Standard Operating Procedure-Instrument Preparation for Lipidomics Analysis

1. Purpose

Standardize LC-MS and/or LC-MS/MS lipidomics analysis of ceramides and sphingolipids.

2. Scope

This SOP applies to all LC-MS samples submitted for ceramide and sphingolipid quantification. Samples may come from academic laboratories or outside companies.

3. Prerequisites

Agreement between the client and our lab.

4. Responsibilities

Dr. Alan Maschek is the primary researcher responsible for this SOP and the procedures involved herein. Dr. James Cox, Steven Bell and Spencer Thompson are also covered in this SOP.

5. Procedures

Maintenance of LC-MS instrument

a. The instrument source will be wiped-down using isopropanol and a lint free cloth.
b. Each day the instrument is calibrated in both positive and negative mode using the Agilent check tune procedure. It must pass the Agilent tune prerequisites prior to analysis.
c. Standards purchased from Avanti Polar Lipids are analyzed to determine if the instrument is at the optimal sensitivity and the chromatographic column is performing optimally. If not instrument will be further cleaned using the manufacturer recommended procedure or the column is replaced.

LC-MS lipid analysis

a. All samples contain the same purchased isotopically labelled internal standards for quantification as above.
b. Lipid extracts are separated on an Acquity UPLC CSH C18 1.7 µm 2.1 x 50 mm column maintained at 60 °C connected to an Agilent HiP 1290 Sampler, Agilent 1290 Infinity pump, equipped with an Agilent 1290 Flex Cube and Agilent 6530 Accurate Mass Q-TOF dual ESI mass spectrometer or an Agilent

6490 triple quadrupole mass spectrometer. For positive mode analysis, the source gas temperature is set to 350 °C, with a gas flow of 11.1 (L/min) and a nebulizer pressure of 24 psig. VCap voltage is set at 5000 V, fragmentor at 250 V, skimmer at 74.4 V and Octopole RF peak at 750 V. For negative mode, the source gas temperature is set to 325 °C, with a drying gas flow of 12 L/min and a nebulizer pressure of 30 psig. VCap voltage is set at 4000 V, fragmentor at 225 V, skimmer at 75 V and Octopole RF peak at 750 V. Reference masses in positive mode (m/z 121.0509 and 922.0098) are infused when using the 6530 mass spectrometer with nebulizer pressure at 2 psig, in negative mode (1033.988, 966.0007, 112.9856 and 68.9958) are infused with a nebulizer pressure at 5 psig. Samples are analyzed in a randomized order in both positive and negative ionization mode in separate experiments acquiring with the scan range between m/z 100 – 1700. Mobile phase A consists of ACN:H2O (60:40 v/v) in 10 mM ammonium formate and 0.1% formic acid, and mobile phase B consists of IPA:ACN:H2O (90:9:1 v/v) in 10 mM ammonium formate and 0.1% formic acid. The chromatography gradient for both positive and negative modes starts at 15% mobile phase B then increases to 30% B over 4 min, it then increases to 52% B from 4-5 min, then increases to 82% B from 5-22 min, then increases to 95% B from 22-23 min, then increases to 99% B from 23-27 min. From 27-38 min it's held at 99%B, then decreases to 15% B from 38-38.2 min and is held there from 38.2-44 min. Flow is 0.35 mL/min throughout, injection volume is 1 µL for positive mode and 5 µL for negative mode. Tandem mass spectrometry is conducted using the same LC gradient at collision energies of 10 V, 20 V and 40 V.

c. Pooled samples are made from a fraction of each sample and run at the beginning, in between 4-6 samples and at the end of analysis to monitor instrument performance. The percent coefficient of variation is determined for each metabolite in the QC samples throughout the analytical run. Each metabolite must have a %CV less than 20% to pass quality control.

Data analysis

a. Results from LC-MS experiments are collected using Agilent Mass Hunter Workstation and analyzed using the software packages Mass Hunter Qual B.05.00 (Agilent Technologies, Inc.) and MZmine 2 (version 2.10).
b. Using Mass Hunter Qual, raw data files are exported as mzData files using the following parameters:peak filters (MS) absolute height >= 1000 counts and/or limit (by height) to the largest 100.
c. Using mzMine 2 chromatograms are processed in a chromatogram dependent manner as follows: mass detection, chromatogram builder, chromatogram deconvolution, deisotoper, join / RANSAC aligner, peak list row filter, duplicate removal and gap-filled thereby generating peak lists.
d. Data (m/z, RT, intensity) is subjected to different statistical approaches (e.g., PCA analysis) and peak lists are exported to Excel and sorted. Based on

identified m/z & RT pairs, these values are then used to build preferred lists for subsequent tandem mass spectrometry (MS-MS) experiments on appropriate samples.

Waste disposal

a. Chemical waste generated from sample extraction and LC-MS analysis are collected and pooled for collection from University of Utah Environmental Health and Safety.

6. Definitions

LC: Liquid Chromatography
MS: Mass Spectrometry
IPA: isopropyl alcohol
MeOH: methanol
MTBE: methyl *tert*-butyl ether
ACN: acetonitrile
%CV: percent co-efficient of variation