Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Salmon Calcitonin
NIBSC code: 98/586
Instructions for use
(Version 5.0, Dated 03/03/2009)

1. INTENDED USE

This consists of a batch of ampoules, coded 98/586, containing synthetic salmon calictonin analysed by international collaborative study and established as the Third International Standard (IS) for Calcitonin, Salmon by the Expert Committee on Biological Standardisation of the World Health Organisation (1). This preparation replaces the Second International Standard of Calcitonin, (coded 87/788) (2), stocks of which are now exhausted.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The material is not of human or bovine origin. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

Each ampoule of the Third IS contains 138 International Units (IU).
(This value is equivalent to 23µg calcitonin per ampoule).

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution which contained:

Salmon Calcitonin	approximately 30mg/L
Mannitol	2g/L
Acetic acid	0.001M

And pure dry nitrogen at slightly less than atmospheric pressure.

5. STORAGE

Unopened ampoules should be stored at –20ºC
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

For practical purposes each ampoule contains the same quantity of calcitonin. The entire contents of each ampoule should be completely dissolved in an accurately measured amount of suitable diluent, with carrier protein where extensive dilution is required. No attempt should be made to weigh out any portion of the freeze-dried powder. The material has not been sterilized and the ampoules contain no bacteriostat. Unopened ampoules should be stored at –20°C in the dark.

PREPARATION OF AMPOULES

A batch of highly purified synthetic peptide, lot number JO 1997007, was donated for ampouling as the replacement standard by Dr H-B. Jenny and Dr U. Merz, Novartis Pharma AG, Basle, Switzerland. Analytical data provided by Novartis stated that the peptide was >96% monocomponent by HPLC. The preparation contained 3.0% moisture and 7.9% acetic acid, had a peptide content of 90.5% by amino acid analysis and a biological activity of 5365IU/mg.
The preparation was received as a lyophilised white powder; 151mg were initially dissolved in 10ml pre-filtered (0.22µm) excipient solution (0.2% mannitol/1.74mM acetic acid), filtered (0.22µm) and added to 4.3 L excipient solution along with filter washings. The filling solution was transferred to a 4°C cold room and stirred gently overnight to equilibrate. The solution was distributed into ampoules as 1.0ml aliquots which were then lyophilized and sealed according to procedures described by WHO for International Biological Standards (3) and stored at –20°C in the dark.
Check weights carried out during the filling process gave a mean filling weight of 1.0083g (CV 0.98%) and the residual moisture content was 0.45%. Each ampoule coded 98/586, has a predicted content of 30µg salmon calcitonin (but see results of international collaborative study), 2mg mannitol and acetic acid (0.001M), thereby maintaining similarity with the Second IS.

COLLABORATIVE STUDY

Aims of the study

The preparation in ampoules coded 98/586 was evaluated by international collaborative study in which twelve laboratories in nine countries took part. The study was designed:

1)to determine the activity of sCT (98/586) by in vivo bioassay in terms of the second IS for sCT (87/788)
2)to assess the stability of the preparation after accelerated thermal degradation
3)to estimate the purity of the ampouled candidate preparation and to determine the sCT content in gravimetric units by HPLC.

Activity of ampoule contents

Five laboratories contributed bioassay data using the *in vivo* rat hypocalcaemic method with one of the laboratories also contributing *in vitro* data. Analysis of all bioassay data gave a homogeneous data set with a geometric mean of 140.4 (95% fiducial limits 130.6-150.8) IU per ampoule.
Twelve laboratories provided HPLC data on purity and ampoule content using methodology based on the EP method (4) or an in-house system. The mean of individual laboratory estimates for purity by all methods was 91.9% (CV4.9%). Using the salmon calcitonin CRS as a reference, the mean of individual laboratory estimates for ampoule content was 23.12µg per ampoule with a relative standard deviation of 3.8% Since the predicted content of the ampoules was 30µg, based on dilution of a known mass of sCT and volume of formulated solution delivered to the ampoule, this indicates that material may have been lost, either prior to filling, possibly when stirring overnight, or during the filling process itself.

Assignment of unitage

On the basis of the consistency of estimates and predicted stability on storage, it was proposed that the gravimetric content of 98/586 be assigned in terms of HPLC measurements, and that the biological activity of the ampoule contents in IU be determined from this value and the internationally agreed figure of 6000 IU per mg for the specific activity of salmon calcitonin. Therefore using the mean laboratory estimate of ampoule content of 23µg per ampoule and a specific activity of 6000IU per mg, 98/586 was assigned an ampoule content of 138 IU per ampoule. Although from a relatively small data set, the bioassay estimate of 140 IU per ampoule was in excellent agreement with this value.

Participants

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8. STABILITY

Ampoule of sCT 98/586 stored at elevated temperatures for 8 months showed little evidence of any significant loss of activity by bioassay and only a modest increase in degradation products by HPLC. The results indicate that sCT 98/586 is sufficiently stable to serve as a standard.

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

1. Report 1999. WHO Expert Committee on Biological Standardisation.
2. Zanelli, J.M., Gaines Das R.D & Corran P.H. International Standards for salmon calcitonin, eel calcitonin and the Asu[1-7] analogue of eel calcitonin: calibration by international collaborative study. Bone and Mineral 1990 11: 1-17.
3. WHO Technical Report Series No. 800, 1990 181-214.
4. European Pharmacopoeia, 2000 Addendum

10. ACKNOWLEDGEMENTS

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11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties	
Physical appearance: Freeze dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Can react with oxidising materials. Avoid contact with acids and alkalis.	
Toxicological properties	
Effects of inhalation: Not established, avoid inhalation	
Effects of ingestion: Not established, avoid ingestion	
Effects of skin absorption: Not established, avoid contact with skin	
Suggested First Aid	
Inhalation: Seek medical advice	
Ingestion: Seek medical advice	
Contact with eyes: Wash with copious amounts of water. Seek medical advice	
Contact with skin: Wash thoroughly with water.	
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 2 mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.