Certificate of analysis

Certified reference material code: CRM-02-DA

Name: Certified Reference Material of Domoic acid Batch: 25-001

Description: This Certified Reference Material (CRM) is a solution of Domoic acid and C5'-epi-domoic acid (CAS Number 14277-97-5 for Domoic acid) in water with 5% of acetonitrile.

Intended use: Calibration of equipment or a measurement procedure. Establishing metrological traceability. Method validation. Quality control of a measurement or measurement procedure. For laboratory use only.

Certified values and uncertainties: The certified concentration given below is based on results obtained from the gravimetric preparation of this solution previous quantity determination by ^{1}H-qNMR in Bruker NEO 750 MHz equipment (Universidad de Santiago de Compostela).

Concentration of Domoic acid and C5'-epi-domoic acid (95% Confidence Interval)	**(329 ± 26) µmol / kg**
	(102.5 ± 8.2) µg / g

The starting material is measured by ^{1}H-qNMR against a Sucrose certified reference material followed by gravimetric preparation using high precision balances calibrated with SI-traceable weights. Consequently, the quantified values are traceable to the International System of Units (SI) via an unbroken chain of comparisons through validated methodology.

Homogeneity was evaluated by analysis of variance on 2% of stratified randomly selected ampoules over the entire batch (6 replicate analyses per ampoule). A possible heterogeneity is hidden by the method repeatability. Therefore, a maximum possible heterogeneity was calculated as u_{bb}, 0.14%. The Stability studies after storage of selected units were performed at 4°C for an expected storage time of 12 months. Using the data from the long-term study, the uncertainty due to possible degradation was calculated as u_{lts}, 0.67%. Both studies were verified against an independently prepared calibration solution.

The components of Standard Uncertainty include related uncertainties due to characterization, heterogeneity, long term instability, short term instability (dispatch), and bulk assay, Table 1.

The results are expressed as the certified value ± the expanded uncertainty (calculated by combination of the squared contribution values). Estimated expanded uncertainty with a coverage factor k = 2, corresponding to a level of confidence of about 95 %, as defined in the Guide to the expression of uncertainty in measurement, ISO 33405.

The certificate is a summary of an extensive program of work involving selection and purification of the material, assessing its suitability and measuring the properties to be certified.

Expiration of Certification: *This certificate of CRM-02-DA, 25-001 batch is valid 12 months after purchase date within the measurement uncertainties specified*, provided the CRM is handled and stored in accordance with the instructions given in this certificate. This validity may be extended if further evidence of stability becomes available.

Maintenance of Certification: The material will be subjected to regular Laboratorio CIFGA stability monitoring programme to control its further stability over the period of its

certification. If substantive changes occur that affect the certification before the expiration date, Laboratorio CIFGA will notify the purchaser.

Instructions for proper handling use: This material has been classified as non-hazardous, but it should still be handled with care. Read MSDS before using. Material Safety Data sheet for information regarding CRM-02-DA is enclosed in the package. Use proper disposal methods.

Before opening an ampoule, it must be guaranteed that the content is a liquid and properly mixed to ensure homogeneity. Sample aliquots for analysis should be handled at 15°C to 25°C immediately after opening the ampoules and should be processed without delay for the certified value to be valid within the stated uncertainty. Ampoule should not remain open longer than five minutes due to solvent volatility and air contamination.

Storage conditions: The original sealed ampoules should be stored at temperatures below/at 4 ºC (refrigerator) until use. Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied.

Source and Preparation of Material: The Domoic acid used in the preparation of this CRM was isolated from samples of scallops collected during outbreak in the coast of Galicia and purified via HPLC preparative. The final solution was prepared by weighing and mixing a DA solution into aqueous 5% acetonitrile until completely homogenization. Identity of the material was confirmed by ^{1}H-NMR (Figure 1), HPLC-UV and HPLC-MS(Figure 2). Finally, 0.5 mL aliquots of toxin solution were dispensed into 1.5 mL amber argon-filled glass ampoules, which were then flame sealed.

[#] Non-certified and non-accredited data:

Taking into account the density of 5% acetonitrile aqueous solution at 20 ºC ($\rho^{20} = 0.993\ \mathrm{g/mL}$), *non-certified volumetric concentration* values are:

Concentration of Domoic acid and C5'-epi-domoic acid (95% Confidence Interval)	**(327 ± 26) µM**
	(101.8 ± 8.1) µg / mL

Concentration of Domoic acid and impurities are neglected in order to apply solution density value.

