Sample preparation and LC-MS/MS procedures

Jan-29-2024

Sample Treatment with human islets as an example

2,000 human Islet Equivalents (IEQs) (~200,000 cells), void of acinar tissue, were treated under low- (LG) or High-Glucose (HG) conditions for 30 minutes to trigger insulin secretion before being flash frozen.

Metabolite Extraction from Islets

The frozen islet pellet was first resuspended in 300 µl of cold methanol and transferred to a bead beating kit (Bertin Corp, USA). The islets were homogenized three times for 30 seconds, with cooling on ice for 1 minute on ice. The homogenized islets were then transferred to a 1.5 ml Eppendorf tube. 900 µl of cold methyl-tert-butyl-ether (MTBE) was then added to the homogenized islets. The sample was then mixed for 10 minutes at room temperature using a rotator. 250 µl of pure water was then added to the sample to initiate phase separation. The sample was then put on ice for 10 minutes for equilibration. After this step, the sample was centrifuged at 21,100 × g for 10 minutes at 4°C. The top non-polar phase (i.e., MTBE) was transferred to a glass vial, and the bottom polar phase (i.e., methanol and water) was transferred to a separate glass vial. Both fractions were then subjected to vacuum concentration using SpeedVac. The non-polar residue was then reconstituted in 160 µl of 1:8 toluene: methanol (v/v), while the polar residue was reconstituted in 60% acetonitrile: water (v/v). 40 µl of each sample was pooled to generate a quality control sample. Samples were transferred to mass spectrometry vials for LC-MS analysis.

LC-MS Conditions

Mobile phase A consisted of 0.1% formic in water (v/v). Mobile phase B consisted of 0.1% formic acid in acetonitrile (v/v). The column temperature was 40 °C and the Autosampler temperature: 4 °C. The sample injection volume was 5 µl. The flow rate of mobile phase A versus B over time was as follows:

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	0.4	99	1
1	0.4	99	1
15	0.4	0	100
19	0.4	0	100
20	0.4	99	1

The ESI source was operated in the positive/negative mode with the following parameters: capillary temperature, 350°C; source voltage and spray voltage, 4.0 KeV (positive); sheath gas (nitrogen) flow, 55 arbs (arbitrary units); and aux gas flow, 10 arbs. Probe heater temperature 300 °C; S-lens RF level 55.

Data was acquired using full MS scan (resolution: 70,000; AGC target: 1 × 106; Maximum IT: 200 ms; scan range: m/z 70-1000. Acquisition was performed in positive ion mode and negative ion mode separately. The spectra are recorded in profile type.

For data independent MS2 acquisition (DIA) mode, the DIA method consisted of a survey scan at resolution 35,000 from 70 to 1000 m/z (automatic gain control target of 1e6 or 100 ms maximum injection time). Then, 10 DIA windows are acquired at resolution 17,500 with automatic gain control target 2e5. Ten viable isolation windows and inclusion list are set up according to the feature distribution.