Description of water	**GM (general monograph) 1.2.3.002.15** **Instead of SPh X** **Instead of SPh X, ed. 1**

1. K. Fischer method (semi-micro method)

This method is based on interaction between water and components of K. Fischer reagent.

K. Fischer reagent. K. Fischer reagent is composed of solution of sulphur dioxide, iodine and pyridine (or another base, imidazole, for example) in methanol. Interaction between water and the reagent proceeds in two stages stoichiometrically:

$$H_2S + SO_2 + I_2 + 3C_5H_5N \rightarrow 2C\ C_5H_5N \square HI + C_5H_5NSO_3$$
$$C_5H_5NSO_3 + CH_3OH \rightarrow C_5H_5N \square HSO_4CH_3$$

Used solutions and reagents must be anhydrous. They must be handled and stored under conditions that protect them from interaction with atmospheric moisture.

Iodine sulphur reagent is composed of anhydrous pyridine, monomethyl alcohol, iodine and sulphur dioxide. Pyridine is often substituted for a different base in these reagents. Use of reagents with this composition should be validated in order to confirm reaction stoichiometry in each individual case and to rule out incompatibility between the test substance and the reagent.

When determining water content in solid substances that are insoluble in methanol mix finely ground accurately weighed sample of the substance with methanol and shake, after that titrate using Fischer reagent. Some substances and mixtures can be dissolved in anhydrous acetic acid, chloroform, pyridine and other solvents.

Propanol and other alkanols have a great solvent ability for long-chain molecules and may be used alone or in combination with methanol when analysing high-molecular compounds.

2-methoxyethanol (ethylene glycol monomethyl ether) is used in cases when methanol causes side reactions (etherification, formation of ketals etc). However, titration in this solvent is slower compared to methanol. Chloroform is a good solvent for lipids and may be used in combination with methanol, concentration of which is usually 50%, but no less than 25%. Formamide increases solubility of polar substances and may be added to methanol to determine water content in proteins. Pure aprotic solvents that interrupt stoichiometry of Fischer reaction are not recommended as a working medium.

Weight of the accurately weighed sample, shake-time for the accurately weighed sample and solvent mixture, as well the name of the solvent used must be indicated in the general monograph.

Both hygroscopic and crystallisation water may be determined using Fischer titration method. With that, water can be determined in organic and nonorganic substances alike, in various solvents and volatile substances.

Device. The device for Karl Fischer titration is a closed system consisting of a burette equipped with a drying tube filled with drying agent (for example, molecular sieves), a vial for reagent addition and a titration tube that are connected to the burette. Titration tube includes is a 60-100 mL vial with two platinum electrodes, a tube for nitrogen delivery, a drying tube filled with drying agent (for example, molecular sieves) and a cork into which the end of the burette is inserted. The test substance is placed into the vial through a tube on the opposite side from the drying tube with a friction-fitted lid. During titration process the solution is mixed with help of magnetic mixer or by blowing dried nitrogen through.

The titration endpoint is determined amperometrically. Electric circuit consists of a 2000 Ohm potentiometer connected to a 1.5 V constant power source that provides necessary difference of potentials. This difference is calibrated in such manner that a low initial electric current passes through platinum electrodes consecutively connected to a microampere. When a reagent is added, the microampere pointer moves slightly but immediately returns to the initial position. At the end of the reaction the pointer should move and remain in an unchanged position for at least 30 sec.

The titration endpoint may be determined visually based on change in colour of titration liquid from yellow to reddish-brown given that necessary accuracy has been provided. Control test should be conducted.

Automatic titrators may be used according to manufacturer’s instructions.

Unless otherwise specified, use method A indicated in the general monograph.

Method A. Place an accurately weighed sample of the test substance containing approximately 30-50 mg if water into a titration vial containing 5.0 mL of anhydrous methanol. Stir for 1 minute and titrate using Fischer reagent adding it closer to the endpoint 0.1-0.05 mL at a time.

Control test should be performed parallel to the main test (titrate 5.0 mL of anhydrous methanol).

Method B. Place approximately 20 mL of anhydrous methanol or a solvent indicated in the general monograph into a titration vial and titrate with Fischer reagent; titration endpoint is determined amperometrically. Then add an accurately weighed sample of the test substance indicated general monograph into the titration vial. Stir for 1 minute and titrate again using Fischer reagent; titration endpoint is determined amperometrically.

Method C. Place approximately 10 mL of anhydrous methanol or a solvent indicated in the general monograph into a titration vial and titrate using iodine sulphur reagent; titration endpoint is determined amperometrically.

Then quickly add the indicated amount of the test substance and accurately measured amount of iodine sulphur reagent with about 1 mL of excess or an amount indicated in the

general monograph. Close the vial with a cork, let it sit in a dark place for about 1 minute or for a period of time indicated in the general monograph and stir the contents periodically. Titrate the excess amount of iodine sulphur reagent to the original current using anhydrous methanol or a solvent indicated in the general monograph, to which a known amount of water was added equal to about 2.5 mg/mL.

