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Information Sheet

Reference Material

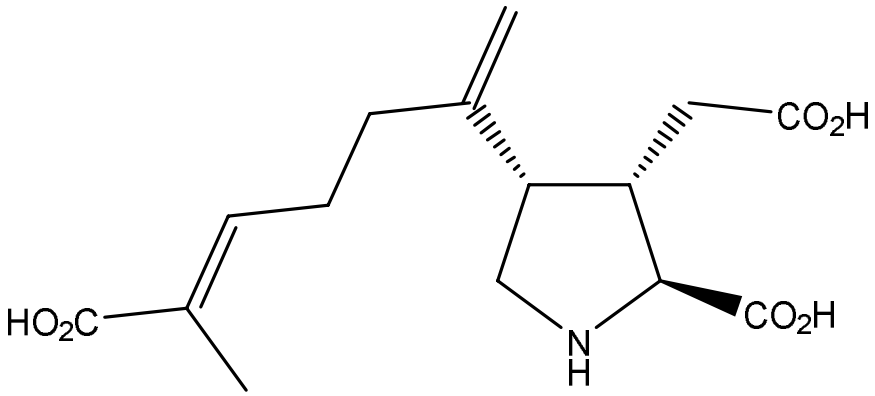
RM-iDAc (Lot# 20051028)

Calibration Solution for Isodomoic Acid C

Isodomoic acid C (iDAc) is an isomer of domoic acid (DA) that has been found in shellfish samples and is shown to be produced by the diatom *Pseudo-nitzschia australis* [1,2]. RM-iDAc is a non-certified calibration solution of iDAc in acetonitrile/water (1:9, v/v).

Table 1: Concentration values for RM-iDAc.

Compound	µmol/L (at +20 °C)	µg/mL (at +20 °C)	µg/g
Isodomoic Acid C	12.7 ± 1.0	4.0 ± 0.3	4.0 ± 0.3



Isodomoic acid C
CAS registry No.: 101899-45-0
InChIkey: OKFIHTSZDXUGQB-PGPNUJALSA-N
Molecular formula: $C_{15}H_{21}NO_6$
Molecular weight: 311.3 g/mol
$[M+H]^+$ *m/z* 312.14

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

Canada

Intended Use

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

Instructions for Storage and Use

To ensure the stability of RM-iDAc, ampoules should be stored at +4°C.

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents thoroughly mixed. The ampoules should be opened at the pre-scored mark. Once an ampoule has been opened, accurate aliquots should be removed with calibrated volumetric equipment and transferred to volumetric flasks or vials. An increase in concentration due to evaporation of solvent will occur if the solution is left opened for more than a few minutes. Note: The volume of the solution is not certified. Therefore, the entire contents of the ampoule should not simply be transferred to a volumetric flask and diluted to volume.

Preparation of RM-iDAc and Value Assignment

iDAc was isolated at the Cawthron Institute (Nelson, New Zealand) from a culture of *Pseudo-nitzschia australis*, purified using chromatography, and dried under vacuum to the anhydrous form. The structure and purity of iDAc was evaluated using ^{1}H NMR, liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [3], LC-MS (Figures 1 and 2) [4] and LC-UV (Figure 3) [5]. Low levels of additional DA analogues are present in RM-iDAc (< 0.1 µg/mL each).

A stock solution was prepared by diluting the purified iDAc in deionized water. Quantitation was performed using ^{1}H NMR (qNMR) [6] and LC-CLND . RM-iDAc was prepared by diluting the stock with high purity acetonitrile in deionized water (1:9, v/v). Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Concentration values (Table 1) were assigned using caffeine (USP grade) as an external calibrant for measurements by qNMR and LC-CLND.

Homogeneity

No heterogeneity was detected in the LC-MS analyses of ampoules of RM-iDAc selected from across the fill series.

Stability

No detectable changes to the concentration of RM-iDAc have been observed during long-term storage. DA and iDAc are closely related analogues [7]. As the stability of DA has been studied extensively in the same diluent, similar good stability of RM-iDAc is inferred at the recommended storage condition.

Safety Instructions

The toxicity of iDAc has been found to be lower than DA [8]. However, to avoid injury, only qualified personnel should handle the solution and appropriate disposal methods should be used. Heavy gloves and eye protection should be used when opening the ampoule in the event the glass shatters. A safety data sheet (SDS) is available for RM-iDAc.

Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the concentration of RM-iDAc is valid for 1 year from the date of sale.

