NCASI METHOD RSC-02.02

REDUCED SULFUR COMPOUNDS BY DIRECT INJECTION GC/PFPD

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REDUCED SULFUR COMPOUNDS BY DIRECT INJECTION GC/PFPD

1.0 SCOPE AND APPLICATION

1.1 This method is used for the determination of the reduced sulfur compounds (RSCs) total sulfide as hydrogen sulfide (H_2S) [7783-06-4], methyl mercaptan (MeSH) [74-93-1], dimethyl sulfide (DMS) [75-18-3], dimethyl disulfide (DMDS) [624-92-0], and dimethyl trisulfide (DMTS) [3658-80-8] in wastewaters from pulp and paper mills. The RSCs are measured by direct aqueous injection gas chromatography with pulsed flame photometric detection (GC/PFPD).

1.2 The concentration of sulfide (H_2S) measured using this method represents the total amount of sulfide in the sample volatile at pH 2.5. It is believed that this includes all freely dissolved sulfide plus sulfide weakly associated with either dissolved organic matter or certain transition metals. If native sample pH is greater than 2.5, the actual total sulfide concentration in solution might be less than the concentration measured by this method.

1.3 The method has been applied to influent to wastewater treatment, samples from within the wastewater treatment system, and effluent from wastewater treatment.

1.4 This method has been validated for a single laboratory.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs and skilled in the interpretation of chromatograms. Each analyst must demonstrate an ability to generate acceptable results with this method.

2.0 SUMMARY OF THE METHOD

2.1 Samples are collected directly from the aqueous process stream or wastewater basin using appropriate collection vessels. Samples require two different preservation techniques to preserve all analytes. Samples are kept refrigerated until analysis.

2.2 In the laboratory, an aliquot of the sample is transferred to a 2-mL sealed vial. An aliquot of an internal standard solution is added to each of the vials. The sample is acidified (total sulfide only) and injected into the GC with a split injection. The GC column is temperature programmed to separate the analytes from other compounds which may be present in the sample. The analytes are selectively detected with a PFPD.

2.3 Identification of the RSCs is determined by comparison of their relative retention times with the relative retention times of an internal standard. If the results are questionable, confirmation using a second column may be necessary.

2.4 The RSCs are quantified by comparison with liquid standards using the internal standard technique. Multiple standards are analyzed to cover a calibration range of 20 to 1000 µg S/L. Calibration to lower concentrations may be possible for some compounds. Dilution is required to analyze samples with concentrations above 1000 µg S/L.

2.5 The method detection limit was calculated using the USEPA procedure in 40 CFR Part 136 Appendix B (Federal Register 1984) in a final effluent collected from an unbleached kraft mill after allowing the sulfide level to drop to less than 50 µg S/L. The method detection limit determined for total sulfide was 32.0 µg S/L. The sensitivity of the method has not been determined for MeSH, DMS, DMDS, and DMTS, and the detection limits have not been established. MeSH, DMS, DMDS, and DMTS have been successfully calibrated down to concentrations of 20 µg S/L.

2.6 Data quality is assured with ongoing recovery assessments, duplicate analyses, surrogate recovery experiments, matrix spike experiments, and blank analyses. MeSH, DMS, and DMDS standards are checked by comparing the results with an independently prepared standard. The sulfide standard is verified by independent analysis using EPA Methods 376.1 and 376.2.

3.0 DEFINITIONS

3.1 The definitions below are specific to this method, but conform to common usage as much as possible.

3.1.1 µg/L – micrograms of compound per liter

3.1.2 µg S/L – micrograms of sulfur per liter

3.1.3 May – this action, activity, or procedural step is neither required nor prohibited

3.1.4 Must not – this action, activity, or procedural step is prohibited

3.1.5 Must – this action, activity, or procedural step is required

3.1.6 Should – this action, activity, or procedural step is suggested, but not required

4.0 INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, injection port liners, and other sample processing hardware. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analyses by running laboratory blanks.

4.2 Glassware must be scrupulously cleaned, and glassware that comes in contact with concentrations less than 50 µg S/L may need to be deactivated. Glassware can be deactivated either by soaking in acid followed by silylation or by Siltek™ coating as described in Section 6.1.1. After use, clean all glassware by washing with mild

detergent in hot water and rinsing with tap water. The glassware should then be drained until completely dry.

4.3 It is required that all metal surfaces that come in contact with the sample be deactivated. This includes injection port liners, seals, and syringe needles. Deactivate the metal surfaces as described in Section 6.1.1.3.

4.4 The internal standard, thiophene, may be present in some pulp mill process streams. If the composition on a matrix is unknown, a sample analyzed without internal standard should be examined for the presence of thiophene. The surrogate, thioanisole, can be used as an internal standard if interference with thiophene is identified.

4.5 Some compounds can interfere with the chromatography if the separation is not efficient. Specific interference includes partial coelution of carbon disulfide with dimethyl disulfide. When performed properly, this method separates these compounds sufficiently. During the development of the method, carbon disulfide was not detected in any of the wastewater samples analyzed.

4.6 After a number of injections of samples, a sulfur dioxide artifact peak can interfere with methyl mercaptan. A clean, deactivated injection port liner should be installed after approximately 20 sample injections. The injection port gold seal should also be cleaned with deionized water, methanol, and acetone using a long cotton swab prior to inserting the clean injection port liner during liner changes.

5.0 SAFETY

5.1 All chemicals should be treated as potential health hazards. It is recommended that prudent practices for handling chemicals in the laboratory be employed (NRC 1995).

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness of OSHA regulations regarding safe handling of chemicals used in this method. Material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.3 The RSCs are either flammable gases or liquids that may be harmful if inhaled or ingested. These compounds can also cause a considerable nuisance odor. Use them in a laboratory fume hood and wear appropriate gloves, eye protection, and other protective clothing.

