Certificate of Analysis

NRC·CNRC Certified Reference Material

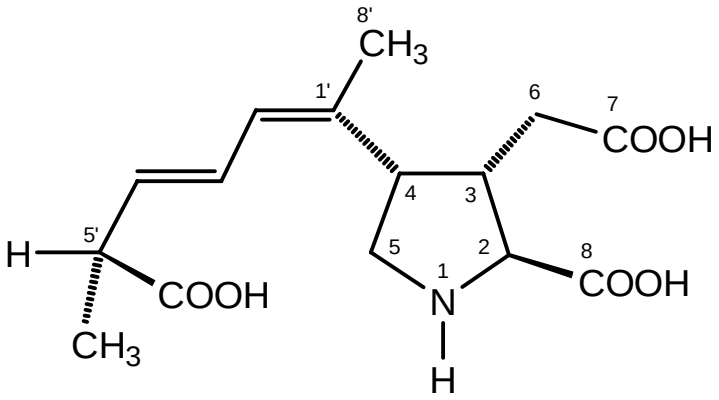
CRM-DA-g (Lot# 20140730)

Certified Calibration Solution for Domoic Acid

Domoic Acid (DA) is a toxin responsible for incidents of amnesic shellfish poisoning (ASP) [1, 2] (see Figure 1). CRM-DA-g is a certified instrument calibration solution prepared to aid the analyst in the determination of DA. Each ampoule contains approximately 0.5 mL of a solution of DA 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. Table 1 lists the certified values along with their expanded uncertainties (U_{CRM}). Table 2 shows information values for the individual DA isomers.

Table 1: Certified concentration values and associated uncertainties for CRM-DA-g.

Compound	µmol/L (at +20 °C)	µg/mL (at +20 °C)	µg/g
Domoic Acid + *epi*-Domoic Acid	332 ± 11	103.3 ± 3.4	104.2 ± 3.5



Domoic acid
CAS registry no: 14277-97-5
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 °C

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

Compound	λ_{max} (nm)	ε_{max}[7] ($L\ cm^{-1}\ mol^{-1}$)	Molecular Weight	Concentration* (µg/mL) (at +20 °C)
Domoic acid	242	26,300	311.3	102.4
C5'-*epi*-domoic acid	242	26,300	311.3	0.88
Isodomoic acid E	241	~ 26,000	311.3	0.20
Isodomoic acid D	244	~ 26,000	311.3	0.76
Isodomoic acid A	220	~ 8,300	311.3	1.1

* These concentrations are not certified

Intended Use

CRM-DA-g is designed for use as an instrument calibration solution to aid the analyst in the determination of DA in plankton and shellfish sample extracts. The concentration of DA in CRM-DA-g is suitable for preparing a dilution series for calibration of instrumentation, such as liquid chromatography with detection by ultraviolet absorbance (LC-UV) and mass spectrometry (LC-MS), as well as for spiking shellfish control samples for recovery experiments.

Instructions for Storage and Use

CRM-DA-g should be stored at +4 ºC in the dark. Stability studies have revealed that there may be a very gradual decomposition when the solution is kept in a conventional freezer (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 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 opened for more than a few minutes. It is recommended that CRM-DA-g should not be evaporated to dryness because of the potential of losses on glass surfaces and increased isomerization of DA. 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 the diluent (the density of the CRM solution is 0.9917 g/mL at +25.5 ºC). Diluted solutions should be at a pH from 5 to 7 and stored in a vial with minimum headspace, preferably with no oxygen present.

