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# Proposal: criteria for positive identification in LC-MS analysis

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Any analytical methodology should comply with several quality requirements, in order to generate reliable data for accurate quantification and safe identification. Method validation is obviously required, and the application of realistic criteria based on the acquisition of several MS/MS transitions considering their specificity, or criteria based on mass measurement accuracy is also necessary. Thus, the accomplishment of retention time and ion intensity ratios when compared with the reference standard is mandatory for reliable identification of the compound. Quality procedures applied by researchers in other fields of investigation i.e. food safety, are used to set up the following proposal.

***Quantification***. An analytical methodology needs to be fully validated to ensure the quality of the results. The performance of the methods should be validated in terms of linearity, LOQ, LOD, accuracy and precision. Validation should be performed prior to the analysis of samples at analyte concentrations close to the expected concentrations in real samples, since matrix effects may depend on the concentration of the analyte[1].

***Confirmation of the identity***. Confirmation of analyte identity is necessary to avoid reporting false positives and/or false negatives. Analytical chemists have to develop reliable methods that allow not only accurate quantification of targeted analytes but also, and even more importantly, their unequivocal identification. Ideally, confirmation of the identity should be objective and safe, not depending on the subjective interpretation of the analyst, so predefined, efficient confirmation rules are necessary.

***Low resolution (LR) MS/MS*** systems typically operate under SRM mode, where a precursor ion is selected in the first quadrupole, fragmented in the collision cell, and a "specific" product ion is selected using the second quadrupole. The selection of a specific SRM transition, avoiding the use of common losses (e.g.

H2O, CO2 and HCl), is important in order to prevent the reporting of false positives or false negatives. However, even with this approach, there are some probabilities that other compounds, not related to the analyte, can share the same transition. Therefore, a second SRM transition is often monitored and the presence of a compound is only considered to be confirmed if both transitions produce chromatographic peaks with retention times corresponding to that of the investigated analyte in pure standard. In addition, the ratio of the intensities of the two recorded SRM transitions must be similar to that obtained for the reference standard. Thus, in order to correctly identify a compound the relative ion intensities between both recorded transitions (q/Q ratio) need to comply.

Slightly different tolerances are proposed for the analysis of wastewater and biological samples as we selected the criteria usually applied in each research field. For wastewater analysis, the ion ratio should coincide with the ion ratio obtained from the reference standard, with maximum permitted tolerance between 20 and 50% (**Table 1**)[1, 2]. These criteria were proposed by SCORE (Sewage Analysis CORe group Europe)[3, 4].

**Table 1**: Maximum permitted tolerances for relative ion intensities (wastewater analysis).

| **Relative Abundance (% of base peak)** | **q/Q ratio value** | **Tolerance (%)** |
|---|---|---|
| > 50% | 0.5 - 1 | 20 |
| > 20 to 50% | 0.5 - 0.2 | 25 |
| > 10 to 20% | 0.2 - 0.1 | 30 |
| ≤ 10% | < 0.1 | 50 |

For biological samples, the relative intensities of any of the ions shall not differ by more than the amount in **Table 2** from the relative intensities of the same ions acquired from the reference material analyzed contemporaneously. These criteria are proposed by the WADA (World Anti-Doping Agencia)[5].

**Table 2** - Maximum tolerance windows for relative ion intensities to ensure appropriate confidence in identification (biological samples).

| **Relative Abundance (% of base peak)** | **Maximum Tolerance Window** |
|---|---|
| > 50% | ±10% (absolute) |
| 25% to 50% | ± 20% (relative) |
| 5% to <25% | ±5% (absolute) |
| <5% | ± 50% (relative) |

In addition, the maximal accepted deviation in retention time is two percent or ± 0.1 min between the compound in sample and the reference standard. Alternatively, the laboratory may choose to use relative retention time (RRT) as an acceptance criterion, where the retention time of the peak of interest is measured relative to a chromatographic reference compound (internal standard). The RRT shall not differ by more than ±1% from that of the reference material analyzed contemporaneously. The signal-to-noise of the least intense diagnostic ion shall be greater than three-to-one (3:1). Identification criteria according to the recommendations of the International Standard for Laboratories - technical document - TD2010IDCR from the international organization WADA[5].

One of the issues that still remains without broad consensus is the criteria applied for confident identification/confirmation when using ***high resolution MS***. Accurate-mass measurements give more confidence for a reliable identification than nominal mass data, and this factor is recognized in several guidelines, which, for example, give more identification points to HRMS ions than LRMS ions[1]. It is widely accepted that at least two accurate-mass ions are required for a confident identification with HRMS. However, two main issues need to be considered: 1) what is the acceptable mass error? 2) what is the maximum deviation acceptable in the ion ratio? Moreover, another parameter helpful in the process is

the isotopic distribution, especially when abundant isotope ions such as chlorine, sulphur, or bromine are present. Several situations might occur that lead to different degrees of confidence in identification. For example, Schymanski et al.[6] propose up to 5 levels of confidence in a non-target analysis. These levels range from only exact mass to unequivocal molecular formula, and then tentative candidate(s) followed by probable structure to a fully confirmed structure with a reference standard.

