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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 sulphur 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.

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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 *sulphuric 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 sulphuric 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 sulphate* 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.