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Analysis of confiscated products with suspiciously doping relevant ingredients by SDS-PAGE, LC-MS(/MS), GC-MS/NPD

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Issue

Sixty nine confiscated black market products were analyzed for their content to provide qualitative and quantitative information for the bureau of customs investigation to confirm the suspicion of German Drug Law violations. The seized material included labeled as well as unlabeled (14) products of different formulation such as oily solutions and suspensions, tablets, powders, or gels. The analysis of confiscated products in general provides interesting data as developments in trafficking of drugs relevant for doping controls are indicated, which can support anti-doping efforts.

Results

In total 22 different drugs were detected; 49 products showed 17 doping-relevant ingredients. The products contained steroid esters (9), steroids and their derivatives (4), agents with anti-estrogenic activity (1), stimulants (1), growth factors (2), virilizing drugs (3) and dermatologic agents (2). The labeled and identified drugs matched only in 50% of the cases

and the quantified amounts differed from 0 to 300% from the declared content. An outstanding discrepancy between labeling and analytical result was the frequent finding of methyltestosterone (6-112mg/ml) in 29% of the analyzed products, especially in those cases where 17-methylated steroid were not supposed to be delivered. This is particularly important given the considerable liver toxicity of 17-alkylated steroids.

Identified drug	**total found**	**labelled - found**	**labelled - not found**	**not labelled - found**	
Steroid esters					**doping relevant**
Boldenone undecylenate	5	4	1	1	
Drostanolone propionate	1	1	2	0	
Nandrolone decanoate	8	6	0	2	
Testosterone					
-propionate	4	2	0	2	
-enantate	12	6	0	6	
-isocaproate	4	1	0	3	
-phenylpropionate	1	0	1	1	
Trenbolone					
-acetate	4	3	0	1	
-enantate	6	3	2	1	
Steroids and their derivatives					
Metandienone	1	1	0	0	
Methyltestosterone	21	1	1	20	
Stanozolol	4	3	0	1	
Testosterone	1	1	0	0	
Anti-estrogenic agents					
Clomiphene	1	1	0	0	
Stimulants					
Synephrine	1	0	0	1	
Growth factors					
FGF-1	2	0	0	2	
HGH	1	0	0	1	
49 Products with 17 drugs	**77**	**33**	**7**	**42**	
Virilizing agents					**actually not doping relevant**
Sildenafil	6	5	0	1	
Tadalafil	8	7	0	1	
Yohimbine	1	1	0	0	
Dermatologic agents					
Isotretinoin	1	1	0	0	
Melanotan	1	0	0	1	
Total 69 Products with 22 drugs	**94**	**47**	**7**	**45**	

