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Bridging the Gap

Choosing an Automated Sample Prep Technique to Match your Analytes and Matrix

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Samples for Analysis

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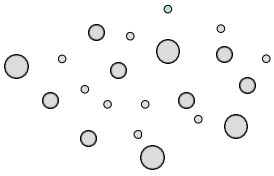
- A sample is made up of:
  - Analytes = compounds of interest
  - Matrix = other components not interested in
  - Matrix interference = matrix component(s) which interfere with the analysis of analytes
- Most analytical techniques require the sample to be prepared for analysis, which sample prep technique is best depends on:
  - Sample phase: Solid, Liquid or Gas
  - Volatility of analytes & matrix
  - Concentration of analytes
  - Analytical technique to be used



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GC 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 vaporise sample

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

LC Analytes

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Are organic compounds of interest which:

- Are soluble in mobile phase
- Have a lower vapour pressure than sample solvent & mobile phase
- No upper mass limit (within reason!)
- Detectable!
  - Need chromophore for UV-Vis
  - Need to know absorbance & emission spectra for fluorescence & therefore contain a fluorophore
  - Must be ionisable for LC-MS

Sample Preparation, Why?

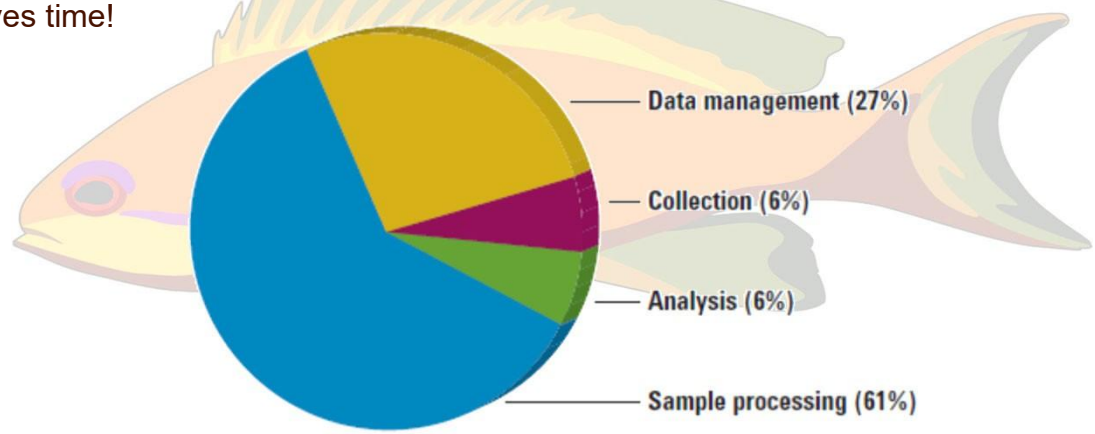
- Sample preparation is key to success
  - Results can only be as good as samples that are prepared for analysis
- Samples for LC analysis must be
  - Soluble in solvent compatible with mobile phase, ideally the same solvent system
  - Not contain particles that can block the system
- Samples for GC analysis must
  - Not exceed volume of inlet liner when injected & heated (i.e. be concentrated enough)
  - Be compatible with stationary phase

Sample Preparation, Pros & Cons

- By removing interfering matrix molecules from a sample, chromatography & associated method development will be:
  - **Easier:** less peaks to resolve
  - **Quicker:** matrix more/less polar or of different volatility than analyte(s) can be selectively removed, reducing run times
  - **Cleaner:** less cleaning required of GC, LC or MS system
- Care must be taken:
  - Not to lose target analyte molecules
    - Extraction efficiency should be measured & considered
    - Recovery >80% is good but should also be reproducible
  - Not to create target analyte molecules through reactions

Sample Analysis Steps

- Time spent completing sample processing is a significant portion of total analysis time!
- Therefore, automated sample prep, especially if hyphenated to analytical technique saves time!



