METHOD 509. DETERMINATION OF ETHYLENE THIOUREA (ETU) IN WATER USING GAS CHROMATOGRAPHY WITH A NITROGEN-PHOSPHORUS DETECTOR

Revision 1.1

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METHOD 509

DETERMINATION OF ETHYLENE THIOUREA (ETU) IN WATER USING GAS CHROMATOGRAPHY WITH A NITROGEN–PHOSPHORUS DETECTOR

1. SCOPE AND APPLICATION

1.1 This method utilizes gas chromatography (GC) to determine ethylene thiourea (ETU, Chemical Abstracts Registry No. 96-45-7) in water.

1.2 This method has been validated in a single laboratory during development. The method detection limit (MDL) has been determined in reagent water (1) and is listed in Table 2. Method detection limits may vary among laboratories, depending upon the analytical instrumentation used and the experience of the analyst. In addition to the work done during the development of this method and its use in the National Pesticide Survey: an interlaboratory method validation study of this method has been conducted.

1.3 This method is restricted to use by or under the supervision of analysts experienced in the use of GC and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

1.4 When a tentative identification of ETU is made using the recommended primary GC column (Sect. 6.7.1), it must be confirmed by at least one additional qualitative technique. This technique may be the use of the confirmation GC column (Sect. 6.7.2) with the nitrogen-phosphorus detector or analysis using a gas chromatograph/mass spectrometer (GC/MS).

2. SUMMARY OF METHOD

2.1 The ionic strength and pH of a measured 50-mL aliqupt of sample are adjusted by addition of ammonium chloride and potassium fluoride. The sample is poured onto a column of kieselguhr diatomaceous earth. ETU is eluted from the column with 400 mL of methylene chloride. A free radical scavenger is then added in excess to the eluate. The methylene chloride eluant is concentrated to a volume of 5 mL after solvent exchange with ethyl acetate. Gas chromatographic conditions are described which permit the separation and measurement of ETU with a nitrogen-phosphorus detector (NPD).

3. DEFINITIONS

3.1 ARTIFICIAL GROUND WATER -- An aqueous matrix designed to mimic a real ground water sample. The artificial ground water should be reproducible for use by others.

3.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.4 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.5 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.6 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.7 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.10 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amounts(s) before extraction or other processing and is measured with the same procedures used to measure other

sample components. The purpose of the SA is to monitor method performance with each sample.

4. INTERFERENCES

4.1 Method interferences from contaminants in solvents, reagents, glassware and other sample processing apparatus may cause discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Sect; 9.2.

4.1.1 Glassware must be scrupulously cleaned (2). Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hr. Do not heat volumetric ware. Thermally stable materials might not be eliminated by this treatment. Thorough rinsing with acetone and methylene chloride may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvent; helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interfering contamination may occurwhen a sample containing a low concentration of ETU is analyzed immediately following a sample containing a relatively high concentration of ETU. Thorough between-sample rin'sing of the sample syringe and associated equipment with ethyl acetate can minimize sample cross contamination. After analysis of a sample containing high concentrations of ETU, one or more injections of ethyl acetate should be made to ensure that accurate values are obtained for the next sample.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences may vary considerably from source to source, depending upon the sample. Tentative identifications must be confirmed using the confirmation column (Sect. 6.7.2) and the conditions in Table 1.

4.4 Studies have shown that persistent ETU decomposition is circumstantially linked to free radical mechanism. Addition of a free radical scavenger is necessary to prohibit any free radical reactions.

4.5 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is

not the case, chromatographic comparability of standards to sample may be affected.

5. SAFETY

5.1 ETU is a suspected carcinogen and teratogen. Primary standards of ETU should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of ETU. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (3-5) for the information of the analyst.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 SAMPLING CONTAINERS -- 60-mL screw cap vials equipped with, Teflon-faced silicone septa. Prior to use, wash vials and septa with detergent and'rinse with tap and distilled water. Allow the septa to air dry at room temperature, place in a 105°C oven for 1 hr, then remove and allow to cool in an area known to be free of organics. Heat vials at 400°C for 1 hr to remove organics.

6.2 GLASSWARE

6.2.1 Concentrator tube, Kuderna-Danish (K-D) - 10-mL or 25-mL, graduated. Calibration must be checked at the volumes employed in the test. Ground glass stoppers are used to prevent evaporation of extracts.

