



Certified Reference Material

CERTIFICATE OF ANALYSIS

NRC CRM-DA-f

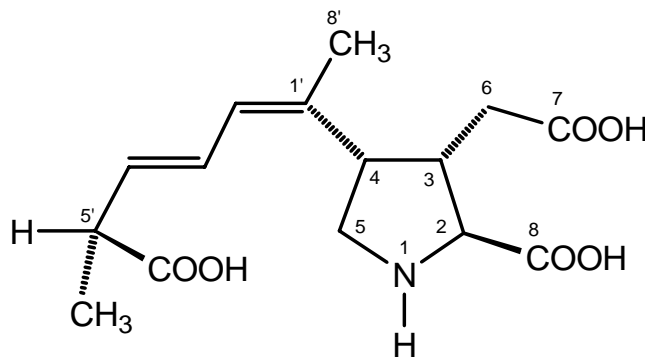
Lot#20071205

Certified Calibration Solution for Domoic Acid

Domoic Acid (DA) is a toxin responsible for incidents of amnesic shellfish poisoning (ASP) on the east and west coasts of North America [1,2]. NRC CRM-DA-f is a certified instrument calibration solution prepared to aid the analyst in the determination of domoic acid. Each ampoule contains approximately 0.5 mL of a solution of domoic acid dissolved in acetonitrile/water (1:19, v/v) at a concentration suitable for calibration of liquid chromatography experiments and for spiking shellfish control samples for recovery experiments. This is a replacement calibration solution for NRC CRM-DA-e, which was released in 2004.

Table 1. Certified concentration values for NRC CRM-DA-f.

Compound	µmoles/L (at 20°C)	µg/mL (at 20°C)	µg/g
Domoic acid + epi-domoic acid	327.1 ± 6.8	101.8 ± 2.1	102.7 ± 2.1



Domoic acid
 Molecular formula: C₁₅H₂₁NO₆
 Molecular weight: 311.33 g/mol
 Monoisotopic mass: 311.14 Da
 CAS registry no: 14277-97-5

Expiry date: 1 year from date of sale.
 Storage conditions: 4°C



1. Intended Use

NRC CRM-DA-f is a calibration solution CRM, designed for analytical method development and accurate quantitation of domoic acid. The concentration of domoic acid in CRM-DA-f is suitable for preparing a dilution series for calibration of LC-UV and LC-MS instrumentation, as well as for spiking shellfish control samples for recovery experiments.

While an accurate standard is essential for determination of calibration factors, detector response linearity and other instrument characteristics, it is not in itself sufficient to assure accurate determinations. For adequate QA/QC to be attained the complete determinative procedure (extraction, cleanup, separation and measurement) needs to be verified and this can only be done through the use of a matrix-based certified reference material (CRM). NRC CRM-ASP-Mus (domoic acid in mussel tissue, also available from the NRC Certified Reference Materials Program) is therefore recommended for testing the entire analytical procedure.

2. Source of domoic acid and structural confirmation

Domoic acid was prepared by BioVectra DCL (Charlottetown, PEI). This material was extracted from contaminated cultured blue mussels (*Mytilus edulis*), purified by chromatography and crystallization [2], and dried *in vacuo* to the anhydrous form. The molecular structure of the domoic acid used in CRM-DA-f was confirmed by NMR and UV spectroscopy, as well as tandem mass spectrometry. The NMR, UV and product ion mass spectra of domoic acid are shown in Figure 1, 2 and 3.

3. Purity assessment

The purity of the domoic acid was checked by LC with UV detection [3,4], LC combined with ion-spray mass spectrometry [5], and 500 MHz proton NMR [2]. Analyses revealed the presence of small amounts of 4 domoic acid isomers [3] (Figure 4) at approximately <2.5% (see Figure 5 and Table 2).

4. Preparation of the CRM-DA-f

The CRM-DA-f solution was prepared in degassed aqueous 5% acetonitrile at pH 5 and dispensed into amber ampoules pre-filled with argon, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

5. Homogeneity assessment

Approximately 1.2% of all ampoules produced were randomly selected and domoic acid was measured by LC-UV using the certified CRM-DA-e as a calibrant. These data were also used to verify the assigned concentration of DA in CRM-DA-f. The between-ampoule variation was measured to be ca. 1%, similar to the variation for replicate analyses of one solution, thus demonstrating acceptable homogeneity over the entire ampoule range.

