PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

GENERAL TESTING PROCEDURE

Title: Limit Test for Heavy Metals

SOP No.:		**Department:**	QC
Effective Date:		**Review Date:**	
Revision No.:	00	**Page No.:**	1 of 10
Supersede SOP No.:	Nil		

1.0 OBJECTIVE:

1.1 To lay down a procedure for limit test for Heavy metals.

2.0 SCOPE:

2.1 It is applicable for the estimation of Raw material**.**

3.0 RESPONSIBILITY:

3.1 Analyst / Officer / Executive follow the procedure.

3.2 Head-QC are responsible for effective implementation of this SOP.

4.0 REFERENCE:

4.1 Ph. Eur. method

5.0 DEFINITION:

5.1 Arsenic is a chemical present in the environment as a naturally occurring substance and as a result of human activity. It is found in water, air, food and soil.

6.0 PROCEDURE:

6.1 METHOD A:

Test solution: 12 mL of the prescribed aqueous solution of the substance to be examined. **Reference solution (standard):** A mixture of 10 mL of lead standard solution (1 ppm Pb) or lead standard solution (2 ppm Pb), as prescribed, and 2 mL of the prescribed aqueous solution of the substance to be examined.

Blank solution: A mixture of 10 mL of water and 2 mL of the prescribed aqueous solution of the substance to be examined.

To each solution, add 2 mL of buffer solution pH 3.5. Mix and add to 1.2 mL of thioacetamide reagent. Mix immediately. Examine the solutions after 2 min.

System suitability The reference solution shows a slight brown colour compared to the blank solution.

Result: Any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 urn), 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

6.2 Method B:

Test solution: 12 mL of the prescribed solution of the substance to be examined prepared using an organic solvent containing a minimum percentage of water (for example, dioxan containing 15 per cent of water or acetone containing 15 per cent of water).

Reference solution (standard): A mixture of 10 mL of lead standard solution (l or 2 ppm Pb), as prescribed, and 2 mL of the prescribed solution of the substance to be examined in an organic solvent. Prepare the lead standard solution (l or 2 ppm Pb) by dilution of lead standard solution (100 ppm Pb) R with the solvent used for the substance to be examined.

Blank solution: A mixture of 10 mL of the solvent used for the substance to be examined and 2 mL of the prescribed solution of the substance to be examined in an organic solvent.

To each solution, add 2 mL of buffer solution pH 3.5 R.Mix and addto 1.2 mL of thioacetamide reagent R.Mix immediately. Examine the solutions after 2 min.

System suitability: The reference solution shows a slight brown colour compared to the blank solution.

Result: Any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge. filter the solutions through a suitable membrane filter (nominal pore size 0.45 urn). 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.

6.3 Method-C:

Test 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 in dilute sulfuric acid. 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 dilute sulfuric acid. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of 5 mL, of dilute hydrochloric acid. Add 0.1 mL of phenolphthalein solution, then concentrated ammonia until a pink colour is obtained. Cool, add glacial acetic acid until the solution is decolorised and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute to 20.mL with water.

Reference solution (standard): Prepare as described for the test solution, using the prescribed volume of lead standard solution (10 ppm Pb) instead of the substance to be examined. To 10 mL of the solution obtained add 2 mL of the test solution. Monitor solution Prepare as described for the test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb) R prescribed for preparation of the reference solution. To 10 mL of the solution obtained add 2 mL of the test solution.

Blank solution: A mixture of 10 mL of water and 2 mL of the test solution. To 12 mL of each solution, add 2 mL of buffer solution pH 3.5 R. Mix and add to 1.2 mL of thioacetamide reagent. Mix immediately. Examine the solutions after 2 min.

System suitability: The reference solution shows a slight brown colour compared to the blank solution,

The monitor solution is at least as intense as the reference solution.

Result: Any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 urn). 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.

6.4 **Method-D: Test solution:** In a silica crucible, mix thoroughly the prescribed quantity of the substance to be examined with 0.5 g of magnesium oxide. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 min 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 1 h. Take up the residue in 2 quantities, each of 5 ml, of a mixture of equal volumes of hydrochloric acid and water. Add 0.1 mL of phenol phthalein solution and then concentrated ammonia until a pink colour is obtained. Cool, add glacial acetic acid until the solution is decolorised and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute to 20 mL with water.

Reference solution (standard): Prepare as described for the test solution using the prescribed volume of lead standard solution (10 ppm Pb)instead "of the substance to be examined and drying in an oven at 100-105 "C. To 10 mlof the solution obtained add 2 mL of the test solution.

Monitor solution: Prepare as described for the test solution, adding to the substance to be examined the volume of lead standard solution (10 ppmPb) R prescribed for preparation of the reference solution and drying in an oven at 100-105 "C. To 10 mL of the solution obtained add 2mL of the test solution.

Blank solution: A mixture of 10 mL of water and 2 ml of the test solution. To 12 mL of each solution, add 2 mL of buffer solution pH 3.5. Mix and add to 1.2 mL of thioacetamide reagent. Mix immediately, Examine the solutions after 2 min.