6.2.2 Evaporative flask, K-D - 500-mL Attach to concentrator tube with springs.

6.2.3 Snyder column, K-D - three-ball macro to which a condenser can be connected to collect solvent.

6.2.4 Vials - Glass, 5 to 10-mL capacity with Teflon lined screw caps.

6.3 Boiling stones - carborundum, #12 granules, heat at 400°C for 30 min prior to use. Cool and store in a desiccator.

6.4 Water bath - Heated, capable of temperature control (±2°C). The bath should be used in a hood.

6.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.6 Tube heater - Capable of holding 8 K-D concentrator tubes and heating the mid-section of the tubes to 35-40°C while applying a nitrogen stream.

6.7 GAS CHROMATOGRAPH - Analytical system complete with GC equipped with a nitrogen-phosphorus detector, split/splitless injector for capillary columns and all required accessories. A data system is recommended for measuring peak areas. An autoinjector is recommended to improve precision of analyses.

6.7.1 Primary column - DB-Wax or equivalent, 10 m x 0.25 mm I.D. bonded fused silica column, 0.25 µm film thickness. Validation data presented in this method were obtained using this column. Alternative columns may be used provided equal or better peak separation and peak shape are obtained.

6.7.2 Confirmation column - DB-1701 or equivalent, 5 m x 0.25 mm I.D. bonded fused silica column, 0.25 µm film thickness.

6.7.3 Detector - Nitrogen-phosphorus (NPD). This detector has proven effective in the analysis of ETU in fortified reagent and artificial ground waters.

7. REAGENTS AND STANDARDS

7.1 REAGENT WATER -- Reagent water is defined as water in which an interference is not observed at the retention time for ETU at the method detection limit. A Millipore Super-Q Water System or its equivalent may be used to generate reagent water. Water that has been charcoal filtered may also be suitable.

7.2 Methylene chloride, ethyl acetate -- distilled-in-glass quality or equivalent.

7.3 Nitrogen gas - high purity.

7.4 Extrelut QE Extraction column - Obtained from EM Science (Catalog No. 902050-1) or equivalent. Extrelut QE columns contain a specially modified form of large pore Kieselguhr with a granular structure.

7.5 Ammonium chloride, granular, ACS grade -- for pH and ionic strength adjustment of samples.

7.6 Potassium fluoride, anhydrous, ACS grade -- for ionic strength adjustment of sample.

7.7 Dithiothreitol (DTT) (Cleland's reagent) - for use as a free-radical scavenger (available from Aldrich Chemical Co.).

7.7.1 DTT in ethyl acetate, 1000 µg/mL - May be prepared by adding 1 g DTT to a l-L volumetric flask and diluting to volume with

ethyl acetate. Smaller amounts may be prepared if only a small number of samples are to be analyzed. Store at room temperature.

7.8 Propylene thiourea (PTU) - For use as a surrogate standard. Prepared from carbon disulfide and 1,2-diaminopropane using the procedure published by Hardtmann, et. al. (Journal of Medicinal Chemistry, 18(5), 447-453, 1975), or purchase from commercial sources.

7.9 3,4,5,6-Tetrahydro-2-pyrimidinethiol (THP) - >98% purity, for use as an internal standard (available from Aldrich Chemical Co.).

7.10 STOCK STANDARD SOLUTION (0.10 µg/µL) - The stock standard solution may be purchased as a certified solution or prepared from pure standard material using the following procedure:

7.10.1 Prepare stock standard solution by accurately weighing 0.0010 g of pure ETU. Dissolve the ETU in ethyl acetate containing 1000 µg/mL of DTT and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If ETU purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.10.2 Transfer the stock standard solution into a Teflon sealed screw cap vial. Store at room temperature and protect from light.

7.10.3 The stock standard solution should be replaced after two weeks or sooner if comparison with laboratory control standards indicates a problem.

7.11 INTERNAL STANDARD FORTIFYING SOLUTION -- Prepare an internal standard fortifying solution by accurately weighing 0.0010 g of pure THP. Dissolve the THP in ethyl acetate containing 1000 µg/mL of DTT and dilute to volume in a 10-mL volumetric flask. Transfer the solution to a Teflon sealed screw cap bottle and store at room temperature. Addition of 50 µL of the internal standard fortifying solution to 5 mL of sample extract results in a final internal standard concentration of 1.0 µg/mL.

