Searching and finding unusual fatty acids and compounds of the unsaponifiable matter

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AK Vetter

polyhalogenated compounds

countercurrent chromatography

lipid analysis

halogenated natural producs

methyl-branched fatty acids

furan fatty acids

halogenated flame retardants

unsaponifi-able matter

enantiomer separations

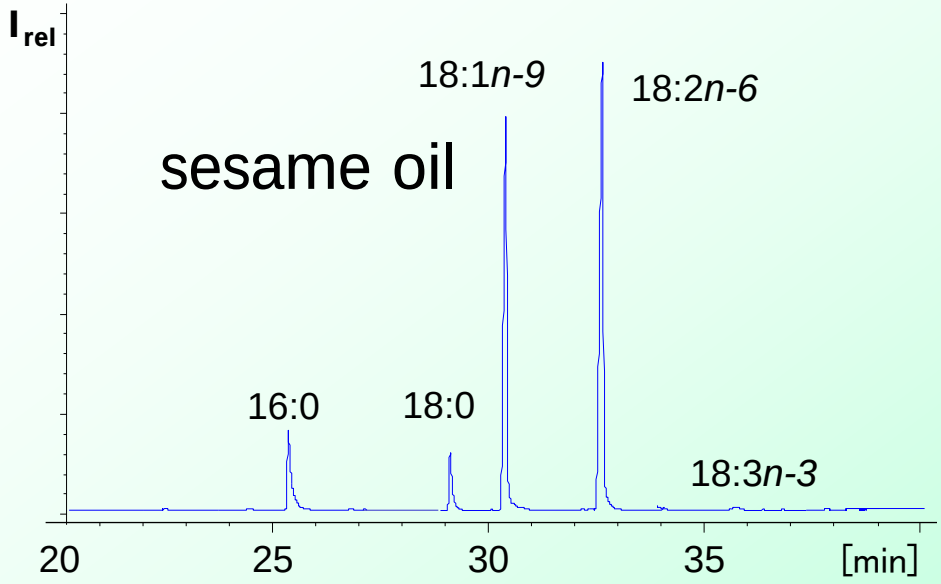
stable isotope mass spectrometry (IRMS)

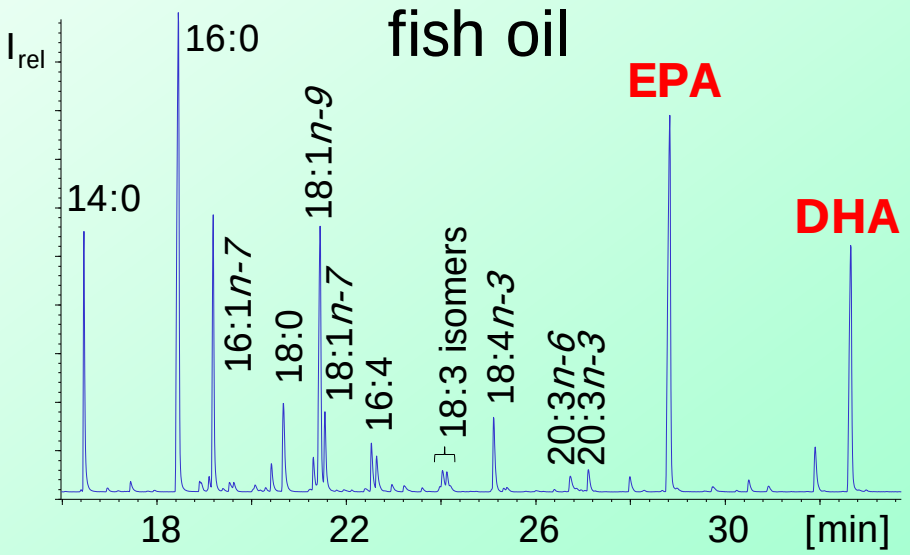
standard compounds

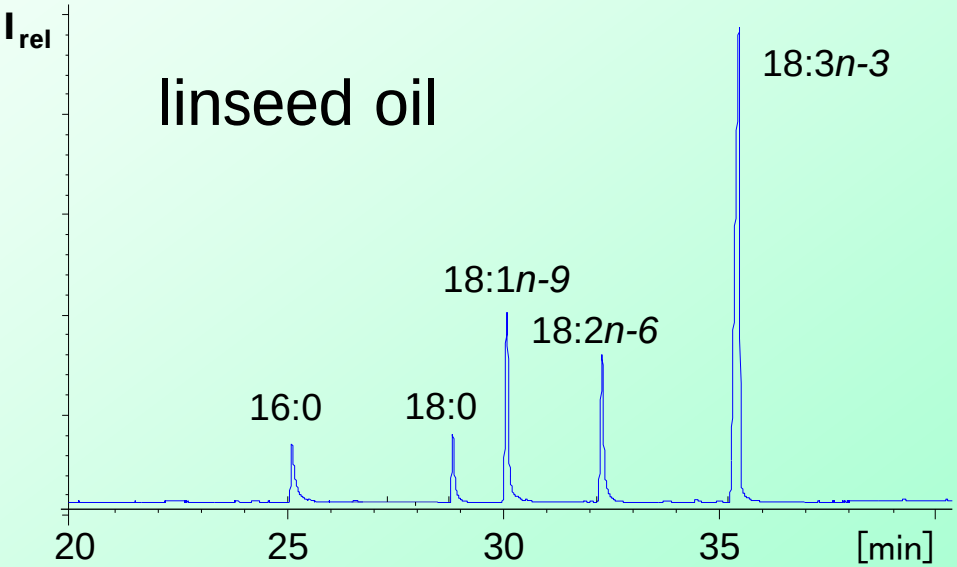
GC/MS

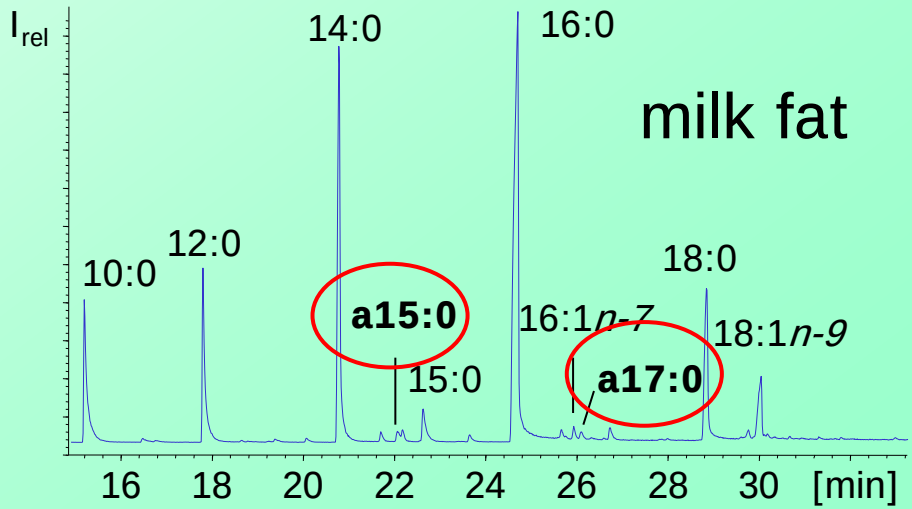
food authenticy

Gas chromatograms of fatty acids (as methyl esters) in food samples



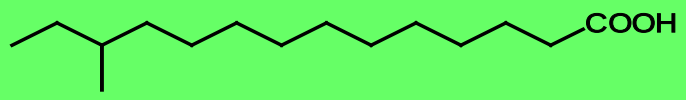






anteiso-fatty acids acids

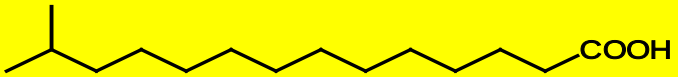
- methyl substitutent at (*n-2*) carbon ⇒ anteiso-fatty acid



12-methyl tetradecanoic acid (a15:0)

- typical chain length: C_{14} and C_{16}, odd carbon number (due to methyl group)
- usually found in ruminants and fish (barely in plants)
- usually occurring together with iso-fatty acids

(methyl-branch on second last carbon)

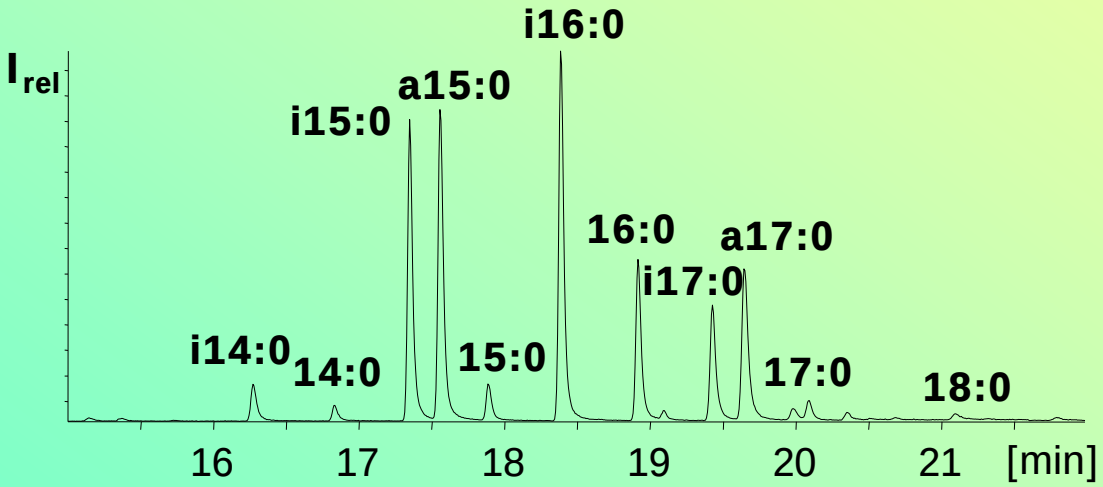


13-methyl tetradecanoic acid (i15:0)

Bacterial lipids

- anteiso- and iso-fatty acids dominate (in gram-positive bacteria)

  ⇒ up to 80% contribution to the total fatty acids



GC / MS analysis of the fatty acids of *Streptomyces avermitilis*

(Thurnhofer & Vetter)

- occurrence of anteiso-fatty acids in food is mostly linked with the presence of bacteria

  ⇒ in gnotobiologic (germ-free) rats only present at traces [1]

[1] Y. Demarne, E. Sacquet, M. J. Lecourtier, J. Flanzy. *Am. J. Clin. Nutr.* 32 (1979) 2027-2032

Stable isotope analysis: GC-IRMS method

- eluate from the GC column led to combustion unit
- organic carbon is transferred into CO_2
- exact determination of the $^{13}C/^{12}C$ ratio is difficult

  $\Rightarrow$ instead, measurement relative to reference standard

$$\delta^{13}C\ [\,‰\,] = \frac{[^{13}C/^{12}C]_{sample} - [^{13}C/^{12}C]_{standard}}{[^{13}C/^{12}C]_{standard}} \times 1000$$

- typical $\delta^{13}C$ values in the range -10 to -40 ‰

Ref.: W. G. Mook WG (ed) (2000) Environmental isotopes in the hydrological cycle, principles and applications, Vol I: Introduction: theory, methods, review. UNESCO/IAEA, Vienna/Paris

$\delta^{13}C$ values [‰] of fatty acids in suet

fatty acid*	$\delta^{13}C$ value [‰]
12:0	-26.6
14:0	-27.2
i15:0	-36.7
a15:0	-35.9
15:0	-30.5
i16:0	-37.6
16:0	-27.1
i17:0	-36.2
a17:0	-35.5

* measured as methyl ester

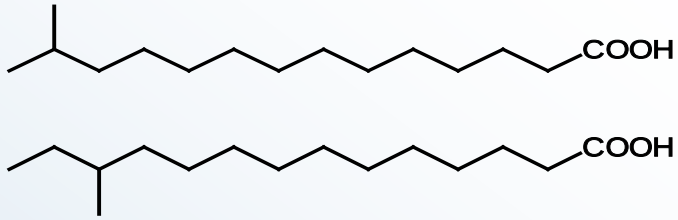
$\delta^{13}C$ values verify:

- methyl-branched fatty acids are depleted in ^{13}C compared to straight-chain fatty acids

$\Rightarrow$ different sources!

Ref.: W. Vetter, S. Gaul, S. Thurnhofer, K. Mayer, *Anal. Bioanal. Chem*. 389 (2007) 597-604

Properties of methyl-branched fatty acids

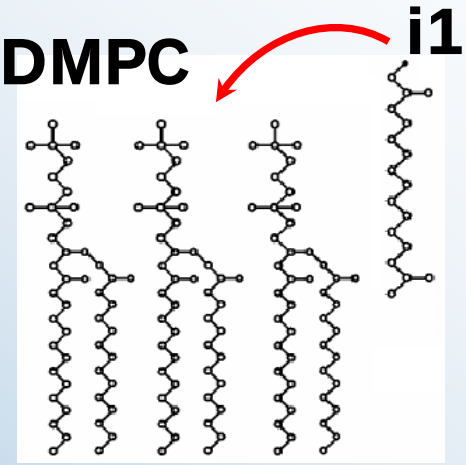


- inertness towards oxidation
- low melting point
- changing physiological properties of lipids

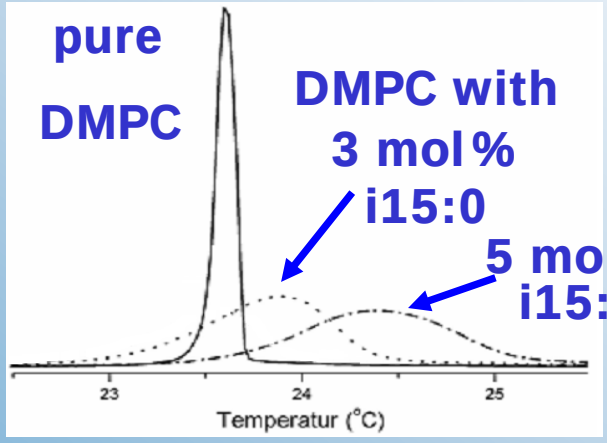
  ⇒ increase of the membrane fluidity

- positive effect on the penetration

  of other compounds into the skin



1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC)



Differential scanning calorimetry (DSC):
DSC thermograms of lipids

Ref.: F. Lindström, S. Thurnhofer, W. Vetter, G. Gröbner, *Phys. Chem. Chem. Phys.* 8 (2006) 4792-4797

A thought during a student lecture

- amino acids – building blocks of proteins – are chiral (typically L-form)

- sugars – building blocks of carbohydrates – are chiral (typically D-form)

- fatty acids – building blocks of lipids – are usually non-chiral

  ⇒ one notable exception: anteiso-fatty acids

Chirality of *anteiso*-fatty acids

- 1950ies: one-time investigation of suet and shark liver oils [1,2]

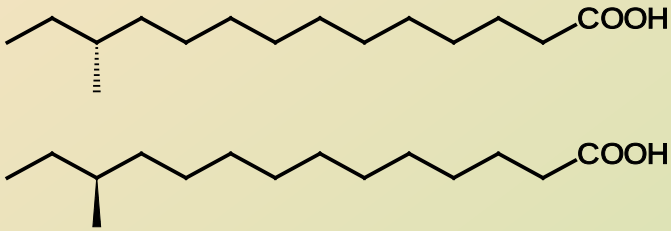
  ⇒ ~ 10 kg fat/oil converted into methyl esters

  ⇒ fractionation via distillation

  ⇒ repeated crystallization

  ⇒ x-ray analysis, optical rotation

S-a15:0



R-a15:0

enantiopure standards
not commercially available

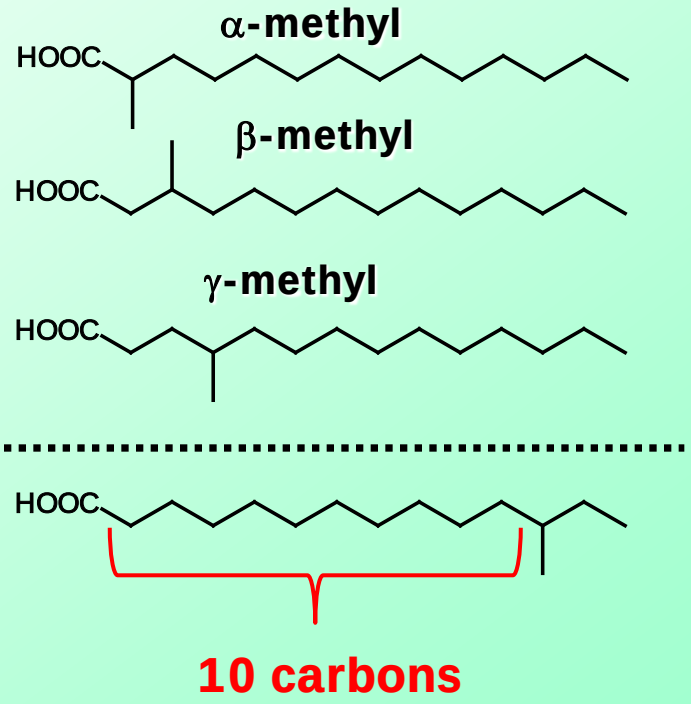
result:

- all samples exclusively featured the (+)-enantiomer

Ref.: [1] R. P. Hansen, F. B. Shorland, N. J. Cooke, *Biochem. J.* 52 (1952) 203-207
[2] I. M. Morice, F. B. Shorland, *Biochem. J.* 64 (1956) 461-464

GC enantiomer separation of anteiso-fatty acids

- not reported when we started
- only of α-, β- and γ-methyl-substituted acids resolved [1][2]
- the more remote the methyl group from the head group, the more difficult the enantiomer separation is [1][2]



[1] V. Karl, J. Gutser, A. Dietrich, B. Maas, A. Mosandl, *Chirality* 6 (1994) 427-434
[2] R. Stritzel, B. Dobner, F. Bringezu, P. Nuhn, J. *High Res. Chrom.* 19 (1996) 121-123

Small problems had to be solved…

- **synthesis of enantiopure standards [1]**

  ⇒ Wittig reaction (olefination of chiral aldehydes via ylides)

  ⇒ hydrogenation of the resulting double bond

  1 year work

- **searching for a chiral stationary GC phase [2]**

  ⇒ testing of ~20 chiral stationary phases

  ⇒ improvement of the only promising one

  1 year work

- **development of sensitive and selective method [3]**

  ⇒ development of a GC/MS-SIM method

  ⇒ enrichment/isolation of anteiso-fatty acids by hydrogenation, urea complexation and/or (Ag^+) HPLC fractionation

  1 year work

Ref.: [1] S. Thurnhofer, W. Vetter, *Tetrahedron* 63 (2007) 1140-1145

[2] S. Thurnhofer, G. Hottinger, W. Vetter, *Anal. Chem*. 79 (2007) 4696-4701

[3] S. Thurnhofer, W. Vetter, *J. Agric. Food Chem*. 53 (2005) 8896-8903

Fatty acid analysis

- important routine task in food science (and life sciences)
- classic method using GC/FID after formation of fatty acid methyl esters (**FAME**)

  ⇒ peak abundance correlates with amount

  ⇒ determination of relative contributions (“100% method“)

