

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 hour, 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.

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 one 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 hours. 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.

Residue on Ignition in Reagents

PROCEDURE—Unless otherwise directed, determine the residue on ignition as follows: Weigh accurately 1 to 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 $800 \pm 25^\circ$.

Sulfate in Reagents

STANDARD SULFATE SOLUTION—Dissolve 181.4 mg of potassium sulfate (dried at 105° for 2 hours) 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 minutes, 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 to boiling the solution, 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 hours, 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.

REAGENT SPECIFICATIONS

Absolute Ether—See *Ethyl Ether, Anhydrous*.

Absorbent Cotton—Use *Purified Cotton* (USP monograph).

Acetal, $\text{C}_6\text{H}_{14}\text{O}_2$ —118.2—Use a suitable grade.

Acetaldehyde (*Ethanal; Acetic Aldehyde*), CH_3CHO —44.05 [75-07-0]—Colorless liquid. Miscible with water and with alcohol. Use ACS reagent grade.

Acetanilide (*Phenylacetamide; Antifebrin*), $\text{C}_8\text{H}_9\text{NO}$ —135.16 [103-84-4]—White, shiny crystals, usually in scales, or a white, crystalline powder. Is stable in air. Freely soluble in alcohol and in chloroform; soluble in boiling water, in ether, and in glycerin; slightly soluble in water.

Melting range (741): between 114° and 116° .

Reaction—Its saturated solution is neutral to litmus.

Loss on drying (731)—Dry it over sulfuric acid for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition (Reagent test): not more than 0.05%.

Acetic Acid (6 N Acetic Acid)—Use *Acetic Acid* (NF monograph) or prepare a suitable dilution of glacial acetic acid in such a way as to obtain a final concentration of acetic acid between 36.0% and 37.0%, by weight.

Acetic Acid, Diluted (1 N Acetic Acid)—Dilute 60.0 mL of glacial acetic acid with water to make 1000 mL.

Residue on evaporation—Evaporate 50 mL on a steam bath, and dry the residue at 105° for 2 hours: the residue weighs not more than 1 mg (0.002%).

Chloride (Reagent test)—Five mL shows not more than 0.01 mg of Cl (2 ppm).

Sulfate (Reagent test, *Method I*)—Ten mL shows not more than 0.5 mg of SO_4 (50 ppm).

Heavy metals (Reagent test)—Evaporate 20 mL on a steam bath to dryness. Add to the residue 2 mL of the acid, dilute with water to 25 mL, and add 10 mL of hydrogen sulfide TS: any brown color produced is not darker than that of a control containing 0.04 mg of added Pb and 2 mL of the diluted acetic acid (2 ppm).

Acetic Acid, Glacial, CH_3COOH —60.05 [64-19-7]—Use ACS reagent grade.

Acetic Anhydride (*Acetic Oxide; Acetyl Oxide*), $(\text{CH}_3\text{CO})_2\text{O}$ —**102.09** [108-24-7]—Use ACS reagent grade.

Acetone (*Propanone; Dimethylformaldehyde*), CH_3COCH_3 —**58.08** [67-64-1]—Use ACS reagent grade. [NOTE—For UV spectrophotometric determinations, use ACS reagent grade Acetone Suitable for Use in UV Spectrophotometry.]

Acetone, Anhydrous, CH_3COCH_3 —**58.08**—Use ACS reagent grade Acetone.

Acetone, Neutralized—To a suitable quantity of acetone add 2 or 3 drops of phenolphthalein TS and a sufficient amount of 0.02 or 0.01 N sodium hydroxide to produce a faint pink color. Prepare neutralized acetone just prior to use.

Acetonitrile (*Methyl Cyanide; Cyanomethane*), CH_3CN —**41.05** [75-05-8]—Use ACS reagent grade.

Acetonitrile, Spectrophotometric—Use ACS reagent grade, which meets also the requirements of the following test.

Spectral purity—Measure in a 1-cm cell between 250 nm and 280 nm, with a suitable spectrophotometer, against air as the blank: its absorbance is not more than 0.01.

Acetophenone (*Phenylethanone; Phenyl Methyl Ketone*), $\text{CH}_3\text{COC}_6\text{H}_5$ —**120.15** [98-86-2]—Liquid. Slightly soluble in water, freely soluble in alcohol and in ether.

Melting range (741): between 19° and 20°.

Refractive index (831): about 1.534 at 20°.

Specific gravity (841): about 1.03.

p-Aacetoluidide, $\text{C}_9\text{H}_{11}\text{NO}$ —**149.19** [103-89-9]—White to off-white powder.

Assay—Inject an appropriate volume into a gas chromatograph (see *Chromatography* (621)) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-μm layer of phase G2; the injection port temperature is maintained at 230°; the detector temperature is maintained at 300°; and the column temperature is maintained at 130° and programmed to rise 10° per minute to 280°. The area of the $\text{C}_9\text{H}_{11}\text{NO}$ peak is not less than 98.5% of the total peak area.

Melting range (741): between 145° and 151°.

Acetylacetone (*2,4-Pentanedione; Diacetyl methane*), $\text{C}_5\text{H}_8\text{O}_2$ —**100.12** [123-54-6]—Clear, colorless to slightly yellow, flammable liquid. Soluble in water; miscible with alcohol, with chloroform, with acetone, with ether, and with glacial acetic acid.

Assay—Not less than 98% of $\text{C}_5\text{H}_8\text{O}_2$, a suitable gas chromatograph equipped with a flame-ionization detector being used and helium being used as the carrier gas. The following conditions have been found suitable: a 3-mm × 1.83-m stainless steel column containing 10% phase G43 on support S1A; the injection port and detector temperatures are maintained at 250° and 310°, respectively; the column temperature is programmed to rise 8° per minute, from 50° to 220°.

Refractive index (831): between 1.4505 and 1.4525, at 20°.

Acetyl Chloride, CH_3COCl —**78.50** [75-36-5]—Clear, colorless liquid. Is decomposed by water and by alcohol. Miscible with benzene and with chloroform. Use ACS reagent grade.

Specific gravity (841): about 1.1.

Acetylcholine Chloride (*Trimethyllethanaminium Chloride; Acecoline*), $[\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3]\text{Cl}$ —**181.66** [60-31-1]—White, crystalline powder. Very deliquescent; very soluble in water; freely soluble in alcohol.

Melting range (741)—When previously dried at 110° in a capillary tube for 1 hour, it melts between 149° and 152°.

Reaction—A solution (1 in 10) is neutral to litmus.

Residue on ignition (Reagent test): negligible, from 200 mg.

Solubility in alcohol—A solution of 500 mg in 5 mL of alcohol is complete and colorless.

Percent of acetyl (CH_3CO)—Weigh accurately about 400 mg, previously dried at 105° for 3 hours, and dissolve in 15 mL of water in a glass-stoppered conical flask. Add 40.0 mL of 0.1 N sodium hydroxide VS, and heat on a steam bath for 30 minutes. Insert the stopper, allow to cool, add phenolphthalein TS, and titrate the excess alkali with 0.1 N sulfuric acid VS. Determine the exact normality of the 0.1 N sodium hydroxide by titrating 40.0 mL after it has been treated in the same manner as in the test. Each mL of 0.1 N sodium hydroxide is equivalent to 4.305 mg of CH_3CO . Between 23.2% and 24.2% is found.

