



Certificate of Analysis

Certified Reference Material

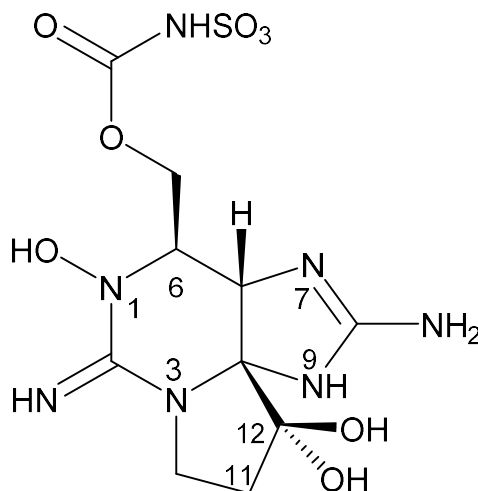
CRM-GTX6-b (Lot# 20181005)

Certified Calibration Solution for Gonyautoxin-6

Gonyautoxin-6 (GTX6) is a paralytic shellfish toxin [1] which has been found in both marine microalgae [2] and freshwater cyanobacteria [3]. CRM-GTX6-b is a certified calibration solution of GTX6 in dilute acetic acid (pH 6). It is designed as an instrument calibration solution to aid in the identification and quantitation of GTX6. This is a replacement calibration solution for CRM-GTX6.

Table 1: Certified concentration and uncertainty for CRM-GTX6-b.

Compound	$\mu\text{mol/L}$ (at +20 °C)
GTX6	13.2 ± 1.3



Gonyautoxin-6

CAS No.: 82810-44-4 (free base)

InChIKey: ALRRPAKWGUBPBK-HGRQIU PRSA-N (free base)

Molecular formula: C₁₀H₁₇N₇O₈S₁

Molecular weight: 395.35 g/mol

Period of validity: 1 year from date of sale

Storage conditions: -12 °C or below

Intended Use

CRM-GTX6-b is a certified calibration solution designed for analytical method development and accurate quantitation of GTX6. 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-GTX6-b, ampoules should be stored at -12 °C or below.

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-GTX6-b

Gonyautoxin-6 was isolated from cultured biomass of *Alexandrium catenella*. The extraction and isolation was carried out at the Cawthron Institute, New Zealand. Final purification by preparative scale chromatography was performed at the National Research Council Canada (NRC) [4]. The structure and purity of GTX6 was confirmed by LC-MS/MS [5] (Figures 1 and 2) and NMR. A measured accurate m/z of 396.0926 ($\Delta = -1.4$ ppm for $C_{10}H_{18}N_7O_8S_1^+$) was obtained for the $[M+H]^+$ ion of GTX6 using LC-high resolution MS (LC-HRMS). Purity was further assessed by LC-ox-FLD [6] (Figure 3), capillary electrophoresis with UV (CE-UV) [7] and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [8]. A low relative level of gonyautoxin-5 (GTX5) is present in CRM-GTX6-b (Table 2). Trace amounts of gonyautoxin-1 and -4 were also detected.

The stock solution was prepared by diluting the purified GTX6 in H₂O for quantitative ¹H-NMR (qNMR) [9]. The CRM-GTX6-b solution was prepared by making an accurate dilution of the qNMR stock solution in filtered (0.2 µm) degassed deionized water adjusted to pH 6 with acetic acid. Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Table 2: Information value for GTX5 present in CRM-GTX6-b assigned by LC-MS.

Compound	$[M+H]^+$, m/z	Concentration (µmol/L)*
Gonyautoxin-5 (GTX5)	380.1	0.07

* Concentration is not certified.

Analytical Methods and Value Assignment

The certified value for CRM-GTX6-b (Table 1) is based on results obtained at the NRC using three analytical methods: qNMR using potassium hydrogen phthalate (NIST SRM 84L) for calibration [9]; LC-CLND using caffeine (NMIA M724c) for calibration [8]; and LC-MS/MS using NRC CRM-GTX6 for calibration [5].

Homogeneity

A representative number of CRM-GTX6-b ampoules were selected from across the fill series and GTX6 response was measured by LC-MS/MS. No heterogeneity was observed.

