



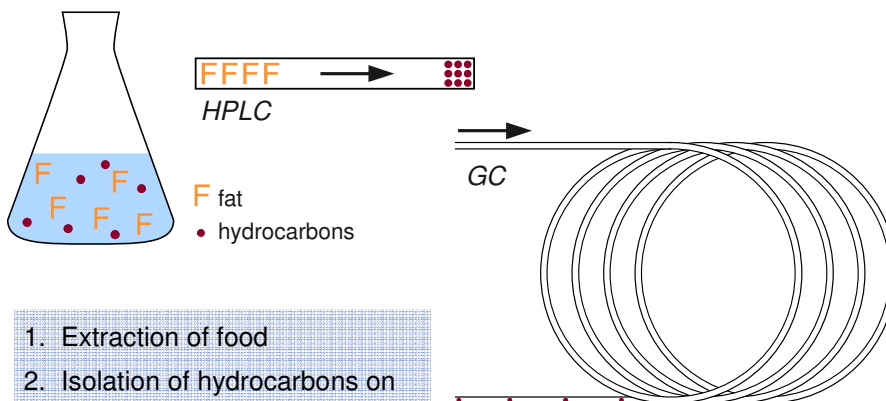
Mineral oil contaminated food: analysis by on-line HPLC-GC

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Principle of on-line HPLC-GC



1. Extraction of food
2. Isolation of hydrocarbons on silica gel, separation from fat
3. Transfer to GC by large volume on-column injection
4. GC-FID, quantification

Large volume on-column transfer

How to transfer 200-2000 μ l organic solvent into GC?

Key elements:

- Solvent trapping
- Retention gap effect
- Solvent vapor exit
- Partial and fully concurrent solvent evaporation
- Interface between HPLC and GC

HPLC-GC, LV on-column injection, LV splitless injection with concurrent solvent recondensation (CSR)



The flooding process

Video:

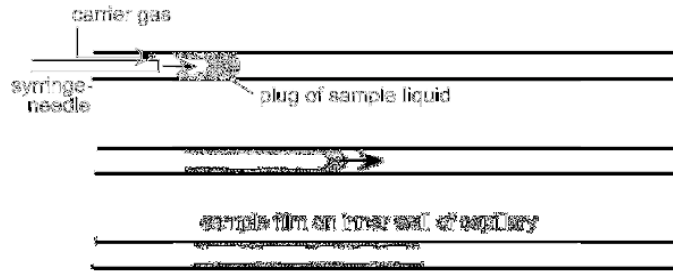
On-column injection:

- 50 μ l pentane
- 5 μ l/sec
- room temperature
- 50 kPa hydrogen

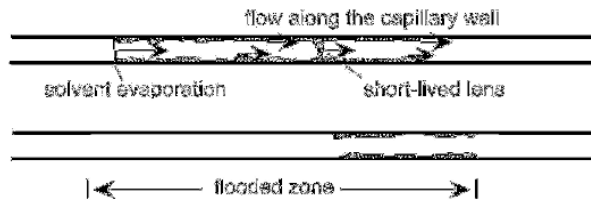


The flooding process

Primary flow of liquid

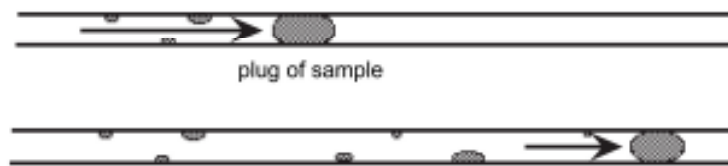


Secondary flow of liquid



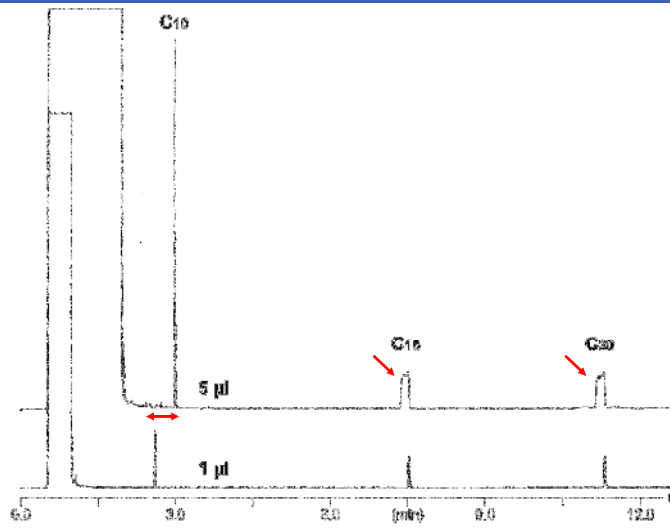
Wettability

Non-wetting sample liquid



The wettability is linked to the polarity of the solvent and the surface of the capillary wall. If the solvent does not wet the column wall the length of flooded zone increases by a factor up to 10!

