Introduction to sample clean-up procedures

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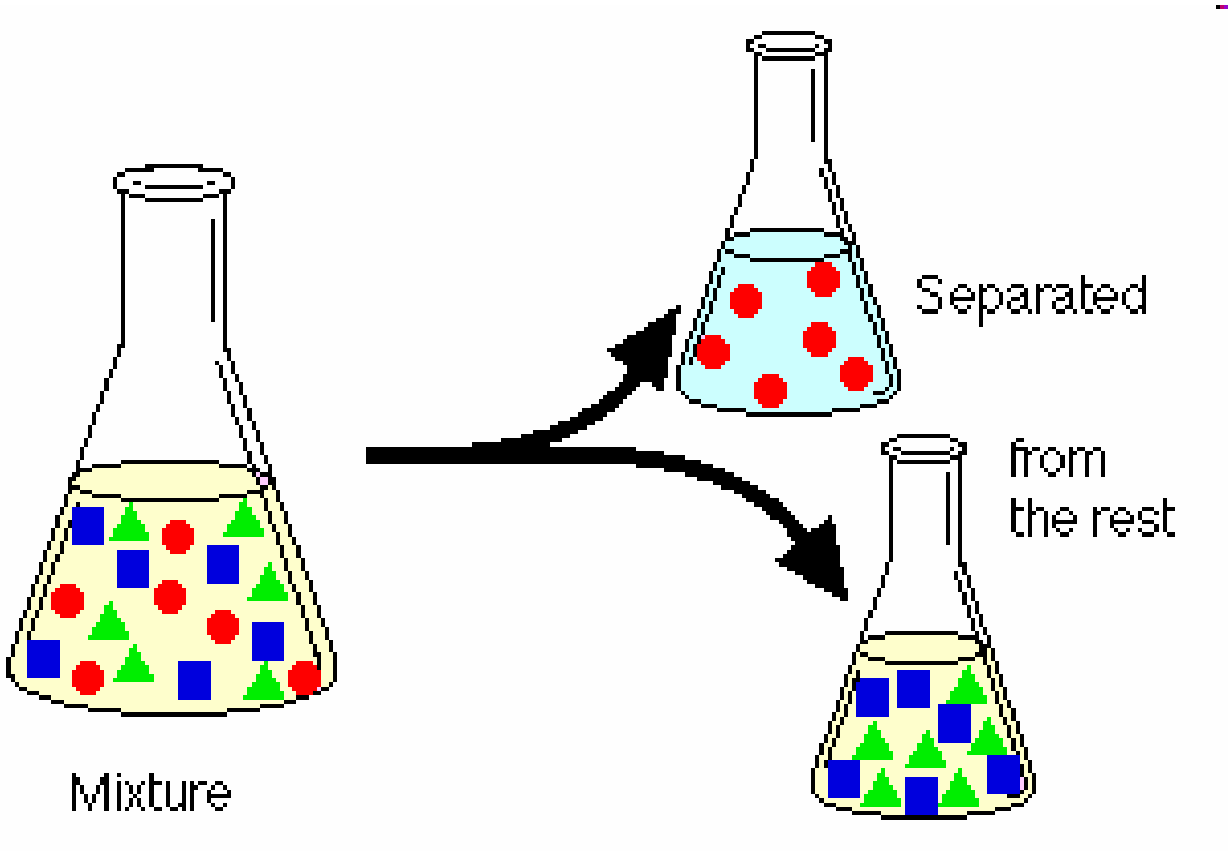
Sample extract (mixture)



Sample clean-up - Aims

To purify or to enrich - Why?