Percent of chlorine (Cl)—Weigh accurately about 400 mg, previously dried at 105° for 3 hours, and dissolve in 50 mL of water in a glass-stoppered, 125-mL flask. Add with agitation 30.0 mL of 0.1 N silver nitrate VS, then add 5 mL of nitric acid and 5 mL of nitrobenzene, shake, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS: each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 19.3% and 19.8% of Cl is found.

3-Acetylthio-2-methylpropanoic Acid, $\text{C}_6\text{H}_{10}\text{O}_3\text{S}$ —**162.21**—Use a suitable grade.

[NOTE—A suitable grade is available as β -(Acetylmercapto)isobutyric Acid, catalog number 39059, from Senn Chemicals AG www.sennchem.com.]

N-Acetyl-L-tyrosine Ethyl Ester, $\text{C}_{13}\text{H}_{17}\text{NO}_4$ —**251.28**—Determine the suitability of the material as directed in the *Assay* under *Chymotrypsin* (USP monograph).

Acrylic Acid (*2-Propenoic Acid; Vinylformic Acid*), $\text{C}_3\text{H}_4\text{O}_2$ —**72.06** [79-10-7]—Colorless liquid. Miscible with water, with alcohol, and with ether.

Assay—Inject an appropriate specimen into a gas chromatograph (see *Chromatography* (621)), equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with a 1-μm layer of phase G2; the injection port temperature is maintained at 150°; the detector temperature is maintained at 300°; and the column temperature is maintained at 50° and programmed to rise 10° per minute to 200°. The area of the $\text{C}_3\text{H}_4\text{O}_2$ peak is not less than 99% of the total peak area.

Refractive index (831): between 1.419° and 1.423° at 20°.

Activated Alumina—See *Alumina, Activated*.

Activated Charcoal—See *Charcoal, Activated*.

Activated Magnesium Silicate—See *Magnesium Silicate, Activated*.

Adamantane, $\text{C}_{10}\text{H}_{16}$ —**136.23** [281-23-2]

Melting range (741): between 270° and 271°.

Adenine Sulfate, $(\text{C}_5\text{H}_5\text{N}_5)_2 \cdot \text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$ —**404.36**—White crystals or crystalline powder. Melts, after drying at 110°, at about 200° with some decomposition. One g dissolves in about 160 mL of water; less soluble in alcohol. Soluble in solutions of sodium hydroxide. It is not precipitated from solution by iodine TS or mercuric-potassium iodide TS, but a precipitate is produced with trinitrophenol TS.

Residue on ignition (Reagent test): negligible, from 100 mg.

Water—Dry it at 105° to constant weight: it loses not more than 10.0% of its weight.

Adipic Acid (*Hexanedioic Acid; 1,4-Butanedicarboxylic Acid*), $\text{C}_6\text{H}_{10}\text{O}_4$ —**146.14** [124-04-9]—Colorless to white, crystalline powder. Slightly soluble in water and in cyclohexane; soluble in alcohol, in methanol, and in acetone; practically insoluble in benzene and in petroleum benzin.

Assay—Weigh accurately about 0.3 g, and dissolve in 50 mL of alcohol. Add 25 mL of water, mix, and titrate with 0.5 N sodium hydroxide VS to a pH of 9.5. Perform a blank determination, and make any necessary correction. Each mL

of 0.5 N sodium hydroxide is equivalent to 36.54 mg of $C_6H_{10}O_4$. Not less than 98% is found.

Melting range (741): between 151° and 155°, but the range between beginning and end of melting does not exceed 2°.

Agar—Use *Agar* (NF monograph). When used for bacteriological purposes, it is to be dried to a water content of not more than 20%.

Agarose [9012-36-6]—Polysaccharide consisting of 1,3-linked β -D-galactopyranose and 1,4-linked 3,6-anhydro- α -L-galactopyranose. Use a suitable grade.

Air–Helium Certified Standard—A mixture of 1.0% air in industrial grade helium. It is available from most suppliers of specialty gases.

Albumin Bovine Serum [9048-46-8]—Almost colorless to faintly yellow powder. Not less than 95% pure. Solubility, 40 mg in 1 mL of water. Molecular weight is approximately 66,000. Use a suitable grade. Store between 2° and 8°.

Alcohol, Ethanol, Ethyl Alcohol, C_2H_5OH —46.07 [64-17-5]—Use a suitable grade with a content of NLT 92.3% and NMT 93.8%, by weight, corresponding to NLT 94.9% and NMT 96% by volume, at 15.56°.

Alcohol, 70 Percent, 80 Percent, and 90 Percent—Prepare by mixing alcohol and water in the proportions given, the measurements being made at 25°.

		Relative Proportions		Volume in ml of Alcohol, 94.9% v/v, Required for 100 mL
Percent by Volume of C_2H_5OH at 15.56°	Specific Gravity at 25°	Alcohol, mL	Water, mL	
70	0.884	38.6	15	73.7
80	0.857	45.5	9.5	84.3
90	0.827	51	3	94.8

The proportions of alcohol and water taken to prepare these or any other percentage (v/v) solutions may be determined as follows. Calculate the amount, in mL, of water to be mixed with 100 mL of alcohol taken by the formula:

$$[94.9(d/c) - 0.8096]100$$

in which 94.9 is the percentage (v/v) of C_2H_5OH in alcohol, 0.8096 is the specific gravity of 94.9% alcohol, d is the specific gravity, obtained from the Alcoholometric Table (see *Reference Tables*), of the solution containing c% (v/v) of C_2H_5OH , and 100 is the volume, in mL, of alcohol taken.

Alcohol, Absolute, C_2H_5OH —46.07—Use ACS reagent grade Ethyl Alcohol, Absolute.

Alcohol, Aldehyde-free—Dissolve 2.5 g of lead acetate in 5 mL of water, add the solution to 1000 mL of alcohol contained in a glass-stoppered bottle, and mix. Dissolve 5 g of potassium hydroxide in 25 mL of warm alcohol, cool the solution, and add it slowly, without stirring, to the alcohol solution of lead acetate. After 1 hour shake the mixture vigorously, allow it to stand overnight, decant the clear liquid, and recover the alcohol by distillation.

Alcohol, Amyl—See *Amyl Alcohol*.

Alcohol, Dehydrated (Absolute Alcohol), C_2H_5OH —46.07—Use ACS reagent grade Ethyl Alcohol, Absolute.

Alcohol, Dehydrated Isopropyl—See *Isopropyl Alcohol, Dehydrated*.

Alcohol, Denatured—It is ethyl alcohol to which has been added some substance or substances which, while allowing the use of the alcohol in most applications, renders it entirely unfit for consumption as a beverage. The most common denaturants used, either alone or in combination, are the following: methanol, camphor, aldehol, amyl alcohol, gasoline, isopropanol, terpineol, benzene, castor oil, acetone, nicotine, aniline dyes, ether, cadmium iodide, pyridine bases, sulfuric acid, kerosene, and diethyl phthalate. Use a suitable grade.

Alcohol, Diluted—Use *Diluted Alcohol* (NF monograph).

