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Mass spectrometric analysis of black market products by HPLC-(HR)MS, GC-(HR)MS and 1D-gel electrophoresis-UPLC-MSⁿ

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Abstract

In order to prevent deception by abuse of drugs in sport terms and its health-risk, the World-Anti-Doping-Agency (WADA) publishes the annually updated Prohibited List. Not only top athletes, but also the athletes of mass sport succumb the abuse of performing enhancing agents. In total 185 black market products as well as products confiscated by the police or the bureau of customs investigation were analysed qualitatively and quantitatively since 2010. The samples contained 38 different drugs with doping relevancy and in most cases unlabeled drugs could be confirmed. An outstanding result was the identification of methyltestosterone in 21 samples, which contained up to 112 mg/mL. Furthermore the steroidal derivatives 5 α -androstano-(2,3-c)-furazan-17 β -tetrahydropyranol and 17-methyl-stenbolone were identified.

Introduction

The endeavor to success in sporting terms leads unfortunately to abuse of performance enhancing agents like anabolic steroids, stimulants, growth hormone, etc., which are in most cases developed for therapeutic use against diseases and are sold as medicaments. Not only top athletes but also the athletes of mass sport succumb the abuse of performing enhancing agents. This opens an enormous black market for original and faked medicaments or drugs from underground laboratories. The aim of the European Monitoring Center for Emerging Doping Agents (EuMoCEDA) is to observe the doping community and identify trends within hence anticipate new developments for preventive actions. 185 Black market products as well as products confiscated by the police or the bureau of customs investigation were analysed qualitatively and quantitatively since 2010.

Experimental

The high performance liquid chromatography / high resolution mass spectrometry (HPLC-HRMS) -experiments were conducted in full-scan mode. Qualification and quantification of analytes were obtained by conducting product-ion scans with substance specific fragmentation pathways [1]. Included agents were anabolic agents, stimulants, growth factors, natural and synthetic insulins, IGF-1 and synthetic analogous as well as growth hormone releasing factors. For gas chromatography/(high resolution) mass spectrometry (GC-(HR)MS)-experiments the analytes were derivatised and measured in full-scan mode. Qualitative and quantitative Analytes were accomplished by using reference substances and/or reference databases. Included substances were anabolic agents, stimulants, beta-2-agonists and narcotics. For the analysis of peptides and proteins aliquots were separated by polyacrylamide gelelectrophoresis and protein specific colouring. By bottom-up analysis, the identities of analytes were confirmed with nano liquid chromatography / tandem mass spectrometry. Included analytes were human growth hormone (hGH), growth factors (e.g.: FGF, MGF, etc.), various erythropoietins (EPO), and growth hormone releasing factors [2].

Results and Discussion

In total 44 active agents were found, 38 of these have doping relevancy. 86% accounted for steroids and their derivatives, 11% accounted for growth hormones, 3% of all analyzed products contained stimulants, and antiestrogenic agents (Fig.1).

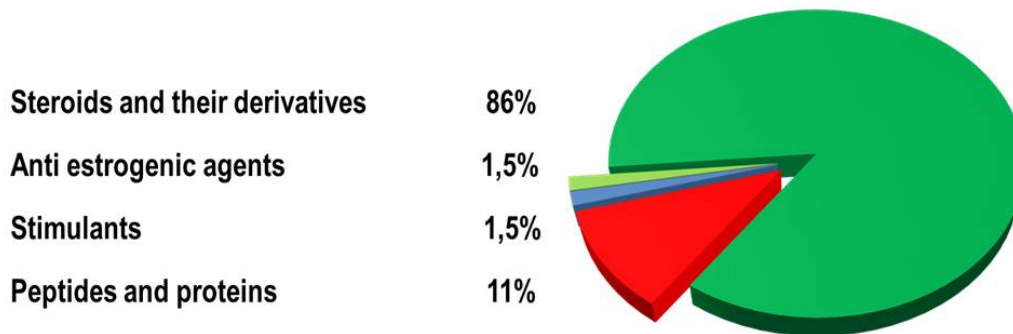


Fig.1: Apportionment of identified drugs in analyzed black market products

Analytes without doping relevancy are virilising and dermatologic agents, which shall eliminate the side effects of steroid applications like loss of libido and dermatologic irritations. An outstanding finding was the discovery of non-labeled 17-methyl-Testosterone in 21 cases in consideration of the health risk owing to its liver toxicity (Fig.2).