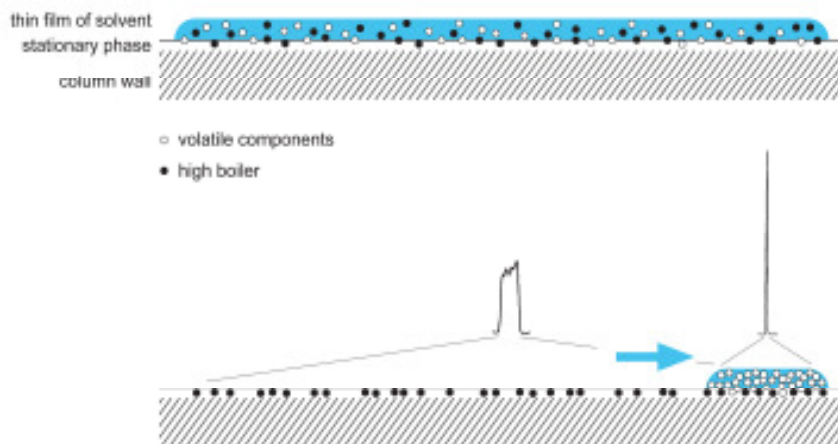
Broadening of the initial band



Sample: mix of C₁₀, C₁₆, C₂₀ alkanes
 Concentration: 4 mg/l each in hexane
 Separation column: 15 m, 0.25 mm i.d., PS 255, 0.2 µm
 Inlet pressure, carrier gas: hydrogen: 35 kPa
 Temperature program: 50 °C (1 min), 15 °/min, 220 °C (1 min)

Width of the initial band

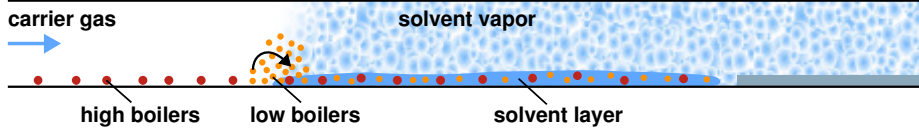
volatile components: → focused by solvent trapping → **sharp peaks**
 high boilers: → spread over the whole flooded zone → **broad peaks**



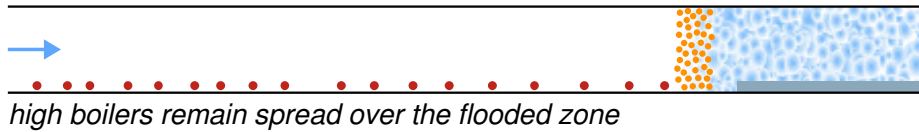
Focusing of the initial band

animation

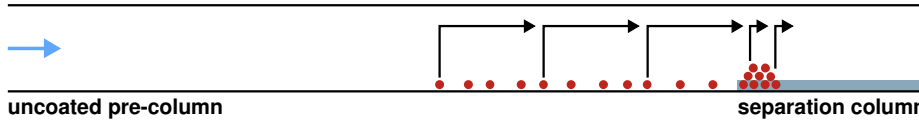
solvent trapping of low boiling components



release of low boilers with the last portion of solvent



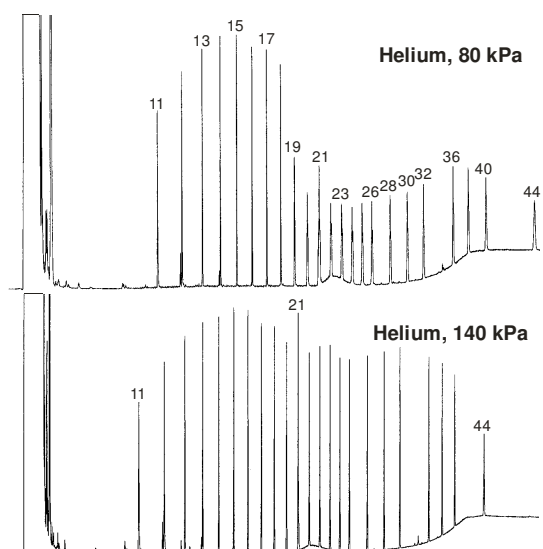
reconcentration of high boilers by the retention gap effect