Alcohol, Isobutyl—See *Isobutyl Alcohol*.

Alcohol, Isopropyl—See *Isopropyl Alcohol*.

Alcohol, Methyl—See *Methanol*.

Alcohol, Neutralized—To a suitable quantity of alcohol add 2 or 3 drops of phenolphthalein TS and just sufficient 0.02 N or 0.1 N sodium hydroxide to produce a faint pink color. Prepare neutralized alcohol just prior to use.

Alcohol, n-Propyl—See *n-Propyl Alcohol*.

Alcohol, Secondary Butyl—See *Butyl Alcohol, Secondary*.

Alcohol, Tertiary Butyl—See *Butyl Alcohol, Tertiary*.

Aldehyde Dehydrogenase—A white powder. One mg contains not less than 2 enzyme activity units.

Assay—Transfer about 20 mg, accurately weighed, to a 200-mL volumetric flask, dissolve in 1 mL of water, dilute with an ice-cold solution of bovine serum albumin (1 in 100) to volume, and mix. Use this solution as the *Assay preparation*. Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol, and 40 mg of edetate disodium in 70 mL of water, adjust with citric acid monohydrate solution (2.1 in 10) to a pH of 9.0 ± 0.1, dilute with water to 100 mL, and mix to obtain a pH 9.0 buffer. Dissolve an accurately weighed quantity of β -nicotinamide adenine dinucleotide (β -NAD) in water to obtain a β -NAD solution having a known concentration of about 20 mg per mL. Pipet 0.1 mL of the *Assay preparation* into a 1-cm spectrophotometric cell. Pipet 0.1 mL of water into a second 1-cm spectrophotometric cell to provide the reagent blank. Add 2.5 mL of pH 9.0 buffer, 0.2 mL of β -NAD solution, and 0.1 mL of pyrazole solution (0.68 in 100) to each cell, and mix. Stopper the cells, and allow to stand for 2 minutes at 25 ± 1°. Add 0.01 mL of acetaldehyde solution (0.3 in 100) to each cell, and mix. Stopper the cells, and determine the absorbance of the solution obtained from the *Assay preparation* at a wavelength of 340 nm, using the solution obtained from the reagent blank as the reference. Calculate the change, ΔA , in absorbance per minute for the solution obtained from the *Assay preparation*, starting at the point when the absorbance and time relationship becomes linear. One enzyme activity unit is defined as the amount of enzyme that oxidizes 1 μ mol of acetaldehyde per minute when the test is conducted under the conditions described herein. Calculate the enzyme activity units in each mg of aldehyde dehydrogenase taken by the formula:

$$[(2.91)(200)/(6.3)(0.1)(1000)](\Delta A/W)$$

in which ΔA is as defined above and W is the weight, in g, of aldehyde dehydrogenase taken.

Alkaline Phosphatase Enzyme—See *Phosphatase Enzyme, Alkaline*.

Alkylphenoxypolyethoxyethanol—A nonionic surfactant. Use a suitable grade.

[NOTE]—A suitable grade is available commercially as “Triton X-100” from Sigma-Aldrich, www.sigma-aldrich.com.]

Alpha-Chymotrypsin—25 kDa [9004-07-3]—Use a suitable salt-free grade for protein sequencing.

[NOTE]—A suitable grade is available as catalog number 4423 from www.sigma-aldrich.com.]

Alpha-Cyclodextrin Hydrate (*Alpha-Schardinger Dextrin; Cyclohexaamylose*), $C_{36}H_{60}O_{30} \cdot xH_2O$ [51211-51-9]—Use a suitable grade with a content of NLT 98%.

[NOTE]—A suitable grade is available as catalog number 22729 from www.acros.com.]

Alpha-(2-(methylamino)ethyl)benzyl alcohol—Use a suitable grade.

Alphanaphthol—See *1-Naphthol*.

Alprenolol Hydrochloride, $C_{15}H_{23}NO_2 \cdot HCl$ —285.8 [13707-88-5]—Use a suitable grade.

Alum (*Ammonium Alum, Aluminum Ammonium Sulfate*), $AlNH_4(SO_4)_2 \cdot 12H_2O$ —453.33 [7784-26-1]—Large, colorless crystals or crystalline fragments or a white powder. Soluble in 7 parts of water and in about 0.5 part of boiling water; insoluble in alcohol. Use ACS reagent grade.

Ammonium Alum—See *Alum*.**Alumina**—See *Aluminum Oxide, Acid-washed*.**Alumina, Activated (Aluminum Oxide)**, [1344-28-1]—Use a suitable grade.**Alumina, Anhydrous (Aluminum Oxide; Alumina specially prepared for use in chromatographic analysis)** [1344-28-1]—A white or practically white powder, 80- to 200-mesh. It does not soften, swell, or decompose in water. It is not acid-washed. Store it in well-closed containers.**Aluminon (Aurin Tricarboxylic Acid, [tri]Ammonium Salt), C₂₂H₂₃N₃O₉**—**473.43** [569-58-4]—Yellowish-brown, glassy powder. Freely soluble in water. Use ACS reagent grade.**Aluminum, Al—At. Wt. 26.98154** [7429-90-5]—Use ACS reagent grade, which also meets the requirements of the following test.**Arsenic**—Place 750 mg in a generator bottle (see *Arsenic in Reagents* under *General Tests for Reagents*), omitting the peldorf of cotton. Add 10 mL of water and 10 mL of sodium hydroxide solution (3 in 10), and allow the reaction to proceed for 30 minutes: not more than a barely perceptible stain is produced on the mercuric bromide test paper.**Aluminum Oxide, Acid-Washed (Alumina specially prepared for use in chromatographic analysis)** [1344-28-1]—White or practically white powder or fine granules. Very hygroscopic. Store in tight containers.**pH of Slurry**—The pH of a well-mixed slurry of 5 g in 150 mL of ammonia-free and carbon dioxide-free water, after 10 minutes' standing, is between 3.5 and 4.5.**Loss on ignition**—Weigh accurately about 1 g, and ignite, preferably in a muffle furnace at 800° to 825°, to constant weight: it loses not more than 5.0% of its weight.**Silica**—Fuse 500 mg with 10 g of potassium bisulfate for 1 hour in a platinum crucible, cool, and dissolve in hot water: not more than a small amount of insoluble matter remains.**Suitability for chromatographic adsorption**—Dissolve 50 mg of o-nitroaniline in benzene to make 50.0 mL. Dilute 10 mL of the resulting solution with benzene to 100.0 mL, and mix (*Solution A*).**Weigh** quickly about 2 (± 0.005) g of specimen in a glass-stoppered weighing bottle, and rapidly transfer it to a dry, glass-stoppered test tube. Add 20.0 mL of *Solution A*, insert the stopper, shake vigorously for 3 minutes, and allow to settle.**Pipet** 10 mL of the clear supernatant into a 100-mL volumetric flask, dilute with benzene to volume, and mix (*Solution B*).**Determine** the absorbances of *Solutions A* and *B* at 395 nm, with a suitable spectrophotometer, using benzene as the blank. Calculate the quantity, in mg, adsorbed per g of test specimen by the formula:

$$[2(1 - A_B/A_A)]/W$$

in which A_A and A_B are the absorbances of *Solutions A* and *B*, respectively; and W is the weight, in g, of the aluminum oxide. Not less than 0.3 mg of o-nitroaniline is adsorbed for each g of the aluminum oxide.

