Certificate of Analysis

NRC·CNRC Certified Reference Material

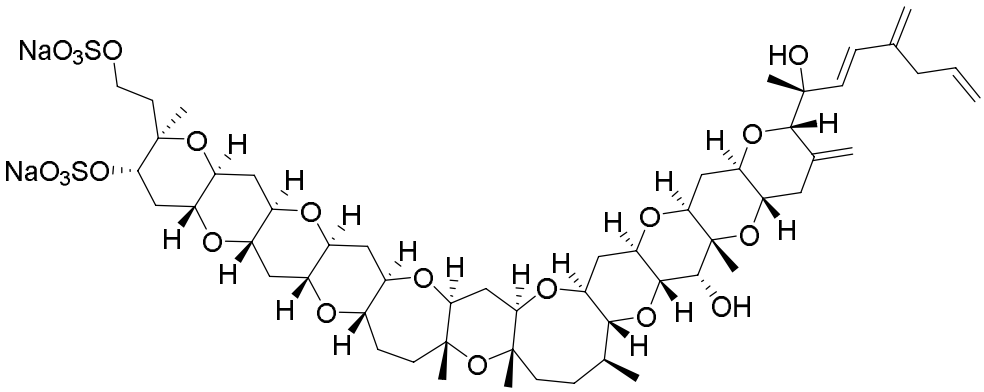
CRM-YTX-c (Lot # 20151125)

Certified Calibration Solution for Yessotoxin

The yessotoxins (YTXs) are a group of polycyclic ether compounds produced by various dinoflagellate algae including *Protoceratium reticulatum*, *Lingulodinium polyedrum* and *Gonyaulax spinifera* [1]. The maximum allowable levels of certain YTXs in shellfish for human consumption is regulated [2]. CRM-YTX-c is a certified calibration solution of YTX in methanol and is a replacement for CRM-YTX-b, which was released in 2012.

Table 1: Certified concentration value for CRM-YTX-c

Compound	µmol/L (at +20 °C)
Yessotoxin (protonated form)	4.3 ± 0.2



Yessotoxin (protonated form)
CAS registry No.: 112514-54-2
Molecular formula: $C_{55}H_{82}O_{21}S_2$
Molecular weight: 1143.4 g/mol
$[M-H]^-$: *m/z* 1141.4717
$[M-2H]^{2-}$: *m/z* 570.2322

Yessotoxin (sodium salt form)
Molecular formula: $C_{55}H_{80}O_{21}S_2Na_2$
Molecular weight: 1187.3 g/mol

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

Intended Use

CRM-YTX-c is a certified calibration solution designed for analytical method development and accurate quantitation of YTX. The concentration of CRM-YTX-c makes it suitable for preparing a dilution series for calibration of liquid chromatography (LC) and liquid chromatography-mass spectrometry (LC-MS) instrumentation, as well as for spiking shellfish control samples for recovery experiments.

Instructions for Storage and Use

To ensure the stability of CRM-YTX-c, ampoules should be stored at -12 °C or below.

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents thoroughly mixed. The ampoules 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 methanol will occur if the solution is left opened for more than a few minutes. It is recommended that solutions of CRM-YTX-c should not be evaporated to dryness, due to potential 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-YTX-c

YTX was isolated from a dinoflagellate *(Protoceratium reticulatum*) cultured at Cawthron Institute, New Zealand. The preparative extraction and purification of YTX was carried out at the Cawthron Institute [3]. Final purity steps and assessment was done at NRC, Halifax. The structure and purity of YTX was confirmed by ^{1}H NMR and high resolution LC-MS. A measured accurate *m/z* of 1141.4704 ± 0.0001 (Δ = -1.1 ppm for $C_{55}H_{81}O_{21}S_2^-$) was obtained for the $[M-H]^-$ ion of YTX (Figure 1A). The collision induced dissociation spectrum (Figure 1B) matched those of previous YTX CRMs and literature reports [4].

A stock solution was prepared by dissolving the purified YTX in CD_3OH for quantitative NMR (qNMR) [5]. The CRM solution was prepared by making an accurate dilution of the qNMR stock solution in degassed, high purity methanol. Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed.

Analytical Methods and Value Assignment

The certified value for CRM-YTX-c is based on the results obtained at NRC using two methods: qNMR on the stock YTX solution using a NIST benzoic acid SRM as a calibrant and LC-MS/MS with an acidic pH mobile phase using CRM-YTX-b (Lot # 20120328) for calibration. CRM-YTX-c contains trace amounts of other YTX analogues (<3% in total, relative to YTX) (Table 2, Figure 2).

