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Long-term detection of metandienone abuse by means of the new metabolite 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one

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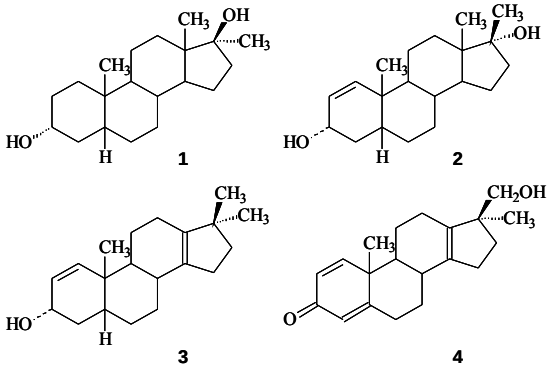
Introduction

Metandienone is classified as an anabolic androgenic steroid and it is prohibited in amateur and professional sports since many years. Numerous studies on the metabolism of metandienone in humans have been performed focusing on the detection of its urinary metabolites [1-3]. During the past years up to today, in doping control laboratories the major metabolic products 17α-methyl-5β-androstane-3α,17β-diol, 17ß-methyl-5ß-androst-1-ene-3α,17α-diol (epimetendiol) and 18-nor-17,17-dimethyl-5β-androst-1,13-diene-3α-ol (18-norepimetendiol) (Scheme 1, **1-3**) have been used as target analytes for the detection of this drug. In 2006, Schänzer et al. [4] succeeded in the identification and characterization of the new long-term metabolite 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (Scheme 1, **4**). Compared with commonly detected metabolites this new compound extends the time frame for the determination of metandienone abuse by several days [4]. In the Cologne Laboratory for Doping analysis the implementation of this new metabolite in GC/MSMS and LC/MSMS anabolic steroid screening assays resulted in a strong increase of adverse findings concerning metandienone misuse. In this article this effect is illustrated by means of various statistics.

Sample preparation

The samples were prepared according to the standard operating procedure for anabolic steroids described by Geyer et al. [5]. Conjugated and unconjugated steroids were extracted from urine at pH 9.6 with TBME following enzymatical hydrolysis of the glucuronides at pH 7. After centrifugation the organic layer was transferred in a fresh glass tube and evaporated to dryness. The dry residue was derivatized with 100 µL MSTFA/NH_4I/ Ethanethiole 1000:2:3.

Chemical structures



Scheme 1: Chemical structures of the main urinary metabolites of metandienone: 17α-methyl-5β-androstane-3α,17β-diol (**1**), 17ß-methyl-5ß-androst-1-ene-3α,17α-diol (epimetendiol) (**2**), 18-nor-17,17-dimethyl-5β-androst-1,13-diene-3α-ol (18-norepimetendiol) (**3**) and 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (**4**).

GC/MSMS analysis

The GC/EI-MSMS experiments were performed using a Thermo TraceGC Ultra gas chromatograph interfaced to a PolarisQ ion trap mass spectrometer. The GC system was equipped with a Varian VF-1ms capillary column (length 25 m, i.d. 0.2 mm, film thickness 0.1 μm). A volume of 2 μL of the sample was injected in the GC system, which was operated in the split (1:10) mode. The GC oven temperature program started at 185°C was increased at 5°C/min to 240°C, and then at 20°C/min to 310°C using helium as carrier gas (0.9 mL/min constant pressure). The injector and interface temperatures were set to 300°C and the ion source was operated at 225°C. Ionization was accomplished using electron ionization (EI) (70 eV). The main EI-MS/MS parameters for the detection of trimethylsilylated 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one are shown in Table 1.

Precursor ion: m/z 442.3	**Precursor ion: m/z 339.3**
Excitation voltage (V): 1.0	Excitation voltage (V): 1.0
Product ion range: m/z 120-445	Product ion range: m/z 100-340
Fragment ions: m/z 339, 236, 133, 243	m/z 243, 193, 159, 245, 145, 133, 177

Table 1: EI-MS/MS parameters for the detection of 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one .

Results

Anabolic androgenic steroids are the most frequently misused drugs in amateur and professional sports. In addition to testosterone, nandrolone and stanozolol metandienone is the most detected substance in the class of anabolic agents [6]. Within 2003 to 2005 the total number of positive metandienone cases worldwide varied between 56 and 63 (Fig.1), whereof a nearly constant rate of about 20% was contributed by the Cologne doping control laboratory.

