



# Certificate of Analysis

Certified Reference Material

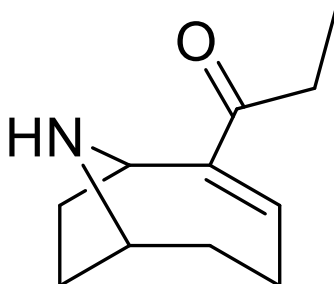
## CRM-hATX (Lot# 20230928)

Calibration Solution Certified Reference Material for Homoanatoxin-a

Homoanatoxin-a (hATX) is a cyanobacterial toxin linked to animal deaths worldwide [1, 2]. CRM-hATX is a certified reference material (CRM) calibration solution of hATX in methanol/water (9:91, v/v) with 0.01 % acetic acid, designed to aid in the identification and quantitation of hATX.

Table 1: Certified values and expanded uncertainties ( $k = 2$ ) for CRM-hATX

Compound	Mass fraction $\mu\text{g/g}$	Mass concentration $\mu\text{g/mL}$ (15-30 °C)	Concentration $\mu\text{mol/L}$ (15-30 °C)
homoanatoxin-a	$5.13 \pm 0.26$	$5.05 \pm 0.26$	$28.2 \pm 1.4$



### (+) homoanatoxin-a

CAS registry number: [142926-86-1](#)

InChIKey: [VVMQRZZXKNDPOT-PSASIEDQSA-N](#)

Molecular formula:  $\text{C}_{11}\text{H}_{17}\text{NO}$

Molar mass: 179.26 g/mol

$[\text{M}+\text{H}]^+$ :  $m/z$  180.1383

Period of validity: 1 year from date of sale

Storage conditions:  $-12$  °C or below

## Intended use

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

## Preparation of material

hATX was isolated from a culture of the cyanobacterium *Oscillatoria formosa* strain NIVA-CYA 92 [3]. The toxin was extracted from the culture medium, purified by open column and semi-preparative chromatography, and then dried *in vacuo*.

The structure and purity of hATX were confirmed by LC with high-resolution MS (LC–HRMS, Figures 1 and 2) [4,5], charged aerosol detection (LC–CAD), chemiluminescence nitrogen detection (LC–CLND) [6], LC–UV and 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. A measured accurate  $m/z$  of 180.1383 ( $\Delta = 0.07$  ppm for  $C_{11}H_{18}NO^+$ ) was obtained for the  $[M+H]^+$  ion of hATX using LC–HRMS.

A stock solution was prepared by dissolving the purified hATX in  $D_2O$  for quantitation using  $^1H$  NMR (qNMR) [7]. The CRM-hATX solution was prepared by accurately diluting the stock solution in degassed methanol/water (9:91, v/v) with 0.01 % acetic acid. Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL.

## Characterization of material

The certified value for CRM-hATX (Table 1) is based on results obtained at the National Research Council Canada (NRC) using qNMR [7]. A potassium hydrogen phthalate certified reference material (NIST SRM 84L) was used for calibration. Supporting values were obtained by LC–HRMS using an in-house reference material for hATX (NRC RM-hATX) and LC–UV using CRM-ATX (Lot# 20100721) [8].

Low levels of dihydrohomoanatoxin-a (*cis*- $H_2$ hATX and *trans*- $H_2$ hATX) and 10-hydroxy-homoanatoxin-a (10-OH-hATX) [4] at  $m/z$  182.1539, as well as several oxidation products of hATX ( $m/z$  196.1332) are present in CRM-hATX (Figure 2). The total contribution of structurally related impurities is estimated at 1.5 % by LC–HRMS, assuming similar molar responses to hATX.

## Metrological traceability

Results presented in this certificate are traceable to the International System of Units (SI) through gravimetrically prepared standards of a potassium hydrogen phthalate CRM (NIST SRM 84L).

## Homogeneity

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

## Stability

Studies on CRM-hATX have demonstrated stability at temperatures up to 23 °C for one year, including at the recommended storage temperature of –12 °C.

## Uncertainty

The expanded uncertainty ( $U$ ) for all values is equal to  $U = ku_c$ , where  $u_c$  is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [9] and  $k$  is the coverage factor. A coverage factor of  $k = 2$  was applied which corresponds to a level of confidence of approximately 95 %.

All reasonable sources of uncertainty related to the certified values in Table 1 were considered. Included in the combined uncertainty estimate are uncertainties in the batch characterization, uncertainties related to possible between-unit variation, and uncertainties related to stability.

## Storage

The material shall be stored at  $-12\text{ }^{\circ}\text{C}$  or below. The ampoule should be stored unopened.

## Instructions for handling and use

Prior to opening, each ampoule should be allowed to equilibrate to room temperature and the contents thoroughly mixed. The ampoule should be opened at the pre-scored mark immediately prior to use. The CRM solution should be transferred using calibrated equipment for accuracy. The certified value is only guaranteed if the ampoule is sampled immediately after opening in order to limit solvent evaporation. It is important to note that the volume of the solution is not certified; only the mass fraction and concentration are certified. Therefore, the entire contents of the ampoule should not be diluted to volume.

