REFERENCE MATERIAL CERTIFICATE

ERGOCORNININE

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:	Ergocorninine
CAS number:	564-37-4
Catalog number:	DRE-C13201210
Lot #:	L19041A
Certificate version:	1
Expiry date:	21.01.2022
Starting material:	Ergocorninine, Lot# S17023E
Physical description of RM:	Thin film, dried down standard
Packaging and amount of RM:	DRE-C13201210: Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps 5 mL
Amount of RM:	0.125 mg dried down, 25.0 µg/mL after reconstitution with 5 mL solvent
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 Reconstitution instruction

The standard that you have received may appear at first glance, as an empty vial. The target compound (s) is (are) in a film at the bottom of the vial. Do not open the vial until you are ready to reconstitute.

To reconstitute this RM use the following procedure:

1. Add 5 mL ± 0.014 mL of acetonitrile with a graduated syringe or a volumetric pipette
2. Cap vial tightly
3. **Mix vigorously on a vortex mixer and repeat for several times over a longer period** or sonicate at room temperature
4. Always keep vial tightly capped.
5. Store the reconstituted standard at -20°C in a dark environment for **max. 8 weeks**.
6. **Store immediately at -20°C after usage to avoid degradation**

Note:

Thorough mixing and sufficient mixing time is required to ensure complete reconstitution of the dried down standard!

Ergot alkaloids are highly sensitive to temperature, light and oxygen!

2.3 Instruction for the correct use of the RM

The dried down standard should be stored at 2-8°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.

2.4 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).

3. Certified values and their uncertainties

Ergocorninine		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Ergocorninine	25.0 µg/mL	± 1.3 µg/mL

[a] *After reconstitution with 5 mL solvent*
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 the calibrant solution was calculated on the basis of preparation [5].

Uncertainty components	**Description**	**Standard uncertainty (u)**	
Purity (P) of solid Ergocorninine	P = 95.6 ± 4.4 %	**u (P) = 2.5 %**	a
Weighing procedure weighted sample: m_{ws}= 2.616 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 = 100 mL pipette: V_p = 5 mL	calibration: 100 mL ± 0.1 mL repeatability: 0.04 mL volume expansion solvent calibration pipette: 5 mL ± 0.005 mL volume expansion solvent pipette	u (cal) = 0.04 mL u (rep) = 0.04 mL u (Vol. exp.) = 0.24 mL **u (V) = 0.244 mL** u (cal2) = 0.002 mL u (Vol. exp.2) = 0.012 mL **u (V_p) = 0.01 mL**	c d e f g h i

[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, g] *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, h] *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, i] *The three 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

$$c_{Toxin} = \frac{10 \times m_{ws} \times P}{V_f} = \frac{10 \times 2.616 \times 95.6}{100} = 25.0\ mg/L$$

$$\frac{u_c(c_{Toxin})}{c_{Toxin}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{ws}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2 + \left[\frac{u(Vp)}{V_{fp}}\right]^2} = \sqrt{\left[\frac{2.5}{95.6}\right]^2 + \left[\frac{0.0007}{2.616}\right]^2 + \left[\frac{0.3}{100}\right]^2 + \left[\frac{0.01}{5}\right]^2} = 0.025$$

$$u_c(c_{Toxin}) = c_{Toxin} \times 0.025 = 25.0 \times 0.025 = 0.63\ mg/L$$

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

$$U(c_{Toxin}) = u_c(c_{Toxin}) \times 2 = 0.63 \times 2 = 1.26\ mg/L = 1.3\ \mu g/mL$$

4. Discussion of traceability

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

The certified concentration of Ergocorninine of the gravimetric prepared solution was confirmed by HPLC-FLD against an independently prepared reference batch.

column	Phenomenex Luna 3µ Phenyl-Hexyl, 150 x 4.60 mm	
Sample dilution	undiluted	
injection volume	2 µL sample	
solvent A	200mg/L ammonium carbonate in water	
solvent B	acetonitrile	
flow rate	1.0 mL / min	
gradient	time in minutes (min)	% solvent B
	0 – 5	50
	5 – 12.5	50 - 70
	12.5 – 14.5	70
	14.5 – 14.6	70 – 50
	14.6 - 20	50
FLD	λ_{EX} = 330 nm, λ_{EM} = 415 nm	

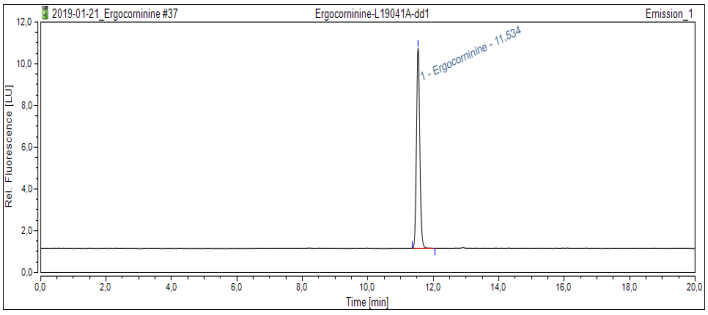

Figure 1: HPLC-FLD chromatogram of Ergocorninine, Lot# L19041A

	time [min]	area	height	concentration [a]
Ergocorninine	11.534	1.149	9.692	24.9 ± 0.4 µg/mL

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

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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:* 21.01.2019

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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, **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