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Results of several (small) research projects at DoCoLab in 2006

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1. Introduction

The paper is a summary of two research projects that were initiated in 2006 at DoCoLab and which deal with quality assurance, improvements in detection methods and analysis of new prohormones.

2. Prohormones

The aims of the WADA funded project are to monitor the internet through websites and news groups as well as specialised media to detect new supplements possibly containing prohibited substances.

Once such products have been spotted, we will purchase them, analyze the supplements and disseminate the obtained information together with samples of the obtained material. Furthermore, it is the objective to try and find scientific information on these substances, their effects, side-effects and metabolism and distribute this to the laboratories as well.

2.1. *2α,17α-dimethyldihydrotestosterone*

2α,17α-dimethyldihydrotestosterone is sold under several commercial names, including superdrol, methylmasteron, methasteron, methylmasterdrol, methylsupervol and methyl-drol. The synthesis of 2α,17α-dimethyldihydrotestosterone and limited data on biological activity have been published in the 1950's. It has been reported that unesterified 2α,17α-dimethyldihydrotestosterone is a potent orally active anabolic agent exhibiting only relatively weak androgenic activity [1, 2].

This steroid is largely excreted unchanged, although a minor dihydro metabolite (2α,17α-dimethyl-5α-androstan-3α,17β-diol) was also detected in in-vivo studies. In-vitro studies resulted in the additional detection of 2α,17α-dimethyl-5a-androstan-3α,16ζ,17β-triol [3-5].

2.2. *4-chloro-17α-methyl-androst-4-ene-3ζ,17β-diol*

Promagnon-25 is a product containing several steroids that are structurally related to clostebol and oral turinabol and several substances including one with the code name "STS 482" that were previously synthesised and used in the East German Doping system [6, 7]. The most important steroid in the supplement is 4-chloro-17α-methyl-androst-4-ene-3ζ,17β-diol, although other chlorinated steroids are present as well.

The mass spectrum of the underivatised substance with molecular weight of 338 amu as well as the mass spectrum of the TMS-derivative have previously been reported [6].

Fragmentation is fairly simple; showing cleavage of the chlorine atom, as well as loss of two hydroxy-TMS groups and the fragment at a mass to charge ratio of 143 amu which is a typical D-ring fragment for 17-hydroxylated and methylated steroids.

Some variability in the content of the capsules was noticed and among the contaminants, metandienone and mibolerone were detected [8].

Preliminary excretion studies have shown that this steroid is also excreted largely unchanged, and detection of its misuse can be based upon the parent compound.

2.3. *6ζ-bromo-androst-4-ene-3,17-dione*

Both 6α- and 6β-bromo-androstenedione have been reported as aromatase inhibitors. 6α–bromo-androstenedione has been reported as an irreversible aromatase inhibitor. Due to the inherent chemical reactivity of this substance it has only been examined in vitro [9-12].

Currently it is marketed under the tradenames Restore and Hyperdrol. The mass spectrum of the 6-bromo-androstenedione-bis-TMS is shown in Figure 1. However, for its detection LC-MS is advisable, since this product seems to degrade during the analysis to form androstenedione-bis-TMS.