6.0 EQUIPMENT AND SUPPLIES

***Note**: Brand names and suppliers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and material other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sampling Equipment

6.1.1 Samples are to be collected in amber glass bottles with minimal headspace. It is recommended that 40-mL amber, borosilicate glass vials with Teflon™ faced silicone backed lids (VOA vials) be used. Although passivation of glassware for RSC compounds is common practice, passivation of sample containers during this study has not been found to be necessary in the standard operating range of this method. Some improvement of the lower level calibration response has been found when using passivated autosampler vials. If passivation of glassware is desired, one of the following techniques can be used.

6.1.1.1 Soak clean glassware in a 10% HCl solution for at least one hour. Rinse the glassware thoroughly with water, followed by an acetone rinse, air drying, and treatment with 5% dimethyldichlorosilane in toluene. Rinse the glassware with toluene, methanol, and water, then air dry it.

6.1.1.2 Treat clear VOA vials with the Siltek deactivation process (Restek Corporation, Bellefonte, PA). Caution: strong caustic detergents will remove the Siltek coating.

6.1.1.3 Treat syringe needles by slowly pumping a 15% solution of BSTFA in hexane three times followed by a rinse with acetone, methanol, and water.

6.1.2 The use of automatic sample collection equipment has not been validated for this method and should not be incorporated until its effectiveness has been proven.

6.2 Laboratory Glassware and Supplies

6.2.1 Amber 2-mL autosampler vials deactivated if desired by one of the methods described in Section 6.1.1

6.2.2 Volumetric flasks (10-mL, 50-mL)

6.2.3 Syringes (including gas-tight syringes) deactivated by methods described in Section 6.1.1.3.

6.3 Analytical Equipment

6.3.1 Gas chromatography system – gas chromatography analytical system complete with a cryogenically cooled, temperature programmable gas chromatograph with a split/splitless injection port and all required accessories including syringes, analytical columns, and gases

6.3.2 Injection port liner – 4-mm deactivated (silanized or Siltek) straight glass liner lightly packed with a plug of deactivated (silanized) quartz two-thirds the distance from the septum end of the liner (Section 17, Figure 1)

6.3.3 Column – 30 m x 0.25 mm x 1.4 μm, 6% cyanopropylphenyl 94% dimethylpolysiloxane bonded phase (624 phase) fused silica capillary column

6.3.4 GC detector – pulsed flame photometric detector (OI Analytical or equivalent) with appropriate data system

7.0 REAGENTS AND STANDARDS

7.1 Reagents

7.1.1 Deionized (DI) water should be tested immediately before use to verify the absence of any target analytes. If the water is contaminated, it may be necessary to prepare fresh deionized water, purge the water with nitrogen or helium, or boil the water to remove the contaminant(s).

7.1.2 Prepare phosphoric acid solution by combining one part of phosphoric acid (reagent grade) with three parts deionized water.

7.1.3 Prepare acidified DI water by adding phosphoric acid solution (Section 7.1.2) to DI water (Section 7.1.1) until the pH is between 2.3 and 2.7. It takes approximately 0.5 mL of acid in 1 L of water to reach this pH.

7.1.4 L-Ascorbic acid (ACS reagent grade)

7.1.5 Methanol (distilled in glass)

7.1.6 Prepare the zinc acetate solution (40 mmole/L) by adding 1.75 g of zinc acetate dehydrate (reagent grade) to 200 mL of DI water. Slowly adjust the pH drop wise by adding 1N NaOH while stirring the DI water containing the zinc acetate (this takes 20 to 30 minutes). Dropwise addition is important up to pH 8.0 in order to produce small crystals of the resulting salt which will homogenize upon shaking. Once pH 8.0 is achieved dropwise addition is not longer required. Finish adjusting the pH to between 12 and 12.5 using the 1N NaOH solution (total 1N NaOH required is approximately 20 mL). This solution should produce a fine, even suspension which does not settle rapidly. If you shake the container and then let it sit, it will usually remain in suspension for over 20 minutes.

7.1.7 Prepare dimethyldichlorosilane (DMDCS) 5% in toluene by adding 25 mL of DMDCS to 475 mL of toluene. It is also available as a mixture from Supelco as Sylon CT.

7.1.8 Prepare N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) 15% in hexane by adding 1.5 mL of BSTFA to 8.5 mL of hexane.

7.1.9 Toluene (distilled in glass)

7.1.10 Hexane (distilled in glass)

7.1.11 $CaCl_2$ desiccant, 96%+ ACS reagent grade

7.1.12 Prepare NaOH 1 N by dissolving 40 g of pellets (97+%) into 1 L of DI water.

7.2 Analytical Standards

Analytical standards are prepared from pure standards. Reported purity should be greater than 95% for all the neat material used.

7.2.1 Prepare the internal standard primary solution by weighing 26 mg (to the nearest 0.1 mg) of thiophene and diluting to 10 mL in volumetric flasks with methanol. Prepare the primary standard at a concentration of approximately 1 mg S/mL . Calculate the actual concentration using Equation 1.

Equation 1

$$C_S = \frac{(m * FS)}{V_S}$$

where: C_S *is the concentration of sulfur in the standard (mg S/mL)*
m is the mass of the compound added to the standard (mg)
FS is the fraction of sulfur in the compound (Section 17, Table 6 except for $NaS_2 \bullet 9H_2O$*, which is 0.1335)*
V_S *is the total volume of the standard (mL)*

7.2.2 Prepare the surrogate standard primary solution by weighing, to the nearest 0.1 mg, 40 mg of thioanisole and diluting to 10 mL in volumetric flasks with methanol. Prepare the primary standard at a concentration of approximately 1 mg S/mL. Calculate the actual concentration using Equation 1.

7.2.3 Prepare a combined internal standard and surrogate working solution by adding 400 µL of each primary stock (Sections 7.2.1 and 7.2.2) to a 10-mL volumetric flask and diluting to the mark with methanol. The concentration in the solution is approximately 40 µg S/mL for each compound.

