REFERENCE MATERIAL CERTIFICATE

U-[$^{13}C_{20}$] – OCHRATOXIN A 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-[$^{13}C_{20}$]-Ochratoxin A in acetonitrile
CAS number:	911392-42-2
Catalog number:	DRE-A15670010AL-10
Lot #:	I19084O
Certificate version:	1
Expiry date:	21.08.2020
Starting material:	U-[$^{13}C_{20}$]-Ochratoxin A, Lot #IS13313O, Romer Labs Diagnostic GmbH
Physical description of RM:	Solution of U-[$^{13}C_{20}$]-Ochratoxin A in acetonitrile
Packaging and amount of RM:	DRE-A15670010AL-10: 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 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

U-[$^{13}C_{20}$]-Ochratoxin A in acetonitrile		
Compound	**Mass concentration** [a]	
	Certified value [b]	Uncertainty [c]
U-[$^{13}C_{20}$]-Ochratoxin A, 99.4 atom % ^{13}C	10.10 µg/mL	± 0.2 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 [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-[$^{13}C_{20}$]-Ochratoxin A, 99.4 atom % ^{13}C	P = 98.7 ± 1.3 %	**u (P) = 0.8 %**	a
Weighing procedure weighted sample: m_{ws}= 2.559 mg	U(m) = 0.0014 mg + 7.72 * 10^{-6} * m_{Toxin} u(m) = U(m)/2	**u (m) = 0.0007 mg**	b
Dilution procedure volumetric flask: V_f = 250 mL	calibration: 250 mL ± 0.15 mL repeatability: 0.03 mL volume expansion solvent	u (cal) = 0.06 mL u (rep) = 0.03 mL u (Vol. exp.) = 0.59 mL **u (V) = 0.6 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 250 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 [8], 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

$$c_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 2.559 \times 98.7}{250} = 10.10\ mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.8}{98.7}\right]^2 + \left[\frac{0.0007}{2.559}\right]^2 + \left[\frac{0.6}{250}\right]^2} = 0.008$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.008 = 10.10 \times 0.008 = 0.08\ mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.08 \times 2 = 0.16\ mg/L = 0.2\ \mu g/mL$$

4. Isotopic enrichment and isotope pattern

Isotope pattern [a]		
Compound	**amu [M-H]⁻**	**Isotopic distribution [%]**
[$^{13}C_{18}$]-Ochratoxin	420.16	0.5
[$^{13}C_{19}$]-Ochratoxin	421.18	6.5
[$^{13}C_{20}$]-Ochratoxin	422.08	73.6
[$^{13}C_{19}$, ^{37}Cl+ ^{18}O]-Ochratoxin	423.08	4.3
[$^{13}C_{20}$, ^{37}Cl+ ^{18}O]-Ochratoxin	424.04	15.2
Calculated isotopic enrichment level [a] : 99.4 atom % ^{13}C		

[a] Approximation based on LC-MS/MS data

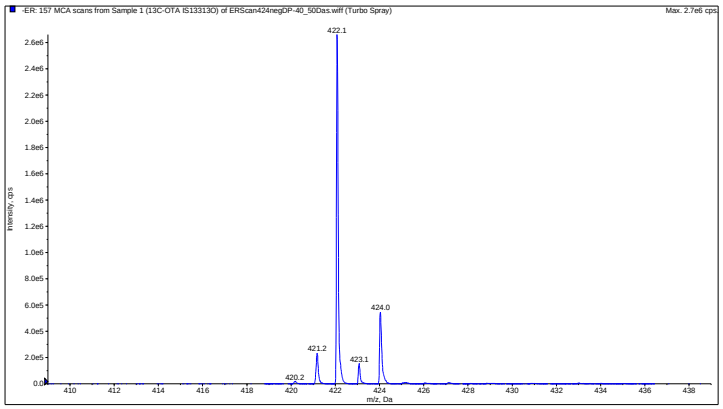

Figure 1: Enhanced resolution scan of U-[$^{13}C_{20}$]-Ochratoxin A for determination of isotope pattern

5. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [7]. Thus the certified value (mass concentration of U-[$^{13}C_{20}$]-Ochratoxin A, 99.4 atom % ^{13}C) 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-FLD

The certified concentration of U-[$^{13}C_{20}$]-Ochratoxin A, 99.4 atom % ^{13}C of the gravimetric prepared solution was confirmed by HPLC-FLD against an independently prepared reference batch of Ochratoxin A calibrant.

column	Phenomenex Luna C18(2), 250 x 3 mm, 5µ	
injection volume	20 µl	
solvent A	0.1 % H_3PO_4	
solvent B	acetonitrile	
sample dilution	1:2 with 0.1 % H_3PO_4	
flow rate	0.5 mL / min, oven at 25°C	
gradient	time in minutes (min)	% solvent B
	0	45 %
	1.5 – 11.5	45 – 65 %
	11.5 – 14.5	65 %
	14.5 – 14.6	65 – 45 %
	14.6 – 18.5	45 %
FLD settings	λ_{EX} = 330 nm, λ_{EM} = 460 nm, response time: 0.5 sec	

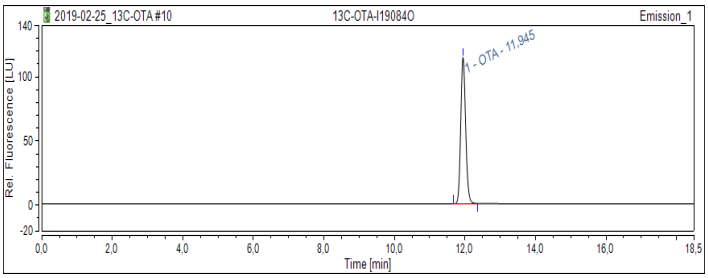

Figure 1: HPLC-FLD chromatoram of U-[$^{13}C_{20}$]-OTA, Lot# I18422A

	Time [min]	area	height	Concentration [a]
U-[$^{13}C_{20}$]-Ochratoxin A	11.945	18.278	113.411	10.19 ± 0.3 µg/mL

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

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7. Further information

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approved for release by: *Laurence Treccani-Chinelli, Global Supply Chain Manager - LGC Standards* *date:* 25.02.2019

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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] 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 ($^{13}C_{15}$)-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