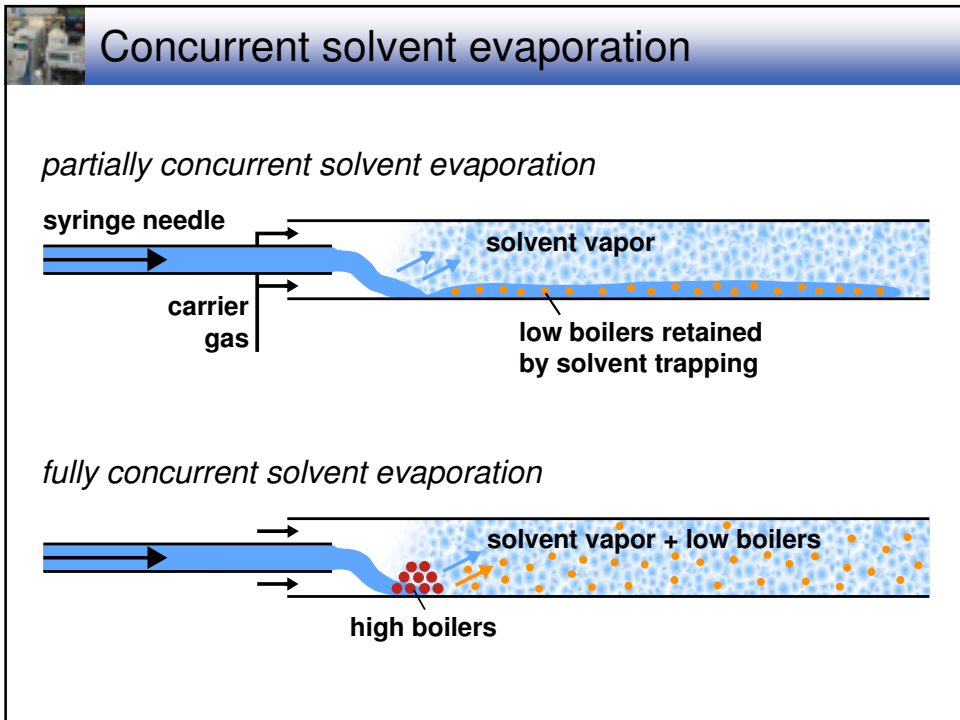
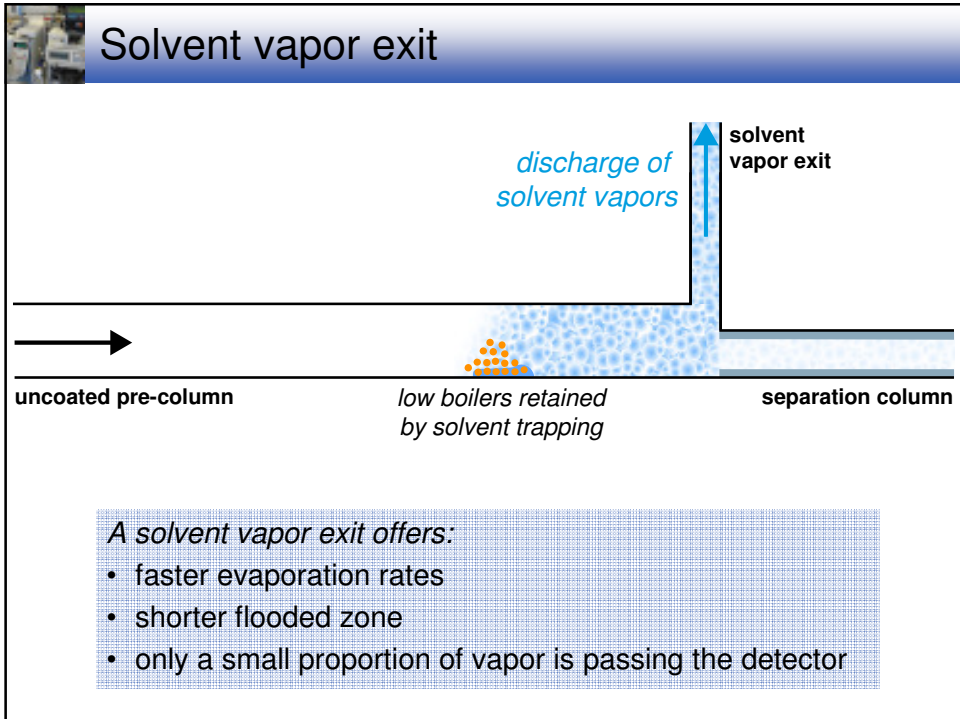


Weak retention gap effect

- High GC temperature program rate and helium as carrier gas
→ elevated inlet pressure or hydrogen as carrier gas

- Too much retention in uncoated pre-column
→ raw or silylated fused silica







Partial vs. fully concurrent solvent evaporation

Partially concurrent solvent evaporation

- uncoated pre-columns, “retention gaps” of 7-15 m, 0.53 mm i.d.
- volatiles are retained by solvent trapping
- injection volume and evaporation rate has to be adjusted to the dimension of the pre-column

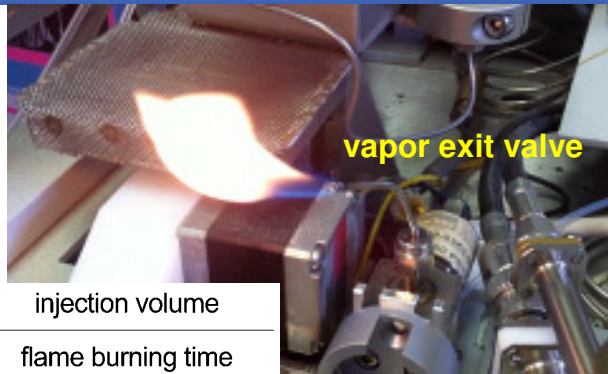
Fully concurrent solvent evaporation

- short (0.5-1 m, 0.53 mm i.d.) coated pre-columns
- flexible selection of injection volumes
- losses of volatiles



