Lipidomics, Metabolomics, Single Cell

Nano-LC-MS based Lipidomics for Single Cell Applications

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ABSTRACT

Purpose: Establish a workflow for lipidomic analysis for single cells/ limited sample utilizing nano-LC-MS (nLC-MS).

Methods: Experiments were conducted to benchmark sensitivity, robustness and reproducibility against analytical flow LC-MS lipidomic methods. Dilution series of lipid standards and lipid extracts (Liver and Cells) were analyzed and compared.

Results: Initial results show an increase in the number of lipids identified and increase in sensitivity as determined by limit of detection of standards utilizing nLC-MS. 30-50 abundant lipids were identified from analysis of single cell equivalent amount of lipid.

INTRODUCTION

Lipidomics is the comprehensive analysis of molecular lipid species. Separation of lipids using liquid chromatography followed by analysis by mass spectrometry has become the method of choice for lipidomic studies. Many biological specimens including single cell extracts are limited in sample amounts and hence require highly sensitive analysis methods. Population studies hide information regarding heterogeneity in cells and hence it is important to have analytics for measurement of single cells. Conventional LC-MS based lipidomics does not offer the sensitivity required for the comprehensive lipidomic analysis of these samples. Nano-LC-MS offers high sensitivity but is technically challenging to implement in terms of robustness and reproducibility. This work describes the development and optimization of nano-LC-MS for robust and reproducible lipidomic analysis.

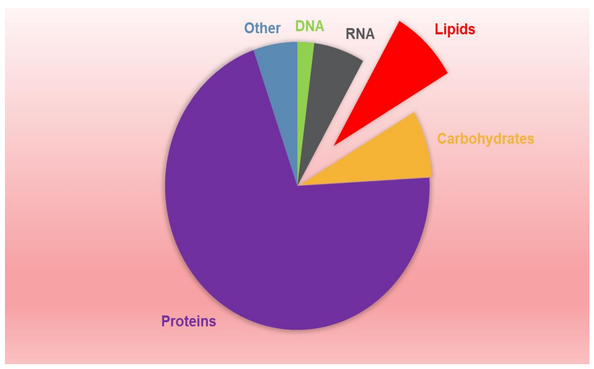

Figure 1. Average cellular composition of a mammalian cell. The percentage of lipids can vary from 1% for erythrocytes to 99% for adipocytes. Hela Cells which can weigh around 600 pg can have around 50 pg of lipids.

MATERIALS AND METHODS

Chemicals and Standards

Lipid standards and bovine liver lipid extract were purchased from Avanti Lipids

HPLC Conditions

Buffer A: 60:40 Acetonitrile: Water with 10 mM Ammonium Formate with 0.1% Formic Acid

Buffer B: 90:8:2 Isopropanol: Acetonitrile: Water with 10 mM Ammonium Formate with 0.1% Formic Acid

Conventional Flow
HPLC: Thermo Scientific™ Vanquish™ UHPLC
Column: Thermo Scientific™ Accucore™ C30 (2.1 x 150 mm, 2.7 µm)
Temperature: 45ºC
Injection Volume: 2 µL
Flow: 0.26 mL/min
Total run time: 30 min

Nano-flow
HPLC: Thermo Scientific™ EASY-nLC™ 1200
Column: Thermo Scientific™ EASY-Spray™ column (2 µm,15 cm × 150 µm)
Temperature: 45ºC
Injection Volume: 2 µL
Flow: 200 nL/min
Total run time: 45 min

Mass Spectrometer

Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer was used.

MS Conditions

MS^1 at 240K resolution (FWHM @ m/z 200) and data-dependent HCD MS^2 experiments (15K resolution) were performed.

The mass spectrometer parameters were optimized for low flow and high sensitivity by adjusting the ion injection time.

Data Processing

Thermo Scientific™ LipidSearch™ 5 was used for lipid identification.

RESULTS

REPRODUCIBILITY AND ROBUSTNESS

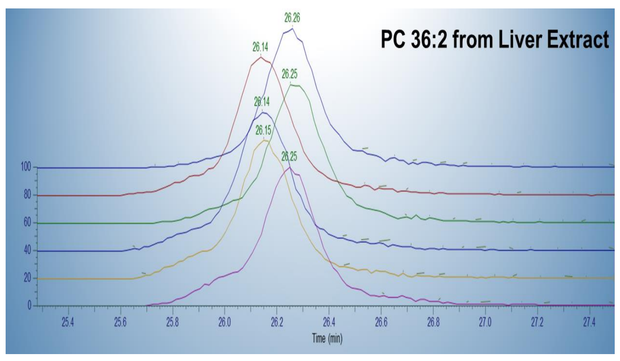

Figure 2. The nLC runs are highly reproducible in retention time. Extracted ion chromatogram of PC 36:2. from six QC runs of liver lipid extract shows a maximum RT shift of 0.1 minute and a peak area CV of 6%.

SENSITIVITY

Lipid Standards belonging to different lipid class were run at various concentrations and the sensitivity of nLC was compared to conventional LC. nLC increased the sensitivity by 1-3 orders of magnitude depending on the lipid class. At higher concentrations the detector was saturated during the nLC analysis indicating a greater ionization efficiency at nanoflow compared to conventional flow. A couple of examples are shown in the figure.