Table 2: Information values for additional YTX analogues present in CRM-YTX-c

Analogue	$[M-H]^-$	RRT**	µmol/L*
9-Me-41a-homoyessotoxin	1169.6	1.01	0.06
41a-homoyessotoxin	1155.6	1.00	0.02
Nor-ring-A-yessotoxin	1085.6	1.00	0.05

* These concentrations are not certified.
** Relative retention times (RRT) compared to YTX (Figure 2).

Homogeneity

A representative number of CRM-YTX-c ampoules (n = 25) were selected from across the fill series and YTX concentrations measured by LC-MS/MS. Results were evaluated using ANOVA and no significant heterogeneity was detected.

Stability Study

Short and long-term stability studies were conducted for CRM-YTX (Lot # 20060308). Because the production process and concentration range are equivalent for CRM-YTX-c, comparable stability is reasonably assumed. Stability studies showed no significant degradation of YTX in methanol at -12 °C over a period of one year.

Uncertainty

All reasonable sources of error related to the characterization of CRM-YTX-c 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}), between-bottle variation (u_{hom}), and instability during long-term storage (u_{stab}) [6-8]. 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-YTX-c

Uncertainties	[µmol/L]
u_{char}	0.09
u_{hom}	negligible
u_{stab}	0.09
U_{CRM} (k = 2)	0.2

Safety Instructions

YTXs were initially classified as diarrhetic shellfish toxins, but the biological activity of these compounds lack any diarrhetic effects and YTXs have since been classified as a separate group of algal toxins [9]. No human toxicity has been reported as a result of consumption of YTX contaminated shellfish [10]. Nevertheless, precautions should be taken when handling CRM-YTX-c. Inhalation and ingestion of methanol is harmful; ingestion may cause blindness or fatality, while prolonged skin contact may result in dermatitis and/or kidney damage. 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-YTX-c.

Period of Validity

If stored unopened at the recommended condition of -12 °C or below, the certified concentration of CRM-YTX-c 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.

References

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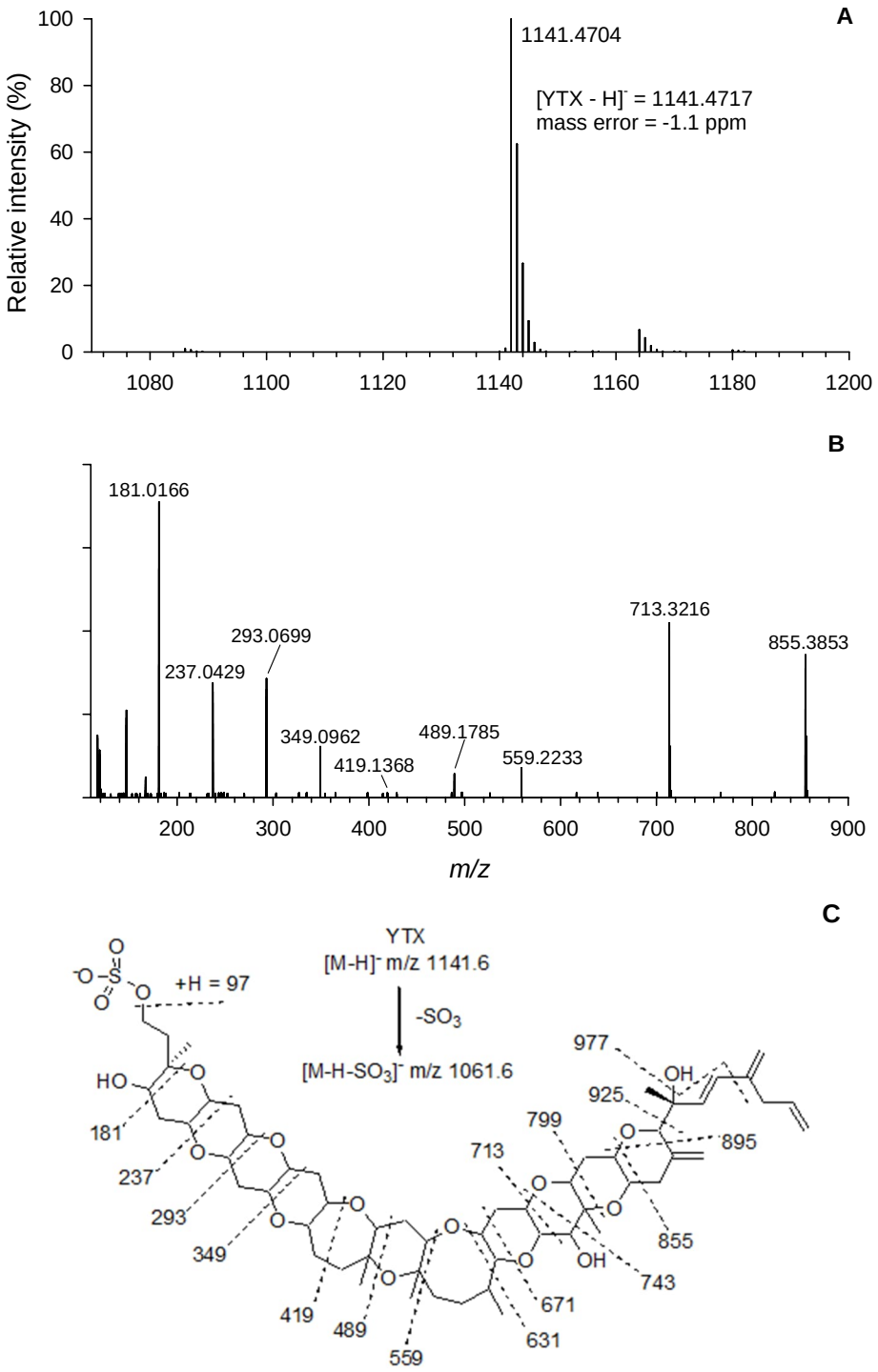

