REFERENCE MATERIAL CERTIFICATE

MYCOTOXIN MIX 1 (AFLATOXINS)

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:	Mycotoxin Mix 1 (Aflatoxins)
Catalog number:	DRE-V30000001AL
Lot #:	1000002267
Certificate version:	1
Expiry date:	15.07.2021
Starting material 1:	Aflatoxin B1 Lot #S16251B, Aflatoxin B2, Lot #0404049 Aflatoxin G1, Lot # S19022G, Aflatoxin G2 Lot #0504071 Romer Labs Diagnostic GmbH
Physical description of RM:	Solution of 4 different Aflatoxins in acetonitrile
Packaging and amount of RM:	DRE-V30000001AL: Amber glass ampoules fitted with teflon faced butyl septa and aluminium crimp cap, solution of 5 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
- calibration of analytical instruments

2.2 Instruction for the correct use of the RM

The ampoules should be stored at -18 to -22°C or below 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

3. Certified values and their uncertainties

Mycotoxin Mix 1 (Aflatoxins)		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Aflatoxin B1	2.01 µg/mL	± 0.03 µg/mL
Aflatoxin B2	0.505 µg/mL	± 0.01 µg/mL
Aflatoxin G1	2.02 µg/mL	± 0.07 µg/mL
Aflatoxin G2	0.510 µg/mL	± 0.01 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 steps*

[c] *Expanded uncertainty U (k = 2) of the value u_c according to GUM [4]*

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 Aflatoxin B1 Aflatoxin G1 Aflatoxin B2 Aflatoxin G2	P_1 = 99 ± 1 % P_2 = 97 ± 3 % P_3 = 99 ± 1 % P_4 = 99 ± 1 %	**$u(P_{1+3+4})$ = 0.6 %** **$u(P_2)$ = 1.7 %**	a
Weighing procedure weighted sample: m_{wsAFB1}= 10.154 mg m_{wsAFB2}= 2.551 mg m_{wsAFG1}= 10.412 mg m_{wsAFG2}= 2.576 mg	$U(m) = 0.0026\ mg + 9.51 * 10^{-6} * m_{Toxin}$ $u(m) = U(m)/2$	**u (m) = 0.0013 mg**	b
Aflatoxin B1 + B2 + G1 + G2 stock solution: volumetric flask 1: V_{f1} = 250 mL Aflatoxin mixture working solution: volumetric flask 2: V_{f2} = 1000 mL one-mark glass pipette: V_p = 50 mL	calibration flask 1: 250 mL ± 0.15 mL repeatability flask 1: 0.03 mL volume expansion solvent flask 1 calibration flask 2: 1000 mL ± 0.4 mL repeatability flask 2: 0.1 mL volume expansion solvent flask 2 calibration pipette: 50 mL ± 0.075 mL volume expansion solvent pipette	u (cal1) = 0.06 mL u (rep1) = 0.03 mL u (Vol. exp.1) = 0.59 mL **u (V1) = 0.6 mL** u (cal2) = 0.2 mL u (rep2) = 0.1 mL u (Vol. exp.2) = 2.4 mL **u (V2) = 2.4 mL** u (cal3) = 0.03 mL u (Vol. exp.3) = 0.12 mL **u (V3) = 0.1 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 and 1000 mL flask; the value was used directly as a standard deviation*

[e,i,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 [6], volume expansion term (rectangular distribution) was divided by* $\sqrt{3}$

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

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Calculation of the combined uncertainty u_c and the expanded standard uncertainty U for Aflatoxin B1 as example

$$c_{Toxin} = \frac{10 \times m_{WAFB1} \times P_1 \times V_p}{V_{f1} \times V_{f2}} = \frac{10 \times 10.154 \times 99 \times 50}{250 \times 1000} = 2.01\ mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P_1)}{P_1}\right]^2 + \left[\frac{u(m)}{m_{WAFB1}}\right]^2 + \left[\frac{u(V1)}{V_{f1}}\right]^2 + \left[\frac{u(V2)}{V_{f2}}\right]^2 + \left[\frac{u(V3)}{V_p}\right]^2} = \sqrt{\left[\frac{0.6}{99}\right]^2 + \left[\frac{0.0013}{10.154}\right]^2 + \left[\frac{0.6}{250}\right]^2 + \left[\frac{2.4}{1000}\right]^2 + \left[\frac{0.1}{50}\right]^2} = 0.007$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.007 = 2.01 \times 0.007 = 0.014\ mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.014 \times 2 = 0.028\ mg/L = 0.03\ \mu g/mL$$

4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [5]. Thus the certified values (mass concentrations of 4 different Aflatoxins) are based on the weighed amount of the starting materials and are therefore traceable to the stated purity of the solid raw materials. High purity materials represent a practical realization of concentration units, through conversion of mass to molar quantity.

5. Confirmation of certified value by HPLC-UV

The certified concentrations of the 4 different Aflatoxins of the gravimetric prepared solution were confirmed by HPLC-UV against an independently prepared reference batch.

column	Phenomenex Luna C18(2), 250 x 3.0 mm, 5µ
injection volume	100 µL sample
solvent A	water / acetonitrile / methanol 57/17/26
oven	30°C
flow rate	0.5 mL / min
DAD settings	365 nm
sample dilution	1:5 with water

	Time [min]	area	concentration [a]
Aflatoxin G2	8.223	1.202	0.507 µg/mL ± 0.02 µg/mL
Aflatoxin G1	9.857	4.226	1.98 µg/mL ± 0.06 µg/mL
Aflatoxin B2	11.150	1.614	0.506 µg/mL ± 0.02 µg/mL
Aflatoxin B1	13.570	5.566	2.00 µg/mL ± 0.06 µg/mL

[a] Mean of 6 replicate measurements against reference batch, confidence interval with P = 95 %

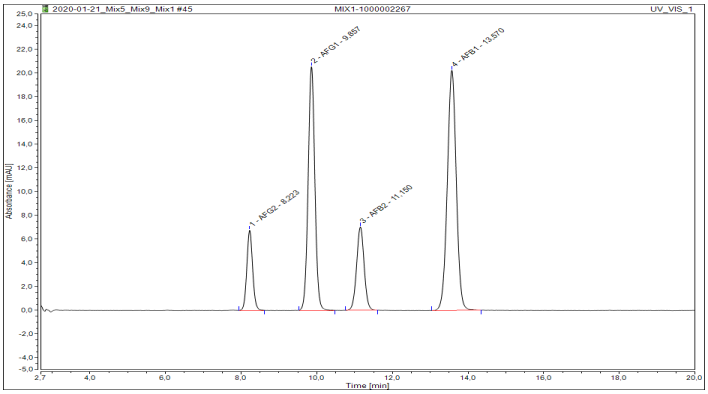

Figure 1: HPLC-UV chromatogram of Mycotoxin Mix 1 (Aflatoxins)

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6. Further information

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approved for release by: *Laurence Treccani-Chinelli, Global Supply Chain Manager - LGC Standards* *date:* 21.01.2020

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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] International Organization for Standardization (ISO), (2008), *"Guide to the expression of uncertainty in measurement"*, (GUM 1995 with minor corrections) 1st 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"*, 5th Ed., Noyes Data Corp. Westwood NJ