

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Mancozeb (Ethylenethiourea, Etu)

MRID #: 448804-01

Matrix: Water

Analysis: LC/MS/MS

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

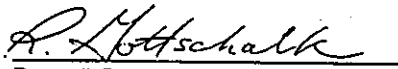
If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

METHOD MS178.00, Revision 1

ENVIRO-TEST LABORATORIES (ETL)
9936 - 67 AVENUE
EDMONTON, ALBERTA T6E 0P5

METHOD TITLE: Method for the Analysis of Ethylenethiourea (ETU) in Water by LC/MS/MS (MS178.00)


AUTHORS:




Russell Gottschalk
Development Chemist
Enviro-Test Labs



Date




Ron Tauber
Principal Investigator
Enviro-Test Labs

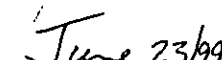


Date

APPROVAL:



Gary Bruns
Manager, Pesticide Division
Enviro-Test Labs



Date

DATE ISSUED: June 23, 1999

TOTAL NO. PAGES: 27

TABLE OF CONTENTS

TITLE PAGE	1
TABLE OF CONTENTS	2
1.0 INTRODUCTION.....	3
1.1 Scope	3
1.2 Principle.....	3
1.3 Method Limits (LOD & LOQ).....	3
1.4 Summary Flow Chart	4
2.0 MATERIALS.....	5
2.1 Reagents/Solvents.....	5
2.2 Equipment and Supplies	5
2.3 Solutions.....	6
2.4 Analytical Standards and Chemical Structures.....	6
3.0 FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS	7
3.1 Preparation	7
3.2 Stability	8
4.0 METHOD PROCEDURES.....	8
4.1 Analysis of ETU in Ground Water	8
4.2 Analysis of ETU in Ground Water	9
5.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/TRIPLE QUAD MASS SPECTROMETRY	10
5.1 PE-Sciex API-III LC/MS/MS Condition	10
5.2 Performance Criteria (LC/MS/MS)	11
6.0 CALCULATIONS.....	12
7.0 SAFETY	13
8.0 EXAMPLE LINEARITY CURVES, WORKSHEETS AND CHROMATOGRAMS (FROM DAY 2, REPEATED) USING THE C8 HPLC COLUMN AND MODIFIED MOBILE PHASE	14

1.0 INTRODUCTION

1.1 Scope

This method set forth the procedure for determining residues of Ethylenethiourea (ETU) in ground water. It is suitable for residue analysis to levels of 0.1 ppb (ng/mL). Revision 1 modified the method to allow for the addition of ammonium hydroxide to stabilize the final extract. The use of a C8 HPLC column and modified mobile phase was also incorporated into the method.

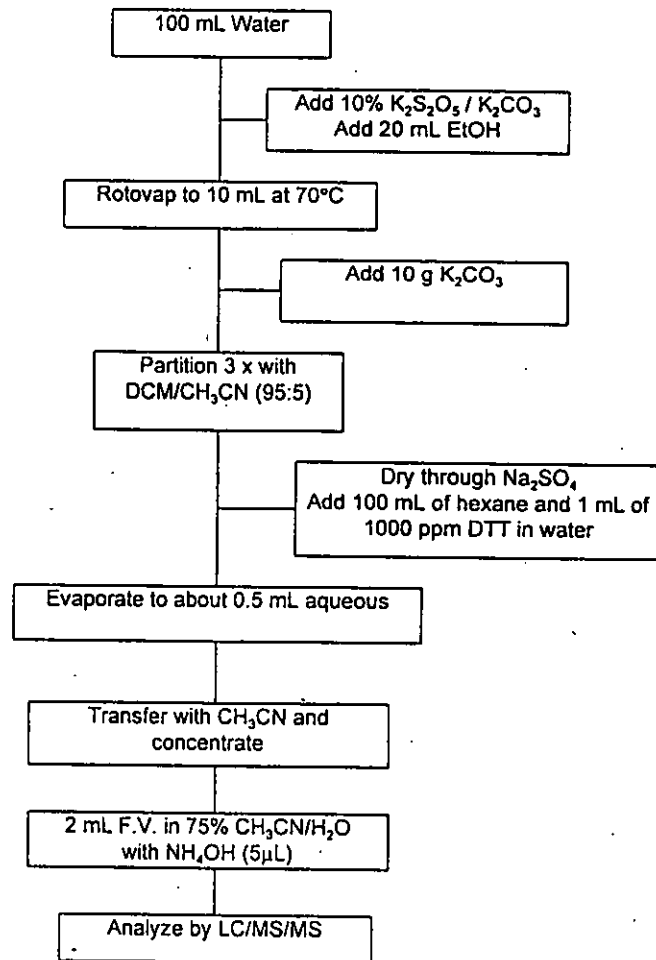
1.2 Principle

An analytical method is described for the determination of residues of ETU in water. The procedure involves the pre-concentration of the water sample (treated with potassium metabisulfite/potassium carbonate) by rotary evaporation followed by saturation with potassium carbonate and liquid/liquid partition with 5% acetonitrile in dichloromethane. The organic phase extract is concentrated, brought up into 75% acetonitrile/water and analyzed by LC/MS/MS. The addition of dithiothreitol (DTT) prevents the oxidation of the ETU during the concentration and evaporation steps and does not interfere in the LC/MS/MS chromatograms.

1.3 Method Limits (LOD & LOQ)

For a 100 mL water sample the proposed limit of detection (LOD) is 0.03 ppb and the limit of quantitation (LOQ) is 0.1 ppb. LOD and LOQ will be determined during the method validation.

1.4 Method Flowchart of Analytical Method for ETU



2.0 MATERIALS

2.1 Reagents and Solvents

(Equivalent or better grade reagents/solvents may be substituted.)

Acetic acid glacial - ACS grade, Fisher Scientific

Acetonitrile (CH_3CN) - glass distilled, EM Science, OmniSolv®

Ammonium acetate - BDH, Analar

Ammonium Hydroxide - ACS grade, BDH, 28-30% solution.

