Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (10)

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5α-Androstan-3,17β-diol and 1,4-Androstadien-3,17-dione, New Anabolic Steroids Available on the InterNet as Nutritional Supplements. Metabolism and Detection in Urine
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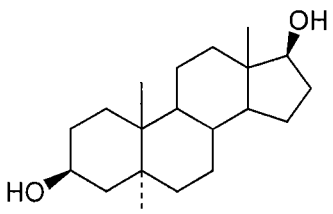
V.P. Uralets, P.A. Gillette

New Anabolic Steroids Available as Nutritional Supplements: 5α-Androstan-3β,17β-diol, 1,4-Androstadien-3,17-dione and 5α-Androst-1-en-17β-ol-3-one

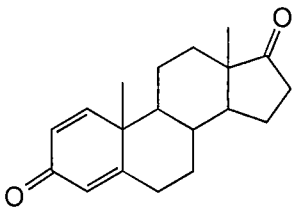
Quest Diagnostics, San Diego, California, USA

Introduction

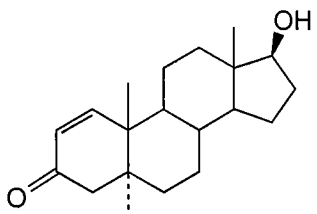
The first generation of over-the-counter (OTC) anabolic steroids (AS) was introduced in 1997. 4-Androsten-3,17-dione, 4-androsten-3β,17β-diol, their 19-nor and Δ^5 analogues are precursors (prohormones) from which body produces testosterone and nandrolone (1-5). The newer 5α-androstan-3β,17β-diol and 1,4-androstadien-3,17-dione, available on the Internet and in the "health" stores, are designed for endogenous conversion into 5α-dihydrotestosterone (DHT) and 1,4-androstadien-17β-ol-3-one (boldenone). They are advertised as highly anabolic/androgenic compounds promoting dense lean muscularity, enhancing strength, and overall physical performance. Another new supplement, 5α-androst-1-en-17β-ol-3-one is offered as "the most potent muscle building agent of all time". It was originally developed as an anabolic drug, but not released to the market. Structurally it relates to DHT and boldenone, suggesting high anabolic potency, which was shown in earlier studies (6). None of three steroids is listed as a controlled substance. They can be legally manufactured and sold in the US as nutritional supplements.



5α-androstan-3β,17β-diol



1,4-androstadien-3,17-dione



5α-androst-1-en-17β-ol-3-one

The IOC has added older OTC AS such as androstendione to the list of banned drugs, emphasizing their harmful effect and wide availability. Newer steroid supplements, described in this paper, are likely to be added to this list too.

Isotope ratio mass spectrometry would be ideal for the OTC steroids, while current routine testing detects them using existing criteria for traditional steroids: nandrolone metabolites or elevated T/E ratio. The parent drug may not be precisely determined. Specific markers for some OTC steroids are recently proposed (7,8).

Experimental

Excretion studies

5α-Androstan-3β,17β-diol (Maxterone™) and 1,4-androstadien-3,17-dione (Equi-Bolan™) both from Impact Nutrition, Inc. (Aurora, CO) were purchased via the internet, *www.AnabolicStore.com*, and checked for purity by GC/MS. Excretion studies were performed with four healthy male subjects. Each took orally a single 50 mg dose with sufficient time interval between two supplements. 5α-androst-1-en-17β-ol-3-one in pure form was purchased from Steraloids (Newport, RI) and supposedly in form of 17- tetrahydropyranyl (THP) ether (TestEtherone) in combination with 4-androstendiol (Test250™) from Funk Laboratories (Aurora, CO). One 5 mg dose was administered orally to two healthy male individuals. Urine specimens were collected for one week after administration and were stored refrigerated.

Reagents and materials

β-Glucuronidase/arylsulfatase type HP-2 (Cat.# G7017) from *Helix pomatia* were purchased from Sigma. β-Glucuronidase type K12 from *Escherichia coli* was supplied by Fluka (Milwaukee, WI). N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was purchased from Campbell Science Corp. (Rockton, IL), ammonium iodide 99+% from Aldrich (Milwaukee, WI). C_{18} solid phase (200 mg) extraction cartridges were purchased from Varian (Harbor City, CA).

