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nano-lipidomics employing silica based monolithic columns

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Nano-lipidomics for improved sensitivity

Nano-liquid chromatography is the state of the art in the proteomics field, yet lipidomics applications that make use of the vastly increased efficiency of electrospray ionization at sub-µL flow rates are still considered exotic. A handful of recent studies have shown that additional experimental efforts that are commonly associated with chromatographic downsizing are vastly outmatched by the gain of sensitivity and concomitant achieved lipid species coverage. Considering the dynamic concentration range of the lipidome which is spanning from the pico- to the millimolar range, it is obvious that analysis of low abundant lipids will greatly benefit from improved sensitivity. Also, single-cell lipidomics is an upcoming application that will greatly benefit from employing nanoscale liquid chromatography.

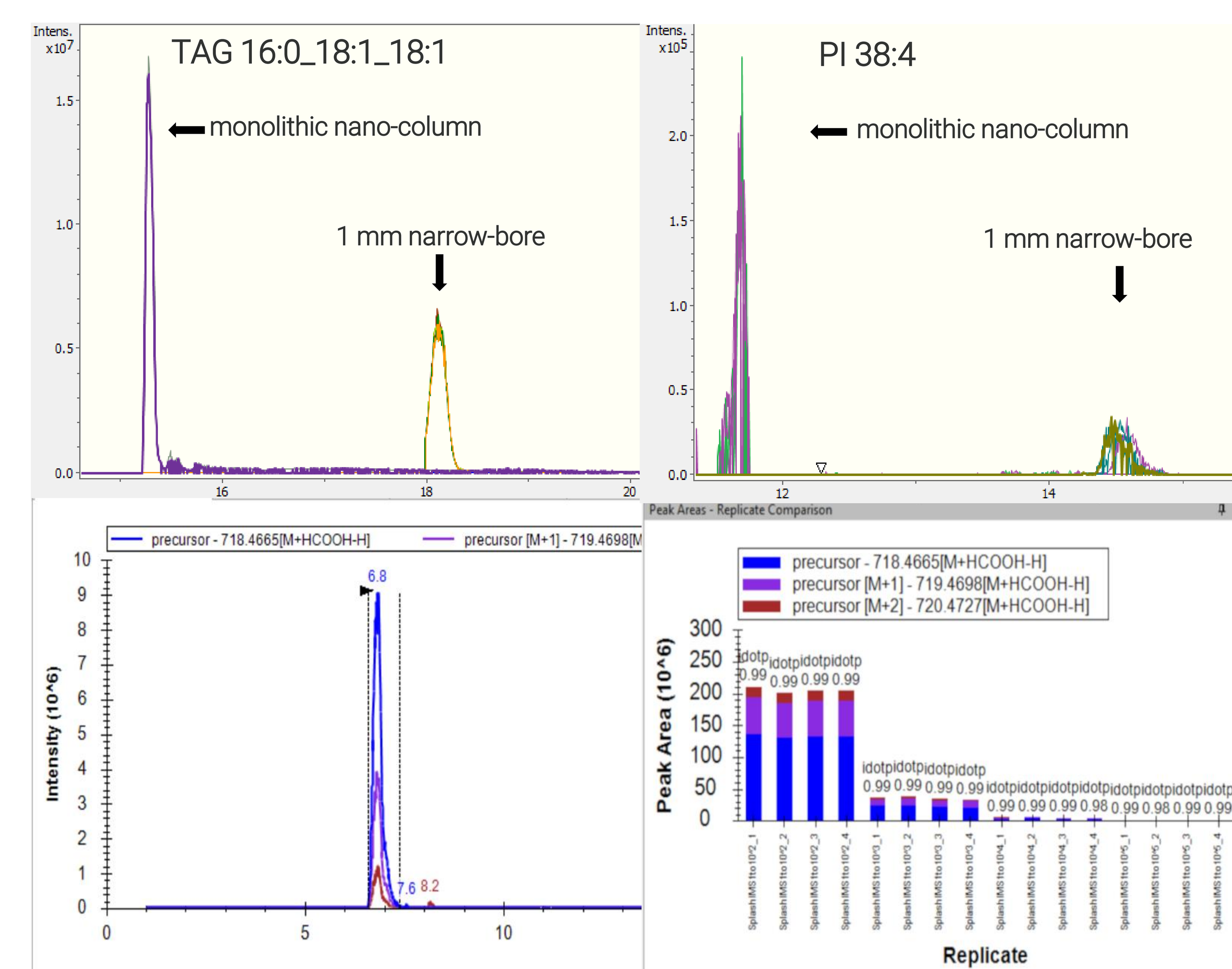

Fig. 1: Top: Significant sensitivity gains are achieved in plasma by employing nano-lipidomics, especially for low abundant lipid species. Bottom half: internal PI standard diluted over 4 orders of magnitude

Methods

Nano-lipidomics analysis on a novel silica monolithic column (C18, 75 µm, 150 mm length) employing a timsTOF Pro was compared to a conventional microbore setup (ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 1 mm X 150 mm). Folch extracts of NIST 1950 plasma were subjected to PASEF lipidomics analysis. Samples were analyzed in both polarities utilizing either the Bruker VIP-HESI source or the captive spray source employing the 4D-lipidomics PASEF method. Lipidomes were annotated employing the lipid annotation tool in Metaboscape 2023.

