MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Determination of water **GPM**.1.2.3.0002.15

Replaces the State Pharmacopoeia of the Russian Federation XI Monograph

1. The Karl Fischer method (semi-micro-method)

This method is based on the chemical interaction between water and the components of the Karl Fischer reagent.

Karl Fischer reagent. The Karl Fischer reagent is a solution of sulphur dioxide, iodine, and pyridine (or a different base, such as imidazole) in methanol. The interaction between the reagent and water unfolds stoichiometrically in two stages according to the following equations:

$$H_2O + SO_2 + I_2 + 3C_5H_5N \rightarrow 2C_5H_5N \cdot HI + C_5H_5NSO_3$$

$$C_5H_5NSO_3 + CH_3OH \rightarrow C_5H_5N \cdot HSO_4CH_3$$

Solutions and reagents used should be anhydrous. They should be stored and used in conditions precluding the possibility of any effects of atmospheric moisture on them.

The iodine sulphur reagent is a solution that contains anhydrous pyridine, monomethyl ester of ethylene glycol, iodine, and sulphur dioxide. Pyridine is often replaced was other bases in iodine sulphur reagents. Use of reagents with this composition should be validated in advance with the aim to demonstrate the stoichiometry of the reaction in each individual case and that the tested substance and the reagent are compatible.

When water is determined in solid substances insoluble in methyl alcohol, a finely comminuted weight of the substance is shaken with methyl alcohol and then

titrated with the Karl Fischer reagent. Some substances or mixtures may be dissolved in anhydrous acetic acid, chloroform, pyridine, and other solvents.

Propanol and other alkanols have considerable dissolving capacity for long-chain molecules, and thus may be used, alone or in a mixture with methanol, for analysis of high-molecular-weight compounds. 2-Methoxyethanol (monomethyl ester of ethylene glycol) is used when side reactions occur in the presence of methanol (esterification, ketal formation, etc.). However, titration in this solvent goes on slower compared with methanol. Chloroform is a good solvent for fats, and may be used in a mixture was methanol, which should usually have a content of 50 % (not less than 25 % in any case). Formamide improves solubility of polar substances, and may be added to methanol for the determination of water in proteins. Pure non-protonic solvents, which impair the stoichiometry of the Karl Fischer reaction, are not recommended as a working medium.

The sample weight and the shaking time for the sample and solvent, as well as the name of the solvent, should be specified in the Pharmacopoeia Monograph.

The Karl Fischer reagent may be used to determine both hygroscopic and crystallized water. Water may be determined both in organic and inorganic compounds, in different solvents, and in volatile substances.

Apparatus. The titration apparatus for the Karl Fischer method is a closed system that consists of a burette equipped with a drying tube filled with a desiccant (such as molecular sieves), a vessel to supply the reagent and a titration flask, both of which are connected with the burette. The titration flask is a receptacle with a capacity of 60 to 100 mL, equipped with two platinum electrodes, a nitrogen supplying tube, a drying tube filled with a desiccant (such as molecular sieves), and a stopper holding the tip of the burette. The tested substance is introduced into the vessel through the tube located on the opposite side to the drying tube and closed with a ground stopper. The solution is stirred in the course of titration using a magnetic mixer or by blowing dried nitrogen through the solution.

The endpoint of the titration is determined by amperometry. The electric circuit consists of a potentiometer with a resistance of 2000 Ohm connected to a

source of constant current (voltage, 1.5 V) and producing the required potential difference. The potential difference is so regulated that a low baseline current passes through the platinum electrodes consecutively connected with the microamperometer. The microamperometer cursor deviates upon addition of the reagent but returns to the home position immediately. At the end of the reaction, the obtained deviation should remain stable for at least 30 seconds.

The endpoint of the titration may be determined visually, by the change in the colour of the titrated colour from yellow to reddish-brown, provided that the required accuracy is ensured. A control experiment should be carried out simultaneously.