Urine sample preparation

Sequential urine hydrolysis with *Escherichia coli* (*E.C.*) and *Helix pomatia* (*H.P.*) enzymes, intermediate clean up on C_{18} extraction cartridges, solvolysis (with 1mL of ethyl acetate and 2μL of 4M H_2SO_4) and separation of urinary steroid fractions were performed as described earlier (9). The dry residues of each fraction were derivatized with 75 μL of MSTFA-NH_4I-Ethanethiol (1000:2:3 v/w/w) for 15 minutes at 70°C. Samples were transferred into vials, 1 μL was injected into the GC/MS.

GC/MS conditions (10)

GC/MS was Agilent Technologies 6890/5973 with 7683A autoinjector. Column: Chrompack CP-Sil5CB fused silica, crosslinked methylsilicone, 10m, 0.15 mm i.d., 0.10 μm film thickness. Hydrogen carrier gas was supplied by Whatman 75-32-V452 hydrogen generator. Total flow was 26.4 mL/min, average carrier gas linear velocity in the column was 93 cm/sec with constant flow of 0.9 mL per minute. Splitless injection with 0.3 minutes purge time was used. Oven temperature program: hold at 150°C for 0.32 min; raise at 50°C/min to 190°C; then 5.4°C/min to 217°C, and 43°C/min to 310°C, hold for 0.02 min. Injector temperature was 270°C, transfer line 280°C.

Results and Discussion

Maxterone™ contained a relatively pure form of 5α-androstan-3β,17β-diol. Typical excretion profiles are shown in fig. 1. Concentrations of 5α-steroid urinary metabolites rise after administration as expected. Androsterone, 5α-androstan-3α,17β-diol, 5α-androstan-3β,17β-diol and DHT in the form of their glucuronic conjugates prevail over 5β- isomers, similar to DHT administration (11). Epiandrosterone (sulfate), at a certain point is the most abundant metabolite (fig. 1), which follows from its position in 5α-androstan-3β,17β-diol metabolic pathway, see fig. 2.

