National Institute for Environmental Studies, Japan

Date

No.

National Institute for Environmental Studies

Certificate of Analysis

NIES CRM No. 34 Water Bloom (^{15}N-MC)

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 microcystin (MC), a toxic substance in cyanobacteria that forms water bloom. The certified values are provided for MCs labeled with ^{15}N, and the extract solution of this CRM can be used as an internal standard solution for microcystin analysis.

Certified Values

Compound	Mass fraction		Analytical method *
	Unit	Certified value ± Uncertainty	
^{15}N-MC-RR	mg/g	1.63 ± 0.23	ID-LC-MS, ID-LC-MS/MS
^{15}N-MC-LR	mg/g	0.542 ± 0.046	ID-LC-MS, ID-LC-MS/MS

All certified values were determined on an "as received" basis, without drying the material.

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

Certified reference materials of MC-RR, LR and 7dmLR were added to the samples, the mixtures were extracted with solvent (such as 50 % MeOH), and ^{15}N-MCs were 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

Reference Value

Compound	Mass fraction		Analytical method *
	Unit	Reference value	
^{15}N-MC-dmLR	mg/g	0.077	ID-LC-MS, ID-LC-MS/MS

Reference value was determined on an "as received" basis, without drying the material.

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

Characterization

The property values of the material were determined statistically based on chemical analyses by 5 organizations (7 laboratories) using isotope dilution mass spectrometry. A property value satisfying the following conditions was

accepted as a certified value:

1) the relative standard deviation associated with the mean of the laboratory means was 15 % 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 values is the expanded uncertainty using a coverage factor $k = 2$, corresponding to the half-width of a confidence interval of approximately 95 %. A property value failing to satisfy one or two of the NIES criteria for certification but supplying valuable additional information about the material is given as a reference value. All certified and reference values were determined on an "as received" basis, that is, the values were 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 three strains of the cyanobacterium *Microcystis aeruginosa*. The strains were separately cultured at the algae culture management facility of NIES and then freeze-dried. The mixed dry alga (ca. 3 g), powdered by sieving through a 63-μm 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 MC-RR, LR and 7dmLR, ^{15}N-microcystins were 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 values 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 values and reference value for this CRM were 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

Appendix

This CRM contains ^{15}N-MC-FR, WR, LA, LBu, LY, LW and LF in addition to the three ^{15}N-microcystins, i.e. MC-RR, LR and dmLR.

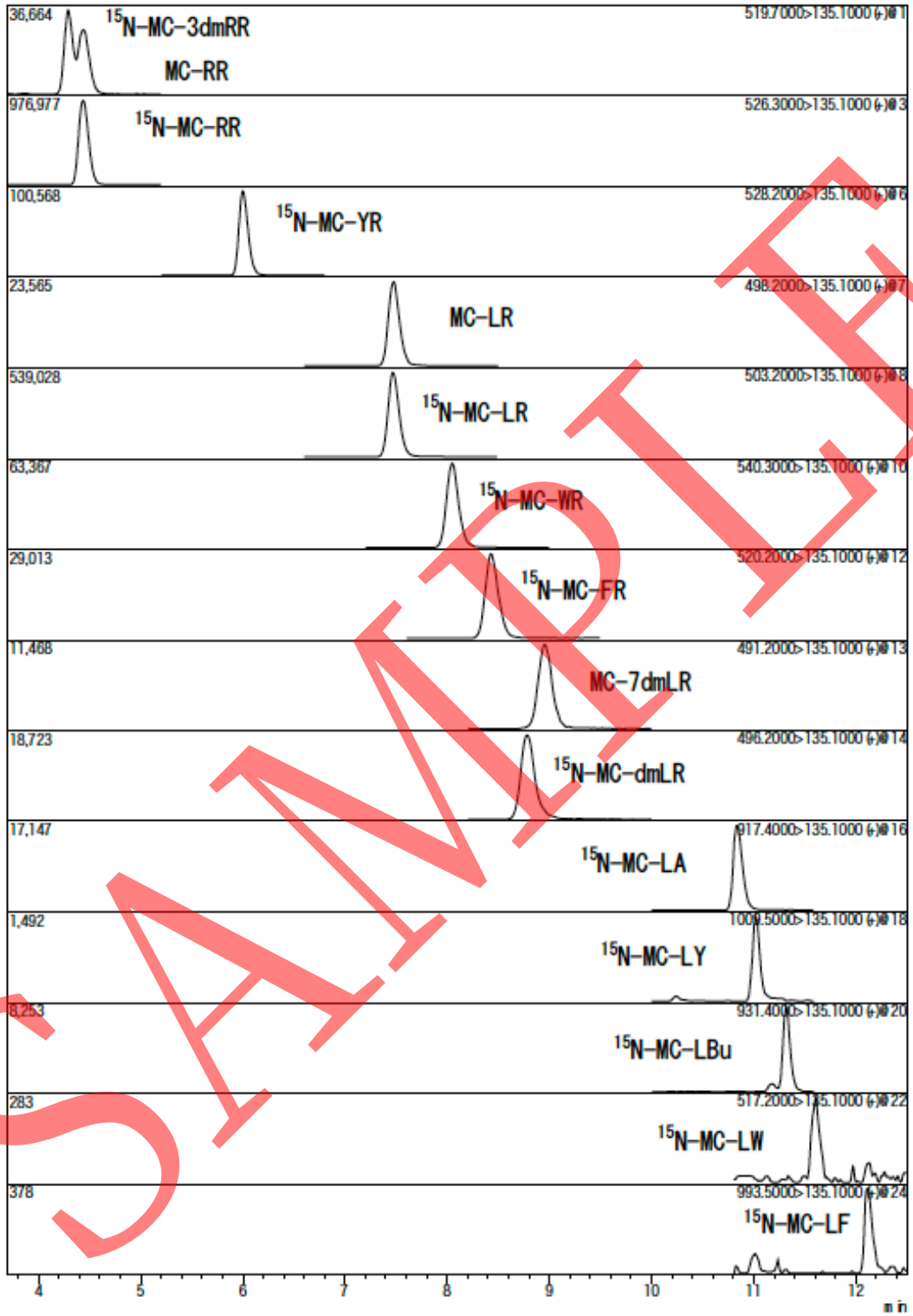

Fig. LC-MS/MS chromatograms after addition of MC-RR, LR and 7dmLR (Column: Ascentis Express C18 2.1 mm × 100 mm, 2.6 µm. LC-MS/MS: Shimadzu LCMS-8040 with Nexera)