

# MICOTOX LTDA



## DETERMINATION OF OCHRATOXIN A (OTA) IN CEREAL GRAINS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

AOAC Official Methods 973.37 and 991.44

### EXTRACTION

1. Weigh out 25 g of ground sample into a 250 mL Erlenmeyer flask.
2. Add 12.5 mL of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) 0.1M (3.42 mL of 85% phosphoric acid in 500 mL water) and swirl to wet sample.
3. Add 125 of methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) and shake for one hour on gyratory shaker.
4. Filter through quantitative filter paper (e.g. S&S 589-3) and pipet exactly 5 mL of extract into a 15 x 85 mm test tube.

### PURIFICATION

5. Place a solid phase Micotox® M2200 ochratoxin A column onto a solid phase extraction apparatus. Pour the 5 mL extract into the column (no preconditioning is necessary). Drain by gravity (<2.0 mL/min)\* and discard solution.

\*NOTE: Solution has to drain through the column at a maximum flow rate of 2 mL/min. Before using the M2200 Micotox® column, press firmly the top frit of the column with a plunger. This will prevent the sample extracts from draining through the column too fast.

6. When top of solution reaches top of packing add 5 mL of hexane followed by 15

mL of CH<sub>2</sub>Cl<sub>2</sub>, added in three successive volumes of 5 mL each. Do not let the column run dry. Discard solution.

7. Elute the ochratoxin A with 20 mL of CH<sub>2</sub>Cl<sub>2</sub>/HCOOH (formic acid) 99+1 in two successive volumes of 10 mL. Use an appropriate test tube or container to collect the eluate (e.g. a 40 mL capacity conical test tube).
8. Evaporate the eluate to dryness under nitrogen or in a 60°C waterbath using vacuum.
9. Dissolve the dry residue with 1.0 mL mobile phase (acetonitrile-water-acetic acid, 50+50+1). Filter through a 0.45 µm pore membrane filter.

### CHROMATOGRAPHIC CONDITIONS

- Column: RP-18, 12.5 cm x 4 mm I.D.
- Temperature: 40°C.
- Mobile phase: Isocratic mixture of acetonitrile-water-acetic acid (50+50+1). Flow rate: 0.6 mL/min.
- Detector: fluorescence. Excitation: 330 nm; emission: 460 nm.
- Approximate retention time: 7.7 min.

### CALIBRATION

10. Pipet 10 µL of standard solution (0.1 µg OTA) into an autosampler vial and add 990 µL of mobile phase. Standard solution contains 10 µg/mL ochratoxin A in methanol (available at Micotox Ltda.). Final calibration solution contains 100 ng/mL ochratoxin A. Inject 50 µL (5 ng) into LC.
11. Calibrate HPLC integrator using the external standard calibration method.

### CALCULATIONS

25 g sample are extracted with 125 mL extraction solvent; 5 mL from the extract are loaded onto column, taken to dryness and then dissolved with 1.0 mL mobile phase; 50 µL are injected into LC.

Sample equivalent injected into LC is:

$$25 \text{ g} \times 5/125 \text{ mL} \times 0.05/1.0 \text{ mL} = 0.05 \text{ g}$$

$$\text{ng/g (ppb)} = \frac{\text{ng of toxin injected}}{0.05 \text{ g}}$$

Amount of standard injected into LC (ng):

Vol. (µL)	Ochratoxin A
50	5 ng

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

Vol. (µL)	Ochratoxin A
50	100 ppb

Limit of detection: <5 ppb.

Limit of quantitation: 5 ppb.

### CONFIRMATION

If necessary, confirm ochratoxin A identity by converting the ochratoxin A into its methyl-ester. To the dry residue (step 8) add 100 µL of 14% boron trifluoride (BF<sub>3</sub>) in methanol (Sigma B-1252). Heat at 80°C for 10 min. Let cool and add 400 µL mobile phase. Mix well, filter, and inject 50 µL into LC. Simultaneously, derivatize 100 ng of ochratoxin A standard in another vial. Ochratoxin A methyl-ester elutes at approximately 17.5 min.

OCHRATOXIN A BY HPLC - MICOTOX