MISCELLANEOUS DETERMINATIONS AND TESTS

Water/Moisture content, Loss on Drying, Evaluation of Ointments, Ash contents and Alkalinity of Glass.

WATER DETERMINATION

Many Pharmacopeial articles either are hydrates or contain water in adsorbed form. As a result, the determination of the water content is important in demonstrating compliance with the Pharmacopeial standards. Generally one of the methods given below is called for in 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.

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. 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 of about 200 mV of applied potential between a pair of platinum electrodes 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 2 to 250 mg of water. The amount of water depends on the water equivalency factor of the Reagent and on the method of endpoint determination. In most cases, the minimum amount of specimen, in mg, can be estimated using the formula:

FCV / KF

in which F is the water equivalency factor of the Reagent, in mg per mL; C is the used volume, in percent, of the capacity of the buret; V is the buret volume, in mL; and KF is the limit or reasonable expected water content in the sample, in percent. C is between 30% and 100% for manual titration, and between 10% and 100% for the instrumental method endpoint determination.

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 fewer than 4 capsules.

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

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%), it is preferable to use Reagent with a water equivalency factor of not more than 2.0. Sodium tartrate may be used as a convenient water reference substance. Quickly add 75 to 125 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. 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.

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.

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.

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.

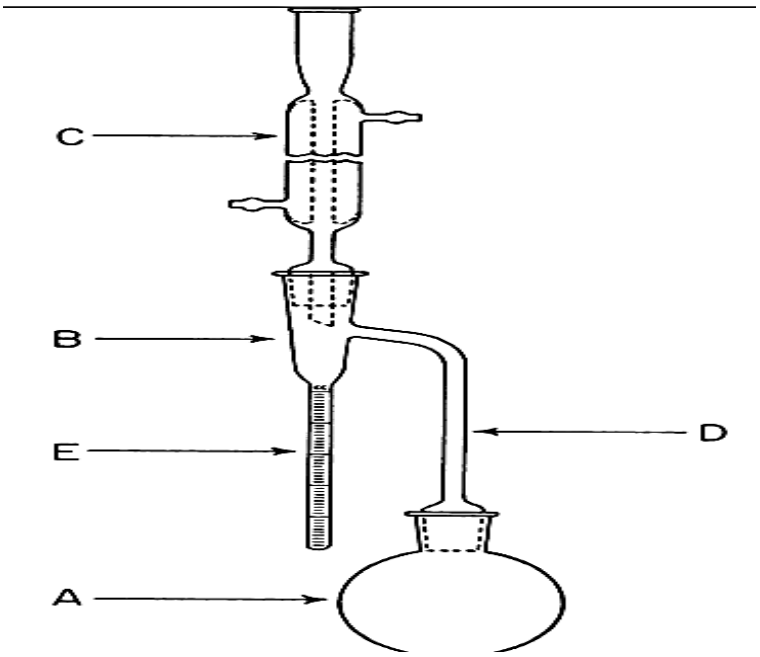
Test Preparation— Where the specimen is a soluble solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or other suitable solvents. Liquids may be used as such or as accurately prepared solutions in appropriate anhydrous solvents.

Where the specimen is an insoluble solid evaporation technique may be used, in which the sample is heated in a tube and water is evaporated and carried into the reaction cell by means of stream of dry inert gas.

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 Distillation Apparatus

The connecting tube D. The receiving tube E. 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—

Weigh accurately a quantity of the sample which is expected to give 2-4 mL of water, transfer to the flask and add about 200 mL of toluene into it. When necessary, add a few pieces of glass beads. After assembly of the apparatus, fill in the narrow part of the receiving tube with toluene through the condenser, then heat the flask gently by using an electric heater or other appropriate means. When the toluene begins to boil, adjust the temperature to allow the distillation proceed at a rate of 2 drops per second until the water has been completely distilled. Rinse the inside of the condenser with toluene. Continue the distillation for five more minutes, then remove the apparatus away from the heat and allow it to cool to room temperature. Disconnect the apparatus and dislodge any droplets of water that adhere to the wall of the receiving tube. Allow the receiving tube to stand a while until the water and toluene are completely separated [Note]. Record the volume of water and calculate the percentage of water content in the weight of sample.