6. Stability Studies

Extensive studies have been conducted to determine the stability of domoic acid in various solvents and under various conditions. Domoic acid is unstable in strongly acidic solutions and is sensitive to light and oxygen. A 9-month stability study was performed on domoic acid solutions stored at a variety of temperatures (-80°C, -16°C, 4°C, 20°C and 50°C). Aqueous acetonitrile at pH 5 to 7 was determined to be an appropriate solvent and no significant loss of domoic acid was observed at 4°C during the course of that study. Less than 1% degradation was observed at 20°C, although 12% loss of material was observed at 50°C after 8 months. At high temperatures, there is also a gradual isomerization of



domoic acid, particularly to *epi*-domoic acid. It is important to note that a very gradual decomposition occurred when the solution was kept in a conventional freezer (approximately -12°C), probably due to freeze/thaw events. Other more extended studies have demonstrated excellent long term stability of DA solutions stored at 4°C.

7. Certified value

The certified value of $327.1 \pm 6.8 \mu\text{mol/L}$ (Table 1) for the sum of domoic acid and its epimer, C5'-*epi*-domoic acid, is based on the results obtained at NRC by two independent analytical methods: gravimetric measurement and quantitative nuclear magnetic resonance (QNMR) spectroscopy [6]. QNMR measurements were performed relative to USP-grade caffeine solutions. Liquid chromatography with UV absorbance detection (LC-UV) quantitation was also performed on the CRM as a check, with calibration based on NRC CRM-DA-e, which was certified in 2004 by the same analytical methods.

Pure domoic acid in solution has been found to gradually isomerize, especially to the diastereomer, C5'-*epi*-domoic acid (*epi*-DA); therefore, a mixture will inevitably result on long-term storage of any standard. Since *epi*-DA has a UV spectrum identical with that of DA, the relative molar response factors in LC with UV detection are identical and relative proportions can be recalculated at any time. Under some LC conditions, domoic acid and *epi*-DA do not resolve; this does not present a problem, and in fact makes analysis simpler. Analysts are advised to always base their instrument calibration on the sum of the peak areas of domoic acid and the diastereomer. The certified total concentration of DA and *epi*-DA is reported in Table 1. The relative concentrations of individual isomers are provided in Table 2 as non-certified information values and were determined from LC-UV peak areas corrected by the published extinction coefficients of domoic acid (26,300) and its isomers [7].

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

Compound	λ_{max} (nm)	ϵ_{max}^7 (L cm ⁻¹ mole ⁻¹)	Molecular Weight	Concentration ($\mu\text{g/mL}$) (at 20°C)
Domoic acid	242	26300	311.3	100.7
C5'- <i>epi</i> -domoic acid	242	26300	311.3	1.1
Isodomoic acid E	241	26000 (est)	311.3	0.013
Isodomoic acid D	244	26000 (est)	311.3	0.41
Isodomoic acid A	220	8300 (est)	311.3	0.52

8. Uncertainty

In general, guidelines for CRM producers suggest that the overall uncertainty estimate for a CRM (U_{CRM}) should include uncertainties associated with batch characterization (u_{char}), between-bottle variation (u_{hom}), and instability associated with long-term storage and short term transport (u_{stab}) [8-9]. These components can be combined as:

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

where k is the coverage factor (generally 2 or 3).



All of these sources of uncertainty were considered for the estimate of the final uncertainty in the certified value of CRM-DA-f. However, only the uncertainty associated with batch characterization (u_{char}) was deemed significant in this case. As this CRM is a true solution, u_{hom} can be considered insignificant and safely ignored, as solutions are inherently homogenous [10]. Nevertheless, an extensive homogeneity study was performed (see Section 6), and the between-bottle variance was determined to be similar to the measurement variance. The uncertainty due to stability (u_{stab}) was also disregarded as stability studies have been performed on this CRM and other CRM-DA preparations that extend past the specified shelf life of the CRM, with no observable loss of material [11]. In addition, recent enhancements to CRMP's shipping and packaging practices have significantly reduced any chance of decomposition due to elevated temperatures during transport. Finally, domoic acid exhibits reasonable stability at room temperature (see Section 6), in the event that a delay in shipping occurs.

