



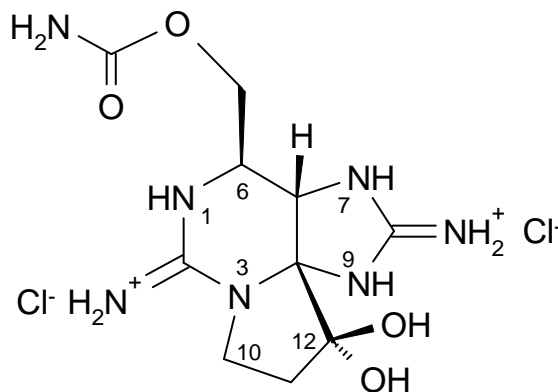
CERTIFICATE OF ANALYSIS

NRC CRM-STX-e

Lot#20060419

Certified Calibration Solution for Saxitoxin Dihydrochloride

Saxitoxin (STX) is one of the toxins associated with paralytic shellfish poisoning (PSP) [1]. **CRM-STX-e** is designed for use as an instrument calibration solution to aid the analyst in the determination of STX in plankton and shellfish samples. Each ampoule contains approximately 0.5 mL of solution with $65 \pm 3 \mu\text{M}$ of STX in 3 mM hydrochloric acid.



Saxitoxin (STX) dihydrochloride

Molecular Formula: $\text{C}_{10}\text{H}_{19}\text{N}_7\text{O}_4\text{Cl}_2$

Molecular Weight: 372.2

CA Registry Number: 35554-08-6

1. Intended Use

CRM-STX-e is designed for use as an instrument calibration solution to aid the analyst in the determination of the paralytic shellfish poisoning (PSP) toxin [1], saxitoxin (STX), in plankton and shellfish samples. The concentration of **CRM-STX-e** is suitable for the calibration of LC instruments, as well as for spiking shellfish control samples for recovery experiments. An example LC-FLD chromatogram showing the analysis of **CRM-STX-e** is shown in Figure 1.

2. Preparation of CRM-STX-e

The starting material was a large-scale laboratory culture of *Alexandrium tamarense* (NRC strain AL18b). The principal toxin in this source material, N-sulfocarbamoyl-gonyautoxin-3 (C2), was extracted, purified and then chemically converted to STX [2]. The final product was purified by preparative scale chromatography [3] and dissolved in 3 mM HCl to give a stock solution with a concentration of approximately 2 mM. The purity of the toxin was checked by the following techniques: liquid chromatography with fluorescence detection (LC-FLD) [4] (see Figure 1), capillary electrophoresis with UV detection (CE-UVD) [5] (see Figure 2), liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [6] (see Figure 3), liquid chromatography with mass spectrometry detection (LC-MS) [7] (see Figure 4), and 500 MHz proton NMR spectroscopy [8] (see Figure 5).

The concentration of STX in the stock solution was determined accurately using quantitative NMR and LC-CLND. An aliquot of this stock solution was weighed and diluted in filtered and degassed 3 mM HCl. After weighing, this solution was mixed thoroughly and dispensed into argon-filled glass ampoules, which were then immediately flame-sealed. Each ampoule contains approximately 0.5 mL.

3. Storage Instructions

Proper attention should be given to storage conditions. After receipt, the ampoules should be stored in the dark in a refrigerator (ca. +4°C). The toxin was found to be stable under these conditions. Solutions are also stable when stored in a freezer, preferably at -20°C or less. Solutions stored in self-defrosting freezers that undergo a periodic freeze-thaw cycle may cause decomposition (G. Boyer, pers. comm.). Bacterial contamination of solutions has also been observed to lead to decomposition of PSP toxins, especially at room temperature (G. Boyer, pers. comm.). However, the CRM has been prepared under conditions that minimize the chance of bacterial contamination. It is recommended that dilutions of the CRM be stored frozen in a freezer (<-20°C).

4. Results of Certification Analyses

4.1 Volume of Standards

Each ampoule contains *approximately* 0.5 mL of solution. This volume is not certified, as only the concentration is certified.

4.2 Homogeneity

Approximately 1.4% of the ampoules produced were selected for homogeneity testing and analyzed by LC-FLD. The inter-ampoule variation was estimated to be less than 1%, approximately the same as the coefficient of variation for replicate analyses of one solution, thus indicating good sample homogeneity.

