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

Certificate of analysis

Certified reference material code: CRM-00-STX

Name: Saxitoxin Dihydrochloride in dilute aqueous hydrochloric acid. Batch: 16-001

Description: This Certified Reference Material (CRM) is a solution of Saxitoxin Dihydrochloride (CAS Number 35554-08-06) in aqueous [HCl] = 0.003 M, pH = 2.63.

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 (USC).

Concentration of Saxitoxin Dihydrochloride (95% Confidence Interval)	**(55.1 ± 2.8) µmol / kg**
	(20.5 ± 1.0) µg / g

The starting material is measured by ^{1}H-qNMR against a Sucrose NIST certified reference material (SRM 17f) 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}, 0.74%. 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}, 1.21%. 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, ISO35.

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-STX, 16-001 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.

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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-STX 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 withdrawn 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 few 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 *Alexandrium tamarense*. After purification, identity of the material was confirmed by ^{1}H-NMR and LC-MS/MS, Figures 1 and 2. Finally, 0.5 mL aliquots of toxin solution were dispensed into 2-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 Saxitoxin Dihydrochloride (95% Confidence Interval)	**(55.1 ± 2.8) µM**
	(20.5 ± 1.0) µg / mL

Concentration of Saxitoxin Dihydrochloride, HCl and impurities are neglected in order to apply solution density value.

Purity analysis was performed by qNMR and UPLC MS/MS. Both analyses indicated that there was no significant contribution from other analogues. Results of nuclear magnetic resonance (NMR) analysis were consistent with structure and a molar ratio purity of principal components ≥ 99 %, see Table 2. *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 Guide 35 *Reference materials - Guidance for characterization and assessment of homogeneity and stability*.

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Figures

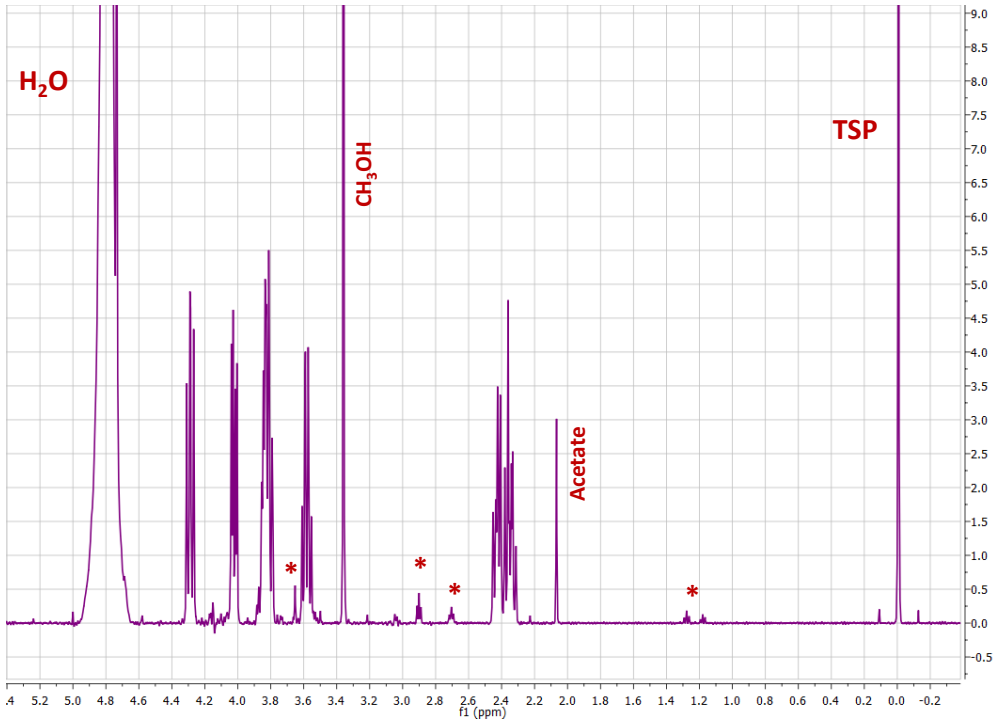

Figure 1. ^{1}H-NMR spectrum of Saxitoxin solution, Varian 500 MHz, solution of STX (5.0 mM) in H_2O used in preparation of CRM-00-STX batch 16-001. *No identified impurities are highlighted. As the structure of these impurities is not known, their exact amount cannot be calculated. Because the impurities usually are smaller molecules (otherwise, more NMR signals would arise), it can be estimated from the corresponding integrals, but this purity is not relative to close-related impurities to Saxitoxin analogues.

