

Introducing Supercritical Fluid Chromatography in the Community of Multidimensional Chromatography.

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Analysis of natural resources
sources and/or cultivation conditions

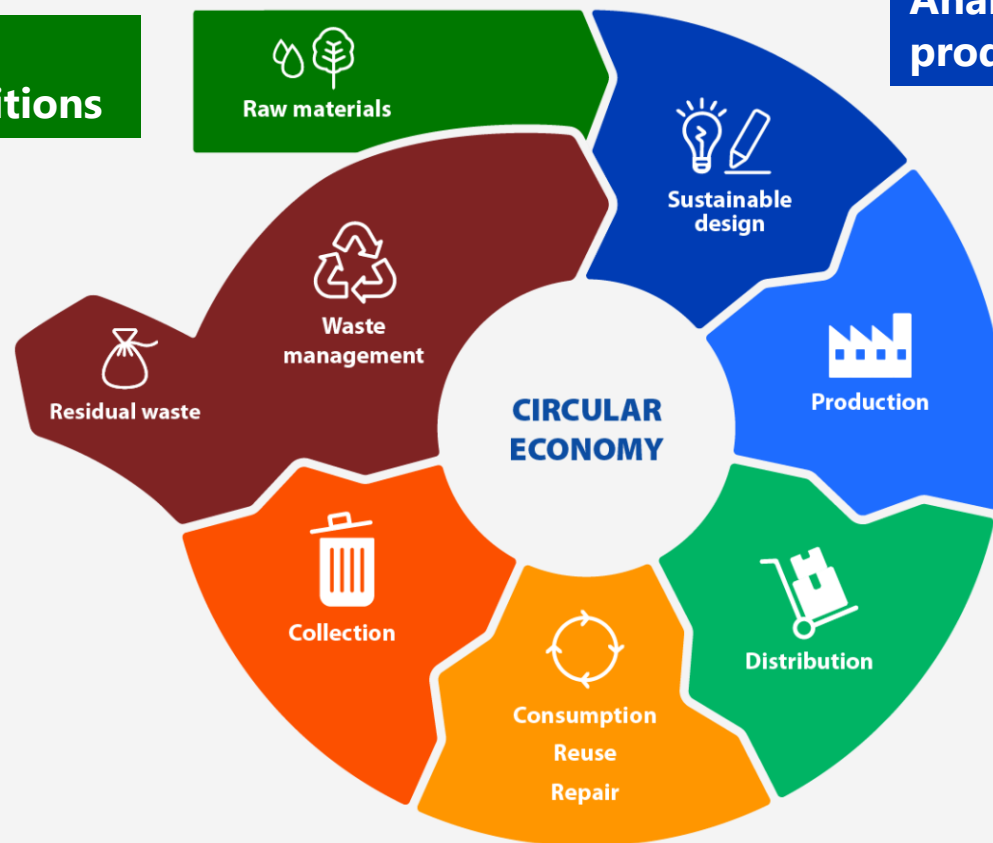
Looking for valuable
molecules in waste



Need for non-target analysis
of highly complex samples:

2D Chromatography – HRMS
(of course...)

The circular economy model:
less raw material, less waste, fewer emissions



Source: European Parliament Research Service

Analysis of transformation
products during recycling paths



Checking the proper
incorporation of plant-based
molecules in industrial products





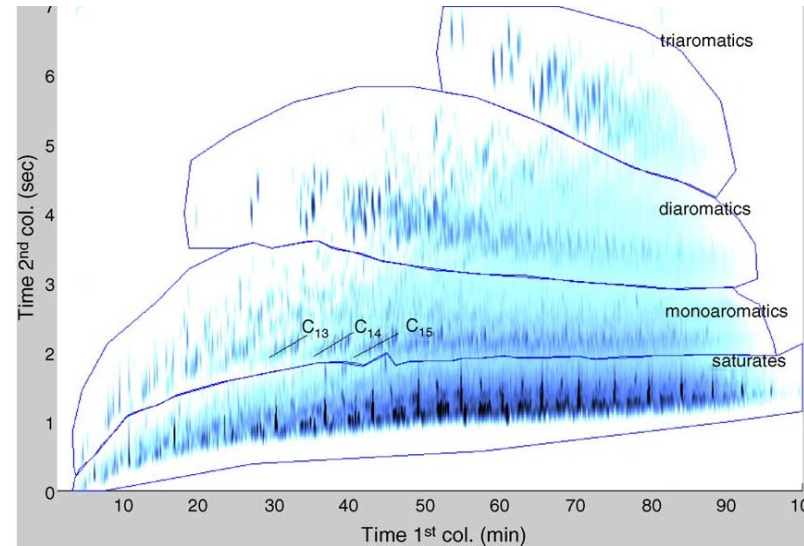
Orthogonality is essential in 2D chromatography

(semi) volatile compounds



GC × GC

apolar column x polar column

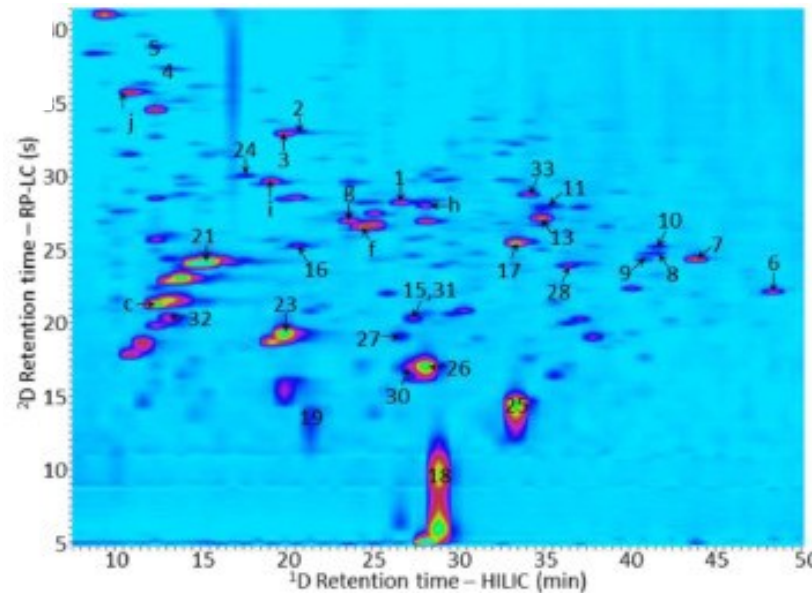


diesel cut
Vandeuve et al.
J.Chromatogr. A 1086 (2005) 21-28

non volatile compounds



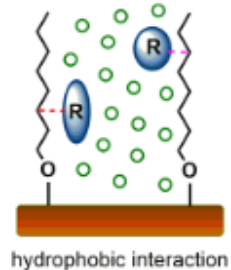
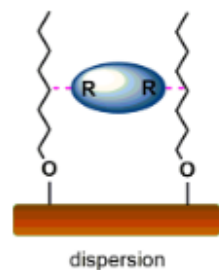
LC × LC



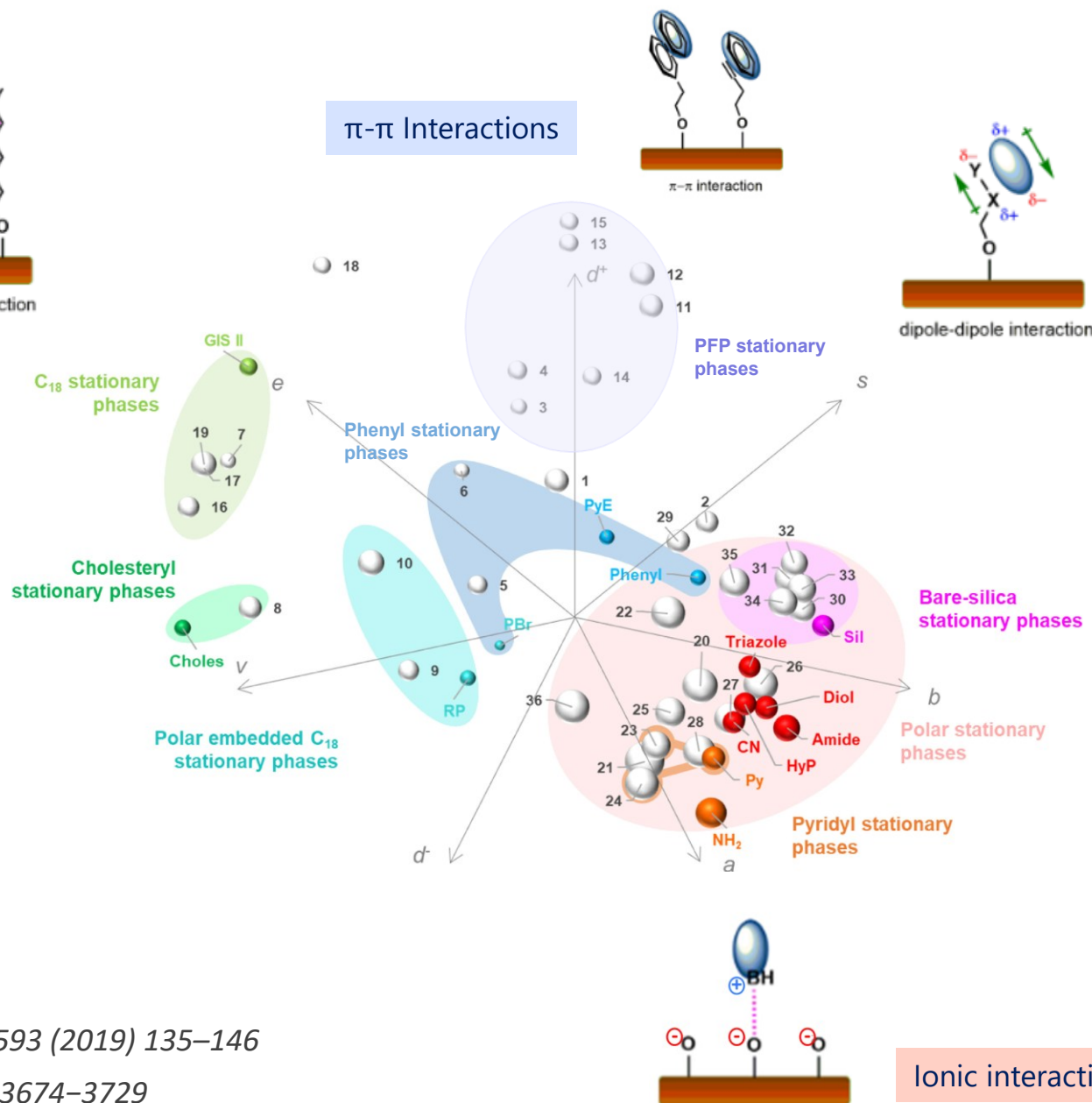
phenolic acids in red wine
Muller et al. .
J.Chromatogr. A 1692 (2023) 463843



Chemical interactions in liquid chromatography



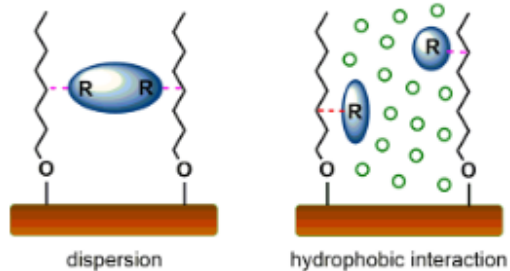
Hydrophobic Interactions



West et al., *J. Chromatogr. A* 1593 (2019) 135–146

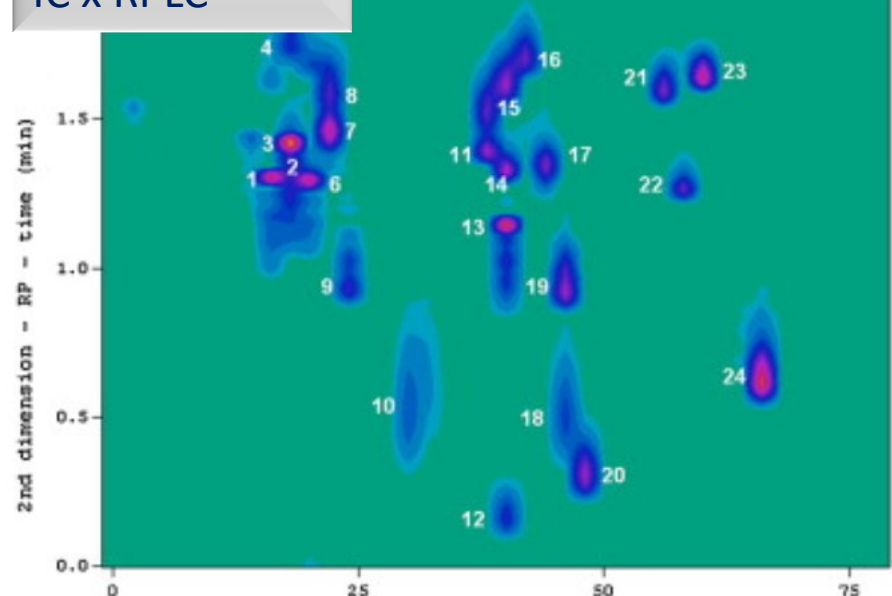
Zuvela *Chem. Rev.* 119 (2019) 3674–3729