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-g

Purified DA was prepared by BioVectra DCL (Charlottetown, PEI). The molecular structure was confirmed by NMR and UV spectroscopy, as well as tandem mass spectrometry. The NMR, UV and product ion mass spectra of DA are shown in Figures 2, 3 and 4, respectively. The purity of the DA was checked by LC-UV [3,4], LC-MS/MS [5], and 500 MHz proton NMR [2]. Analyses revealed, in addition to C5'-*epi*-domoic acid (*epi*-DA), the presence of small amounts of 3 additional DA isomers [3] at approximately 2% of the total mass (see Figures 5 and 6). 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 DA (26,300) and its isomers [7]. The CRM-DA-g solution was prepared in degassed aqueous 5% acetonitrile at pH 7 and dispensed into amber ampoules pre-filled with argon, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Analytical Methods and Value Assignment

The certified value was obtained using three independent analytical methods: gravimetric measurement, quantitative nuclear magnetic resonance spectroscopy [6] and LC-UV (which used CRM-DA-f Lot 20071205 for calibration). The certified value is comprised of the combined concentration of DA and its C5'-diastereomer, C5'-epiDA. Since DA has been found to isomerize gradually in solution to its diastereomer, *epi*-DA, 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-UV are identical and relative proportions can be recalculated at any time. Analysts are advised to base their instrument calibration on the sum of the peak areas of DA and *epi*-DA.

Homogeneity

A representative number (n = 26) of ampoules produced were randomly selected and analyzed by LC-UV. Results were evaluated using ANOVA and no heterogeneity was detected across the entire fill series.

Stability Studies

Extensive studies have been conducted to determine the stability of DA in various solvents and under various conditions. DA is unstable in strongly acidic solutions and is sensitive to light and oxygen. A 9 month stability study was performed on DA solutions stored at a variety of temperatures (-80, -16, +4, +20 and +50 °C). Aqueous acetonitrile at pH 5 to 7 was determined to be an appropriate solvent and no significant loss of DA 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 DA, particularly to *epi*-DA. 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. Extended studies on previous CRM-DA preparations have demonstrated excellent long-term stability of DA solutions stored at +4 °C.

Uncertainty

All reasonable sources of error related to the characterization of CRM-DA-g were considered and quantitated. A combined uncertainty component relating to the three analytical methods used is included (u_{char}). The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}), between-bottle variation (u_{hom}) and instability during long-term 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-g.

Uncertainties	[µmol/L]
u_{char}	4.5
u_{hom}	negligible
u_{stab}	3.1
U_{CRM} (k=2)	11

Safety Instructions

If sufficient quantities are ingested, DA 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-g.

Period of Validity

If stored unopened at the recommended storage condition of +4 °C, the certified concentration of CRM-DA-g is valid for 1 year from the date of sale**.**

Metrological Traceability

Results presented in this certificate are traceable to the SI (*Le Système international d'unités*) through gravimetrically prepared standards of established purity.

Quality Management (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented NRC MSS Quality System, which conforms with the requirements of ISO/IEC 17025 and ISO Guide 34.

The Quality Management System supporting 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 certificate of approval is available upon request.

References

1. Quilliam MA, Wright JLC (1989). The amnesic shellfish poisoning mystery. *Anal Chem* 61: 1053-1060.
2. Wright JLC, Boyd RK, de Freitas ASW, Falk M, Foxall RA, Jamieson WD, Laycock MV, McCulloch AW, McInnes AG, Odense P, Pathak VP, Quilliam MA, Ragan MA, Sim PG, Thibault P, Walter JA, Gilgan M, Richard DJA, Dewar D (1989). Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. *Can J Chem* 67: 481-490.
3. Quilliam MA, Sim PG, McCulloch AW, 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.
4. Quilliam MA, Xie M, Hardstaff WR (1995). Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood. *J AOAC Int* 78: 543-554.
5. Quilliam MA, Thomson BA, Scott GJ, Siu KWM (1989). Ion-spray mass spectrometry of marine neurotoxins. *Rapid Commun Mass Spectrom* 3: 145-150.
6. Burton IW, Quilliam MA, 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. Falk M, Walter JA, Wiseman PW (1989). Ultraviolet spectrum of domoic acid. *Can J Chem* 67: 1421-1425.
8. Pauwels J, Lamberty A, Schimmel H (2000). Evaluation of uncertainty of reference materials. *Accred Qual Assur* 5: 95-99.

