



Certificate of Analysis

Certified Reference Material

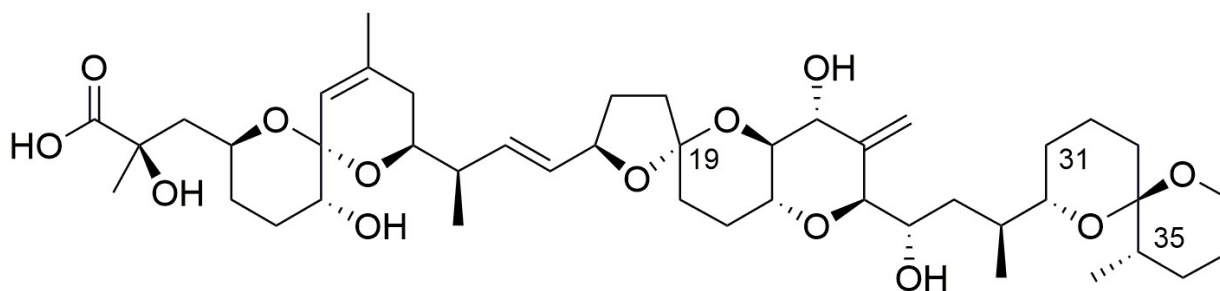
CRM-DTX2-c (Lot# 20221102)

Certified Calibration Solution for Dinophysistoxin-2

Dinophysistoxin-2 (DTX2) is a lipophilic toxin produced by marine dinoflagellates that can accumulate in shellfish and cause diarrhetic shellfish poisoning [1]. DTX2 is a regulated structural analogue of okadaic acid (OA), with a methyl group at position C35 instead of C31 [2]. CRM-DTX2-c is a certified calibration solution of DTX2 in methanol and is a replacement for CRM-DTX2-b [3].

Table 1: Certified concentration and uncertainty for CRM-DTX2-c.

Compound	$\mu\text{g/g}$	$\mu\text{g/mL}$ (15 - 30 °C)	$\mu\text{mol/L}$ (15 - 30 °C)
Dinophysistoxin-2	4.86 ± 0.23	3.83 ± 0.18	4.76 ± 0.23



Dinophysistoxin-2

CAS registry No.: 139933-46-3

InChIKey: BRFKTXCAUCYQBT-AKHROESGSA-N

Molecular formula: $\text{C}_{44}\text{H}_{68}\text{O}_{13}$

Molar mass: 805.00 g/mol

$[\text{M}+\text{H}]^+$: m/z 805.4733

$[\text{M}-\text{H}]^-$: m/z 803.4587

Period of validity: 2 years from date of sale

Storage conditions: -12 °C or below

Intended Use

CRM-DTX2-c is a certified calibration solution for analytical method development and accurate quantitation of DTX2. The concentration is suitable for preparing a dilution series for calibration of instruments such as liquid chromatography–mass spectrometry (LC–MS), and for spiking control samples for recovery experiments.

Instructions for Storage and Use

To ensure the stability of CRM-DTX2-c, ampoules should be stored at $-12\text{ }^{\circ}\text{C}$ or below.

It is important to note that the 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-DTX2-c

DTX2 was isolated from contaminated mussel (*Mytilus edulis*) tissue harvested on the southwest coast of Ireland. The preparative extraction and purification of DTX2 was carried out at the Marine Institute (Rinville, Oranmore, Co. Galway, Ireland) using previously described procedures [4].

The structure and purity of DTX2 was confirmed by LC–MS (Figures 1 and 2), LC with charged aerosol detection (LC–CAD) and 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. A measured accurate m/z of 803.4592 ($\Delta = 0.6\text{ ppm}$ for $\text{C}_{44}\text{H}_{67}\text{O}_{13}^{-}$) was obtained for the $[\text{M}-\text{H}]^{-}$ ion of DTX2 using LC–high-resolution MS (LC–HRMS), and MS/MS data (Figure 1B) matched that of CRM-DTX2-b and literature reports [5].

