

***“Evaluation of different off-line immunocapture techniques for efficient purification of large peptides in human urine”***

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**Project overview**

Recently a wide variety of peptide hormones were included in the World Anti-Doping Agency List of prohibited substances and methods. The analytical approach of choice to detect these substances in biological fluids is most of the times linked to (i) the matrix selected, (ii) the concentration of both the analyte of interest and the interferences, (iii) the natural occurrence in human body and finally (iv) the molecular weight of the compound under investigation. Concerning, specifically, those peptide hormones with a molecular weight in the interval of 2000-10000 Da (i.e. insulins, GHRHs, synacthen or IGF-1 and its analogues), they are excreted in urine, either as parent compounds and/or as metabolites/degradation products, in very low levels.

Different analytical approaches have already been developed and published for the detection of these agents in doping control specimens (plasma/serum or urine samples) employing immunoaffinity purification and liquid chromatographic-mass spectrometric-based techniques. The sample pre-treatment protocols proposed in literature are mainly based on ultrafiltration followed by the use of immunoaffinity purification: magnetic beads, immune-affinity chromatography or antibody pre-coated ELISA plates. Although better recoveries were achieved by using immune-affinity chromatography (>70%), the strategies currently adopted by the anti-doping laboratories are based on the use of magnetic beads (<30%) or of antibody pre-coated ELISA plate, mainly due to their simpler protocols and to the possibility to fully automate.

This project is focused on the evaluation of alternative off-line immunocapture techniques, such as for example, pre-coated pipette tips or spin trap column with the aim to improve the efficacy, repeatability and robustness of the protocols currently used by the anti-doping laboratories to extract large peptides from biological fluids.