Procedure for GC/MS Analysis of
Ground Wheat/Barley Samples

Extraction

1. Weigh 1.00g from each bulk sample of ground grain and place in a 15mL screw cap conical tube. Assign a "Vial ID" number for the day's analysis and record the number on both the tube and lab prep documentation.
2. Add 8 mL of acetonitrile:water (86:14) to each tube. Mix thoroughly to ensure all ground flour is dislodged and wetted.
3. Shake extraction tubes horizontally at room temperature for 1 hour at 200rpm.

Sample Cleanup

4. Decant approximately 3ml of each sample extraction into a C18: Aluminum oxide (1:3) cleanup column that has been conditioned at 55°C overnight. Collect the flow through in a clean glass tube labeled with the appropriate vial number. For high throughput, a maximum of 5 in. Hg pressure is applied briefly using a vacuum manifold.
5. Transfer 2mL of each cleaned extract flow through to a new glass tube labeled with the corresponding vial number.
6. Dry each 2mL sample to complete dryness under N_2 flow and 55°C incubation.

Derivatization

7. Add 100uL TMSI:TMCS (100:1) to each dried sample. Vortex and incubate at room temperature for 20 minutes.
8. Add 500uL isooctane + Mirex to each tube. Mirex is present as an internal standard at a concentration of 0.5ppm.
9. Add 500uL ddH_2O to each tube and vortex to mix well. Incubate at room temperature for 5 minutes or until two distinct layers appear.
10. Carefully transfer 150uL of the top isooctane layer of each sample into a clean GC/MS assay vial labeled with corresponding vial number. Top with septum caps and proceed with GC/MS analysis.

GC/MS Analysis

Samples are assayed to quantitate the presence of deoxynivalenol (DON), 3ADON, 15ADON and nivalenol (NIV). All analyses are performed on an Agilent 6890N / 5975 GC/MS. Samples are assayed concurrently with standards of each toxin, ranging from 0.025ppm – 15ppm. Internal checks are assayed throughout each batch run for quality control purposes.

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