Purity analysis was performed by qNMR and HPLC. Results of nuclear magnetic resonance (NMR) analysis were consistent with structure. Both analyses indicated that there was no significant contribution from other analogues. Under chromatographic analyses the presence of small amounts of domoic acid isomers (Figure 2) are revealed.

Purity determination was performed by impurities quantification and subtracting the sum from 100%, the final molar ratio purity is ≥ 98%. *No purity correction over certified concentration should be done.*

Additional lectures:

1. UNE-EN ISO 17034 *General Requirements for the competence of reference material producers.*

2. ISO 33405 *Reference materials - Approaches for characterization and assessment of homogeneity and stability.*

Figures

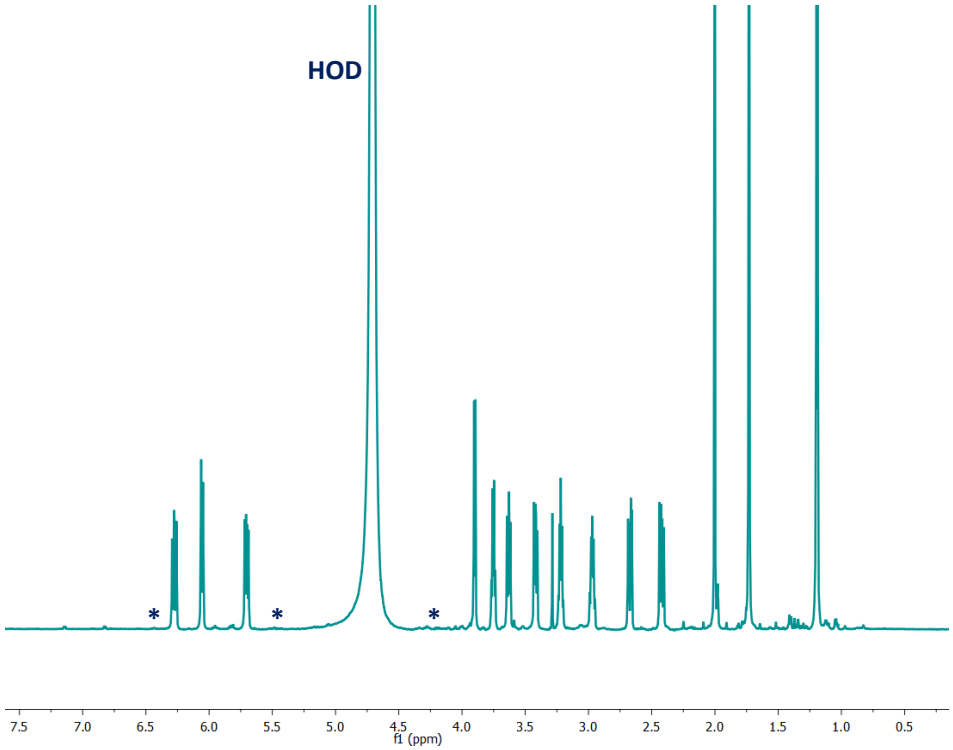


Figure 1. ^{1}H-NMR spectrum of Domoic acid, Bruker NEO 750 MHz, 13mM solution in D_2O used in preparation of CRM-02-DA batch 25-001. (*) Tentative Isodomoic acid E identification according to chemical shift and coupling pattern.