Dichloromethane (DCM) - glass distilled, EM Science, OmniSolv®

Dithiothreitol (DTT) - >99%, Sigma

Ethanol (EtOH) - glass distilled, EM Science, OmniSolv®

Hexane - glass distilled, EM Science, OmniSolv®

Sodium chloride - ACS grade, Fisher Scientific

Potassium carbonate (K_2CO_3)- ACS grade, Fisher Scientific

Potassium metabisulfite ($\text{K}_2\text{S}_2\text{O}_5$) - ACS grade, Fisher Scientific

Sodium sulfate, baked (Na_2SO_4)- ACS grade, Fisher Scientific; heat to 500°C for 2 hours in a muffle furnace

Water, deionized - Millipore MilliQ Purification System

2.2 Equipment and Supplies (Equivalent equipment may be substituted.)

Balance - Sartorius 1206 MP, VWR Scientific

Centrifuge - Sorvall, RC2-B with 250 mL rotor head, DuPont Instruments

Centrifuge-HN-S with 8 position head, International Equipment Co. (for 40 mL tubes)

Culture tubes, - 80 mL, screw-top tube, 16 x 60 mm, Kimble Glass Inc.

Cylinders, graduated - 100, 250 and 500 mL, Kimble Glass Inc.

Flasks, Erlenmeyer - 250 mL, Kimble Glass Inc.

Flasks, round bottom (RB) - 50, 250 and 500 mL, Kimble Glass Inc.

Flasks, separatory - 250 mL, Kimble Glass Inc.

Flasks, volumetric - 10, 25, 50 and 100 mL, Class A, Kimble Glass Inc.

Funnels, wide-mouth - polypropylene, VWR Scientific

Glass wool - VWR Scientific

HPLC Column - Merck Lichrospher 100 RP-8, 5 μm , 4 x 250 mm

Nitrogen evaporator with water bath - Organomation Assoc. Inc., Model No.111

Pipettes, volumetric - 1.0 and 10 mL, Kimble Glass Inc.

Rotovap with water bath - RV 06-ML IKA Rotary Evaporator, IKA Switzerland

Shaker, platform or wrist - Psychotherm

Syringe, Hamilton, 10 μL

Ultrasonicator - Fisher Scientific, FS-28

2.3 Solutions

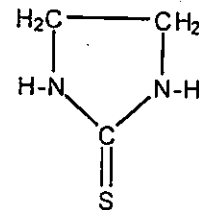
The following solutions are required:

- 2.3.1 75% acetonitrile in water (v/v): Add 750 mL of acetonitrile to 250 mL of deionized water and mix.
- 2.3.2 5% acetonitrile/dichloromethane (DCM): Add 50 mL of acetonitrile to 950 mL of DCM and mix.
- 2.3.3 10% Potassium metabisulfite and 10% potassium carbonate (w/v): Add 100g of $K_2S_2O_5$ and 100g of K_2CO_3 to about 800 mL of deionized water and mix to dissolve. Bring to 1 L with deionized water and mix.
- 2.3.4 1000 ppm Dithiothreitol (DTT) in water solution: Dissolve 0.1 g DTT in 100 mL of deionized water. Adjust to pH 8-9 with ammonium hydroxide and mix.
- 2.3.5 100 ppm DTT in 5% acetonitrile/DCM. Dissolve 0.01 g of DTT in 100 mL of 5% acetonitrile/DCM and mix.
- 2.3.6 0.001 M Ammonium acetate (NH_4OAc)/water: Add 0.308 g of NH_4OAc to 4 L of deionized water and mix. Adjust to pH 5.0 with glacial acetic acid. De-gas by placing 4 L bottle in sonic bath and apply vacuum for at least 5 minutes.
- 2.3.7 5% acetonitrile in 0.001 M NH_4OAc /water: Add 50 mL of acetonitrile to every 950 mL of 0.001 M NH_4OAc /water and mix. De-gas as above (2.3.6)

2.4 Analytical Standards and Chemical Structures:

The following analytical standard of ETU was supplied by the Rohm and Haas Company. References to derivation (batch number), characterization, and certificates of purity can be supplied by the Rohm and Haas Company.

ETU (Ethylenethiourea)
 Chemical Name:
 2-Imidazolidinethione; imidazoline-2-thiol
 Physical State: Solid
 Molecular Weight: 102
 CAS No.: 96-45-7
 Lot #: 08002DT
 Purity: 100%
 Expiry Date: February 5, 2002



Ethylenethiourea

3.0 FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

3.1 Preparation

All the standard solutions must be stored in glass at or below 4°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example procedure for preparing a standard solution. Alternate or additional standards of appropriate weight and volume may be prepared as needed. The "~" symbol indicates approximately.

- 3.1.1 Accurately weigh ~ 0.010 g (10,000 µg) of 100% ETU into a 10 mL volumetric flask and dilute to the mark with acetonitrile. Cap and mix by inversion. The concentration of this stock standard is ~1000 µg/mL.
- 3.1.2 For the preparation of the fortification or spiking standard for ETU, transfer 100 µL (0.1 mL, 100 µg ETU) of the ~1000 µg/mL standard via volumetric class "A" pipettes, to a 10 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~10 µg/mL ETU.
- 3.1.3 Calibration standards for sample analysis are prepared by serial dilution of an exact 100 µg/mL standard in 75% acetonitrile/water. Prepare the 100 µg/mL stock solution by dilution of the ~1000 µg/mL standard. Working standards are prepared by serial dilution in 75% acetonitrile/water from this 100 µg/mL stock. They should be made in the concentration range of 0.003 to 0.1 µg/mL.

3.2. Stability

3.2.1 To evaluate the stability of the standard solutions over the length of a study, the following formula will be used:

$$\% \text{ Stability} = 1 - \frac{\text{'old stock' standard solution}}{\text{'new stock' standard solution}} \times 100$$

A standard curve will be run using the new standard solutions. The old standard solutions will be injected at various concentrations within the standard curve. The old standard solutions should be within 15% deviation of the standard curve.

