FEDERAL INSTITUTE FOR RISK ASSESSMENT

Analysis of mineral oil:

„Manual" pre-separation, followed by

LV-GC/FID

Off-line HPLC-GC/FID

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Topics

Design of the manual methods

Large Volume GC injections
CSR
Cool on-column
Validation data

Evaporisation / Enrichment

„Manual" separation of MOSH and MOAH (SPE)
Development of the method
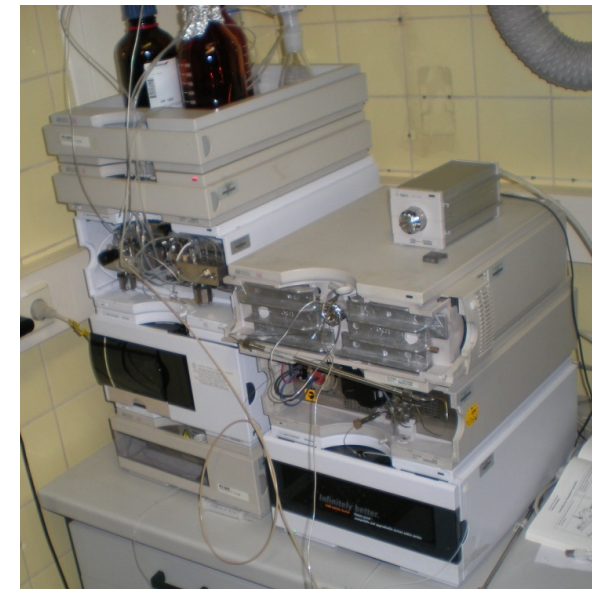Validation data
Chromatograms (vs. LC-GC)

Offline-NP-HPLC
Principle
Pitfalls

Analytical Equipment used for analysis of mineral oil

- LC-GC (Brechbühler)

- NP-HPLC (Agilent 1100)

- 2 GC-FID (Agilent 6890)

Challenges in analytics of mineral oil

Flame ionization detector (FID)

- The only detector with equal response for all hydrocarbons (Calibration!)

- **But**: low sensitivity, no selectivity

- GC-Analysis:

Necessary for differentiation to n-alkanes from biological sources

- Characterization of mineral oils is important → possible Information about the source of the contamination

- Broad humps of compounds.

→ MOSH and MOAH have to be separated from each other and from other disturbing compounds

Possible Interferences by Olefines (Terpenes, Squalenes und Carotinoides)

- Must be considered for MOAH.

Interferences by herbal paraffine

Design of the manual methods: Separation of MOSH and MOAH

• Samples are diluted by chromatographic separation of MOSH and MOAH

• Enrichment like at the LC-GC-Interface is missing for manual methods

→ Need for discrimination free enrichment of the sample
→ Need of large volume (LV) injection techniques

• Commercial available cartridges are often made of polyolefines (blank value - contamination!)

→ Need to use glass columns without turning valve (lubrication grease)

• Silica gel has to be activated

→ limited stability

Design of the manual methods: GC-parameters

- Fast temperature program: 15-25 °/min

→ Narrow and high humps

- Plane baseline (allows low attenuation)

→ Short analytical column (e.g. 10 m)
→ Small inner diameter of the analytical column (e.g. 0.25 mm I.D.)
→ Thin film of the stationary phase (e.g. 0.10 µm)
→ Apolar stationary phase with low bleeding (e.g. Dimethylpolysiloxane)

- High carrier gas flow, preferable hydrogen

→ Low temperatures for elution

Techniques for Large Volume (LV)-GC-injection

Cool On-Column

- Powerful, but allows no high boiling matrix
- With classical retention gap technique: 50 µl
- With vapor exit up to 1000 µl

Programmed Temperature Vaporizing (PTV)

- splitless 30 µl
- solvent splitting: up to 500 µl
- Cool on column adapters for different PTV

Hot Splitless (SSL)

- Concurrent Solvent Recondensation Technique (CSR): 50 µl
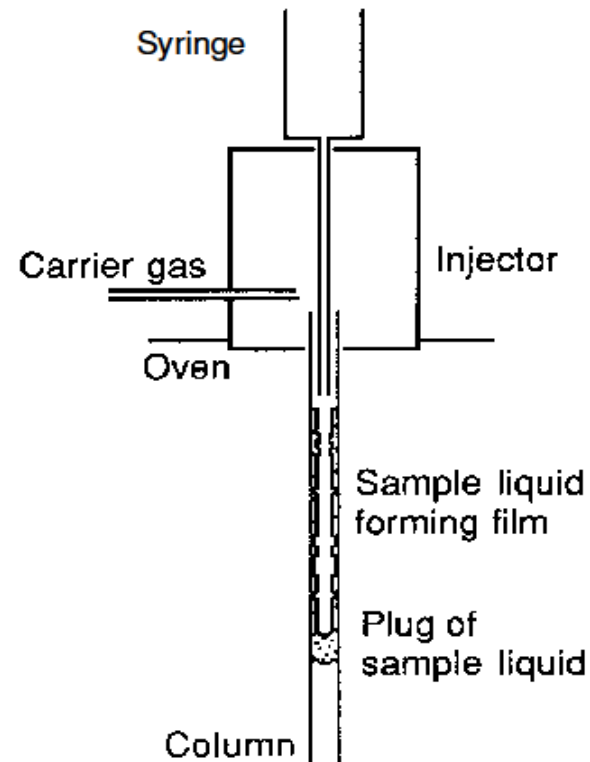- With vapor exit up to 1000 µl

BfR

GC-FID: Techniques for large volume injection

- On-column

  – Retention gap technique: 50 µl

  – with vapor exit : 300-1000 µl

On-column injection

Slow injection of the sample
onto the pre-column heated
by the GC-oven.

No discrimination for the
evaporisation process.

Sensitive to non volatile
residues.

Large Volume Splitless Injection – Concurrent Solvent Recondensation (CSR)

• „Normal" Splitless Injection:
Maximum Injection Volume (normally 1-2 µl) is determined by the Volume of the evaporisation-chamber (= Liner), in which the vapor (mostly solvent) is stored for a short time.

• CSR
Transfer of the Vapor simultaneously to the evaporization, the vapor is not stored in The evaporisation-chamber. A fast transfer of the vapor to the column is needed for that reason. The solvent-recondensation in the entrance of the precolumn is the driving force for the fast transfer.

Concurrent solvent recondensation large sample volume splitless injection. P. Magni and T. Porzano. J. Sep. Sci. 17 (2003) 1491-1498

Large volume splitless injection with concurrent solvent recondensation: keeping the sample in place in the hot vaporizing chamber. M. Biedermann, A. Fiscalini and K. Grob. J. Sep. Sci. 27 (2004) 1157-1165

CSR splitless injection

Expanding vapors

Evaporating sample

Split outlet closed

Injection

Carrier gas transfers sample vapors into the column

Sample transfer (splitless period)

Residual vapors discharged through split outlet

Injector purge
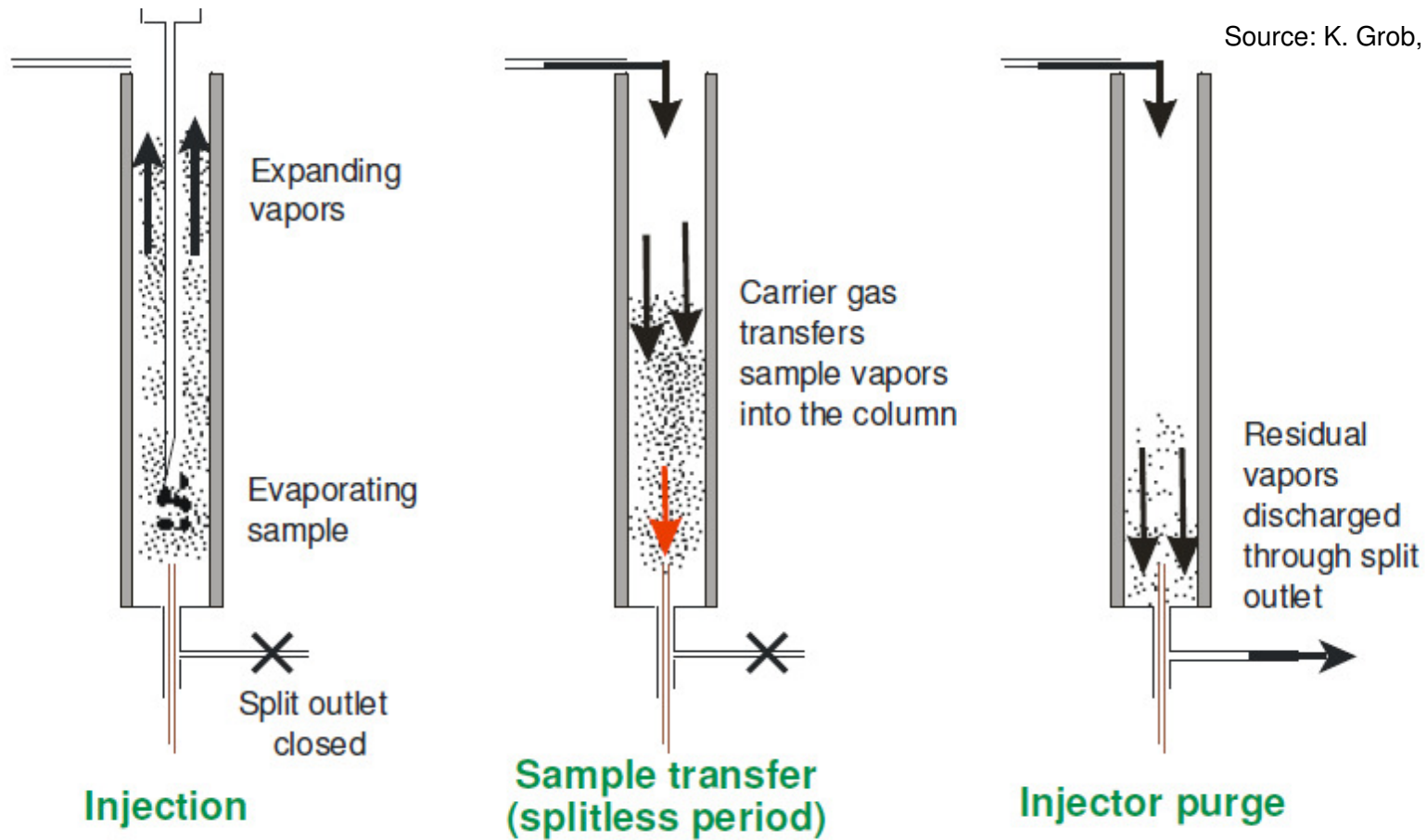
CSR splitless injection

Stopper for the liquid

- Stream of liquid routes itself to the bottom of the chamber
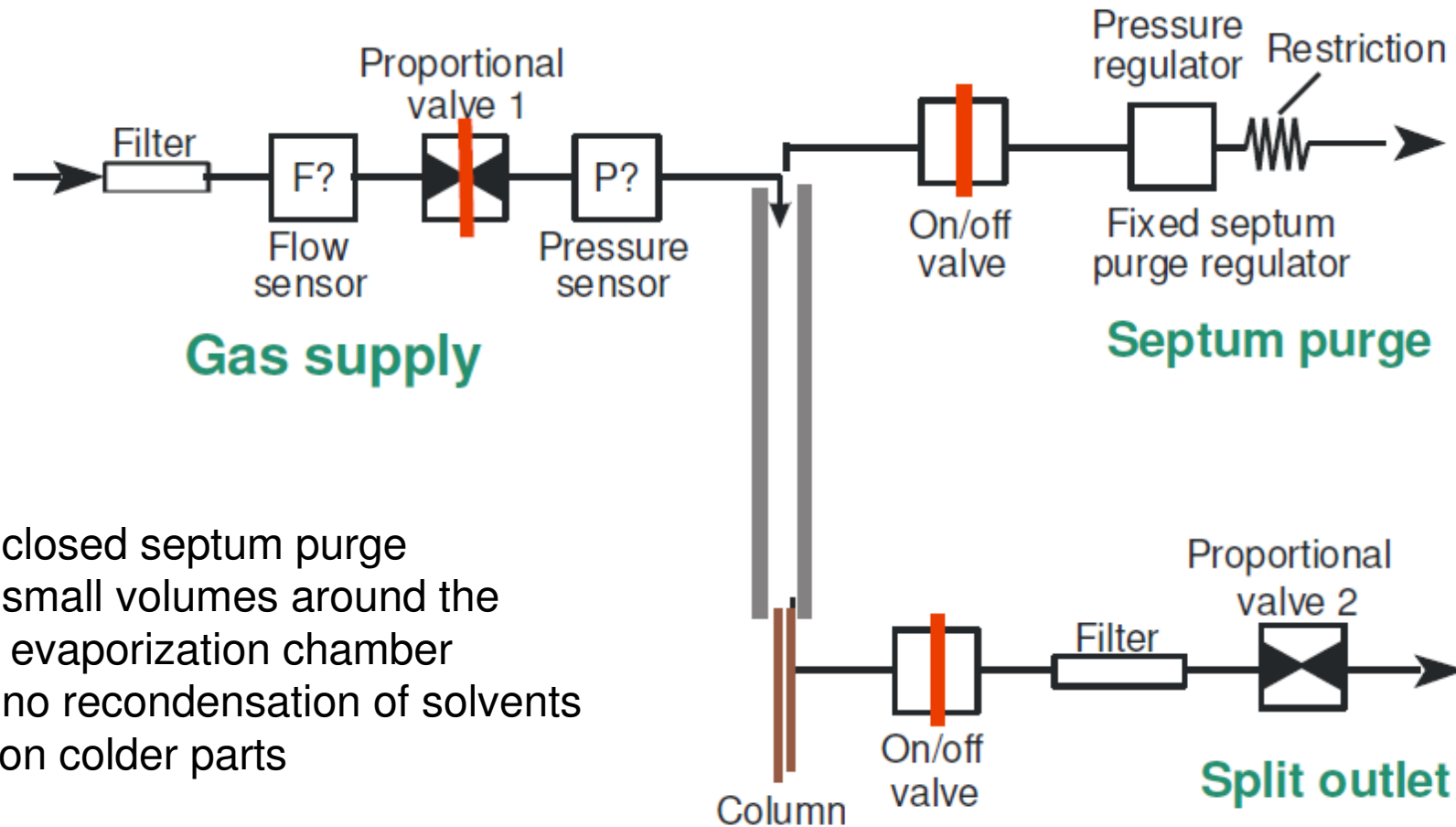- Rejection from the hat chamber wall
- High speed, fast injection

- The stream of liquid has to be stopped and retained
- Glass wadding (fast cooling, low thermal mass)
- Easy to place in narrow, also in narrow sites

Large volume splitless injection with concurrent solvent recondensation: keeping the sample in place in the hot vaporizing chamber. M. Biedermann, A. Fiscalini and K. Grob. J. Sep. Sci. 27 (2004) 1157-1165.

