U-[$^{13}C_{22}$]-HT2 Toxin in acetonitrile

This document is designed, and the certified values and uncertainty are determined in accordance with ISO Guide 31, ISO Guide 34, ISO Guide 35, AOAC, and Eurachem/ CITAC Guides.

Description of the Reference Material (RM)

	Product name:	U-[$^{13}C_{22}$]-HT2 toxin in acetonitrile			
	CAS number:	1486469-92-4			
	Formula:	$^{13}C_{22}H_{32}O_9$			
	Molecular weight:	446.32			
	Product number:	FIA000155			
	Lot number:	HT213C16111503			
	Expiry date:	15-May-25			
	Certified value (s):	[$^{13}C_{22}$]-HT2	25,04	± 0,87	µg/mL
	Isotope incorporation by mass spectrometry	13C/molecule	98%		
	Physical description:	Clear solution of [$^{13}C_{22}$]-HT2 Toxin in acetonitrile			
	Packing and amount:	Amber glass vial filled with 10 mL of solution			
	Storage conditions	≤-10°C			
	Matrix and starting material:	This material was prepared with/from:			
		Acetonitrile LCMS grade		Internal ID:	000120410BS
		[$^{13}C_{22}$]-HT2 toxin (crystalline)		Internal ID:	HT213C-16051302

Intended use of the RM:

For laboratory use for R&D purposes only. The main purpose of this material is for analytical instrument calibration (e. g. external calibration, standard addition). Not for drug, household or other uses.

Instruction for the correct use of the RM:

The vial should be stored in a dark place at ≤-10°C. Before usage of the RM, allow the vial to warm to room temperature. The expiry date of this RM is based on the current knowledge and holds only for proper storage conditions in the originally closed vials / packages. Solutions prepared for calibration purpose should be protected from exposure to light. Discard solutions after use in accordance with appropriate safety regulations for chemical substances

Hazardous situation:

Flammable liquid, Category 2, H225 (Highly flammable liquid and vapour)
Acute toxicity, Oral, Category 4; H302 (Harmful if swallowed)
Acute toxicity, Dermal, Category 4; H312 (Harmful in contact with skin)
Eye irritation, Category 2; H319 (Causes serious eye irritation)
Acute toxicity, Inhalation, Category 4; H332 (Harmful if inhaled)
In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid exposure. Wear suitable protective clothing.

Traceability

The certified values are based on the results of analytical techniques previously used for purity assessment of solid mycotoxins. High purity material represents a practical realization of concentration units, through conversion of mass to molar quantity.

Calculation of certified values and associated uncertainties

The standard solution of the mycotoxin is made according to AOAC Official Method 970.44. Crystalline toxin (approx. 1 mg) is dissolved in methanol and after one dilution the concentration of toxin solution is determined by measuring absorbance (A) at wavelength of maximum absorption (near 202 nm) and using the following equation:

$$C\ (\mu g/mL) = \frac{A \times m.w \times 1000}{\varepsilon}$$

Once the exact concentration of toxin solution is established, a fixed amount (µg) is transferred to an amber glass vial. The solvent is evaporated under a gentle stream of nitrogen and immediately the toxins are dissolved in acetonitrile, vortexed and sonicated to obtain a standard at the desired concentration.

Toxin	Source				Standard uncertainty
HT2-Toxin (ε 1959 in MeOH)	Purity of solid HT2-Toxin				0,577
	Calibration UV-VIS	A =202 nm	0,494	Abs	0,008
	Dilution 1	Volume	0,500	mL	0,002
	Dilution 2	Volume	1,000	mL	0,005
	Dilution 3	Volume	4,845	mL	0,004
	Dilution 4	Volume	50,000	mL	0,034
$Combined_{u_c} = \sqrt{\left(\frac{u_A}{A}\right)^2 + \left(\frac{u_{V1}}{V_1}\right)^2 + \left(\frac{u_{V2}}{V_2}\right)^2 + \left(\frac{u_{V3}}{V_3}\right)^2 + \left(\frac{u_{V4}}{V_4}\right)^2 + \left(\frac{u_{Purity}}{A_{Purity}}\right)^2}$					0,017
$Concentration_{Stock} = \frac{A \times mw \times V_{S2} \times V_{S3}}{\varepsilon \times V_{S1} \times V_{S4}}$					25,04
Total expanded uncertainty (using a coverage factor k=2)					0,67

Notes: The purity of the mycotoxin used for this RM was determined by chromatographic and spectrophotometric techniques. The UV absorption spectrum showed no detectable impurities.
Following the Guide to the Expression of Uncertainty in measurement (GUM) the expanded uncertainty of toxin a level is obtained by multiplication with a coverage factor K for which 2 is usually chosen to obtain a confidence level of 95 %.