Ionisable compounds separation in LCxLC



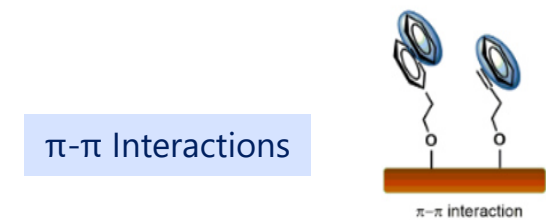
Hydrophobic Interactions

IC x RPLC

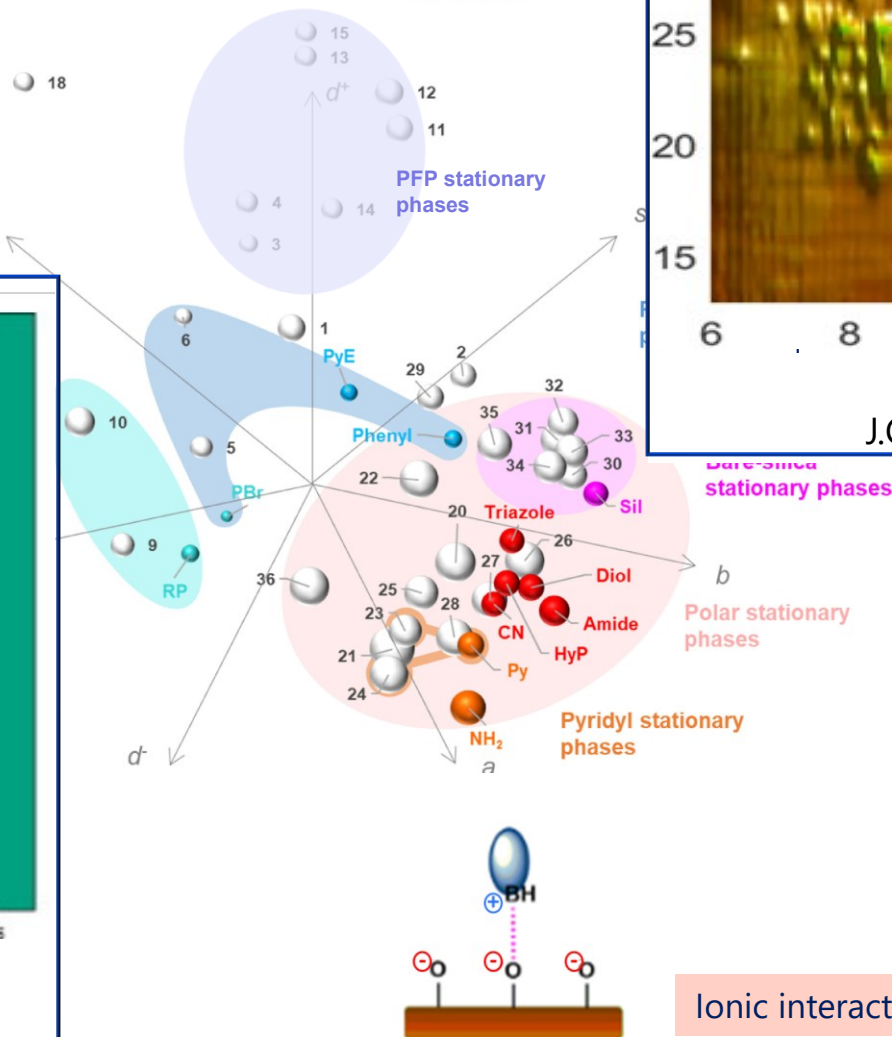


Brudin et al.
J.Chromatogr. A 1217 (2010) 6742

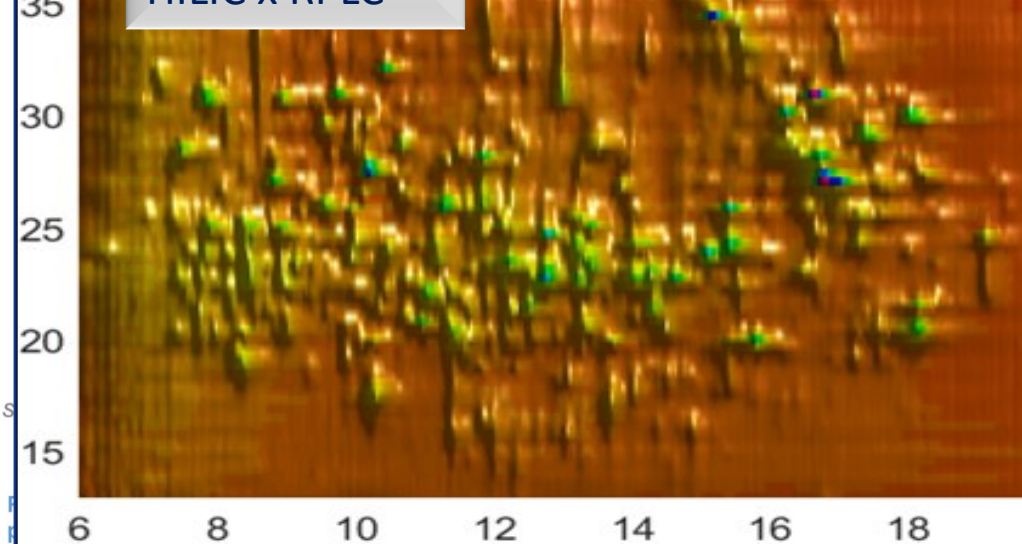
π - π Interactions



C₁₈ stationary phases

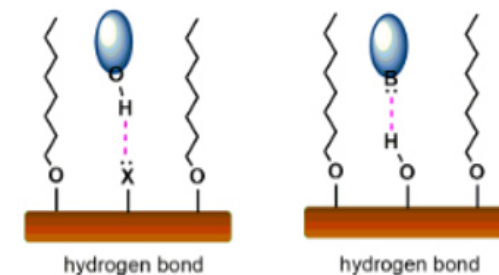


HILIC x RPLC

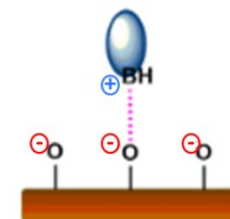


Chapel et al.
J.Chromatogr. A 1615 (2020) 460753

Hydrogen bonding interactions

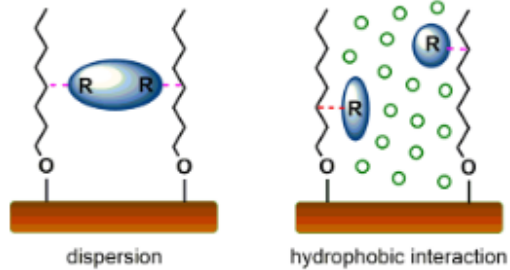


Ionic interactions



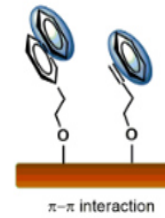


What about neutral compounds ?

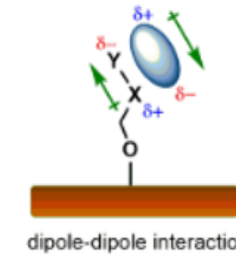


Hydrophobic Interactions

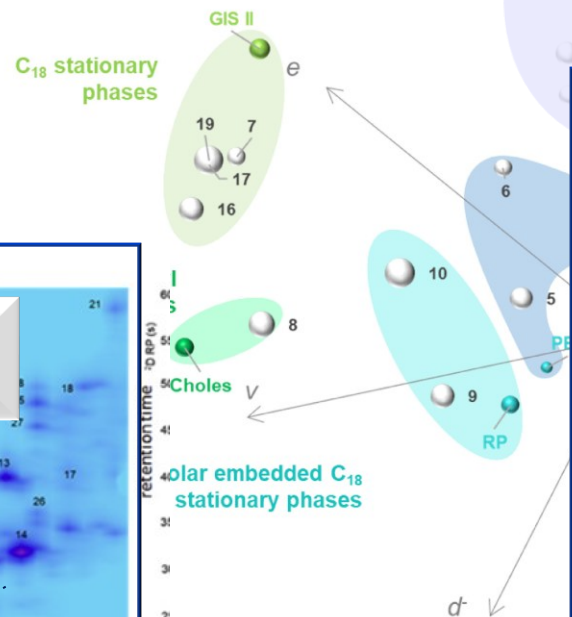
π - π Interactions



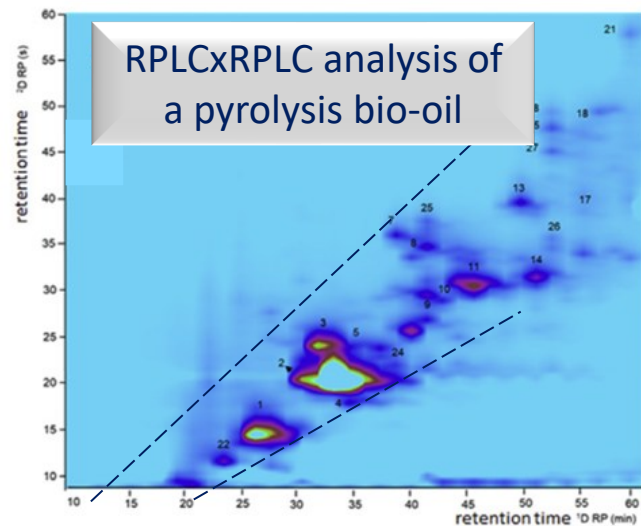
Dipole-dipole Interactions



C_{18} stationary phases

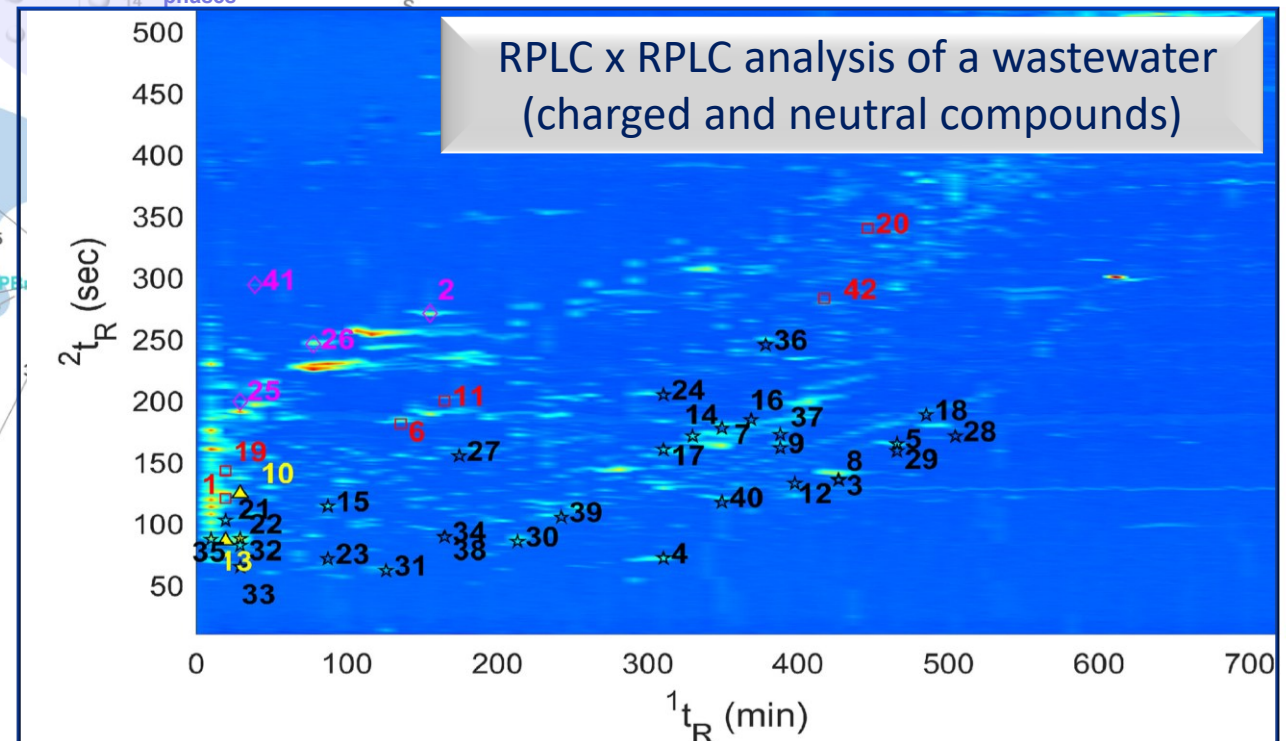


PFP stationary phases



Lazzari et al.