7.2.4 Prepare a primary and working standard of sulfide from sodium sulfide nonahydrate (Na_2S). The $Na_2S \bullet 9H_2O$ should be either opaque or white crystals. This material is hydroscopic and will turn into a slurry if not stored in a dry environment such as a desiccator containing anhydrous $CaCl_2$ and wrapped with tape to seal the bottle. It will also turn yellow or green (elemental sulfur) in storage. Prepare the working solution by adding 340 mg of zinc acetate dihydrate to 40 mL of purged DI water. Slowly adjust the pH drop wise by adding 1N NaOH while stirring the water containing the zinc acetate (this takes 10 to 20 minutes). Dropwise addition is important up to pH 8.0 in order to produce small crystals of the resulting salt which will homogenize upon shaking. Once pH 8.0 is achieved dropwise addition is not longer required. Finish adjusting to between 10.5 and 11 using the 1 N NaOH solution. Add 38 mg of $Na_2S \bullet 9H_2O$, weighed to the nearest 0.1 mg, while continuing to stir for 5 minutes. This solution should be a well dispersed suspension with no visible clumping of the solids. Transfer the solution quantitatively into a 50-mL volumetric flask and dilute to the mark with purged DI water. The concentration in the solution will be approximately 100 µg S/mL, with an equivalent total sulfide concentration of 106 µg/mL.

Calculate the actual concentration using Equation 1. The fraction of sulfur (FS) in $Na_2S \bullet 9H_2O$ is 0.1335.

7.2.5 Prepare a primary solution of MeSH by slowly bubbling MeSH gas into a tared 10-mL volumetric flask containing methanol. Allow the MeSH to dissolve into the methanol until approximately 15 mg (weighed to the nearest 0.1 mg) has been added. This corresponds to approximately 7.5 mL of pure gas at room temperature. Use a thin (1/16 inch) Teflon line to transfer the MeSH into the methanol and be sure that any methanol clinging to the line is knocked back into the volumetric flask before measuring the final weight. Dilute to the mark with methanol for a concentration of approximately 1 mg S/mL or 1.6 mg/mL as MeSH. Calculate the actual concentration using Equation 1.

7.2.6 Prepare a primary solution of DMS by weighing 19 mg (to the nearest 0.1 mg) of DMS into a 10-mL volumetric flask containing methanol. Dilute to the mark with methanol for a concentration of approximately 1 mg S/mL or 1.9 mg/mL as DMS. Calculate the actual concentration using Equation 1.

7.2.7 Prepare a primary solution of DMDS by weighing 15 mg (to the nearest 0.1 mg) of DMDS into a 10-mL volumetric flask containing methanol. Dilute to the mark with methanol for a concentration of approximately 1 mg S/mL or 1.5 mg/mL as DMDS. Calculate the actual concentration using Equation 1.

7.2.8 Prepare a primary solution of DMTS by weighing 13 mg (to the nearest 0.1 mg) of DMTS into a 10-mL volumetric flask containing methanol. Dilute to the mark with methanol for a concentration of approximately 1 mg S/mL or 1.3 mg/mL as DMTS. Calculate the actual concentration using Equation 1.

7.2.9 Prepare a working solution of MeSH by adding 1.0 mL of the primary solution (Section 7.2.4) to a 10-mL volumetric flask and diluting with methanol. MeSH is not stable when mixed with the other standards.

7.2.10 Prepare a primary solution of carbon disulfide (CS_2) by weighing 12 mg (to the nearest 0.1 mg) of CS_2 into a 10-mL volumetric flask containing methanol. Dilute to the mark with methanol for a concentration of approximately 1 mg S/mL or 1.2 mg/mL as CS_2. Calculate the actual concentration using Equation 1.

7.2.11 Prepare a working solution of mixed RSCs and CS_2 by adding 1.0 mL of the primary solutions of DMS (Section 7.2.6), DMDS (Section 7.2.7), DMTS (Section 7.2.8), and CS_2 (Section 7.2.10) to a 10-mL volumetric flask and diluting with methanol.

7.3 Calibration Standards

7.3.1 Prepare a multilevel calibration working solution by adding 500 µL of each of the individual working solutions of sulfide (Section 7.2.4), MeSH (Section 7.2.9), and mixed RSCs (Section 7.2.11) to a 5-mL volumetric flask. Dilute to the mark with purged DI water and adjust the pH to around 2.5 with

phosphoric acid solution. The calibration working solution has limited stability and should be prepared the day it is used.

7.3.2 Prepare a nominal 20 µg S/L calibration standard by adding 4.0 µL of the multipoint calibration solution (Section 7.3.1) to 1.8 mL of pH 2.5 adjusted DI water (Section 7.1.3) in a 2-mL autosample vial. Add 9 µL of the internal standard working solution (Section 7.2.3) for a nominal internal standard concentration of 200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

Equation 2

$$C_{cal} = \frac{C_{WS} * V_{WS}}{V_{cal}}$$

where: C_{cal} *is the concentration of the analyte/internal standard in the calibration standard (µg S/L)*
C_{WS} *is the concentration of the analyte in the working solution (µg S/mL)*
V_{WS} *is the volume of working solution added to the calibration standard (mL)*
V_{cal} *is the volume of the calibration standard (0.002 L)*

7.3.3 Prepare a nominal 50 µg S/L calibration standard by adding 10 µL of the multipoint calibration solution (Section 7.3.1) to 1.8 mL of pH 2.5 adjusted DI water (Section 7.1.3) in a 2-mL autosample vial. Add 9 µL of the internal standard working solution (Section 7.2.3) for a nominal internal standard concentration of 200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

7.3.4 Prepare a nominal 200 µg S/L calibration standard by adding 40 µL of the multipoint calibration solution (Section 7.3.1) to 1.8 mL of pH 2.5 adjusted DI water in a 2-mL autosampler vial. Add 9 µL of the internal standard working solution (Section 7.2.3) for a nominal internal standard concentration of 200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

7.3.5 Prepare a nominal 500 µg S/L calibration standard by adding 100 µL of the multipoint calibration solution (Section 7.3.1) to 1.7 mL of pH 2.5 adjusted DI water in a 2-mL autosample vial. Add 9 µL of the internal standard working solution (Section 7.2.3) for a nominal internal standard concentration of 200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

7.3.6 Prepare a nominal 1000 µg S/L calibration standard by adding 200 µL of the multipoint calibration solution (Section 7.3.1) to 1.6 mL of pH 2.5 adjusted DI water in a 2-mL autosample vial. Add 9 µL of the internal standard working solution (Section 7.2.1) for a nominal internal standard concentration of

200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

7.3.7 Prepare a daily calibration check standard (200 µg S/L) by adding 4.0 µL of the working standards of sulfide (Section 7.2.4), MeSH (Section 7.2.9), and mixed RSCs (Section 7.2.11) to 1.8 mL of pH 2.5 adjusted DI water (Section 7.1.3) in a 2-mL autosample vial. Add 9 µL of the internal standard working solution (Section 7.2.1) for a nominal internal standard concentration of 200 µg S/L. Calculate the concentration of each of the analytes and the internal standard using Equation 2.

