USATHAMA METHOD NUMBER: LW18
ANALYSIS OF THIODIGLYCOL AND CHLOROACETIC ACID IN ENVIRONMENTAL SOIL SAMPLES

I. SUMMARY

A. ANALYTES

This method is applicable to the quantitative determination of thiodiglycol and chloroacetic acid in environmental soil samples.

B. MATRIX

This method is applicable to all environmental soil matrices.

C. GENERAL METHOD

A measured weight of sample is extracted with alkaline methanol on a wrist-action shaker. A portion of the methanol is filtered, and removed by evaporation under a nitrogen stream. The extract is acidified, buffered, and brought to volume with water. Chromatographic conditions described in this method permit the separation and measurement of the two analytes in the methanol extract. Analyte identification is performed using retention times, and quantitative analysis is performed using a standard curve of area counts.

II. APPLICATION

A. TESTED CONCENTRATION RANGE

The tested concentration ranges in "standard soil" samples are:

Analyte	Tested Concentration Range (ug/g)*
Thiodiglycol	2.55 to 102.0
Chloroacetic acid	7.54 to 302.0

*ug/g = micrograms per gram.

B. SENSITIVITY

The normalized response (integrator counts corrected for attenuation) at the "standard soil" reporting limits are:

Analyte	Area Counts
Thiodiglycol	630,000
Chloroacetic acid	680,000

C. REPORTING LIMIT

The certified reporting limits in standard soil as calculated according to the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) certified reporting limit program are:

Analyte	Reporting Limit (ug/g)	Upper Certified Range (ug/g)
Thiodiglycol	3.94	102.0
Chloroacetic acid	18.0	302.0

D. INTERFERENCES

1. Interferences in methods employing ultraviolet (UV) detection at short wavelengths can pose problems in analysis of this method. The interference can usually be minimized by preventing contact of reagents, glassware, apparatus, and samples with any plastic materials.
2. Solvents, reagents, glassware, and other sample processing equipment may yield chromatograms with interfering peaks. All reagents, glassware, and sample handling equipment must be demonstrated to be free from interferences that have retention times equal to those of the compounds of interest.

E. ANALYSIS RATE

After instrument calibration, one analyst can analyze eight samples in an 8-hour day. One analyst can perform

approximately eight extractions in an 8-hour day.

F. SAFETY INFORMATION

This method involves the use of methanol and acid. Adequate dermal and eye protection should be used, and proper ventilation available. Chloroacetic acid is highly toxic and z strong irritant and should be handled with care.

III. APPARATUS AND CHEMICALS

A. GLASSWARE/HARDWARE

1. Eight 50 milliliter (mL) centrifuge tubes, screw top;
2. Eight 25 mL pipettes;
3. Eight 10 mL Gastight syringes;
4. Gelman Acrodisc CR filter assemblies, 0.45 micrometer (um);
5. Eight 10 mL pipettes;
6. Eight scintillation vials;
7. Nitrogen manifold (8 ports);
8. 0.5 mL pipettes; and
9. 15 mL graduated centrifuge tubes.

B. INSTRUMENTATION AND OPERATING PARAMETERS

1. Altex Model 322 gradient high-pressure liquid chromatograph (HPLC) equipped with a Perkins Elmer LC-75 variable wavelength UV visible detector and interfaced to a Shimadzu C-R3A computing integrator.
2. IEC Model 5000CU centrifuge.
3. Burrell Model 75 wrist-action shaker.
4. Detector: Perkin Elmer LC-75 variable wavelength detector [= 215 nanometers (nm)].
5. Column: Ultrasphere octadecylsilane (ODS) [4.6-millimeter (mm) inside diameter (ID) by 25 centimeters (cm)].
6. Particle Size: 5 um.

7. Guard column: 2.6 mm ID by 10 cm packed with Whatman Co: Pell ODS.
8. Silica precolumn: 2.6 mm ID by 10 cm packed with 60-230 mesh silica gel (ICN Pharmaceuticals, Inc.).
9. Flow rate and mobile phase: 1 milliliter per minute (mL/min) of 0.06 molar (M) phosphate buffer [7.94 grams (g) of sodium dihydrogen phosphate hydrate ($NaH_2PO_4 \cdot H_2O$) and 0.16 mL of 85-percent phosphoric acid (H_3PO_4) dissolved in 1 liter (L) of water].
10. Temperature: Room temperature.
11. Injection volume: 250 microliters (uL) fixed loop.
12. Retention times: Thiodiglycol -- 11.9 minutes (min) $\pm$ 0.4 min; chloroacetic acid -- 4.5 min $\pm$ 0.4 min. Retention time windows represent 3 standard deviations of a control spike measured during the 4 days of certification.

