

## ALTERNARI OLMETHYLETHER

### 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 / CITAC Guides [3,4].

### 2. Description of the Reference Material (RM)

Name:	Alternariolmethylether
CAS number:	26894-49-5
Catalog number:	B-MYC5120-1
Lot #:	L12465B
Expiry date:	16.11.2015
Starting material 1:	Alternariolmethylether, Lot#S08242Z
Physical description of RM:	Thin film, dried down standard
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps (1 mL)
Name and address of the manufacturer:	Romer Labs Diagnostic GmbH Technopark 1, 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 1.000 mL  $\pm$  0.014 mL of acetonitrile (HPLC grade quality) with a graduated syringe or a volumetric pipette
2. Cap vial tightly
3. Mix on a vortex mixer for 3 minutes, or shake vigorously by hand for 5-8 minutes.
4. Always keep vial tightly capped.
5. Store the reconstituted standard at +2-8°C in a dark place

#### 2.3 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 100  $\mu$ L. 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

Alternariolmethylether		
Compound	Mass concentration <sup>a</sup>	
	Certified value <sup>b</sup>	Uncertainty <sup>c</sup>
Alternariolmethylether	100.2 µg/mL	± 2.0 µg/mL
<sup>a</sup> Values are based on preparation data and confirmed experimentally by HPLC-UV <sup>b</sup> Mass concentration based on weighed amount, purity and dilution step <sup>c</sup> Expanded uncertainty U (k = 2) of the value u <sub>c</sub> 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 Alternariolmethylether	P = 99.2 % ± 0.8 %	<b>u (P) = 0.5 %</b>	a
Weighing procedure weighted sample: m <sub>ws</sub> = 5.052 mg	repeatability: 0.03 mg linearity: 0.012 mg	<b>u (m) = 0.03 mg</b>	b
Dilution procedure volumetric flask: V <sub>f</sub> = 50 mL pipette: V <sub>p</sub> = 1 mL	calibration: 50 mL ± 0.1 mL repeatability: 0.04 mL volume expansion solvent  calibration pipette: 1 mL ± 0.014 mL volume expansion solvent pipette	u (cal1) = 0.04 mL u (rep1) = 0.04 mL u (Vol. exp.1) = 0.12 mL <b>u (V<sub>f</sub>) = 0.1 mL</b> u (cal2) = 0.006 mL u (Vol. exp.2) = 0.002 mL <b>u (V<sub>p</sub>) = 0.006 mL</b>	c d e f g h i

<sup>a</sup> Maximum tolerance of purity (rectangular distribution) was divided by  $\sqrt{3}$

<sup>b</sup> Estimation of this u-value is based upon the values for repeatability and linearity described in the user manual of the microbalance

<sup>c, g</sup> A triangular distribution (division by  $\sqrt{6}$ ) was chosen for the calculation of u (cal)

<sup>d</sup> Based on a series of ten fill and weigh experiments on a typical 50 mL flask; the value was used directly as a standard deviation

<sup>e, h</sup> Based on the density of 0.7857 g/cm<sup>3</sup> at temperature T = 20°C and a maximum temperature variation of ± 3°C, of volume expansion, relative volume expansion coefficient of acetonitrile is  $1370 \cdot 10^{-6}/^{\circ}\text{C}$  [7], volume expansion term (rectangular distribution) was divided by  $\sqrt{3}$

<sup>f, i</sup> The three contributions are combined to give the u (V) =  $\sqrt{u(\text{cal})^2 + u(\text{rep})^2 + u(\text{Vol. exp.})^2}$

#### Calculation of the combined uncertainty u<sub>c</sub> and the expanded standard uncertainty U

$$c_{\text{Toxin}} = \frac{10 \times m_{\text{ws}} \times P}{V_f} = \frac{10 \times 5.052 \times 99.2}{50} = 100.2 \text{ mg / L}$$

$$\frac{u_c(c_{\text{Toxin}})}{c_{\text{Toxin}}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{\text{ws}}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2 + \left[\frac{u(V_p)}{V_p}\right]^2} = \sqrt{\left[\frac{0.5}{99.2}\right]^2 + \left[\frac{0.03}{5.052}\right]^2 + \left[\frac{0.1}{50}\right]^2 + \left[\frac{0.006}{1}\right]^2} = 0.01$$

$$u_c(c_{\text{Toxin}}) = c_{\text{Toxin}} \times 0.01 = 100.2 \times 0.01 = 1.0 \text{ mg / L}$$

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

$$U(c_{\text{Toxin}}) = u_c(c_{\text{Toxin}}) \times 2 = 1.0 \times 2 = 2.0 \text{ mg / L} \approx 2.0 \mu\text{g / mL}$$

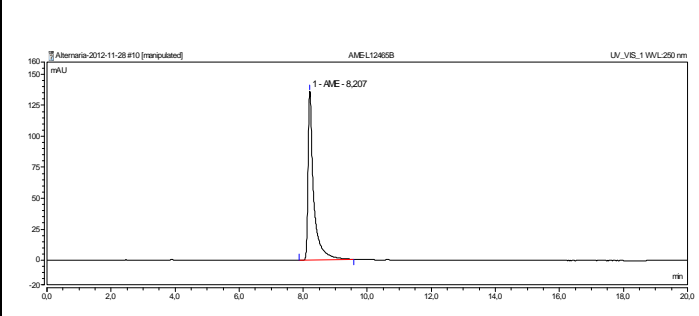
## 4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified value (mass concentration of Alternariolmethylether) 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 Alternariolmethylether of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch (Biopure Lot# L12022G).

column	Phenomenex Luna C18(2), 250x3.00 mm, 5 $\mu$	
flow rate	0.5 mL / min	
injection volume	25 $\mu$ L sample	
solvent A	Acetonitrile / water (10 / 90)	
solvent B	Acetonitrile	
oven	30°C	
gradient	time in minutes (min)	% solvent B
	0 – 2.0	50
	2.0 – 10.0	50-70
	10.0 – 15.0	70
	15.0 – 15.1	70-50
	15.1 – 20.0	50
DAD settings	250 nm	
sample dilution	1:20 with water	



*Figure 1: HPLC-UV chromatogram of Alternariolmethylether, Lot# L12465B*

	time [min]	area	concentration <sup>a</sup> [ $\mu$ g/mL]
Alternariolmethylether	8.207	27.691	101.2 $\pm$ 2.9

<sup>a</sup> Mean of 7 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: Dipl. Chem. Ulrich Berger

date: 28.11.2012

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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 / CITAC Guide, 1-37, (2003), "Traceability in Chemical Measurement"
- [4] Eurachem / CITAC Guide, 1-120, (2000), "Quantifying Uncertainty in Analytical Measurement"
- [5] International Organization for Standardization (ISO), (1995), "Guide to the Expression of Uncertainty in Measurement", 1<sup>st</sup> Ed. Geneva, Switzerland
- [6] 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"
- [7] E.W. Flick, (1996), "Industrial Solvents Handbook", 3<sup>rd</sup> Ed., Noyes Data Corp. Westwood NJ