

Two-dimensional liquid chromatography

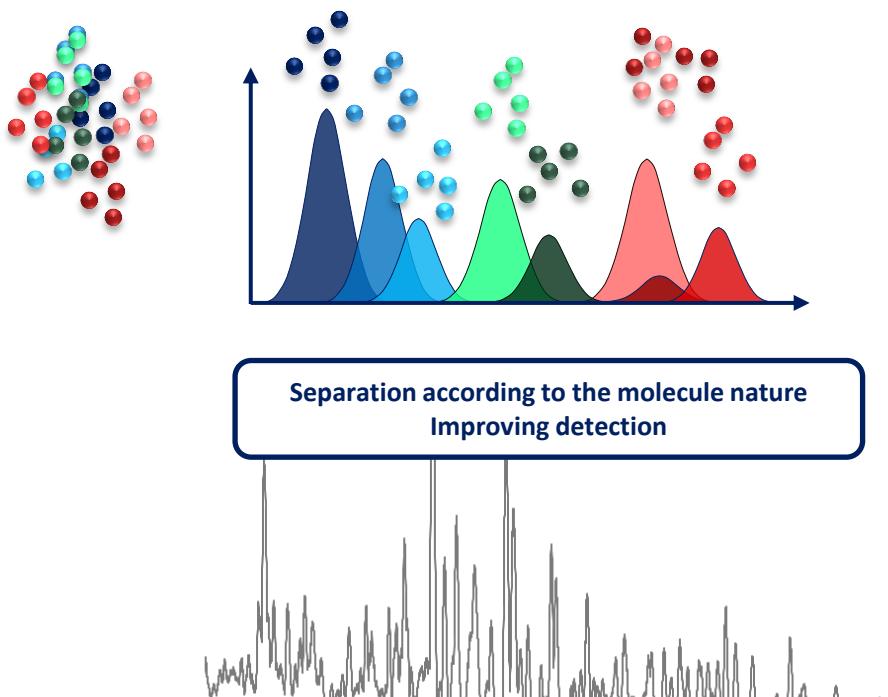
TUNTWIN summer school



Funded by the Horizon 2020 Framework Programme of the European Union
under the grant N° 952306



Liquid chromatography



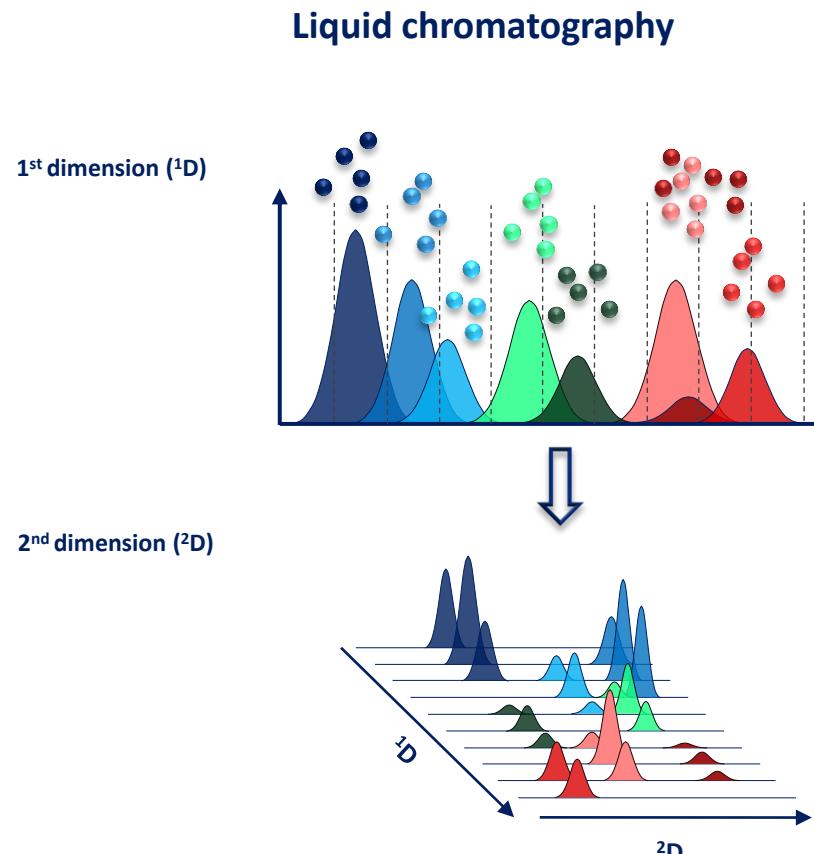
Séparation de dérivés de lignine par RPLC

High resolution mass spectrometry (HRMS)



Mass accuracy
High acquisition frequency

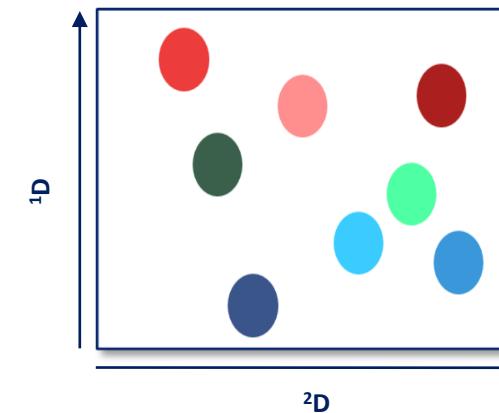
- Increase **peak capacity**
- Distinguish **isomers**
- Confirm the **molecule identity**
- Improve **sensitivity**



High resolution mass spectrometry (HRMS)



