

# Certificate of Analysis

**NRC-CMRC**

Certified Reference Material

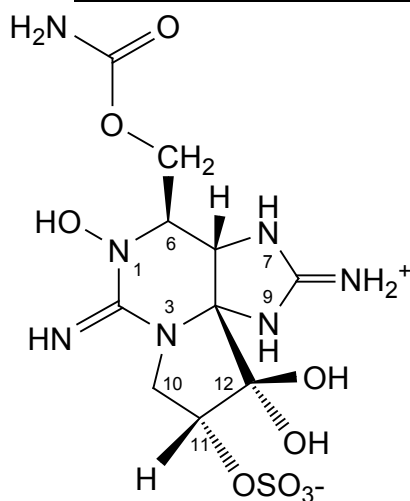
## CRM-GTX1&4-d (Lot # 20160608)

Certified Calibration Solution for Gonyautoxins-1 and -4

Gonyautoxins-1 and -4 (GTX1 and GTX4) are two of the toxins responsible for incidents of paralytic shellfish poisoning (PSP) [1]. CRM-GTX1&4-d is a certified instrument calibration solution prepared to aid the analyst in the determination of GTX1 and GTX4. Each ampoule contains a solution of GTX1 and GTX4 dissolved in filtered, aqueous 0.5 mM hydrochloric acid (HCl). This is a replacement calibration solution for CRM-GTX1&4-c, which was released in 2008.

**Table 1:** Certified concentration values for CRM-GTX1&4-d.

Compound	$\mu\text{mol/L}$ (at +25°C)
GTX1	$57.2 \pm 1.7$
GTX4	$18.0 \pm 0.9$
GTX1 + GTX4	$75.1 \pm 2.1$



### Gonyautoxin-1

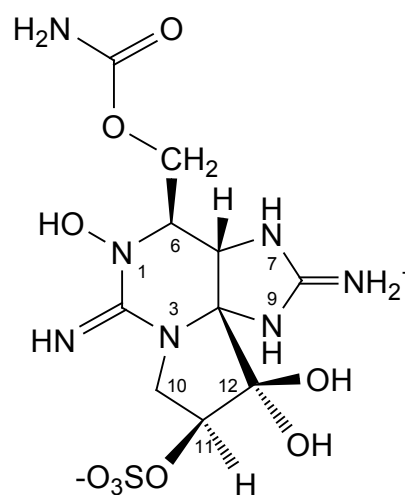
CAS registry no.: 60748-39-2 (free base)

Molecular formula:  $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_9\text{S}_1$

Molecular weight: 411.4 g/mol

Period of validity: 1 year from date of sale

Storage conditions: +4 °C or below



### Gonyautoxin-4

CAS registry no.: 64296-26-0 (free base)

Molecular formula:  $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_9\text{S}_1$

Molecular weight: 411.4 g/mol



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## Intended Use

CRM-GTX1&4-d is a calibration solution CRM designed for analytical method development and accurate quantitation of GTX1 and GTX4. 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-GTX1&4-d, ampoules should be stored in a refrigerator (~ +4 °C or lower).

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-GTX1&4-d

GTX1&4 was isolated from cultured *Alexandrium fundyense* using preparative scale chromatography [2]. Purified GTX1&4 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 (Figure 2), LC-ox-FLD [3] (Figure 3), LC-MS/MS [4] (Figure 4), capillary electrophoresis with UV detection (CE-UVD) [5], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [6].

The CRM-GTX1&4-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-GTX1&4-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 NIST potassium hydrogen phthalate (KHP) SRM (84L) as the calibrant [7], and LC-CLND using accurate caffeine solutions as the calibrant.

Gonyautoxin-2 (GTX2) and gonyautoxin-3 (GTX3) are present in CRM-GTX1&4-d and were measured by LC-ox-FLD with calibration based on the NRC CRM for those analogues (Table 2). An unidentified PSP compound was observed by LC-MS (Figure 3).

**Table 2:** Information values for other compounds present in CRM-GTX1&4-d at the time of packaging.

Compound	[M+H] <sup>+</sup> , m/z	Concentration (µmol/L)*
Gonyautoxin-2 (GTX2)	396.1	0.13
Gonyautoxin-3 (GTX3)	396.1	0.05

\* These concentrations are not certified.