To remove non-target bulk matrix interferences

- protect your analytical system
- effectively increase the concentration of the target analytes to improve sensitivity

To remove specific matrix interferences that will mask or suppress your analyte signal(s)

Sample

Sample extract

Cleaned-up extract ready for instrumental analysis (GC or LC)

Removal of non-specific bulk interferences

Example: Analysis of cheese for fat-soluble organochlorine pesticide residues (OCs)

Extract the OCs with solvent, also extracts the fat

Cheese contains around 30% fat, so if looking for 0.01mg/kg in the cheese - 0.033 mg/kg in the fat

The fat could block the column and will contaminate the detector

Bulk interferences

Fruits & vegetables

Lettuce, cabbage – chlorophyll, waxes
Carrots – carotenoids
Beetroot, red wine – anthocyanins
Avocados, Kiwi fruit – oil

Fruits and vegetables – high levels of sugars

Underlying mechanisms

Differentiate using the different physico-chemical properties of the target analyte(s) and the bulk interferences

- volatility
- size
- polarity
- shape
- reactivity

Clean-up strategy

- Additional step added to the method
- Combine with the extraction stage
- Combine with the determination stage

Last two could ‘on-line’

Physico-chemical properties

How does the analyte differ from the co-extractives ?

volatility

- headspace, purge-trap, steam distillation, SPME

size

- size-exclusion chromatography (GPC)

polarity

- liquid-liquid extraction, SPE

shape

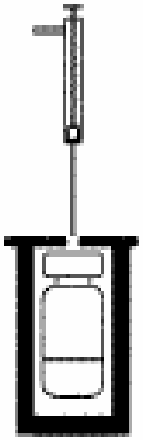
- immunoaffinity, MIPS (molecular imprinted polymers)

reactivity

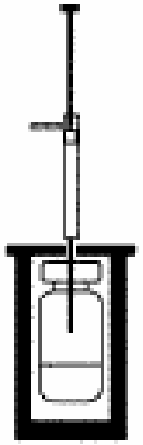
- acid-base ionisation, destruction, precipitation, binding, derivatisation (methylation) etc

Volatility – Headspace analysis

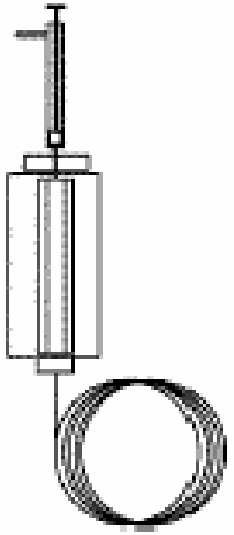
Step 1
Sample reaches equilibrium



Step 2
Sample is extracted from headspace



Step 3
Sample is injected



Volatility

- purge-trap
- ➢ take a greater fraction of the vapour phase, trap the analyte (e.g. cryotrap) then thermal desorb into the GC

- SPME (solid phase microextraction)
- ➢ replace the needle with a wire coated with an absorbent
  Expose this to the headspace vapour for some minutes then thermally desorb in the hot GC inlet

