From extraction to characterization: development of an online tool

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November 25th, 2021

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Close to the Versailles castle

Founded in 1970 by Jean-Jacques GUERLAIN.

Research team

6 persons : 3 PhD, 2 Engineers, 1 Technician

2 main areas of research:

- Sensory analysis
- Physical-chemical analysis: including "green" chemistry

Introduction : context

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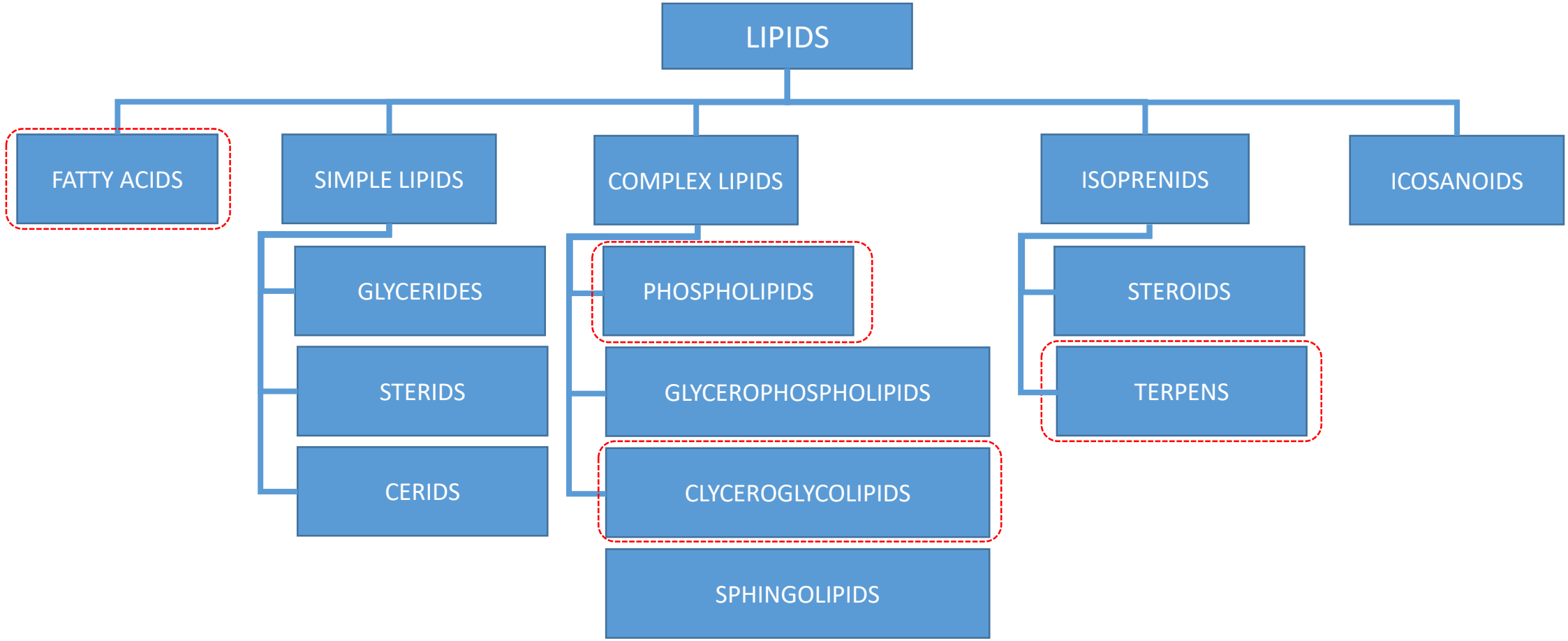
Introduction : project goals

- LIPOCOSM2 project: lipids interest and more particularly polar lipids (MGDG, DGDG, Phospholipids, SQDG, Sphingolipids, glycoglycerolipids ...),

- Processes (extraction, control and pre-formulation): green chemistry and more particularly supercritical CO2, custom circuit and recovery of co-products (waste),

- Use of cosmetic actives: make-up products and more particularly hybrid make-up.

Introduction : lipid families



Industrial applications of CO_2

Remove caffein from coffee without dichloromethane

Remove « cork taste », 2,4,6-trichloroanisole (TCA)

Textile-Dying Processes

Waterless and dryerless cleaning technology

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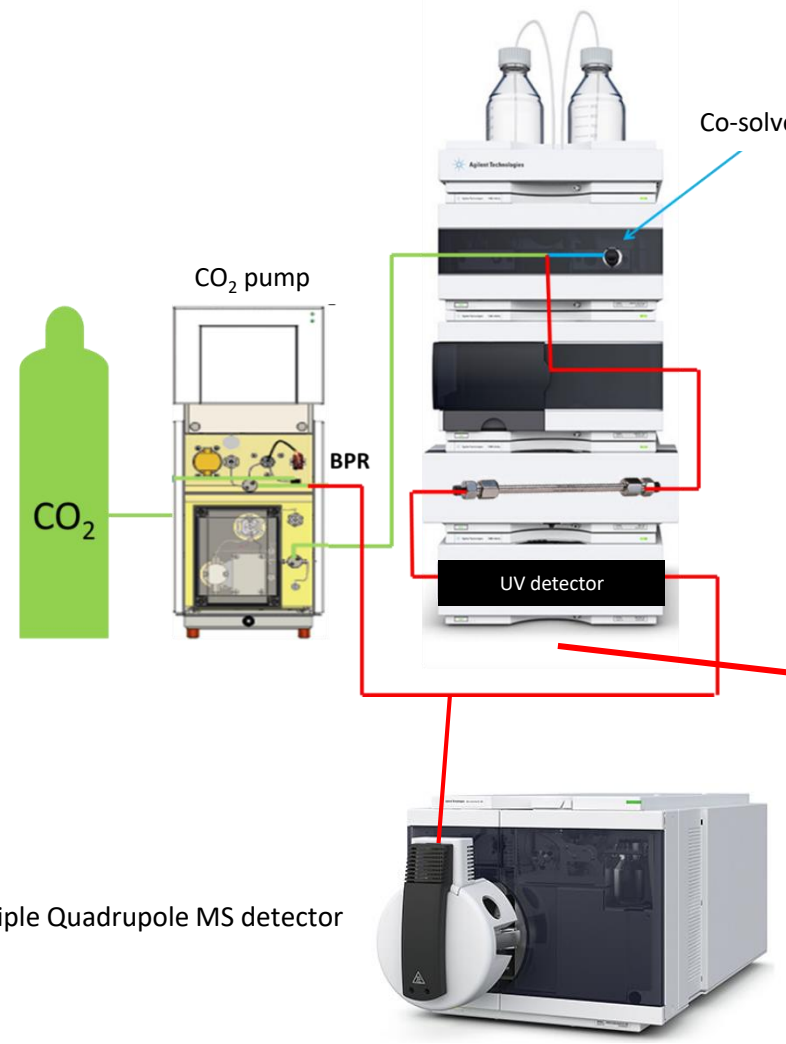
SFC-UV-MS



SFE (extraction)-SFF (fractionation)

2 meters length column for Supercritical Fluids Fractionation

100 ml cell for SFE or PLE until 150 bar with US (750W max, frequency 20kHz)

Two pumps : CO_2 and co-solvent
Max pressure 1000 bar

Oven, max operational temp : 150°C
Extraction cells sizes : 25, 50, 100 ml
SFE or PLE

Part I : Pepper SFE extracts

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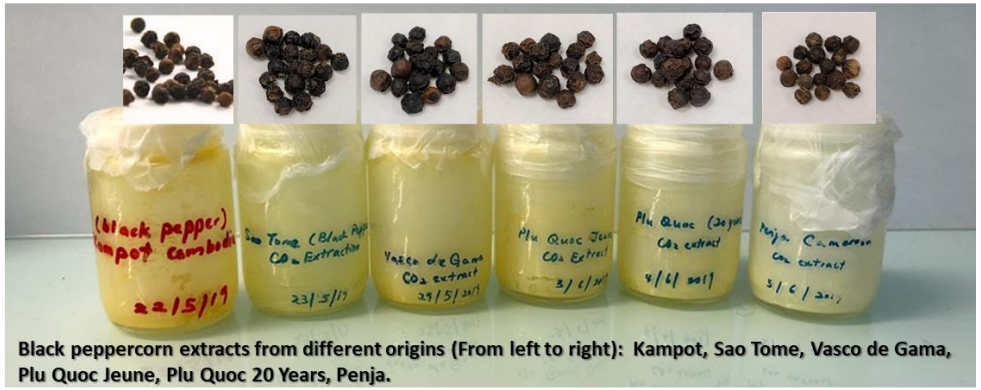
What kind of peppers?