4.3 Certified Concentration

Due to its hygroscopic nature and the possible presence of associated salts, STX cannot be weighed with sufficient accuracy. Therefore, the concentration of STX in a concentrated stock solution was determined by a combination of quantitative NMR and LC-CLND, both of which can be calibrated by an external standard. USP-certified caffeine was used as the calibrant for these experiments. The concentration of STX in the final diluted **CRM-STX-e** solution was also measured with LC-CLND. The certified concentration of **CRM-STX-e** is based upon the results of all of these analyses.

It should be noted that small amounts of neosaxitoxin (NEO), 11-hydroxy-saxitoxin and decarbamoylsaxitoxin (dcSTX) are present as impurities at concentrations of *ca* 0.4, 0.4 and 0.6 μM , respectively (these values are not certified). Trace levels of gonyautoxin-5 (GTX5) and gonyautoxin-2 (GTX) are also detectable by LC-MS analysis (Figure 4).

Table 1: Certified concentration for CRM-STX-e.

Compound	Concentration $\mu\text{mol/L}$ at 20°C ^{a,b}
STX-dihCl	65 \pm 3

^a The estimated uncertainty was derived from uncertainty of measurements and evaluation of possible systematic errors and stability.

^b Date of original certification: July 2006

5. Expiration of Validity

If stored unopened under the conditions recommended in Section 3, the certified concentration of **CRM-STX-e** is valid for 1 year from the date of sale. Please refer to the label on the original packaging for the expiration date.

6. Safety Instructions

Saxitoxin and its analogues are responsible for incidents of paralytic shellfish poisoning. Only qualified personnel should handle these solutions. Heavy gloves and eye protection should be used when opening the ampoule in case the glass shatters. Appropriate disposal methods should be used. A Material Safety Data Sheet (MSDS) is available for **CRM-STX-e**.

7. Instructions for Use of CRM

Prior to opening, each ampoule should be allowed to warm to room temperature. The ampoule contents should be thoroughly mixed by inverting end-to-end, and then tapped to ensure that most of the solution drains to the bottom. The ampoules should be opened at the pre-scored mark. An increase in concentration due to evaporation of water will occur if the solution is left exposed to air for more than several minutes. This can also occur if the solution is transferred to screw-cap vials with a large headspace volume. It is also important to be aware that screw caps can loosen when placed in a freezer, resulting in subsequent loss of solvent. If the solution must be stored, try to use a storage vial with minimum headspace and wrap the cap with parafilm.

Note: the volume of the solution is not certified, only the concentration. Therefore you should NOT simply transfer the entire contents of the ampoule and dilute to volume.

Our recommended procedure for preparation of a dilution series is the following. Once an ampoule has been opened, accurate aliquots should be removed with calibrated volumetric equipment and transferred to volumetric flasks. A useful procedure that ensures accurate dilutions is to use a balance to determine weights of the empty flask and then the dispensed aliquot, as well as the final diluted solution, by difference. This approach is only valid if water is used for dilutions.

8. References

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9. Acknowledgements

M. Quilliam designed and supervised the production of **CRM-STX-e**. Plankton biomass was produced under the supervision of M. Bricelj. The toxin was isolated and purified by M. Laycock, P. LeBlanc, and K. Thomas. NMR analyses were conducted by J. Walter and I. Burton, LC-FLD analyses were conducted by K. Reeves and G. Pitcher, and CE-UV, LC-MS and LC-CLND analyses were performed by K. Thomas. The final solution was prepared and ampouled by K. Thomas, K. Reeves, W. Hardstaff, P. Blay, S. Crain, G. Pitcher, and C. Trudeau. K. Thomas and D. Marciniak assisted with documentation.

Feedback, comments and inquiries from users will be welcomed. Please contact:

NRC Certified Reference Materials Program
Institute for Marine Biosciences
National Research Council of Canada
1411 Oxford Street
Halifax, Nova Scotia, Canada B3H 3Z1
Tel: (902)-426-8281
FAX: (902)-426-5426
E-mail: crm.imb@nrc.ca

