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Certificate of Analysis

Certified Reference Material

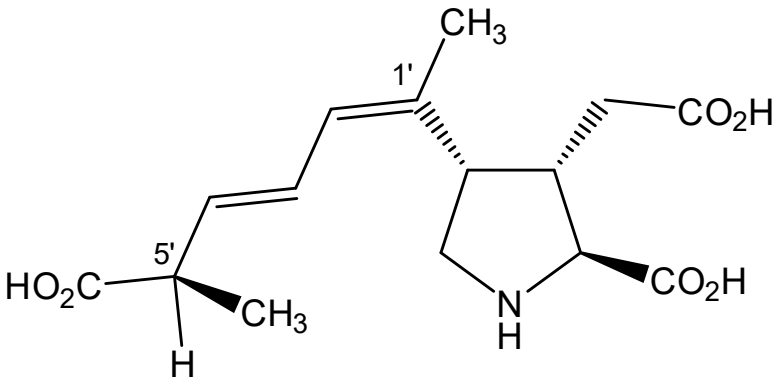
CRM-DA-h (Lot# 20210922)

Certified Calibration Solution for Domoic Acid

Domoic Acid (DA) is a toxin responsible for incidents of amnesic shellfish poisoning (ASP) [1, 2]. CRM-DA-h is a certified calibration solution of DA in acetonitrile/water (1:19, v/v) designed to aid in the identification and quantitation of DA. This is a replacement calibration solution for CRM-DA-g.

Table 1: Certified concentration and uncertainty for CRM-DA-h.

Compound	µg/g	µg/mL (15 - 30 °C)	µmol/L (15 - 30 °C)
Domoic Acid + C5'-*epi*-Domoic acid	97.9 ± 5.5	97.1 ± 5.5	312 ± 18



Domoic acid
CAS registry no: 14277-97-5
InChIKey: VZFRNCSOCOPNDB-AOKDLOFSSA-N
Molecular formula: $C_{15}H_{21}NO_6$
Molecular weight: 311.33 g/mol
$[M+H]^+$: *m*/*z* 312.1442

Period of validity: 1 year from date of sale
Storage conditions: +4 ºC

Canada

Intended Use

CRM-DA-h is a certified calibration solution designed for analytical method development and accurate quantitation of DA. 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-DA-h, ampoules should be stored at +4 °C. Stability studies have shown that there may be a very gradual decomposition when the solution is kept frozen (approximately -12 ºC), probably due to freeze/thaw events. Therefore, do not freeze the solution.

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 of losses on glass surfaces and increased isomerization of DA. *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-DA-h

Purified DA was obtained from BioVectra DCL (Charlottetown, PE, Canada). The structure and purity of DA was confirmed by LC–MS [3] (Figures 1 and 2), LC–UV [4, 5] (Figure 3), NMR spectroscopy and LC with charged aerosol detection (LC–CAD). Total purity was assessed using quantitative NMR (qNMR) with benzoic acid as an internal standard [6]. A measured accurate *m*/*z* of 312.1431 (Δ = -3 ppm for $C_{15}H_{22}NO_6^+$) was obtained for the $[M+H]^+$ ion of DA using LC–high-resolution MS (LC–HRMS).

The stock solution was prepared gravimetrically by dissolving the purified DA in deionized water, the pH was adjusted to 5 with diluted sodium hydroxide to avoid decomposition of the DA. The CRM-DA-h solution was prepared by accurately diluting the stock solution in degassed aqueous 5% acetonitrile. 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-DA-h (Table 1) is based on results obtained at the NRC using qNMR of the source material and LC–UV analyses of the CRM solution. The certified value is the combined concentration of DA and its C5'-diastereomer, C5'-*epi*-DA. The relative concentrations of individual isomers (Table 2) are provided as non-certified information values which were determined using LC–UV analyses and corrected using the published extinction coefficients of DA and its isomers [7]. DA has been shown to isomerize slowly in solution to C5'-*epi*-DA. Therefore, because the combined total of DA and C5'-*epi*-DA is certified, analysts should base instrument calibrations on the sum of their peak areas.

Table 2: Information values for isomers of DA present in CRM-DA-h at the time of packaging.

