Lipidomics

Nano flow lipidomics: sensitivity, reliability, and practical implementation

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Abstract

Introduction: Untargeted lipidomics is typically performed using high flow liquid chromatography, which requires more sample and solvent and may limit sensitivity. Nano flow separations can improve electrospray ionization efficiency, reduce solvent consumption, and enable lower on column loads, but adoption in lipidomics has been limited by concerns around robustness, column availability, and method complexity. This work evaluates the performance benefits of nano flow lipidomics compared to high flow and highlights practical considerations for routine implementation.

Methods: Bovine liver total lipid extract spiked with SPLASH internal standards was analyzed using nano flow and high flow liquid chromatography coupled to a Thermo Scientific™ Orbitrap™ Excedion™ Hybrid Mass Spectrometer. Full scan MS¹ polarity switching was used for untargeted profiling, and the Thermo Scientific™ AcquireX™ Deep Scan workflow was applied to increase data-dependent MS/MS coverage. A SPLASH dilution series was analyzed in triplicate to evaluate quantitative performance and limits of quantitation.

Results: Nano flow lipidomics increased lipid annotation depth at lower on column loads compared with high flow analysis and produced more high confidence lipid annotations after class-specific filtering. Nano flow conditions also reduced unintentional MS¹ in-source fragmentation across multiple lipid classes and improved quantitative performance across the replicated SPLASH dilution series. Overall, nano flow improved sensitivity and reliability for untargeted lipidomics while reducing sample and solvent requirements.

Introduction

Lipids play key roles in membrane structure, cellular signaling, and energy storage, and altered lipid metabolism is linked to diseases including neurodegeneration, diabetes, and cancer. LC-MS with high resolution accurate mass (HRAM) mass spectrometry is widely used for untargeted lipidomics due to its broad coverage and sensitivity, but conventional analytical flow methods require more sample and solvent and may limit sensitivity. Nano flow separations can improve ionization efficiency, reduce on column load and solvent consumption, and increase lipid annotations, but require optimization for robust routine use.

Materials and methods

Sample Preparation

Bovine liver total lipid extract and SPLASH Lipidomix internal standards were diluted in isopropanol (50:50, v/v). On column loads were 25 ng and 5 ng for nano flow analyses and 100 ng for high flow analyses. A SPLASH Lipidomix dilution series from 10% to 0.001% was analyzed in triplicate.

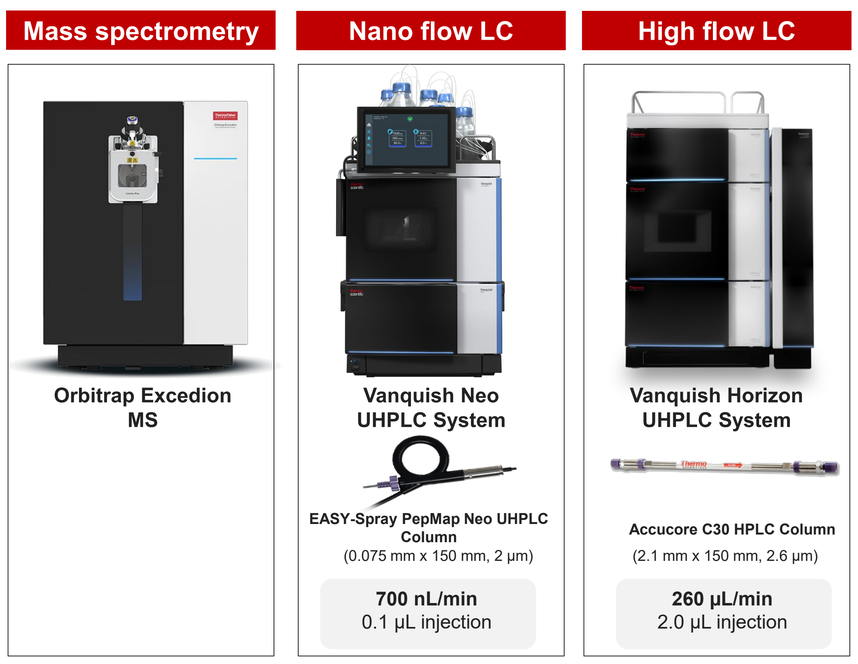
Methods

Nano flow LC was performed using a Thermo Scientific™ Vanquish™ Neo UHPLC System with a Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC Column. High flow LC was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC System with a Thermo Scientific™ Accucore™ C30 HPLC Column. Both methods used the same mobile phases and were coupled to an Orbitrap Excedion Mass Spectrometer with MS¹ polarity switching and AcquireX Deep Scan data-dependent MS/MS.

