

PROJECT REVIEW

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

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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 ($^{13}\text{C}/^{12}\text{C}$), 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 projects 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.