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# RECENT ADVANCES IN DOPING ANALYSIS (9)

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# Excretion studies with 7-keto-dehydroepiandrosterone

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## Introduction

7-keto-dehydroepiandrosterone is an anabolic steroid and is also referred to as 7-oxo-dehydroepiandrosterone. It has been characterized as a metabolite of dehydroepiandrosterone in in-vitro studies[1,2].

On the internet this product is widely available and although at present, the emphasis in the marketing campaign of most suppliers is put on its so-called anti-ageing effects, the product has been mentioned in body builder newsgroups. It is therefore likely that 7-keto-DHEA is being misused.
The marketing profile so far is that 7-keto-DHEA acts in the same way as DHEA, but that it has less undesirable side-effects.

Scientific studies on the effects of 7-keto-DHEA in mice showed that 7-keto-DHEA improves the memory[3] and immune response[4] of these animals.
7-keto-DHEA is claimed to be zero percent androgenic and is not converted into testosterone or DHEA[5].

Until now, most of the scientific work on the effects of 7-keto-DHEA has been done by one group of scientists[3,5].

Although 7-keto-DHEA is available in different formats from a range of suppliers, the substance itself seems to be manufactured by Humanetics corporation (http://www.7keto.com/).

In the United States, 7-keto-DHEA is regarded as a dietary supplement and sold as an over the counter product.
Other anabolic agents including androstenedione and some norsteroids have illustrated that this approach results in a global distribution of these products via the internet[6,7]. The internet serves as a medium which allows for fast direct distribution of the products or provides addresses of regionally based suppliers in different parts of the world.
In most cases, customs control does not have the necessary means to stop the import of internet purchased products in countries where they are prohibited. In this case, we ordered the Enzymatic Therapy 7 keto-product from a local representative of this company. Although in direct violation with European and Belgian laws, we received the product without any problems, illustrating the ease in obtaining this kind of substances in Europe and probably also in the rest of the world.

Because 7-keto-DHEA is not metabolised into endogenous steroids commonly screened for during doping control, misuse of this substance remains undetected. In order to identify possible markers for 7-keto-DHEA misuse, an excretion study was set up.

## Material and methods

*Administration*
Prior to the excretion study, the capsules were tested for the presence of 7-keto-DHEA by comparison with an authentic standard obtained from Steraloids and checked for the presence of endogenous and other exogenous steroids by GC/MS[8].

During the excretion study, five male volunteers (aged 23 to 55) took two capsules of the Enzymatic Therapy 7-keto product (total dose: 50 mg). Urine was collected 0, 2, 4, 6, 9, 12, 24, 30, 36, 48, 72, 96 and 120 h post administration.

*Analysis*

For the determination of the steroid profile and the detection of possible metabolites, the normal 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 $Na_2SO_4$ and dried under OFN.

All samples were analysed in duplicate. One residue was derivatised with 100 μL MSTFA/NH4I/ethanethiol (380:1:2 vol/wt/vol) for 2 h at 80°C to obtain TMS-enol-TMS-ether derivatives, while the other residue was derivatised with 100 μL MSTFA (2h at 80°C) to obtain TMS-ether derivatives.
Mass spectrometric analysis was performed on a HP-MSD 5973 instrument (Hewlett-Packard, Waldbron, Germany) equipped with a 17 m HP-Ultra 1 column (internal diameter 0.2 mm, film thickness 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**

Analysis of the 7-keto "dietary supplement" did not reveal the presence of other anabolic steroids. The ion chromatograms (m/z=374 and m/z= 348) of a blank sample and a 9h post administration sample after TMS-ether derivatisation are shown in Fig. 1

All samples were analysed as TMS-ether and TMS-enol-TMS-ether derivatives to obtain structural information. In the case of 7-keto-DHEA, we observed that derivatisation as TMS-enol-TMS-ether derivative was incomplete and three derivatisation products were formed. This was not the case for the TMS-ether derivative. The TMS-ether derivatives of 7-keto-DHEA and 7$\alpha$-OH-DHEA are shown in Fig. 2 and Fig. 3 respectively.

