1 Limit Tests

GENERAL QUESTIONS

Q 111 What do you understand by impurity?

Ans: Impurity is the foreign matter like glass particles, plastic fragments, porcelain particles or chemical substances like lead and arsenic salts, chloride and sulphate compounds, iron salts, etc.

Q 112 Is it possible to obtain absolute pure substance?

Ans: A state of absolute purity is almost unattainable. Even analytically pure compounds are having minute traces of impurities.

Q 113 Why it is not desirable to use 100% pure drug?

Ans: Purification of chemicals is an expensive process. Therefore the drug should not be purified more than needed, otherwise the cost of medicines would be beyond the reach.

Q 114 Which factors contribute to the purity of a substance?

Ans: Purity of the substance depends upon method of manufacture, type of crystallization and purification process and stability of the substance.

Q 115 What are the different sources of impurities in pharmaceutical substances?

Ans: The different sources of impurities in pharmaceutical substances are:

1. Raw material.
2. The manufacturing process.
   - Intermediates formed.
   - Reagents, solvents and catalysts used.
   - Metal of the reaction vessel.
3. Chemical decomposition of drug in presence of air, moisture.

4. Improper storage and deliberate adulteration.
5. Accidental substitution.
6. Deleberate adulteration with spurious or useless materials.

Q 116 Name the common impurities for which the pharmacopoeia prescribes limit tests.

Ans: The common impurities for which the pharmacopoeia prescribes limit tests are arsenic, heavy metals, chlorides, sulphates, iron.

Q 117 What is the meaning of limit tests?

Ans: Limit tests are semiquantitative or quantitative tests designed to identify and control small quantities of impurity which are likely to be present in the substance.

Q 118 What does the limit in limit test indicates?

Ans: Limit indicates the level of tolerance of impurity in a given substance. Beyond this level, the impurity would produce the harmful effects.

Q 119 What is the importance of limit tests? Why should limit tests be performed?

Ans: Limit tests should be performed to ensure the quality of the substance. The drugs should be free from physiologically harmful impurities or the impurity level should be acceptable.

Q 120 Which factors are taken into consideration before setting the limits?

Ans: Before setting limit for any impurity following factors are taken into consideration.

1. Intended use of the official compound
2. Safety considerations
3. Cost of production

It is necessary to strike a balance so that the drug is sufficiently pure to ensure reasonable levels of safety and efficacy, yet cost effective.

Q 121 Why does a drug need to pass the limits set in pharmacopoeia?

Ans: Adherence of the drug to official standards ensures consistent therapeutic response, acceptable level of potency and freedom from toxicity.

Q 122 Which apparatus is used for performing limit tests?

Ans: A pair of Nessler cylinders made of clear, lead-free glass is used for performing limit tests.

Q 123 What are the specifications for Nessler cylinders as per IP 2007?

Ans: Nessler cylinders are tubes made from clear, colourless glass with a uniform internal diameter and a flat, transparent base. They should be of 50 ml capacity. The overall height is about 150 mm, the external height to 50 ml mark, is 110 to 124 mm and the thickness of wall is 1.0 to 3.0 mm. The external height to the 50 ml mark of the cylinders, must not vary by more than 1 mm.

Q 124 What do you understand by comparative method of performing limit tests?

Ans: In comparative method, the test is performed on standard solution of impurity and test solution of the sample, under same conditions using same reagents. Here, the extent of reaction (either colouration or turbidity) is determined by direct comparison between the test solution and standard solution with known amount of impurity. If the opalscence/colouration produced by test solution is more than the standard solution, then the amount of impurity present in test sample is more than the specified limit.

Q 125 State the meaning of the term opalescence.

Ans: The milky, iridescent appearance of a dense, transparent medium or colloidal system illuminated by sunlight or visible light is called opalescence.

Q 126 Give the reason for formation of opalescence.

Ans: Opalescence is formed due to the white precipitate produced by the reaction between the impurity and the added reagents.

Q 127 Why the opalescence/colouration produced in two Nessler cylinders should be observed transversely against the dark background?

