Collaborative Study for the Establishment of the Second International Standard for Neomycin B

Sylvie Jorajuria¹, Chantal Raphalen, Valérie Dujardin and Arnold Daas

European Directorate for the Quality of Medicines & HealthCare,
Council of Europe,
7 allée Kastner, CS 30026, F-67031 Strasbourg, France

¹ Coordinator of the study: Dr. Sylvie Jorajuria, EDQM, Council of Europe.
Tel.: +33 3 88 41 24 25, E-mail: sylvie.jorajuria@edqm.eu

Note:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by 01 October 2012 and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Quality Safety and Standards (QSS). Comments may also be submitted electronically to the Responsible Officer: Dr Jongwon Kim at email: kimjon@who.int

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Summary

This report describes the results and the outcome of an international collaborative study organised to establish the second World Health Organization (WHO) International Standard (IS) for Neomycin B. 7 laboratories from different countries participated. Potencies of the candidate material were estimated by microbiological assays with sensitive micro-organisms. To ensure continuity between consecutive batches, the first IS for Neomycin B was used as reference.

This report provides details about the material donated by a manufacturer, the processing involved to generate a candidate batch and the analytical controls to assess its quality. It includes statistical analysis of the results, the conclusions made thereof and a recommendation to the WHO Expert Committee for Biological Standardization (ECBS).

It is proposed that the second WHO International Standard for Neomycin B (EDQM internal code ISA_46104) be assigned an antimicrobiological activity of 17 640 IU/vial.

Introduction

Neomycin B, also called Framycetin, is a broad spectrum aminoglycoside antibiotic derived from Streptomyces decaris or Streptomyces fradiæ, and a major component of neomycin. It is used, usually in combination with other antibacterial agents, in topical preparations to treat infections of the skin, nose, ears and eyes.

The 1st IS for Neomycin B (code-labelled 68_041) was established in 1971 on the basis of an international collaborative study [1]. It was assigned a potency of 16 756 International Units per ampoule.

As stocks of the 1st IS for Neomycin B were becoming exhausted, the European Directorate for the Quality of Medicines & HealthCare (EDQM), which is responsible for the production of WHO International Standards for Antibiotics (ISA), took appropriate steps for its replacement by the establishment of a new batch.

Bulk material, processing and stability

The candidate bulk material was kindly donated by Sanofi Aventis, Romainville, France. About 500 g of Neomycin B sulfate (CAS n°28002-70-2) was received by the EDQM in June 2008. The candidate material was claimed to comply with the quality standards of the European Pharmacopoeia monograph “Framycetin sulphate, 0180”. A certificate of analysis was provided in the batch documentation. The bulk material was stored at -20°C before processing.

Production of the second WHO IS for Neomycin B candidate batch

Due to the hygroscopicity of the Neomycin B powder, it was decided to use freeze-drying rather than powder filling as with the previous IS. The parameters of the freeze-drying process were based on EDQM experience with the Ph. Eur. Chemical Reference Standard for Framycetin sulfate. The processing operations were carried out from 8th to 10th March 2011.
All powder weighing was performed in a glove box under a controlled humidity atmosphere of argon gas. Several vials containing accurately weighed amounts were prepared concomitantly to enable further testing of the bulk powder and to prepare the solution to be freeze dried.

The Neomycin B bulk container was allowed to equilibrate at room temperature and was subsequently homogenised in a Turbula mixer.

Formulation: 91.97 g of Neomycin B bulk was dissolved in purified water to a final weight of 3551 g and stirred until complete dissolution. The nominal concentration of the final solution was 25.9 mg Neomycin B bulk per g of solution.

Filling: The solution was filled into 9.0 mL amber glass vials using automated filling equipment. The nominal fill weight was 1.00328 g. Control of fill weight: 34 vials were randomly sampled across the filling run. Results were as follows: Mean: 1.00487 g; RSD= 0.24 %; SD= 0.002. The fill weight was considered homogeneous. The vials were filled with a nominal content of 25 mg of Neomycin B. No buffer, bulking agents or stabilizer were added.

