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Enhancing structural elucidation of the lipidome through optimized SFC-HRMS/MS hyphenation

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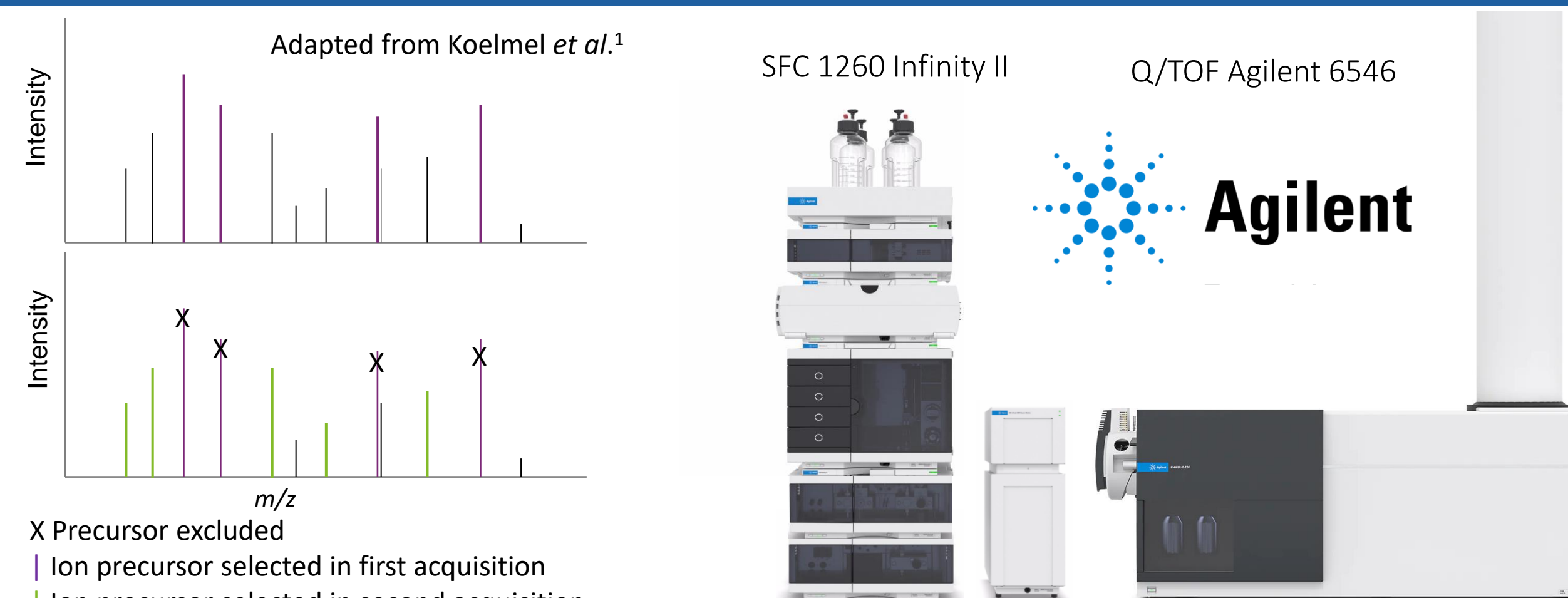
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Introduction

SFC separation on packed column for lipids analysis is well adapted thanks to the interclass lipid species separation as normal phase liquid chromatography¹. The characterization of the lipidome typically requires the use of Data Dependent Acquisition (DDA) MS/MS method, which is limited to the fragmentation to the most intense signals during a cycle time². Considering this issue, the strategy involving the iterative ion exclusion consisted of automatically placing prior fragmented ions on a list along sequential repetitive injections seems well adapted³. However, some MS/MS spectra at lower intensity present fragment ions of interest with weak signal-to-noise ratio. In this context, MS/MS acquisition method parameters are critical to ensure a short cycle time that meets this minimum requirements for an optimal coverage of lipid species in complex biological matrices. SFC gradient and MS/MS parameters of the Q/TOF were optimized to address this issue. A mix of lipid standards representing of 27 lipid classes were injected to determine the optimized parameters for the highly confident structural elucidation of lipid species present in complex biological matrices.



