main therapeutic properties include:
- Analgesic: Reduces pain by numbing nerve endings and diverting pain signals.
- Antipruritic: Alleviates itching through counter-stimulation.
- Rubefacient: Increases local blood flow, which may enhance healing [35].

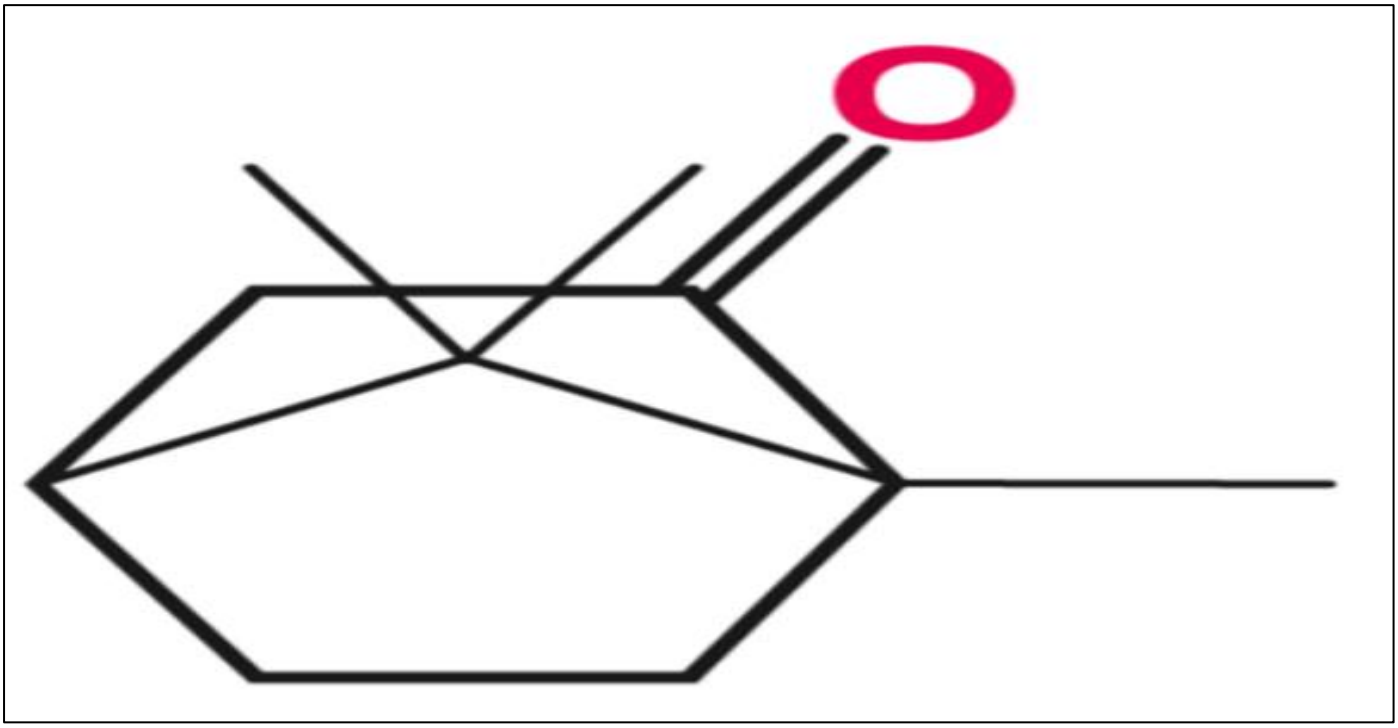

Fig 2 Camphor Molecular Structure [36]

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F. Combined Benefits in Topical Therapy

➢ *When Used in Combination, Hydrocortisone and Camphor Create A Synergistic Therapeutic Effect:*

- *Dual-Action Relief:*

Hydrocortisone targets the underlying inflammatory processes, while camphor provides immediate symptomatic relief through sensory distraction and cooling [37].

- *Enhanced Comfort:*

Camphor's cooling effect may reduce the burning sensation often experienced with corticosteroid use in sensitive individuals.

- *Complementary Effects:*

Camphor provides a rapid onset of sensory relief, and hydrocortisone offers long-term anti-inflammatory control [38].

➢ *This Combination is Particularly useful in Managing Conditions such as:*

- Inflamed insect bites
- Contact dermatitis with intense itching
- Minor burns accompanied by irritation
- Muscle pain with associated redness or rash [39]

Moreover, the inclusion of camphor may improve the patient's perception of efficacy and comfort, encouraging adherence to the treatment regimen.