Aluminum Potassium Sulfate, AlK(SO₄)₂ · 12H₂O—**474.39** [10042-67-1]—Use ACS reagent grade.**Amaranth, C₂₀H₁₁N₂Na₃O₁₀S₃**—**604.48** [915-67-3]—A deep brown or dark reddish-brown fine powder. Use a suitable grade.**Aminoacetic Acid (Glycine), NH₂CH₂COOH**—**75.07**

[56-40-6]—White, crystalline powder. Very soluble in water; slightly soluble in alcohol.

Nitrogen content (Reagent test)—Determine by the Kjeldahl method, using a test specimen previously dried at 105° for 2 hours: between 18.4% and 18.8% of N is found, corresponding to not less than 98.5% of C₂H₅NO₂.**Insoluble matter** (Reagent test): not more than 1 mg, from 10 g (0.01%).**Residue on ignition** (Reagent test): not more than 0.05%.**Chloride** (Reagent test)—One g shows not more than 0.1 mg of Cl (0.01%).**Sulfate** (Reagent test, *Method I*)—Two g shows not more than 0.1 mg of SO₄ (0.005%).**Heavy metals** (Reagent test): 0.001%, 5 mL of 1 N hydrochloric acid VS being used to acidify the solution of the test specimen.**Iron** (241)—One g, dissolved in 47 mL of water containing 3 mL of hydrochloric acid, shows not more than 0.01 mg of Fe (0.001%).**4-Aminoantipyrine, C₁₁H₁₃N₃O**—**203.24** [83-07-8]—Light yellow, crystalline powder. A 500-mg portion dissolves completely in 30 mL of water and yields a clear solution.**Melting range** (741): between 108° and 110°.**p-Aminobenzoic Acid**—See *Para-aminobenzoic Acid*.**2-Aminobenzonitrile (Anthranilonitrile), C₇H₆N₂**—**118.14** [1885-29-6]—Use 2-Aminobenzonitrile 98%.**Melting range** (741): between 49° and 52°.**4-Amino-6-chloro-1,3-benzenedisulfonamide, C₆H₈ClN₃O₄S₂**—**285.73** [121-30-2]—White powder. Insoluble in water and in chloroform; soluble in ammonia TS.**Residue on ignition** (Reagent test): not more than 2 mg from 2 g (0.1%).**Absorbance**—A 1 in 200,000 solution in methanol exhibits absorbance maxima at about 223 nm, 265 nm, and 312 nm. Its absorptivity (see *Spectrophotometry and Light-Scattering* (851)) at 265 nm is about 64.0.**4-Amino-2-chlorobenzoic Acid, C₆H₃Cl(NH₂)(COOH)**—**171.58** [2457-76-3]—White crystals or white, crystalline powder.**Melting range** (741): between 208° and 212°.**2-Amino-5-chlorobenzophenone, C₁₃H₁₀ClNO**—**231.68** [719-59-5]—Use USP 2-Amino-5-chlorobenzophenone RS.**7-Aminodesacetoxycephalosporanic Acid, C₈H₁₀N₂O₃S**—**214.2**—Light yellow powder.**Ordinary impurities** (466)—**Test solution:** 1 N ammonium hydroxide.**Standard solution:** 1 N ammonium hydroxide.**Eluant:** 0.5 N sodium chloride.**Visualization:** 1.**2-Aminoethyl Diphenylborinate**—See *Diphenylborinic Acid, Ethanolamine Ester*.**1-(2-Aminoethyl)piperazine, C₆H₁₅N₃**—**129.20** [140-31-8]—Viscous, colorless liquid.**Assay**—Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm × 30-m capillary column coated with G2. The injection port temperature is maintained at 280°; the column temperature is maintained at 180° and programmed to rise 10° per minute to 280° and held there for 10 minutes. The detector temperature is maintained at 300°. The area of the main peak is not less than 97% of the total peak area.**Refractive index** (831): between 1.4978 and 1.5010 at 20°.**Aminoguanidine Bicarbonate (Aminoguanidine Hydrogen Carbonate), CH₆N₄ · H₂CO₃**—**136.11** [2582-30-1]—White powder.**Assay**—Dissolve about 34 mg, accurately weighed, in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 13.61 mg of CH₆N₄ · H₂CO₃. Not less than 98.5% is found.**Melting point** (741): about 170°, with decomposition.**2-Aminoheptane (2-Heptylamine; 1-Methylhexylamine), C₇H₁₇N**—**115.22** [123-82-0]—Use a suitable grade with a content of not less than 99%.

N-Aminohexamethyleneimine (*N*-Aminohomopiperidine, 1-Aminohomopiperidine), $C_6H_{14}N_2$ —**114.19** [5906-35-4]—Colorless liquid.

Assay—Inject an appropriate specimen into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a flame-ionization detector, helium being used as the carrier gas. The following conditions have been found suitable: a 0.25-mm \times 30-m capillary column coated with G2. The injection port temperature is maintained at 180°; the column temperature is maintained at 80° and programmed to rise 10° per minute to 230° and then maintained at 230° for 5 minutes. The detector temperature is maintained at 300°. The area of the main peak is not less than 95% of the total peak area.

Refractive index (831): between 1.4840 and 1.4860 at 20°.

4-Amino-3-hydroxy-1-naphthalenesulfonic Acid, $C_{10}H_9NO_4S$ —**239.25** [116-63-2]—Light purple powder. Use ACS reagent grade.

8-Amino-6-methoxyquinoline (*6*-Methoxy-8-aminoquinoline), $C_{10}H_{10}N_2O$ —**174.2** [90-52-8]—Use a suitable grade with a content of not less than 98.0%.

[NOTE—A suitable grade is available from www.3bmedical-systems.com, catalog number 3B3-002598.]

1,2,4-Aminonaphtholsulfonic Acid, $C_{10}H_9NO_4S$ —**239.25**—White to slightly brownish pink powder. Sparingly soluble in water.

Sensitiveness—Dissolve 100 mg in 50 mL of freshly prepared sodium bisulfite solution (1 in 5), warming if necessary to effect solution, and filter. Add 1 mL of the filtrate to a solution prepared by adding 2 mL of dilute sulfuric acid (1 in 6) and 1 mL of *Phosphate Reagent A* (see Reagent test) to 20 mL of a 1 in 100 dilution of *Standard Phosphate Solution* (see Reagent test): a distinct blue color develops within 5 minutes.

Solubility in sodium carbonate solution—Dissolve 100 mg in 3 mL of sodium carbonate TS, and add 17 mL of water: not more than a trace remains undissolved.

Residue on ignition (Reagent test)—To 1 g add 0.5 mL of sulfuric acid, and ignite at $800 \pm 25^\circ$ to constant weight: the residue weighs not more than 5 mg (0.5%).

Sulfate (Reagent test, *Method I*)—Heat 500 mg with a mixture of 25 mL of water and 2 drops of hydrochloric acid on a steam bath for 10 minutes. Cool, dilute with water to 200 mL, and filter: 20 mL of the filtrate shows not more than 0.25 mg of SO_4 (0.5%).