Courtesy of Agilent Technologies

Reasons for Sample Preparation

- Sample preparation is used to:
  - Remove particulates
  - De-salt
  - Remove proteins, phospholipids or high molecular weight matrix
  - Switch solvent systems
  - Increase chromatographic column lifetime
  - Concentrate sample
  - Dilute sample
  - Ionise or neutralise (buffer)
  - Extract specific target compounds

Introduction of samples

- Samples very rarely directly introduced onto analytical column
- Even liquid injection often needs some type of pre-extraction of analytes from matrix
- Best sampling technique is determined by:
  - Sample phase: gas/liquid/solid or something in-between?
  - Where is sample?
    - Can a portion be moved to lab or must be sampled in-situ (can instrument be taken to it)?
  - Analytes: volatile/semi-volatile/involatile?
  - Possible to automate sampling/extraction technique?

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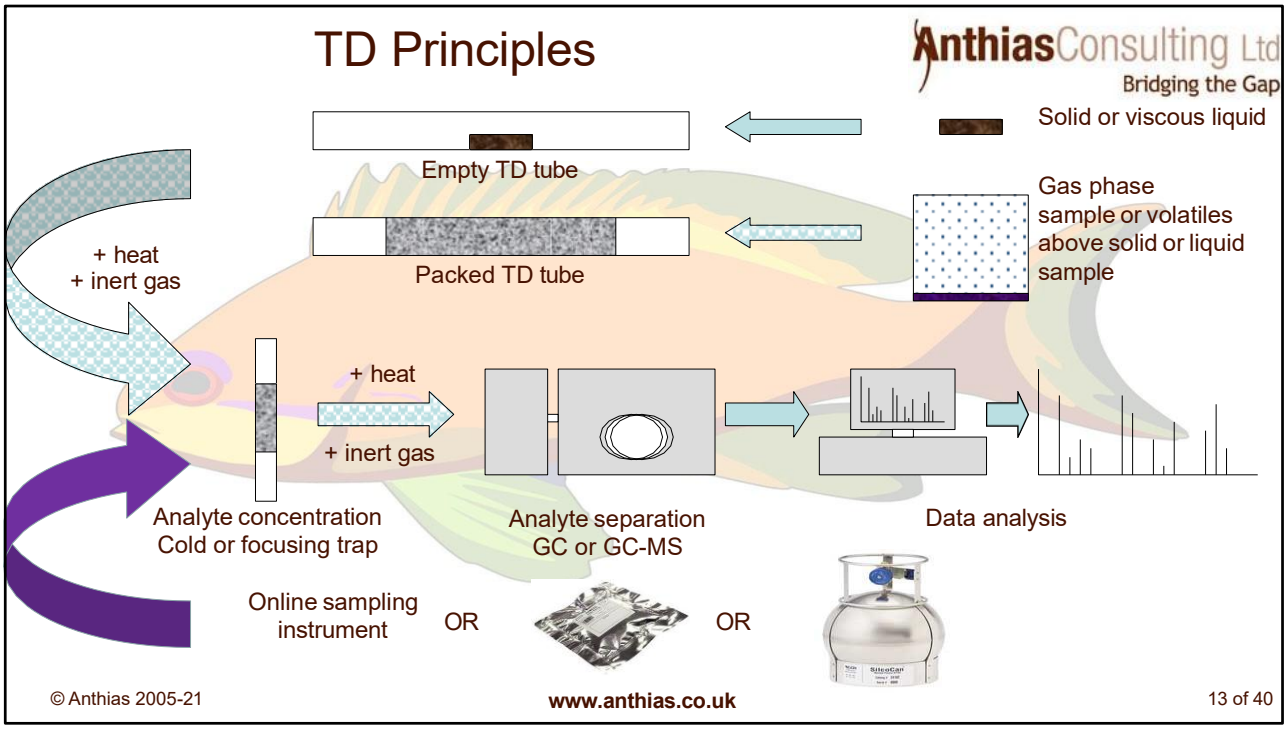
Gas phase samples

