NOVEL COMPREHENSIVE CHROMATOGRAPHIC TECHNIQUES FOR DETAILED EDIBLE OIL AND FAT ANALYSIS

MINOR AND MAJOR COMPOUNDS

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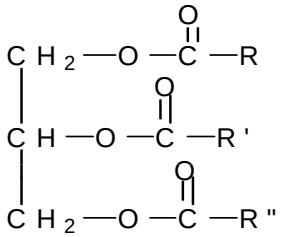
PEOPLE KNOW US BY OUR BRANDS

Unilever

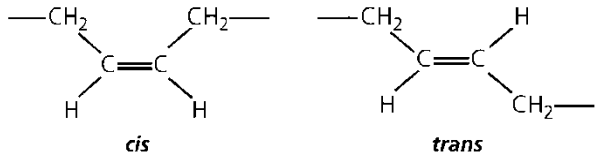
Edible fats and oils (main components)

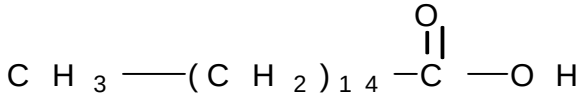


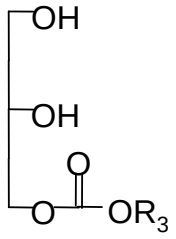
R= CCCCCCCC..
R = CCCC=CCC..
R = CC=CCCC=CC..

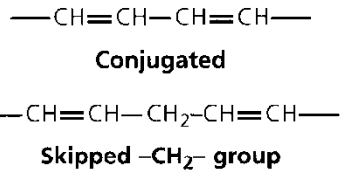
Main compounds

- Triglycerides (approx. 90%)
- Diglycerides (approx. 5%)
- Monoglycerides (approx. 0-5%)
- Free Fatty Acids (approx. 0-1%)

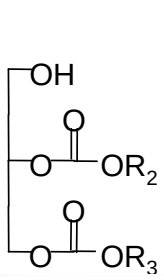


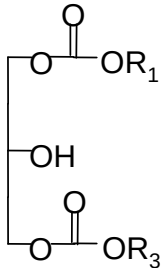


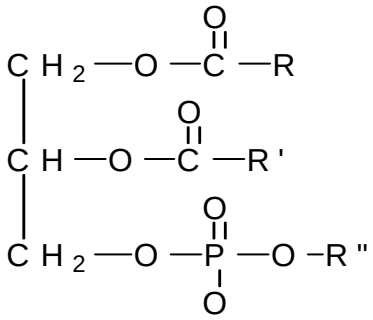


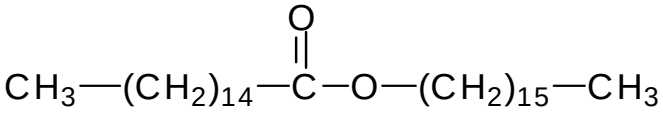


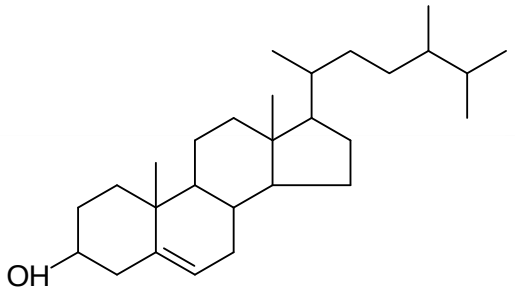
- Phospholipids (approx. 0-2%)
- Sterols (approx. 0-2%)
- Wax esters (approx. 0-300 mg/kg)











Edible fats and oils (minor constituents)

‘Natural’ ingredients

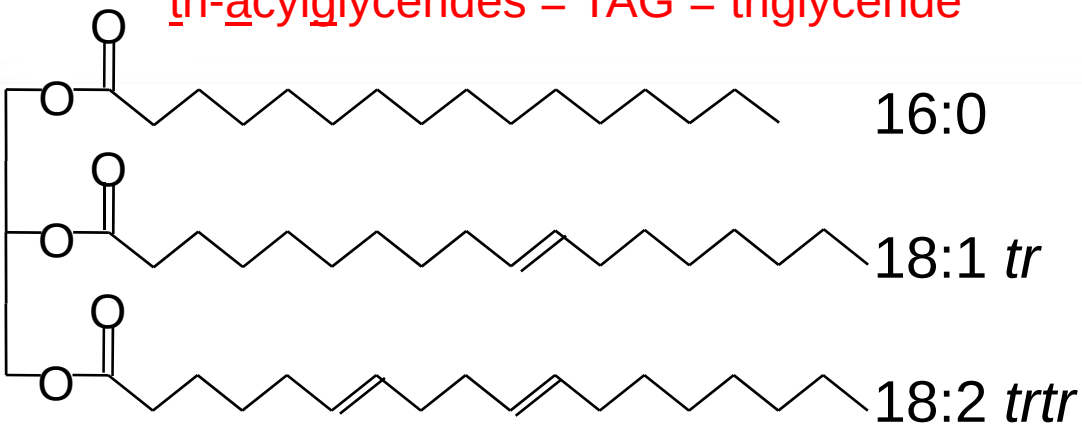
Sterolesters
Glycolipids
Sterolglucosides
Alcohols
Natural antioxidants / vitamins
Carotenoids
Minerals / metals
……..
Steradienes
Alkanes
Oxidized lipids
Polymerised TAGs
……..
Monochloropropanediol esters (MCPD-esters)
Dialkylketones
Glycidyl fatty acid esters

‘Contaminants’

Pesticides
PAHs
Dioxines
Solvents
Stabilisers (BHT, EDTA..)
…….

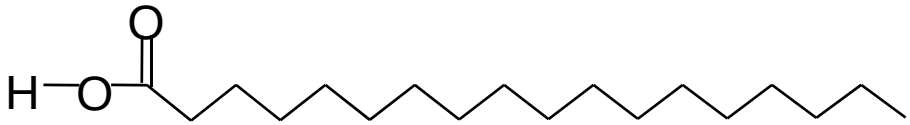
Oils and fats: Structures and reactions

tri-acylglycerides = TAG = triglyceride

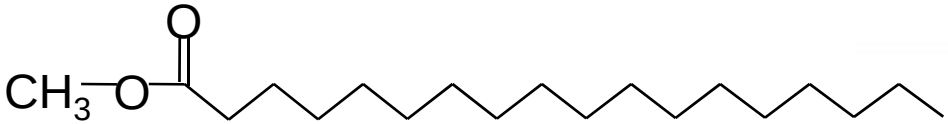


Chain length: 4-**10-20**-24
No. Double bonds: 0-5-10
Positions: All
Orientation: *cis* and *trans*

In vivo, lipase enzyme → FFA



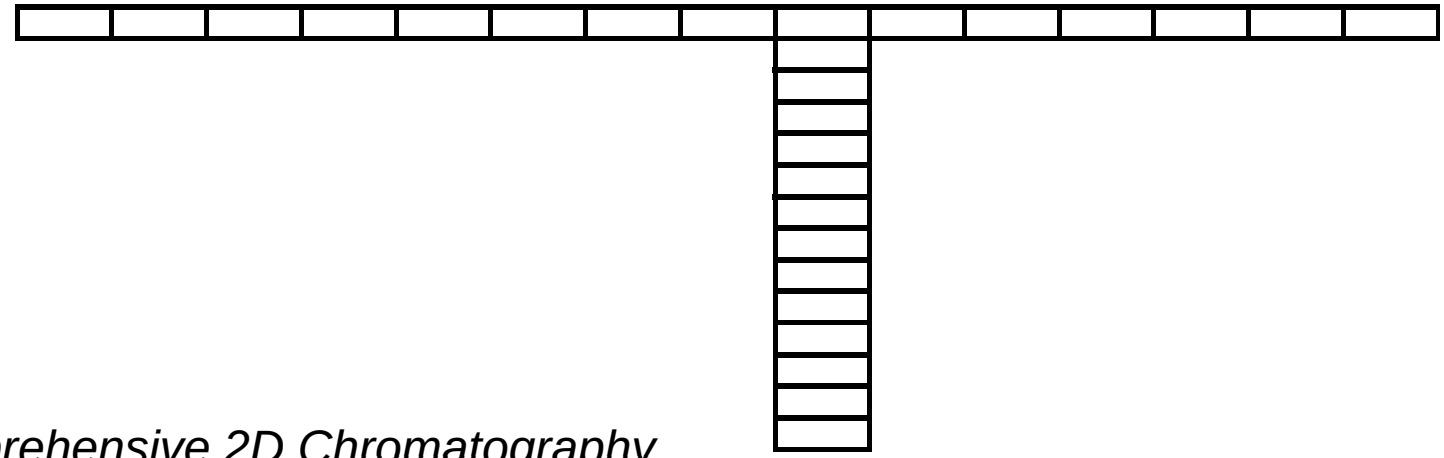
Analysis: Trans-esterification, saponification/methylation → FAME



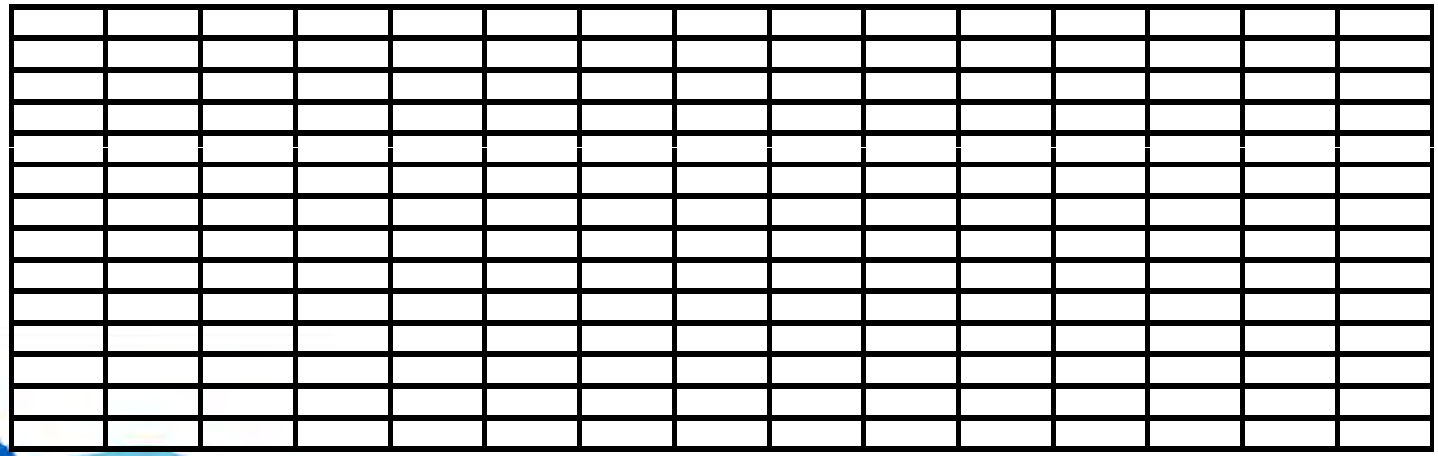
Principle of Comprehensive 2D Chromatography

