USATHAMA METHOD NUMBER: UL04

DETERMINATION OF ORGANOSULFUR COMPOUNDS IN WATER BY GAS CHROMATOGRAPHY

I. SUMMARY

A. ANALYTES

This method is applicable to the Class 1 analysis of the following organosulfur compounds in environmental water samples.

Dimethyldisulfide (DMDS)
1,4-Oxathiane (OXAT)
1,4-Dithiane (DITH)
p-Chlorophenylmethylsulfide (CPMS)
Benzothiazole (BTZ)
p-Chlorophenylmethylsulfoxide (CPMSO)
p-Chlorophenylmethylsulfone (CPMSO2)

B. MATRIX

This method is applicable to all environmental water matrices.

C. GENERAL METHOD

This method employs extraction of the water matrix with methylene chloride, solvent concentration using standard Kuderna-Danish (KD) techniques, and analysis by gas chromatography (GC) using flame-photometric detection (FPD) in the sulfur mode. The method is very similar to Method U8 certified under the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) 1985 Quality Assurance (QA) Plan.

II. APPLICATION

A. TESTED CONCENTRATION RANGE

The tested concentrations in "Standard water" samples are:

Analyte	Tested Concentration Range(ug/L)
DMDS	1.14 to 22.8
OXAT	1.98 to 39.5
DITH	1.11 to 22.2
CPMS	1.26 to 25.3
BTZ	2.11 to 42.2
CPMSO	4.23 to 106
CPMSO2	4.22 to 106

B. SENSITIVITY

The instrumental responses for each compound, reported in peak area units, at the certified reporting limits for the calibration curve were determined by the Hubaux and Vos detection limits from the calibration curve data.

Analyte	Calibration Reporting Limit (ug/L)	Area Counts
DMDS	1.14	57,000
OXAT	1.98	23,200
DITH	1.11	32,600
CPMS	1.26	15,600
BTZ	2.11	26,600
CPMSO	4.23	37,000
CPMSO2	4.72	33,400

C. REPORTING LIMITS

The corresponding upper certified range and reporting limits for each analyte in environmental water samples are:

Analyte	Certified Reporting Limit (ug/L)	Upper Range (ug/L)
DMDS	1.14	22.8
OXAT	1.98	39.5
DITH	1.11	22.2
CPMS	1.26	25.3
BTZ	2.11	42.2
CPMSO	4.23	106
CPMSO2	4.72	106

D. INTERFERENCES

Solvents, reagents, glassware, and other sample processing equipment may yield chromatograms with interfering peaks. All reagents, glassware, and sample handling equipment must be free from interferences that have retention times equal to the retention times of the compounds of interest.

E. ANALYSIS RATE

After instrument calibration, one analyst can analyze 10 sample extracts in an 8-hour day. One analyst can perform approximately 10 extractions in an 8-hour day.

F. SAFETY INFORMATION

The target compounds in this method are toxic. The preparation of all standards should be performed in a laboratory hood. Adequate dermal protection must be used when handling samples and standards.

III. APPARATUS AND CHEMICALS

A. GLASSWARE/HARDWARE

1. 1,000-milliliter (mL) separatory funnel with Teflon stopcock.
2. 500-mL KD concentrator.
3. Class A volumetric flasks (5-, 25-, 50- and 100-mL).
4. Class A volumetric pipettes (0.5- to 25-mL).
5. Microsyringes (250- and 1,000-mL).
6. Pasteur pipettes (disposable).
7. Graduated cylinder (1,000-mL).
8. Amber-glass vials (9-mL with Teflon -lined crimp caps).
9. Snyder columns (KD, 3-ball macro- and modified micro-Snyder).
10. Concentrator tubes (KD, 25-mL graduated, with ground-glass stoppers).
11. Glass funnels [58-millimeter (mm) short-stem].
12. Hengar boiling chips (10/40 mesh, pre-extracted with methylene chloride; available from Hengar Co., Philadelphia, PA, catalog number 136CC).
13. Glass vials (2-mL with Teflon -lined crimp-seal caps for use with an automatic sampler).
14. Amber bottles (60-mL with Teflon -lined screw caps).
15. Hot water bath.
16. Stainless-steel spatulas.
17. Analytical balance [Mettler AE160 or equivalent, with 0.0001-gram (g) sensitivity].
18. Glass wool (silanized).
19. Tipit (50-mL).

