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Prohormones and sport☆

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Abstract

Several precursors of testosterone and nandrolone introduced on the nutritional supplement market as performance enhancing drugs are banned in sports. Until now they are legally sold without a prescription in the US. Results of excretion studies with related compounds including 7-keto-DHEA and 1-androstenediol are presented. The main metaboliltes of 7-keto-DHEA are 7-hydroxylated compounds. The commercial 1-androstenediol preparation was contaminated with several other anabolic steroids. Oxidation of 1-androstenediol to 1-androstenedione seems to be the major renal metabolic pathway.

Additionally contaminated nutritional supplements containing banned substances not indicated on the label were administered. The results of the excretion studies indicate that after the intake of amounts substantially lower than the recommended dose athletes can fail a doping test for periods up to 120 h.

Keywords: Doping analysis; Contamination; Nutritional supplements; 1-Androstenediol; 7-keto-DHEA

1. Introduction

Since the introduction in the 1980s of a general screening method for anabolic steroids in doping analysis, the detection of long acting injectable preparations became rather easy [1–3]. In order to circumvent a positive test athletes therefore switched to injectable testosterone or oral preparations that were rapidly cleared from the body. It was reported that in the 1980s East German scientists, knowing that a test would soon be adopted to detect the use of testosterone, developed short acting testosterone preparations and nasal sprays containing the testosterone precursor androstenedione [4].

Another way to circumvent the doping tests was the use of prohormones, especially precursors of testosterone, while the orally short acting precursors of nortestosterone also seemed to attract the cheating athlete.

One of the first hormonal supplements that became available in the USA was DHEA around 1996. The list steadily expanded and now includes DHEA, 4-androstenedione, 4-androstenediol and 5-androstenediol as well as the nandrolone precursors 19-norandrostenedione and 19-norandrostenediol. Their introduction on the US market and the internet trade were the direct result of the 1994 Dietary Supplement and Health Education Act. Originally this act was designed to allow people the use of common vitamin supplements. However, through several loop-holes in the law, any substance that is natural to the body can be sold according to this act. In this way consumers including professional athletes and people using substances for cosmetic reasons, are being increasingly provided with untested, unproven and potentially lethal products. A strong indication of this trend comes from the increased sales of dietary supplements which have surged in the US from 8.3 billion in 1994 to 14 billion in 1999. Moreover these supplements place athletes at sanctions from international sports authorities.

In this work results of excretion studies with prohormones including 1-androstenediol and 7-keto-DHEA will be presented.

Another problem with prohormones and sport is related to nutritional supplements contaminated with prohormones [5–7]. The aim of this study was to determine detection times for doping substances not listed on the product labels after the ingestion of two contaminated supplements.

2. Experimental

2.1. Prohormones

The prohormone 7-keto-DHEA (7-keto®) was manufactured by Enzymatic Therapy® (Green Bay, WI, USA) and

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was purchased in the Netherlands. The lot number was 25740.

The other prohormone 1-AD was manufactured by LPJ Research Inc. (Seymour, IL, USA) and was labelled to contain 100 mg 1-androstene-3β-17β-diol. A jar containing 60 capsules of this supplement (lot number: DC25301EX04) was purchased via internet.

2.2. Contaminated nutritional supplements

Two different supplements were purchased. The manufacturer's recommended dose for supplement X containing hydroxycitric acid, L-carnitine, L-phenylalanine, vanadyl sulfate and extracts from citrus auranitium and guarana (lot number: 810269) was seven capsules per day. The recommended dose for a pyruvate supplement Y (lot number: EXP5/02-64327) was limited to one tablet per day.

2.3. Analysis of urine samples

Urine samples were analysed for anabolising agents by GC–MS as routinely done for samples collected for doping analysis [8]. Endogenous steroids were quantified by GC–MS (SIM). Low norandrosterone concentrations were measured by GC–MS–MS as described by Van Eenoo et al. [9].

For the identification of metabolites of the 7-keto and 1-AD supplement GC–MS analysis was performed in full scan mode. Urinary extracts in the 7-keto excretion study were derivatised with MSTFA for 20 min at 80 °C.

