Methotrexate

 MEPHOTREXATE

Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetone, in ethanol (95%) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

Identification

(1) Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

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

Water

Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

Residue on ignition

Not more than 0.10% (0.5 g).

Assay

Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and 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

= 14.921 mg of C₆H₇NO₃S

Containers and storage

Containers—Tight containers.

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds. It contains not less than 94.0% and not more than 102.0% of C₉₉H₂₁N₀₅₀₂₃, calculated on the anhydrous basis.

C₂₉H₂₈N₄O₆: 454.44

N-(4-[N-(2,4-Diaminopteridin-6-ylmethyl)-N-methylaminobenzoyl]-L-glutamic acid [59-05-2]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of disodium hydrogenphosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

Selection of column: Dissolve 0.010 g each of Methotrexate and folic acid in 100 mL of the mobile phase.
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

\[
\text{C}_{12}\text{H}_{12}\text{O}_4: 216.19 \\
9\text{-Methoxy-7H-furo[3,2-g]chromen-7-one} \\
[298-81-7]
\]

Methoxsalen contains not less than 98.0% and not more than 102.0% of C\text{12}H\text{12}O\text{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 of these solutions, and dilute each with ethanol (95%) to make exactly 25 mL. Pipet 10 mL of each of these solutions, and dilute each 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.

\[
\text{Amount (mg) of } C_{12}H_{12}O_4 = \frac{A_T}{A_S} \times \text{amount (mg) of Methoxsalen Reference Standard, calculated on the anhydrous basis}
\]

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

**Methylbenactyzium Bromide**

臭化メチルベナクチジウム

\[
\text{C}_{21}\text{H}_{28}\text{BrNO}_5: 422.36 \\
N,N'-Diethyl-N-[2-(hydroxydiphenylacetox)ethyl]-N'-methylammonium bromide [3166-62-9]
\]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of C\text{21}H\text{28}Br\text{NO}_\text{5}.

**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.