**Discussion**

The parent compound and a number of metabolites were detected.
The parent compound remained detectable for up to 9h post administration using both derivatisation methods.
The main metabolites of 7-keto-DHEA are the 7-hydroxylated-DHEA compounds (Fig. 1 and Fig. 3). The 7-hydroxy-DHEA isomers were previously identified as metabolites of DHEA and are endogenously present in very small amounts[9].
After ingestion of 7-keto-DHEA, however these substances are abundantly present. More quantitative analyses on a wide range of samples will be necessary to determine threshold values for absolute concentrations. Nevertheless, substantial amounts of the 7-hydroxy metabolites were present for 30 hours. It has been shown that the ratio of 7β-hydroxydehydroepiandrosterone to 16-OH-androsterone could be used as an indicator for DHEA misuse[10]. In the case of DHEA misuse, this ratio would exceed 1. Because 7-keto-DHEA is partially metabolized to 7-hydroxylated-DHEA, the same ratio could be applied.
Besides the 7-hydroxy-DHEA metabolites a number of other metabolites were detected.
The substance with the mass spectrum shown in Fig.4, was tentatively identified as TMS-derivatised 7 ζ-hydroxy-androstenedione. In accordance with the metabolism of dehydroepiandrosterone, metabolisation of 7-keto-dehydroepiandrosterone to 7 ζ-hydroxy-androstenedione is likely to occur. Definite identification by comparison of this metabolite with a reference standard is in progress.
A number of other metabolites have also been recorded, but not yet identified.
Using the routine screening procedure IV, derivatisation results in the formation of enol-TMS-ether-TMS derivatives and the m/z-values in Table 1 are suggested to monitor the presence of the respective substances.

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Table 1. Suggested m/z for monitoring 7-keto-DHEA and its main metabolites in urine as enol-TMS-ether-TMS derivatives

| substance | m/z |
|---|---|
| 7-keto-DHEA | 518, 429, 323 |
| 7$\zeta$-hydroxy-DHEA | 520, 430, 415 |
| 7$\zeta$-hydroxy-androstenedione | 518, 429, 169 |

Fig. 1 Ion chromatograms of a 7-keto excretion urine (4h post administration)

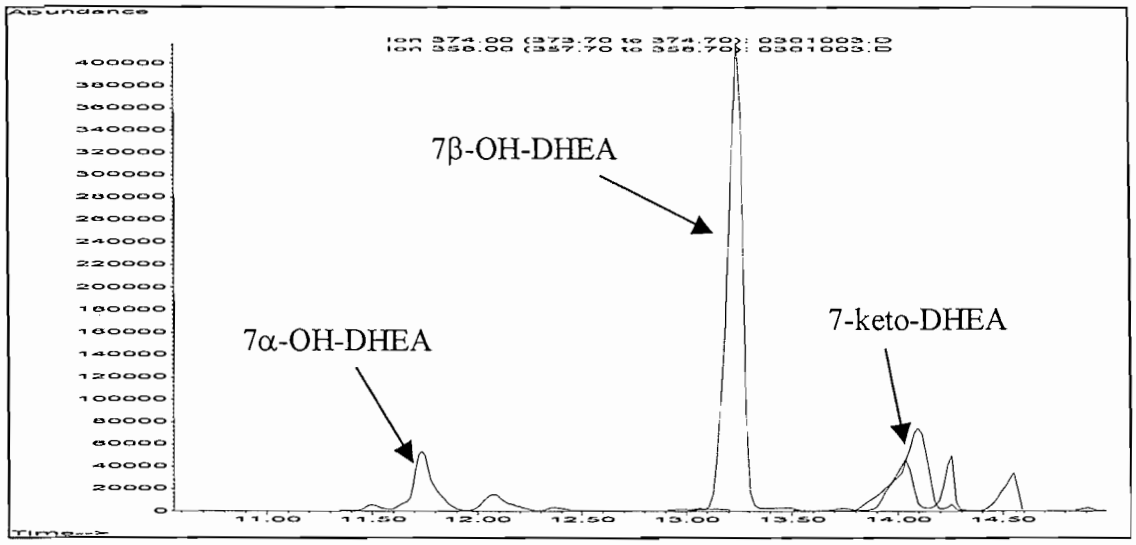


Fig. 2. Mass spectrum of TMS derivatised 7-keto-DHEA in a 7-keto excretion urine (4h post administration)