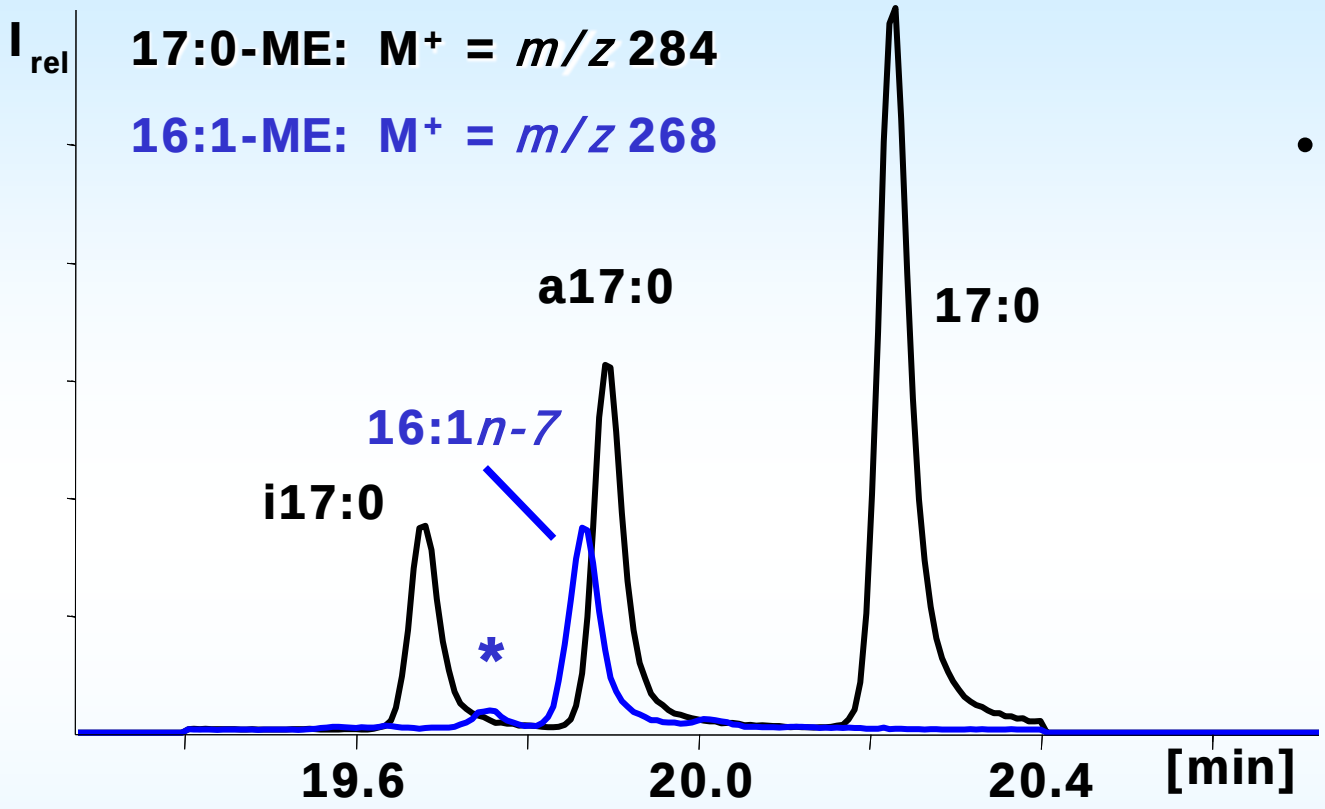
disadvantages of GC/FID

- low selectivity
- co-elutions may be overlooked
- problems with low abundant fatty acids

GC: gas chroma-tography

FID: flame ionisation detector

Coelution of monoenoic and *anteiso*-fatty acids



- co-elutions can hardly be omitted on 50 m columns

GC / MS chromatogram of a milk fat sample

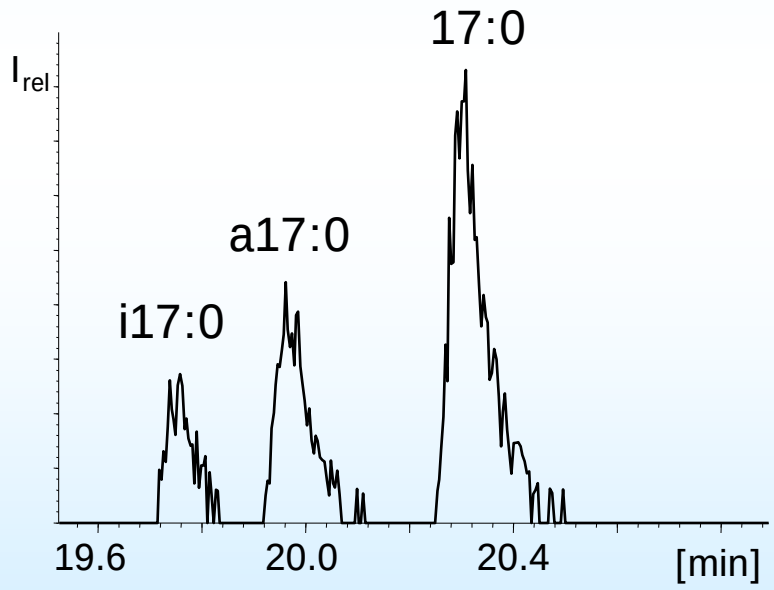
(GC column: 50 m x 0.25 mm i.d. x 0.2 µm 100% cyanopropyl polysiloxane)

Why GC/MS in SIM mode?

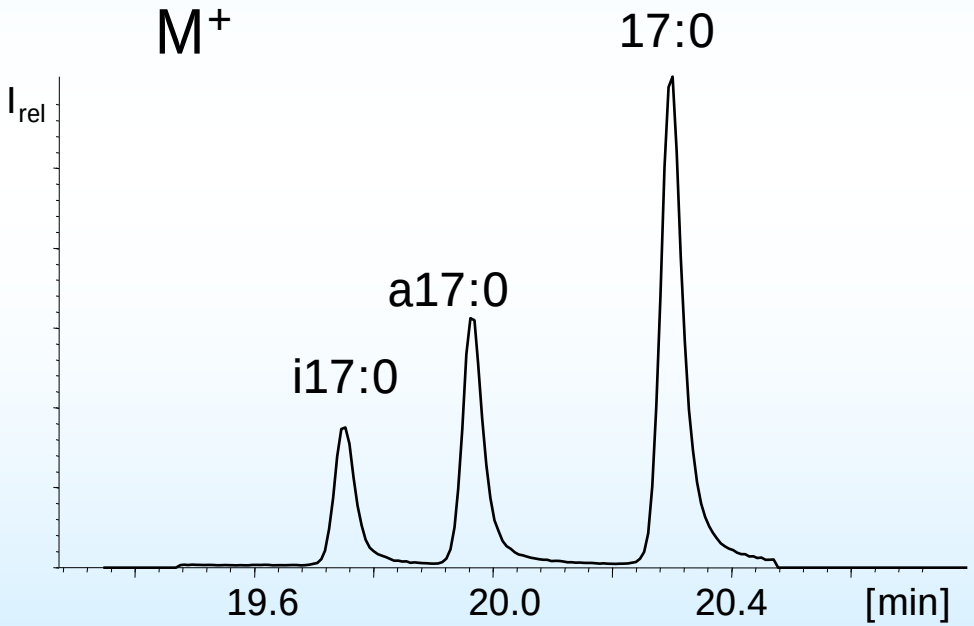
(selected ion monitoring (SIM))

- more sensitive and selective than full scan

m/z 284 extracted from full scan



m/z 284 measured in SIM mode



GC column: 50 m x 0.25 mm i.d. x 0.2 µm 100% cyanopropyl polysiloxane

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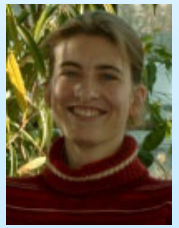
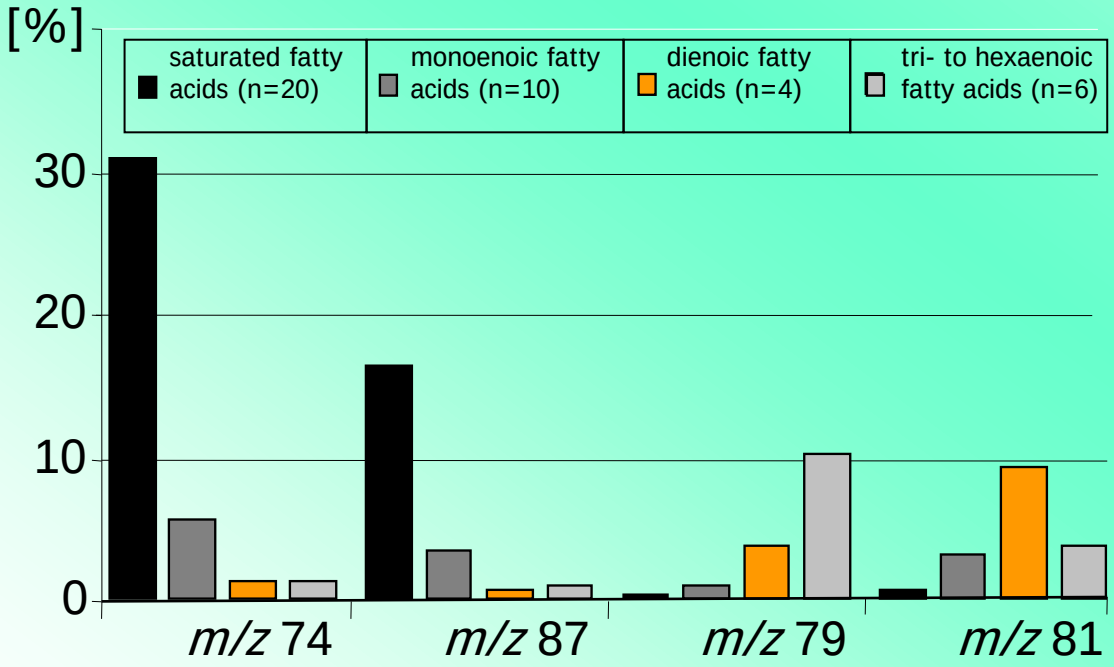
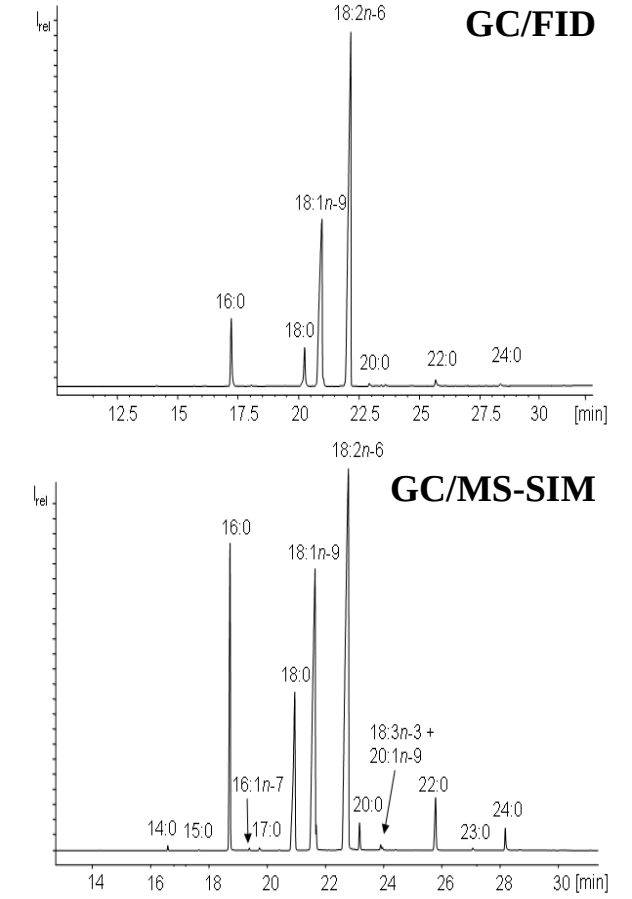


Determination of fatty acid methyl esters by GC/MS-SIM

- sensitivity and selectivity





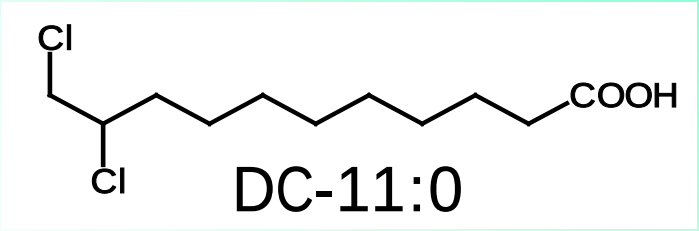
Ref:. W. Vetter, S. Thurnhofer,, *Lipid Technol*. 19 (2007) 184-186

Quantitative determination of individual fatty acids

- quantification requires use of internal standards (IS) not present in the sample

(1) IS for sample cleanup

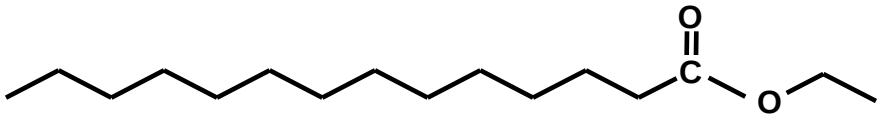
(addition before/after the extraction)



DC-11:0

(2) syringe standard

(addition to GC/MS solution)



14:0-EE

Ref:. S. Thurnhofer, K. Lehnert, W. Vetter, *Eur. Food Res. Technol*. 226 (2008) 975-983

House method

lipid extraction using

accelerated solvent extraction (ASE) for dry samples

or

microwave-assisted extraction (MAE) of aqueous samples

IS, DC-11:0

transesterification

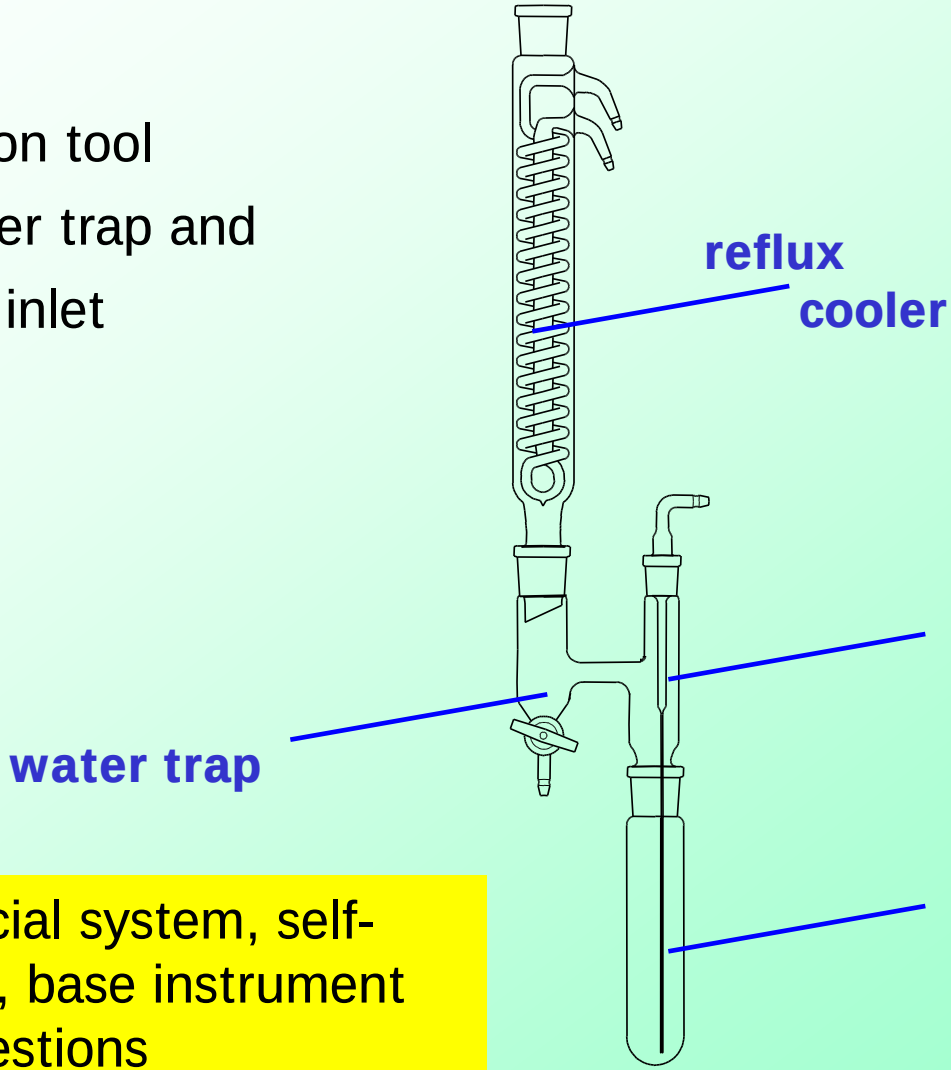
IS, ethyl ester

GC/MS-SIM analysis

Ref:. S. Thurnhofer, K. Lehnert, W. Vetter, *Eur. Food Res. Technol.* 226 (2008) 975-983

Microwave-assisted extraction (focused-open vessel; FOV-MAE)

