Certificate of analysis

Certified reference material code: CRM-03-MCLR

Name: Certified Reference Material of Microcystin-LR. Batch: 24-001

Description: This Certified Reference Material (CRM) is a solution of Microcystin-LR (CAS Number 101043-37-2) in acetonitrile, 50% (V/V) aqueous solution.

Intended use: Calibration of equipment or a measurement procedure. Establishing metrological traceability. Method validation. Quality control of a measurement or measurement procedure. For laboratory use only.

Certified values and uncertainties: The certified concentration given below is based on results obtained from the gravimetric preparation of this solution previous quantity determination by ^{1}H-qNMR in Varian 500 MHz equipment (Universidad de Santiago de Compostela).

Concentration of Microcystin-LR (MC-LR)* (95% Confidence Interval)	**(11.0 ± 0.8) µmol / kg**
	(11.0 ± 0.8) µg / g

***** NOTE: In order to perform the analysis, it should be noted that the certificate value corresponds to the sum of Microcystin-LR and minor analogues concentration. See #Non-certified and non-accredited data section of this certificate.

The starting material is measured by ^{1}H-qNMR against a Benzoic acid standard for quantitative NMR, certified reference material followed by gravimetric preparation using high precision balances calibrated with SI-traceable weights. Consequently, the quantified values are traceable to the International System of Units (SI) via an unbroken chain of comparisons through validated methodology.

Homogeneity was evaluated by analysis of variance on 2% of stratified randomly selected ampoules over the entire batch (6 replicate analyses per ampoule). A possible heterogeneity is hidden by the method repeatability. Therefore, a maximum possible heterogeneity was calculated as u_{bb}, 1.43%. The Stability studies after storage of selected units were performed at -20°C for a storage time of 12 months. Using the data from the long-term study, the uncertainty due to possible degradation was calculated as u_{lts}, 1.40%. Both studies were verified against an independently prepared calibration solution.

The components of Standard Uncertainty include related uncertainties due to characterization, heterogeneity, long term instability, short term instability (dispatch), and bulk assay, Table 1.

The results are expressed as the certified value ± the expanded uncertainty (calculated by combination of the squared contribution values). Estimated expanded uncertainty with a coverage factor k = 2, corresponding to a level of confidence of about 95 %, as defined in the Guide to the expression of uncertainty in measurement, ISO33405.

The certificate is a summary of an extensive program of work involving selection and purification of the material, assessing its suitability and measuring the properties to be certified.

Expiration of Certification: *This certificate of CRM-03-MCLR, 24-001 batch is valid 12 months after purchase date within the measurement uncertainties specified*, provided the CRM is handled and stored in accordance with the instructions given in this certificate. This validity may be extended if further evidence of stability becomes available.

The marked activities (#) are not covered by ENAC accreditation

Maintenance of Certification: The material will be subjected to regular Laboratorio CIFGA stability monitoring programme to control its further stability over the period of its certification. If substantive changes occur that affect the certification before the expiration date, Laboratorio CIFGA will notify the purchaser.

Instructions for proper handling use: This material contains toxic material, and should be handled with care. Read MSDS before using. Material Safety Data sheet for information regarding CRM-03-MCLR is enclosed in the package. Use proper disposal methods.

Before opening an ampoule, it must be guaranteed that the content is a liquid and properly mixed to ensure homogeneity. Sample aliquots for analysis should be handled at 15ºC to 25ºC immediately after opening the ampoules and should be processed without delay for the certified value to be valid within the stated uncertainty. Ampoule should not remain open longer than five minutes due to solvent volatility and air contamination.

Storage conditions: The original sealed ampoules should be stored at temperatures below/at -20 ºC (freezer) until use. Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied.

Source and Preparation of Material: Cyclic heptapeptide toxin was obtained from commercial source, identity of the material was confirmed by ^{1}H-NMR and LC-MS/MS. Finally, 0.5 mL aliquots of MC-LR solution were dispensed into 2-mL amber argon-filled glass ampoules, which were then flame sealed and ready to use for calibration.

[#] **Non-certified and non-accredited data:** Taking into account the density of acetonitrile, 50% (v/v) aqueous solution at 20 ºC ($\rho^{20°C}_{ACN\ 50\%\ V:V} = 0.9109\ g/mL$), *non-certified volumetric concentration* values are:

Concentration of Microcystin-LR (MC-LR) **(95% Confidence Interval)**	**(10.0 ± 0.7) µM**
	(10.0 ± 0.7) µg / mL

Concentration of Microcystin-LR and impurities are neglected in order to apply solution density value.

Purity analysis was performed by combining ^{1}H-qNMR and UPLC MS/MS, ≥ 98 % (molar ratio purity). *Percentage values not certified*.

Low amounts of MC-LR analogues are presented, see [#]Table 2. In order to get a certified concentration, quantification for MC-LR and these analogues have been conducted jointly, therefore certified concentration is the sum of all these compounds. *No purity correction over certified concentration should be done*.

