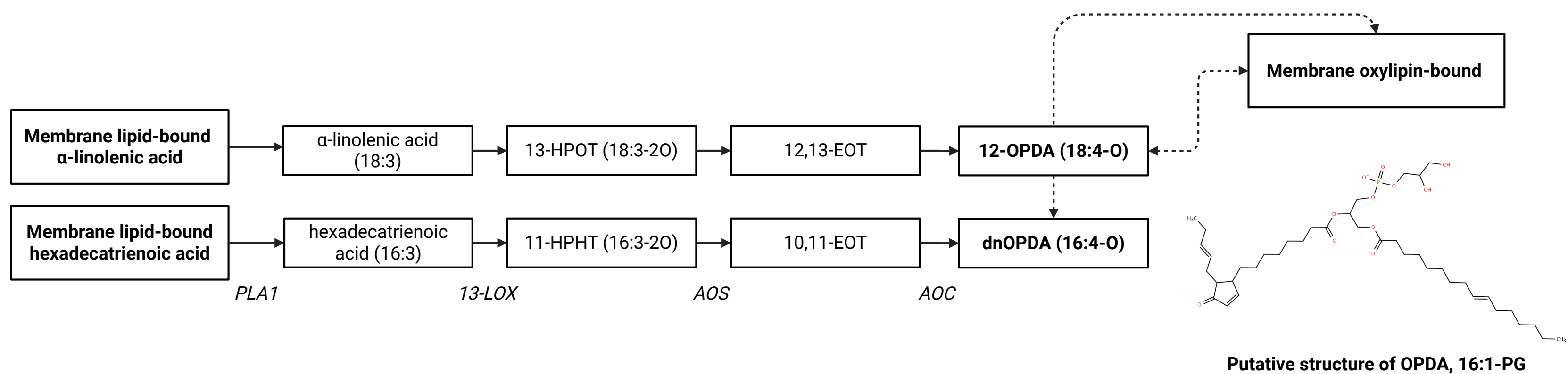


INTRODUCTION

Oxylipins are oxidized lipids derived from polyunsaturated fatty acids that accumulate in plants in response to biotic and abiotic stresses. Among them, jasmonates, including 12-oxo-phytodienoic acid (OPDA), dinor-OPDA (dnOPDA) and jasmonic acid (JA), are key signalling molecules involved in the regulation of plant defence responses. Besides their free forms, oxylipins can bind to membrane phospholipids, giving rise to phosphooxylipins (POLs), a poorly characterized class of esterified oxylipins. However, their low abundance, structural diversity and the lack of dedicated analytical methods make their detection particularly challenging.



12-oxo-phytodienoic acid (OPDA) and dinor-OPDA (dnOPDA) biosynthetic pathway — PLA1: Phospholipase A1, 13-LOX: 13-Lipoxygenase, AOS: Allene Oxide Synthase, AOC: Allene Oxide Cyclase