Labels: Syringe, Septum, Sample liquid travelling as a band, Droplet of sample, Glass wool, Entrance of precoumn
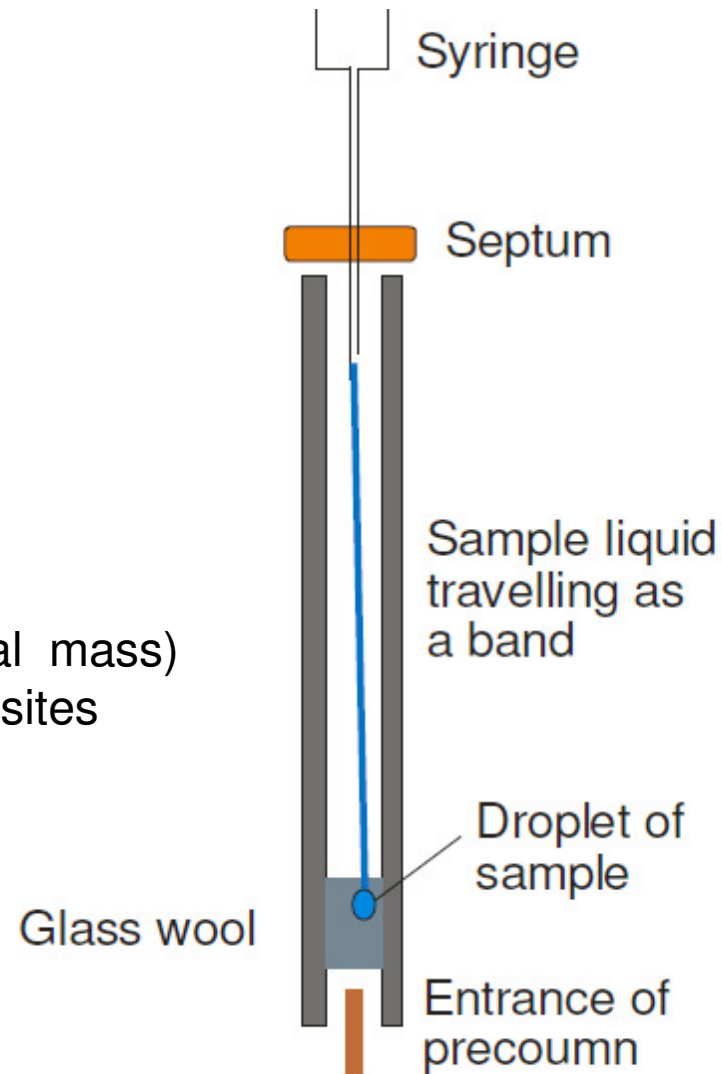
CSR splitless injection: requirements for a pressure increase

Source: K. Grob, Zürich 2010

- closed septum purge
- small volumes around the evaporization chamber
- no recondensation of solvents on colder parts

CSR splitless injection: Feasible with our Agilent GC 6890 ?
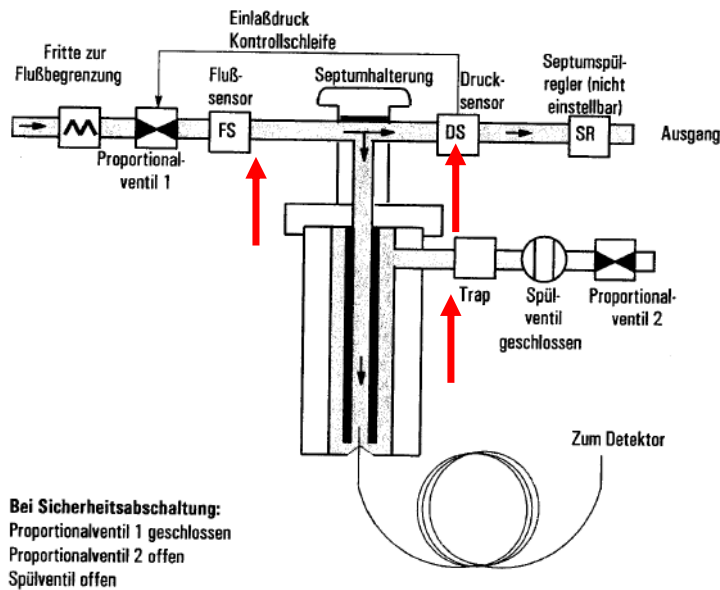
Source: Agilent manual GC 6890
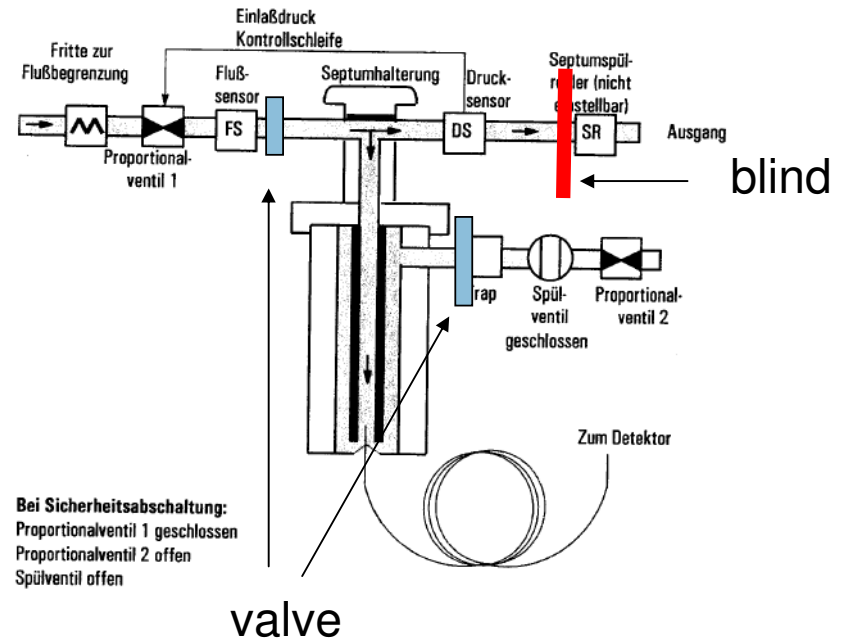
GC-conditions for cool on-column LV-injection

Carrier gas:	Hydrogen	**FID-Detector:**	
		Temp.:	365 °C
Injector:	cool on column	H_2 :	40 mL/min
Volume:	40 µl	Syntetic air:	450 mL/min
Pressure:	24.4 kPa (H_2)	Mode:	constant make up
Total flow:	45 ml/min (H_2)	Makeup flow:	45 mL/min
		Makeup Gas Type:	Nitrogen

Oven:

Start temperature:	75 °C (hold for 9 min)
Ramp1:	22 °C/min to 240 °C
Ramp2:	30 °C/min to 380 °C
(hold for 12 min)	

Column:

Precolumn	i.d. 0.53 mm, 7 m
Analytical column	DB-1HT (15.0 m; 0.32 mm i.d., 10 µ)
Mode:	ramp pressure
Nominal init. pressure:	65 kpa for 3 min, then 35 kPa

LV-GC-injection: cool on column vs CSR

On column: mix of n-alkanes, 40 µl
0.5 µg/ml (n-hexane/toluene 1:1)

CSR: mix of n-alkanes, 40 µl
0.5 µg/ml (n-hexane/toluene 1:1)
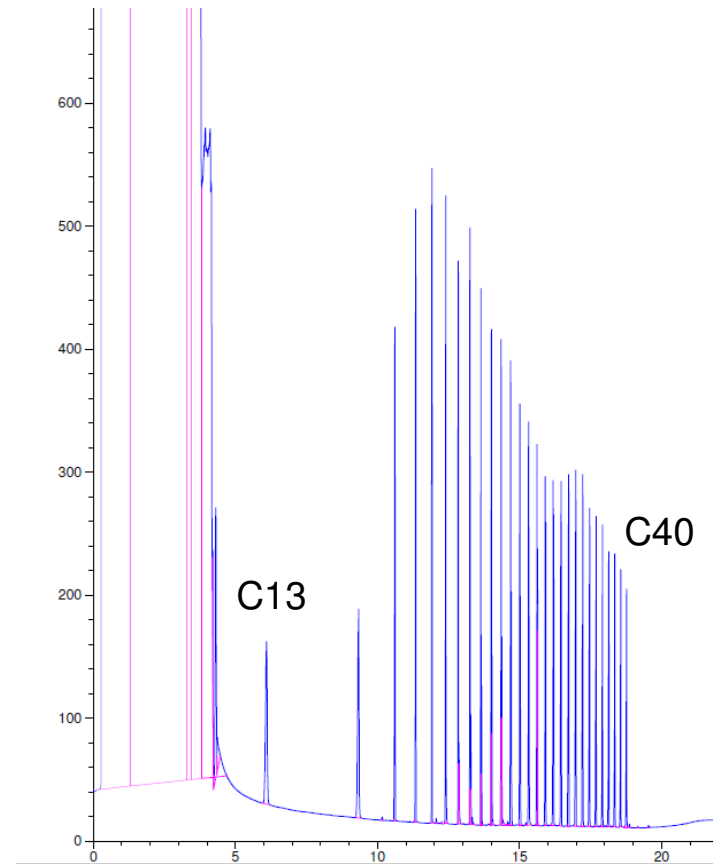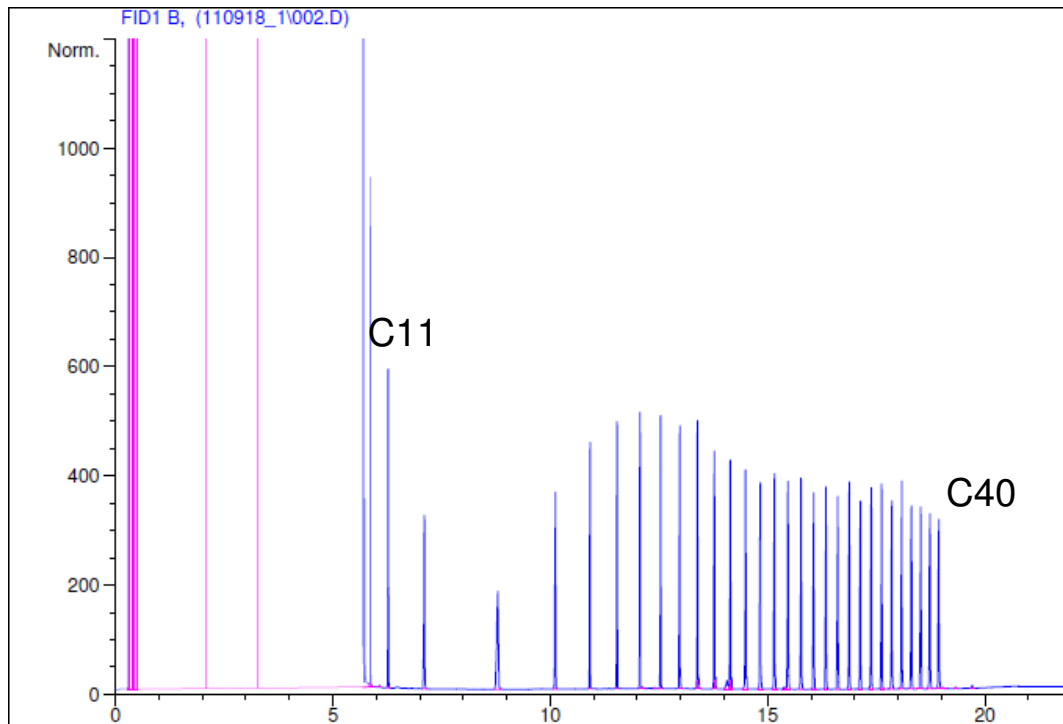
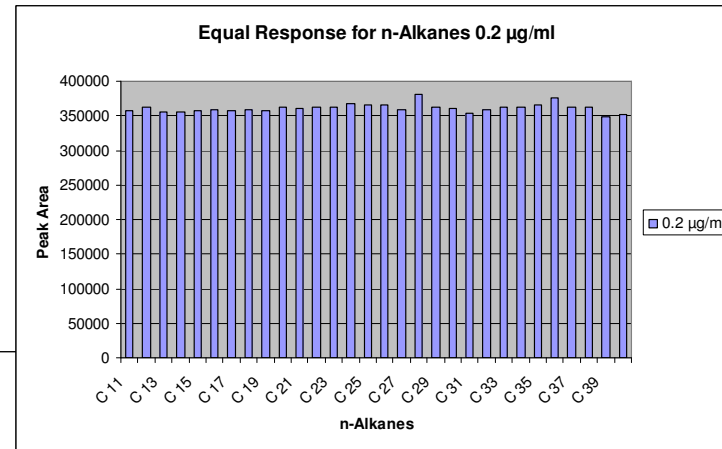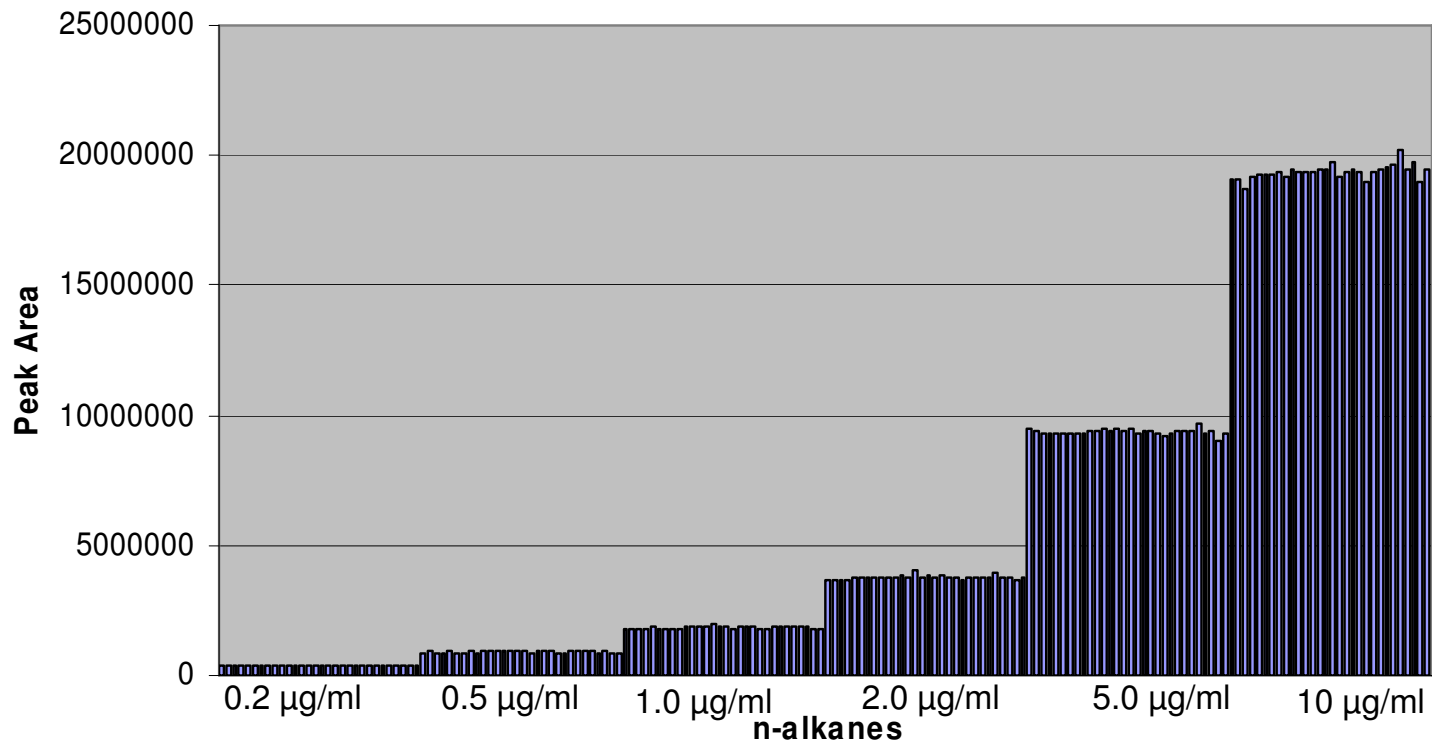# Validation of the LV-GC-method: Linearity with n-alkanes

