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Reagents

1. DEFINITIONS

1.1 BLANK

A blank consists of the same quantities of the same reagents treated in the same manner as the specimen under test.

1.2 CONTROL

A control is a blank to which has been added the limiting quantity of the substance being tested for, or is a specified comparison solution prepared as directed in the particular test.

2. DESCRIPTION OF THE REAGENT ENTRY

See [Figure 1](#).

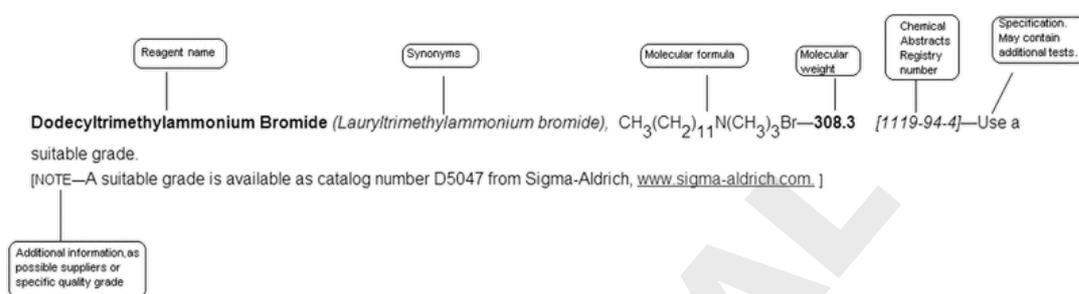


Figure 1. Components of the reagent entry.

3. VISUAL COMPARISONS

For color and turbidity comparisons, proceed as directed in [Visual Comparison \(630\)](#). The tubes used in this procedure are frequently called “Nessler tubes”.

In making visual comparisons of the densities of turbid fluids, compensate for difference in color, if necessary, by viewing the turbidity through a column of water, the depth of which is determined by the volume specified in the individual reagent specification. Place the water in color-comparison tubes, and hold one of the tubes above the control tube and the other below the specimen tube.

4. RETAIN THE FILTRATE

“Retain the filtrate” is to be understood, unless otherwise indicated, that the washings of the residue are not to be added to the filtrate obtained.

5. EXPRESSION R_2O_3

The expression R_2O_3 is intended to indicate the residue on ignition from compounds precipitated upon the addition of ammonium hydroxide, such as iron oxide (Fe_2O_3) and aluminum oxide (Al_2O_3).

6. GENERAL TESTS FOR REAGENTS

The following general test methods are provided for the examination of reagents to determine their compliance with the specifications of the individual reagents and are to be used unless it is otherwise directed in such specifications.

6.1 BOILING OR DISTILLING RANGE FOR REAGENTS

Use the following procedure for determining the boiling or distilling range of reagents, unless otherwise directed in the individual specifications:

APPARATUS: Use apparatus similar to that specified for [Distilling Range \(721\), Method I](#), except that the distilling flask is to be of 250-mL capacity, to have a short neck, and to be connected to the condenser by means of a three-way connecting tube fitted with standard-taper ground joints.

PROCEDURE: Place the distilling flask in an upright position in the perforation in the asbestos board, and connect it to the condenser. Measure 100 mL of the liquid to be tested in a graduated cylinder, and transfer to the boiling flask together with some device to prevent bumping. Use the cylinder as the receiver for the distillate. Insert the thermometer, and heat so as to distill at the rate of 3–5 mL/min. Make a preliminary trial, if necessary, to determine the adjustment for the proper rate of heating. Read the thermometer when about 20 drops have distilled and thereafter at volumes of distillate of 5, 10, 40, 50, 60, 90, and 95 mL. Continue the distillation until the dry point is reached.

The *Boiling or Distilling Range* is the interval between the temperatures when 1 mL and 95 mL, respectively, have distilled.

