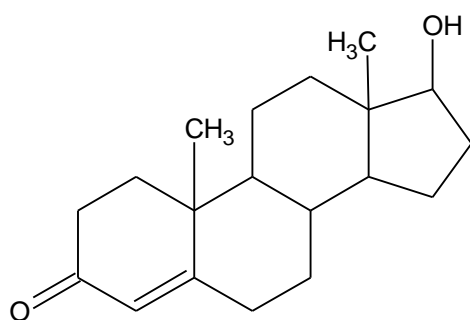


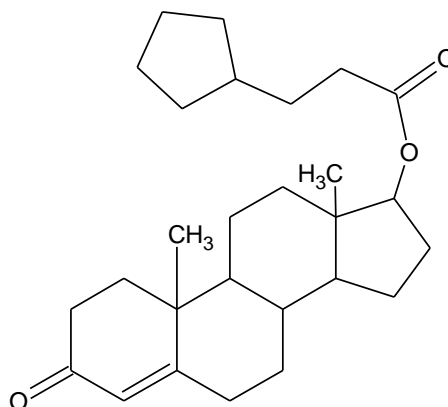
## TESTOSTERONE AND ESTERS\*

Latest Revision: June 23, 2005

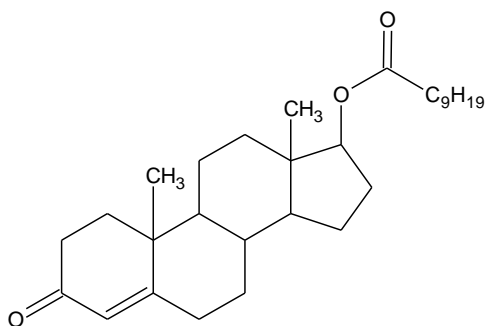
\*Other esters of testosterone have been synthesized and identified; however, only the following are treated in this monograph:



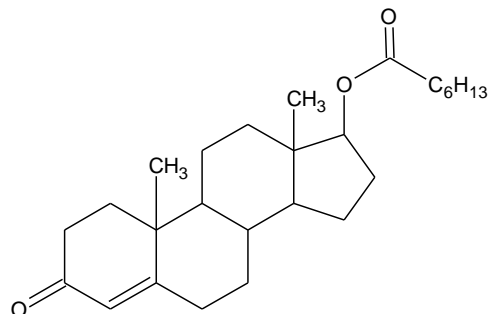
Testosterone



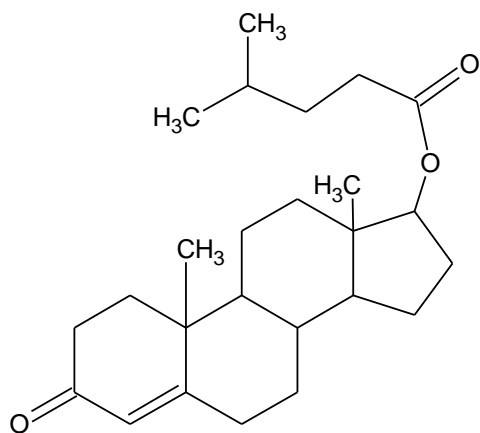
Testosterone Cypionate



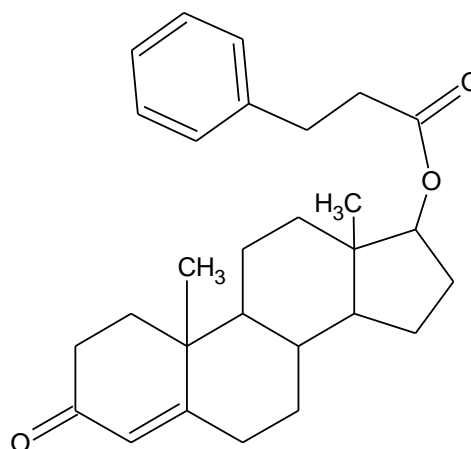
Testosterone Decanoate



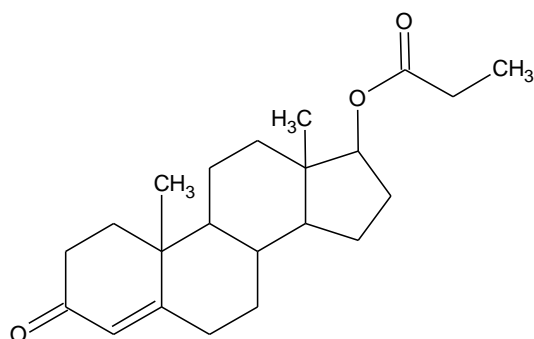
Testosterone Enanthate



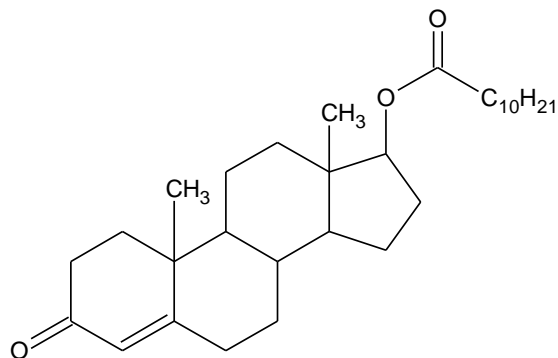
Testosterone Isocaproate



Testosterone Phenylpropionate



Testosterone Propionate



Testosterone Undecanoate

## 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 #:

Testosterone	58-22-0
Testosterone cypionate	58-20-8
Testosterone decanoate	5721-91-5
Testosterone enanthate	315-37-7
Testosterone isocaproate	15262-86-9
Testosterone phenylpropionate	1255-49-8
Testosterone propionate	57-85-2
Testosterone undecanoate	5949-44-0

### 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 cyclopentylpropionate

Depo-Testosterone  
depAndro  
Virolon

**Testosterone decanoate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one decanoate  
Testosterone caprate

**Testosterone enanthate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one enanthate  
17 $\beta$ -Hydroxyandrost-4-ene-3-one-17-enanthate  
Testosterone heptanoate  
Andro LA  
Delatesteryl  
Testinon  
Testo-Enant

**Testosterone isocaproate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one isocaproate  
Testosterone 4-methylvalerate

**Testosterone phenylpropionate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one Phenylpropionate  
Testosterone phenpropionate  
Testosterone hydrocinnamate  
Retandrol

**Testosterone propionate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one propionate  
Testosterone-17-propionate  
17-(1-Oxopropoxy)-(17 $\beta$ )-androst-4-en-3-one

**Testosterone undecanoate:**

17 $\beta$ -Hydroxyandrost-4-ene-3-one undecanoate  
4-Androsten-17 $\beta$ -ol-3-one undecanoate  
17-[(1-Oxoundecyl)oxy]-androst-4-en-3-one

## 2. CHEMICAL AND PHYSICAL DATA

### 2.1. CHEMICAL DATA

COMPOUND	Chemical Formula	Molecular Weight	Melting Point (°C)
Testosterone	C <sub>19</sub> H <sub>28</sub> O <sub>2</sub>	288.4	154
Testosterone cypionate	C <sub>27</sub> H <sub>40</sub> O <sub>3</sub>	412.6	101-102
Testosterone decanoate	C <sub>29</sub> H <sub>46</sub> O <sub>3</sub>	442.6	48-54

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

COMPOUND	A	C	E	H	M	W
testosterone (T)	S	VS	PS	SS	FS	I
T. cypionate	FS	FS	FS	PS	FS	I
T. decanoate	VS	VS	VS	VS	VS	I
T. enanthate	VS	VS	VS	S	VS	I
T. isocaproate	S	VS	VS	PS	S	I
T. phenylpropionate	FS	S	PS	I	S	VSS
T. propionate	FS	VS	FS	PS	FS	I
T. undecanoate	VS	VS	VS	VS	VS	I

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

COMPOUND	SULFURIC ACID	MANDELIN'S
Testosterone	N/R	faint orange
Testosterone cypionate	N/R	N/R
Testosterone decanoate	slow orange	faint orange
Testosterone enanthate	N/R	N/R
Testosterone	slow orange	slow orange

isocaproate		
Testosterone phenylpropionate	N/R	faint aqua
Testosterone propionate	N/R	N/R
Testosterone undecanoate	N/R	N/R

### 3.2. CRYSTAL TESTS

Currently, there are no reliable crystal tests.

### 3.3. THIN-LAYER CHROMATOGRAPHY

#### Visualization

Sulfuric acid: ethanol spray (1:9)

COMPOUND	RELATIVE R <sub>f</sub> SYSTEM TLC19
testosterone	1.00
testosterone undecanoate	0.17
testosterone decanoate	0.22
testosterone cypionate	0.35
testosterone enanthate	0.40
testosterone isocaproate	0.50
testosterone phenylpropionate	0.55
testosterone propionate	0.72

### 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.

COMPOUND	RRT
testosterone	1.0 (5.81 min.)
testosterone propionate	1.1
testosterone isocaproate	1.3
testosterone enanthate	1.5
testosterone cypionate	1.9
testosterone phenylpropionate	2.2
testosterone decanoate	2.3
testosterone undecanoate	2.6

### 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:H<sub>2</sub>O

**Mobile Phase:** 90:10 MeOH:H<sub>2</sub>O

Samples are to be dissolved in methanol and filtered with a 0.45-micron filter.

COMPOUND	RRT
testosterone	1.00 (2.9 min)
testosterone propionate	1.62
testosterone phenylpropionate	2.47
testosterone isocaproate	2.86
testosterone enanthate	3.45
testosterone cypionate	3.79
testosterone decanoate	8.11
testosterone undecanoate	10.65

### 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 ( $\text{CDCl}_3$ ) 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*

##### *$^1\text{H}$ 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

##### *$^{13}\text{C}$ 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

Decoupler modulation frequency: 10100 Hz  
Variable temperature set @: 25°C  
Number of transients: 1024

#### **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].)

<b><i>Instrument:</i></b>	High performance liquid chromatograph equipped with diode array
<b><i>Column:</i></b>	Phenomenex Aqua C-18 5 µm; 150 mm x 4.6 mm
<b><i>Detector:</i></b>	UV, 240 nm
<b><i>Flow:</i></b>	1.00 mL/min
<b><i>Injection Volume:</i></b>	5.0 µL
<b><i>Buffer:</i></b>	90:10 MeOH:H <sub>2</sub> O



**Mobile Phase:** 90:10 MeOH:H<sub>2</sub>O

**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%

COMPOUND	RRT
testosterone	1.00 (2.9 min)
testosterone propionate	1.62
testosterone enanthate	3.45
testosterone cypionate	3.79

## 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.

Analytical Profiles of Anabolic Steroids; CND Analytical: Auburn, AL, 1989.

Budavari, S., The Merck Index, 13 Edition, Merck and Co., Inc., 2001, p.1638

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Moffat A.C., Sr. Ed., Clarke's Isolation and Identification of Drugs, The Pharmaceutical Press, London, Second Edition 1986, p.1003.

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.

Zaretskii, V.I. Mass Spectrometry of Steroids; New York, Wiley; 1976.

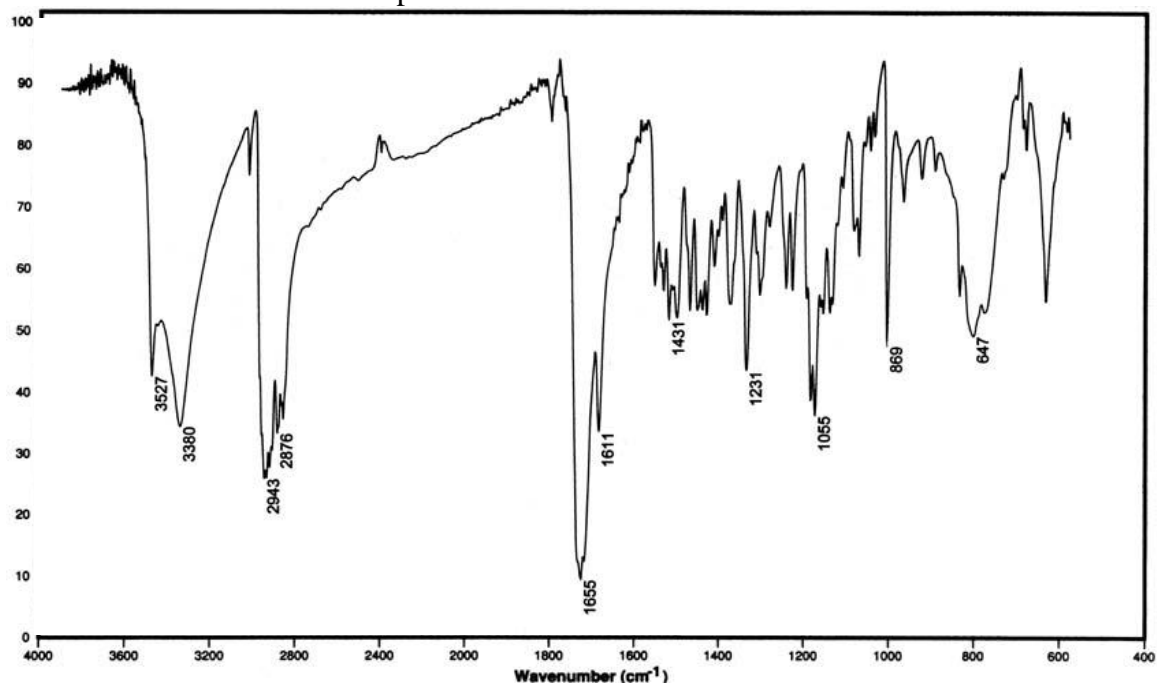
## **8. ADDITIONAL RESOURCES**

[Forendex](#)

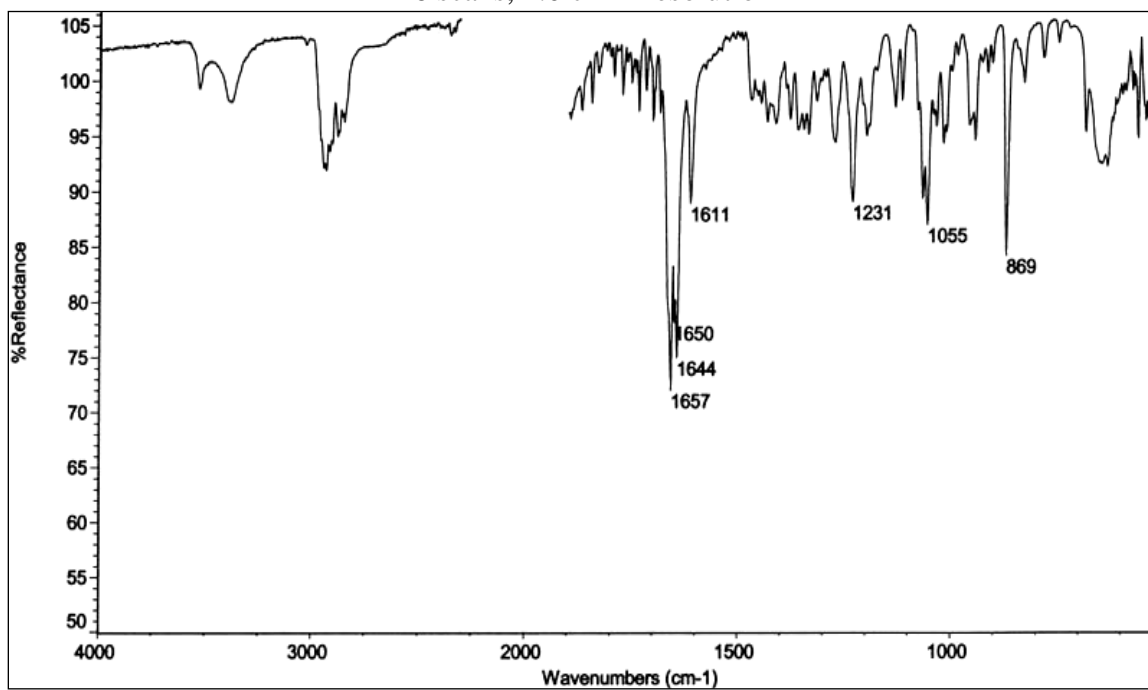
[Wikipedia](#)