Normal Chromatography

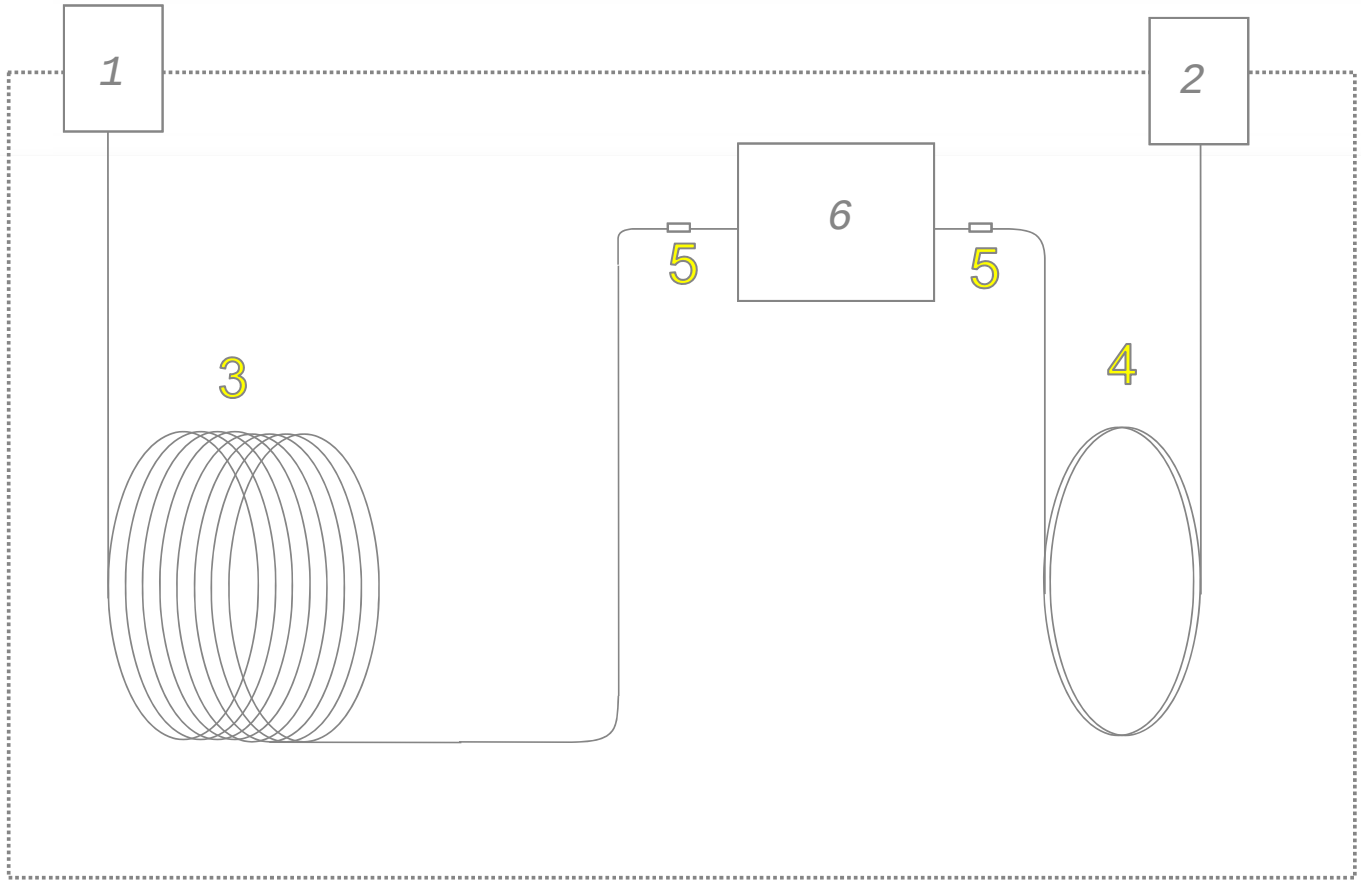
Heart-cut 2D Chromatography



Comprehensive 2D Chromatography

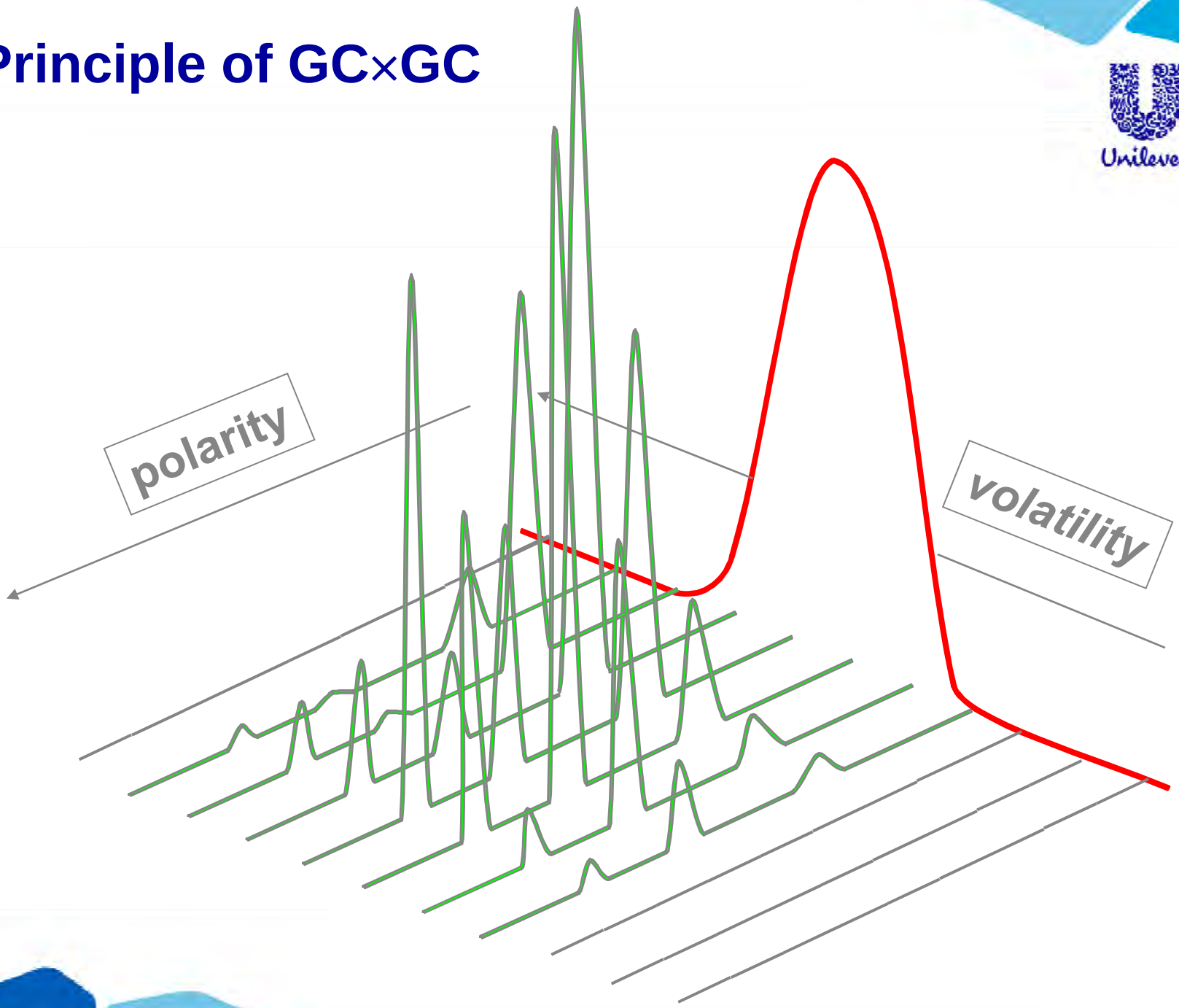


Schematic diagram of a comprehensive GC×GC system



1. Injector, 2. Detector, 3. 1st column, 4. 2nd column,
5. Column connection, 6. modulator

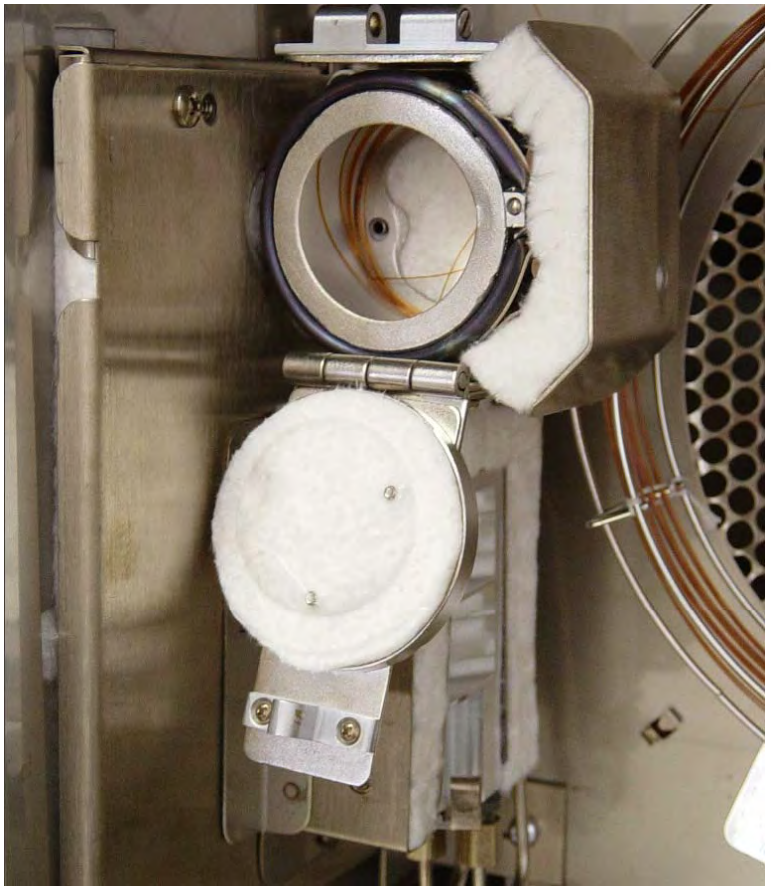
Principle of GC×GC



GC×GC hardware

Modulator

2nd oven



Advantages of GC×GC

Improved chromatographic resolution

Increased peak capacity

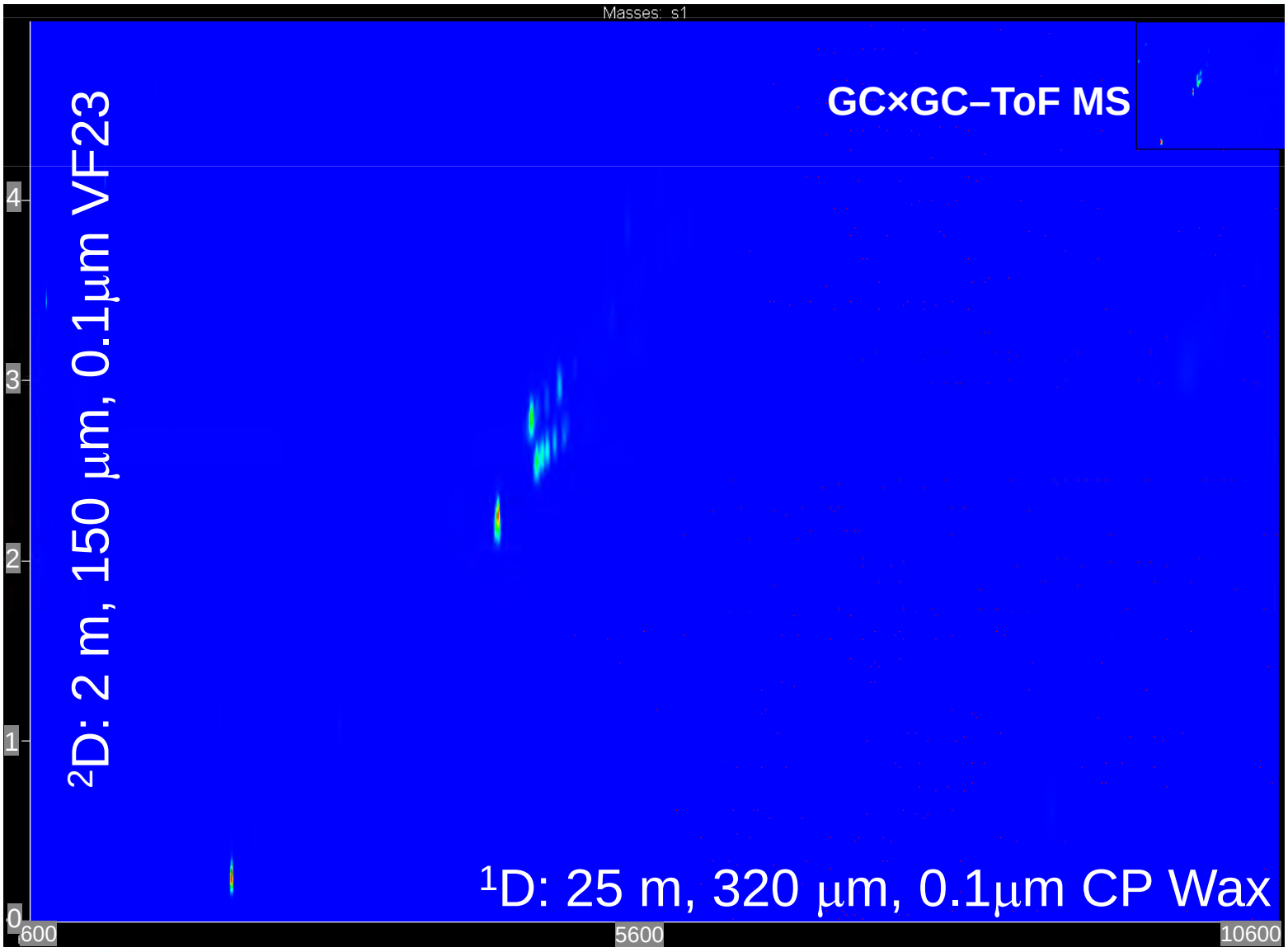
Enhanced signal-to-noise ratios

