

FEB 28, 2024

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocols.io. 81wgbxe2ylpk/v1

Protocol Citation: John Chen, Romeo Maciuca, Paul Benton, Jung Suh, Sonnet Davis, Sarah Huntwork-Rodriguez, Ning Xia, Michael A. Schwarzschild 2024. LC/MS analysis of plasma samples from PPMI. protocols.io https://dx.doi.org/10.17504/protoc ols.io.81wgbxe2ylpk/v1

MANUSCRIPT CITATION:

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

C) LC/MS analysis of plasma samples from PPMI

John Chen¹, Romeo Maciuca¹, Paul Benton¹, Jung Suh¹, Sonnet Davis¹, Sarah Huntwork-Rodriguez¹, Ning Xia^{2,3}, Michael A. Schwarzschild^{2,3}

ASAP Collaborative Research Network

LRRK2



sarfraz.ahmed

ABSTRACT

Plasma samples from PPMI were analyzed by liquid chromatography with mass spectrometry (LC/MS) for a variety of metabolites (including piperine) and lipids as interrelated markers of Parkinson's disease and its pathophysiology.

¹Denali Therapeutics Inc, San Francisco, CA, USA;

²Molecular Neurobiology Laboratory, Department of Neurology, Massachusetts General Hospital, Boston, MA, USA;

³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network



Protocol status: Working We use this protocol and it's

working

Created: Jan 25, 2024

Last Modified: Feb 28, 2024

PROTOCOL integer ID: 94181

Keywords: LC/MS analysis, plasma samples, PPMI

Funders Acknowledgement:

Aligning Science Across Parkinson's (ASAP)

Grant ID: Grant ID: ASAP-000312

Plasma sample preparation for LC/MS analysis

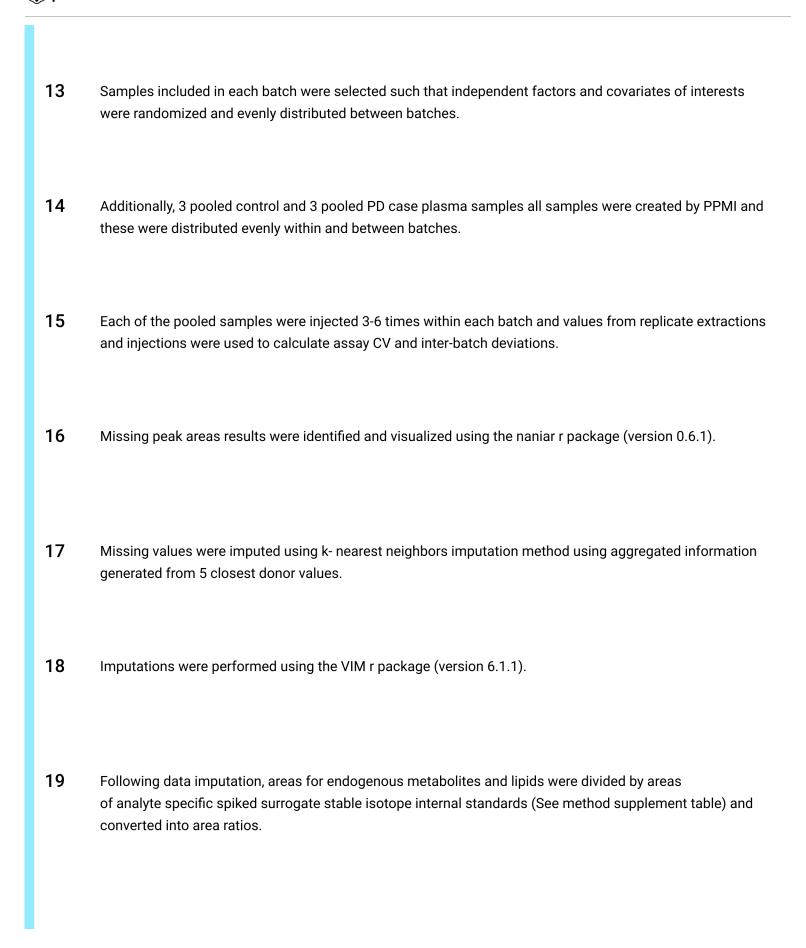
Thawed plasma samples were spun down at 3.5k*g for 10 min at 4°	1	Thawed plasma	samples were	spun down at	3.5k*q for	10 min at 4°
---	---	---------------	--------------	--------------	------------	--------------

