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Certificate of Analysis

Product Name:	Aflatoxin Mix 4 solution analytical standard
Product Number:	34036
Batch Number:	BCCM0156
CAS Number:	
Formula:	
Formula Weight:	
Storage Temperature:	-20 C
Expiration Date:	JUN 2026
Quality Release Date:	28 JUN 2024

TEST	SPECIFICATION	RESULT
APPEARANCE (FORM)	LIQUID	LIQUID
CONCENTRATION COMP1	~ 2UG/ML B1	2.093 UG/ML
CONCENTRATION COMP2	~ 2UG/ML G1	2.084 UG/ML
CONCENTRATION COMP3	~ 0.5UG/ML B2	0.526 UG/ML
CONCENTRATION COMP4	~ 0.5UG/ML G2	0.521 UG/ML
MEASURING TOLERANCE P±	0.20 UG/ML FOR B1/G1	± 0.042 UG/ML
	0.05 UG/ML FOR B2/G2	± 0.031 UG/ML
SOLVENT	ACETONITRILE	ACETONITRILE
PURITY (HPLC) COMP1	≥ 98% B1	99 %
PURITY (HPLC) COMP2	≥ 98% G1	99 %
PURITY (HPLC) COMP3	≥ 98% B2	99 %
PURITY (HPLC) COMP4	≥ 98% G2	99 %
MEASURING TOLERANCE P± COMP1	≤ 1.0% FOR ALL AFLATOXINS	0.6 %

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Aflatoxin Mix 4 solution OEKANAL®, analytical standard
2 µg/mL B1 and G1 in acetonitrile 0.5 µg/mL B2 and G2 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], ISO Guide 35 [2] and Eurachem / CITAG Guides [3,4].

2. Description of the Reference Material (RM)

Name:	Aflatoxin Mix 4 solution OEKANAL®
Catalog number:	34036-1ML-R
Batch / Lot #:	BCCM0156
Date of production / Expiry date:	26.06.2024 / 26.06.2026
Starting materials:	Aflatoxin B1 A6636 Lot# 020M4075, Aflatoxin B2 A9887 Lot# 079K4041 Aflatoxin G1 A0138 Lot# 050M4071, Aflatoxin G2 A0263 Lot# 030M4043, Sigma-Aldrich™
Physical description of RM:	Solution of 4 different aflatoxins in acetonitrile
Packaging of RM:	Amber glass Certan® ampoules
Name and address of the supplier:	Sigma-Aldrich Chemie GmbH Kappelweg 1 91625 Schnelldorf Germany www.sigma-aldrich.com

2.1 Intended use of the RM

- calibration of analytical instruments
- validation of analytical methods
- determination of detection limits and linearity studies
- recovery experiments

2.2 Instruction for the correct use of the RM

The ampoules should be stored at approximately -20°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 0.5 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 material safety data sheet (MSDS).

Hazardous Ingredients	Concentration in %	Hazard symbol	Risks (R-phrases)
acetonitrile	> 99.9	Xn	11-20/21/22-36

3. Certified values and their uncertainties

Aflatoxin Mix 4 solution OEKANAL®		
Compound	**Mass concentration [a]**	
	Certified value [b]	Uncertainty [c]
Aflatoxin B1	2.093 µg/mL	± 0.042 µg/mL
Aflatoxin G1	2.084 µg/mL	± 0.042 µg/mL
Aflatoxin B2	0.526 µg/mL	± 0.032 µg/mL
Aflatoxin G2	0.521 µg/mL	± 0.031 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 steps*
[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 Aflatoxins	P = 99 % ± 1 %	**u (P) = 0.6**
Weighing procedure weighted sample: m_{wsAFB1}= 5.285 mg, m_{wsAFG1}= 5.263 mg m_{wsAFB2}= 1.328 mg; m_{wsAFG2}= 1.316 mg	repeatability: 0.030 mg linearity: 0.012 mg	**u (m) = 0.032 mg**
Dilution procedure steps	volumetric flask 1: V_{f1} = 250 mL volumetric flask 2: V_{f2} = 500 mL one-mark glass pipette: V_p = 50 mL	**u (V1) = 0.6 mL** **u (V2) = 1.2 mL** **u (V3) = 0.1 mL**

4. Discussion of traceability

This calibrant is certified on the basis of gravimetric preparation [6]. Thus the certified values (mass concentrations of 4 different aflatoxins) are based on the weighed amount of the starting materials and are therefore traceable to the stated purity of the solid mycotoxins. High purity materials represent a practical realization of concentration units, through conversion of mass to molar quantity.

5. Confirmation of certified value by HPLC-UV

The concentration values of the 4 different aflatoxins of the gravimetric prepared solution was confirmed by HPLC-UV against an independently prepared reference batch.

column	Phenomenex Luna C18(2), 250 x 3.0 mm, 5µ
injection volume	100 µL sample
solvent A	Water/acetonitrile/methanol = 57/17/26
oven	30°C
flow rate	0.5 mL / min
VWD settings	365 nm
sample dilution	1:5 with water

	time [min]	area	concentration [a] [µg/mL]
Aflatoxin G2	7.7	52.0	0.520 ± 0.042
Aflatoxin G1	9.1	220.0	2.080 ± 0.064
Aflatoxin B2	10.3	80.3	0.528 ± 0.042
Aflatoxin B1	12.4	299.3	2.091 ± 0.064

[a] Mean of 7 replicate measurements against reference batch, confidence intervall with P = 95 %

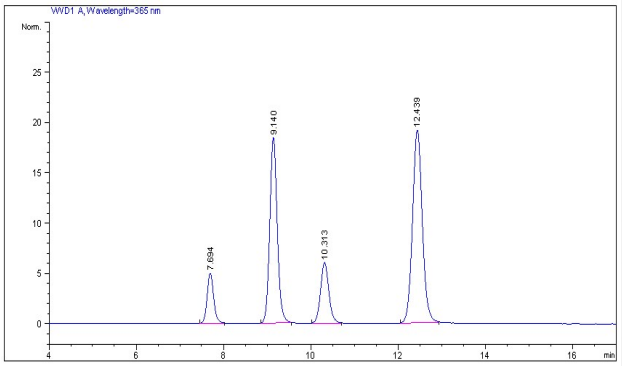

Figure 1: HPLC-UV chromatogram of Aflatoxin Mix 4 solution

6. Further information

The purchaser must determine the suitability of this product for its particular use. Sigma-Aldrich Chemie GmbH makes no warranty of any kind, express or implied, other than it`s products meet all quality control standards set by Sigma-Aldrich Chemie GmbH. We do not guarantee that the product can be used for a special application.

This document has been computer generated and is valid without a signature.

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"*