6.2 AMINO NITROGEN TEST IN REAGENTS

Determine the percentage of loss on drying of the sample in appropriate conditions. Transfer about 500 mg of the sample to a 100-mL beaker. Add 20 mL of water. Adjust the pH potentiometrically with 0.1 N hydrochloric acid or 0.2 N sodium hydroxide to 6.0. Add 10 mL of formaldehyde solution. Titrate the solution potentiometrically with 0.2 N sodium hydroxide to a pH of 9.0. Calculate the percentage of amino nitrogen:

$$\% \text{ amino nitrogen} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times 14 \times 100 \times f}{\text{mg sample} \times (1.0 - \% \text{LOD}/100)}$$

where f is the correction factor obtained in the standardization of 0.2 N sodium hydroxide and %LOD is the percentage of loss on drying.

Change to read:

6.3 ARSENIC IN REAGENTS

Select reagents for this test for a low arsenic content, so that a blank test results in either no stain or one that is barely perceptible.

APPARATUS: Prepare a generator by fitting a 1-hole rubber stopper into a wide-mouth bottle of about 60-mL capacity. Through the perforation insert a vertical exit tube about 12 cm in total length and 1 cm in diameter along the entire upper portion (for about 8 cm) and constricted at its lower extremity to a tube about 4 cm in length and about 5 mm in diameter. The smaller portion of the tube should extend to just slightly below the stopper. Place washed sand or a pledget of purified cotton in the upper portion to about 3 cm from the top of the tube. Moisten the sand or cotton uniformly with lead acetate TS, and remove any excess or adhering droplets of the latter from the walls of the tube. Into the upper end of this tube fit a second glass tube 12 cm in length, with an internal diameter of 2.5–3 mm, by means of a rubber stopper. Just before running the test, place a strip of mercuric bromide test paper (see *Indicator and Test Papers*) in this tube, crimping the upper end of the strip so that it will remain in position about 2 cm above the rubber stopper. Clean and dry the tube thoroughly each time it is used.

STANDARD ARSENIC SOLUTION: Use the *Standard Preparation*, prepared as directed in [▲Arsenic \(211\), Procedures, Procedure 1▲](#) (CN 1-Jun-2023) .

TEST PREPARATION: Add 1 mL of sulfuric acid to 5 mL of a solution of the chemical substance (1 in 25), unless another quantity is directed in the individual reagent specification. Omit its addition entirely in the case of inorganic acids. Unless especially directed otherwise, add 10 mL of sulfurous acid. Evaporate the liquid in a small beaker, on a steam bath, until it is free from sulfurous acid and has been reduced to about 2 mL in volume. Dilute with water to 5 mL to obtain the *Test preparation*. Substances subjected to special treatments specified in the individual reagent specification may be used directly as the *Test preparation*. [NOTE—Solutions prepared by the dissolving of the chemical substances in dilute acids are not considered to have undergone special treatment.]

STANDARD STAIN: Place in the generator bottle 5 mL of potassium iodide TS, 2.0 mL of *Standard arsenic solution*, 5 mL of acid stannous chloride TS, and 28 mL of water. Add 1.5 g of granulated zinc (in No. 20 powder), and immediately insert the stopper containing the exit tube. Keep the generator bottle immersed in water at 25° during the period of the test to moderate the reaction so that the stain will take the form of a distinctive band to facilitate the comparison of color intensity. When evolution of hydrogen has continued for 1 h, remove the mercuric bromide test paper for comparison. This stain represents 2 µg of arsenic.

PROCEDURE: Pipet into the generator bottle 5 mL of potassium iodide TS and 5 mL of the *Test preparation*, and add 5 mL of acid stannous chloride TS. Set the apparatus aside at room temperature for a period of 10 min, then add 25 mL of water and 1.5 g of granulated zinc (in No. 20 powder), and proceed as directed for the *Standard stain*. Remove the mercuric bromide test paper, and compare the stain upon it with the *Standard stain*: the stain produced by the chemical tested does not exceed the *Standard stain* in length or in intensity of color, indicating NMT 10 parts of arsenic per million parts of the substance being tested. Because light, heat, and moisture cause the stain to fade rapidly, place the papers in clean, dry tubes, and make comparisons promptly.