System suitability: The reference solution shows a slight brown colour compared to the blank solution,

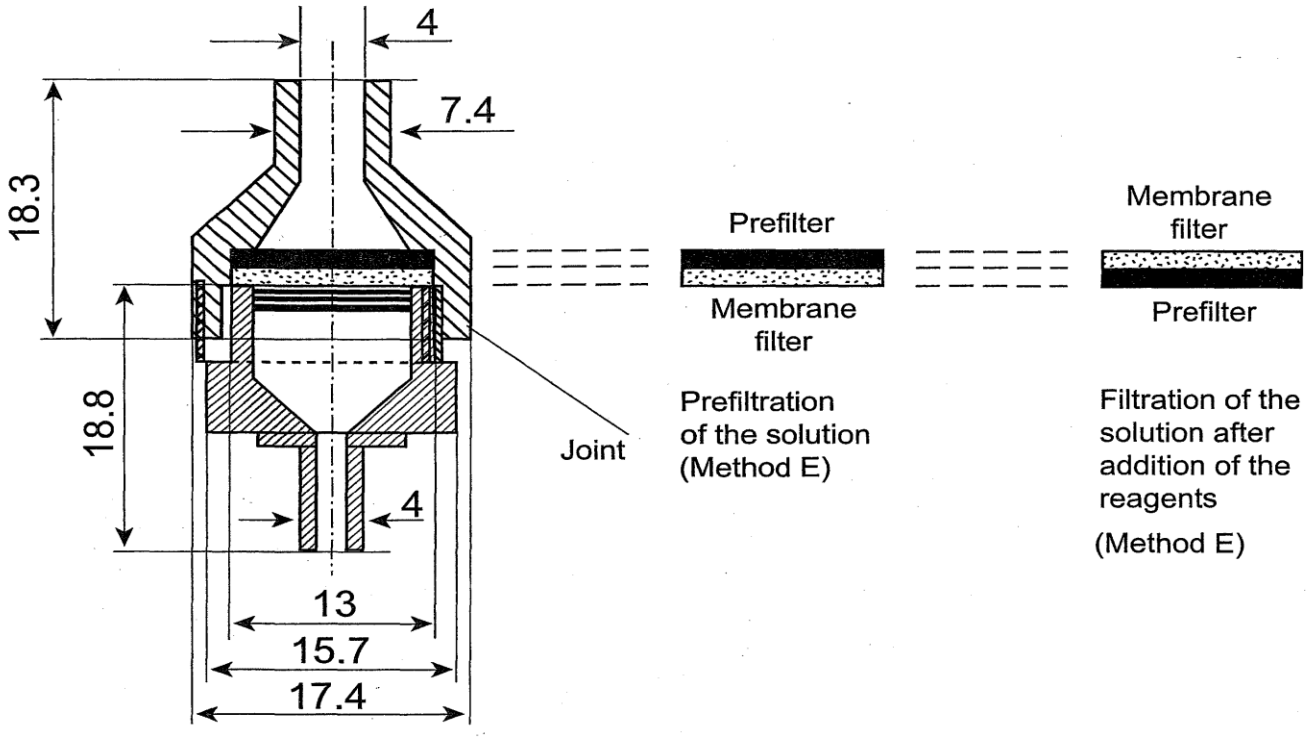
The monitor solution is at least as intense as the reference solution.

Result: Any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 um). 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.

6.5 **Method E: Test solution:** Dissolve the prescribed quantity of the substance to be examined in 30 mL of waterR or the prescribed volume.

Reference solution (standard) Unless otherwise prescribed, dilute the prescribed volume of lead standard solution (1 ppm Pb) R to the same volume as the test solution. Prepare the filtration apparatus by adapting the barrel of a SO mL syringe without its piston to a support containing, on the plate, a membrane filter (nominal pore size 3 urn) and above it a prefilter (Figure 2.4.8.-1). Transfer the test solution into the syringe barrel, put the piston in place and then apply an even pressure on it until the whole of the liquid has been filtered. In opening the support and removing the prefilter, check that the membrane filter remains uncontaminated with impurities. If this is not the case replace it with another membrane filter and repeat the operation under the same conditions. To the prefiltrate or to the prescribed volume of the prefiltrate add 2 mL of buffer solution pH 3.5 R. Mix and add to 1.2 mL of thioacetamide reagent R. Mix immediately and allow to stand for 10 min and again filter as described above, but inverting the order of the filters, the liquid passing first through the membrane filter before passing through the prefilter (Figure 2.4.8.-1). The filtration must be carried out slowly and uniformly by applying moderate and constant pressure to the piston of the syringe. After complete filtration, open the support, remove the membrane filter, and dry using filter paper. In parallel, treat the reference solution in the same manner as the test solution.

