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

**50 panel standards**

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

**LC optimization**

Column screening

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

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

Single pulse vs. 4-bit multiplexing

Evaluation of trap filling and trap release times

**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.

**Application**

- 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.