C. ANALYTES

Analyte	CHEMICAL ABSTRACT Service (CAS) Registry Number
Thiodiglycol	111-48-8
Chloroacetic acid	79-11-8

D. REAGENTS

1. HPLC-grade methanol (Burdick and Jackson);
2. 4 normal (N) sodium hydroxide -- 160 g of sodium hydroxide dissolved in 1 L of HPLC water;
3. HPLC water (Burdick and Jackson);
4. Nitrogen gas;
5. Basic methanol solution -- mix 40 mL of 4 N sodium hydroxide and 1,000 mL of methanol;
6. Acid solution -- 1:4 (volume:volume) mixture of concentrated H_2SO_4 and HPLC water;
7. Concentrated buffer solution -- 79.4 g of $NaH_2PO_4 \cdot H_2O$ and 1.6 mL of 85-percent H_3PO_4 in one liter of HPLC water;

8. Chloroacetic acid (Aldrich Gold Label), 99+ percent purity.
9. 2,2'-Thiodiethanol (Thiodiglycol) (Aldrich), 99+ percent purity.

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards

Combined primary stock calibration standards are prepared by weighing approximately 0.5 grams (g) of thiodiglycol and 3.5 g of chloroacetic acid into a 100-mL volumetric flask; then dilute to volume with HPLC water. The concentrations of each compound in the primary stock calibration standard prepared this way is 5,000 ug/mL and 35,000 ug/mL, respectively.

A composite secondary stock calibration standard is prepared by adding 10 mL of the primary stock calibration standard to volume with HPLC water in a 100-mL volumetric flask. The concentration in the composite secondary stock calibration standard for each analyte is 500 and 3,500 ug/mL, respectively.

Composite working calibration standards are prepared using HPLC water, the composite secondary stock calibration standard and volumetric pipettes as shown in Table IV-1.

2. Instrument Calibration

To calibrate the instrument, at least 250 uL of each standard in Table IV-1 is injected into the instrument in the same manner as a sample extract. Duplicate composite calibration standards are analyzed during precertification calibration, and the single dilutions of the composite standards are analyzed during initial calibration. Currently an independent reference standard is not available for thiodiglycol and chloroacetic acid. Meanwhile an independent stock will be prepared to serve as

Table IV-1. Preparation of Initial Instrument Calibration Standards

Calibration Standard	Volume Composite 2° Stock Standard Used	Final Volume (mL)	Concentration of Each Analyte in Instrument Calibration Standard (ug/L)	
			Thiodiglycol	Chloroacetic Acid
A	1.0	10	50	350
B	1.0	25	20	140
C	0.5	25	10	70
D	0.2	25	4.0	28
E	0.1	25	2.0	14
F	0.05	25	1.0	7.0
G	0.025	25	0.50	3.5
H	0	25	0	0

Source: Hunter/ESE, 1989.

a reference standard. The reference must be analyzed along with the initial and precertification calibration standards, and the results must be within ± 25% of the true value for the calibration to be considered valid. If the analysis of the reference standard fails, the source of the problem must be identified and corrected. The initial calibration and analysis of the independent reference standard must be repeated. The result of the second analysis of the independent reference standard must be within the acceptable limits, as specified by the source of the standard, before the analysis of samples may proceed. Since initial calibrations are performed daily, a reference is required at least weekly.

3. Analysis of Calibration Data

After analyzing the standards (i.e., one blank and nine standards) in duplicate, the data are tabulated and graphed. Data are analyzed using the lack of fit (LOF) and zero intercept (ZI) tests (USATHAMA QA Plan, 2nd Edition, March 1987). All pre-certification calibration data passed the LOF-ZI tests, therefore calibrations are linear.

4. Calibration Checks

At the end of the daily instrumental analysis, the highest working calibration standard is injected into the instrument. The response or the recovery of this end-of-day analysis should be ± 25% of the response or recovery obtained from the analysis of the same working calibration standard curve analyzied that day. If the first calibration check does not pass this criterion, then the standard may be reinjected and compared to the initial response. The post run standard data is documented and the percent drift is calculated on the chromatographic logsheet. An explanation must be provided if the calibration check is not acceptable for the data to be reported, otherwise the samples are rerun.

B. DAILY CALIBRATION

For daily calibration an initial calibration curve and QC checks will be performed as stated in Section IV.A.

V. CERTIFICATION TESTING

A. PREPARATION OF CONTROL SPIKES FOR METHOD CERTIFICATION

1. A combined primary certification spiking solution was prepared by weighing 0.509 g of thiodiglycol and 1.510 g of chloroacetic acid into a 100-mL volumetric flask and diluting to volume with HPLC water. The concentration of the primary certification spiking solution was 5,090 ug/mL and 15,100 ug/mL, respectively.
2. A secondary certification spiking solution was prepared by pipetting 10.0 mL of the primary certification spiking solution to a 100-mL volumetric flask and diluting to volume with HPLC water. The concentration of the secondary certification spiking solution was 509 and 1,510 ug/mL, respectively.
3. Certification spikes were prepared as shown in Table V-1 and were used to determine the accuracy, range, and reporting limits of the analytes to which this method applies. In each case, 10 g of standard soil was spiked with the appropriate certification spiking solution. The standard soil used for certification in this method was an uncontaminated background soil from the Rocky Mountain Arsenal (RMA) area in Denver, CO., rather than the USATHAMA standard soil. (see Attachment 3 for USATHAMA soil spike data.)