Strategy for iterative list exclusion DDA Top N based method

SFC-Q/TOF system used in this study

Materials and Methods

Mobile phase : MeOH + 20 mM AcNH₄ supercritical CO₂

Make-up : MeOH + 20 mM AcNH₄

Column : Diethylamine Torus 1.7 μm, 130 Å, 3x100 mm (Waters)

Flow rate : 1.4 mL/min

Column temperature : 60 °C

Solvent injection : Isopropanol

Back Pressure Regulator pressure : 130 bar

Back Pressure Regulator temperature : 60 °C

Ionization source : Electrospray Ionization (ESI)

Resolution : 50-60 000 @ *m/z* 922

Activation energy : Collision Induced Dissociation

Determination of Optimal Collision Energies by ERMS

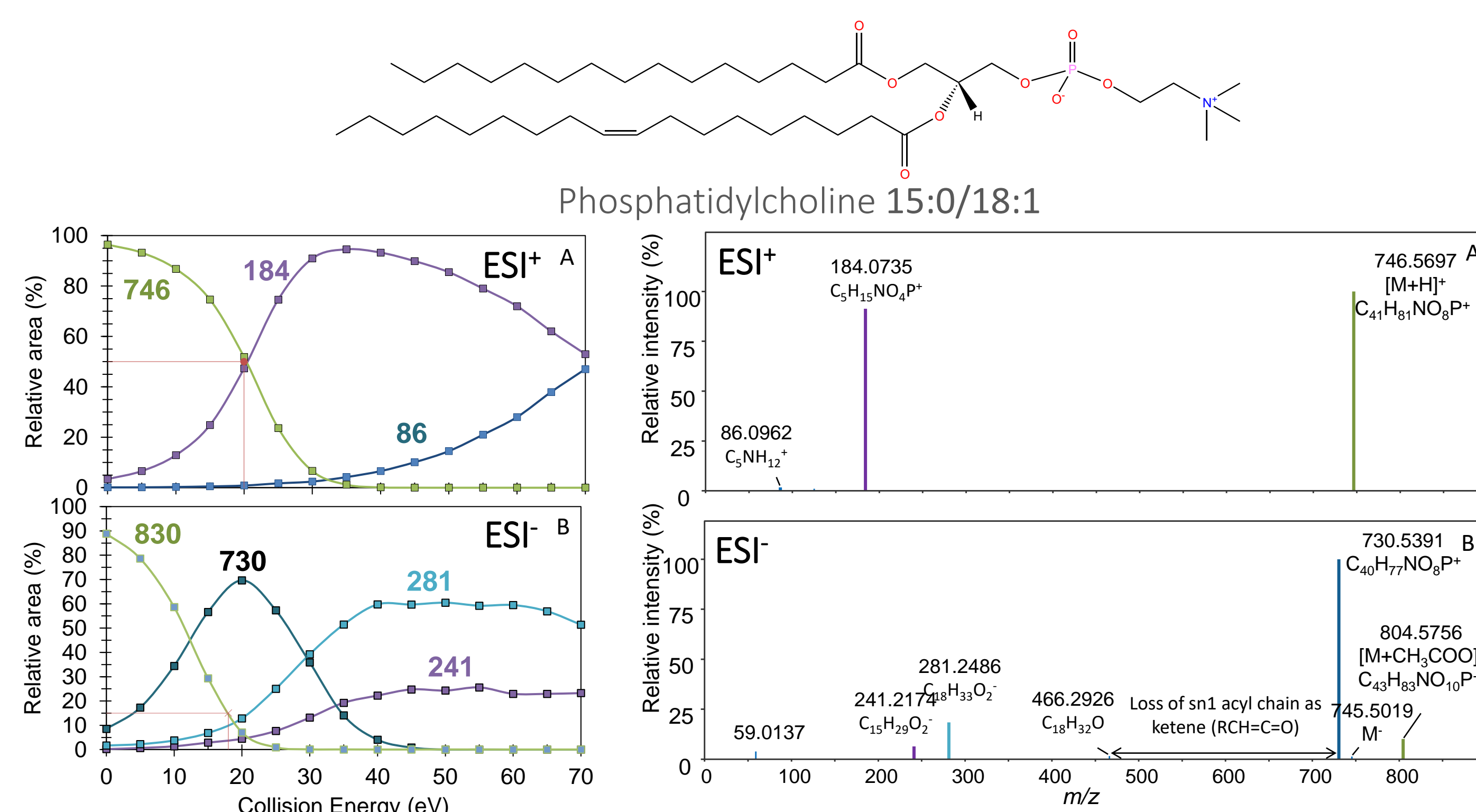


Fig. 3 : Energy Resolved curves of PC 15:0_18:1 in both positive (A) and negative (B) ionization mode.

Fig. 4 : MS/MS spectra of PC 15:0_18:1 in both positive (A) and negative (B) ionization mode⁵.

Chromatographic method optimization

- The elution gradient initially established on a DEA column by Chollet *et al.*⁴ was evaluated for the elution of high-polar lipid species. The introduction of shorter DEA columns has facilitated an increase in the proportion of the modifier.

