Certificate of Analysis

NRC·CNRC Certified Reference Material

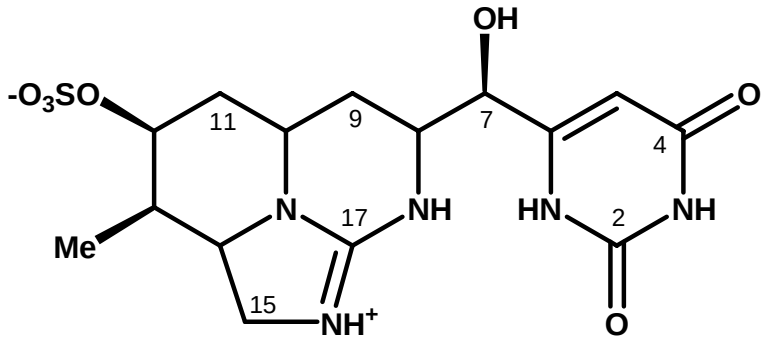
CRM-CYN (Lot# 20050531)

Certified Calibration Solution for Cylindrospermopsin

Cylindrospermopsin (CYN) is a cyanobacterial toxin associated with poisoning incidents [1]. CRM-CYN is a certified instrument calibration solution prepared to aid the analyst in the determination of CYN. Each ampoule contains approximately 0.5 mL of a solution of CYN dissolved in filtered, deionized water at a concentration suitable for calibration of liquid chromatography experiments and for spiking control samples in recovery experiments.

Table 1: Certified concentration values for CRM-CYN.

Compound	µmol/L (at +20 °C)	µg/mL (at +20 °C)	µg/g
CYN	30 ± 2	12.6 ± 0.8	12.7 ± 0.8



Cylindrospermopsin
CAS registry no: 143545-90-8
Molecular formula: $C_{15}H_{21}N_5O_7S$
Molecular weight: 415.42 g/mol
$[M+H]^+$: *m/z* 416.12345

Expiry date: 1 year from date of sale.
Storage conditions: +4 °C

Intended Use

CRM-CYN is a calibration solution CRM designed for analytical method development and accurate quantitation of CYN. The concentration of CYN in this CRM is suitable for preparing a dilution series for calibration of instrumentation, such as liquid chromatography with detection using ultraviolet absorbance (LC-UV) or mass spectrometry (LC-MS).

Preparation of the CRM-CYN

The starting material was a large-scale laboratory culture of the algae *Cylindrospermopsis raciborskii* [2,3]. The CYN was extracted, purified by preparative scale chromatography [4], dried under vacuum, and dissolved in deionized water to give a stock solution. The CRM-CYN solution was prepared in filtered (0.2 µm) and degassed deionized water and dispensed into amber ampoules pre-filled with argon, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Structural Confirmation and Purity Assessment

The molecular structure of CYN was confirmed by NMR spectroscopy and tandem mass spectrometry. The NMR and product ion mass spectra are shown in Figures 1 and 2, respectively.

The purity of CRM-CYN was checked using the following techniques: 500 MHz proton NMR spectroscopy, LC-UV (Figure 3), LC-MS [5] (Figure 2), capillary electrophoresis with UV detection (CE-UVD) [6], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [7].

Homogeneity

As this CRM is a true solution, it is expected to be homogeneous. To confirm this, the concentration of CYN in randomly selected ampoules representing 1.2% of those produced was measured by LC-UV. The between-ampoule variation was measured to be no greater than the variation for replicate analyses of one solution, which demonstrates acceptable homogeneity over the entire ampoule range.

Stability Study

Stability studies have demonstrated excellent long-term stability of CYN solutions stored at +4 °C. Solutions are also stable when stored in a reliable, non-defrosting freezer (preferably ≤ -20 °C). It has further been determined that CRM-CYN solutions exhibit good stability at room temperature, with no detectable decomposition observed after twelve months.