Gas Phase Samples

- Usually analysed by GC
- Must be sampled in-situ:
  - e.g. Air, breath from patient, process plant
- Sample can be taken:
  - e.g. Cylinder of industrial gas
  - Canister/bag of sample collected from above
- Analytes: usually volatile
- Techniques available:
  - Thermal desorption (TD)
  - Solid phase micro-extraction (SPME)
  - Stir bar sorptive extraction (SBSE)

Thermal Desorption (TD)

- Definition: thermal means 'heat' & desorption means 'changing from an adsorbed state on a surface, to a gaseous or liquid state'
- Analyte range:
  - C_2 (acetylene) freons → C_{40}, PAHs & phthalates
  - Not good for some inorganic gases, thermally unstable or high MW analytes with b.p. > 525°C
- Sample types:
  - **Gas sample** *or* headspace above a fixed or **large solid sample**
    → Sampled onto adsorbent in a packed TD tube
    → Thermal desorption of adsorbent
  - **Small solid sample** *or* **viscous liquid sample**
    → Placed in empty TD tube
    → Direct thermal extraction of sample



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TD Tube Packing

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- Sorbent beds:
  - Single (one sorbent)
  - Multi-bed (multiple sorbents)
- Packing material (sorbent): type is dependent on target analytes
  - Must trap target analytes at ambient temperature
    - *Consider using multi-bed trap*
  - Must easily release target analytes when rapidly heated
    - *No catalytic breakdown or irreversible adsorption*
  - Maximum temperature of packing material must not be exceeded!

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TD Instrumentation

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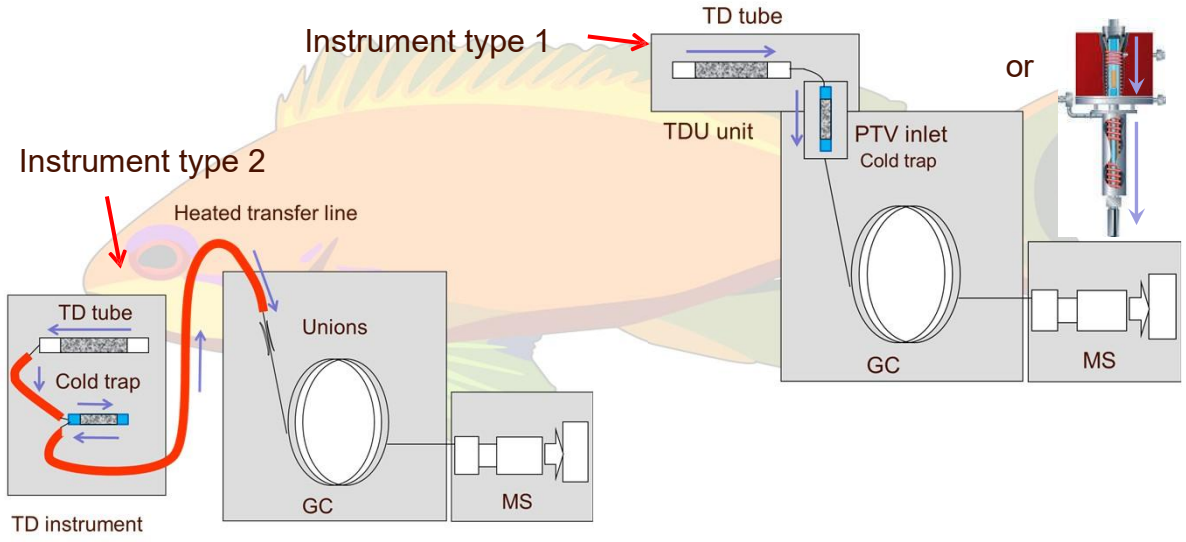