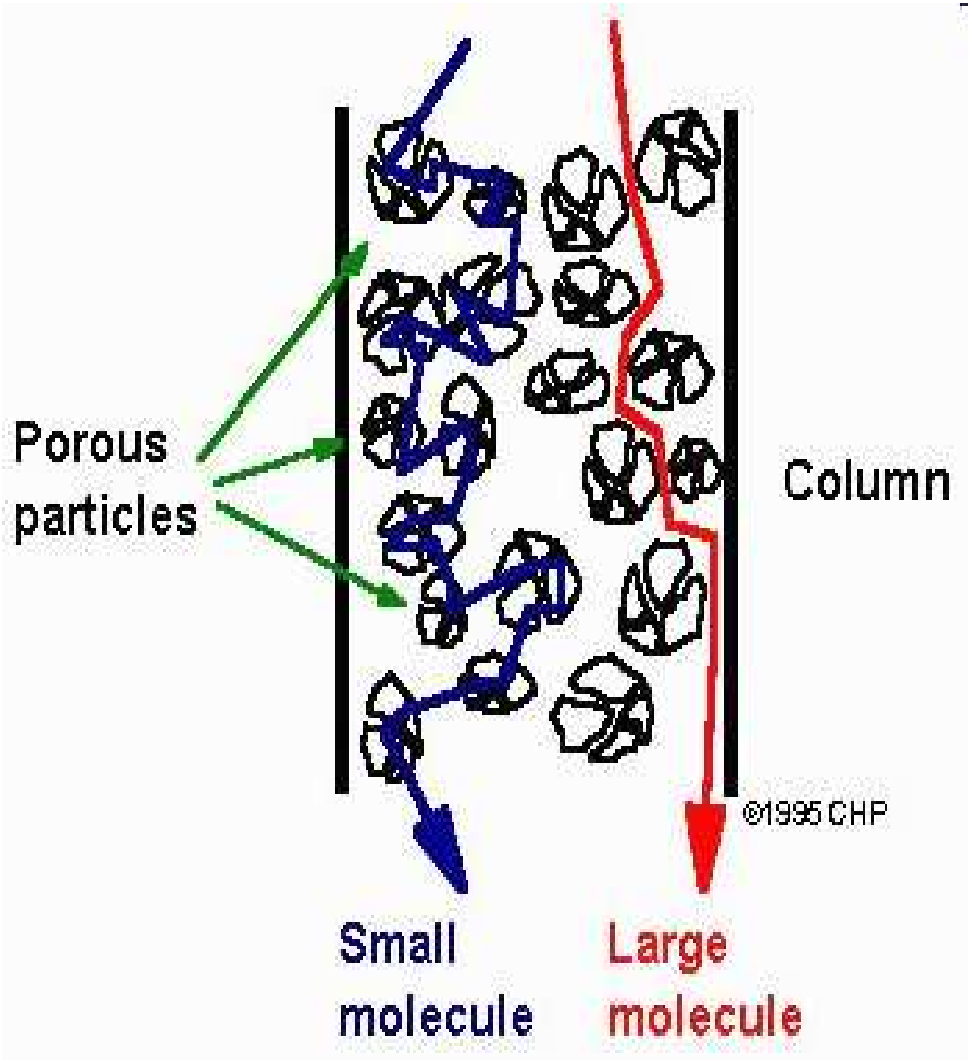
- co-sweep distillation
- ➢ drive-off the volatiles by heating, collect on an adsorbent

SEC/GPC/HPGPC

Size-exclusion chromatography (SEC), also called gel-filtration or gel-permeation chromatography (GPC), uses porous particles to separate molecules of different sizes.

Molecules that are smaller than the pore size can enter the gel and therefore have a longer path and longer transit time than larger molecules that cannot enter the gel.