Certified value

The certified value for CRM-CYN, 30 ± 2 µmol/L (at +20 °C) (Table 1), is based on results obtained at the NRC using two independent analytical methods: LC-CLND and quantitative NMR spectroscopy [8]. Calibrations of both LC-CLND and qNMR were performed using accurate solutions of USP-grade caffeine.

The results shown in this certificate are traceable to the SI standard through gravimetrically prepared standards of established purity. This product serves as a suitable reference material for laboratory quality assurance programs.

UV Molar Absorptivity Coefficient

Several different values for the molar absorptivity coefficient of CYN have been reported in the literature. Using CRM-CYN, the following absorbance data were measured:

Table 2: Molar absorptivity coefficient value for CYN.

λmax (nm)*	ε (M^{-1} cm^{-1})*
262	9800 ± 300

* Measurements made with water as solvent at pH 7 and +23 °C. Please note that different pHs can result in different values.

Uncertainty

The overall uncertainty estimate (U_{CRM}) for CRM-CYN includes uncertainties associated with batch characterization (u_{char}), between-bottle variation (u_{hom}), and instability associated with long-term storage (u_{stab}) [9,10]. These components can be combined as:

$$U_{CRM} = k\sqrt{\mu_{char}^2 + \mu_{hom}^2 + \mu_{stab}^2}$$

where *k* is the coverage factor (= 2).

All of these sources of uncertainty were considered for the estimate of the final uncertainty in the certified concentration of CRM-CYN [11,12]. Tests of homogeneity within and between ampoules were performed (see section Homogeneity) and were deemed to be negligible. Stability studies which extend past the specified shelf life of CRM-CYN have been completed and no significant loss of material was observed. CYN exhibits reasonable stability at room temperature (see Section Stability Study), in the event that a delay occurs during shipping. It follows that the relative contribution to uncertainty due to stability during storage (u_{stab}) was determined to be negligible.

All reasonable sources of error related to the characterization of CRM-CYN were considered and quantified. The certified value for the concentration of CYN is based on quantitative measurements by qNMR and LC-CLND, which together contribute a relative uncertainty of 0.030 (calculated from the standard uncertainty of the average of method means and the uncertainty of the calibrations). The relative uncertainty associated with the dilution of the stock to the final CRM solution is a negligible due to the use of gravimetric procedures. Applying a coverage factor of 2 resulted in a final relative standard uncertainty for the certified concentration of CYN in CRM-CYN of 0.061.

Storage Instructions

To ensure the long-term stability of CRM-CYN, the unopened ampoule should be stored in the dark in a refrigerator at approximately +4 °C. The toxin has been found to be stable under these conditions. Solutions are also stable when stored in a reliable, non-defrosting freezer (preferably ≤ -20 °C). The CRM has been prepared under conditions that minimize the chance of bacterial contamination, but it is recommended that aliquots and dilutions of the CRM be stored frozen (≤ -20 °C).

Expiry

If stored unopened at the recommended storage conditions (Section Storage Instructions), the certified concentration of the CRM is valid for 1 year from the date of sale**.**

Instructions for Use

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents should be thoroughly mixed. The ampoule should be inverted several times, then held upright, tapped to ensure that the solution drains to the bottom, and opened at the pre-scored mark. Once an ampoule has been opened, accurate aliquots should be taken 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 open for more than a few minutes. It is recommended that the CRM not be evaporated to dryness due to potential for losses on glass surfaces. A useful procedure that ensures accurate dilutions involves using a balance to determine weights of the dispensed aliquot and the final diluted solution, assuming that water is used as diluent. *Please Note*: The volume of the solution is not certified. Only the concentration is certified.

Safety Instructions

CYN irreversibly inhibits protein synthesis and may also be genotoxic [4,13]. 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 for protection should the glass shatter. A material safety data sheet (MSDS) is available for CRM-CYN.

