Modern methods of sample preparation

Objective of sample preparation

To transfer analyte into a form suitable for analysis

Main steps of sample prep.

Extraction (liquid-liquid, solid-liquid, liquid-gas, solid-gas)

Concentration

Derivatization

Cleanup

Methods to be considered

Liquid-liquid extraction

Solid phase extraction

Soxhlet extraction

Accelerated solvent extraction

Microwave digestion

Static and dynamic headspace extraction

Solid phase microextraction

Derivatization

Evaporative concentration

Liquid-liquid extraction (LLE)

Goal – to transfer analyte from aqueous to organic phase (or reversely)

When the volume of extractant is lower than sample volume, combines concentration of analyte

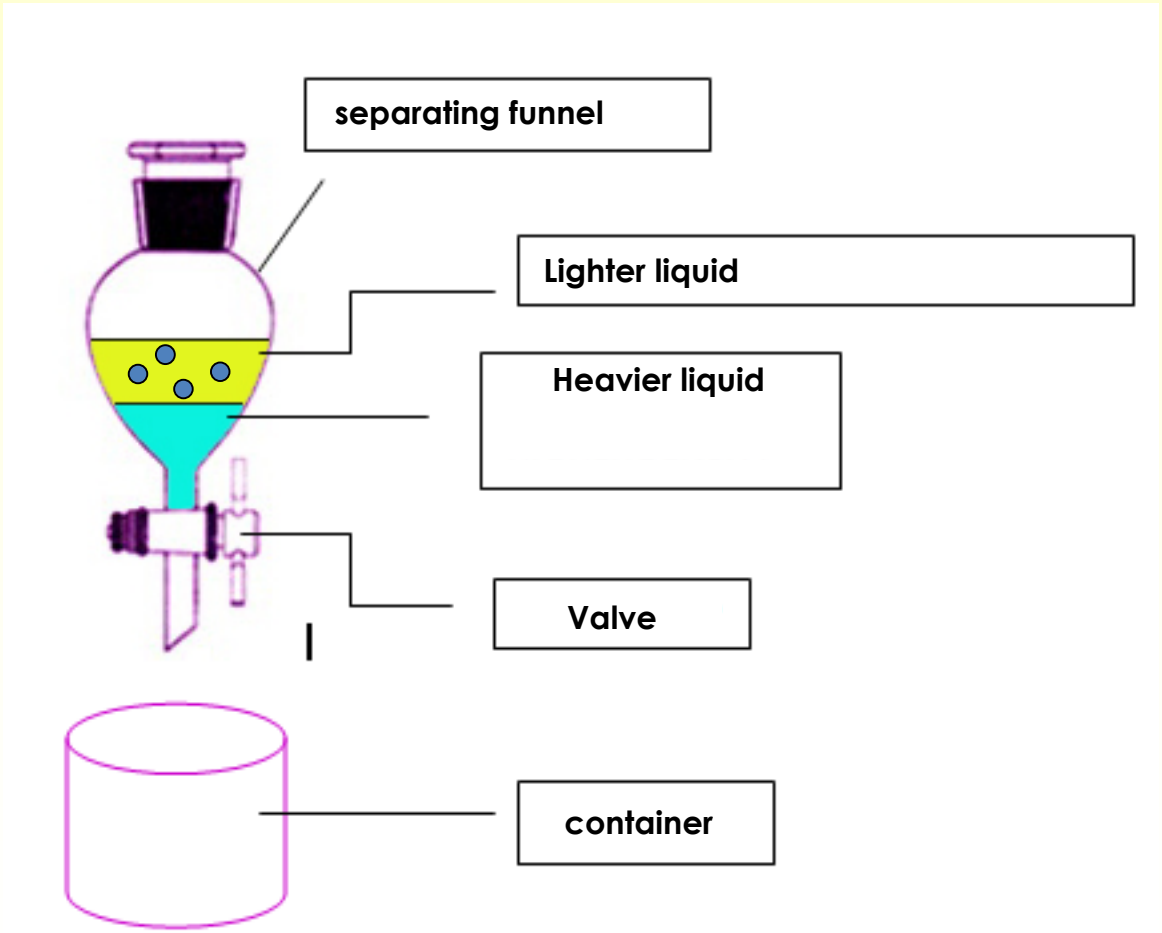
Old and reliable extraction method

Widely applied for extraction of organic analytes from aqueous samples. The are a number of methods for selective extraction of metals from aqueous to organic phase.

$$X_A \rightleftharpoons X_B$$

$$K_D = \frac{[X]_B}{[X]_A}$$

How it works


separating funnel
Lighter liquid
Heavier liquid
Valve
container

Hydrophobicity

A physical property of a molecule that wants to avoid a contact with water

Hydrophobicity is expressed as octanol-water partitioning coeffient, or its decimal logarithm (log K_{ow})

$$K_{OW} = K_D = \frac{[X]_O}{[X]_W}$$

Highly hydrophilic molecules have log $K_{ow} < 1$

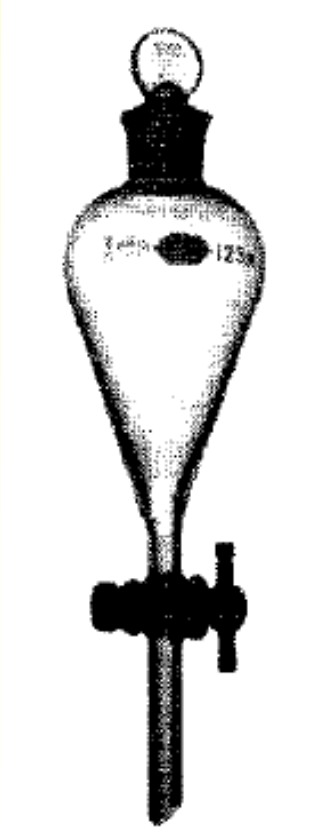
Highly hydrophobic molecules have log $K_{ow} > 3$

Hydrophobicity of few compounds

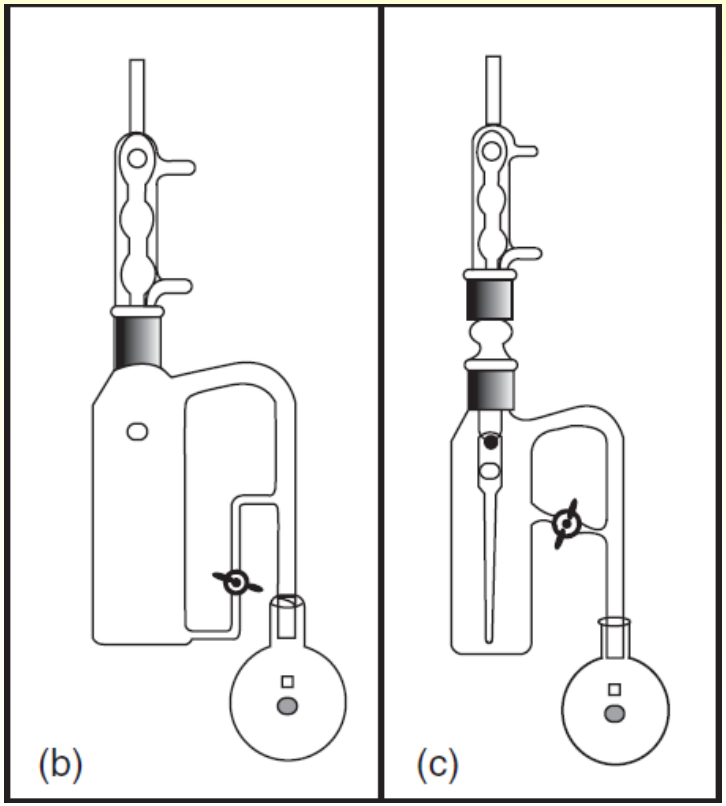
Compound	Log K_{ow}	Water solubility (mg/L)
Benzo[a]pyrene	6.13	0.11
Hexane	3.9	13
Benzene	2.13	1800
Chloroform	1.97	8000
Acetone	-0.24	mixed

LLE equipment

Separatory funnel



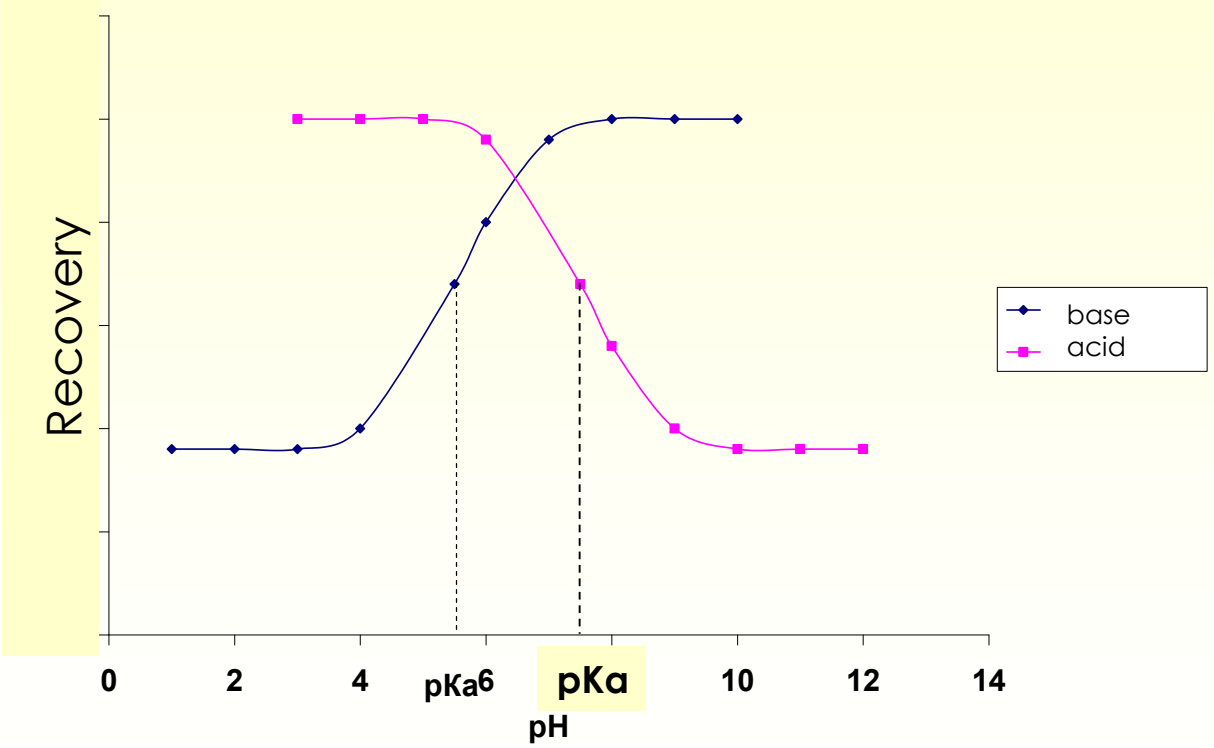
Continuous LLE apparatus



pH effect of LLE recovery

$$HA \rightleftharpoons H^+ + A^-$$

$$BH^+ \rightleftharpoons H^+ + B$$



Exercise

Sample of water (V = 50 mL) containing phenol was extracted by 10 mL of methylene chloride. Distribution constant for phenol between water and methylene chloride is 8.7. Extract was analyzed by GC-MS, phenol concentration was 22 µg/L. Calculate concentration of phenol in a sample of water and recovery of phenol from water sample