METHOD III (GRAVIMETRIC)

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.

Procedure for Biologics— Proceed as directed in the individual monograph.

Procedure for Articles of Botanical Origin— Place about 10 g of the drug, prepared as directed and accurately weighed, in a tared evaporating dish. Dry at 105 for 5 hours, and weigh. Continue the drying and weighing at 1-hour intervals until the difference between two successive weighings corresponds to not more than 0.25%.

Loss on drying

The procedure set forth in this chapter determines the amount of volatile matter of any kind that is driven off under the conditions specified. For substances appearing to contain water as the only volatile constituent, the procedure given in the chapter, Water Determination 921 , is appropriate, and is specified in the individual monograph.

Mix and accurately weigh the substance to be tested, and, unless otherwise directed in the individual monograph, conduct the determination on 1 to 2 g. If the test specimen is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes under the same conditions to be employed in the determination. Put the test specimen in the bottle, replace the cover, and accurately weigh the bottle and the contents. By gentle, sidewise shaking, distribute the test specimen as evenly as practicable to a depth of about 5 mm generally, and not more than 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the test specimen at the temperature and for the time specified in the monograph. [note—The temperature specified in the monograph is to be regarded as being within the range of ±2 of the stated figure.] Upon opening the chamber, close the bottle promptly, and allow it to come to room temperature in a desiccator before weighing.

a) If the substance melts at a lower temperature than that specified for the determination of Loss on drying, maintain the bottle with its contents for 1 to 2 hours at a temperature 5 to 10 below the melting temperature, then dry at the specified temperature.
b) Where the specimen under test is Capsules, use a portion of the mixed contents of not fewer than 4 capsules.
c) Where the specimen under test is Tablets, use powder from not fewer than 4 tablets ground to a fine powder. Where the individual monograph directs that loss on drying be determined by thermogravimetric analysis, a sensitive electrobalance is to be used.
d) Where drying in vacuum over a desiccant is directed in the individual monograph, a vacuum desiccator or a vacuum drying pistol, or other suitable vacuum drying apparatus, is to be used.
e) Where drying in a desiccator is specified, exercise particular care to ensure that the desiccant is kept fully effective by frequent replacement.
a) Where drying in a capillary-stoppered bottle in vacuum is directed in the individual monograph, use a bottle or tube fitted with a stopper having a 225 ± 25 µm diameter capillary, and maintain the heating chamber at a pressure of 5 mm or less of mercury. At the end of the heating period, admit dry air to the heating chamber, remove the bottle, and with the capillary stopper still in place allow it to cool in a desiccator before weighing.

Ash content determinations

Sulfated Ash test

Sulfated Ash test uses a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid

according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

Procedure— Ignite a suitable crucible (for example, silica, platinum, quartz, or porcelain) at 600 ± 50 for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant), and weigh it accurately. Weigh accurately 1 to 2 g of the substance, or the amount specified in the individual monograph, in the crucible.
Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. Cool; then, unless otherwise directed in the individual monograph, moisten the residue with a small amount (usually 1 mL) of sulfuric acid; heat gently until white fumes are no longer evolved; and ignite at 600 ± 50 , unless another temperature is specified in the individual monograph, until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately, and calculate the percentage of residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and igniting as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

Conduct the ignition in a well-ventilated hood, but protected from air currents, and at as low a temperature as is possible to effect the complete combustion of the carbon.