Additional lectures:

1. UNE-EN ISO 17034 *General Requirements for the competence of reference material producers*.

2. ISO 33405 *Reference materials - Approaches for characterization and assessment of homogeneity and stability*.

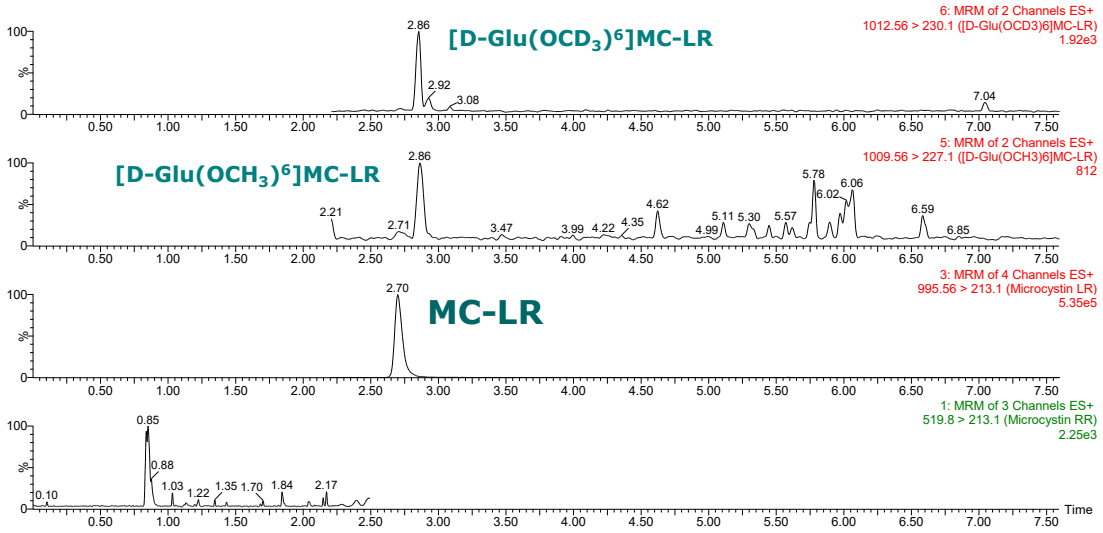

Figure 1. Analysis of the **CRM-03-MCLR** (24-001) by UPLC-MS/MS. Conditions: ACQUITY UPLC HSS T3 1.7µm, 100x2.1 mm, UPLC column. Main elution conditions, gradient mode with mobile phases: (A) Water and (B) acetonitrile, both with 0.1% formic acid; Gradient: 0 min 30% B; 0.8 min 30% B; 2.5 min 60% B; 5.0 min 100% B; 5.5 min 100% B; 5.6 min 30% B; flow rate: 0.3 mL/min. Temperature: 30°C. MS detector: Waters XEVO TQ-MS using the typical MRM transitions of the most relevant Microcystins and main expected impurities.

Table 1. Uncertainties contribution for the certification of **CRM-03-MCLR (24-001)**. ***Char**: qNMR quantification; **ba**: bulk assay; **bb**: homogeneity test; **lts**: long term stability test; **sts**: short term stability test.*

Uncertainty contribution parameter	Uncertainty values
	MC-LR + minor analogues*
u_{char}/%	2.38
u_{ba}/%	0.63
u_{bb}/%	1.43
u_{lts}/%	1.40
u_{sts}/%	1.41
Expanded uncertainty (*k*=2) / %	6.95

Short-term stability study. The uncertainty due to transport instability was estimated from the test for slope significance at 40°C. The results indicated continued stability, no change is expected in the property values of the CRM. The combined uncertainty budget of the assigned value was upgraded to incorporate short-term stability at 40°C for 9 days.

***** Microcystin-LR and structural closely related analogues have been quantified jointly.

[#] **Table 2.** Non-certified reference values of impurities concentration (µM) determined by UPLC MS/MS. Assigned analogues entries for compounds must be regarded as tentative according to MRM transitions agree. Assumption of equimolar response at sulphate loss MRM was used. Estimated uncertainty corresponds to a level of confidence in terms of one standard deviation. *Tentative analogue assignment.

Toxin	Concentration / µM	%
MC-LR	**10.0 ± 0.7**	≥ 98%
[D-Asp^3]MC-LR	-	0.2%
[D-(GluOCD$_3$)^6]MC-LR	**0.09 ± 0.02**	0.9%
[D-(GluOCH$_3$)^6]MC-LR	-	0.2%

Inquiries concerning this Reference Material should be directed to:

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RM PRODUCER'S APPROVING OFFICER:

Certification Manager: Álvaro Antelo

This Certificate is only valid if the product was obtained directly from Laboratorio CIFGA S.A. or one of our authorized distributors.

Certificate Revision History:

November, 29th 2024 (Original certificate issue date, revision 1).

January, 12th 2026. Revised and added associated uncertainties following additional testing. Minor typographical errors *(certificate rev. 2).*

The marked activities ([#]) are not covered by ENAC accreditation