Bulk Untargeted LC-MS/MS Lipidomics

Forked from Bulk Untargeted LC-MS/MS Lipidomics for Small Tissue Amounts

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APR 05, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ewov1ob5klr2/v1

Protocol Citation: Madeline E. Colley, Jamie Allen, Katerina V Djambazova, Angela Kruse, melissa.a.farrow, Jeff Spraggins 2023. Bulk Untargeted LC-MS/MS Lipidomics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1ob5klr2/v1

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

Created: Mar 30, 2023

Last Modified: Apr 05, 2023

PROTOCOL integer ID: 79793

ABSTRACT

This protocol is the detailed methodology for extracting lipids from tissue, connecting a Waters UHPLC to a timsTOF mass spectrometer via contact closure and performing LC-MS/MS analysis.

MATERIALS

EXTRACTION

Glass vials: https://www.fishersci.com/shop/products/shell-vials/p-6062401#?keyword=thermo%20shell%20vials
Autosampler vials: https://www.waters.com/nextgen/us/en/shop/vials-containers--collection-plates/186002803-amber-glass-12-x-32-mm-screw-neck-qsert-vial-300--l-volume-100-p.html
CSH Column: https://www.waters.com/nextgen/us/en/shop/columns/186009461-acquity-premier-csh-c18-column-17--m-21-x-100-mm-1-pk.html
Drummond Pipettes: https://www.drummondsci.com/product/microdispensers/fixed-volume-microdispenser/

All solvents brand: LiChrosolv by EMD Millipore

STANDARDS

Avanti Equisplash: https://avantilipids.com/product/330731
C24:1 sulfatide-d7 https://avantilipids.com/product/860736

Instrumentation

Bruker TimsTOF FleX Mass Spectrometer
Waters Premier QSM UHPLC with FTN and 2 column manager
Benchtop Sonicator
Benchtop, hanging bucket centrifuge

SAFETY WARNINGS

methyl tert-butyl ether and chloroform are toxic to inhale! Do all procedures in a fume hood.

Preamble

1 Please reference the materials section for details on materials, consumables, equipment details and solvents used. In the protocol, they will be referred to in layman terms (ie "glass vial", "mobile phase A", etc.)

All pipettes used in this protocol are Drummond positive displacement with borosilicate tips.

Tissue Collection

2 Tissue is sectioned at 10 um width in a cryostat and (1-3) scrolls of tissue is placed into a thermo glass shell vial.

Keep one empty vial with a random, known number on it without tissue inside for a sample blank.

Note

A scroll of tissue is generally called a "section". Sections most adjacent to those used for other technologies will be most relevant to your analysis.

Good tissue archival technique, storage, and cryopreservation are critical to analysis.

Lipid Extraction by MTBE

3 Spike 5 uL Avanti Equisplash internal standard into your sample vial using the 1-25 uL Drummond pipette.

4 Add enough cold methanol to cover your tissue in the vial (400 uL for small sample amounts).

Note

Tissue sections will often stick to the sides of the vial. Try to get cold methanol into your vial asap and knock down the tissue into the methanol by tapping on the benchtop or scraping the side of the vial with a sterile needle tip.

5 Add 2 large and 3 medium sized stainless steel beads from a navy RINO homogenizer tube to your glass vials and vortex sample for 1 minute.

6 Place samples on dry ice for 5 minutes.

7 Place samples on wet ice for 5 minutes and sonicate on ice for 30 minutes.

8 Repeat steps 6 and 7.

9 Add 1600 uL cold tert-butyl methyl ether (MTBE) to the vial and vortex for 30 seconds.

10 Sonicate for 30 minutes on ice.

11 Add 400 uL of cold water for phase separation and vortex for 30 seconds.

12 Cetrifuge for 10 minutes at 100 xg

13 Allow to separate further for 10 minutes on ice.

14 Collect 1500 uL of the top layer (MTBE) with the Drummond 250 uL pipette into an autosampler vial.

15 For small sample amounts, repeat steps 9-14 an additional time and add the top layer to the same new autosampler vial from step 14.

16

Note

Lipids are prone to radical oxidation under flourescent lights and general atmosphere, so work as much as possible in a fume hood and keep caps on tightly between steps. For highly labile species such as fatty acids, do your work with a constant flow of nitrogen over your working area.

