

Certificate of Analysis

NRC-CMRC

Certified Reference Material

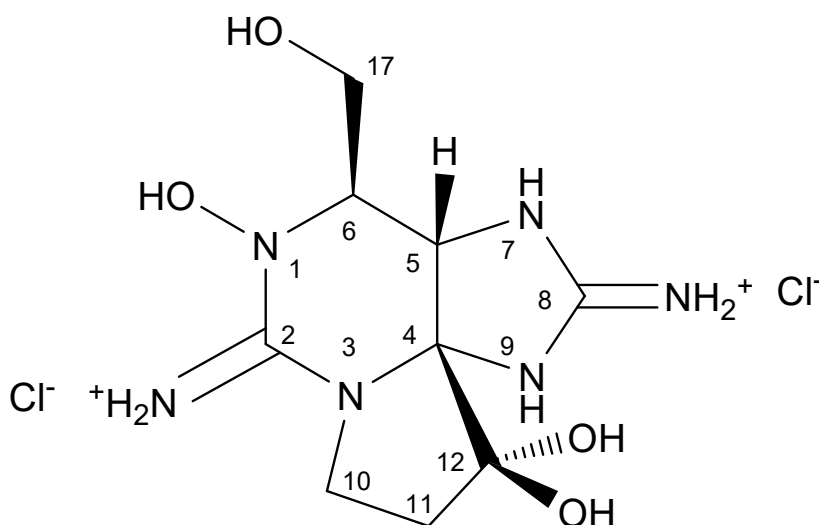
CRM-dcNEO-d (Lot# 20170413)

Certified Calibration Solution for Decarbamoylneosaxitoxin

Decarbamoylneosaxitoxin (dcNEO) is one of the toxins responsible for incidents of paralytic shellfish poisoning (PSP) [1]. CRM-dcNEO-d is a certified instrument calibration solution prepared to aid the analyst in the determination of dcNEO. Each ampoule contains a solution of dcNEO dissolved in filtered, aqueous 0.5 mM hydrochloric acid (HCl). This is a replacement calibration solution for CRM-dcNEO-c.

Table 1: Certified concentration value for CRM-dcNEO-d.

Compound	$\mu\text{mol/L}$ (at +25°C)
dcNEO	30.4 ± 1.7



Decarbamoylneosaxitoxin dihydrochloride

CAS registry no.: 68683-58-9 (free base)

Molecular formula: C₉H₁₈N₆O₄Cl₂

Molecular weight: 345.2 g/mol

Period of validity: 1 year from date of sale

Storage conditions: -12 °C or below



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Intended Use

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

Instructions for Storage and Use

To ensure the stability of CRM-dcNEO-d, ampoules should be stored in a freezer (-12 °C or below). Prior to opening, each ampoule should be allowed to warm to room temperature and the contents mixed thoroughly. The ampoule 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 solvent will occur if the solution is left opened for more than a few minutes. It is recommended that the CRM should not be evaporated to dryness because of the potential of 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-dcNEO-d

NEO was isolated from cultured *Alexandrium tamarense* using preparative scale chromatography [2]. Purified NEO was chemically converted to dcNEO, which was then purified by preparative scale chromatography, dried *in vacuo* and dissolved in filtered, aqueous 0.5 mM HCl to give a stock solution. The purity of the toxin was checked by 500 MHz proton nuclear magnetic resonance (NMR) spectroscopy, LC-ox-FLD [3] (Figure 1), LC-MS/MS [4] (Figures 2 and 3), capillary electrophoresis with UV detection (CE-UVD) [5], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [6].

The CRM-dcNEO-d solution was prepared in filtered (0.2 µm) and degassed 0.5 mM HCl at pH 3.4 and dispensed into amber ampoules pre-filled with argon, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Analytical Methods and Value Assignment

Certified values for CRM-dcNEO-d (Table 1) are based on results obtained at NRC using two analytical methods: quantitative ¹H-NMR (qNMR) with accurate solutions of potassium hydrogen phthalate (NIST SRM 84I) as the calibrant [7], and LC-CLND using accurate solutions of caffeine (NMIA M724c) as the calibrant.