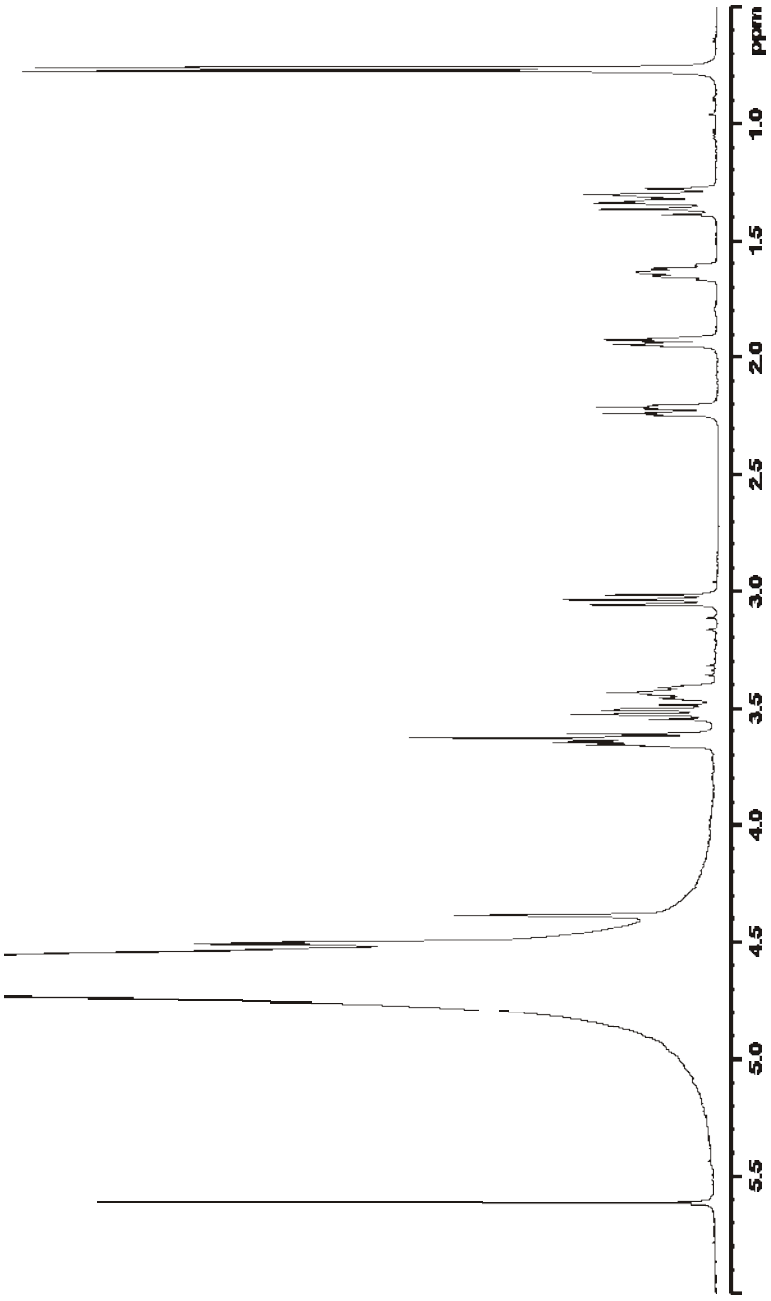


Figure 1: Proton NMR spectrum (500 MHz) of the stock solution of CYN (in H_2O) used for preparation of CRM-CYN.