J Chromatogr. A 1602 (2019) 359–367



Kronik et al.

J.Chromatogr. A 1730 (2024) 465079



π - π Interactions

Dipole-dipole Interactions

RPLC x NPLC

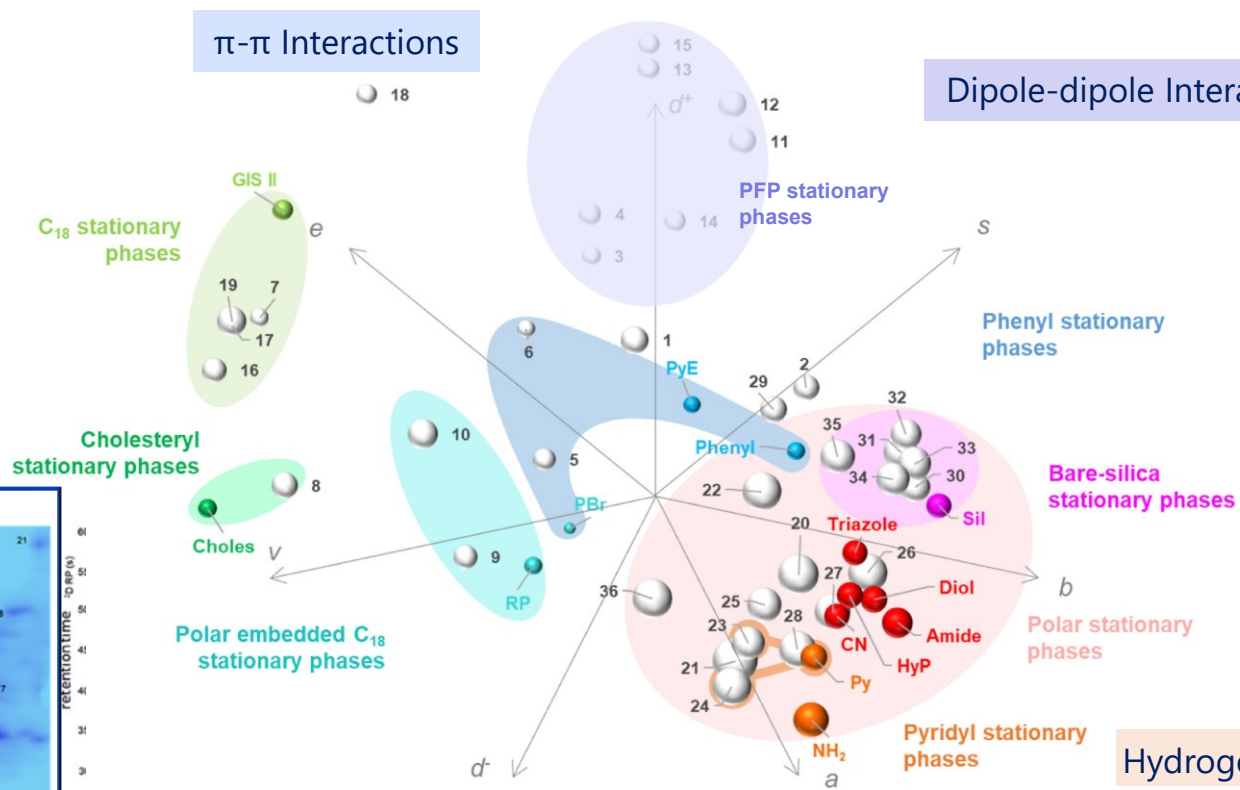
RPLC x SFC

RPSFC x SFC

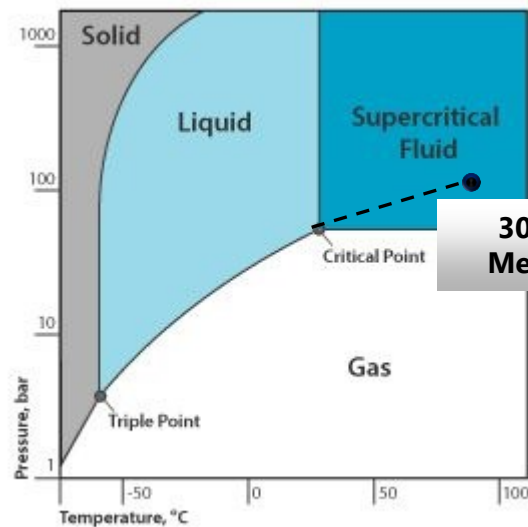
Hydrogen bonding interactions



J Chromatogr. A 1602 (2019) 359–367

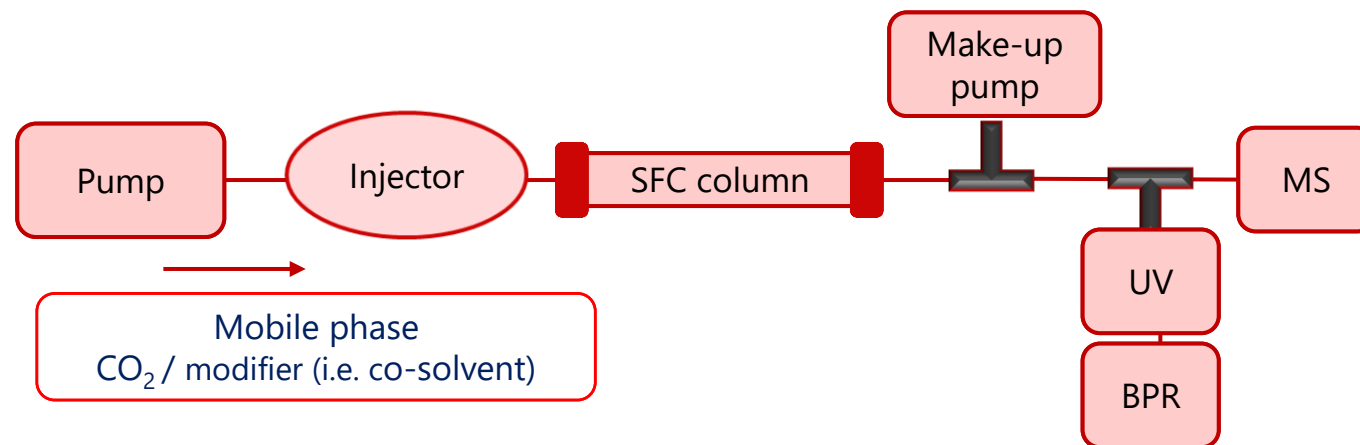


Supercritical fluid chromatography (SFC)

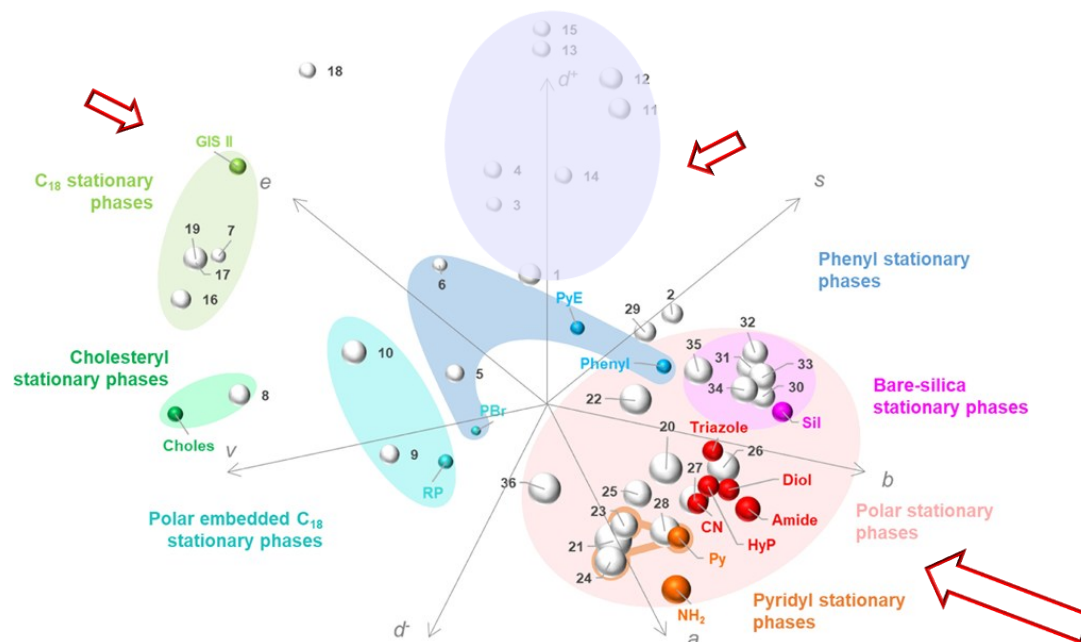


30 % MeOH
170 bars 125 $^{\circ}\text{C}$

CO_2 pur
73 bars 31 $^{\circ}\text{C}$

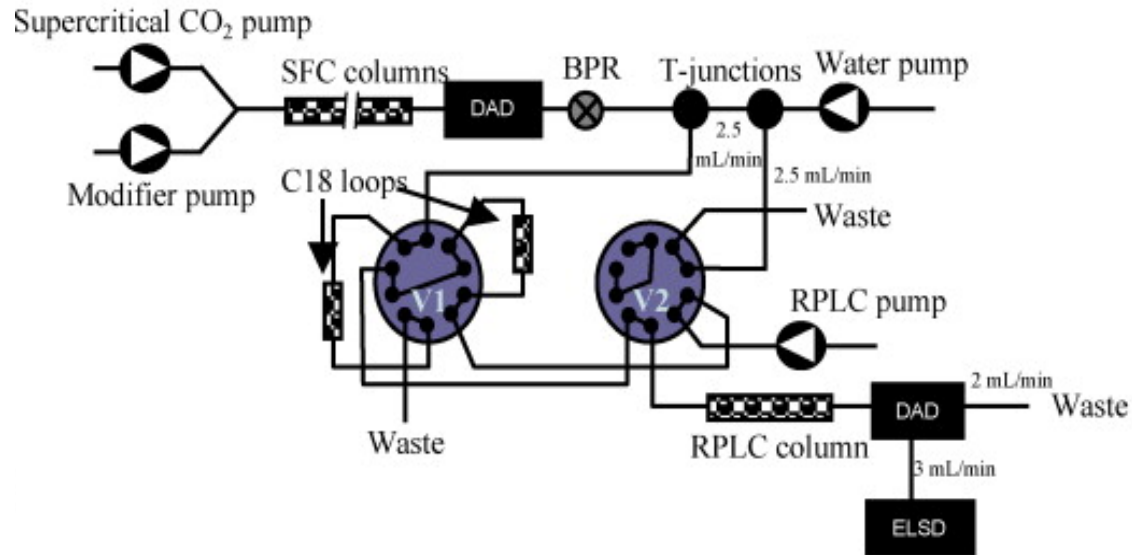


Mobile phase
 CO_2 / modifier (i.e. co-solvent)



