

High-performance liquid chromatography/mass spectrometry (LC/MS)

Mass spectrometry (MS) provides us with uniquely valuable information: molecular mass (via mass to charge (m/z) ratio); molecular structural information and quantitative data, all at high sensitivity. However, it is best to apply separation techniques to complex mixtures before mass spectrometry is undertaken. High-performance liquid chromatography (LC) is excellent for separating mixtures but generally poor at identification of compounds. The combination of these two techniques (LC/MS) thus provides an extraordinarily powerful analytical tool.

Initially the marriage of MS and LC was uneasy and many interfaces have been tried over the last 20 years. Nowadays the relationship is more harmonious, thanks to the development of electrospray ionisation, the best means currently available for transferring molecules from solution into the gas phase for MS analysis.

LC/MS Instrumentation

Figure 1 shows a schematic diagram of a mass spectrometer linked to LC. The mass spectrometer separates the ionised molecules that have been transferred to the gas phase, after desolvation of the LC eluent. The transfer takes place in the interface and may occur at atmospheric pressure or in vacuum, depending on the type of ionisation employed. Ions are then separated on the basis of their mass and charge (m/z) at high vacuum in the mass analyser. There are currently about 60 different mass spectrometers on the market, available from about a dozen manufacturers, with various ionisation methods, mass analysers and detector types, and a wide range of prices.

Methods of ionisation

The coupling of LC with mass spectrometers has been an important development and continues to evolve. Interfacing began in the early 1970s and involved techniques for evaporating solvent and splitting the flow from LC columns to admit eluted compounds into the high vacuum of the spectrometer. However, it was not until 1987 and the commercialisation of atmospheric pressure ionisation (API) that LC/MS became prominent. Interfaces involving sources at vacuum (such as thermospray, particle-beam and continuous-flow fast atom bombardment) are still in use, but API interfaces are by far the most widely used, being the most inherently suitable for LC/MS coupling. API involves 'soft' ionisation, producing little fragmentation of molecules so providing molecular weight information. However, fragmentation may be induced subsequently to obtain molecular structural information. There are three main API techniques, electrospray ionisation (ESI), atmospheric pressure

chemical ionisation (APCI) and the relatively new technique of atmospheric pressure photo-

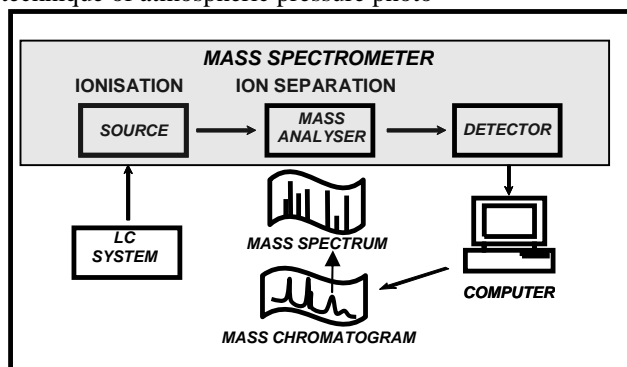


Figure 1. Schematic diagram of mass spectrometer linked to LC.

ionisation (APPI). Figure 2 shows the applicability of these ionisation techniques for analytes of different polarity and molecular weight. Gas chromatography/MS (GC/MS) is included for comparison.

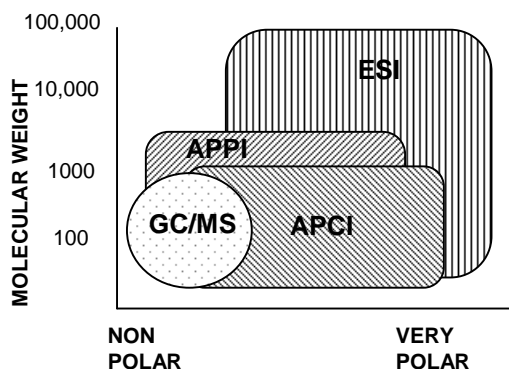


Figure 2. Applicability of ionisation techniques to molecules of various polarities and molecular weights.

