The Quality of Phospholipids

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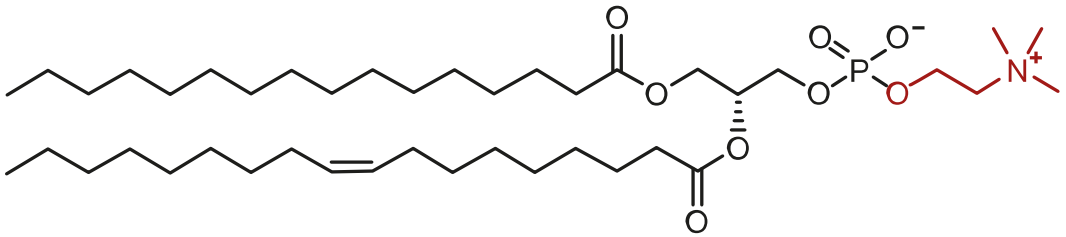
NEXT-GENERATION DELIVERY INNOVATIONS

Phospholipids

Definition: phosphor containing amphiphilic lipids



Analytical Methods

Index values / sum parameters

Biological methods (bioburden testing, endotoxins, …)

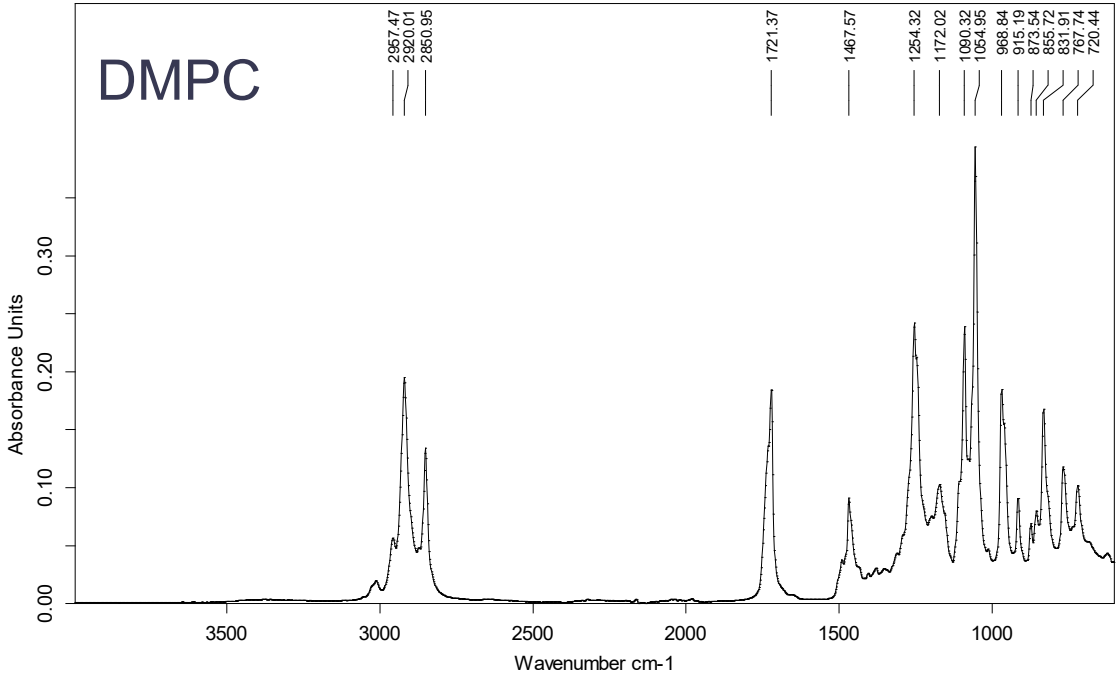
Spectroscopy

Chromatography

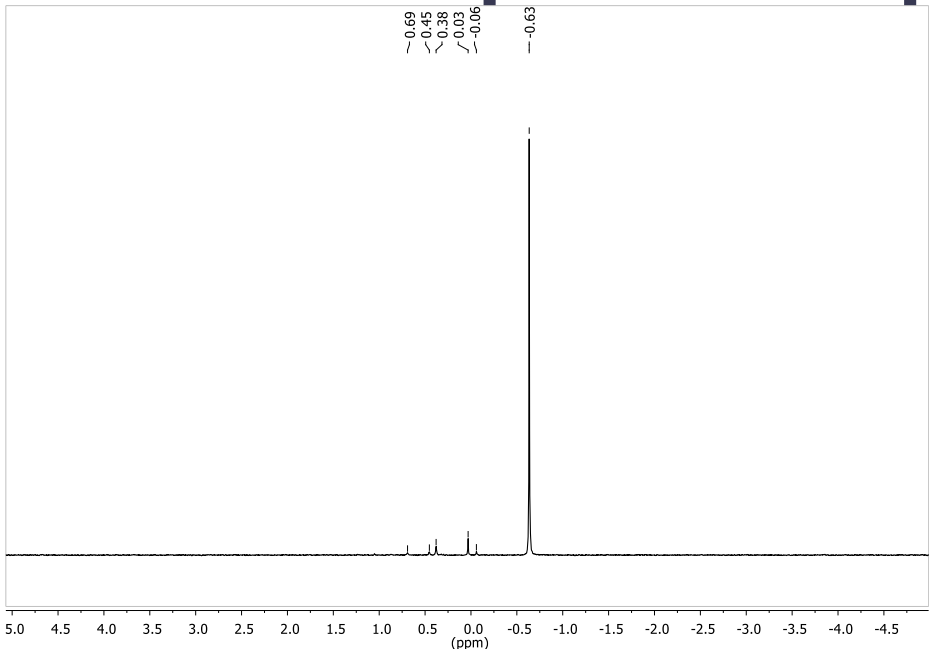
