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Metabolism of Anabolic Steroids in Man: Synthesis and Use of Reference Substances for Identification of Anabolic Steroid Metabolites*

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Abstract

The use of anabolic steroids was banned by the International Olympic Committee for the first time at the Olympic Games in Montreal in 1976. Since that time the misuse of anabolic steroids by athletes is controlled by gas chromatography-mass spectrometry (GC-MS) analysis of urine extracts. The excreted steroids and/or their metabolites are isolated from urine by XAD-2 adsorption, enzymatic hydrolysis of conjugated excreted metabolites with ß-glucuronidase from Escherichia coli, liquid/liquid extraction with diethyl ether, and derivatized yielding trimethylsilyl derivatives.

The confirmation of an anabolic steroid misuse is based on comparison of the EI-spectrum and GC retention time of the isolated steroid and/or its metabolite with the EI-spectrum and GC retention time of authentic reference substances. For this purpose excretion studies with the most common anabolic steroids were performed and the main excreted metabolites were synthesized for bolasterone, boldenone, 4-chloro-dehydromethyltestosterone, clostebol, drostanolone, fluoxymesterone, formebolone, mestanolone, mesterolone, metandienone, methandriol, metenolone, methyltestosterone, nandrolone, norethandrolone, oxandrolone and stanozolol.

The metabolism of anabolic steroids, the synthesis of their main metabolites, their GC retention and EI mass spectra as TMS-derivatives are discussed.

Table 1 presents the actual list of anabolic steroids and synthesized metabolites which are used for screening of an anabolic steroid misuse.

Table 2 shows the temperature programmed Kovats indices of the anabolic steroids and the synthesiszed metabolites as TMS derivatives used for screening and conformation. The indices were estimated on six different columns (A-F) by GC/FID and on column A by GC/MSD.

GC-MS parameters
GC-MSD HP 5890 / HP 5970
carrier gas: helium 1 ml/min at 180°C, split 1:10, 15 psi pressure
Column A': same column as GC/FID column A (16.5 m)
temperature program: 180°C / 5°C/min / 320°C

GC/FID parameters
HP 5880, carrier gas: helium 1.5 ml/min at 180°C, split 1:10,
27 psi pressure, temperature program: 180°C - 5°C/min - 320°C
Column A: Hewlett-Packard; Ultra-1 fused silica, crosslinked methyl silicone (OV 1), 17 m, i.D. 0.2 mm, film thickness 0.11 μm.
Column B: Macherey-Nagel; Permabond, fused silica, methyl silicone (OV 1), 17 m, i.D. 0.25 mm, film thickness 0.25μm.
Column C: Hewlett&Packard; HP-1 fused silica, crosslinked methyl silicone (OV 1), 17 m, i.D. 0.25 mm, film thickness 0.33 μm.
Column D: Chrompack; WCOT fused silica CP-SIL 8CB, crosslinked 5% phenyl methyl silicone (SE 54) , 17m, i.D. 0.25 mm, film thickness 0.25 μm.
Column E: Macherey-Nagel; Permabond, fused silca, crosslinked 5% phenyl methyl silicone (SE 54), 17 m, i.D. 0.25 mm, film thickness 0.25 μm.
Column F: Hewlett & Packard; HP-5, fused silica, 5% phenyl methyl silicone (SE 54), 17 m, i.D. 0.2 mm, film thickness 0.33μm.

Table 1 Screening for Anabolic Steroids

(Status Cologne Laboratory 1st of March 1992)

Anabolic steroid	main excreted substance: parent and/or metabolite	origin of substance used for conformation	excretion into urine
Bolasterone	7α,17α-dimethyl-5β-androstane-3α,17β-diol **I**	synthesized	conjugated
Boldenone	boldenone **II**	parent	conjugated
	5β-androst-1-en-17β-ol-3-one **III**	synthesized	conjugated
4-Chloro-dehydro-methyltestosterone	6β-hydroxy-4-chloro-dehydromethyl-testosterone **IV**	synthesized	'free'
Clostebol	4-chloro-androst-4-en-3α-ol-17-one **V**	synthesized	conjugated
Drostanolone	2α-methyl-5α-androstan-3α-ol-17-one **VI**	synthesized	conjugated
Fluoxymesterone	9α-fluoro-18-nor-17,17-dimethyl-androsta-4,13-dien-11β-ol-3-one **VII**	synthesized	'free'
	9α-fluoro-17α-methyl-androst-4-ene-3α,6β,11β,17β-tetrol **VIII**	synthesized	'free'
Formebolone	2-hydroxymethyl-17α-methyl-androsta-1,4-diene-11α,17β-diol-3-one **IX**	synthesized	'free'
Furazabol	16z-hydroxy-furazabol	urine ex. study	conjugated
Mestanolone	17α-methyl-5α-androstane-3α,17β-diol **X**	synthesized	conjugated
Mesterolone	1α-methyl-5α-androstan-3α-ol-17-one **XI**	synthesized	conjugated
Metandienone	17-epimetandienone **XII**	synthesized	'free'
	6β-hydroxy-metandienone **XIII**	synthesized	'free'
	17α-methyl-5β-androstane-3α,17β-diol **XIV**	synthesized	conjugated
	17β-methyl-5β-androst-1-ene-3α,17α-diol **XV**	synthesized	conjugated
Methandriol	17α-methyl-5β-androstane-3α,17β-diol **XIV**	synthesized	conjugated
Metenolone	metenolone **XVI**	parent	conjugated
	1-methylen-5α-androstan-3α-ol-17-one **XVII**	synthesized	conjugated
Methyltestosterone	17α-methyl-5α-androstane-3α,17β-diol **X**	synthesized	conjugated
	17α-methyl-5β-androstane-3α,17β-diol **XIV**	synthesized	conjugated
Nandrolone	5α-estran-3α-ol-17-one **XVIII**	synthesized	conjugated
	5β-estran-3α-ol-17-one **XIX**	synthesized	conjugated
Norethandrolone	17α-ethyl-5β-estrane-3α,17β-diol **XX**	synthesized	conjugated
Oxandrolone	oxandrolone **XXI**	parent	'free'
	17-epioxandrolone **XXII**	synthesized	'free'
Oxymesterone	oxymesterone **XXIII**	parent	conjugated
Oxymetholone	17α-methyl-5α-androstane-3α,17β-diol **X**	synthesized	conjugated
	2-hydroxymethyl-17α-methyl-5α-androstane-3z,z,17β-triol	urine ex. study	conjugated
Stanozolol	3'-hydroxy-stanozolol **XXIV**	synthesized	conjugated and 'free'
	3'-hydroxy-17-epistanozolol **XXV**	synthesized	'free'
	4β-hydroxy-stanozolol **XXVI**	synthesized	conjugated
	16β-hydroxy-stanozolol **XXVII**	synthesized	conjugated