Compound	λ_{max} (nm)	ε_{max} (L cm^{-1}mol^{-1})	Concentration* (µg/mL)
Domoic acid	242	26,300	96.6
C5'-epi-domoic acid	242	26,300	0.55
Isodomoic acid E	241	~26,000	0.19
Isodomoic acid D	244	~26,000	0.82
Isodomoic acid A	220	~8,300	1.1

*These concentrations are not certified

Homogeneity

A representative number of CRM-DA-h ampoules were selected from across the fill series and DA response was measured by LC–UV. No heterogeneity was observed.

Stability

Stability studies performed on previous CRM-DA preparations have demonstrated good stability for DA in 5 % acetonitrile stored in sealed ampoules at temperatures of +4 °C.

Uncertainty

All reasonable sources of uncertainty related to the characterization of CRM-DA-h were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}) and instability during storage (u_{stab}) [8]. These components are listed in Table 3, 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 3: Uncertainty components for the certified value of CRM-DA-h.

Uncertainties	Relative*
u_{char}	0.020
u_{hom}	negligible
u_{stab}	0.020

*Relative to concentration shown in Table 1.

Safety Instructions

If sufficient quantities are ingested, DA can cause severe neurological symptoms such as short-term memory loss and even death. 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 the glass shatters. A safety data sheet (SDS) is available for CRM-DA-h.

Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-DA-h 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 gravimetrically prepared standards of benzoic acid (NIST PS1) and NRC CRM-DA-g (lot # 20140730).

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

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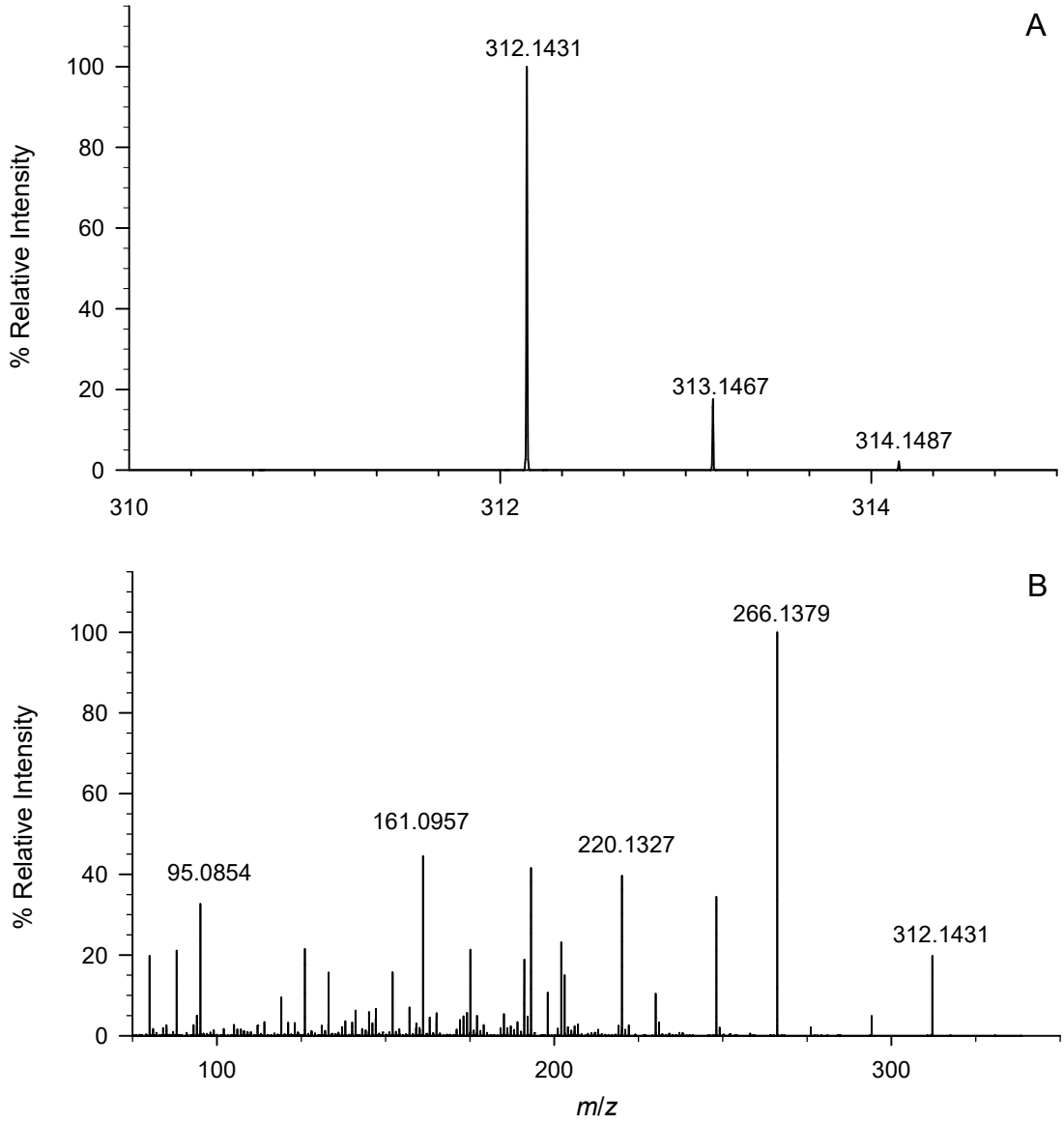