SEC/GPC theory



Clean-up by GPC

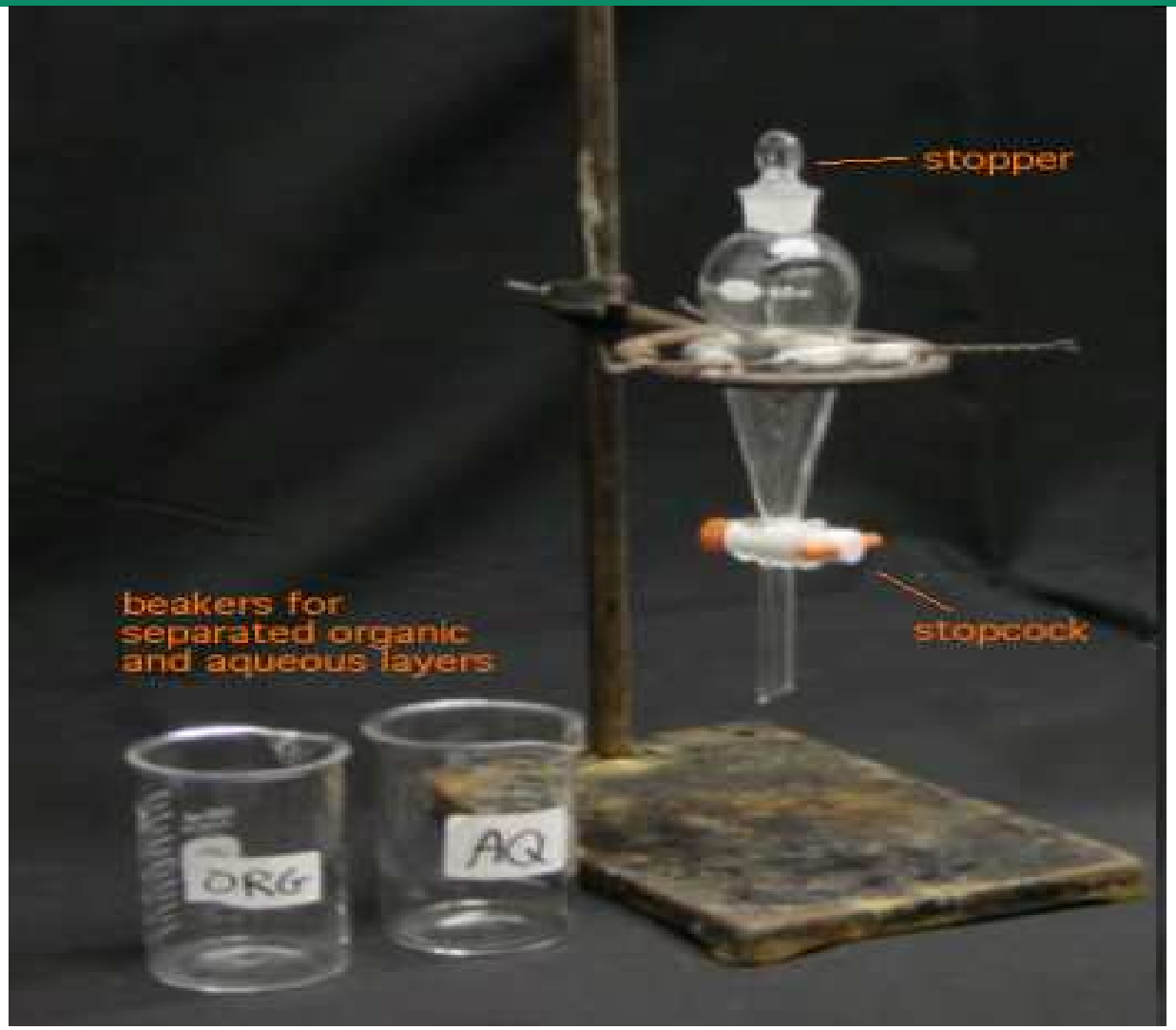
OC pesticides in from co-extracted fat

pesticides molecular weight 200-300

Fat (triglycerides) molecular weight around 900

- Can be automated (autosampler & fraction collector)
- Predictable retention/collection times
- Columns last indefinitely

LLE in its simplest form



Polarity - LLE

Use two immiscible phases and partition the solute (pesticides) from one phase to the other.

acetone/water extract - use dichloromethane or cycloexane/ethyl acetate to solvent exchange