TD Applications

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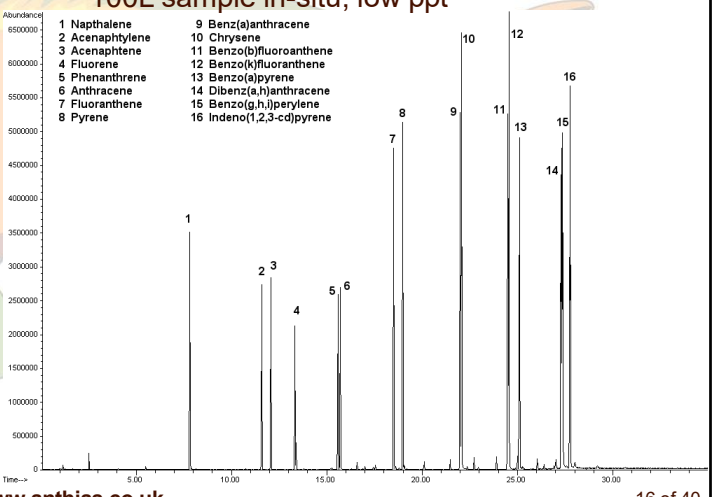
- Air monitoring:
  - Atmospheric, occupational hygiene, indoor, landfill
  - Defence
  - Health
- Material emissions:
  - Buildings, vehicle interior & trim materials, electronic components,
  - Plants & living organisms (biomarkers)
- Thermal extraction (solids):
  - Forensics, polymers, pharmaceuticals,
  - Flavour & fragrance

Quantitative analysis of semi-volatile polyaromatic hydrocarbons from ambient air

100L sample in-situ, low ppt



Solid Phase Micro-Extraction (SPME)

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- Developed in early 1990s at University of Waterloo by Professor Pawliszyn's group
- Fibre coated with stationary phase similar to GC column phase:
  - Polymer (liquid) or sorbent (solid)
- Used to extract & concentrate analytes from liquid or gas phase
- Fibre desorbed thermally in GC inlet or by liquid desorption in solvent or HPLC interface
- Fast & simple technique (no solvents)
- Manual or automated
- Onsite sampling possible if fibres appropriately stored
- Good for rapid screening & quantitative analysis

SPME principles

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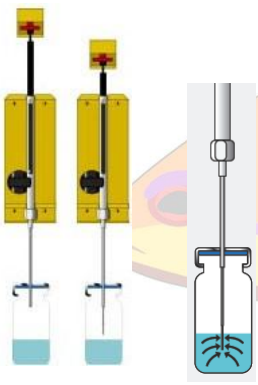

1) SPME fibre conditioned @ recommended temperature & time for phase
2) Sample pre-shaken & heated
   - HS: Equilibrium established between sample & headspace
   - Immersion: Only if temperature adjustment required
3) Fibre inserted into headspace or sample & heated/gently shaken for 2-30 minutes
4) Fibre & needle retracted & inserted into GC inlet
5) Analytes quickly desorbed onto column (1-2 mins)

SPME fibres

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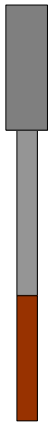
- 1cm fused silica coated with stationary phase bonded to SS plunger
- Plunger held within needle
  → Withdrawn to protect fibre when piercing septa, etc.
- Phase type & thickness chosen to match characteristics of analytes (selectivity)
- Amount of analyte adsorbed depends on thickness of phase & partition coefficient of analyte
  – Thick phase: volatile analytes
  – Thin phase: semi-volatile analytes



SPME phases

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- Select SPME phase to match chemistry of analytes trying to extract:
  – Molecular weight & polarity
  – Consider chemistry of matrix too!
- Small changes in stationary phase polarity = no large selectivity differences
- Addition of sorbent to coating e.g. strongly polar Carbowax PEG onto DVB polymer → increases surface area & improves extraction efficiency of polar molecules
- Adsorption type fibres better for extracting low conc. analytes e.g. carboxen, DVB
- Absorptive fibres have greater capacity & linearity as they use partitioning for the extraction, e.g. carbowax, PDMS, polyacrylate

SPME Instrumentation

- Possible to do manual SPME
- Automated systems allow:
  - Sample preparation in tray (ambient temperature)
  - In incubator with heating and/or shaking
  - A derivatisation reagent to be added to fibre prior to extraction step
- Some autosamplers have a multi-fibre exchange
  - Good if analysing many samples
  - Good for method development to try different phases
- Syringe kits allow autosampler to be used for liquid or headspace injections too, or even SPE & liquid-liquid extract too!

