P. VAN EENOO, F.T. DELBEKE, N. DESMET:
Excretion Studies with a Supplement Containing 1(5a)-Androstene-3β,17β-diol
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Excretion studies with a supplement containing 5α-androst-1-ene-3β,17β-diol

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Introduction
The anabolic steroid 5α-androst-1-ene-3β,17β-diol, also referred to as 1-androstenediol, is available from the internet. It has been mentioned in body builder newsgroups and has been reported in specialized body builder magazines.

1-Androstenediol is marketed as an extremely potent anabolic steroid which is seven times more potent than testosterone (1). Moreover it is claimed, that the oral bioavailability is remarkably high as compared to the other prohormones because of the unsaturation at the C1 position.

In order to convince those people who still hesitate to buy this product because of possible health risks, the manufacturers are very reassuring in stating that the product is all natural and safe. The commercial name of 1-androstenediol is 1-AD® and it is produced by Ergopharm, the same company that put 4-androstenedione, 4-androstenediol and 19-nor-4-androstenediol on the prohormone market.
In the United States, 1-androstenediol is regarded as a dietary supplement and is sold as an over the counter product.

Based upon the chemical structure of the compound, misuse of 1-androstenediol might remain undetected with the current doping control procedures.

In order to detect possible markers for misuse of this compound an excretion study was set up.
Material and methods

Administration

The content of the capsules was checked for the presence of 1-androstenediol and other anabolic steroids by GC/MS (2).

A male volunteer (aged 29) took one capsule of the 1-AD supplement (total declared dose: 100 mg). Urine was collected 0, 1, 2, 4, 6, 9, 12, 24, 30, 36, 48, 72, 96, 120, 144 and 168 h post administration.

Analysis

For the determination of the steroid profile and the detection of possible metabolites, the screening procedure IV was used.

Briefly, 1 mL of sodium acetate buffer (pH 5.2) and 50 μL β-glucuronidase (type HP-2, Sigma Co, St-Louis, MO, USA) were added to 2 mL of urine and the mixture was hydrolysed for 2.5 h at 56°C. After addition of the internal standard (50 μL of a 2 μg/mL 17α-methyltestosterone solution in methanol), extraction was performed by rolling (20 min) with 5 mL freshly distilled diethylether, followed by centrifugation. The organic layer was separated, dried over anhydrous Na₂SO₄ and dried under OFN.

The dried residue was derivatised with 100 μL MSTFA/NH₄I/ethanethiol (380:1:2 vol/vol/vol) for 1 h at 80°C to obtain TMS-enol-TMS-ether derivatives.

Mass spectrometric analysis was performed on a Agilent-MSD 5973 instrument (Agilent, Waldbronn, Germany) equipped with a 17 m HP-Ultra 1 column (internal diameter 0.2 mm, film thickness 0.11 μm). The GC temperature program was as follows: 120°C (1 min) - 70°C/min → 182°C - 4°C/min → 235°C - 30°C/min → 300°C (3 min). Injection volume was 0.5 μL, splitless mode.
The instrument was operated in full scan mode for identification of metabolites and in SIM-mode for steroid profiling and determination of detection times.

**Results**

The weight of ten capsules was tested and varied between 700 and 750 mg.

1-androstenediol could not be purchased from the regular suppliers of reference substances (NARL, Steraloids, Sigma, Radian, Research plus, etc). Hence the definite identification of 1-androstenediol in the capsules was impossible. Nevertheless, analysis of the capsules revealed the presence of a compound in large quantities with a mass spectrum similar to that of 4-androstendiol and 5-androstenediol, but with a different retention time. The results of the analysis of the urine samples suggest that the capsules do indeed contain 1-androstenediol.

The same analysis however also showed the presence of boldenone, dehydroepiandrosterone, 19-nor-4-androstenediol, testosterone and 5α-androstane-3α, 17β-diol.

The detection times, according to the decision criteria used for screening of regular doping control samples, for the different steroids that contaminated the 1-AD prohormone preparation are shown in Table 1.

The mass spectrum of the substance eluting after 10.96 min (RRT=0.76) is shown in Fig. 1. The administration of the 1-AD capsule resulted in the detection of a second GC-peak at a retention time of 11.62 min (RRT=0.82) The mass spectrum of this substance is shown in Fig. 2.

The excretion profile of 1-androstenedione is shown in Fig. 3.

**Discussion**

The variation in weight of the capsules, can lead to substantial differences in the daily administered dose, especially if the recommendations of the manufacturer are followed (1-3 capsules, three times per day). The occurrence of other anabolic steroids in the different capsules
can be regarded as even more worrisome. The presence of boldenone and testosterone (both registered drugs) is even a direct violation of the US laws on food supplements.

As shown in Table 1, analysis of the excretion urines using screening procedure 4 resulted in positive findings for boldenone as a parent compound from 2 to 6 h post administration. 17-β-hydroxy-5β-androst-1-ene-3-one was detected from 2 to 36 h post-administration. This substance was previously reported as a major metabolite of boldenone (3). Norandrosterone, the main urinary metabolite of the norsteroids was detected in concentrations exceeding 2 ng/ml from 2 until 24 h post administration.

Although a number of substances were present that could influence the testosterone to epitestosterone ratio, the results indicated that this ratio was only slightly affected. In contrast to the T/E-ratio, the ratio of androsterone to etiocholanolone increased from approximately 1 to 5, in compliance with previous studies that determined the influence of the intake of DHEA on the steroid profile. This ratio returned to its basal value after 24 hours.

Because 1-androstenediol could not be purchased from the regular suppliers of reference substances, the definite identification of 1-androstenediol in the capsules was impossible. Based upon the similarity of the mass spectrum and the different GC retention time compared to those of 4-androstendiol and 5-androstenediol and taking into account the results of the analysis of the urine samples, it is likely that the capsules contained 1-androstenediol.

A substance with the mass spectrum shown in Fig. 1 was detected in several excretion urines. Based on data from literature, the proposed structure of this substance is 3α-hydroxy-5α-androst-1-ene-17-one. This substance remained detectable for about 72 hours and can be used to monitor the use of the 1-AD product.

The mass spectrum of a second metabolite is shown in Fig. 2. This substance was identified as 5α-androst-1-ene-3,17-dione, by comparison with a reference standard, and was previously identified as the sole metabolite of boldenone with a 5α-configuration (3).

The maximum urinary concentration of 1-androstenedione was attained 9 h post administration.
and was approximately 700 ng/ml (Fig. 3). These high concentrations suggest at least a partially other origin than as a metabolite of the boldenone contaminant.

Taking into account the metabolisation pathways of 4-androstenediol and 5-androstenediol (4-5), the metabolism of 5α-androst-1-ene-3β,17β-diol to 5α-androst-1-ene-3,17-dione is very likely to occur.

As shown in Fig. 3, analysis of the excretion urines in the SIM-mode (m/z=430, 415, 194, 325) resulted in the detection of this substance up to 120 h post administration, which makes it the evident choice for monitoring 1-AD misuse.
Acknowledgements

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References


Table 1. Detection times for anabolic steroids as contaminants in 1-AD using the routinely used screening methods (+: positive GC/MS-result; -: negative GC/MS-result)

<table>
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<tr>
<th>Post administration time (h)</th>
<th>Boldenone</th>
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Fig. 1. Mass spectrum of TMS-derivatised 3α-hydroxy-5α-androst-1-ene-17-one (proposed)

Fig. 2. Mass spectrum of TMS-derivatised 1(5α)-androstene-3,17-dione

Fig. 3. Excretion profile of 1-androstenedione after intake of one 1-AD capsule