Figure 1: High-resolution full scan (A) and collision induced dissociation (B) mass spectra of the $[M-H]^-$ ion, *m/z* 1141 of YTX in CRM-YTX-c measured on a Thermo Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization probe. Data acquired in negative ion mode with a -2.7 kV spray voltage, +360 °C capillary temperature, and +250 °C heater temperature. HCD collision energy in (B) was 120 V. Fragmentation mechanism for collision induced dissociation of the $[M-H]^-$ ion (*m/z* 1141) of YTX (C).

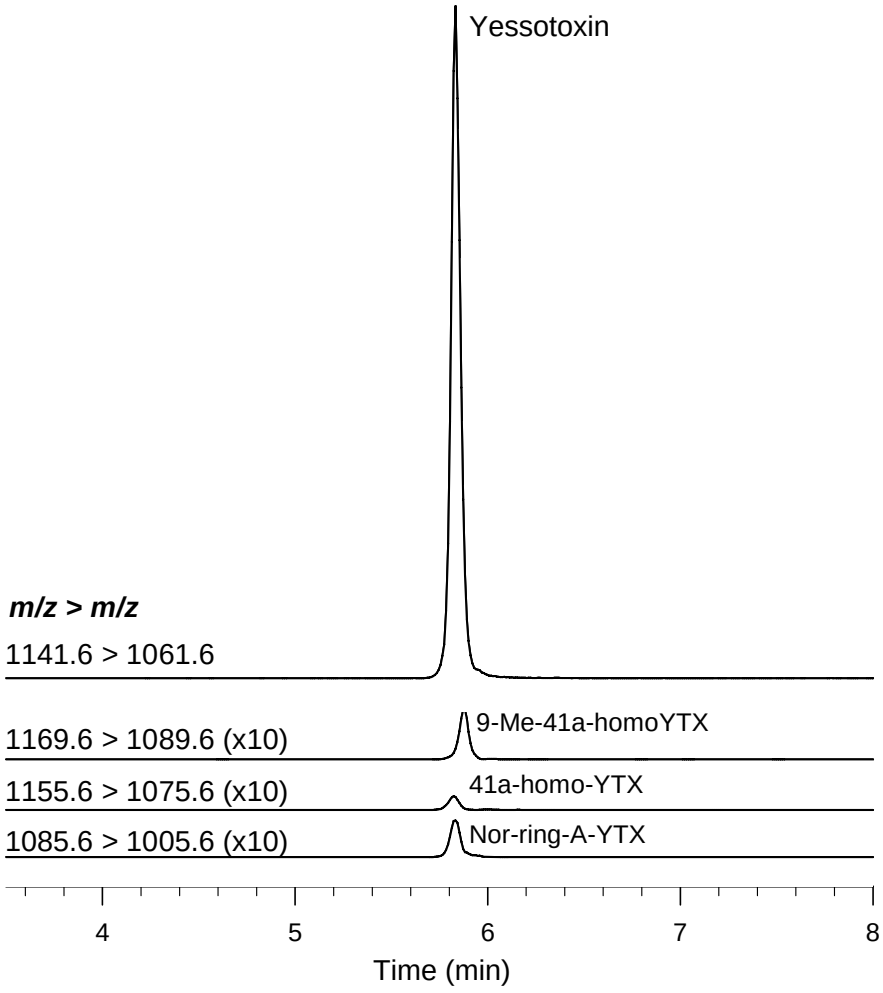

Figure 2: LC-MS analysis of CRM-YTX-c. Conditions: 2.5 µm Phenomenex Luna C18(2) column, 50 × 2.1 mm, maintained at +20°C; mobile phase: 50 mM formic acid and 2 mM ammonium formate in both A) water and B) 95% acetonitrile with gradient elution of 5 to 50% B in 1 min then 50 to 100% B in 4 min at 0.3 mL/min; 2 µL injection; MS operated in negative selected reaction monitoring mode.

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