INTERFERING CHEMICALS: *Antimony*, if present in the substance being tested, produces a gray stain. *Sulfites, sulfides, thiosulfates*, and other compounds that liberate hydrogen sulfide or sulfur dioxide when treated with sulfuric acid must be oxidized by means of nitric acid and then reduced by means of sulfur dioxide as directed for the *Test preparation* before they are placed in the apparatus. Certain *sulfur compounds*, as well as *phosphine*, give a bright yellow band on the test paper. If sulfur compounds are present, the lead acetate-moistened cotton or sand will darken. In that case, repeat the operation as directed for the *Test preparation* upon a fresh portion of the solution being tested, and use greater care in effecting the complete removal of the sulfurous acid. In testing hypophosphites, observe special care to oxidize completely the solution being tested as directed; otherwise the evolution of phosphine may result in a yellow stain that may be confused with the orange-yellow color produced by arsine. The stain produced by phosphine may be differentiated from that given by arsine by means of moistening it with 6 N ammonium hydroxide. A stain caused by arsine becomes dark when so treated, but a stain produced by phosphine does not materially change in color.

6.4 CHLORIDE IN REAGENTS

STANDARD CHLORIDE SOLUTION: Dissolve 165.0 mg of dried sodium chloride in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of chlorine (Cl) in each mL.

PROCEDURE: Neutralize, if alkaline, a solution of the quantity of the reagent indicated in the test in 25 mL of water, or a solution prepared as directed in the test, with nitric acid, litmus paper being used as the indicator, and add 3 mL more of nitric acid. Filter the solution, if necessary, through a filter paper previously washed with water until the paper is free from chloride, and add 1 mL of silver nitrate TS. Mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced in a control made with the same quantities of the same reagents as in the final test and a volume of *Standard chloride solution* equivalent to the quantity of chloride (Cl) permitted by the test. Adjust the two solutions with water to the same volume before adding the silver nitrate TS, and compare the turbidities.

In *testing barium salts*, neutralize, if alkaline, the solution containing the reagent, with nitric acid, and add only 3 drops more of nitric acid.

Conduct the remainder of the test as described previously.

In *testing salts giving colored solutions*, dissolve 2 g of the reagent in 25 mL of water, and add 3 mL of nitric acid. Filter the solution, if necessary, through a filter paper previously washed with water, and divide the filtrate into two equal portions. Treat one portion with 1 mL of silver nitrate TS, allow to stand for 10 min, and, if any turbidity is produced, filter it through a washed filter paper until clear, and use the filtrate as a blank. Treat the other portion with 1 mL of silver nitrate TS, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity with that produced in the blank by the addition of a volume of *Standard chloride solution* equivalent to the quantity of chloride (Cl) permitted in the test, both solutions being adjusted with water to the same volume.

6.5 FLAME PHOTOMETRY FOR REAGENTS

The use of flame photometric procedures to determine traces of calcium, potassium, sodium, and strontium is called for in some of the reagent specifications. The suitability of such determinations depends upon the use of adequate apparatus, and several instruments of suitable selectivity are available. The preferred type of flame photometer is one that has a red-sensitive phototube, a multiplier phototube, a monochromator, an adjustable slit-width control, a selector switch, and a sensitivity control. Other types of photometers may be used, provided the operator has proved that the instrument will determine accurately the amount of impurities permitted in the reagent to be tested.

The flame photometric procedures depend upon the use of semi-internal standards, and thus require both a *Sample solution* and a *Control solution*. For the *Sample solution*, a specified weight of specimen is dissolved and diluted to a definite volume. For the *Control solution*, the same amount of specimen is dissolved, the limiting amounts of the suspected impurities are added, and the solution is then diluted to the same definite volume as the *Sample solution*. The flame photometer is set as directed in the general procedures and then adjusted to give an emission reading as near 100% transmittance as is possible with the *Control solution* at the wavelength specified for the particular impurity concerned. With the instrument settings left unchanged, the emission from the *Sample solution* is read at the same wavelength and at a specified background wavelength. The background reading is then used to correct the observed emission of the *Sample solution* for the emission due to the specimen and the solvent. The specimen being tested contains less than the specified limit of impurity if the difference between the observed background and total emissions for the *Sample solution* is less than the difference between the observed emissions for the *Control solution* and the *Sample solution* at the wavelength designated for the particular impurity.

