Optimization of a liquid chromatography-ion mobility-high resolution mass spectrometry (LC-IM-HRMS) platform for untargeted lipidomics

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INTRODUCTION

Lipidomics, the full characterization of the lipid molecular species of a biological system, has significantly grown in recent years due to advances in technology and the increasing number of applications in pathogenesis elucidation, biomarker discovery, and toxicity testing.

Different biochemical function

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Analytical challenges

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Multidimensional techniques

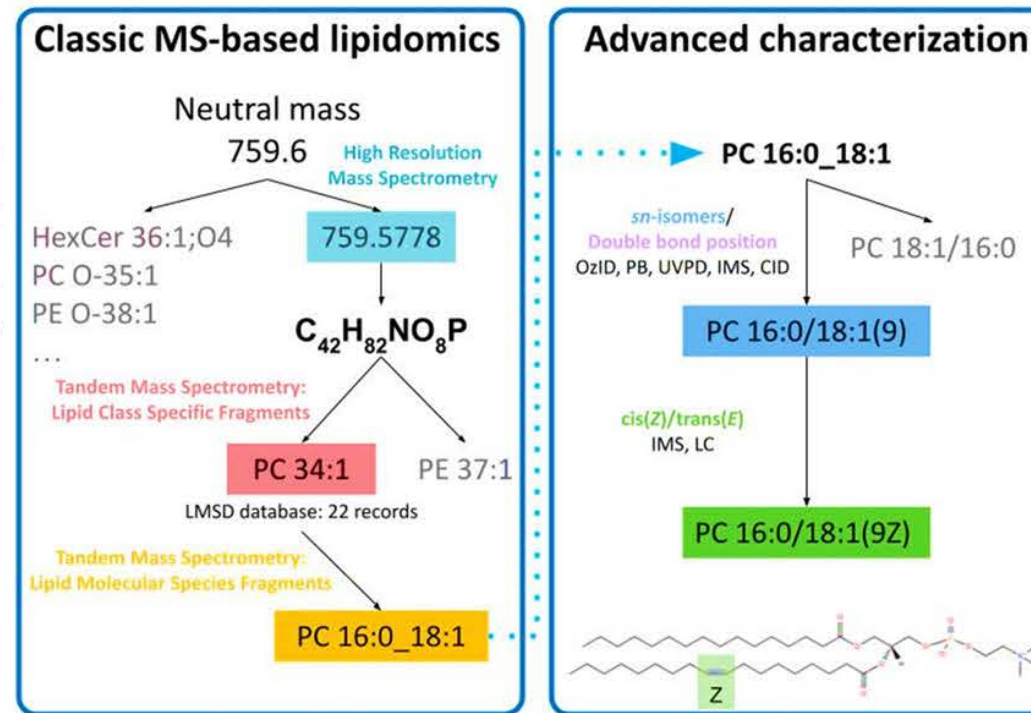

Figure 1. The diversity of lipid classes and structures requires different analytical techniques for full characterization.

A gas-phase separation method, **ion mobility** (**IM**) spectrometry hyphenated to LC-HRMS can increase peak capacity and confidence in annotation by using **collision cross section** (**CCS**) information.

The goal of this study was to optimize an LC-IM-HRMS platform with a high lipid coverage and annotation confidence for untargeted in vitro cell-based experiments.

APPROACH

Drift tube ion mobility

Acquisition

Single pulse vs 4-bit multiplexing

Trapping filling and release times

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Maximizing sensitivity

Box-Behnken design

1. Drift entrance voltage (DEV),
2. Drift exit voltage (DXV),
3. Rear funnel entrance (RFE) voltage,
4. Rear funnel exit (RFX) voltage

Liquid chromatography

RPLC screening

1. Kinetex XB-C18
2. Acquity HSS T3 C18
3. Acquity BEH C18

↓

Step-wise optimization

Stationary phase > Mobile phase pH and modifier > Additional parameters (temperature, gradient, flow)

Proof-of-concept

Liver cell extracts (HepaRG)

Lipid profiling

Liquid-liquid extraction ($MeOH/H_2O/CHCl_3$, 3/2/2)

