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

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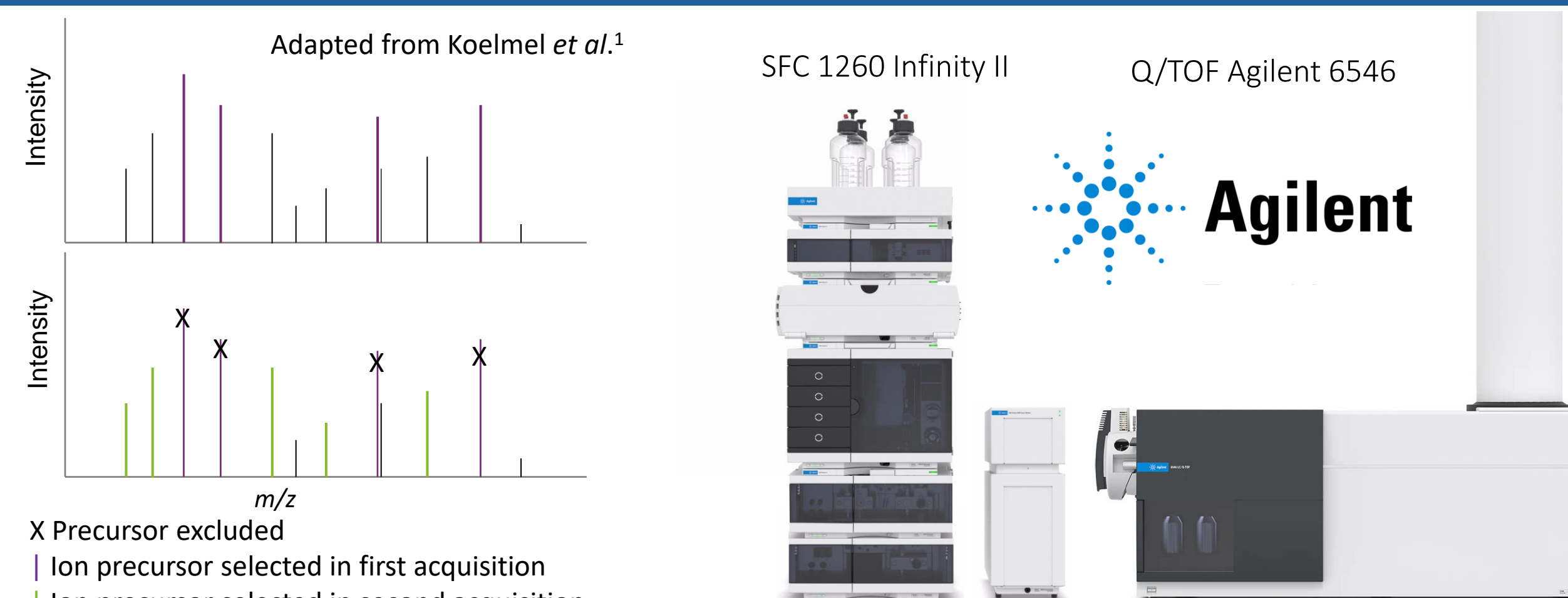
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## Introduction

SFC separation on packed column for lipids analysis is well adapted thanks to the interclass lipid species separation as normal phase liquid chromatography<sup>1</sup>. 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 time<sup>2</sup>. 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 adapted<sup>3</sup>. 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 AcNH<sub>4</sub> supercritical CO<sub>2</sub>