2.4. Analysis of nutritional supplements

The determination of anabolic steroids in nutritional supplements was done by a slightly different method than previously described [6]. Briefly, 5 ml NaOH (1 M) was added to 1 g of the nutritional supplement. After addition of 10 μl of the internal standard (17α-methyltestosterone, 100 μg/ml MeOH) extraction was performed by rolling (1 h) with 5 ml *n*-pentane. After centrifugation, the organic layer was separated and evaporated under OFN at 40 °C. The residue was derivatised with 100 μl of a mixture containing MSTFA/NH_4I/ethanethiol (380:1:2, v/w/v) for 1 h at 80 °C.

GC–MS analysis was carried out on a HP 6890 gas chromatograph directly coupled to a HP 5973 mass selective detector (HP, Palo Alto, USA). The GC column was a HP-Ultra 1 (J&W, Folsom, USA), 100% methylsilicone with a length of 17 m, an internal diameter of 0.2 mm and a film thickness of 0.11 μm. The oven temperature program was as follows: 120 °C (0 min) – 70 °C/min – 181 °C (0 min) – 4 °C/min – 234 °C (0.1 min) – 30 °C/min – 300 °C (3 min). Injection (0.5 μl) was splitless. The mass spectrometer was operated in the full scan mode.

2.5. Excretion studies

Prior to the excretion study with 7-keto-DHEA, the capsules were tested for the presence of 7-keto-DHEA by comparison with an authentic standard obtained from Steraloids (Newport, RI, USA) and checked for the eventual presence of other steroids by GC–MS.

Five male volunteers (aged 23–55) took two capsules of the 7-keto product (total dose: 50 mg). Urine was collected before and 2, 4, 6, 9, 12, 24, 30, 36, 48, 72, 96 and 120 h post-administration.

The content of the 1-AD capsules was also checked for the presence of 1-androstenediol and other anabolic steroids by GC–MS analysis.

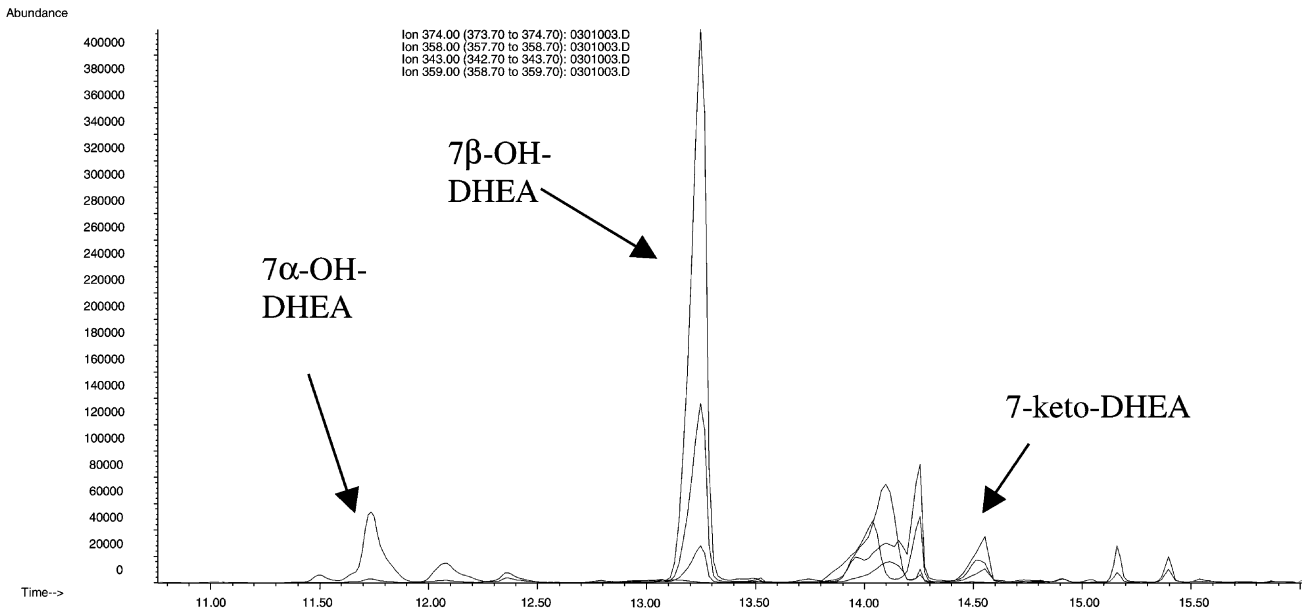

Fig. 1. Ion chromatograms (*m/z* 374, 358, 343 and 359) of an excretion urine (4 h post-administration of 7-keto-DHEA).

