National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

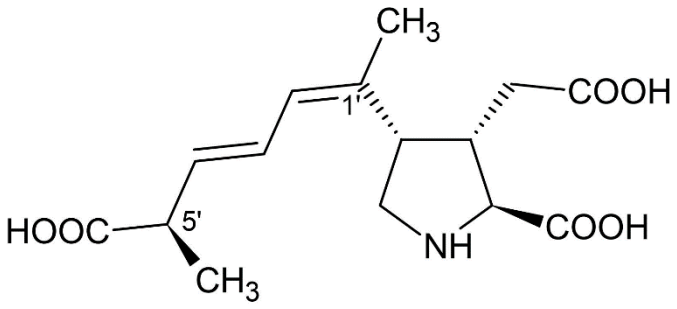
CRM-ASP-Cla (Lot# 20230222)

Clam Tissue Certified Reference Material for Domoic Acid

Domoic acid (DA) is a toxin responsible for incidents of amnesic shellfish poisoning (ASP) [1]. CRM-ASP-Cla is a Pacific razor clam (*Siliqua patula*) homogenate containing DA, as well as low levels of several isomers. A certified value and expanded uncertainty have been assigned for the sum of DA and the epimeric C5'-*epi*-DA in CRM-ASP-Cla (Table 1).

Table 1: Certified value and expanded uncertainty (*k* = 2) for CRM-ASP-Cla

Compound	Mass fraction µg/g
domoic acid + C5′-*epi*-domoic acid	3.2 ± 0.2



domoic acid

CAS registry number: 14277-97-5
InChIKey: VZFRNCSOCOPNDB-AOKDLOFSSA-N
Molecular formula: $C_{15}H_{21}NO_6$
Molar mass: 311.33 g/mol
$[M+H]^+$: *m/z* 312.1442

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

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Table 2: Information values for CRM-ASP-Cla

Compound	Mass fraction* µg/g
domoic acid	3.1
C5′-*epi*-domoic acid	0.1
isodomoic acid E	0.1
isodomoic acid D	0.2

*Not certified

Intended use

CRM-ASP-Cla is a clam tissue matrix certified reference material (CRM) designed to test the accuracy of entire analytical methods for the quantitation of DA. This may include, but is not limited to, sample extraction procedures, separation methods, and evaluating instrumental techniques including liquid chromatography with detection by ultraviolet absorbance (LC–UV) or mass spectrometry (LC–MS).

Preparation of material

Clam tissues (*Siliqua patula*) naturally contaminated with DA were provided by the Washington State Department of Health (USA). The tissues were processed using a Polytron homogenizer and autoclaved at 120 °C for 20 min. Distilled water was added to adjust the water content to approximately 84 %. Ethoxyquin, oxytetracycline, erythromycin and ampicillin were added as stabilizers (0.02 % (*w/w*) [2]). The homogenate was de-aerated, purged with nitrogen, and dispensed as 4 g (± 0.7 g) aliquots into 5 mL polypropylene bottles which were flushed with nitrogen and heat sealed. The seals were inspected and bottle caps were attached. The bottles were heat sealed in trilaminate pouches prior to shipment.

Characterization of material

The certified value for CRM-ASP-Cla (Table 1) is based on results obtained at the National Research Council Canada (NRC) and is the combined mass fraction of DA and its C5′-epimer, C5′-*epi*-DA, which has a UV spectrum identical to that of DA. The certified value is a combination of results from LC–UV [3] and LC–MS/MS using NRC CRM-DA-h for external calibration (Figure 1).

Samples (approximately 4 g) were prepared using an exhaustive four-step liquid-solid extraction method, by vortex mixing with 10 mL of 1:1 methanol/water (v/v) at each step. The extracts were combined and brought to a final volume of 50 mL with 50 % methanol, and filtered prior to analysis. DA has been shown to isomerize under strongly acidic conditions or heat-treatment [4]. CRM-ASP-Cla contains isomeric forms of DA and information values (Table 2) were measured using LC–MS/MS (Figure 1B).

Metrological traceability

Results presented in this certificate are traceable to the International System of Units (SI) through gravimetrically prepared standards of NRC CRM-DA-h (Lot# 20210922) [5].

Homogeneity

A representative number of CRM-ASP-Cla bottles were selected from across the fill series and analyzed by LC–UV. A small uncertainty (< 0.5 % relative) due to variation between bottles was included in the final combined uncertainty.

Stability

Transportation stability was assessed using an isochronous study and showed good stability after four weeks at temperatures up to 4 °C. A long-term stability study on CRM-ASP-Cla showed good stability for DA at −12 °C or below over 1 year.

Uncertainty

The expanded uncertainty (U) for the value is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [6] and k is the coverage factor. A coverage factor of $k = 2$ was applied which corresponds to a level of confidence of approximately 95 %.
All reasonable sources of uncertainty related to the certified value in Table 1 were considered. Included in the combined uncertainty estimate are uncertainties in the batch characterization, uncertainties related to possible between-unit variation, and uncertainties related to stability.

Storage

The material shall be stored at –12 °C or below. The bottle should be stored unopened.

Instructions for handling and use

Each bottle contains approximately 4 g (± 0.7 g) of tissue. The mass of homogenate is not certified. Prior to opening, each bottle should be allowed to equilibrate to room temperature. Remove hermetic seal carefully, mix contents thoroughly and weigh the sample on an analytical balance. It is not recommended that sub-samples be taken from individual bottles of CRM-ASP-Cla. The entire contents of the bottle should be used for analysis.

