Assay preparation—Weigh accurately about 100 mg of Methylprednisolone Sodium Succinate, dissolve it in alcohol to make 200.0 mL, and mix. Pipet 5 mL of this solution into a 200-mL volumetric flask, add alcohol to volume, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.

Procedure—To each of the flasks containing the *Assay preparation* and the *Standard preparation*, and to a similar flask containing 20.0 mL of alcohol, to provide the blank, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of alcohol, and mix. Then to each flask add 4.0 mL of a mixture of 1 volume of tetramethylammonium hydroxide TS and 9 volumes of alcohol. Mix, allow to stand in the dark for 90 minutes, add 1.0 mL of glacial acetic acid, mix, and proceed as directed for *Procedure* under *Assay for Steroids* ⟨351⟩, beginning with "Concomitantly determine the absorbances." Calculate the quantity, in mg, of $C_{26}H_{33}NaO_8$ in the portion of Methylprednisolone Sodium Succinate taken by the formula:

$$8.37C(A_U / A_S).$$

Methylprednisolone Sodium Succinate for Injection

» Methylprednisolone Sodium Succinate for Injection is a sterile mixture of Methylprednisolone Sodium Succinate with suitable buffers. It may be prepared from Methylprednisolone Sodium Succinate or from Methylprednisolone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the volume of constituted solution designated on the label.

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Fluorometholone RS
USP Methylprednisolone RS
USP Methylprednisolone Hemisuccinate RS

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Identification—It meets the requirements of *Identification* test *A* under *Methylprednisolone Sodium Succinate*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.17 USP Endotoxin Unit per mg of methylprednisolone.

pH ⟨791⟩: between 7.0 and 8.0, in a solution containing about 50 mg of methylprednisolone sodium succinate per mL.

Loss on drying ⟨731⟩**—**Dry it at 105° for 3 hours: it loses not more than 2.0% of its weight.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Free methylprednisolone—Using the chromatograms obtained in the *Assay*, measure the areas of the peaks from the internal standard and free methylprednisolone. Calculate the ratio of the area of the free methylprednisolone peak to that of the internal standard in the chromatogram obtained from the *Standard preparation*, S_S, and the same ratio in the chromatogram obtained from the *Assay preparation*, S_U. Calculate the quantity, in mg, of free methylprednisolone in the *Assay preparation* taken by the formula:

$$100C(S_U / S_S)$$

in which C is the concentration, in mg per mL, of USP Methylprednisolone RS in the *Standard preparation;* and S_U and S_S are the ratios as defined above. The amount of free methylprednisolone is not more than 6.6% of the labeled amount of methylprednisolone.

Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* under *Injections* ⟨1⟩.

Assay—

Internal standard solution—Prepare a solution of USP Fluorometholone RS in tetrahydrofuran containing about 3 mg per mL.

Mobile phase—Prepare a filtered mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Weigh accurately about 32.5 mg of USP Methylprednisolone Hemisuccinate RS, and transfer it to a 50-mL volumetric flask. Add by pipet 5.0 mL of *Internal standard solution* and 5.0 mL of a solution of glacial acetic acid in chloroform (3 in 100) containing in each mL an accurately known quantity of about 0.30 mg of USP Methylprednisolone RS. Dilute with glacial acetic acid in chloroform (3 in 100) to volume, and mix.

Assay preparation—Mix the constituted solutions prepared from the contents of 10 vials of Methylprednisolone Sodium Succinate for Injection. Transfer an accurately measured volume of the resulting constituted solution, equivalent to about 50 mg of methylprednisolone, to a suitable flask containing 10.0 mL of *Internal standard solution*, and dilute with glacial acetic acid in chloroform (3 in 100) to 100.0 mL. Shake thoroughly for 5 minutes, then allow the phases to separate, discarding the upper phase.

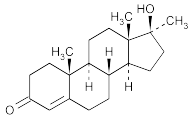
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the order of elution of peaks is the internal standard peak, methylprednisolone hemisuccinate peak, and successive smaller peaks of free methylprednisolone and methylprednisolone 17-hemisuccinate.

Procedure—Separately inject equal volumes (about 6 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the internal standard, methylprednisolone hemisuccinate, and methylprednisolone 17-hemisuccinate. Calculate the quantity, in mg, of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of constituted solution taken by the formula:

$$0.789(100C)(R_U / R_S)$$

in which 0.789 is the ratio of the molecular weight of methylprednisolone to that of methylprednisolone hemisuccinate; C is the concentration, in mg per mL, of USP Methylprednisolone Hemisuccinate RS in the *Standard preparation;* and R_U and R_S are the ratios of the sum of the peak areas for methylprednisolone hemisuccinate and methylprednisolone 17-hemisuccinate to the peak area of the internal standard obtained from the *Standard preparation* and the *Assay preparation*, respectively. To this quantity add the amount, in mg, of free methylprednisolone found in the test for *Free methylprednisolone.*

Methyltestosterone



$C_{20}H_{30}O_2$ 302.45
Androst-4-en-3-one, 17-hydroxy-17-methyl-, (17β)-.
17β-Hydroxy-17-methylandrost-4-en-3-one [58-18-4].

» Methyltestosterone contains not less than 97.0 percent and not more than 103.0 percent of $C_{20}H_{30}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Methyltestosterone RS
USP Testosterone RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: alcohol.

Melting range ⟨741⟩: between 162° and 167°.

Specific rotation ⟨781S⟩: between +79° and +85°.

Test solution: 10 mg per mL, in alcohol.

Loss on drying ⟨731⟩**—**Dry it at 105° for 4 hours: it loses not more than 2.0% of its weight.

Chromatographic purity—

Solution A—Prepare a filtered and degassed mixture of methanol and water (55:45).

Solution B—Use methanol.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dilute a volume of the *Test solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 0.005 mg of methyltestosterone per mL.

Test solution—Dissolve an accurately weighed quantity of Methyltestosterone in methanol to obtain a solution containing about 0.5 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–20	100→60	0→40	linear gradient
20–40	60→0	40→100	linear gradient
40–45	0	100	isocratic
45–60	0→100	100→0	re-equilibration

Chromatograph the *Test solution* and the *System suitability solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 33,000 theoretical plates; and the relative standard deviation for replicate injections for the methyltestosterone peak in the chromatogram of the *Test solution* is not more than 2.0%; and the signal-to-noise ratio of the methyltestosterone peak in the chromatogram of the *System suitability solution* is not less than 100.

Procedure—Inject a volume (about 5 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Methyltestosterone taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks, disregarding any impurity having a peak less than 0.05%. Not more than 0.5% of any individual impurity is found, and not more than 1.0% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 25 mg of USP Methyltestosterone RS, accurately weighed, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pipet 8 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain the *Standard preparation* having a known concentration of about 20 µg per mL.

Assay preparation—Transfer about 50 mg of Methyltestosterone, accurately weighed, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pipet 8 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Prepare a solution of testosterone in methanol containing about 250 µg per mL. Dilute 4 mL of this solution with the *Standard preparation* to 50 mL, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 241-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for testosterone and 1.0 for methyltestosterone; the resolution, *R,* between testosterone and methyltestosterone is not less than 2.0; the column efficiency determined from the analyte peak is not less than 2000 theoretical plates; the tailing factor for the analyte peak is not more than 2.7; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{30}O_2$ in the portion of Methyltestosterone taken by the formula:

$$2500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Methyltestosterone RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Methyltestosterone Capsules

» Methyltestosterone Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Methyltestosterone RS