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Food Safety and Inspection Service, Office of Public Health Science**

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A. INTRODUCTION

1. Theory

This method measures free sulfite plus reproducible portion of bound sulfites, such as carbonyl addition products, in meat products. A test portion is heated with refluxing hydrochloric acid to convert sulfite to sulfur dioxide (SO₂) gas. A stream of nitrogen introduced below the surface of the refluxing solution sweeps SO₂ through a water-cooled condenser. SO₂ is trapped by bubbling through a 3% hydrogen peroxide solution, where it is oxidized to sulfuric acid. Sulfite content is directly proportional to generated sulfuric acid, which is determined by titration with standardized sodium hydroxide solution. For verification, sulfate is determined gravimetrically as barium sulfate.

2. Applicability

This method is applicable to fresh and processed meat and poultry products at levels of ≥ 15 ppm. This method is applicable in the presence of other volatile sulfur compounds. It is not applicable to dried onions, leeks, and cabbage.

B. EQUIPMENT

Note: Equivalent equipment may be substituted for any of the following.

1. Apparatus

- a. Distillation apparatus - Refer to Figures 1 and 2, section K.
- b. Burette - 10 mL, Cat. No. 17124-F, Kimble Glass, Inc.
- c. Flasks - Erlenmeyer, with screw caps or stoppers.
- d. Chilled water circulator - Chill condenser with coolant, such as methanol-water (20 + 40, v/v), maintained at <15 °C. Circulating pump, Neslab Coolflow 33.
- e. Micropipettors - (100 to 1000 μ L), Cat. EP2500, Rainin.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted for any of the following.

1. Reagents

- a. Hydrochloric Acid (HCl) - 12N, reagent grade, Cat. No. JT9530, VWR.
- b. Hydrogen peroxide (H₂O₂) - 30% ACS reagent, Cat. No. JT2186, VWR.
- c. Diethyl Ether (C₄H₁₀O) anhydrous - Cat. No. JT9244, VWR.
- d. Ethanol (EtOH) absolute - Cat. No. MK618310, VWR.

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- e. Nitrogen (N₂) - High purity, Used with regulator to maintain flow of 200 mL/min.

Note: To guard against oxygen in N₂ gas, use GC-type trap (Oxy-Purge N Alltech-Applied Science Laboratories, Inc.).

Also, an indicating oxygen trap Phenomenex P/N AGO 4776 can be used to indicate when to change the trap.

- f. Barium Chloride (BaCl₂) reagent grade - Cat. No. MK375602, VWR.

- g. 0.010N Sodium Hydroxide (NaOH) Solution - Cat. No. JT5663, VWR.

- h. Water - deionized (18 megohm), prepared from distilled water.

Note: Optional - Water - deionized, deoxygenated. Sparge ~4L deionized water with an inert gas (N₂, He) at 250-300 mL/minute for at least 15 minutes. Store in airtight container.

- i. Sodium Hydroxymethylsulfonate (HMS) - Cat. No. 112704, Aldrich Chemical Co.

- j. Sodium monohydrogen phosphate heptahydrate (Na₂HPO₄•7H₂O) - Cat No. MK791404, VWR.

- k. D-mannitol (C₆H₁₄O₆) - Cat. No. M-9546, Sigma.

- l. Methyl red - Cat. No. 25,019-8, Aldrich Chemical Co.

2. Solutions

- a. 4N HCl :

Add 30 mL of 12N HCl to 60 mL deionized water and carefully mix.

- b. Methyl red indicator:

Dissolve 250 mg methyl red in 100 mL ethanol.

- c. Standardized titrant (0.010N NaOH):

Certified reagent may be used. Alternatively, prepare from a 1N NaOH solution (4.0 g NaOH diluted to 100 mL with distilled water) by diluting 1:100 with distilled water. Standardize solution with reference standard potassium acid phthalate, to 3 significant figures (nearest 0.0001N). Store titrant tightly sealed in suitable plastic container.

- d. 3% Hydrogen peroxide solution:

For each analysis, dilute 3 mL 30% H₂O₂ to 30 mL with deionized water. Just prior to use, add 3 - 5 drops methyl red indicator and titrate with 0.01N NaOH just to a yellow end point. If end point is exceeded, discard solution.

- e. 10% Barium Chloride:

Dilute 5.0 g BaCl₂ to 50 mL with deionized water.