	Origin	Family	Gender	Species
True peppers	Kampot	Piperaceae	*Piper*	*nigrum*
	Ceylan			
	Pondichery			
	Phu Quoc			
	Kerala			
	Vasco de gama			
	Sao tome			
	Penja			
	Voatsiperifery			*borbonense*
False peppers	Sancho	Rutaceae	*Zanthoxylum*	*piperitum*
	Timut			*armatum*

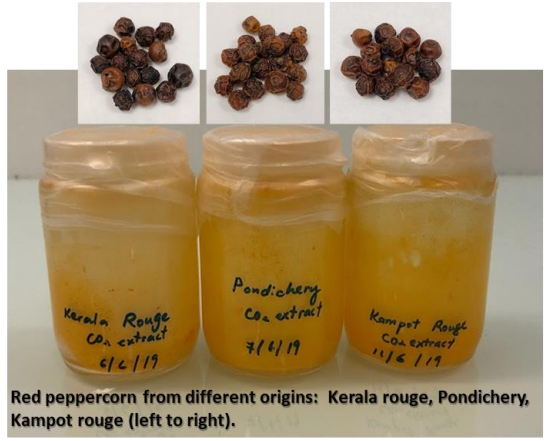
	Solvent	Pressure (bars)	Temperature (°C)	Time (min)	Mode	CO_2 Density (kg/m^3)
Extraction Condition	Pure CO_2	300	50	45	Dynamic	≈900

Peppers extracts

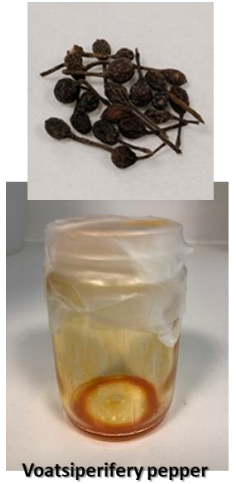
White peppercorn extracts from different origins: Ceylan White, Kampot White, Penja White (left to right).



Black peppercorn extracts from different origins (From left to right): Kampot, Sao Tome, Vasco de Gama, Plu Quoc Jeune, Plu Quoc 20 Years, Penja.



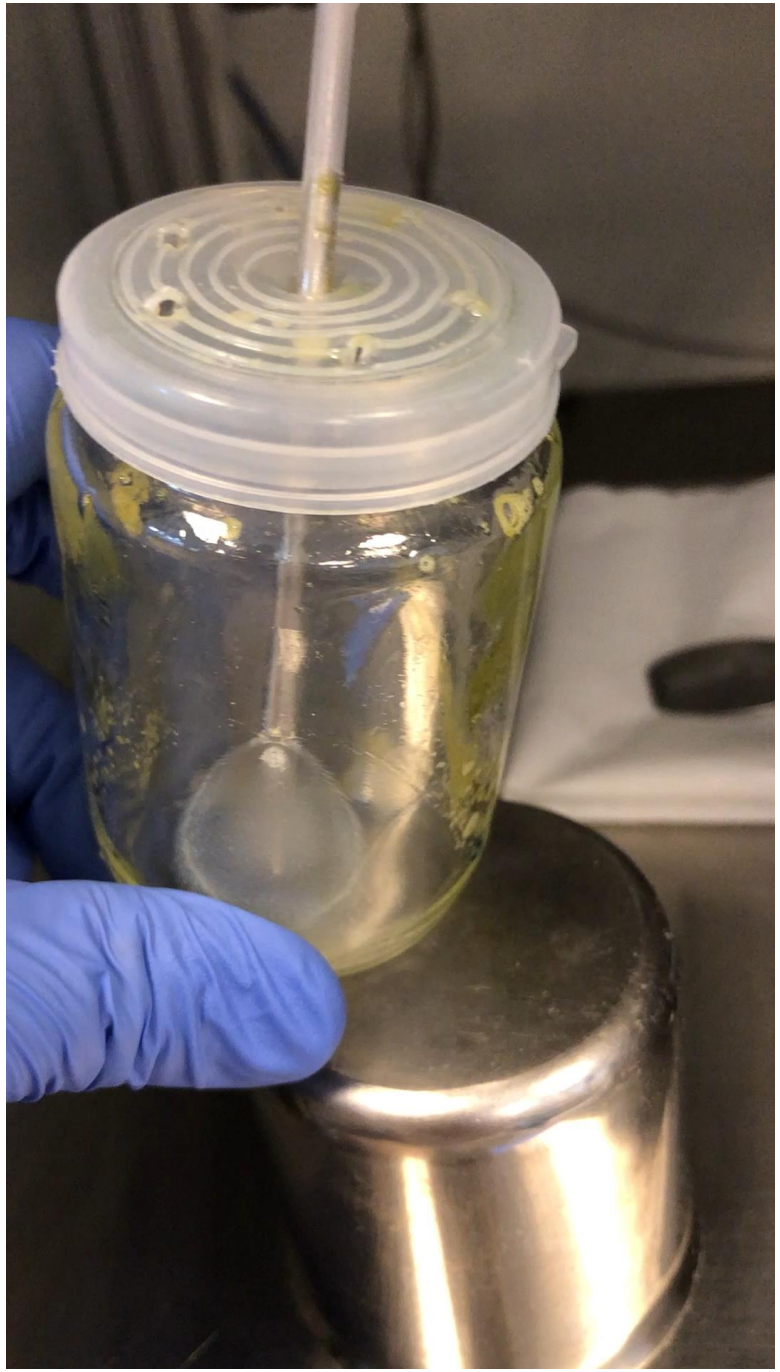
Red peppercorn from different origins: Kerala rouge, Pondichery, Kampot rouge (left to right).


Voatsiperifery pepper


Timut Nepal


Green Sancho

- **Green pepper is harvested from September** onwards, when it is still **young and tender**.
- **Black pepper is** actually **green pepper** which, once removed, **becomes black by a natural oxidation process**.
- The **pepper which has been allowed to mature** (and under **sufficient sunlight**) takes on a **red color**, it is then **picked by hand in March and left to dry for eight days**.
- **White pepper** is actually **dried red** peppercorns from which the **flesh has been removed** to **have only the core** of the pepper.



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Extraction yields

Origin	Extraction yield (%)	Extraction recovery (%)
Kampot Cambodia	2.3	39.6
Sao tome	2.2	24.9
Vasco de Gama	1.9	48.3
Plu Quoc Young	2.9	27.1
Plu Quoc 20 Years	2.0	35.7
Penja	2.4	39.0

Origin	Extraction yield (%)	Extraction recovery (%)
Kerala red	2.6	50.0
Kondichery	1.9	45.5
Kampot red	2.3	75.7

Origin	Extraction yield (%)	Extraction recovery (%)
Ceylan White	3.1	51.6
Kampot White	2.6	69.5
Penja White	3.8	46.9

Origin	Extraction yield (%)	Extraction recovery (%)
Voatsiperifery pepper	5.5	55.1
Timut Nepal	7.9	68.3

Origin	Extraction yield (%)	Extraction recovery (%)
Red Sancho	5.5	60.5
Green Sancho	4.8	64.2

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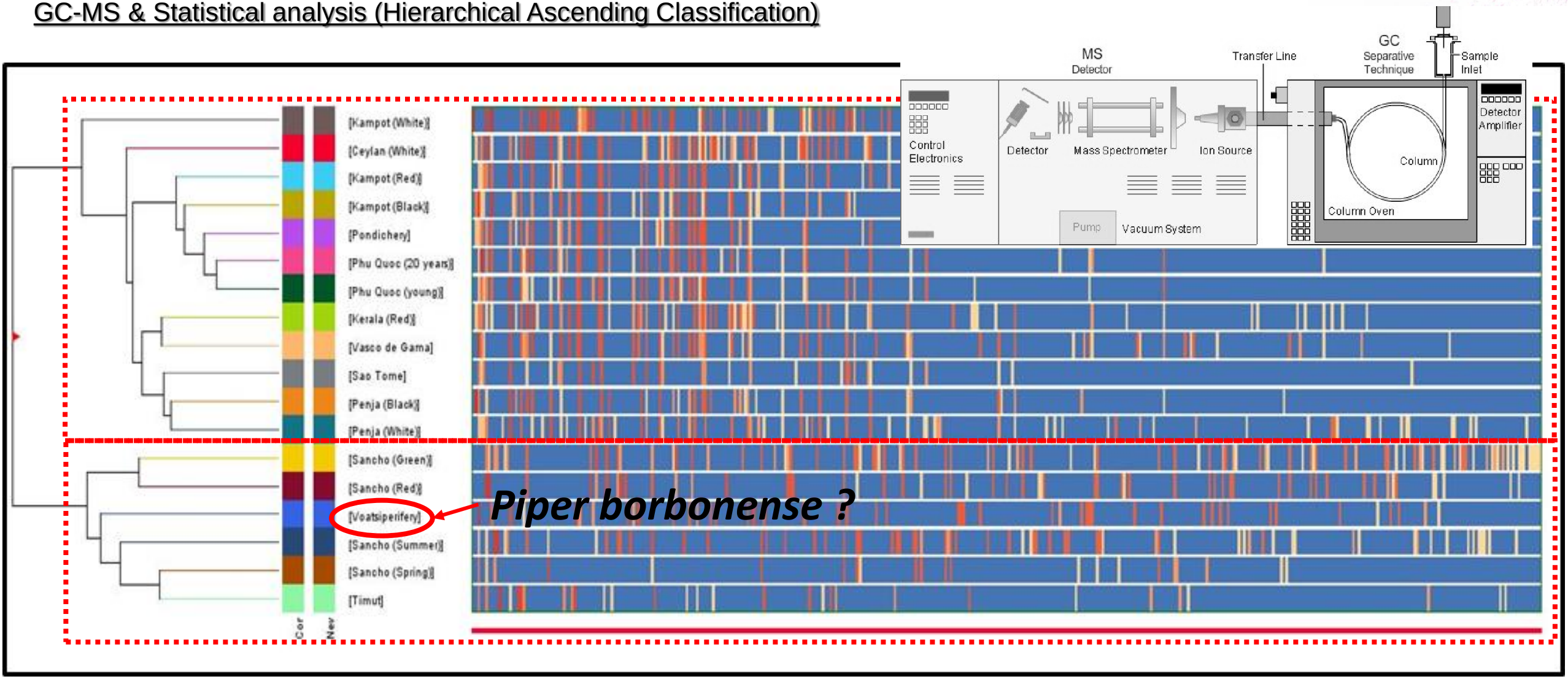
GC-MS & Statistical analysis (Hierarchical Ascending Classification)



