Sample preparation

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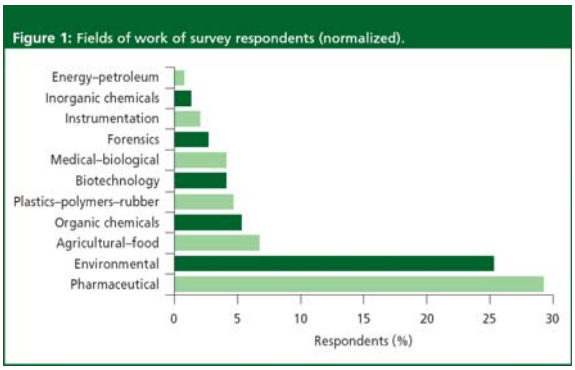
What type of samples are common?



Number of samples?

Table 2: Number of samples analysed per instrument per week.

Number of samples	Respondents in 2001 (%)	Respondents in 1996 (%)
Less than 20	33.2	29.7
21–50	27.5	36.7
51–100	21.8	18.4
101–150	7.0	5.8
151–200	2.8	4.4
More than 200	7.7	4.9

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Time spent on different operations during LC analyses

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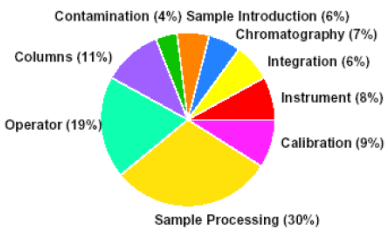
Sources of error



(R.E. Majors, LCGC Magazine, 1991, 1997, 2002)

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Aims

- Sample has to be representative
- Sample has to be homogenous and reproducible
- Should not contain interfering substances
- Sample components/solvent should not damage column or detector
- Suitable solvent should be used: soluble in mobile phase without influencing analytes retention
- Concentrating and derivatization may be needed

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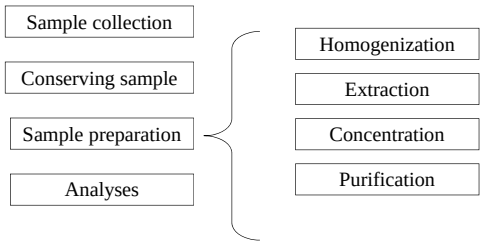
Stages



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Gasses and suspensions

- Volatile organic substances
  - Solid phase trapping
  - Liquid phase trapping
- Suspensions
  - Filtration
  - Centrifugation
  - Sedimentation

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Liquids

- Solid phase extraction
- Liquid-liquid extraction
- Dilution (to avoid overloading or to dilute into linear range).
- Vaporization.
  - Sample is heated at atmospheric pressure or in a inert gas flow. Also vacuum can be used.
    - Boiling should be avoided because analyte may be lost then.

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Extraction

Extraction is physical-chemical method for separating compounds from mixtures or solutions. Extraction is based on compounds different solubility in different solvents.

Extraction aim is to:

- Removing interfering compounds
- Concentrating samples
- Changing the solvent

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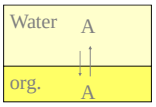
Theory of extraction

Partitioning of compound A between two phases (organic and water) can be described with K_d:



$$K_d = \frac{[A]_{org}}{[A]_{vesi}}$$

Extraction is more efficient if K_d increases

Amount of analyte extracted into the organic phase :

$$E = \frac{K_d V}{1 + K_d V} \qquad V = \frac{V_{org}}{V_{vesi}}$$

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Extraction: one or several times?

- Several extractions with small amount of solvent are more efficient then one extraction with large amount of solvent.

For example: Substance A concentration in 100 ml is 1 unit and partitioning coefficient is 5 then:

1 x 100 ml - 17% of the substance is left into water phase

2 x 50 ml – 8%

4 x 25 ml – 4%

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Choosing the extraction solvent

- Has to dissolve substance of interest very well
- Preferably dissolve poorly interfering substances
- Solubility of solvents in each other should be low (less then 10%).
- Solvents density should be very different.
- Solvent should be inert.
- Cheep and nontoxic.

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Optimization

- Extracting solvent
- pH and ionic strength
  - Acidic compounds
  - Basic compounds
  - Neutral compounds

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Problems

- Large solvent consumption
- Work extensive
- Slow separation of phases and possibility of forming emulsion.

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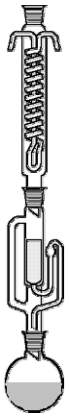
Aparatus

- Separation funnel – periodic extraction, cheep and easy.
  - glass
  - plastic (polypropylene, teflon)
- Soxhleti extractor – half continuous, concentrates, also suitable for extraction of solid samples.
- More and more extraction in tube is used.



