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## Total Sulfur Dioxide by Aeration-Oxidation

### Scope and Application

Sulfite is routinely used in the production of wine to reduce the risk of spoilage. It may also be present in beers and some distilled spirits such as brandy. Amounts of sulfite determined as total sulfur dioxide >350 ppm is indicative of excessive processing and may have a negative impact on consumer health. Most traditionally processed wines contain from 0 – 150 ppm total sulfur dioxide. Consumers who may have an allergic reaction to sulfites are made aware of its presence through mandatory labeling requirements provided under 27CFR.4.32(e) as follows:

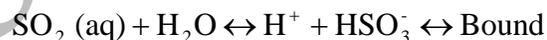
There shall be stated on a front label, back label, strip label or neck label, the statement “Contains sulfites” or “Contains (a) sulfiting agent(s)” or a statement identifying the specific sulfiting agent where sulfur dioxide or a sulfiting agent is detected at a level of 10 or more parts per million, measured as total sulfur dioxide.

Winemakers seeking to label their product “Contains No Detectable Sulfites” must have a product with no detectable sulfur dioxide present. For this method, the reported result must be “not detected” (ND) for a 50 mL test aliquot. Wines with detectable sulfur dioxide less than 10 ppm do not need to have the sulfite declaration on their label or they can label the product “contains less than 10 ppm sulfites”. These wines require a sulfur dioxide (SO<sub>2</sub>) waiver for such labeling and must be tested each year in which the claim is made.

Most traditional malt beverages do not have a sulfite declaration on their label since sulfites are not typically used in processing and are present at levels less than 10 ppm. If malt beverages, like other wine and distilled products, contain sulfites at a level of 10 or more ppm, the presence of sulfites must be declared on the label.

### SO<sub>2</sub> Equilibria in Wine

**Depends on SO<sub>2</sub> concentration, pH and temperature**



### Titration Equation



### Regulatory Tolerances:

Sulfite levels should not exceed 350 ppm total sulfur dioxide (27CFR 4.22(b)(1)).

Wine produced “from organically grown grapes” must contain less than 100 ppm SO<sub>2</sub> (27 CFR 4.101, 7 CFR 205).

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Products containing 10 or more ppm total SO<sub>2</sub> need to have the presence of sulfites declared on the label (27CFR 4.32(e), 27 CFR 7.22(b)(6)).

### Method Principle:

In this method, a stream of air is passed through an acidified wine sample, carrying SO<sub>2</sub> through a condenser into a hydrogen peroxide trapping solution containing an acid-base indicator. The trapping solution is then titrated with standardized NaOH to a visible endpoint (Reference: Buechsenstein, 1978).

### Levels and Limitations

Analyte	Sample Size* (mL)	Detection Limit (ppm)	Quantitation Limit (ppm)	Linear Range (ppm)	Interferences
Sulfur Dioxide	10 mL	1	5	5 - 500 ppm	None
Sulfur Dioxide	50 mL	0.3	0.6	0.6-20ppm	None

\*The appropriate sample size is initially chosen based on the product label. A 50 mL aliquot is used for products expected to have < 10 ppm sulfur dioxide, and 10 mL for ≥ 10 ppm.

### Supplemental Documents

BAL-WG-103b Rotary Shaker De-gassing of Beers

CL-WG-103 Sample Pretreatment by Degassing

### Equipment

Glass sulfur dioxide specialty apparatus ( Fig. 1) or equivalent

50 mL pear flasks (see D) (for trapping solution and samples with 10 or more ppm SO<sub>2</sub>)

**OR**

250 mL round bottomed flasks (for samples with less than 10 ppm SO<sub>2</sub>) (see G)

Claissen Adapter #K-273750 (see E)

Pasteur pipet holder GL-14 with adapter (see F)

350 mm Graham condenser #K-43900 (see B)

Connecting adapter #K-275050 (see A)

Vacuum adapter #K-276750 (see C) with sintered bubbling tube end (see H)

10 mL burette TD 20°C, 0.05 mL, Class A

Class A pipets or autopipettor for sample measurement

Recirculating water bath or chilled water (for condenser) maintained at 5 °C ± 5 °C

Micro Bunsen burner

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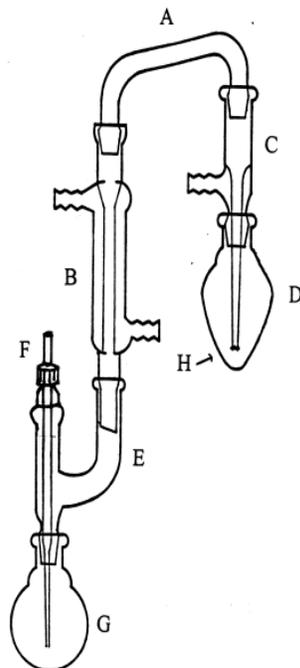
Compressed air source and accompanying regulators/accessories as needed.

