MATERIALS AND METHODS

Solvents

Methyl Acetate (99%, EMD)

Glassware and Miscellaneous Equipment

Air Sample Tubes, SKC Anasorb CSC (Cat. No. 226-09)

Air Sampling Pumps, SKC Universal Pump (44XR or PCXR4)

Balance, Mettler, AT261

Centrifuge, Mistral 3000E Centrifuge

Culture tubes, glass, 15 mL, with PTFE lined screw caps

Electrical Tape

Erlenmeyer Flasks, 500 mL for trapping apparatus (see Figure 1)

Flow Meter, Dry-Cal DC-Lite Model DCL-M

Hamilton microliter syringes, various volumes

Pasteur pipette, 5 inch, 9 inch

Shaker Bath, Lab-Line Shak-R-Bath model 3582

Sonicator (Branson 2510)

Tygon tubing (R-3603)

Vials, Amber glass (2 mL capacity) with Teflon®-lined crimp caps

Volumetric pipette, class-A, 5.0 mL

Volumetric flask, class-A, various volumes

Vortex, Fisher Model 12-810

ANALYTICAL PROCEDURES

Analytical Standard

Dimethyl Disulfide (DMDS), was supplied by Cerexagri, Inc. with the following batch number and PTRL designation (see Appendix C for certificate of analysis):

	Batch No.	PTRL No.	Purity (%)	Expiration Date
Dimethyl Disulfide (DMDS)	06025PB	1419W-001	99.8	March 1, 2006

A stock standard solution of DMDS was prepared at 100.0 mg DMDS/mL in methyl acetate using the formula as described under the "Methods of Calculation" section in this report. 1.0141 g. was dissolved in 10.120 mL of methyl acetate. The DMDS stock standard was stored in an amber glass vial with PTFE lined screw cap. The stock solution was stored in a refrigerator when not in use.

Preparation of Fortification Standards

DMDS fortification standards were prepared in methyl acetate according to the procedure outlined in Reference 1.

A 10.0 mg DMDS/mL solution was prepared by diluting 1.0 mL of the stock standard solution (100mg DMDS/mL) to 10.0 mL with methyl acetate.

A 1.0 mg DMDS/mL solution was prepared by diluting 1.0 mL of the 10 mg DMDS/mL solution to 10.0 mL with methyl acetate.

A 100 µg DMDS/mL fortification solution was prepared by diluting 1.0 mL of 1.0 mg DMDS/mL solution to 10.0 mL with methyl acetate.

A 10.0 µg DMDS/mL fortification solution was prepared by diluting 1.0 mL of 100 µg DMDS/mL fortification solution to 10.0 mL with methyl acetate.

All solutions were prepared with volumetric flasks and Hamilton syringes. Solutions were placed in amber glass vials with PTFE lined screw caps and stored in a refrigerator when not in use.

Preparation of Linearity Standards

Linearity standards ranging from 0.005µg DMDS/mL to 0.2 µg DMDS/mL in methyl acetate were prepared by diluting the 10 µg DMDS/mL fortification solution as follows:

Standard Solution Concentration (µg DMDS/mL)	Volume Used (mL)	Final Volume (mL)	Final Concentration (µg/mL)
10.0	0.025	50.0	0.005
10.0	0.050	50.0	0.010
10.0	0.10	50.0	0.020
10.0	0.15	50.0	0.030
10.0	0.20	50.0	0.040
10.0	0.25	50.0	0.050
10.0	0.50	50.0	0.100
10.0	1.0	50.0	0.200

All linearity solutions were prepared in methyl acetate using volumetric flasks and Hamilton syringes. Calibration solutions were placed in amber glass jars with PTFE lined screw caps, and were stored in a freezer when not in use.

Sample Fortification

Air trapping samples were prepared using DMDS fortification solutions at two concentration levels, five replicates at each level. The low level fortification samples were fortified with 0.1µg DMDS. The high level fortification samples were fortified with 1.0µg DMDS. Fortification solutions were introduced to the air sampling tubes in a gaseous form using an air trapping method. Two control samples were also collected. Two extraction fortification samples were prepared, one at the low level and one at the high level, whereby the front portion (400 mg) charcoal from unused charcoal tubes were fortified directly with fortification solutions, then extracted immediately.

