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Bridging the Gap

A Molecule's Journey Through Gas Chromatography

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A Molecule's Journey Through Gas Chromatography

What is Gas Chromatography?

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Gas Chromatography

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"**Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction."

"Gas Chromatography is a type of chromatography that involves the use of an inert or unreactive gas to separate the chemicals in a mixture."

Generally used in Analytical Chromatography

Sample travels through instrument in gaseous state

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GC Instrumentation

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Samples

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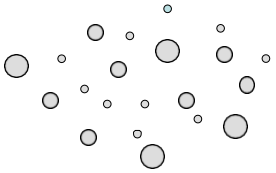
- Can be a Solid a Liquid or a Gas
- A sample is made up of:
  - Analytes = compounds of interest
  - Matrix = other components not interested in
  - Matrix interference = matrix component(s) which interfere with the analysis of analytes





Analytes

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- Are organic compounds of interest which are:
  - Volatile enough to be vapourised & carried by carrier gas through a GC instrument, usually below 400°C
  - Do not decompose at temperature required to vapourise sample

Only around 20% of known organic compounds can be analysed by GC!

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How does Gas Chromatography work?

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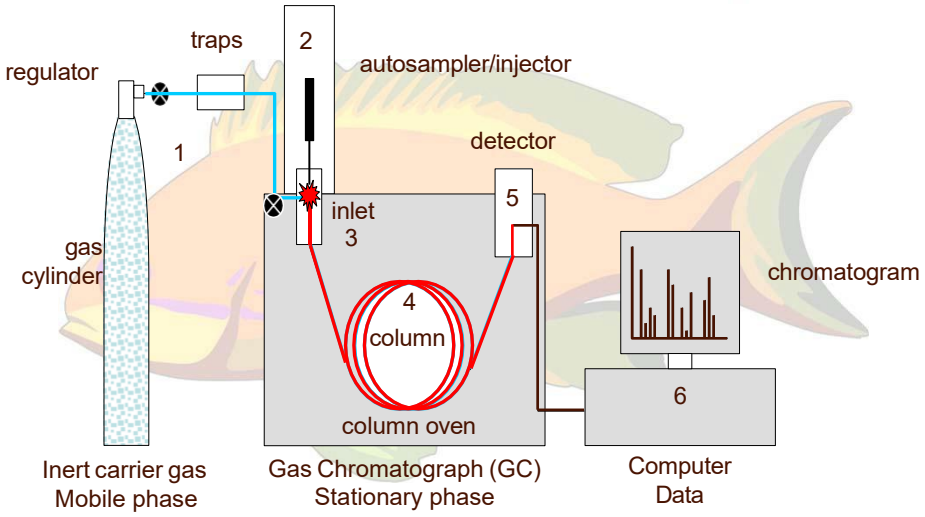
GC Instrumentation



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1) Mobile phase

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- Carrier gas needed to transport vapourised sample through GC
- Inert or unreactive gas
  - *Doesn't react with sample*
- Easily available in high purity
  - *Relatively cheap*
  - *Doesn't add extra peaks to your chromatogram*
  - *Doesn't increase noise levels*
  - Grade 5.0 or higher is recommended
- Commonly used gases:
  - Helium: most common, inert & non-flammable but expensive!
  - Nitrogen: common for volatiles analysis, cheap
  - Hydrogen: becoming more common, cheap but flammable & reactive!

Mobile phase flow rate

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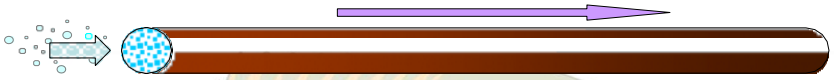
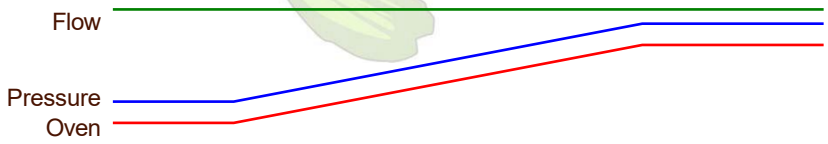


