*Other esters of testosterone have been synthesized and identified; however, only the following are treated in this monograph:

- Testosterone
- Testosterone Cypionate
- Testosterone Decanoate
- Testosterone Enanthate
- Testosterone Isocaproate
- Testosterone Phenylpropionate
1. SYNONYMS

**CFR:**

The Anabolic Steroid Control Act (ASCA) of 1990 amended the Controlled Substances Act to list anabolic steroids as a Schedule III substance. Subsequently, the ASCA of 2003 and 2004 were adopted to clarify the definition of anabolic steroid, listing testosterone and “any salt, ester, or ether of a drug or substance described in this paragraph.”

**CAS #:**

<table>
<thead>
<tr>
<th>Substance</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>58-22-0</td>
</tr>
<tr>
<td>Testosterone cypionate</td>
<td>58-20-8</td>
</tr>
<tr>
<td>Testosterone decanoate</td>
<td>5721-91-5</td>
</tr>
<tr>
<td>Testosterone enanthate</td>
<td>315-37-7</td>
</tr>
<tr>
<td>Testosterone isocaproate</td>
<td>15262-86-9</td>
</tr>
<tr>
<td>Testosterone phenylpropionate</td>
<td>1255-49-8</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>57-85-2</td>
</tr>
<tr>
<td>Testosterone undecanoate</td>
<td>5949-44-0</td>
</tr>
</tbody>
</table>

**Other Names:**

**Testosterone:**

- 17β-Hydroxyandrost-4-ene-3-one
- 4-Androsten-17β-ol-3-one
- *trans*-Testosterone
- Androst-4-en-17β-ol-one
- Andro
- Androderm
- Androlin
- Testoderm
- Testred

**Testosterone cypionate:**

- 17β-Hydroxyandrost-4-ene-3-one cyclopentanepropionate
- Testosterone cyclopentanylpropionate
Depo-Testosterone
depAndro
Virolon

**Testosterone decanoate:**
17β-Hydroxyandrost-4-ene-3-one decanoate
Testosterone caprate

**Testosterone enanthate:**
17β-Hydroxyandrost-4-ene-3-one enanthate
17β-Hydroxyandrost-4-ene-3-one-17-enanthate
Testosterone heptanoate
Andro LA
Delatestryl
Testinon
Testo-Enant

**Testosterone isocaproate:**
17β-Hydroxyandrost-4-ene-3-one isocaproate
Testosterone 4-methylvalerate

**Testosterone phenylpropionate:**
17β-Hydroxyandrost-4-ene-3-one Phenylpropionate
Testosterone phenpropionate
Testosterone hydrocinnamate
Retandrol

**Testosterone propionate:**
17β-Hydroxyandrost-4-ene-3-one propionate
Testosterone-17-propionate
17-(1-Oxopropoxy)-(17β)-androst-4-en-3-one

**Testosterone undecanoate:**
17β-Hydroxyandrost-4-ene-3-one undecanoate
4-Androsten-17β-ol-3-one undecanoate
17-[(1-Oxoundecyl)oxy]-androst-4-en-3-one

---

### 2. CHEMICAL AND PHYSICAL DATA

#### 2.1. CHEMICAL DATA

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>C_{19}H_{28}O_{2}</td>
<td>288.4</td>
<td>154</td>
</tr>
<tr>
<td>Testosterone cypionate</td>
<td>C_{27}H_{40}O_{3}</td>
<td>412.6</td>
<td>101-102</td>
</tr>
<tr>
<td>Testosterone decanoate</td>
<td>C_{29}H_{46}O_{3}</td>
<td>442.6</td>
<td>48-54</td>
</tr>
</tbody>
</table>
Testosterone enanthate  |  C_{26}H_{40}O_{3}  |  400.5  |  36-37
Testosterone isocaproate  |  C_{25}H_{38}O_{3}  |  386.5  |  77-79
Testosterone phenylpropionate  |  C_{28}H_{36}O_{3}  |  420.5  |  116
Testosterone propionate  |  C_{22}H_{32}O_{3}  |  344.4  |  120
Testosterone undecanoate  |  C_{30}H_{48}O_{3}  |  456.6  |  61