Part II : SFE-SFC-MS hyphenation

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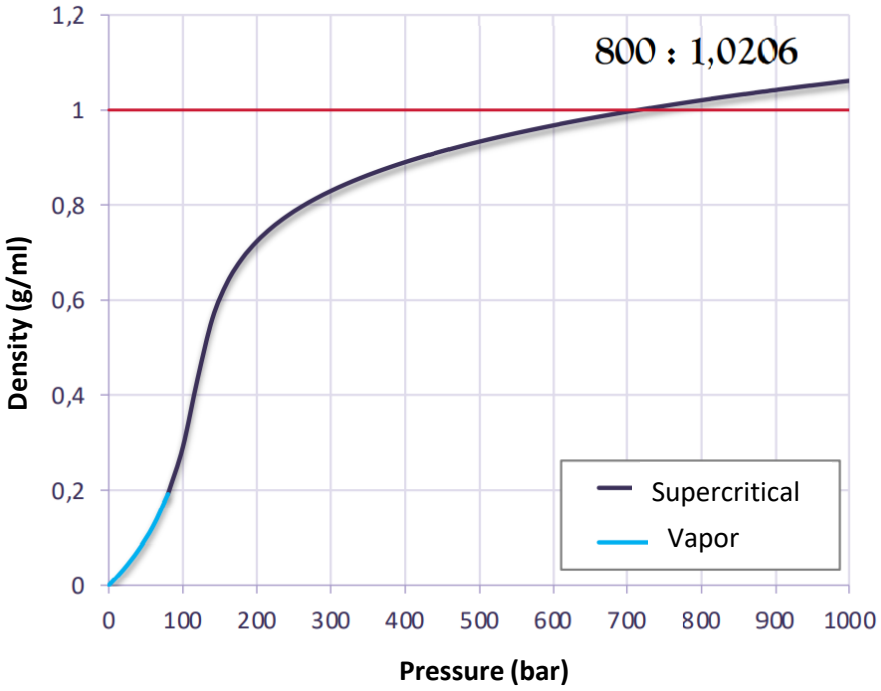
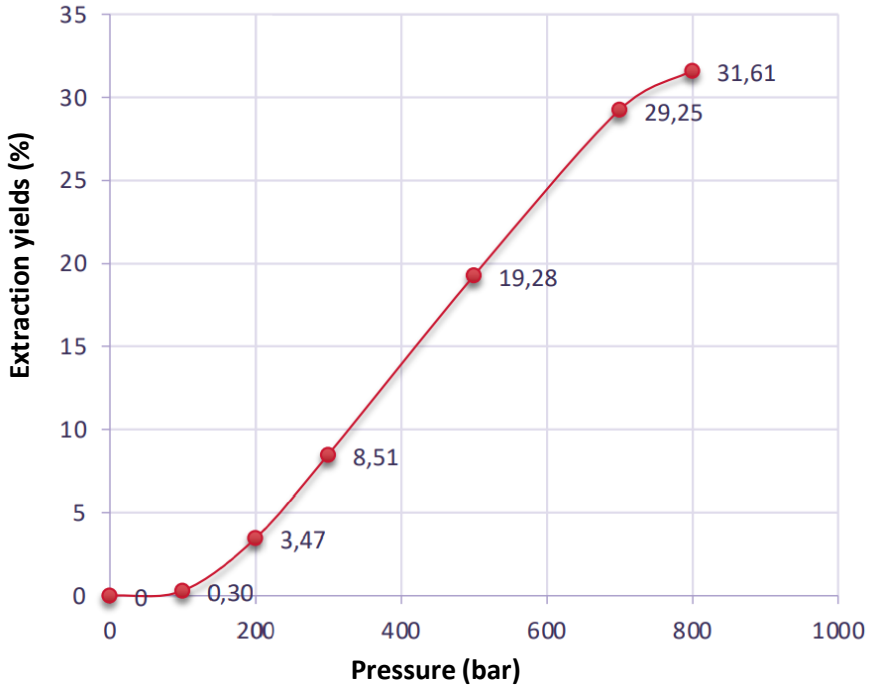
High-pressure SFE extraction : impact on yield

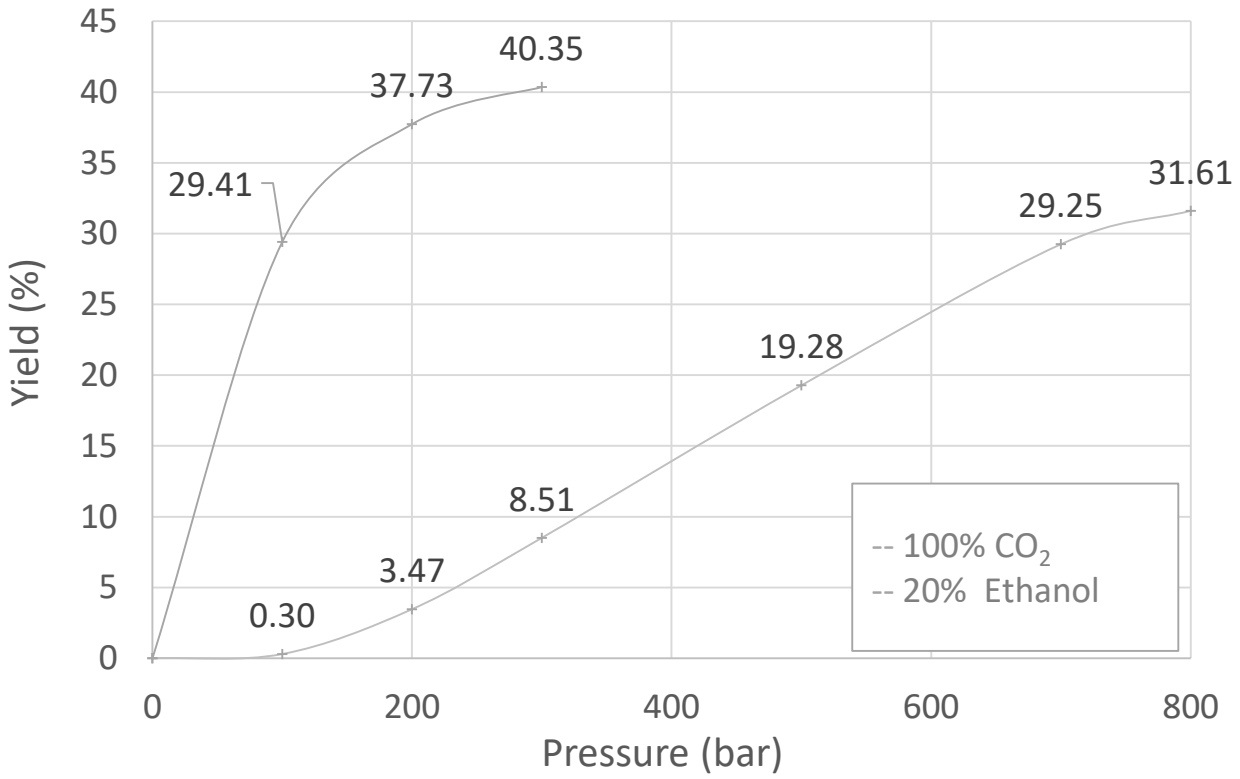


https://webbook.nist.gov/chemistry/fluid/

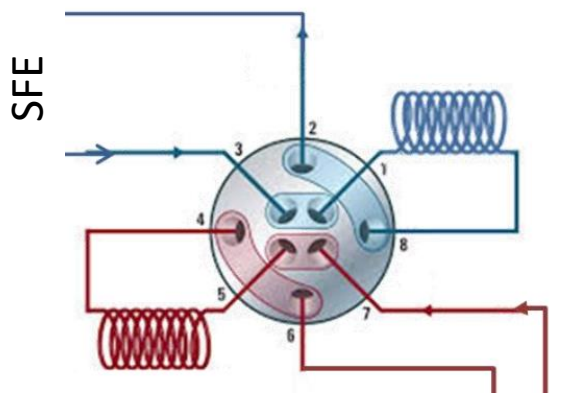


Extraction yield is linked to supercritical solvent density impact (solvent power)