Lyophilisation: The vials were placed onto 12 trays, they underwent lyophilisation, were stoppered with teflon-coated rubber stoppers and sealed under nitrogen. Ampoule integrity after sealing was validated by methylene blue immersion test with UV detection. The vials were stored at -20°C at the EDQM premises and the batch was assigned the production code 11/02-13. A total of 1080 vials were produced.

Selection of a batch suitable as “reference standard” for monitoring purposes

WHO IS are primary reference materials and as such cannot be tested against higher order reference standards. As a consequence, real time stability studies are not usual practice and in many cases, stability of WHO IS is assessed by means of accelerated degradation studies.

Nevertheless, it was decided to store some of the vials of the 1st IS for Neomycin B at -80°C and to use them, at regular intervals in the future, to assess the potency of vials stored at -20°C, the customary storage temperature of the WHO IS batch for Neomycin B. Vials stored at -80°C were registered under EDQM internal number 28388.

Quality control on bulk and final batch

Conformity of the bulk

As described above, precisely weighed samples generated during a single weighing session were submitted to physico-chemical analysis according to the Ph. Eur. monograph “Framycetin sulphate, 0180” to confirm compliance. The results obtained using the analytical methods described under “Identification reactions of ions and functional groups, pH, Specific optical rotation, Related substances, Sulfate content, Loss on drying and Sulfated ash” were compliant to the monograph specifications and were in agreement with those of the certificate of analysis provided by the manufacturer. The bulk material was therefore considered suitable for further processing.

Visual appearance of final vials

Vials were randomly sampled from the freeze-dried batch and inspected visually. The appearance of the cakes was judged satisfactory.
Residual water content
Vials of the candidate batch contain about 25 mg of freeze-dried Neomycin B per vial. It was decided to estimate the residual water content in 6 vials randomly sampled from the batch. The determination of water content was performed as described in the Ph. Eur. general chapter "2.5.32. Water: Micro determination". The residual water content represents less than 1.8 percent of the 25 mg target fill.

Homogeneity of Neomycin B content in final vials
The average content (mg/vial) of Neomycin B was measured by extracting and weighing the content of 10 vials sampled throughout the batch. The weight was determined according to the difference between the full and empty vials, i.e. vials were weighed, emptied, rinsed, dried and weighed again. The SD and RSD were determined. The mean weight was 24.7 mg (RSD= 0.24 %; SD= 0.06). Based on the % RSD result, the degree of homogeneity of the 2nd IS for Neomycin B was considered satisfactory.

Identity of Neomycin B content in final vials
The identity of the 2nd IS for Neomycin B was confirmed by TOF-MS analysis. The results obtained were concordant with the expected monoisotopical mass. The 2nd IS for Neomycin B was considered suitable for the intended use.

Stability studies on the product in the final container
An accelerated degradation study was carried out at the EDQM by storing freeze-dried vials of the candidate batch of the second IS for Neomycin B at +20°C, +37°C and +45°C in different climate chambers (Binder, KBF 720 model) for 1, 3 and 6 months. Both the liquid chromatography (LC) and the microbiological assay were performed.

Accelerated degradation assessed by liquid chromatography
The impurities/degradation products were estimated by using the compendial Ph. Eur. LC method. The EDQM laboratory has a long record of experience with this method which is used internally for monitoring the quality of the corresponding Ph. Eur. Reference Standard.

Two vials at each of the three elevated storage temperatures and two vials kept at the customary storage temperature of -20°C were analysed using the liquid chromatography analytical method described under "Test. Related substances” of the Ph. Eur. monograph "Framycetin sulfate, 0180".

Individual peaks were identified on each chromatogram and their contents were quantified by normalisation calculated from triplicate injections for each vial. Data are presented in Table 1, Figures 1 and 2 after one and three month storage respectively.