Cool on-column, 40 µl, carriergas H$_2$

Linearity n-alkane: C11

$y = 1905888.2912x$
$R^2 = 0.9999$

Linearity n-alkane: C40

$y = 1927562.0511x$
$R^2 = 0.9993$
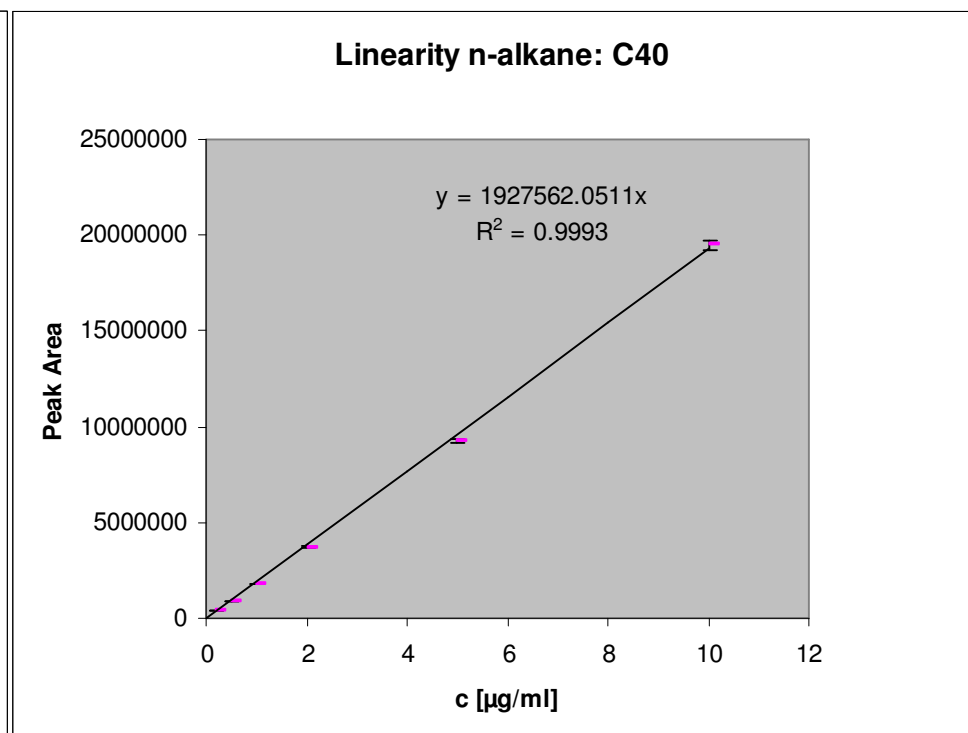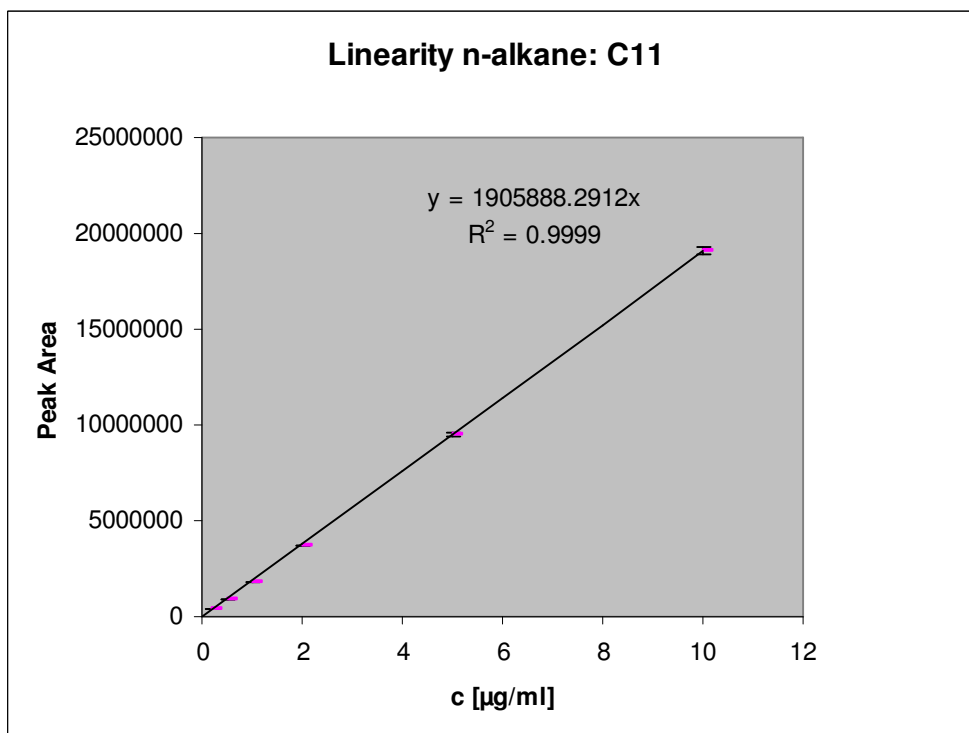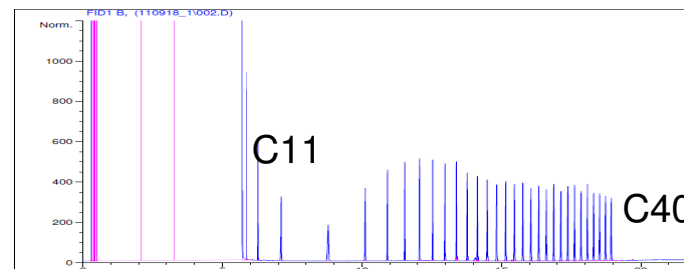
Validation of the LV-GC-method: Response for n-alkanes

Cool on-column, 40 µl, carrier gas H$_2$

Equal Response for n-Alkanes 0.2 µg/ml

Equal Response for n-alkanes

Validation of the LV-GC-method: Response for n-alkanes

Cool on-column, 40µl injection volume, carrier gas H_2

Mix of n-alkanes, conc. = 1 µg/ml for each
Cv below 2% for every analyte from C11 to C40 from 0.2 – 10 µg/ml
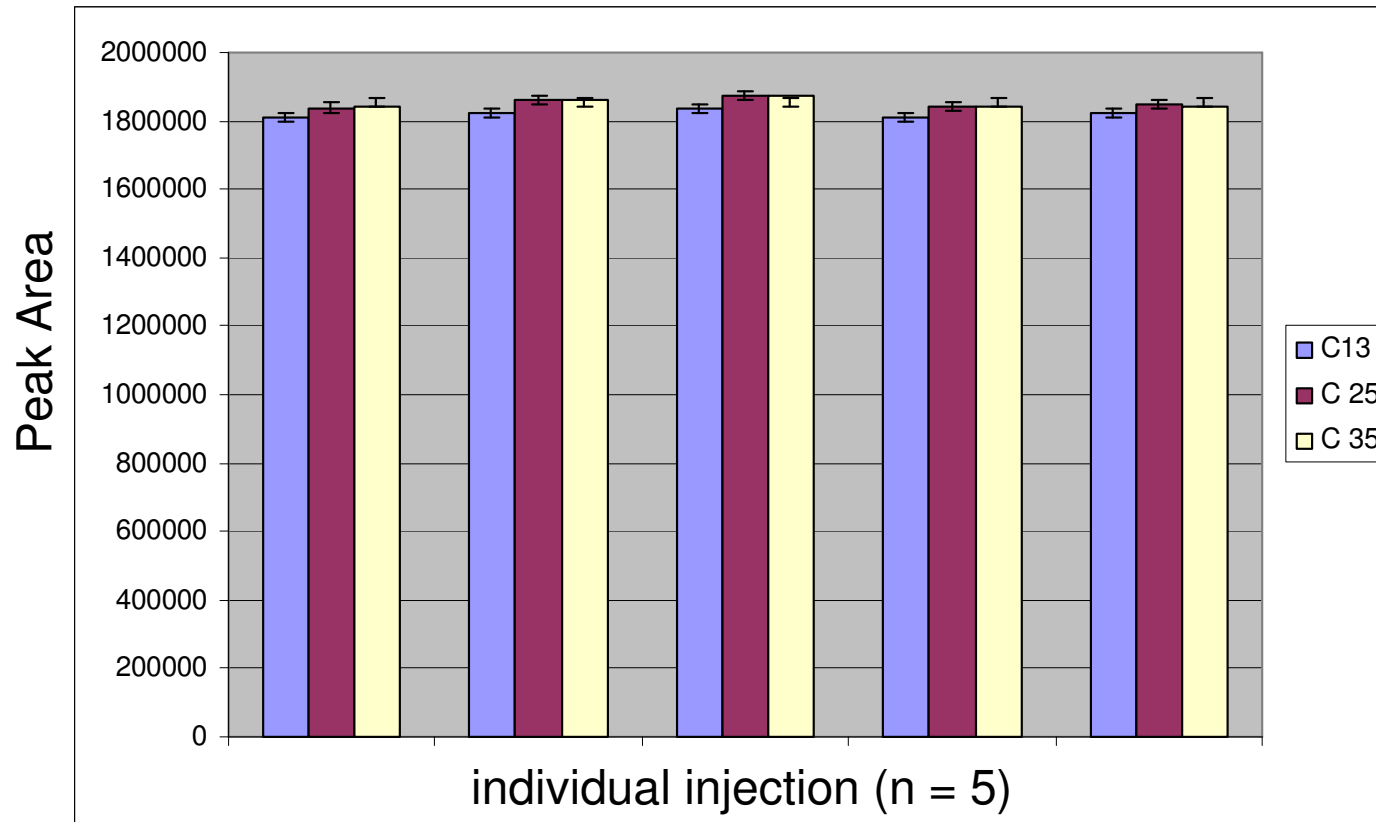
Validation of the LV-GC-method: linearity and Cv with mineral oil

Cool on-column, 40 µl, carrier gas H_2

Linearity Gravex, 40 µl cool on-column

$y = 2E+06x$
$R^2 = 0,9975$
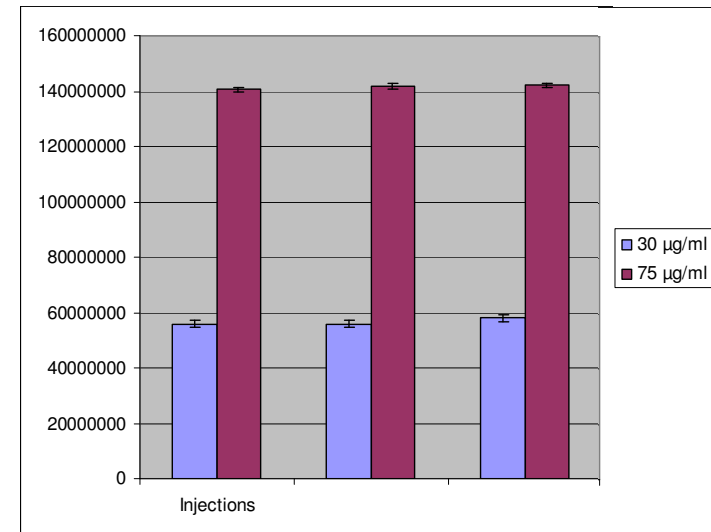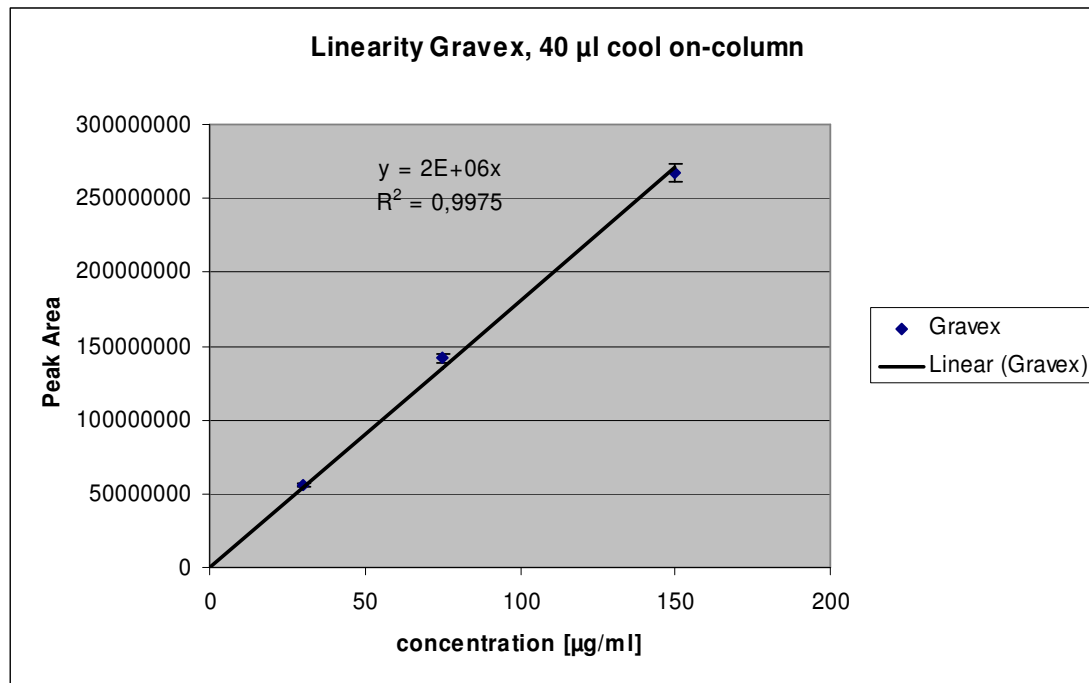
"Manual" pre-separation by SPE: Evaporisation and enrichment of the samples

- MOSH and MOAH-fraction have to be evaporated to 200 to 300 µL.
- 270 µL toluene have to be added to the MOSH-fraction.
- Toluene has to be pre-cleaned on 0.3 % AgNO3/Silicagel
- For concentration a rotary evaporator system is used in two steps:
  Water bath temperature: 55°C

	Time [min]	Vacuum [mbar]
1. Step	3.0	520 – 530
2. Step	3 – 4	350 – 360

- Use of a teflon tubing and an additional glass liner.
- Clean the rotary evaporator system every working day with hexane.
- At least 2 blank values in every series: flask with hexane/toluene.
- The concentrated samples are transferred with a baked out glass pipette into the baked out autosampler vial.

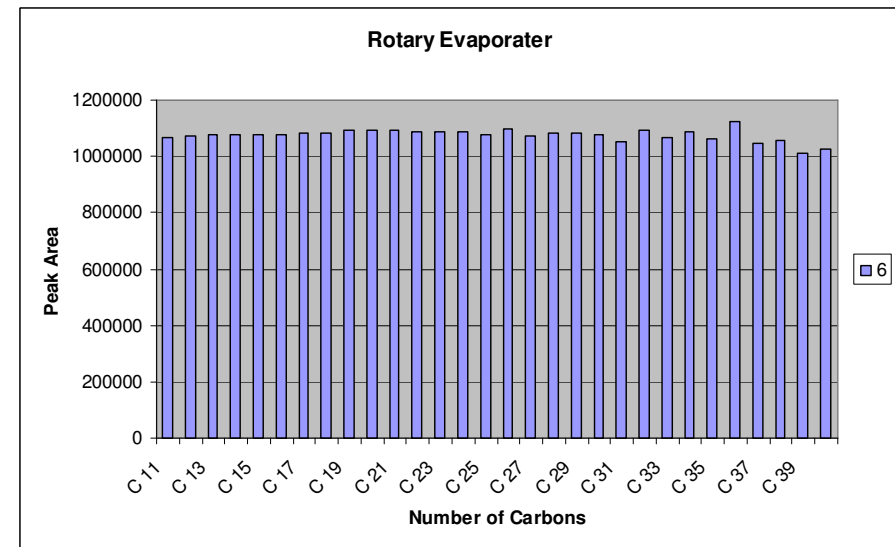# Validation of the evaporisation: Rotary evaporization system

