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USE OF INTERNAL STANDARDS

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the selection of internal standards for the analysis of anabolic androgenic steroids (AAS) by chromatographic-mass spectrometric techniques.

2.0 Analysis and Reporting Requirements

1. Use of **17α-Methyltestosterone** as Internal Standard

Laboratories shall exercise caution regarding the use of 17α-Methyltestosterone (MT) as an internal standard when analyzing steroids by chromatographic-mass spectrometric techniques. This applies, most importantly, to the Confirmation Procedures (CP).

The use of MT as an internal standard may lead to the formation of **17α-Methyl-5α-androstane-3α,17β-diol** and **17α-Methyl-5β-androstane-3α,17β-diol** [1] when microbial activity is present in urine *Samples* and, therefore, to the consequent misinterpretation of the result as an *Adverse Analytical Finding* (*AAF*). In addition to MT, these artifacts are known *Metabolites* of various AAS (*e.g.* **Mestanolone, Metandienone, Oxymetholone, Methandriol, and Methyl-1-testosterone**).

It is highly recommended that CPs for AAS have the following characteristics:

- Avoid metabolic links between the Presumptive *Adverse Analytical Finding* (PAAF) and the internal standard *e.g.* by using deuterated (^{2}H)- or carbon (^{13}C)-labeled internal standards of endogenous steroids instead of MT or any other exogenous AAS;
- Incorporate solid phased extraction (SPE) to clean up the *Sample* prior to the enzymatic hydrolysis, since the direct incubation of already contaminated urine *Samples* may lead to the formation of microbial degradation artifacts and alter the steroid profile.

2. Use of Isotopically Labeled Steroids as Internal Standards

It is recommended that ^{2}H- or ^{13}C-labeled standards of endogenous steroids are utilized for the Initial *Testing* Procedure (ITP) applied for the determination of the steroid profile as well as for the CP of steroids in general. However, it may not be optimal to employ isotopically labeled standards of **Testosterone** and **Epitestosterone** for all *Samples* due to:

i. The potential contribution of the fraction of non-labeled compound when these endogenous steroids are present at low concentrations; and

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ii. The potential interference caused by $^{2}H_{3}$- or $^{13}C_{3}$-E with the detection of the M-15 ion of bis-trimethylsilylated 17α-methylandrostanediols (*m/z* 435).

3. Additional recommendation for qualitative CP

Whenever the internal standard to be used in a qualitative CP has to be identical to the one used in the relevant ITP, and where decomposition or metabolism of the internal standard could potentially contribute to a false positive result for the Non-Threshold Substance subject to the CP, Laboratories should consider the concomitant analysis of one extra Aliquot without the addition of any internal standard.

3.0 References

[1] Schweizer G., Baume N., and Saugy M. Degradation of methyltestosterone in urine samples. *Drug Test Anal.* **6**(11-12): 1170-3, 2014.