A total of 5 peaks were detected in the chromatograms. At either -20°C or +20°C, the mean peak areas corresponding to peaks 5, 6, 7 and impurity C remained unchanged at 1 month. At the same time, a slight increase in these peaks, with the exception of peak 6, was observed at 37°C and 45°C. However, after 3 months storage, while peak 5 exhibited the same profile, peak 7 and impurity C at elevated temperature exhibited no significant difference compared to at -20°C. Moreover peak 8 is detected at 3 months at low levels (0.15%). Taking into account the sum of impurities after 1 and 3 months storage at -20°C and +45°C respectively, no significant changed is observed (4.14% vs 3.97%).
The data obtained at 1 month and 3 months suggest that storage at elevated temperature does not induce a significant degradation of the 2nd IS for Neomycin B. Results at 6 months storage will be available for the ECBS meeting in October 2012.

**Accelerated degradation assessed by microbiological assay**
The degradation of the vials will also be estimated by microbiological assay after 6 months storage at +20°C, +37°C and +45°C. The potencies of these vials will be estimated as the relative potencies against vials of the same batch kept at -20°C. Two vials will be analysed by two independent assays for each temperature using the diffusion method. The data will be available for the ECBS meeting in October 2012.

In addition, potencies of vials stored at -20°C will also be estimated against vials stored at -80°C to generate some baseline data for future monitoring purposes.

**Conclusion from accelerated degradation studies**
Taking into account the accelerated degradation data available so far based on liquid chromatography, it is not considered that storage at an elevated temperature for up to three months significantly affects the potency of the 2nd IS for Neomycin B.

**Collaborative study**

**Participants**
A total of 7 laboratories from different countries around the world volunteered to participate in the study. Each participant is referred to in this report by an arbitrarily assigned number, not necessarily reflecting the order of listing in the Appendix.

**Samples**
Each laboratory was provided with:
- 3 vials of the 1st WHO IS for Neomycin B (68/041), containing approximately 25 mg of freeze-dried powder per ampoule (assigned content: 16 756 IU per vial) (EDQM internal code: 28120),
- 7 vials of the 2nd WHO IS for Neomycin B candidate batch containing approximately 25 mg of freeze-dried powder per vial (activity about 18000 IU per vial) (EDQM internal code: 43407)

**Assay method and study design**
The participants were asked to estimate the potency of the 2nd WHO IS for Neomycin B candidate batch by microbiological activity against target micro-organisms using either the diffusion method or the turbidimetric method or, where possible, both, using the WHO 1st IS for Neomycin B as reference.
A total of six independent assays were to be carried out by each participant.

Prior to carrying out the study, a pilot assay was performed in the EDQM laboratory in order to develop and provide details for the study protocol.

Participating laboratories were requested to follow the study protocol as far as possible.
Results and statistical analysis

Statistical methods
The experimental data obtained in this study were analysed as parallel line assays [2], using the SAS-System [3] (GLM procedure) and CombiStats [4]. Both programs give identical outcomes but the output is somewhat easier to transform to tables with the SAS-system, whereas CombiStats provides a more streamlined output for individual assays.

All assays were submitted to visual inspection of the plots to check for unusual features. Validity of the assays was assessed according to the flow chart in Figure 3. In routine situations where decisions are based on only one assay or only a few assays, the level of significance is usually taken to be $P=0.05$. In collaborative studies with many participants, however, a more conservative level of significance is often used. This is because the level of $P=0.05$ leads to about 10 per cent errors of the first kind (incorrect rejection of assays), whereas errors of the second kind (incorrect acceptance of assays) will not influence the global outcome of the study much because of the large amount of data available. Hence, the level of significance in this study is taken to be $P=0.01$ which would imply an expectation of about 2 per cent incorrect rejections. A slight but significant curvature was not considered reason for rejection if the mean square for quadratic regression was less than $1/100$ of the mean square for linear regression and the difference between preparations was small [5,6].

