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Certificate of Analysis

Certified Reference Material

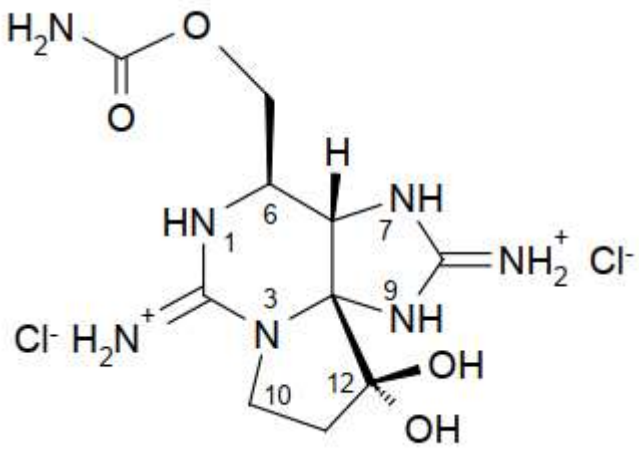
CRM-STX-g (Lot# 20201215)

Certified Calibration Solution for Saxitoxin Dihydrochloride

Saxitoxin (STX) [1] is found in both marine microalgae [2] and freshwater cyanobacteria [3]. CRM-STX-g is a certified calibration solution of STX in aqueous 0.5 mM hydrochloric acid, designed to aid in the identification and quantitation of STX. This is a replacement calibration solution for CRM-STX-f.

Table 1: Certified concentration and uncertainty for CRM-STX-g.

Compound	µmol/L (15 - 30 °C)
Saxitoxin (STX)	61.4 ± 2.4



Saxitoxin dihydrochloride
CAS registry No.: 35554-08-6
InChiKey: YHAHUGQQOBPXOZ-UIPPETONSA-N
Molecular formula: $C_{10}H_{19}N_7O_4Cl_2$
Molecular weight: 372.2 g/mol

Period of validity: 1 year from date of sale.
Storage conditions: +4 °C

Canada

Intended Use

CRM-STX-g is a certified calibration solution designed for analytical method development and accurate quantitation of STX. The concentration is suitable for preparing a dilution series for calibration of instruments such as liquid chromatography with detection by pre/post-column oxidation-fluorescence (LC-ox-FLD) or liquid chromatography-mass spectrometry (LC-MS), as well as for spiking control samples for recovery experiments.

Instructions for Storage and Use

To ensure the stability of CRM-STX-g, ampoules should be stored at +4 °C.

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents thoroughly mixed. The ampoule should be opened at the pre-scored mark. Calibrated volumetric equipment should be used for accurate transfer of aliquots. An increase in concentration due to evaporation of solvent will occur if the solution is left opened for more than a few minutes. It is recommended that the CRM should not be evaporated to dryness because of the potential for losses. *Note:* The volume of the solution is not certified. Only the concentration is certified. Therefore, the entire contents of the ampoule should not simply be transferred to a volumetric flask and diluted to volume.

Preparation of CRM-STX-g

N-sulfocarbamoylgonyautoxin-3 (C2) was isolated from a large-scale laboratory culture of *Alexandrium tamarense,* chemically converted to STX, which was then purified by several chromatographic steps [4]. The structure and purity of STX was confirmed by LC-MS [5, 6] (Figures 1 and 2) and ^{1}H NMR. A measured accurate *m*/*z* of 300.1413 (Δ = -1 ppm for $C_{10}H_{18}N_7O_4^+$) was obtained for the $[M+H]^+$ ion of STX (free base) using LC-high resolution MS (LC-HRMS). Purity was further assessed by LC-ox-FLD [7] (Figure 3), capillary electrophoresis with UV (CE-UV) [8], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [9]. A trace level of decarbamoylsaxitoxin (dcSTX) is present in CRM-STX-g.

The stock solution was prepared by diluting the purified STX in 0.5 mM aqueous hydrochloric acid for quantitation using ^{1}H-NMR (qNMR) [10]. The CRM-STX-g solution was prepared by accurately diluting the stock solution in 0.5 mM aqueous hydrochloric acid. Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL.

Analytical Methods and Value Assignment

The certified value for CRM-STX-g (Table 1) is based on results obtained at the NRC using three analytical methods: qNMR using potassium hydrogen phthalate for calibration, LC-CLND using caffeine for calibration, and LC-MS/MS using CRM-STX-f for calibration.