Solution

Knowing distribution constant and equilibrium concentration of phenol in organic phase, we can find equilibrium concentration in a water phase:

$$K_D = \frac{[C_o]}{[C_w]} \; or \; [C_w] = \frac{[C_o]}{K_D} = \frac{22\,\frac{\mu g}{L}}{8.7} = 2.53\,\frac{\mu g}{L}$$

Now we need to find initial concentration of phenol in water sample using mass conservation law – part of the phenol mass was transferred from water to organic phase: m = $[m_w]$+$[m_o]$

$$m = [C_w] \times V_w + [C_o] \times V_o = 2.53\,\frac{\mu g}{L} \times\ 50\ mL + 22\,\frac{\mu g}{L} \times\ 10\ mL$$
$$= 126.5\ ng + 220\ ng = 346.5\ ng$$

Solution (continued)

Now we can find initial concentration of phenol in water (C=m/V):

$$C = \frac{346.5\ ng}{50\ mL} = 6.93\ \frac{ng}{mL}\ or\ 6.93\frac{\mu g}{L}$$

Recovery can be found as the fraction of the mass that was extracted by organic solvent:

$$R = \frac{[m_o]}{m} = \frac{220\ ng}{346.5\ ng} = 0.635\ or\ 63.5\%$$

Question

How to determine analyte concentration in water if distribution constant is unknown?

Answers

Reach 100% recovery using serial extractions (5-10) with new portions of an organic solvent

Determine recovery and K_D by analyzing serial extracts (2-3) with new portions of solvent. Difference between analyte concentration in serial extracts will show the recovery and K_D

Spike water with an isotopically labeled internal standard of phenol of a known concentration. Then you can find recovery from a water sample and K_D

Make a separate experiment and determine K_D

Question

How to determine analyte concentration if distribution constant is different for different samples (20-90)?

Answer

Use standard addition method to control matrix effect

Spike water with an isotopically labeled internal standard of phenol of a known concentration. Then you can find recovery from a water sample and K_D

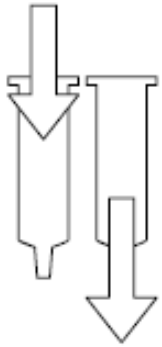
Methods to increase recovery

Add excess salt

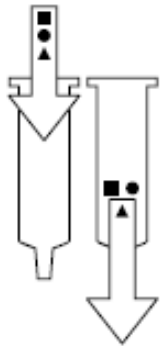
Change pH

Extract by several solvent portions (serial extraction)

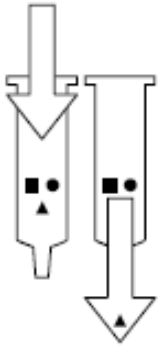
Solid phase extraction (SPE)



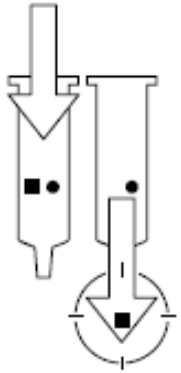
CONDITIONING
Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate).



RETENTION
■ Adsorbed isolate
● Undesired matrix constituents
▲ Other undesired matrix components

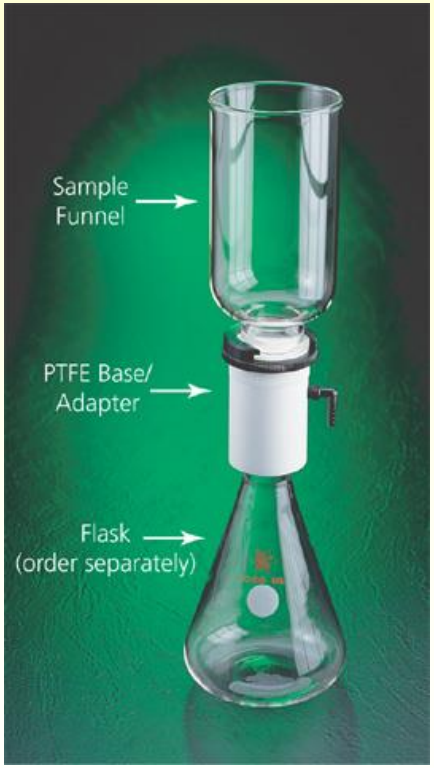


RINSE
▲ Rinse the columns to remove undesired matrix components

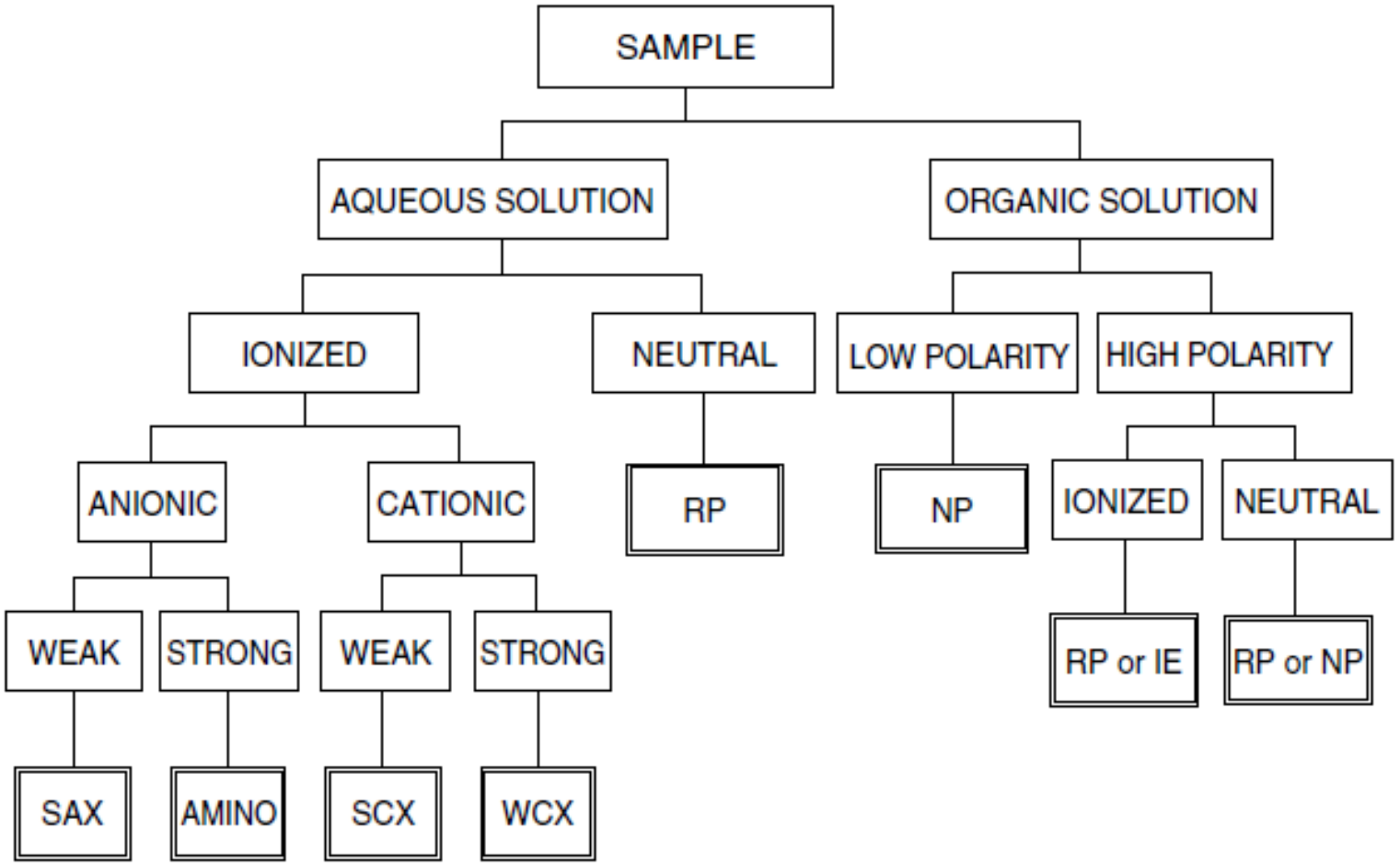


ELUTION
● Undesired components remain
■ Purified and concentrated isolate ready for analysis

SPE equipment



SPE method development



Exercise

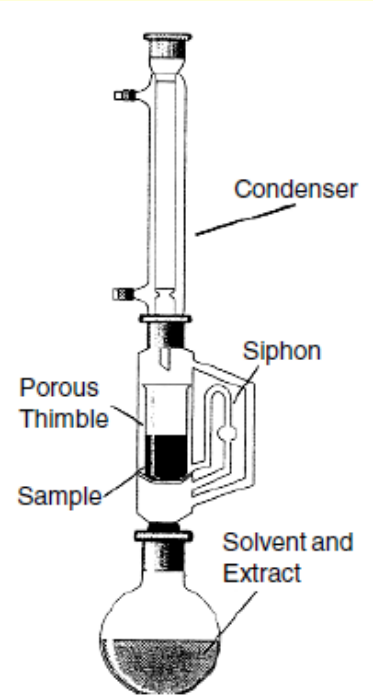
Water sample (V = 50 mL) containing phenol was passed through reversed-phase SPE cartridge. Analyte was 100% retained by a stationary phase and eluted by 2 mL of methylene chloride. To the extract, 50 µL of dodecane was added followed by full evaporation of methylene chloride. Analysis showed phenol concentration in the evaporated extract 230 µg/L. Calculate phenol concentration in a water sample.