z Configuration not identified
urine ex. study = Metabolite obtained from an excretion study
'free' = Unconjugated

Table 2 **Temperature programmed Kovats indices of anabolic steroids and their metabolites**

Steroid	column	A OV 1 0.11µm	A´ OV 1 0.11µm	B OV 1 0.25µm	C OV 1 0.33µm	D SE 54 0.25µm	E SE 54 0.25µm	F SE 54 0.33µm
5α-Estran-3α-ol-17-one,bis-TMS **XVIII**		2440	2435	2441	2453	2451	2454	2468
5β-Androst-1-en-17β-ol-3-one,bis-TMS **III**		2452	2449	2455	2467	2469	2471	2488
17β-Methyl-5β-androst-1-ene-3α,17α-diol,bis-TMS **XV**		2454	2449	2456	2468	2472	2474	2492
5β-Estran-3α-ol-17-one,bis-TMS **XIX**		2490	2485	2492	2505	2502	2504	2520
2α-Methyl-5α-androstan-3α-ol-17-one,bis-TMS **VI**		2555	2549	2558	2574	2563	2565	2586
1-Methylen-5α-androstan-3α-ol-17-one,bis-TMS **XVII**		2583	2577	2586	2604	2599	2602	2623
9α-Fluoro-18-nor-17,17-dimethyl-4,13-dien-11β-ol-3-one,bis-TMS **VII**		2600	2594	2605	2621	2619	2621	2641
17α-Methyl-5β-androst-1-ene-3α,17β-diol,bis-TMS epiXV		2607	2601	2611	2628	2626	2630	2649
1α-Methyl-5α-androstan-3α-ol-17-one,bis-TMS **XI**		2607	2600	2611	2630	2619	2623	2644
17α-Methyl-5α-androstane-3α,17β-diol,bis-TMS **X**		2611	2604	2615	2632	2625	2628	2650
17α-Methyl-5β-androstane-3α,17β-diol,bis-TMS **XIV**		2617	2610	2620	2636	2628	2631	2654
17-Epimetandienone,TMS **XII**		2625	2616	2630	2657	2674	2680	2713
Boldenone,bis-TMS **II**		2648	2640	2652	2671	2671	2674	2698
17-Epioxandrolone,TMS **XXII**		2673	2662	2677	2707	2735	2741	2777
7α,17α-Dimethyl-5β-androstane-3α,17β-diol,bis-TMS **I**		2692	2684	2692	2713	2706	2710	2730
4-Chloro-androst-4-en-3α-ol-17-one,bis-TMS **V**		2693	2687	2696	2712	2720	2724	2746
Metenolone,bis-TMS **XVI**		2694	2687	2700	2718	2716	2721	2743
17α-Ethyl-5β-estrane-3α,17β-diol,bis-TMS **XX**		2695	2589	2698	2715	2710	2714	2733
Methyltestosterone,bis-TMS (Internal Standard)		2754	2745	2759	2778	2775	2779	2803
Oxandrolone,TMS **XXI**		2778	2768	2783	2816	2845	2851	2893
6β-Hydroxy-metandienone,bis-TMS **XIII**		2846	2837	2850	2873	2877	2882	2911
9α-Fluoro-17α-methyl-androst-4-ene-3α,6β,11β,17β-tetrol,tetra-TMS **VIII**		2854	2852	2856	2855	2855	2854	2860
Oxymesterone,tris-TMS **XXIII**		2952	2943	2956	2977	2968	2972	2993
6β-Hydroxy-4-chloro-dehydromethyl-testosterone,bis-TMS **IV**		3007	2996	3011	3039	3044	3048	3081
3´-Hydroxy-17-epistanozolol,tris-TMS **XXV**		3100	3092	3101	3113	3119	3122	3137
2-Hydroxymethyl-17α-methyl-androsta-1,4-diene-11α,17β-diol-3-one,tris-TMS **IX**		3163	3152	3166	3187	3203	3206	3232
3´-Hydroxy-stanozolol,tris-TMS **XXIV**		3219	3208	3221	3238	3242	3245	3266
4β-Hydroxy-stanozolol,tris-TMS **XXVI**		3219	3208	3238	3245	3244	a	3277
4α-Hydroxy-stanozolol,tris-TMS (Internal Standard)		3238	3228	3250	3262	3268	a	3296
16β-Hydroxy-stanozolol,tris-TMS **XXVII**		3334	3320	3351	3360	3368	3402	3397

a Substance showed strong tailing

Indices were estimated on columns A-F (see experimental)

Temperature program 180°C - 5°C/min - 320°C