7.12 SURROGATE STANDARD FORTIFYING SOLUTION - Prepare a surrogate standard fortifying solution by accurately weighing 0.0010 g of pure PTU. Dissolve the PTU in ethyl acetate containing 1000 µg/mL of DTT and dilute to volume in a 10-mL volumetric flask. Transfer the solution to a Teflon sealed screw cap bottle and store at room temperature. Addition of 5 µL of the surrogate standard fortifying solution to a sample prior to extraction results in a surrogate

standard concentration in the sample of 10 µg/L and, assuming quantitative recovery of PTU, a surrogate standard concentration in the final extract of 0.10 µg/mL.

7.13 INSTRUMENT PERFORMANCE CHECK SOLUTION - Prepare the instrument performance check solution by adding 10 µL of the ETU stock standard solution, 1.0 mL of the internal standard fortifying solution, and 100 µL of the surrogate standard fortifying solution to a 100-mL volumetric flask and diluting to volume with ethyl acetate containing 1000 µg/mL of DTT. Transfer the solution to a Teflon sealed screw cap bottle and store at room temperature.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION -- Grab samples must be collected in 60-mL glass containers fitted with Teflon-lined screw caps (Sect. 6.1). Conventional sampling practices (6) should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2 SAMPLE STORAGE -- The samples must be iced or refrigerated at 4°C and protected from light from the time of collection until extraction. Samples should be extracted as soon as possible after collection to avoid possible degradation of ETU. All samples must be extracted within 14 days of collection. Extracts must be stored under refrigeration and protected from light. Extracts must be analyzed within 28 days of extraction.'

8.3 SAMPLE PRESERVATION -- ETU may chemically degrade in some samples even when the sample is refrigerated. When this method was developed, mercuric chloride was used to ensure against biological degradation. No suitable preservation reagent has been found other than mercuric chloride. However, the use of mercuric chloride is not recommended due to its toxicity and potential harm to the environment. Biological degradation may occur only rarely in samples with limited biological activity such as finished drinking waters.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of the following: an initial demonstration of laboratory capability; measurement of the surrogate compound in each sample; analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples, and QC check standards. A MDL for ETU must also be determined.

9.2 LABORATORY REAGENT BLANKS -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control., This is accomplished by analyzing a laboratory reagent blank (LRB). A LRB is a 50-mL aliquot of reagent water, fortified with the internal standard and the

surrogate compound, that is analyzed according to Sect. 11 exactly as if it were a sample. Each time a set of samples is analyzed or reagents are changed, it must be demonstrated that the laboratory reagent blank is free of contamination that would prevent the determination of ETU at the MDL. All interfering contaminants must be eliminated before sample analyses are started.

9.3 Initial Demonstration of Capability.

9.3.1 Select a representative fortified concentration (about 10 times MDL or at a concentration in the middle of the calibration range established in Section 10) for ETU. Prepare a 4-7 replicate LFBs containing ETU at the selected concentration, and analyze each LFB according to procedures beginning in Sect. 11.

9.3.2 The mean recovery value for these samples must fall in the range of ± 20% of the fortified amount. The precision of these measurements, expressed as RSD, must be 20% or less. If the data meet these criteria, performance is considered acceptable. If acceptance criteria is not met, this procedure must be repeated using fresh replicate samples until satisfactory performance has been demonstrated.

9.3.3 To determine the MDL, prepare a minimum of 7 LFBs at a low concentration. The fortification concentration in Table 2 may be used as a guide, or use calibration data obtained in Section 10 to estimate a co'ncentration that will produce a peak with a 3-5 times signal to noise response. Extract and analyze each replicate according to Sections 11 and 12. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day variations are reflected in the precision of the measurements. Calculate mean recovery and standard deviation for each analyte. Use the standard deviation and the equation given in Section 13 to calculate the MDL.

9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.

9.4 The analyst is permitted to modify GC columns or GC conditions to improve the separations, identifications, or lower the cost of measurement. Each time a modification is made, the analyst is required to repeat the procedure in Sect. 9.3.

9.5 ASSESSING SURROGATE RECOVERY

9.5.1 All samples and blanks must be fortified with the surrogate compound according to Sect. 11.1 before extraction to monitor preparation and analysis of samples.

