Institut Kirchhoff Berlin

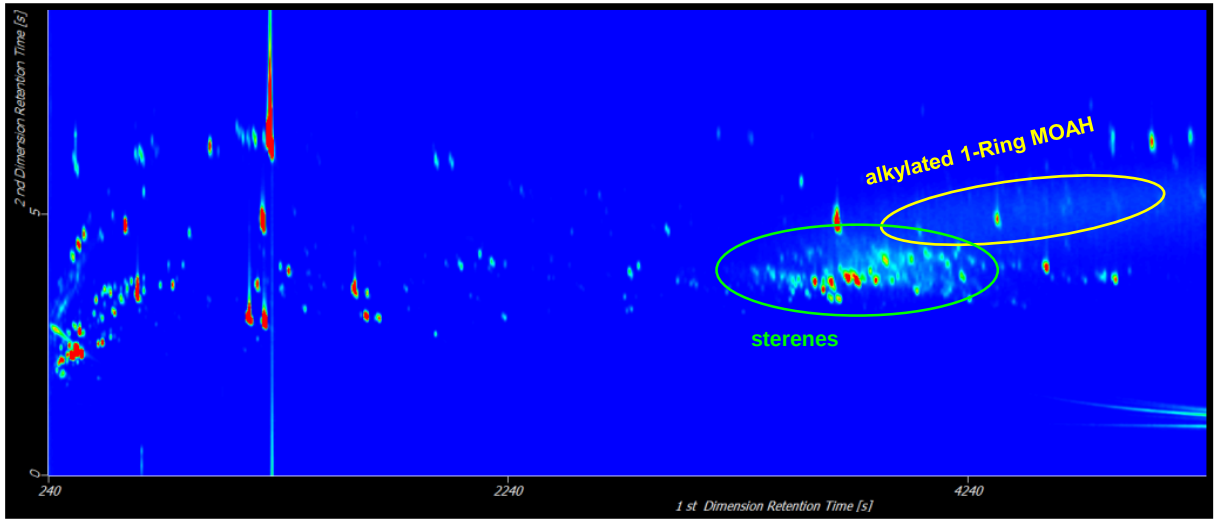
MERIEUX NutriSciences

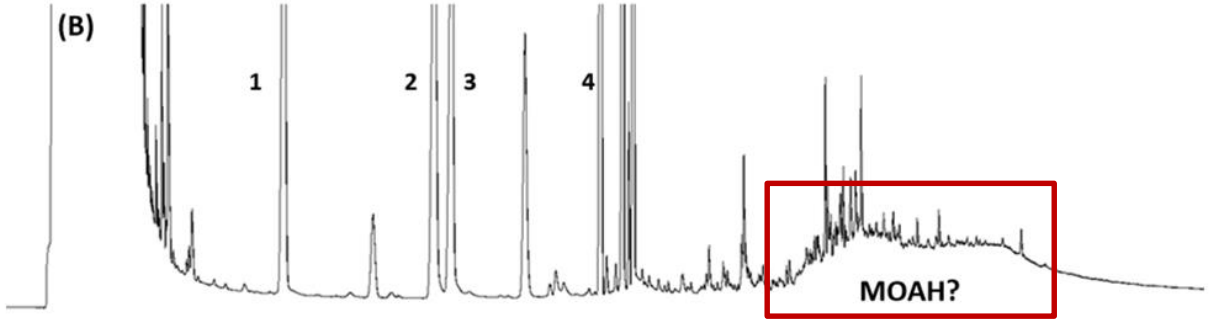
Development of an improved analytical method

Stand: 10.06.2021

Limitations of the current LC-GC-FID method

MOAH vs. Biogenic Components





HPLC-GC-FID-Chromatogramm der MOAH in „Palmöl“: (A) vor Aufreinigung, (B) nach Aufreinigung mittels Epoxidierung; 1 - 5PB, 2 - 1-MN, 3 - 2-MN, 4 – TBB, 5 - Squalen, 6 - Carotinoide

- in case of disturbances due to matrix components, an additional purification step is necessary
- after epoxidation a "hump" remains for certain samples
- no MOAH, but not epoxidized, biogenic substances

-> "Hump" is assigned to the MOAH by laboratories (false-positive)

-> significant consequences for raw material suppliers and food companies

-> Limit of quantification has to be raised

Toxicological Considerations

- according to the BfR contamination of food with MOAH should be avoided (potentially cancerogenic)
- EFSA: carcinogenic potential correlates with increasing number of aromatic ring systems

EFSA Journal 2012; 10(6): 2704
"MOAH with three or more, non- or simple alkylated, aromatic rings may be mutagenic and carcinogenic and therefore of potential concern."

J Agric Food Chem 2018 Jul 11;66(27):6968-6974
"MOAH of at least 3 (conjugated) aromatic rings may include genotoxic constitutents. For this reason, it seems important to distinguish between MOAH of 1-2 and more aromatic rings."

Rapid risk assessment, EFSA, 15.11.2019, doi:10.2903/sp.efsa.2019.EN-1741
"The potential human health impact of MOH varies widely. Mineral oil aromatic hydrocarbons (MOAH), in particular 3-7 ring MOAH, may act as genotoxic carcinogens, while some mineral oil saturated hydrocarbons (MOSH) can accumulate in human tissue and may caus adverse effects in the liver."

-> GCxGC-TOF-MS can identify occurrence of carcinogenic or mutagenic constituents

-> separation of the condensed aromatics

-> limitation due to substance dependent response -> calibration mixture not available

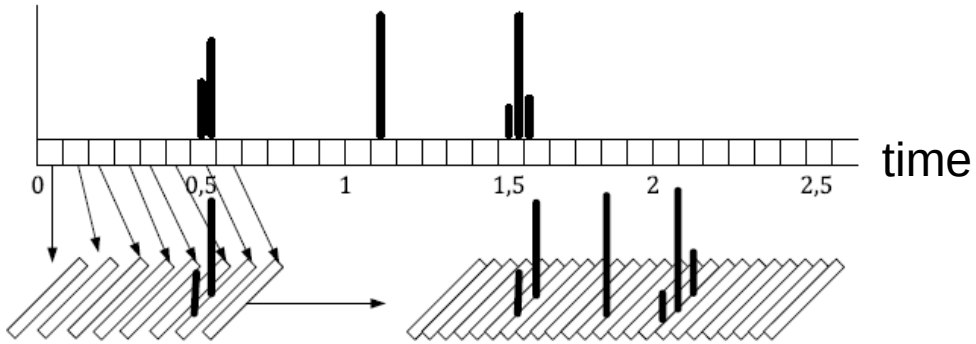
The Solution

- GCxGC can separate the toxicological relevant constituents and is able to eliminate co-elution
- FID can quantify independent of structure