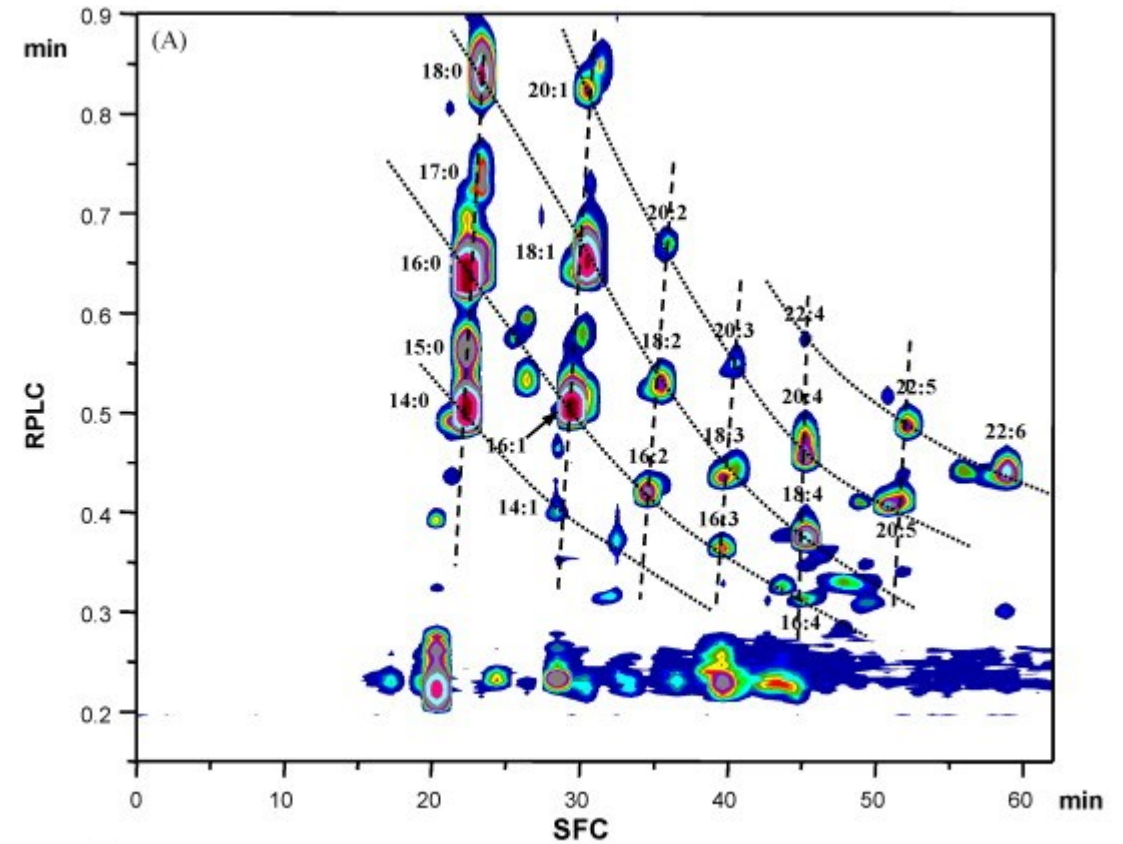


SFC x LC: the early work from Isabelle François and Pat Sandra



Limitations

- trapping loops in the modulation interface
- limited injection volume and injection phase
- risk of CO₂ bubbles inside the LC system : instability

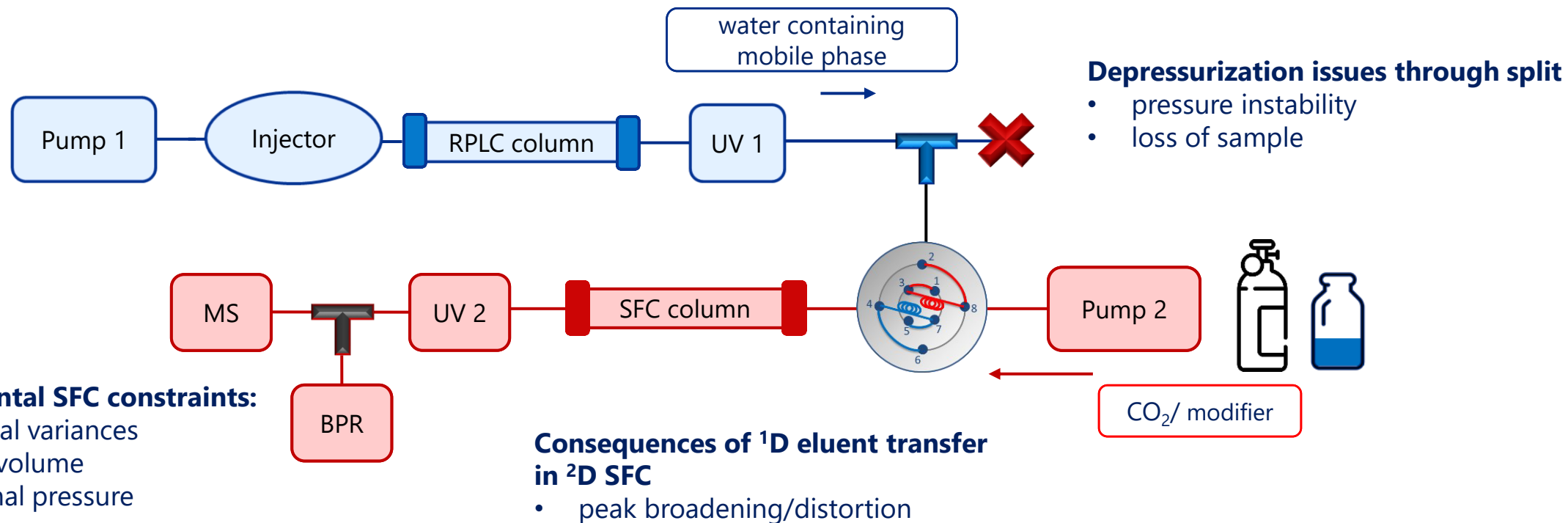


phenacyl esters from fish oil

**Online, comprehensive and orthogonal analysis
15 years ago !!!**



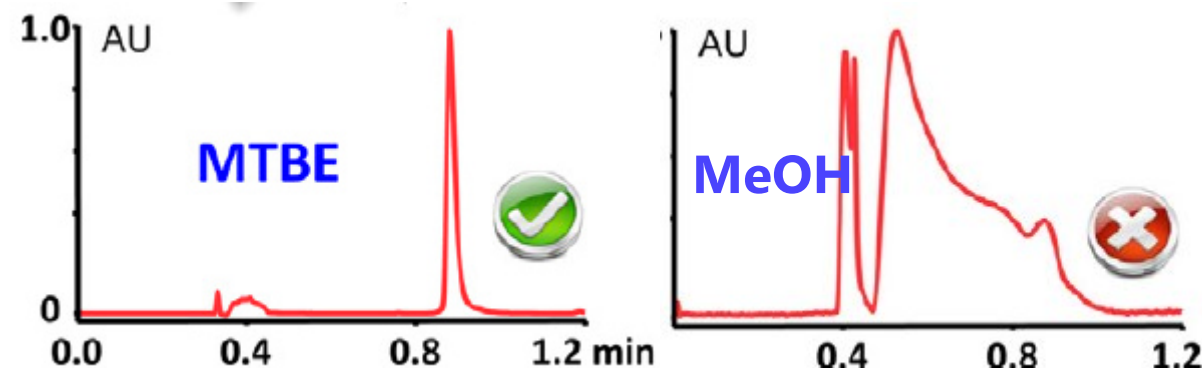
Online LC × SFC: so many technical challenges



A systematic investigation of sample diluents in modern supercritical fluid chromatography[☆]

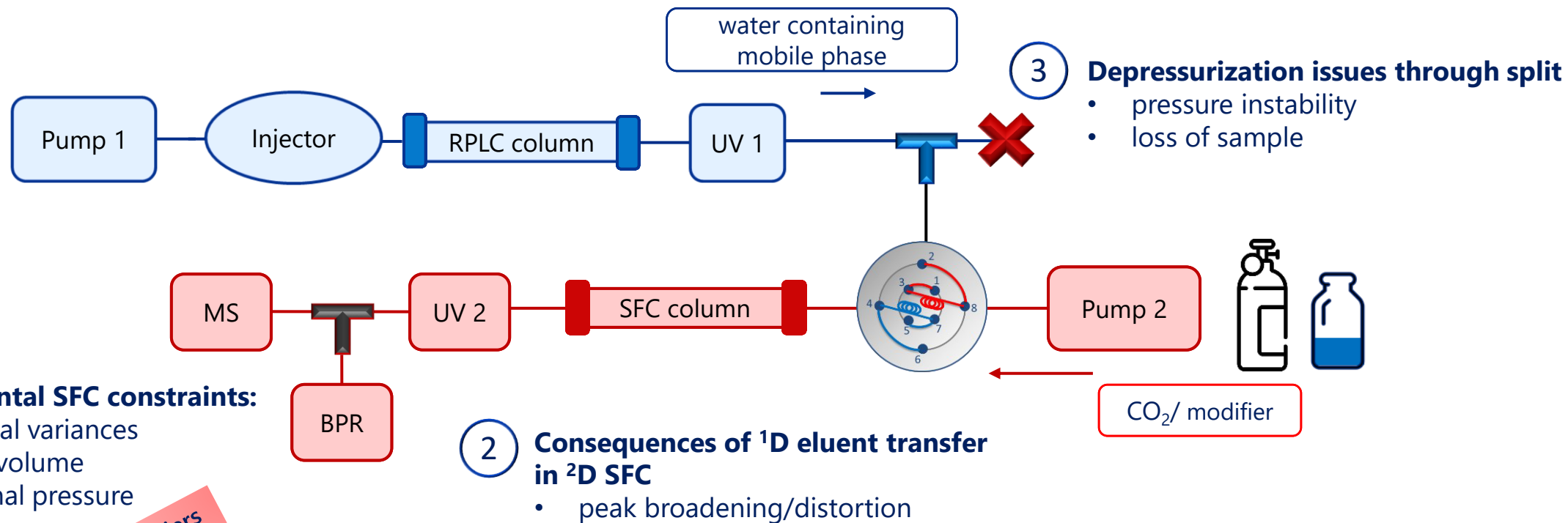
Vincent Desfontaine^a, Abhijit Tarafder^b, Jason Hill^b, Jacob Fairchild^b,
Alexandre Grand-Guillaume Perrenoud^a, Jean-Luc Veuthey^a, Davy Guillarme^{a,*}

Journal of Chromatography A, 1511 (2017) 122–131





Online LC × SFC: so many technical challenges



reduce the impact

Very low ¹D flow rate

Sarrut et al.
J. Chromatogr. A 1402 (2015) 124-133

avoid the problem

Trapping loops

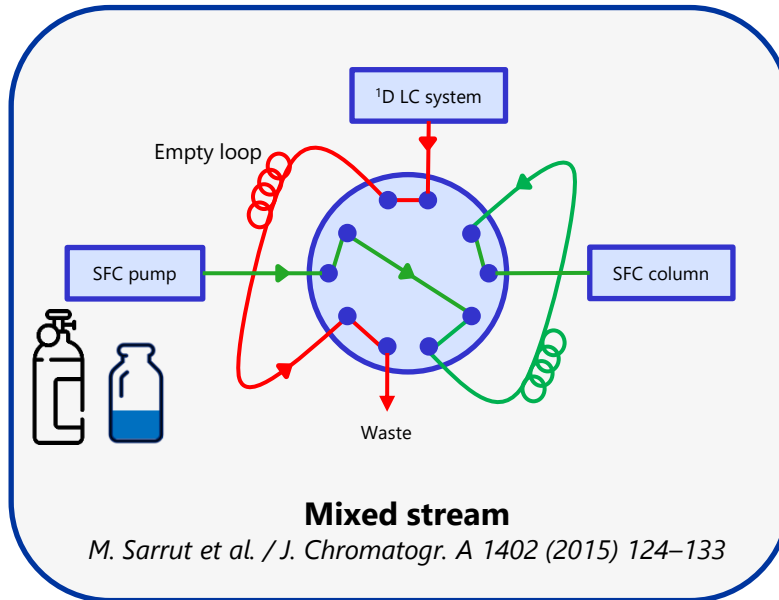
Sun et al.
J. Chromatogr. A 1541 (2018) 21-30



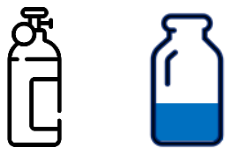
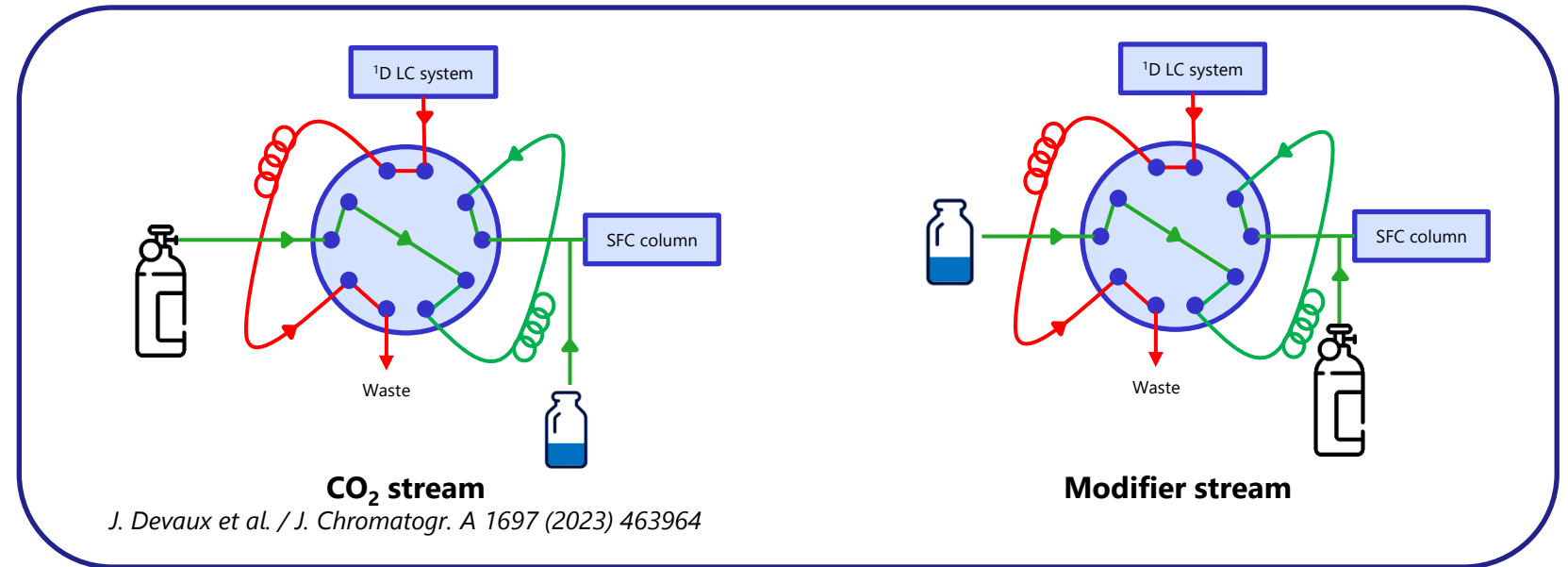
How to transfer LC eluent to SFC ?