- connection tool with water trap and nitrogen inlet



no commercial system, self-constructed, base instrument for acid digestions

Concentrations [g/100 g fat] of methyl-branched fatty acids in food

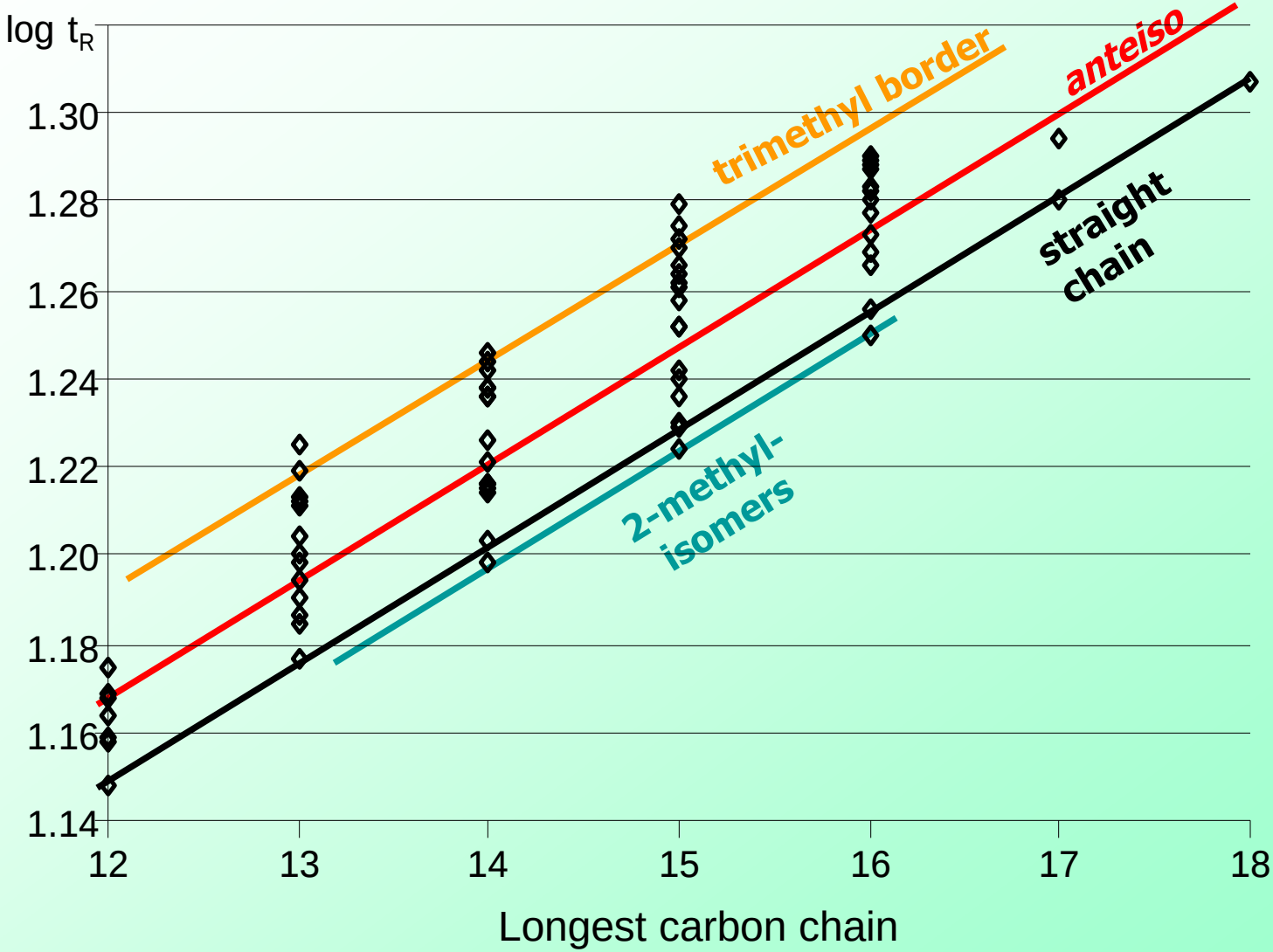
FAME (n = 3)	mozzarella (cow) [g/100 g]	feta (cow) [g/100 g]	feta cheese [g/100 g]	human milk [g/100 g]
i14:0	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.02 ± 0.00
i15:0	0.20 ± 0.01	0.29 ± 0.01	0.21 ± 0.01	0.06 ± 0.00
a15:0	0.30 ± 0.01	0.38 ± 0.01	0.34 ± 0.03	0.08 ± 0.00
i16:0	0.12 ± 0.00	0.09 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
i17:0	0.47 ± 0.01	0.56 ± 0.02	0.37 ± 0.02	0.11 ± 0.02
a17:0	0.39 ± 0.01	0.42 ± 0.02	0.37 ± 0.01	0.14 ± 0.00

- only these fatty acids were quantified using the corresponding ethyl esters as IS

Ref:. S. Thurnhofer, K. Lehnert, W. Vetter, *Eur. Food Res. Technol.* 226 (2008) 975-983

Birth goo (*Vernix caseosa*)

- detection of ~60 methyl-branched fatty acids (although germ-free)

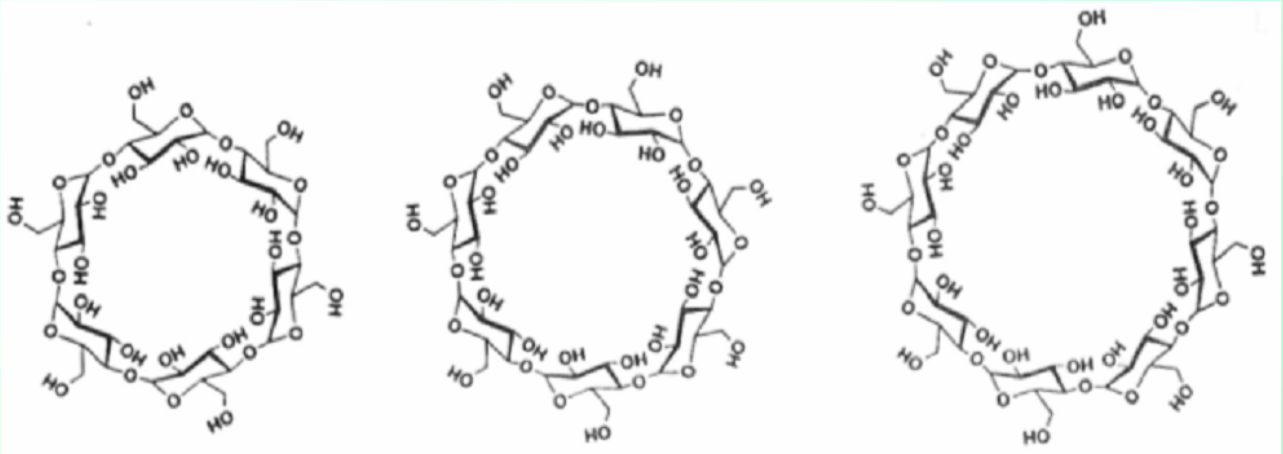


The AOCS Lipid Library is of exceptional help, it´s our first aid kit when there are problems

<u>Ref.</u>: S. Hauff, W. Vetter, *J. Chromatogr. A* 1217 (2010) 8270—8278

Direct GC enantiomer separation of anteiso-fatty acids

- application of modified cyclodextrins



α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
(6 glucose units)	(7 glucose units)	(8 glucose units)

- *O*-derivatisation at C-2, C-3 and C-6 provides a range of modified cyclodextrins suitable as chiral stationary GC phases

6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin (β-TBDM)

- tests of >20 chiral stationary phases only partly successful with β-TBDM

$\Rightarrow$ improvement of the initial phase
(collaboration with G. Hottinger, BGB-Analytik)

10% β-TBDM
thin film

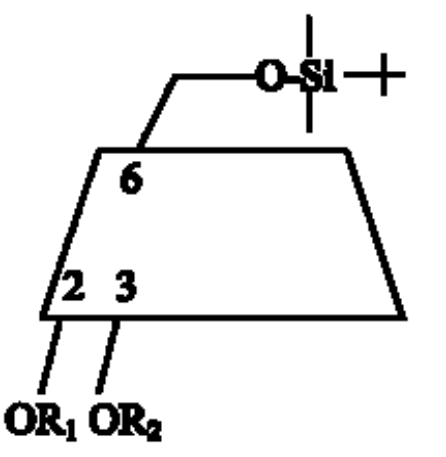
↓

- lowers elutions temperature
- resolution decreases

50% β-TBDM
standard film

↓

- better interaction
- better resolution



β-TBDM:

$R_1 = R_2$ = methyl

Ref: S. Thurnhofer, G. Hottinger, W. Vetter, *Anal. Chem*. 79 (2007) 4696-4701

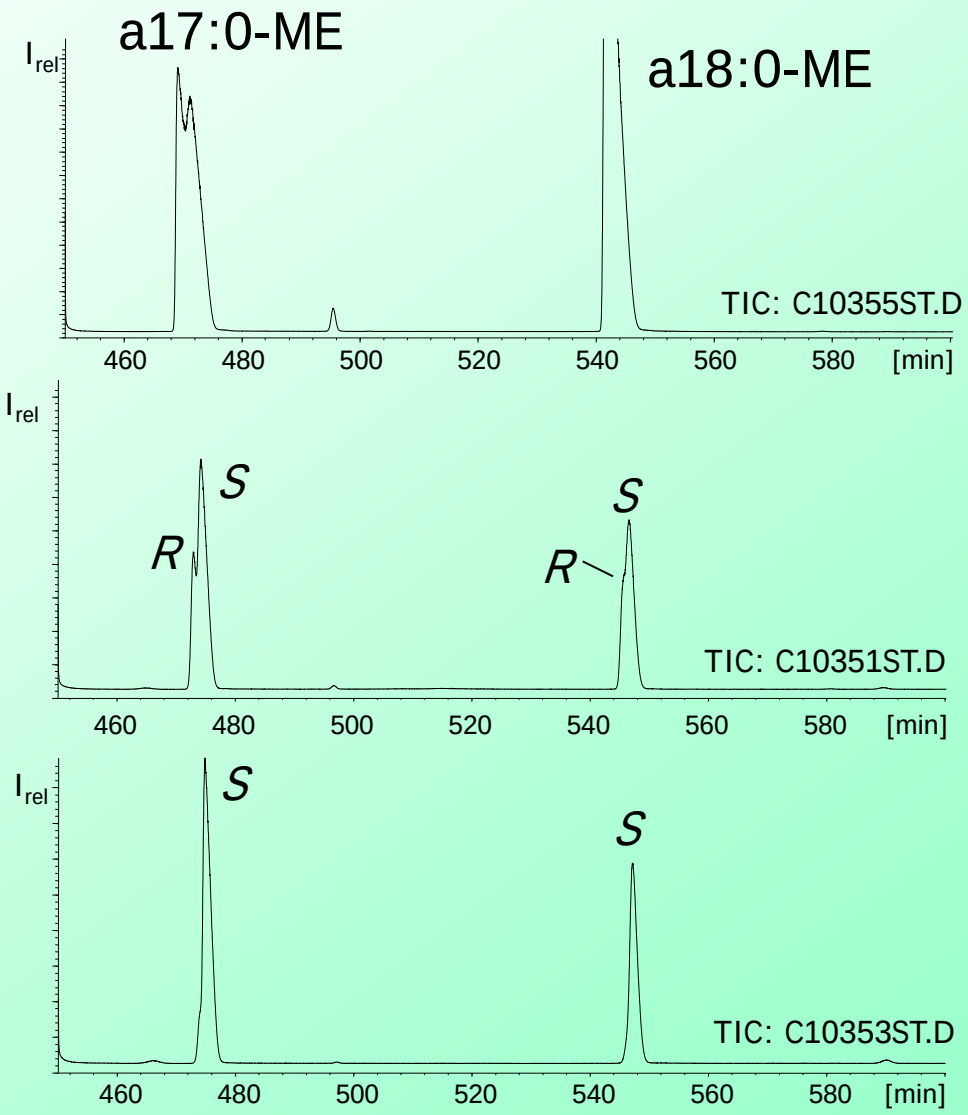
Enantioselective determination of a17:0 on β-TBDM

racemates partly resolved

racemates spiked with *S*-enantiomer
elution order: *R* < *S*

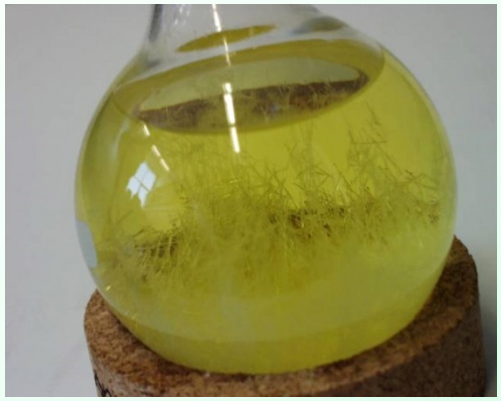
pure *S*-enantiomer

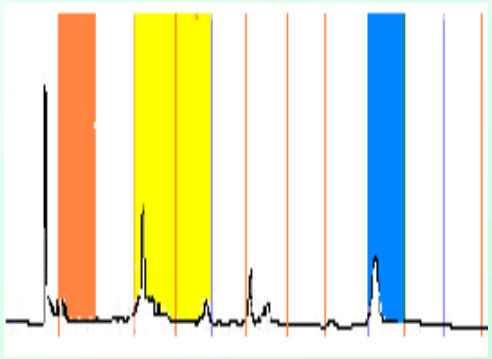
⇒ required GC run time 8–10 h elution time

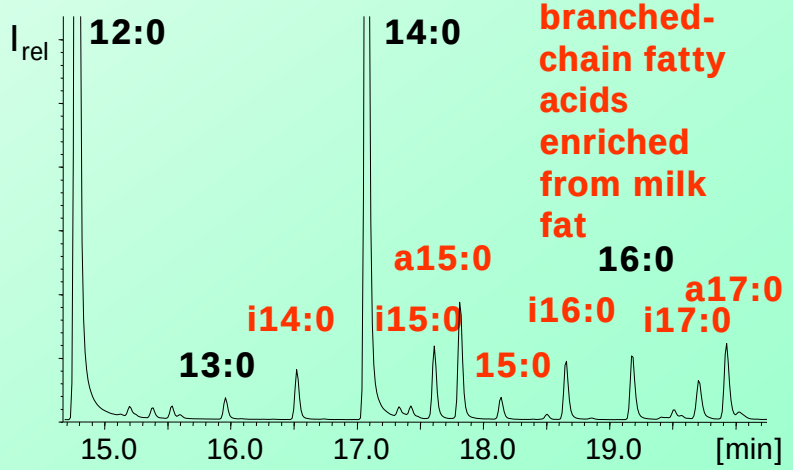


Ref: S. Thurnhofer, G. Hottinger, W. Vetter, *Anal. Chem.* 79 (2007) 4696-4701

Sample preparing for enantiomer separation: (1) urea complexation (2) silver ion chromatography







urea complexation

⇒ separation of major saturated fatty acids

Ag^+-HPLC

⇒ separation of unsaturated fatty acids

Ref: S. Thurnhofer, G. Hottinger, W. Vetter, *Anal. Chem.* 79 (2007) 4696-4701

Results: Chirality of anteiso-fatty acids

- anteiso-fatty acids are predominantly *S*-configurated in milk fat and fish oil

  (S. Thurnhofer, G. Hottinger, W. Vetter, *Anal. Chem*. 79 (2007) 4696-4701)

- yet, up to 10% *R*-anteiso-fatty acids detected in milk fat and fish

- higher share of *R*-anteiso fatty acids in polar lipids

  (S. Hauff, G. Hottinger, W. Vetter, *Lipids* (2010) 357-365

- *R*-anteiso-fatty acids cannot be synthesized by the classical biosynthesis via isoleucine as the primer

  $\Rightarrow$ a hitherto mostly unknown biosynthesis pathway must exist

  (D. Eibler, H. Abdurahman, T, Ruoff, S. Kaffarnik, H. Steingass, W. Vetter, PLOS One 12 (2017) e0170788)

Results: Chirality of anteiso-fatty acids

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- higher shar…
  (S. Hauff, C…
- *R*-anteis… …al biosynthesis …
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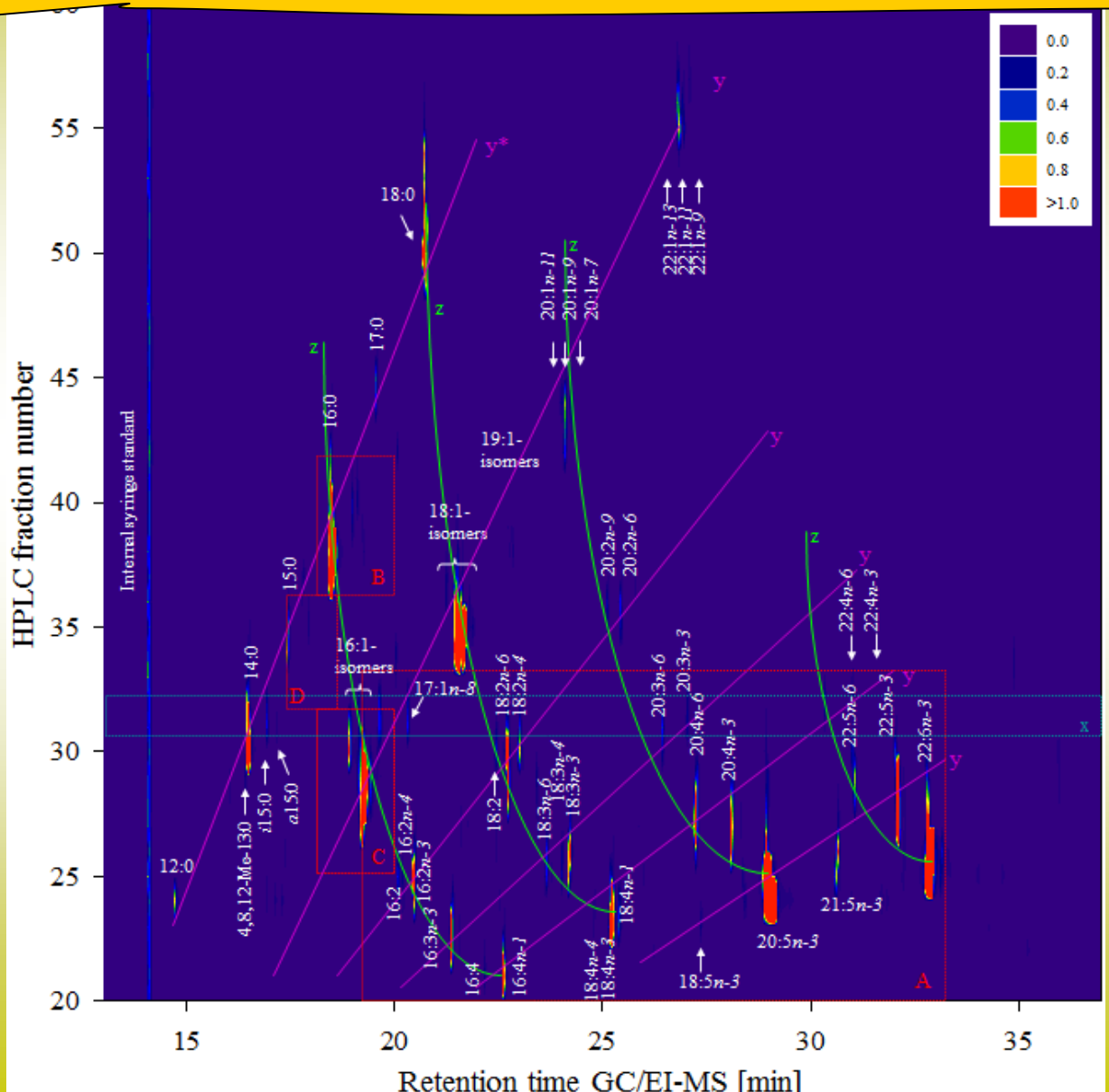
  (D. Eibler, H. Abdurahman, T, Ruoff, S. Kanan… …eingass, W. Vetter, PLOS One 12 (2017) e0170788)

- surprisingly, 4-alkylbranched fatty acids (principal flavor compounds of goat and sheep (4-Me-8:0 and 4-Et-8:0) were recently shown to be *R*-enantiopure
- enantiopure 4-alkylbranched standards were produced via repeated enantioselective esterification

Ref.: D. Eibler, W. Vetter, *J. Chromatogr. A* 1505 (2017) 87-95

4-alkyl: *R*-enantiomers
anteisos: *S*-enantiomers

Two dimensional HPLC/GC chromatogram of fish oil fatty acids (as methyl esters)