B. INSTRUMENTATION

1. Gas chromatograph with an FPD (Varian 3400 or equivalent), equipped with an automatic sampler (Varian 8055 or equivalent) and integrator (Spectra-Physics 4270 or equivalent).

2. Chromatographic conditions:

   a. Column: 5-percent SP-1000 on Chromosorb [6-foot (ft) by 2-mm inside diameter (ID) by 6-mm outside diameter (OD) column].

   b. Injector temperature: 200 degrees Celsius (C).

   c. Temperature program: 80 C, hold 3 minutes (min), heat at 32 degrees Celsius per minute (C/min) to 240 C, hold 7 min.

   d. Detector temperature: 300 C.

   e. Gas flow: Helium at 30 milliliters per minute (mL/min), hydrogen at 140 mL/min, Air 1 at 80 mL/min, air 2 at 170 mL/min.

   f. Injection volume: 5 microliters (uL).

   g. Retention times:

Analyte	Retention Time (min)
DMDS	1.29 ± 0.04
OXAT	4.02 ± 0.12
DITH	6.17 ± 0.19
CPMS	7.42 ± 0.22
BTZ	7.72 ± 0.24
CPMSO	10.07 ± 0.30
CPMSO2	11.20 ± 0.34

C. ANALYTES

Analytes	Chemical Abstract Service (CAS) Number Abbrev.	Boiling Point (C)
DMDS	624-92-0	--
OXAT	15890-15-1	147
DITH	505-29-3	200
CPMS	123-09-1	--
BTZ	95-16-9	231
CPMSO	934-73-6	--
CPMSO2	98-57-7	--

D. REAGENTS AND STANDARD ANALYTICAL REFERENCE MATERIALS (SARMS)

1. Methylene chloride (pesticide-grade);
2. Acetone (pesticide-grade);
3. Sodium chloride (reagent grade);
4. Sodium sulfate [American Chemical Society (ACS), granular, anhydrous, heated at 400 degrees C for 4 hours in a muffle furnace];
5. "Standard water" [distilled water containing 100 milligrams per liter (mg/L) each of sulfate and chloride];
6. DMDS [Standard Analytical Reference Material (SARM) ID No. 1378, obtained from USATHAMA];
7. OXAT (SARM ID No. PA2340, from USATHAMA):
8. DITH (SARM ID No. PA2318, from USATHAMA):
9. CPMS (SARM ID No. PA2320, from USATHAMA):
10. BTZ (from Aldrich Chemical Co. Milwaukee, WI 91928DP)
11. CPMSO (SARM ID No. PA2321, from USATHAMA):
12. CPMSO2 (SARM ID No. PA2322, from USATHAMA):

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards

Individual primary stock calibration standards are prepared by weighing approximately 32 milligrams (mg) of each of the six target analytes into separate 25-mL volumetric flasks, then diluting to volume with methylene chloride. The nominal concentrations of each of the primary stock calibration standards prepared this way for each analyte is 1,280 ug/mL.

A composite primary stock calibration standard is prepared by adding 5 mL of each individual stock calibration standard to methylene chloride in a 50-mL volumetric flask and then diluting to volume with methylene chloride. The nominal concentrations in the composite primary stock calibration standard for each analyte is 128 ug/mL.

A composite secondary calibration stock is prepared by placing 5 mL of the composite primary calibration stock into a 50-mL volumetric flask and diluting to volume with methylene chloride (a 1:10 dilution of the composite primary calibration stock). The nominal concentration in the composite secondary calibration stock for each analyte is 12.8 ug/mL.

