solution of *lithium carbonate R* and dry in an oven before use. Envelop the substance to be examined in the paper and place it in the sample carrier. Introduce into the flask *water R* or the prescribed solution designed to absorb the combustion products, displace the air with oxygen by means of a tube having its end just above the liquid, moisten the neck of the flask with *water R* and close with its stopper. Ignite the paper strip by suitable means with the usual precautions. Keep the flask firmly closed during the combustion. Shake the flask vigorously to completely dissolve the combustion products. Cool and after about 5 min, unless otherwise prescribed, carefully unstopper the flask. Wash the ground parts and the walls of the flask, as well as the sample carrier, with *water R*. Combine the combustion products and the washings and proceed as prescribed in the monograph.

01/2008:20511

2.5.11. COMPLEXOMETRIC TITRATIONS

ALUMINIUM

Introduce 20.0 mL of the prescribed solution into a 500 mL conical flask, add 25.0 mL of *0.1 M sodium edetate* and 10 mL of a mixture of equal volumes of a 155 g/L solution of *ammonium acetate R* and *dilute acetic acid R*. Boil for 2 min, then cool. Add 50 mL of *ethanol R* and 3 mL of a freshly prepared 0.25 g/L solution of *dithizone R* in *ethanol R*. Titrate the excess of sodium edetate with *0.1 M zinc sulfate* until the colour changes from greenish-blue to reddish-violet.

1 mL of *0.1 M sodium edetate* is equivalent to 2.698 mg of Al.

BISMUTH

Introduce the prescribed solution into a 500 mL conical flask. Dilute to 250 mL with *water R* and then, unless otherwise prescribed, add dropwise, with shaking, *concentrated ammonia R* until the mixture becomes cloudy. Add 0.5 mL of *nitric acid R*. Heat to about 70 °C until the cloudiness disappears completely. Add about 50 mg of *xylenol orange triturate R* and titrate with *0.1 M sodium edetate* until the colour changes from pinkish-violet to yellow.

1 mL of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

CALCIUM

Introduce the prescribed solution into a 500 mL conical flask, and dilute to 300 mL with *water R*. Add 6.0 mL of *strong sodium hydroxide solution R* and about 15 mg of *calconecarboxylic acid triturate R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to full blue.

1 mL of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

MAGNESIUM

Introduce the prescribed solution into a 500 mL conical flask and dilute to 300 mL with *water R*. Add 10 mL of *ammonium chloride buffer solution pH 10.0 R* and about 50 mg of *mordant black 11 triturate R*. Heat to about 40 °C then titrate at this temperature with *0.1 M sodium edetate* until the colour changes from violet to full blue.

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

LEAD

Introduce the prescribed solution into a 500 mL conical flask and dilute to 200 mL with *water R*. Add about 50 mg of *xylenol orange triturate R* and *hexamethylenetetramine R* until the solution becomes violet-pink. Titrate with *0.1 M sodium edetate* until the violet-pink colour changes to yellow.

1 mL of *0.1 M sodium edetate* is equivalent to 20.72 mg of Pb.

ZINC

Introduce the prescribed solution into a 500 mL conical flask and dilute to 200 mL with *water R*. Add about 50 mg of *xylenol orange triturate R* and *hexamethylenetetramine R* until the solution becomes violet-pink. Add 2 g of *hexamethylenetetramine R* in excess. Titrate with *0.1 M sodium edetate* until the violet-pink colour changes to yellow.

1 mL of *0.1 M sodium edetate* is equivalent to 6.54 mg of Zn.

01/2008:20512
corrected 6.0

2.5.12. WATER: SEMI-MICRO DETERMINATION

The semi-micro determination of water is based upon the quantitative reaction of water with sulfur dioxide and iodine in a suitable anhydrous medium in the presence of a base with sufficient buffering capacity.

Apparatus

The apparatus consists of a titration vessel with:

- 2 identical platinum electrodes;
- tight inlets for introduction of solvent and titrant;
- an inlet for introduction of air via a desiccant;
- a sample inlet fitted with a stopper or, for liquids, a septum.