Whenever a laboratory performed several assays based on the same weighings, yielding several non-independent estimates of potency, a weighted mean potency of the valid sub-assays was calculated using weights proportional to the reciprocal of the variance. The valid assays per laboratory were combined using the same method of weighted combination, but a semi-weighted combination was used whenever the confidence intervals of the independent potency estimates did not satisfactorily overlap each other by means of a $\chi^2$ test for homogeneity ($P<0.10$). The estimates (one for each of the participants) were then combined into one single estimate with a 95 per cent confidence interval using the same method of semi-weighted combination.

Results
Among the seven participants, five laboratories reported results from assays. One laboratory submitted 2 sets of results obtained by two different operators. In this report they are treated as if they were from different laboratories and therefore assigned two distinct laboratory codes. The laboratories are referred to by their randomly assigned code-numbers (1 to 6), not necessarily corresponding to the order of listing in the list of participants. All participants carried out 6 assays by diffusion except Laboratory 6 which submitted results from 4 assays only. A total of 34 assays were reported or 1360 zone diameter readings.

The complete computer output of the parallel line analyses as performed at the EDQM is available in PDF format to participants of the study (68 pages generated by CombiStats). A summary of the results, as generated by the SAS-System is given in Table 2 (See Annex 1 for the essential SAS-scripts used). Shown are the potency estimates and associated 95 per cent confidence intervals, together with the relevant $P$-values. $P$-values below the significance level of 0.01 are printed on a grey background. The confidence intervals based on calculations by the participants are also listed.

A graphical representation of the confidence intervals of each individual assay is shown in Figure 4 (EDQM calculations) and in Figure 5 (Participants’ calculations). Potency estimates ranged from 16372 IU/vial (Lab 1) to 21149 IU/vial (Lab 4).
Laboratory 1
A log-transformation of the data was used to achieve better linearity. The highest of the 4 doses was excluded in all assays because it did not fall within the linear range. Assay 5 had to be rejected due to significant deviations from linearity. The other 5 assays were statistically valid and the potency estimates were homogeneous (P=0.364). The weighted combined estimate is 17015 IU/vial (±2.3%).

Laboratory 2
The 6 assays were statistically valid and the potency estimates were homogeneous (P=0.997). The weighted combined estimate is 17959 IU/vial (±1.2%).

Laboratory 3
The 6 assays were statistically valid and the potency estimates were homogeneous (P=0.724). The weighted combined estimate is 17724 IU/vial (±2.3%).

Laboratory 4
The 6 assays were statistically valid and the potency estimates were heterogeneous (P<0.001). The semi-weighted combined estimate is 20000 IU/vial (±2.2%).

Laboratory 5
The 6 assays were statistically valid and the potency estimates were heterogeneous (P<0.001). The semi-weighted combined estimate is 17785 IU/vial (±2.5%).

Laboratory 6
The 4 assays were statistically valid and the potency estimates were homogeneous (P=0.456). The weighted combined estimate is 17519 IU/vial (±2.0%).

A histogram of all potency estimates per assay is shown in Figure 6 and a histogram of the mean results per laboratory is shown in Figure 7. The final confidence intervals per laboratory are summarised in Table 3 and a graphical representation is given in Figure 8. The results from Laboratory 4 are on average 13% higher than the average result from the other laboratories. This difference is considered too large so it is excluded as an outlier from the overall combination of potencies. The χ2 value for the remaining between-laboratory homogeneity is highly significant (P=0.002) so a semi-weighted combination was made which yields 17643 IU/vial with 95% confidence limits of 17426 and 17863 IU/vial (which is ±1.2%).

Comments from Participants
None of the participants opposed the conclusions of this report.