Total Ash

Accurately weigh a quantity of the Test Sample, representing 2 to 4 g of the air-dried material, in a tared crucible, and incinerate, gently at first, and gradually increase the temperature to 675 ± 25 , until free from carbon, and determine the weight of the ash. If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness, and heat the whole to a temperature of 675 ± 25 . If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15 mL of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a temperature of 675 ± 25 . Cool in a desiccator, weigh the ash, and calculate the percentage of total ash from the weight of the drug taken.

Acid-Insoluble Ash

Boil the ash obtained as directed under Total Ash, above, with 25 mL of 3 N hydrochloric acid for 5 minutes, collect the insoluble matter on a tared filtering crucible or ashless filter, wash with hot water, ignite, and weigh. Determine the percentage of acid-insoluble ash calculated from the weight of drug taken.

Water-Soluble Ash

Boil the ash obtained as directed for Total Ash with 25 mL of water for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450 . Subtract the weight of

this residue, in mg, obtained under Total Ash, and calculate the percentage of water-soluble ash with reference to the weight of sample as determined under Total Ash.

Glass alkalinity test

Glass containers for pharmaceutical use are intended to come into direct contact with pharmaceutical preparations. Glass used for pharmaceutical containers is either a borosilicate (neutral) glass or a soda-lime glass.

Type I glass : Borosilicate glass contains a significant amount of boric oxide, aluminum oxide, and alkali and/or alkaline earth oxides. Borosilicate glass has a high hydrolytic resistance due to the chemical composition of the glass itself; it is classified as Type I glass.

Type II glass : The inner surface of glass containers may be treated, for example, to improve hydrolytic resistance. The treatment of Type III soda-lime glass containers will raise their hydrolytic resistance from a moderate to a high level, changing the classification of the glass to Type II.

Type III glass : Soda-lime glass is a silica glass containing alkali metal oxides. Soda-lime glass has a moderate hydrolytic resistance due to the chemical composition of the glass itself; it is classified as Type III glass.

The quality of glass containers is defined by measuring their resistance to chemical attack.

Chemical Resistance

The following tests are designed to determine the resistance to water attack of new (not previously used) glass containers. The degree of attack is determined by the amount of alkali released from the glass under the influence of the attacking medium under the conditions specified. This quantity of alkali is extremely small in the case of the more resistant glasses, thus calling for particular attention to all details of the tests and the use of apparatus of high quality and precision. The tests should be conducted in an area relatively free from fumes and excessive dust.

Glass Types— Glass containers suitable for packaging Pharmacopeial preparations may be classified as in Table 1 on the basis of the tests set forth in this section. Containers of Type I borosilicate glass are generally used for preparations that are intended for parenteral administration. Containers of Type I glass, or of Type II glass (i.e., soda-lime glass that is suitably dealkalized) are usually used for packaging acidic and neutral parenteral preparations. Type I glass containers, or Type II glass containers (where stability data demonstrate their suitability), are used for alkaline parenteral preparations. Type III soda-lime glass containers usually are not used for parenteral preparations, except where suitable stability test data indicate that Type III glass is satisfactory for the parenteral preparations that are packaged therein.

Table 1. Glass Types

Type	General Description	Type of Test
I	Highly resistant, borosilicate glass	Powdered Glass
II	Treated soda-lime glass	Water Attack
III	Soda-lime glass	Powdered Glass

Apparatus

Autoclave: For these tests, use an autoclave capable of maintaining a temperature of 121 ± 2.0, equipped with a thermometer, a pressure gauge, a vent cock, and a rack adequate to accommodate at least 12 test containers above the water level.

Mortar and Pestle: Use a hardened-steel mortar and pestle.
Other Equipment: Also required are 20.3-cm (8-inch) sieves made of stainless steel, including the Nos. 20, 40, and 50 sieves, along with the pan and cover); 250-mL conical flasks made of resistant glass aged as specified; a 900-g (2-lb) hammer; a permanent magnet; a desiccator; and an adequate volumetric apparatus.