Peak Area for n-alkanes
Cool on-column, 40 µl, carrier gas H$_2$
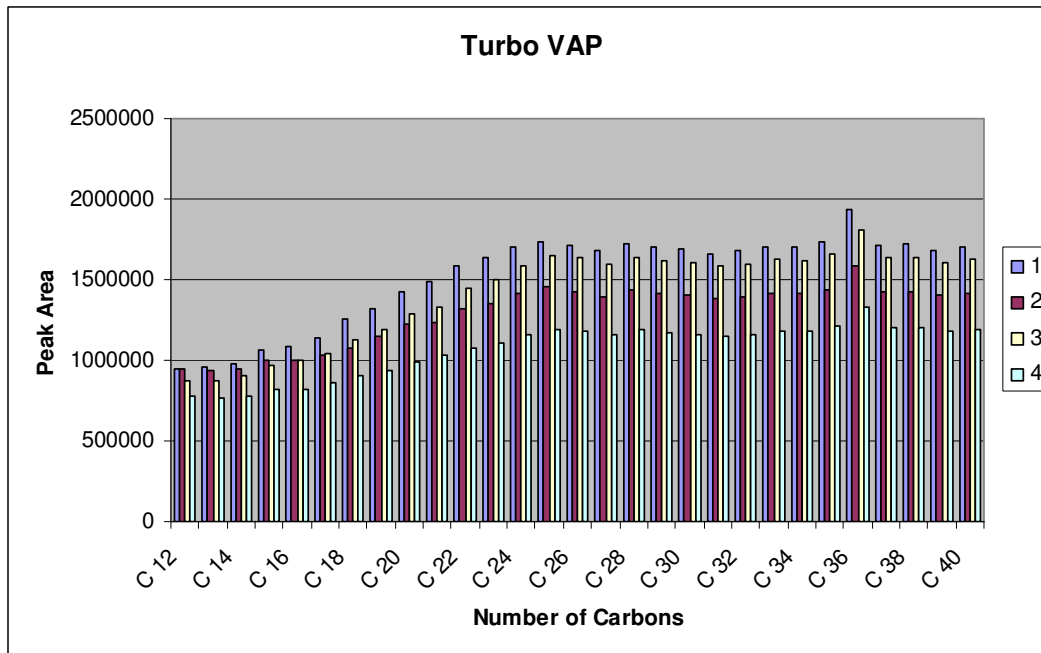**270 µl toluene + 5 ml n-hexane solution**
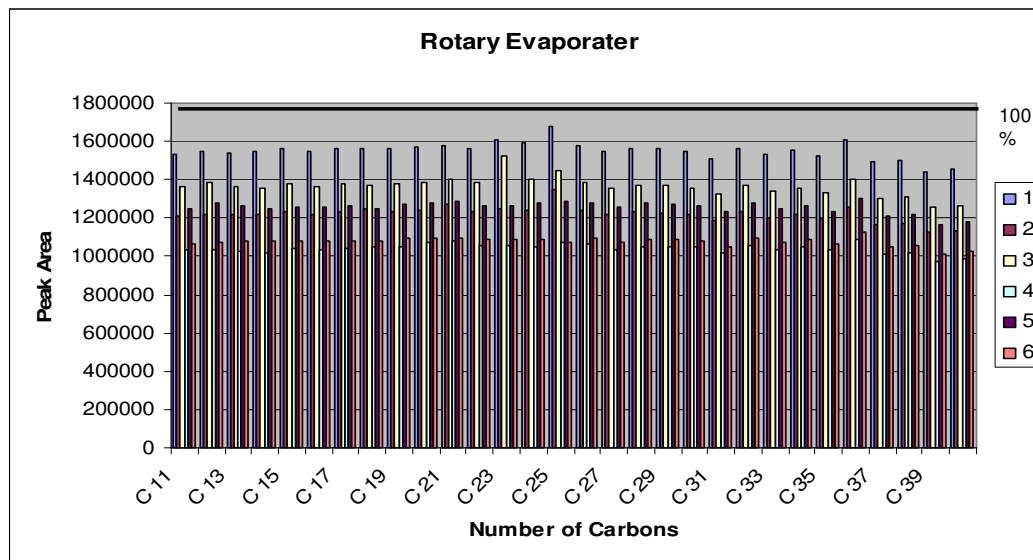
n=6

Single Experiment

-> no discrimination between C11 and C 40
-> variation of the concentrations

Validation of the evaporisation: Turbo® VAP and IR-Dancer
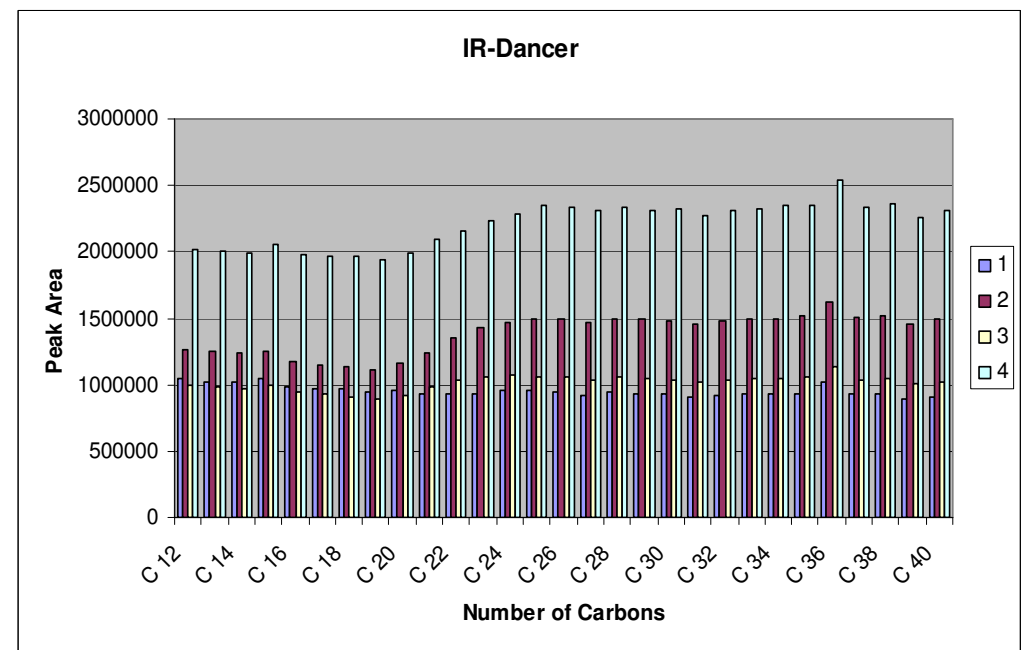
Cool On-Column, 40 µl, carrier gas H_2

45 °C, 2 kPa, 19 min
300 µl n-alkane-mix + 5 ml n-hexane + 270 µl toluene

45 °C, 400 hPa, 2h
300 µl n-alkane-mix + 5 ml n-hexane + 270 µl toluene

-> discrimination of more volatile compounds
-> variation of the concentrations

-> no discrimination
-> variation of the concentrations

Chromatographical Challenge

- Paraffine
- Naphthenes
- Highly alkylated benzenes
- Last MOAH: Perylene
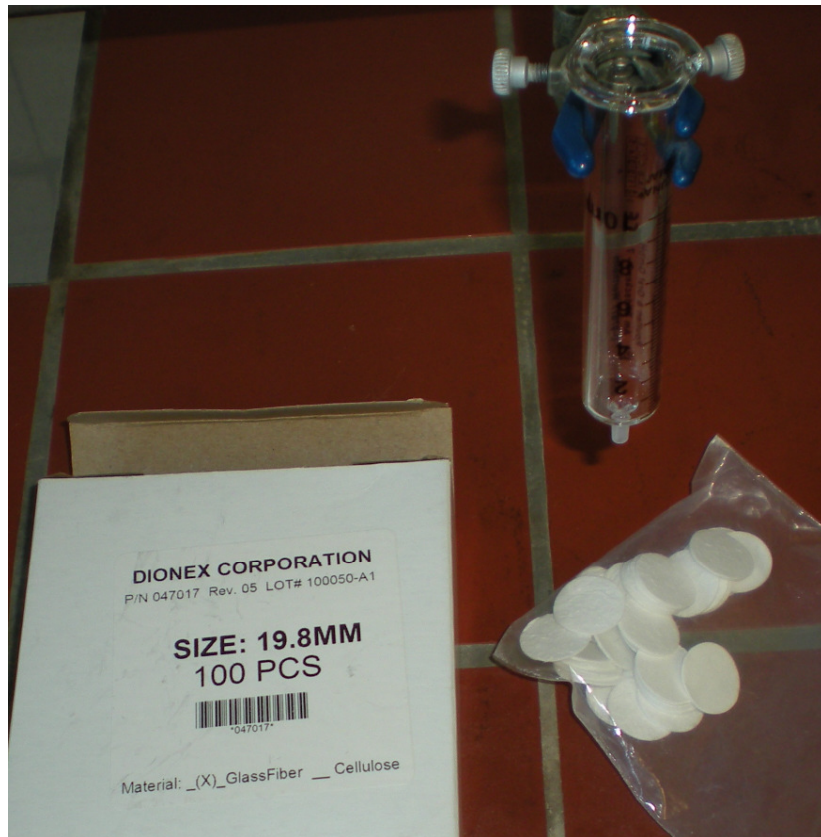
Development of the method with standards.:

- Cholestane (Cho) - tri-tert. Butyl Benzol (TBB)
- Perylene (Per) – satuarated wax esters separate: C18:C17-Ester (WE35) from MOAH
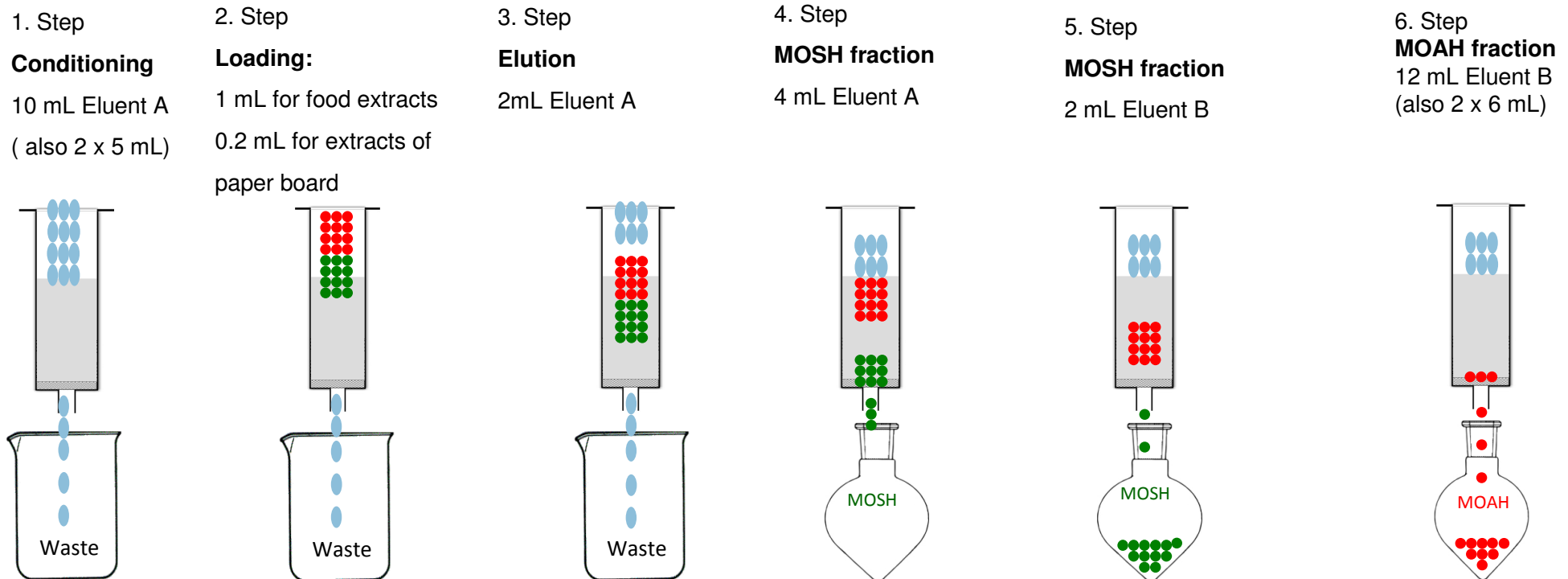
Step-gradient n-hexane / n-hexane + polar solvent

MOSH
Paraffine,
Naphthene

MOAH
alkylated benzenes
alkylated polycycles

Wax esters

injection

Cho

TBB

PER

Source: Grob, Zürich 2010

"Manual" pre-separation by SPE

- Use of a glass column (baked out for 24 h at 400 °C)
- Equipped with a fiber glass filter
- 3.0 g silica gel containing 0.3% $AgNO_3$ are transferred into the glass column

"Manual" pre-separation by SPE

1. Step

Conditioning

10 mL Eluent A

(also 2 x 5 mL)

2. Step

Loading:

1 mL for food extracts

0.2 mL for extracts of

paper board

3. Step

Elution

2mL Eluent A

4. Step

MOSH fraction

4 mL Eluent A

5. Step

MOSH fraction

2 mL Eluent B

6. Step

MOAH fraction

12 mL Eluent B

(also 2 x 6 mL)

Waste

Waste

Waste

MOSH

MOSH

MOAH
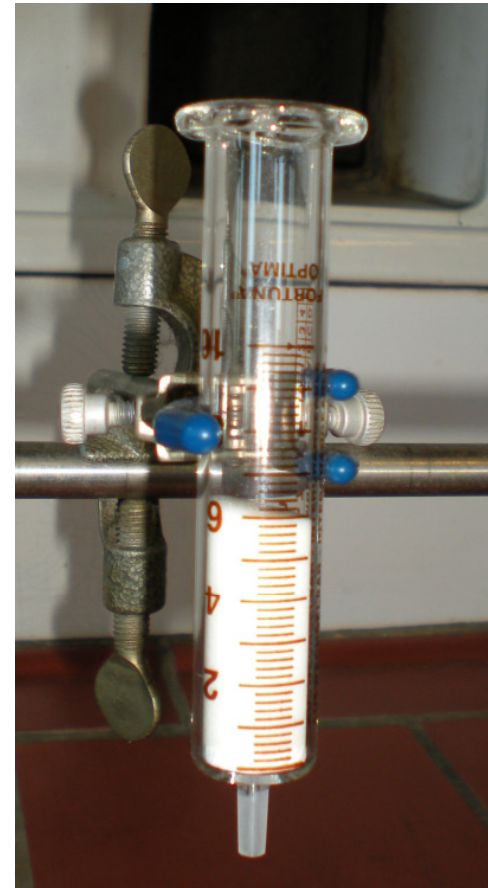
Eluent A: n-hexane

Eluent B: 5 mL toluene + 20 mL DCM ad 100 ml n-hexane

"Manual" pre-separation by SPE: Silica gel with $AgNO_3$

Silica gel containing 1 % $AgNO_3$

• 0.5 g AgNO3 are dissolved 50 mL deionised water.

• The solution is transferred into a round bottomed flask containing 49,5 g silica gel. Use a brown glass aluminum foil to avoid exposure to light.

• The water is removed for 1 h at 70°, 1 h at 80°C and finally for 12 h at 90°C in a laboratory-type drying cabinet. The silica gel hast to be totally dry after this treatment.
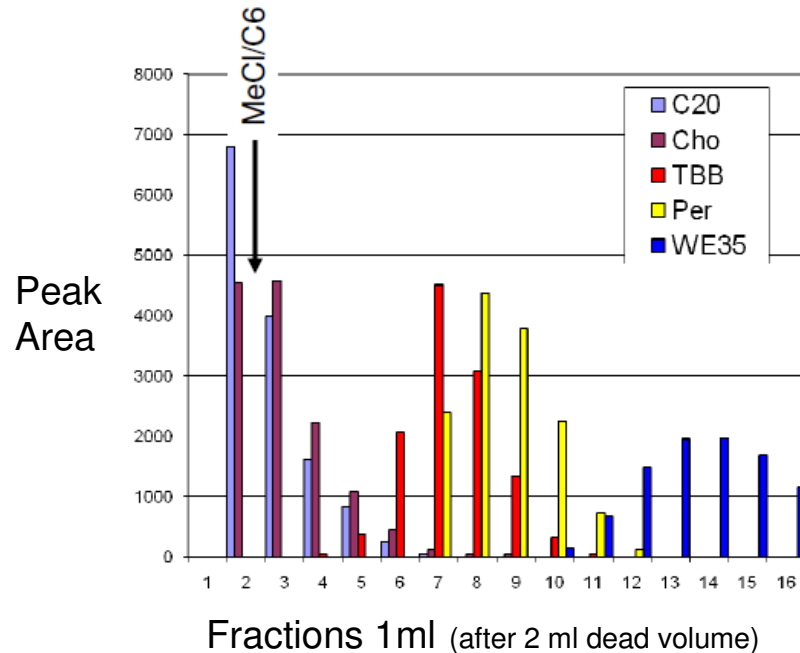
Silica Gel containing 0.3 % $AgNO_3$

• 66 g highly activated silica gel (baked out at 400°C) is mixed with 33 g silica gel containing 1 % $AgNO_3$ for 12 h using a head over heals shaker.

