



DETERMINATION OF AFLATOXINS IN CEREAL GRAINS AND NUTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

AOAC Official Method 994.08

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 about 10 mL of the filter extract into a 15 x 85 mm test tube.

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 about 0.5 mL purified extract are filtered into the column reservoir. 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 200 µL of purified extract into a 1.5 amber vial and then add 700 µL derivatizing solution (trifluoroacetic acid–acetic acid–water, 2+1+7).
7. Close vial with cap and mix. Heat vial in ≥65°C waterbath for ≥8.5 min. Cool to room temperature before opening.
8. Inject 50 µL into LC.

CHROMATOGRAPHIC CONDITIONS

- Column: RP-18, 12.5 cm x 4 mm I.D.
- Temperature: 40°C.
- Mobile phase: isocratic mix of water-methanol (60+40).
- Flow rate: 0.6 mL/min.
- Detector: fluorescence. Excitation: 360 nm; Emission: 440 nm.
- Approximate retention times: aflatoxin G1, 4.1 min; aflatoxin B1, 6.4 min; aflatoxin G2, 8.6 min; aflatoxin B2; 15.0 min.

CALIBRATION

9. Pipet 200 µL of an standard solution containing 25 ng/mL aflatoxins B1 and G1, and 7.5 ng/mL aflatoxins B2 and G2 in acetonitrile (available from Micotox Ltda.), into an autosampler vial. Add 700 µL derivatizing solution and treat as described before (steps 7-8).
10. 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; 0.2 mL from the extract are taken to which 0.7 mL derivatizing solution are added; 50 µL are injected into LC.

Sample equivalent injected into LC is:

$$50 \text{ g} \times 0.2/100 \text{ mL} \times 0.05/0.9 \text{ mL} = 0.005555 \text{ g}$$

$$\text{ng/g (ppb)} = \frac{\text{ng injected into LC}}{0.005555 \text{ g}}$$

Amount of standard injected into LC (ng):

Vol. µL	AFB ₁	AFB ₂	AFG ₁	AFG ₂
50	0.2778	0.0833	0.2778	0.0833

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

Vol. µL	AFB ₁	AFB ₂	AFG ₁	AFG ₂
50	50	15	50	15

Limit of detection: <1 ppb of each aflatoxin.

Limit of quantitation: 1 ppb of each aflatoxin.

CONFIRMATION

The identity of aflatoxins B1 and G1 can be confirmed by injecting 200 µL of sample extract diluted with 700 µL mobile phase. If sample contains aflatoxins B1 and G1, the chromatographic peaks disappear. Alternatively, analyse sample by TLC. Request method: “**Analysis of aflatoxins by TLC**” to the Technical Department of Micotox Ltda.

AFLATOXINS by HPLC - MICOTOX