Neosaxitoxin (NEO) and decarbamoylsaxitoxin (dcSTX) are present in CRM-dcNEO-d. Information values were assigned by LC-ox-FLD with calibration using NRC CRMs for those analogues (Table 2).

Table 2: Information values for other PSP toxins present in CRM-dcNEO-d at the time of packaging.

Compound	[M+H] ⁺ , m/z	Concentration (µmol/L)*
Neosaxitoxin (NEO)	316.1	0.66*
Decarbamoylsaxitoxin (dcSTX)	257.1	0.25*

* These concentrations are not certified.



Homogeneity

A representative number of CRM-dcNEO-d ampoules were selected from across the fill series and dcNEO concentrations measured by LC-ox-FLD. The data was assessed using ANOVA.

Stability

Studies have demonstrated good long-term stability of dcNEO solutions stored in sealed ampoules at -20 °C, with no detectable decomposition observed over a period of 12 months. It has been determined that dcNEO exhibits good stability at +4 °C, with no detectable decomposition observed for periods of one month within the limits of uncertainty of the analytical method (LC-MS, ± 3.8% RSD for replicate injections of a single solution; n = 11).

Uncertainty

All reasonable sources of error related to the characterization of CRM-dcNEO-d 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}), homogeneity (u_{hom}) and instability during storage (u_{stab}) [8-11]. 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 values of CRM-dcNEO-d.

Uncertainties	[µmol/L]
u_{char}	0.80
u_{hom}	0.16
u_{stab}	0.14

Safety Instructions

If sufficient quantities are ingested, PSP toxins can cause paralysis and even death. 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-dcNEO-d.

Period of Validity

If stored unopened at the recommended storage condition of -12 °C or below, the certified concentration of CRM-dcNEO-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 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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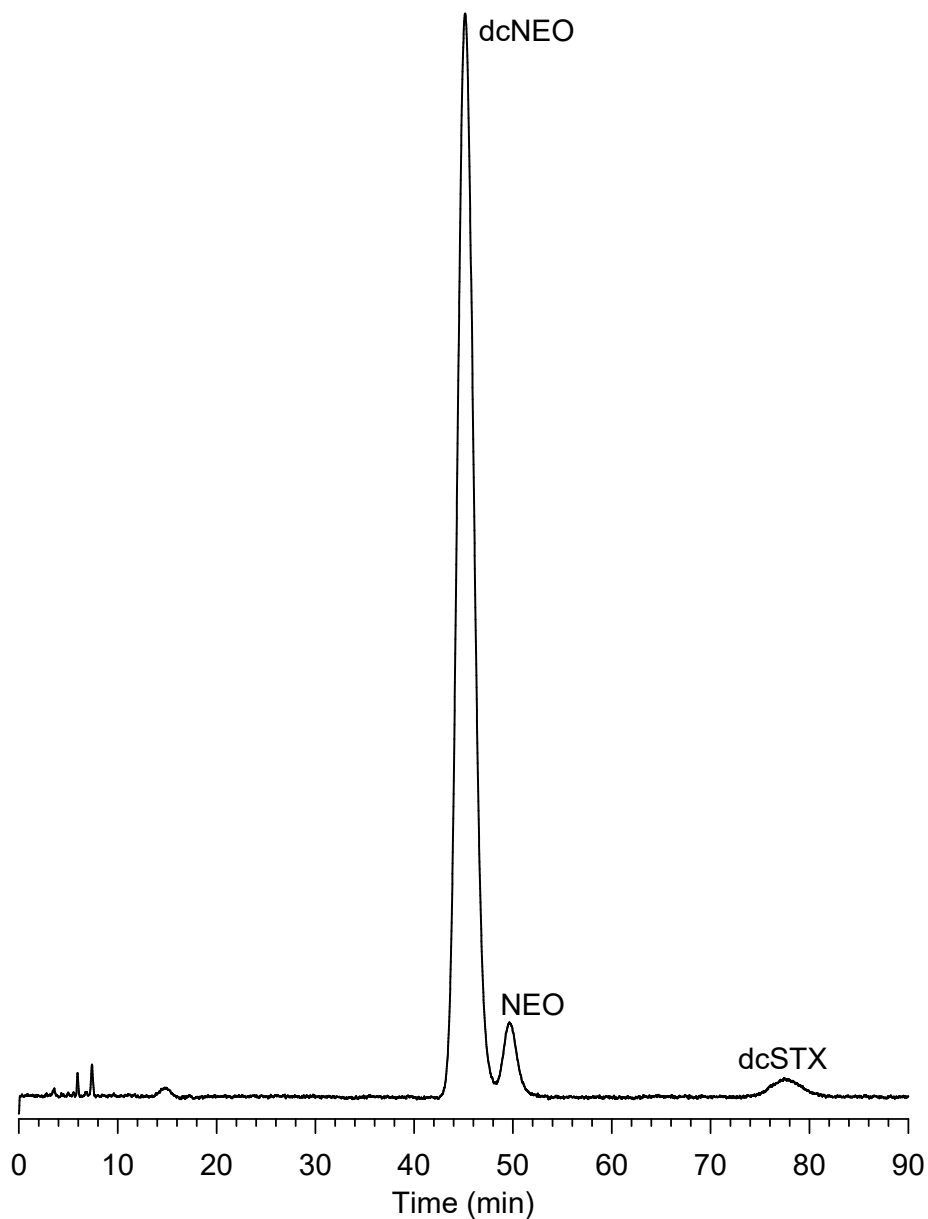