Stability

Studies performed on CRM-GTX6-b demonstrated good stability with no detectable decomposition at -12 °C or +4 °C after one month of storage.

Uncertainty

All reasonable sources of error related to the characterization of CRM-GTX6-b were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}) and instability during storage (u_{stab}) [10-13]. These components are listed in Table 3, 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 3: Uncertainty components for the certified value of CRM-GTX6-b.

Uncertainties	Relative*
u_{char}	0.05
u_{hom}	negligible
u_{stab}	0.02

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, GTX6 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 the glass shatters. A safety data sheet (SDS) is available for CRM-GTX6-b.

Period of Validity

If stored unopened at the recommended storage condition of -12 °C or below, the certified concentration of CRM-GTX6-b 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 (NIST SRM 84L), an NMIA caffeine certified reference material (NMIA M724c.2014.01), and a certified reference material for gonyautoxin-6 (NRC CRM-GTX6).

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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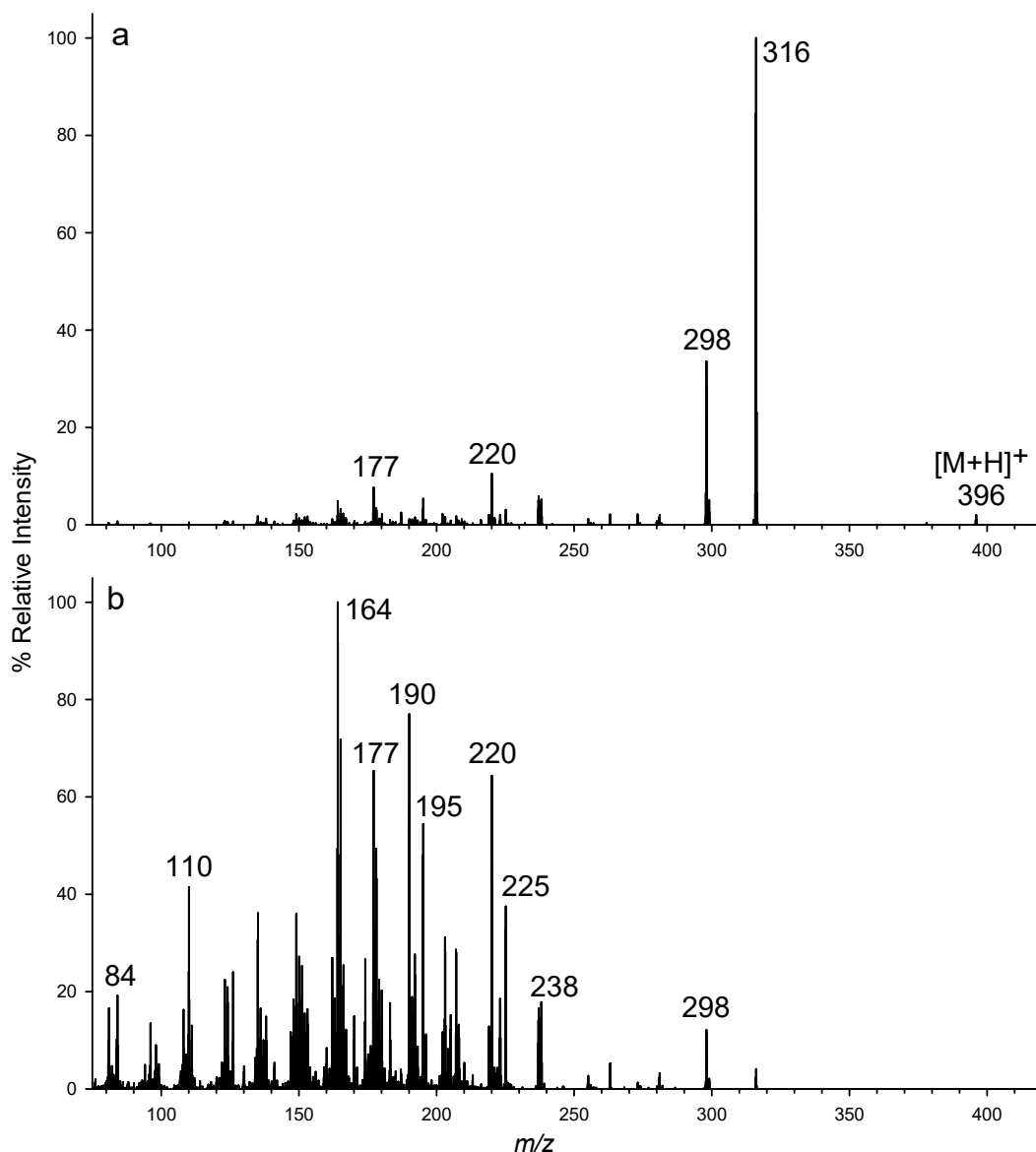


