

Report concerning the collaborative study for establish of reference standard efficacy of Rifamycin

Raport asupra studiului colaborativ pentru stabilirea potenței standardului de referință: Rifamicină

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Keywords: *collaborative study, rifamycin, diffusion method, latin squares*

Cuvinte cheie: *studiu colaborativ, rifamicină, metoda difuzimetrică, pătrate latine*

Abstract

The Microbiology Laboratory from the Institute for the Control of Veterinary Biological Products and Medicines participated in a collaborative study in order to determine the potency of rifamycin CRS, batch 4, according to the protocol sent by the EDQM (European Directorate for the Quality of Medicines) - coordinating of the study. The purpose of the study was to establish the potency of the batch 4 rifamycin in the characterization of the substance as reference standard. Potency was determined by microbiological method, based on comparison of the inhibition zones of growth of micro-organisms sensitive to those of a reference standard, as specified in European Pharmacopoeia, Chapter 2.7.2. - Microbiological testing of antibiotics. After analysis of received data from participants and their statistical processing by the EDQM, the potency of the batch 4, rifamycin - reference materials, was established in 870 UI/mg.

Rezumat

Laboratorul Control Microbiologic din cadrul Institutului pentru Controlul Produselor Biologice și Medicamentelor de uz Veterinar a participat la studiul colaborativ pentru determinarea potenței lotului 4 de rifamicină, conform protocolului trimis de EDQM (Directoratul European pentru Calitatea Medicamentelor), coordonatorul studiului. Scopul studiului a fost de a stabili potența rifamicinei, în vederea caracterizării cât mai exacte a lotului 4 de substanță de referință. Potența a fost determinată prin metoda microbiologică, bazată pe compararea zonelor de inhibare a creșterii unui microorganism sensibil, cu cele ale unui standard de referință, conform prevederilor din Farmacopeea Europeană, ediția a 8 a, capitolul 2.7.2. – testarea microbiologică a antibioticelor. În urma analizei și coroborării rezultatelor primite de la participanți și a prelucrării statistice a acestora de către EDQM, valoarea potenței lotului 4 de rifamicină, substanța de referință, a fost stabilită la 870 UI/mg.

Introduction

One of the important activities of the Laboratory of the Microbiological Control from the ICBMV, is the participation in interlaboratory comparison schemes and collaborative studies in Europe.

This participation aims at checking the check laboratory capabilities, and support to

demonstrate competence of the Laboratory of the Microbiological Control.

This paper aims to present important aspects of the Laboratory of the Microbiological Control from the ICBMV participation in the collaborative study to determine the potency of rifamycin CRS, batch 4, according to the protocol sent by EDQM (European Directorate for Quality of Medicines) - coordinator of the study.

Rifamycin is a bactericidal antibiotic, active against *Mycobacterium tuberculosis*, Gram-positive and some Gram-negative bacteria (*Neisseria gonorrhoeae*).

The mechanism of action is inhibition of RNA polymerase, the enzyme involved in the synthesis of bacterial RNA.

The mechanism of rifampicin prevent new biosynthesis of RNA molecules. It also has immunosuppressant action and relatively quickly develops resistance. Rifamycin is indicated especially in the treatment of pulmonary and extrapulmonary tuberculosis, and in sepsis with susceptible Gram negative bacteria (resistant to other antibiotics), serious staphylococcal infections (endocarditis, osteomyelitis, septic arthritis), leprosy (in combination with other substances effective against leprosy),

Legionella pneumonia (associated with erythromycin), gonorrhoea (as a second choice). It is used also in the prevention of meningococcal meningitis.

1. Materials and Methods

Samples and Protocol work were sent to all participating laboratories by EDQM.

