

# Certificate of Analysis

**NRC-CMRC**

Certified Reference Material

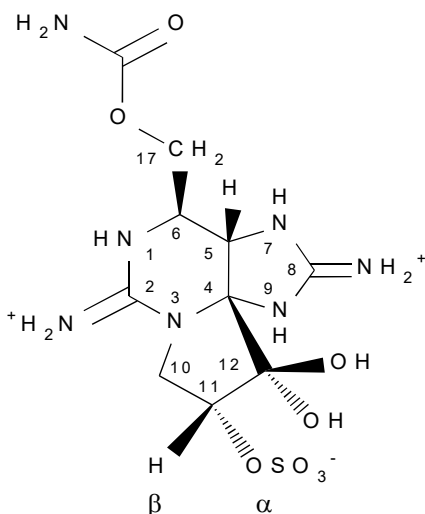
## CRM-GTX2&3-d (Lot# 20170419)

Certified Calibration Solution for Gonyautoxins-2 and -3

Gonyautoxins-2 and -3 (GTX2 and GTX3) are two of the toxins responsible for incidents of paralytic shellfish poisoning (PSP) [1]. CRM-GTX2&3-d is a certified instrument calibration solution prepared to aid the analyst in the determination of GTX2 and GTX3. Each ampoule contains a solution of GTX2 and GTX3 dissolved in filtered, aqueous 0.5 mM hydrochloric acid (HCl). This is a replacement calibration solution for CRM-GTX2&3-c.

**Table 1:** Certified concentration values for CRM-GTX2&3-d.

Compound	$\mu\text{mol/L}$ (at +25 °C)
GTX2	$102.6 \pm 4.0$
GTX3	$43.5 \pm 2.9$
GTX2 + GTX3	$146.1 \pm 6.9$



### Gonyautoxin-2

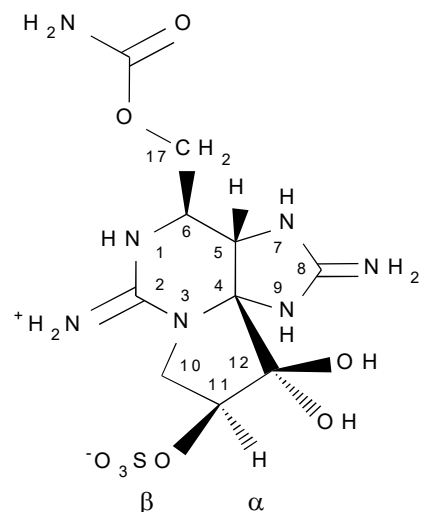
CAS registry no.: 60508-89-6 (free base)

Molecular formula:  $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_8\text{S}_1$

Molecular weight: 395.4 g/mol

Period of validity: 1 year from date of sale

Storage conditions: +4 °C



### Gonyautoxin-3

CAS registry no.: 60537-65-7 (free base)

Molecular formula:  $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_8\text{S}_1$

Molecular weight: 395.4 g/mol



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## Intended Use

CRM-GTX2&3-d is a calibration solution CRM designed for analytical method development and accurate quantitation of GTX2 and GTX3. The concentrations are suitable for preparing a dilution series for calibration of instruments such as liquid chromatography with detection by post-column oxidation-fluorescence (LC-ox-FLD) or liquid chromatography-mass spectrometry (LC-MS).

## Instructions for Storage and Use

To ensure the stability of CRM-GTX2&3-d, ampoules should be stored in a refrigerator (+4 °C). Prior to opening, each ampoule should be allowed to warm to room temperature and the contents mixed thoroughly. The ampoule should be opened at the pre-scored mark. Once an ampoule has been opened, accurate aliquots should be removed with calibrated volumetric equipment and transferred to volumetric flasks or vials. 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 of losses on glass surfaces. *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-GTX2&3-d

N-Sulfocarbamoylgonyautoxins-2 and -3 were extracted from cultured *Alexandrium tamarense* and chemically converted to a GTX2 and GTX3 mixture which was purified using preparative scale chromatography [2]. Purified GTX2&3 was dried *in vacuo* and dissolved in filtered, aqueous 0.5 mM HCl to give a stock solution. The purity of the toxins was checked by 500 MHz proton nuclear magnetic resonance (NMR) spectroscopy, LC-ox-FLD [3] (Figure 1), LC-MS/MS [4] (Figures 2 and 3), capillary electrophoresis with UV detection (CE-UVD) [5], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [6].

