分間かき混ぜた後，激しくかき混ぜながら滴定を行う．別に，試料が溶剤に溶けないとき，又は試料がカールフィッシャー反応を妨害するときは，水分気化装置を用いて試料を加熱し，窒素をキャリヤーとして試料中の水分を滴定フラスコに導入することができる．

なお，滴定は湿度の低い雰囲気下で行う必要があるが，滴定に長時間を要するなど雰囲気中の水分の影響が避けられない場合は，試料を測定したときと同様の操作により空試験を行い，補正する．

水 (H_2O) %

$$= \frac{\text{試料の滴定に要した水分測定用試液の量 (mL)} \times f\ (\text{mg/mL})}{\text{試料の質量 (mg)}} \times 100$$

（2） 逆滴定

別に規定するもののほか，次の方法による．

水分測定用メタノール適量を乾燥滴定フラスコにとり，これをあらかじめ水分測定用試液で終点まで滴定してフラスコ内を無水の状態にしておく．次に水分 5 ～ 30 mg を含むような量の試料を精密に量り，速やかに滴定フラスコに入れ，過量の水分測定用試液の一定量を加え，かき混ぜて溶かし，激しくかき混ぜながら水・メタノール標準液で終点まで滴定する．試料が溶剤に溶けないときは手早く粉末とし，その質量を精密に量り，速やかに滴定フラスコに入れ，過量の水分測定用試液の一定量を加え，湿気を避けて 5 ～ 30 分間かき混ぜた後，激しくかき混ぜながら滴定を行う．

水 (H_2O) %

$$= \frac{\left[\text{水分測定用試液の量(mL)}\right] \times f\,(\text{mg/mL}) - \left[\text{滴定に要した水・メタノール標準液の量(mL)}\right] \times f'\,(\text{mg/mL})}{\text{試料の質量(mg)}} \times 100$$

2．電量滴定法

装 置

通例，ヨウ素発生用電解槽を備えた滴定フラスコ，かき混ぜ機及び定電流分極電位差滴定装置からなる．ヨウ素発生用装置は，隔膜で隔てられた陽極及び陰極より構成され，陽極は水分測定用陽極液（発生液）中に，陰極は水分測定用陰極液（対極液）中に浸される．通例，両極とも白金網が用いられる．

水分測定用陽極液及び水分測定用陰極液は吸湿性が非常に強いので，装置は外部からの吸湿を防ぐようにする．防湿には，シリカゲル又は水分測定用塩化カルシウムなどを用いる．

水分測定用陽極液及び水分測定用陰極液の調製法

水分測定用陽極液及び水分測定用陰極液は，一組の試薬として次に示す．

調製（1），（2）又は（3）のいずれかの方法により調製する．

（1） 調製法 1

水分測定用陽極液　水分測定用イミダゾール 102 g を水

分測定用メタノール 900 mL に溶かし，氷冷し，液温を 30 °C 以下に保ちながら，乾燥二酸化イオウを通じ，その増量が 64 g に達したとき，ヨウ素 12 g を加えて溶かし，かき混ぜながら，液の色が褐色から黄色に変わるまで水を滴加し，水分測定用メタノールを加えて 1000 mL とする．

水分測定用陰極液　塩酸ジエタノールアミン 24 g を水分測定用メタノール 100 mL に溶かす．

（2）　調製法 2

水分測定用陽極液　1,3-ジ-(4-ピリジル)プロパン 40 g 及びジエタノールアミン 30 g を水分測定用メタノール約 200 mL に溶かし，乾燥二酸化イオウを増量が 25 g になるまで通じる．炭酸プロピレン 50 mL を加え，ヨウ素 6 g を溶かした後，水分測定用メタノールを加えて 500 mL とし，液の色が褐色から黄色に変わるまで水を滴加する．

水分測定用陰極液　塩化コリン 30 g を水分測定用メタノールに溶かし 100 mL とする．

（3）　調製法 3

水分測定用陽極液　ジエタノールアミン 100 g を水分測定用メタノール又は水分測定用メタノール/水分測定用クロロホルム混液 (3 : 1) 900 mL に溶かし，冷却しながら，乾燥二酸化イオウを通じ，増量が 64 g に達したとき，ヨウ素 20 g を加えて溶かし，液の色が褐色から黄色に変わるまで水を滴加する．

