

## ***“Profiling of minor metabolites and confirmation by IRMS”***

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### **Project Overview**

Because of the advances in analytical methodology, it has become possible to screen for compounds in complex biological matrices at very low concentrations. As a result, during the last decade, the role of minor steroid metabolites has gained importance in steroid detection due to their more specific nature. In this light, our group developed an analytical screening method that encompasses minor androgenic steroid metabolites. Some of these minor steroid metabolites are promising new biomarkers in steroid profiling and can contribute substantially to the evidence of misuse with various endogenous steroids. The endogenous concentrations of these minor metabolites are very low and a strong increase in concentration is obtained when anabolic steroids are administered. This makes these minor metabolites, theoretically, excellent compounds to confirm on IRMS because the endogenous dilution is very small. During this project, a method will be developed that is able to analyze some of these minor metabolites on IRMS. Urinary concentrations beneath a certain concentration threshold are considered as endogenous, urine samples with a higher concentration are suspicious and will be forwarded to IRMS for confirmation. This approach is complementary to the present classic markers (of which the T/E ratio is the most important one) and will reduce the number of false negatives on two levels. It has been shown in the past that the T/E ratio does not always increase with the administration of testosterone. In such cases, minor metabolites might trigger an IRMS confirmation, whereas the classic markers do not. On a second level, elevated values of classic markers might still lead to a negative IRMS result if the endogenous dilution is too big. For minor metabolites this effect can almost be neglected.

### **Result and Conclusion:**

#### Screening:

In most excretion studies, the minor metabolites had shorter detection times than at least one of the traditional screening markers that can trigger on IRMS confirmation (an elevated T/E ratio, elevated concentration of androsterone, etiocholanolone,...). This means that all samples that had elevated concentrations for a minor metabolite, would have been labelled 'suspicious' and forwarded to IRMS anyway because at least one other marker had a suspicious value. Nevertheless, when dealing with steroid profiles, one must always keep the large inter-individual differences in mind, meaning that this observation is not necessarily applicable for every individual. In the past, our lab received a sample with perfectly normal values for the traditional steroid profile parameters according to population based reference intervals. However, the 6 $\alpha$ OH-ADION concentration was elevated, leading to an IRMS confirmation and consequently an adverse analytical finding was revealed. Based on the traditional parameters alone

there would not have been an IRMS confirmation, illustrating the usefulness of monitoring 6 $\alpha$ OH-ADION in the steroid profile.

#### IRMS analysis

In general, the IRMS detection time of the minor metabolites does not seem to outperform the traditional IRMS target compounds. There is a sharp concentration increase of minor metabolites after administration, but the concentrations also drop again relatively fast and this neutralizes the advantage of lower endogenous dilution. As a result, the traditional IRMS target compounds have a longer window in which their  $\delta^{13}\text{C}$  values remain in the exogenous range.

#### Publications:

- **M. Polet**, P. Van Renterghem, W. Van Gansbeke, P. Van Eenoo Profiling of urinary formestane and confirmation by isotope ratio mass spectrometry. *Steroids*, 78, 11 (2013), p 1103-9.
- **M. Polet**, P. Van Renterghem, W. Van Gansbeke, P. Van Eenoo. Studies on the minor metabolite 6 $\alpha$ -hydroxy-androstenedione for doping control purposes and its contribution to the steroid profile. *Drug Testing and Analysis*, 6, 10 (2014), p 978-84.

#### Presentation of data:

- Profiling of urinary formestane and confirmation by isotope ratio mass spectrometry. **M. Polet**, W. Van Gansbeke, P. Van Renterghem and P. Van Eenoo. 31<sup>th</sup> Cologne workshop on Dope Analysis 24/02/2013 – 01/03/2013
- Studies on the minor metabolite 6 $\alpha$ -hydroxy-androstenedione for doping control purposes and its contribution to the steroid profile. **M. Polet**, P. Van Renterghem, W. Van Gansbeke and P. Van Eenoo 32<sup>nd</sup> Cologne workshop on Dope Analysis 30/03/2014 – 04/04/2014.