Recommendation
The proposed candidate batch is suitable for its intended purpose. It is proposed that the 2nd WHO International Standard for Neomycin B (EDQM internal code ISA_46104) be assigned an antimicrobiological activity of 17 640 IU per vial.
Acknowledgements
On behalf of EDQM, the Study Director wishes to express her sincere thanks to all participants for their valuable contribution to this study. Special thanks go to Sanofi Aventis, for their well-appreciated donation of candidate material.

Traceability of data
This study has been conducted by EDQM (project code ISA012). Data and protocols are filed under studies numbers 07169 and 07205.
Date of reporting: June 2012.

References
LIST OF PARTICIPANTS

By alphabetical order of contact person

P. CSOKÁN
Directorate of Veterinary Medicinal Products - Central
Szállás utca 8
HU - 1107 Budapest

D. PIVODOVA, L. HROMÁDKOVÁ
Institute for the State Control of Veterinary Biologicals and Medicaments
Laboratory Control Section
Hudcova 56A
CZ - 621 00 Brno

S. JORAJURIA, V. DUJARDIN, C. RAPHALEN
EDQM, DLAB
7, Allée Kastner
F - 67085 Strasbourg Cedex

A. KISO
Agency for Medicinal Products and Medical Devices
Control Laboratory
Titova 9
BA – 71000 Sarajevo

K. LONGSTAFF
Therapeutic Goods Administration Laboratories
Office of Laboratories and Scientific Services – Microbiology Section
136 Narrabundah Lane
AU – Symonston act 2609

L. MEIRINHOS-SOARES, M. JOÃO PORTELA
INFARMED
National Authority of Medicines and Health Products, I.P.
Parque de Saúde de Lisboa, Av. do Brasil, nº 53
PO – 1749-004 Lisboa

G. PIANETTI
Laboratório de Controle de Qualidade
Faculdade de Farmácia da UFMG
BR – 31270-901 - Belo Horizonte - Minas Gerais
Avenida Presidente Antônio Carlos, 6627 - Campus Pampulha
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Table 1: Accelerated Degradation. Liquid Chromatography Results.
Figure 1

Impurity profile 1 month

Figure 2

Impurity profile 3 months
Figure 3
Flow chart for assay validity check

START

Significant deviations from parallelism?
Yes → Reject the assay
No

Significant deviations from linearity?
No → Accept the assay
Yes

Significant lack of quadratic fit?
Yes → Reject the assay
No

Significant quadratic curvature?
No → Accept the assay
Yes

Ratio MS curvature/regression small?
No → Reject the assay
Yes

Difference between preparations small?
Yes → Accept the assay
No → Reject the assay
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<th>Table 2: Overview of results</th>
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**Results:**
- **Lab:** Assay
- **Cumulative:** Count time spot density, count time spot density
- **Area:** Overview of results
Figure 4 - Individual estimates per assay (calculated at EDM)

Laboratory

The numbers below the assay code identify the laboratory codes followed by the assay number. The assay results are shown with an error bar.
Figure 5 - Individual estimates per assay (calculated by participants)
Figure 6
Histogram of potencies per independent assay

Figure 7
Histogram of combined potencies per laboratory
### Table 3 - Combined potency estimates per laboratory

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* Excluding laboratory 4
Figure 8 - 95% confidence intervals per laboratory
Annex 1: SAS-Script used for the calculations

/* This is the essential script to perform the analysis of variance. It expects a SAS-data-set "Neomycin B" with the following fields: prep: 1 for standard, 2 for test. dose: on log-scale with the primary dose set to 0. p: copy of dose. row: indicates the row in Latin square designs. block: the column in Latin square designs or the petri-dish in randomized block designs. block and/or row are set to 1 if not applicable for their respective designs. obs: the value of the observation (possibly transformed). */

ods select none;
proc glm data=Neomycin B;
   /* Perform the Anova by progressively relaxing model assumptions */
   class prep dose block row;
   model obs=block row prep dose dose*prep dose*dose*prep / ss1;
   ods output OverallAnova=OverallAnova ModelAnova=ModelAnova;