All reasonable sources of error related to the characterization of CRM-DA-f were considered and quantified, whereby quantitative measurements by QNMR contributed the most significantly with a relative standard uncertainty of 0.0104. Applying a coverage factor of 2 resulted in a final relative standard uncertainty in the certified value of 0.021.

9. Storage Instructions

To ensure the stability of domoic acid, this material should be stored at +4°C in the dark at a pH from 5 to 7 in a vial with preferably no oxygen present. As indicated above, our stability studies have revealed that there is a very gradual decomposition when the solution is kept in a conventional freezer (approximately -12°C), probably due to freeze/thaw events. Therefore, do not freeze the solution.

10. Expiry

If stored unopened under the conditions recommended in Section 9, the certified concentration of CRM-DA-f is valid for 1 year from the date of sale. Please refer to the label on the original packaging for the expiration date.

11. Instructions for Use

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents should be thoroughly mixed. The ampoule should be inverted several times, then held upright, tapped to ensure that most of the solution drains to the bottom, and 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 unopened for more than a few minutes. It is recommended that CRM-DA-f should not be evaporated to dryness because of the potential of losses on glass surfaces and increased isomerization of domoic acid. A useful procedure that ensures accurate dilutions involves using a balance to determine weights of the dispensed aliquot and the final diluted solution, assuming that acetonitrile/water (1:19) is used as diluent (the density of the CRM solution is 0.9915 at 23.5°C).

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.

12. Safety Instructions

If sufficient quantities are ingested, domoic acid can cause severe neurological symptoms such as short term memory loss and even death. Inhalation or ingestion of acetonitrile is harmful and may be fatal.



Inhalation may cause pulmonary edema and cyanosis. 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 material safety data sheet (MSDS) is available for CRM-DA-f.

13. References

1. Quilliam, M.A. and Wright, J.L.C., "The amnesic shellfish poisoning mystery", *Analyt. Chem.* 61: 1053A-60A (1989).
2. Wright, J.L.C., Boyd, R.K., deFreitas, A.S.W., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCulloch, A.W., McInnes, A.G., Odense, P., Pathak, V., Quilliam, M.A., Ragan, M., Sim, P.G., Thibault, P., Walter, J.A., Gilgan, M., Richard, D.J.A., and Dewar, D., "Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern P.E.I.", *Can. J. Chem.* 67: 481-490 (1989).
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4. Quilliam, M.A., Xie, M., and Hardstaff, W.R., "Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood", *J. AOAC Int.* 78: 543-554 (1995).
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6. Burton, I.W., Quilliam, M.A., and Walter, J.A. (2005). "Quantitative ^1H NMR with external standards: Use in preparation of calibration solutions for algal toxins and other natural products", *Analyt. Chem.* 77: 3123-3131 (2005).
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11. Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993).