Index Values

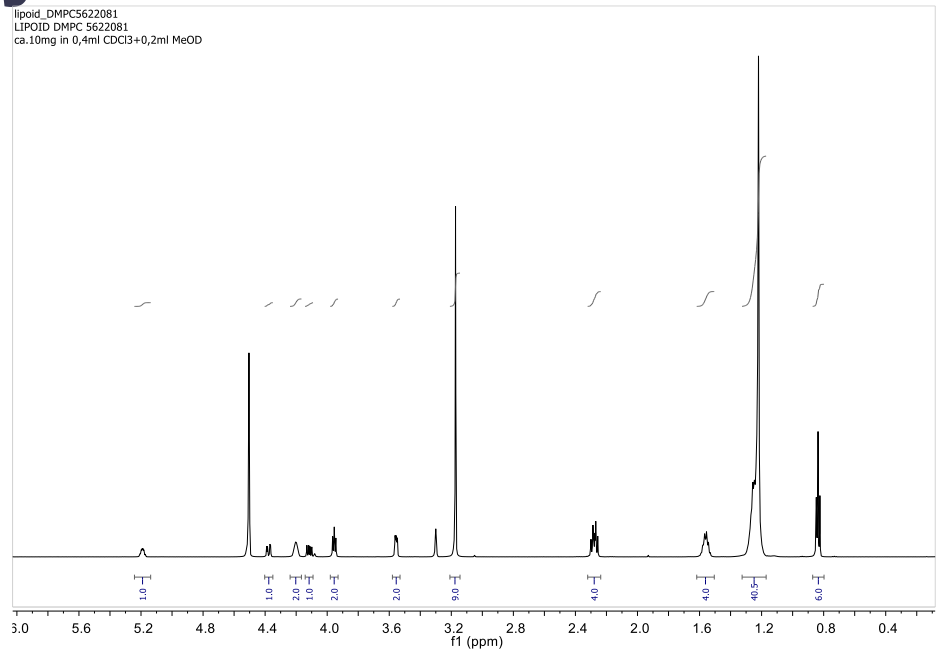
- Peroxide Value (POV)
  → Rancidity
- Iodine Value (IV)
  → Degree of unsaturation (e.g., DSPC: ~ 0, POPC: ~33)
- Acid Value (AV)
  → Free fatty acids, acidic compounds (PE, PA)

IR Spectroscopy



NMR Spectroscopy

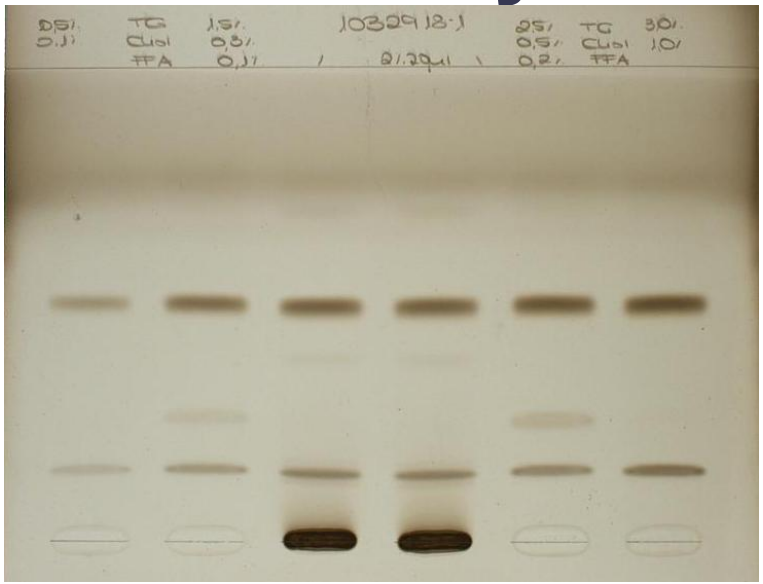




Chromatography

- Thin layer chromatography (TLC)
- Gas chromatography (GC)
- Liquid chromatography (HPLC)