Determination of the evaporation rate

movie



$$\text{evaporation rate } [\mu\text{l/s}] = \frac{\text{injection volume}}{\text{flame burning time}}$$

Example:

HPLC column flow rate:	300 $\mu\text{l}/\text{min}$
Transfer time:	60 sec
Transfer volume:	300 μl
Flame visible = evaporation time:	70 sec
Evaporation rate:	300 μl / 70 sec = 4.3 $\mu\text{l}/\text{sec}$ = 258 $\mu\text{l}/\text{min}$
Amount of flooding solvent:	300 μl – 258 μl = 42 μl



Parameters determining evaporation rate

- boiling point and specific vapor volume of solvent
- resistance to gas flow of pre-column system
 - carrier gas flow rate through the vapor exit
- inlet pressure
- oven temperature/dilution of the vapors with carrier gas

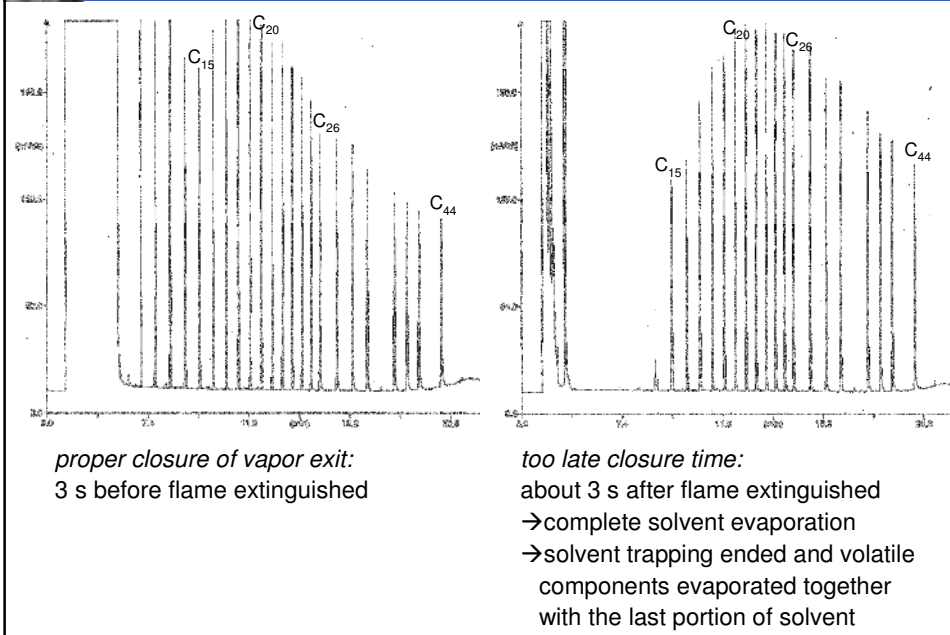


Capacities of “retention gaps”

The capacity of uncoated pre-columns for retaining wetting liquids is influenced by the carrier gas flow rate

- **no vapor exit** (flow rate <10 ml/min)
 - 80 μ l / 10 m x 0.53 mm i.d.
- **with vapor exit** (flow rates >50 ml/min)
 - increase by a factor 3 (3x thicker film is formed)
 - e.g. 170 μ l / 7 m x 0.53 mm i.d.

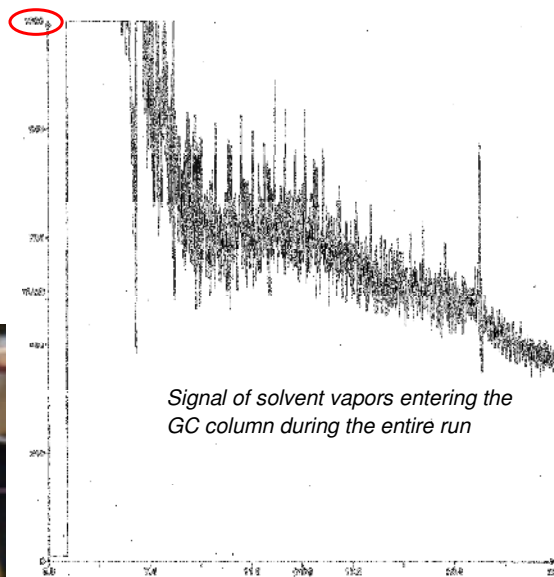
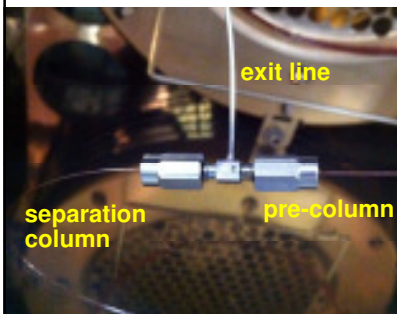
Closure of vapor exit



Purge of vapor exit line

A permanent small purge flow is required to remove solvent vapors from the T-piece and the vapor exit valve:
capillary of 0.5-1 m x 50 μm i.d.

