

# Certificate of Analysis (Ver.2.0)

## Aflatoxins 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<sup>[1]</sup>.

### 2. Description of the Reference Material (RM)

<b>Product Name:</b>	Aflatoxins in Acetonitrile								
<b>Product Number:</b>	STD#1086								
<b>Lot#:</b>	2A01A23								
<b>CAS number:</b>	Aflatoxin B <sub>1</sub> : 1162-65-8; Aflatoxin B <sub>2</sub> : 7220-81-7; Aflatoxin G <sub>1</sub> : 1165-39-5; Aflatoxin G <sub>2</sub> : 7241-98-7 ; Aflatoxin M <sub>1</sub> : 6795-23-9; Aflatoxin M <sub>2</sub> : 6885-57-0;								
<b>Formula:</b>	Aflatoxin B <sub>1</sub> : C <sub>17</sub> H <sub>12</sub> O <sub>6</sub> ; Aflatoxin B <sub>2</sub> : C <sub>17</sub> H <sub>14</sub> O <sub>6</sub> ; Aflatoxin G <sub>1</sub> : C <sub>17</sub> H <sub>12</sub> O <sub>7</sub> ; Aflatoxin G <sub>2</sub> : C <sub>17</sub> H <sub>14</sub> O <sub>7</sub> ; Aflatoxin M <sub>1</sub> : C <sub>17</sub> H <sub>12</sub> O <sub>7</sub> ; Aflatoxin M <sub>2</sub> : C <sub>17</sub> H <sub>14</sub> O <sub>7</sub> ;								
<b>Formula Weight:</b>	Aflatoxin B <sub>1</sub> : 312.27; Aflatoxin B <sub>2</sub> : 314.29 ; Aflatoxin G <sub>1</sub> : 328.27; Aflatoxin G <sub>2</sub> : 330.29; Aflatoxin M <sub>1</sub> : 328.27; Aflatoxin M <sub>2</sub> : 330.29;								
<b>Result Concentration:</b>	<table border="0" style="width: 100%;"> <tr> <td style="width: 50%;">Aflatoxin B<sub>1</sub>: <b>10.12±0.14</b> µg/mL</td> <td style="width: 50%;">Aflatoxin B<sub>2</sub>: <b>10.01±0.14</b> µg/mL</td> </tr> <tr> <td>Aflatoxin G<sub>1</sub>: <b>10.00±0.14</b> µg/mL</td> <td>Aflatoxin G<sub>2</sub>: <b>10.00±0.26</b> µg/mL</td> </tr> <tr> <td>Aflatoxin M<sub>1</sub>: <b>10.02±0.14</b> µg/mL</td> <td>Aflatoxin M<sub>2</sub>: <b>10.00±0.14</b> µg/mL</td> </tr> </table> <p>Aflatoxin B<sub>1</sub>, lot# <b>19704P</b>, Pribolab Pte. Ltd. ; Aflatoxin B<sub>2</sub>, lot# <b>19704P</b>, Pribolab Pte. Ltd. ; Aflatoxin G<sub>1</sub>, lot# <b>19704P</b>, Pribolab Pte. Ltd. ; Aflatoxin G<sub>2</sub>, lot# <b>19704P</b>, Pribolab Pte. Ltd. ;</p>			Aflatoxin B <sub>1</sub> : <b>10.12±0.14</b> µg/mL	Aflatoxin B <sub>2</sub> : <b>10.01±0.14</b> µg/mL	Aflatoxin G <sub>1</sub> : <b>10.00±0.14</b> µg/mL	Aflatoxin G <sub>2</sub> : <b>10.00±0.26</b> µg/mL	Aflatoxin M <sub>1</sub> : <b>10.02±0.14</b> µg/mL	Aflatoxin M <sub>2</sub> : <b>10.00±0.14</b> µg/mL
Aflatoxin B <sub>1</sub> : <b>10.12±0.14</b> µg/mL	Aflatoxin B <sub>2</sub> : <b>10.01±0.14</b> µg/mL								
Aflatoxin G <sub>1</sub> : <b>10.00±0.14</b> µg/mL	Aflatoxin G <sub>2</sub> : <b>10.00±0.26</b> µg/mL								
Aflatoxin M <sub>1</sub> : <b>10.02±0.14</b> µg/mL	Aflatoxin M <sub>2</sub> : <b>10.00±0.14</b> µg/mL								
<b>Starting material:</b>	Aflatoxin M <sub>1</sub> , lot# <b>20501P</b> , Pribolab Pte. Ltd. ; Aflatoxin M <sub>2</sub> , lot# <b>20724P</b> , Pribolab Pte. Ltd.								
<b>Matrix:</b>	Acetonitrile, LiChrosolv <sup>®</sup> , Merck								
<b>Amount:</b>	1.2mL								
<b>Production date:</b>	23/Nov/2021								
<b>Expiry date:</b>	22/May/2023								
<b>Name of the supplier:</b>	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°C or below in a dark place. Before usage of the RM, the compound should be allowed to warm to temperature (20±3°C). 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 handling 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

