FEB 28, 2024

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/protocols.io.81wgbxe2ylpk/v1

MANUSCRIPT CITATION:

LC/MS analysis of plasma samples from PPMI

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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.

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

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

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).

3 Bravos Low Volume Plasma Metabolite/Lipid protocols were applied. 1:1 methanol/ethanol (v/v) (112.5 µL) 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.

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.

13 Samples included in each batch were selected such that independent factors and covariates of interests were randomized and evenly distributed between batches.

14 Additionally, 3 pooled control and 3 pooled PD case plasma samples all samples were created by PPMI and these were distributed evenly within and between batches.

15 Each of the pooled samples were injected 3-6 times within each batch and values from replicate extractions and injections were used to calculate assay CV and inter-batch deviations.

16 Missing peak areas results were identified and visualized using the naniar r package (version 0.6.1).

17 Missing values were imputed using k- nearest neighbors imputation method using aggregated information generated from 5 closest donor values.

18 Imputations were performed using the VIM r package (version 6.1.1).

19 Following data imputation, areas for endogenous metabolites and lipids were divided by areas of analyte specific spiked surrogate stable isotope internal standards (See method supplement table) and converted into area ratios.

20 Subsequently, inter-batch variances of area ratios were corrected by using the Combat function provided in the sva r package (version 3.38).

21 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.

22 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.

23 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.

24 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

26 Data files were extracted from Thermo ‘raw’ format to mzML using proteowizard msconvert (version-3.0.21334).

27 A table of files and batch number was produced using the file name nomenclature.

28 This was feed into the apLCMS algorithm using the latest update (version 6.6.8) for multi-batch processing (two.step.hybrid), as described in Liu et al.(1)

29 Briefly, this method performs peak detection on each file within a batch, while also gain accuracy and power by using knowledge from other samples within the batch.

30 Once peak detection is finished retention time alignment is performed.

31 The algorithm finds a medium retention time deviation sample within the batch and uses this sample to align the other samples to this medium sample.

32 When all the batches have been processed a pairwise retention time alignment (similar to the above method) and weak-signal recovery is performed across the batches.

33 The weak-signal recovery adds accuracy to downstream data analysis while also adding alignment across batches.