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LC-MS/MS Analysis

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We use this protocol and it's working

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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 $\times$ 180 μm trapping column at a flow rate of 5 $\mu\text{L}/\text{min}$ 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 $\times$ 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.