Determination of Ethylene Thiourea (ETU) in Water Using Gas Chromatography with a Nitrogen-Phoshorus Detector

UCT Product Numbers:
CLEAN-ELUTE™ (25,000 mg diatomatious earth, 200 mL cartridge)

EPA Method 509

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Method 509 is a gas chromatographic method for the determination of ethylene thiourea (ETU) (CAS 96-45-7) a metabolic byproduct of the ethylene bisdithiocarbonate (EBDC) fungicides in water. Toxicological studies indicate that ETU may produce goitrogenic, tumorigenic, and teratogenic effects in laboratory animals, raising the concern that residues may be found in agricultural commodities. The method uses a packed column of diatomaceous earth to capture the analyte before elution with methylene chloride. Confirmation is made using a nitrogen-phosphorous detector or a mass spectrometer.

Method Summary

A 50 mL water sample is adjusted to ionic strength and pH by the addition of ammonium chloride (NH_4Cl) and potassium fluoride (KF). The sample is poured into a UCT CLEAN-ELUTE™ column and the ETU is eluted from the column using 400 mL of methylene chloride. An excess of a free radical scavenger is added to the eluate. The methylene chloride eluant is concentrated to 5 mL after exchange into ethyl acetate. GC analysis with a nitrogen-phosphorous detector or mass spectrometer is used for quantitation.

Safety

- ETU is a suspected carcinogen. Prepare all standards in a fume hood

Sample Collection and Preservation

- Grab samples must be collected in 60 mL glass containers fitted with Teflon-lined crew caps
- Do not pre-rinse with sample before collection
- After collection shake the sample bottle for 1 minute
- ETU may degrade in water even during refrigeration. Mercuric chloride has been used as a preservative but due to its toxicity and harm to the environment is not recommended
- Store sample on ice or in refrigerator at 4°C and protected from light. Extract as soon as possible after collection

Interferences

Method interferences arise from contaminated glassware, solvents, reagents and other laboratory apparatus in which the sample may come in contact. All reagents and glassware must be shown to be free from interferences under analysis conditions.

- Glassware must be scrupulously clean
- Clean glassware by rinsing with the last solvent used followed by hot water and detergent. Rinse with reagent water, dry and heat in an oven at 400°C for one hour. Do not heat volumetric flasks

- Always use high purity reagents and solvents
- Interfering contamination may occur when a low concentration sample is analyzed after a high concentration sample. Complete rinsing of the syringe using ethyl acetate may be required

Procedure

1. Sample Preparation

a) Pipette 50 mL of the water sample into a clean bottle

b) Add 1.5 grams ammonium chloride (NH_4Cl)

c) Add 25 grams potassium fluoride (KF)

d) Seal bottle and shake until salts are completely dissolved

2. Sample Extraction

a) Add 5 mL of 1000 g/mL of dithiothreitol (DTT, Cleland's Reagent) in ethyl acetate as a free radical scavenger to a 500 mL Kuderna-Danish K-D concentrator tube

b) Support a CLEAN-ELUTE™ 200 mL cartridge using a clamp over a (K-D) tube

c) Add the entire contents of the bottle from step 1) d) above

d) Do not use vacuum but allow the cartridge to stand for 15 minutes

3. Sample Collection

a) Add 400 mL of methylene chloride in 50 mL aliquots to the CLEAN-ELUTE™ column

b) Collect the eluant in the K-D apparatus

4. Extract Concentration

The following steps must be conducted in a fume hood

a) Add two boiling chips to the K-D apparatus and attach a macro Snyder column

b) Attach a condenser to the Snyder column to collect solvent

c) Place the K-D apparatus in a 65-70°C water bath so that the K-D tube is partially submerged in the water

d) Once liquid volume had been reduced to 5 mL remove from the water bath

e) Continue to reduce the liquid volume to < 1 mL in an analytical evaporator at 35-40°C under a stream of nitrogen

f) Dilute sample to 5 mL with ethyl acetate rinsing the walls of the K-D apparatus

g) Add 50 µL of internal standard and agitate

h) Transfer to a GC vial

i) Sample is ready for analysis

5. GC Analysis Conditions

Primary Conditions:

Column: 10 m long x 0.25 mm I.D. DB-Wax bonded fused
Carrier Gas: He @ 30 cm/sec linear velocity
Makeup Gas: He @ 30 mL/min flow
Detector Gases: Air @ 100 mL/min flow; H2 @ 3 mL/min flow
Injector Temperature: 220°C
Detector Temperature: 230°C
Oven Temperature: 220°C isothermal
Sample: 2 µL splitless; nine second split delay
Detector: Nitrogen-phosphorus

Confirmation Conditions:

Column: 5 m long x 0.25 mm I.D. DB-1701 bonded fused
Carrier Gas: He @ 30 cm/sec linear velocity
Makeup Gas: He @ 30 mL/min flow
Detector Gases: Air @ 100 m:/min flow; H_2 @ 3 mL/min flow
Injector Temperature: 150°C
Detector Temperature: 270°C
Oven Temperature: 150°C isothermal
Sample: 2 µL splitless; nine second split delay
Detector: Nitrogen-phosphorus

Analyte	Primary Column RT (min)	Confirmation Column RT
ETU	3.5	4.5
THP internal standard	5.1	5.0
PTU surrogate standard	2.7	2.2

*The analyst should refer to EPA Method 509 “Determination of Ehtylene Thiourea (ETU) in Water Using Gas Chromatography with a Nitrogen-Phoshorus Detector”, Revision 1.0 Issued 1992, By DJ Munch and RL Graves, US EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268
and TM Engel and ST Champagne, Battelle, Columbus Division