Isotopic incorporation	
Compound	Isotopic distribution
$^{13}C_{22}$-HT2 Toxin	37%
$^{13}C_{22}$-HT2 Toxin	63%
Calculated isotopic incorporation (^{13}C/molecule)	***98%***

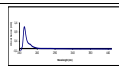
The calculation are based on LC-MS/MS data

Quality control

UV spectrum of starting material and expected chromatogram of the RM

$[^{13}C_{22}]$-HT2

Molar absorptivity in MeOH
ε= 1959 L/mol*cm
Max absorbance at ~202 nm = see uncertainty table
Optical path length d = 1 cm



LC-MS/MS analysis of $[^{13}C_{22}]$-HT2 toxin

Chromatogram
Chromatographic conditions:

Column:	C18 column; 100 mm x 2.1 mm I.D. / 1.8 µm
Mobile phase:	Water * 0.1% Formic Acid + 5 mM Ammonium Acetate : Methanol (Gradient)
Flow:	0.3 mL/min
Temperature:	30°C
	MS/MS
Detector	MRM transition: 464,1>229,1

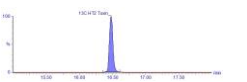
LC-MS/MS chromatogram of $[^{13}C_{22}]$-HT2 toxin

Confirmation of the certified concentration by LC-MS/MS

The certified concentrations of the prepared solution was confirmed by LC-MS/MS against a reference batch.

Chromatogram
Chromatographic conditions:

Column:	C18 column, 100 mm x 2.1 mm I.D. / 1.8 µm
Mobile phase:	Water + 0.1% Formic Acid + 5 mM Ammonium Acetate : Methanol (Gradient)
Flow:	0.3 mL/min
Temperature:	35°C
Detector	MS/MS
$(^{13}C_{24})$-HT2	24.66 ± 0.52 µg/mL
	Mean of 5 replicates measurement against reference batch, confidence interval with P = 95%



LC-MS/MS chromatogram of Toxin

Safety measures:

Special care must be taken when manipulating this standard. Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Use only in a chemical fume hood. Safety shower and eye bath must be near. In case of spills, cover and absorb with an inert dry material such as dry-lime, sand or soda ash and place in an appropriate waste disposal container.
Keep container tightly closed. Do not store in direct sunlight. Keep away from heat, sparks, flame and incompatible material. Storage area should be cool, dry and away from incompatible materials.

Further information:

Further information is available in the MSDS provided along with this certificate. Final users should make their own investigations to determine the suitability of the information for their particular research purposes. In no event the supplier of this RM shall be held liable for any damage resulting from handling or from contact with the product.

References:

a- AOAC Official Method 970.44 – Preparation of Standards for Mycotoxins.
b- ISO Guide 31, 1–7, (2000), "Reference Materials–Contents of certificates and labels".
c- ISO Guide 35, 1–7 (2000) "Certification of Reference Materials – General and Statistical Principles".
d- Eurachem/CITAC guide, 1–37 (2003) "Traceability in Chemical Measurement".
e- Eurachem/CITAC guide, 1–120 (2000) "Quantifying Uncertainty in Analytical Measurement".
f- Bennett, G.A., and Shotwell, O.L. 1990. Criteria for determining the purity of Fusarium mycotoxins. J. Assoc. Off. Anal. Chem., 73:270-275.
g- Kriska, R., Szente, E., Freudenschuss, M., Harnetner, C., and Zöllner, P. 2004. Purity assessment of commercially available crystalline HT2 toxin. J. AOAC Int., 87:909-919.
h- Sydenham, E.W., Thiel, P.G., and Vleggaar, R. 1996. Physicochemical data for some selected Fusarium toxins. J. AOAC Int., 79:1365-1379.

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Quality Control

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