REFERENCE MATERIAL CERTIFICATE

MYCOTOXIN MIX 3 (FUMONISINS)

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 3 (Fumonisins)
Catalog number:	DRE-A30000003WL
Lot #:	L19443M
Certificate version:	1
Expiry date:	29.04.2021
Starting material:	Fumonisin B1, Lot # S18322A, Romer Labs Diagnostic GmbH Fumonisin B2, Lot # S18322F, Romer Labs Diagnostic GmbH
Physical description of RM:	Solution of Fumonisin B1 and Fumonisin B2 in acetonitrile – water (1:1)
Packaging and amount of RM:	DRE-A30000003WL: Amber glass ampoules fitted with teflon faced butyl septa and aluminium crimp cap, solution of 1 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 2-8°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	> 50		Danger	H225, H302, H312, H319, H332

3. Certified values and their uncertainties

Mycotoxin Mix 3 (Fumonisins)		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Fumonisin B1	50.0 µg/mL	± 1.2 µg/mL
Fumonisin B2	50.2 µg/mL	± 0.6 µg/mL

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

[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 [4]

3.1 Calculation of uncertainty

The uncertainty of each certified value was calculated on the basis of preparation [5].

Uncertainty components	Description	Standard uncertainty (u)	
Purity (P) of solid Fumonisin B1 Fumonisin B2	 P_1 = 98.0 ± 2.0 % P_2 = 99.1 ± 0.9 %	 **u (P_1) = 1.2 %** **u (P_2) = 0.5 %**	a
Weighing procedure weighted sample: m_{wsFB1}= 25.516 mg m_{wsFB2}= 25.320 mg	U(m) = 0.0026 mg + 9.51 * 10^{-6} * m_{Toxin} u(m) = U(m)/2	**u (m) = 0.0014 mg**	b
Dilution procedure volumetric flask: V_f = 500 mL	calibration: 500 mL ± 0.25 mL repeatability: 0.1 mL volume expansion solvent	u (cal) = 0.1 mL u (rep) = 0.1 mL u (Vol. exp.) = 1.2 mL **u (V) = 1.2 mL**	c d e 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 500 mL flask; the value was used directly as a standard deviation

*[e] Estimation based on the density of pure acetonitrile = 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] The three 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 for Fumonisin B1 as example

$$c_{Toxin} = \frac{10 \times m_{wsFB1} \times P}{V_f} = \frac{10 \times 25.516 \times 98.0}{500} = 50.0\, mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P_1)}{P_1}\right]^2 + \left[\frac{u(m)}{m_{wsFB1}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{1.2}{98.0}\right]^2 + \left[\frac{0.0014}{25.516}\right]^2 + \left[\frac{1.2}{500}\right]^2} = 0.012$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.012 = 50.0 \times 0.012 = 0.6\, mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.6 \times 2 = 1.2\, mg/L = 1.2\, \mu g/mL$$

4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [5]. Thus the certified values (mass concentration of Fumonisin B1 and Fumonisin B2) 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 material represents a practical realization of concentration units, through conversion of mass to molar quantity.

5. Confirmation of certified value by LC-MS:

The certified concentration of Fumonisin B1 and Fumonisin B2 of the gravimetric prepared solution was confirmed by LC-MS against an independently prepared reference batch.

column	Agilent ZOBAX Eclipse XDB-C8 3,0 x 100mm, 3,5µm	
injection volume	2 µL	
solvent A	5 mM ammonium acetate buffer pH 3.1	
solvent B	acetonitrile	
flow rate	0.4 mL / min, oven at 40°C	
	time in minutes (min)	% solvent
isocratic	0	35 % B
	10	35 % B
source type	HESI, positive mode	
sample dilution	1:10 with water / acetonitrile = 50:50	

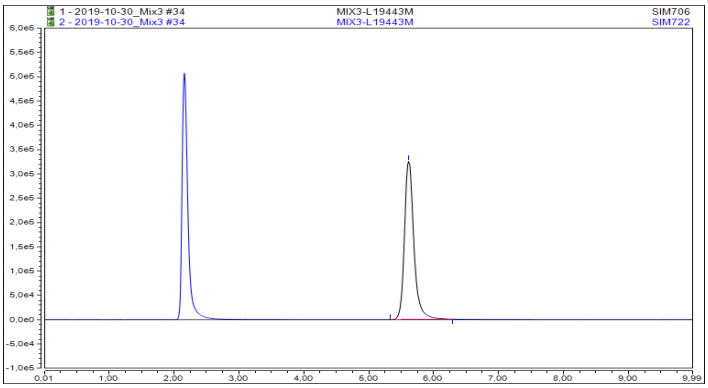

Figure 1: SIM 722 $[M+H]^+$ of Fumonisin B1 and SIM 706 $[M+H]^+$ of Fumonisin B2

Analyte Peak Name	Peak Area (counts*min)	Retention Time (min)	concentration [a] [µg/mL]
Fumonisin B1	5.22E+04	2.159	50.5 ± 1.5
Fumonisin B2	5.49E+04	5.613	51.0 ± 1.5

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

6. 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:* 30.10.2019

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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