**Note:** When the apparatus is initially set up, make absolutely sure there are no leaks. Maximize the recovery of standards by varying the flow rate for air. Measure the flow rate as it exits the side arm of the vacuum adapter (Figure 1:C) and record in the logbook.

Precision gas flow meter ( $\pm 1\%$  in L/min) - to monitor flow at apparatus-only required during initial set-up or trouble shooting.

Pinch clamps or equivalent (optional) - These may be needed to control the gas flow through tubing at apparatus.

Figure 1: Example of Apparatus Set-up for Aeration-Oxidation Method



**Note: Make sure joints are properly greased.**

Figure 1. apparatus for SO<sub>2</sub> determination by aeration-oxidation

- A) Connecting adapter, 19/22 #K-275050
- B) Condenser Graham 300mm, 19/22 #K-43900
- C) Vacuum adapter, 19/22 #K-276750
- D) 50-ml pear-shaped flask 19/22
- E) Claisen adapter 19/22 #K-273750
- F) Pasteur pipette sealed using a GL-14 with adapter 19/22
- G) 50-ml pear shaped flask or 100-ml round-bottom flask 19/22
- H) sintered end of vacuum adaptor.

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## Reagent and Sample Preparation and Handling

### Reagents:

Methyl Red (Certified ACS)  
Methylene Blue (Certified ACS)  
30% Hydrogen Peroxide (ACS grade, no stabilizing agent)  
Concentrated Hydrochloric Acid (certified ACS)  
0.01±0.0001 N sodium hydroxide solution (certified NIST traceable)  
Sodium phosphate dibasic heptahydrate (certified ACS)  
D-mannitol (certified ACS)  
Sodium metabisulfite (certified ACS )  
200 proof ethanol  
DI water  
50% Ethanol/water (Use 200° EtOH and DI water 1:1 by volume)

### Reagent Preparation:

1. Prepare an indicator of 1000 ppm methyl red and 500 ppm methylene blue with a 50% ethanol/water solution. The shelf life is one year stored at room temperature.  
**Example:** 0.10 g methyl red and 0.05 g methylene blue in 100 mL total volume.
2. Prepare 0.3 % hydrogen peroxide by diluting with DI water. Make fresh daily.  
**Example:** 5 mL of 30% hydrogen peroxide in 500 mL total volume.
3. Prepare a 4 M hydrochloric acid solution using concentrated (12 M) HCl and DI water. The shelf life is one year stored at room temperature.  
**Example:** 333 mL of concentrated HCl in 1 L total volume.
4. Standardized 0.01 ± 0.0001N sodium hydroxide solution. Store under manufacturer's recommended conditions.
5. Prepare a 20 mM sodium phosphate dibasic heptahydrate, 10 mM mannitol buffer in 15% ethanol/DI water. The shelf life is six months at room temperature.  
**Example:** 10.7 grams sodium phosphate dibasic heptahydrate and 3.6 grams D-mannitol in 300 mL of 200 proof ethanol then Q.S. to 2 L with DI water.

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## Stock and Working Standards:

1. The primary stock standard is a 1000 ppm sulfite as sulfur dioxide solution prepared with  $\text{Na}_2\text{S}_2\text{O}_5$  (certified ACS) in mannitol buffer or DI water. The shelf life when prepared in buffer is 1 month refrigerated. The shelf life when prepared in DI water is 1 day.

**Example:** 0.1493  $\pm$  0.001 grams  $\text{Na}_2\text{S}_2\text{O}_5$  in 100 mL volumetric and bring to volume with buffer or DI water.

2. Working standards:
  - a. For samples expected to contain  $\geq 10$  ppm sulfite, prepare 100 ppm working standard.

Example: Transfer 10 mL of 1000 ppm stock standard into 100 mL volumetric and bring to volume with buffer or DI water.

- b. For samples expected to contain  $< 10$  ppm sulfite, prepare 10 ppm working standard.

Example: Transfer 5 mL of 1000 ppm stock standard into 500 mL volumetric and bring to volume with buffer or DI water.

Standards prepared in buffer are stable when refrigerated for no more than 2 days. Bring to room temperature before use.

Standards prepared in DI water are stable for 1 day.

## Procedures

**NOTE: If sample is carbonated, de-gas before testing per lab procedure.**

1. Turn on chiller.
2. Transfer 10 mL 0.3% hydrogen peroxide into a 50 mL pear shaped flask. Add approximately 1-5 drops, as needed of indicator.
3. Adjust color of the solution from deep blue/purple to olive green/aqua with a drop or two of 0.01 N NaOH if necessary. Attach the flask to the end of the vacuum adapter.
4. For samples expected to contain 10 or more ppm  $\text{SO}_2$ , transfer 10 mL of standard or sample and 10 mL of 4M hydrochloric acid into a 50 mL pear shaped flask and connect to the apparatus. For samples expected to contain less than 10 ppm  $\text{SO}_2$ , transfer 50 mL of standard or sample and 50 mL of 4 M hydrochloric acid into a 250 mL round bottomed flask.
5. Make sure the pipette tip (Fig 1:F) is immersed to about the center of the solution. Adjust accordingly.

