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Review Article

Advances in Analytical Techniques for the Simultaneous Quantification of Azole Antifungals and Corticosteroids in Topical Formulations

Imad Osman Abu Reid*, Sayda Mohamed Osman, Somia Mohammed Bakheet

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International University of Africa, Khartoum, Sudan

Abstract

Background. The combination of azole antifungals and topical corticosteroids is widely used in treating inflamed superficial mycoses infections. These combinations provide both antifungal activity and anti-inflammatory effects, making them effective for managing various dermatological conditions. *Objective*. This review explores various quantitative analytical methods used for assessing azole antifungal and corticosteroid combinations in topical formulations. It evaluates techniques employed in studies published between 1999 and 2024, highlighting their advantages and limitations. *Main ideas and results.* The review examines various analytical techniques, including ultraviolet (UV) spectroscopy, high-performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE), and high-performance thin-layer chromatography (HPTLC). Among these methods, HPLC was the most frequently employed, accounting for approximately 60% of published studies. While HPLC offers high sensitivity and reliability, its dependence on organic solvents and costly instrumentation presents limitations. Alternative methods such as thin-layer chromatography (TLC) and UV spectrophotometry were also widely utilized. TLC, representing about 14% of studies, was preferred for its simplicity and cost-effectiveness, while UV spectrophotometry, making up 24.5% of reported methods, was favored for its accessibility and ease of optimization. In contrast, capillary zone electrophoresis (CZE) was the least commonly used, appearing in only one study (less than 2%), despite its eco-friendly nature and procedural simplicity. *Conclusion.* Among the various analytical techniques reviewed, HPLC remained the most preferred method due to its accuracy and sensitivity. However, the continued use of UV spectrophotometry and TLC highlights the importance of cost-effective and accessible methods. The minimal adoption of CZE suggests an opportunity for further exploration into eco-friendly analytical alternatives.

Keywords

Azole Antifungals, Topical Corticosteroids, Combination, Determination

1. Introduction

Following a superficial fungal infection or dermatomycosis, the breakdown of the keratin layer often leads to inflammation, resulting in symptoms such as itching, redness, swelling, and a burning sensation at the affected site. These symptoms can

*Corresponding author: iabureid@hotmail.com (Imad Osman Abu Reid)

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cause significant discomfort, leading to scratching and, in some cases, pain. Such irritation may reduce patient compliance with treatment, leaving the skin vulnerable to further complications, including secondary fungal or bacterial infections. To counteract the inflammation associated with superficial mycoses, topical corticosteroids are commonly incorporated into treatment regimens. [1-4]

Azole antifungals are classified into two main groups: imidazoles (e.g., miconazole and ketoconazole) and triazoles (e.g., itraconazole, fluconazole, and voriconazole). The key structural difference between them lies in the number of nitrogen atoms in the azole ring—imidazoles contain two, while triazoles contain three. These antifungals function by inhibiting ergosterol biosynthesis, a vital component of fungal cell membranes. They achieve this by targeting lanosterol 14-alpha-demethylase, a cytochrome P450-dependent enzyme. Disrupting this pathway leads to ergosterol depletion and the accumulation of methylated sterol precursors, which alters membrane integrity and impairs the activity of membrane-associated enzymes such as chitin synthase. These effects inhibit fungal proliferation and prevent replication. Additionally, azoles interfere with yeast-to-mycelial transformation, reduce fungal adhesion, and exert toxic effects on membrane phospholipids. [5]

Mineralocorticoids primarily regulate the hydroelectrolytic balance by retaining sodium and depleting potassium, with aldosterone being the most significant endocrine mineralocorticoid. Glucocorticoids play a crucial role in the metabolism of carbohydrates, proteins, and calcium, and possess strong anti-inflammatory and immunosuppressive properties. Due to their potent immunomodulatory and anti-inflammatory effects, glucocorticoids are used to treat a variety of inflammatory, malignant, and allergic conditions, including rhinitis, asthma, dermatological, rheumatic, ophthalmic, and neurological diseases, as well as following organ transplants. They are among the most widely prescribed drugs globally; however, their therapeutic use is limited by significant side effects, particularly when administered in high doses or over extended periods. [6]

When two or more drugs are combined in specific proportions to create a single product, they are referred to as fixed-dose combinations (FDCs) or combined products in general. [7] FDCs offer several benefits, including enhanced therapeutic efficacy, improved patient compliance due to a reduced number of administrations [8], lower individual drug doses, and fewer side effects [9]. They also exhibit better pharmacokinetic profiles and minimize the risk of developing resistance. [7, 10] Additionally, the production cost of an FDC product is generally lower than the total cost of producing the individual drugs separately, thanks to streamlined manufacturing processes and reduced packaging and distribution needs. [11]

FDC products are typically more complex than formulations containing a single active pharmaceutical ingredient (API). The quality control (QC) requirements for FDCs, such as active pharmaceutical ingredient assays and related substance tests, are more extensive compared to single-ingredient products. This complexity increases the QC burden on manufacturers, necessitating a higher number of tests. However, developing selective, sensitive, reliable, and robust analytical methods for estimating impurities, degradation products, excipients, and individual active pharmaceutical ingredients (APIs) in combined drug products presents significant challenges. Typically, the API assay, determination of the degradation products and related substances (RS) tests are conducted using separation techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and capillary electrophoresis (CE).

This literature review presents a detailed analysis of the advancements in analytical methodologies for evaluating topical formulations containing azole antifungals and corticosteroids from 1999 to the present. The research was conducted using leading scientific databases, including Science Direct, Taylor & Francis, Springer Link, PubMed, Scopus, Google Scholar, and Wiley. The search strategy incorporated the keywords: "determination azole antifungals AND corticosteroids dosage form combination."

