Protocol MSU_MSMC_007a
Gas chromatography-mass spectrometry profiling of central metabolites derivatized by methoximation/trimethylsilylation (GC/MS operating procedures)

Materials
Gas chromatography-mass spectrometry instrument (e.g. Agilent 7890 GC coupled to Agilent 5975 (or 7010) mass spectrometer with autosampler (e.g. CTC PAL)
Retention index mix of n-alkanes, C10-C40, all even, 50 ng/µL (Sigma-Aldrich #68281-2ML-F)
Sample metadata should be provided with each sample

Experiment design
Each batch of samples to be analyzed should include the following:

- One vial containing retention index mix of n-alkanes (50 ng/µL)
- One vial (for each 20 samples) containing a procedural blank prepared with derivatizing agents (methoxyamine hydrochloride in pyridine and MSTFA + 1% TMSCl (see Protocol MSU_MSMC_007)
- Metabolite extracts prepared and derivatized according to Protocol MSU_MSMC_007 and randomized in order of analysis (e.g. use RAND function in Microsoft Excel)
- One vial containing hexanes (solvent blank)

Order of sample analyses

1. Hexanes solvent blank
2. Procedural blank
3. n-alkanes retention mix standard solution
4. Derivatized metabolite extracts

Experimental parameters for GC/MS analysis

Column:	Agilent VF-5ms + 10 m EZ-Guard (30 m long, 0.25 mm I.D., 0.25 µm film; 10 m uncoated retention gap); part #CP9013
Mobile phase:	Helium
Mobile phase flow rate	1.2 mL/min (flow controlled)
Injection volume	1.0 µL
Injector type	Mixed mode injector (MMI) or programmed temperature vaporizer (PTV)
Injector temperature (program)	50˚C ramped to 250˚C at 12˚/min; hold at 250˚ until 50 minutes post-injection
Injection type	Splitless; purge at 0.4 min; purge flow 5 mL/min
Column temperature	50˚C (1 min hold), increase at 6˚/min to 330˚; hold at 320˚ for 5 minutes (total acquisition time: 52.67 minutes)
Ionization method	Electron ionization (70 eV)
Ion source temperature	250˚C
Transfer line temperature	280˚C

Data acquisition type	Scan
Scan range	*m/z* 80-600
Solvent delay (min)	3.0
Scan time (ms)	250
Detector gain	1
Mass calibration procedure	Tune must have been evaluated within the past seven days
Autosampler procedures	
PAL Cycle	GC-Liq4-V3
Wash solvent 1	Ethyl acetate
Wash solvent 2	Hexanes
Pre-clean with solvent 1	3
Pre-clean with solvent 2	3
Preclean with sample	1
% Syringe fill for cleaning	80
Syringe fill volume	8 µL
Sample amount for cleaning (µL)	1
Filling speed (µL/s)	5
Filling strokes	6
Volume for filling strokes	6
Injection speed (µL/s)	50
Pre-inject delay (ms)	500
Post-inject delay (ms)	500
Post clean with solvent 1	3
Post clean with solvent 2	3

Data processing to be described in a separate protocol.