WADA Technical Letter – TL25 Tramadol

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Written by: Reviewed by:	*WADA* Science WADA Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
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Minimum Reporting Level for Tramadol

1.0 Introduction

WADA wishes to draw the attention of the Laboratories and *Anti-Doping Organizations* (*ADO*s) to the following requirements for the analysis and reporting of tramadol findings in urine *Samples*.

Tramadol is prohibited *In-Competition* as of 1 January 2024, and as such is included in the *WADA Prohibited List* under section S7. Narcotics.

2.0 Analytical Requirements

2.1 Initial *Testing* Procedure (ITP):

- The Laboratory's method validation of the ITP shall include the estimation of the Limit of Detection (LOD) for, at least, the tramadol free parent compound.
- The estimated LOD of the ITP for the tramadol free parent compound shall be less than or equal to (≤) 20 µg/mL, which constitutes the Minimum Required Performance Level (MRPL) and the *Minimum Reporting Level* (*MRL*).

2.2 Confirmation Procedure (CP):

- The Laboratory shall document that the CP allows the identification of the tramadol free parent compound in compliance with the effective TD IDCR [1].
- The Limit of Identification (LOI) of the CP shall be less than (<) the MRPL of 20 µg/mL.

3.0 Reporting Requirements

WADA wishes to draw the attention of the Laboratories to the following observation: O-desmethyl-venlafaxine, a *Metabolite* of the non-prohibited antidepressant venlafaxine, may coelute with and interfere in the detection of free tramadol parent compound under certain chromatographic conditions [3]. Both compounds have the same molecular formula and produce the same product ion at m/z 58 (m/z 264 > 58 transition by MS/MS analysis). However, the additional product ion resulting from water loss (m/z 264 > 246) has a different relative abundance, being more abundant for O-desmethyl-venlafaxine than for tramadol.

In addition, the presence of the tramadol *Metabolite* O-desmethyl-tramadol (ODMT), identified using the ion transitions m/z 250 > 58 and m/z 250 > 232, is also indicative of the presence of tramadol in the *Sample*. Potential di-desmethyl-*Metabolites* of venlafaxine would have the same molecular formula as ODMT; however, they would

not produce the ion at m/z 58.

For these reasons, when the ITP produces a Presumptive *Adverse Analytical Finding* (PAAF) for tramadol, Laboratories should follow these steps to exclude the use of venlafaxine as the cause of the finding:

- Check the *Sample Doping Control* Form for a declaration of use of venlafaxine;
- Apply during the CP chromatographic conditions allowing the separation of tramadol and O-desmethyl-venlafaxine [1];
- Also consider the monitoring of ODMT during the CP [2] .

3.1 "A" *Sample*:

- The "A" CP shall confirm the presence of the tramadol free parent compound in compliance with the effective TD IDCR [1].
- In addition, to estimate the concentration of the tramadol free parent compound in the "A" *Sample*, the CP shall follow the requirements established in the effective TD MRPL [2] for Non-Threshold Substances with an *MRL*.
- The *MRL* for reporting an *Adverse Analytical Finding* (*AAF*) for tramadol in a urine "A" *Sample* is set at 20 µg/mL, applicable to the tramadol free parent compound only (without hydrolysis of phase II *Metabolites*).

[As per the TD MRPL reporting requirements for Non-Threshold Substances with an *MRL* [2], a finding for tramadol in an "A" urine *Sample* shall be reported as an *AAF* if the tramadol free parent compound (obtained without hydrolysis of phase II *Metabolites*) is confirmed in the "A" *Sample* at an estimated concentration (adjusted for specific gravity (SG), if needed), which is confidently higher (as determined by comparison with a 120% *MRL* single point calibrator – see TD MRPL) than (>) the corresponding *MRL* of 20 µg/mL.]

[1] The chromatographic separation of tramadol and O-desmethyl-venlafaxine could also be implemented during the ITP, at the Laboratory's discretion. This would avoid the unnecessary confirmatory analyses of putative findings related to the permitted use of venlafaxine.

[2] The monitoring of ODMT serves to further confirm an *AAF* based on the identification of the presence of the free tramadol parent compound in the *Sample* at levels higher than the *MRL* of 20 µg/mL. However, this *MRL* is not to be applied to ODMT.

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3.2 "B" *Sample*:

- The "B" *Sample* CP shall only confirm the presence, at any concentration, of the tramadol free parent compound (in compliance with the TD IDCR [1]) for the *AAF* to be valid. No quantification or estimation of the concentration is necessary.

4.0 References

[1] *WADA Technical Document* TD IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

[2] *WADA Technical Document* TD MRPL: Minimum Required Performance Levels and Applicable *Minimum Reporting Levels* for Non-Threshold Substances Analyzed by Chromatography-Mass Spectrometric Analytical Methods.

[3] Allen KR. Interference by Venlafaxine Ingestion in the Detection of Tramadol by Liquid Chromatography Linked to Tandem Mass Spectrometry for the Screening of Illicit Drugs in Human Urine. *Clin Toxicol*, **44**:2, 147-153, 2006.