2.2. SOLUBILITY

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>A</th>
<th>C</th>
<th>E</th>
<th>H</th>
<th>M</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone (T)</td>
<td>S</td>
<td>VS</td>
<td>PS</td>
<td>SS</td>
<td>FS</td>
<td>I</td>
</tr>
<tr>
<td>T. cypionate</td>
<td>FS</td>
<td>FS</td>
<td>FS</td>
<td>PS</td>
<td>FS</td>
<td>I</td>
</tr>
<tr>
<td>T. decanoate</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>I</td>
</tr>
<tr>
<td>T. enanthate</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>S</td>
<td>VS</td>
<td>I</td>
</tr>
<tr>
<td>T. isocaproate</td>
<td>S</td>
<td>VS</td>
<td>VS</td>
<td>PS</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>T. phenylpropionate</td>
<td>FS</td>
<td>S</td>
<td>PS</td>
<td>I</td>
<td>S</td>
<td>VSS</td>
</tr>
<tr>
<td>T. propionate</td>
<td>FS</td>
<td>VS</td>
<td>FS</td>
<td>PS</td>
<td>FS</td>
<td>I</td>
</tr>
<tr>
<td>T. undecanoate</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>I</td>
</tr>
</tbody>
</table>

A = acetone, C = chloroform, E = ether, H = hexane, M = methanol and W = water, VS = very soluble, FS = freely soluble, S = soluble, PS = sparingly soluble, SS = slightly soluble, VSS = very slightly soluble and I = insoluble

3. SCREENING TECHNIQUES

3.1. COLOR TESTS

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>SULFURIC ACID</th>
<th>MANDELIN’S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>N/R</td>
<td>faint orange</td>
</tr>
<tr>
<td>Testosterone cypionate</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Testosterone decanoate</td>
<td>slow orange</td>
<td>faint orange</td>
</tr>
<tr>
<td>Testosterone enanthate</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Testosterone</td>
<td>slow orange</td>
<td>slow orange</td>
</tr>
<tr>
<td>COMPOUND</td>
<td>RELATIVE R1 SYSTEM TLC19</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>testosterone</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>testosterone undecanoate</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>testosterone decanoate</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>testosterone cypionate</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>testosterone enanthate</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>testosterone isocaproate</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>testosterone phenylpropionate</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>testosterone propionate</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

3.2. CRYSTAL TESTS

Currently, there are no reliable crystal tests.

3.3. THIN-LAYER CHROMATOGRAPHY

Visualization

Sulfuric acid: ethanol spray (1:9)

3.4. GAS CHROMATOGRAPHY

Method Test-GCS1

**Instrument:**
Gas chromatograph operated in split mode with FID

**Column:**
5% phenyl/95% methyl silicone 12 m x 0.2 mm x 0.33 µm film thickness

**Carrier gas:**
Helium at 1.0 mL/min

**Temperatures:**
Injector: 250°C
Detector: 280°C
Oven program:
1) 180°C initial temperature for 2.0 min
2) Ramp to 280°C at 25°C/min
3) Hold final temperature for 12.0 min

Injection Parameters: Split Ratio = 60:1, 1 μL injected

Samples are to be dissolved in appropriate solvent (chloroform) and filtered.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone</td>
<td>1.0 (5.81 min.)</td>
</tr>
<tr>
<td>testosterone propionate</td>
<td>1.1</td>
</tr>
<tr>
<td>testosterone isocaproate</td>
<td>1.3</td>
</tr>
<tr>
<td>testosterone enanthate</td>
<td>1.5</td>
</tr>
<tr>
<td>testosterone cypionate</td>
<td>1.9</td>
</tr>
<tr>
<td>testosterone phenylpropionate</td>
<td>2.2</td>
</tr>
<tr>
<td>testosterone decanoate</td>
<td>2.3</td>
</tr>
<tr>
<td>testosterone undecanoate</td>
<td>2.6</td>
</tr>
</tbody>
</table>

3.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method Test-LCS1

Instrument: High performance liquid chromatograph equipped with diode array

Column: Phenomenex Aqua C-18 5 μm; 150 mm x 4.6 mm

Detector: UV, 240 nm

Flow: 1.0 mL/min

Injection Volume: 5.0 μL

Buffer: 90:10 MeOH:H₂O

Mobile Phase: 90:10 MeOH:H₂O

Samples are to be dissolved in methanol and filtered with a 0.45-micron filter.
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone</td>
<td>1.00 (2.9 min)</td>
</tr>
<tr>
<td>testosterone propionate</td>
<td>1.62</td>
</tr>
<tr>
<td>testosterone phenylpropionate</td>
<td>2.47</td>
</tr>
<tr>
<td>testosterone isocaproate</td>
<td>2.86</td>
</tr>
<tr>
<td>testosterone enantheate</td>
<td>3.45</td>
</tr>
<tr>
<td>testosterone cypionate</td>
<td>3.79</td>
</tr>
<tr>
<td>testosterone decanoate</td>
<td>8.11</td>
</tr>
<tr>
<td>testosterone undecanoate</td>
<td>10.65</td>
</tr>
</tbody>
</table>

### 3.6 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

**Sample preparation:**
Use ca. 5 to 10 mg of sample for proton NMR and 30 mg for carbon NMR. Dissolve sample in chloroform-d (CDCl₃) with the internal reference standard tetramethylsilane (TMS). Filter all preparation solutions before analysis.

