


High-coverage targeted lipidomics analysis by LC-MS/MS in mouse brain tissue prepared by laser-capture microdissection

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ABSTRACT

Understanding metabolic changes at the cellular level can help to reveal the underlying molecular mechanisms involved in complex biological systems. Here, a detailed LC-MRM using multiple reaction monitoring (MRM) method is provided that will enable comprehensive coverage of many of the multiple lipid classes present in trace tissue samples. The sample was collected by laser-capture microdissection and analyzed with the Triple Quad version of a SCIEX 7500 system coupled with an ExionLC AD system. More than 2000 MRM transitions were simultaneously acquired in both positive and negative mode simultaneously in a single run. This method detected 285 from 7 different classes in trace tissue samples. These results indicate that the method met the spatial lipidomics analysis requirements.

INTRODUCTION

Cells are the basic structural and functional units of living organisms. Understanding metabolic changes at the cellular level can help to reveal the underlying molecular mechanisms involved in complex biological systems. For single-cell or micro-scale spatial metabolomics, trace sample preparation, identification of intracellular metabolites, and data analysis all require sophisticated techniques and models. Since cell metabolism responds to environmental changes, a major challenge to single-cell sample preparation is impact on cell metabolism during sample preparation. One strategy is to maintain cells in the natural environment as much as possible during the preparation. Alternatively, reducing the number of cells for analysis from large populations of cells to tens of cells or to a single cell is helpful for many prospective studies.[1]

Laser-capture microdissection is a state-of-the-art technique that provides the scientific community with a rapid and reliable method to isolate a homogeneous population of cells from heterogeneous tissue specimens. This enables investigators to analyze the metabolome accurately from pure populations of cells with a highly sensitive analysis system.

Unlike DNA and RNA, cellular metabolites cannot be amplified by PCR, the concentration of metabolites in a trace tissue sample is low and some extremely rare metabolites require more sensitive detection methods. The Triple Quad version of the SCIEX 7500 system offers a new generation of high-end quantitative work platform. The SCIEX 7500 system offers the durability of previous products, in addition to improved sensitivity that can address extremely challenging analytical efforts, such as metabolomics.

MATERIALS AND METHODS

Sample preparation:
The 40 µm × 50 µm tissue samples were dissected from the mouse brain with an LMD-7 system. The dissected sample was extracted with a 30 µL solution containing by volume 30% isopropanol, 65% acetonitrile and 5% water. After centrifugation, the supernatant was analyzed by a triple quadrupole mass spectrometer.

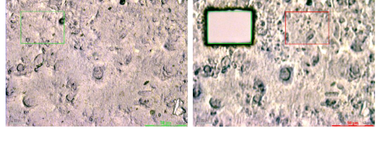

Figure 1. The brain tissue section sample was used for laser microdissection system. An area of approximately 2000 µm² was selected at 40 × magnification (left) for subsequent analysis. Additional samples were later selected from the same brain section (right).

HPLC conditions:
A 17-minute gradient was used on the Phenomenex Kinetex C18 column (2.6 µm, 100 × 2.0 mm). Mobile phase A was 5mM ammonium acetate in a solvent containing equal volumes of water, methanol and acetonitrile and mobile phase B was 5mM ammonium acetate in isopropanol.

MS/MS conditions:
The Triple Quad version of a SCIEX 7500 system with a Turbo V ion source and electrospray ionization (ESI) probe was used. More than 2000 MRM transitions of lipid species were simultaneously acquired in both positive and negative mode with scheduled MRM (sMRM) mode and processed using SCIEX OS software.
The sMRM algorithm was used for best accuracy and reproducibility.

RESULTS

The target list of lipids was comprehensive, covering most major lipid classes and categories. MRMs were selected to cover lipids containing fatty acids with 12-26 carbons and 0-6 double bonds, including odd chain lipids. There were 1408 lipid molecular species detected in a representative sample of plasma (Figure 2). According to plasma results, the lipid species in a trace tissue sample can be identified with the same MRM transitions and LC retention time in plasma. The addition of isotopic internal standards (Internal Standards kit for the Lipidyzer platform, SCIEX) enabled the lower limit of detection for each type of lipid species to be determined.

