

## WADA Technical Document – TD2003IDCR

Document Number:	TD2003IDCR	Version Number:	1.2
Written by:	WADA Project Team	Approved by:	
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### IDENTIFICATION CRITERIA FOR QUALITATIVE ASSAYS INCORPORATING CHROMATOGRAPHY AND MASS SPECTROMETRY

The appropriate analytical characteristics must be documented for a particular assay. The Laboratory must establish criteria for identification of a compound. Examples of acceptable criteria are:

#### Chromatographic separation

For capillary gas chromatography, the retention time (RT) of the analyte shall not differ by more than one (1) percent or  $\pm 0.2$  minutes (whichever is smaller) from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed contemporaneously. In those cases where shifts in retention can be explained, for example by sample overload, the retention time criteria may be relaxed. For high performance liquid chromatography, the RT of the analyte shall not differ by more than two (2) percent or  $\pm 0.4$  minutes (whichever is smaller) from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed in the same analytical batch.

#### Mass Spectrometric Detection

**Full scan mode:** A full or partial **scan** is the preferred approach to identification. A partial **scan** may begin at an m/z value greater than any abundant ion due to the derivatizing agent or chemical ionization reagent.

When a full or partial **scan** is acquired, all **diagnostic ions** with a **relative abundance** greater than 10% in the reference spectrum obtained from a positive control urine, a Reference Collection sample, or a Reference Material must be present in the spectrum of the unknown peak. In addition, the **relative abundance** of three **diagnostic ions** shall not differ by more than the amount shown in Table 1 from the relative intensities of the same ions from that of a spiked urine, a Reference Collection sample, or a Reference Material. The **relative abundance** of the **diagnostic ions** may be obtained from single or averaged spectra or integration of peak areas of extracted ion profiles.

Background subtraction, if applied, should be performed uniformly on all *Samples* analyzed contemporaneously and used to make decisions regarding the presence of a *Prohibited Substance* or *Method*, its *Metabolite*, or *Marker*.

The use of computer-based mass spectral library searching or matching is permitted. The laboratory must establish criteria for acceptance of compound identification based on spectral match quality. Since the match factor of a reverse search does not guarantee identification, all spectral library matches must be reviewed by a qualified scientist.

If three **diagnostic ions** with a **relative abundance** greater than 5% are not available, a second derivative shall be prepared, or a second ionization or fragmentation technique shall be used. The second derivative should yield different

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**diagnostic ions.** The second ionization technique must be based on a different physical principle, i.e., chemical ionization vs. electronic ionization and again should provide different diagnostic ions. It is not acceptable to utilize a technique that changes only the **relative abundance** of the same mass ions. In any case, a minimum of two **diagnostic ions** is mandatory in each mass spectrum.

**Selected Ion Monitoring Mode:** In some cases, it may be necessary to monitor selected ions in order to detect the substance at the Minimum Required Performance Limits. When selected ions are monitored, at least three **diagnostic ions** must be acquired. The **relative abundance** of a **diagnostic ion** shall preferably be determined from the peak area or height of integrated selected ion chromatograms. The **signal-to-noise ratio** of the least intense **diagnostic ion** must be greater than three to one (3:1). The relative intensities of any of the ions shall not differ by more than the amount in Table 1 from the relative intensities of the same ions acquired from a spiked urine, Reference Collection sample, or Reference Material. For a **diagnostic ion** with a **relative abundance** of less than 5% in the reference, the ion must be present in the unknown. The concentration of *Prohibited Substance*, or its *Metabolite*, or its *Marker* should be comparable in the *Sample* and the spiked urine, Reference Collection sample, or Reference Material.

**Table 1**  
**Maximum Tolerance Windows for Relative Ion Intensities**  
**to Ensure Appropriate Uncertainty in Identification**

Relative Abundance (% of base peak)	EI-GC/MS	CI-GC/MS; GC/MS <sup>n</sup> ; LC/MS ; LC/MS <sup>n</sup>
> 50%	±10% (absolute)	± 15% (absolute)
25% to 50%	± 20% (relative)	±25% (relative)
< 25%	±5% (absolute)	±10% (absolute)

If the Laboratory protocol requires three ions to be within a tolerance window to identify a substance, it is not permissible to collect additional ions and select those ion ratios that are within tolerance and ignore others that would not result in meeting identification criteria without a valid explanation.

If three **diagnostic ions** are not available, a second derivative shall be prepared, or a second ionization or fragmentation technique shall be used. The second derivative should yield different **diagnostic ions**. The second ionization technique must be based on a different physical principle, i.e., chemical ionization vs. electronic ionization and again should provide different **diagnostic ions**. It is not acceptable to utilize a technique that changes only the **relative abundance** of the

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same mass ions. In any case a minimum of two **diagnostic ions** must be present in each mass spectrum.