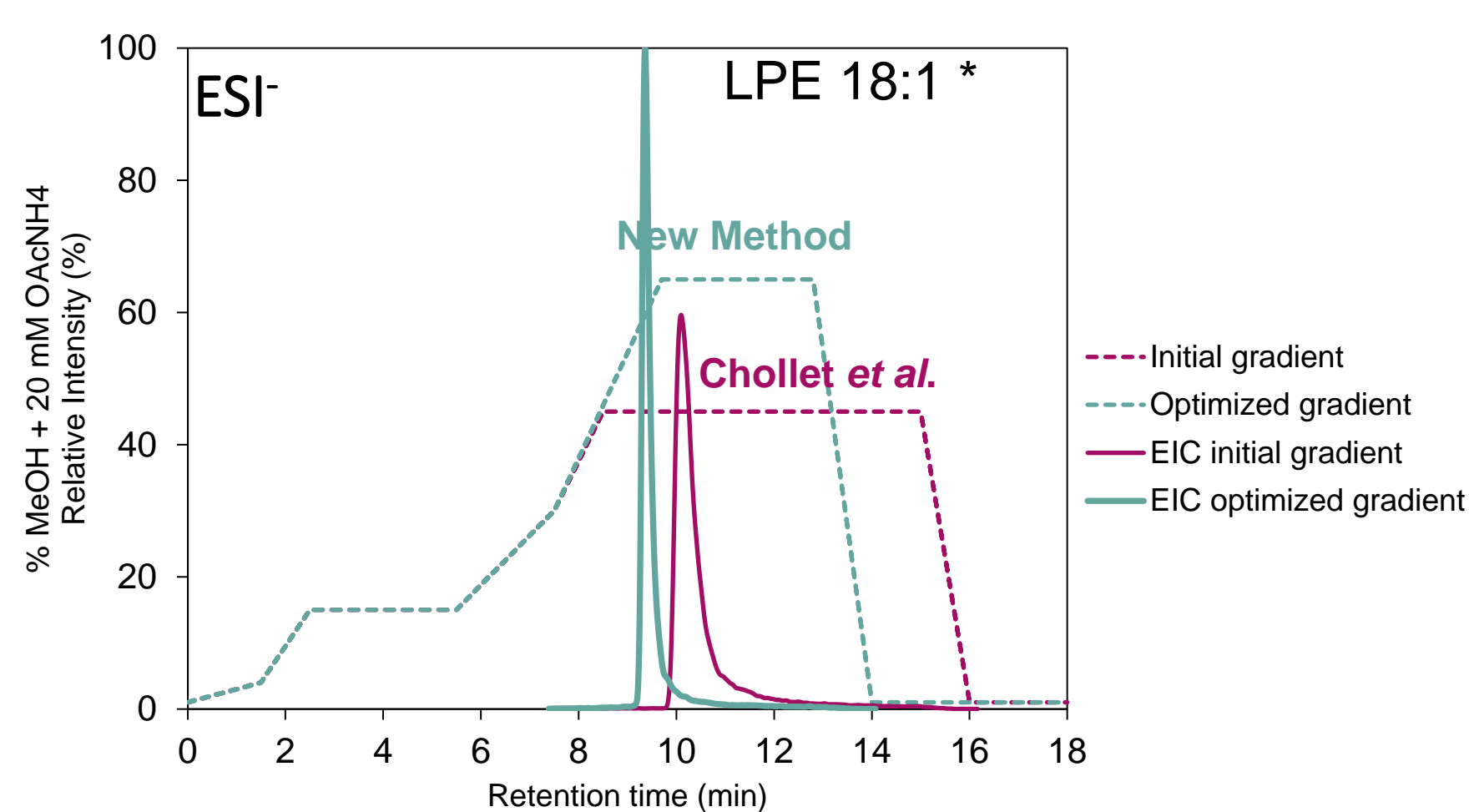


Fig. 2 : Modifier gradient solvent and extracted ion chromatogram at 5ppm IC of Lysophosphatidylethanolamine 18:1.

- Increasing the modifier proportion by 65% in the mobile phase during elution gradient, enhances the chromatographic peak quality :

→ For LPE 18:1, height ratio is improved by 2 while full width at half maximum is reduced by 2.