Ans: We must observe the opalescence/colouration of the Nessler cylinders transversely (placed equidistant from eye) to avoid the judgement error. When we view transversely, the distance from our eye and the cylinders is same which helps in correct judgement. If it is observed from an angle other than 90°C (between eye and the cylinders) then the distance between the eye and the cylinders change giving false denseness of opalescence/colouration, and thus faulty judgement.

Dark background will make it easy to understand the denseness/intensity of the opalescence.

Q 128 How do you draw inferences in limit tests?

Ans: If the opalescence/colouration produced by test solution is equal or less than the opalescence/colouration produced by the standard solution, the sample passes the limit test, indicating that the quantity of impurity is within the prescribed limit.

Q 129 How should we decide the borderline cases of the comparison?

Ans: Borderline cases may be decided by comparison over the printed page of a book, when the definition of the print is readily discernible and can be used as the criterion of the depth of opalescence.

Q 130 Why are the limits for some impurities generous while for others stringent?

Ans: Some impurites like arsenic and lead are potent nerve poisons even in small amounts. To prevent their dangerous effects, stringent limits are fixed. For other impurities like chlorides, sulphates, the harmful effects are not as prominent as for arsenic, cadmium and other heavy metals and thus the limits are generous.

Q 131 Why is the limit set for same impurity different for different substances?

Ans: In fixing the limits for impurities many points are taken into consideration, like how much of the impurity is likely to be harmful or to cause problems in formulation, intended duration of therapy, etc. In long-term therapy, cumulative effect of impurity may cause problem. Presence of iron in a sample of copper sulphate does not make its presence so objectionable as the same amount would in sodium salicylate. Thus, limit set for same impurity is different for different substances.

Q 132 How does the different limits for same impurity are tested even when the standard solution used is same?

Ans: As per pharmacopoeia, the standard solution for testing a specific impurity remains same for different drugs but the limit for impurity for different drugs is different. We change the amount of drug to be used for preparing test solution as

per the set limit. Variations in the permissible limits for different substances are arranged for, by varying the quantity of the substance used in the test, and not by varying the standard opalescence/turbidity or colouration.

Q 133 What is the unit used to express the limit of impurity in IP 2007?

Ans: 'ppm' is the unit used to express the limit of impurity in I.P. 2007.

Q 134 What do you understand by 200 ppm limit?

Ans: The meaning of 200 ppm limit for a particular impurity is 200 parts of impurity are permitted in 1 million parts of the sample.

Q 135 How would you prepare 100 ml of 25 ppm solution of a substance?

Ans: When 1 mg of substance is dissolved in 1000 ml, we get 1 ppm solution of the substance. We need to dissolve 2.5 mg of substance in 100 ml to get 25 ppm solution of the substance.

LIMIT TEST FOR CHLORIDES

Q 136 Write the principle of limit test for chlorides.

or

What is the chemical basis for the limit test for chlorides?

Ans: In this test, the white precipitate of silver chloride is formed by reaction between chloride ions and silver nitrate, in the presence of nitric acid. This precipitate causes the opalescence in the test solution and sample solution.

Q 137 State the chemical equation of chloride limit test.

Ans:

$$\underset{\text{Silver nitrate}}{Cl^- + AgNO_3} \xrightarrow{HNO_3} \underset{\text{Silver chloride white ppt}}{AgCl\downarrow} + NO_3^-$$

Q 138 Give the reason for formation of opalescence.

Ans: The opalescence is observed due to the formation of insoluble silver chloride, after reaction between chloride ions and silver nitrate. The denseness depends upon the amount of silver chloride formed.

Q 139 Why is nitric acid added in chloride limit test?

Ans: Nitric acid is added to acidify the solution before adding silver nitrate (If the solution is alkaline, nitric acid neutralises the alkali). Nitric acid is used to prevent the precipitation of other

acid radicals (like hydroxides, carbonates present as impurity) with silver nitrate solution. It acts by providing common ion, i.e. nitrate.

Q 140 Give the procedure for preparation of standard opalescence for chloride limit test.

Ans: The standard opalescence for chloride limit test is prepared as follows:

1. Take 10 ml of chloride standard solution (25 ppm Cl^-) in a Nessler cylinder.
2. Mix it with 5 ml of water.
3. Add 10 ml of dilute nitric acid.
4. Dilute to 50 ml with water.
5. Add 1 ml of 0.1 M silver nitrate solution.
6. Stir.
7. Allow to stand for 5 minutes protected from sunlight.