4.0 METHOD PROCEDURES

4.1 Analysis of ETU in Ground Water

- 4.1.1 Measure 100 ± 1 mL of ground water into a 500 mL round bottomed flask.
- 4.1.2 Add 1.0 mL of solution containing 10% $K_2S_2O_5$ and 10% K_2CO_3 . Spike samples at this point.
- 4.1.3 Add 20 ± 1 mL of EtOH.
- 4.1.4 Concentrate on a rotovap (15 in. Hg) with waterbath @ $70 \pm 5^\circ C$ to about 5 mL.
- 4.1.5 Quantitatively Transfer with rinses (2×1 mL) of deionized water to a 80 mL culture tube and bring to 10 ± 0.5 mL with deionized water.
- 4.1.6 Add 10 ± 0.2 g of K_2CO_3 and shake slowly to dissolve. Vent tubes cautiously.
- 4.1.7 Partition 3 times with 30 ± 3 mL of 5% acetonitrile/DCM. Transfer by pipette and combine the organic extracts in a 250 mL Erlenmeyer flask.

4.1 Analysis of ETU in Ground Water cont'd

- 4.1.8 Prepare drying funnels of about 20 g of baked Na_2SO_4 (section 2.1). Wash the baked Na_2SO_4 with about 50 mL of 100 $\mu\text{g}/\text{mL}$ DTT in 5% acetonitrile/DCM (discard). Pass the organic extract through the washed baked Na_2SO_4 into a 500 mL round bottomed flask. Rinse the Erlenmeyer flask at least 2 times with 2 mL of 5% acetonitrile/DCM and pass through the Na_2SO_4 . Then rinse the Na_2SO_4 with about 10 mL of the 5% acetonitrile/DCM.
- ◆4.1.9 Add about 100 mL of hexane and 1.0 mL of 1000 $\mu\text{g}/\text{mL}$ DTT in water solution to the 500 mL round bottom flask.
- 4.1.10 Remove the organic extract by concentration on a rotovap (15 in. Hg) with waterbath @ $40 \pm 5^\circ\text{C}$ until only the aqueous layer remains. (about 0.5 mL). Note: Do not let go to dryness.
- 4.1.11 Transfer the aqueous residue to a calibrated 4 mL vial with 2 rinses of 0.5 mL acetonitrile and concentrate to about 0.3-0.4 mL using a nitrogen evaporator with waterbath @ $40 \pm 5^\circ\text{C}$.
- 4.1.12 Bring to 0.5 mL with deionized water.
- ◆4.1.13 Add 1.5 mL of acetonitrile to give a 2.0 mL FV. If particulates are present, filter the final extract using a 0.45 μm nylon syringe filter.
- 4.1.14 Add 5 μL of conc. NH_4OH (28-30%) using a glass 10 μL syringe.
- ◆4.1.15. Analyze by LC/MS/MS with heated nebulizer ionization.

4.2 General Notes

- 4.2.1 The "◆" symbol indicates an optional stopping point after completing the indicated step. Samples may be stored overnight in a refrigerator (at or below 5°C). Final extracts should be stored in a freezer at $-20 \pm 5^\circ\text{C}$.
- 4.2.2 EtOH is added to the water to lower the boiling point and increase the speed of the water concentration by rotary evaporation.

4.2 General Notes: cont'd

- 4.2.3 The potassium metabisulfite ($K_2S_2O_5$) and DTT solution (1000 $\mu\text{g/mL}$) are added to prevent oxidation of ETU in solution. Municipal tap waters or "dirty" ground waters containing significant levels of dissolved oxygen may require a greater amount of DTT (step 4.1.9) to prevent destruction of ETU. (Amount to be determined during validation.)
- 4.2.4 Pre-wash the anhydrous sodium sulfate with 100 $\mu\text{g/mL}$ DTT in 5% acetonitrile/DCM to prevent oxidation of the ETU by reactive residues present in the Na_2SO_4 .
- 4.2.5 The hexane is added to cause the aqueous layer to sink to the bottom of the RB flask. If a fine emulsion develops after evaporation of the hexane/DCM, add an additional 10 mL of hexane and repeat the evaporation.
- 4.2.6 The final extract should be in 75% acetonitrile/water. If dilutions are required, dilute the extract with 75% acetonitrile/water.

5.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS/MS)**5.1 Instrumental Analysis by LC/MS/MS**

Instrument: PE SCIEX API III
HPLC: Varian 9012, Solvent Delivery System
Autoinjector: Rainin AI-200
Data System: MacIntosh IICI with OS 7.5
Operating System: API (Version 2.7)

HPLC column: Merck Lichrospher 100 RP-8 (4 x 250 mm)
Flow: 0.6 mL/min
Eluent: 5% Acetonitrile in 0.001M Ammonium Acetate, pH 5
Injection Volume: 25 μL

Samples and standards were analysed using the PE Sciex API III heated nebulizer interface.

Heated nebulizer ionization parameters:

Nebulizer Temperature: 500°C

Auxiliary flow: 5.0 (L/min)

Nebulizer Gas: Nitrogen @ 60 psi

5.1 Instrumental Analysis by LC/MS/MS cont'd

The instrument was operated in the MRM mode.

MRM parameters:

Curtain Gas: Nitrogen @ 1.2 L/min

ISV Voltage: 6000 V

Orifice: 55 V

Interface Setpoint: 65°C

Collision Gas: Argon at ~275 cgt

MRM MASS TRANSITION		
Compound	m/z Parent	m/z Daughter
Ethylenethiourea	103.1	44.0

ETU retention time is about 5.5 min.

Example chromatograms are attached. (See Section 8.0). Note that the retention times will vary from system to system and may require gradient or flow optimization.

5.2 Performance Criteria (LC/MS/MS)

First Criterion:

Run a standard solution on LC/MS/MS corresponding to a level at or below the estimated LOQ and obtain a signal to noise ratio of at least 9:1.

If this criterion cannot be met optimize and/or change instrument operating parameters.

Second Criterion:

Run a set of ETU standards of four to five concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. Generate a constrained quadratic calibration curve. The samples are run with standards interspersed.

5.2 Performance Criteria (LC/MS/MS) cont'd

A typical set may consist of a high standard, low standard, fortified samples, control, standard, 4-5 treated (or full) samples, standard, 4-5 treated samples, standard, 4-5 treated samples, etc., ending with 2 standards at 2 levels. There are typically 4-5 levels of standards used throughout the run to generate the linearity curve.