Homework

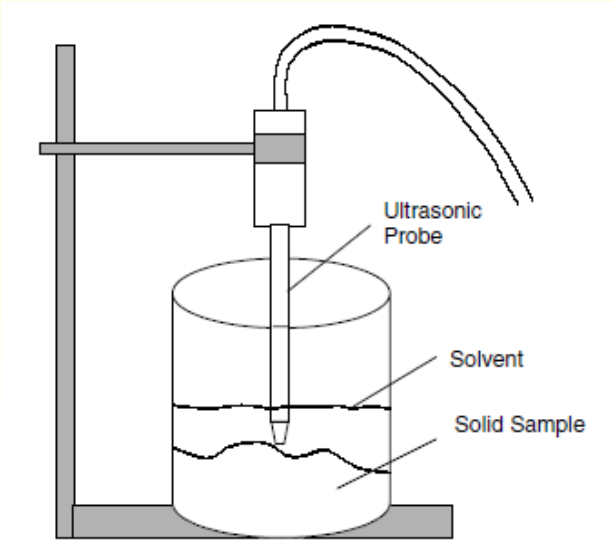
Water sample (V = 50 mL) containing phenol was passed through reversed-phase SPE cartridge. Analyte was 100% retained by a stationary phase and eluted by 2 mL of methylene chloride. To the extract, 10 µL of bromophenol (C = 25 µg/mL) was added. Analysis of the extract by GC-MS showed the ratio of analyte and internal standard concentrations 5.6. Calculate phenol concentration in water.

Solid-liquid extraction of organic analytes

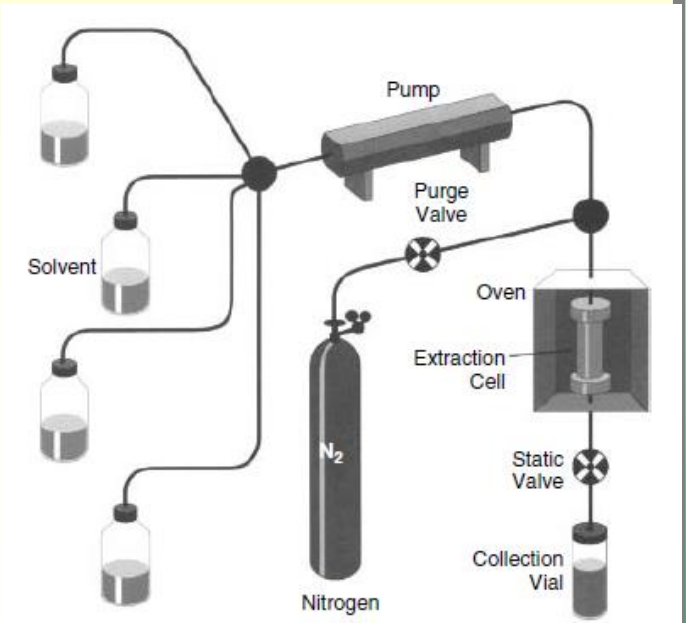
Soxhlet extraction



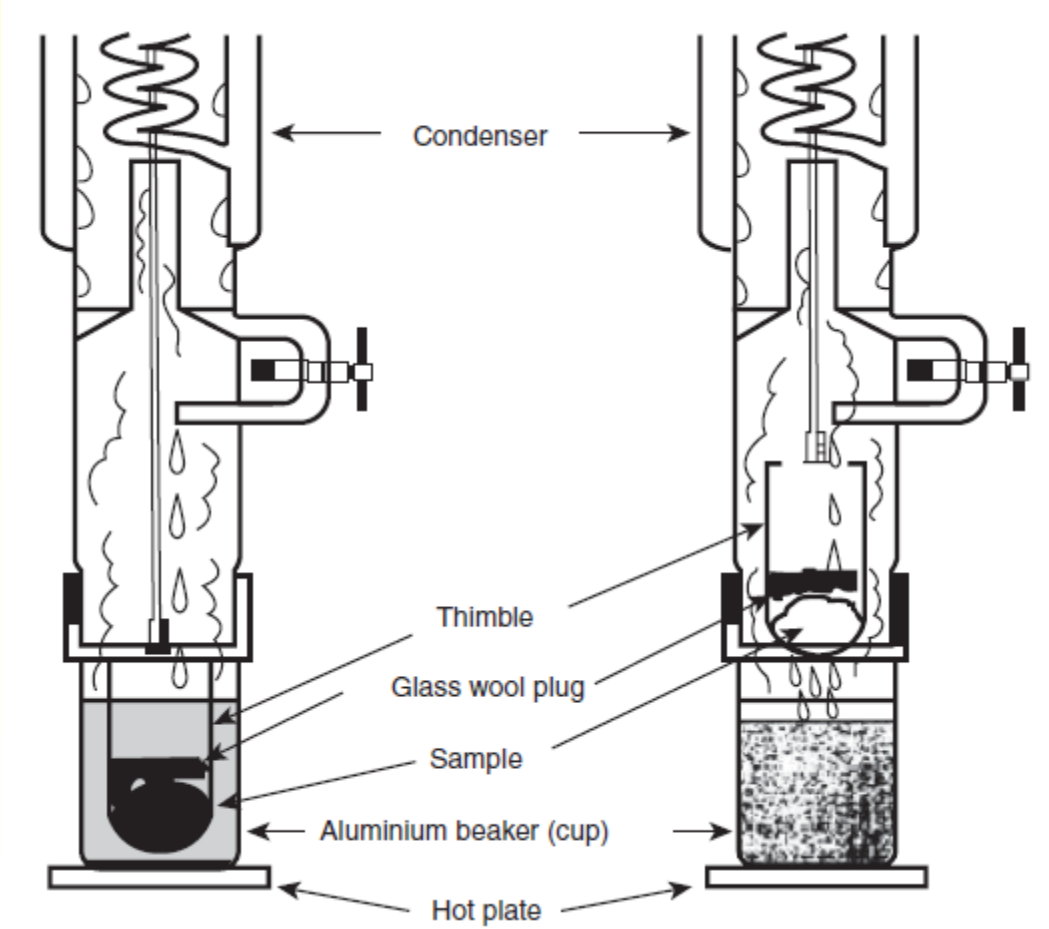
Ultrasonic extraction



Accelerated solvent extraction (pressurized liquid extraction)



Automated Soxhlet (Soxtec)



Pressurized liquid extraction – leading extraction method around the world

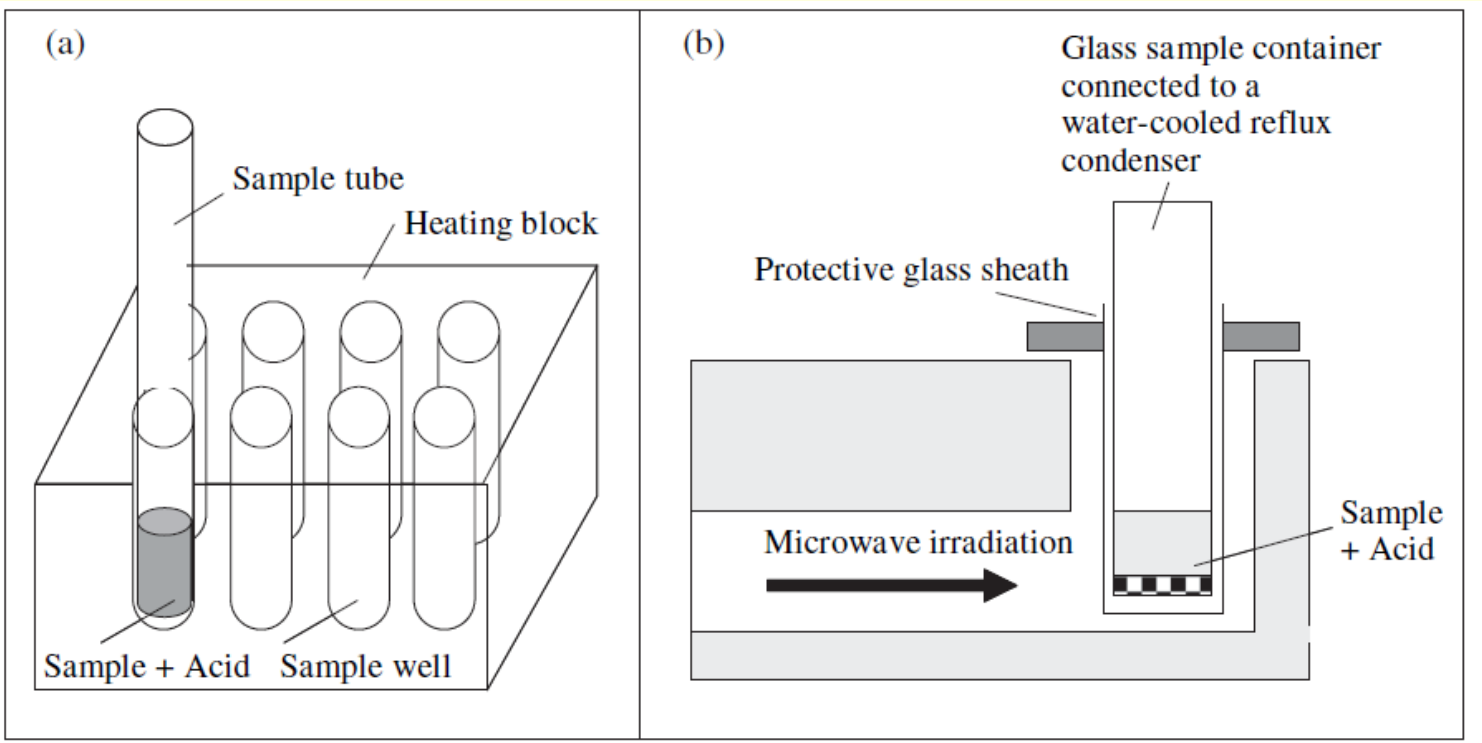
Dionex ASE-150

Dionex ASE-350

Digestion of solid samples for inorganic analysis

Digestion at elevated temperature

Microwave digestion by acids



Digestion of metals from solid matrices

For clean and easily oxidizing matrices HNO_3 is utilized

For fast oxidizing matrices – mixtures HNO_3-HCl or HNO_3-H_2SO_4

For hardly oxidizing samples – mixture HNO_3-$HClO_4$

For silicate-containing samples - mixture HNO_3-$HClO_4$-HF

For high concentration of mercury – mixture HNO_3-H_2SO_4 at a presence of $KMnO_4$ и $K_2S_2O_8$