More effective automated qualitative and quantitative data processing

More information per sample

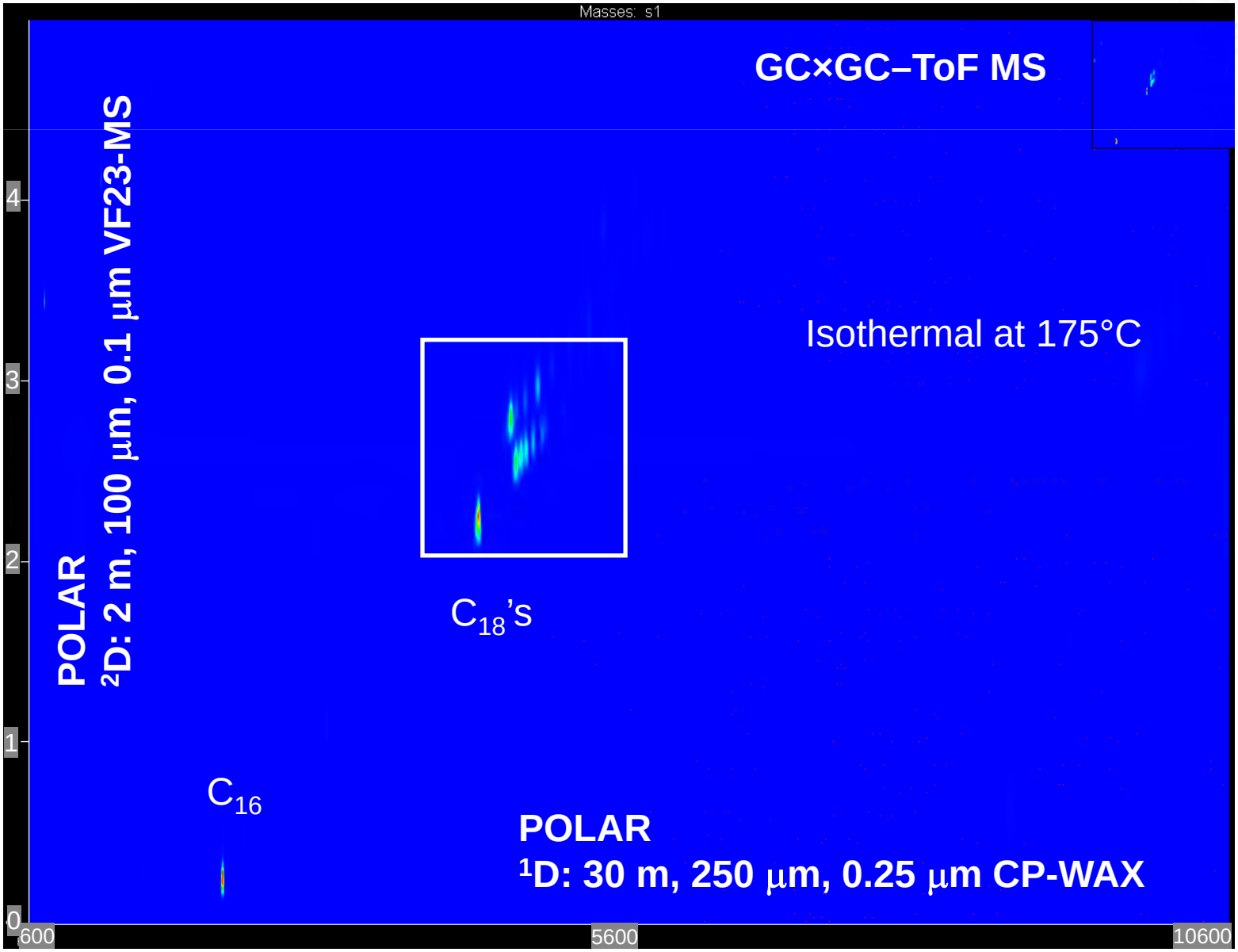
Minimizes dynamic range problems

Comprehensive GC×GC in (*trans*) fatty acid analysis

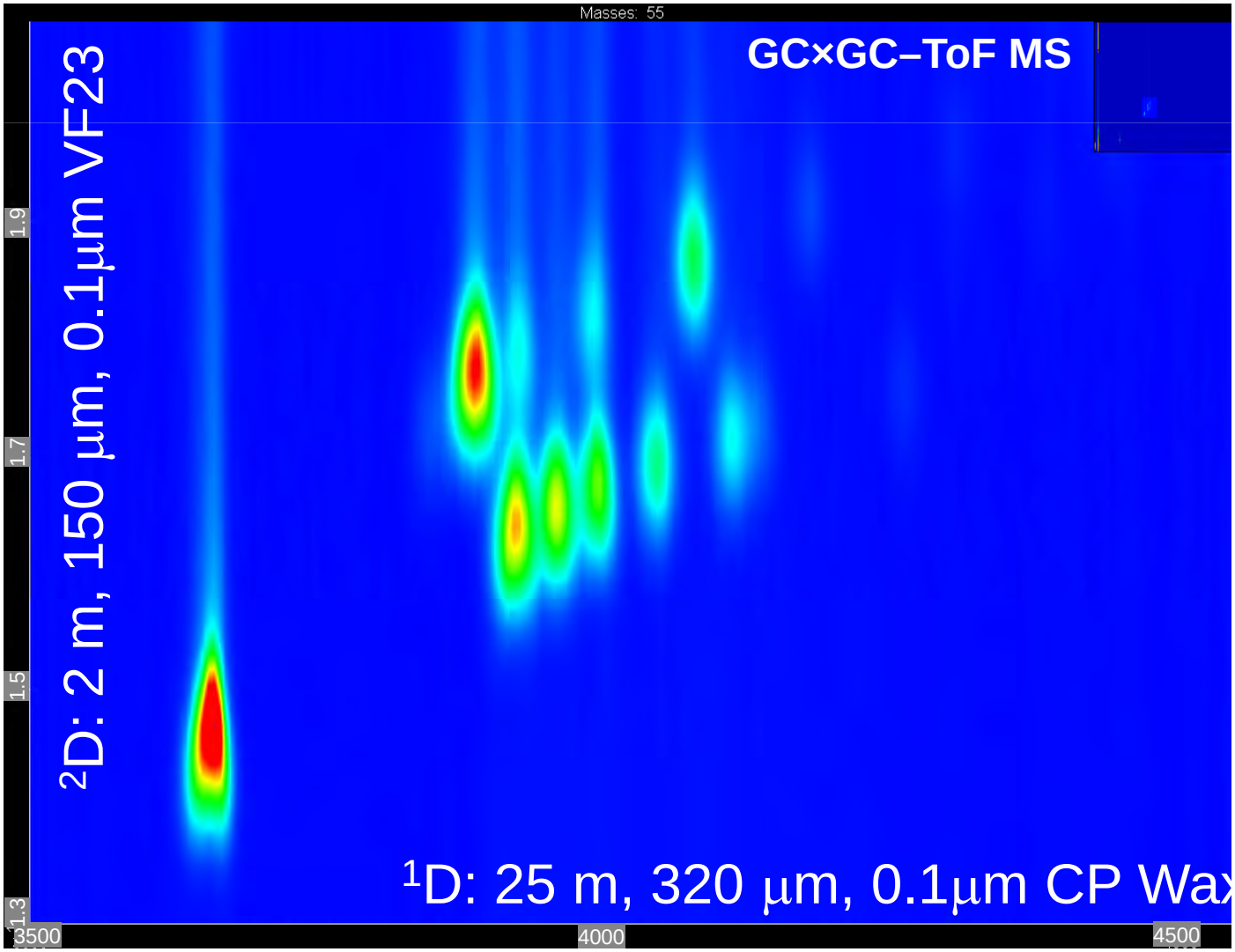


C16 region C18 region

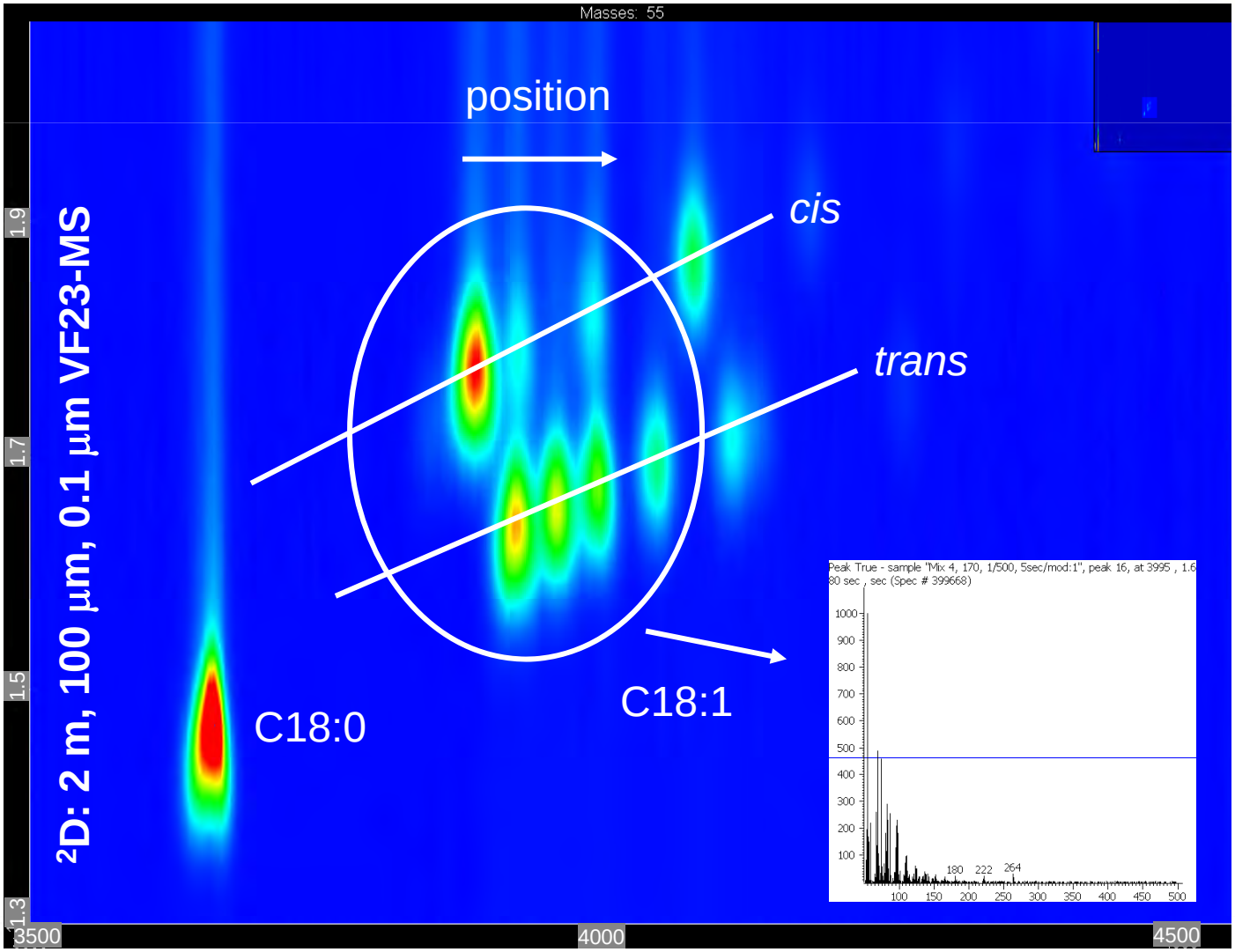
Comprehensive GC×GC in (*trans*) fatty acid analysis



Comprehensive GC × GC in (*trans*) fatty acid analysis



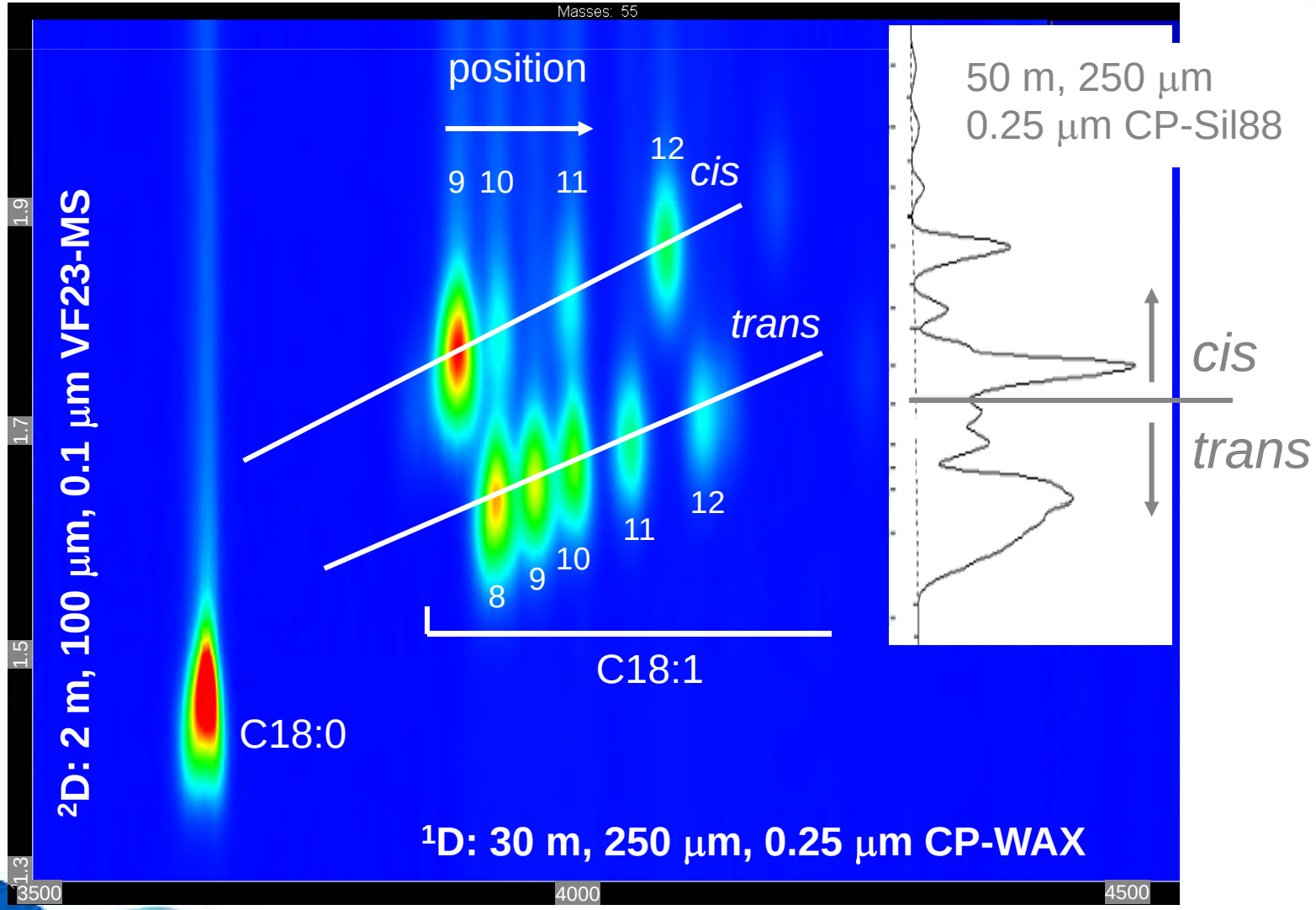
Comprehensive GC×GC in (*trans*) fatty acid analysis