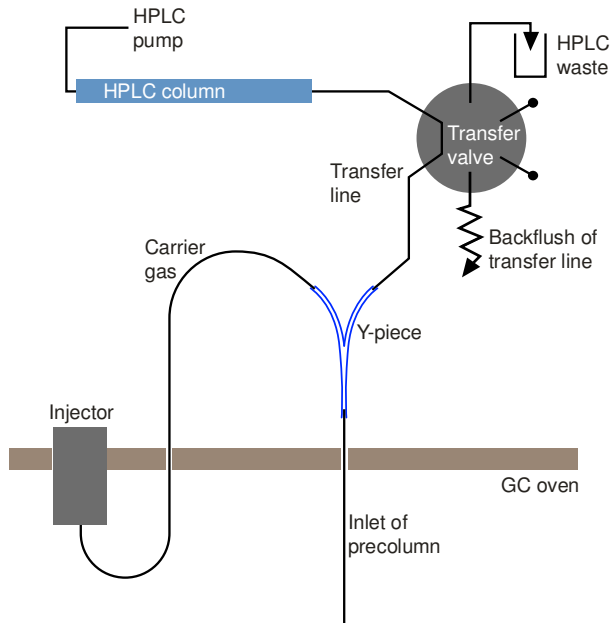
metal T-piece



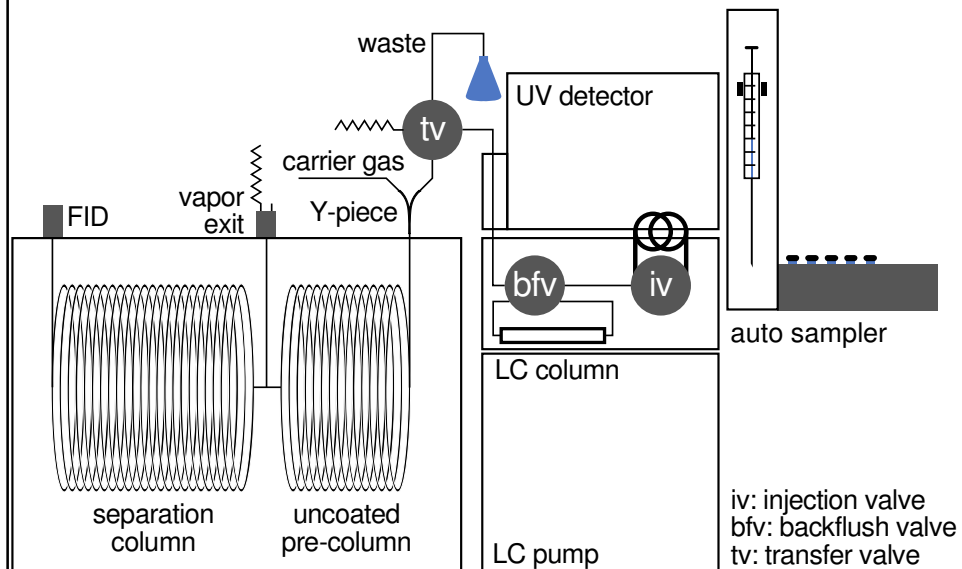
Y-piece HPLC-GC interface (since 2009)

During transfer the eluent is fed into the GC pre-column through a Y-pressfit connector.

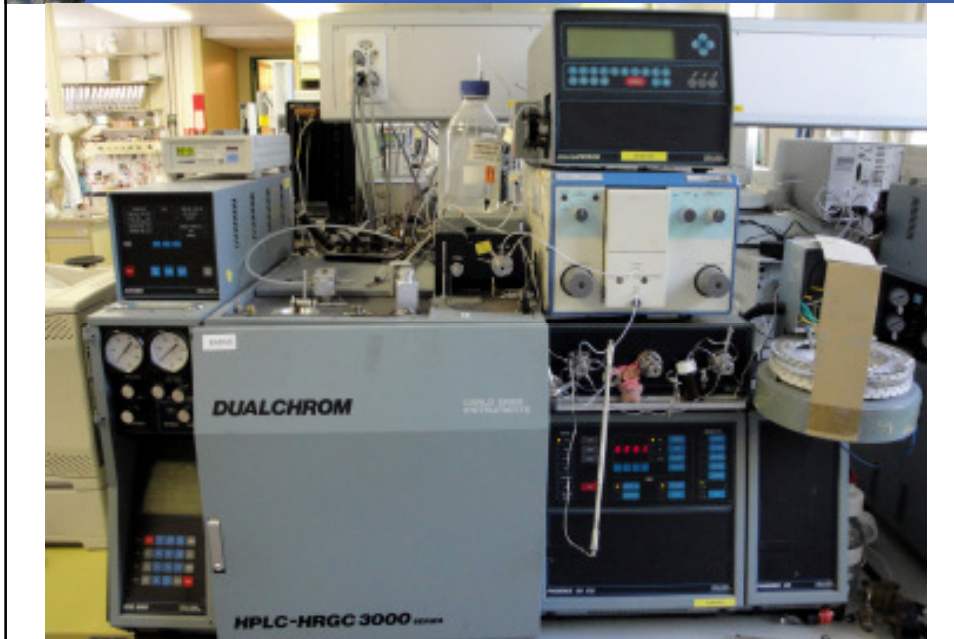
→ Carrier gas and solvent is mixed without entering a dead volume.