Reagents

High-Purity Water— The water used in these tests has a conductivity at 25° , as measured in an in-line cell just prior to dispensing, of not greater than 6.67 Megohm-cm. There must also be an assurance that this water is not contaminated by copper or its products. The water may be prepared by passing distilled water through a deionizer cartridge packed with a mixed bed of nuclear-grade resin, then through a cellulose ester membrane having openings not exceeding 0.45 µm. Do not use copper tubing. Flush the discharge lines before water is dispensed into test vessels. When the low conductivity specification can no longer be met, replace the deionizer cartridge.

Carbon Dioxide–Free Water— This is Purified Water that has been boiled vigorously for 5 minutes or more and allowed to cool while protected from absorption of carbon dioxide from the atmosphere, or Purified Water that has a resistivity of not less than 18 Mohm-cm.

Methyl Red Solution (Powdered Glass Test and Water Attack at 121)— Dissolve 24 mg of methyl red sodium in Purified Water to make 100 mL. If necessary, neutralize the solution with 0.02 N sodium hydroxide, or acidify it with 0.02 N sulfuric acid so that the titration of 100 mL of High-Purity Water, containing 5 drops of indicator, does not require more than 0.020 mL of 0.020 N sodium hydroxide to effect the color change of the indicator, which should occur at a pH of 5.6.

Methyl Red Solution (Surface Glass Test)— Dissolve 50 mg of methyl red solution in 1.86 mL of 0.1 M sodium hydroxide and 50 mL of ethanol (96%) and dilute to 100 mL with Purified Water. To test for sensitivity, add 100 mL of Carbon Dioxide-Free Water and 0.05 mL of 0.02 M hydrochloric acid to 0.1 mL of the methyl red solution (the solution should be red). Not more than 0.1 mL of 0.02 M sodium hydroxide is required to change the color to yellow. Color change: pH 4.4 (red) to pH 6.0 (yellow).

Powdered Glass Test

Rinse thoroughly with Purified Water six or more containers selected at random, and dry them with a current of clean, dry air. Crush the containers into fragments about 25 mm in size, divide about 100 g of the coarsely crushed glass into three approximately equal portions, and place one of the portions in the special mortar. With the pestle in place, crush the glass further by striking 3 or 4 blows with the hammer. Nest the sieves, and empty the mortar into the No. 20 sieve. Repeat the operation on each of the two remaining portions of glass, emptying the mortar each time into the No. 20 sieve. Shake the sieves for a short time, then remove the glass from the Nos. 20 and 40 sieves, and again crush and sieve as before. Repeat again this crushing and sieving operation. Empty the receiving pan, reassemble the nest of sieves, and shake by mechanical means for 5 minutes or by hand for an equivalent length of time. Transfer the portion retained on the No. 50 sieve, which should weigh in excess of 10 g, to a closed container, and store in a desiccator until used for the test.

Spread the specimen on a piece of glazed paper, and pass a magnet through it to remove particles of iron that may be introduced during the crushing. Transfer the specimen to a 250-mL conical flask of resistant glass, and wash it with six 30-mL portions of acetone, swirling each time for about 30 seconds, and carefully decanting the acetone. After washing, the specimen should be free from agglomerations of glass powder, and the surface of the grains should be practically free from adhering fine particles. Dry the flask and contents for 20 minutes at 140° , transfer the grains to a weighing bottle, and cool in a desiccator. Use the test specimen within 48 hours after drying.

