



# Certificate of Analysis

## Certified Reference Material

### CRM-YTX-d (Lot# 20231207)

#### Certified Calibration Solution for Yessotoxin

The yessotoxins (YTXs) are a group of polycyclic ether compounds produced by dinoflagellate algae including *Protoceratium reticulatum*, *Lingulodinium polyedrum* and *Gonyaulax spinifera* [1]. Levels of YTXs in shellfish for human consumption are regulated in some areas [2,3]. CRM-YTX-d is a certified calibration solution of YTX in methanol and is a replacement for CRM-YTX-c.

**Table 1:** Certified values and uncertainties for CRM-YTX-d

Compound	$\mu\text{g/g}$	$\mu\text{g/mL}$ (15 - 30 °C)	$\mu\text{mol/L}$ (15 - 30 °C)
Yessotoxin	$6.01 \pm 0.32$	$4.74 \pm 0.26$	$4.15 \pm 0.23$

#### Yessotoxin

CAS registry No.: 112514-54-2

InChIKey: HCYDZFGUKMTQB-AVHIVUAZSA-N

Molecular formula:  $\text{C}_{55}\text{H}_{82}\text{O}_{21}\text{S}_2$

Molar mass: 1143.4 g/mol

[M-H]<sup>-</sup>:  $m/z$  1141.4717

[M-2H]<sup>2-</sup>:  $m/z$  570.2322

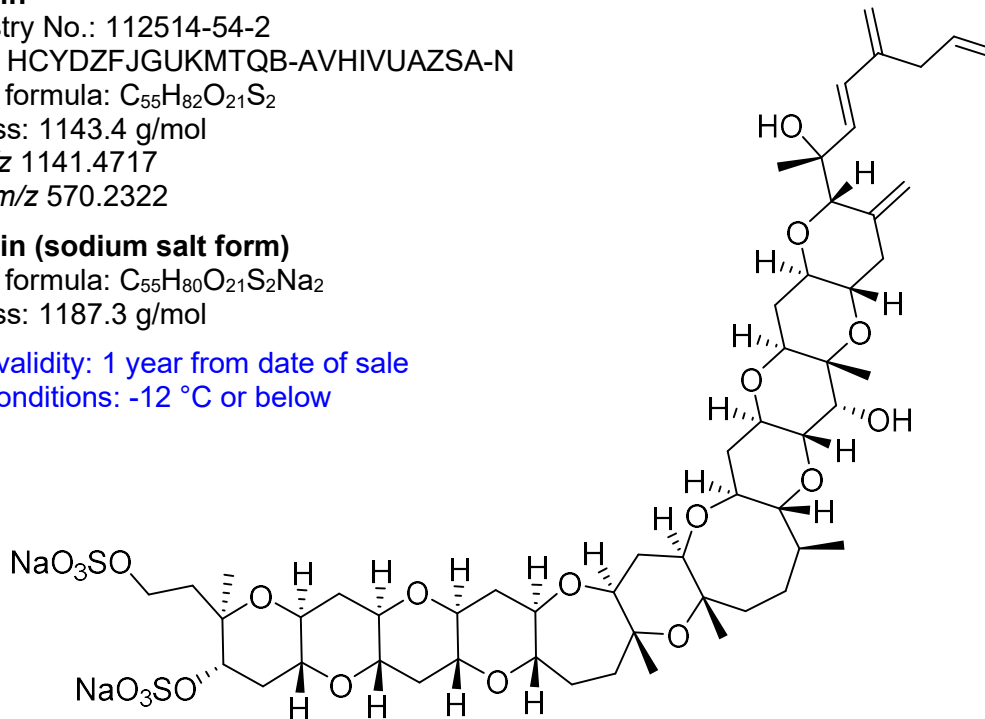
#### Yessotoxin (sodium salt form)

Molecular formula:  $\text{C}_{55}\text{H}_{80}\text{O}_{21}\text{S}_2\text{Na}_2$

Molar mass: 1187.3 g/mol

Period of validity: 1 year from date of sale

Storage conditions: -12 °C or below



## Intended Use

CRM-YTX-d is a certified calibration solution for analytical method development and accurate quantitation of YTX. 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-YTX-d, 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-YTX-d

YTX was isolated from a dinoflagellate (*Protoceratium reticulatum*) cultured at the Cawthron Institute, New Zealand [4]. The structure and purity of YTX was confirmed by  $^1\text{H}$  NMR and LC–MS. A measured accurate  $m/z$  of  $1141.4751 \pm 0.0004$  ( $\Delta = +2.9$  ppm for  $\text{C}_{55}\text{H}_{81}\text{O}_{21}\text{S}_2^-$ ) was obtained for the  $[\text{M}-\text{H}]^-$  ion of YTX using high-resolution MS (Figure 1A). The collision induced dissociation spectrum (Figure 1B) matched literature reports and the previous YTX CRM [4,5].