6.0 CALCULATIONS

A constrained quadratic regression (zero intercept) should be used to generate calibration curves for ETU. After the instrument performance criteria are met, a minimum of four standards over a range of concentration levels should be included with a set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Samples should be diluted so that any ETU peak areas or peak heights are within the area or height range between the lowest and highest standard injected.

Quadratic regression coefficients should be calculated from peak area or peak height versus standard concentration (ng/mL). The data from the analytical standards should then be fit to a suitable linear model.

$$y = Ax^2 + Bx + C$$

Where:

- y = peak area or peak height
- x = standard concentration (ng/mL)
- A, B, C = variables dependent on data points entered
- C = zero for a constrained curve.

The equation to be used to estimate the residues in the samples is:

$$\text{Conc. (ppb)} = \frac{-B + \text{SQRT}(B^2 - 4 \times A \times (C - \text{Peak Area}))}{2A} \times \frac{1}{\text{S.V. (mL)} \times \text{F.V. (mL)}}$$

Where:

- F.V. = Final sample volume (mL)
- S.V. = Starting volume of sample (mL)

95

Protocol No: ETL99RH01.PRO
ETL Report No.: 99RHC42.REP

13

Analytical Method
MS 178.00, Revision 1

7.0 SAFETY

All available appropriate MSDS's should be available to the study personnel during the conduct of the study. General laboratory safety precautions should be taken. This method does not present any specific risks.

96

Protocol No: ETL99RH01.PRO
ETL Report No.: 99RHC42.REP

14

Analytical Method
MS 178.00, Revision 1

**8.0 EXAMPLE LINEARITY CURVES, WORKSHEETS AND
CHROMATOGRAMS (FROM DAY 2, REPEATED) USING THE C8 HPLC
COLUMN AND MODIFIED MOBILE PHASE (REVISION 1)**

PE SCIEX API III BIOMOLECULAR MASS ANALYZER
LC/MS/MS CONDITIONS

DATE: June 18/99
OPERATOR: Russell G

COLUMN CONDITIONS:

Guard Column: _____
Column I.D.: LiChrospher 100 RP-8 Serial No.: 50982
Length (cm): 25 I.D.(mm): 4.6
Particle Size (µm): 5 Temperature (°C): Ambient

LC CONDITIONS: AG-0730 = 5% ACN in 0.001M Am Ac pH 5.0

Eluent A: AG-0730 Eluent B: _____
Eluent C: _____ Eluent D: _____
Post Column Eluent: _____ at a flow rate (mL/min): _____

GRADIENT:

TIME (min)	FLOW RATE (mL/min)	COMPOSITION (%)
0	0.600	100% A
8.0	0.600	100% A

Injection Volume (µL): 25

API INTERFACE CONDITIONS:

ION SPRAY/TURBO ION SPRAY/HEATED NEBULIZER ION SPRAY TIP POSITION
Temperature (°C): 500 Horizontal: _____
Auxiliary Flow (L/min): 5 (N₂) Vertical: _____
Nebulizer Flow (psi): 60 (N₂) Away from Orifice: _____
Curtain Gas Flow (L/min): 1.2 (N₂) Angular: _____
Split: _____

MS/MS CONDITIONS:

RAD Method: ETU method
Acquisition Mode: MRM Dwell Time (ms): 999
Pause Time (ms): 0.05 Single Scan Duration (s⁻¹): 0.50
Acquisition Delay (min): 2.00

MASS:

Mass Transitions (m/z)	State File Name	Period	Ion Mode
103.1 → 44.0	ETU State	2.0 → 8.0 min	+ve ion
/	/	/	/
/	/	/	/
/	/	/	/
/	/	/	/

STATE FILE: *attached sheets trial 1 ETU state: ETUMSMST*

DI	5	M3	1000
ISV	1000	RE3	128.5
IN	650	DM3	0.100
OR	55	RX	5
RO	50	R3	10
M1	1000	L9	0
RE1	120.5	FP	-200
DM1	0.150	MU	-3500
R1	26	CC	1
L7	20	CG	12
R2	15	CGT	254

Q1 Calibration File: 990514

Q3 Calibration File: 990514

Comments:

- no guess column

RESIDUE ANALYSIS WORKSHEET									
Title: Validation of ETU In Groundwater By LC/MS/MS Protocol No.: ETL99RH01.PRO Matrix: Water Analytical Method: Method Summary: MS 178.00 Analysis Date: 06/19/1999 Analyst: RG Set #: Trial 6 EE RG 06/21/99									
					Equation of the Curve: $Y = AX^2 + BX + C$ where: Y = Peak Area X = Concentration				
					ETU Regression Parameters: A= 0.190 B= 1260 C= 0				
ETL Standard/ Sample I.D.	Client Sample I.D.	LC/MS/MS File Name	Sample Volume (mL)	Final Volume (mL)	Peak Area	Fort. Level (ppb)	Calculated Conc. (ppb)	Recovery (%)	
0.00	ETU standard	RG0618A001	-	-	0	0	-	-	
2.50	ETU standard	RG0618A002	-	-	2299	5	-	-	
5.00	ETU standard	RG0618A003	-	-	5611	10	-	-	
E9-04-864-19AB	Well#1-Control - Day 2(rep)	RG0618A004	100	2.0	298	-	0.00473	-	
E9-04-864-20AB	Well#1-0.1 ppb - Day 2(rep)	RG0618A005	100	2.0	4043	0.100	0.0594	59.4	
E9-04-864-21AB	Well#1-0.5 ppb - Day 2(rep)	RG0618A006	100	2.0	26493	0.500	0.414	82.8	
7.50	ETU standard	RG0618A007	-	-	9110	-	-	-	
10.0	ETU standard	RG0618A008	-	-	12851	-	-	-	
E9-04-864-22AB	Well#2-Control - Day 2(rep)	RG0618A009	100	2.0	299	-	0.00475	-	
E9-04-864-23AB	Well#2-0.1 ppb - Day 2(rep)	RG0618A010	100	2.0	6391	0.100	0.0966	96.6	
E9-04-864-24AB	Well#2-0.5 ppb - Day 2(rep)	RG0618A011	100	2.0	32104	0.500	0.503	101	
20.0	ETU standard	RG0618A012	-	-	25156	-	-	-	
35.0	ETU standard	RG0618A013	-	-	45000	-	-	-	
50.0	ETU standard	RG0618A014	-	-	63180	-	-	-	
Comments: Standard Curve Type: Quadratic/Constrained Residues(ppb) from controls were subtracted from the fortifications. Calculated Conc. = $(-B + \sqrt{B^2 - 4 * A * (C - \text{Peak Area})}) / (2A * \text{Sample Volume} * \text{Final Volume})$ Data Entered By: RG Date: 06/21/99 GC:									