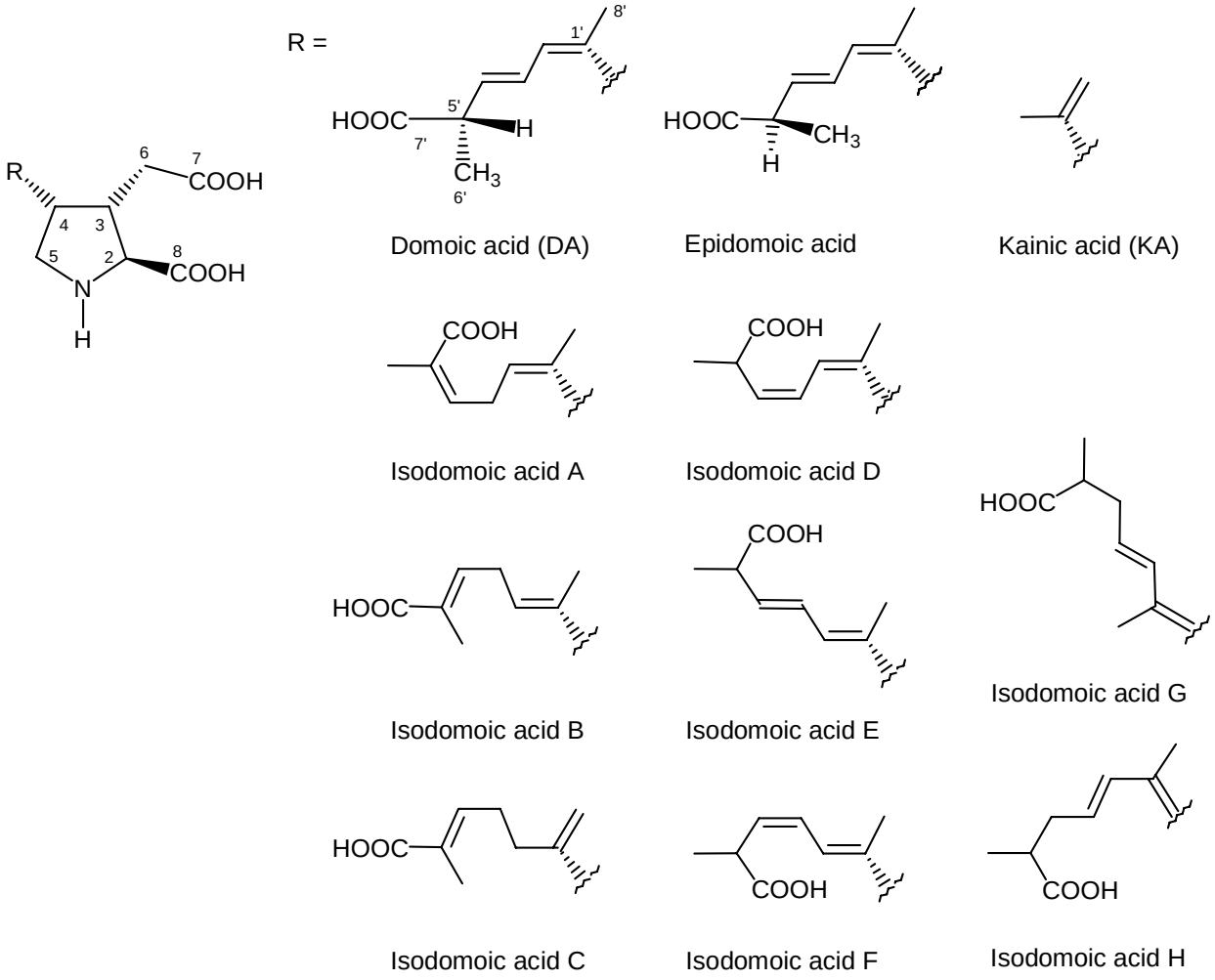


Figure 1: Structures of DA and its known isomers reported in the literature.