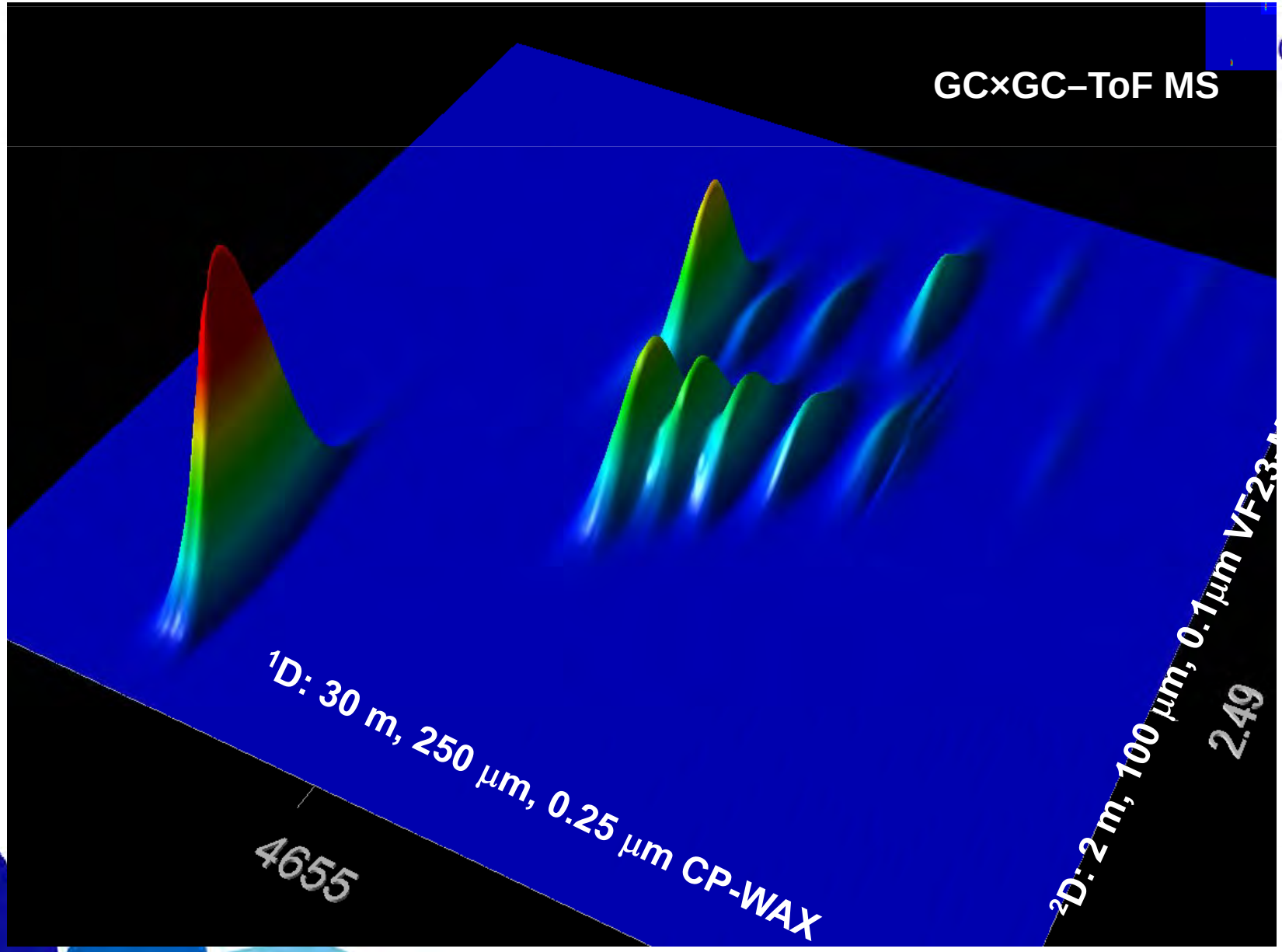
Comprehensive GC×GC in (*trans*) fatty acid analysis

Comprehensive GC×GC

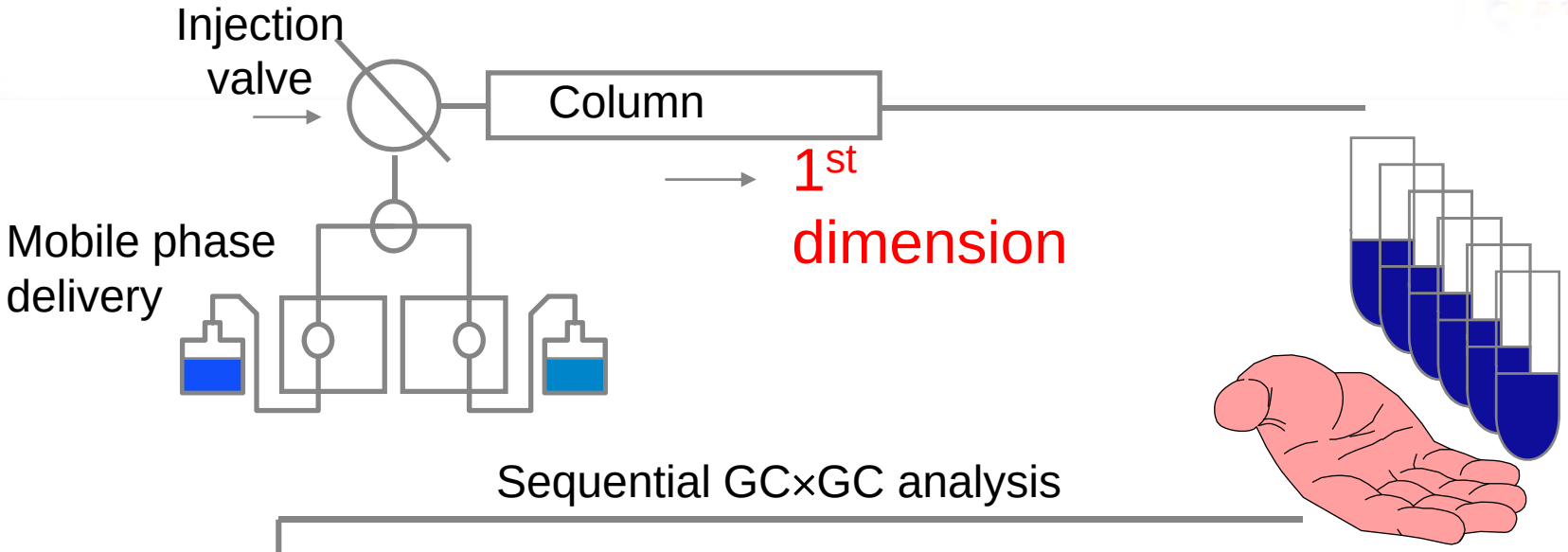
'Normal' analysis



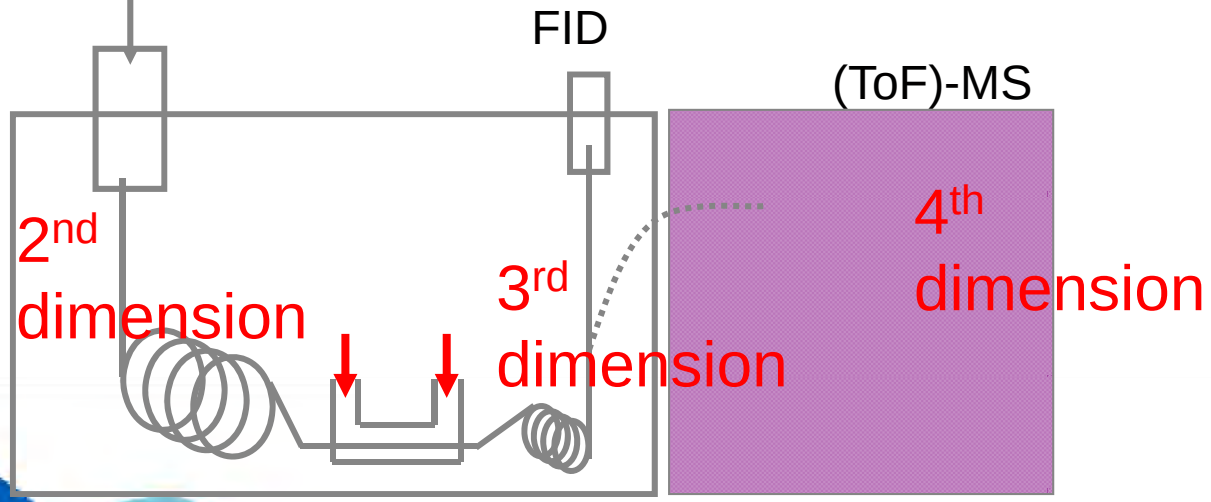
Comprehensive GC×GC in (*trans*) fatty acid analysis



Instrumentation for Comprehensive (off-line) LC×GC (×GC)(-MS)