Figure 1: LC-ox-FLD analysis of CRM-dcNEO-d. Retention times of related toxins are indicated. Conditions: Zorbax Bonus-RP column (250 × 4.6 mm i.d., 5 µm) at +10 °C; 1.0 mL/min of 18.9 mM ammonium phosphate with 7.6 mM sodium heptane sulphonate, pH 7.1 with 2% acetonitrile; 25 µL injection; post column oxidation using 0.4 mL/min 5 mM periodic acid in 100 mM sodium phosphate at pH 7.8; reaction coil at +80 °C; effluent acidified with 0.4 mL/min 0.75 M nitric acid; fluorescence detection with excitation at 330 nm and emission at 390 nm.

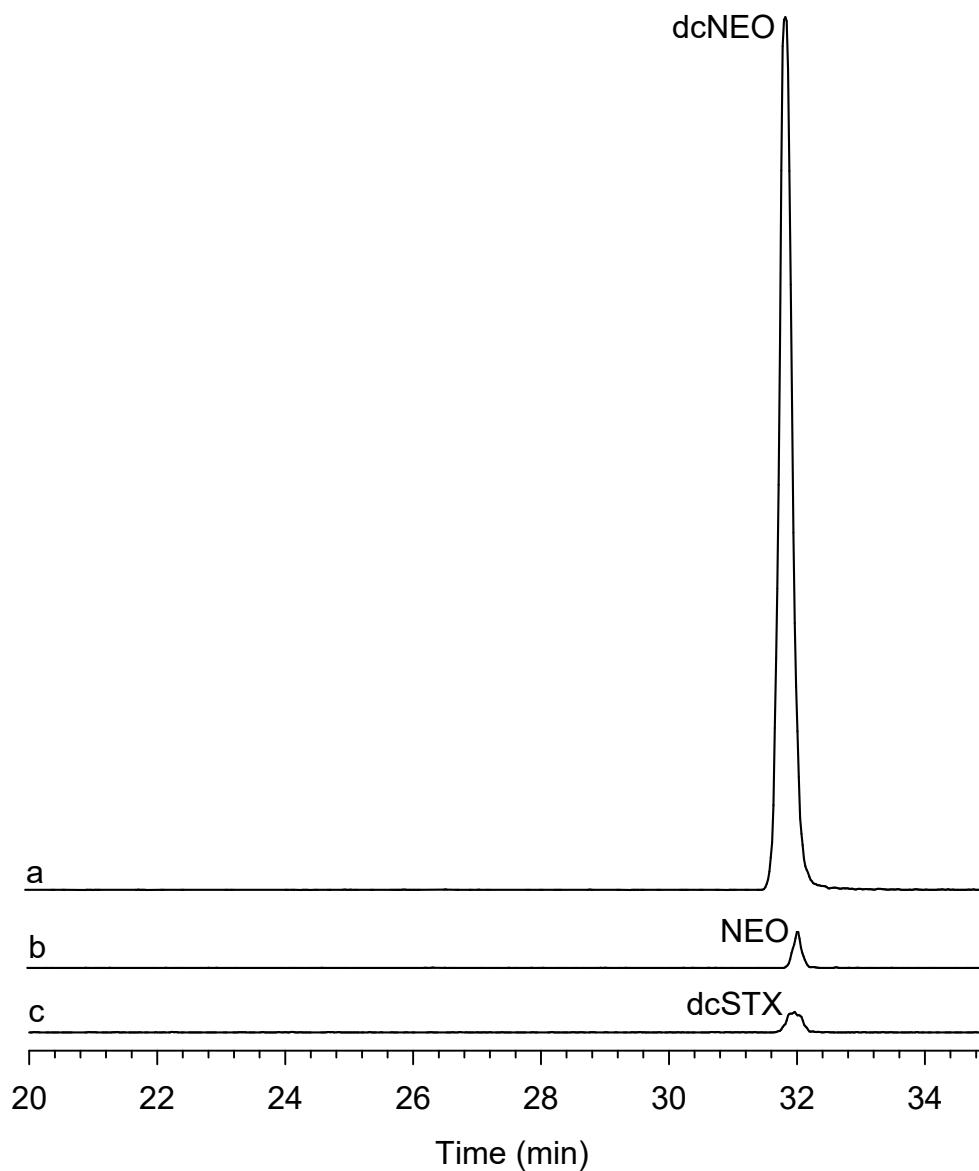


Figure 2: LC-MS/MS analysis of CRM-dcNEO-d using selected reaction monitoring: (a) summed signals for m/z 273 > 190 and m/z 273 > 225; (b) summed signals for m/z 316 > 177 and m/z 316 > 220; and (c) summed signals for m/z 257 > 180 and m/z 257 > 239. Conditions: Agilent 1200 LC and Sciex API4000 QTRAP MS; 2.5 μ L injection; 5 μ m TOSOH Amide-80 column (250 mm \times 2 mm i.d.) at +40 $^{\circ}$ C; 0.2 mL/min gradient elution from 10 to 45% A over 25 min, hold to 40 min. Mobile phase A: water with 2 mM ammonium formate and 50 mM formic acid; mobile phase B: acetonitrile.

