

REFERENCE MATERIAL CERTIFICATE

U-[¹³C₁₇] – AFLATOXIN B2 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] and Eurachem / CITAC Guides [2,3].

2. Description of the Reference Material (RM)

Name:	U-[¹³ C ₁₇]-Aflatoxin B2 in acetonitrile
CAS number:	1217470-98-8
Catalog number:	DRE-A10047250AL-0.5
Lot #:	1000005303
Certificate version:	1
Expiry date:	10.03.2023
Starting material:	U-[¹³ C ₁₇]-Aflatoxin B2, Lot # IS19203A, Romer Labs Diagnostic GmbH
Physical description of RM:	Solution of U-[¹³ C ₁₇]-Aflatoxin B2 in acetonitrile
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and aluminium crimp cap, solution of 1.2 mL
Name and address of the manufacturer:	Romer Labs Diagnostic GmbH Technopark 5, 3430 Tulln, Austria www.romerlabs.com
Name and address of the supplier:	LGC Standards GmbH Mercatorstraße 51, 46485 Wesel, Germany Tel +49(0)2 81 98 87 0, Fax +49(0)2 81/98 87 199 www.lgcstandards.com

2.1 Intended use of the RM

- for laboratory use only
- internal standard [4, 5]

2.2 Instruction for the correct use of the RM

The ampoules should be stored at -18 to -22°C in a dark place. Before usage of the RM, the ampoules should be allowed to warm to room temperature. 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 (SDS).

Hazardous Ingredients	Concentration in %	Pictograms	Signal word	Hazard statement(s)
Acetonitrile	> 99.9	 	Danger	H225, H302, H312, H319, H332

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3. Certified values and their uncertainties

U-[¹³ C ₁₇]-Aflatoxin B2 in acetonitrile		
Compound	Mass concentration ^a	
	Certified value ^b	Uncertainty ^c
U-[¹³ C ₁₇]-Aflatoxin B2, 99.1 atom % ¹³ C	0.506 µg/mL	± 0.008 µg/mL
^a Values are based on preparation data and confirmed experimentally by HPLC-UV		
^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 [6]		

3.1 Calculation of uncertainty

The uncertainty of the calibrant solution was calculated on the basis of preparation [7].

Uncertainty components	Description	Standard uncertainty (u)	
Purity (P) of solid U-[¹³ C ₁₇]-Aflatoxin B2, 99.1 atom % ¹³ C	P = 99 ± 1 %	u (P) = 0.58	a
Weighing procedure weighted sample: m _{ws} = 1.278 mg	U(m) = 0.0026 mg + 9.51 * 10 ⁻⁶ * m _{Toxin} u(m) = U(m)/2	u (m) = 0.0013 mg	b
Dilution procedure volumetric flask 1: V _{f1} = 250 mL volumetric flask 2: V _{f2} = 250 mL one-mark glass pipette: V _p = 25 mL	calibration flask 1: 250 mL ± 0.15 mL repeatability flask 1: 0.03 mL volume expansion solvent flask 1 calibration flask 2: 250 mL ± 0.15 mL repeatability flask 2: 0.03 mL volume expansion solvent flask 2 calibration pipette: 25 mL ± 0.03 mL volume expansion solvent pipette	u (cal1) = 0.06 mL u (rep1) = 0.03 mL u (Vol. exp.1) = 0.59 mL u (V1) = 0.59 mL u (cal2) = 0.06 mL u (rep2) = 0.03 mL u (Vol. exp.2) = 0.59 mL u (V2) = 0.59 mL u (cal3) = 0.01 mL u (Vol. exp.3) = 0.06 mL u (V3) = 0.06 mL	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 250 mL flask; the value was used directly as a standard deviation

^{e,l} Based on the density of 0.7857 g/cm³ at temperature T = 20°C and a maximum temperature variation of ± 3°C, of volume expansion, relative volume expansion coefficient of acetonitrile is 1370 * 10⁻⁶/°C [8], volume expansion term (rectangular distribution) was divided by $\sqrt{3}$

^{i,j,m} All contributions are combined to give the $u(V) = \sqrt{u(cal)^2 + u(rep)^2 + u(Vol. exp.)^2}$