- excel-programmed 2D evaluation (T. Kapp, W. Vetter, *J. Chromatogr. A* 1216 (2009) 8391-8397)
- similar approach as GCxGC

disadvantage

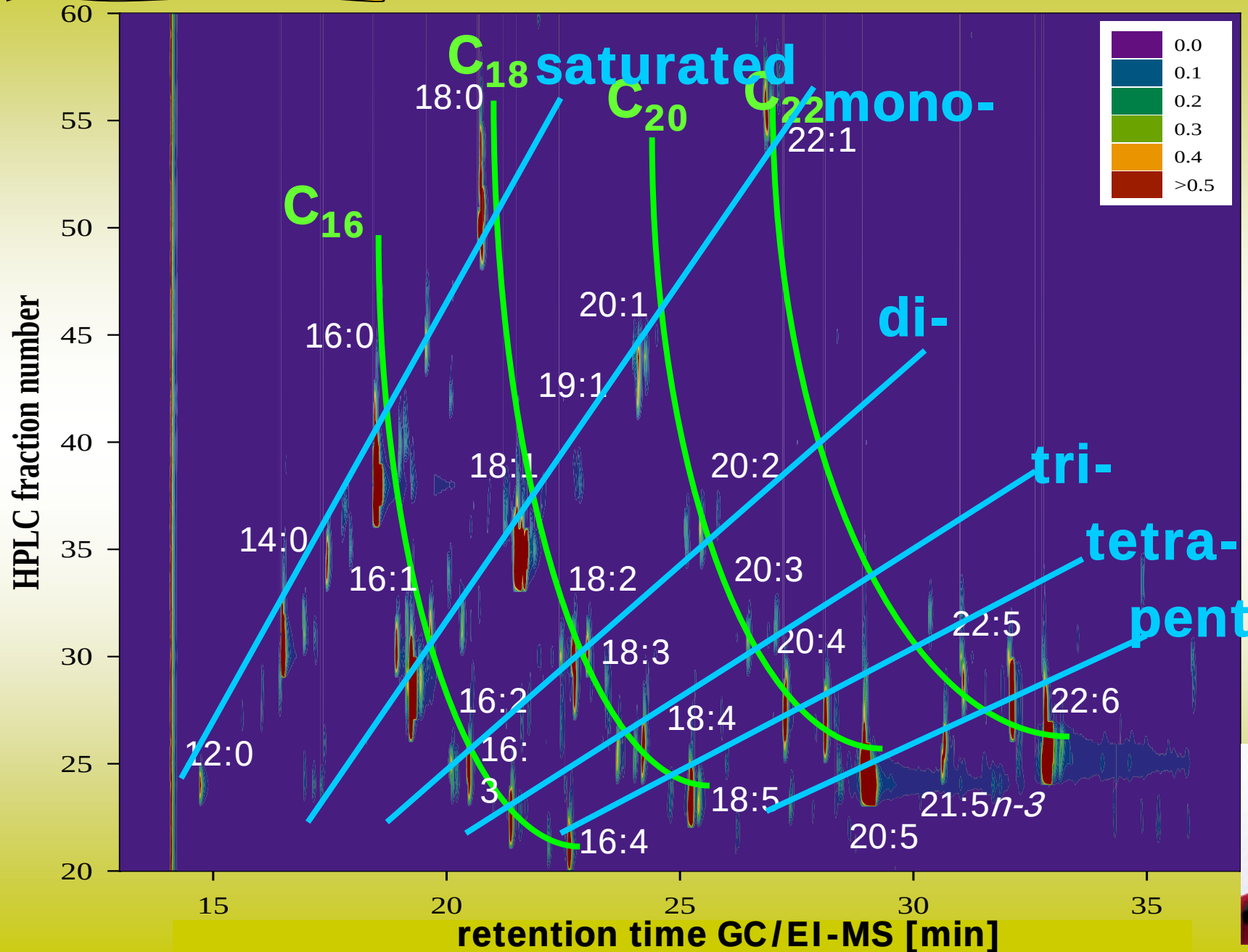
- time consuming (~2 days per sample)

advantage

- good orthogonality
- higher amounts (post analysis possible)

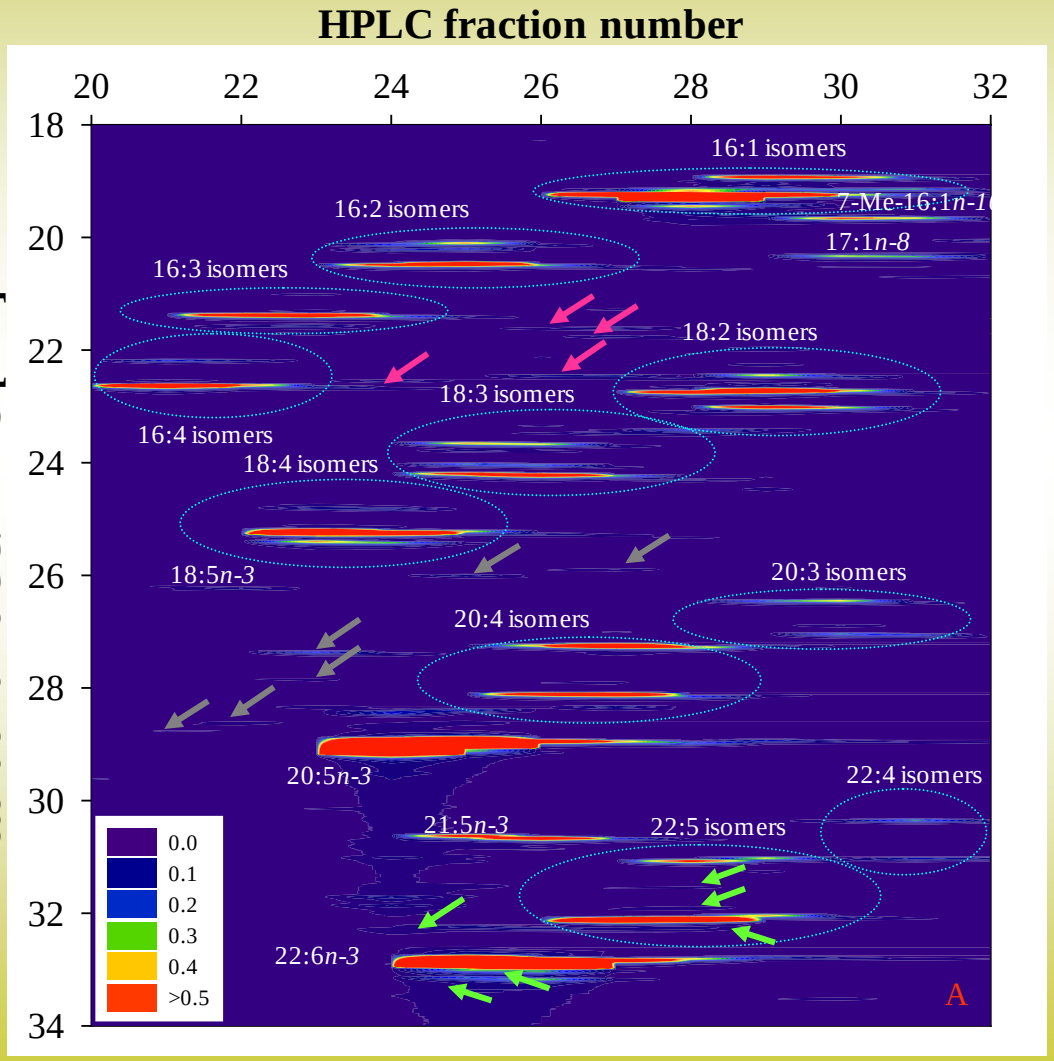
Ref.: S. Hauff, W. Vetter, *Anal. Bioanal. Chem*. 396 (2010) 2695-2707

HPLC/GC - 2D Konturplot



Ref.: S. Hauff, W. Vetter, *Anal. Bioanal. Chem.* 396 (2010) 2695-2707

PUFAs in the HPLC/GC plot of a fish oil

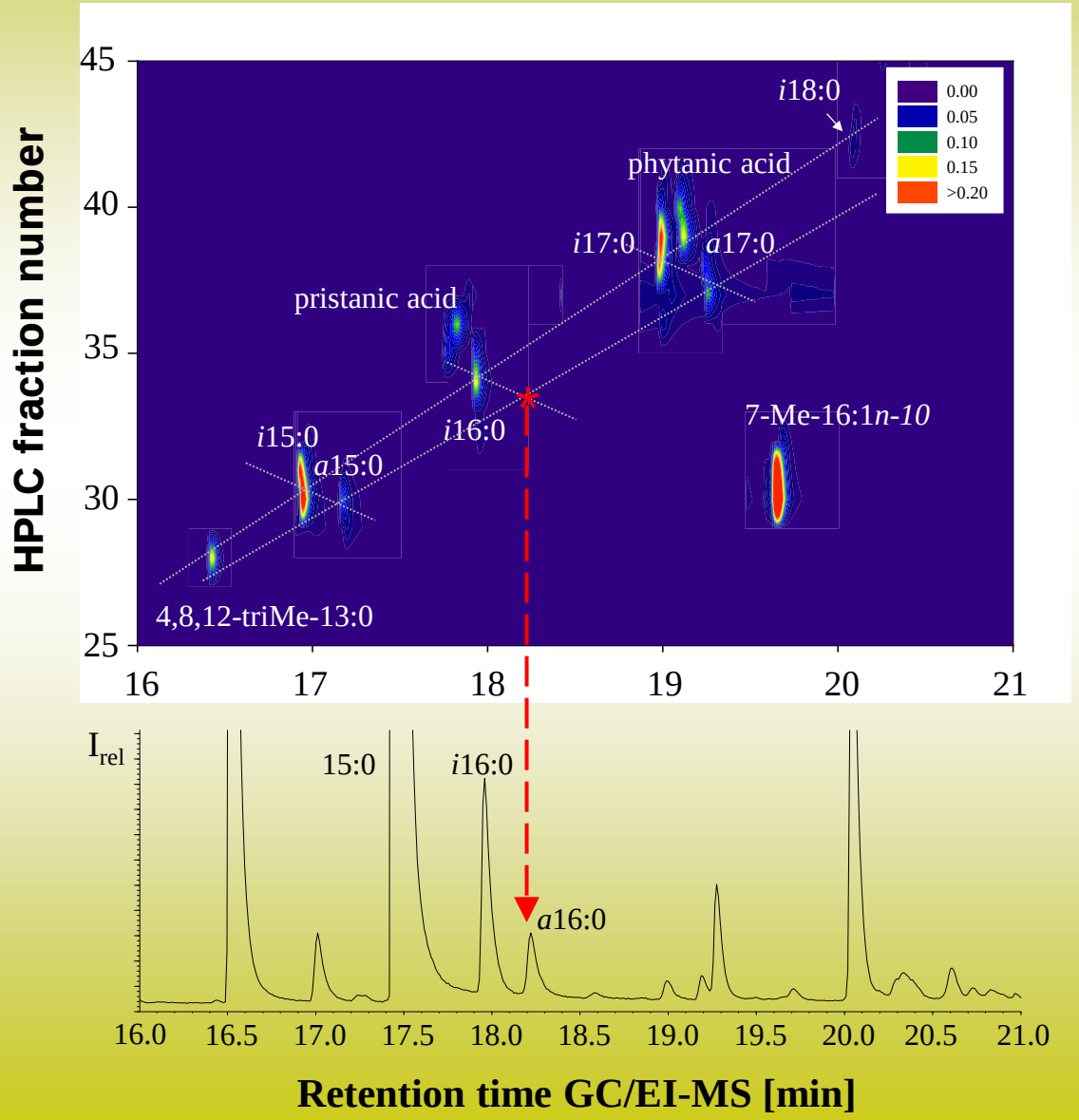


- many, many PUFAs

- non-aqueous RP-HPLC with three C_{18} columns

Ref.: S. Hauff, W. Vetter, *Anal. Bioanal. Chem.* 396 (2010) 2695-2707

HPLC/GC plots of branched chain fatty acids



- HPLC fractionation allowed to detect traces of a16:0
- most likely produced by α-oxidation of a17:0

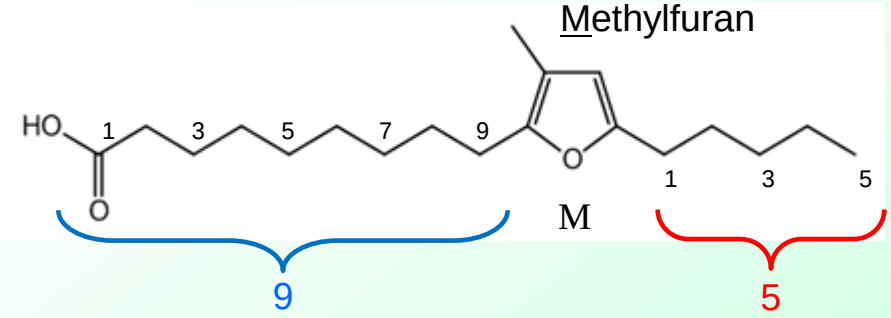
Valuable minor fatty acids in milk

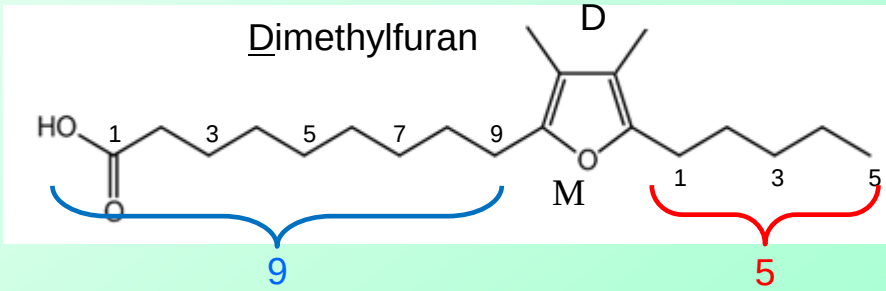
parameter	organic milk*
phytanic acid	+
PUFA (ALA, EPA)	+
CLA	+
furan fatty acids	+

“+“ higher content in organic milk than in conventional milk due to green feed

Features of furan fatty acids

- structural feature: furan moiety in the carbon chain
- substituted with one (“M“) or two (“D“) methyl groups





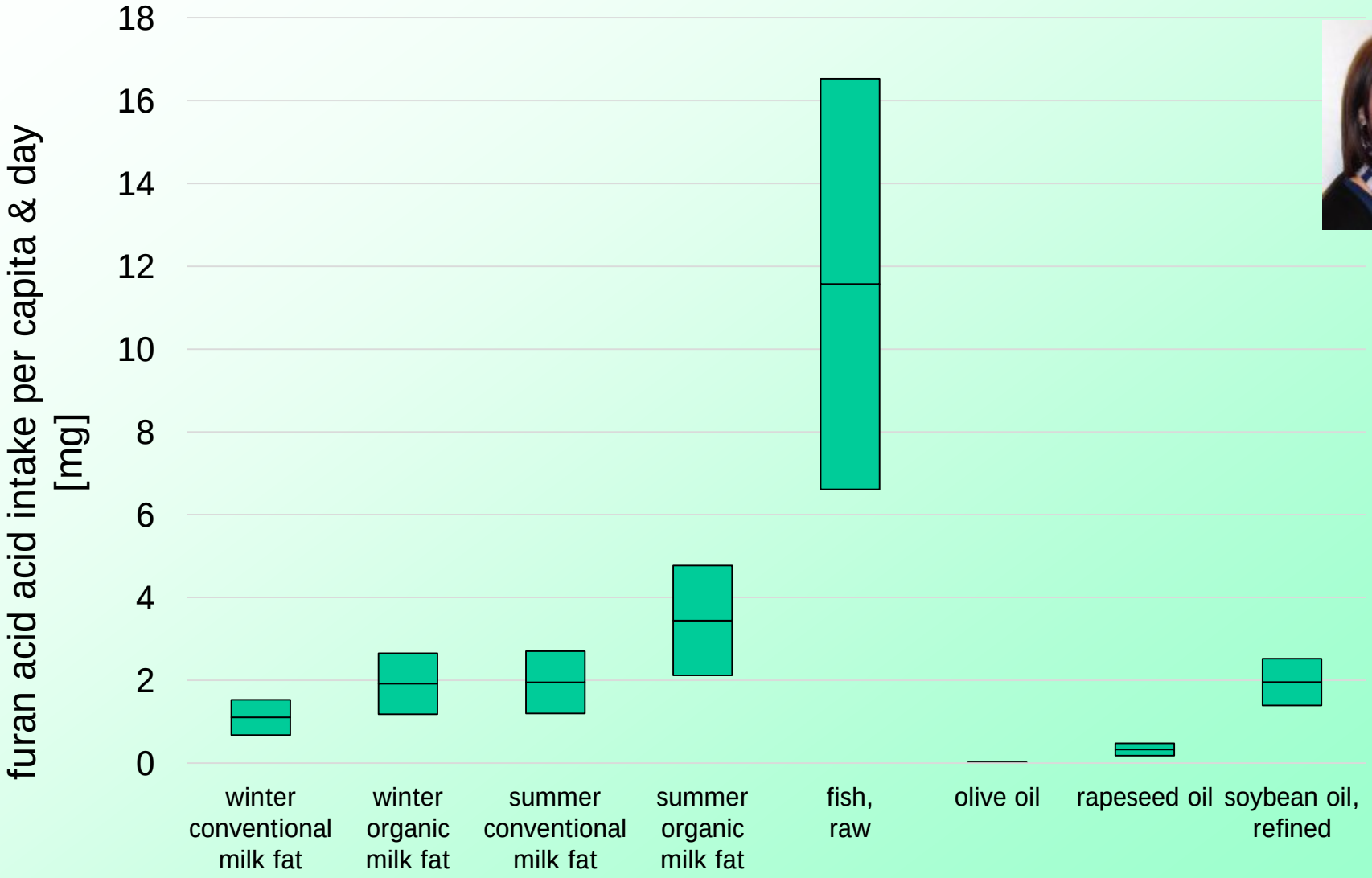
- short terms: **9M5** **9D5**

- “D“-furan fatty acids are more widespread but much less stable

  ⇒ partly absent in processed or stored samples
- excellent antioxidants (very effective protectors of PUFAs)
- degradation products responsible for off flavor of soy (among other)