Figure 1: Full-scan (A), and tandem (B) LC–HRMS spectra of CRM-DA-h, analyzed on a Q Exactive-HF mass spectrometer equipped with an electrospray ionization probe in positive mode. A resolution setting of 60 000 was used with a collision energy of 35 V for the tandem MS spectrum.

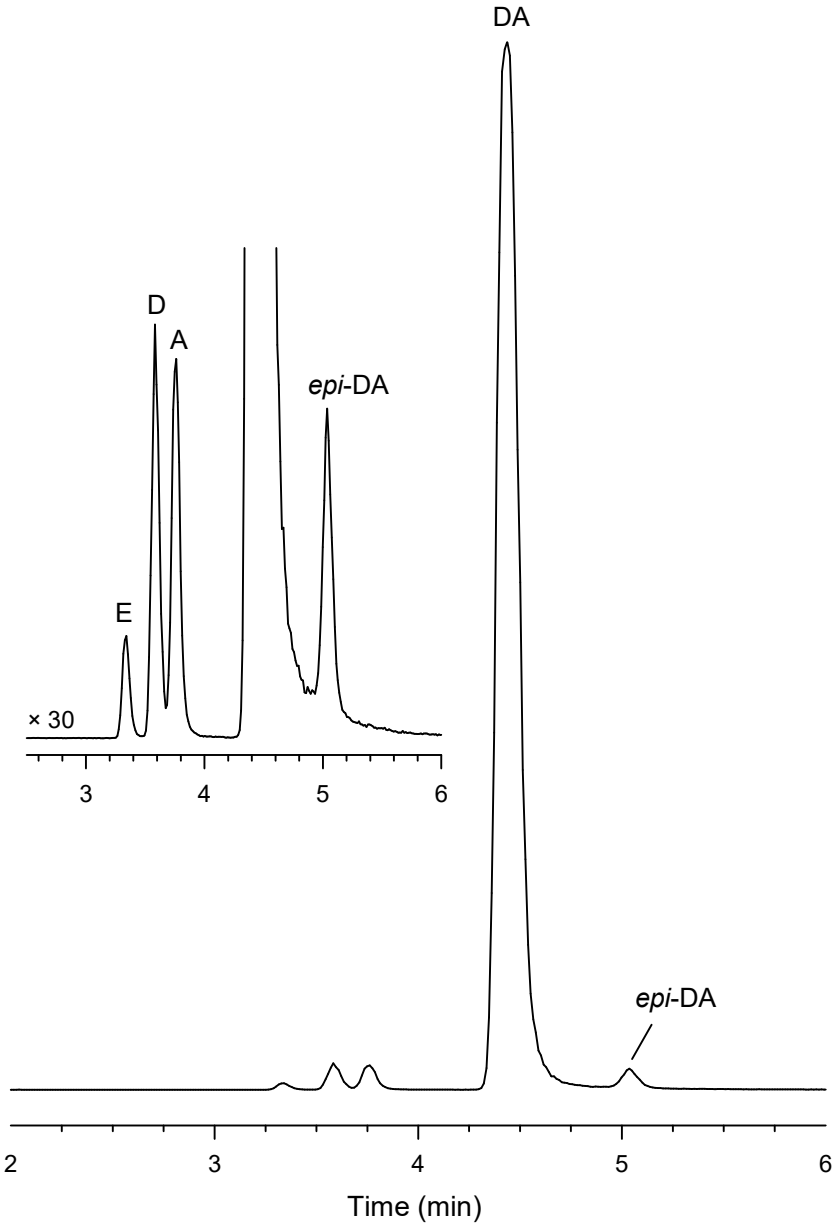

Figure 2: LC–MS/MS analysis of CRM-DA-h using selected reaction monitoring (*m*/*z* 312 → 166) on an Agilent1290 LC connected to a Sciex 5500 QTRAP with electrospray ionization. Chromatographic conditions: Waters Acquity 1.8 µm HSS T3 column (100 mm × 2.1 mm) at +45 °C; mobile phase: 0.1% formic acid in deionized water (A), and 0.1% formic acid in acetonitrile (B); isocratic elution with 10% B at 0.30 