7.3.8 When preparing standards or samples, the autosampler vial has an air bubble after being sealed. This is important so that the analyte and internal standard spikes can be mixed well before analyzing the sample or standard. At least three good inverted shakes should be performed before injecting the standard or sample.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Collection

Collect grab samples directly from the process liquid stream using appropriate collection vessels, typically 40-mL VOA amber vials. Fill each vial with the sample, leaving minimum headspace. Collect a separate sample for analyzing total sulfide because of the preservation technique. A substantial quantity of preservative is required, so a dilution factor is needed to correct for dilution due to preservation. This can be accomplished by measuring the volume of preservative added and the final volume of the sample including preservative.

8.2 Preservation

8.2.1 Preservation for the analysis of MeSH, DMS, DMDS, and DMTS requires the addition of 120 mg of ascorbic acid to a 40-mL VOA vial (3 g/L) and pH adjustment to <2.5 with phosphoric acid solution. To adjust the pH, add a representative sample to an extra vial containing ascorbic acid. Measure the volume of phosphoric acid required to reach the target pH and discard that sample. Use that volume of acid to adjust the samples to be analyzed. If the volume of acid needed is less than 2 mL, no correction for dilution is required.

8.2.2 Preservation for the analysis of total sulfide requires the addition of 5 mL of zinc acetate solution (Section 7.1.6) to a 40-mL VOA vial. The final pH of the sample should be greater than 10. Adjust the pH with 1 N NaOH solution if necessary. A correction for the dilution of the sample by the preservative must be made. For example, if 35 mL of sample is diluted to 40 mL, the measured concentration should be multiplied by a dilution factor of 1.14. Sample volumes can be measured gravimetrically or using calibrated glassware (graduated cylinder).

8.3 Storage

All samples must be stored in a refrigerator (4°C) until analysis. Storage stability has been found to be matrix dependent. Using the prescribed preservation techniques, greater than 80% recovery was found for all compounds in both a bleached kraft mill effluent and an unbleached kraft mill effluent after 14 days of storage. Storage of zinc acetate preserved samples with native concentrations of <0.1 mg S/L collected in highly aerated portions of WWTP have yielded increasing concentrations of total sulfide over time.

9.0 QUALITY CONTROL

To control the quality of the data generated using this method, an initial calibration check, independent standard check, daily blank checks, daily calibration checks, surrogate recovery experiments, periodic duplicates, and periodic matrix spikes should be performed.

9.1 Initial Calibration Check

A multipoint internal standard calibration should be performed covering the operating range of the method (20 to 1000 µg S/L). A wider or narrower range is acceptable if all sample concentrations fall within that range. The criterion for acceptable linearity is a mean absolute percent error (MAPE) for the curve of less than or equal to 20% (Section 10.2.3).

9.2 Independent Standard Check

When a primary standard is prepared for calibration and matrix spike experiments, it should be compared with an independent standard either prepared from another source of compound or obtained from a certified standard vendor. Only methyl mercaptan, dimethyl sulfide, and dimethyl disulfide are commercially available as solutions in methanol at this time (Crescent Chemicals). The independent standard should match the primary standard used for calibration and matrix spikes within 30%. This check will minimize bias due to errors in standard preparation.

9.3 Daily Blank Checks

A daily blank check should be performed before running samples. A blank check should be performed if carryover is suspected (e.g., after running a sample outside the calibration range). A blank check consists of analyzing 1.8 mL of purged DI water with internal standard and surrogate as described in Section 11.1. The RSC level in the blank should not exceed 20% of the lowest calibration point (4 µg S/L for MeSH, DMS, DMDS, and DMTS; 6 µg S/L for total sulfide).

9.4 Daily Calibration Checks

Prepare and analyze a mid-level calibration point every day that samples are analyzed. The percent recovery of each compound in the standard should be within 20% of the percent recovery of the same calibration level in the multipoint calibration. If the daily calibration check fails, it should be repeated. If it fails a second time, the standards (working, primary, internal standard) should be

reprepared. If it continues to fail, the multipoint calibration should be repeated. A summary of single laboratory daily calibration checks for this method is provided in Section 17, Table 1.

9.5 Surrogate Recovery Check

In this method thioanisole is utilized as a surrogate for the reduced sulfur compounds. All samples are spiked with 9 μL of the thioanisole spiking solution (Section 7.2.1) to monitor surrogate recovery. The percent recovery of the surrogate should be determined and the results charted to document the surrogate recovery of the method. Performance criteria for acceptable surrogate recovery, as determined during a single-laboratory validation of this method, are presented in Section 17, Table 2.

9.6 Duplicate Analyses

A duplicate sample should be analyzed with each set of samples (batch of samples no greater than 20). Duplicate analysis requires the analyses of separate aliquots of the sample. The relative percent difference between the two samples should be calculated and charted to estimate the method's precision. Section 17, Table 3 lists the relative percent differences found during a single laboratory validation of the method.

9.7 Matrix Spike Analyses

A matrix spike analysis should be performed with each set of samples (batch of samples no greater than 20). A known amount of the RSC working solutions should be added to a sample so that the native plus the spike level of each RSC is at least one times the native level. The percent recovery of the matrix spike should be determined and the results charted to document the recovery of the method. Section 17, Table 3 lists the recovery found during single laboratory validation studies.