Homogeneity

A representative number of CRM-STX-g ampoules were selected from across the fill series and STX response was measured by LC-ox-FLD. No heterogeneity was observed.

Stability

Stability studies have demonstrated good stability for STX in aqueous hydrochloric acid stored in sealed ampoules at temperatures of +4 °C and below.

Uncertainty

All reasonable sources of error related to the characterization of CRM-STX-g were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}) and instability during storage (u_{stab}) [11-14]. These components are listed in Table 2, and are combined and expanded as follows:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{hom}^2 + u_{stab}^2}$$

where k is the coverage factor for a 95% confidence level (= 2).

Table 2: Uncertainty components for the certified value of CRM-STX-g.

Uncertainties	Relative*
u_{char}	0.020
u_{hom}	negligible
u_{stab}	0.002

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, STX and related toxins can cause paralysis and even death. Only qualified personnel should handle the solution and appropriate disposal methods should be used. Suitable personal protective equipment should be used when opening the ampoule in the event glass shatters. A safety data sheet (SDS) is available for CRM-STX-g.

Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-STX-g is valid for 1 year from the date of sale**.**

Metrological Traceability

Results presented in this certificate are traceable to the SI (*Système international d'unités*) through gravimetrically prepared standards of a NIST potassium hydrogen phthalate certified reference material (SRM 84L), a NMIA caffeine certified reference material (M724c), and a NRC certified reference material for STX (CRM-STX-f, lot # 20110316).

Quality Management System (ISO 17034, ISO/IEC 17025)

This material was produced in compliance with the National Research Council of Canada (NRC) Metrology Quality Management System, which conforms to the requirements of ISO 17034 and ISO/IEC 17025.

The Metrology Quality Management System supporting the NRC Calibration and Measurement Capabilities, as listed in the *Bureau international des poids et mesures* (BIPM) Key Comparison Database (http://kcdb.bipm.org/), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM) and found to be in compliance with the expectations of the *Comité international*

des poids et mesures (CIPM) Mutual Recognition Arrangement. The SIM approval is available upon request.

References

1. Hall S, Strichartz GR, Moczydlowski E, Ravindran A, Reichardt PB (1990). The saxitoxins: sources, chemistry, and pharmacology. In: Hall S and Strichartz GR (Eds) *Marine Toxins. ACS Symposium Series* 418: 29-65. Washington DC, *Am Chem Soc.*
2. Shumway SE (1990). A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquacult Soc* 21 (2): 65-104.
3. D'Agostino PM, Boundy MJ, Harwood TD, Carmichael WW, Neilan BA, Wood SA (2019). Re-evaluation of paralytic shellfish toxin profiles in cyanobacteria using hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Toxicon* 158: 1-7.
4. Laycock MV, Thibault P, Ayer SW, Walter JA (1994). Isolation and purification procedures for the preparation of paralytic shellfish poisoning toxin standards. *Nat Toxins* 2: 175-183.
5. Dell'Aversano, C, Hess, P, & Quilliam, MA (2005). Hydrophilic interaction liquid chromatography-mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. *J. Chromatogr. A* 1081: 190-201.
6. Thomas K, Beach DG, Reeves KL, Gibbs RS, Kerrin ES, McCarron P, Quilliam MA (2017). Hydrophilic interaction liquid chromatography-tandem mass spectrometry for quantitation of paralytic shellfish toxins: validation and application to reference materials. *Anal Bioanal Chem* 409: 5675-5687.
7. Anon. AOAC Official Method 2011.02. Paralytic shellfish toxins in mussels, clams, oysters, and scallops; post-column oxidation (PCOX) method (2009). *Official Methods of Analysis* 18th Ed. AOAC International, Gaithersburg, MD.
8. Thibault P, Pleasance S, Laycock MV (1991). Analysis of paralytic shellfish poisons by capillary electrophoresis. *J Chromatogr A* 542: 483-501.
9. Thomas K, Wechsler D, Chen YM, Crain S, Quilliam MA (2016). Analysis of natural toxins by liquid chromatography-chemiluminescence nitrogen detection and application to the preparation of certified reference materials. *J AOAC Int* 99: 1173-1184.
10. Burton IW, Quilliam MA, Walter JA (2005). Quantitative ^{1}H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. *Anal Chem* 77: 3123-3131.
11. Pauwels J, Lamberty A, Schimmel H (2000). Evaluation of uncertainty of reference materials. *Accred Qual Assur* 5: 95-99.
12. Pauwels J, Lamberty A, Schimmel H (1998). The determination of the uncertainty of reference materials certified by laboratory intercomparison. *Accredit Qual Assur* 3: 180-184.
13. Ellison SLR, Burke S, Walker RF, Heydorn K, Mansson M, Pauwels J, Wegscheider W, te Nijenhuis B (2001). Uncertainty for reference materials certified by interlaboratory study: Recommendations of an international study group. *Accredit Qual Assur* 6: 274-277.
14. Evaluation of measurement data – Supplement 1 to the "Guide to the expression of uncertainty in measurement" (GUM) – Propagation of distributions using a Monte Carlo method. JCGM 101:2008, www.bipm.org.