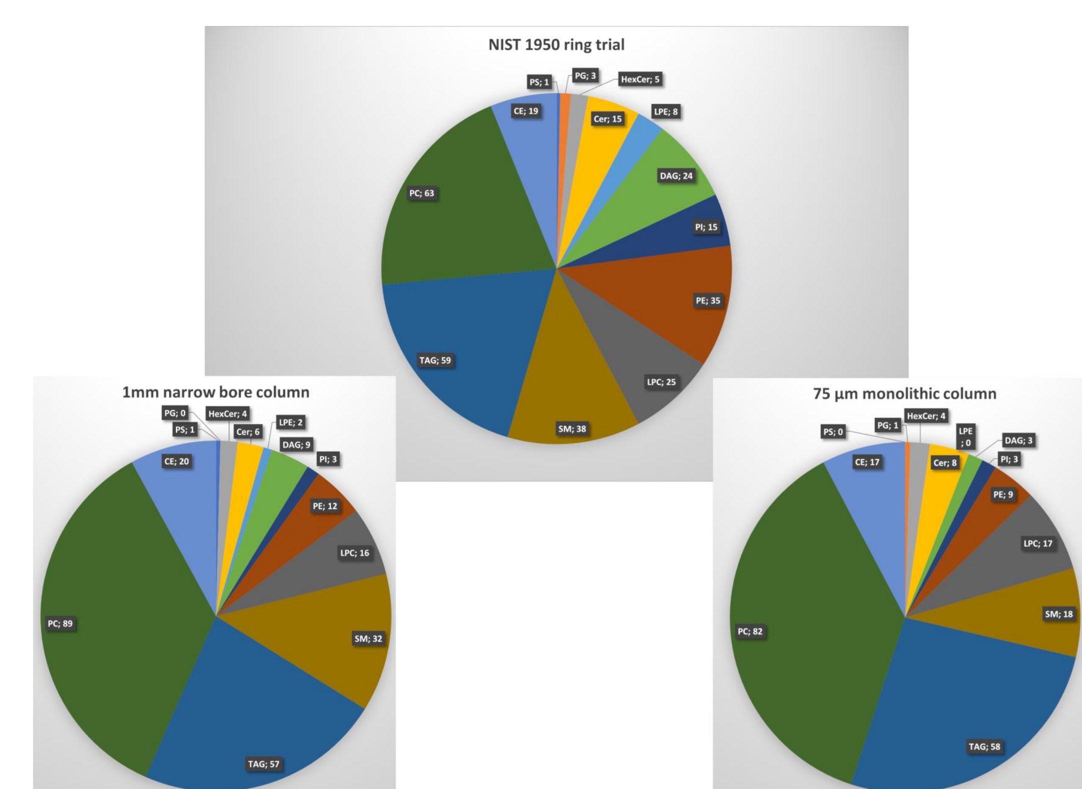

Fig. 2: Lipid species sorted by classes identified in NIST 1950 ring trial paper[1] and in this study on narrow bore and monolithic nano column.

Results

- Nano-lipidomics analysis of NIST1950 plasma and internal standard dilutions on a novel monolithic nano-reversed phase column.
- Equal amounts of lipid injected, comparable 25 min gradients, (25 min, 10 mM $NH_4(HCOO)$, 60% ACN → 90 % IPA/10 %ACN)
- Narrow-bore (Acquity BEH C18, 1 x 150 mm, 1.7 µm) vs. monolithic nano (C18, silica based, 0.075 x 150 mm).
- Significantly lower LOD on monolithic nano-column
- Low backpressures generated by monolith (< 400 bar) despite the highly viscous solvent enables further improvement of throughput.