G. Need For Accurate Estimation And Quality Control

Accurate quantification of APIs such as hydrocortisone and camphor is essential for ensuring the efficacy and safety of topical formulations. Due to the complexity of topical matrices and the presence of multiple excipients, the analytical method must be robust and sensitive. High-Performance Liquid Chromatography (HPLC) serves as a powerful tool for such estimations, offering high precision, reproducibility, and the ability to separate compounds even in complex mixtures [40]. Importance of quality estimations are mentioning as follows,

- Ensures therapeutic effectiveness
- Prevents under- or over-dosing
- Complies with pharmacopeial standards
- Supports product stability and shelf-life determination

Quality control through validated analytical techniques like HPLC is therefore indispensable in the pharmaceutical industry for product approval and batch-to-batch consistency [41].

H. Topical Formulations Containing Hydrocortisone And Camphor

Topical formulations combining hydrocortisone and camphor are commonly used in dermatology to provide dual therapeutic effects: anti-inflammatory and analgesic. These formulations are developed in various dosage forms such as creams, ointments, gels, and lotions. Each form is designed based on the target skin condition, site of application, and desired drug release profile. For instance, creams and lotions are ideal for acute, moist conditions and have better cosmetic acceptability, whereas ointments are suitable for chronic, dry skin due to their occlusive properties. Gels provide rapid absorption and are preferred for hairy areas or oily skin types [42].

I. Creams, Gels, Ointments, Lotions

Topical formulations containing hydrocortisone and camphor are widely available in the forms of creams, gels, ointments, and lotions, each tailored for specific skin conditions and patient preferences. The choice of formulation depends on the desired absorption rate, the nature of the skin lesion (dry, moist, or hairy), and the drug release profile [43].

➢ Creams are semi-solid emulsions (oil-in-water) that offer good spreadability and fast absorption, making them suitable for inflamed or weeping skin conditions.
➢ Gels are water- or alcohol-based systems that dry quickly and provide a cooling effect, making them ideal for oily skin or hairy areas like the scalp.
➢ Ointments are greasy, occlusive bases (water-in-oil) suitable for chronic, dry, or scaly conditions where hydration and prolonged contact with the skin are desired.
➢ Lotions are liquid emulsions or suspensions with lower viscosity than creams and are useful for large surface areas or sensitive skin, especially in pediatric or elderly patients [44].

J. Excipients Used

Excipients play a vital role in determining the stability, efficacy, texture, and sensory appeal of topical products. For formulations with hydrocortisone and camphor, excipients are selected based on their compatibility with both APIs and their ability to maintain the integrity of the product during storage and use [45].

➢ *Common Excipients Include:*

- Emulsifiers such as polysorbate 60, cetostearyl alcohol, and glyceryl monostearate help maintain stable emulsions.
- Humectants like glycerin and propylene glycol draw moisture to the skin and improve spreadability.
- Preservatives such as parabens and phenoxyethanol prevent microbial growth.
- Gelling agents like carbomer or xanthan gum are used in gel formulations for viscosity control.
- Stabilizers and antioxidants, such as tocopherol or butylated hydroxytoluene (BHT), may be added to minimize oxidation of camphor and hydrocortisone [46].

K. Stability and pH Considerations

Maintaining the chemical and physical stability of hydrocortisone and camphor in a formulation is essential for product efficacy and safety. Hydrocortisone is sensitive to pH and degrades in highly acidic or alkaline environments, while camphor is volatile and prone to sublimation [47].

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The ideal pH range for topical formulations containing hydrocortisone is between 4.0 and 6.0, which aligns with the skin's natural pH and ensures stability. Camphor requires packaging that limits exposure to air and light to reduce evaporation and degradation.

Additionally, temperature control during manufacturing and storage is crucial. Both APIs can degrade under high heat or in presence of strong oxidizing agents. Using air-tight, opaque containers helps in extending shelf life [48].

L. Regulatory Requirements

Topical formulations must adhere to stringent regulatory guidelines set by authorities like the USFDA (United States), EMA (Europe), and CDSCO (India). These regulations ensure that the product is safe, effective, and consistent in quality.

Hydrocortisone is usually allowed up to 1% for OTC (over-the-counter) use, whereas camphor is permitted up to 3% in topical applications. Exceeding these concentrations requires prescription-based dispensing and additional safety evaluations [49].

➢ *Formulations must undergo*
- Stability studies (ICH guidelines),
- Microbial limit tests,
- Irritation and sensitivity testing, and
- Labeling compliance (INCI names, expiry date, batch number) [50].

