Certificate of analysis

Certified reference material code: CRM-00-hYTX

Name: Certified Reference Material of 1a-homoyessotoxin di-sodium salt Batch: 21-001

Description: This Certified Reference Material (CRM) is a solution of 1a-homoyessotoxin di-sodium salt (CAS Number 196309-94-1 acidic form) in methanolic solution.

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 equipment (Universidad de Santiago de Compostela).

Concentration of 1a-homoyessotoxin di-sodium salt and isomers. (95% Confidence Interval)	**(6.70 ± 0.50) µmol / kg**
	(8.05 ± 0.60) µg / g

NOTE: In order to perform the analysis, it should be noted that the certificate value corresponds to the sum of 1a-homoyessotoxin and minor analogues concentration and therefore the sum of the areas of the chromatographic signals should be considered. See #Non-certified and non-accredited data section of this certificate.

The starting material is measured by ^{1}H-qNMR against a Benzoic acid 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}, 1.67%. 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}, 2.19%. 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-hYTX, 21-001 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.

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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 contains toxic material, and should be handled with care. Read MSDS before using. Material Safety Data sheet for information regarding CRM-00-hYTX 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 15ºC to 25ºC 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: 1a-homoyessotoxin di-sodium salt was isolated from large scale culture of *Protoceratium reticulatum* and purified via preparative HPLC–MS/MS. After purification, identity of the material was confirmed by ^{1}H-NMR and UPLC-MS/MS, Figure 1. Finally, 0.5 mL aliquots of hYTX solution were dispensed into 2-mL amber argon-filled glass ampoules, which were then flame sealed.

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

The certified concentration of 1a-homoyessotoxin presents a low level (0.04 ± 0.01) µg/g (0.5%) of tentative 32-arabinoside-1a-homoyessotoxin. *Percentage value not certified*.

Taking into account the density of methanol at 20 ºC ($\rho^{20} = 0.7915$ g/mL), *non-certified volumetric concentration* values are:

Concentration of 1a-homoyessotoxin di-sodium salt and isomers (95% Confidence Interval)	**(5.30 ± 0.40) µM**
	(6.37 ± 0.48) µg / mL

Concentration of 1a-hYTX and impurities are neglected in order to apply solution density value.

Purity analysis was performed by qNMR and UPLC MS/MS. This material presents yessotoxin isomers at low level (<0.1 %). Material molar ratio purity is ≥ 99 %. *Percentage values not certified. 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.*

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Figures

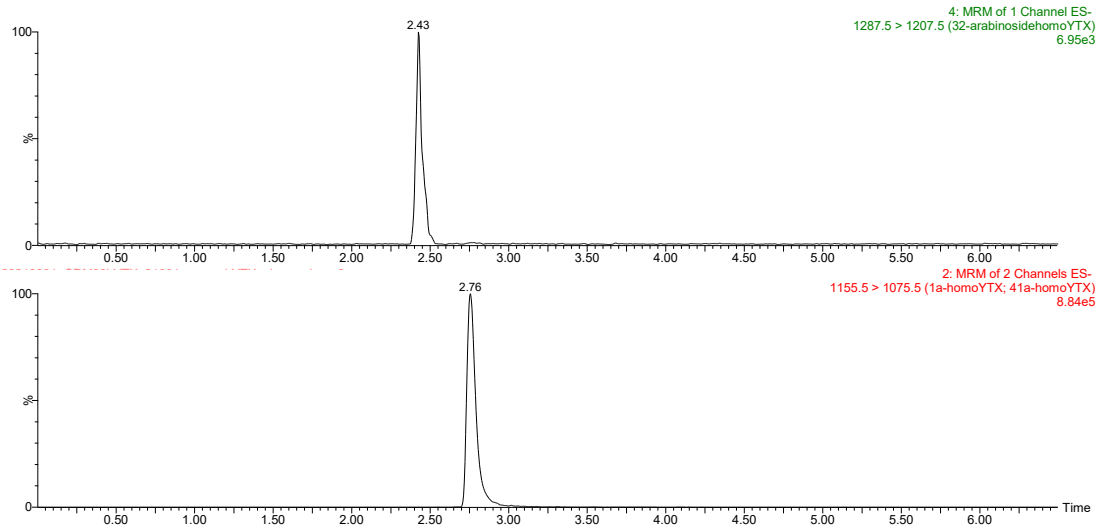


Figure 1. Analysis of the **CRM-00-hYTX** (21-001) by UPLC-MS/MS. Conditions: ACQUITY UPLC BEH C18 1.7µm, 100x2.1 mm, UPLC column. Elution conditions, gradient mode with mobile phases: (A) Water and (B) Aqueous 95% acetonitrile, both with 2mM ammonium formate and 50mM formic acid; Gradient: 0.00 min 30% B; 3.00 min 90% B; 4.50 min 90% B; 4.51 min 30% B; 6.50 min 30% B; flow rate: 0.40 mL/min. Temperature: 30°C. MS detector: Waters XEVO TQ MS using the typical MRM transitions of yessotoxin family (sulphate loss).

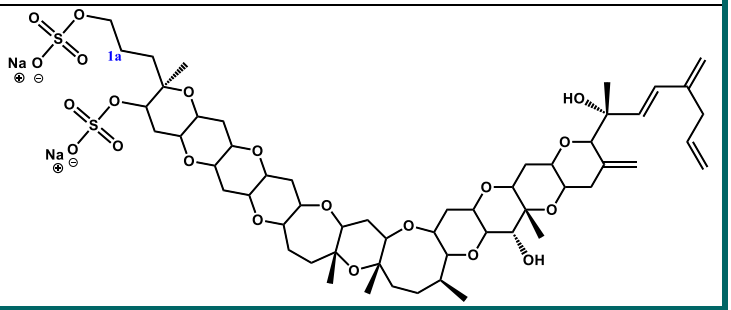
Table 1. Uncertainties contribution for the certification of **CRM-00-hYTX (21-001)**. ***Char**: qNMR quantification*; ***ba**: bulk assay*; ***bb**: homogeneity test*; ***lts**: long term stability test*; ***sts**: short term stability test*.

Uncertainty contribution parameter	Uncertainty values 1a-homoyessotoxin di-sodium salt *
$u_{char}/\%$	2.23
$u_{ba}/\%$	0.60
$u_{bb}/\%$	1.67
$u_{lts}/\%$	2.19
$u_{sts}/\%$	1.09
Expanded uncertainty (*k*=2) / %	7.50

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 9 days.

***** 1a-homoyessotoxin di-sodium salt and isomers detected have been quantified jointly

Table 2. Molecular information.

Name	1a-homoyessotoxin di-sodium salt
CAS NUMBER	196309-94-1 acidic form
Molecular formula	$C_{56}H_{82}Na_2O_{21}S_2$
Molecular Weight	1201.347 g·mol^{-1}
Monoisotopic molecular weight	1200.459 g·mol^{-1}
2D structure	

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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:

March, 08th 2021 (Original certificate issue date, revision 1).

January, 12th 2026. Editorial changes. Address update. Revised and added associated uncertainties following additional testing. *(certificate rev. 2)*.

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