- f. HMS Diluent (0.04M Na₂HPO₄•7H₂O + 0.10M d-mannitol):

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Weigh 10.7 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ + 18.2 g d-mannitol into a 1L volumetric flask. Dissolve and dilute to volume with deionized water. Store in refrigerator. Stable for 1 year.

g. 1N HCl:

Add 1 volume 12N HCl to 11 volumes deionized water and mix.

D. STANDARDS

1. Potassium acid phthalate (If required for standardization of NaOH titrant), Cat. No. P1190, Spectrum.
2. Sodium hydroxymethylsulfonate (HMS) - ~95% purity, Cat. No. 11,270-4, Aldrich.
3. HMS fortification solution (5 $\mu\text{g}/\mu\text{L}$ as SO_2):

Weigh approximately 110 mg HMS into small beaker. Dissolve in HMS diluent (C.2.f) and quantitatively transfer to a 10 mL volumetric flask. Dilute to volume with HMS diluent. Calculate equivalent SO_2 concentration using the formula:

$$\text{Conc. SO}_2, \mu\text{g}/\mu\text{L} = (\text{g HMS weighed} \times 0.4778) \times (\% \text{Purity HMS}) / 1000$$

$$[\text{Example: SO}_2, \mu\text{g}/\mu\text{L} = 110.0 \times 0.4778 \times 95 / 1000 = 4.99 (5.0)]$$

Note: On the basis of a 50 g sample weight, each 10 μL of this solution added equals approximately 1 ppm SO_2 added to the recovery.

E. SAMPLE PREPARATION

Samples should be cold when received and stored cold (preferably frozen) in airtight containers to minimize degradation of labile sulfites. If samples are ground prior to analysis, care should be taken to minimize exposure to air and heat.

F. ANALYTICAL PROCEDURE

Caution: Carry out test sample preparation and analysis as quickly as possible to avoid loss of labile forms of sulfite.

Refer to section I.6 for information regarding controls that must be included as part of every sample set.

1. Weigh approximately 50 g product, or quantity that contains 500 - 1500 μg SO_2 , to nearest 0.1 g, into three-neck round bottom distillation flask.

Note: A recovery should be prepared at this time by fortifying blank tissue with an appropriate amount of HMS solution (D.3). If SO_2 content of the blank tissue has not been previously determined, it must be analyzed as part of the set.

Note: Titration and gravimetric readings from a tissue blank may be used in place of

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those from a reagent blank, in calculations described in section G parts 2 and 3, if the tissue blank is known to contain <1 ppm SO₂, using gravimetric quantitation.

2. Add 400 - 500 mL H₂O to flask, taking care to rinse down any residual sample that may cling to the neck or walls of the flask. Dry neck of flask with tissue. Proceed immediately to next step.

3. Assemble apparatus described in B.1.a (refer to Figure 1, Section K). Apply stopcock grease or Teflon[®] sleeves to all joints and clamp where practical.

Note: Most assembly may take place prior to start of analysis in order to save time. Order of steps a-c below is not critical.

- a. Place flask (C), containing sample, into heating mantle controlled by power-regulating device (rheostat).
 - b. Close stopcock of separatory funnel (B) and add 90 mL 4N HCl to funnel.
 - c. Add 30 mL 3% H₂O₂, which has been titrated to yellow end point with 0.01N NaOH, to vessel (G).
 - d. Before putting vessel (G) into place, adjust nitrogen flow to 190 - 210 mL/min using a flowmeter. Count bubble rate in flask (C). After attaching vessel (G), readjust nitrogen flow, if necessary to maintain the same bubble rate in flask.
4. Purge apparatus and check for leaks
 - a. Initiate condenser coolant flow at this time. Allow 15 min for N₂ flow to purge system of oxygen.
 - b. Inspect system to verify that all joints are leak free.
5. Generate and distill SO₂
 - a. Apply positive pressure to separatory funnel using a rubber bulb or other apparatus. Open stopcock and let HCl flow into flask. Maintain sufficient pressure to force acid solution into flask without allowing gases from flask to bubble into funnel. Stopcock may be closed, if necessary, to pump up pressure above acid, and then opened again.
 - b. Close stopcock before last 2 - 3 mL drain out of separatory funnel to guard against escape of SO₂ into separatory funnel.
 - c. Apply power to the heating mantle, using a power setting that causes 80 - 90 drops/min of condensate to return to flask from condenser.
 - d. Let contents of flask boil 1 hour 45 minutes, then remove vessel (G) and turn off power to mantle.
 - e. Quantitatively transfer contents of vessel to a 125 mL Erlenmeyer flask using a small volume of deionized water. Note: Solution is stable for several hours when

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flask is capped, but following step must be done on day of distillation.