• During mixing the used bottle has to be covered with aluminum foil to avoid exposure to light.

Note: The silica gel containing 0.3 % $AgNO_3$ can be used for two weeks while storing at room temperature in the dark.

Development of the SPE procedure (**S**olid **P**hase **E**xtraction)

activated silica

1% AgNO$_3$

Peak Area

MeCl/C6

Legend:
- C20
- Cho
- TBB
- Per
- WE35

Fractions 1ml (after 2 ml dead volume)

Peak Area

Legend:
- C20
- Cho
- TBB
- **WE 35**

Fractions 1ml (after 2 ml dead volume)
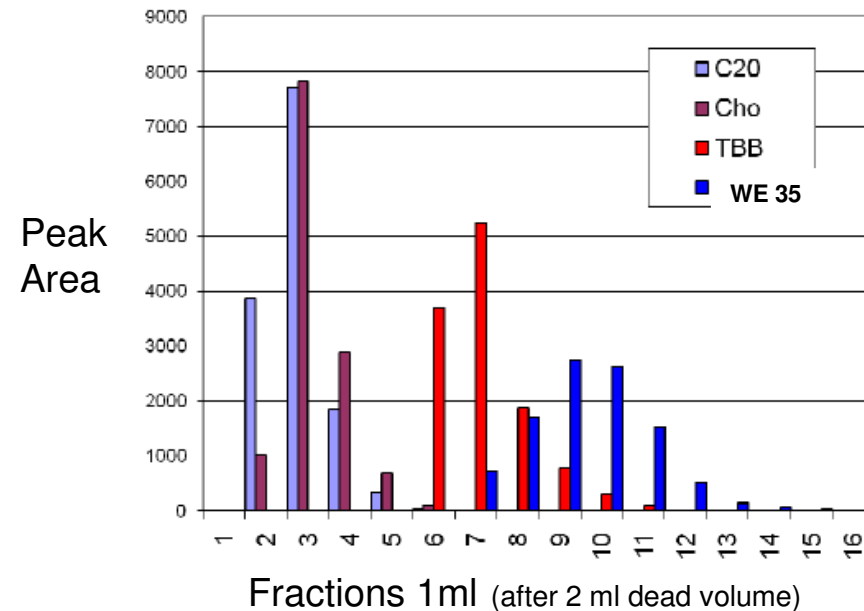
Experiment:
• silica gel 400 °C (act) / 24 h
• 50% DCM for MOAH
 starting from fraction 3

Result:
• Cho/TBB not efficient separated
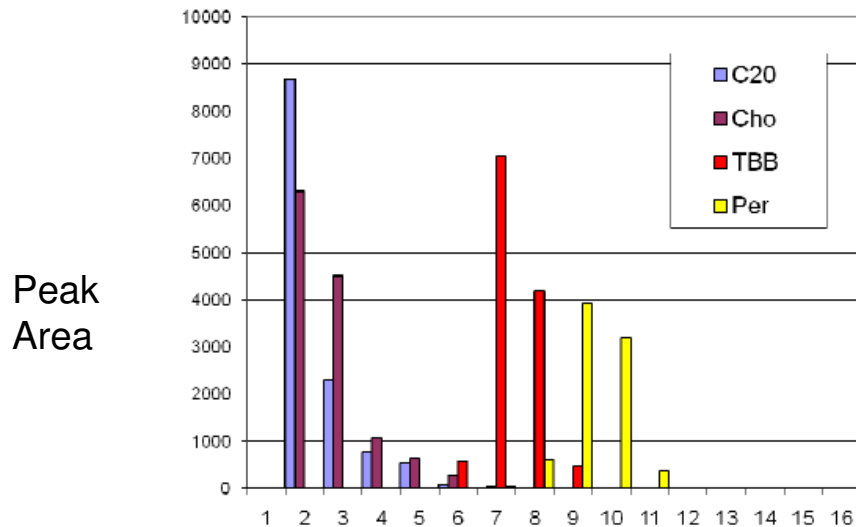• Per/WE35 inadequate separated

Experiment:
• silica gel 150 °C 1% AgNO3
• 100% DCM for MOAH
 starting from fraction 3

Result:
• Cho/TBB ok
• no Per, but WE 35

Development of the SPE procedure

Use of toluene to elute the MOAH-fraction

Peak Area

Fractions 1 ml (after 2 ml dead volume)

Experiment:
- silica gel / 0.1% AgNO3
- 50% toluene for MOAH

Result:
- Cho/TBB narrow
- Per near to TBB
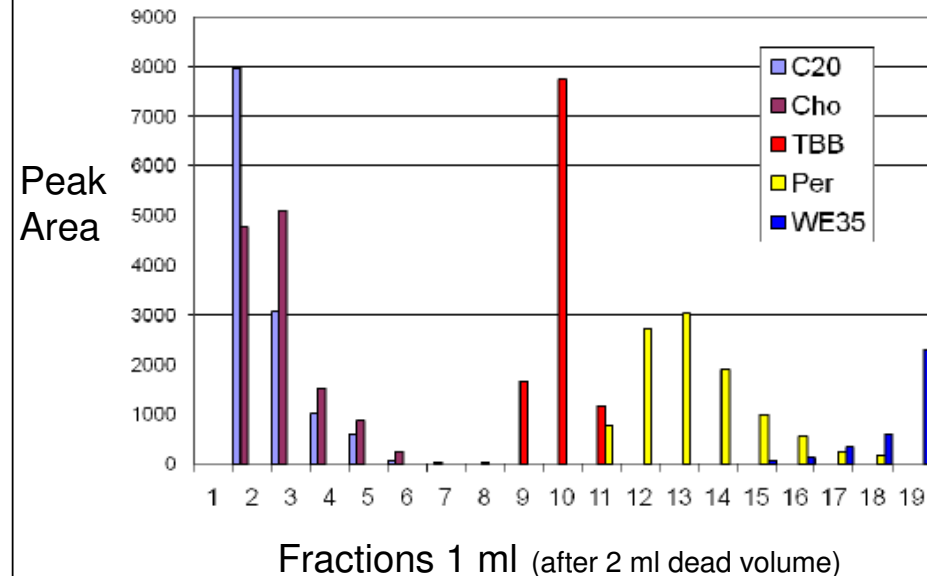- no WE35

Gradient later
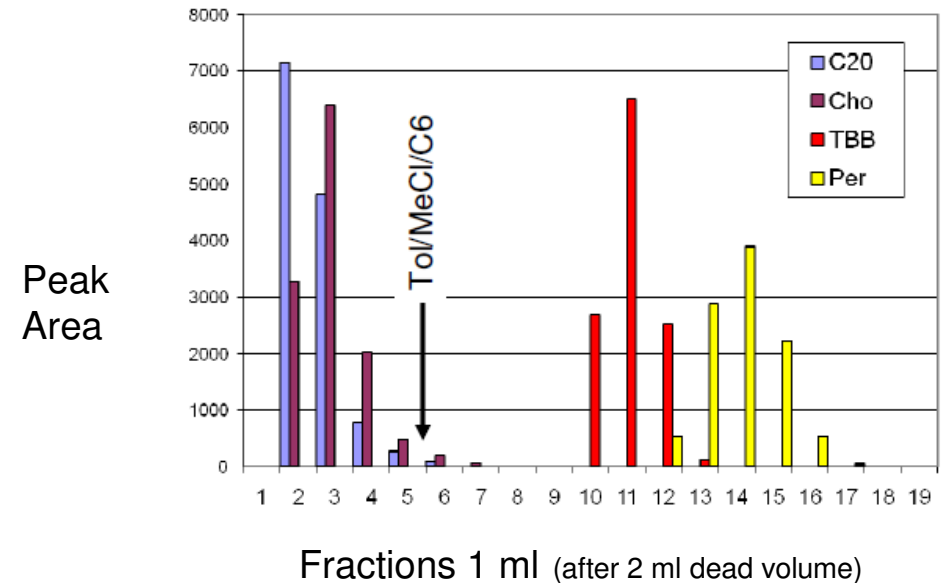
Peak Area

Fractions 1 ml (after 2 ml dead volume)

Experiment:
- silica gel 1% AgNO3 + act silica gel 1 + 2
- 30 % toluene for MOAH starting from fraction 6

Result:
- Cho/TBB very good
- Per near to TBB
- no WE35
- Problem: evaporisation of toluene

Development of the SPE protocol

Partial replacement of toluene by DCM

Peak Area

Fractions 1 ml (after 2 ml dead volume)
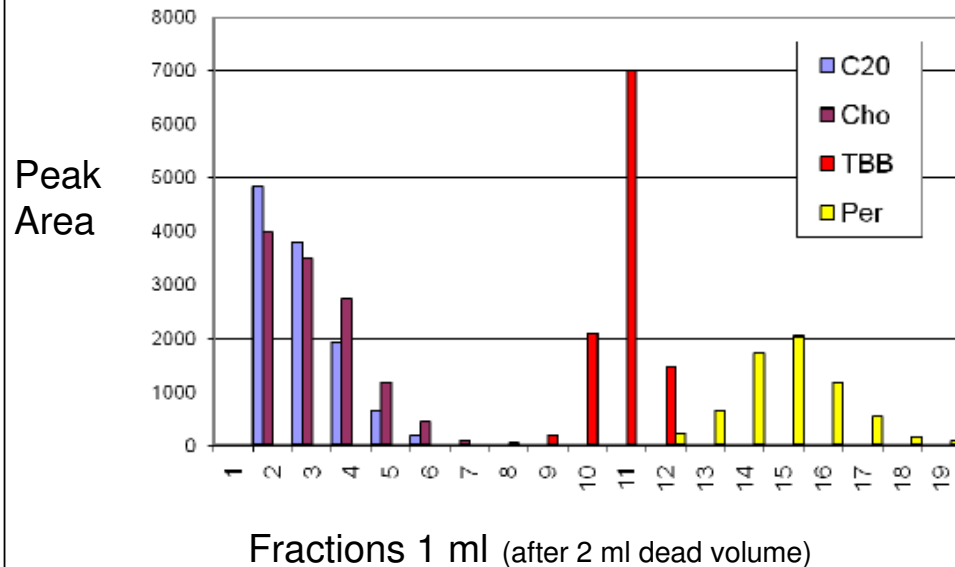
Experiment:
- silica gel 1% AgNO3 + act silica gel 1 + 2
- 20 % DCM and 5% toluene
for MOAH starting from fraction 6

Result:
- Cho/TBB ok
- Per broad
- no WE35

30% DCM

Peak Area

Fractions 1 ml (after 2 ml dead volume)

Experiment:
- silica gel 1% AgNO3 + act silica gel 1 + 2
- 30 % DCM and 5%toluene
for MOAH starting from fraction 6

Result:
- Cho/TBB good
- Per broad
- WE35 visible

Validation of the SPE-Method

Gravex 913 1000 ppm in n-hexane

Direct injection without SPE -separation

963 ppm
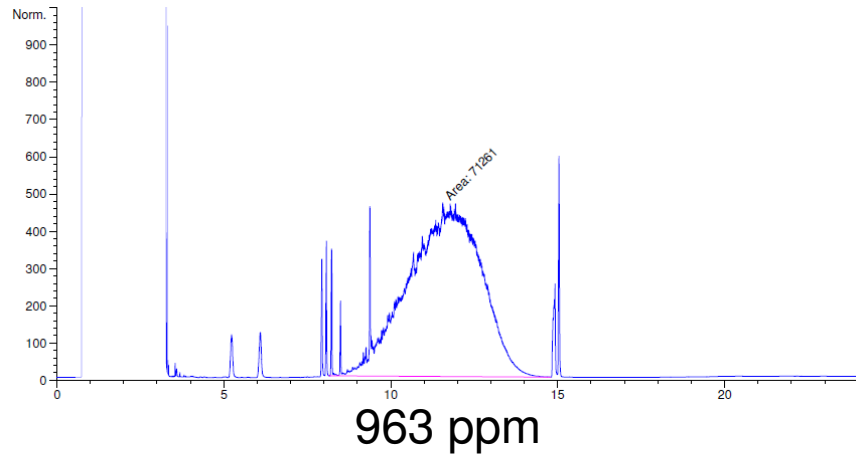
Recovery of 99.9% of the Gravex-Peak after
SPE separation (n=4):
72.5 ± 0.5 % MOSH   (LC-GC: 72.9)
27.5 ± 1.7 % MOAH   (LC-GC: 27.1)

Recovery related to the initial weight: 96 %

MOSH

694 ppm

MOAH

264 ppm
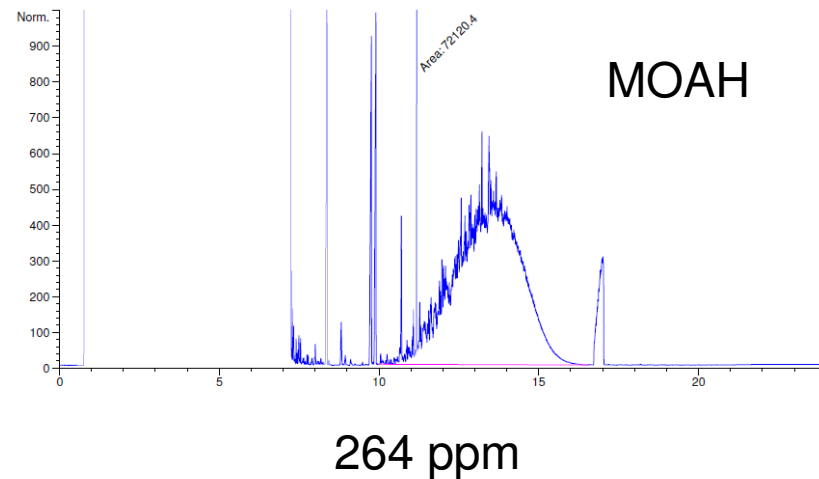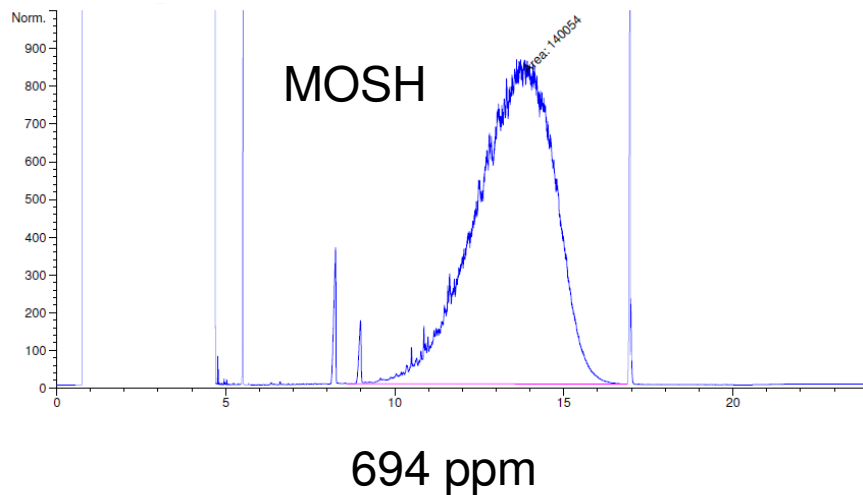
Extraction of samples

10 g dry food

10 ml n-hexane
10 µl IS-mix
12 h
1 ml auf SPE

2 g paper/paper board

10 ml n-hexane / EtOH (1:1)
20 µl IS-mix
12 h
4 ml extract is shaken with 10 ml water
0.2 ml upper phase on SPE