**Make-up :** MeOH + 20 mM AcNH<sub>4</sub>

**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

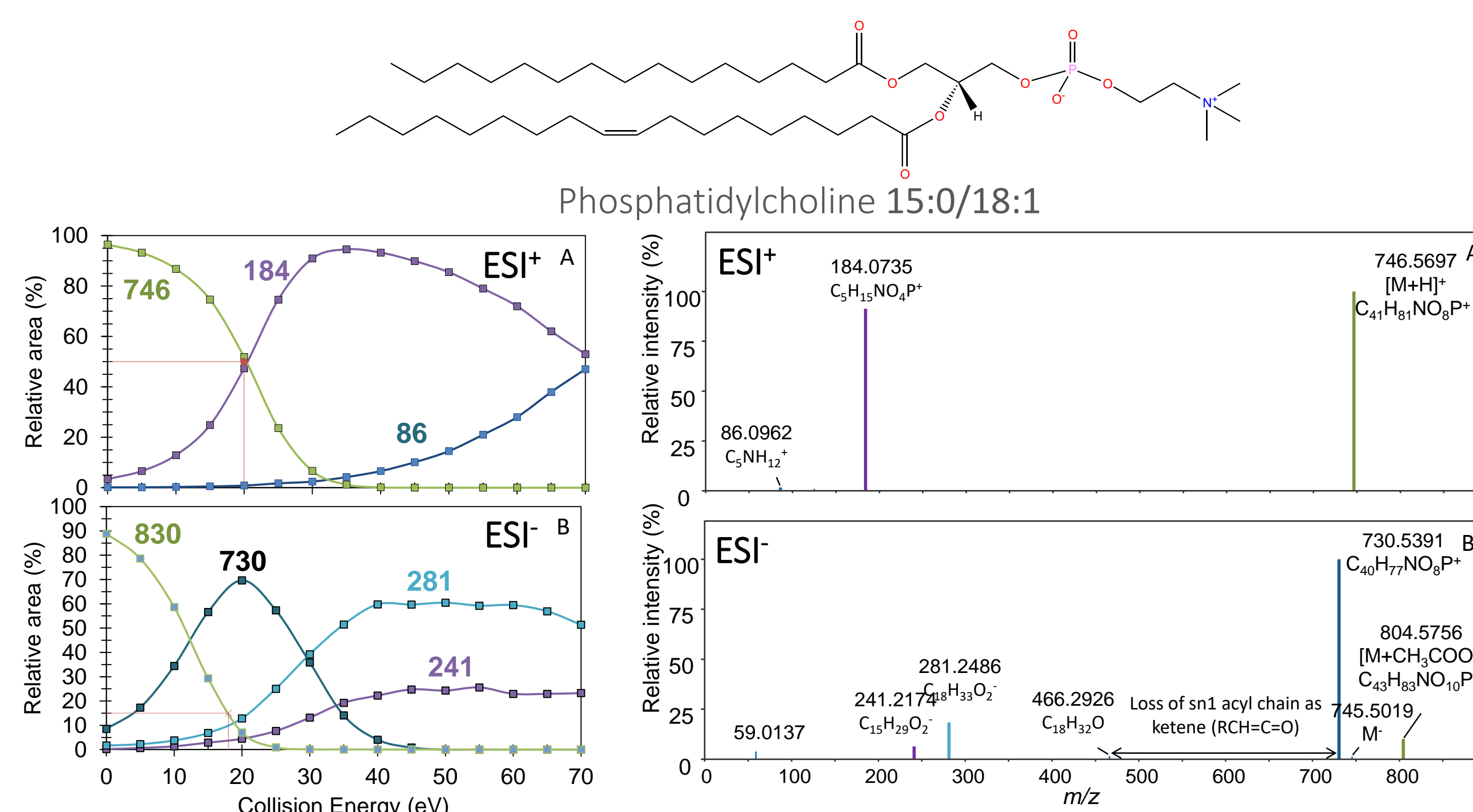


Fig. 3 : Energy Resolved curves of PC 15:0\_18:1 in both positive (A) and negative (B) ionization mode.

Fig. 4 : MS/MS spectra of PC 15:0\_18:1 in both positive (A) and negative (B) ionization mode<sup>5</sup>.

## Chromatographic method optimization

- The elution gradient initially established on a DEA column by Chollet *et al.*<sup>4</sup> 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.