## Homogeneity

A representative number of CRM-GTX1&4-d ampoules were selected from across the fill series and GTX1&4 concentrations measured by LC-ox-FLD. No heterogeneity was observed.

## Stability Studies

Stability studies have demonstrated good long-term stability of GTX1&4 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 (~ -20 °C). It has been determined that GTX1&4 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-ox-FLD, ± 3.2% RSD for replicate injections of a single solution; n = 5).

## Uncertainty

All reasonable sources of error related to the characterization of CRM-GTX1&4-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 ( $u_{char}$ ) and instability during storage ( $u_{stab}$ ) [8-11]. The between-bottle variation ( $u_{hom}$ ) was found to be negligible. 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-GTX1&4-d.

Uncertainties	GTX1	GTX4	GTX1&4
$u_{char}$	0.82	0.44	1.02
$u_{hom}$	negligible	negligible	negligible
$u_{stab}$	0.16	0.06	0.24
$U_{CRM} (k = 2)$	1.7	0.9	2.1

## Safety Instructions

If sufficient quantities are ingested, PSPs 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-GTX1&4-d.

## Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-GTX1&4-d is valid for 1 year from the date of sale. The label on the original packaging includes the period of validity.



**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.

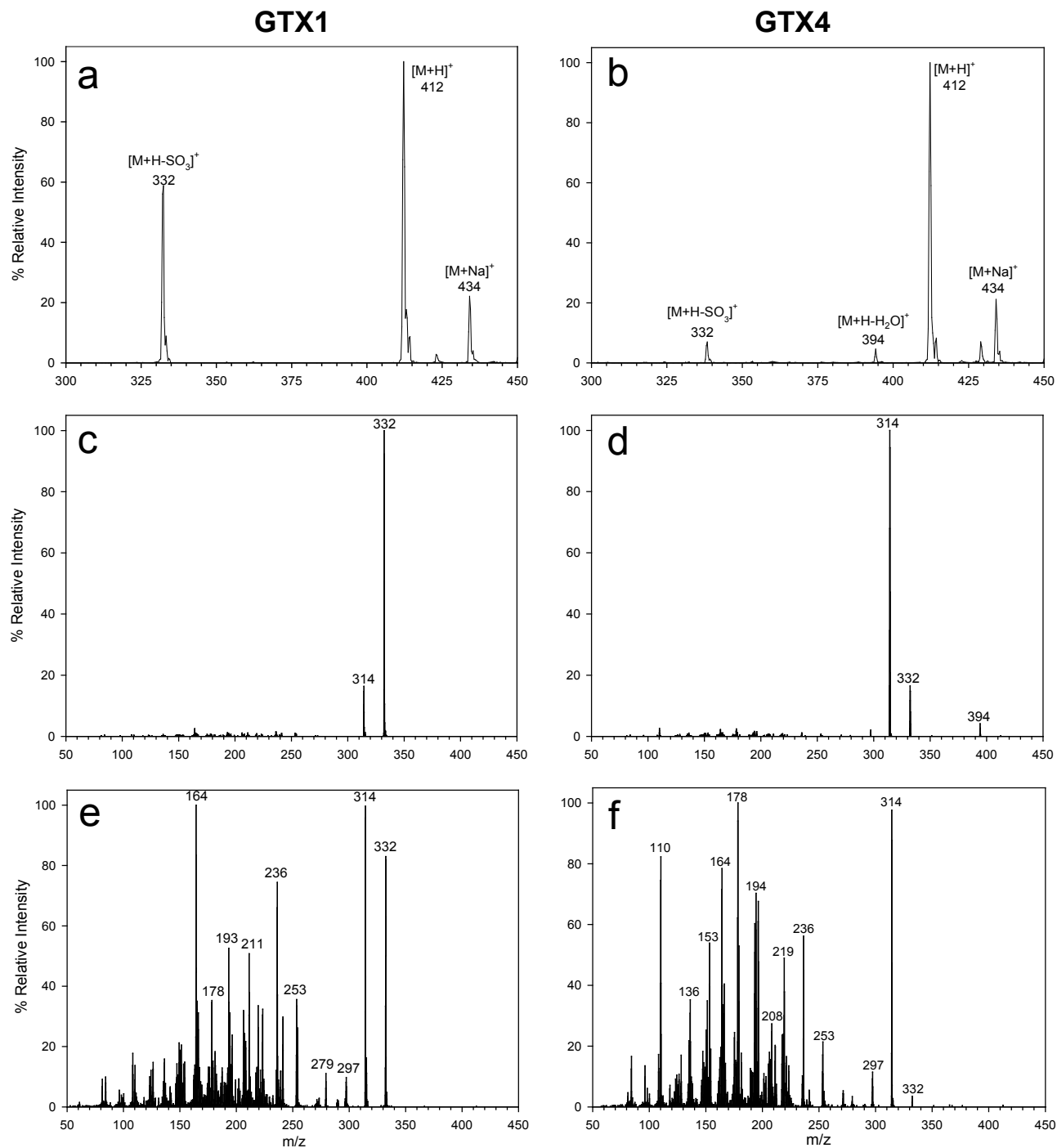
The MSS Quality System supporting NRC calibration and measurement capabilities, as listed in the Bureau international des poids et mesures (BIPM) key comparison database (<http://kcdb.bipm.org/>), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM) and found 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.



