Interreg France-Wallonie-Vlaanderen

GoToS3 ALPO

Lipid profiling of *Nannochloropsis gaditana*: milling or not milling the microalgae biomass?

3rd Workshop – ALPO project
Teams plateform
28/01/2021

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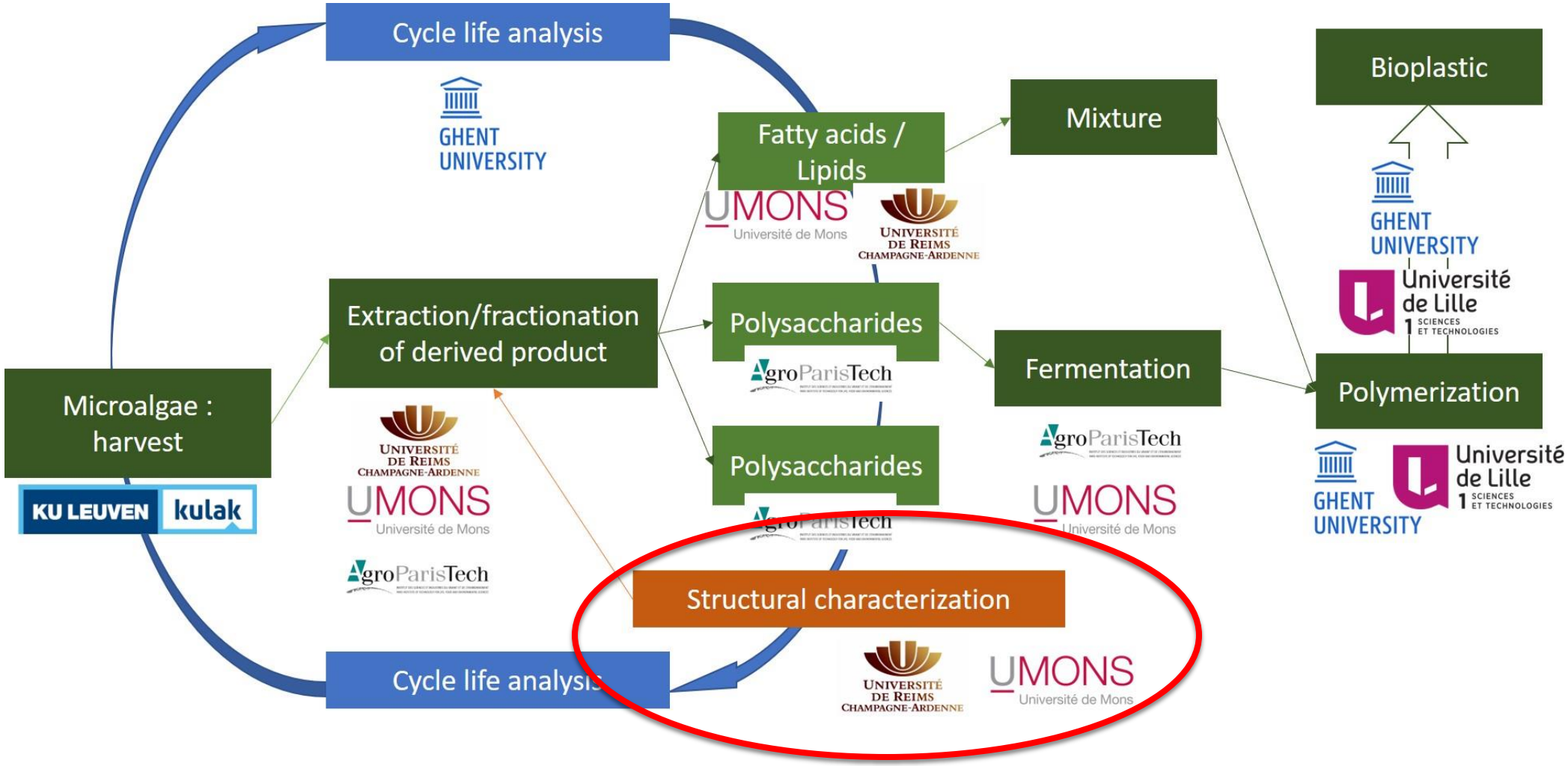
UMONS Université de Mons

S²MOs

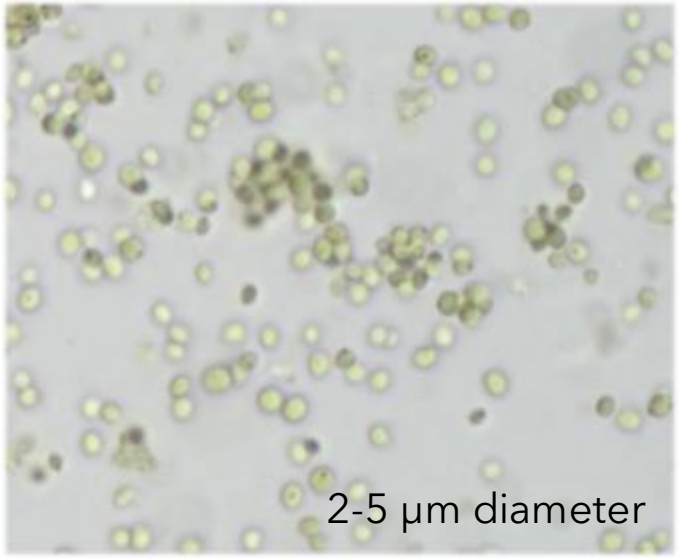
Université de Reims Champagne-Ardenne

ICMR Institut de Chimie Moléculaire de Reims UMR CNRS 7312

ALPO project : Bioplastics – biobased building blocks



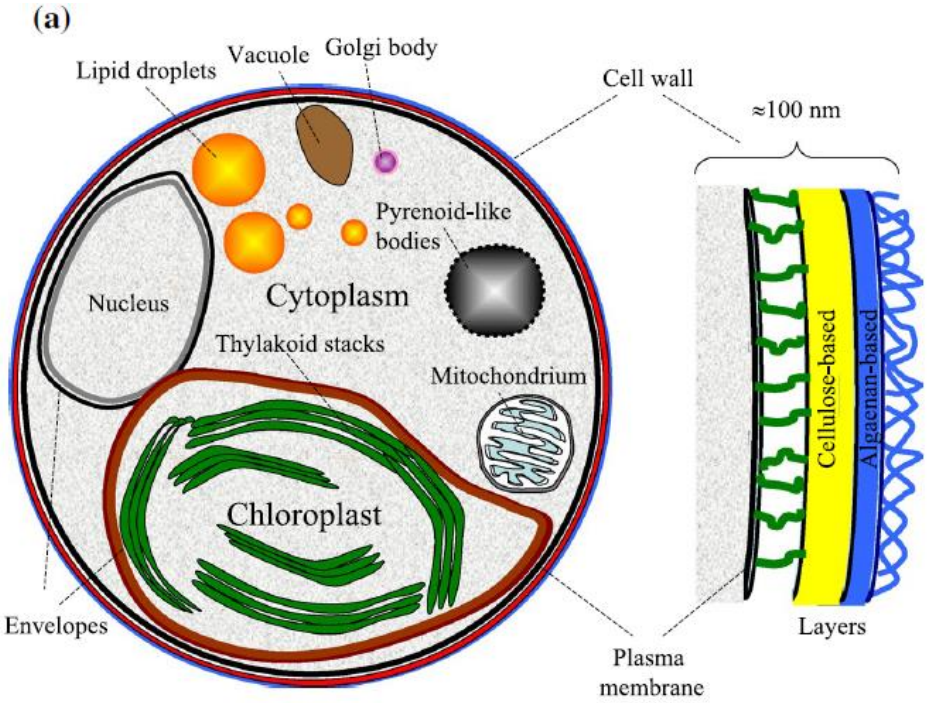
Nannochloropsis gaditana



Described by Lubián in 1982

Rich in lipids

necton



Zhang, R. *et al. Bioprocess Biosyst. Eng.* 2019 , *42*, 173–186.

Composition from litterature (Becker, 2007):

- Carotenoids
- Proteins
- Carbohydrates (starch, sugars, polysaccharides)
- Lipids (glycerol, fatty acids…)
- Salts

Becker, E. W. Micro-Algae as a Source of Protein. *Biotechnol. Adv*. 2007, *25* (2), 207–210.

ALPO project

Interreg France-Wallonie-Vlaanderen

GoToS3

ALPO

UNIVERSITÉ DE REIMS CHAMPAGNE-ARDENNE

ICMR Institut de Chimie Moléculaire de Reims UMR CNRS 7312

REIMS

- Microalgae extractions
- CPE of the polar extracts
- NMR analysis of the CPE fractions

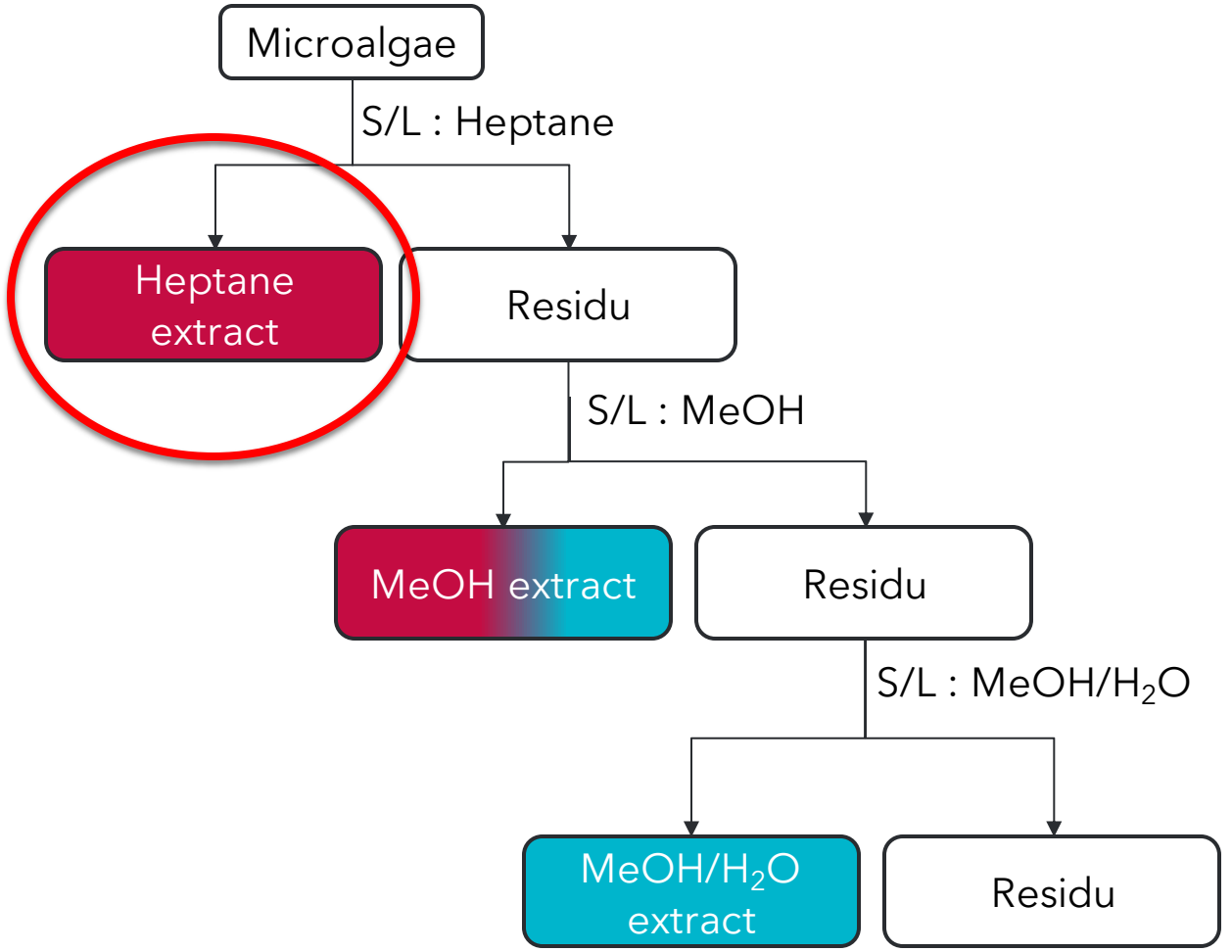
UMONS Université de Mons

S²MOs

MONS

- Microalgae extractions
- LC-MS analysis of the apolar extracts
- Ion mobility analysis of the lipids

