

CERTIFICATE OF ANALYSIS
Alternaria ALT, AOH, AME, TEN mixture in Methanol LCMS grade

The certified values and uncertainty are determined in accordance with NF ISO 33401, ISO 17034, ISO/IEC 17025, ISO33405, ISO TR 16476 and JCGM 100.

Description of the standard

| | | | | | |
|--------------------------------------|---|-------------|---|------|-----------------------------|
| Product name: | Alternaria ALT, AOH, AME, TEN mixture | | | | |
| Product number: | FIA000405 | | | | |
| CAS number: | Altenuene | 889101-41-1 | | | |
| | Alternariol Monomethyl Ether | 26894-49-5 | | | |
| | Alternariol | 641-38-3 | | | |
| | Tentoxin | 28540-82-1 | | | |
| Batch: | MALT18092302 | | | | |
| Expiry date: | 24-Mar-2026 | | | | |
| Certified value (s): | Altenuene | 10,00 | ± | 0,48 | µg/mL |
| | Alternariol Monomethyl Ether | 10,00 | ± | 0,30 | µg/mL |
| | Alternariol | 9,99 | ± | 0,50 | µg/mL |
| | Tentoxin | 10,00 | ± | 0,46 | µg/mL |
| Physical description: | Clear solution of toxins mixture in Methanol LCMS grade | | | | |
| Packing | Amber glass vial filled with 5 mL of solution | | | | |
| Storage conditions | ≤ -10°C | | | | |
| Matrix and starting material: | This material was prepared with/from: | | | | |
| | Methanol LCMS Grade | | | | Batch: P4A580164 |
| | Altenuene | | | | Internal ID: ALT18091701 |
| | Alternariol Monomethyl Ether | | | | Internal ID: AME17110701 |
| | Alternariol | | | | Internal ID: SS-AOH17101701 |
| | Tentoxin | | | | Internal ID: TEN18091701 |

Intended use of the standard:

For laboratory use only. Not for drug, household or other uses. The main purpose of this material is :

- Demonstrate mastery of a measurement process within a laboratory over a given period;
- Check the performance of the instrument;
- Repeatability and reproducibility studies: repeated use over a long period of time, instruments, operators, etc., to estimate the long-term reproducibility or robustness of a measuring process or that of a laboratory;
- Confirm the degree of equivalence of measurement results from at least two laboratories (e.g. supplier and user);
- Check variability due to the operator;
- Study the impact of any variation in environmental conditions (e.g. temperature, humidity).

Instruction for the correct use of the standard:

The vial should be stored in a dark place at ≤ -10°C . Before usage of the standard, allow the vial to warm to room temperature. If condensation is present on the bottle, the bottle should be wiped before opening. Homogenization can be done by vortexing for at least 10 seconds. There is no indication as to the vortex speed, but the vortex must be visible to the user. The bottle should not be left open on the bench, it should be opened only to take the necessary quantity and immediately closed. The expiry date of this standard is based on the current knowledge and holds only for proper storage conditions in the originally closed vials / packages.

Hazardous situation:

H225 : Flammable liquid - Category 2 - Highly flammable liquid and vapour

H301 : Acute toxicity - Oral - Category 3 - Toxic if swallowed

H311 : Acute toxicity - Dermal - Category 3 - Toxic in contact with skin

H331 : Acute toxicity - Inhalation - Category 3 - Toxic if inhaled

H370 : Acute toxicity - Organs - Category 1 - Causes damage to organs

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 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.

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 standard be held responsible for any damage resulting from handling or contact with the product.

More information are available on the SDS online on www.fianovis.com/documentation.

Commutability

As part of the standards produced by Fianovis, the property values are guaranteed for chromatography analysis. For another use, the user must make additional qualification to use it in this context.

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 Methanol LCMS grade .

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

| Toxin | Source | | | | Standard uncertainty |
|---|---------------------------|---------------|-------|------------------|----------------------|
| Altenuene | Liquid solution C_{ss} | concentration | 50,91 | $\mu\text{g/mL}$ | 1,12 |
| | Volumetry procedure V_p | volume | 3,93 | mL | 0,00 |
| | Dilution V_D | volume | 20,00 | mL | 0,03 |
| $\text{Combined}_u = \sqrt{\left(\frac{u_{C_{ss}}}{V_{C_{ss}}}\right)^2 + \left(\frac{u_{V_p}}{V_p}\right)^2 + \left(\frac{u_{V_D}}{V_D}\right)^2}$ | | | | | 0,02 |
| $\text{Concentration}_{\text{roxin}} = \frac{\text{Concentration stock solution}}{V_D} \quad \mu\text{g/mL}$ | | | | | 10,00 |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | 0,48 |

