

(231) HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* (851)) with a control prepared from a *Standard Lead Solution*. [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I*, or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III*, a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Lead Nitrate Stock Solution—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard Lead Solution—On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Method I

pH 3.5 Acetate Buffer—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Test Preparation—Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve in and dilute with water to 25 mL the 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. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Monitor Preparation—Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation*, and add 2.0 mL of *Standard Lead Solution*. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Procedure—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, 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 solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation*. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation*, use *Method II* instead of *Method I* for the substance being tested.]

Method II

NOTE—This method does not recover mercury.

pH 3.5 Acetate Buffer—Prepare as directed under *Method I*.

Standard Preparation—Prepare as directed under *Method I*.

Test Preparation—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, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as an external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Procedure—To each of the tubes containing the *Standard Preparation* and the *Test Preparation*, 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 solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

Method III

pH 3.5 Acetate Buffer—Prepare as directed under *Method I*.

Standard Preparation—Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-

* In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.

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 [$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$] 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.