Supplemental Material:

Qualitative and Semi-Quantitative Analysis of seized Doping Products in Switzerland

Materials and Methods

Except of minor changes in the protocols, this part was transcribed according to Krug et al., 2014 [[1](#_ENREF_1)].

**Chemicals**

Glacial acetic acid and ammonium acetate were purchased from Merck (Darmstadt, Germany). 12% Bis-Tris gels (8 cm x 8 cm x 0.1 cm, 10-well), MOPS running buffer (10x) as well as lithium dodecyl sulfate (LDS) buffer (4x) came from Life Technologies (Darmstadt, Germany). GelCode Blue Stain Regent (Coomassie G-250) was supplied by Pierce (Rockford, IL, USA) and N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) by Chem. Fabrik Karl Bucher (Waldstetten, Germany). Trypsin was obtained from Promega (Madison, WI), and dithiothreitol (DTT), all buffer ingredients came from Sigma-Aldrich (Steinheim, Germany). Access2 total βhCG Assay system inclusive total β-hCG-Test (2x50) N° 33500, Access2 buffer N° A16792 and alkaline phosphatase substrate N° 81906 came from Beckman Coulter (Krefeld, Germany). Acentonitrile (ACN) was bought from VWR International GmbH (Darmstadt, Germany) and were of analytical or high-performance liquid chromatography (HPLC) grade. Aqueous buffers were prepared by using deionized water (Sartorius Stedim Biotech S.A., Aubagne, France).

**LC-MS/MS screening**

The initial LC-MS/MS screening was performed in selected reaction monitoring (SRM) mode including ion transitions for the most common anabolic androgenic steroids and their esters and stimulants. These analyses were conducted on a TSQ Vantage (Thermo Fisher Scientific, Dreieich, Germany) coupled to an Agilent (Walbronn, Germany) 1100 HPLC system equipped with an heated electrospray ionization (HESI) source and a C-18 column (2.1 x 50 mm, particle size 3.0 mcm, Macherey Nagel, Düren, Germany). For LC, aqueous formic acid 0.2 % was used as eluent A and ACN as eluent B. A gradient is set as follows: starting conditions 0 % B; increasing to 100 % within 5 min, remaining at 100 % for 4 min, 5 min re-equilibration with 0 % B. The flow was set to 250 mcL/min and the injection volume to 10 mcL. The ESI source is operated in positive mode at a source temperature of 350°C. Identified analytes are confirmed and semi-quantified by using the corresponding reference standard solutions and product ion scan experiments.

**LC-HRMS (Full-scan mode)**

Samples were analysed by LC-HRMS on an Agilent 6550 iFunnel Q-TOF connected to an Agilent 1290 Infinity HPLC system. In order to detect unusual or unknown ingredients, measurements were conducted in full-scan mode and MS/MS from *m/z* 50 to 1700. For LC, aqueous formic acid 0.1 % was used as eluent A and formic acid 0.1 % in ACN as eluent B. A gradient was set as follows: Starting conditions 2 % B; increasing to 100 % B within 5 min, remaining at 100 % for 3 min, 5 min re-equilibration with 2 % B. The flow was set to 300 mcL/min and the injection volume to 5 mcL. The HESI source was operated in positive mode at a source temperature of 350°C.

Full-scan data were manually assessed and formula are generated in silico with GPMAW 8.0 Software with regard to unfamiliar drugs or drug candidates including anabolic agents, stimulants, growth factors and growth hormone releasing factors and natural and synthetic insulins.

**SDS-PAGE**

Lyophilisates with assumed peptidic content were subjected to SDS-PAGE analysis on 12 % Bis-Tris gels. A total of 20 mcL of the dissolved lyophilisates are mixed with 7 mcL of LDS sample buffer as well as 3 mcL of DTT (1M) and subsequently heated for 10 min at 70°C to achieve denaturation of proteins and reduction of disulphide bonds according to standard protocols. Gels were run in MOPS electrophoresis buffer for 90 min at a constant voltage of 125 V (XCell Surelock Mini-Cell, Life Technologies, Darmstadt, Germany). Following Coomassie staining, gels were digitalized with a light transmission scanner (GE Healthcare, Munich, Germany) operated in transparent mode using a red filter and a resolution of 300 dpi.

For protein identification, gel bands were excised from the gel and subjected to in-gel tryptic digestion and nano-LC-HRMS. Following destaining with 200 mcL of 100 mM NH4HCO3/ACN (1:1) (2 x 10 min, 750 rpm, RT), gel slices were dehydrated in 100 mcL of ACN and dried under reduced pressure for 15 min (Joua 10.22, Thermo Fisher Scientific). Afterwards, bands were rehydrated in 10 mcL of a trypsin solution (20 mcg/mL in 50 mM NH4HCO3 , pH 8) for 45 min at 4°C and incubated for 3 - 16 hours at 37°C. Tryptic peptides were extracted with 100 mcL of 50 % ACN/1 % TFA (3 x 10 min, 1000 rpm) and combined extracts dried in a vacuum concentrator. Dried extracts were then reconstituted in 50 mcL of 2 % acetic acid and subjected to LC-MS/MS. Alternatively, in solution digestion was performed after ultra-filtration (10 kDA Filter, 15 min, 140000 x g).

**ELICA**

Lyophilisates with declared human choriogonatropine content were subjected to an electro chemiluminescence immuno assay (ELICA). The Access Total βhCG assay was a one‐step immunoenzymatic (“sandwich”) assay, N° 151. A total of 200 mcL of the dissolved lyophilisate was added to a reaction vessel along with the Access2 buffer solution.

1. Krug, O., et al., *Identification of black market products and potential doping agents in Germany 2010-2013.* Eur J Clin Pharmacol, 2014.