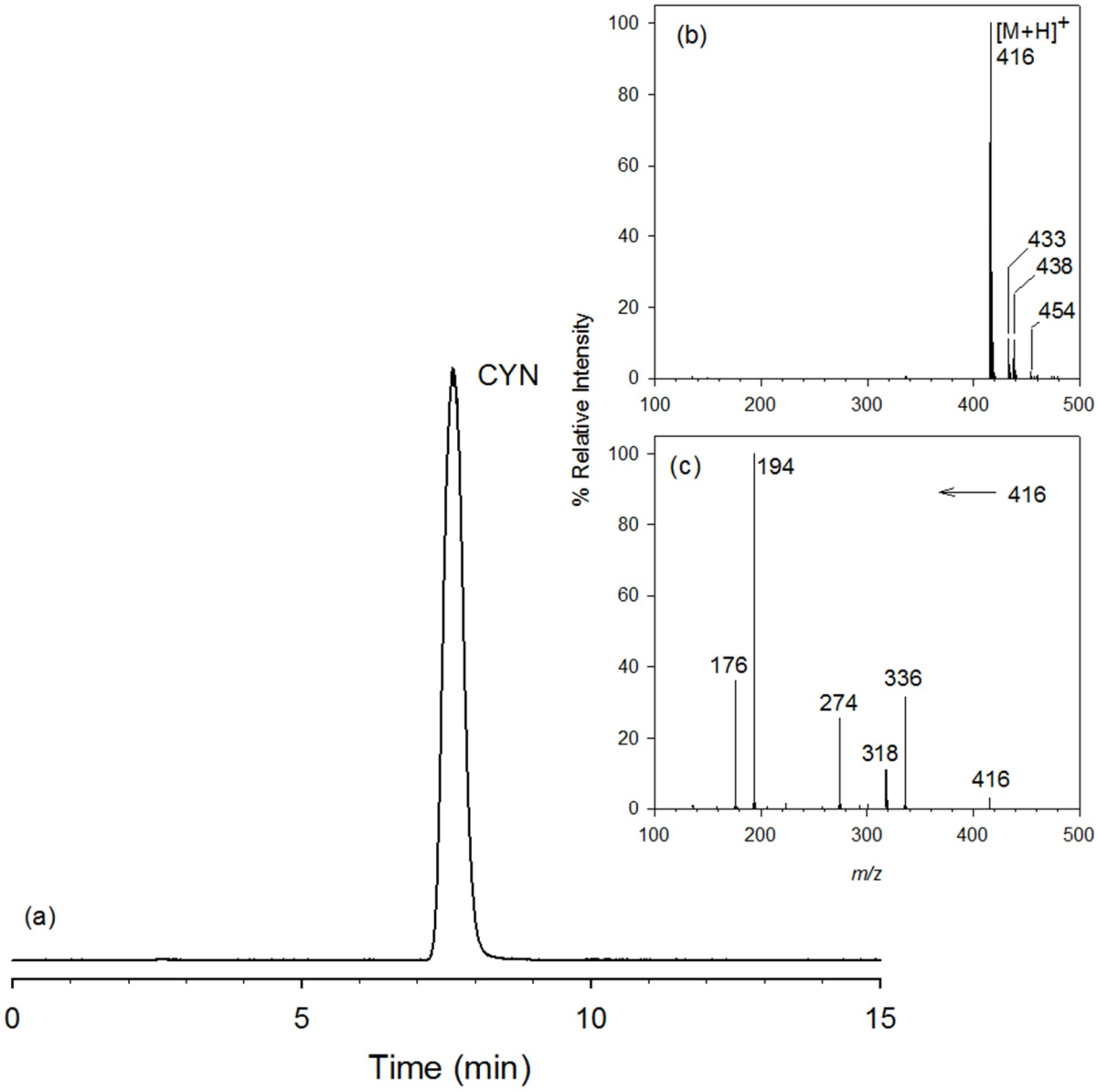


Figure 2: Analysis of the CRM-CYN by LC-MS. Conditions: column = TosoHaas TSKgel Amide-80, 2.1 × 250 mm at +30 °C; mobile phase = 71% CH_3CN/H_2O, 3.6 mM formic acid, 2 mM ammonium formate (isocratic); flow rate = 0.2 mL/min; 10 µL injected. (a) API-165 single quadrupole mass spectrometer, selected ion monitoring (*m/z* 416.4) with positive electrospray ionization, dwell = 150 msec; (b) electrospray mass spectrum; (c) product ion spectrum of *m/z* 416.

