ADNI Alzheimer's Disease Neuroimaging Initiative

UPLC/MS-MS Analysis of Human Serum Samples from the Alzheimer's Disease Neuroimaging Initiative using the Biocrates® Bile Acids Kit

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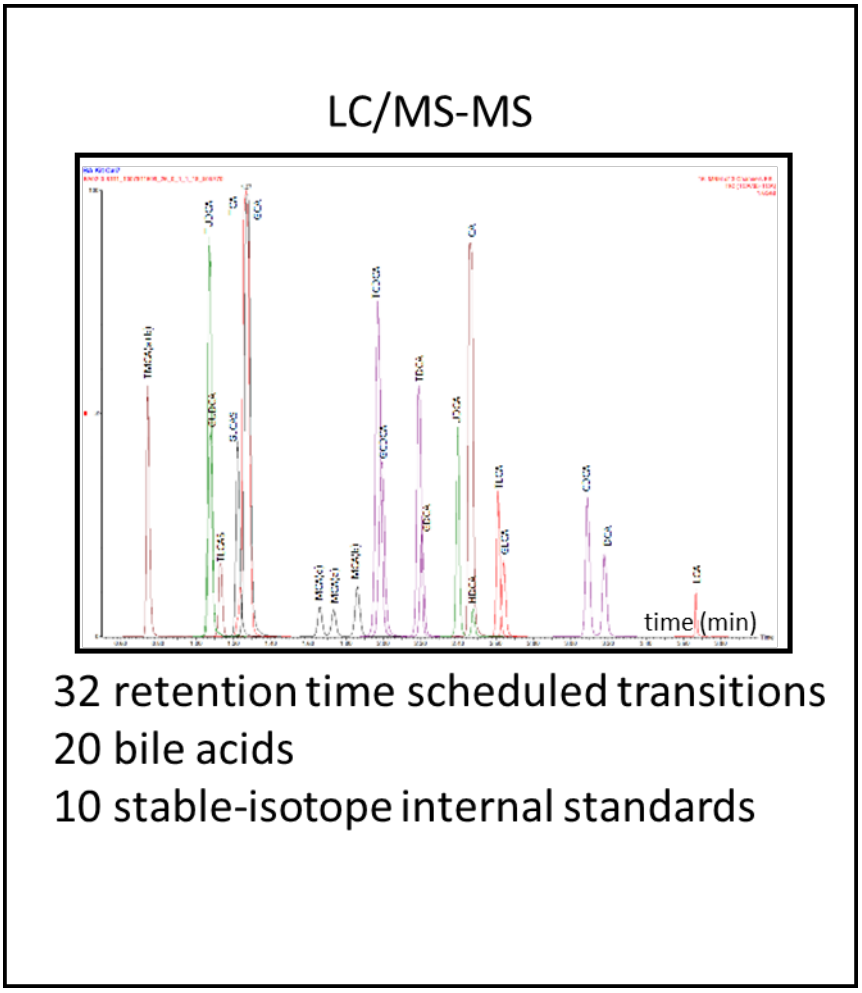
Objective

Measure the levels of selected bile acids in human serum samples from the ADNI 1 cohort using the Biocrates Bile Acids kit.

