AOAC Official Method 990.28
Sulfites in Foods

Optimized Monier–Williams Method
First Action 1990
Final Action 1994

(Applicable of determination of ≥10 ppm (μg/g) sulfites in foods. Applicable in presence of other volatile sulfur compounds; not applicable to dried onions, leeks, and cabbage.)

Results of the interlaboratory study supporting the acceptance of the method:

Hominy, 9.17 ppm (μg/g) sulfites:
s_r = 1.33; s_R = 1.42; RSD_r = 14.5%; RSD_R = 15.5%

Fruit juice, 8.05 ppm (μg/g) sulfites:
s_r = 1.36; s_R = 1.62; RSD_r = 16.9%; RSD_R = 20.1%

Protein (seafood), 10.41 ppm (μg/g) sulfites:
s_r = 1.47; s_R = 2.77; RSD_r = 14.1%; RSD_R = 26.6%

A. Principle

Method measures free sulfite plus reproducible portion of bound sulfites, such as carbonyl addition products, in foods. Test portion is heated with refluxing HCl (ca 1M) to convert sulfite to SO_2. Stream of N_2 introduced below surface of refluxing solution sweeps SO_2 through water-cooled condenser and, via bubbler attached to condenser, with 3% H_2O_2 solution, where SO_2 is oxidized to H_2SO_4. Sulfite content is directly related to generated H_2SO_4, which is determined by titration with standardized NaOH solution. For verification, sulfate can be determined gravimetrically as $BaSO_4$.

B. Apparatus

(**a**) *Distillation apparatus*.—(*Note:* In this method, back pressure inside apparatus is limited to unavoidable pressure due to height of 3% H_2O_2 solution above tip of bubbler (F). Keep back pressure as low as possible to avoid loss of SO_2 through leaks. Use thin film of stopcock grease on sealing surfaces of all joints except joint between separatory funnel and flask. Clamp together each joint to ensure complete seal throughout analysis.) Assemble apparatus (Figure **990.28A**), which includes (*1*) inlet adapter (A) with hose connector (Kontes 183000). Adapter provides means of applying head pressure above solution. Use of pressure-equalizing dropping funnel is not recommended because condensate, perhaps containing SO_2, is deposited in funnel and side arm. (*2*) Separatory funnel (B), ≥100 mL capacity. (*3*) Round-bottom flask (C), 1 L, with three 24/40 tapered joints. (*4*) Gas inlet tube (D) (Kontes 179000) of sufficient length to permit introduction of N_2 within 2.5 cm of bottom of flask. (*5*) Allihn condenser (E) (Kontes 431000-2430), jacket length 300 mm. (*6*) Bubbler (F), fabricated from glass according to dimensions in Figure **990.28B**. (*7*) Vessel (G), ca 2.5 cm id and 18 cm deep.

(**b**) *Buret*.—10 mL (Kimble Glass, Inc., No. 17124-F) with overflow tube and hose connections for Ascarite tube or equivalent air-scrubbing apparatus to permit maintenance of CO_2-free atmosphere over standardized 0.010M NaOH.

(**c**) *Chilled water circulator*.—Chill condenser with coolant, such as methanol-water (20 + 40, v/v), maintained at ≤15°C. Circulating pump, Neslab Coolflow 33 (Neslab Instruments, Inc., PO Box 1178, Portsmouth, NH 03801, USA), or equivalent, is suitable.

C. Reagents

(**a**) *Aqueous hydrochloric acid*.—4M. For each analysis, prepare 90 mL solution by adding 30 mL HCl to 60 mL deionized (18 megohm) water.

(**b**) *Methyl red indicator*.—Dissolve 250 mg methyl red in 100 mL ethanol.

(**c**) *Standardized titrant*.—0.010M NaOH. Certified reagent may be used (Fisher SO-5-284). Standardize solution with reference standard potassium acid phthalate.

(**d**) *Hydrogen peroxide solution*.—3%. For each analysis, dilute 3 mL ACS reagent grade 30% H_2O_2 to 30 mL with deionized (18 megohm) water. Just prior to use, add 3 drops methyl red indicator and titrate with 0.010M NaOH to yellow end point. If end point is exceeded, discard solution.

