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Excretion study of furazabol, an anabolic steroid, in human urine

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Abstract

Furazabol and 16-hydroxyfurazabol represented the most abundant peaks in urinary metabolic profiles for two men obtained by gas chromatography–mass spectrometry (GC–MS) after oral administration of 5 mg furazabol. The excreted amounts of unchanged furazabol were determined and its response in GC–MS was compared with that of the 16-hydroxy metabolite. The maximum excretion rates of these compounds were reached 2–3 h after oral administration. The half-lives of unchanged furazabol for two human subjects, were 1.87 and 1.29 h respectively and the recovered amount in 48 h was 24% (33% for one, 15% for the other). Also the spectrum of an unidentified metabolite is reported.

Keywords: Furazabol; Anabolic steroids; Steroids

1. Introduction

Furazabol (17β-hydroxy-17α-methyl-5α-androstano[2,3-c]furazan) is a possible anabolic steroid doping agent, although no misuse of it has been reported by any International Olympic Committee accredited laboratory [1]. Its chemical structure is the same as that of stanozolol except for a furazan ring fused to the steroidal skeleton. Because its myotrophic/androgenic ratio (5.8), like that of stanozolol, is higher than that of testosterone (1) in animal studies [2], it has been used clinically as an anabolic and hypolipidaemic agent [3–6], and some track and field athletes allegedly used it in the 1980's. Studies on the detection of furazabol metabolites in human urine using gas chromatography–mass spectrometry (GC–MS) have been recently reported. Particularly, Gradeen et al. [7] have reported a urinary excretion study of 16-hydroxyfurazabol by area ratios and Schänzer and Donike [8] have reported that furazabol and 16-hydroxyfurazabol were identified as metabolites of furazabol in man.

In this paper, we describe a simple and specific extraction method for the detection of furazabol, based on the previous report of Schänzer and Donike. Using this method, we report an excretion study of furazabol involving a quantitative analysis of unchanged furazabol and the urinary excretion pattern by peak-height ratios of a major metabolite (16-hydroxyfurazabol). Then, we present the data from a study of two subjects and discuss the resulting variations.

2. Experimental

2.1. Reagents

Furazabol was supplied by Daiichi Seiyaku (Tokyo, Japan) and Amberlite XAD-2 resin (particle size 0.15–0.2 mm) was supplied by Serva (Heidelberg, Germany). β-Glucuronidase (activity 200 U/ml) was purchased from Boehringer (Mannheim,

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Germany). The derivatizing agent MSHFB (N-methyl-N-trimethylsilylheptafluorobutyramide) was obtained from Macherey and Nagel (Düren, Germany) and TMS-Cl (trimethylchlorosilane) and TMS-imidazole (trimethylsilylimidazole) from Sigma (St. Louis, MO, USA). A methanolic solution of 10 mg/ml calusterone (Sigma) was prepared and stored at 4°C. All other chemicals and solvents were of the highest purity grade available and used without further purification.

2.2. Calibration

Calibration samples were prepared by adding the appropriate amounts of furazabol and internal standard to 5 ml of blank urine. The concentration range of the standards was 0.08, 0.2, 0.4, 1 and 2 μg/ml for furazabol. The internal standard (calusterone) was added to each sample at a concentration of 80 ng/ml.

2.3. Instrumentation

A Hewlett-Packard (Palo Alto, CA, USA) 5890 gas chromatograph coupled with 5970B mass selective detector with a crosslinked 5% phenylmethylsilicone capillary column (length 17 m, I.D. 0.2 mm, film thickness 0.33 μm) was used. Helium, at a flow-rate of 0.73 ml/min, was used as carrier gas. Samples were injected in the splitless mode. Temperatures of the injector and transfer line were set to 280 and 300°C, respectively. Oven temperature was initially 180°C, then ramped at 25°C/min to 300°C, and held for 5 min. The quantitation of samples was carried out in the selected ion monitoring mode. Ions monitored for furazabol, 16-hydroxyfurazabol and calusterone were *m/z* 143, 218 and 315, respectively, which were selected on the basis of maximum abundance without interferences.

2.4. Drug administration

Five milligrams of furazabol (five 1-mg tablets, Miotolon) was orally administered to two healthy males (K and M, 65 kg). Urine samples were collected up to 48 h post-dose and stored at 4°C.

