National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

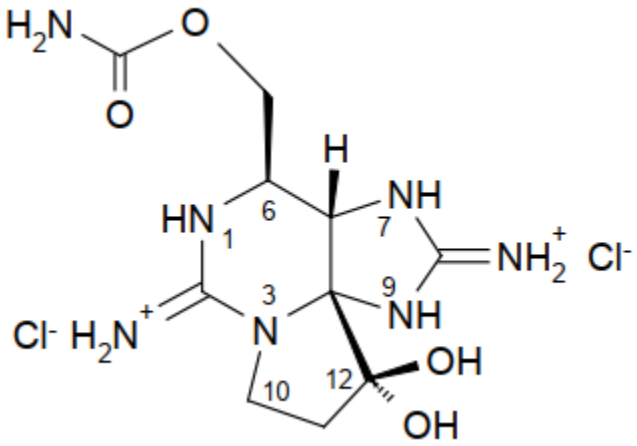
CRM-STX-g (Lot# 20201215)

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 reference material (CRM) 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 value and expanded uncertainty (*k* = 2) for CRM-STX-g

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



saxitoxin dihydrochloride

CAS registry number: 35554-08-6
InChIKey: YHAHUGQQOBPXOZ-UIPPETONSA-N
Molecular formula: $C_{10}H_{19}N_7O_4Cl_2$
Molar mass: 372.2 g/mol

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

DOI: doi.org/10.4224/crm.2021.stx-g.20201215

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.

Preparation of material

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.

Characterization of material

The certified value for CRM-STX-g (Table 1) is based on results obtained at the National Research Council Canada (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.

Metrological traceability

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

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 uncertainty related to the certified value in Table 1 were considered. Included in the combined uncertainty estimate are uncertainties in the batch characterization, uncertainties related to possible between-unit variation, and uncertainties related to stability [11-14].

The expanded uncertainty (U) for the value is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [14] and k is the coverage factor. A coverage factor of $k = 2$ was applied which corresponds to a level of confidence of approximately 95 %.

Storage

The material shall be stored at +4 °C. The ampoule should be stored unopened.

Instructions for handling and use

Prior to opening, each ampoule should be allowed to equilibrate to room temperature and the contents thoroughly mixed. The ampoule should be opened at the pre-scored mark immediately prior to use. The CRM solution should be transferred using calibrated equipment for accuracy.
The certified value is only guaranteed if the ampoule is sampled immediately after opening in order to limit solvent evaporation. It is important to note that the volume of the solution is not certified; only the concentration is certified. Therefore, the entire contents of the ampoule should not be diluted to volume.

Repeated sub-sampling and storage of the CRM solution after initial opening may impact the certified value. Users shall take responsibility for demonstrating that their sub-sampling and storage procedures do not impact the certified value.

Health and safety information

If sufficient quantities are ingested, STX and related toxins can cause paralysis and even death. Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at doi.org/10.4224/crm.2021.stx-g.20201215. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified value is valid for 1 year from the date of sale, provided the storage and instructions for handling and use specified in this certificate are followed.

Quality Management System

The NRC is Canada's national metrology institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and

comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

References

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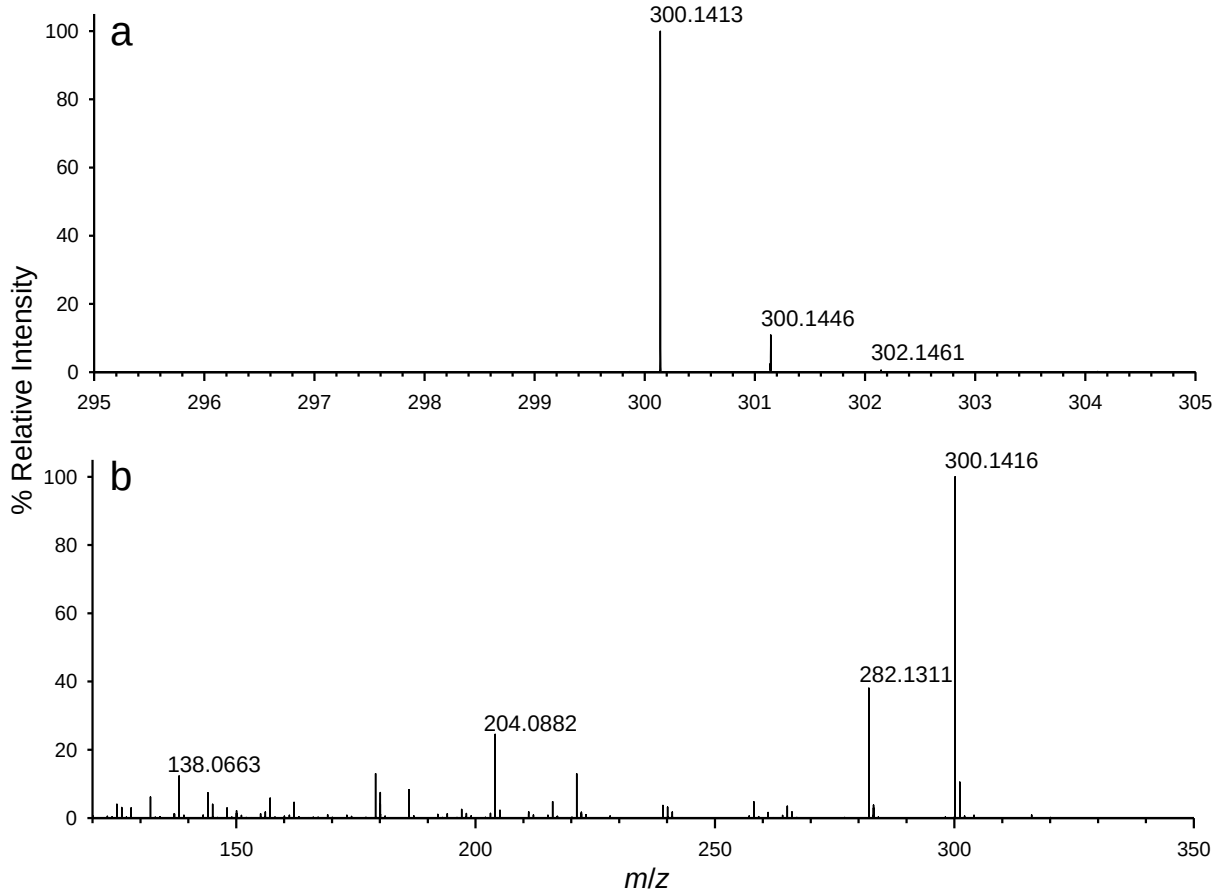

