



Recently, anabolic steroids have come into public prominence. These steroids are used in both meat production and sport. In human sport, they are sometimes used in large quantities for increasing the body bulk of athletes. In horse racing, they are used to improve a horse's appetite and produce behavioral changes of the masculinizing type. Because of their long-term deleterious effects on physiologic processes and unfair use in sport, control agencies have begun to assess their use.

Testosterone is the principal hormone of the testes. In humans, the major metabolites are androstanediol, androsterone, and androstenedione. In horses, the major metabolite is androstanediol, which is excreted in the urine as the glucuronide or sulfate conjugate.

Analysis of the conjugated steroids requires hydrolysis of the sample. An enzymatic and an acid hydrolysis procedure are given below. There is evidence that the enzymatic procedure will hydrolyze only the glucuronide. The sulfate conjugates require acid hydrolysis.

The procedure described here may be used for the total or conjugated steroid content of urine, plasma, or amniotic fluid. Total steroid content is obtained by hydrolyzing the sample. Free steroid content can be determined by extraction of the unhydrolyzed sample. The con-

Compound	Retention Factor
Dihydroepiandrosterone	0.59
Androstenedione	0.55
Epitestosterone	0.48
Testosterone	0.47
Androsterone	0.35

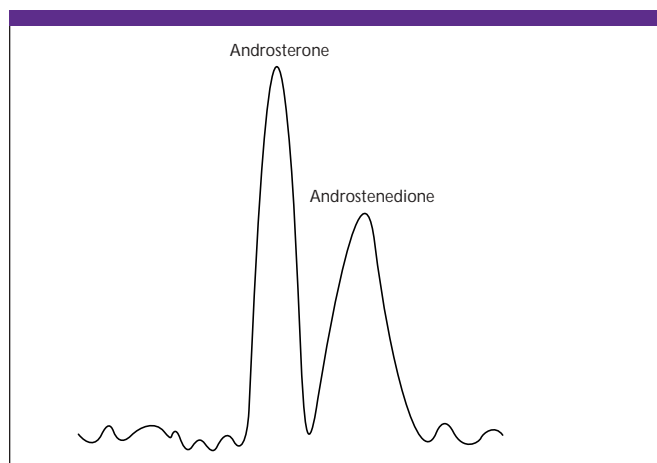


Figure 1: Scan of some androgens for fluorescence at 366 nm.

Anabolic Steroids

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jugated portion is then the difference between the hydrolyzed and unhydrolyzed amounts.

Sample Preparation

Buffer preparation:

1. pH 5.0 acetate buffer — 14.8 mL of 0.2 M acetic acid plus 35.2 mL of 0.2 M sodium acetate diluted to 100 mL with water.

Hydrolysis procedures:

1. Acid hydrolysis: Add 0.75 mL hydrochloric acid per 5 mL sample (urine, plasma, or amniotic fluid) in a screw-capped test tube. Screw the tube top on loosely and heat it in a boiling water bath for 60 min. Adjust to pH 7 with 1.0 N sodium hydroxide.
2. Enzymatic hydrolysis: Adjust 10 mL urine to pH 4.5 with hydrochloric acid. Add 1 mL acetate buffer (pH 5) containing 1000 units glucuronidase. Incubate at 37 °C for 24 h. (Note: An expedited procedure is to heat at 55 °C for 60 min.)

Extraction procedure:

1. Condition a SPICE C18 cartridge in sequence with 2 mL of 8:2 (v/v) water–acetone, 2 mL of methanol, and 2 mL of water. Allow each solvent to pass through before adding the next.
2. Add the sample from the hydrolysis procedure (or unhydrolyzed sample) to the cartridge. Allow to completely flow through under vacuum and discard the eluent.
3. Wash the cartridge in sequence with 2 mL of water and 2 mL of 8:2 (v/v) water–acetone. Discard all eluents.
4. Elute the steroids with 1 mL of methanol. Collect the eluent, evaporate it, and reconstitute it in 100 μ L of methanol.

Recovery:

1. The recovery of androsterone and androstenedione from SPICE sample extraction of urine spiked with radiolabeled steroid was 99% and 72%, respectively.

Thin-Layer Chromatography

Separation:

1. Apply 10 and 20 μ L of the reconstituted sample to an Analtech silica gel G layer. Apply standard androstanediol (1 μ g/ μ L methanol) to the reference lane.
2. Develop the chromatogram in a mobile phase of 8:2 (v/v) isopropyl ether–acetone.

Visualization:

1. Examine the plates under UV at 254 nm. Most of the steroids quench at the short UV wavelength.
2. Spray with 10% sulfuric acid in ethanol. Heat at 150 °C for 2–3 min.
3. Examine the plate under UV at 366 nm. The sulfuric acid spray yields fluorescent spots with colors leading to some specificity.

Quantitation

Figure 1 is a scan of some androgens for fluorescence at 366 nm. The mobile phase for this separation was 95:5 (v/v) isopropyl ether–acetone.

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