# Certificate of analysis

## Certified reference material code: CRM-00-C3&4

Name: Certified reference material of N-sulfocarbamoyl gonyautoxin-1 and N-sulfocarbamoyl gonyautoxin-4.

Batch: 25-001

**Description:** This Certified Reference Material (CRM) is a solution of N-sulfocarbamoyl gonyautoxin-1 (CAS Number 89614-45-9) and N-sulfocarbamoyl gonyautoxin-4 (CAS Number 89674-98-6) in aqueous [AcOH] = $25 \cdot 10^{-6}$ M, pH = 4.90.

**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 Varian WB 500 MHz equipment (Universidad de Santiago de Compostela).

| | |
|---|---|
| **Concentration of N-sulfocarbamoyl gonyautoxin-1 (C3) (95% Confidence Interval)** | **(23.9 ± 2.0) µmol / kg** |
| | **(11.7 ± 1.0) µg / g** |
| **Concentration of N-sulfocarbamoyl gonyautoxin-4 (C4) (95% Confidence Interval)** | **(7.00 ± 0.67) µmol / kg** |
| | **(3.43 ± 0.33) 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 $u_{bb}$, 0.37% for N-sulfocarbamoyl gonyautoxin-1 (C3) and 0.77% for N-sulfocarbamoyl gonyautoxin-4 (C4). The Stability studies after storage of selected units were performed at -20°C for a storage time of 12 months. Using the data from the long-term study, the uncertainty due to possible degradation was calculated as $u_{lts}$, 2.60% for C3 and 3.05% for C4. 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, ISO33405.

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-00-C3&4, 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.



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

**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-00-C3&4 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 -20 ºC (freezer) until use. Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied.

**Source and Preparation of Material:** The raw material used in the preparation of this CRM was obtained from large-scale culture of *Gymnodinium catenatum*. After purification, identity of the material was confirmed by $^{1}$H-NMR and LC-MS/MS. 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 water at 20 ºC ($\rho^{20} = 0.9982$ g/mL), *non-certified volumetric concentration* values are:

| | |
|---|---|
| **Concentration of N-sulfocarbamoyl gonyautoxin-1 (C3) (95% Confidence Interval)** | **(23.8 ± 2.0) µM** |
| | **(11.7 ± 1.0) µg / mL** |
| **Concentration of N-sulfocarbamoyl gonyautoxin-4 (C4) (95% Confidence Interval)** | **(6.97 ± 0.67) µM** |
| | **(3.43 ± 0.33) µg / mL** |

Concentration of N-sulfocarbamoyl gonyautoxin-1 & 4, AcOH and impurities are neglected in order to apply solution density value.

Purity analysis was performed by qNMR and UPLC MS/MS. UPLC MS/MS additional analyses indicated that there was no significant contribution from other analogues, low amounts of Decarbamoylgonyautoxin 1 (dcGTX1), Decarbamoylgonyautoxin 4 (dcGTX4), N-sulfocarbamoyl gonyautoxin-2 (C1), N-sulfocarbamoyl gonyautoxin-3 (C2), Decarbamoylgonyautoxin 2 (dcGTX2) and Decarbamoylgonyautoxin 3 (dcGTX3) are revealed, see [#]Table 2. Concentration values are presented as non-certified.

Purity determination was performed by impurities quantification and subtracting the sum from 100%, the final molar ratio purity is ≥ 97%, relative to close-related impurities to saxitoxin analogues. *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*.



