

Method suitability validation for determination of microbiological purity of Sildenafil film-coated tablet

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Introduction

Sildenafil is a medication used to treat pulmonary arterial hypertension and erectile dysfunction. It is taken by mouth or by injection into a vein. Onset is typically within twenty minutes and lasts for about two hours. Mechanism of act of sildenafil is by blocking phosphodiesterase 5 (PDE5), an enzyme that promotes breakdown of cGMP, in the smooth muscle cells lining the blood vessels supplying various tissues. It also results in dilation of the blood vessels in the lungs (Cocci et al., 2017).

All pharmaceutical forms are subject to chemical and microbiological quality control. A microbiological quality control method that will be used in routine analysis for determination of product microbiological purity must be subject of validation. Microbiological quality control parameters for Sildenafil film coated tablets 100 mg as non-aqueous pharmaceutical preparation for oral use are: Total Aerobic Microbial Count (TAMC), Total Yeasts and Molds Count (TYMC) and Absence of *Escherichia coli* (Ph.Eur. 10.0, 2019).

Materials and methods

Standard laboratory equipment was used during the method validation: Biosafety Cabinet Class II A, microbiological incubators Binder within three temperature intervals 20-25 °C, 30-35 °C and 42-44 °C, Bunsen burner, Orbital shaker, and standard, sterile laboratory glass for microbiological use. In addition to the validation of the method, 10 g of Sildenafil film-coated tablet were weighted using analytical balance. Buffered sodium chloride-peptone solution- Pharmacopeia diluent pH 7.0, 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 test microorganisms that were part of this validation are standard microorganisms specified by the current European Pharmacopoeia for method suitability test: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 (Ph.Eur. 10.0, 2019).

Method

For determination of the antimicrobial activity and validation of the test method, challenge test was conducted with above mentioned microorganisms. Surface-spread method using secondary suspension of the product to be examined (1:100 dilution) is determined to be validated as suitable for the conduction of the quantitative tests for determination of TAMC and TYMC, as defined in Ph. Eur. 2.6.12. Test for specified microorganisms using primary suspension of the product to be examined (1:10 dilution) is determined to be validated as suitable for the conduction of the qualitative tests for determination of absence of *Escherichia coli*, as defined in Ph. Eur. 2.6.13.

The number of all microorganisms applied in the challenge tests from 10 – 100 cfu/mall Each test was conducted in duplicate and the temperature and the time of incubation depended of the type of the medium. TSA were incubated for 3 days at 30-35 °C, SDA were incubated for 5 days at 20-25 °C, TSB was incubated for 24 h at 30-35 °C, MCK broth was incubated for 24 h at 42-44 °C and MCK agar was incubated for 24 h at 30-35 °C. After the incubation time the relation between the

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number of each microorganism dissolved in Pharmacopeia diluent pH 7.0 and the number of the same microorganism in the presence of the product was calculated. The recovery percent is limited by the values of 50-200% on all tested microorganisms.

Calculating the results and evaluating the recovery percent and recovery factor for compliance to the reference requirements.

When verifying the suitability of the plate-count method, a mean of any of the test organisms not differing by a factor greater than 2 must be obtained (Ph.Eur. 10.0, 2019).

Results and discussion

The chosen test for determination of microbiological purity as suitable, must mimic the proposed microbial limit test - the sample preparation, types of media and buffers as well as incubation conditions must be reproduced during validation. In order to demonstrate that the proposed method is capable of recovering viable microbes that might be present in the product sample, the method suitability protocol requires the use of representative microorganisms to challenge the microbial recovery methods.

Our results show that the product, Sildenafil film-coated tablet, doesn't demonstrate antimicrobial properties against any microorganisms recommended by the European Pharmacopeia while using primary suspension (1:10 dilution). The primary suspension (1:10 dilution) demonstrated product matrix-spread effect on motile microorganisms *P. aeruginosa* and *B. subtilis*, which resulted in inability to determine the exact microbial count for the two specified microorganisms. The count is reported as TNTC and the recovery factor is incalculable. Compared to the Control, demonstration of successfully overpassed matrix-spread effect of the product on subjected microorganisms was achieved using secondary suspension (1:100 dilution). A product negative control was performed to evaluate any inherent product bio burden that might interfere with the recovery challenge studies (Ph.Eur. 10.0, 2019).

Conclusion

The product, Sildenafil 100 mg film-coated tablet, demonstrates product matrix-spread effect on motile microorganisms *P. aeruginosa* and *B. subtilis* while using Pharmacopeia diluent pH 7.0 for the preparation of Primary suspension (1:10 dilution). The successfully overpassed matrix-spread effect of the product on subjected microorganisms were eliminated in the further

dilution prepared with same Pharmacopeia diluent pH 7.0. Compared to the Control, countable microbial growth was detected using secondary suspension (1:100 dilution).

Secondary suspension (1:100 dilution) demonstrates absence of product matrix-spread interference and compliant recovery factor values ≤ 2 on all tested microorganisms.

References

- Cocci, A., Capece, M., Cito, G., Russo, G.I., Falcone, M., Timpano, M., Rizzo, M., Della Camera, P.A., Morselli, S., Campi, R., Sessa, F., 2017. Effectiveness and safety of orodispersible sildenafil in a new film formulation for the treatment of erectile dysfunction: comparison between sildenafil 100-mg film-coated tablet and 75-mg orodispersible film. *The Journal of Sexual Medicine*, 14(12), 1606-1611. <https://doi.org/10.1016/j.jsxm.2017.10.066>
- European Pharmacopoeia- 10th edition, Volume 1, 2019. Council of Europe, 67075 Strasbourg, France, pp, 185-194.