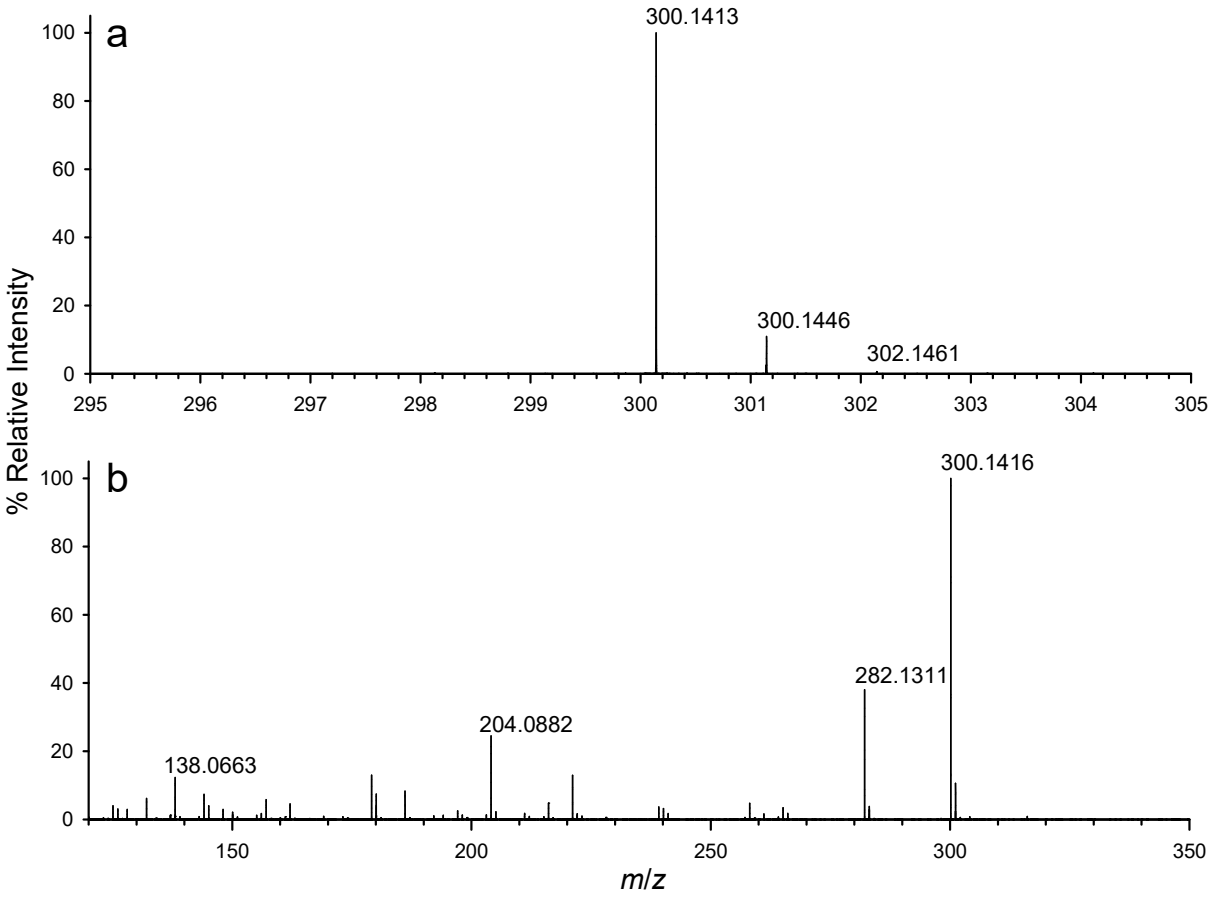


Figure 1: Full scan (a) and collision induced dissociation (b) (MS/MS) LC-HRMS spectra of STX used for CRM-STX-g analyzed on a Thermo Q Exactive-HF mass spectrometer equipped with a heated electrospray ionization probe. Data was collected in positive mode with a 2500 V spray voltage, +275 °C capillary temperature, and a +375 °C heater temperature. Full scan data was acquired with a resolution setting of 120 000. MS/MS data was acquired in parallel reaction monitoring scan mode with the same resolution setting and a normalized collision energy of 30 V.