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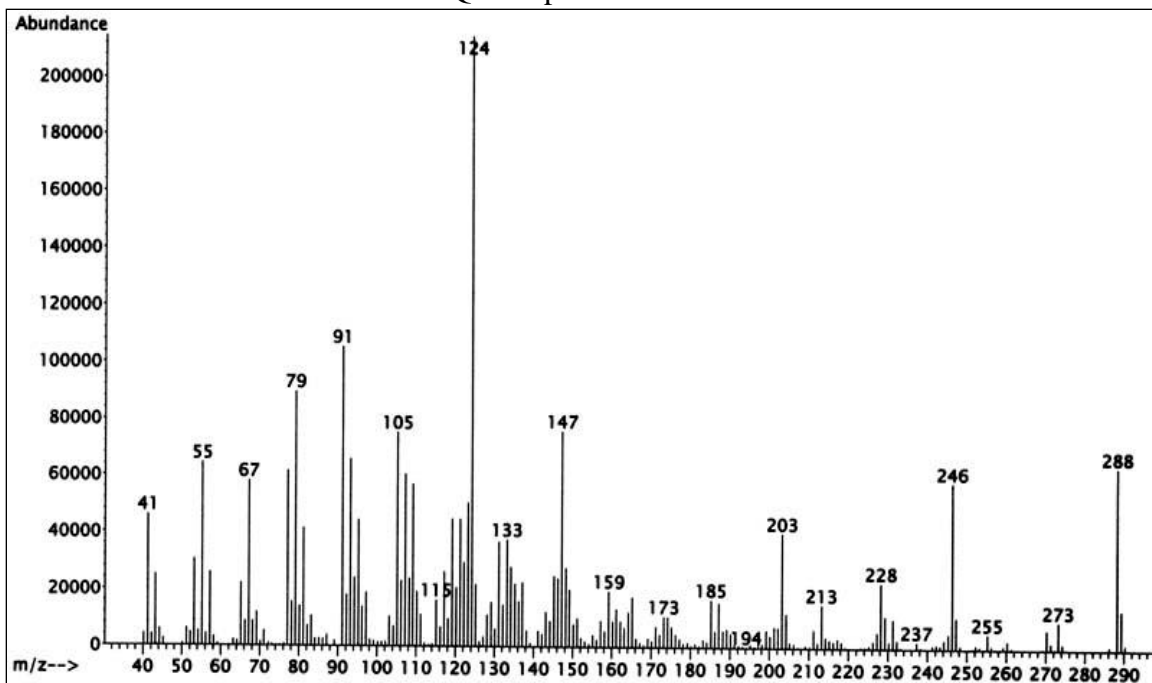
Transmission IR: Testosterone  
16 scans, 4.0 cm<sup>-1</sup> resolution  
Sample in Potassium Bromide matrix



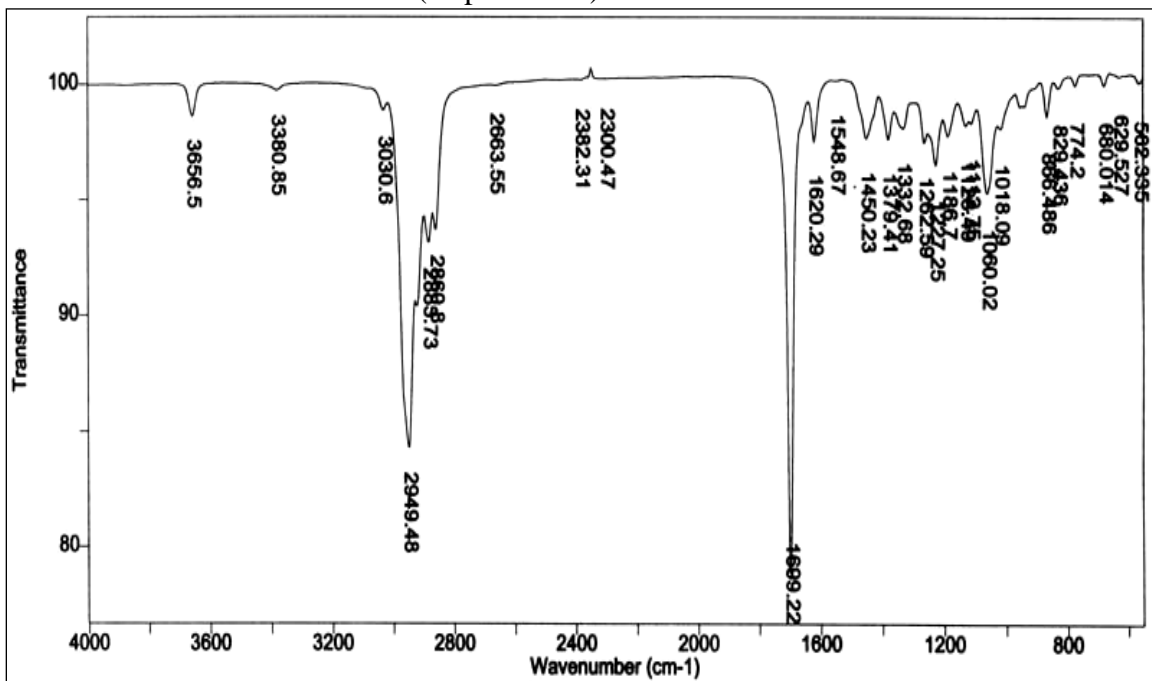
IR (ATR, 3-bounce, diamond device): Testosterone  
16 scans, 4.0 cm<sup>-1</sup> resolution



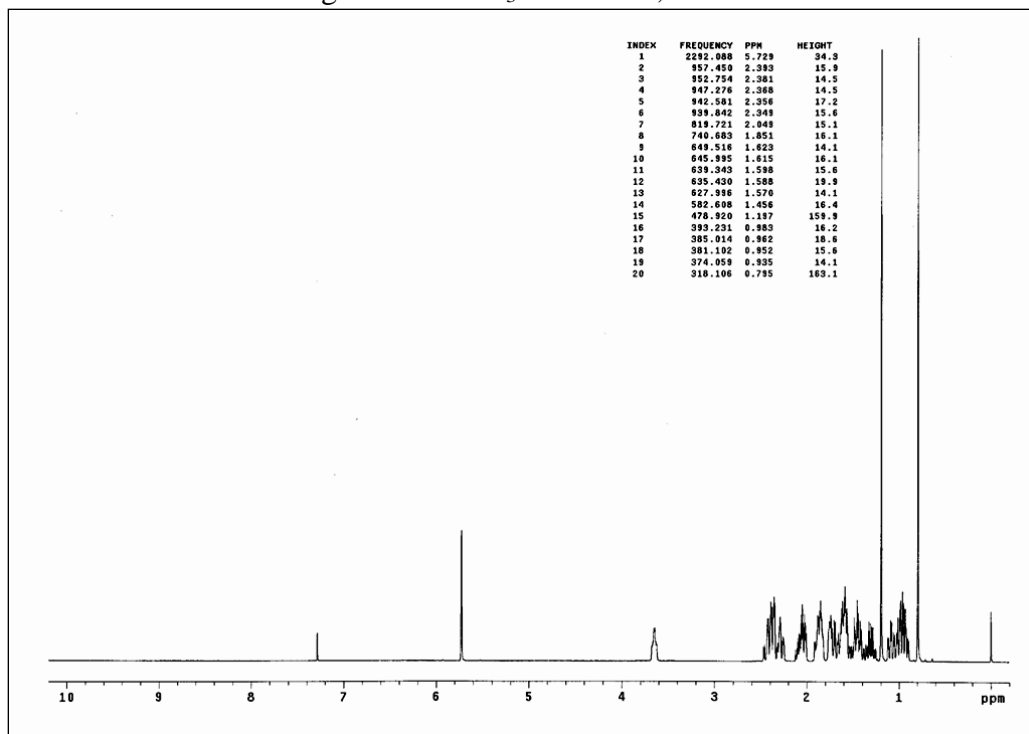
MS (EI): Testosterone  
Quadrupole Detector



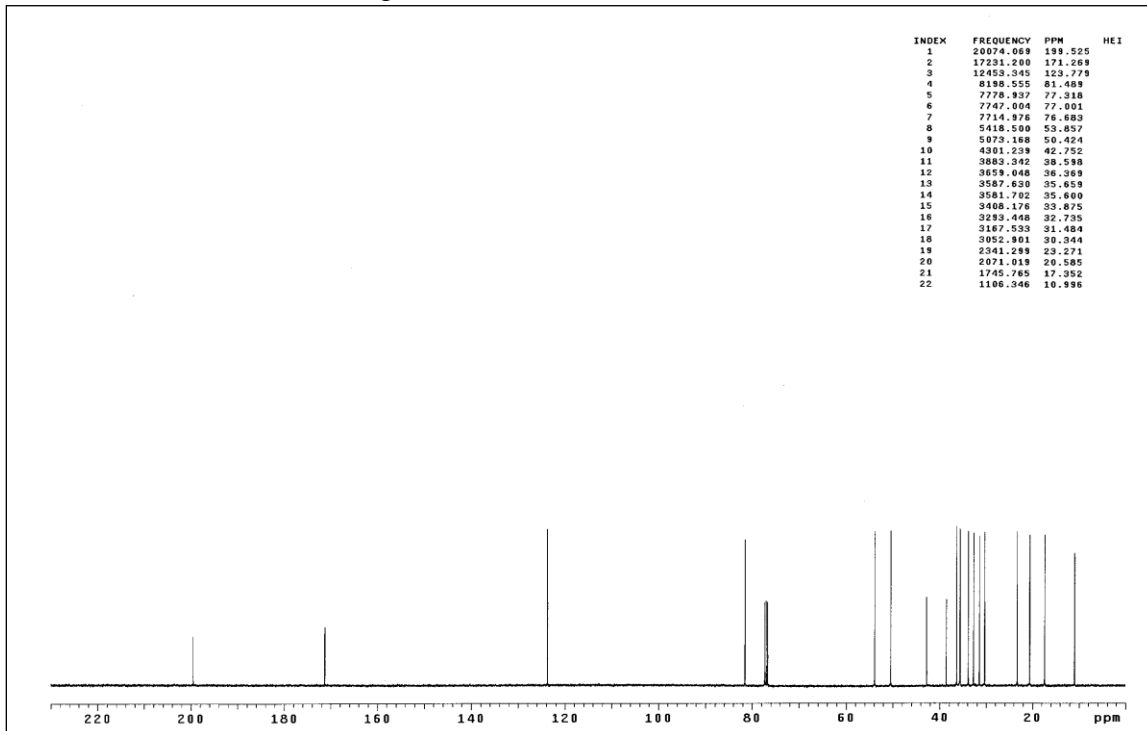
IR (Vapor Phase): Testosterone



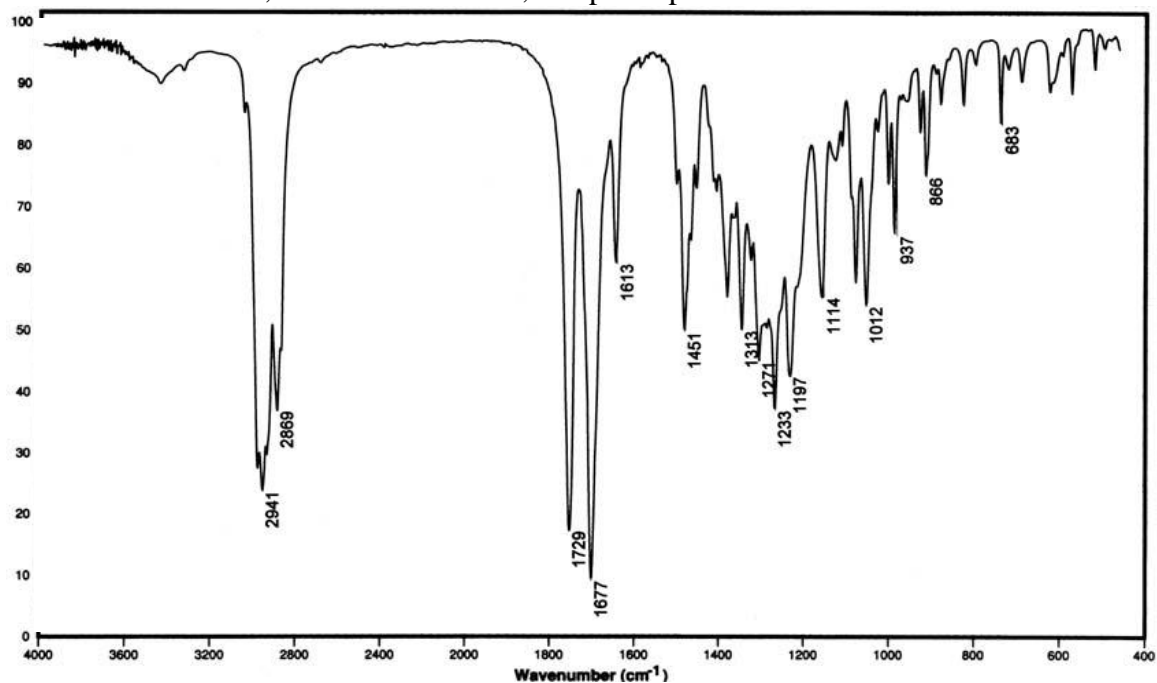
NMR (PROTON): Testosterone  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



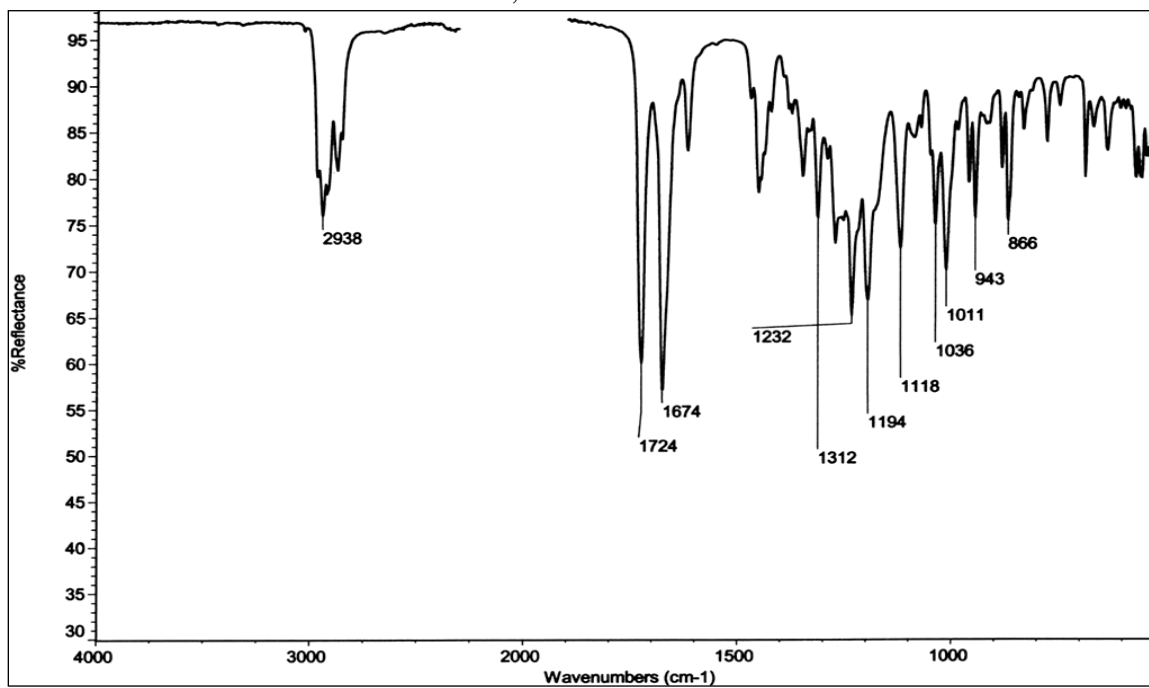
NMR (CARBON): Testosterone  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



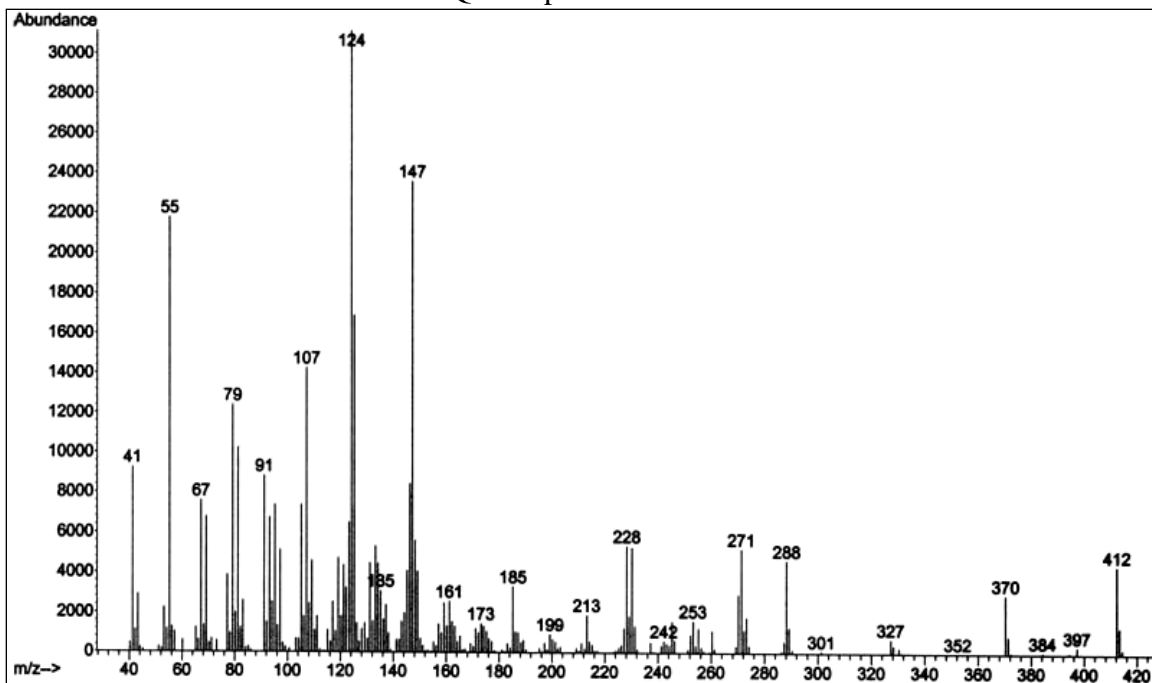
Transmission IR: Testosterone cypionate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution, sample in potassium bromide matrix



