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 SANCO and 2002/657/EC 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 ($m/z > 100$) ions are more specific than low m/z ions.
  - Product ions by 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.
  - 2002/657/EC tolerance limits for relative ion intensities.

Relative intensity (% of base peak)	LC-MS, LC-MS^n (relative)
> 50%	± 20%
> 20% to 50%	± 25%
> 10% to 20%	± 30%
≤ 10%	± 50%

  - SANCO sets tolerance limit of ± 30% (relative) independent of peak intensity.

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 mode	Identification requirements
Single stage MS (low resolution)	≥ 3 diagnostic ions
Single stage high resolution MS	≥ 2 diagnostic ions; at least one fragment
MS/MS	≥ 2 product ions

  - For all diagnostic/product ions criteria for ion intensity ratios must be met.

Required number of ions

- 2002/657/EC uses system of identification points.
  - 4 points for substances having anabolic effect and unauthorized substances.
  - 3 points for veterinary drugs and contaminants.
- Number of identification points earned depends on MS technique.
  - For example:

MS technique	Identification points earned per ion
MS^n precursor ion	1.0
MS^n transition products	1.5
High resolution MS^n precursor ion	2.0
High resolution MS^n transition product	2.5

Example of identification points

- Calculate the number identification points earned for LC-MS/MS method with 2 transitions for an analyte.
  - 1 precursor ion → 1 point.
  - 2 transition products, 1.5 points each → 3 points.
  - Total identification points earned: 4.