RESIDUE ANALYSIS WORKSHEET

Title: Validation of ETU
In Groundwater By LC/MS/MS
Protocol No.: ETL99RH01.PRO
Matrix: Water
Analytical Method:
Method Summary: MS 178.00
Analysis Date: 05/19/1999
Analyst: RG
Set #: Trial 6
EE. R2-06/21/99

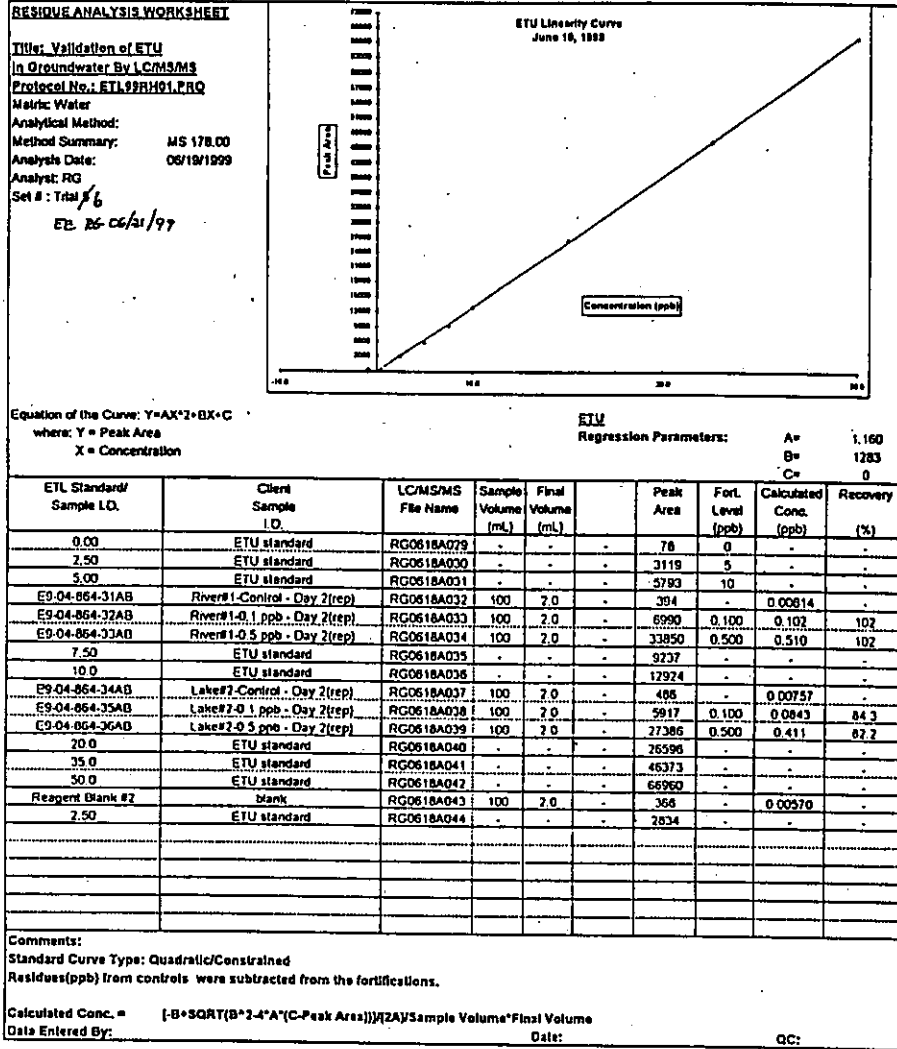
Equation of the Curve: $Y = AX^2 + BX + C$
where: Y = Peak Area
X = Concentration

ETU Regression Parameters:
A= 2.120
B= 1225
C= 0

ETL Standard/ Sample I.D.	Client Sample I.D.	LC/MS/MS File Name	Sample Volume (mL)	Final Volume (mL)	Peak Area	Fort. Level (ppb)	Calculated Conc. (ppb)	Recovery (%)
0.00	ETU standard	RG0618A015	-	-	45	0	-	-
2.50	ETU standard	RG0618A016	-	-	2442	5	-	-
5.00	ETU standard	RG0618A017	-	-	5034	10	-	-
E9-04-864-25AB	Chlorinated#1-Control - Day 2(rep)	RG0618A018	100	2.0	452	-	0.00737	-
E9-04-864-26AB	Chlorinated#1-0.1 ppb - Day 2(rep)	RG0618A019	100	2.0	5518	0.100	0.0820	87.0
E9-04-864-27AB	Chlorinated#1-0.5 ppb - Day 2(rep)	RG0618A020	100	2.0	35084	0.500	0.540	106
7.50	ETU standard	RG0618A021	-	-	7989	-	-	-
10.0	ETU standard	RG0618A022	-	-	11799	-	-	-
E9-04-864-28AB	Chlorinated#2-Control - Day 2(rep)	RG0618A023	100	2.0	340	-	0.00555	-
E9-04-864-29AB	Chlorinated#2-0.1 ppb - Day 2(rep)	RG0618A024	100	2.0	5500	0.100	0.0836	83.8
E9-04-864-30AB	Chlorinated#2-0.5 ppb - Day 2(rep)	RG0618A025	100	2.0	31560	0.500	0.489	97.8
20.0	ETU standard	RG0618A026	-	-	25081	-	-	-
35.0	ETU standard	RG0618A027	-	-	45773	-	-	-
50.0	ETU standard	RG0618A028	-	-	65074	-	-	-

Comments:
Standard Curve Type: Quadratic/Constrained
Residues(ppb) from controls were subtracted from the fortifications.