To enhance clarity and accessibility, the collected data is systematically categorized based on the analytical techniques employed, offering a structured comparison of various approaches used to assess these pharmaceutical combinations. By compiling insights from numerous published studies, this review serves as a valuable resource for pharmaceutical researchers and professionals, enabling them to quickly identify relevant analytical methods applicable to their work.

2. Analytical Methods

2.1. Official Analytical Methods

The British Pharmacopoeia outlines two high-performance liquid chromatography (HPLC) methods for analyzing various pharmaceutical formulations containing clotrimazole (CLT), miconazole (MCN), and hydrocortisone acetate (HCA) [12]. These methods utilize different chromatographic columns and mobile phase compositions to achieve effective separation of the active ingredients.

The first method is designed for the analysis of clotrimazole and hydrocortisone acetate in creams, as well as miconazole and hydrocortisone acetate in both creams and ointments. It employs a base-deactivated, endcapped octadecylsilane (C18) column (10 cm × 4.6 mm, 3 µm) and a binary gradient elution. The mobile phase consists of 0.01 M ammonium dihydrogen orthophosphate (adjusted to pH 6.0 with dilute ammonia) as phase A, and a mixture of 10% tetrahydrofuran and 90% acetonitrile as phase B. Initially, the gradient starts with 80% phase A, decreasing to 20% over 20 minutes, followed by a 5-minute hold. The composition then reverts to 80% phase A and is re-equilibrated for 5 minutes before the next injection. The system operates at a flow rate of 1.5 mL/min, with de-

tection at 235 nm.

The second method focuses on the determination of clotrimazole and hydrocortisone acetate in cream formulations. This approach utilizes an octadecylsilane column (25 cm × 4.6 mm, 5 μm) maintained at 40 ℃, with a ternary gradient elution program. The mobile phases include (A) 1.5 g/L potassium dihydrogen orthophosphate in water (adjusted to pH 3.0 with 10% v/v orthophosphoric acid), (B) acetonitrile, and (C) methanol. The initial conditions consist of 90% phase A and 10% phase B, held for 2 minutes. The gradient then transitions to 25% phase A and 75% phase B over 35 minutes, followed by a 5-minute hold. Next, the composition shifts to an equal mixture of phases B and C, before reverting to the initial conditions over 5 minutes and maintaining equilibrium for 10 minutes before the subsequent injection. This method also uses a flow rate of 1.5 mL/min, with detection at 235 nm.

In addition, the United States Pharmacopoeia (USP) provides an HPLC method for the simultaneous quantification of clotrimazole and betamethasone dipropionate (BMD) in cream formulations [13]. The separation is carried out using a C18 column (4.6 mm × 25 cm, 10 μm) and a mobile phase composed of methanol and 0.05 M dibasic ammonium phosphate (7:3 ratio), adjusted to pH 7.0. The chromatographic system operates at a flow rate of 1.7 mL/min, with analyte detection performed at 254 nm.

2.2. Separation Methods

2.2.1. Thin-layer Chromatography (TLC)

Over the past two decades, thin-layer chromatography (TLC) has gained recognition as a valuable tool, particularly for qualitative analysis and preparative separations. Traditionally, its use for quantitative analysis was limited compared to gas chromatography and high-performance liquid chromatography due to difficulties in sample application, chromatographic development, and result evaluation. However, recent technological advancements in TLC instrumentation have significantly improved its precision and accuracy. As a result, TLC is now increasingly utilized for the quantitative analysis of pharmaceuticals in various dosage forms, including tablets, capsules, solutions, and ointments. [14]

Various thin-layer chromatography (TLC) methods have been documented for the simultaneous determination of azole antifungals and corticosteroids in combination [15-22]. Among these, mometasone (MOM) and miconazole (MCN) of NAD [15, 16] or mupirocin (MUP) have been successfully separated on a normal phase thin-layer chromatographic system applying densitometric scanning in the ultraviolet region. The method developed by Patel et al. [15] stands out as the most sensitive, with limits of detection (LOD) of 44.77 μg/mL for MCN and 7.03 μg/mL for MOM. Additionally, this method benefits from being systematically developed and optimized using a fractional factorial experimental design. Meanwhile, the method reported by Zanwar et al. [17] is noted for its eco-friendly approach. The determination of other combinations has also been performed using TLC. However, since these combinations contain dissimilar active ingredients, direct comparison is not feasible. A detailed description of the reported TLC methods is provided in Table 1.

2.2.2. Capillary Zone Electrophoresis (CZE)

A capillary zone electrophoresis (CZE) method has been developed and validated for determining a mixture of MCN and HCA in a cream formulation. The optimal conditions included a sodium dihydrogen phosphate buffer (50 mM, pH 4) and an applied voltage of 30 kV in an 85 cm × 75 μm ID capillary. Direct UV detection was performed at 230 nm, where both MCN and HCA exhibit maximum absorbance. The effects of pH, phosphate buffer concentration, voltage, temperature, sample injection pressure, and time on peak elution time and symmetry were studied and optimized. The validated method complies with ICH guidelines. [23]

2.2.3. High-performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is an essential technique for analyzing the purity and composition of pharmaceutical formulations, particularly in cases where gas-liquid chromatography (GLC) is unsuitable due to the instability or low volatility of certain compounds. As a result, HPLC has become the preferred method for quality control in pharmaceutical production and is extensively referenced in international pharmacopeias. The continuous development of highly selective adsorbents and enhancements in the sensitivity of spectrophotometric, fluorometric, and electrochemical detectors have further broadened its applications in pharmaceutical analysis.

Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely utilized for determining azole antifungals in combination with corticosteroids in pharmaceutical products. Most analytical methods employ isocratic elution with reversed-phase columns, such as C8 or C18, using mobile phases consisting of organic solvent-buffer mixtures that are pH-adjusted to optimize separation efficiency.

