Certificate of Analysis(Ver.2.0)

U-[$^{13}C_{22}$]-Oxytetracyline

1. General information

This document is designed and the certified value(s) and uncertainty(ies) are determined in accordance with ISO Guide 31[1].

2. Description of the Reference Material (RM)

Name:	**U-[$^{13}C_{22}$]-Oxytetracyline**
Catalog number:	**STD#7071U**
CAS number:	**79-57-2(Unlabeled)**
Formula:	**$^{13}C_{22}H_{24}N_2O_9$**
Formula weight:	**483.26**
Lot #:	**2B00E10**
Starting material：	**U-[$^{13}C_{22}$]-Oxytetracyline,lot#D21213P,Pribolab Pte.Ltd.**
Amount:	**2.50μg dried down, 2.50μg/mL after reconstitution with 1mL solvent**
Production date:	**10,May,2022**
Expiry date:	**09,May,2023**
Name of the supplier:	**Pribolab Pte.Ltd.**

2.1 Intended use of the RM

- for laboratory use only
- internal standard[2]

2.2 Reconstitution instruction

The standard that you have received may appear at first glance, as an empty vial. The target compound (s) is (are) in a film at the bottom of the vial. Do not open the vial until you are ready to reconstitute

To reconstitute this RM use the following procedure:

1. Add 1mL solvent with a graduated syringe or a volumetric pipette.
2. Cap vial tightly.
3. Mix vigorously on a vortex mixer and repeat for several times over a longer period or sonicate at room temperature.
4. Always keep vial tightly capped.
5. Store the reconstituted standard at -20°C in a dark environment for max. 8 weeks.
6. Store immediately at -20°C after usage to avoid degradation.

Note: Thorough mixing and sufficient mixing time is required to ensure complete reconstitution of the dried down standard!

2.3 Instruction for the correct use of the RM

The compound should be stored at -20℃ in a dark place.Before usage of the RM,the compound should be allowed to warm to temperature(20±3℃).The recommended minimum sub-sample amount for all kinds of application is 100 µL.The expiry date of this RM is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/packages.

2.4 Hazardous situation

The normal laboratory safety precautions should be observed when working with this RM.Further details for the handing of this RM are available as safety data sheet.

3. Certified values and their uncertainties

U-[$^{13}C_{22}$]-Oxytetracyline		
Compound	**Mass concentration [a]**	
U-[$^{13}C_{22}$]-Oxytetracyline,96.02 atom% ^{13}C	Certified value [b]	Uncertainty [c]
	2.50µg/mL	±0.09µg/mL
a Values are based on preparation data and confirmed experimentally by HPLC-DAD		
b Mass concentration based on weighed amount ,purity and dilution step		
c Expanded uncertainty U(k=2) of the value u_c according to GUM[3]		

3.1 Calculation of uncertainty

The uncertainty of the calibrant solution was calculated on the basis of preparation [4].

Uncertainty components	**Description**	**Standard uncertainty$_{(U)}$**	
Purity (P) of solid U-[$^{13}C_{22}$]-Oxytetracyline,96.02 atom% ^{13}C	P=96.6±3.4%	**u(P)=1.7%**	a
Weighing procedure Weighted sample: m_{ws}=1.294mg	$U_{(m)}$=0.0000008g+1.30*10^{-5}*m Toxin $u_{(m)}=U_{(m)}/2$	**$u_{(m)}$=0.0004mg**	b
Dilution procedure Volumetric flask1:V_f=500mL	Calibration:500mL ± 0.25mL Repeatability : 0.1mL Volume expansion solvent	u(cal)=0.1mL u(rep)=0.1mL u(Vol.exp.1)=1.0mL **u(V)=1.0mL**	c d e f

a Maximum tolerance of purity was divided by $\sqrt{3}$

b Calculation of this u-value is based upon the uncertainty formula for the weighed amount as given in the calibration report from annual balance calibration

c A triangular distribution(division by $\sqrt{6}$)was chosen for the calculation of u(cal)

d Based on a series of ten fill and weigh experiments on a typical 250mL flask; the value was used directly as a standard deviation

e Based on the density of 0.7918 g/cm^3 at temperature T=20℃ and a maximum temperature variation of ±3℃,of volume expansion, relative volume expansion coefficient of methanol is 1190*10^{-6}/℃[7],volume expansion term(rectangular distribution)was divided by $\sqrt{3}$

f The three contributions are combined to give the u(V)= $\sqrt{u(cal)^2+u(rep)^2+u(Vol.exp)^2}$

