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Certificate of Analysis

Certified Reference Material

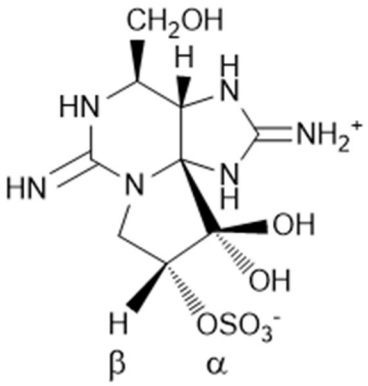
CRM-dcGTX2&3-d (Lot# 20220630)

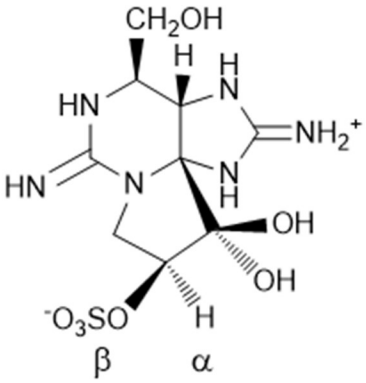
Certified Calibration Solution for Decarbamoylgonyautoxins-2 and -3

Decarbamoylgonyautoxins-2 and -3 (dcGTX2 and dcGTX3) are saxitoxin analogues found in both marine microalgae [1] and freshwater cyanobacteria [2]. CRM-dcGTX2&3-d is a certified calibration solution of dcGTX2 and dcGTX3 in 0.5 mM hydrochloric acid, designed to aid in the identification and quantitation of dcGTX2 and dcGTX3. This is a replacement calibration solution for CRM-dcGTX2&3-c.

Table 1: Certified concentrations and uncertainties for CRM-dcGTX2&3-d.

Compound	µmol/L (15 - 30 °C)
dcGTX2	107.1 ± 6.2
dcGTX3	21.0 ± 2.0
dcGTX2&3	128.1 ± 9.4





Decarbamoylgonyautoxin-2
CAS No.: 86996-87-4 (free base)
InChIKey: AJLCXXKDNUGKKH-WVBYAZCYSA-N
Molecular formula: $C_9H_{16}N_6O_7S$
Molecular weight: 352.3 g/mol

Decarbamoylgonyautoxin-3
CAS No.: 87038-53-7 (free base)
InChIKey: AJLCXXKDNUGKKH-RGDLXGNYSA-N
Molecular formula: $C_9H_{16}N_6O_7S$
Molecular weight: 352.3 g/mol

Period of validity: 1 year from date of sale
Storage conditions: -12 °C or below

Canada

Intended Use

CRM-dcGTX2&3-d is a certified calibration solution designed for analytical method development and accurate quantitation of dcGTX2&3. 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 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-dcGTX2&3-d, ampoules should be stored at -12 °C or below.

It is important to note that volume of the solution is not certified. Only the concentration is certified. Therefore, the ampoule contents should not simply be transferred to a volumetric container and diluted to volume.

Prior to opening, each ampoule should be allowed to equilibrate to room temperature and the contents thoroughly mixed. The CRM solution should be transferred using calibrated equipment for accuracy. Repeated sub-sampling and storage of the CRM solution after initial opening may impact concentration values. However, users may take responsibility for demonstrating that their sub-sampling and storage procedures do not impact concentrations.

Preparation of CRM-dcGTX2&3-d

N-sulfocarbamoylgonyautoxin-2 and -3 (C1&2) were isolated from laboratory cultures of *Alexandrium sp.*, chemically converted to dcGTX2 and dcGTX3, then purified by several chromatographic steps [3]. The structure and purity of dcGTX2&3 were confirmed by LC-MS/MS [4] (Figures 1 and 2) and ^{1}H NMR. A measured accurate *m*/*z* of 353.0873 (Δ = 0.3 ppm for $C_9H_{17}N_6O_7S^+$) was obtained for the $[M+H]^+$ ion of both dcGTX2 and dcGTX3 using LC-high resolution MS (LC-HRMS). Purity was further assessed by LC-ox-FLD [5] (Figure 3), capillary electrophoresis with UV (CE-UV) and LC with chemiluminescence nitrogen detection (LC-CLND) [6].

The stock solution was prepared by diluting the purified dcGTX2&3 in deionized water for quantitation. The CRM-dcGTX2&3-d solution was prepared by making an accurate dilution of the stock solution in degassed 0.5 mM HCl (pH 3.5). 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 values for CRM-dcGTX2&3-d (Table 1) are based on results obtained at the NRC using two analytical methods: qNMR [7] using caffeine (NMIA M724c) for calibration, and LC-MS/MS using CRM-dcGTX2&3-c as the calibrant.

