

# Certificate of Analysis (Ver.2.0)

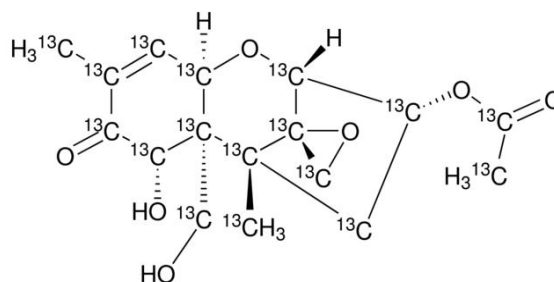
## U-[<sup>13</sup>C<sub>17</sub>]-3-Acetyldeoxynivalenol in Acetonitrile

### 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)

|                              |   |
|------------------------------|---|
| <b>Name:</b>                 | U-[ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol in Acetonitrile              |
| <b>Catalog number:</b>       | STD#3106U   |
| <b>CAS number:</b>           | 1217476-81-7  |
| <b>Formula:</b>              | <sup>13</sup> C <sub>17</sub> H <sub>22</sub> O <sub>7</sub>                            |
| <b>Formula weight:</b>       | 355.23  |
| <b>Lot #:</b>                | 2A01J19   |
| <b>Starting material :</b>   | U-[ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol,lot#T18917,Pribolab Pte.Ltd. |
| <b>Solvent:</b>              | Acetonitrile,LiChrosolv®,Merck  |
| <b>Amount:</b>               | 1.2mL   |
| <b>Production date:</b>      | 22/Oct/2021   |
| <b>Expiry date:</b>          | 21/Apr/2023   |
| <b>Name of the supplier:</b> | 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°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

| U-[ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol in acetonitrile   |                                 |                          |
|--|---------------------------------|--------------------------|
| Compound   | Mass concentration <sup>a</sup> |                          |
| U-[ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol, 99.51 atom% <sup>13</sup> C  | Certified value <sup>b</sup>    | Uncertainty <sup>c</sup> |
|  | 10.08 μg/mL                     | ±0.30 μg/mL              |
| <p>a Values are based on preparation data and confirmed experimentally by HPLC-DAD</p> <p>b Mass concentration based on weighed amount, purity and dilution step</p> <p>c Expanded uncertainty U(k=2) of the value u<sub>c</sub> according to GUM[4]</p> |                                 |                          |

### 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<br>U-[ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol, 99.51 atom% <sup>13</sup> C | P=99.1±0.9%   | <b>u(P)=0.5%</b>                | a |
| Weighing procedure<br>Weighted sample: m <sub>ws</sub> =2.531mg  | U <sub>(m)</sub> =0.0000008g+1.21*10 <sup>-5</sup> *m <sub>Toxin</sub><br>u <sub>(m)</sub> =U <sub>(m)</sub> /2 | <b>u<sub>(m)</sub>=0.0004mg</b> | b |
| Dilution procedure<br>Volumetric flask: V <sub>f</sub> =100mL  | calibration: 100±0.1mL<br>repeatability: 0.04mL<br>volume expansion solvent                                     | u(cal)=0.04mL                   | c |
|  |   | u(rep)=0.04mL                   | d |
|  |   | u(Vol.exp.)=0.24mL              | e |
|  |   | <b>u(v)=0.25mL</b>              | 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 100mL 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 [6], 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

$$C_{\text{Toxin}} = \frac{10 \times m_{\text{ws}} \times P}{V_f} = \frac{10 \times 2.531 \times 99.1}{100} = 25.08 \text{ mg / L}$$

$$\frac{u_c(C_{\text{Toxin}})}{C_{\text{Toxin}}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{\text{ws}}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.5}{99.1}\right]^2 + \left[\frac{0.0004}{2.531}\right]^2 + \left[\frac{0.25}{100}\right]^2} = 0.006$$

$$u_c(C_{\text{Toxin}}) = C_{\text{Toxin}} \times 0.006 = 25.08 \times 0.006 = 0.15 \text{ mg / L}$$

