National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

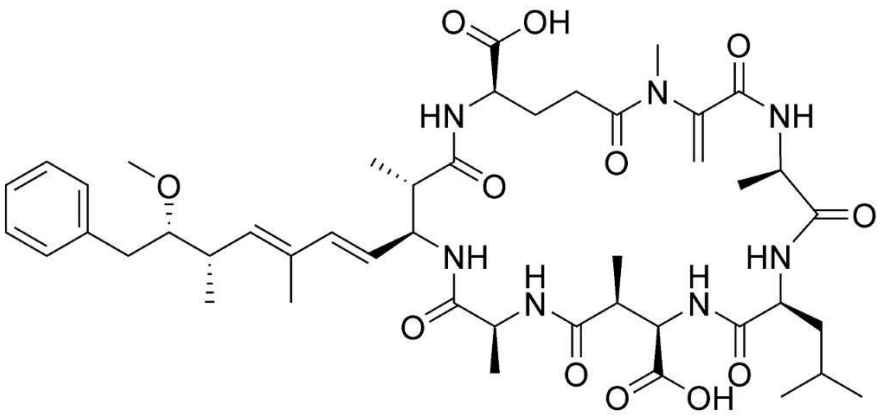
CRM-MCLA (Lot# 20210128)

Certified Calibration Solution for Microcystin-LA

Microcystin-LA (MC-LA) is a cyclic peptide toxin produced by freshwater cyanobacteria [1] that has been associated with domestic and wild animal poisonings and poses a threat to human health through contamination of drinking water supplies [2]. CRM-MCLA is a certified calibration solution of MC-LA in aqueous methanol (1:1, v/v), designed to aid in the identification and quantitation of MC-LA.

Table 1: Certified concentration and uncertainty for CRM-MCLA.

Compound	µg/g	µg/mL (15 - 30 °C)	µmol/L (15 - 30 °C)
Microcystin-LA	5.07 ± 0.20	4.69 ± 0.19	5.15 ± 0.21



Microcystin-LA
CAS registry No.: 96180-79-9
InChIKey: DIAQQISRBBDJIM-DRSCAGMXSA-N
Molecular formula: $C_{46}H_{67}N_7O_{12}$
Molecular weight: 910.06 g/mol

Period of validity: 1 year from date of sale.
Storage conditions: −12 °C or below

Canada

Intended Use

CRM-MCLA is a certified calibration solution designed for analytical method development and accurate quantitation of MC-LA. The concentration is suitable for preparing a dilution series for calibration of instruments such as liquid chromatography with detection by ultraviolet absorbance (LC–UV) or mass spectrometry (LC–MS), as well as for spiking control samples for recovery experiments.

Instructions for Storage and Use

To ensure the stability of CRM-MCLA, ampoules should be stored at −12 °C or below.

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents thoroughly mixed. The ampoule should be opened at the pre-scored mark. Calibrated equipment should be used for accurate transfer of aliquots. An increase in concentration due to evaporation of solvent will occur if the solution is left opened for more than a few minutes. It is recommended that the CRM should not be evaporated to dryness because of the potential for losses. *Note:* The volume of the solution is not certified. Only the concentration is certified. Therefore, the entire contents of the ampoule should not simply be transferred to a volumetric flask and diluted to volume.

Preparation of CRM-MCLA

MC-LA was obtained from Beagle Bioproducts Inc. (Columbus, OH, USA) and further purified in-house using semi-preparative liquid chromatography. The structure and purity of MC-LA was confirmed by LC–MS (Figures 1 and 2), LC–UV (Figure 3) and 1D and 2D NMR spectroscopy. A measured accurate *m*/*z* of 910.4910 (Δ = 1.2 ppm for $C_{46}H_{68}N_7O_{12}^+$) was obtained for the $[M+H]^+$ ion of MC-LA using LC–high-resolution MS (LC–HRMS). Purity was further assessed by LC with chemiluminescence nitrogen detection [3] and charged aerosol detection.