In LCxLC, the modulation system is a valve with 2 storage loops

"Commercial" system



LCxSFC specific interfaces



CO₂ modifier



Impact of the chemical nature of diluent (LC mobile phase) and modifier (SFC co-solvent)

Mixed stream



CO₂ stream



Modifier stream



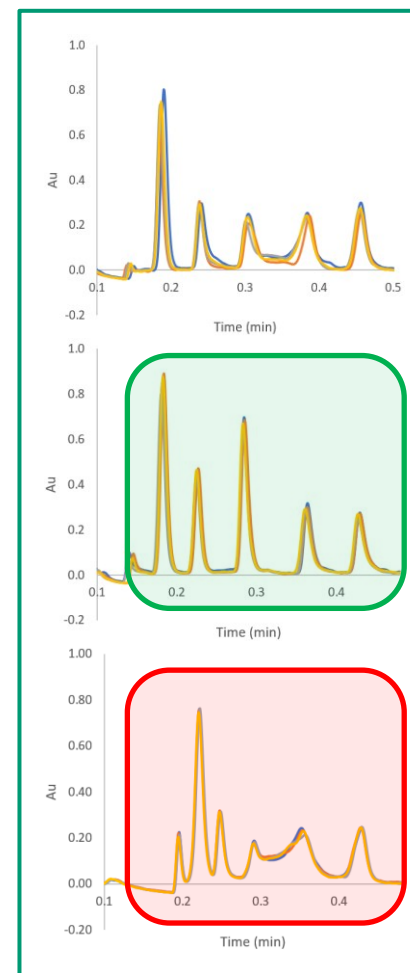
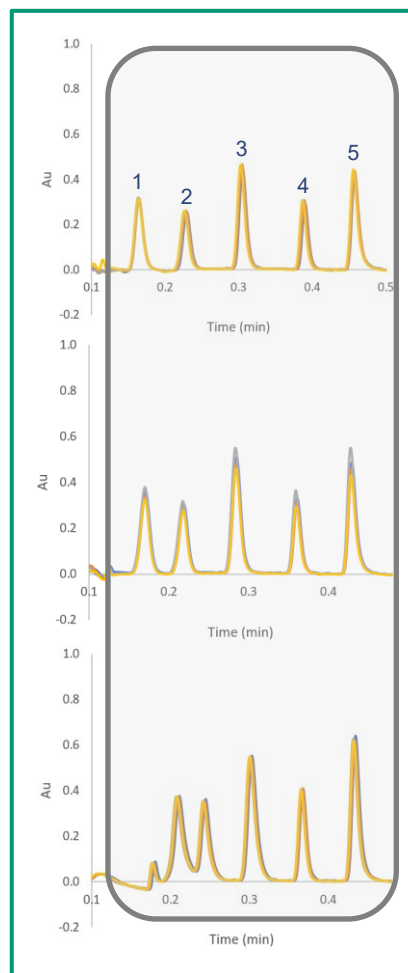
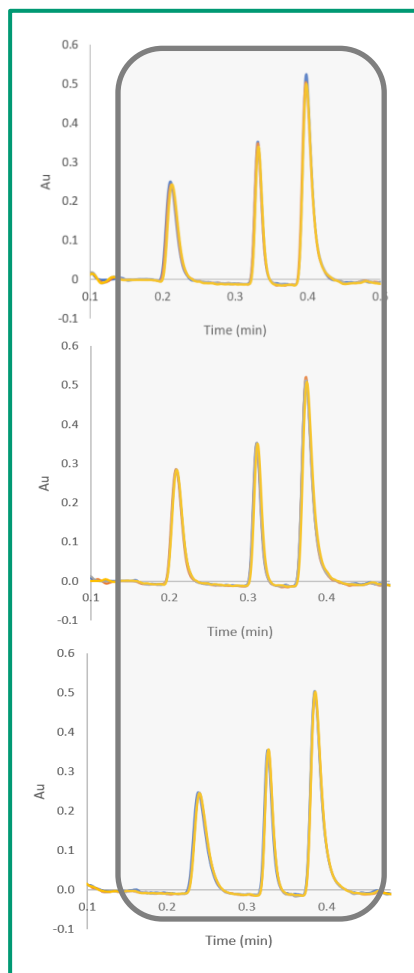
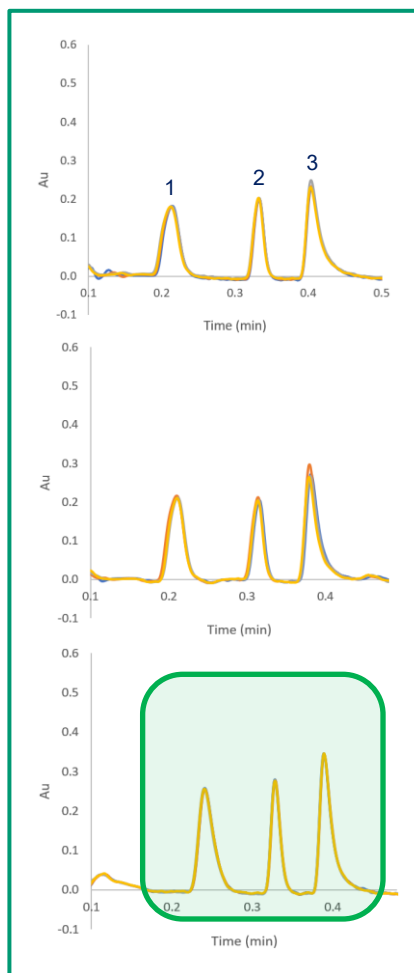
LC
SFC

ACN/MTBE 50/50
ACN

ACN/H₂O 50/50
ACN

ACN/MTBE 50/50
MeOH

ACN/H₂O 50/50
MeOH



Never the best



Preferential configuration

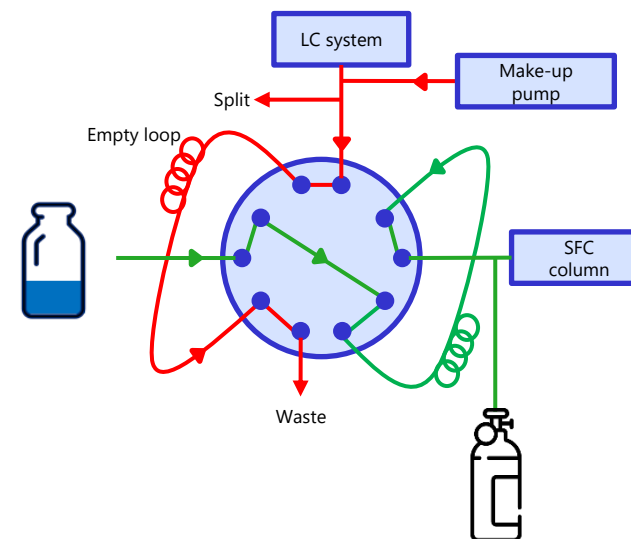
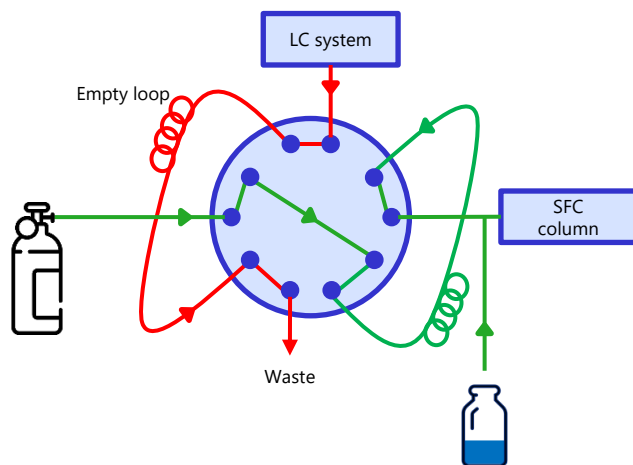
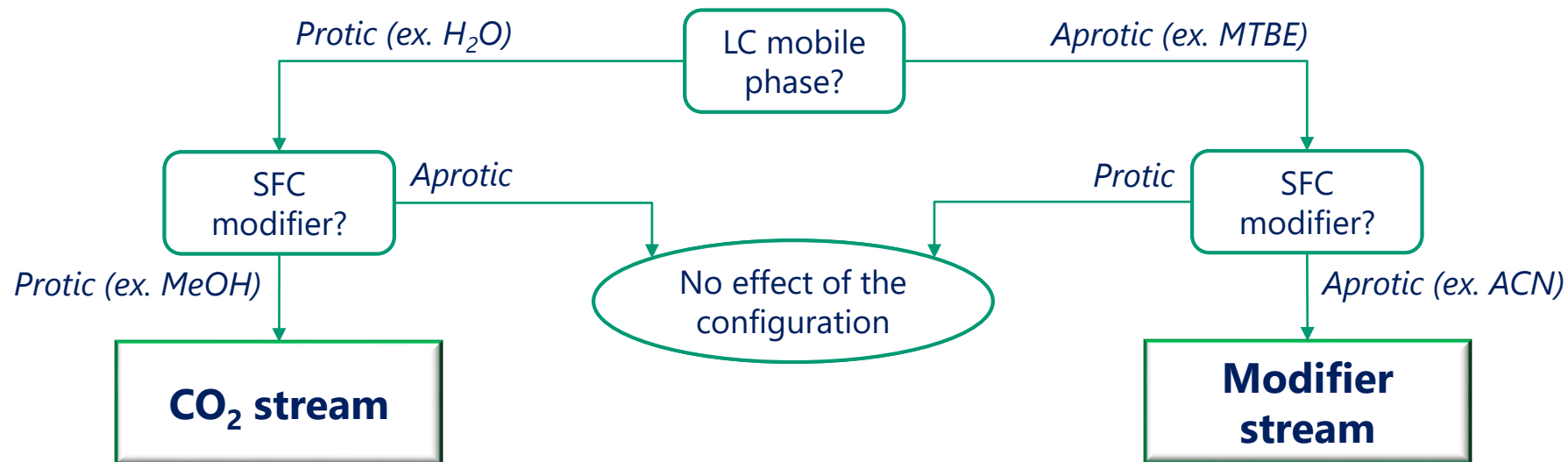
No effect of the configuration

