National Institute of Standards & Technology

Report of Investigation

Reference Material® 8642

FDA Saxitoxin Dihydrochloride Solution

This Reference Material (RM) is intended for use in calibrating the mouse bioassay used in AOAC International Official Method 959.08 Paralytical Shellfish Poison [1] and for other similar uses. RM 8642 FDA Saxitoxin Dihydrochloride Solution was prepared by the U.S. Food and Drug Administration's (FDA's) Center for Food Safety and Applied Nutrition (CFSAN), where it was identified as Lot 089. The RM is saxitoxin dihydrochloride (CAS No. 35554-08-6) in a solution containing a hydrochloric acid concentration of 5 mmol/L in 20 % ethanol in water (volume fraction). A unit of RM 8642 consists of ten amber, borosilicate glass ampoules each containing approximately 1.2 mL of solution.

Reference Value of Saxitoxin Hydrochloride: 103 µg/g ± 4 µg/g

Reference Mass Fraction Value: The reference value for saxitoxin hydrochloride in solution in RM 8642 is identified by FDA as Lot 089. Reference values are noncertified values that are estimates of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [2]. The reference mass fraction value is based on the gravimetric preparation of a stock solution and gravimetric dilution to produce the final material, and uncertainties associated with the associated weighings. The uncertainty is expressed as an expanded uncertainty, $U = ku_c$, at the 95 % level of confidence, $k = 2$, and includes a 2 % Type B purity uncertainty component as well as the gravimetric uncertainty [3]. Values are reported on an "as-received" basis in mass fraction units [4]. Since a single primary method was used, the measurand is the total mass fraction listed based on the gravimetric preparation. Metrological traceability is to the unit microgram analyte per gram sample on an "as-received" basis.

Expiration of Value Assignment: RM 8642 is valid, within the measurement uncertainty specified, until **01 July 2018**, provided the RM is handled and stored in accordance with instructions given in this Report of Investigation (see "Instructions for Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM: NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The technical and support aspects involved in the preparation and issuance of this RM were coordinated through K.E. Sharpless of the NIST Chemical Sciences Division and M.P. Cronise of the NIST Office of Reference Materials.

The solution was prepared and characterized by S. Hall of the Division of Bioanalytical Chemistry, Office of Regulatory Science, CFSAN, FDA.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved with the issuance of this RM were coordinated through the NIST Office of Reference Materials.

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Gaithersburg, MD 20899
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Report Revision History on last page.

Robert L. Watters, Jr., Director
Office of Reference Materials

NOTICE AND WARNING TO USERS

Warning: For laboratory use only.

Storage: Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied by NIST.

INSTRUCTIONS FOR USE

Gently tap the ampoule prior to opening to allow any solution in the tip to drain into the body of the ampoule.

Prepare a working solution as follows: On a top-loading balance, record the tare weight of an appropriate plastic bottle to 0.1 g or better. To the bottle, add approximately 100 mL water that has been acidified to pH 3 with hydrochloric acid. To minimize error due to evaporation, be prepared to immediately transfer the RM solution to this bottle after opening the ampoule. To open, hold the ampoule steady and grasp the stem at the metallic band with thumb and forefinger; **minimal** thumb pressure should be applied to the stem to snap it. Correctly done, the stem should break easily where pre-scored. Aspirate the RM solution into a dry, clean, disposable plastic syringe, 2 mL to 5 mL capacity, fitted with a suitable needle (such as 18 G × 1 ½″), weigh the syringe and its contents to 1 mg or better, and dispense the solution into the bottle of acidified water. Do not rinse the syringe. Reweigh the emptied syringe to determine the mass of RM solution transferred to the bottle. Add sufficient acidifed water (pH 3, HCl) to adjust the concentration to 1 µg/g. Weigh the bottle and its contents to determine the mass of solution prepared and the exact concentration of the working solution.

Because of the volatility of ethanol, the reference value is not applicable to material in ampoules that have been previously opened. The concentration of the working solution should be stable for more than one month if the solution is protected from evaporation. Dilution by mass is preferred but, if dilution by volume must be performed, the density of the solution is 0.971 g/mL and the concentration of this standard is 100 µg/mL with an expanded uncertainty of 4 µg/mL. This uncertainty is calculated as described above.

Source and Preparation of Material: Saxitoxin was extensively purified on three low-pressure preparative columns, each containing a different stationary phase. The saxitoxin was converted to the dihydrochloride form by passage through an ion exchange resin in the chloride form. Purity was assessed at FDA by proton nuclear magnetic resonance spectroscopy, combustion analysis, and optical rotation. RM 8642, identified by FDA as Lot 089, was prepared by dissolving the saxitoxin dihydrochloride in a solution of hydrochloric acid (5 mmol/L) in 20 % ethanol in water (volume fraction).

REFERENCES

[1] AOAC International; *Official Methods of Analysis of AOAC International*, 18th ed.; Gaithersburg, MD (2005).

[2] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Sep 2013).

[3] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement;* (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Sep 2013); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); http://www.nist.gov/pml/pubs/index.cfm (accessed Sep 2013).

[4] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at: http://www.nist.gov/pml/pubs/sp811/index.cfm (accessed Sep 2013).

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