LIPID ID

A seven level double dilution series of liver lipid extract starting at 250 ng/mL was analyzed using nLC-MS as conventional LC-MS. The data was processed using LipidSearch 5. nLC was able to detect almost twice the number of lipids **(1468 to 578)** and more lipid classes. nLC was also able to detect analytes at very low levels.

Figure 3: LPC (18:0) was detected at different concentrations in nLC but not in LC

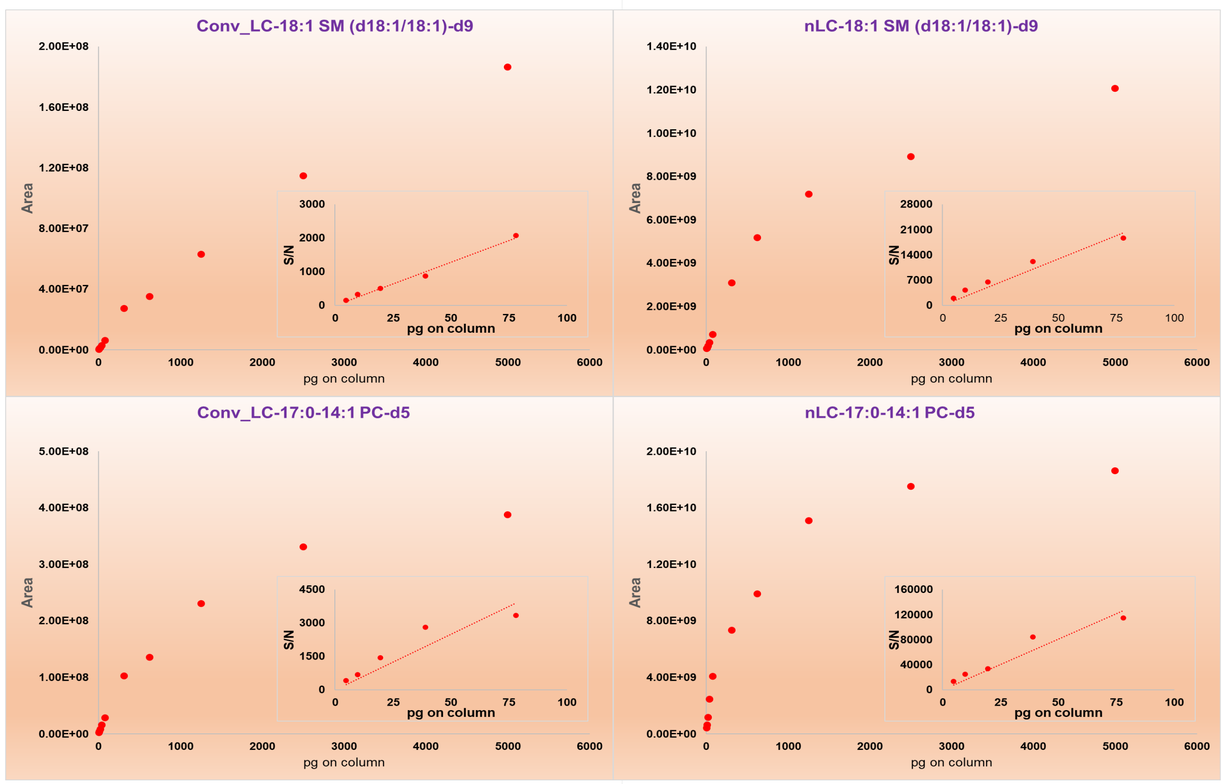

Figure 4. Deuterated standard of lipid species from class Sphingomyelin (SM) and Phosphatidylcholine (PC) show much greater sensitivity (higher S/N) at nanoflow compared to conventional flow.

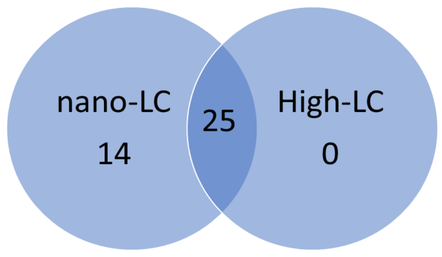

Figure 5. Lipid Classes detected using nLC vs conventional LC

Single Cell Equivalent Lipid Extract

Dilution series of the HeLa cell lipid extract were analyzed on nLC-MS system. Lipid equivalent to 10 cells was able to id 45 lipids. The top identified lipids include many PC's as well as other phospholipids as well as monoacylglycerol.

HeLa Cell Extract (cell on column)	Lipid Identified
2500	296
250	96
25	45
2.5	31

Top 10 Lipids
PC 34:1
PC 36:2
PC 32:1
PC 36:1
PI 36:2
PS 36:2
ChE 16:1
MG 18:0
MG 18:1
PC 34:2

Figure 6. LipidSearch 5 was used to analyze data obtained from the nLC runs. ddMS2 was performed on the bulk (2500 cells) sample and the lipids in other dilutions were identified by MS^1 match.

OUTLOOK

More optimization on the nLC as well as Mass Spec parameters need to be done for increasing the number of lipid identified.

CONCLUSION

A workflow for nanoflow LC-MS is described for analysis of limited samples/single cells.

1. Robustness and reproducibility of nLC is shown.
2. nLC is shown to be more sensitive than conventional LC
3. Applicability for single cell equivalent lipid amount is shown.

The challenge of analysis of limited samples can be addressed using nLC.

ACKNOWLEDGEMENT

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