Calculated Conc. = $\frac{[-B + \sqrt{B^2 - 4 \cdot A \cdot (C - \text{Peak Area})}]}{2 \cdot A} \cdot \text{Sample Volume} \cdot \text{Final Volume}$
Data Entered By: RG Date: 06/21/99 QC:

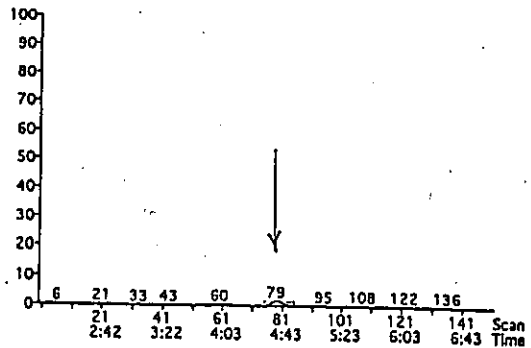


MacQuan, version 1.6
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acad Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A009 E904864-22AB Fri, Jun 18, 1999 23:48
WELL #2 CONTROL DAY2 ETL99RH01.PRO

7:01 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

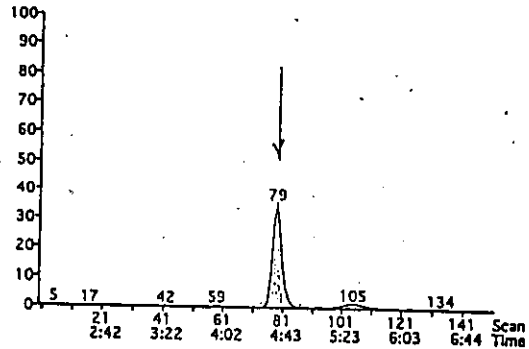
1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 299
Height 42
Start Time 4:33
End Time 4:47
Integration Width 0:14.0
Retention Time 4:39
Integration Type A - BB



RG0618A010 E904864-23AB Fri, Jun 18, 1999 23:56
WELL #2 0.1 PPB DAY2 ETL99RH01.PRO

7:02 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:50 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 6391
Height 890
Start Time 4:31
End Time 4:51
Integration Width 0:20.1
Retention Time 4:39
Integration Type A - BB

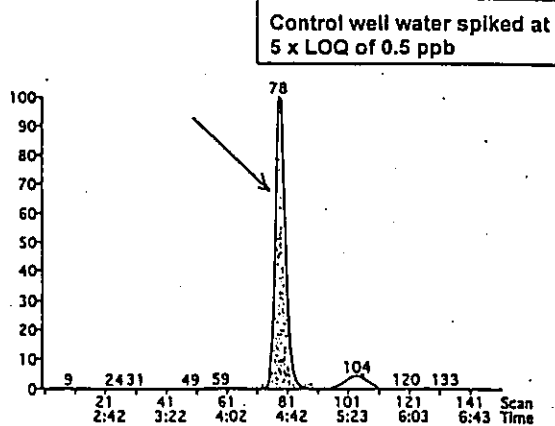


MacQuan, version 1.6
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A011 E904864-24AB Sat, Jun 19, 1999 00:05
WELL #2 0.5 PPB DAYZ ETL99RH01.PRO

7:01 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

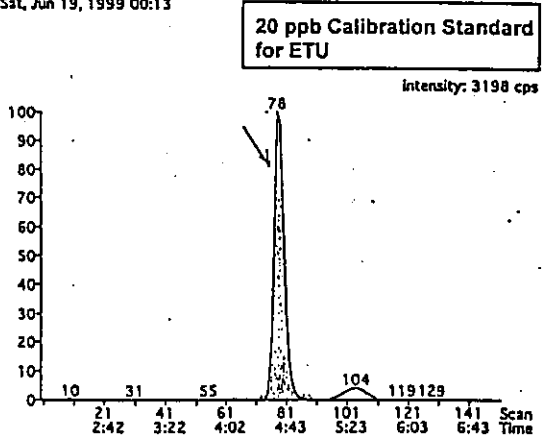
1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Wln. (secs) 10
Smooth 3
Expected RT 4:38
Area 32104
Height 4040
Start Time 4:28
End Time 4:55
Integration Width 0:26.3
Retention Time 4:36
Integration Type A - BB



RG0618A012 ETU STD 20 PPB Sat, Jun 19, 1999 00:13
. STD A382 06/07/99 75% ACN

7:01 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Wln. (secs) 10
Smooth 3
Expected RT 4:38
Area 25156
Height 3197
Start Time 4:28
End Time 4:55
Integration Width 0:26.1
Retention Time 4:36
Integration Type A - BB



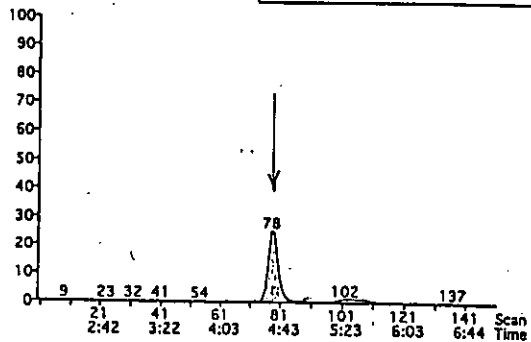
MacQuan, version 1.6
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU In WATER Method Validation ETL99RH01.PRO

RG0618A017 ETU STD 5.0 PPB Sat, Jun 19, 1999 00:55
STD A382 06/07/99 75% ACN

7:02 In 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:60 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 5034
Height 627
Start Time 4:29
End Time 4:59
Integration Width 0:30.2
Retention Time 4:37
Integration Type A - BB