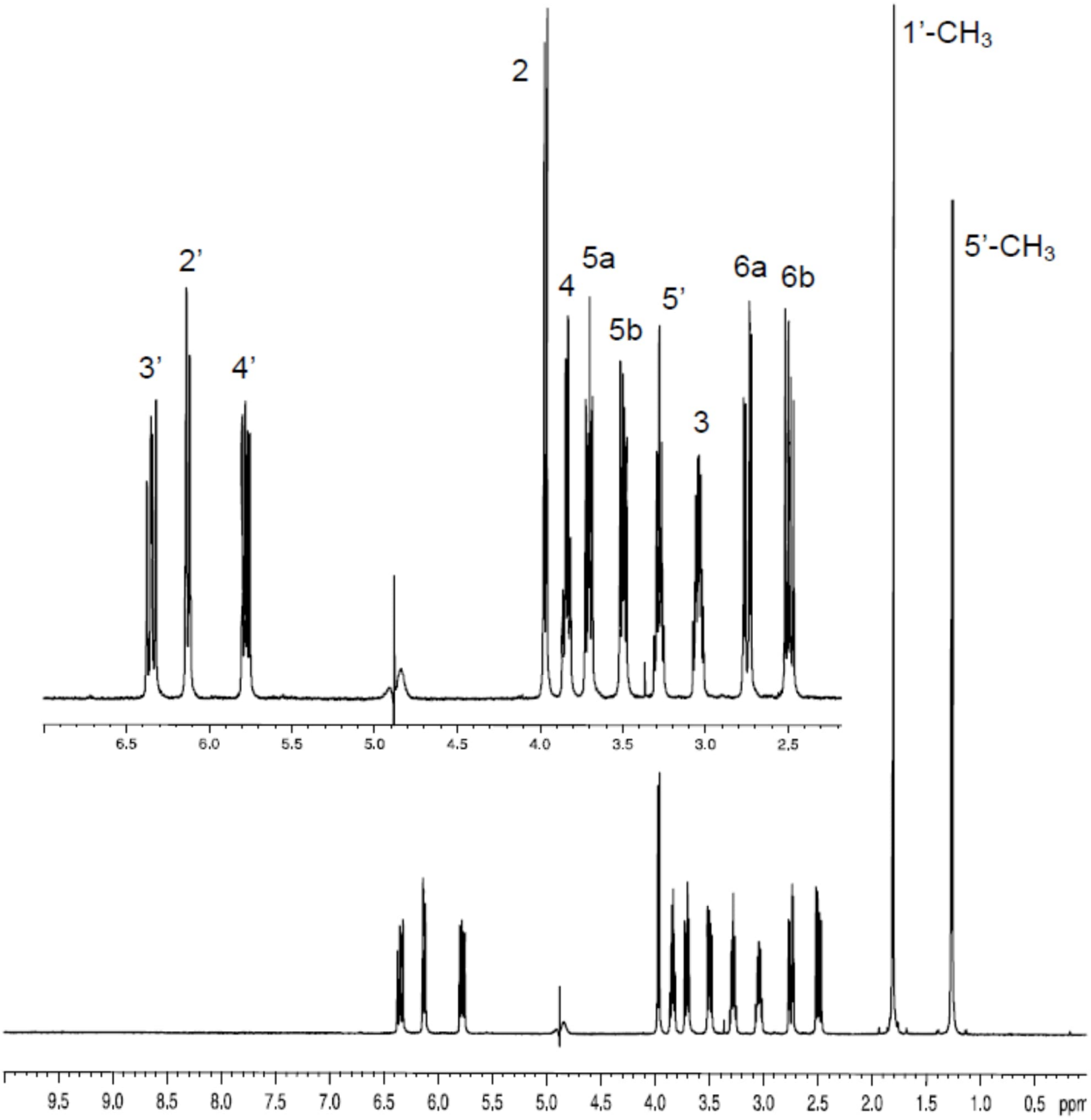


Figure 2: Proton NMR spectrum of DA.