Thin layer chromatography

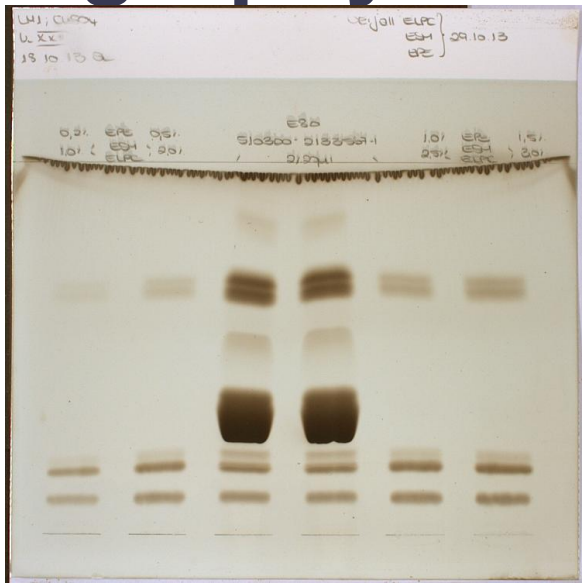


Triglycerides

Tocopherol
FFA

Cholesterol

Non-polar lipids
Hexane/Ether/HOAc 70/30/1



PE

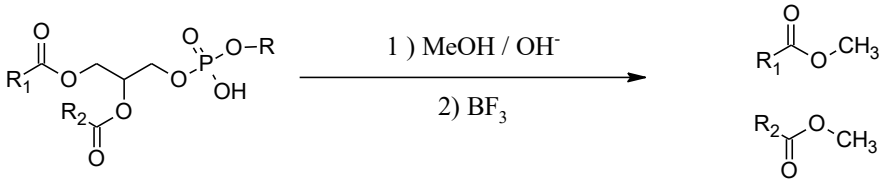
PC

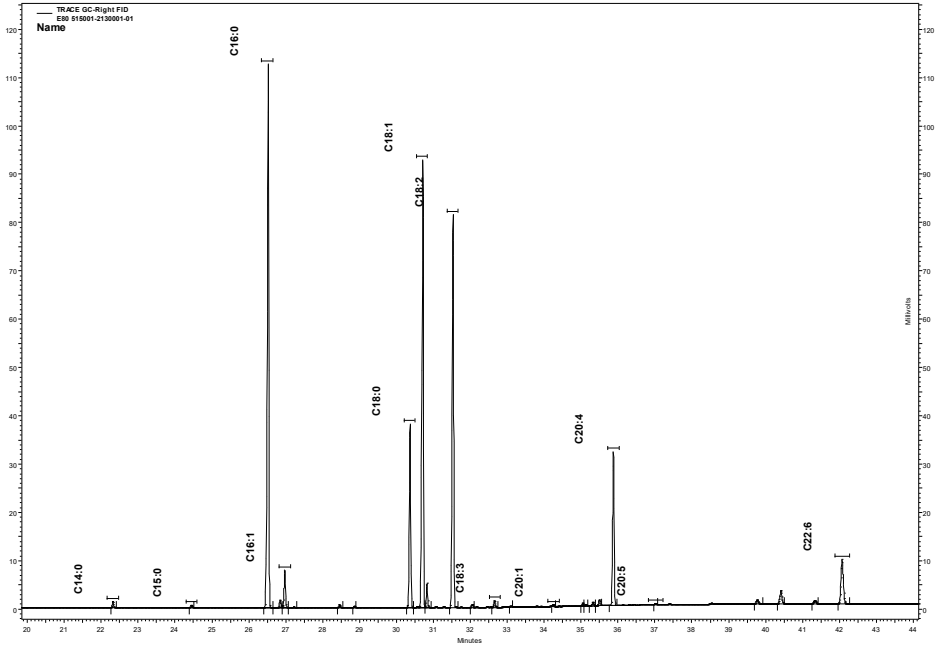
SM
LPC

Polar lipids
$CHCl_3/MeOH/H_2O/[NH_3]$ 65/25/4

Gas chromatography (GC)

- FAME analysis
  (Fatty Acid Methyl Esters)





Liquid Chromatography (HPLC)