Extraction procedure

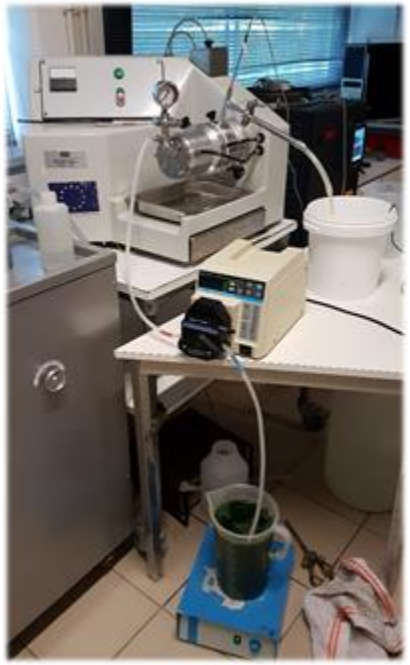


MS analysis

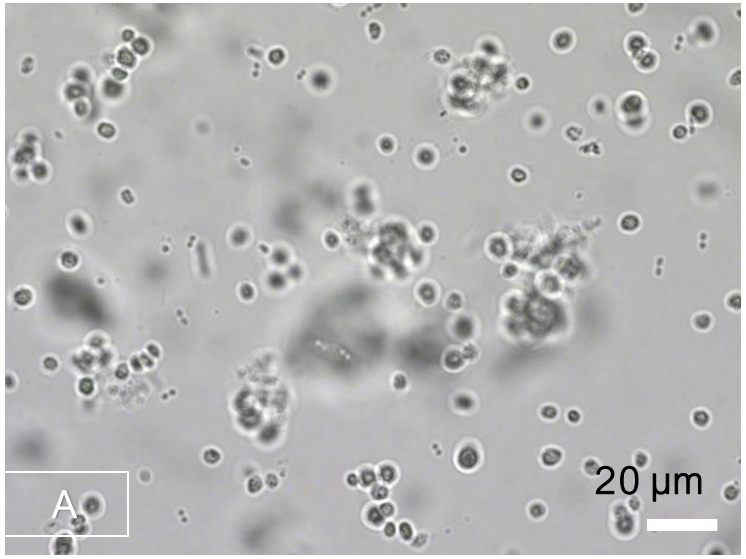
NMR analysis

Bead milling

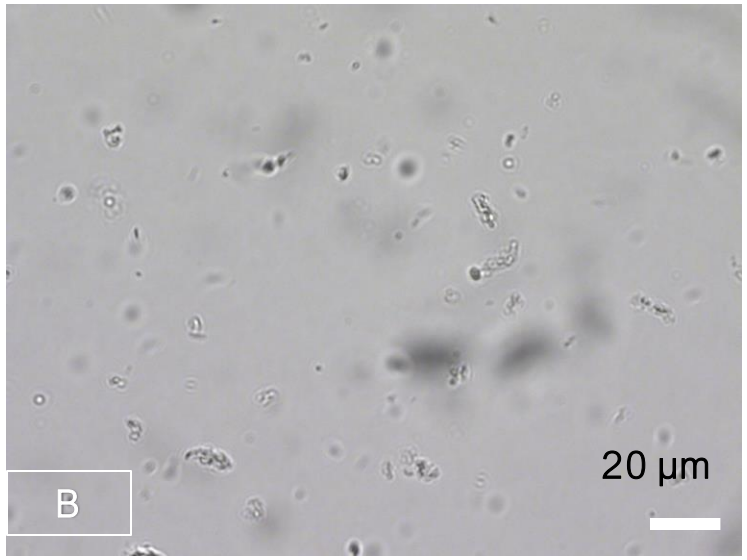
GEPEA



Intact cells



Milled cells



BOMB

Guillaume Caulier

Extraction yield

Ratio biomass/solvent
1/15 (w/v)

Gravimetric yield

3.1% (± 0.9)

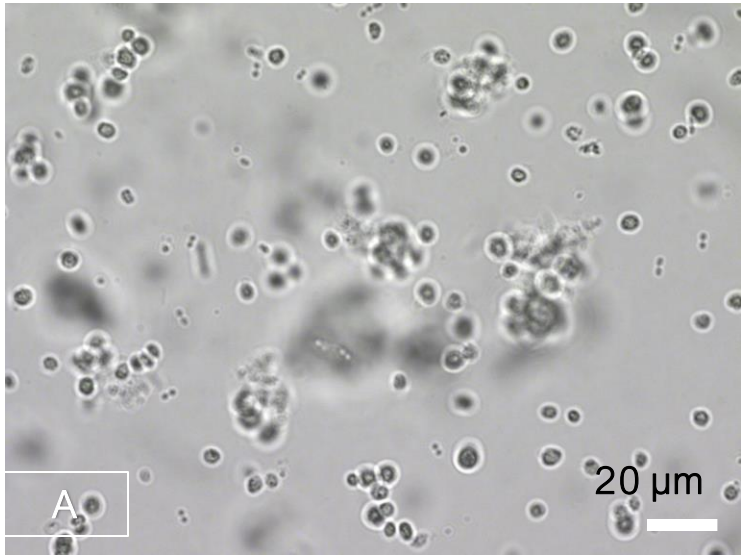
11.8% (± 0.1)