- Pressure applied to head of analytical column to produce flow of carrier gas through column
- Head pressure controlled by Electronic Pressure Control module (EPC) or regulator
- Making changes to head pressure changes flow of gas through column
- Flow rate depends on:
  - Head pressure, column dimensions, carrier gas type & oven temperature
    - Higher temperature → gas more viscous = slower flow rate

Flow & Pressure

- **Constant pressure**
  - o Head pressure held constant throughout GC run
  - × As oven temperature increases gas viscosity increases so flow reduces
  - × Peaks broaden & take longer to elute
- **Constant flow**
  - o EPC increases head pressure as oven temperature increases
  - ✓ Column flow is maintained at a constant
  - ✓ Peaks elute faster & broaden far less



2) Autosamplers

- Simple autosamplers to inject pre-prepared liquid samples
  - – Washes syringe with solvents to prevent carryover
  - – Eliminates air bubbles & uses pull-up delays to ensure full sample volume is pulled into syringe
  - – Fast injection & post-injection delay to ensure full volume is injected into GC
  - – Accurate measurement of sample volume
  - – Reproducible
- Advanced autosamplers can prepare solid, liquid or gas-phase samples for analysis

3) Inlets

How do you get your sample into analytical column?

- Aims:
  - To introduce analytes in a tight sample band into analytical column (to obtain sharp peaks)
  - To be representative of the sample
  - To not introduce any chemical change
  - To be repeatable & reproducible in doing so

Injection onto the column

- We need a set-up to:
  - Stop mobile phase escaping
  - Keep the pressure constant
  - Stop air & contaminants getting onto analytical column
- Therefore we use an inlet :
  - Split / splitless
  - Cool on-column
  - Programmable
- And an injection technique
  - Manual
  - Or automated

Vapourising injections

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What happens if we have a dirty sample?

- Vapourise sample before putting onto analytical column
- Hot injection: inject sample into a hot inlet so that it immediately vapourises
- Cold injection: inject sample into cold inlet below solvent b.p. then heat it to vapourise sample
- Transfer vapourised sample onto column
- Dirt is left inside inlet liner

Hot splitless principles

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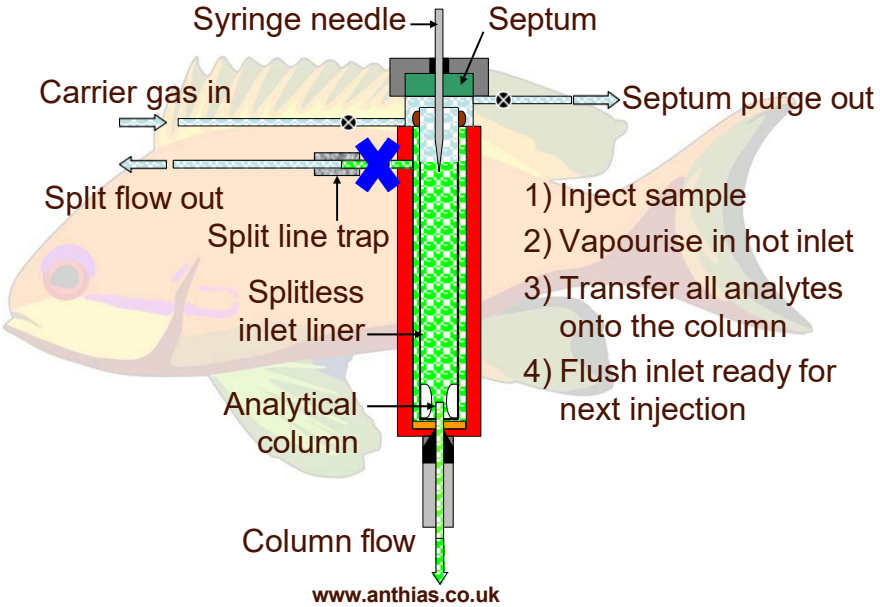