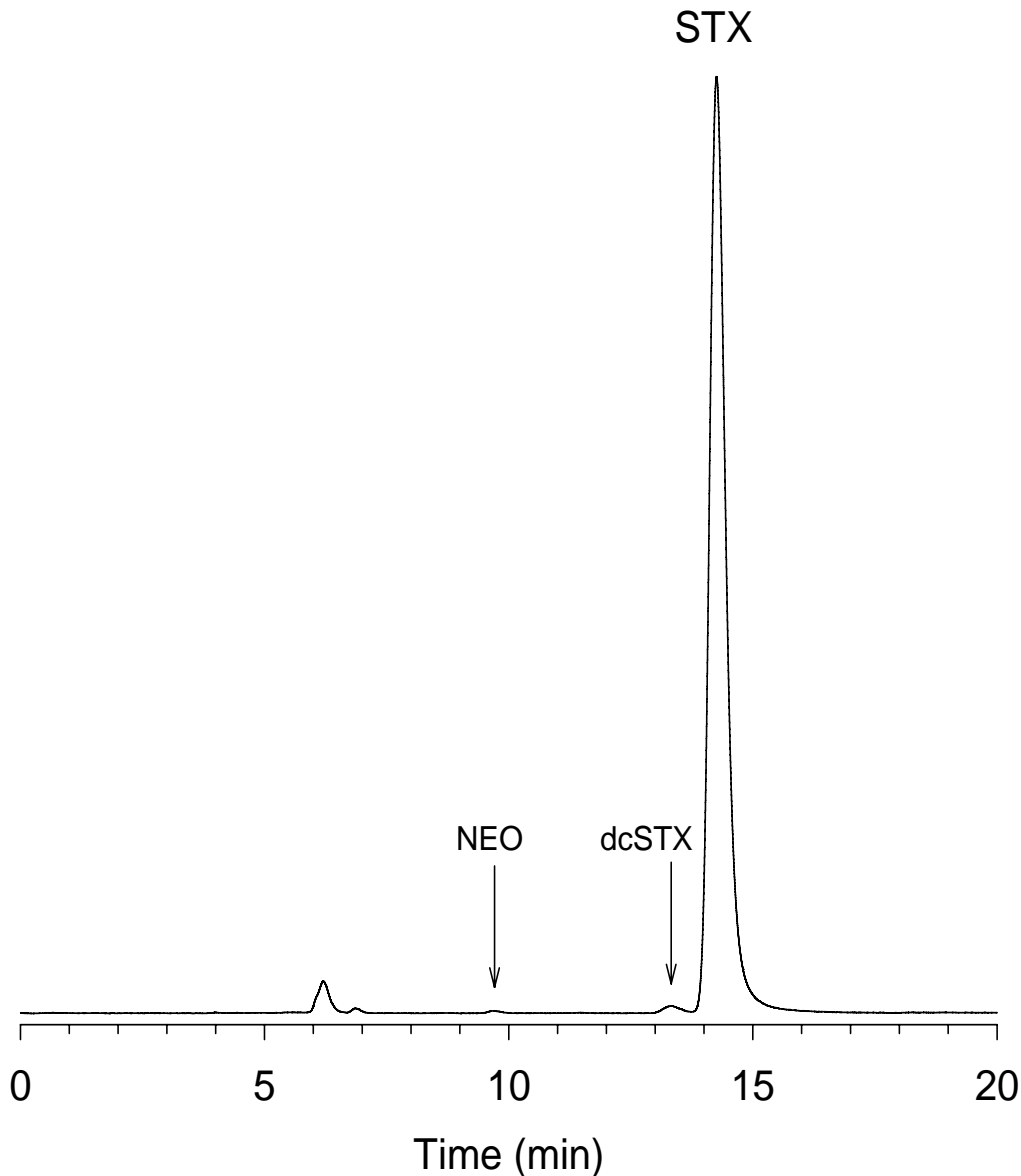


Figure 1: Analysis of the final **CRM-STX-e** by liquid chromatography with post-column oxidation and fluorescence detection (LC-FLD). Conditions: column = Keystone Betabasic-C8, 250 x 4.6 mm i.d. at 37°C; flow = 0.8 mL/min; mobile phase = 30 mM ammonium phosphate with 2mM sodium heptane sulphonate, pH 7.1, and 4% acetonitrile; 10 μ L injection; post column oxidation using 5 mM periodic acid in 0.1 M sodium phosphate, pH 7.8, 0.4 mL/min, reaction coil at 85°C, effluent acidified with 0.75 M nitric acid 0.4 mL/min; detection = fluorescence, ex 330nm, em 390nm. Retention times of related toxins are indicated: dcSTX = decarbamoylsaxitoxin, NEO = neosaxitoxin.