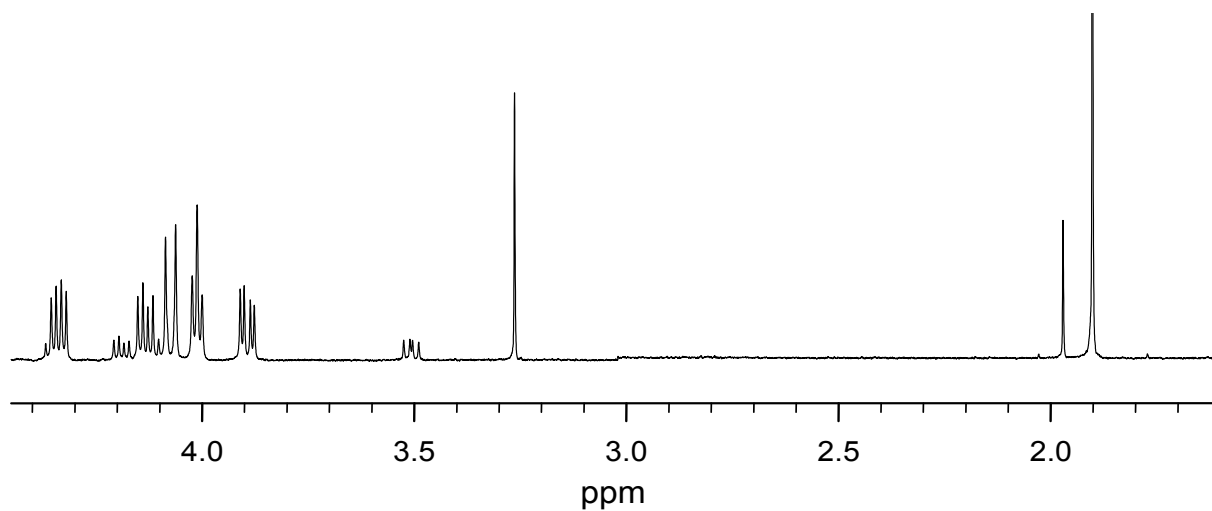
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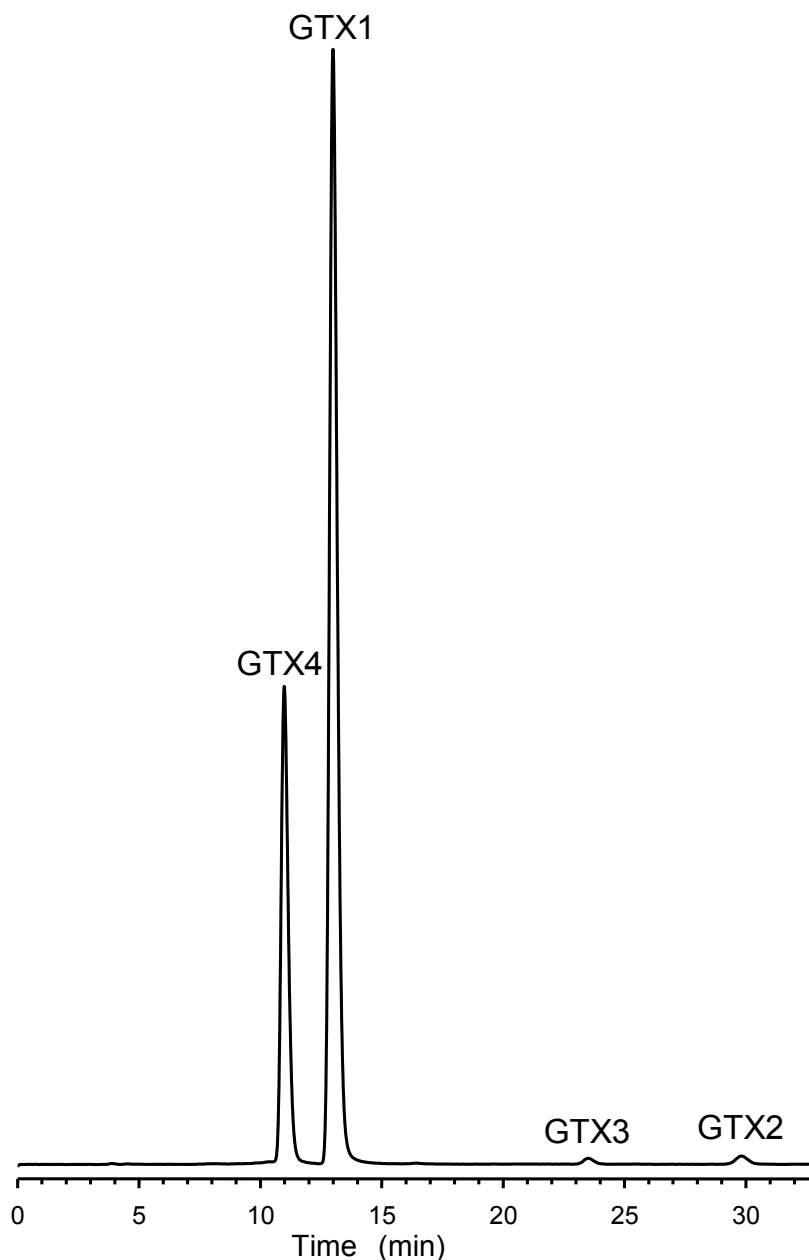