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6. Aspirate compressed air at optimum flow rate (established during initial apparatus setup) through the system for 20 minutes while heating the sample flask with a micro Bunsen flame.
7. Turn off flame.
8. Remove the trapping flask, rinse off the end of the bubbler into the trapping flask using minimum amount of DI water.
9. Titrate with standardized 0.01N NaOH back to the original olive green/aqua endpoint. Record volume of NaOH used for the titration.
10. If the endpoint is not reached or the calculated  $\text{SO}_2$  result is beyond the linear range for the aliquot sized used, dilute the sample with water or use the other aliquot size to get within the method's valid linear range. Adjust for dilution factor in final calculation.

## Quality Control

- To avoid loss of  $\text{SO}_2$ , samples should be tested within 3 days of opening.
- Before running samples expected to contain 10 or more ppm sulfite, run the 100 ppm working standard on each apparatus to be used. The standard results must be  $95 \text{ ppm} \pm 10\%$  in order to proceed with sample analysis. (The sulfite standard degrades over time such that a 95 ppm result is typical as opposed to 100 ppm.)
- Before running samples expected to contain less than 10 ppm sulfite, run the 10 ppm working standard on each apparatus to be used. If only one apparatus will be used, run the standard in duplicate. Since a suitable wine LCS is not available at this level, this standard is considered the LCS. If more than one apparatus is used, the minimum and maximum results are used for precision control charting and must meet the established limits. If only one apparatus is used, the duplicate results are precision control charted and must meet the established limits. All results must meet established accuracy control chart limits.
- For wines expected to contain 10 or more ppm sulfite, a wine Laboratory Control Sample (LCS) is run in duplicate per sample batch. Results must meet established control chart limits.
- If the standard or LCS fail, the troubleshooting notes provided below should be consulted and the standard or LCS should be re-run.
- Samples can be analyzed only when acceptable values have been achieved for the working standards and the LCSs.

## Sources of Uncertainty/Troubleshooting Notes

If the optimum recovery is not obtained for either standard or LCS:

1. Check preparation of standards and reagents.
2. Ensure all joints on the apparatus are greased to prevent any potential system leaks.

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3. Check the system for leaks.
4. Make sure the condenser water is between  $5 \pm 5$  °C.
5. Ensure the height of the sample flask is adjusted to allow enough heating of the sample from the Bunsen burner.
6. Check the air flow rates using the flow meter. If the value has drifted by more than 20%, re-optimize the flow rate.
7. Consult with the primary analyst to obtain information on previous problems and/or advice.

## Calculations

Total Sulfur Dioxide =  $(V_b \times N_b \times 32000)/V_s$

where:  $V_b$  = volume of base to titrate to endpoint (mL)

$N_b$  = actual normality of 0.01 N NaOH

$V_s$  = volume of sample used (mL)

## Reporting Results

Report the results as ppm to the nearest whole number (xx).

## Required Training, Certification and Re-certification

1. In-house training by a certified chemist.
2. Analyst has demonstrated competency after successfully obtaining values of four replicates of the LCS within established control chart limits.
3. Chemists will be recertified periodically using proficiency test results and/or re-demonstration of competence.

## Safety Notes

Wear Personal Protective Equipment: Safety goggles, lab coat

Concentrated  $H_2O_2$  is extremely irritating to eyes, skin and respiratory tract. Avoid inhalation of vapor and contact of liquid with skin.  $H_2O_2$  is a powerful oxidizing material and potential explosion hazard. Avoid contact with metals.

Hydrochloric acid is a strong irritant to eyes, skin and mucous membranes. Wear proper gloves when handling hot wine/hydrochloric acid solutions. If contact is made with eyes, skin and mucous membranes rinse with copious amounts of water. Clean all spills immediately neutralizing with a weak base such as sodium carbonate.

Sodium metabisulfite is a mild irritant to eyes, skin and mucous membranes. It may cause allergic respiratory reactions.

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Sample/Hydrochloric acid solutions should be neutralized before disposing down the drain in compliance with local regulations. Waste solutions can also be collected into a labeled waste disposal container.

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## Revision History

Rev. 4 – Removal of designation "Sulfite Free" from Scope and Application; Addition of class A requirement to buret in Equipment; addition of comment to check note regarding change to linear range for samples using 50 mL.

Rev. 5 – Extensive revision

Rev. 6 – Updated Linear Range for 50 mL samples in the Levels and Limitations section