TRAPPING/EXTRACTION METHOD

The following method was followed as close as possible to that described in Reference 1. Modifications to that method are detailed in method modifications section to follow. DMDS was trapped from the vapor phase onto the sorbent tubes by drawing air through the trapping apparatus at 2L/min for a 15 minute trapping interval.

A sealed SKC Anasorb SCS sample tube was opened at each end by breaking the tips and introducing the tube in line on the sampling apparatus as shown in Figure 1. The tube was aligned such that the 400 mg charcoal portion of the tube faced the 500 mL flask. An air sampling pump was turned on and set to 2L/min. The flow rate for each sample apparatus was set just before introducing DMDS to the system and recorded, then recorded again during the last minute of trapping using Bios DryCal DC-Lite flow meter. An average flow rate was determined for each sample.

With air flow on and set to the correct flow rate, DMDS was introduced to the sampling apparatus using 10µL Hamilton syringes. For control samples, 10µL of methyl acetate was introduced to the sampling apparatus. For low level fortification samples (LOQ) 10µL of 10µg/mL DMDS fortification solution was introduced (0.1 µg DMDS). For high level fortification samples (10xLOQ) 10µL of 100µg/mL DMDS fortification solution was introduced (1.0µg DMDS). Fortification solutions were injected into the side-arms of the flasks as shown in Figure 1. After 15 minutes the sample tubes were removed from the apparatus and capped until extraction. The front end (~400 mg) portion from each sample tube was removed from the tubes and placed in a 15 mL glass culture tube. The front portion also included both glass wool plugs (one in front and one separating the front and back portions). The back portion (~200mg) was placed in a separate 15 mL glass culture tube. The back portion did not include the foam plug at the end of the sample tube (the foam plug was discarded)

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Front and back portions from each sample tube were extracted by adding 5.0 mL methyl acetate via a volumetric pipette. The tubes were capped and vortexed for ~ 30 seconds. The samples were sonicated for ~ 2 minutes, then shaken on a shaker bath at room temperature for 30 minutes. After shaking, the samples were sonicated a second time for ~ 2 minutes. Finally, the samples were centrifuged at 2500 rpm for 5 minutes. The supernatant was removed from the charcoal and an aliquot was taken for GC-MSD (gas chromatography with mass selective detection) analysis.

GC/MS ANALYSIS

Instrumentation: Model No. 5890 Hewlett Packard Gas Chromatograph (GC) equipped with Mass Selective Detector (MSD) HP5971A

Column: Supelco SPB-1 Sulfur Capillary Column

30m x 0.32mm i.d. x 4.0 µm film thickness

Carrier Gas: Helium, Column Head Pressure = 5.0 psi.

Injector Temperature: 280°C

Detector Temperature: 300°C

MS Detector Settings: Selected Ion Mode (SIM)
Ions selected: m/z = 45, 61, 79 and 94 (dwell time = 50 ms)
(Ions entered 3x each)
Electron Multiplier Voltage offset = ~400
MS detector on @ 12.5 minutes, off @ 16.5 minutes.

Injection Volume: 2 µL; splitless, by Hewlett Packard 7673A Autosampler, silanized straight injection port liner (4mm I.D., splitless). Purge valve on at 2 minutes.

Oven Temperature:

Initial Temperature: 35°C for 3 minutes
Ramp: 35°C to 75°C at 3°C/minute
75°C to 250°C at 50°C/minute, 250°C for 2 minutes
Retention Time Dimethyl Disulfide: ~15 minutes

Prior to analysis the MSD is tuned using a manual tune file at 240°C oven temperature. The manual tuning procedure was used to optimize MS parameters using m/z ions 50, 69 and 100 from the instruments' PFTBA internal standard.

Separation of the analyte was achieved by capillary gas chromatography. The analyte was identified by the coincidence of its retention time with the calibration standard and quantified by integration of the peak areas relative to the calibration standard linearity curve. DMDS was quantified based on detection of ions at m/z 45, 61, 79 and 94. An electron impact-mass spectrum (EI-MS) was generated on a 100 µg/mL solution of DMDS to confirm ion abundance for analyte fragments.

