mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation.* Heat the solution to the production of dense, white fumes; cool; cautiously add 10 mL of water; and, if hydrogen peroxide was used in treating the *Test Preparation,* add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested. Boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation—Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L)$$

in which L is the *Heavy metals* limit, as a percentage.

If the substance is a solid—Transfer the weighed quantity of the test substance to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside, and add portions of the same acid mixture, heating after each addition, until a total of 18 mL of the acid mixture has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 mL of nitric acid, and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30 percent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 mL. If the solution is still yellow, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid—Transfer the weighed quantity of the test substance to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid,* beginning with "add portions of the same acid mixture."

Monitor Preparation—Proceed with the digestion, using the same amount of sample and the same procedure as directed in the subsection *If the substance is a solid* in the section *Test Preparation,* until the step "Cool, dilute cautiously with a few mL of water." Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Procedure—Treat the *Test Preparation,* the *Standard Preparation,* and the *Monitor Preparation* as follows. Using a pH meter or short-range pH indicator paper as external indicator, adjust the solution to a pH between 3.0 and 4.0 with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer,* then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface*: the color of the *Test Preparation* is not darker than that of the *Standard Preparation,* and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation.*

〈241〉 IRON

This limit test is provided to demonstrate that the content of iron, in either the ferric or the ferrous form, does not exceed the limit for iron specified in the individual monograph. The determination is made by concomitant visual comparison with a control prepared from a standard iron solution.

Special Reagents—

STANDARD IRON SOLUTION—Dissolve 863.4 mg of ferric ammonium sulfate [$FeNH_4(SO_4)_2 \cdot 12H_2O$] in water, add 10 mL of 2 N sulfuric acid, and dilute with water to 100.0 mL. Pipet 10 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, dilute with water to volume, and mix. This solution contains the equivalent of 0.01 mg (10 µg) of iron per mL.

AMMONIUM THIOCYANATE SOLUTION—Dissolve 30 g of ammonium thiocyanate in water to make 100 mL.

Standard Preparation—Into a 50-mL color-comparison tube pipet 1 mL of *Standard Iron Solution* (10 µg of Fe), dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

Test Preparation—Into a 50-mL color comparison tube place the solution prepared for the test as directed in the individual monograph and if necessary dilute with water to 45 mL; or, dissolve in water, and dilute with water to 45 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$1.0/(1000L)$$

in which L is the *Iron* limit in percentage. Add 2 mL of hydrochloric acid, and mix.

Procedure—To each of the tubes containing the *Standard Preparation* and the *Test Preparation* add 50 mg of ammonium peroxydisulfate crystals and 3 mL of *Ammonium Thiocyanate Solution*, and mix: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation.*

〈251〉 LEAD

The imposition of stringent limits on the amounts of lead that may be present in pharmaceutical products has resulted in the use of two methods, of which the one set forth following depends upon extraction of lead by solutions of dithizone. For determination of the content of heavy metals generally, expressed as a lead equivalent, see *Heavy Metals* 〈231〉.

Select all reagents for this test to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Rinse thoroughly all glassware with warm dilute nitric acid (1 in 2), followed by water.

Special Reagents—

AMMONIA-CYANIDE SOLUTION—Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

AMMONIUM CITRATE SOLUTION—Dissolve 40 g of citric acid in 90 mL of water. Add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a reddish color. Remove any lead that may be present by extracting the solution with 20-mL portions of *Dithizone Extraction Solution* (see below), until the dithizone solution retains its orange-green color.

DILUTED STANDARD LEAD SOLUTION—Dilute an accurately measured volume of *Standard Lead Solution* (see *Heavy Metals* ⟨231⟩) [containing 10 µg of lead per mL], with 9 volumes of dilute nitric acid (1 in 100) to obtain a solution that contains 1 µg of lead per mL.

DITHIZONE EXTRACTION SOLUTION—Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of alcohol. Store the solution in a refrigerator.

Before use, shake a suitable volume of the dithizone extraction solution with about half its volume of dilute nitric acid (1 in 100), discarding the nitric acid.

HYDROXYLAMINE HYDROCHLORIDE SOLUTION—Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make approximately 65 mL. Transfer to a separator, add 5 drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 minutes. Extract this solution with successive 10- to 15-mL portions of chloroform until a 5-mL portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 3 N hydrochloric acid until the solution is pink (if necessary, add 1 or 2 drops more of thymol blue TS), and then dilute with water to 100 mL.