水分測定用陰極液　塩化リチウム 25 g を水分測定用メタノール/ニトロメタン混液 (4 : 1) 1000 mL に溶かす．

操作法

滴定フラスコ中に水分測定用陽極液を入れた後，この液中に定電流分極電位差滴定装置の一対の白金電極又は双白金電極を浸す．別に，水分測定用陰極液を満たしたヨウ素発生用装置を水分測定用陽極液中に浸す．あらかじめ電解電流を流して，滴定フラスコ内を無水の状態にしておく．次に水分 0.2 ～ 5 mg を含むような量の試料を精密に量り，速やかに滴定フラスコに入れ，かき混ぜて溶かし，激しくかき混ぜながら終点まで滴定する．試料が陽極液に溶けないときは，手早く粉末とし，水分 0.2 ～ 5 mg を含むような量の試料を精密に量り，速やかに滴定フラスコに入れ，湿気を避けて 5 ～ 30 分間かき混ぜた後，激しくかき混ぜながら滴定を行う．別に，試料が溶剤に溶けないとき，又は試料がカールフィッシャー反応を妨害するときは，水分気化装置を用いて試料を加熱し，窒素をキャリヤーとして試料中の水分を滴定フラスコ中に導入することができる．

滴定開始より終点に至るまでのヨウ素の発生に要した電気量 (C)〔電流 (A) ×時間 (秒)〕を測定し，次の式より試料中の水分量 (%) を求める．

なお，滴定は湿度の低い雰囲気下で行う必要があるが，滴定に長時間要するなど雰囲気中の水分の影響が避けられない場合は，試料を測定したときと同様の操作により空試験を行い，補正する．

$$\text{水 } (H_2O)\ \% = \frac{\text{ヨウ素の発生に要した電気量 (C)}}{10.72 \times \text{試料の質量 (mg)}} \times 100$$

10.72 : 水 (H_2O) 1 mg に対応する電気量 (C/mg)

⟨921⟩ WATER DETERMINATION

Many Pharmacopeial articles either are hydrates or contain water adsorbed form. As a result, the determination of the water content is important in demonstrating compliance with the Pharmacopeia standards. Generally one of the methods given below is called for the individual monograph, depending upon the nature of the article. In rare cases, a choice is allowed between two methods. When the article contains water of hydration, the Method I (Titrimetric), the Method II (Azeotropic), or the Method III (Gravimetric) is employed as directed in the individual monograph, and the requirement is given under the heading *Water*.

The heading *Loss on drying* (see *Loss on Drying* ⟨731⟩) is used in those cases where the loss sustained on heating may be not entirely water.

METHOD I (TITRIMETRIC)

Determine the water by *Method Ia*, unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle—The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the *Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the *Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Apparatus—Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes (about 5 mm^2 in area and about 2.5 cm apart) immersed in the solution to be titrated.

At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds to 30 minutes, depending upon the solution being titrated. The time is shortest for substances that dissolve in the reagent. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. Commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant, and the titration vessel may be purged by means of a stream of dry nitrogen or current of dry air.

Reagent—Prepare the Karl Fischer Reagent as follows. Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and, keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 hour before use, or daily if in continuous use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light, and under refrigeration.

A commercially available, stabilized solution of Karl Fischer type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine or alcohols other than methanol may be used also. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol or other suitable solvent, such as ethylene glycol monomethyl ether, may be used as the diluent.

Test Preparation—Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 10 to 250 mg of water.

Where the specimen under test is an aerosol with propellant, store it in a freezer for not less than 2 hours, open the container, and test 10.0 mL of the well-mixed specimen. In titrating the specimen, determine the endpoint at a temperature of 10° or higher.

Where the specimen under test is capsules, use a portion of the mixed contents of not less than 4 capsules.

Where the specimen under test is tablets, use powder from not less than 4 tablets ground to a fine powder in an atmosphere of temperature and relative humidity known not to influence the results.

Where the monograph specifies that the specimen under test is hygroscopic, use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into a tared container, and shake to dissolve the specimen. Using the same syringe, remove the solution from the container and transfer it to a titration vessel prepared as directed for *Procedure*. Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured, add this washing to the titration vessel, and immediately titrate. Determine the water content, in mg, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed for *Standardization of Water Solution for Residual Titrations*, and

subtract this value from the water content, in mg, obtained in the titration of the specimen under test. Dry the container and its closure at 105° for 3 hours, allow to cool in a desiccator, and weigh. Determine the weight of specimen tested from the difference in weight from the initial weight of the container.