Low levels of gonyautoxin-2, gonyautoxin-3 (GTX2&3), and decarbamoylgonyautoxin-1 (dcGTX1) are present in CRM-dcGTX2&3-d and were assigned information values by LC-MS/MS with calibration using NRC CRM-GTX2&3-d (assuming a similar molar response) (Table 2). Trace levels of decarbamoylgonyautoxin-4 and M5 [8] were also present (below the limits of quantitation).

Table 2: Information values for other saxitoxins present in CRM-dcGTX2&3-d at the time of packaging.

Compound	$[M+H]^+$, *m/z*	Concentration (µmol/L)*
Gonyautoxin-2 (GTX2)	396.1	0.62
Gonyautoxin-3 (GTX3)	396.1	0.06
Decarbamoylgonyautoxin-1	369.1	0.35

* These concentrations are not certified.

Homogeneity

A representative number of CRM-dcGTX2&3-d ampoules were selected from across the fill series and dcGTX2&3 responses were measured by LC-MS/MS. No heterogeneity was observed.

Stability

Stability studies demonstrated good stability for dcGTX2&3 in aqueous 0.5 mM HCl stored in sealed ampoules at -12 °C or below.

Uncertainty

All reasonable sources of error related to the characterization of CRM-dcGTX2&3-d were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}) [9] and instability during storage (u_{stab}). 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-dcGTX2&3-d.

Uncertainties	dcGTX2*	dcGTX3*	dcGTX2&3*
u_{char}	0.03	0.04	0.03
u_{hom}	negligible	negligible	negligible
u_{stab}	0.01	0.02	0.02

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, dcGTX2&3 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-dcGTX2&3-d.

Period of Validity

If stored unopened at the recommended storage condition (- 12 °C or below), the certified concentration of CRM-dcGTX2&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 caffeine CRM (NMIA M724c), and a CRM for dcGTX2&3 (NRC CRM-dcGTX2&3-c).

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

1. Qiu J, Rafuse C, Lewis NI, Li A, Meng F, Beach DG, and McCarron P (2018). Screening of cyclic imine and paralytic shellfish toxins in isolates of the genus *Alexandrium* (*Dinophyceae*) from Atlantic Canada. *Harmful Algae* 77: 108-118. doi: 10.1016/j.hal.2018.05.005.
2. D'Agostino PM, Boundy MJ, Harwood TD, Carmichael WW, Neilan BA, and Wood SA (2019). Re-evaluation of paralytic shellfish toxin profiles in cyanobacteria using hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Toxicon* 158: 1-7. doi: 10.1016/j.toxicon.2018.11.301.
3. Laycock MV, Thibault P, Ayer SW, and Walter JA (1994). Isolation and purification procedures for the preparation of paralytic shellfish poisoning toxin standards. *Nat Toxins* 2: 175-183. doi: 10.1002/nt.2620020405.
4. Thomas KM, Beach DG, Reeves KL, Gibbs RS, Kerrin ES, McCarron P, and Quilliam MA (2017). Hydrophilic interaction liquid chromatography-tandem mass spectrometry for quantitation of paralytic shellfish toxins: validation and application to reference materials. *Anal Bioanal Chem* 409: 5675-5687. doi: 10.1007/s00216-017-0507-3.
5. Anon. AOAC Official Method 2011.02. Paralytic shellfish toxins in mussels, clams, oysters and scallops; post-column oxidation (PCOX) method. in *AOAC International, Gaithersburg, MD, USA*.
6. Thomas K, Wechsler D, Chen YM, Crain S, and Quilliam MA (2016). Analysis of natural toxins by liquid chromatography-chemiluminescence nitrogen detection and application to the preparation of certified reference materials. *J AOAC Int* 99: 1173-1184. doi: 10.5740/jaoacint.16-0146.

7. Burton IW, Quilliam MA, and Walter JA (2005). Quantitative ^{1}H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. *Anal Chem* 77: 3123-3131. doi: 10.1021/ac048385h.

8. Dell'Aversano C, Walter JA, Burton IW, Stirling DJ, Fattorusso E, and Quilliam MA (2008). Isolation and structure elucidation of new and unusual saxitoxin analogues from mussels. *J Nat Prod* 71: 1518-1523. doi: 10.1021/np800066r.

9. Pauwels J, van der Veen A, Lamberty A, and Schimmel H (2000). Evaluation of uncertainty of reference materials. *Accred Qual Assur* 5: 95-99. doi: 10.1007/s007690050020.