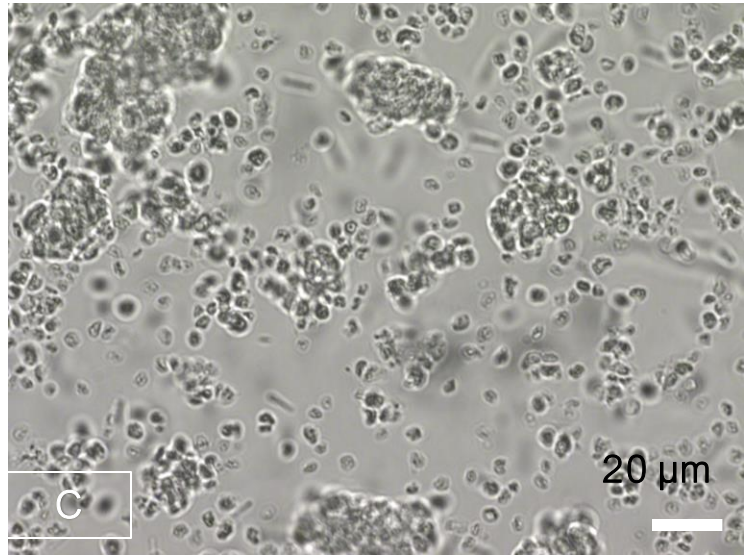
No extraction

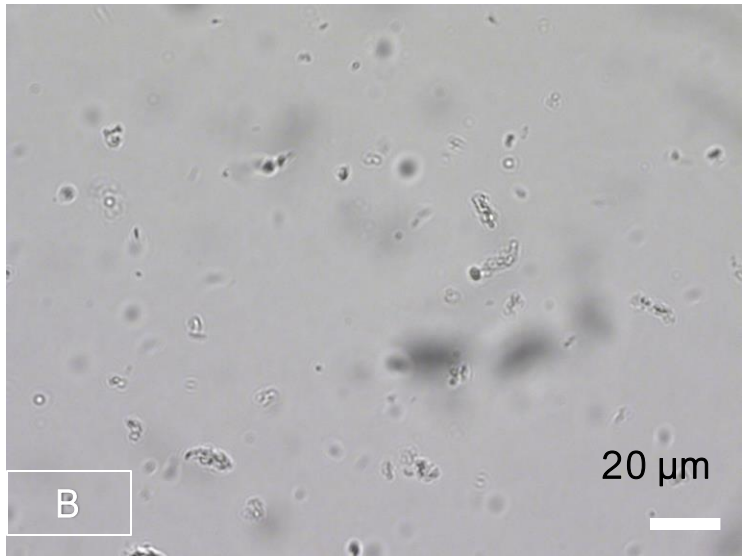
n-heptane extraction

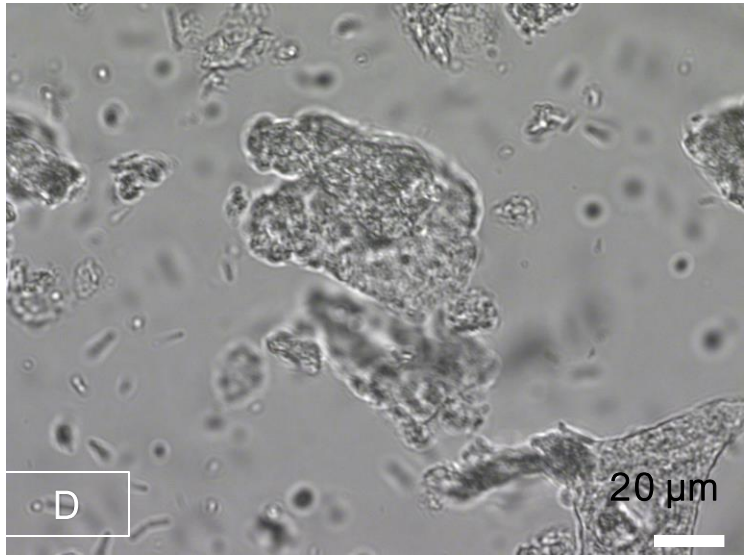
Intact cells

Milled cells

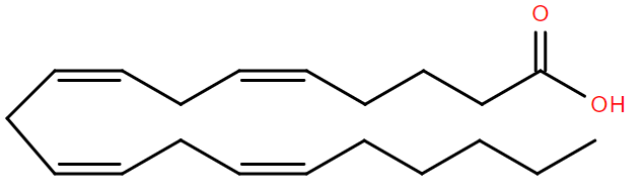


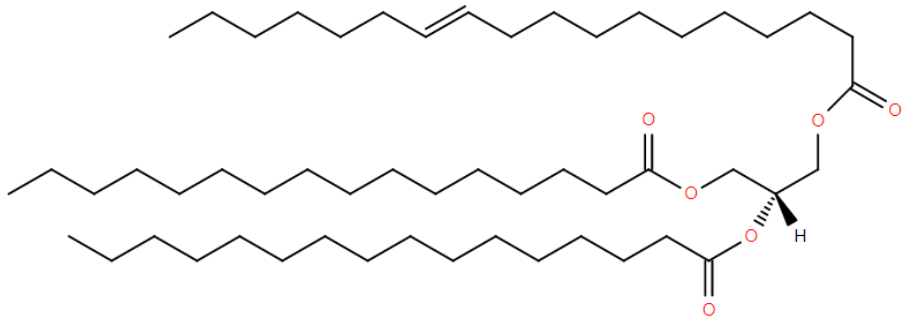


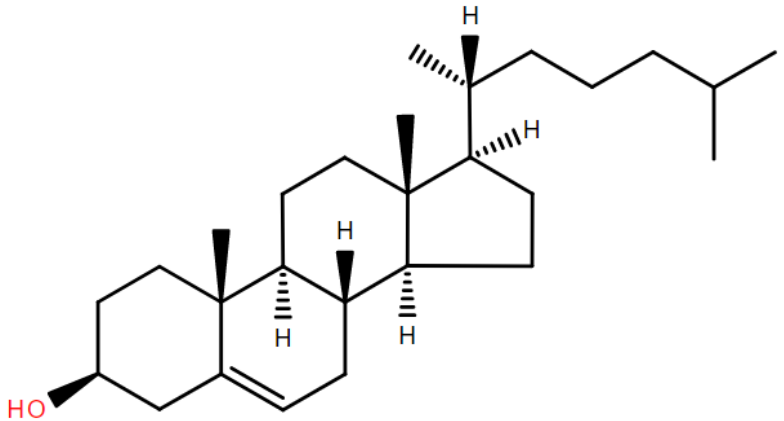


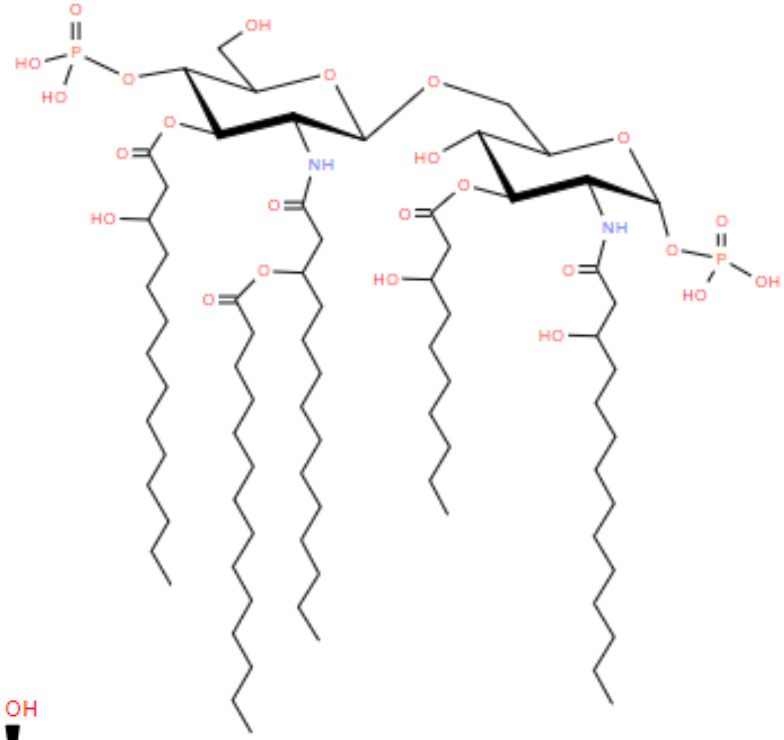


Lipids

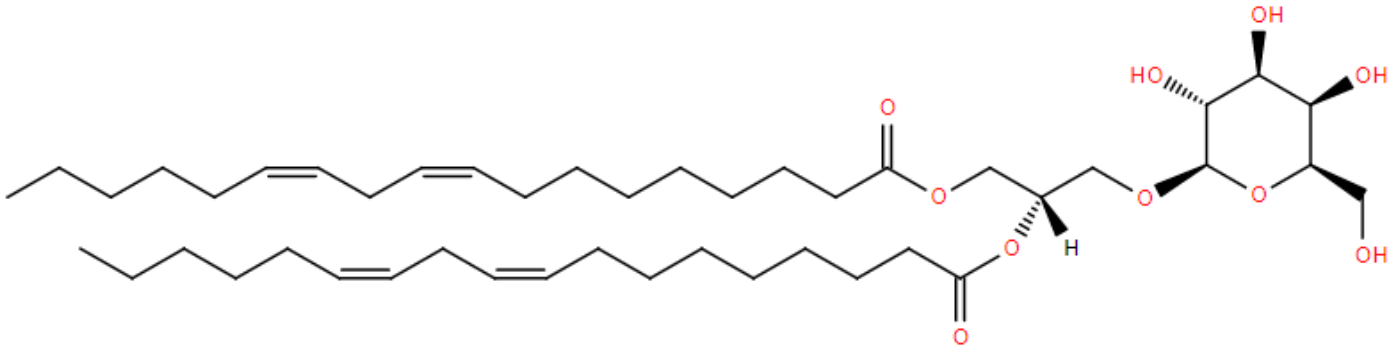








- Fatty acyls
- Glycerolipids
- Glycerophospholipids
- Sphingolipids
- Sterol Lipids
- Prenol Lipids
- Saccharolipid
- Polyketides



Fahy, E. *et al*; Update of the LIPID MAPS Comprehensive Classification System for Lipids. *J. Lipid Res.* **2009**, *50* (Supplement), S9–S14.

UPLC-MS

<u>UPLC :</u>

Waters Acquity H-class liquid chromatography device

Column : Acquity UPLC BEH C18 130 Å, 1,7 µm,
2,1 mm x 150 mm

<u>UPLC mobile phases :</u>

Phase A : MeCN/H_2O (50:50, v/v) + 0,1% FA

Phase B : iPrOH/MeCN (90:10, v/v) + 0,1% FA

Flow rate : 200 µL/min

<u>MS conditions :</u>

Waters Synapt G2-Si mass spectrometer

Capillary voltage : 3.1 kV (**positive**) and 2.5 kV (**negative**)

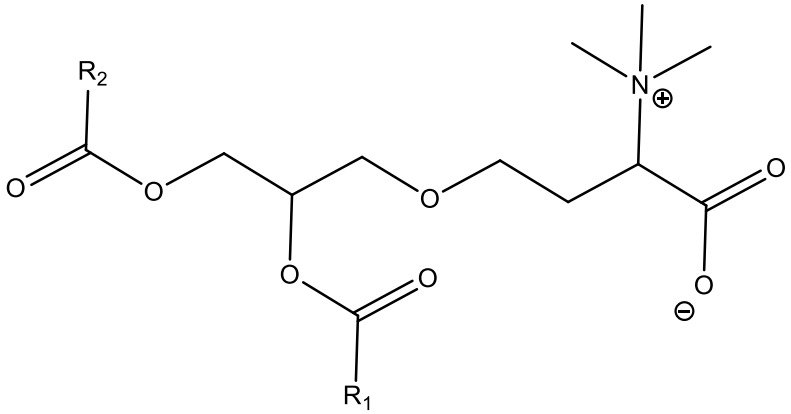
Positive and negative mode

Source : 150°C

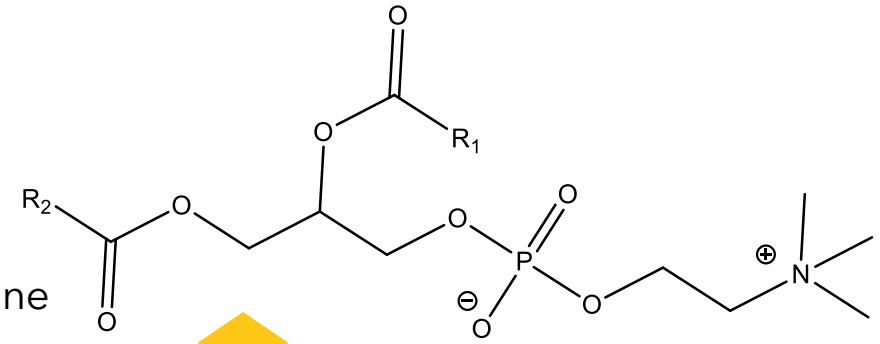
Desolvation : 300°C

Servaes, K. *et al*, Polar Lipid Profile of Nannochloropsis oculata Determined Using a Variety of Lipid Extraction Procedures. J. Agric. Food Chem. 63, 3931–3941 (2015).

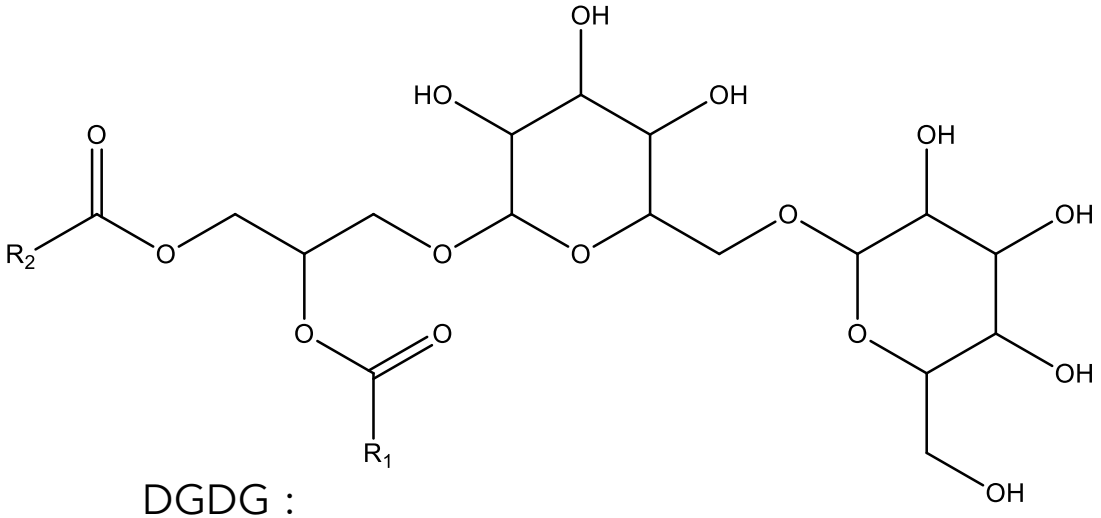
Lipids ESI(+)



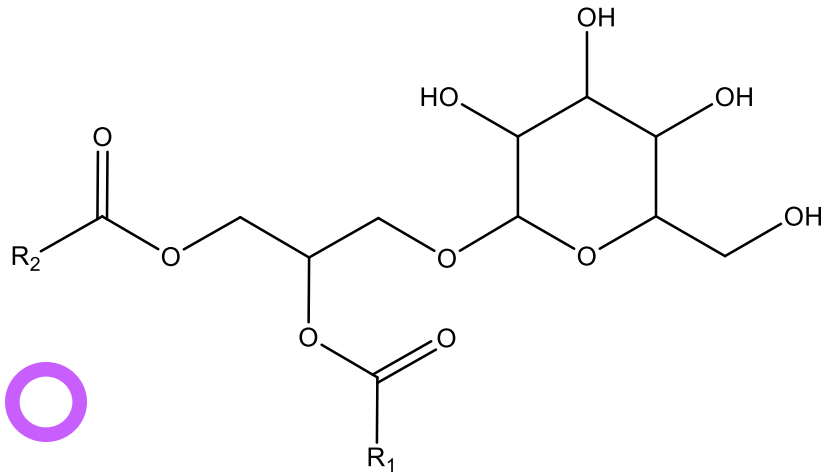
DGTS :
diacyl**g**lyceryl**t**rimethylhomo**s**erine



PC :
phosphatidyl**c**holine

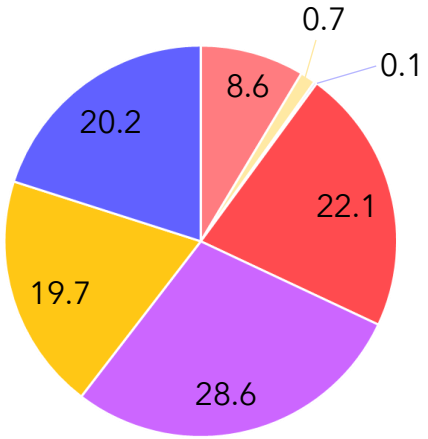


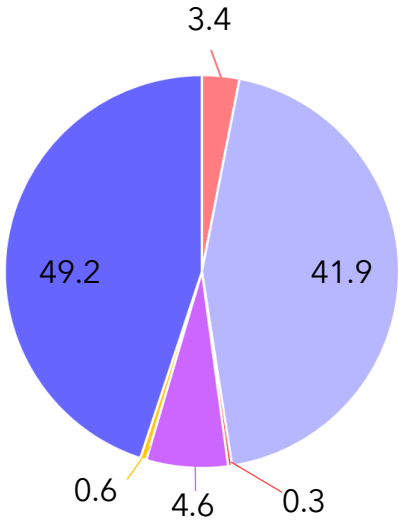
DGDG :
digalactosyl**d**iacyl**g**lycerol