Introduction

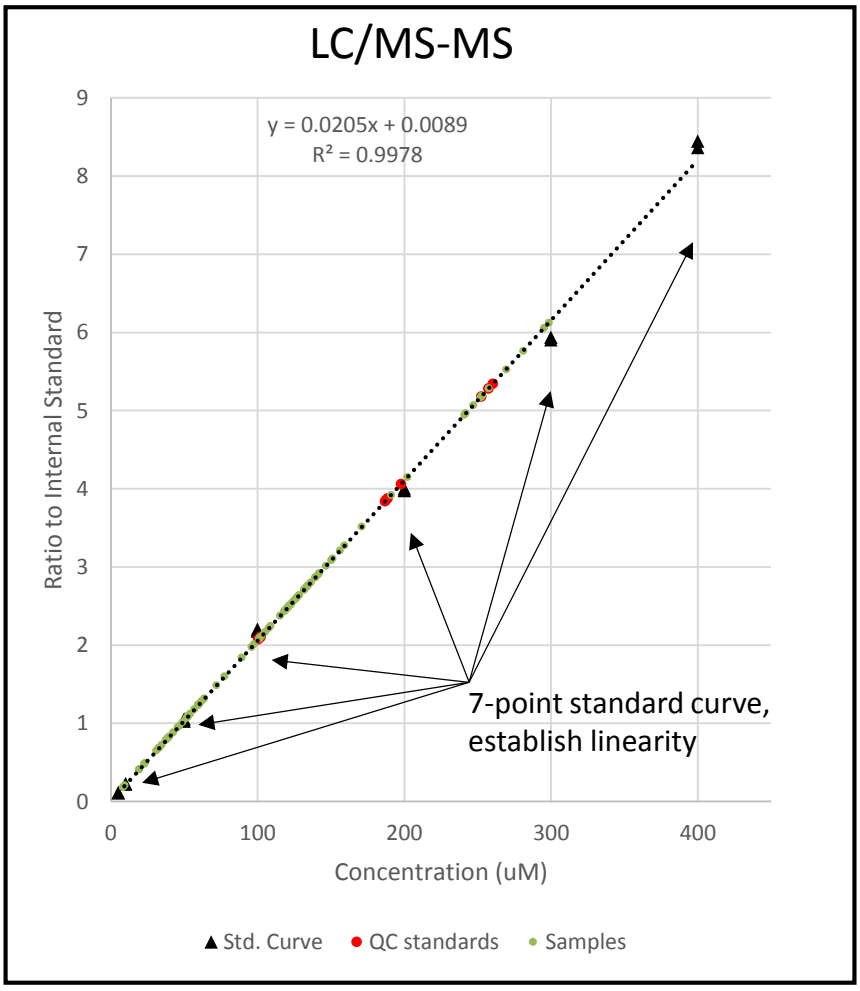
The Biocrates Bile Acids assay quantifies 20 bile acids, 16 of which are normally detected in human serum. Two sulphates (GLCAS and TLCAS) are also measured; however, Biocrates have not analytically validated the results for these compounds. The bile acids kit includes all requisite calibration standards, internal standards, and QC samples. The use of these standards according to the detailed analysis protocol which was validated in Biocrates' laboratory in Austria assures assay harmonization and standardization within a project, across projects, and across laboratories. Sample analysis of bile acids are performed by a UPLC (ultra-high pressure liquid chromatography) tandem mass spectrometry (MS) method using a reversed phase analytical column for analyte separation (LC/MS-MS, Figure 1). Selective analyte detection is accomplished by use of a triple quadrupole tandem mass spectrometer operated in Multiple Reaction Monitoring (MRM) mode, in which specific precursor to product ion transitions are measured for every analyte and stable isotope labeled internal standard.

Figure 1. Example of a liquid chromatography-tandem mass spectrometry (LC/MS-MS) analysis of bile acids using the Biocrates Bile Acids kit.



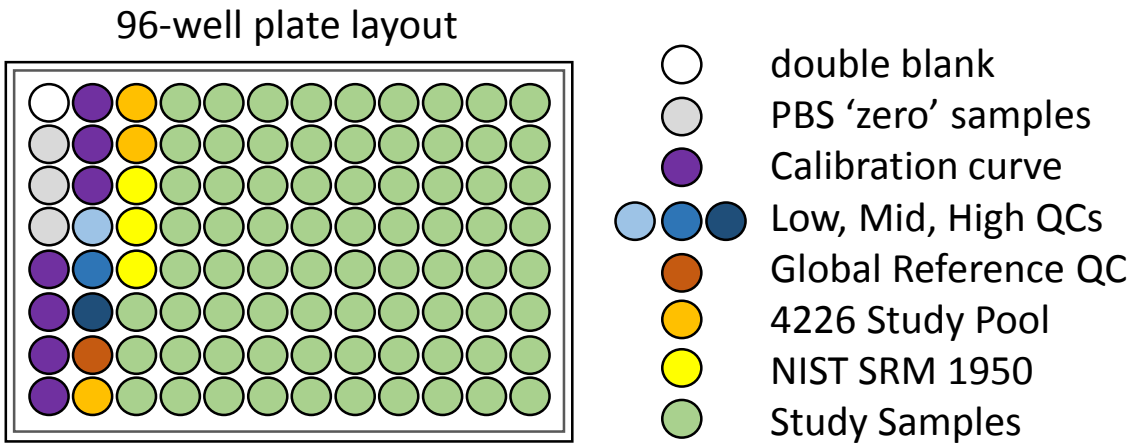
The seven calibration standards provided in the Biocrates Bile Acids kit were used for highly accurate and reproducible quantitation of the bile acids. **Figure 2** shows a schematic and representative examples of how the calibration curve was used to back-calculate QC and sample concentrations.