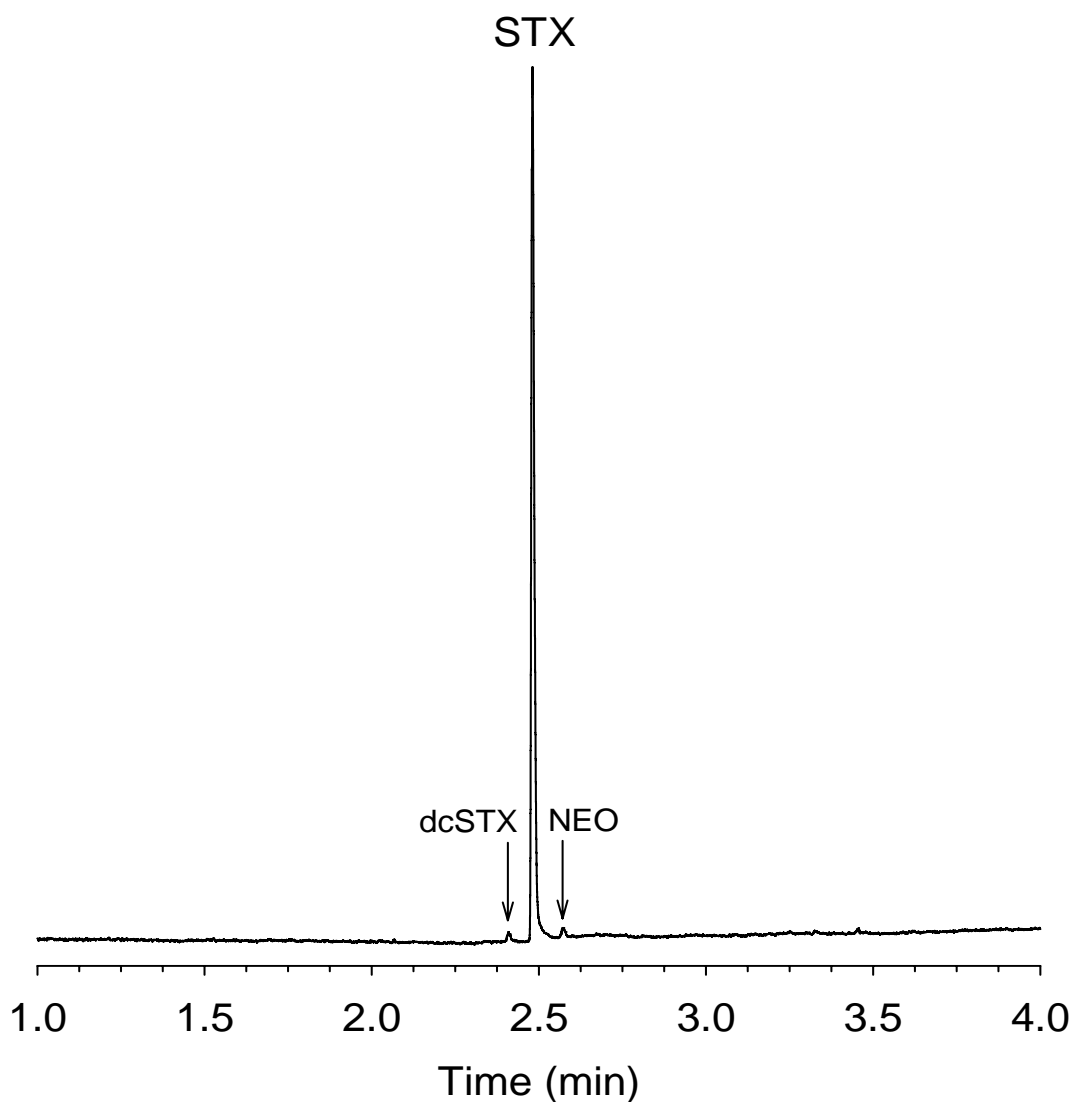


Figure 2: Analysis of a 1/10 dilution of the STX stock solution (ca. 2 mM) by capillary electrophoresis with UV absorbance detection (CE-UVD). Conditions: fused silica capillary with extended light path, 360 μm o.d. x 50 μm i.d.; buffer = 75mM morpholine formate, pH 4.9; sample loaded with 50 mbar pressure for 5 seconds; voltage applied = +30kV; UV detection at 210 nm. Migration times of related toxins are indicated: dcSTX = decarbamoylsaxitoxin, NEO = neosaxitoxin.

