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BAF_Protocol_012_Lipidomics LC-MS(/MS): Vanquish UPLC and Orbitrap ID-X

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

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Abstract

This protocol is the basic LC and MS running parameters for lipids or very hydrophobic molecules. Most are singly charged. Lipids can be more challenging as more mass isoforms exist.

Guidelines

These are our general settings as a starting point for untargeted lipidomics experiments. Specific adjustments need to be made to meet specific samples or type of data required.

Materials

Thermo Orbitrap ID-X - FETD1-10001
Thermo Vanquish Duo UHPLC
Sigma - Ascentis Express 90A C18 2.7 um, 10 cm x 2.1 mm - 53823-U
Thermo Optima 0.1% FA (formic acid) in water - LS118-4
Thermo Optima Methanol - A456-212
Thermo Optima Isopropanol - A461-4
Thermo Optima Acetonitrile- A955-4
Thermo Autosampler glass ambar vials 2 mL – 6PK1655
WHEATON MicroLiter insert, 300uL, conical with spring Part number: 11-0000-100
Fisherbrand Standard Pipette Tips (200 uL - Yellow) – 53503-065
SPLASH LipidoMIX Internal Standard - Part number 330707, Avanti Ploar Lipids, Inc.

Troubleshooting

Prepare samples for injection

1 Add 10 uL of Splash Mix standard to the extracted lipids. Dry samples under liquid nitrogen at 40C.

2 Suspend dried extracted samples with 100 µL of 1:1 IPA/ACN

3 Mix well using a "figure-*eight*" movement of the vials in the bench. Transfer the sample to a new vial with borosilicate inserts.

4 Make sure there is no air in the bottom of the inserts add the vials into the autosampler of the UPLC Vanquish system.

5 In the sequence setup view of the Xcalibur software include each sample in a row filling with: file name, sample ID, folder directory to save results, directory for the acquisition method, vial position and injection volume. Normally use 10% of each sample for injection.

LC parameters 15 min gradient

6 33m

Speed limits: draw speed 1 uL/s; dispense speed 1 uL/s.
Injection wash parameters: Wash mode: both; Wash time: 10.0s; Wash speed: 10.0 uL/s.
Column temperature: 55 °C
Run time: 00:33:00
Solvents: %A1: 60% ACN in 10 mM Ammonium Formate with 0.1%FA.
%A2: 90% IPA, 10% ACN, 10 mM Ammonium Formate with 0.1% FA.
Upper limit pressure: 1500 bar
Flow = 0.260 ml/min
Equilibration: -3min, 32% B, curve = 5.
Gradient (all steps curve = 5): 0 -1.5 min 32%B; 4 min 45% B; 5 min 52% B; 8 min 58% B; 11min 66% B; 14min 70%B; 18 min 75%B; 21min 97%B; 25 min 97%B; 25.01 min 32% B, 25.01-33 min 32%B.

General Instrument (Orbitrap ID-X) – Positive and Negative modes

7 Ion Source Type: H-ESI
Spray Voltage Positive 3500V
Spray Voltage Negative 2500V

Ion Transfer Tube Temperature Temperature 275 °C , Sheath gas 35, Aux Gas 7, Sweep gas 1, Vaporizer Temp Temperature 350 °C

Default charge state: 1

Full scan parameters

8 Scan 200-2000 m/z, resolution 120K, Quadrupole isolation: True, normalized AGC 50%, RF lens 40%, Max. Inj. Time 50ms, 1 microscan, Data Type Profile, Polarity Positive or Negative (create two methods one for Positive acquisition and another for Negative acquisition)

MS/MS parameters

9 Min intensity 5E4, Dynamic exclusion - 1 time, 5s, 10ppm, isolation 1.5 m/z, resolution 15K, HCD NCE stepped 25, 30, 35%, Max. Inj. Time 50ms, normalized AGC 50%, 1 microscan, Data Type Centroid.