Current Role of LC-MS/MS in Sports Drug Testing

Chromatographic and Mass Spectrometric techniques have become an invaluable tool in the continuing fight against illegal doping in sport. The great majority of current assays employed in this field rely on the power of identification obtained from retention times and (product ion) mass spectra derived from hundreds of target analytes. The inventive nature of cheating athletes and the growing pool of drugs and therapeutics have necessitated the need for comprehensive, sensitive and specific detection methods and LC combined with MS has been shown to provide the necessary characteristic for the detection of low and high molecular weight compounds. Professor Mario Thevis here outlines the problems, challenges and successes in this continuing war against the cheats.

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Bernie Monaghan (BM): *Could you tell us a little about the group in which you work in Cologne and what are the aims and objectives of the group?*

Mario Thevis (MT): I am working at the Institute of Biochemisty and Center for Preventive Doping Research at the German Sport University in Cologne. Besides routine doping controls (approx. 13,000 samples/year), we develop and establish new doping control strategies and methods to enable the identification of emerging drugs before these compounds enter the pharmaceutical market. This should reduce the window of opportunity that cheating athletes' might have when abusing a new, probably not even approved drug.

BM: *Which Instruments do you have available to allow you to meet these objectives? Are there particular technological reasons for one manufactures equipment, maybe sensitivity or interface characteristics which make your job easier?*

MT: Our laboratory is equipped with various different LC and MS systems, all of which provide characteristics that are particularly useful for our work. The systems include regular (normal flow) liquid chromatography as well as micro- and nanoflow LC provided by Agilent, Thermo, and Waters. The mass spectrometers range from triple quadrupole, over quadrupole-linear ion trap, Orbitrap, to linear ion trap-Orbitrap systems to ensure robust target screening, quantitation, and allow general unknown analyses.

Figure 1. Agilent 1100 series HPLC coupled to Applied Biosystems API4000 Q Trap.

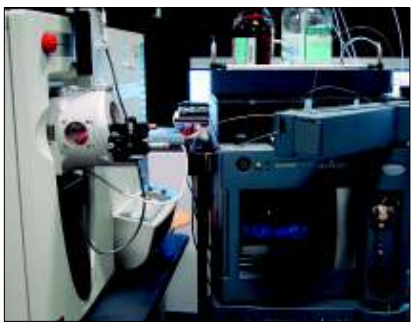

Figure 2. Waters Acquity nano UPLC with Thermo Fisher LTQ Orbitrap Mass Spectrometer.

Figure 3. Thermo Fisher Exactive MS and Accela LC with Advion Triversa Nanomate interface.

BM: *Which were the first types of drugs that were successfully detected using the LC-MS/MS type of detection*

MT: One of the first applications using LC-MS(/MS) in our laboratory was established concerning corticosteroids and those anabolic steroids that are hardly analysed by means of GC-MS due to thermal instability and formation of artefacts. The gain in sensitivity was enormous.

A little later, the issue of detecting peptide hormones in sports drug testing samples was addressed using modern LC-MS/MS systems, and we successfully established procedures to measure physiological levels of insulins and respective metabolites in plasma and urine. [1]

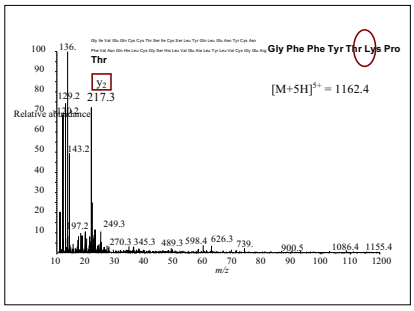

Figure 4. Mass spectral information on Humalog LisPro, a synthetic derivative of human insulin

BM: *'Designer Steroids' were a type of drug that had a very high public profile and sounded very glamorous and almost 'side effect free'. How did you go about designing assays for these? What are the side effects of these products on the human body?*

MT: Designer steroids have challenged the doping control community for several years, and most of them are hardly (if at all) clinically or pharmacologically tested; hence, undesired effects are likely and presumably very dangerous, but that did not stop athletes from taking these products.

There are several options to approach the problem of unknown, modified steroidal agents, and the one including mass spectrometry focuses on conserved steroid nuclei. Those generate common product ions under ESI/CID conditions, and screening for steroid-typical fragments allows to 'profile' urine samples, for example, if unusual signals are found further studies on the analyte behind are required to prove whether it is a new steroid derivative or not. [2]

BM: *Athletes are frequently led by their coach/trainers in to taking the performance enhancing drugs in cocktails. These cases must be difficult to analyse. Are their any high profile success stories where you have identified these cocktails?*

MT: The most prominent case of designer steroid detection was probably the so-called BALCO affair. The compound tetrahydrogestrinone (THG) was discovered and identified by the doping control laboratory in Los Angeles, CA, which was provided with a syringe containing the, at that time unknown, substance THG. [3] Based on the knowledge that this compound exists, detection assays were adjusted and numerous elite athletes were convicted of having used the designer steroid.

