Macedonian pharmaceutical bulletin, 68 (Suppl 1) 121 - 122 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.056
Short communication

Method suitability validation for determination of microbiological purity of Ibuprofen film coated tablets 400mg

Damjan Shushleski*, Marjan Velkovski, Irena Slaveska Spirevska, Elena Metcheva, Silvana Ristevska, Tatjana Dimitrieva

Replek Farm, st. Kozle 188, 1000 Skopje, N. Macedonia

Introduction

Ibuprofen (IPB), α-methyl-4-(2-methylpropyl)-benzene acetic acid, is one of the most common non-steroidal anti-inflammatory drugs (NSAIDs). It is widely used as an analgesic in mild to moderate pain, and in the treatment of rheumatoid arthritis and osteoarthritis (Zhao et al., 2005). The conventional daily dose of this NSAID is 600–1200 mg per day (De Brabander et al., 2004). It was the 28th most dispensed drug in the USA in 2017 (Wong et al., 2021). The antimicrobial potency of Ibuprofen has been demonstrated in its ability to reverse resistance related to efflux pump activity (Ogundeji, 2016).

All pharmaceutical forms are subject to chemical and microbiological quality control. A microbiological quality control method that will be used in routine for determination of product microbiological purity must be subject of validation. Microbiological quality control parameters for Ibuprofen film coated tablets 400mg 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

Materials

During the method validation, standard laboratory equipment was used: Biosafety Cabinet Class II A, microbiological incubators Binder within tree temperature intervals 20-25ºC, 30-35ºC and 42-44ºC, Bunsen burner, Orbital shaker, and standard, sterile laboratory glass for microbiological use. In addition to the validation of the method, 10g of Ibuprofen film coated tablets were weighted using Kern 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., 5-2-2-2. 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., 4-2.

The number of all microorganisms applied in the challenge tests from 10 – 100 cfu/mL. Each test was conducted in duplicate and the temperature and the time

*damjan.susleski@replek.mk

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 24h at 30-35˚C, MCK broth was incubated for 24h at 42-44˚C and MCK agar was incubated for 24h at 30-35˚C. After the incubation time the relation between the number of the 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, Ibuprofen film coated tablets, demonstrates significant antimicrobial properties against almost all microorganisms recommended by the European Pharmacopeia while using primary suspension (1:10 dilution) resulting in Recovery factor >2. Compared to the Control, demonstration of successfully overpassed antimicrobial properties 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, Ibuprofen film coated tablets 400mg, demonstrates significant antimicrobial properties against test microorganism: *S. aurues*, *B. subtilis*, *C. albicans* and *A. brasiliensis* while using Pharmacopeia diluent pH 7.0 for the preparation of Primary suspension (1:10 dilution). The antimicrobial properties of the product on subjected microorganisms were eliminated in the further dilution prepared with same Pharmacopeia diluent pH 7.0. Compared to the Control, positive microbial growth was detected using secondary suspension (1:100 dilution).

Secondary suspension (1:100 dilution) demonstrates absence of product microbial interference and compliant Recovery Factor values ≤2 on all tested microorganisms.

References

De Brabander, C., Vervaet, C., Van Bortel, L., Remon, J.P., 2004. Bioavailability of ibuprofen from hot-melt extruded mini-matrices. Int. J. Pharm. 271(1-2), 77-84. 10.1016/j.ijpharm.2003.10.029

European Pharmacopoeia- 10th edition, Volume 1, 2019. Council of Europe, 67075 Strasbourg, France, pp, 185-194.

Ogundeji, A.O., Pohl, H.C., Sebolal, M.O., 2016. Repurposing of aspirin and ibuprofen as candidate anti-Cryptococcus drugs. Antimicrob. Agents Chemother. 60(8), 4799808. 10.1128/AAC.02810-15

Wong, A.Y., MacKenna, B., Morton, C.E., Schultze, A., Walker, A.J., Bhaskaran, K., Brown, J.P., Rentsch, C.T., Williamson, E., Drysdale, H., Croker, R., 2021. Use of non-steroidal anti-inflammatory drugs and risk of death from COVID-19: an OpenSAFELY cohort analysis based on two cohorts. Annals of the rheumatic diseases 80 (7), 943-951. 10.1136/annrheumdis-2020-219517

Zhao, X.G., Wei, D.Z., Song, Q.X., 2005. A facile enzymatic process for thepreparation of ibuprofen ester prodrug in organic media. J. MolCatal. B: Enzym. 36(1-6), 47-53. https://doi.org/10.1016/j.molcatb.2005.08.005