Standardization of the Reagent—Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Reagent* to give the characteristic endpoint color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential.

For determination of trace amounts of water (less than 1%), sodium tartrate may be used as a convenient water reference substance. Quickly add 150 to 350 mg of sodium tartrate ($C_4H_4Na_2O_6 \cdot 2H_2O$), accurately weighed by difference, and titrate to the endpoint. The water equivalence factor F, in mg of water per mL of reagent, is given by the formula:

$$2(18.02/230.08)(W/V),$$

in which 18.02 and 230.08 are the molecular weights of water and sodium tartrate dihydrate, respectively. W is the weight, in mg, of sodium tartrate dihydrate, and V is the volume, in mL, of the *Reagent* consumed in the second titration.

For the precise determination of significant amounts of water (1% or more), use *Purified Water* as the reference substance. Quickly add between 25 and 250 mg of water, accurately weighed by difference, from a weighing pipet or from a precalibrated syringe or micropipet, the amount taken being governed by the reagent strength and the buret size, as referred to under *Volumetric Apparatus* (31). Titrate to the endpoint. Calculate the water equivalence factor, F, in mg of water per mL of reagent, by the formula:

$$W/V,$$

in which W is the weight, in mg, of the water, and V is the volume, in mL, of the reagent required.

Procedure—Unless otherwise specified, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the *Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed, since it does not enter into the calculations.) Quickly add the *Test Preparation*, mix, and again titrate with the *Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$SF,$$

in which S is the volume, in mL, of the *Reagent* consumed in the second titration, and F is the water equivalence factor of the *Reagent*.

Method Ib (Residual Titration)

Principle—See the information given in the section *Principle* under *Method Ia*. In the residual titration, excess *Reagent* is added to the test specimen, sufficient time is allowed for the reaction to reach completion, and the unconsumed *Reagent* is titrated with a standard solution of water in a solvent such as methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, Reagent, and Test Preparation—Use *Method Ia*.

Standardization of Water Solution for Residual Titration—Prepare a *Water Solution* by diluting 2 mL of water with methanol or other suitable solvent to 1000 mL. Standardize this solution by

titrating 25.0 mL with the *Reagent*, previously standardized as directed under *Standardization of the Reagent*. Calculate the water content, in mg per mL, of the *Water Solution* taken by the formula:

$$V'F/25,$$

in which V' is the volume of the *Reagent* consumed, and F is the water equivalence factor of the *Reagent*. Determine the water content of the *Water Solution* weekly, and standardize the *Reagent* against it periodically as needed.

Procedure—Where the individual monograph specifies that the water content is to be determined by *Method Ib*, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the *Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation*, mix, and add an accurately measured excess of the *Reagent*. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$F(X' - XR),$$

in which F is the water equivalence factor of the *Reagent*, X' is the volume, in mL, of the *Reagent* added after introduction of the specimen, X is the volume, in mL, of standardized *Water Solution* required to neutralize the unconsumed *Reagent*, and R is the ratio, $V'/25$ (mL *Reagent*/mL *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration*.

Method Ic (Coulometric Titration)

Principle—The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the endpoint. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer solution after each determination is not necessary since individual determinations can be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen is compatible with the other components, and no side reactions take place. Samples are usually transferred into the vessel as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system; thus, the introduction of solids into the cell is not recommended, unless elaborate precautions are taken, such as working in a glove-box in an atmosphere of dry inert gas. Control of the system may be monitored by measuring the amount of baseline drift. This method is particularly suited to chemically inert substances like hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micro-method. The method utilizes extremely small amounts of current and is used to determine water content in the range of 100% to 0.0001%.

Apparatus—Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary, as the current consumed can be measured absolutely.

Reagent—See *Reagent* under *Method Ia*.

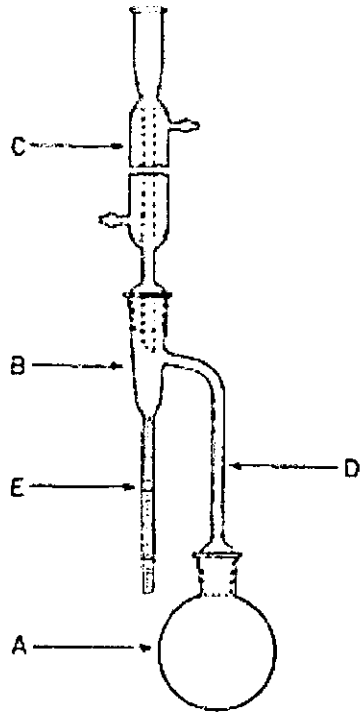
Test Preparation—Where the specimen is a soluble solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or other suitable solvents. Liquids may be used as such or as accurately prepared solutions in appropriate anhydrous solvents.

