butylamine R and 0.1 mL of *triethanolamine R*. Adjust the solution, if necessary, to pH 10.5 to pH 11.5. Add 4 mL of the solution of hydroxyquinoline in chloroform, shake for 1 min, allow to stand and separate. Use the lower layer for comparison. Prepare a standard in the same manner using a mixture of 1 mL of *magnesium standard solution (10 ppm Mg) R* and 9 mL of *water R*.

Any colour in the solution obtained from the substance to be examined is not more intense than that in the standard.

01/2008:20407

2.4.7. MAGNESIUM AND ALKALINE-EARTH METALS

To 200 mL of *water R* add 0.1 g of *hydroxylamine hydrochloride R*, 10 mL of *ammonium chloride buffer solution pH 10.0 R*, 1 mL of *0.1 M zinc sulfate* and about 15 mg of *mordant black 11 triturate R*. Heat to about 40 °C. Titrate with *0.01 M sodium edetate* until the violet colour changes to full blue. To the solution add the prescribed quantity of the substance to be examined dissolved in 100 mL of *water R* or use the prescribed solution. If the colour of the solution changes to violet, titrate with *0.01 M sodium edetate* until the full blue colour is again obtained.

The volume of *0.01 M sodium edetate* used in the second titration does not exceed the prescribed quantity.

07/2010:20408

2.4.8. HEAVY METALS

The methods described below require the use of *thioacetamide reagent R*. As an alternative, *sodium sulfide solution R1* (0.1 mL) is usually suitable. Since tests prescribed in monographs have been developed using *thioacetamide reagent R*, if *sodium sulfide solution R1* is used instead, it is necessary to include also for methods A, B and H a monitor solution, prepared from the quantity of the substance to be examined prescribed for the test, to which has been added the volume of lead standard solution prescribed for preparation of the reference solution. The test is invalid if the monitor solution is not at least as intense as the reference solution.

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) R* or *lead standard solution (2 ppm Pb) R*, 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 R* 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 R*. Mix and add to 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 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.

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 (1 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 (1 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 add to 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 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.

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 R* in *dilute sulfuric acid R*. 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 R*. 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 R*. Add 0.1 mL of *phenolphthalein solution R*, then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* 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 R*.

Reference solution (standard). Prepare as described for the test solution, using the prescribed volume of *lead standard solution (10 ppm Pb) R* 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 R* 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 R*. 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 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.

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 R1*. 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 R1* and *water R*. Add 0.1 mL of *phenolphthalein solution R* and then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* 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 R*.

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

Blank solution. A mixture of 10 mL of *water R* 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 R*. 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 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.

METHOD E

Test solution. Dissolve the prescribed quantity of the substance to be examined in 30 mL of *water R* 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 50 mL syringe without its piston to a support containing, on the plate, a membrane filter (nominal pore size 3 µm) 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.

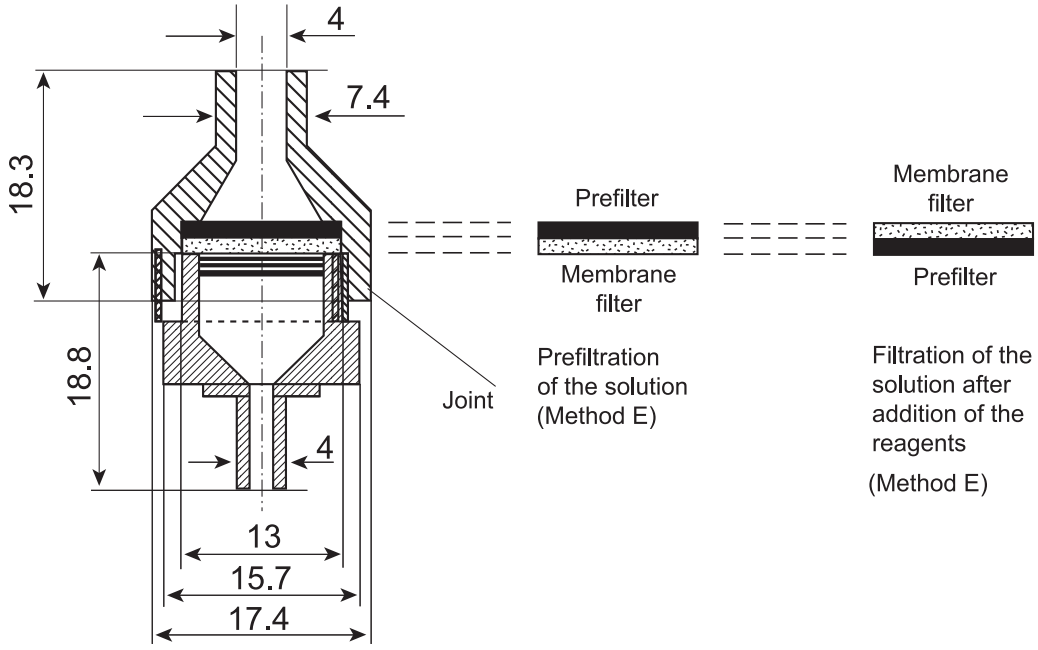

Figure 2.4.8.-1. – *Apparatus for the test for heavy metals*
Dimensions in millimetres

