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Results of several (small) research projects at DoCoLab in 2008

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1. Introduction

The paper is a summary of two research projects that were initiated in 2008 at DoCoLab and which deal with the analysis of new prohormones and excretion studies with pseudoephedrine.

2. Prohormones

Similar as last year supplements containing “new” prohormones have been purchased through a WADA funded project and these supplements have been analyzed for the presence of prohibited or new steroids.

2.1. Formadrol extreme

The commercial preparation Formadrol extreme was analyzed by GC-MS and LC-MS. The analysis of this product from LG Sciences confirmed the presence of androsterone and epiandrosterone, as claimed on the label.

Using the current doping control methods the use of this preparation should therefore be routinely detected through alteration of the steroid profile. However, although the efficacy of androsterone as a supplement can be argued, it should be noted that the alterations in the steroid profile will most probably not resemble those of other known endogenous steroids (testosterone, DHEA, DHT or androstenedione) but primarily result in raised androsterone levels.

This should also be taken into account during GC/C/IRMS-analysis.

2.2. 1,4 AD BOLD

This product from iFORCE Nutrition, contains boldione (1,4-androstadiene-3,17-dione) an orally active precursor of boldenone. Even though it is listed on the Anabolic Steroid Control Act, it is available as a nutritional supplement in the United States. In cattle it was shown to be a metabolite of boldenone [3] and endogenous [4]. In man, 1,4-androstadiene-3,17-dione was identified as a metabolite and precursor of boldenone [2]. Two excretion studies with orally administered 1,4-androstadiene-3,17-dione have been performed so far and have shown that this steroid is partially excreted unchanged [1, 5]. Besides the major metabolite boldenone, two other unidentified metabolites were detected by Kim et al. [5]. Uralets and Gilette [1] however identified 5 β -androst-1-ene-3 α -ol,17-one as the major metabolite and 5 β -androst-1-ene-17 β -ol-3-one, 5 β -androst-1-ene-3 α ,17 β -diol, 5 β -androst-1-ene-3,17dione and 5 β -androst-1-ene-6 β -ol-3,17-dione as other metabolites. These metabolites were previously identified as metabolites of boldenone [2].

2.3. AromX

GC-MS and LC-MS analysis of AromX from Advanced muscle Science revealed the presence of two steroids: 1,4,6-androstatriene-3,17-dione and ethyl esterified 3 α -hydroxyandrostane, as claimed on the label (Figure 1).

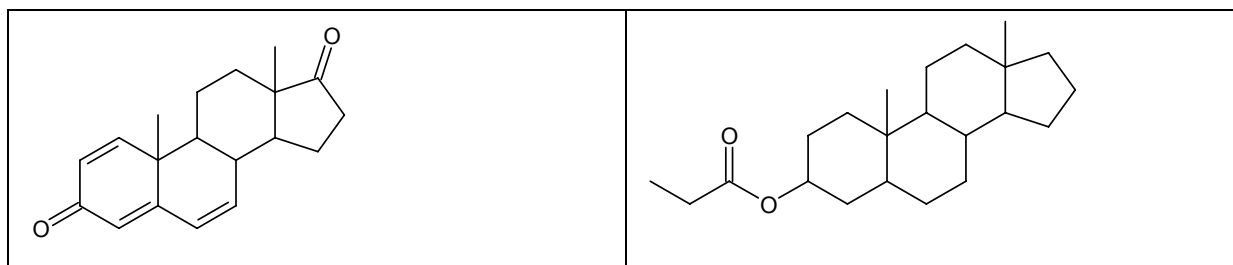


Figure 2: Substances present in Arom X

Data on 1,4,6-androstatriene-3,17-dione, which is sold under several commercial names, including novedex and recently 4AD has been reported previously [7-10]. "Attitude" is a preparation combining 1,4,6-androstatriene-3,17-dione and 3-hydroxy-4-androstene-6,17-dione. Both were distributed in a previous prohormone pack. Data on the elimination and detection of 1,4,6-androstatriene-3,17-dione were published, and showed that it is largely excreted unchanged, yet also difficult to detect via GC-MS due to derivatisation problems [8, 9].