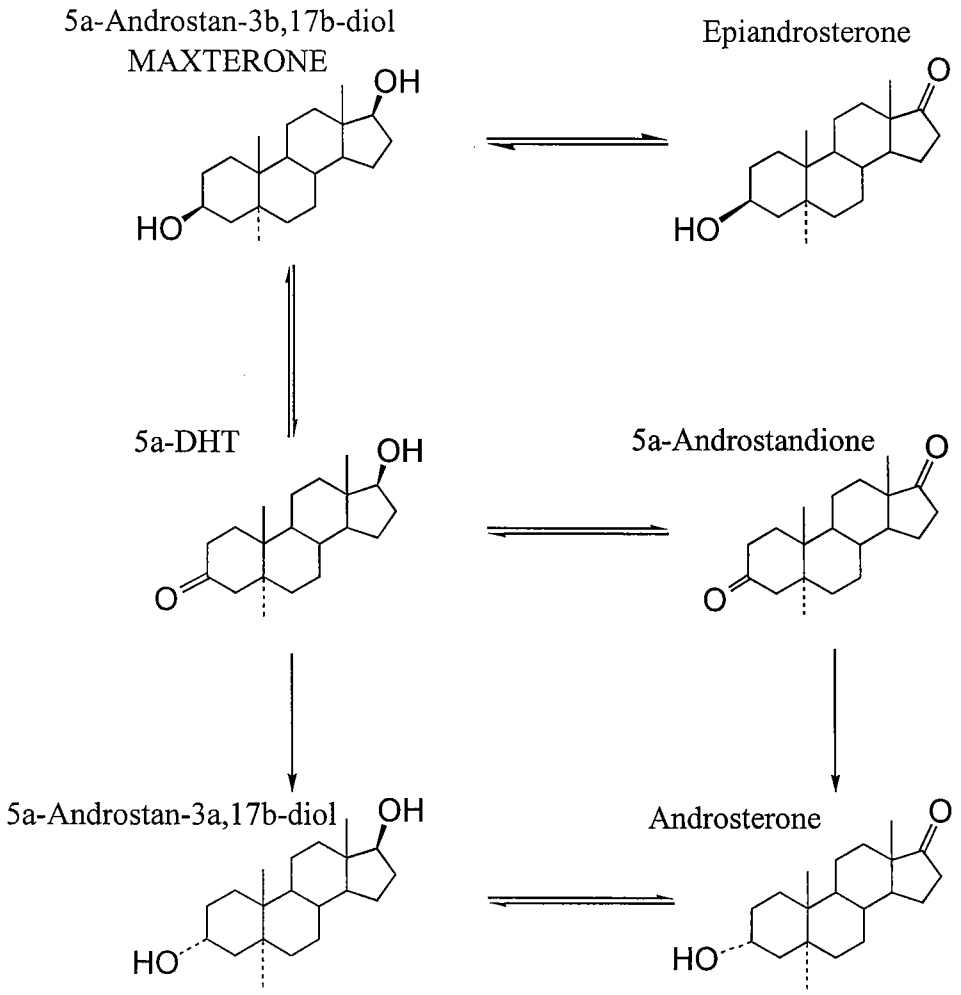


Figure 2. 5α-Androstan-3β,17β-diol metabolic pathway.

Detection of 5α-androstan-3β,17β-diol ingestion should be based on the current criteria applied for DHT with, perhaps, emphasis on the parent 17-glucuronide and epiandrosterone sulfate. 5α-Androstan-3β,17β-diol effect on steroid profile may vary from one individual to another and may be traced for three days.

Oral 1,4-androstadien-3,17-dione produces classic urinary excretion patterns characteristic of boldenone (12), where 5β- reduced metabolites are found as glucuronides: 5β-androst-1-en-3α-ol-17-one (major); 5β-androst-1-en-17β-ol-3-one; 5β-androst-1-en-3α,17β-diol and 5β-androst-1-en-3,17-dione (fig. 3). A chromatogram showing main metabolites is presented in fig. 4A. Hydroxylated 1,4-androstadien-3,17-dione, 5β-androst-1-en-6β-ol-3,17-dione and another unidentified long-term metabolite appear after solvolysis of their sulfates.

Some elevation of T/E ratio is observed. Boldenone (1,4-androstadien-17β-ol-3-one) was not detected, indicating little or no conversion into boldenone, which makes 1,4-androstadien-3,17-dione not a precursor, but rather an immediate active metabolite of boldenone.