ESI (+) and (-) modes

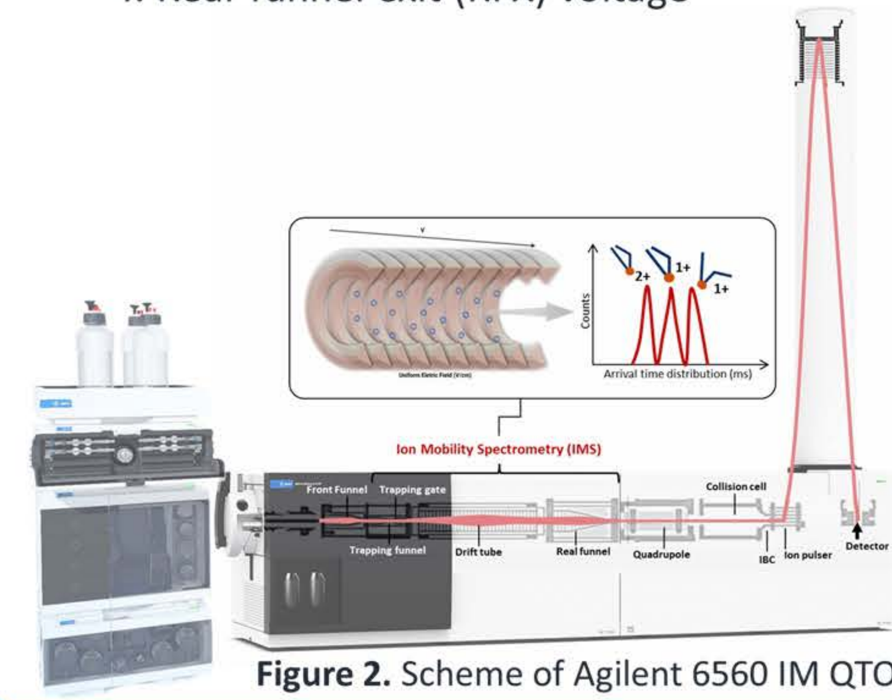

Figure 2. Scheme of Agilent 6560 IM QTOF.

RESULTS AND DISCUSSIONS

RPLC METHOD

Using the ACQUITY UPLC BEH C18 (2.1 × 150 mm, 1.7 µm), all panel lipid standards could be detected with an excellent peak shape.