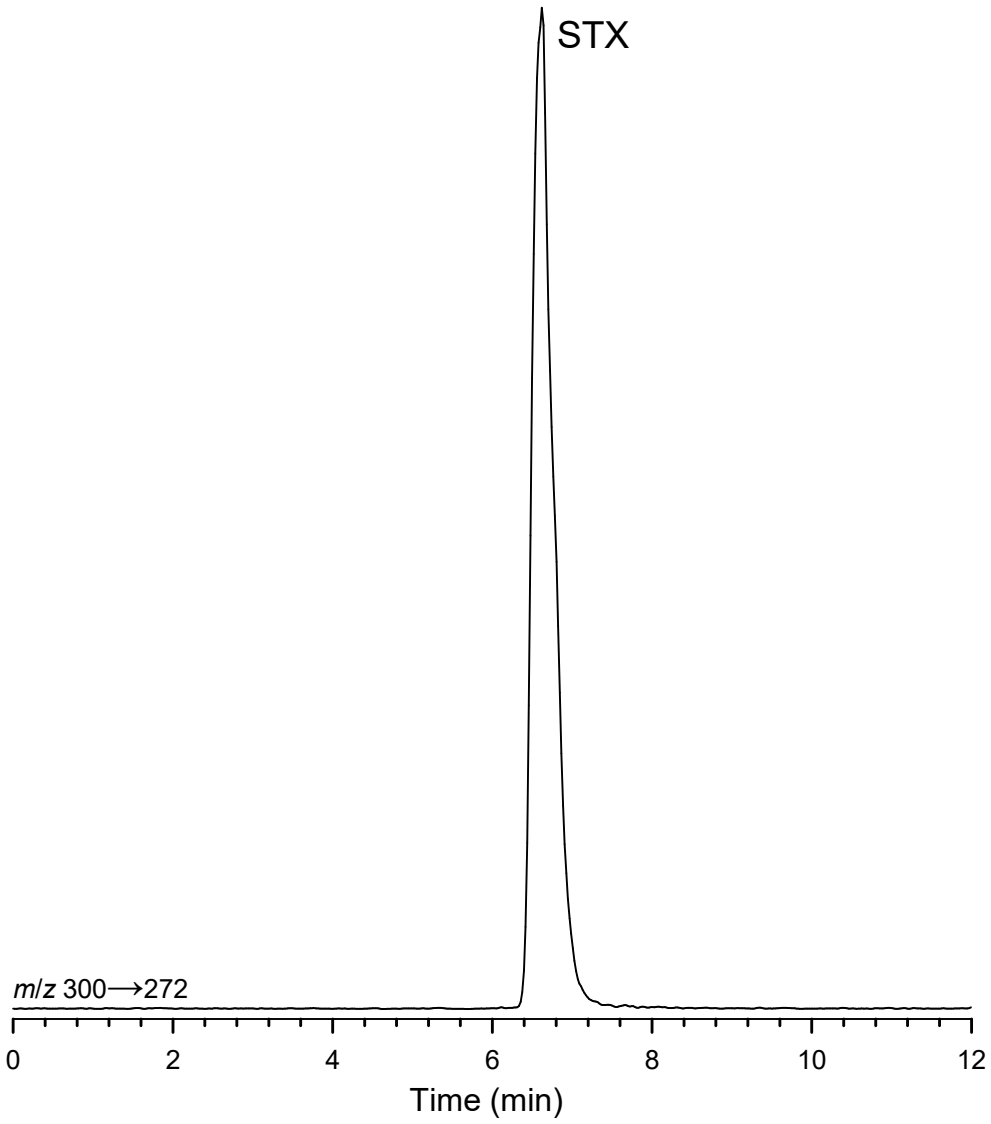


Figure 2: LC-MS/MS analysis of CRM-STX-g using selected reaction monitoring on an Agilent 1260 LC connected to a Sciex 4000 QTRAP with electrospray ionization. Chromatographic conditions: Toso-Haas Amide-80 column (250 mm × 2 mm, 5 µm) at +40 °C; mobile phase: 50% acetonitrile in water with 2 mM ammonium formate and 50 mM formic acid, 0.3 mL/min; injection volume: 5 µL. MS conditions: collision energy 25 V; declustering potential 40 V, and source temperature of +275 °C.