Q 141 Give the procedure for preparation of sodium acetate test solution for chloride limit test.

Ans:

1. Dissolve 1 gram of sodium acetate in sufficient carbon dioxide free water to produce 10 ml.
2. Transfer to a Nessler cylinder.
3. Add 10 ml of dilute nitric acid.
4. Dilute to 50 ml with water.
5. Add 1 ml of 0.1 M silver nitrate.
6. Stir and allow to stand for 5 minutes protected from sunlight.

Q 142 What is the limit of chloride for sodium acetate sample?

Ans: 250 ppm is the limit of chloride for sodium acetate.

Q 143 How limit test for chlorides is performed?

Ans: Limit test for chlorides is performed as follows.

1. Prepare standard opalescence.
2. Prepare test opalescence.
3. Test opalescence is compared with standard opalescence by viewing transversely against the suitable background.
   - If the test opalescence is denser than the standard opalescence, the quantity of chloride in the test sample is more than the limit prescribed by pharmacopoeia.
   - If the test opalescence is equal to standard opalescence, the quantity of chloride is equal in test and standard solution.

- If the test opalescence is less than the standard opalescence the quantity of chloride is less than the limit prescribed by pharmacopoeia.

4. The sample passes the limit test for chlorides, if the turbidity is equal or less than standard turbidity.

Q 144 How much quantity of sample should be taken to prepare test solution for limit test of chloride, when 200 ppm is the limit and standard opalescence is produced using 10 ml of 25 ppm standard solution?

Ans: 1g of sample ≅ 200 ppm

How many g of sample ≅ 250 ppm

$$= \frac{250}{200} = 1.25 \text{ g}$$

We should take 1.25 g of sample.

Q 145 What is the limit set for chloride for a sample when 2 g of sample is used for preparing test solution and 10 ml of 25 ppm solution is used for preparing standard opalescence?

Ans: 2 g of sample ≅ 10 ml of 25 ppm chloride

2 g of sample ≅ 250 ppm of chloride

2 g of sample ≅ 0.00025 g of chloride

$$1 \text{ g of sample} \cong \frac{0.00025}{2} \text{ g of chloride}$$

$$\cong 0.000125 \text{ g of chloride}$$

1000000 g of sample ≅ 125 g of chloride

∴ 1g of sample ≅ 125 ppm of chloride

The limit for chloride is 125 ppm.

Q 146 State the quantity of sodium carbonate taken for limit test for chlorides.

Ans: 1.25 g of sodium carbonate is taken.

Q 147 What is the limit of chlorides in sodium carbonate?

Ans: The prescribed limit for chloride is 200 ppm for sodium carbonate.

Q 148 State the quantity of sodium hydroxide required for limit test for chlorides.

Ans: 2 g of sodium hydroxide needs to be taken for limit test for chlorides.

Q 149 What is the limit of chlorides in sodium hydroxide?

Ans: The prescribed limit for chloride is 125 ppm.

Q 150 Which salt is used for preparing standard chloride solution?

Ans: Standard chloride solution is prepared using sodium chloride.

LIMIT TEST FOR SULPHATES

Q 151 What is the chemical basis for the limit test for sulphates?

Ans: Limit test for sulphate depends upon the precipitation of the sulphate with barium chloride in the presence of hydrochloric acid and traces of barium sulphate. The barium sulphate is prepared *in situ* by precipitation from ethanolic potassium sulphate. It assists rapid and complete precipitation by seeding. The opalescent solution thus obtained is compared with standard turbidity.

Q 152 Write chemical equation for limit test for sulphates.

Ans: $SO_4^{2-} + BaCl_2 \longrightarrow BaSO_4\downarrow + 2Cl^-$

Barium chloride — Barium sulphate white ppt

Q 153 Why ethanolic sulphate standard solution is added while preparing test opalescence in limit test for sulphate?

or

Why potassium sulphate is added in limit test for sulphate?

Ans: Ethanolic sulphate standard solution contains potassium sulphate. The potassium sulphate increases the sensitivity of the test by giving ionic concentrations in the reagent which just exceed the solubility product of barium sulphate. This leads to rapid and complete precipitation of sulphates.