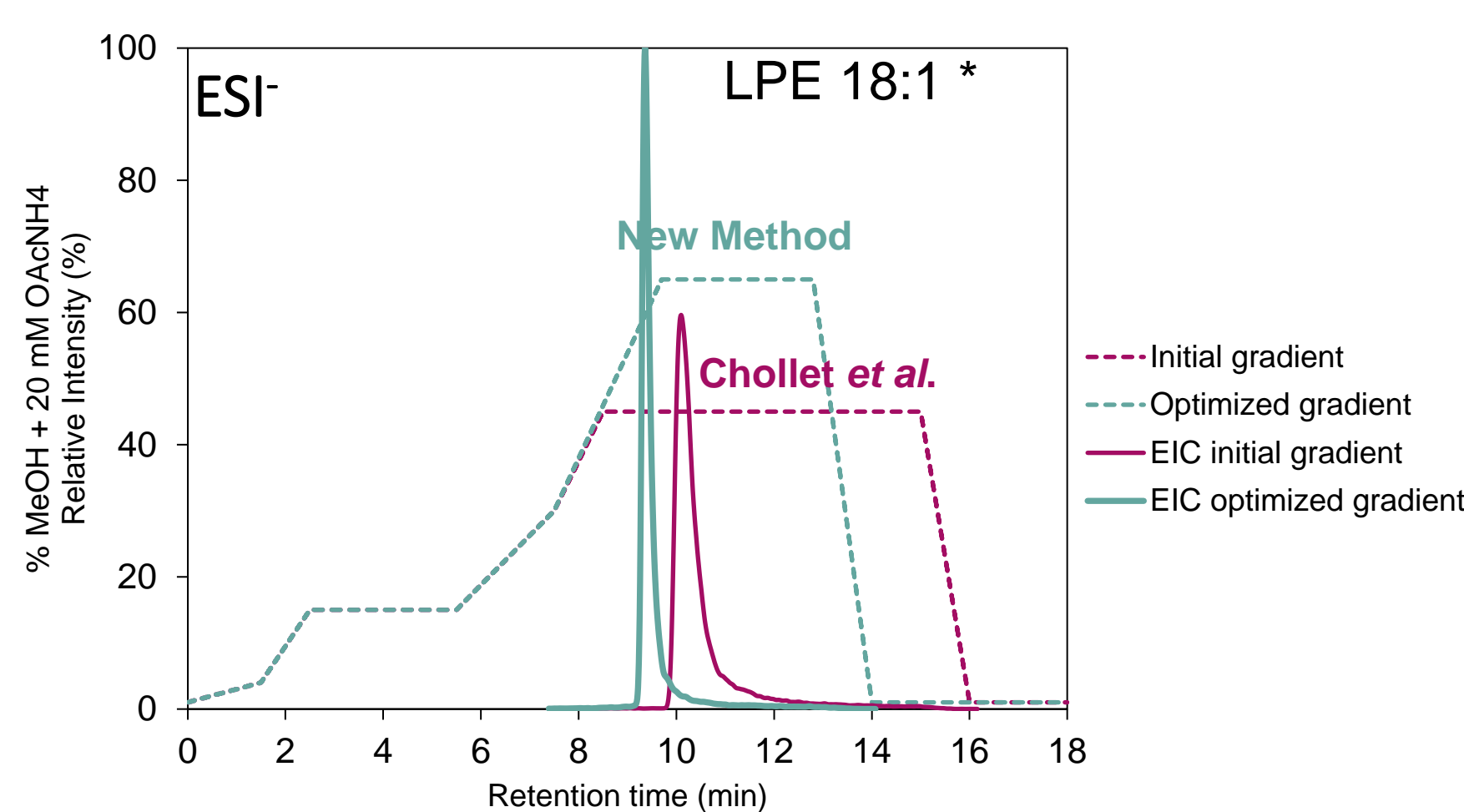


Fig. 2 : Modifier gradient solvent and extracted ion chromatogram at 5 ppm IC of Lysophosphatidylethanolamine 18:1.

