



**DETERMINATION OF ZEARELENONE
IN CEREAL GRAINS BY HIGH-
PERFORMANCE LIQUID
CHROMATOGRAPHY (HPLC)**

Standard NTC 4881

EXTRACTION

1. Weigh out 50 g of ground sample into a 250 mL glass blender jar or 250 mL Erlenmeyer flask.
2. Add 100 mL of 84+16 acetonitrile-water.
3. Blend at high speed for 2 minutes (blender) or shake for one hour on gyratory shaker.
4. Filter through fast qualitative filter paper and pipet 5 mL of the filter extract into a 15 x 85 mm test tube. Add 50 µL glacial acetic acid and homogenize.

PURIFICATION

5. Slowly push a Micotox® M2004 cleanup column (rubber flange end) into the test tube creating a tight seal between rubber flange and glass wall of tube. Push until just over 0.5 mL purified extract are filtered into the column reservoir and homogenize with a pipet. For complex matrices (e.g. complete feeds) use a Micotox® M2006 column, which contains double packing and more cleanup capacity. **NOTE: Passage through the column should last for 30 to 40 seconds.**

6. Pipet exactly 0.5 mL of homogenized purified extract into a vial or test tube.
7. Add 0.5 mL of HPLC water and mix.
8. Filter through a 0.45 µm pore membrane filter.
9. Inject 50 µL into LC.

CHROMATOGRAPHIC CONDITIONS

- Column: RP-18, 150 x 4.6 mm I.D.
- Temperature: ambient.
- Mobile phase: isocratic mix of water:methanol:acetic acid (65+35+1).
- Flow rate: 0.6 mL/min.
- Detector: fluorescence. Excitation: 275 nm; Emission: 470 nm.
- Approximate retention time: 11 min.

CALIBRATION

10. Pipet 10 µL of standard solution (100 ng zearalenone) into an autosampler vial. Add 900 µL water. The standard solution contains 10.0 µg/mL zearalenone in methanol (available from Micotox Ltda.). Final calibration standard contains 100 ng/mL. Inject 50 µL standard solution into LC (5 ng of zearalenone on column).
11. Calibrate HPLC integrator using the external standard calibration method. Construct a calibration curve with several points if desired.

CALCULATIONS

50 g sample are extracted with 100 mL extraction solvent; 0.5 mL from the extract are diluted with 0.5 mL HPLC water; 50 µL are injected into LC.

Sample equivalent injected into LC is:

$$50 \text{ g} \times 0.5/100 \text{ mL} \times 0.05/1.0 \text{ mL} = 0.0125 \text{ g}$$

$$\text{ng/g (ppb)} = \frac{\text{ng injected into LC}}{0.0125 \text{ g}}$$

Amount of standard injected into LC (ng):

Vol. µL	Zearalenone
50	5 ng

Equivalent in ppb of zearalenone standard injected into LC (ng/g):

Vol. µL	DON
50	400 ppb

Limit of detection: ≤50 ppb.

Limit of quantitation: 100 ppb.

CONFIRMATION

Identity of zearalenone can be confirmed by TLC analysis using the same extract. Ask for the 3-toxin analysis method: **“Analysis of aflatoxin B1, zearalenone and DON by TLC”** to the Technical Department of Micotox Ltda.

ZEARELENONE by HPLC V01-07