Table V-1. Preparation of Certification Control Spikes

Certification Control Spike Sample Prepared	Control Stock Used	Volume Spiked* (mL)	Concentration of Each Analyte in the Prepared Sample (ug/g)	
			Thiodiglycol	Chloroacetic Acid
Blk	----	0	0	0
1S	2°	0.05	2.55	7.54
2S	2°	0.10	5.09	15.1
3S	2°	0.20	10.2	30.2
4S	2°	0.50	25.5	75.4
5S	1°	0.10	50.9	151
6S	1°	0.20	102	302
7S	1°	0.50	255	754

* to 10 g of standard soil

Source: Hunter/ESE 1989

B. ANALYSIS OF CERTIFICATION SPIKES

Certification Control Spikes are analyzed by the procedures outlined in Section VII. Instrumentation is calibrated as in Sec. IV.

VI. SAMPLE HANDLING AND STORAGE

A. SAMPLING PROCEDURE

There are no special considerations required due to the nature of these compounds. Soil samples may be collected as grab samples or cores. The samples need to be chilled to 4 deg. C immediately following sampling.

B. CONTAINERS

Sampling containers used are 1.2 Litre glass amber jars with a teflon-lined cap for grab samples, or polybutyrate tubes for core samples.

C. STORAGE CONDITIONS

Samples are to be kept on ice in coolers until shipped to the analytical lab. Upon arrival, the samples are stored at 4 deg. C in a walk-in refrigerator.

D. HOLDING TIME LIMITS

The holding time limit is seven days from the time of sampling to extraction of the sample and 40 days from extraction to analysis.

E. SOLUTION VERIFICATION

Calibration standards are verified with daily control spikes and analysis of reference samples. When fresh control spike stock solutions are prepared. They must be verified to determine that:

1. the previous spiking solution had or had not deteriorated

2. the new solution was correctly prepared

Therefore, the combined primary spike stock solutions (IX.A.1) will be checked against working standards before initial use and again within seven days before subsequent use.

VII. PROCEDURE

A. EXTRACTIONS

1. Extraction of Certification Control Spikes Only
   The certification control spikes are extracted beginning with Sec. VII.2.d.
2. Extraction of Environmental Samples
   a. Place 10 g of soil into a 50-mL centrifuge tube.
   b. Add 25.0 mL of basic methanol (Sec. III.D.5.) solution to each centrifuge tube.
   c. Shake the samples on a wrist-action shaker for 20 min.
   d. Centrifuge the samples at approximately 2,000 revolutions per minute (rpm) for 5 min.
   e. Equip a 10-mL Gastight syringe with a 0.45-um Acrodisc CR filter assembly. Pipette 10.0 mL of the extract into the syringe, pass it through the filter, and collect the filtrate in a 15 mL graduated centrifuge tube.
   f. Reduce the extract volume to exactly 1 mL using a nitrogen stream without heating. Note: Final volume critical. Extra methanol can cause chromatographic problems and going below 1 mL causes loss of chloroacetic acid by volitization.
   g. Add approximately 4 mL of HPLC water and mix in the centrifuge tube. Acidify the extract to pH 2 with the 1:4 acid solution (approximately 8 drops).
   h. Add 0.5 mL of the concentrated buffer solution to the extract. Bring to 10-mL final volume with HPLC water and mix thoroughly. In some cases (i.e. USATHAMA

Standard soil) a second filtration is needed.

1. Transfer some of the extract to a 5-mL, amber-glass, septum-sealed vial for storage at 4 degrees Celsius (C). The solution is now ready for analysis by HPLC.

B. CHEMICAL REACTIONS

There is no chemical reation stage.

C. INSTRUMENT ANALYSIS

Daily calibration performed as described in Sec. IV.A. and B. with instrument condition described in Sec. III.B. For daily control spikes of USATHAMA Standard soil a 12 min. delay is required after each run because of a late eluting broad peak. Caution should be used with environmental samples in case a broad eluting peak is present.

VIII. CALCULATIONS

Determine the concentration of each analyte according to the following formula:

$$\text{Concentration (ug/mL)} = \frac{(A)(Vt)}{(Vs)(1 - M)}$$

where: A = Concentration (ug/mL) of each analyte found in the extract by comparison with the appropriate standard curve (ug/mL),

Vt = Final volume of extract solution (25 mL), and

Vs = Weight of initial sample extracted (10 g).