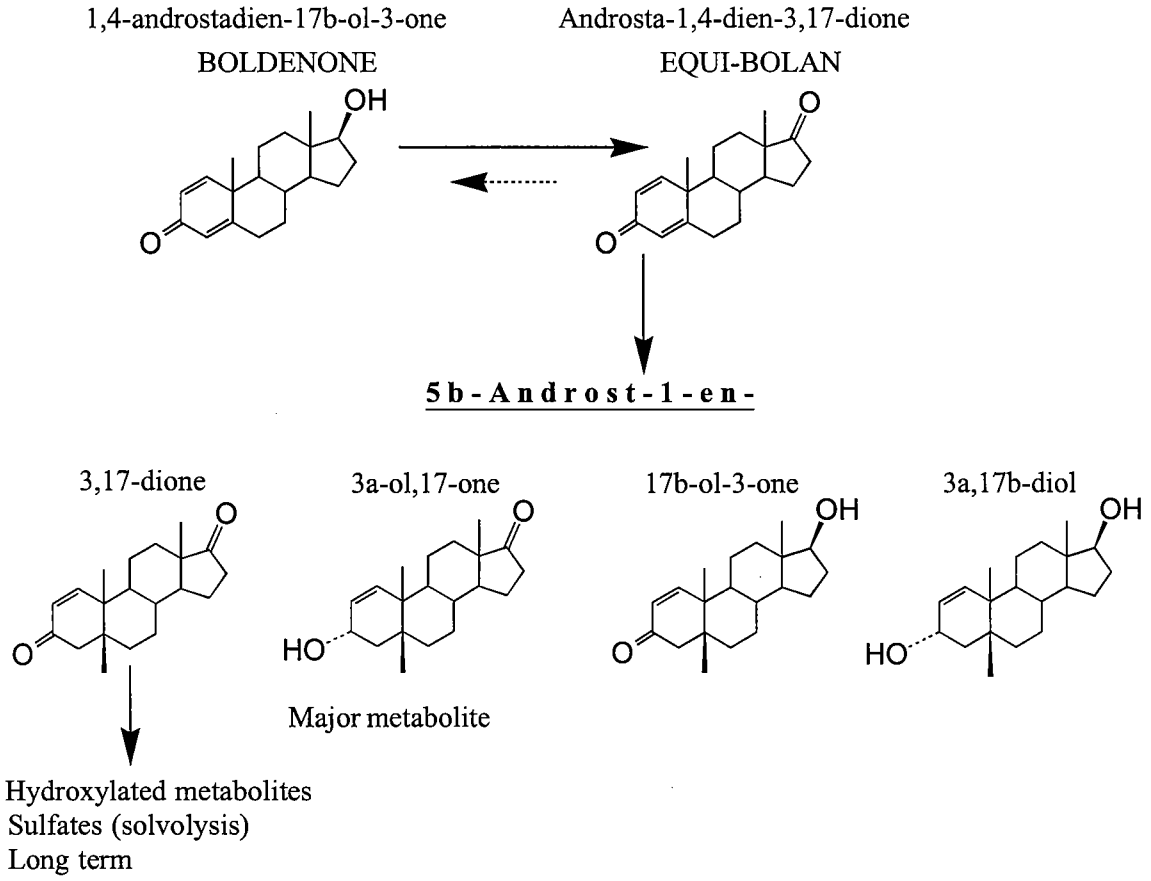


Figure 3. 1,4-Androstadien-3,17-dione metabolic pathway.

Detection criteria used for boldenone should be applied for 1,4-androstadien-3,17-dione in routine testing.

Typical 5α-androst-1-en-17β-ol-3-one ("1-Testosterone") metabolic profiles were first detected and identified in our laboratory during routine testing in summer 2001. Excretion study with 5 mg of pure substance in controlled conditions gave steroid profiles with elevated 5α-androst-1-en-3α-ol-17-one (major metabolite) and 5α-androst-1-en-3,17-dione in addition to parent drug, all found as glucuronides (figure 5). According to classic studies by Schänzer and Donike (12,13) these metabolites

cannot be formed in the body from 1,4-dien steroids, such as boldenone and 1,4-androstadien-3,17-dione (fig. 4A.). Δ^1 Metabolites with a predetermined 5α-configuration in fig. 4B have mass spectra similar to classic 5β- (boldenone) metabolites, but markedly different retention times (compare A and B in fig. 4). 5β-Androst-1-en-3α-ol-17-one, coelutes with androsterone, while its 5α- isomer appears on the tail of etiocholanolone. Reduction of Δ^1 double bond yields DHT and a consequent rise of androsterone and other 5α- steroids:

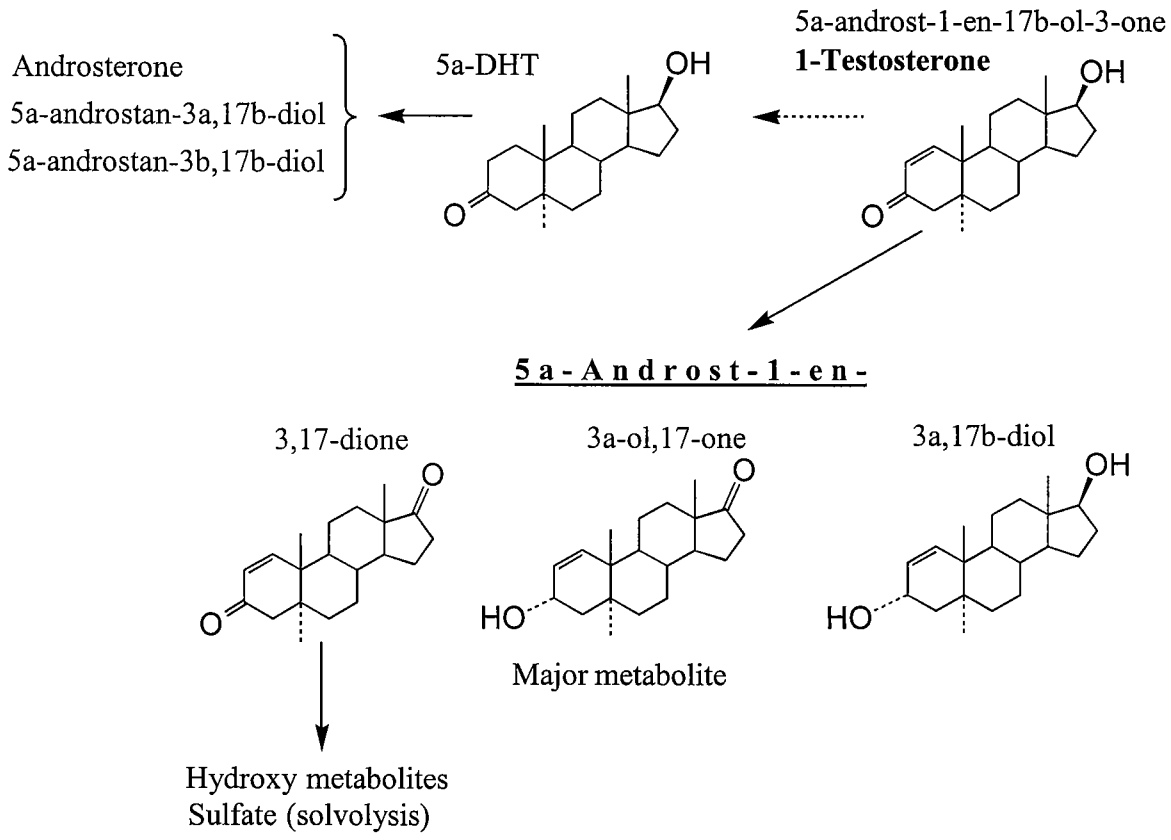


Figure 5. 5α-Androst-1-en-17β-ol-3-one ("1-Testosterone") metabolic profile.

Similar metabolites are identified by Van Eenoo et.al. in their study of a corresponding diol, 5β-androst-1-en-3β,17β-diol (14). Routine screening procedure 4 (anabolic steroids) must be modified for detection of 5α-androst-1-en- metabolites.

12. Schänzer W and Donike M. Metabolism of Boldenone in Man: Gas Chromatographic/Mass Spectrometric Identification of Urinary Excreted Metabolites and Determination of Excretion Rates. *Biol Mass Spectrom* 1992; **21**:3-16.
13. Schänzer W. Metabolism of Anabolic Androgenic Steroids: 5α- and 5β-Reduction of 3-Keto-4-ene Steroids. In Schänzer W, ed. *Recent Advances in Doping Analysis (4). Proceedings of the Manfred Donike 14th Cologne Workshop on Dope Analysis,* 1996. Cologne: Sport und Buch Strauß, 1997, 185-201.
14. Van Eenoo P, Delbeke FT, Desmet N. Excretion Studies with the Supplement Containing 1(5α)-Androstene-3β,17β-diol. In Schänzer W, ed. *Recent Advances in Doping Analysis (10). Proceedings of the Manfred Donike 20th Cologne Workshop on Dope Analysis,* 2002. Cologne: Sport und Buch Strauß, 2002,