9.8 Field Replicates and Field Spikes

Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of sampling and sample transporting techniques.

9.9 Resolution Checks

The resolution of the separation should be checked periodically (ideally on a daily basis) by measuring the valley between the DMS and CS_2 peaks. The valley should be less than 10% of the average peak heights of the two peaks. If the valley is 10% or greater, maintenance of the injection port and/or column is necessary.

10.0 CALIBRATION AND STANDARDIZATION

10.1 GC/PFPD Operating Conditions

Assemble the GC/PFPD and establish the operating conditions outlined in Section 17, Table 4. Use the conditions specified by the PFPD manufacturer to optimize for the detection of sulfur compounds. Once the GC/PFPD system is optimized for

analytical separation and sensitivity, the same operating conditions must be used to analyze all samples, blanks, calibration checks, and quality assurance samples.

If excessive peak broadening is observed for sulfide and MeSH, a pressure pulse during the injection might keep the injection focused on the column. This has been necessary when using autoinjectors with a rapid injection stroke. An initial pressure of 30 psi for 0.2 min followed by a rapid drop back to a constant flow of 1.2 mL/min sharpened the early eluting peaks. Keep the pressure pulse time to a minimum because the PFPD loses its sulfur response at high carrier gas flow rates.

10.2 Initial Multipoint Calibration

The square root of the PFPD response for sulfur is approximately linear with respect to concentration over the operating range of the method. To demonstrate this and establish a calibration function for the method, prepare and analyze calibration standards to cover this range. The internal standard calibration approach should be used for this method. Calibrate the RSCs using concentrations normalized to the sulfur content of the standard. The use of sulfur concentrations ensures that the concentrations prepared cover the operating range of the detector. It also allows the relative response factors to be checked, because, theoretically, they should all be 1.

10.2.1 Determine the retention times of the analytes by analyzing a daily calibration solution (Section 7.3.7). A chromatogram similar to that shown in Section 17, Figure 2 should be obtained. Identify the peaks and determine their relative retention times using Equation 3. Section 17, Table 6 lists the relative retention times for the RSCs using this method.

Equation 3

$$RRT_i = \frac{RT_i}{RT_{IS}}$$

where: RRT_i *is the relative retention time for compound i*
RT_i *is the retention time for compound i*
RT_{IS} *is the retention time for the internal standard*

10.2.2 Prepare a five point calibration curve to determine the relationship between instrument response and concentration over the operating range for each analyte. Analyze each of the calibration standards prepared as described in Sections 7.3.2 through 7.3.7.

10.2.3 The results of the calibration standard analyses for each compound are either fitted to a quadratic equation or described by an average relative response factor using internal standard calibration techniques. To find the best quadratic fit for the data, plot the response ratio of each compound as calculated in Equation 4 versus the ratio of the standard concentration versus the internal standard concentration. Curve fitting software either in the data system (e.g., Agilent Chemstation) or external to the data system (e.g., Excel) can be used to fit the best quadratic equation in the form of Equation 5.

Equation 4

$$RR = \frac{A_i}{A_{IS}}$$

where: RR is the response ratio
A_i is the area of the peak for compound i
A_{IS} is the area of the internal standard peak

Equation 5

$$RR_i = a + b * C_R + c * C_R^2$$

where: RR is the response ratio
a is the y-intercept from the quadratic regression
b is the linear constant from the quadratic regression
C_R is the ratio of the compound concentration versus the internal standard concentration
c is the quadratic constant from the quadratic regression

If the calibration criteria cannot be met using a quadratic fit, the average response factor can be used. Calculate the average response factor by finding the mean of the relative response factors calculated for each concentration of standard, as shown in Equation 6.

Equation 6

$$RRF_i = \left(\frac{A_i \times C_{IS}}{A_{IS} \times C_{cal}} \right)$$

where: RRF_i is the relative response factor for compound i
A_i is the area of the peak for compound i
A_{IS} is the area of the internal standard peak
C_{cal} is the concentration as sulfur in the calibration standard (µg S/L)
C_{IS} is the concentration of internal standard as sulfur (µg S/L)

To evaluate the closeness of the fit for the calibration, use the calibration model chosen (quadratic curve or average response factor) to calculate the concentration for each calibration level. Use Equation 7 to calculate the concentration using the quadratic model or Equation 8 to calculate the average response factor model. Determine the error for each level and calculate the mean absolute percent error (MAPE) as shown in Equation 9. The MAPE is used by software packages such as SAS and Statgraphics to evaluate the fit between a model prediction and the measured values.

Equation 7

$$C_i = \frac{\left(-b + \sqrt{b^2 - 4c(a - RR)}\right)}{2c} * C_{IS}$$

where: C_i *is the measured concentration of compound i (µg S/L)*
a is the y-intercept from the quadratic regression
b is the linear constant from the quadratic regression
c is the quadratic constant from the quadratic regression
RR is the response ratio
C_{IS} *is the concentration of internal standard as sulfur (µg S/L)*

Equation 8

$$C_i = \frac{RR}{RRF} * C_{IS}$$

where: C_i *is the measured concentration of compound i (µg S/L)*
RR is the response ratio
RRF is the relative response factor
C_{IS} *is the concentration of internal standard as sulfur (µg S/L)*

Equation 9

$$MAPE = \frac{\sum \left| \frac{C_{cal} - C}{C_{cal}} \right| * 100}{n}$$

where: MAPE is the mean absolute percent error
C_{cal} *is the concentration in the calibration standard*
C is the concentration measured for the calibration level
n is the number of calibration levels

The MAPE should be below 20% for each compound. Section 17, Table 5 lists the MAPE found for several calibrations using both an average and a quadratic calibration model. Section 17, Figure 3 shows a typical calibration curve for the PFPD response with a quadratic fit.

If a 20% MAPE cannot be achieved, one or more of the following actions should be taken.