**Method Test-NMRS1**

**Instrument:**
400 MHz Nuclear magnetic resonance spectrometer

**Probe:**
5 mm indirect detection gradient NMR probe

**Parameters**

**¹H NMR:**
- Observed frequency: 400.1 MHz
- Pulse angle: 30°
- Acquisition time: 1.995 s
- Acquisition delay: 1.000 s
- Spectral window: 6410 Hz
- Transmitter power: 57 dB
- Variable temperature set @: 25°C
- Number of transients: 16

**¹³C NMR:**
- Observed frequency: 100.6 MHz
- Pulse angle: 45°
- Acquisition time: 1.202 s
- Acquisition delay: 1.000 s
- Spectral window: 25062 Hz
- Transmitter power: 61 dB
- Decoupler: on
- Decoupler modulation mode: Waltz
4. SEPARATION TECHNIQUES

Testosterone and its esters are generally encountered in one of three forms: tablets, suspensions or dissolved in oils. Isolation from tablets is achieved by direct extraction with chloroform or methylene chloride. Following evaporation of the solvent, the residue may be suitable for infrared identification or mass spectrometer identification in the case of multi-entity preparations. Isolation of the steroid(s) from suspensions is achieved by separating the liquid and drying the resultant powder. The powder is then analyzed similarly as above. Testosterone and its esters, when dissolved in an oil matrix, are impossible to identify by infrared and can cause fouling of other types of instrumentation. Solid-phase extraction is a simple and effective means to isolate the steroid(s) for identification. Approximately five to seven drops of the oil solution is dissolved in pet ether or hexanes and added to a solid-phase extraction cartridge filled with silica. After washing the cartridge with the same solvent, the steroid(s) are eluted from the cartridge with acetone and analyzed as above.

5. QUANTITATIVE PROCEDURES

5.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method Test-LCQ1

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of the appropriate testosterone ester at approximately 0.5 mg/mL using methanol.

Sample Preparation:
For powder or other solid dosage forms, accurately weigh an amount of sample into a volumetric flask and dilute with methanol. For aqueous suspensions, insure that the sample is well mixed, then pipette an aliquot into a volumetric flask and dilute with methanol. If necessary, dilute the sample so the final concentration approximates the standard concentration. Filter sample with a 0.45-micron filter. (Recovery studies have not been performed for steroids dissolved in oil matrices, so this method is not validated for testosterone and/or its esters dissolved in oil. Literature suggests that quantitative recoveries can be obtained by multiple extractions with methanol [Walters, et al].)

Instrument: High performance liquid chromatograph equipped with diode array

Column: Phenomenex Aqua C-18 5 µm; 150 mm x 4.6 mm

Detector: UV, 240 nm

Flow: 1.00 mL/min

Injection Volume: 5.0 µL

Buffer: 90:10 MeOH:H₂O
**Mobile Phase:** 90:10 MeOH:H₂O

**Typical Retention Time:**
- Testosterone: 2.9 min.
- Testosterone enanthate: 10.0 min
- Testosterone cypionate: 11.0 min
- Testosterone propionate: 4.7 min

**Linear Range:** 0.25 - 1.0 mg/mL

**Repeatability:** RSD less than 0.5%

**Correlation Coefficient:** 0.9999

**Accuracy:** Error less than 5%

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone</td>
<td>1.00 (2.9 min)</td>
</tr>
<tr>
<td>testosterone propionate</td>
<td>1.62</td>
</tr>
<tr>
<td>testosterone enanthate</td>
<td>3.45</td>
</tr>
<tr>
<td>testosterone cypionate</td>
<td>3.79</td>
</tr>
</tbody>
</table>

6. **QUALITATIVE DATA**

See spectra on the following pages for FT-IR, Mass Spectrometry, Nuclear Magnetic Resonance, and Vapor Phase IR.