Co-solvent SFE extraction : impact on yield



Extraction yield is linked to co-solvent percentage



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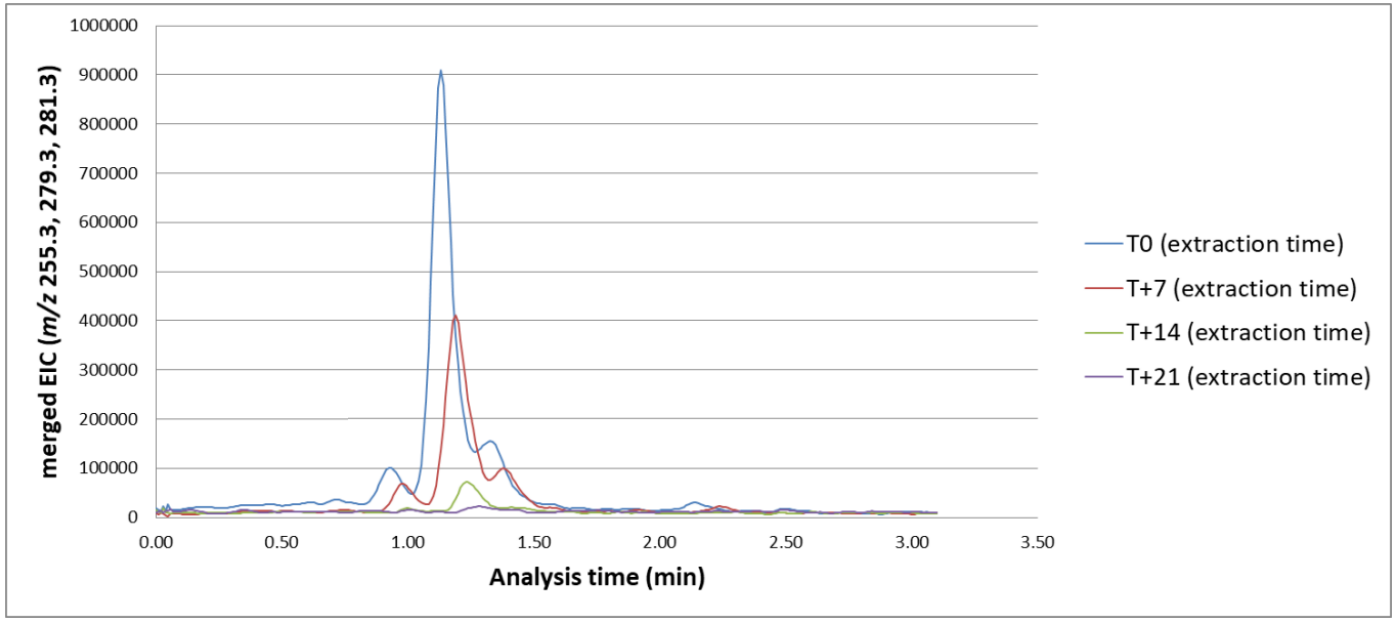
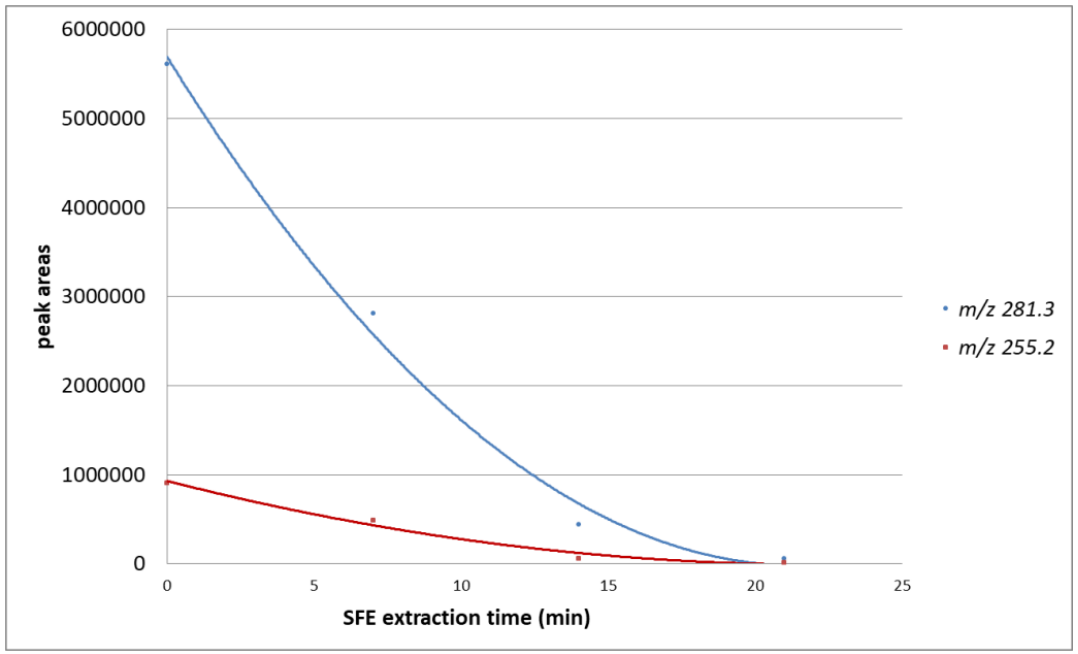
Proof of concept : Online analysis of sunflower extracts



Fatty acids	Caproidic	Caprylic	Capric	lauric	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Gadoleic	Behenic	Erucic	Lignoceric
	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
Sunflower	Nd	Nd	Nd	Nd	<0.5	6	Trace	5	18	69	<0.5	<0.5	<0.5	<1	Nd	Nd



Jane ROCHE, Composition de la graine de tournesol (Helianthus annuus L.) sous l'effet conjugué des contraintes agri-environnementales et des potentiels variétaux, thèse de doctorat de l'institut national polytechnique de Toulouse, sous la direction de Andrée BOUNIOLS, 2005, 305 p.

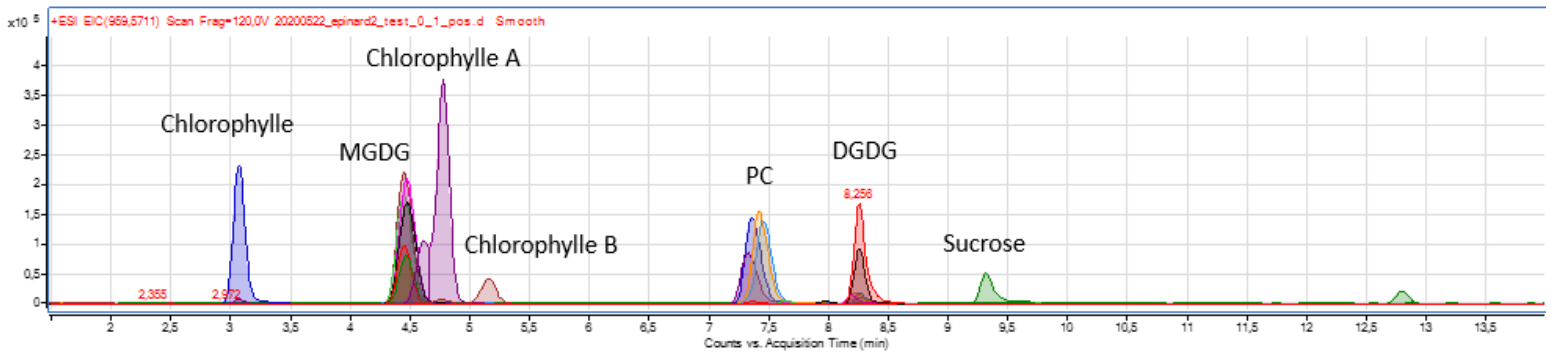
SFC method for Polar lipids analysis

Same chromatographic parameters as ICSN published method.