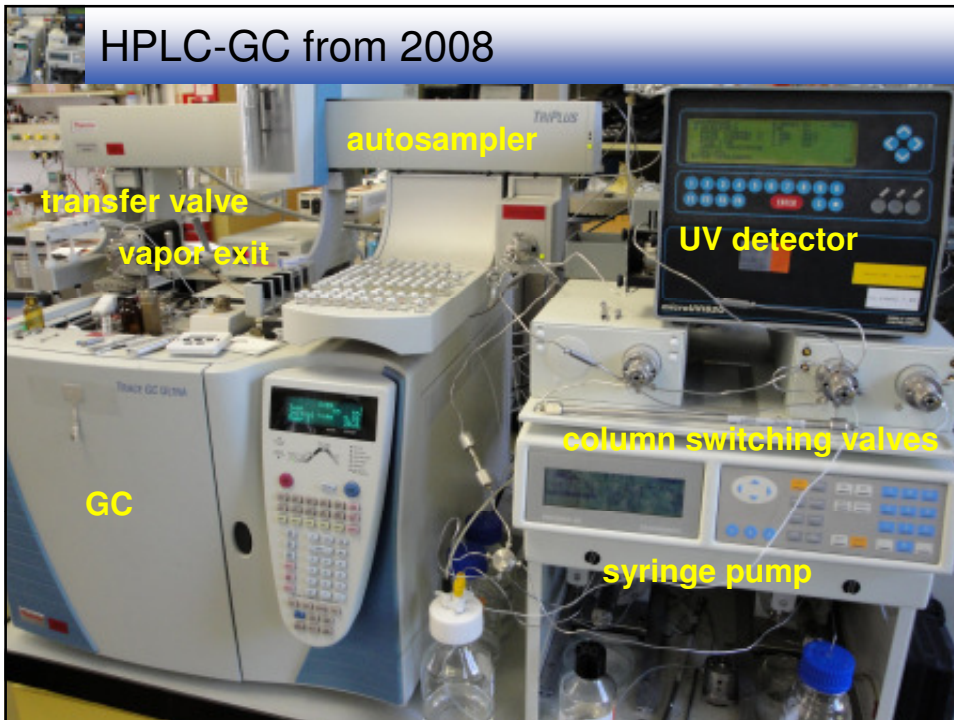
HPLC-GC components



HPLC-GC instrument from 1990

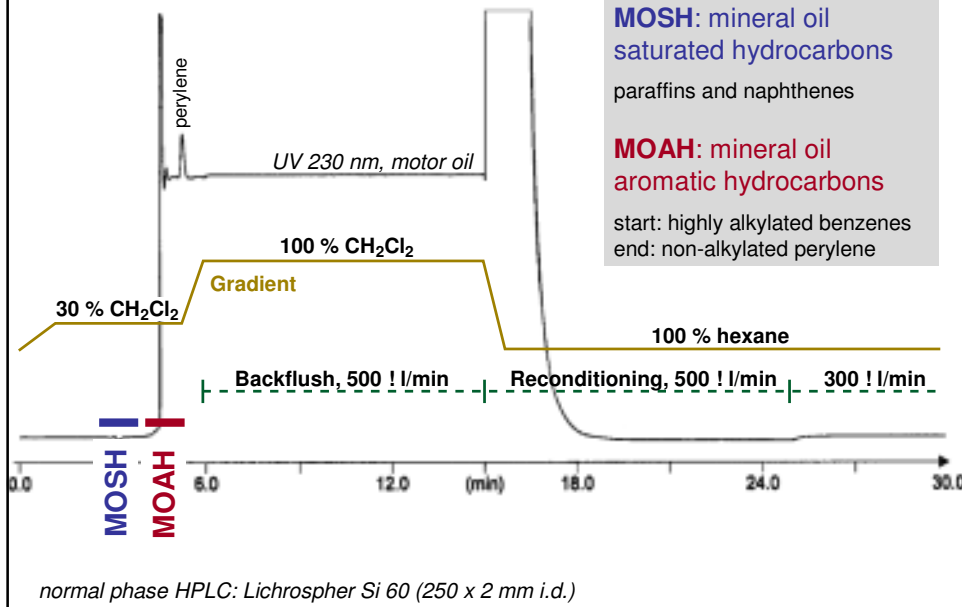


HPLC-GC from 2008

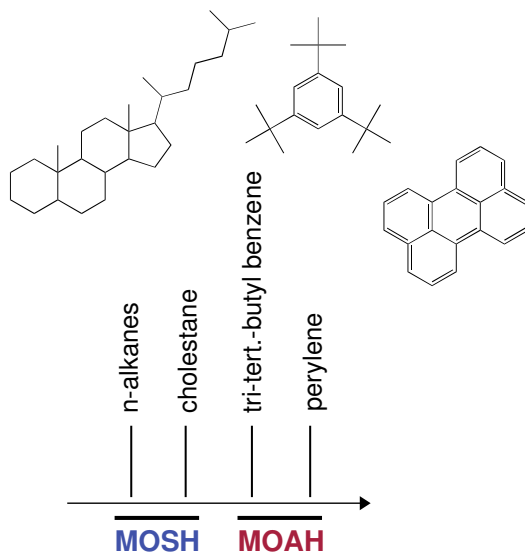




HPLC isolation of MOSH and MOAH fraction



Verification of LC fraction windows



MOSH: mineral oil saturated hydrocarbons

paraffins and naphthenes

MOAH: mineral oil aromatic hydrocarbons

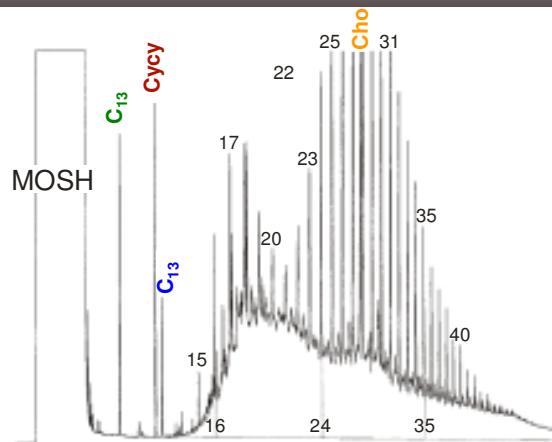
start: highly alkylated benzenes
end: non-alkylated perylene

→ verification standards must be completely included within the corresponding fraction

→ gap between two fractions, no overlapping



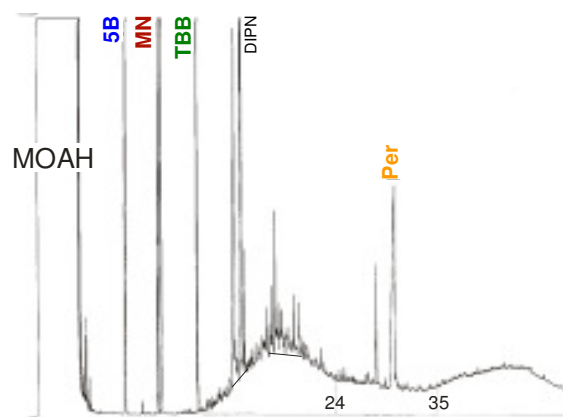
MOSH fraction, paperboard extract



- Cyclohexyl cyclohexane (**Cycy**): standard for quantification → not present in mineral oil products
- Tridecane (**C₁₃**): proves no co-elution with Cycy; identification of peaks (peak pair)
- Undecane (**C₁₁**): detection of losses of volatile components: transfer or reconcentration of sample extract
- Cholestane (**Cho**): end of MOSH fraction



MOAH fraction, paperboard extract



- 1-/2-methyl naphthalene (**MN**): standard for quantification; peak pair for easy identification