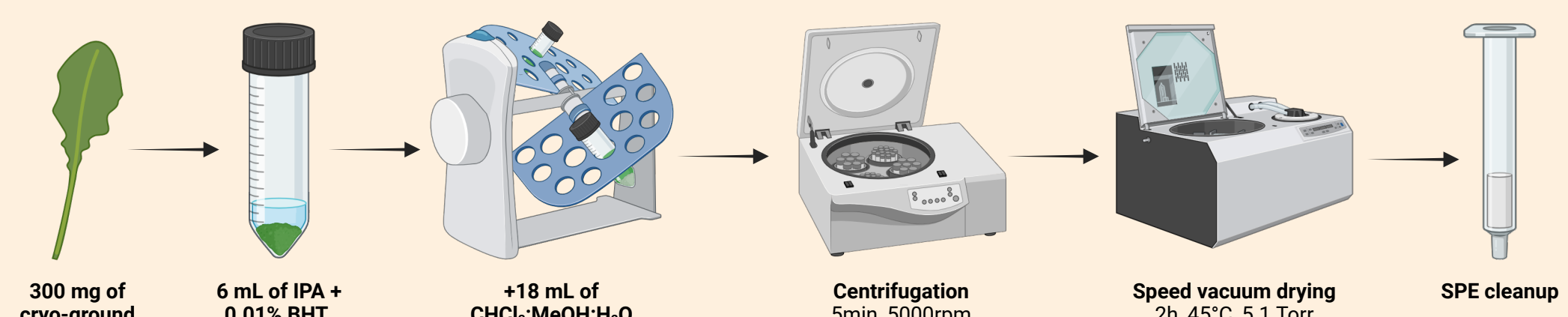
OBJECTIVES

- Develop a single UHPLC method enabling efficient separation of lysophospholipids, phospholipids and oxidized phospholipids (phosphooxylipins)
- Optimize UHPLC and ESI–MS/MS parameters to maximize chromatographic resolution, sensitivity and selectivity
- Develop a targeted MRM acquisition method for the selective detection of phosphooxylipins (POLs)
- Apply the optimized method to detect phosphooxylipins in *Arabidopsis thaliana* subjected to stress conditions.