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

## Figures

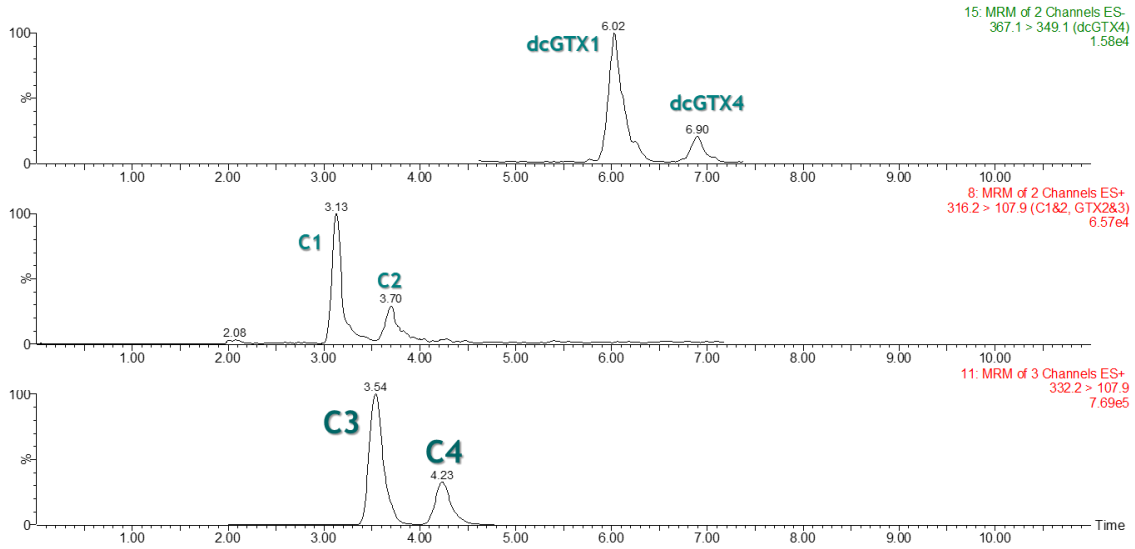



**Figure 1.** Analysis of the CRM-00-C3&4 (25-001) solution by UPLC-MS/MS. Conditions: ACQUITY UPLC BEH AMIDE 1.7µm, (150 x 2.1) mm, UPLC column. Elution conditions, gradient mode with mobile phases: (A) [$FmONH_4$] = 1.0 mM (B) Acetonitrile 95% with 0.01% FmOH; Gradient: 0 min 73% B; 5.0 min 73% B; 7.5 min 40% B; 7.6 min 20% B; 9.5 min 20% B; 9.6 min 73% B; 11.0 min 73% B; flow rate: 0.40 mL/min. Temperature: 55°C. MS detector: Waters XEVO TQ MS using the typical MRM transitions for PSPs analogues. Mayor impurities are highlighted.

**Table 1.** Uncertainties contribution for the certification of **CRM-00-C3&4 (25-001)**. ***Char**: qNMR quantification; **ba**: bulk assay; **bb**: homogeneity test; **lts**: long term stability test; **sts**: short term stability test.*

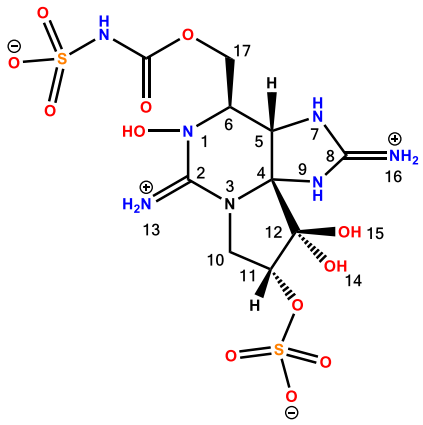
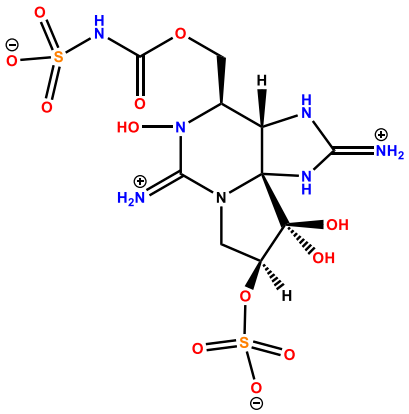
| Uncertainty contribution parameter | Uncertainty values | |
|---|---|---|
| | C3 | C4 |
| $u_{char}$/% | 2.46 | 3.41 |
| $u_{ba}$/% | 0.13 | 0.13 |
| $u_{bb}$/% | 0.37 | 0.77 |
| $u_{lts}$/% | 2.60 | 3.05 |
| $u_{sts}$/% | 2.03 | 1.03 |
| Expanded uncertainty (*k*=2) / % | 8.27 | 9.50 |

**Short-term stability study**. The uncertainty due to transport instability was estimated from the test for slope significance at 40°C. The results indicated continued stability, no change is expected in the property values of the CRM. The combined uncertainty budget of the assigned value was upgraded to incorporate short-term stability at 40°C for 6 days.

[#] **Table 2.** Non-certified reference values of impurities concentration (µmol/kg) determined by UPLC MS/MS. Assigned analogues entries for compounds must be regarded as tentative according to MRM transitions agree. Assumption of equimolar response. Estimated uncertainty corresponds to a level of confidence in terms of one standard deviation.

| Toxin | Concentration / µmol/kg | % |
|---|---|---|
| Decarbamoylgonyautoxin-1 | 0.022 ± 0.002 | 0.1 % |
| Decarbamoylgonyautoxin-4 | 0.026 ± 0.002 | 0.1 % |
| C1 | 0.54 ± 0.05 | 1.7 % |
| C2 | 0.12 ± 0.01 | 0.4 % |
| Decarbamoylgonyautoxin-2 | < 0.01 | < 0.1 % |
| Decarbamoylgonyautoxin-3 | < 0.01 | < 0.1 % |

**Table 3.** Molecular information.

| Name | C3 | C4 |
|---|---|---|
| CAS NUMBER | 89614-45-9 | 89674-98-6 |
| Molecular formula | $C_{10}H_{17}N_7O_{12}S_2$ | |
| Molecular Weight | 491.403 g·mol$^{-1}$ | |
| Monoisotopic molecular weight | 491.038 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:**

*December, 18th 2025* (Original certificate issue date, revision 1).



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