Q 154 What is the significance of alcohol in limit test for sulphates?

Ans: Alcohol prevents supersaturation of barium sulphate.

Q 155 What is supersaturation?

Ans: A supersaturated solution is one that contains a greater concentration of solute than that corresponds to equilibrium solubility at the temperature under consideration. Supersaturation is therefore an unstable state which may be brought to a state of stable equilibrium by addition of crystal of solute (seeding the solution) or by mechanical means such as shaking or stirring.

Q 156 Why acetic acid is added in limit test for sulphates?

Ans: The solubility of the barium sulphate depends on the concentration of acid. Acetic acid is used to acidify the solution.

Q 157 Give the procedure for preparation of standard opalescence in the limit test for sulphates.

Ans:

- Take 1 ml of 25% w/v solution of barium chloride in Nessler cylinder.
- Add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix.
- Allow to stand for 1 minute.
- Add 15 ml of sulphate standard solution (10 ppm SO_4).
- Add 0.15 ml of 5 M acetic acid.
- Add sufficient water to produce 50 ml and stir.
- Allow to stand for 5 minutes.

Q 158 How would you prepare test opalescence in the limit test for sulphates?

Ans:

- Take 1 ml of a 25% w/v solution of barium chloride in a Nessler cylinder.
- Add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), and mix.
- Allow to stand for one minute.
- In another beaker, suspend desired quantity of sample in 10 ml of distilled water. Neutralise with hydrochloric acid and dilute to 15 ml with distilled water.
- Transfer this solution to Nessler cylinder.
- Add sufficient water to produce 50 ml and stir.
- Allow to stand for five minutes.

Q 159 How limit test of sulphate is performed?

Ans: Same as described for chlorides.

Q 160 What is the prescribed limit for sulphate in sodium bicarbonate?

Ans: 150 ppm is the limit for sulphate in sodium bicarbonate.

Q 161 What is the prescribed limit for sulphate in sodium dihydrogen phosphate dihydrate?

Ans: It is 300 ppm.

Q 162 What is the quantity of sample that should be used in preparing test opalescence in limit test for sulphate, where limit is 300 ppm? The standard solution is prepared using 15 ml of 10 ppm SO_4^{2-}.

Ans: 300 ppm ≅ 1 g of sample

(15 × 10) ppm ≅ ?

$$\text{Quantity of sample} = \frac{150}{300} \times 1 = 0.5 \text{ g}$$

Q 163 What is the limit set for sulphate for a sample when 3 g of sample is used for preparing test opalescence? 15 ml of 10 ppm SO_4^{2-} is used for preparing standard opalescence.

Ans: 3 g of sample ≅ 15 ml of 10 ppm SO_4^{2-}

≅ 150 ppm SO_4^{2-}

1 g of sample ≅ 50 ppm SO_4^{2-}

So, the limit for sulphate in that sample is 50 ppm.

Q 164 What quantity of sodium carbonate is used for preparing test opalescence in limit test for sulphates?

Ans: 1 g of sodium carbonate is used for preparing test opalescence for limit test for sulphates.

Q 165 What quantity of sodium dihydrogen phosphate dihydrate is used for limit test for sulphates?

Ans: For limit test for sulphates 0.5 g of sodium dihydrogen phosphate dihydrate is used.

Q 166 Which salt is used for preparation of sulphate standard solution?

Ans: Potassium sulphate is used for preparation of sulphate standard solution.

LIMIT TEST FOR IRON

Q 167 What is the chemical basis for the limit test for iron?

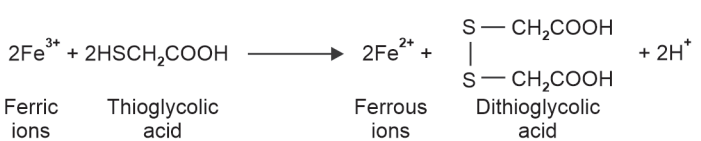
Ans: In the limit test for iron, thioglycolic acid reacts with iron, in a solution made alkaline with ammonia, and containing citric acid. Thioglycollic acid reduces ferric ions to ferrous ions and then further the ferrous ions react with thioglycolic acid to form coordination compound, ferrous thioglycolic, which has purple colour.