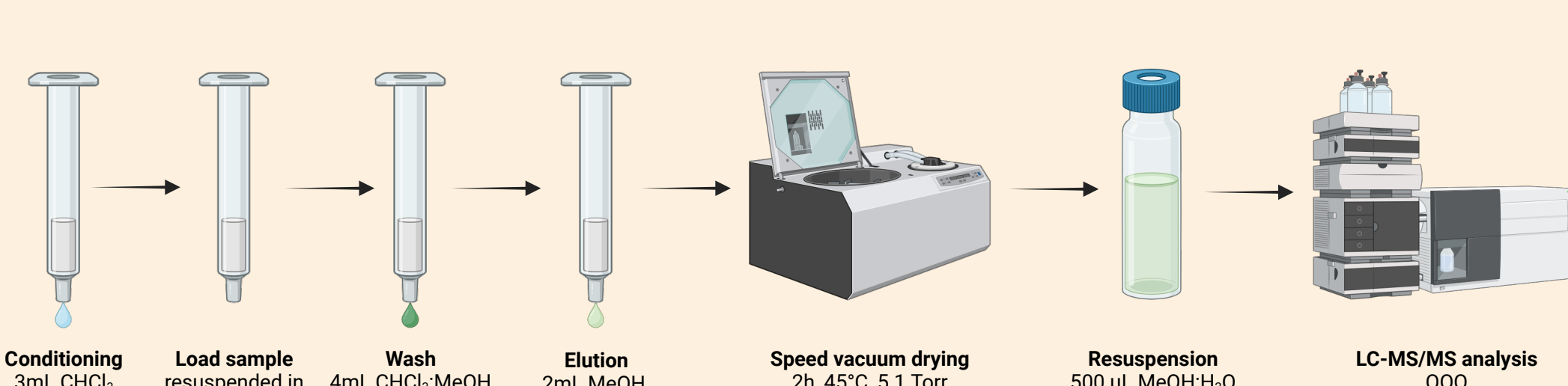
METHODS

Sample preparation

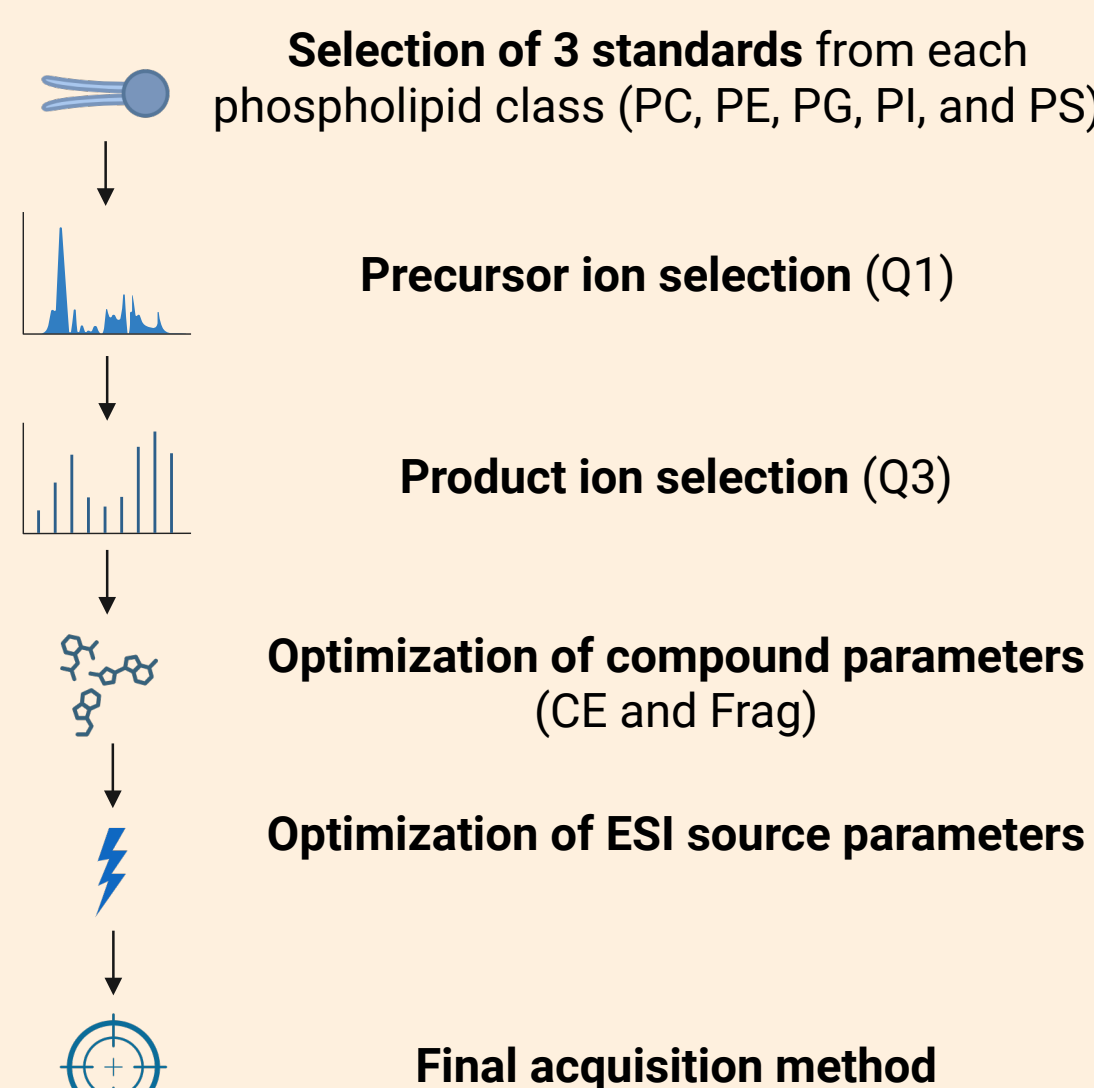
Lipid extraction method



SPE cleanup (silica cartridge)