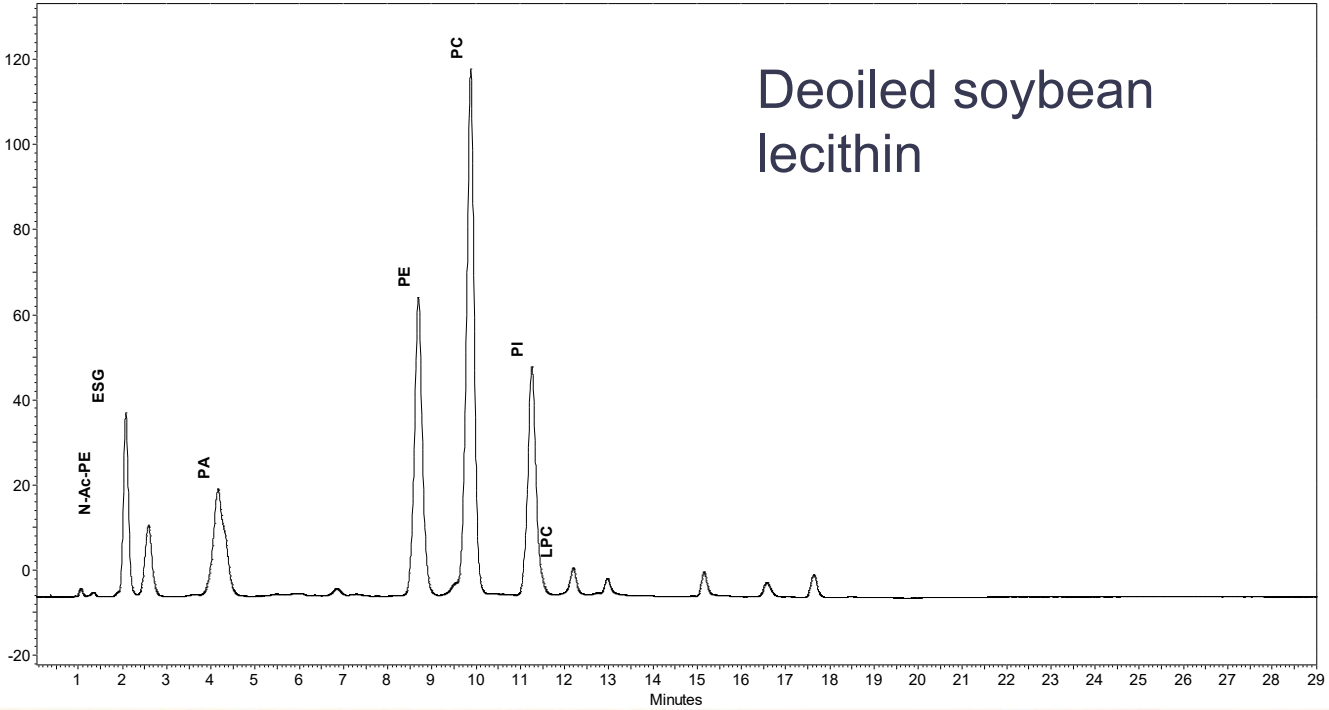
- In principle two methods
  - Normal phase conditions (NP)
  - Reversed phase conditions (RP)
- Detection techniques
  - Evaporative light scattering detector (ELSD)
  - Charged Aerosol Detector (CAD)
  - UV-Detector
  - Mass spectrometer

Normal Phase HPLC

- Many methods are based on the DGF-method F-I 6a(00)
- Stationary phase: „Lichrospher Diol“
- Mobile phase: binary gradient of Hexane / 2-propanol / water / triethylamine / acetic acid
- Detector: ELSD
- Separation for „head group“
- No or only little separation for different fatty acids

Normal Phase HPLC

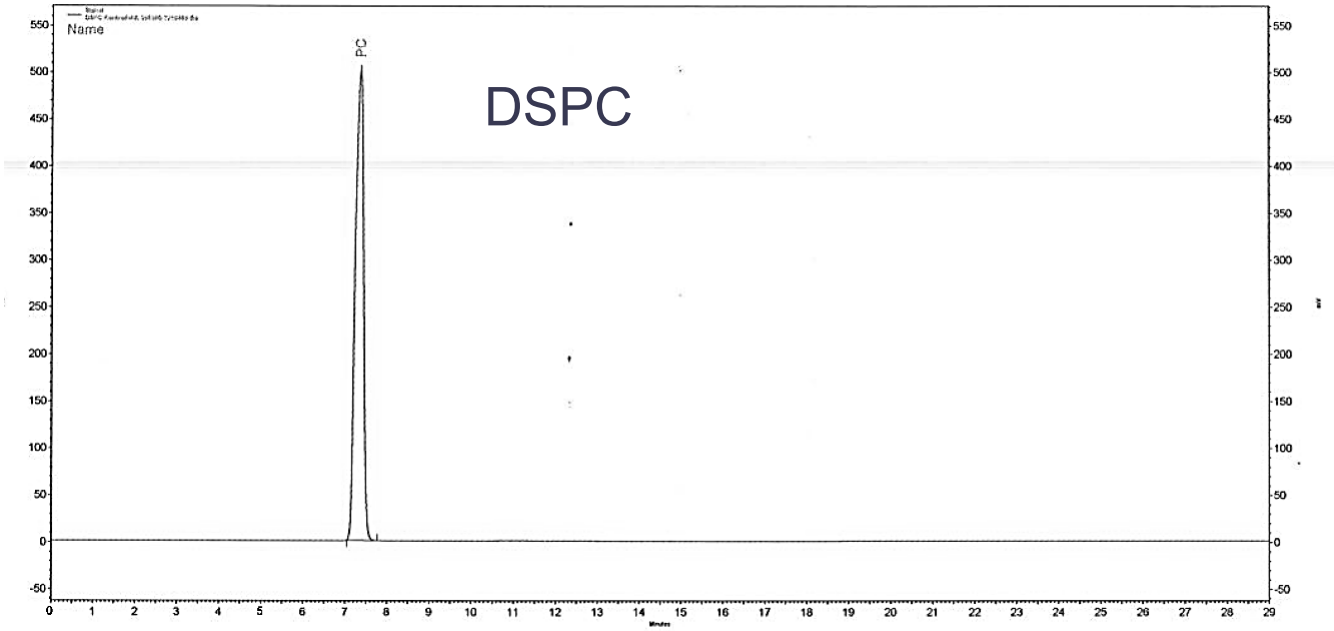
Condition:
ELSD, USP L20 (Diol), Hexane / 2-propanol / water / triethylamine / acetic acid



