

Certificate of Analysis

Certified Reference Material

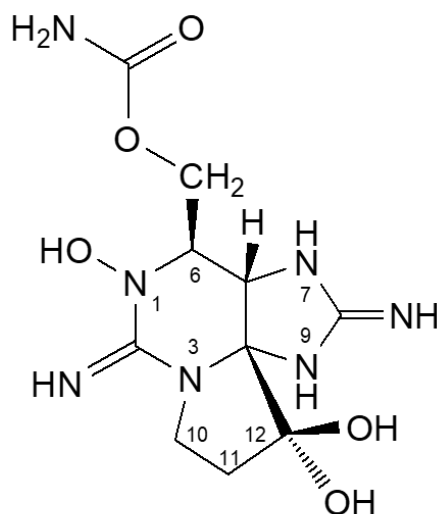
CRM-NEO-e (Lot# 20251218)

Calibration Solution Certified Reference Material for Neosaxitoxin

Neosaxitoxin (NEO) is a saxitoxin analogue found in both marine microalgae [1] and freshwater cyanobacteria [2]. CRM-NEO-e is a certified reference material (CRM) calibration solution of NEO in aqueous 0.5 mM hydrochloric acid (HCl).

Table 1: Certified value and expanded uncertainty ($k = 2$) for CRM-NEO-e

Compound	Concentration $\mu\text{mol/L}$ (15-30 °C)
NEO	59.6 ± 1.5



neosaxitoxin

CAS registry number: [64296-20-4](#)

InChIKey: [PPEKGEBBBBNZKS-HGRQIUPRSA-N](#)

Molecular formula: $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_5$

Molar mass: 315.3 g/mol

$[\text{M}+\text{H}]^+$: m/z 316.1364

Period of validity: 1 year from date of sale

Storage conditions: +4 °C

Table 2: Information value for CRM-NEO-e

Compound	Concentration $\mu\text{mol/L}$ (15-30 °C)
STX	0.06

Intended use

CRM-NEO-e is a certified calibration solution designed for analytical method development and accurate quantitation of NEO. The concentration is suitable for preparing a dilution series for calibration of instrumentation, such as liquid chromatography with detection by post-column oxidation–fluorescence (LC–ox–FLD) or liquid chromatography–mass spectrometry (LC–MS).

Preparation of material

Neosaxitoxin was extracted from cultured *Alexandrium tamarense* cell biomass, purified by open column and preparative scale chromatography, and then dried *in vacuo* [3].

The structure and purity of NEO were confirmed using nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography with low [4] and high-resolution MS (Figures 1 and 2), LC–ox–FLD [5] (Figure 3) and charged aerosol detection [6]. A measured accurate m/z of 316.1363 ($\Delta = 0.3$ ppm for $\text{C}_{10}\text{H}_{18}\text{N}_7\text{O}_5^+$) was obtained for the $[\text{M}+\text{H}]^+$ ion of NEO using LC–HRMS.

A stock solution was prepared by dissolving the purified NEO in aqueous 0.5 mM HCl for quantitation using ^1H NMR (qNMR) [7]. The CRM-NEO-e solution was prepared by accurately diluting the stock solution in degassed 0.5 mM HCl at pH 3.4. 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-NEO-e (Table 1) is based on results obtained at the National Research Council of Canada (NRC) by ^1H qNMR using potassium hydrogen phthalate for calibration and LC–HRMS using NRC CRM-NEO-d for calibration. An information value (Table 2) for saxitoxin (STX) was assigned by LC–MS.

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 (Inmetro MRC 8943.0001) and a neosaxitoxin CRM (NRC CRM-NEO-d, Lot# 20170411).

Homogeneity

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

Stability

Studies on NEO in 0.5 mM HCl have demonstrated stability at the recommended storage temperature of +4 °C and also in a freezer (-12 °C or below). No detectable decomposition was observed at room temperature for one month.