Data Analysis

Bovine liver extract data were processed in Thermo Scientific™ LipidSearch™ Software using consistent workflows for both flow regimes. Lipid peak areas were class-normalized to SPLASH internal standards and filtered by expected main ions and LipidSearch Software grades A, B, or C. SPLASH dilution data were evaluated in Thermo Scientific™ TraceFinder™ Software to determine limit of quantitation (LOQ).

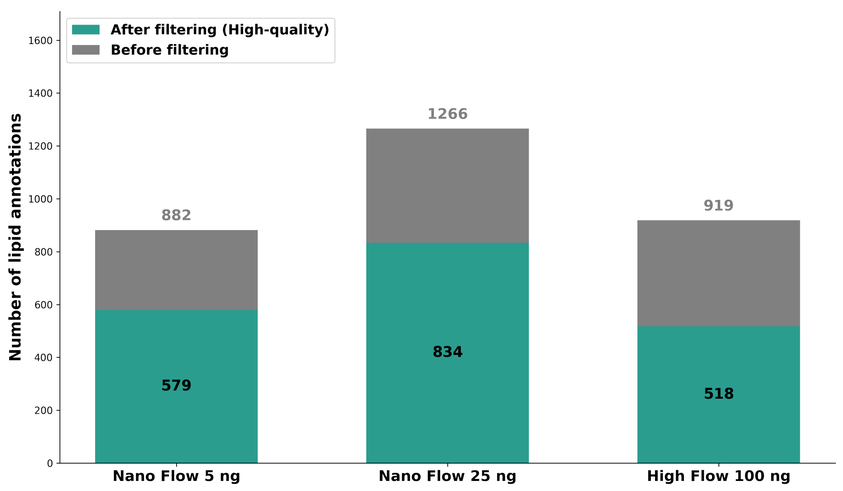
Figure 1. Instrumentation and LC-MS workflow for nano flow and high flow untargeted lipidomics



Results

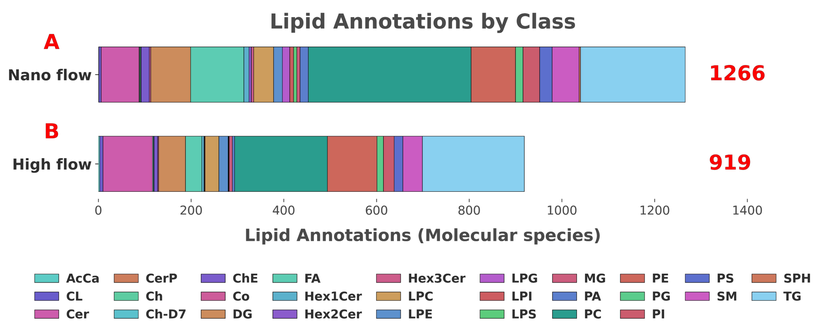
Lipid annotations were higher with nano flow despite substantially lower on column loads (Figure 2). At 25 ng, nano flow yielded 1266 total lipid annotations and 834 high-confidence annotations after class-specific filtering, compared with 919 total and 518 high-confidence annotations for high flow at 100 ng. Even at 5 ng, nano flow produced 579 high-confidence annotations, comparable to high flow at 100 ng, demonstrating improved sensitivity under low-loading conditions.

Figure 2. Total annotations before and after filtering for high-quality lipids across column loads for nano flow and high flow LC–MS



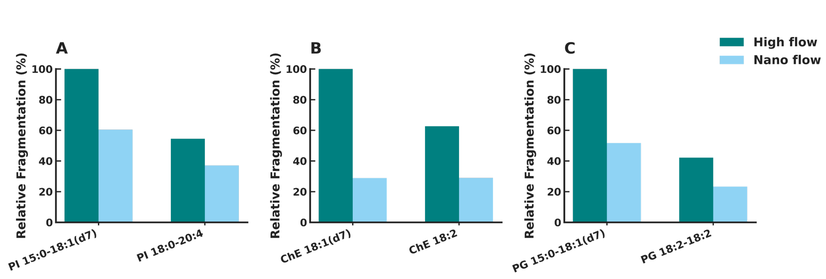
Nano flow expanded lipidome coverage while preserving a similar overall class distribution compared with high flow (Figure 3). At 25 ng, nano flow detected additional lipid classes not observed with high flow, including ceramide-1-phosphate (CerP), sphingosine (SPH), and lysophosphatidylserine (LPS), and increased molecular species annotations across most shared classes. This indicates that improved sensitivity enabled deeper within-class coverage across the lipidome rather than shifting the lipid class profile.