Table 1: Identified drugs in black market products

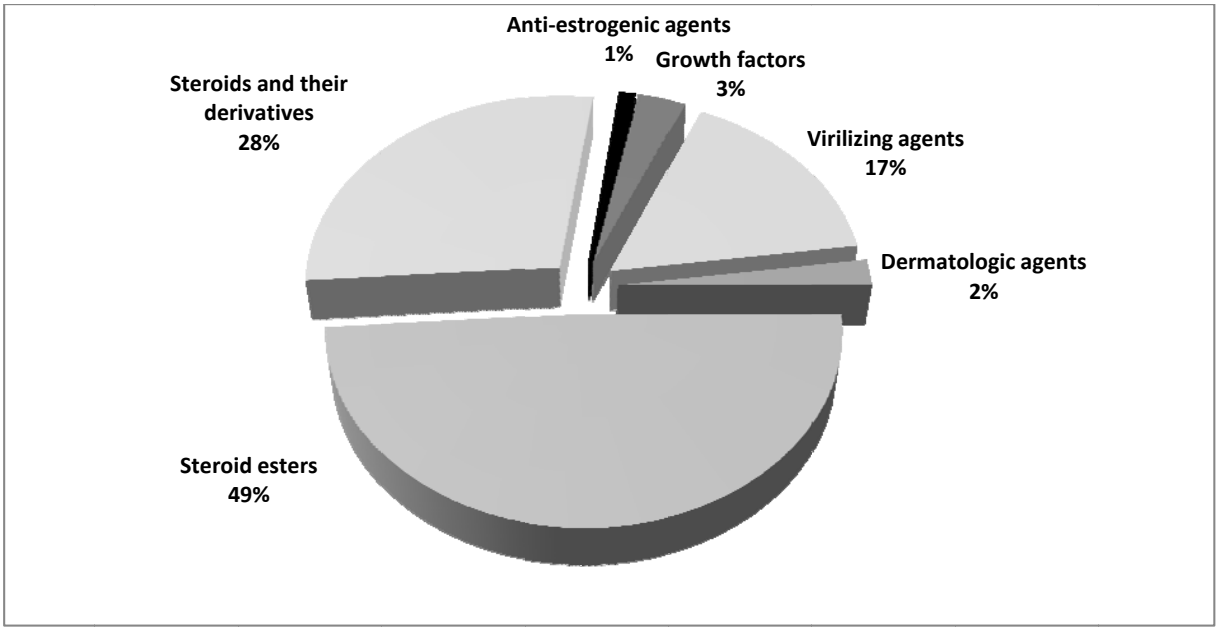

Figure 2: Apportionment of identified drugs in analyzed black market produkts

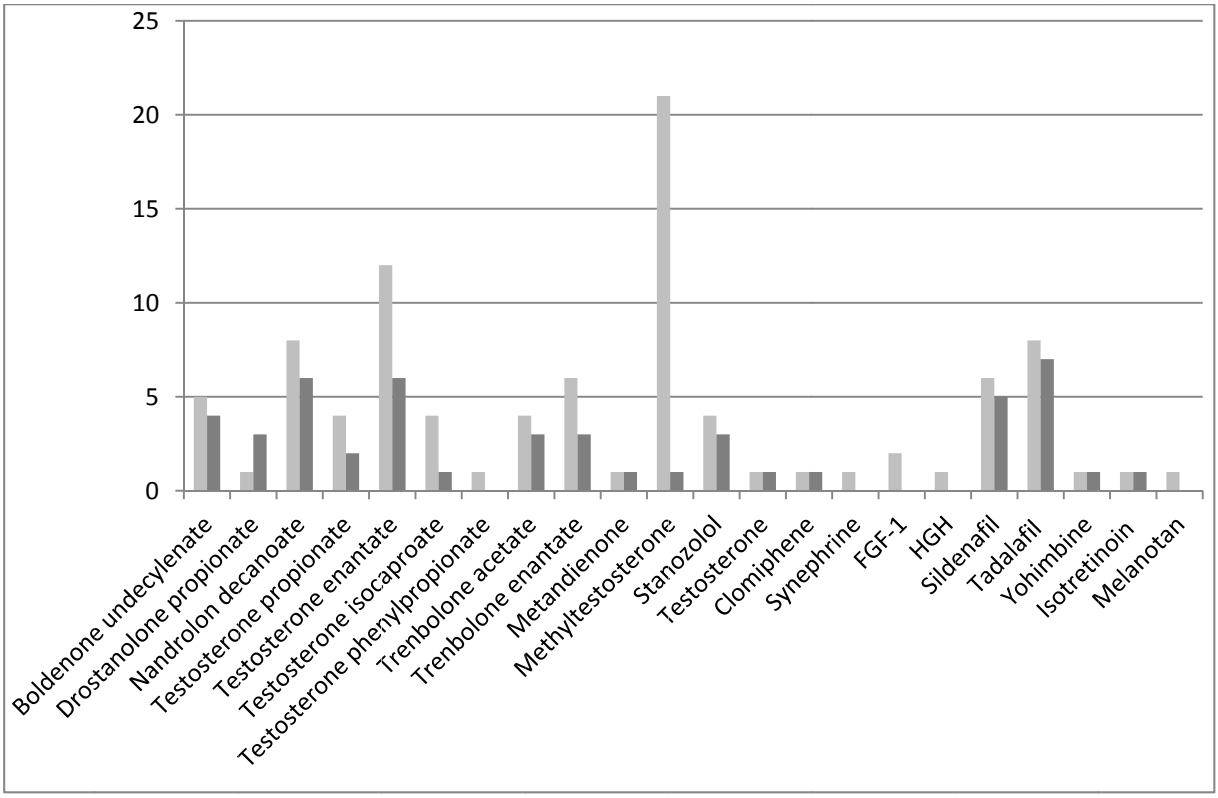

Figure 3: Discrepancy of identified (bright) and labelled (dark) drugs

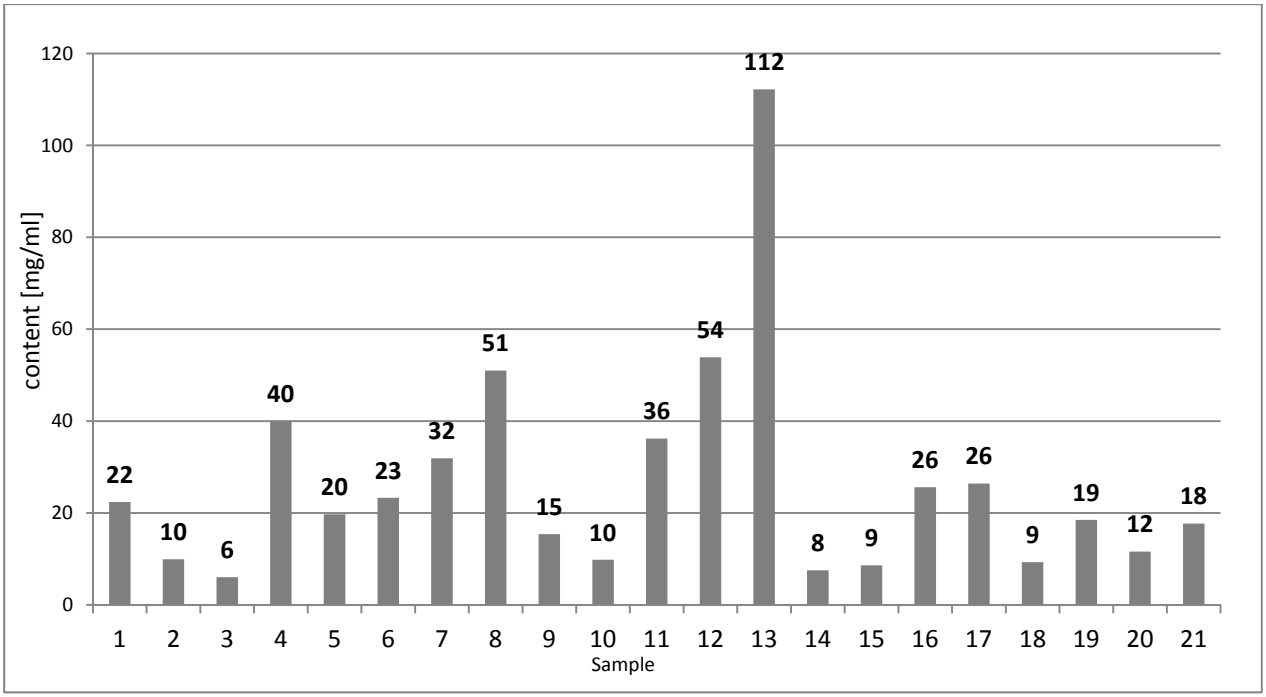

Figure 4: Amounts of quantified methyltestosterone

Methods

Sample preparation: The samples were dissolved or extracted with water/acetonitrile (50:50 v/v) or methanol and subsequently diluted to yield a 10 ppm concentration of labelled drug content.

Measurement: For identification and quantification all samples were screened by HPLC-ESI-MS consisting of an Agilent 1100 series HPLC interfaced via electrospray to an Applied Biosystems API 2000 Q Trap. In those cases where no drug could be identified, further experiments were performed with GC–MS/NPD consisting of a HP 6890 Series GC-System and a 5973 Mass Selective Detector. Here the samples were treated with MSTFA and MBTFA for derivatization.

To identify peptides, additional experiments were performed by electrophoretic separation, trypsin digestion, and nano-UPLC-high resolution/high accuracy mass spectrometry.

Reference

Thevis M, Schrader Y, Thomas A, Sigmund G, Geyer H, Schänzer W. (2008) Analysis of confiscated black market drugs using chromatographic and mass spectrometric approaches. J Anal Toxicol, Vol. 32, 232-40