Where the specimen is an insoluble solid, the water may be extracted using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas, this gas being then passed into the cell.

Procedure—Using a dry syringe, quickly inject the *Test Preparation*, accurately measured and estimated to contain 0.5 to 5 mg of water, or as recommended by the instrument manufacturer, into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, and make any necessary corrections.

METHOD II (AZEOTROPIC—TOLUENE DISTILLATION)

Apparatus—Use a 500-mL glass flask *A* connected by means of a trap *B* to a reflux condenser *C* by ground glass joints (see figure).



Toluene Moisture Apparatus

The critical dimensions of the parts of the apparatus are as follows. The connecting tube *D* is 9 to 11 mm in internal diameter. The trap is 235 to 240 mm in length. The condenser, if of the straight-tube type, is approximately 400 mm in length and not less than 8 mm in bore diameter. The receiving tube *E* has a 5-mL capacity, and its cylindrical portion, 146 to 156 mm in length, is graduated in 0.1-mL subdivisions, so that the error of reading is not greater than 0.05 mL for any indicated volume. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Clean the receiving tube and the condenser with chromic acid cleansing mixture, thoroughly rinse with water, and dry in an oven. Prepare the toluene to be used by first shaking with a small quantity of water, separating the excess water, and distilling the toluene.

Procedure—Place in the dry flask a quantity of the substance, weighed accurately to the nearest centigram, which is expected to yield 2 to 4 mL of water. If the substance is of a pasty character, weigh it in a boat of metal foil of a size that will just pass through the neck of the flask. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask, or a number of capillary melting-point tubes, about 100 mm in length, sealed at the upper end. Place about 200 mL of toluene in the flask, connect the apparatus, and fill the receiving tube *E* with toluene poured through the top of the condenser. Heat the flask gently for 15 minutes and, when the toluene begins to boil, distil at the rate of about 2 drops per second until most of the water has passed over, then increase the rate of distillation to about 4 drops per second. When the water has apparently all distilled over, rinse the inside of the condenser tube with toluene while brushing down the tube with a tube brush attached to a copper wire and saturated with toluene. Continue the distillation for 5 minutes.

remove the heat, and allow the receiving tube to cool to room erature. If any droplets of water adhere to the walls of the ving tube, scrub them down with a brush consisting of a rubber wrapped around a copper wire and wetted with toluene. When ater and toluene have separated completely, read the volume of r, and calculate the percentage that was present in the substance.

METHOD III (GRAVIMETRIC)

rocedure for Chemicals—Proceed as directed in the individual ograph preparing the chemical as directed under *Loss on Drying* ⟨1⟩.

rocedure for Biologics—Proceed as directed in the individual ograph.

rocedure for Articles of Botanical Origin—Place about 10 g of rug, prepared as directed (see *Methods of Analysis* under *Articles Botanical Origin* ⟨561⟩) and accurately weighed, in a tared orating dish. Dry at 105° for 5 hours, and weigh. Continue the ng and weighing at 1-hour intervals until the difference between successive weighings corresponds to not more than 0.25%.

C. Determination of Water

Use Method IA unless otherwise directed.

METHOD I

(Ph. Eur. method 2.5.12)

Apparatus

The apparatus consists of a titration vessel of about 60-ml capacity fitted with two platinum electrodes, a nitrogen inlet tube, a stopper which accommodates the burette tip and a vent tube protected by a suitable desiccant. The substance being examined is introduced through an inlet tube or side arm which can be closed by a ground-glass stopper. Stirring is effected magnetically or by means of a stream of dried nitrogen passed through the solution during the titration. The end point is determined by amperometry. A suitable circuit consists of a potentiometer of about 2000 ohms resistance connected across a 1.5 volt battery to supply a variable potential. The potential is adjusted so that a low initial current passes through the platinum electrodes connected in series with a microammeter. After addition of the reagent the microammeter shows a deflection but returns immediately to its original position. At the end of the reaction a deflection is obtained which persists for not less than 30 seconds.