2-Aminophenol (*o*-Aminophenol; 2-Hydroxyaniline), C_6H_7NO —**109.13** [95-55-6]—Off-white powder. Use a suitable grade with a content of not less than 99%.

m-Aminophenol (*3*-Amino-1-Hydroxybenzene), C_6H_7NO —**109.13** [591-27-5]—Cream-colored to pale yellow flakes. Sparingly soluble in cold water; freely soluble in hot water, in alcohol, and in ether.

Assay—Dissolve about 1.5 g, accurately weighed, in about 400 mL of water in a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to an iodine flask, add 50.0 mL of 0.1 N bromine VS, dilute with 50 mL of water, add 5 mL of hydrochloric acid, and immediately insert the stopper in the flask. Shake for 1 minute, allow to stand for 2 minutes, and add 5 mL of potassium iodide TS through the slightly loosened stopper. Shake thoroughly, allow to stand for 5 minutes, remove the stopper, and rinse it and the neck of the flask with 20 mL of water, adding the rinsing to the flask. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. From the volume of 0.1 N sodium thiosulfate used, calculate the volume, in mL, of 0.1 N bromine consumed by the test specimen. Each mL of 0.1 N bromine is equivalent to 1.819 mg of C_6H_7NO : not less than 99.5% is found.

Melting range (741): between 121° and 123°.

Loss on drying (731)—Dry it over calcium chloride for 4 hours: the loss in weight is negligible.

Residue on ignition (Reagent test): negligible, from 2 g.

p-Aminophenol (*p*-Hydroxyaniline), C_6H_7NO —**109.13** [123-30-8]—Fine, yellowish, crystalline powder. Slightly soluble in water and in alcohol. Use a suitable grade with a content of not less than 99%.

3-Amino-1-propanol, $H_2N(CH_2)_3OH$ —**75.11** [156-87-6]—Liquid.

Boiling range (Reagent test): between 184° and 188°.

Refractive index (831): between 1.461 and 1.463 at 20°.

3-Aminopropionic Acid (β -Alanine), $NH_2CH_2CH_2COOH$ —**89.09** [107-95-9]—Use a suitable grade.

3-Aminosalicylic Acid, $C_7H_7NO_3$ —**153.14** [570-23-0]—Tan-grey powder. Use a suitable grade with a content of not less than 97%.

Ammonia Detector Tube—A fuse-sealed glass tube so designed that gas may be passed through it and containing suitable absorbing filters and support media for the indicator bromophenol blue.

Measuring range: 5 to 70 ppm.

[NOTE—Available from Draeger Safety, Inc., www.draeger.com, or from Gastec Corp., www.gastec.co.jp, distributed in the USA by www.nexteq.com.]

Ammonia Solution, Diluted—Use *Ammonia TS*.

Ammonia Water, 25 Percent [1336-21-6]—Use a suitable grade.

Ammonia Water, Stronger (*Ammonium Hydroxide*)—[1336-21-6]—Use ACS reagent grade *Ammonium Hydroxide*.

Ammonium Acetate, $NH_4C_2H_3O_2$ —**77.08** [631-61-8]—Use ACS reagent grade.

Ammonium Bicarbonate (*Ammonium Hydrogen Carbonate*), NH_4HCO_3 —**79.06** [1066-33-7]—Use a suitable grade with a content of NLT 99.0%.

Ammonium Bisulfate (*Ammonium Hydrogen Sulfate*), NH_4HSO_4 —**115.11** [7803-63-6]—White crystals. Freely soluble in water; practically insoluble in alcohol, in acetone, and in pyridine.

Assay—Dissolve about 300 mg, accurately weighed, in 50 mL of a mixture of water and alcohol (25:25). Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 11.51 mg of NH_4HSO_4 . Not less than 98% is found.

Ammonium Bromide, NH_4Br —**97.94** [12124-97-9]—Use ACS reagent grade.

Ammonium Carbonate (*Hartshorn Salt*) [506-87-6]—Use ACS reagent grade.

Ammonium Chloride (*Salmiac*), NH_4Cl —**53.49** [12125-02-9]—Use ACS reagent grade.

Ammonium Citrate, Dibasic (*Citric Acid Diammonium Salt*), $(NH_4)_2H_2C_6O_7$ —**226.18** [3012-65-5]—Use ACS reagent grade.

Ammonium Dihydrogen Phosphate—See *Ammonium Phosphate, Monobasic*.

Ammonium Fluoride, NH_4F —**37.04** [12125-01-8]—Use ACS reagent grade.

Ammonium Formate (*Formic Acid Ammonium Salt*), CH_5NO_2 —**63.06** [540-69-2]—Use a suitable grade.

Ammonium Hydroxide (*Ammonium Aqueous*), [1336-21-6]—Use ACS reagent grade.

Ammonium Hydroxide, 6 N—Prepare by diluting 400 mL of *Ammonia Water, Stronger* (see *Reagents* section) with water to make 1000 mL.

Ammonium Molybdate, $(NH_4)_6Mo_7O_24 \cdot 4H_2O$ —**1235.86** [12054-85-2]—Use ACS reagent grade.

Ammonium Nitrate, NH_4NO_3 —**80.04** [6484-52-2]—Use ACS reagent grade.

Ammonium Oxalate, $(NH_4)_2C_2O_4 \cdot H_2O$ —**142.11** [6009-70-7]—Use ACS reagent grade.

Ammonium Persulfate (*Ammonium Peroxydisulfate*), $(NH_4)_2S_2O_8$ —**228.20** [7727-54-0]—Use ACS reagent grade *Ammonium Peroxydisulfate*.

Ammonium Phosphate, Dibasic (*Diammonium Hydrogen Phosphate*), $(\text{NH}_4)_2\text{HPO}_4$ —132.06 [7783-28-0]—Use ACS reagent grade.

Ammonium Phosphate, Monobasic (*Ammonium Dihydrogen Phosphate*), $\text{NH}_4\text{H}_2\text{PO}_4$ —115.03 [7722-76-1]—Use ACS reagent grade.

Ammonium Pyrrolidinedithiocarbamate (*1-pyrrolidine-carbodithioic acid, ammonium salt*), $\text{C}_5\text{H}_{12}\text{N}_2\text{S}_2$ —164.29 [5108-96-3]—Use a suitable grade.

Ammonium Reineckate (*Reinecke Salt*), $\text{NH}_4[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4] \cdot \text{H}_2\text{O}$ —354.44 [13573-16-5]—Dark red crystals or red, crystalline powder. Moderately soluble in cold water; more soluble in hot water. Gradually decomposes in solution.

Sensitivity—Dissolve 50 mg in 10 mL of water. Add 0.2 mL of the solution to 1 mL of a solution of 10 mg of chlorine chloride in 20 mL of water, and shake gently: a distinct precipitate forms within 5 to 10 seconds.

Ammonium Sulfamate, $\text{NH}_4\text{OSO}_2\text{NH}_2$ —114.13 [7773-06-0]—Use ACS reagent grade.

Ammonium Sulfate, $(\text{NH}_4)_2\text{SO}_4$ —132.14 [7783-20-2]—Use ACS reagent grade.