Deoiled soybean lecithin

Normal Phase HPLC

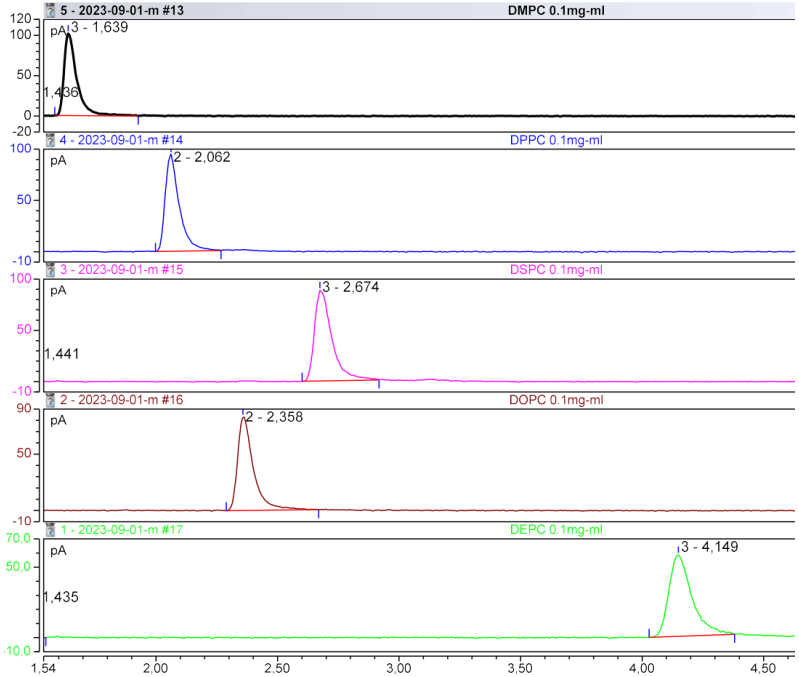
Condition:
ELSD, USP L20 (Diol), Hexane / 2-propanol / water / triethylamine / acetic acid



Reversed Phase HPLC

- Stationary phase: C_8 or C_{18} RP material
- Mobile phase: methanol / water / buffer
- Detection: ELSD/ CAD / UV / MS
- Separation of different fatty acids and different head groups
- The longer and the more saturated the fatty acid, the longer the retention time