- n-Pentyl benzene (**5B**): detection of losses of volatile components
- Tri-tert.-butyl benzene (**TBB**): start of MOAH fraction
- Perylene (**Per**): end of MOAH fraction; determination also through HPLC-UV



HPLC column and conditions

Packaging material: Lichrosphere Si 60, 5 μm

- high internal surface \rightarrow high retention power for MOAH

Dimensions: 25 cm x 2 mm i.d.

- flow rate of 300 $\mu\text{l}/\text{min}$ \rightarrow well fits to the evaporation rate required by GC pre-column system
- column has the capacity of retaining about 20 mg of lipids

Hexane eluting the MOSH fraction

- no polar impurities \rightarrow if required clean-up by chromatography on activated silica gel and distillation

Dichloromethane to elute the MOAH fraction

- methyl. tert.-butyl ether was not successful, since it took too much hexane during reconditioning
- Backflush by 100 % dichloromethane



GC columns

Analysis of MOSH/MOAH starting from C_{10} \rightarrow partially concurrent solvent evaporation

Pre-column: 7 m x 0.53 mm i.d., e.g. raw fused silica

- evaporation rate: 200-250 $\mu\text{l}/\text{min}$
- deactivation of the pre-column must not exhibit too high retention power \rightarrow weak retention gap effect

