Optimization of a Liquid Chromatography-Ion Mobility-Mass Spectrometry Platform in Untargeted Lipidomics Exploring Ion Mobility Sensitivity

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Introduction

Analytical methods providing high lipidome coverage and high feature quality are vital to ensure comprehensive profiling and reliable structural elucidation. Implementation of drift tube ion mobility spectrometry (DTIMS) in liquid chromatography-high resolution mass spectrometry platforms has the ability to increase peak capacity and confidence during annotation by providing collision cross section (CCS) values for the analytes under investigation, but may result in reduced sensitivity due to impaired duty cycles.

Objective

Development of a LC-DTIM-QToF-MS platform with high coverage and sensitivity for lipids

Approach

50 panel standards

Wide range of metabolic classes including **fatty acyls**, **glycerolipids**, **glycerophospholipids**, **prenol lipids**, **sphingolipids** and **sterol lipids.**

LC optimization

Column screening

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

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Step-wise optimization

Order of effects

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

Evaluation

Peak shape (FWHM, tailing factor)
Intensity (S/N)
Retention factor

DTIM optimization

Maximizing sensitivity

Box-Behnken design combined with maximized desirability function

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

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Single pulse vs. 4-bit multiplexing

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Evaluation of trap filling and trap release times

Proof-of-concept: analysis of human plasma and HepaRG extracts

Results and Discussions

LC optimization

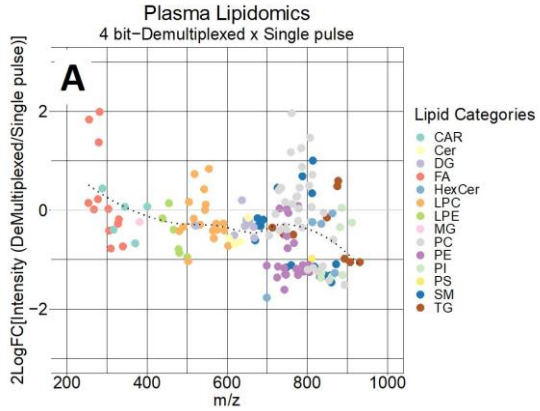
- Column screening was used to select the column for future optimization.
- All 50 **panel standards** could be detected and separated by the optimized methods.
- Separation of *sn*-positional isomers.
- Biological samples: **3912** features (HepaRG), **2855** features (plasma)

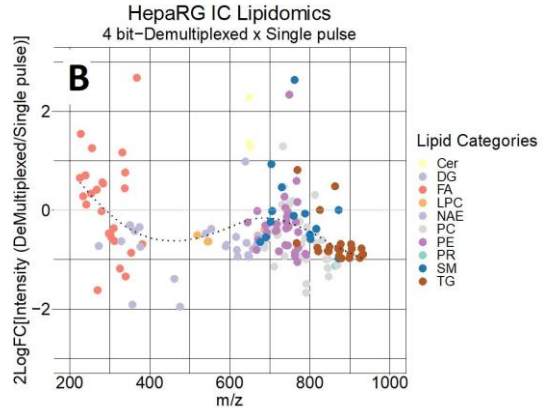
DTIM optimization

- Maximized desirability function, which provided optimal voltages in the scope of **maximal sensitivity** for lipidomics.

- Sensitivity increases with **longer trap filling** in single pulse mode ($p < 0.05$, ANOVA).
- Sensitivity is not significantly altered by increasing trap filling time in 4-bit Hadamard multiplexing mode or by increasing trap release time.

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





- **Annotation** of lipid species with **high confidence** through MS-DIAL and manual confirmation.
  - Accurate mass, isotopic pattern, MS/MS, CCS error < 3%.
  - Biological samples: **170** lipid species (HepaRG), **162** lipid species (plasma).

DTIM improves separation capacity and enables a more reliable structural elucidation