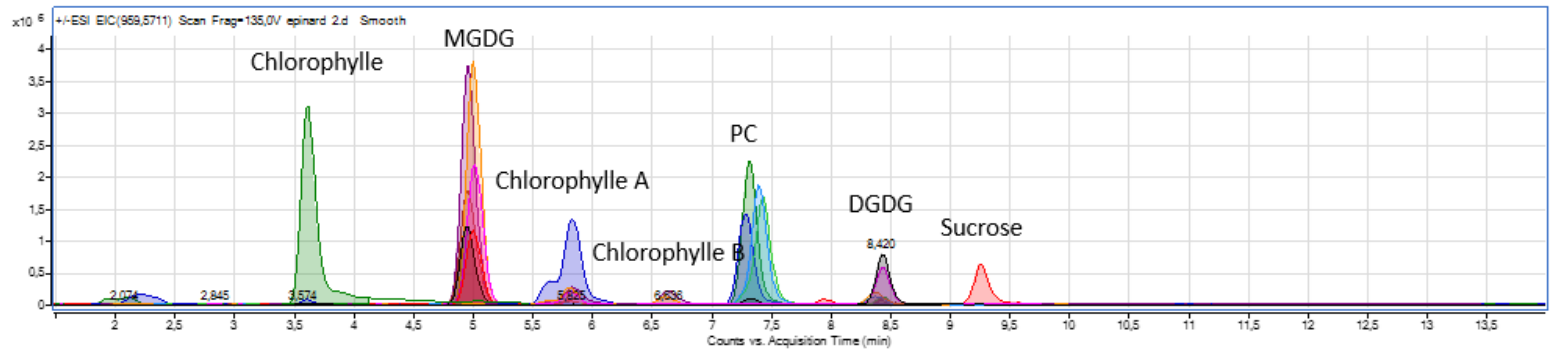
Only one modification :

✓ column size 2.1 mm x 100 mm, 1.7 µm to reduce pressure inside SFC

Chromatogram obtained using ICSN method



Chromatogram obtained using ISIPCA modified method



Similar retention times and shape's peaks

Céline Chollet, Stéphanie Boutet-Mercey, Laurent Laboureur, Carlos Rincon, Marie Méjean, Juliette Jouhet, François Fenaille, Benoit Colsch, David Touboul, Supercritical fluid chromatography coupled to mass spectrometry for lipidomics, Journal of Mass spectrometry, Volume54, Issue10, October 2019, Pages 791-801

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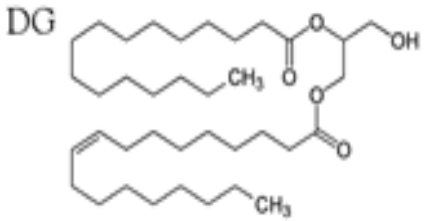
SFC method : Fatty acids analysis

Nom AG	AG théorique	100 bar		200 bar		300 bar		500 bar		700 bar	
C6:0		GC		GC							
C8:0		GC		GC							
C12:0											
C14:0											
C16:1		GC	SFC	GC	SFC	GC		GC		GC	
C16:0		GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC
C17:0											
C18:3											
C18:2		GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC
C18:1		GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC
C18:0		GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC
C20:1		GC	SFC	GC	SFC	GC		GC		GC	
C20:0		GC	SFC	GC		GC		GC		GC	
C22:0		GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC
C24:3									SFC		SFC
C24:2			SFC		SFC				SFC		SFC
C24:0			SFC		SFC		SFC		SFC		SFC

SFC method : other lipids analysis



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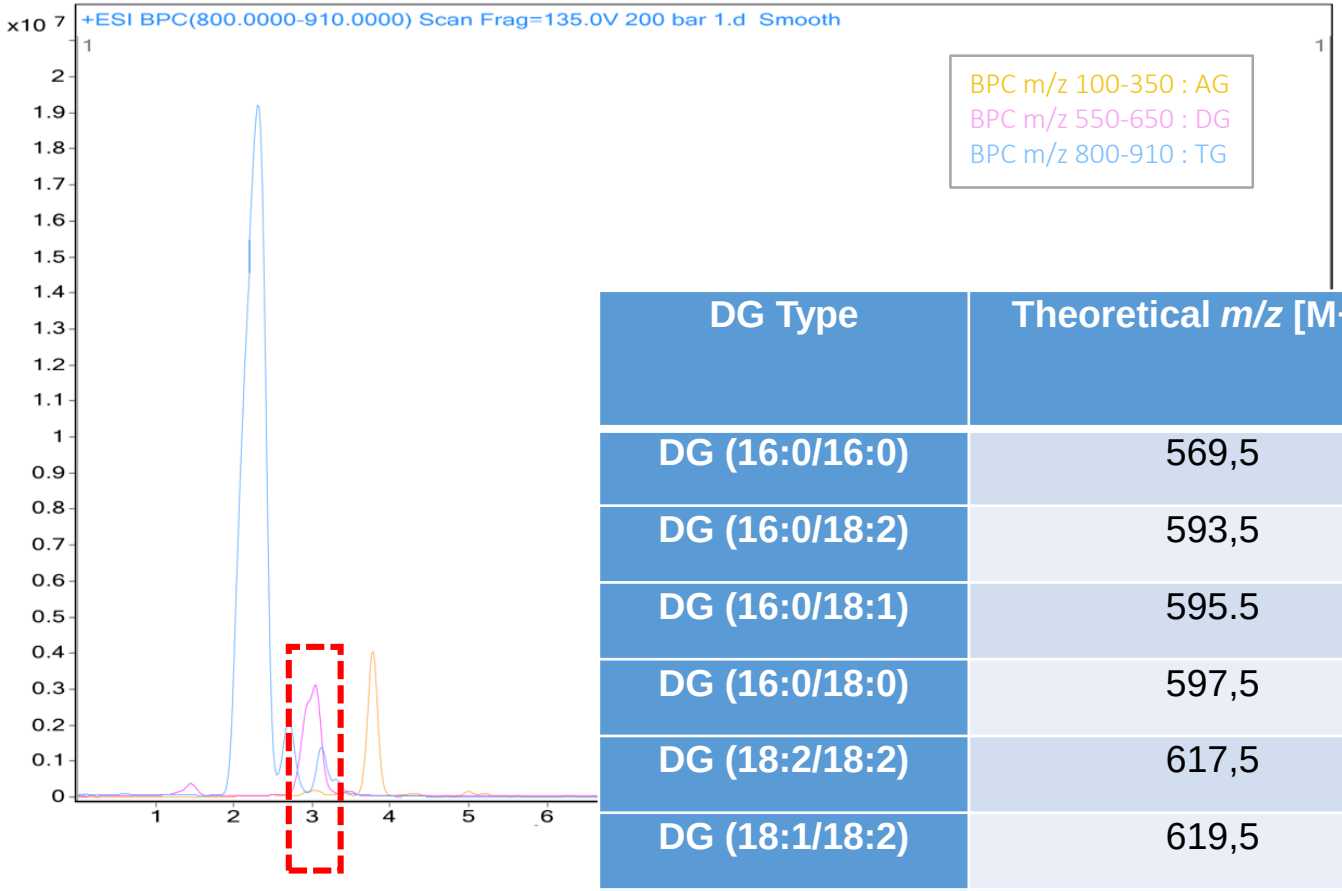
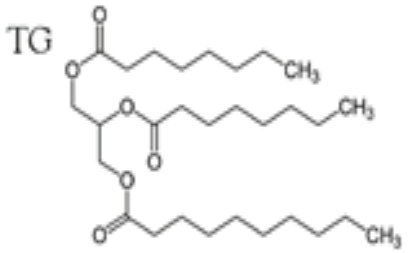


DG Type	Theoretical *m/z* $[M+H]^+$	Theoretical *m/z* $[M+NH_4]^+$	Annotated adduct in sunflower extract
DG (16:0/16:0)	569,5	586,5	$[M+H]^+$
DG (16:0/18:2)	593,5	610,5	$[M+H]^+$
DG (16:0/18:1)	595.5	612,5	$[M+H]^+$
DG (16:0/18:0)	597,5	614,5	$[M+H]^+$
DG (18:2/18:2)	617,5	634,5	$[M+NH_4]^+$
DG (18:1/18:2)	619,5	636,5	$[M+H]^+$
DG (18:0/18:1)	623,5	640,5	$[M+H]^+$
DG (18:0/18:0)	625,5	642,5	$[M+H]^+$