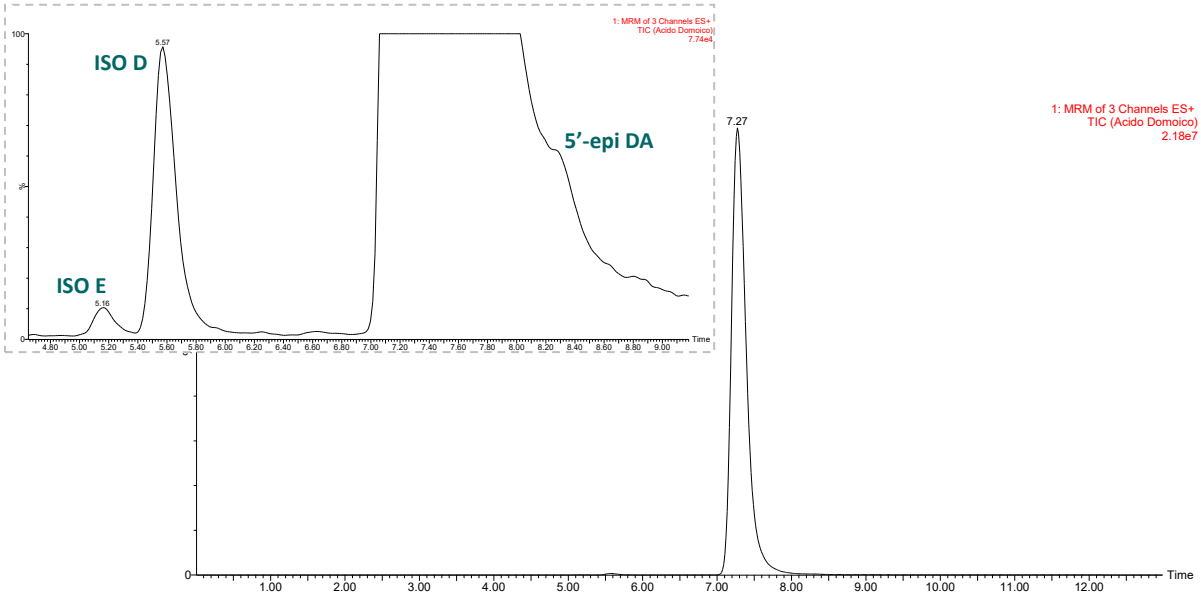


Figure 2. Analysis of the CRM-02-DA (25-001) by UPLC-MS/MS. Conditions: ACQUITY UPLC HSS T3 1.7µm, 100x2.1 mm. Mobile phases: (A) Water and (B) acetonitrile, both with 0.1% formic acid. Isocratic elution with 92% mobile phase A; flow rate: 0.3 mL/min. Temperature: 45°C. MS detector: Waters XEVO TQ-MS.

The marked activities (#) are not covered by ENAC accreditation

Table 1. Uncertainties contribution for the certification of **CRM-02-DA (25-001).** ***Char**: qNMR quantification; **ba**: bulk assay; **bb**: homogeneity test; **lts**: long term stability test; **sts**: short term stability test.*

Uncertainty contribution parameter	Uncertainty values
	Domoic acid and C5'-epi-domoic acid*
$u_{char}/\%$	2.51
$u_{ba}/\%$	1.46
$u_{bb}/\%$	0.14
$u_{lts}/\%$	0.67
$u_{sts}/\%$	2.66
Expanded uncertainty (k=2) / %	7.99

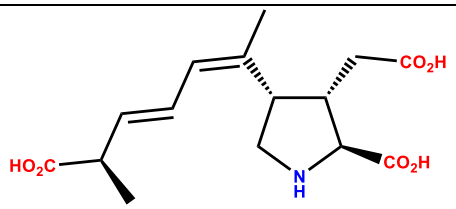
Short-term stability study. The uncertainty due to transport instability was estimated from the test for slope significance at 40°C for 9 days. The results indicated continued stability, no change is expected in the property values of the CRM.

***** Domoic acid and C5'-epi-domoic acid isomer have been quantified jointly.

[#]**Table 2.** Non-certified reference values of isomers concentration (µg·g^{-1}) determined by HPLC-UV. **Assumption of equimolar response at λ = 242 nm was used.** Estimated uncertainty corresponds to a level of confidence in terms of one standard deviation.

Toxin	Concentration / µg·g^{-1}	%
Isodomoic acid E	< 0.1	< 0.1
Isodomoic acid D	< 0.2	< 0.2
Isodomoic acid A	-	-
Domoic acid	**102.5 ± 8.2**	**≥ 98**
C5'-epi-domoic acid	< 0.2	< 0.2

Table 3. Molecular information.

Name	Domoic acid
CAS NUMBER	14277-97-5
Molecular formula	$C_{15}H_{21}NO_6$
Molecular Weight	311.33 g·mol^{-1}
Monoisotopic molecular weight	311.14 g·mol^{-1}
2D structure	

Inquiries concerning this Reference Material should be directed to:

Laboratorio CIFGA S.A.

Rúa do Vidro, 117D, parcelas 3-6

27003 • Lugo • Spain

Tel. 0034 982 816 715

E-mail: info@cifga.com

RM PRODUCER'S APPROVING OFFICER:

Certification Manager: Álvaro Antelo

This Certificate is only valid if the product was obtained directly from Laboratorio CIFGA S.A. or one of our authorized distributors.

Certificate Revision History:

April, 6th 2026 (Original certificate issue date, revision 1).

June, 30th 2026. Update of purity statement. Revision of Figure 2. Inclusion of an impurity summary table. Minor typographical errors (*certificate rev. 2*).