Numerous HPLC techniques have been developed for the simultaneous quantification of azole antifungals and corticosteroids in combination formulations [17, 18, 22, 24-57]. Reversed-phase HPLC (RP-HPLC) has proven effective for separating mometasone (MOM) and miconazole (MCN) in topical products, with several studies reporting successful applications [17, 24-30]. Among these, Shah et al. [28] presented the most sensitive approach, achieving detection limits of 0.048 μg/mL for MOM and 1.31 μg/mL for MCN. Despite validation, concerns persist regarding the reliability of these methods due to a lack of systematic optimization. Notably, Fahmy et al. introduced the only environmentally friendly method for this combination. [25]

The RP-HPLC analysis of beclomethasone (BEC) and clotrimazole (CLT) in semisolid formulations has been ex-

plored in multiple studies [31-36], with Dhudashia et al. [31] offering a method characterized by superior sensitivity and an extensive linear range. This approach achieved limits of detection of 1.24 µg/mL for CLT and 0.039 µg/mL for BEC, making it applicable across a broad spectrum of formulations. However, only Bhaskararao et al. have confirmed the stability-indicating capability of their method through forced degradation testing. [34]

For betamethasone (BMD) and clotrimazole (CLT), RP-HPLC methods exhibit variability in sensitivity, and some studies do not report detection limits, complicating direct comparisons. Lin et al. [39] demonstrated the stability-indicating nature of their method by profiling impurities and conducting forced degradation experiments.

The determination of isoconazole (ISO) and diflucortolone (DIF) in cream formulations has been carried out using RP-HPLC and ultra-performance liquid chromatography (UPLC). [18, 40-42] Ozen et al. [41] developed a highly sensitive UPLC method with detection limits of 0.11 µg/mL for ISO and 0.35 µg/mL for DIF, while Gundogdu et al. [42] validated their RP-HPLC method as stability-indicating.

Simultaneous quantification of ketoconazole (KET) and beclomethasone dipropionate (BMD) has been primarily assessed based on linearity, as some studies lack detection limit data. The method proposed by Kannaiah et al., [43] which incorporates a central composite design for optimization, stands out for its improved reliability and eco-friendliness.

In the case of miconazole (MCN) and hydrocortisone (HCA) in creams, three HPLC methods have been documented, with the approach by Manikanta et al. [45] being particularly notable for its broad linear range and high sensitivity, achieving detection limits of 0.56 µg/mL for HCA and 0.57 µg/mL for MCN.

Table 1. *Thin-layer chromatographic methods used for the analysis of azole antifungals combinations with corticosteroids.*

No.	Analytes	Plate	Mobile Phase	Detection λ (nm)	Working range (ng/spot)	LOD (ng/spot)	Ref.
1	mometasone and miconazole in cream	Silica gel $60F_{254}$	methanol, ethyl acetate, toluene, acetonitrile, and 3 M ammonium formate in water (1:2.5:6.0:0.3:0.2 v/v/v/v/v)	224	400-2400 NAD and MCN 100-600 MOM	44.77 MCN 7.03 MOM	[15]
2	mometasone and miconazole in cream	Silica gel $60F_{254}$	toluene, ethyl acetate, ethanol, and formic acid (10:3:2:0.5 v/v/v/v)	235	60-220 MOM 1,200-4,400 MCN 600-2,200 NAD	14.07 MOM 326.94 MCN	[16]
3	mometasone and miconazole in cream	Silica gel $60F_{254}$	toluene, ethylacetate, and ethanol (10:3:2; v/v/v)	240	1200–5200 MCN and MUP 60–260 MOM	19.04 MOM 296.23 MCN	[17]
4	isoconazole nitrate and diflucortolone valerate in cream	Silica gel $60F_{254}$	ethyl acetate, chloroform, and toluene (60:10:10 v/v/v)	215	0.1-4 ISO and 0.1-1.4 DIF	NA	[18]
5	miconazole and clobetasol in cream	Silica gel $60F_{254}$	toluene, Chloroform, Methanol, and Ammonia (5:4:1:0.1 v/v/v/v)	235	20-100 MCN 800-4000 CLB	1.21 CLB 63.88 MCN	[19]
6	betamethasone and miconazole in cream	Silica gel $60F_{254}$	chloroform, acetone, and glacial acetic acid (34:4:3 v/v/v)	233	300-1200 BET 5-20 MCN	50.9 BET 0.68 MCN	[20]
7	fluocinolone and miconazole in ointment	Silica gel $60F_{254}$	n-hexane: ethyl acetate (1:9, v/v)	254	200-700 FLC 40,000-140,000 MCN	11.54 FLU 2887.5 MCN	[21]
8	Triamcinolone and econazole in the presence of its impurities and degradation products in cream	Silica gel $60F_{254}$	ethylacetate-tetrahydrofuran-ammonia (10.0:7.0:0.1, v/v/v)	225	0.20-28.00 TRI 0.50-55.00 ECO	0.056 TRI 0.16 ECO	[22]

Econazole (ECO) and triamcinolone (TRI) have been analyzed using both isocratic and gradient elution HPLC methods.

The gradient elution technique effectively separates ECO and TRI from excipients and degradation products within a reasonable timeframe. Meanwhile, Abbas et al. [22] developed a method demonstrating high selectivity and sensitivity in resolving TRI impurities and degradation products, making it well-suited for routine quality control applications.

For ketoconazole (KET) and clobetasol (CLB) combinations in creams, both isocratic and gradient elution methods have been explored. The gradient elution approach ensures better separation of analytes from related substances [50], while the isocratic method offers practical advantages for routine analysis, achieving detection limits of 0.7 µg/mL for KET and 2.0 µg/mL for CLB [49].