The ILV set injection sequence was: conditioning standard, solvent blank, 0.005µg/mL linearity standard, control 1-front, control 1-back, control 2-front, control 2-back, 0.010 µg/mL linearity standard, low fort.1-front, low fort. 1-back, low fort. 2-front, low fort. 2-

back, 0.02 µg/mL linearity standard, low fort. 3-front, low fort. 3-back, low fort. 4-front, low fort. 4-back, 0.03 µg/mL linearity standard, low fort. 5-front, low fort. 5-back, high fort. 1-front, solvent blank, high fort. 1-back, 0.04 µg/mL linearity standard, high fort. 2-front, high fort. 2-back, high fort. 3-front, high fort. 3-back, 0.05 µg/mL linearity standard, high fort. 4-front, high fort. 4-back, high fort. 5-front, high fort. 5-back, 0.10 µg/mL linearity standard, low extraction fort., high extraction fort., 0.20 µg/mL linearity standard, linearity standard check sample (0.05µg/mL), linearity standard check sample 2 (0.05µg/mL).

Method Modifications

The modifications below are changes made from the method described in Reference 1.

1. The air sampling apparatus glassware was modified from that used by Enviro-Test Laboratories (Referred hereafter as Cerexagri apparatus, see Figure 2). The apparatus used in this study was shown to be equally effective to the apparatus used in the reference method. A comparison of the two apparatus systems was conducted and the data is discussed in the Results and Discussion section of this report. See Figures 1 and 2 for a comparison of the apparatus.

2. The reference method uses a Varian 3400CX gas chromatograph and Saturn 2000 mass spectra detector. This study used a HP5890 gas chromatograph and HP5971A mass selective detector. Because of the different instrumentation, the injection volume was reduced from 5µL to 2µL and a straight (splitless 4mm i.d.) injection port liner was used to avoid excessive vapor volume in the injection port. The detector temperature was also raised to 300°C. To maintain adequate sensitivity with the lower injection volume it was necessary to tune the detector on masses m/z 50, 69 and 100 prior to analysis.

METHODS OF CALCULATION

Preparation of Stock Standard

$$\text{Volume of solvent (mL)} = \frac{(W)x(P)}{(FC)}$$

where W = Milligrams of neat standard
P = Purity of neat standard
FC = Final Concentration (mg/mL)

Regression analysis with standard solutions

The peak area of DMDS of the calibration solutions is the dependent variable (y) and the concentration of DMDS of the calibration solutions is the independent variable (x). These values are used to generate a linear regression equation to determine the intercept, slope and correlation (r^2).

Peak area (y) = Intercept (b) + Slope (m) x concentration (x)

The slope and intercept obtained from this curve are used for the calculation of final extract concentration (µg/mL of DMDS) in the samples.

Calculation of Final Extract Concentration

The concentration of DMDS in the sample extracts were calculated as follows:

Linear regression formula from calibration curve y = mx + b (generated with Excel® program)

$$x = \mu g/mL = \frac{y - b}{m}$$

where y = Sample peak area
b = Calibration intercept
m = Slope

Calculation of DMDS Recovered (µg)

µg DMDS recovered = Final Ext. Conc. (µg/mL) x Final Volume (mL)

Calculation of % Recovery

$$\text{Percent Recovery} = \frac{\text{DMDS recovered } (\mu g)}{\text{DMDS fort. level } (\mu g)} \times 100$$

Sample Calculation

An example calculation from low fort.1-front:

Calibration curve : y = 56,717,978 x – 102,209
Final Extract Concentration = [821,961 – (-102,209)] ÷ 56,717,978
= 0.0163 µg/mL DMDS
where 821,961 = peak area sample
and –102,209 = linear regression intercept
and 56,717,978 = linear regression slope

DMDS Recovered = 0.0163 µg/mL DMDS x 5.0 mL
= 0.082 µg DMDS

Percent Recovery = (0.082 µg recovered ÷ 0.10 µg fortified) x 100
= 82%

Statistical Analysis

The residue data included the following statistical calculations: means, averages, relative standard deviations, percent deviations and linear regression analyses.

Time Required for Analysis

Time required per sample set, where a sample set consists of twelve (12) matrix samples extracted in two separate portions, two extraction fortification samples, 8 standards and 1 solvent blank:

Trapping and Extraction take approximately 8 hours for two analysts

GC/MS analysis takes approximately 16 hours

TOTAL = approximately 24 hours (2 calendar days)