II. ANALYTICAL METHODS FOR ESTIMATION

A. Overview of Analytical Techniques (UV, TLC, HPLC, GC)

Various analytical techniques are employed to quantify and identify active pharmaceutical ingredients (APIs) in topical formulations. These include UV spectrophotometry, Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography (GC). Each method has its strengths and limitations based on the chemical nature of the analyte and formulation complexity [51].

➢ UV Spectrophotometry is simple, cost-effective, and useful for preliminary analysis. However, it lacks specificity, especially when analyzing mixtures or components with overlapping absorption spectra.
➢ TLC provides qualitative and semi-quantitative data, suitable for rapid screening and fingerprinting. It is simple but not as accurate or sensitive for quantitative analysis.
➢ HPLC is widely used due to its precision, reproducibility, and ability to simultaneously estimate multiple components, including in complex matrices.
➢ GC is suitable for volatile compounds like camphor, but hydrocortisone's thermal instability limits its use in this context [52].

B. Importance of HPLC over Other Chromatographic Techniques

High-Performance Liquid Chromatography (HPLC) is the preferred method for estimating hydrocortisone and camphor in topical formulations due to its high sensitivity, selectivity, and versatility. It can efficiently separate components, even in complex, multi-ingredient formulations, without the need for prior extraction or derivatization [53].

➢ *HPLC Allows For*

➢ Simultaneous estimation of hydrocortisone and camphor in a single run.
➢ Adaptability to various detectors (UV, PDA, fluorescence).
➢ Accurate quantification even at low concentrations.
➢ Minimal interference from excipients and formulation additives.

Moreover, HPLC can be validated as per ICH Q2(R1) guidelines, covering accuracy, precision, linearity, and robustness—making it acceptable for regulatory and industrial use [54].

C. Challenges in Simultaneous Estimation

Despite HPLC's advantages, simultaneous estimation of hydrocortisone and camphor presents unique challenges. The major issue lies in the differences in polarity, solubility, and chemical behavior of the two compounds [55].

➢ Hydrocortisone is relatively polar and UV-absorbent, whereas camphor is volatile and less polar, requiring careful optimization of mobile phase and detection wavelength.
➢ Formulation excipients can interfere with the chromatographic peaks, especially in creams and ointments, demanding rigorous sample pretreatment and column selection.
➢ The retention time overlap and peak resolution can be problematic, necessitating fine-tuning of mobile phase composition (e.g., acetonitrile-water ratio, buffer pH).
➢ Additionally, ensuring peak stability for camphor during analysis is crucial due to its volatile nature [56].

III. HPLC METHOD DEVELOPMENT FOR HYDROCORTISONE AND CAMPHOR

A. Selection of Mobile Phase

The mobile phase is a critical component in the HPLC method, significantly affecting the separation quality, retention times, and peak resolution of the analytes. For simultaneous estimation of hydrocortisone and camphor, the mobile phase must provide an optimal environment to separate two compounds that differ in polarity [57]. Hydrocortisone, being moderately polar, and camphor, which is less polar, require a balanced mobile phase. Typically, mixtures of aqueous buffers (such as phosphate buffer with pH adjusted between 3 and 6) combined with organic solvents like acetonitrile or methanol is used. The buffer helps maintain a consistent pH to control the ionization state of hydrocortisone, enhancing peak shape and reproducibility.

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An isocratic mobile phase system, often with 60:40 or 70:30 ratios of acetonitrile to buffer, is preferred for its simplicity and reproducible retention times. This selection ensures sharp, well-resolved peaks with minimal tailing, which is essential for accurate quantification [58].

B. Column Type and Stationary Phase

The choice of column directly influences the separation efficiency and resolution of hydrocortisone and camphor in an HPLC analysis. Reversed-phase chromatography is the method of choice for these compounds due to its compatibility with a wide range of analytes and its ability to separate molecules based on hydrophobic interactions. A C18 column, packed with octadecylsilane bonded silica particles, is commonly used because its non-polar stationary phase provides strong retention and good peak shapes for both hydrocortisone and camphor [59]. Columns with dimensions around 150 mm in length and 4.6 mm internal diameter, with particle sizes between 3 to 5 µm, offer an excellent balance between resolution and analysis time. Maintaining the column temperature at 25–30°C helps in achieving consistent retention times and reproducible results across different runs [60].