2.5. Extraction method

The procedure was adapted from the method of Schänzer and Donike [8]. Urine samples (5 ml), spiked with 40 μl of internal standard (calusterone, 10 μg/ml in methanol), were applied to a preconditioned XAD-2 column. The column was washed with 3 ml of water, then eluted with 3 ml of methanol. The methanolic solution was evaporated, then 1 ml of 0.2 *M* phosphate buffer (pH 7.0) and 25 μl of β-glucronidase were added and the mixture heated at 55°C for 1 h. After the solution was cooled to room temperature, 100 mg of potassium carbonate and 5 ml of diethyl ether were added. The mixture was shaken mechanically for 5 min and centrifuged at 850 *g* for 5 min. The organic layer was transferred to another tube and the solvent was evaporated to dryness. The residue was dissolved in 50 μl of silylating reagent mixture MSHFB–TMS-Cl–TMS-imidazole (100:5:2, v/v) and heated at 80°C for 15 min. After heating, the solution was analyzed by GC–MS.

3. Results and discussion

3.1. Analytical considerations

Considering the fact that the majority of furazabol was detected in the conjugated fraction as the glucuronide form [8], we determined the amounts of unchanged furazabol and the excretion patterns of 16-hydroxyfurazabol after enzyme hydrolysis (β-glucuronidase), based on the extraction method of Schänzer and Donike. Unchanged furazabol, its metabolite identified as 16-hydroxyfurazabol and an unidentified metabolite were detected at retention times of 8.59, 11.52 and 8.77 min, respectively by comparing total ion chromatograms of blank urine and samples. The spectra of TMS derivatives of unchanged furazabol and its metabolites are shown in Fig. 1. Furazabol-O-TMS gave ions *m/z* 143, 387 ($[M-CH_3]^+$) and 402 (M^+) and the ion *m/z* 143 originated from a typical D-ring (cyclopentane ring) cleavage of 17α-methyl-17β-hydroxy anabolic steroids. The major metabolite 16-hydroxyfurazabol gave ions *m/z* 218, 231 and 490 (M^+). The furazan ring moiety of furazabol has been reported to be little