5.0 ppb Calibration
Standard for ETU

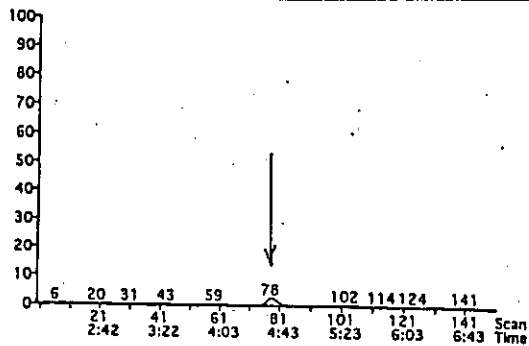


RG0618A018 E904864-25AB Sat, Jun 19, 1999 01:04
CHLORINATED #1 CONTROL DAY2 ETL99RH01.PRO

7:02 In 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 452
Height 59
Start Time 4:31
End Time 4:45
Integration Width 0:14.1
Retention Time 4:37
Integration Type A - BB

Control chlorinated tap
water

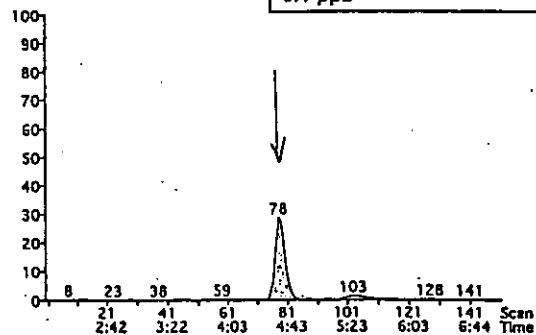


MacQuan, version 1.6.
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A019 E904864-26AB Sat, Jun 19, 1999 01:12
CHLORINATED #1 0.1 PPB DAY2 ETL99RH01.PRO

7:02 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

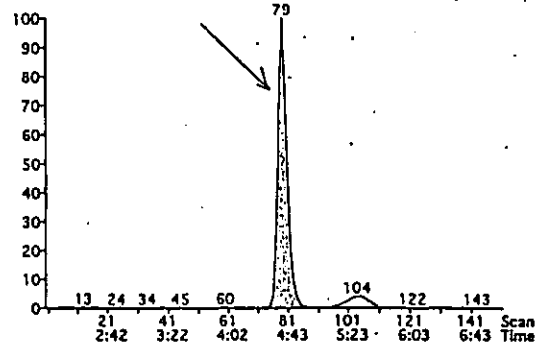
1: 4:60 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Wln. (secs) 10
Smooth 3
Expected RT 4:38
Area 5518
Height 717
Start Time 4:29
End Time 4:49
Integration Width 0:20.2
Retention Time 4:37
Integration Type A-BB



RG0618A020 E904864-27AB Sat, Jun 19, 1999 01:21
CHLORINATED #1 0.5 PPB DAY2 ETL99RH01.PRO

7:01 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Wln. (secs) 10
Smooth 3
Expected RT 4:38
Area 35084
Height 4868
Start Time 4:30
End Time 4:57
Integration Width 0:26.1
Retention Time 4:39
Integration Type A-BB



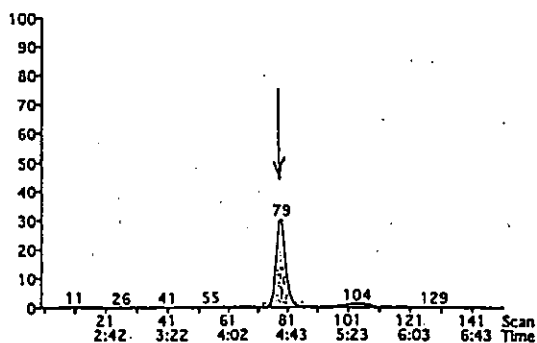
MacQuan, version 1.6
 Printed: Sat, Jun 19, 1999 08:36
 Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
 Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A031 ETU STD 5.0 PPB Sat, Jun 19, 1999 02:54
 . STD A382 06/07/99 75% ACN

7:01 in 1 period
 ETHYLENE THIOUREA
 No Internal Standard
 Use Area

1: 4:59 MRM, 150 scans
 103.1->44.0
 Noise Thres. 1.0
 Quant Thres. 1.0
 Min. Width 5
 Mult. Width 5
 Base. Width 15
 RT Win. (secs) 10
 Smooth 3
 Expected RT 4:38
 Area 5793
 Height 752
 Start Time 4:29
 End Time 4:49
 Integration Width 0:20.1
 Retention Time 4:39
 Integration Type A - BB

5.0 ppb Calibration
 Standard for ETU

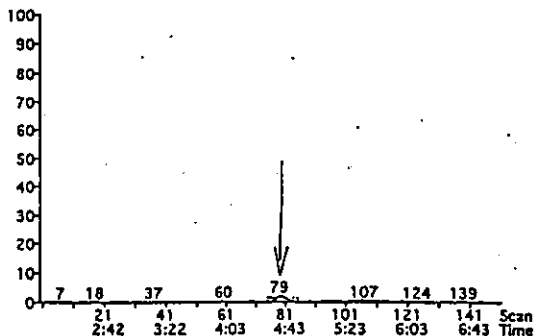


RG0618A032 E904864-31A8 Sat, Jun 19, 1999 03:02
 RIVER #1 CONTROL DAY2 ETL99RH01.PRO

7:01 in 1 period
 ETHYLENE THIOUREA
 No Internal Standard
 Use Area

1: 4:59 MRM, 150 scans
 103.1->44.0
 Noise Thres. 1.0
 Quant Thres. 1.0
 Min. Width 5
 Mult. Width 5
 Base. Width 15
 RT Win. (secs) 10
 Smooth 3
 Expected RT 4:38
 Area 394
 Height 51
 Start Time 4:33
 End Time 4:47
 Integration Width 0:14.0
 Retention Time 4:39
 Integration Type A - BB

Control river water

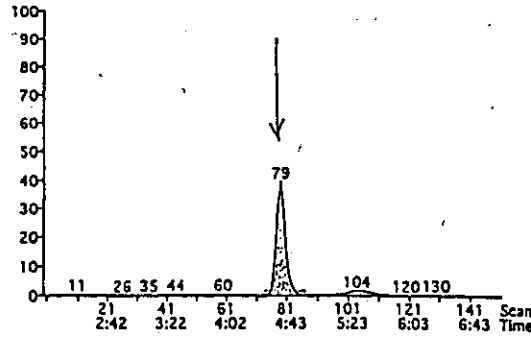


MacQuan, version 1.6
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A033 E904864-32AB Sat, Jun 19, 1999 03:11
RIVER #1 0.1 PPB DAY2 ETL99RH01.PRO

