Certificate of Analysis(Ver.2.0)

U-[$^{13}C_{34}$]-Fumonisin B_2 in Acetonitrile/Water (1:1)

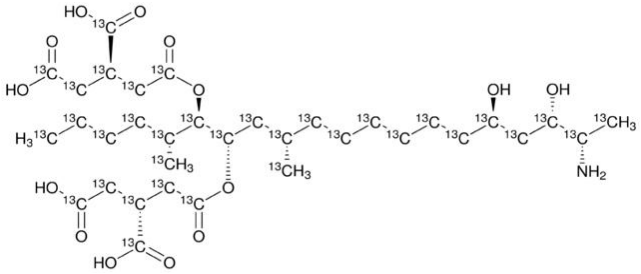
1. General information

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-[$^{13}C_{34}$]-Fumonisin B_2 in Acetonitrile/Water(1:1)**

Catalog number: **STD#2040U**



CAS number: **1217481-36-1**

Formula: **$^{13}C_{34}H_{59}NO_{14}$**

Formula weight: **739.58**

Lot #: **2A01J26**

Starting material: **U-[$^{13}C_{34}$]-Fumonisin B_2,lot#RI18313G,Pribolab Pte.Ltd.**

Solvent: **Acetonitrile,LiChrosolv®;Merck**

Amount: **1.2mL**

Production date: **26/Oct/2021**

Expiry date: **25/Apr/2023**

Name of the supplier: **Pribolab Pte.Ltd.**

2.1 Intended use of the RM

- for laboratory use only
- internal standard[2,3]

2.2 Instruction for the correct use of the RM

The compound should be stored at 2-8℃ 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.

2.3 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.

Hazardous Ingredients	Concentration in%	Pictograms	Signal word	Hazard statement(s)
Acetonitrile	>50		Danger	H225,H302,H312,H319,H332

3. Certified values and their uncertainties

U-[$^{13}C_{34}$]-Fumonisin B_2 in Acetonitrile/Water		
Compound	**Mass concentration [a]**	
U-[$^{13}C_{34}$]-Fumonisin B_2,97.99 atom % ^{13}C	Certified value [b]	Uncertainty [c]
	10.00µg/mL	±0.16µg/mL
a Values are based on preparation data and confirmed experimentally by LC-MS		
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[4]		

3.1 Calculation of uncertainty

The uncertainty of the calibrant solution was calculated on the basis of preparation [5].

Uncertainty components	**Description**	**Standard uncertainty(U)**	
Purity (P) of solid U-[$^{13}C_{34}$]-Fumonisin B_2,97.99 atom% ^{13}C	P=98.7±1.3%	**u(P)=0.8%**	a
Weighing procedure Weighted sample: m_{ws}=2.026mg	$U_{(m)}$=0.0000008g+1.30*10^{-5}*m $_{Toxin}$ $u_{(m)}=U_{(m)}/2$	**$u_{(m)}$=0.0004mg**	b
Dilution procedure Volumetric flask:V_f =200mL	calibration: 200mL±0.15mL repeatability: 0.06mL volume expansion solvent	u(cal)=0.06mL U(rep)=0.06mL u(Vol.exp.)=0.47mL **u(v)=0.5mL**	c d e f

a Maximum tolerance of purity (rectangular distribution) 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 200mL flask; the value was used directly as a standard deviation

e Based on the density of 0.7857 g/cm^3 at temperature T=20℃ and a maximum temperature variation of ±3℃,of volume expansion, relative volume expansion coefficient of acetonitrile is 1370*10$^{-6/℃}$[6],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

$$C_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 2.026 \times 98.7}{200} = 10.00\ mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.8}{98.7}\right]^2 + \left[\frac{0.0004}{2.026}\right]^2 + \left[\frac{0.5}{200}\right]^2} = 0.008$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.008 = 10.00 \times 0.008 = 0.08\ mg/L$$

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

$$U_{(c_{Toxin})} = u_c(c_{Toxin}) \times 2 = 0.08 \times 2 = 0.16\ mg/L = 0.16\ \mu g/mL$$

4.Isotopic enrichment and isotope pattern

Isotope pattern [a]	
Compound	**Isotopic distribution**
[$^{13}C_{34}$]-Fumonisin B_2	54.95%
[$^{13}C_{33}$]- Fumonisin B_2	26.92%
[$^{13}C_{32}$]- Fumonisin B_2	13.19%
[$^{13}C_{31}$]- Fumonisin B_2	4.94%
Calculated isotopic enrichment level [a]:97.99 atom % ^{13}C	
[a] Approximation based on LC-MS/MS data	

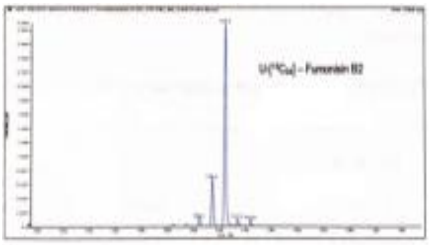


Figure1: Enhanced resolution scan of [$^{13}C_{34}$]-Fumonisin B_2 for determination of isotope pattern

5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified value(mass concentration of U-[$^{13}C_{34}$]-Fumonisin B_2,97.99 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 quantity.

6. Confirmation of certified value by LC-MS

The certified concentration of U- [$^{13}C_{34}$]-Fumonisin B_2,97.99 atom % ^{13}C of the gravimetric prepared solution was confirmed by LC-MS against an independently prepared reference batch of U-[$^{13}C_{34}$]-Fumonisin B_2.

column	Agilent XDB-C8,3.0×100mm 3.5μm
injection Volume	2μL
solvent A	5mM ammonium acetate buffer pH 3.1
solvent B	acetonitrile
flow rate	0.4mL/min, oven at 40℃
gradient	time in minutes(min) %solvent
	0 35% B
	10 35 % B
source type	HESI positive mode
sample dilution	1:2 with solvent A

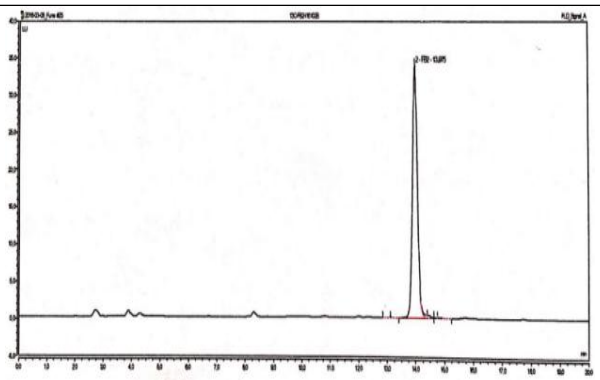


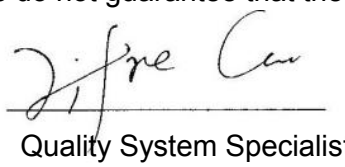
Figure 1:SIM 740 $[M+H]^+$ of U- [$^{13}C_{34}$]-Fumonisin B_2 calibrante

	Peak Area	Retention Time	concentration [a]
U- [$^{13}C_{34}$]-Fumonisin B_2	2.6E+04	14.008	10.01±0.3μg/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]G. Häubl, F.Berthiller, J. Rechthaler,G. Jaunecker, E.M. Binder,R.Krska,R.Schuhmacher,(2006),” Characterization and application of isotope- substituted ($^{13}C_{15}$)-Deoxynivalenol (DON)as an internal standard for the determination of DON”,Food Addit Contam.23,(2006),1187-1193(2006)

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

[5] 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“

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