14. Figures

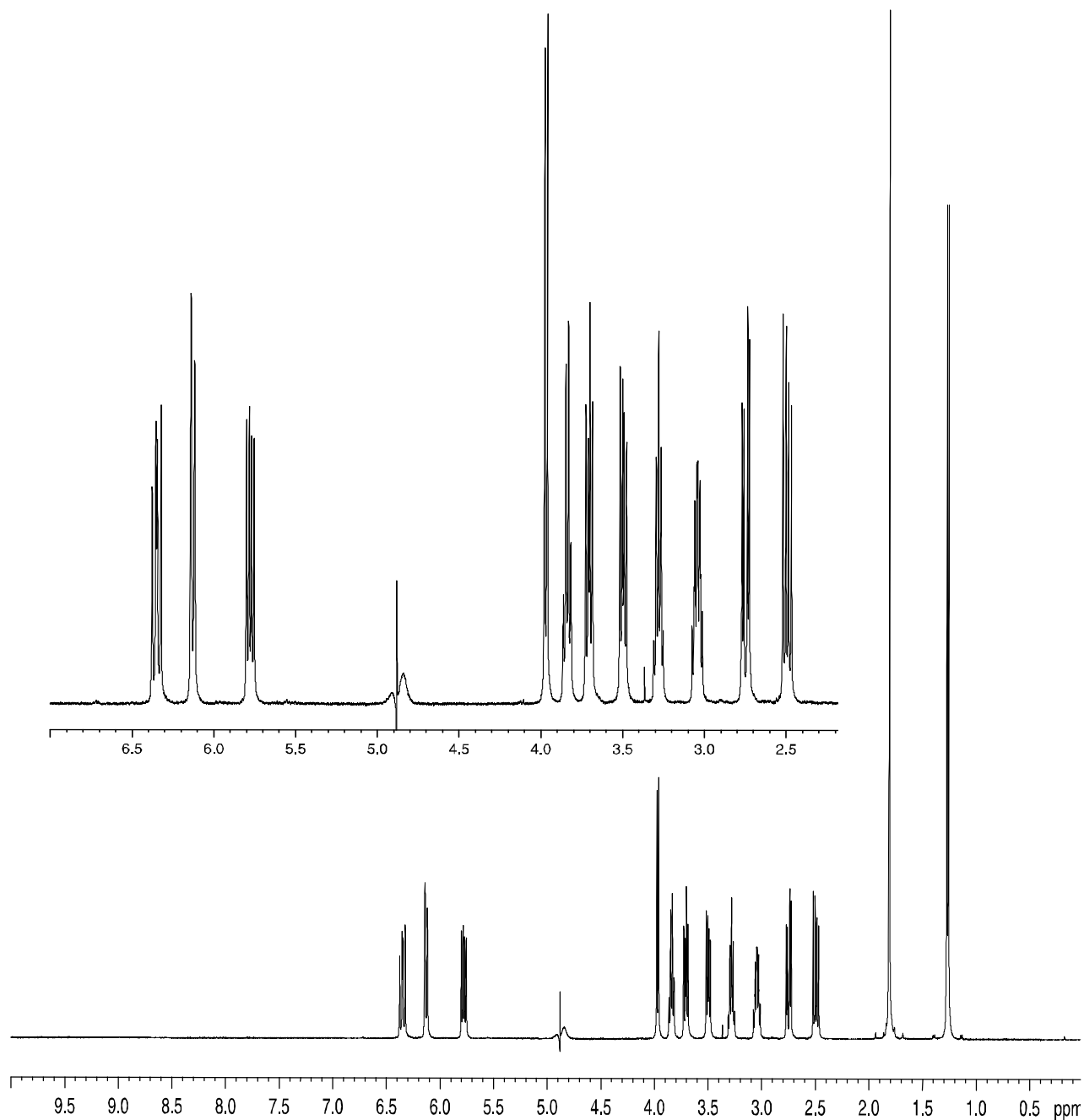


Figure 1. Proton NMR spectrum of domoic acid.