Composite working calibration standards are prepared using methylene chloride, the composite primary and secondary stock calibration standards and volumetric pipettes as shown in Table IV-1.

2. Instrument Calibration

To calibrate the instrument, 5 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 organosulfur compounds in water. Meanwhile an independent stock will be prepared to serve as 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. Not all data from the standards analyzed in Table IV-1 are used for the regressions (i.e. Standard A would be used for CPMSO and CPMSO2 only, while Standards G-J would not be used for CPMSO and CPMSO2).

4. Calibration Checks

At the end of the daily instrumental analysis, the highest

working calibration standard is injected into the GC. The response of 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.

Table IV-1. Preparation of Initial Calibration Standards.

Standard	Composite Standard Used	Vol. Used (mL)	Final Vol. (mL)	Nominal Concentration (ug/L)	Nominal Extract Concentration
Blank	-	0	25	0.0	0.0
A	1	5.0	25	25,600	160.0
B	1	4.0	50	10,240	64.0
C	1	2.0	50	5,120	32.0
D	1	1.0	50	2,560	16.0
E	2	4.0	50	1,024	6.40
F	2	5.0	100	640	4.00
G	2	2.0	50	512	3.20
H	2	2.5	100	320	2.00
I	2	1.0	50	256	1.60
J	2	0.8	50	205	1.28

Source: Hunter/ESE, 1988.

V. CERTIFICATION TESTING

A. PREPARATION OF CERTIFICATION SPIKES

1. Individual primary stock control spiking solutions are prepared by weighing approximately 50 mg of each of the analytes into separate 50-mL volumetric flasks, then diluting each to volume with acetone. The nominal concentrations in the individual primary stock spiking solutions were 1,000 ug/mL.
2. A composite primary stock control spiking solution is prepared (in a 100-mL volumetric flask) by diluting 1 mL of each of the individual primary stock control spiking solutions (2 mL for BTZ) with acetone to the final volume of 100 mL. The nominal concentrations in the composite primary stock control spiking solution prepared as above were 10 ug/mL (20ug/mL for BTZ).
3. The spiking procedure in Table V-1 was implemented to prepare control spike samples to determine the accuracy and reporting limits for each analyte. In each case, 800 mL of "standard water" in separate, 1-liter separatory funnels were spiked with the appropriate volume of the composite secondary control spiking solution and sufficiently mixed prior to extraction. Actual concentrations during certification are presented in Table V-1.

B. ANALYSIS OF CERTIFICATION SPIKES

Certification control spikes are analyzed by the procedures outlined is Sec. VII.

Table V-1: Preparation of Certification Control Spike Samples

Control Spike Samples Prepared	Volume of Composite Primary Control Spiking Solution Used (mL)	Volume of Standard Water Used (mL)	Concentration of Control Spike Samples (ug/L)						
			DMDS	CXAT	DITH	CRMS	BTZ	CRMS02	CRMSO
Blank	0	800	0	0	0	0	0	0	0
2	0.08	800	1.14	0.99*	1.11	1.26	2.11	1.06*	1.13*
3	0.16	800	2.28	1.98	2.22	2.53	4.22	2.12*	2.26*
4	0.32	800	4.56	3.95	4.44	5.06	10.6	4.23	4.22
5	0.80	800	11.4	9.88	11.1	12.6	21.1	10.6	10.6
6	1.6	800	22.8	19.8	22.2	25.3	42.2	21.2	21.2
7	3.2	800	NA+	39.5	NA	NA	NA	42.3	42.2
8	8.0	800	NA	NA	NA	NA	NA	106	106

*Not detected during certification and, consequently, data are not presented in Att. 1.
+NA = not analyzed. Spikes were made at higher concentrations to suplement the non-detected data (see * above) for some analytes; only selected higher concentration spikes were analyzed since only selected data needed to be supplemented.

Source: Hunter/ ESE, 1988.