10.2.3.1 Standards should be reanalyzed if the analysis appears to be suspect due to large variation from predicted response.

10.2.3.2 Standards should be reprepared if they appear to be suspect after reanalysis.

10.2.3.3 System maintenance should be performed, including replacing the injection port liner, replacing the septum, clipping the column, checking the split ratio, and checking the detector parameters.

10.2.3.4 The calibration range may be reduced by eliminating the low level or high level calibration standard. If the calibration range is changed, do not report values that are measured outside this range. This is especially true for the quadratic model, where large errors can occur.

10.3 Daily Calibration Check

Prior to analyzing samples each day, a daily calibration check should be prepared (Section 7.3.7) and analyzed. Calculate the percent recovery of the standard using Equation 10 to verify the calibration. In-house percent recovery control limits should be determined, and should not exceed ±20%. If the calibration check does not pass, the action items in Section 10.2.3 should be repeated. If these fail, the initial multipoint calibration should be repeated. Section 17, Table 1 summarizes the results for daily calibration checks during the method evaluation and subsequent single laboratory analyses.

Equation 10

$$R = \left(\frac{C_i}{C_{IC}} \right) \times 100$$

where: R is the recovery in percent
C_i is the measured concentration for compound i (µg S/L)
C_{IC} is the concentration measured during the initial calibration (µg S/L)

10.4 Blank Analysis

A method blank should be prepared and analyzed with the initial calibration and every day on which samples are analyzed. Prepare the blank the same as the calibration standards, but only add the internal standard solution (Section 7.3). The blank concentration should be less than 20% of the lowest calibration point. High blank levels can be caused by contaminated reagent water/acid, contaminated internal standard, contaminated glassware or syringes, and dirty injection ports. Resolution of sulfur dioxide, a common contaminate, from methyl mercaptan is critical for meeting the blank criteria. Section 17, Figure 4 shows a typical sample with MeSH resolved from the artifact peak.

11.0 PROCEDURE

11.1 Sample Analysis

Transfer a known volume (1.8 mL) of the sample to a autosample vial using a deactivated gas-tight syringe. If the sample is preserved at pH 2.5 no pH adjustment is required. If the sample is preserved at pH 10, phosphoric acid solution should be added to bring the pH to between 1.5 and 2.5. Determine the amount of acid needed using a trial sample, then add the determined amount to the sample to be analyzed (typically 15 to 20 µL). Add 9 µL (assuming a sample volume of 1.8 mL) of the internal standard solution (40 mg S/L thiophene and thioanisole) to the vial. Be sure that the spike goes into the sample liquid and that it is well mixed (Section 7.3.8). Inject the sample using the exact instrumental conditions used for the analysis of the calibration standards (Section 10.1). Calculate the concentration of each RSC using Equation 7 or 8, depending on the calibration model. If the concentration is above the calibration range, the sample must be diluted and reanalyzed.

11.2 Dilution

If dilution is necessary, inject some fractional volume less than 1.8 mL into the vial using a deactivated gas-tight syringe, bring it to 1.8 mL with DI water pH adjusted to 2.5, and analyze it as described in Section 11.1. Calculate the dilution factor by dividing 1.8 mL by the volume of sample used. For samples preserved for total sulfide analysis, dilution by the preservative must also be accounted for by multiplying the two dilution factors together.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Identification of Compounds

An analyte is identified by comparison of the relative retention time of the sample with the relative retention time of an authentic standard of the target compound analyzed using the same analytical conditions. Section 17, Table 6 lists the relative retention time windows for the RSCs and the absolute retention time windows for the internal standards.

12.2 Quantification of Compounds

Measure the concentration of each analyte as sulfur using Equation 7 or 8, then adjust for dilution and percent sulfur using Equation 11 to report the concentration as mass of compound instead of sulfur. The fraction of sulfur in each compound can be found in Section 17, Table 6.

Equation 11

$$C = \frac{C_i * DF}{FS}$$

where: C is the concentration of compound in the sample (µg/L)
C_i is the measured concentration for compound i (µg S/L)
DF is the dilution factor
FS is the fraction of sulfur in the compound

12.3 Duplicate Precision Estimate

Duplicate samples should be analyzed with each set of samples. Calculate the relative percent difference (RPD) for each duplicate pair as shown in Equation 12.

Equation 12

$$RPD = \frac{2 * |C_1 - C_2|}{(C_1 + C_2)} \times 100$$

where: RPD is the relative percent difference in the two determinations
C_1 is the first concentration measured (µg/L)
C_2 is the second concentration measured (µg/L)

12.4 Matrix Spike Calculation

A matrix spike experiment should be performed with each set of samples analyzed. Calculate the percent recovery using Equation 13.

Equation 13

$$R = \frac{(C_{MS} - C)}{C_S} \times 100$$

where: R is the percent recovery
C_{MS} is the concentration measured in the matrix spiked sample (µg/L)
C is the concentration measured in the unspiked sample (µg/L)
C_S is the theoretical concentration of the spiked compound (µg/L)

13.0 METHOD PERFORMANCE

13.1 Single laboratory performance of this method is detailed in Section 17, Tables 2 and 3. Single laboratory precision is estimated to be 12.3% MeSH and 10% or less for the other RSCs. The average matrix spike recoveries ranged from 93 to 112% for all target analytes. The average surrogate spike recovery was 106%.

13.2 Interlaboratory precision estimates have not been determined for this method.

14.0 POLLUTION PREVENTION

14.1 The laboratory should check state and local requirements to determine if pollution prevention equipment is required or recommended in its area.

15.0 WASTE MANAGEMENT

15.1 It is the responsibility of the laboratory to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and lands by minimizing releases into the environment. Compliance with all sewage discharge permits and regulations is also required.