Figure 2. Schematic depicting the quantitative methodology used in the Biocrates Bile Acids kit.



The samples were prepared in a 96-well plate format using the layout shown in **Figure 3**.

Figure 3. Schematic depicting the 96-well plate layout for the analysis of study samples including: blanks, calibration standards, and QC samples from Biocrates, and three additional QC samples: the DPMCF Global Reference QC, the 4226 study pool, and the NIST SRM 1950 reference plasma standard (https://www-s.nist.gov/srmors/view_detail.cfm?srm=1950)



Sample Preparation

Samples were prepared using the Bile Acids kit (Biocrates Life Sciences AG, Innsbruck, Austria) in strict accordance with their detailed protocol. Addition of 10 µL of the supplied internal standard solution to each well of the 96-well extraction plate was followed by drying under a gentle stream of nitrogen. Study samples, calibration standards, and QCs were added in 10 µL aliquots to the appropriate wells. The plate was then dried a second time under a gentle stream of nitrogen. The samples were eluted with methanol then diluted with water for UPLC analysis.

To allow cross-plate normalization using a set of samples representative of the cohort, a study pool QC ("4226 study pool") was created by combining the unused portion of the serum samples from Plate 1 (samples F-001 through 076 excluding F-019 which was not provided). The 4226 study pool sample was divided into aliquots of 45 µL each, and frozen. For the analysis of each plate, a single 45 µl aliquot of the 4226 study pool was thawed with the study samples, prepared in triplicate (10 µL aliquots in three wells) and analyzed in the same way as the study samples. Replicates of this study pool were injected once before, once during, and once after the appropriate group of samples in order to measure the performance of the assay across the sample set.

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Sample Analysis

UPLC separation of bile acids was performed using a Waters (Milford, MA) Acquity UPLC with a proprietary reversed-phase UPLC column and guard column provided by Biocrates. Analytes were separated using a gradient from 10mm ammonium acetate, 0.015% formic acid in water to 10mm ammonium acetate, 0.015% formic acid in acetonitrile (65%) and methanol (30%). Total UPLC analysis time was approximately 6 minutes per sample. Using electrospray ionization in negative ion mode, samples were introduced directly into a Xevo TQ-S triple quadrupole mass spectrometer (Waters) operating in the Multiple Reaction Monitoring (MRM) mode. MRM transitions (compound-specific precursor to product ion transitions) for each analyte and internal standard were collected over the appropriate retention time. The UPLC-MS/MS data were imported into Waters application TargetLynx™ for peak integration, calibration, and concentration calculations. The UPLC-MS/MS data from TargetLynx™ were analyzed using Biocrates Me*tIDQ*™ software.

Dataset Information

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

Dataset Name	**Date Submitted**
ADMC Bile Acids Analysis Version XXX	XX January 2016

About the Authors

This document was prepared by: Lisa St. John-Williams (sample preparation, data collection, data analysis, report writing), Will Thompson (study design, scientific oversight, report writing), and Arthur Moseley (scientific oversight, report writing). For more information please contact Lisa St. John-Williams at +1 919 681 0327 or by email at lisa.stjohn-williams@duke.edu.