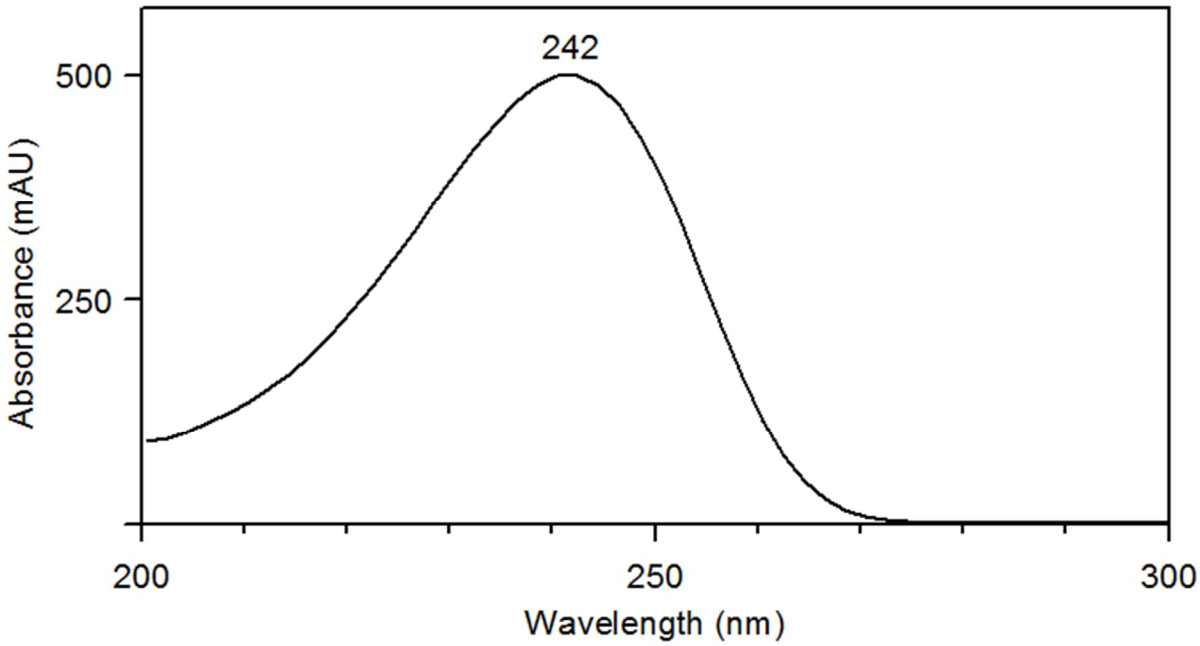


Figure 3: UV absorbance spectrum of DA.

