Monolithic Column for Improved 4D-Lipidomics Analysis

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Introduction

Large-scale lipidomics studies are often performed by LC-MS/MS to identify and quantify as many lipids as possible and thus associate them with physiological processes and diseases. Lipids exhibit large structural diversity and dynamic range of concentrations. Capturing low abundance lipids in the presence of higher abundant species requires efficient and reproducible chromatography as well as a sensitive high-resolution MS. Most lipidomics experiments are performed using analytical flow, which suffers from dilution effects and high backpressure when using high flow rates. Monolithic columns provide a unique skeletal stationary phase structure that reduces backpressure for viscous mobile phases such as isopropanol enabling improved efficiency for nanoflow LC lipidomics.

Methods

A comprehensive investigation was conducted based on the evaluation of lipid extraction methods, LC mobile phase compositions, columns for both analytical and nano flow LC with particle-based and monolithic columns. The analytical

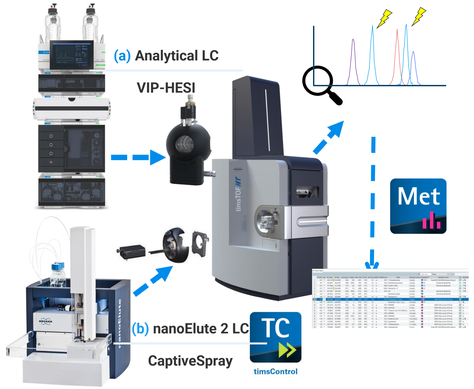

Figure 1. Schemes illustrates (a) analytical LC-VIP-HESI-timsTOF HT; (b) nanoLC-Captive Spray-timsTOF HT

(a) YMC (2.1 mm x 100 mm, 1.9 µm), (b) Bruker Bio-LP (2.1 mm x 100mm, 1.9µm), and (c) Acquity BEH C18 (1mm x 150mm, 1.7 µm) were assessed. The monolithic analytical and nanoLC columns were acquired from Kyoto Monotech (analytical C8 at 1.0mm x 150mm, 11nm; nanoLC C18 at 75 µm x 150mm, 1.5µm). Agilent 1290 Infinity II UHPLC with VIP-HESI source were used for analytical workflow; nanoElute 2 with CaptiveSpray source was applied for nanoLC workflow. Both were interfaced with Bruker timsTOF HT mass spectrometry system (Figure 1).

The modified mobile phases were freshly prepared with 2:1:1 H2O:IPA:ACN with 0.05% acetic acid, 20 µM phosphoric acid and 5 mM ammonium acetate in channel A (MP-A), 50:50 isopropanol/ACN with 0.05% acetic avid and 5 mM ammonium acetate in channel B (MP-B). The standard analytical LC was optimized at 400 µL/min or 150 µL/min for 1.0 mm i.d. monolithic column, and 1.85 µL/min for nanoLC column over 15 min gradient.

NIST SRM-1950 extract was spiked with Avanti EquiSplash internal standards from two different lipid extraction methods. (a) MTBE extraction: 225 µL of cold methanol was added into 30 µL of NIST SRM 1950 human plasma, vortexed for 10 s, 750 µL of methyl tertiary-butyl ether (MTBE) was added, vortexed for 10s, shaken for 60 min at room temperature; 190 µL of water was added, vortexed for 10s; centrifuge for 5 min at 1000 g; collect all supernatant or aliquots from the non-polar layer; evaporate to dry in a SpeedVac; the dried extract was resuspended using 300 µL of mixture of methanol/dichlormethane (9:1). (b) IPA extraction: 30 µL of NIST SRM 1950 human plasma was aliquoted into 30 µL of water, mix for 5 min at 1400 rpm; add 240 µL of IPA and mix well at 2-8^{0}C for 2 hours, centrifuge at 4^{0}C at 10,000rpm for 10 min; transfer 200 µL of the supernatant to the analytical vial for injection. 2 µL injection volumne for analytical LC and 1 µL for nanoLC. Data was analyzed in DataAnalysis 6.2 and MetaboScape 2025b. Both rule-based lipid species annotation and MS/MS spectral library annotation were applied for unknow lipid identfication. The results from different lipid extraction methods, different mobile phase compositions, analytical LC columns, different LC workflows were compared based on Bruker ion mobility ON with LC-timsTOF HT DDA-PASEF lipidomics workflow.