Figure 1: Collision induced dissociation mass spectrum of the $[M+H]^+$ ion (m/z 396) of GTX6 measured on a Sciex 4000 QTRAP mass spectrometer with electrospray ionization. Chromatographic conditions: Toso-Haas Amide-80 column (250 mm \times 2 mm, 5 μ m) at +40 $^{\circ}$ C; mobile phase: 62% acetonitrile in water with 2 mM ammonium formate and 3.6 mM formic acid, 0.2 mL/min; injection volume: 1 μ L. MS conditions: collision energies of 30 V (a) and 50 V (b); declustering potential +70 V, and source temperature of 475 $^{\circ}$ C.

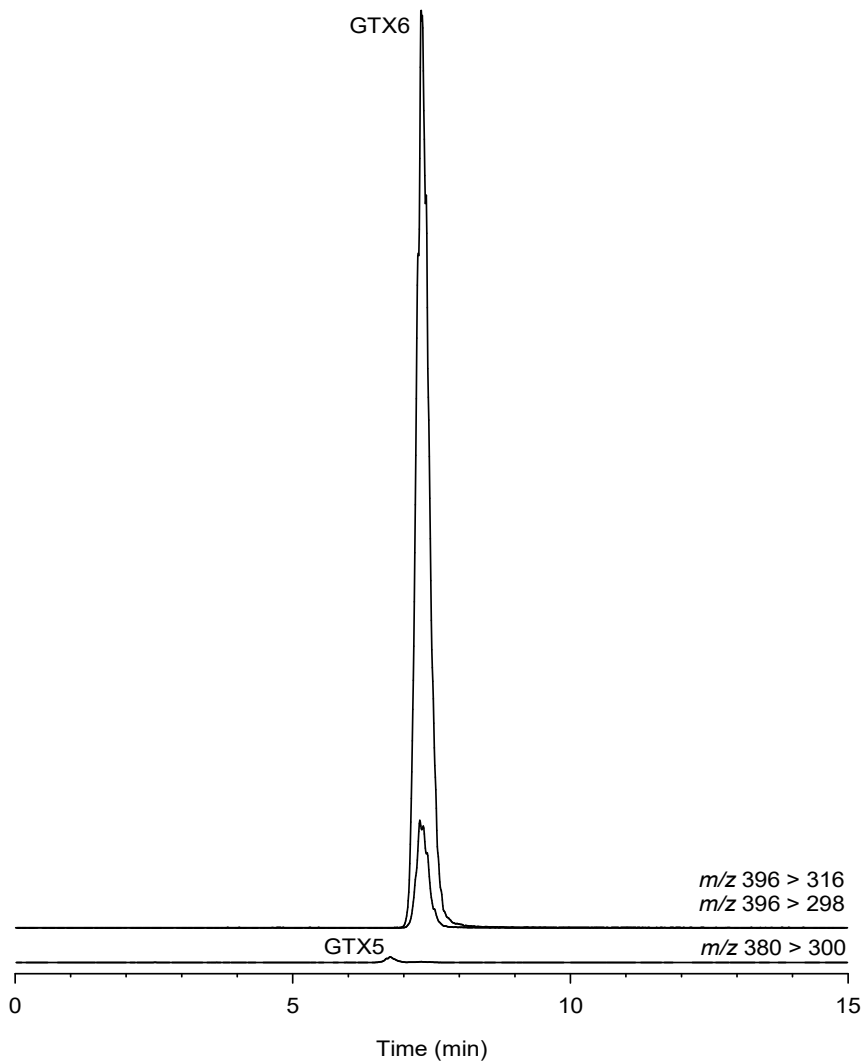


Figure 2: LC-MS/MS analysis of CRM-GTX6-b using selected reaction monitoring on an Agilent 1290 LC connected to a Sciex 5500 QTRAP MS with electrospray ionization. Chromatographic conditions: Toso-Haas Amide-80 column (250 mm × 2 mm, 5 μ m) at +40 °C; mobile phase: 62% acetonitrile in water with 2 mM ammonium formate and 3.6 mM formic acid, 0.3 mL/min; injection volume: 5 μ L. MS conditions: collision energy spread 20-30 V; declustering potential +100 V, and source temperature of 275 °C.