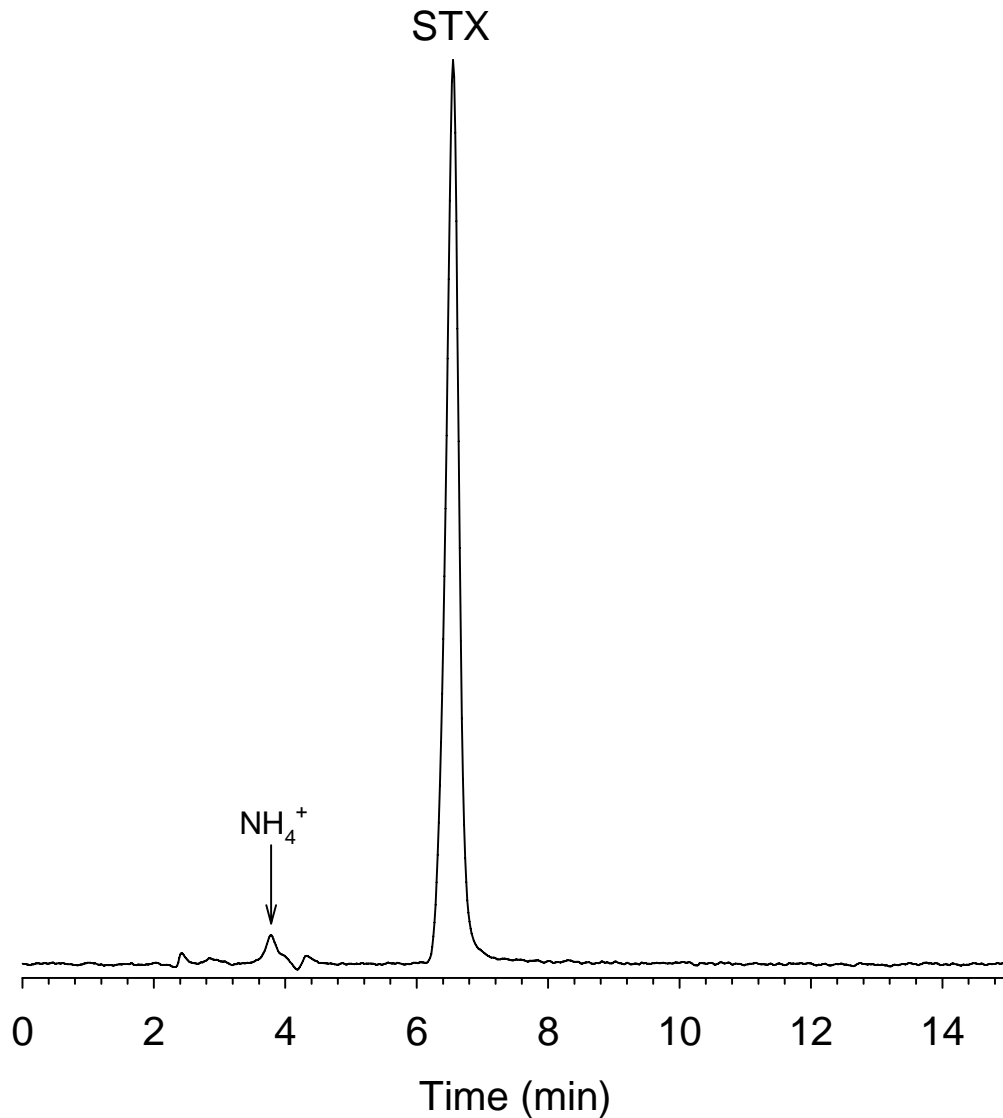


Figure 3: Analysis of the final **CRM-STX-e** by liquid chromatography with chemiluminescence nitrogen detection (LC-CLND). Conditions: column = Inertsil ODS-3, 250 mm x 2 mm i.d. at 20°C; flow = 0.2 mL/min; mobile phase = tetrahydrofuran/water (20:80) with 2 mM heptanesulfonic acid (pH 2.1); 5 μ L injection; detection = Antek 8060 CLND.

