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Short communication

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

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Introduction

Ciprofloxacin belongs to the group of fluoroquinolone antibiotics, used to treat a number of bacterial infections. This includes bone and joint infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others (Solomkin et al. 2010). Ciprofloxacin occupies an important role in treatment guidelines issued by major medical societies for the treatment of serious infections, especially those likely to be caused by Gram-negative bacteria, including *Pseudomonas aeruginosa*. For example, ciprofloxacin in combination with metronidazole is one of several first-line antibiotic regimens recommended by the Infectious Diseases Society of America for the treatment of community-acquired abdominal infections in adults (Solomkin et al., 2010). It also features prominently in treatment guidelines for acute pyelonephritis, complicated or hospital-acquired urinary tract infection, acute or chronic prostatitis (Grabe et al., 2013).

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 Ciprofloxacin 500 mg film-coated tablet 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 tree temperature intervals 20-25 ºC, 30-35 ºC and 42-44 ºC, Bunsen burner, Orbital shaker, and standard, sterile laboratory glass for microbiological use. In addition to the validation of the method, 10 g of Ciprofloxacin film-coated tablets 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. Membrane filtration method using tertiary suspension of the product to be examined (1:1000 dilution), supplemented with 10 mL 1 M $MgCl_2$ solution to obtain 1:1000 dilution, is determined to be validated as suitable for the conduction of the quantitative tests for determination of TAMC as defined in Ph. Eur. 2.6.12. Surface-spread method using secondary suspension of the

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product to be examined (1:100 dilution) supplemented with 10 mL 1M $MgCl_2$ solution to obtain 1:100 dilution is determined to be validated as suitable for the conduction of the quantitative tests for determination of TYMC, as defined in Ph. Eur. 2.6.12., 5-2-2-2. Test for specified microorganisms using membrane filtration of secondary suspension of the product to be examined (1:100 dilution) supplemented with 10 mL 1M $MgCl_2$ solution to obtain 1:100 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 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.

The supplementation of the Pharmacopeia diluent pH 7.0 (PD90) with 10 mL 1M $MgCl_2$, as well as the supplementation of TSA media with 1 mL 1 M $MgCl_2$ demonstrates absence of neutralization toxicity and compliant Recovery Factor values ≤2 on all tested microorganisms.

Membrane filtration of Test Solution 3 (1:1000) demonstrates absence of significant product microbial interference and compliant Recovery Factor values ≤ 2 on all tested microorganisms for TAMC.

Spread plating of Test Solution 2 (1:100) demonstrates absence of significant product microbial interference and compliant Recovery Factor values ≤ 2 on all tested microorganisms for TYMC.

The supplementation of the Pharmacopeia diluent pH 7.0 (PD90) with 10 mL 1 M $MgCl_2$ solution, as well as the supplementation of Tryptic Soya Broth (TSB100) with 10 mL 1 M $MgCl_2$ doesn't demonstrate neutralization toxicity properties against specified test microorganism: *E.coli*. Membrane filtration technique of 100 mL of Test Solution 2 (1:100), corresponding to 1 g of product to be examined was used during the method validation procedure for determination of absence of *Escherichia coli.*

Conclusion

Usage of inorganic compounds for chelation of the Ciprofloxacin as active pharmaceutical ingredient in Ciprofloxacin 500 mg film-coated tablets, it is effective method in overcoming antimicrobial properties of quinolone antibiotics. In our study, usage of 1 M $MgCl_2$ accompanied with membrane filtration, proved as effective method for neutralizing antimicrobial properties of ciprofloxacin.

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