SFC method : other lipids analysis



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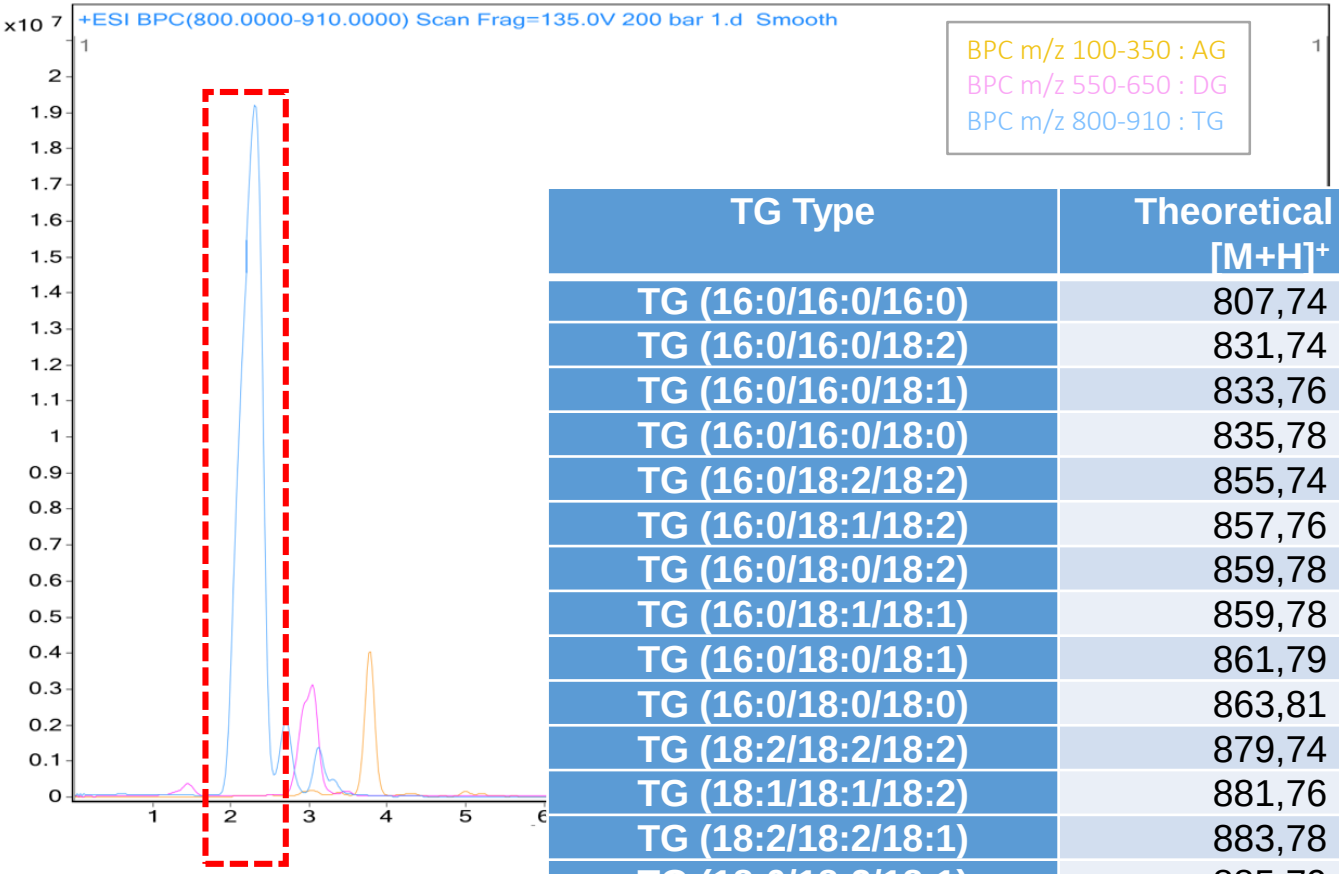

TG Type	Theoretical *m/z* $[M+H]^+$	Theoretical *m/z* $[M+NH_4]^+$	Annotated adduct in sunflower extract
TG (16:0/16:0/16:0)	807,74	824,77	$[M+NH_4]^+$
TG (16:0/16:0/18:2)	831,74	848,77	$[M+NH_4]^+$
TG (16:0/16:0/18:1)	833,76	850,79	$[M+NH_4]^+$
TG (16:0/16:0/18:0)	835,78	852,8	$[M+NH_4]^+$
TG (16:0/18:2/18:2)	855,74	872,77	$[M+NH_4]^+$
TG (16:0/18:1/18:2)	857,76	874,79	$[M+NH_4]^+$
TG (16:0/18:0/18:2)	859,78	876,80	$[M+NH_4]^+$
TG (16:0/18:1/18:1)	859,78	876,80	$[M+NH_4]^+$
TG (16:0/18:0/18:1)	861,79	878,82	$[M+NH_4]^+$
TG (16:0/18:0/18:0)	863,81	880,83	$[M+NH_4]^+$
TG (18:2/18:2/18:2)	879,74	896,77	$[M+NH_4]^+$
TG (18:1/18:1/18:2)	881,76	898,79	$[M+NH_4]^+$
TG (18:2/18:2/18:1)	883,78	900,8	$[M+NH_4]^+$
TG (18:0/18:2/18:1)	885,79	902,82	$[M+NH_4]^+$
TG (18:1/18:1/18:1)	885,79	902,82	$[M+NH_4]^+$
TG (18:0/18:0/18:2)	887,81	904,83	$[M+NH_4]^+$
TG (18:0/18:0/18:1)	889,82	906,85	$[M+H]^+$
TG (18:0/18:0/18:0)	891,84	908,86	$[M+NH_4]^+$

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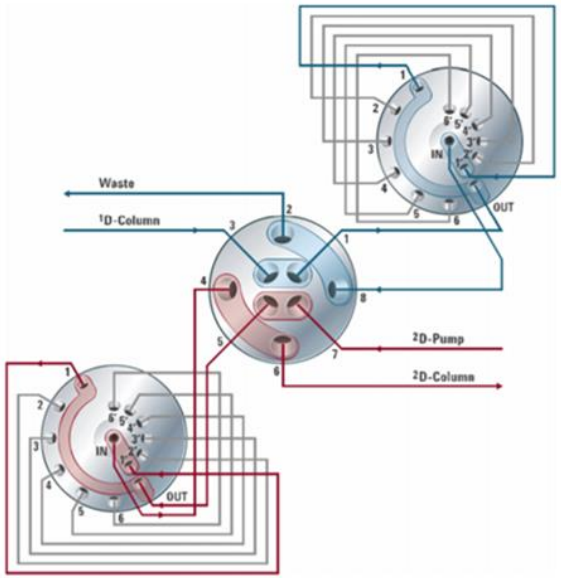
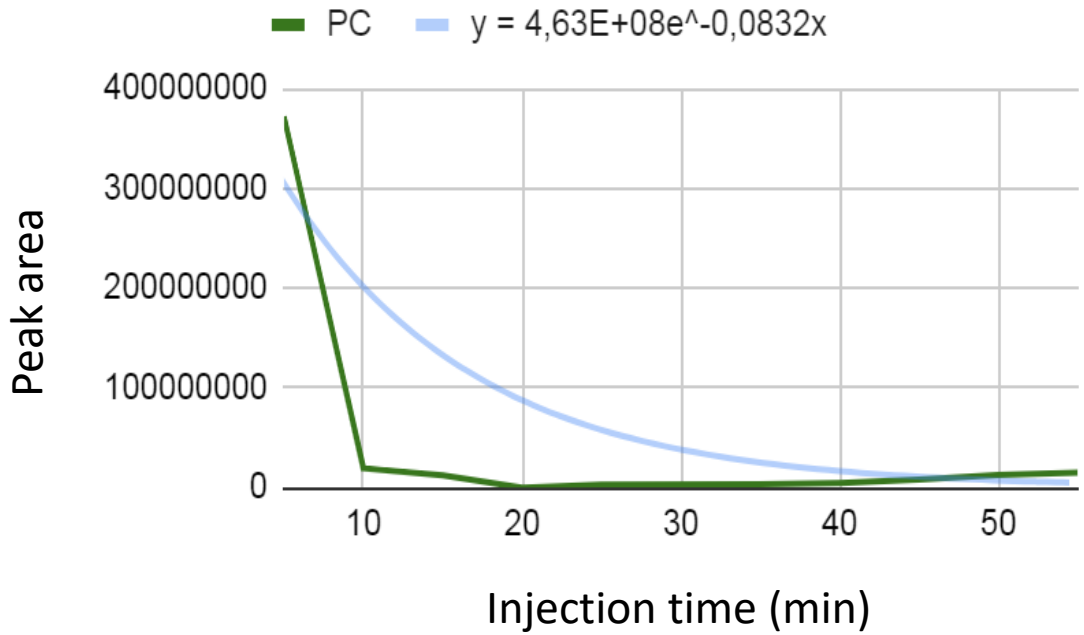
Second step: Online analysis of spinach CO_2/EtOH extracts

