DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR MICROBIOLOGICAL QUALITY CONTROL OF KETOCONAZOLE SHAMPOO 2%

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INTRODUCTION

Ketoconazole 2% shampoo is a non-sterile topical pharmaceutical preparation containing 20 mg/g micronized ketoconazole, used in the treatment of Malassezia-associated skin disorders. Microbiological quality control is essential for topical preparations, as microbial contamination may affect both product quality and patient safety.

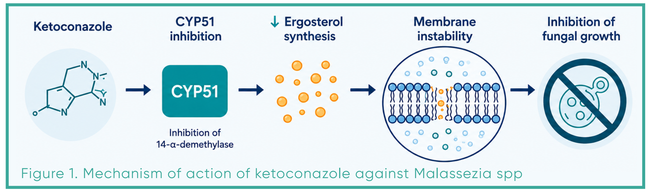


Figure 1. Mechanism of action of ketoconazole against Malassezia spp

CYP51 inhibition disrupts ergosterol biosynthesis, impairing fungal membrane function and inhibiting fungal growth.

The intrinsic antifungal activity of ketoconazole, together with surfactants, preservatives and other formulation excipients, may interfere with microbial recovery. Therefore, effective matrix neutralization and demonstration of method suitability are essential for reliable determination of TAMC, TYMC and specified microorganisms according to Ph. Eur. requirements.

AIM OF THE STUDY

To develop and validate a microbiological quality-control method for Ketoconazole Shampoo 2% in accordance with Ph. Eur. requirements.

MATERIALS & METHODS

Materials & Sample Preparation

Pharmacopoeial approach

- *Ph. Eur. 2.6.12 and 2.6.13*
- *Low-level inocula: <100 CFU*
- *0.45 µm PVDF membrane filters*

Neutralizing system

Buffered sodium chloride-peptone diluent with neutralizers

Sample preparation

10 mL shampoo → ***PDN neutralizers***

Quantitative Method Suitability

PD90 viability control | PDN90 control | Product test

Surface-Spread Method	*Membrane-Filtration Method*
TAMC Test suspension → TSA→ 30–35 °C / 3 d	Test suspension → 0.45 µm PVDF membrane → Rinsing → membrane transfer → **TSA/TAMC 30–35 °C / 3 d**; **SDA/TYMC 20–25 °C / 5 d**
TYMC Test suspension → SDA→ 20–25 °C / 5 d	

Qualitative Method Suitability

Parallel enrichment and selective subculturing for specified microorganisms using low-level inocula (<100 CFU). TSB enrichment: 30–35 °C / 18–24 h.

Staphylococcus aureus

Product / Controls + inoculum → TSB Enrichment → MSA



Pseudomonas aeruginosa

Product / Controls + inoculum → TSB Enrichment → CTRA



RESULTS

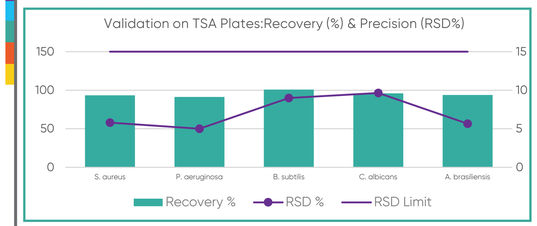


Figure 2. TAMC recovery and precision on TSA.

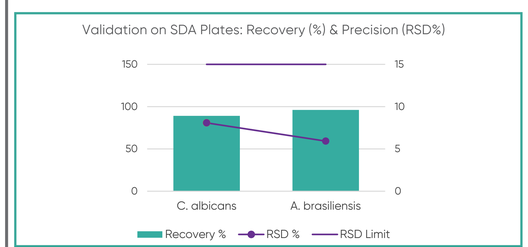


Figure 3. TYMC recovery and precision on SDA.

TAMC recovery ranged from 91.37% to 100.80% across the tested microorganisms.
TYMC recovery ranged from 89.14% to 96.10% for C. albicans and A. brasiliensis.

KEY CRITERIA

50–200%
Recovery

≤ 2
Recovery factor

<15%
RSD

CONCLUSION

The obtained recovery and precision results demonstrate effective neutralization of the intrinsic antimicrobial activity of the Ketoconazole 2% shampoo matrix, without relevant inhibition of the challenge microorganisms. The developed microbiological method demonstrated adequate recovery, precision, and effective neutralization of the intrinsic antimicrobial activity of Ketoconazole 2% shampoo. The method was suitable for both quantitative enumeration and detection of specified microorganisms and is therefore considered reliable for routine microbiological quality control in aaccordance with Ph. Eur. requirements.

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