

color fades, and a gray, flocculent precipitate is produced.

(2) Dissolve 0.01 g of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Methylprednisolone in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation**  $[\alpha]_D^{20}$ : +79 – +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity** Other steroids—Dissolve 0.050 g of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10  $\mu$ L each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** Not more than 0.2% (0.2 g).

**Assay** Weigh accurately about 0.01 g of Methylprednisolone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance  $A$  at the wavelength of maximum absorption at about 243 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\begin{aligned} & \text{Amount (mg) of } C_{22}H_{30}O_5 \\ &= \frac{A}{400} \times 1000 \end{aligned}$$

**Containers and storage** Containers—Tight containers.

## Methylrosanilinium Chloride

### Crystal Violet

塩化メチルロザニン

$C_{25}H_{30}ClN_3$ : 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride ( $C_{25}H_{30}ClN_3$ )], calculated on the dried basis.

**Description** Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

It is soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

**Identification** (1) To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

(2) Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

(3) To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

**Purity** (1) Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

(2) Heavy metals—Proceed with 1.0 g of Methylrosanilinium Chloride according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) Arsenic—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3, and perform the test using Apparatus B (not more than 5 ppm).

**Loss on drying** Not more than 7.5% (1 g, 105°C, 4 hours).

**Residue on ignition** Not more than 1.5% (0.5 g).

**Assay** Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate the excess titanium (III) chloride with 0.05 mol/L ammonium iron (III) sulfate VS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L titanium (III) chloride VS} \\ &= 20.399 \text{ mg of } C_{25}H_{30}ClN_3 \end{aligned}$$

**Containers and storage** Containers—Tight containers.