Stir-Bar Sorptive Extraction (SBSE)

- SBSE developed at Eindhoven University of Technology in late 90s
- Analyte enrichment technique similar to SPME but with 50-250x more PDMS phase → up to 1000x more sensitivity
- Phase is coated onto stir-bar → used to stir aqueous samples or can extract from headspace
- Analytes are thermally desorbed
- Basic principles are same as SPME
- Can be used for quantitation with both external & internal standardisation

SPME of Tequila & SBSE of Urine

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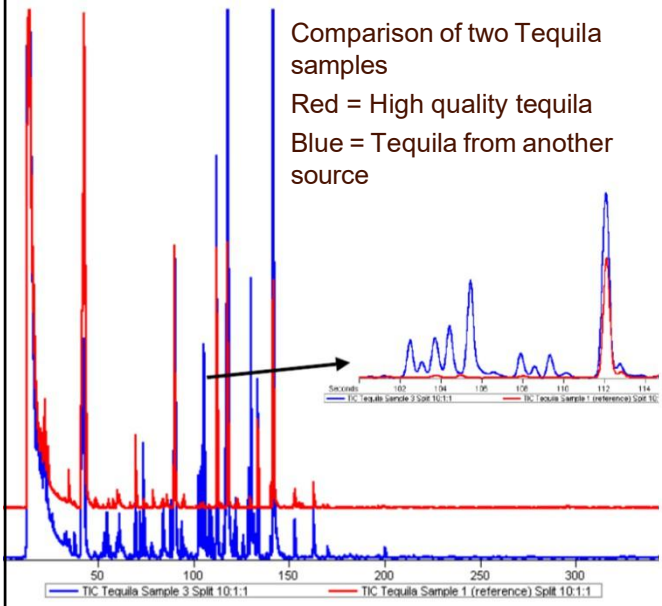
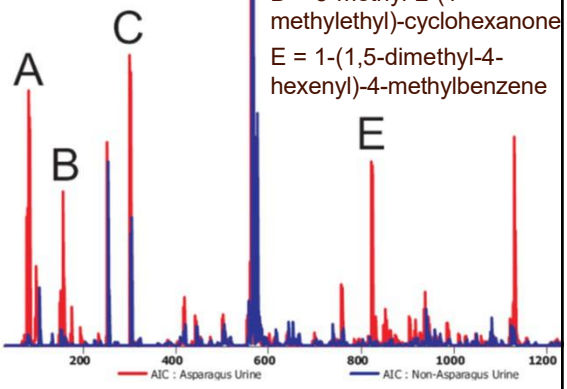
Comparison of two Tequila samples

Red = High quality tequila

Blue = Tequila from another source



Comparison of urine samples before & after consumption of asparagus



D

A = acetone

B = acetic anhydride

C = 4-heptanone

D = 5-methyl-2-(1-methylethyl)-cyclohexanone

E = 1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene

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Courtesy of Leco Instruments

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Liquid phase samples

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Liquid Phase Samples

- Sampled in-situ:
  - e.g. Process & waste streams
- Sample can be taken:
  - e.g. Water, chemical, urine, fuel
- Analytes: volatile to involatile
- Techniques available:
  - Headspace (HS), purge & trap (P&T), solid phase micro-extraction (SPME), stir-bar sorptive extraction (SBSE), solid phase extraction (SPE) or liquid-liquid extraction (LLE),
  - *Plus* thermal desorption (TD) or pyrolysis (Py) (soluble, dispersed or viscous liquids only)