VI. SAMPLE HANDLING STORAGE

A. SAMPLING PROCEDURE

There are no special considerations required due to the nature of organosulfur compounds. 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.

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

When fresh control spiking 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, dilute working spike solutions will be checked against working standards before initial use and again within seven days before subsequent use. The spike solutions should use the same instrumentation used on the samples.

VII. PROCEDURE

Daily quality control spikes (Section IX.A) and environmental samples are prepared for analysis and analyzed as follows:

A. SEPARATIONS

1. Using a 1-liter graduated cylinder, transfer 800 mL of sample (or standard water for daily control spikes) to a 1-liter separatory funnel.
2. Daily control spikes are spiked as specified in Table IX-1.
3. Add 50 mL of methylene chloride to each separatory funnel.
4. Extract the sample by shaking the funnel for 2 min, with periodic venting to release solvent vapor pressure.
5. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must mechanically complete the phase separation. The optimum technique depends on the sample but may include either sonication or stirring, filtering, and filtration of the emulsion through glass wool.
6. Pass the methylene chloride extract through unwashed, baked (400 C, 4 hours) sodium sulfate into a 50-mL KD apparatus. Rinse the sodium sulfate with methylene chloride after the extract has dried.
7. Add a second 50-mL volume of methylene chloride to the separatory funnel and complete the extraction procedure a second time, combining the extracts in the KD apparatus.
8. Perform a third extraction in the same manner.
9. Add 1 to 2 clean boiling chips to the KD apparatus and attach a 3-ball Snyder column. Prewet the Snyder column by adding approximately 1 mL of methylene chloride to the top.
10. Place the KD apparatus on the hot water bath (70 C to 80 C)

so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches approximately 5 mL, remove the KD apparatus and allow it to drain for at least 10 min while cooling.

11. Rinse the Snyder column, remove the Snyder column, and rinse the flask into the concentrator tube with approximately 5 mL of methylene chloride. Rinse the lower joint into the concentrator tube when separating it from the flask.
12. Attach a modified micro-Snyder column to the concentrator tube in the hot water bath. When the apparent volume of the liquid reaches 2 mL, remove the concentrator tube and allow it to cool for approximately 10 min.
13. Adjust the final volume to 5 mL by adding methylene chloride. Transfer approximately 1 mL to an autosampler vial and seal (if another method requiring the same extraction is being performed by the laboratory, the extract may be split into roughly equal portions). Save the remaining extract at 4 C as a backup. Sample extracts must be analyzed within 40 days of extraction.

B. CHEMICAL REACTIONS

No chemical reactions are required by this method.

C. INSTRUMENTAL ANALYSIS

1. Perform daily instrument calibration as described in Sec. IV.B. Use the instrument conditions listed in Sec. III.B.

2. Place the sample extracts in the autosampler tray and inject 5 uL of each sample extract into the instrument under exactly the same conditions as those under which it was calibrated.

VIII. CALCULATIONS

A linear calibration curve is constructed from the calibration data by plotting the response versus the concentration of each standard. The calibration curve slope and intercept are determined by linear regression. The concentration of a target compound in the extract is calculated by substituting the response into the calibration curve equation. The concentration of each analyte in the original water sample is determined by the following formula:

$$\text{Concentration (ug/L)} = \frac{\text{Extract Conc. (ng/L)} \times \text{DF} \times 5\ \text{mL}}{V_S\ \text{(L)} \times 1{,}000\ \text{mL/L}}$$

where Extract Conc. = Calculated concentration in the extract in nanograms per milliliter (ng/mL);
DF = Dilution factor, if required, to get the response into linear calibration range;
10 mL = Final extract volume in milliliters;
V_S = Sample volume in liters (nominally 0.8 L).

