Report concerning the colaborative study for establish of reference standard eficacity of Kanamycin

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

Simona Sturzu, Daniela Tîrsînoagă, Ioana Tihulcă, Mariana Dumitrache, Alina Drăghici, Cristina Popica,Valentina Năstase

Institute for Control of Veterinary Biological Products and Medicines

Corespondența: sturzu.simona@icbmv.ro

Keywords: *collaborative study, kanamycin, diffusion method , Combistats*
Cuvinte cheie: *studiu colaborativ, kanamicina, metoda difuzimetrică, pătrate latine*

Abstract

The Microbiology Laboratory from the Institute for the Control of Veterinary Biological Products and Medicines participated to a collaborative study in order to determine the potency of kanamycin CRS, batch 3, 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 3 for 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 3, kanamycin - reference materials, was established in 790 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 3 de kanamicină, conform protocolului trimis de EDQM (Directoratul European pentru Calitatea Medicamentelor), coordonatorul studiului. Scopul studiului a fost stabilirea potenței kanamicinei, in vederea caracterizării cât mai exacte a seriei de substanță de referință. Potența a fost determinată prin metoda microbiologică, bazată pe compararea zonelor de inhibare a cresterii unui microorganism sensibil, cu cele ale unui standard de referin_ă, conform prevederilor din Farmacopeea Europeană, editia a 8 a, capitolul 2.7.2. – testarea microbiologică a antibioticelor. În urma analizei si coroborării rezultatelor primite de la participanți și a prelucrării statistice a acestora de către EDQM, valoarea potenței lotului 3 de kanamicină, substanța de referinta, a fost stabilită la 790 UI/mg.

Introduction

In recent years, the collaborative studies have gained a remarkable importance, both as a verification instrument for the trasability in quality assurance process as well as a validation instrument for the working procedures of the activities in testing laboratories, accredited or on the way to be accredited.

Participation in such actions can be done by enlisting the candidate laboratories in interlaboratories comparision schemes or collaborative studies in order to do an independent competence verification regarding the analyzes which they are performing.

The Institute for Control of Veterinary Biological Products and Medicines has partipated in the collaborative study to determine the potency for CRS kanamycin, batch 3, according to the protocol sent by EDQM (European Directorate for the Quality of Medicines) – the study coordinator.

Kanamycin is an aminoglycoside antibiotic, having a bactericidal spectrum of action particularly including Gram-negative bacilli. Also, a part of E. coli strains, Enterobacter, Klebsiella, Proteus, Serratia, M. tuberculosis are sensitive.

Kanamycin is indicated in serious infections with Gram-nagative bacilli, sensitive to kanamycin but resistant to less toxic antibiotics: pyelonephritis, colibacilli nosocomial pneumonia, Proteus mirabilis infections (in association with ampicillin), Klebsiella (in association with a cephalosporin), Gram-negative sensitive bacilli meningitis; infected burns, usual tuberculostatics-resistant germs tuberculosis. Also, kanamycin is indicated in the treatment of external eyeball and anexes infections produced by kanamycin-sensitive germs: conjuctivitis, keratitis, keratoconjunctivitis, corneal ulcer, blepharitis, blepharoconjunctivitis, dacryocystitis as well as in ophtalmological surgical infection prophylaxis.

Kanamycin administration is contraindicated in association with other aminoglycosides simultaneously administered but it is synergist with the penicillines and cephalosporines.

It is reccomended to avoid association of the aminoglycosides, not even topically, with polymixins, cephalothin, furosemide, etacrynic acid, amphotericin B, cyclosporine, cisplatin or with other medicines having nephrotoxic and ototoxic potential (as it rises the nephro and ototoxic risks).

The aminoglycoside antibiotics should not be mixed with other medicines, especially with β-lactamase antibiotics (in vitro studies showed reciprocal inactivation).

1. Materials and method

Seven laboratories from different UE countries had registered to the study and they received participant codes from 1-7.

The sample and the testing protocol were provided by the EDQM – the study coordinator.

The testing method was applied in the Service for Microbiological Control and Biocidal Products Efficacy, Microbiological Control Laboratory, in accordance with the imposed requirements of the Study Testing Protocol.