Result: The colour of the spot obtained with the test solution is not more intense than that obtained with the reference solution



Apparatus for the test for heavy metals Dimensions in millimetres

6.6 **Method F: Test Solution:** Place the prescribed quantity or volume of the substance to be examined in a clean, dry, 100 mL long necked combustion flask (a 300 mL flask may be used if the reaction foams excessively). Clamp. the flask at an angle of 45°. If the substance to. be examined is a solid, add a sufficient volume of a mixture of 8 mL of sulfuric acid and 10 mL of nitric add R to moisten the substance thoroughly; if the substance to be examined is a liquid, add a few millilitres of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently unti the reaction commences, allow the reaction to subside and add additional 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 the addition of nitric acid until no. further darkening occurs, then heat strongly until dense, white fumes are produced. Cool, cautiously add 5 mL of water, boil gently until dense, white fumes are

produced and continue heating to reduce to 2-3 ml. Cool, cautiously add 5 mL of water and examine the colour of the solution. If the colour is yellow, cautiously add 1 mL of strong hydrogen peroxide solution and again evaporate until dense, white fumes are produced and reduce to a volume of 2-3 ml. If the solution is still yellow in colour, repeat the addition of 5 mL of water and 1 mL of strong hydrogen peroxide solution until the solution is colourless. Cool, dilute cautiously with water and rinse into a 50 mL colour comparison tube, ensuring that the total volume does not exceed 25 ml. Adjust the solution to pH 3.0-4.0, using short range pH indicator paper as external indicator, with concentrated ammonia (dilute ammonia may be used, if desired, as the specified range is approached), dilute with water to 40 mL and mix. Add 2 mL of buffer solution pH3.5. Mix and add to 1.2 mL of thioacetamide reagent. Mix immediately. Dilute to 50 mL with water and mix.

Reference solution (standard): Prepare at the same time and in the same manner as the test solution, using the prescribed volume of lead standard solution (10 ppm Pb). Monitor solution Prepare as described for the test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb) prescribed for the preparation of the reference solution.

Blank solution: Prepare as described for the test solution, omitting the substance to be examined. Examine the solutions vertically against a white background after 2 min.

System suitability: The reference solution shows a brown colour compared to the blank solution, The monitor solution is at least as intense as the reference solution.

Result: Any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 µl). 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.

6.7 **Method G:** 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 (Jor example, energy-controlled microuaoe opens, temperature-controlled microwave ovens or high-pressure ovens). The cycle must conform to the manufacturer's instructions. The digestz'on cycle is suitable if a

clear solution is obtained. rest solution 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 sulfuric acid, 3.3 mL of nitric acid and 2.0 mL of strong hydrogen peroxide solution using a magnetic stirrer. Allow the substance to react with a reagent before adding the next one. Transfer the mixture to a dry high-pressure-resistant digestion vessel (fluoropolymer or quartz glass).

Reference solution (standard): Prepare as described for the test solution, using the prescribed volume of lead standard solution (10 ppm Pb) instead of the substance to be examined. Monitor. solution. Prepare as prescribed for the test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb) prescribed for the preparation of the reference solution.

Blank solution: Prepare as described for the test 'solution, omitting the substance to be examined. Close the vessels and place in a laboratory microwave oven. Digest using a sequence of 2 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 strong hydrogen peroxide solution 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 strong hydrogen peroxide solution and the second digestion programme. Cool, dilute cautiously with water and rinse into a flask, ensuring that the total volume does not exceed 25 mL. Using short-range pH indicator paper as external indicator, adjust the solutions to pH 3.0-4.0 with concentrated ammonia (dilute ammonia 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 and mix. Add 2 mL of buffer solution pH 3.5 R. Mix and add to 1.2 mL of thioacetamide reagent. Mix immediately. Dilute to 50 mL with water, mix and allow to stand for 2 min. Filter the solutions through a suitable membrane filter (nominal pore size 0.45 11m). 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.

System suitability: The spot obtained with the reference solution shows a brown colour compared to the spot obtained with the blank solution.

The spot obtained with the monitor solution is at least as intense as the spot obtained with the reference solution.

Result: The brown colour of the spot obtained with the test solution is not more intense than that of the spot obtained with the reference solution.

6.8 **Method H: Test solution**: Dissolve the prescribed quantity of the substance to be examined in 20 ml of the solvent or solvent mixture prescribed.

Reference solution: Dilute the prescribed volume of leadstandard solution (10 ppm Pb) R to 20 mL with the solvent or solvent mixture prescribed.

Blank solution: 20 mL of the solvent or solvent mixture prescribed. To each solution, add 2 mL of buffer solution pH 3.5 R. Mix. (In some cases precipitation occurs, in which case the Specific monograph would describe re-dissolutionin a defined volume of a given solvent.) Add to 1.2 mL of thioacetamide reagent R. Mix immediately and allow to stand for 2 min. Filter the solutions through a suitable membrane filter (nominal pore size 0.45µl). Compare the spots on the filters obtained with the different solutions.

System suitability: The spot obtained with the reference solution shows a brownish-black colour compared to the spot obtained with the blank solution.

Result: The brownish-black colour of the spot obtained with the test solution is not more intense than that of the spot obtained with the reference solution.

7.0 **Annexures:** NA

8.0 **Distribution:**

8.1 Display copy 1: Quality Control Lab

9.0 **Abbreviation:**

GTP : General Test Procedure

QC : Quality Control laboratories

10.0 Revision History:

10.1 Revision history table:

Document Number	CC Number/Date	Brief Description of Change