High-throughput UHPLC-TIMS-based lipidomics: Sub-5 min screening of plasma, cells, stool and tissues

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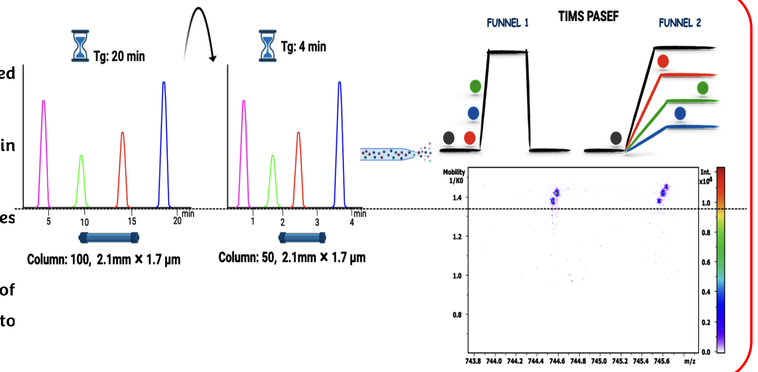
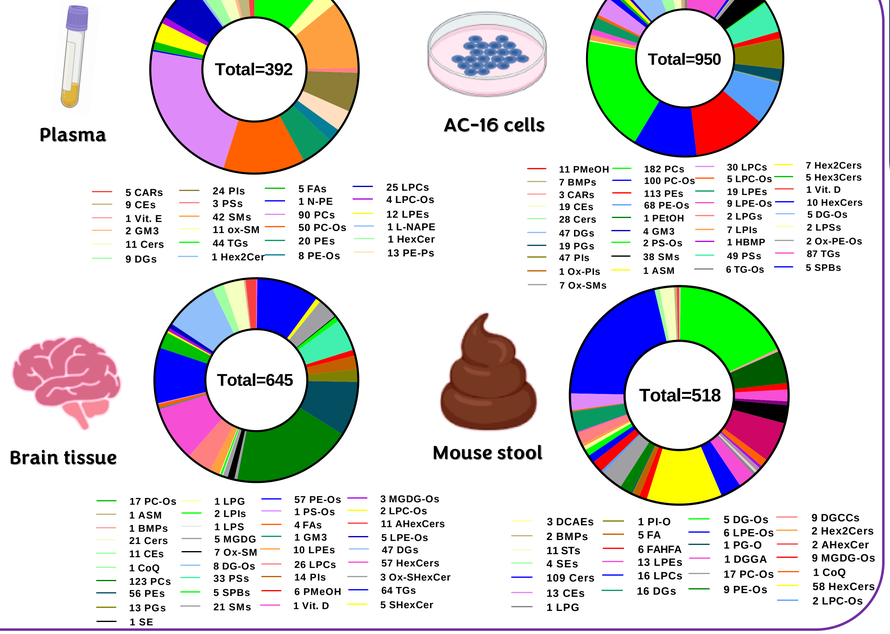
Introduction

- In the last years ion mobility mass spectrometry has emerged as an interesting alternative to perform lipid profiling.
- Reversed-phase UHPLC-MS represents the golden standard in untargeted lipidomics.
- Conventional methods are characterized by long analyses times that are not suitable for large cohort screening.
- In this contribution we evaluated the implementation of trapped ion mobility mass spectrometry (TIMS) as tool to reduce analysis time while keeping accuracy and coverage.



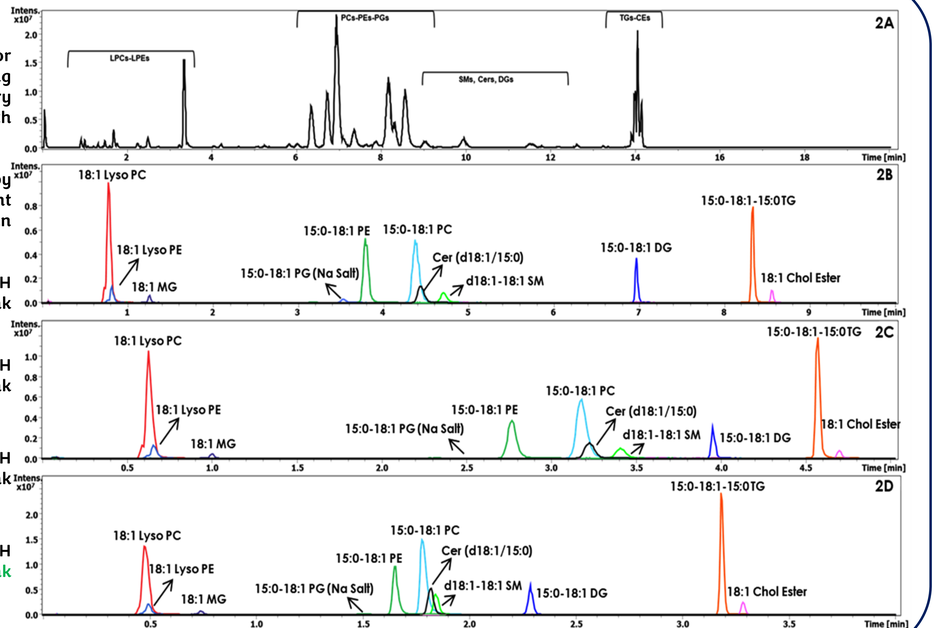
Results

- The developed platform was first challenged against human plasma extract.
- Following careful manual curation, the method showed average MS/MS scores, Δppm and ΔCCS (Å²) errors of: 876.5, -0.57 and 1.47, respectively.
- We evaluated the coefficient of variation (CV%) of retention times and CCS values for more than 800 lipids over 6 consecutive days achieving average values of 0.54 % and 0.34 %, respectively.
- The method was extended to different biological specimens, such as AC-16 cell lines, mouse stool and brain homogenates, with 950, 518 and 645 lipids monitored respectively, and covering more than 30 subclasses.



Methods

- We started from popular condition for untargeted lipidomics, such as using charged surface hybrid stationary phase (CSH) and 20 minutes length gradients.
- The analysis time was scaled-down by reducing column (5 cm) and gradient length to 4 minutes, comprising column re-equilibration.
- 2A) Flow: 0.4 mL/min; Column: CSH C18 10 cm; Run time: 20 min; Peak Capacity: 65.57.
- 2B) Flow: 0.4 mL/min; Column: CSH C18 5 cm; Run time: 10 min; Peak Capacity: 38.58.
- 2C) Flow: 0.5 mL/min; Column: CSH C18 5 cm; Run Time: 5 min; Peak capacity: 24.45.
- 2D) Flow: 0.55 mL/min; Column: CSH C18 5 cm; Run time: 4 min; Peak Capacity: 28.73.



Conclusions

- The 4 min UHPLC-TIMS-MS method has been applied to investigate the plasma lipid profiles of a small cohort of amyothrophic lateral sclerosis patients (ALS, n = 15) and healthy controls (CTRL, n = 15).
- Principal component analysis (PCA) build on lipidomics profile is shown in Fig. 4A.
- The most perturbed and statistically significant lipid subclass was represented by ether-linked lipids. As can be observed from heatmap in Fig. 4B, ALS patients were characterized by decreased concentrations of numerous PC-Os and PE-Ps, while several LPC-Os were found increased. This could suggest an extensive remodeling of glycerophospholipids metabolism, that plays a key role in membrane homeostasis.