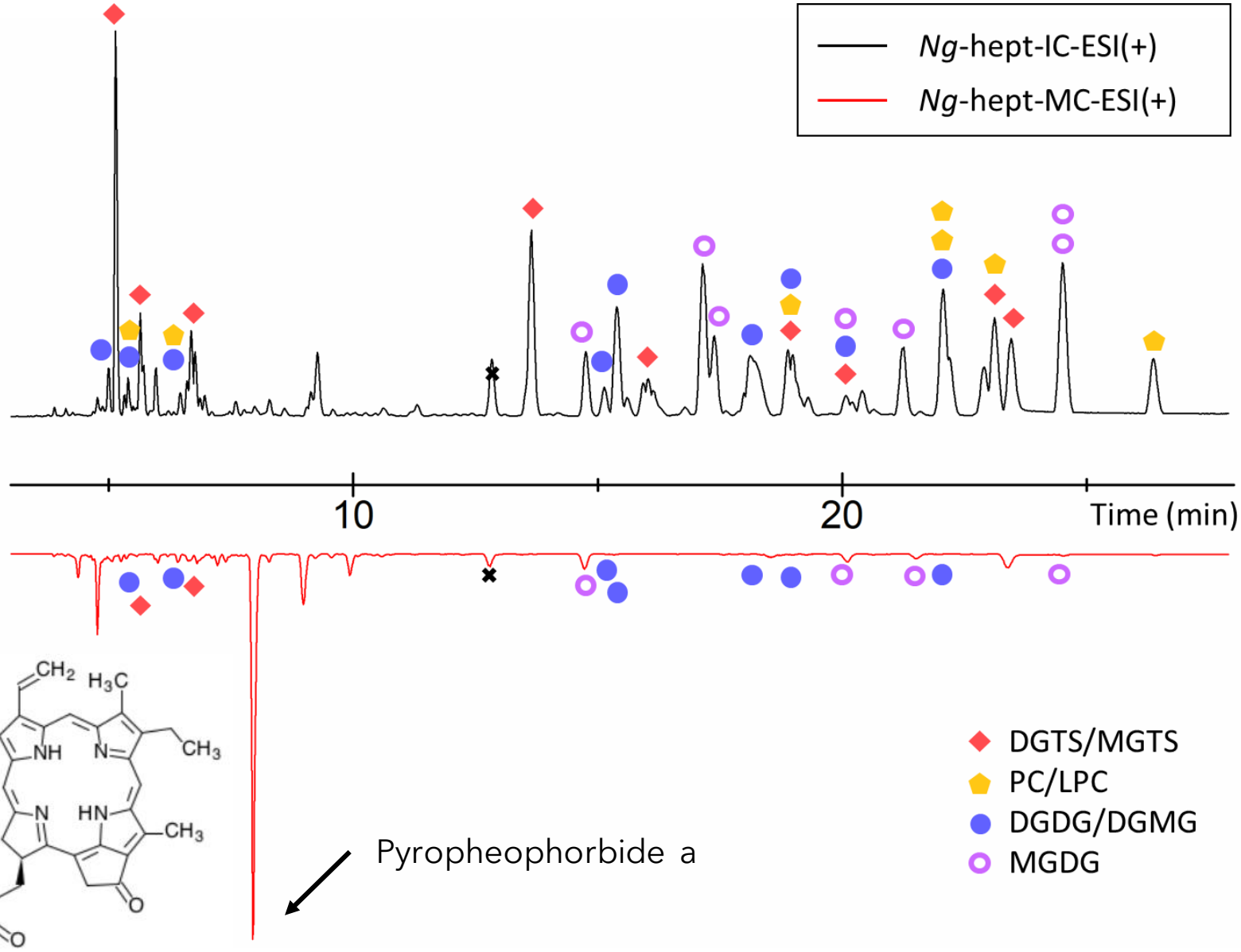


MGDG :
mono**g**alactosyl**d**iacyl**g**lycerol

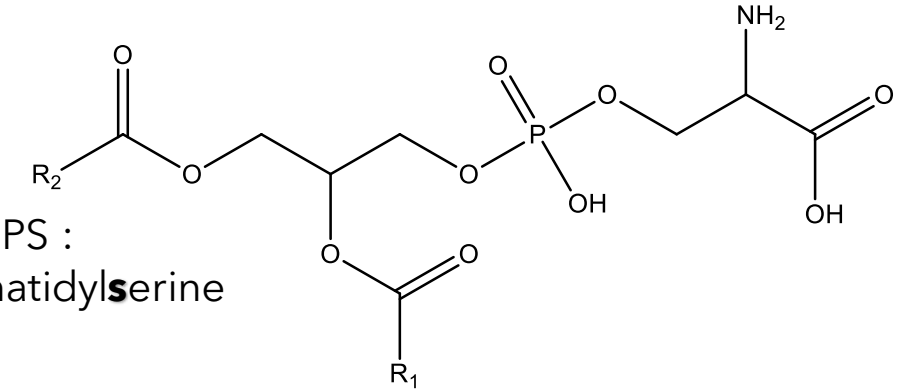
n-heptane – ESI(+)



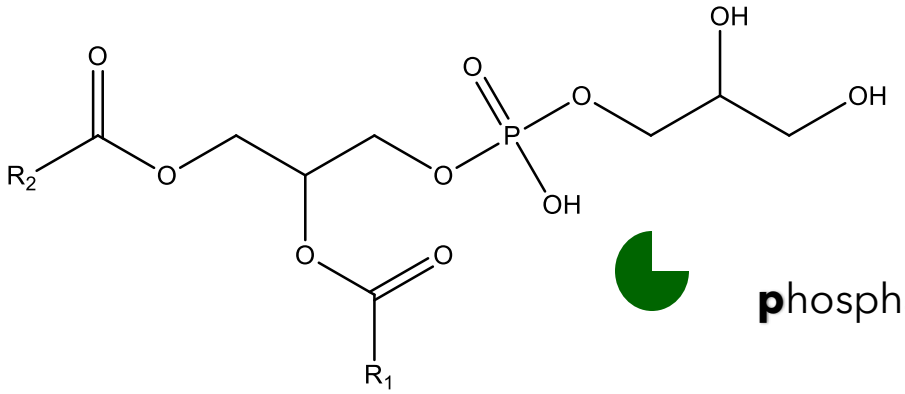




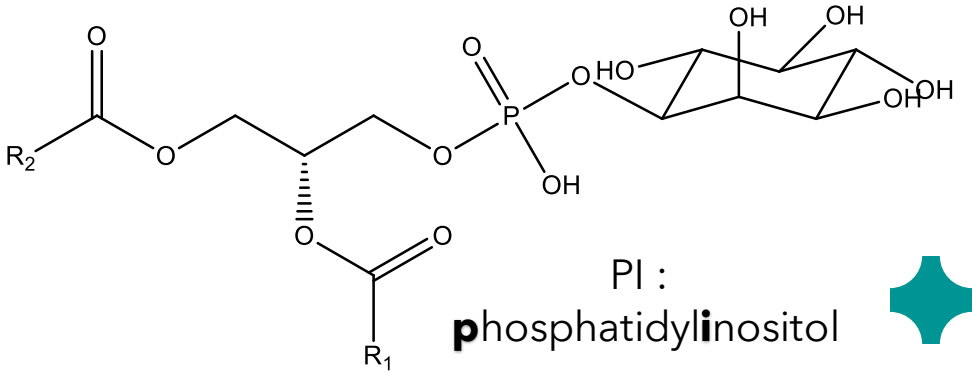
Lipids ESI(-)



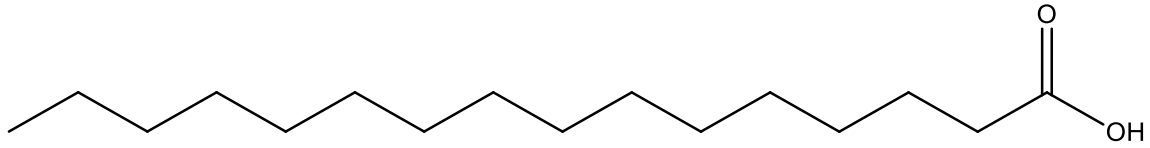
PS :
phosphatidyl**s**erine



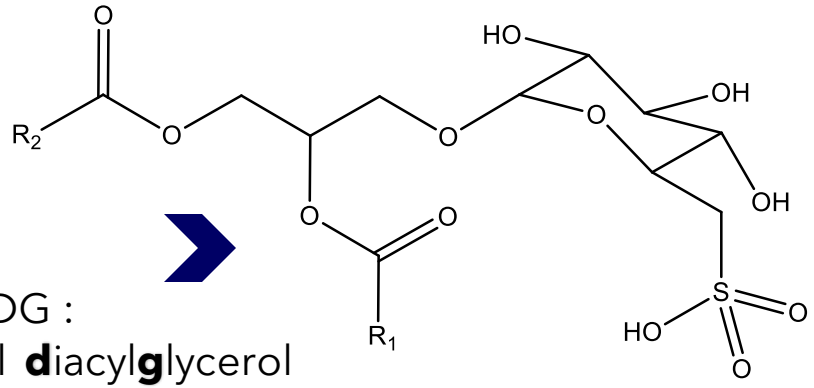
PG :
phosphatidyl**g**lycerol



PI :
phosphatidyl**i**nositol

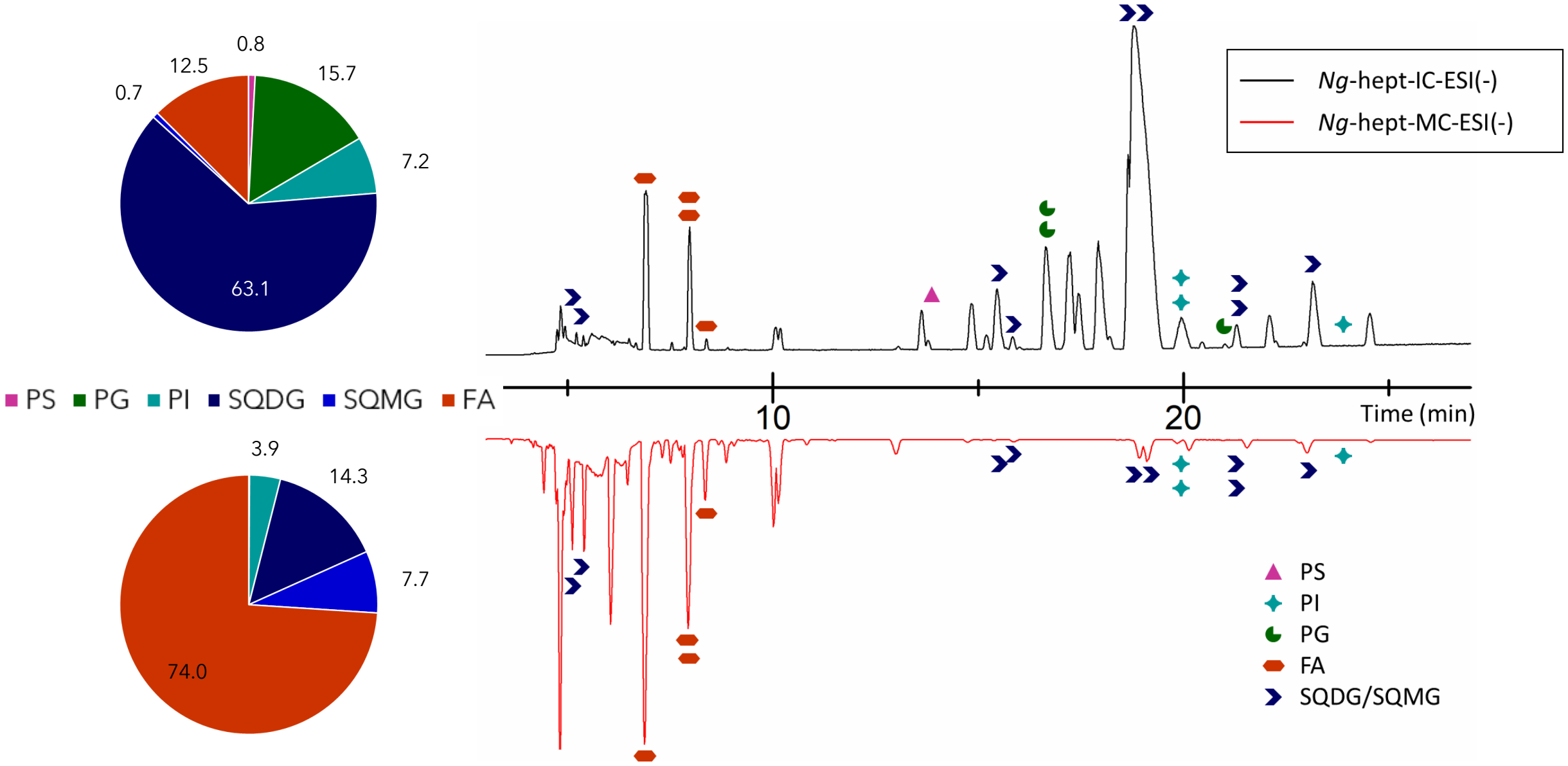


FA :
fatty **a**cid
saturated/insaturated



SQDG :
sulfo**q**uinovosyl **d**iacyl**g**lycerol

n-heptane – ESI(-)