16.0 REFERENCES

16.1 National Research Council (NRC) 1995. *Prudent Practices in the Laboratory*. National Academy Press. Washington, DC

16.2 Taylor, J.K. 1987. *Quality Assurance of Chemical Measurements.* Lewis Publishers. Chelsea, Michigan

17.0 TABLES AND DIAGRAMS

Table 1. Results of Daily Calibration Checks

Compound	Mean Recovery	RSD (%)	n
Total sulfide	106	11.2	94
Methyl mercaptan	95.0	10.6	42
Dimethyl sulfide	100	10.5	42
Dimethyl disulfide	111	8.3	42
Dimethyl trisulfide	102	11.5	42

Table 2. Surrogate Recovery

Compound	Mean Recovery	RSD (%)	n
Thioanisole	106	6.7	1077

Table 3. Duplicate Results and Matrix Spike Recovery

	Duplicate Precision		Matrix Spike Recovery		
Compound	Pooled RSD[a] (%)	n	Mean Recovery (%)	RSD (%)	n
Total sulfide	9.4	87	93	20.7	70
Methyl mercaptan	12.3	33	106	20.0	33
Dimethyl sulfide	5.6	34	102	11.7	34
Dimethyl disulfide	7.0	33	112	16.5	34
Dimethyl trisulfide	4.7	25	96	24.1	34

[a] equation for pooled relative standard deviation can be found in Taylor 1987

Table 4. GC/PFPD Operating Conditions for Measuring Reduced Sulfur Compounds

Injection port	split (15:1 ratio)
Injection volume	2 µL
Split vent flow rate	16 mL/min helium
Injector temperature	110°C
Injection liner	4 mm id with fused silica wool packing (deactivated, either Siltek or Silanized)
Carrier gas	helium
Carrier gas flow rate	constant flow mode at 1.2 mL/min (pressure pulse at injection might be necessary see Section 10.1)
Column	J&W DB-624, 30 m x 0.25 mm id with 1.4 µm film fused silica capillary column or equivalent
Oven temperature program	
Initial	10°C
Ramp 1	6°C/min to 35°C for 2 minutes
Ramp 2	8°C/min to 170°C
Ramp 3	40°C/min to 250°C for 3 minutes
Detector	PFPD (OI model 5380 or equivalent)
Temperature	250°C
Combustion tube	2 mm
Optical filter	BG-12 (purple)
Hydrogen flow	11 mL/min
Air flows	optimized as described by manufacturer
Pulse rate	3.1 Hz
Signal	square root of PMT signal

Table 5. Summary of Initial Calibration Results

	Average Response Factor		Quadratic Fit			
Compound	Mean RRF[a]	Mean MAPE[b]	Mean a[c]	Mean b[d]	Mean c[e]	Mean MAPE[b]
Total sulfide	0.641	30.2	-0.073	0.838	-0.006	20.7
Methyl mercaptan	0.673	21.5	-0.074	0.906	-0.025	14.8
Dimethyl sulfide	0.887	18.0	-0.062	1.094	-0.013	14.8
Dimethyl disulfide	0.983	16.8	-0.092	1.385	-0.083	13.0
Dimethyl trisulfide	0.989	16.3	-0.092	1.401	-0.089	12.1

[a] average of eight calibration sets' mean relative response factors
[b] average of fifteen calibration sets' mean absolute percent errors
[c] average of eight calibration sets' y-intercepts from a quadratic regression
[d] average of eight calibration sets' linear constants from a quadratic regression
[e] average of eight calibration sets' quadratic constants from a quadratic regression

Table 6. Retention Time Statistics for RSCs and Sulfur Fraction

Compound	Mean[a] RRT	RSD[b] (%)	Relative Retention Time Window[c]	Fraction Sulfur
Total sulfide	0.192	1.12	0.186 – 0.198	0.9408
Methyl mercaptan	0.318	0.84	0.310 – 0.326	0.6665
Dimethyl sulfide	0.527	0.57	0.518 – 0.536	0.5160
Dimethyl disulfide	1.217	0.11	1.213 – 1.221	0.6808
Dimethyl trisulfide	1.748	0.19	1.738 – 1.758	0.7618
Internal standards	Mean RT[d] (min)	RSD[b] (%)	Retention Time Window	Fraction Sulfur
Thiophene	11.37	0.37	11.24 – 11.49	0.3810
Thioanisole	22.41	0.17	22.52 – 22.29	0.2581

[a] mean relative retention time (relative to thiophene) for 30 calibration standard analyses
[b] relative standard deviation for 30 calibration standard analyses
[c] windows are calculate from the mean value ± three times the standard deviation
[d] mean retention time for 30 calibration standard analyses

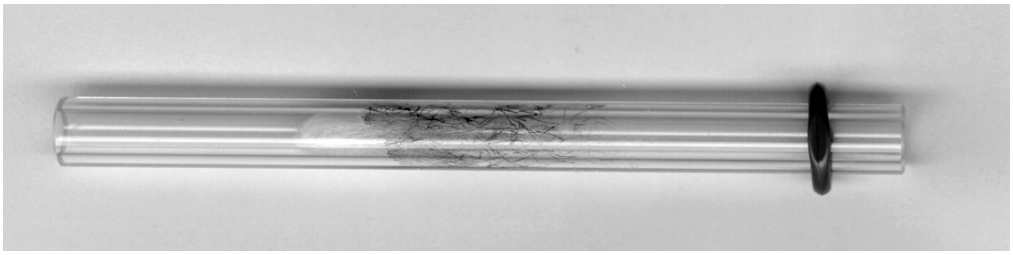

Figure 1. Injection Port Liner with Glass Wool Plug and Deposits from Approximately 20 injections Containing 3 g/L Ascorbic Acid

