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Metabolites of methylnortestosterone

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Abstract

This preliminary study was aimed to investigate the excretion of methylnortestosterone and its metabolites in order to improve the detectability of a potential misuse. Compounds of interest were isolated from urine following methods commonly used in the WADA accredited laboratories. Various derivatization methods were used and GC-MS and GC-MS/MS analyses were employed. After a single oral dose 17α-methyl-5β-estrane-3α,17β-diol and 17α-methyl-5α-estrane-3α,17β-diol (as reported previously) were detected together with the parent compound. In addition, further so far unknown compounds were detected. Using GC-MS/MS technique the detection time window could be improved compared to conventional GC-MS.

Introduction

Methylnortestosterone (17α-MeNT, methylestrenolone, normethandrolone, 17β-hydroxy-17α-methylestr-4-en-3-one) is a progestin and an anabolic steroid [1,2] and as such it is on the WADA Prohibited List. Its distribution as pharmaceutical preparation (“Orgasteron” by Organon) had been discontinued decades ago. However, recently it became available again through the internet market.
LC-MS/MS analysis has been applied for the detection of the parent compound in horse urine and plasma [3-5]. 17α-methyl-5β-estrane-3α,17β-diol and 17α-methyl-5α-estrane-3α,17β-diol were reported as metabolites in human urine after analysis by GC-MS(/MS) [6,7]. The goal of this study was to find out which of the metabolites represents the most suitable target analyte for 17α-MeNT detection and to incorporate it into the existing screening method accordingly.

Experimental

Excretion study

6.2 mg of 17α-MeNT substance (“methyloestrenolon CH:8537/82”, Organon) dissolved in 10 mL of 40% ethanol were administered to a male volunteer (46 years old, 70 kg body weight), followed by 250 mL of water. 25 urine samples were collected and analyzed over the time range from 0 to 175 hours.

Experimental

Compounds of interest were isolated from 2 mL urine following methods commonly used for steroid analysis. Enzymatic hydrolysis (glucuronidase from E. coli) and liquid – liquid extraction (by MTBE at pH 9.6) were employed. D_3-testosterone was used as internal standard. Agilent GC-MSD 6890+/5975 and Agilent GC-MS Triple Quad 7890A/7000 systems were used for analyses. GC separation was achieved on a Phenomenex ZB-1ms column (10 m x 0.18 mm and 0.18 µm film thickness) applying temperature programming. The conditions for GC-MS and GC-MS/MS were as follows: 130°C to 185°C at 55°C/min, then to 235°C at 5°C/min and then to 325°C at 30°C/min (held 2 min), the carrier gas He flow 0.7 mL/min and 130°C to 186°C at 56°C/min, then to 206°C at 2°C/min, then to 221°C at 5°C/min and then to 326°C at 35°C/min (held 1.5 min), the back flush of a ZB-1ms pre-column (1 m) 2.5 mL/min at 16 min, the He flow 1.0 mL/min, resp.
In the first step the per-TMS derivatives were prepared (by mixture of MSTFA/NH_4I/propane-2-thiol 1000:5:1 v/m/v) and GC-MS in the scan and SIM mode and GC-MS/MS in the MRM mode were used to detect and identify 17α-MeNT metabolites.

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In the second step TMS (without enolization using a mixture of MSTFA and trimethylsilylimidazol, 100:5 v/v) and TBDMS (by mixture of MBDSTFA/NH_4I/propane-2-thiol 1000:5:1 v/m/v) derivatives of the compounds of interest were prepared to elucidate the 17α-MeNT metabolites structure. The mass-spectral behaviour of TBDMS derivatives of methyltestosterone and 17α-methyl-5β-androstane-3α,17β-diol as model compounds was also studied.

