



Certificate of Analysis

Certified Reference Material

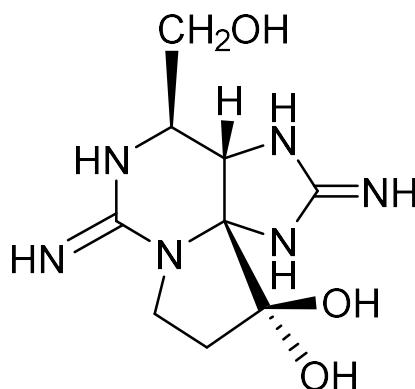
CRM-dcSTX-c (Lot# 20201125)

Certified Calibration Solution for Decarbamoysaxitoxin

Decarbamoysaxitoxin (dcSTX) is a saxitoxin analogue [1] found in both marine microalgae [2] and freshwater cyanobacteria [3]. CRM-dcSTX-c is a certified calibration solution of dcSTX in aqueous 0.5 mM hydrochloric acid, designed to aid in the identification and quantitation of dcSTX. This is a replacement calibration solution for CRM-dcSTX-b.

Table 1: Certified concentration and uncertainty for CRM-dcSTX-c.

Compound	$\mu\text{mol/L}$ (15 - 30 °C)
Decarbamoysaxitoxin (dcSTX)	65.3 ± 2.6



Decarbamoysaxitoxin (dcSTX)

CAS registry No.: 58911-04-9 (free base)

InChiKey: VRRIZJUSNMZMP-PJPYAQQDSA-N (free base)

Molecular formula: C₉H₁₆N₆O₃

Molecular weight: 256.3 g/mol

Period of validity: 1 year from date of sale.

Storage conditions: +4 °C

Intended Use

CRM-dcSTX-c is a certified calibration solution designed for analytical method development and accurate quantitation of dcSTX. The concentration is suitable for preparing a dilution series for 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-dcSTX-c, 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-dcSTX-c

N-sulfocarbamoylgonyautoxin-2 and -3 (C1&2) were isolated from a large-scale laboratory culture of *Alexandrium tamarense*, chemically converted to dcSTX, which was then purified by several chromatographic steps [4]. The structure and purity of dcSTX was confirmed by LC-MS/MS [5, 6] (Figures 1 and 2) and ¹H nuclear magnetic resonance (NMR). A measured accurate *m/z* of 257.1356 ($\Delta = -0.2$ ppm for C₉H₁₇N₆O₃⁺) was obtained for the [M+H]⁺ ion of dcSTX (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].

The stock solution was prepared by diluting the purified dcSTX in 0.5 mM aqueous hydrochloric acid for quantitation using ¹H-NMR (qNMR) [10]. The CRM-dcSTX-c solution was prepared by making an accurate dilution of 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 of solution.

Analytical Methods and Value Assignment

The certified value for CRM-dcSTX-c (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-dcSTX-b for calibration.

Homogeneity

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

Stability

Stability studies have demonstrated good stability for dcSTX 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-dcSTX-c 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-dcSTX-c.

Uncertainties	Relative*
u_{char}	0.020
u_{hom}	negligible
u_{stab}	0.001

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, dcSTX 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-dcSTX-c.

Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-dcSTX-c 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 CRM (NIST SRM 84L), a NMIA caffeine CRM (M724d), and a NRC CRM for dcSTX (CRM-dcSTX-b, lot # 20110713).