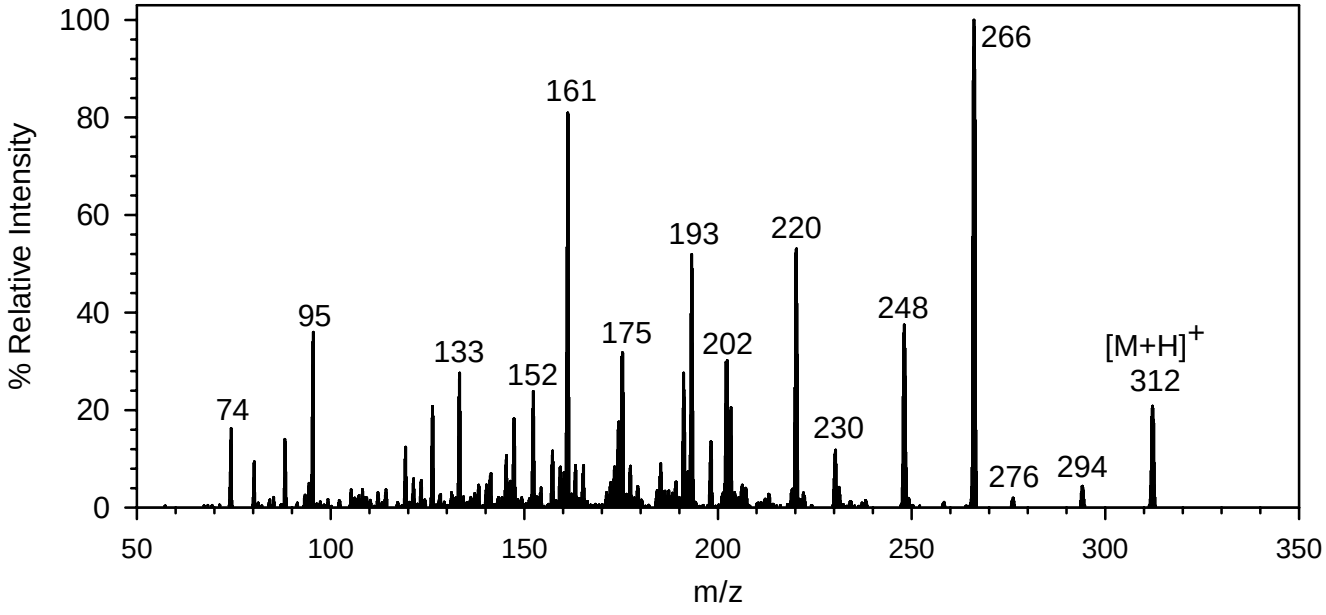


Figure 4: Product ion mass spectrum of DA.