Automatic titrators may be employed, in accordance with the manufacturer's Instructions for Use.

Unless otherwise specified in the Pharmacopoeia Monograph, Method A should be used.

Technique A. An accurately measured weight of the tested substance containing approximately 30 to 50 mg of water is transferred into the titration receptacle already containing 5.0 mL of anhydrous methanol. After 1-minute mixing, titration with the Karl Fischer reagent follows, which should be added in portions of 0.1 to 0.05 mL towards the endpoint of the titration.

A control experiment should be carried out simultaneously (titration of a 5.0 mL portion of anhydrous methanol).

Technique B. Approximately 20 mL of anhydrous methanol or the solvent specified in the Pharmacopoeia Monograph is transferred into the titration receptacle and titrated with the Karl Fischer reagent, determining the endpoint of the titration by amperometry. After that, the accurately measured weight of the tested substance specified in the Pharmacopoeia Monograph is introduced into the titration receptacle. The mixture is then stirred for 1 minute and titrated again with the Karl Fischer reagent, determining the endpoint of the titration by amperometry.

Technique C. Approximately 10 mL of anhydrous methanol or the solvent specified in the Pharmacopoeia Monograph is transferred into the titration recepta-

cle and titrated with the iodine sulphur reagent, determining the endpoint of the titration by amperometry.

After that, the specified amount of the tested substance and an accurately measured volume of the iodine sulphur reagent taken with an excess of approximately 1 mL or the volume specified in the Pharmacopoeia Monograph are quickly introduced into the titration receptacle. The receptacle is closed with a stopper and left to stand away from light for 1 minute or for the amount of time specified in the Pharmacopoeia Monograph, while the contents of the receptacle are stirred from time to time. The iodine sulphur reagent is titrated to the original current value, using anhydrous methanol or the solvent specified in the Pharmacopoeia Monograph mixed with an accurately measured amount of water equivalent to approximately 2.5 mg/mL.

2. The micro-method for the determination of water (coulometric)

During coulometric titration, the iodine required for the Karl Fischer reaction is produced by anodic oxidation of the iodide ion:

$$2J^- - 2e \rightarrow J_2$$

The produced iodine reacts with the present water and sulphur dioxide in the presence of a base. Iodine is consumed until water is present in the medium. Iodine excess indicates that the endpoint of the titration has been achieved. The amount of titrated water is proportionate to the amount of electricity directed through the cell.

1 mole of iodine corresponds to 1 mole of water, and 10.71 C of electricity corresponds to 1 mg of water.

Since the titration current is low, coulometric determination is used to quantify small amounts of water: from 10 μg to 10 mg.

Precision and accuracy of this method should be ensured by elimination of the atmospheric moisture from the system.

Equipment

The main element of the apparatus is a coulometric cell. The most frequently used type of cell consists of an anodic compartment, in which the Karl Fischer reaction takes place, and a cathodic compartment of a smaller volume, in which the

cathodic reduction reaction unfolds. Each of the compartments contains a platinum electrode. The anodic compartment is filled with an anolyte, the modified Karl Fischer reagent containing an iodide anion instead of the iodine. The cathodic compartment is filled with an appropriate catholyte, which usually contains ammonium salts as an active component. The two compartments are separated by a diaphragm, which prevents mixing of the two solutions. As diffusion of the active components cannot be completely ruled out by the diaphragm, the catholyte components should be compatible with the anolyte. Single-chamber cells without a diaphragm may be used as well. In this case, the anodic and cathodic reactions develop in the same volume of the electrolyte, and therefore the cathodic reduction reaction should not give products that may be oxidized on the anode, which may produce false high results in this test.

The reaction cell should be maintained absolutely dry. The reagent is poured into the anodic compartment through a dry funnel, and after that the cell should be immediately pressurized. The reagent may become discoloured at this stage. The moisture should be removed from the system by means of preliminary electrolysis.