GCxGC-FID

- **advantages**: comprehensive separation efficiency

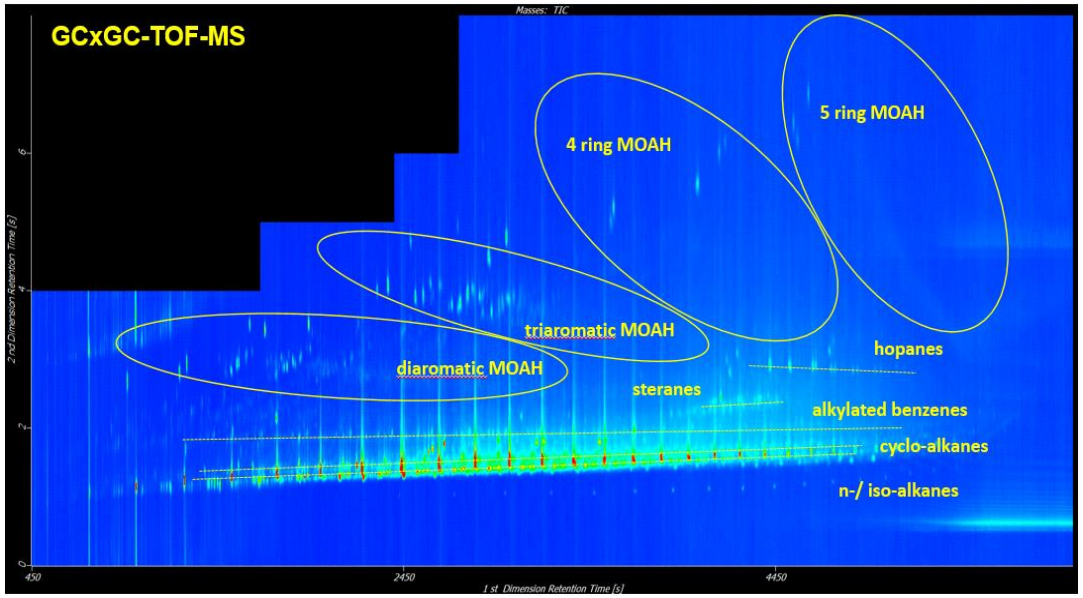
  non-selective detector



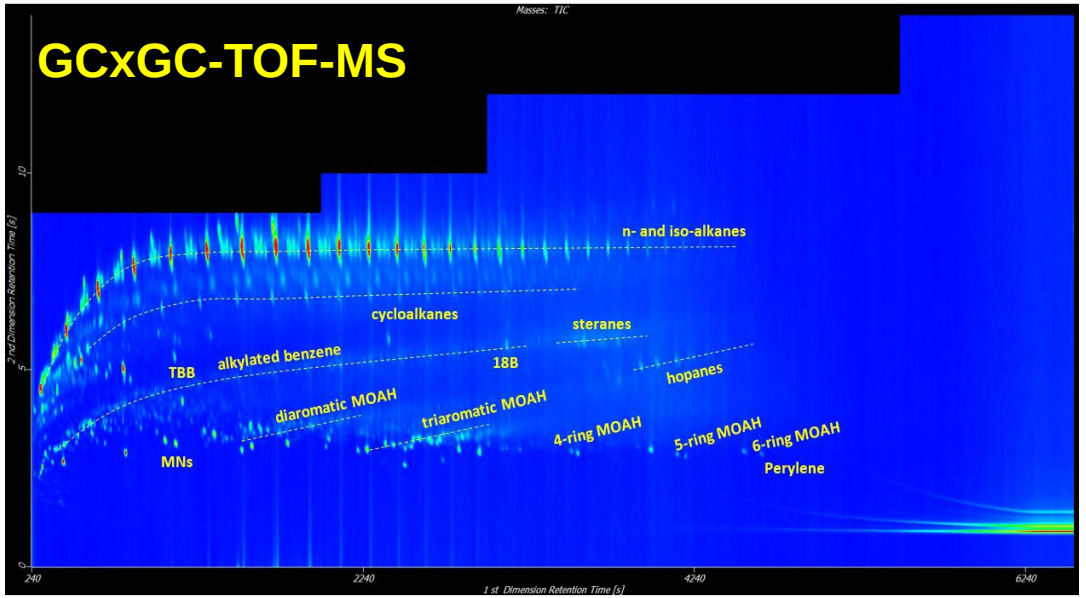
Choices

- two main strategies are used within the GCxGC community

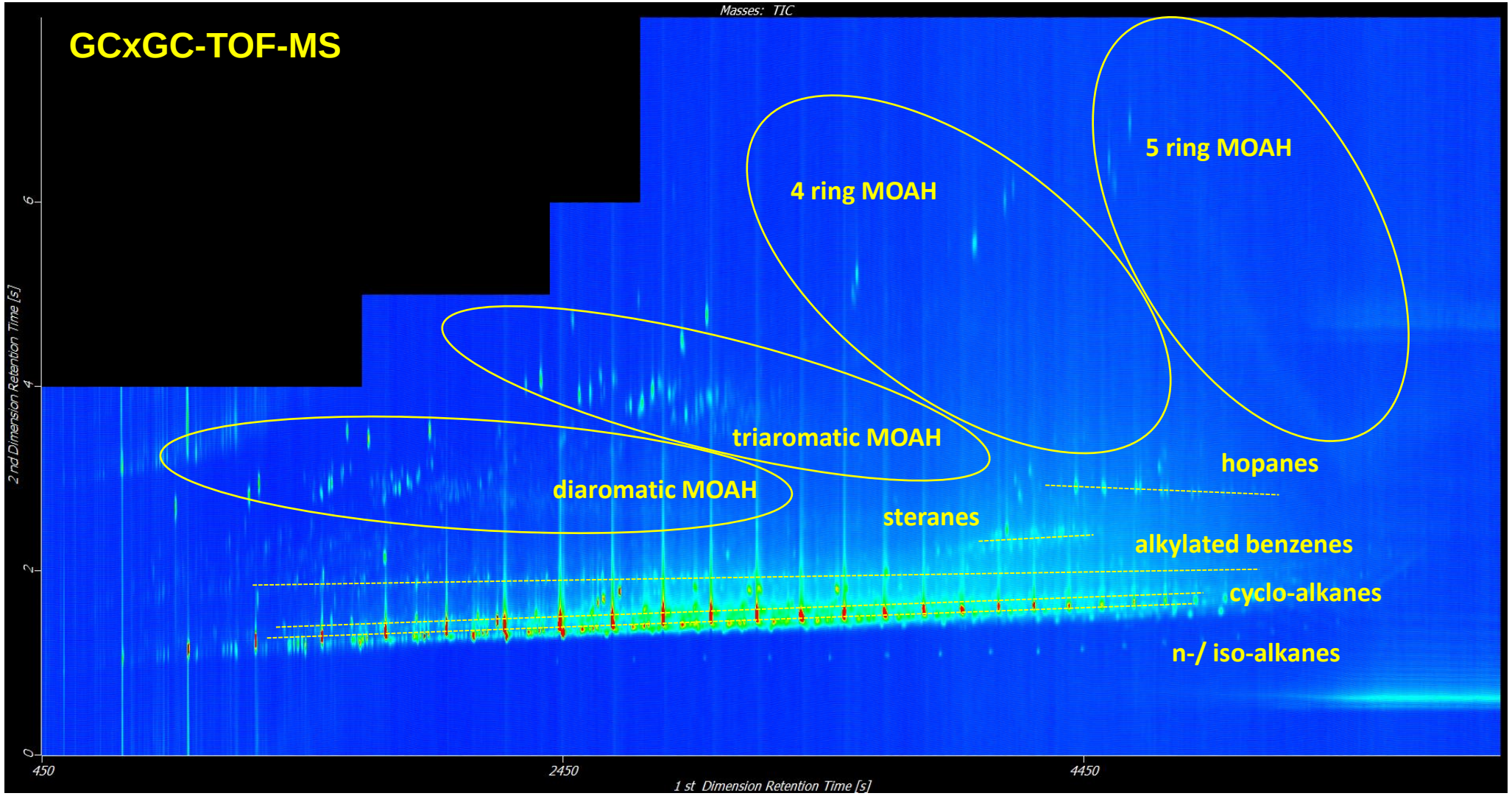
Normal Setup



Reverse Setup



Normal Setup



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Reverse Setup