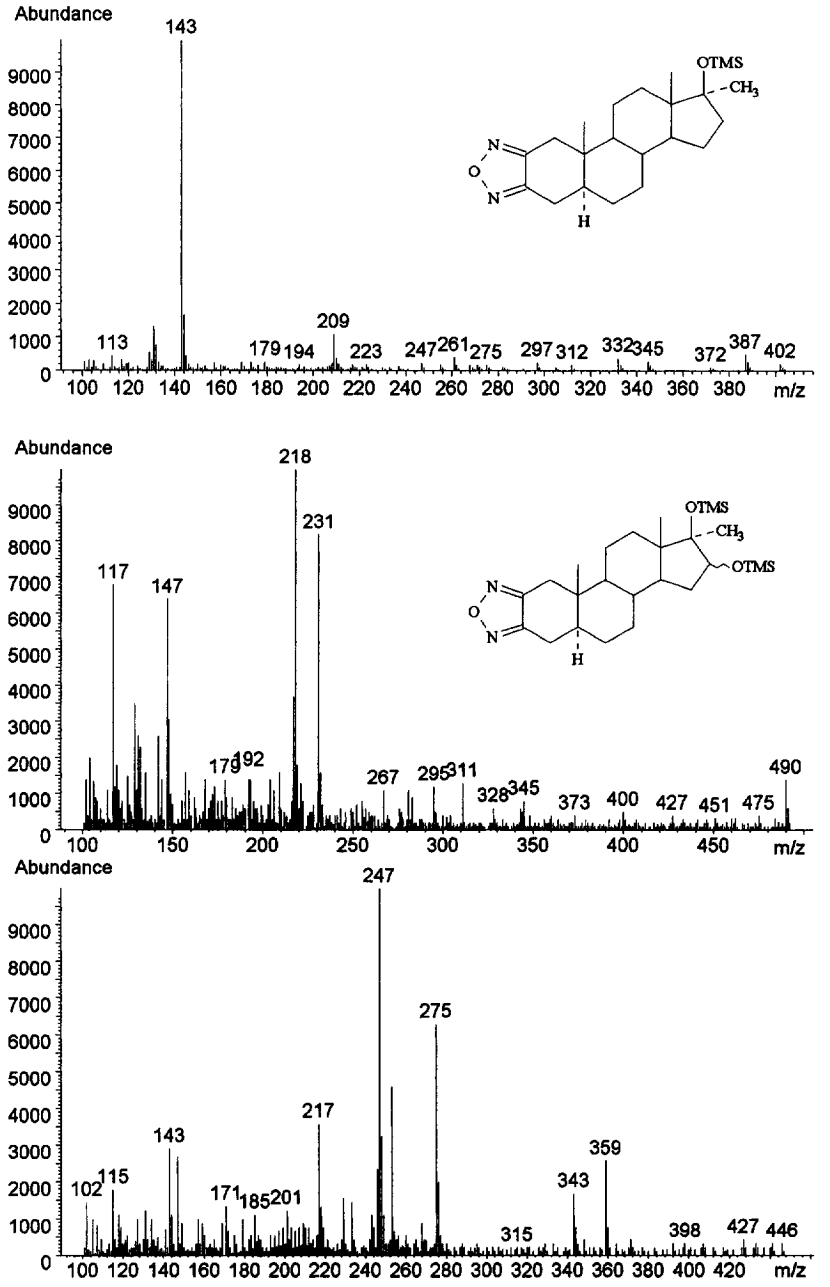

Fig. 1. Mass spectra of TMS derivatives of furazabol (upper), 16-hydroxyfurazabol (middle) and an unidentified metabolite (lower).

affected in urinary metabolic processes [5]. Ions *m/z* 218, 231 may also be explained by typical D-ring cleavages of 16,17-dihydroxy-17-methyl steroids. The unidentified metabolite gave ions *m/z* 247, 275 and 359, but its structure has not yet been elucidated.

The TMS derivative of calusterone used as internal standard gave ions *m/z* 143 (D-ring cleavage) and 315 ($M^+ - D$-ring). For the mass spectral quantitation, ions *m/z* 143, 218 and 315 were selected for furazabol 16-hydroxyfurazabol and calusterone, re-

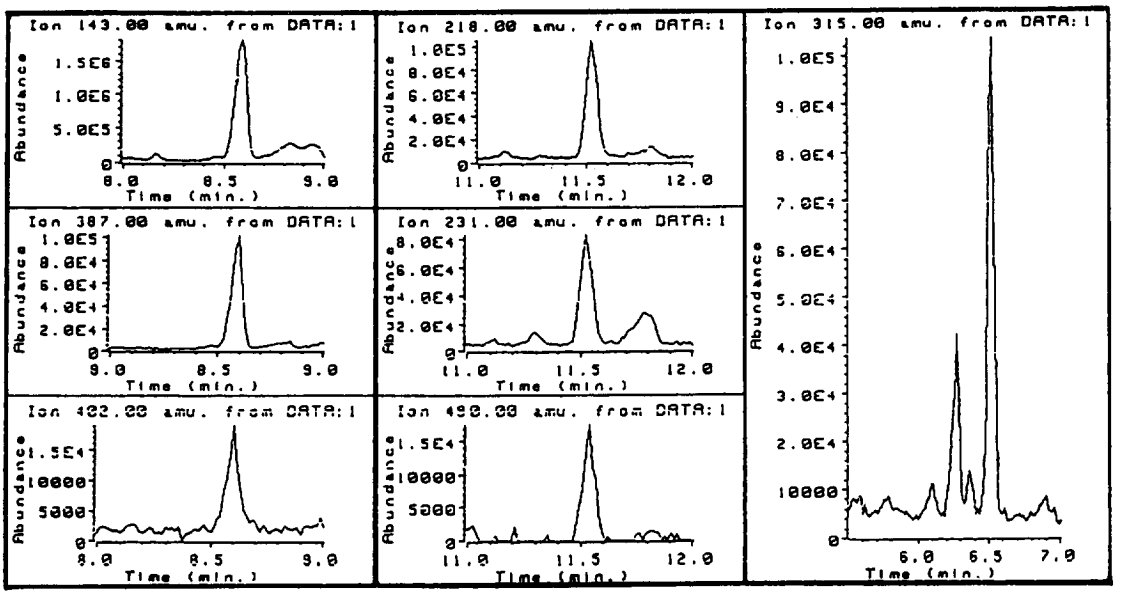

Fig. 2. Ion chromatograms of TMS derivatives of furazabol (left), 16-hydroxyfurazabol (middle) and calusterone (right).

spectively, on the basis of maximum abundance without interferences.