Inlet systems for introduction of dry nitrogen or for aspiration of solvents may also be fitted.

The titration is carried out according to the instrument supplier's instructions. Care is taken throughout the determination to avoid exposure of reagents and solvents to atmospheric moisture. The end-point is determined using 2 identical indicator electrodes connected to an electrical source that maintains between the electrodes either a constant current or a constant voltage. Where direct titration is used (method A), addition of titrant causes either a decrease in voltage where constant current is maintained or an increase in current where constant voltage is maintained, until the end-point is reached. Instruments with automatic end-point detection are commonly used.

Standardisation. To the titration vessel, add *methanol R*, dried if necessary, or the solvent recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce a suitable amount of water in an appropriate form (*water R* or a certified reference material) and carry out the titration, stirring for the necessary time. The water equivalent is not less than 80 per cent of that indicated by the supplier. Standardise the titrant before the first use and at suitable intervals thereafter.

Unless otherwise prescribed, use Method A.

Method A. Introduce into the titration vessel *methanol R*, or the solvent indicated in the monograph or recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce the substance to be examined rapidly and carry out the titration, stirring for the necessary extraction time.

Method B. Introduce into the titration vessel *methanol R*, or the solvent indicated in the monograph or recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce the substance to be examined rapidly and in a suitable state of division. Add an accurately measured volume of the titrant, sufficient to give an excess of about 1 mL or the prescribed volume. Allow to stand protected from light for 1 min or the prescribed time, with stirring. Titrate the excess of reagent using *methanol R* or the prescribed solvent, containing an accurately known quantity of water.

Suitability. The accuracy of the determination with the chosen titrant must be verified for each substance to be examined. The following procedure, given as an example, is suitable for samples containing 2.5-25 mg of water.

The water content of the substance to be examined is determined using the reagent/solvent system chosen. Thereafter, sequential known amounts of *water R* are added in an appropriate form (at least 5 additions) and the cumulative water content determined after each addition. Calculate the percentage recovery (*r*) at each point using the following expression:

$$r = 100\frac{W_2}{W_1}$$

W_1 = amount of water added, in milligrams;

W_2 = amount of water found, in milligrams.

Calculate the regression line of the cumulative water determined against the water added. Calculate the slope (*b*), the intercept with the *y*-axis (*a*) and the intercept of the extrapolated calibration line with the *x*-axis (*d*).

Calculate the percentage mean recovery ($\overline{r}$). Calculate the percentage errors (e_1 and e_2) using the following expressions:

$$e_1 = 100\frac{a - M}{M}$$

$$e_2 = 100\frac{|d| - M}{M}$$

a = the *y*-axis intercept, in milligrams of water;

d = the *x*-axis intercept, in milligrams of water;

M = water content of the substance, in milligrams of water.

The reagent/solvent system is considered to be acceptable if:

- $|e_1|$ and $|e_2|$ are not greater than 2.5 per cent;
- b is between 0.975 and 1.025 (deviation ± 2.5 per cent);
- $\overline{r}$ is between 97.5 per cent and 102.5 per cent.

01/2008:20513

2.5.13. ALUMINIUM IN ADSORBED VACCINES

Homogenise the preparation to be examined and transfer a suitable quantity, presumed to contain 5 mg to 6 mg of aluminium, to a 50 mL combustion flask. Add 1 mL of *sulfuric acid R*, 0.1 mL of *nitric acid R* and some glass beads. Heat the solution until thick, white fumes are evolved. If there is charring at this stage add a few more drops of *nitric acid R* and continue boiling until the colour disappears. Allow to cool for a few minutes, carefully add 10 mL of *water R* and boil until a clear solution is obtained. Allow to cool, add 0.05 mL of *methyl orange solution R* and neutralise with *strong sodium hydroxide solution R* (6.5 mL to 7 mL). If a precipitate forms dissolve it by adding, dropwise, sufficient *dilute sulfuric acid R*. Transfer the solution to a 250 mL conical flask, rinsing the combustion flask with 25 mL of *water R*. Add 25.0 mL of *0.02 M sodium edetate*, 10 mL of *acetate buffer solution pH 4.4 R* and a few glass beads and boil gently for 3 min. Add 0.1 mL of *pyridylazonaphthol solution R* and titrate the hot solution with *0.02 M copper sulfate* until the colour changes to purplish-brown. Carry out a blank titration omitting the vaccine.

1 mL of *0.02 M sodium edetate* is equivalent to 0.5396 mg of Al.

01/2008:20514

2.5.14. CALCIUM IN ADSORBED VACCINES

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

Determine the calcium by atomic emission spectrometry (*2.2.22, Method I*). Homogenise the preparation to be examined. To 1.0 mL add 0.2 mL of *dilute hydrochloric acid R* and dilute to 3.0 mL with *water R*. Measure the absorbance at 620 nm.

01/2008:20515

2.5.15. PHENOL IN IMMUNOSERA AND VACCINES

Homogenise the preparation to be examined. Dilute an appropriate volume with *water R* so as to obtain a solution presumed to contain 15 µg of phenol per millilitre. Prepare a series of reference solutions with *phenol R* containing 5 µg, 10 µg, 15 µg, 20 µg and 30 µg of phenol per millilitre respectively. To 5 mL of the solution to be examined and to 5 mL of each of the reference solutions respectively, add 5 mL of *buffer solution pH 9.0 R*, 5 mL of *aminopyrazolone solution R* and 5 mL of *potassium ferricyanide solution R*. Allow to stand for 10 min and measure the intensity of colour at 546 nm.

Plot the calibration curve and calculate the phenol content of the preparation to be examined.

01/2008:20516

2.5.16. PROTEIN IN POLYSACCHARIDE VACCINES

Test solution. Use a volumetric flask with a suitable volume for preparation of a solution containing about 5 mg per millilitre of dry polysaccharide. Transfer the contents of a container quantitatively to the flask and dilute to volume with *water R*. Place 1 mL of the solution in a glass tube and add 0.15 mL of a 400 g/L solution of *trichloroacetic acid R*. Shake, allow to stand for 15 min, centrifuge for 10 min at 5000 r/min and discard the supernatant. Add 0.4 mL of *0.1 M sodium hydroxide* to the centrifugation residue.

Reference solutions. Dissolve 0.100 g of *bovine albumin R* in 100 mL of *0.1 M sodium hydroxide* (stock solution containing 1 g of protein per litre). Dilute 1 mL of the stock solution to 20 mL with *0.1 M sodium hydroxide* (working dilution 1: 50 mg of protein per litre). Dilute 1 mL of the stock solution to 4 mL with *0.1 M sodium hydroxide* (working dilution 2: 250 mg of protein per litre). Place in 6 glass tubes 0.10 mL, 0.20 mL and 0.40 mL of working dilution 1 and 0.15 mL, 0.20 mL and 0.25 mL of working dilution 2. Make up the volume in each tube to 0.40 mL using *0.1 M sodium hydroxide*.

Prepare a blank using 0.40 mL of *0.1 M sodium hydroxide*.

Add 2 mL of *cupri-tartaric solution R3* to each tube, shake and allow to stand for 10 min. Add to each tube 0.2 mL of a mixture of equal volumes of *phosphomolybdotungstic reagent R* and *water R*, prepared immediately before use. Stopper the tubes, mix by inverting and allow to stand in the dark for 30 min. The blue colour is stable for 60 min. If necessary, centrifuge to obtain clear solutions.

Measure the absorbance (*2.2.25*) of each solution at 760 nm using the blank as the compensation liquid. Draw a calibration curve from the absorbances of the 6 reference solutions and the corresponding protein contents and read from the curve the content of protein in the test solution.