

ride to volume, and mix. These preparations contain, in each mL, 25 µg of *n*-tricosane and, respectively, 20, 30, 40, and 50 µg of each toluenesulfonamide isomer.

**Test preparation**—Prepare as directed under *Column Partition Chromatography* (see *Chromatography* (621)), employing a chromatographic tube fitted with a porous glass disk in its base, a plastic stopcock on the delivery tube, and a reservoir at the top. Add a mixture consisting of 12 g of *Solid Support* and a solution of 2.0 g, accurately weighed, of Saccharin with 12 mL of filtered sodium bicarbonate solution (1 in 11). Add about 200 mg of sodium bicarbonate to effect complete solution of the saccharin. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 mL of methylene chloride in the reservoir, and adjust the stopcock so that 50 mL of eluate is collected in 20 to 30 minutes. To the eluate add 25 µL of *Internal standard solution*, mix, and concentrate the solution, by suitable means, to a volume of 1.0 mL.

**Chromatographic system** (see *Chromatography* (621))—Under typical conditions, the instrument is equipped with a flame ionization detector, and contains a 1.8 m × 3.2 mm glass column packed with 10% liquid phase G3 on 100 to 120 mesh support SLAB, utilizing a glass-lined sample introduction system or on-column injection. The injector port, column, and detector block are maintained at temperatures of about 225°, 210°, and 250°, respectively, and dry helium is used as the carrier gas at a flow rate of about 30 mL per minute.

**Procedure**—Inject portions (about 2.5 µL) of the *Standard preparations*, successively, into a gas chromatograph, and record each chromatogram so as to obtain at least 50% of maximum recorder response. Measure the areas under the first (*o*-toluenesulfonamide), second (*p*-toluenesulfonamide), and third (*n*-tricosane) peaks, and for each chromatogram record the values as  $A_o$ ,  $A_p$ , and  $A_n$ , respectively. Calculate the ratios  $R_o$  and  $R_p$  taken by the equations:

$$R_o = A_o/A_n \text{ and } R_p = A_p/A_n$$

and prepare standard curves by plotting the concentrations, in µg per mL, of USP *o*-Toluenesulfonamide RS and of USP *p*-Toluenesulfonamide RS in the *Standard preparations* versus  $R_o$  and  $R_p$ , respectively. [NOTE—Relative retention times are, approximately, 0.39 for *o*-toluenesulfonamide, 0.46 for *p*-toluenesulfonamide, and 1.0 for *n*-tricosane.] Similarly inject a portion (about 2.5 µL) of the *Test preparation*, and record the chromatogram. Measure the areas under the first (*o*-toluenesulfonamide), second (*p*-toluenesulfonamide), and third (*n*-tricosane) peaks, and record the values as  $a_o$ ,  $a_p$ , and  $a_n$ , respectively. Calculate the ratios  $r_o$  and  $r_p$  taken by the equations:

$$r_o = a_o/a_n \text{ and } r_p = a_p/a_n$$

and, from the standard curve, determine the concentration, in µg per mL, of each toluenesulfonamide isomer in the *Test preparation*; the total amount of toluenesulfonamides in the specimen taken is not more than 0.0025%.

**Selenium** (291):—0.003%, a 100 mg specimen, mixed with 100 mg of magnesium oxide, being used.

**Heavy metals, Method II** (231):—0.001%.

**Benzoic and salicylic acids**—To 10 mL of a hot, saturated solution of it add ferric chloride TS, dropwise; no precipitate or violet color appears in the liquid.

**Organic volatile impurities, Method V** (467):—meets the requirements.

**Solvent**—Use dimethyl sulfoxide.

**Assay**—Accurately weigh about 500 mg of Saccharin, dissolve in 40 mL of alcohol, add 40 mL of water, mix, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 18.32 mg of  $C_7H_5NO_3S$ .

## ■ Saccharin



$C_7H_5NO_3S$  183.19

1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide.

1,2-Benzisothiazolin-3-one 1,1-dioxide [81-07-2].

» Saccharin contains not less than 99.0 percent and not more than 101.0 percent of  $C_7H_5NO_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at room temperature.

**USP Reference standards** (11)—USP Saccharin RS. USP *o*-Toluenesulfonamide RS. USP *p*-Toluenesulfonamide RS.

**Clarity of solution**—[NOTE—The *Test solution* is to be compared to *Reference suspension A* and to water in diffused daylight 5 minutes after preparation of *Reference suspension A*.]

**Hydrazine solution**—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours.

**Methenamine solution**—Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension**—[NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before

use.] Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 hours.

*Opalescence standard*—[NOTE—This suspension should not be used beyond 24 hours after preparation.] Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

*Reference suspensions*—Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain *Reference suspension A*. Transfer 10.0 mL of the *Opalescence standard* to a second 100-mL volumetric flask, dilute with water to volume, and mix to obtain *Reference suspension B*.

*Test solution*—Dissolve 5.0 g of test material in about 20 mL of a 200 g per L solution of sodium acetate, dilute with the same solution to 25 mL, and mix.

*Procedure*—Transfer a sufficient portion of the *Test solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Reference suspension A*, *Reference suspension B*, water, and a 200 g per L solution of sodium acetate to separate matching test tubes. Compare the *Test solution*, *Reference suspension A*, *Reference suspension B*, water, and a 200 g per L solution of sodium acetate in diffused daylight, viewing vertically against a black background (see *Visual Comparison* under *Spectrophotometry and Light-Scattering* (851)). [NOTE—The diffusion of light must be such that *Reference suspension A* can readily be distinguished from water and that *Reference suspension B* can readily be distinguished from *Reference suspension A*.] The *Test solution* shows the same clarity as that of water, or the 200 g per L solution of sodium acetate, or its opalescence is not more pronounced than that of *Reference suspension A*.

