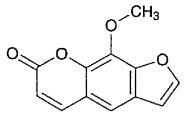
Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of folic acid and methotrexate in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 2.5%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Methoxsalen

メトキサレン



$C_{12}H_8O_4$: 216.19
9-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one
[*298-81-7*]

Methoxsalen contains not less than 98.0% and not more than 102.0% of $C_{12}H_8O_4$, calculated on the anhydrous basis.

Description Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) To 0.01 g of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2) To 0.01 g of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3) Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point 145 - 149°C

Purity (1) Heavy metals—Proceed with 1.0 g of Methoxsalen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

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

(3) Related substances—Dissolve 0.050 g of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40:10:3) 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 the spot from the standard solution.

Water Not more than 0.5% (1 g, direct titration).

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

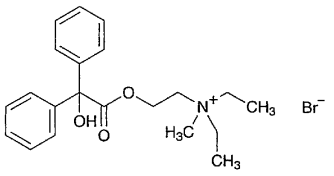
Assay Weigh accurately about 0.05 g each of Methoxsalen and Methoxsalen Reference Standard, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 300 nm as directed under the Ultraviolet-visible Spectrophotometry.

Amount (mg) of $C_{12}H_8O_4$
= amount (mg) of Methoxsalen Reference Standard, calculated on the anhydrous basis
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methylbenactyzium Bromide

臭化メチルベナクチジウム



$C_{21}H_{28}BrNO_3$: 422.36
N,*N*-Diethyl-*N*-[2-(hydroxydiphenylacetoxy)ethyl]-*N*-methylammonium bromide [*3166-62-9*]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of $C_{21}H_{28}BrNO_3$.

Description Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 50) is between 5.0 and 6.0.

Identification (1) Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2) To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3) Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests (1) for bromide.

Melting point 168 - 172°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals—Proceed with 2.0 g of Methylbenactyzium Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying Not more than 0.5% (2 g, 105°C, 2 hours).

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

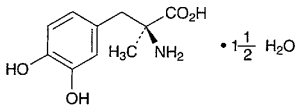
Assay Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.24 mg of $C_{21}H_{28}BrNO_3$

Containers and storage Containers—Tight containers.

Methyldopa

メチルドパ



$C_{10}H_{13}NO_4.1½H_2O$: 238.24
(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate [*41372-08-1*]

Methyldopa contains not less than 98.0% of $C_{10}H_{13}NO_4$ (mol.wt.: 211.21), calculated on the anhydrous basis.

Description Methyldopa occurs as a white to pale grayish white, crystalline powder.

It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification (1) To 0.01 g of Methyldopa add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

(2) Determine the absorption spectrum of a solution of Methyldopa in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

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

Optical rotation $[\alpha]_D^{20}$: −25 - −28° (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

Purity (1) Acid—Shake 1.0 g of Methyldopa with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

(2) Chloride—Perform the test with 0.5 g of Methyldopa. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals—Proceed with 2.0 g of Methyldopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic—Prepare the test solution with 1.0 g of Methyldopa in 5 mL of dilute hydrochloric acid, and perform the test using Apparatus B (not more than 2 ppm).

(5) 3-*O*-Methylmethyldopa—Dissolve 0.10 g of Methyldopa in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the sample solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

Water 10.0 - 13.0% (0.2 g, direct titration).

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

Assay Weigh accurately about 0.3 g of Methyldopa, dissolve in 80 mL of acetic acid (100), and titrate with 0.1