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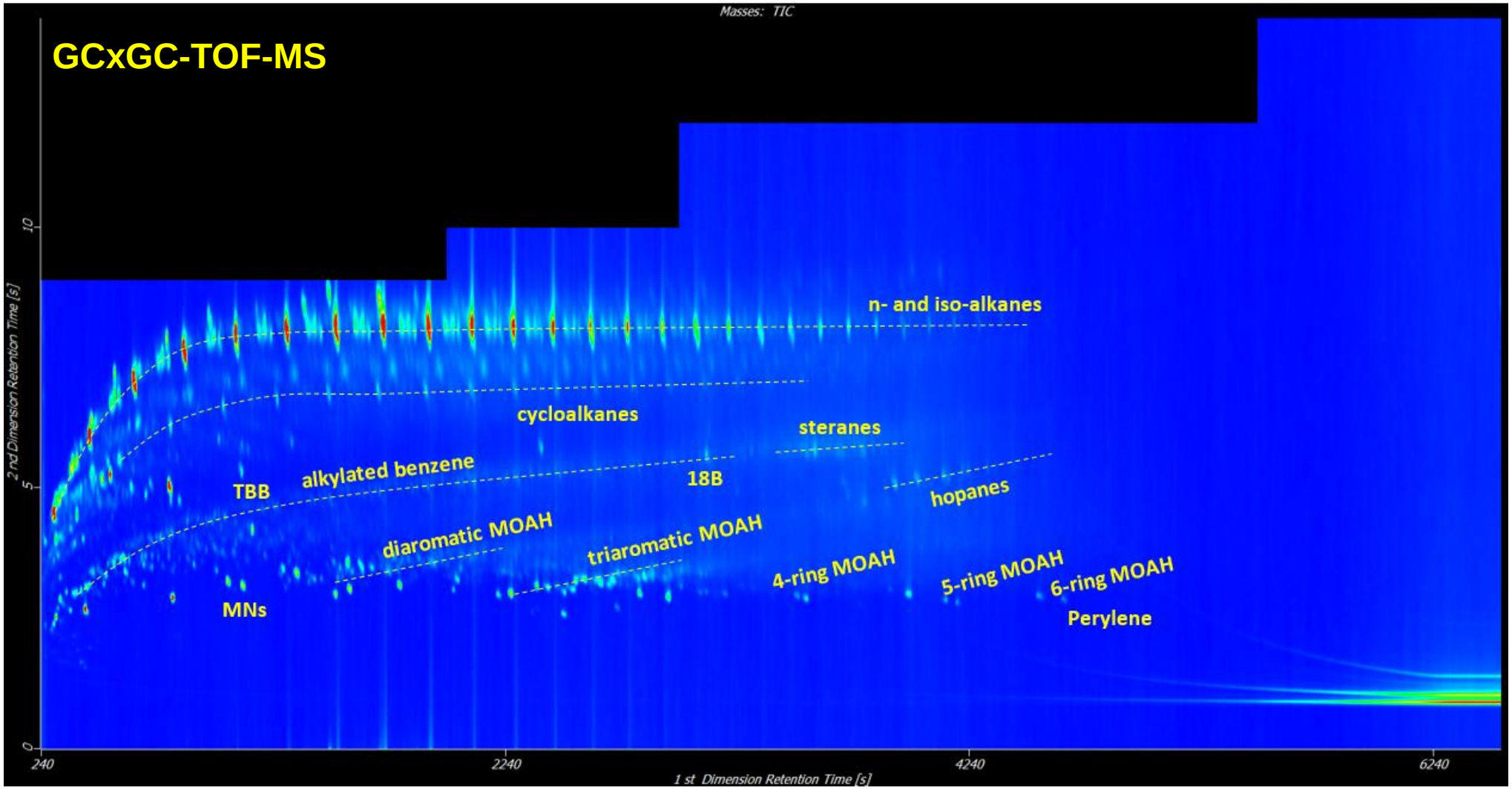
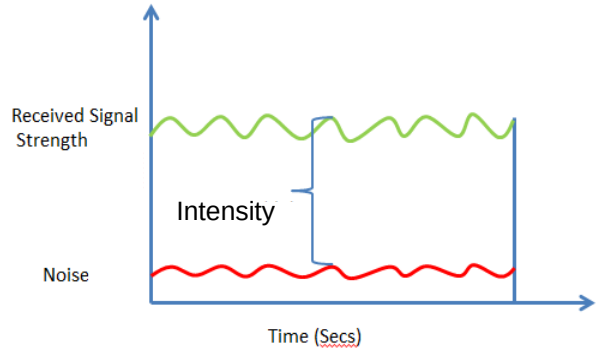
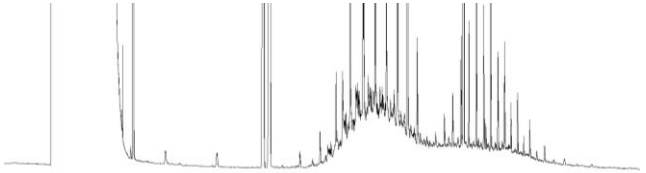


Difficulties



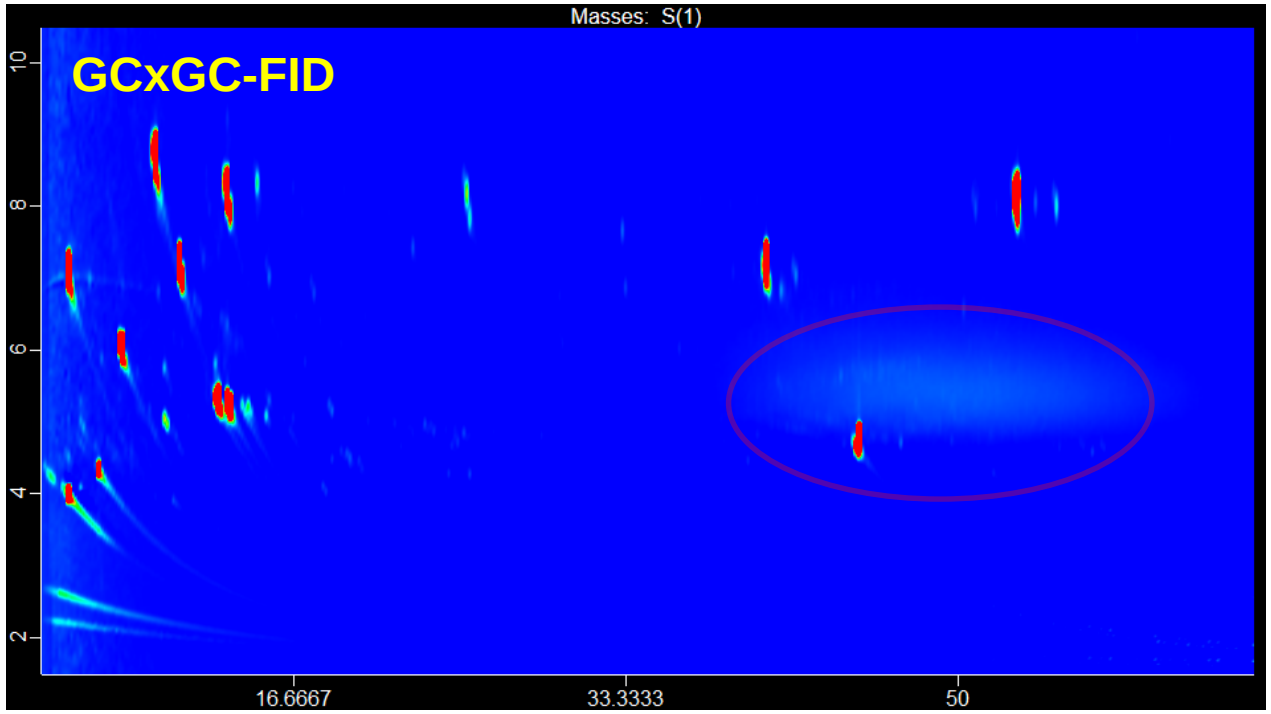
- **sensitivity**
- consider and if possible eliminate the **blank**
- in case of reverse setup: are the n-alkanes still valid as **fraction markers?** What is the influence of the polar first dimension?