Uncertainty

The expanded uncertainty (U) for all values is equal to $U = k u_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [8] 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 +4 °C. 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 concentration is 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 [10.4224/crm.2026.neo-e.20251218](https://www.nrc.gov/reading-rm/doc-collections/sds/sds-10.4224/crm.2026.neo-e.20251218). For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified value is 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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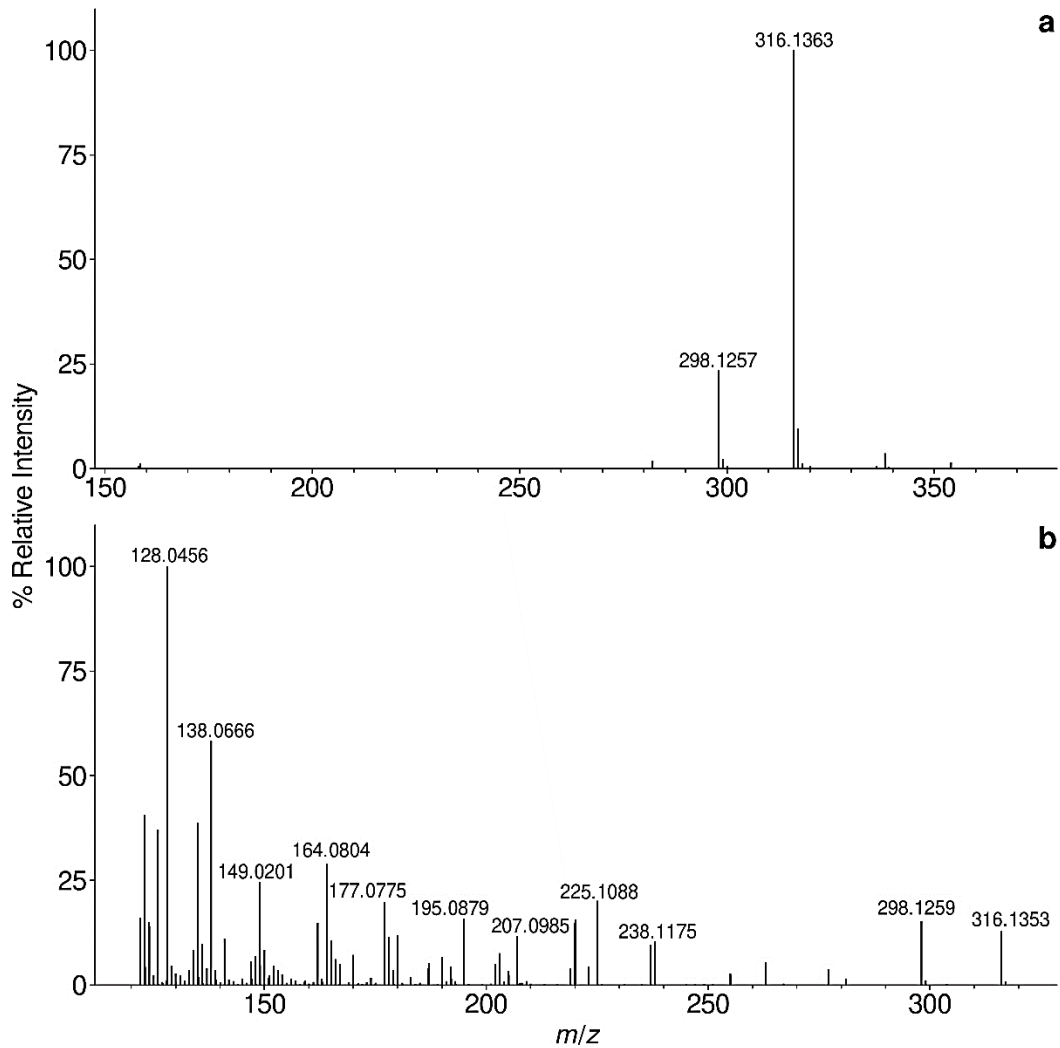


Figure 1: Full-scan (a) and collision-induced dissociation (MS/MS) (b) spectra from LC–HRMS analysis of CRM-NEO-e acquired using a Thermo QExactive-HF mass spectrometer in positive mode. All data was acquired with a resolution setting of 60 000. MS/MS data was acquired in parallel reaction monitoring scan mode using a normalized collision energy of 60 eV.