Results and Discussion

After a single dose oral administration of 17α-MeNT the A-ring reduced metabolites 17α-methyl-5α-estrane-3α,17β-diol (**M1**, relative retention time RRT 0.817) and 17α-methyl-5β-estrane-3α,17β-diol (**M2**, 0.889) were detected together with the parent compound (**PC**, 1.064) as per-TMS derivatives (Figure 1 b, c, a). The RRTs refer to the GC-MS/MS system (IS at 13.45 min). In addition, further so far unknown compounds were detected. For the most abundant ones their structures were tentatively proposed as 16z,17β-dihydroxy-17α-methylestr-4-en-3-one (**M3**, 1.181), 17α-methyl-5β-estr-3-ene-17β-ol (**M4**, 0.473) and 4,17β-dihydroxy-17α-methylestr-4-en-3-one (**M5**, 1.155) (Figure 1 d, e, f). **M5** could be detected only in the first two urines after administration. 17β-hydroxymethyl-17α-methyl-18-norestra-4,13-dien-3-one (analogous to the so-called “night watchman” metabolite), 17,17-dimethyl-18-norestra-4,13-dien-3-one as also reported by Parr et al. [7] and 17β-hydroxymethyl-17α-methyl-18-nor-5β-estr-13-en-3α-ol could not be found in the post administration urines.

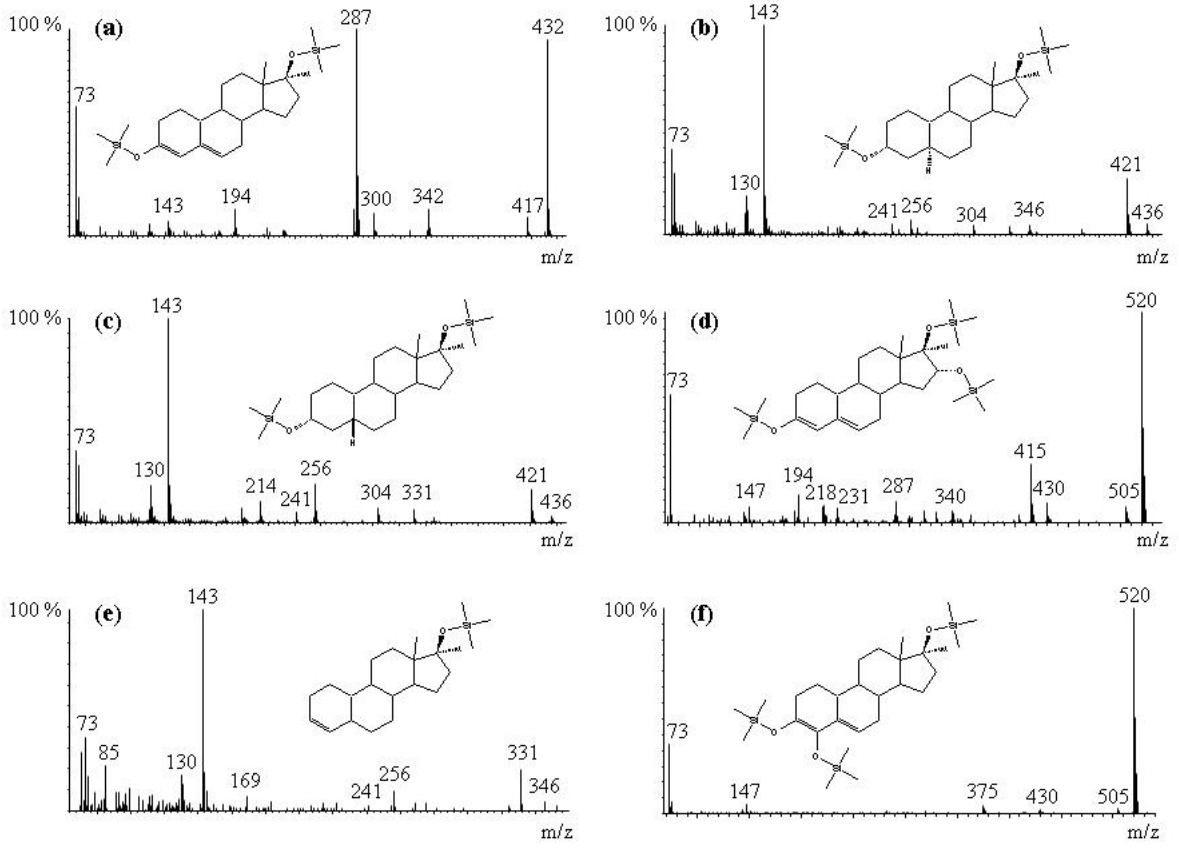

Figure 1. Mass spectra of per-TMS derivatives of (a) **PC**, (b) **M1**, (c) **M2**, (d) **M3**, (e) **M4** and (f) **M5**