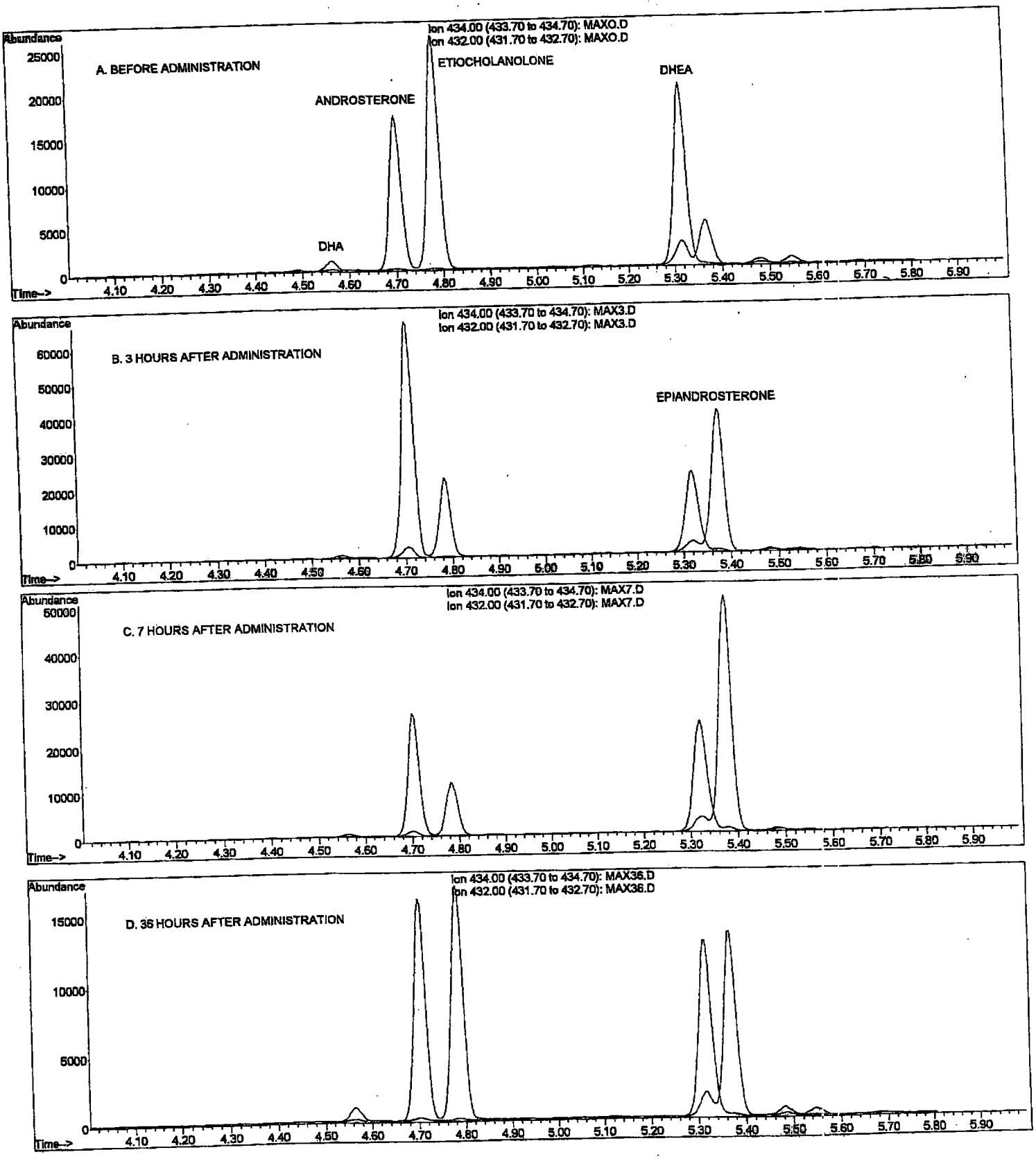


Figure 1. Chromatograms of selected urinary steroids before (A) and after (B,C,D) oral administration of 5α-androstan-3β,17β-diol (50 mg).