References

1. Holland PT, Selwood AI, Mountfort DO, Wilkins AL, Mcnabb P, Rhodes LL, Doucette GJ, Mikulski CM, and King KL (2005). Isodomoic acid C, an unusual amnesic shellfish poisoning toxin from *Pseudo-nitzschia australis*. *Chem. Res. Toxicol.* 18: 814-816.
2. Olesen AJ, Leithoff A, Altenburger A, Krock B, Beszteri B, Eggers SL, and Lundholm N (2021). First evidence of the toxin domoic acid in Antarctic diatom species. *Toxins (Basel)* 13.
3. Thomas K, Wechsler D, Chen YM, Crain S, and Quilliam MA (2016). Analysis of natural toxins by liquid chromatography-chemiluminescence nitrogen detection and application to the preparation of certified reference materials. *J AOAC Int* 99: 1173-1184.
4. Quilliam MA, Thomson BA, Scott GJ, and Siu KWM (1989). Ion-spray mass spectrometry of marine neurotoxins. *Rapid Commun. Mass Spectrom.* 3: 145-150.
5. Quilliam MA, Sim PG, McCulloch AW, and McInnes AG (1989). High-performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. *Int. J. Environ. Anal. Chem.* 36: 139-154.
6. Burton IW, Quilliam MA, and Walter JA (2005). Quantitative ^{1}H NMR with external standards: Use in preparation of calibration solutions for algal toxins and other natural products. *Anal Chem* 77: 3123-3131.
7. Thomas K, Perez-Calderon RA, Crain S, and Quilliam MA (2014). CRM-DA-g, a certified calibration solution for domoic acid, Biotoxin Metrology Technical Report CRM-DA-g. National Research Council Canada, Halifax.
8. Munday R, Holland P, McNabb P, Selwood A, and Rhodes L (2008). Comparative toxicity to mice of domoic acid and isodomoic acids A, B and C. *Toxicon* 52: 954-956.

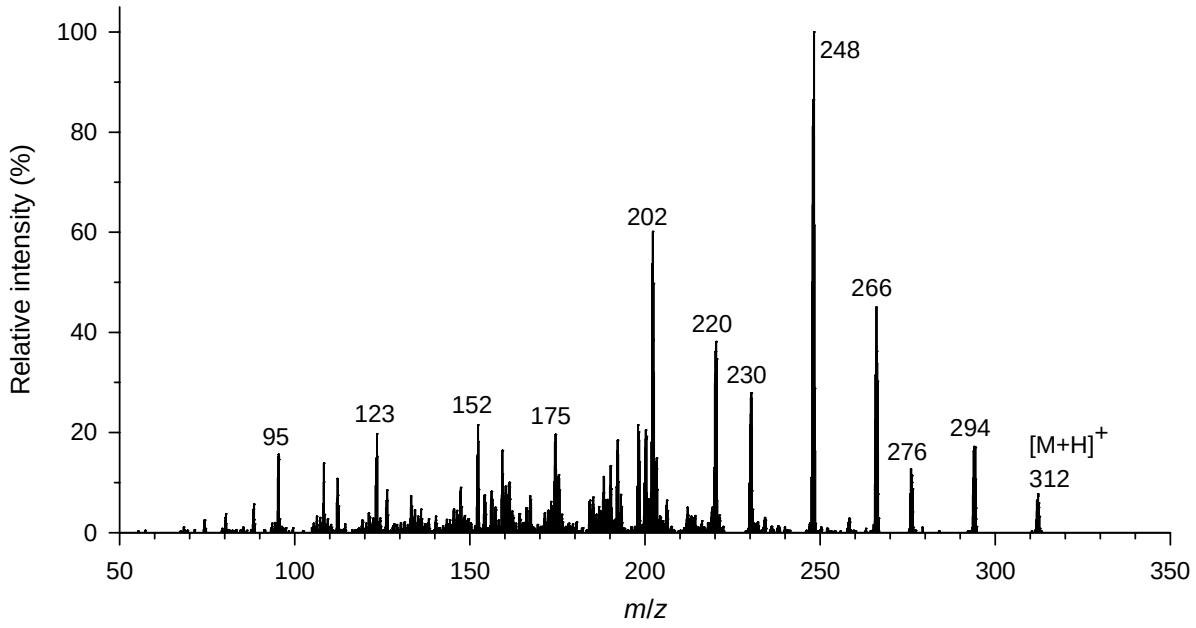


Figure 1: Product ion spectrum of iDAc from LC-MS/MS analyses of RM-iDAc. Conditions: Agilent 1100 LC and AB Sciex 4000 MS; Luna C8(2) column (150 × 2 mm, 3 µm) at +30 °C; 0.2 mL/min; isocratic elution with 15% acetonitrile/water with 0.2% formic acid; 2 µL injection volume ; *m/z* 312 with 50 V declustering potential and 25 V collision energy.