Procedure— Transfer 10.00 g of the prepared specimen, accurately weighed, to a 250-mL conical flask that has been digested (aged) previously with High-Purity Water in a bath at 90° for at least 24 hours or at 121° for 1 hour. Add 50.0 mL of High-Purity Water to this flask and to one similarly prepared to provide a blank. Cap all flasks with borosilicate glass beakers that previously have been treated as described for the flasks and that are of such size that the bottoms of the beakers fit snugly down on the top rims of the containers. Place the containers in the autoclave, and close it securely, leaving the vent cock open. Heat until steam issues vigorously from the vent cock, and continue heating for 10 minutes. Close the vent cock, and adjust the temperature to 121°, taking 19 to 23 minutes to reach the desired temperature. Hold the temperature at 121° ± 2.0 for 30 minutes, counting from the time this temperature is reached. Reduce the heat so that the autoclave cools and comes to atmospheric pressure in 38 to 46 minutes, being vented as necessary to prevent the formation of a vacuum. Cool the flask at once in running water, decant the water from the flask into a suitably cleansed vessel, and wash the residual powdered glass with four 15-mL portions of High-Purity Water, adding the decanted washings to the main portion. Add 5 drops of Methyl Red Solution, and titrate immediately with 0.020 N sulfuric acid. If the volume of titrating solution is expected to be less than 10 mL, use a microburet. Record the volume of 0.020 N sulfuric acid used to neutralize the extract from 10 g of the prepared specimen of glass, corrected for a blank. The volume does not exceed that indicated in Table 2 for the type of glass concerned.

Table 2. Test Limits for Powdered Glass Test

			Limits	
Type	General Description [a]	Type of Test	Size, [b] mL	mL of 0.020 N Acid
I	Highly resistant, borosilicate glass	Powdered Glass	All	1.0
III	Soda-lime glass	Powdered Glass	All	8.5

a The description applies to containers of this type of glass usually available.
b Size indicates the overflow capacity of the container.

Surface Glass Test

Determination of the Filling Volume— The filling volume is the volume to be filled with Purified Water in the container for the purpose of the test. For vials and bottles the filling volume is 90% of the brimful capacity. For ampules it is the volume up to the height of the shoulder.

Vials and Bottle— Select, at random, 6 containers from the sample lot, or 3 if their capacity exceeds 100 mL, and remove any dirt or debris. Weigh the empty containers with an

accuracy of 0.1 g. Place the containers on a horizontal surface, and fill them with Purified Water to about the rim edge, avoiding overflow and introduction of air bubbles. Adjust the liquid levels to the brimful line. Weigh the filled containers to obtain the mass of the water, expressed to 2 decimal places, for containers having a nominal volume less or equal to 30 mL, and expressed to 1 decimal place for containers having a nominal volume greater than 30 mL. Calculate the mean value of the brimful capacity in mL, and multiply it by 0.9. This volume, expressed to 1 decimal place, is the filling volume for the particular container lot.

Ampules— Place at least 6 dry ampules on a flat, horizontal surface, and fill them with Purified Water from a buret until the water reaches point A, where the body of the ampule decreases to the shoulder of the ampule. Read the capacities, expressed to 2 decimal places, and calculate the mean value. This volume, expressed to 1 decimal place, is the filling volume for the particular ampule lot. The filling volume may also be determined by weighing.

Test— The determination is carried out on unused containers. The volumes of the test liquid necessary for the final determination are indicated in Table 3.

Table 3. Volume of Test Liquid and Number of Titrations

Filling Volume (mL)	Volume of Test Liquid for One Titration (mL)	Number of Titrations
Up to 3	25.0	1
Above 3 and up to 30	50.0	2
Above 30 and up to 100	100.0	2
Above 100	100.0	3

Cleaning— Remove any debris or dust. Shortly before the test, rinse each container carefully at least twice with Purified Water, and allow to stand. Immediately before testing, empty the containers, rinse once with Purified Water, then with Carbon Dioxide-Free Water and allow to drain. Complete the cleaning procedure from the first rinsing in not less than 20 minutes and not more than 25 minutes. Heat closed ampules in a water bath or in an air-oven at about 50 for approximately 2 minutes before opening. Do not rinse before testing.

