

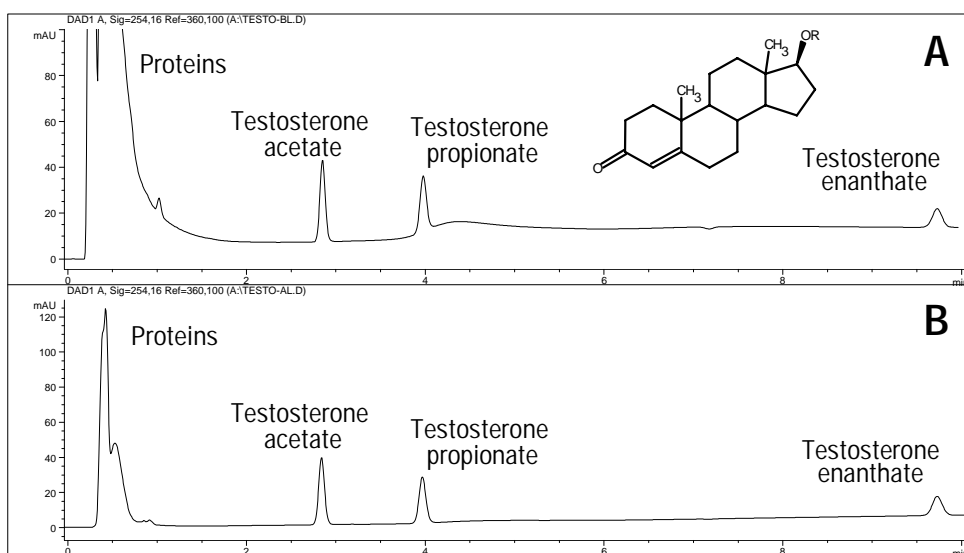
# Analysis of Testosterones in Serum: Zorbax SPE C18 Cartridge

## Application Brief

### Pharmaceutical

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Testosterone and related compounds are present in the bloodstream at various levels, both naturally, and as a result of medical treatment, and occasionally, due to misuse. It is of interest to determine levels of testosterone-like compounds in the bloodstream with minimal background from the matrix (e.g., blood proteins). As shown below, it is possible to significantly reduce levels of matrix compounds through the use of a Zorbax SPE C18 cartridge. The analytes of interest are then easily quantitated by reversed-phase HPLC. Recovery of testosterone acetate and propionate were very high, *see Recovery*.



**Figure 1.** Comparison of spiked serum sample before (panel A) and after (panel B) extraction using the Zorbax SPE C18 cartridge. Notice effectiveness of the extraction. In panel A, the large amount of protein in the sample interferes with optimal quantitation of testosterone propionate. Once the sample has passed through the cartridge, analytes are clearly detected and quantifiable (panel B). Testosterone enanthate is included in samples as an internal standard.

### Solid Phase Extraction Method

- **CARTRIDGE:** 100 mg, 1 ml Zorbax SPE C18 (P/N 5184-3590, Box of 100)
- **CONDITION:**
  - 1.0 mL Methanol
  - 1.0 mL Deionized Water
- **LOAD:**
  - 1.0 mL Sample (see sample preparation)
- **WASH:**
  - 1.0 mL 5% Methanol in Water
- **ELUTE:**
  - 1.0 mL Methylene Chloride/Methanol (50/50, v/v)
- **EVAPORATE and RECONSTITUTE:**
  - Room Temperature nitrogen stream
  - Add 200  $\mu$ L of Methanol with 10  $\mu$ L (100  $\mu$ g/mL) testosterone enanthate as internal standard.

### Sample Preparation

- Testosterone acetate and testosterone propionate (1mg/mL each) in Methanol.
- Spike 10  $\mu$ L above solution with:
  - 8 mL bovine serum
  - 2 mL Deionized Water
- **Internal Standard:**
  - Prepare a 1 mg/ml solution of testosterone enanthate in Methanol.



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## HPLC Method

LC Instrument:

Agilent 1100

Column:

Zorbax XDB C8, 4.6 mm x 75 mm, 3.5  $\mu$ m  
(P/N 966967-906)

Eluent:

A: Acetonitrile/Water 60/40

B: Acetonitrile

Gradient: 0% B - 60% B in 12 min.

UV: 254 nm

Flow: 1.5 mL/min.

Temperature: 25°C

Inj. Vol.: 20  $\mu$ L

## Recovery

- Testosterone Acetate (1  $\mu$ g/mL):

$X = 95\%$ ,  $n = 4$

- Testosterone Propionate (1  $\mu$ g/mL):

$X = 92\%$ ,  $n = 4$

*Recovery of analyte in these experiments is an indication of 'goodness' of technique, as well as quality of the SPE packing. For best results, solutions should not be drawn completely through the cartridge until the final elute step.*

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