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**CERTIFICATE OF ANALYSIS**

# Aflatoxins B1, B2, G1, G2 Mixture in Acetonitrile LCMS grade

This document is designed, and the certified values and uncertainty are determined in accordance with ISO Guide 31, ISO Guide 34, ISO Guide 35, AOAC, and Eurachem/ CITAC Guides.

## Description of the Reference Material (RM)

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Product name:** | Aflatoxins B1, B2, G1, G2 Mixture | | | | |
| | **Product number:** | FIA000223 | | | | |
| | **CAS number:** | Aflatoxin B1 | 1162-65-8 | | | |
| | | Aflatoxin B2 | 7220-81-7 | | | |
| | | Aflatoxin G1 | 1165-39-5 | | | |
| | | Aflatoxin G2 | 7241-98-7 | | | |
| | **Lot number:** | AFBG18040801 | | | | |
| | **Expiry date:** | 08-Apr-2026 | | | | |
| | **Certified value (s):** | Aflatoxin B1 | **25,04** | **±** | **0,28** | **µg/mL** |
| | | Aflatoxin B2 | **24,75** | **±** | **0,33** | **µg/mL** |
| | | Aflatoxin G1 | **25,00** | **±** | **0,44** | **µg/mL** |
| | | Aflatoxin G2 | **25,13** | **±** | **0,57** | **µg/mL** |
| | **Physical description:** | Clear solution of toxins mixture in Acetonitrile LCMS grade | | | | |
| | **Packing** | Amber glass vial filled with 10 mL of solution | | | | |
| | **Storage conditions** | ≤ -10°C | | | | |
| | **Matrix and starting material:** | This material was prepared with/from: | | | | |
| | | Acetonitrile LCMS Grade | | Batch: | 0001204102BS | |
| | | Aflatoxin B1 | | Internal ID: | SS-AFB1-18040401 | |
| | | Aflatoxin B2 | | Internal ID: | SS-AFB2-18040401 | |
| | | Aflatoxin G1 | | Internal ID: | SS-AFG1-18040401 | |
| | | Aflatoxin G2 | | Internal ID: | SS-AFG2-18040401 | |

## Intended use of the RM:

For laboratory use for R&D purposes only. The main purpose of this material is for analytical instrument calibration (e. g. external calibration, standard addition). Not for drug, household or other uses.

## Instruction for the correct use of the RM:

The vial should be stored in a dark place at Acetonitrile LCMS Grade. Before usage of the RM, allow the vial to warm to room temperature. The expiry date of this RM is based on the current knowledge and holds only for proper storage conditions in the originally closed vials / packages. Solutions prepared for calibration purpose should be protected from exposure to light. Discard solutions after use in accordance with appropriate safety regulations for chemical substances.

## Hazardous situation:

H225 : Flammable liquid - Category 2 - Highly flammable liquid and vapour
H302 : Acute toxicity - Oral - Category 4 - Harmful if swallowed
H312 : Acute toxicity - Dermal - Category 4 - Harmful in contact with skin
H319 : Eye irritation - Category 2 - Causes serious eye irritation
H332 : Acute toxicity - Inhalation - Category 4 - Harmful if inhaled

In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid exposure. Wear suitable protective clothing.

## Safety measures:

Special care must be taken when manipulating this standard. Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Use only in a chemical fume hood. Safety shower and eye bath must be near. In case of spills, cover and absorb with an inert dry material such as dry-lime, sand or soda ash and place in an appropriate waste disposal container.
Keep container tightly closed. Do not store in direct sunlight. Keep away from heat, sparks, flame and incompatible material. Storage area should be cool, dry and away from incompatible materials.

## Further information:

Further information is available in the SDS available online (downloading page: www.fianovis.com/documentation (documentation section)). Final users should conduct their own investigations to determine the suitability of the information for their particular research purposes. Under no circumstances will the supplier of this RM be held responsible for any damage resulting from handling or contact with the product.