Can get poor recoveries with polar compounds

LLE

Advantages

1. High sample capacity
2. Low cost (no capital investment)
3. Quick and easy to set up

Disadvantages

1. Difficult to automate
2. Solvents must be immiscible and may be costly

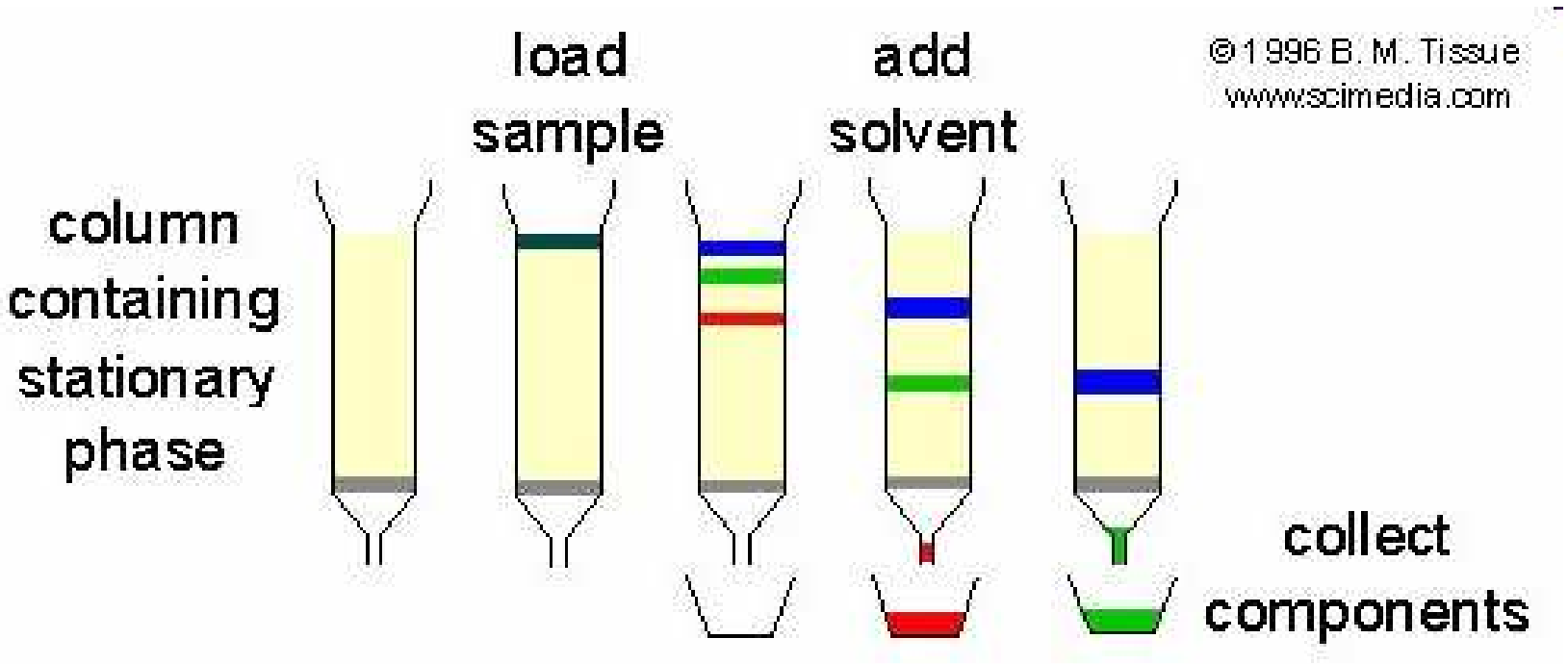
Solid phase (micro)extraction (SPE)

