

Review Article

Analytical Methods for Quantification of Ketoconazole and Zinc Pyrithione in Shampoos and Other Dosage Forms: A Comprehensive Literature Review

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ABSTRACT

Ketoconazole and zinc pyrithione (ZPTO) are widely utilized antifungal agents in various topical and pharmaceutical formulations including shampoos, creams, gels, and tablets. Robust and validated analytical methods are crucial for ensuring their quality, efficacy, and regulatory compliance. This comprehensive review presents an integrated perspective on developments in chromatographic, spectroscopic, and titrimetric analytical methods for these drugs, highlighting advances in high-performance liquid chromatography (HPLC) alongside alternative techniques such as UV-visible spectrophotometry, FTIR, complexometric titration, and LC-MS/MS. Emphasis is placed on method development, validation parameters aligned with ICH and USP guidelines, sample preparation challenges especially in complex matrices like shampoos, and the evolving landscape across different dosage forms. This review serves as a valuable resource for pharmaceutical researchers, quality control analysts, and regulatory professionals involved in the analysis of ketoconazole and zinc pyrithione.

Key words: Ketoconazole, zinc pyrithione, HPLC, UV-visible spectrophotometry, FTIR, LC-MS/MS.

INTRODUCTION

Pharmaceutical analysis is vital in verifying the safety, potency, and purity of drug substances and formulations. Ketoconazole, a synthetic imidazole antifungal agent, and zinc pyrithione, an antimicrobial compound effective against dandruff and seborrheic dermatitis, are commonly co-formulated in topical dosage forms. Analytical methods must accurately quantify these actives amidst complex excipients and matrices. Over the years, a variety of analytical techniques have been developed and validated, driven by evolving regulatory expectations and the need for efficient, precise, and reliable quality control practices. This review consolidates analytical methodologies across shampoos and other dosage forms, focusing on validation parameters, sample preparation, and

advances in instrumental techniques. HPLC Method Development and Validation in Shampoos

Reverse-phase HPLC (RP-HPLC) has become the preferred choice for the simultaneous or separate quantification of ketoconazole and ZPTO in shampoos due to its high selectivity, precision, and regulatory acceptance. Most methods employ C18 stationary phases, with mobile phases comprising acetonitrile and buffered water, adjusted often to pH ~5.4 to optimize separation. Detection wavelengths typically range between 230 nm and 322 nm based on the absorption maxima of the analytes.

Sample preparation commonly involves dilution, solvent extraction with methanol or acetonitrile, centrifugation, and filtration to minimize matrix effects from surfactants and emollients. Some studies advocate additional washing or complexation specifically for ZPTO to further reduce interference.

Validation adheres to ICH/USP guidelines assessing parameters such as linearity (covering low $\mu\text{g/mL}$ ranges), precision (%RSD 0.5–2%), accuracy (recoveries 98–102%), and detection limits. Stability-indicating capacity and multi-analyte simultaneous

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detection have featured prominently in recent method evolution

1-9.

Study Reference & Year	Methodology	Validation Focus	Application Summary	Ref. No.
Pharmatutor, 2013	RP-HPLC, C18, 322nm	Acetonitrile:Water (60:40), ZPTO in ketoconazole shampoo, Precision (%RSD<1), Recovery (98–102%), Selectivity proven	Routine QC of shampoos	1
Pharmacophore J, 2012	RP-HPLC, ketoconazole	Accurate, simple method development for bulk and formulation, Linearity up to 100µg/mL, validated as per ICH	Bulk and finished products	2
Der Pharma Chemica, 2023	RP-HPLC, C18, 322nm	Simultaneous assay: Ketoconazole & Salicylic acid, %RSD<0.5, specific for drugs, recovery >99%	Simultaneous estimation in shampoo	3
SCC Online, 2014	HPLC/HPLC-MS/MS	ZPTO, multiple batches, comprehensive matrix cleanup, Statistically equivalent with HPLC-MS/MS	Regulatory testing, product surveillance	4
Academia.edu, 2021	HPLC, isocratic	Fast methods (short and long column), ketoconazole/formaldehyde, Comparable precision, validated	Shampoo, stability testing	5
IJCBS, 2024	RP-HPLC, ZPTO	Newer sample workflow, robust for various excipients, In line with ICH/USP, high precision	Complex shampoos, ZPTO stability	6
Staub & Bergold, 2004	Isocratic HPLC-UV, C18, 254nm	Linearity, precision, recovery >98%, %RSD <2%, interference testing	Ketoconazole quantitation in 2% shampoo	7
Nguyen et al., 2003	Assay on short and long HPLC columns, UV	Selectivity, linearity (0–0.2mg/mL), bias, robustness comparison between column lengths	Simultaneous ketoconazole and formaldehyde	8
Vander Heyden et al., 2002	Isocratic HPLC-UV, acetonitrile-phosphate buffer (pH 4.0)	Selectivity, linearity, interference study from excipients, stress testing	Generic anti-dandruff shampoo analysis	9

