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The 2ND International Standard for Bacitracin

NB: Since April 2006, the European Directorate for the Quality of Medicines & HealthCare (EDQM-Council of Europe) is in charge of the establishment and distribution of International Standards for Antibiotics instead of the National Institute for Biological Standards and Control (NIBSC-UK).

1. Introduction

The 2nd International Standard for Bacitracin was established by the WHO Expert Committee on Biological Standardisation at its 15th meeting in 1963[1]. It consists of ampoules each containing approximately 100 mg of zinc bacitracin, 74 International Units per mg and the International Unit of Bacitracin is defined as the activity contained in 0.01351 mg of the 2nd International Standard for Bacitracin.

2. Biological Activity

The 2nd International Standard for Bacitracin (code-labelled 62/003) was calibrated by 7 laboratories against the 1st International Standard for Bacitracin in an international collaborative study[2]. A total of 246 individual assays were carried out by plate diffusion methods. Five different test organisms were used in the study. The potency agreed by the participants was **74 International Units per mg.**

3. Caution

For laboratory use only. Handle in accordance with good occupational hygiene, safety and laboratory practices and take precautions to avoid exposure. This material is not for administration to humans or animals. The corresponding safety data sheet, when applicable, can be accessed via the EDQM website (Reference Standards Database) or is available upon request from the EDQM (Helpdesk-FAQ section).

4. Preparation of solutions

Since zinc bacitracin has a very low solubility in water at normal pH values, care has to be taken to ensure complete solution. The following method, which had been recommended by the manufacturer and found satisfactory in this laboratory is recommended for use.

The zinc bacitracin is dissolved at 1000 units/mL in a solution which is 0.5 N with respect to H2SO4 and 0.375 N with respect to NaOH, i.e. three-quarters neutralised; the temperature is maintained at 10°-15°C (running tap- water); under these conditions the zinc bacitracin dissolves within 15 minutes. The 1000 units/mL solution is diluted immediately to 100 units/mL using 1% phosphate buffer pH 6.0 (pH of solution containing zinc bacitracin at this stage found to be approximately 2.8). Further dilutions may be made using 1% phosphate buffer pH 6.0 to give solutions of zinc bacitracin of a concentration appropriate to the particular assay conditions. At a concentration of 10 units/mL the pH is approximately 6.0[2].

5. Further Information

Preparation of the International Standard

The bulk material consisted of 500gm of a single batch of zinc bacitracin, stored in sealed containers at -10°C in the dark until required for filling. In March 1962 it was allowed to warm to room temperature overnight being thoroughly mixed during that period. It was then distributed in approximately 100 mg quantities into suitable glass ampoules. After filling the ampoules were dried over P_2O_5 *in vacuo* at room temperature to constant weight. After being filled with pure dry nitrogen, the ampoules were sealed by fusion of the glass, tested for leaks and finally placed on storage at -10°C and at a later date, at -20°C in the dark.

The ampouled material
The batch consisted of about 4000 ampoules, each ampoule containing approximately 100 mg of zinc bacitracin. The mean residual moisture content of the material in the sealed ampoules was found to be 0.33% w/w[2]. The contents of the ampoule are hygroscopic. When exposed to an atmosphere of 49% relative humidity, samples increased in weight by up to 0.13% w/w in 5 minutes and to approximately 0.80% w/w in 45 minutes[2]. Care should therefore be exercised in weighing out material from the ampoule to avoid error due to uptake of moisture.

Stability and Storage
It is the policy of WHO not to assign an expiry date to their International Standards. They remain valid with the assigned potency and status until withdrawn or amended.

The 2nd International Standard for Bacitracin is a stable preparation when stored in unopened ampoules in the dark at -20°C or below.

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References
1) World Health Organisation, Expert Committee on Biological Standardisation, WHO Technical Report Series, 1963, **259**, 8.
2) Lightbown, J.W., de Rossi, P. and Issacson, P., (1979), Journal of Biological Standardisation, **7**, 239-246.

6. Citation
In all publications (or data sheets for kits) in which this Standard is used as an assay calibrant, it is more important that the title of the Standard, code and the name and addresses of EDQM are cited correctly.

7. Product liability
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9. Signature
This document is electronically signed by:

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