The stock solution was prepared by dissolving the purified DTX2 in CD_3OD for quantitation using ^1H NMR (qNMR) [6]. The CRM-DTX2-c solution was prepared by accurately diluting the stock solution in degassed high purity methanol. Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL.

Analytical Methods and Value Assignment

The certified value for CRM-DTX2-c (Table 1) is an uncertainty-weighted mean of results obtained at the NRC with qNMR using benzoic acid for calibration, LC-MS/MS using CRM-DTX2-b as the calibrant, and LC-CAD using CRM-DTX1-c as the calibrant.

19-*epi*-DTX2 present in CRM-DTX2-c was assigned an information value of $0.12 \pm 0.03\text{ }\mu\text{g/g}$ by qNMR and LC–HRMS. It is common for 19-*epi*-DTX2 to co-elute with OA in reverse phase chromatography [7]. This should be considered before using CRM-DTX2-c in a mixed solution with OA. Trace amounts ($\sim 1.4\%$ total) of other structurally related impurities are present in the material.

Homogeneity

A representative number of CRM-DTX2-c ampoules were selected from across the fill series and analyzed by LC–MS/MS. No heterogeneity was observed.

Stability

Studies with DTX2 in methanol have demonstrated good stability when stored in sealed ampoules at temperatures of $-12\text{ }^{\circ}\text{C}$ and below [7].

Uncertainty

All reasonable sources of uncertainty related to the characterization of CRM-DTX2-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}) [8]. These components are listed in Table 2, and are combined and expanded as follows:

$$U_{\text{CRM}} = k\sqrt{u_{\text{char}}^2 + u_{\text{hom}}^2 + u_{\text{stab}}^2}$$

where k is the coverage factor for a 95 % confidence level (= 2).

Table 2: Uncertainty components for the certified value of CRM-DTX2-c.

Uncertainties	Relative*
u_{char}	0.014
u_{hom}	negligible
u_{stab}	0.019

*Relative to concentration shown in Table 1.

Safety Instructions

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 is available for CRM-DTX2-c.

Period of Validity

If stored unopened at the recommended storage condition of $-12\text{ }^{\circ}\text{C}$, the certified concentration of CRM-DTX2-c is valid for 2 years from the date of sale.

Metrological Traceability

Results presented in this certificate are traceable to the SI (*Système international d'unités*) through a gravimetrically prepared standard of NIST Benzoic acid certified reference material (PS1), NRC CRM-DTX2-b (Lot# 20150819), and NRC CRM-DTX1-c (Lot# 20191010).

