PROJECT REVIEW

"Synthesis of peptide hormone metabolites for inclusion LC-MS/MS detection methods"

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The detection of the misuse of these peptides is challenging due to a rapid elimination and consequently low concentrations in body fluids. Despite the analytical challenge, screening procedures have already been established, targeting numerous GHRPs in urine, employing liquid chromatographic/mass spectrometric detection. Thus, the focus has mainly been on the detection of the parent compounds. The additional incorporation of metabolites in routine detection methods would improve detectability and furthermore increase the detection window of the peptides. For most of these compounds, no or only limited pharmacokinetic data are available on human excretion. Moreover, little is known about the toxicology of the non-approved compounds and human studies are challenged by ethical considerations. Nevertheless, animal in vivo studies, in addition to in vitro simulations of metabolic reactions, have identified metabolites which represent promising targets in doping control analysis. Unfortunately, the majority of these metabolites are not commercially available.

Thus, in the current project, we plan to synthesize six proposed human metabolites of different small peptide hormones, purify and structurally characterize them by high resolution high accuracy mass spectrometry (Orbitrap FT-MS/MS; e.g. Q Exactive). Furthermore, the synthesized metabolites will be incorporated in our peptide LC-MS/MS protocol in order to proof their detectability in a single run and together with their parent compounds as well as other prohibited small peptides. Since such peptide hormones are commonly administered in low doses and additionally show extensive metabolic degradation, well-characterized reference standards of targeted metabolites are of utmost importance for a sensitive and specific detection of this group of doping agents.

Results and Conclusions:

In this study, eight proposed peptide metabolites were successfully synthesized employing microwave peptide synthesis, including Fmoc-based peptide synthesis methods. Subsequently, the purified peptides were characterized by high-resolution high-accuracy mass spectrometry. The peptide structures were confirmed by the accurate peptide mass (mass error ± 0 ppm - 0.7 ppm), in addition to structural information from MS-MS analysis.

Metabolites synthesized : GHRP M1, M3, M4, M5, alexamorelin M1 to M3 and leuprolide M1