MS/MS optimization



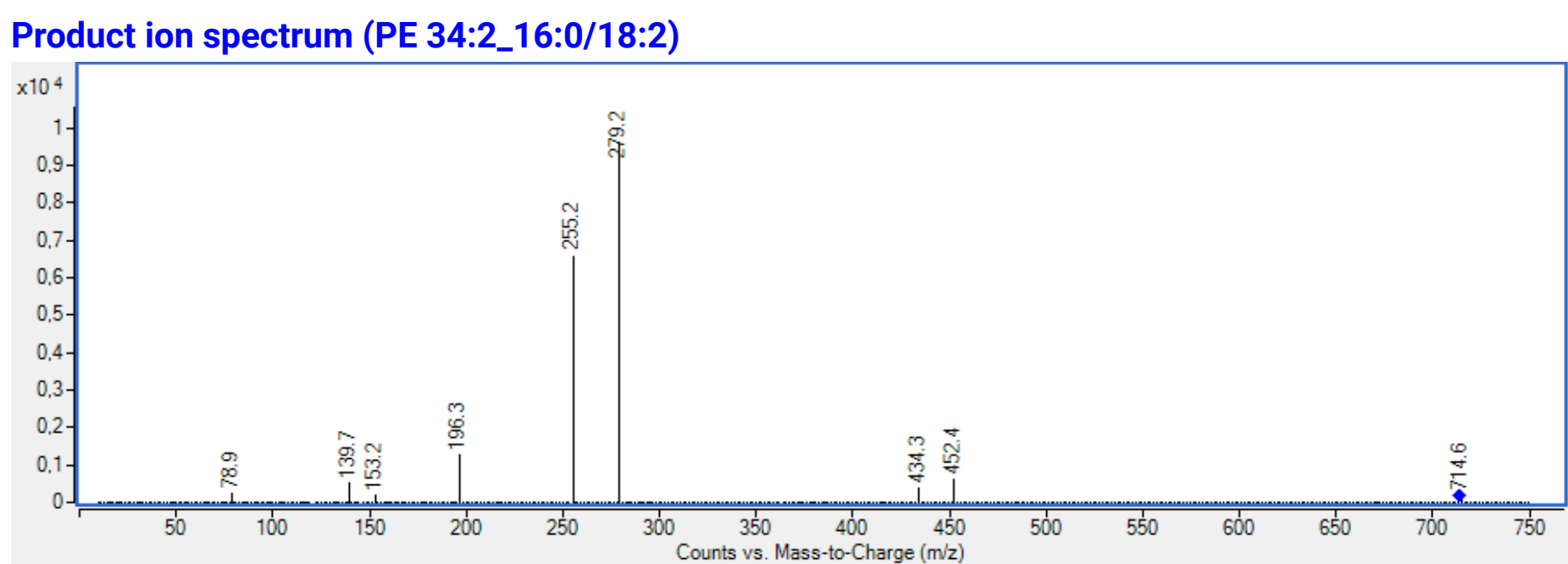
Parameter	Values Tested	Step
Fragmentor voltage (Frag, V)	70-320	10
Collision energy (CE, eV)	10-65	5
Capillary voltage (V)	2500-5000	500
Gas Flow (L/min)	5-11	2
Gas Temperature (°C)	250-350	50
Nebulizer (psi)	25-50	5
Nozzle Voltage (V)	0-2000	500
Sheath Gas Flow (L/min)	8-12	2
Sheath Gas Temperature (°C)	250-400	50

