

National Institute for Environmental Studies
Certificate of Analysis
NIES CRM No. 35 Water Bloom (¹⁵N-CYN)

This environmental certified reference material (CRM) was developed and certified by the National Institute for Environmental Studies (NIES) for use in controlling and improving the accuracy of chemical analysis of cylindrospermopsin (CYN), a toxic substance in cyanobacteria that forms water bloom. The certified value is provided for CYN labeled with ¹⁵N, and the extract solution of this CRM can be used as an internal standard solution for cylindrospermopsin analysis.

Certified Value

Compound	Mass fraction		Analytical method *	
	Unit	Certified value		Uncertainty
¹⁵ N-CYN	mg/g	0.607	0.193	ID-LC-MS, ID-LC-MS/MS

The certified value was determined on an “as received” basis, without drying the material.

The uncertainty attached to the certified value is the expanded uncertainty using a coverage factor $k = 2$, corresponding to the half-width of a confidence interval of approximately 95 %.

Certified reference material of CYN was added to the samples, the mixtures were extracted with solvent (such as 1 % acetic acid) and ¹⁵N-CYN was then analysed by isotope dilution mass spectrometry.

* ID-LC-MS, isotope dilution-liquid chromatography-mass spectrometry

ID-LC-MS/MS, isotope dilution-liquid chromatography-tandem mass spectrometry

Characterization

The certified value of the material was determined statistically based on chemical analyses by 5 organizations (7 laboratories) using isotope dilution mass spectrometry. The certified value satisfied the following conditions:

- 1) the relative standard deviation associated with the mean of the laboratory means was 20 % or less,
- 2) the number of laboratories contributing to the mean of the laboratory means was at least seven, and
- 3) the number of analytical methods contributing to the mean of the laboratory means was at least three.

The uncertainty attached to the certified value is the expanded uncertainty using a coverage factor $k = 2$, corresponding to the half-width of a confidence interval of approximately 95 %. The certified value was determined on an “as received” basis, that is, the value was determined without drying the material.

Description of the Material

The CRM is supplied as fine green powder in a glass centrifuge tube.

Preparation of the CRM

The starting material for this CRM was a mixture of two strains of the cyanobacterium *Raphidiopsis raciborskii*. The strains were separately cultured at the algae culture management facility of NIES and were then freeze-dried. The mixed dry alga (ca. 3 g), powdered by sieving through a 63- μ m screen, was packed into glass centrifuge tubes (130 tubes) with individual sample sizes of 10 mg. All procedures complied with ISO Guide 34.

Homogeneity

Homogeneity tests were carried out on 10 sample tubes selected by stratified random sampling. After addition of cylindrospermopsin, ^{15}N -cylindrospermopsin was extracted with solvent and measured by ID-LC-MS. The CRM was confirmed to be homogeneous within the uncertainty of the certified value by the one-way analysis of variance (ANOVA).

Instructions for Use

1. This CRM should be stored in a freezer at ≤ -20 °C.
2. This CRM contains toxic substances. Precautions must therefore be taken to avoid inhalation of, and skin and eye contact with, the sample powder.
3. Precautions must be taken to avoid contamination of the immediate environment when taking a sample.
4. The whole quantity in a tube (about 10 mg) should be used for analysis.
5. Do not use for purposes other than research. When disposing of samples, adhere strictly to local laws concerning processing and disposal of waste materials.

Expiry Date of Certification

The expiry date for the certified value of this CRM is November 2026, assuming that above mentioned storage conditions are adhered to. NIES will announce via its website if any changes in the contents are noticed within the term of validity.

Collaborating Laboratories in Analysis

The certified value for this CRM was based on analytical values from the following participating organizations: National Institute for Environmental Studies; National Institute of Health Sciences; Chemicals Evaluation and Research Institute, Japan; Japan Food Research Laboratories; Fukuoka Institute of Health and Environmental Sciences

Technical Information

Technical information and the latest reports regarding this material can be obtained from the website.

<http://www.nies.go.jp/labo/crm-e/index.html>

November 1, 2021
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Original certificate date: November 1, 2021

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Appendix

This CRM contains ^{15}N -deoxycylindrospermopsin (^{15}N -dCYN) in addition to ^{15}N -CYN.

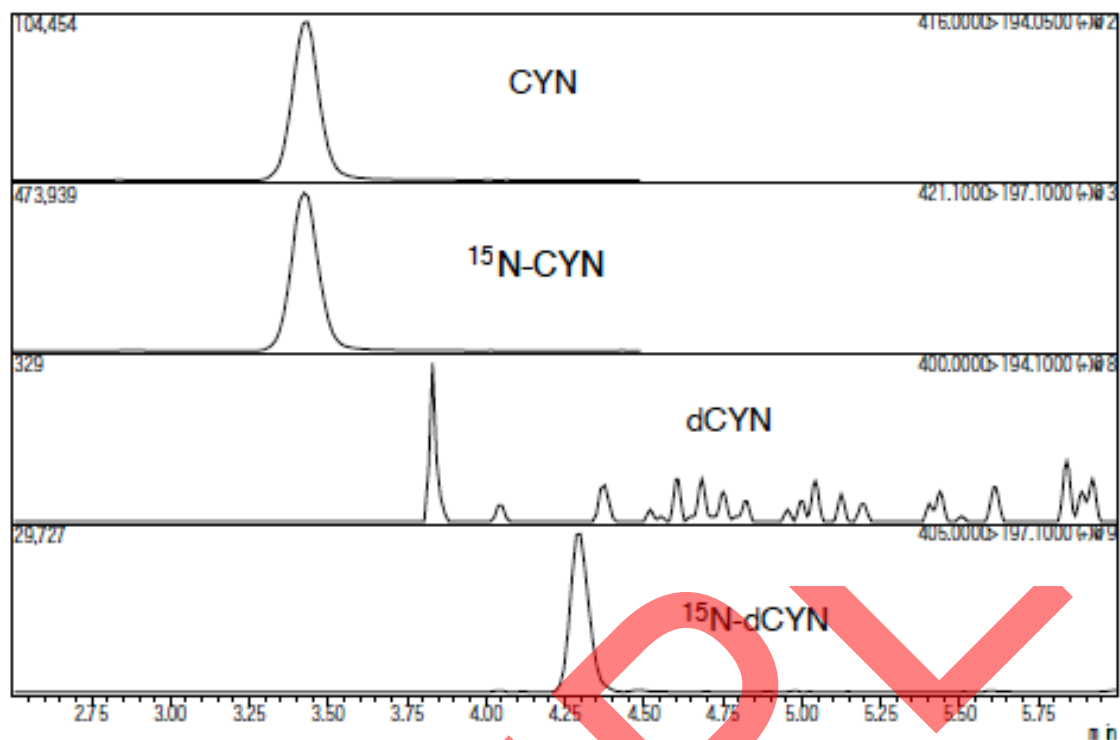


Fig. LC-MS/MS chromatograms after addition of CYN (Column: Shiseido Capcell Core ADME 2.1 mm \times 100 mm, 2.6 μm . LC-MS/MS: Shimadzu LCMS-8040 with Nexera)