AFLATOXIN 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], ISO Guide 35 [2] and Eurachem / CITAG Guides [3,4].

2. Description of the Reference Material (RM)

Name:	Aflatoxin B1 in acetonitrile
Catalog number:	BRM 002017 (5 mL) BRM S02017 (1 mL)
Lot #:	L10203A
Expiry date:	19.11.2011
Starting material 1:	Aflatoxin B1, Lot #03016Z, Romer Labs Diagnostic GmbH
Matrix:	Acetonitrile, Ultra Gradient HPLC Grade Lot #0814308957, J.T. Baker, catalog Nr. 9017
Physical description of RM:	Solution of aflatoxin B1 in acetonitrile
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps, solution of 1 mL and 5 mL
Name and address of the supplier:	Romer Labs Diagnostic GmbH Technopark 1 3430 Tulln, Austria www.romerlabs.com, office-europe@romerlabs.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 -20°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 1 mL. 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 %	Hazard symbol	Risks (R-phrases)
acetonitrile	> 99.9	Xn	11-20/21/22-36

3. Certified values and their uncertainties

Aflatoxin B1 in acetonitrile		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Aflatoxin B1	2.02 µg/mL	± 0.04 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 [5]*

3.1 Calculation of uncertainty

After the concentration of the gravimetric prepared solution was confirmed by HPLC-UV, the uncertainty of the calibrant solution was calculated on the basis of preparation [6].

Uncertainty components	Description	Standard uncertainty (u)	
Purity (P) of solid Aflatoxin B1	P = 99 % ± 1 %	**u (P) = 0.6**	a
Weighing procedure weighted sample: m_{ws} = 5.108 mg	repeatability: 0.03 mg linearity: 0.012 mg	**u (m) = 0.03 mg**	b
Dilution procedure volumetric flask 1: V_{f1} = 250 mL volumetric flask 2: V_{f2} = 500 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: 500 mL ± 0.25 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) = 0.56 mL **u (V1) = 0.6 mL** u (cal2) = 0.1 mL u (rep2) = 0.1 mL u (Vol. exp.2) = 1.2 mL **u (V2) = 1.2 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] *Estimation of this u-value is based upon the values for repeatability and linearity described in the user manual of the microbalance*

[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 typical 250 and 500 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 [7], volume expansion term (rectangular distribution) was divided by $\sqrt{3}$*

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

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

$$c_{Toxin} = \frac{10 \times m_{ws} \times P \times V_p}{V_{f1} \times V_{f2}} = \frac{10 \times 5.108 \times 99 \times 50}{250 \times 500} = 2.02 mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V1)}{V_{f1}}\right]^2 + \left[\frac{u(V2)}{V_{f2}}\right]^2 + \left[\frac{u(V3)}{V_p}\right]^2} = \sqrt{\left[\frac{0.6}{99}\right]^2 + \left[\frac{0.03}{5.108}\right]^2 + \left[\frac{0.6}{250}\right]^2 + \left[\frac{1.2}{500}\right]^2 + \left[\frac{0.1}{50}\right]^2} = 0.01$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.01 = 2.02 \times 0.01 = 0.02\ mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.02 \times 2 = 0.04 mg/L \approx 0.04\ mg/L = 0.04 \mu g/mL$$

4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified value (mass concentration of aflatoxin B1) is based on the weighed amount of the starting material 1 and is therefore traceable to the stated purity of the solid mycotoxin. High purity material represents a practical realization of concentration units, through conversion of mass to molar quantity.

5. Confirmation of certified value by HPLC-UV

The concentration value of aflatoxin B1 of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch (Biopure Lot# L09513A).

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	area	height	concentration [a]
Aflatoxin B1	12.67	5.341	19.43	2.09 ± 0.06 µg/mL

[a] Mean of 7 replicate measurements against reference batch, confidence interval with P = 95 %

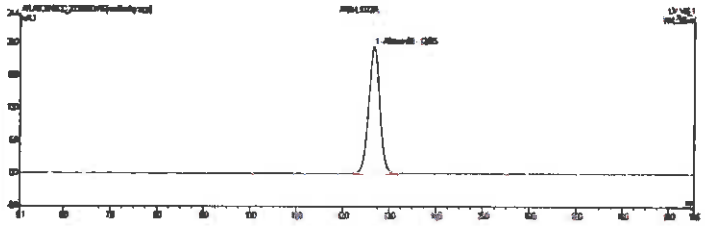

Figure 1: HPLC-UV chromatogram of aflatoxin B1 calibrant, Lot# L10203A

6. Further information

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

approved for release by: Michaela Streicher date: 28.05.2010

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References:

[1] ISO Guide 31, 1-7, (2000), *"Reference Materials – Contents of Certificates and Labels"*

[2] ISO Guide 35, 1-7, (2000), *"Certification of Reference Materials – General and Statistical Principles"*

[3] Eurachem / CITAG Guide, 1-37, (2003), *"Traceability in Chemical Measurement"*

[4] Eurachem / CITAG Guide, 1-120, (2000), *"Quantifying Uncertainty in Analytical Measurement"*

[5] International Organization for Standardization (ISO), (1995), *"Guide to the Expression of Uncertainty in Measurement"*, 1st Ed. Geneva, Switzerland

[6] 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"*

[7] E.W. Flick, (1996), *"Industrial Solvents Handbook"*, 3rd Ed., Noyes Data Corp. Westwood NJ