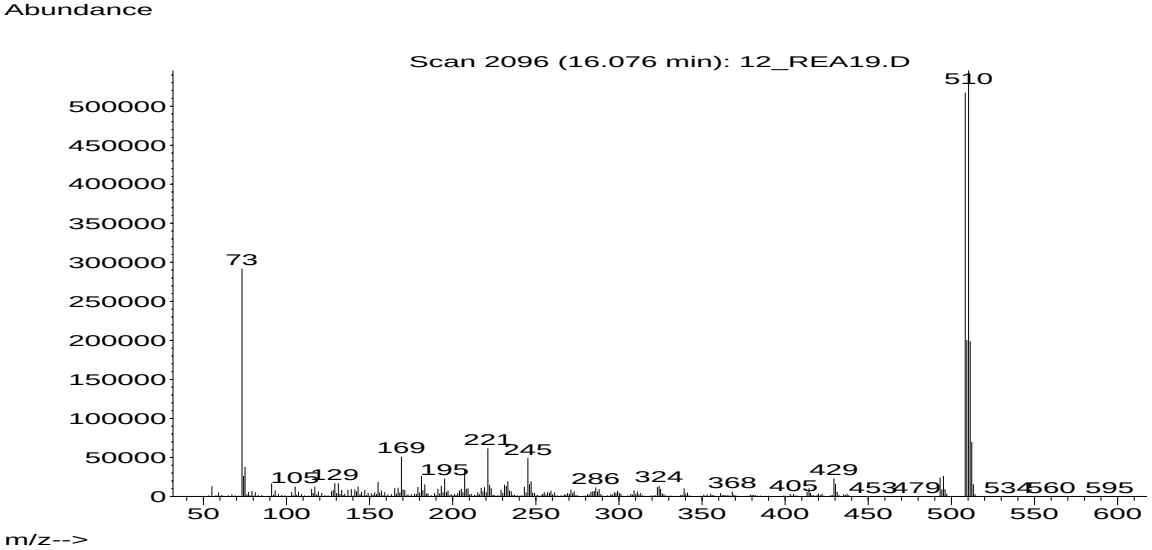

Figure 1. GC-MS mass spectrum of 6-bromo-androstenedione-bis-TMS

2.4. *Prostanozol*

Orastan-e contains two steroids, [3,2-c]-5α-androstan-17β-ol and the tetrahydropyranol esterified analogue at C-17.

This steroid is intensively metabolised to proposed structures as 3'OH-prostanozol, 4-OH-prostanozol and 16β-OH-prostanozol (main metabolite). LC-MS/MS analysis should be preferred instead of GC-MS analysis after TMS-derivatisation in order to obtain the required sensitivity [5, 13].

2.5. ***17α-methyl-5α-androstane-3α,17β-diol***

17α-methyl-5α-androstane-3α,17β-diol is sold under the trade name M-5AA from underground laboratories and is a urinary metabolite of mestanolone, methyltestosterone and oxymetholone [14]. As such it is normally routinely screened for by doping control laboratories. No elimination data after oral intake is however available.

2.6. *6-oxo-androstenedione*

6-oxo-androstenedione is marketed as an aromatase inhibitor and several in-vitro experiments have shown that the anabolic steroid androst-4-ene-3,6,17-trione exhibits aromatase inhibiting properties.

Administration of 6-oxo-androstenedione results in the detection of 6-oxo-androstenedione, 6α-OH-testosterone (minor metabolite), 6α-OH-etiocholanolone and 6α-OH-androstenedione (major metabolite) [15-17].

An increase in the urinary concentration of androstenedione was also noticed after intake of 6-OXO®, indicating a possible aromatase inhibition.

3. Ephedrine as artefact from pseudo-ephedrine

Quantitation of ephedrines has been performed routinely at our laboratory using GC-NPD for over a decade. This was traditionally followed by a qualitative analysis, confirming the presence of the respective ephedrines by GC-MS after derivatisation with TFAA [18].

Last year, similar to observations in many laboratories, it was noticed that cathine could be detected in concentrations exceeding the threshold in samples containing huge amounts of pseudoephedrine. However, ephedrine was –unexpectedly- also detected in these urine samples using our methodology as shown in Figure 2.