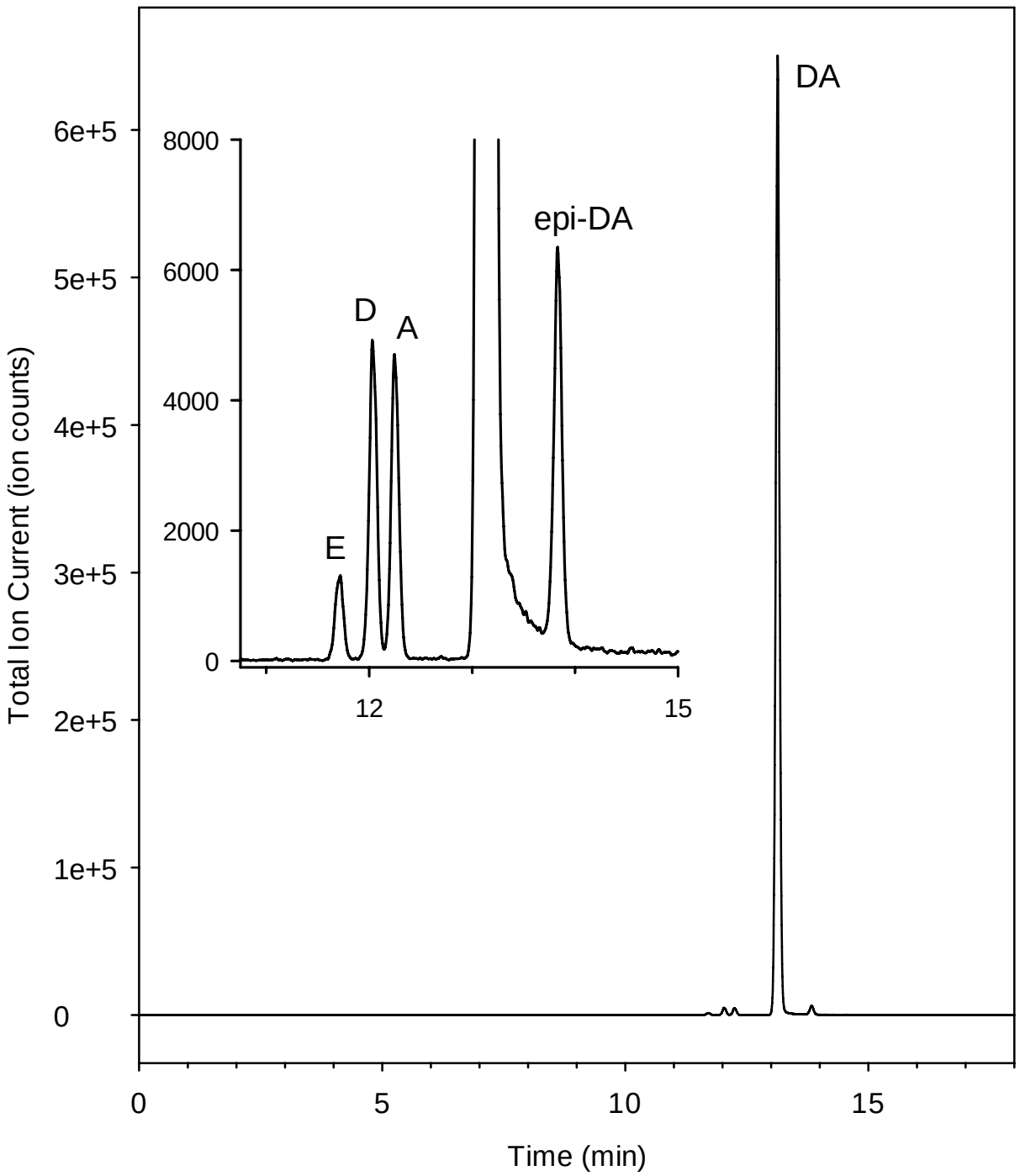


Figure 5: Analysis of CRM-DA-g by gradient elution LC-MS/MS. Conditions: electrospray ionization; selected reaction monitoring, *m/z* 312 > 266; 15 cm x 4.6 mm i.d. column packed with 3 µm Luna C18(2) (Phenomenex) maintained at +30 °C; gradient from 5 to 25% B over 25 min with A = water with 0.1% formic acid and B = acetonitrile with 0.1% formic acid. Peak identities: DA = domoic acid; E, D, A = isodomoic acids E, D and A; *epi*-DA = C5'-diastereomer of DA (Figure 1).

