

Certificate of Analysis (Ver.1.0)

U-[¹³C₃₆]-Enniatin A,U-[¹³C₃₅]-Enniatin A₁,

U-[¹³C₃₃]-Enniatin B,U-[¹³C₃₄]-Enniatin B₁ 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)

Name:	U-[¹³ C ₃₆]-Enniatin A,U-[¹³ C ₃₅]-Enniatin A ₁ ,U-[¹³ C ₃₃]-Enniatin B,U-[¹³ C ₃₄]-Enniatin B ₁ in Acetonitrile
Catalog number:	STD#3219U
CAS number:	U-[¹³ C ₃₆]-Enniatin A:2503-13-1(unlabeled);U-[¹³ C ₃₅]-Enniatin A ₁ :4530-21-6(unlabeled); U-[¹³ C ₃₃]-Enniatin B:917-13-5(unlabeled);U-[¹³ C ₃₄]-Enniatin B ₁ :19914-20-6(unlabeled)
Formula:	U-[¹³ C ₃₆]-Enniatin A: ¹³ C ₃₆ H ₆₃ N ₃ O ₉ ;U-[¹³ C ₃₅]-Enniatin A ₁ : ¹³ C ₃₅ H ₆₁ N ₃ O ₉ ; U-[¹³ C ₃₃]-Enniatin B: ¹³ C ₃₃ H ₅₇ N ₃ O ₉ ;U-[¹³ C ₃₄]-Enniatin B ₁ : ¹³ C ₃₄ H ₅₉ N ₃ O ₉
Formula weight:	U-[¹³ C ₃₆]-Enniatin A:717.90;U-[¹³ C ₃₅]-Enniatin A ₁ :702.87; U-[¹³ C ₃₃]-Enniatin B:672.82;U-[¹³ C ₃₄]-Enniatin B ₁ :687.85
Lot #:	2A01A16
Starting material :	U-[¹³ C ₃₆]-Enniatin A:lot# J200110P ,Pribolab Pte. Ltd. ; U-[¹³ C ₃₅]-Enniatin A ₁ :lot# J200110P ,Pribolab Pte. Ltd. ; U-[¹³ C ₃₃]-Enniatin B,lot# J200110P ,Pribolab Pte. Ltd. ; U-[¹³ C ₃₄]-Enniatin B ₁ ,lot# J200110P ,Pribolab Pte. Ltd.
Solvent:	Acetonitrile,LiChrosolv [®] ,Merck
Amount:	1.2mL
Production date:	16/Nov/2021
Expiry date:	16/Nov/2022
Name of the supplier:	Pribolab Pte.Ltd.

2.1 Intended use of the RM

- for laboratory use only
- internal standard[2]

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.

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-[¹³ C ₃₆]-Enniatin A, U-[¹³ C ₃₅]-Enniatin A ₁ , U-[¹³ C ₃₃]-Enniatin B, U-[¹³ C ₃₄]-Enniatin B ₁ in Acetonitrile		
Compound	Mass concentration ^a	
	Certified value ^b	Uncertainty ^c
U-[¹³ C ₃₆]-Enniatin A, 99.15 atom% ¹³ C	2.02µg/mL	±0.04µg/mL
U-[¹³ C ₃₅]-Enniatin A ₁ , 98.86 atom% ¹³ C	2.03µg/mL	±0.04µg/mL
U-[¹³ C ₃₃]-Enniatin B, 98.64 atom% ¹³ C	2.12µg/mL	±0.03µg/mL
U-[¹³ C ₃₄]-Enniatin B ₁ , 98.52 atom% ¹³ C	2.12µg/mL	±0.04µg/mL

a Values are based on preparation data and confirmed experimentally by HPLC-DAD
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[3]