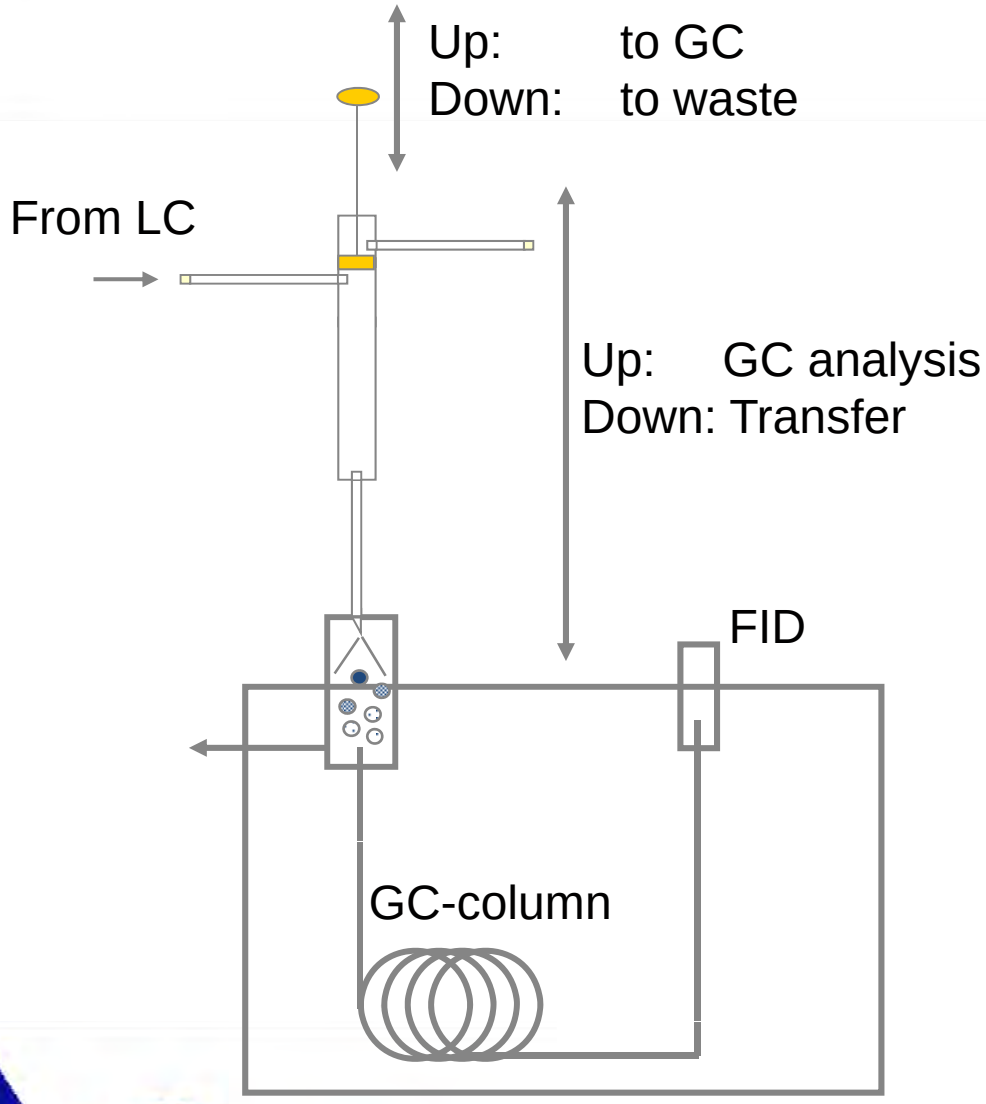


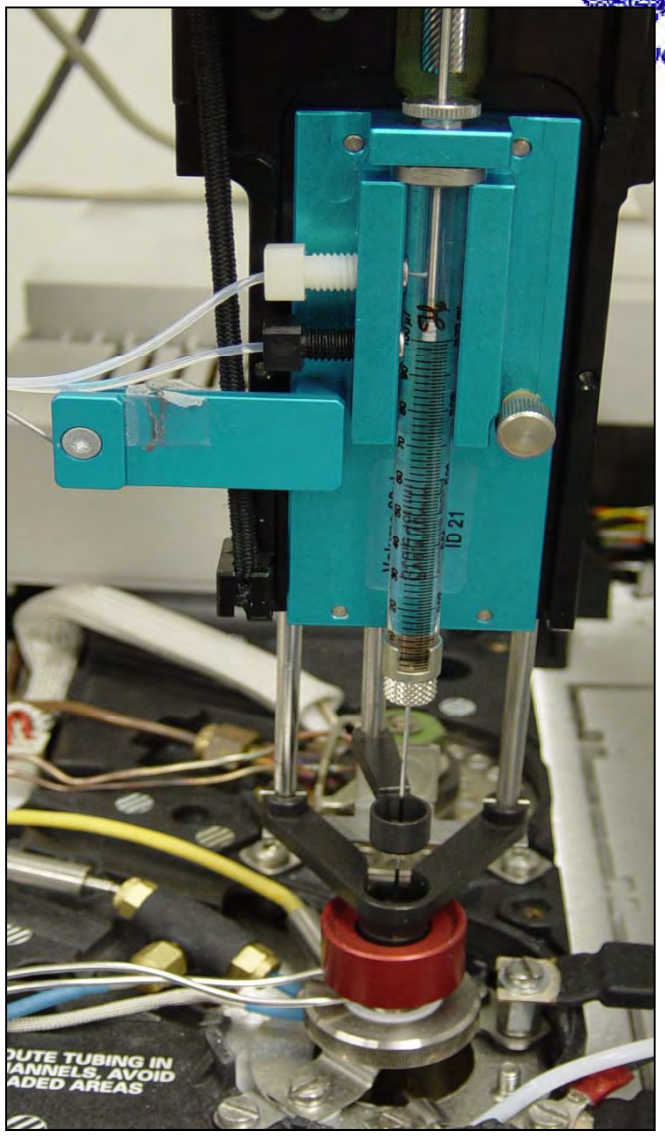
Sequential GC×GC analysis



LC×GC×GC -ToF MS set-up: Syringe interface

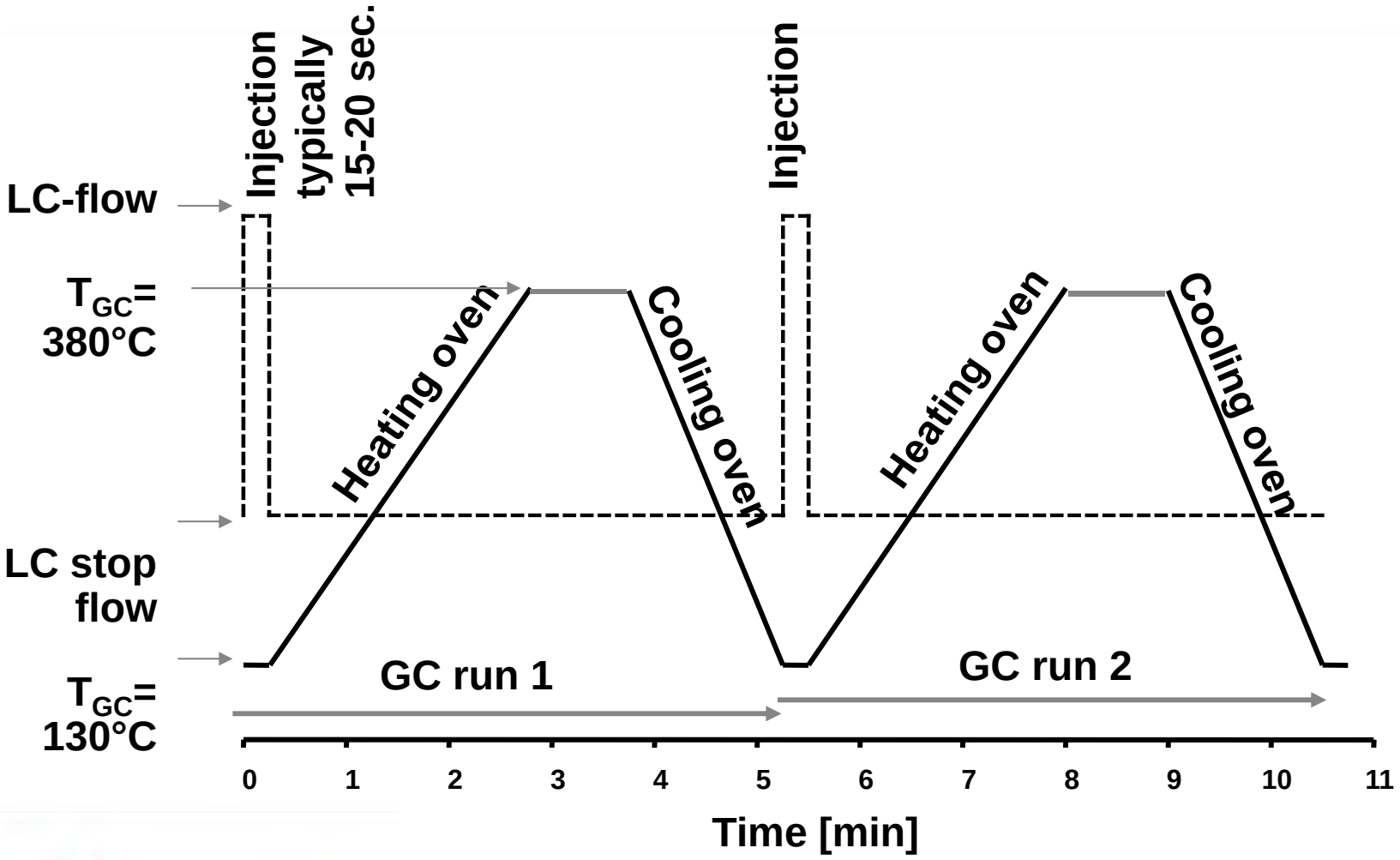
Hardware-interfacing for LC×GC





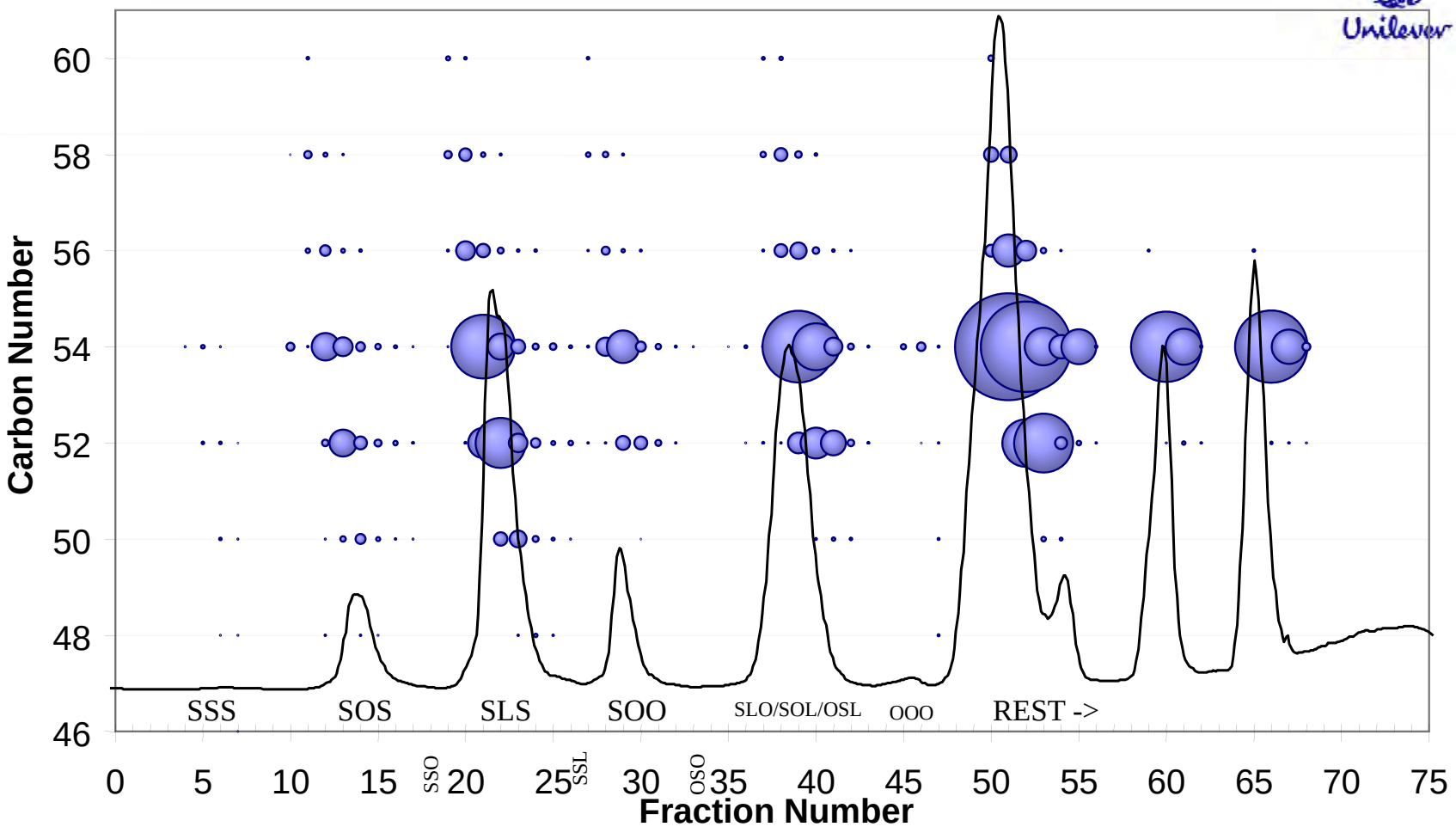
Journal of Separation Science, 27 (**2004)** 397-409

Injection diagram for stop-flow operation



Off-line comprehensive LC×GC

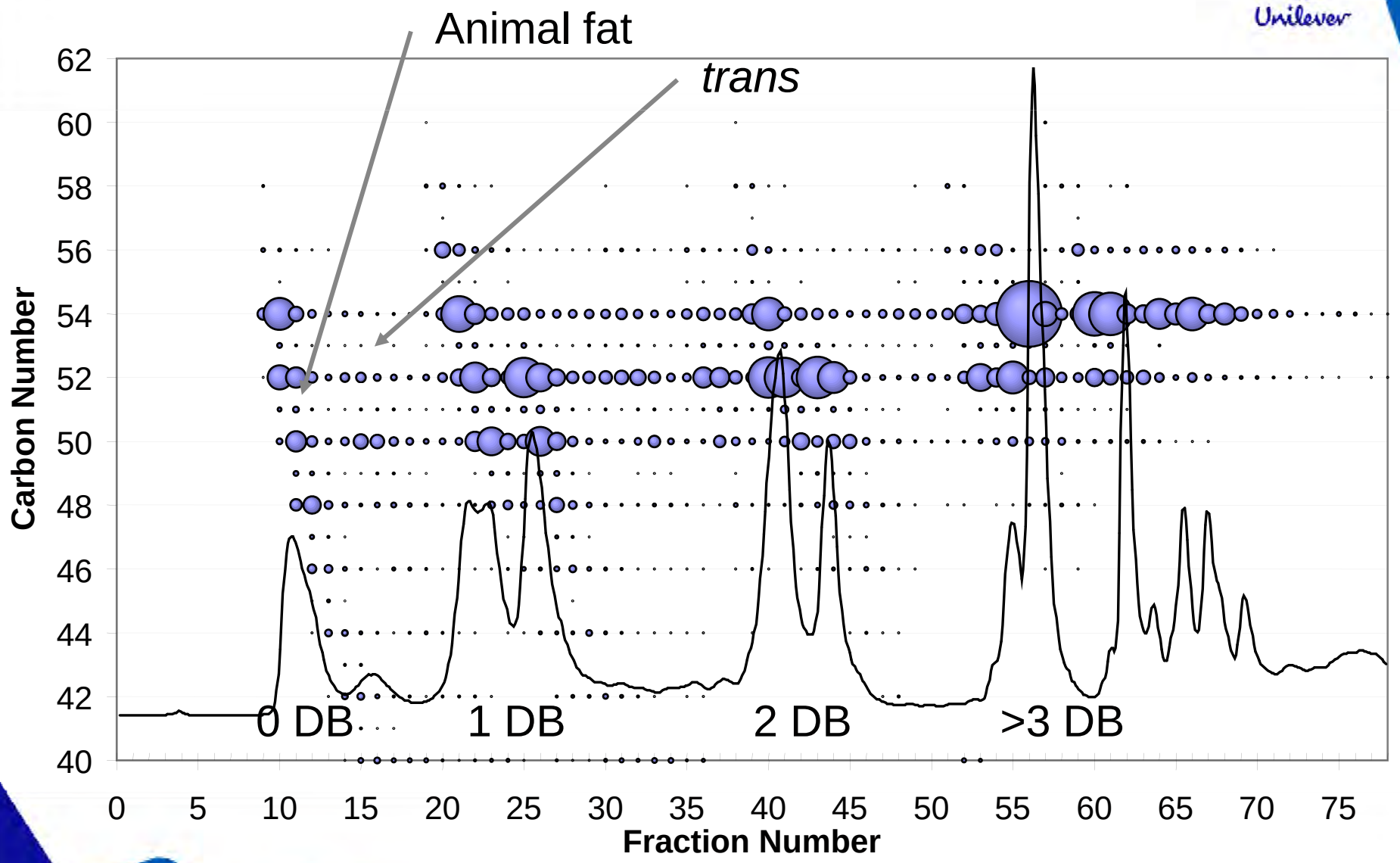
AgLC×Carbon number GC (TAG)



Conditions: AgLC: Ag loaded Silica, 4.6 mm, 10 cm, 3 μm,
From Hx/Tol/EtAC (48.5/50.75/0.75) to Hx/Tol/EtAC (5/72.5/22.5) at 2 ml/min.

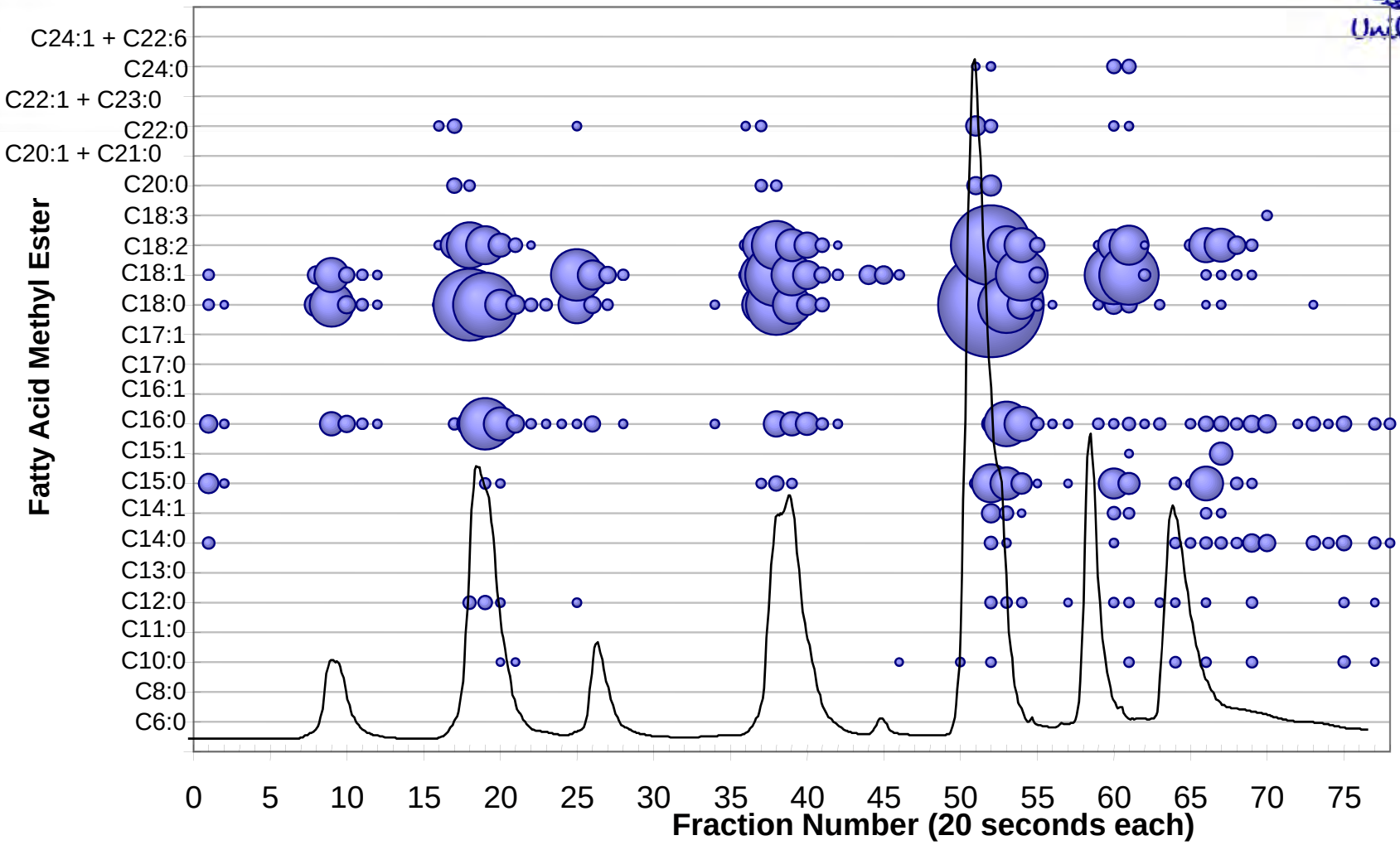
GC: CP-Sil 5 CB, Ultimetal, 10 m, 530 μm, 0.1 μm, 12 ml/min (H_2), 94°C (2 min), 20°C/min, 385°C.