Aflatoxins in acetonitrile					
Compound	Mass concentration <sup>a</sup>		Compound	Mass concentration <sup>a</sup>	
	Certified value <sup>b</sup>	Uncertainty <sup>c</sup>		Certified value <sup>b</sup>	Uncertainty <sup>c</sup>
Aflatoxin B <sub>1</sub>	10.12µg/mL	±0.14 µg/mL	Aflatoxin G <sub>1</sub>	10.01µg/mL	±0.14µg/mL
Aflatoxin B <sub>2</sub>	10.00µg/mL	±0.14 µg/mL	Aflatoxin G <sub>2</sub>	10.00µg/mL	±0.26µg/mL
Aflatoxin M <sub>1</sub>	10.02µg/mL	±0.14 µg/mL	Aflatoxin M <sub>2</sub>	10.00µg/mL	±0.14µg/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<sub>c</sub> according to GUM<sup>[2]</sup>

### 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<sup>[3]</sup>.

Uncertainty components	Description	Standard uncertainty(u)	
Purity(P)of solid Aflatoxin B <sub>1</sub> Aflatoxin B <sub>2</sub> Aflatoxin G <sub>1</sub> Aflatoxin G <sub>2</sub> Aflatoxin M <sub>1</sub> Aflatoxin M <sub>2</sub>	P <sub>1</sub> =99.0±1.0% P <sub>2</sub> =98.9±1.1% P <sub>3</sub> =98.9±1.1% P <sub>4</sub> =98.0±2.0% P <sub>5</sub> =98.9±1.1% P <sub>6</sub> =98.9±1.1%	<b>u(P<sub>1</sub>)=0.6%</b> <b>u(P<sub>2</sub>)=0.6%</b> <b>u(P<sub>3</sub>)=0.6%</b> <b>u(P<sub>4</sub>)=1.2%</b> <b>u(P<sub>5</sub>)=0.6%</b> <b>u(P<sub>6</sub>)=0.6%</b>	a
Weighing procedure: Weighted sample: m <sub>Aflatoxin B1</sub> =25.556mg m <sub>Aflatoxin B2</sub> =25.303mg m <sub>Aflatoxin G1</sub> =25.278mg m <sub>Aflatoxin G2</sub> =25.510mg m <sub>Aflatoxin M1</sub> =25.329mg m <sub>Aflatoxin M2</sub> =25.278mg	U <sub>(m)</sub> =0.0000008g+1.30*10 <sup>-5</sup> *m <sub>Toxin</sub> u <sub>(m)</sub> =U <sub>(m)}/2</sub>	<b>u<sub>(m)</sub>=0.0006mg</b>	b
Dilution procedure Volumetric flask1:V <sub>f1</sub> =250mL Volumetric flask2:V <sub>f2</sub> =250mL One-mark glass pipette:V <sub>p</sub> =25mL	calibration flask1: 250mL±0.15mL repeatability flask1: 0.03mL volume expansion solvent flask1  calibration flask2: 250mL±0.15mL repeatability flask2: 0.03mL volume expansion solvent flask2  Calibration pipette: 25mL±0.03mL volume expansion solvent pipette	u(cal1)=0.06mL u(rep1)=0.03mL u(Vol.exp.1)=0.59mL <b>u(V1)=0.59mL</b> u(cal2)=0.06mL u(rep2)=0.03mL u(Vol.exp.2)=0.59mL <b>u(V2)=0.59mL</b> u(cal3)=0.01mL u(Vol.exp.3)=0.06mL <b>u(V3)=0.06mL</b>	c d e f g h i j k l m

