2.2.3 Limit test for heavy metals

2019-01

Note: *The Guideline for Elemental Impurities Q3D, published by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), presents a process to assess and control elemental impurities in finished pharmaceutical products using the principles of risk assessment. It is within a regulatory authority's remit to decide whether or not they apply this guideline for the assessment of elemental impurities. If ICH Q3D is implemented, compliance of pharmaceutical substances with the limit test for heavy metals will no longer be required. However, performing the limit test for heavy metals does not ensure compliance with the limits of ICH Q3D.*

The limit test for heavy metals is provided to demonstrate that the content of metallic impurities that are precipitated as coloured sulfides by thioacetamide does not exceed the heavy metals limits given in the individual monographs in terms of micrograms of lead per gram of the test substance.

The test consists of three consecutive operations: preparation of the test solutions (Procedures 1 to 5), development of the coloured precipitate by reaction with thioacetamide, and comparison of the colours thus obtained, either by directly comparing the coloration of liquids in suitable comparison tubes (Method A) or by comparing the intensity of coloured residues obtained by filtering the liquid using an appropriate apparatus (Methods B or C). Method A is generally applicable only when the amount of heavy metals in the weight of the test substance used exceeds 5 µg; Methods B or C can also be used for amounts of 2 to 5 µg of heavy metals.

PREPARATION OF THE TEST SOLUTIONS

For the standard solution, unless otherwise specified, dilute lead PbTS containing 10 µg of lead per mL solvent to obtain a solution containing 1 µg of lead per mL or 2 µg of lead per mL, depending on the limit prescribed in the monograph. Use the solvent used to prepare the sample solution.

Procedure 1. For the sample solution, unless otherwise specified in the monograph, weigh the quantity of the substance to be examined and dissolve it in 25 mL of water R. For the reference solution, add 2 mL of the sample solution to 10 mL of the standard solution. For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

Procedure 2. For the sample solution, unless otherwise specified in the monograph, weigh the quantity of the substance to be examined and dissolve it in 25 mL of the organic solvent specified in the monograph, containing a minimum percentage of water R (for example, dioxan R containing 15% of water R or acetone R containing 15% of water R). For the reference solution, add 2 mL of the sample solution to 10 mL of the standard solution. For the blank solution, add 2 mL of the sample solution to 10 mL of the solvent used to prepare the sample solution.

Procedure 3. For the sample solution, place the prescribed quantity (not more than 2 g) of the substance to be examined in a silica crucible with 4 mL of a 250 g/L solution of magnesium sulfate R in sulfuric acid (~98 g/L) TS. Mix using a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water bath. Progressively heat to ignition and continue heating until an almost white or, at most, greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Moisten the residue with a few drops of sulfuric acid (~98 g/L) TS. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed two hours. Take up the residue in two quantities, each of 5 mL, of hydrochloric acid (~70 g/L) TS. Add 0.1 mL of diluted phenolphthalein/ethanol TS, then ammonia (~35 g/L) TS, until a pink colour is obtained. Cool, add anhydrous acetic acid R until the solution is decolorized and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute with water R to 20 mL.

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead PbTS containing 10 µg of lead per mL instead of the substance to be examined. To 10 mL of the solution obtained, add 2 mL of the sample solution.

For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution. To 10 mL of the solution obtained, add 2 mL of the sample solution.

For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

Procedure 4. For the sample solution, unless otherwise specified in the monograph, mix thoroughly in a silica crucible the prescribed quantity of the substance to be examined with 0.5 g of magnesium oxide R1. Ignite to a dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 minutes of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary, repeat the operation. Heat at 800 °C for about one hour. Take up the residue in two quantities, each of 5 mL, of a mixture of equal volumes of hydrochloric acid (~250 g/L) TS and water R. Add 0.1 mL of diluted phenolphthalein/ethanol TS and then ammonia (~35 g/L) TS until a pink colour is obtained. Cool, add anhydrous acetic acid R until the solution is decolorised, then add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute with water R to 20 mL.

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead

PbTS containing 10 µg of lead per mL instead of the substance to be examined and drying in an oven at 100 °C to105 °C. To 10 mL of the solution obtained, add 2 mL of the sample solution.

For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution and drying in an oven at 100 °C to105 °C. To 10 mL of the solution obtained, add 2 mL of the sample solution.

