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Focus
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Mass spectrometry: do we need it?

Mass spectrometry

Mass spectrometry is an analytical technique that determines the molecular mass of compounds while also identifying and quantifying the compounds under analysis.

The mass spectrometer is an instrument made of many modules arranged in series, which sequentially ensure:

- The loading of the sample;
- The evaporation and ionisation of the molecules within a module known as the ion **source** (this results in the conversion of molecules in their natural state into gas phase ions);
- The acceleration of these ions;
- The sorting and separation of these ions within a module known as the **mass analyser**, which sorts the ions in function to their m/z ratio (mass-to-charge ratio);
- The detection, i.e. obtaining a mass spectrum.

The sources used depend on the compounds under analysis

Under low pressure

1. **Ionisation and fragmentation using electric collision:** This technique is adapted to compounds of a molecular mass of less than 1,000 daltons, which are easily volatile and stable at a high temperature.
2. **Positive chemical ionisation:** This technique is softer than the previously mentioned method and is suitable for the same type of compounds.
3. **Matrix-assisted laser desorption/ionization (MALDI):** This is a soft ionisation technique using a metal plate made of a co-crystallised mix (molecule matrix + analyte). This technique is rarely used for the analysis of organic molecules with a mass of below 500 daltons.

NB : a similar technique **(SELDI-TOF: Surface enhanced laser desorption-ionisation at time-of-flight)** is combined with a flat surface chromatography protein and peptide separation system (this technique can have low reproducibility).

At atmospheric pressure

1. Electrospray nebulisation **(ESI)** : Suitable technique for an analyte introduced after liquid chromatography or capillary zone electrophoresis. Compatible with small outputs.
2. Atmospheric pressure chemical ionization **(APCI)** : This technique can be used following liquid chromatography (complementary with ESI technique)

Mass analysers

a) The TOF analyser (TOF =time-of-flight)

The application of an electric field means that the distance travelled by an ion to the detector (time-of-flight) depends on the m/z ratio (lighter ions will reach the detector **avant** the heavier ions).

b) Analysers using an oscillating electrical field:

- Quadrupole mass filter analysers: These systems select ions with a defined m/z ratio. These ions can pass through the detector without losing their charge on contact with the quadrupole rods.
- Ion trap analysers. In these systems, the electric field confines ions with different m/z ratio in defined spaces within the analyser. The spectrum is obtained by ejecting the ions in function to their m/z ratio.

Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) usually combines the use of two analysers. The formed ions found at the source enter into the first analyser MS1, where only ions of a defined m/z ratio can pass through. In a collision cell, these selected ions are split into ion fragments that can then be analysed by the second analyser MS2. In laboratories, the triple quadrupole mass spectrometer is the model most frequently encountered.

The MS/MS has three major advantages:

- The capacity to study numerous molecules regardless of whether they are from the same structural family or not;
- The capacity to highlight the specific metabolites of a disease;
- It's an automated technique offering the possibility of large-scale analysis.

Combined with separation techniques

1. Coupled gas chromatography techniques

With separation of thermally vaporised molecules and carried by a neutral gas into a MS or a MS/MS. Sample preparation is a complicated procedure.

2. Coupled liquid chromatography techniques (or capillary zone electrophoresis)

Once released from the analytical column, ionisation is performed by either **ESI ou APCI**.
These techniques have enormous potential; the ions are not molecular fragments; the efficiency of the ionisation is molecule dependant; there is a risk of charge transfer between compounds.

3. 2D gel electrophoresis

2D gel electrophoresis is the main tool used for the separation of thousands of proteins (proteomic analysis). The proteins within the biological sample are initially separated by isoelectrofocalisation in function to their isoelectric point and then by sodium dodecyl sulphate polyacrylamide gel electrophoresis (PAGE-SDS) in function to their mass. The areas of interest are cut out of the gels and then treated with a protease. The peptide masses are obtained by **ESI or MALDI-TOF mass spectrometry**. However, this technique is not suitable for proteins expressed at a low level, highly hydrophobic proteins or membrane proteins.

Applications in molecular biology

Note: proteomic analysis also relies on the use of mass spectrometry; this area has not been covered here as its objectives are less directly associated with routine clinical needs; indeed, it is used more for the collection of data for fundamental biology, to identify sensitive and specific disease markers or therapeutic intervention targets.

1. Application in bacteriology and mycology

Rapid identification of microorganisms by MALDI-TOF MS relies on the analysis of ribosomal proteins and membrane-associated proteins following removal from a colony or protein extraction. This identification procedure uses a comparison spectrum obtained through the use of valid reference spectra. In regards to performance, publications state that it is at least 90% as performant as conventional identification techniques.

2. Applications en biochimie

Generally, this is the reference technique.

- Vitamin D: Possible demonstration of both D2 (medication) and D3 forms (endogenous and nutritional synthesis).
- Steroids: Non-conjugated steroids by GC-MS/MS; conjugated forms by LC-MS/MS. NB: access to bioavailable testosterone.
- Metabolic disorders: Mass spectrometry forms part of the group of essential methods for the diagnosis and follow-up of metabolic hereditary diseases, including: aminoacidopathies, urea cycle disorders, organic acidurias and fatty acid beta-oxidation disorders (acylcarnitine profile).
- Bile acids, free fatty acids and sterols.

3. Pharmacological and therapeutic follow-ups

Examples: immunosuppressive agents (cyclosporin, tacrolimus, sirolimus, everolimus and mycophenolic acid), neuroleptic agents (clozapine, haloperidol, penfluridol, thioridazine, flupentixol and zuclopenthixol), hypnotics such as benzodiazepine compounds (lorazepam, bromazepam, flunitrazepam, clonazepam, alprazolam, zopiclone and zolpidem), antimycotic agents (posaconazole), medication used for the treatment of acute myeloid leukaemia: erufosine (acylphosphocholine), antihistaminics and antiretroviral agents.

4. Toxicology (toxic agents, performance drugs and narcotics)

- Toxic agents: metals (up to 34 elements) and cyanides.
- Performance drugs: detection and identification of anabolic steroids (nandrolone and metabolites: norandrosterone and norethiocholanolone).
- Narcotics and drug-facilitated crime: opiates, cocaine, buprenorphine and norbuprenorphine, cannabis, LSD, ecstasy derivatives, GHB and ketamine.

Note: Mass spectrometry is also applied in veterinary medicine; it is also frequently used in the agri-foodstuffs sector and for the protection of the environment.

Conclusion

The advantages of mass spectrometry:

- Access to important assays (when in a competitive market): medications (such as immunosuppressive agents and antiretroviral agents), drugs, steroids + vitamin D and hereditary metabolic disease etc.;
- This technique is also essential for certain clinical trial investigations;
- Beyond the field of human clinical pathology, MS could prove to be an asset in veterinary pathology as well as in the agri-foodstuffs and environmental sectors.

The disadvantages of mass spectrometry:

- The cost, requiring a significant materials/equipment budget.
- Multifunctional systems do not exist. As we have already seen, prior to result release, the analytical chain can vary in function to the molecule under investigation (GC or LC-MS, type of ionisation, working under low or atmospheric pressure, TOF assay or the use of oscillating electric fields, MS or tandem MS).
- MS must be performed by highly specialised technicians.

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