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

One capsule of the food supplement X was taken orally by five volunteers. Urine was collected quantitatively during the first 12 h (i.e. 2, 4, 6, 9, 12 h). Additional urine samples were collected after 24, 48, 72, 96, 120, 144 and 168 h.

Two tablets of the supplement Y were ingested by two volunteers. Sample collection was as above.

3. Results

3.1. Prohormones

Analysis of the 7-keto dietary supplement did not reveal the presence of other anabolic steroids.

The ion chromatograms (*m/z* 374 and 358) obtained after the analysis of extracts from a 4 h post-administration sample after TMS-ether derivatisation are shown in Fig. 1.

The main metabolites of 7-keto-DHEA are 7-hydroxylated-DHEA compounds. Mass spectra of TMS-ether derivatives of 7α-OH-DHEA and 7-keto-DHEA are shown in Figs. 2 and 3, respectively. Besides 7-hydroxy-DHEA metabolites a number of other metabolites were detected including a substance tentatively identified as TMS-derivatised 7ζ-hydroxy-androstenedione (Fig. 4).

As 1-androstene-3β,17β-diol could not be purchased from suppliers of reference substances, a definite identification of 1-androstenediol in the 1-AD capsules was not possible. 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 same analysis however also showed the presence of boldenone, dehydroepiandrosterone, 19-nor-4-androstenediol, testosterone and 5α-androstane-3α, 17β-diol in the capsule.

Table 1
Detection times for the anabolic steroids contaminating the 1-AD prohormone (intake one capsule)

Post-administration time (h)	Boldenone	5-β-Androst-1-ene-1-β-ol-3-one	19-Norandrosterone (>2 ng/ml)
0	−	−	−
1	−	−	−
2	+	+	+
4	+	+	+
6	+	+	+
9	−	+	+
12	−	+	+
24	−	+	+
30	−	+	−
36	−	+	−
48	−	−	−

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

The mass spectra of the substances eluting after 10.96 min (RRT = 0.76) and 11.62 min (RRT = 0.82) in a urine sample taken 9 h post-administration of 1-AD are shown in Figs. 5 and 6. The excretion profile of 1-androstenedione is shown in Fig. 7.

3.2. Contaminated nutritional supplements

The presence of 19-nor-4-androstene-3-17-dione (nor-4-adione) and 4-androstenedione (4-adione), both not listed on the label of supplement X, was confirmed by GC–MS