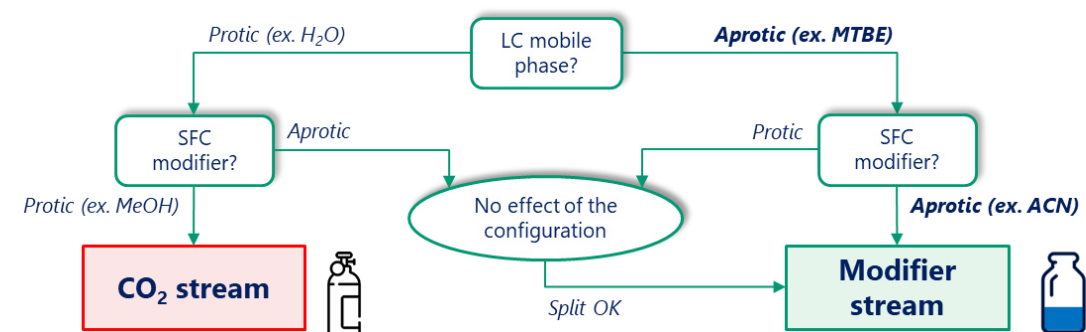
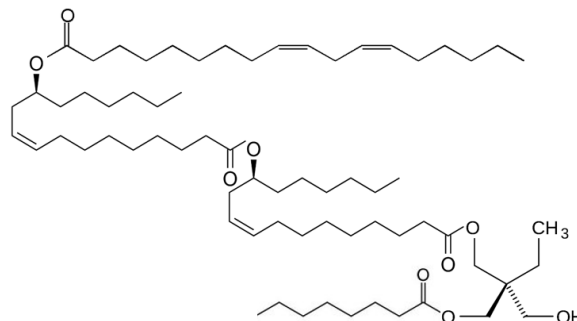
Configuration to be avoided

- 1 : Eugenol
- 2 : Phenol
- 3 : Syringaldehyde
- 4 : Hydroquinone
- 5 : Pyrogallol



Guidelines to select LCxSFC interface (modulation system)



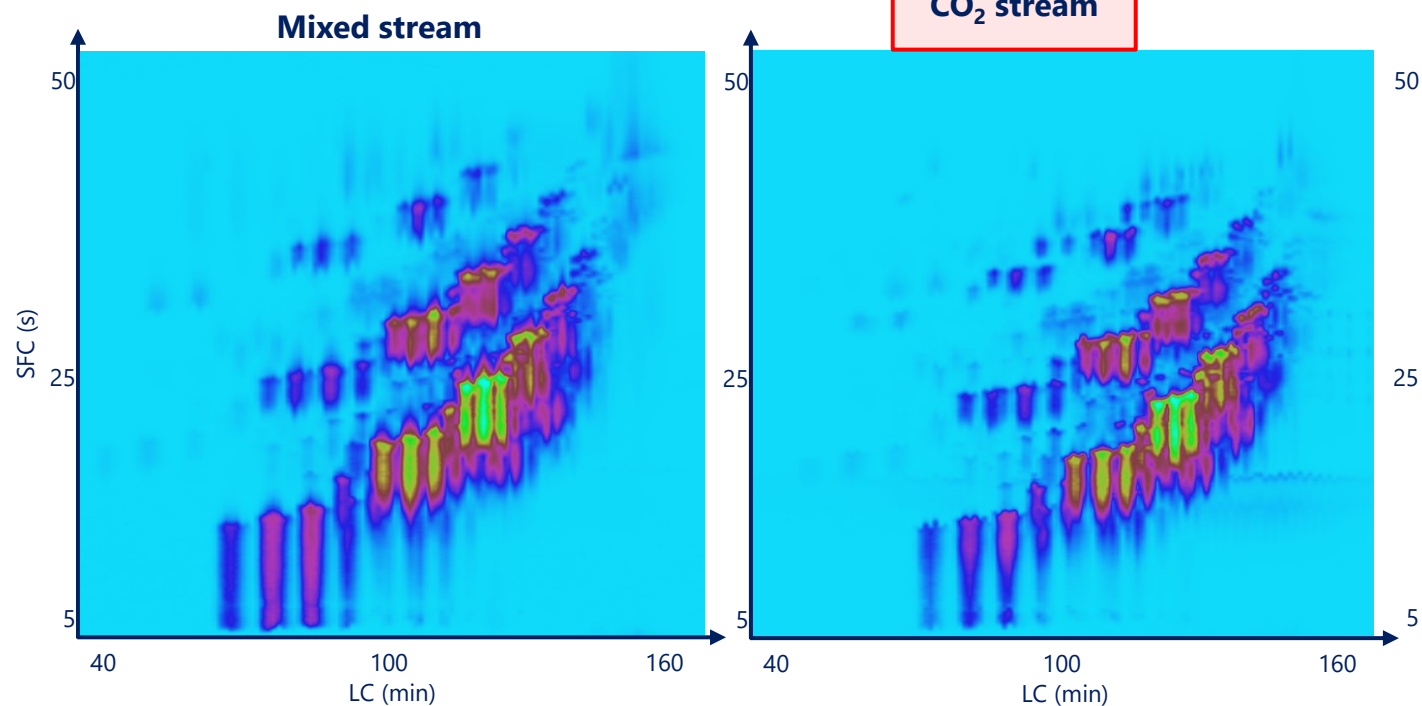


¹LC

**aprotic eluent
(ACN/MtBE)**

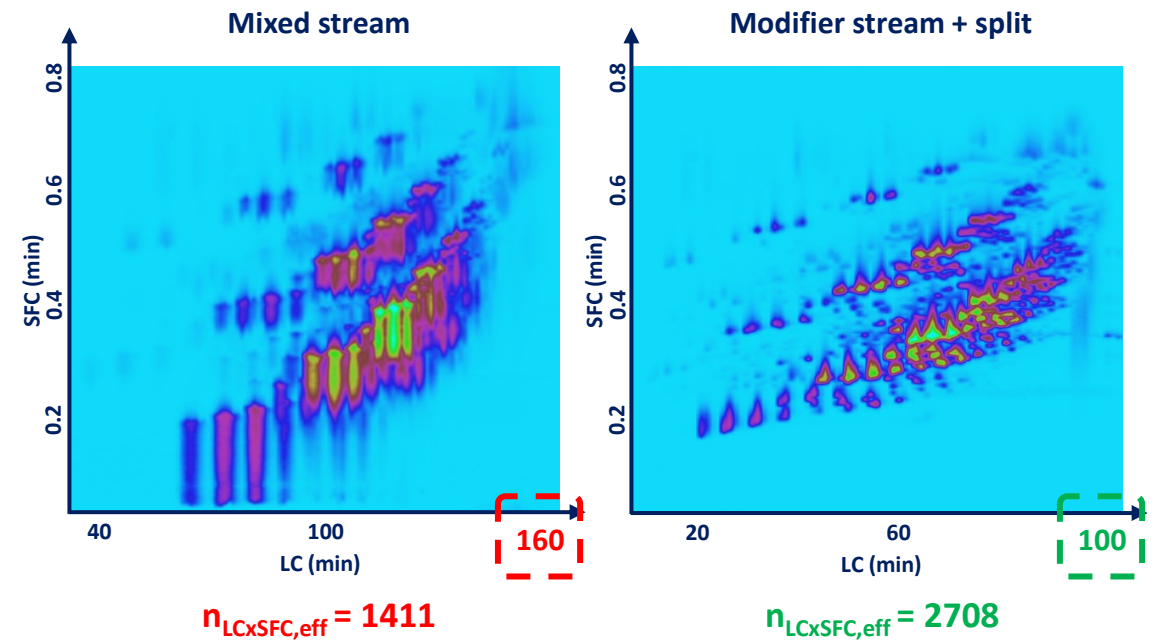
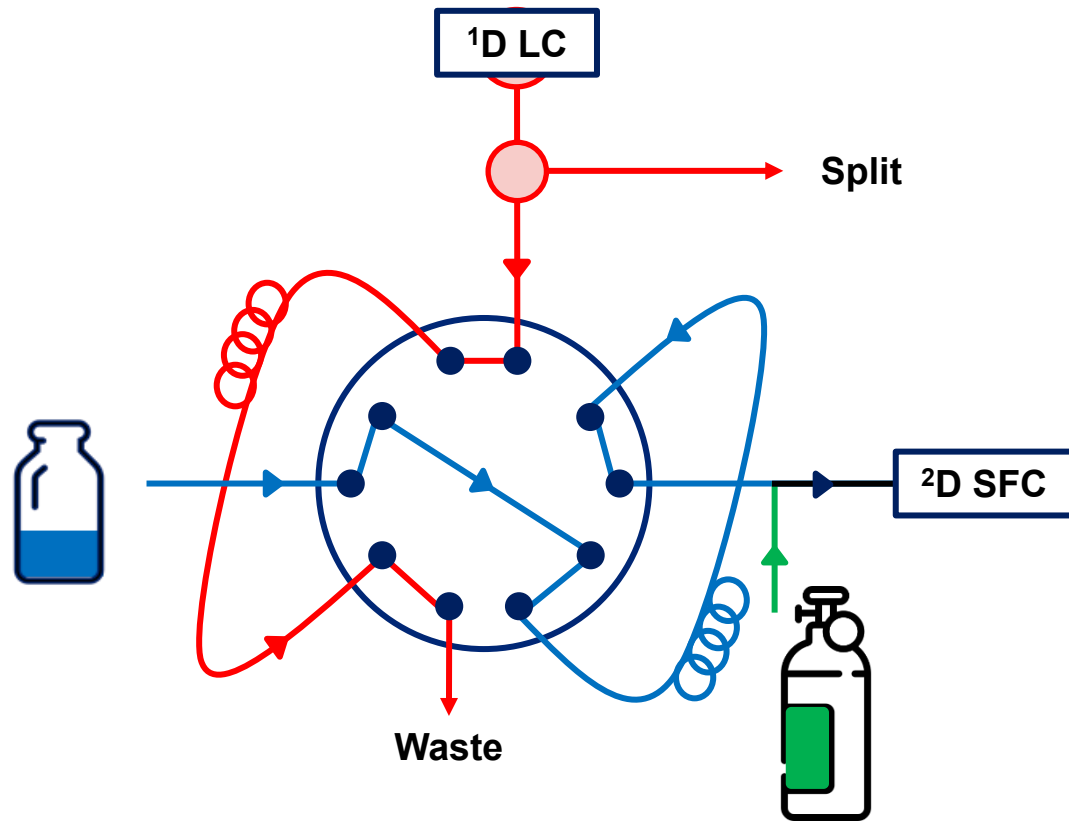
2SFC

**aprotic modifier
(ACN)**



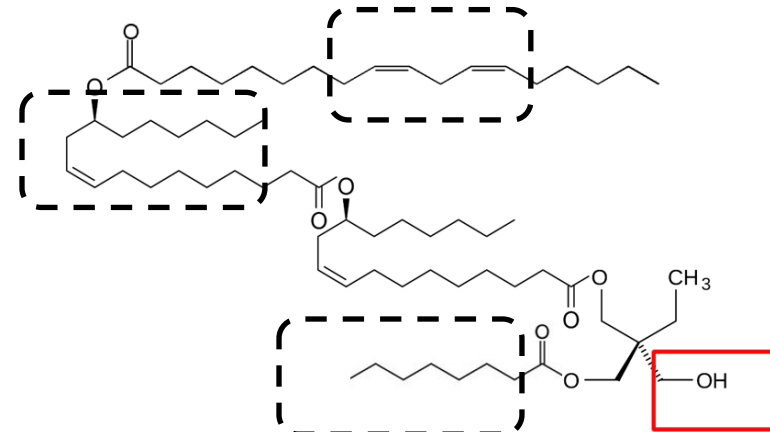
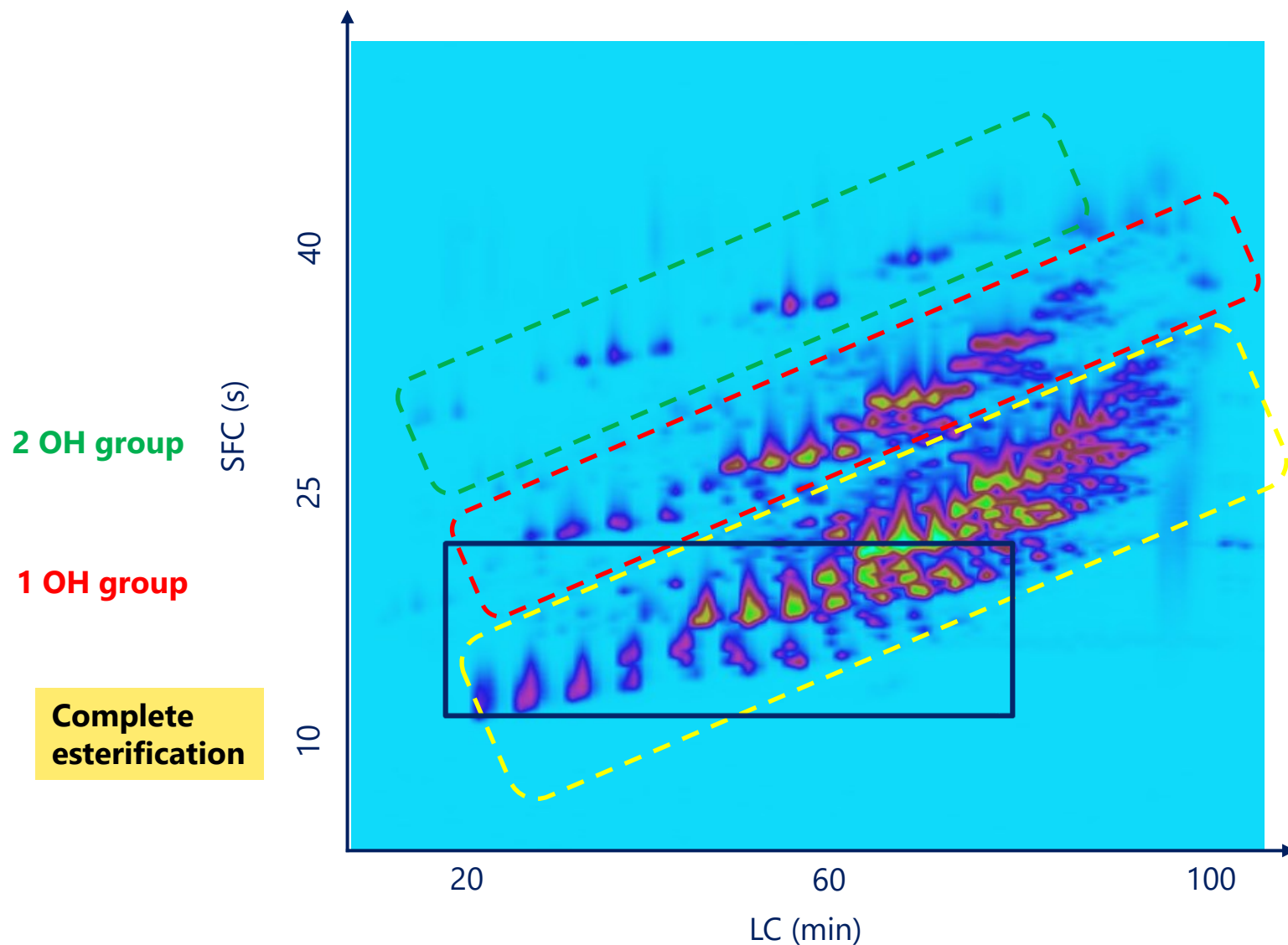