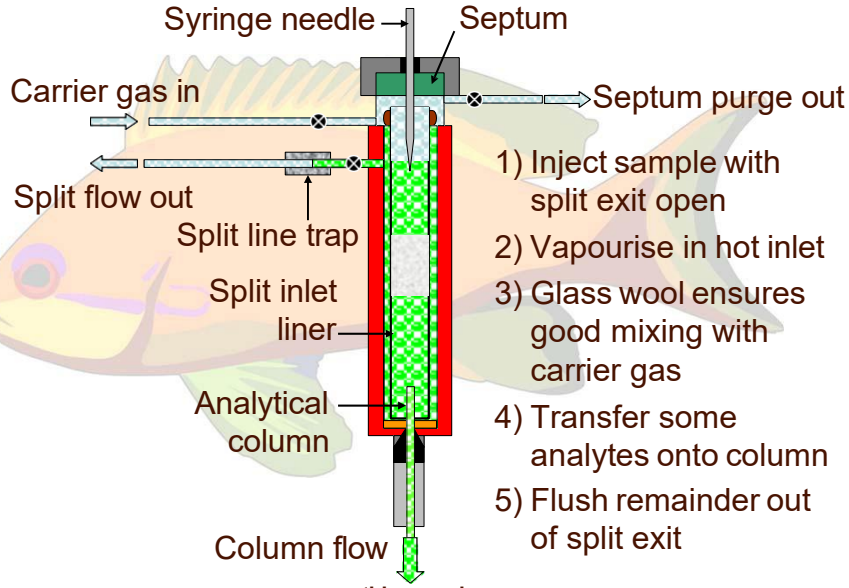
Splitless injection

- ✓ Any dirt & involatile matrix stays within inlet liner (doesn't dirty the column as much – depends on temperature)
- ✓ Easy to set-up (optimise temperature & splitless time)
- ✓ Can use any column i.d.
- × Hot injection causes problems for thermally labile or high molecular weight analytes (they can break down or get stuck in syringe or inlet)
- ✓ Cold injection is better for these analyte types
- × But takes a special inlet - a Programmable Temperature Vapouriser (PTV)

Splitless & Split injections

- ✓ Splitless injection transfers all of sample onto column, good for trace analysis
- × What happens if we have high concentration samples?
  - × The column would overload resulting in poor chromatography
- ✓ Use a split injection
  - ➢ Only put a small proportion of sample onto column
  - ➢ Remainder is flushed out of split exit

Hot split principles



1) Inject sample with split exit open
2) Vapourise in hot inlet
3) Glass wool ensures good mixing with carrier gas
4) Transfer some analytes onto column
5) Flush remainder out of split exit

Split injection

- ✓ Any dirt & involatile matrix stays within inlet liner
- ✓ Very easy to set-up & rugged method (optimise inlet temperature & split ratio)
- ✓ Majority of sample goes to waste - good for high concentration samples
- × Not good for trace analysis
- ✓ Can be hot or cold injections
- o Split ratio is: proportion of sample leaving split exit compared to proportion going onto column e.g. 10:1 or 100:1

4) Analytical column

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- Where separation occurs in gas chromatograph
- Holds stationary phase
- Allows mobile phase to sweep through it to separate analytes




