

Identity confirmation by MS

MS data for identity confirmation

- MS provides the most valuable information for analyte identity confirmation.
- Identity confirmation data from MS:
 - m/z -s of quasimolecular ion, adduct ions and product ions.
 - m/z -s from high resolution (high mass accuracy) MS are particularly valuable.
 - Ion intensity ratios.
- With respect LC-MS analysis validation guides by SANTE and 2021/808 are most specific – give guidelines and set criteria.

Requirements for mass spectrometry

- Reference spectra for the analyte.
 - Use the same instrument and operating mode as for samples.
 - Preferably, record within the same analysis batch with samples.
- Diagnostic (characteristic) ions.
 - The quasimolecular ion should be involved in identification procedure.
 - High m/z ($m/z > 100$) product ions are more specific than low m/z ions.
 - Product ions due to common losses (eg H_2O , NH_3) are of little diagnostic value.
 - The choice of diagnostic ions depends on matrix interferences.

Requirements for mass chromatograms

- Extracted ion chromatograms for the analyte.
 - Analyte peaks in sample should have similar retention time, peak shape and response ratio as in calibration standard.
 - Chromatographic peaks on extracted ion chromatograms for the same analyte must overlap.
 - For quantitation use the ion that shows the highest S/N ratio and no chromatographic interference.

Ion intensity ratios

- In addition to presence of specific ions in mass spectrum, also their **relative intensity** must match that of standard substance.
 - Intensities are found from mass spectra or by integrating respective chromatographic peaks.
- Tolerance limits are set for relative intensities by two guideline documents:
 - 2021/808: $\pm 40\%$
 - SANTE: $\pm 30\%$
- In both cases $S/N \geq 3$ for all ions.

Required number of ions

- Number of ions required for analyte identification depends on used MS technique and operating mode.
 - For example, identification criteria of SANCO.

MS detection	Identification requirement
Single stage low resolution MS	≥ 3 diagnostic ions
Low resolution MS/MS	≥ 2 product ions
High resolution MS	≥ 2 diagnostic ions with mass accuracy ≤ 5 ppm

- For diagnostic/product ions recorded on low resolution MS, criteria for ion intensity ratios must be met.

Required number of ions

- 2021/808 uses system of identification points.
 - 4 points for authorized substances.
 - 5 points for unauthorized or prohibited substances.
- Number of identification points earned depends on technique.
 - For example:

Technique	Identification points earned
Chromatographic separation	1.0
MS precursor	1.0 (indirect)
MS ⁿ product	1.5
High resolution MS ion	1.5
High resolution MS ⁿ product	2.5

Example of identification points calculation

- Calculate the number identification points earned for LC-MS/MS method with 2 transitions for an analyte.
 - Chromatographic separation → 1 point
 - 1 precursor ion → 1 point
 - 2 transition products, 1.5 points each → 3 points
 - Total identification points earned: 5