

B. Analytical Method for Ethion and its Oxygen Analogs
in Soil

1. Analytical Procedure:

- a. Weigh about 50.0 grams (\pm 0.1 gram) of soil/sand into a 250 ml polypropylene centrifuge container.
- b. Reference standards are added to check samples (for method recovery determination) using a Hamilton syringe. After samples have been fortified, allow the solvent to evaporate.
- c. Add 100 ml of 9:1 acetone:water (v:v) to each container and seal the container with a screw cap top fitted with an O-ring to prevent leakage.
- d. Place the samples, horizontally, on a reciprocal laboratory shaker and shake for 30 minutes at high speed.
- e. After shaking is completed, centrifuge the samples for 3 minutes at 2000 rpm.
- f. A 10.0 ml aliquot of the solvent extract from each sample is taken and transferred to a 25mm X 150mm screw cap test tube.
- g. The samples are then placed on the robotics system for a liquid/liquid partition (can be done manually).
 1. Each 10 ml sample (all samples are done one at a time) is evaporated under nitrogen to approximately 1 ml.
 2. Add 9.0 ml of aqueous 5% NaCl and 10.0 ml of 3:1 hexane:ethyl acetate (v:v) to the sample.
 3. Vortex to provide a good whirl for 13 seconds followed by a 2-second pause. This cycle is repeated four times.
 4. A 7.0 ml aliquot is taken from the organic layer and transferred to a clean 25mm X 150mm test tube.

5. An additional 7.0 ml of the 3:1 hexane:ethyl acetate solvent is added to the tube containing the salt solution. Repeat steps 3, 4, and 5 twice for a total of three partitions.

6. After partitioning is completed, the 21.0 mls of 3:1 hexane:ethyl acetate is evaporated under nitrogen to approximately 1 ml. (Robotics phase is complete.)

h. The 1 ml sample from the liquid:liquid partition is transferred to a 15 ml graduated centrifuge tube. Rinse the 25mm X 150mm tube two times with approximately 1 ml of ethyl acetate and place in centrifuge tube. Adjust the volume to 10.0 ml with ethyl acetate.

i. The sample is concentrated to approximately 1.0 - 0.5 ml and final volume is adjusted to 1.0 ml using ethyl acetate.

j. Samples are analyzed and quantified using a GC equipped with an N-P detector.

2. Gas Chromatographic System:

Hewlett-Packard (HP) 5890 GC
HP 7673A Autosampler
HP 3396 Integrator

3. GC-NPD Operating Conditions:

Gas Flow Rates: He, carrier, -7.75 ml/min
H₂, -4 ml/min
He, make-up, -30 ml/min
Air, -100 ml/min

Inlet: Splitless injection fitted with a Gooseneck Splitless Sleeve (4mm I.D.); 225°C

Column: 15 m x 0.53 mm, 1.5 µm film thickness, DB-5 capillary column (J & W Scientific).

Oven Temp: 210°C Isothermal

Detector: HP Nitrogen/Phosphorus

Detector Temp: 280°C

Injection Volume: 2 μ l
Run Time: 10 min
Chart Speed: 0.5 cm/min

4. Quantitation

A Hewlett-Packard 3396A Integrator was used to quantitate both ethion and metabolites of ethion by peak area integration and external standard calibration. Prior to analysis, the HP 5890A was calibrated with the proper standards and showed a linear response between ng injected and area counts. An external standard calibration method was entered at the start of analysis of a series of samples. The method was verified by injecting a standard after every two samples. The amount of both ethion and metabolites of ethion in each sample was reported in parts per million (ppm) using appropriate dilution factors calculated from sample aliquot, amount injected, and final volume of samples.

5. Zymate[®] Robotic System (FMC-1)
Capacity - 20 Samples/Run

Zymate II Robot
ZyMark Max-12 Computer System
EasyLab Controller
Printer, H-P ThinkJet[®]
Liquid/Liquid station
Evaporation station for 25 x 150 tubes
Capping/Uncapping station
Vortex station (25 x 150 mm tubes) and liquid
dispenser nozzles
Evaporation station for 15 ml centrifuge tubes
Hand A
Hand B
Power and Event Controller (PEC)
Master Lab Stations (MLS) with 3 syringes
Masterflex[®] pump

6. Reagents

Acetone, Resi-Analyzed, JT Baker
Sodium Chloride, Reagent Grade, JT Baker
Ethyl Acetate, Resi-Analyzed, JT Baker
Hexane, Burdick & Jackson
Hexanes, Resi-Analyzed, JT Baker