Stationary phase

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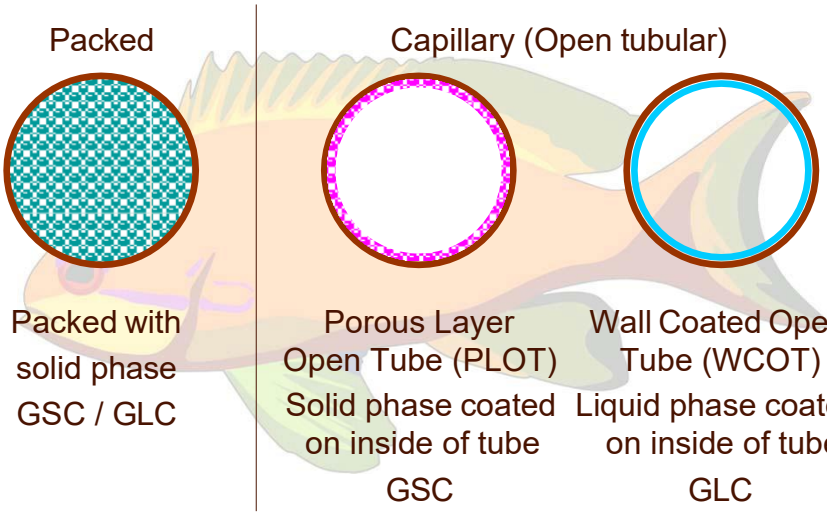
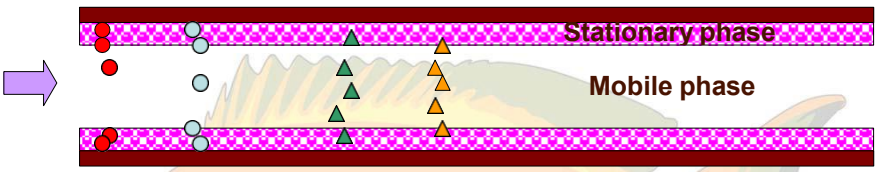
- Stationary phase interacts with analytes to varying degrees depending on both their chemical & physical properties
- If interaction is equivalent for all analytes → no separation!
- Stationary phase can be:
  - A liquid adsorbed on a solid = gas-liquid chromatography (GLC or GC) → Partitioning
  - A solid = gas-solid chromatography (GSC) → Adsorption


GC columns

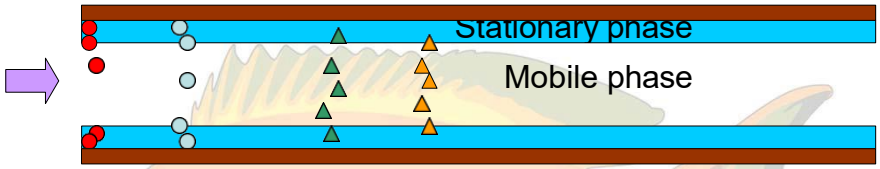


Capillary PLOT columns

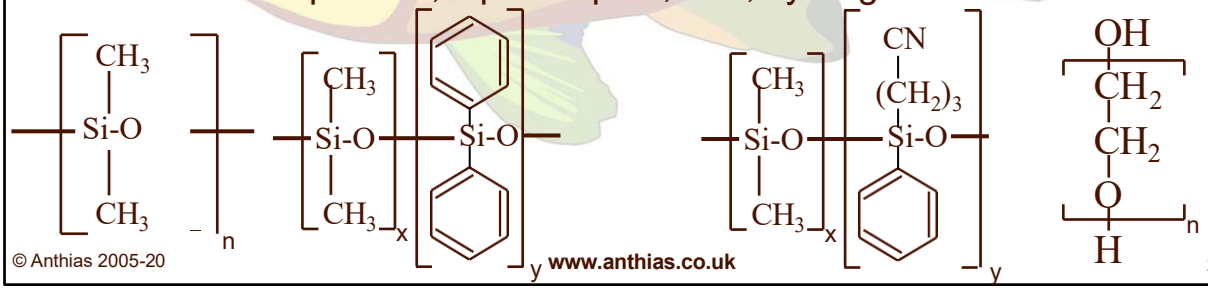


- Column: usually fused silica
- Phase = solid particles affixed to inner walls of capillary, available in different thicknesses
- Typical adsorbents or porous polymers: molecular sieve, divinylbenzene (DVB), carboxen, aluminium oxide
- Separation through adsorption (GSC)