Despite the abundance of reported HPLC methods, only one has been optimized using an experimental design approach [43]. Many methods proceed directly to validation without proper optimization or inaccurately equate preliminary adjustments with true optimization. Ideally, optimization should be a distinct step following method development, integrated into the broader analytical lifecycle. While these methods comply with international validation guidelines such as ICH Q2 (R1) [58], they often lack robustness in real-world quality control settings and may not be suitable for their intended purpose [59]. Given the increasing regulatory emphasis on analytical quality by design (AQbD), there is a growing need for stricter publication standards to ensure the development of reliable and high-quality analytical methods.

2.3. Spectrophotometric Methods

UV-Vis spectroscopy is a widely used analytical technique for both qualitative and quantitative analysis of compounds that contain chromophores capable of absorbing light in the ultraviolet (UV) or visible spectrum. The Beer–Lambert law establishes a direct relationship between light absorption and the concentration of the analyte. This method is valued for its ease of use, minimal sample preparation, affordability, and rapid analysis time.

However, the application of direct spectrophotometry in the analysis and quality control of complex mixtures presents significant challenges, particularly when spectral overlap occurs between components. To address this issue, various mathematical approaches have been developed to process spectral data, enhancing resolution and improving accuracy. The effectiveness of these computational techniques largely depends on the degree of spectral interference and the number of substances present in the mixture.

Different methods have been reported by Abbas et al. [60] and Abou-elkheir et al. [61] for determining miconazole (MCN) and hydrocortisone (HCA) in topical dosage forms. Both researchers have utilized the absorption correction method, with no significant distinction between the two. However, when comparing these methods, the bivariate method reported by Abou-elkheir et al. [61] is the most sensitive for MCN, with LOD values of 0.3 µg/mL for MCN and 0.81 µg/mL for HCA. These methods cover a wide concentration range, from as low as 1 µg/mL up to 40 µg/mL, depending on the method and analyte. Notably, the first derivative of the ratio spectra method and the bivariate method offer broader ranges, making them versatile for various sample types. [61] These methods are applicable to a variety of forms, including pharmaceutical dosage forms, bulk powders, and topical creams. The choice of method should be guided by specific application needs, such as the required sensitivity or the analyte concentration range.

Ramzia et al. [24] have reported derivative and derivative of the ratio spectrophotometric methods, while Merey et al. [62] have introduced three spectrophotometric and two chemometric methods for the determination of miconazole and mometasone in cream preparations. The linear ranges of all these methods are comparable and fall within the typical ranges encountered in spectrophotometric analysis. Direct comparison of the methods' sensitivity is not feasible since Merey et al. [62] did not report LOD values., however among the methods reported by Ramzia et al. [24], the ratio derivative method was more sensitive than the first derivative method, with LOD values of 1.44 µg/mL for MCN and 1.78 µg/mL for MOM. It is worthmentionening that none of these methods has undergone any validation step.

Chemometric methods (PCR, PLS, and ANN) [63] and spectrophotometric methods (Q-absorption ratio, dual wavelength, and first-order derivative) [64] have been described for determining betamethasone (BMD) and clotrimazole (CLT) in creams. A direct comparison of the methods' sensitivity is challenging because Darwish et al. [63] did not report LOD values. However, among the methods reported by Dhudashia et al. [64], the first derivative method proved to be the most sensitive, with LOD values of 0.22 µg/mL for BMD and 0.58 µg/mL for CLT, surpassing the sensitivity of the other two methods.

Fares et al. [68] have reported five spectrophotometric methods for the determination of clotrimazole and hydrocortisone in topical creams. These methods share the same linear range and exhibit nearly identical sensitivity values.

Several researchers have utilized advanced chemometric techniques and mathematical processing of spectrophotometric data to address challenges posed by overlapping spectral bands [66-69]. Although these approaches significantly enhance spectral resolution and accuracy, their implementation requires in-depth mathematical knowledge, access to high-performance spectrophotometers, and specialized software for data analysis. Consequently, their widespread adoption in routine quality control remains limited. A summary of the reported spectrophotometric methods is provided in Table 3.

Table 2. HPLC methods for azole antifungals combinations with corticosteroids.