For extraction of Cr (VI) – basic solution of 0.28M Na_2CO_3 and 0.5M NaOH

Static headspace (HS) extraction

Goal – to transfer analyte from solid or liquid to gaseous phase for subsequent analysis (typically by GC)

Requires heat of the sample (max to 200°C)

Does not require any solvents of adsorbents

Can be fully automated

Efficient sample cleanup from non-volatile compounds (perfect for GC)

Low detection limits are achievable with cryogenic focusing

Standard method for alcohol in blood analysis

Vapor pressure and Henry's Law constant

Vapor pressure – pressure of a vapor (gas) of a substance in equilibrium with pure, condensed liquid or solid phase at a given temperature

Henry's Law constant shows evaporability of a substance from aqueous solution at a given temperature

$$H' = K_D = \frac{[\mathrm{X}]_\mathrm{G}}{[\mathrm{X}]_\mathrm{L}}$$

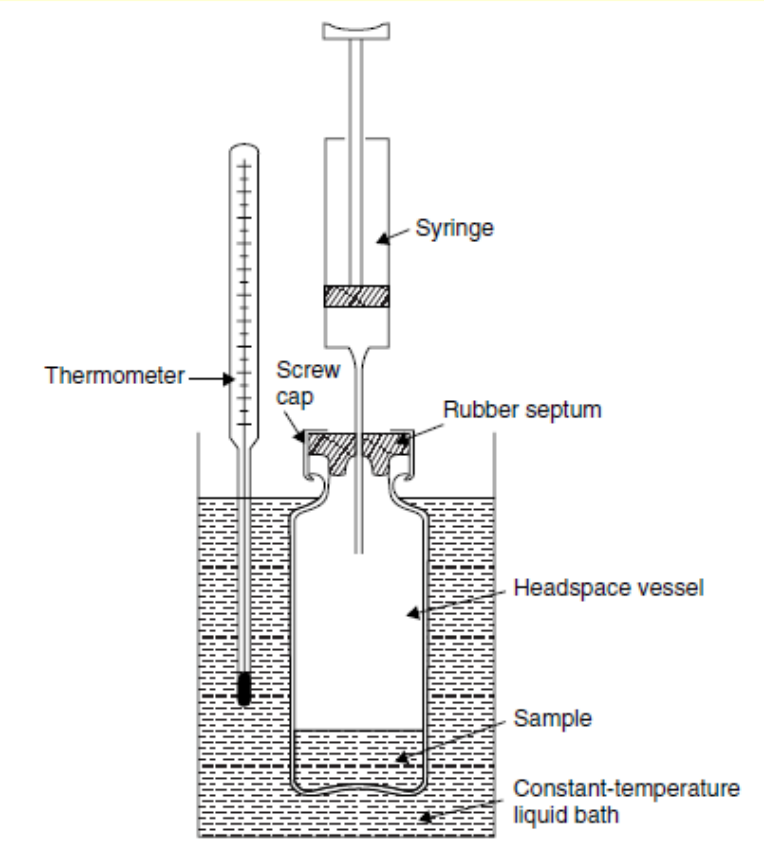
For highly diluted samples

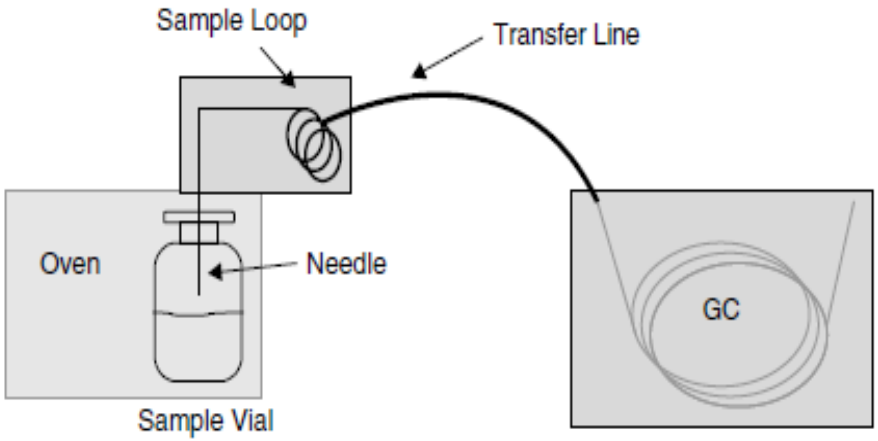
$$H = \frac{P_{\mathrm{vp}}}{S}$$

- vapor pressure

- water solubility

Static HS instrumentation





Exercise

Sample of blood (V = 1.00 mL) of a drunk driver was diluted by distilled water to a final volume of 100.0 mL. Obtained solution (V = 5.00 mL) was introduced into a 20 mL headspace vial. Vial was sealed, kept at 25°C and the headspace was analyzed by GC-FID. Concentration of ethanol in the headspace was 970 µg/L. Calculate ethanol concentration in blood sample if Henry's law constant of ethanol at 25°C is 5.00×10^{-3} atm m^3 / moL. Was the driver drunk? If yes, at what level?

Solution

Henry's law constant represents equilibrium between gas (HS) and aqueous (W) phases:

$$HLC = \frac{[C_{HS}]}{[C_w]}$$

The constant has been given in atm m^3 / moL. It means that the equilibrium concentration of ethanol in water is represented in (mol/m^3). Concentration of ethanol in gas phase is represented in pressure units (atm) as a partial pressure.

Concentration of ethanol in the headspace is 970 µg/L. We need to convert this concentration to atm.

Solution (continued)

Pressure and concentration of compounds in gas phase are connected via the Ideal Gas Law:

$$pV = \frac{mRT}{M} \textbf{ or } \frac{pM}{RT} = \frac{m}{V} \textbf{ or } \frac{pM}{RT} = C\left(\frac{ug}{L}\right) \textbf{ or } p = \frac{CRT}{M}$$

$$p = \frac{970\ \cancel{\mu g} \times 8.31\ \cancel{L}\ kPa \times 298\ \cancel{K\ mol}}{\cancel{L\ mol\ K}\ 46\ \cancel{g}} \times \frac{1\ \cancel{g}}{10^6 \cancel{\mu g}} = 0.0522\ kPa$$

Let's convert this pressure to atm (1 atm = 101.325 kPa):

$$p = 0.0522\ kPa \times \frac{1\ atm}{101.325\ kPa} = 5.15 \times 10^{-4} atm$$

Solution (continued)

Now let's find the equilibrium concentration of ethanol in water:

$$HLC = \frac{[C_{HS}]}{[C_w]} \textbf{ or } [C_w] = \frac{[C_{HS}]}{HLC} = \frac{5.15 \times 10^{-4} \cancel{atm}\ mol}{5.00 \times 10^{-3} \cancel{atm}\ m^3} = 0.103 \frac{mol}{m^3}\ or \frac{mmol}{L}$$

Initial ethanol concentration will be higher because part of ethanol mass was transferred from water to headspace. We need to use mass conservation law to find the total mass of ethanol: $m_0=m_{HS}+m_w$

$$[m_{HS}] = [C_{HS}] \times V_{HS}$$

Volume of headspace is unknown, but we know that total volume (of vial) is 20 mL and volume of water is 5.00 mL: V_{HS} = 20 mL – 5.00 mL

$$[m_{HS}] = 970 \frac{\mu g}{L} \times 15\ mL \times \frac{1L}{1000mL} = 14.6\ \mu g$$

Solution (continued)

Now let's find the equilibrium mass of ethanol in water:

$$[m_W] = [C_W] \times V_w = 0.103 \frac{\cancel{mmol}}{\cancel{L}} \times 5.00\ \cancel{mL} \times \frac{46\ g}{1\ \cancel{mol}} \times \frac{1\ \cancel{L}}{1000\ \cancel{mL}} \times \frac{1\ \cancel{mol}}{1000\ \cancel{mmol}} = 23.7\ \mu g$$

Now we can find the initial concentration: (C = m/V):

$$C_0 = \frac{23.7\ \mu g + 14.6\ \mu g}{5.00\ mL} = 7.65\ \frac{\mu g}{mL}$$

To find the concentration of ethanol in blood sample, we need to take into account a dilution using the formula $C_1V_1=C_2V_2$:

$$C_b = \frac{7.65\ \mu g\ \times 100\ mL}{mL\ 1.00\ mL} = 765\ \frac{\mu g}{mL}$$

Solution (continued)