So, to perform the test were received:

- 3 ampoules with kanamycin powder, reference substance WHO standardized (approximately 10345 IU/ampoule), 13 mg/ampoule
- 6 ampoules of kanamycin powder, batch 4, to determine the potency (estimated potency 765 IU/mg), 150 mg/ampoule

The principle of the method is based on the dose-response model in which the antibiotic concentration is directly proportional with the growth inhibition zone of the test-microorganism.

The assessment of the potency was performed on 6 samples by diffusion method using:

- Test microorganism – Bacillus subtilis ATCC 6633
- Culture medium - medium A, prepared according to the European Pharmacopoeia requirements
- Buffer solution, pH=8

Working samples and the reference stock-substance dilutions were prepared in keeping with the protocol received from EDQM.

To validate the test 3 different doses were used, for the test sample and for the reference used material, respectively 1/6.75, 1/4.5 and 1/3 dilutions (1.5 dilution rate).

The plates were prepared with the necessary media and test-microorganism for each sample. After medium solidification 4 metallic cylinders were placed on the medium surface using sterile forceps.

After the working dilution' preparing 0.4 ml of standard solution and of sample were allocated to the corresponding cylinders. The prepared plates were left for 1-4 hours at room temperature (pre-incubation time), and after that they were incubated for 18 hours at 35^0 C (+/- 1^0C). A special attention was paid to the manipulation and to the transfer of the plates from working table to the incubator.

The inhibition zones' diameters were visually measured using the digital micrometer. The sample' concentration was calculated using System SAS program and CombiStats.

2. Results and discussions

The reporting of the results was done through e-mail, according to the testing protocol specifications using an Excel file, separately for each of the 6 individual tests. The used calculation method was the parallel lines method. The combined result for the 6 tested samples was 852 IU/mg and the obtained results for the 6 individual tested samples, sent by Microbiological Control Laboratory, are presented in Table 1.

Table 1.

The obtained results by Microbiological Control Laboratory by comparison with those obtained by EDQM

Crt. No.	*Calculated results by Microbiological Control Laboratory – ICVBPM (IU/mg)*		*Calculated results by EDQM (IU/mg)*	
	Estimated potency	**Confidence limits 95 %**	**Estimated potency**	**Confidence limits 95 %**
1	849.9	96.0- 104.2%	849.9	96.0 - 104.2%
2	849.9	96.0 - 104.2%	849.9	96.0 - 104.2%
3	866.4	96.4 - 103.7%	866.4	96.4 - 103.7%
4	861.4	97.6 - 102.5%	861.4	97.6 - 102.5%
5	843.4	97.5 - 102.5%	843.4	97.5 - 102.5%
6	849.1	98.2 - 102.1%	849.1	98.2 - 102.1%

Thereby, it can be noticed that sample no.1 and sample no.2, for which the obtained value was 849.9 IU/mg, the deviation from the EDQM established value of potency was 4.2%.

Sample no.3 for which the obtained value was 866.4 IU/mg had a deviation of 3.7%.

For sample no.4 and for sample no.5 the deviation was of 2.5% and for sample no.6 for which a value of 849.1 IU/mg was obtained the deviation was 2.1%.

All 6 results sent by Microbiological Control Laboratory were accepted by EDQM as valid and were statistically processed.

For the other 6 results, accepted in terms of homogeneity the average value for the potency was 852 IU/mg +/- 1.1 % (95% confidence limits with values in the 96.66 - 103.2% interval).

The results obtained by the participant laboratories were analyzed by comparison with those obtained by EDQM laboratories.

The EDQM report in which the results obtained by the participants, accompanied by the study organizers comments about the confidence interval, the potency value, the deviation from linearity, the homogeneity of the readings, the average of the results, the standard deviation, the relative standard deviation, etc was submitted to each participant laboratory.

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

3. Conclusions

As a result of analyzing and corroborating the received results from the participants and of statistical processing of those by EDQM, the potency value for batch 3 of kanamycin – reference substance, was established at 790 IU/mg.

Bibliography

1. European Pharmacopoeia, 8-th edition: chapter 2.7.2. Biological tests – Microbiological assay of antibiotics
2. European Directorate for the Quality of Medicines (EDQM), Testing protocol for determination of the potency of kanamycin CRS, batch 3
3. European Directorate for the Quality of Medicines (EDQM), Testing report for determination of the potency of kanamycin CRS, batch 3