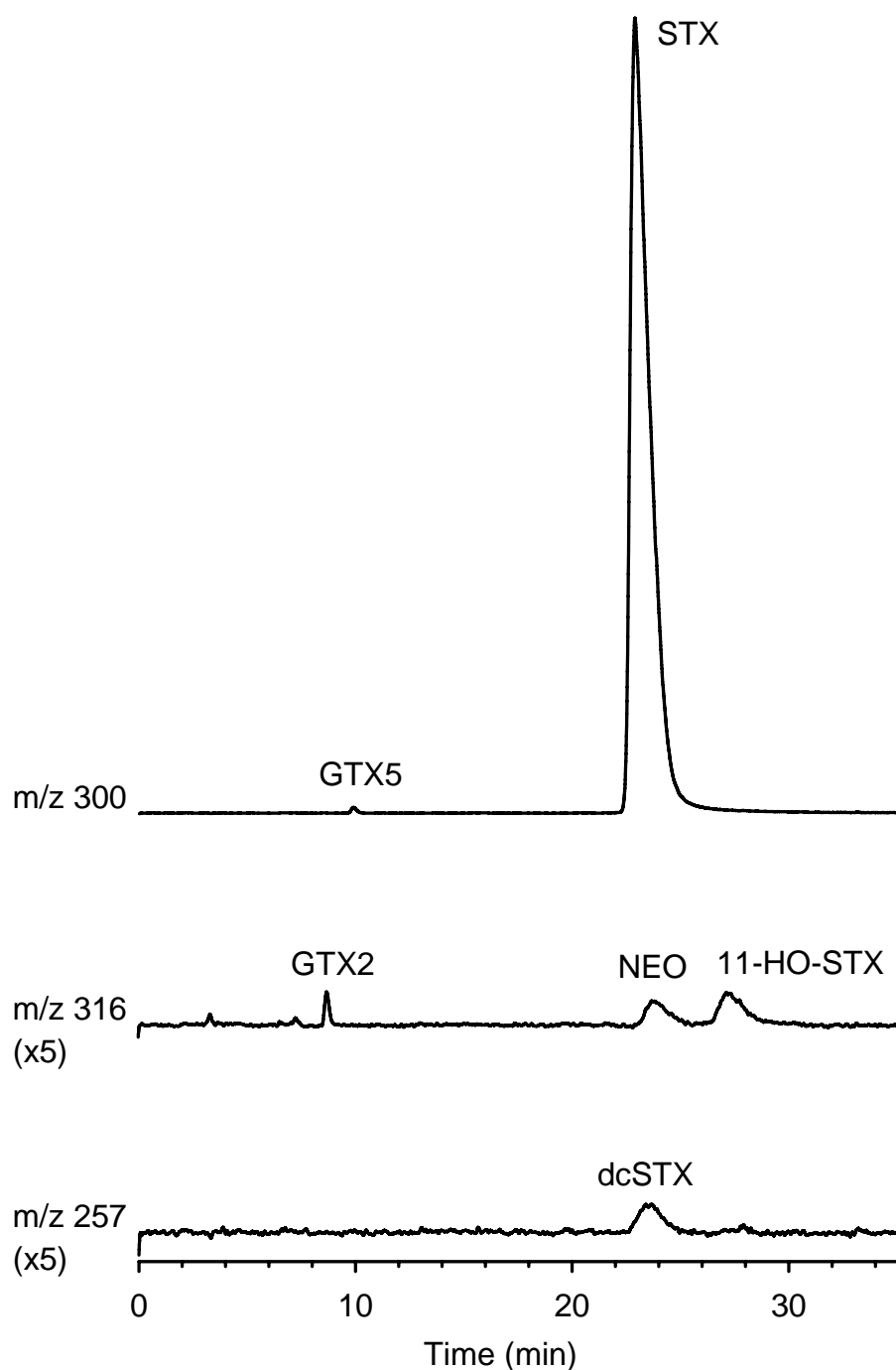


Figure 4: Analysis of the CRM-STX-e by liquid chromatography with mass spectrometry (LC-MS). Conditions: column = TosoHaas TSKgel 5 μ m Amide-80, 2.1 x 250 mm i.d. at 40°C; mobile phase = 52% CH₃CN/H₂O, 3.6 mM formic acid, 2mM ammonium formate (isocratic); flow rate= 0.2 mL/min; 2 μ L injection. MDS-SCIEX/Applied Biosystems API-165 single quadrupole mass spectrometer with selected ion monitoring, electrospray ionization, positive mode.