The stock solution was prepared by dissolving the purified YTX in  $\text{CD}_3\text{OD}$  for quantitation using  $^1\text{H}$  NMR (qNMR) [6]. The CRM-YTX-d 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-YTX-d (Table 1) is an uncertainty-weighted mean of results obtained at the National Research Council Canada (NRC) with qNMR using benzoic acid (NIST PS-1) for calibration, LC–MS/MS using CRM-YTX-c for calibration, and LC charged aerosol detection using CRM-hYTX-b as the calibrant. Low levels ( $\sim 2\%$ ) of tentatively identified YTX impurities are present in CRM-YTX-d (Figure 2).

## Homogeneity

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

## Stability

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

## Uncertainty

All reasonable sources of uncertainty related to the characterization of CRM-YTX-d were considered and measured. The overall uncertainty estimate ( $U_{CRM}$ ) includes uncertainties associated with batch characterization ( $u_{char}$ ) and instability during storage ( $u_{stab}$ ) [7]. These components are listed in Table 2, 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 2:** Uncertainty components for the certified values of CRM-YTX-d

Uncertainties	Relative*
$u_{char}$	0.015
$u_{hom}$	negligible
$u_{stab}$	0.022

\*Relative to values 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 (SDS) is available for CRM-YTX-d.

## Period of Validity

If stored unopened at the recommended storage condition of  $-12\text{ }^{\circ}\text{C}$  or below, the certified concentration of CRM-YTX-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 a gravimetrically prepared standard of NIST Benzoic acid certified reference material (PS1), NRC CRM-YTX-c (Lot# 20151125), and NRC CRM-hYTX-b (Lot# 20190716).

## Quality Management System (ISO 17034, ISO/IEC 17025)

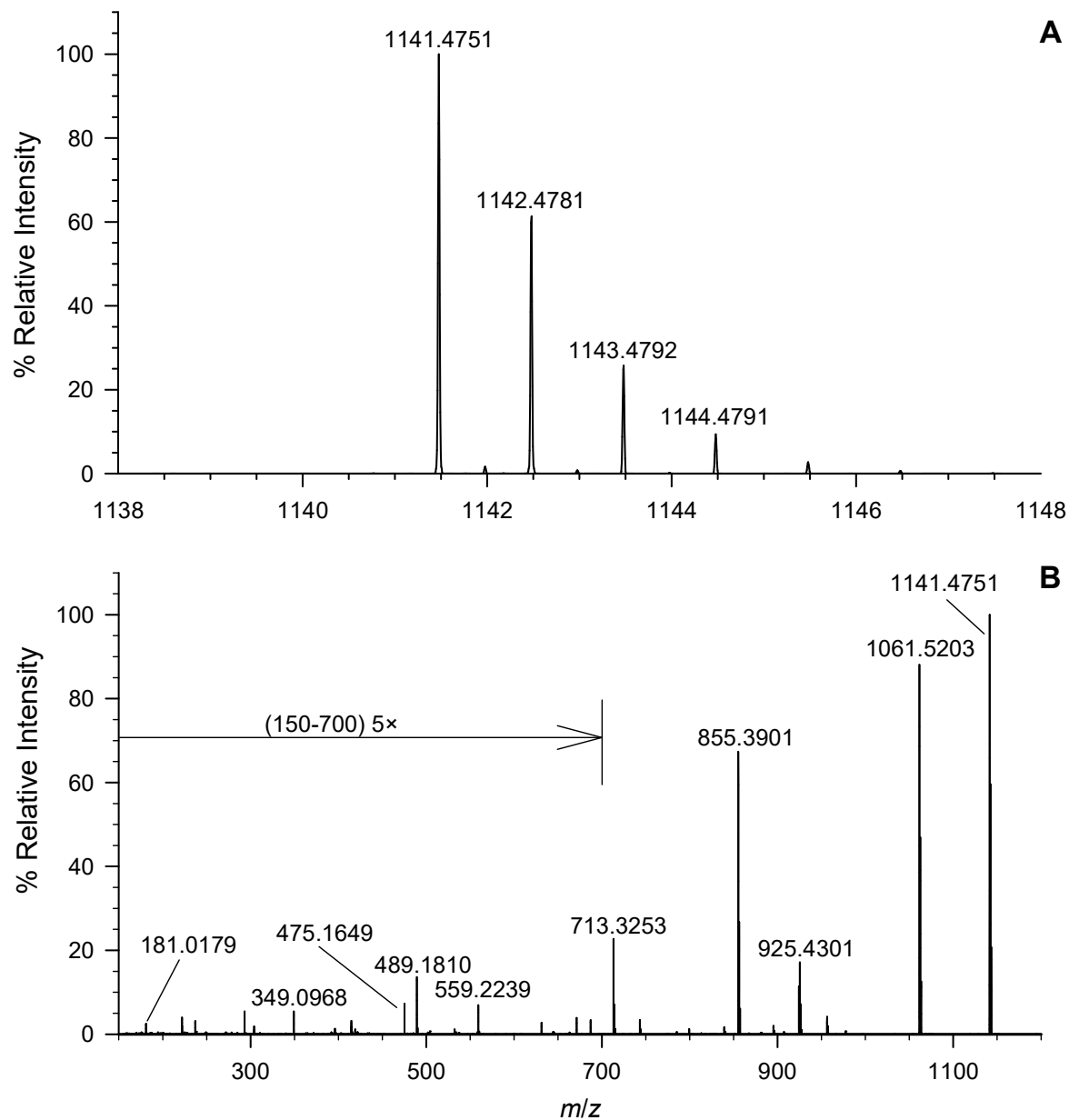
This material was produced in compliance with the National Research Council of Canada (NRC) Metrology Quality Management System, which conforms to the requirements of ISO 17034 and ISO/IEC 17025.