| Toxin | Source | | | | Standard uncertainty |
|---|---------------------------|---------------|-------|------------------|----------------------|
| Alternariol Monomethyl Ether | Liquid solution C_{ss} | concentration | 46,64 | $\mu\text{g/mL}$ | 1,12 |
| | Volumetry procedure V_p | volume | 4,29 | mL | 0,00 |
| | Dilution V_D | volume | 20,00 | mL | 0,03 |
| $\text{Combined}_u = \sqrt{\left(\frac{u_{C_{ss}}}{V_{C_{ss}}}\right)^2 + \left(\frac{u_{V_p}}{V_p}\right)^2 + \left(\frac{u_{V_D}}{V_D}\right)^2}$ | | | | | 0,02 |
| $\text{Concentration}_{\text{roxin}} = \frac{\text{Concentration stock solution}}{V_D} \quad \mu\text{g/mL}$ | | | | | 10,00 |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | 0,30 |

| Toxin | Source | | | | Standard uncertainty |
|---|---------------------------|---------------|--------|------------------|----------------------|
| Alternariol | Liquid solution C_{ss} | concentration | 498,32 | $\mu\text{g/mL}$ | 11,36 |
| | Volumetry procedure V_p | volume | 0,40 | mL | 0,00 |
| | Dilution V_D | volume | 20,00 | mL | 0,03 |
| $\text{Combined}_u = \sqrt{\left(\frac{u_{C_{ss}}}{V_{C_{ss}}}\right)^2 + \left(\frac{u_{V_p}}{V_p}\right)^2 + \left(\frac{u_{V_D}}{V_D}\right)^2}$ | | | | | 0,02 |
| $\text{Concentration}_{\text{roxin}} = \frac{\text{Concentration stock solution}}{V_D} \quad \mu\text{g/mL}$ | | | | | 9,99 |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | 0,50 |

| Toxin | Source | | | | Standard uncertainty |
|--|------------------------------------|---------------|-------|-------|----------------------|
| Tentoxin | Liquid solution C _{SS} | concentration | 48,62 | µg/mL | 1,01 |
| | Volumetry procedure V _p | volume | 4,12 | mL | 0,00 |
| | Dilution V _D | Volume | 20,00 | mL | 0,03 |
| $Combined_u = \sqrt{\left(\frac{u_{C_{SS}}}{C_{SS}}\right)^2 + \left(\frac{u_{V_p}}{V_p}\right)^2 + \left(\frac{u_{V_D}}{V_D}\right)^2}$ | | | | | 0,02 |
| $Concentration_{Tentoxin} = \frac{Concentration\ stock\ solution}{V_D}$ µg/mL | | | | | 10,00 |
| Total expanded uncertainty (using a coverage factor k=2) | | | | | 0,46 |

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 LC-MS/MS | | | | |
|--|--|--------|-------|------------------------|
| The certified concentrations of the prepared solution was confirmed by LC-MS/MS against a reference batch. | | | | |
| Chromatographic conditions | | | | Chromatogram of Toxins |
| Column : | Acquity UPLC BEH C18 150 x 2,1 mm 1,7 µm | | | |
| Mobile phase : | H2O + 1mM ammonium acetate + ammonium hydroxyde (pH=9) / MeOH/Isopropanol (90/10) / Gradient | | | |
| Flow (mL/min) : | 0,20 | | | |
| Temperature (°C) : | 30,00 | | | |
| Detector : | PDA | | | |
| Altenuene | 9,41 | ± 0,44 | µg/mL | |
| Alternariol Monomethyl Ether | 9,95 | ± 0,19 | µg/mL | |
| Alternariol | 9,22 | ± 0,09 | µg/mL | |
| Tentoxin | 10,33 | ± 0,21 | µg/mL | |
| Mean of 6 replicates measurement against reference batch, confidence interval with P = 95% | | | | |

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- References:
- NF ISO 33401 (2024), Reference Materials - Contents of certificates, labels and accompanying documentation.
 - ISO 17034 (2016) General requirements for the competence of reference material producers.
 - ISO/IEC 17025 (2017) General requirements for the competence of testing and calibration laboratories.
 - ISO 33405 (2024), Reference Materials - Approaches for characterization and assessment of homogeneity and stability.
 - ISO TR 16476 (2016) Reference Materials – Establishing and expressing metrological traceability of quantity values assigned to reference materials.
 - JCGM 100(2008) (E) – Evaluation of measurement data - Guide to the expression of uncertainty in measurement.

Control and Certification

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Date: 26-May-2025

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