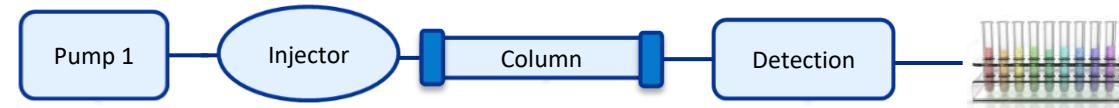
Compare
Quantify
Identify



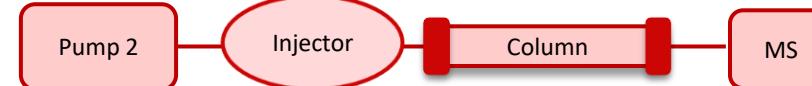
Soraya Chapel@ISA

Off-line
2D LC

1st Dimension
LC

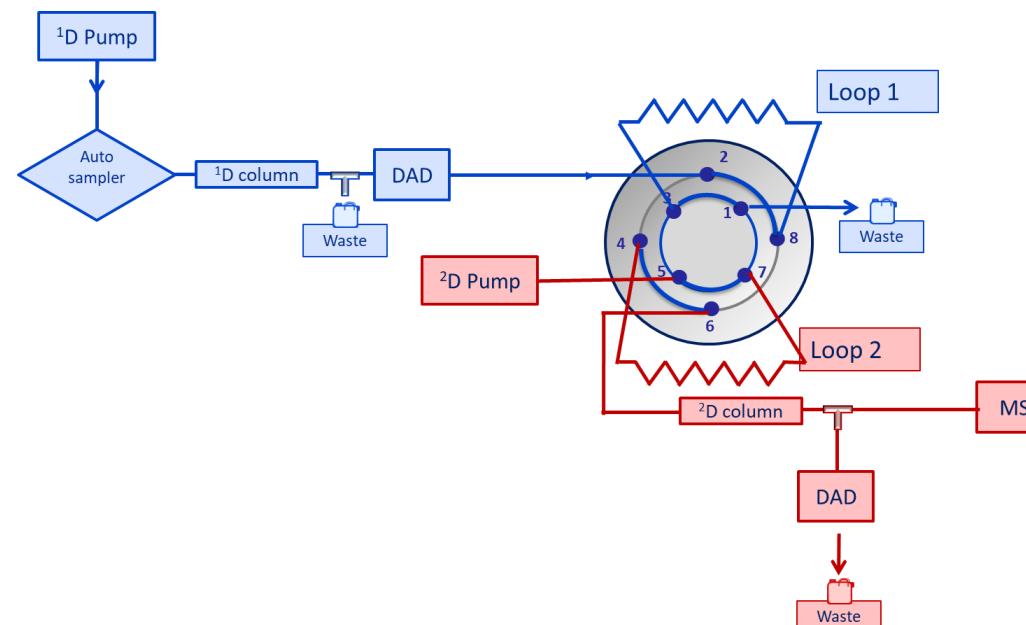


2nd Dimension
LC or SFC



$$t_{\text{analysis}} = {}^1t_{\text{analysis}} + n_{\text{fractions}} * {}^2t_{\text{analysis}}$$

Online 2D LC



$$t_{\text{analysis}} = {}^1t_{\text{analysis}}$$

$${}^2V_{\text{injection}} = {}^1V_{\text{fraction}} \times z_{\text{split}}$$

$${}^2t_{\text{analysis}} = t_{\text{sampling}}$$

$${}^2V_{\text{injection}} = {}^1V_{\text{fraction}} = {}^2t_{\text{analysis}} \times {}^1F$$

Nature of interactions

Hydrophobic

Hydrophilic/ionic

Polar

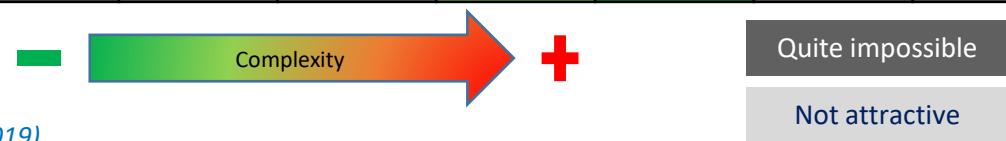
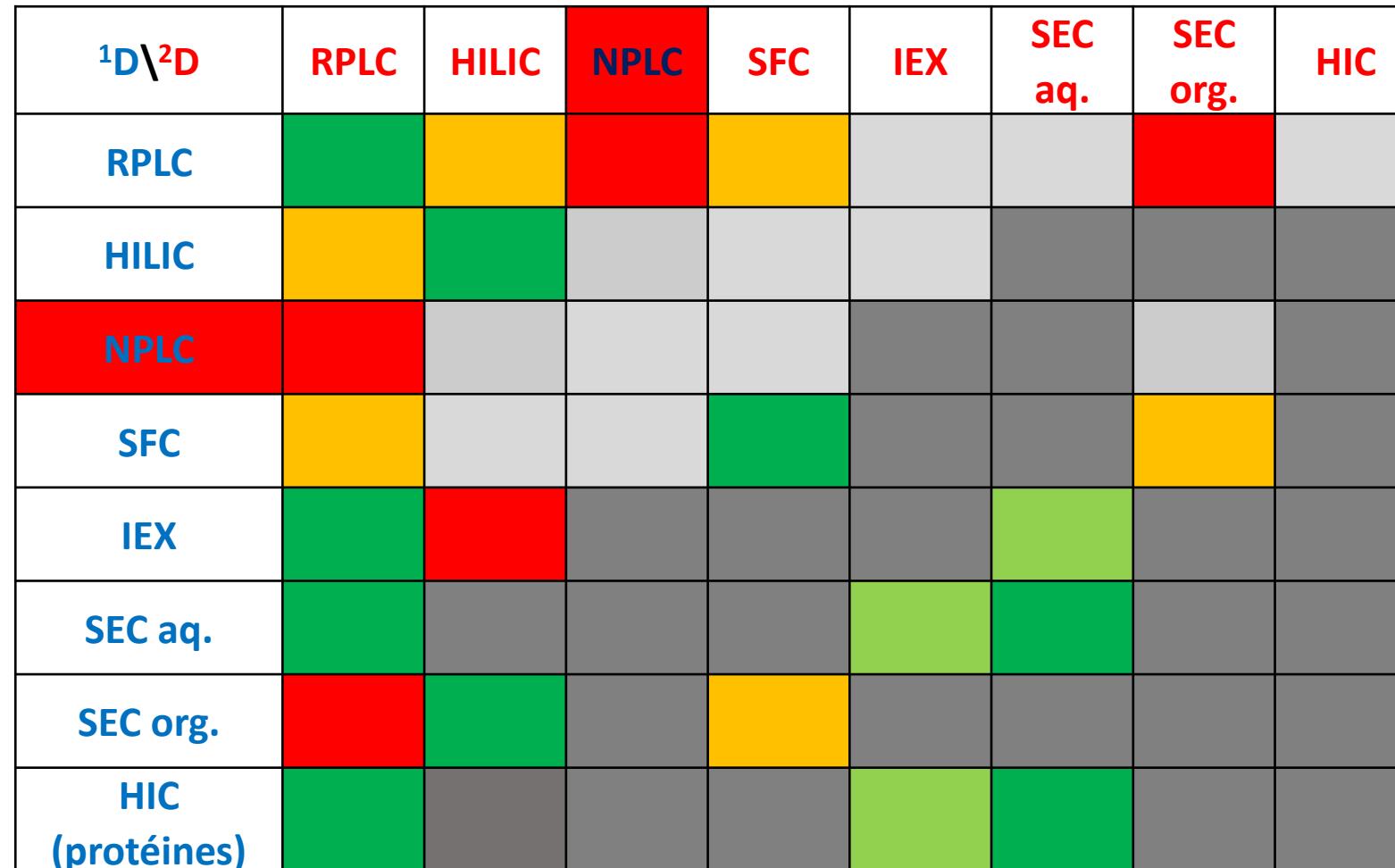
Polar

