TD2015IDCR

Summary of Major Modifications

Based on new technological developments and the experience accumulated during the last 5 years on the application of chromatographic-mass spectrometric methods for the confirmatory identification of analytes in doping control analyses, the Technical Document on IDENTIFICATION CRITERIA FOR QUALITATIVE ASSAYS INCORPORATING COLUMN CHROMATOGRAPHY AND MASS SPECTROMETRY (TD2010IDRC) has undergone revision by the *WADA* Laboratory Expert Group (LabEG).

The revised MINIMUM CRITERIA FOR CHROMATOGRAPHIC-MASS SPECTROMETRIC CONFIRMATION OF THE IDENTITY OF ANALYTES FOR DOPING CONTROL PURPOSES (TD2015IDCR) is focused on the chromatographic and mass spectrometric criteria to be met in order to ensure the unequivocal identification of the target analyte(s). The document has been substantially simplified taking in consideration technical elements which are shared between different chromatographic methods (*e.g.* gas and liquid chromatography), while refining the requirements for different chromatographic conditions. On the other hand, the common information contained in the application of different MS approaches (*e.g.* scanning or non-scanning techniques, bottom-up and top-down methods, single- or multiple-stage MS) has been put together in a common approach to the identification of analytes irrespective of their molecular mass.

Title:

The word "Minimum" has been added to emphasize that these are the minimum criteria to be met by the Laboratories to reduce the risk of false identification when applying chromatographic-mass spectrometric methods for confirmation analyses. It has also been specified that these are criteria to be used for confirmation purposes.

Title:

It is emphasized that the identification criteria described in this document shall be applicable to the differentiation between isomers of the same substance (if required for the unequivocal identification of a *Prohibited Substance*).

1.0 Sample Preparation

This section of the TD2010IDCR has been deleted as there are no criteria related with this section and it has been considered to be too basic in its content.

2.0 Chromatographic Separation

- In this section, the common criteria applicable to gas and liquid chromatography (*e.g.* criteria for retention factors, ΔRT or ΔRRT) have been merged into one common section on Chromatographic Criteria.
- It has been specified that the ΔRT between the analyte's chromatographic peak in the *Sample* and that of the same analyte in a spiked sample, Reference Collection sample, or Reference Material analyzed in the same analytical batch shall not be greater than the peak's full-width-at-half-maximum, FWHM. This has been done to account for very narrow chromatographic peaks, when the "whichever is greater" criterion (between 1% and $\pm$ 0.1 minutes) may result in a ΔRT unrealistically large: for example, when the peak width is too narrow, the $\pm$ 0.1 min (6 s) criterion would not exclude the possibility of other non-related peaks eluting in between.
- The ΔRRT criterion when a stable isotope-labeled analyte is used as the chromatographic reference compound (CRC) has been increased from ±0.1% to ±0.5%, since the RRT is often calculated close to 1 and the ±0.1% ΔRRT criterion is often not attainable.

3.0 Mass Spectrometric Detection and Identification of Molecules with Mass Less than 800 Da and

4.0 Mass Spectrometric Detection and Identification of Molecules with Mass between 800 and 8000 Da

Sections 3.0 and 4.0 in the TD2010IDCR have been completely reworked and merged into one common section 2.0 Mass Spectrometric Identification Criteria, which describes criteria applicable to commonly employed strategies for the identification of analytes, including "top-down" and "bottom-up" MS approaches from a more general perspective, and defining, when needed, specific criteria for scanning or non-scanning techniques and single- or multiple-stage MS.

- The rather arbitrary division between the analysis of molecules with molecular masses less than 800 Da or between 800-8000 Da has been removed.
- Specific identification criteria are provided:

  - It is specified that the maximum difference in mass between the ion acquired in the *Sample* and the same ion acquired from a spiked sample, Reference Collection sample, or Reference Material analyzed in the same analytical batch shall not exceed ±0.5 Da.

  - At least 3 diagnostic ions shall be acquired when using single-stage MS, whereas at least 2 precursor-product ion transitions shall be monitored when using multiple-stage MS.

  - The abundance of diagnostic ions shall always be determined from the peak area or height of integrated selected ion chromatograms, irrespective of the scanning or non-scanning acquisition mode employed.

- Relative Abundances shall be calculated by dividing the peak area or height of the ion trace of each diagnostic ion by the corresponding peak area or height of the ion trace of the most abundant diagnostic ion taken as the base peak. Maximum tolerance windows for comparing Relative Abundances are provided in the table 1.

Table of Maximum Tolerance Windows

- This table has been modified to ensure that identical tolerance windows are obtained when tolerance criteria are applied to limiting values of Relative Abundances. For example, when applying tolerance window criteria for a RA of 50%, the ±10 (absolute) criterion and the ± 20% (relative) criterion would produce the same range of acceptable abundances, *i.e.* 40 – 60. The same holds true for the ± 20% (relative) and the ±5 (absolute) criteria when applied to a RA of 25%: the resulting tolerance window is 20-30.
- It has been specified (footnote 6) that the tolerance window of RA must be always >0, since the diagnostic ions must always be detected in the *Sample.*

Definitions

The list of definitions has been updated in accordance with the changes made to the document.