Certificate of Analysis(Ver.2.1)

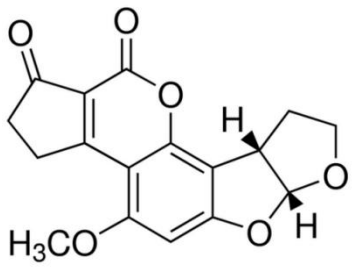
Aflatoxin B_2 in Acetonitrile

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)

Product name:	**Aflatoxin B_2 in Acetonitrile**
Product number:	**STD#1051**
CAS number:	**7220-81-7**
Formula:	**$C_{17}H_{14}O_6$**
Formula weight:	**314.29**
Lot#:	**2B00E06**
Result concentration:	**25.00±0.35μg/mL**
Starting material:	**Aflatoxin B_2,lot#J200249P,Pribolab Pte. Ltd.**
Matrix:	**Acetonitrile, LiChrosolv®, Merck**
Amount:	**5.2mL**
Production date:	**06,May,2022**
Expiry date:	**05,Nov,2023**
Name of the supplier:	**Pribolab Pte. Ltd.**



2.1 Intended use of the RM

- for laboratory use only
- calibration of analytical instruments

2.2 Instruction for the correct use of the RM

The compound should be stored at -20℃ or below 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	>99.9		Danger	H225,H302,H312,H319,H332

3. Certified values and their uncertainties

Aflatoxin B_2 in Acetonitrile		
Compound	**Mass concentration [a]**	
Aflatoxin B_2	Certified value [b]	Uncertainty [c]
	25.00μg/mL	±0.35μg/mL

a Mass concentration based on weighed amount ,purity and dilution steps
b Values are based on preparation data and confirmed experimentally by HPLC-DAD
c Expanded uncertainty U(k=2) of the value u_c according to GUM[2]

3.1 Calculation of uncertainty

After the concentration of the gravimetric prepared solution was confirmed by HPLC-DAD,the uncertainty of the calibrant was calculated on the basis of preparation[3].

Uncertainty components	**Description**	**Standard uncertainty(U)**	
Purity(P)of solid Aflatoxin B_2	P=99.0±1.0%	**u(P)=0.6%**	a
Weighing procedure Weighted sample: m_{ws}=1.263mg	$U_{(m)}$=0.0000008g+1.30*10^{-5}*m $_{Toxin}$ $u_{(m)}=U_{(m)}/2$	**$u_{(m)}$=0.0004mg**	b
Dilution procedure Volumetric flask :V_f=50mL	Calibration:50mL ± 0.05mL Repeatability : 0.03mL Volume expansion solvent	u(cal)=0.02mL u(rep)=0.03mL u(Vol.exp.1)=0.12mL **u(v)=0.13mL**	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 50mL 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}/℃[7],volume expansion term(rectangular distribution)was divided by $\sqrt{3}$

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

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

$$C_{Toxin}=\frac{10\times m_{ws}\times P}{V_f}=\frac{10\times 1.263\times 99.0}{50}=25.00\ mg/L$$

$$\frac{u_c(C_{Toxin})}{C_{Toxin}}=\sqrt{\left[\frac{u(P)}{P}\right]^2+\left[\frac{u(m)}{m_{ws}}\right]^2+\left[\frac{u(V)}{V_f}\right]^2}=\sqrt{\left[\frac{0.6}{99.0}\right]^2+\left[\frac{0.0004}{1.263}\right]^2+\left[\frac{0.13}{50}\right]^2}=0.007$$

$$u_c(C_{Toxin})=C_{Toxin}\times 0.007=25.00\times 0.007=0.175 mg/L$$

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

$$U(C_{Toxin})=u_c(C_{Toxin})\times 2=0.175\times 2=0.35\ \mu g/mL$$

4.Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation[4]. Thus the certified value mass concentration of Aflatoxin B_2 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.

5. Confirmation of certified value by HPLC-DAD

The certified concentration of Aflatoxin B_2 of the gravimetric prepared solution was confirmed by HPLC-DAD against an independently prepared reference batch of Aflatoxin B_2.

column	C_{18},250×4.6mm, 5μm
injection Volume	100μL
solvent	Water/Acetonitrile/Methanol (57/17/26)
oven	30℃
flow rate	0.5mL/min
DAD settings	365nm
Sample dilution	1:5 with water

	time[min]	concentration [a]
Aflatoxin B_2	11.418	25.01μg/mL

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

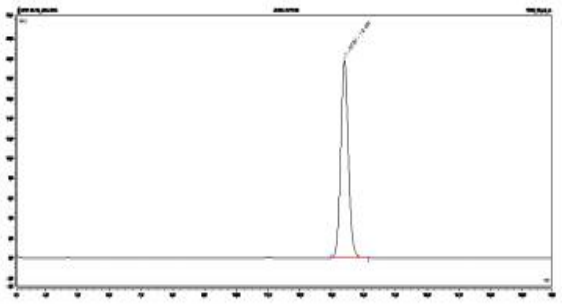


Figure 1:HPLC-DAD chromatogram of Aflatoxin B_2

6.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, 1-7, (2000), “Reference Materials - Contents of Certificates and Labels”

[2] .International Organization for Standardization (ISO), (2008), “Guide to the Expression of Uncertainty in Measurements ”, (GUM 1995 with minor corrections) 1st Ed. Geneva, Switzerland

[3] 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”

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