6. Titrate H₂SO₄ from vessel G.
Titrate contents of Erlenmeyer flask with 0.010N NaOH to yellow end point that persists at least 20 seconds.
7. Gravimetric determination (Required if titration indicates reportable amounts of SO₂ detected).
 - a. Add 4 drops 1N HCl and an excess of filtered 10% BaCl₂ solution to flask, and let mixture stand overnight.
 - b. Swirl flask contents to suspend precipitate and pour through a tared Gooch crucible. Quantitatively transfer residual precipitate in flask using three approximately 20 mL portions of hot water, and allow to drain. Wash crucible with 20 mL absolute alcohol and 20 mL ether, and dry for at least 2 hours at 105 - 110 °C.
 - c. Allow crucible to cool, then reweigh. Calculate weight of BaSO₄ in milligrams by subtracting the tare.

G. CALCULATIONS

1. A reagent blank must be run and calculated for both titration (as mL NaOH) and gravimetric quantitation (as mg BaSO₄) for each sample set.
2. Titration Quantitation: Compute sulfite content, expressed in µg SO₂/g food (ppm), as follows:
$$\text{SO}_2 \text{ (ppm)} = (32.03)(V_{\text{Corr}})(N)(1000) / W_{\text{Sample}}$$
 Where
32.03 = milliequivalent weight of SO₂
N = Normality of NaOH titrant.
V_{Corr} = volume (mL) of NaOH of normality N required to reach end point, minus the NaOH volume required to titrate the reagent blank.
1000 = factor to convert milliequivalents to microequivalents.
W_{Sample} = Sample weight, in grams.
3. Gravimetric Quantitation: Compute sulfite content, expressed in µg SO₂/g food (ppm), as follows:
$$\text{SO}_2 \text{ (ppm)} = (\text{mg BaSO}_4 \text{ Sample} - \text{mg BaSO}_4 \text{ Blank})(274.46) / W_{\text{Sample}}$$
 Where
mg BaSO₄ Sample and mg BaSO₄ Blank are the weights of BaSO₄ precipitates recorded for the sample and reagent blank, respectively.
274.46 = Conversion factor: (1000) (FW SO₂) / FW BaSO₄ (FW = formula wt.)
W_{Sample} = Sample weight, in grams.

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4. Recovery Calculation:

a. Determine the SO₂ concentration in the recovery (SO₂ found). Correct for tissue blank.

b. Determine recovery's fortified level (SO₂ added) using

$$\text{SO}_2 \text{ added, (ppm)} = (C_{\text{Fort}})(V_{\text{Fort}}) / W_{\text{Sample}}$$

Where:

C_{Fort} = Concentration of fortification Solution (D.3) in µg SO₂/µL

V_{Fort} = Volume of fortification solution added, in µL.

W_{Sample} = Sample weight, in grams.

c. Calculate % Recovery as:

$$\% \text{ Recovery} = 100\% \times (\text{SO}_2 \text{ found} / \text{SO}_2 \text{ added}).$$

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H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Lab coat, safety glasses, plastic gloves, thermal gloves.
2. Hazards

Reagent or Action	Hazard	Recommended Safe Procedures
Hydrogen Peroxide	Corrosive to skin.	Avoid contact with skin.
Hydrochloric Acid, Sodium Hydroxide	Irritating to mucus membranes, corrosive to skin	Avoid breathing vapors. Avoid contact with skin.
Diethyl Ether	Highly flammable. Explosive hazard, vapors, mixed with air, will explode if ignited. Inhalation of high concentration will cause narcosis, unconsciousness.	Keep tightly closed and away from fire. Use under fume hood. Avoid breathing vapor.
Ethanol	Flammable	Keep tightly closed and away from fire.
Barium Chloride	May cause skin irritation. May cause eye irritation. May be harmful if absorbed through the skin. Harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract. Toxic if swallowed.	Keep tightly closed. Do not breathe dust. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure.