Ref.: G. Spiteller, *Lipids* 40 (2005) 755-771

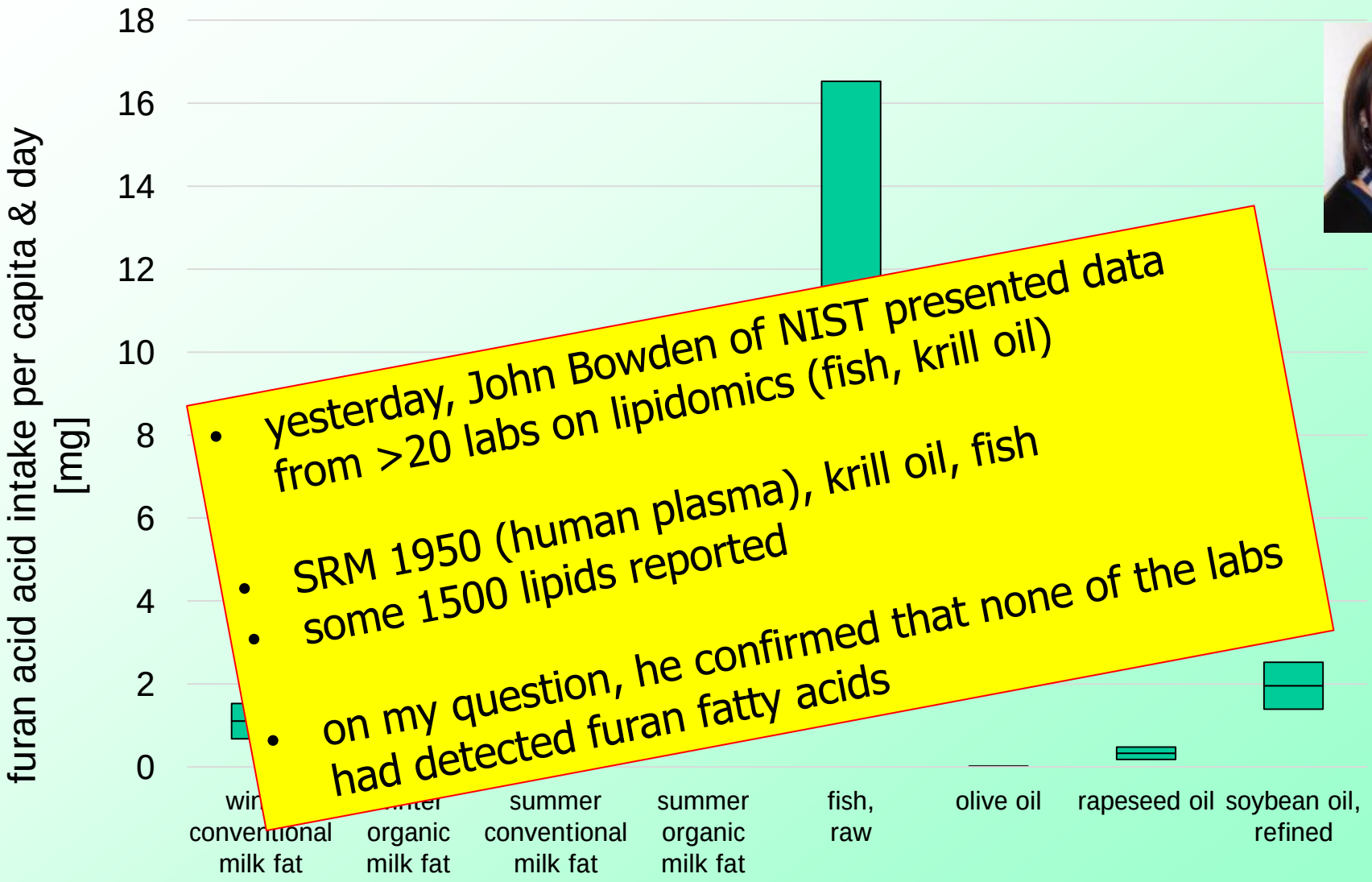
Calculated daily intake of furan fatty acids per capita [mg], in Germany



Ref.: C. Wendlinger, W. Vetter, *J. Agric. Food Chem.* 62 (2014) 8740-8744

Calculated daily intake of furan fatty acids per capita [mg], in Germany

- yesterday, John Bowden of NIST presented data from >20 labs on lipidomics (fish, krill oil)
- SRM 1950 (human plasma), krill oil, fish
- some 1500 lipids reported
- on my question, he confirmed that none of the labs had detected furan fatty acids



Ref.: C. Wendlinger, W. Vetter, *J. Agric. Food Chem.* 62 (2014) 8740-8744

Furan fatty acids

- minor fatty acids, low concentrations (typically <0.1% contribution to the total fatty acids
- but: widely spread in virtually all plants and animals
- discovered, studied in the 1970s (R.L. Glass, H. Schlenk, F.D. Gunstone)
- mostly forgotten, studied again ~1985-1995 (G. Spiteller, W. Grosch)
- almost forgotten since the 2000s
- no reference standards commercially available

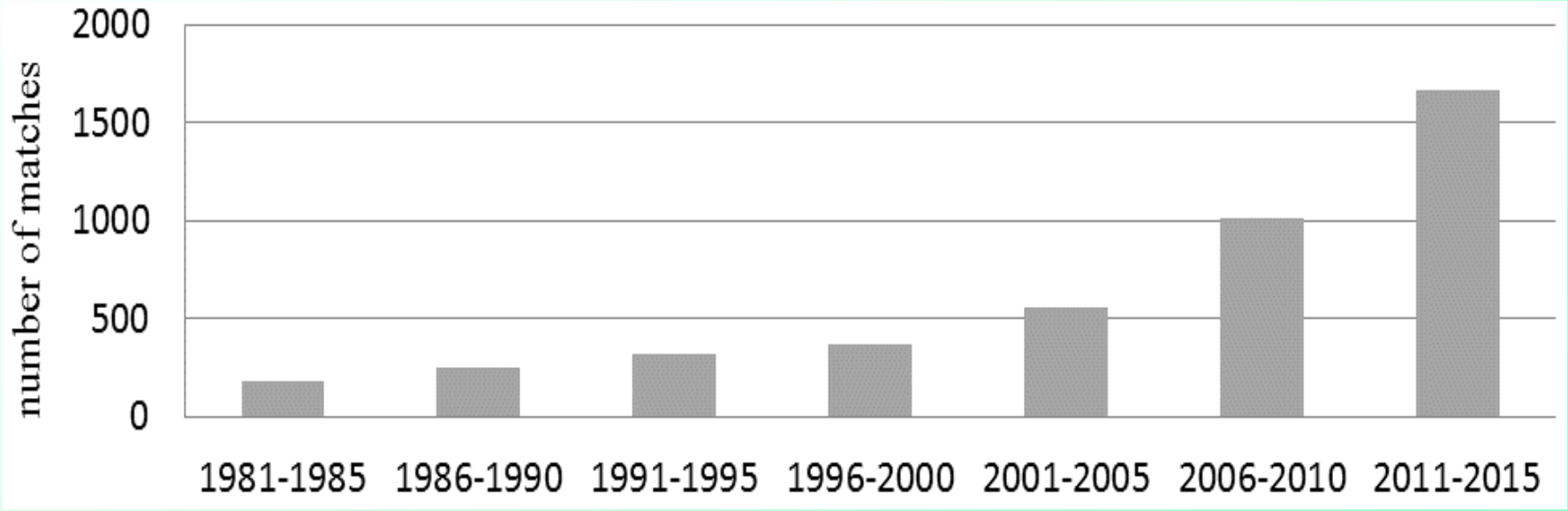
$\Rightarrow$ research only possible with house-prepared standards

Compound isolation using countercurrent chromatography (CCC)

- CCC is a well-established method in natural product isolation [1][2]
- all liquid based chromatographic method (no solid support) [3]
- allows injection and isolation of gram-amounts of analytes [3]
- barely used in field of lipid compounds [1]

Ref.: [1] J. B. Friesen, J. B. McAlpine, S.-N. Chen, G. F. Pauli, *J. Nat. Prod*. 78 (2015) 1765-1796
[2] I. A. Sutherland, D. Fisher, *J. Chromatogr. A* 1216 (2009) 740-753
[3] Y. Ito, *J. Chromatogr. A* 1065 (2005)145-168

Applications of countercurrent chromatography (CCC) according to SciFinder (1981-2015)

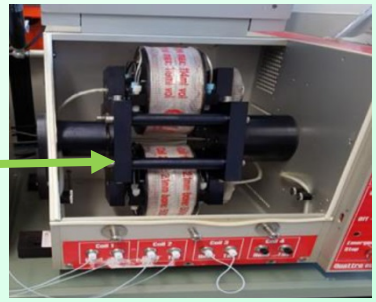


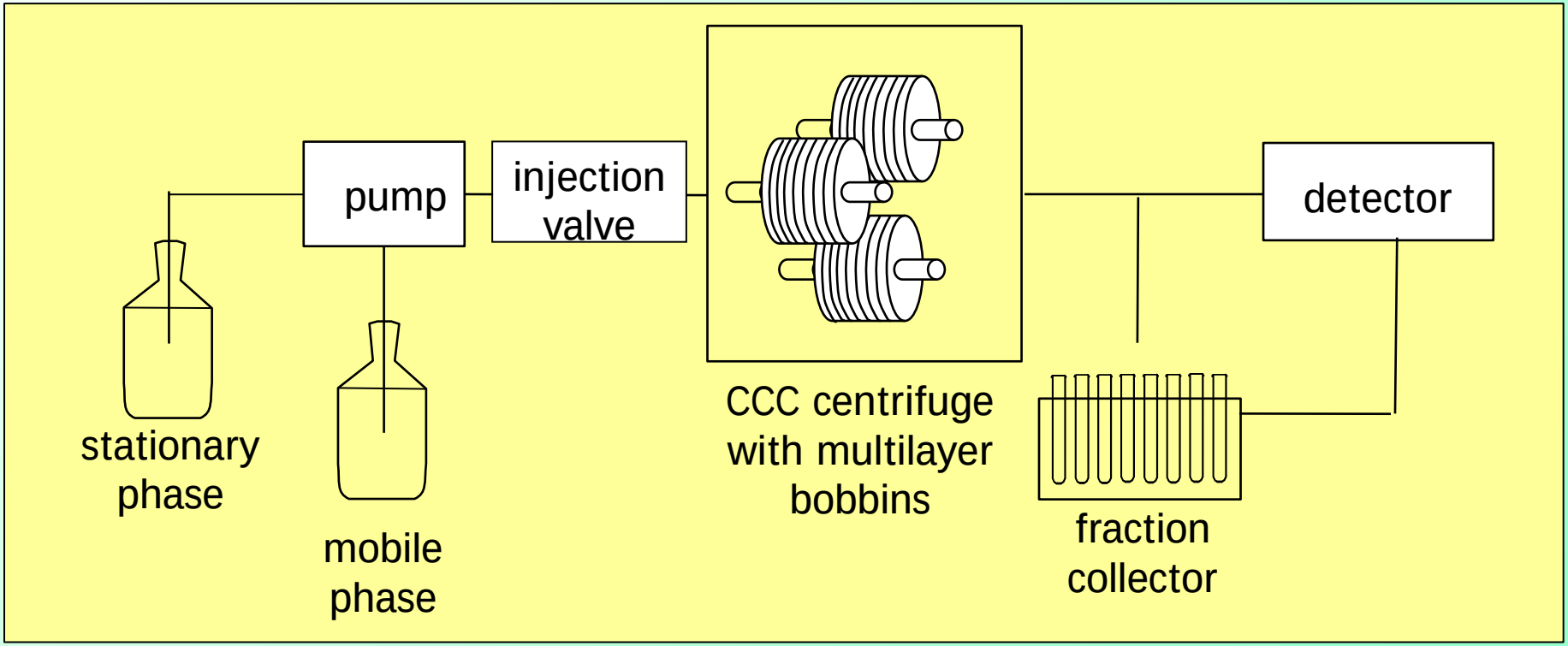
- currently >300 CCC papers/year, number is increasing
- ~3% in the field of lipid compounds

CCC instrumentation

- **CCC systems same setup as HPLC instruments except the column**

  ⇒ instead: CCC centrifuge with multilayer coils





CCC method development

- **CCC is different to liquid-liquid extractions** as it aims to **distribute the analyte evenly between both phases**

  ⇒ determination of the partitioning factor

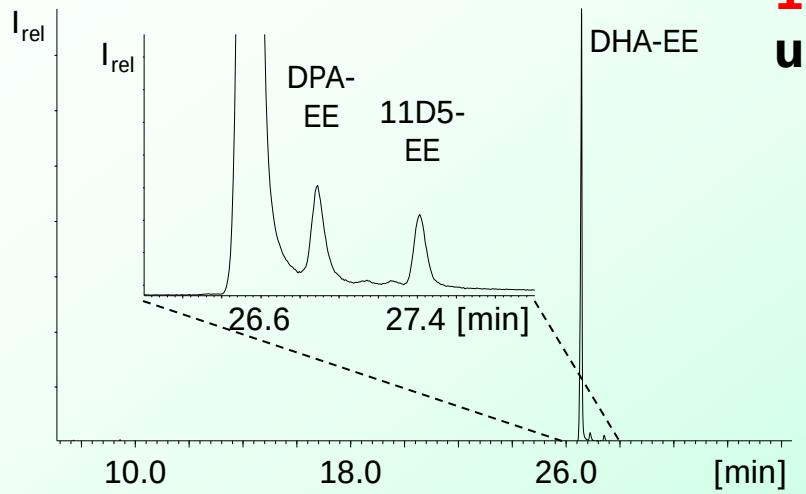
$$\mathbf{K_{U/L}} = \frac{\text{[concentration in upper phase]}}{\text{[concentration in lower phase]}} \approx \mathbf{0.4 - 2.5}$$

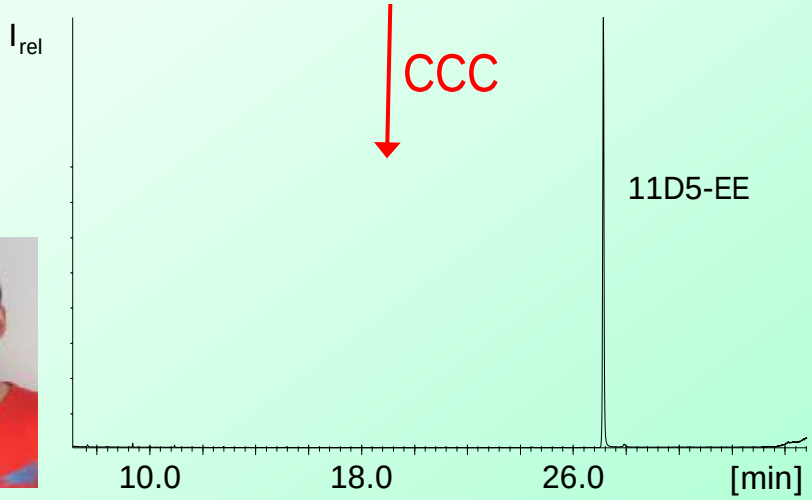
- even distribution: $K_{U/L} = 1$; acceptable range: $K_{U/L} = 0.4 - 2.5$

  ⇒ the ~~goal~~ challenge is to find a biphasic solvent system in which the analytes are ~ evenly distributed (and resolved)

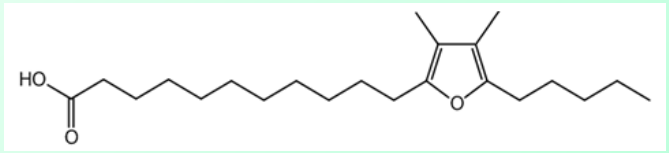
⇒ see tutorial on CCC in the AOCS Lipid Library

Isolation of the valuable furan fatty acid 11D5





11-(3,4-dimethyl-5-pentylfuran-2-yl)-undecanoic acid (11D5)



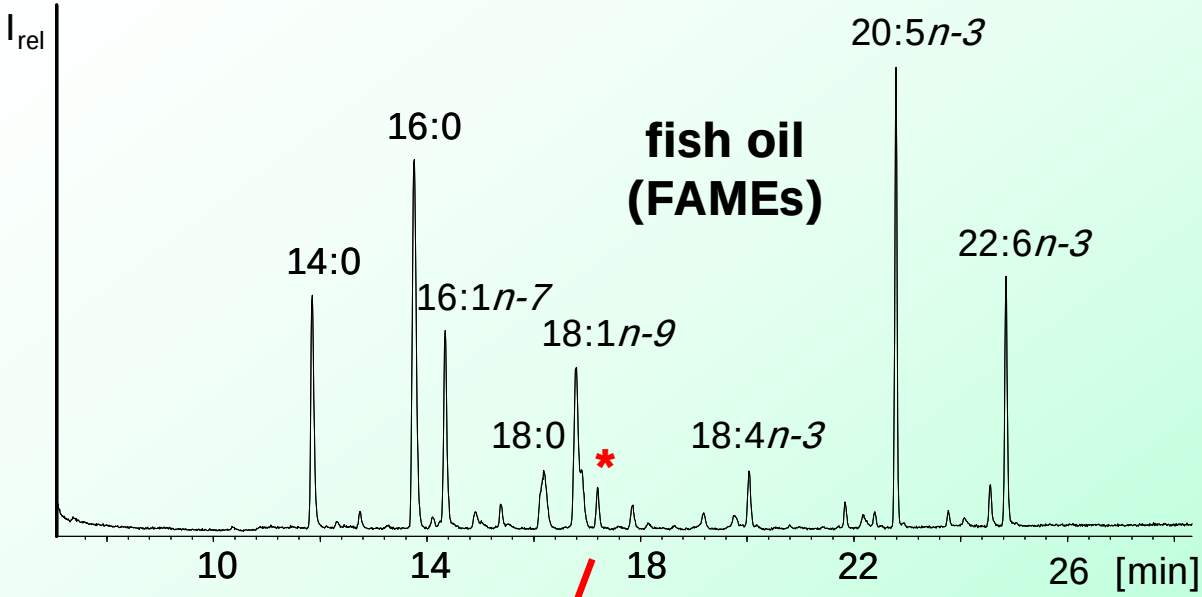
- excellent antioxidant
- no standards available
- limited research

injection:	1 g
yield:	19 mg 11D5
purity:	99%
solvent consumption:	100 mL
time per mg 11D5:	3.4 min

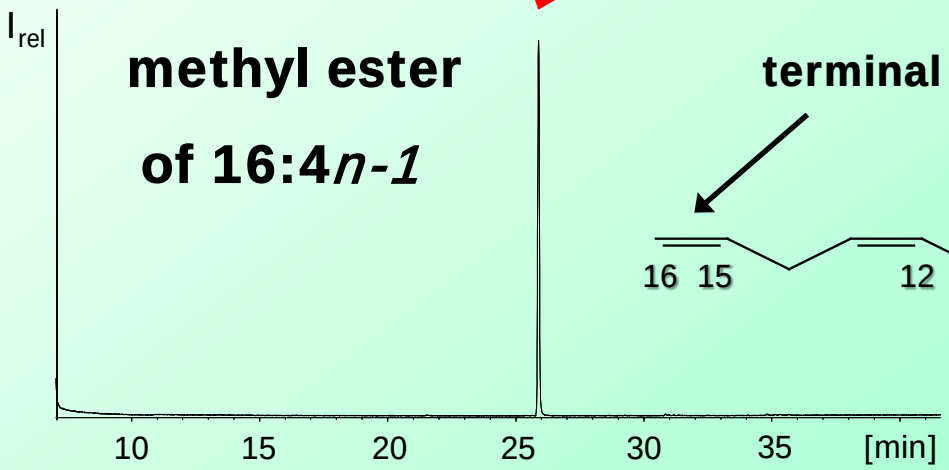
Ref: M. Müller, K. Wasmer, W. Vetter, *J. Chromatogr. A* (2018), in press (DOI: 10.1016/j.chroma.2018.04.069)