GC-FID : Total lipid

Pr. Anne-Lise Hantson
Guillaume Delfau-Bonnet

GC-FID:

Shimadzu GC-2010 Plus Gas Chromatography (GC)

Detector : Flame Ionization dectector (FID)

Column : RT-2560 (100m lenght, 0.25 mm diameter, Restek)

Modified AOAC method 966.06 used for the result

Internal standard : C9

n-heptane extract

→ Methanolic HCl 3M, 6h at 50°C

→ Fatty acids methyl esters (FAMEs)

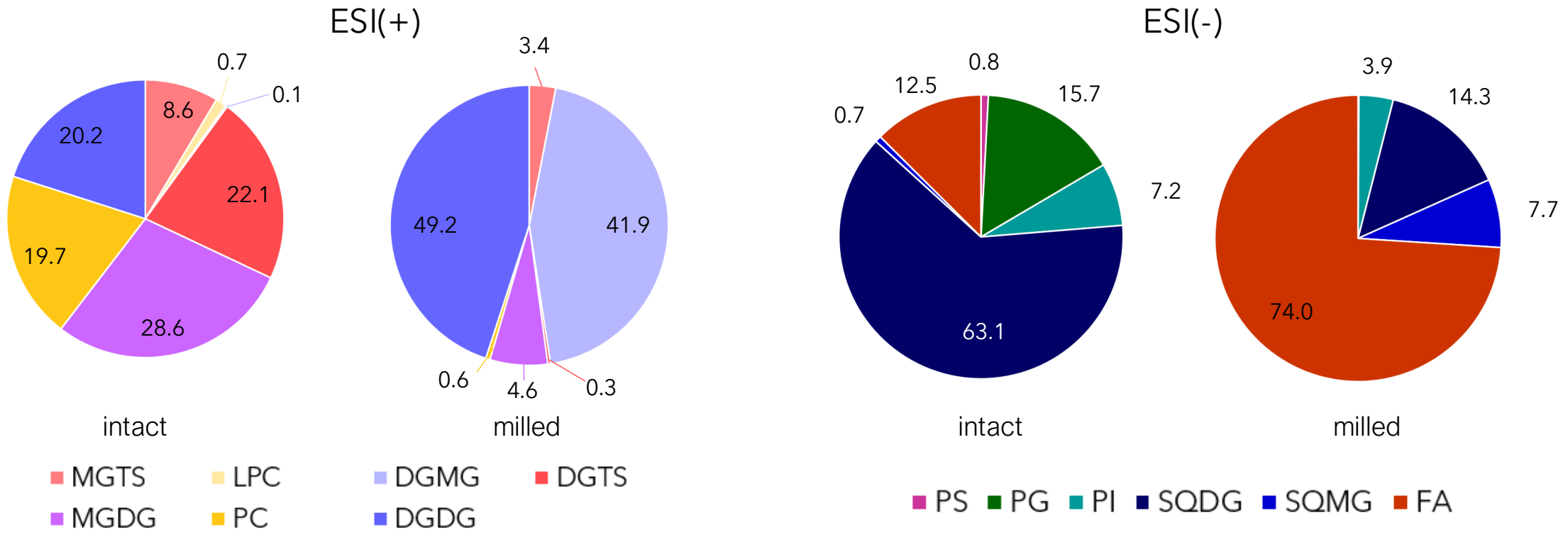
↓ L/L extraction *n*-heptane HPLC

Only FAMEs

	FAMEs
Intact cells	36.3% (±1.0)
Milled cells	42.0% (±5.2)

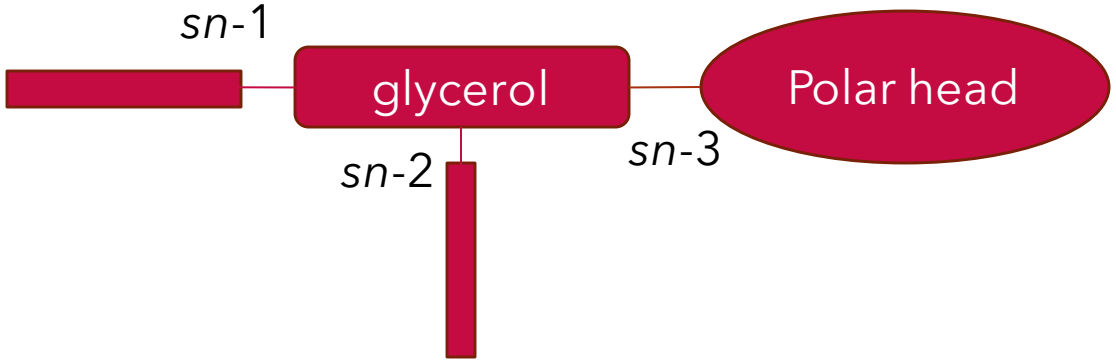
Conclusion

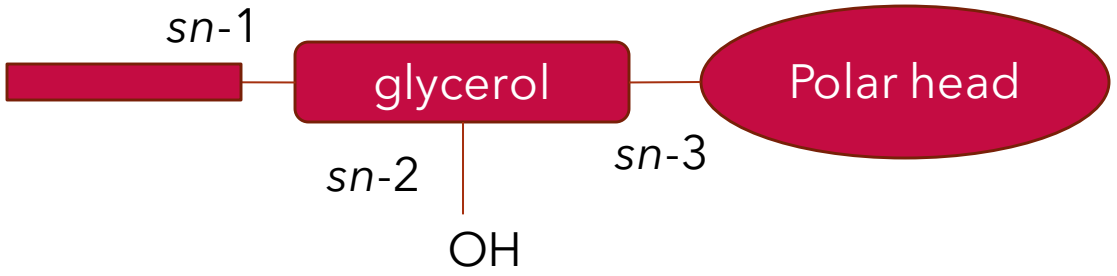
n-heptane	Extraction yield	GC-FID : FAMEs
Intact cells	3.1% (± 0.9)	36.3% (±1.0)
Milled cells	11.8% (± 0.1)	42.0% (±5.2)