Ammonium Thiocyanate (*Ammonium Rhodanide*), NH_4SCN —76.12 [1762-95-4]—Use ACS reagent grade.

Ammonium Vanadate (*Ammonium Metavanadate*), NH_4VO_3 —116.98 [7803-55-6]—White, crystalline powder. Slightly soluble in cold water; soluble in hot water and in dilute ammonia TS.

Assay—Weigh accurately about 500 mg, transfer to a suitable container, add 30 mL of water and 2 mL of dilute sulfuric acid (1 in 4), swirl to dissolve, and pass sulfur dioxide gas through the solution until reduction is complete and the solution is bright blue in color. Boil gently while passing a stream of carbon dioxide through the solution to remove any excess sulfur dioxide, then cool, and titrate with 0.1 N potassium permanganate VS. Each mL of 0.1 N potassium permanganate consumed is equivalent to 11.7 mg of NH_4VO_3 . Not less than 98.0% is found.

Solubility in ammonium hydroxide—Dissolve 1 g in a mixture of 3 mL of ammonium hydroxide and 50 mL of warm water: the solution is clear and colorless.

Carbonate—To 500 mg add 1 mL of water and 2 mL of diluted hydrochloric acid: no effervescence is produced.

Chloride—Dissolve 250 mg in 40 mL of hot water, add 2 mL of nitric acid, and allow to stand for 1 hour. Filter, and to the filtrate add 0.5 mL of silver nitrate TS: any turbidity produced does not exceed that of a blank containing 0.5 mg of added Cl (0.2%).

Sulfate—Dissolve 500 mg in 50 mL of hot water, and add 2 mL of diluted hydrochloric acid and 1.5 g of hydroxylamine hydrochloride. Heat at 60° for 3 minutes, filter, cool, and add to the filtrate 2 mL of barium chloride TS: no turbidity or precipitate is produced within 30 minutes.

Amyl Acetate (*Isoamyl Acetate*), $\text{CH}_3\text{CO}_2\text{C}_5\text{H}_{11}$ —130.18 [2308-18-1]—Clear, colorless liquid. Slightly soluble in water. Miscible with alcohol, with amyl alcohol, with benzene, and with ether.

Specific gravity (841): about 0.87.

Boiling range (Reagent test, Method I): not less than 90%, between 137° and 142°.

Solubility in diluted alcohol—A 1.0-mL portion dissolves in 20 mL of diluted alcohol to form a clear solution.

Acidity—Add 5.0 mL to 40 mL of neutralized alcohol, and, if the pink color is discharged, titrate with 0.10 N sodium hydroxide: not more than 0.20 mL is required to restore the pink color (about 0.02% as CH_3COOH).

Water—A 5-mL portion gives a clear solution with 5 mL of carbon disulfide.

Amyl Alcohol (*Isoamyl Alcohol*), $\text{C}_5\text{H}_{11}\text{OH}$ —88.15 [598-75-4]—Use ACS reagent grade Isopentyl Alcohol.

tert-Amyl Alcohol, $\text{C}_5\text{H}_{12}\text{O}$ —88.15 [75-85-4]—Clear, colorless, flammable, volatile liquid.

Specific gravity (841): about 0.81.

Boiling range (Reagent test): not less than 95%, between 100° and 103°.

Residue on evaporation—Evaporate 50 mL (40 g) on a steam bath, and dry at 105° for 1 hour: the residue weighs not more than 1.6 mg (0.004%).

Acids and esters—Dilute 20 mL with 20 mL of alcohol, add 5.0 mL of 0.1 N sodium hydroxide VS, and reflux gently for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.1 N hydrochloric acid VS: not more than 0.75 mL of the 0.10 N sodium hydroxide is consumed, correction being made for the amount consumed in a blank (0.06% as amyl acetate).

Aldehydes—Shake 5 mL with 5 mL of potassium hydroxide solution (30 in 100) in a glass-stoppered cylinder for 5 minutes, and allow to separate: no color develops in either layer.

α -Amylase—Use a suitable grade. It can be from vegetal or animal or microbiological origin.

(E)-Anethole (*1-Methoxy-4-(1-propenyl)benzene*), $\text{C}_{10}\text{H}_{12}\text{O}$ —148.20 [4180-23-8]—Use a suitable grade of transisomer.

Anhydrous Alumina—See *Alumina, Anhydrous*.

Anhydrous Barium Chloride—See *Barium Chloride, Anhydrous*.

Anhydrous Calcium Chloride—See *Calcium Chloride, Anhydrous*.

Anhydrous Cupric Sulfate—See *Cupric Sulfate, Anhydrous*.

Anhydrous Dibasic Sodium Phosphate—See *Sodium Phosphate, Dibasic, Anhydrous*.

Anhydrous Magnesium Perchlorate—See *Magnesium Perchlorate, Anhydrous*.

Anhydrous Magnesium Sulfate—See *Magnesium Sulfate, Anhydrous*.

Anhydrous Methanol—See *Methanol, Anhydrous*.

Anhydrous Potassium Carbonate—See *Potassium Carbonate, Anhydrous*.

Anhydrous Sodium Acetate—See *Sodium Acetate, Anhydrous*.

Anhydrous Sodium Carbonate—See *Sodium Carbonate, Anhydrous*.

Anhydrous Sodium Sulfate—See *Sodium Sulfate, Anhydrous*.

Anhydrous Sodium Sulfite—See *Sodium Sulfite, Anhydrous*.

Aniline, $\text{C}_6\text{H}_5\text{NH}_2$ —93.13 [62-53-3]—Use ACS reagent grade.

Aniline Blue (*Certified Biological Aniline Blue*) [8004-91-9]—A water-soluble dye consisting of a mixture of the tri-sulfonates of triphenylpararosaniline and of diphenylrosaniline.

Aniline Sulfate, $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot \text{H}_2\text{SO}_4$ —284.33 [542-16-5]—Use a suitable grade.

Anion-Exchange Resin, Chloromethylated Polystyrene-Divinylbenzene—Strongly basic, cross-linked resin containing quaternary ammonium groups. It consists of small, moist, yellow beads having a characteristic amine odor. It is available in the chloride form which can be converted to the hydroxide form by regeneration with sodium hydroxide solution (1 in 4). For satisfactory regeneration a contact time of about 25 minutes is required, after which it must be washed with water until neutral. Suitable for use in column chromatography.

[**NOTE**—A suitable resin is “Amberlite IRA-400,” available from Sigma-Aldrich, www.sigma-aldrich.com.]

Anion-Exchange Resin, Strong, Lightly Cross-Linked, in the Chloride Form—Use a suitable grade.

[**NOTE**—A suitable resin is “AG 1-X4, catalog number 140-1331,” produced by BioRad Laboratories, www.biорad.com.]

Anion-Exchange Resin, Styrene-Divinylbenzene—Strongly basic, cross-linked resin containing quaternary ammonium groups and about 8% of divinylbenzene. It is available in the chloride form in the 50- to 100-, 100- to 200-,

and 200- to 400-mesh sizes. It can be converted to the hydroxide form by regeneration with a sodium hydroxide solution (5 in 100). Insoluble in water, in methanol, and in acetonitrile. Suitable for use in column chromatography.

