AAFCO MEETING
3 August 2008

Sample Processing for Micro Assay for DDG Samples:

1. Routine feed assay – 2 ppm LOQ
   - 1 ppm LOD

2. FDA Letter – November 22, 1993

    a. Listed 0.5 ppm as an acceptable DDG concentration.

3. Method quoted in letter used MIBK shake extraction, Rotovap, dilute with assay solvent, wash with Pet Ether and cyclohexane.

4. Limited success with method quoted in #3:

    a. Corn model okay however unreliable with DDG.
        i. Poor recovery, fuzzy zones

5. Change solvent to methanol, shake with buffer dilution, centrifuge, filter, SepPak C18 cleanup.

6. Method okay on corn model

    a. Variable results with DDG
        i. poor handling on initial extraction
        ii. low solvent recovery
        iii. poor Vm recovery
        iv. fuzzy zones
7. Current processing method back to the future:

    a. Use a Soxhlet extraction with methanol
        i. Good handling and good solvent recovery
    b. Rotovap to oily residue
    c. 2X hexane washes
    d. Re-suspend with methanol-buffer
    e. Filter with paper and 0.45 µm membrane filter
    f. SepPak C18 SPE cleanup and wash with assay solvent
    g. Elute with 70% MeOH, reconstitute and plate.

8. This approach handles most samples (damp and dry).

    a. Assay at mid-range of Vm micro assay
    b. Sharp zones with light yellow edge haze
    c. Recoveries 85-90% @ 0.5 ppm
    d. Reproducible
    e. Processing is slow and labor-intensive.
    f. Needs good lab hands.