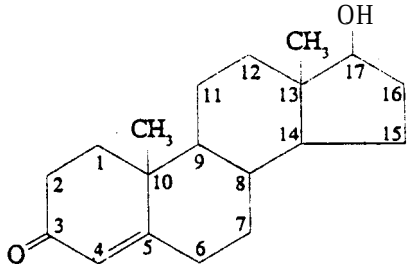
Clinical Corner

Analysis of Anabolic Steroids

Steroids are a group of polycyclic compounds that have a wide variety of functions in human biochemistry. They have been identified as precursors to vitamins and bile acids as well as forming the base structure for many different hormones. The anabolic steroids, or androgens, are responsible for many of the virilizing characteristics associated with male development. Increases in height and muscle development can be linked to increased androgen production during the onset of puberty in boys. The potential for abuse of anabolic steroids is linked to this developmental phenomenon. Athletes have used anabolic steroids since the 1950's (1) to tty to add lean body mass and. subsequently enhance athletic performance. The abuse of steroids today has spread from the competitive arena to those that are just seeking to improve their own physical appearance by "bulking up". Because of the increased use of anabolic steroids and the potential for harmful side effects when used in large doses, anabolic steroids were classified as a Controlled Subs&e and placed under Schedule III effective February 27, 1991 (2). The Controlled Substances Act regulates the manufacture, distribution or dispensing of anabolic steroids in bulk form, whereas the use and misuse of anabolic steroids is usually-controlled by the different sports regulating bodies.

Figure **1** - Chemical structure of testosterone.



The anabolic steroids are all structurally related to testosterone (Figure 1). Modifications at the 3,5,9 and 17 positions give a variety of androgens that not only differ in molecular weight and boiling point, but also in pharmacological activity. The abundance of structurally related compounds multiplies the difficulty of detecting and confirming the identity of anabolic steroids in both clinical samples and bulk formulations. Analysis of anabolic steroids is most commonly performed by gas chromatography with detection by mass spec. Some of the higher molecular weight steroids, likeI-dehydrotestosterone benzoate and 1-dehydrotestosterone undecylenate require high temperature for extended periods of time in order to be eluted from standard film thickness columns. In order to reduce the effective elution temperature and reduce the overall analysis time, columns made with thii films of non-polar stationary phases should be used.

Retention time for the steroids . , . wil l be affected by the choice of column length, stationary film thickness and stationary phase polarity.

