REFERENCE MATERIAL CERTIFICATE

OCHRATOXIN-ALPHA 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:	Ochratoxin-alpha in acetonitrile
CAS number:	16281-39-3
Catalog number:	DRE-A15670400AL-10
Lot #:	L18213B
Certificate version:	2
Expiry date:	22.09.2020
Starting material 1:	Ochratoxin-alpha, Lot #S07353Z, Romer Labs Diagnostic GmbH
Physical description of RM:	Solution of Ochratoxin-alpha in acetonitrile
Packaging and amount of RM:	DRE-A15670400AL-10: 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	> 99.9		Danger	H225, H302, H312, H319, H332

3. Certified values and their uncertainties

Ochratoxin-alpha in acetonitrile		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Ochratoxin-alpha	10.2 µg/mL	± 0.1 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 [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 Ochratoxin-alpha	P = 98.9 ± 1.1 %	**u (P) = 0.6 %**	a
Weighing procedure weighted sample: m_{ws}= 1.032 mg	U(m) = 0.0000008g + 1.26 * 10^{-5} * m_{Toxin} u(m) = U(m)/2	**u (m) = 0.0004 mg**	b
Dilution procedure volumetric flask: V_f = 100 mL	calibration: 100 mL ± 0.1 mL repeatability: 0.04 mL volume expansion solvent	u (cal) = 0.04 mL u (rep) = 0.04 mL u (Vol. exp.) = 0.24 mL **u (V) = 0.3 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 100 mL flask; the value was used directly as a standard deviation

*[e] 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] 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

$$c_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 1.032 \times 98.9}{100} = 10.2\ mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.6}{98.9}\right]^2 + \left[\frac{0.0004}{1.032}\right]^2 + \left[\frac{0.3}{100}\right]^2} = 0.007$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.007 = 10.2 \times 0.007 = 0.07\ mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.07 \times 2 = 0.14\ mg/L = 0.1\ \mu g/mL$$

4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [5]. Thus the certified value (mass concentration of Ochratoxin-alpha) 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.

5. Confirmation of certified value by HPLC-UV

The certified concentration of Ochratoxin-alpha of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch of Ochratoxin-alpha.

column	Phenomenex Luna C18(2), 250x3.00 mm, 5µ	
injection volume	20 µL sample	
solvent A	0.1% H_3PO_4 in water	
solvent B	acetonitrile	
flow rate	0.5 mL / min	
gradient	time in minutes (min)	% solvent B
	0 – 1.5	45
	1.5 – 11.5	45 - 65
	11.5 – 14.5	65
	14.5 – 14.6	65 - 45
	14.6 – 18.5	45
DAD settings	222 nm	
sample dilution	1:2 with 0.1% H_3PO_4 in water	

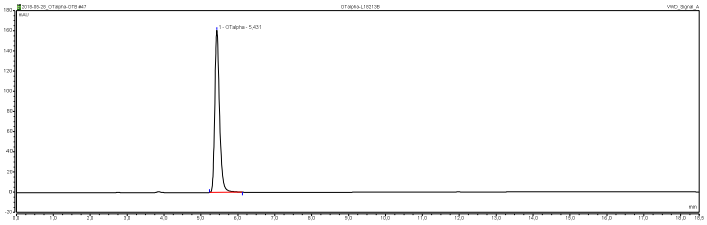

Figure 1: HPLC-UV separation of Ochratoxin-alpha calibrant Lot# L18213B

Analyte	time [min]	area	concentration [a] [µg/mL]
Ochratoxin-alpha	5.431	24.041	10.11 ± 0.30

[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:* 14.05.2019

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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