## Traceability

The values are based on the chromatographic determination of the concentration of the stock solution. The chromatographic assay method was demonstrated to be selective through validation of the analytical method. Pipette calibration is verified by an accredited external calibration service. Production is carried out with specially dedicated glassware. Only Class A glassware is used for volumetric measurements.

## Calculation of certified values and associated uncertainties

This calibrant is certified on solution preparation. Mass concentration calculation is based on certified concentration and dilution step. Toxin is pipetted and diluted in Acetonitrile LCMS grade .

$$C\ (\mu g/mL) = \frac{C_{ss} \times V_p}{V_D}$$

| Toxin | Source | | | | Standard uncertainty |
|---|---|---|---|---|---|
| **Aflatoxin B1** | Liquid solution $C_{ss}$ | concentration | 252,49 | µg/mL | 0,82 |
| | Volumetry procedure $V_p$ | volume | 5,42 | mL | 0,00 |
| | Dilution $V_D$ | volume | 54,65 | mL | 0,06 |
| $Combined_u = \sqrt{\left(\frac{u_p}{P}\right)^2 + \left(\frac{u_{Cm}}{V_{Cm}}\right)^2 + \left(\frac{u_{Vp}}{V_p}\right)^2 + \left(\frac{u_{V1}}{V_1}\right)^2}$ | | | | | 0,01 |
| $Concentration_{Toxin} = \frac{Concentration\ mother}{V_{D1}}$ | | | | µg/mL | **25,04** |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | **0,28** |

| Toxin | Source | | | | Standard uncertainty |
|---|---|---|---|---|---|
| **Aflatoxin B2** | Liquid solution $C_{ss}$ | concentration | 98,42 | µg/mL | 0,82 |
| | Volumetry procedure $V_p$ | volume | 13,74 | mL | 0,00 |
| | Dilution $V_D$ | volume | 54,65 | mL | 0,06 |
| $Combined_u = \sqrt{\left(\frac{u_p}{P}\right)^2 + \left(\frac{u_{Cm}}{V_{Cm}}\right)^2 + \left(\frac{u_{Vp}}{V_p}\right)^2 + \left(\frac{u_{V1}}{V_1}\right)^2}$ | | | | | 0,01 |
| $Concentration_{Toxin} = \frac{Concentration\ mother}{V_{D1}}$ | | | | µg/mL | **24,75** |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | **0,33** |

| Toxin | Source | | | | Standard uncertainty |
|---|---|---|---|---|---|
| **Aflatoxin G1** | Liquid solution $C_{ss}$ | concentration | 258,06 | µg/mL | 1,94 |
| | Volumetry procedure $V_p$ | volume | 5,30 | mL | 0,00 |
| | Dilution $V_D$ | volume | 54,65 | mL | 0,06 |
| $Combined_u = \sqrt{\left(\frac{u_p}{P}\right)^2 + \left(\frac{u_{Cm}}{V_{Cm}}\right)^2 + \left(\frac{u_{Vp}}{V_p}\right)^2 + \left(\frac{u_{V1}}{V_1}\right)^2}$ | | | | | 0,01 |
| $Concentration_{Toxin} = \frac{Concentration\ mother}{V_{D1}}$ | | | | µg/mL | **25,00** |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | **0,44** |

| Toxin | Source | | | | Standard uncertainty |
|---|---|---|---|---|---|
| **Aflatoxin G2** | Liquid solution $C_{ss}$ | concentration | 298,22 | µg/mL | 3,09 |
| | Volumetry procedure $V_p$ | volume | 4,61 | mL | 0,00 |
| | Dilution $V_D$ | Volume | 54,65 | mL | 0,06 |
| $Combined_u = \sqrt{\left(\frac{u_P}{P}\right)^2 + \left(\frac{u_{Cm}}{V_{Cm}}\right)^2 + \left(\frac{u_{Vp}}{V_p}\right)^2 + \left(\frac{u_{V1}}{V_1}\right)^2}$ | | | | | 0,01 |
| $Concentration_{Toxin} = \frac{Concentration\ mother}{V_{D1}}$ | | | | µg/mL | **25,13** |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | **0,57** |

Notes: The purity of the mycotoxin used for this RM was determined by liquid chromatography.
Following the Guide to the Expression of Uncertainty in measurement (GUM) the expanded uncertainty of toxin level is obtained by multiplication with a coverage factor K for which 2 is usually chosen to obtain a confidence level of 95 %.