- 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.

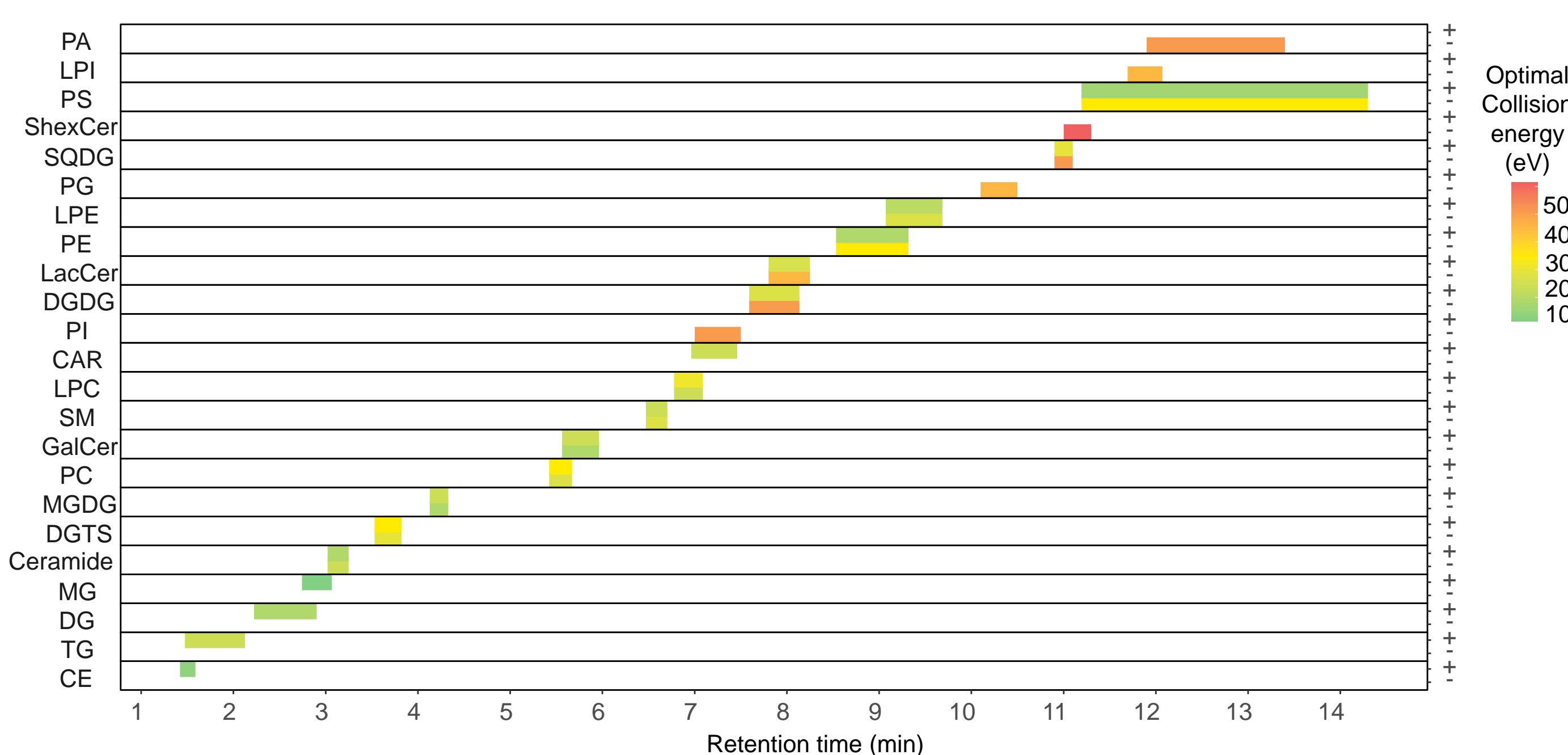


Fig. 5 : Optimal collision energies of lipid species belonging to 21 lipid classes in both positive and negative ionization mode.