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LLE in a tube

- If K is large it might be possible to carry out extraction once in a tube
  - Phases are separated with centrifugation
  - Time efficient
  - Smaller sample volumes
- Samples are often „solids“
  - Plant parts, honey, tissue

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LLE in a tube

- Often extraction is followed by additional cleaning and drying step
- After phase separation
  - Dispersive sorbents (slaid 41), adsorbs interfering compounds from matrix but does not adsorb analyte
  - Hydroscopic salts (eg $MgSO_4$)
- Extraction in tube does not allow concentrating the sample in a large scale

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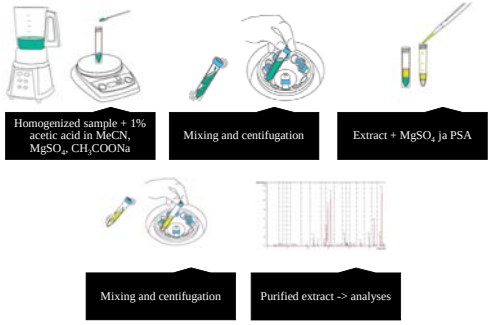

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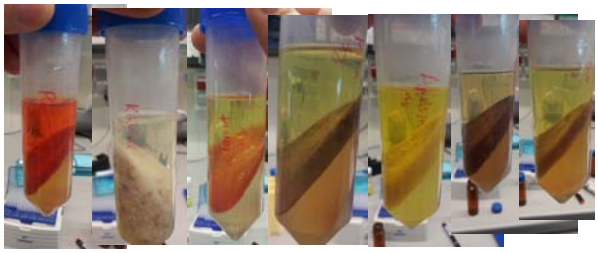
LLE in a tube



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Solide phase extraction- SPE

SPE (*solid phase* extraction) is a type of extraction that uses solid and liquid phase to remove same component from the solution.

- Sample purification
- Concentrating sample
- Solvent exchange

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Procedure

1. Conditioning of SPE tube
   1. Wetting the sorbent
   2. Washing with sample solvent
2. Introduction of the sample to SPE tube.
3. Interfering compounds are eluted from SPE.
4. Analyte is eluted with a different solvent.

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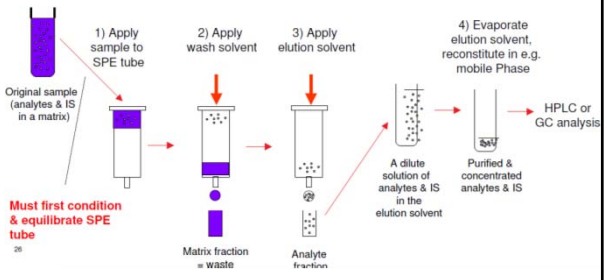
Procedure



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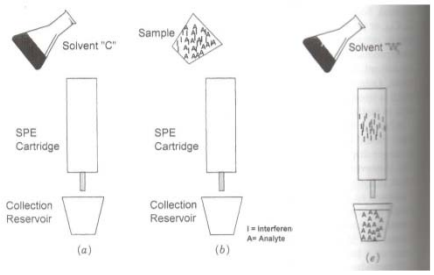
Alternative procedure



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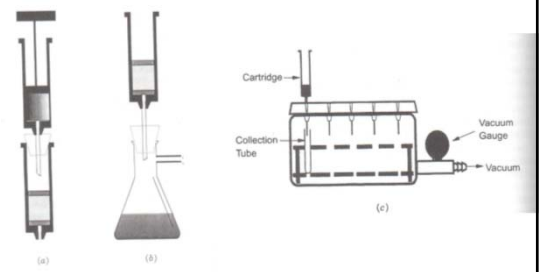
Apparatus



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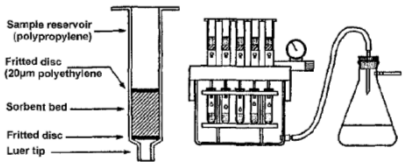
Apparatus



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Retention

- Hydrophilic
- Hydrophobic
- Ionic
- Retention must be absolute when sample in introduced k > 1000
- and elution must also be absolute k < 0,1

For comparison in HPLC ..... < k < .....

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How to choose sorbent?

- It is wise to work with a different retention mechanism then in HPLC
  - Eg SPE at ion exchange, HPLC as reversed phase
  - Not always possible
- Both analyte type and matrix components need to be considered

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Sorbent types

- Inorganic oxides
- Sorbents with bonded phase
- Ion exchange
- Mixed- type sorbents
- Internal surface reversed phase

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Inorganic oxides

- Silica, aluminum oxide, Florisil, Diatomite
- Samples in organic solvents
- Strong retention for
  - Hydrogen bonding compounds
  - Compounds wit polar groups
  - Compounds with polarizable groups
- Problem: adsorption
  - Can be reduced if small amount of water is added to the sample

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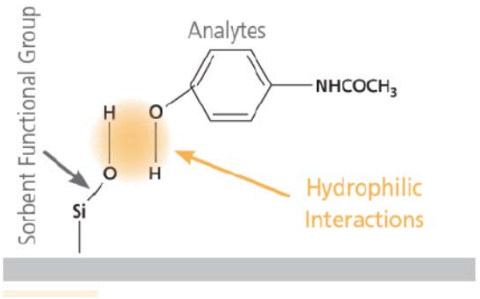

Matrix can not give hydrophilic interactions!