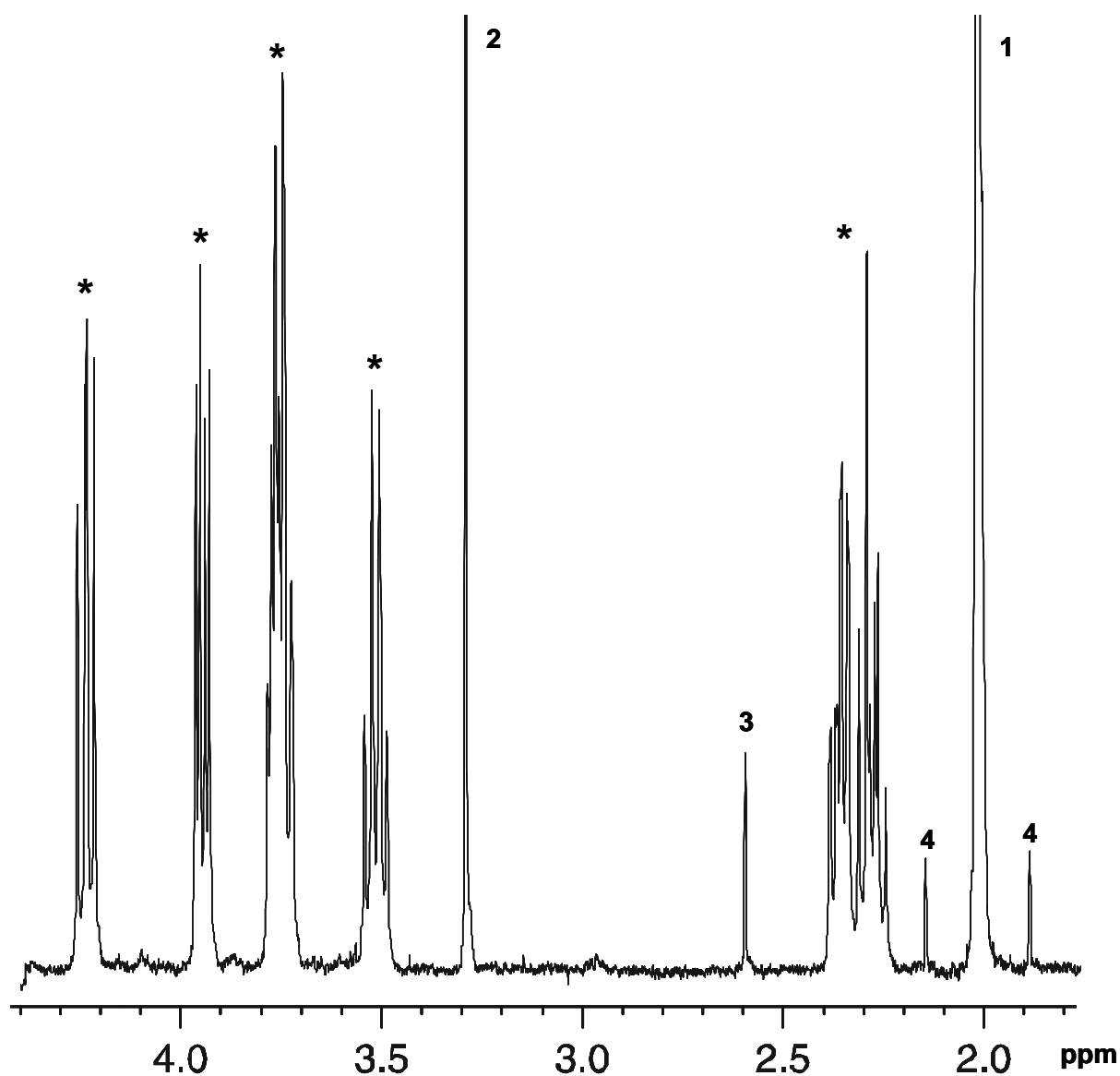


Figure 5: ^1H -NMR spectrum of saxitoxin stock solution in H_2O . Saxitoxin resonances used for quantitation are marked with asterisks (*). Other peak identities: 1 = CH_3COOH ; 2 = CH_3OH ; 3 = CH_3CN ; 4 = ^{13}C satellite peaks from CH_3COOH . CH_3OH and CH_3CN are residues left from final column chromatography.