IX. DAILY QUALITY CONTROL

A. CONTROL SAMPLES

The daily control spikes required are a Standard Matrix Method Blank, one low level Standard Matrix Spike at approximately twice the CRL, and two high-level Standard Matrix Spikes at

five times the low level spike. Individual stock spiking solutions are prepared as discussed in Sec. V.A.1. A composite daily control spike solution is prepared (in a 100-mL volumetric flask) by diluting the following volumes of the individual primary stock control spiking solutions to volume with acetone:

Analyte	mLs Stock	Concentration (ug/mL)
DMDS	1.5	15.0
OXAT	2.0	20.0
DITH	1.5	15.0
CPMS	1.5	15.0
BTZ	2.0	20.0
CPMSO	4.0	40.0
CPMSO2	5.0	50.0

The spike solution is verified when needed as described in Sec. VI.E. From the composite daily control spike solution, the daily control spikes are prepared 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,
2. Difference (R) between the percent recoveries for the two high concentration spiked QC samples in each lot,
3. Three-point moving average (x) spikke 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.

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

Attachment 1

B. PRE-CERTIFICATION CALIBRATION

Attachment 2

C. DAILY CALIBRATION AND CHROMATOGRAM

Attachment 3 (Reference check sample not required during time of original certification)

D. CERTIFICATION DATA

Attachment 4

Table IX-1. Preparation of Daily Control Spike Samples

Daily Control Spike Number	Volume of Daily Composite Control Spiking Solution Used* (mL)	Volume of Standard Water Used (mL)	Nominal Concentration of Daily Control Spike Samples (ug/L)						
			DMDS	CXAT	DITH	CRMS	BIZ	CRMSO	CRMSO2
Blank	0	800	0	0	0	0	0	0	0
Low-Level Spike	0.16	800	3.0	4.0	3.0	3.0	4.0	8.0	10.0
High-Level Spike A	0.80	800	15.0	20.0	15.0	15.0	20.0	40.0	50.0
High-level Spike B	0.80	800	15.0	20.0	15.0	15.0	20.0	40.0	50.0

Source: Hunter/ESE, 1988.

Table IX-2. Initial Control Limits for Ogranosulfur Compounds in Water

ESE CERTIFICATION LIMITS

METHOD: OS in Water
UNITS: ug/L

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

CODE	CONC.	UCL $\bar{X}$	UWL $\bar{X}$	$\bar{\bar{X}}$	LWL $\bar{X}$	LCL $\bar{X}$	UCL R	UWL R	$\bar{R}$
DMDS	3.42	87.5	85.2	80.5	75.8	73.5	17.6	14.0	6.8
OXAT	3.95	88.4	85.7	80.2	74.7	71.9	20.7	16.5	8.1
DITH	2.22	97.1	92.2	82.6	72.9	68.1	36.4	29.0	14.1
CPMS	2.53	101.3	95.5	83.9	72.3	66.5	43.8	34.8	17.0
BTZ	4.22	105.8	103.9	100.2	96.5	94.6	14.0	11.2	5.4
CPMSO	4.23	82.1	79.6	74.6	69.6	67.1	18.9	15.1	7.3
CPMSO2	9.04	85.6	83.8	80.1	76.4	74.6	13.9	11.1	5.4

SINGLE DAY $\bar{X}$ - R

CODE	CONC.	UCL $\bar{X}$	UWL $\bar{X}$	$\bar{\bar{X}}$	LWL $\bar{X}$	LCL $\bar{X}$	UCL R	UWL R	$\bar{R}$
DMDS	17.1	82.8	80.1	74.6	69.2	66.4	14.2	10.9	4.3
OXAT	19.8	82.1	80.8	78.3	75.8	74.5	6.5	5.0	2.0
DITH	11.1	83.8	81.3	76.3	71.2	68.7	13.1	10.0	4.0
CPMS	12.6	82.9	80.6	75.9	71.3	69.0	12.1	9.3	3.7
BTZ	42.2	99.1	97.5	94.4	91.3	89.7	8.2	6.3	2.5
CPMSO	21.2	85.9	83.6	79.0	74.5	72.2	11.9	9.2	3.7
CPMSO2	45.2	98.6	95.4	89.1	82.7	79.5	16.7	12.8	5.1