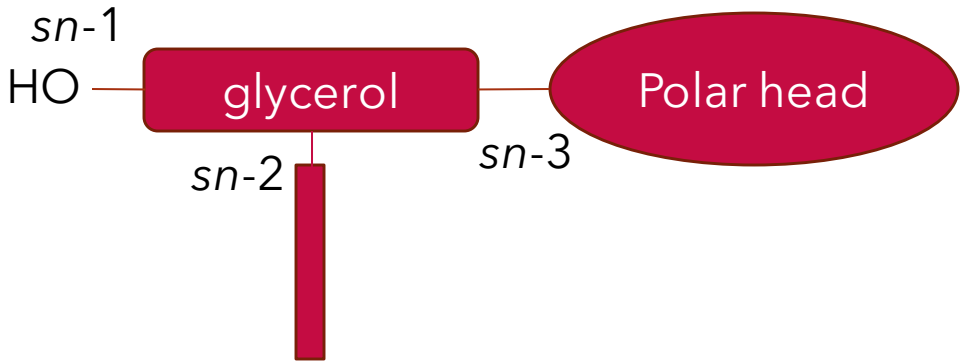


sn-position

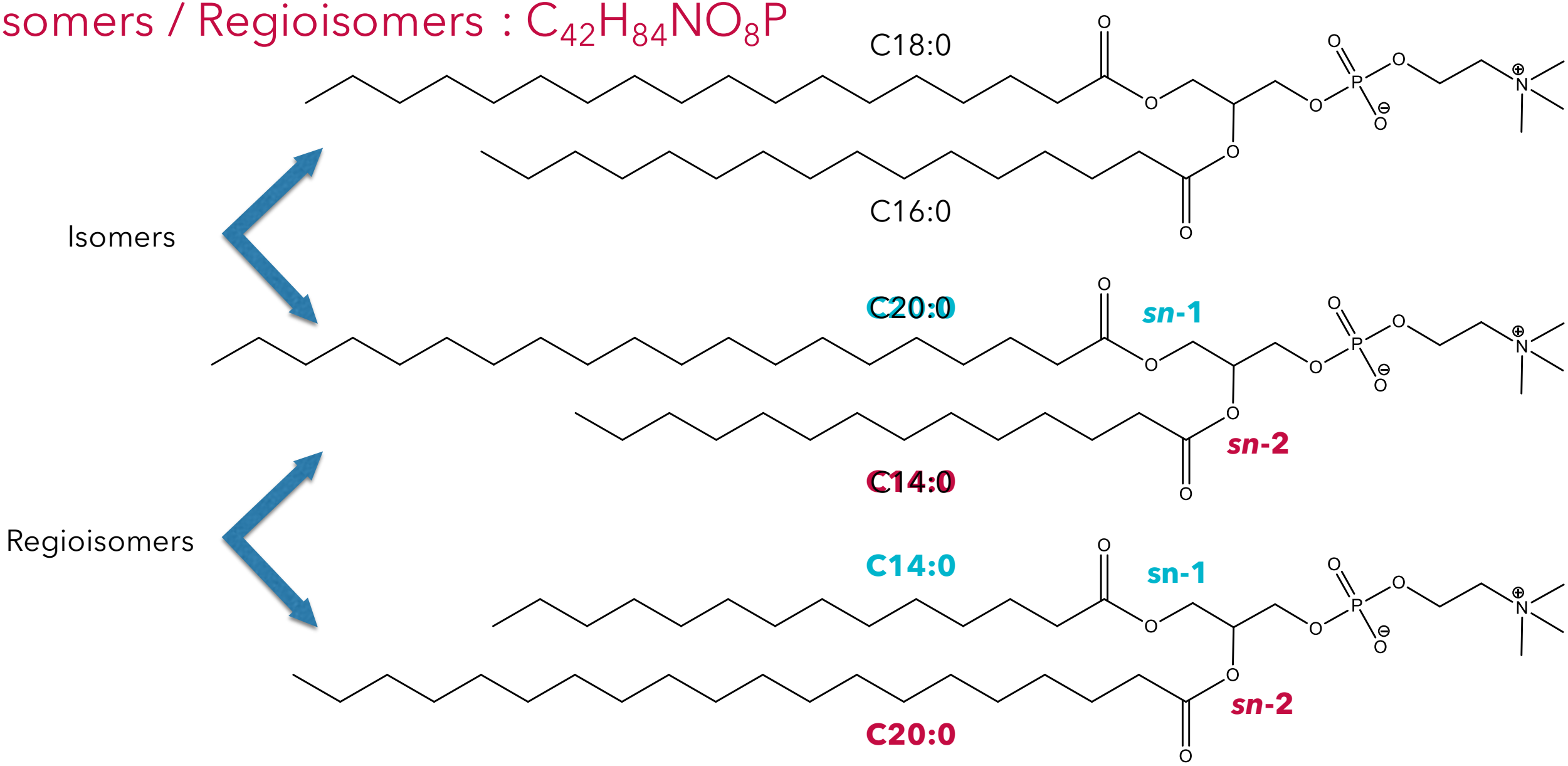
Example with PC and LPC





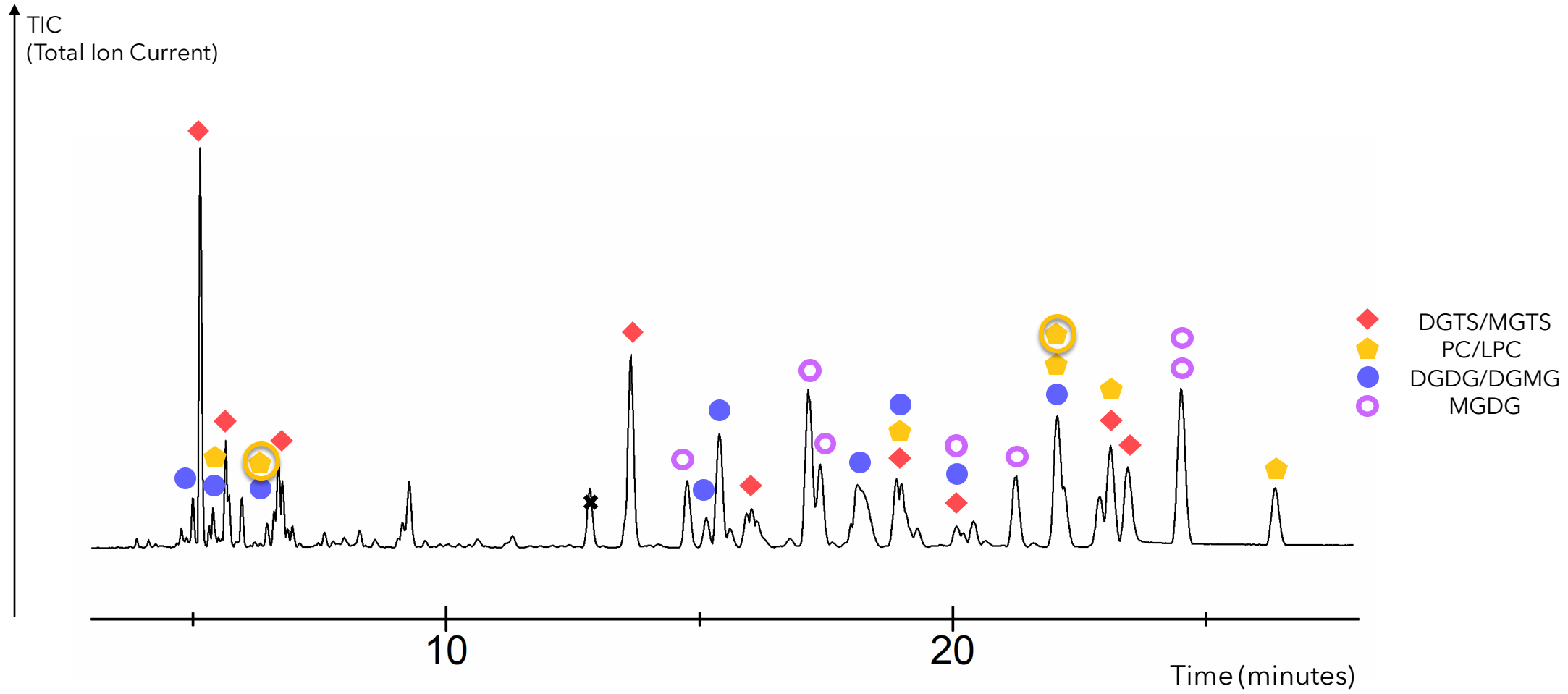


Isomers / Regioisomers : $C_{42}H_{84}NO_8P$



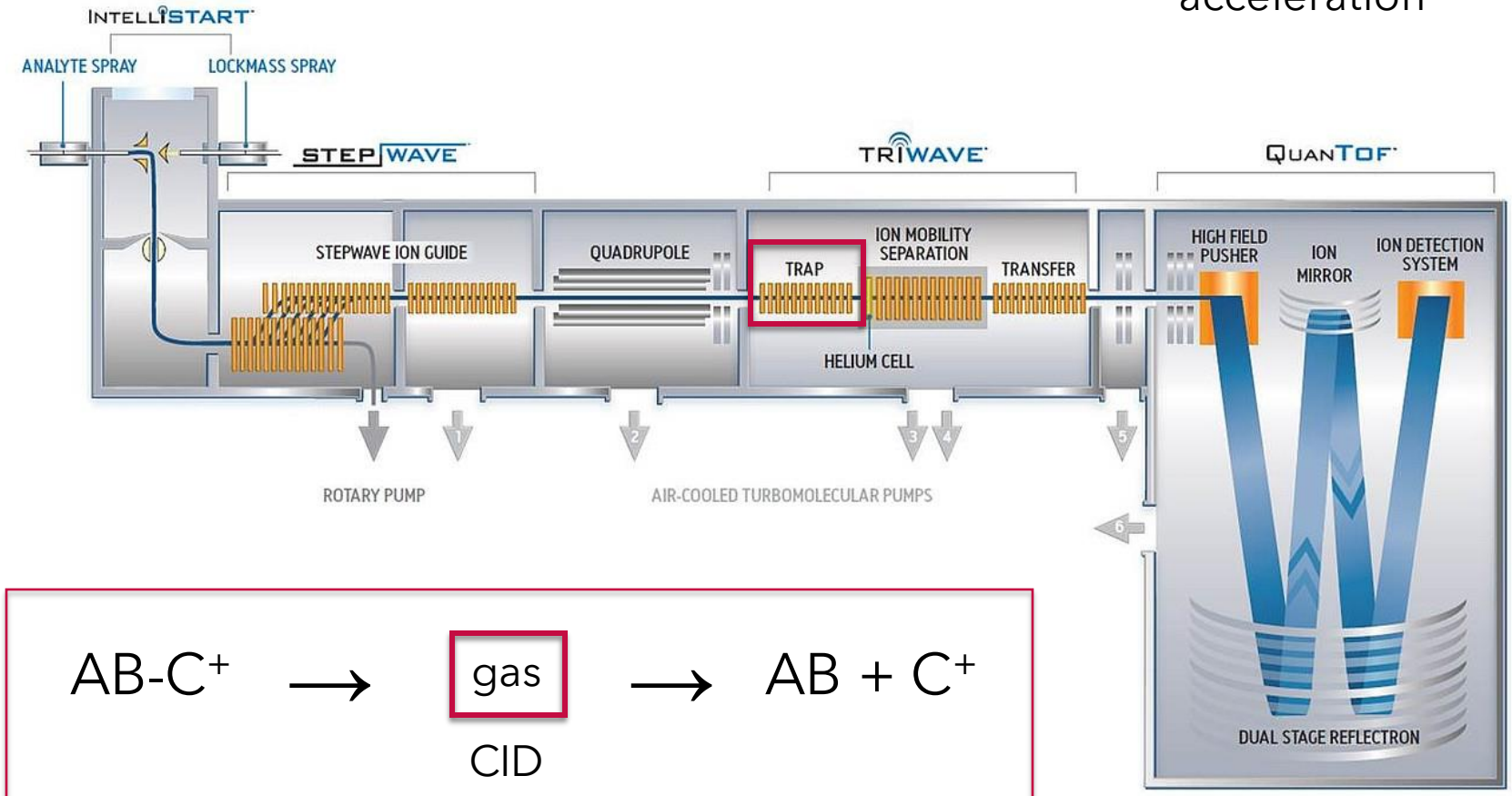
What about MS for isomer and regioisomer distinction ?

UPLC-MS *n*-heptane extract ESI(+)



MSMS - Principle

$$M^+ + \text{gas} \xrightarrow{\text{acceleration}} (M^+)^* \longrightarrow \text{fragments}$$



Structural information
Isomer distinction

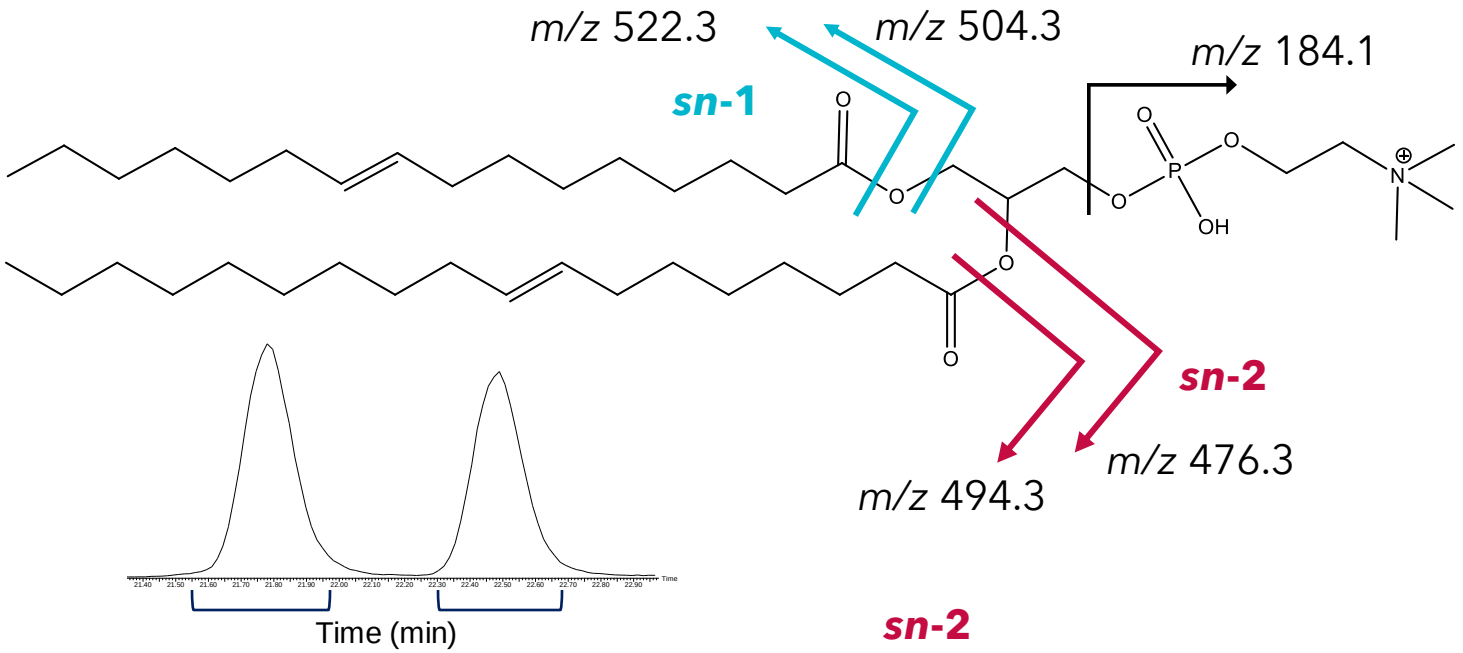
$$AB\text{-}C^+ \longrightarrow \boxed{\text{gas}} \longrightarrow AB + C^+$$