Ionic

Size filtering

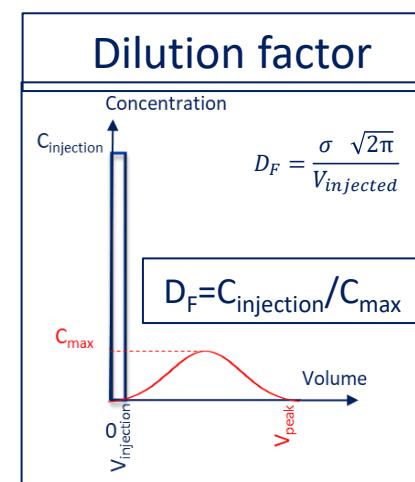
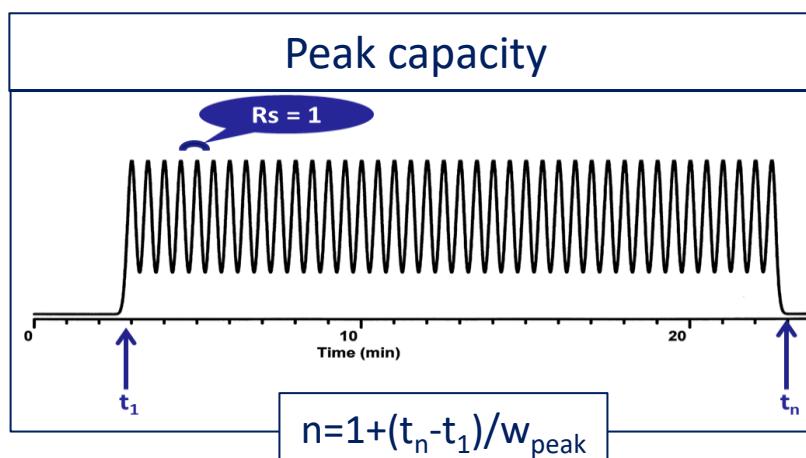
Size filtering

Hydrophobic

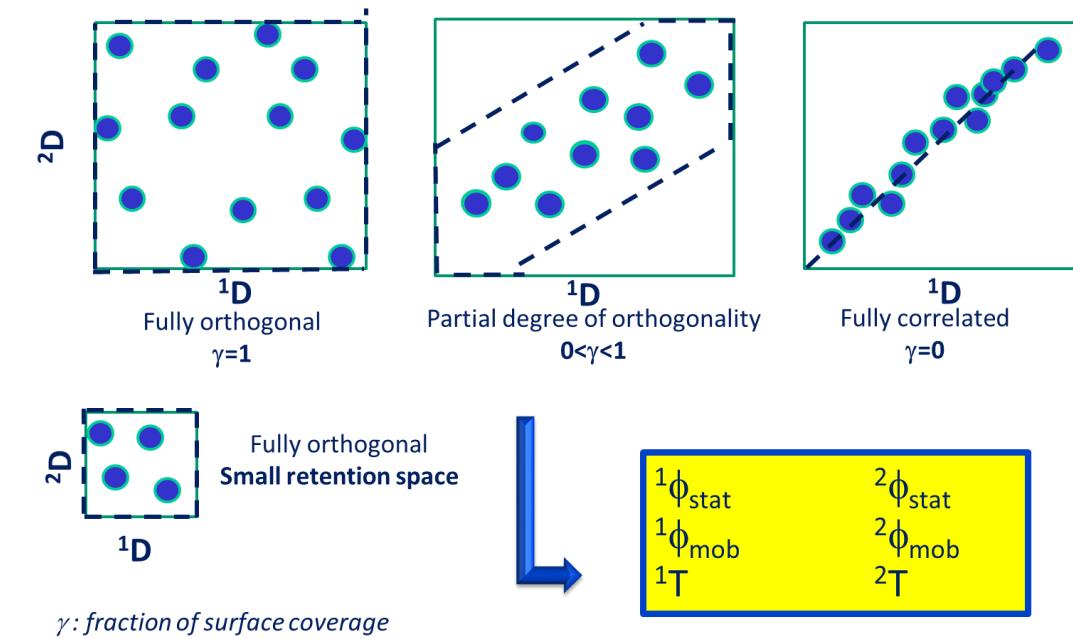


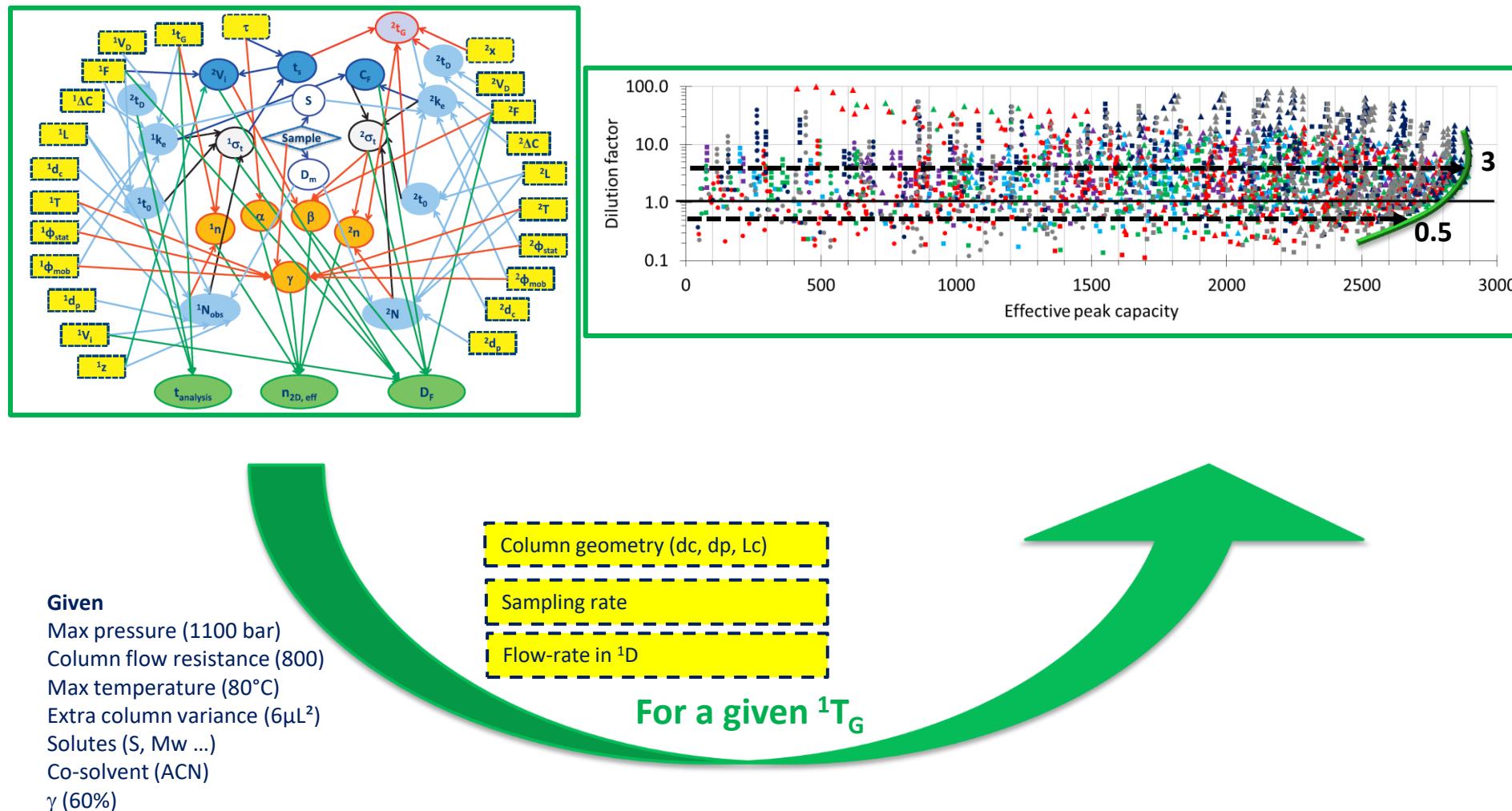
Adapted from Pirok and Schoenmakers J. Sep. Science (2019)