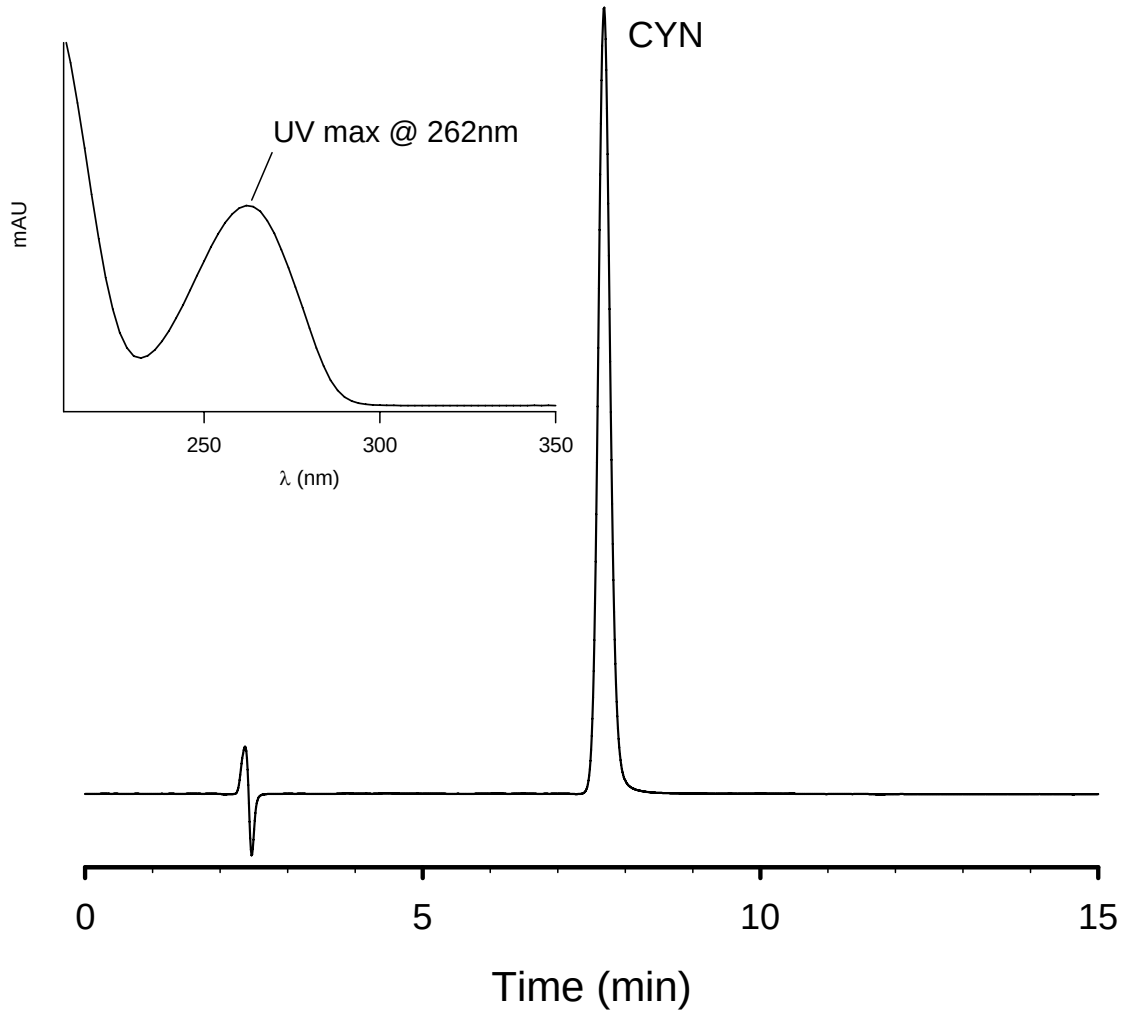


Figure 3: Analysis of CRM-CYN by LC-UV at 262 nm. Conditions: column = Zorbax SB-C8, 2.1 × 150 mm, held at +30 °C; mobile phase = 5% CH_3OH/H_2O with 2 mM heptanesulfonic acid (isocratic); flow rate = 0.2 mL/min; 10 µL injected.

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Acknowledgements

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Signed **:** 

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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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