



**DETERMINATION OF DEOXY-NIVALENOL (DON) IN CEREAL GRAINS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)**

AOAC Method PVM 2:1997

### EXTRACTION

1. Weigh out 25 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® M2005 cleanup column (rubber flange end) into the test tube creating a tight seal between rubber flange and glass wall of tube. Push until about 2.0 mL purified extract are filtered into the column reservoir. For complex matrices (e.g. complete feeds) use a Micotox® M2007 column, which contains double packing and more cleanup capacity.  
**NOTE: Passage through the column should last for 30 to 40 seconds.**

6. Pipet exactly 2.0 mL of purified extract into a clean test tube. Evaporate to dryness under nitrogen or in a 60°C waterbath using vacuum.
7. Dissolve the dry residue with 500 µL mobile phase (water-methanol-acetonitrile, 90+5+5) and filter through a 0.45 µm pore membrane filter.
8. Inject 100 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-acetonitrile (90+5+5).
- Flow rate: 0.6 mL/min.
- Detector: UV at 220 nm.
- Approximate retention time: 7.9 min.

### CALIBRATION

9. Pipet 20 µL of standard solution (1.0 µg DON) into an autosampler vial and add 980 µL water. The standard solution contains 50 µg/mL DON in methanol (available from Micotox Ltda.). Final calibration standard contains 1.0 µg/mL DON. Inject 100 µL standard solution into LC (0.1 µg of DON on column).
10. Calibrate HPLC integrator using the external standard calibration method. Construct a calibration curve with several points if desired.

### CALCULATIONS

25 g sample are extracted with 100 mL extraction solvent; 2 mL from the extract are taken to dryness and then dissolved with 500 µL mobile phase; 100 µL are injected into LC.

Sample equivalent injected into LC is:

$$25 \text{ g} \times 2/100 \text{ mL} \times 0.1/0.5 \text{ mL} = 0.1 \text{ g}$$

$$\mu\text{g/g (ppm)} = \frac{\text{ng injected into LC}}{0.1 \text{ g}}$$

Amount of standard injected into LC (µg):

Vol. µL	DON
100	0.1 µg

Equivalent in ppm of DON standard injected into LC (µg/g):

Vol. µL	DON
100	1 ppm

Limit of detection: <0.1 ppm.

Limit of quantitation: 0.1 ppm.

### CONFIRMATION

Identity of DON 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.

DON by HPLC - MICOTOX