- FWHM <0.2, tailing factor <2 and >0.8, and no elution close to t_0

Separation of *sn*-positional isomers

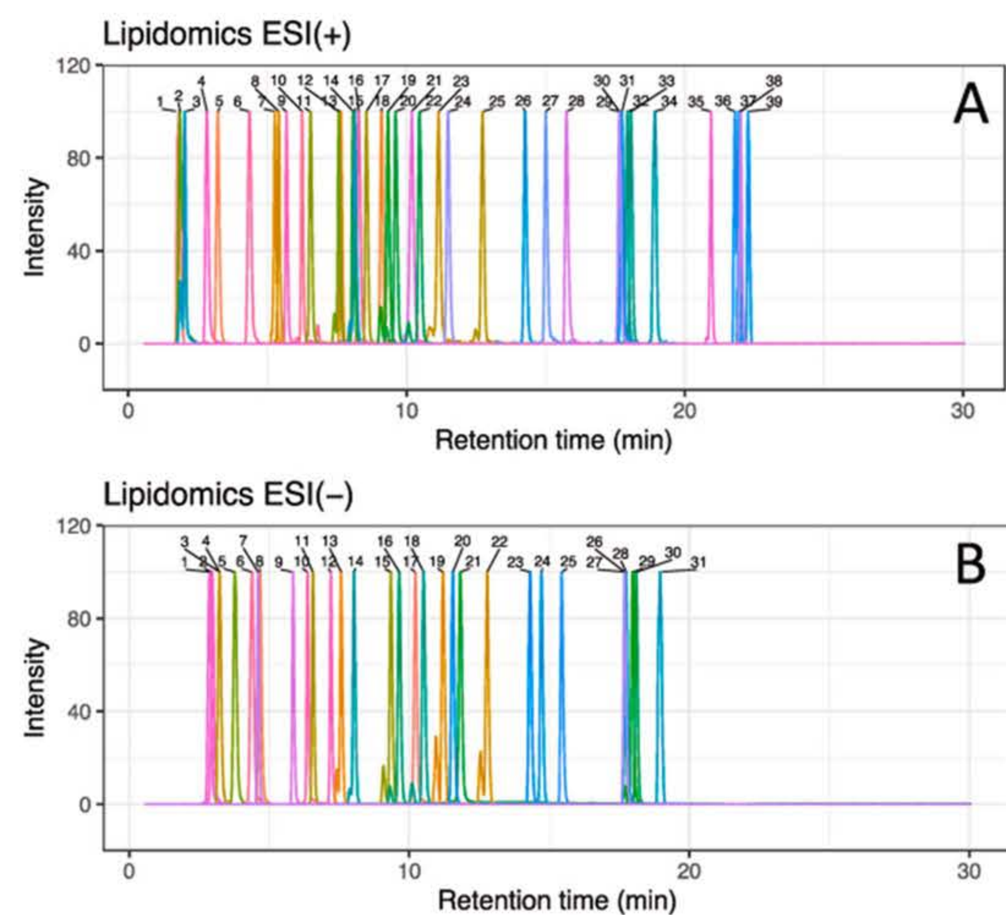

Figure 3. Chromatographic separation of panel lipid standards in positive (A) and negative (B) ionization modes.

IM OPTIMIZATION

The chemometrics approach (BBD design and desirability) provided optimal voltages for improving sensitivity taking into consideration detector saturation.

Table 1. Optimized drift tube parameters.

ESI	DEV	DXV	RFE	RFX
+	1221	300	200	49
-	-1273	-300	-216	-47

Trap filling time

Sensitivity increases with longer trap filling in single pulse mode ($p < 0.05$, ANOVA). No difference using 4-bit Hadamard multiplexing mode or by increasing trap release time.