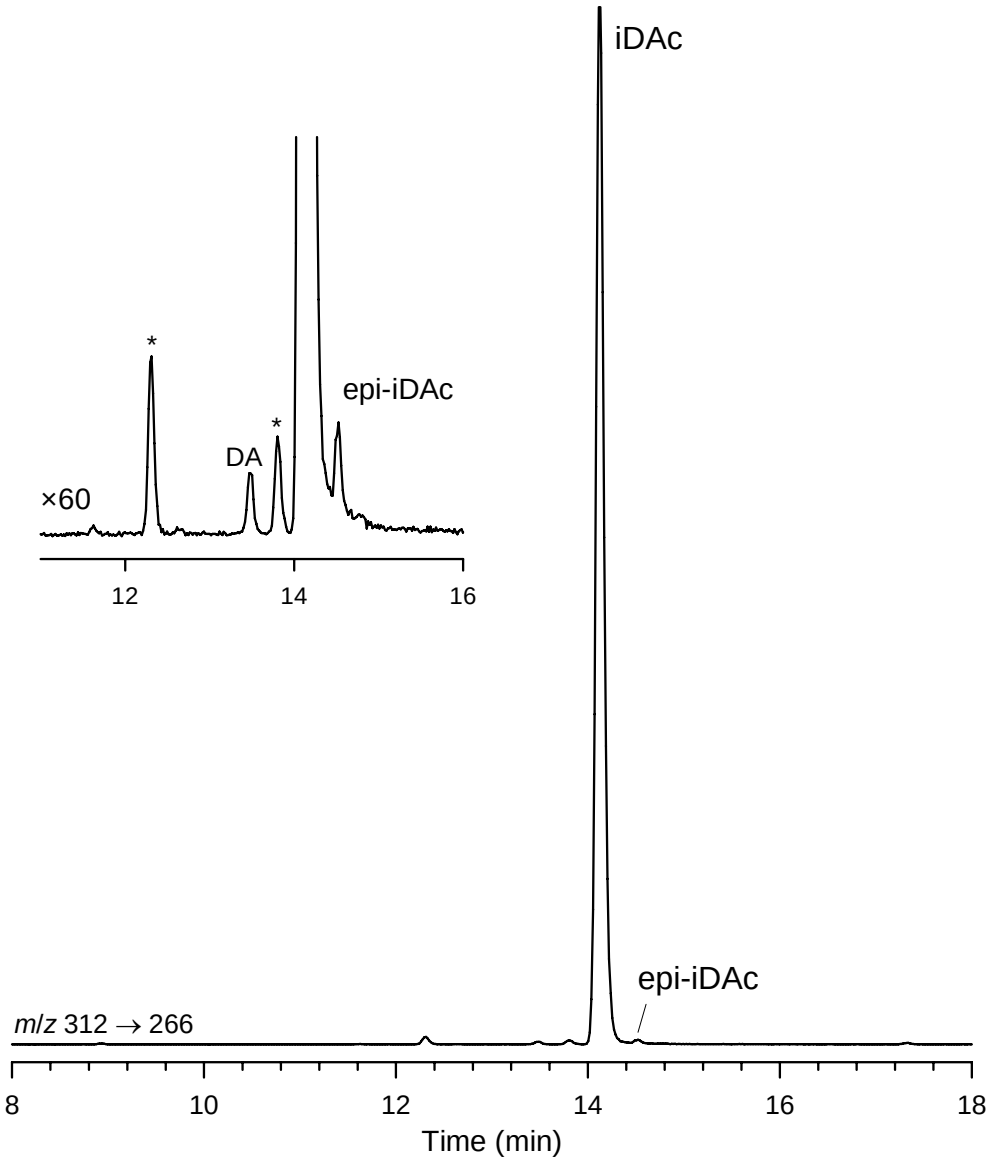


Figure 2: LC-MS/MS chromatogram of RM-iDAc. Conditions: Luna C18(2) column (4.6 × 150 mm, 3 µm); mobile phase: (A) deionized water with 0.2% formic acid; (B) acetonitrile with 0.2% formic acid; gradient: 5 to 25% B over 25 min, 0.9 mL/min at +30 °C; 25 µL injection volume. Peaks marked with an asterisk (*) are unidentified isomers of domoic acid.