Headspace & Purge-and-Trap Analysis

- "The 'headspace' is the gas space in a chromatography vial above the sample. Headspace analysis is therefore the analysis of the components present in that gas."
- Sample: anything that fits into vial & releases volatile analytes
  - Liquid or viscous liquid
  - Solid analysed directly or after crushing (HS only)
  - Solid mixed/dissolved in a liquid
- Analytes: volatile analytes which are released from sample, usually after applying some heat
  - How much heat can be applied depends on
    - Solvent (if any) boiling point
    - Maximum temperature of instrument & vial
    - In thermal desorption region <350°C where C-C bonds not broken

HS and P&T

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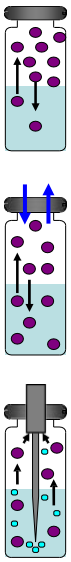
- **Static headspace**: sample heated/shaken in sealed vial & portion of gas phase injected
- **Dynamic headspace**: sample swept with gas & analytes trapped on sorbent trap, thermally desorbed transferring analytes to GC
- **Purge & Trap**: gas bubbled through sample & analytes trapped on sorbent trap, thermally desorbed transferring analytes to GC
- Sensitivity: P&T > DynamicHS > StaticHS



Partition Coefficient (K)

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- Ratio of analyte in headspace (C_g) to analyte in sample (C_s)
- If K is large (>1) analytes prefer sample → will NOT get a good recovery
- If K is small (<1) analytes prefer headspace → will get a good recovery
- Lowering K → increases concentration in headspace → improves detection limits
- K can be lowered by:
  1. Raising equilibration temperature
  2. Changing composition of sample to push analytes into headspace
     - Adding salt, adjusting pH, adding co-solvent, derivatisation
  3. Changing phase ratio

Typical Partition Coefficients

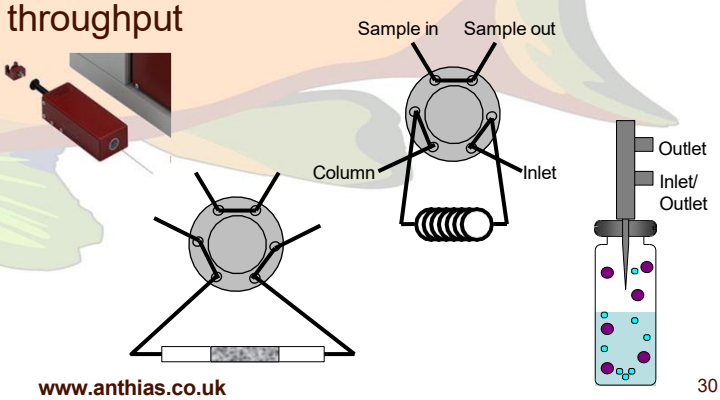
Analyte	K value	Analyte	K value
Cyclohexane	0.077	Dichloromethane	5.65
n-Hexane	0.14	Ethyl acetate	62.4
Tetrachloroethylene	1.48	Methylethyl ketone (MEK)	139.5
1,1,1-Trichloromethane	1.65	n-Butanol	647
o-Xylene	2.44	Isopropanol	825
Toluene	2.82	Ethanol	1355
Benzene	2.90	Methanol	1670

Typical partition coefficients in water at 40°C

- Raising sample temperature reduces solubility
- At 80°C ethanol has K = 328

HS & P&T Instrumentation

- Most instrumentation can retrofit to GC or GC-MS with a standard split/splitless inlet
- Can have added functionality e.g. automatic addition of IS
- Usually can prepare samples (at least up to 6) while previous samples are analysed increasing sample throughput
- HS has three main types:
  - Gas-tight syringe
  - Balanced pressure system
  - Pressure loop system