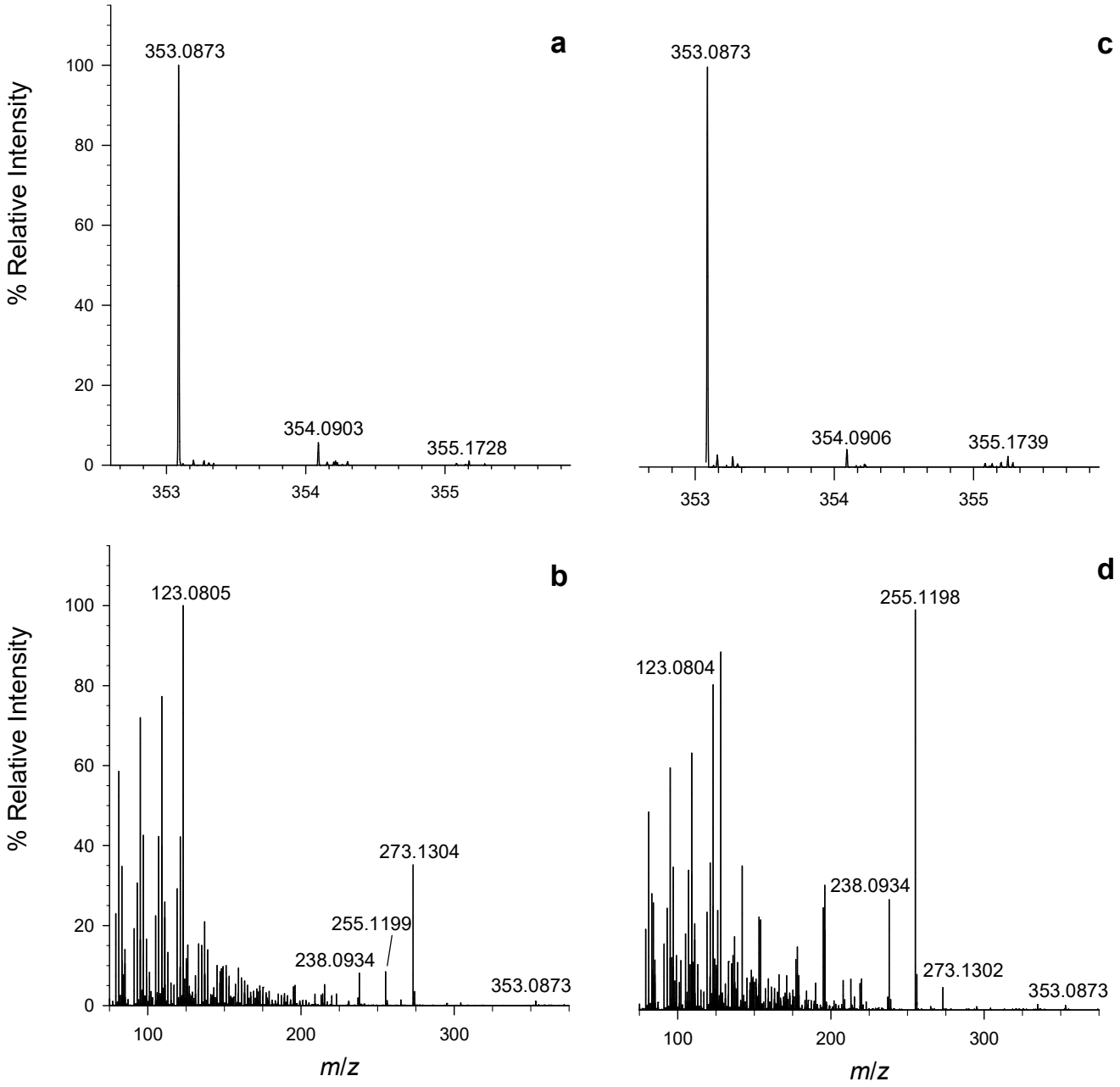


Figure 1: Full scan and collision induced dissociation LC–HRMS spectra of dcGTX2 (a and b, respectively) and dcGTX3 (c and d, respectively) used for CRM-dcGTX2&3-d analyzed on a Thermo QExactive-HF mass spectrometer equipped with a heated electrospray ionization probe. Data was collected in positive mode with a 4000 V spray voltage, +300 °C capillary temperature, and a +300 °C heater temperature. Full scan data was acquired with a resolution setting of 60 000. MS/MS data was acquired in parallel reaction monitoring scan mode with the same resolution setting and a normalized collision energy (30-60 V).