A chromatographic clean-up technique

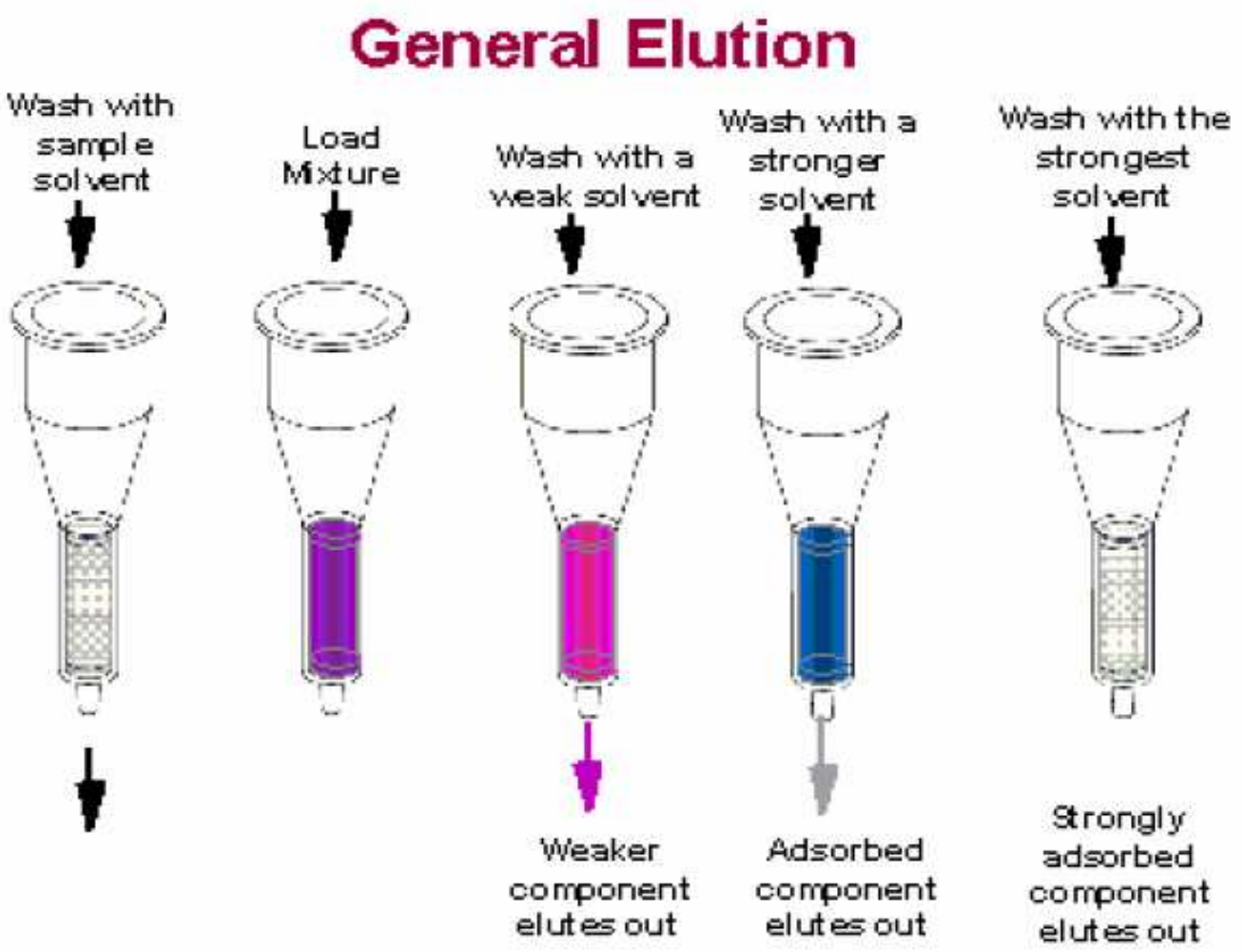
Various forms

columns, cartridges, syringe barrel columns
dispersive powders,disc membranes, etc

SPE using diposable columns/cartridges



SPE – gradient elution of components



SPE formats

SPE modes

Many different phases are available

- Reverse-phase (partition) chromatography
- Normal phase (partition) chromatography
- Adsorption chromatography
- Ion chromatography

SPE versus LLE

- Better removal of interferences – more specificity
- Easier to automate
- Less solvent use
- No emulsions

- Lower sample capacity
- Longer to set up

Automated SPE – Gilson Aspec

Clean-up by destruction

The analyte(s) have to be inert compared to the matrix that is to be eliminated

- OCs in fatty extracts – destroy the fat by using sulfuric acid – *cyclodienes rings are opened*

Conclusions

‘Clean-up’ or ‘No clean-up’?

Have to decide based on:

- The matrices that are to be analysed
- The range of analytes to be sought
- The chromatographic detection system to be used

Often have to check chromatograms to see if a clean-up step is necessary