Figure 1: Full scan (a) and collision induced dissociation (b) (MS/MS) LC-HRMS spectra of STX used for CRM-STX-g analyzed on a Thermo Q Exactive-HF mass spectrometer equipped with a heated electrospray ionization probe. Data was collected in positive mode with a 2500 V spray voltage, 275 °C capillary 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 eV.

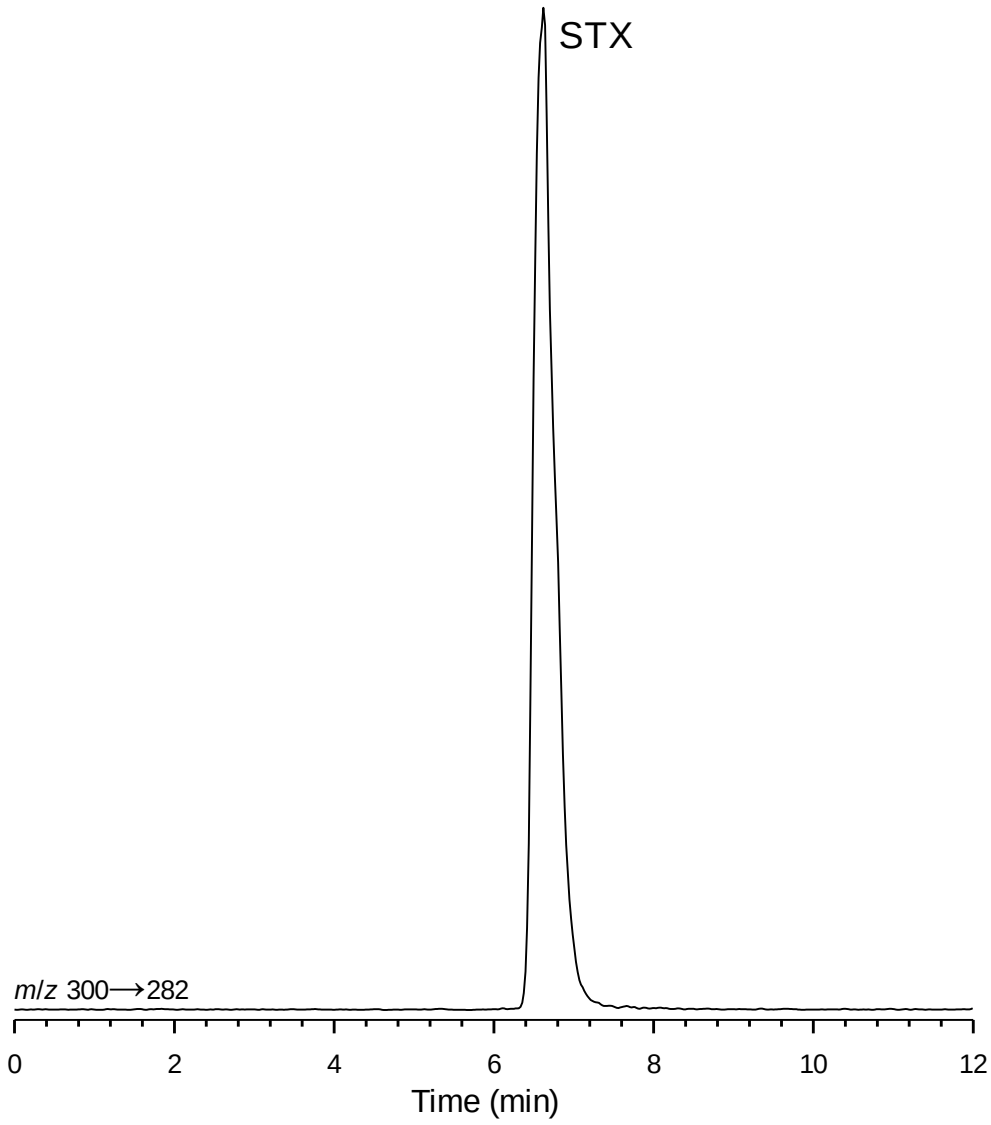

Figure 2: LC-MS/MS analysis of CRM-STX-g using selected reaction monitoring on an Agilent 1260 LC connected to a Sciex 4000 QTRAP with electrospray ionization. Chromatographic conditions: Toso-Haas Amide-80 column (250 mm × 2 mm, 5 µm) at 40 °C; mobile phase: 50% acetonitrile in water with 2 mM ammonium 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.

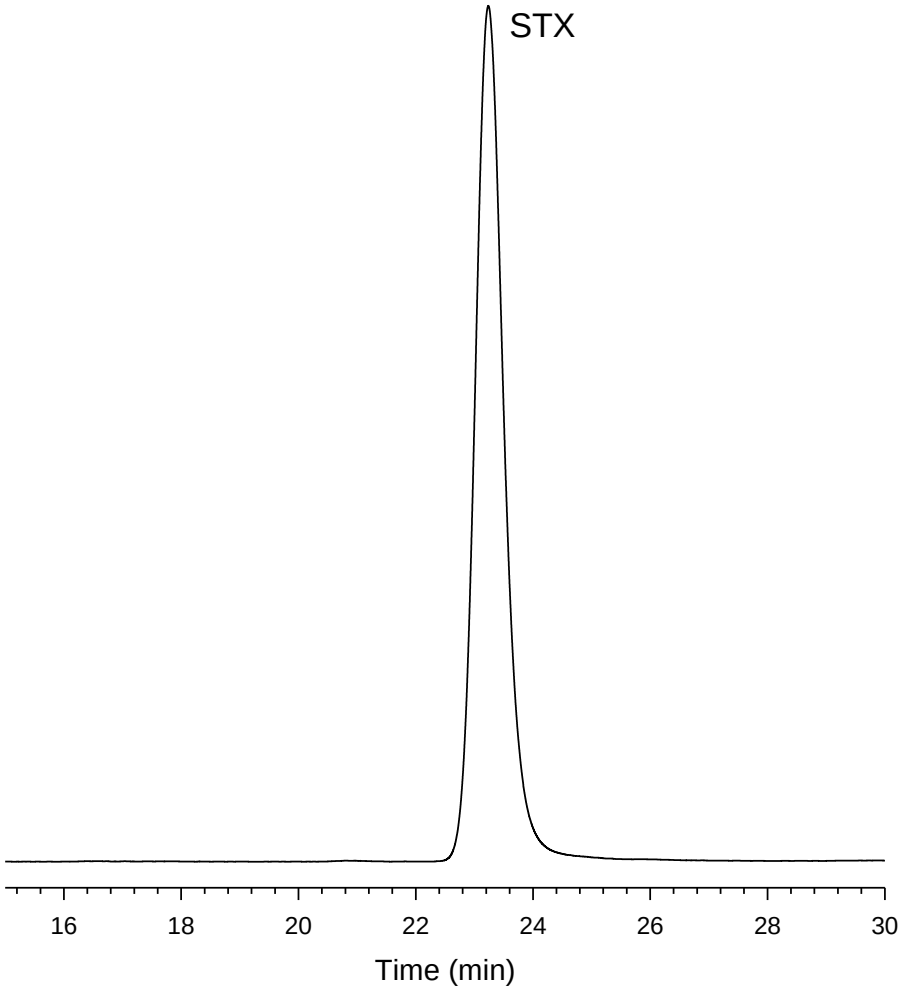

Figure 3: LC-ox-FLD analysis of CRM-STX-g. Conditions: Zorbax Bonus-RP column, 250 × 4.6 mm i.d. at 30 °C; mobile phase: water with 20 mM phosphoric acid, 8 mM heptane sulfonate, and 9% acetonitrile at pH 7.1, 0.8 mL/min; 10 µL injection volume; post column oxidation with 0.4 mL/min 5 mM periodic acid, 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.

Authorship

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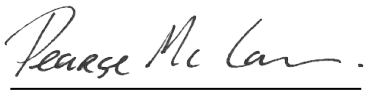
Approved by: 

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This certificate is only valid if the corresponding material was obtained directly from the NRC or an authorized reseller. Users should ensure that the certificate they have is current. For updates, please refer to doi.org/10.4224/crm.2021.stx-g.20201215.

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