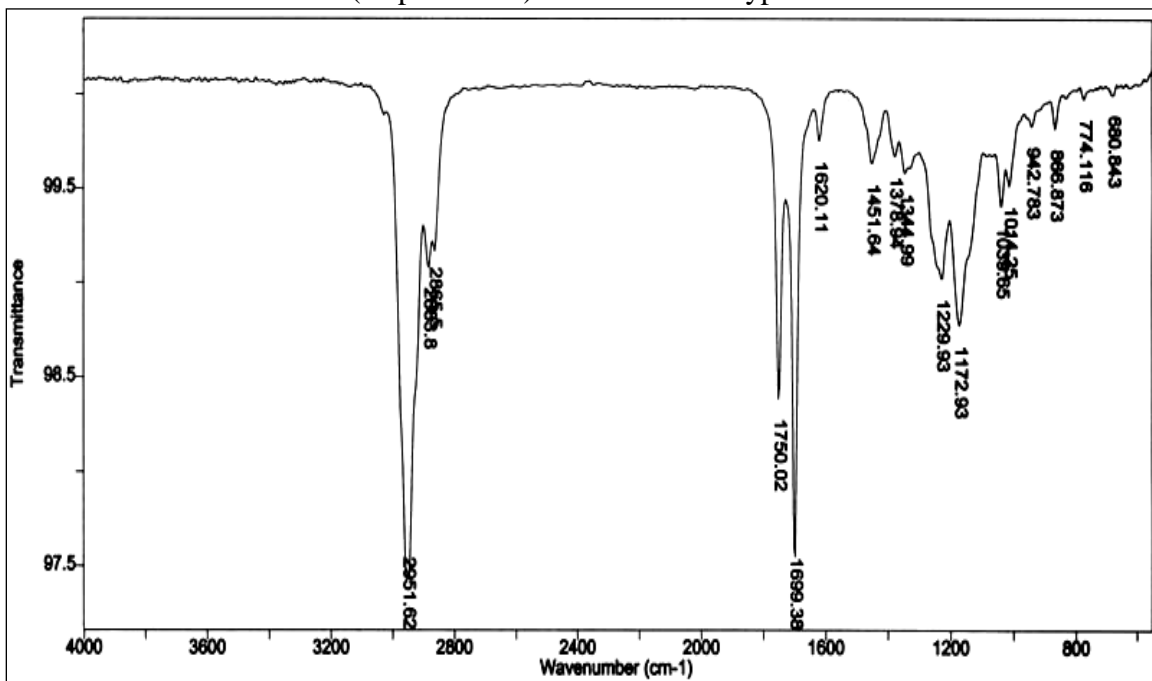
IR (ATR, 3-bounce, diamond device): Testosterone cypionate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution



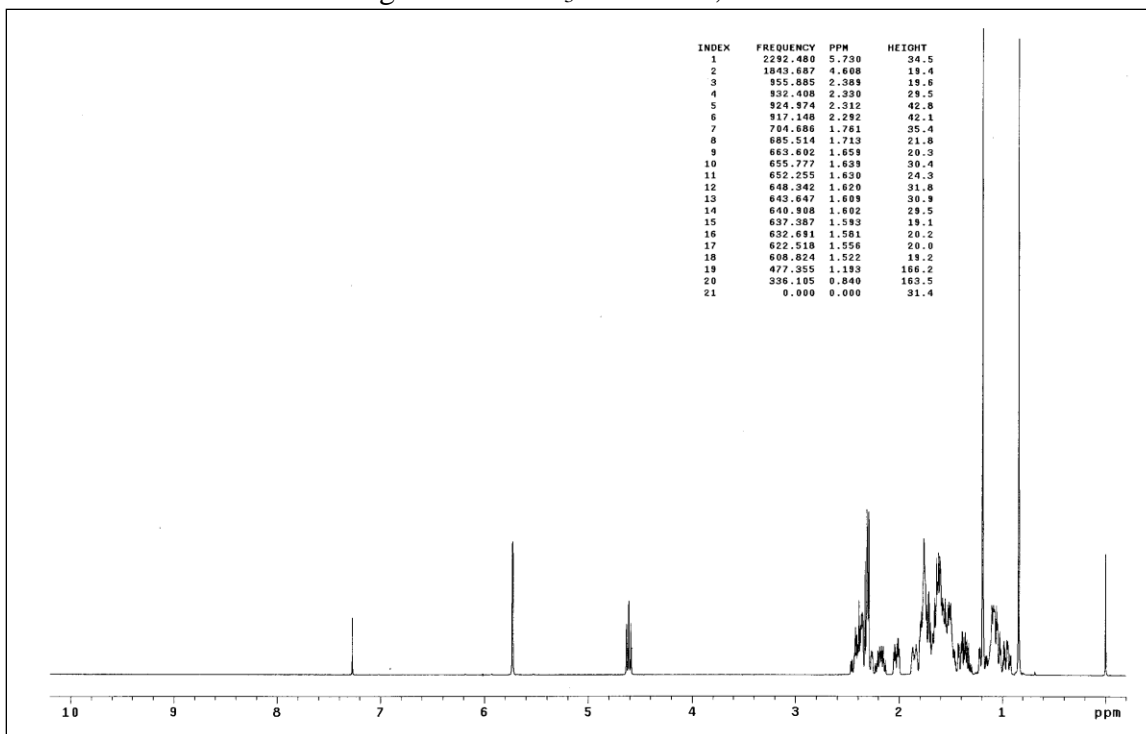
MS (EI): Testosterone cypionate  
Quadrupole Detector



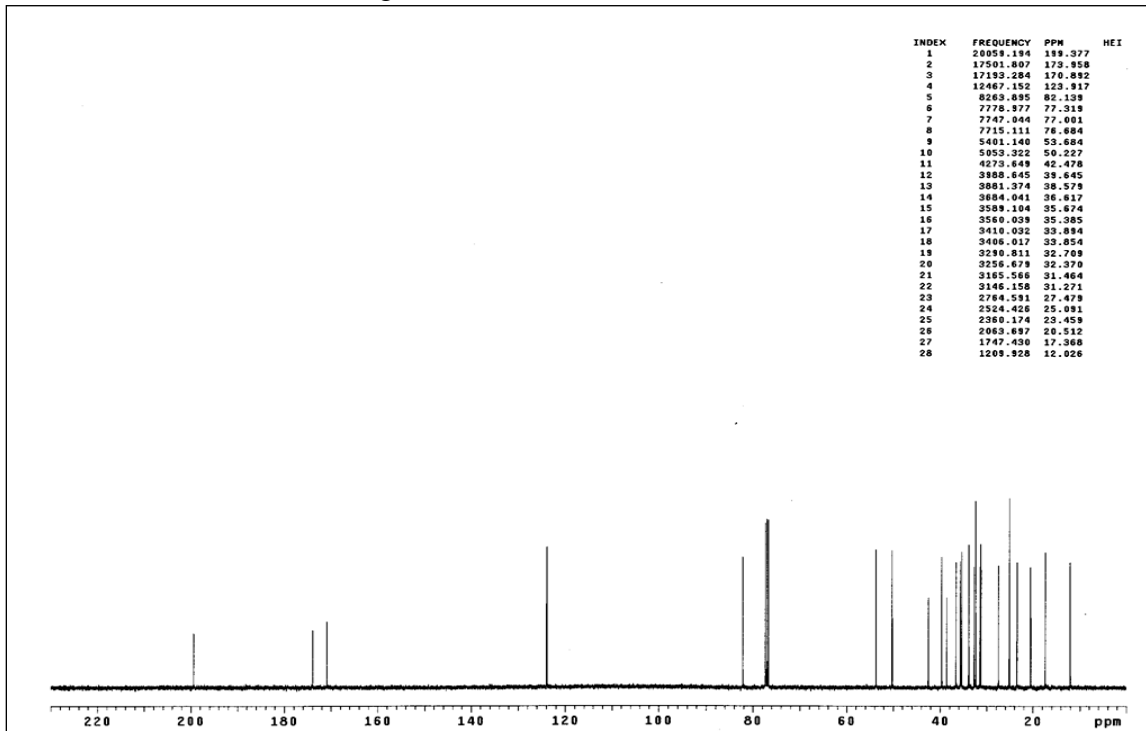
IR (Vapor Phase): Testosterone cypionate



NMR (PROTON): Testosterone cypionate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz

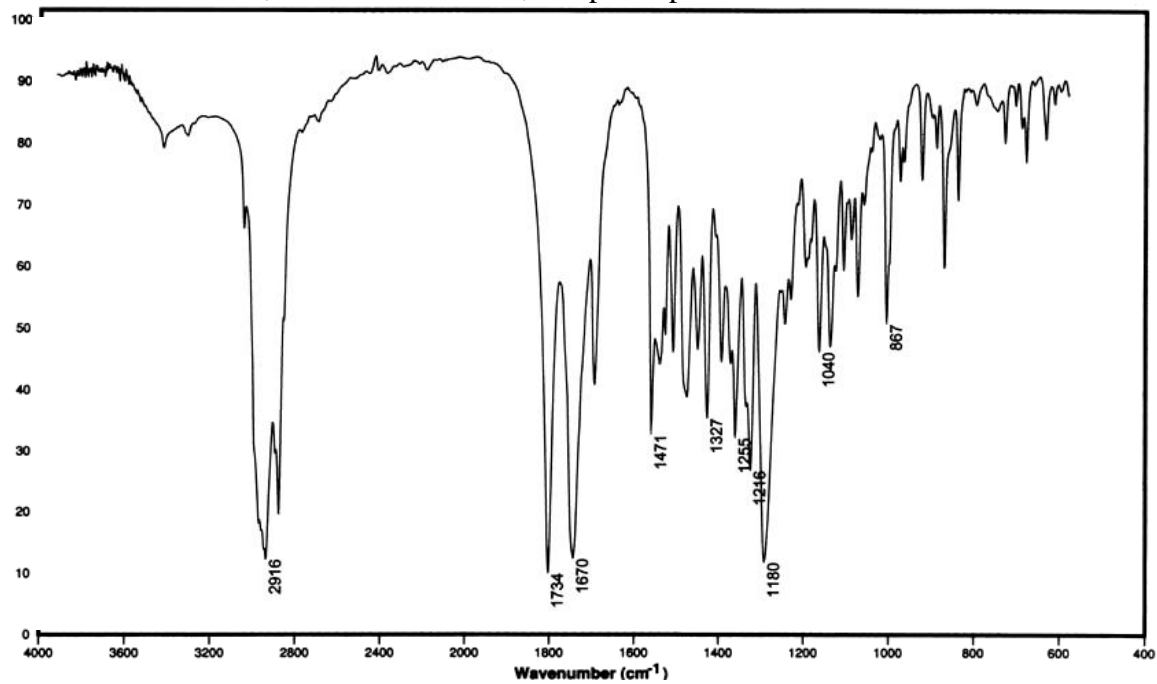


NMR (CARBON): Testosterone cypionate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz

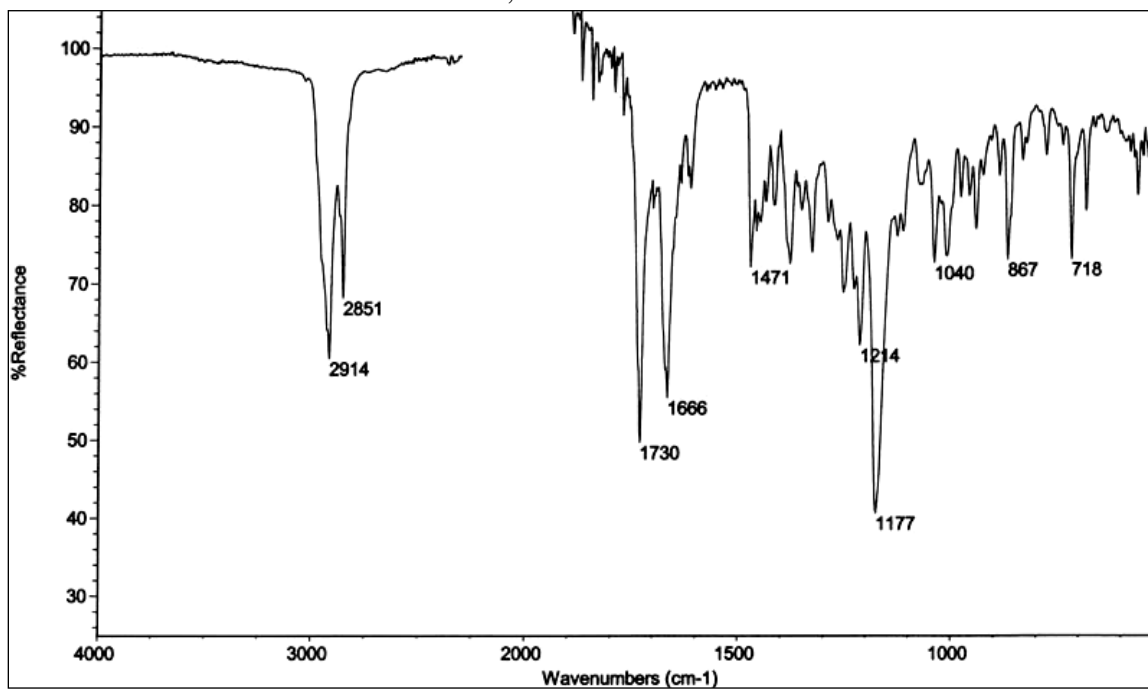




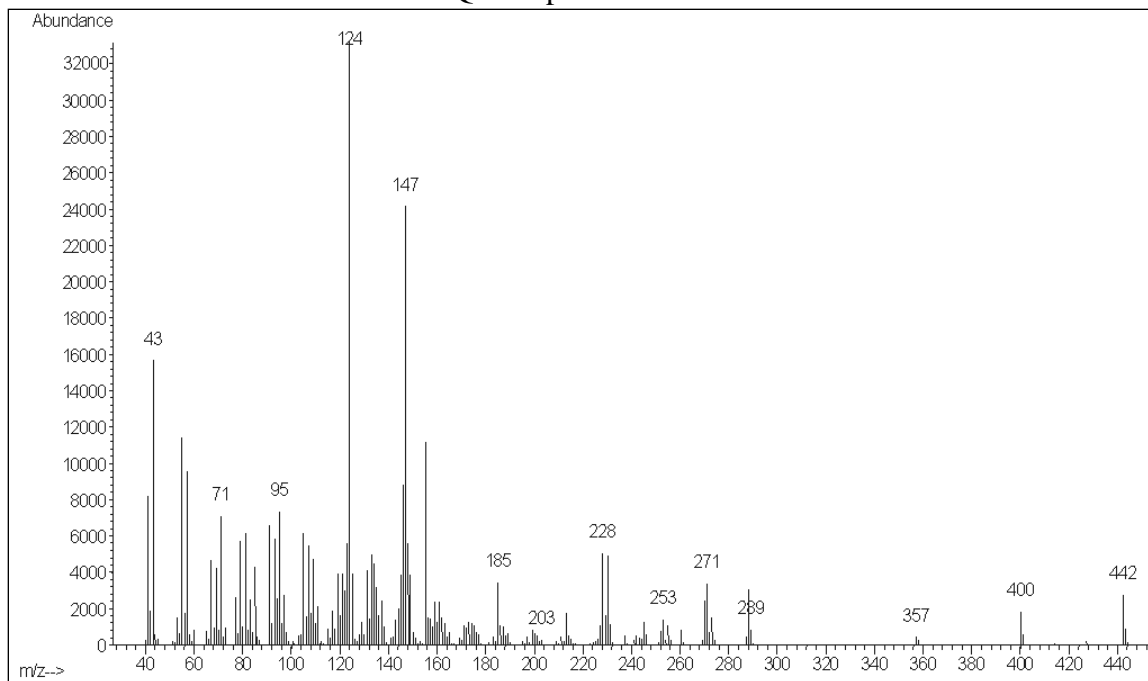
Transmission IR: Testosterone decanoate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution, sample in potassium bromide matrix