C. Detection Wavelength

Detection wavelength selection is pivotal for sensitive and selective quantification of hydrocortisone and camphor. Both compounds absorb ultraviolet (UV) light, but their maximum absorbance peaks occur at different wavelengths—hydrocortisone around 240 nm and camphor closer to 210 nm. To enable simultaneous detection, a compromise wavelength in the range of 220 to 230 nm is typically chosen. This range allows for adequate absorbance of both compounds with reasonable sensitivity [61]. Using a UV detector set to this wavelength enables effective monitoring during the run. Additionally, employing a photodiode array (PDA) detector offers the advantage of scanning multiple wavelengths simultaneously, enhancing the reliability of detection and identification of peaks, particularly in complex topical formulations where excipients might interfere [62].

D. Sample Preparation Techniques

Sample preparation is a crucial step to ensure the analytes are effectively extracted from the topical formulations and that impurities or excipients do not interfere with the analysis. For hydrocortisone and camphor, the topical preparation (cream, gel, or ointment) is accurately weighed and dissolved in a suitable solvent system, commonly methanol or a methanol-water mixture, which dissolves both the active pharmaceutical ingredients efficiently [63]. The solution is often sonicated to enhance dissolution and break down any particulate matter, ensuring a homogeneous sample. After dissolution, the sample is filtered through a 0.45 µm membrane filter to remove any undissolved particles that could clog the HPLC column or interfere with detection. Proper dilution of the filtered sample to fall within the linear dynamic range of the method is necessary for accurate quantification and reproducibility [64].

E. Run Time and Retention Time Data

The run time and retention time are essential parameters reflecting the efficiency and practicality of the developed HPLC method. Typically, camphor, being less polar, elutes faster, with retention times ranging from 1 to 3 minutes, while hydrocortisone elutes later, typically between 3 to 6 minutes. The total run time is generally kept under 10 minutes to enable rapid analysis, making the method suitable for routine quality control where high sample throughput is required [65]. Well-defined, sharp, and symmetrical peaks with baseline separation confirm the effectiveness of the chromatographic conditions chosen. Short run times combined with reliable retention times and peak shapes contribute to enhanced productivity and lower operational costs in analytical laboratories [66].

IV. CHALLENGES IN ANALYSIS OF TOPICAL DOSAGE FORMS

Table 1 Challenges in Analysis of Topical Dosage Forms [67-71]

Challenge	Description
Matrix Interference	Complex excipients in topical formulations (emulsifiers, preservatives, oils, polymers) may cause overlapping peaks, baseline noise, or signal suppression/enhancement, leading to inaccurate quantification of hydrocortisone and camphor. Selective and sensitive analytical methods are needed to overcome this [72].
Sample Extraction Complexities	Semi-solid nature and complex composition require efficient extraction techniques (solvent extraction, sonication, and centrifugation) to isolate active ingredients without degradation or loss. Choosing an appropriate solvent system is critical to ensure high recovery and minimize excipient interference [73].
Viscosity and Spreadability Impact	High viscosity and poor spreadability affect uniform sampling, dilution, and homogenization during sample preparation. This can cause inconsistent analyte concentration and reproducibility issues, often requiring additional steps like heating or vigorous mixing to obtain representative samples [74].
Chemical Stability of Analytes	Hydrocortisone and camphor can degrade due to exposure to light, temperature variations, and pH changes during sample preparation and storage, resulting in inaccurate quantification. Stability-indicating methods and proper sample handling are essential to preserve analyte integrity [75].
Interference from Degradation Products	Degradation products of hydrocortisone and camphor may co-elute with parent compounds or cause additional peaks, complicating chromatographic interpretation and leading to potential misidentification or quantification errors. Forced degradation studies help identify these impurities [76].
Low Analyte Concentration	Low concentration levels of hydrocortisone and camphor in certain formulations can challenge detection limits and precision, requiring optimization of chromatographic parameters and use of sensitive detectors to ensure reliable quantification [77].

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Sample Homogeneity Issues	Uneven distribution of active ingredients in semi-solid topical formulations may result in sample variability and inconsistent assay results. Thorough mixing and validated sampling protocols are necessary to obtain representative and reproducible samples [78].
Instrumental Variability	Variations in HPLC system components such as pump pressure, detector sensitivity, and column efficiency can affect retention times and peak shapes, impacting method reproducibility. Regular calibration, maintenance, and system suitability tests are critical to ensure consistent performance [79].

