"On-line multidimensional GC as clean-up step for IRMS and quadrupole MS measurements of endogenous anabolic steroids in urine"

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Project Overview:

Urine, as a biological waste material, is an extremely complex fluid counting thousands of components belonging to more than 200 different chemical classes. It is conventionally adopted for investigating the endogenous steroids profile in athletes to prevent doping. Isotopic Ratio Mass Spectrometry (IRMS) is the technique able to distinguish the source of the steroid via carbon isotopic measurements (13C/12C), differentiating the exogenous from the endogenous ones. Reliable IRMS determinations strongly depend on adequate purity of the investigated steroids. This demand is guaranteed by labor-intensive and time-consuming preliminary steps (i.e. sample preparation, derivatization, liquid chromatography fractionation).
Multidimensional gas chromatography (MDGC) is a consolidated technique for the separation of complex matrices as well as the investigation of target compounds. In such approach, two columns are arranged in a series (e.g. non-polar stationary phase in the first dimension, followed by a polar stationary phase in the second dimension). The principle is to select the peak of interest in the first dimension and send it (heart-cut) into the second - ideally orthogonal - dimension for further separation.

This project aims to develop, a multidimensional method before the IRMS measurement to speed-up the sample preparation and increase the automation of the process. The clean-up conventionally obtained by time-consuming analytical steps (e.g. liquid chromatography fractionations) is replaced by two GC columns. Additionally, a quadrupole mass spectrometer, which detect simultaneously with the IRMS, guarantees spectra quality confirmation of the peak delta values measurement.

Further planned activities comprise to carefully validate the method to be compliant with the WADA requirements. Special attention will be dedicated to the evaluation of the LOQ and robustness of the method. Finally, the potential of a MDGC-MS/C/IRMS method for measurements of Boldenone, Boldenone M1, 19-Norandrosterone and Formestane will be investigated.

Results and Conclusions:

Distinguishing endogenous anabolic steroids from their synthetic copies in urine samples from athletes requires a specific analysis by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS, IRMS). Such analysis is mandatory to be implemented in a doping control laboratory, since many of the adverse analytical findings involve endogenous steroids whose administration cannot be characterized by other techniques.

In order to obtain suitable and consistent results by IRMS, a laborious and time-consuming sample treatment is required due to both the natural presence of the target analytes and the high complexity of the matrix, ensuring the convenient purification of the steroids prior to the analysis. Many steps are involved, including one or more preparative high-performance liquid chromatography (HPLC) to isolate the steroids from matrix interference. Besides a long time, preparative HPLC necessitates many sample transfer steps, which represents potential losses of the amount of steroids to be analyzed and possible contribution to the measurement uncertainties.

In order to accelerate the sample preparation and increase the automation of the process, the use of multidimensional gas chromatography (MDGC) prior to IRMS experiments has been investigated as an alternative to preparative HPLC. After preliminary studies already published, a full validation was performed in two doping control laboratories accredited by the World Anti-Doping Agency (WADA) to conclude about the advantages in replacing preparative HPLC with MDGC in the routine confirmatory analysis. A well-established instrumental configuration based on two independent GC ovens and one heart-cutting device was used. The first dimension (^{1}D) separation was obtained by a non-polar column which assured high efficiency and good loading capacity, while the second dimension (^{2}D), based on a mid-polar stationary phase, provided good selectivity. The assembled MDGC set-up was applied for measuring testosterone, 5α- and 5β-androstanediol, androsterone and etiocholanolone as target compounds, and pregnanediol, 11-ketoetiocholanolone and 16-androstenol as endogenous reference compounds. One additional solid phase extraction was included at the end of the conventional sample treatment already implemented in the two laboratories, at which two fractions were obtained to be analyzed, minimizing matrix effects and column overloads. Following WADA regulations, the experiments comprised linearity of the instrument, repeatability, linear mixing models, method performance assessment and limit of quantification.