data Anova(keep=source df ss ms value probf);
   /* Non-linearity has to be calculated in a separate dataset */
   retain dfLin ssLin msLin;
   set ModelAnova OverallAnova;
   if df>0 then output;
   if Source="dose" then do; dfLin=df; ssLin=SS; end;
   if Source="prep" then do; dfLin=dfLin+dF; ssLin=ssLin+SS; end;
   if Source="Error" then do;
      Source="Non-linearity";
      FValue=(ssLin/dfLin)/ms;
      ProbF=1-ProbF(FValue,dfLin,df);
      ss=ssLin; df=dfLin; ms=ms/df;
      if df>0 then output;
   end;
ods select all;
proc print data=Anova noobs;
run;

/* This is the essential script to perform the potency calculations. It expects a SAS-data-set "info" with the following fields: Assigned: The assigned potency of the standard mgS: weight taken of the Standard mlS: Dilution used to prepare the primary dose of the Standard. mgT: weight taken of the Test mlT: Dilution used to prepare the primary dose of the Test. */

ods select none;
proc glm data=Neomycin B;
   /* Fit the parallel line model and output the parameter estimates and covariance matrix */
   class block row;
   model obs=prep dose block row / inverse solutions;
   ods output InvFXX=CovB ParameterEstimates=FarmEst;

data Estimate(keep=Low Est High);
   /* calculate the relative potency [m] */
   set FarmEst where Parameter='prep'; a=Estimate;
   set FarmEst where Parameter='dose'; b=Estimate;
   m=a/b;
   /* Use Fieller's theorem to compute the confidence limits */
   set CovB where Parameter='prep'; v11=prep;
   set CovB where Parameter='dose'; v21-prep; v22=dose;
   set FarmEst where source='Error'; t=tinv(0.975,df); s=sqrt(ms);
   g=(t*s*s*v22)/(b*b);
   root=v11-2*m*v12*m*m*v22-g*(v11-v12*v12/v22);
   ml=m-g*(v11/v22-t*s*b)*sqrt(root)/(1-g);
   ml=m-g*(v11/v22-t*s*b)*sqrt(root)/(1-g);
   /* Transform the relative potency to IU by correcting for the pre-dilutions */
   set info; Correction= Assigned*mgs/mlS*mlT/mgT;
   Low=Correction*exp(ml1); Est=Correction*exp(m1); High=Correction*exp(ml2);
   output;
ods select all;
proc print data=Estimate noobs;
run;
Annex 2: Safety Data Sheet and Leaflet

**PROPOSAL**

SECTION 1 Identification of the substance/mixture and of the company/undertaking

Trade name: NEOMYCIN B
Use: For laboratory tests and assays only.
Directions for use: For any questions: www.edqm.eu/hdl (HelpDesk)
Company identification: European Directorate for the Quality of Medicines & Healthcare
EDQM, Council of Europe,
7 Allée Kastner CS 30026
F-67081 Strasbourg FRANCE
Tel. +33 (0)3 88 41 20 35
Fax. +33 (0)3 88 41 27 71
Emergency phone: +44 (0) 1235 238670

SECTION 2 Hazards identification

Risk Phrases: Irritating to eyes, respiratory system and skin. - May cause sensitization by inhalation and skin contact. - Possible risk of harm to the unborn child.

Adverse human health effects: Exposure may produce an allergic reaction. This material may induce blood disorders and/or aggravate pre-existing blood disorders. Gastrointestinal disorders.

Note: This substance is not classified in the table 3.1 of Annex VI of Regulation (EC) No 1272/2008.