#### Color of solution—

*Standard stock solution*—Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 g per L).

*Standard solution*—[NOTE—Prepare the *Standard solution* immediately before use.] Transfer 1.0 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with dilute hydrochloric acid (10 g per L) to volume, and mix.

*Test solution*—Use the *Test solution* from *Clarity of solution*.

*Procedure*—Transfer a sufficient portion of the *Test solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution*, a 200 g per L solution of sodium acetate, and water to separate matching test tubes. Compare the *Test solution*, the *Standard solution*, a 200 g per L solution of sodium acetate, and water in diffused daylight, viewing vertically against a white background (see *Visual Comparison* under *Spectrophotometry and Light-Scattering* (851)). The *Test solution* has the appearance of water or the 200 g per L solution of sodium acetate, or is not more intensely colored than the *Standard solution*.

**Identification**, *Infrared Absorption* (197K).

**Melting range** (741): between 226° and 230°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Readily carbonizable substances** (271)—Dissolve 200 mg in 5 mL of sulfuric acid (between 94.5% and 95.5% [w/w] of H<sub>2</sub>SO<sub>4</sub>), and keep at a temperature of 48° to 50° for 10 minutes: the solution has no more color than *Matching Fluid A*, when viewed against a white background.

**Residue on ignition** (281): not more than 0.2%. Ignition temperature: 600 ± 50°.

**Heavy metals, Method II** (231): 0.001%.

**Limit of toluenesulfonamides—**

*Internal standard solution*—Dissolve 25 mg of caffeine in methylene chloride, and dilute with the same solvent to 100 mL.

*Reference solution*—Dissolve 20.0 mg of USP *o*-Toluenesulfonamide RS and 20.0 mg of USP *p*-Toluenesulfonamide RS in methylene chloride, and dilute with the same solvent to 100.0 mL. Dilute 5.0 mL of the solution with methylene chloride to 50.0 mL. Evaporate 5.0 mL of the final solution to dryness in a stream of nitrogen. Dissolve the residue in 1.0 mL of the *Internal standard solution*.

*Test solution*—Suspend 10.0 g of the substance to be examined in 20 mL of water, and dissolve using 5 mL to 6 mL of 10 N sodium hydroxide. If necessary adjust the solution with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 7 to 8, and dilute with water to 50 mL. Shake the solution with four quantities each of 50 mL of methylene chloride. Combine the lower layers, dry over anhydrous sodium sulfate, and filter. Wash the filter and the sodium sulfate with 10 mL of methylene chloride. Combine the solution and the washings, and evaporate almost to dryness in a water bath at a temperature not exceeding 40°. Using a small quantity of methylene chloride, quantitatively transfer the residue into a suitable 10 mL tube, evaporate to dryness in a stream of nitrogen, and dissolve the residue in 1.0 mL of the *Internal standard solution*.

*Blank solution*—Evaporate 200 mL of methylene chloride to dryness in a water bath at a temperature not exceeding 40°. Dissolve the residue in 1 mL of methylene chloride.

*Chromatographic system* (see *Chromatography* (621))—The instrument is equipped with a flame-ionization detector and contains a 0.53-mm × 10-m fused silica column, coated with G3 phase (film thickness 2 μm). The injector port, column, and detector temperatures are maintained at

about 250°, 180°, and 250°, respectively; and nitrogen is used as the carrier gas at a flow rate of about 10 mL per minute. The injector employs a split ratio of 1 : 2.

*Procedure*—Inject about 1 μL of the *Reference solution*. Adjust the sensitivity of the detector so that the height of the peak due to caffeine is not less than 50% of the full scale of the recorder. The substances are eluted in the following order: *o*-toluenesulfonamide, *p*-toluenesulfonamide, and caffeine. The test is not valid unless the resolution between the peaks due to *o*-toluenesulfonamide and *p*-toluenesulfonamide is at least 1.5. Inject about 1 μL of the *Blank solution*. In the chromatogram obtained, verify that there are no peaks with the same retention times as the internal standard, *o*-toluenesulfonamide, and *p*-toluenesulfonamide. Inject about 1 μL of the *Test solution* and 1 μL of the *Reference solution*. If any peaks due to *o*-toluenesulfonamide, and *p*-toluenesulfonamide appear in the chromatogram obtained with the *Test solution*, the ratio of their areas to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the *Reference solution* (10 ppm of *o*-toluenesulfonamide and 10 ppm of *p*-toluenesulfonamide).

**Limit of benzoate and salicylate**—To 10 mL of a hot, saturated solution of it add ferric chloride TS, dropwise: no precipitate or violet color appears in the liquid.

**Organic volatile impurities, Method V** (467): meets the requirements.

*Solvent*—Use dimethyl sulfoxide.

**Assay**—Accurately weigh about 500 mg of Saccharin, dissolve in 40 mL of alcohol, add 40 mL of water, mix, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Perform a blank titration, if necessary, and make the appropriate correction. Each mL of 0.1 N sodium hydroxide is equivalent to 18.32 mg of C<sub>7</sub>H<sub>5</sub>NO<sub>3</sub>S. ■<sub>1S</sub> (NF23)