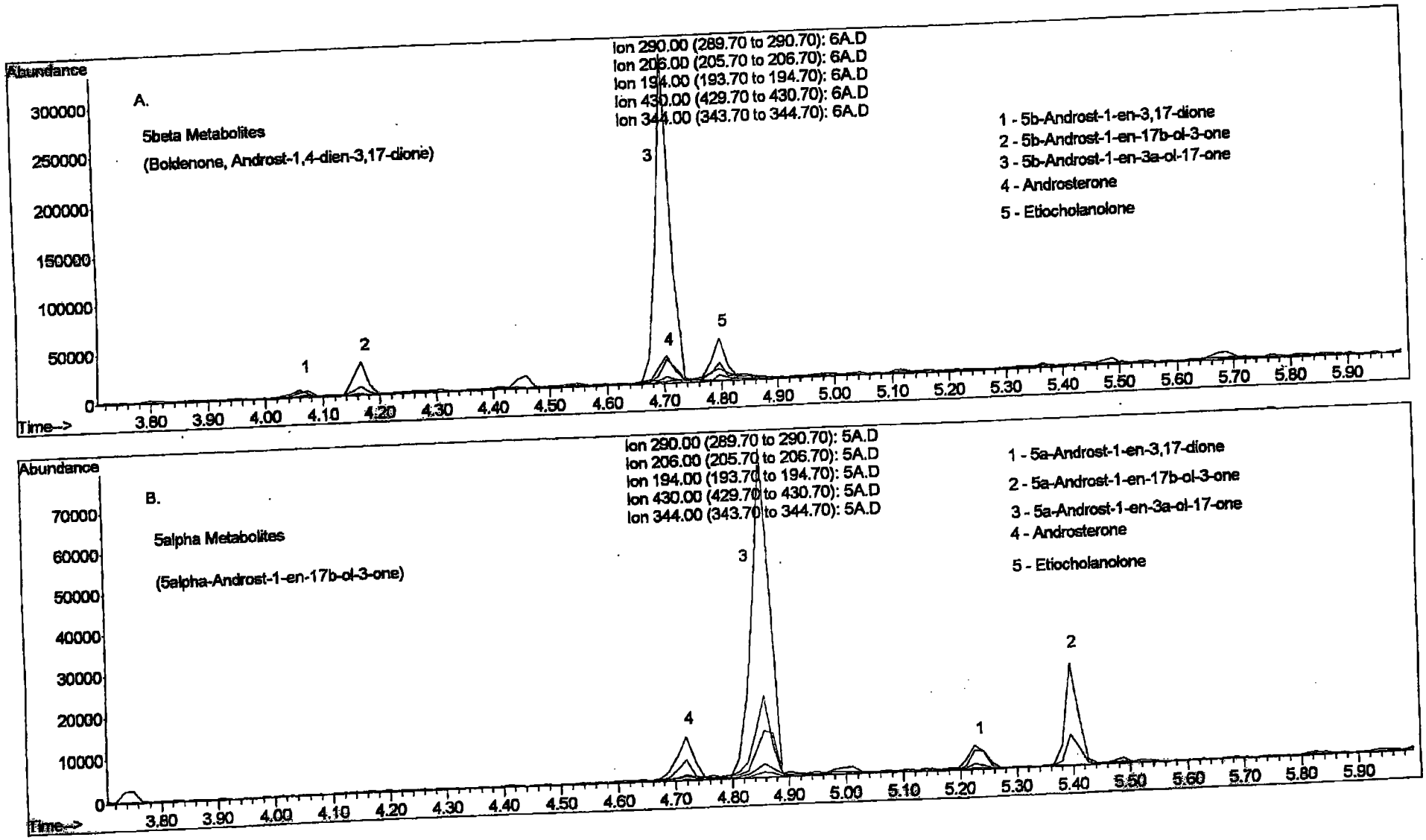


Figure 4. Chromatograms of selected urinary metabolites of 1,4-androstadien-3,17-dione (A); and 5α-androst-1-en-17β-ol-3-one (B).