For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

Procedure 5. For the sample solution, unless otherwise specified in the monograph, place the prescribed amount of the substance to be examined (not more than 0.5 g) in a suitable, clean beaker. Add successively 2.7 mL of cadmium-free and lead-free sulfuric acid (~1760 g/L) TS, 3.3 mL of cadmium-free and lead-free nitric acid (~1000 g/L) TS, and 2.0 mL of hydrogen peroxide (~330 g/L) TS using a magnetic stirrer. Allow the substance to react with the reagent before adding the next one. Transfer the mixture to a dry high-pressure digestion vessels (fluoropolymer or quartz glass).

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead PbTS containing 10 µg of lead per mL instead of the substance to be examined.

For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution.

For the blank solution, prepare the solution as described for the sample solution, omitting the substance to be examined.

CAUTION: When using high-pressure digestion vessels, the safety precautions and operating instructions given by the manufacturer must be followed. The digestion cycles have to be elaborated depending on the type of microwave oven to be used (for example, energy-controlled microwave ovens, temperature-controlled microwave ovens or high-pressure ovens). The cycle must conform to the manufacturer's instructions. The digestion cycle is suitable if a clear solution is obtained.

Close the vessels and place them in a laboratory microwave oven. Digest using a sequence of two separate suitable programmes. Design the programmes in several steps in order to control the reaction, monitoring pressure, temperature or energy depending on the type of microwave oven available. After the first programme, allow the digestion vessels to cool before opening.

Add to each vessel 2.0 mL of hydrogen peroxide (~330 g/L) TS and digest using the second programme. After the second programme, allow the digestion vessels to cool before opening. If necessary to obtain a clear solution, repeat the addition of hydrogen peroxide (~330 g/L) TS and the second digestion programme.

Cool, dilute cautiously with water R and rinse into a flask, ensuring that the total volume does not exceed 25 mL.

Colour development and measurement

For Procedures 1 to 4

Method A. Use matched flat-bottomed comparison tubes of transparent glass with a uniform internal diameter of 16 mm for the comparison of the colours. "Matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects.

Transfer 12 ml of each of the test solutions prepared as described under *Preparations of the test solutions* to comparison tubes, add 2 mL of acetate buffer, pH 3.5, TS and mix. Add 1.2 mL of freshly prepared thioacetamide reagent TS, mix and allow to stand for two minutes.

Compare the colours of the solutions by viewing down the vertical axis of the tube in diffused light against a white or, if necessary, a black background, or by another suitable method. The test is not valid unless the colour of the reference solution is more intense than the colour of the blank solution. If the use of a monitor solution is prescribed, the colour of the monitor solution is at least as intense as the colour of the reference solution.

The sample complies with the requirements of the test when the colour of the test solution is not darker than the reference solution.

Method B. Transfer 12 ml of each of the test solutions prepared as described under *Preparations of the test solutions* to a beaker, add 2 mL of acetate buffer, pH 3.5, TS and mix. Add 1.2 mL of freshly prepared thioacetamide reagent TS, mix and allow to stand for two minutes.

Filter the solutions through a suitable membrane filter (nominal pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston.

Compare the intensity of the coloration of the residues obtained with the different test solutions on the membrane filters. The test is not valid unless the coloured residue obtained with the reference solution is more intense than the coloured residue obtained

with the blank solution. If the use of a monitor solution is prescribed, the coloured residue obtained with the monitor solution is at least as intense as the coloured residue obtained with the reference solution.

The sample complies with the requirements of the test when the coloured residue obtained from the test solution is not more intense than the coloured residue from the lead standard.

For Procedure 5:

Method C. Using short-range pH indicator paper, adjust the test solutions to pH 3.0-4.0 with ammonia (~260 g/L) TS. (Ammonia (~100 g/L) TS may be used as the specified range is approached). To avoid heating of the solutions, use an ice bath and a magnetic stirrer. Dilute to 40 mL with water R and mix. Add 2 mL of acetate buffer, pH 3.5, TS and mix. Add to 1.2 mL of thioacetamide reagent TS. Mix immediately. Dilute to 50 mL with water R, mix and allow to stand for two minutes. Filter the solutions through a suitable membrane filter (nominal pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston.

Compare the spots on the filters obtained with the different solutions. The test is not valid unless the coloured residue obtained with the reference solution is more intense than the coloured residue obtained with the blank solution. The coloured residue obtained with the monitor solution is at least as intense as the coloured residue obtained with the reference solution.

The sample complies with the requirements of the test when the coloured residue obtained with the sample solution is not more intense than the coloured residue obtained with the reference solution.