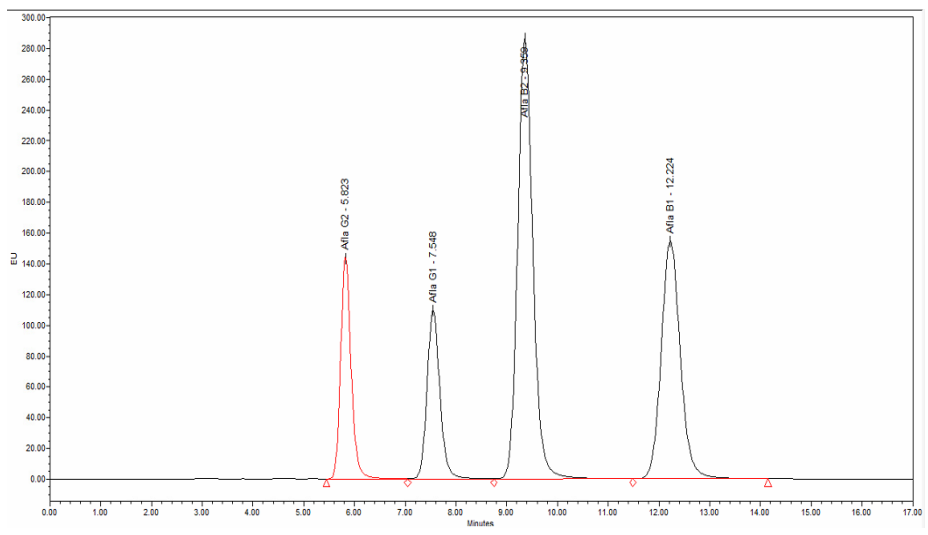
## Quality control

### Confirmation of the certified concentration by HPLC-FLD & cell

The certified concentrations of the prepared solution was confirmed by HPLC-FLD & cell against a reference batch.

Chromatogram
Chromatographic conditions:

| | | | | |
|---|---|---|---|---|
| Column : | InertSustain C18 250 x 4,6 mm5 µm | | | |
| Mobile phase : | MeOH / H2O + HNO3 +KBr / Isocratic : 35%A / 65%B | | | |
| Flow (mL/min) : | 1,80 | | | |
| Temperature (°C) : | 50,00 | | | |
| Detector : | FLD with post-column electrochemical with bromide using FARLIB® ECD Cell | | | |
| Aflatoxin B1 | 25,57 | ± | 1,34 | µg/mL |
| Aflatoxin B2 | 25,01 | ± | 1,29 | µg/mL |
| Aflatoxin G1 | 25,06 | ± | 1,29 | µg/mL |
| Aflatoxin G2 | 25,20 | ± | 1,31 | µg/mL |
| Mean of 6 replicates measurement against reference batch, confidence interval with P = 95% | | | | |




Chromatogram of Toxins

## References:


a-ISO GUIDE 31:2015, Reference Materials - Contents of certificates, labels and accompanying documentation.
b-ISO GUIDE 34:2009, General requirements for the competence of reference material producers
c-ISO GUIDE 35:2006, Reference materials - General and Statistical Principles.
d-ISO/IEC Guide 98-3:2008 Uncertainty of measurement-Part 3 : Guide to the expression of uncertainty in measurment (GUM:1995)
e-Eurachem/CITAC guide (2019), Traceability in Chemical Measurement.
f-Eurachem/CITAC guide (2012), Quantifying Uncertainty in Analytical Measurement.
g-AOAC Official Method 970.44-1971 - Preparation of Standards for Mycotoxins.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Quality Control

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