

# LC-MS/MS Method for the Determination of Testosterone using an Accucore C8 HPLC Column

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## Key Words

Accucore C8, Testosterone

## Abstract

A liquid chromatography-tandem mass spectrometry method for the analysis of testosterone was carried out on a Thermo Scientific Accucore C8 HPLC Column, 2.6  $\mu\text{m}$  50 x 2.1 mm resulting in a fast separation with a cycle time of 1.5 minutes while maintaining excellent peak shape.

## Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6  $\mu\text{m}$  diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks with very low tailing. Accucore C8 uses a shorter alkyl chain length designed to have lower hydrophobic retention than an equivalent C18 phase. The tightly controlled 2.6  $\mu\text{m}$  diameter of Accucore particles results in much lower backpressures than typically seen with sub-2  $\mu\text{m}$  materials.

Testosterone is a steroid hormone from the androgen group, it is found in mammals and most vertebrates (Figure 1). Androgen steroids promote protein synthesis and tissue growth within areas that contain androgen receptors. These are also known as anabolic steroids. Testosterone levels need to be monitored in testosterone deficient males to observe the effects of hormone replacement therapy. Testosterone is also used by athletes to promote muscle growth and protein synthesis for recovery. Most sports deem this to be doping and routinely test athletes.

The analysis of testosterone is demonstrated in this application.

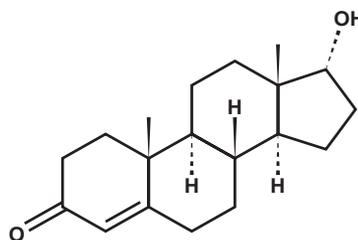


Figure 1. Testosterone

## Experimental Details

Consumables	Part Number
Fisher Scientific LCMS grade water	W/011217
Fisher Scientific LCMS grade methanol	M/4062/17
Fisher Scientific LCMS grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Separation Conditions	Part Number	
Instrumentation:	Thermo Scientific Accela 600	
Column:	Accucore C8, 2.6 $\mu\text{m}$ , 50 x 2.1 mm	17226-052130
Mobile phase A:	water + 0.1 % formic acid	
Mobile phase B:	acetonitrile + 0.1 % formic acid	
Gradient:	5 to 95 % B in 0.8 minutes	
Flow rate:	1.5 mL/min	
Column temperature:	60 $^{\circ}\text{C}$	
Injection volume:	5 $\mu\text{L}$	
Injection wash solvent 1:	80:20 (v/v) water / acetonitrile	
Injection wash solvent 2:	45:45:10 (v/v/v) IPA / acetonitrile / acetone	

### MS Conditions

Instrumentation:	Thermo Scientific TSQ Vantage
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TSQ Vantage™ Conditions	
Ionization conditions	HESI
Polarity	Positive
Spray voltage (V)	4000
Vaporizer temperature ( $^{\circ}\text{C}$ )	425
Sheath gas pressure (Arb)	60
Aux gas pressure (Arb)	50
Capillary temp ( $^{\circ}\text{C}$ )	350
Collision pressure (mTorr)	1.5
Scan time(s)	0.02
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Compound Transition Details	
Compound	Testosterone
Parent (m/z)	289.14
Products (m/z)	97.08, 109.06
Collision energy (V)	20, 22
S-lens (V)	80

### Data Processing

Software:	Thermo Scientific LC QUAN
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## Results

### Chromatography

Accucore C8 gave excellent peak shape.

The chromatography of 20 ng/mL is shown in Figure 2.

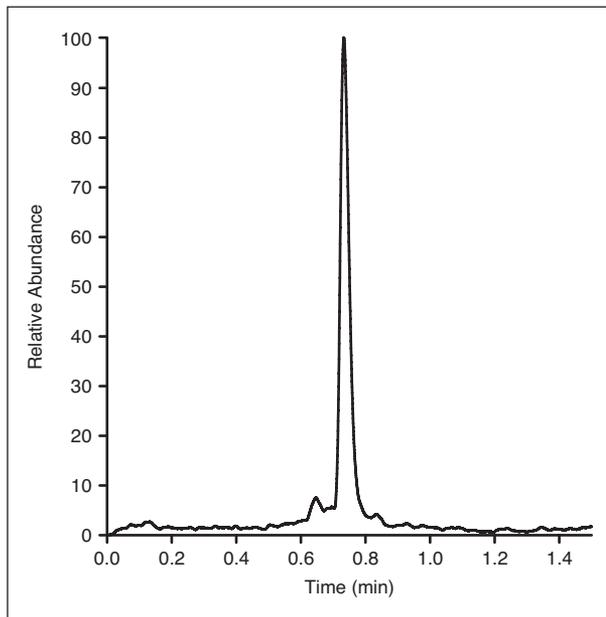


Figure 2. Representative chromatogram of Testosterone SRM at 20 ng/mL

Replicate injections of testosterone showed that Accucore C8 produced stable and reproducible results (Table 1).

Results	Peak 1: Testosterone
Retention Time, $t_R$ /min	0.73
%RSD $t_R$	0.22
%RSD Area	3.01

Table 1. Statistical assessment based upon data derived from 6 replicate injections

## Conclusion

Accucore C8 HPLC column gives a fast run time, excellent peak shape and performs reproducibly for the analysis of testosterone.

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