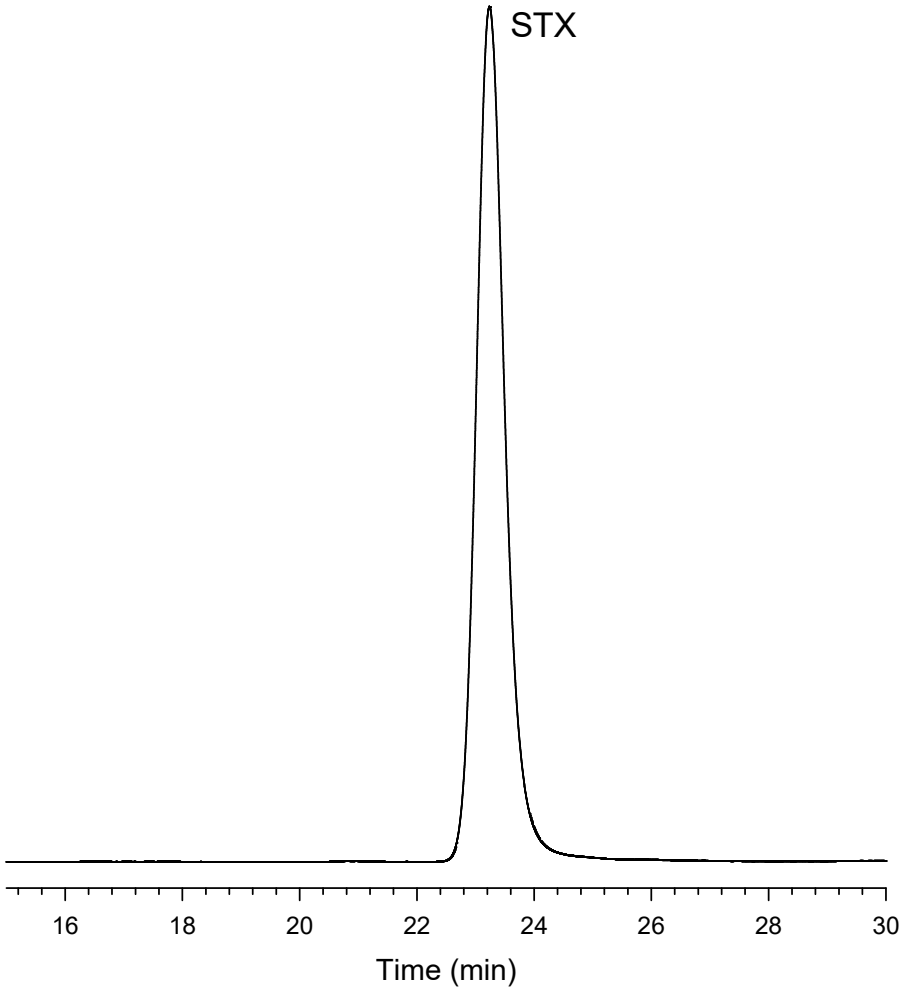


Figure 3: LC-ox-FLD analysis of CRM-STX-g. Conditions: Zorbax Bonus-RP column, 250 × 4.6 mm i.d. at +30 °C; mobile phase: water with 20 mM phosphoric acid, 8 mM heptane sulfonate, and 9% acetonitrile at pH 7.1, 0.8 mL/min; 10µL injection volume; post column oxidation with 0.4 mL/min 5 mM periodic acid, 100 mM phosphoric acid in water at pH 7.8; 1 mL reaction coil at +80 °C; effluent acidified with 0.4 mL/min 0.75 M nitric acid; fluorescence detection with excitation at 330 nm and emission at 390 nm.

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This Certificate is only valid if the corresponding material was obtained directly from the NRC or an Authorized Reseller.

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