UHPLC optimization

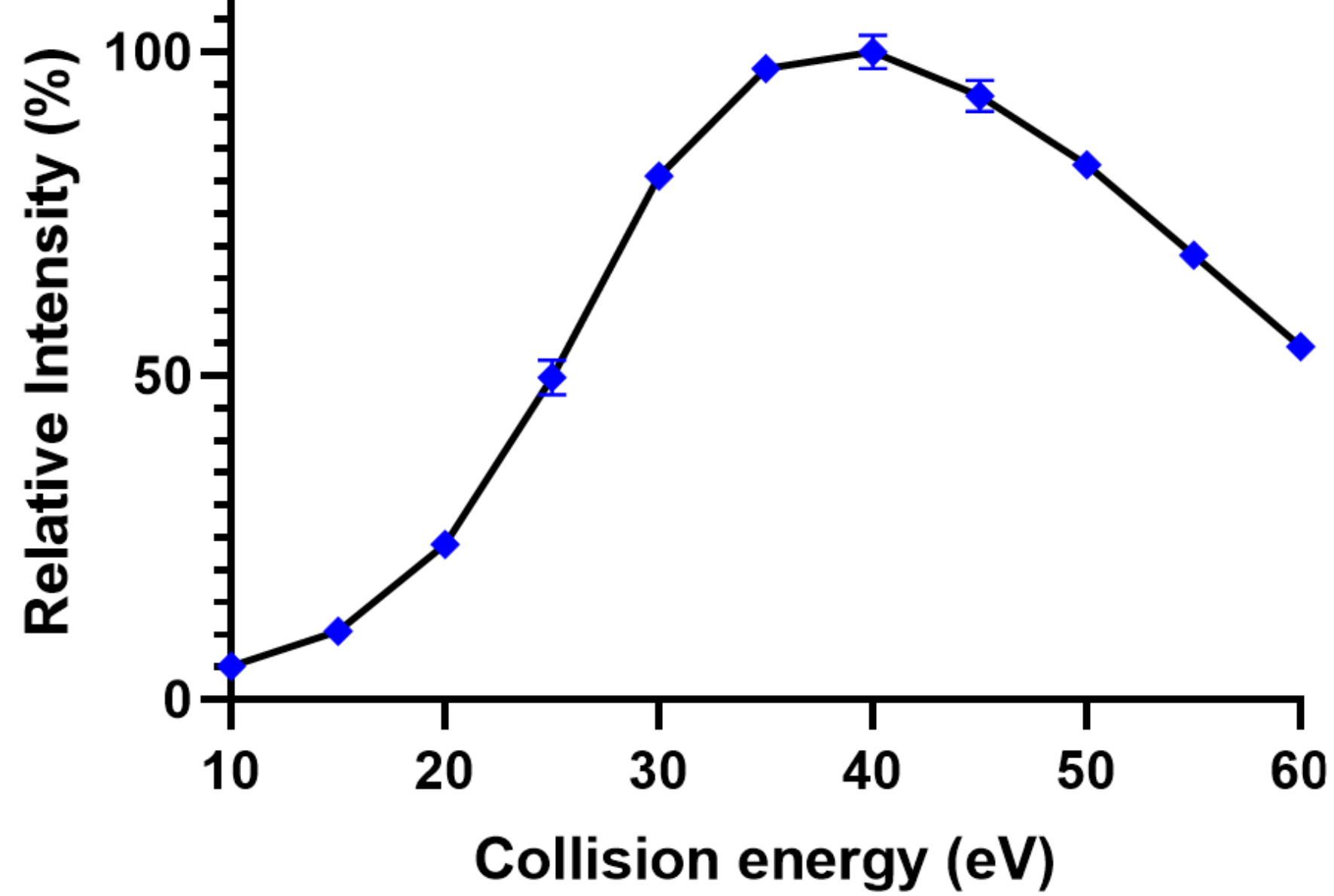
Column	ACQUITY UPLC C18, 2.1x50mm, 1.7µm
Mobile phase A	H ₂ O:ACN (70:30, v/v) + 0.1% FA + 10 mM ammonium formate
Mobile phase B	IPA:ACN (80:20, v/v) + 0.1% FA + 10 mM ammonium formate
Column temperature	30 - 40°C
Gradient	Several gradients tested
Flow rate	0.4 - 0.6mL/min
Injection volume	2.5 - 20µL

