Supporting Information

High-throughput bioaffinity mass spectrometry for screening and identification of designer anabolic steroids in dietary supplements

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Chemicals

17α-testosterone (4-androsten-17α-ol-3-one, α-T), 17β-testosterone (4-androsten-17β-ol-3-one, β-T), 17β-testosterone-D3 (4-androsten-17β-ol-3-one-16,16,17-D3, T-D3), 17β-estradiol (1, 3, 5(10)-estratrien-3,17β-diol, β-E2), 17α-estradiol (1, 3, 5(10)-estratrien-3,17α-diol, α-E2), 17β-estradiol-3-glucuronide (1, 3, 5(10)-estratrien-17β-ol-3-glucuronide, β-E2-glu), 17β-estradiol-3-sulphate (1, 3, 5(10)-estratrien-17β-ol-3-sulfate, β-E2-sul), 17β-testosterone-glucuronide (4-androsten-3-one-17β-glucuronide, β-T-glu), dihydrotestosterone (5α-androstan-17β-ol-3-one, DHT), zearalenone (zea) α-zearalanol (α-zear), β- zearalanol (β-zear), 4-chloro-testosterone (4-androsten-4-chloro-17β-ol-3-one, Cl-T), equol (3,4-dihydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol), estrone (1, 3, 5(10)-estratrien-3-ol-17-one, E1), testosterone-17-acetate (4-androsten-3-one-17β-acetate, T-Ac), testosterone-17-cypionate (4-androsten-3-one-17β-cypionate, T-cyp), testosterone-17-decanoate (4-androsten-3-one-17β-decanoate, T-dec), progesterone (4-pregnen-3,20-dione, proges.), 16-dehydroprogesterone (4,16-pregnadien-3,20-dione, dehydroproges.) and cortisol (4-pregnene-11β,17α,21-triol-3,20-dione) were purchased from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands). 17α-1-testosterone (1,(5α)-androsten-17α-ol-3-one, α-1-T), 17β-1-testosterone (1,(5α)-androsten-17β-ol-3-one, β-1-T), (4-androsten-4-chloro-3,17-dione, CLAD), 17α-19-nortestosterone (4-estren-17α-ol-3-one, α-norT), 17β-19-nortestosterone (4-estren-17β-ol-3-one, β-norT), 17β-boldenone (1,4-androstadien-17β-ol-3-one, β-bol), 17α-boldenone (1,4-androstadien-17α-ol-3-one, α-bol), dehydroepitestosterone (5-androsten-3β-ol-17-one DHEA), androst-4-ene-3β-17β-diol, tetrahydrogestrinone 13,17-diethyl-17-hydroxy-18,19-dinor-17-pregn-4,9,11-trien-3-one, THG) and 5α-androstane-3β-17β-diol and its seven other isomers were purchased from Steraloids (Newport, RI, US). Acetonitrile (ACN) and methanol (MeOH) were from Biosolve (Valkenswaards, The Netherlands). Formic acid (HCOOH), ethanol (EtOH), EDTA (Triplex), HEPES, sodium azide (NaN_3), imidazole and

sodium chloride (NaCl) were purchased from Merck (Whitehouse Station, NJ, USA). The 2-(N-Morpholino) ethanesulfonic acid (MES) and N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) were from Sigma Aldrich Chemie (Zwijndrecht, The Netherlands). Six times his-tagged full-length recombinant human SHBG (purified, 96 kDa dimer) was purchased from Generi Biotech s.r.o. (Hrdadec Kralove, Czech Republic).