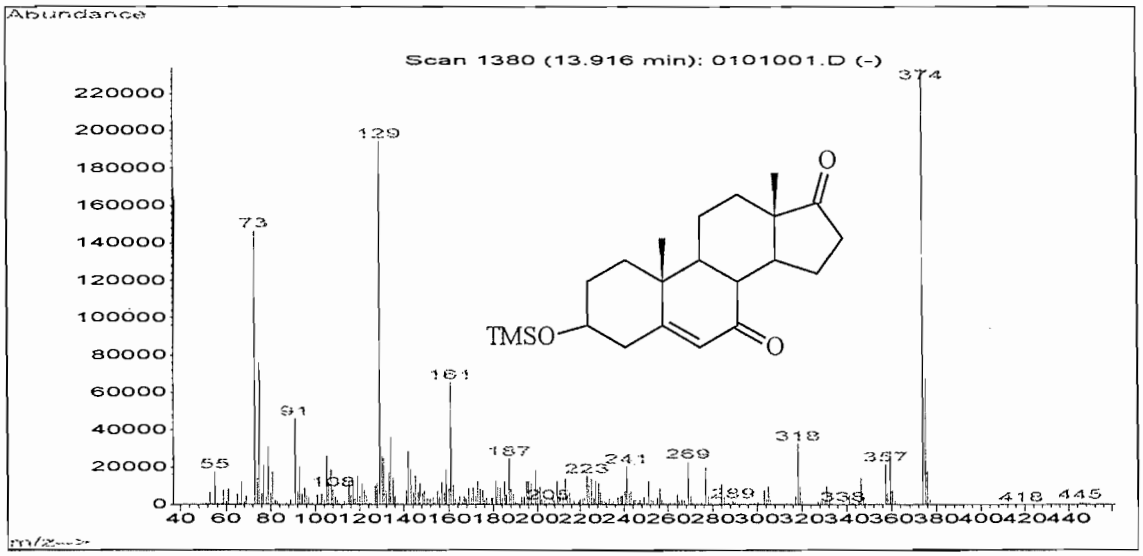

Fig. 3. Mass spectrum of TMS derivatised 7ζ-OH-DHEA in a 7-keto excretion urine (4h post administration)

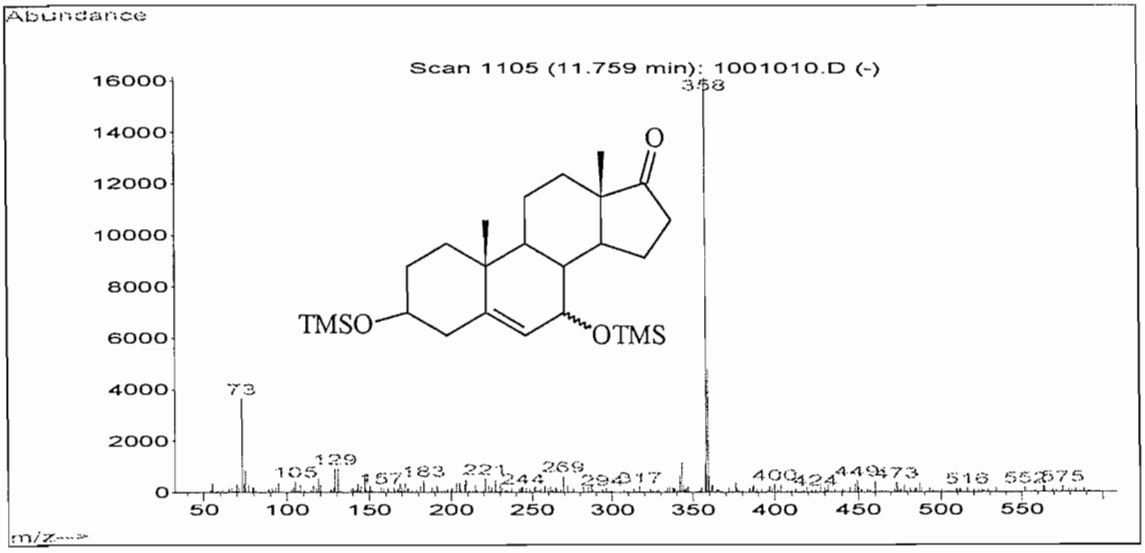


Fig. 4. Mass spectrum of the substance, tentatively identified as TMS derivatised 7ζ-OH-androstenedione in a 7-keto excretion urine (4h post administration)