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BAF_Protocol_010 Metabolomics 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 metabolite runs. The samples typically have fewer components (ions) than proteomics so the gradients are shorter and most molecules are singly charged.

Guidelines

These are our general settings as a starting point for untargeted metabolomics 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
Waters ACQUITY UHPLC BEH C18 1.7 um, 2.1 x 150 mm - 186002353
Thermo Optima 0.1% FA (formic acid) in water - LS118-4
Thermo Optima Methanol - A456-212
Thermo Autosampler
vials 0.25 mL – 14-823-136
Thermo Orange
caps – 14-823-380
Fisherbrand Standard Pipette Tips (200 uL - Yellow) – 53503-065
Cambridge Isotope Laboratories QRESS heavy labeled standards - MSK-QRESS-KIT

Prepare samples for injection

1 Suspend dried metabolite extracted samples with 100 µL of 0.1% Formic acid in water containing 100X diluted Metabolomics QRESS heavy labeled standards.

2 Vortex, microfuge for 00:15:00 at max speed, and take off 50 µL into autosampler vial (do not touch bottom of tube). 15m

3 Make sure there is no air in the bottom of the vial, carefully add the vial into the autosampler of the UPLC Vanquish system.

4 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 20% of sample.

LC parameters 15 min gradient

5 Speed limits: draw speed 1 uL/s; dispense speed 1 uL/s. 15m
Injection wash parameters: Wash mode: both; Wash time: 10.0s; Wash speed: 10.0 uL/s.
Column temperature: 30 °C
Run time: 00:15:00
Solvents: %A1: Water, 0.1%FA.
%A2: Methanol, 0.1% FA.
Upper limit pressure: 1500 bar
Flow = 0.250 ml/min
Equilibration: -3min, 0% B, curve = 5.
Gradient (all steps curve = 5): 0 min 5%B; 8 min
50% B; 8-9 min 98% B; 9-13 98% B; 13-13.1
min 0%B; 13.1-15 min 95%B.

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

6 Ion Source Type: H-ESI
Spray Voltage Positive 3500V
Spray Voltage Negative 2500V
Ion Transfer Tube Temperature 275 °C, Sheath gas 35, Aux Gas 7, Sweep gas 2, Vaporizer Temp 320 °C
Default charge state: 1

Full scan parameters

7 Scan 67-1000 m/z, resolution 60K, Quadrupole isolation: True, normalized AGC 25%, RF lens 35%, Max. Inj. Time 50ms, 1 microscan, Data Type Profile, Polarity Positive or Negative (create two methods one for Positive acquistion and another for Negative acquisition)

MS/MS parameters

8 Min intensity 2E4, Dynamic exclusion - 1 time, 2.5s, 10ppm, isolation 1.5 m/z, resolution 15K, HCD NCE stepped 20, 35, 50%, Max. Inj. Time 25ms, normalized AGC 25%, 1 microscan, Data Type Centroid.