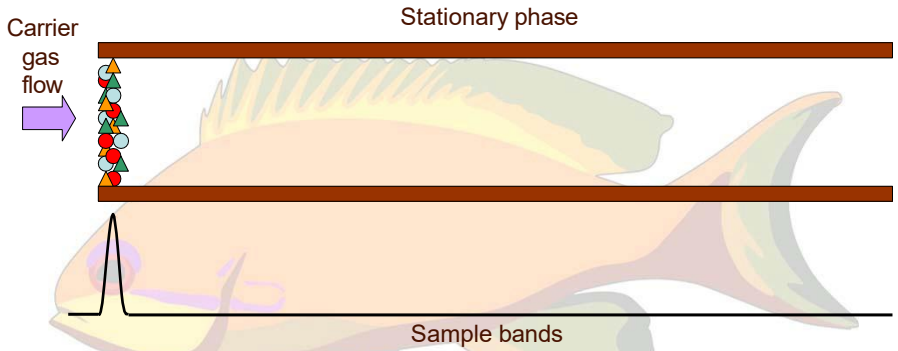
Capillary (WCOT) columns



- Column: fused silica or metal
- Stationary phase: siloxane-based polymers or polyethylene glycol (PEG)
- Interactions: dispersion, dipole-dipole, π-π, hydrogen bonds

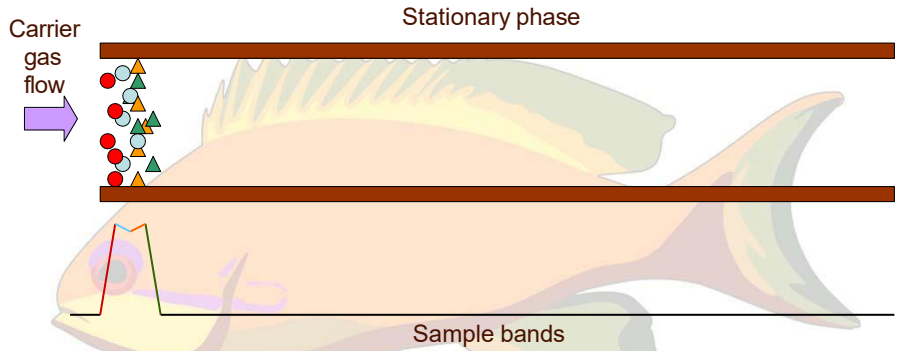


Separation process (1)



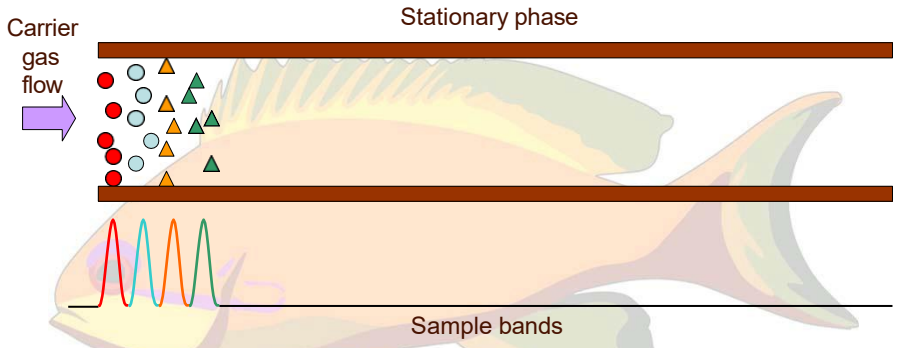
- Chromatography is a physical & chemical separation process
- First vapourise sample & introduce onto analytical column for separation in narrow sample band

Separation process (2)



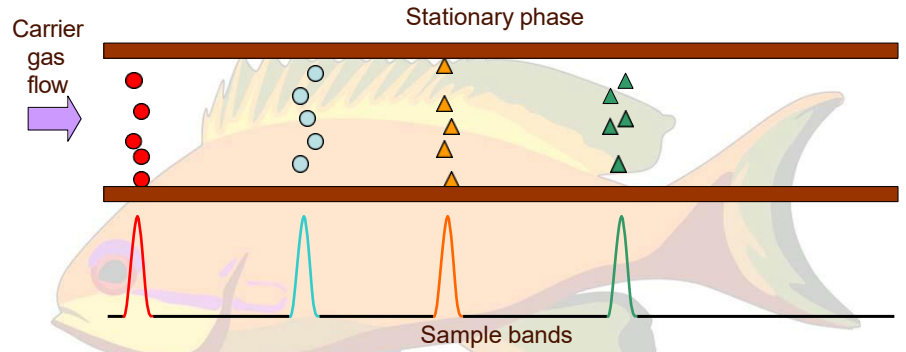
- Mobile phase (inert gas) sweeps vapourised sample over stationary phase (adsorbent/liquid)
- Analytes separated by differences in their distribution between the two phases and volatility