### Tandem mass spectrometric (MS<sup>n</sup>) detection:

**Tandem mass spectrometry** data can be acquired in either the full **scan** or selected reaction monitoring (SRM) mode. The combination of mass selection of the precursor ion followed by a potentially unique collision-induced dissociation and mass selection or scanning of the product ion gives **tandem mass spectrometry** increased specificity. Collision conditions should be selected to ensure that the precursor ion is present in the **MS/MS scan** or SRM acquisition. In some cases, the combination of a single precursor-product ion pair may be sufficiently unique to be definitive. When monitoring one precursor ion to yield one product ion, the mass resolution of the first mass analyzer should be set to unity. When monitoring more than one product ion, the relative intensities of any of the ions shall not differ by more than the amount in Table 1 from the relative intensities of the same ions acquired from a spiked urine, Reference Collection sample, or Reference Material analyzed contemporaneously. The **signal-to-noise** of the least intense **diagnostic ion** must be greater than three-to-one (3:1). The **relative abundance** of a diagnostic ion shall preferably be determined from the peak area or height of integrated selected ion chromatograms. For a diagnostic ion with a **relative abundance** of less than 5% in the reference, the ion must be present in the unknown.

If unique **diagnostic** product **ion(s)** are not available, a second derivative shall be prepared, or a second ionization or fragmentation technique shall be used. The second derivative should yield different precursor and/or product ions. The second ionization technique may use a different chemical ionization reagent, but should provide different precursor or product ions. It is not acceptable to utilize a technique that changes only the relative abundance of the same mass ions.

### Estimation of concentration

The concentration may be estimated by any of the above techniques by taking the ratio of the peak height (or peak area) obtained at the retention time for the analyte of interest compared to that obtained from an internal standard. An appropriately deuterated internal standard is preferred but not required. The peak height (or peak area) ratio may then be compared to a standard or positive control urine. The use of a single ion at the appropriate mass-to-charge ratio (e.g. m/z 405 for 19-norandrosterone di-TMS derivative) taken from an extracted ion chromatogram or from a selected ion monitoring chromatogram is sufficient for the estimation of concentration. Additional ions must be used for meeting identification criteria.

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### Definitions

**Diagnostic ion(s):** Molecular ion or fragment ions whose presence and abundance are characteristic of the substance and thereby may assist in its identification. A second ion belonging to the same isotopic cluster may also be used as diagnostic only when the peculiarity of the atomic composition of the fragment so justifies (e.g. presence of Cl, Br, or other elements with abundant isotopic ions).

**High resolution mass spectrometry (HRMS):** For the purposes of the *International Standards for Laboratories*, HRMS is defined as mass spectrometry at a resolving power (10 % valley definition) in excess of 3,000.

**Low resolution mass spectrometry (LRMS):** LRMS is defined as mass spectrometry at a resolving power (10 % valley definition) lower than 3,000.

**Relative abundance (mass spectrometry):** The abundance of a particular ion relative to the most abundant ion monitored expressed as a percentage.

**Maximum difference in relative abundance:** The maximum permitted difference between the relative abundance of a particular ion obtained from the *Sample* and that obtained from the positive control urine. This may be expressed in ABSOLUTE or RELATIVE terms.

**Absolute difference:** Calculated by subtracting the stated percentage from the relative abundance obtained for the studied ion from the positive control urine or Reference Material. For example, if the relative abundance of an ion in the chromatographic peak of interest in the positive control urine or Reference Material is measured as 20%, then the observed relative abundance for the same ion in the peak of interest in the unknown urine sample would be required to be in the range of 15-25% ( $20\% \pm 5\%$ ) for the ion to contribute to an acceptable identification.

**Relative difference:** Calculated by multiplying the stated percentage by the relative abundance obtained for the studied ion from the positive control urine or Reference Material. For example, if the relative abundance of an ion in the chromatographic peak of interest in the positive control urine or Reference Material appears as 30 % and the stated maximum permitted difference is 20 % (relative), then the observed relative abundance for the same ion in the peak of interest in the unknown urine sample would be required to be in the range of 24-36% ( $30\% \pm (30 \times 20\%)$ ) for the ion to contribute to an acceptable identification.

**Scan:** Acquisition of ions of a continuous range of m/z values.

**Selected ion monitoring (SIM):** Acquisition of ions of one or more pre-determined discrete m/z values for specified dwell times.

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**Signal-to-Noise Ratio:** Magnitude of the instrument response to the analyte (signal) relative to the magnitude of the background (noise).

**Tandem mass spectrometry (MS/MS or MS<sup>n</sup>):** A technique in which a precursor ion is isolated in a mass analyzer, fragmented by collision with a collision gas, and the product ions collected in a second mass analyzer. This process can be applied multiple times, each application being reflected in the “n” exponent. The technique may be accomplished either in space (e.g. triple quadrupole MS) or in time (e.g. ion trap MS)