Waters™

Application Note

Testosterone Esters in Serum

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

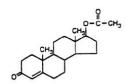
Abstract

This application brief demonstrates analysis of testosterone esters in serum.

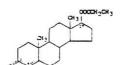
Introduction

The compounds used in this study are -

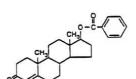
- 1. Testosterone acetate
- 2. Testosterone propionate
- 3. Testosterone benzoate
- 4. Testosterone enanthate (I.S.)



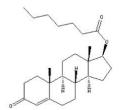
1. Testosterone acetate



2. Testosterone propionate



3. Testosterone benzoate



4. Testosterone enanthate(I.S.)

Experimental

HPLC Method

Column: Symmetry C_8 , 4.6 x 75 mm, 3.5 μm

Guard column: Sentry Guard Column, 3.9 x 20 mm, 5 µm

Part numbers: Column - WAT066224, Guard - WAT054225

Mobile phase: Acetonitrile/water 60:40

Flow rate: 1.5 mL/min

Detection:

UV @ 254 nm

Oasis® HLB Extraction Method

Oasis® HLB 1 cc/30 mg Extraction Cartridge Part Number WAT094225

Condition

1 mL methanol/1 mL water

Load

1 mL sample with 800 μ L spiked porcine serum and 200 μ L of methanol

Wash

1 mL 5% methanol in water

Elute

1 mL methylene chloride: methanol 50:50 (v/v)

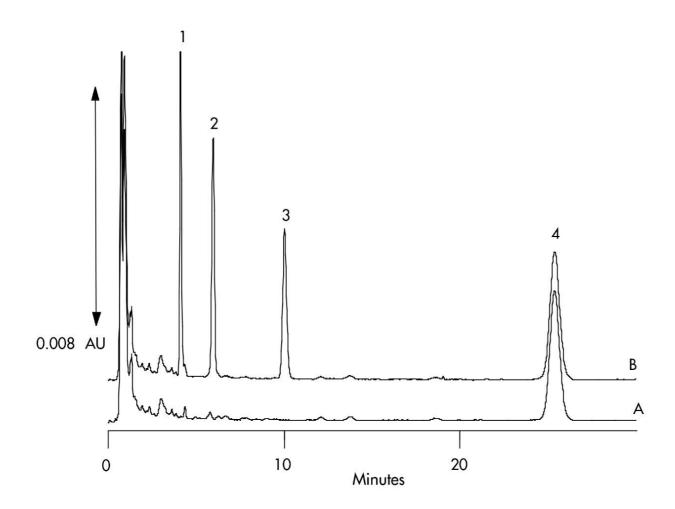
Evaporate and Reconstitute

40 °C under nitrogen stream 500 µL methanol with 5 µg/mL of testosterone enanthate

Results and Discussion

Compound	Concentration µg/mL	% Recovery	%RSD (n=6)
Testosterone	1.00	98%	1.2%
Acetate	0.200	94%	3.7%
Testosterone	1.00	100%	1.9%
Propionate	0.200	94%	4.3%
Testosterone	1.00	94%	1.6%
Benzoate	0.200	87%	3.2%

Chromatogram of Serum Extracts: A) Blank B) Spiked Sample



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