The stock solution was prepared by dissolving the purified MC-LA in CD_3OH for quantitation using ^1H NMR (qNMR) [4]. The CRM-MCLA solution was prepared by accurately diluting the stock solution in degassed CH_3OH–H_2O (1:1, v/v). Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL.

Analytical Methods and Value Assignment

The certified value for CRM-MCLA (Table 1) is based on results obtained at the NRC with qNMR using caffeine for calibration, and LC-UV using CRM-MCLR as the calibrant.

A low level of the methyl ester of MC-LA ([Glu(OMe)^6]MC-LA, exact mass $[M+H]^+$ *m/z* 924.5067; 0.02 µmol/L) is present in CRM-MCLA. The concentration was estimated by LC–MS using selected ion monitoring with an accurate dilution of CRM-MCLA as the calibrant. Analyses also revealed small amounts of other partially identified MC-LA related impurities (exact masses $[M+H]^+$ *m/z* 840.4899 and 926.4889) which, combined, were below 0.5 % of the MC-LA concentration.

Homogeneity

A representative number of CRM-MCLA ampoules were selected from across the fill series and MC-LA response was measured by LC–MS/MS. No heterogeneity was observed.

Stability

Stability studies have demonstrated good stability for CRM-MCLA stored in sealed ampoules at temperatures of −12 °C and below.

Uncertainty

All reasonable sources of error related to the characterization of CRM-MCLA were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}) and instability during storage (u_{stab}) [5-6]. These components are listed in Table 2, and are combined and expanded as follows:

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

where k is the coverage factor for a 95 % confidence level (= 2).

Table 2: Uncertainty components for the certified value of CRM-MCLA.

Uncertainties	Relative*
u_{char}	0.017
u_{hom}	negligible
u_{stab}	0.010

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, MC-LA and related microcystins are known to be hepatotoxic and may also be genotoxic [7].
Only qualified personnel should handle the solution and appropriate disposal methods should be used. Suitable personal protective equipment should be used when opening the ampoule in the event glass shatters. A safety data sheet (SDS) is available for CRM-MCLA.

Period of Validity

If stored unopened at the recommended storage condition of −12 °C, the certified concentration of CRM-MCLA is valid for 1 year from the date of sale.

Metrological Traceability

Results presented in this certificate are traceable to the SI (*Système international d'unités*) through a gravimetrically prepared standard of NMIA caffeine certified reference material (M724c), and NRC CRM-MCLR (lot # 20070131).

Quality Management System (ISO 17034, ISO/IEC 17025)

This material was produced in compliance with the National Research Council of Canada (NRC) Metrology Quality Management System, which conforms to the requirements of ISO 17034 and ISO/IEC 17025.

The Metrology Quality Management System supporting the NRC Calibration and Measurement Capabilities, as listed in the *Bureau international des poids et mesures* (BIPM) Key Comparison Database (http://kcdb.bipm.org/), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM) and found to be in compliance with the expectations of the *Comité international des poids et mesures* (CIPM) Mutual Recognition Arrangement. The SIM approval is available upon request.

References

1. Carmichael WW (1994). The toxins of cyanobacteria. *Sci Am* 270:78–86. DOI:10.1038/scientificamerican0194-78
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7. Arman T, Clarke JD (2021). Microcystin toxicokinetics, molecular toxicology, and pathophysiology in preclinical rodent model and humans. *Toxins* 13: 537–557.

