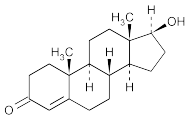
Testosterone



$C_{19}H_{28}O_2$ 288.42
Androst-4-en-3-one, 17-hydroxy-, (17β)-.
17β-Hydroxyandrost-4-en-3-one [58-22-0].

» Testosterone contains not less than 97.0 percent and not more than 103.0 percent of $C_{19}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

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

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: methanol.

Melting range ⟨741⟩**:** between 153° and 157°.

Specific rotation ⟨781S⟩**:** between +101° and +105°.

Test solution: 10 mg per mL, in dioxane.

Loss on drying ⟨731⟩**—**Dry it in vacuum over phosphorus pentoxide for 4 hours: it loses not more than 1.0% of its weight.

Assay—

Standard preparation—Prepare as directed under *Single-Steroid Assay* ⟨511⟩, using USP Testosterone RS.

Assay preparation—Accurately weigh about 20 mg of Testosterone, previously dried; dissolve in a sufficient quantity of a mixture of equal volumes of alcohol and chloroform to make 10.0 mL; and mix.

Procedure—Proceed as directed for *Procedure* under *Single-Steroid Assay* ⟨511⟩, using a solvent system consisting of a mixture of benzene and ethyl acetate (1:1), through the fourth sentence of the second paragraph under *Procedure.* Then centrifuge the tubes for 5 minutes, and determine the absorbances of the supernatant in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{19}H_{28}O_2$ in the portion of Testosterone taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Testosterone RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Testosterone Injectable Suspension

» Testosterone Injectable Suspension is a sterile suspension of Testosterone in an aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{28}O_2$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

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

Identification—The testosterone obtained by filtration and washing, as directed in the *Assay*, and dried at 105° to constant weight, meets the requirements for *Identification* tests *A* and *B* under *Testosterone.*

Bacterial endotoxins ⟨85⟩**—**It contains not more than 3.5 USP Endotoxin Units per mg of testosterone.

Uniformity of dosage units ⟨905⟩: meets the requirements.

pH ⟨791⟩: between 4.0 and 7.5.

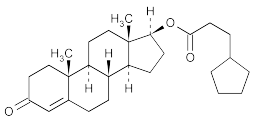
Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—Transfer an accurately measured volume of previously well-mixed Injectable Suspension, equivalent to about 100 mg of testosterone, to a fine-porosity, sintered-glass filtering crucible, previously dried at 105° for 1 hour, and filter with suction. If the filtrate is not clear, again pass it through the same filter into a second receiver. Wash the residue in the filter with several 5-mL portions of water until 2 mL of the last washing, when evaporated on a steam bath, leaves a negligible residue. [NOTE—If the Injectable Suspension is passed through the filter twice, rinse the first receiver with the portions of water before passing them through the filter.] Dry the crucible and the collected testosterone at 105° for 1 hour. Completely dissolve the testosterone with five 25-mL portions of methanol, passing each portion through the crucible under gentle suction, and transfer the combined methanol solution to a 200-mL volumetric flask. Rinse the crucible and receiver with two 25-mL portions of methanol, add the rinsings to the main solution, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Testosterone RS, in the same medium having a known concentration of about 10 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{19}H_{28}O_2$ in each mL of the Injectable Suspension taken by the formula:

$$(10C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Testosterone RS in the Standard solution, V is the volume, in mL, of Injectable Suspension taken, and A_U and A_S are the absorbances of the solution from the Injectable Suspension and the Standard solution, respectively.

Testosterone Cypionate



$C_{27}H_{40}O_3$ 412.60
Androst-4-en-3-one, 17-(3-cyclopentyl-1-oxopropoxy)-, (17β)-.
Testosterone cyclopentanepropionate [58-20-8].

» Testosterone Cypionate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{40}O_3$, calculated on the dried basis.

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

USP Reference standards ⟨11⟩—
USP Cholesteryl Caprylate RS
$C_{35}H_{60}O_2$ 512.86
USP Testosterone Cypionate RS

Identification, *Infrared Absorption* ⟨197K⟩.

Melting range ⟨741⟩: between 98° and 104°.

Specific rotation ⟨781S⟩: between +85° and +92°.

Test solution: 20 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Free cyclopentanepropionic acid—Dissolve 500 mg in 10 mL of alcohol that previously has been neutralized to a faint blue color following the addition of 2 or 3 drops of bromothymol blue TS, and promptly titrate with 0.01 N sodium hydroxide VS: not more than 0.70 mL of 0.01 N sodium hydroxide is required (0.20% of cyclopentanepropionic acid).

Assay—

Internal standard solution—Dissolve 80 mg of USP Cholesteryl Caprylate RS in a mixture of methanol and chloroform (4:1) in a 100-mL volumetric flask, then add the same solvent mixture to volume.

