ADNI Alzheimer's Disease Neuroimaging Initiative

Targeted UHPLC-MS analysis of High-Value Metabolites in Serum Samples

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Summary

A high-throughput LC-MS based targeted quantitative assay for serum samples has been developed and applied to measure high-value metabolites (high-abundance) in ADNI serum samples. The samples were analyzed using a kit-based assay (96-well plate format).

Method

1. **Reagents and Materials**
1.1. Phenomenex Kinetex C18, 3.0 x 100 mm, 2.6 µm particle size
1.2. 0.45 µm or 0.22 µm membrane filter
1.3. Acetonitrile
1.4. Methanol
1.5. Ammonium acetate
1.6. Formic acid
1.7. Screw top glass vials and inserts
1.8. Eppendorf tubes
1.9. 96-well plate

2. Equipment
2.1. AB Sciex QTRAP® Mass Spectrometer
2.2. Agilent 1100/1200 Series LC
2.3. Analytical balance
2.4. pH meter
2.5. Centrifuge
2.6. Pipettes

3. **Standards (Analytes)/Internal Standards**

Standards	Internal Standards
ADMA	ADMA-d6
Beta-hydroxybutyrate	Beta-Hydroxybutyrate-d4
Betaine	Betaine-d9

Choline	Choline-d9
Creatinine	Creatinine-d3
Glucose	Glucose-13C6
Glutamate	Glutamic acid-d5
Glutamine	Glutamic acid-d5
Lactate	Lactate-d3
Leucine	Leucine-13C
Methionine	Methionine-d3
Phenylalanine	Phenylalanine-13C
Serine	Serine-13C
Taurine	Taurine-13C2
TMAO	TMAO-d9
Tryptophan	Tryptophan-d5
Tyrosine	Tyrosine-13C

4. Solutions

4.1. Extraction solvent: Methanol, 0.1% formic acid
4.2. Sample diluent: HPLC water, 0.1% formic acid

5. Samples

5.1. NIST SRM-1950 Reference Plasma – This commercial plasma sample was used as a quality control sample.
5.2. Duke-ADNI serum samples

6. Sample Preparation

Serum samples were thawed on ice on. Ten microliters of standards, QC samples and serum samples mixed with 10 µL internal standards were placed into the wells of a 96-well filter plate and extracted with 150 µL of extraction solvent (0.1% formic acid in methanol). The samples were vortexed at 150 rpm at 4°C for 30 minutes to facilitate protein precipitation. The samples were then filtered by centrifugation at 800 rpm and 4°C for 30 minutes. 130 µL of sample diluent (0.1% formic acid in water) was added to each well and the samples were subjected to LC-MS/MS analysis.
Following standards and QC samples were used in each plate : one reagent blank, 7 calibration stds, 3 QC stds (low, medium, high), 3 NIST SRM-1950 Reference Plasma, 2 QC pooled Human Serum. Batch effect and plate to plate variabilities were checked using those standards/QC samples.

7. LC/MS/MS Analysis

LC-MS/MS analysis was carried out on an Agilent 1290 UHPLC system coupled to an AB Sciex QTRAP® mass spectrometer equipped with a TurboIon spray. LC separation was carried out on a Phenomenex Kinetex C18 column (2.6 µm, 3.0 x 100 mm) with a flow rate of 0.3 mL/min and mobile phases 10 mM ammonium acetate, pH 3(mobile phase A) and 90:10 acetonitrile: 10 mM ammonium acetate, pH 3(mobile phase B). The gradient was as follows: 4 % eluent B for 2.5 min; 4-100 %B from 2.5 – 3.5 min; and held at 100 % from 3.5 – 5 min. From 5 - 9 min, the column was re-equilibrated to 4% B. The autosampler was cooled at 4°C.

8. **MS/MS Detection**

The QTRAP was operated in the positive mode for the analysis of the following analytes: ADMA, betaine, choline, creatinine, glutamic acid, glutamine, leucine, methionine, phenylalanine, serine, taurine, TMAO, tryptophan, and tyrosine and in the negative mode for the following analytes: β-hydroxybutyrate, glucose and lactic acid. The parameters of the source using nitrogen as a curtain gas were as follows: capillary ion spray voltage +4800 V in positive and −3000 V in negative modes, respectively; temperature 600◦C; curtain gas 10 psi, GS1 50 psi and GS2 50 psi. The entrance potential was set to 10V and collision cell exit potentials were optimized for each analyte. The analytes were measured in the multiple reaction monitoring mode (MRM). Declustering potential and collision energy were optimized for each analyte. Data were acquired and processed using Analyst software, v. 1.6.

Please note that we don't have any published paper yet for this method, as this is a part of new method development.

Version Information

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
Wishart Lab – High-value high-abundance metabolites	June 17, 2016

References

1. NA

About the Authors

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