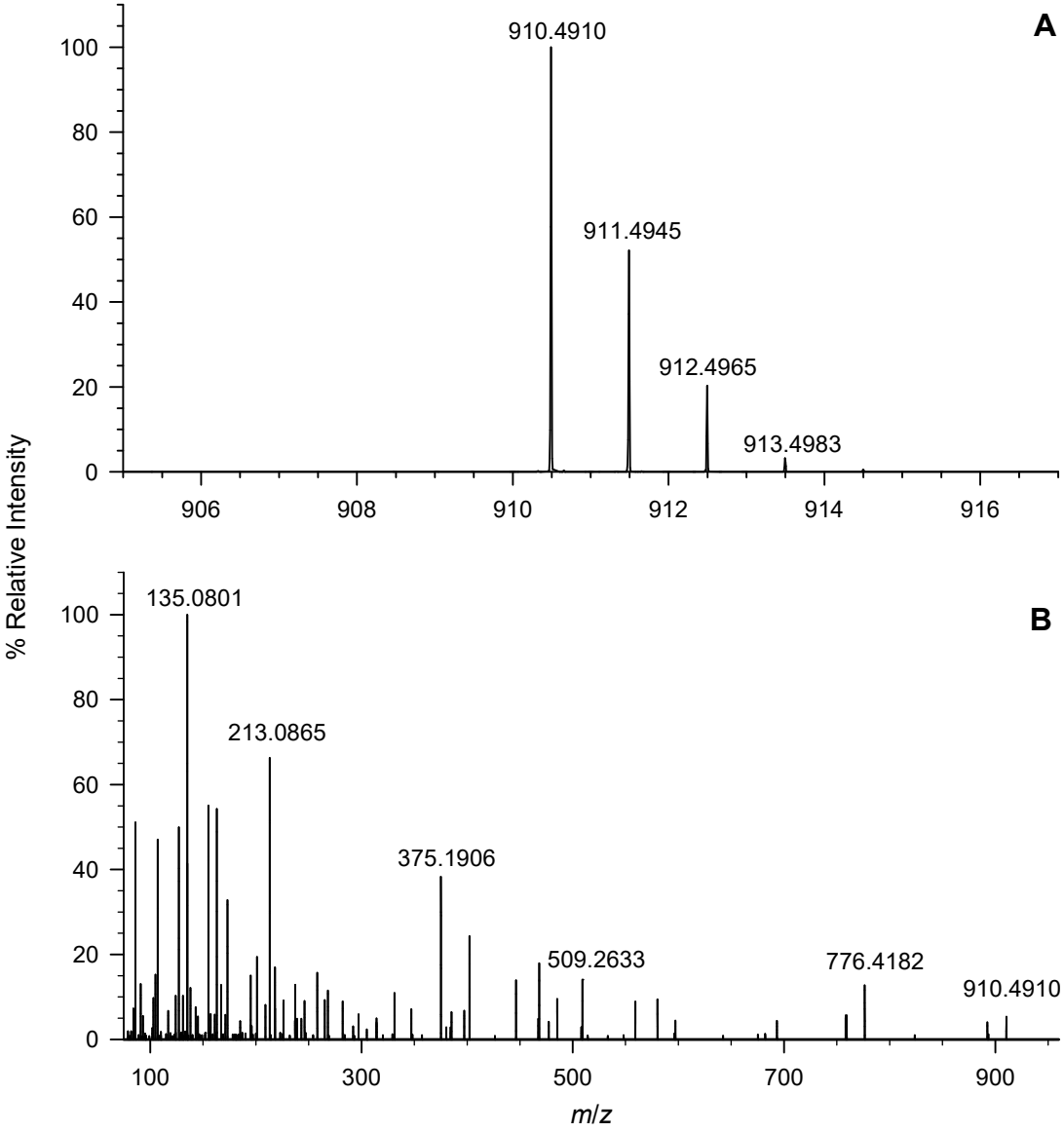

Figure 1: Full-scan (A), and collision-induced dissociation (B) (MS/MS), LC–HRMS spectra of MC-LA used for preparation of CRM-MCLA, analyzed on a Q Exactive-HF mass spectrometer equipped with a heated electrospray ionization probe. Data was collected in positive mode with a 2500 V spray voltage, +275 °C capillary temperature, and a +375 °C heater temperature. Full-scan data was acquired with a resolution setting of 120 000. MS/MS data was acquired in parallel reaction monitoring scan mode with the same resolution setting and a normalized collision energy of 30 V.