- **comparability** to LC-GC-FID (How to handle sharp peaks on the hump?)
- ensure that no **discrimination** occurs (ratio n-C10/n-C20 and n-C50/n-C20 not less than 80 %)

Sensititvity

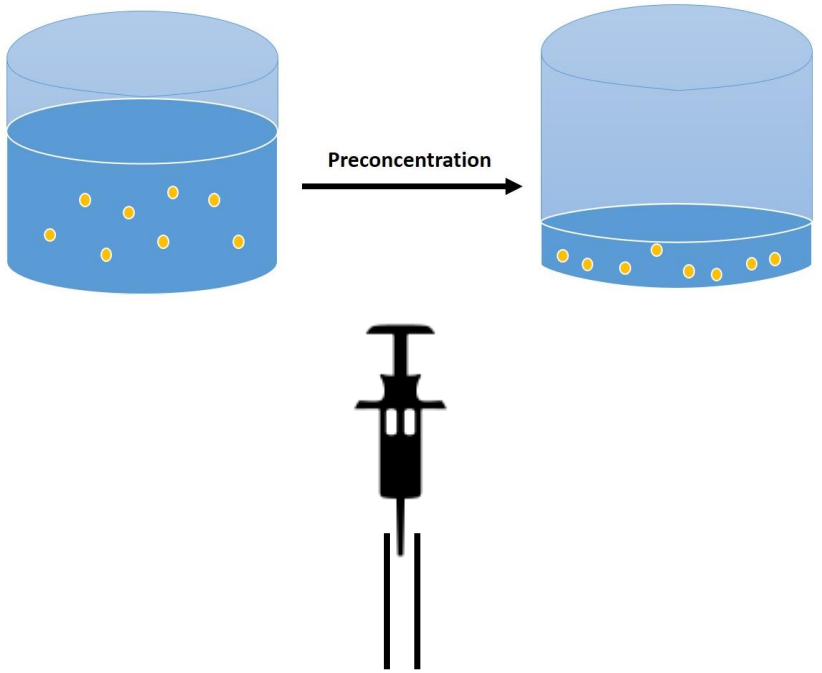
- Minimum amount of MOH for the FID to be detectable circa **25 ng absolute**



contour plot of
20 ng MOAH absolute
after blank subtraction

Sensitivity

- different techniques to achieve the needed sensitivity:
  - concentration of sample prior to injection
  - variety of different injection techniques:
    - splitless injection
    - large volume injection via PTV (MMI or Optic injector)
    - large volume on column injection
    - retention gap technique using SVE



Different ways to reach the goal!

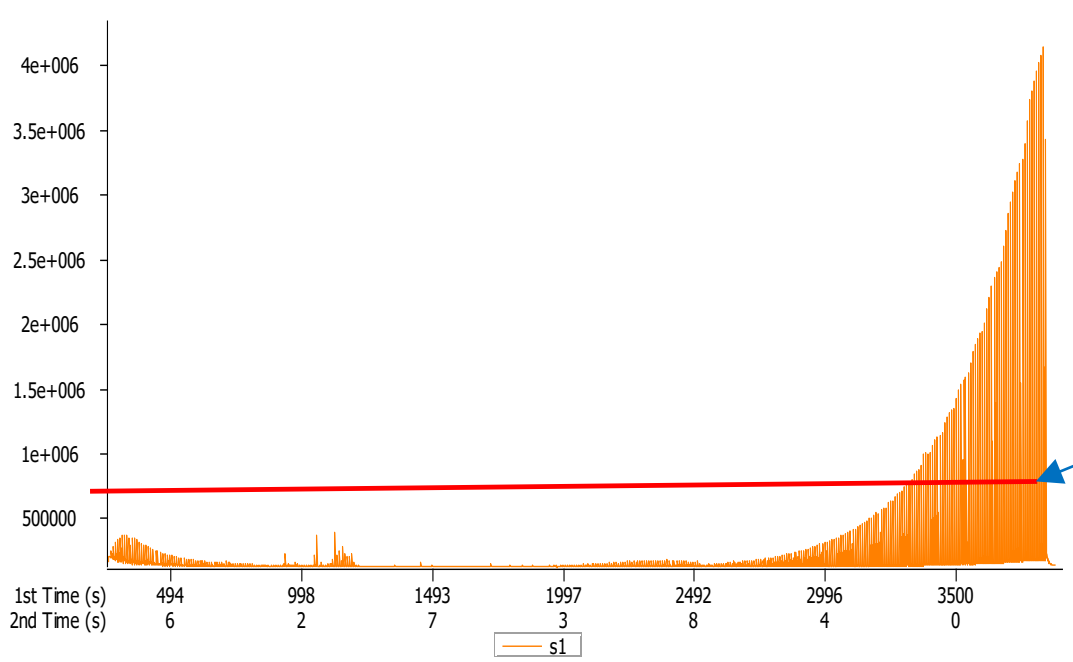
Blank

- humps are detected with non selective detector → blank can lead to incorrect quantification
- is depending on choice of columns
- normal setup is in advantage due to available low bleeding MXT-1 steel capillary columns
- for reverse setup high temperature polar columns often higher column bleeding
- choice has to be carefully made

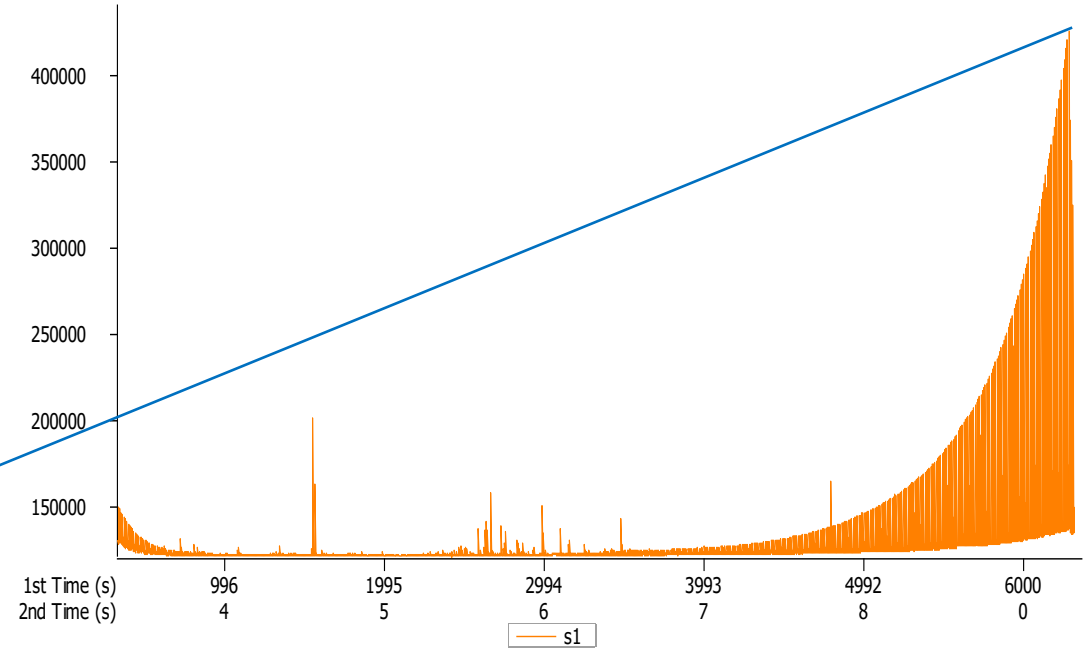
Blank
GCxGC-FID

- Example for two different polar columns in the first dimension:

Column 1

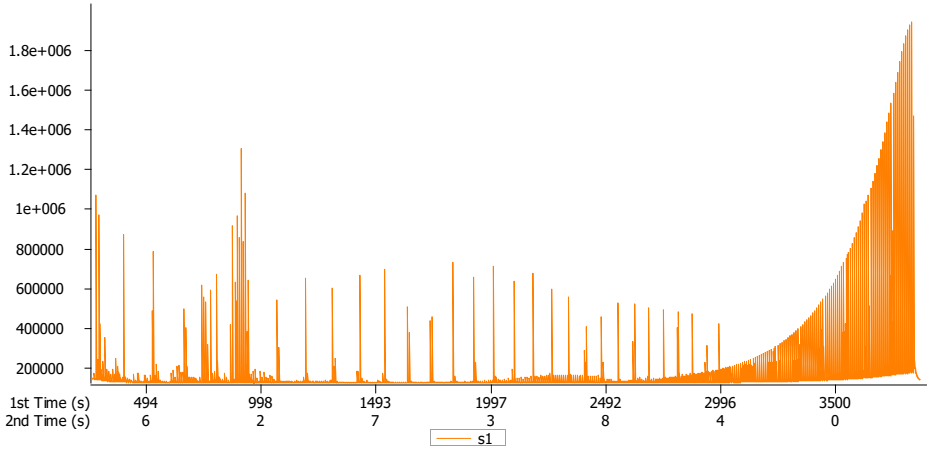


Column 2

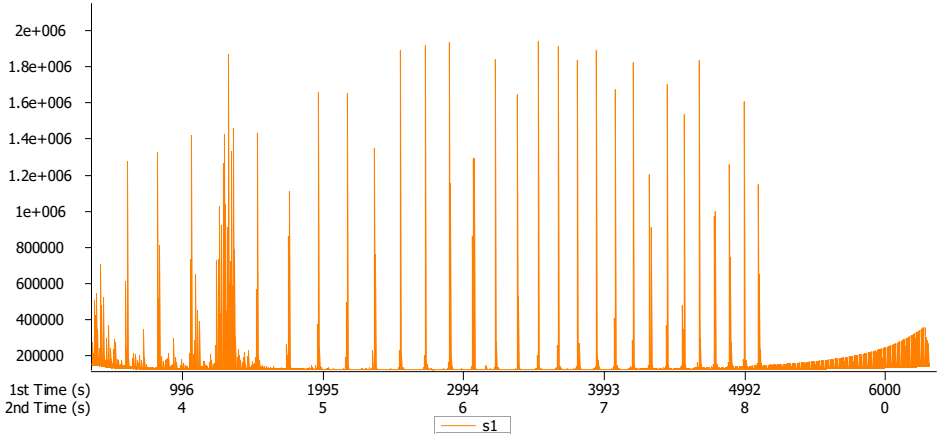


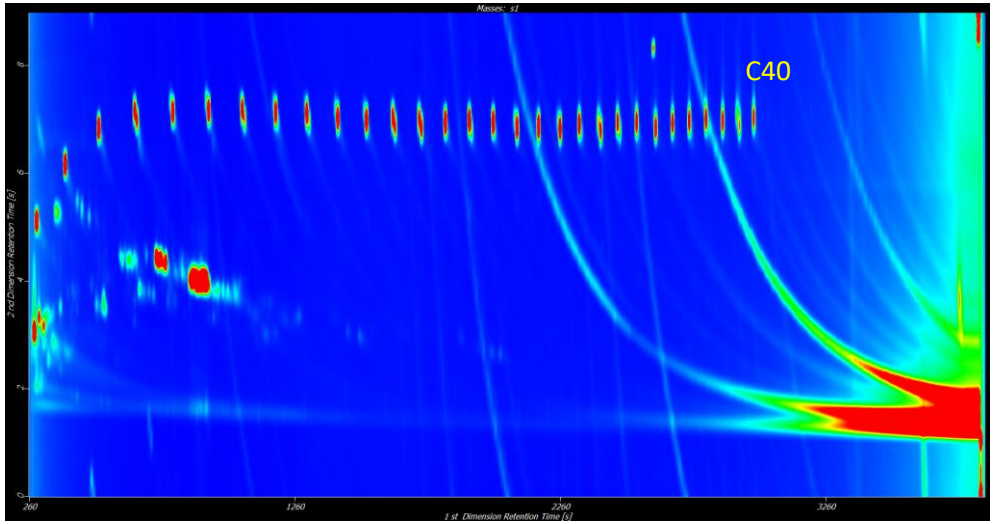
n-C_7 – n-C_{40} Standard
GCxGC-FID

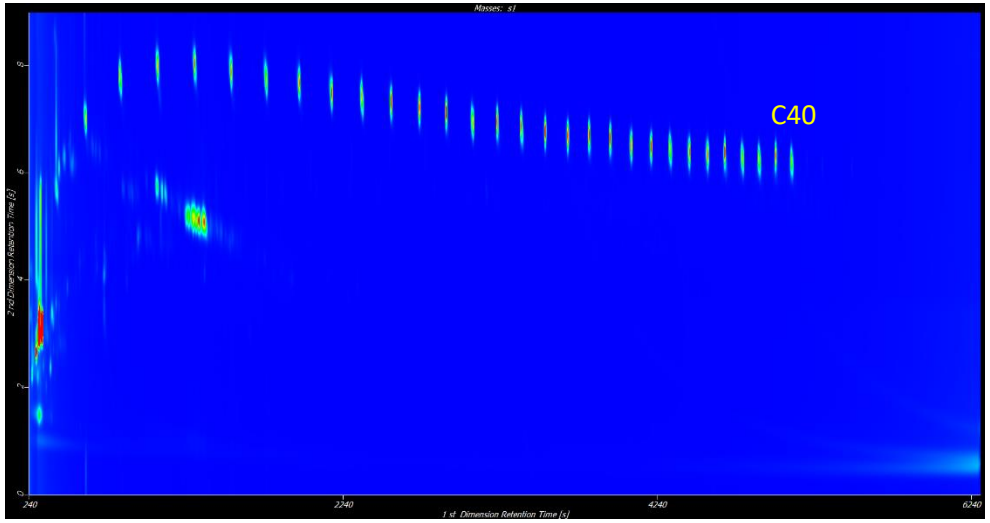
Column 1



Column 2







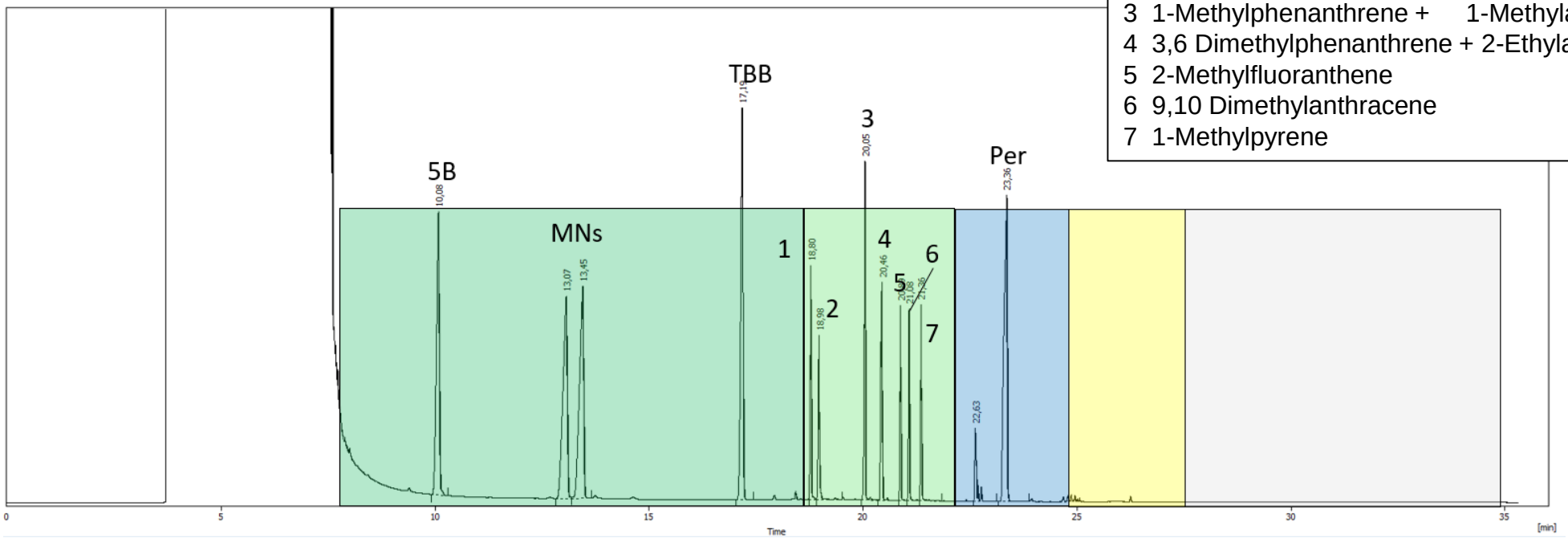
Influence of Polar Column

LC-GC-FID

- **Question:** What is the effect of polar first dimension on first dimension retention time of MOAH?

