REFERENCE MATERIAL CERTIFICATE

NIVALENOL

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:	Nivalenol hydrate
CAS number	23282-20-4
Catalog number:	DRE-C15618100-5MG; DRE-C15618100-10MG
Lot #:	S19101N
Certificate version:	2
Expiry date:	04.03.2024
Physical description of RM:	White crystals of nivalenol hydrate
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps, quantities of 5 mg or 10 mg of RM
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 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 mg. 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. Solutions prepared for calibration purposes should be protected from exposure to light. Discard solutions after use in accordance with appropriate safety regulations for chemical substances

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).

3. Certified values and their uncertainties

Nivalenol hydrate		
Compound	**Purity**	
	Certified value [a]	Uncertainty [b]
Nivalenol hydrate	98.2 %	± 1.8 %

[a] *The certified value is based upon the results from several analytical techniques*

[b] *Expanded uncertainty U (k = 2) of the value u_c according to GUM [4]*

4. Discussion of traceability

The value (purity of nivalenol hydrate) is based on the results of several independent analytical techniques previously used for purity assessment of solid mycotoxins [**Fehler! Textmarke nicht definiert.**]. High purity material represents a practical realization of concentration units, through conversion of mass to molar quantity.

5. Purity assessment of Nivalenol

5.1. UV-spectrophotometry

The wavelength scale accuracy in both UV and visible regions of the applied spectrophotometer was controlled with holmium oxide in dilute perchloric acid [5]. The absorbance scale and the linearity of the apparatus were validated with potassium dichromate in dilute sulfuric acid [6]. All measurements were performed at 22 ± 3 °C.

The UV absorption spectrum of nivalenol was consistent with literature data [7] and showed no detectable impurities.

molecular mass	m	=	330,32	g/mol
molar absorptivity in ACN [7]	ε	=	6955	L mol^{-1} cm^{-1}
wavelength	λ	=	218	nm
dilution factor	f	=	4	
absorbance at λ = 218 nm [a]	A	=	0.520	
optical path length	d	=	1	cm
mass concentration [b]	c_m		96.70 ± 2.2	µg/mL
analytical concentration [c]	c	=	98.78 ± 2.4	µg/mL

$$c = \frac{A \times m \times 1000 \times f}{\varepsilon \times d} = \frac{0.520 \times 330.32 \times 1000 \times 4}{6955 \times 1} = 98.78 \mu g/mL$$

[a] Mean of 6 replicate measurements

[b] Mass concentration calculated on weighed amount of sample with expanded uncertainty according to GUM [4]

[c] confidence interval with P = 95 %

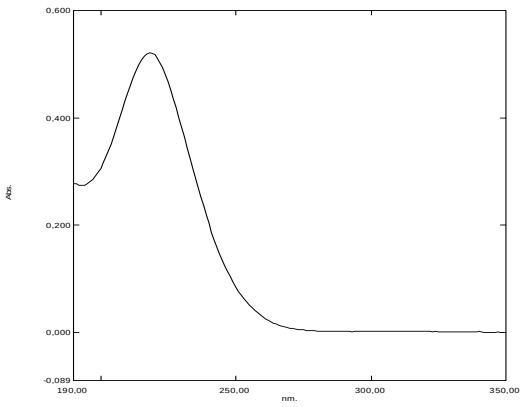

Figure 4: UV-absorption spectrum of nivalenol sample

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5.2. HPLC-DAD

The purity check using gradient LC-DAD of the nivalenol sample showed one main peak and no other impurities after blank subtraction. The peak purity of the main signal was examined by diode array spectra of the nivalenol peak and led to the conclusion that this peak consists only of nivalenol.

Column	Phenomenex Luna C18(2), 250x3.00 mm, 5µ					
injection volume	100 µL sample					
solvent A	acetonitrile / water (10 / 90)					
solvent B	acetonitrile					
flow rate	0.5 mL / min					
gradient	time in minutes (min)	% solvent B				
	0 – 3	0				
	3 – 6	0 - 11				
	6 – 20	11				
	20 – 21	11 - 0				
	21 – 30	0				
DAD settings	λ	=	218 nm			
mass concentration	c_m	=	98.7	±	2.2 µg/mL	
sample dilution	1:20 with water					

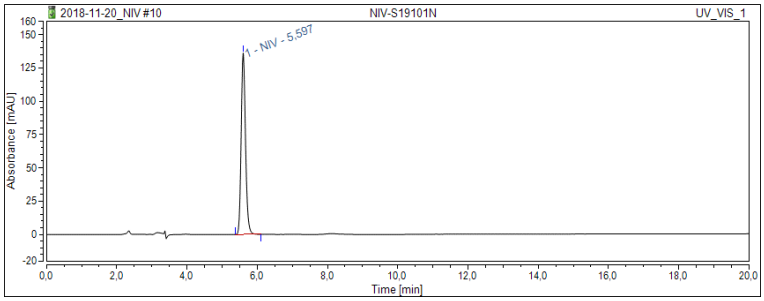

Figure 1: HPLC-UV chromatogram of Nivalenol

	time	area	height
Nivalenol hydrate	5.597	19.496	136.2

no impurity peaks detectable in the LC-DAD chromatogram

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:* 18.03.2019

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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), (1995), *"Guide to the Expression of Uncertainty in Measurement"*, 1st Ed. Geneva, Switzerland

[5] J.C. Travis, J.C. Zwinkels, F. Mercader, A. Ruiz, E.A. Early, M.V. Smith, M. Noel, M. Maley, G.W. Kramer, K.L. Eckerle, D.L. Duewer, Anal Chem., **74**, 3408-3415, (2002) "*An International Evaluation of Holmium Oxide Solution Reference Materials for Wavelength Calibration in Molecular Absorption Spectrophotometry"*

[6] Official Methods of Analysis, **970.44**, 1185-1186, (1990) 16th Ed., *"Preparation of Standards for Mycotoxins"*, AOAC Int. Arlington VA

[7] Krska et al.(2007) "*Determination of molar absorptivity coefficients for major type-B trichothecenes and certification of calibrators for deoxynivalenol and nivalenol" Anal Bioanal Chem (2007) 388:1215-1226, 11 pp.*