V. VALIDATION OF HPLC METHOD (AS PER ICH GUIDELINES)

A. *Linearity*

Linearity assesses the ability of the HPLC method to produce results directly proportional to the concentration of analytes within a given range. For hydrocortisone and camphor, a series of standard solutions with known concentrations are prepared, typically spanning from 50% to 150% of the expected sample concentration. These standards are analysed, and the peak area is plotted against concentration to generate calibration curves. A linear relationship with a correlation coefficient (R^2) greater than 0.999 indicates excellent linearity. This confirms that the detector response is consistent and reliable over the concentration range, ensuring accurate quantification of the drugs in topical formulations [80].

B. *Accuracy*

Accuracy refers to the closeness of the measured value to the true value or standard. It is evaluated by recovery studies where known amounts of hydrocortisone and camphor are spiked into the placebo matrix or formulation samples at multiple concentration levels (typically 80%, 100%, and 120%). The percentage recovery is calculated by comparing the measured amount with the actual spiked amount. An acceptable accuracy range is typically between 98% and 102%. High accuracy ensures the method can reliably quantify the analytes without interference from formulation excipients or matrix effects [81].

C. *Precision (Intra-Day & Inter-Day)*

Precision evaluates the reproducibility of the HPLC method under normal operating conditions and is divided into intra-day (repeatability) and inter-day (intermediate precision) assessments. Intra-day precision involves analysing multiple replicates of the same concentration within a single day, while inter-day precision involves repeating the analysis on different days. The results are expressed as the relative standard deviation (% RSD) of peak areas or concentrations. A % RSD value below 2% is generally considered acceptable, indicating the method produces consistent and reliable results over time [82].

D. *Specificity*

Specificity is the method's ability to measure the analytes distinctly in the presence of other components such as excipients, impurities, or degradation products. It is confirmed by analysing blank samples, placebo samples, and samples containing known impurities or degradation products. The hydrocortisone and camphor peaks should be well resolved without interference or overlapping peaks. This ensures the method is selective and suitable for analysing complex topical formulations without false readings [83].

E. *Limit of Detection (LOD) & Limit of Quantification (LOQ)*

LOD is the lowest concentration of the analyte that can be detected but not necessarily quantified, while LOQ is the lowest concentration that can be quantitatively measured with acceptable precision and accuracy. These limits are typically calculated based on the signal-to-noise ratio method, with LOD corresponding to a ratio of 3:1 and LOQ to 10:1. Low LOD and LOQ values demonstrate the method's sensitivity, which is essential for detecting trace amounts of hydrocortisone and camphor in formulations or during stability testing [84]...

F. *Robustness and System Suitability*

Robustness evaluates the method's reliability under small deliberate variations in chromatographic conditions, such as slight changes in mobile phase composition, flow rate, column temperature, or pH. The method should maintain consistent results and peak parameters despite these changes, confirming its practical applicability in routine analysis. System suitability tests are performed before sample analysis to ensure the HPLC system is functioning correctly. Parameters such as retention time, resolution, theoretical plates, tailing factor, and repeatability of peak area are checked. Meeting predefined criteria ensures the system's performance is optimal, guaranteeing accurate and precise analysis throughout the batch [85-101].

VI. CONCLUSION

Validated HPLC methods are indispensable in the pharmaceutical industry for the accurate, precise, and reliable quantification of active pharmaceutical ingredients (APIs) such as hydrocortisone and camphor in topical formulations. These methods ensure product quality, safety, and efficacy by providing critical data for formulation development, quality control, and regulatory compliance. The robustness and specificity of HPLC allow for the detection of impurities and degradation products, thus safeguarding patient safety and supporting shelf-life determination.

The growing interest in advanced drug delivery systems presents significant opportunities for the application of validated analytical techniques. Nano-formulations, such as nanoparticles, liposomes, and transferosomes, are being increasingly explored for topical delivery of hydrocortisone and camphor, offering improved skin penetration, controlled release, and enhanced therapeutic outcomes. HPLC methods will play a vital role in the characterization and quality assessment of these novel delivery systems, including the quantification of encapsulated drugs and their release profiles.

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Future research should focus on developing more sensitive and rapid analytical methods tailored for complex nano-formulations and bioanalytical matrices. Integration of hyphenated techniques, such as HPLC coupled with mass spectrometry (LC-MS), can provide deeper insight into drug metabolism and pharmacokinetics in topical applications. Additionally, exploring green chemistry principles in method development can reduce solvent use and environmental impact. Continuous advancements in chromatographic technologies and sample preparation methods will further streamline quality control processes and foster innovation in topical pharmaceutical formulations.

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