3. Disposal Procedures

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Diethyl Ether	See Above	Dispose according to appropriate local, state and federal regulations
Ethanol	See Above	Dispose according to appropriate local, state and federal regulations

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Diethyl Ether	See Above	Dispose according to appropriate local, state and federal regulations
Barium Sulfate	See Above	Dispose according to appropriate local, state and federal regulations
Hydrochloric Acid	See Above	Dispose according to appropriate local, state and federal regulations. May be neutralized and disposed through the sanitary sewer system.
Sodium Hydroxide	See Above	May be neutralized and disposed through the sanitary sewer system. Dispose according to appropriate local, state and federal regulations
Hydrogen Peroxide	See Above	Dispose according to appropriate local, state and federal regulations

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I. QUALITY ASSURANCE PLAN

1. Performance Standard

<i>Analyte</i>	<i>Analytical Range</i>	<i>Acceptable Recovery, Titrimetric Analysis</i>	<i>Acceptable Recovery Gravimetric Analysis)</i>
Sulfite	≥15 ppm	60 - 95%	60 -105%

2. Critical Control Points and Specifications

<i>Record</i>	<i>Acceptable Control</i>
a. Sample weight	Amount to contain 500 - 1500 µg SO ₂
b. Apparatus integrity	Verify apparatus is completely sealed
c. Distillation Rate	Adjust to 80 - 90 drops per minute
d. Distillation Time	1 hour 45 minutes
e. Titration	End point must persist at least 20 seconds

3. Readiness To Perform (FSIS Training Plan)

Before beginning familiarization exercises listed below, trainee should read and understand method and observe experienced analyst, if possible.

a. Familiarization

- i. Phase I: N/A
- ii. Phase II: Analysis of fortified samples - Minimum of 3 replicates fortified at 15 - 100 ppm over a period of 3 different days (9 samples total).
- iii. Phase III: Check samples, analytes concentrations unknown to the analyst.
 - (a) Minimum 6 recoveries fortified at 15 - 100 ppm levels.
 - (b) Report analytical findings to Supervisor/Quality Manager (QAM)
 - (c) Letter from QAM is required to commence official analysis.

b. Acceptability criteria.

Refer to section I.1 above.

4. Intralaboratory Check Samples

- a. System, minimum contents.

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- i. Frequency: One per week per analyst when samples analyzed.
 - ii. Records are to be maintained.
 - b. Acceptability criteria.
Refer to section I. 1.
If unacceptable values are obtained, then:
 - i. Stop official analyses by that analyst.
 - ii. Take corrective action.
- 5. Sample Acceptability and Stability
 - a. Matrix: Fresh and processed meat and poultry products.
 - b. Minimum Sample Size: 500 g.
 - c. Condition upon receipt: Sealed from air, and unspoiled.
 - d. Sample storage:
 - i. Time: Stability unknown, likely to vary with sample type and condition.
Samples should be analyzed as soon as possible.
 - ii. Condition: Frozen and stored in airtight container.
- 6. Sample Set
 - a. Reagent blank and/or tissue blank (see section F.1.)
 - b. Recovery (fortified blank tissue) must be included in each sample set. If the tissue used to prepare the recovery has not been previously analyzed, it must also be included in the sample set.
 - c. Test samples to be analyzed.
- 7. Sensitivity
Minimum proficiency level (MPL): 15 ppm.

J. WORKSHEET

The following is an example of a worksheet.

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Analyst: _____	Date Started: _____	Date Completed: _____
Fortification Std. ILN: _____	HOCH ₂ SO ₃ Na %Purity: _____	Crucible Balance #: _____
STD Balance: _____	30%H ₂ O ₂ #: _____	1N HCl #: _____
Weight (g): _____	Methyl Red #: _____	10% BaCl ₂ : _____
µL Fortified: _____	NAOH #: _____	EtOH: _____
ppm: _____	NAOH (N): _____	Ethyl Ether: _____
Sample Balance #: _____	4N HCl #: _____	Crucible-Reweigh Balance #: _____
Buffer #: _____	Standardization book #: _____	Sol'n Book #: _____
		Oven: _____
		Time In: _____
		Temp °C: _____
		Time Out: _____
		Temp °C: _____
		Thermometer #: _____
		Pipette #: _____

Volumetric NaOH Titration

Sample	Serial #	Weight (g)	Start ml	End ml	Total ml	ppm SO ₂	% Rec
TissueBlank	_____						
Rec.#	_____						%

Gravimetric

Sample	Crucible #	Crucible wt (g)	Total wt (g)	Sample (mg):	ppm SO ₂	% Rec
Tissue Blank	_____					
Rec.#	_____					%

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K. APPENDIX

1. References

- a. AOAC Official Method 990.28.
- b. "Optimized Monier-Williams Method for Determination of Sulfites in Foods: Collaborative Study" (1989), *J. Assoc. Off. Anal. Chem.* 72, 470-475.