9.5.2 Surrogate recovery must be evaluated for acceptance by determining whether the measured surrogate concentration (expressed as percent recovery) falls within the required recovery limits. Performance-based recovery criteria for PTU has been generated from single-laboratory results. Measured recovery of PTU must be between 70 and 130 percent.

9.5.3 If the surrogate recovery for a sample or blank is outside of the required surrogate recovery limits specified in Sect. 9.5.2, the laboratory must take the following actions:

(1) Check calculations to make sure there are no errors.

(2) Check internal standard and surrogate standard solutions for degradation, contamination, or other obvious abnormalities.

(3) Check instrument performance.

Reinject the extract if the above steps fail to reveal the cause of the problem. The problem must be identified and corrected before continuing. Reanalyzing the sample or blank, if possible, may be the only way to solve the problem.

9.6 ASSESSING THE INTERNAL STANDARD

9.6.1 The analyst is must monitor the internal standard peak area in all samples and blanks during each analysis day. The IS response for any sample chromatogram should not deviate from the IS response of the most recent daily calibration check standard by more than 20%.

9.6.2 If >20% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract. If the reinjected aliquot produces an acceptable IS response, report results for that injection. If a deviation >30% is obtained for the reinjected extract, reanalyze the sample beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but mark them as suspect.

9.6.3 If consecutive samples fail the IS response acceptance criteria, immediately analyze a medium calibration check standard. If the check standard provides a response for the IS within 20% of the predicted value, then follow procedures

. itemized in Sect. 9.6.2 for each sample failing the IS response criteria. If the check standard provides a response which deviates more than 20% from the predicted value, then the analyst must recalibrate.

9.7 ASSESSING LABORATORY PERFORMANCE

9.7.1 The laboratory must analyze at least one laboratory fortified blank (LFB) per sample set. The ETU fortifying concentration in the LFB should be 10 times the MDL or at a concentration near the middle of the calibration range demonstrated by the laboratory. Calculate the percent recovery of the ETU. If the recovery falls outside the control limits (see Sect. 9.7.2), the system is judged out of control and the source of the problem must be identified and resolved before continuing analyses.

9.7.2 Until sufficient LFB data become available, usually a minimum of 20 to 30 results, the laboratory should assess its performance against the control limits described in Sect. 9.3.2. When sufficient laboratory performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

$$\text{Upper Control Limit} = R + 3S$$
$$\text{Lower Control Limit} = R - 3S$$

After five to ten new recovery measurements are made, control limits should be recalculated using only the most recent 20 to 30 data points. Control limits must not exceed the fixed acceptance limits in Section 9.3.2.

9.8 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

9.8.1 The laboratory must add a known concentration to a minimum of 5% of the routine samples or one sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 9.3.1). Over time, samples from all routine sample sources should be fortified.

9.8.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.,:

$$P = 100\ (X - b)\ /\ \text{fortifying concentration},$$

and compare these values recoveries listed in Table 2. The calculated value of P must fall in the range of ± 25% of the amount fortified. If P exceeds this control limit the results for that analyte in the unfortified matrix must be listed as suspect due to matrix interference.

9.9 ASSESSING INSTRUMENT PERFORMANCE -- Instrument performance should be monitored on a daily basis by analyzing the instrument performance check solution (IPC). The IPC solution contains compounds monitor instrument sensitivity and column performance. The IPC components and performance criteria are listed in Table 4. Inability to demonstrate acceptable instrument performance indicates the need for remedial action on the GC-NPD system. A chromatogram from the analysis of the IPC is shown in Figure 1. The sensitivity requirements are set according the' MDL. MDLs will vary somewhat in different laboratories according to instrument capabilities. The laboratory should adjust the amount of ETU in the IPC based on the demonstrated sensitivity of the instrumentation used.

9.10 QC Samples- It is recommended that the laboratory periodically (at least quarterly), analyze one or more standard materials from an outside source to validate performance.

9.11 ADDITIONAL QC -- It is recommended that the laboratory adopt additional quality assurance practices for use with this method., The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples.

10. CALIBRATION AND STANDARDIZATION

10.1 Establish GC operating parameters equivalent to those indicated in Table 1. Ensure that the gas chromatographic system is working properly by injecting the instrument performance check solution (Sect. 7.14) and checking for proper peak shapes, reasonable retention times, and sufficient sensitivity. The GC system is calibrated using the internal standard technique (Sect. 10.2).