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Sorbents with bonded phase

- Same as in HPLC (-C18, -CN, -phenyl)
- Both silica and polymer are used as sorbent material
- $-C_{18}$ and $-C_8$ are suitable for organic matter determination in water rich sample (including biological fluids)
- -CN, $-NH_2$ and diool
  - If matrix is organic solvent

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Ion exchange

- Separation of both organic and inorganic ions
  - For strong acids weak anion exchange (WAX) is used
  - For weak acids strong anion exchange (SAX) is used
- Both silica and polymer based sorbents
  - Polymer can be used on a wider pH range

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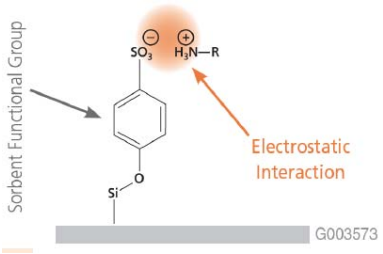

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Mixed- type sorbents

- Usually ion exchange groups and alkyl chains are bonded together
- Very popular for determination of medicines with ionizable groups
- Allows to bond analyte with two different mechanisms and wash out interferences with both types (analyte is bond with opposite mechanism)

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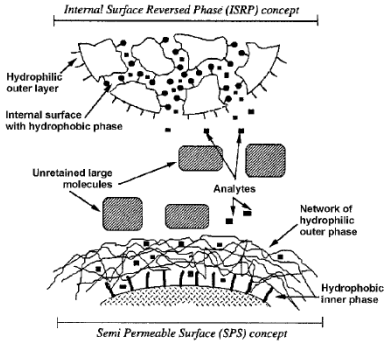
Internal surface reversed phase



SPE tubes

Tubes with different volume and sorbent mass are available.

Bed Weight	Tube Volume	Minimum Elution Vol.	Bed Capacity*
50-100 mg	1 mL	100-200 µL	2.5-10 mg
500 mg	3 mL	1-3 mL	25-100 mg
0.5-1 g	6 mL	2-6 mL	25-100 mg
2 g 5 g 10 g	12 mL 20 mL 60 mL	10-20 mL 20-40 mL 40-100 mL	0.1-0.2 g 1.25-2.5 g 0.5-1 g

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SPE tubes

- Sorbent should not be saturated with analyte and matrix

1. Large amount of analyte
2. Large amount of matrix
3. Matrix bonds strongly to sorbent
4. Too small amount of sorbent

→

1. Use SPE with different mechanism
2. Use SPE tube with larger sorbent mass
3. Reduce sample amount

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SPE vs. liquid-liquid extraction

- SPE uses less solvents
  - Comparable with LLE in a tube
- SPE extract is more concentrated
- SPE needs to have liquid samples
  - LLE in a tube also uses „suspensions“
- SPE is considered more specific
  - Also opposite examples are in the literature
- SPE can be automated

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SPME

- Solid-Phase Micro extraction
- Sorbent is bonded to a inert carrier.
- Sorbent is introduced into the sample
  - Analytes bond to the sorbent
  - Latter analytes are desorbed with a small amount of strong solvent
- Very fast and can be used in both lab and *in situ*

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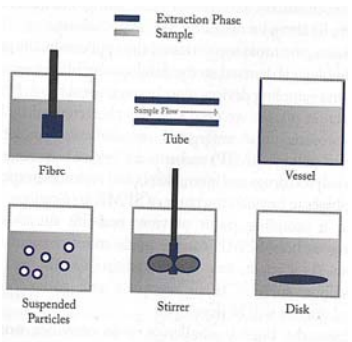

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Dialysis

- Molecules diffuse through walls (membrane) of dialysis column according to the size of molecules
- Dialysis column is putted into a dialyses cell, which is filled with solution of appropriate pH.
- Biological and herbal samples

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Solid samples

- Solid - liquid extraction.
  - Room temperature or heated. Solid part is removed with filtration.
- Soxhlet extraction.
- *Forced-flow leaching.*
  - Sample is placed into a flow-through tube. Tube is heated ta a temperature near solvent boiling point.
- Homogenization.
- Sonication.
- Dissolving.

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Solid samples 2

- Accelerated solvent extraction (ASE) or pressurized solvent extraction (PSE).
  - Sample and solvent are heated in a closed vessel at high pressure to temperature above solvent boiling point.
- Automated Soxhlet (*aka* Soxtec).
- Supercritical fluid extraction (SFE).
- Extraction with microwaves.
  - In a closed (if solvent adsorbs microwaves) or oppened (if solvent does not adsorb microwaves) vessel.

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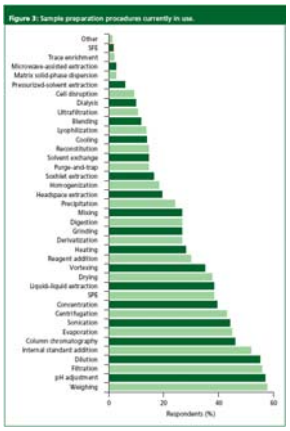

Table 1: Number of sample preparation techniques required per sample.

Number of techniques	Respondents (%)
1	19.3
2	28.6
3	20.7
4	15.0
5	8.6
6	2.1
7	0.7
More than 7	5.0

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