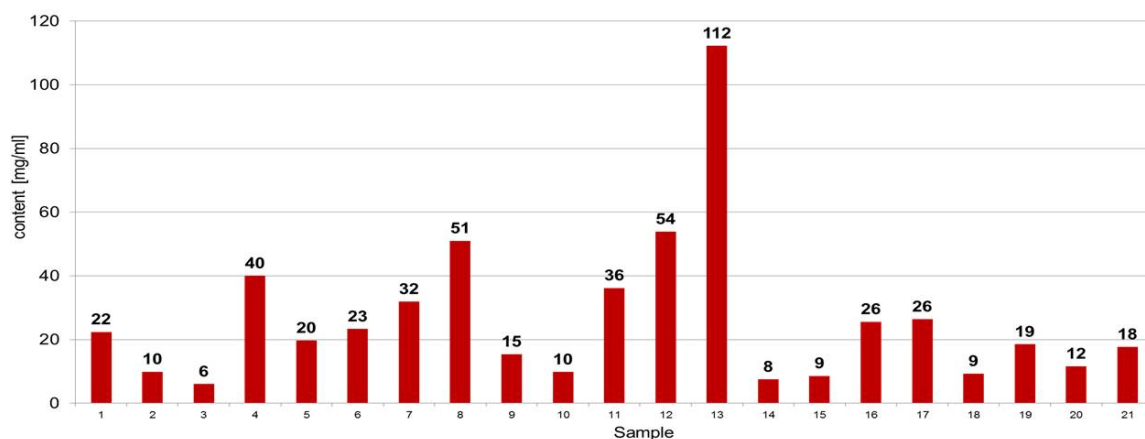


Fig.2: Amounts of undeclared methyltestosterone in oily solutions [3]

By means of LC-MS³ - and GC-QToF-HRMS-experiments the structure of two further steroidal derivatives, which were not known yet as relevant targets in doping analysis, could be identified. a: 5 α -androstano-(2,3-c)-furazan-17 β -tetrahydropyranol and b: 17-methyl-stenbolone [4] (Fig.3+4), which was characterized also by NMR.

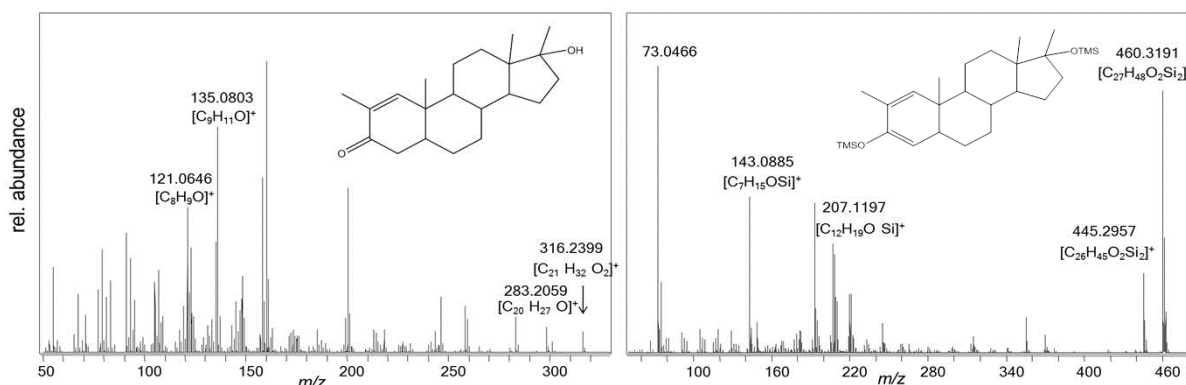


Fig.3: High resolution / high accuracy EI mass spectra of (a) 17-methylstenbolone at m/z 316.2397; (B) 17-methylstenbolone-bis-TMS at m/z 460.3191 from GC-Q-TOF

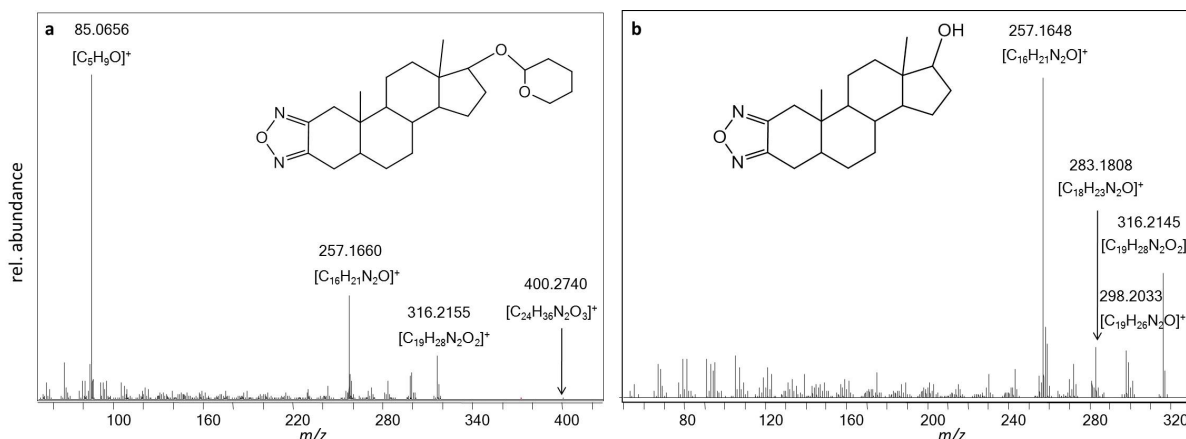


Fig.4: High resolution / high accuracy EI mass spectra of (a) 5α-androstano-(2,3-c)-furazan-17β-tetrahydropyranol at m/z 400.2740; (b, reference) 17-nor-furazabol at m/z 316.2145 obtained from GC-Q-TOF

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Acknowledgements

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