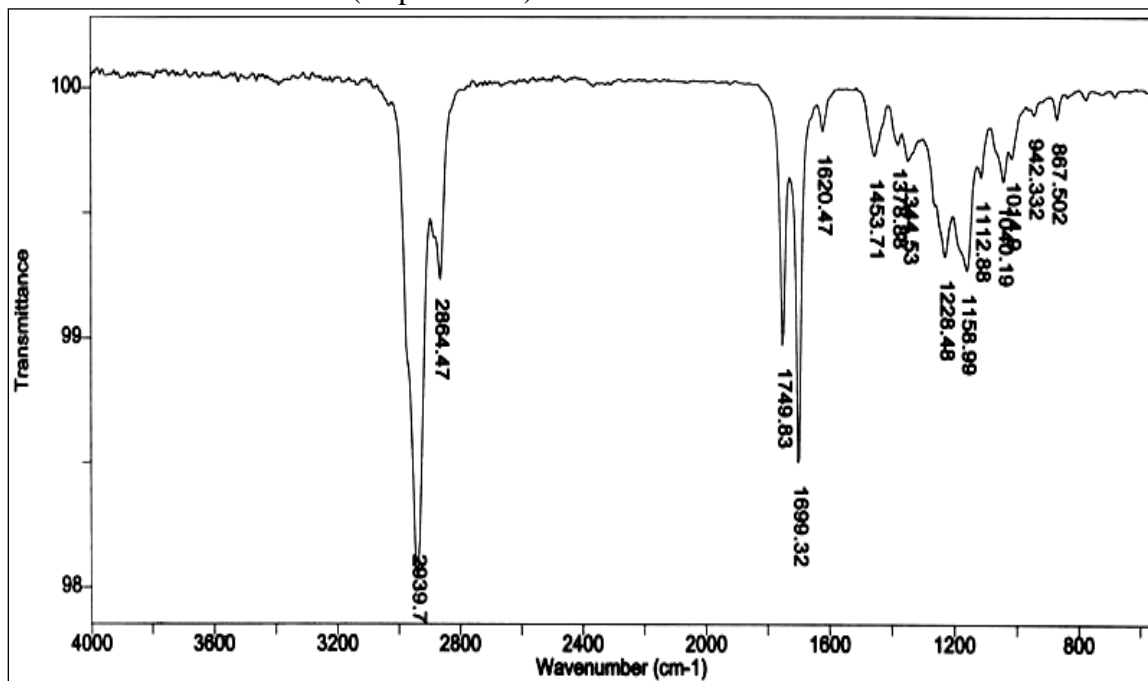
IR (ATR, 3-bounce, diamond device): Testosterone decanoate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution



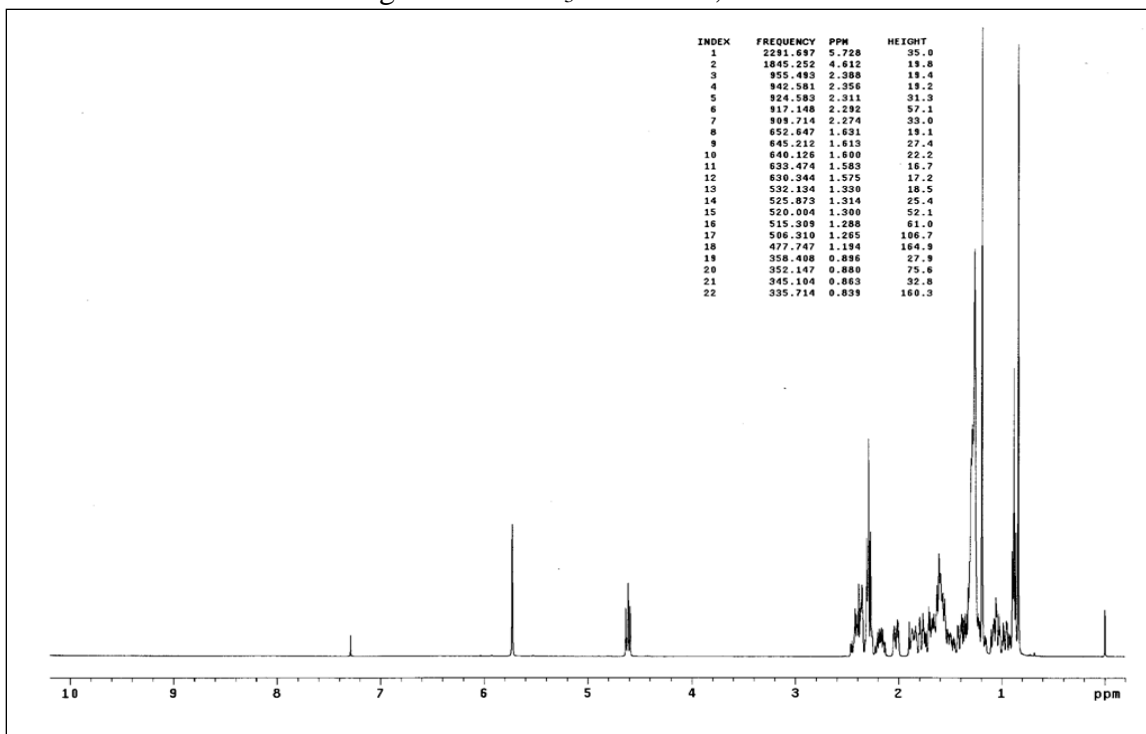
MS (EI): Testosterone decanoate  
Quadrupole Detector



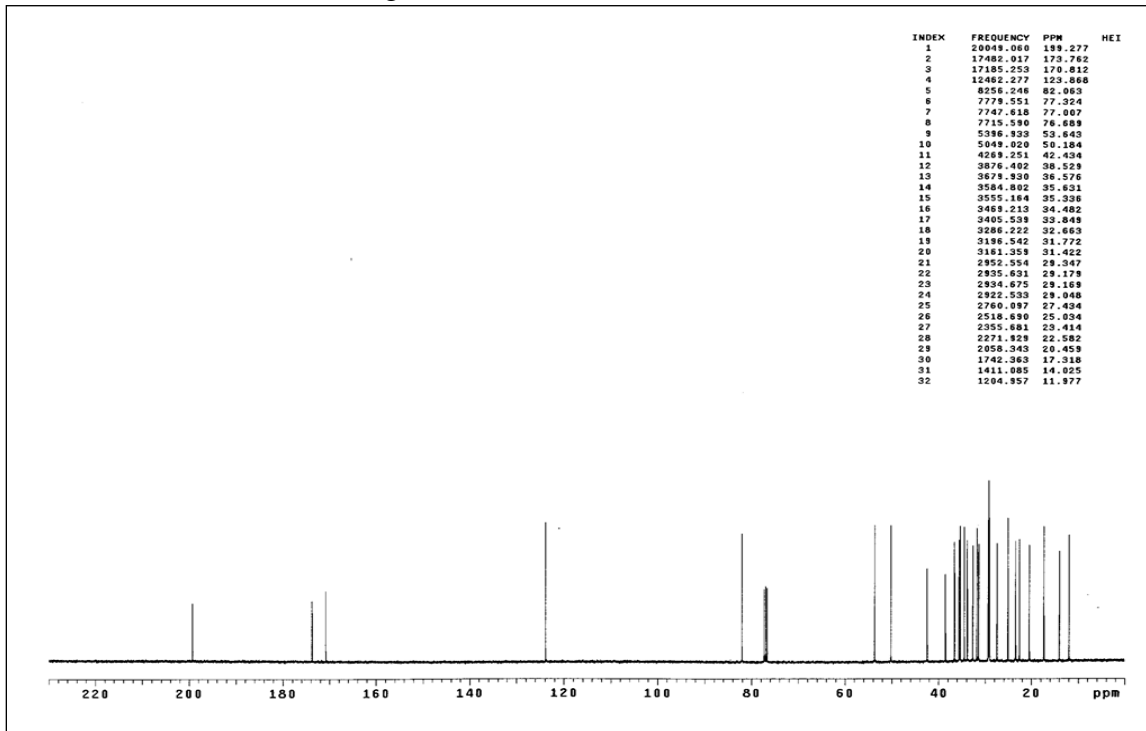
IR (Vapor Phase): Testosterone decanoate



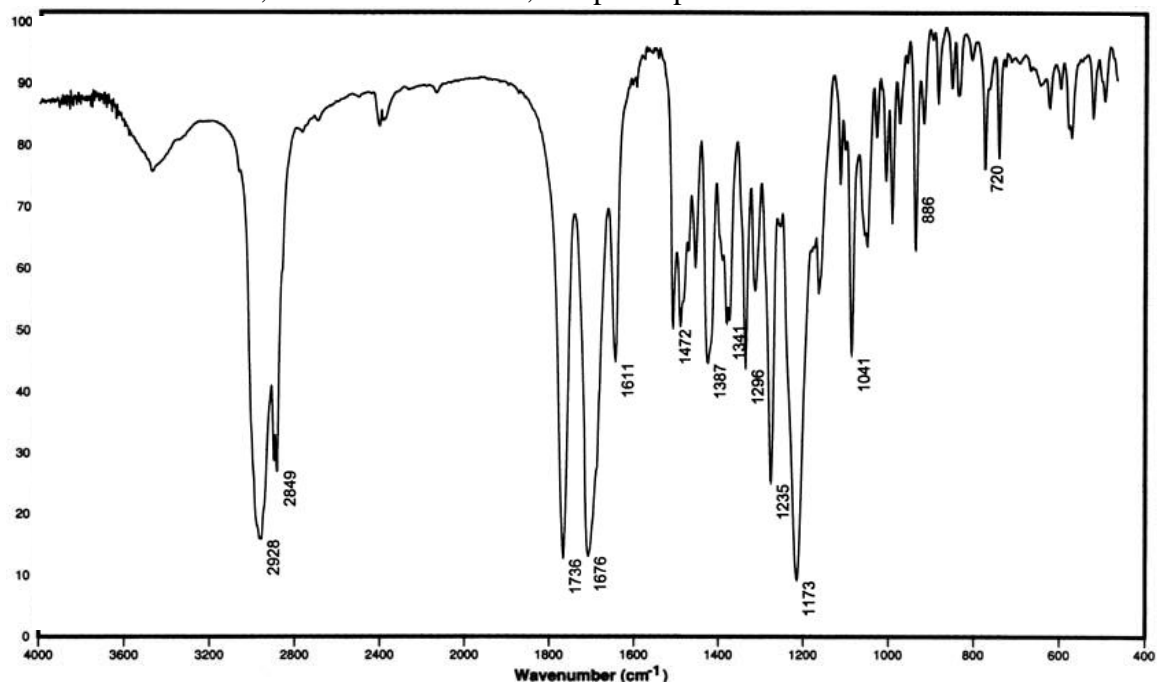
NMR (PROTON): Testosterone Decanoate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



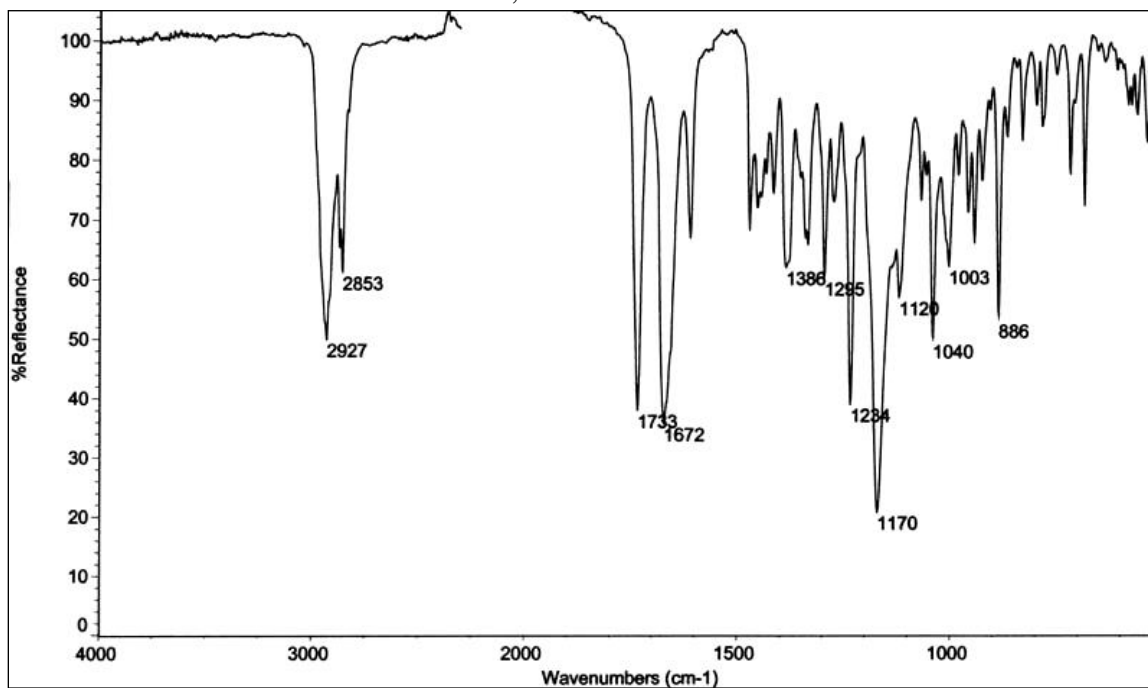
NMR (CARBON) Testosterone decanoate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



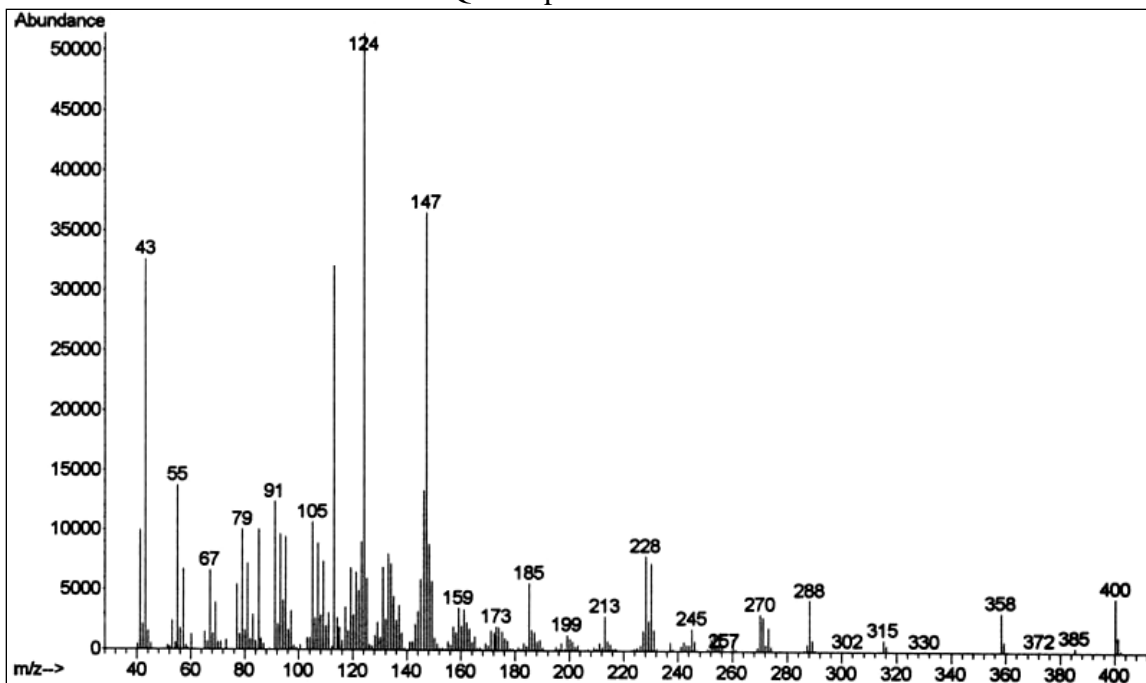
Transmission IR: Testosterone enanthate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution, sample in potassium bromide matrix



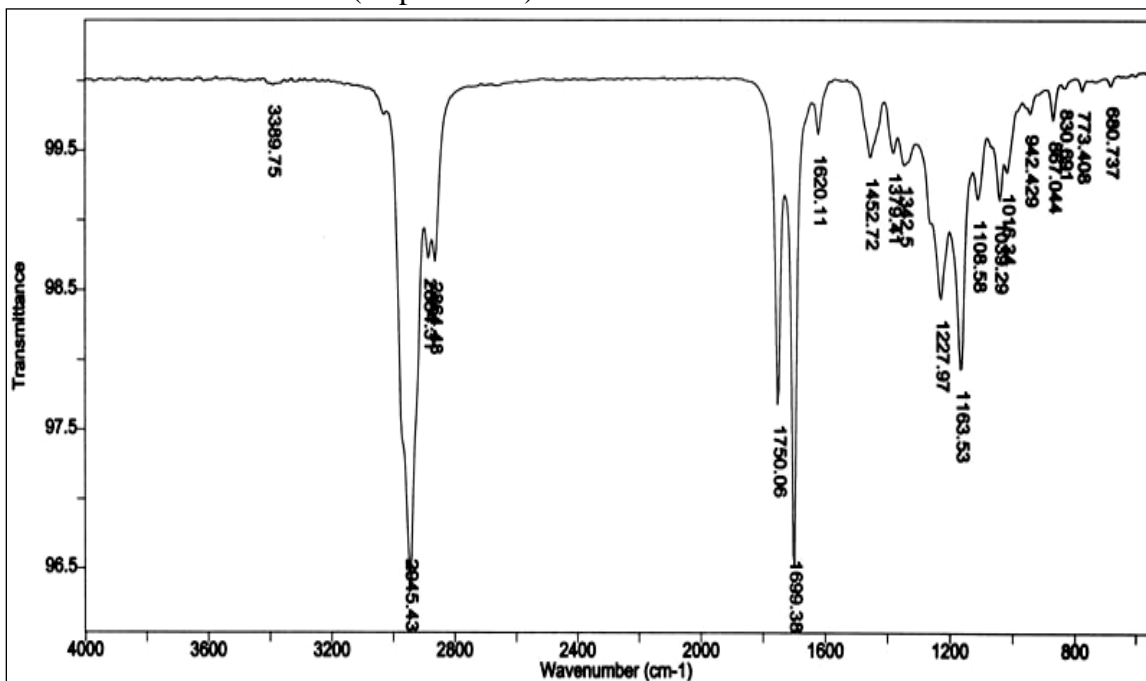
IR (ATR 3-bounce, diamond device): Testosterone enanthate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution



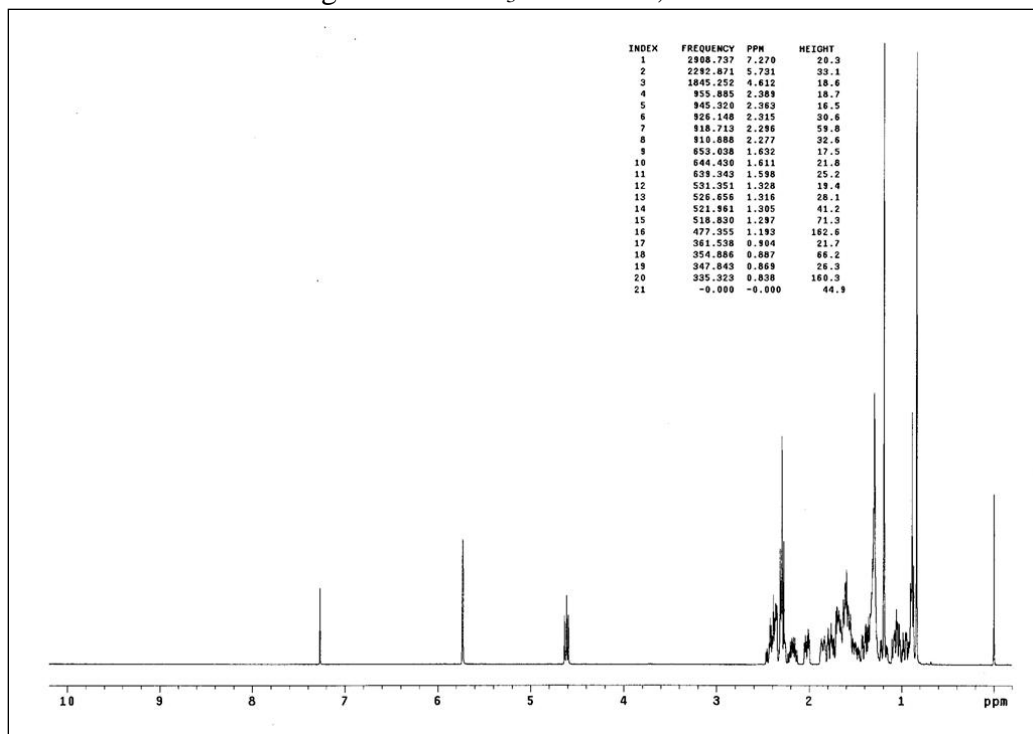
Mass Spectrum (EI): Testosterone enanthate  
Quadrupole Detector



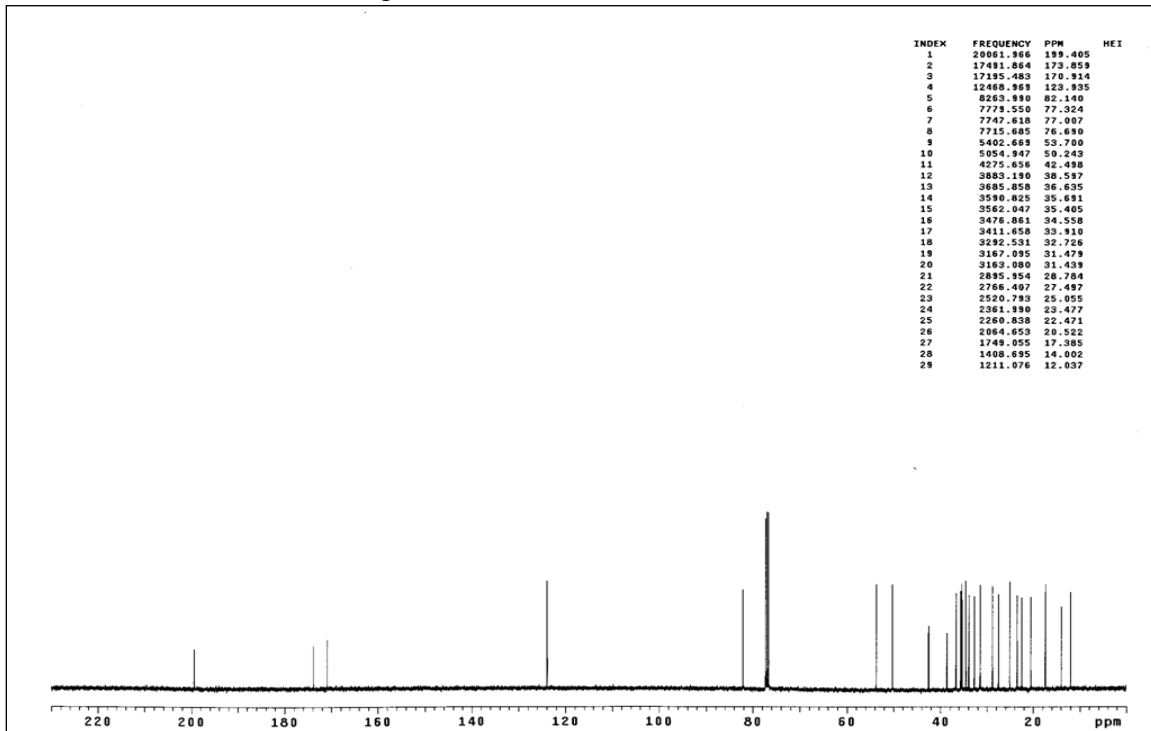
IR (Vapor Phase): Testosterone enanthate



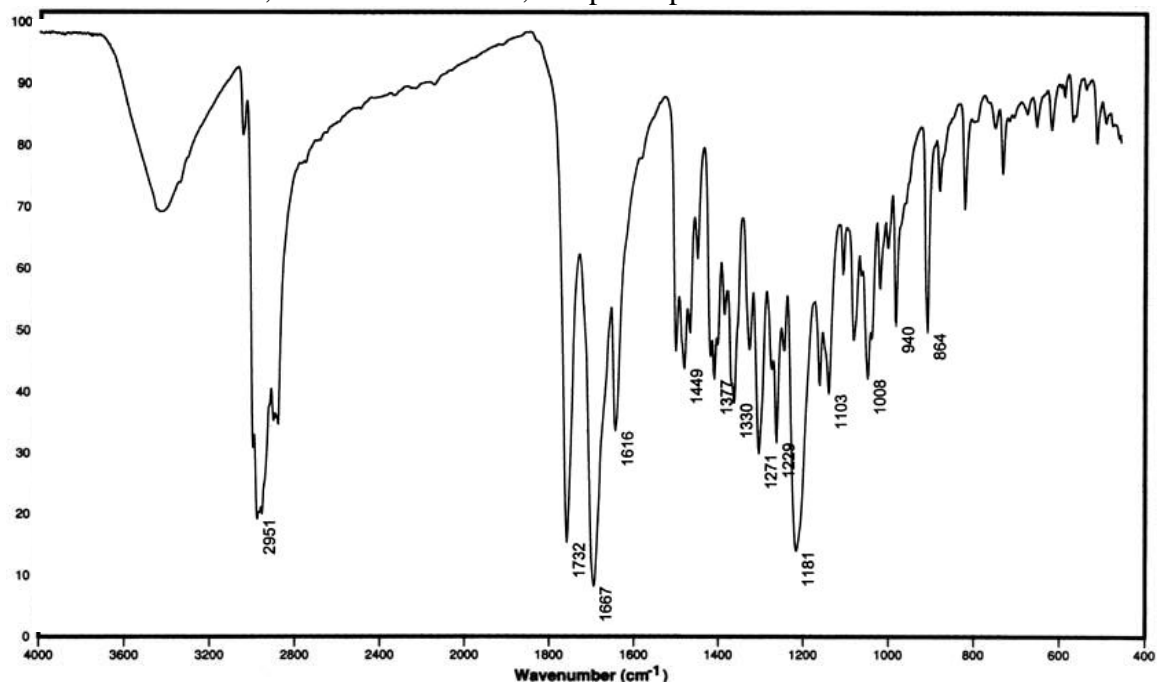
NMR (PROTON): Testosterone enanthate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



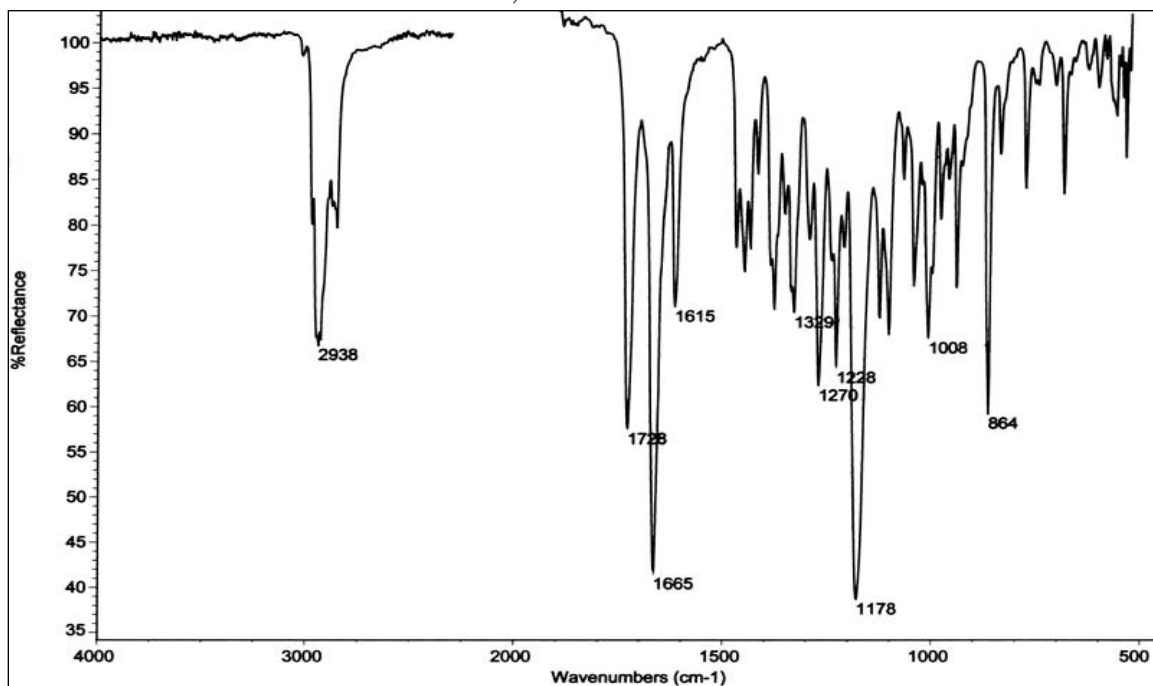
NMR (CARBON): Testosterone enanthate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



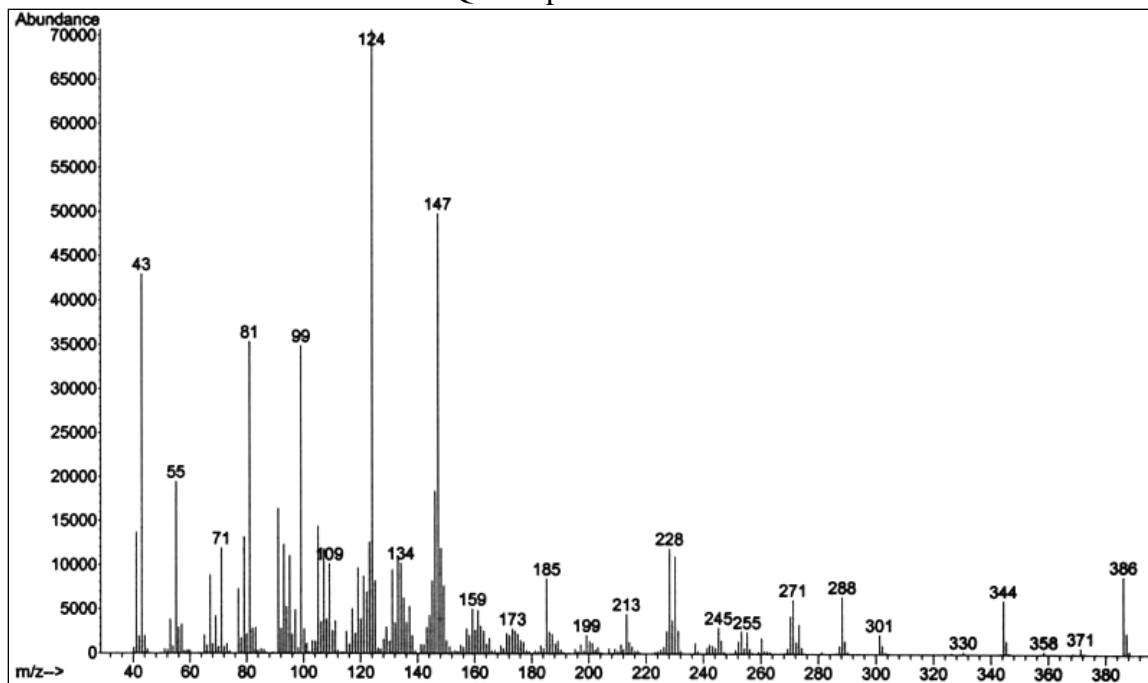
Transmission IR: Testosterone isocaproate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution, sample in potassium bromide matrix



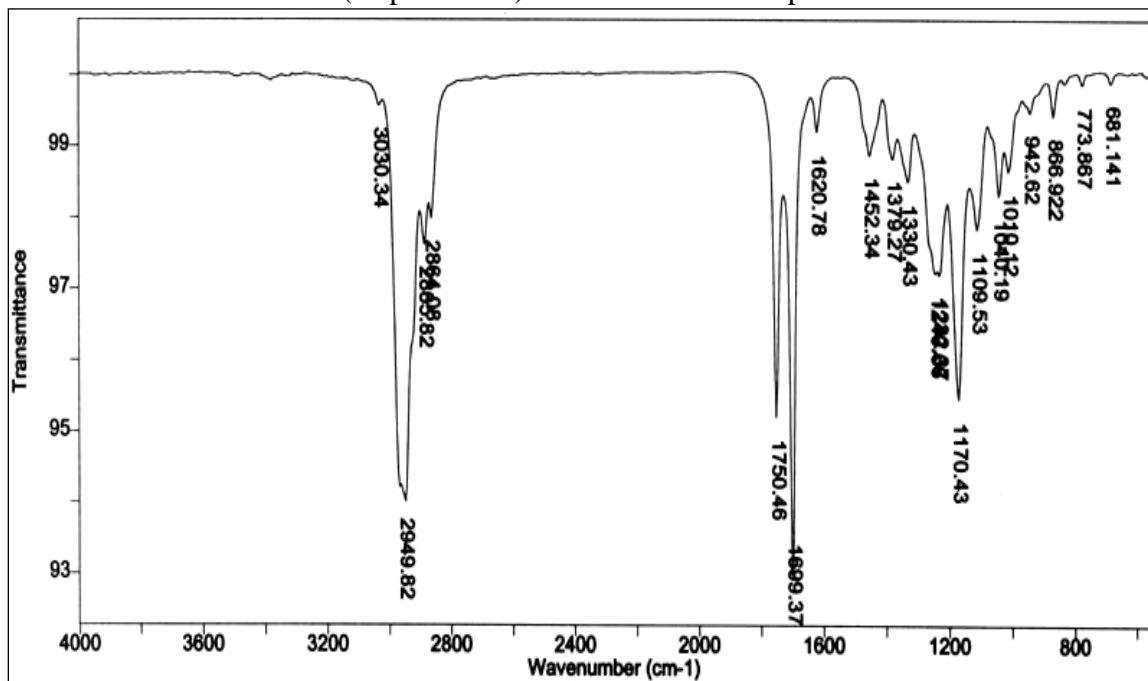
IR (ATR, 3-bounce diamond device): Testosterone isocaproate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution



MS (EI): Testosterone isocaproate  
Quadrupole Detector

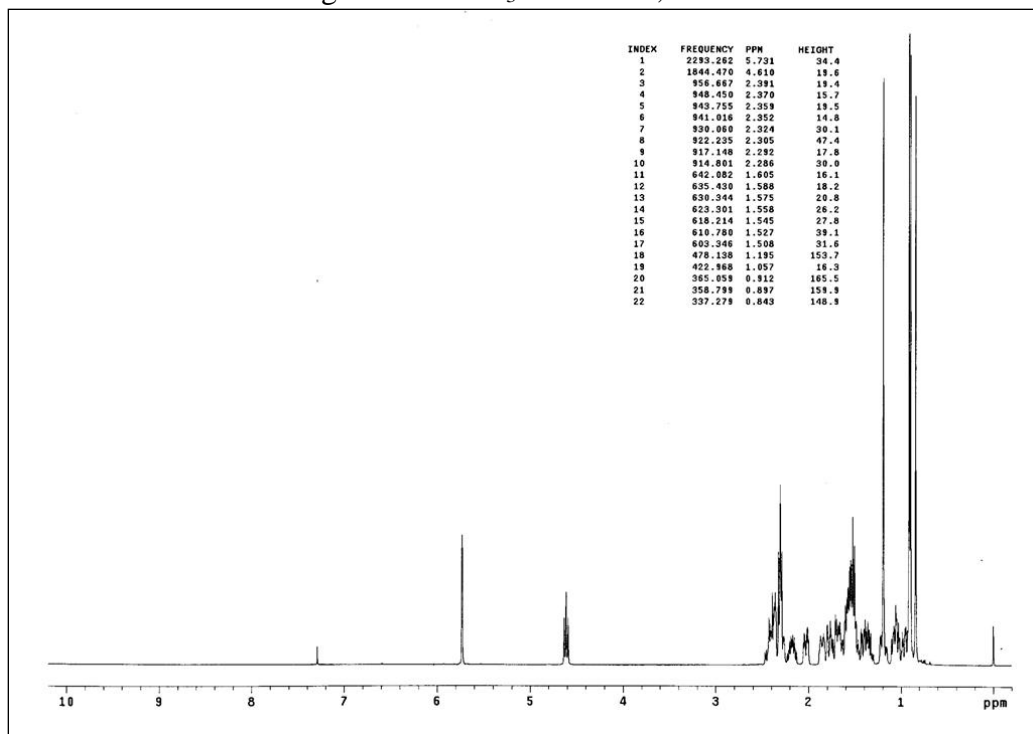


IR (Vapor Phase): Testosterone isocaproate

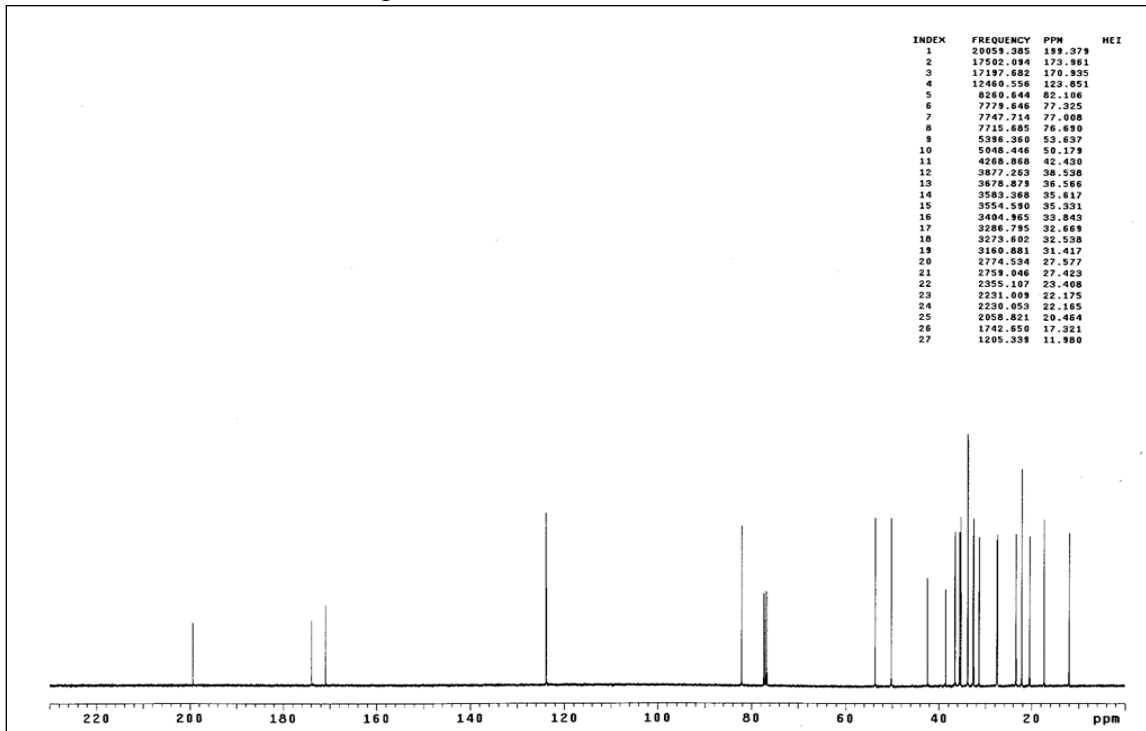




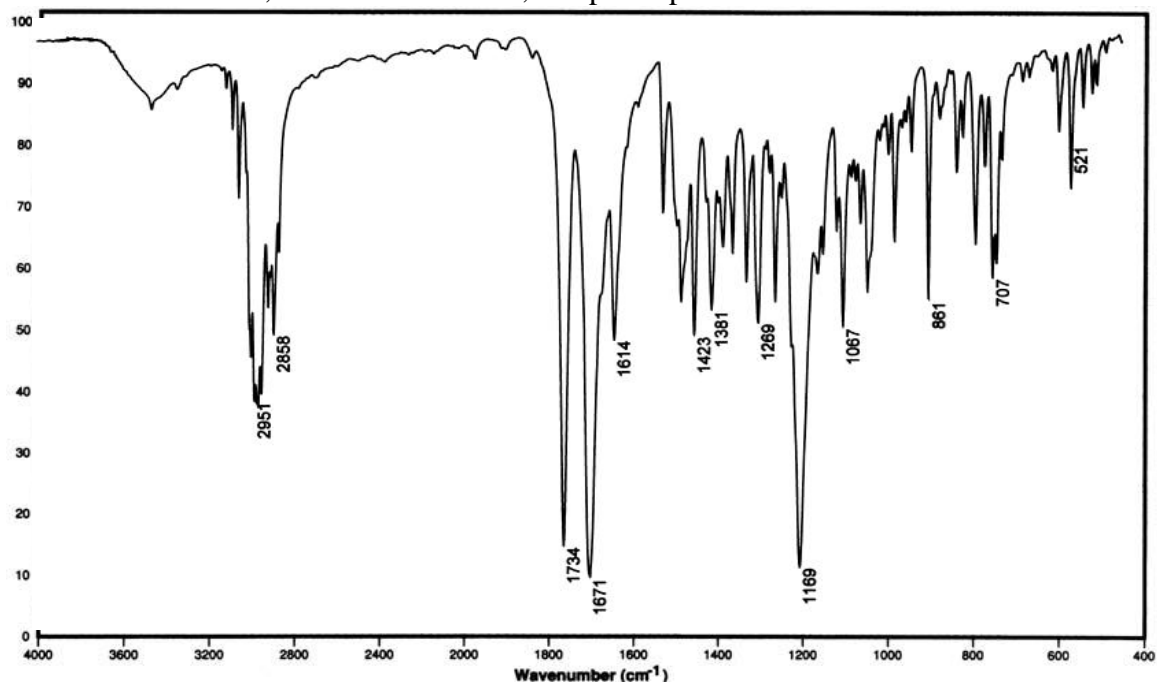
NMR (PROTON): Testosterone isocaproate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



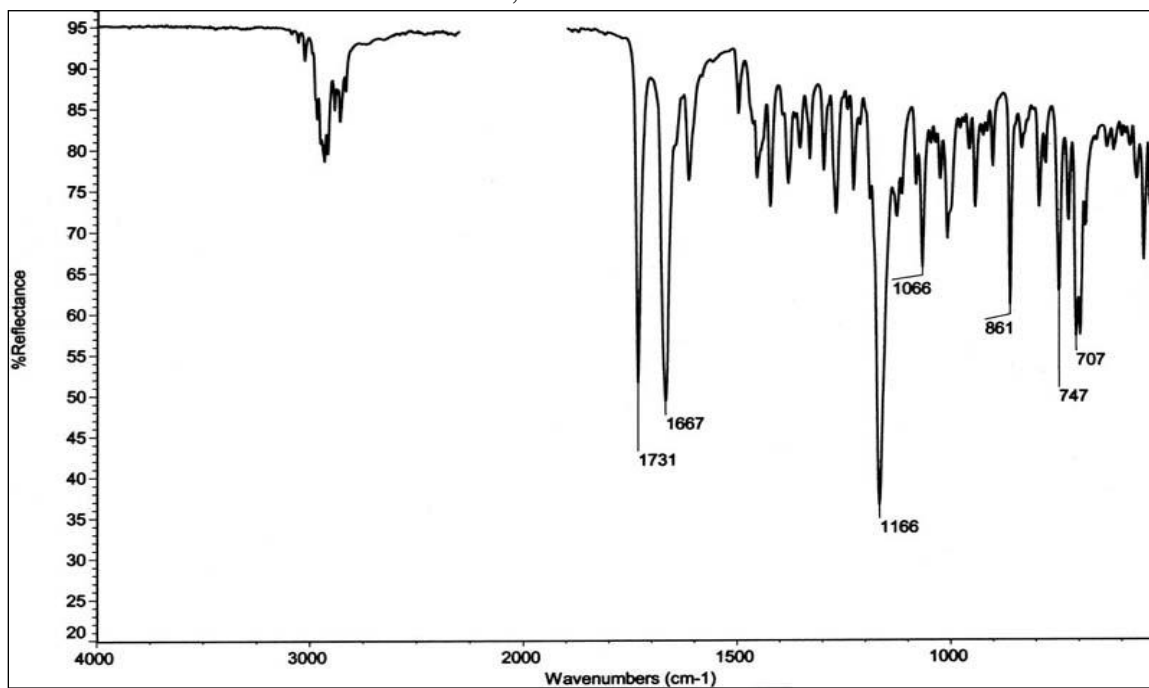
NMR (CARBON): Testosterone isocaproate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



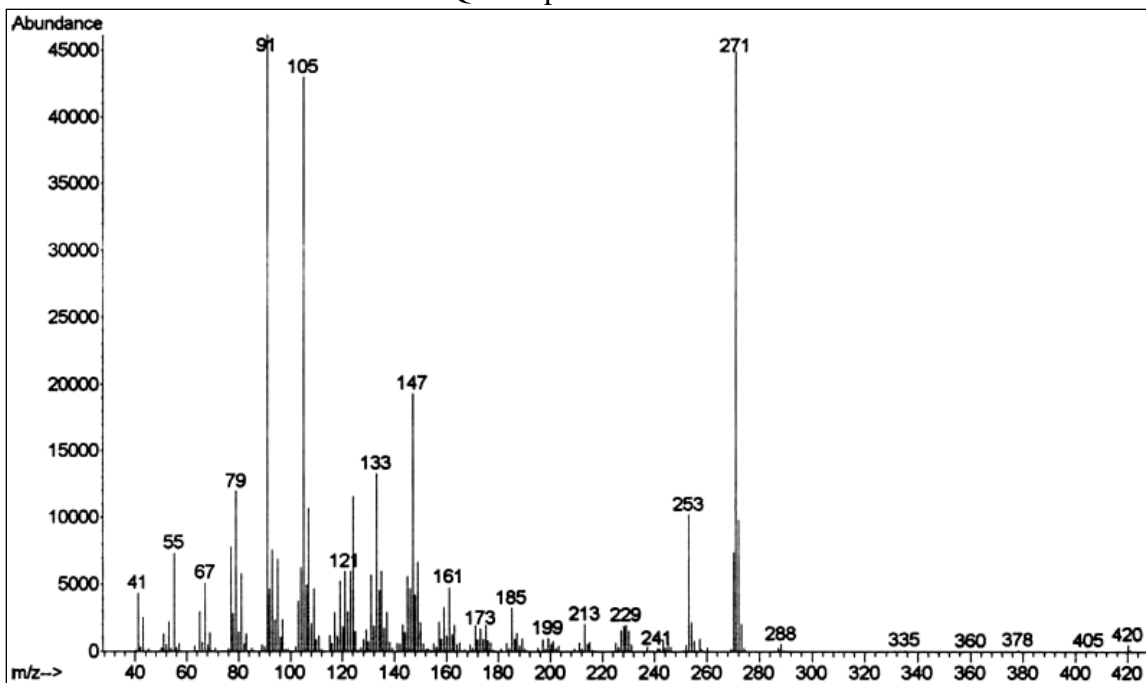
Transmission IR: Testosterone phenylpropionate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution, sample in potassium bromide matrix



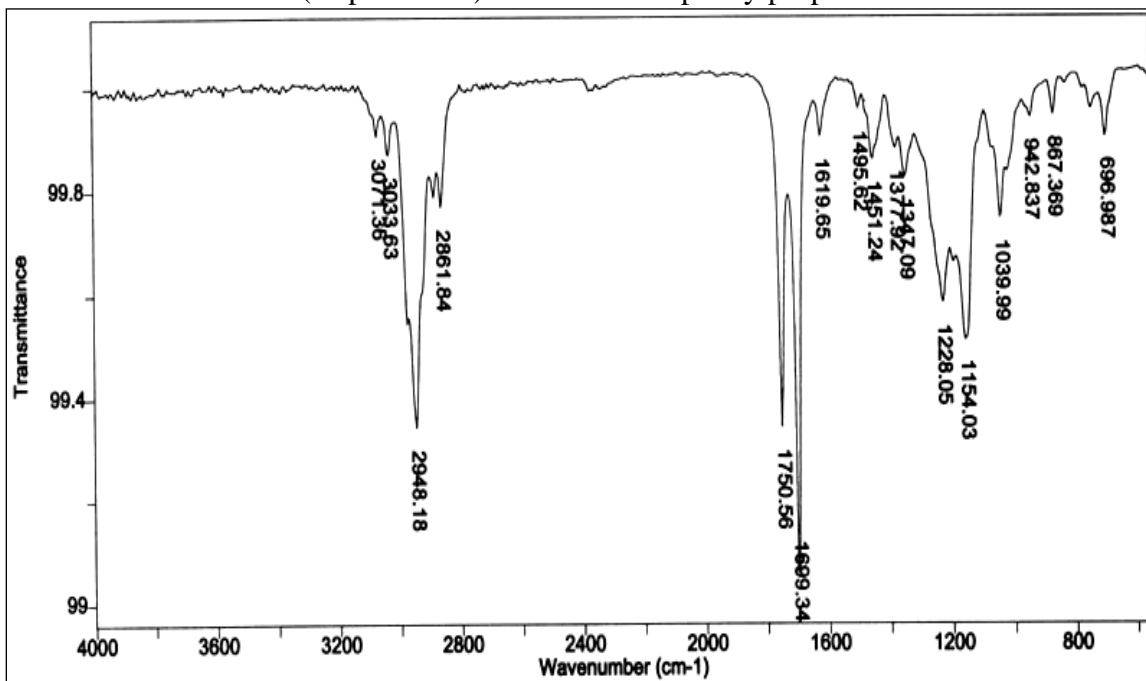
IR (ATR, 3-bounce diamond device): Testosterone phenylpropionate  
16 scans, 4.0  $\text{cm}^{-1}$  resolution



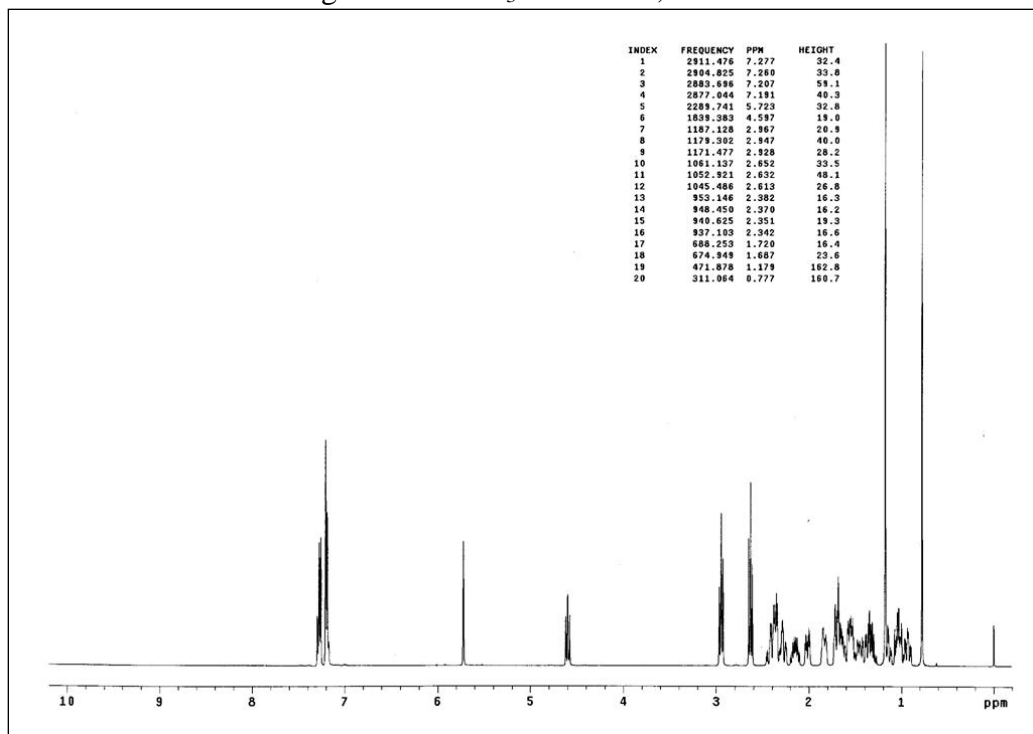
MS (EI): Testosterone phenylpropionate  
Quadrupole Detector