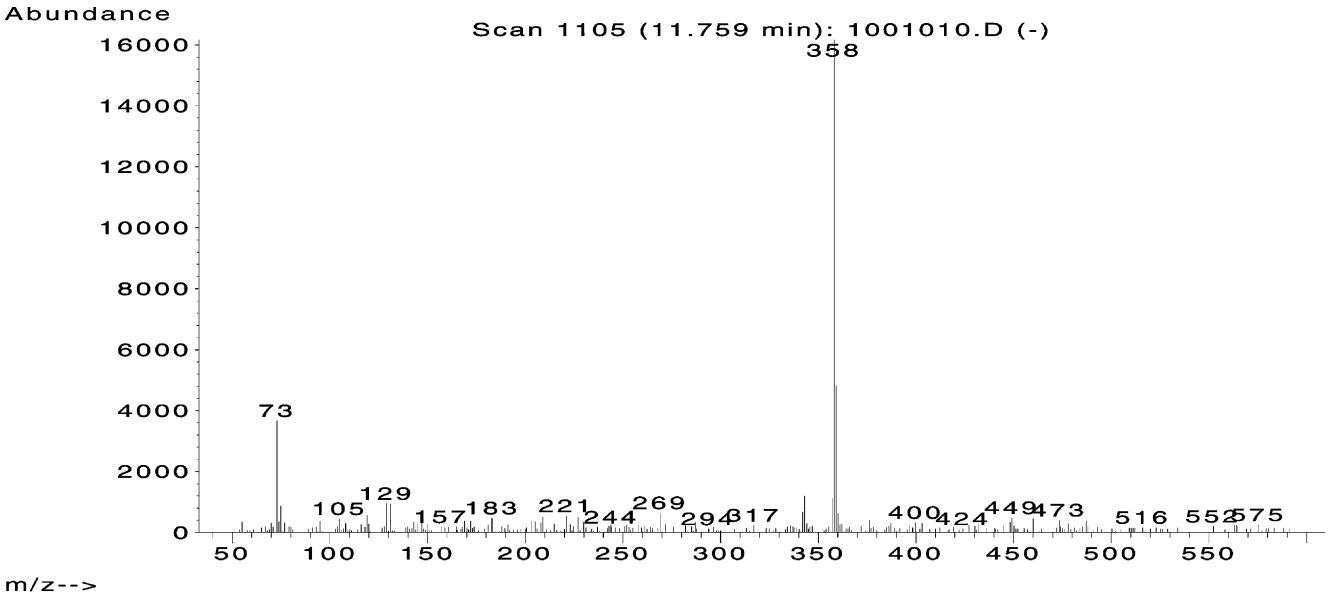

Fig. 2. Mass spectrum of mono-TMS-derivatised 7α-OH-DHEA in an excretion urine (4 h post-administration of 7-keto-DHEA).

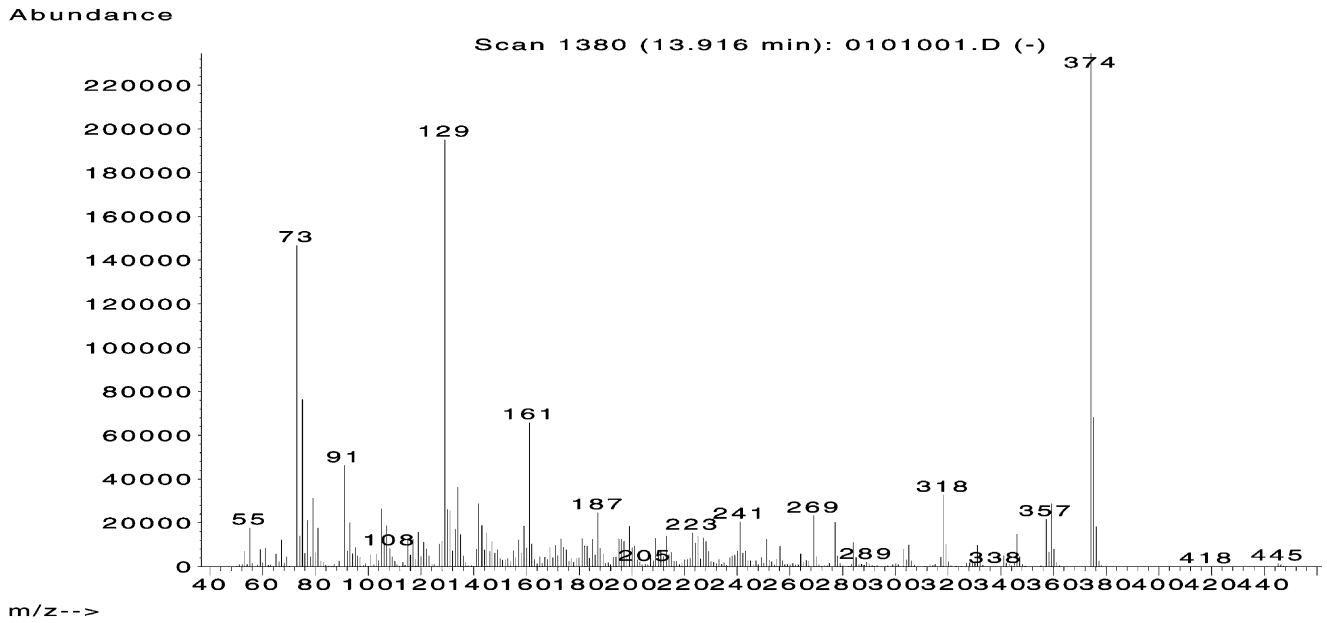

Fig. 3. Mass spectrum of mono-TMS-derivatised 7-keto-DHEA in an excretion urine (4 h post-administration of 7-keto-DHEA).