Modulation using modifier stream: let's split !

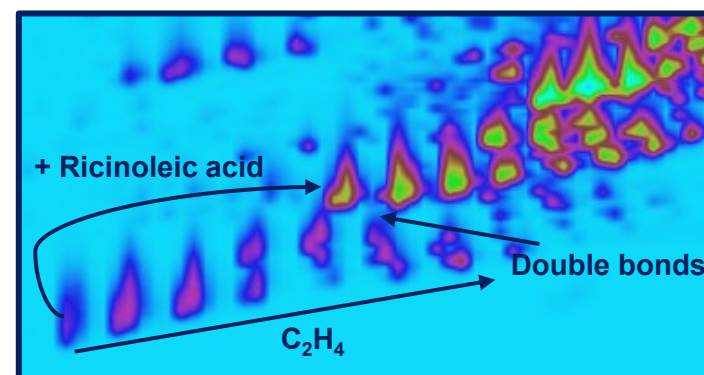




LCxSFC-HRMS classification of chemical structures

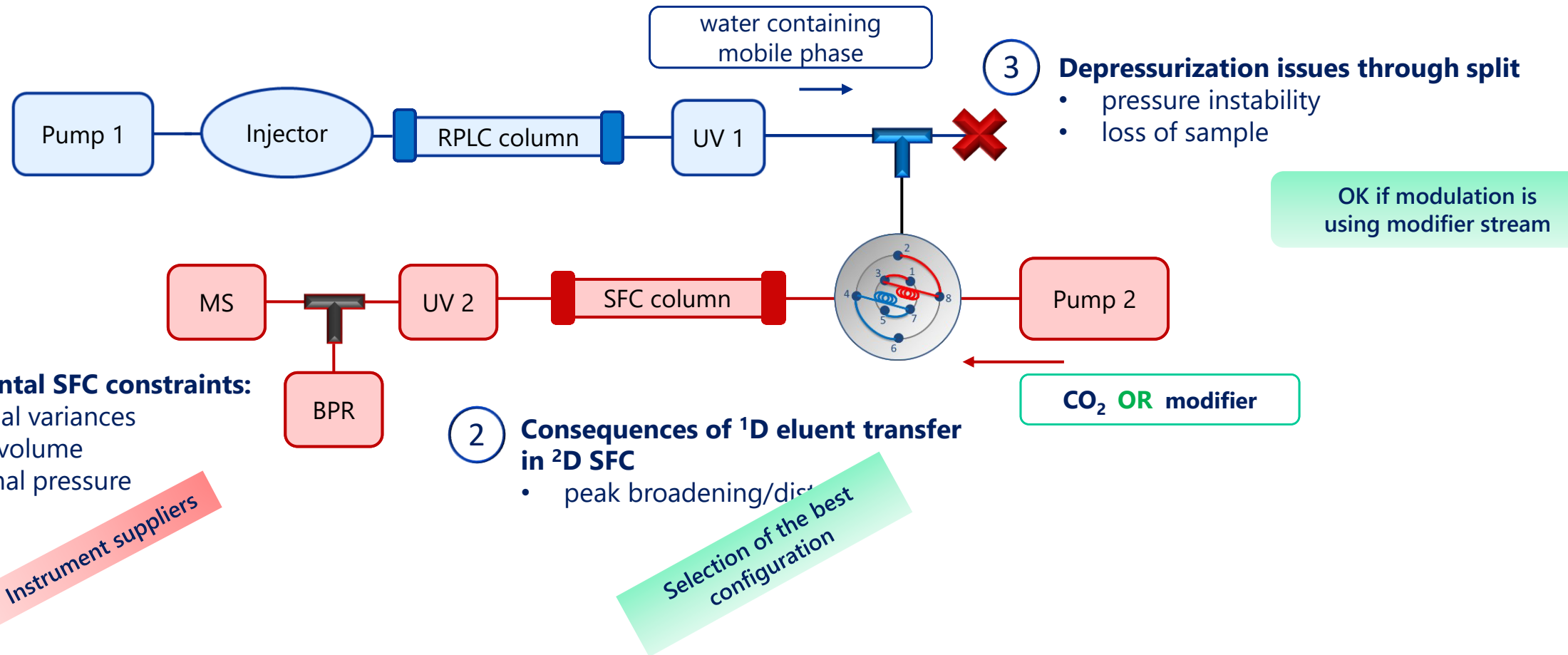


Complex Polyols Polyesters



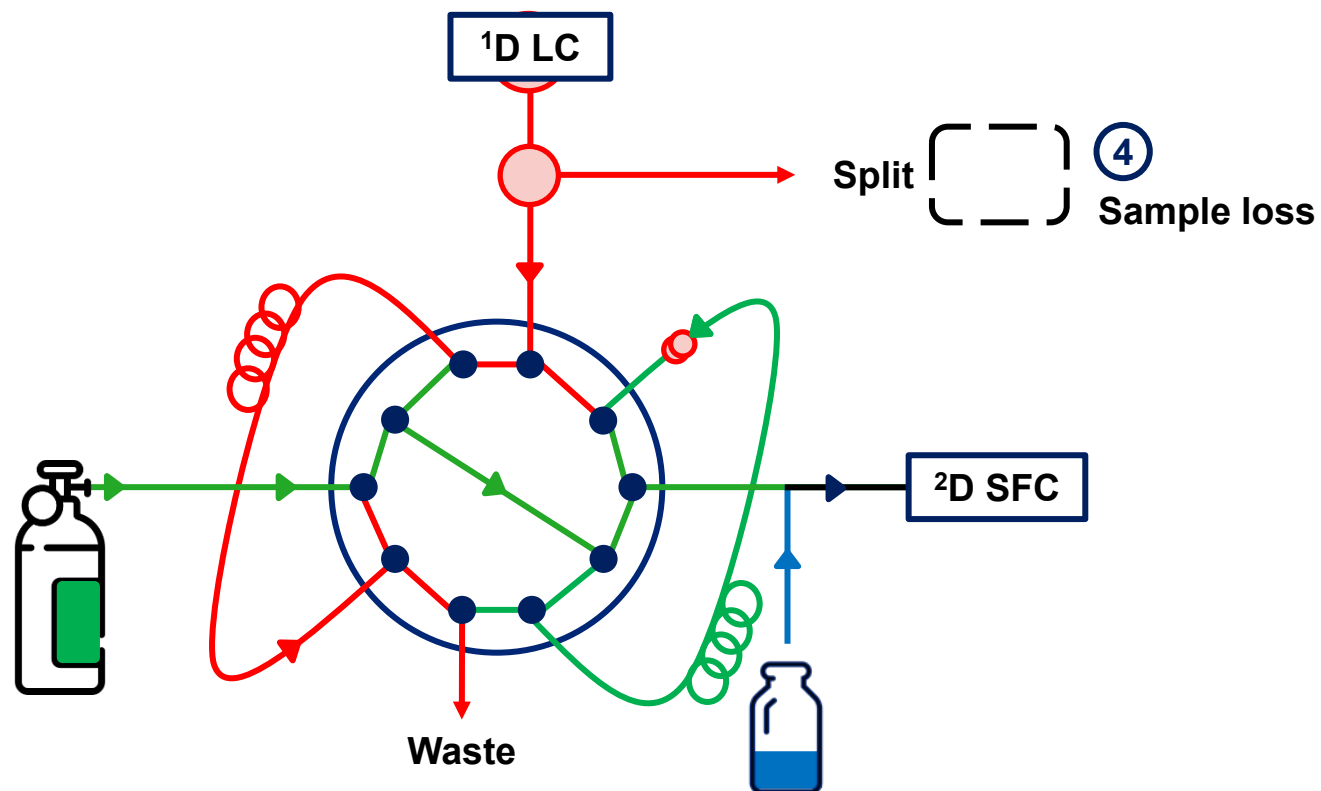


Online LC × SFC: so many technical challenges





Split in CO₂ stream



- ① 2D analysis → loop full of CO₂
- ② Depressurization step → loop empty
- ③ Loading → CO₂ expand





Spent coffee grounds : a valuable source of bioactives



World coffee production reached **178.0 million bags** in 2023/2024

Spent coffee grounds (SCGs) about **6 million tons / year**

Spent coffee grounds

- Carbohydrates
- Phenolic compounds
- Nitrogen compounds
- Fats
- Non saponifiable lipids

Sterols

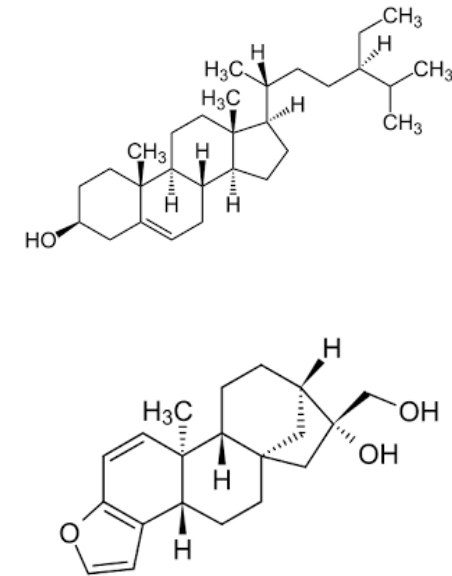


Anticancer, anti-inflammatory, hepatoprotective abilities [2]

Diterpenes

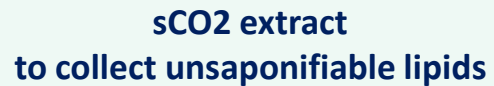


UVB skin protection, anticancer, anti-inflammatory, antioxidant activities [2]