The CRM-GTX2&3-d solution was prepared in filtered (0.2 µm) and degassed 0.5 mM HCl at pH 3.4 and dispensed into amber ampoules pre-filled with argon, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

## Analytical Methods and Value Assignment

Certified values for CRM-GTX2&3-d (Table 1) are based on results obtained at NRC using two analytical methods: quantitative <sup>1</sup>H-NMR (qNMR) with accurate solutions of potassium hydrogen phthalate (NIST SRM 84I) as the calibrant [7], and LC-CLND using accurate solutions of caffeine (NMIA M724c) as the calibrant.

Low levels of decarbamoylgonyautoxin-2 (dcGTX2), decarbamoylgonyautoxin-3 (dcGTX3), gonyautoxin-1 (GTX1), and gonyautoxin-4 (GTX4), are present in CRM-GTX2&3-d. Information values were assigned by LC-ox-FLD with calibration using NRC CRMs for those analogues (Table 2).



**Table 2:** Information values for other PSP toxins present in CRM-GTX2&3-d at the time of packaging.

Compound	[M+H] <sup>+</sup> , m/z	Concentration (µmol/L)*
Gonyautoxin-1 (GTX1)	412.1	0.21
Gonyautoxin-4 (GTX4)	412.1	0.03
Decarbamoylgonyautoxin-2 (dcGTX2)	353.1	0.23
Decarbamoylgonyautoxin-3 (dcGTX3)	353.1	0.13

\* These concentrations are not certified.

### Homogeneity

A representative number of CRM-GTX2&3-d ampoules were selected from across the fill series and GTX2&3 concentrations measured by LC-ox-FLD. The data was assessed using ANOVA.

### Stability

Studies have demonstrated good long-term stability of GTX2&3 solutions stored in sealed ampoules at +4 °C, with no detectable decomposition observed over a period of one year. Solutions are also stable when stored in the freezer (-12 °C or lower). It has been determined that GTX2&3 exhibits good stability at room temperature, with no detectable decomposition observed for periods of one month within the limits of uncertainty of the analytical method (LC-MS, < 1% RSD for replicate injections of a single solution; n = 6).

### Uncertainty

All reasonable sources of error related to the characterization of CRM-GTX2&3-d were considered and quantitated. A combined uncertainty component relating to the two analytical methods used is included ( $u_{char}$ ). The overall uncertainty estimate ( $U_{CRM}$ ) includes uncertainties associated with batch characterization ( $u_{char}$ ), instability during storage ( $u_{stab}$ ) and between-bottle variation ( $u_{hom}$ ) [8-11]. 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 values of CRM-GTX2&3-d.

Uncertainties (µmol/L)	GTX2	GTX3	GTX2&3
$u_{char}$	1.96	1.43	3.36
$u_{hom}$	0.47	0.20	0.68
$u_{stab}$	0.07	0.05	0.11



## Safety Instructions

If sufficient quantities are ingested, PSP toxins can cause paralysis and even death. Only qualified personnel should handle the solution and appropriate disposal methods should be used. Heavy gloves and eye protection should be used when opening the ampoule in the event the glass shatters. A safety data sheet (SDS) is available for CRM-GTX2&3-d.

## Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-GTX2&3-d 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 established purity.

## Quality System (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented National Research Council of Canada (NRC) Measurement Science and Standards (MSS) Quality System, which conforms with the requirements of ISO/IEC 17025 and ISO Guide 34.

The MSS Quality System supporting 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 certificate of approval is available upon request.