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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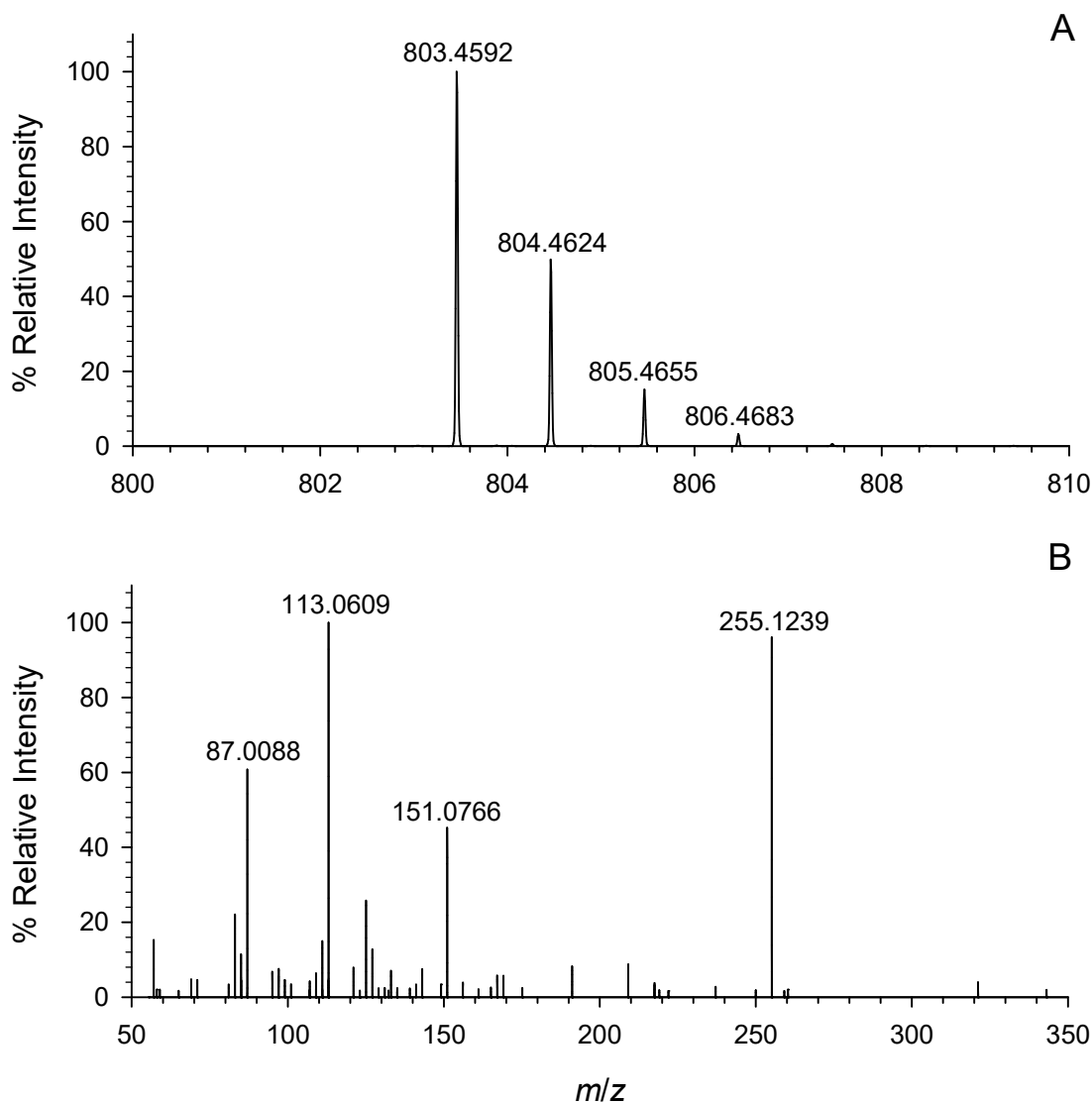


Figure 1: Full-scan (A) and low m/z range collision-induced dissociation (MS/MS) (B) spectra from LC–HRMS analysis of DTX2 used for preparation of CRM-DTX2-c, acquired using a Q Exactive-HF mass spectrometer in negative mode. Full-scan data was acquired with a resolution setting of 60 000. MS/MS data was acquired in parallel reaction monitoring scan mode with a 15 000 resolution setting using a stepped collision energy of 50, 65, 80 eV.