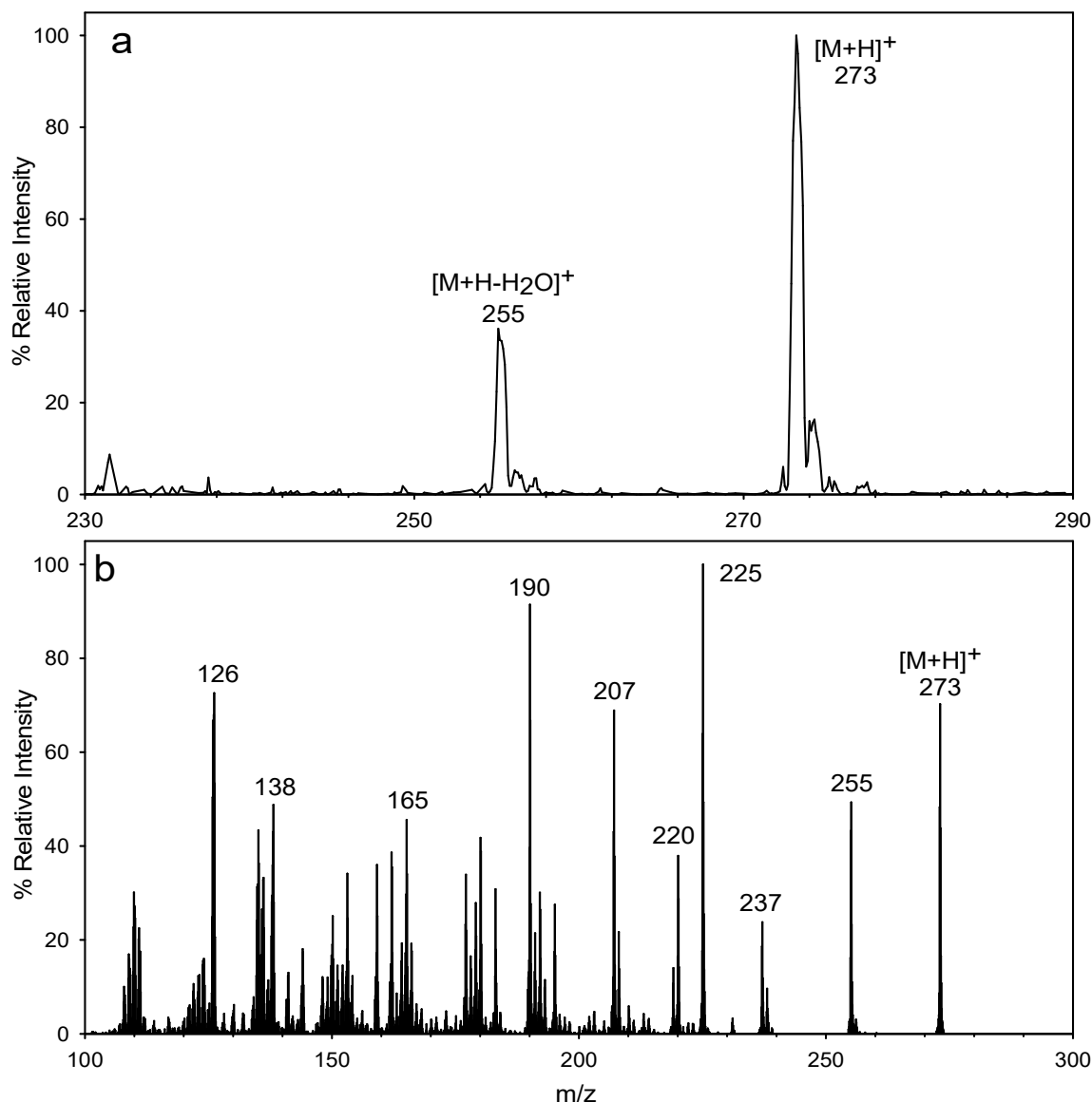


Figure 3: Mass spectra of dcNEO: (a) full Q1 scan positive ion ESI mass spectrum; and (b) product ion spectrum of $[M+H]^+$ ion at m/z 273 with a collision energy of 35 V. Conditions: Agilent 1200 LC and Sciex QTRAP 4000 MS; declustering potential = 70 V; injection volume = 5 μ L; column = TOSOH Amide-80, 250 mm \times 2 mm i.d. at +40 $^{\circ}$ C; flow = 0.2 mL/min; isocratic elution with 45% A and 55% B, where A is water, B is 95% acetonitrile, both with 2 mM ammonium formate and 3.6 mM formic acid.

Acknowledgements

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This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our qualified vendors.

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