Got Gas? Try GCMS!
What you need to know before method development

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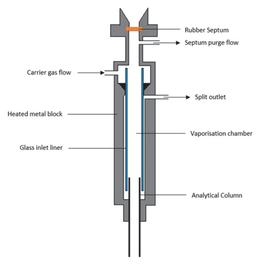
GCMS is a hybrid system with a Gas Chromatograph (GC) combined with a Mass Selective Detector (MS). Gas chromatography is a separation technique whereby sample components are separated by their retention in the analytical column. This is determined by their affinities for the stationary phase and their volatility. More volatile species will generally elute first and reach the MS. The MS detects the analyte by first ionising the molecules and then distinguishing them based on their mass-to-charge ratios (m/z).

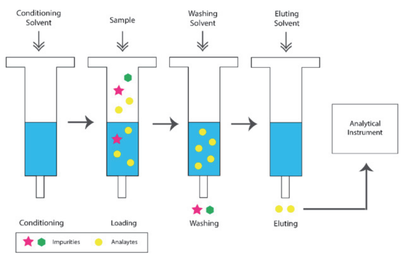
Sample preparation

Sample preparation techniques for direct injection

- If the target analytes are not volatile enough for headspace analysis, you may consider direct injection.
- As the name implies, the sample is injected directly onto the column through the inlet – therefore the sample should be in a volatile solvent.
- Complex sample matrices, including aqueous and solid pharmaceutical formulations, will often require clean-up to remove excipients and other components that are not of interest.
- Here are the two most common extraction techniques, which are used in a variety of analytical settings:



Solid phase extraction



In SPE, samples are loaded onto a cartridge containing a stationary phase (SP) designed to adsorb target analytes based on their chemistry.

The cartridge is often pre-treated, for example, with acid, to prime the SP to best adsorb analytes.

A washing solvent is applied to preferentially elute impurities.

Next, a solvent with similar characteristics to the analytes is pulled through the cartridge (sometimes under vacuum) to elute the targets in a more suitable matrix for analysis.

Particularly complex matrices may require additional clean-up.

Liquid-liquid extraction

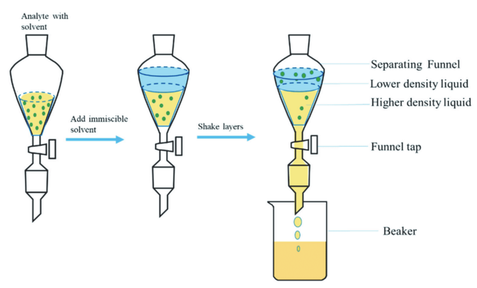
LLE proceeds by selecting a solvent that is immiscible with the sample formulation.

Repeated washings of the sample formulation with the second solvent will cause molecules to migrate from one solvent to the other based on hydrophilic or hydrophobic character, until equilibrium is reached.

In this way, target analytes can be extracted into an excess of organic solvent from an aqueous matrix. Typically, three extractions are performed and combined.

The addition of salts, acids, or bases can be used to tailor the extraction to specific analytes.

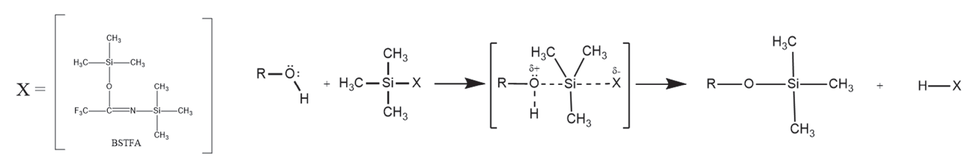
The sample can then be concentrated by evaporating excess solvent (e.g.: under nitrogen stream).



Derivatisation – for pesky analytes

Hydrophilic species exhibiting dipole interactions are usually non-volatile and have poor ionisation efficiency by electron impact (EI) – for example, sugars. In these cases, it may be best to derivatise the functionality of the target species to bestow it with more favourable characteristics (greater hydrophobicity and volatility).

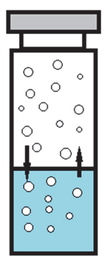
A commonly used reagent for derivatising hydroxyl groups is BSTFA. BSTFA works by replacing acidic protons on hydroxyl groups with trimethyl silyl groups. The resulting molecules are branched, non-polar, and more volatile, making them better candidates for EI GC-MS. MSTFA works by the same mechanism.