RESULTS

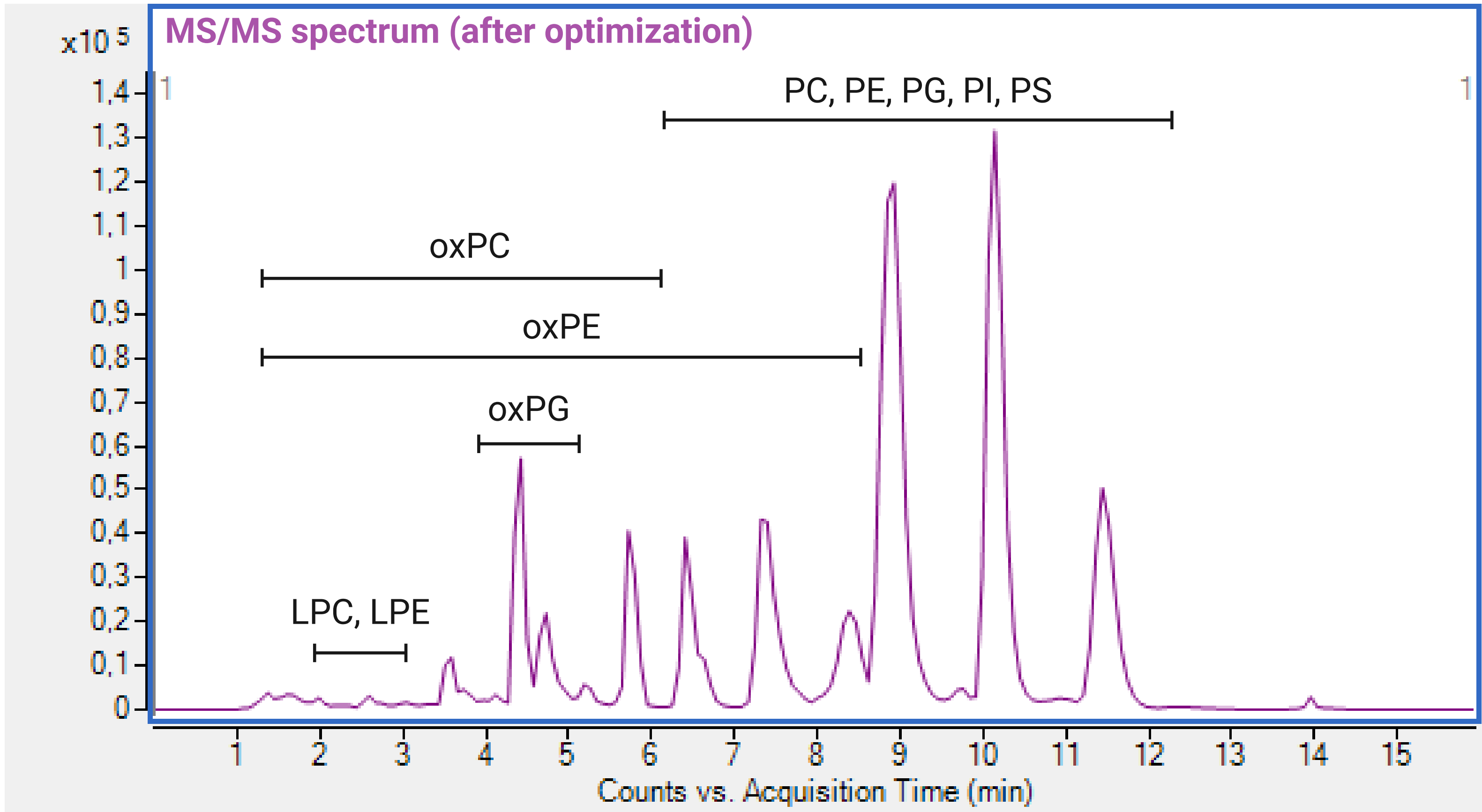
MS/MS optimization



CE optimization for PE 34:2, 16:0/18:2 (m/z 714.5 → 279.2)