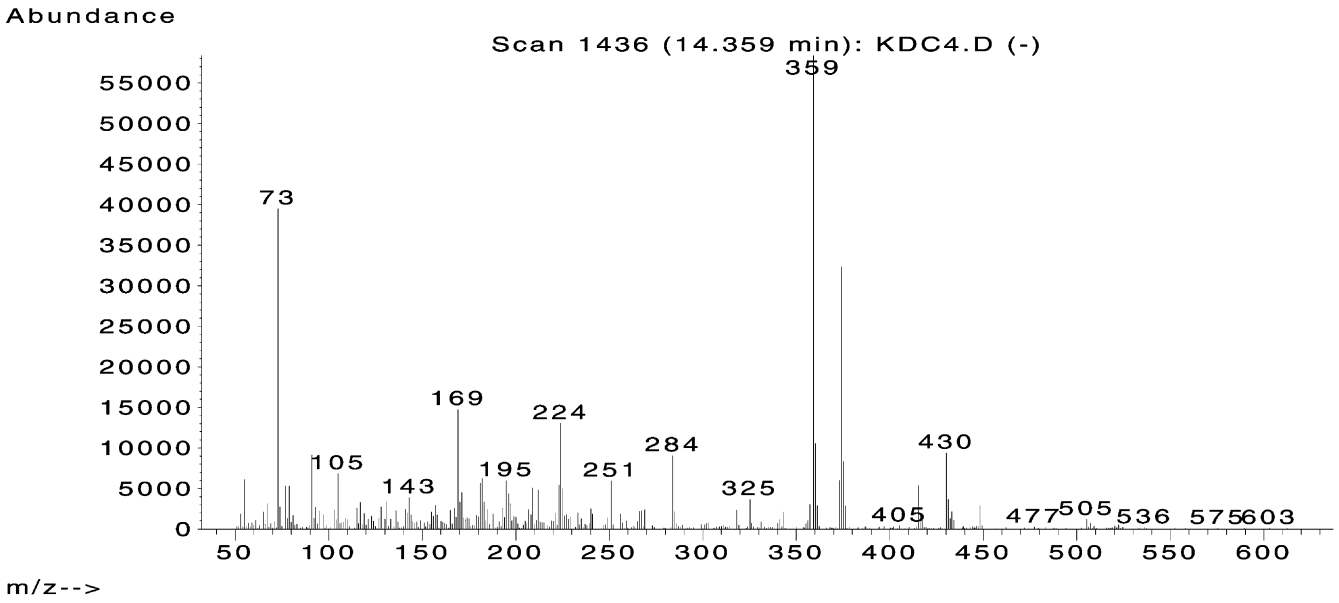

Fig. 4. Mass spectrum of 7ζ-hydroxy androstenedione-bis-TMS in an excretion urine (4 h post-administration of 7-keto-DHEA).

in full scan mode. Concentrations of approximately 0.9 and 6.3 mg/g were found for, respectively, 4-adione and nor-4-adione. Hence, one capsule of the nutritional supplement contained 0.7 mg 4-adione and 4.8 mg nor-4-adione.

Prohormones not listed on the label were also found in supplement Y. The concentrations were as follows: DHEA 159 ng/g, 4-adione 78 ng/g, testosterone 243 ng/g and 4-nor-adione 189 ng/g.

The excretion profile of norandrosterone after the ingestion of one capsule of the contaminated supplement is given in Fig. 8.

After the intake of two capsules of the supplement Y no significant changes were detected in the steroid profile. Norandrosterone could only be detected in the 2 h sample in both subjects. The concentration was 0.11 and 0.23 ng/ml, respectively.