17 Dry samples down to completion under nitrogen without heat using a sample concentrator.

18 Store lipids in -80 C dried down.

19 Prior to analysis, resuspend sample in methanol to volume appropriate for analysis. For small sample amounts, we use 50 uL. For bulk samples, we use 500 uL.

Waters Premier Liquid Chromatography Preparation

20 Prepare your mobile phases using only UHPLC grade solvents and additives. Additives must be stored in a dry environment.

Mobile Phase A: 60% ACN, 50% H2O, 10 mM ammonium formate, 0.1% formic acid
Mobile Phase BL 90% IPA, 10% ACN, 10 mM ammonium formate, 0.1% formic acid

Degas solvents for 20 minutes by sonication.

Note

Ammonium formate takes a long time to go into IPA solution! Prepare your solvents well in advance and let the ammonium formate go into solution overnight. If there is a rush, you can gently heat the solution on a hot plate.

21 **Perform system checks:**

1. Check solvent levels
   - SM = needle wash (IPA)
   - SW = seal wash (90:10 ACN:H2O)
   - PURGE = (90:10 ACN:H2O)
   - A = desalting columns (H2O)
   - B = desalting columns (ACN)
   - C = Mobile phase A
   - D = mobile phase B

22 Restart system starting with turning on the sample manager near the mass spectrometer

23 Connect the contact closure RS232 cable from the back of the LC system to the back of the instrument computer. Connect ethernet cable to university internet.

24 Open MassLynx and Console

25 Change sample manager temp to 8C. Change column temperature to 55C.

26 Connect your column (Waters Premier C18 CSH 2.1 mm x 100 mm) to the column manager and condition by running mobile phase a for 20 minutes.
Ensure pressure delta goes to low double or single digits.

Bruker timsTOF FleX Mass Spectrometer Preparation

27 1. Open the relevant method for analysis
4D-lipidomics positive or negative with MS/MS Stepping

28 Install appropriate ion source (ViP-hESI or Apollo)

29 Adjust all temperatures and flow rates appropriate for analysis and wait 30 minutes for TIMS Tunnel In pressure to stabilize.

30 Attack PEEK tubing from syringe pump to the source inlet and flow Agilent low concentration tune mix and 180 uL/min.

31 **Calibrate mass and mobility**: Adjust funnel pressure by selecting the lightning bolt icon in timsControl software (Display mobilogram in [V]. Adjust funnel pressure until the calibrant is at the appropriate [V].

Positive mode: m/z 622.029 should have 132.5 [V]
Negative mode: m/z 601.979 should have 118.5 [V]

Calibrate m/z dimension.

Repeat mobility calibration.

Wait 20 minutes to assess drift. If system has not drifted in pressure, do not recalibrate. If system has drifted in pressure, recalibrate.

32 Open the configuration editor software and select "WATERSRS232" method.
This method has the timsTOF mass spectrometer as the instrument and the COM2 channel active for input with RS232.

Set method to active.

33 Open Bruker HyStar software

34 Create matching sample tables in HyStar and MassLynx

35 Right click on the first line of your HyStar stample table and click "upload sample conditions"

System Suitability Test (SST)

36 Prepare 5 serial dilutions of Avant Equisplash Mix and Sulfatide standard within the linear range of your sample concentration

37 Run all dilutions in RANDOM order with the same chromatography and mass spectrometer methods as your samples

38 Open data in skyline and ensure that retention times do not drift, area under the chromatographic curves are linear for all dilutions, and that all expected species with adducts are detected.

39 Ensure that your blank is "blank".

Sample Table Setup

40 Prepare your sample table so that you randomly sample across your QCs, blanks and samples.

41 You should have a solvent blank - in our case, methanol.
A sample blank - a blank vial that endured the same extraction protocol as your samples.
A QC sample - take 5 uL off the top of all samples and add then to the same vial

Data Analysis

42 Files are converted to IBF format using the converter provided in MS-DIAL 4.90

43 Data is input into MS-DIAL with the pooled reference sample being the "QC", any random blank being the "blank" and the samples being separated by their category (in our case by patient or condition).

44 Our MS Accuracy tolerance is 0.01 Da, with 0.05 MS2 tolerance. We allow for 500 minimum peak height.

45 Once data is processed we filter by "Ref. matched" only and export this as our final list.