The reagents and solutions used must be kept anhydrous and precautions must be taken throughout to prevent exposure to atmospheric moisture. *Karl Fischer reagent VS (iodosulphurous reagent R)* should be protected from light and preferably stored in a bottle fitted with an automatic burette. Its water-equivalent is determined before use.

The composition of commercially available reagents may differ from that of *Karl Fischer reagent VS* by the replacement of pyridine with various other basic compounds. The use of these reagents must be validated in order to verify in each case the stoichiometry and the absence of incompatibility between the substance being examined and the reagent.

Method IA

Unless otherwise prescribed, add about 20 ml of *anhydrous methanol* or the solvent prescribed in the monograph to the titration vessel and titrate to the amperometric end point with *Karl Fischer reagent VS*. Quickly add the prescribed amount of the substance being examined, stir for 1 minute and again titrate to the amperometric end point with *Karl Fischer reagent VS*.

Method IB

Unless otherwise prescribed, add about 10 ml of *anhydrous methanol* or the solvent prescribed in the monograph to the titration vessel and titrate to the amperometric end point with *Karl Fischer reagent VS*. Quickly add the prescribed amount of the substance being examined in a suitable state of division followed by sufficient *Karl Fischer reagent VS*, accurately measured, to give an excess of about 1 ml, or the volume prescribed in the monograph. Unless otherwise prescribed, allow the closed vessel to stand for 1 minute,

protected from light, stirring occasionally. Unless otherwise directed, titrate the excess of *Karl Fischer reagent VS* with *anhydrous methanol* to which has been added an accurately measured amount of *water* equivalent to about 0.25% w/v, until the low initial current is attained.

METHOD II

(Ph. Eur. method 2.2.13)

Apparatus

The apparatus (Fig. 9C–1) consists of a glass flask (A) connected by a tube (D) to a cylindrical tube (B) fitted with a graduated receiving tube (E) and reflux condenser (C). The receiving tube (E) is graduated in 0.1-ml increments. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

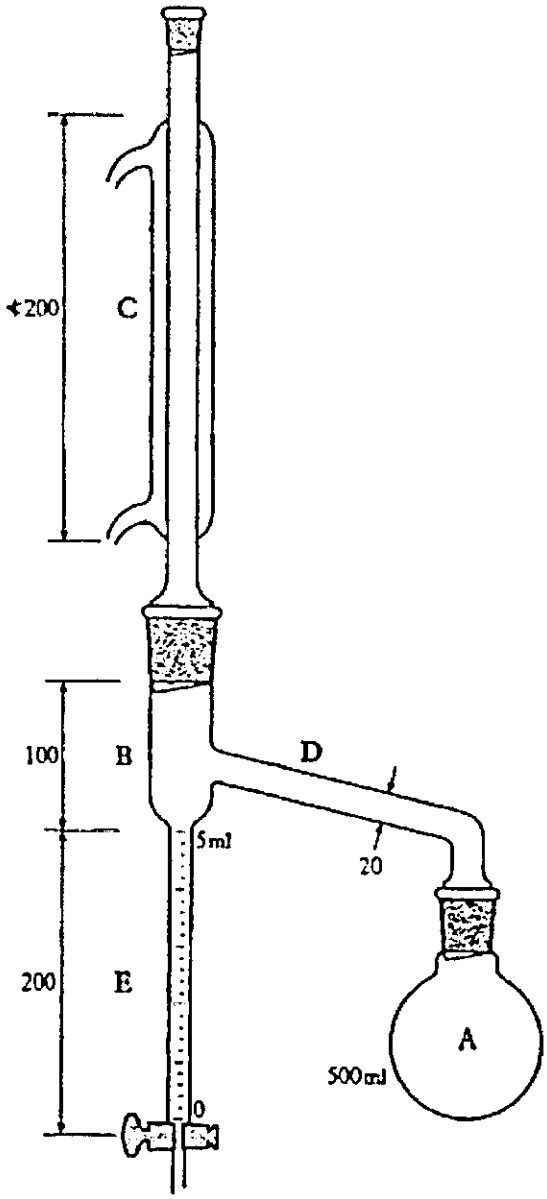


Fig. 9C–1 Apparatus for the Determination of Water (Method II)
Dimensions in mm unless otherwise stated