Figure 3. Number of lipid annotations assigned to each class for nano flow (A) and high flow (B). Each color represents a lipid class, and the total number of lipid annotations is displayed on the right in red



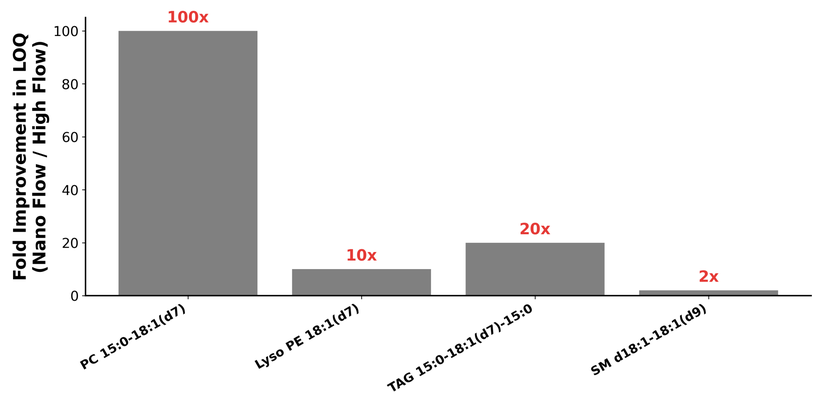
Nano flow reduced unintentional MS¹ fragmentation across representative lipid classes compared with high flow (Figure 4). Both SPLASH internal standards and endogenous bovine liver lipids showed lower fragmentation under nano flow conditions, with the largest differences observed for cholesteryl esters (ChE). Fragmentation decreased by 71a% for ChE 18:1 (d7) and 54% for endogenous ChE 18:2, with similar reductions observed for phosphatidylinositol (PI) and phosphatidylglycerol (PG) supporting improved annotation confidence.

Figure 4. Evaluation of unintentional MS¹ fragmentation for representative lipid classes



The SPLASH dilution series demonstrated improved quantitative sensitivity under nano flow conditions, with LOQ improvements shown for selected standards from the SPLASH Lipidomix (Figure 5). Lower LOQs were observed compared with high flow, with improvements ranging from 2-fold to 100-fold depending on lipid class. The largest gain was observed for phosphatidylcholine (PC), which decreased from 15.06 pg to 0.151 pg on-column. LOQs also improved for lysophosphatidylethanolamine (LPE), triacylglycerol (TG), and sphingomyelin (SM), supporting improved quantitative performance under nano flow conditions.

Figure 5. Fold improvement in LOQ using nano flow compared to high flow for representative SPLASH Lipidomix internal standards



Nano flow sensitivity gains translate into practical workflow advantages. When sample amount is limited, lower on column loads enable less starting material to be used while maintaining sensitivity. When sample amount is not limited, the same sample preparation approach as high flow can be maintained by reducing injection volume or diluting the sample to avoid column overloading (Table 1). Test injections at several dilution levels are recommended before running a full batch, with 10–100-fold dilution serving as a practical starting point. Robust nano flow performance requires optimized wash and re-equilibration steps, including weak and strong wash solvents, a zebra wash sequence to reduce carryover and buildup, and extended equilibration to maintain stable retention times and peak shapes.

Key consideration	Recommended practice for nano flow lipidomics
Sample preparation and injection	Use 10–100 × less starting material or dilute the final extract 10–100 × (equivalently inject 10–100 × less). Run a small set of test injections before starting a full batch.
Wash solvents	Use a weak wash solvent matching initial mobile phase and a strong wash solvent to remove retained lipids and minimize carryover.
Wash strategy	Include a “zebra” wash sequence of at least 3 iterations alternating between mobile phases at the end of the gradient to remove strongly retained lipids before re-equilibration.
Re-equilibration	Extend re-equilibration after zebra wash until retention times and peak shapes are stable (typically longer than high flow). At least 5 column volumes.

Table 1. Considerations for nano flow lipidomics compared with high flow

Conclusions

- Nano flow enabled greater sensitivity and deeper lipidome coverage at lower on column loads compared with high flow.
- Increased lipid annotations, reduced unintentional MS¹ fragmentation, and lower LOQs demonstrated improved performance for untargeted lipidomics.
- Lower sample requirements may expand lipidomics workflows to limited biological samples, including small tissue inputs or low-abundance sample types.
- Solvent consumption was reduced by 99.7% for a 30-minute gradient, from 7.8 mL with high flow to 0.021 mL with nano flow, supporting lower solvent costs and reduced waste.
- Robust implementation requires careful optimization of sample loading, wash strategy, and re-equilibration to avoid column overloading, carryover, and retention time drift.

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