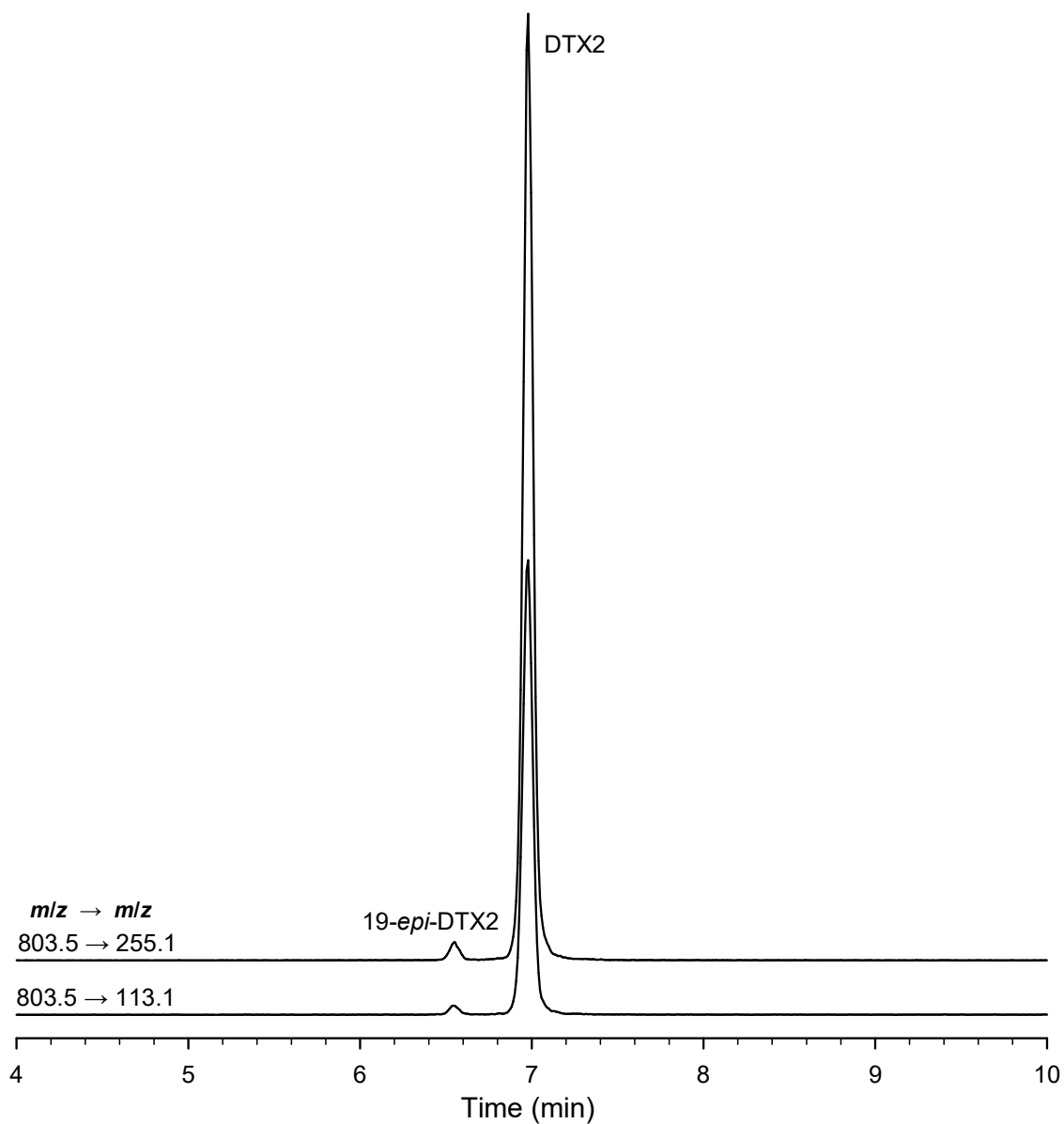


Figure 2: LC-MS/MS analysis of CRM-DTX2-c using an acidic pH mobile phase, showing resolution of 19-*epi*-DTX2. Chromatographic conditions: Luna 2.5 μ m C18(2) column (2.0 \times 50 mm); mobile phase: 2 mM ammonium formate and 50 mM formic acid (pH 2.3) in both deionised water (A) and 95% acetonitrile (B); gradient: 25 to 100% B over 8 min, 250 μ L/min at +20 $^{\circ}$ C; injection volume: 1 μ L. MS conditions included negative electrospray ionization (-4.5 kV) and selected reaction monitoring scan mode with declustering potential -80 eV for both and collision energies of -65 and -85 eV for the 255.1 and 113.1 transitions respectively.

Acknowledgements

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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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