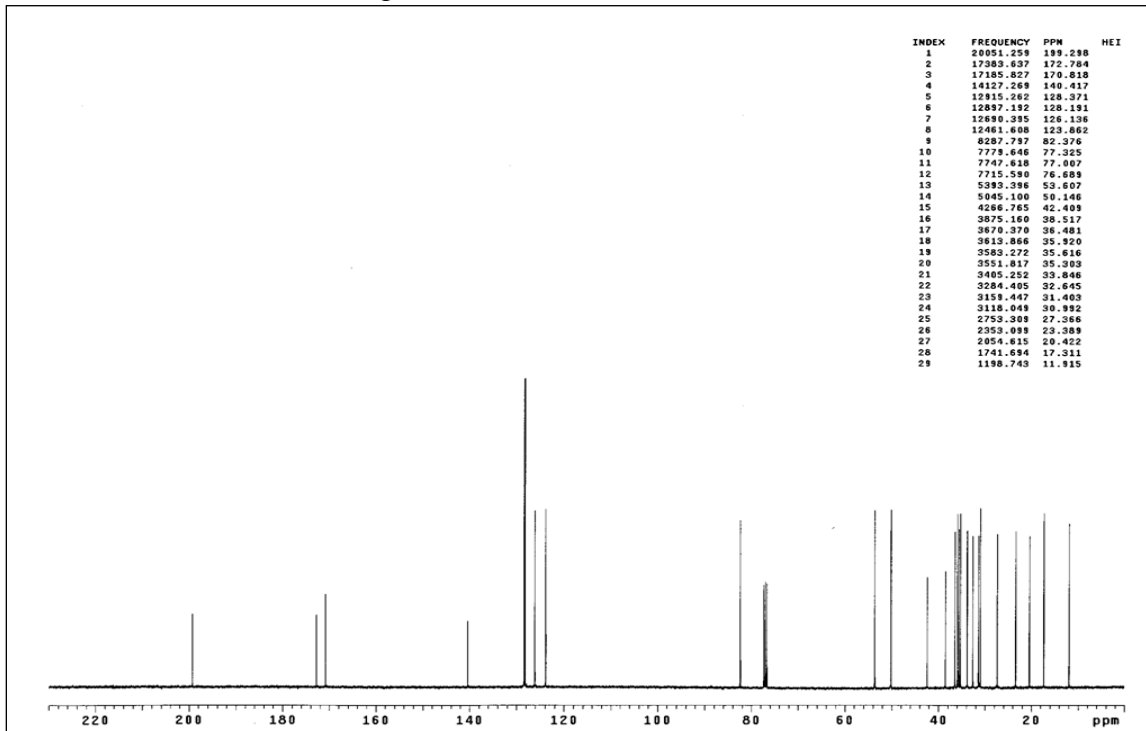
IR (Vapor Phase): Testosterone phenylpropionate



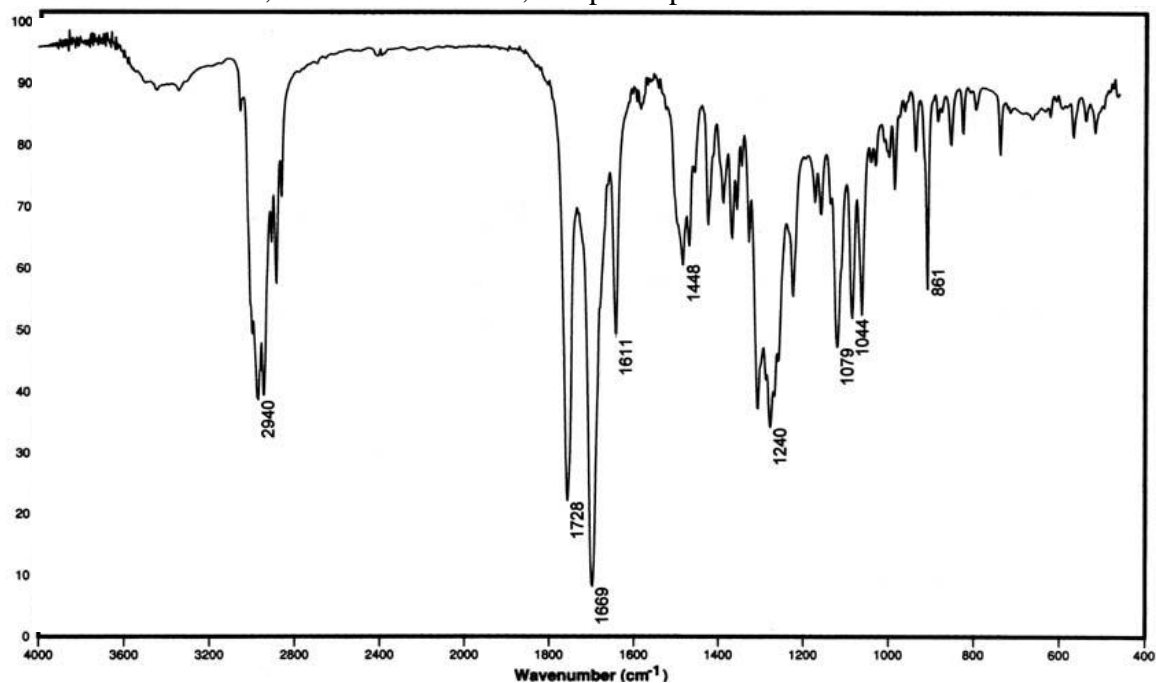
NMR (PROTON): Testosterone phenylpropionate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



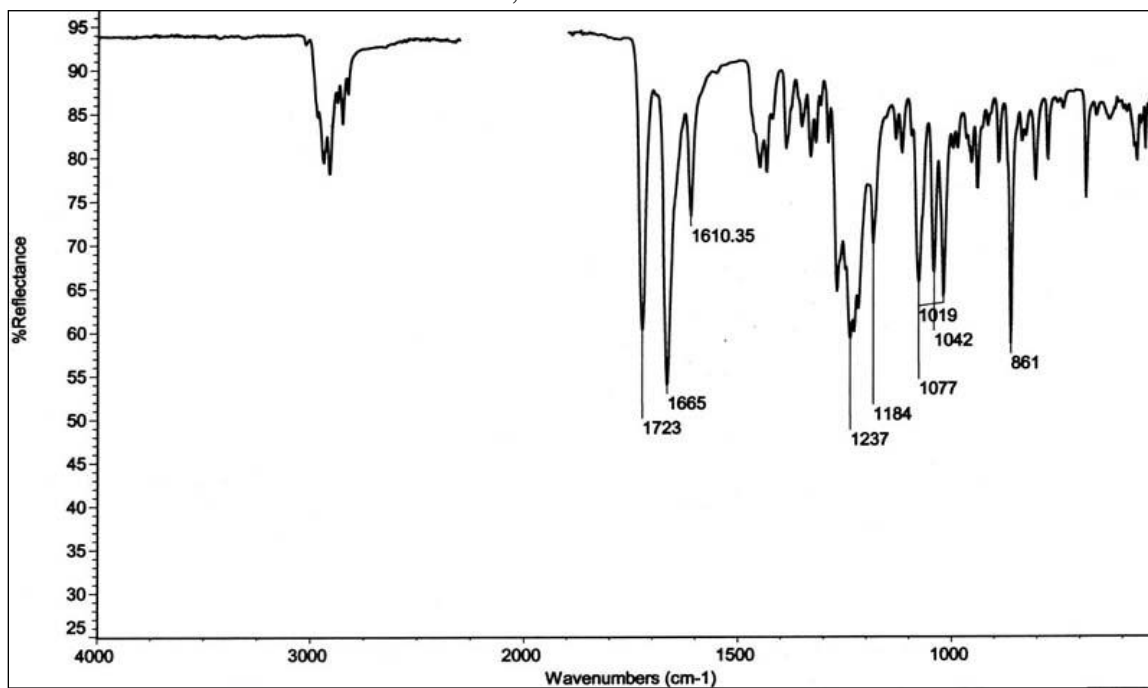
NMR (CARBON): Testosterone phenylpropionate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



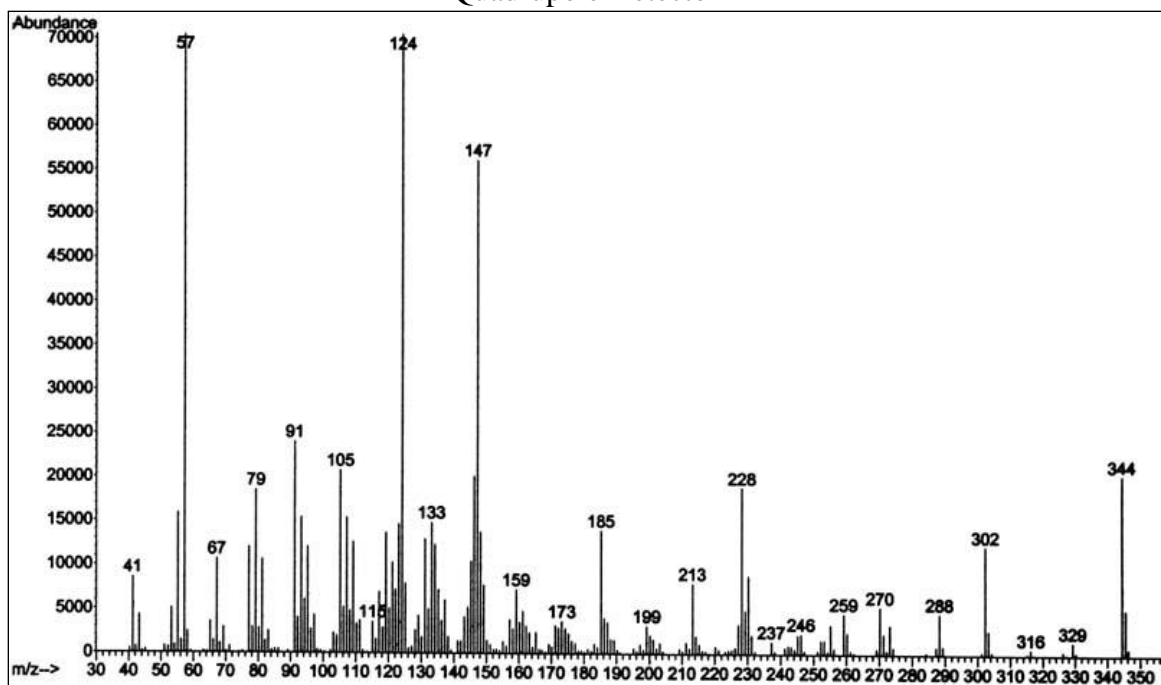
Transmission IR: Testosterone propionate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution, sample in potassium bromide matrix



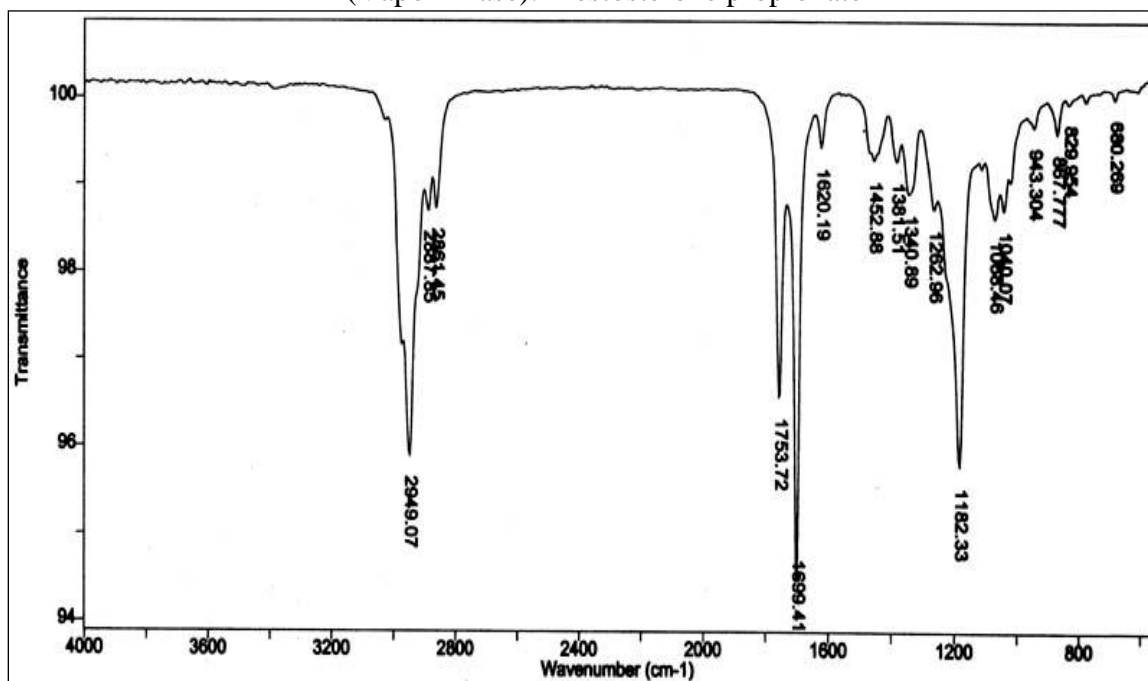
IR (ATR 3-bounce diamond device): Testosterone propionate  
16 scans,  $4.0\text{ cm}^{-1}$  resolution



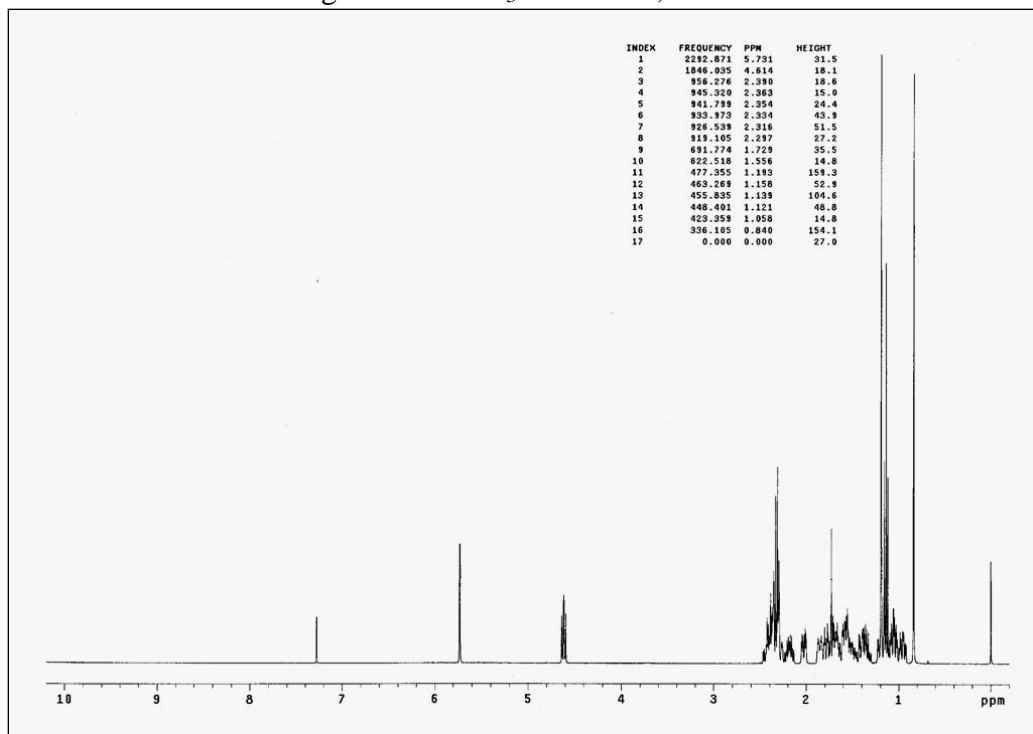
MS (EI): Testosterone propionate  
Quadrupole Detector