[NOTE—A suitable resin is Dowex 1X8, produced by Dow Chemical Co. (www.dow.com) and available through Sigma-Aldrich (www.sigma-aldrich.com).]

Anion-Exchange Resin, 50- to 100-Mesh, Styrene-Divinylbenzene—Strongly basic, cross-linked resin containing quaternary ammonium groups and about 4% of divinylbenzene. It consists of tan-colored beads that may be relatively free flowing. It is available in the chloride form which can be converted to the hydroxide form by regeneration with a sodium hydroxide solution (5 in 100). For satisfactory regeneration a contact time of at least 30 minutes is required after which it must be washed free of excess alkali. Insoluble in water, in methanol, and in acetonitrile. Suitable for use in column chromatography.

[NOTE—A suitable resin is "Dowex 1X4," available from Sigma-Aldrich, www.sigma-aldrich.com.]

Moisture content of fully regenerated and expanded resin—Transfer 10 to 12 mL of the resin (as received) to a flask, and convert it completely to the chloride form by stirring with 150 mL of hydrochloric acid (5 in 100) for not less than 30 minutes. Decant the acid, and wash the resin in the same manner with distilled water until the wash water is neutral to litmus.

Transfer 5 to 7 mL of the regenerated resin to a glass filtering crucible, and remove only the excess surface water by very careful suction filtration. Transfer the conditioned, dried resin to a tared weighing bottle, and weigh. Dry in a vacuum oven at 100° to 105° and at a pressure of 50 mm of mercury for 16 hours. Transfer from the vacuum oven to a desiccator, and cool to room temperature. Reweigh. The loss in weight is between 50% and 65%.

Total new volume capacity—Transfer 2.5 to 3 mL of the conditioned, undried (See *Moisture content*, above) resin to a 5-mL graduated cylinder, and fill it with water. Remove any air bubbles from the resin bed with a stainless steel wire, and settle the resin to its minimum volume by tapping the graduated cylinder. Record the volume of the resin.

Transfer the resin with 100 mL of water to a 250-mL flask. Add 2 mL of sulfuric acid, heat to 70° to 80°, and hold at that temperature for 5 minutes with occasional stirring (do not boil). Cool to room temperature, and add 2.5 mL of nitric acid (1 in 2), 2 mL of ferric ammonium sulfate TS, and 0.20 mL of 0.1 N ammonium thiocyanate. Titrate with 0.1 N silver nitrate VS until the solution turns colorless, and add a measured excess (1 to 5 mL). Heat to boiling to coagulate the silver chloride precipitate. Cool to room temperature, add 10 mL of nitrobenzene, shake vigorously, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS.

$$(\text{net mL AgNO}_3 \times \text{N}) / (\text{mL of resin}) = \text{mEq/mL}$$

The total exchange capacity of the regenerated, wet resin is more than 1.0 mEq per mL.

Wet screen analysis—The purpose of this test is to identify properly the mesh size of the resin. To obtain an accurate screen analysis requires special apparatus and technique.

Add 150 mL of resin to 200 mL of distilled water in an appropriate bottle, and allow it to stand at least 4 hours to completely swell the resin.

Transfer by means of a graduated cylinder 100 mL of settled and completely swollen resin to the top screen of a series (20-, 50-, 100-mesh) of 20.3-cm brass screens. Thoroughly wash the resin on each screen with a stream of distilled water until the resin is completely classified, collecting the wash water in a suitable container. Wash the beads remaining on the respective screens back into the 100-mL cylinder, and record the volume of settled resin on each screen: not less than 80% of the resin is between 50- and 100-mesh.

p-Anisaldehyde (*4-Methoxybenzaldehyde*), C₈H₈O₂—

136.15 [123-11-5]—Clear, colorless liquid.

Boiling temperature: 248°.

Density: between 1.119 and 1.123.

Refractive index $\langle 831 \rangle$: between 1.5725 and 1.5730 at 20°.

p-Anisidine, C₇H₉NO—**123.06** [104-94-9]—Brown crystals. Use a suitable grade.

Anisole, CH₃OC₆H₅—**108.14** [100-66-3]—Colorless liquid.

Assay—Inject an appropriate specimen (about 0.5 μ L) into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a flame-ionization detector, nitrogen being used as the carrier gas. The following conditions have been found suitable: a 30-m capillary column is coated with phase G3; the injection port and detector temperatures are maintained at 140° and 300°, respectively; the column temperature is maintained at 70° and programmed to rise 10° per minute to 170°. The area of the anisole peak is not less than 99% of the total peak area.

Refractive index $\langle 831 \rangle$: 1.5160 at 20°.

Anthracene, C₁₄H₁₀—**178.23** [120-12-7]—White to off-white crystals or platelets. Darkens in sunlight. Insoluble in water; sparingly soluble in alcohol, in benzene, and in chloroform.

Melting range $\langle 741 \rangle$: between 215° and 218°.

Anthrone, C₁₄H₁₀O—**194.23** [90-44-8]—Use ACS reagent grade.

Anti-D Reagent—The reagent can be monoclonal (low protein) or polyclonal (high-protein) and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results. Note that this reagent is different from Anti-D (Rh₀) Reagent. Consult manufacturer's package insert to ensure that the reagent is suitable for the Weak Anti-D test and does not contain other antibodies that will react when antihuman immunoglobulin is added.

[NOTE—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Anti-D (Rh₀) Reagent—The reagent can be monoclonal or polyclonal and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration for use in microplate tests. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results. Note that this reagent is different from Anti-D Reagent. Consult manufacturer's package insert to ensure that it is Anti-D (Rh₀) Reagent and not Anti-D Reagent.

[NOTE—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Antifoam Reagent—A 10% silicone-glycol emulsion, white in appearance. Nonionic but miscible with cool water.

[NOTE—A suitable grade is available as "Antifoam Reagent," catalog number 2210, from Dow Corning Corporation, www.dowcorning.com.]

Antihuman Globulin Reagent—The reagent can be poly-specific or anti-immunoglobulin (Anti-IgG) and must be obtained from manufacturers or suppliers licensed by the Center for Biologics Evaluation and Research, Food and Drug Administration. The use of reagents from an unlicensed manufacturer or supplier may invalidate the results.

[NOTE—There are many manufacturers and suppliers of these reagents that are licensed by the Center for Biologics

Evaluation and Research, Food and Drug Administration. Some examples of licensed manufacturers or suppliers are the following: Gamma Biologics, Houston, TX; and Ortho Diagnostics, Raritan, NJ.]

Antimony Pentachloride, $SbCl_5$ —**299.02** [7647-18-9]—Clear, reddish-yellow, oily, hygroscopic, caustic liquid. Fumes in moist air and solidifies by absorption of one molecule of water. Is decomposed by water; soluble in dilute hydrochloric acid and in chloroform. Boils at about 92° at a pressure of 30 mm of mercury and has a specific gravity of about 2.34 at 25° .

[**CAUTION**—Antimony pentachloride causes severe burns, and the vapor is hazardous.]

