Guidance document on identification of mycotoxins in food and feed

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Preamble
Identification is an integral part of confirmatory analysis of mycotoxins in food and feed. This document has been established by and has been discussed in the EURL/NRL mycotoxin network, taking existing criteria from other domains and literature data into account [1-5]. It provides guidance criteria for identification that should be taken into account during method validation and mycotoxin analysis. This guidance supplements the “Specific requirements for confirmatory methods” from Annex II of Commission Regulation (EC) No 401/2006 [6].

Identification requirements for mycotoxins

For identification of mycotoxins, chromatography combined with mass spectrometry is the method of choice. Alternatively, liquid chromatography with fluorescence detection may be applied, but only when an immunoaffinity-based cleanup specific for the targeted mycotoxin(s) has been employed during sample preparation. The use of methods based on UV detection is discouraged, however already established methods for which adequate selectivity has been demonstrated may continue to be used, this typically applies to patulin and deoxynivalenol.

The criteria provided below are default guidance criteria that should be met in order to achieve proper identification. During validation of the method, it should be verified that the criteria are met within the concentration range of the method, using spiked samples or certified reference materials. This should include the lowest level for which results will be reported, and the legislative maximum levels. Furthermore, it should be verified that for blank samples no false positive identifications are obtained.

Requirements for chromatography
The minimum acceptable retention time for the analyte under examination should be twice the retention time corresponding to the void volume of the column. The retention time of the analyte in the sample extract should correspond to that of the average of the calibration standards measured in the same sequence with a tolerance of ±0.2 min or ±50% of the peak width at half height (whichever is larger), for both gas chromatography and liquid chromatography. With UHPLC and GC, deviations are typically within ±0.1 min.

In case a 13C-isotopically labelled analogue of an analyte (internal standard) has been added to the sample or extract, the retention time of the analyte should correspond to that of its labelled internal standard with a tolerance of ±0.05 min.

Requirements for fluorescence detection
This applies to molecules that exhibit native fluorescence and to molecules that exhibit fluorescence after either transformation or derivatisation. The selection of the excitation and emission wavelengths in combination with the chromatographic conditions should be done in such a way as to minimise the occurrence of interfering components in blank sample extracts. The nearest peak maximum in the chromatogram should be separated from the designated analyte peak by at least one full peak width at 10% of the maximum height of the analyte peak.

Requirements for mass spectrometric detection
Identification relies on proper selection of ions that are selective and specific for the analyte. Molecular ions or (de)protonated molecules (or, if not available, adducts) should be included in the measurement and identification procedure whenever possible. Low m/z fragments (<100) and fragment ions arising from loss of water or common moieties are often less specific, and may therefore be less useful for identification purposes.

Identification should be based on chromatographic peaks observed in the extracted ion chromatograms of two or more (see Table 1) ions that are specific for the analyte. The peaks must have a similar peak shape, overlap with each other, and the ion ratio (defined as the response of the
peak with the lower area divided by the response of the peak with the higher area) should be within ±30% (relative) to that obtained from the average of the calibration standards from the same sequence. The peaks need to be within the linear range of the detector and have an S/N ratio of at least 3. Where an extracted ion chromatogram shows evidence of significant interference, it must not be relied upon for identification.

In addition to the degree of selectivity of the ions measured, different types and modes of mass spectrometric detection provide different degrees of selectivity and specificity, which relates to the confidence in identification. The requirements for identification are given in Table 1. They should be regarded as guidance criteria, not as absolute criteria to prove presence or absence of an analyte.

*Table 1. Identification requirements for different MS techniques*¹

<table>
<thead>
<tr>
<th>MS detector / characteristics</th>
<th>Typical systems (examples)</th>
<th>Acquisition</th>
<th>Requirements for identification</th>
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<tbody>
<tr>
<td>Unit mass resolution</td>
<td>quadrupole, ion trap, TOF</td>
<td>full scan, limited m/z range, SIM</td>
<td>minimum number of ions: 3 ions</td>
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<tr>
<td>MS/MS</td>
<td>triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap</td>
<td>selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 product ions: 2 ions&lt;sup&gt;a&lt;/sup&gt; with mass accuracy ≤ 5 ppm for m/z ≤ 200, ≤ 1 mDa for m/z ≤ 200</td>
</tr>
<tr>
<td>Accurate mass measurement</td>
<td>High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS</td>
<td>full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof</td>
<td>2 ions&lt;sup&gt;b&lt;/sup&gt;: 1 molecular ion, (de)protonated molecule or adduct ion with mass accuracy ≤ 5 ppm (or ≤ 1 mDa for m/z ≤ 200) plus 1 MS/MS product ion&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
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</table>

<sup>a</sup> preferably including the molecular ion, (de)protonated molecule or adduct ion
<sup>b</sup> including at least one fragment ion
<sup>c</sup> no specific requirement for mass accuracy
<sup>d</sup> in case noise is absent, a signal should be present in at least 5 subsequent scans

References


