National Institute for Environmental Studies, Japan

Date

No.

National Institute for Environmental Studies

Certificate of Analysis

NIES CRM No. 26 Water Bloom

This environmental certified reference material (CRM) was developed for use in calibrating and managing the precision of analytical equipment used for the chemical analysis of microcystins contained in blue-green algae, and for the analysis of the elemental composition of the algae. The target chemical substances are the microcystins and the constituent inorganic elements found in *Microcystis aeruginosa*, the main cyanobacterium involved in the formation of blue-green algal blooms in freshwater.

Certified Values

Compound	Mass fraction			Analytical method
	Unit	Certified value	Uncertainty	
Microcystins	mg/g	4.5	0.4	a, b

The microcystins were determined in accordance with the manual for examination of substances requiring investigation by the Ministry of Environment, Japan (March 2003). This method followed those in reports for the determination of total microcystins[1-3]. As described in the manual, microcystins were oxidatively decomposed to MMPB (3-methoxy-2-methyl-4-phenyl-butylic acid), which was determined using HPLC-mass spectrometry (a), or gas chromatography-mass spectrometry after esterification (b).

Reference

1) T. Sano, K. Nohara, F. Shiraishi, and K. Kaya, (1992). Intern. J. Environ. Anal. Chem. 49, 163-170.

2) K. Kaya and T. Sano, (1999). Anal. Chim. Acta 386, 107-112.

3) H. Takagi, M. Shirai, T. Sano, and K. Kaya, (2004). J. Environ. Chem. 14, 587-596 (in Japanese).

Element	Mass fraction			Analytical method *
	Unit	Certified value	Uncertainty	
Calcium (Ca)	%	0.56	0.02	AAS, ICP-OES, INAA, XRF
Iron (Fe)	%	0.086	0.006	ICP-OES, ID-ICP-MS, INAA
Magnesium (Mg)	%	0.44	0.03	AAS, ICP-OES, XRF
Potassium (K)	%	0.90	0.05	AAS, ICP-OES, INAA, XRF
Sodium (Na)	%	0.12	0.02	AAS, ICP-OES, INAA
Manganese (Mn)	mg/kg	39	3	HR-ICP-MS, ICP-MS, ICP-OES, INAA

Strontium (Sr)	mg/kg	4.5	0.3	ICP-MS, ICP-OES, ID-ICP-MS
Zinc (Zn)	mg/kg	13	2	HR-ICP-MS, ICP-MS, ICP-OES, ID-ICP-MS, INAA

* AAS, atomic absorption spectroscopy
HR-ICP-MS, high resolution-inductively coupled plasma-mass spectrometry
ICP-MS, inductively coupled plasma-mass spectrometry
ICP-OES, inductively coupled plasma-optical emission spectrometry
ID-ICP-MS, isotope dilution-inductively coupled plasma-mass spectrometry
INAA, instrumental neutron activation analysis
XRF, X-ray fluorescence spectroscopy

Reference Values

Element	Mass fraction		Analytical method *
	Unit	Reference value	
Phosphorus (P)	%	0.89	ICP-OES, Molybdenum blue-FIA, XRF
Sulfur (S)	%	0.82	ICP-OES, XRF
Cobalt (Co)	mg/kg	0.75	ICP-MS, ICP-OES, INAA
Copper (Cu)	mg/kg	2.3	GFAAS, ICP-MS, ICP-OES, ID-ICP-MS, INAA, XRF
Nickel (Ni)	mg/kg	2.2	ICP-MS, ICP-OES, ID-ICP-MS
Lead (Pb)	mg/kg	4.3	ICP-MS, ICP-OES, ID-ICP-MS

* GFAAS, Graphite furnace atomic absorption spectrometry
ICP-MS, inductively coupled plasma-mass spectrometry
ICP-OES, inductively coupled plasma-optical emission spectrometry
ID-ICP-MS, isotope dilution-inductively coupled plasma-mass spectrometry
INAA, instrumental neutron activation analysis
Molybdenum blue-FIA, Molybdenum blue flow injection analysis
XRF, X-ray fluorescence spectroscopy

Preparation of the CRM

The starting material for this CRM was a mixture of two strains of the cyanobacterium *Microcystis aeruginosa* that were isolated from natural water blooms. The strains were separately cultured at the algae culture management facility within the National Institute for Environmental Studies (NIES), then freeze-dried. The mixed dry alga (ca. 40 g), powdered by sieving through a 63-μm screen, was packed into amber glass bottles (636 bottles) with individual sample sizes of 54 mg. After vacuum drying the bottles were filled with an inert gas (argon), capped, and sealed in aluminum packs containing desiccants.