M = Moisture expressed as a fraction

Wet weight and moisture results are reported to the USATHAMA data management system for dry weight calculations.

IX. DAILY QUALITY CONTROL

A. CONTROL SAMPLES

1. Preparation of the Combined Primary Spike Stock Solution (CPSSS): The CPSSS is prepared by weighing .75 g of thiodiglycol and 3.5 g of chloroacetic acid into a 100 mL volumetric flask and diluting to volume with HPLC water. The concentrations of the CPSSS are 7,500 and 35,000 ug/mL respectively. (prepare fresh monthly)
2. Preparation of the Secondary Spike Stock Solution (SSSS): An intermediate spike solution is made by diluting 10 mL of the CPSSS (1) with HPLC water into a 100 mL volumetric flask. The concentrations of this solution are 750 and 3,500 ug/mL. (prepare fresh daily)
3. Using the SSSS, the Daily Control Spikes are made by spiking the appropriate volume of stock solutions to 10 g of standard soil as shown in Table IX - 1.

B. CONTROL CHARTS

In the initial construction of the control charts, data from the laboratory certification analysis will be used. Data from spiked QC samples, within a lot, will be compared to control chart limits to demonstrate that analysis of the lot are under control and will be used to update the charts. X-R control charts will be used in the Quality Assurance (QA) Program. Control charts are prepared for each target analyte using percent recovery (found concentration - method blank/ spiked concentration x 100) data from the duplicate spiked QC samples variations in spiking solution concentrations.

To prepare control charts, the analyst should have access to the following data:.

1. Average (x) percent recovery for the two high concentration spiked QC samples in each lot,

Table IX-1. Preparation of Daily Control Spikes

Daily Control Spike	Control Stock Used	Volume Spiked* (mL)	Concentration of Each Analyte in the Prepared Sample (ug/g)	
			Thiodiglycol	Chloroacetic Acid
Blank	None	0	0	0
Low	SSSS	0.1	5	35
High	CPSSS	0.05	25	175
High	CPSSS	0.05	25	175

* to 10g of standard soil

Source: Hunter/ESE, 1989.

2. Difference (R) between the percent recoveries for the two high concentration spiked QC samples in each lot,
3. Three-point moving average (x) spkie recovery of the low-concentration spike QC sample, and
4. Three-point moving difference (R) between the percent recoveries for the low-concentration spike QC sample.

The initial control chart shall be prepared using the four pairs of certification data closest to the spiking concentration used during analysis. The average (x), average range (R), and control limits for x and R shall be updates after each lot for the first 20 lots. Limits established after Lot 20 will be used for the next 20 lots. Control charts will be updated after each 20 lots thereafter using the most recent 40 lots of QC data. In updating the control charts, the new data must be combined with the individual values of previous percent recoveries and not the mean of all previous data. Initial control chart limits are presented in Table IX-2.

Table IX-2. Initial Control Limits for Thiodiglycol in Soil

METHOD:
COMPOUNDS: Thiodiglycol and Chloroacetic acid in Soil
UNITS: UGG

3 PT. MOVING AVERAGE $\bar{X}$ - R

CODE	CONC.	UCL $\bar{X}$	UWL $\bar{X}$	$\bar{X}$	LWL $\bar{X}$	LCL $\bar{X}$	UCL R	UWL R	$\bar{R}$
TDGCL	4.46	127.4	119.9	104.7	89.6	82.1	57.0	45.4	22.1
CLC2A	35.0	111.6	102.7	84.7	66.8	57.8	67.7	53.9	26.3

SINGLE DAY $\bar{X}$ - R

CODE	CONC.	UCL $\bar{X}$	UWL $\bar{X}$	$\bar{X}$	LWL $\bar{X}$	LCL $\bar{X}$	UCL R	UWL R	$\bar{R}$
TDGCL	22.3	109.3	105.1	96.6	88.0	83.8	22.2	17.1	6.8
CLC2A	175	185.0	158.0	103.9	49.9	22.9	140.8	108.2	43.1

X. REFERENCES

U.S. Army Toxic and Hazardous Materials Agency, (USATHAMA). 1987. QA Plan (December 1985, 2nd Edition, March 1987).

XI. DATA

A. OFF-THE-SHELF ANALYTICAL REFERENCE MATERIALS CHARACTERIZATION

These compounds cannot be seen on GC/MS and GC/FID. Therefore, standards can only be verified by independently prepared references.

B. PRE-CERTIFICATION CALIBRATION

Attachment 1

C. DAILY CALIBRATION AND CHROMATOGRAM

Attachment 2

D. CERTIFICATION DATA (RMA Standard Soil)

Attachment 3

E. ONE DAY CERTIFICATION WITH USATHAMA STANDARD SOIL

Attachment 4