Repeated sub-sampling and storage of the CRM solution after initial opening may impact certified values. Users shall take responsibility for demonstrating that their sub-sampling and storage procedures do not impact certified values.

## Health and safety information

Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at [doi.org/10.4224/crm.2024.hatx.20230928](https://doi.org/10.4224/crm.2024.hatx.20230928). For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

## Period of validity

The certified values are valid for 1 year from the date of sale, provided the storage and instructions for handling and use specified in this certificate are followed.

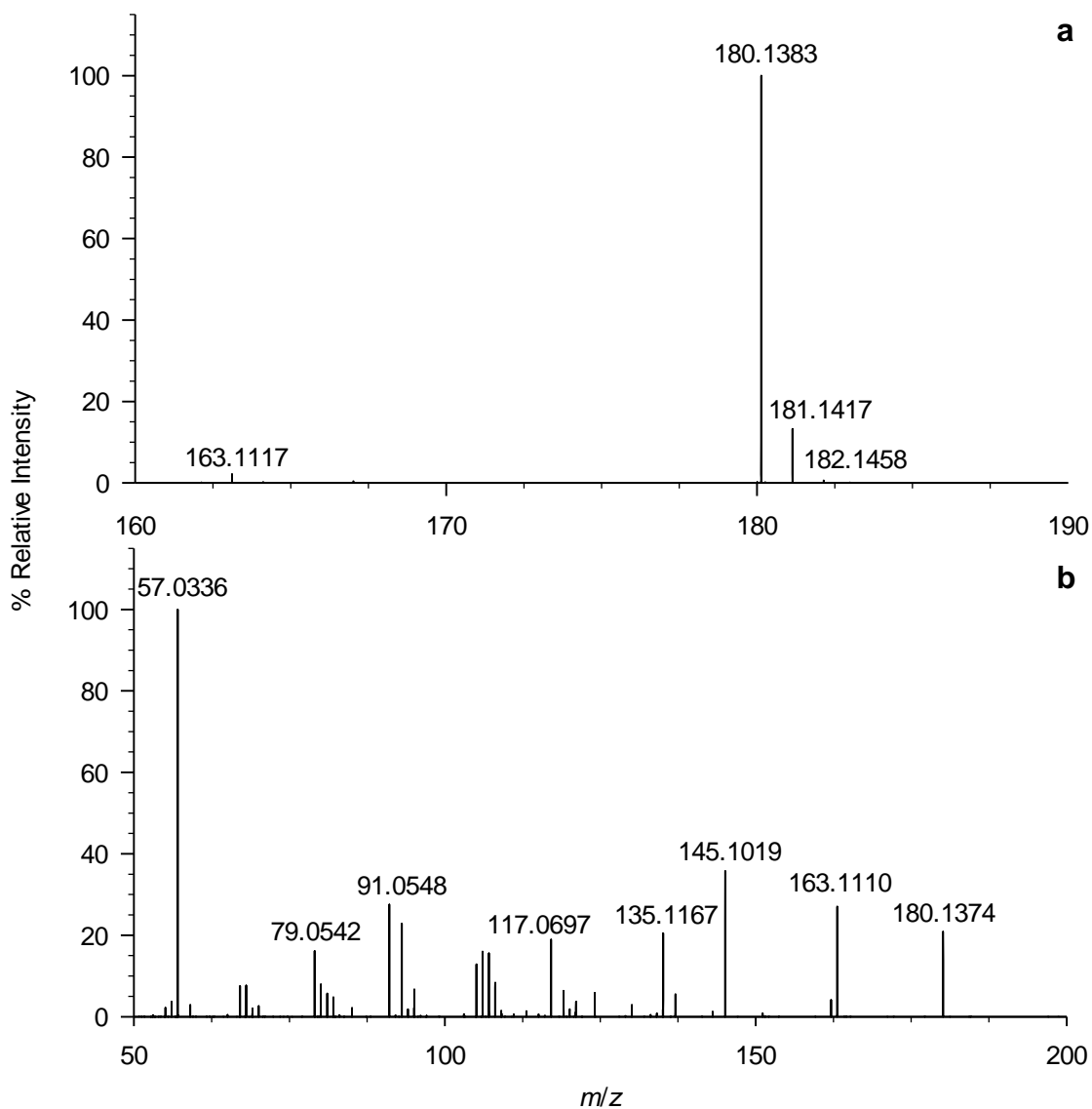
## Quality Management System

The NRC is Canada's national metrology institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated