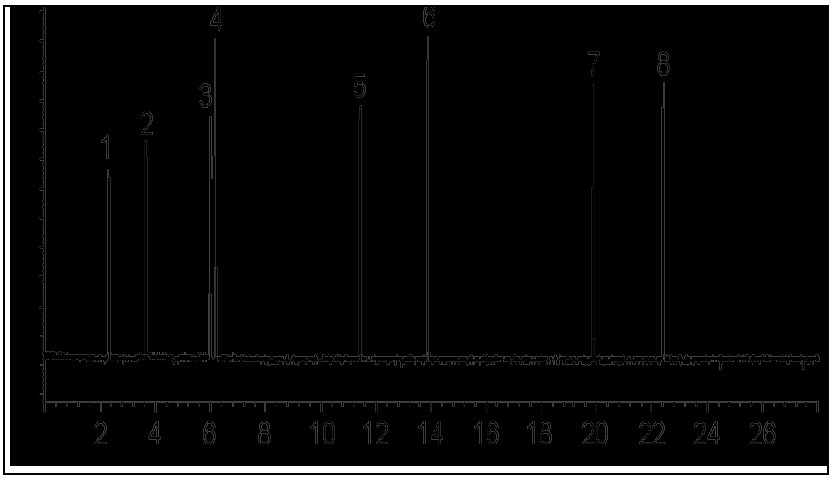

Figure 2. Chromatogram of 200 µg S/L Standard Containing (1) Total Sulfide; (2) MeSH; (3) DMS; (4) CS_2 (resolution check compound); (5) Thiophene (internal standard); (6) DMDS; (7) DMTS; (8) Thioanisole (internal standard)

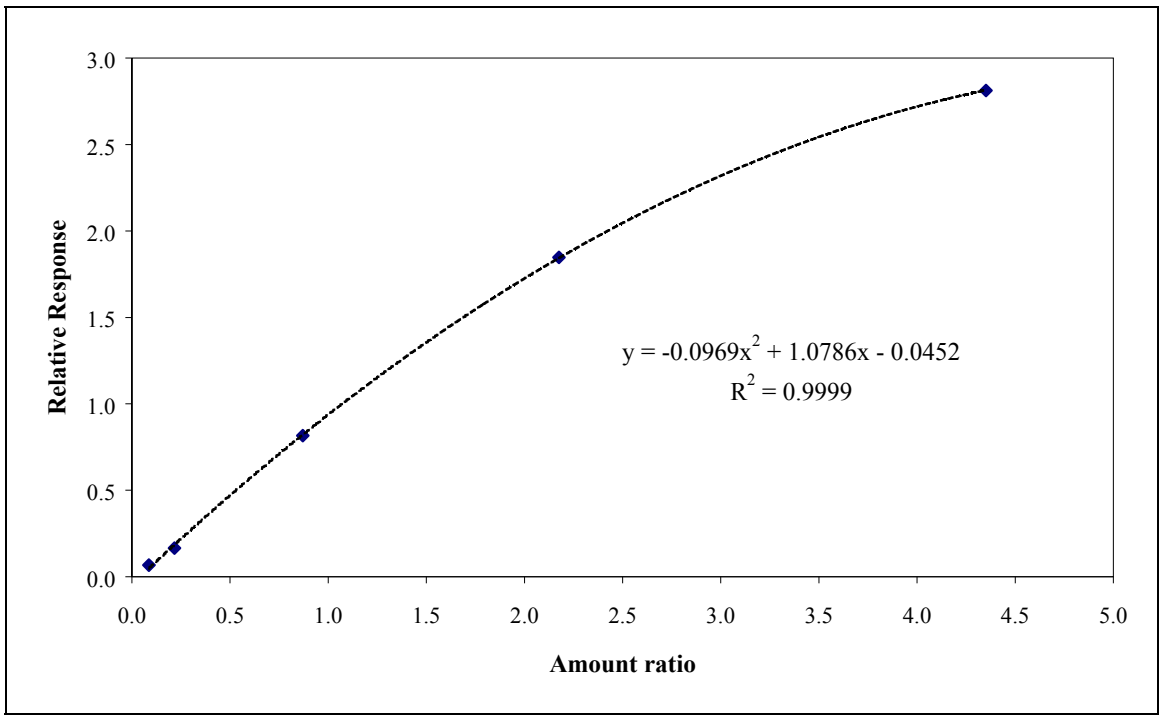

Figure 3. Typical Calibration Curve for Total Sulfide with Quadratic Equation

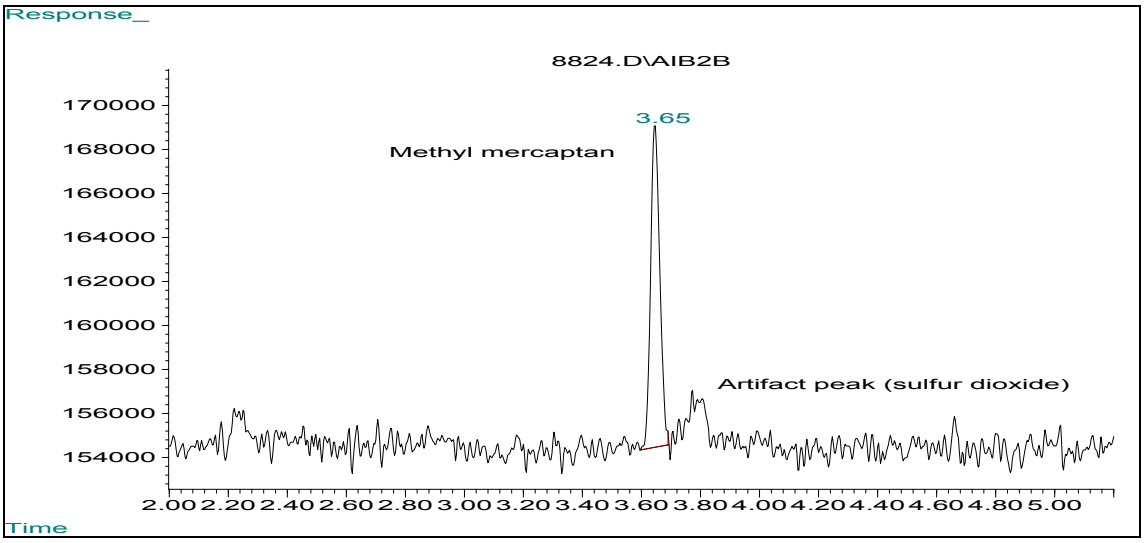

Figure 4. Separation of Methyl Mercaptan (100 µg S/L) from Artifact Peak in Pulp Mill Effluent