Separation techniques allied to mass spectrometry:

Mass spectrometry lends itself to being linked directly to various separation techniques, most notably gas chromatography (GC) and liquid chromatography (LC). With both techniques, material eluting from the GC or LC column is taken directly into the ion source of the mass spectrometer, where it is ionised in the normal way. Normally, the mass range is being repeatedly scanned during the time compounds are eluting, so that complete spectra of all separated components are obtained.

Some restrictions on the combination of separation and ionisation methods apply:

	E.I.	C.I.	FAB	MALDI	Electrospray	APcI
GC	Yes	Yes	No	No	No	No
LC	Possible	No	Possible	No	Yes	Yes

Supercritical Fluid Chromatography (SFC) and Capillary Electrophoresis (CE) may also be interfaced to mass spectrometers.

Derivatisation:

Compounds for analysis by GC/MS must be volatile enough to be amenable to GC, and also sufficiently thermally stable to survive the extended time the sample must spend in the gas phase. Many compounds which do not meet these criteria can be modified by derivatisation to increase their volatility and thermal stability.

Trimethyl silylation is one of the most common derivatisations used for GC/MS. It is applicable to alcohols, phenols, acids, amides and amines.

Methylation using diazomethane is another common derivatisation method, and results in methyl ethers and methyl esters when applied to phenols and acids respectively.

Involatility is a result of either high molecular weight or strong inter-molecular forces, usually caused by hydrogen bonding, or both. Replacement of active hydrogen atoms will increase volatility, even though the molecular weight of the resulting derivative is higher than that of the original compound.

In general, increasing volatility consists of replacing polar functional groups in a molecule with groups of lower polarity.

Derivatisation may also be used to increase the likelihood of an intact molecular ion being observed in the E.I. mass spectrum.

Quantitative analysis:

Selected Ion Recording (SIR):

When a mass spectrometer analyser is scanned through a range of masses, a very short time is spent at each mass. This can result in very few ions of a required mass being collected, limiting sensitivity. If, instead of scanning a wide mass range, a few ions can be monitored, greatly enhanced sensitivity results. This technique (SIR) is commonly used allied to GC/MS or LC/MS to detect and quantify known compounds in mixtures.

The specificity of the technique is increased by monitoring more than one mass known to be present in the spectrum of the target compound.

In a high-resolution instrument, it is possible to reduce any cross-contamination between masses by performing SIR at high resolution, thus increasing further the specificity.
The highest level of selectivity is achieved by monitoring ions which have been formed by a known fragmentation pathway in the target molecule. This is possible using MS/MS instrumentation.

Using selected ion recording, it is possible to determine the quanity of specific compounds in mixtures. This technique is normally carried out in conjunction with gas chromatography (GC/MS) using EI or CI, but quantitation is also possible using LC, and also other ionisation methods.

The intensity of an ion measured by SIR is a measure of the response of a compound to the conditions in the mass spectrometer. In order to measure the quantity of a compound in a sample, a calibration must be performed to measure the response of the instrument to a differing quantities of the compound to be measured. The unknown quantity may then be introduced, and the instrument response used to determine the quantity present. This is an **external** standard calibration method, and is prone to considerable error, due to variations in instrument conditions between calibration and measurement, and variations introduced during sample work-up.

For greater accuracy, an **internal** standard is used to eliminate instrumental and sample preparation errors. Calibration is carried out with known quantities of analyte mixed with a fixed quantity of standard. The ratio of the responses of the sample and the standard are plotted against the molar ratio of the two compounds in the mixture. The unknown sample is then 'spiked' with a the same amount of the internal standard, and the calibration curve used to determine the quantity of the compound.