PROJECT REVIEW

"Development of a universal screening procedure for acidic, neutral and basic doping agents in urine."

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The appearance of new abused molecules (e.g. HES, THG, adrafinil, modafinil) has increased the number of substances on the list and has given to the doping control laboratories a big challenge to keep their analytical procedures updated. Occasionally totally new analytical methods should be designed which is time consuming and may also require additional, expensive instrumentation or reagents. Furthermore, the increase in the number of separate analytical procedures renders the laboratory analysis more complex, delays reporting, increases the workload, and raises the cost of one test. Inclusion of new drugs and their metabolites in screening procedures is sometimes slow or impossible due to the lack of reference substances.

For a long time, the analytics has mainly been based on different gas chromatography – mass spectrometric techniques However, recently, the excellent suitability of liquid chromatography – mass spectrometry (LC/MS) has been demonstrated for multi-analyte screening of many classes of prohibited substances (e.g. anabolic steroids, beta-adrenergic drugs, diuretics and glucocorticosteroids). Actually, many new compounds added to the list of banned substances can be effectively screened only by LC/MS. In theory, LC/MS would have capabilities "almost for an all-in-one screening procedure". Unfortunately, at the moment, this approach is greatly restricted by the use of non-universal sample preparation procedures and by the use of scanning-type of mass spectrometers.

Recently, a novel toxicological screening method for urine samples based on liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) has been established. In the method, acidic, neutral, and basic drugs are extracted in urine and analyzed by LC/TOFMS with positive-ion ionspray and continuous accurate mass measurement. The method has been used effectively for screening of several different drugs, metabolites, and pesticides.

In this project a general LC/TOFMS-based screening method will be developed and its effectiveness will be evaluated for several chemically and pharmacologically different doping agents. Compounds will be identified based on their monoisotopic masses and retention times. A substance database containing accurate masses and retention behaviour of the prohibited drugs will be built up and will be accessible without charge for all WADA/IOC accredited doping control laboratories. The method will be validated with respect to limit of detection,

repeatability, extraction recovery and specificity. To investigate the efficiency of the method for formula-based metabolite identification, excretion studies with different drugs will be carried out.

Results and Conclusions

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The demanding task of the anti-doping laboratories is to detect substances on the prohibited list of World Anti-Doping Agency (WADA).The constant appearance of new prohibited substances challenges the laboratories to update their methods. Today, doping analysis requires the use of several different chromatographic and mass spectrometric methods. Consequently, a large number of separate analytical methods results in a more complex, time-consuming and laborious screening strategy.

The aim of our research was to develop and validate a general liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) –based screening method for several chemically and pharmacologically different doping agents, in order to reduce the number of separate screening procedures used at present in antidoping laboratories. Included in the study were the following classes of prohibited subtances: agents with anti-estrogenic activity, aromatase inhibitors, cannabinoids, beta-blockers, beta-2-adrenergic agonists, diuretics, narcotics, and stimulants. The project began on March 2005 and terminated in September 2007.

The method consisted of enzymatic hydrolysis of urine samples, solid phase extraction, separation of compounds on a reversed phase column and continuous accurate mass measurement by positive-ion ionspray TOFMS. Compounds were identified based on their monoisotopic masses, isotopic patterns and retention times.

The method was validated with 124 different substances. The minimum required performance limit (MRPL) established by WADA was attained to 97 substance. The maximum mass error of the method was 0.7 mDa. Extraction recoveries varied between 33 and 98 %. Repeatability of the method for spiked urine samples (median of relative standard deviations at concentrations of MRPL and 10 times MRPL) were 14% and 9%, respectively. The method exhibited high specificity and proved to be suitable also for formula-based metabolite identification enabling preliminary identification of new drugs and their metabolites even without reference substances. A substance database containing chromatographic and mass spectrometric behaviour of all investigated substances was built up and will be accessible for all anti-doping laboratories for their method development.

The developed LC/TOFMS method allowed identification of chemically and pharmacologically different drugs in urine in the same run and has great potential in doping analysis and simplifies analytical screening strategies in anti-doping laboratories.