*Note: This derivatisation process **must** be conducted in dry, aprotic solvent. Aqueous or protic solvents will consume the derivatising agent by the same mechanism and prevent the target species from reacting to completion.*

Preparation techniques for headspace

- If the target analytes are volatilisable at low temperatures (e.g. <100 °C), and the sample matrix is a complex liquid solution, headspace analysis may be a good option.
- Headspace analysis works by heating the liquid sample such that an equilibrium forms between the liquid phase and the gas phase (headspace) of a vial. This equilibrium can be controlled to optimise the amount of analyte in the gas phase, by changing headspace parameters, by altering the pH, or by 'salting out' analytes. A gas tight syringe then pierces the vial septum and pulls a set volume of gas to inject onto the column.
- This procedure cuts down on sample preparation requirements by only extracting volatile species. Water and non-volatile impurities are left in the liquid phase and are not injected onto the column, and therefore presents the instrument with a cleaner sample.



Internal standards – a silver bullet?

Internal standards can be used to compensate for some of the impacts of sample matrix, extraction procedures, and instrument effects (such as ionisation method) on quantitation.

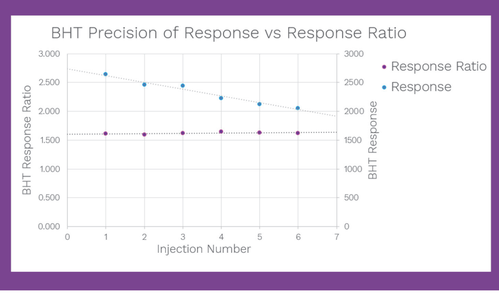
The theory is that chemically homologous species will respond equally to sample matrix conditions, extraction procedures, and instrumentation. Typically, deuterated or C_{13} – labelled compounds of the target species are used, as they are chemically identical but have different molecular masses – and can therefore be distinguished by a mass spectrometer.

These internal standard species are spiked into the sample formulation before sample preparation, and therefore undergo the same losses in recovery as the targets. Thus, the ratio of target species to internal standard species in the prepared sample should be unaffected by matrix, prep, or instrument bias.

Case study: Use of deuterated internal standards to correct for drift in the quantitative analysis of BHT

Butylated hydroxytoluene (BHT) showed a considerable negative drift. This reduces confidence in quantifying unknown samples.

The solution was to spike the samples and standards with an equal concentration of deuterated BHT (d21-BHT). The internal standard drifts in the same manner as BHT, giving a consistent response ratio (RR) between them.



Instrument parameters

The GC inlet

The inlet is the interface which allows transfer of sample onto the analytical column. Many types of GC inlets exist. For capillary columns, the most common type is split/splitless (S/SL).

Parameters to consider include injection volume, inlet temperature, split ratio, splitless time, and inlet pressure/flow, which may help to inform the desired sample concentration to get the appropriate mass on-column for detection and identification.

Split injection	Splitless injection
Uses a split ratio (e.g.: 1:50) to remove a portion of vaporised sample from the inlet. This can be optimised to reduce the amount of matrix injected and maintain sensitivity.	In splitless injection, the split valve is kept closed initially, allowing the majority of sample to enter the column.
High carrier flow through the inlet pushes sample vapour onto the column faster than in splitless injection, resulting in narrow analyte bands on-column.	Sample vapour moves very slowly onto the column due to lower carrier flow through the liner, meaning broader analyte bands.
Requires higher sample concentrations to achieve sensitivity as most of the sample is purged through the split vent.	Allows for lower sample concentrations as a greater portion enters the column.
Allows for higher initial oven temperatures as solvent re-focusing is unnecessary.	Requires a cooler initial oven temperature to re-focus analytes on the column.
Small sample residence time in the inlet can lead to poor mixing and inlet discrimination for high boilers.	Purge time must be optimised to avoid inlet discrimination and excessive solvent tailing.

For **splitless** injection, it is important to avoid a solvent/stationary phase polarity mismatch. In cases where this is unavoidable, a guard column with similar polarity to the solvent may be used between the inlet and the analytical column.