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

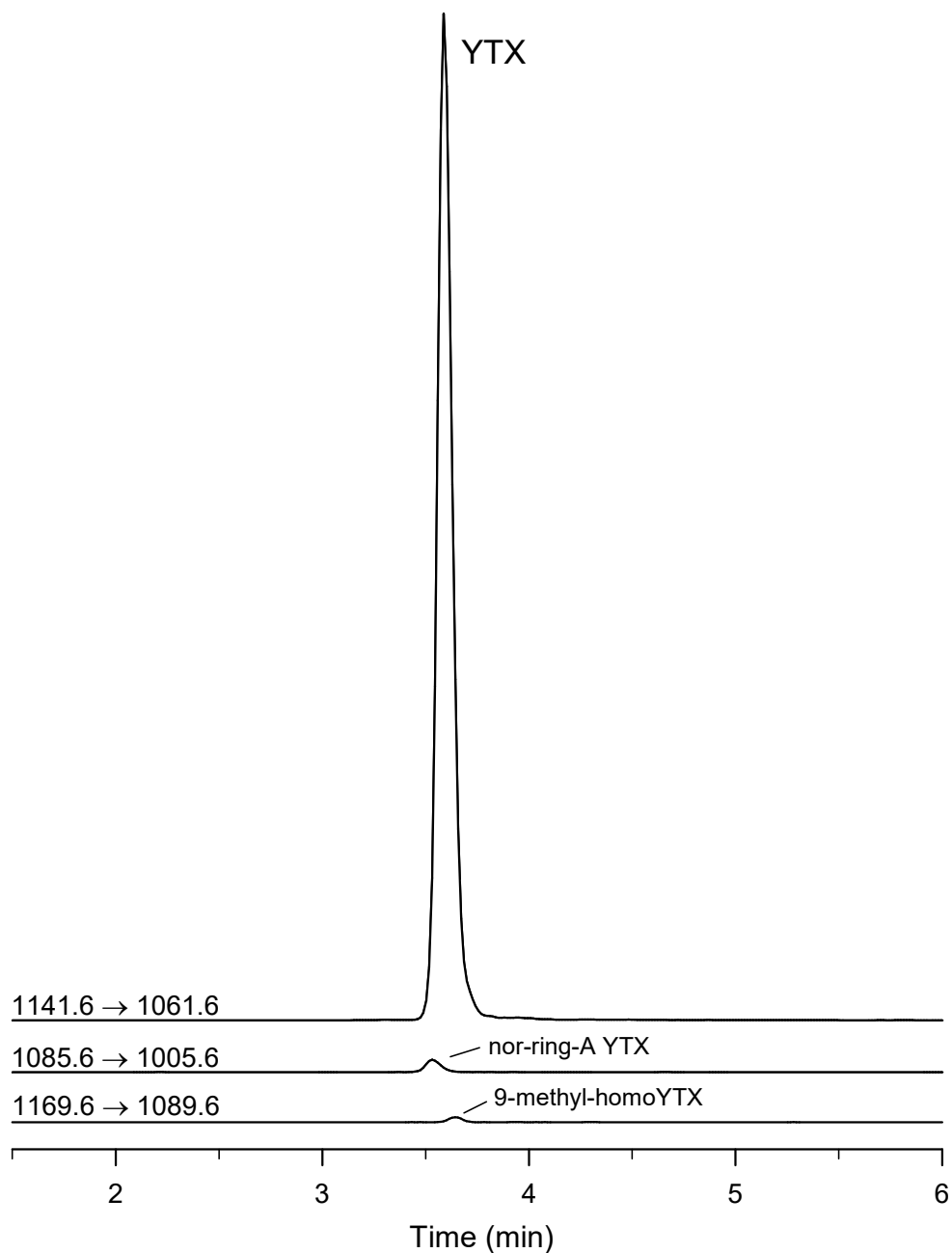
the *Comité international des poids et mesures* (CIPM) Mutual Recognition Arrangement. The SIM approval is available upon request.