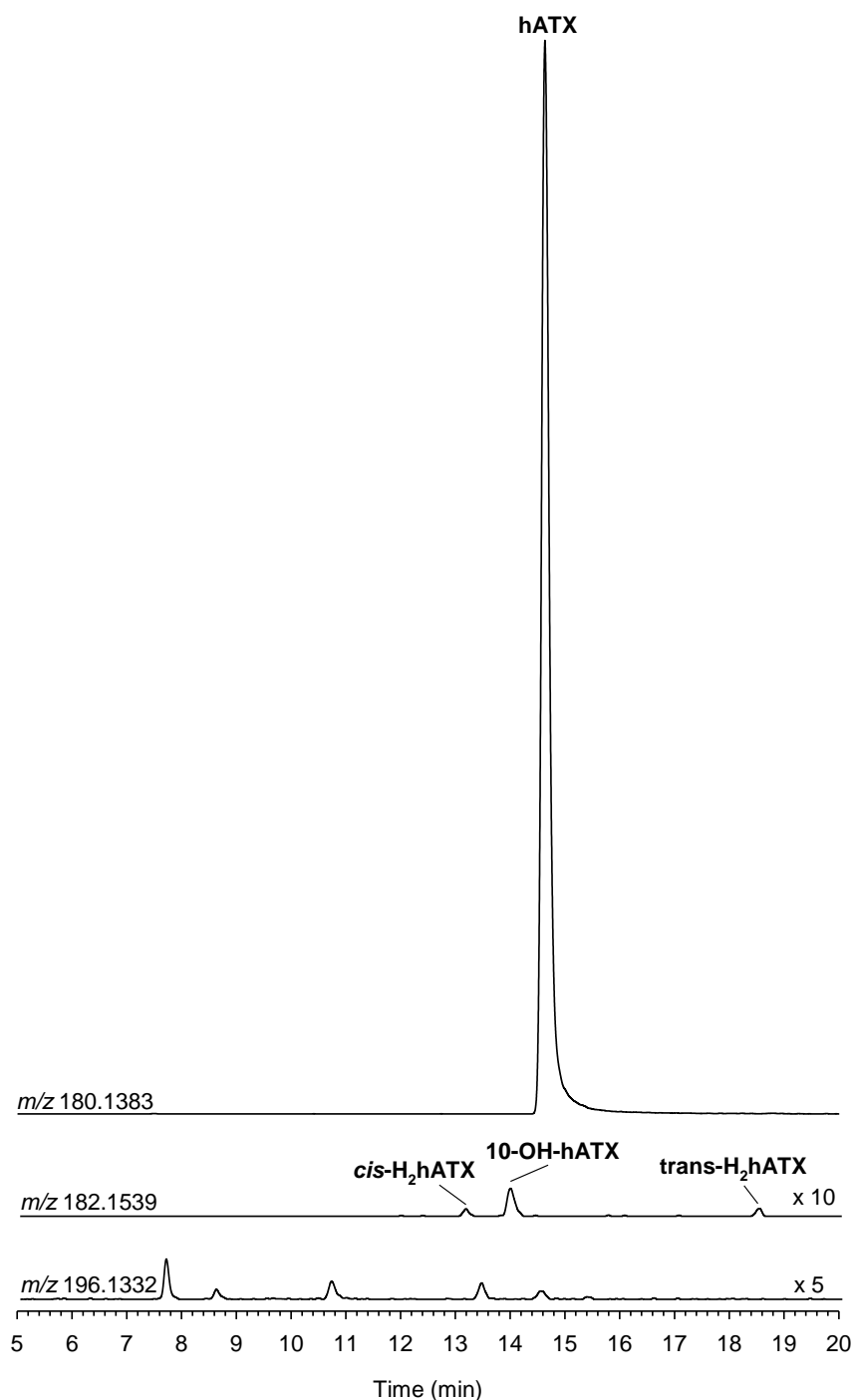
for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

## References

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**Figure 1:** Full scan (a) and collision induced dissociation (MS/MS) (b) spectra from LC-HRMS analysis of hATX used for the preparation of CRM-hATX acquired using a Thermo QExactive-HF mass spectrometer in positive mode. All data was acquired with a resolution setting of 120 000. MS/MS data was acquired in parallel reaction monitoring scan mode using a normalized collision energy of 35 eV.



**Figure 2:** LC–HRMS analysis of CRM-hATX using an Agilent 1200 LC coupled to a QExactive–HF mass spectrometer. LC conditions: Waters Acquity 1.8  $\mu$ m HSS T3 (2.1 mm  $\times$  150 mm) at 40  $^{\circ}$ C; mobile phase: 0.1 % formic acid in both water (A) and acetonitrile (B); 0.2 mL/min gradient elution from 2 % to 11 % B at 25 min, at 25.1 min B increased to 95 % and held to 30 min, followed by 10 min re-equilibration with 2 % B; injection volume 0.2  $\mu$ L. Extracted-ion chromatograms ( $\pm$  5 ppm) showing hATX, 10-OH-hATX, *cis*-H<sub>2</sub>hATX, *trans*-H<sub>2</sub>hATX, and oxidation products of hATX at *m/z* 196.1332.

## Authorship

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## Approved by:

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This certificate is only valid if the corresponding material was obtained directly from the NRC or an authorized reseller. Users should ensure that the certificate they have is current. For updates, please refer to [doi.org/10.4224/crm.2024.hatx.20230928](https://doi.org/10.4224/crm.2024.hatx.20230928).

## Comments, information, and inquiries should be addressed to:

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The logo for Canada, featuring the word "Canada" in a serif font with a small Canadian flag icon above the letter "a".