Silver phase AgLC×Carbon Number GC: Competitor product research (TAGs)



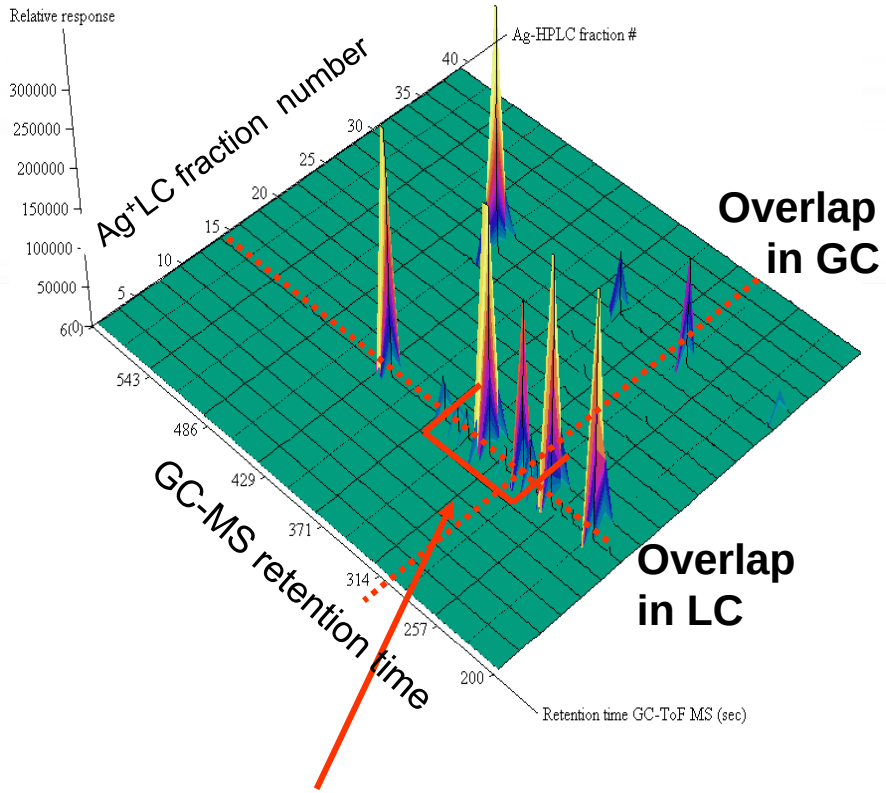
Off-line comprehensive LC×GC

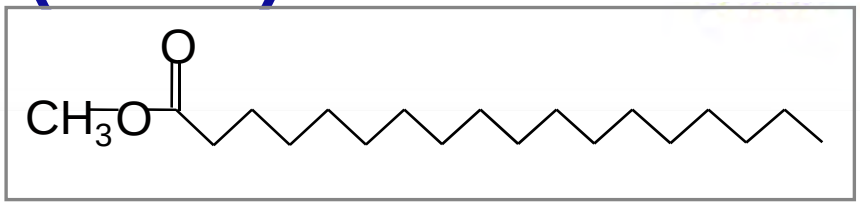
AgLC×FAME GC (in-tact×in pieces)



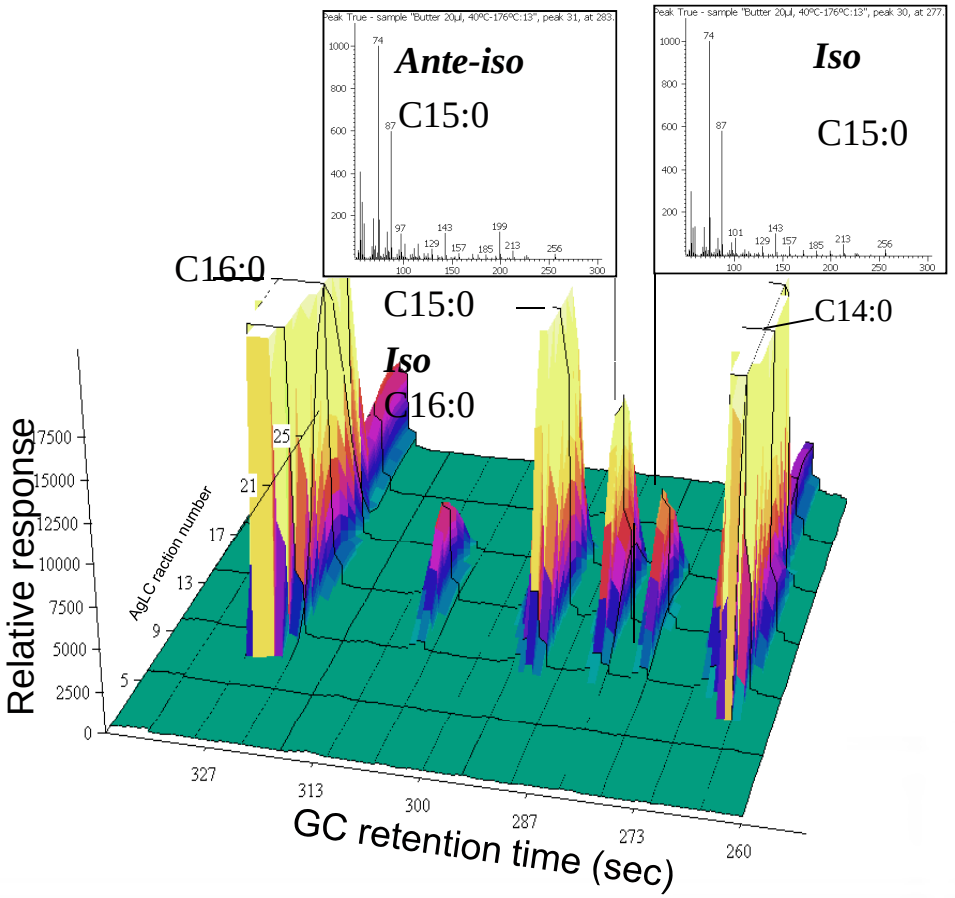
Conditions: AgLC: Ag loaded Silica, 4.6 mm, 10 cm, 3 μm,
From Hx/Tol/EtAC (48.5/50.75/0.75) to Hx/Tol/EtAC (5/72.5/22.5) at 2 ml/min.
GC:(TMSH methylation), CP-wax, 10 m, 100 μm, 0.15 μm, Split, 1 μl, 75°C (2 min), 20°C/min, 265°

Enhanced resolution in $Ag^+LC \times GC$-ToF MS (FAME)





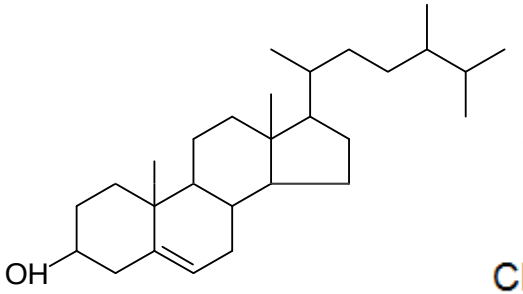
Zoom in + identify

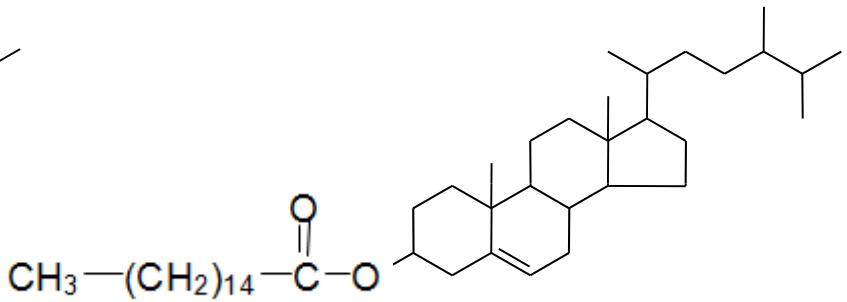


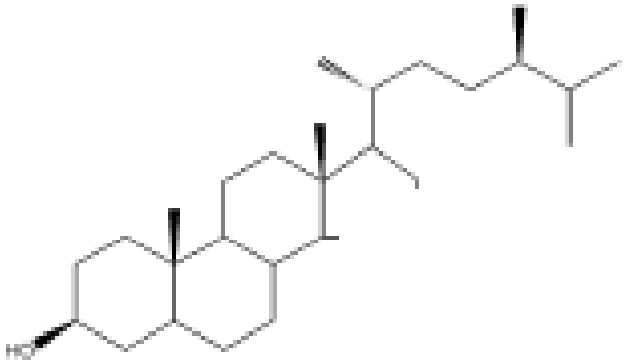
Comprehensive LC×GC better than 120 m GC column

or 5 LC columns!

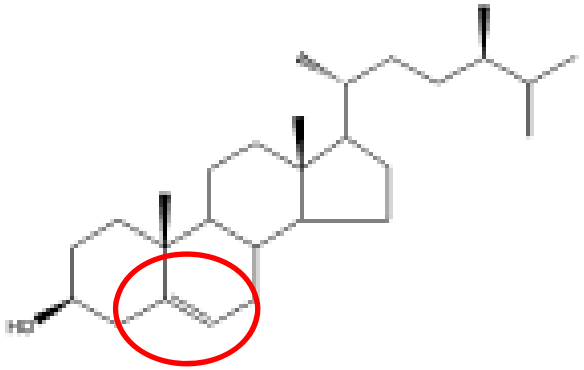
Sterols and sterol esters



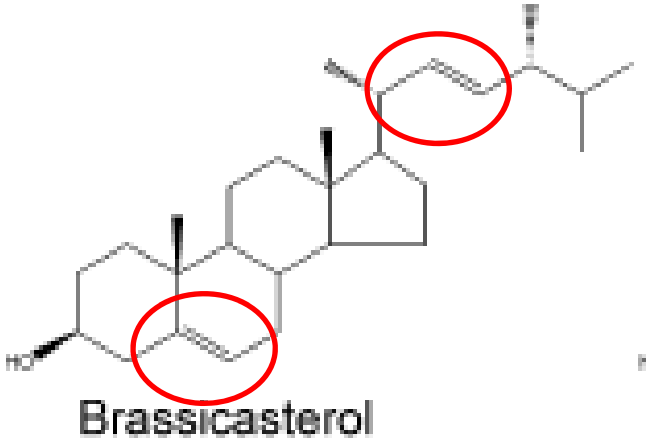




Campestano



Campesterol



Brassicasterol

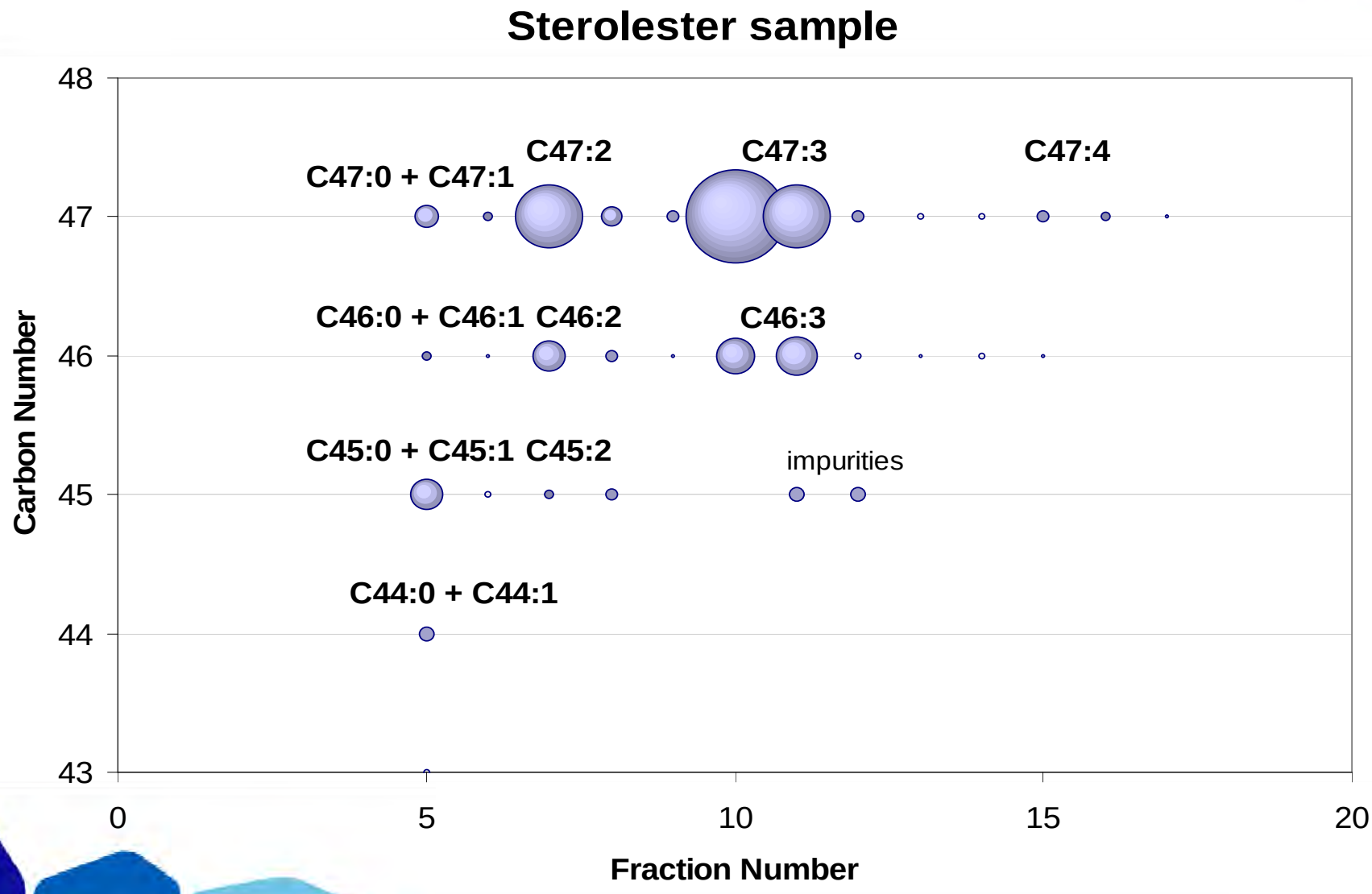
And in the fatty acid chain:

R= CCCCCCCC..

R = CCCC=CCC..

R = CC=CCCC=CC..

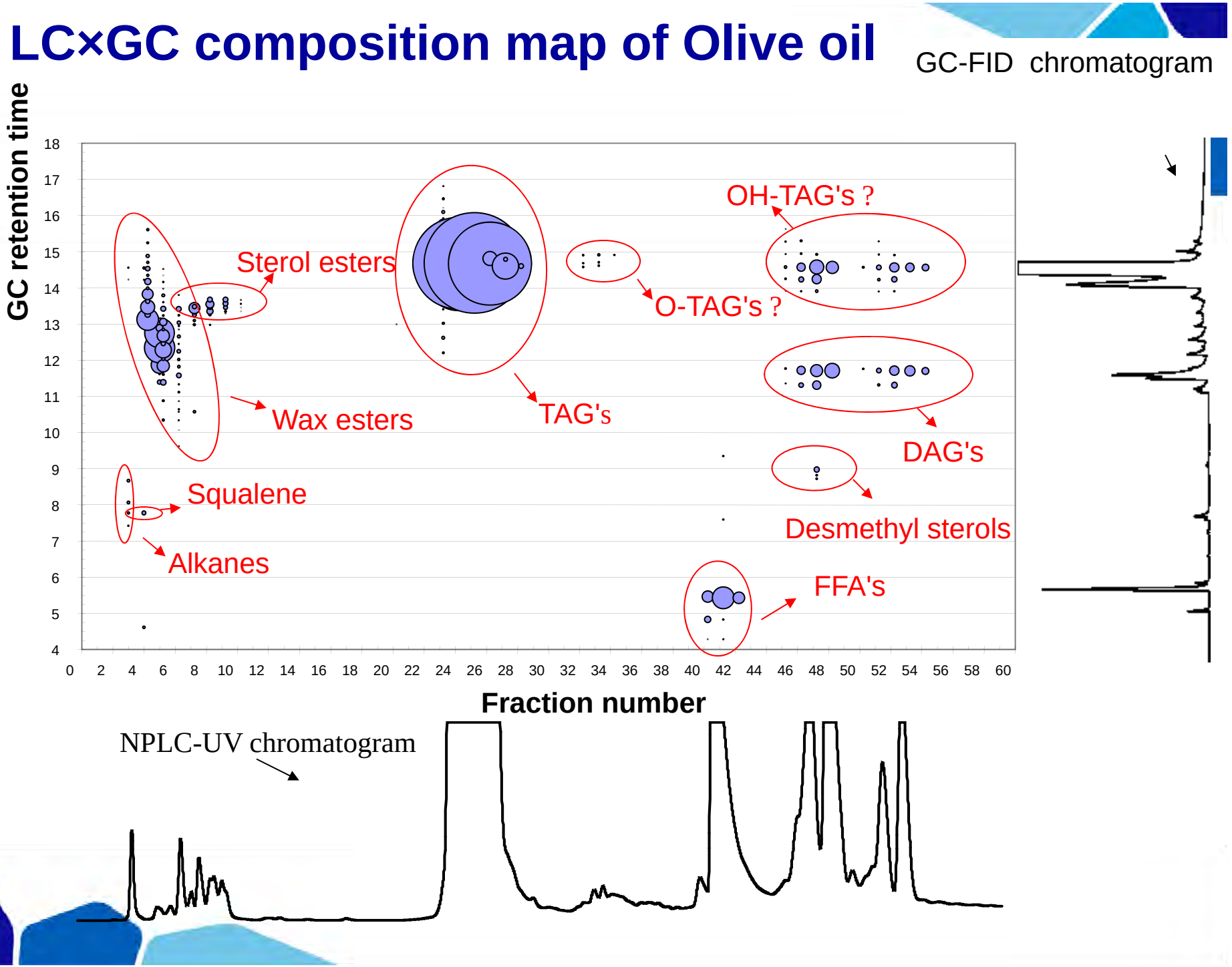
AgLC×GC of a sterolester sample



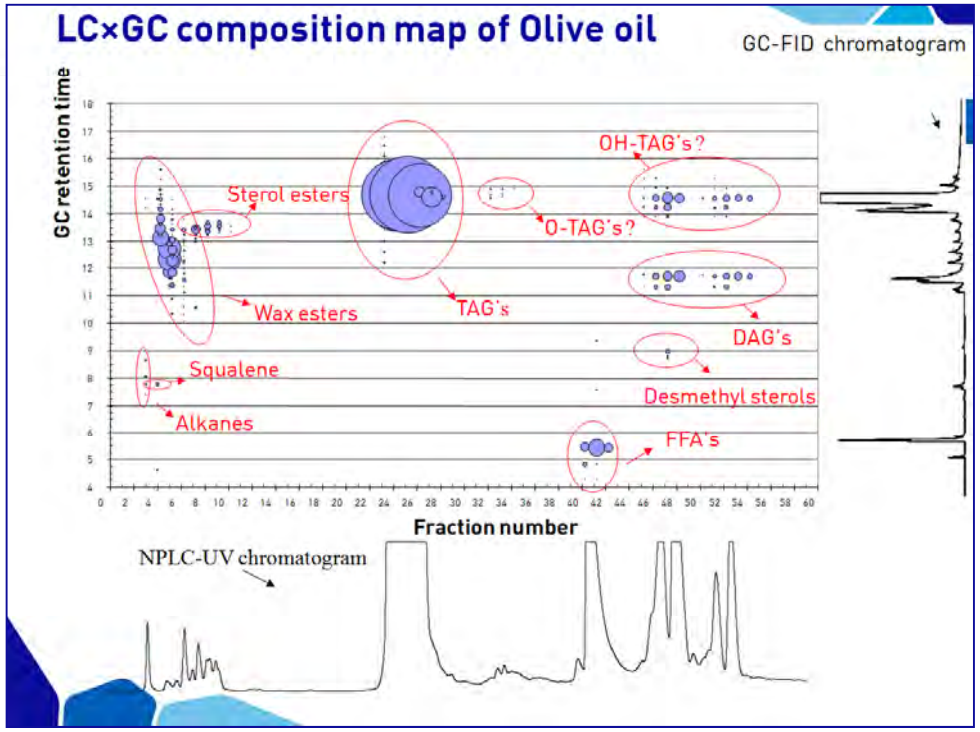
Detailed sterolester composition

Fatty acids :		C16:0	C18:0	C18:1	C18:2
		6.20%	3.80%	29.60%	60.30%
		CN 16	CN 18	CN 18	CN 18
Sterols		No of DB: 0	No of DB: 0	No of DB: 1	No of DB: 2
Campestanol	**0.7%**	0.04%	0.03%	0.21%	0.42%
	CN 28	44	46	46	46
	No of DB: 0	0	0	1	2
Stigmasterol	0.8%	0.05%	0.03%	0.24%	0.48%
	CN 29	45	47	47	47
	No of DB: 2	2	2	3	4
D5-avenasterol	1.1%	0.07%	0.04%	0.33%	0.66%
	CN 29	45	47	47	47
	No of DB: 2	2	2	3	4
Brassicasterol	2.8%	0.17%	0.11%	0.83%	1.69%
	CN 29	45	47	47	47
	No of DB: 2	2	2	3	4
Sitostanol	6.4%	0.40%	0.24%	1.89%	3.86%
	CN 29	45	47	47	47
	No of DB: 0	0	0	1	2
Campesterol	15.9%	0.99%	0.60%	4.71%	9.59%
	CN 28	44	46	46	46
	No of DB: 1	1	1	2	3
Sitosterol	72.2%	4.48%	2.74%	21.37%	43.54%
	CN 29	45	47	47	47
	No of DB: 1	1	1	2	3

LC×GC composition map of Olive oil



NPLC as sample preparation for GC (off-line or on-line)



Aim: isolate specific compound classes for futher separation and quantification by GC-MS

<u>Target compound groups:</u>

- Sterol
- Sterolesters
- Waxesters
- Partial glycerides
- **Glycidyl fatty acid esters**

Experimental conditions: Glycidylesters by GC

Standards
GE-C12:0, GE-C14:0, GE-C16:0-d31, GE-C16:0, GE-C18:0, GE-C18:1, GE-C18:2, GE-C18:3.

Equipment
Agilent 7890A GC with cold-on-column and split/splitless injector.
Agilent 5975C inert XL mass selective detector.

Columns
On-column: 15 m x 0.25 mm x 0.10 µm DB-5ms (pre-column 1 m x 0.53 mm apolar deactivated).
Splitless: 5 m x 0.10 mm x 0.2 µm Carbowax or 15 meter x 0.25 mm x 0.50 µm Carbowax.

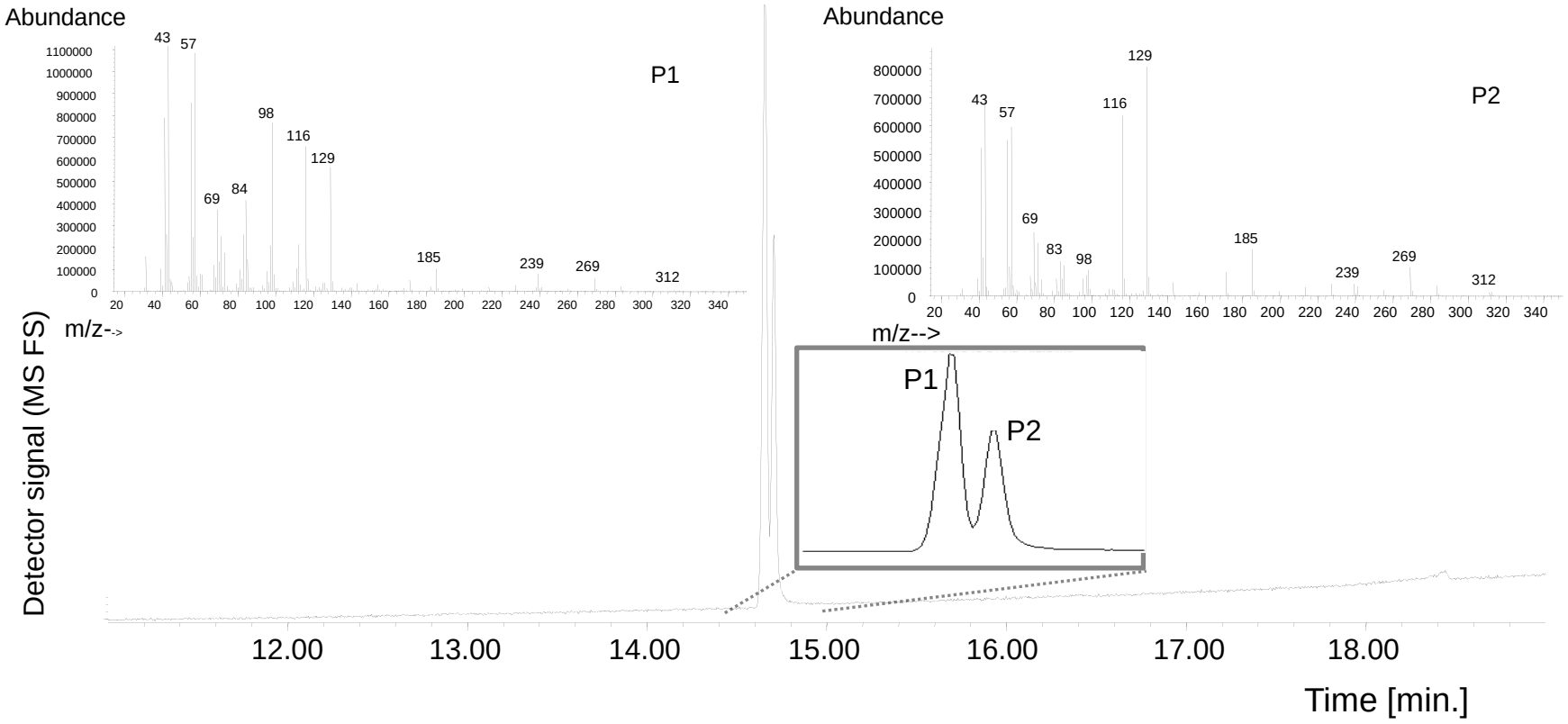
Operating conditions
Helium at 150 kPa (splitless injection) or 2 ml/min (on-column)
Injection volume 1 µL.
Temperature from 60 °C (on-column) or 110 °C (splitless) to 260 °C at 10 °C/min.