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

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 R* and 10 mL of *nitric acid 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 R* and 10 mL of *nitric acid R*. Warm gently until 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 R* and heat again until the solution darkens. Continue the heating, followed by the addition of *nitric acid R* until no further darkening occurs, then heat strongly until dense, white fumes are produced. Cool, cautiously add 5 mL of *water R*, boil gently until dense, white fumes are produced and continue heating to reduce to 2-3 mL. Cool, cautiously add 5 mL of *water R* and examine the colour of the solution. If the colour is yellow, cautiously add 1 mL of *strong hydrogen peroxide solution R* 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 R* and 1 mL of *strong hydrogen peroxide solution R* until the solution is colourless. Cool, dilute cautiously with *water R* 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 R1* (*dilute ammonia R1* may be used, if desired, as the specified range is approached), dilute with *water R* to 40 mL and mix. Add 2 mL of *buffer solution pH 3.5 R*. Mix and add to 1.2 mL of *thioacetamide reagent R*. Mix immediately. Dilute to 50 mL with *water R* 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) R*.

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 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 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.

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 (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.

Test 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 R*, 3.3 mL of *nitric acid R* and 2.0 mL of *strong hydrogen peroxide solution R* 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) R* 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) R* 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 R* 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 R* 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.

Using short-range pH indicator paper as external indicator, adjust the solutions to pH 3.0-4.0 with *concentrated ammonia R1* (*dilute ammonia R1* 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 *buffer solution pH 3.5 R*. Mix and add to 1.2 mL of *thioacetamide reagent R*. Mix immediately. Dilute to 50 mL with *water R*, mix and allow to stand for 2 min.

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.

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.

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 *lead standard 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-dissolution in 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 0.45 µm). 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.

01/2008:20409

2.4.9. IRON

Dissolve the prescribed quantity of the substance to be examined in *water R* and dilute to 10 mL with the same solvent or use 10 mL of the prescribed solution. Add 2 mL of a 200 g/L solution of *citric acid monohydrate R* and 0.1 mL of *thioglycollic acid R*. Mix, make alkaline with *ammonia R* and dilute to 20 mL with *water R*. Prepare a standard in the same manner, using 10 mL of *iron standard solution (1 ppm Fe) R*.

After 5 min, any pink colour in the test solution is not more intense than that in the standard.

01/2008:20410

2.4.10. LEAD IN SUGARS

Determine the lead by atomic absorption spectrometry (*2.2.23, Method II*).

Test solution. Dissolve 20.0 g of the substance to be examined in a mixture of equal volumes of *dilute acetic acid R* and *water R* and dilute to 100.0 mL with the same mixture of solvents. Add 2.0 mL of a clear 10 g/L solution of *ammonium pyrrolidinedithiocarbamate R* and 10.0 mL of *methyl isobutyl ketone R* and then shake for 30 s protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solutions. Prepare 3 reference solutions in the same manner as the test solution but adding 0.5 mL, 1.0 mL and 1.5 mL respectively of *lead standard solution (10 ppm Pb) R* in addition to the 20.0 g of the substance to be examined.

Set the zero of the instrument using *methyl isobutyl ketone R* treated as described for the test solution without the substance to be examined. Measure the absorbance at 283.3 nm using a lead hollow-cathode lamp as source of radiation and an air-acetylene flame.

The substance to be examined contains not more than 0.5 ppm of lead, unless otherwise prescribed.

01/2008:20411

2.4.11. PHOSPHATES

To 100 mL of the solution prepared and, if necessary, neutralised as prescribed add 4 mL of *sulfomolybdic reagent R3*. Shake and add 0.1 mL of *stannous chloride solution R1*. Prepare a standard in the same manner using 2 mL of *phosphate standard solution (5 ppm PO_4) R* and 98 mL of *water R*. After 10 min, compare the colours using 20 mL of each solution.

Any colour in the test solution is not more intense than that in the standard.

01/2008:20412

2.4.12. POTASSIUM

To 10 mL of the prescribed solution add 2 mL of a freshly prepared 10 g/L solution of *sodium tetraphenylborate R*. Prepare a standard in the same manner using a mixture of 5 mL of *potassium standard solution (20 ppm K) R* and 5 mL of *water R*.

After 5 min, any opalescence in the test solution is not more intense than that in the standard.

01/2008:20413 corrected 8.0

2.4.13. SULFATES

All solutions used for this test must be prepared with distilled water R.

Add 3 mL of a 250 g/L solution of *barium chloride R* to 4.5 mL of *sulfate standard solution (10 ppm SO_4) R1*. Shake and allow to stand for 1 min. To 2.5 mL of this suspension add 15 mL of the prescribed solution and 0.5 mL of *acetic acid R*. Prepare a standard in the same manner using 15 mL of *sulfate standard solution (10 ppm SO_4) R* instead of the prescribed solution.

After 5 min, any opalescence in the test solution is not more intense than that in the standard.

04/2010:20414

2.4.14. SULFATED ASH(1)

Ignite a suitable crucible (for example, silica, platinum, porcelain or quartz) at 600 ± 50 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant and weigh. Place the prescribed amount of the substance to be examined in the crucible and weigh. Moisten the substance to be examined with a small amount of *sulfuric acid R* (usually 1 mL) and heat gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of *sulfuric acid R* (usually 1 mL), heat gently until white fumes are no longer evolved and ignite at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue.

If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with *sulfuric acid R* and ignition, as previously, for 30 min periods until 2 consecutive weighings do not differ by more than 0.5 mg or until the percentage of residue complies with the prescribed limit.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation.*