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OXILOFRINE

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the following observations and instructions on the analysis and reporting of **Oxilofrine** (methysynephrine).

After the intake of **Ephedrine**, oxilofrine, which is a Non-Threshold Substance, can be found in urine at levels higher than (>) the *Minimum Reporting Level (MRL)* of 50 ng/mL ^[1], whereas ephedrine, a Threshold Substance, may be present at levels not higher than (\leq) the *Decision Limit (DL)* of 11 μ g/mL (or the SG-adjusted *DL*, if needed) ^[2]. Nevertheless, excretion studies of therapeutic doses of ephedrine indicate that oxilofrine free form levels never exceed 1,000 ng/mL in urine ^[3,4].

In addition, **Hydroxy-pseudoephedrine** ('pseudo-oxilofrine') — a minor *Metabolite* of **Pseudoephedrine** — under certain conditions may co-elute with oxilofrine and show an identical fragmentation pattern in Liquid Chromatography-Mass Spectrometry (LC-MS) analyses.

These two scenarios may lead to the incorrect reporting of an *Adverse Analytical Finding (AAF)* for oxilofrine.

2.0 Analysis and Reporting Requirements

2.1 Detection of Oxilofrine in the Presence of Ephedrine at Levels \leq *DL*

If oxilofrine is detected in a urine *Sample* at a concentration higher than (>) the *MRL* and ephedrine is also present in the *Sample* at a concentration which is not higher than (\leq) the *DL* or SG-adjusted *DL*, if applicable, Laboratories shall evaluate whether the finding is consistent with the formation of oxilofrine through the metabolism of ephedrine:

- Report the result as a Negative Finding if:
 - The estimated concentration of oxilofrine is not higher than (\leq) 1,000 ng/mL;
- Report the result as an *AAF* for oxilofrine if:
 - The estimated concentration of oxilofrine is greater than (>) 1,000 ng/mL, irrespective of the ephedrine concentration in the *Sample*.

[This MRL applies to the determination of the parent compound excreted in its free form only and shall not be applied to the sum of concentrations of phase-II Metabolites of oxilofrine.]

These conditions do not apply to the reporting of oxilofrine findings in the absence of ephedrine. In such cases, the finding should be reported as an AAF for oxilofrine if the estimated concentration is higher than (>) 50 ng/mL, which is the MRL for stimulants ^[2].

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2.2 Chromatographic Resolution of Oxilofrine and Hydroxy-pseudoephedrine

Laboratories shall implement procedures that allow the proper chromatographic separation and identification of oxilofrine and hydroxy-pseudoephedrine, for example by performing Gas Chromatography (GC)-MS analysis of the per-trimethylsilyl (TMS) derivatives, prior to reporting an AAF for oxilofrine. Oxilofrine and hydroxy-pseudoephedrine can also be separated by Ultra High-Performance Liquid Chromatography (UHPLC).

3.0 References

- [1] WADA *Technical Document* TD DL: Decision Limits for the Confirmatory Quantification of Exogenous Threshold Substances by Chromatography-based Analytical Methods.
- [2] WADA *Technical Document* TD MRPL: Minimum Required Performance Levels for Detection and Identification of Non-Threshold Substances by Chromatographic-Mass Spectrometric Analytical Methods.
- [3] Espinosa P. *et al.* Oxilofrine: ephedrine minor metabolite. In *Recent Advances in Doping Analysis* (26), Schanzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 2018; 139-144.
- [4] Orlovius A.-K. *et al.* Detection of urinary phase-I and phase-II metabolites of ephedrine and oxilofrine by LC-MS/MS. In *Recent Advances in Doping Analysis* (18), Schanzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds). Sport und Buch Strauß: Köln, 2010; 153-155.

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