NANO-LC-MS BASED LIPOIDOMICS FOR SINGLE CELL APPLICATIONS

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ABSTRACT

Purpose: Establish a workflow for lipidomic analysis for single cells/tissue sample using 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 standards using 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 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 hold 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.

RESULTS

REPRODUCIBILITY AND ROBUSTNESS

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: 90:10 Isopropanol: Acetonitrile: Water with 10 mM Ammonium Formate with 0.1% Formic Acid

Column: Thermo Scientific™ Vanquish™ UHPLC
Column: Thermo Scientific™ Accucore™ C18 (2.1 x 150 mm, 2.7 μm)
Injection Volume: 2 μL
Flow Rate: 0.26 mL/min
Total run time: 30 min

Mass Spectrometer

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

MS Conditions

MS: 50, 240 m/z resolution (FWHM @ m/z 200) and data-dependent HCD MS2 experiments (15k resolution) were performed.

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

ACKNOWLEDGEMENT

Thermo Fisher Scientific, San Jose, USA, Bremen, Germany

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.