## References

1. Hess P, Aasen J (2007). Chemistry, origins, and distribution of yessotoxin and its analogues, in Botana LM (Ed). *Phycotoxins: Chemistry and Biochemistry* (Blackwell Publishing, Oxford):187-202. <https://doi.org/10.1002/9780470277874.ch10>
2. Anonymous (2004). Regulation (EC) No 853/2004 of the European parliament and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union L 139 of 30 April 2004. <https://eur-lex.europa.eu/eli/reg/2004/853/oj>
3. Anonymous (2013). Commission Regulation (EU) No 786/2013 of 16 August 2013 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the permitted limits of yessotoxins in live bivalve molluscs. Official Journal of the European Union L 220 of 16 August 2013. <https://data.europa.eu/eli/reg/2013/786/oj>
4. Miles CO, Wilkins AL, Jensen DJ, Cooney JM, Quilliam MA, Aasen J, MacKenzie AL (2004). Isolation of 41a-homoyessotoxin and the identification of 9-methyl-41a-homoyessotoxin and nor-ring A-yessotoxin from *Protoceratium reticulatum*. *Chem Res Toxicol* 17 (11):1414-1422. <https://doi.org/10.1021/tx049832r>
5. Giddings SD, Crain S, McCarron P (2016). CRM-YTX-c, certified calibration solution for Yessotoxin. National Research Council Canada, Halifax. <https://doi.org/10.4224/crm.2016.ytx-c.20151125>
6. Burton IW, Quilliam MA, Walter JA (2005). Quantitative <sup>1</sup>H NMR with external standards: Use in preparation of calibration solutions for algal toxins and other natural products. *Anal Chem* 77:3123-3131. <https://doi.org/10.1021/ac048385h>
7. Pauwels J, Lamberty A, Schimmel H (2000). Evaluation of uncertainty of reference materials. *Accreditation and Quality Assurance* 5:95-99. <https://doi.org/10.1007/s007690050020>



**Figure 1:** Full-scan (A) and collision induced dissociation (MS/MS) (B) spectra from LC-HRMS analysis of YTX used for preparation of CRM-YTX-d, acquired using a Q Exactive-HF mass spectrometer in negative mode. Full-scan data was acquired with a resolution setting of 120 000. MS/MS data was acquired in parallel reaction monitoring scan mode with a 30 000 resolution setting using a stepped collision energy of -25 and -80 eV.



**Figure 2:** LC-MS/MS analysis of CRM-YTX-d. Chromatographic conditions: Phenomenex Synergi Max-RP 2.5  $\mu\text{m}$  column (2.0  $\times$  50 mm); 2 mM ammonium acetate (pH 6.8) in both deionised water (A) and 95% acetonitrile (B); gradient: 25 to 100% B over 5 min, 300  $\mu\text{L}/\text{min}$  at 20  $^{\circ}\text{C}$ ; injection volume: 5  $\mu\text{L}$ . MS conditions included negative electrospray ionization ( $-4.5$  kV) and selected reaction monitoring scan mode with declustering potential and collision energies of  $-80$  eV and  $-55$  eV, respectively. Trace level impurities are tentatively identified.

## Acknowledgements

The following staff members at the NRC contributed to the production and certification of CRM-YTX-d: Crain S, Giddings SD, Gillies JR, Gray TA, LeBlanc P, McAulay CJ, Lewis N, McCarron P, Mudge EM, Perez Calderon RA, Rafuse C, Reeves KL and Thomas K.

### This document should be cited as:

Giddings SD, Crain S, McCarron P "CRM-YTX-d, a certified calibration solution reference material for yessotoxin", Biotoxin Metrology Certificate of Analysis CRM-YTX-d-20231207, National Research Council Canada, Halifax.

DOI: <https://doi.org/10.4224/crm.2024.ytx-d.20231207>

*Date of issue: June 2024*

*Document version: 20240612*

### 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](mailto:CRM-MRCHalifax@nrc-cnrc.gc.ca)