Project ID: DFI – HMMF – 708
Submitter, Submitter PI: Jeffrey Dewey, Dion Antonopoulos
Panel(s) Requested: TMS-MOX-3
Project Completed: March 28, 2023

Experiment Details

TMS-MOX

a. Chemistry and sample processing overview

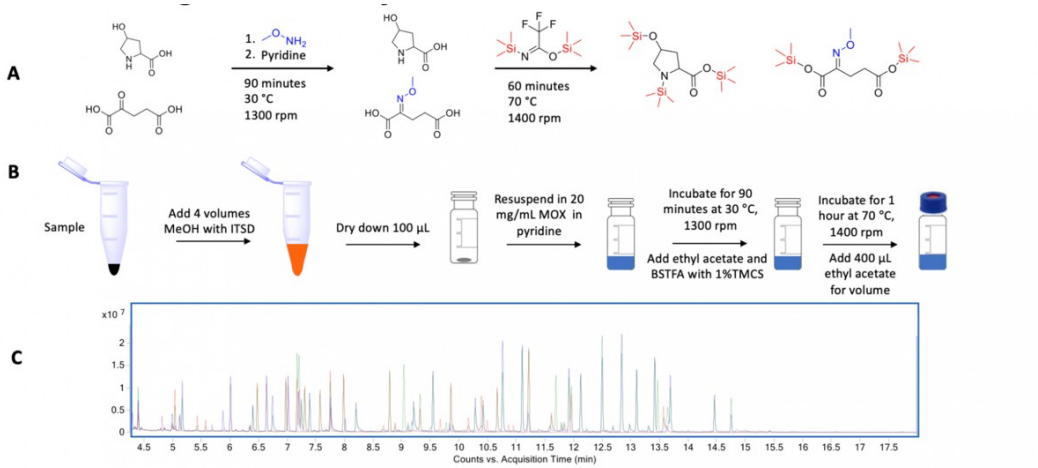

Figure 1. Trimethylsilane (TMS) and Methoxyamine (MOX) Derivatization. A) Hydroxyl groups and primary and secondary amines are derivatized with TMS, while keto and aldehyde carbonyls are derivatized with MOX. Derivatized analytes are subjected to Gas Chromatography Mass Spectrometry (GCMS) with Electron Impact Ionization (EI) and positive mode detection. This derivatization, ionization and detection enables clean, accurate, and robust detection of a large panel of diverse metabolites. B) Sample preparation workflow for metabolite extraction and derivatization. C) Example chromatogram showing peaks for a number of compounds in the panel.

b. Instrumentation used for analyses
    i. Positive electron impact - gas chromatography - mass spectrometry (+EI-GC-MS, Agilent, 7890B)
c. Files reported and analysis details
    i. Qualitative files: Qualitative values are calculated from the raw peak area of the endogenous compound normalized to the median raw peak area of two internal standards.
        1. Excel file of qualitative values
        2. Heatmap of the $\log_2$ fold change of the median-normalized peak areas for each compound. Grey indicates compound in sample was below minimum threshold.

HMMF Quality Control

Quality control samples:

- Control biological samples: These samples are used to evaluate all HMMF processes. Specifically, metabolite extraction efficiency and instrument performance. The samples are extracted with solvent containing known internal standard (IS) concentrations. These samples are processed and analyzed alongside project runs. Recovery, retention time and %CV are calculated for ISs.
- Method blanks (Labeled "MB" in datasets): These samples do not contain any metabolites and are included in datasets to indicate instrument noise for the m/z at the retention time extracted.
- PooledQC: A small aliquot of submitter samples are combined to create a PooledQC sample that is run to determine if the matrix submitted impacts processes.

Quality control ions:

- All submitted samples are extracted with solvent that includes deuterated or heavy atom standards with known concentrations to evaluate metabolite extraction efficiency and instrument performance.
- Quality control ions are tracked in all control samples and the submitted samples

Methods

Metabolite Extraction from Fecal/Cecal Material

Extraction solvent (80% methanol spiked with internal standards and stored at -80 ºC) was added to pre-weighed fecal/cecal samples at a ratio of 100 mg of material/mL of extraction solvent in beadruptor tubes (Fisherbrand; 15-340-154). Samples were homogenized at 4 ºC on a Bead Mill 24 Homogenizer (Fisher; 15-340-163), set at 1.6 m/s with 6 thirty-second cycles, 5 seconds off per cycle. Samples were then centrifuged at -10 ºC, 20,000 x *g* for 15 min and the supernatant was used for subsequent metabolomic analysis.

Metabolite Extraction from Plasma/Serum/Culture Supernatant

Samples were incubated at -80 ºC for at least one hour, or up to overnight. Extraction solvent (4 volumes of 100% methanol spiked with internal standards and stored at -80 ºC) was added to the liquid sample (1 volume) in a microcentrifuge tube. Tubes were then centrifuged at -10 ºC, 20,000 x *g* for 15 min and supernatant was used for subsequent metabolomic analysis.

Metabolite Analysis using GC-EI-MS and Methoxamine and TMS Derivatization

Metabolites were analyzed using GC-MS with electron impact ionization. The metabolite extract (100uL) was added to prelabeled mass spectrometer autosampler vials (Microliter; 09-1200) and dried down completely under nitrogen stream at 30 L/min (top) 1 L/min (bottom) at 30 ºC (Biotage SPE Dry 96 Dual; 3579M). To dried samples, 50 µL of freshly prepared 20 mg/mL methoxamine (Sigma; 226904) in pyridine (Sigma; 270970) was added and incubated in a thermomixer C (Eppendorf) for 90 min at 30 °C and 1400 rpm. After samples are cooled to room temperature, 80 µL of derivatizing reagent (BSTFA + 1% TMCS; Sigma; B-023) and 70 µL of ethyl acetate (Sigma; 439169) were added and samples were incubated in a thermomixer at 70 ºC for 1 hour and 1400rpm. Samples were cooled to RT and 400 µL of ethyl acetate was added to dilute samples. Turbid samples were transferred to microcentrifuge tubes and centrifuged at 4 ºC, 20,000 x g for 15 min. Supernatants were then added to mass spec vials for analysis using a GC-MS (Agilent 7890A GC system, Agilent 5975C MS detector) operating in electron impact ionization mode, using a HP-5MSUI column (30 m x 0.25 mm, 0.25 µm; Agilent Technologies 19091S-433UI) and 1 µL injection. Oven ramp parameters: 1 min hold at 60 ºC, 16 ºC per min up to 300 ºC with a 7 min hold at 300 ºC. Inlet temperature was 280 ºC and transfer line was 300 ºC. Data analysis was performed using MassHunter Quantitative Analysis software (version B.10, Agilent Technologies) and confirmed by comparison to authentic standards. Normalized peak areas were calculated by dividing raw peak areas of targeted analytes by averaged raw peak areas of internal standards.