(**e**) *Nitrogen*.—High purity, used with regulator to maintain flow of 200 mL/min. To guard against oxygen in N_2 gas, use GC-type trap (Oxy-Purge N [Alltech-Applied Science Laboratories, Inc.], or equivalent).

Alternatively, oxygen-scrubbing solution, such as alkaline pyrogallol, in gas-washing bottle (Kimble Glass, Inc.) may be used. Prepare trap as follows: (*1*) Add 4.5 g pyrogallol to trap. (*2*) Purge trap with N_2 for 2–3 min. (*3*) Prepare KOH solution by adding 65 g

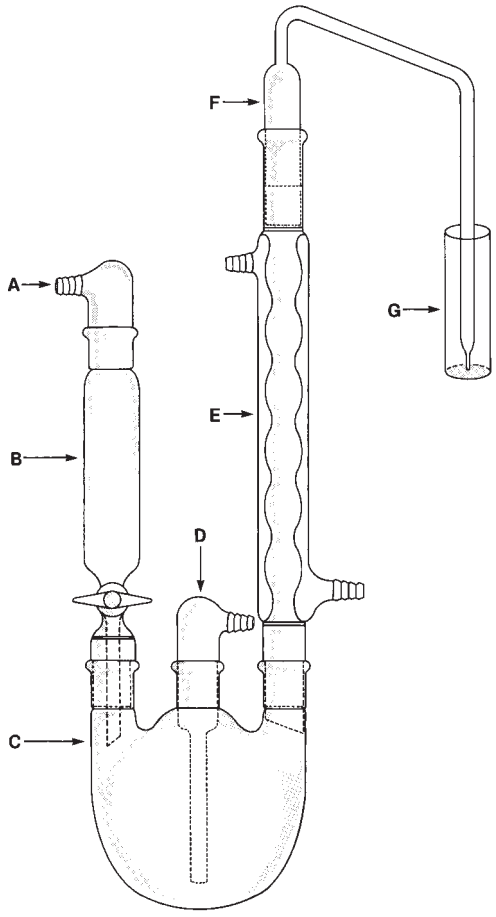

Figure 990.28A—Apparatus for optimized Monier-Williams method: A, inlet adapter; B, separatory funnel; C, round-bottom flask; D, gas inlet tube; E, Allihn condenser; F, bubbler; G, vessel.

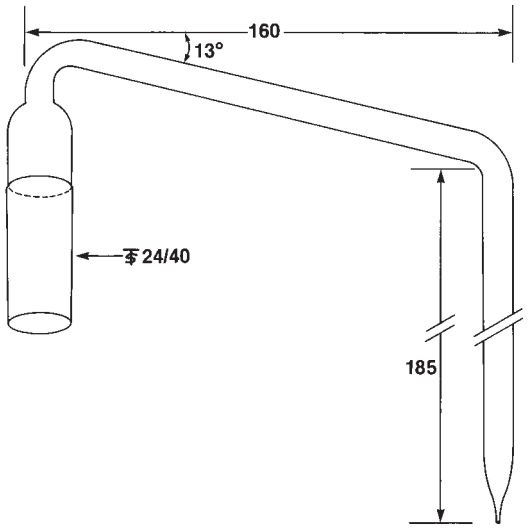

Figure 990.28B—Enlarged diagram of bubbler for Monier-Williams apparatus (lengths in mm).

KOH to 85 mL H_2O. (*Caution:* Heat is generated.) (*4*) Add KOH solution to trap while atmosphere of N_2 is maintained in trap.

D. Test Sample Preparation

(**a**) *Solids*.—Transfer 50 g food, or quantity that contains 500–1500 μg SO_2, to food processor or blender. Add 100 mL ethanol–water (5 + 95, v/v) and briefly grind mixture. Continue grinding or blending only until food is chopped into pieces small enough to pass through standard taper 24/40 joint of flask (C).