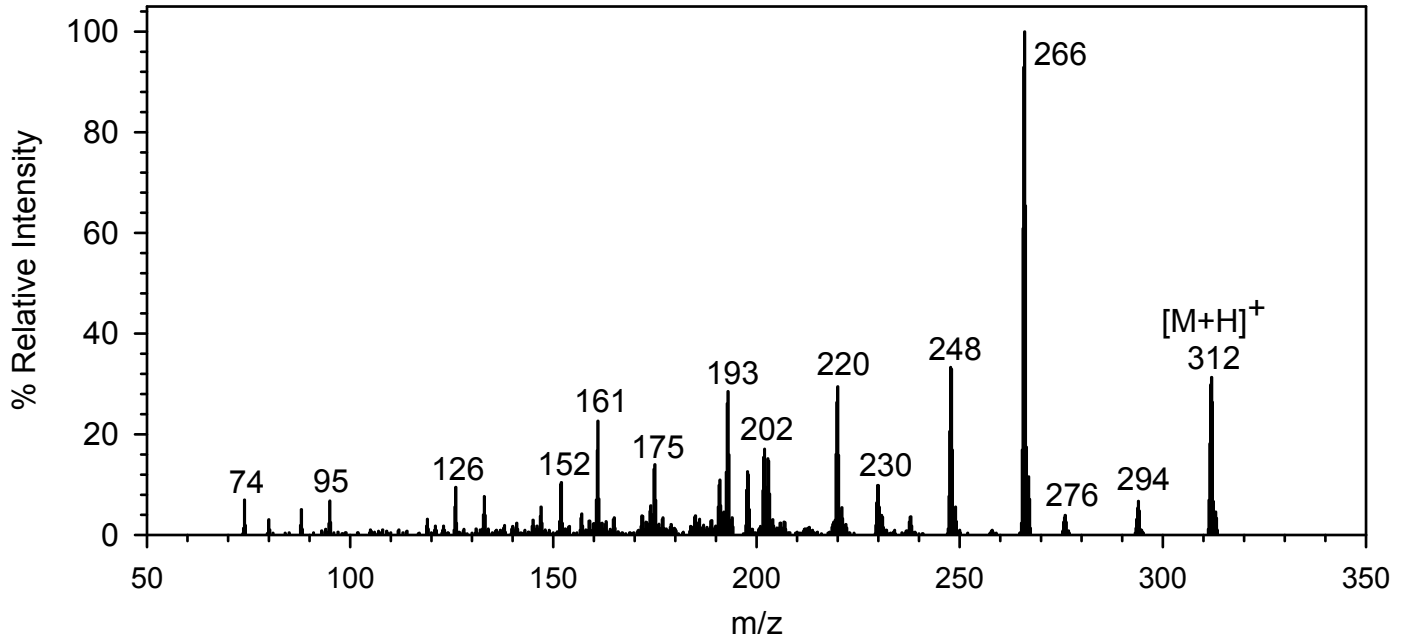


Figure 2. Product ion mass spectrum of domoic acid.

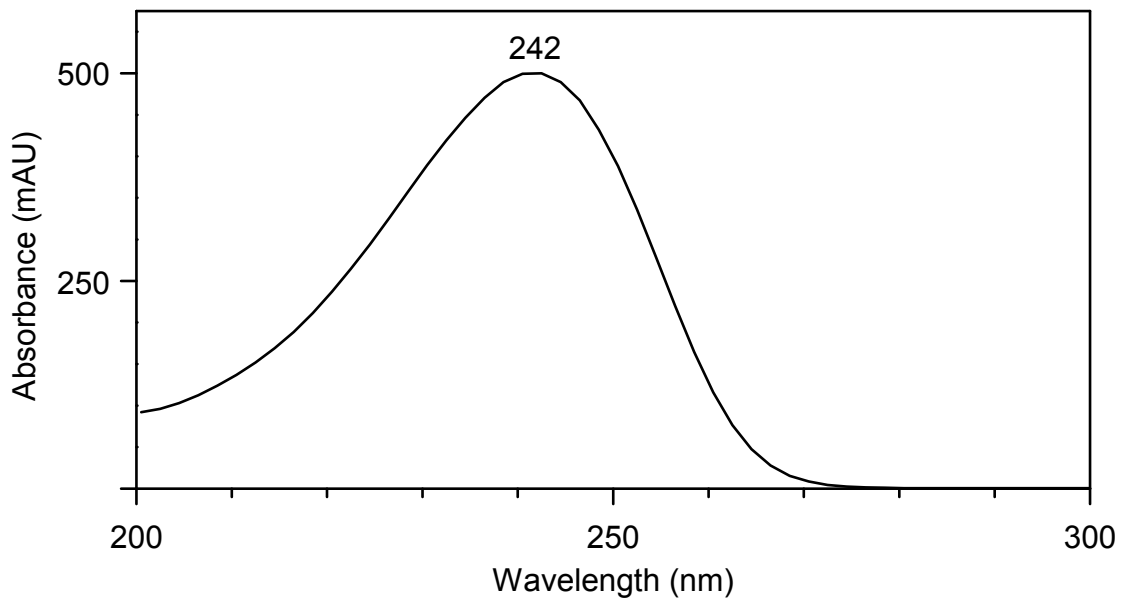


Figure 3. UV absorbance spectrum of domoic acid.