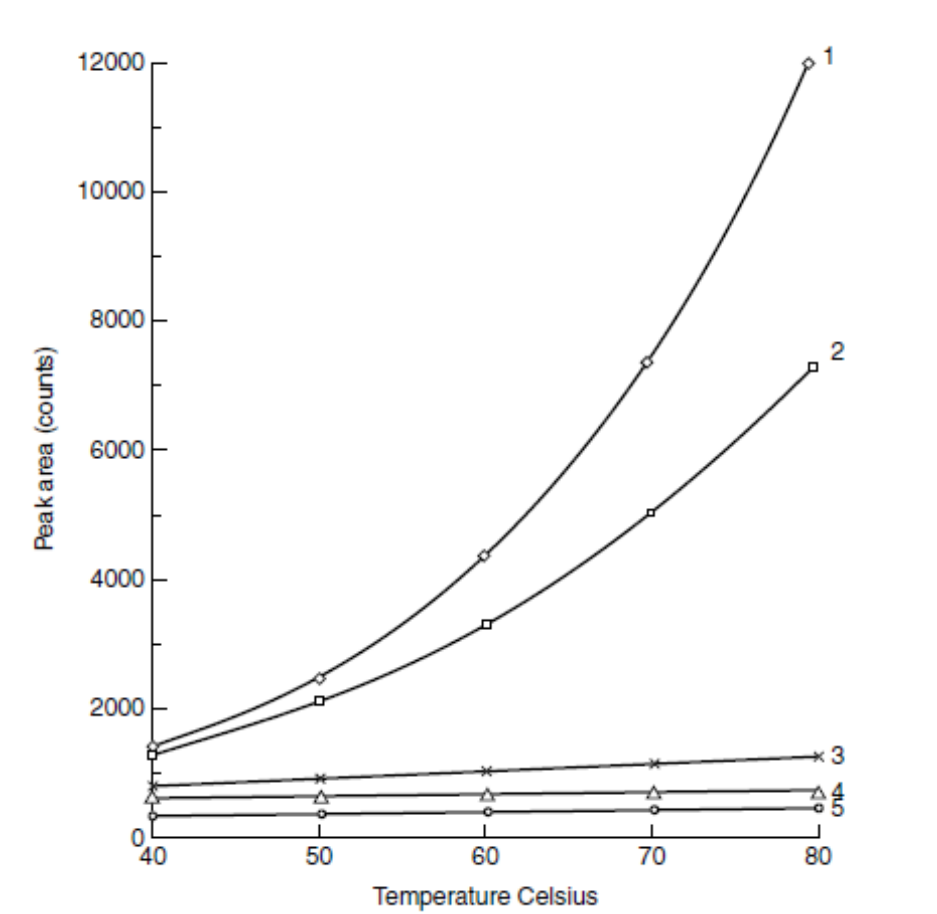
To check if the driver was drunk, we need to find concentration in promille (mL of ethanol in 1 L of blood – mL/L). We can convert mass of ethanol to a volume using its density (0.789 g/mL)

$$C = \frac{765\ \cancel{\mu g}\ mL}{\cancel{mL}\ 0.789\ \cancel{g}} \times \frac{1\ \cancel{g}}{10^6 \cancel{\mu g}} \times \frac{10^3\ \cancel{mL}}{L} = 0.97 \frac{mL}{L}$$

According to the legislation of Kazakhstan, such concentration corresponds to a light degree of drunkenness. In all countries around the world, such level of ethanol in blood of drivers is not permitted!

Answer: concentration of ethanol in blood of a driver is 0.97 mL/L corresponding to a light level of drunkenness. It is prohibited to drive at such level of ethanol in blood.

Effect of temperature for water samples



1 – ethanol;

2 – methyl ethyl ketone;

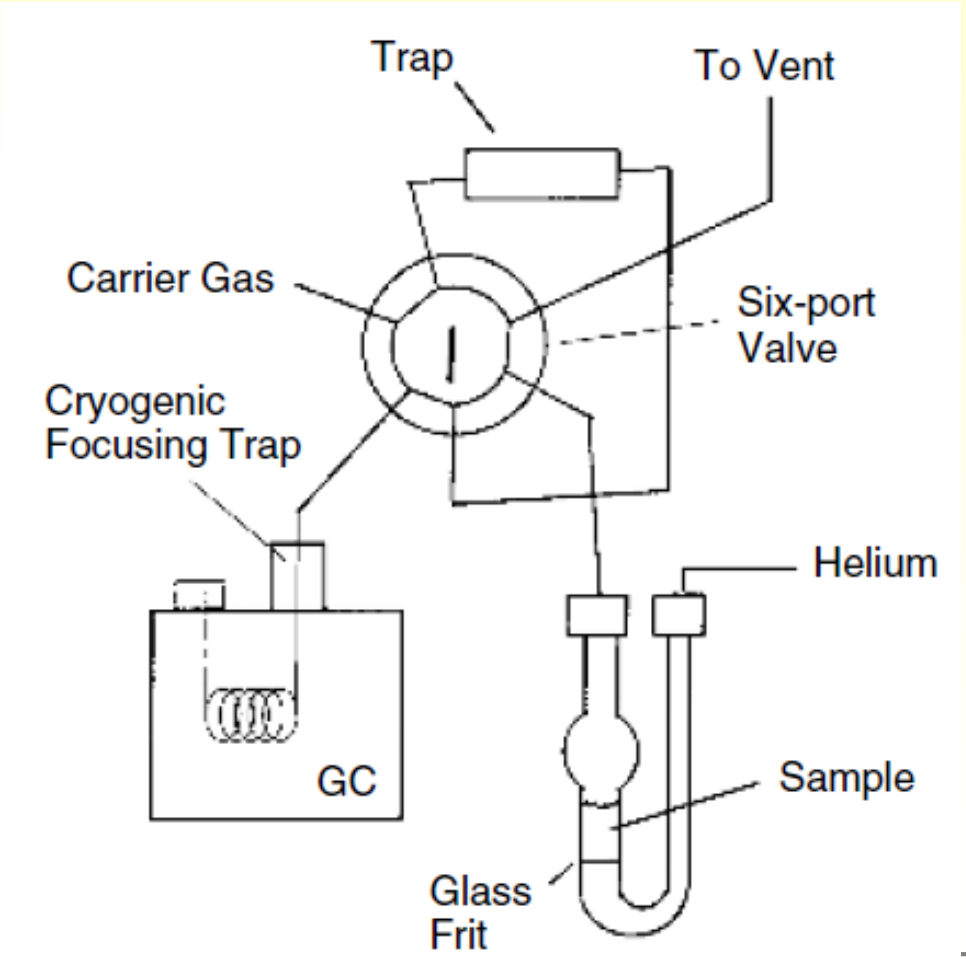
3 – toluene;

4 – n-hexane;

5 - tetrachloroethylene

Static HS autosamplers

Dynamic headspace extraction (Purge and Trap)



Exercise

Sample of drinking water (V = 2.00 mL) was introduced into a 20 mL headspace vial. Vial was sealed and purged by helium during 30 min till the full transfer of analytes into a thermal desorption tube packed with Tenax adsorbent. Sorbent tube was completely desorbed to a GC-MS instrument. According to external standard calibration, 380 pg of tetrachloroethylene reached the detector. Calculate concentration of tetrachloroethylene in the analyzed water sample. Did the concentration exceed regulatory threshold for TCE in water (1 µg/L)?

Solution

From the method principle, we know that 100% of TCE was transferred from sample to sorbent and later to GC column and detector. So, the mass measured by the detector is equal to the mass in the sample.

$$C = \frac{m}{V} = \frac{380\ pg}{2.00\ mL} = 190\ \frac{pg}{mL}\ or\ 190\ \frac{ng}{L}\ or\ 0.190\ \frac{\mu g}{L}$$

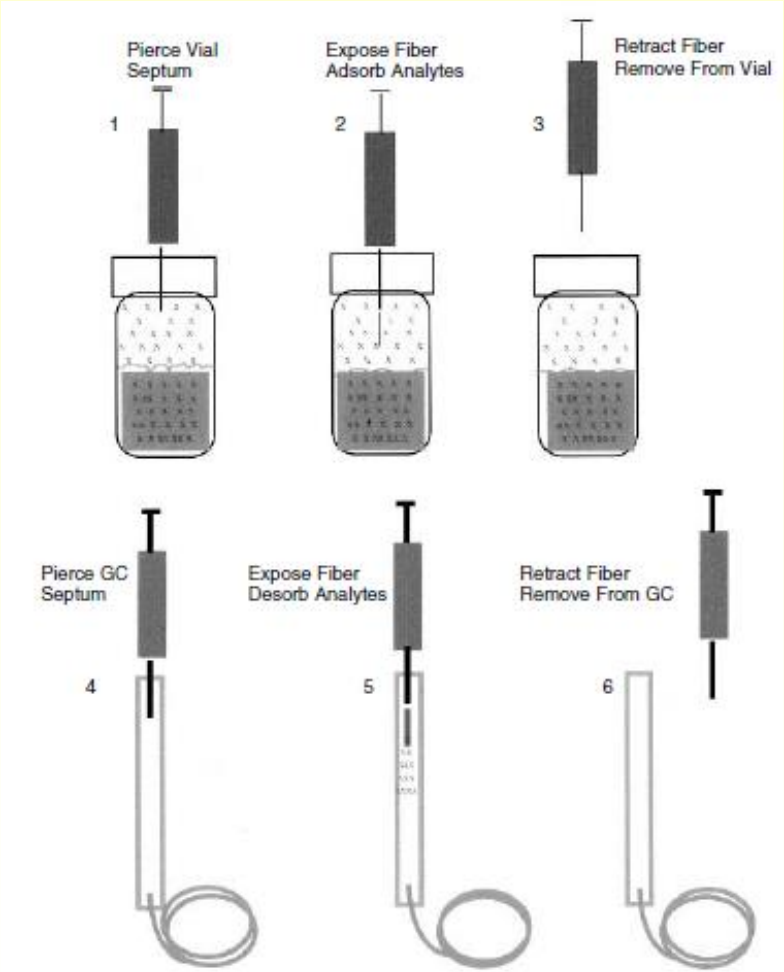
Determined concentration (0.190 µg/L) is lower than a regulatory threshold (1 µg/L).