1,4,6-androstatriene-3,17-dione is mainly excreted unchanged and as its 17-hydroxy analogue. In addition, several reduced metabolites are detected in the post-administration urines, namely 17-hydroxyandrost-1,4-dien-3-one (boldenone), 17-hydroxy-5-androst-1-en-

3-one (boldenone metabolite), 17-hydroxyandrosta-4,6-dien-3-one, and androsta-4,6-diene-3,17-dione [8].

For 3 α -hydroxy-androstane ethyl ester no scientific data on excretion is available. However, it can be expected -based upon “general knowledge” on steroid metabolism- that :

- The ester at 3 α -position will be hydrolyzed
- C-17 will be hydroxylated

Therefore, intake of this substance will influence the steroid profile traditionally monitored in doping control.

2.4. 11-OXO

11-OXO is manufactured by Ergopharm, the company from P. Arnold, a lead convict in the Balco case. The supplement contains androstene-4,11,17-trione, an endogenous steroid.

An administration study with 11-OXO by Booker et al. showed that it is largely metabolized to 11-OH-androsterone and 11-OH-etiocholanolone, which are often included in screening procedures of doping control laboratories or used as endogenous reference compounds in GC/C/IRMS analysis. The most specific parameter for misuse was identified as 5 β -androsta-3 α ,17 β -diol-11-one [10]

3. Excretion studies with pseudoephedrine

Three different pseudoephedrine preparations were administered to 6 healthy volunteers. The administered dose was 240 mg, which is equivalent to the maximum daily therapeutic dose, but this dose was given in a single administration. The results were described (partially) in Drug Testing and Analysis [12].

The maximum urinary concentration of pseudoephedrine was as high as 233 μ g/ml and the highest maximum urinary cathine concentration was 12 μ g/ml. These results indicate that the therapeutic use of a large-but therapeutic dose of pseudo-ephedrine- can result in a concentration of pseudoephedrine that is higher than the threshold.

The results also indicated that there was a correlation between the urinary cathine and pseudoephedrine concentrations after oral administration of pseudoephedrine, as shown in Figure 2. However, this correlation is too low for the introduction of a threshold based upon this

concentration ratio to unequivocally establish that a cathine finding was caused by pseudoephedrine misuse.