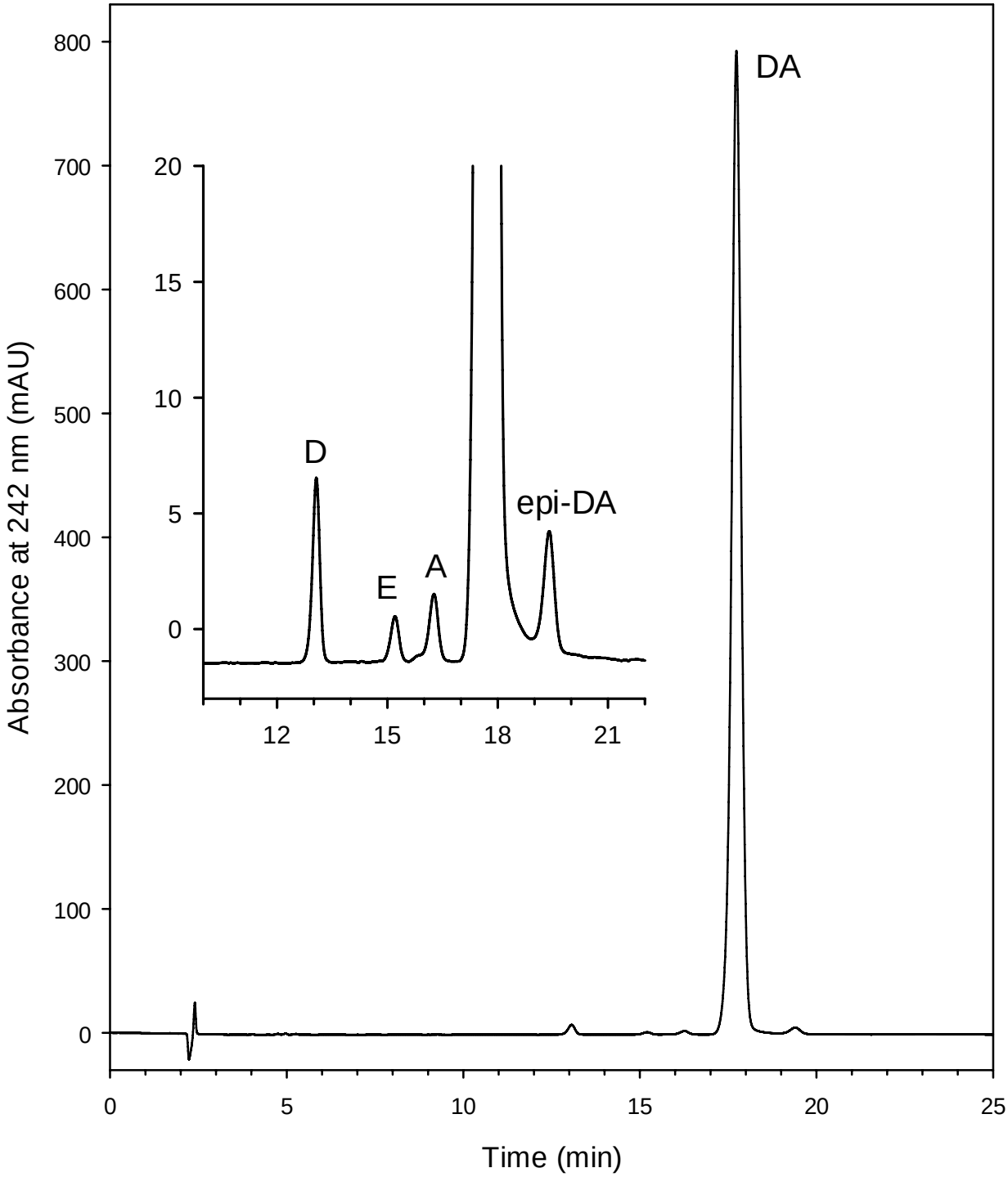


Figure 6: Analysis of CRM-DA-g by isocratic elution LC-UV. Conditions: 242 nm absorbance detection; 15 cm x 4.6 mm i.d. column packed with 3 µm Luna C18(2) (Phenomenex) maintained at +30 °C; 3 µL injection volume; 900 µL/min of 10% acetonitrile in water with 0.1% trifluoroacetic acid. Peak identities: DA = domoic acid; D, E, A = isodomoic acids D, E and A; *epi*-DA = C5'-diastereomer of DA (Figure 1).

Acknowledgements

The following staff members of the Measurement Science and Standards portfolio at NRC contributed to the production and certification of CRM-DA-g: D. Beach, S. Crain, S. Giddings, W. Hardstaff, P. LeBlanc, N. Lewis, P. McCarron, R.A. Perez, M. Quilliam, K. Reeves, K. Thomas.

This document should be cited as:

K. Thomas, R.A. Perez, S. Crain and M.A. Quilliam, “CRM-DA-g, certified calibration solution for domoic acid”, Biotoxin Metrology Technical Report CRM-DA-g-20140730, National Research Council Canada, Halifax, November 2014.
DOI https://doi.org/10.4224/crm.2014.da-g.20140730

Date of issue: November 2014
Document version: 20220321

Revised: March 2022 (DOI added and editorial updates)

Approved by:

Pearse McCarron, Ph.D.
Discipline Leader, Biotoxin Metrology
Measurement Science and Standards

This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our qualified vendors.

Comments, information and inquiries should be addressed to:

National Research Council Canada
Measurement Science and Standards
1411 Oxford Street
Halifax, Nova Scotia B3H 3Z1

Telephone: 1-902-426-8281
Fax: 1-902-426-5426
Email: CRM-MRCBiotoxin-Biotoxines@nrc-cnrc.gc.ca