GC Metabolomics Procedures

Aliquots of 150 µL human saliva were mixed with 1000 µL of degassed 3:3:2 Acetonitrile:Isopropanol:Water solution in a 2 mL snap cap tube. Samples are vortexed, shaken for 5 minutes then centrifuged at 4⁰C for 10 minutes at 14000rcf. The supernatant was recovered and transferred into a new 2mL Eppendorf tube. Samples were completely dried by vacuum centrifuge and reconstituted with 450 µL 1:1 acetonitrile:water solution. Samples were shaken for 5 minutes and centrifuged for 10 minutes at 14000rcf at 4°C and transferred supernatant to new snap cap tubes followed by complete evaporation by vacuum centrifuge. Dried samples were spiked with 10 µL of Methoxyamine Hydrochloride (MeOx) in pyridine (40 mg/mL) and placed onto a Thermomixer for 90 minutes at 30⁰C at maximum speed. After cooling to room temperature, samples were spiked with 91 µL of MSTFA+FAME mix (1 mL (N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) + 10 µL fatty acid methyl ester (FAME) retention index marker solution; FAME mix contains C8, C9, C10, C12, C24, C26, C28, and C30 fatty acid methyl esters)) and placed onto a Thermomixer for 45 minutes at 70⁰C at maximum speed. Samples were cooled to room temperature before transferring to GC vials with inserts. Sample preparation procedures were similar to those established by Dr. Oliver Fiehn’s Lab at UC Davis.

Each day samples were randomized to ensure that phenotypes were not grouped and to ensure that the holding time on the GC was random. QC pooled samples were interspersed during the analytical sequence. Samples were analyzed on an Agilent 7890 GC and a Leco Pegasus IV TOF-MS. Compounds were separated and characterized on a Restek RXI-5Silms capillary column (30 m X .25 mm X .25 µm) under the instrument acquisition parameters located in Table 1. Acquisition parameters were similar to parameter established by Dr. Oliver Fiehn’s lab.

Table 1. Instrument Acquisition Parameters

|  |  |
| --- | --- |
| Carrier Gas | Helium |
| Front Inlet Mode | Splitless |
| Front Inlet Temperature | 250⁰C |
| Front Inlet Purge Time | 25 sec after injection |
| Front Inlet Purge Flow | 40 mL/min |
| Injection Volume | 0.5 µL |
| Column Flow Rate | 1 mL/min constant flow |
| Oven Temp Initial | 75⁰C hold for 1 min |
| Oven Temp Ramp | 20⁰C to 330⁰C, hold for 3 min |
| Transfer Line Temp | 280⁰C |
| Source Temp | 250⁰C |
| Scan Range | 50 – 750 m/z |
| Scanning Cycle | 20 spectra/sec |

Following data acquisition, data files were processed for peak deconvolution by Leco’s ChromatTOF software and transferred to Binbase for spectral identification, peak retention index calculations and generation of a table of peak identifications and intensities.

References:

Fiehn, O. (2012*). QC and FAME Prep for GCTOF*. UC Davis West Coast Metabolomics Center.

Fiehn, O. (2012). *Sample Preparation of Blood Plasma or Serum Samples for GCTOF Analysis*. UC Davis West Coast Metabolomics Center.

Fiehn, O. (2012). *Primary Metabolism Analysis by GCTOFMS*. UC Davis West Coast Metabolomics Center.

O Fiehn, G. Wohlgemuth, M Scholz, T Kind, DY Lee, Y Lu, S Moon and B Nikolau: Quality control for plant metabolomics: reporting MSI-compliant studies. The Plant Journal2008; 53:691-704.