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

$$c_{Toxin} = \frac{10 \times m_{ws} \times P \times V_p}{V_{f1} \times V_{f2}} = \frac{10 \times 1.278 \times 99 \times 25}{250 \times 250} = 0.506 \text{ mg/L}$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V1)}{V_{f1}}\right]^2 + \left[\frac{u(V2)}{V_{f2}}\right]^2 + \left[\frac{u(V3)}{V_p}\right]^2} = \sqrt{\left[\frac{0.58}{99}\right]^2 + \left[\frac{0.0007}{1.278}\right]^2 + \left[\frac{0.59}{250}\right]^2 + \left[\frac{0.59}{250}\right]^2 + \left[\frac{0.06}{25}\right]^2} = 0.007$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.007 = 0.506 \times 0.007 = 0.004 \text{ mg/L}$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.004 \times 2 = 0.008 \text{ mg/L} = 0.008 \text{ µg/mL}$$

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4. Isotopic enrichment and isotope pattern

Isotope pattern ^a	
Compound	Isotopic distribution
[¹³ C ₁₇]-Aflatoxin B2	85.6 %
[¹³ C ₁₆]-Aflatoxin B2	12.9 %
[¹³ C ₁₅]-Aflatoxin B2	1.5 %
Calculated isotopic enrichment level ^a : 99.1 atom % ¹³ C	
^a Approximation based on LC-MS/MS data	

5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [7]. Thus the certified value (mass concentration of U-[¹³C₁₇]-Aflatoxin B2, 99.1 atom % ¹³C) 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-UV

The certified concentration of U-[¹³C₁₇]-Aflatoxin B2, 99.1 atom % ¹³C of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch of unlabeled Aflatoxin B2 calibrant.

column	Phenomenex Kinetex C18, 100 x 3.0 mm, 2.6μ			
injection volume	25 μL sample			
solvent A	water / acetonitrile / methanol 57/17/26			
oven	35°C			
flow rate	0.3 mL / min			
DAD settings	365 nm			
sample dilution	1:5 with solvent A			
	time	area	height	concentration ^a
U-[¹³ C ₁₇]-Aflatoxin B2	4.627	0.653	3.290	0.507 ± 0.02 μg/mL
^a Mean of 6 replicate measurements against reference batch, confidence interval with P = 95 %				

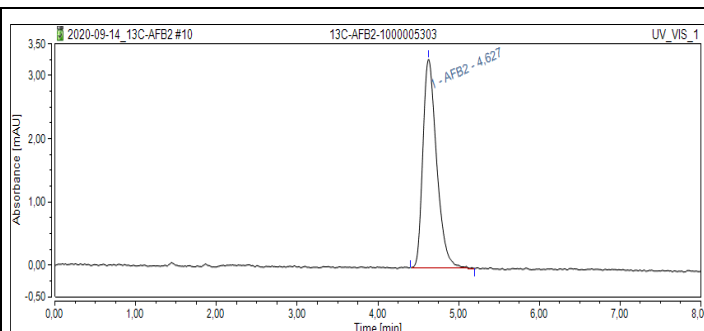


Figure 1: HPLC-UV chromatogram of U-[¹³C₁₇]-Aflatoxin B2

7. Further information

The purchaser must determine the suitability of this product for its particular use. LGC Standards GmbH makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by LGC Standards GmbH. We do not guarantee that the product can be used for a special application.

approved for release by: Laurence Treccani-Chinelli, Global Supply Chain Manager - LGC Standards

date: 17.09.2020

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References:

- [1] ISO Guide 31:2015 - 1-18, "Reference materials – contents of certificates, labels and accompanying documentation"
- [2] Eurachem / CITAC Guide, 1-37, (2003), "Traceability in Chemical Measurement"
- [3] Eurachem / CITAC Guide CG4, 1-133, (QUAM:2012.P1), "Quantifying Uncertainty in Analytical Measurement", 3rd Ed.
- [4] 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
- [5] 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
- [6] International Organization for Standardization (ISO), (2008), "Guide to the expression of uncertainty in measurement", (GUM 1995 with minor corrections) 1st Ed. Geneva, Switzerland
- [7] 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"
- [8] E.W. Flick, (1998), "Industrial Solvents Handbook", 5th Ed., Noyes Data Corp. Westwood NJ