mL/min; injection volume 0.5 µL. MS conditions: collision energy +25 V; declustering potential +50 V, and source temperature +375 °C.

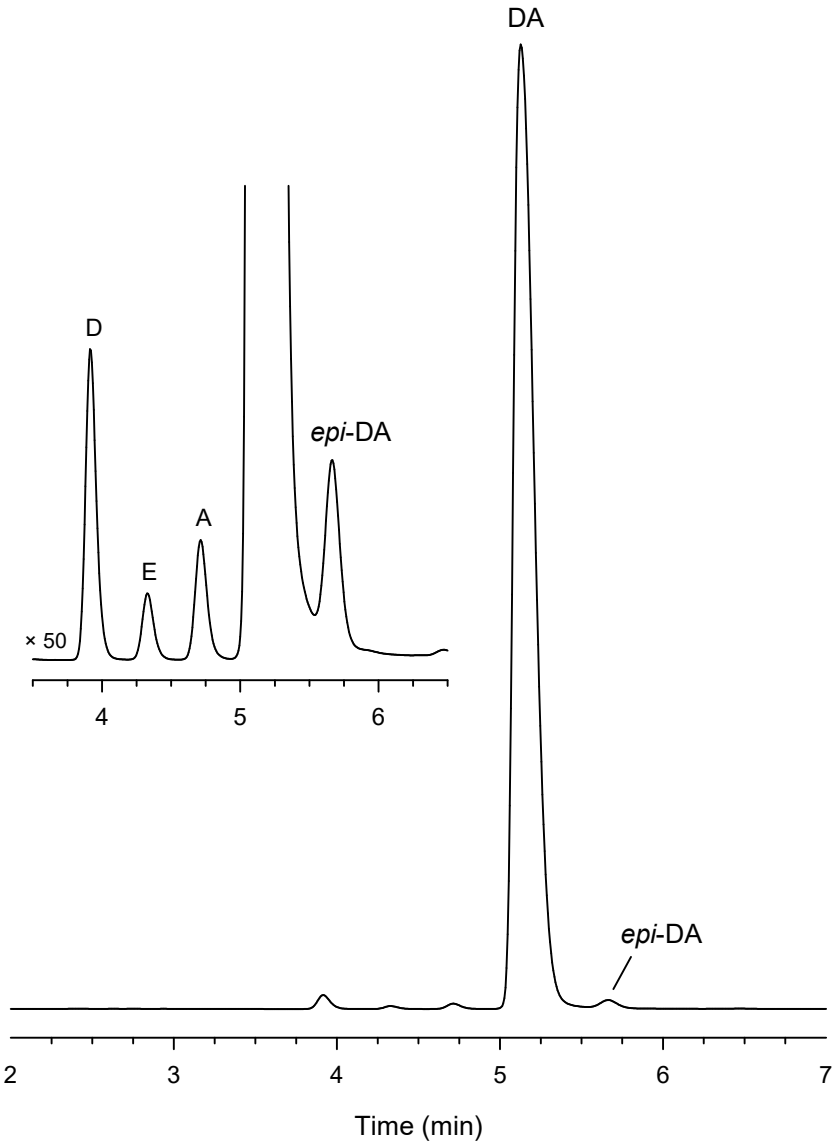

Figure 3: LC–UV analysis of CRM-DA-h. Conditions: Waters Acquity 1.8 µm HSS T3 column (100 mm × 2.1 mm) at +40 °C; mobile phase, 0.1% trifluoracetic acid in deionized water (A), and in acetonitrile (B); isocratic elution with 10% B, at 0.40 mL/min; injection volume, 5 µL ; UV detection at 242 nm.

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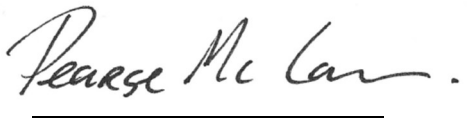
Approved by: 

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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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