Procedure: Bayer RA-362/1823,
as used by Chemonics Labs

AMITROLE SOIL ANALYSIS PROCEDURE

Extraction

50 gm of soil is shaken for 2 hours with a mixture of 150 ml Ethanol (EM Science, Omni-Solv, #EX-0278) and 100 ml distilled water in a one quart Mason Jar covered with aluminum foil. The sample is shaken on an orbital soil-extraction table (Model 0S-42, Fermentation Design Inc. Allentown, PA). Check soils will have been spiked with a suitable concentration of Amitrole in Methanol added with first extractant.

The supernatant is decanted through doubled 2V filter paper (32 cm) in a large funnel into a 1000 ml boiling flask (clay soil will not settle out, even overnight, so much of the clay will be poured onto the filter. Subsequent extractants will percolate through this and continue the extraction). The extraction is repeated twice more with fresh Ethanol and water.

The combined filtrates are concentrated on a vacuum rotary evaporator to final volume of 10 ml (add 3 drops of 1% Mineral Oil in Benzene keeper and 2 boiling chips).

The water bath temperature should not exceed 70 degrees C (push 65 degrees for time's sake).

Standard

A 100 mcg Amitrole standard in Ethanol is pipetted into a 250 ml boiling flask. Five ml of water and 20 ml Ethanol are added, and the standard is taken to the Acetylation step.

Acetylation

The concentrated sample extracts at 10 ml are allowed to cool, and 20 ml of Ethanol is added. One ml of Acetic Anhydride (Mallinckrodt, AR #2420) is added to samples and the acetylation standard, and allowed to react for 10 minutes at room temperature with occasional swirling to mix.

Ten ml of water is added to quench the reaction, followed by 50 ml of Chloroform (MBC, Omnisolve, glass distilled, #CX1054-3).

Partition

The acetylated extracts are transferred to 500 ml separatory funnels and shaken out twice with 50 ml of chloroform each time (shake 2 minutes, vigorously, and use the second Chloroform to rinse the flask into the funnel).

Each Chloroform phase is filtered over 5 gm Sodium Sulfate (Mallinckrodt, anhydrous, granular, AR, #8024) on 2V filter paper (15 cm fluted) into a 250 ml boiling flask, and rinsed through with about 40 ml of Chloroform.

The combined Chloroform phases are concentrated (add 3 drops keeper and one boiling chip) under rotary vacuum (bath temperature not to exceed 50 degrees C) until an oily residue of about 0.5 ml remains.

Finishing drying under stream of Nitrogen on a rotary evaporator, without waterbath or vacuum (Acetic fumes! Use hood!).

Transfer quantitatively with Acetone (EM Science, Omnisolve, glass distilled, #AX0116-1) to 15 ml graduated centrifuge tubes. Add one drop keeper and blow to dryness with Nitrogen (the Meyer N-Evap Analytical Evaporator, Model 112, Organomation Associates Inc., P. O. Box 159, South Berlin, MA 01549, has been used with tubes out of the water bath). Final dryness may be hard to achieve, so one ml of Acetone is added one or more times to sweep out the remains.

Two ml of Acetone is added to dissolve the residue in the tubes, which are then capped and held for analysis.

Gas Chromatographic Measurement

Chromatograph: Tracor, Model 560, with Model 702 N-P detector.

Separation Column: 240 cm x 2 mm I.D. x 6 mm O.D. glass column packed with 15% Carbowax 20M + 5% OV-61 on 80/100 mesh Gas Chrom Q.

Column Temperature: 190° C.

Carrier Gas: 45 ml/min. at 40 psig U.H.P. Helium.

Injection Block Temperature: 250° C.

Detector Type: Nitrogen (N-P), 290° C, U.H.P. Hydrogen at 3 ml/min. at 40 psig, Air 40 psig through fixed orifice, power to bead for 60% F.S.D. at 1 x 4 attenuation.

Injection Volume: 4 microliters for >10 cm deflection at 10 ng Acetylated Amitrole. Detection Limit = .02 ppm at 50 gm at 4 ml shooting 4 microliters.

Retention Time: About 7.5 min.

Chart Speed: 0.25 in./min.