Capacity for fat: 200 mg

Validation of the SPE-method: internal standards (IS)

Old set of IS (Biedermann and Grob, 2010):

MOSH: n-C12   (C12)
        n-C14   (C14)
        n-C16   (C16)
        Cholestane   (Cho)

MOAH: Hexyl benzene   (6B)
        Nonyl benzene   (9B)
        Tritert.butyl benzene (TBB)
        Biphenyle   (BP)
        Perylene   (Per)
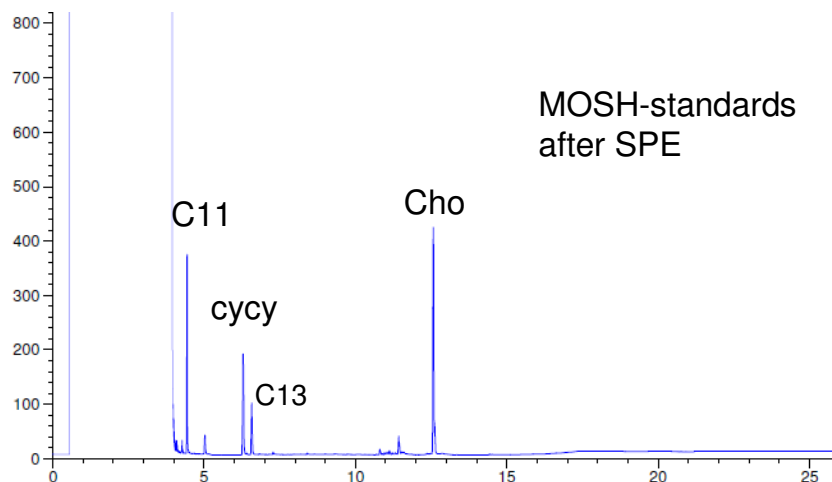
New set of IS:

MOSH: n-C11   (C11)
        Bicyclohexyl   (cycy)
        n-C13   (C13)
        Cholestane   (Cho)

MOAH: Pentyl benzene   (5B)
        1-Methyl naphthalene (2MN)
        2-Methyl naphthalene (1MN)
        Tritert.butyl benzene (TBB)
        Perylene   (Per)

MOSH-standards after SPE

MOAH-standards after SPE
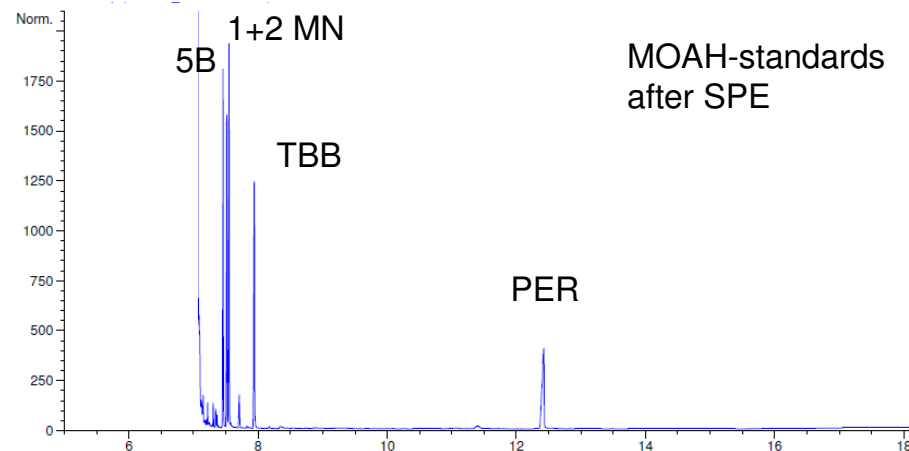
Use of commercial available silica gel containing AgNO$_3$
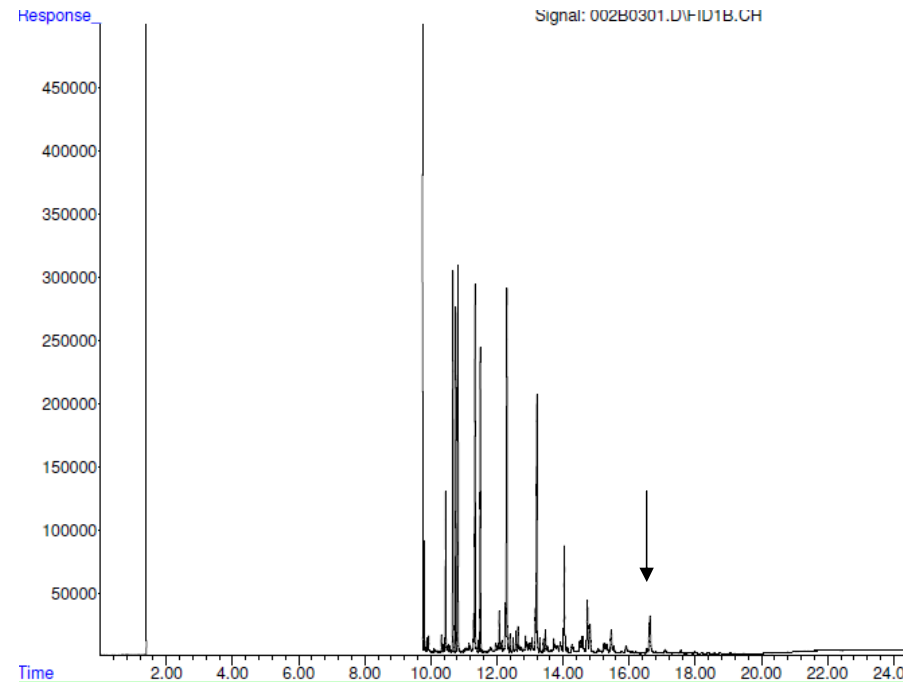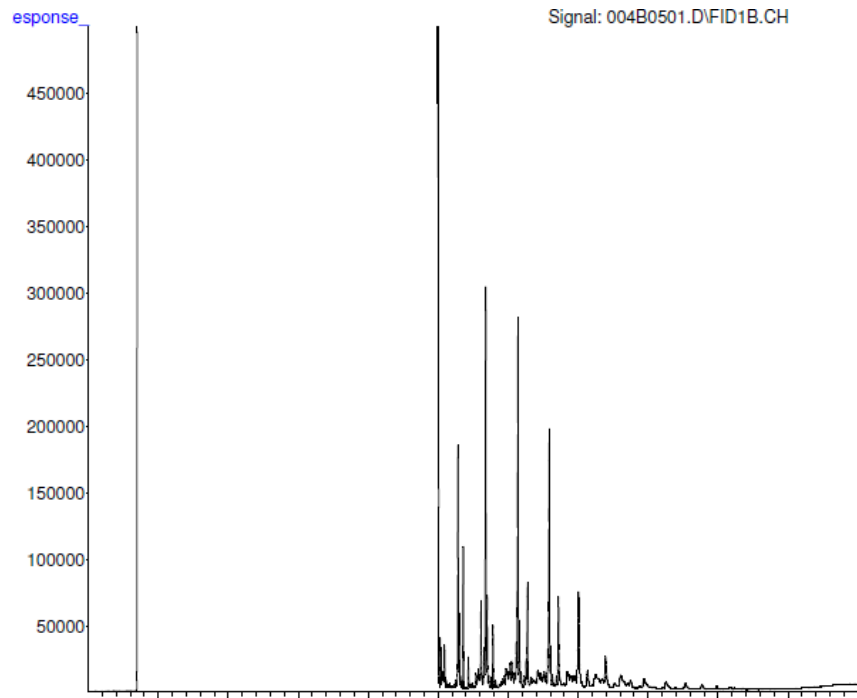
Extract of the silica gel with toluene/hexane   MOAH-Fraction after SPE

⟶ Disturbing substances from the silica gel, from the caps ?

Validation of the SPE-method: further verification standards tested

MOSH: Alpha-homo-cholestane   → not detected in MOAH-fraction

MOAH: Hexaethylbenzene (1)   →
       1,4-Bis(2-Ethyl-hexyl)-benzene (2)   →
       1,4-Bis(3,7-dimethyloctyl)-benzene (3) →   → not detected in MOSH-fraction
       1-Phenyloctadecane (4)   →
       1,2,3,5-Tetracyclohexylbenzene (5)   →

Chromatogram of MOAH-fraction
after SPE separation
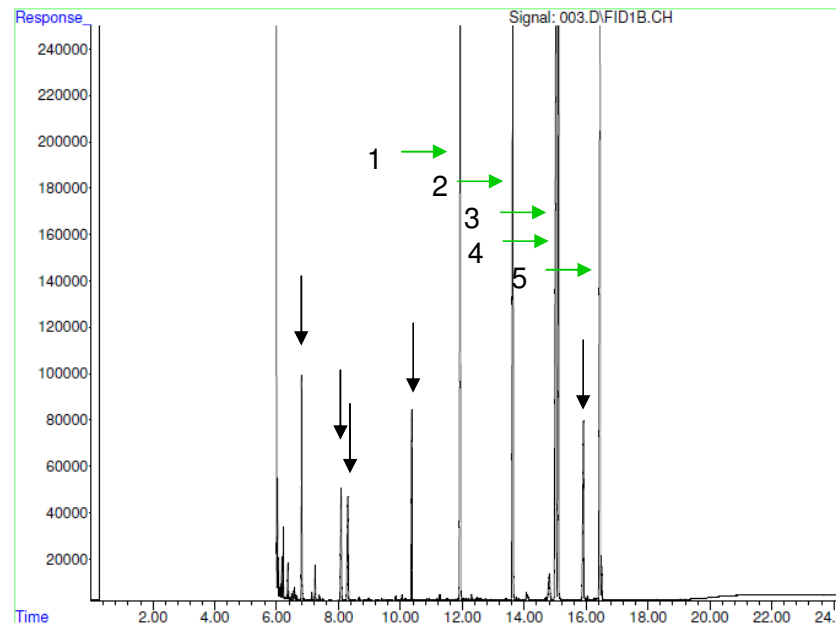
Mineral oil chromatograms – "manual" method vs. LC-GC

Contaminated rice

SPE / LV-GC

LC-GC

MOSH

MOAH

0.9 11 1.5 [ppm]

0.8 11.2 1.4 [ppm]

2.6 0.1 [ppm]

2.1 [ppm]
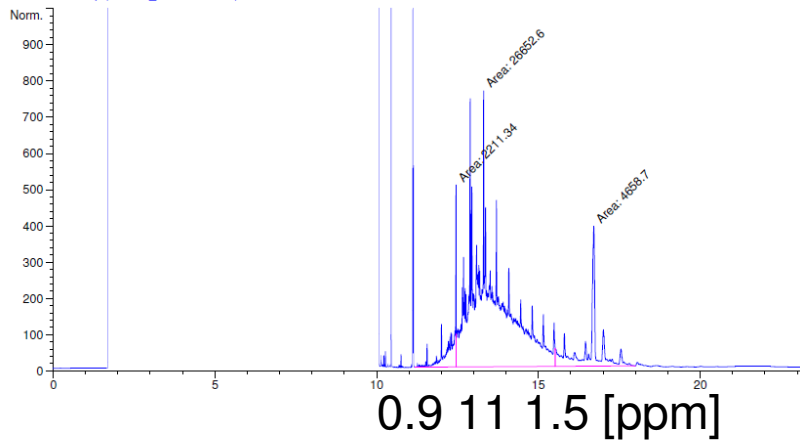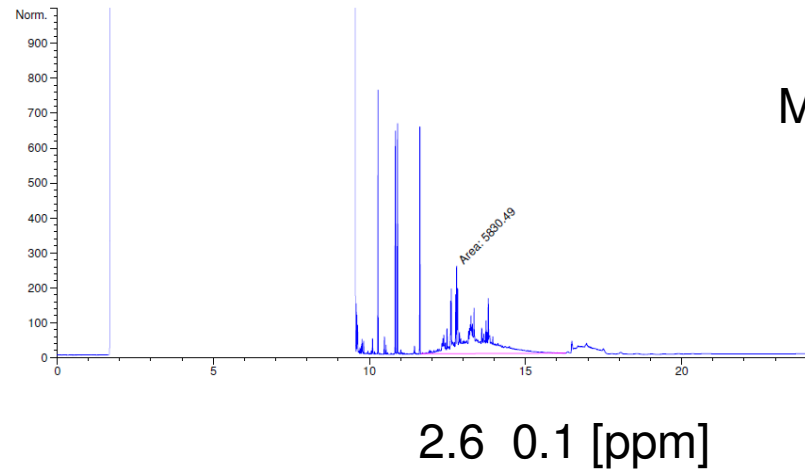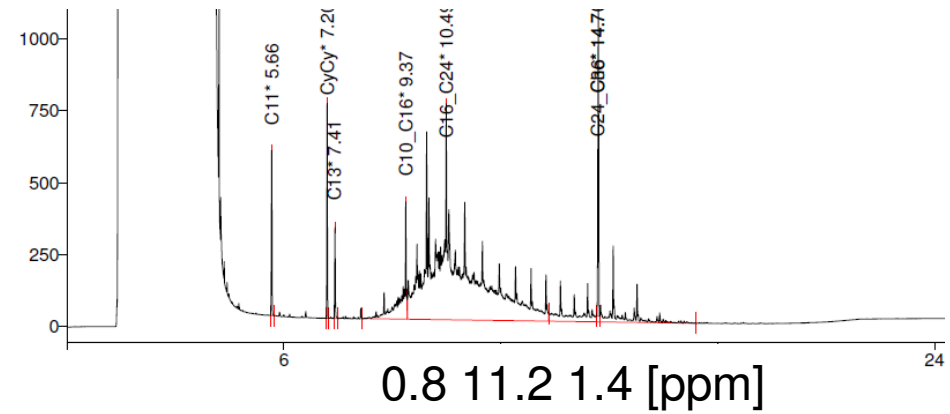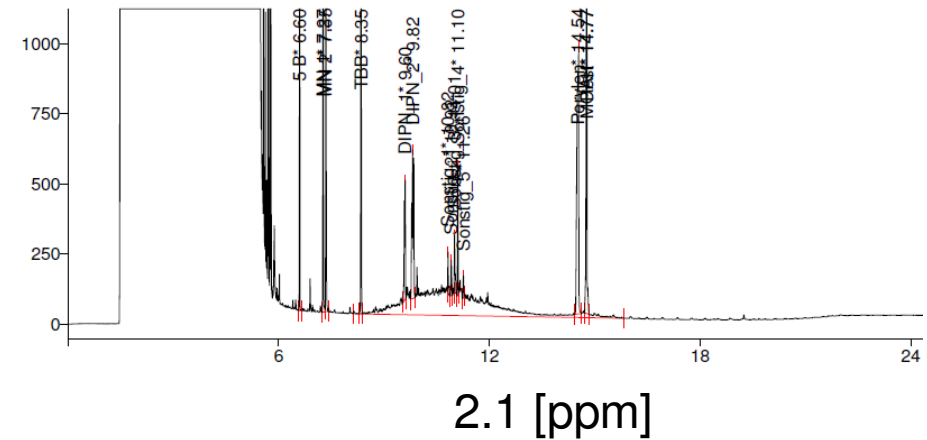
Mineral oil chromatograms – "manual" method vs. LC-GC

