INTRODUCTION

The method for determining amitrole residue in soil is presented. The methodology employed is novel and achieves detection limits lower than previously reported. The method was validated with soil collected from sites in Oregon and Washington (soil characterization in Tables 1 and 3). The method is flexible enough to allow for the variable matrix associated with soil.

PRINCIPLE AND APPLICATION

The method for determining Amitrole residue in soil is presented. The method was validated with soil collected from sites in Oregon and Washington. The method is flexible enough to allow for the variable matrix associated with soil. Amitrole [1H-1,2,4-Triazol-3-ylamine] is extracted and concentrated from soil by methanol/aqueous potassium bromide partition; the soil–solvent mixture is sonic disrupted and then centrifuged. The methanolic extract is then rotary evaporated to dryness and the residues dissolved in E-Pure® water. An aliquot of this solution is analyzed by isocratic elution HPLC using electrochemical detection. Quantification of Amitrole is effected by point to point analysis of the peak heights for samples and reference standards.

ANALYTICAL METHOD

Reagents

Amitrole, Lot No. L1-4420, 98.2% active ingredient, supplied by CFPI
Methanol, Burdick and Jackson "or equivalent", HPLC grade
Acetonitrile, Burdick and Jackson "or equivalent", HPLC grade
Potassium bromide, Aldrich Chemical, AR grade
Sodium dodecylsulfate, Aldrich Chemical, AR grade
Potassium phosphate monobasic, Aldrich Chemical, AR grade
Phosphoric acid, Mallinckrodt, AR grade
E-Pure water ®, Barnstead "or equivalent", ASTM type II

Equipment

Balance, Ohaus Galaxy 160 "or equivalent", four-place analytical balance
Flasks, volumetric, assorted sizes
Sonic disruptor, Branson Model 450, 3/4 inch horn
Centrifuge, Beckman GPR (or equivalent), 0-5000 rpm
Centrifuge bottles, 500 mL, Nalgene
Beakers, Pyrex, 250 mL
Roundbottom flasks, Pyrex, 1000 mL
Filter flasks, Pyrex, 1000 mL
Filter paper, Whatman, glass microfiber, 9.0 cm, 0.7 μm
Pipets, volumetric, assorted sizes

Serum bottles, Wheaton, assorted sizes, with Teflon-lined lids and metal crimp caps
Syringes, Hamilton, assorted sizes

Apparatus

High Performance Liquid Chromatographic System

Liquid chromatograph solvent pump, Waters Model 590
Autosampler, Waters Model 712 Intelligent Sample Processor
Detector, ESA Coulochem II electrochemical
Integrator, Hewlett-Packard Model 3396 A
Pulse Damper, SSI Model LP - 21
Chromatographic Column, Phenomenex Ultramex NH_2, 5µm, 250 mm x 4.6 mm I.D.

High Performance Liquid Chromatographic Conditions

Mobile phase: 20% CH_3CN : 80% aqueous 5.00 mM sodium dodecylsulfate/12.5 mM KH_2PO_4/15.0 mM H_3PO_4
Flowrate: 1.5 mL/minute
Injection volume: 60 µL
Autosampler runtime: 30 minutes
Sensitivity: 1 Volt output
Electrochemical conditions:
Channel 1: 550 mV, 50 µA range, 5 seconds filter
Channel 2: 600 mV, 50 µA range, 5 seconds filter
Attenuation (integrator): 2^5
Threshold (integrator): 6
Peak width (integrator): 0.20

Detailed Procedure

I. **Preparation of Stock and Standard Solutions**

A. **Amitrole Stock Solutions:**

1. Weigh 100 milligrams (a.i.) of Amitrole on an analytical balance.
2. Transfer the Amitrole to a 100-mL volumetric flask and dissolve to the mark with methanol.
3. In order to prepare appropriate stocks for fortification, make serial dilutions (in methanol) as follows:

Concentration of stock (mg/L)	Volume of stock used (mL)	Final volume of dilution (mL)	Final concentration of dilution (mg/L)
1000	10.0	100	100
100	10.0	100	10.0
10.0	10.0	100	1.00
1.00	10.0	100	0.100
0.100	10.0	100	0.0100

4. Transfer each stock solution to its properly labeled 100-mL amber serum vial and seal with a Teflon-lined crimp cap.
5. Store all stock solutions in a refrigerator maintained at 4°C.