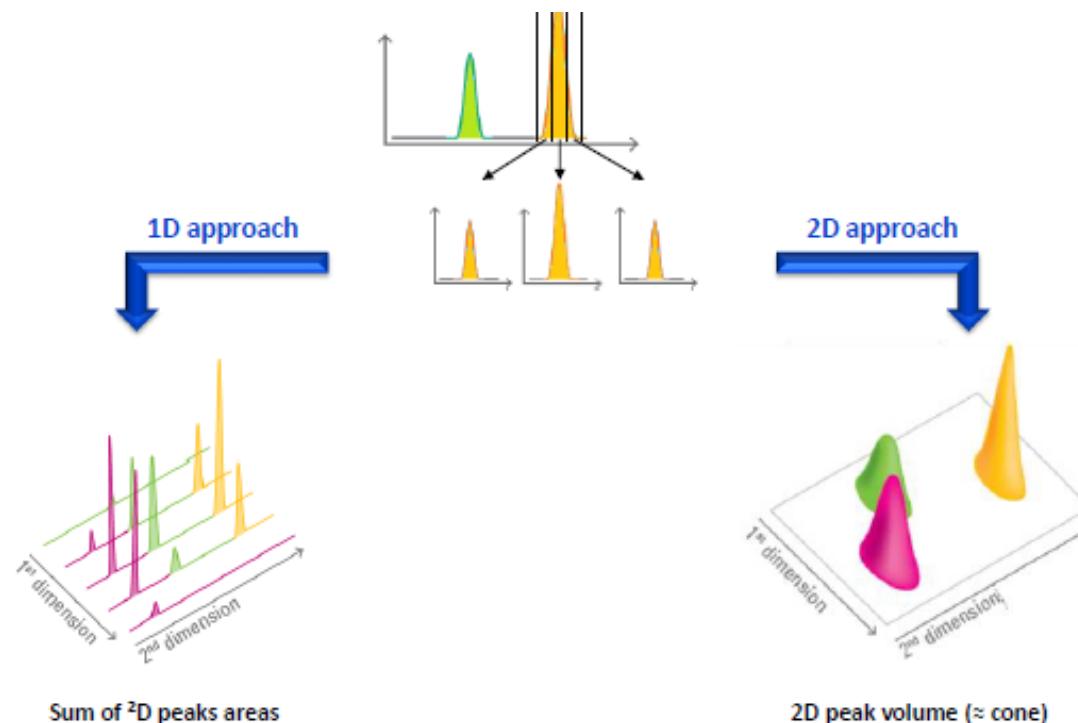
1D-LC		
Analysis time	Gradient time	T_G
Separation power	Peak capacity	n
Sensitivity	Dilution factor	D_F



$w_{\text{peak}} = \text{peak width}$

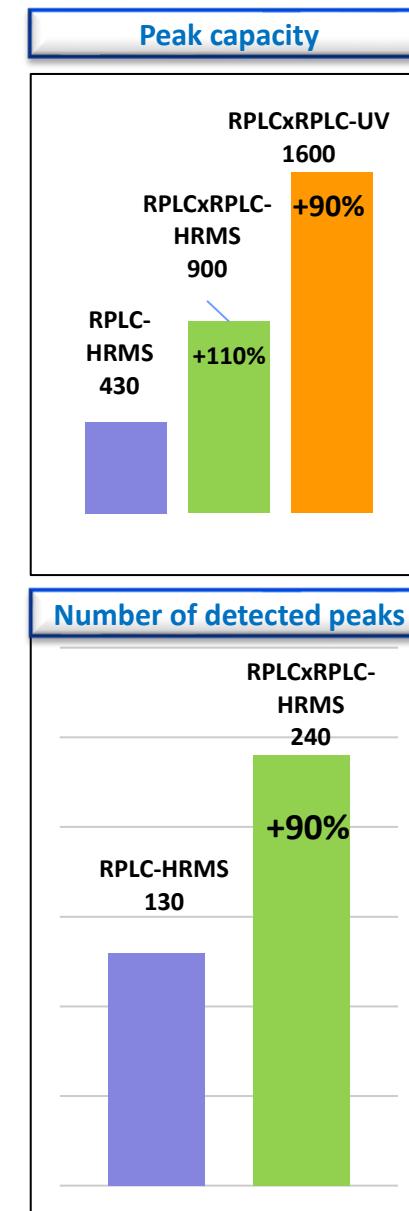
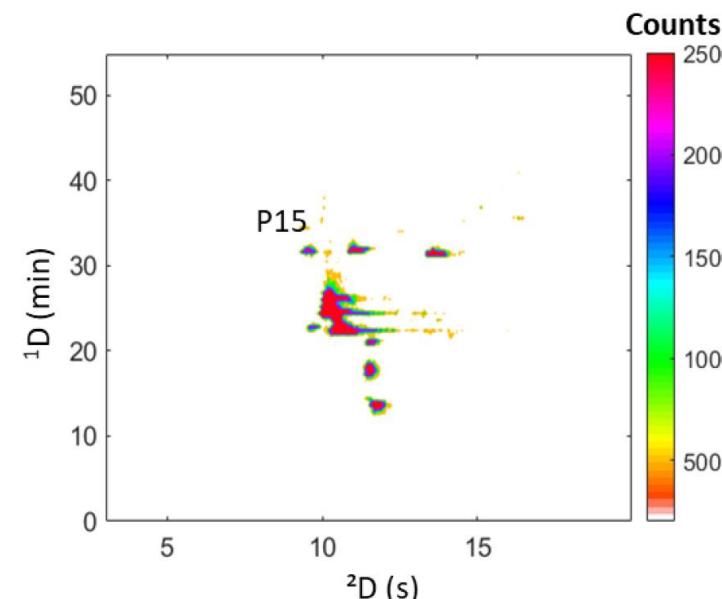
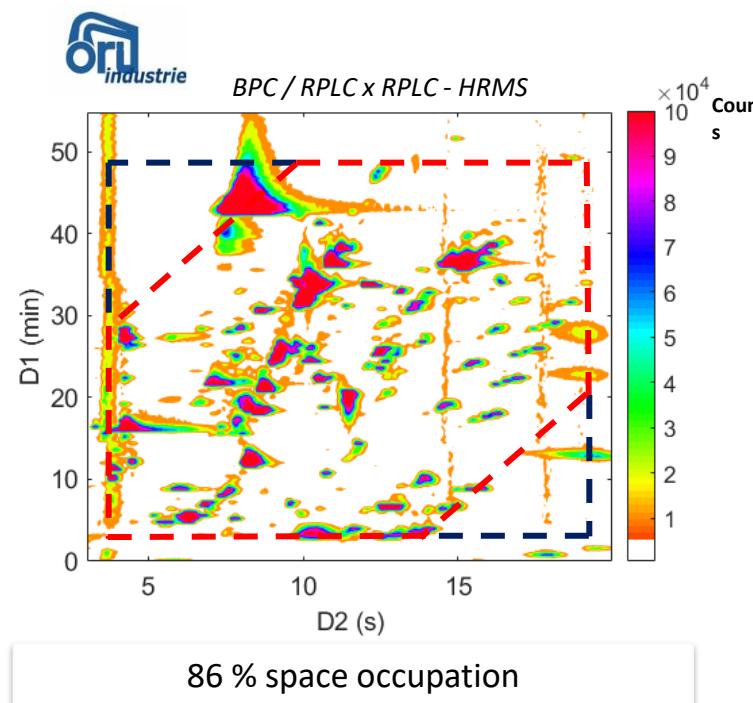


