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Short communication

Development and validation of analytical method for microbiological quality control of Ketoconazole cream 2%

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Introduction

Ketoconazole is a well-established antifungal active substance widely used in topical formulations, particularly 2% creams and shampoos (Choi et al., 2019). As a lipophilic imidazole derivative with weak basic properties (Beggs, 1991), it may present challenges during microbiological examination due to its intrinsic antimicrobial activity. This activity can inhibit microbial growth during testing and may compromise microorganism recovery, creating a potential risk of false-negative results if not adequately neutralized or controlled. Therefore, method suitability testing is essential to confirm that the applied microbiological method can reliably detect microorganisms in the presence of the product matrix.

For non-sterile topical formulations, microbiological quality control includes TAMC, TYMC, and absence of specified microorganisms, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in accordance with European Pharmacopoeia requirements (Ph. Eur. 12.0, 2025). Therefore, the aim of this study was to validate the microbiological method for microbiological quality control of ketoconazole 2% cream.

Materials and methods

Materials

During the method validation, standard laboratory equipment was used: Biosafety Cabinet Class II A, microbiological incubators Binder within temperature intervals 30-35 ºC and 20-25 ºC, Bunsen burner, Orbital shaker and standard, sterile laboratory glass for microbiological use. A 10 g of Ketoconazole 2% cream was weighed using Kern analytical balance. Pharmacopeia diluent, supplemented with neutralizers: Buffered sodium chloride-peptone solution pH 7.0 +3% Polysorbate 80 + 0.3% Lecithin + 0.1% Histidine, was chosen as medium for dissolving and dilution of the sample for culture suspensions. Ready -to -use nutrient media from BioMérieux, Oxoid and Merck were used during this validation.

The microbiological strains that were used in this microbiological validation are standard microorganisms specified by the current European Pharmacopoeia for method suitability test: *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404, prepared at low inoculum levels (<100 CFU/0.1 ml) in accordance with pharmacopeia guidelines (Ph. Eur. 12.0, 2025).

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Method

For the determination of antimicrobial activity and validation of the test method, challenge testing was performed using the specified reference microorganisms.

The surface-spread method was applied using a primary suspension prepared by diluting 10 g of product in 90 mL of Pharmacopeial Diluent with neutralizers (PDN) (1:10 dilution). The method was validated for quantitative determination of Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) in accordance with Ph. Eur. 2.6.12.

Samples were individually inoculated with reference microorganisms at concentrations of 10–100 CFU/ml. All tests were performed in duplicate on three product batches. Incubation conditions were as follows: TSA at 30–35 °C for 3 days, SDA at 20–25 °C for 5 days, and TSB at 30–35 °C for 24 h.

The primary suspension was also validated for qualitative detection of the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* according to Ph. Eur. 2.6.13. Ten milliliters of the suspension were transferred into TSB, inoculated with the specified microorganisms, incubated at 30–35 °C for 24 h, and subcultured on selective media: Mannitol Salt Agar (MSA) for *S. aureus* and Cetrimide Agar for *P. aeruginosa* incubated for 24h at 30-35 ˚C (Ph. Eur. 12.0, 2025)

Results and discussion

The microbiological method used for the analysis of Ketoconazole cream 2% was validated according to European Pharmacopeia requirements. The results showed that the method specificity is demonstrated through the ability of culture media and test conditions to support the growth of the selected microorganism without interference from the product matrix. Characteristic colony morphology and expected microbial responses were obtained. Accuracy was evaluated through recovery percentages with limit values of 50-200%, and recovery factor values under ≤2 on all tested microorganisms. Precision was asserted by repeated analysis under the same experimental conditions. The results were presented by SD or relative standard deviation of a series of measurements. The repeatability results showed RSD below <15%, acceptable according to Ph. Eur. criteria.

Conclusion

The method suitability validation for Ketoconazole 2% cream, a pharmaceutical preparation for topical use, demonstrated no significant product matrix interference against the reference microorganisms when the primary suspension of the product to be examined (1:10 dilution in PDN) was used. The antimicrobial activity of the product was effectively neutralized due to the presence of neutralizers in the dilution medium. The challenge tests for the qualitative detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* demonstrated positive recovery in all three testing iterations for both specified microorganisms, compared to the Product-Negative Control Group. Therefore, the method is considered suitable for routine microbiological quality control and complies with pharmacopeial requirements for non-sterile pharmaceutical products.

References

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