Calculation of the combined uncertainty u_c and the expanded standard uncertainty U

$$C_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 1.294 \times 96.6}{500} = 2.50 mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{1.7}{96.6}\right]^2 + \left[\frac{0.0004}{1.294}\right]^2 + \left[\frac{1.0}{500}\right]^2} = 0.018$$

$$u_C(c_{Toxin}) = c_{Toxin} \times 0.018 = 2.50 \times 0.018 = 0.045 mg/L$$

calculation of expanded standard uncertainty U using a coverage factor k=2

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.045 \times 2 = 0.09 \mu g/mL$$

4.Isotopic enrichment and isotope pattern

Isotope pattern [a]	
Compound	**Isotopic distribution**
U-[$^{13}C_{22}$]-Oxytetracyline	47.55%
U-[$^{13}C_{21}$]-Oxytetracyline	24.73%
U-[$^{13}C_{20}$]-Oxytetracyline	20.28%
U-[$^{13}C_{19}$]-Oxytetracyline	7.44%
Calculated isotopic enrichment level [a]:96.02 atom % ^{13}C	
[a] Approximation based on LC-MS/MS data	

5.Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [5]. Thus the certified value(mass concentration of U-[$^{13}C_{22}$]-Oxytetracyline,96.02 atom% ^{13}C is based on the weighed amount of the starting material and is therefore traceable to the stated purity of the solid raw material.High purity material represents a practical realization of concentration units,through conversion of mass to molar quality.

6.Confirmation of certified value by HPLC-DAD

The certified concentration of U-[$^{13}C_{22}$]-Oxytetracyline,96.02 atom% ^{13}C of the gravimetric prepared solution was confirmed by HPLC-DAD against an independently prepared reference batch of unlabeled Oxytetracyline .

column	C_{18},150×4.6mm, 5µm
injection Volume	10µL
solvent	acetonitrile/0.01mol/L disodium hydrogen phosphate buffer solution=20/80 (PH=2)
oven	30℃
flow rate	1mL/min
DAD settings	269nm
Sample dilution	methanol

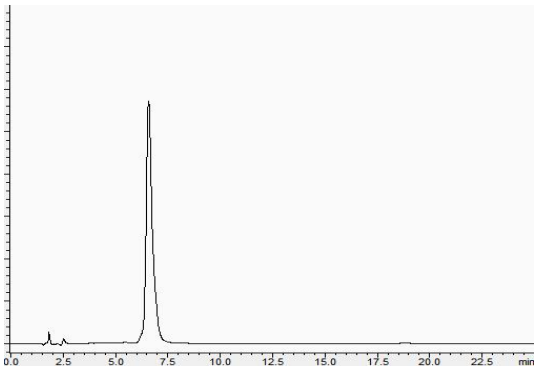

Figure 1:HPLC-DAD chromatogram of U-[$^{13}C_{22}$]-Oxytetracyline

	time[min]	concentration [a]
U-[$^{13}C_{22}$]-Oxytetracyline	6.579	2.52 µg/mL

[a] Mean of 6 replicate measurements against reference batch,confidence interval with P=95%

7.Further information

The purchaser must determine the suitability of this product for its particular use. Pribolab makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by Pribolab. We do not guarantee that the product can be used for a special application.

Inspected by

Quality System Specialist

References:

[1]ISO Guide 31:2015 - 1-18, ”Reference materials – contents of certificates, labels and accompanying documentation“

[2]G. Häubl, F. Berthiller, R. Krska, R. Schuhmacher, ”Suitability of a fully ^{13}C isotope labelled internal standard for the determination of the mycotoxin deoxynivalenol by LC-MS/MS without clean-up“, Anal. Bioanal. Chem. 384 (3), (2006), 692-696

[3] International Organization for Standardization (ISO), (2008), ”Guide to the expression of uncertainty in measurement“, (GUM 1995 with minor corrections) 1st Ed. Geneva, Switzerland

[4] R.D. Josephs, R. Krska, S. MacDonald, P. Wilson, H. Pettersson, J. AOAC Int. 86, 50-60, (2003), ”Preparation of a Calibrant as Certified Reference Material for Determination of the Fusarium Mycotoxin Zearalenone“

[5] E.W. Flick, (1998), ”Industrial Solvents Handbook“, 5th Ed., Noyes Data Corp. Westwood NJ