low color is produced gradually.

(2) To 0.02 g of Acetazolamide add 2 mL of dilute hydrochloric acid, boil for 10 minutes, cool, and add 8 mL of water: this solution responds to the Qualitative Tests for primary aromatic amines.

(3) To 0.2 g of Acetazolamide add 0.5 g of granulated zinc and 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moistened lead (II) acetate paper.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Acetazolamide in 10 mL of sodium hydroxide TS: the solution is clear and colorless to pale yellow.

(2) Chloride—To 1.5 g of Acetazolamide add 75 mL of water, and warm at 70°C for 20 minutes with occasional shaking. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Sulfate—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals—Proceed with 1.0 g of Acetazolamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Silver-reducing substances—Wet 5 g of Acetazolamide with 5 mL of aldehyde-free ethanol, and add 125 mL of water, 10 mL of nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS. Stir for 30 minutes by protecting from light, filter through a glass filter (G3), and wash the residue on the glass filter with two 10-mL portions of water. Combine the filtrate with the washings, to the solution add 5 mL of ferric ammonium sulface TS, and titrate with 0.1 mol/L ammonium thiocyanate VS: not less than 4.8 mL of 0.1 mol/L ammonium thiocyanate VS is consumed.

Loss on drying Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.15 g of Acetazolamide, and dissolve in 400 mL of water in a water bath by heating. After cooling, add water to make exactly 1000 mL. Pipet 5 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS, and then add water to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 265 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of } C_4H_6N_4O_3S_2 = \frac{A}{474} \times 200{,}000$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Acetohexamide

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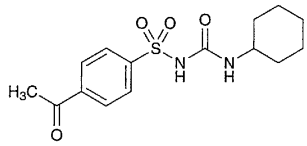

$C_{15}H_{20}N_2O_4S$: 324.40

4-Acetyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide

[*968-81-0*]

Acetohexamide, when dried, contains not less than 98.0% of $C_{15}H_{20}N_2O_4S$.

Description Acetohexamide occurs as a white to yellowish white powder.

It is freely soluble in dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point: about 185°C (with decomposition).

Identification (1) Dissolve 0.10 g of Acetohexamide in 100 mL of methanol. To 5 mL of the solution add 20 mL of 0.5 mol/L hydrochloric acid TS and 75 mL of methanol, and use the solution as the sample solution (1). Determine the absorption spectrum of the sample solution (1) as directed under the Ultraviolet-visible Spectrophotometry, using methanol as the blank, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to exactly 10 mL of the sample solution (1) add methanol to make exactly 50 mL, and use the solution as the sample solution (2). Determine the absorption spectrum of the sample solution (2) as directed under the Ultraviolet-visible Spectrophotometry, using methanol as the blank, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acetohexamide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride—Dissolve 1.5 g of Acetohexamide in 40 mL of dimethylformamide, add 6 mL of dilute nitric acid and dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and dimethylformamide to make 50 mL (not more than 0.011%).

(2) Sulfate—Dissolve 2.0 g of Acetohexamide in 40 mL of dimethylformamide, and add 1 mL of dilute hydrochloric acid and dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and dimethylformamide to make 50 mL (not more than 0.010%).

(3) Heavy metals—Proceed with 1.0 g of Acetohex-

amide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic—Prepare the test solution with 1.0 g of Acetohexamide according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) Related substances—Cyclohexylamine—To 0.20 g of Acetohexamide add exactly 2 mL of a mixture of *N,N*-dimethylformamide and acetone (1:1) to dissolve, and use this solution as the sample solution. Separately, dissolve 0.020 g of cyclohexylamine for thin-layer chromatography in a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate for 30 minutes or more. Then, develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) to a distance of about 10 cm, and heat the plate at 100°C for 10 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 120°C for 10 minutes: the spot from the sample solution corresponding to the spot obtained from the standard solution is not more intense than the spot from the standard solution.

Dicyclohexylurea—Dissolve 0.20 g of Acetohexamide in exactly 2 mL of a mixture of *N,N*-dimethylformamide and acetone (1:1), and use this solution as the sample solution. Separately, dissolve 0.020 g of dicyclohexylurea for thin-layer chromatography in a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate for 30 minutes or more. Develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) to a distance of about 10 cm, and heat the plate at 120°C for 10 minutes. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 120°C for 10 minutes: the spot from the sample solution corresponding to the spot obtained from the standard solution is not more intense than the spot from the standard solution.

Other related substances—Dissolve 0.10 g of Acetohexamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet two 1-mL portions of this solution, add acetone to make exactly 10 mL and 25 mL, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than spot from the standard solution (1), and the number of them which are intense than the spot from the standard solution (2) is not more than 4.

Loss on drying Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.3 g of Acetohexamide, previously dried, dissolve in 30 mL of dimethylformamide, add 10 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination using a solution prepared by adding 19 mL of water to 30 mL of dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 32.440 mg of $C_{15}H_{20}N_2O_4S$

Containers and storage Containers—Well-closed containers.

Acetylcholine Chloride for Injection

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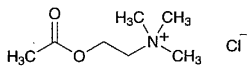

$C_7H_{16}ClNO_2$: 181.66
N-(2-Acetoxyethyl)-*N,N,N*-trimethylammonium chloride
[*60-31-1*]

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use. It contains not less than 98.0% and not more than 102.0% of acetylcholine chloride ($C_7H_{16}ClNO_2$), and not less than 19.3% and not more than 19.8% of chlorine (Cl: 35.45), calculated on the dried basis. It contains not less than 93% and not more than 107% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

Method of preparation Prepare as directed under Injections.

Description Acetylcholine Chloride for Injection occurs as white crystals or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is extremely hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Acetylcholine Chloride for Injection, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Acetylcholine Chloride for Injection (1 in 10) responds to the Qualitative Tests (2) for chloride.

Melting point 149 - 152°C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point immediately after drying both of the sample and the tube at 105°C for