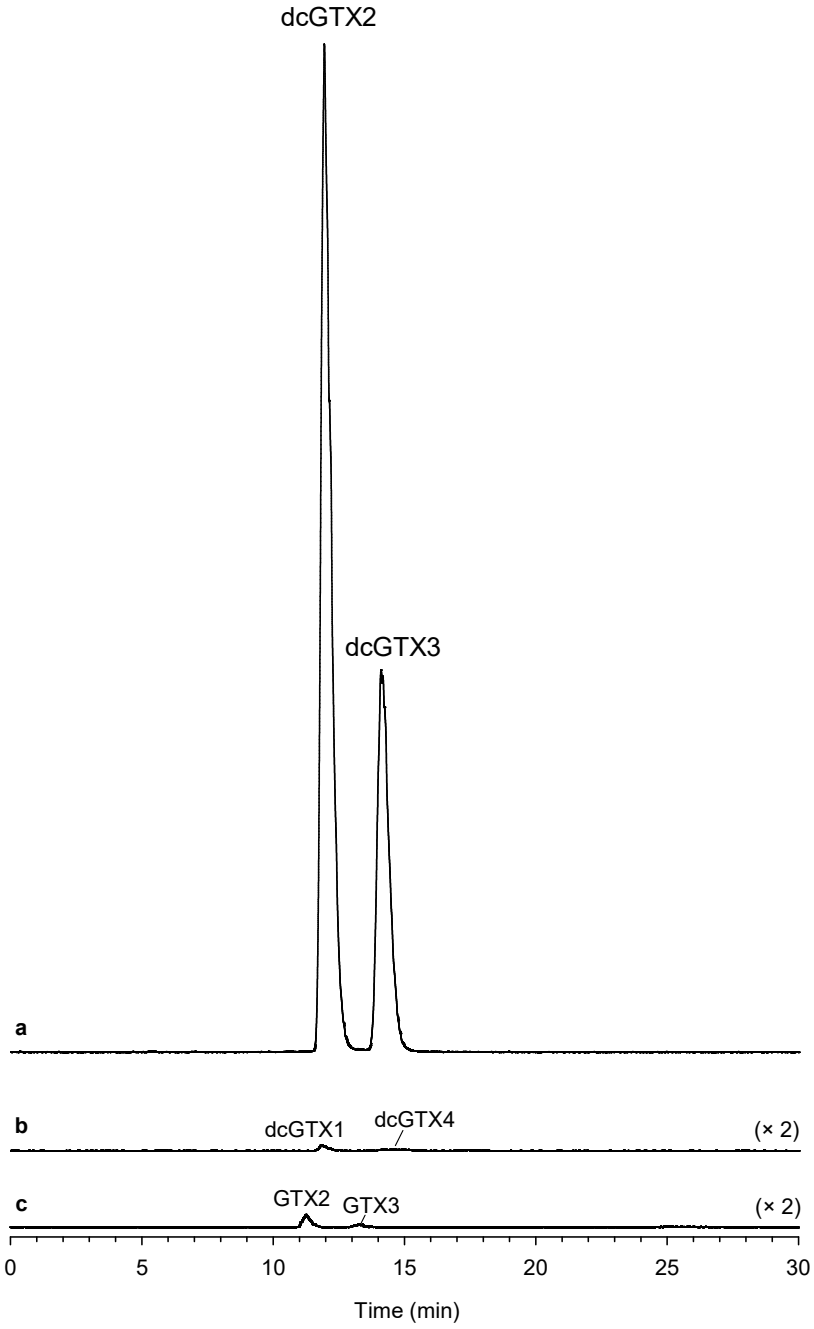


Figure 2: LC–MS/MS analysis of CRM-dcGTX2&3-d using selected reaction monitoring with summed ion transitions: (a) *m*/*z* 353 → 273 and *m*/*z* 353 → 255; and (b) *m*/*z* 369 → 289 and *m*/*z* 369 → 271, and (c) *m*/*z* 396 → 316 and *m*/*z* 396 → 298. Conditions: Agilent 1260 LC connected to a Sciex 4000 QTRAP MS with electrospray ionization. Column: Toso-Haas Amide-80 (250 mm × 2 mm, 5 µm) at +40 °C; mobile phase: 69.5% 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 +20 and +35 V, declustering potential +25 and +55 V for dcGTX2 and dcGTX3 (respectively), and source temperature +275 °C.