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

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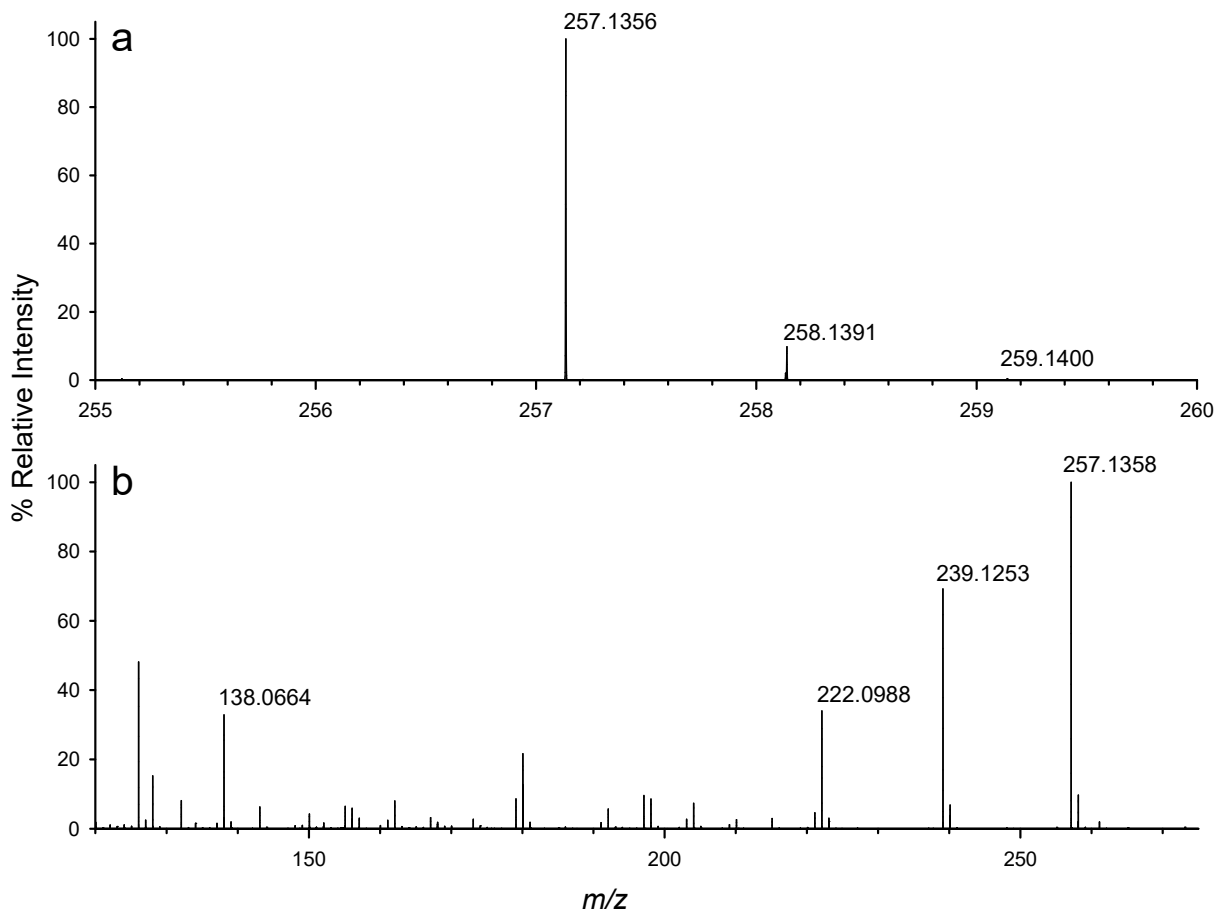


Figure 1: Full scan (a) and collision induced dissociation (MS/MS) (b) LC-HRMS spectra of dcSTX used for CRM-dcSTX-c analyzed on a Thermo QExactive-HF mass spectrometer equipped with a heated electrospray ionization probe. Data was collected in positive mode with a 2500 V spray voltage, +275 °C capillary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