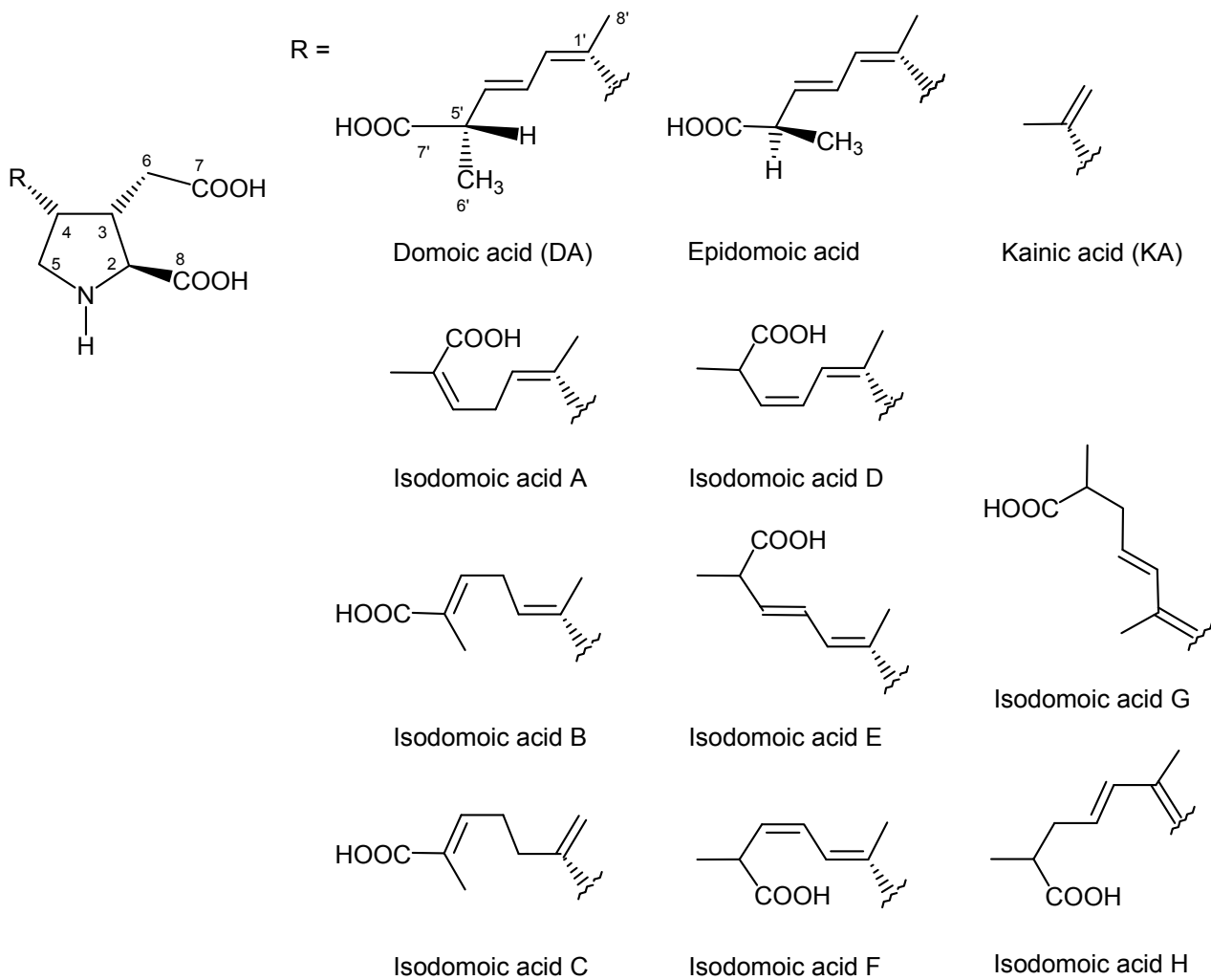


Figure 4: Structures of domoic acid and its known isomers reported in the literature.