Standard preparation—Weigh accurately about 10 mg of USP Testosterone Cypionate RS into a suitable vial, add by pipet 10 mL of *Internal standard solution*, and mix.

Assay preparation—Prepare as directed for *Standard preparation*, using an accurately weighed portion of about 10 mg of Testosterone Cypionate instead of the Reference Standard.

Procedure—Inject 1 µL of the *Assay preparation* and the *Standard preparation*, successively, into a suitable gas chromatograph fitted with a flame-ionization detector. Under typical conditions, the instrument contains a 3-mm × 1.2-m glass column packed with 1% (w/w) phase G6 on packing S1AB. The column temperature is maintained at 260° and the helium carrier gas flows at 50 mL per minute. In a suitable chromatogram, the resolution factor, *R* (see *Chromatography* ⟨621⟩), is not less than 3 between the internal standard and testosterone cypionate peaks, and five replicate injections of a single *Standard preparation* show a coefficient of variation of not more than 2% in the peak area ratio of testosterone cypionate to internal standard. Measure the areas under the peaks for testosterone cypionate and cholesteryl caprylate in each chromatogram. Calculate the ratio, R_U, of the area of the testosterone cypionate peak to the area of the internal standard peak in the chromatogram from the *Assay preparation*, and similarly calculate the ratio, R_S, in the chromatogram from the *Standard preparation*. Calculate the quantity, in mg, of $C_{27}H_{40}O_3$ in the portion of Testosterone Cypionate taken by the formula:

$$W(R_U / R_S)$$

in which *W* is the weight, in mg, of USP Testosterone Cypionate RS in the *Standard preparation*, and the other terms are as defined therein.

Testosterone Cypionate Injection

» Testosterone Cypionate Injection is a sterile solution of Testosterone Cypionate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{27}H_{40}O_3$. It may contain a suitable solubilizing agent.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Cholesteryl Caprylate RS
$C_{35}H_{60}O_2$ 512.86
USP Testosterone Cypionate RS

Identification—Dilute a suitable volume of Injection with chloroform to obtain a solution having a concentration of about 400 µg of testosterone cypionate per mL. Prepare a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic siliceous earth, by placing it in a developing chamber containing and equilibrated with a mixture of chloroform and corn oil (90:10), and allowing the solvent front to move about three-fourths of the length of the plate. Remove the plate, and allow the chloroform to evaporate. Apply 10 µL each of the solution under test and of a solution of USP Testosterone Cypionate RS in chloroform containing about 400 µg per mL on the plate, on a line about 2.5 cm from the bottom edge and about 1.5 cm apart. Place the plate in a developing chamber that contains and has been equilibrated with a mixture of methanol and water (90:10) previously saturated with corn oil. Develop the chromatogram until the solvent front has moved to about 10 cm above the line of application. Remove the plate, and heat in an oven at 105° for a few minutes. Spray the plate with a mixture of alcohol and sulfuric acid (3:1), and heat in an oven at 105° for 1 to 2 minutes. Observe the plate under long-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Internal standard solution and *Standard preparation*—Prepare as directed in the *Assay* under *Testosterone Cypionate.*

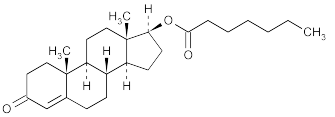
Assay preparation—Transfer 1 mL of Injection, accurately measured, into a glass-stoppered, 50-mL centrifuge tube. Add 30 mL of a mixture of methanol and water (9:1), insert the stopper, and shake for 15 minutes. Centrifuge, remove the dilute methanol layer without disturbing the oil, and transfer it to a 200-mL volumetric flask. Repeat the extraction with three additional 30-mL portions of the dilute methanol, collecting the combined extracts in the volumetric flask. Dilute the combined extracts with the dilute methanol to volume, mix, and chill the contents of the flask to –8°. Remove the flask from the freezer, and immediately filter a portion of the contents. Allow the filtrate to reach room temperature, transfer a portion of it, equivalent to about 3 mg of testosterone cypionate, to a suitable vial, and evaporate to dryness. Add by pipet 3 mL of *Internal standard solution*, and shake vigorously to dissolve the residue.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Testosterone Cypionate.* Calculate the quantity, in mg, of $C_{27}H_{40}O_3$ in the portion of Injection taken by the formula:

$$600(C / V)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Testosterone Cypionate RS in the *Standard preparation;* and *V* is the volume, in mL, of the filtrate used in the *Assay preparation.*

Testosterone Enanthate



$C_{26}H_{40}O_3$ 400.59
Androst-4-en-3-one, 17-(1-oxoheptyl)oxy-, (17β)-.
Testosterone heptanoate [315-37-7].