Homework

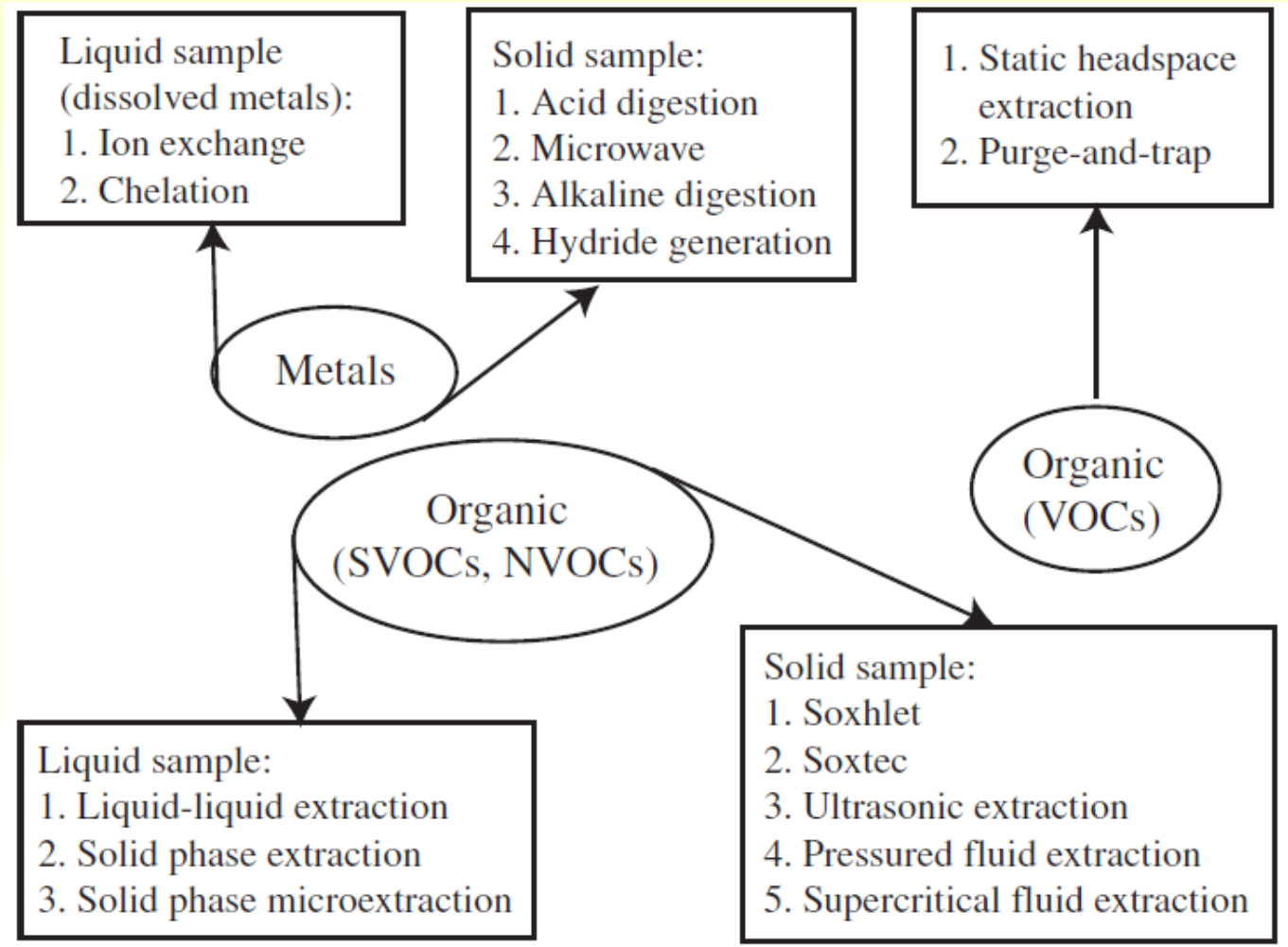
Is it possible to detect TCE in water at such a level by analysis of 100 µL of the headspace above 10 mL of sample in 20 mL vial? Detector sensitivity is 2 pg.

First student who solve 100% correctly this task and send me a solution to e-mail will get 4 extra points

Solid phase microextraction



Method selection



Evaporative concentration

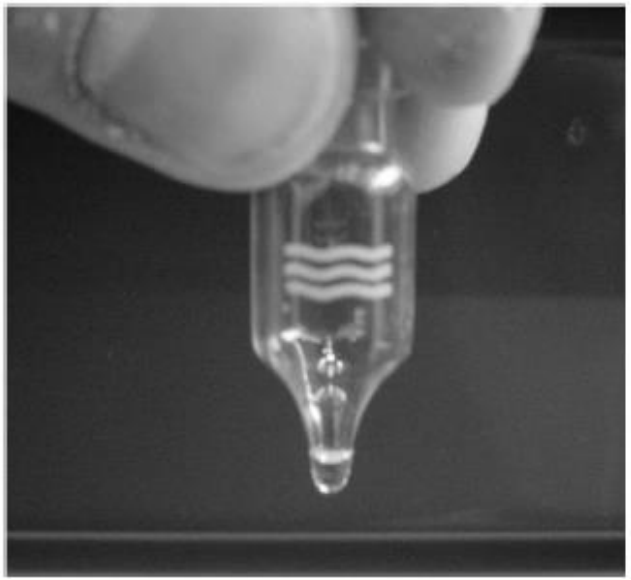
Goal – to decrease sample volume by solvent evaporation leading to an increase of analyte concentration

Simple, reliable and efficient concentration method

Solvent can be regenerated

Concentration can be increased 1000-fold

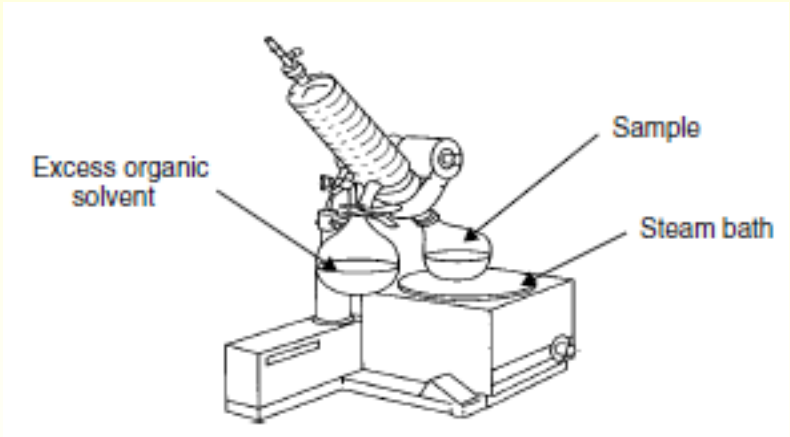
Concentration degree



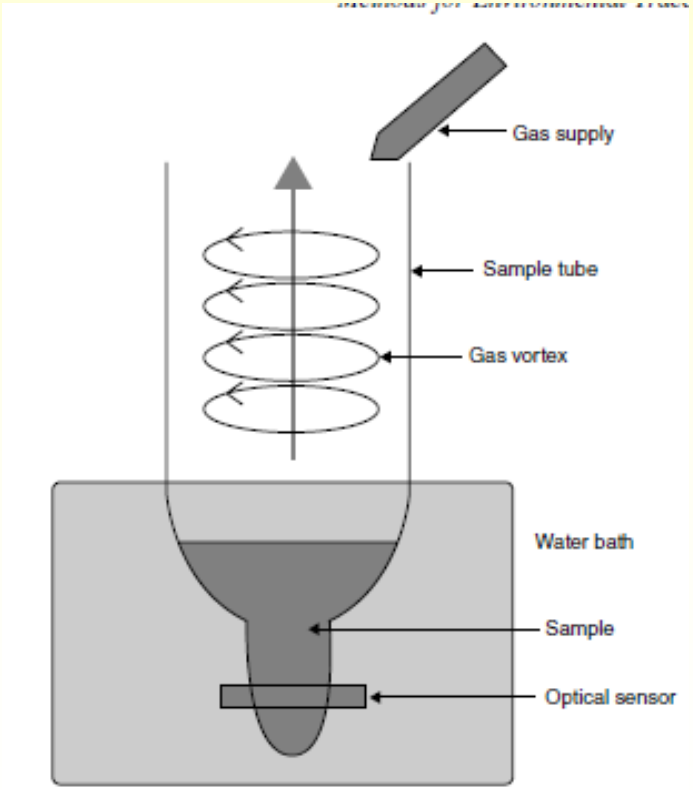
***Thanks to JF Focant**

Equipment for concentration

Rotary evaporator

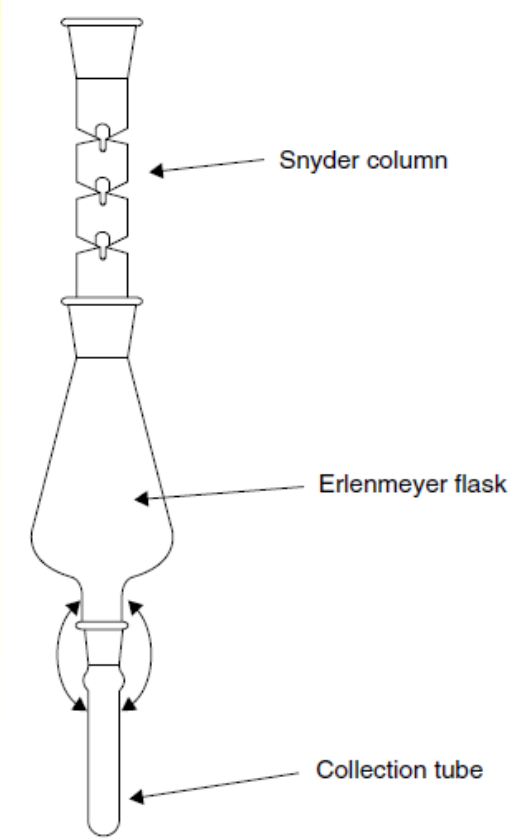


Solvent gas purge system

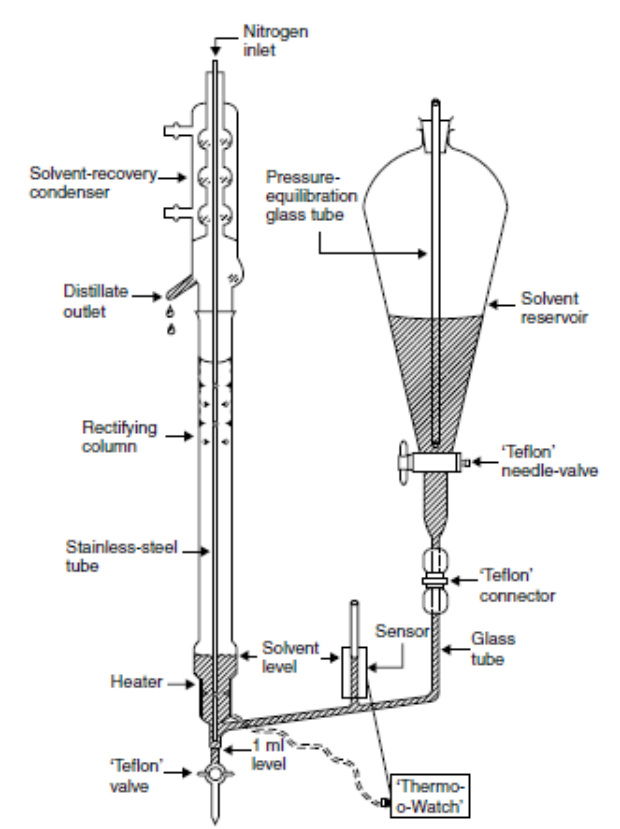


Equipment for concentration to decrease losses of analytes

Kuderna-Danish concentrator



Automated evaporation system (EVACS)



Exercise

10.0 g of soil containing N-nitrosodimethylamine (NDMA) was extracted by two portions of acetone (25 mL each). Extracts were combined to the final volume 42±2 mL and evaporated to the final volume 1.00±0.02 mL. Analysis of the evaporated extract by GC-MS showed NDMA concentration 27±1 ng/mL. Calculate NDMA concentration in soil sample and its uncertainty.