Separation process (3)



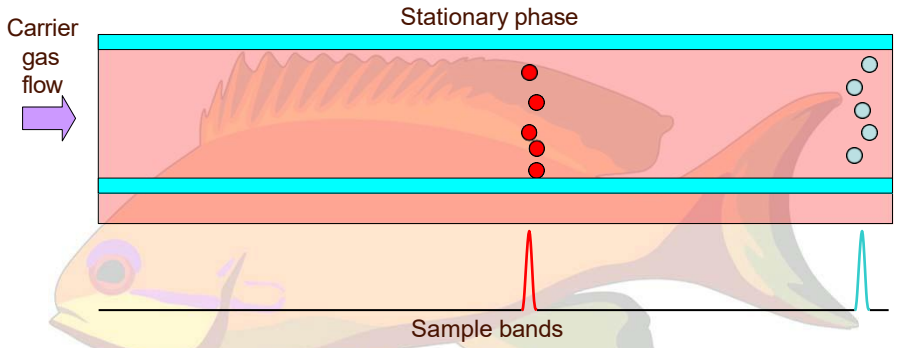
- Analytes must interact with stationary phase to be retained & separated by it
- The more the interaction the longer it takes for an analyte to progress through the column

Separation process (4)



- Analytes having least interaction with stationary phase (spend more time in mobile phase) elute 1st
- Peaks are detected to produce a chromatogram

Separation process (5)



- Heating GC oven helps to elute less volatile analytes
- Warmer temperature means that the molecules spend more time in the mobile phase

GC oven

- Isothermal temperature program
  - o Oven temperature held constant through GC run
  - ✓ Good when separating analytes of similar volatility
  - × Non-volatile analytes will not move through analytical column or will take a long time & result in broad peaks!
- Ramped temperature program
  - o Oven is heated during GC run
  - ✓ Good when separating analytes of different volatilities – less volatiles elute faster & are therefore sharper
  - × Takes time to cool oven after analysis

Oven temperature program

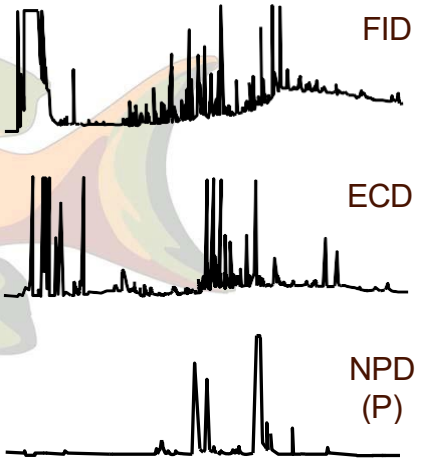
4) Detection

I've now put my sample on the column, separated it, how do I detect it?

- Many different types of detectors on the market
- ➢ Some universal – see all organic compounds
- ➢ Some specific – see specific atoms or bonds
- ➢ Some less sensitive or highly sensitive
- ➢ Some can be used over a wide concentration of analytes, others very narrow
- Choose detector for application

Detector types

- Universal:
  - FID, TCD
  - MS (in scan mode)
- Selective:
  - ECD, TID, FPD, NPD, SCD, ELCD, NCD, IRD
  - MS (in SIM or SIR mode)