Evaluation: Sample peak ht./10 ng std. peak ht.
x 10 ng = ng found.
ng found x factor = ppm Amitrole
(factor = ml of sample/gm of sample x microliters injected)

<u>or</u>

ng found/mg injected = ppm

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PROTOCOL AMENDMENT

Amendment Number: 1
Protocol Sponsor: Mobay Corporation
Protocol Number : PR91004
Protocol Title : NTN 33893 Residue Analysis, Soil dissipation

1.) Change the original study number N302013 to number N3022103. (Reason: Typographical error)

2.) The Protocol is being changed to include the amendments made to the methodology reported in MOBAY method 99619 with attachments 1.2-1.3. (Reason: To create a working assay for the analysis of NTN 33893 residues in soil.)

METHOD ADDENDA

Extraction of NTN 33893 Residue from Soil is as follows:

1.) Weigh nominal 40.0-gram soil sample into a 500-ml boiling flask, add Teflon® covered egg stirring bar. Spike for concurrent recovery. Add 200 ml of acetonitrile and reflux for seven hours.

2.) Filter under vacuum through a 12.5-cm Whatman GF/A filter paper into a 1-liter flat bottom flask. Rinse the 500-ml flask with 5 X 10-ml portions of acetonitrile.

3.) Rotary evaporate the filtrate at 25°C and 26.5" Hg vacuum. The temperature is raised to 40°C over the course of 20 minutes. Evaporate to a volume of about 5 ml.

4.) Quantitatively transfer the 5 ml to a 250-ml separatory funnel that has a mark indicating a volume of 50 ml. Add 2 X 20-ml portions of Reverse Osmosis water to the 1-liter flask and decant into the separatory funnel. Bring the aqueous phase to the mark with RO water.

5.) Add 10 ml of saturated NaCl solution to the funnel. Rinse the 1-liter flask with 2 X 25-ml portions of 20% MTBE/DCM and add these rinses to the separatory funnel. Shake for 60 seconds and allow the phases to separate. The organic will be on the bottom.

6.) Take the organic phase off through 1 teaspoon of anhydrous sodium sulfate in a #5 Whatman filter paper into a 500-ml boiling flask. Rinse the sodium sulfate with 2 X 10-ml portions of 20% MTBE/DCM.

7.) Add 100 µl of ethylene glycol to the organic extract and swirl to mix. Rotary evaporate to the ethylene glycol at 25°C and 20-22" Hg vacuum. Transfer to a 250-ml separatory funnel with 2 X 50-ml portions of DCM. Partition with 50 ml of 0.05 M potassium carbonate.

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8.) Drain the DCM phase through 15-20 g prewashed anhydrous sodium sulfate supported in a powder funnel by a glass wool plug. Collect the DCM in a 250-ml flat bottom flask. Rinse the filter bed with 2 X 10-ml portions of DCM. Add 100 µl ethylene glycol and swirl to mix. Concentrate to 5 ml at 25°C and 20-22" Hg vacuum.

9.) Quantitatively transfer to a 15-ml centrifuge tube using DCM. Rinse the 250-ml flask with 2 X 3 ml of DCM and transfer the rinse to the centrifuge tube. Blow down to the ethylene glycol with a gentle stream of nitrogen.

10.) Add RO water to a volume of 2.0 ml. Filter through a 0.45 µ PTFE filter into a 1.5 ml sample vial and inject.

The following HPLC parameters were used for the analysis of NTN 33893:

Mobile Phase Gradient Program

- A— filtered reverse osmosis water with 1.0 g NaH_2PO_4/liter
  Pump A - Shimadzu LC-6A or equivalent
- B— filtered UV - grade acetonitrile
  Pump B - Shimadzu LC-6A or equivalent

Flow rate: 1.5 ml/min throughout

Initial:	90%A / 10%B
Gradient 1:	linear from 0 to 20 min. to 75%A / 25%B
Gradient 2:	linear from 20 to 25 min. to 10%A / 90%B
Plateau:	from 25 to 39 min. at 10%A / 90%B
Gradient 3:	linear from 39 to 43 min. to 90%A / 10%B
Re-equilibrate:	from 43 to 50 min. at 90%A / 10%B

Autosampler: Shimadzu SIL-6A or equivalent
Controller: Shimadzu SCL-6A or equivalent

Injection Volume: 25 µl

Column: 15 cm x 4.6 mm ID Supelco LC-8DB or equivalent
Column Heater: Eldex III or equivalent
Temperature: 40 °C

Detector: Shimadzu SPD-6A or equivalent
UV absorbance at 270 nm
Response: standard
0.08 AUFS; 0.8 V/AU

Note: These typical parameters are subject to change to optimize chromatographic response and separation during this study on these instruments or their equivalents.