All ions fragmentation

Data independent acquisition

Low and high-energy IM frames

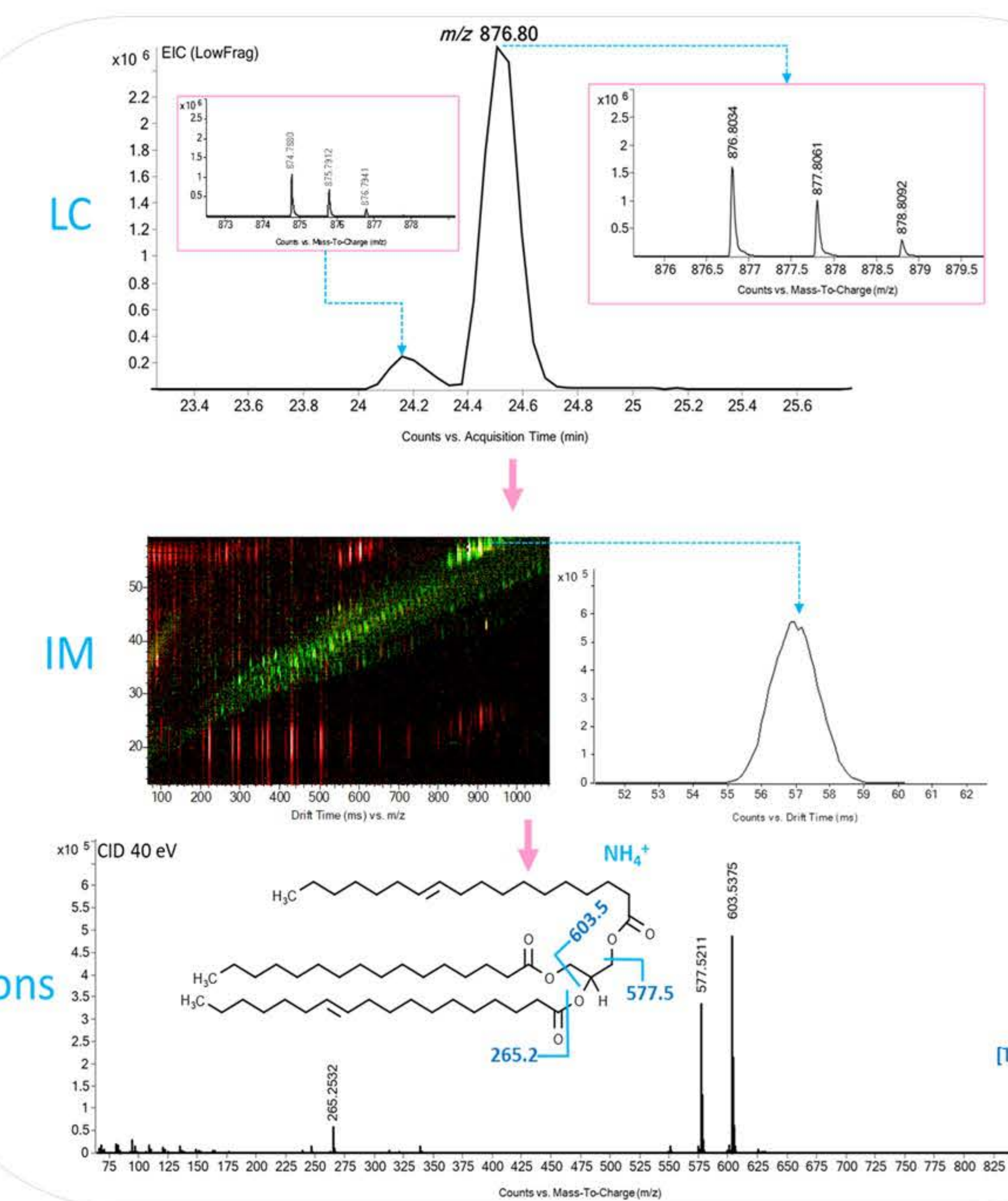

Figure 4. Three-dimensional separation strategy to annotate lipids in HepaRG extracts.

LIPID ANNOTATION

Annotation of lipid species with high confidence through MS-DIAL and manual confirmation.

- Accurate mass, isotopic pattern, MS/MS, CCS error < 3%.
- 169 lipid species were annotated in ESI (−) and 267 in ESI (+)

