"Simplifying the sample preparation for routine IRMS analysis using immunoaffinity purification"

Project Overview

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Testosterone misuse still remains a challenging task for doping control laboratories as this steroid is produced naturally by each and everybody. As it is detectable in all urine samples, concentration-based thresholds have been established to uncover testosterone administration. As soon as these thresholds are exceeded, samples are forwarded to isotope ratio mass spectrometry determinations (IRMS) to elucidate the steroid´s source.

These IRMS determinations require highly purified analytes, which results in high requirements concerning sample preparation and necessitates time-consuming clean-up steps using high performance liquid chromatography. Immunoaffinity chromatography relies on gels produced from (polyclonal) antibodies that are specific against individual analytes, i.e. ideally only this particular analyte is retained on the gel and can be recovered. For testosterone, several gels are commercially available and one of these was successfully tested regarding its potential to support the clean-up of urine samples for IRMS analyses.

Within this project gels will be produced specific against other steroids relevant for IRMS in sports drug testing that are currently not available. These gels will be tested and used to develop a simplified and much faster clean-up procedure for IRMS analysis of doping control samples found beyond the established thresholds. Particular attention will be paid to fulfill all recent WADA requirements for IRMS.

Results and Conclusions:

Detecting the administration of naturally occurring but synthetically derived steroids (e.g. testosterone) in routine doping controls is particularly laborious and time-consuming. Carbon isotope signatures determined by isotope ratio mass spectrometry (IRMS) have been established as the method of choice to generate confirmatory evidence in case of suspicious or atypical findings in steroid profile analyses; however, IRMS measurements require sophisticated sample preparation methods employing up to two HPLC (high performance liquid chromatography) purification steps.

Here, an alternative sample preparation approach is presented. Immunoaffinity chromatography (IAC) was employed to reduce the batch analysis time by omitting the time-consuming HPLC purification steps, while pre- and post-IAC sample handling followed published protocols. IAC exploits specific antibody-immunogen interactions, and the option of combining three immunoaffinity gels containing specific antibodies for testosterone, pregnanediol and 11-ketoetiocholanolone into a

multi-immunoaffinity sample preparation approach was assessed. Due to cross reactivities, also etiocholanolone, androsterone, 5β-androstanediol and 5α-androstanediol were co-extracted and included in the testing protocol.

The method was validated by determining precision, recovery, and carry over, and performing linear mixing models. IAC was found to be applicable to the determination of carbon isotope ratios in doping controls and the approach allowed for an accelerated sample preparation.

Publications:

- Putz M, Piper T, Dubois M, Delahaut P, Thevis M. Analysis of endogenous steroids in urine by means of multi-immunoaffinity chromatography and isotope ratio mass spectrometry for sports drug testing. *Anal Bioanal Chem* 2019. https://doi.org/10.1007/s00216-019-02169-3.