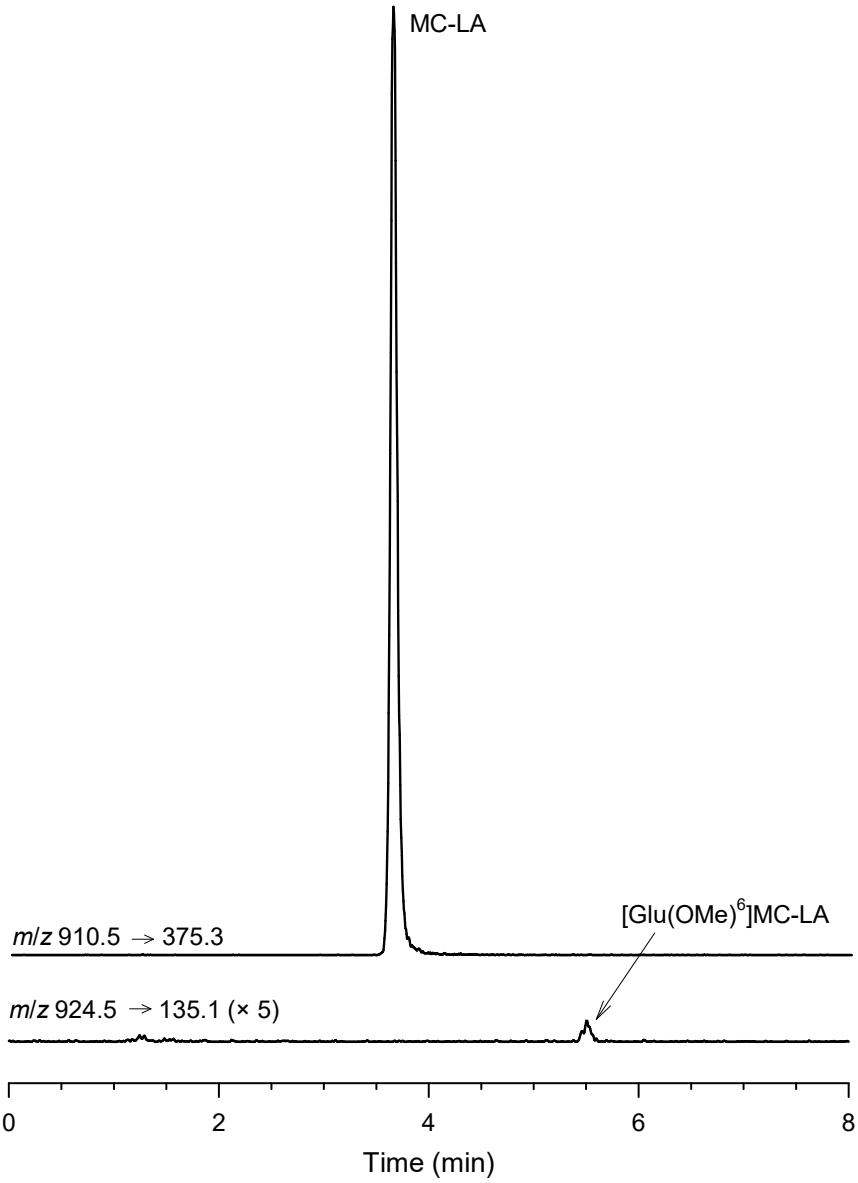

Figure 2: LC–MS/MS analysis of CRM-MCLA using selected reaction monitoring on an Agilent1290 LC connected to a Sciex 5500 QTRAP with electrospray ionization. Chromatographic conditions: Poroshell 120 SB C18 column (150 mm × 2.1 mm, 2.7 µm) at +40 °C; mobile phase, 2 mM ammonium formate and 50 mM formic acid in deionized water (A), and 95 % acetonitrile (B); isocratic elution with 50 % B, at 0.25 mL/min; injection volume, 6 µL. MS conditions: collision energy +80 V; declustering potential +100 V, and source temperature +575 °C.