Method

Clean the receiving tube and the condenser of the apparatus, thoroughly rinse with *water* and dry. Add 200 ml of *toluene* and about 2 ml of *water* to the dry flask. Distil for 2 hours, allow to cool for about 30 minutes and read the water volume to an accuracy of 0.05 ml. Add to the flask a quantity of the substance being examined, weighed with an accuracy of 1%, expected to give about 2 to 3 ml of water. If the substance is of a pasty consistence, weigh it in a boat of metal foil. Add a few pieces of porous material and heat the flask gently for 15 minutes. When the toluene begins to boil, distil at a rate of 2 drops per second until most of the water has distilled over and then increase the rate of distillation to about 4 drops per second. When the water has completely distilled, rinse the inside of the condenser tube with *toluene*. Continue the distillation for 5 minutes, remove from the heat, allow the receiving tube to cool to room temperature and dislodge any droplets of water that adhere to the walls of the receiving tube. When the water and toluene have completely separated, record the volume of water and calculate the content of water in the substance being examined in millilitres per kilogram (ml/kg) using the formula

$$1000\ (n_2 - n_1)/w$$

where n_1 = the number of ml of water obtained in the first distillation,

n_2 = the number of ml of water obtained in the two distillations,

w = the weight, in g, of substance taken.

METHOD III (COULOMETRIC TITRATION)

(Ph. Eur. method 2.5.32)

Principle

The coulometric titration of water is based upon the quantitative reaction of water with sulphur dioxide and iodine in an anhydrous medium in the presence of a base with sufficient buffering capacity. In contrast to the volumetric method described under Method I (*2.5.12*), iodine is produced electrochemically in the reaction cell by oxidation of iodide. The iodine produced at the anode reacts immediately with the water and the sulphur dioxide contained in the reaction cell. The amount of water in the substance is directly proportional to the quantity of electricity up until the titration end point. When all of the water in the cell has been consumed, the end point is reached and thus an excess of iodine appears. 1 mole of iodine corresponds to 1 mole of water, a quantity of electricity of 10.71 C corresponds to 1 mg of water.

Moisture is eliminated from the system by pre-electrolysis. Individual determinations can be carried out successively in the same reagent solution, under the following conditions:

— each component of the test mixture is compatible with the other components,

— no other reactions take place,

— the volume and the water capacity of the electrolyte reagent are sufficient.

Coulometric titration is restricted to the quantitative determination of small amounts of water, a range of 10 µg up to 10 mg of water is recommended.

Accuracy and precision of the method are predominantly governed by the extent to which atmospheric moisture is excluded from the system. Control of the system must be monitored by measuring the amount of baseline drift.

Apparatus

The apparatus consists of a reaction cell, electrodes and magnetic stirrer. The reaction cell consists of a large anode compartment and a smaller cathode compartment.

Depending on the design of the electrode, both compartments can be separated by a diaphragm. Each compartment contains a platinum electrode. Liquid or solubilised samples are introduced through a septum, using a syringe. Alternatively, an evaporation technique may be used in which the sample is heated in a tube (oven) and the water is evaporated and carried into the cell by means of a stream of dry inert gas. The introduction of solid samples into the cell should in general be avoided. However, if it has to be done it is effected through a sealable port; appropriate precautions must be taken to avoid the introduction of moisture from air, such as working in a glove box in an atmosphere of dry inert gas. The analytical procedure is controlled by a suitable electronic device, which also displays the results.

Method

Fill the compartments of the reaction cell with *electrolyte reagent for the micro determination of water* according to the instructions of the manufacturer and perform the coulometric titration to a stable endpoint. Introduce the prescribed amount of the substance to be examined into the reaction cell, stir for 30 seconds, if not otherwise indicated in the monograph, and titrate again to a stable endpoint. In case an oven is used, the prescribed sample amount is introduced into the tube and heated. After evaporation of the water from the sample into the titration cell, the titration is started. Read the value from the instrument's output and calculate if necessary the percentage or amount of water that is present in the substance. When appropriate to the type of sample and the sample preparation, perform a blank titration.

Verification of the accuracy

Between two successive sample titrations, introduce an accurately weighed amount of water in the same order of magnitude as the amount of water in the sample, either as *water* or in the form of *standard solution for the micro determination of water*, and perform the coulometric titration. The recovery rate is within the range from 97.5% to 102.5% for an addition of 1000 µg of H_2O and in the range from 90.0% to 110.0% for the addition of 100 µg of H_2O.