2. Micro-method of water determination (coulometric)

For coulometric titration, iodine needed for Fischer reaction is formed during an anodic oxidation of iodine-ion:

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

Iodine reacts with water and sulphur dioxide with presence of a base. Iodine is used as long as the medium contains water. Iodine excess indicates that titration endpoint has been reached. Amount of titrated water is proportionate to the amount of electricity sent through a cell.

1 mol of iodine corresponds to 1 mol of water, 1 mg of water corresponds to a consumption of 10.71 C electrical current.

Ensure the validity and accuracy of the test by eliminating moisture from the system.

Equipment

Coulometric cell constitutes the primary block of the device. The most commonly used cell consists of an anode compartment where the Fischer reaction occurs and a smaller cathode compartment where reduction reaction takes place. Each compartment contains a platinum electrode. Anode compartment is filled with an anolyte: modified Fischer reagent containing iodide-anion instead of iodine. Cathode compartment is filled with a suitable catolyte typically containing ammonium salts as an active component. The compartments are separated by a diaphragm that prevents the two solutions from mixing. Considering that diffusion of active components cannot be completely excluded even with help of the diaphragm, components of catolyte should be compatible with anolyte. In this case, anode and cathode reactions take place in the same electrolyte volume, therefore the cathode reduction reaction must not produce products that can oxidise on the anode, which may lead to higher results regarding water content.

Reaction cell must be kept completely moisture-free. Addition of reagent into anode compartment is performed through a dry funnel, after which the cell is immediately sealed hermetically. This may lead to reagent discolouration. Moisture is removed from the system through pre-electrolysis.

Cathode compartment must also be kept completely moisture-free. A small excess of elementary iodine in catolyte does not affect the titration.

Liquid sample for analysis is injected into the cell containing analyte using a syringe through a silicone septum. Injection of solid samples into the cell should be avoided. Although, if testing needs to be performed on solid samples, they are injected into the cell through the hermetically sealable shutter; with that, take precautions to avoid intrusion of moisture into the

cell, for example, work in a glove box with dry inert gas. Solid substances maybe dissolved in a suitable solvent prior to injection or water can be extracted from the sample by heating it in a thermostat after which it is transferred to the titration cell in a current of dry inert gas. Gases are transferred into the cell through a gas bubbler.

Sample volume should not exceed 10 mL. Typically, 0.5-5.0 mL is injected into the cell. Gas samples are typically 100 mL-10 L.

Method

Cuolonometric titration is performed until cuolonometric endpoint is determined.

Fill the reaction cell with electrolyte for micro-determination of water according to manufacturer's instructions. Moisture is removed from the system through pre-electrolysis.

Add an exact amount of test substance indicated in the general monograph into the reaction cell and stir for 30 seconds or for a period of time indicated in the general monograph. Titrate until the determined endpoint.

If a thermostat is used: place an accurately weighted sample of the test substance indicated in the general monograph into a tube and heat it up. Titrate after the water is vaporised from the sample into the cell.

Perform control test and calculate water content (in %).

Accuracy test. In-between two consecutive titrations inject an accurately weighed amount of water – same as in the test sample – and perform coulonometric titration. The results should be within the range of 97.5-102.5% for 1,000 μg of water in a sample and 90.0-110.0% for 100 μg.

3. Distillation method

Device. Determination is performed in a device (fig. 1) consisting of a 250-500 mL glass round-bottom vial (1), receptacle (2): 6-10 mL graduated test tube or burette with a 0.1 mL grading division, and a cooler (3).

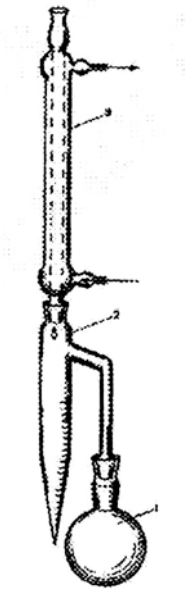

Fig. 1. Device for determining water content using distillation method.
1 – tube, 2 – receptacle, 3 – cooler

Method

Weigh an exact amount of the test substance with 1% accuracy indicated in the general monograph (10.0-20.0 g (accurate weigh) containing 2-3 mL of water) and place it into the tube (1), add 100 mL of toluene or xylene and a few pieces of porous material (for example, pumice). Heat the tube on an electrical stove or a sand bath until boiling point is reached. Make sure that during the boiling process the condensable solvent does not accumulate in the cooler but smoothly runs off towards the rising liquid vapours at a rate of 2-4 drops per second. Continue boiling until the amount of water in the receptacle stops increasing and the upper layer of the solvent becomes clear. Wash out the inner tube of the cooler with toluene and continue heating up for another 5 minutes, after that cool the receptacle to room temperature and shake all water drops off the walls.

All distilled water will collect at the bottom of the receptacle. Note the amount of distilled water after complete separation of layers.