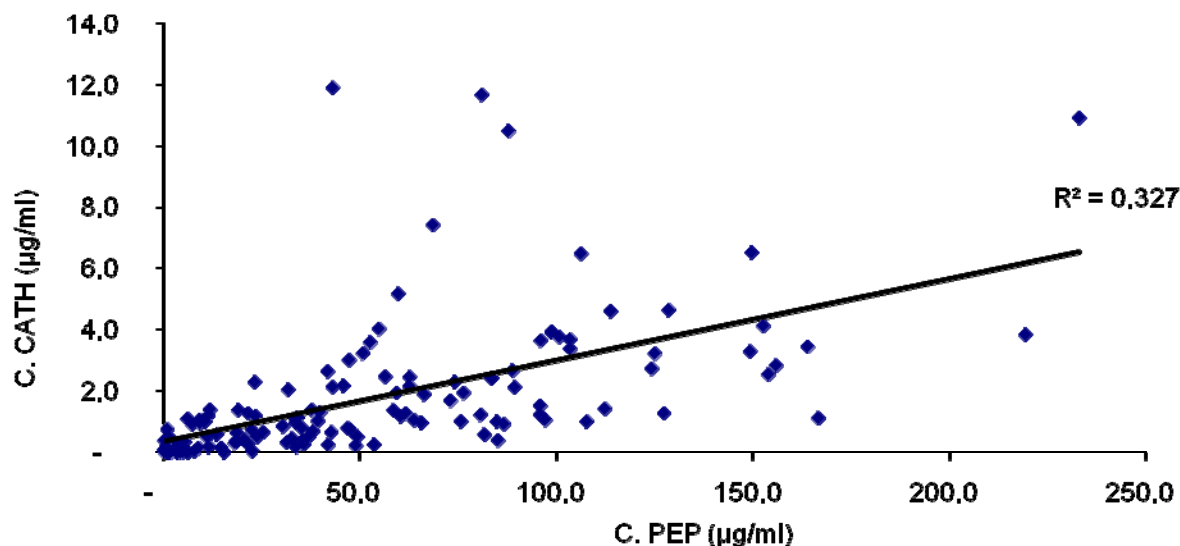


Figure 2. Concentration of cathine (CATH) and pseudoephedrine (PEP) in the samples from the administration studies (240 mg of pseudoephedrine, orally)

Over the period 10/200/-12/2008 all routine samples where pseudoephedrine was detected during screening, were reanalyzed and pseudoephedrine was quantified.

A statistical analysis (t-test, $\alpha=0.05$) showed significant differences in the frequency of the detection of pseudo-ephedrine in urine samples according to sport (major sports, number of samples analyzed >25). Ice hockey, cycling and rugby had the highest percentage of samples in which pseudo-ephedrine was detected. Additionally, the statistical evaluation showed an increase in the occurrence of pseudoephedrine in urine samples before and after its removal from the list of prohibited substances.

A comparison of the dataset obtained after administration of pseudoephedrine and the data from routine-analysis is shown in Figure 3. From these results, it is clear that there are no indications that pseudo-ephedrine and cathine were used simultaneously by athletes. Indeed, no differentiation between both populations (therapeutic use in this study) and the samples derived from routine doping control can be made.

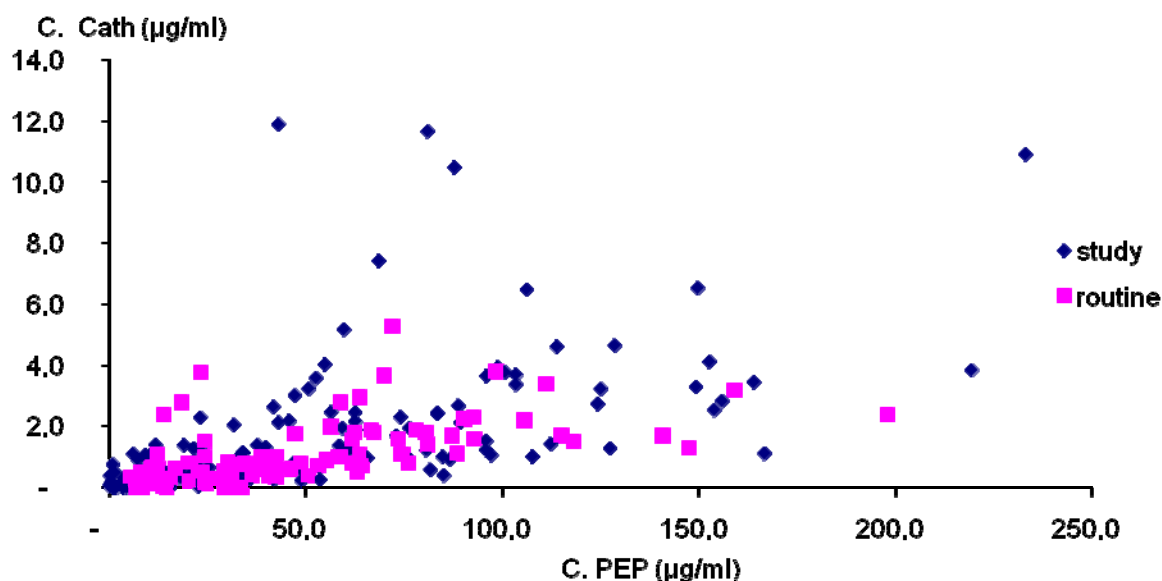


Figure 3. Overlay of the determined concentrations of pseudo-ephedrine (PEP) and cathine (CATH) in the samples from the administration study and all regular doping control samples in which cathine was detected.

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