No.	Drug	Column/Stationary phase	Mobile phase	Working range (µg/mL)	Detection (nm)	Detection limit (µg/mL)	Ref.
1	Mometasone furoate and miconazole nitrate in cream	C_{18} 3.9 ×300 mm, 10 µm	A 1.5% w/v aqueous ammonium acetate buffer (pH 7.6) and acetonitrile were used as the mobile phase, with a flow rate of 2 mL/min.	5-320 MOM 25-800 MCN	240 MOM 230 MCN	1.61 MCN 3.5 MOM	[24]
2	Hydrocortisone, mometasone, beta-methasone, clotri-mazole and micon-azole in creams	C_{18} 150 ×4.6 mm, 5 µm	methanol–water (80: 20%, v/v), at a flow rate of 1 mL/min.	0.05–9.00 HCA, 0.1–9.0 MOM, 0.2–9.0 BET, 0.6–9.0 CLT	photo-diode array de-tector	NA	[25]
3	Mometasone furoate, nadifloxacin and miconazole nitrate in their combined dos-age form	C_{18} 2.1 ×100 mm, 2.2 µm 30 ℃	methanol, acetonitrile and water with ratio (50:20:30; v/v/v) and 0.1 g ammonium acetate, then pH was adjusted to (7.00) using acetic acid, at a flow rate of 0.6 mL/min.	5-400 MCN 20 - 500 MOM	220	5.0 MOM 1.0 MCN	[26]
4	Mometasone furoate and miconazole nitrate in semisolid dosage form	C_{18} 4.6 ×100 mm, 5 µm	0.1% v/v triethylamine: methanol: acetonitrile (40:30:30 V/V/V; pH 3.5). The flow rate was 0.6 mL/min	200-600 MCN 10 - 30 MOM	235	1.48 MOM 1.20 MCN	[27]
5	Mometasone and miconazole cream	C_{18} 250 ×4.6 mm, 5 µm	0.02% triethylamine: metha-nol (15:85;% v/v), at a flow rate of 0.9 mL/min	20–180 MCN 1–9 MOM	240		[17]
6	Mometasone furoate and miconazole nitrate in cream formulation	C_{18} 150 mm x 4.6 mm, 5 µm	acetonitrile: water (80:20 v/v) as mobile phase, having flow rate 0.6 mL/min	2-7 MOM 40-140 MCN	229	0.048 MOM 1.31 MCN	[28]
7	Mometasone furoate, nadifloxacin and miconazole nitrate in semisolid dosage form	C_{18} 4.6 ×250 mm, 5 µm	methanol and 0.02% tri-ethylamine in an 80:20 (v/v) ratio was used, with the pH adjusted to 4.2 using ortho-phosphoric acid and a flow rate set at 0.7 mL/min.	1-9 MOM 20-180 MCN	225	NA	[29]
8	Miconazole and Hydrocortisone cream Miconazole and mometasone cream	C_{18} 4.6 ×250 mm, 5 µm	2.22 mM NaH_2PO_4 (Tri-ethylamine 0.2%): acetonitrile (45:55, v/v) at a flow rate of 0.9 mL/min acetonitrile: water (Tri ethylamine 0.2%) (70:30, v: v) at a flow rate of 0.9 mL/min	300 - 800 MCN 40 - 800 HCA 100 - 2000 MIC 20 - 600 MOM	215 MCN 245 HCA 215 MCN 250 MOM	3.18 MCN 1.55 MOM 2.15 MCN 1.30 MOM	[30]
9	Clotrimazole and beclomethasone dipropionate in lo-tion and cream	C_{18} 150 mm ×4.6 mm, 5 µm	acetonitrile–water (70:30, v/v) was used as the mobile phase, at a flow rate of 1.0 mL/min	2-16 BEC 80-640 CLT	254	1.24 CLT 0.039 BEC	[31]
10	Beclomethasone	C_{18} 150 mm ×4.6	acetonitrile and buffer (30:70)	0.05-0.38	239	NA	[32]

No.	Drug	Column/Stationary phase	Mobile phase	Working range (µg/mL)	Detection (nm)	Detection limit (µg/mL)	Ref.
	dipropionate, clotrimazole and neomycin sulphate in bulk and pharmaceutical dosage forms	mm, 5 µm	v/v; at a flow rate of 1.0 mL/min.	BEC 0.200-1.506 CLT			
11	Beclomethasone Dipropionate and clotrimazole from dosage form	C_{18} 250 ×4.6 mm, 3 µm 40 ̊C	methanol: buffer, 60:40, v/v (20 mM potassium dihydrogen orthophosphate at pH 6.8 maintained with diluteorthophosphoric) at flow rate of 1.0 mL/min	100- 300 for both	254	10.0 CLT 10.0 BMD	[33]
12	Beclomethasone, neomycin, lignocaine and clotrimazole dosage ear drops formulation	C_{18} 150 ×4.6 mm, 3 µm	of 0.01 M KH_2PO_4 (pH 3.5) and acetonitrile in the ratio of 50: 50 v/v as mobile phase, with flow rate of 0.7 ml/min.	50-300 LIG 25-150 CLT, 12.5-75 NEO 0.625-3.75 BEC	215	NA	[34]
13	Beclomethasone dipropionate) and clotrimazole in bulk and cream	C_{18} 4.6 ×250 mm, 5 µm	1 mM ammonium acetate buffer and acetonitritle (10:90 v/v) as mobile phase at flow rate of 1.0 mL/min	10-60 BEC 5-30 CLT	223	0.773 BEC 0.121 CLT	[35]
14	Beclomethasone dipropionate, clotrimazole, chloramphenicol and lidocaine in ear drops	C18 4.6 ×250 mm, 5 µm	Solution A (1.6 gm CH_3COONH_2 in 1 L water, add 1o ml trimethylamine and adjust the to the pH to 6.4±0.1 with diluted acetic acid) and Solution B: acetonitrile, at a flow rate of 1.0 mL / min	1-6 BMD 37.5-225.0 CLT	254	NA	[36]
15	Betamethasone dipropionate (BTD) and clotrimazole with benzyl alcohol as preservative	C_{18} 250 ×4.6 mm, 5 µm	water: acetonitrile (35:65, v/v) as mobile phase at flow rate of 1 mL/min	0.5-20 BMD 2-300 CLT	230	0.065 BMD 0.503 CLT	[37]
16	Betamethasone and clotrimazole in cream formulations	C_{18} 4.6 ×250 mm, 5 µm	methanol-acetate buffer-acetonitrile (33:27:40, v/v)	25-75 for both	254	NA	[38]
17	Betamethasone Dipropionate, Clotrimazole and their related substances	C_8 3.9×150 mm, 5 µm	1.8 volume of methanol and 1.0 volume of 25 mM K_2HPO_4 adjusted with phosphoric acid to an apparent pH of 7.0. The flow rate 1.2 mL/min	400–2000 MCN 40-200 BMD	254	0.13 CLT 0.34 BMD	[39]
18	Diflucortolone valerate and isoconazole nitrate in creams	C_{18} 250 ×4.6 mm, 5 µm	methanol–water (95: 5, v/v)	4.00–48.0 DIF 50.0–400.0 ISO	280	0.76 DIF 0.22 ISO	[40]
19	Isoconazole nitrate and diflucortolone valerate in pharma-	C_{18} 250 ×4.6 mm, 5 µm C_{18} (UPLC) 50 x	methanol: water (80:20, v/v) The flow rate 1.0 mL/min methanol: water (61:39, v/v)	15-240 ISO 6-60 DIF	220	0.25 ISO 0.45 DIF 0.11 ISO	[41]