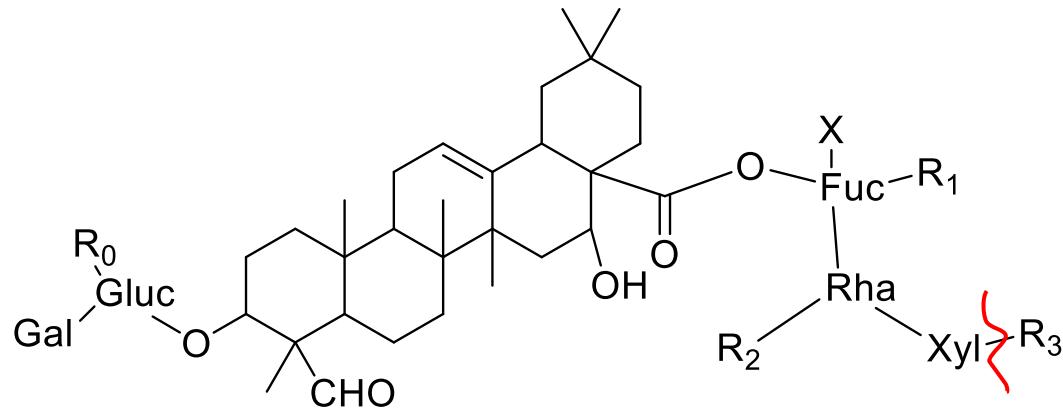




Reference ratio	Sum of peak areas		Peak volume	
	calculated ratio	Error (%)	calculated ratio	Error (%)
Sample #1	47.9%	0.02	48.8%	1.9
Sample #2	0.89%	2.2	1.03%	15.7
Sample #3	0.04%	2.5	0.05%	32.5

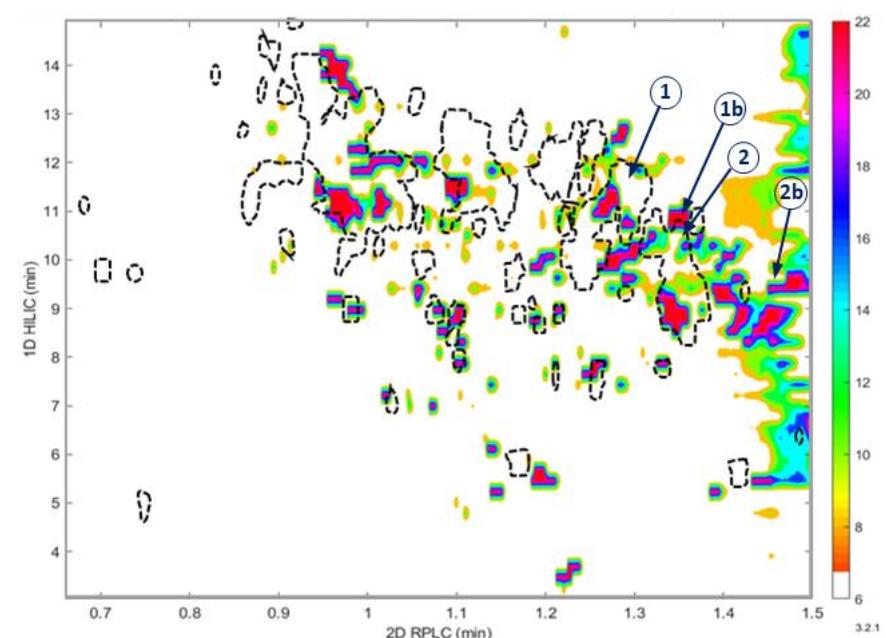
The sum of peak areas is more accurate

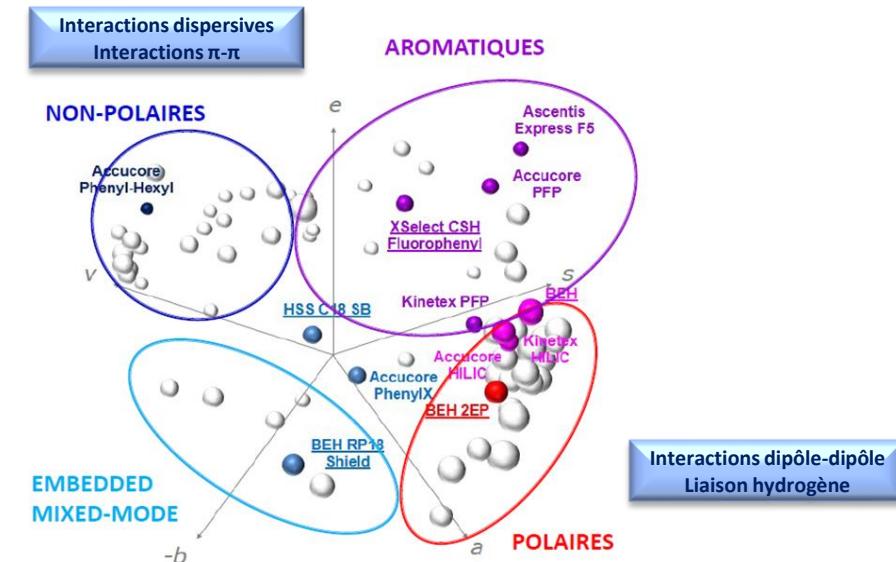
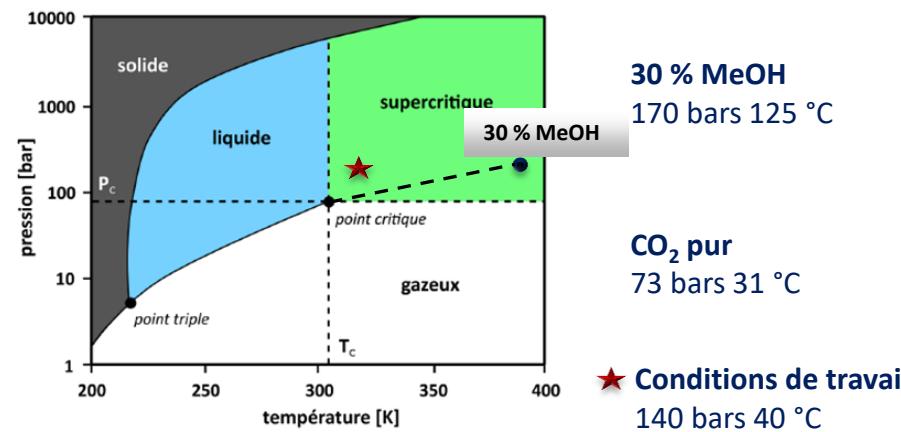