HS & P&T Applications

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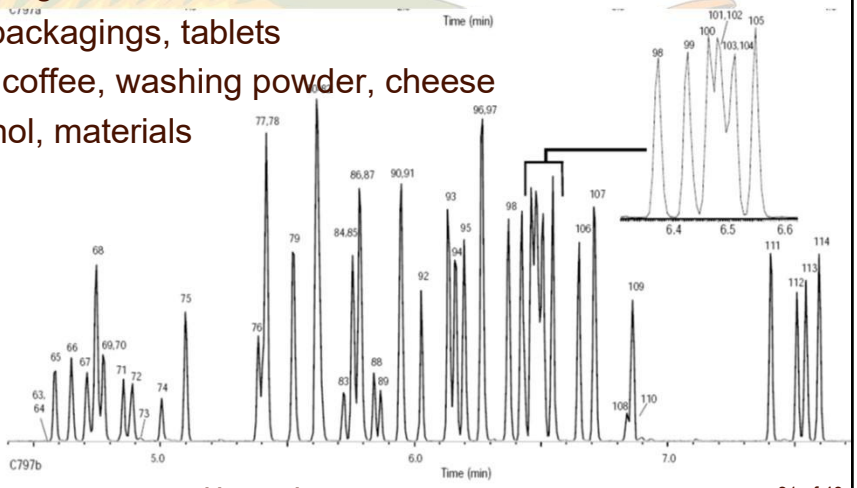
- Volatile Organic Compound (VOC) analysis in:
  - Environmental monitoring: waters, soils
  - Out-gassing studies: packagings, tablets
  - Flavour & fragrances: coffee, washing powder, cheese
  - Forensics: blood alcohol, materials

114 VOCs in water by P&T

Halogenated & aromatic compounds at 40 ppb

Ethers, alcohols & ketones at 100 ppb

GC runtime 8 minutes



SPE & LLE

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- Samples: liquid (solid/viscous - dissolve/dilute)
- Analytes: volatile to involatile
- Purpose:
  - Extract target analytes
  - Remove interfering matrix components & protect analytical system from contaminants
  - Selectively isolate & concentrate analytes: 100-5000x enrichment
  - Change solvent: e.g. water samples cannot be injected into GC
- **Solid phase extraction (SPE)**: select a sorbent to trap analytes (or matrix) then elute using a different solvent
- **Liquid-liquid extraction (LLE)**: a non-miscible solvent used, efficiency improved by increasing temperature, matrix modification

SPE & LLE Applications

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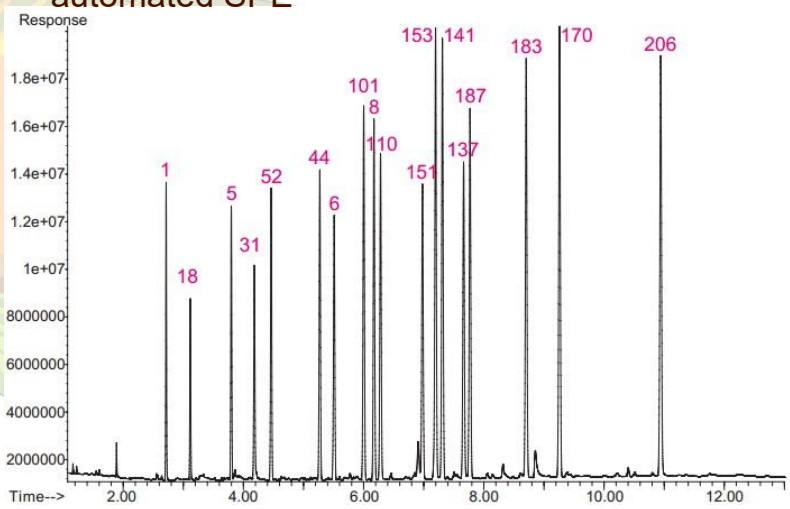
- Forensics (drugs) in biological fluids
- Pharmaceutical compounds & metabolites in biological fluids
- Environmental pollutants in waste & drinking water
- Pesticides & other targets in food & drinks