No.	Drug	Column/Stationary phase	Mobile phase	Working range (µg/mL)	Detection (nm)	Detection limit (µg/mL)	Ref.
	ceutical creams	2.1 mm, 1.8 µm 40 °C	The flow rate 0.5 mL/min			0.35 DIF	
20	Isoconazole nitrate and diflucortolone valerate cream	C_{18} 150 ×4.6 mm, 5 µm at 40 °C	0.03 M NaH_2PO_4 buffer and methanol mixture in the ratio of 27: 73, v/v. The flow rate was 1.5 mL/min	2-12 DIF 20-123 ISO	240	0.2 DIF 1.0 ISO	[42]
21	Isoconazole nitrate and diflucortolone valerate cream	C_{18} 250 ×4.6 mm, 5 µm	acetonitrile: methanol 80:20%v/v. The flow rate 1 mL/min	10–90 ISO 2–18 DIF	230	NA	[18]
22	Ketoconazole and beclomethasone in bulk and cream	C_{18} 250 ×4.6 mm, 5 µm	ethanol: 0.1 M KH_2PO_4 buffer (pH 2.5) 33: 67 v/v. The flow rate was 1.0 mL/min	140–260 KET 1.75–3.75 BMT		NA	[43]
23	Beclomethasone dipropionate and ketoconazole in combined dosage form	C_{18} 250 ×4.6 mm, 5 µm	acetonitrile: acidic water (pH is adjusted 6.0 with acetic acid) (57: 43, v/v) at flow rate of 0.8 mL/min.	0.5-50 for both	254	0.108 BEC 0.161 KET	[44]
24	Hydrocortisone and miconazole in topical dosage forms	C_{18} 250 ×4.6 mm, 5 µm	Methanol: Buffer (KH_2PO_4 0.1 M) 80:20 v/v, at a flow rate of 1.0 mL/min.	20-80 for both	239	0.56 HCA 0.57 MCN	[45]
25	Hydrocortisone acetate and miconazole nitrate in cream	C_{18} 4.6 ×250 mm, 5 µm	0.1 M ammonium acetate (80:20, v/v) at a flow rate of 1.0 ml/min	10-60 MCN 20-60 HCA	225	0.87 HCA 2.03 MCN	[46]
26	Econazole nitrate, triamcinolone acetonide and benzoic acid in ternary mixture	C_{18} 4.6 ×150 mm, 5 µm	methanol: 50 mM potassium dihydrogen phosphate buffer (pH 2.60 ±1), (70:30, v/v) Isocratic flow rate at 1.0 mL/min	10-200 ECO 1-20 TRI	230	NA	[47]
27	Econazole nitrate, triamcinolone acetonide, benzoic acid and butylate hydroxyanisole	C_8 4.6 ×150 mm, 5 µm	Gradient elution of the mobile phase composed of 0.2% w/v phosphoric acid (adjusted to pH 3.0 using ammonia solution) and methanol. 0 min; 75% A at 8 min; 40% A, at 10 min; 40% A at 11-15 min; 35% A. The flow rate was 2.0 mL/min. At 19-20 min; 30% A. The flow rate was 2.0 mL/min.	1.5–300 ECO 1–200 TRI 0.6–100 BA 1–100 BHA	225 ECO 242 TRI 290 BHA	0.15 ECO 0.14 TRI	[48]
28	Triamcinolone and econazole in the presence of impurities and degradation products	C_{18} 4.6 ×250 mm, 5 µm	acetonitrile–methanol–0.05 M potassium dihydrogen phosphate mobile phase, pH 3.0 (25.0 + 15.0 + 60.0, v/v/v)	0.05–30.00 TRI 1.00–40.00 ECO	225	0.015 TRI 0.317 ECO	[22]
29	Clobetasol and ketoconazole in cream	C_{18} 4.6 ×250 mm, 5 µm	methanol: acetonitrile: phosphate buffer (50:20:30 v/v/v) as mobile phase composition. The flow rate	6-14 CLB 120-280 KET	254	0.7 CLB 2.0 KET	[49]

No.	Drug	Column/Stationary phase	Mobile phase	Working range (µg/mL)	Detection (nm)	Detection limit (µg/mL)	Ref.
			was adjusted to 1.5 mL/min A gradient elution was performed using acetonitrile (A) and 0.01 M ammonium acetate (pH 7.5) in purified water (B), following the specified gradient program. 0-7 min→ 25%-30% A 7-8 min →30-34% A 8-25 min→34-40%A 25-35 min→40-48%A 35-42 min→48–55% A 42-47 min→55% A. The flow rate was 1.0 mL/min.				
30	Ketoconazole and clobetasol propionate cream	C_8 4.6 mm x 100 mm, 2.7 µm			MS	0.06 for both	[50]
31	Miconazole and Clobetasol and Characterization of Hydrolytic Degradation Products	C_{18} 150 ×4.6 mm, 5 µm Column temperature 40 °C	ammonium acetate buffer (10 mm, pH 4.2 adjusted with acetic acid) and acetonitrile (43:57 v/v). The flow rate was 0.75 ml/min.	100-600 MCN 5-30 CLB	240 MS	3.85 MCN 0.29 CLB	[51]
32	Hydrocortisone and clotrimazole in topical dosage	C_{18} 250 ×4.6 mm, 5 µm	acetonitrile: buffer (dissolve 4.35 g of dibasic potassium phosphate in water to make 1000 mL of solution) 75:25%, v/v The flow rate was kept at 1.0 mL/minute.	100–300 for both	254	14.39 HCA 11.23 CLT	[52]
33	Halobetasol propionate and miconazole nitrate in marketed formulation	C_{18} 250 ×4.6 mm, 5 µm	A mixture of 0.2% orthophosphoric acid in water and acetonitrile (40:60 v/v) was used as the mobile phase, with a flow rate of 1.0 mL/min.	8-12 HAL 320-480 MCN	291	0.87 HAL 14.7 MCN	[53]
34	Methylparaben, ketoconazole, and mometasone furoate in topical pharmaceutical dosage formulation	C_{18} 4.6 ×250 mm, 5 µm	0.2% v/v triethyl amine in water, pH adjusted to 6.5 with glacial acetic acid): acetonitrile (40:60, v/v). The flow rate was adjusted to 1.5 mL/minute	0.67–149.4 KET 0.42–7.6 MOM	250	0.204 KET 0.12 MOM	[54]
35	Luliconazole and clobetasol propionate in synthetic mixture	C_{18} 50 ×4.6 mm, 5 µm	Acetonitrile: Water pH adjusted with H_3PO_4 (60: 40)	10-200 LUL 5-100 CLB	264	0.032 LUL 0.02 CLB	[55]
36	Miconazole, triamcinolone, methylparaben and propylparaben in cream	C_8 (150 x 4.6 mm, 5 µm) Finasteride internal standard	5 mM trichloroacetic acid in 0.05% phosphoric acid and acetonitrile (52: 48% v/v) at a flow rate of 0.9 mL/min	50-500 MCN 5-50 TRI	264	NA	[56]
37	Marbofloxacin, dexamethasone and clotrimazole in a liquid pharmaceuti-	C18 75 ×4.6 mm, 4 µm 35 ℃	(A) buffer solution (acetic acid/ ammonium acetate, pH 4)/(B) acetonitrile, delivered by gradient elution, as fol-	60-140 DEX 600-1400	260	NA	[57]