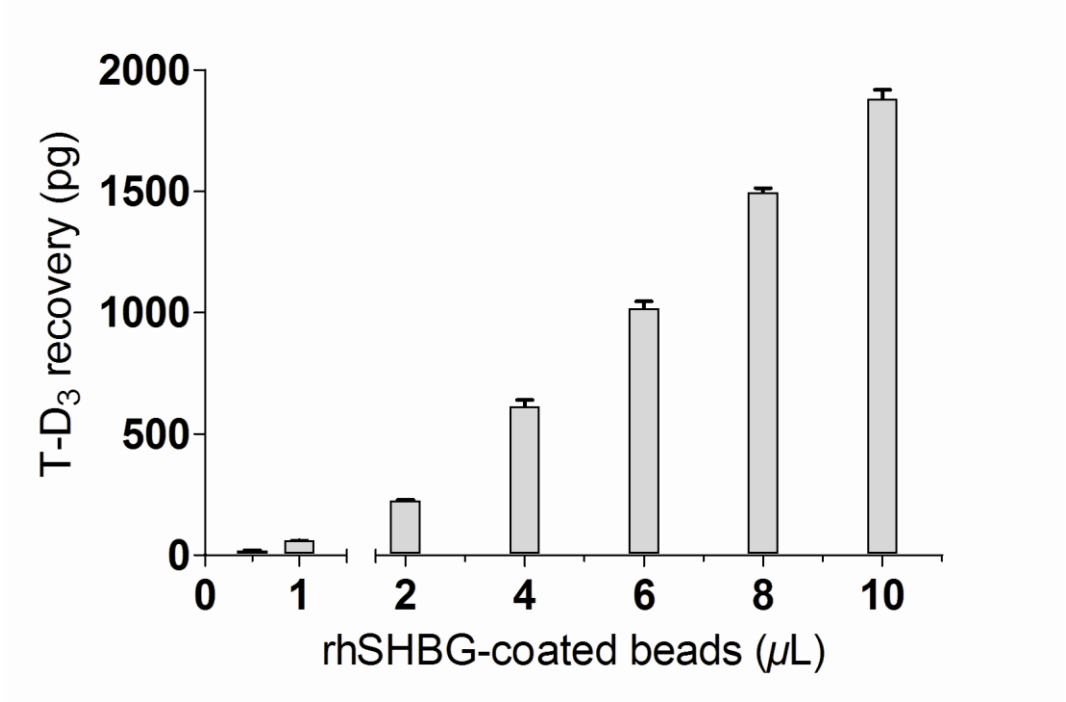

Figure S-1. Average amounts (n=2) of T-D_3 label recovered from different volumes of rhSHBG-coated beads after pre-incubation with 5 ng of the label and measured by UPLC-QqQ-MS.

Table S-2. Theoretical and experimental exact masses, mass errors, retention times and elemental composition of β-norT, α-T, β-T, androst-4-ene-3β-17β-diol and 1-β-T in standard solutions and in biopurified dietary supplement extracts with chip-UPLC-Q-ToF-MS following screening.

Measurement	T_R (min)	Experimental $[M+H]^+$	Theoretical $[M+H]^+$	Mass error (ppm)	Elemental composition $[M+H]^+$	Accurate mass product ion scanning (m/z)
			β-T			
standard	6.07	289.217	289.217	0	$C_{19}H_{29}O_2$	97.063, 109.065
sample 15	6.07	289.216		-3	$C_{19}H_{29}O_2$	97.061, 109.061
			β-norT			
standard	5.52	275.203	275.201	+7	$C_{18}H_{27}O_2$	109.063, 145.061
sample 16	5.53	275.203		+7	$C_{18}H_{27}O_2$	109.061, 145.060
			β-T			
standard	6.07	289.217	289.217	0	$C_{19}H_{29}O_2$	97.064, 109.062
sample 17	6.06	289.216		-3	$C_{19}H_{29}O_2$	97.065, 109.065
			androst-4-ene-3β-17β-diol			
standard	5.83	273.221	$[M-H_2O+H]^+$	-4	$C_{19}H_{29}O_1$	255.210, 81.072
sample 18	5.82	273.221	273.222	-4	$C_{19}H_{29}O_1$	255.209, 81.073
			β-T			
standard	6.06	289.217	289.217	0	$C_{19}H_{29}O_2$	97.068, 109.064
sample 19	6.06	289.220		+10	$C_{19}H_{29}O_2$	97.067, 109.066
			β-T			
standard	6.06	289.217	289.217	0	$C_{19}H_{29}O_2$	97.065, 109.061

sample 20	6.08	289.216		-3	$C_{19}H_{29}O_2$	97.067, 109.063
			β-1-T			
standard	6.51	289.216	289.217	-3	$C_{19}H_{29}O_2$	187.145, 205.155
sample 20	6.49	289.216		-3	$C_{19}H_{29}O_2$	187.150, 205.157
			β-T			
standard	6.08	289.217	289.217	0	$C_{19}H_{29}O_2$	97.064, 109.061
sample 21	6.08	289.216		-3	$C_{19}H_{29}O_2$	97.067, 109.067
			α-T			
standard	6.70	289.216	289.217	-3	$C_{19}H_{29}O_2$	97.063, 109.067
sample 21	6.72	289.216		-3	$C_{19}H_{29}O_2$	97.065, 109.066
			β-1-T			
standard	6.52	289.217	289.217	0	$C_{19}H_{29}O_2$	187.146, 205.155
sample 22	6.48	289.216		-3	$C_{19}H_{29}O_2$	187.142, 205.153
			THG			
standard	7.33	313.215	313.217	-6	$C_{21}H_{29}O_2$	241.161, 159.083
spiked sample	7.35	313.215		-6	$C_{21}H_{29}O_2$	241.155, 159.086

3800 GC Varian 1200 L GC-MS/MS conditions

Derivatization of β-E2. Twenty-five microliters of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide solution was added to dried samples and incubated during 1 hour at 60°C. After incubation the mixture is evaporated to dryness under a stream of nitrogen at 55°C and the residue was dissolved in 50 µL of isooctane.

GC conditions.

- injection 1 µl pulsed splitless at 260°C
- initial oven temperature 110°C (1 minute)
- temperature is increased by 20°C/min to 260°C, remaining time : 0.5 minutes.
- Increasing 1°C/min to 266°C, followed by increasing of 20°C/min to 340°C. Total runtime: 20 minutes.
- temperature transfer line 330°C
- Constant flow mode, 1.1 ml/min helium
- Temperature ion volume 250°C

GC column: stationary phase: VF-17ms, film thickness (µm): 0.25 L (m) x ID (mm) x OD (mm): 30 x 0.25 x 0.39, from Agilent Technologies (Santa Clara, CA, USA).