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

$$U(C_{\text{Toxin}}) = u_c(C_{\text{Toxin}}) \times 2 = 0.15 \times 2 = 0.3 \text{ mg / L} = 0.3 \mu\text{g / mL}$$

#### 4. Isotopic enrichment and isotope pattern

| Isotope pattern <sup>a</sup>  |                       |
|---|-----------------------|
| Compound  | Isotopic distribution |
| [ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol                            | 93.9%                 |
| [ <sup>13</sup> C <sub>16</sub> ]-3-Acetyldeoxynivalenol                            | 3.94%                 |
| [ <sup>13</sup> C <sub>15</sub> ]-3-Acetyldeoxynivalenol                            | 2.16%                 |
| <b>Calculated isotopic enrichment level <sup>a</sup>:99.51 atom% <sup>13</sup>C</b> |                       |
| <sup>a</sup> Approximation based on LC-MS/MS data                                   |                       |

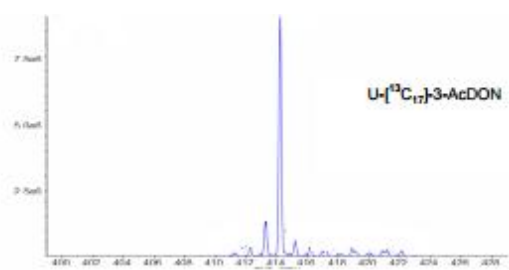


Figure1: Enhanced resolution scan of [<sup>13</sup>C<sub>17</sub>]-3-Acetyldeoxynivalenol for determination of isotope pattern, negative mode

#### 5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified value (mass concentration of U- [<sup>13</sup>C<sub>17</sub>]-3-Acetyldeoxynivalenol, 99.51 atom% <sup>13</sup>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 HPLC-DAD

The certified concentration of U- [<sup>13</sup>C<sub>17</sub>]-3-Acetyldeoxynivalenol, 99.51 atom% <sup>13</sup>C of the gravimetric prepared solution was confirmed by HPLC-DAD against an independently prepared reference batch of unlabeled 3-Acetyldeoxynivalenol.

|                  |  |            |
|------------------|--|------------|
| column           | Phenomenex Luna C <sub>18</sub> , 250×3.0mm, 5μm |            |
| injection Volume | 100μL sample                                     |            |
| solvent A        | acetonitrile/water(10/90)                        |            |
| solvent B        | acetonitrile                                     |            |
| flow rate        | 0.5mL/min  |            |
| gradient         | time in minutes(min)                             | %solvent B |
|                  | 0-3  | 0          |
|                  | 3-6  | 0-11       |
|                  | 6-20   | 11         |
|                  | 20-21  | 11-0       |
|                  | 21-30  | 0          |
| DAD settings     | 218nm  |            |
| Sample dilution  | acetonitrile/water(10/90)                        |            |

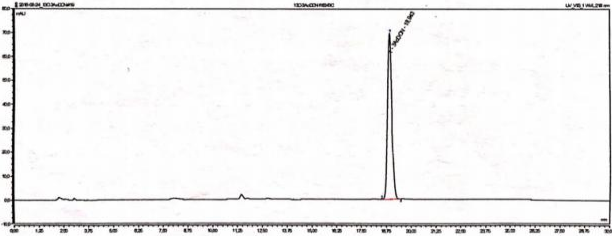


Figure 2: HPLC-DAD chromatogram of U- [<sup>13</sup>C<sub>17</sub>]-3-Acetyldeoxynivalenol

| Time [min]  | area   | concentration <sup>a</sup> [μg/mL] |          |
|---|--------|------------------------------------|----------|
| U- [ <sup>13</sup> C <sub>17</sub> ]-3-Acetyldeoxynivalenol | 18.923 | 17.423                             | 1015±0.8 |

<sup>a</sup> 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  $^{13}\text{C}$  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 ( $^{13}\text{C}_{15}$ )-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) 1<sup>st</sup> 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", 5<sup>th</sup> Ed., Noyes Data Corp. Westwood NJ