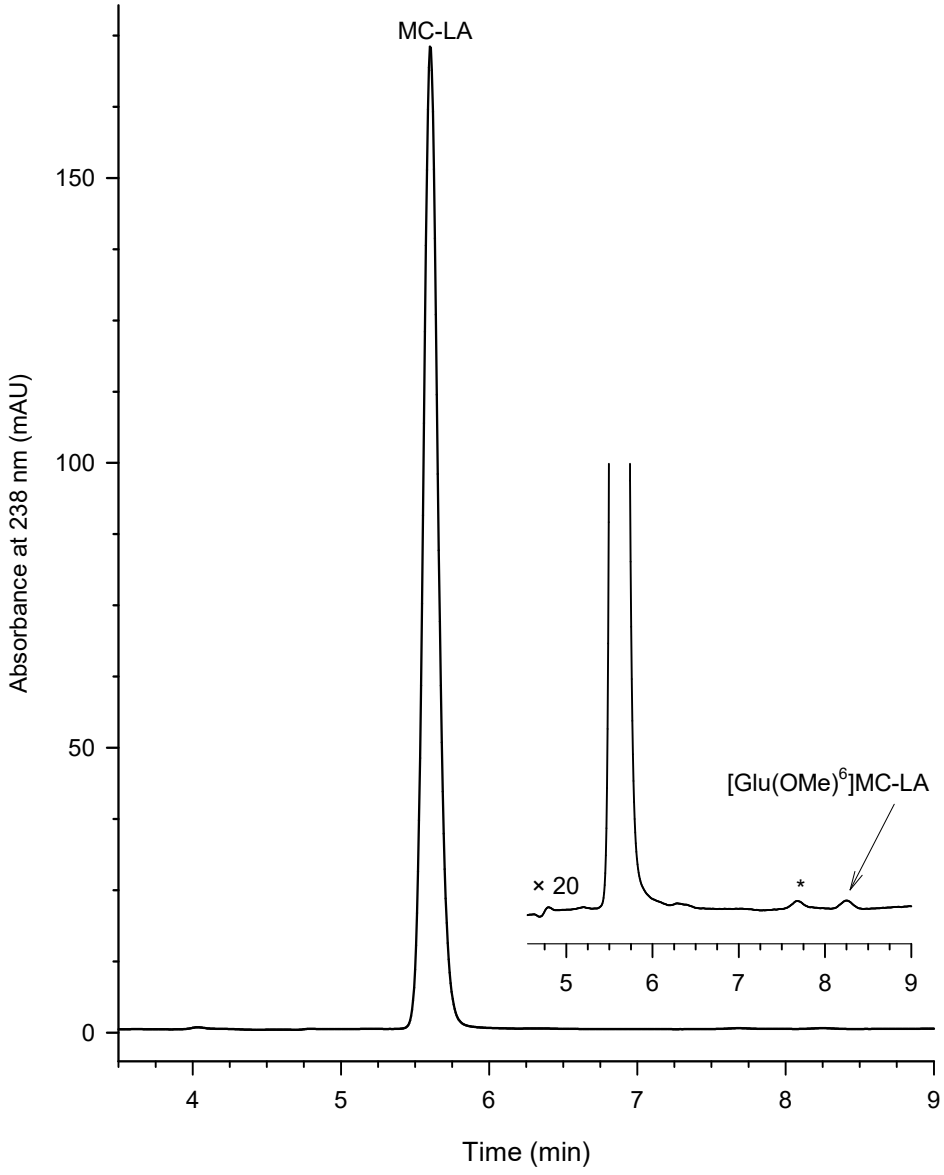

Figure 3: LC–UV analysis of CRM-MCLA. Conditions: Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 µm) at +40 °C; mobile phase, 0.1 % trifluoroacetic acid in deionized water (A), and 0.1 % in acetonitrile (B); isocratic elution with 45 % B, at 0.4 mL/min; injection volume, 7 µL; UV detection at 238 nm. A low level of [Glu(OMe)^6]MC-LA is present in the CRM. The asterisk (*) indicates a trace level impurity.

Acknowledgements

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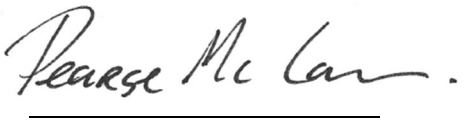
Approved by: 

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This Certificate is only valid if the corresponding material was obtained directly from the NRC or an Authorized Reseller.

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