Integrating shotgun lipidomics and HPLC separation of lipids into a new platform for lipid analysis

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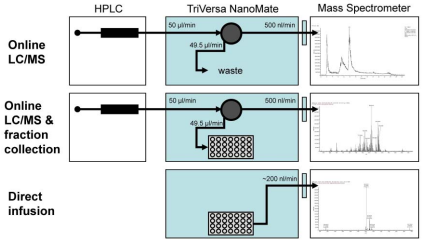
Summary

- Development of a lipidomics platform based on direct infusion and LC MS analysis
- LC MS analysis by normal phase chromatography and nanoflow split to a chip-based infusion device
- Implementation of a fraction collection strategy for in-depth profiling of lipid species by direct infusion analysis
- This platform is a powerful tool for arraying the lipidome of poorly characterised organisms

Methodology

Overview



HPLC: Lipid extracts were analyzed by a Dionex LC system equipped with a FAMOS autosampler. Samples were kept at 10°C and injection volume was 0.5-1 µl. Chromatographic separation of lipids was achieved using a normal phase YMC-Pack PVA-Sil column (150x1.0 mm) and a ternary gradient composed of A: Hexane/IPA (98/2); B: MTBE/IPA (45/55); C: MeOH; where B and C contained 5 mM AA. The flow rate was 50 µL/min.

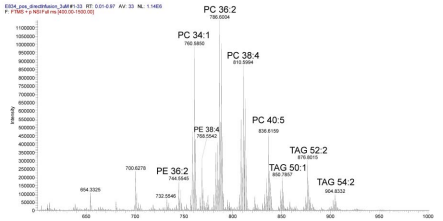
Ion source/Fraction collector: NanoMate Triversa Sample analysis was performed by LC-coupling/fraction collection mode using a post-column split where 50 nl/min was directed to the ESI-chip and 49.5 µl/min was use for parallel fraction collection. The LC-spray coupler was set at ±1.7 kV. Direct infusion was done using ±0.96 kV and 0.6-1.25 psi.

MS instrumentations: LTQ Orbitrap XL

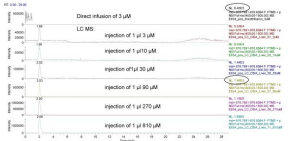
Method development

Comparison of direct infusion and LC MS analysis

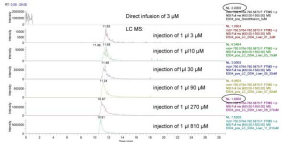
Direct infusion of 3 µM liver lipid extract, +FT MS spectrum:



XIC of **TAG** 52:2 *m/z* 876.8014:

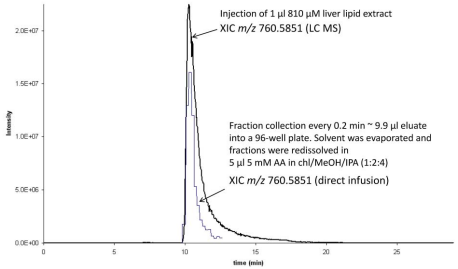


XIC of **PC** 34:1 *m/z* 760.5851:



→ direct infusion analysis is more sensitive than the LC MS analysis
→ the LC system has a high loading capacity and is reproducible

Accurate fraction collection and parallel LC MS analysis using the NanoMate Triversa



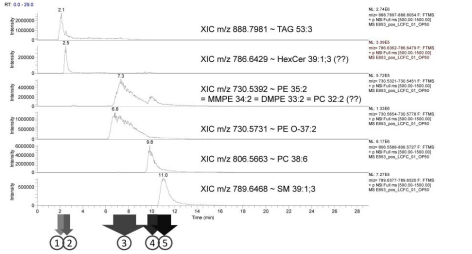
→ Fraction collection can be used for accurate isolation of lipids

Application

- **AIM:** characterise the molecular lipid composition of *Caenorhabditis elegans*
- *C. elegans* is a powerful model organism for genetic studies of lipid function. The lipidome is relatively complex due its complex lipid metabolic network and the uptake of *E. coli* lipids with odd-chain fatty acid moieties
- To array the *C. elegans* lipidome we analysed lipid extracts by LC MS combined with lipid fractionation and direct infusion analysis

LC MS analysis/fractionation of C. *elegans* lipid extracts

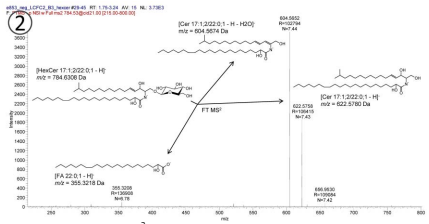
XIC of selected lipid precursors, +FT MS analysis (R = 100,000):



Lipid fractions → direct infusion and tandem mass analysis

Lipid identification

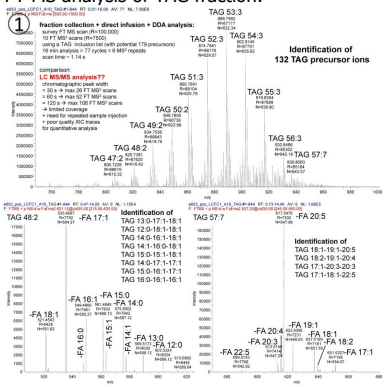
m/z 786.6429 is a hexosylceramide:



Polarity shift: -FT MS^2 analysis of the lipid fraction

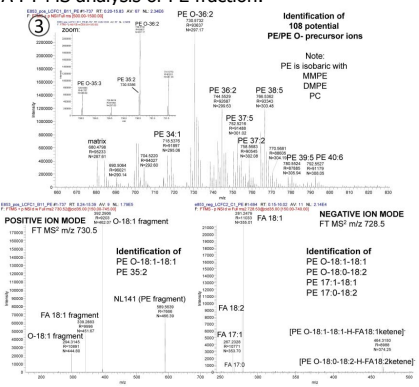
Profiling the molecular composition of TAG species

+DDA FT MS analysis of TAG fraction:



Profiling the molecular composition of PE species

±DDA FT MS analysis of PE fraction:



→ fraction collection enables accurate characterisation of lipid fractions by DDA analysis (~30 min stable ion spray)