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WATER DETERMINATION

Method I (Karl Fischer Titrimetric Method) Determine the water by *Method Ia*, unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle The titrimetric determination of water is based on the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as *Karl Fischer Reagent*, the sulfur dioxide and iodine are dissolved in pyridine and methanol. Pyridine-free reagents are more commonly used now. The test specimen may be titrated with the *Karl Fischer Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of the determination depends on such factors as the relative concentrations of the *Karl Fischer Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, the apparent pH of the final mixture, and the technique used in the particular determination. Therefore, an empirically standardized technique is used to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Substances that may interfere with the test results are ferric ion, chlorine, and similar oxidizing agents, as well as significant amounts of strong acids or bases, phosgene, or anything that will reduce iodide to iodine, poison the reagent, and show the sample to be bone dry when water may be present (false negative). 8-Hydroxyquinoline may be added to the vessel to eliminate interference from ferric ion. Chlorine interference can be eliminated with SO_2 or unsaturated hydrocarbon. Excess pyridine or other amines may be added to the vessel to eliminate the interference of strong acids. Excess acetic acid or other carboxylic acid can be added to reduce the interference of strong bases. Aldehydes and ketones may react with the solution, showing the sample to be wet while the detector never reaches an endpoint (false positive).

Apparatus Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes (about 5 mm^2 in area and about 2.5 cm apart) immersed in the solution to be titrated. At the endpoint of the titration, a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 s to 30 min, depending on the solution being titrated. The time is shortest for substances that dissolve in the reagent. The longer times are required for solid materials that do not readily go into solution in the *Karl Fischer Reagent*. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. A commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant such as phosphorus pentoxide, and the titration vessel may be purged by means of a stream of dry nitrogen or a current of dry air.

Reagent The *Karl Fischer Reagent* may be prepared as follows: Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250 mL graduated cylinder, and keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 h before use, or daily in continual use. Protect the solution from light while in use. Store any bulk stock of the solution in a suitably sealed, glass-stoppered container, fully protected from light and under refrigeration.

A commercially available, stabilized solution of a Karl Fischer-type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine and/or alcohols other than methanol also may be used. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Karl Fischer Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol, or another suitable solvent such as ethylene glycol monomethyl ether, may be used as the diluent.

Test Preparation Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 10 to 250 mg of water.

Where the monograph specifies that the specimen under test is hygroscopic, accurately weigh a sample of the specimen into a suitable container. Use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into the container and shake to dissolve the specimen. Dry the syringe, and use it to remove the solution from the container and transfer it to a titration vessel prepared as directed under *Procedure*. Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured; add this washing to the titration vessel; and immediately titrate. Determine the water content, in mg, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed under *Standardization of Water Solution for Residual Titrations*, and subtract this value from the water content, in mg, obtained in the titration of the specimen under test.

Standardization of the Reagent Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Karl Fischer Reagent* to give the characteristic color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential. Pure methanol can make the detector overly sensitive, particularly at low ppm levels of water, causing it to deflect to dryness and slowly recover with each addition of reagent. This slows down the titration and may allow the system to actually pick up ambient moisture during the resulting long titration. Adding chloroform or a similar nonconducting solvent will retard this sensitivity and can improve the analysis.

For determination of trace amounts of water (less than 1%), quickly add 25 µL (25 mg) of pure water, using a 25- or 50-µL syringe, and titrate to the endpoint. The water equivalence factor F, in mg of water per mL of reagent, is given by the formula

$$25/V,$$

in which V is the volume, in mL, of the *Karl Fischer Reagent* consumed in the second titration.

For the precise determination of significant amounts of water (more than 1%), quickly add between 25 and 250 mg (25 to 250 µL) of pure water, accurately weighed by difference from a weighing pipet or from a precalibrated syringe or micropipet, the amount of water used being governed by the reagent strength and the buret size, as referred to under *Volumetric Apparatus*.

Titrate to the endpoint. Calculate the water equivalence factor, F, in mg of water per mL of reagent by the formula

$$W/V,$$

in which W is the weight, in mg, of the water, and V is the volume, in mL, of the *Karl Fischer Reagent* required.

Procedure Unless otherwise specified, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed since it does not enter into the calculations.) Quickly add the *Test Preparation*, mix, and again titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, by the formula

$$SF,$$

in which S is the volume, in mL, of the *Karl Fischer Reagent*, consumed in the second titration, and F is the water equivalence factor of the *Karl Fischer Reagent*.