Separation column: 10-15 m x 0.25 mm i.d., 0.13 μm 100 % dimethyl polysiloxane

- thin film results in low column bleeding
- whole range of MOSH and MOAH up to C35 should elute before base line starts shifting (column bleeding)
- drawbacks of a thin film coating:
 - less capacity \rightarrow overloading of dominant peaks
 - weaker reconcentration by the retention gap effect



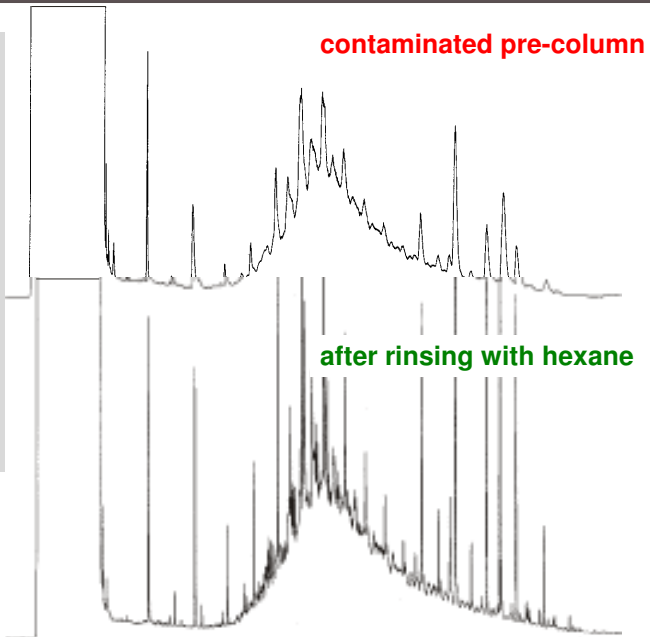
Rinsing of matrix loaded pre-column

Extracts of paperboard and plastic may contain non-evaporating hydrocarbons, which accumulate in the pre-column (on-column transfer) and built up retention

- peak broadening
- rinsing of the pre-column with hexane or dichloromethane removes the non-volatile components

contaminated pre-column

after rinsing with hexane

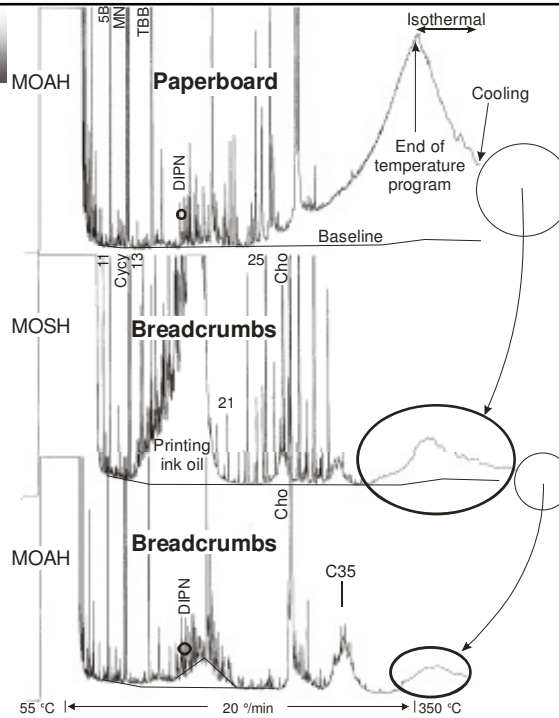


Delayed elution

High boiling material may remain in the column after the first transfer

- carry-over from one analysis to the next analyses

→ important to keep the delayed eluted material ("grand-fathers") beyond the elution of the C₃₅





GC conditions

Carrier gas: hydrogen (60 kPa, constant pressure)

- faster chromatography at optimal separation conditions
- an efficient reconcentration in the inlet of the pre-column requires fast carrier gas velocity in the pre-column

Transfer temperature/pressure: 55 °C, 80-105 kPa

- optimized for evaporation rates of 200-250 $\mu\text{l}/\text{min}$
- 10-15 kPa lower pressure for the transfer of the MOAH fraction (mixture of hexane and dichloromethane)
- closure of vapor exit about 3 sec before flame extinguishes

Temperature rate: 20-25 %/min (up to 350 °C)

- narrow humps of mineral oil isomers \rightarrow better sensitivity
- short analysis time



On-line HPLC-GC provides:

- High efficiency in pre-separation \rightarrow efficient sample clean-up
- On-line detection \rightarrow accurate determination of the HPLC elution windows
- Whole fraction of sample material (no aliquot) is transferred to GC \rightarrow low detection limit
- Close system \rightarrow rules out sample contamination during preparation
- HPLC enables reuse of the same column for many analyses
- Clean-up can largely be automated \rightarrow a minimum of manpower recommends the method for routine use (e.g. MOSH/MOAH analysis: 35 injections/day)