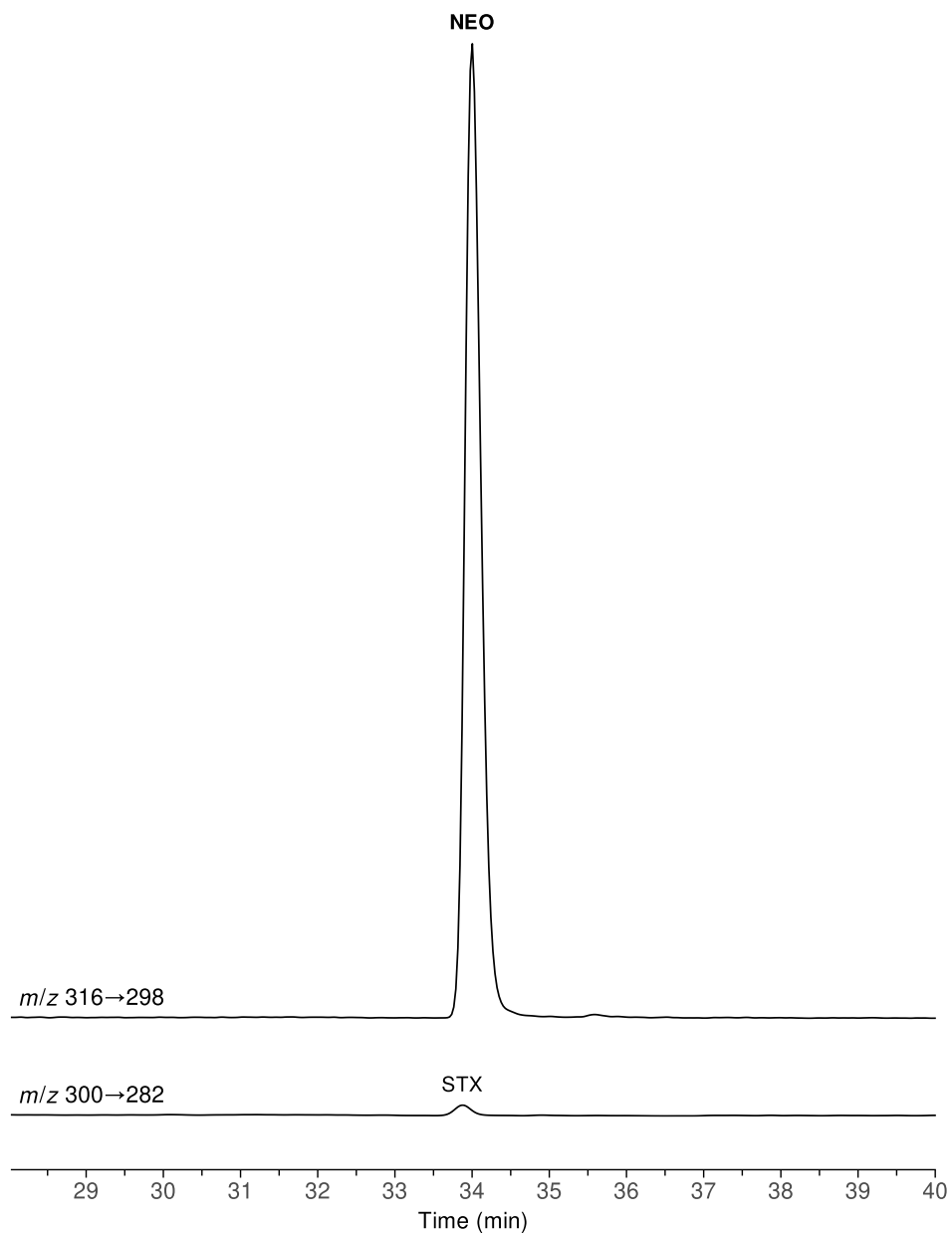


Figure 2: LC–MS/MS analysis of CRM-NEO-e using selected reaction monitoring. LC conditions: TOSOH 5 μ m TSKgel Amide-80 (2.1 \times 250 mm) at 40 $^{\circ}$ C; mobile phase: (A) water with 2 mM ammonium formate and 50 mM formic acid, and (B) 100 % acetonitrile; gradient: 10 % to 45 % A over 25 min, to 70 % A at 27 min, hold to 40 min; 0.2 mL/min; injection volume: 1 μ L.