The cathodic compartment should contain no water as well. The small excess of elemental iodine in the catholyte has no effect on the course of the titration.

The analyzed liquid sample is introduced into the anolyte-containing cell using a syringe, through the silicone separator. Injection of solid samples into the cell should be avoided. Nevertheless, if solid samples have to be analyzed, they should be introduced through a hermetically closable inlet, and measures should be taken to protect the cell from contact with atmospheric moisture: for instance, the operator may work in a glove cabinet, in a dry inert gas atmosphere. Solid samples may be also be introduced as a solution, following dissolution in an appropriate solvent, or else water may be released from the sample in a pipe furnace during heating and transferred to the anolyte with a current of a dry inert gas. Gasses are introduced into the anolyte through a gas introducing tube (bubbler).

The sample volume should not exceed 10 mL. The cell injection volume is usually 0.5 mL to 5.0 mL for liquid samples. Appropriate volumes for gaseous samples range from 100 mL to 10 L.

Procedure description

Coulometric titration should be performed until the endpoint of the titration is achieved.

The reaction cell compartment is filled with the water determination microtest electrolyte according to the Instructions for Use of the manufacturer. The moisture should be removed from the system by means of preliminary electrolysis.

The accurately measured amount of the tested substance specified in the Pharmacopoeia Monograph is introduced into the reaction cell and mixed for 30 seconds or for the period of time specified in the Pharmacopoeia Monograph. Titration should be performed until the endpoint of the titration is achieved.

If an evaporator is used, the accurately measured weight of the tested substance specified in the Pharmacopoeia Monograph is introduced into the tube and heated. After the water has evaporated from the sample into the cell, the titration is performed.

A control experiment is carried out, and the content of water in the tested substance is calculated as a percentage.

Accuracy check. An accurately measured weight of water (the same as in the tested sample) is introduced between two successive titration procedures, and coulometric titration is performed. The result should lie in the range of 97.5 % to 102.5 % for samples containing 1000 μg of water and in the range of 90.0 % to 110.0 % for samples containing 100 μg of water.

3. Determination of water by the distillation method

Apparatus. The determination is carried out in an apparatus (Figure 1) consisting of a glass round-bottomed volumetric flask (1) with a volume in the range of 250 mL to 500 mL, a receptacle (a graduated tube or burette with a capacity of 6 to 10 mL and a 0.1 mL division) (2), and a refrigerator (3).

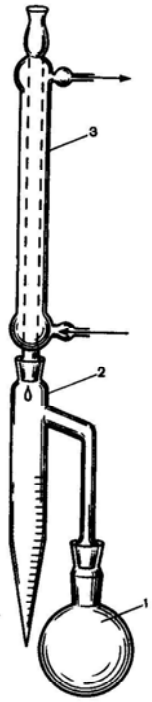

Figure 1. Apparatus for the determination of water by the distillation method
1 – flask, 2 – receptacle, 3 – refrigerator.

Procedure description

The tested substance quantity specified in the Pharmacopoeia Monograph (from 10.0 g to 20.0 g (accurately measured weight), containing from 2 to 3 mL of water) is transferred into the volumetric flask (1) within an accuracy of up to 1 %, then 100 mL of toluene or xylene is added, as well as a few pieces of a porous material (for instance, a few pieces of pumice stone). The volumetric flask is heated on an electric stove or sand bath to boiling. Boiling should be carried out so that the condensing solvent does not accumulate in the refrigerator but flows down freely towards the rising vapour of the liquid at a rate of 2 to 4 drops per second. Boiling is discontinued once the water volume in the receptacle has stopped to increase and the upper layer of the solvent in the receptacle has become clear. The internal tube of the refrigerator is washed with toluene, and heating is continued

for another 5 minutes, after which the receptacle is cooled down to room temperature and all drops of water are shaken off the receptacle walls.

All distilled water is collected in the lower portion of the receptacle. Once the layers have completely formed, the volume of the distilled water is measured.