Another finding was remarkable and concerned the steroid methyltrienolone (or methyltrenbolone), a drug that was developed in the 1960s and immediately discontinued due to severe side effects, particularly liver toxicity. It was never approved, neither for veterinary nor for human use; however, it was detected in 11 elite weight lifters' urine samples in 2008 and further to that in an Olympic doping control sample in Beijing. [4]

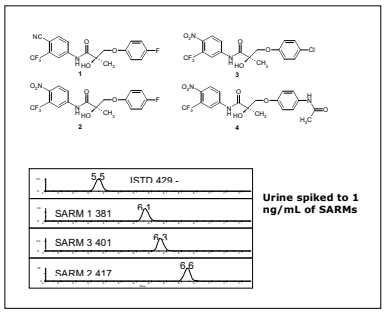

Figure 5. Model aryl-propionamide derived SARMs

BM: *What are the next generation drugs that the cheats are using and are you already able to tackle this trend?*

MT: There are numerous new drug candidates that possess potential for misuse in sports. These include for instance so-called selective androgen receptor modulators (SARMs), which could be referred to as the anabolic agents of the future. Although structurally different from steroids, as can be seen in *Figure 4*, they selectively stimulate the steroid receptor and have several beneficial effects for the ageing person; in addition, they are certainly drugs of interest for cheating athletes as a gain in muscle mass and performance is very likely. Other therapeutics developed to treat the metabolic syndrome such as GW1516 might be misused in sports due to their ability to stimulate fat utilisation and to mimic exercise on a genetic level. In both cases, methods to detect these drugs and/or metabolites have been established and are further optimised to ensure utmost retrospective. [5]

BM: *There appears to be room for much more research into methods of testing and screening. What are you currently working on?*

MT: We have focused our work on various new classes of compounds such as releasing hormones, for example, luteinizing hormone releasing hormone, growth hormone releasing hormone), hypoxia-inducible factor (HIF)-stabilisers, and drugs stimulating mitochondrial biogenesis (e.g., AICAR). These include the peptide hormones LH-RH (or gonadorelin) 6 and Geref (sermorelin), the prolyl hydroxylase inhibitor FG-2216, and the endogenous compound AICAR.5 LH-RH triggers the production and release of endogenous testosterone and might increase plasma levels of testosterone that a) increases athletic performance, and b) interferes with detection assays developed to test for administered synthetic testosterone. The analysis of LH-RH in urine is shown in *Figure 5*. Geref stimulates the secretion of human growth hormone, which is also desirable for athletes, FG-2216 mimics hypoxia and causes an increased production of erythropoietin with subsequently increased amounts of erythrocytes, and AICAR was shown to improve endurance in laboratory rodents due to the elevated amount of mitochondria in skeletal muscle tissue.

BM: *How do you see the spectroscopic methods of detection advancing in, say, the next 3 – 5 years? What room for improvement is there still to be made in the hyphenated sequence you have used? Is the sensitivity and ruggedness of your methods enough to keep you ahead of the cheats?*

MT: Chromatographic-mass spectrometric methods will still be the most important tool in doping control laboratories but might be complemented by other techniques. With the increasing number of analytes, faster analyses without loss of sensitivity and specificity would be desirable, and the instrumental developments seem to support these needs with ultrahigh performance LC and constantly reduced duty cycle times of modern mass spectrometers. This should allow us to efficiently conduct our research and routine doping controls.

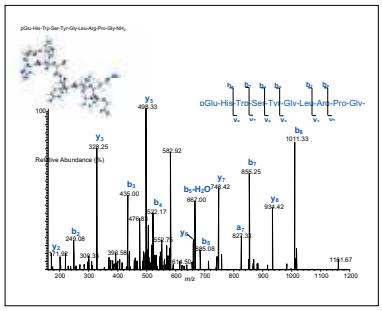

Figure 6. Gonadorelin (LH-RH) and its analysis from urine (3 pg/mL).

ABOUT THE AUTHOR

Mario Thevis graduated in Chemistry (University of Technology Aachen, Germany) and Sports Science (German Sport University Cologne, Germany) in 1998. He earned his PhD in Biochemistry in 2001 at the German Sport University Cologne with special focus on the chemical synthesis and characterisation of steroid glucuronide conjugates using GC-MS/MS, LC-MS/MS and NMR. In the same year, He received the MANFRED-DONIKE AWARD for excellence in anti-doping research. In 2002, he did post-doctoral research at the Department of Chemistry and Biochemistry of the University of California Los Angeles (UCLA) in the group of Joseph A Loo working on chemical modifications of intact proteins and their identification using proteomics strategies and high resolution/high accuracy mass spectrometry. Between 2003 and 2005 he was employed as research scientist at the Institute of Biochemistry (German Sport University Cologne), and since February 2006 he is Professor for Preventive Doping Research at the German Sport University Cologne. Current primary fields of research include method development for the detection of new compounds relevant for sports drug testing, mass spectrometric characterisation of low molecular weight drugs, interpretation of dissociation routes of small molecules using different ionisation and fragmentation techniques, and determination of peptides and proteins in doping controls with high resolution / high accuracy mass spectrometry.

Being a board member of RAPID COMMUNICATIONS IN MASS SPECTROMETRY and CURRENT PROTEOMICS since 2006, He was assigned Editor-in-Chief of the Journal DRUG TESTING & ANALYSIS. He supported the doping control laboratories in Athens, Torino, and Beijing during the Olympic Games in 2004, 2006, and 2008 and has been active as advisor for numerous international anti-doping organisations.

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