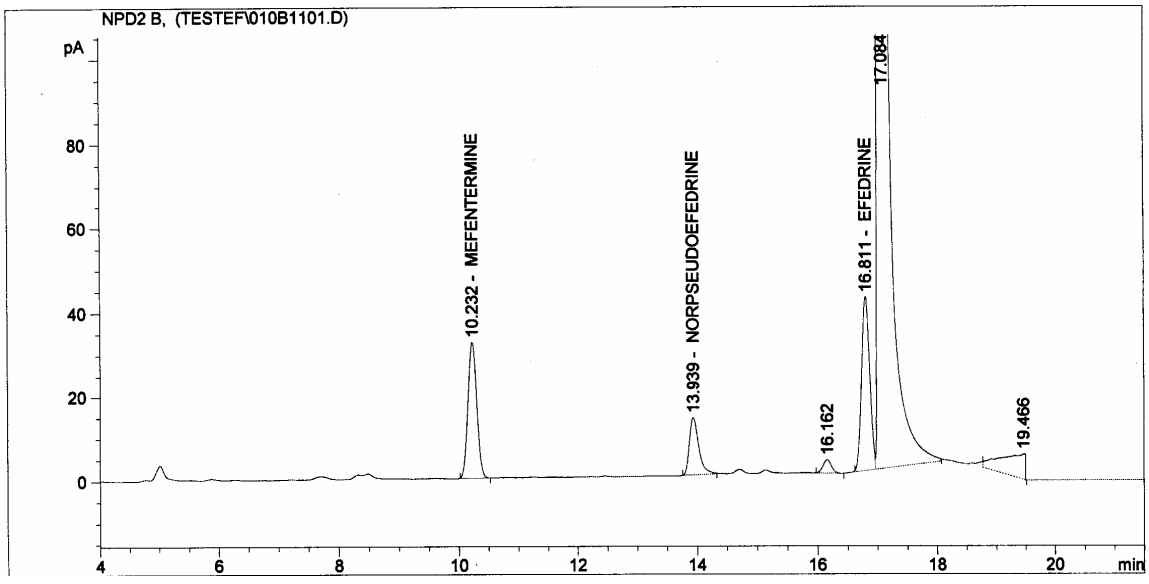

Figure 2. GC-NPD chromatogram of a urine sample after intake of high quantities of pseudoephedrine

Based upon the declarations on the doping control forms of several athletes, the pseudo-ephedrine tablets that lead to these findings were analyzed and – surprisingly - ephedrine was detected in these pills as well (Figure 3). The relative abundance of ephedrine in these pills ranged from 0.5 up to 10 percent using the GC-NPD. Analysis by GC-MS after TFAA derivatisation resulted in the detection of ephedrine as well, but in different ratios.

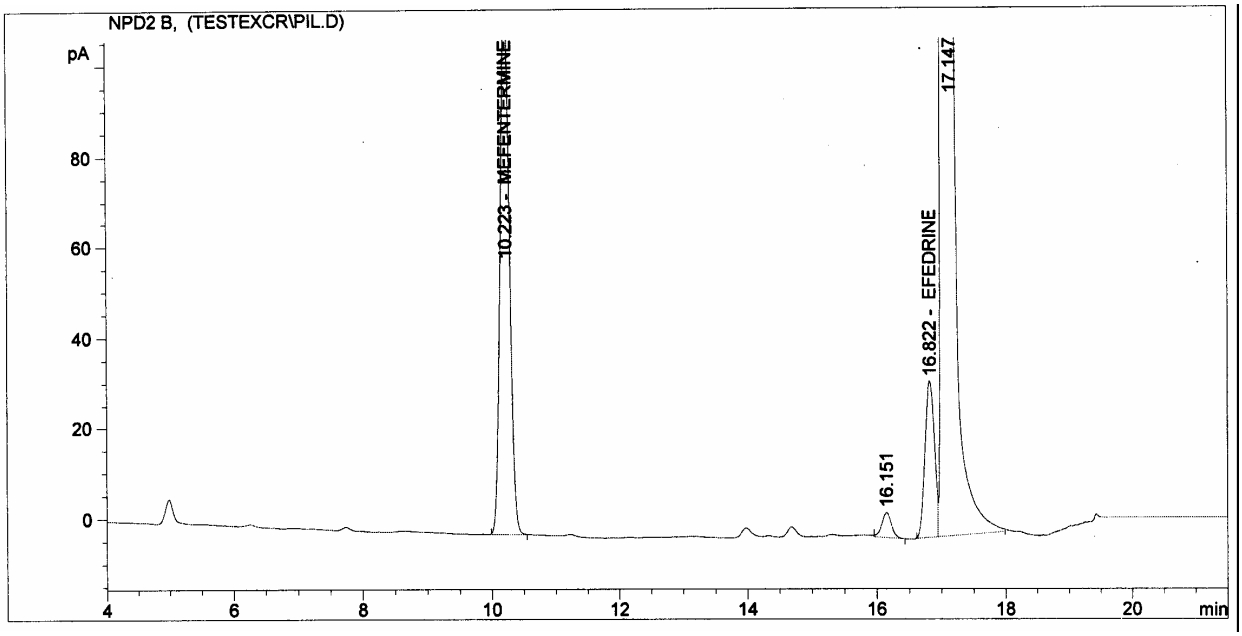

Figure 3. GC-NPD chromatogram of a pseudoephedrine tablet

Direct analysis of the urine samples by LC-MS [19] and using the same extraction method as in the GC-NPD method showed the absence of ephedrine. Hence, ephedrine is formed as an artefact in the GC-NPD and in the GC-MS methods. So far, no explanation has been found for this phenomenon, but it should be considered when reporting adverse analytical findings for ephedrine in the presence of vast quantities of pseudoephedrine.

4. Acknowledgements

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