10.2 INTERNAL STANDARD CALIBRATION PROCEDURE -- This approach requires the analyst to use at least one internal standard compatible in analytical behavior to the compound of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. In developing this method, THP (3,4,5,6-tetrahydro-2-pyrimidinethiol) was found to be a suitable internal standard.

10.2.1 Prepare ETU calibration stahdards at five concentration levels by adding volumes of the ETU stock standard solution to five volumetric flasks. To each flask, add a known constant amount of internal standard and dilute to volume with ethyl acetate containing 1000 µg/mL of DTT. One of the standards should be representative of an ETU concentration near, but above, the MDL. The other concentrations should

correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

10.2.2 Inject each calibration standard and tabulate the relative response for ETU to the internal standard (RR_a) using the equation:

$$RR_a = A_a/A_{is}$$

where: A_a = the peak area of ETU, and
A_{is} = the peak area of the internal standard.

Generate a calibration curve of RR_a versus ETU concentration in the sample in µg/L.

10.2.3 The working calibration curve must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hrs.), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the ETU response varies from the predicted response by more than 20%, the test should be repeated using a fresh calibration standard. Alternatively, a new ETU calibration curve should be prepared. Any sample extracts analyzed since the last acceptable calibration check should be considered suspect, and should be reanalyzed after calibration is restored.

PROCEDURE

11.1 SAMPLE EXTRACTION

11.1.1 Pipet a 50-mL aliquot of water sample into a sample bottle (Sect. 6.1) containing 1.5 g of ammonium chloride and 25 g of potassium fluoride. Seal the bottle and shake vigorously until salts are dissolved. Fortify the sample with 5 µL of the surrogate standard fortifying solution (Sect. 7.13).

11.1.2 Pour contents of the bottle onto the Extrelut (sorbent) column (Sect. 7.4). Allow the column to stand undisturbed for 15 min.

11.1.3 Add 5 mL of 1000 µg/mL DTT in ethyl acetate to a K-D concentrator tube equipped with a 500-mL flask.

11.1.4 Add 400 mL of methylene chloride in 50-75 mL portions to the Extrelut column and collect the eluant in the K-D apparatus (Sect. 11.1.3).

11.2 EXTRACT CONCENTRATION

11.2.1 Conduct the following work in a fume hood which is properly vented. Add 1 or 2 boiling stones to the K-D apparatus and attach a macro Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Attach a condenser to the Snyder column to recover the methylene chloride as it escapes the column. Place the K-D apparatus in a 65-70°C water bath so that the K-D tube is partially immersed in the hot water,, and the entire lower rounded surface of the flask is bathed with hot vapor. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

11.2.2 Reduce the liquid volume in the K-D tube to approximately 1 mL by placing the sample ih a tube heater at 35-40°C under a stream of nitrogen. The tube heater heats the solvent in the K-D tube at volume markings between 1 and 10 mL.

11.2.3 Dilute sample to 5 mL with ethyl acetate; rinse walls of K-D tube while adding ethyl acetate. Immediately fortify the sample with 50 µL of internal standard fortifying solution (Sect. 7.12). Agitate sample to disperse internal standard. Transfer sample to a GC vial and determine ETU by GC-NPD as described in Sect. 11.3.

11.3 GAS CHROMATOGRAPHY

11.3.1 Table 1 summarizes the recommended GC operating conditions. Included in Table 1 are retention times observed using this method. An example of the separations achieved using these conditions are shown in Figure 1. Other GC columns or chromatographic conditions may be used if the requirements of Sect. 9.3 are met.

11.3.2 Calibrate or verify the system calibration daily as described in Sect. 10. The standards and extracts must be in ethyl acetate.

11.3.3 Inject 2 µL of the sample extract. Record the resulting peak size in area units.

11.3.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.5 Confirmatory techniques such as chromatography with a dissimilar column, or an alternate technique such as particle

beam/HPLC/mass spectrometry (EPA Method 553) may be used for confirmation of ETU in extracts prepared by this method. A suggested confirmation column is described in Table 1.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Calculate the ETU concentration in the sample from the ETU relative response (RR_a) to the internal standard using the multi-point calibration curve described in Sect. 10.2.2. Do not use the daily calibration verification standard to quantitate ETU in samples. Do not extrapolate beyond the linear range established during calibration.