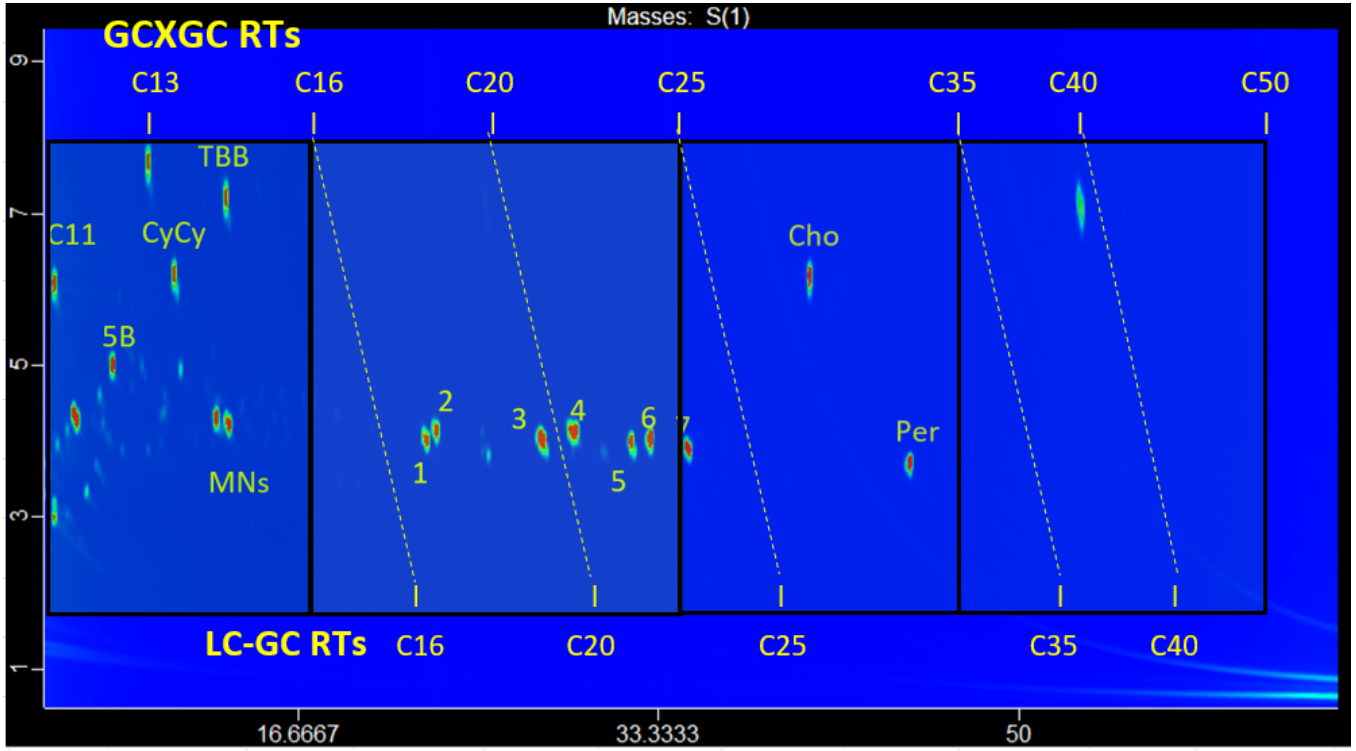
- mix of alkylated aromatic compounds was measured by **LC-GC-FID** to determine MOAH fraction they belong to and relative retention time

1 9,10 Dihydroanthracene
2 1-Methylfluorene
3 1-Methylphenanthrene + 1-Methylanthracene
4 3,6 Dimethylphenanthrene + 2-Ethylanthracene
5 2-Methylfluoranthene
6 9,10 Dimethylanthracene
7 1-Methylpyrene



