GC/MS Sample Preparation

Standard Operating Procedure-Sample Preparation for GC-MS

1. Purpose

To prepare samples for GC-MS analysis. This procedure covers the preparation of FAMES (Fatty Acid Methyl Ester) GC reference standard, MOX (Methoxyamine) derivatization of GC samples and setup of the instrument for analysis.

2. Scope

This SOP applies to all GC-MS samples submitted for GC metabolomics analysis. Samples may come from academic laboratories or outside companies.

3. Prerequisites

Agreement between the client and our lab.

4. Responsibilities

Quentinn Pearce is the primary researcher responsible for this SOP and the assays involved herein. Dr. Alan Maschek and Dr. James Cox are also covered in this SOP.

5. Procedures

MOX (Methoxyamine) derivatization

a) Determine the quantity of MOX solution needed for the sample derivatization. For each sample 40 µL will be added. **Example:** If one has 20 samples, 20 X 40 µL = 800 µL MOX is needed; make 1000 µL for extra samples. MOX solution consists of 40 mg/ml MOX in dry pyridine. **ATTENTION:** keep in mind that dryness is crucial for GC-MS analysis as water will consume the derivatization reagent. Pyridine used for making MOX (and FAMES solution) is DriSolv Pyridine from EMD. Before using the syringe to remove pyridine, briefly dry the syringe with a heat gun. Keep a positive flow of dry nitrogen through the solvent bottle (using another syringe) to keep out the moisture throughout the solvent-removing process. Weigh the appropriate amount of MOX (e.g. 40 mg for making 1000 µL MOX solution) in a microcentrifgue tube and add needed amount of pyridine. Briefly warm the solution with heat gun, dissolve MOX by inverting the tube several times.

b) To each sample tube, add 40 µL MOX solution prepared in a). Always use freshly-prepared MOX solution. Vortex the tube for 30 seconds and put into 40 ?C sand bath incubator for 1 hour. Use timer to keep track of the time.

c) After 1 hour incubation, take out the sample tubes and remove the sand stuck to the outside of the tubes. Spin at 14,000 rpm for 5 min with the Eppendorf 5418 microcentrifuge.

d) Prepare a set of GC glass vials and inserts. Glass vial used for GC-MS is Agilent Part # 5181-3376 (Vial, crimp, 2ml ambr, WrtOn, cert, 100PK), insert used for GC-MS is Agilent Part # 5183-2086 (400 µL Silanized Flat bottom Insert). Put an insert into each glass vial then label the vials appropriately.

e) Transfer 25 µL of cleared supernatant obtained after centrifugation in c) into corresponding labeled vial. Seal the vial using a crimper with the vial cap, using Agilent Part # 5188-5386 (Crimp cap, 11mm Magnetic CTC, 100pk;

golden-colored). The sample vials are ready to be loaded onto the autosampler sample tray.

Instrument Setup

f) Change liner and tune/calibrate the GCT Premier if necessary. Normally the liner should be changed after running 20 or more samples; GCT Premier should be tuned/calibrated once a week. Please refer to SOP (Instrument Preparation _ GCT Premier) for detailed procedures.

g) Add sample information to the corresponding sample list in Mass Lynx software. Sample lists are named as: GCT_Month_YEAR. Add appropriate number of sample files to the sample list. For routine sample run, required number of sample files to be added will be 2XN+N/6 (N is the number of samples to be run, 2XN is because a defaultShort and a defaultLong will be run for each sample; N/6 is because a hexane blank needs to be run every 6 samples). **Example:** total samples are 17, sample files to be added will be 2X17 + 17/6 = 34 + 17/6 (here in the case of non-integer, round up to next integer, 17/6 = 2.83, round up to 3) = 34 + 3 = 37. Need to add 37 sample files to the sample list.

h) **Randomize the samples** and put them into the sample tray. In the sample list add the sample information into the sample file following the sample order. **Example:** first sample is D4-suc/AA-IS, a defaultShort and a defaultLong method will be run on this sample; then the first sample file will be named as: 100:1 D4 AA (100:1 is the split ratio for default short method, meaning this is a default short run), the second sample file will be named as: 10:1 D4 AA (10:1 is the split ratio for default long method, meaning this is a default long run). Remember to add the hexane blank every 6 samples. Return the sample tray to the autosampler tray holder.

i) Check the inlet methods for defaultShort and defaultLong. The split ratio for defaultShort should be 100:1 and the split ratio for defaultLong should be 10:1.

j) In MassLynx select the samples files that will be run, start the sample list run. (Note: at this time no sample injection will occur as it will be waiting for the autosampler.)

k) In the MassHunter software that controls the autosampler select the appropriate prepSequence file, for the first run, add 60 uL MSTFA, for subsequent runs, add 20 uL of MSTFA.

l) Make sure the MSTFA (Vials 2 and 3) vials contain sufficient reagents for the run. It is critical that MSTFA + 1% TMCS is used. Also make sure the washing solution bottles Acetonitrile and hexanes (vials A1 and B1) in both towers contain enough washing reagents.

m) Start the Sequence from the MassHunter software.

Waste disposal

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

6. Definitions

GC: Gas Chromatography

MS: Mass Spectrometry

MOX: Methoxyamine

FAMES: Fatty Acid Methyl Ester

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