7. **REFERENCES**

Analytical Methods for the Analysis of Steroids; (FFS-630-01); DEA Special Testing and Research Laboratory, February 22, 1991.


Walters, M.J.; Ayers, R.J.; Brown, D.J. "Analysis of Illegally Distributed Steroid Products by Liquid Chromatography with Identity Confirmation by Mass Spectrometry or Infrared Spectrometry"; JAOAC; 1990, Vol. 73, No.6, pp 904-926.


8. ADDITIONAL RESOURCES

Forendex

Wikipedia
Transmission IR: Testosterone
16 scans, 4.0 cm⁻¹ resolution
Sample in Potassium Bromide matrix

IR (ATR, 3-bounce, diamond device): Testosterone
16 scans, 4.0 cm⁻¹ resolution
MS (EI): Testosterone
Quadrupole Detector

IR (Vapor Phase): Testosterone
NMR (PROTON): Testosterone
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone cypionate
16 scans, 4.0 cm\(^{-1}\) resolution, sample in potassium bromide matrix

IR (ATR, 3-bounce, diamond device): Testosterone cypionate
16 scans, 4.0 cm\(^{-1}\) resolution
MS (EI): Testosterone cypionate
Quadrupole Detector

IR (Vapor Phase): Testosterone cypionate
NMR (PROTON): Testosterone cypionate
10 mg/mL in CDCl$_3$ with TMS, 400 MHz

NMR (CARBON): Testosterone cypionate
50 mg/mL in CDCl$_3$ with TMS, 100 MHz
Transmission IR: Testosterone decanoate
16 scans, 4.0 cm\(^{-1}\) resolution, sample in potassium bromide matrix

IR (ATR, 3-bounce, diamond device): Testosterone decanoate
16 scans, 4.0 cm\(^{-1}\) resolution
MS (EI): Testosterone decanoate

Quadrupole Detector

IR (Vapor Phase): Testosterone decanoate
NMR (PROTON): Testosterone Decanoate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON) Testosterone decanoate
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone enanthate
16 scans, 4.0 cm⁻¹ resolution, sample in potassium bromide matrix

IR (ATR 3-bounce, diamond device): Testosterone enanthate
16 scans, 4.0 cm⁻¹ resolution
Mass Spectrum (EI): Testosterone enanthate
Quadrupole Detector

IR (Vapor Phase): Testosterone enanthate
NMR (PROTON): Testosterone enanthate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone enanthate
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone isocaproate
16 scans, 4.0 cm$^{-1}$ resolution, sample in potassium bromide matrix

IR (ATR, 3-bounce diamond device): Testosterone isocaproate
16 scans, 4.0 cm$^{-1}$ resolution
MS (EI): Testosterone isocaproate
Quadrupole Detector

IR (Vapor Phase): Testosterone isocaproate
NMR (PROTON): Testosterone isocaproate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone isocaproate
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone phenylpropionate
16 scans, 4.0 cm$^{-1}$ resolution, sample in potassium bromide matrix

IR (ATR, 3-bounce diamond device): Testosterone phenylpropionate
16 scans, 4.0 cm$^{-1}$ resolution
MS (EI): Testosterone phenylpropionate

Quadrupole Detector

IR (Vapor Phase): Testosterone phenylpropionate
NMR (PROTON): Testosterone phenylpropionate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone phenylpropionate
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone propionate
16 scans, 4.0 cm\(^{-1}\) resolution, sample in potassium bromide matrix

IR (ATR 3-bounce diamond device): Testosterone propionate
16 scans, 4.0 cm\(^{-1}\) resolution
MS (EI): Testosterone propionate
Quadrupole Detector

IR (Vapor Phase): Testosterone propionate
NMR (PROTON): Testosterone propionate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone propionate
50 mg/mL in CDCl₃ with TMS, 100 MHz
Transmission IR: Testosterone undecanoate
16 scans, 4.0 cm$^{-1}$ resolution, sample in potassium bromide matrix

IR (ATR 3-bounce diamond device): Testosterone undecanoate
16 scans, 4.0 cm$^{-1}$ resolution

MS (EI): Testosterone undecanoate
Quadrupole Detector
IR (Vapor Phase): Testosterone undecanoate
NMR (PROTON): Testosterone undecanoate
10 mg/mL in CDCl₃ with TMS, 400 MHz

NMR (CARBON): Testosterone undecanoate
50 mg/mL in CDCl₃ with TMS, 100 MHz