

# Certificate of Analysis (Ver.1.0)

## 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)

Product name:	Deoxynivalenol, Nivalenol ,3-Acetyl-Deoxynivalenol,15-Acetyl-Deoxynivalenol in Acetonitrile	
Product number:	STD#3110	
Lot#:	2B00B24	
CAS number:	Deoxynivalenol:51481-10-8;	Nivalenol:23282-20-4;
	3-Acetyl-Deoxynivalenol:50722-38-8;	15-Acetyl-Deoxynivalenol:88337-96-6
Formula:	Deoxynivalenol:C <sub>15</sub> H <sub>20</sub> O <sub>6</sub> ;	Nivalenol:C <sub>15</sub> H <sub>20</sub> O <sub>7</sub> ;
	3-Acetyl-Deoxynivalenol:C <sub>17</sub> H <sub>22</sub> O <sub>7</sub> ;	15-Acetyl Deoxynivalenol:C <sub>17</sub> H <sub>22</sub> O <sub>7</sub>
Formula weight:	Deoxynivalenol:296.32;	Nivalenol:312.32;
	3-Acetyl-Deoxynivalenol:338.35;	15-Acetyl-Deoxynivalenol:338.35
Result concentration:	Deoxynivalenol:100.4±1.4µg/mL;	
	Nivalenol:100.2±1.4µg/mL;	
	3-Acetyl-Deoxynivalenol:100.4±1.2µg/mL;	
	15-Acetyl-Deoxynivalenol:100.6±1.4µg/mL;	
Starting material:	Deoxynivalenol:lot#19724P,Pribolab Pte. Ltd.;	
	Nivalenol:lot#03410Z, Pribolab Pte. Ltd.;	
	3-Acetyl-Deoxynivalenol:lot#J200110P,Pribolab Pte. Ltd.;	
	15-Acetyl-Deoxynivalenol:lot#17117P,Pribolab Pte. Ltd.	
Matrix:	Acetonitrile, LiChrosolv®, Merck	
Amount:	1.2mL	
Production date:	24, Feb, 2022	
Expiry date:	23, Aug, 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 2-8°C 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

Deoxynivalenol, Nivalenol ,3-Acetyl-Deoxynivalenol,15-Acetyl-Deoxynivalenol 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>
Deoxynivalenol	100.4µg/mL	±1.4µg/mL	3-Acetyl Deoxynivalenol	100.4µg/mL	±1.2µg/mL
Nivalenol	100.2µg/mL	±1.4µg/mL	15-Acetyl Deoxynivalenol	100.6µg/mL	±1.4µg/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 Deoxynivalenol Nivalenol 3-Acetyl-Deoxynivalenol 15-Acetyl-Deoxynivalenol	P <sub>1</sub> =99.0±1.0% P <sub>2</sub> =99.0±1.0% P <sub>3</sub> =99.1±0.9% P <sub>4</sub> =99.0±1.0%	u(P <sub>1</sub> )=0.6% u(P <sub>2</sub> )=0.6% u(P <sub>3</sub> )=0.5% u(P <sub>4</sub> )=0.6%	a
Weighing procedure: Weighted sample: Deoxynivalenol=10.141mg Nivalenol=10.121mg 3-Acetyl-Deoxynivalenol=10.131mg 15-Acetyl-Deoxynivalenol=10.161mg	U <sub>(m)</sub> =0.0000008g+1.30*10 <sup>-5</sup> *m <sub>Toxin</sub> u <sub>(m)</sub> =U <sub>(m)</sub> /2	u <sub>(m)</sub> =0.0005mg	b
Dilution procedure Volumetric flask :V <sub>f</sub> =100mL	Calibration:100mL ± 0.1mL Repeatability : 0.04mL Volume expansion solvent	u(cal)=0.04mL u(rep)=0.04mL u(Vol.exp.1)=0.24mL u(v)=0.25mL	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<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<sup>[7]</sup>, volume expansion term (rectangular distribution) was divided by  $\sqrt{3}$

f 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<sub>c</sub> and the expanded standard uncertainty U**

$$C_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 10.141 \times 99.0}{100} = 100.4 \text{ mg / L}$$

$$\frac{u_c(C_{Toxin})}{C_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.6}{99.0}\right]^2 + \left[\frac{0.0005}{10.141}\right]^2 + \left[\frac{0.25}{100}\right]^2} = 0.007$$

$$u_c(C_{Toxin}) = C_{Toxin} \times 0.007 = 100.4 \times 0.007 = 0.7 \text{ mg / L}$$

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

$$U(C_{Toxin}) = u_c(C_{Toxin}) \times 2 = 0.7 \times 2 = 1.4 \text{ mg / L} = 1.4 \mu\text{g / mL}$$

#### 4. Discussion of traceability

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

column	C <sub>18</sub> , 250×4.6mm, 5μm	
injection Volume	20μL	
Solvent A	Water	
Solvent B	Acetonitrile	
oven	30°C	
flow rate	1.0mL/min	
DAD settings	218nm	
Sample dilution	1:10 with water	
gradient	time in minutes(min)	% solvent B
	0-5	10
	5-10	10-20
	10-20	20
	20-30	20-10




Figure: HPLC-DAD chromatogram of B-Trichothecenes

	Time(min)	area	concentration
DON	6.166	30.452	100.1±1.4μg/mL
NIV	7.805	26.219	100.0±1.4μg/mL
15-DON	16.811	27.352	100.3±1.4μg/mL
3-DON	17.522	43.743	100.5±1.4μg/mL

## 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