7:01 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

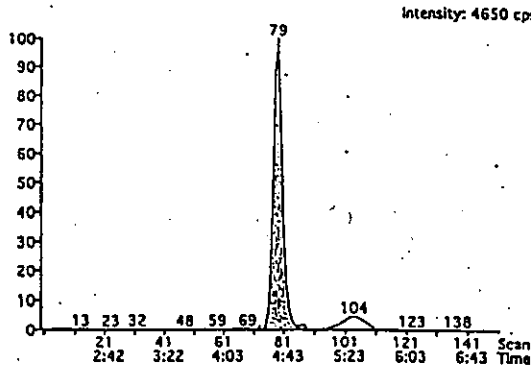
1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 6990
Height 985
Start Time 4:30
End Time 4:51
Integration Width 0:20.1
Retention Time 4:38
Integration Type A - BB



RG0618A034 E904864-33AB Sat, Jun 19, 1999 03:19
RIVER #1 0.5 PPB DAY2 ETL99RH01.PRO

7:02 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 33850
Height 4647
Start Time 4:29
End Time 4:53
Integration Width 0:24.1
Retention Time 4:39
Integration Type A - BB



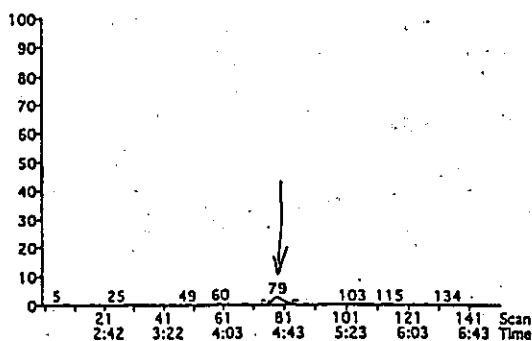
MacQuan, version 1.6
 Printed: Sat, Jun 19, 1999 08:36
 Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
 Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A037 E904864-34AB Sat, Jun 19, 1999 03:45
 LAKE #1 CONTROL DAY2 ETL99RH01.PRO

7:02 In 1 period
 ETHYLENE THIOUREA
 No Internal Standard
 Use Area

1: 4:59 MRM, 150 scans
 103.1->44.0
 Noise Thres. 1.0
 Quant Thres. 1.0
 Min. Width 5
 Mult. Width 5
 Base. Width 15
 RT Win. (secs) 10
 Smooth 3
 Expected RT 4:38
 Area 486
 Height 62
 Start Time 4:31
 End Time 4:49
 Integration Width 0:18.1
 Retention Time 4:39
 Integration Type A-BB

Control lake water

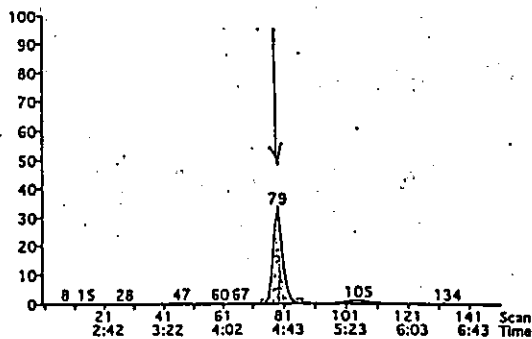


RG0618A038 E904864-35AB Sat, Jun 19, 1999 03:53
 LAKE #1 0.1 PPB DAY2 ETL99RH01.PRO

7:01 In 1 period
 ETHYLENE THIOUREA
 No Internal Standard
 Use Area

1: 4:59 MRM, 150 scans
 103.1->44.0
 Noise Thres. 1.0
 Quant Thres. 1.0
 Min. Width 5
 Mult. Width 5
 Base. Width 15
 RT Win. (secs) 10
 Smooth 3
 Expected RT 4:38
 Area 5917
 Height 837
 Start Time 4:31
 End Time 4:51
 Integration Width 0:20.1
 Retention Time 4:39
 Integration Type A-BB

Control lake water spiked at
 LOQ of 0.1 ppb



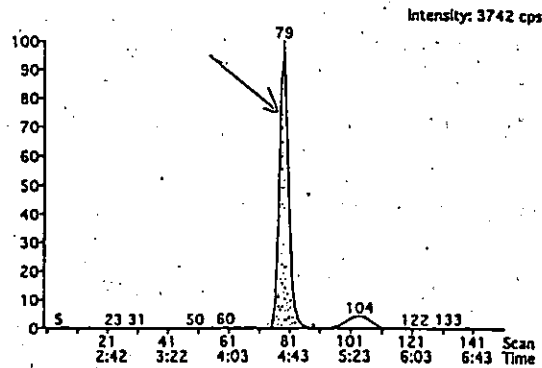
MacQuan, version 1.6
Printed: Sat, Jun 19, 1999 08:36
Calibration File: 0618Acal Path: 2 G backup:DATA:ETU LC/MS/MS:WATER:0618A99:
Comments: ETU in WATER Method Validation ETL99RH01.PRO

RG0618A039 E904864-36AB Sat, Jun 19, 1999 04:01
LAKE #1 0.5 PPB DAY2 ETL99RH01.PRO

7:02 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 27386
Height 3741
Start Time 4:31
End Time 4:55
Integration Width 0:24.1
Retention Time 4:39
Integration Type A - BB

Control lake water spiked at
5 x LOQ of 0.5 ppb



RG0618A040 ETU STD 20 PPB Sat, Jun 19, 1999 04:10
STD A382 06/07/99 75% ACN

7:02 in 1 period
ETHYLENE THIOUREA
No Internal Standard
Use Area

1: 4:59 MRM, 150 scans
103.1->44.0
Noise Thres. 1.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 5
Base. Width 15
RT Win. (secs) 10
Smooth 3
Expected RT 4:38
Area 26596
Height 3735
Start Time 4:30
End Time 4:53
Integration Width 0:22.1
Retention Time 4:38
Integration Type A - BB

20 ppb Calibration Standard
for ETU

