Mass Spectrometry in Sports Drug Testing

Detection of New Anabolic and Endurance Enhancing Agents

Liquid chromatography interfaced to tandem mass spectrometry (LC-MS/MS) has become an important tool in modern doping control analysis [1]. The flexible and robust nature of LC-MS/MS systems is used to determine known drugs and, more recently, also for emerging therapeutics [2]. Detection assays for new anabolic agents (selective androgen receptor modulators) [3] and endurance-enhancing compounds (benzothiazepines) [4] not having received clinical approval, were established for preventive sports drug testing.

Selective Androgen Receptor Modulators (SARMs)

The class of selective androgen receptor modulators comprises new anabolic agents that have demonstrated great potential for various clinical purposes such as the treatment and prevention of osteoporosis, frailty, muscle wasting, etc. Due to structures independent from anabolic androgenic steroids, SARMs are tissue-selective and have shown much less of those undesirable effects that are commonly associated with steroid replacement therapies. However, their therapeutic benefits may tempt cheating athletes to misuse these agents to illegally and artificially increase their performance, which urged the World Anti-Doping Agency (WADA) to add SARMs to the list of prohibited compounds in 2008. First approaches to screen for representatives, the chemical structures of which are of considerable diversity (fig. 1), were reported in 2006 and have been expanded ever since. Depending on the physicochemical properties, SARMs are isolated from urine specimens either by solid-phase extraction (SPE) or liquid-liquid extraction (LLE) as routinely employed for sample preparation purposes in sports drug testing. Samples are subsequently analyzed on LC-MS/MS systems that are programmed to sensitively measure the active drugs as well as metabolites that were identified in in vitro metabolism studies. These assays require utmost comprehensiveness to cover also unknown analogues as designer derivatives might be introduced via the black market also. Consequently, various mass spectrometric studies on common dissociation routes of
typical SARMs were conducted, and mass analyzers are operated in specific multiple reaction monitoring (MRM) and, simultaneously, more general precursor ion scan modes.

The latter is focused on conserved product ions indicating molecules with a core structure closely related to particular SARM drugs. In figure 2, the product ion mass spectrum of a typical 2-quinolinone-based SARM (LGD-2226) is depicted, which contains several characteristic product ions for instance at m/z 306 and 241. These are employed for the specific detection of LGD-2226 in urine samples as well as for precursor ion scan experiments allowing for the determination of 1 and 50 ng/mL of urine, respectively. Although drugs such as LGD-2226 have not received clinical approval, drug testing authorities are alerted due to the considerable misuse potential resulting from the stimulating effect on muscle growth and corresponding strength.

**Benzothiazepine-derived Drugs**

Recent studies on cardiac arrhythmia outlined the importance of the ryanodine receptor-based Ca\(^{2+}\) channel and the affinity to calstabin1, which was found impaired in cardiac arrhythmia patients. New drug candidates based on a benzothiazepine structure such as S-107 and JTV-519 (fig. 3), which correct the underlying altered function of Ca\(^{2+}\) channels and, thus, local subcellular Ca\(^{2+}\) release events ("leaking"), also demonstrated to increase skeletal muscle endurance in laboratory rodents. This is due to the fact that both phenomena, skeletal muscle fatigue and cardiac arrhythmia, result from leaky Ca\(^{2+}\) channels caused by progressive modification including hyperphosphorylation and S-nitrosylation. The administration of compounds such as S-107 and JTV-519 improves the affinity of calstabin1 to the ryanodine receptor and, thus, the closed state of the Ca\(^{2+}\) channel. In proof-of-concept studies, mice demonstrated significantly improved
endurance capacities, which raise concerns in the drug testing community that these agents might also be misused in elite sport. Also here, preventive doping control approaches were taken and methods to detect the active drugs as well as putative metabolites were established. First, the compounds of interest were synthesized and studied using state-of-the-art mass spectrometric methods to identify compound-specific product ions and conserved nuclei that potentially enable comprehensive detection assays. In figure 4, the product ion mass spectra of JTV-519 and S-107 are illustrated, which contain abundant product ions at m/z 188 (C_{13}H_{18}N, attributed to the 4-benzyl-1-methylene piperidine residue) and m/z 153 (C_{8}H_{9}OS, suggestewd to result from the loss of 1-methyl-aziridine), which are considered highly characteristic for the particular structures. Using MRM and precursor ion scan experiments, the active drugs and related compounds can be measured in doping control specimens and, thus, allow the determination of the prevalence of these agents. Also here, clinical approval is not obtained but the simple nature of the drug candidates and comparably easy route of synthesis would allow an illegal preparation and distribution to cheating athletes, which necessitates an early implementation of these potentially misused compounds into doping control assays.

The early (and hence preventive) incorporation of new, emerging drugs that potentially increase athletic performance into sports drug testing procedures supports the efforts of doping control authorities to minimize pharmaceutical options of cheating sportsmen. Although based on assumptions that either the active drug or a metabolite with conserved core structure is eliminated into the urine, the use of LC-MS/MS for preventive doping research has demonstrated great utility in modern doping controls and allows for the comprehensive detection of known as well as unknown substances.

References