Results

Current MTBE lipid extraction method involves multiple steps, manual supernatant transfer, drying and reconstituting steps which are time consuming. The IPA lipid extraction method was found more straightforward and easier to operate while keep the same sample dilution time as in MTBE method. It was also noticed the lipid annotation numbers increased by 6.75% using the new IPA extraction; About 16.9% more lipids were annotated when using the modified mobile phases with YMC LC column which was also confirmed by using the monolithic analytical LC column.

IPA was used in mobile phase preparation for comprehensive lipidomic profiling due to its unique properties to solubilize both polar and non-polar lipids and facilitate the separation and detection of a wide range of lipids, Because its high viscosity, significant high backpressure were noticed even at high column temperature which limits sample analysis throughput on both reversed-phase LC and nanoLC methods. Monolithic column is a single piece of high porous materials offer high permeability and low backpressure enabling faster separation at higher flow rates, it also helps to reduce possible column clogging. Several regular analytical LC columns and the nanoLC monolithic columns were evaluated for the lipid profiling (Figure 2). The backpressure on the packed monolithic columns were significantly reduced even for the nanoLC at a much high flow-rate at 1.85 µL/min achieving 15-min run time at maximum backpressure of ~550 bar which indicates the monolithic column would be well suited for microflow and nanoLC application. The annotated lipid results

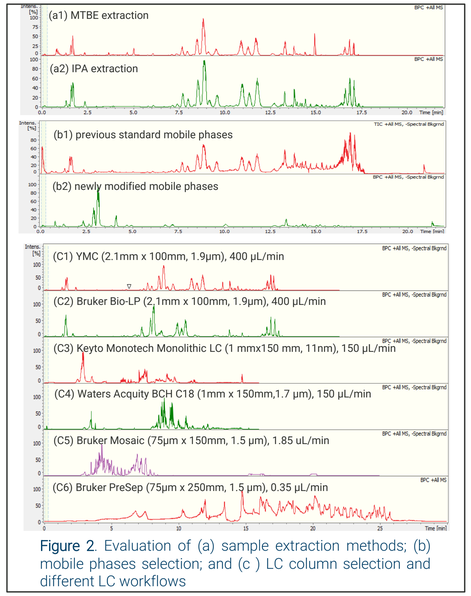

Figure 2. Evaluation of (a) sample extraction methods; (b) mobile phases selection; and (c) LC column selection and different LC workflows

based on different columns were carefully assessed using same feature finding parameters and the narrow and wide windows for mass accuracy <2.0 ppm to <10.0 ppm, isotope fidelity mSigma <25 to <1000, MS/MS cosine similarity >900 to >600, and CCS <1.0% to <3.0%. The spiked internal calibration in each sample as a typical 4D-Lipidomics experiment analysis showed good reproducibility between injections with best lipid annotations found from nanoLC monolithic column. The Kendrick Mass Defect plots were used to easily visualize lipid classes across the samples (Figure 3).

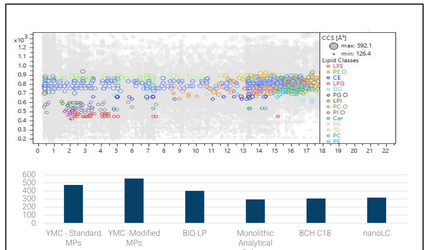

Figure 3. 4D-lipidomics feature finding and annotation for lipid identification on different columns and workflows

Summary

1. Both analytical and nanoLC analytical workflows have been proved monolithic columns are effective tool for high throughput lipidomics sample analysis.
2. The modified IPA sample lipid extraction method was proved to be an alternative fast and easy to operate lipid extraction procedure.

Conclusion

- IPA lipid extraction was found to be an easy to operate, fast and efficient method for lipidomics analysis.
- Results between analytical LC and nanoLC methods were evaluated for best lipid profiling.
- Monolthic nanoLC column was proved to be a high-throughput lipidomics application workflow.

Lipidomics on timsTOF HT

X.P., B.W., M.K., R.M., and E.F. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.