CCC isolation of fatty acid methyl esters

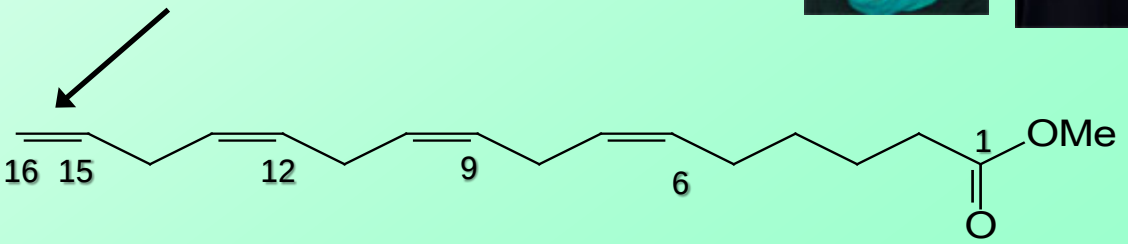
hexane/methanol/water (350/175/2) for fatty acid methyl esters (FAME)



CCC



terminal DB



- <u>equivalent chain length (ECL) rule:</u>

  ⇒ 2 carbons ~ 1 DB

- no problem with 16:4 (ECL: 12:2 / 10:1 / 8:0)

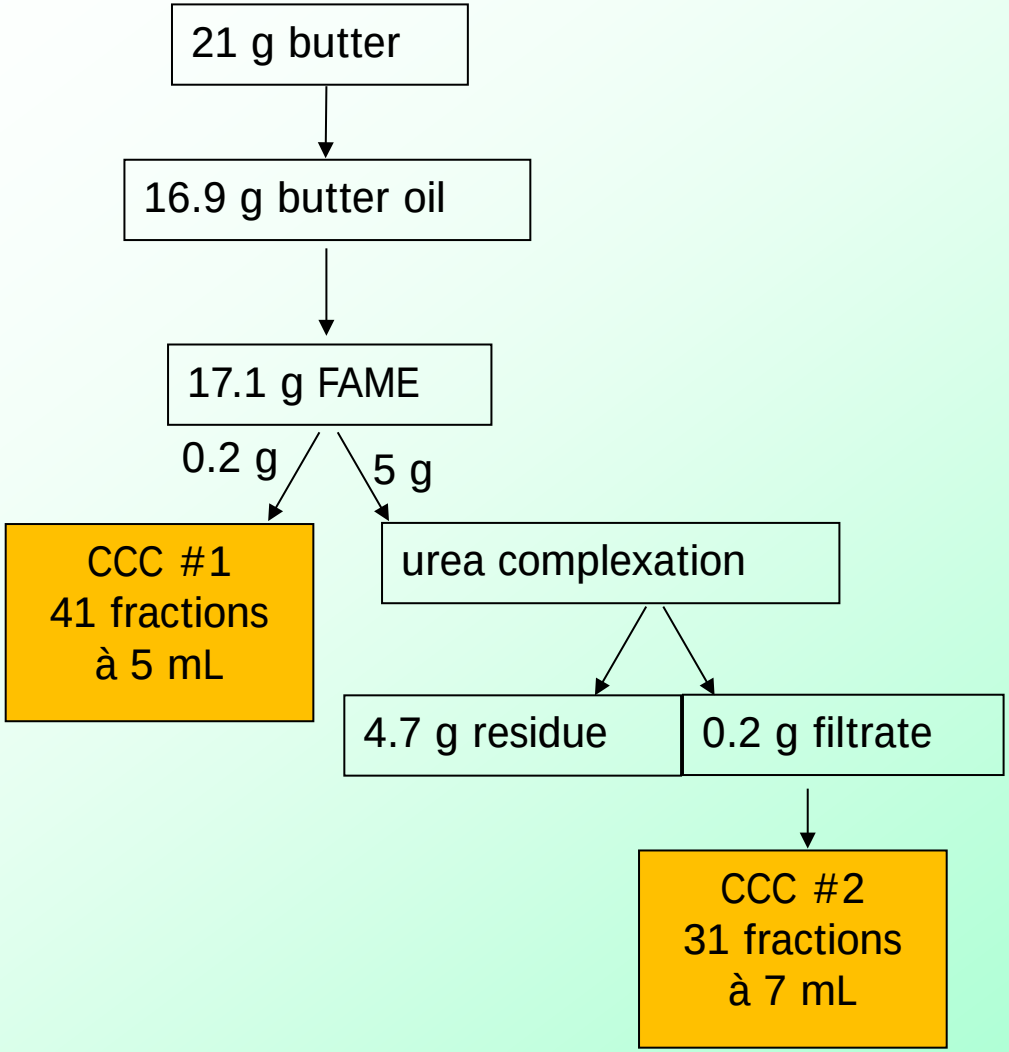
<u>Ref.</u>: D. Li, M. Schröder, W. Vetter, *Chromatographia* 75 (2012) 1-6

Sample fractionation and analyte isolation via countercurrent chromatography (CCC)

1. **isolation of lipid compounds for use as standards / in biotests**
   - examples: - isolation of uncommon fatty acids
     - isolation of phytosterols, tocopherols, carotenes etc.

2. **fractionation of lipids or lipid fractions by CCC**
   - detection of minor compounds usually “invisible“ without fractionation
   - examples: - detection of 430 fatty acids in one butter sample
     - discovery of aromatic fatty acids in milk fat

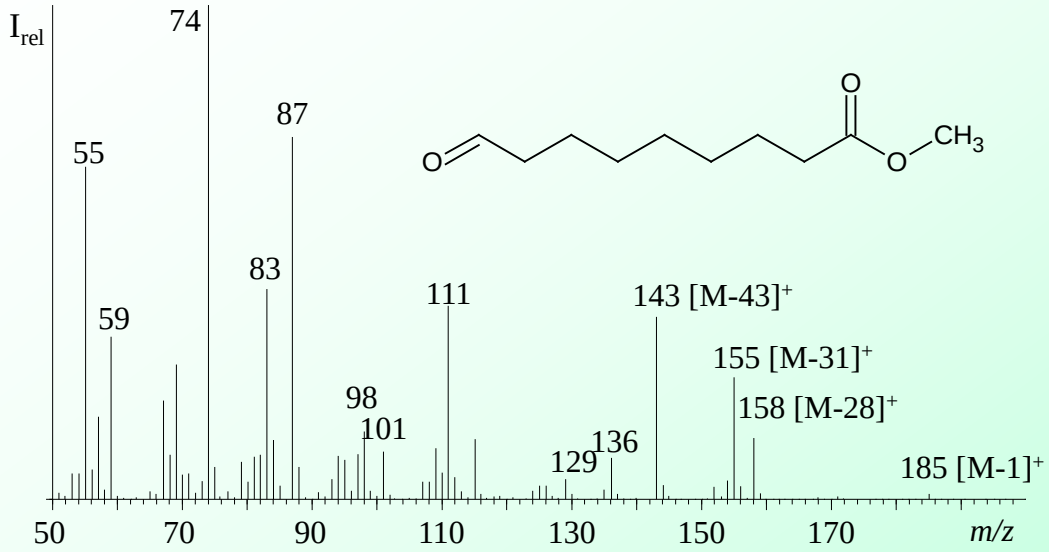
Detailed analysis of a butter sample

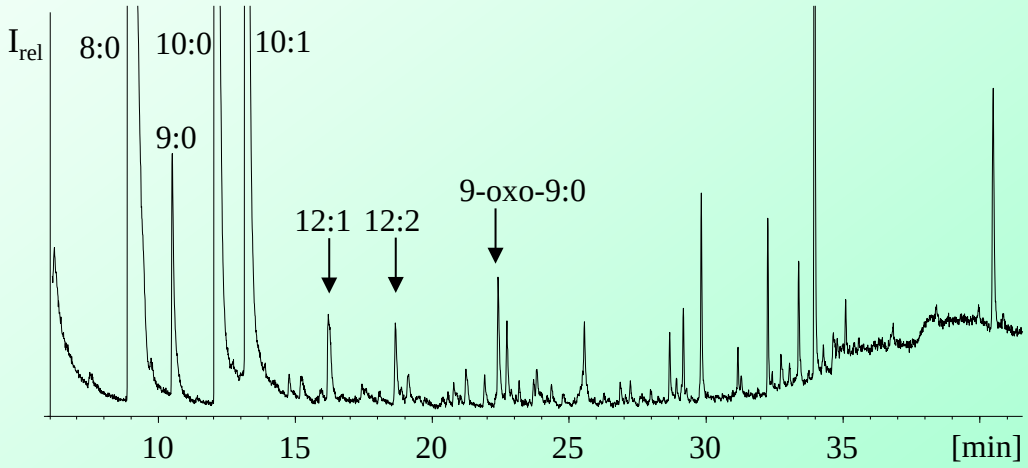


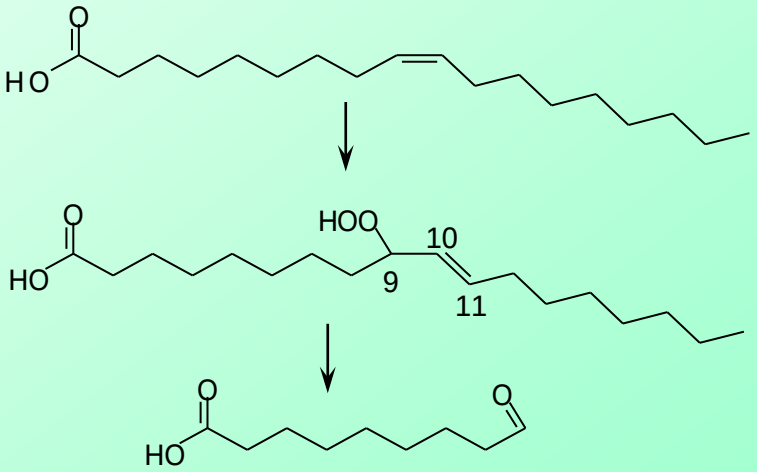
- CCC #1 from FAME fraction (major fatty acids)
- CCC #2 from filtrate of urea complexation (rare trace fatty acids)
- 430 different fatty acids
- >100 PUFAs
- several rare fatty acids

Ref.: M. Schröder, W. Vetter, *J. Am. Oil Chem. Soc*. 90 (2013) 771-790

Detailed analysis of a butter sample



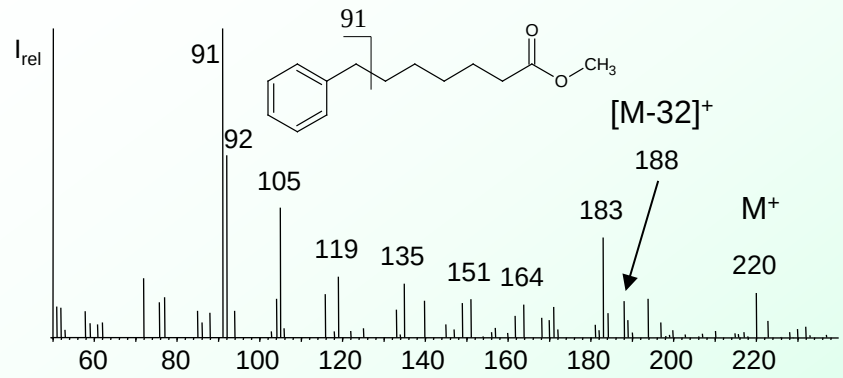


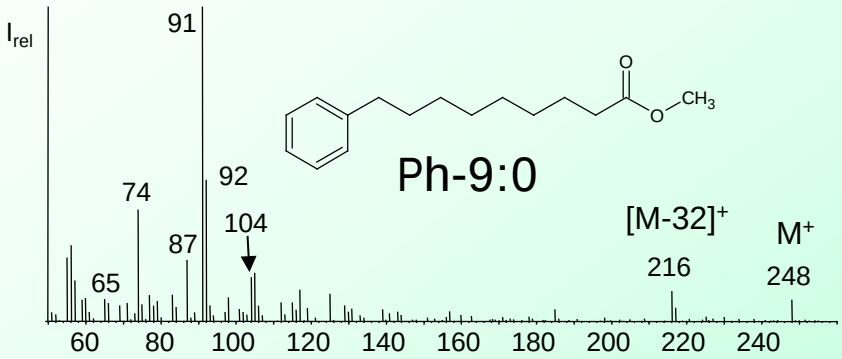


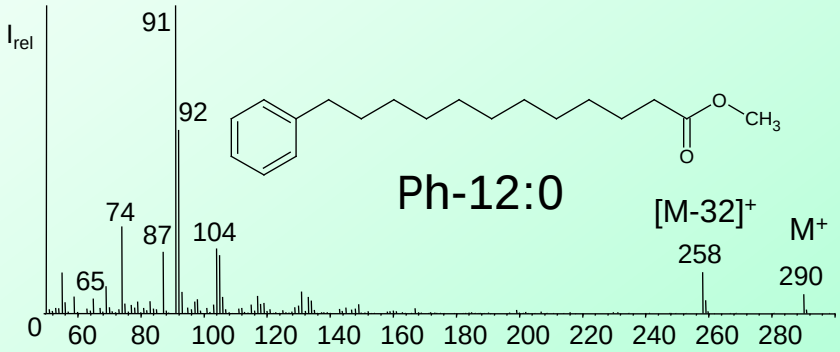
- potential formation from oleic acid (lipid oxidation)

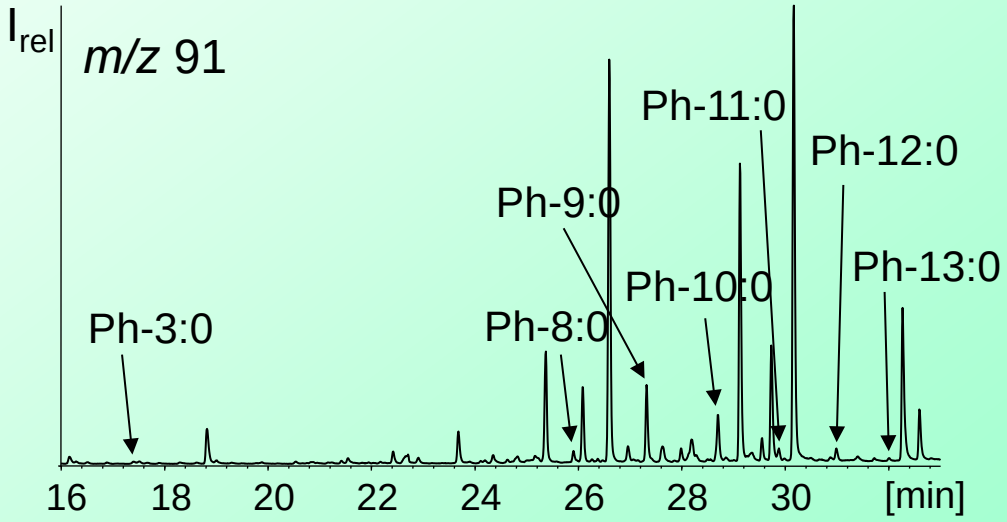
Ref.: M. Schröder, W. Vetter, *J. Am. Oil Chem. Soc*. 90 (2013) 771-790

Aromatic fatty acids in butter





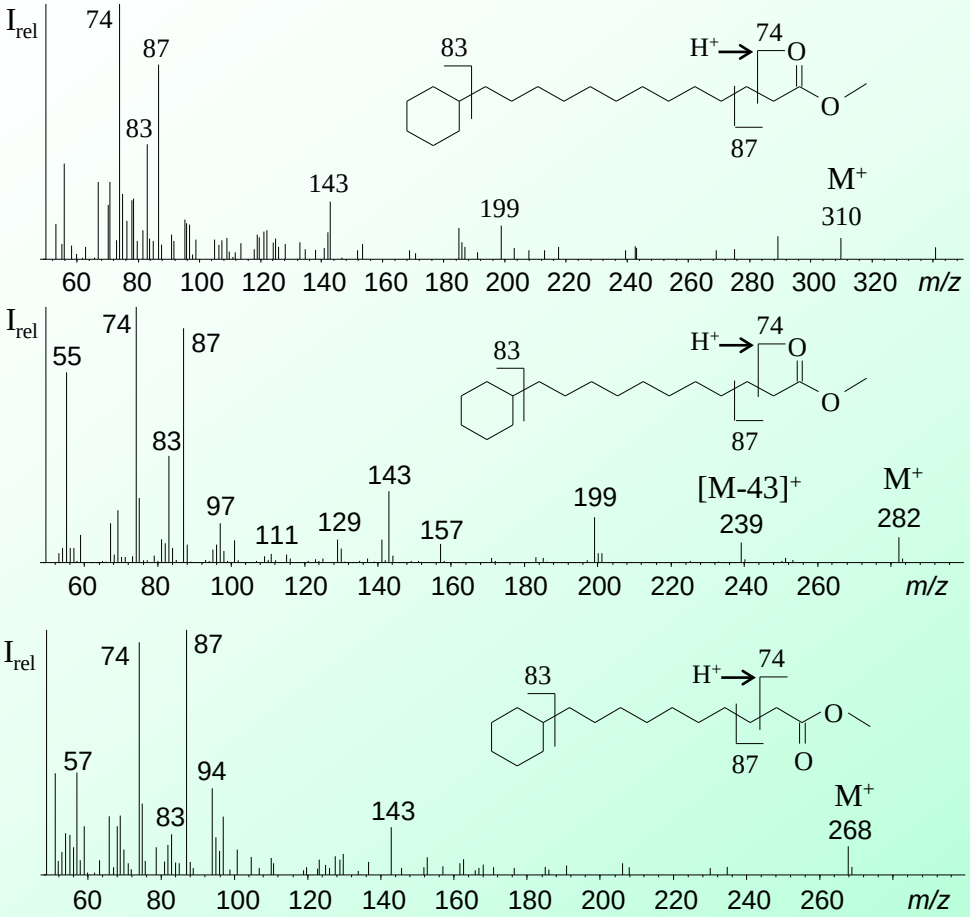




- previously not known to occur in milk (and other food)
- potential formation from phenylalanine as the primer

Ref.: M. Schröder, W. Vetter, *J. Am. Oil Chem. Soc*. 91 (2014) 1695-1702

Cyclic fatty acids

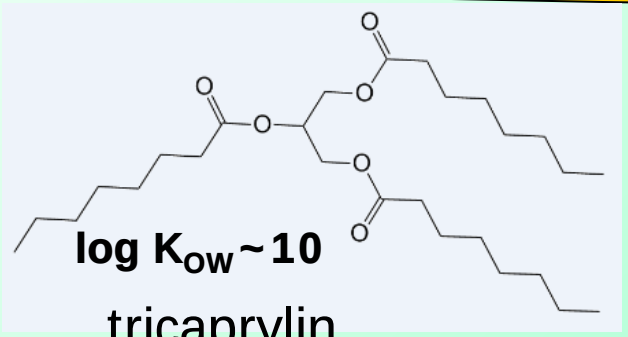


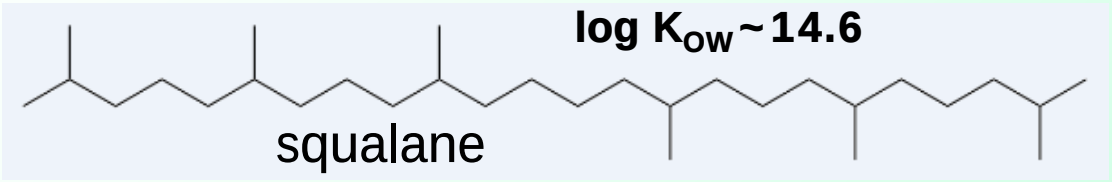
- previously known to occur in milk
- potential formation with aromatic fatty acids by hydrogenation?