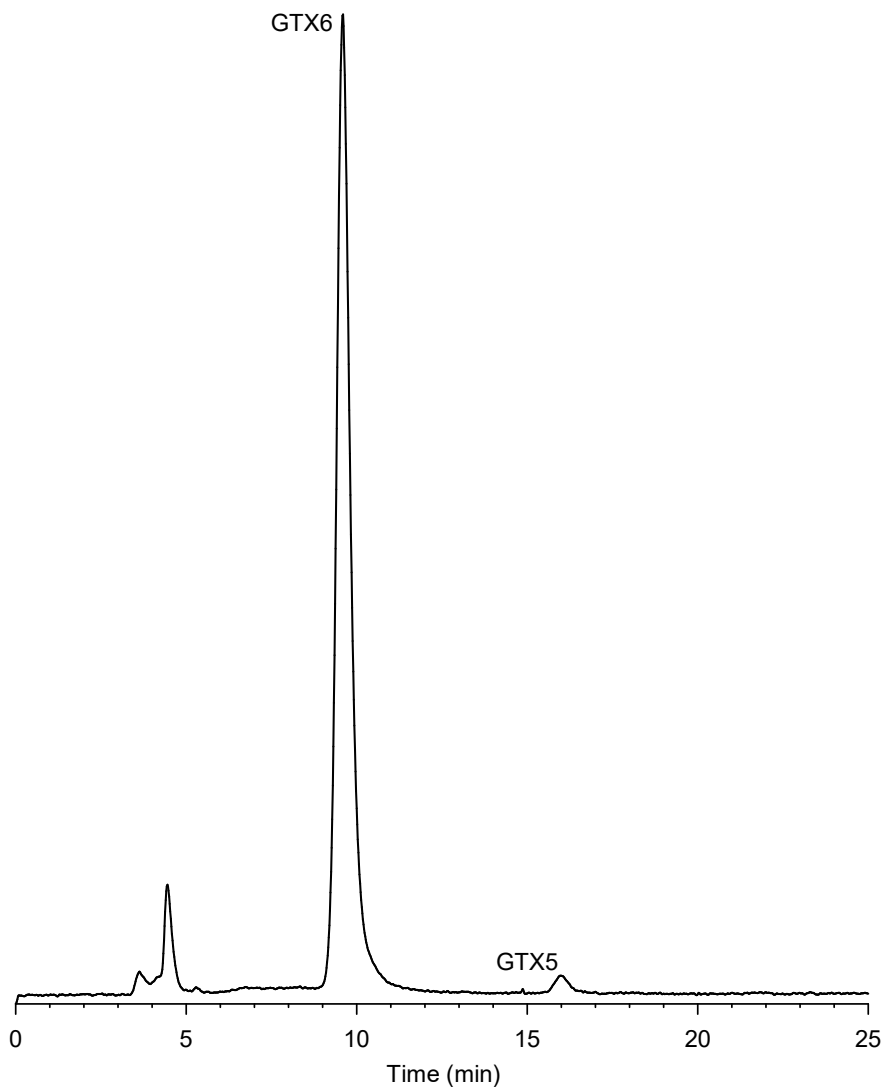


Figure 3: LC-ox-FLD analysis of CRM-GTX6-b. Conditions: Zorbax Bonus-RP column (250 mm × 4.6 mm i.d., 5 µm) at +35 °C; mobile phase: 5.5 mM ammonium phosphate with 11 mM sodium heptane sulfonate in water (pH 7.1), 0.8 mL/min; injection volume 25 µL; post-column oxidation: 0.4 mL/min 5 mM periodic acid in 100 mM sodium phosphate at pH 7.8 with reaction coil at +80 °C; effluent acidified with 0.4 mL/min 0.75 M nitric acid; detection: fluorescence 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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