

Jul 10, 2024

## LC-MS/MS Analysis

DOI

[dx.doi.org/10.17504/protocols.io.q26g71388gwz/v1](https://dx.doi.org/10.17504/protocols.io.q26g71388gwz/v1)

Shiyi Wang<sup>1</sup>

<sup>1</sup>Duke University

ASAP Collaborative Rese...



Shiyi Wang

Duke University

OPEN  ACCESS



DOI: [dx.doi.org/10.17504/protocols.io.q26g71388gwz/v1](https://dx.doi.org/10.17504/protocols.io.q26g71388gwz/v1)

**Protocol Citation:** Shiyi Wang 2024. LC-MS/MS Analysis. protocols.io <https://dx.doi.org/10.17504/protocols.io.q26g71388gwz/v1>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this protocol and it's working**

**Created:** July 10, 2024

**Last Modified:** July 10, 2024

**Protocol Integer ID:** 103179

**Keywords:** ASAPCRN

**Funders Acknowledgement:**  
**Aligning Science Across**  
**Parkinson's (ASAP) initiative**  
**Grant ID: ASAP-020607**



## Disclaimer

### DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

## Abstract

LC-MS/MS Analysis

- 1 **\*\*Sample Injection\*\*** - Inject 3  $\mu\text{L}$  of each sample into the MClass UPLC system (Waters Corp).
- 2 **\*\*Sample Trapping\*\*** - Trap the sample on a Symmetry C18 20 mm  $\times$  180  $\mu\text{m}$  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  $\mu\text{m}$  Acquity HSS T3 C18 75  $\mu\text{m}$   $\times$  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.