B. Amitrole Standards:

1. In order to prepare representative standards add approximately 50 mL of E-Pure® water to each 100 mL volumetric flask. Prepare standards as follows:

Concentration of stock (mg/L)	Volume of stock used (µL)	Final volume of dilution (mL)	Final concentration of dilution (µg/L)
100	200	100	200
100	150	100	150
100	100	100	100
100	50.0	100	50.0
100	20.0	100	20.0

2. Following fortification, adjust each sample to the 100 mL line with E-Pure® water.
3. Transfer standards to bottles, cap and refrigerate as described for stocks; see section IA.

II. Control Sample Fortification

A. Processing and Dry-Weight Determination of Soil Samples:

1. Rinse all glassware with reagent grade methanol.
2. Remove the appropriate sets of soil cores from the freezer. Document (in the Amitrole logbook) the time of and person responsible for the removal of cores from the freezer.
3. Determine the upper end of each soil core.
4. Using a PVC tube cutter, measure, mark and remove the top 15 cm of each core and combine the appropriate cores in a mixing bowl. Allow the soil sections to thaw.

5. Homogenize until a uniform mixture is obtained (ca 10-20 minutes).
6. Remove a 50-gram portion of the mixed soil into a tared, prelabeled, 250 mL Pyrex beaker. Document this analytical weight.
7. Remove a second soil sample (approximately 10–15 grams) and determine its soil (dry–weight) content.

8. Quality Control Sample Fortification

For preparation of quality control or method validation samples, fortify each preweighed soil sample (contained in a beaker) with Amitrole by volumetric addition of the prepared stock solutions.

III. Extraction

1. To each soil sample add 100 mL of 10 mM KBr in 80% CH_3OH:20% H_2O.
2. Sonic disrupt the sample for 5 minutes at 80% output and 50% duty cycle.
3. Transfer the sample (rinsing with methanol) to a 500 mL Nalgene centrifuge bottle. Centrifuge at 2000 rpm for 10-15 minutes.
4. Filter the extract through a Whatman 9.0 cm., 0.7μm glass microfiber filter into a 1000 mL roundbottom flask.
5. Add an additional 100 mL of the extraction solvent to the soil contained in the centrifuge bottle. Firmly tighten the cap and shake vigorously while holding the bottle in an inverted position.
6. Empty the contents of the centrifuge bottle into the original 250 mL beaker. Rinse the tube with methanol; add this rinsate to the beaker.
7. Repeat steps 2-4 combining the filtrates in the roundbottom flask.
8. Rotary evaporate the extract to dryness at 80°C.
9. Dissolve the residues in E-Pure® water.
10. Proceed to Section IV, High Performance Liquid Chromatography.

IV. High Performance Liquid Chromatography

A. Method: High performance liquid chromatographic conditions for the analysis of Amitrole standards and samples have been determined. Close adherence to these parameters is necessary in order to obtain adequate sensitivity and resolution.

B. Analysis:

1. Prepare standard solutions containing Amitrole. Standard solution concentrations used for the recovery study were 20.0, 50.0, 100, 150 and 200 μg/L.
2. Inject 60 μL of the 20.0 μg/L standard solution. Identify the Amitrole peak by its retention time and document the peak height. Adjust the attenuation so that the peak signal results in at least a ten percent deflection from the baseline.

3. Inject 60 µL of each of the standards, document the peak heights, and determine the coefficient of determination for the line. The coefficient of determination should be greater than or equal to 0.985.
4. Inject 60 µL of a standard that reflects a final extract concentration.
5. Identify the Amitrole peak by its retention time and document the peak height.
6. Inject 60 µL of the extract and document the peak height.
7. Repeat steps 4-6 until all samples have been injected.
8. Inject 60 µL of each of the standards, document the peak heights, and their linearity.
9. In order to determine the analytical result for each sample, the following equation is used:

$$\text{Analytical Result } (\mu g/g) = (H_{sam}-B_y) \times C_{std} \times D.F./ (H_{std}-B_y)$$

Analytical Result = soil concentration of Amitrole
H_{sam} = peak height of Amitrole sample (extract)
B_y = The mean y intercept from the two linear regressions of the standards, analyzed at the beginning and end of the sample run.
C_{std} = concentration (µg/L) of Amitrole standard
D.F. = dilution factor, ratio of the final volume (L) of the sample to the initial mass (g) of sample used
H_{std} = peak height of Amitrole standard analyzed immediately prior to sample