[1] <https://icocoffee.org/>

[2] Trends in Food Science & Technology 143 (2024) 104312

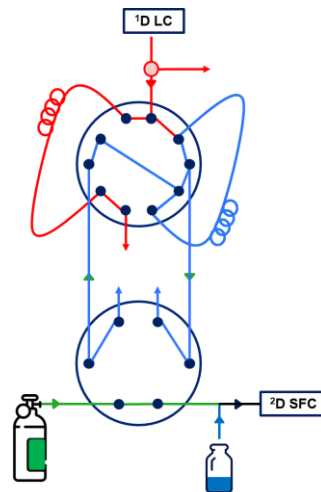


¹LC C18

**protic eluent
(ACN/iPrOH)**

2SFC 1-AA

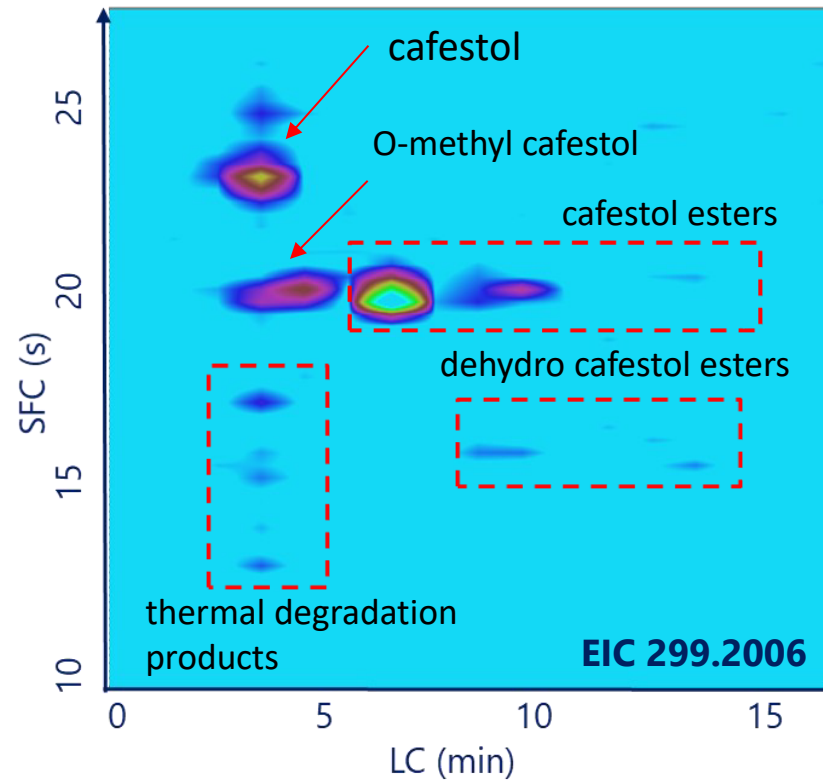
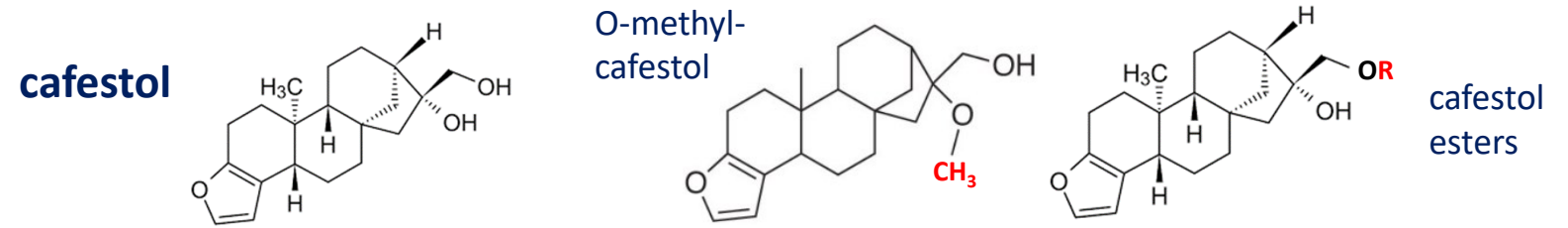
**protic modifier
(EtOH)**



LCxSFC-(APCI)-HRMS

modulation: CO₂ stream + split

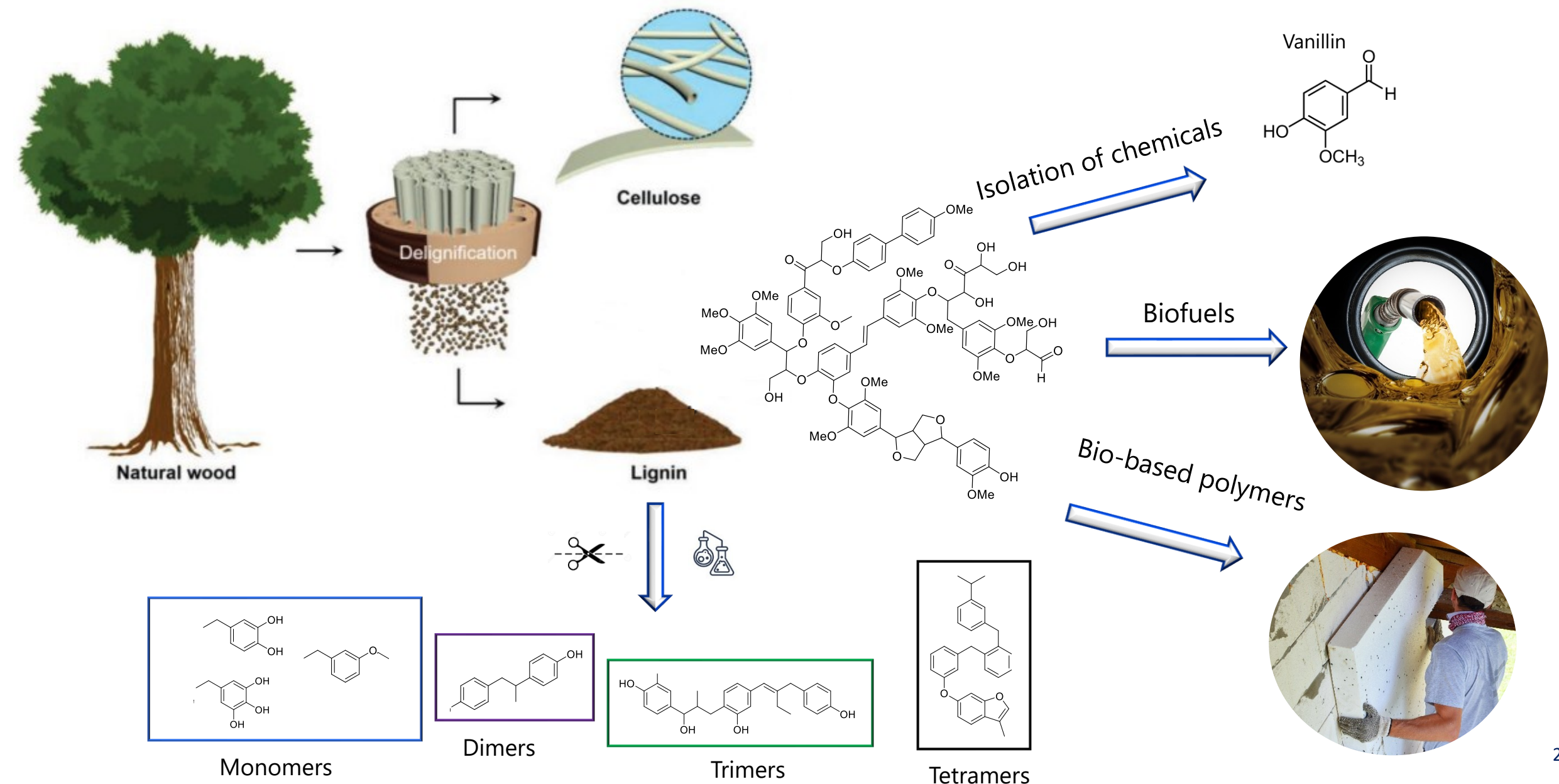
total gradient time: 20 min



The same behavior for the derivatives of

- kahweol
- 20 different sterols

Lignin valorization as renewable material





Non targeted analysis of depolymerized lignin



alcohol extract
to collect depolymerized oligomers

¹LC F5

protic eluent

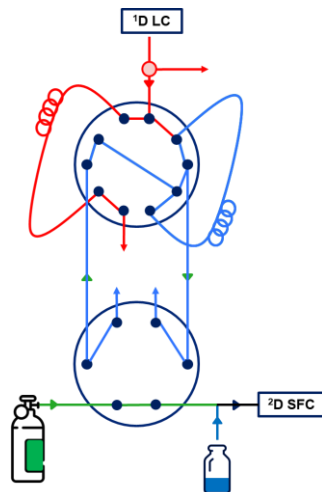
(Water/ACN)

starting at 100 % water

²SFC 1-AA

protic modifier

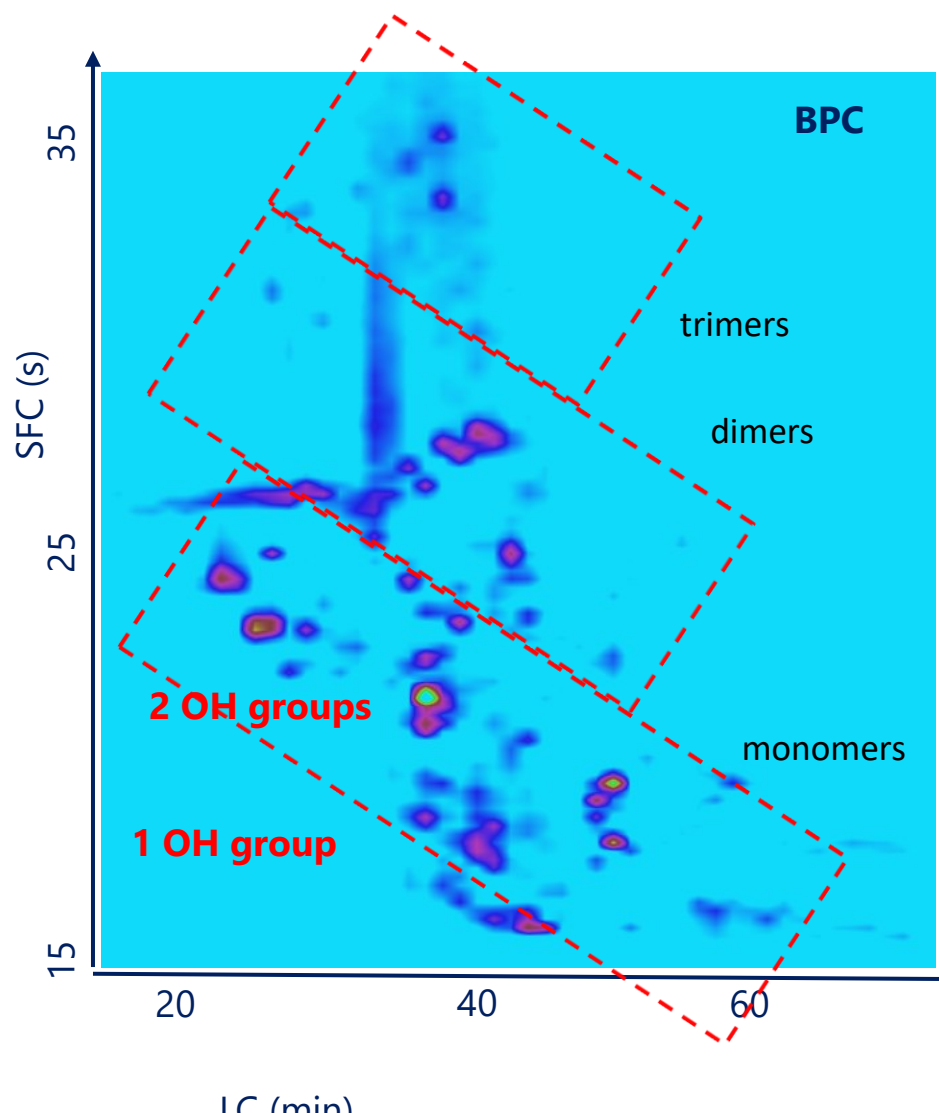
(MeOH)



LCxSFC-(ESI)-HRMS

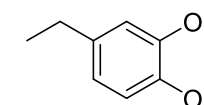
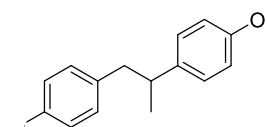
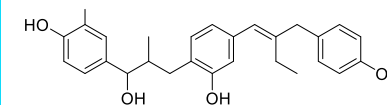
modulation: CO₂ stream + split

total gradient time: 60 min



384 C_xH_yO_z compounds

70 % of compounds had
at least one isomer





No Conclusion.... It is just the beginning...

Research and Innovation on two-dimensional chromatography for natural, recycled and bio-based products



Thanks to the creative stubborn students

Irene
Spent coffee Grounds



Eliise
Lignin oligomers

Margaux
LCxSFC interface(s)



Spent Coffee grounds



Lignin oligomers



PhD grant M. Sanchez



SFC joining the 2D world ?

