PROJECT REVIEW

Development of a screening tool for peptide hormones using immunoaffinity purification and LC-MS/MS

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This project comprises a combination of the analysis of different prohibited peptide hormones (for example, but not limited to: synthetic insulin analogues, synacthen, gonadorelin, synthetic IGF analogues) in plasma and/or urine samples. Former studies provided information about the expected concentrations that were estimated in low fmol/mL range, their chromatographic separation and their mass spectrometric characterisation. Thus, an efficient and highly specific sample preparation procedure with nanotechnology-based immunoaffinity purification employing coated magnetic beads and appropriate primary antibodies will be developed. The subsequent determination and identification of peptides will be performed by means of liquid chromatography – tandem mass spectrometry.

The major goal of the planned project is the simultaneous purification and determination of a variety of structurally and physiologically different bioactive peptide hormones from biological fluids, and the installation of a comprehensive screening tool dedicated to the detection of prohibited compounds with peptide-based structure using mass spectrometric approaches is aimed. The assay shall utilize commercially available reagents and equipment only in order to allow a rapid and facile transfer to other anti-doping laboratories, and detailed instructions and protocols shall enable a fast implementation of a complementary and highly specific screening tool in sports drug testing laboratories.

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Results and Conclusions

Bioactive peptides such as insulins, the synthetic adrenocorticotrophic hormone analogue Synacthen, Gonadorelin (LHRH), Growth Hormone Releasing Hormones (GHRHs) and insulin-like growth factors (IGF-1 and derivatives) provide a reasonable potential for the misuse as performance enhancing agents and are prohibited in elite sports according to the list of banned substances established by the World Anti-Doping Agency. Currently, the determination of these analytes is possible by dedicated assays only or methods are even not at hand so far.

In the present project, a procedure to determine several prohibited peptides, which are excreted into urine (e.g. Gonadorelin, Human insulin, Humalog (Insulin Lispro), Apidra (Insulin Glulisine), Novolog (Insulin Aspart), Lantus (Insulin Glargine), Porcine Insulin, Synacthen, IGF-1, longR^3-IGF-1, Geref and CJC-1295), was developed. The method enables the effective, highly sensitive and specific screening for several different target analytes that are simultaneously purified and analysed by means of immunoaffinity purification, subsequent liquid-chromatographic separation and high resolution / high accuracy mass spectrometric determination at low pg/mL concentrations. Central aspect of the approach is the combination of immunoaffinity purification with mass spectrometry. Employing different specific antibodies coupled to paramagnetic beads, the simultaneous isolation of all targets in one sample preparation procedure was accomplished. In general, the approach is extendable to any banned peptide if adequate antibodies are available. At the present status of the project the above mentioned analytes (11 prohibited peptides, 5 internal standards) are covered and the method is fully validated under consideration of qualitative result interpretation.