Paper board

SPE / LV-GC

LC-GC
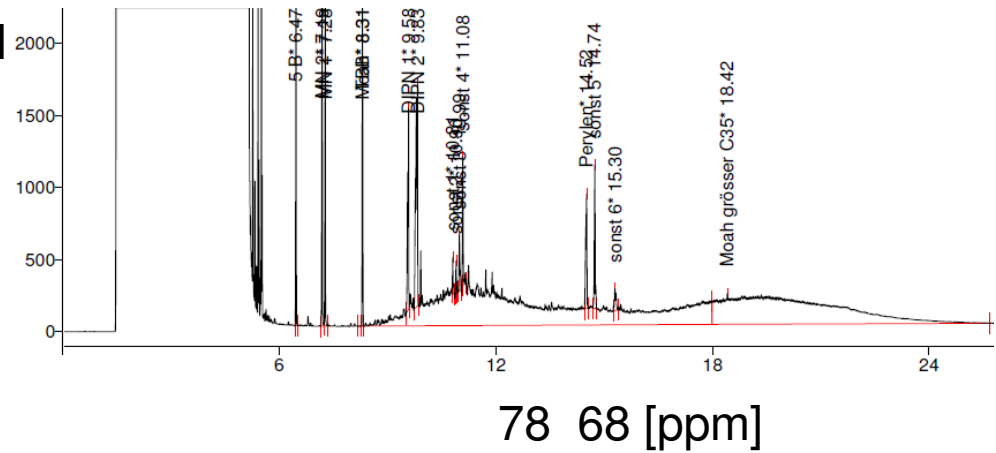

MOSH

9.8 195 171 57 [ppm]

10.3 202 171 55 [ppm]

MOAH

103 61 [ppm]

78 68 [ppm]
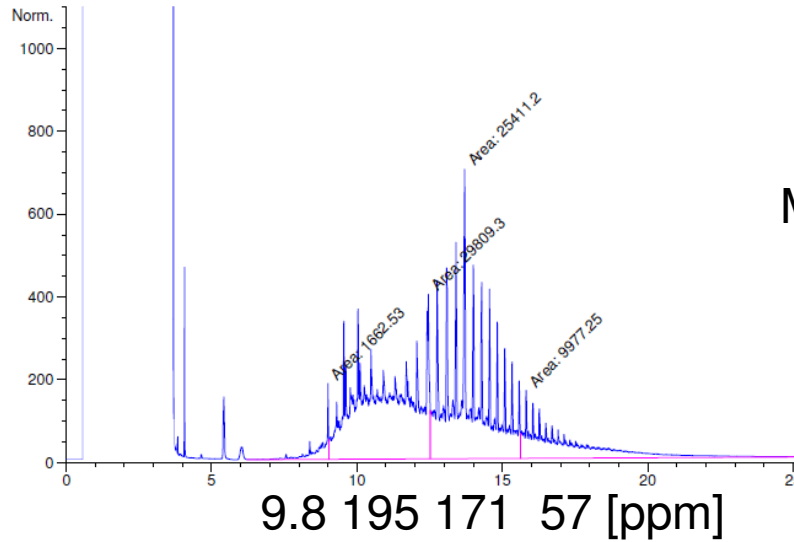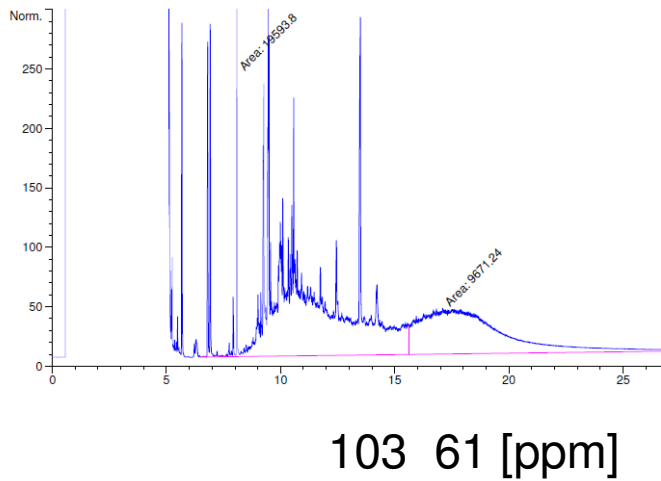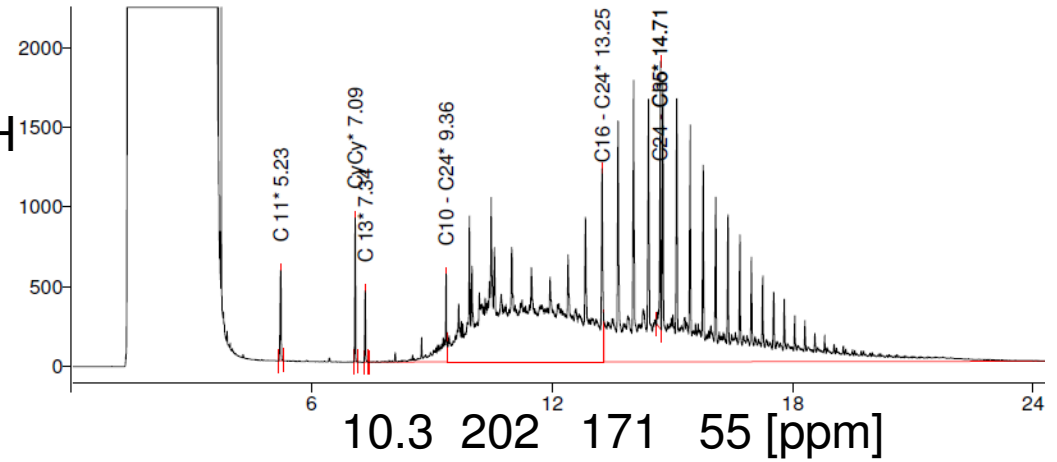
Mineral oil chromatograms – "manual" method vs. LC-GC

Gravex 913 in n-hexane (1000 ppm)

SPE / LV-GC

LC-GC
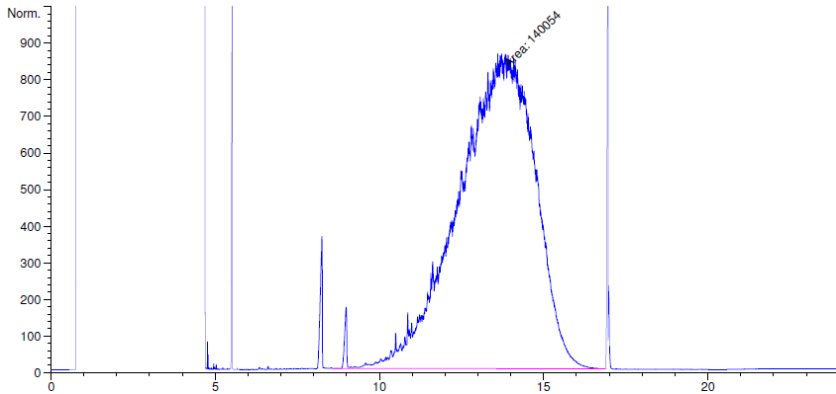
MOSH

694 [ppm]
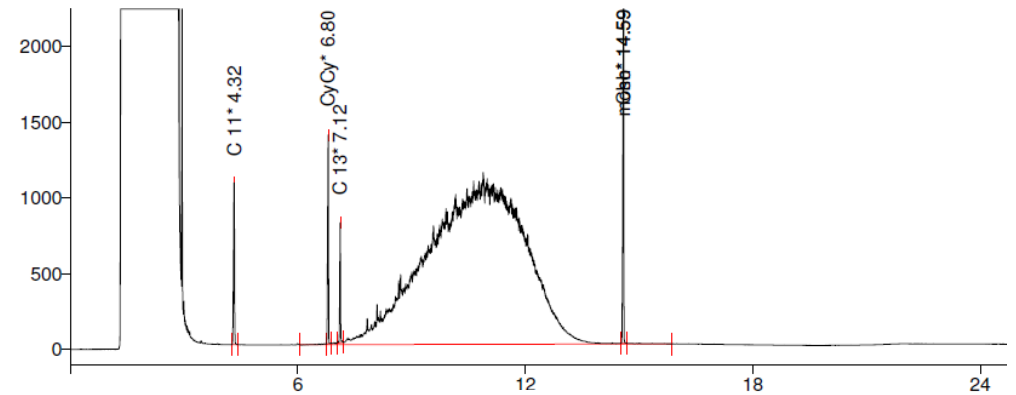
731 [ppm]

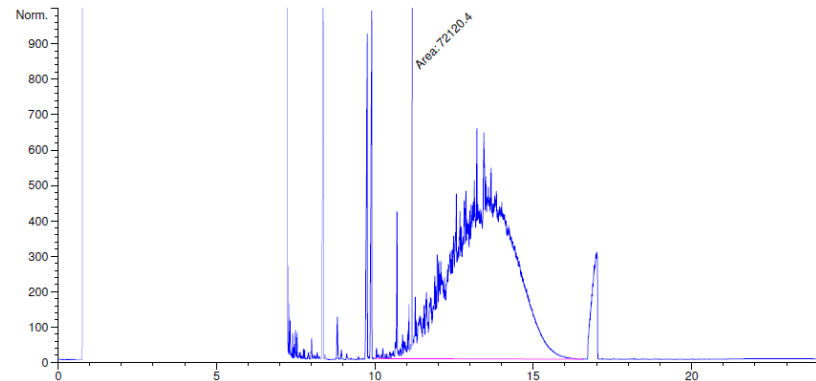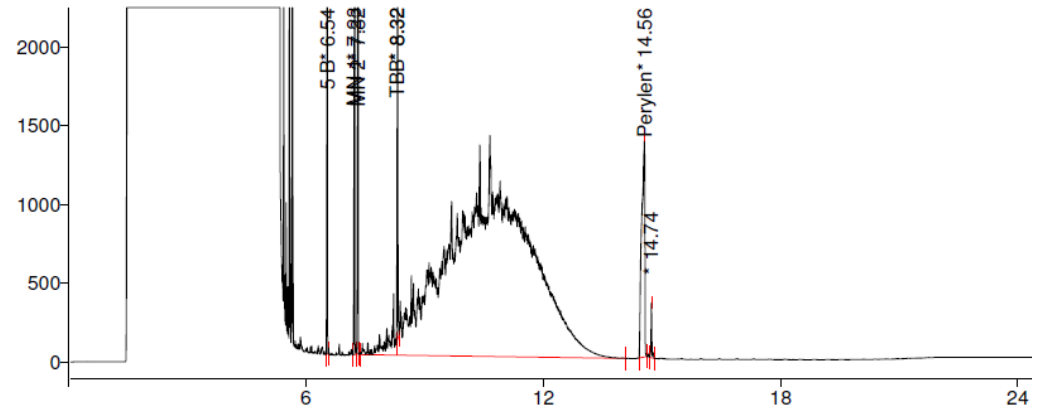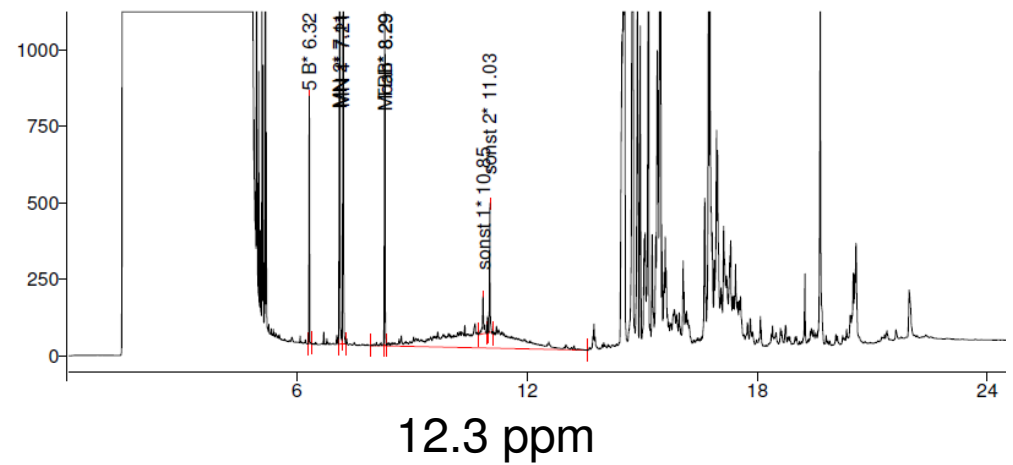MOAH

264 [ppm]
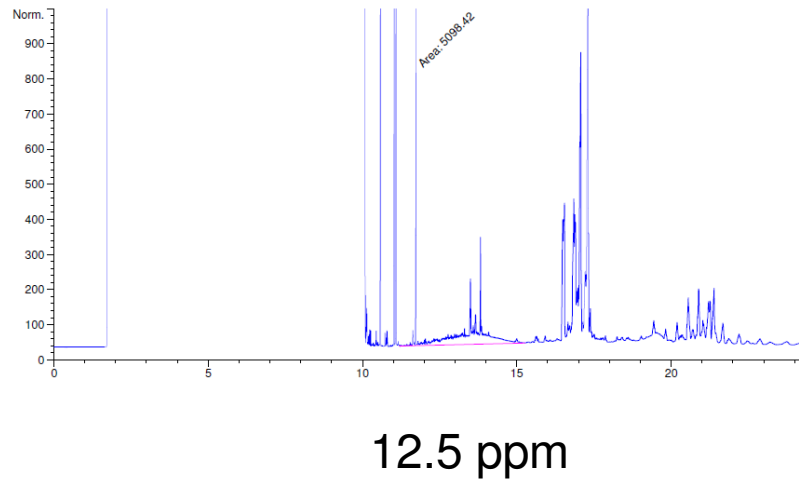
272 [ppm]

Mineral oil chromatograms – "manual" method vs. LC-GC

Gravex 913 in rapeseed oil (50 ppm)