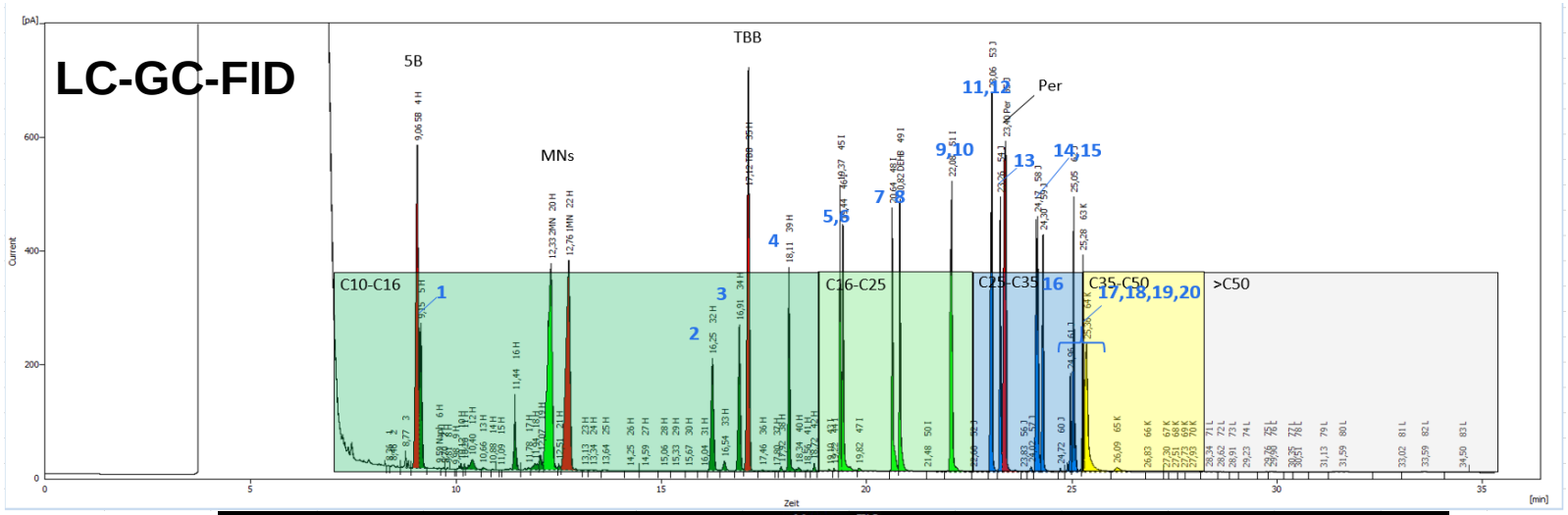
Influence of Polar Column
GCxGC-FID

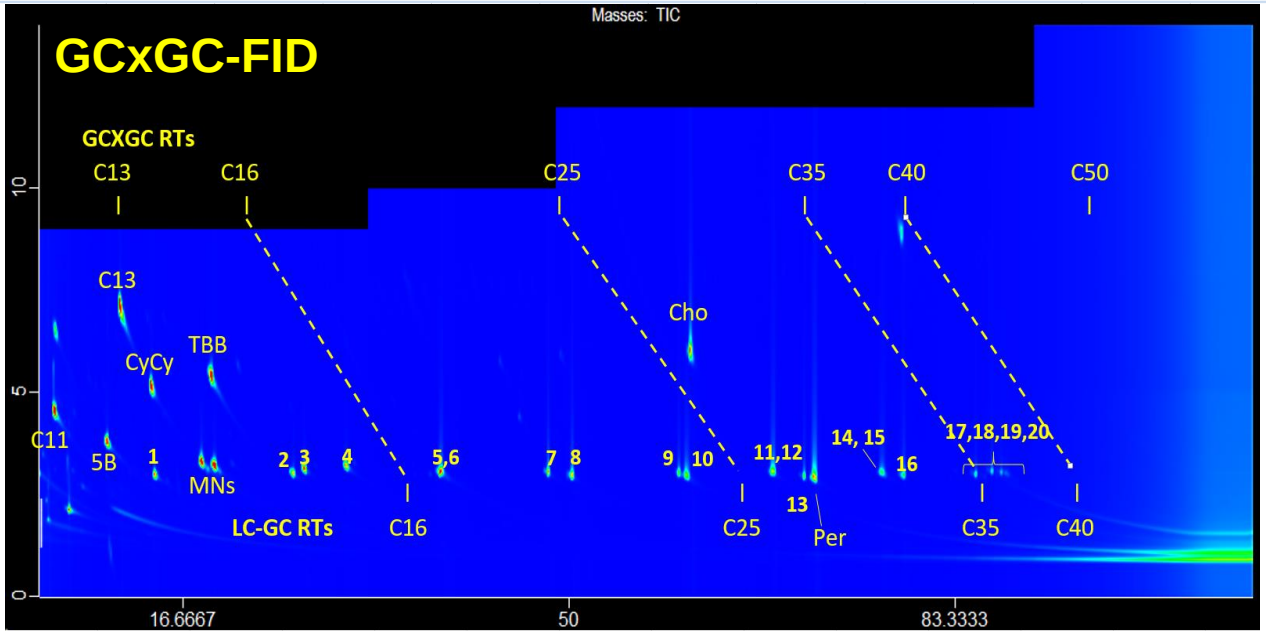
- experiment repeated on **GCxGC-FID**:
- n-alkanes define start of fraction at upper part of contour plot
- shift of polar compounds observed



1 9,10 Dihydroanthracene
2 1-Methylfluorene
3 1-Methylphenanthrene + 1-Methylanthracene
4 3,6 Dimethylphenanthrene + 2-Ethylanthracene
5 2-Methylfluoranthene
6 9,10 Dimethylanthracene
7 1-Methylpyrene

Influence of Polar Column

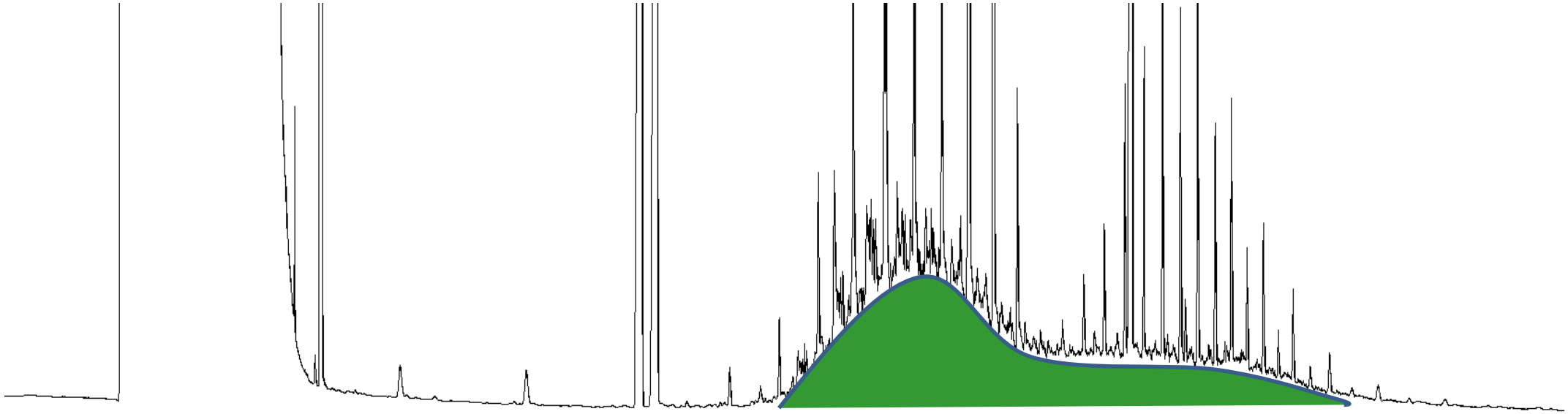




1	Naphthalene
2	Acenaphtylene
3	Acenaphthene
4	Fluorene
5	Phenanthrene
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benzanthracen
10	Chrysen
11	Benzo(b)fluoranthen
12	Benzo(k)fluoranthen
13	Benzo(a)pyren
14	Indeno(1,2,3-cd)pyren
15	Dibenz(a,h)anthracen
16	Benzo(g,h,i)perylen
17	Dibenzo(a,e)pyren
18	Dibenzo(a,i)pyren
19	Dibenzo(a,h)pyren
20	Dibenzo(a,l)pyren

Peak subtraction

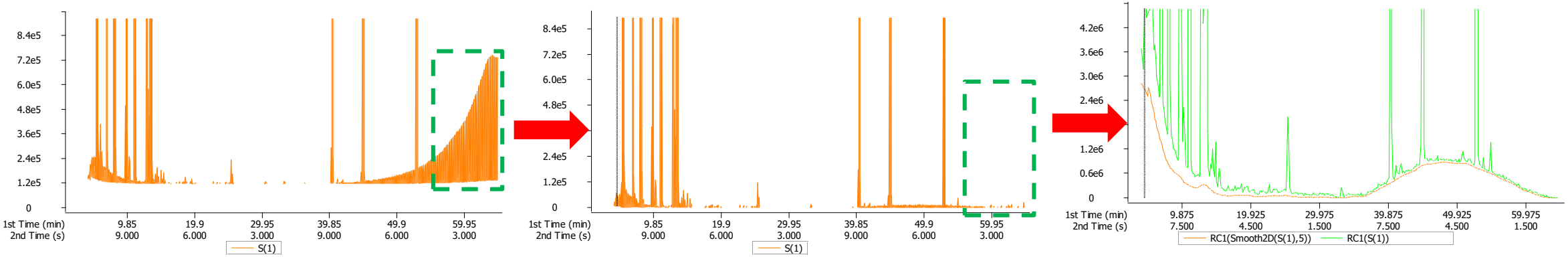
LC-GC-FID



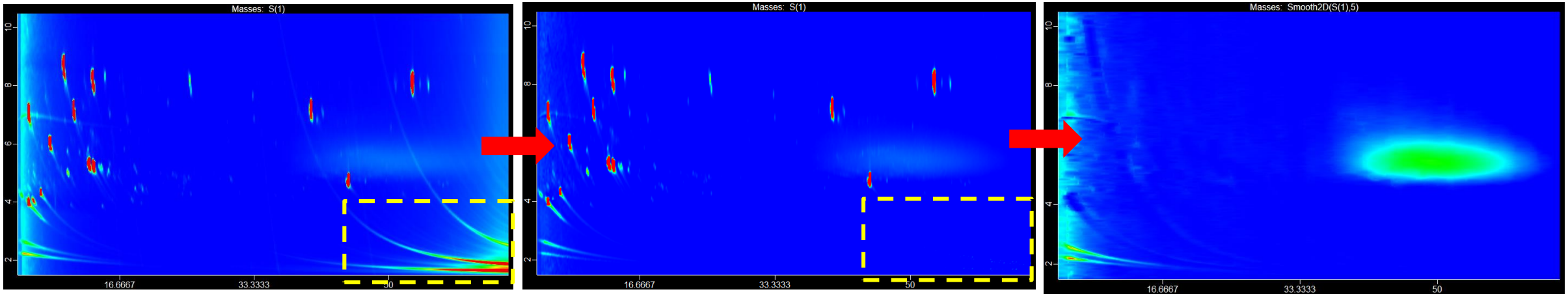
- DIN EN 16995 demands all sharp peaks on top of the hump have to be subtracted for quantification of MOSH and MOAH

