

MELAMINE IN ACETONITRILE - WATER

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 / CITAG Guides [3,4].

2. Description of the Reference Material (RM)

Name:	Melamine in acetonitrile - water
Catalog number:	CMT001 (5 mL)
Lot #:	L08403Z
Expiry date:	01.10.2009
Starting material 1:	Melamine, FLU 1289303
Matrix:	Acetonitrile, Ultra Gradient HPLC Grade Lot #0720609018, J.T. Baker, catalog Nr. 9017 MilliQ water, Biopure Referenzsubstanzen GmbH
Physical description of RM:	Solution of Melamine in acetonitrile – water (84:16)
Packaging and amount of RM:	Amber glass ampoules fitted with teflon faced butyl septa and PP screw caps, solution of 5 mL
Name and address of the supplier:	Biopure Referenzsubstanzen GmbH Technopark 1 3430 Tulln, Austria www.biopure.at, office@biopure.at

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 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.

2.3 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) at www.biopure.at.

Hazardous Ingredients	Concentration in %	Hazard symbol	Risks (R-phrases)
acetonitrile	84	Xn	11-20/21/22-36



3. Certified values and their uncertainties

Melamine in acetonitrile – water (84:16)		
Compound	Mass concentration ^a	
	Certified value ^b	Uncertainty ^c
Melamine	103.8 µg/mL	± 1.0 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 [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 Melamine	P = 99.5 % ± 0.5 %	u (P) = 0.3 %	a
Weighing procedure weighted sample: m _{ws} = 10.43 mg	repeatability: 0.03 mg linearity: 0.012 mg	u (m) = 0.03 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 Estimation of this u-value is based upon the values for repeatability and linearity described in the user manual of the microbalance

^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 $1370 \cdot 10^{-6}/^{\circ}\text{C}$ [7], volume expansion term (rectangular distribution) was divided by $\sqrt{3}$

^f 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_c and the expanded standard uncertainty U

$$c_{\text{Toxin}} = \frac{10 \times m_{\text{ws}} \times P}{V_f} = \frac{10 \times 10.43 \times 99.5}{100} = 103.8 \text{ mg / L}$$

$$\frac{u_c(c_{\text{Toxin}})}{c_{\text{Toxin}}} = \sqrt{\left[\frac{u(P)}{P}\right]^2 + \left[\frac{u(m)}{m_{\text{ws}}}\right]^2 + \left[\frac{u(V)}{V_f}\right]^2} = \sqrt{\left[\frac{0.3}{99.5}\right]^2 + \left[\frac{0.03}{10.43}\right]^2 + \left[\frac{0.3}{100}\right]^2} = 0.005$$

$$u_c(c_{\text{Toxin}}) = c_{\text{Toxin}} \times 0.005 = 103.8 \times 0.005 = 0.52 \text{ mg / L}$$

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

$$U(c_{\text{Toxin}}) = u_c(c_{\text{Toxin}}) \times 2 = 0.52 \times 2 = 1.04 \text{ mg / L} \approx 1.0 \mu\text{g / mL}$$

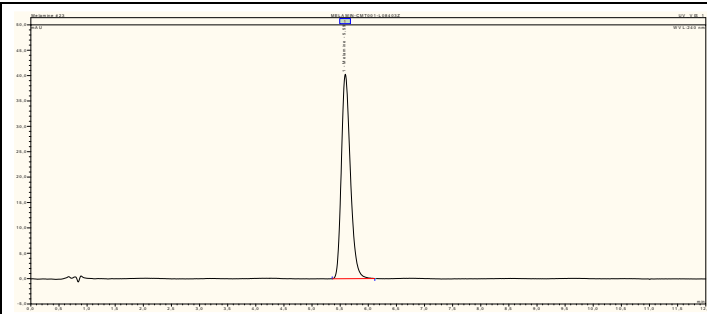


4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified value (mass concentration of Melamine) 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 certified concentration of Melamine of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch of Melamine calibrant Lot# L08391Z.

column	Zorbax Eclipse XDB-C18, 2.1 x 150 mm, 5 μ	 <p>Figure 1: HPLC-UV chromatogram of Melamine calibrant Lot # L08403Z</p> <table border="1"> <thead> <tr> <th></th> <th>time [min]</th> <th>area</th> <th>concentration ^a [μg/mL]</th> </tr> </thead> <tbody> <tr> <td>Melamine</td> <td>5.59</td> <td>7.499</td> <td>102,6 \pm 2.3</td> </tr> </tbody> </table>			time [min]	area	concentration ^a [μ g/mL]	Melamine	5.59	7.499	102,6 \pm 2.3
	time [min]			area	concentration ^a [μ g/mL]						
Melamine	5.59			7.499	102,6 \pm 2.3						
injection volume	5 μ L sample										
column thermostat	40°C										
solvent A	buffer: 10 mM citric acid, 10 mM 1-octanesulfonic acid sodium salt in water, adjusted to pH 3.0										
solvent B	acetonitrile										
mobile phase	90:10, buffer: acetonitrile										
flow rate	0.5 mL / min										
DAD settings	240 nm										
sample dilution	1:10 with buffer										

^a 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. Biopure Referenzsubstanzen GmbH makes no warranty of any kind, express or implied, other than it's products meet all quality control standards set by Biopure Referenzsubstanzen GmbH. We do not guarantee that the product can be used for a special application.

approved for release by: Dr. Freudenschuss

date: 01.10.2008

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