

MICOTOX LTDA



DETERMINATION OF TYPE-A TRICHOHECENES (T-2 TOXIN, DAS, HT-2 TOXIN AND NEOSOLANIOL) IN CEREAL GRAINS BY THIN-LAYER CHROMATOGRAPHY - TLC

EXTRACTION AND CLARIFICATION

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.
5. Add 0.2 g of charcoal, mix using a vortex mixer, and let stand for 5-10 minutes.

PURIFICATION

6. 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 2.5 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.

7. Pipet exactly 2.5 mL of purified extract to a clean test tube. Evaporate purified extract to dryness under nitrogen or in a 60°C waterbath using vacuum.

THIN LAYER CHROMATOGRAPHY

8. Dissolve the dry residue with 100 µL of toluene–acetonitrile, 95+5 (add the solvent mixture, stopper the test tube and mix well on vortex for 30 seconds).
9. 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 5 µg/mL of each T-2 toxin and HT-2 toxin, and 10 µg/mL of each DAS and neosolaniol in toluene–acetonitrile, 95+5 (available from Micotox Ltda.).
10. Develop plate in chloroform to about 1 cm from the top of the plate. Let plate air dry in a fume hood. NOTE: Chloroform does not cause analytes to migrate, it only removes liposoluble interferences that might be present, improving sensitivity.
11. Develop plate in toluene–ethyl acetate–formic acid (5+4+1) to about 1 cm from the top of the plate. Let plate air dry in a fume hood.
12. Spray or dip TLC plate in 10% sulfuric acid (H₂SO₄) in methanol. Let air dry. Heat at about 130°C using a heat plate or oven (better an oven), until the standard spots are visible to the naked eye (5-10 minutes). Avoid overheating the TLC plate.
13. View TLC plate under long wave UV light (365 nm) and interpret as follows:

Trichothecene	Rf	Fluorescence
T-2 toxin	0.45	Light blue
DAS	0.35	Light orange
HT-2 toxin	0.20	Light blue
Neosolaniol	0.15	Light yellow

CALCULATIONS

50 g of sample are extracted with 100 mL of extraction solvent; 2.5 mL of the extract are taken to dryness and dissolved with 100 µL; 20 µL are spotted on the TLC plate. Sample equivalent in g is:

$$50 \text{ g} \times 2.5/100 \text{ mL} \times 0.02/0.1 \text{ mL} = 0.25 \text{ g}$$

$$\text{ng/g (ppb)} = \frac{\text{ng of toxin on plate}}{0.25 \text{ g}}$$

Amount of standard spotted on TLC plate (ng):

Vol. µL	T-2	DAS	HT-2	NEO
5	25	50	25	50
10	50	100	50	100
20	100	200	100	200

Equivalent concentration of standards spotted on TLC plate (ng/g):

Vol. µL	T-2	DAS	HT-2	NEO
5	100	200	100	200
10	200	400	200	400
20	400	800	400	800

Limits of detection: <100 ppb of T-2 and HT-2 toxins, <200 ppb of DAS and neosolaniol.
Limits of quantitation: 100 ppb of T-2 and HT-2 toxins, 200 ppb of DAS and neosolaniol.

Type A TCT by TLC – MICOTOX