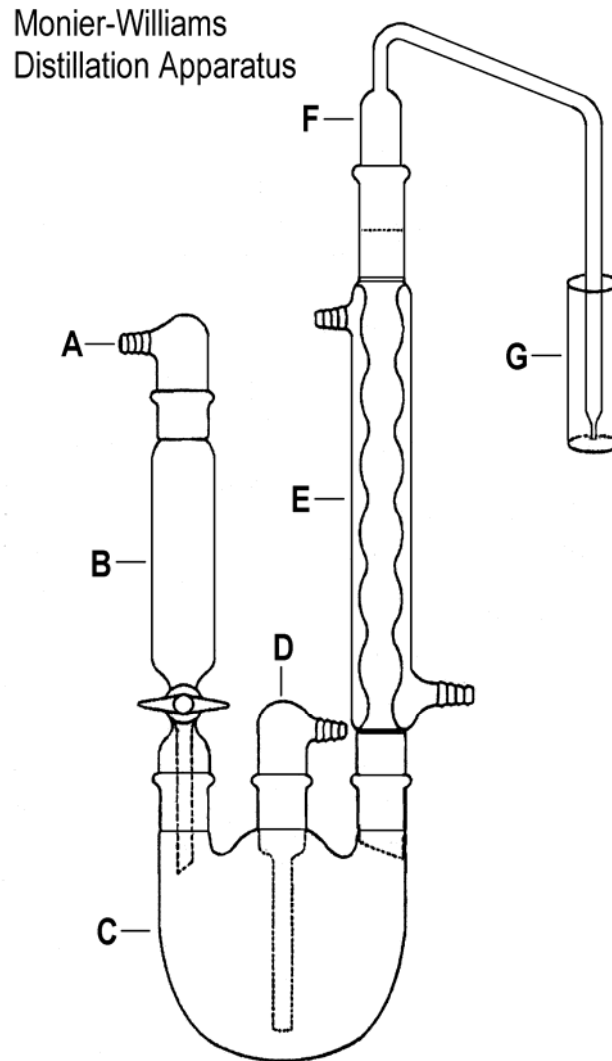
2. Figure 1. Monier Williams SO₂ Distillation Apparatus.

Figure 2. Bubbler.

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Figure 1.

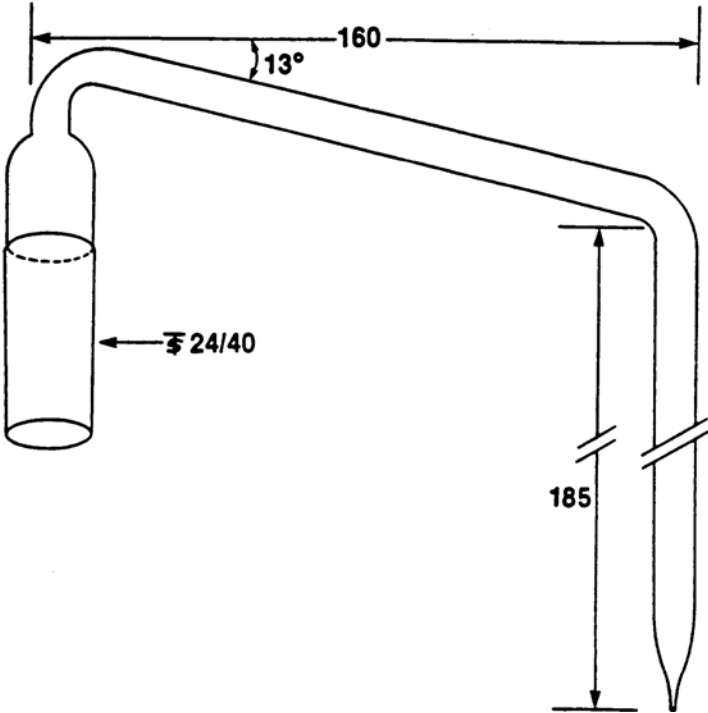


- (A) Inlet adapter with hose connector (Kontes 183000);
- (B) Separatory funnel, ≥ 100 mL capacity;
- (C) Round-bottom flask, 1 L, with three 24/40 tapered joints;
- (D) Gas inlet tube (Kontes 179000) of sufficient length to permit introduction of N_2 within 2.5 cm of bottom of flask;
- (E) Allihn condenser, (Kontes 431000-2430), jacket length 300 mm.
- (F) Bubbler, fabricated from glass according to dimensions in Figure 2;
- (G) Receiving vessel, ca 2.5 cm id and 18 cm deep.

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Figure 2. (See K.2. Figure 1.F)



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L. APPROVALS AND AUTHORITIES

Approvals on file.

Issuing Authority: Laboratory Quality Assurance Division.