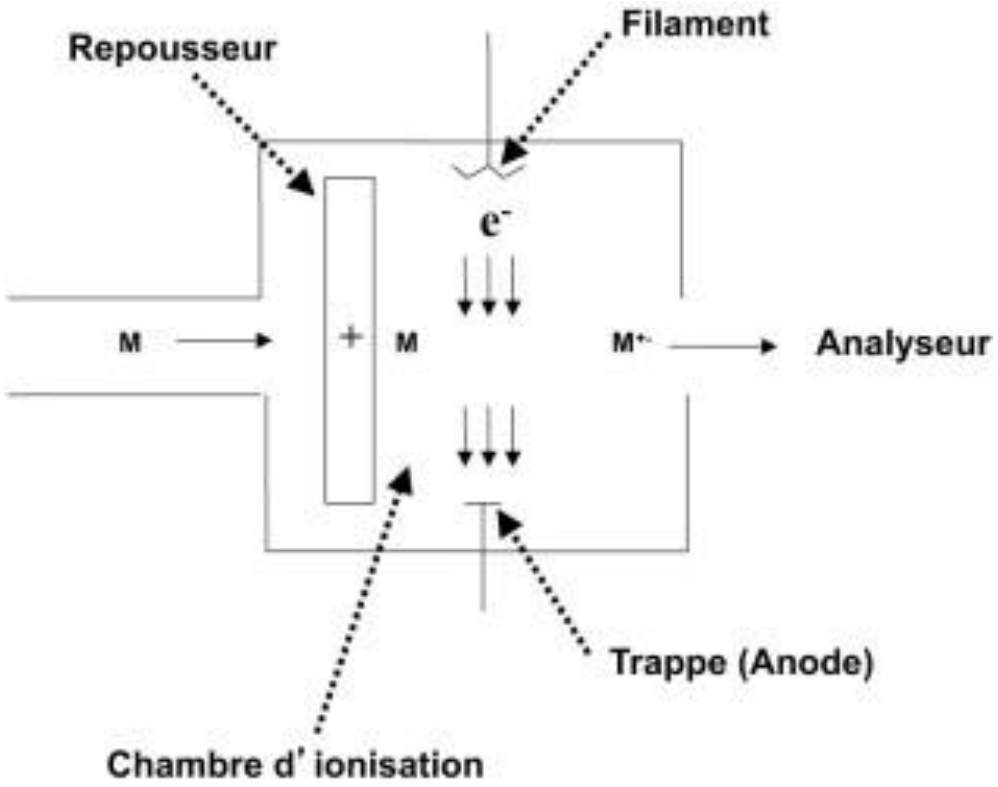
Polar lipds	m/z $[M+H]^+$	m/z $[M+Na]^+$	m/z $[M+NH_4]^+$
MGDG (36:6)	775,6	797,6	792,6
DGDG (36:6)	937,6	959,6	954,6
PC (34:3) Or PC (32:0)	756,6	778,6	773,6
	734,6	756,6	751,6