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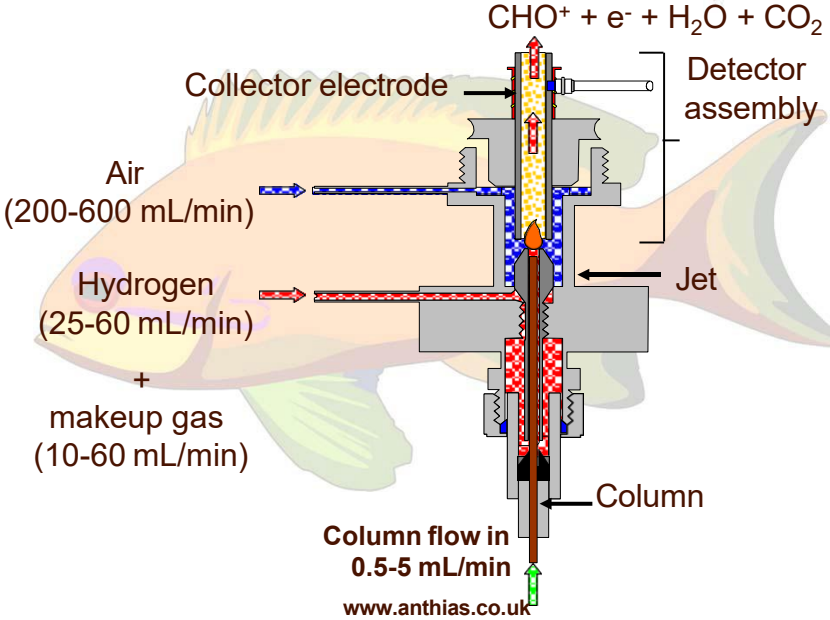
Flame Ionisation Detector



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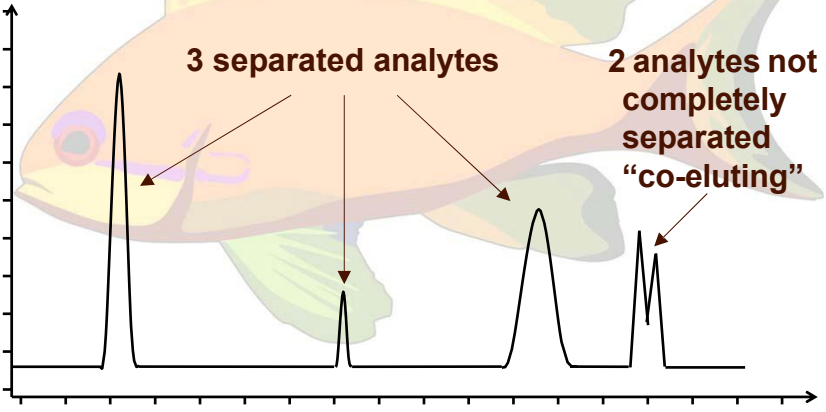
The FID

- Non-selective detector: gives information of retention time & response
  - Column effluent mixed with hydrogen & air (& make up gas e.g. nitrogen to increase flow through jet) then ignited
  - Organic compounds burn, producing cations (CHO^+) & electrons, water & CO_2
  - Cations are collected & produce a signal (measured by collector electrode)
- Response proportional to number of C-H bonds
- Compounds with little/no response include:
  - Carbonyls, COH, COOH
  - Alcohols, halogens, amines
  - Non-combustible gases, H_2O, CO_2, CO, SO_2, NO_x, N_2, O_2, NH_3, rare gases

6) Chromatogram

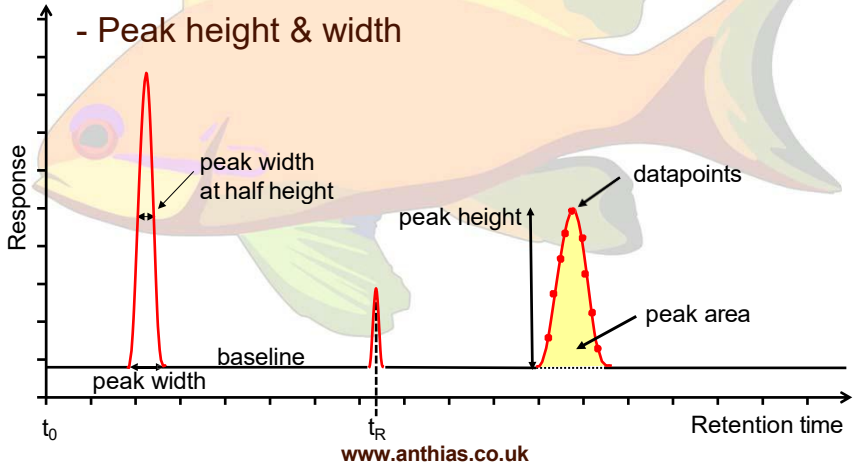
Output from gas chromatograph of separated analytes identified as “peaks”