SPE / LV-GC

LC-GC

MOSH

37.0 ppm

36.3 ppm
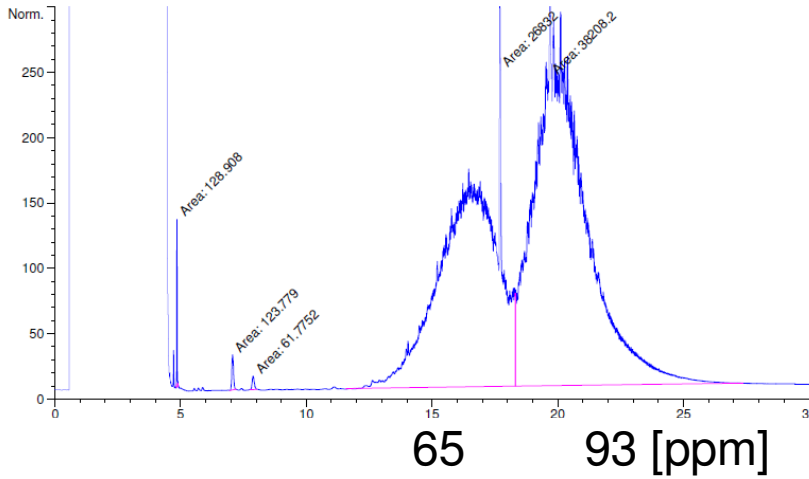
MOAH

12.5 ppm

12.3 ppm
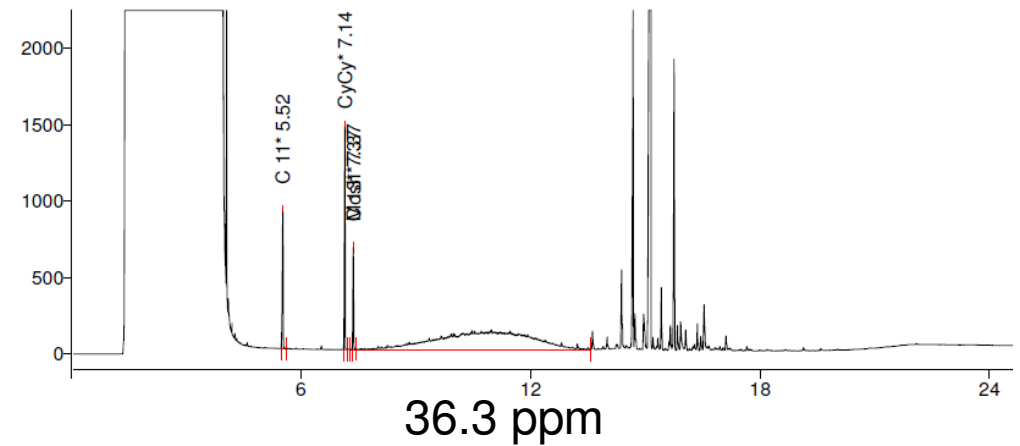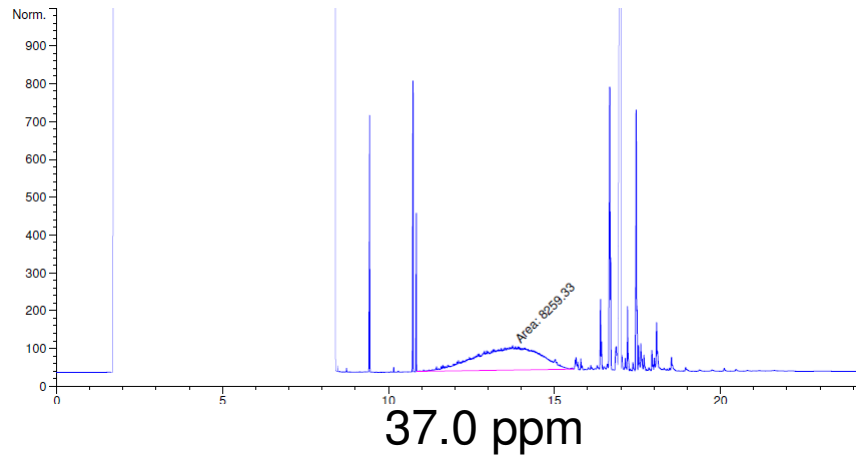
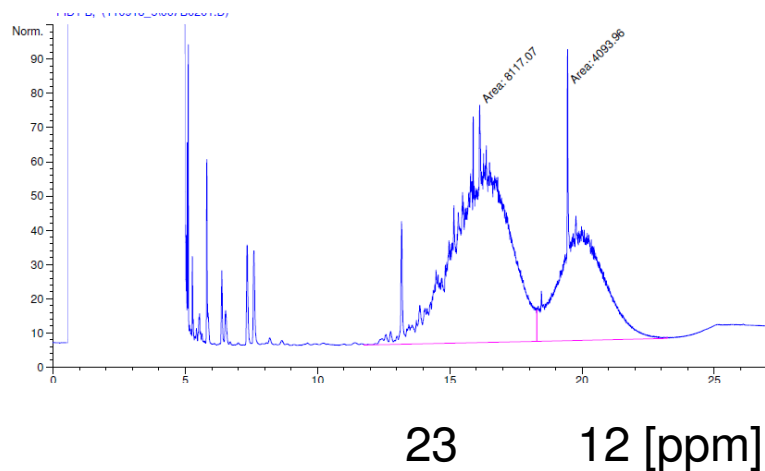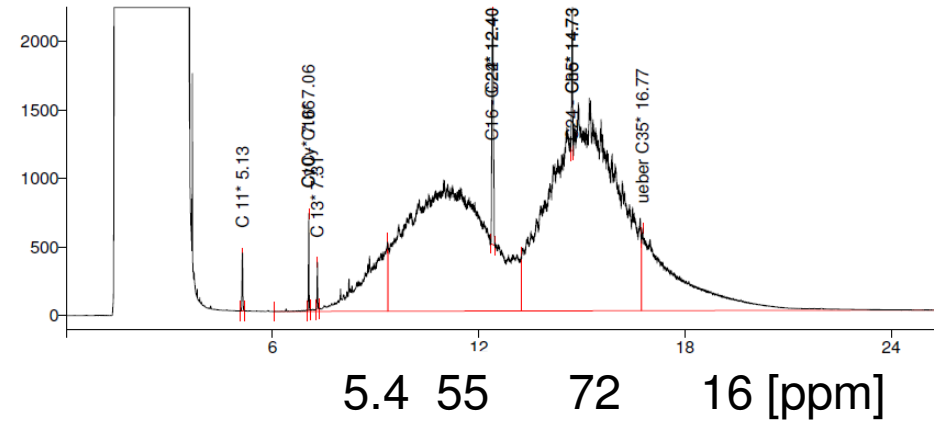# Mineral oil chromatograms – "manual" method vs LC-GC

SPE / LV-GC

Mix of different mineral oils

LC-GC

MOSH

MOAH
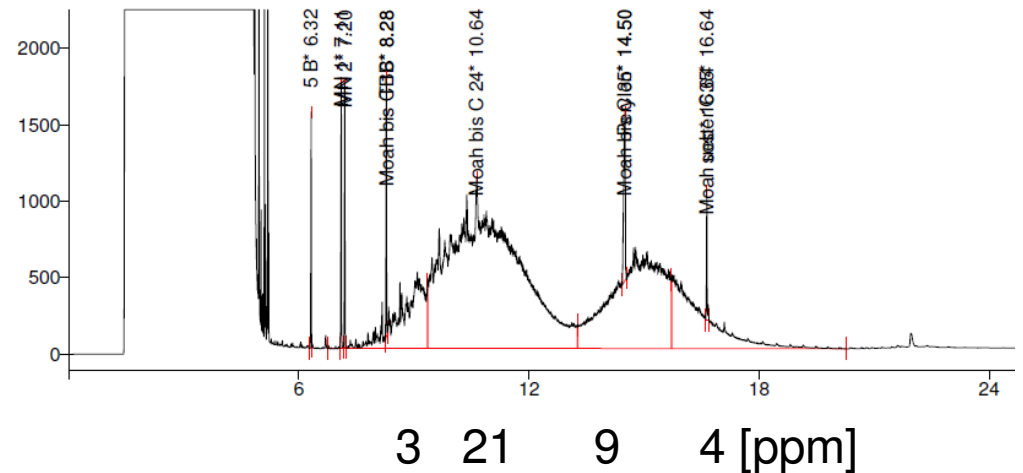
Analysis using on-line HPLC-GC-FID vs. off-line HPLC

Reproducible and highly resoluted separation

- Control by on-line UV-detector **+**

Automatable Process

- High efficiency, high sample output **0**
- Highly reproducible results **?**

Complete Transfer of one HPLC-Fraction into the GC-System **+**

→ **higher sensitivity**

Discrimination-free solvent evaporisation during the transfer **-**

No loss of volatile mineral oil compounds, no discrimination

Closed system: no cross-contamination of the samples **-**

Low solvent consume (<10 mL/sample) **0**

Analytical Equipment for the mineral oil analysis (Off-line LC-GC)

Off-line NP-HPLC Sampling

HPLC Agilent Technologies 1100 Series

(Degaser, binary Pump, auto sampler (with Cooling Unit), column oven, UV-detector, fraction collector)

HPLC-column: LiChrospher Si 60 5µm; 2 mm x 250mm

Quantification with GC –FID

GC: Agilent 6890

Autosampler: Agilent 6890

GC-Column: DB-1HT (15 m x 0,32 mm x 0,10 µm) and 7 m

            unlined (dectivated) pre column

Injection cool on-column: 40 µL

MOSH-samples after off-line HPLC-Separation

NP-HPLC with manual or automatic collection of fractions

LV-GC-FID analysis

Literature method needs adaption to our HPLC-system.

MOSH ok

MOAH: Perylene not found, shift in retention time

-> larger volume for MOAH-fractions

Recovery of standards after LC-separation:
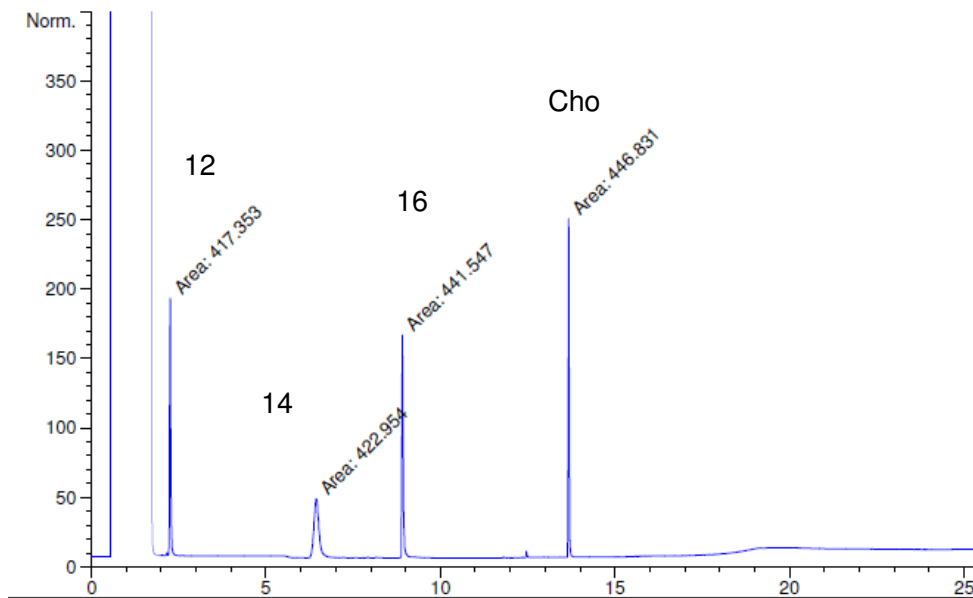Transfer of the published LC-method from LC-GC from Biedermann and Grob (2010)

C 12	109%
C 14	105%
C 16	99%
Cho	104%
9B	101%
6B	103%
TBB	102%
BP	93%
Per	shifted

Off-line HPLC separation of standards
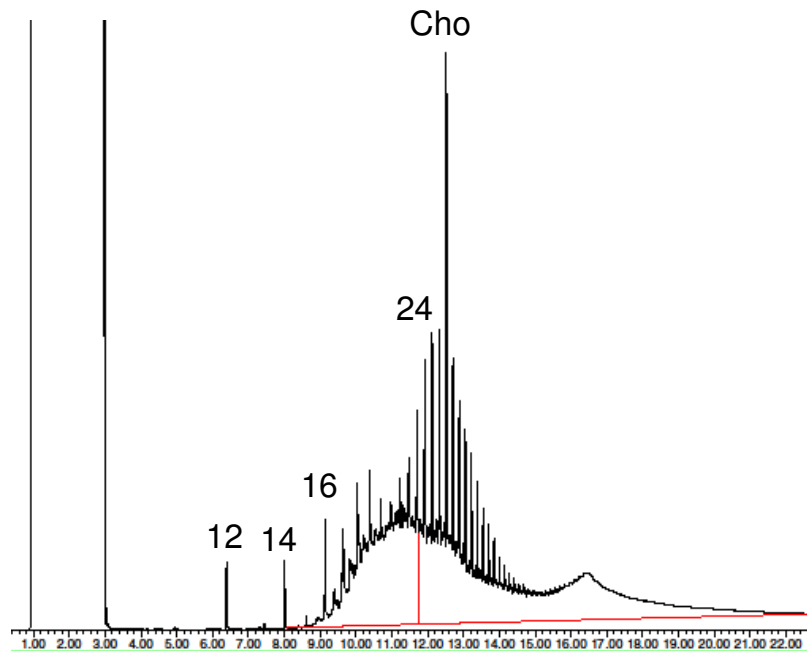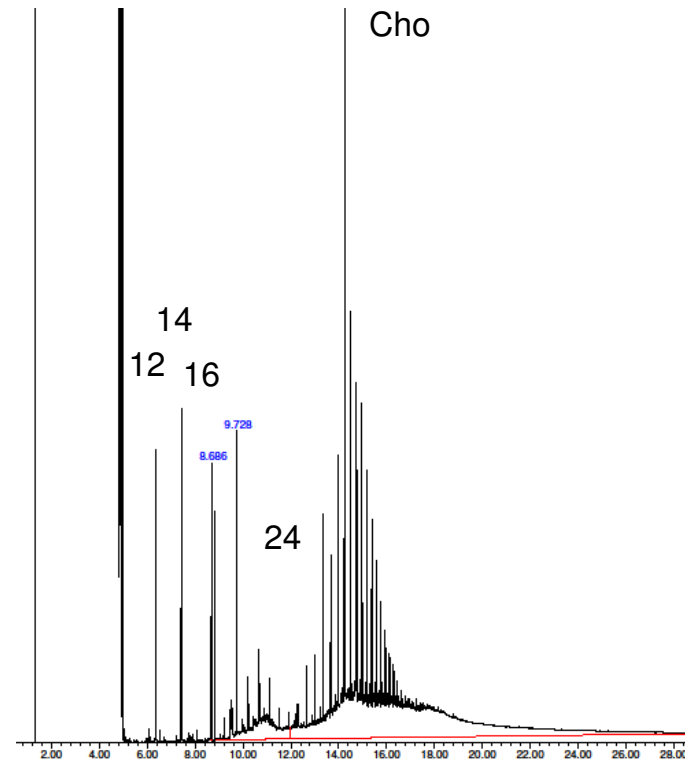
MOSH

MOAH

Disturbances in MOAH-fraction
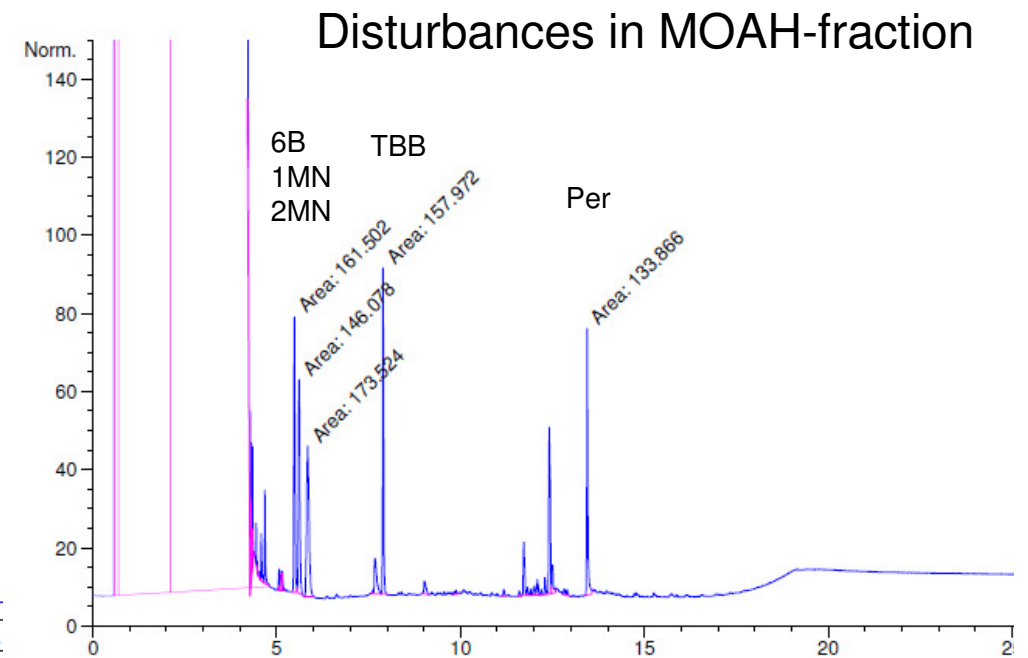
Source of the contaminations in the MOAH-fraction?
Switching valve?

MOSH-samples after off-line HPLC-Separation

MOSH-
hygiene paper from
recycled material

464   ppm MOSH < C24

776   ppm MOSH > C24

MOSH
Paper board from food packaging

40   ppm MOSH < C24

306   ppm MOSH > C24

MOSH-samples after off-line HPLC-Separation

Possible pitfalls and disadvantages related to offline-HPLC-separation of mineral oil-samples

- Parts of the LC-system may be not stable against DCM
- Up scaling of the column dimensions requires higher flow rates -> higher dilution
- Adaption of the published LC-Method for LC-GC to the used HPLC-System is necessary
- When using a automatic fraction collector, additional tubing (and dead volume) is necessary
-> Chromatographic resolution will decrease

- Need for enrichment before GC-analysis, keeper for evaporisation to avoid loss of volatile compounds

- It's easy to prepare MOSH-fractions with offline-HPLC-separation, **but** for MOAH-analysis more time and effort has to be spent.

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Bundesinstitut für Risikobewertung

Thank you for your attention

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