

Guidance document on identification of mycotoxins and plant toxins in food and feed

Implemented by 01/01/2023

Guidance document on identification of mycotoxins and plant toxins in food and feed

Preamble

Identification is an integral part of confirmatory analysis of mycotoxins and plant toxins in food and feed. This document has been established by and has been discussed in the EURL/NRL mycotoxin/plant toxin 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 sample analysis. This guidance supplements the "Specific requirements for confirmatory methods" from Annex II of Commission Implementing Regulation (EU) No xxx/xxx [6] and Commission Implementing Regulation (EU) xxx/xxx [7].

Identification requirements

For identification of mycotoxins and plant toxins, 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 selective for the targeted toxin(s) has been employed during sample preparation. The use of methods based on UV or FID 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, and also for erucic acid detected by GC-FID.

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 ± 0.1 min in both gas chromatography and contemporary (ultra) high performance liquid chromatography ((U)HPLC). For classical HPLC and/or isocratic elution, ± 0.2 min, or $\pm 50\%$ of the peak width at half height can be acceptable when the ± 0.1 min criterion proves too strict.

In case a ^{13}C -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. There should be a minimum of 50 nanometres between the excitation and emission wavelength. 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 [1].

Requirements for mass spectrometric detection

Identification relies on proper selection of ions. They must be sufficiently selective for the analyte in the matrix being analysed, and in the relevant concentration range. Molecular ions, (de)protonated molecules, or adducts are highly characteristic and should be included in the measurement and identification procedure whenever possible. In general, and especially in single-stage unit resolution MS, high m/z ions are more selective than low m/z ions (e.g. $m/z < 100$). However, high mass m/z product ions arising from loss of water, common moieties or loss of adduct may be of little use because they are often less specific, and may therefore be less useful for identification purposes. The choice of

ions for identification may change depending on background interferences. In high resolution MS, the selectivity of an ion of the analyte is determined by the narrowness of the mass extraction window (MEW) that is used to obtain the extracted ion chromatogram. The narrower the MEW, the higher the selectivity.

Identification should be based on chromatographic peaks observed in the extracted ion chromatograms of two or more (see Table 1) selective ions. 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 $\pm 30\%$ (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 a signal-to-noise (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, which relates to the confidence in identification. The requirements for identification are given in Table 1 [2]. 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 techniques¹

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		Minimum number of ions	Additionally
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3 ^{d)} Analyte peaks from ions in the extracted ion chromatograms must fully overlap.
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	Selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	Ion ratio from sample extracts should be within $\pm 30\%$ (relative) of average of calibration standards from same sequence
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap	Full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm ^{a, b, c)}	S/N ≥ 3 ^{d)} Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: e)

^{a)} preferably including the molecular ion, (de)protonated molecule or adduct ion

^{b)} including at least one fragment ion

^{c)} <1 mDa for $m/z < 200$

^{d)} Based on visual evaluation; in case noise is absent, a signal should be present in at least 5 subsequent scans

^{e)} depending on the acquisition conditions, higher variability may be observed, especially in case of wide precursor-ion selection windows. Therefore, deviations exceeding 30% may be acceptable.

¹ For definition of terms relating to mass spectrometry see Murray et al. (2013) Pure Appl. Chem., 85:1515–1609.

Provisions for specific toxins

For certain toxins multiple isomers exist. These may yield the same MS/MS transitions, but not always in the same ion ratios. When no suitable alternative transitions are available, and when the isomers cannot be chromatographically separated, issues may arise regarding meeting the ion ratio criteria for identification. In such cases, deviations from the $\pm 30\%$ relative ion ratio criterion are acceptable.

An known example of this situation are certain isomers of the pyrrolizidine alkaloids.

References

[1] Commission Implementing Regulation (EU) 2021/808 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. Off. J. Eur. Union L180 (21.5.2021) 84–109.

[2] SANTE/11312/2021 Analytical quality control and method validation procedures for pesticide residue analysis in food and feed.

[3] S.J. Lehotay, Y. Sapozhnikova, H.G.J. Mol, Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry, Trends in Analytical Chemistry 69 (2015) 62–75.

[4] H.G.J. Mol, P. Zomer, M. García López, R.J. Fussell, J. Scholten, A. de Kok, A. Wolheim, M. Anastassiades, A. Lozano, A. Fernandez Alba. Identification in residue analysis based on liquid chromatography with tandem mass spectrometry: Experimental evidence to update performance criteria, Analytica Chimica Acta 873 (2015) 1–13.

[5] B.J.A. Berendsen, T. Meijer, R. Wegh, H.G.J. Mol, W.G. Smyth, S.A. Hewitt, L. van Ginkel, M.W.F. Nielen, A critical assessment of the performance criteria in confirmatory analysis for veterinary drug residue analysis using mass spectrometric detection in selected reaction monitoring mode, Drug Test. Analysis 8 (2016) 477–490.

[6] Commission Implementing Regulation (EU) No xxx/xxx [revision 401/2006]

[7] Commission Implementing Regulation (EU) xxx/xxx [new plant toxin regulation on sampling & analysis methods]