HKU Med LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心

Proteomics & Metabolomics
Core

Report

Untargeted lipidomics service
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Basic information

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed at Centre for PanorOmic Sciences - Proteomics and Metabolomics Core, LKS Faculty of Medicine, University of Hong Kong.

Materials

Water, methanol and ammonium formate were purchased from Wako. Acetonitrile was purchased from VWR. Isopropanol and chloroform were purchased from Labscan. Formic acid was purchased from ThermoFisher. Internal standard SPLASH LipidoMix was purchased from Avanti Polar Lipids. All reagents were in HPLC grade equivalent or higher.

Sample preparation

2mL chloroform:methanol (2:1, v/v) was added to user provided tissue. The sample was then sonicated under ice chilled probe sonicator for 20 sec, cooled down for 10 sec and another sonication for 20 sec. The sample was then centrifuged at 3000 g for 5 min. Supernatant was aliquot out and dried under nitrogen. The sample was then reconstituted with IPA:MeOH:chloroform (1:1:0.2, v/v) in 1mg to 5uL. Then, 3 µL was injected into the LC-MS/MS system.

Data acquisition

LC-MS/MS

The chromatographic separation was carried out on an Vanquish UPLC (Thermo Fisher, Waltham, MA, USA). Mobile phases used were 10mM ammonium formate with 0.1% formic acid in acetonitrile and water, v/v 6:4 (A) and 10mM ammonium formate with 0.1% formic acid in acetonitrile and IPA 1:9 (B). The column was a ThermoFisher Accucore C30 (2.1×150 mm, 2.6 µm). An injection volume of 3 µL and a flow rate of 0.26 mL min^{-1} were used. The column oven temperature was set at 45°C. The gradient started at 30% B and was increased to 43% B in 2 min, then increased to 55% B in 2.1 min, 65 % B in 12 min, 85% B in 18 min and 100 % B in 20 min, then held for 5min, and decreased linearly to 30% B for re-equilibration time at starting conditions.

The mass spectrometry analysis was processed using an Orbitrap Exploris 120 mass spectrometer Thermo Fisher (Waltham, MA, USA) equipped with a HESI II probe in polar switching mode with source parameters set as follows: sheath gas flow rate, 60; auxiliary gas flow rate, 17; sweep gas flow rate, 1; spray voltage, +3.5/-3.0 kV; capillary temperature, 275 ^{o}C; S-lens RF level, 70; and heater temperature, 325 °C. Data was collected at dd-MS2 mode.

Data analysis

Data analysis was performed using Lipidsearch (Thermofisher Scientific/Mitsui Knowledge Industries) with the default parameters for Orbitrap MS Product Search and Alignment. After alignment, raw peak areas for all identified lipids were exported to Excel files.

Reference

Nature volume 543, pages681–686 (2017)
Nature volume 609, pages1005–1011 (2022)