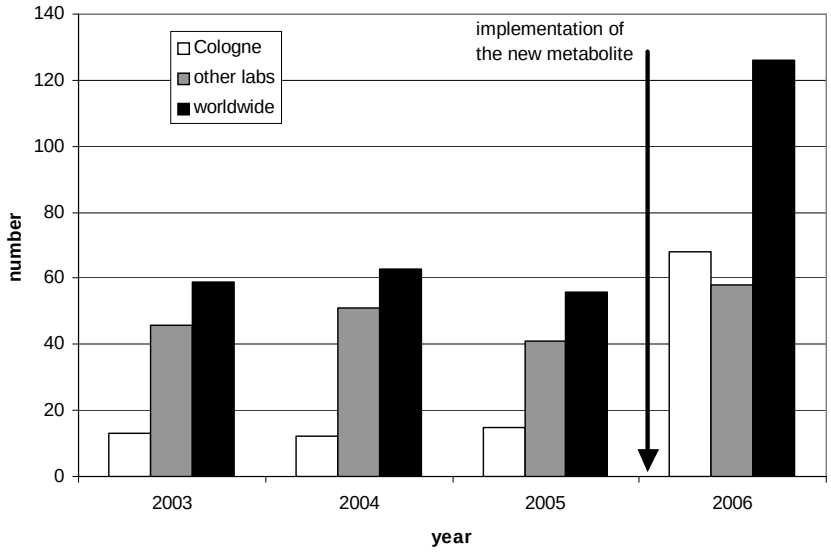


Figure 1: Comparison of the number of metandienone findings in Cologne laboratory with those of the other doping control laboratories.

Since the implementation of the new metabolite 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one in anabolic steroid screening assays in the early 2006, the total number of metandienone findings in the Cologne doping control laboratory has strongly increased by a factor of four to 68 samples (Fig. 1). This value is even higher than the quantity of worldwide findings in 2003, 2004 and 2005, respectively. Additionally, in 2006 it even exceeds the quantity of positive metandienone cases reported by the other doping control laboratories.

Closer examination of the 68 adverse findings in 2006 reveals the great importance of the new long-term metabolite: whereas in all suspicious samples the metabolite 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (**4**) has been detected, the commonly applied target analytes 17α-methyl-5β-androstane-3α,17β-diol (**1**), epimetendiol (**2**) and 18-norepimetendiol (**3**) were found at the most in 30% of the samples (Fig.2).

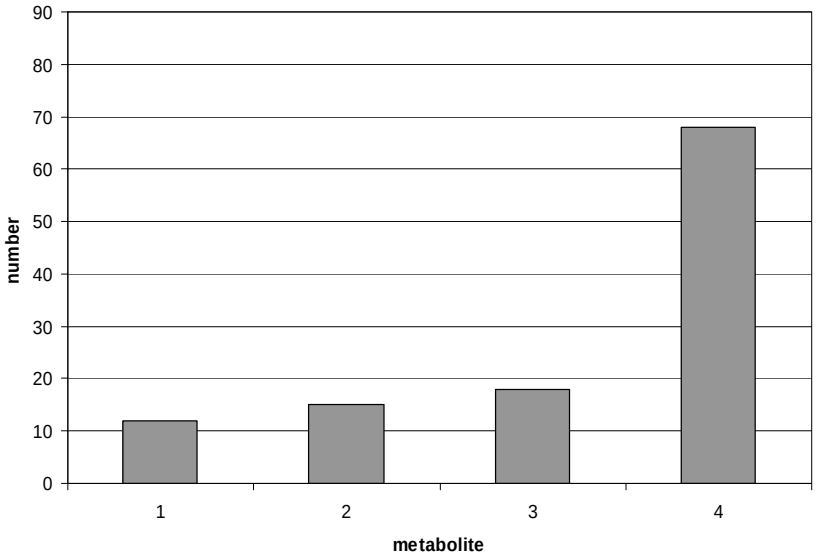


Figure 2: Comparison of the traceability of the different metandienone metabolites in all 68 positive samples.

Conclusions

The new metabolite 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one improves the existing doping control strategies for metandienone misuse in sports to an extremely high degree. Compared to commonly detected target analytes this metabolite provides a prolonged detection period and is a new powerful tool for the long-term detection of metandienone abuse. Since its implementation into screening assays the total number of metandienone findings in the Cologne doping control laboratory is increased by several hundred percent.

References

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