MS/MS conditions: Electron Impact (EI) source was at -70 eV and 250°C while following multiple reaction monitoring (MRM) transitions were measured for β-E2 using argon as collision-induced dissociation gas; m/z 416.2 → m/z 285.2, m/z 416.2 → m/z 326.2 (using collision energy 7 and 6 V respectively).

Table S-3

Theoretical and experimental exact masses, mass errors, retention times and elemental composition of β-bol, β-norT, α-T, β-T, β-E2, 1-β-T, THG, proges. and androst-4-ene-3β-17β-diol in standard solutions and in biopurified dietary supplement extracts with chip-UPLC-Q-ToF-MS following adjusted BioMS identification procedure.

Measurement	T_R (min)	Experimental $[M+H]^+$	Theoretical $[M+H]^+$	Mass error (ppm)	Elemental composition $[M+H]^+$	Accurate mass product ion scanning (m/z)
			β-T			
standard	6.07	289.220	289.217	-10	$C_{19}H_{29}O_2$	97.065, 109.063
sample 15	6.07	289.220		-10	$C_{19}H_{29}O_2$	97.068, 109.065
			β-bol			
standard	5.44	287.200	287.201	-3	$C_{19}H_{27}O_2$	135.082, 121.063
sample 16	5.44	287.204		+10	$C_{19}H_{27}O_2$	135.085, 121.065
			β-norT			
standard	5.52	275.202	275.201	+4	$C_{18}H_{27}O_2$	109.064, 145.061
sample 16	5.52	275.203		+7	$C_{18}H_{27}O_2$	109.062, 145.063
			β-bol			
standard	5.44	287.200	287.201	-3	$C_{19}H_{27}O_2$	135.081, 121.063
sample 17	-	-		-	-	-
			β-T			
standard	6.00	289.217	289.217	0	$C_{19}H_{29}O_2$	97.065, 109.068
sample 17	6.00	289.214		+10	$C_{19}H_{29}O_2$	97.066, 109.069

		$[M-H_2O+H]^+$	androst-4-ene-3β-17β-diol		$[M-H_2O+H]^+$	
standard	5.82	273.221	$[M-H_2O+H]^+$	-4	$C_{19}H_{29}O_1$	255.211, 81.074
sample 18	5.81	273.220	273.222	-7	$C_{19}H_{29}O_1$	255.210, 81.071
			β-E2*			
standard	13.6		416.2			285.2, 326.2
sample 19	13.6					285.2, 326.2
			β-T			
standard	6.04	289.215	289.217	-7	$C_{19}H_{29}O_2$	97.065, 109.065
sample 19	6.07	289.214		-10	$C_{19}H_{29}O_2$	97.067, 109.061
			β-norT			
standard	5.51	275.202	275.201	+3	$C_{18}H_{27}O_2$	109.061, 145.061
sample 19	5.53	275.203		+7	$C_{18}H_{27}O_2$	109.064, 145.062
			β-1-T			
standard	6.51	289.214	289.217	-10	$C_{19}H_{29}O_2$	187.143, 205.153
sample 19	6.49	289.215		-7	$C_{19}H_{29}O_2$	187.145, 205.155
			β-T			
standard	6.00	289.214	289.217	-10	$C_{19}H_{29}O_2$	97.069, 109.068
sample 20	6.01	289.215		-7	$C_{19}H_{29}O_2$	97.066, 109.064
			β-1-T			
standard	6.51	289.214	289.217	-10	$C_{19}H_{29}O_2$	187.149, 205.159
sample 20	6.49	289.216		-3	$C_{19}H_{29}O_2$	187.150, 205.160
			β-T			
standard	6.01	289.217	289.217	0	$C_{19}H_{29}O_2$	97.067, 109.062
sample 21	6.01	289.219		+7	$C_{19}H_{29}O_2$	97.069, 109.064
			α-T			

standard	6.70	289.216	289.217	-3	$C_{19}H_{29}O_2$	97.062, 109.069
sample 21	6.72	289.218		+3	$C_{19}H_{29}O_2$	97.063, 109.067
			proges.			
standard	8.06	315.230	315.232	-6	$C_{21}H_{31}O_2$	97.065, 109.063
sample 21	8.06	315.231		-3	$C_{21}H_{31}O_2$	97.061, 109.061
			β-1-T			
standard	6.51	289.219	289.217	+7	$C_{19}H_{29}O_2$	187.151, 205.153
sample 22	6.51	289.220		+10	$C_{19}H_{29}O_2$	187.145, 205.155
			THG			
standard	7.32	313.215	313.217	-6	$C_{21}H_{29}O_2$	241.155, 159.082
spiked sample	7.36	313.216		-3	$C_{21}H_{29}O_2$	241.151, 159.085

* β-E2 identified on basis of retention time and specific product ions with GC-MS/MS following derivatization with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide.