

WADA Technical Letter – TL08

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Written by:	WADA LabEG	Approved by:	WADA LabEG*
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*The approval by the WADA Executive Committee is applicable only to Technical Letters issued after November 2019.

USE OF INTERNAL STANDARDS

The *World Anti-Doping Agency* wishes to draw the attention of the Laboratories to the following issues that may affect Laboratory operations. This pertains, in particular, to the selection of internal standards for the analysis of androgenic anabolic steroids (AAS) by chromatographic-mass spectrometric techniques:

1. Use of 17 α -Methyltestosterone as internal standard

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

The use of MT as an internal standard may lead to the formation of 17 α -methyl-5 α -androstane-3 α ,17 β -diol and 17 α -methyl-5 β -androstane-3 α ,17 β -diol¹ when microbial activity is present in urine *Samples* and, therefore, to the consequent misinterpretation of the result as an *Adverse Analytical Finding*. In addition to MT, these artifacts are known *Metabolites* of various exogenous steroids (e.g. mestanolone, metandienone, oxymetholone, methandriol, and methyl-1-testosterone).

Therefore, it is highly recommended that Laboratory Confirmation Procedures for AAS have the following characteristics:

- Avoid metabolic links between the Presumptive Adverse Analytical Finding and the internal standard e.g. by using deuterated (²H)- or carbon (¹³C)-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 incubating directly already contaminated urine *Samples* may lead to the formation of microbial degradation artifacts and alter the “steroid profile”.

2. Use of deuterated steroids as internal standards

It is recommended that ²H- or ¹³C-labeled standards of endogenous steroids are utilized for the Initial Testing Procedure applied for the determination of the “steroid profile” as well as for the Confirmation Procedures of steroids in general. However, it may not be optimal to employ isotopically labeled standards of T and E 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

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

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- ii. the potential interference caused by $^2\text{H}_3$ - or $^{13}\text{C}_3$ -E with the detection of the M-15 ion of bis-trimethylsilylated 17α -methylandrostanediols (m/z 435).

3. Additional recommendation for qualitative Confirmation Procedures

Whenever the internal standard to be used in a qualitative Confirmation Procedure has to be identical to the one used in the relevant Initial Testing Procedure, 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 Confirmation Procedure, Laboratories should consider the concomitant analysis of one extra Sample Aliquot without the addition of any internal standard.

Should you have any further questions, please do not hesitate to contact the WADA Science Department.