Assay ($SbCl_5$)—Accurately weigh a glass-stoppered, 125-mL flask, quickly introduce about 0.3 mL of the test specimen, and reweigh. Dissolve with 20 mL of diluted hydrochloric acid (1 in 5), and add 10 mL of potassium iodide solution (1 in 10) and 1 mL of carbon disulfide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. The brown color will gradually disappear from the solution, and the last traces of free iodine will be collected in the carbon disulfide, giving a pink color. When this pink color disappears the endpoint has been reached. Each mL of 0.1 N sodium thiosulfate is equivalent to 14.95 mg of $SbCl_5$; not less than 99.0% of $SbCl_5$ is found.

Sulfate (Reagent test, *Method II*)—Dissolve 4.3 mL (10 g) in the minimum volume of hydrochloric acid, dilute with water to 150 mL, neutralize with ammonium hydroxide, and filter. To the filtrate add 2 mL of hydrochloric acid; the solution, 10 mL of barium chloride TS being used, yields not more than 1.3 mg of residue, correction being made for a complete blank test (0.005%).

Arsenic—Add 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid to 100 mg of specimen dissolved in 5 mL of hydrochloric acid. Mix, transfer to a color-comparison tube, and allow to stand for 30 minutes. Any color in the solution of the specimen should not be darker than that in a control containing 0.02 mg of arsenic (As), which has been treated in the same manner as the test specimen, when viewed downward over a white surface (0.02% of As).

Substances not precipitated by hydrogen sulfide (as SO_4)—Dissolve 0.90 mL (2 g) in 5 mL of hydrochloric acid, and dilute with 95 mL of water. Precipitate the antimony completely with hydrogen sulfide, allow the precipitate to settle, and filter, being careful not to transfer much of the precipitate to the filter paper. (Retain the precipitate.) To 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate in a tared porcelain crucible to dryness, and ignite at $800 \pm 25^\circ$ for 15 minutes. (Retain the residue.) The weight of the ignited residue should not be more than 0.0010 g greater than the weight obtained in a complete blank test (0.10%).

Iron <241>—To the residue from the test for *Substances not precipitated by hydrogen sulfide* add 2 mL of hydrochloric acid and 5 drops of nitric acid, and evaporate on a steam bath to dryness. Take up the residue in 2 mL of hydrochloric acid, and dilute with water to 47 mL; the solution shows not more than 0.01 mg of Fe (0.001%).

Other heavy metals (as Pb)—Dissolve the precipitate on the filter paper from the test for *Substances not precipitated by hydrogen sulfide*, with 75 mL of a solution containing 6 g of sodium sulfide and 4 g of sodium hydroxide dissolved in and diluted with water to 100 mL. Collect the filtrate in the original flask containing the remainder of the sulfide precipitate. Warm the solution to dissolve the soluble sulfides, and allow the insoluble sulfides to settle. Filter, wash thoroughly with hydrogen sulfide TS, and dissolve any precipitate remaining on the filter paper with 10 mL of hot diluted hydrochloric acid. Dilute the filtrate with water to 50 mL. Neutralize a 25-mL portion of this solution with 1 N sodium hydroxide, and add 1 mL of 1 N acetic acid and 10 mL of hydrogen sulfide TS. Any brown color should not exceed that produced by 0.05 mg of lead ion in an equal volume

of solution containing 1 mL of 1 N acetic acid and 10 mL of hydrogen sulfide TS (0.005%).

Antimony Trichloride (*Antimonous Chloride*), $SbCl_3$ —**228.12** [10025-91-9]—Use ACS reagent grade.

Antithrombin III—Antithrombin III human (heparin cofactor, factor II_a inhibitor, and factor X_a inhibitor) is a serine protease inhibitor. It is a glycoprotein having a molecular weight of 58,000 Da.

One Antithrombin III Unit is the amount found in 1 mL of normal human plasma. The potency of antithrombin III is not less than 4.0 Antithrombin III Units per mg of protein when tested in the presence of heparin. It exhibits 90% homogeneity when tested by SDS-PAGE.

Antithrombin III for test or assay purposes contains no detectable heparin. Test as follows. To a solution containing 1 Antithrombin III Unit per mL, add 1 μ L of toluidine blue solution. In the presence of heparin, the color changes from blue to purple.

Aprobarbital, $C_{10}H_{14}N_2O_3$ —**210.23** [77-02-1]—Fine, white crystalline powder. Slightly soluble in cold water; soluble in alcohol, in chloroform, and in ether.

Assay—Dissolve about 200 mg, previously dried at 105° for 2 hours and accurately weighed, in 20 mL of dimethylformamide in a 100-mL conical flask. Add 4 drops of thymol blue solution (1 in 200 in methanol), and titrate with 0.1 N lithium methoxide VS using a 10-mL buret, a magnetic stirrer, and a cover for the flask to protect against atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 21.02 mg of $C_{10}H_{14}N_2O_3$. Between 98.5% and 101.0% of $C_{10}H_{14}N_2O_3$ is found.

Melting range <741>: between 140° and 143° .

Arsenazo III Acid, $C_{22}H_{18}As_2N_4O_{14}S_2$ —**776.38** [1668-00-4]—Brown powder. Stable in air. Store at room temperature in a dry area.

Melting temperature <741>: greater than 320° .

Arsenic Trioxide, As_2O_3 —**197.84** [1327-53-3]—Use ACS reagent grade.

[**NOTE**—Arsenic Trioxide of a quality suitable as a primary standard is available from the National Institute of Standards and Technology, Office of Standard Reference Materials, www.nist.gov, as standard sample No. 83.]

L-Asparagine (*L-2-Aminosuccinic Acid*), $COOCH(NH_2)CH_2CONH_2 \cdot H_2O$ —**150.13** [70-47-3]—Colorless crystals. One g dissolves in 50 mL of water; soluble in acids and in alkalies; insoluble in alcohol and in ether. Its neutral or alkaline solutions are levorotatory; its acid solutions are dextrorotatory.

Specific rotation <781>: between $+31^\circ$ and $+33^\circ$, determined in a solution in diluted hydrochloric acid containing the equivalent of 5 g (on the anhydrous basis, as determined by drying at 105° for 5 hours) in each 100 mL.

Residue on ignition (Reagent test): not more than 0.1%.

Chloride (Reagent test)—One g shows not more than 0.03 mg of Cl (0.003%).

Sulfate (Reagent test, *Method I*)—One g shows not more than 0.05 mg of SO_4 (0.005%).

Heavy metals (Reagent test): 0.002%.

Nitrogen content, *Method II* <461>: between 18.4% and 18.8% of N is found.

L-Aspartic Acid, $C_4H_7NO_4$ —**133.1** [56-84-8]—White to off-white powder. Use a suitable grade.

Azure A, $C_{14}H_{14}ClN_3S$ —**291.80** [531-53-3]—Use a suitable grade.

Bacterial Alkaline Protease Preparation—Use a suitable grade.

[**NOTE**—A suitable grade is commercially available as “Protex 6L” from Genencor, www.genencor.com, or as “Optimase Enzyme” from Solvay Enzymes Inc., www.solvaypharmaceuticals.com.]

Barbital Sodium, $C_8H_{11}N_2NaO_3$ —**206.2** [144-02-5]—White, crystalline powder or colorless crystals. Freely soluble