- 2 Plasma (10 µL) samples were transferred to an Agilent Captiva collection plate and processed via the Agilent Bravo Metabolomics Sample Prep Platform (Agilent Technologies, Santa Clara, CA).
- Bravos Low Volume Plasma Metabolite/Lipid protocols were applied. 1:1 methanol/ethanol (v/v) (112.5 μ L) containing internal standards were added into each.
- 4 Plates were shaken for 1 min at 1000 rpm and incubated for 10 min at room temperature.

protocols.io

5	Water (82.5 µL) was added to each well, shaken for 1 min at 500 rpm, and incubated for 10 min at room temperature.
6	The sample (200 µL) was transferred to a Captiva EMR-Lipid plate to collect metabolite filtrate and remove protein and lipids.
7	The Captiva EMR-Lipid plate was washed twice with 250 μL of 1:1:1 water/ethanol/methanol (v/v/v).
8	The metabolite filtrate was dried under N ₂ gas for 4 h then reconstituted with 200 µL methanol.
9	To collect the lipids retained on the Captiva EMR-Lipid plate, 1:2 dichloromethane/methanol (v/v) (1.8 mL) was added to the Captiva EMR-Lipid plate for elution.
10	Lipid filtrate was collected in a 2mL glass coated microplate, dried under N ₂ gas for 4 h, and reconstituted with 200 μL methanol.
11	For analysis of the GlcCer/GalCer panel, a 25 µL aliquot from the methanol lipid fraction was dried under N2 gas for 4 h and resuspended in 50 µL of 92.5/5/2.5 acetonitrile/isopropanol/water) with 5mM ammonium formate and 0.5% formic Acid.
	Data reporting: Targeted LC-MS/MS data analysis

12 Both targeted and untargeted metabolomics and lipidomics analysis were analyzed in 8 different batches.

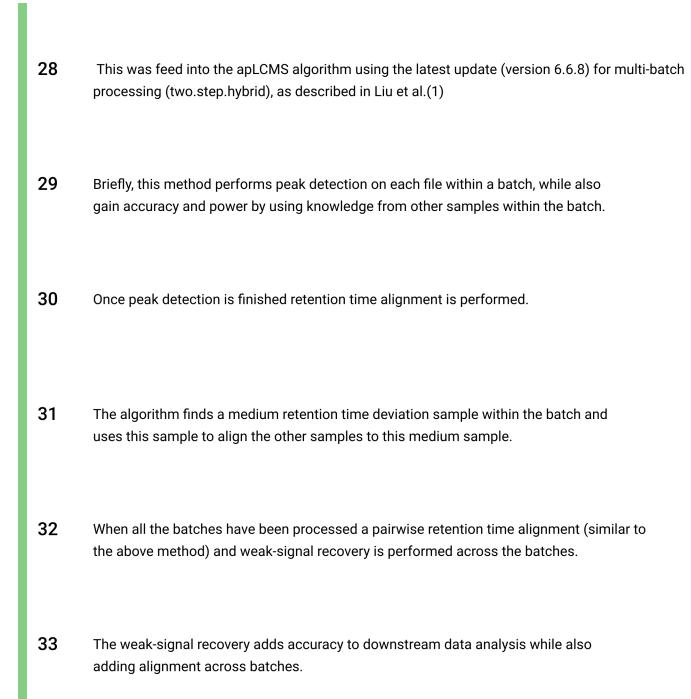


protocols.io

- Subsequently, inter-batch variances of area ratios were corrected by using the Combat function provided in the sva r package (version 3.38).
- Relative log expression plots of area ratios were plotted to visualize variations within and across batches. Following correction for between batch effects, imputed values were removed.
- For each non-imputed quantified analyte, un-normalized peak area (UNITS = area), ratios of endogenous metabolite area to surrogate internal standards (UNITS = area_ratio) and batch adjusted area ratios (UNITS = adjusted_area_ratio) were reported.
- Batch adjusted area ratios are recommended for downstream analysis. However, unnormalized areas and area ratios provided should allow for different normalization schemes to be adopted by end data consumers.
- Note that analytes with missing values in >70% of the samples were not reported.
- 25 Data analysis and reports were generated using R version 4.0.2.

Untargeted LC-MS data analysis

- Data files were extracted from Thermo 'raw' format to mzML using proteowizard msconvert (version-3.0.21334).
- A table of files and batch number was produced using the file name nomenclature.



Oct 28 2024