Peak subtraction
GCxGC-FID

- Software assisted handling of sharp peaks on to of the hump available



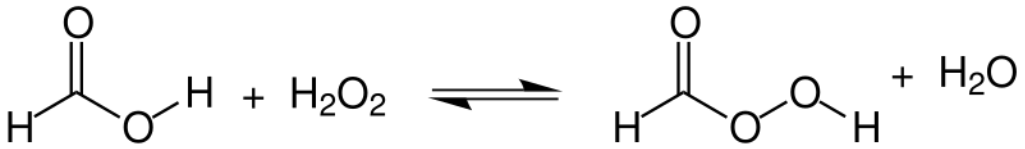
GCxGC-FID contour plot | after blank subtraction | after smoothing



New Epoxidation Technique

- Marco Nestola improved the automated epoxidation using performic acid
- robust technique, applicable in automated or manual mode
- improved removal of biogenic interferences in MOAH fraction compared to current epoxidation methods using m-CPBA (in ethanol or dichloromethane)
- less interferences from epoxidising agent

→ in combination with GCxGC-FID a further tool to lower the LOQ for interfered samples



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New Epoxidation Technique

Strongly Interfered Palm Olein

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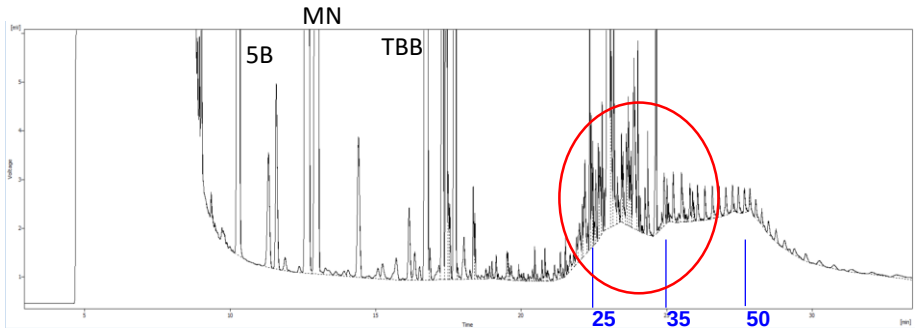
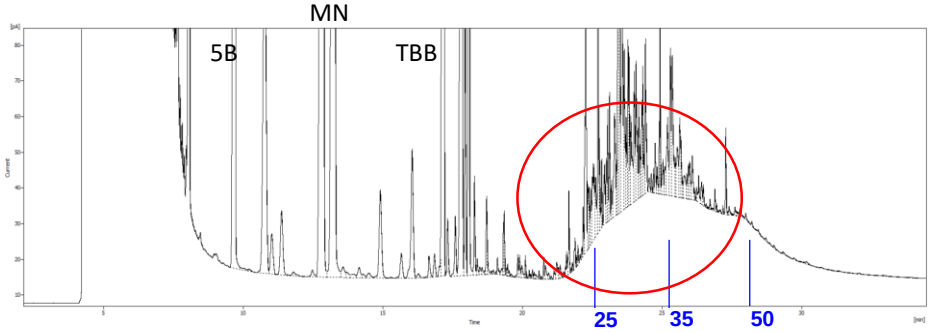
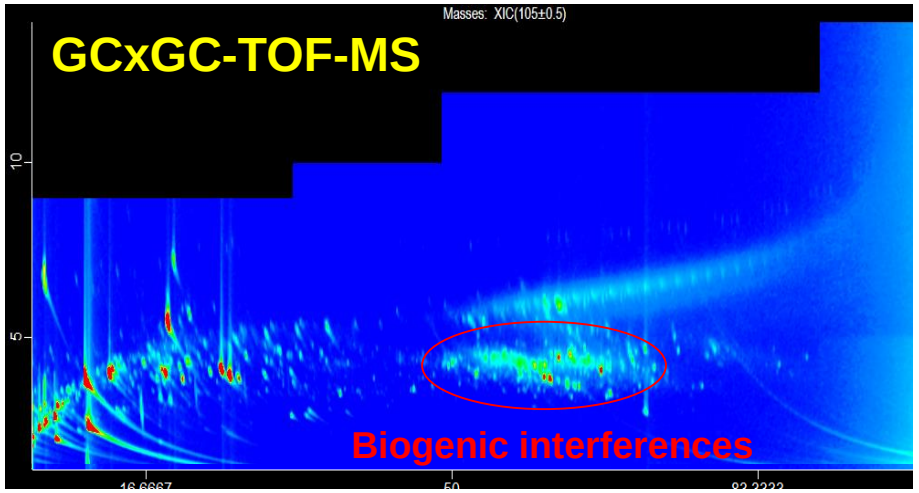
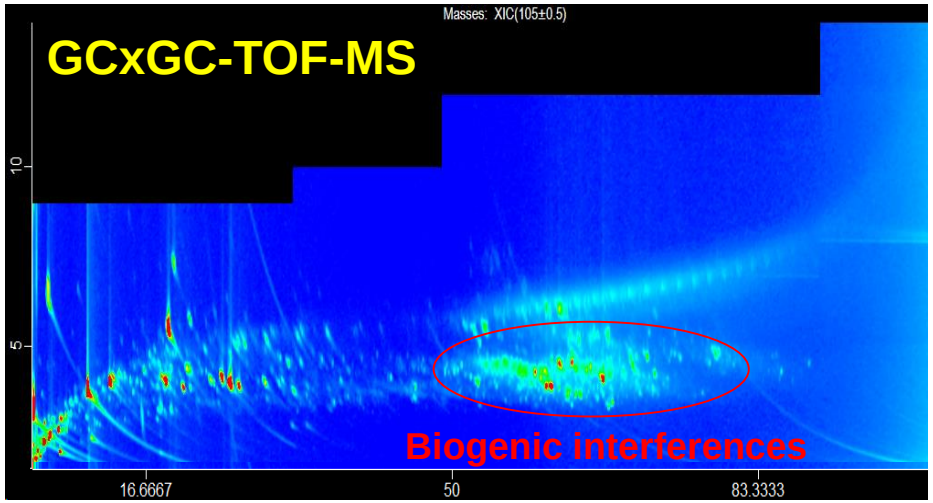
	Epoxidation using performic acid	Epoxidation using m-CPBA
LC-GC-FID		
GCxGC-TOF-MS (m/z 105)		

Conclusion and Outlook

- GCxGC-FID good tool to overcome current problems of LC-GC-FID technique
- applicable especially for **fat/oil samples** and to asses **toxicological relevance** of MOAH fraction
- different techniques to achieve needed sensitivity
- blank can be minimized
- n-alkanes invalid as fraction markers for the complete contour plot (find the right angle)
- discrimination and sharp peaks on the hump can be handled

  (Bauwens, Panto, Purcaro, J Chrom. A 1643 (2021) 462044)

Acknowledgements

Thanks to:

- **Axel Semrau** scientific discussions and cooperation on the new Nestola epoxidation technique
- **LECO** software and technical support
- **Michael Koch (Institut Kirchhoff Berlin part of Mérieux NutriSciences)**
  the work I am honoured to present

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MERIEUX NutriSciences

Knowing
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