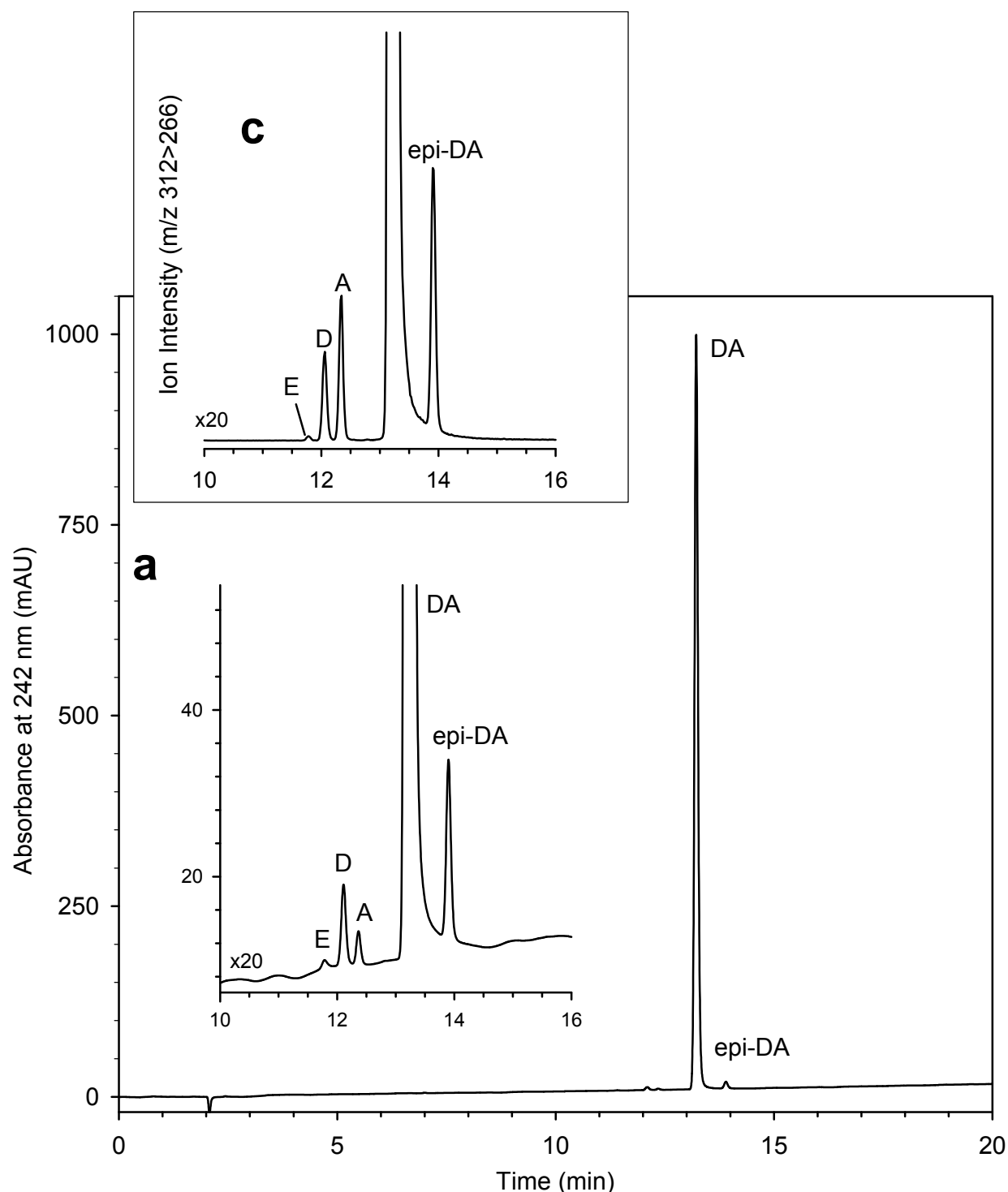


Figure 5: Gradient analysis of CRM-DA-f by HPLC with detection by (a,b) UVD (242 nm) and (c) electropray MS (multiple reaction monitoring, m/z 312.2 > m/z 161.1). Conditions: 15 cm x 4.6 mm i.d. column packed with 3 μ m Luna C18(2) (Phenomenex), maintained at 30°C; 0.9 mL/min acetonitrile/water/formic acid from 5.0:94.8:0.2 to 25.0:74.8:0.2 over 25 min; 10 μ L injection volume for (a) and 25 μ L for (b) and (c). Peak identities: DA = domoic acid; A, D, E = isodomoic acids A, D and E; *epi*-DA = C5'-diastereomer of domoic acid (see Figure 1). Instrumentation: Agilent 1100 LC and UVD with standard flow cell; Sciex API 4000 triple quadrupole MS.

15. Acknowledgements

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16. This document should be cited as:

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