The study enrolled six laboratories from different EU countries, which received participant codes, respectively from number 1 to 6. Each laboratory study participant received:

- 3 vials of powder rifamycin, WHO standardized reference substance (approximately 887 IU / mg), 100 mg / vial
- 6 vials of rifamycin powder, batch 4, in order to determine the potency (the potency estimated at 850 IU / vial), 300 mg / vial

The working method was applied in the Microbiological Control and Effective Biocides Service, Laboratory of the Microbiological Control, in agreement with the conditions imposed by the work protocol of the study.

The principle of the method is based on the dose-response model in which the concentration of antibiotic is proportional to inhibiting growth of the test microorganism.

Potency estimation was conducted on 6 samples by diffusion method, using:

- *Kocuria rhizophila* test microorganism (*Micrococcus luteus*) ATCC 9341,
- cultivation medium – A medium, prepared in accordance with the requirements of the European Pharmacopoeia
- pH 7 buffer.

Samples of the working and reference substance stock dilution were prepared according to the protocol received from the EDQM. For the validity of the test were used three different doses for both the test sample and the reference material used, that dilutions of 1 / 33.75, 1 / 22.5 1/15 (dilution ratio 1.5).

Plates were prepared with the media and test microorganisms needed for each sample separately. After solidification environment were placed four metal cylinders/plate, on the plate surface using sterile forceps.

After preparing the working dilutions were dispensed 0.4 ml/ cylinder of standard solution and sample in the corresponding cylinders.

The plates thus prepared were left for 1-4 hours at room temperature (pre-incubation period), after which they were carefully transferred to an incubator where they stayed for 18 hours at 35°C (± 1°C). The diameters of the zones of inhibition were measured visually using digital micrometer. Sample concentration was calculated using an Excel sheet.

2. Results and Discussion

Reporting of results was done by e-mail, as specified in the test protocol using an Excel file separately for each of the six individual tests, the calculation method that uses Latin squares. The results obtained for the 6 samples worked, sent by the Laboratory of the Microbiological Control from the ICBMV, are presented below in [Table 1](#).

Thus it can be seen that:

- sample no. 1, the value obtained was 921 IU/ml, had a deviation value determined by the potency of EDQM 4.5%.

- sample no. 2 that the value obtained was 904 IU/ml, had a deviation of 3.7%;
 - sample no. 3 deviation was 3.0%, f
 - or sample no. 4 deviation was 3.1%;
 - for sample no. 5 deviation was 2.9% and
 - for sample no. 6 deviation was 4.0%.
- The combined result for the 6 samples was worked 892 IU / ml.
- All 6 results sent by the Laboratory of the Microbiological Control were accepted as valid and were statistically processed.

Table 1

The results of Laboratory of the Microbiological Control compared with those obtained by the EDQM

Crt. No.	Results calculated by the Laboratory of the Microbiological Control - ICBMV (IU / ml)		Results calculated by the EDQM (IU / ml)	
	Estimated potency	Confidence Limits 95 %	Estimated potency	Confidence Limits 95 %
1	921	95.7 - 104.5%	921	95.7 - 104.5%
2	904	96.4 - 103.7%	904	96.4 - 103.7%
3	897	97.1 - 103.0%	897	97.1 - 103.0%
4	890	97.0 - 103.1%	890	97.0 - 103.1%
5	873	97.2 - 102.9%	873	97.2 - 102.9 %
6	889	96.2 - 104.0%	889	96.2 - 104.0 %

For the other 6 acceptable results in terms of homogeneity, the average potency was 892 IU / ml \pm 1.3% (95% confidence limits with values of 96.6 - 103.53%).

The results obtained by the participating laboratories were analyzed compared with those obtained in the EDQM laboratories.

EDQM report which contains the results of participants with comments organizers of study related confidence interval, potency value, deviation from linearity, homogeneity readings, results average, standard deviation, relative standard deviation, etc. was submitted to each participating laboratory.

Evaluation of the results was done using statistical calculation model parallel lines using SAS System program and CombiStats.

3. Conclusion

Following analysis and collaboration results received from the participants and statistical processing by EDQM, the value of batch 4 of rifamycin potency - reference substance, was set at 870 IU / ml.

Bibliography

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