SECTION 3 Composition/information on ingredients

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<th>Substance name</th>
<th>Contents</th>
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<th>Annex No</th>
<th>REACH</th>
<th>Classification</th>
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<td>28000-79-2</td>
<td>224-770-9</td>
<td></td>
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</table>

SECTION 4 First aid measures

First aid measures
- Inhalation: Assure fresh air breathing. Rest. If you feel unwell, seek medical advice.
- Skin contact: Remove affected clothing and wash all exposed skin areas with mild soap and water, followed by warm water rinse.
- Eye contact: Rinse immediately with plenty of water. Obtain medical attention if pain, blinking, tears or redness persist.
- Ingestion: Rinse mouth. If swallowed, seek medical advice immediately and show this container or label.

In case of reactions described in hazards identification or other severe, immediate or persisting symptoms seek medical advice and call the nearest poison centre. Show the label and this safety data sheet.
**NEOMYCIN B**

**SAFETY DATA SHEET**

**ISA_68_041**

Revised edition no : 3  
Date : 12 / 8 / 2009  
Supersedes : 0 / 0 / 0

### SECTION 6 Fire-fighting measures

- **Extinguishing media**
- **Unsuitable extinguishing media**
  - Not fully tested.
- **Surrounding fires**
  - Use water spray or fog for cooling exposed containers.
- **Protection against fire**
  - Do not enter fire area without proper protective equipment, including respiratory protection.
- **Hazardous combustion products**
  - Incomplete combustion will generate carbon monoxide and other toxic gases.

### SECTION 6 Accidental release measures

**General precautions**
- Remove ignition sources. Evacuate area.

**Personal precautions**
- Spill should be handled by trained cleaning personnel properly equipped with respiratory, skin and eye protection.

**Clean up methods**
- Clean spills promptly. To clean the floor and all objects contaminated by this material, use Sodium hypochlorite solution. Ensure adequate ventilation.

### SECTION 7 Handling and storage

**Personal protection**
- Avoid all unnecessary exposure. Ensure prompt removal from eyes, skin and clothing.

**Technical protective measures**
- Material should be handled in a laboratory hood, glove box or similar whenever possible.

**Handling**
- Handle in accordance with good industrial hygiene and safety procedures.

**Storage**
- NEOMYCIN B is not intended for long-term storage. Keep container tightly closed in a cool, well ventilated place.

**Storage - away from**
- All heat sources, including direct sunlight. Open flame. Sources of ignition. Sparks. Incompatible materials, see §10

### SECTION 8 Exposure controls/personal protection

**Personal protection**

- Respiratory protection
  - Wear approved mask. (P1)
  - In case of insufficient ventilation, wear suitable respiratory equipment.

- Hand protection
  - Wear suitable gloves resistant to chemical penetration.

- Skin protection
  - Wear suitable protective clothing.

- Eye protection
  - Chemical goggles or safety glasses.

**Industrial hygiene**
- Provide local exhaust or general room ventilation.
- Material should be handled in a laboratory hood, glove box or similar whenever possible.

### SECTION 9 Physical and chemical properties

- **Chemical formula**
  - C23H46N6O13·xH2SO4
- **Molecular weight**
  - 615
**NEOMYCIN B**

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**SECTION 9 Physical and chemical properties (continued)**

- **Appearance**: White to light yellow. Powder.
- **Physical state at 20 °C**: Powder.
- **pH value**: No data available.
- **Melting point [°C]**: No data available.
- **Boiling point [°C]**: No data available.
- **Solubility in water**: Complete.
- **Solubility**: No data available.
- **Flash point [°C]**: No data available.
- **Log P octanol / water at 20°C**: No data available.

**SECTION 10 Stability and reactivity**

- **Stability and reactivity**: Stable under normal conditions.
- **Materials to avoid**: Strong oxidizers.
- **Conditions to avoid**: Moisture, Light.
- **Hazardous reactions**: None under normal conditions.
- **Hazardous polymerization**: Will not occur.