4. Isotopic enrichment and isotope pattern

Isotope pattern ^a			
Compound	Isotopic distribution	Compound	Isotopic distribution
U-[¹³ C ₃₆]-Enniatin A	73.10%	U-[¹³ C ₃₅]-Enniatin A ₁	66.23%
U-[¹³ C ₃₅]-Enniatin A	23.25%	U-[¹³ C ₃₄]-Enniatin A ₁	27.81%
U-[¹³ C ₃₄]-Enniatin A	3.65%	U-[¹³ C ₃₃]-Enniatin A ₁	5.96%
U-[¹³ C ₃₃]-Enniatin B	63.29%	U-[¹³ C ₃₄]-Enniatin B ₁	59.88%
U-[¹³ C ₃₂]-Enniatin B	28.48%	U-[¹³ C ₃₃]-Enniatin B ₁	29.94%
U-[¹³ C ₃₁]-Enniatin B	8.23%	U-[¹³ C ₃₂]-Enniatin B ₁	10.18%

Calculated isotopic enrichment level ^a: U-[¹³C₃₆]-Enniatin A 99.15 atom% ¹³C; U-[¹³C₃₅]-Enniatin A₁ 98.86 atom% ¹³C; U-[¹³C₃₃]-Enniatin B 98.64 atom% ¹³C; U-[¹³C₃₄]-Enniatin B₁ 98.52 atom% ¹³C

^a Approximation based on LC-MS/MS data

5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [4]. Thus the certified value (mass concentration of U-[¹³C₃₆]-Enniatin A, U-[¹³C₃₅]-Enniatin A₁, U-[¹³C₃₃]-Enniatin B, U-[¹³C₃₄]-Enniatin B₁) 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 the gravimetric prepared solution was confirmed by HPLC-DAD against an independently prepared reference batch of unlabeled Enniatin A, Enniatin A₁, Enniatin B, Enniatin B₁.

column	C ₁₈ , 250×4.6mm, 5µm	
injection Volume	10µL	
solvent	acetonitrile/water=80/20	
oven	30°C	
flow rate	1mL/min	
DAD settings	206nm	
Sample dilution	acetonitrile	
	time [min]	concentration ^a
U-[¹³ C ₃₆]-Enniatin A	15.913	2.03 µg/mL
U-[¹³ C ₃₅]-Enniatin A ₁	13.200	2.06 µg/mL
U-[¹³ C ₃₃]-Enniatin B	9.441	2.25 µg/mL
U-[¹³ C ₃₄]-Enniatin B ₁	11.115	2.05 µg/mL
^a Mean of 6 replicate measurements against reference batch, confidence interval with P=95%		

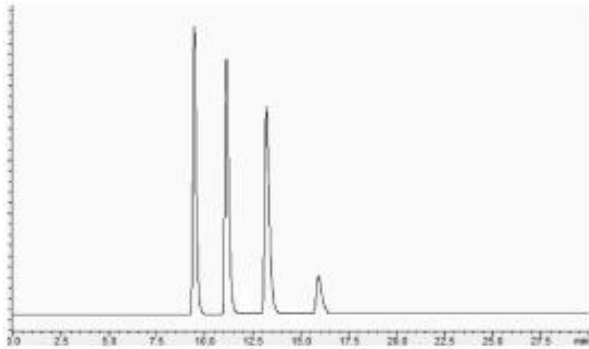
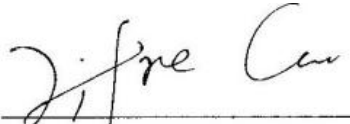


Figure 1: HPLC-DAD chromatogram of U-[¹³C₃₆]-Enniatin A, U-[¹³C₃₅]-Enniatin A₁, U-[¹³C₃₃]-Enniatin B, U-[¹³C₃₄]-Enniatin B₁

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] International Organization for Standardization (ISO), (2008), "Guide to the expression of uncertainty in measurement", (GUM 1995 with minor corrections) 1st Ed. Geneva, Switzerland
- [4] E.W. Flick, (1998), "Industrial Solvents Handbook", 5th Ed., Noyes Data Corp. Westwood NJ