4. Discussion

4.1. Prohormones

The main metabolites of 7-keto DHEA are the 7-hydroxylated-DHEA compounds. 7-Hydroxy DHEA isomers were previously identified as metabolites of DHEA and are endogenously present in very small amounts [8]. After

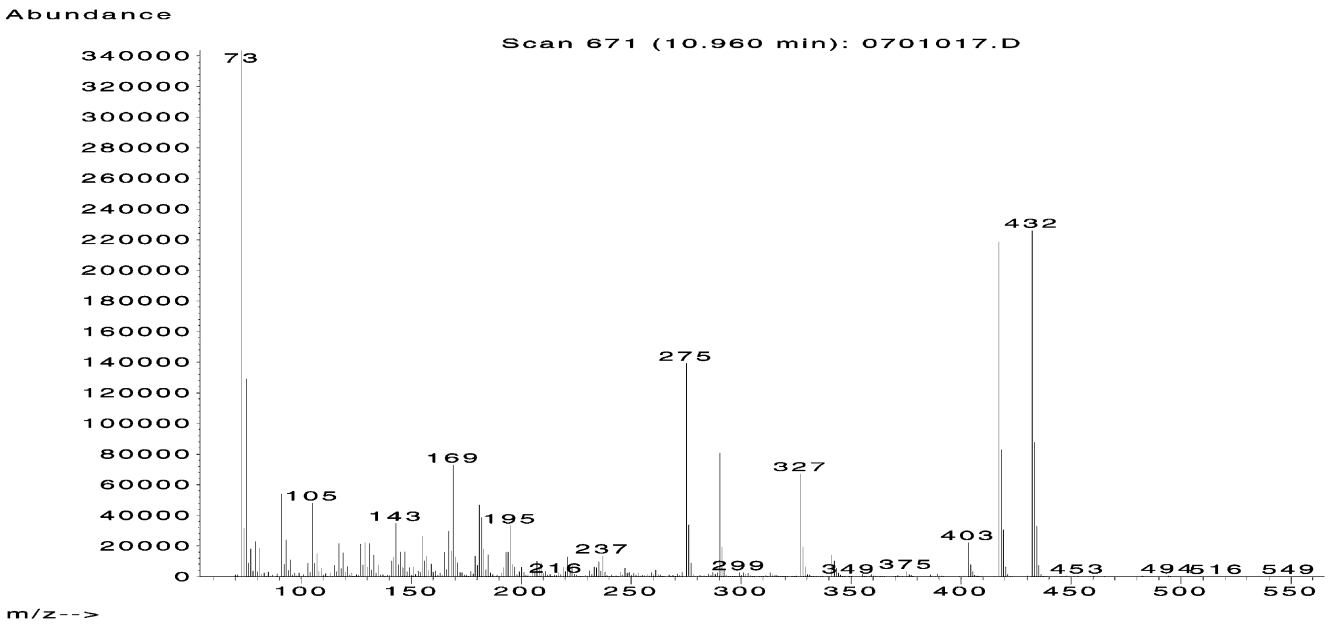

Fig. 5. Mass spectrum of 5α-androst-1ene-3α-ol-17-one-bis-TMS in an excretion urine (9 h post-administration of the 1-AD supplement).

ingestion of 7-keto DHEA, however, these substances are abundantly present. Regarding doping analysis more quantitative analyses on a wide range of samples from excretion studies will be necessary to determine appropriate threshold values.

Besides the 7-hydroxy-DHEA metabolites, one metabolite was tentatively identified as 7ζ-hydroxy-androstenedione. According to the metabolism of DHEA, metabolism of 7-keto-DHEA to 7ζ-hydroxy androstenedione is likely to occur.

From this study it seems that some of the over the counter prohormones are contaminated with anabolic steroids, regulated substances, including testosterone and veterinary drugs such as boldenone. In addition DHEA, 19-norandrostenediol and 5-α-androstane-3α-17β diol were found in this 1-AD supplement. As indicated in Table 1 the intake of one capsule can result in positive doping tests for boldenone 2–6 h post-administration.

