



Metabolite extraction (modified Bligh and Dyer extraction method) and Untargeted LC-MS

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ABSTRACT

Extraction of tissue metabolites for untargeted LC-MS on LC-QTOF.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|----------------------|--------------------------|
| Methanol | PA-33900HPLCCS4L | P212121 |
| Chloroform | BP1145-1 | Fisher Scientific |
| Formic acid, LC-MS grade | 28905 | Thermo Fisher Scientific |
| Acetonitrile, LC-MS grade | 51101 | Thermo Fisher Scientific |
| Zorbax Eclipse plus C-18 Rapid Resolution High Throughput (RRHT) 2.1x 100mm 1.8 mm column | View | Agilent Technologies |

BEFORE STARTING

The tissue, disposable pestle and 1.5 ml-centrifuge tube in liquid nitrogen were chilled in liquid nitrogen.

Metabolite extraction

- 1 The tissue was pulverized in the presence of liquid nitrogen to fine powder. 100 ml of chloroform and 200 ml of methanol were added to the fine powder and resuspended by vigorous vortexing. The mixture was stored at room temperature for 30 min. Subsequently, 100 ml of chloroform and 100 ml of water were added and mixed. The tube was centrifuged at 12,000 xg for 10 min. The biphasic solutions were separated into two separate tubes without disturbing the protein precipitate at the interface. The samples were vacuum concentrated to dryness in a Refrigerated CentriVap concentrator (Labconco, USA) at 4°C. The samples were reconstituted with 20 ml of mobile phase (95% water:5% ACN), vortexed and centrifuged at 12,000 xg for 10 minutes at 4°C.

Untargeted metabolomics by LC/MS

- 2 The samples were analyzed on an Agilent 1260 Infinity-6540 UHD Accurate-Mass Quadrupole-Time-of-Flight (Q-TOF) LC/MS system coupled with Dual Agilent Jet Stream Electrospray Ionization source. The injection volume was 3 ml of sample and separation was using a Zorbax Eclipse plus C-18 Rapid Resolution High Throughput (RRHT) 2.1x 100mm 1.8 mm column. The separation was performed at a flow rate of 0.45 mL/min with linear gradient program. Mobile phase A composed of 0.1% formic acid in Milli-Q water and mobile phase B composed of 0.1% formic acid in acetonitrile. The gradient program was set as follows: t= 0 min, 5% B; t=2 min, 5% B; t=15 min, 98% B; t=18min, 98%; t=20 min, 5% B and the final stop time, t=25 min, 5% B.

For positive ionization mode, two reference masses of (i) 121.0509 m/z and (ii) 922.0098 m/z were measured continuously while for negative ionization mode, the reference masses were (i) 112.9855 and (ii) 1033.9881. Reference mass correction was enabled. The gas temperature was maintained at 300°C, drying gas flow was set at the rate of 8 L/min, sheath gas temperature and sheath gas flow at 350°C and 11 L/min respectively. The capillary voltage was 3500 V. The nebulizer pressure was set at 35 psi. The MassHunter Workstation software B.05.01 (Agilent Technologies, USA) was applied for instrument control and data acquisition. The data was analyzed using the Mass Profiler Professional software version 12.6.1.



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