M. Schröder, W. Vetter, *J. Am. Oil Chem. Soc*. 90 (2013) 771-790

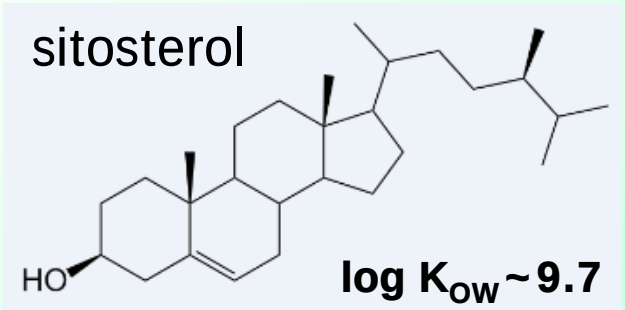
Very nonpolar lipid compounds

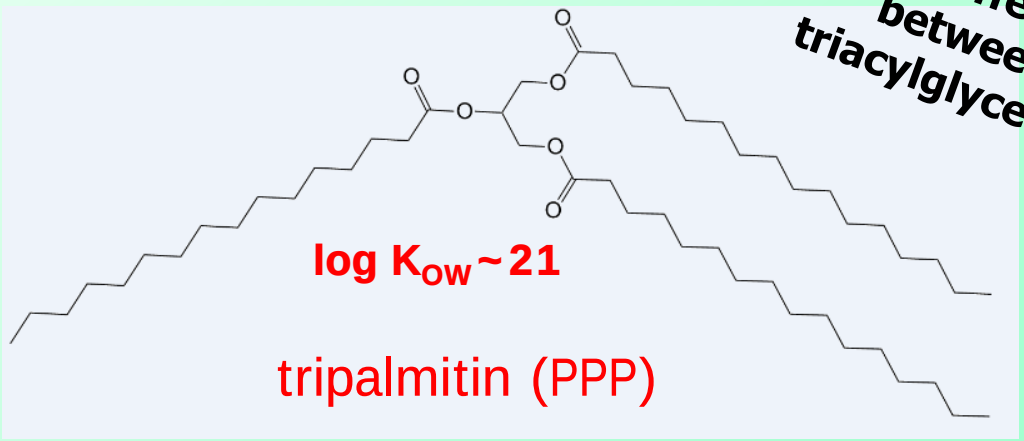
- log K_{OW} 7 -20
- main interest in this study: log K_{OW} >15



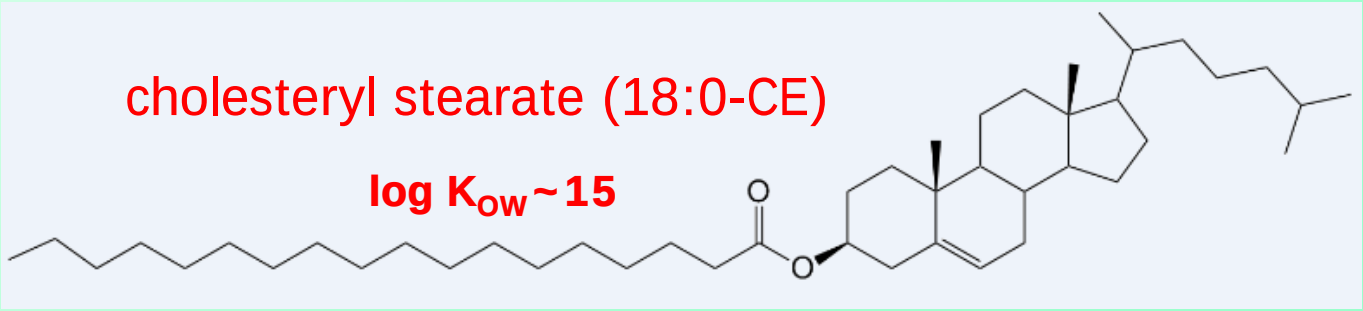


huge difference between triacylglycerols

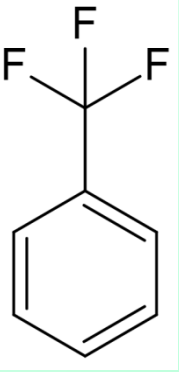




- except sitosterol no polar groups (-OH)

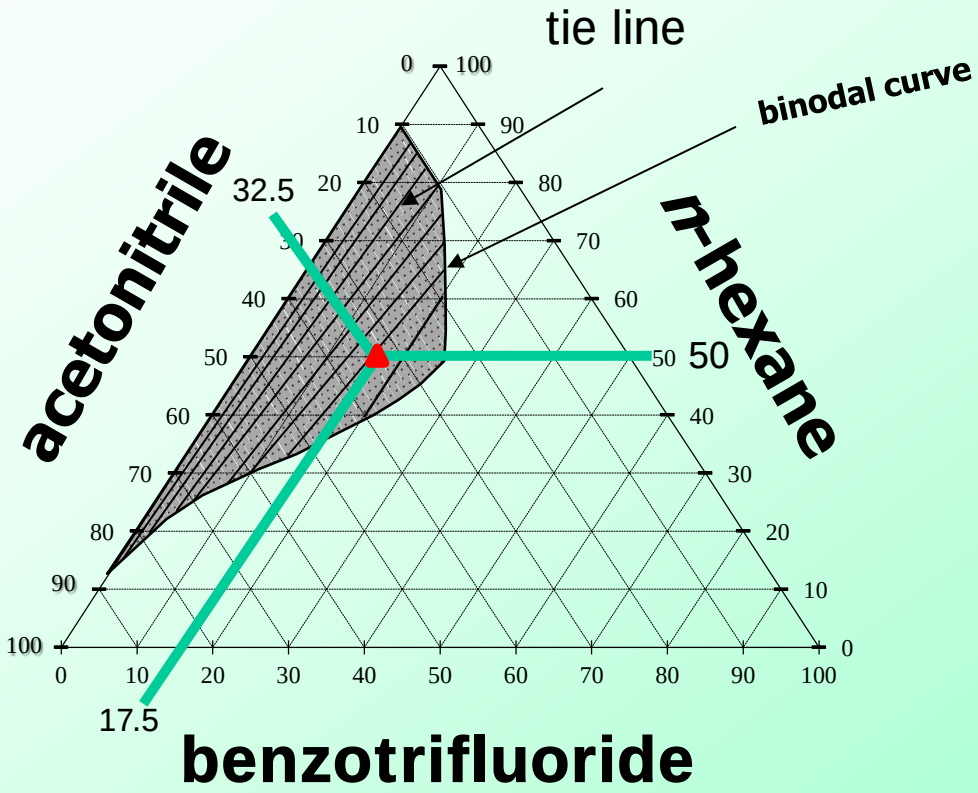


Introduction of benzotrifluoride as modifier in solvent systems



- bridging solvents between the biphasic system [1]

ternary phase diagram

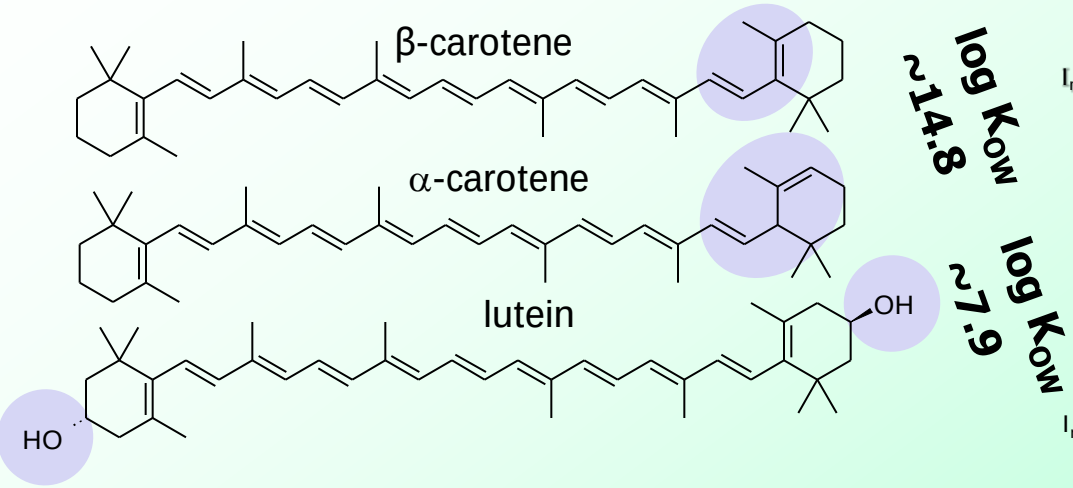


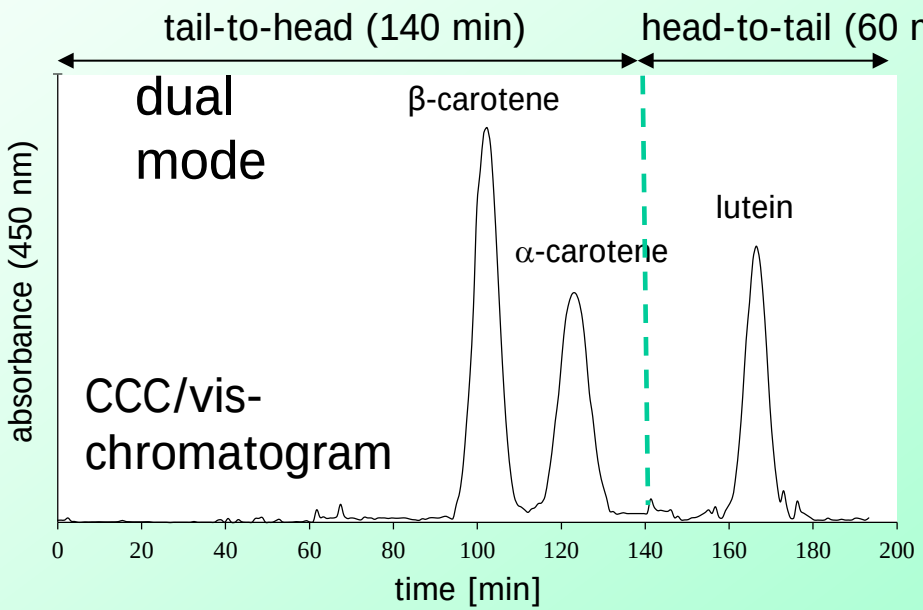
- **well suited composition and properties:**
- Hex/ACN/BTF: 10 / 6.5 / 3.5
- settling time: < 20 sec

Ref.: [1] M. Englert, S. Hammann, W. Vetter, *J. Chromatogr. A* 1388 (2015) 119-125

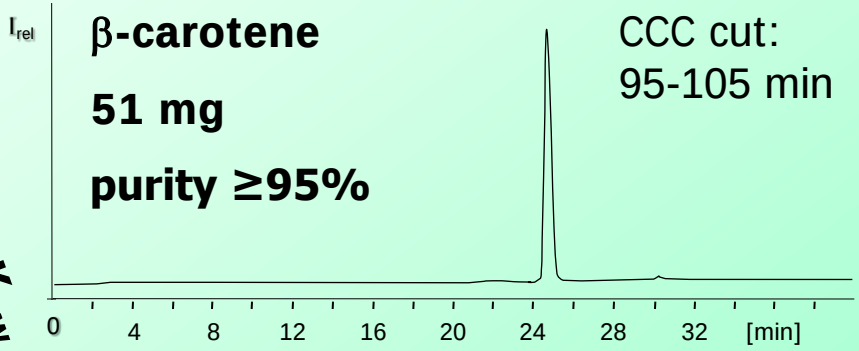
Isolation of carotenoids from carrot juice using hexane / acetonitrile / benzotrifluoride (BTF)

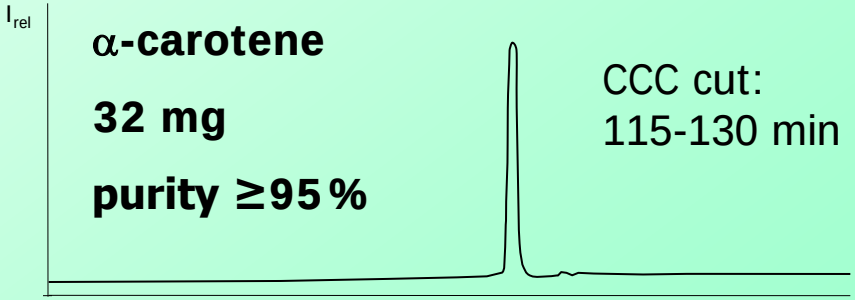
π-π interactions

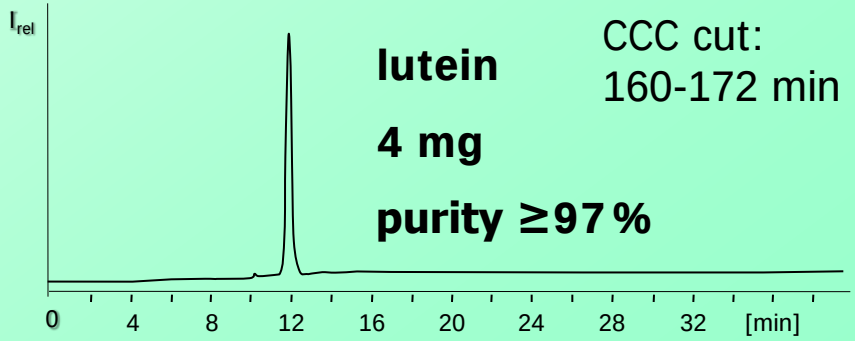




100 mg injected







HPLC/UV chromatograms (450 nm)

Ref.: M. Englert, S. Hammann, W. Vetter, *J. Chromatogr. A* 1388 (2015) 119-125

Difference between “Hex / ACN“ and the “Hex / ACN / BTF“ solvent system

Phase composition of solvent systems determined by GC/FID [1]

solvent system	lower	upper
hexane / ACN [1]	**1.2 / 98.8**	**99.5 / 0.5**
hexane / ACN / BTF [2]	**29.0 / 56.7 / 14.3**	**76.0 / 12.5 / 11.6**

partitions stronger into lower phase

equal distribution

- >1/4th hexane partitions into lower phase
- difference in polarity decreased

Ref.: [1] M. Englert, W. Vetter, *J. Chromatogr. A* 1342 (2014) 54–62
[2] M. Englert, S. Hammann, W. Vetter, *J. Chromatogr. A* 1388 (2015) 119-125

$K_{U/L}$ of lipid compounds in the BTF system (Hex/ACN/BTF, 10:6.5:3.5)

lipid compound	log K_{OW}	HEMWAT -7 $K_{U/L}$	BTF $K_{U/L}$
oleic acid	7.7	29	0.57
oleic acid methyl ester	7.5	41	2.4
sitosterol	9.7	16	2.2
squalane	14.6	550	62
cholesteryl stearate	15	1260	80
tripalmitin (PPP)	21	1490	60

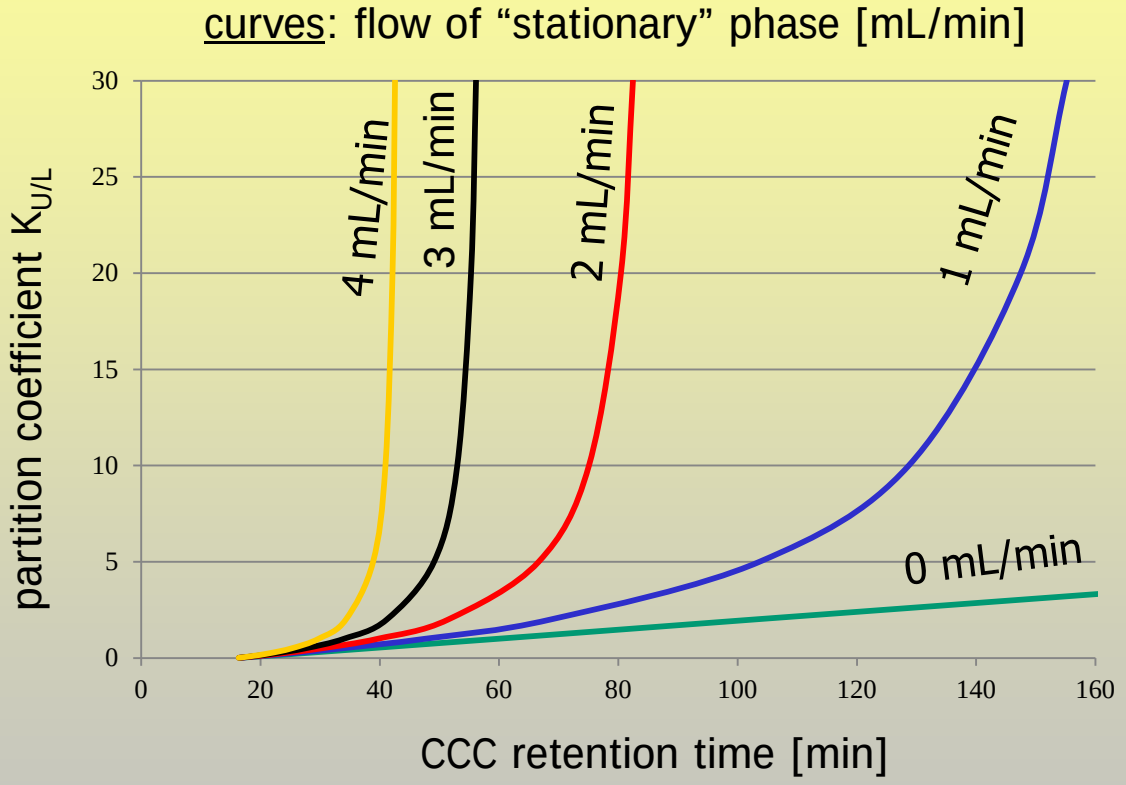
excellent for FAMEs, sterols, tocopherols

Ref.: M. Englert, W. Vetter, *J. Chromatogr. A* 1342 (2014) 54–62

BTF system in co-current* CCC mode

* introduced by Sutherland *et al*., theory by Berthod *et al*.