CALCIUM IN REAGENTS

Standard calcium solution: Dissolve 250 mg of calcium carbonate in a mixture of 20 mL of water and 5 mL of diluted hydrochloric acid, and when solution is complete, dilute with water to 1 L. This solution contains 0.10 mg of calcium (Ca) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.03 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 422.7-nm calcium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 422.7 nm. Change the monochromator to the wavelength specified in the individual test procedure, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 422.7 nm and at the background wavelength is not greater than the difference between transmittances observed at 422.7 nm for the *Sample solution* and the *Control solution*.

POTASSIUM IN REAGENTS

Standard potassium solution: Dissolve 191 mg of potassium chloride in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of potassium (K) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

[NOTE—In testing calcium salts, use an oxyhydrogen burner.]

Set the slit-width control of a suitable flame photometer equipped with a red-sensitive detector at 0.1 mm, unless otherwise directed, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 766.5-nm potassium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 766.5 nm. Change the monochromator to 750 nm, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 766.5 and 750 nm is not greater than the difference between transmittances observed at 766.5 nm for the *Sample solution* and the *Control solution*.

SODIUM IN REAGENTS

Standard sodium solution: Dissolve 254 mg of sodium chloride in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of sodium (Na) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.01 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 589-nm sodium line, and record the transmittance. Without changing any of the instrument settings, record the transmittance for the emission of the *Sample solution* at 589 nm. Change the monochromator to 580 nm, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 589 and 580 nm is not greater than the difference between transmittances observed at 589 nm for the *Sample solution* and the *Control solution*.

STRONTIUM IN REAGENTS

Standard strontium solution: Dissolve 242 mg of strontium nitrate in a few mL of water, and dilute with water to 1 L. Dilute a portion of this solution with water in the ratio of 1:10 to obtain a concentration of 0.01 mg of strontium (Sr) per mL.

Procedure: Use the *Sample solution* and the *Control solution* prepared as directed in the individual test procedure.

Set the slit-width control of a suitable flame photometer at 0.03 mm, and set the selector switch at 0.1. Adjust the instrument to give the maximum emission with the *Control solution* at the 460.7-nm strontium line, and record the transmittance. Without changing any of the

instrument settings, record the transmittance for the emission of the *Sample solution* at 460.7 nm. Change the monochromator to the wavelength specified in the individual test procedure, and record the background transmittance for the background emission of the *Sample solution*: the difference between the transmittances for the *Sample solution* at 460.7 nm and at the background wavelength is not greater than the difference between transmittances observed at 460.7 nm for the *Sample solution* and the *Control solution*.

6.6 HEAVY METALS IN REAGENTS

STANDARD LEAD SOLUTION: Use standard lead solution TS. Each mL of this solution contains the equivalent of 0.01 mg of lead (Pb).

PROCEDURE: Unless otherwise directed, test for heavy metals as follows:

(a) If the heavy metals limit is 0.0005% (5 ppm), dissolve 6.0 g of the specimen in water to make 42 mL.

(b) If the heavy metals limit is 0.001% (10 ppm) or more, or in the event of limited solubility, use 4 g, and dissolve in water to make 40 mL, warming, if necessary, to aid solution.

For the control, transfer 7 mL of the solution from (a) to a color-comparison tube, and add a volume of *Standard lead solution* equivalent to the amount of lead permitted in 4 g of the reagent. Dilute with water to 35 mL, and add diluted acetic acid, or ammonia TS, until the pH is about 3.5, determined potentiometrically, then dilute with water to 40 mL, and mix. Transfer the remaining 35 mL of the solution from (a) to a color-comparison tube closely matching that used for the control, and add diluted acetic acid, or ammonia TS, until the pH is about 3.5, determined potentiometrically, then dilute with water to 40 mL, and mix. Then to each tube add 10 mL of hydrogen sulfide TS, mix, and compare the colors by viewing through the color-comparison tube downward against a white surface. The color in the test specimen is not darker than that of the control.