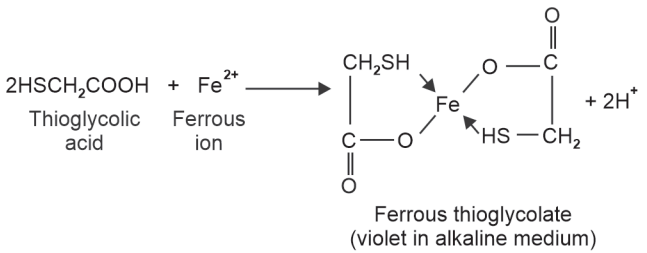
Q 168 Write the chemical equations for limit test for iron.

or

Explain the role of thioglycolic acid in limit test for iron.

Ans:





Ferrous thioglycolate
(violet in alkaline medium)

Q 169 Why is deep reddish purple colour developed in iron limit test?

Ans: Deep reddish purple colour is developed in iron limit test due to the formation of ferrous thioglycolic in alkaline medium.

Q 170 Why is citric acid added in limit test for iron?

Ans: Citric acid prevents precipitation of iron with ammonia and keeps iron in solution by forming a soluble complex with iron.

Q 171 Why is the solution made alkaline in limit test for iron?

Ans: Ferrous thioglycolic formed in the reaction is colourless in acidic medium while it is violet in alkaline medium. So, it is made alkaline.

Q 172 Give the procedure for preparing standard colouration in limit test for iron.

Ans: Take 2 ml of iron standard solution (20 ppm Fe) in a Nessler cylinder.

- Add 2 ml of 20% solution of iron free citric acid.
- Add 0.1 ml of thioglycolic acid.
- Mix.
- Make alkaline with iron free ammonia solution.
- Dilute to 50 ml with water.
- Allow to stand for 5 minutes.

Q 173 Describe the procedure for preparing test colouration.

Ans:

- Dissolve the prescribed quantity of sample in 20 ml of water, in a Nessler cylinder.
- Add 4 ml of hydrochloric acid.
- Dilute to 40 ml with water.
- Add 2 ml of a 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycollic acid.
- Mix.

- Make alkaline with iron-free ammonia solution.
- Dilute to 50 ml with water.
- Allow to stand for 5 minutes.

Q 174 How is limit test for iron performed?

Ans: Same as the answer under limit test for chlorides.

Q 175 What is the prescribed IP limit for iron in the samples of sodium bicarbonate?

Ans: 20 ppm

Q 176 What is the prescribed IP limit for iron in the sample of calcium carbonate?

Ans: 200 ppm

Q 177 What quantity of calcium carbonate should be used in preparing test colouration in limit test for iron, if the limit for iron is 160 ppm? The standard colouration is produced using 2 ml of 20 ppm Fe.

Ans: 1 g of sample ≅ 160 ppm of Fe

How many g of sample ≅ 40 ppm of Fe

$$= \frac{40}{160} \times 1 = 0.25 \text{ g of sample}$$

Q 178 What is the limit set for iron when 2 g of sample is used for preparing test colouration when standard colouration is prepared using 2 ml of 20 ppm Fe solution?

Ans: 2 g of sample ≅ 2 ml of 20 ppm Fe

≅ 40 ppm Fe

1 g of sample $\cong \frac{40}{2}$ ppm of Fe

≅ 20 ppm of Fe

Q 179 What quantity of calcium carbonate is used for limit test for iron as per IP?

Ans: IP prescribes 0.2 g of calcium carbonate when the limit is 200 ppm.

Q 180 What quantity of sodium bicarbonate is prescribed for limit test for iron in IP?

Ans: IP prescribes 2 g of sodium bicarbonate.

Q 181 Which salt is used for preparation of iron standard solution?

Ans: Ferric ammonium sulphate is used for preparation of iron standard solution.

LIMIT TEST FOR HEAVY METALS

Q 182 Name five heavy metals.

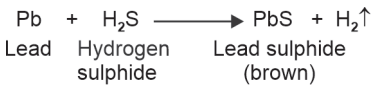
Ans: Lead, iron, copper, nickel, cobalt, and bismuth are some heavy metals.

