"Expanding the testing capability of immunopurification assisted analytical methods for peptides > 2kDa by means of mass spectrometry"

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Project Overview

A variety of prohibited peptide hormones potentially enhancing athletic performance is still lacking sufficient detection methods in accredited anti-doping laboratories. This is mainly due to a yet missing adequate analytical methodology, which is crucial for detection of these very efficient (and therefore low-dosed) therapeutics and drug candidates in biological fluids (urine / blood).

Examples for new peptide-based compounds, which are explicitly mentioned on the Prohibited List, are Corticorelin (CRH) and Mechano Growth Factors (MGFs). Other peptidic compounds (e.g. Thymosin beta-4) share the same status, but are not named on the list so far although their misuse has been reported. They all are available as approved drugs (CRH) or at least subject of different clinical trials (Thymosin beta-4, MGF with different analogues).
Essential requirement for the mass spectrometric detection is the appropriate extraction of the target peptides from biological matrices to meet the required limits of detection in the low pg/mL range. This will be achieved by applying the established coated magnetic beads technology (which has been successfully applied to other peptide hormones such as insulin analogues, CJC-1295, Tesamorelin, Geref, Synacthen, etc.) with respective antibodies. The identification of isolated and enriched analytes will be realized after liquid chromatographic separation by means of high resolution mass spectrometry. Due to the unknown metabolic fate of the peptides after administration to humans, in-vitro experiments are planned, which will help to characterize amino acid sequences serving as additional analytical targets in routine doping controls. The method will be validated and the implementation into existing screening procedures (if possible) for peptides will be realized.

Results and Conclusions:

While the analysis of small prohibited peptides < 2 kDa is well established in doping control laboratories worldwide, the detection of peptides > 2 kDa still remains challenging due to low analyte concentrations, unstable target peptides, an unknown metabolism and a complex sample preparation. However, recent developments in analytical chemistry can help to overcome these issues and facilitate the development of mass spectrometric initial testing procedures for large peptide hormones.

The aim of this research project was to optimize current methodologies for the detection of peptide-based drugs > 2 kDa. For that purpose, new

metabolites were identified by using in-vitro experiments and the sample preparation procedure was additionally modified in order to implement further prohibited peptides. For several IGF-I and MGF analogues as well as CRH, relevant metabolites were identified, comprehensively characterized, and valid analytical procedures were developed. All metabolites comprised N- or C-terminal truncated amino acid sequences and resulted from exoproteolytic processes. In principle, the developed assays are applicable to both urine and blood (plasma) specimens, but as the renal clearance of some peptides/metabolites remains to be elucidated, blood represents the preferred biological matrix.