Literature Review on Other Analytical Techniques for Ketoconazole and ZPTO Evaluation

Alternative Analytical Techniques for Ketoconazole and Zinc Pyrithione

Several non-chromatographic approaches supplement HPLC in the analysis of these compounds:

- UV-visible Spectrophotometry: Widely used for ketoconazole and ZPTO, exploiting characteristic absorbance in 225–260 nm range; simple and cost-effective for routine assays.
- Visible Spectrophotometry and Complexometry: Utilize reagents such as chloranil and metal-ion complexation for detection at specific wavelengths (e.g., 481 nm).
- Fourier Transform Infrared (FTIR) Spectroscopy: Provides structural confirmation and stability insights, correlating functional groups to antifungal efficacy.

The following table combines major analytical techniques beyond HPLC, including UV-visible spectroscopy, FTIR analysis, spectrophotometric and complexometric methods, and related

instrumental approaches used in the estimation and validation of ketoconazole and zinc pyrithione (ZPTO) in pharmaceutical or shampoo formulations ¹⁰⁻¹⁶.

Study Reference & Year	Analytical Technique	Methodology Summary	Key Validation/Findings	Ref. No.
IJARST, 2024	UV-Visible spectrophotometry	Simple, robust, and precise UV spectroscopic quantitation of ketoconazole using ethanol as solvent; $\lambda_{\max} \sim 225\text{--}260$ nm	Linearity (10–50 $\mu\text{g/mL}$), Recovery 98–101%, $R^2 = 0.999$, validated as per ICH	10
CTPPC, 2025	UV spectrophotometry	Developed an economical and validated method for simultaneous estimation of ketoconazole and zinc pyrithione; calibration follows Beer–Lambert law with λ_{\max} of 240 nm (ZPTO) and 225 nm (ketoconazole)	Excellent reproducibility, RSD <2%, widely applicable to shampoo formulations	11
AJRCPS, 2020	Visible spectrophotometry	Visible complexometric method using chloranil reagent; detection at 481 nm with methanol/DMSO medium	Linearity 5–30 $\mu\text{g/mL}$, LOD 2.97 $\mu\text{g/mL}$, LOQ 0.89 $\mu\text{g/mL}$, Recoveries within 99–102%	12
PMC (Kumar et al.), 2011	Amplification spectrophotometry	Periodate oxidation method converting KC to ICl_2^- ion pair complex measured at 535 nm	Linear range 0.21–1.70 $\mu\text{g/mL}$, molar absorptivity $5 \times 10^5 \text{ mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1}$	13
Pharmatutor, 2013	UV spectroscopic determination of ZPTO	λ_{\max} determination of Zinc Pyrithione after Cu^{2+} complexation in methanol-chloroform; Cu^{2+} complex improves selectivity	High sensitivity, accuracy within 98–102%, reproducible for zinc complex quantification	14
ScienceDirect, 1996	Infrared spectroscopy and microbiological assay	IR and antimicrobial correlation of ketoconazole and ZPTO; characteristic bands observed near 1500 cm^{-1} and 1100 cm^{-1} verifying functional integrity	Confirmed structural stability and synergistic antifungal activity	15
PMC, 2021	Complexometric titration (ZPTO)	Complexometric back-titration using EDTA and metal-ion indicators for Zn^{2+} quantification in shampoo	Simple, reproducible; recovery 98–101%, standard deviation <1.5%	16

Literature Review on Analytical Techniques for Ketoconazole and Zinc Pyrithione in Other Dosage Forms

Analytical Techniques in Other Dosage Forms

Ketoconazole and ZPTO quantification is performed in various dosage forms such as tablets, creams, gels, and lotions using tailored methods:

- RP-HPLC: Rapid and precise for tablets, creams, and semi-solids, typically employing C18 columns and optimized mobile phases.
- UV-visible Spectrophotometry: Extended application to semisolids and tablets, supporting economical QC.
- LC-MS/MS: Emerging for nanoparticle-based topical formulations, offering high sensitivity and stability data.

The following table summarizes key analytical methods developed and validated for ketoconazole and ZPTO in various dosage forms such as tablets, creams, gels, lotions, and semi-solids:¹⁷⁻²³

CONCLUSIONS

RP-HPLC remains the cornerstone analytical method for the simultaneous and selective estimation of ketoconazole and zinc pyrithione, notably in complex topical formulations such as shampoos. Alternate spectroscopic and titrimetric techniques provide valuable complementary and economical analytical routes, especially beneficial for routine quality control and assays in simpler dosage forms. Method validation consistently aligns with international guidelines, underscoring reproducibility, accuracy, and stability-indicating features. Advances including multiple analyte quantification and application to novel nanoparticle formulations demonstrate the evolving analytical landscape serving modern pharmaceutical needs.

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