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LC-MS/MS Analysis

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We use this protocol and it's working

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Disclaimer

Abstract

LC-MS/MS Analysis

1 **Sample Injection** - Inject 3 µL of each sample into the MClass UPLC system (Waters Corp).

2 **Sample Trapping** - Trap the sample on a Symmetry C18 20 mm × 180 µm trapping column at a flow rate of 5 µL/min with a solvent composition of 99.9/0.1 v/v water/acetonitrile.

3 **Analytical Separation** - Perform analytical separation using a 1.8 µm Acquity HSS T3 C18 75 µm × 250 mm column (Waters Corp.). - Use a 90-minute linear gradient of 5 to 30% acetonitrile with 0.1% formic acid at a flow rate of 400 nanoliters/minute (nL/min). - Maintain the column temperature at 55°C.

4 **Mass Spectrometry Data Collection** - Perform data collection on the Thermo Orbitrap Fusion Lumos mass spectrometer equipped with a FAIMSPro device via a nanoelectrospray ionization source. - Use three different compensation voltages (-40V, -60V, -80V).

5 **Full MS Scan** - Use data-dependent acquisition (DDA) mode for each compensation voltage (CV). - Perform full MS scan from m/z 375 – 1500 with a resolution of 120,000 (@ m/z 200) and a target AGC value of 4e5 ions. - Set the max fill time to 35 ms.

6 **MS/MS Scan** - Acquire MS/MS scans with HCD settings of 30% in the linear ion trap in “rapid” mode. - Use a target AGC value of 1e4. - Set the max fill time to 35 ms. - Employ a 20-second dynamic exclusion to increase the depth of coverage.

7 **Cycle Time** - Set the total cycle time for each CV to 0.66 seconds. - Set total cycle times to 2 seconds between like full MS scans.

8 **Total Analysis Time** - The total analysis cycle time for each sample injection is approximately 2 hours.