Filling and Heating— The containers are filled with Carbon Dioxide-Free Water up to the filling volume. Containers in the form of cartridges or prefilled syringes are closed in a suitable manner with material that does not interfere with the test. Each container, including ampules, shall be loosely capped with an inert material such as a dish of neutral glass or aluminum foil previously rinsed with Purified Water. Place the containers on the tray of the autoclave.
Place the tray in the autoclave containing a quantity of water such that the tray remains clear of the water. Close the autoclave, and carry out the following operations:

1. Heat the autoclave to 100° and allow the steam to issue from the vent cock for 10 minutes;
2. Close the vent cock and raise the temperature from 100° to 121° at a rate of 1° per minute;
3. Maintain the temperature at 121° ± 1 for 60 ± 1 minutes;

4. Lower the temperature from 121° to 100° at a rate of 0.5° per minute, venting to prevent a vacuum;
5. Do not open the autoclave before it has cooled down to 95°;
6. Remove the containers from the autoclave using normal precautions, place them in a water bath at 80°, and run cold tap water, taking care that the water does not contact the loose foil caps to avoid contamination of the extraction solution;
7. Cooling time does not exceed 30 minutes. The extraction solutions are analyzed by titration according to the method described below.

Method— Carry out the titration within 1 hour of removal of the containers from the autoclave.
Combine the liquids obtained from the containers, and mix. Introduce the prescribed volume indicated in Table 3 into a conical flask. Place the same volume of Carbon Dioxide-Free Water into a second similar flask as a blank. Add 0.05 mL of Methyl Red Solution to each flask for each 25 mL of liquid. Titrate the blank with 0.01 M hydrochloric acid. Titrate the test liquid with the same acid until the color of the resulting solution is the same as that obtained for the blank. Subtract the value found for the blank titration from that found for the test liquid, and express the results in mL of 0.01 M hydrochloric acid per 100 mL. Express titration values of less than 1.0 mL to 2 decimal places and titration values of more than or equal to 1.0 mL to 1 decimal place.

Limits— The results, or the average of the results if more than one titration is performed, are not greater than the values stated in Table 4.
Table 4. Test Limits for Surface Glass Test

	Maximum Volume of 0.01 M HCl per 100 mL of Test Liquid (mL)	
Filling Volume (mL)	Types I and II	Type III
Up to 1	2.0	20.0
Above 1 and Up to 2	1.8	17.6
Above 2 and Up to 5	1.3	13.2
Above 5 and Up to 10	1.0	10.2
Above 10 and Up to 20	0.80	8.1
Above 20 and Up to 50	0.60	6.1
Above 50 and Up to 100	0.50	4.8
Above 100 and Up to 200	0.40	3.8
Above 200 and Up to 500	0.30	2.9
Above 500	0.20	2.2

Water Attack at 121°

Option— The Water Attack at 121° test can be used to qualify Type II glass.
Rinse thoroughly 3 or more containers, selected at random, twice with High-Purity Water.

Procedure— Fill each container to 90% of its overflow capacity with High-Purity Water, and proceed as directed for Procedure under Powdered Glass Test, beginning with "Cap all flasks," except that the time of autoclaving shall be 60 minutes instead of 30 minutes, and ending with "to prevent the formation of a vacuum." Empty the contents from 1 or more containers into a 100-mL graduated cylinder, combining, in the case of smaller containers,

the contents of several containers to obtain a volume of 100 mL. Place the pooled specimen in a 250-mL conical flask of resistant glass, add 5 drops of Methyl Red Solution, and titrate, while warm, with 0.020 N sulfuric acid. Complete the titration within 60 minutes after opening the autoclave. Record the volume of 0.020 N sulfuric acid used, corrected for a blank obtained by titrating 100 mL of High-Purity Water at the same temperature and with the same amount of indicator. The volume does not exceed that indicated in Table 5.

Table 5. Test Limit for Water Attack at 121

Type	General Description[a]	Type of Test	Limits	
			Size,[b] mL	mL of 0.020 N Acid
II	Treated soda-lime glass	Water Attack	100 or less	0.7
			Over 100	0.2

a The description applies to containers of this type of glass usually available.
b Size indicates the overflow capacity of the container.

