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Short communication

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 pharmaceutical preparation for cutaneous use, containing micronized ketoconazole as the active antifungal substance at a concentration of 20 mg/g. The product is intended for topical dermatological application and is used in conditions associated with *Malassezia* species, including dandruff, seborrheic dermatitis (Piérard-Franchimont et al., 2002), and pityriasis versicolor (Lange et al., 1998). Microbiological quality control is particularly important for preparations applied to the skin, as contamination may compromise both product stability and patient safety. Therefore, microbiological quality assessment of Ketoconazole 2% shampoo should include determination of Total Aerobic Microbial Count (TAMC), Total Yeasts and Moulds Count (TYMC), and confirmation of the absence of specified microorganisms relevant to cutaneous preparations, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa* in accordance with the requirements of the European Pharmacopoeia (Ph. Eur. current). Shampoo formulations may contain surfactants, preservatives, and other excipients that can interfere with microbial recovery. Therefore, validation of the microbiological method for this specific product matrix is essential before implementation in routine quality control.

Materials and methods

Materials

The validation of the microbiological quality control method for Ketoconazole 2% shampoo, was performed under aseptic conditions using standard microbiological laboratory equipment, including a Class II A biosafety cabinet, incubators at 20–25 °C and 30–35 °C, membrane-filtration apparatus, orbital shaker, Bunsen burner, and sterile glassware. Buffered sodium chloride-peptone solution pH 7.0, supplemented with 3% polysorbate 80, 0.3% lecithin, and 0.1% histidine, was used as a pharmacopoeial diluent with neutralizing properties. Sterile 0.45 µm PVDF membrane filters, sterile 0.45% sodium chloride solution, and ready-to-use culture media from BioMérieux, Oxoid, and Merck were applied during the validation.

For sample preparation, 10 mL of shampoo was transferred into the selected diluent and mixed until a homogeneous dispersion was obtained.

Method suitability was evaluated using pharmacopoeial reference strains: *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis*

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ATCC 16404, in order to confirm suitability for TAMC, TYMC, and detection of specified microorganisms in non-sterile cutaneous preparations.

Method

The microbiological method was validated in accordance with the European Pharmacopoeia requirements for non-sterile pharmaceutical preparations, using low-level inocula (<100 CFU/0.1 mL) of pharmacopoeial reference microorganisms. Viability control, product-negative control, and test groups were included to evaluate the potential antimicrobial activity of the product matrix and the effectiveness of the selected neutralizing diluent.

The suitability of the quantitative methods for TAMC and TYMC was assessed by surface-spread and membrane-filtration techniques, performed in duplicate. TAMC was determined on Tryptic Soy Agar after incubation at 30–35 °C for 3 days, while TYMC was determined on Sabouraud Dextrose Agar after incubation at 20–25 °C for 5 days, in accordance with Ph. Eur. 2.6.12. Method suitability was confirmed when microbial recovery in the presence of the product did not differ by more than a factor of 2 from the control, corresponding to an acceptable recovery range of 50–200%.

Qualitative testing for the absence of specified microorganisms was performed according to Ph. Eur. 2.6.13. Samples inoculated with *Staphylococcus aureus* or *Pseudomonas aeruginosa* were enriched in Tryptic Soya Broth and subcultured on Mannitol Salt Agar or Cetrimide Agar, respectively. The Absence of characteristic colonies confirmed that *S. aureus* and *P. aeruginosa* were not detected, demonstrating compliance of the validated microbiological control method with pharmacopoeial requirements.

Results and discussion

The results confirmed that the selected culture media, incubation conditions, and neutralizing system were suitable for the recovery of reference microorganisms, without significant interference from the shampoo matrix. Method specificity was demonstrated by characteristic colony morphology and expected microbial growth. Accuracy was confirmed by acceptable recovery values within the range of 50–200% and recovery factors not exceeding 2%, according to Ph. Eur. criteria. Precision was evaluated by repeated testing under the same conditions, with RSD values below 15%, confirming method repeatability and reliability.

Overall, the validated method is suitable for routine microbiological quality control of Ketoconazole 2% shampoo, including determination of TAMC and TYMC, and verification of the Absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Conclusion

The validated microbiological quality control method for Ketoconazole 2% Shampoo demonstrated suitability for routine analysis of this non-sterile cutaneous pharmaceutical preparation. The applied procedures for TAMC, TYMC, and detection of specified microorganisms confirmed adequate microbial recovery, effective neutralization of the product matrix, and absence of interference under the defined test conditions. Compliance with European Pharmacopoeia requirements supports the reliability of the method and confirms its applicability for regulatory quality control, contributing to the microbiological safety and overall quality assurance of the finished product.

References

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