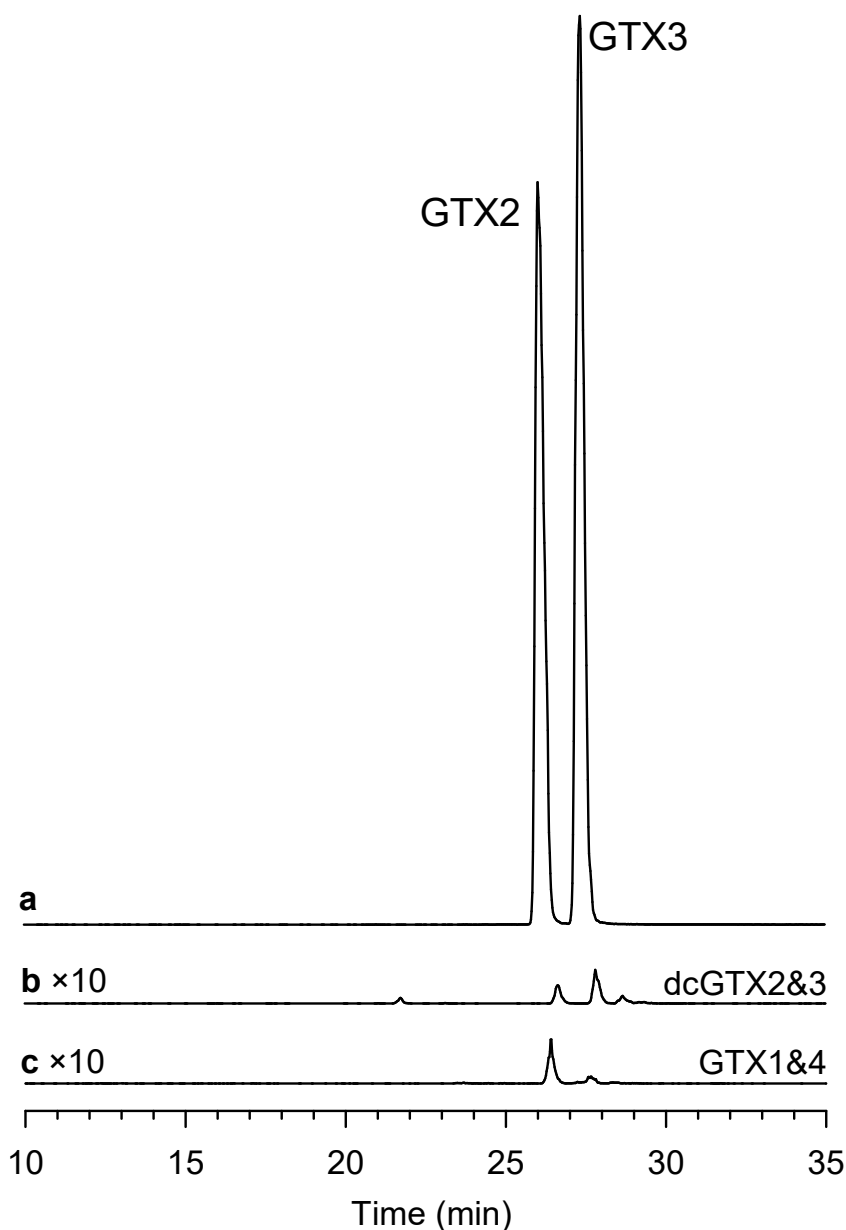
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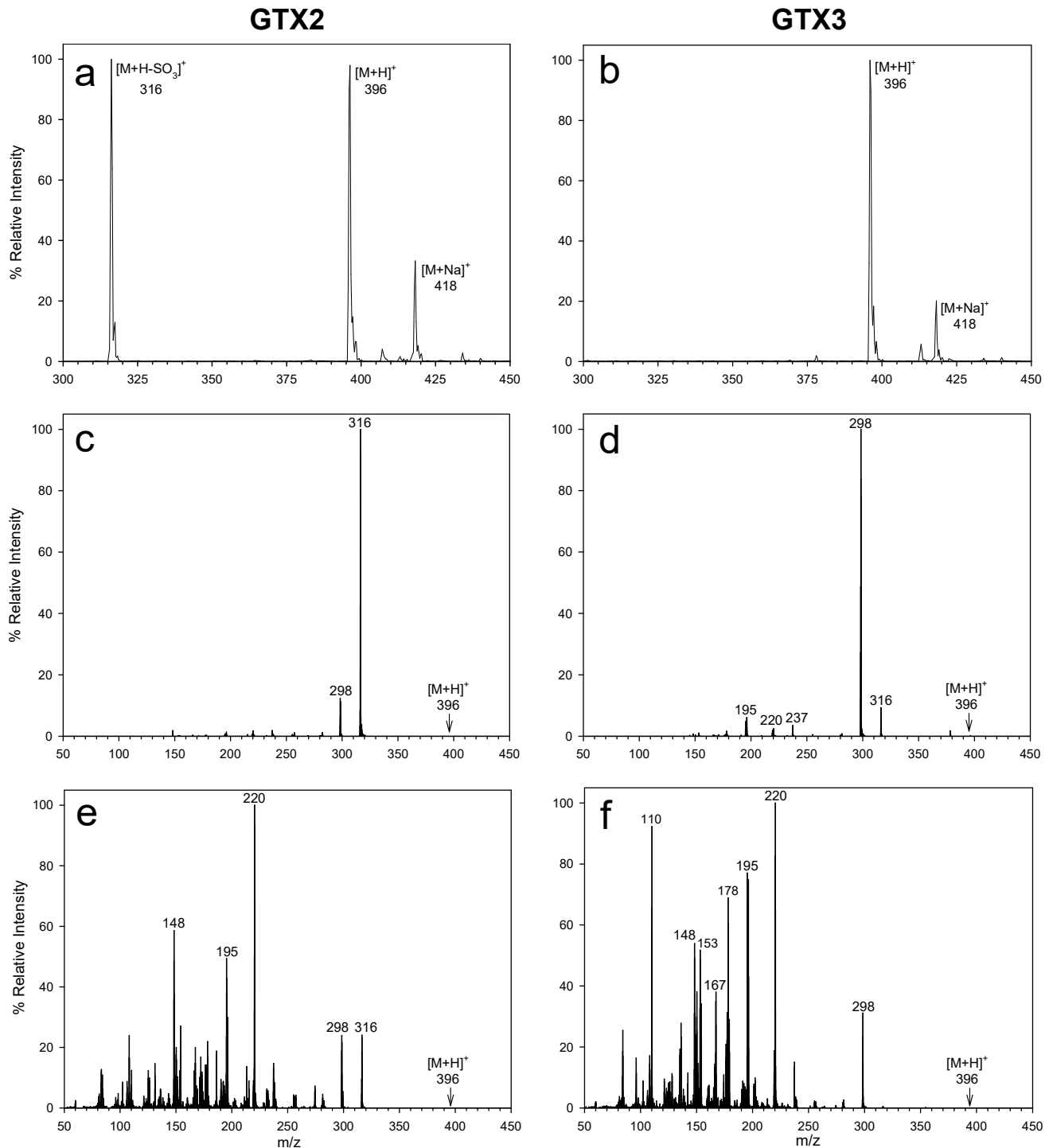