The major boldenone metabolite was detected till 36 h. Norandrosterone was found in concentrations exceeding

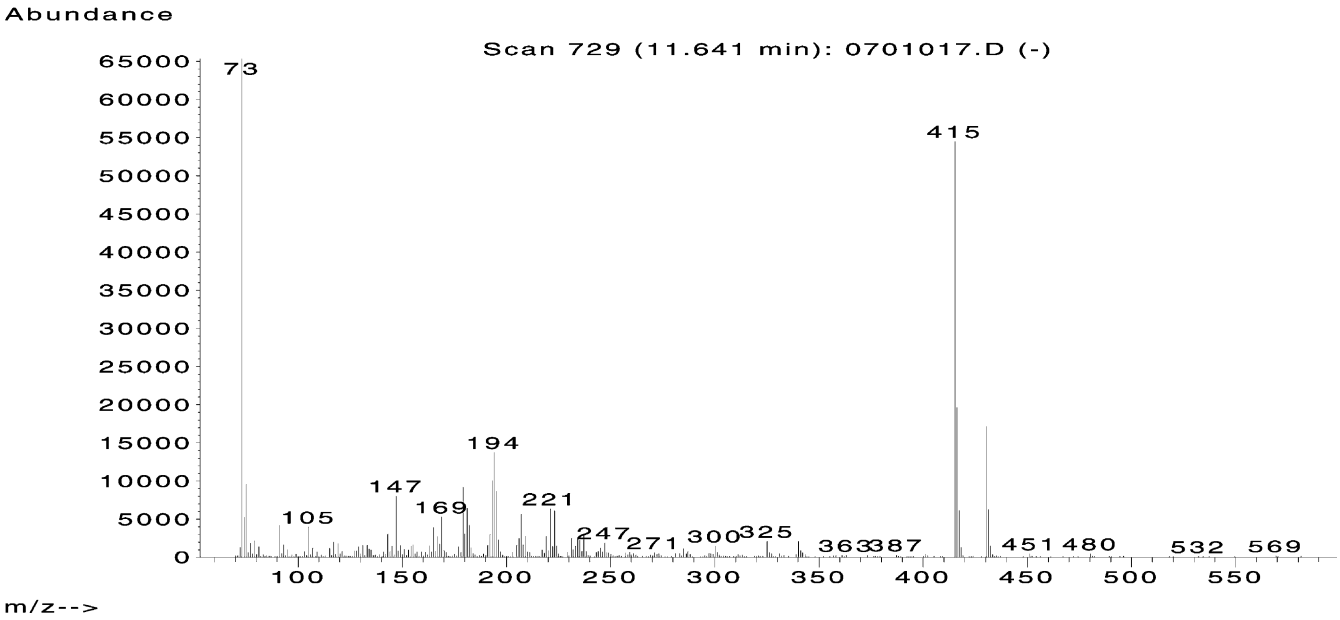

Fig. 6. Mass spectrum of 1(5α)-androstene-3,17-dione-bis-TMS in an excretion urine (9 h post-administration of the 1-AD supplement).

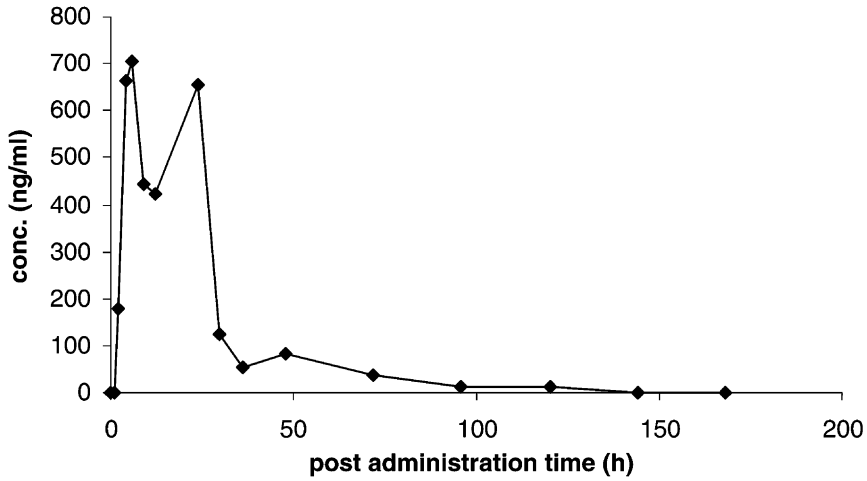

Fig. 7. Excretion profile of 1-androstene-3,17-dione after administration of one 1-AD capsule.