Column selection

The four main factors in column selection are:

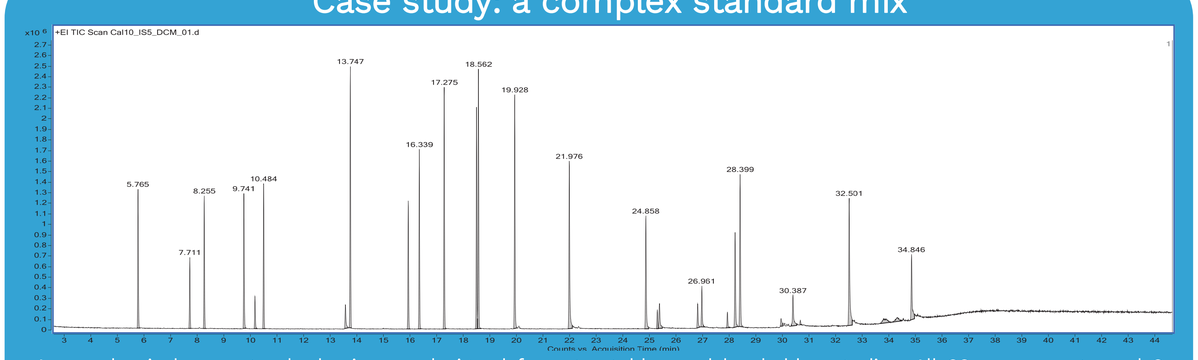
1. Stationary phase: Targeted analyses will require some knowledge of analyte chemistry. Most important are polarity/polarizability. In general, try to choose stationary phases with similar characteristics to the targets. Generally, bonded phases are preferred as they resist column bleed.
2. Internal diameter: For GCMS, column diameter is usually 0.32 mm or less due to the need to maintain a vacuum at the MS. For most applications the optimum trade-off between column efficiency and sample capacity is around 0.25 mm.
3. Film thickness: Thinner films generally mean narrower peak widths, shorter retention times, and reduced column bleed, at the expense of analyte interaction with the exterior tubing.
4. Column length: Greater column length gives greater resolution and longer retention times but requires greater column head pressure. 30 m is a good compromise for most applications.

The GC oven ramp

The initial temperature will be selected to ensure retention of the most volatile species in the analysis. For splitless injection, the aim is to condense the solvent onto the front of the column, and so the initial temperature is usually held at least 10 °C below the solvent boiling point.

Typically, the oven ramp is devised to elute all of the sample components quickly while ensuring peak resolution. Carrier flow and column pressure will also affect retention and column efficiency. It is important to note that due to the need to maintain a vacuum in the MS, carrier flows are generally much slower in GCMS than in conventional GC setups.

Case study: a complex standard mix



A complex in-house standard mix was designed for extractables and leachables studies. All 23 components and 3 internal standard components have been resolved without excessive run-time. Increasing the ramp rate would sacrifice resolution for closely-eluting peaks (e.g.: at 18.5 mins). Full separation allows for reliable identification using NIST library search.

The mass selective detector: ion sources

The two most common source types are Electron Impact (EI) and Chemical Ionisation (CI):

Electron impact:

EI is a hard ionisation technique. Electrons are emitted and accelerated to 70 eV towards the sample gas. The resulting interactions produce unique and highly reproducible fragmentation patterns. Spectra obtained in the lab can therefore be compared directly to large external databases (e.g.: NIST) for simple identification.

Chemical ionisation:

CI is a softer ionisation technique than EI, allowing for the preservation of the molecular ion and adducts of the molecular ion, making it a useful technique for determining the molecular weight of analyte species. Negative Chemical Ionisation (NCI) is a highly selective technique, especially useful for halogenated species.

Types of mass spectrometer: a brief overview

MS experiments can be run in scan mode across a range of mass-to-charge ratios (m/z), e.g.: 30 – 500, or in Selected Ion Monitoring (SIM) mode, whereby only ions of specific m/z are recorded by the detector. Tandem mass spectrometers can also run Multiple Reaction Monitoring experiments (MRM), whereby selected parent ions are filtered through and fragmented into parent ions reproducibly to give increased selectivity.

The most common mass analyser is the Quadrupole. Quadrupoles can be used alone in single quadrupole (SQ) systems combined in tandem in triple quadrupole (QQQ) systems whereby the second quadrupole acts as a collision cell to further fragment precursor ions for MRM experiments. Time-of-flight (ToF) systems can give accurate mass data to confirm molecular formulae, and when combined with two quadrupoles (Q-ToF), in a similar setup to QQQ instruments, can also run MRM experiments.

System	Scan	SIM	MRM	Accurate mass
SQ	✓	✓	×	×
QQQ	✓	✓	✓	×
ToF	✓	✓	×	×
Q-ToF	✓	✓	✓	✓

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