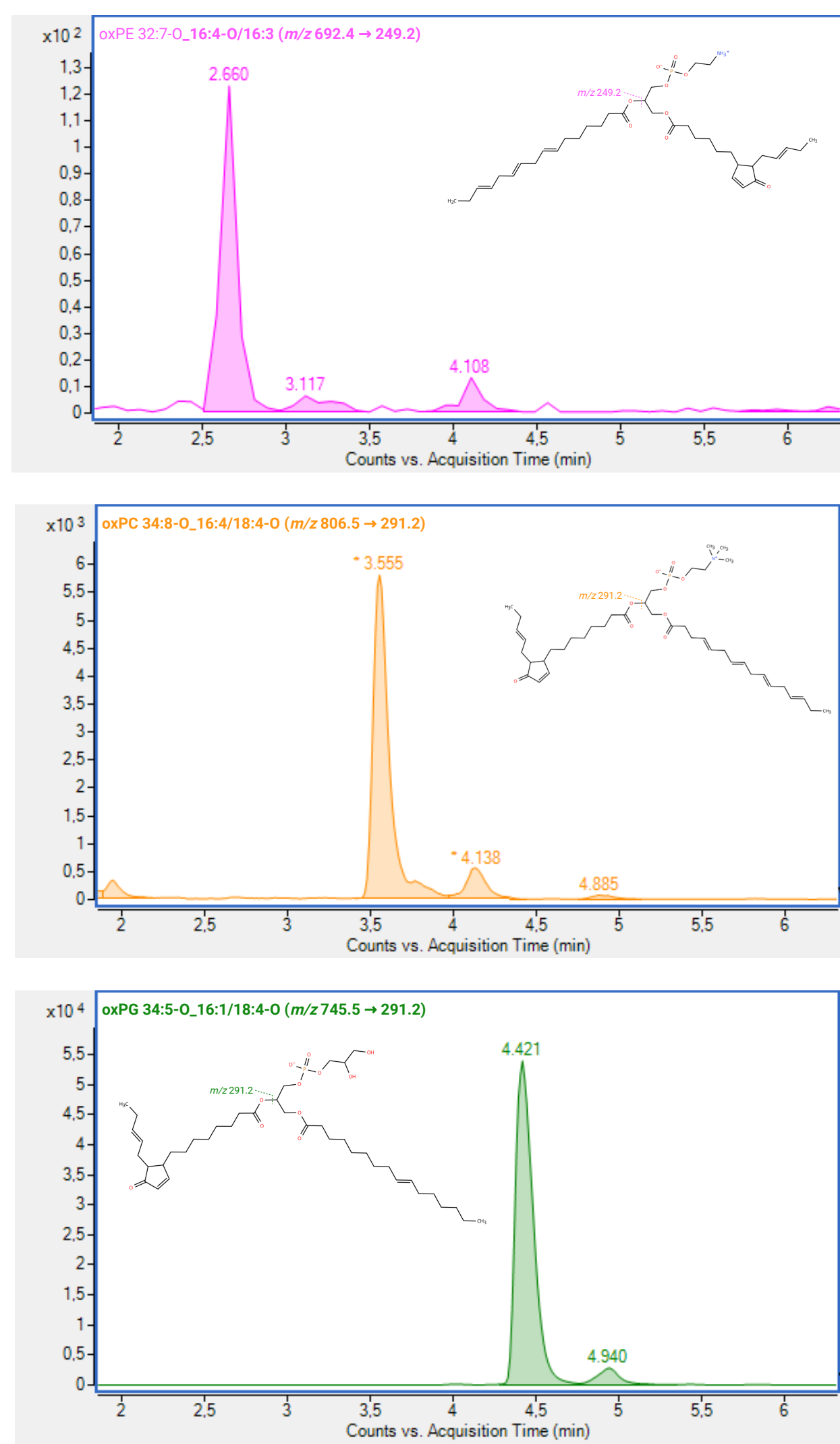


UHPLC optimization



Detection of POLs in stressed *A. thaliana*

MRM chromatograms (optimized method)



CONCLUSION

The targeted UHPLC–ESI(–)–MS/MS method was successfully developed for the analysis of plant phosphooxylipins. Optimization of both the C18 chromatographic gradient and ESI source parameters enabled efficient separation of the major phospholipid classes (PC, PE, PG, PI and PS) within a 16 min analysis, while providing sensitive and selective detection of putative phosphooxylipins. The optimized workflow constitutes a robust platform for future analytical validation and quantitative plant lipidomics applications.

PERSPECTIVES

- Optimization of MS/MS and ESI source parameters using oxidized standards
- Optimization of SPE cleanup conditions to enhance selectivity
- Improvement of lipid extraction protocols
- Full analytical validation (LOD, LOQ, linearity, accuracy, precision)
- Application to additional biotic stress conditions in *Arabidopsis thaliana*

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