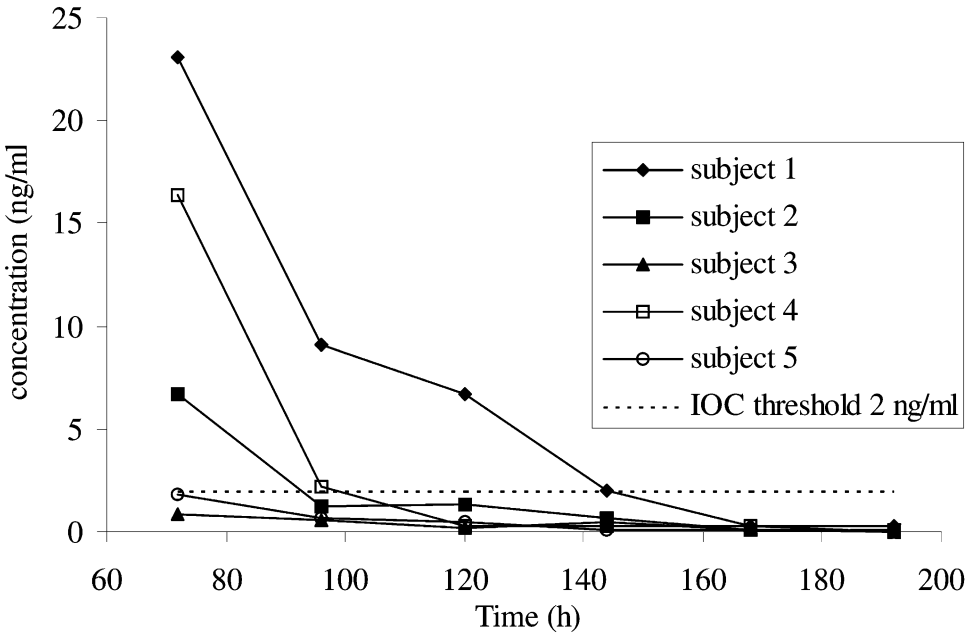

Fig. 8. Urinary concentrations of 19-norandrosterone in five male volunteers from 60–120 h after the ingestion of a contaminated food supplement.

2 ng/ml, the IOC doping threshold in males [9] from 2 until 24 h after the intake of one capsule.

1-Androstenediol, the major component of the 1-AD supplement was metabolised to androstenolone (5α-androst-1-ene-3α-ol-17-one) and 1-androstenedione. Using the latter metabolite, the misuse of only one capsule can be detected for 120 h.

4.2. Contaminated food supplements

In a study sponsored by the EU in which different supplements purchased in fitness centres were analysed, 19-norandrostenedione was found in a branched chain amino acid preparation [10]. Geyer et al. [5] found anabolic steroids in nutritional supplements including a chrysin product, tribulus terrestris and guarana supplements. In a Swiss study Kamber et al. [7] found that 7 out of 74 supplements bought through the internet contained other hormone substances than indicated on the label.

In this study, a supplement was found to contain norandrostenedione and androstenedione in relatively high amounts. After the ingestion of one capsule athletes could test positive until 48–144 h. The manufacturer's recommended dose was seven capsules per day.

5. Conclusion

Except in very special circumstances requiring medical care nutritional supplements are not required in addition

to a normal balanced diet. Athletes needing supplements should be very careful as some supplement may contain prohormones not declared on the label. Moreover following international rules athletes remain responsible for what is found in their bodyfluids, irrespective the origin.

As long as initiatives to legally control the production and distribution of prohomones are not instigated the possibility to ingest contaminants or by-products from the synthesis constitutes a very real health risk for the consumer including athletes or people urine supplements or prohormones for cosmetic reasons.

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