MS SIM ions

	Target ion	Qualifier 1	Qualifier 2
GE-C16:0-d31	119.1	133.0	X
GE-C12:0	116.0	129.0	183.1
GE-C14:0	116.0	129.0	185.1
GE-C16:0	116.0	129.0	X
GE-C18:0	129.0	116.0	185.1
GE-C18:1	129.0	116.0	185.0
GE-C18:2	67.1	79.1	95.0
GE-C18:3	79.1	67.1	95.0

Method development GC analysis II: GC-MS of intact glycidyl esters

Understanding the degradation behaviour of glycidyl esters. Deliberately select conditions where degradation occurs (i.e. on-column injection on a 1 m x 0.53 mm apolar retention gap, press-fit connector, 15 m x 0.25 mm x 0.10 µm DB-5ms analytical column).

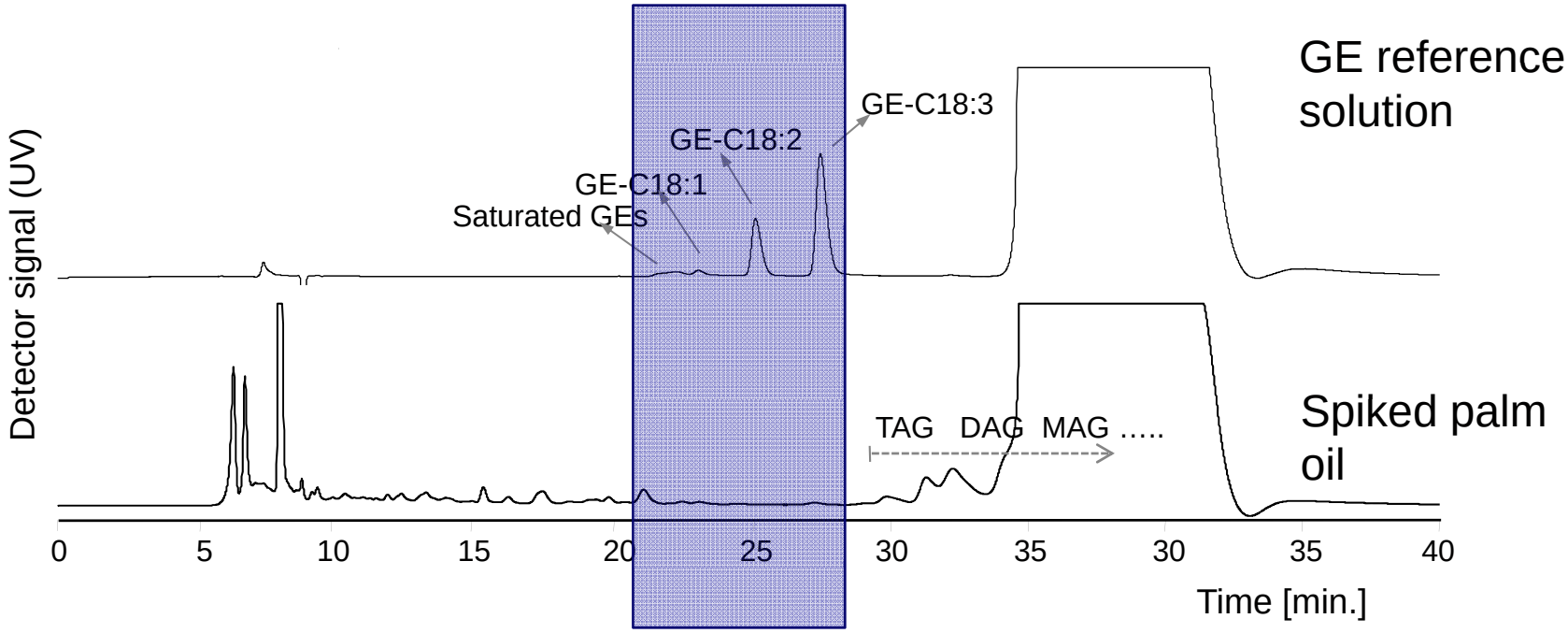


Double peaks are seen. MS spectra show identical mass fragments, albeit at different relative abundances. Structures and degradation routes are yet unknown.

Method development sample preparation II: NPLC method

Normal phase TLC, SPE and LC are widely used for isolating specific compound-classes from edible oils and fats.

Glycidyl ester are slightly less polar than triacylglycerides.



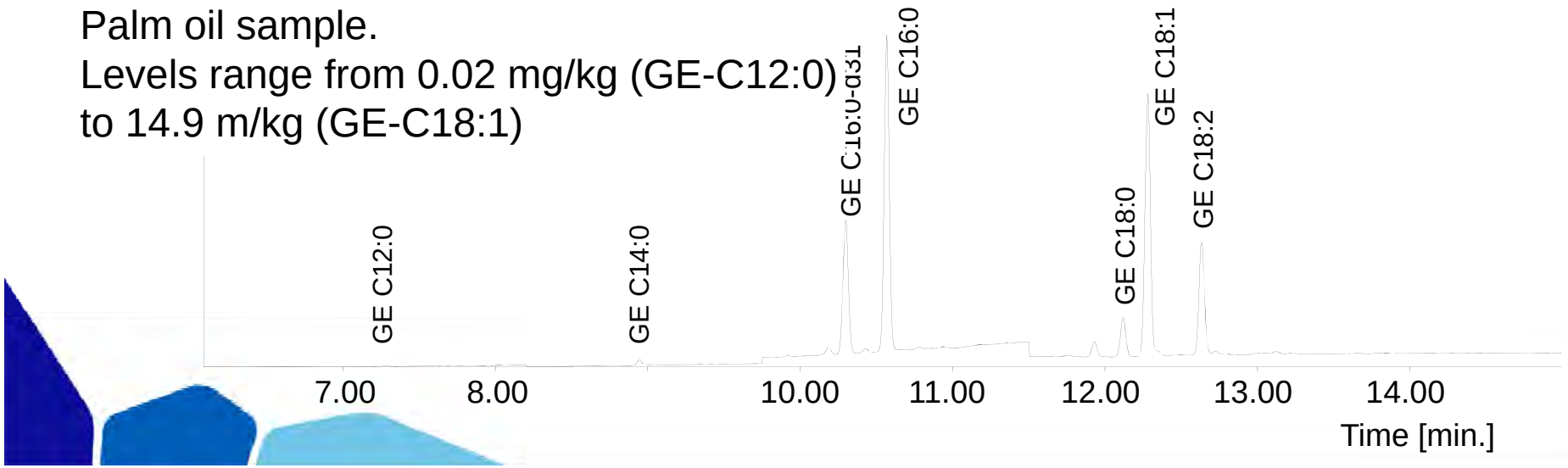
8 min = 8 mL

The NPLC step provides efficient isolation of the glycidyl-esters, but unfortunately only at low injected amounts. Enrichment prior to NPLC isolation is needed.

GC-MS analysis: the final method (submitted)

1. 100 mg of oil (containing GE-C16:0-d 31) is dispersed in 4 mL of acetonitrile.
2. The oil is slightly warmed and vigorously mixed for 20 second.
3. The acetonitrile phase is washed with 2 mL of heptane.
4. Coextracted glycidyl-esters are recovered from the heptane by acetonitrile extraction.
5. The solvent is evaporated under nitrogen at 35 °C.
6. The residue is redissolved in 1 mL hexane/isopropanol (85/15 v/v).
7. 100 µl of the extract are separated by gradient HPLC.
8. The glycidyl ester fraction is collected and evaporated (under nitrogen, at 35 °C).
9. The residue is redissolved in 40 µl chloroform.
10. 1 µL of the final sample is injected in GC-MS using splitless injection. MS detection is by SIM.

Palm oil sample.
Levels range from 0.02 mg/kg (GE-C12:0) to 14.9 m/kg (GE-C18:1)



LC-GC-MS method for glycidyl ester analysis
Validation results – Detection limits

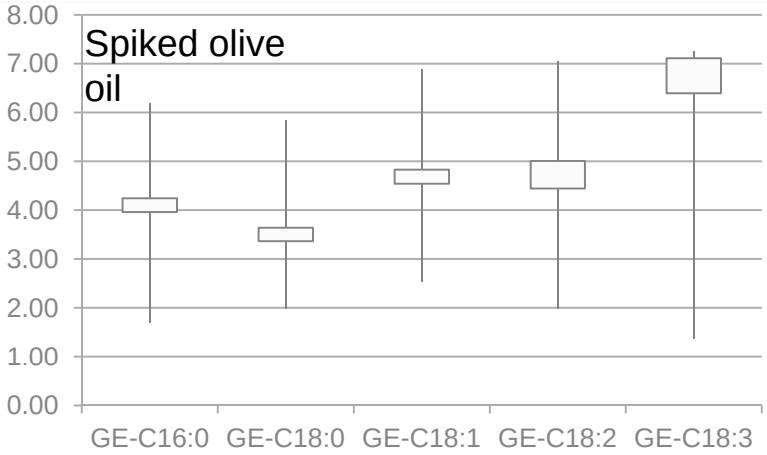
The LOD and LOQ were estimated from a concentration level that gives a peak with a signal-to-noise ratio of 4:

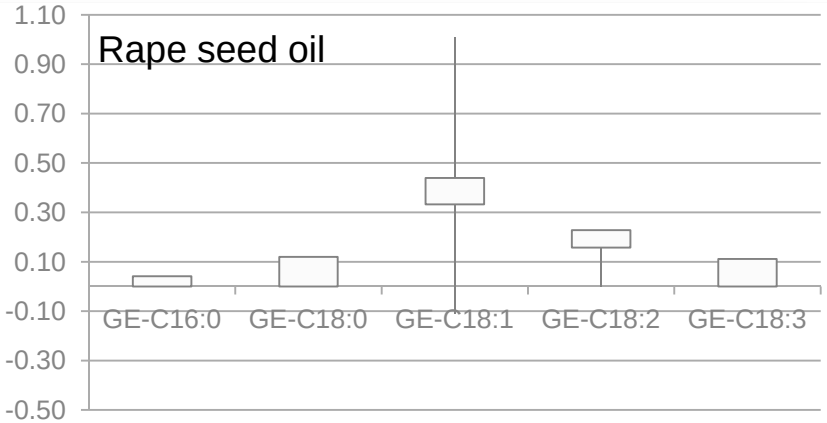
LOD ≈ 0.01 mg/kg per individual glycidyl ester.
LOQ ≈ 0.05 mg/kg per individual glycidyl ester (≈ 0.01 mg/kg as free glycidol).

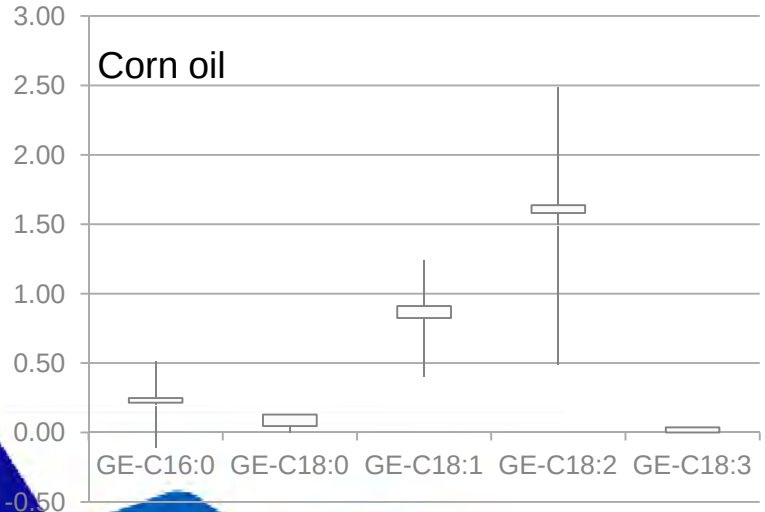
Detection limits are highest (poorest) for GE-C18:3 as a result of the extensive MS fragmentation. The lower MW ions suffer more from interferences.

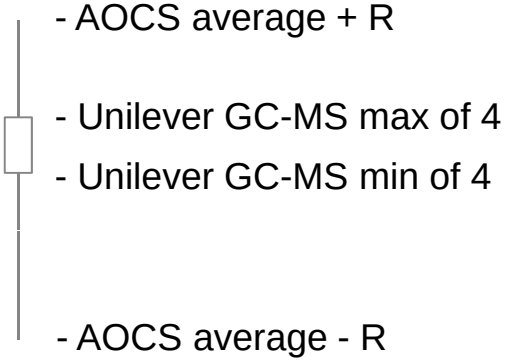
GC-MS method: Validation results – Trueness

The trueness of the method was assessed by re-analyzing the samples from the collaborative AOCS ringtrial at different time points (Aug. 2012 - April 2013)









Collaborative study for the analysis of glycidyl fatty acid esters in edible oils using LC-MS. Blumhorst et al., J Am Oil Chem Soc 2013 90:493-500

Conclusions Glycidyl esters by GC-MS

- GC-MS can be reliably used to quantify intact glycidyl esters in edible oils.
- NPLC isolation after ACN extraction gives very clean fractions.
- The final method is rather similar to other methods used in edible oil analysis (e.g. sterol analysis, waxesters, partial acylglycerides etc.)
- Detection limits are better than 0.05 mg/kg glycidol.
- Quantitative data from our new method agree very well with data from the AOCS ringtrial.
- The method proofed to be robust: so far over 450 samples were analysed without problems.

Overall Conclusions