Visual monitoring of saponin extract from *Quillaja Saponaria*

N°	m/z	Name	R ₀	R ₁	R ₂	R ₃	X
1	2151	QS18	Xyl	H	Glc	Api	FA-Araf
2	1989	QS21	Xyl	H	H	Api/Xyl	FA-Araf
1a	2019	QS18-Api	Xyl	H	Glc	coupé	FA-Araf
2a	1857	QS21-Api	Xyl	H	H	coupé	FA-Araf

HILIC x RPLC - MS



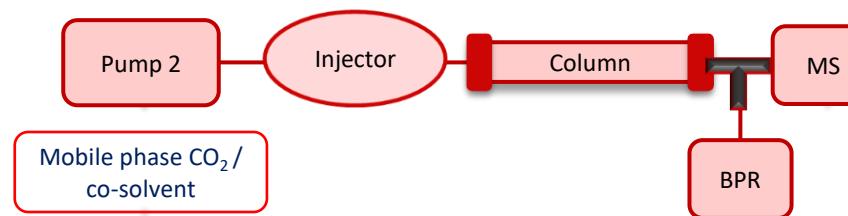


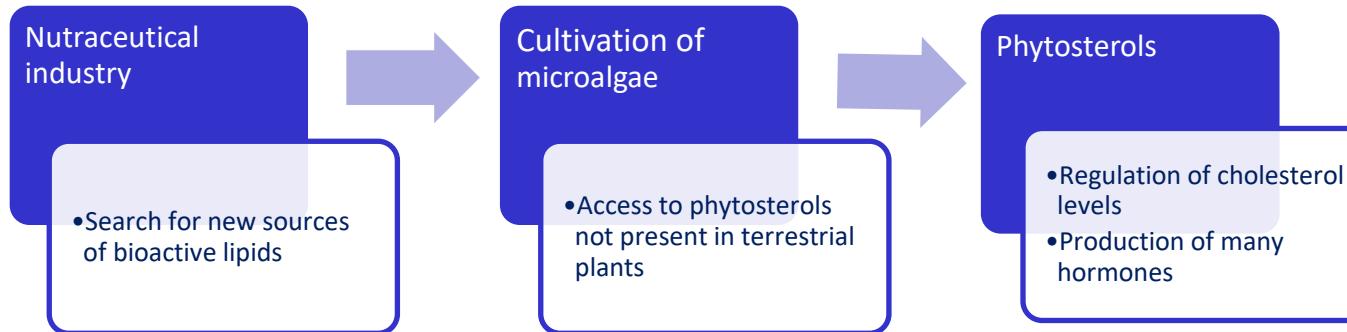
E. Lemasson et al., J. Chromatogr. A 1408 (2015) 227 – 235

1st Dimension
LC



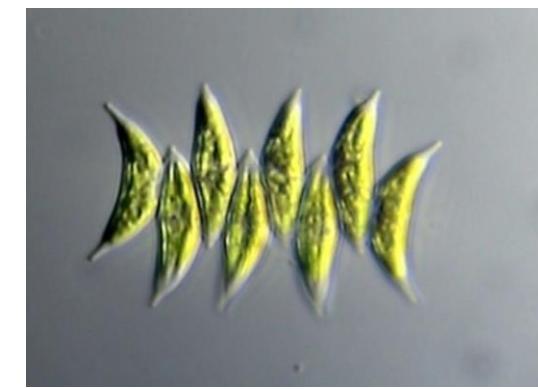
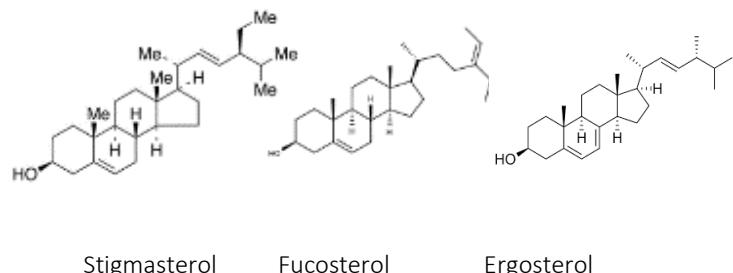
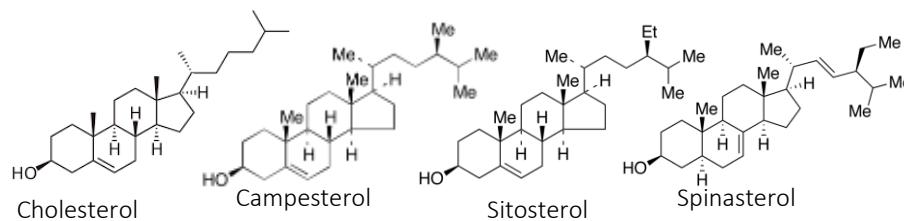
2nd Dimension
LC or SFC