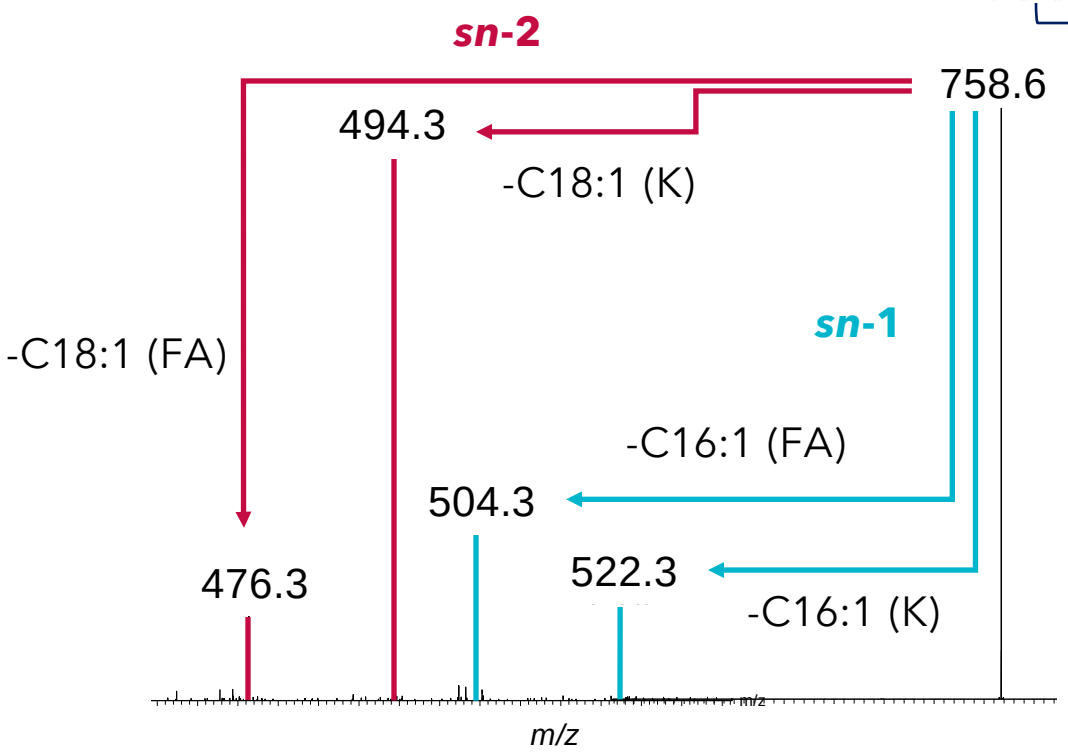
CID

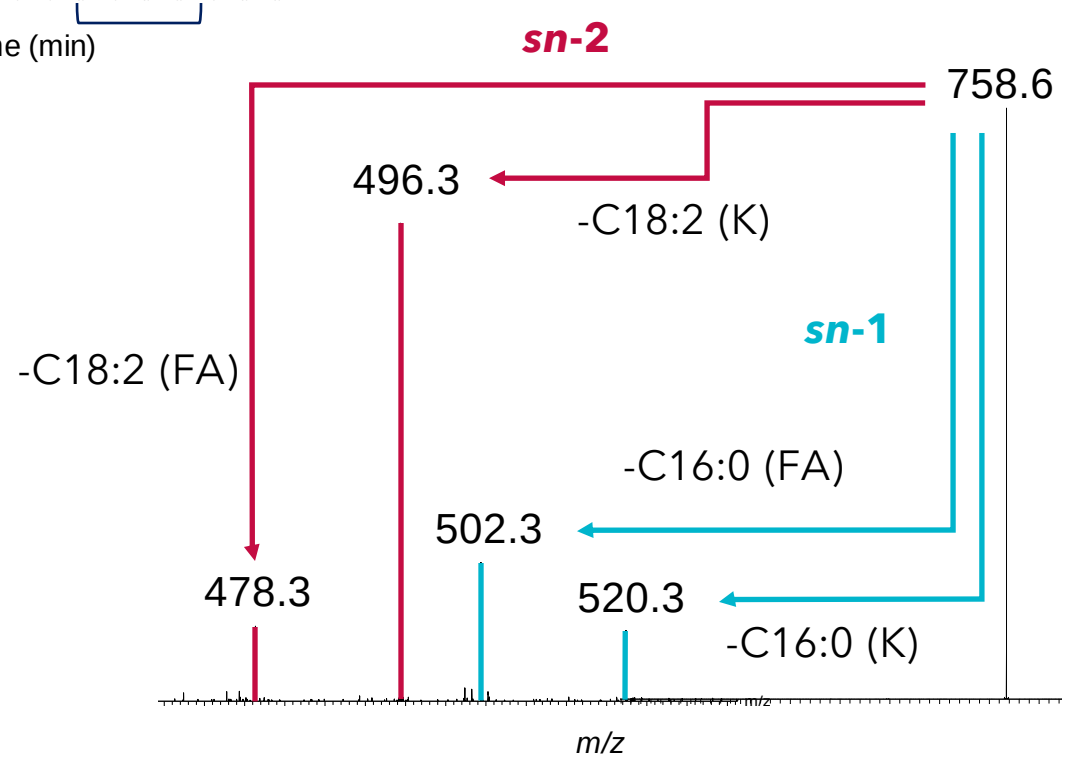
$$AC\text{-}B^+ \longrightarrow \boxed{\text{gas}} \longrightarrow AC + B^+$$

Isomeric PC : LC-MSMS

Litterature :
« In the case of the loss of the acyl group at sn-2, the abundances of the ketene ion were higher than the corresponding loss of the FA, but, in the case of sn-1 substituents, the reverse was true. »
Houjou, 2004



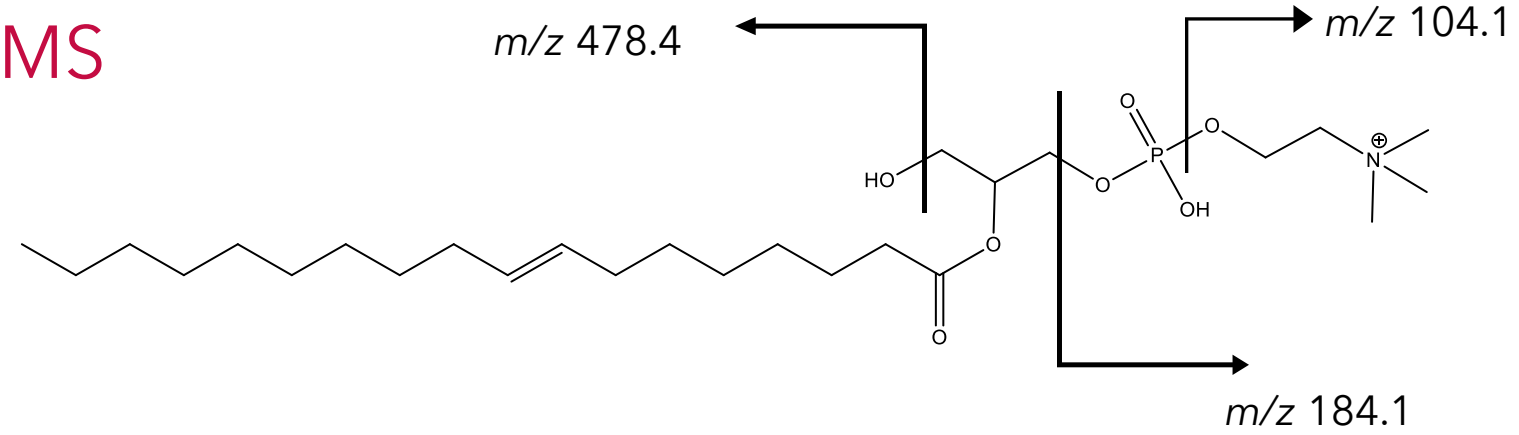


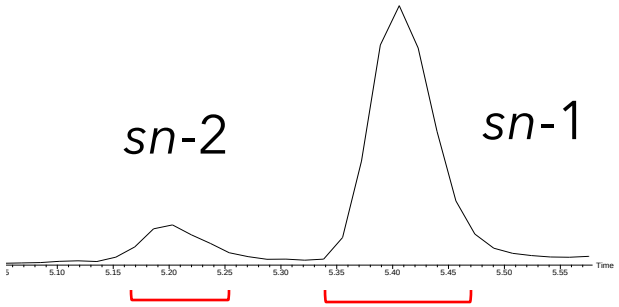


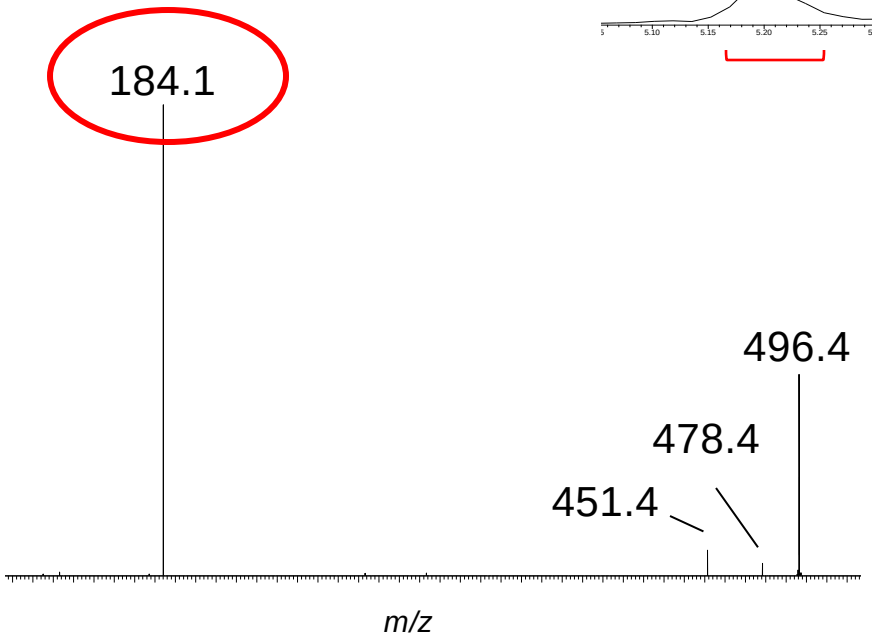
Regioisomeric LPC : LC-MSMS

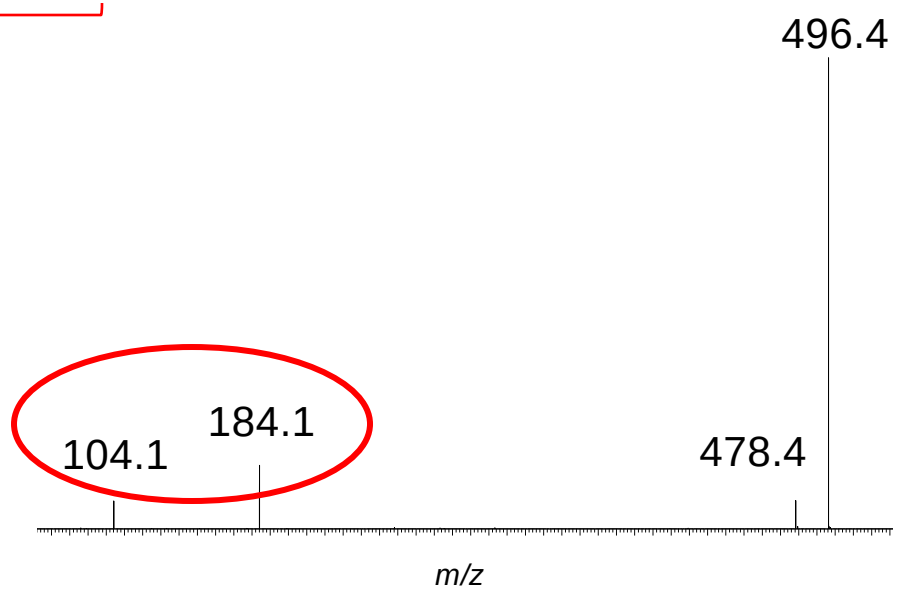
" As the *sn-2* regioisomer of LPC is relatively unstable, especially during incubation at 37°C (acyl migration generates a 9:1 excess of the *sn-1* form) "

Journal of Lipid Research Volume 52, 2011







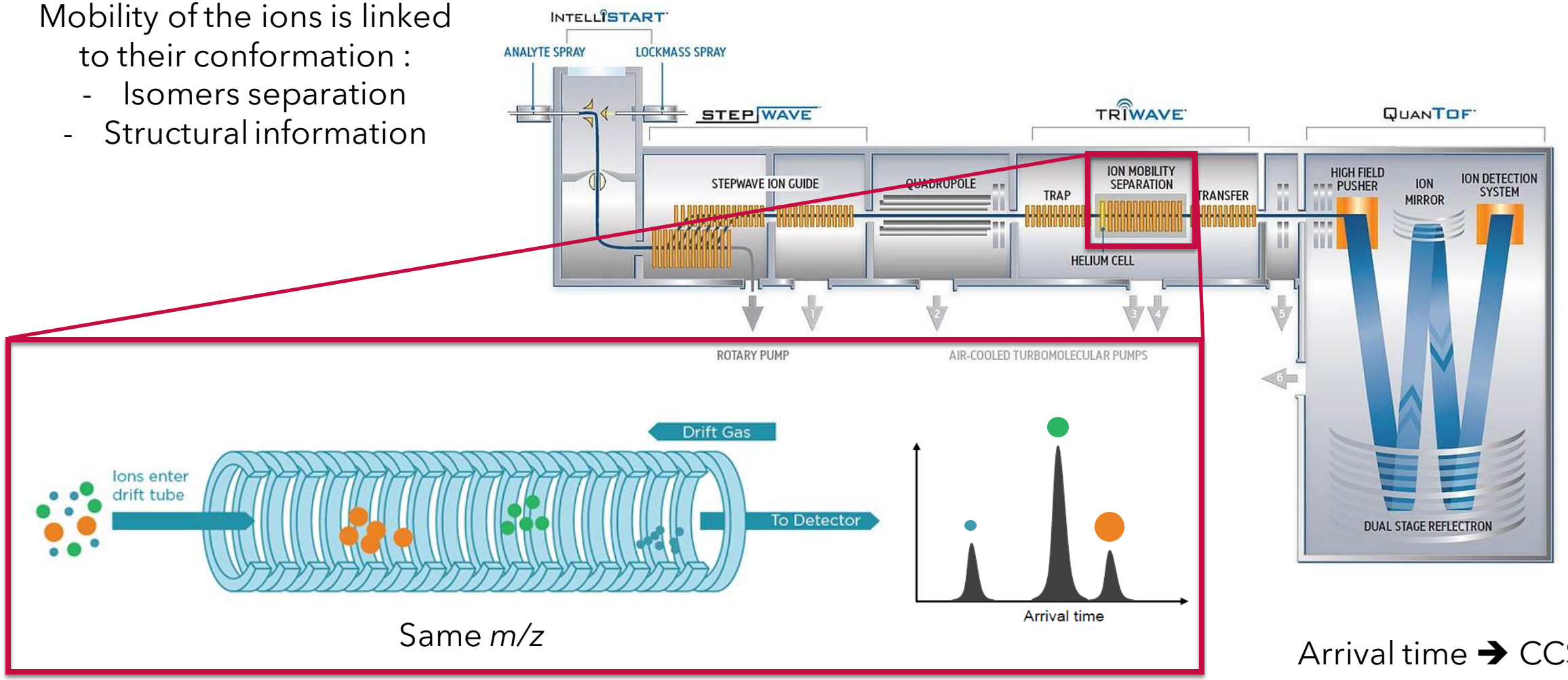


Ion Mobility Spectrometry (IMS)

IMS is an analytical technique based on the separation of ions according to their mobility in interaction with an inert gas, in an electric field.