No.	Drug	Column/Stationary phase	Mobile phase	Working range (μg/mL)	Detection (nm)	Detection limit (μg/mL)	Ref.
	cal dosage form		lows: (B) 0–3 min 17%, 4 min 50%, 4–9 min 50%, 10 min 70%, 11 min 70%, 12 min 95%, 14 min 95%, 15 min 17%, 20 min 17%. The flow rate was 1.7 mL/ min	CLT			

Table 3. *Methods for spectrophotometric methods analysis of azole antifungals combinations with corticosteroids.*

No.	Analytes/Dosage form	Method	λ (nm)	Working range (μg/mL)	LOD (μg/mL)	Ref.
1	miconazole nitrate and hydrocortisone acetate in pharmaceutical dosage form	Absorbance correction	205 MCN 249 HCA	1-12 MCN 1-40 HCA	0.4944 MCN 1.5081 HCA	[60]
2	miconazole nitrate and hydrocortisone in bulk powder and in topical cream	First derivative of the ratios spectra	233, 285 MCN 240, 256 HCA	5-30 MCN 5-30 HCA	0.5 MCN 1.2 HCA	[61]
		Absorbance correction	231.5 total 241.5 HCA	5-30 5-30	1.3 MCN 0.81 HCA	
		Factorized absorptivity Method	217.5, 224 nm (two isosbestic points) 241.5 HCA	3-25 3-15	0.81 MCN 0.79 HCA	
		Bivariate method	223 and 237	10-30 10-35	0.3 MCN 0.81 HCA	
3	mometasone furoate and miconazole nitrate in cream	First derivative	270.5 MCN 282.1 MOM	5-80 MCN 50-500 MOM	0.93 MCN 8.25 MOM	[24]
		Derivative ratio	267.1 MCN 281.2 HCA	5-50 MCN 30-600 MOM	1.44 MCN 1.78 MOM	
		Derivative ratio spectra (DD)	265.5 MOM 284.2 MCN	6-33 MOM 180-840 MCN		
4	mometasone furoate and miconazole nitrate in topical pharmaceuticals	Derivative of double divisor of ratio spectra (DD-RS)	251.5 MOM 262.3 MCN	3-33 MOM 60-840 MCN	NA	[62]
		Mean centering	249 MOM 279 MCN	6-33 MOM 60-480 MVN		
		Partial Least Squares (PLS) method	200-400	6-33 MOM 60-840 MCN		
		Principal component regression (PCR) method		6-33 MOM 60-480 MVN		
5	betamethasone dipropionate, clotrimazole and benzyl alcohol in cream	Principal component regression (PCR) Partial Least Squares (PLS) Artificial Neural Net-	235 – 275	8-16 BMD 120-200 MCN 200-400 BA	NA	[63]

No.	Analytes/Dosage form	Method	λ (nm)	Working range (µg/mL)	LOD (µg/mL)	Ref.
		works (ANN)				
6	clotrimazole and beclomethasone dipropionate in their combined dosage forms	Q-absorption ratio	260.6 (λ_{max} of CLT) 250.8 (Isoabsorptive point)	100-450 CLT 6-34 BMD	1.65 CLT 0.32 BMD	[64]
		Dual Wavelength	237, 241 BMD 259, 264 CLT		1.7 CLT 0.41 BMD	
		First Order Derivative	260.8 CLT 239 BMD		0.58 CLT 0.22 BMD	
7	betamethasone, clotrimazole and neomycin in topical cream	Simultaneous equation	240 and 300	10-50 CLT 2 – 10 BMD	1.25 CLT 0. 99 BMD	[65]
8	clotrimazole and dexamethasone in cream	Mean centering	CLT: 205–240 measuring amplitude 234 DEX: 205–240 dividing by amplitude 234	1-12 CLT 2-20 DEX	NA	[66]
		Partial Least Squares (PLS) method	233–278	1-12 CLT 2-20 DEX		
		Principal component regression (PCR) method				
9	clotrimazole with dexamethasone in cream	Constant center	260 CLT 239 DEX	75–550 CLT 2–20 DEX	NA	[67]
		Constant value via amplitude difference	247 CLT 266.6 DEX			
		Ratio difference	247 and 266.6 CLT 270 nm and 247 DEX			
		Derivative ratio	272 CLT P_{242_252} DEX			
10	clotrimazole and hydrocortisone in a topical cream	First derivative	247.4 CLT 236.2 HCA	5 - 40 CLT 5 - 45 HCA	1.21 CLT 1.26 HCA	[68]
		Second derivative of ratio	225.4 CLT 269 HCA		1.13 CLT 1.38 HCA	
		Dual wavelength	225.4 and 264 CLT 228 and 247 HCA		1.56 CLT 1.14 HCA	
		Advanced absorbance subtraction	225.4 and 264		1.39 CLT 1.44 HCA	
		Mean centering of ratio spectra	232-265		1.43 CLT 1.18 HCA	
11	diflucortolone valerate and isoconazole nitrate in cream	Constant center	238 DIF 272.2 ISO	5-60 DIF 65-850 ISO	NA	[69]
		Ratio difference	245.5 and 255 DIF 260 and 270 ISO			