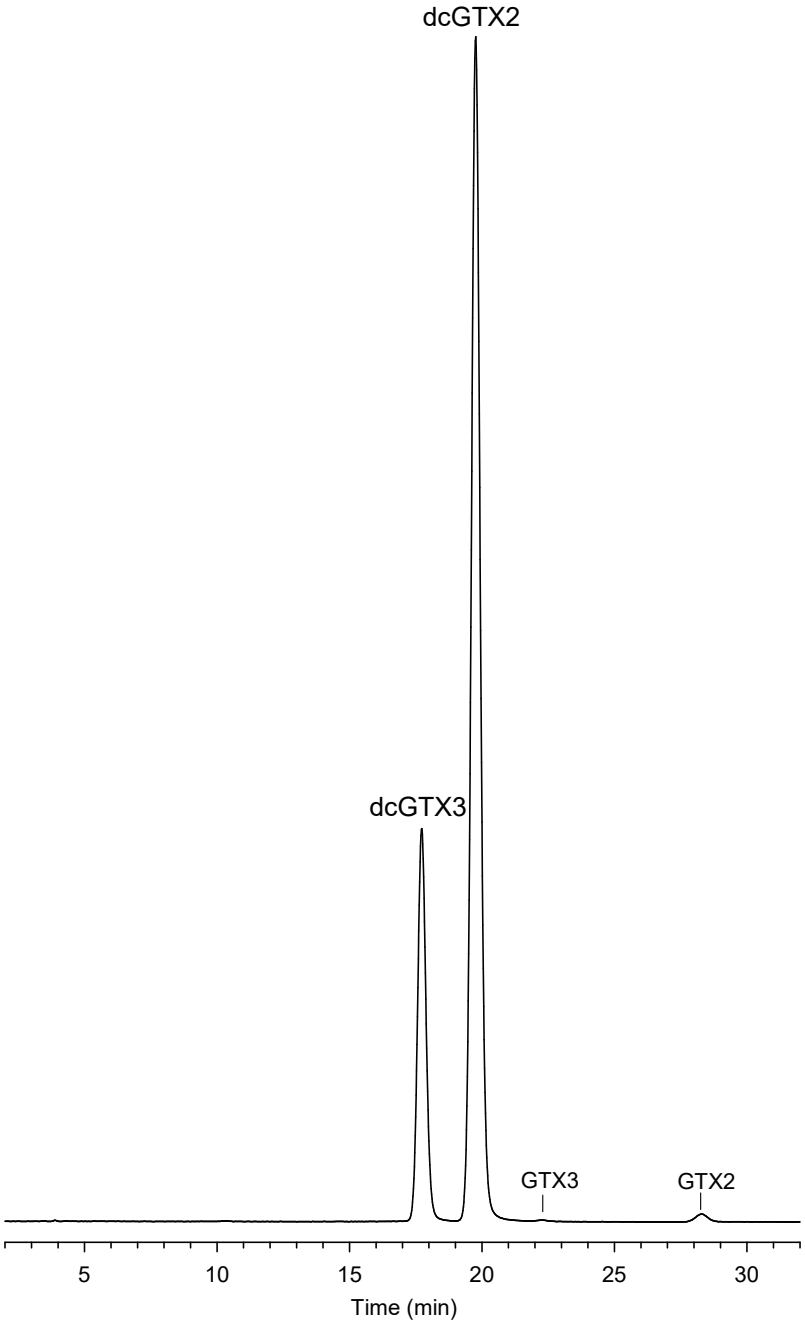


Figure 3: LC–ox–FLD analysis of CRM-dcGTX2&3-d. Conditions: Zorbax Bonus-RP column, 250 × 4.6 mm i.d. at +35 °C; mobile phase: 5.5 mM ammonium phosphate with 11 mM sodium heptane sulphonate, pH 7.1: 0.8 mL/min; injection volume 5 µL; post-column oxidation: 0.4 mL/min 5 mM periodic acid in 100 mM sodium 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.

Acknowledgements

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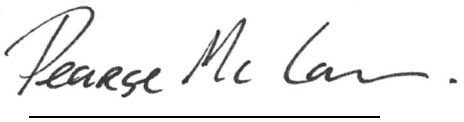
Approved by: 

Pearse McCarron, Ph.D.
Team Leader, Biotoxin Metrology

This Certificate is only valid if the corresponding material was obtained directly from the NRC or an Authorized Reseller.

Comments, information and inquiries should be addressed to:

National Research Council Canada
1411 Oxford Street
Halifax, Nova Scotia B3H 3Z1
Canada

Telephone: 1-902-426-8281
Fax: 1-902-426-5426
Email: CRM-MRCHalifax@nrc-cnrc.gc.ca

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