Reversed Phase HPLC



PC(14:0/14:0) - DMPC

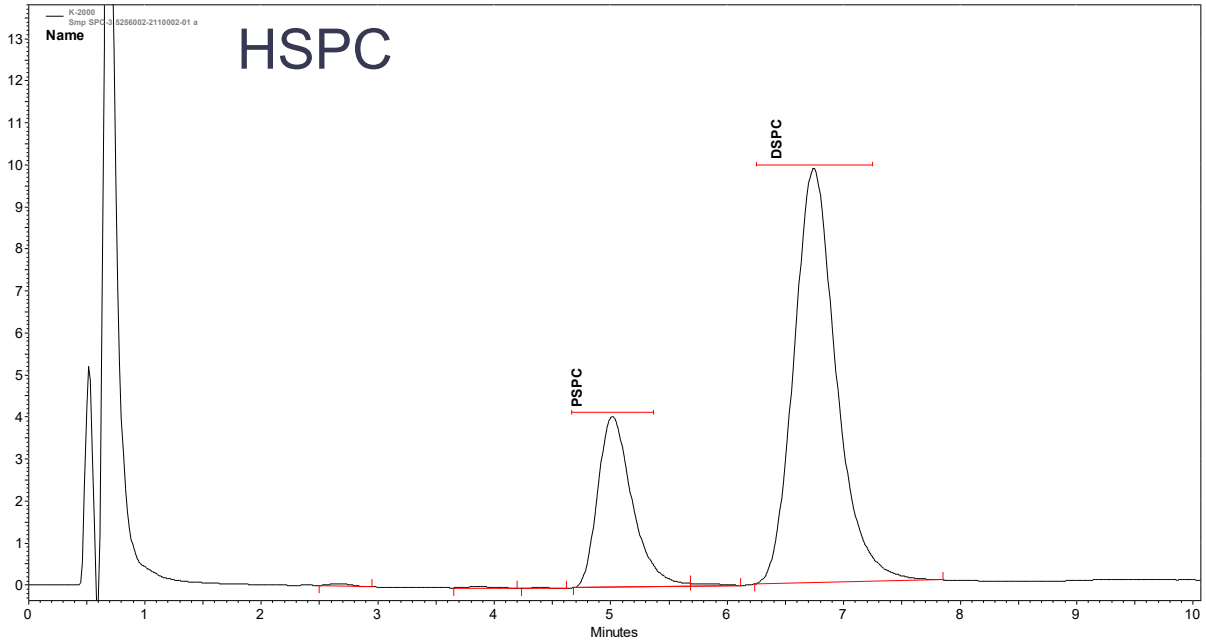
PC(16:0/16:0) - DPPC

PC(18:0/18:0) - DSPC

PC(18:1/18:1) - DOPC

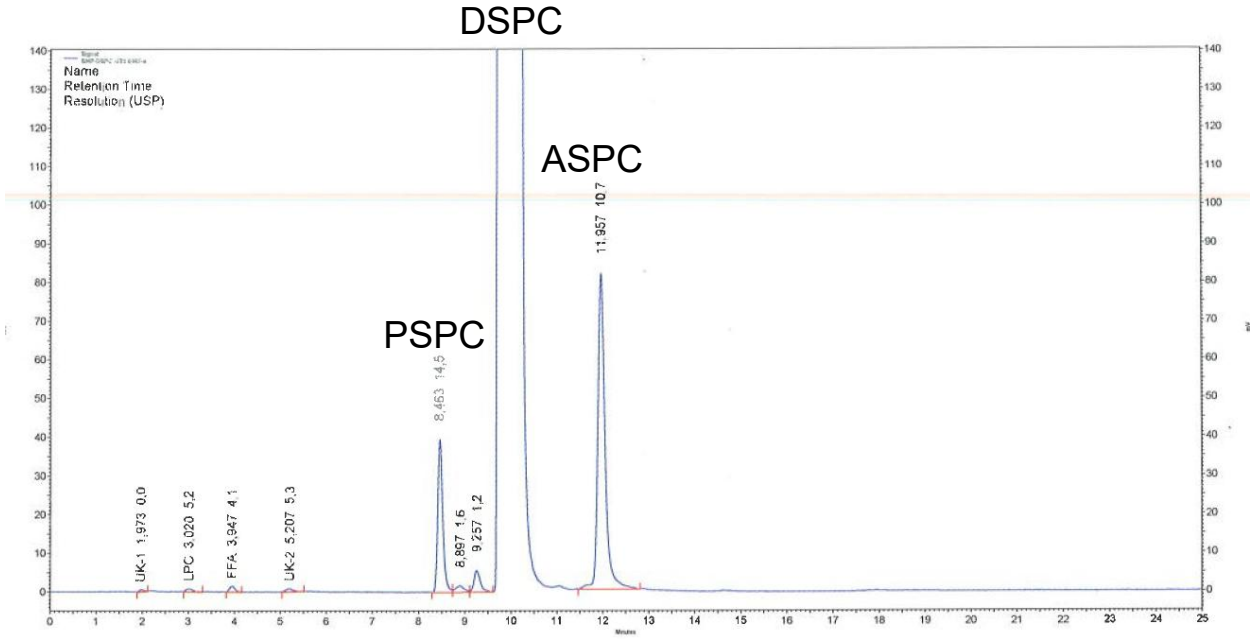
PC(22:1/22:1) - DEPC

Reversed Phase HPLC



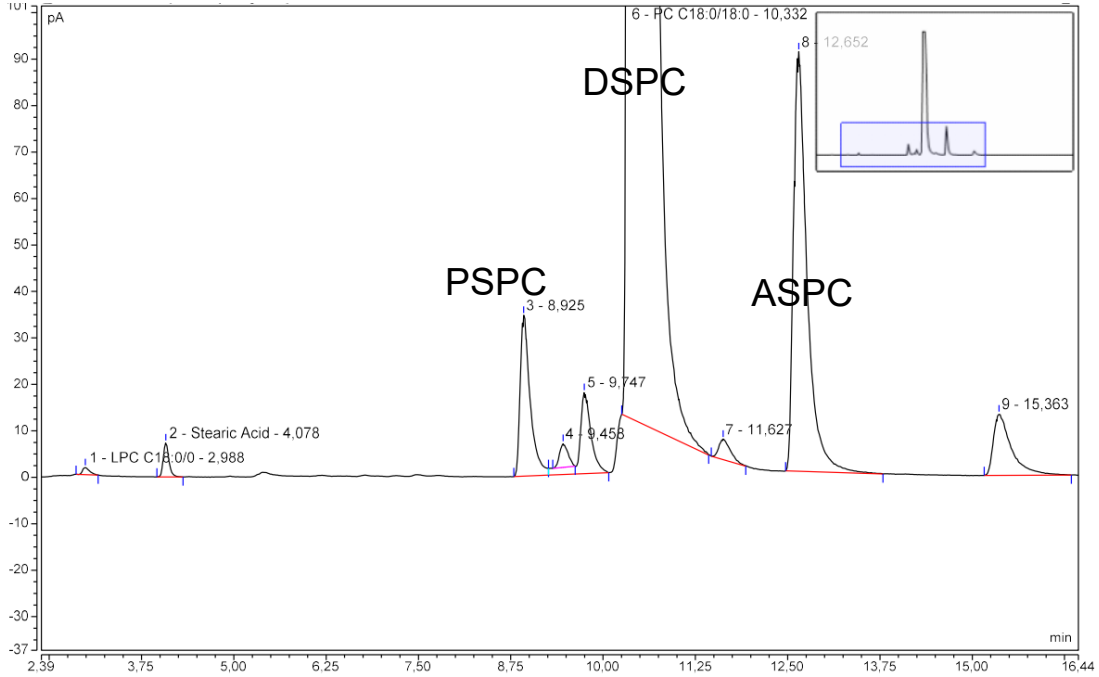
Reversed Phase HPLC

Conditions:
ELSD, USP L1 (C_{18}),
MeOH, H_2O, acetic acid



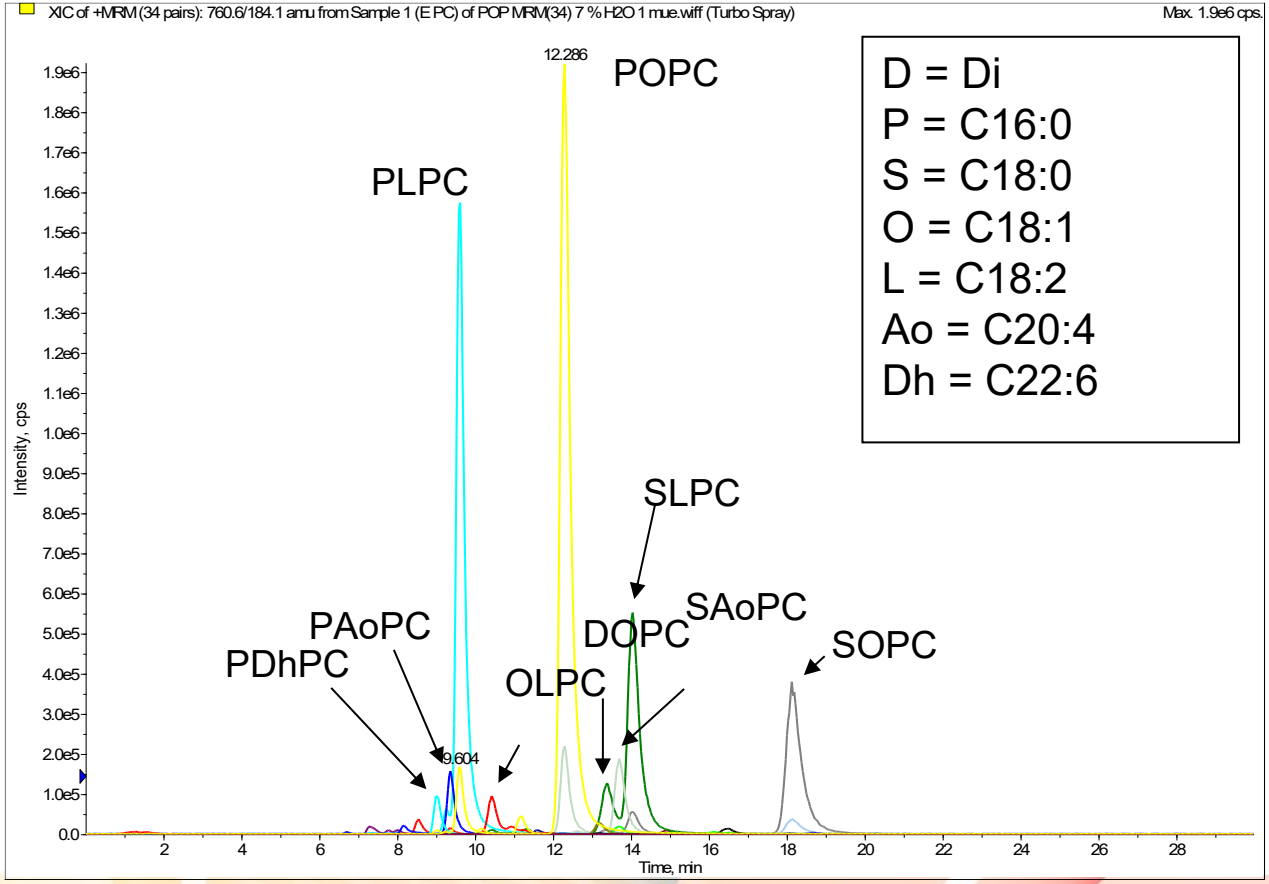
