"Simplifying and expanding the screening for peptides < 2 kDa by means of liquid chromatography mass spectrometry"

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Project Overview

The number of samples and target analytes is constantly increasing, necessitating all analytical methods to be optimized with regards to speed and costs effectiveness to follow the challenges of the modern anti-doping fight. Low molecular mass peptidic drugs (< 2kDa) have become more and more prominent on the Internet and in black markets, and their misuse in elite sport has been proven in the past.

The analysis of these new compounds is possible and methods are installed in the accredited anti-doping laboratories worldwide. Unfortunately, these methods are largely designed to cover small peptidic drugs only in exclusive categories or after dedicated sample preparation as shown for instance for growth hormone releasing peptides, gonadorelins, desmopressin, TB-500, AOD-9604 and ARA-290. This is mainly due to the required sensitivity of these methods that enables the detection of the drugs in very low concentrations (sub-ng/mL). Within this study, a generic sample preparation strategy (dilute and inject) which is valid for several classes of compounds (diuretics, stimulants, anabolic agents etc.), will be evaluated for the detection of low molecular mass peptide hormones in urine even in the sub-ng/mL-levels. Therefore, several types of mass spectrometers and liquid chromatography methodologies will be assessed to find the best option for the aimed combination. This will enable a comprehensive multi-target screening within one analysis.

Results and Conclusions:

The number of small peptides prohibited in sports is steadily increasing, as well as the number of samples that have to be analyzed in sports drug testing programs. Consequently, fast, comprehensive, sensitive, and reliable new methods are needed to accordingly cover all these challenges. "Dilute-and-inject" approaches for urine analysis by liquid chromatography/mass spectrometry represent the most simplified way with a minimum of sample preparation workload. But due to the missing pre-concentration and purification steps, sophisticated chromatographic and mass spectrometric conditions are required to enable sensitive initial testing procedures. All of these aspects were taken into consideration when developing this new screening procedure by direct urine injection with online pre-concentration using a trapping column, liquid chromatographic separation, and subsequent detection by means of ion mobility coupled to high resolution mass spectrometry.

Additionally, the in-vitro metabolism of selected model peptides such as ARA-290 and GHRP-3 was evaluated and new metabolites were identified by using different strategies as for example incubation with plasma, serum, human liver microsomes, and specific enzymes (e.g. amidases).