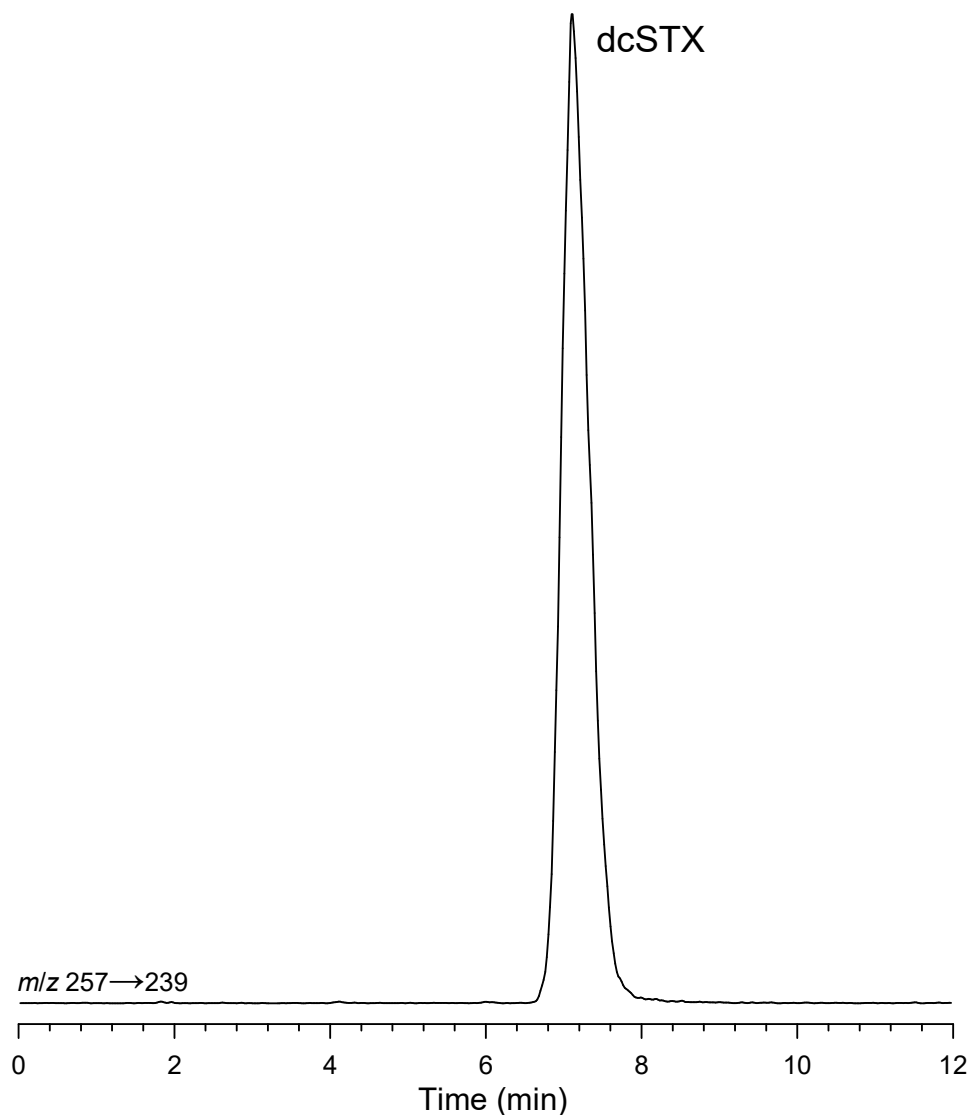


Figure 2: LC-MS/MS analysis of CRM-dcSTX-c using selected reaction monitoring on an Agilent 1260 LC connected to a Sciex 4000 QTRAP MS with electrospray ionization. Chromatographic conditions: Toso-Haas Amide-80 column (250 mm × 2 mm, 5 μm) at +40 °C; mobile phase: 50% acetonitrile in water with 2 mM ammonium formate and 50 mM formic acid, 0.3 mL/min; injection volume: 2 μL. MS conditions: collision energy +25 V; declustering potential +60 V, and source temperature of +275 °C.

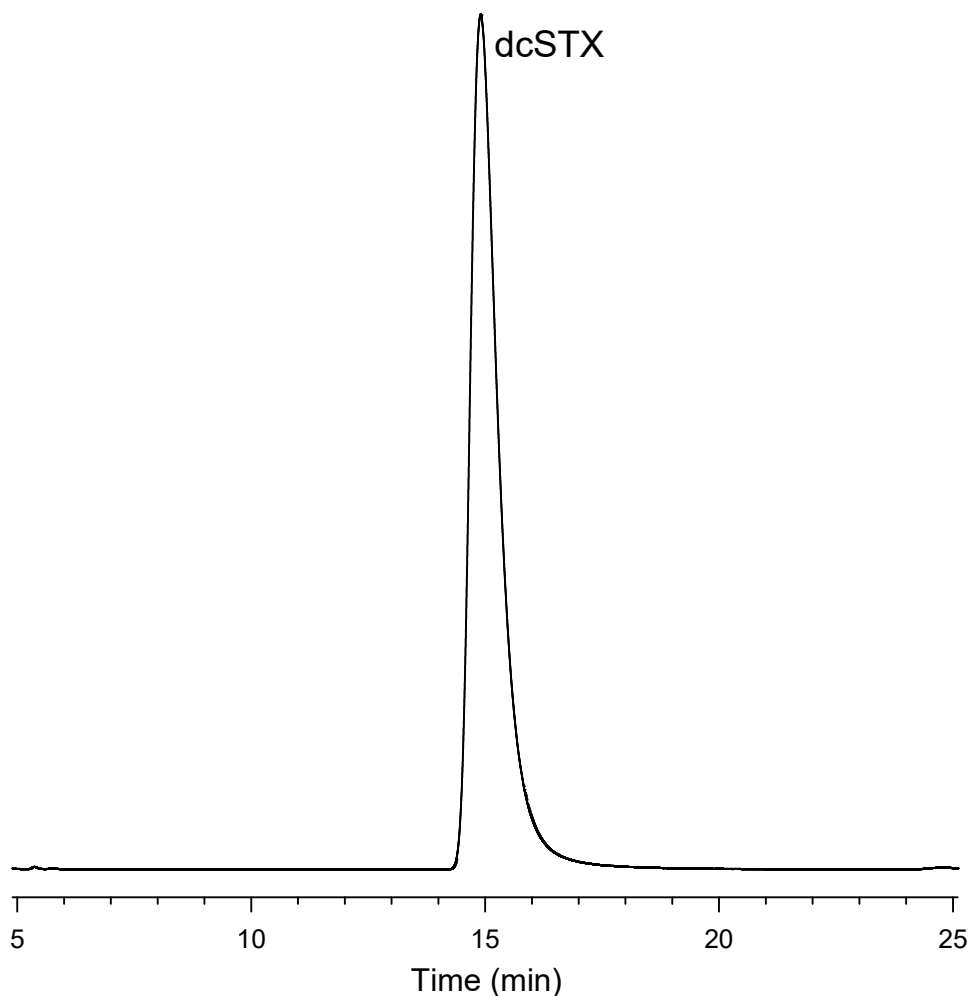


Figure 3: LC-ox-FLD analysis of CRM-dcSTX-c. Conditions: Zorbax Bonus-RP column (250 × 4.6 mm, 5 μm) at +37 °C; mobile phase: water with 20 mM phosphoric acid, 8 mM heptane sulfonate, and 11% acetonitrile at pH 7.1, 0.8 mL/min; 20 μL injection volume; post column oxidation with 0.4 mL/min 5 mM periodic 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.

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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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