Homogeneity

Homogeneity tests were carried out on 5 sample bottles selected at random. The major elements (Ca, Fe, K, Mg, Mn, and Na) in the samples were determined by plasma emission spectrometry after acid digestion. Microcystins were extracted with solvent and subjected to oxidative decomposition. The MMPB thereby formed was measured by HPLC-mass spectrometry[1-3]. Analysis of variance of the analytical results showed the relative standard deviation of all samples was within 4 %, and the variation between bottles was within the range of uncertainty for the certified values. It was adjudged that the material was sufficiently homogenous for its intended purpose.

Certified and Reference Values

Of the measured values reported by 14 organizations, those with *z*-scores of 2 or more, obtained by use of robust statistics, were rejected. Certified values for microcystins, Ca, Fe, K, Mg, Mn, Na, Sr, and Zn were decided in accordance with ISO Guide 35. 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 %. Reference values as additional information were given for S, P, Co, Cu, Ni, and Pb.

Precautions for Storage and Handling for Analysis

1. All certified and reference values refer to the vacuum-dried contents as supplied. Thorough attention must be paid to moisture because the properties of the samples preclude the possibility of drying them with heat.
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. At least 10 mg of sample should be used for analysis.
5. As this CRM absorbs moisture, it is desirable to use up the sample as soon as possible after opening. If storage is necessary, re-seal the bottle containing the remaining sample, and store it in a desiccator at ambient temperature. It is desirable that samples stored for prolonged periods be vacuum-dried before being used for analysis.
6. 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 August 2032, 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 values for this CRM were based on analytical values from the following participating organizations:

National Institute for Environmental Studies; Tohoku University Graduate School; Nara Prefectural Institute for Hygiene and Environment; Institute for Environmental Sciences; Tohoku Nuclear Services Co., Ltd.; Environmental Control Center Co., Ltd.; Environmental Research Center Co., Ltd.; Shimadzu Techno-Research, Inc.; Sumika Chemical Analysis Service, Ltd.; Chikyu Kagaku Kenkyusho Co., Ltd.; Naitoh Environmental Science Co., Ltd.; Japan Chemical Analysis Center; China National Research Center for Environmental Analysis and Measurements; China Institute of Atomic Energy.

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

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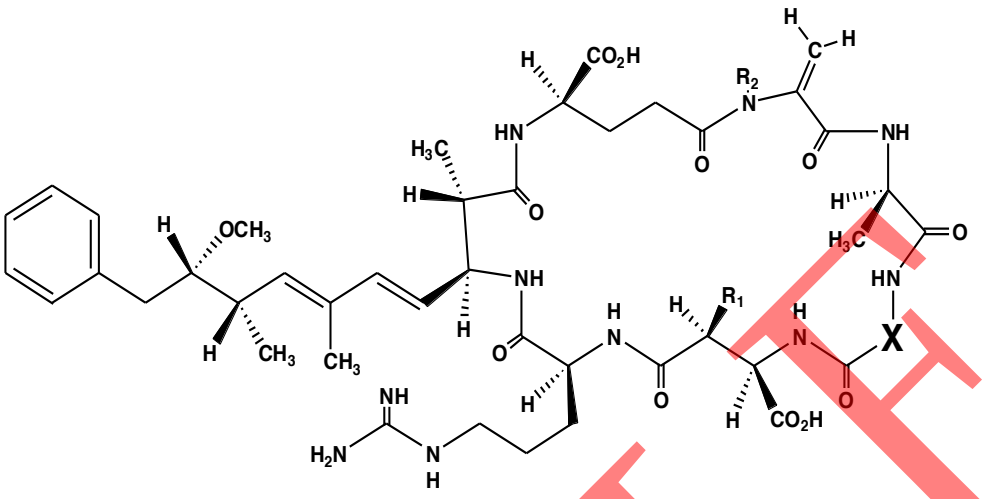
Original certificate date: July 1, 2007
Certificate revision date: July 25, 2012 (Update of expiry date)
Certificate revision date: April 1, 2021 (Editorial changes)
Certificate revision date: July 1, 2022 (Update of expiry date)

Appendix

Information that could be useful for handling this material is provided, though the values are non-certified.