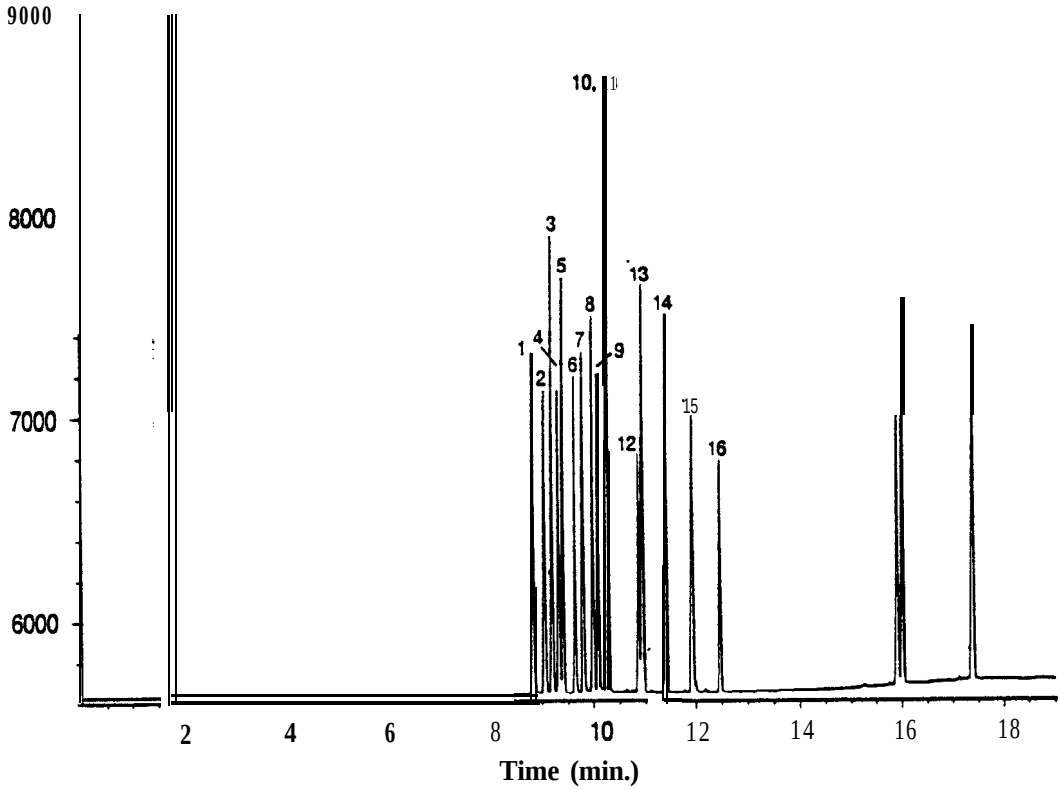
Figure 2 shows the analysis of a mixture of anabolic steroids on an Rtx-5 column. An analysis time of less than 18 minutes was achieved by using a 0.10 micron film thickness. When film thicknesses greater than 0.10 microns were used, the last three steroids could not be eluted except by using final hold times in excess of twenty minutes with a corresponding deterioration in peak shape. Retention time is also strongly influenced by the choice of stationary phase. Although lower polarity stationary phases are needed to maintain reasonable analysis times and peak shapes, non-polar stationary phases do not provide enough resolution to maintain separation between all of the steroids. A 5% phenyl stationary phase provides enough polarity to improve the resolution of the early eluting steroids without substantially lengthening the retention time of the latest eluting steroids. A 30-meter length column was used to minimize retention times without sacrificing resolution.

Several factors must be taken into account when selecting a column for anabolic steroids. Retention time for the steroids, as well as resolution, will be affected by the choice of column length, stationary film thickness, and stationary phase polarity. By combining a low polarity stationary phase like the Rtx-5 with a thin film, minimal length format(30m, 0.25mm ID, 0. l0um), reasonable analysis times can be achieved while maximizing the resolution between most of the anabolic steroids. ■

References

J. Wright, J. *Anabolic Steroids and Sports.* Natick, MA: Sports Science Consultants 3.1978.
2. Federal Register, Vol. 56 No. 30. p. 98-99.

Figure 2 - The analysis of a mixture of anabolic steroids on an Rtx®-5 column.



COMPOUNDS	
1	5-androstene-3b-17b-diol
2	17a-methyl-5-androstene-3b-17b-diol
3	5a-andratan-17b-ol-3-one
4	19-nortestosterone
5	17a-methylandrostan-l7b-ol-3-one
6	mesterolone
7	testosterone
8	17a-methyltestosteronc
9	I-dehydrotestosterone
10	l-dehydro-17a-methyltestosterone
11	bolasterone
12	oxymethalone
13	19-nortestosterone-17-propionatc
14	testosterone propionate
15	fluoxymcsterone
16	4-chlorotestosterone-17-acetate
17	testosterone-17b-cypionate
18	1-dehydrotestosterone benzoate
19	1-dehydrotestosterone undecylenate

30m. 0.25mm ID, 0.10um Rtx-5 (cat-# 10208)
0.1ul split injection of anabolic steroids concentration - 1000ng/ul
Oven temp. 180°C to 340C @ 10C/min hold 3min

In] temp.:	280°C
Det. type:	FID
Det. temp.:	340C
carrier gas:	helium
Linear velocity:	35cm/sec set @ 180C
FID sensitivity:	128 x 10-10 AFS
Split ratio:	50:1

*Maximum operating temperatures on the 30m 0.10um Rtx-5 have been raised to 340°C to provide faster analysis times for high molecular weight compounds.

Ordering Information

Rtx-5 30m, 0.25mm ID, 0.10um Cat.# 10208

Restek : Australian Distributors - Chromalytic Technology Pty Ltd Fax : +61 3 9761 1169