

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 PO₄) 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 SO₄) 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 SO₄) 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*.

The amount of substance used for the test (usually 1–2 g) is chosen so that at the prescribed limit the mass of the residue (usually about 1 mg) can be measured with sufficient accuracy.

01/2008:20415
corrected 7.0



2.4.15. NICKEL IN POLYOLS

Determine the nickel 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 saturated solution of *ammonium pyrrolidinedithiocarbamate R* (about 10 g/L) 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 *nickel standard solution (10 ppm Ni) 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 preparation of the test solution omitting the substance to be examined. Measure the absorbance at 232.0 nm using a nickel hollow-cathode lamp as source of radiation and an air-acetylene flame.

The substance to be examined contains not more than 1 ppm of nickel, unless otherwise prescribed.



01/2008:20416

2.4.16. TOTAL ASH

Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator and weigh. Unless otherwise prescribed, evenly distribute 1.00 g of the substance or the powdered herbal drug to be examined in the crucible. Dry at 100 °C to 105 °C for 1 h and ignite to constant mass in a muffle furnace at 600 °C ± 25 °C, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up with hot water, filter through an ashless filter paper and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant mass.



01/2008:20417

2.4.17. ALUMINIUM

Place the prescribed solution in a separating funnel and shake with 2 quantities, each of 20 mL, and then with one 10 mL quantity of a 5 g/L solution of *hydroxyquinoline R* in *chloroform R*. Dilute the combined chloroform solutions to 50.0 mL with *chloroform R* (test solution).

Prepare a standard in the same manner using the prescribed reference solution.

Prepare a blank in the same manner using the prescribed blank solution.

Measure the intensity of the fluorescence (2.2.21) of the test solution (I_1), of the standard (I_2) and of the blank (I_3) using an excitant beam at 392 nm and a secondary filter with a transmission band centred on 518 nm or a monochromator set to transmit at this wavelength.

The fluorescence ($I_1 - I_3$) of the test solution is not greater than that of the standard ($I_2 - I_3$).

01/2008:20418



2.4.18. FREE FORMALDEHYDE

Use method A, unless otherwise prescribed. Method B is suitable for vaccines where sodium metabisulfite has been used to neutralise excess formaldehyde.

METHOD A

For vaccines for human use, prepare a 1 in 10 dilution of the vaccine to be examined. For bacterial toxoids for veterinary use, prepare a 1 in 25 dilution of the vaccine to be examined. To 1 mL of the dilution, add 4 mL of *water R* and 5 mL of *acetylacetone reagent R1*. Place the tube in a water-bath at 40 °C for 40 min. Examine the tubes down their vertical axes. The solution is not more intensely coloured than a standard, prepared at the same time and in the same manner, using 1 mL of a dilution of *formaldehyde solution R* containing 20 µg of formaldehyde (CH₂O) per millilitre, instead of the dilution of the vaccine to be examined.

METHOD B

Test solution. Prepare a 1 in 200 dilution of the vaccine to be examined with *water R*. If the vaccine is an emulsion, prepare an equivalent dilution using the aqueous phase separated by a suitable procedure (see below). If one of the methods described below is used for separation of the aqueous phase, a 1 in 20 dilution of the latter is used.

Reference solutions. Prepare solutions containing 0.25 g/L, 0.50 g/L, 1.00 g/L and 2.00 g/L of CH₂O by dilution of *formaldehyde solution R* with *water R*. Prepare a 1 in 200 dilution of each solution with *water R*.

To 0.5 mL of the test solution and of each of the reference solutions in test-tubes, add 5.0 mL of a freshly prepared 0.5 g/L solution of *methylbenzothiazolone hydrazone hydrochloride R*. Close the tubes, shake and allow to stand for 60 min. Add 1 mL of *ferric chloride-sulfamic acid reagent R* and allow to stand for 15 min. Measure the absorbance (2.2.25) of the solutions at 628 nm. Calculate the content of formaldehyde in the vaccine to be examined from the calibration curve established using the reference solutions. The test is invalid if the correlation coefficient (r) of the calibration curve is less than 0.97.

Emulsions. If the vaccine to be examined is an emulsion, the aqueous phase is separated using a suitable procedure and used for preparation of the test solution. The following procedures have been found suitable.

(a) Add 1.0 mL of the vaccine to be examined to 1.0 mL of *isopropyl myristate R* and mix. Add 1.3 mL of 1 M *hydrochloric acid*, 2.0 mL of *chloroform R* and 2.7 mL of a 9 g/L solution of *sodium chloride R*. Mix thoroughly. Centrifuge at 15 000 g for 60 min. Transfer the aqueous phase to a 10 mL volumetric flask and dilute to volume with *water R*. If this procedure fails to separate the aqueous phase, add 100 g/L of *polysorbate 20 R* to the sodium chloride solution and repeat the procedure but centrifuge at 22 500 g.

(b) Add 1.0 mL of the vaccine to be examined to 1.0 mL of a 100 g/L solution of *sodium chloride R* and mix. Centrifuge at 1000 g for 15 min. Transfer the aqueous phase to a 10 mL volumetric flask and dilute to volume with *water R*.