- Optimal collision energies are determined on standards to provide the presence of the precursor ion for at least 15% and the ion fragments ions. The fragmentation of lipid classes at optimal collision energy enables the complete putative elucidation structure.

## Evaluation of MS/MS acquisition rate

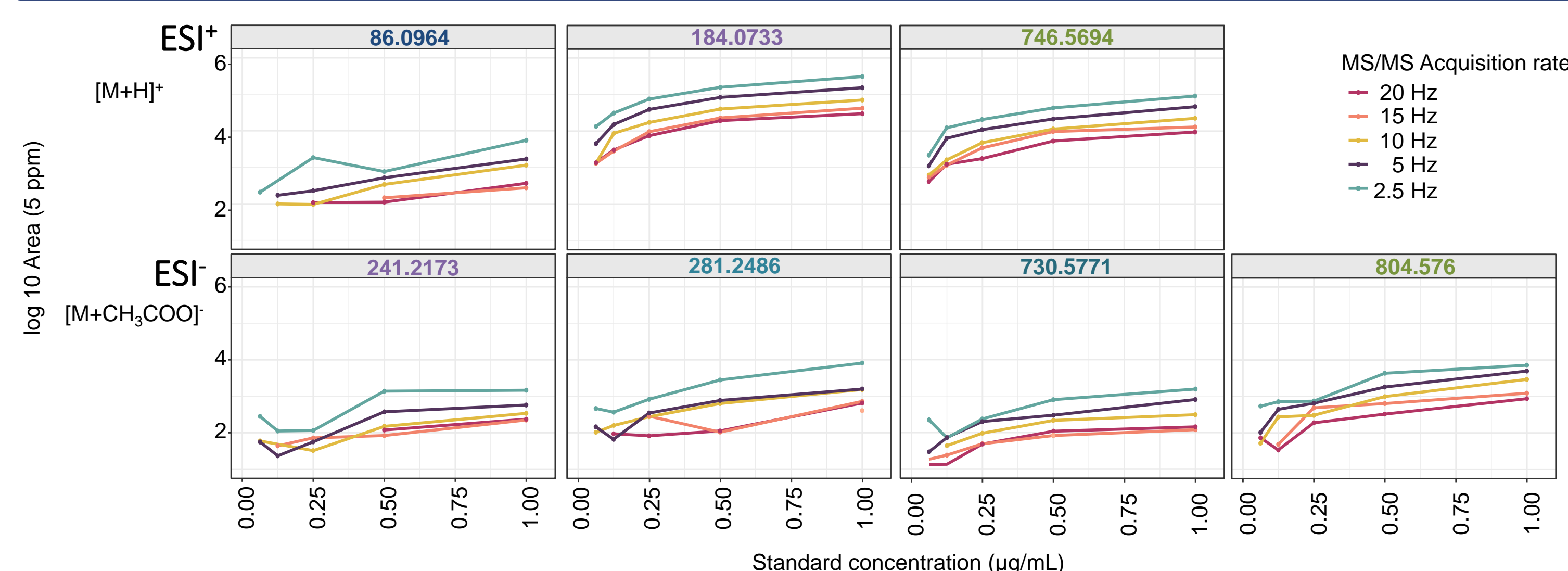


Fig. 6 : Intensity of fragment of MS/MS spectra of PC 15:0\_18:1 in positive and negative ionization mode

- With a fragmentation of a PC 15:0\_18:1 with an optimal collision energy of 18 eV (Fig.5) in negative ionization mode, the carboxylate anion at *m/z* 241.2173 is absent under conditions of high acquisition rate and low mass concentration. While more intense ions are detected even when utilizing a high acquisition rate for both positive and negative ionization mode.
- The most appropriate MS/MS acquisition rate is 10 Hz to promote the presence of fragments for the complete putative elucidation structure in both positive and negative ionization modes.

## 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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