Fig. 2 shows the typical selected ion monitoring chromatograms with m/z 143, 387 and 402 for furazabol and m/z 490, 232 and 218 for 16-hydroxyfurazabol in human urine sample. What is noted in Fig. 2 is the significant abundance of unchanged furazabol as well as that of 16-hydroxyfurazabol. The detection of these substances will be necessary for the more accurate screening of drug abuse.

3.2. Excretion in human urine

The amounts of the unchanged furazabol and peak height ratios of 16-hydroxyfurazabol excreted in urine after oral administration of 5 mg furazabol are shown in Table 1. The mean total amount of furazabol recovered after 48 h was 1.2 mg (1.7 mg for K, 0.7 mg for M) and this amount is about 24% of the administered drug (33% for K, 15% for M). Of the total secreted, 77% was secreted after 24 h and 92% within 36 h. In the same way, 83% of the total excreted amount of 16-hydroxyfurazabol was obtained within 24 h, 95% within 36 h. The two cases (K and M) showed different excretion amounts of both furazabol and its metabolite. The urinary excretion half-lives of unchanged furazabol for both persons were 1.87 and 1.29 h and the maximum excretion rates of furazabol and 16-hydroxyfurazabol after oral administration were reached after 2–3 h.

4. Conclusion

The analytical method described in this paper showed good specificity and sensitivity for the detection and quantitation of furazabol and 16-hydroxyfurazabol in human urine using GC–MS. The excretion amount of furazabol and the excretion patterns of 16-hydroxyfurazabol were determined. The urinary excretion half-lives, based on the above method, were determined and subject variations in an excretion study of furazabol were found.

This method will be used in an excretion study of 16-hydroxyfurazabol with synthesized 16-hydroxyfurazabol at a later date. We hope that this procedure can also be used for the analysis of similar steroids in biological fluids.

Table 1
Excretion profile of unchanged furazabol and 16-hydroxyfurazabol after oral administration

Time (h)	16-OH-F[a] / ISTD (K)	Parent[b] / ISTD	Concentration[c] (μg/ml)	Amount[d] (μg)	Excretion[e] rate (μg/l)	16-H-F[a] / ISTD (M)	Parent[b] / ISTD	Concentration[c] (μg/ml)	Amount[d] (μg)	Excretion[e] rate (μg/h)
1.0	0.03	1.7	0.2 (1.3)	15	15	0.0	0.0	0.0 (0.0)	0	0
2.0	1.2	12	1.1 (1.1)	298	298	0.28	4.2	0.4 (1.3)	41	41
4.0	0.45	25	2.4 (0.7)	361	180	0.55	13.1	1.3 (1.2)	238	119
6.0	1.0	17	1.6 (2.1)	98	49	0.38	9.8	0.9 (0.8)	98	49
8.0	0.45	7.2	0.7 (1.0)	85	42	0.14	4.2	0.4 (1.2)	50	25
12.0	0.38	9.4	0.9 (2.5)	119	30	0.03	1.4	0.1 (2.3)	33	8.4
20.0	0.21	14	1.4 (2.7)	234	26	0.03	1.3	0.1 (2.4)	55	6.8
24.0	0.45	9.9	0.9 (0.9)	75	25	0.07	4.1	0.4 (1.2)	50	12
28.0	0.14	3.7	0.4 (0.8)	107	27	0.03	1.5	0.1 (0.9)	33	8.3
32.0	0.24	6.6	0.6 (2.7)	75	19	0.03	2.0	0.2 (3.0)	19	4.9
36.0	0.10	3.1	0.3 (0.9)	55	14	0.03	2.6	0.2 (1.4)	40	10
45.0	0.14	5.3	0.5 (0.8)	111	12	0.03	2.9	0.3 (1.0)	59	7.4
48.0	0.24	7.5	0.7 (0.9)	34	11	0.07	2.5	0.2 (0.8)	20	5.1

[a] Peak-height ratio of ion m/z 218 of 16-hydroxyfurazabol to ion m/z 315 of internal standard (calusterone).
[b] Peak-height ratio of ion m/z 143 of parent furazabol to ion m/z 315 of internal standard (calusterone).
[c] Concentration of parent furazabol in urine (relative standard deviation, $n=3$).
[d] Excretion amount of parent furazabol, concentration×urine volume.
[e] Excretion rate of parent furazabol, amount/ΔT.

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