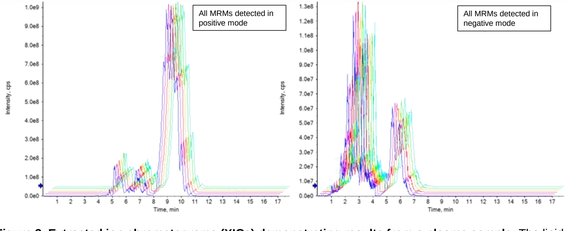

Figure 2. Extracted ion chromatograms (XICs) demonstrating results from a plasma sample. The lipid molecular species of 1408 MRM transitions were detected simultaneously in both positive and negative ion modes in a single run of a plasma sample. The RSD value of more than 88% of all MRM responses were < 30% across 6 replicate runs. The retention time for each peaks was within 0.1 min across runs.

According to the MRM response of lipid standards, the limit of detection was between 0.04 and 5 pg on-column for all lipid compounds analyzed from 13 classes. These lipid classes included ceramides (Cer), dihydroceramides (DHCer), glucosylceramides (GlcCer), lactosylceramides (LacCer), cholesterol esters (CE), diacylglycerols (DAG), triacylglycerols (TAG), sphingomyelins (SM), free fatty acids (FFA), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), phophatidylcholine (PC) and phosphatidylethanolamines (PE).
The RSD values of the peak areas for more than 88% of the 1408 of lipid compounds detected in the plasma samples were below 30% and the difference in retention times of all compounds did not exceed 0.1 min during the whole batch. These results indicate that the method was qualified for lipidomics analysis.

In isolated trace cells (about 2000 µm² in tissue sections), 285 lipid compounds were detected, including SM, CE, TAG, DAG, PE, PC and Cer (Figure 3)

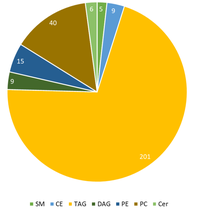

Figure 3. The number of different types of lipid compounds measured in trace brain tissue sections. The targeted lipidomics method was based on rapid analysis of multiple lipids in the matrix by LC-MS/MS.

The determination of lipid compounds detected in trace tissue samples was mainly based on the blank extraction solvent (negative control) and plasma lipid extraction samples (positive control). These results were combined to determine all kinds of lipids based on standard retention time (Figure 4).

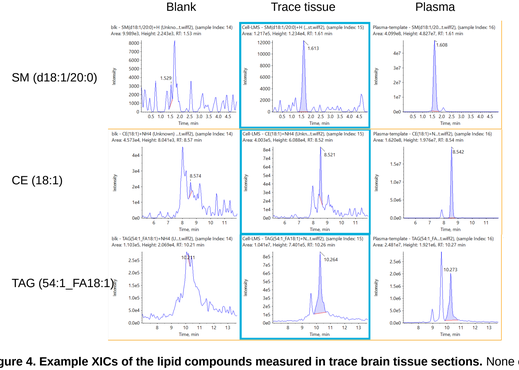

Figure 4. Example XICs of the lipid compounds measured in trace brain tissue sections. None of the 7 lipid compounds measured by LC-M S/MS were present in the blank extraction solvent (left), consistent with the peak emergence behavior in the positive control plasma samples (right).

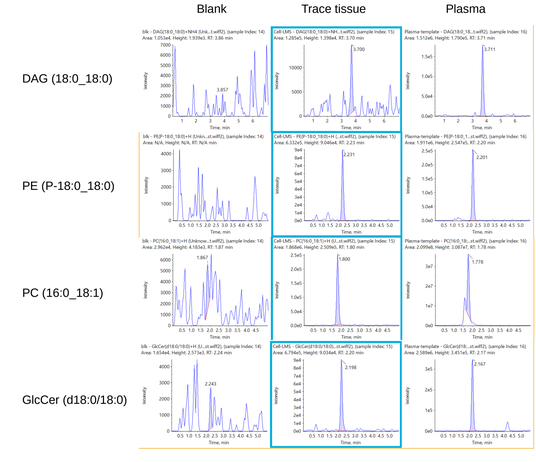

Figure 4 (continued). Example XICs of the lipid compounds measured in trace brain tissue sections. None of the 7 lipid compounds measured by LC-M S/MS were present in the blank extraction solvent (left), consistent with the peak emergence behavior in the positive control plasma samples (right).

CONCLUSIONS

The acquisition of microsamples by laser-capture microdissection was combined with the highly sensitive detection of a LC-MS/MS system. This analysis detected 285 lipid compounds in a 2000 µm² tissue section and met the requirements for compound coverage in lipidomics analysis. The excellent sensitivity of the system enabled the reliable detection of trace compounds. This method can be applied to other cell slice samples to facilitate the development of precision and spatial metabolomics research.

REFERENCES

1. Domenick, T. M.; Gill, E. L.; Vedam-Mai, V.; Yost, R. A. Anal. Chem. 2021, 93, 546−566

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