If the solution of the reagent is prepared as in (b), use for the control 10 mL of the solution, and add to it a volume of *Standard lead solution* equivalent to the amount of lead permitted in 2 g of the reagent. Dilute the remaining 30 mL of solution (b) with water to 35 mL, and proceed as directed in the preceding paragraph, beginning with "add diluted acetic acid, or ammonia TS," in the second sentence.

If the reagent to be tested for heavy metals is a salt of an aliphatic organic acid, substitute 1 N hydrochloric acid for the diluted acetic acid specified in the foregoing method.

6.7 INSOLUBLE MATTER IN REAGENTS

Dissolve the quantity of reagent specified in the test in 100 mL of water, heat to boiling unless otherwise directed, in a covered beaker, and warm on a steam bath for 1 h. Filter the hot solution through a tared sintered-glass crucible of fine porosity. Wash the beaker and the filter thoroughly with hot water, dry at 105°, cool in a desiccator, and weigh.

6.8 LOSS ON DRYING FOR REAGENTS

Determine as directed in [Loss on Drying \(731\)](#).

6.9 NITRATE IN REAGENTS

STANDARD NITRATE SOLUTION: Dissolve 163 mg of potassium nitrate in water, add water to make 100 mL, and dilute 10 mL of this solution with water to 1 L to obtain a solution containing the equivalent of 0.01 mg of nitrate (NO_3) per mL.

BRUCINE SULFATE SOLUTION: Dissolve 600 mg of brucine sulfate in 600 mL of nitrate-free, dilute sulfuric acid (2 in 3) that previously has been cooled to room temperature, and dilute with the acid to 1 L. [NOTE—Prepare the nitrate-free sulfuric acid by adding 4 parts of sulfuric acid to 1 part of water, heating the solution to dense fumes of sulfur trioxide, and cooling. Repeat the dilution and heating three or four times.]

SAMPLE SOLUTION: To the weight of sample specified in the individual reagent specification, dissolved in the designated volume of water, add *Brucine sulfate solution* to make 50 mL.

CONTROL SOLUTION: To a volume of *Standard nitrate solution* equivalent to the weight of nitrate (NO_3) specified in the individual reagent specification, add the weight of sample specified in the individual reagent specification, and then add *Brucine sulfate solution* to make 50 mL.

BLANK SOLUTION: Use 50 mL of *Brucine sulfate solution*.

PROCEDURE: Heat the *Sample solution*, *Control solution*, and *Blank solution* in a boiling water bath for 10 min, then cool rapidly in an ice bath to room temperature. Adjust a suitable spectrophotometer to zero absorbance at 410 nm with the *Blank solution*. Determine the absorbance of the *Sample solution*, note the result, and adjust the instrument to zero absorbance with the *Sample solution*. Determine the absorbance of the *Control solution*: the absorbance reading for the *Sample solution* does not exceed that for the *Control solution*.

6.10 NITROGEN COMPOUNDS IN REAGENTS

PROCEDURE: Unless otherwise directed, test for nitrogen compounds as follows: dissolve the specified quantity of test specimen in 60 mL of ammonia-free water in a Kjeldahl flask connected through a spray trap to a condenser, the end of which dips below the surface of 10 mL of 0.1 N hydrochloric acid. Add 10 mL of freshly boiled sodium hydroxide solution (1 in 10) and 500 mg of aluminum wire, in small pieces, to the Kjeldahl flask, and allow to stand for 1 h, protected from loss of and exposure to ammonia. Distill 35 mL, and dilute the distillate with water to 50 mL. Add 2 mL of freshly boiled sodium hydroxide solution (1 in 10), mix, add 2 mL of alkaline mercuric-potassium iodide TS, and again mix: the color produced is not darker than that of a control containing the amount of added N (as ammonium chloride) specified in the individual test procedure.