**SECTION 11 Toxicological information**

- **RTECS nr**: WK2323000 (See actual entry in RTECS for complete information.)
- **Rat oral LD50 [mg/kg]**: No data available.
- **Mouse oral LD50 [mg/kg]**: >5000
- **Rabbit dermal LD50 [mg/kg]**: No data available.
- **Rat inhalation LC50 [mg/L/4h]**: No data available.
- **Acute toxicity**: This material may induce blood disorders and/or aggravate pre-existing blood disorders. Gastrointestinal disorders. Exposure may produce an allergic reaction.
- **Chronic toxicity**: No data available.
- **Toxic for reproduction : unborn child**: Category 3: Substances which cause concern for humans owing to possible developmental toxic effects.

**SECTION 12 Ecological information**

- **Ecological effects information**
  - **LC50-96 Hour - fish [mg/L]**: No data available.
  - **EC50-48 Hour-Daphnia magna [mg/L]**: No data available.
  - **IC50-72h-Algae [mg/L]**: No data available.
  - **Biodegradation [%]**: No data available.
  - **Persistence - degradability**: No data available.
  - **Log P octanol / water at 20°C**: No data available.
  - **Bioaccumulative potential**: No data available.
NEOMYCIN B
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SECTION 13 Disposal considerations

General : Dispose of this material and its container at hazardous or special waste collection point.
Dispose in a safe manner in accordance with local/national regulations.

SECTION 14 Transport information

General information : Not classified.

SECTION 16 Regulatory information

Symbol(s)

R Phrase(s) : R36/37/38 : Irritating to eyes, respiratory system and skin.
R42/43 : May cause sensitization by inhalation and skin contact.
R63 : Possible risk of harm to the unborn child.

S Phrase(s) : S22 : Do not breathe dust.
S36/37 : Wear suitable protective clothing and gloves.
S45 : In case of accident or if you feel unwell, seek medical advice immediately (show the label when possible).
S53 : Avoid exposure - obtain special instructions before use.

Toxic for reproduction : unborn child : Category 3 : Substances which cause concern for humans owing to possible developmental toxic effects.

SECTION 16 Other information

Further information : Revision - See :
List of relevant R phrases : R36/37/38 : Irritating to eyes, respiratory system and skin.
R42/43 : May cause sensitization by inhalation and skin contact.
R63 : Possible risk of harm to the unborn child.

The contents and format of this SDS are in accordance with REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, as amended.

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End of document
The 2nd International Standard for Neomycin B

1. The Standard

The 2nd International Standard (IS) for Neomycin B (ISA_46104) consists of vials of freeze-dried neomycin B sulfate. This preparation was established as the 2nd IS for Neomycin B by the Expert Committee on Biological Standardization of the World Health Organization in 2012.

2. Biological Activity

The standard was calibrated in an international collaborative study involving 7 laboratories from different countries, against the 1st IS for Neomycin B.

The assigned potency is 17640 IU per vial for the 2nd IS for Neomycin B.

3. Use of the Standard

Dissolve the entire content of the vial with an exact amount of solvent using gentle shaking. Transfer the solution to a plastic tube and keep at room temperature during the assay. The solution should be used as soon as possible and should be kept at 25°C maximum during assays. Unused material must be discarded and not frozen for later use. Unopened vials should be stored at -20°C.

The product in the vial is freeze-dried. Do not weigh out portions of the product; dissolve it preferably by injecting solvent through the rubber stopper while avoiding the generation of pressure within the vial which might lead to a loss of material when retracting the needle. The cake should dissolve rapidly. Care should be taken to avoid any loss and rinsing steps are recommended to ensure quantitative transfer into the volumetric flask.

4. Stability

Accelerated degradation studies have shown that the standard is stable when stored in unopened vials at -20°C, with no predictable loss of potency over a period of 36 months. It is therefore recommended that the unopened vials are stored at -20°C or below until immediately before use.

5. References

Collaborative Study for the Establishment of the Second International Standard for Neomycin B, WHO/BS/12.xxxx

6. Caution

This material is not for administration to humans. Safety Data Sheet is available on the EDQM website (www.edqm.eu) or on request.

7. Citation

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

8. Product liability

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