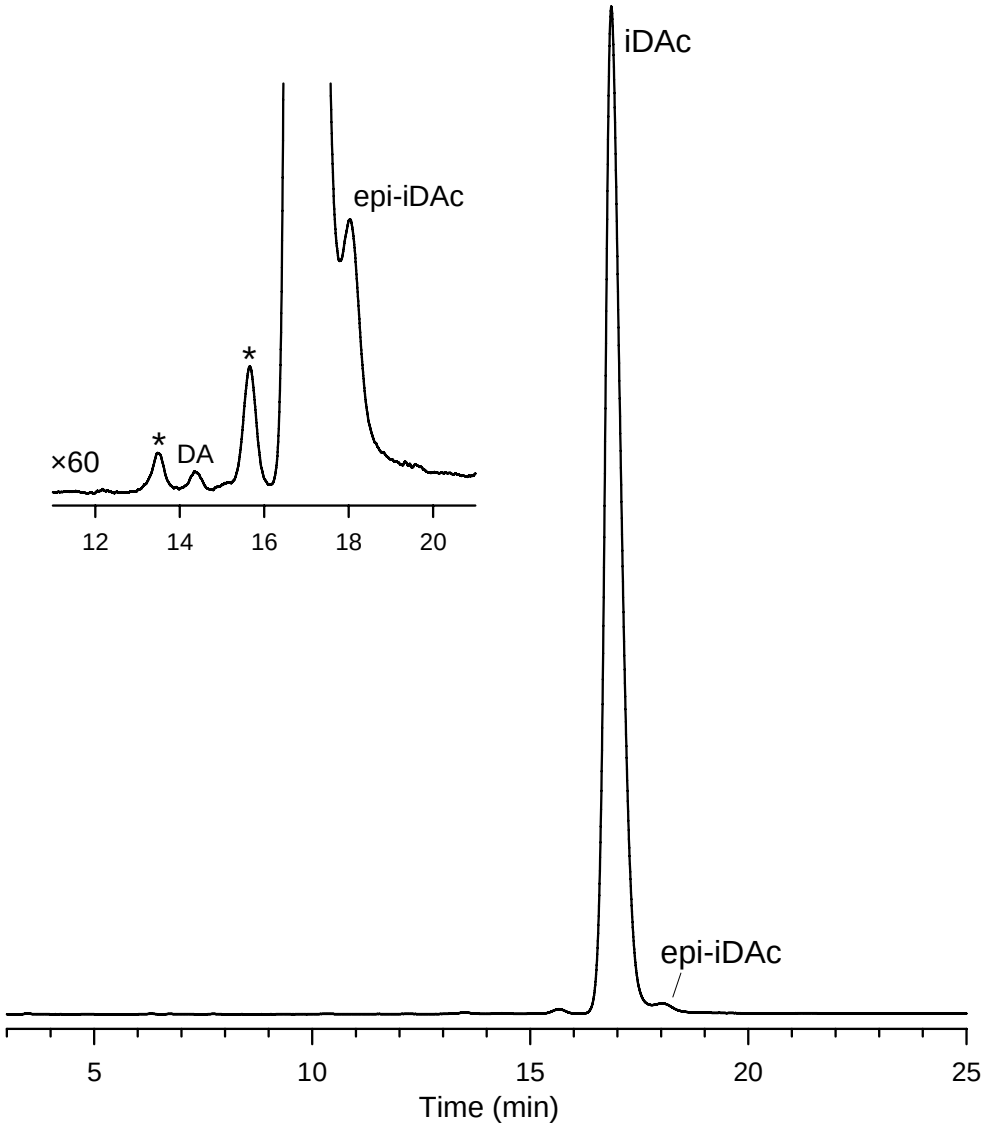


Figure 3: LC-UV chromatogram of the stock solution used for RM-iDAc. Conditions: Luna C18(2) column (2.1 × 100 mm, 3 µm); mobile phase: (A) deionized water with 0.1% trifluoroacetic acid; (B) acetonitrile with 0.1% trifluoroacetic acid; isocractic 10% B, 0.2 mL/min at +30 °C; 2 µL injection volume. UV absorbance measured at 220 nm. Peaks marked with an asterisk (*) are unidentified isomers of domoic acid.

Acknowledgements

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