6.11 PHOSPHATE IN REAGENTS

STANDARD PHOSPHATE SOLUTION: Dissolve 143.3 mg of dried monobasic potassium phosphate (KH_2PO_4) in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate (PO_4) in each mL.

PHOSPHATE REAGENT A: Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.

PHOSPHATE REAGENT B: Dissolve 200 mg of *p*-methylaminophenol sulfate in 100 mL of water, and add 20 g of sodium bisulfite. Store this reagent in well-filled, tightly stoppered bottles, and use within 1 month.

PROCEDURE: [NOTE—The tests with the specimen and the control are made preferably in matched color-comparison tubes.] Dissolve the quantity of the reagent specified in the test, or the residue obtained after the prescribed treatment, in 20 mL of water, by warming, if necessary, add 2.5 mL of dilute sulfuric acid (1 in 7), and dilute with water to 25 mL. (If preferable, the test specimen or the residue may be dissolved in 25 mL of approximately 0.5 N sulfuric acid.) Then add 1 mL each of *Phosphate reagents A and B*, mix, and allow to stand at room temperature for 2 h. Compare any blue color produced with that produced in a control made with the same quantities of the same reagents as in the test with the specimen, and a volume of *Standard phosphate solution* equivalent to the quantity of phosphate (PO_4) designated in the reagent specifications.

6.12 RESIDUE ON IGNITION IN REAGENTS

PROCEDURE: Unless otherwise directed, determine the residue on ignition as follows: accurately weigh 1–2 g of the substance to be tested in a suitable crucible that previously has been ignited, cooled, and weighed. Ignite the substance, gently and slowly at first and then at a more rapid rate, until it is thoroughly charred, if organic in nature, or until it is completely volatilized, if inorganic in nature. If the use of sulfuric acid is specified, cool the crucible, add the specified amount of acid, and ignite the crucible gently until fumes no longer are evolved. Then ignite the crucible at $800 \pm 25^\circ$, cool in a suitable desiccator, and weigh. If the use of sulfuric acid is not specified, the crucible need not be cooled but can be ignited directly at $800 \pm 25^\circ$ once the charring or volatilization is complete. Continue the ignition until constant weight is attained, unless otherwise specified.

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. A muffle furnace may be used, if desired, and its use is recommended for the final ignition at $800 \pm 25^\circ$.

6.13 SULFATE IN REAGENTS

STANDARD SULFATE SOLUTION: Dissolve 181.4 mg of potassium sulfate (dried at 105° for 2 h) in water to make 1000 mL. This solution contains the equivalent of 0.10 mg of sulfate (SO_4) per mL.

PROCEDURE

Method I: Neutralize, if necessary, a solution of the quantity of the reagent or residue indicated in the test in 25 mL of water, or a solution prepared as directed in the test, with hydrochloric acid or with ammonia TS, litmus paper being used as the indicator, and add 1 mL of 1 N hydrochloric acid. Filter the solution, if necessary, through a filter paper previously washed with water, and add 2 mL of barium chloride TS. Mix, allow to stand for 10 min, and compare the turbidity, if any, with that produced in a control containing the same quantities of the same reagents used in the test and a quantity of *Standard sulfate solution* equivalent to the quantity of sulfate (SO_4) permitted in the test. Adjust the two solutions with water to the same volume before adding the barium chloride TS.

Method II: Heat the solution to boiling, prepared as directed in the individual test procedure, or the filtrate designated in the procedure, and add 5 mL of barium chloride TS. Then digest the solution on a steam bath for 2 h, and allow to stand overnight. If any precipitate is formed, filter the solution through paper, wash the residue with hot water, and transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Perform a blank determination concurrently with the test specimen determination, and subtract the weight of residue obtained from that obtained in the test specimen determination to obtain the weight of residue attributable to the sulfate content of the specimen.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
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