**Figure 1:** Mass spectra of GTX1 (a,c,e) and GTX4 (b,d,f) generated from an LC-MS/MS analysis of GTX1&4: full Q1 scan positive ion ESI mass spectra (a, b); product ion spectra of  $m/z$  412 with collision energy (CE) = 25 V (c, d); and product ion spectra of  $m/z$  412 with CE = 50 V (e, f). Conditions: Agilent 1200 LC and Sciex API4000 QTRAP MS; 35 V declustering potential; 2.5  $\mu$ L injection volume; Tosoh-Haas 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.



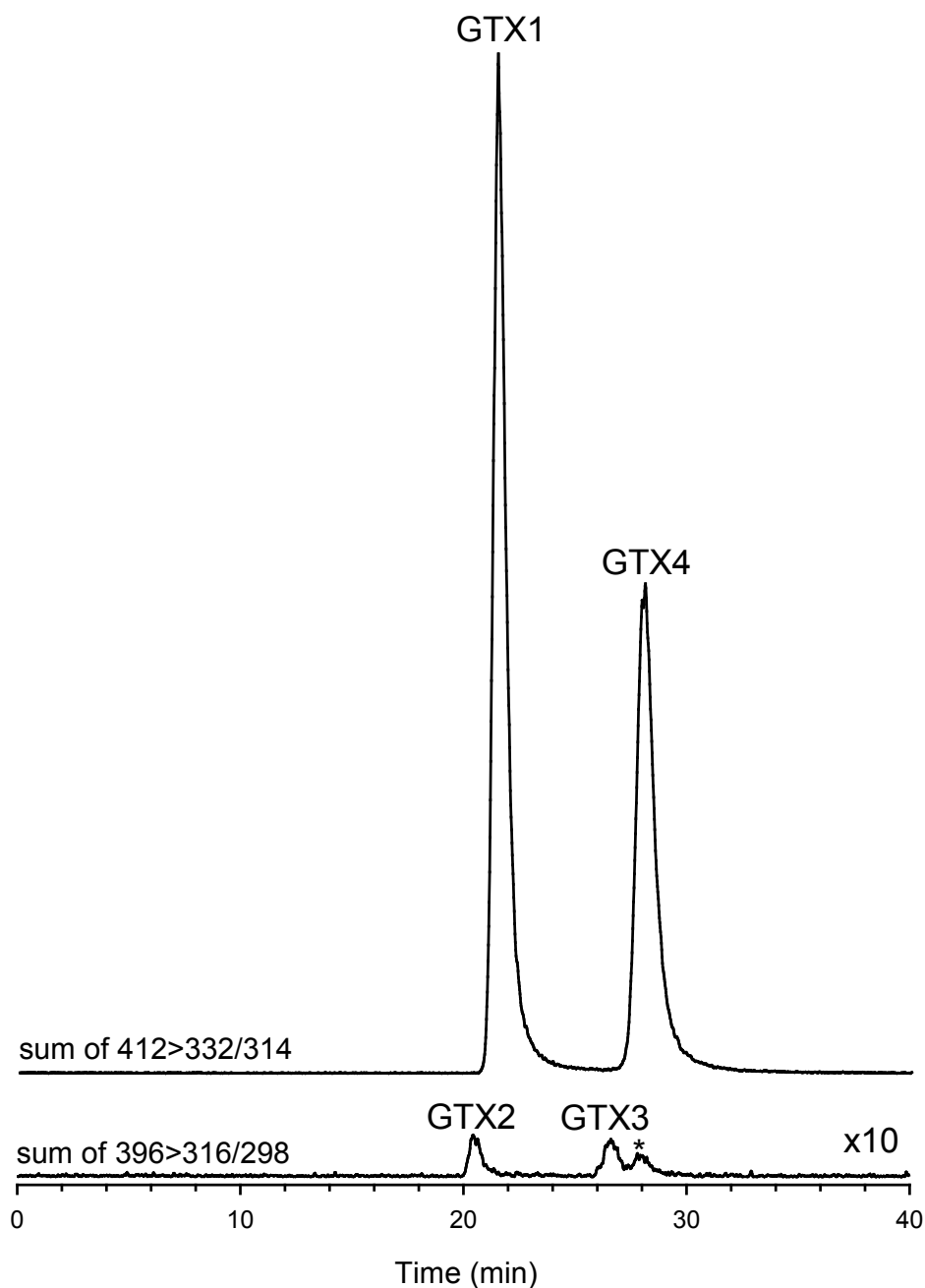
**Figure 2:** Proton NMR spectrum of the stock solution used for CRM-GTX1&4-d in 0.5 mM HCl.



**Figure 3:** LC-ox-FLD analysis of CRM-GTX1&4-d. Retention times of related toxins are indicated. Conditions: Zorbax Bonus-RP column (250 mm × 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; 2 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 4:** LC-MS/MS analysis of CRM-GTX1&4-d using selected reaction monitoring. Conditions: Agilent 1100 LC and Sciex API4000 MS; 25 V collision energy; 35 V declustering potential; 5  $\mu$ L injection volume; Tosoh-Haas Amide-80 column (250 mm  $\times$  2 mm i.d) at +40  $^{\circ}$ C; 0.4 mL/min isocratic elution with 20% A (water) and 80% B (95% acetonitrile), both with 2 mM ammonium formate and 3.6 mM formic acid. Unknown indicated by asterisk (\*).

## Acknowledgements

The following staff members of Measurement Science and Standards at NRC contributed to the production and certification of CRM-GTX1&4-d: T. Chan, S. Crain, W. Hardstaff, P. LeBlanc, N. Lewis, M. MacArthur, P. McCarron, R. Perez, M.A. Quilliam, K. Reeves, K. Thomas.

### This document should be cited as:

K. Reeves, T. Chan, M. MacArthur, S. Crain, K. Thomas, M.A. Quilliam, P. McCarron "CRM-GTX1&4-d, a certified calibration solution reference material for gonyautoxins-1 and -4", Biotoxin Metrology Technical Report CRM-GTX1&4-d-20160608, National Research Council Canada, Halifax.

*Date of issue: December 2016*

*Document version: 20161202*

**Approved by:**



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**This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our qualified vendors.**

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