

**Aim:** Limit Test for Heavy Metals

**References:**

1. Indian Pharmacopoeia (IP) 2022, Appendix 2.3.5, Limit Tests for Heavy Metals.
2. British Pharmacopoeia (BP) 2023, Appendix VIII, Limit Test for Heavy Metals.
3. United States Pharmacopoeia (USP) 43-NF 38, General Chapter <231> Heavy Metals.

**Objective:**

To determine the presence and permissible limit of heavy metals (e.g., lead, mercury, cadmium) in a pharmaceutical substance to ensure it meets pharmacopeial standards for safety and purity.

**Materials and Reagents**

**1. Reagents:**

- Lead standard solution (10 ppm Pb)
- Dilute acetic acid (6% v/v)
- Hydrogen sulfide (H<sub>2</sub>S) solution or Sodium sulfide (Na<sub>2</sub>S, 10% w/v)
- Ammonium acetate buffer solution (pH 3.5)
- Hydrochloric acid (HCl, 1% v/v)
- Purified water

**2. Apparatus:**

- Nessler cylinders (50 mL)
- Pipettes (1 mL, 5 mL, 10 mL)
- Glass rods
- Measuring cylinders
- Beakers (100 mL)
- Analytical balance

**Principle:**

The limit test for heavy metals is based on the reaction of heavy metal ions (like lead) with hydrogen sulfide ( $\text{H}_2\text{S}$ ) or sodium sulfide ( $\text{Na}_2\text{S}$ ) in a mildly acidic solution to produce a brown or black precipitate of metal sulfides. The intensity of color is compared visually against a standard solution containing a known amount of lead (Pb) to determine if the sample meets the specified limit.

## **Procedure:**

### **1. Preparation of Standard Lead Solution:**

- **Standard Lead Solution (10 ppm Pb):** Dissolve 159.8 mg of lead nitrate [ $\text{Pb}(\text{NO}_3)_2$ ] in 100 mL of water containing 1 mL of nitric acid ( $\text{HNO}_3$ ). Dilute to 1000 mL with purified water to obtain a 100 ppm Pb solution. From this, take 10 mL and dilute to 100 mL with purified water to make a 10 ppm Pb solution.

### **2. Preparation of the Test Solution:**

- Weigh and dissolve the specified quantity of the test substance in 25 mL of purified water.
- Add 2 mL of dilute acetic acid and adjust the pH to 3.0-4.0 using ammonia or additional acetic acid.
- Dilute the solution with purified water to 40 mL.

### **3. Preparation of the Standard Solution:**

- Transfer 2 mL of the standard lead solution (10 ppm Pb) to a Nessler cylinder.
- Add 25 mL of purified water and 2 mL of dilute acetic acid, adjusting the pH to 3.0-4.0.
- Dilute with purified water to 40 mL.

### **4. Addition of Reagent:**

- To both the test solution and the standard solution, add 10 mL of freshly prepared hydrogen sulfide solution ( $\text{H}_2\text{S}$ ) or sodium sulfide solution ( $\text{Na}_2\text{S}$ , 10% w/v).
- Mix well and allow to stand for 5 minutes.

### **5. Comparison:**

- Compare the intensity of any color produced in the test solution with that of the standard solution against a white background.

**Observation:**

- The color intensity of the test solution should not be darker than that of the standard solution, indicating that the heavy metal content is within the permissible limit.

**Result:** The sample passes the limit test if the color of the test solution is not more intense than that of the standard lead solution, confirming that the heavy metal content is within the acceptable limit.

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