Solution

From the available data, we can calculate the total mass of analyte in concentrated extract:

$$m = V \times C = 27 \frac{ng}{mL} \times 1.00\ mL = 27\ ng$$

If no analyte was lost during evaporation, this analyte mass was also present in the extract before evaporation. Extraction recovery was not provided. Some portion of solvent remained in soil duiring filtration. But probably, the mass of NDMA in this part is negligible and recovery is 100%.

$$C = \frac{m_{NDMA}}{m_{soil}} = \frac{27\ ng}{10.0\ g} = 2.7\ \frac{ng}{g}$$

Uncertainty

$$e_{rel}^{total} = \sqrt{e_{rel1}^2 + e_{rel2}^2 + e_{rel3}^2}$$

$$e_{rel1} = \frac{1}{27} \times 100\% = 3.7\%$$

$$e_{rel2} = \frac{0.02}{1} \times 100\% = 2\%$$

$$e_{rel3} = \frac{0.1}{10.0} \times 100\% = 1\%$$

Uncertainty

$$e_{rel}^{total} = \sqrt{3.7^2 + 2^2 + 1^2} = 4.3\%$$

$$e_{abs} = 2.7\frac{ng}{g} \times \frac{4.3\%}{100\%} = 0.1\frac{ng}{g}$$

Answer: NDMA concentration in analyzed soil sample is 2.7±0.1 ng/g

Question

Part of NDMA was lost during evaporation. How to determine how much was lost? How to improve the accuracy of the analysis?

Answer

The amount of NDMA lost during evaporation may be determined usign internal standard having similar boiling point.

Using internal standard will increase total accuracy of the measurement

Derivatization

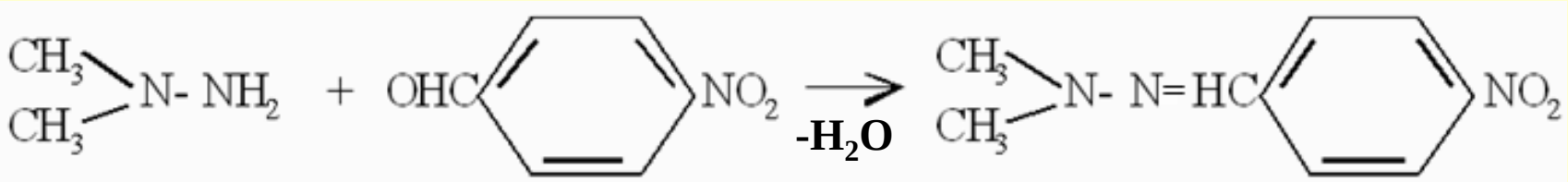
Chemical transfer of analytes into the form suitable for analysis for:

- Enhancement of analytical signal
- Increase/decrease of volatility
- Enhancement of thermal and chemical stability
- Increase of hydrophobicity

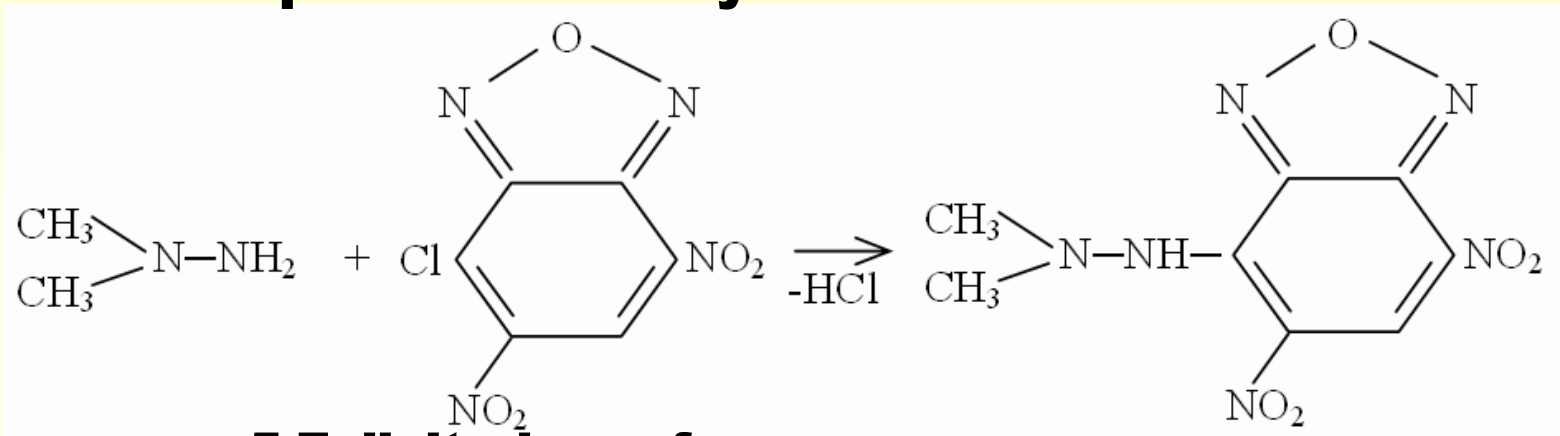
Allows to do any analyses on the available equipment (e.g., photo-colorimeter) – just find the proper agent!

Many agents are developed and commercially available

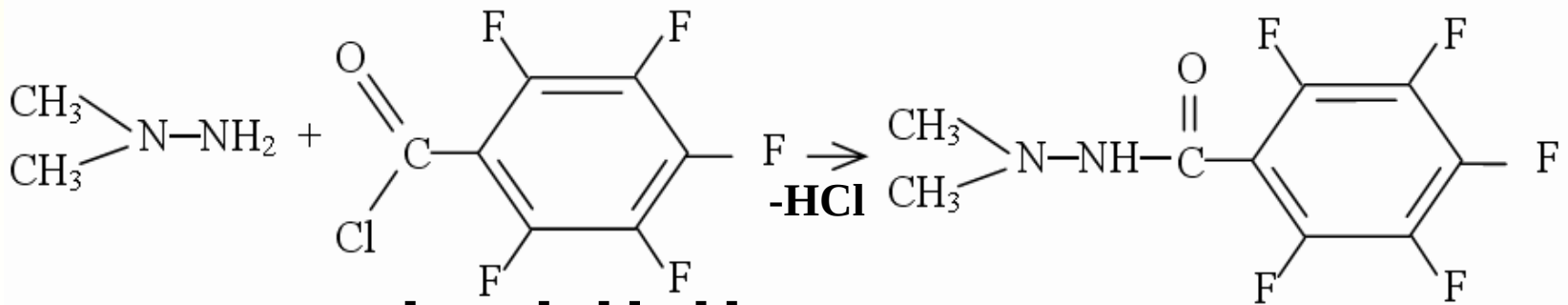
Derivatization of 1,1-dimethylhydrazine



p-nitrobenzaldehyde



5,7-dinitrobenzofurazane



dancyl chloride

Other examples

Fatty esters – methylation (fatty acids methyl esters)

Amino acids – o-phtaldialdehyde (OPA)

Phenolics, monosaccharides – BSTFA, BSA

Aldehydes – dinitrophenylhydrazine (DNPA)

Requirements for derivatization

Complete (yield close to 100%)

Fast and simple

Stable derivatization product

High analytical signal

Sample cleanup

Separation of analytes from interfering compounds:

- Particulates
- Non-volatile compounds
- Signal interferences
- Other matrix components

This step is required for trace analysis

Required in the case of analysis of dirty matrices – soil, plants, tissues, food, biological liquids