**Figure 1:** LC-ox-FLD analysis of CRM-GTX2&3-d. Retention times of related toxins are indicated. Conditions: Zorbax Bonus-RP column (250 × 4.6 mm i.d., 5 μm) at +35 °C; 0.8 mL/min of 5.5 mM ammonium phosphate with 11 mM sodium heptane sulphonate, pH 7.1; 5 μL injection; post column oxidation using 0.4 mL/min 5 mM periodic acid in 100 mM sodium phosphate at pH 7.8; 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.



**Figure 2:** LC-MS/MS analysis of CRM-GTX2&3-d stock solution using selected reaction monitoring: (a) summed signals for  $m/z$  396 > 316 and  $m/z$  396 > 298; (b) summed signals for  $m/z$  353 > 273 and  $m/z$  353 > 255; and (c) summed signals for  $m/z$  412 > 332 and  $m/z$  412 > 314. Conditions: Agilent 1200 LC and Sciex API4000 QTRAP MS; 2.5  $\mu$ L injection; 5  $\mu$ m TOSOH Amide-80 column (250 mm  $\times$  2 mm i.d.) at +40  $^{\circ}$ C; 0.2 mL/min gradient elution from 10 to 45% A over 25 min, to 70% A at 27 min, hold to 40 min. Mobile phase A: water with 2 mM ammonium formate and 50 mM formic acid; mobile phase B: acetonitrile.



**Figure 3:** Mass spectra of GTX2 (a,c,e) and GTX3 (b,d,f) generated from an LC-MS/MS analysis of GTX2&3: full Q1 scan positive ion ESI mass spectra (a,b); product ion spectra of  $m/z$  396 with collision energy (CE) = 25 V (c,d); and product ion spectra of  $m/z$  396 with CE = 50 V (e,f). Conditions: Agilent 1200 LC and Sciex API4000 QTRAP MS; 35 V declustering potential; 2.5  $\mu$ L injection; TOSOH Amide-80 column (150 mm  $\times$  2 mm i.d.) at +40  $^{\circ}$ C; flow = 0.2 mL/min; isocratic elution with 30% A (water) and 70% B (95% acetonitrile), both with 2 mM ammonium formate and 3.6 mM formic acid.

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