Oliver Krug, Andreas Thomas, Katja Walpurgis, Gerd Sigmund, Wilhelm Schänzer, Mario Thevis

Analysis of confiscated products with suspiciously doping relevant ingredients by GC-MS, LC-HRMS and LC-MS^n

Center for Preventive Doping Research - Institute of Biochemistry / European Monitoring Center for Emerging Doping Agents, German Sport University, Cologne, Germany

Abstract

The summary of analytical results of forty seven confiscated black-market products in 2011 is presented. Different formulations (oily solutions, tablets, and powders) in labeled and unlabeled packages were dissolved or extracted and diluted followed by measurements conducted with LC-MS^n, LC-HRMS, and GC-MS.
Thirty nine products showed 21 doping-relevant ingredients, furthermore labelled and identified drugs matched in only 25% of all cases. Additionally nearly half of the products contained non-declared doping-relevant drugs in trace amounts. An appreciable challenge was the identification of a phosphorylated compound, which was identified as Coenzyme A (CoA).

Introduction

Forty seven confiscated black market products were analyzed for their content to provide qualitative and quantitative information for the Bureau of Customs Investigation to verify or falsify the suspicion of German Drug Law violations. The seized material included labeled as well as unlabeled (35) products of different formulation such as oily solutions, tablets, or powders (e.g. lyophilisates). 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.

Experimental

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 (Waldbronn, Germany) interfaced via electrospray to an Applied Biosystems API 2000 Q Trap (Darmstadt, Germany). 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 (Waldbronn, Germany). Here the samples were treated with MSTFA and MBTFA for derivatization. In order to get more structural information of residues of an unknown analyte MS^3 experiments were carried out on an AB Sciex Q-Trap 5500 (Darmstadt, Germany) with ESI in positive mode.

Results and Discussion

In total, 25 different drugs were detected; 39 products showed 21 doping-relevant ingredients. The products contained steroid esters (10), steroids and their derivatives (9), agents with anti-estrogenic activity (1), stimulants (1), virilizing drugs (2), dermatologic agents (1), and vitamin precursors (1). The labeled and identified drugs matched in only 25% of the cases. Additionally nearly half of the products contained non-declared doping-relevant drugs in trace amounts.

 ISBN 978-3-86884-038-4

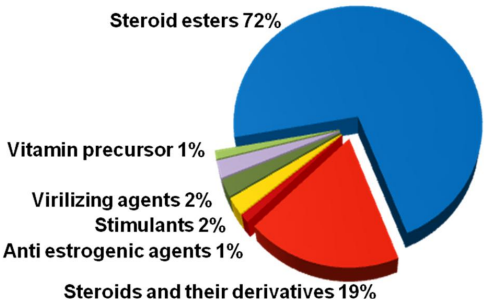

Figure 1: Apportionment of identified drugs in analyzed black-market products

A considerable challenge was the identification of a phosphorylated compound, which turned out to be Coenzyme A. First LC-MS/MS experiments showed an analyte with *m/z* 768 $[M+H]^+$ and its product ion mass spectra contained fragments indicating the loss of 80 Da. Further LC-HRMS experiments verified the presence of one or more phosphate residues, and by means of MS^3 experiments the coenzyme´s structure was fully elucidated. For the identification of coenzyme A (CoA) comparison analysis with LC-HRMS and LC-MS/MS were conducted (Fig.3). The high-resolution high-accuracy MS data were generated on an Accela LC system connected to a Thermo Exactive mass spectrometer (Bremen, Germany) (Fig.3A and Tab.1). The analysis were carried out in positive mode with electrospray ionization. Fragment ions were generated with higher collisional energy dissociation (hcd).