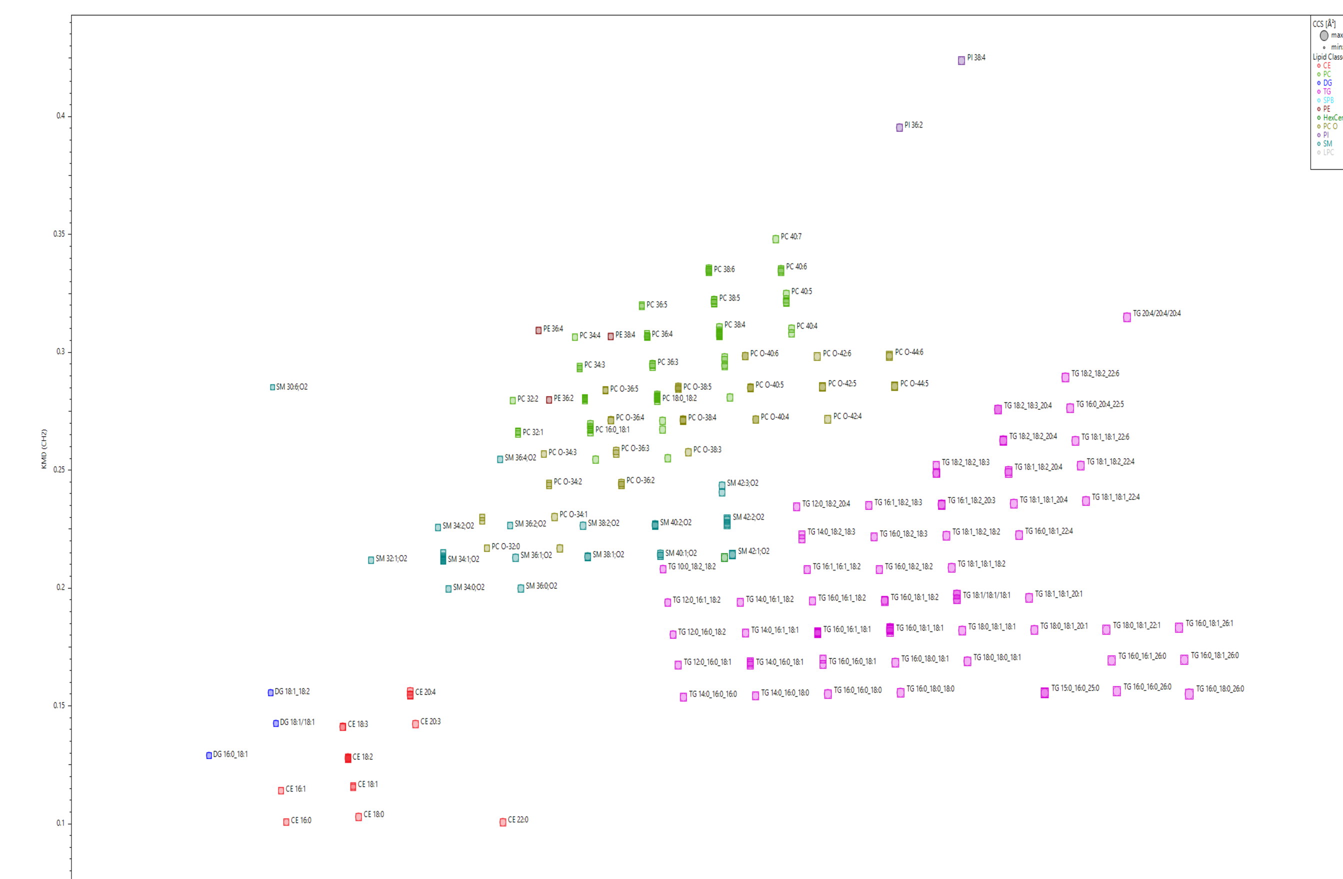

Fig. 3: Kendrick mass defect plot of NIST 1950 plasma lipids identified employing the monolithic nano-column (blue)

Equal amounts of each extract were injected in 4 technical replicates on both front ends from identical vials. Both setups showed comparable chromatographic performance and quantitative accuracy in the upper concentration range. As expected, peak areas were on average higher on the nano-lipidomics setup and hence data quality at low concentrations was significantly improved (Fig. 1).

Distribution of the identified lipid species matched well between the narrow-bore and the nano-lipidomics setup and also (for classes covered by our rule based approach) with a ring trial conducted on NIST1950 plasma extract.

Sensitivity was also tested by diluting internal standards in the extracts. At the lowest tested concentration (femtomol range OC) for most lipid species M+2 was at or below the LOD on the microbore setup, leading to a dropoff of idotp score while the nano-lipidomics setup yielded consistent values > 0.9. The most pronounced effect was observed for PI 28:2 where even the monoisotopic peak was below LOD on the microbore setup whereas in the nano-lipidomic setup the M+2 peak was readily detectable and the overall isotopic pattern was scored at 0.95.

Summary

Straight forward transfer of existing analytical flow applications to nano-lipidomics

Captive-spray prov stable nano-spray in negative polarzitation

Greatly improved sensitivity: The combination of PASEF and nano-chromatography yields LOD of most lipid species in the low fmol on column range

Comparable coverage of lipid classes to state of the art narrow bore system

Comparable retention time and quantitative reproducibility

Improved chromatographic peak shapes with the possibility to further improve throughput or resolution due to low backpressure of the monolithic column

1. Bowden, J.A., Heckert, et al., 2017. Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950–Metabolites in Frozen Human Plasma[S]. J. Lipid Res. 58, 2275–2288. https://doi.org/10.1194/jlr.M079012.

Conclusion

- Monolithic reversed phase column nano-lipidomics approach
- Very good lipid species coverage
- Improved LOD for low abundant lipid species

Technology

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