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Short communication

Antimicrobial efficacy testing of Methadone hydrochloride 10 mg/mL oral solution

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Introduction

Antimicrobial preservatives are substances added to pharmaceutical products to inhibit the growth of microorganisms that may be introduced inadvertently during the manufacturing process or through repeated product use, thereby extending the shelf life and ensuring the quality of the product. However, preservatives may also cause adverse effects such as allergic reactions, irritation and toxic side effects, therefore it is important to determine the minimum effective concentration that is sufficient and complies to pharmacopoeial requirements.

Antimicrobial efficacy testing is a procedure performed to evaluate the effectiveness of antimicrobial preservatives added to medicinal products, by challenging the formulation of the product under controlled conditions. The use of antimicrobial agents is particularly important in liquid/aqueous and multi-dose formulations which are more susceptible to microbial contamination.

The aim of this study was to confirm the efficacy of antimicrobial preservation in Methadone hydrochloride 10 mg/mL oral solution, a widely used and globally recognized medicinal product. Methadone hydrochloride is primarily used for the treatment of opioid dependence and severe pain management that does not respond to other non-narcotic analgesics (Anderson, 2000; Council of Europe, 2025; Tyski et al., 2025).

Materials and methods

The antimicrobial efficacy testing was performed using the surface spread method. The test was conducted in sterile, capped containers into which an adequate volume of the test product was transferred. Each container was inoculated with one of the prepared and standardized inocula in accordance with the requirements of the European Pharmacopoeia (Ph. Eur) Chapter 5.1.3 “Efficacy of antimicrobial preservation”. The final concentration of the test preparation after inoculation is between 1 x 10^5 and 1 x 10^6 CFU/mL of the product. The containers were stored at 20-22 °C and sampled at 0, 14 and 28 days.

For performance of the test, the following strains of test microorganisms from the American Type Culture Collection (ATCC), were used: *Pseudomonas paraeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404, *Burkholderia cepacia* ATCC 25416, *Burkholderia cenocepacia* BAA 245 and *Burkholderia multivorans* BAA 247.

Dilutions and suspensions were prepared using buffered sodium chloride-peptone solution (pH 7.0), 9 g/L sodium chloride solution and suspending fluid

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containing 9 g/L sodium chloride and 0.5 g/L polysorbate 80. Casein soya bean digest agar (TSA) and Sabouraud-dextrose agar (SDA) were used for the viable count determination.

Results and discussion

The antimicrobial efficacy test was conducted on Methadone HCl 10 mg/mL oral solution, 1000 mL, at in-use conditions (90 days), the in-use stability study was performed at the beginning of shelf-life and the following results were obtained: at the start of the analysis (time 0) microbial growth was observed for all of the tested microorganisms (*P. paraeruginosa, S.aureus, E. coli, B. cepacia, B. cenocepacia, B. multivorans, C. albicans, A. brasiliensis*) within the limits specified in Ph. Eur. 5.1.3. In the other time intervals, 14th day and 28th day, growth was observed only for *A. brasiliensis*. However, there was no increase in the number of viable microorganisms on the 28th day compared to the previous interval.

The antimicrobial efficacy test was also conducted on Methadone HCl 10 mg/mL oral solution, 1000 mL, at in-use conditions (90 days, stability condition below 25 °C); this in-use stability study was performed at the end of shelf-life (36 months). The obtained results are similar to the previous antimicrobial efficacy testing, 14th day and 28th day, growth was observed only for *A. brasiliensis,* in accordance with the requirements for log $_{10}$ reduction and acceptance criteria for oral preparations specified in Ph.Eur. 5.1.3.

The obtained results obtained from antimicrobial efficacy testing at the beginning of shelf life and at the end of shelf life confirm that the formulation of Methadone HCl 10 mg/mL oral solution is highly robust, ensuring patient safety. The product remained effective even when challenged with objectionable microorganisms from the *Burkholderia cepacia* Complex, recognized as major contaminants of sterile and non-sterile pharmaceuticals, being the cause of numerous nosocomial outbreaks in the last two decades. A notable finding was the persistent growth of *A. brasiliensis* at all of the sampling points throughout the test, despite compliance with the established acceptance criteria, which suggests that although the preservative system is effective, optimization may be possible and continued vigilance against fungal contamination remains necessary (Tavares et al., 2020).

Conclusion

Contaminated pharmaceutical products are a serious risk to public health, especially for immunocompromised individuals. For liquid oral medications like methadone solution, the risk of contamination is amplified due to the potential for microbial introduction during repeated patient dosing and the usage of medical tools for product administration. Pharmaceutical companies are constantly revising and upgrading existing formulations and rapidly inventing new drugs. As a result, the significance of antimicrobial efficiency testing continues to grow, helping to ensure the quality and stability of the product over time while safeguarding patients' health.

References

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