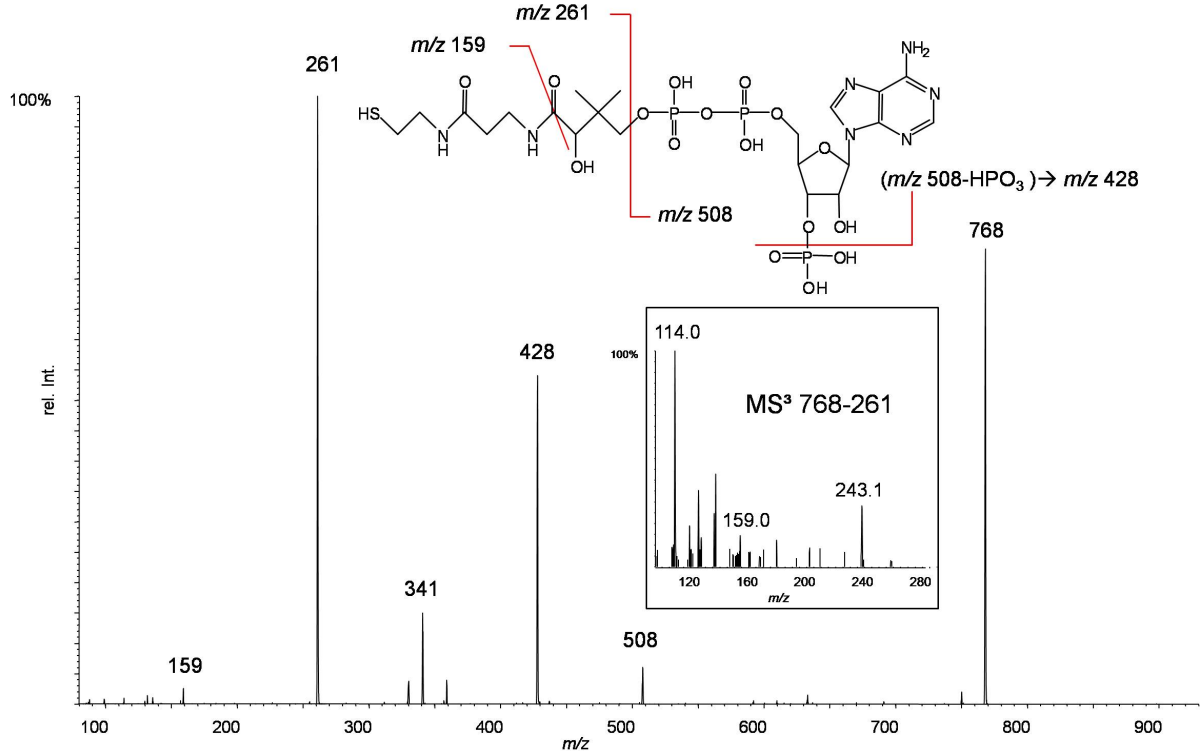

Figure 2: Enhanced produt ion spectrum of Coenzyme A m/z 768 [M+H]+ (ESI, CE=30V); Inlet: MS^3 spectrum of ion transition m/z 768-261 (ESI, CE=35V)

 ISBN 978-3-86884-038-4

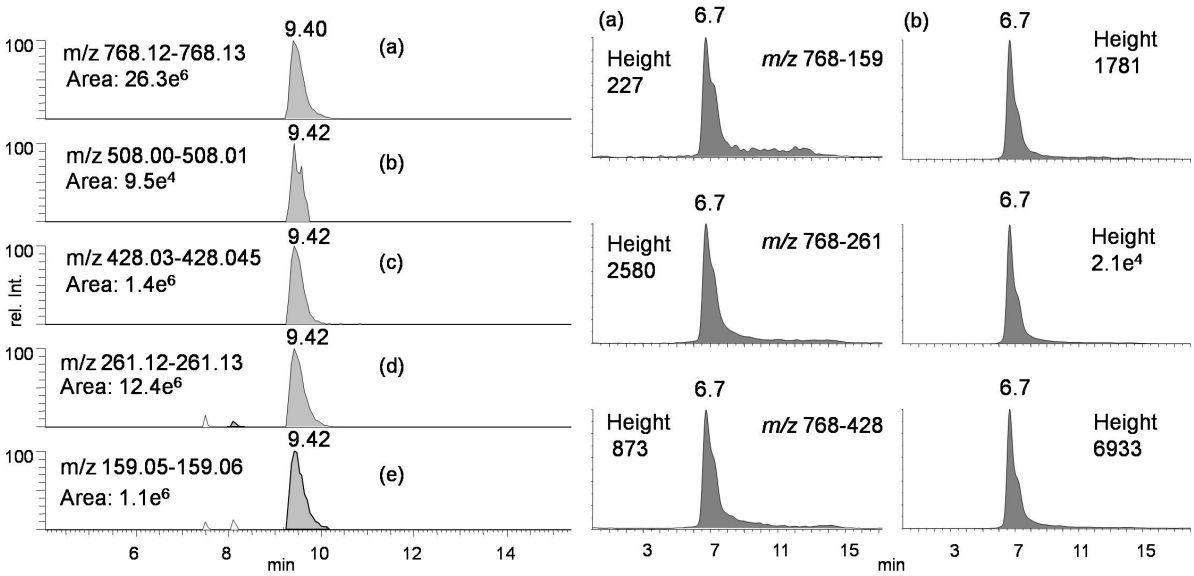

Figure 3: (I) Extracted ion chromatograms of (a) CoA m/z 768.12 [M+H]+, (b-e): diagnostic CoA fragments (HRMS [M+H]+ (ESI+; hcd 50 eV; full scan) (II) MRM chromatograms of CoA with ion transitions m/z 768-159, 768-261, and 768-428 (a) reference (50 ng/mL), (b) confiscated product (1:1000 diluted in H2O)

compound	Precursor/Product Ions $[M+H]^+$	Elemental Composition	m/z (Exp.)	Error (ppm)
Coenzyme A	768 / 768	$C_{21}H_{37}O_{16}N_7P_3S$	768.1225	-2.3
	508	$C_{10}H_{17}O_{13}N_5P_3$	508.0017	-2.6
	428	$C_{10}H_{16}O_{10}N_5P_2$	428.0353	-3.2
	261	$C_{11}H_{21}O_3N_2S$	261.1260	-3.0
	159	$C_6H_{11}ON_2S$	159.0584	-1.4

Table 1: Experimental elemental composition of CoA and its hcd generated fragment ions

References

- 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* 32(3), 232-40
- Krug O, Thomas A, Sigmund G, Walpurgis K, Beuck S, Laussmann T, Schänzer W, Thevis M. (2011) Analysis of confiscated products with suspiciously doping relevant ingredients by SDS-PAGE, LC-MS(/MS), GC-MS/NPD. In Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (19), Köln, pp 288-91

Acknowledgements

The authors thank the Federal Ministry of the Interior of the Federal Republic of Germany and Manfred-Donike-Institute for Doping Analysis, Cologne, for supporting the presented work.