No.	Analytes/Dosage form	Method	λ (nm)	Working range (µg/mL)	LOD (µg/mL)	Ref.
		First derivative ratio spectra	241.1 DIF 279.8 ISO			
		Mean centering of ratio spectra	245.6 DIF 282 nm ISO			
12	isoconazole nitrate and diflucortolone valerate in creams	Second derivative	289.2 ISO 262 DIF	100-500 ISO 5-25 DIF	2.63 ISO 0.54 DIF	[41]
13	betamethasone and luliconazole in synthetic mixture	Simultaneous equations	243.20 LUL 225.00 BET	10-50 LUL 20-100 BET	0.083 LUL 0.249 BET	[70]

3. Conclusion

This review provides an in-depth analysis of the progression of analytical methods used for quantifying azole antifungal agents combined with corticosteroids in topical pharmaceutical formulations from 1999 to 2024. High-performance liquid chromatography (HPLC) has emerged as the most widely utilized technique, featuring in nearly 60% of the reported studies. Its popularity is largely due to its exceptional sensitivity, reliability, and capability to effectively separate complex mixtures, although it requires organic solvents and involves high operational costs. Other commonly employed methods include UV-visible spectrophotometry (24.5%) and thin-layer chromatography (≈14%), both valued for their simplicity, affordability, and ease of method optimization. These attributes make them particularly attractive for routine quality control applications in pharmaceutical laboratories where resources or access to advanced instrumentation may be limited. Capillary zone electrophoresis (CZE), recognized for its environmentally friendly nature and minimal solvent consumption, remains the least utilized technique, with only a single study (<2%) documented over the 25-year review period. This limited adoption may be due to challenges related to reproducibility, method development complexity, and a lack of widespread availability in pharmaceutical laboratories.

Figure 1 presents the distribution of analytical techniques employed for the determination of azole-corticosteroid combinations throughout the study period. Moving forward, research efforts should prioritize the refinement of analytical methodologies by integrating the quality-by-design (QbD) approach to improve method reliability and efficiency. Furthermore, a stronger focus on green analytical chemistry is essential, promoting the adoption of environmentally friendly solvents and miniaturized techniques to minimize ecological impact while preserving analytical accuracy. Advancing these aspects will support the development of more sustainable and effective quality control strategies for azole antifungal-corticosteroid formulations.

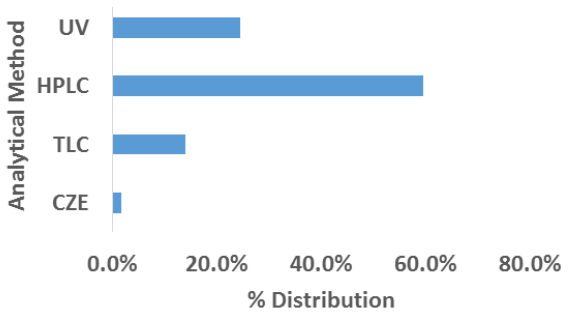

Figure 1. *Reported analytical techniques for the quantification of azole antifungal and corticosteroid combinations: percentage distribution of from 1999 to 2024.*

Abbreviations

FDC	Fixed-dose Combination
QC	Quality Control
RPHPLC	Reverse-phase High-performance Liquid Chromatography
HPLC	High-performance Liquid Chromatography
TLC	Thin-layer Chromatography
HPTLC	High-performance Thin-layer Chromatographic
CZE	Capillary Zone Electrophoresis
Rf	Retardation Factor
BP	British Pharmacopoeia
USP	United States Pharmacopoeia
ICH	International Conference on Harmonization
AQbD	Analytical Quality by Design
PCR	Principal Component Regression
PLS	Partial Least Squares
ANN	Artificial Neural Networks
CLT	Clotrimazole

HCA	Hydrocortisone Acetate
MCN	Miconazole
FLC	Fluocinolone Acetonide
MOM	Mometasone Furoate
NAD	Nadifloxacin
ISO	Isoconazole
DIF	Diflucortolone
CLB	Clobetasol Propionate
BET	Betamethasone Valerate
ECO	Econazole Nitrate
TRI	Triamcinolone Acetonide
MUP	Mupirocin
BMD	Betamethasone Dipropionate
BA	Benzyl Alcohol
DEX	Dexamethasone Acetate
LUL	Luliconazole
HAL	Halobetasol Propionate
LID	Lidocaine
CHL	Chloramphenicol
LIG	Lignocaine
KET	Ketoconazole
BEC	Beclomethasone Dipropionate
BAA	Benzoic Acid
BHA	Butylate Hydroxyanisole

Author Contributions

Imad Osman Abu Reid: Conceptualization, Supervision, Writing – review & editing

Sayda Mohamed Osman: Data curation, Formal analysis

Somia Mohammed Bakheet: Methodology, Writing – original draft

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Conflicts of Interest

The authors declare no conflicts of interest.

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