Reversed Phase HPLC

Conditions:
CAD, USP L1 (C_{18}),
MeOH, H_2O, acetic acid



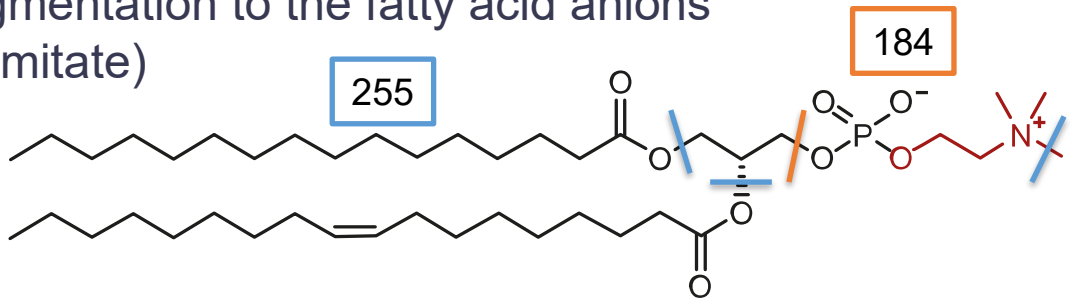
Reversed Phase HPLC

Conditions:
MS, USP L1 (C_{18}),
MeOH, H_2O, formic acid

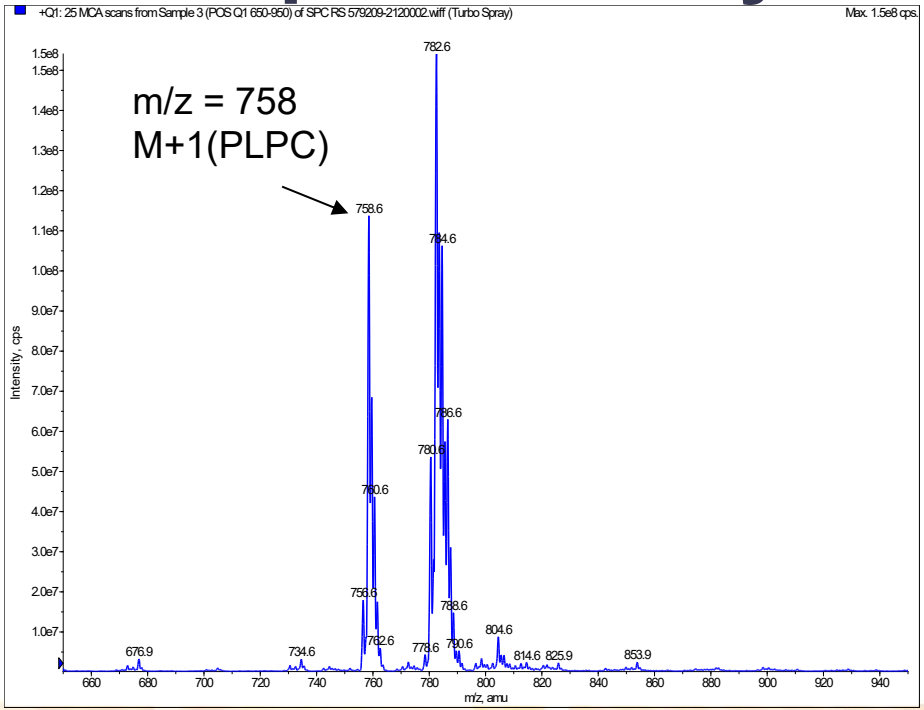


Mass spectrometry

- With positive ionization: M+1 ions
- Fragmentation of the M+1 ions results in an intense fragment of the head group
  (m/z = 184 → Phosphocholine)
