

PROJECT REVIEW

“Analysis of 19-Norsteroids, Testosterone and Precursors Metabolites in Human Urine”

Detecting the use of androgenic anabolic steroids, potentially endogenous in humans, and which are prohibited substances in sport doping control programmes, still represents a major challenge to the analysts. These steroids include testosterone, its precursors androstenedione and dehydroepiandrosterone, and the 19-norsteroids equivalents, some of which are commercially available for oral self-administration.

This project is aimed at applying the IRMS (isotope ratio mass spectrometry) to the detection of the “natural” testosterone and 19-nortestosterone anabolic agents. Complement of the existing GC/MS methods, the GCIC/IRMS permits the differentiation of the exogenous or endogenous origin of urinary androgens metabolites, by measuring the isotopic content of their carbon atoms. That novel application of an otherwise well-known technique, now requires its validation in different experienced laboratories, the determination of international reference ranges of the metabolites of endogenous origin and the documentation of the changes commonly observed following the administration of these steroids. Ultimately, one of its most direct outcomes will be to verify that although different methodological approaches and different equipment are used, the same determination is made from the analysis of common specimens.

Testosterone and 19-nortestosterone (nandrolone) are potent androgenic anabolic steroids of known abuse in sport. Anabolic steroids are banned in Olympic sports for more than 20 years and since 1986, the highest number of positive cases reported are due to these two steroids. The administration of testosterone and its precursors, androstenedione and DHEA has been described to significantly alter the parameters of the urinary androgens steroid profile measured by GC/MS. The administration of testosterone is first detected in human urine by the GC/MS measurement of a testosterone/epitestosterone (*T/E*) value higher than 6, which is caused by the relative increase of excreted testosterone glucuronide (Donike, (1983)). The oral intake of androstenedione and DHEA was shown to transiently increase the excreted *T/E* value in females and in some male volunteers, from whom *T/E* values slightly higher than one were measured (Uralets (1999); Lévesque (2000); Bowers (1999); Garle (1998)). Other alterations of the urinary steroid profile, such as abnormally high concentration of androsterone and etiocholanolone and the presence of the characteristic hydroxylated metabolites glucuro- and sulfoconjugated, 6 α -androstenedione, 6 β -epiandrosterone, had permit to report positive findings (Lévesque, (1999)).

Disruption of the normal urinary profiles of androgens metabolites can be demonstrated by comparison to the described population reference ranges

and to the individual's norm (Donike (1993); Ayotte (1997) and reference cited therein). That requires the investigation of the athlete's previous or subsequent tests results in order to exclude the few individuals who naturally produce urine samples in which elevated *T/E* values are systematically measured. Although successfully applied in many cases, this method is time-consuming and complex. Considering only the *T/E* values above 6 also leads to false negative results since it is known that the limit will not be exceeded following the administration of testosterone and precursors, when the basal values are lower than one, which is a characteristic but not exclusively, of the Asian population (Shackleton, (1997)). The level of androgens in female samples is generally very low and the uncertainty of the measurements may represent a problem to which must be add reports of alteration of the normal values attributed to other sources than the administration of androgens.

The administration of 19-nortestosterone and of its precursors, 19-norandrostenedione and 19-norandrostenediol, which are available for oral self-administration, results in the excretion of 19-norandrosterone and 19-noretiocholanolone, mostly found in the glucuroconjugated form. The period, during which the metabolites can be detected, is drastically reduced when the oral preparations are taken (Engel (1958); Masse (1985); Ayotte (1996); Schanzer (1996); Kintz (1999)). In the last years, many positive cases were reported with low levels of the urinary metabolites. Extremely low levels of 19-norandrosterone can be endogenously excreted in human urine, and that has prompted the IOC to safely recommend a threshold in males and females. However, as it is the case with the androgens, natural factors are systematically invoked to challenge the positive test results.