13. METHOD PERFORMANCE

13.1 In a single laboratory, ETU recovery and precision data from reagent water were determined at four concentration levels. Results were used to determine the MDL and demonstrate method range. These data are given in Table 2. The equation used to calculate the MDL are as follows:

$$MDL = S\ t_{(n-1,1-alpha = 0.99)}$$

where:

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

S = standard deviation of replicate analyses.

13.2 In a single laboratory, ETU recovery and precision data from two artificial ground waters were determined at a single concentration level of 10 µg/L. Results were used to demonstrate applicability of the method to different ground water matrices. These data are listed in Table 3.

14. POLLUTION PREVENTION

14.1 Although this method requires 400 mL methylene chloride extracting solvent per sample, no pollution of the environment will occur due to the recovery of the solvent during the extract concentration procedure. Very little solvent will escape the fume hood. No other solvents are utilized in this method except for the very small amount of ethyl acetate needed to make up calibration and fortification standards. These small amounts of solvent pose no threat to the environment.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical

Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules, and land disposal restrictions. The laboratory has the responsibility to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Sect. 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. PRIMARY AND CONFIRMATION CHROMATOGRAPHIC CONDITIONS

Analyte	Retention Time, min	
	Primary column	Confirmation column
ETU	3.5	4.5
THP (internal standard)	5.1	5.0
PTU (surrogate standard)	2.7	2.2

Primary conditions:

Column:	10 m long x 0.25 mm I.D. DB-Wax bonded fused silica column (J&W), 0.25 m film thickness
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; 9 sec split delay
Detector:	Nitrogen-phosphorus

Confirmation conditions:

Column:	5 m long x 0.25 mm I.D. DB-1701 bonded fused silica column (J&W), 0.25 m film thickness
Carrier gas:	He @ 30 cm/sec linear velocity
Makeup gas:	He @ 30 mL/min flow
Detector gases:	Air @ 100 m:/min flow; H2 @ 3 mL/min flow
Injector temperature:	150°C
Detector temperature:	270°C
Oven temperature:	150°C isothermal
Sample:	2 µL splitless; 9 sec split delay
Detector:	Nitrogen-phosphorus

TABLE 2. RESULTS FROM MDL AND METHOD RANGE STUDIES (a)

Fortified Level, µg/L	Amt in Blank, µg/L	n(d)	R(e)	S(f)	RSD(g)	MDL
5.0	0.492	7	97 (c)	0.845	17	2.7,
10	ND (b)	7	102	0.886	9	-
25	ND	7	94	1.31	6	-
100	ND	7	97	5.96	6	-

(a) Studies conducted in reagent water; average recovery of PTU surrogate from seven fortified reagent water samples was 100%'(RSD) was 8.5%.

(b) ND = not detected.

(c) Data corrected for amount detected in blank.

(d) n = number of recovery data points.

(e) R = average percent recovery.

(f) S = standard deviation.

(g) RSD = percent relative standard deviation.

TABLE 3. RESULTS FROM MATRIX EVALUATION STUDIES (a)

Matrix	Amt. in Blank, µg/L	n(e)	R(f)	S(g)	RSD(h)
Hard (b)	ND (d)	7	93	0.372	4
Organic-contaminated (c)	ND	7	93	0.253	3

(a) Samples were fortified at 10 µg/L level with ETU.

(b) Absopure Natural Artesian Spring water obtained from the Absopure Water Company in Plymouth, Michigan.

(c) Reagent water fortified with fulvic acid at the 1 mg/L concentration level. A well-characterized fulvic acid, available from the International Humic Substances Society (associated with the United States Geological Survey in Denver, Colorado), was used.

(d) ND = not detected.

(e) n = number of recovery data points.

(f) R = average percent recovery.

(g) S = standard deviation.

(k) RSD = percent relative standard deviation.

TABLE 4. LABORATORY PERFORMANCE CHECK

Test	Analyte	Conc. µg/mL	Requirements(a)
Sensitivity	Ethylene thiourea (ETU)	0.01	Detection of analyte; S/N > 3
Chromatographic performance	3,4,5,6-Tetrahydro-2-pyrimidinethiol (THP)	1	PSF between 0.95 and 1.05 (a) PGF between 0.93 and 1.07 (b)

(a) PSF = peak symmetry factor. Calculated using the equation.

$$PSF = \frac{w(\frac{1}{2})}{0.5 \times W(\frac{1}{2})}$$

where $w(\frac{1}{2})$ is the width of the front of the peak at half height in secs. and $W(\frac{1}{2})$ is the peak width in secs. at half height.