Mobility of the ions is linked to their conformation :

- Isomers separation
- Structural information



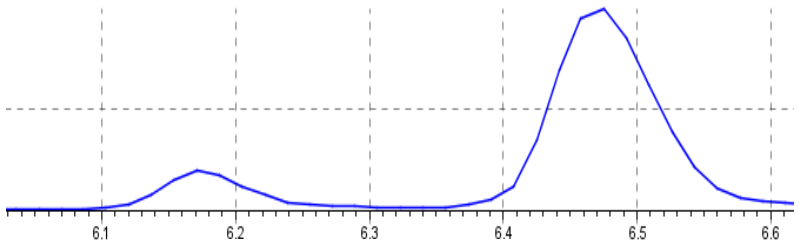
Same *m/z*

Arrival time ➔ CCS

LC-IMS

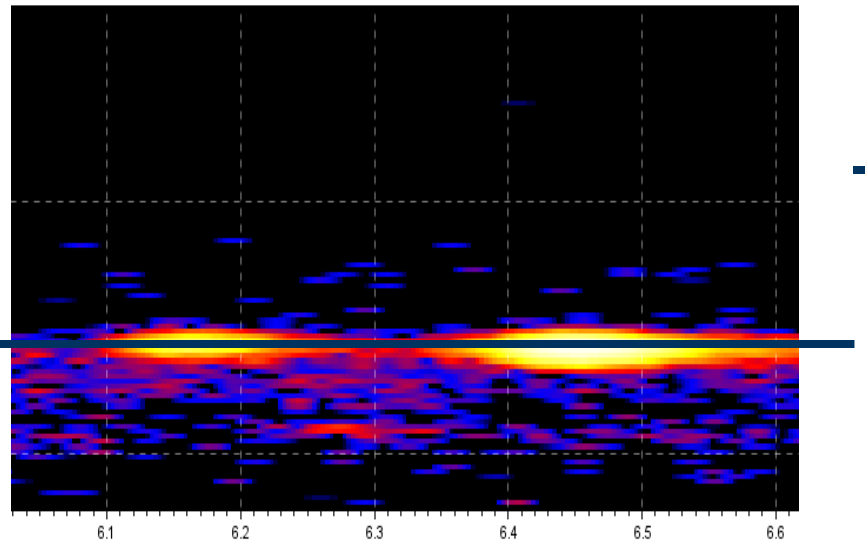
LPC

sn-2 *sn*-1



Retention time LC (min)

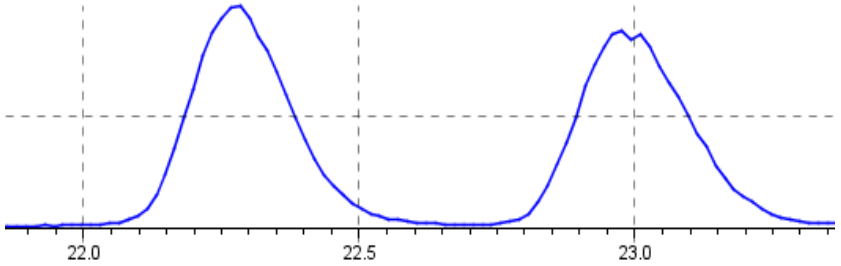
Arrival time (ms)



Same arrival time

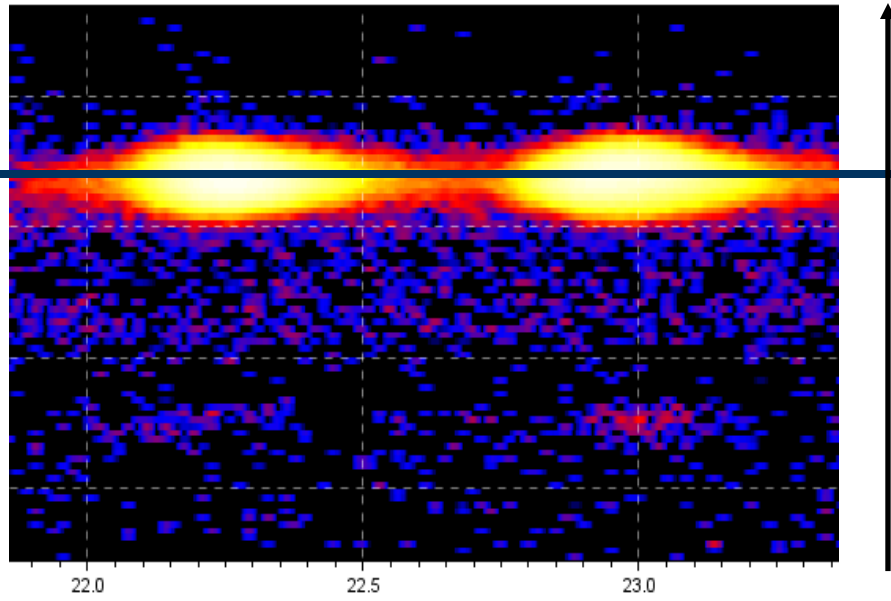
Retention time LC (min)

PC



Retention time LC (min)

Arrival time (ms)



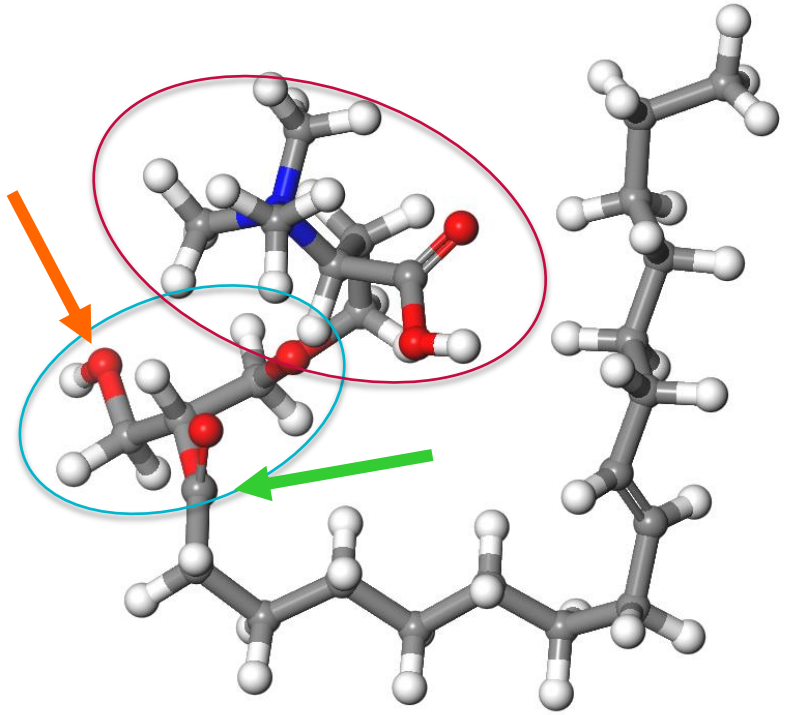
Same arrival time

Retention time LC (min)

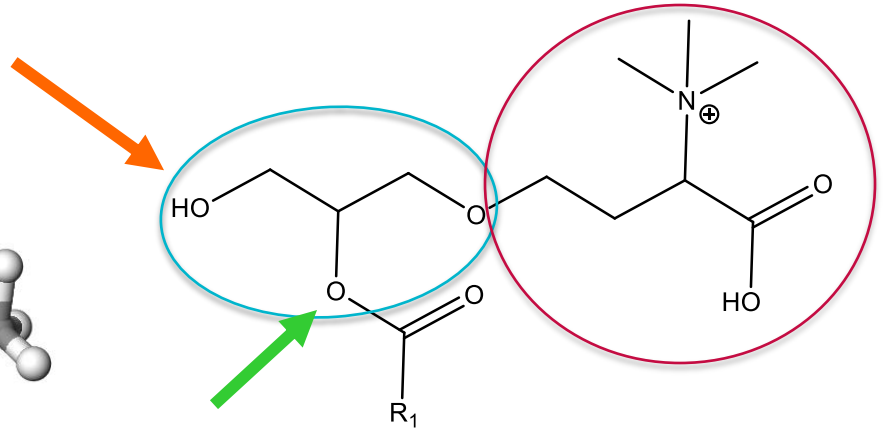
No separation with IMS technique.

Computational analysis – molecular dynamics

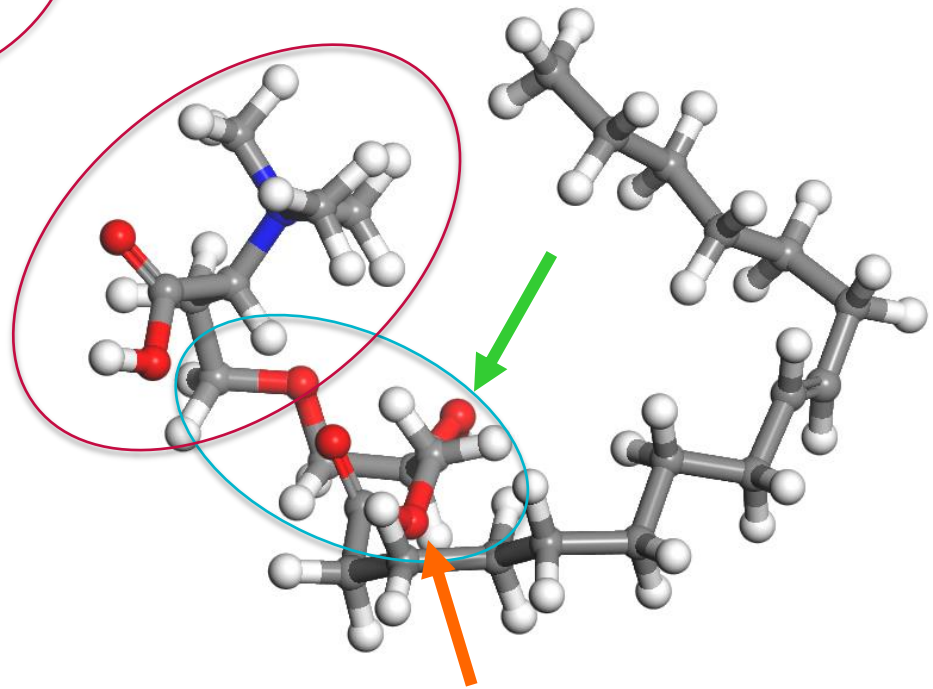
MGTS *sn*-2
$[M+H]^+$



CCS 168 Å²



MGTS *sn*-1
$[M+H]^+$



CCS 167 Å²

Quentin Duez

Conclusion & Perspectives

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GoToS3 ALPO

- Microalgae : *N. gaditana* ➔ other microalgae : *Chlorella vulgaris*
- UPLC-MS and MSMS : lipids ; relative quantification
- GC-FID : total lipid
- *sn*-position : LPC, PC, DGDG, MGDG
- Ion mobility spectrometry : same arrival time due to ion folding
- Storage : effect on the chemical profile ?
- *n*-heptane vs Bligh and Dyer ?
- Intact cells vs Milled cells ?

Thank you for your attention.