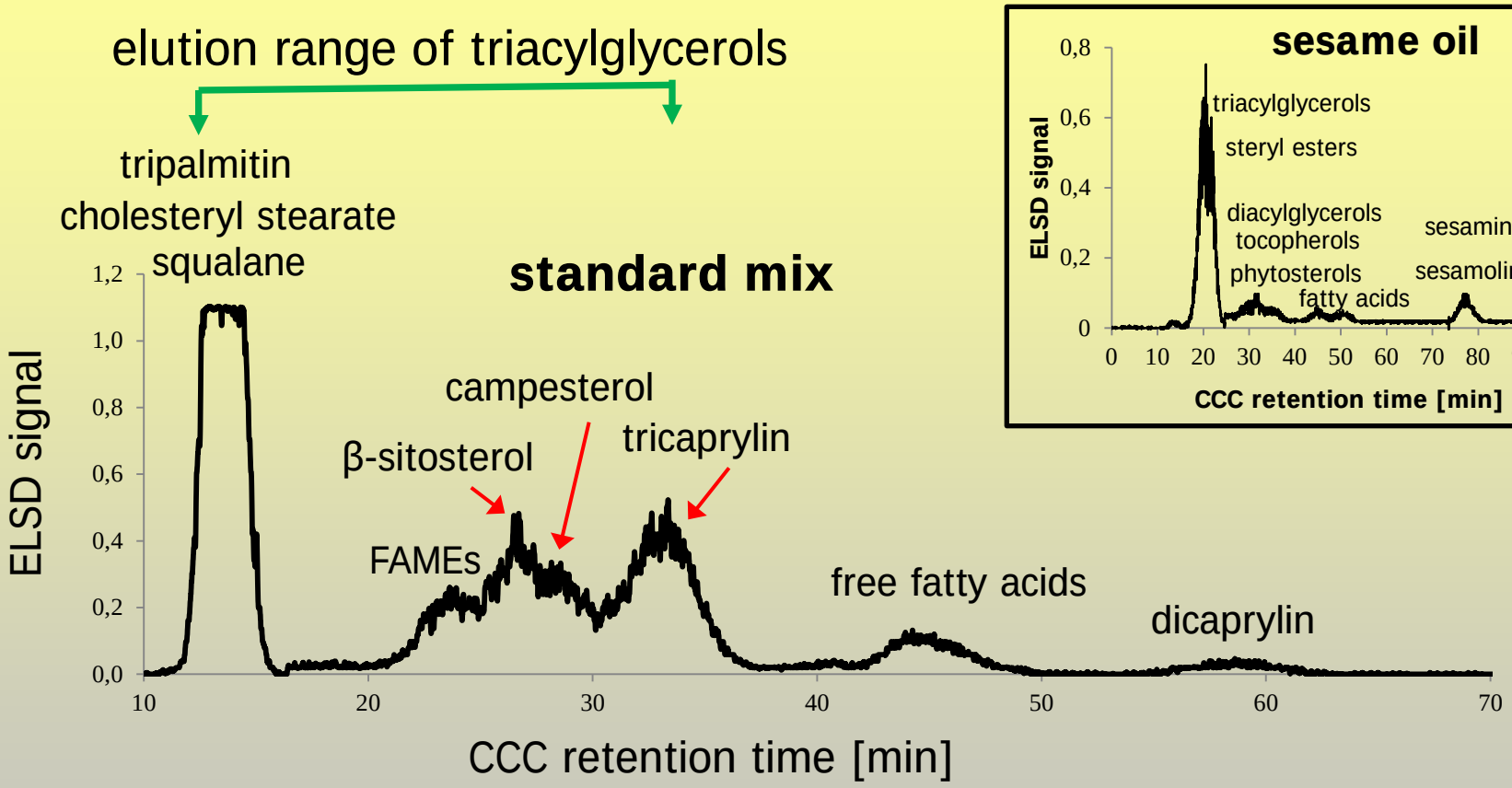
- both phases are moved
- accelerates elution of analytes with high $K_{U/L}$
- exponential increase leads to co-elution of analytes with high $K_{U/L}$, even if $\Delta K_{U/L}$ is high



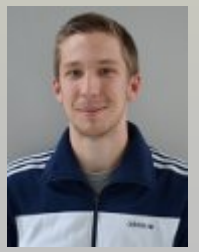
mobile phase: 4 mL/min

Ref.: S. Hammann, M. Englert, M. Müller, W. Vetter, *Anal. Bioanal. Chem*. 407 (2015) 9010-9027

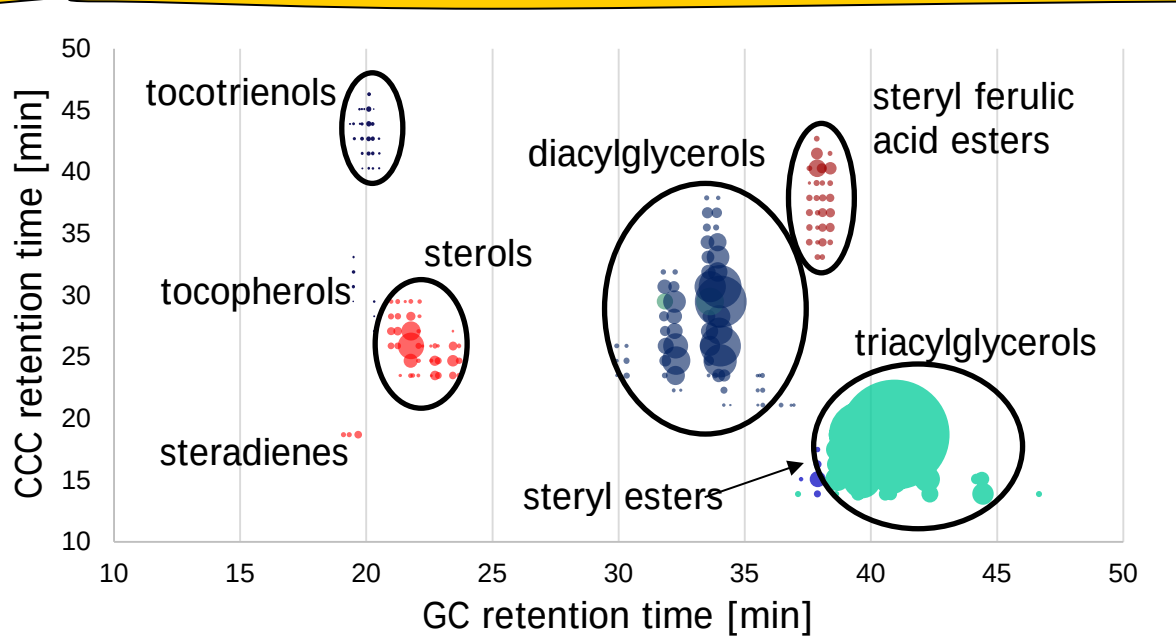
Co-current CCC mode with the BTF system



- elution of lipid compounds spreading from log K_{OW} 3 – 30 within acceptable run time
- no separation of extremely nonpolar lipid compounds

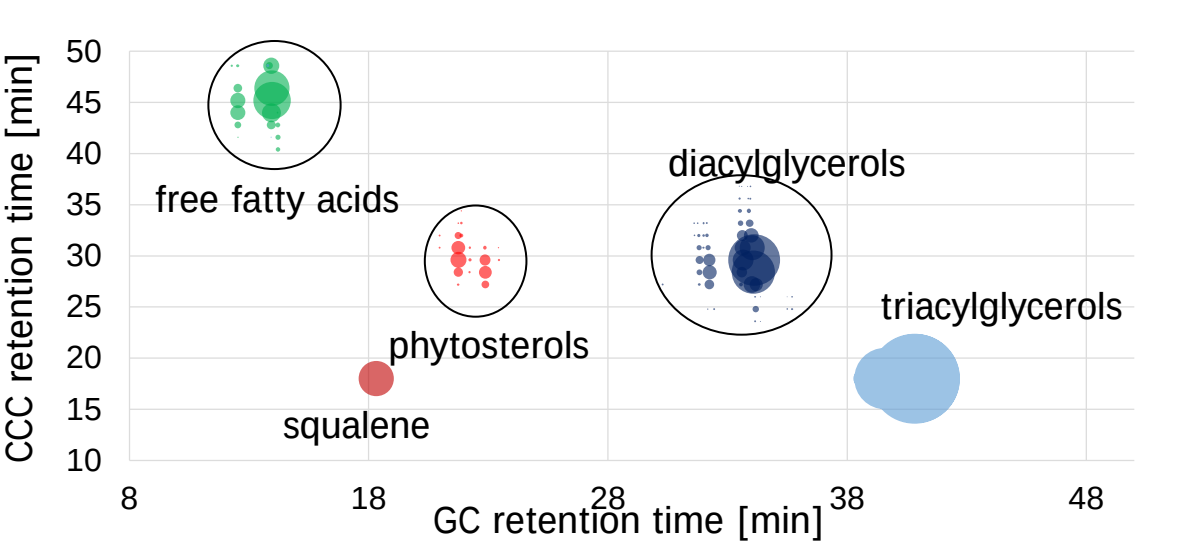


Co-current CCC mode with the BTF system



bubble blot of the co-current CCC separation of 0.5 g rice bran oil

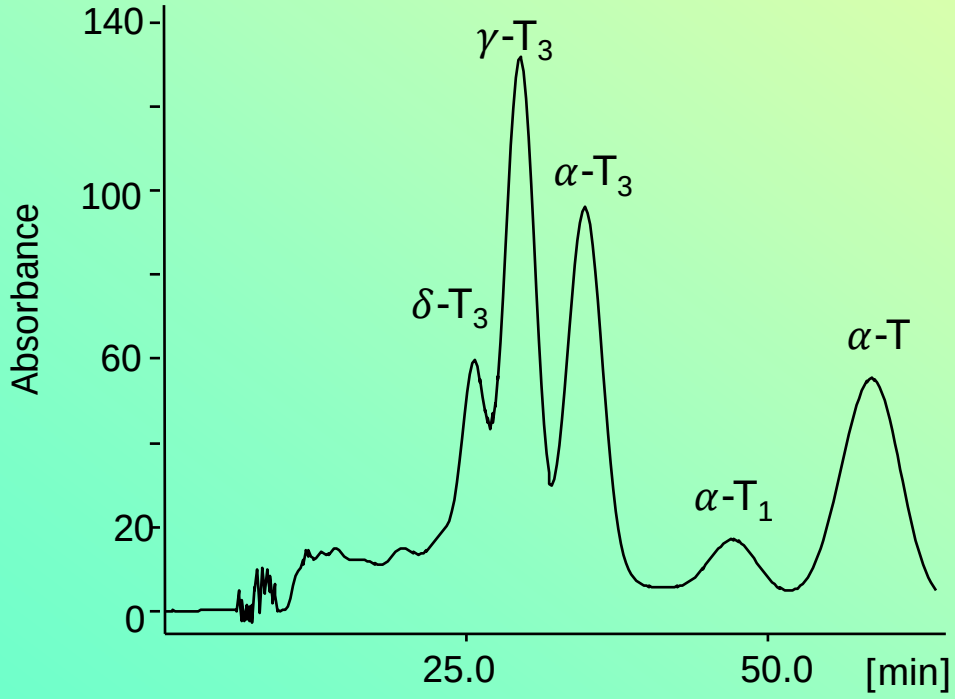
Ref.: S. Hammann, A. Kröpfl, W. Vetter, J. Chromatogr. A 1476 (2016) 77-87

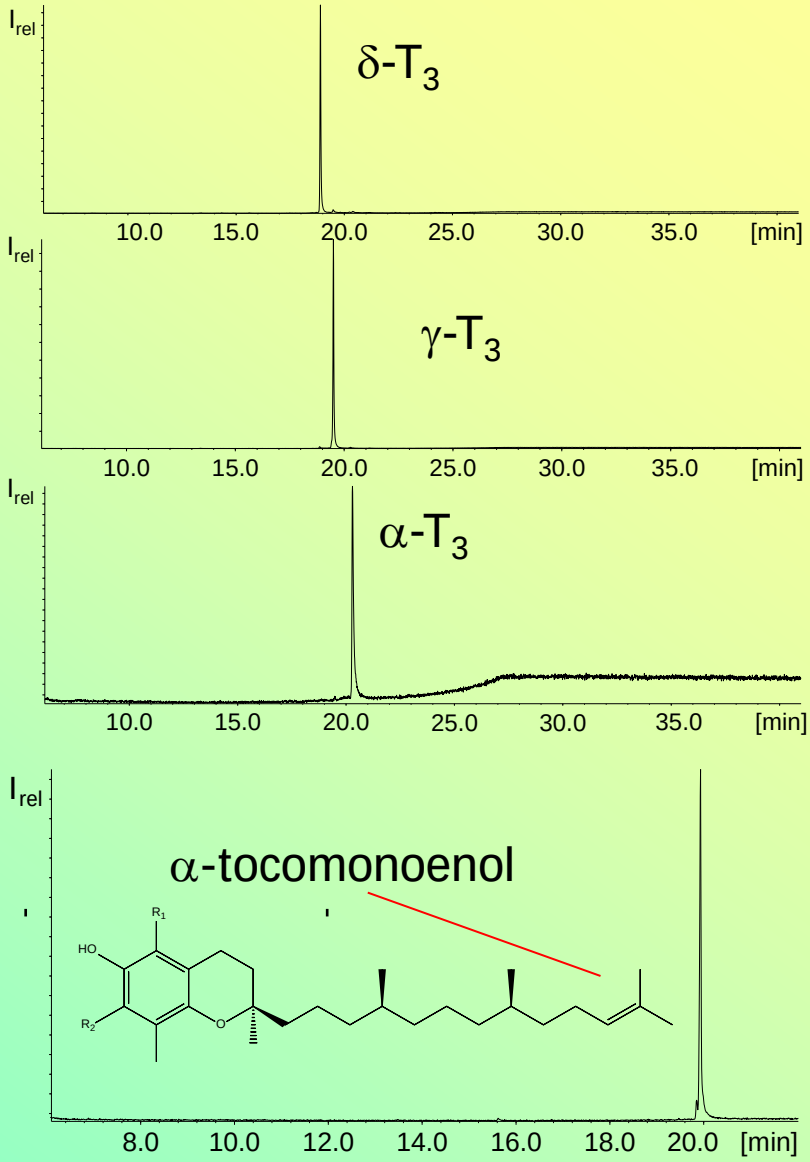


Ref.: S. Hammann, M. Englert, M. Müller, W. Vetter, *Anal. Bioanal. Chem*. 407 (2015) 9010-9027

CCC isolation of vitamin E compounds

- dietary supplementary capsule made from palm oil

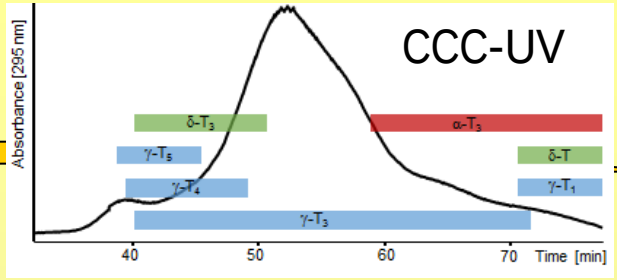


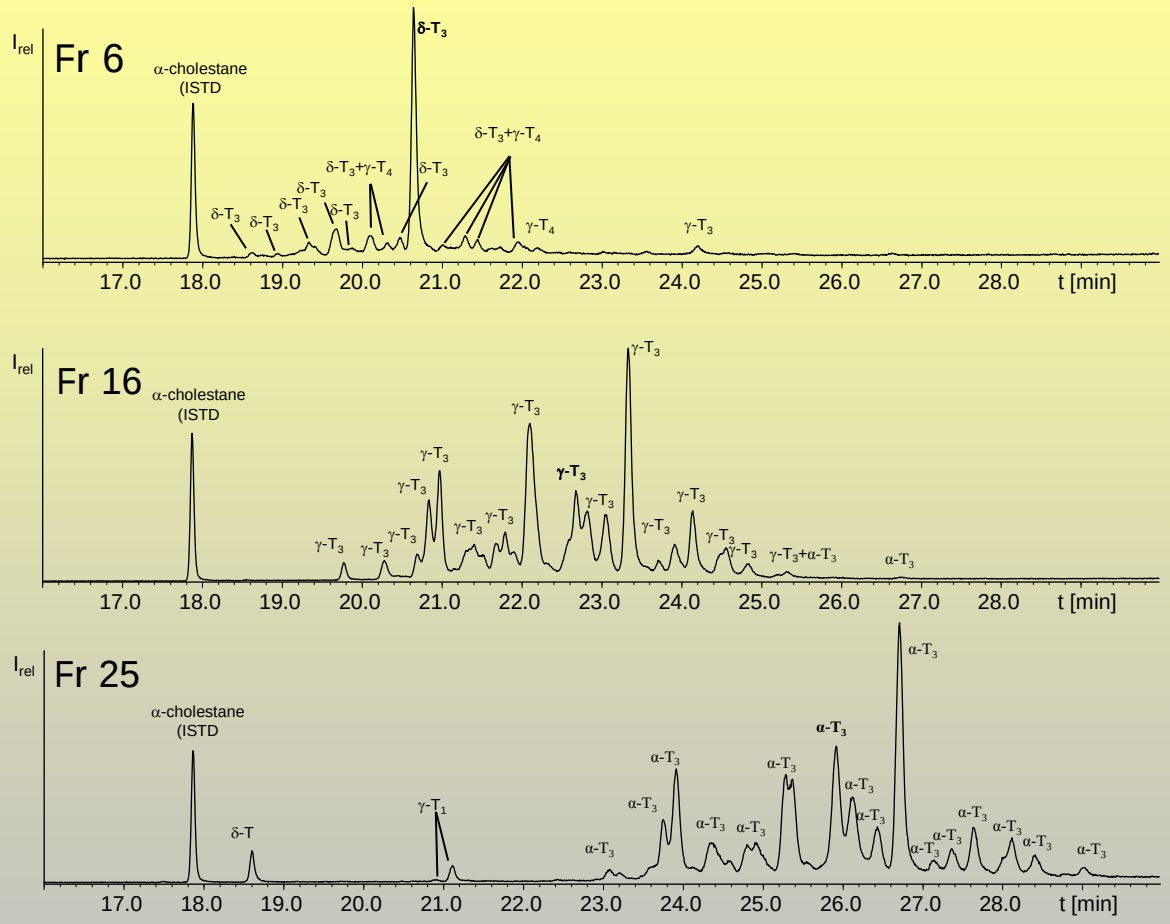


- each isolated at 10-65 mg/run (purity 99%)

Ref.: M. Müller, S. Hammann, W. Vetter, *Food Chem.* 256 (2018) 327-332

Tocotrienol artefacts

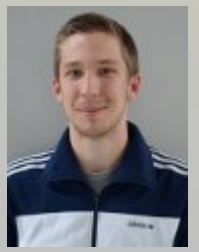




- 170 non-natural vitamin E compounds in a palm-oil based dietary supplementary oil

- ~80 tocotrienol isomers
- tocotetra- & pentaenols
- ...

- formed during inadequate sample processing



Ref.: S. Hammann, A. Kröpfl, W. Vetter, *J. Chromatogr. A* 1476 (2016) 77-87

Summary

- analysis of minor lipid compounds is a fascination and varied research field
- the actual relevance of minor fatty acids may currently be underrated
- unavailability of standards frequently hampers progress in the field
  (no standard = no research = no knowledge)
- lipid standards can be isolated by countercurrent chromatography
- our work is a mixture of basic research and applications
- and sometimes ...

  ... it´s like a road movie (the journey is the goal)

Thousand thanks!

- to my former and previous ph. d. students, master and bachelor students, especially those pictured in this presentation (people first)!
- to our research partners here, there and everywhere!
- to our funders (without money, no research)!
- to Analytical Division of AOCS for honoring our research with the Herbert J. Dutton Award
- to Dr. Perluigi Delmonte, US FDA, for inviting me to the AOCS meeting in Minneapolis
- to **YOU** for your attention