(b) PGF = peak Gaussian factor. Calculated using the equation

$$PGF = \frac{1.83 \times w(\frac{1}{2})}{W(1/10)}$$

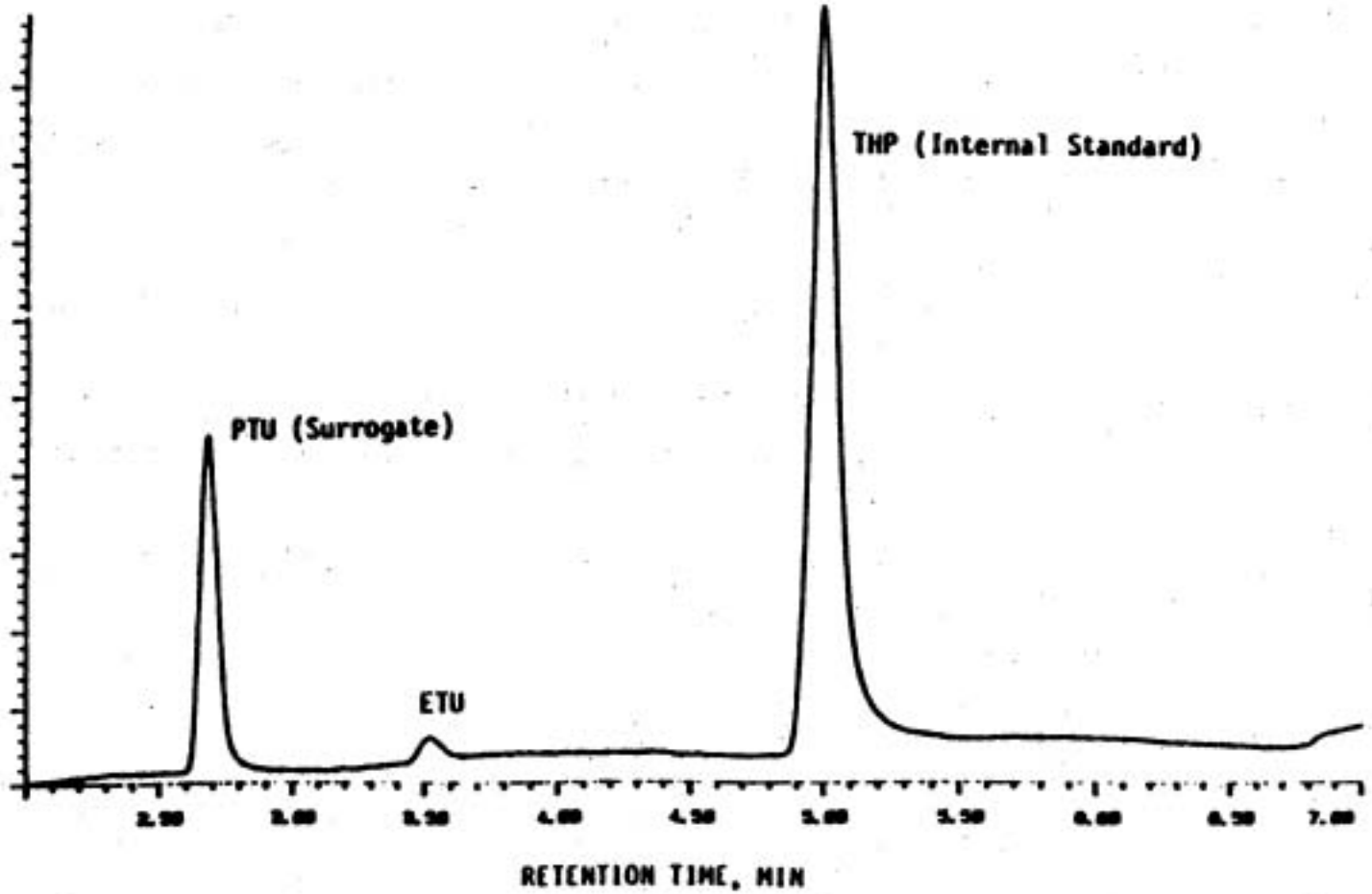

FIGURE 1. GC-NPD CHROMATOGRAM OF QUALITY CONTROL STANDARD