Polychlorinated biphenyls (PCBs) in waste oil by automated SPE



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Solid phase samples

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Solid Samples

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- Sampled in-situ:
  - e.g. Building materials, car interiors, plants
- Sample can be taken:
  - e.g. Soil, drugs, washing powder, meteorite, food
- Analytes: volatile to involatile
- Techniques available:
  - Headspace (HS), thermal desorption (TD) or pyrolysis (Py)
  - *Plus* purge & trap (P&T), solid phase micro-extraction (SPME), stir-bar sorptive extraction (SBSE), solid phase extraction (SPE) or liquid-liquid extraction (LLE) after mixing/dissolving in a liquid

Thermal Extraction Techniques

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- Techniques which use heat to directly extract analytes from sample then are swept onto GC column include:
  - Headspace analysis (volatiles only)
  - Thermal desorption (up to around 350°C): no chemical bonds broken
  - Analytical pyrolysis (above 350°C, some pyrolysers heat in excess of 1400°C!): breaks chemical bonds

Analytical Pyrolysis

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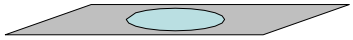
- "Pyrolysis is the chemical decomposition of organic materials by heating in an inert atmosphere"
- Samples: solid or dispersed (solvent must be evaporated first)
- Analytes: non-volatile or trapped
- Sample is heated to high temperatures (500-1400°C) where bonds are broken
- Small amount is placed on filament or in quartz tube & placed in pyrolyser
- Pyrolysis takes place in an inert atmosphere (carrier gas)
- A pyrogram is produced which is a fingerprint of the sample



Pyrolysis Applications

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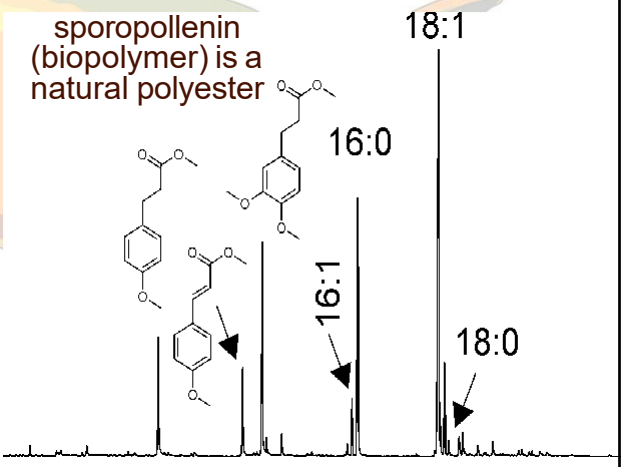
- Pyrolysis can used for analysis of natural & artificial macromolecules
- Natural
  - PVC, acrylics, varnishes, etc.
  - Lignin, cellulose, chitin, etc.
- Food, flavour & fragrance
  - Components of biopolymers
- Chemical
  - Paints, coatings, plastics
- Kinetic studies of thermal degradation products
- Product quality control

Automated Thermochemolysis-GC-MS of solvent extracted *Lycopodium* spores



Courtesy of Jon Watson, The Open University

Summary of sample prep techniques

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Technique	Sample phase	Analytes	In-situ?
TD	G & S (L)	Volatiles & semi-volatiles	Yes
Pyrolysis	S (G, L)	Volatiles-involatiles, bound & free	No
HS	L & S	Volatiles	No
P&T	L (S)	Volatiles+	No
SPE	L (S)	All	No
SPME	G & L (S)	Volatiles & semi-volatiles	Yes
SBSE	G & L (S)	Volatiles & semi-volatiles	Yes
LLE	L & S	All	No

Key message: understand the chemistry of your analytes & matrix: volatility, polarity, functional groups => match technique & consumables required!

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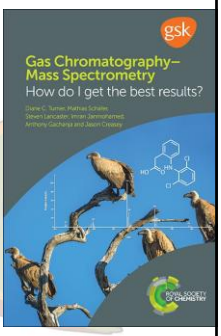
Thank you for listening!



Many thanks to:
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Colleagues at Anthias Consulting Ltd.
Dr Geraint Morgan & colleagues – SPS, The Open University
RSC Analytical Division, PACN and Books
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