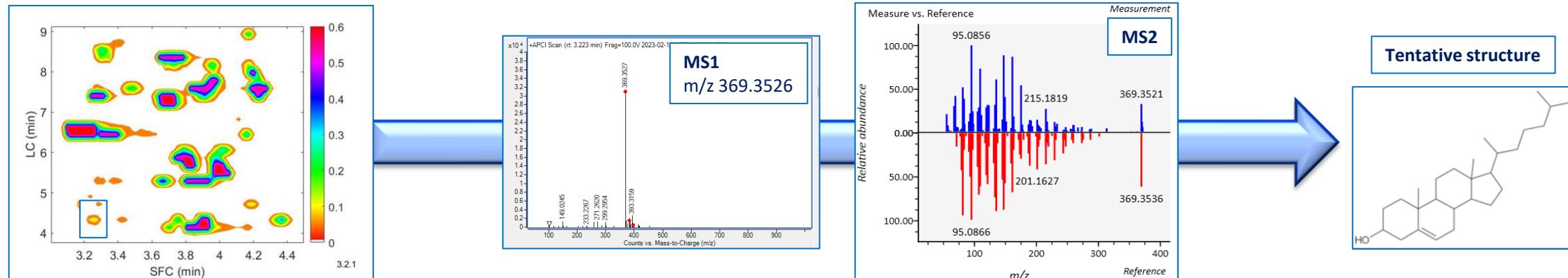
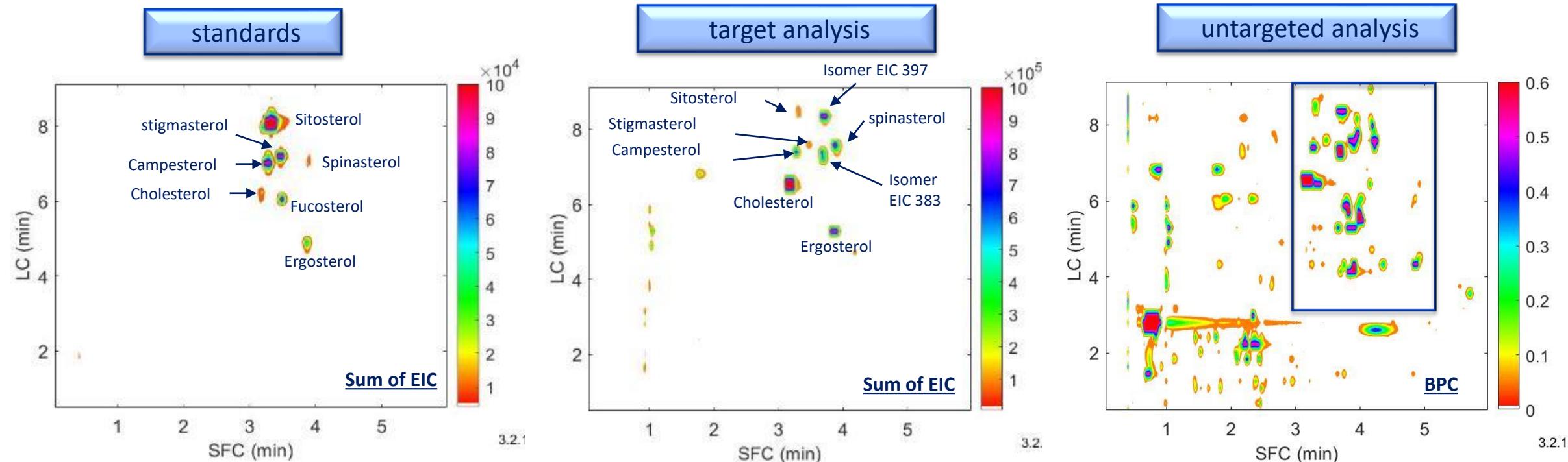


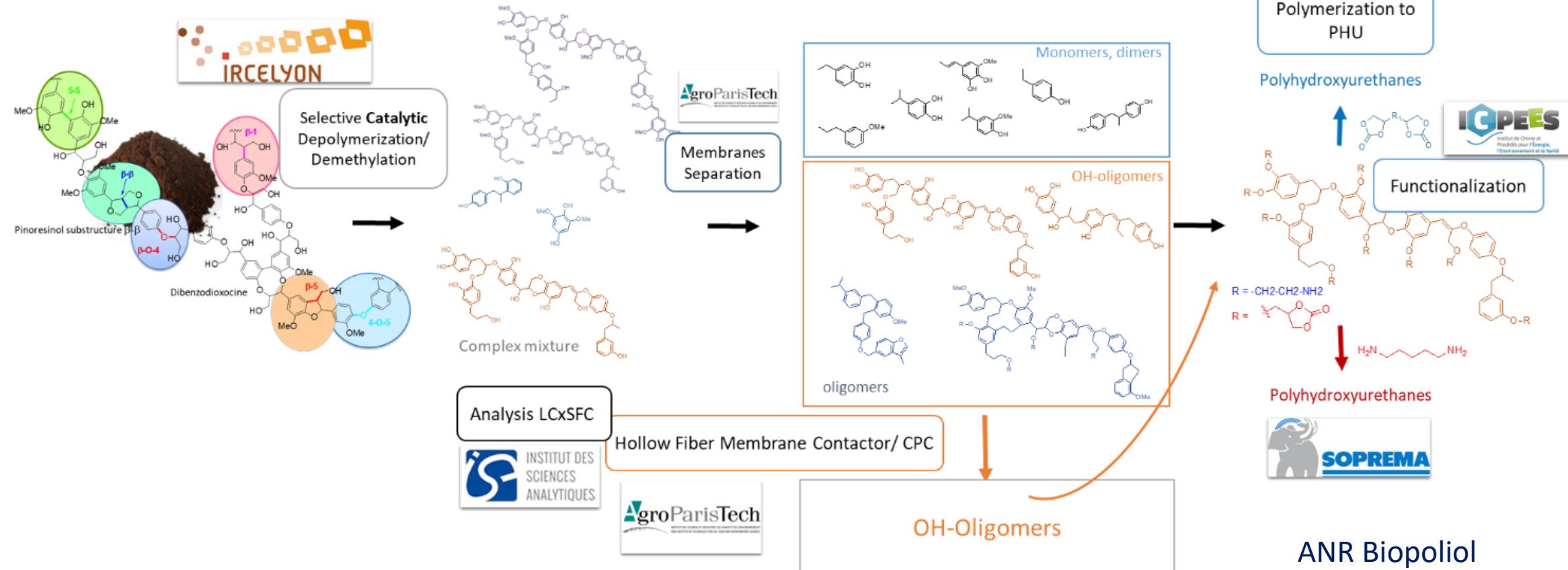
Phytosterols :