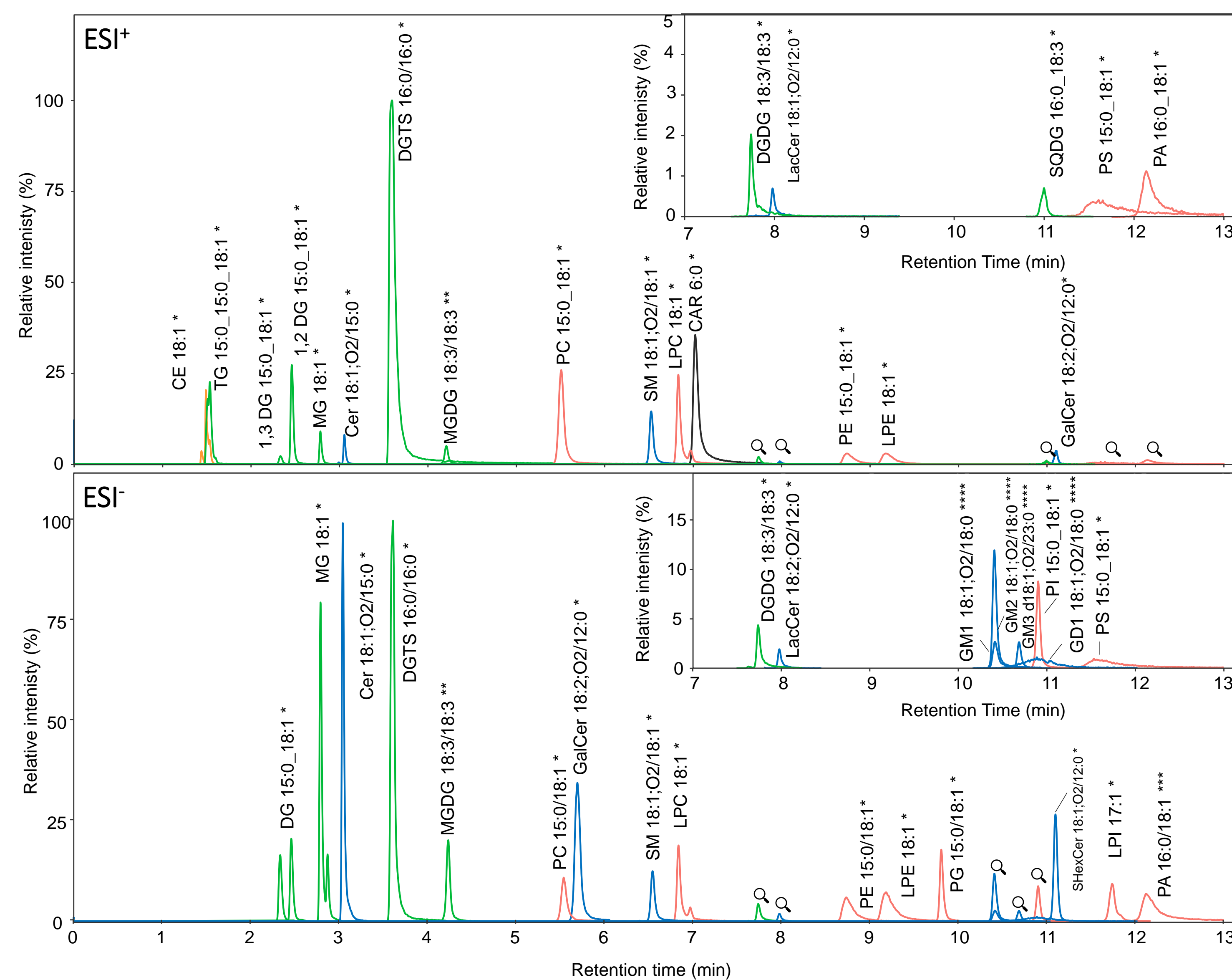


Fig.1 : Extracted ion chromatograms at 5 ppm. For the visualization of all EICs, concentrations of standards vary. Standards specified by * are at 10 μg/mL, by ** are at 15 μg/mL, *** are at 20 μg/mL, **** are at 100 μg/mL
Glycerolipids : Tri/Di/Monoacylglycerol (TG/DG/MG), Diacylglyceroltrimethylhomoserine (DGTG), Di/Monogalactosyldiacylglycerol (D/MGDG) ; **Sphingo(glyco)lipids** : Ceramide (Cer), Lactosyl ceramide (LacCer), Galactose ceramide (GalCer), Sphingomyelin (SM), Ganglioside (GM, GD) ; **Fatty acyl** : Acylcarnitine (CAR) ; **Sterol lipids** : Cholesteryl Ester (CE) ; **Glycerophospholipids** : (Lyso)Phosphatidylcholine ((L)PC), (Lyso)Phosphatidylethanolamine ((L)PE); Phosphatidylglycerol (PG); (Lyso)phosphatidylinositol ((L)PI), Phosphatidic acid (PA).

- 27 lipid classes belonging to 5 distinct lipid categories are eluted along 13 minutes on the diethylamine stationary phase.
- Ionization efficiencies are variable according to the polar headgroups of lipid species.

References

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Conclusion and Perspectives

The optimized SFC gradient enhances the sensitivity of polar lipid species characterized by low ionization efficiency in ESI. Application of optimal collision energies with an acquisition rate of 10 Hz promotes the MS/MS spectra enabling an highly confident annotation of low abundant lipid species.

Those optimized parameters for MS/MS acquisition will be further evaluated on diverse biological matrices such as the human plasma and vegetal lipidic extracts.

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