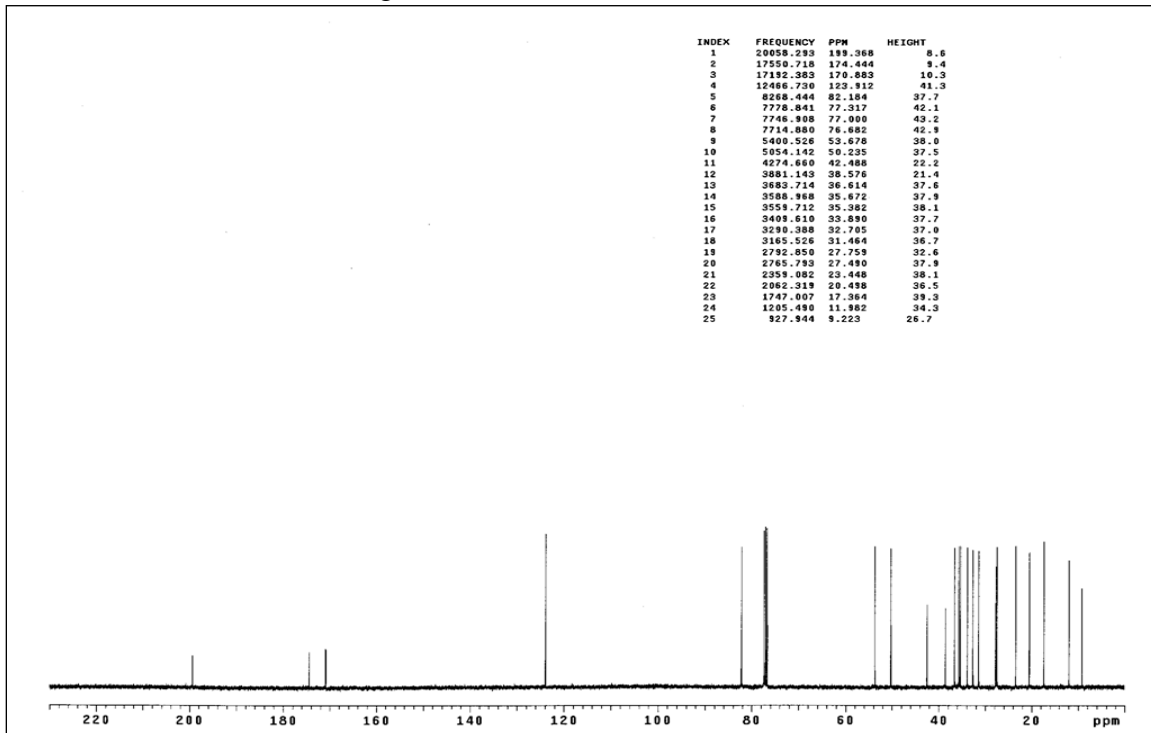
IR (Vapor Phase): Testosterone propionate



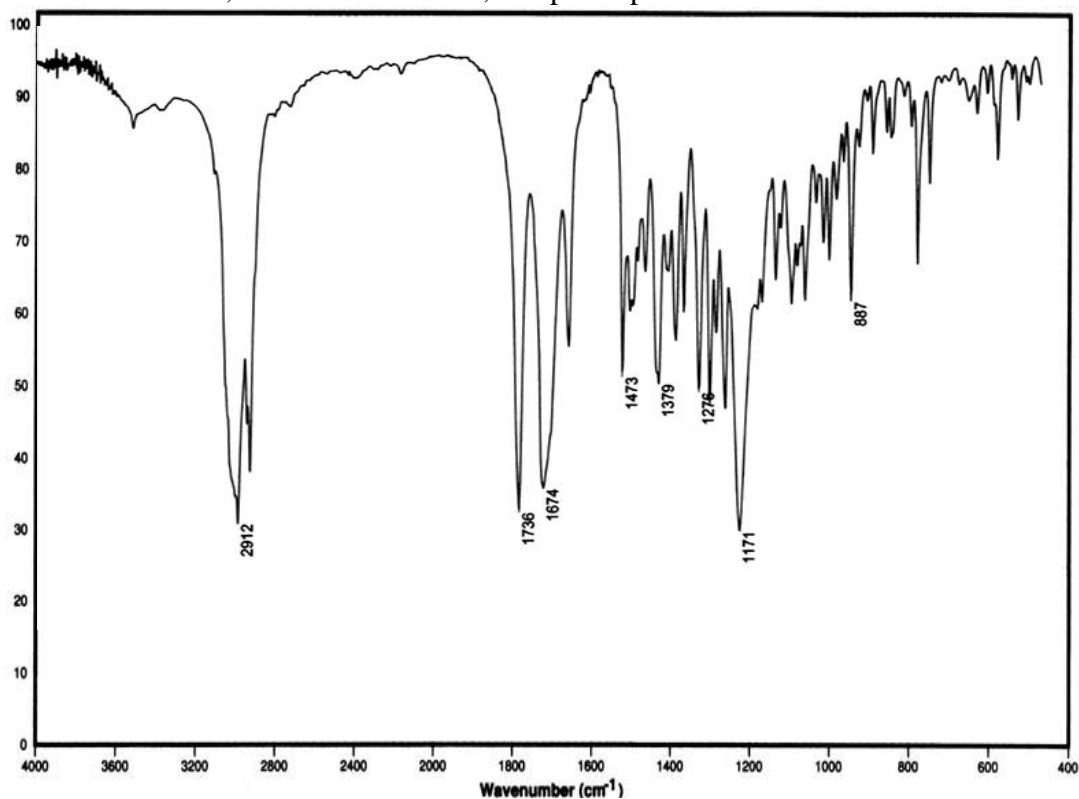
NMR (PROTON): Testosterone propionate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



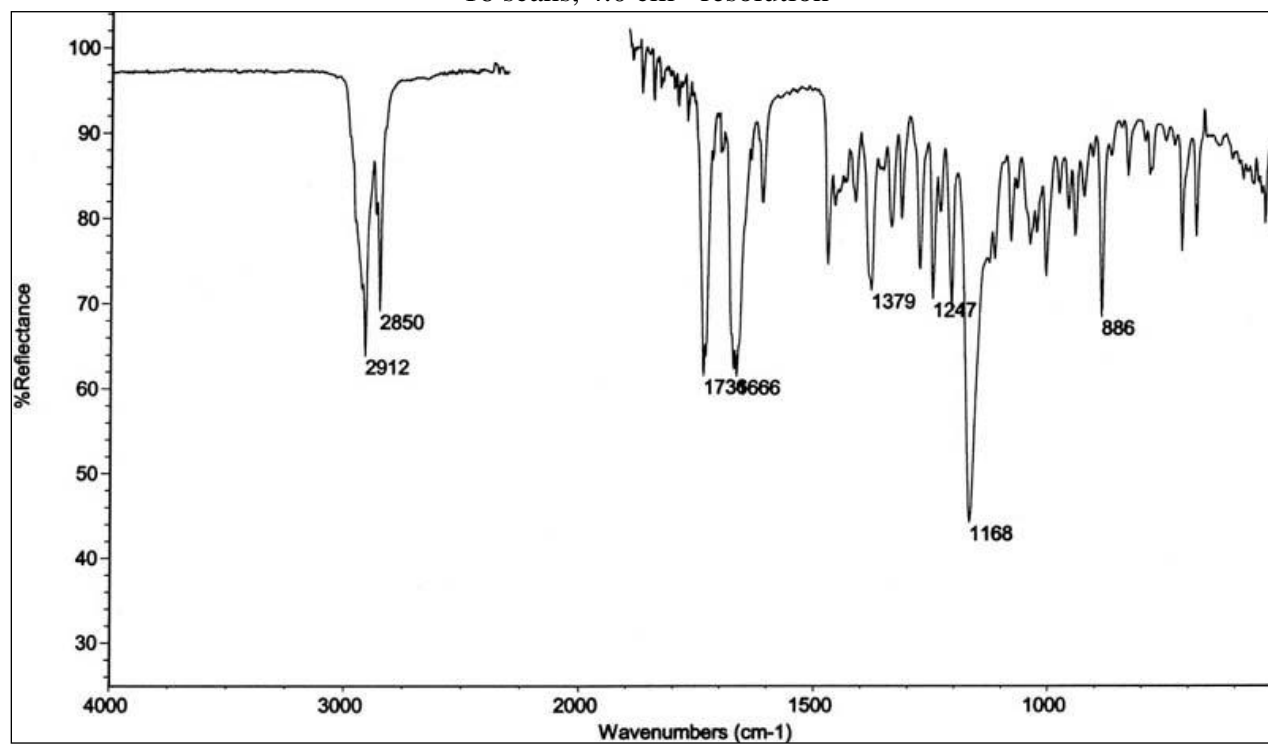
NMR (CARBON): Testosterone propionate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz



Transmission IR: Testosterone undecanoate  
16 scans, 4.0 cm<sup>-1</sup> resolution, sample in potassium bromide matrix

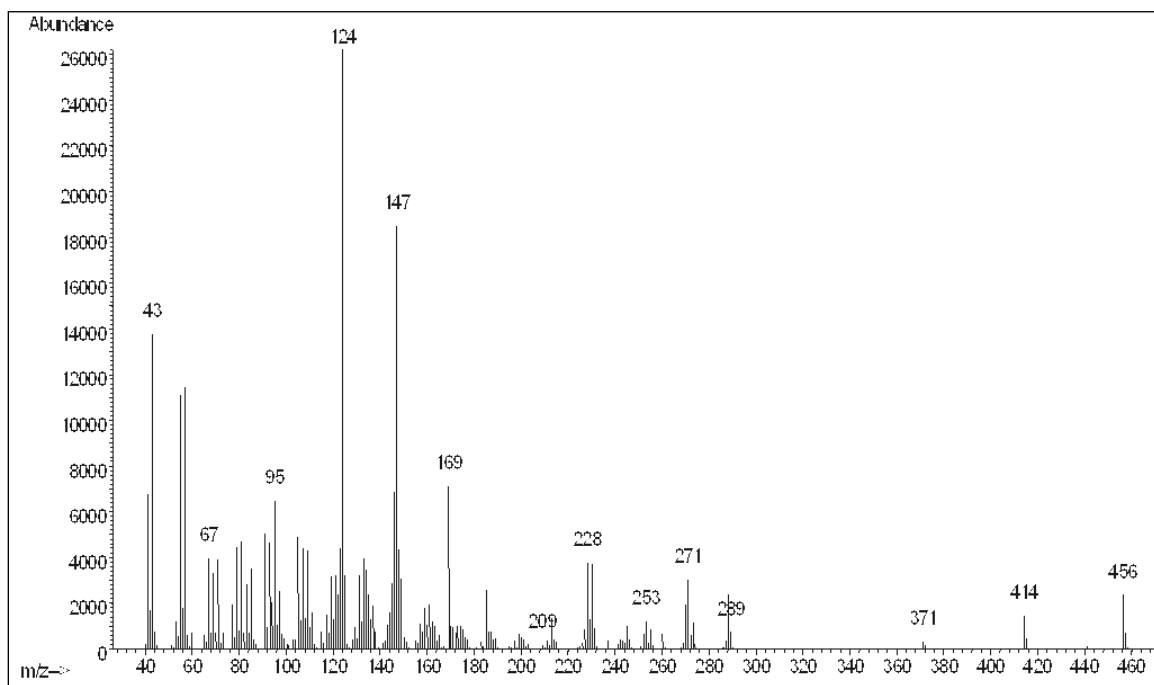


IR (ATR 3-bounce diamond device): Testosterone undecanoate  
16 scans, 4.0 cm<sup>-1</sup> resolution

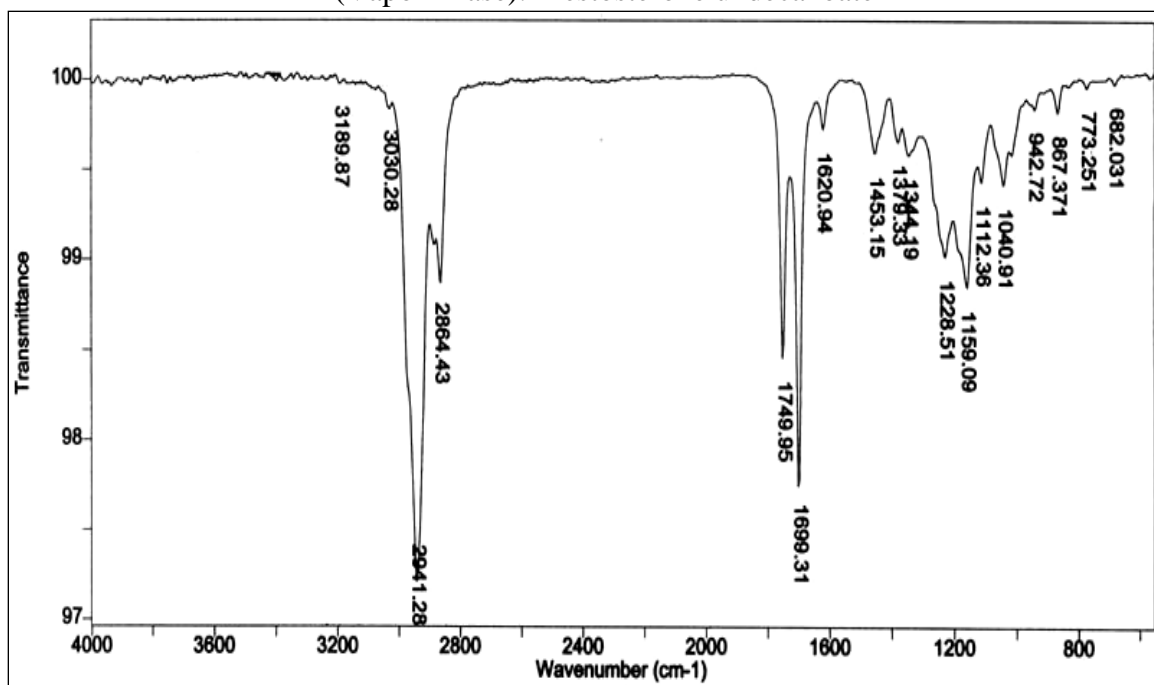


MS (EI): Testosterone undecanoate  
Quadrupole Detector

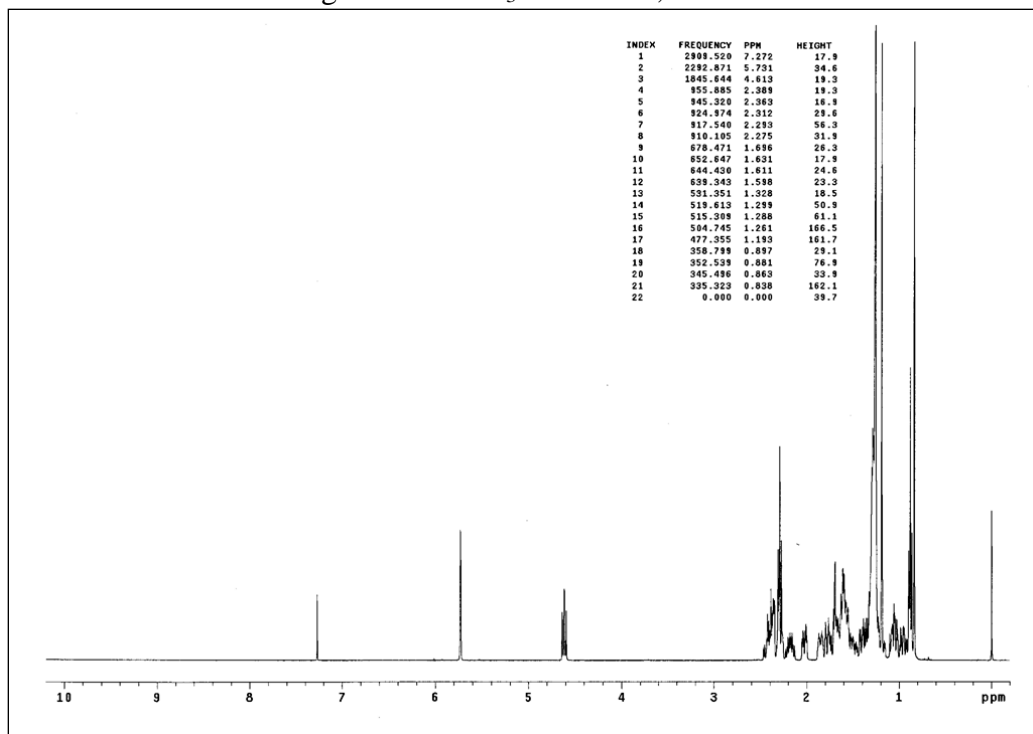




IR (Vapor Phase): Testosterone undecanoate



NMR (PROTON): Testosterone undecanoate  
10 mg/mL in CDCl<sub>3</sub> with TMS, 400 MHz



NMR (CARBON): Testosterone undecanoate  
50 mg/mL in CDCl<sub>3</sub> with TMS, 100 MHz