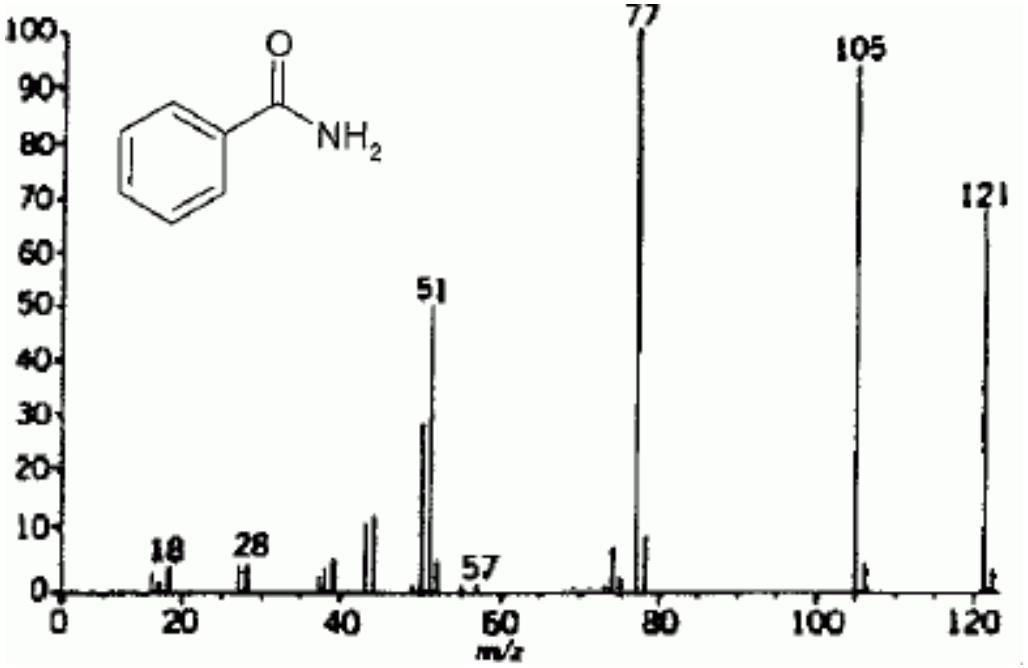


Phospholipids analysis : Profile



Part III : SFE-EI-MS hyphenation





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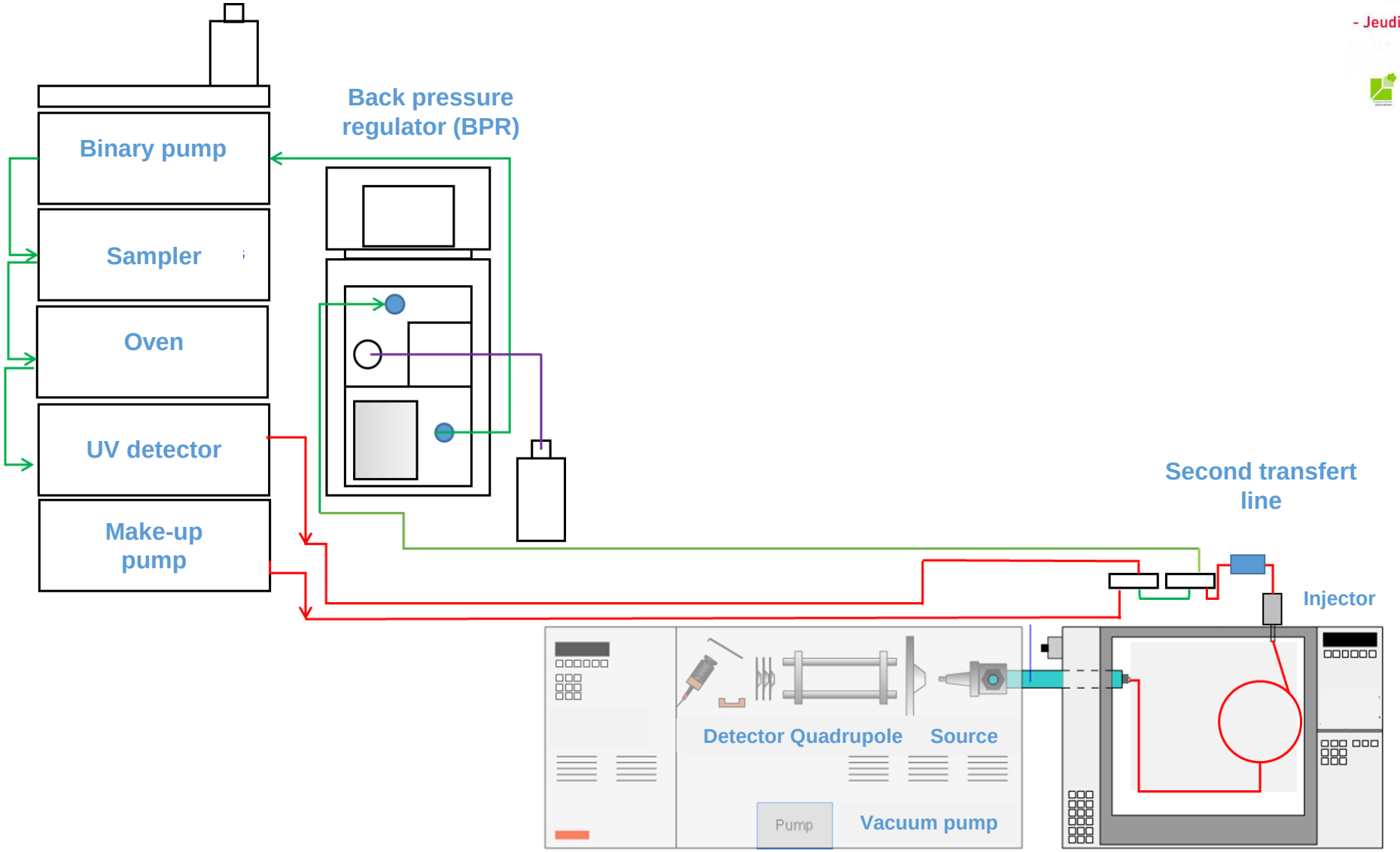
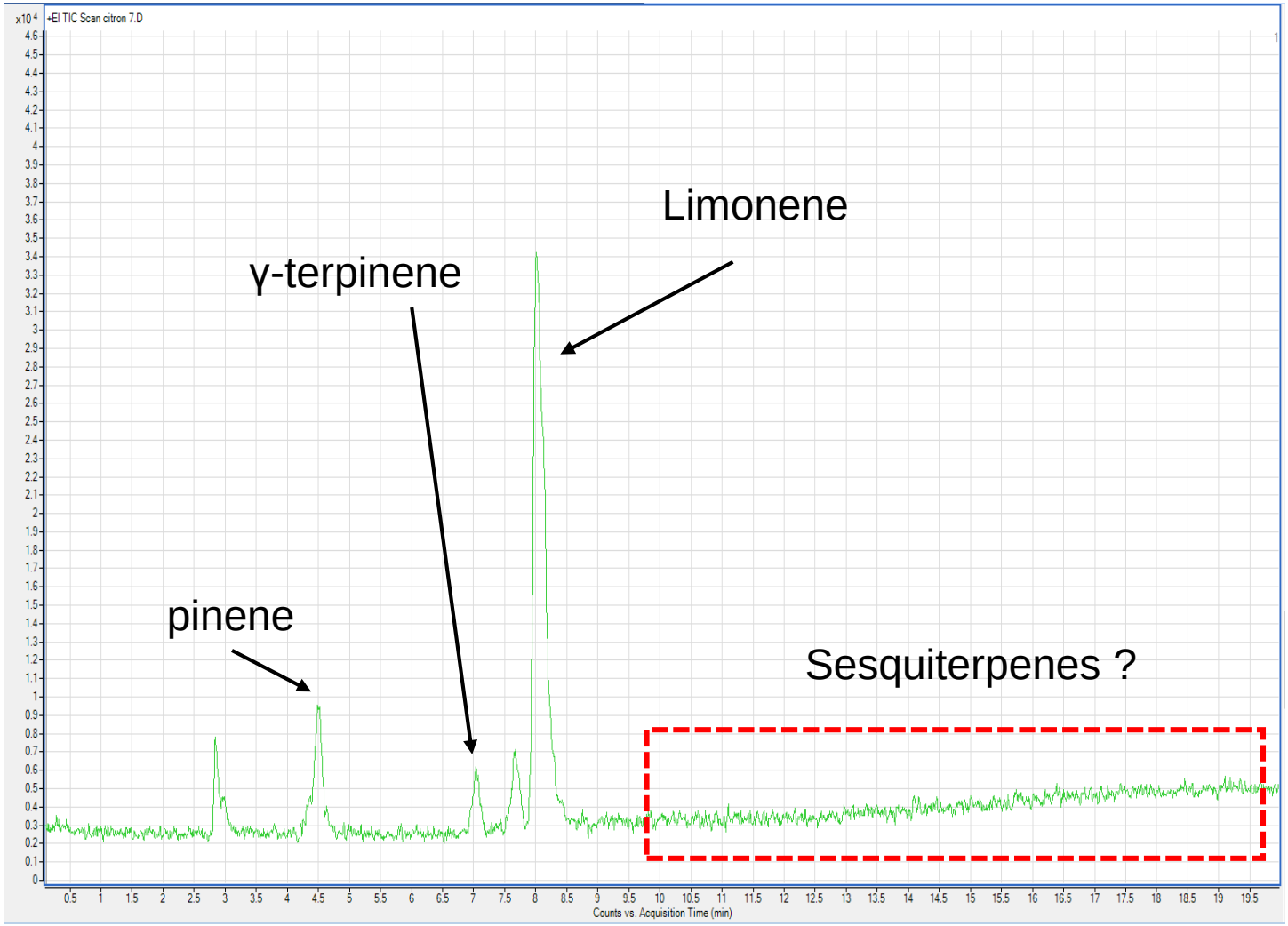
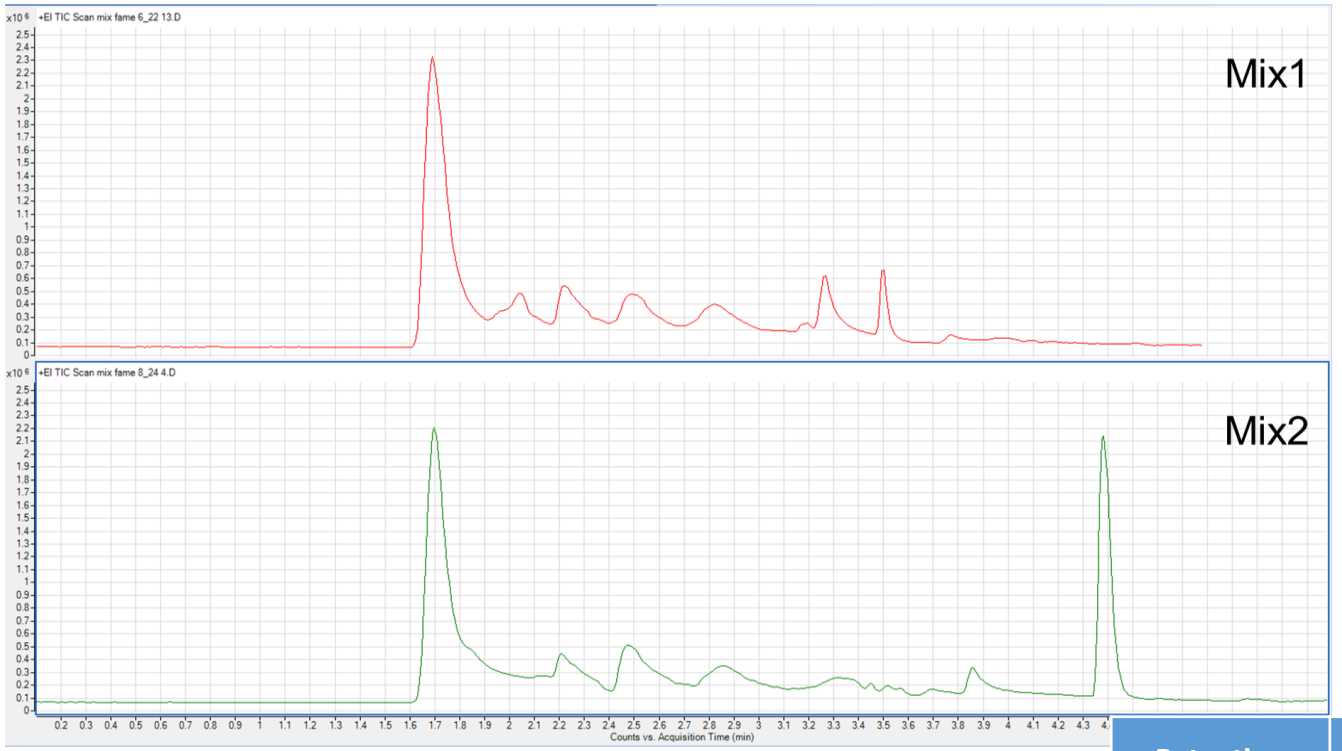

Back pressure regulator (BPR)
Binary pump
Sampler
Oven
UV detector
Make-up pump
Second transfert line
Injector
Detector Quadrupole
Source
Pump
Vacuum pump

Lemon essential oil analysis



- Detection and identification of limonene,
- Detection without identification of γ-terpinene and pinene,
- Non detection of sesquiterpenes.



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Fatty Acids Metyl Ester (FAME) analysis

Retention time (min)	Fatty Acids Metyl Ester (FAME)	Formula	Similarity score
Mix1			
2.0	Hexanoic acid methyl ester	$C_7H_{14}O_2$	84.0
2.2	Decanoic acid methyl ester	$C_{11}H_{22}O_2$	89.8
2.5	Myristic acid methyl ester	$C_{15}H_{30}O_2$	89.9
2.8	Stearic acid methyl ester	$C_{19}H_{38}O_2$	87.1
3.3	Docosanoic acid methyl ester	$C_{23}H_{46}O_2$	85.3
Mix2			
2.2	Octanoic acid methyl ester	$C_9H_{18}O_2$	93.4
2.5	Dodecanoic acid methyl ester	$C_{13}H_{26}O_2$	86.0
2.9	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	84.1
3.8	Eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	85.3

Conclusion

- Clustering for peppers is to be investigated
- First results are encouraging
- Improve the robustness of the multi-loop system
- SFE-SFC : Analyze other lipids and/or other matrix
- First results using SFC-EI-MS are encouraging

Acknowledgment

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Thank you for your attention. Any questions?