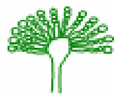
MICOTOX LTDA 

DETERMINATION OF OCHRATOXIN A (OTA) IN CEREAL GRAINS BY THIN LAYER CHROMATOGRAPHY (TLC)

AOAC Official Method 973.37

EXTRACTION

1. Weigh out 25 g of ground sample into a 250 mL Erlenmeyer flask.

2. Add 12.5 mL of phosphoric acid (H_3PO_4) 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_2Cl_2) 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 extract 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_2Cl_2, added in three successive volumes of 5 mL each. Discard solution.

7. Elute the ochratoxin A with 20 mL of CH_2Cl_2/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.

THIN LAYER CHROMATOGRAPHY

- Dissolve the dry residue with 100 µL toluene-acetic acid, 99+1 (add the solvent mixture, stopper the test tube and mix well on vortex for 30 seconds).

- Spot 20 µL of each sample along with 5, 10, and 20 µL of working standard on a 10 x 10 cm silicagel 60 plate (Merck 1.05553). Working standard contains 1.0 µg/mL ochratoxin A in toluene-acetic acid, 99+1 (available from Micotox Ltda.).

- Develop plate in toluene-methanol-acetic acid (90+5+5) to about 1 cm from the top of the plate. Let plate air dry in a fume hood.

- View TLC plate under long wave UV light (365 nm) and determine ochratoxin A content by comparison with the standard spots. Ochratoxin A has a blue-green fluorescence and its approximate Rf is 0.6.

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 100 µL toluene-acetonitrile; 20 µL are spotted on TLC plate.

Sample equivalent in g is:

25 g x 5/125 mL x 0.02/0.1 mL = 0.2 g

$$\text{ng/g (ppb)} = \frac{\text{ng of toxin on plate}}{0.2\ \text{g}}$$

Amount of ochratoxin A standard spotted on plate (ng):

Vol. (µL)	Ochratoxin A
5	**5 ng**
10	**10 ng**
20	**20 ng**

Equivalent concentration of ochratoxin A standard on TLC plate (ng/g):

Vol. (µL)	Ochratoxin A
5	**25 ppb**
10	**50 ppb**
20	**100 ppb**

Limit of detection: <5 ppb.
Limit of quantitation: 10 ppb

Confirmation: spray TLC plate with an alcoholic solution of sodium bicarbonate (6.0 g of $NaHCO_3$ in 100 mL H_2O + 20 mL ethanol) and let dry. View under long wave UV light. The blue-green fluorescence of ochratoxin A turns to blue and becomes more intense.

www.micotox.com info@micotox.com micotox@cable.net.co Telefax(+57-1) 2870572 Bogotá, D.C., Colombia