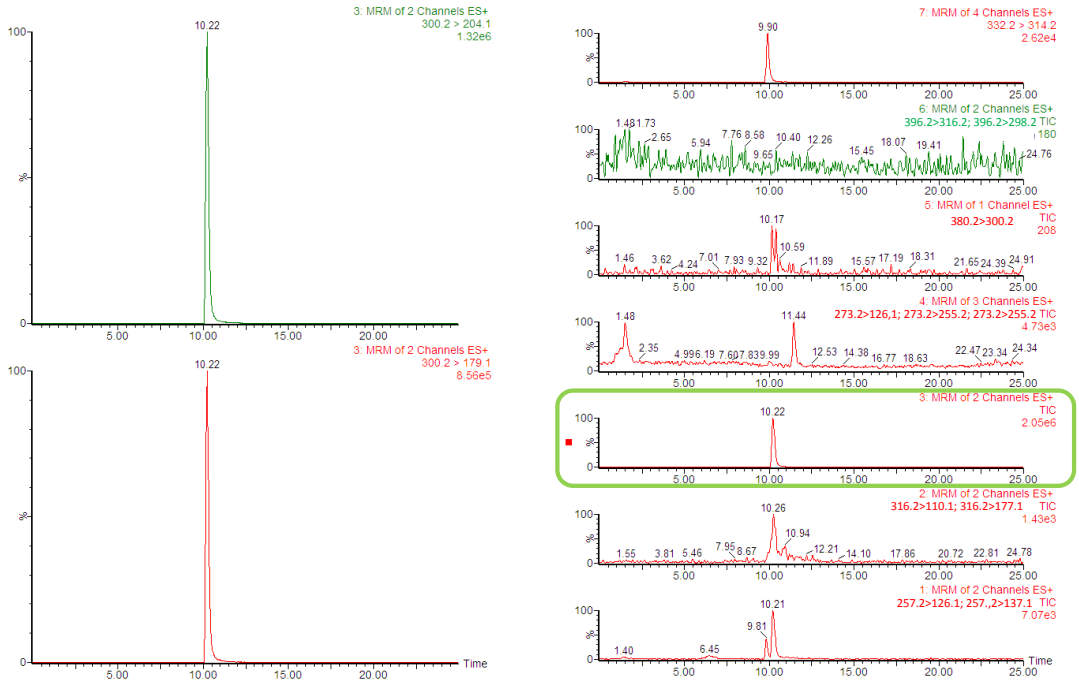

Figure 2. Analysis of the CRM-00-STX solution by HPLC-MS/MS. Internal SOP for PSPs analysis by HPLC-MS/MS using the typical MRM transition of STX anologues.

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Table 1. Uncertainties contribution for the certification of CRM-00-STX, 16-001. ***Char**: qNMR quantification; **ba**: bulk assay; **bb**: homogeneity test; **lts**: long term stability test; **sts**: short term stability test.*

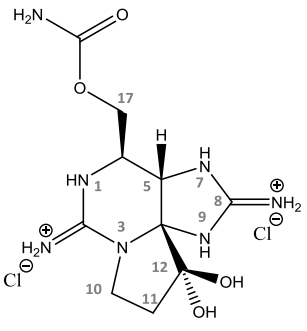
Uncertainty contribution parameter	Uncertainty values
	STX
u_{char}/%	2.08
u_{ba}/%	0.13
u_{bb}/%	0.74
u_{lts}/%	1.21
Expanded uncertainty (*k*=2) / %	5.05

Short-term stability study, test for slope significance, has been carried out to simulate the conditions of transport. Packaged vials were exposed for 12 days, 9 days, 6 days, 3 days and 0 hr (-80 ºC, control), with an isochronous design being used to enable subsequent LC-MS/MS analysis of CRMs to be conducted under repeatability conditions. After 12 days at 37 ºC – 40ºC temperature results indicated continued stability. With these conditions, no change is expected in the property values of the CRM. The short-term stability uncertainty (u_{sts}) was not considered relevant as an uncertainty component and it was not included in the u_{CRM} uncertainty calculation.

#Table 2. Non-certified reference values of impurities concentration (µmol/L) determined by UPLC MS/MS.

Toxin	Concentration / µM	%
dcGTX2	0.11 ± 0.01	0.2 %
dcSTX		< 0.1 %
332.2 > 314.2 257.2 > 126.1	HPLC MS/MS analysis show signals for these ion transitions, similar to another PSPs analogues. But retention times are not matched with known PSPs analogues.	

Table 3. Molecular information.

Name	Saxitoxin
CAS NUMBER	35554-08-6
Molecular formula	$C_{10}H_{17}N_7O_4Cl_2$
Molecular Weight	372.21 g·mol^{-1}
Monoisotopic molecular weight	300.12 [Saxitoxin free base + H^+]
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: *August, 22nd 2016 (Original certificate issue date, revision 1).*

March, 07th 2017. Introduction of the accreditation mark, stability uncertainty changes and editorial changes. Format F-01-08, revision 1 *(certificate rev. 2).*

May, 30th 2017. Minor editorial changes. No attempt was made to re-evaluate the certificate values or any technical data presented on this certificate *(certificate rev. 3).*

January, 15th 2019. Intended use update. Editorial changes. Uncertainty update following long term stability testing *(certificate rev. 4).*

January, 28th 2019. Minor typographical corrections. No attempt was made to re-evaluate the certificate values or any technical data presented on this certificate *(certificate rev. 5).*