Method Ib (Residual Titration)

Principle See the information in the section entitled *Principle* under *Method Ia*. In the residual titration, add excess *Karl Fischer Reagent* to the test specimen, allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Karl Fischer Reagent* with a standard solution of water in a solvent such as methanol. The residual titration procedure is generally applicable and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, Reagent, and Test Preparation Use *Method Ia*.

Standardization of Water Solution for Residual Titration Prepare a *Water Solution* by diluting 2 mL of pure water with methanol or another suitable solvent to 1000 mL. Standardize this solution by titrating 25.0 mL with the *Karl Fischer Reagent*, previously standardized as directed under *Standardization of the Reagent*. Calculate the water content, in mg/mL, of the *Water Solution* with the formula

$$VF/25,$$

in which V is the volume of the *Karl Fischer Reagent* consumed, and F is the water equivalence factor of the *Karl Fischer Reagent*. Determine the water content of the *Water Solution* weekly, and standardize the *Karl Fischer Reagent* against it periodically as needed. Store the *Water Solution* in a tightly capped container.

Procedure Where the individual monograph specifies the water content is to be determined by *Method Ib*, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation*, mix, and add an accurately measured excess of the *Karl Fischer Reagent*. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Karl Fischer Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, with the formula

$$F(X' - XR),$$

in which F is the water equivalence factor of the *Karl Fischer Reagent*; X' is the volume, in mL, of the *Karl Fischer Reagent*, added after introduction of the specimen; X is the volume, in mL, of standardized *Water Solution* required to neutralize the unconsumed *Karl Fischer Reagent*; and R is the ratio $V/25$ (mL *Karl Fischer Reagent*/mL *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration*.

Method Ic (Coulometric Titration)

Principle Use the Karl Fischer reaction in the coulometric determination of water. In this determination, iodine is not added in the form of a volumetric solution, but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with the water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which can be detected potentiometrically, thus indicating the endpoint. Pre-electrolysis, which can take several hours, eliminates moisture from the system. Therefore, changing the *Karl Fischer Reagent* after each determination is not practical. Individual determinations may be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen be compatible with the other components and that no side reactions take place. Samples may be transferred into the vessel as solids or as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. For the water determination of solids, another common technique is to dissolve the solid in a suitable solvent and then inject a portion of this solution into the cell. In the case of insoluble solids, water may be extracted using suitable solvents, and then the extracts injected into the coulometric cell. Alternatively, an evaporation technique may be used in which the sample is heated in a tube and the water is evaporated and carried into the cell by means of a stream of dry, inert gas. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system. Control of the

system may be monitored by measuring the amount of baseline drift. The titration of water in solid test specimens is usually carried out with the use of anhydrous methanol as the solvent. Other suitable solvents may be used for special or unusual test specimens. This method is particularly suited to chemically inert substances such as hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micromethod. The method uses extremely small amounts of current. It is predominantly used for substances with a very low water content (0.1% to 0.0001%).

Apparatus Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary as the current consumed can be measured absolutely. Proper operation of the instrument can be confirmed by injecting 1 µL of water into the vessel. The instrument should read 1000 µg of water on reaching the endpoint.

Reagent See *Reagent* under *Method Ia*.

Test Preparation Using a dry syringe, inject an appropriate volume of test specimen estimated to contain 0.5 to 5 mg of water, accurately measured, into the anolyte solution. The sample may also be introduced as a solid, accurately weighed, into the anolyte solution. Perform coulometric titration and determine the water content of the specimen under test.

Alternatively, when the specimen is a suitable solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or another suitable solvent, and inject a suitable portion into the anolyte solution.

When the specimen is an insoluble solid, extract the water by using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively use an evaporation technique.

Procedure Quickly inject the *Test Preparation*, or transfer the solid sample, into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance.

Method II (Toluene Distillation Method)

Principle This method determines water by distillation of a sample with an immiscible solvent, usually toluene.

Apparatus Use a glass distillation apparatus (see Fig. 8) provided with 24/40 ground-glass connections. The components consist of a 500-mL short-neck, round-bottom flask connected by means of a trap to a 400-mm, water-cooled condenser. The lower tip of the condenser should be about 7 mm above the surface of the liquid in the trap after distillation conditions have been established (see *Procedure*).

The trap should be constructed of well-annealed glass, the receiving end of which is graduated to contain 5 mL and subdivided into 0.1-mL divisions, with each 1-mL line numbered from 5 mL beginning at the top. Calibrate the receiver by adding