POTASSIUM CYANIDE SOLUTION—Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from this solution by extraction with successive portions of *Dithizone Extraction Solution*, as described under *Ammonium Citrate Solution* above, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

STANDARD DITHIZONE SOLUTION—Dissolve 10 mg of dithizone in 1000 mL of chloroform. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light, and store in a refrigerator.

Test Preparation—[NOTE—If, in the following preparation, the substance under test reacts too rapidly and begins charring with 5 mL of sulfuric acid before heating, use instead 10 mL of cooled dilute sulfuric acid (1 in 2), and add a few drops of the hydrogen peroxide before heating.] Where the monograph does not specify preparation of a solution, prepare a *Test Preparation* as follows. [*Caution—Exercise safety precautions in this procedure, as some substances may react with explosive violence when digested with hydrogen peroxide.*] Transfer 1.0 g of the substance under test to a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest on a hot plate in a hood until charring begins. Other suitable means of heating may be substituted. (Add additional sulfuric acid, if necessary, to wet the substance completely, but do not add more than a total of 10 mL.) Add, dropwise and with caution, 30 percent hydrogen peroxide, allowing the reaction to subside and again heating between drops. Add the first few drops very slowly, mix carefully to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls of the flask. [NOTE—Add peroxide whenever the mixture turns brown or darkens.] Continue the digestion until the substance is completely destroyed, copious fumes of sulfur trioxide are evolved, and the solution is colorless. Cool, cautiously add 10 mL of water, evaporate until sulfur trioxide again is evolved, and cool. Repeat this procedure with another 10 mL of water to remove any traces of hydrogen peroxide. Cautiously dilute with 10 mL of water, and cool.

Procedure—Transfer the *Test Preparation,* rinsing with 10 mL of water, or the volume of the prepared sample specified in the monograph to a separator, and, unless otherwise directed in the monograph, add 6 mL of *Ammonium Citrate Solution* and 2 mL of *Hydroxylamine Hydrochloride Solution.* (For the determination of lead in iron salts use 10 mL of *Ammonium Citrate Solution.*) Add 2 drops of phenol red TS, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution if necessary, and add 2 mL of *Potassium Cyanide Solution.* Immediately extract the solution with 5-mL portions of *Dithizone Extraction Solution*, draining off each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 seconds with 20 mL of dilute nitric acid (1 in 100), and discard the chloroform layer. Add to the acid solution 5.0 mL of *Standard Dithizone Solution* and 4 mL of *Ammonia-Cyanide Solution*, and shake for 30 seconds: the color of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *Diluted Standard Lead Solution* equivalent to the amount of lead permitted in the sample under examination, and the same quantities of the same reagents and in the same manner as in the test with the sample.

⟨261⟩ MERCURY

Method I

NOTE—Mercuric dithizonate is light-sensitive. Perform this test in subdued light.

Reagents—

DITHIZONE STOCK SOLUTION—Dissolve 40 mg of dithizone in 1000 mL of chloroform.

DITHIZONE TITRANT—Dilute 30.0 mL of *Dithizone Stock Solution* with chloroform to 100.0 mL. This solution contains approximately 12 mg of dithizone per L.

MERCURY STOCK SOLUTION—Transfer 135.4 mg of mercuric chloride to a 100-mL volumetric flask, and dilute with 1 N sulfuric acid to volume. This solution contains the equivalent of 100 mg of Hg in 100 mL.

MERCURY SOLUTION FOR STANDARDIZING DITHIZONE TITRANT—Transfer 2.0 mL of *Mercury Stock Solution* to a 100-mL volumetric flask, and dilute with 1 N sulfuric acid to volume. Each mL of this solution contains the equivalent of 20 µg of Hg.

The following solutions are called for in the limit test for mercury that is specified in the monographs on Ferrous Fumarate, Ferrous Sulfate, and Dried Ferrous Sulfate.

HYDROXYLAMINE HYDROCHLORIDE SOLUTION—Prepare as directed in the test for *Lead* ⟨251⟩.

STANDARD MERCURY SOLUTION—On the day of use, quantitatively dilute 1.0 mL of *Mercury Stock Solution* with 1 N sulfuric acid to 1000 mL. Each mL of the resulting solution contains the equivalent of 1 µg of mercury.

DITHIZONE EXTRACTION SOLUTION—Prepare as directed in the test for *Lead* ⟨251⟩.

DILUTED DITHIZONE EXTRACTION SOLUTION—Just prior to use, dilute 5 mL of *Dithizone Extraction Solution* with 25 mL of chloroform.

Standardization of Dithizone Titrant—Transfer 1.0 mL of *Mercury Solution for Standardizing Dithizone Titrant* to a