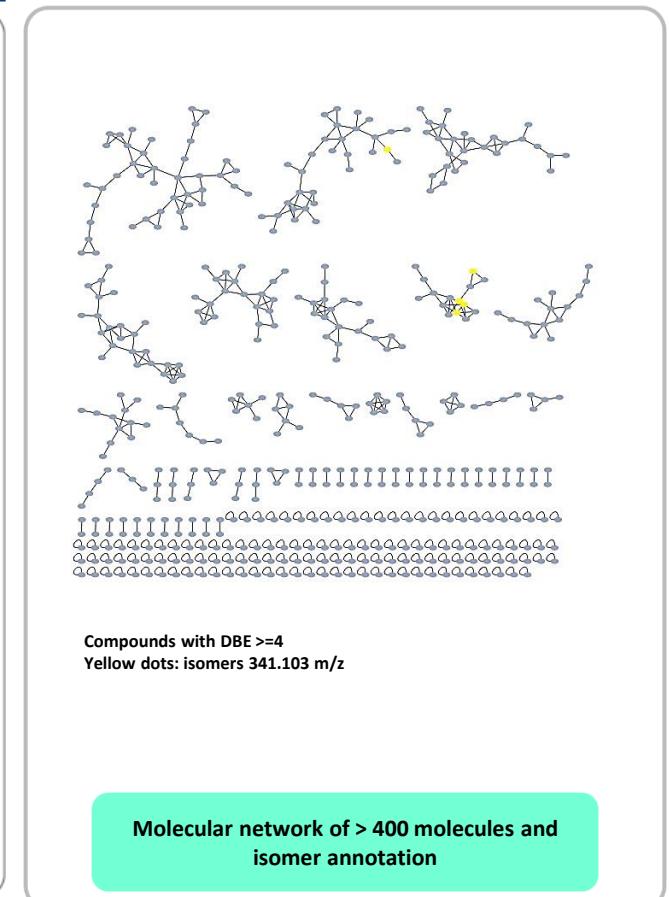
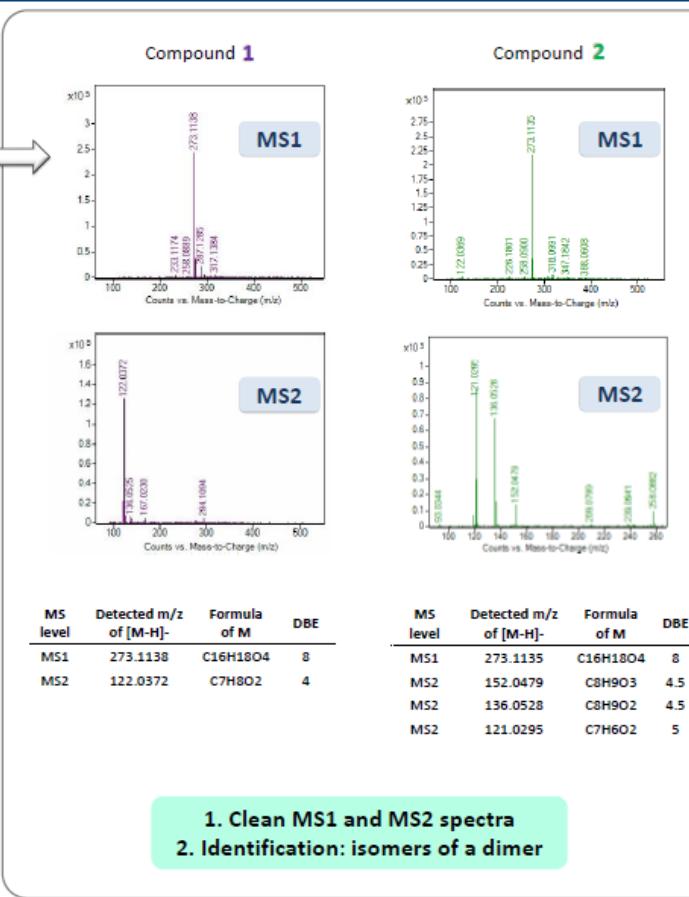
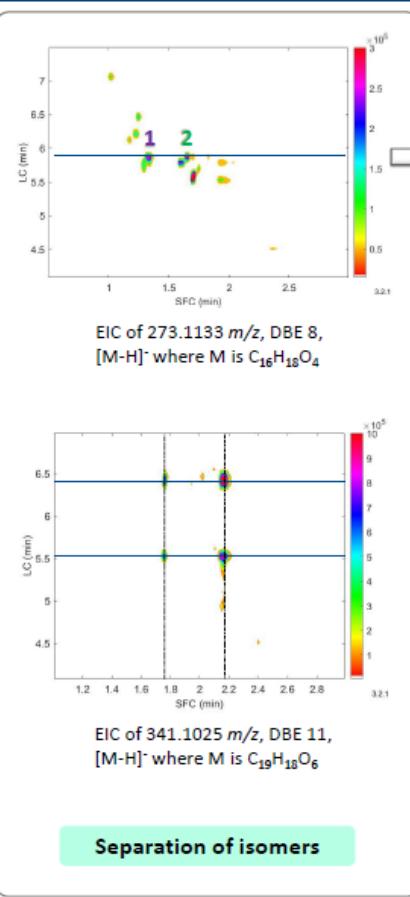
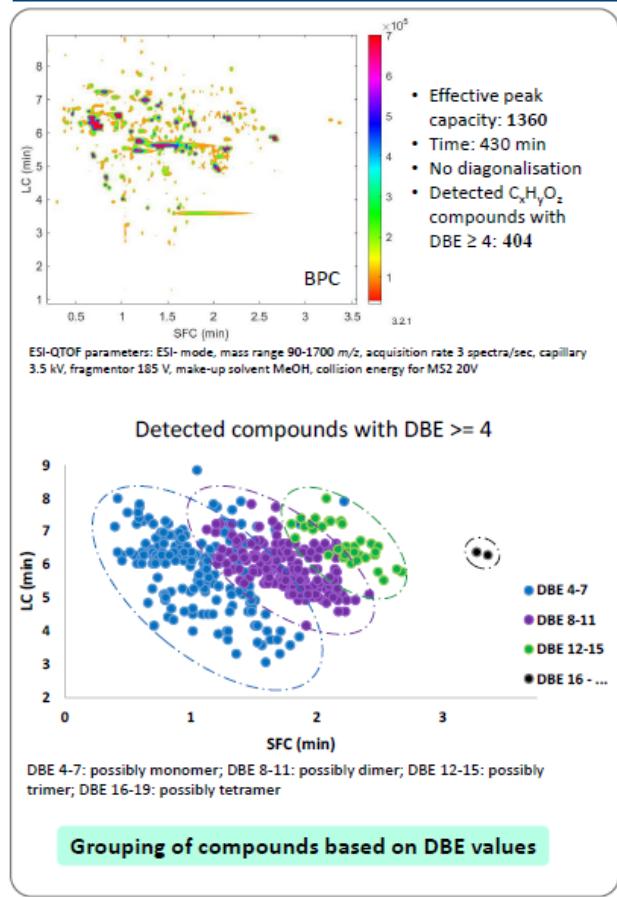
- Analogous compounds
- Great structural similarity → Separation and identification difficult



Chlorophyceae Scenedesmus







Two-dimensional liquid chromatography (coupled to mass spectrometry) offers huge peak capacity essential to the analysis of complex samples, especially for untargeted analysis

RPLC x RPLC is a efficient combination for ionisable compounds

RPLC x HILIC is technically more difficult, but interesting for **very polar compounds** (ionisable molecules or glycosides)

RPLC x SFC (offline or online) is an alternative technique for **neutral compounds**. Especially attractive for positional isomers

2DLC is complex to optimize but generate massive amounts of information on a sample.

1 chercheuse CNRS CRNC (K Faure)

1 directeur de recherche CNRS émérite (A. Berthod)

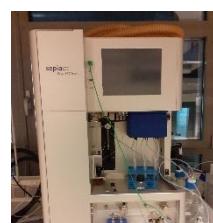
1 ingénieur d'étude CNRS (M. Batteau)

1 assistant ingénieur CNRS (F. Rouvière)

1 ingénieur études CDD projet Arkema (C. Giffard)

2 postdoctorantes (C. Gély – projet Allergan; E. Tammekivi projet Biopoliol)

1 doctorante (M. Sanchez , projet Total)



Semi-Préparative: CPC, LC et SFC



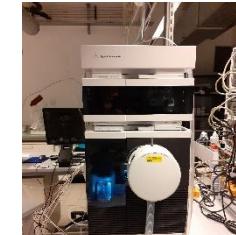
LC x LC

- 2 Agilent Infinity II 1290
- 1 Waters I-Class II



IM-QToF

- Agilent 6560



MS simple quadrupole

- Agilent MSD
- Waters QDA



LC unidimensionnelle

- Waters I-Class
- Waters UPLC
- Agilent 1260



SFC

- Agilent Infinity II 1290 SFC
- Waters UPC²