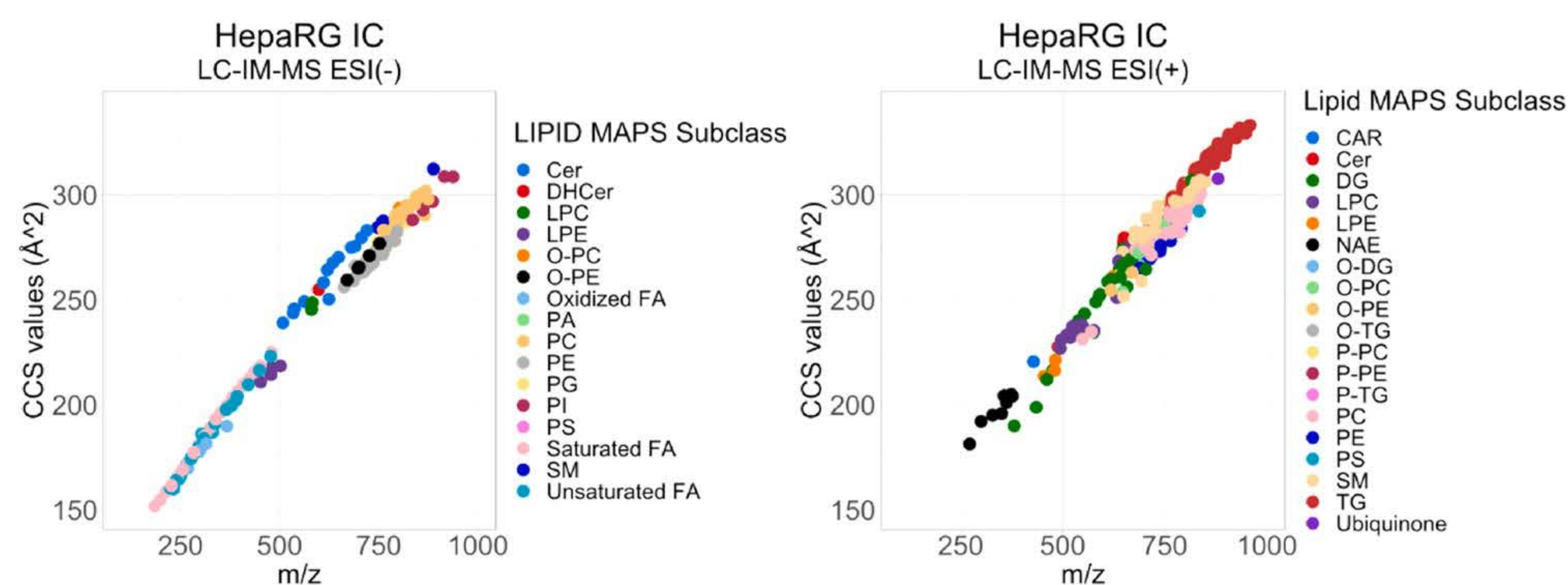

Figure 5. CCS-m/z trendlines of lipid species annotated in intracellular extracts of HepaRG cells.

FUTURE PERSPECTIVES

Explore the potential to separations isomers using high resolution IM approaches to improve peak capacity.

Preliminary results show that the separation of different types of isomers (e.g., chiral, constitutional) is highly dependent on the structure and achievable at a resolution > 150

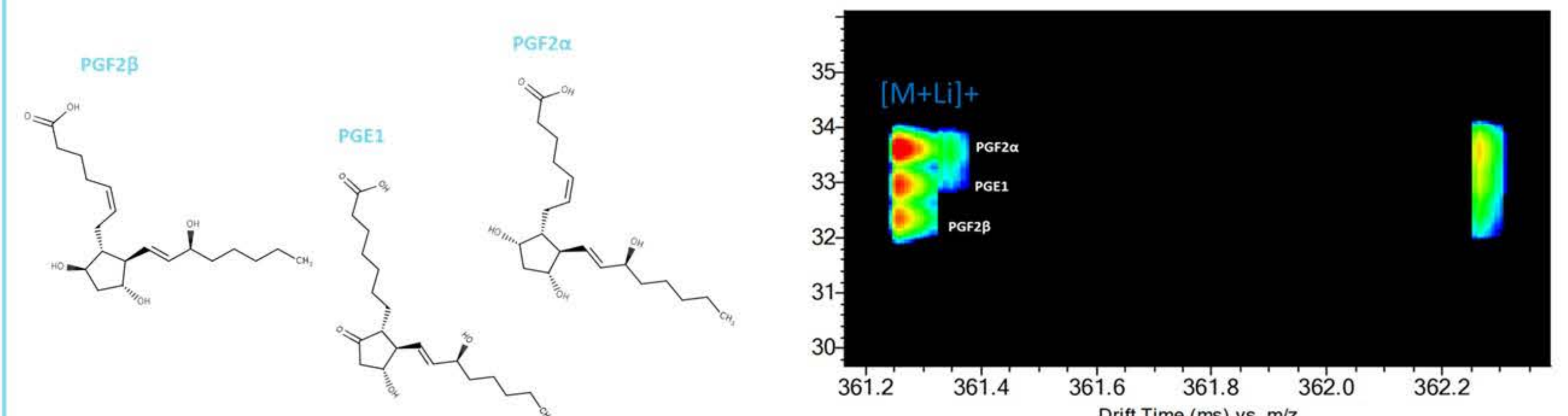

Figure 6. IM spectrum of a mixture of prostaglandins dissolved in 1mM Li+ in 10/90 H_2O/IPA

CONCLUSIONS

LC-IM-MS can be used for lipidomics profiling in complex samples, but data processing is still very time consuming

The BEH C18 column provided satisfactory results in terms of lipid coverage and its ability to separate critical pairs

The current resolution of DTIM (~40-60) does not allow comprehensive separation of isomeric lipids but provides class-based separation and obtention of cleaner MS2 spectra

Dynamic range can be affected by the acquisition and trap filling time.

ACKNOWLEDGMENTS

KMS: BOF DOCPRO 4

EI: FWO-1161620N

More Information?

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