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,g,k A triangular distribution (division by  $\sqrt{6}$ ) was chosen for the calculation of u(cal)  
d,h 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,i,l Based on the density of 0.7857 g/cm<sup>3</sup> at temperature T=20°C and a maximum temperature variation of ±3°C, of volume expansion, relative volume expansion coefficient of acetonitrile is 1370\*10<sup>-6</sup>/°C [7], volume expansion term (rectangular distribution) was divided by  $\sqrt{3}$   
f,j,m The three contributions are combined to give the u(V)= $\sqrt{u(\text{cal})^2 + u(\text{rep})^2 + u(\text{Vol.exp})^2}$

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

$$c_{\text{Toxin}} = \frac{10 \times m_{\text{ws}} \times P_1 \times V_P}{V_{f1} \times V_{f2}} = \frac{10 \times 25.556 \times 99.0 \times 25}{250 \times 250} = 10.12$$

*mg / L*

$$\frac{u_c(c_{\text{Toxin}})}{c_{\text{Toxin}}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{\text{ws}}}\right]^2 + \left[\frac{u(V1)}{V_{f1}}\right]^2 + \left[\frac{u(V2)}{V_{f2}}\right]^2 + \left[\frac{u(V3)}{V_P}\right]^2} = \sqrt{\left[\frac{0.6}{99.0}\right]^2 + \left[\frac{0.0006}{25.556}\right]^2 + \left[\frac{0.59}{250}\right]^2 + \left[\frac{0.59}{250}\right]^2 + \left[\frac{0.06}{25}\right]^2} = 0.007$$

$$u_c(c_{\text{Toxin}}) = c_{\text{Toxin}} \times 0.007 = 10.12 \times 0.007 = 0.07 \text{ mg / L}$$

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

$$U(c_{\text{Toxin}}) = u_c(c_{\text{Toxin}}) \times 2 = 0.07 \times 2 = 0.14 \text{ mg / L} = 0.14 \mu\text{g / mL}$$

#### 4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation<sup>[4]</sup>. Thus the certified value (mass concentration of Aflatoxins) 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 Aflatoxins of the gravimetric prepared solution was confirmed by HPLC-DAD against an independently prepared reference batch of Aflatoxins.

column	C <sub>18</sub> , 250×4.6mm, 5μm	
injection Volume	100μL	
solvent	Water/Acetonitrile/Methanol (57/17/26)	
oven	30°C	
flow rate	1.0mL/min	
DAD settings	365nm	
Sample dilution	1:5 with water	
	time[min]	concentration <sup>a</sup> [μg/mL]
Aflatoxin B <sub>1</sub>	11.521	10.02
Aflatoxin B <sub>2</sub>	9.738	10.22
Aflatoxin G <sub>1</sub>	8.875	10.04
Aflatoxin G <sub>2</sub>	7.672	10.13
Aflatoxin M <sub>1</sub>	6.762	10.15
Aflatoxin M <sub>2</sub>	5.848	10.11
<sup>a</sup> Mean of 6 replicate measurements against reference batch, confidence interval with P=95%		

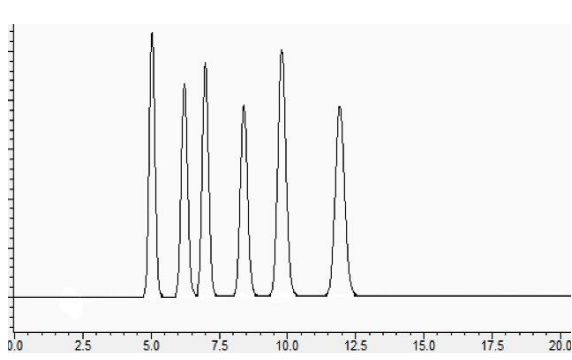


Figure: HPLC-DAD chromatogram of Aflatoxins

## 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) 1<sup>st</sup> 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 ", 5<sup>rd</sup> Ed., Noyes Data Corp. Westwood NJ