Chromatogram

Each peak has:

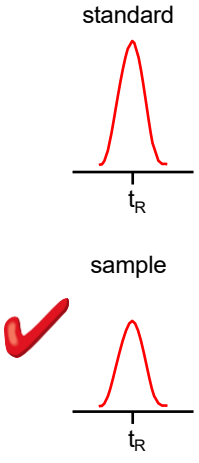
- A retention time (t_R) = time retained by analytical column before being eluted & detected
- Peak height & width



Qualitative data analysis

What analytes are in the sample?

- GC analysis used to confirm presence or absence of analytes in sample whose identities are known
  - Retention time of peak is compared to retention time of a known peak analysed under same conditions
- GC may not lead to positive identification of an analyte, but provides evidence of absence of a species (or it is present below detection limit of method)
  - Failure of sample to produce peak at same retention time as standard obtained under identical conditions is strong evidence of absence



Quantitative data analysis

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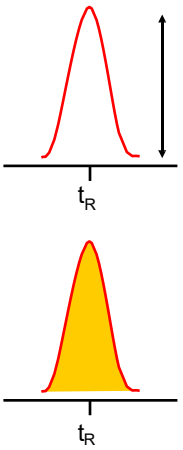
How much of each analyte in sample?

- Compounds must be known (target analytes)
  - Uses retention time (& mass spectrum) to identify analyte
  - Uses peak height or peak area to quantify how much is there, by comparing to height/area of known concentration of analyte



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What next?

GC course flow chart

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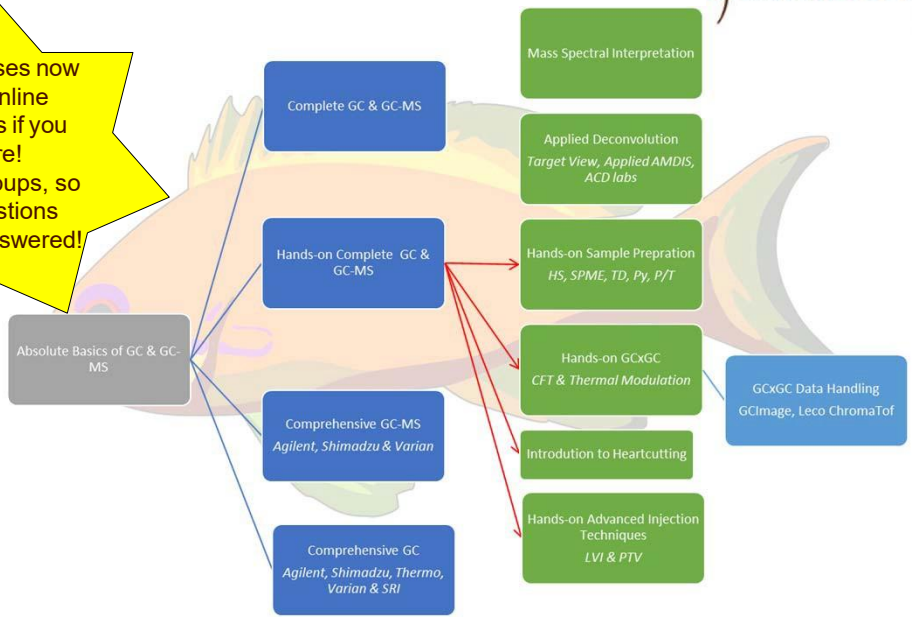
Many courses now available online
- Taught as if you were there!
- Small groups, so your questions will be answered!



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Other courses

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- Liquid Chromatography (HPLC, UHPLC, LCxLC, LC-MS)
- Spectroscopy (ICP-MS, ICP-OES, InfraRed, UV-Vis, AAS)
- Chemometrics
- Method development
- Validation
- Deconvolution
- Interpretation of Mass Spectra
- …and much more!

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Thank you!

Any questions?

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