MISCELLANEOUS DETERMINATIONS AND TESTS

Evaluation of Ointments

Metal Particles In Ophthalmic Ointments

The following test is designed to limit to a level considered to be unobjectionable the number and size of discrete metal particles that may occur in ophthalmic ointments. **Procedure—** Extrude, as completely as practicable, the contents of 10 tubes individually into separate, clear, flat-bottom, 60-mm. Petri dishes that are free from scratches. Cover the dishes, and heat at 85° for 2 hours, increasing the temperature slightly if necessary to ensure that a fully fluid state is obtained. Taking precautions against disturbing the melted sample, allow each to cool to room temperature and to solidify.

Remove the covers, and invert each Petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eye-piece micrometer disk that has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45 angle. Examine the entire bottom of the Petri dish for metal particles. Varying the intensity of the illuminator from above allows such metal particles to be recognized by their characteristic reflection of light.

Count the number of metal particles that are 50 µm or larger in any dimension: the requirements are met if the total number of such particles in all 10 tubes does not exceed 50, and if not more than 1 tube is found to contain more than 8 such particles. If these results are not obtained, repeat the test on 20 additional tubes: the requirements are met if the total number of metal particles that are 50 µm or larger in any dimension does not exceed 150 in all 30 tubes tested, and if not more than 3 of the tubes are found to contain more than 8 such particles each.

Leakage Test

Select 10 tubes of the Ointment, with seals applied when specified. Thoroughly clean and dry the exterior surfaces of each tube with an absorbent cloth. Place the tubes in a horizontal position on a sheet of absorbent blotting paper in an oven maintained at a temperature of 60°

± 3 for 8 hours. No significant leakage occurs during or at the completion of the test (disregard traces of ointment presumed to originate externally from within the crimp of the tube or from the thread of the cap). If leakage is observed from one, but not more than one, of the tubes, repeat the test with 20 additional tubes of the Ointment. The requirement is met if no leakage is observed from the first 10 tubes tested, or if leakage is observed from not more than one of 30 tubes tested.

71 Sterility Tests Ointments And Creams

Diluting And Rinsing Fluids For Membrane Filtration

Fluid K

Dissolve 5.0 g of peptic digest of animal tissue, 3.0 g of beef extract, and 10.0 g of polysorbate 80 in water to make 1 L. Adjust the pH to obtain, after sterilization, a pH of 6.9 ± 0.2. Dispense into containers, and sterilize using a validated process

Procedure

Use for each medium not less than the quantities of the product prescribed in Tables 2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1% in isopropyl myristate as described above, by heating, if necessary, to not more than 40 . In exceptional cases it may be necessary to heat to not more than 44 . Filter as rapidly as possible, and proceed as described below.

Number of Articles to Be Tested

Table 2. Minimum Quantity to be Used for Each Medium

Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)
Insoluble preparations, creams, and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg

Table 3. Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Number of Items in the Batch	Minimum Number of Items to be Tested for Each Medium (unless otherwise justified and authorized)*
Ophthalmic and other noninjectable preparations	
Not more than 200 containers	5% or 2 containers, whichever is the greater
More than 200 containers	10 containers
If the product is presented in the form of single-	

Number of Items in the Batch	Minimum Number of Items to be Tested for Each Medium (unless otherwise justified and authorized)*
dose containers, apply the scheme shown above for preparations for parenteral use.	

Allow the ointment to penetrate the membrane by its own weight, and then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as Fluid K containing a suitable emulsifying agent at a concentration shown to be appropriate in the validation of the test, for example polysorbate 80 at a concentration of 10 g per L. Transfer the membrane or membranes to the culture medium or media, or vice versa, as described and incubate at the same temperatures and for the same times

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However, when thioglycollate medium or other similar medium is used for the detection of anaerobic microorganisms, keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

Observation And Interpretation Of Results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium, and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility

If the test is declared to be invalid, it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility. If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.