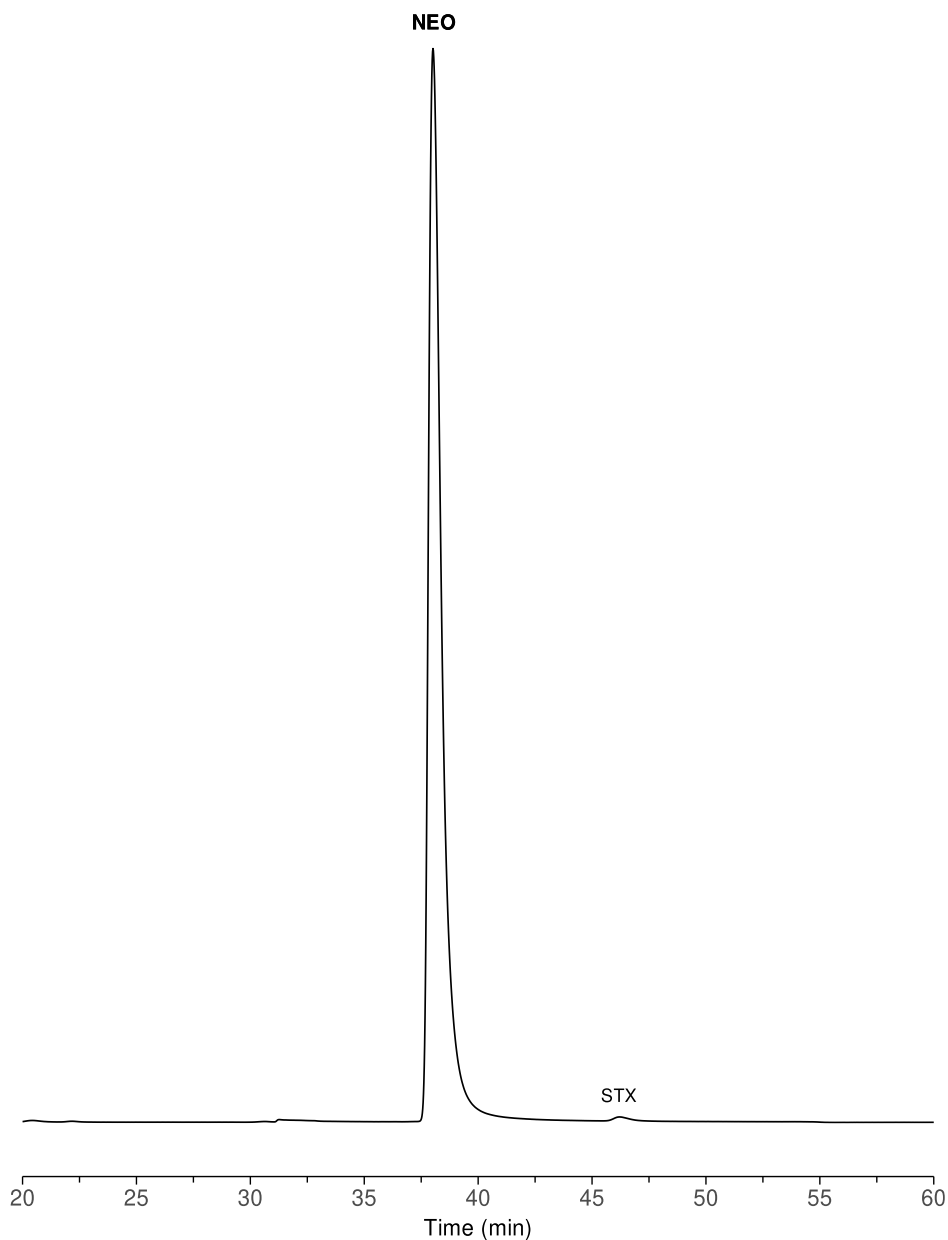


Figure 3: LC-ox-FLD analysis of CRM-NEO-e. Conditions: Agilent 5 μ m Zorbax Bonus-RP, (4.6 \times 250 mm) at 35 $^{\circ}$ C; mobile phase: (A) water with 11 mM heptane sulfonate, 5.5 mM phosphoric acid (pH 7.1), and (B) water with 11 mM heptane sulfonate, 16.5 mM phosphoric acid (pH 7.1) and 9 % acetonitrile; 100 % A, step to 100 % B at 25 min; 0.8 mL/min; injection volume: 25 μ L; post-column oxidation using 5 mM periodic acid in 100 mM sodium phosphate at pH 7.8; 0.4 mL/min; reaction coil at 80 $^{\circ}$ C; effluent acidified with 0.75 M nitric acid; 0.4 mL/min; fluorescence detection with excitation at 330 nm and emission at 390 nm.

Authorship

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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 [10.4224/crm.2026.neo-e.20251218](https://doi.org/10.4224/crm.2026.neo-e.20251218).

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