**Figure 1** summarizes the key parameters in the detection and identification of a compound with HRMS. As shown in this figure, different scenarios might occur as a function on the information provided, and on the availability of reference standard.

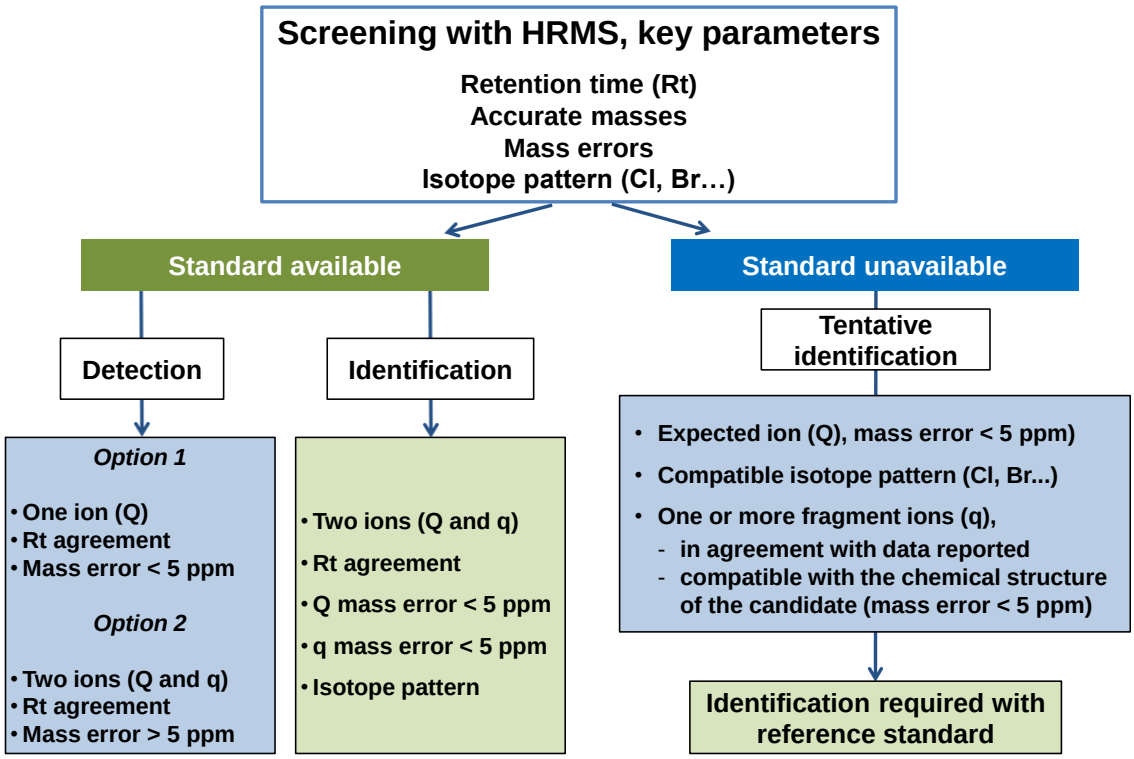


**Figure 1.** Detection and identification criteria in screening of illicit drugs with HRMS[4].

When a reference standard is available, compounds can be detected or identified. Detection is considered satisfactory when the most-abundant ion (Q), commonly the (de)protonated molecule, is found at the expected $t_R$ (± 0.1 min,) and mass error < 5 ppm[7]. Another likely situation for detection is to find two representative ions (*i.e.*, the most-abundant ion (Q) and a fragment/adduct ions(q) at the expected $t_R$), but with mass errors between 5-20 ppm. The latter situation seems to occur when the signal intensity is low (favored at low analyte concentrations). In that case, an additional effort is recommended to investigate more accurate-mass ions and/or repeat sample injection. Identification is based on the presence of at least two representative ions (Q, q) at the expected $t_R$ with mass errors < 5 ppm. Additionally, q/Q ratios should fit with those for reference standards within tolerance limits[7]. Identification under these conditions is highly reliable and can be considered as the ideal situation.

When the reference standard is not available, a tentative identification can be made when an expected ion with mass error < 5 ppm is observed, together with its characteristic isotopic pattern. Subsequently, the fragment ions should be evaluated by comparing the data with, e.g., data reported in the literature or justified by the accurate-mass fragments taking into account the structure of the molecule. However, for structure confirmation, injection of the reference standard is eventually required.

**References:**

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