Q 183 Why limit test for heavy metals is performed?

Ans: Limit test for heavy metals is performed to check that the quantity of heavy metals present in sample is below the threshold level which causes harmful effects.

Q 184 What is the chemical basis for limit test for heavy metals?

Ans: The limit test for heavy metals is indicated in terms of the parts of lead (Pb), per million parts (by weight) of the substance under examination. When the metallic impurities react with hydrogen sulphide, coloured metallic sulphides are precipitated.



Q 185 Why does the colour of solution ranges between black and brown?

Ans: Intensity of the colour produced depends upon the concentration of metallic impurity. Concentrated solutions of lead salts produce black precipitate while dilute solutions produce brown precipitate.

Q 186 Why pH of the solution is adjusted between 3.0 and 4.0?

Ans: Between pH 3.0 and 4.0, the metal sulphides are precipitated in colloidal form, giving uniform colour to the solution, making the comparison between test and the standard possible.

Q 187 How pH is adjusted between 3 and 4?

Ans: The pH is adjusted between 3 and 4 by use of either acetic acid or ammonia solution and confirming the pH by use of pH paper.

Q 188 What harmful effects heavy metals are capable to cause?

Ans: Heavy metals like lead, cadmium and mercury are potent nerve poisons.

Q 189 Why stringent limit is prescribed for heavy metals?

Ans: Stringent limit for heavy metals is prescribed because of severe harmful effects of them. In addition the, cumulative effect of such impurity can occur when patient is on long term/chronic

therapy like anti-rheumatics, heart stimulants and anti-hypertensives.

Q 190 Which salt is used for preparation of lead standard solution?

Ans: Lead nitrate is used for preparation of lead standard solution.

Q 191 Describe the procedure for preparing standard colouration in limit test for heavy metals.

Ans:

- Take 1 ml of lead standard solution (20 ppm Pb) in a Nessler cylinder.
- Dilute with water to 25 ml.
- Adjust the pH between 3.0 and 4.0 with dilute acetic acid or ammonia solution.
- Dilute with water to about 35 ml, and mix. Add 10 ml of freshly prepared hydrogen sulphide solution, and mix.
- Dilute to 50 ml with water.
- Allow to stand for 5 minutes.

Q 192 Why freshly prepared solution of hydrogen sulphide should be used in limit test for heavy metals?

Ans: Hydrogen sulphide solution is a saturated solution of H_2S in cold water. There is a possibility that H_2S gas is lost in storage. To avoid this freshly prepared solution of hydrogen sulphide should be used in limit test for heavy metals.

Q 193 How would you prepare test colouration in limit test for heavy metals?

Ans:

- Dissolve prescribed quantity of sample in 10 ml of water in a Nessler cylinder.
- Add 2 ml of dilute acetic acid and sufficient water to produce 25 ml.
- Adjust pH in between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solution.
- Dilute with water to about 35 ml and mix.
- Add 10 ml of freshly prepared hydrogen sulphide solution and mix.
- Dilute to 50 ml with water.
- Allow to stand for five minutes.

Q 194 How limit test for heavy metals is performed?

Ans: Same as described in limit test for chlorides.

Q 195 What is the prescribed IP limit for heavy metals in the sample of magnesium sulphate?

Ans: 10 ppm

Q 196 What is the prescribed IP limit for heavy metals in the sample of sodium chloride?

Ans: 5 ppm

Q 197 What quantity of sodium chloride should be used in preparing test colouration in limit test for heavy metals if the limit for heavy metal is 5 ppm? The standard colouration is produced using 2 ml of 20 ppm Pb solution. The limit for heavy metals in sodium chloride is 5 ppm.

Ans: 1 g of sample ≅ 5 ppm of Pb

How many g of sample ≅ 40 ppm of Pb

$$= \frac{40}{5} \times 1 = 8 \text{ g of sample}$$

Q 198 What is the limit set for heavy metals when 3 g of sample is used for preparing test colouration? Standard colouration is prepared using 2 ml of 15 ppm solution.

Ans: 3 g of sample ≅ 2 ml of 15 ppm Pb

≅ 30 ppm of Pb

1 g of sample = 10 ppm of Pb