MC1: [D-Asp³, Dha⁷]microcystin-RR: R1=R2=H, X=Arg
MC2: [Dha⁷]microcystin-RR: R1=Me, R2=H, X=Arg
MC3: [Dha⁷]microcystin-ThTyrR: R1=Me, R2=H, X=ThTyr
MC4: [Dha⁷]microcystin-YR: R1=Me, R2=H, X=Tyr
MC5: [Dha⁷]microcystin-LR: R1=Me, R2=H, X=Leu
MC6: [D-Asp³, Dha⁷]microcystin-LR: R1=R2=H, X=Leu
MC7: [Dha⁷]microcystin-HilR: R1=Me, R2=H, X=Hil
(cf. Microcystin-LR: R1=R2=Me, X=Leu)

Fig. A1 Structures of microcystins in NIES CRM No. 26

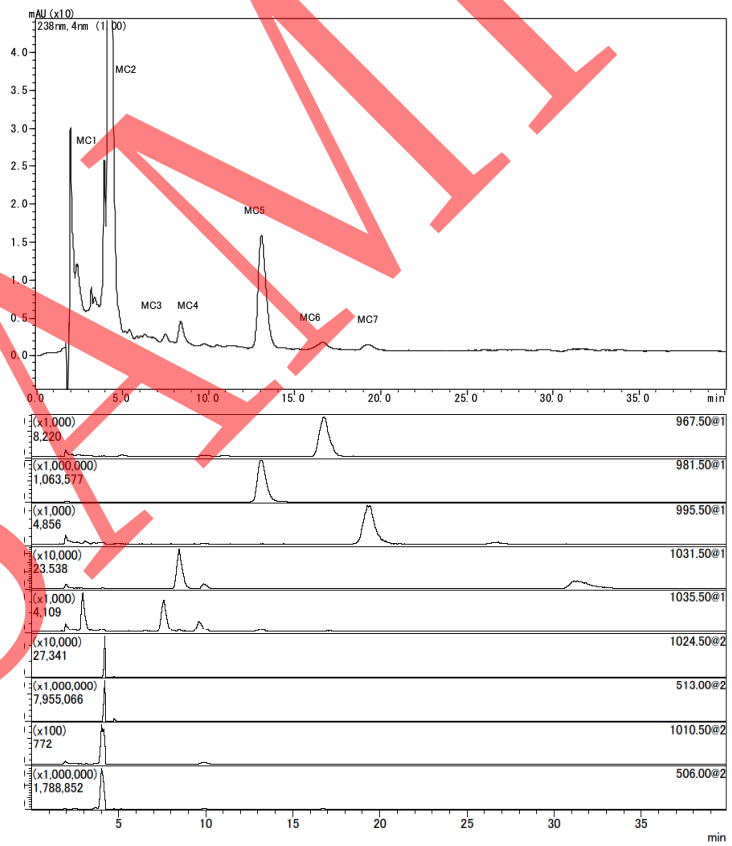


Fig. A2 Examples of HPLC/MS chromatograms of microcystins in NIES CRM No. 26

Column: Zorbax XDB Eclipse C-18 2.1 x150 mm, solvent: 55% MeOH in 0.1% formic acid, flow rate: 0.2 ml / min, temp.: 40 °C, wave length: 238 nm

Table A1 Examples of relative retention time ratios of microcystins in NIES CRM No. 26

Column	MC1	MC2	MC3	MC4	MC5	MC6	MC7
Mightysil RP-18	0.164	0.190	0.653	0.751	1.223	1.540	1.803
Super ODS	0.475	0.513	0.720	0.808	1.221	1.591	1.766
Zorbax XDB C18	0.232	0.264	0.605	0.701	1.211	1.573	1.882
SunFire C18	0.022	0.022	0.606	0.710	1.253	1.587	1.933

Relative retention time ratio: retention time of microcystin / retention time of microcystin-LR

Solvent for HPLC was 55% MeOH in 0.1% formic acid.