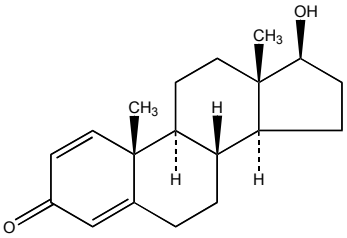
HPLC Separation of Anabolic Steroids on ZirChrom®-PBD

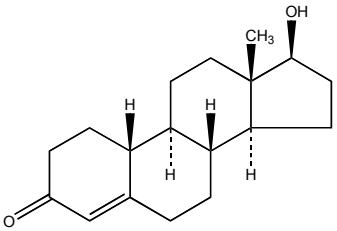
Dwight Stoll and Dr. Clayton V. McNeff
ZirChrom Separations, Inc.

Technical Bulletin #264

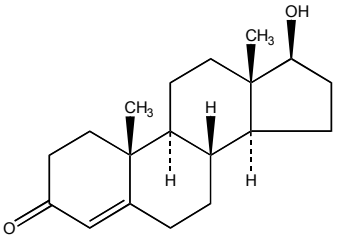
This note shows the separation of three closely related anabolic steroids, boldenone, nandrolone, and testosterone, using a ZirChrom®-PBD column. A typical analysis of these compounds involves derivatization and subsequent quantitation by GC-FID or GC-MS, however these methods tend to be labor intensive, and analytically unreliable. Baseline resolution of all three compounds was obtained on ZirChrom®-PBD at slightly elevated temperature in under 10 minutes using isocratic conditions.



Boldenone



Nandrolone



Testosterone

Figure 1: Structures of Anabolic Steroids

Introduction

The rapid and accurate detection of anabolic steroids is crucial in today's sporting world. Historically the structurally similarity of these compounds has made quantitative analysis by reversed-phase HPLC difficult at best. These steroids are very difficult to separate on silica ODS phases and provide nearly identical mass spectra. This method capitalizes on the unique temperature stability and surface chemistry of a zirconia-based stationary phase to achieve baseline resolution of these compounds in less than 10 minutes.

Experimental

A mixture of anabolic steroids was separated at 60 ºC using a ZirChrom®-PBD column (See Figure 2) using the following conditions.

Column:	150 mm x 4.6 mm i.d. ZirChrom®-PBD
Mobile Phase:	15/85 ACN/Water
Flow Rate:	1.5 ml/min
Injection Vol.:	5 µl
Pressure Drop:	160 bar
Temperature:	60 ºC with Metalox™ 200-C Column Heater
Detection:	UV at 215 nm

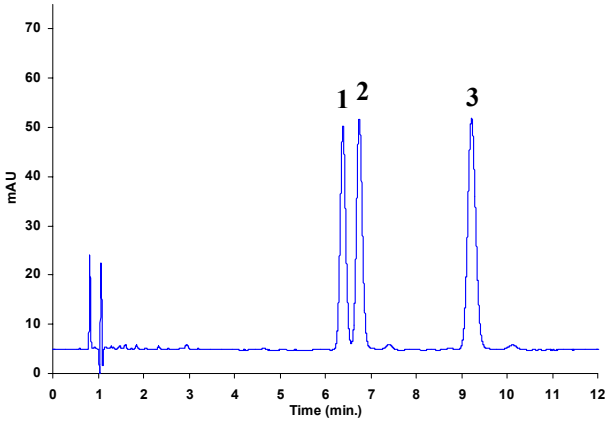


Figure 2: Separation of anabolic steroids on ZirChrom®-PBD. 1-Boldenone, 2-Nandrolone, 3-Testosterone

This separation allows for clear identification and quantification of these compounds without the use of expensive MS detection. The separation also requires no complex buffers and uses a minimum of organic modifier.

Acknowledgments

(1) Walter Hyde, Iowa State University

(2) Metalox Technologies, Inc.

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.