Basic cleanup methods

Size-exclusion chromatography

Solid phase extraction

Adsorption chromatography

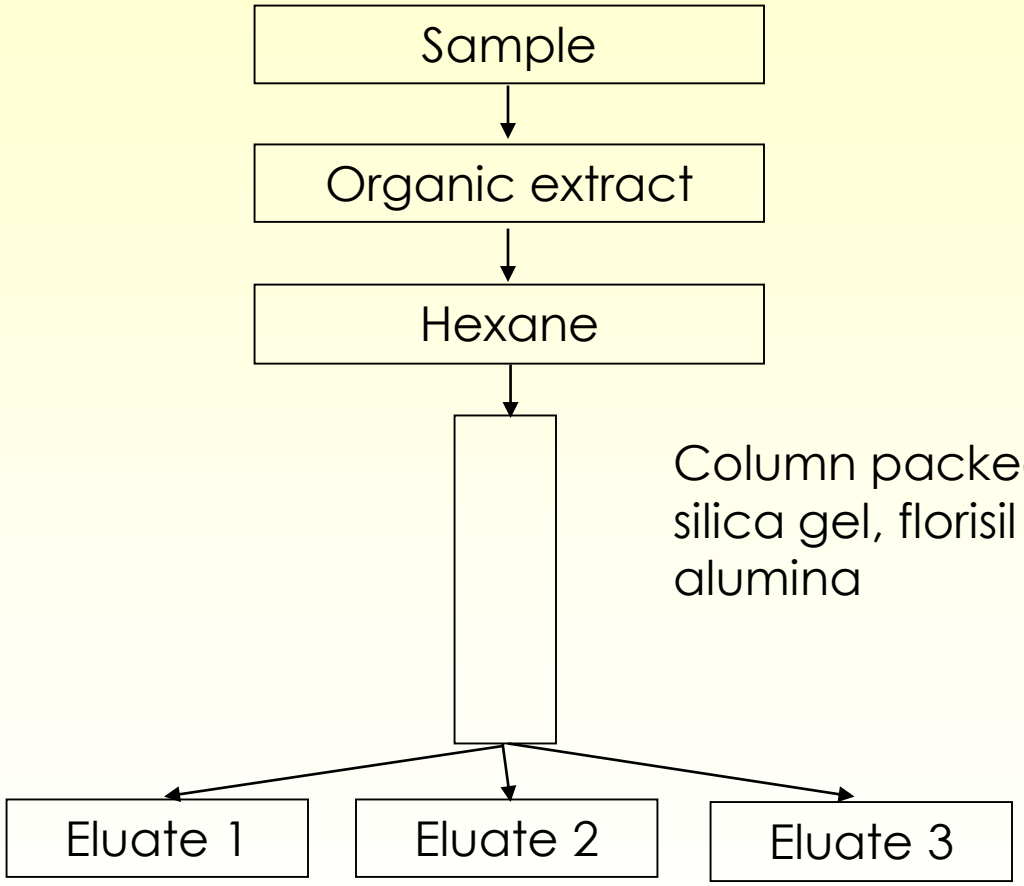
Distillation

Precipitation

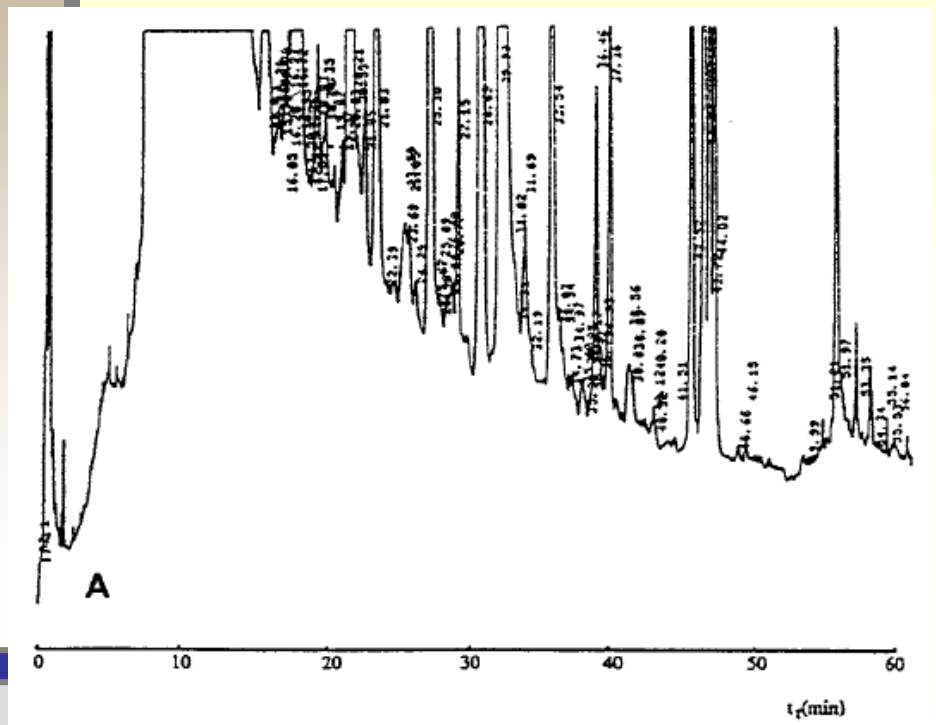
Filtration

LLE, static and dynamic HS, SPME

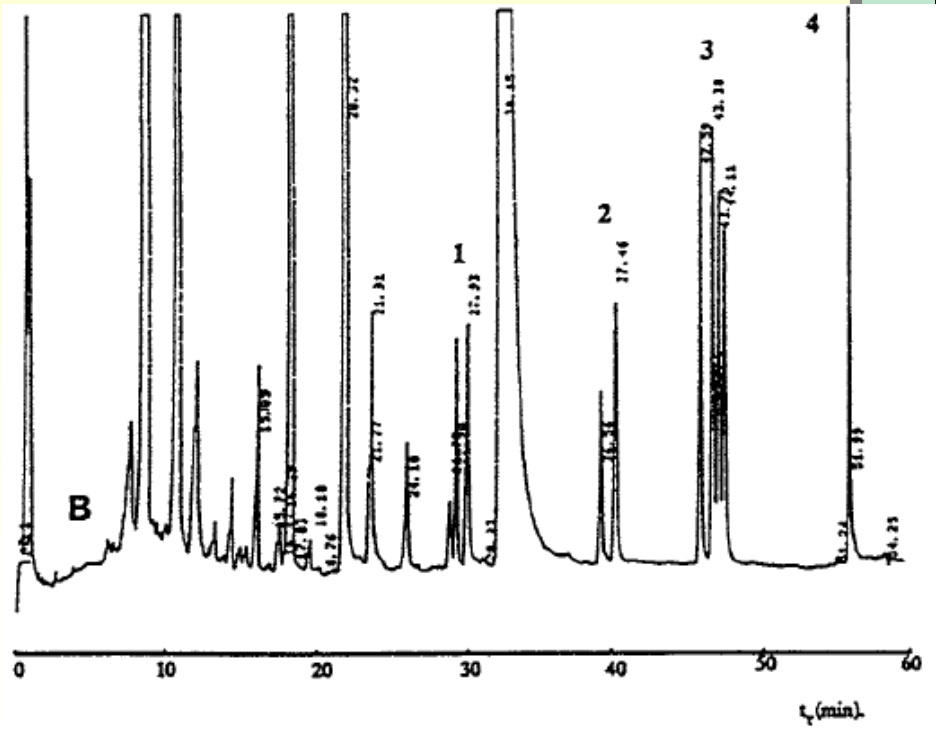
Sample cleanup for analysis of highly hydrophobic compounds



Effect of cleanup on results

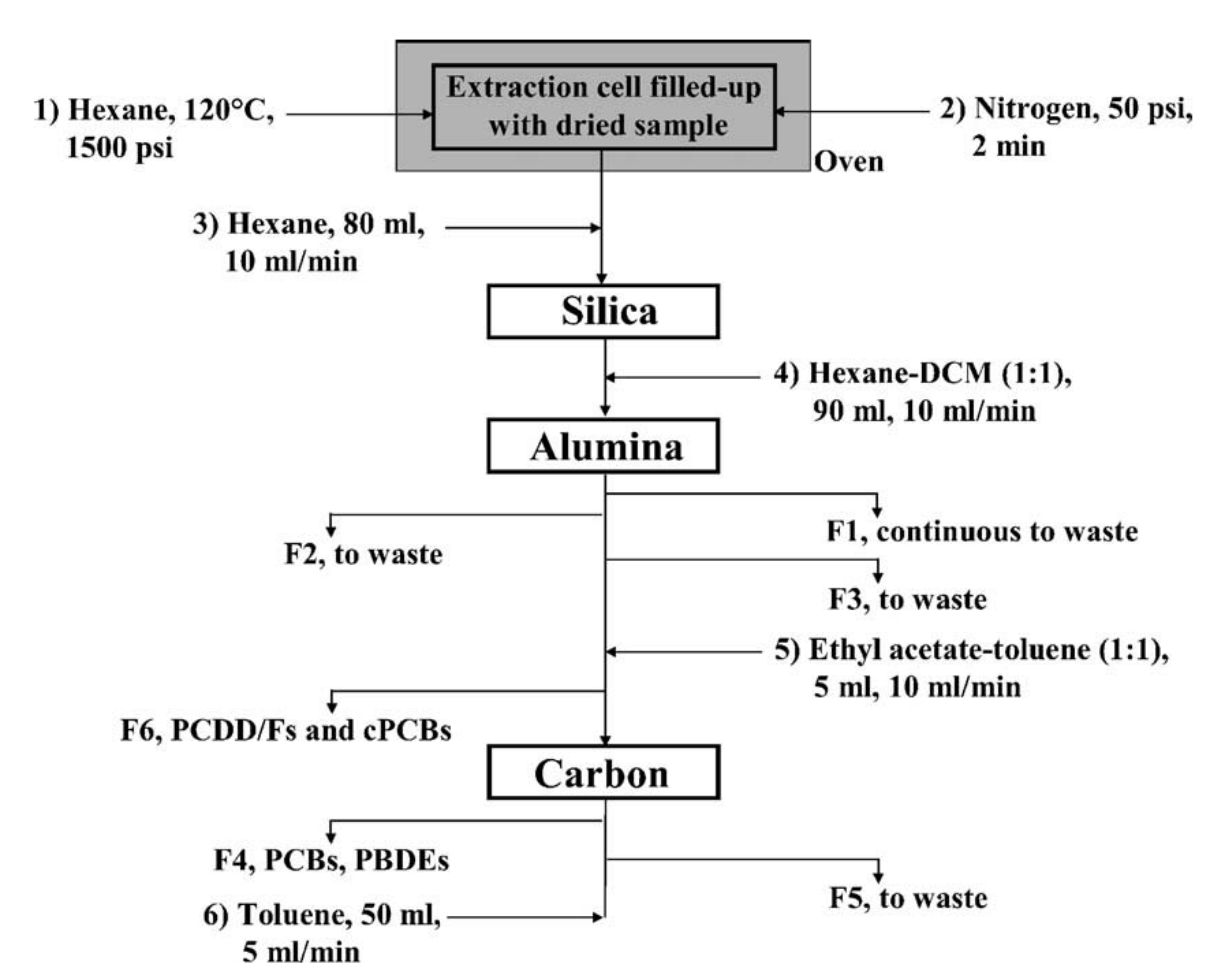


without cleanup



with cleanup

Analysis of food for dioxins, furans and PCBs – example of complex sample preparation



Integrated extraction-cleanup system for POPs analyses