- With negative ionization less intense M-15 ions (for PC), but characteristic fragmentation to the fatty acid anions
  (m/z = 255 → Palmitate)

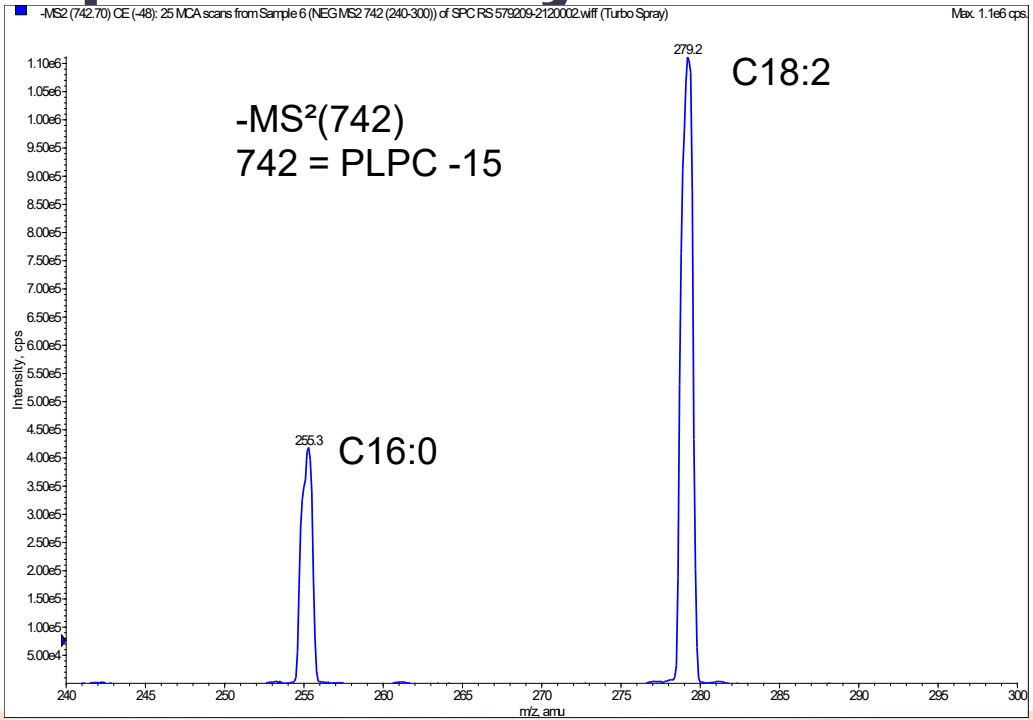


Mass spectrometry



m/z	PC
756	16:0 / 18:3
758	16:0 / 18:2
760	16:0 / 18:1
780	18:2 / 18:3
782	18:2 / 18:2 18:1 / 18:3
784	18:1 / 18:2
786	18:1 / 18:1 18:0 / 18:2

Mass spectrometry



Thank you

for your attention!