Apr 29, 2020

Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) for Bulk Metabolomics

DOI
dx.doi.org/10.17504/protocols.io.be2ajgae

Dusan Velickovic[1], Jessica Lukowski[1], Guanshi Zhang[2], Theodore Alexandrov[3], Christopher R Anderton[1], Kumar Sharma[2]

[1]Pacific Northwest National Laboratory; [2]University of Texas Health San Antonio; [3]European Molecular Biology Laboratory

KPMP | Metabolomics Protocols ...

Christopher R Anderton
Pacific Northwest National Lab

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.be2ajgae

Protocol Citation: Dusan Velickovic, Jessica Lukowski, Guanshi Zhang, Theodore Alexandrov, Christopher R Anderton, Kumar Sharma 2020. Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) for Bulk Metabolomics. **protocols.io https://dx.doi.org/10.17504/protocols.io.be2ajgae**

Protocol status: Working
We use this protocol and it's working

Created: April 14, 2020

Last Modified: April 29, 2020

Protocol Integer ID: 35618

Abstract

Mass spectrometry imaging is an exciting technologywhich enables a simultaneous analysis of multiple molecular components directly from single cells, tissues, and organs,. In combination with histological methods, this technique provides information about the spatial distribution of molecules in various biological tissues. Particularly, MALDI-MS imaging increases the coverage of metabolites by using different matrices and ion modes. In coordination with in situ analysis of proteins, transcripts and epigenetic marks, the complementary spatial information on metabolites will establish metabolic pathways that are dominant and characteristic of disease states. We have recently developed and optimized a spatial metabolomics approach to image small molecules in human kidneys and biopsy sized material. With our combined expertise at UTHSA, PNNL and EMBL and recent advances, we have established methods for identifying metabolites in human kidneys, employed ultra-high mass resolution MS imaging for tissue analysis, and developed a bioinformatics resource (METASPACE) to annotate metabolites for anatomical localization and 3-D reconstruction. Our integrated technology can easily connect with other TIS sites to provide biochemical readouts of genes/proteins in specific tissue and cellular compartments.

Although METASPACE bioinformatics provides metabolite annotations of given reliability, there may be an inherent level of ambiguity due to the lack of untargeted MS/MS fragmentation in MALDI-MSI. Metabolite annotations from MALDI-MSI and METASPACE are structurally validated using orthogonal analytical techniques such as LC-MS/MS due to its high metabolome coverage, sensitivity, and throughput. The work flow for ‘bulk omics’ is shown in Figure 5.

Guidelines

Significance of the data/analysis generated

1. Generated high quality lipidomic and metabolomics data in both ionization polarities
2. Identified all the molecules within the tissue biopsy's (without spatial information)
3. Increased confidence in the annotations of molecules detected by MALDI-MSI
4. Used results to create a kidney database of known molecules within these biopsies that can be used in future studies

QA/QC for LC-MS/MS

1. Ensure LC pump is working correctly, and pressures readbacks are correct.
2. Thermo Velos-ETD Orbitrap mass spectrometer is tuned weekly with Thermo CalMix in ESI direct infusion mode. Injection times (1-2 ms) are similar to those of the S lens configuration that uses a larger ID inlet capillary.
3. Ensure ion trap fill times are > 2 ms for target ion accumulations of 1E5, in effort to see if ion collection portion of mass spectrometer is optimal and clean.
4. Visually observe TIC current as LC-MS/MS of samples are being ran. Large clumps early in the chromatogram indicate either sample degradation or column overloading. Check MS data to confirm which is the issue, detection of small/fragment ions or parent species would indicated either, respectively.

LC-MS/MS to KPMP

Portions of the kidney biopsy not used for MALDI-MSI are homogenized and utilized to determine bulk molecular composition of the tissue via LC-MS/MS. LC-MS/MS is used to validate the MALDI-MSI data of molecular targets for kidney diseases. Altered metabolic pathways identified in MALDI-MSI will be further confirmed by bulk metabolomics analysis. The validated metabolites/markers measured by both MALDI-MSI and LC-MS/MS will help the development of novel therapeutics with precision for kidney diseases.

LC-MS/MS to the kidney atlas

The bulk lipidomic and metabolic data provides more confidence in the putative molecular annotations generated from the MALDI-MSI data. Here, all the classes of metabolites and lipids can be more confidently identified based upon their LC-elution time and the MS and MS/MS spectra. These results will be used to generate a kidney database of known molecular compounds that can be utilized in future MALDI-MSI studies; increasing the specificity of METASPACE for kidney studies. **The high mass accuracy MALDI-MSI data can be correlated with the high mass accuracy LC-MS/MS data, to confidently localize where these molecules are in the kidney tissue, which expands the Kidney Atlas' potential.**

Materials

MATERIALS

Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)

STEP MATERIALS

Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)

Protocol materials

- Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)
- Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)
- Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)

LC- MS/MS

1 Add MilliQ water (200 µL - 300 µL) to biopsy tube containing remaining biopsy sample (material not sectioned) and lyse the remaining biopsy sample (material not sectioned) using a tissue lyser. Quantify the amount of tissue remaining by weight.

2 Place the sample into a 1.7 mL or 2 mL Sorenson MµlTI™ SafeSeal™ Microcentrifuge Tube

> Note
>
> These tubes have been shown to not leach polymers into the lipid layer from the chloroform.

3 Add cold (-20 °C) chloroform:methanol mix (prepared 2:1 v/v) to sample in 4:1 ratio over sample volume and vortex

> Note
>
> For example add 400 µl of the 2:1 Chl:MeOH mixture to 80 µl of sample

3.1 Vortex for 00:00:05 to 00:00:10

3.2 Let stand on ice for 00:05:00

3.3 Vortex for 00:00:05 to 00:00:10

3.4 Centrifuge the sample at 12000 x g, 4°C , 5-10 minutes

4 Carefully removed the upper aqueous metabolite layer until the interphase contracts without disturbing the protein disk, discard.

4.1 Carefully puncturing the protein interphase with a pipette tip, remove the organic lipid phase from the bottom of the tube (be sure to gently push out any protein or upper methanol phase that might have entered the pipette tip) into a conical bottom Waters autosampler vial.

5 The organic layer (containing lipids) is placed into the speed vac to dry.

6 500 µL of 2:1 Chl:MeOH and cap (no septa) is store at -70 °C until ready for analysis (need extra solution volume for safe storage).

7 Prior to LC analysis, dry sample in speed vac and reconstitute in 100 µL of 95:5 MeOH:Chl in an vial.

8 Vial is placed in autosampler of Waters H class LC.

9 Inject 10 µL of sample onto a reversed phase Waters CSH column (3.0 mm x 150 mm x 1.7 µm particle size) with a 34 min gradient (mobile phase A: ACN/H2O (40:60) containing 10 mM ammonium acetate; mobile phase B: ACN/IPA (10:90) containing 10 mM ammonium acetate) at a flow rate of 250 µl/min to separate the lipid molecular species.
Reversed Phase Waters CSH Column (3 mm X 150 mm X 1.7 um particle size)

Note

The gradient should be made fresh for each run.

10 Mass spectrometry is performed using a Thermo Velos-ETD Orbitrap mass spectrometer.

11 Samples and are analyzed in both positive and negative ionization modes using HCD (higher-energy collision dissociation) and CID (collision-induced dissociation) to obtain high coverage of the lipidome

12 The LC-MS/MS raw data files were analyzed using LIQUID and confident lipid identifications were determined as outlined in the main text.

13 LC-MS data is also converted to mzML format and uploaded to LC-METASPACE