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The excretion profiles of the compounds of interest were measured by GC-MS in the SIM mode and GC-MS/MS in the MRM mode. Collision energies for the MRM mode were optimized (Table 1). The results are summarized as detection time windows (Figure 2). Currently, 17α-methyl-5β-estrane-3α,7β-diol (**M2**) seems to be the most suitable target analyte for screening purposes due to the longest time of detection and the absence of major interferences from urine matrix.

Compound	MRM	CE [V]
methylnortestosterone **PC**	432 → 300 432 → 287 432 → 285	15 17 15
17α-methyl-5α/β-estrane-3α,17β-diols **M1** and **M2**	421 → 241 256 → 213 256 → 199	15 12 8
16z,17β-dihydroxy-17α-methylestr-4-en-3-one **M3**	520 → 415 520 → 287 520 → 194	20 17 30
17α-methyl-5β-estr-3-ene-17β-ol **M4**	331 → 241 331 → 143 256 → 143	8 35 20

Table 1. MRMs and collision energies for the selected analytes

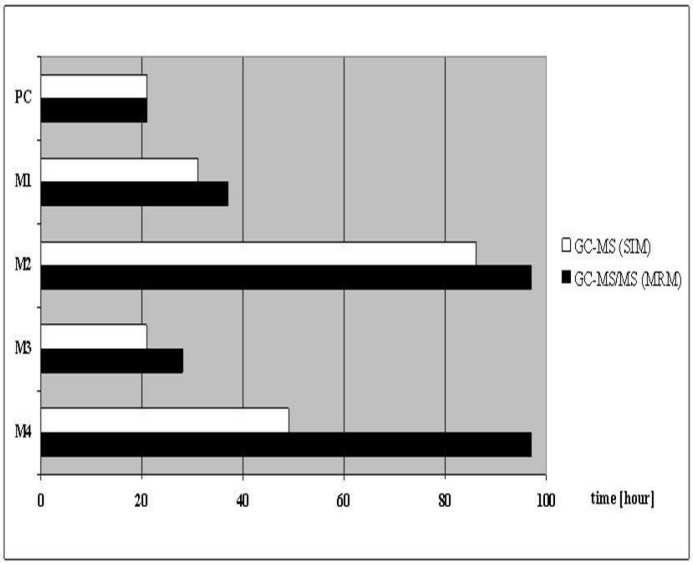

Figure 2. Detection time windows

The derivatization experiments showed that using MSTFA/TMSI mixture same derivatives of **M1**, **M2** and **M4** and as expected mono-TMS derivative of **PC** and bis-TMS derivatives of **M3** and **M5** were obtained. In comparison to the formation of TMS derivatives the tertiary 17β-hydroxyl group is sterically hindered for the larger TBDMS group and so predominantly mono-TBDMS derivatives of the studied compounds were detected. The mass-spectral behaviour of the derivatives of 17α-MeNT and methyl-5α/β-estranediols (**M1** and **M2**) corresponds to that of methyltestosterone and methyl-5α/β-androstanediols derivatives. The obtained mass spectra of the proposed new metabolites were not conclusive enough and the detailed structure of those metabolites remains uncertain.

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Conclusions

Using triple quadrupole GC-MS/MS technique the detection time window could be prolonged compared to single quadrupole GC-MS. MRMs of 17α-methyl-5β-estrane-3α,17β-diol as the so far longest detectable metabolite (97 hours) and due to the absence of interferences in the chromatographic window were implemented into our routine screening method.

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