Repeated sub-sampling and storage of the matrix tissue CRM after initial opening may impact the certified value. Users shall take responsibility for demonstrating that their sub-sampling and storage procedures do not impact the certified value.

Health and safety information

Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at 10.4224/crm.2026.asp-cla.20230222. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified value is valid for 1 year from the date of sale, provided the storage and instructions for handling and use specified in this certificate are followed.

Quality Management System

The NRC is Canada's national metrology institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

The Calibration and Measurement Capabilities (CMC) supporting the result in Table 1 is listed in the International Bureau of Weights and Measures (BIPM) Key Comparison Database (https://www.bipm.org/kcdb/) which recognizes the validity of the measurements performed by NMIs participating in the CIPM MRA. The NRC has the following CMC relevant for this material: measurements applied for determining the certified value of domoic acid and C5′-*epi*-domoic acid (STT01).

References

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4. Zaman L, Arakawa O, Shimosu A, Onoue Y, Nishio S, Shida Y, Noguchi T. Two new isomers of domoic acid from a red alga, *Chondria armata*. Toxicon. 1997; 205-212. https://doi.org/10.1016/s0041-0101(96)00123-7
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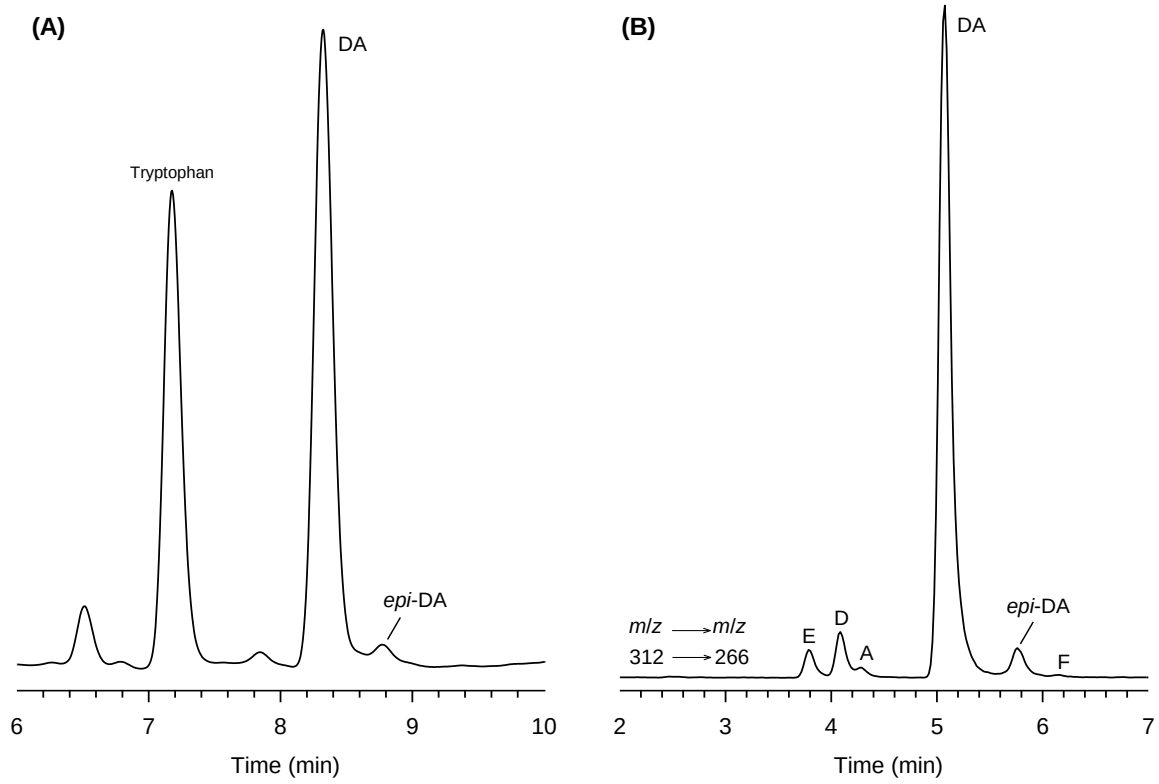

Figure 1: LC–UV (A) and LC–MS/MS (B) analysis of DA and *epi*-DA in CRM-ASP-Cla. LC–UV conditions: Agilent 1290 LC; wavelength: 242 nm; column: Waters ACQUITY 1.8 µm HSS T3 (100 mm × 2.1 mm); mobile phase: A = 0.1 % trifluoroacetic acid in deionized water, B = 0.1 % trifluoroacetic acid in acetonitrile; isocratic 10 % B elution: 0.32 mL/min at 40 °C; injection volume: 3 µL. LC–MS conditions: Agilent 1290 LC and SCIEX QTRAP 5500 with electrospray ionization; column: Waters ACQUITY 1.8 µm HSS T3 (100 mm × 2.1 mm); mobile phase: A = 0.1 % formic acid in deionized water, B = 0.1 % formic acid in acetonitrile; isocratic 10 % B elution: 0.3 mL/min at 45 °C; injection volume: 1 µL. MS operated in positive ion selected reaction monitoring mode; 30 V collision energy; 50 V declustering potential; 375 °C source temperature.

Authorship

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Approved by: Pearse Mc Carron.

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This certificate is only valid if the corresponding material was obtained directly from the NRC or an authorized reseller. Users should ensure that the certificate they have is current. For updates, please refer to 10.4224/crm.2026.asp-cla.20230222.

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