ESI is most widely used in LC/MS and ions are usually formed by protonation of molecules in positive mode and deprotonation in negative mode, the choice being dictated by the characteristics of the analyte. However, some MS instruments can rapidly switch between modes in alternate scans. In ESI, ionised droplets are produced by applying a high voltage (typically 3-5kV) to the outlet of a capillary carrying the LC eluent. A fine mist of charged droplets is produced at atmospheric pressure, aided by nebulisation with nitrogen gas which is also used as a drying gas together with source heating to de-solvate the droplets. The de-solvated ions are guided through 'skimmers' into the high vacuum region of the mass analyser by application of electric fields. 'Nanospray', a development of electrospray, uses flow rates lower than microlitres per

minute, with a narrower capillary outlet, providing smaller droplets and more efficient ionisation. Multiple sprayers (2-8) may be used in either technique: independent liquid streams are fed into the MS source and sampled sequentially into the mass analyser. This allows coupling of up to 8 LC systems into one mass spectrometer and the use of a standard reference solution for accurate mass measurement with high resolution instruments (to determine empirical formulae). ESI flow rates range from nL/min to about 200 μ L/min and response is dependent on the concentration of analyte rather than amount, allowing use of microbore and capillary columns, or even chip-based LC. With larger columns the best performance is obtained by splitting the flow via a simple T-piece. Capillary electrophoresis and electro-chromatography may also be linked to MS.

APCI is a development of ESI, in which the LC eluent is rapidly evaporated on passing through a nebuliser at high temperature. Ionisation is produced by corona discharge in the spray and solvent ions are produced that can react with the analytes in the gas phase (chemical ionisation). Higher flow rates of ml min⁻¹ can be used and a greater degree of fragmentation takes place. APPI is a newer technique which is reported to have fewer matrix effects than ESI or APCI. LC eluent is sprayed with a nebulising gas into a heated probe, as in APCI, and a 'dopant' compound is vaporised and ionised by UV radiation, forming 'photo-ions'. The photo-ions initiate a cascade of ion-molecule reactions, forming ionised analyte.

ESI is generally most suitable for relatively polar molecules, across a wide range of molecular mass, while APCI and APPI are most suitable for small (less than 1000 Da) relatively non-polar molecules.

Mass analysers

The type of mass analyser defines the mass spectrometer. Thus there are sector instruments relying on magnetic and electric fields for m/z ion separation, quadrupole instruments using radio frequency and DC voltage, the related ion-traps, and measurement of ion flight time in time-of-flight (TOF) analysers. It is beyond the scope of this technical brief to describe these in more detail, but all have advantages and disadvantages in coupling with LC, in respect of cost, data acquisition rate, resolution, sensitivity etc. Sector instruments are difficult to couple with LC for several reasons, but the other analysers are well represented in LC/MS instruments. There are other instruments, including FTICR (Fourier transform ion cyclotron resonance) and the 'Orbitrap', but these are not described here.

Mass analysers may be coupled serially to provide a variety of tandem MS/MS experiments, including fragmentation of a selected ion from the first mass analyser by collision with an inert gas in a collision cell, as shown schematically in Figure 3. In tandem MS/MS, separation of the fragments from the selected precursor ion occurs in the second mass analyser, with the resulting product ion spectrum providing structural information. There are several other MS/MS experiments possible, including precursor ion scans, constant neutral loss scans, and single and multiple reaction monitoring, but describing these is beyond the scope of this

brief. Similar or different mass analysers may be coupled for various reasons, for example, quadrupole/quadrupole and quadrupole/TOF are popular combinations for LC/MS/MS. Ion-traps are able to perform multiple mass spectrometry (MS_n) experiments within the space of the trap, rather than MS/MS in time. Quadrupole and ion-trap instruments are currently most common in LC/MS, but there is a trend towards the use of TOF instruments.

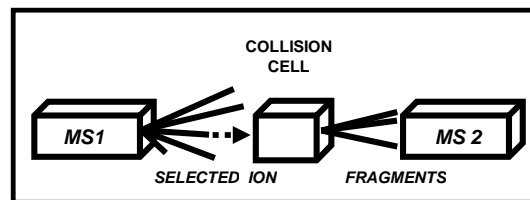


Figure 3. Tandem MS/MS Mass Spectrometer

Applications of LC/MS

Applications of LC/MS cover a vast range, often involving quantification as well as molecular structural studies, and include studies in drug metabolism, clinical chemistry, natural products, trace contaminants in food and the environment, chemical synthesis structure confirmation, and many others. Of particular note is the use of MS and LC/MS in the determination of protein structures in the relatively new field of proteomics: this is a large and fast growing application area. Metabolic products of the proteome are also studied in the new field of metabonomics as are biomarkers as indicators of disease states.

Further reading

Details of mass spectrometers and LC interfaces can be found in many texts on instrumental analysis. Purchase of LC/MS instrumentation requires a critical evaluation of a wide range of instrumental features in relation to the intended application and these are comprehensively covered in a recent report of the Instrumental Criteria Subcommittee:

Evaluation of analytical instrumentation-Part XXII Instrumentation for liquid chromatography/mass spectrometry. Analytical Methods Committee, *Accred Qual Assur*, 2007, **12**, 3-11.

Gwyn Lord

This Technical Brief was prepared for the Analytical Methods Committee by the Instrument Criteria Sub-committee (Chairman Prof. S. Greenfield).

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