(**b**) *Liquids*.—Mix 50 g test portion, or quantity that contains 500–1500 μg SO_2 with 100 mL ethanol-water (5 + 95, v/v).

(*Note:* Carry out test sample preparation and analysis as quickly as possible to avoid loss of labile forms of sulfite.)

E. System Preparation

Using apparatus assembled as shown in Figure **990.28A**, position flask (C) in heating mantle controlled by power-regulating device (rheostat), and add 400 mL H_2O to flask. Close stopcock of separatory funnel (B) and add 90 mL 4M HCl to separatory funnel. Begin N_2 flow at 200 ± 10 mL/min. Initiate condenser coolant flow at this time. To vessel (G) add 30 mL 3% H_2O_2, which has been titrated to yellow end point with 0.010M NaOH. After 15 min, apparatus and water will be thoroughly deoxygenated and prepared test portion may be introduced into system.

F. Sample Introduction and Distillation

Remove separatory funnel (B) and quantitatively transfer test portion in aqueous ethanol to flask (C). Wipe tapered joint clean with laboratory tissue, quickly apply stopcock grease to outer joint of separatory funnel, and return separatory funnel to flask. Nitrogen flow through 3% H_2O_2 solution resumes as soon as separatory funnel is reinserted into appropriate joint in flask. Examine each joint to be sure that it is sealed.

Use rubber bulb equipped with valve to apply head pressure above HCl in separatory funnel. Open stopcock in separatory funnel and let HCl flow into flask. Continue to maintain sufficient pressure above acid solution to force solution into flask. Stopcock may be closed, if necessary, to pump up pressure above acid, and then opened again. Close stopcock before last 2–3 mL drain out of separatory funnel to guard against escape of SO_2 into separatory funnel.

Apply power to heating mantle. Use power setting that causes 80–90 drops/min of condensate to return to flask from condenser. Let contents of flask boil 1.7 h, and then remove vessel (G).

G. Determination

(**a**) *Titration*.—Immediately titrate contents of vessel (G) with 0.010M NaOH to yellow end point that persists ≥20 s. Compute sulfite content, expressed in μg SO_2/g food (ppm), as follows:

$$SO_2,\ \mu g/g\ (ppm) = \frac{32.03 \times V_B \times M \times 1000}{weight}$$

where 32.03 = milliequivalent weight of SO_2; V_B = volume (mL) of NaOH of molarity M required to reach end point; 1000 = factor to convert milliequivalents to microequivalents; weight = weight, g, of test portion introduced into 1 L flask.

(**b**) *Gravimetric determination*.—Optional. Following titration, rinse contents of vessel (G) into 400 mL beaker. Add 4 drops 1M HCl and excess of filtered 10% $BaCl_2$ solution, and let mixture stand overnight. Wash precipitate by decantation 3 times with hot water through weighed Gooch crucible. Wash with 20 mL alcohol and 20 mL ether, and dry at 105–110°C.

$$SO_2,\ \mu g/g\ (ppm) = \frac{mg\ BaSO_4 \times 274.46}{g\ test\ portion}$$

(**c**) *Blank determination*.—Determine blank on reagents both by titration and gravimetrically, and correct results accordingly.

H. Recovery Assays

To become familiar and proficient with method before routine use, analyze food test portions containing known amounts of sulfite. Perform analysis in manner that precludes any loss of sulfite by oxidation or reaction with components in food. Since sulfites are reactive with air and food matrixes and lack stability, fortify portions with stable source of sulfite, not sodium sulfite or similar salts. Sodium hydroxymethylsulfonate (HMS), which is bisulfite addition product of formaldehyde and is structurally similar to some combined forms of sulfite in foods, is useful for preparing stable fortified test materials.

For analysis, transfer 50 g prepared test sample of sulfite-free food to Monier-Williams flask. Add aliquot of aqueous solution of HMS sodium salt. Analyze solution immediately.

HMS recoveries of ≥80% from food matrixes fortified at 10 μg/g are recommended to ensure accurate analytical data.

Reference: *JAOAC* **72**, 470(1989).

CAS-7446-09-5 (sulfur dioxide)