University of Michigan Department of Translational Pathology
Beecher Lab
Traverwood 2900 Huron Parkway Suite 100 Ann Arbor MI 48105

Plasma Preparation Method for LC and GC analysis

Protein Precipitation and filtration

1. Samples transferred from original vial to a 2mL deep well plate. Preferred volume for precipitation is 100µL.
2. Precipitate samples plus 2 Human Plasma Blanks (HB) and 2 Process Blanks (PB) with addition of cold (-20°C) 100% Ethanol with Algal standard already added. Vortex the deep well plate 2 min at 2000RPM. Volume of crash solvent is determined by 1:8 ratio (by volume). Ex: if sample is 100µL then precipitate w/ 800µL of crash solvent.
3. Plate is centrifuged at 4750 RPM 10 minutes temp set to 4°C. Post centrifuge solvent sample is carefully removed from pellet and transferred to protein filter plate and is either centrifuged at the same setting as the precipitation step or a vacuum is applied to the plate until entire sample has passed through filter into a new 2 mL deep well plate.

LC and GC Sample Plate Preparations:

4. Transfer solvent to sample plates (aluminum 96 shallow well with appropriate number of glass inserts. Default volumes are used for the Controls (PB and HB). Default volumes are: GC transfer 240 µL of solvent, for LC transfer 400µL.
   Unknowns solvent transfer volume for GC is determined by the total volume of crash solvent x 44%, for LC the total volume of crash solvent x 50%.
   Ex: solvent volume is 600µL. Solvent volume for dry down=600µL x 0.44=264µL transferred to GC and 600 x 50% = 300µL for LC.
5. Blow down plates under continuous 100%N_2 at RT°C for ~2.5 hr.
   Actual time under N_2 rack: _____

Reconstitution for LC analysis:

6. Remove plate from N_2 rack, add 27µL of H_2O with internal standards to each sample and controls (SB, PB, and HB). Add 27µL of water without internal standard to BB. Plate is ready for LC analysis.

Derivatization for GC analysis:

7. Remove plate from N_2 rack, place plate in Dry box for derivative reagent (DR) addition. Add 60µL of DR to each sample and controls (SB, PB, and HB). Add nothing to the BB controls. Crimp cap all samples, now plate can be safely removed from the Dry box.
8. Incubate the GC sample plate at 60°C for 30 min.
9. Post short cool down (3-5 min.) plate is now ready for GC analysis.

Observations:

Reagent preparations

Derivative Reagent (GC):

1. For each sample you will need 60µL of Derivatization Reagent (DR) plus 6 blanks (SB, PB and HB). *Always add in order of Pyridine, BHT, BSTFA + 1%. Tuning standard FAMES already present in a NEAT format within the 4mL amber vials. Transfer BSTFA +1% directly from primary storage container to derivatization solution as quickly as possible then mix reagents by swirling the container gently in a circular motion. Mix DR components as follows:

	% of Total Vol.	Volume (mL) Needed for 10mL	Actual Volumes (mL) Added
BHT (0.1mg/mL in pyridine)	3.6	.360	
Pyridine (anhydrous)	25	2.5	
BSTFA + 1%[1]	71.4	7.14	
FAMES[2]	NA	NA	

[1] BSTFA is photosensitive and decays when exposed to humidity.

[2] FAMES 60µL of 10mg/mg solution added to 4mL vial then dried under N_2 and stored @-80°C till use

Observations:

LC reconstitution solvent:

T-boc standards are premixed and dried down to neat format then stored at RT°C till use.

2. Remove T-boc vial from storage to make solution A. Add 250µL of 40% EtOH to the vial, vortex. Solution A can be stored at 2-8°C for up to 2 weeks.
3. To make T-boc dosing stock add 12.5µL of solution A to 987.5µL of distilled water, vortex. Dosing stock can be stored at 2-8°C for up to 2 weeks.

Observations:

Reagent or Lab Equipment	Manufacturer/ Supplier	Item #	Description	LOT/ EXP
BSTFA + 1%	Thermo	TS-38831	+1% TMCS ampoules	
Pyridine (anhydrous)				
BHT				
Ethanol (EtOH)				
FAMES	Made in house			
N2 Rack				
Dry Box (N_2)				
Dry Heat Block				
GC glass inserts	MicroLiter	08-0300	325µL cap/100pk	
LC glass inserts	MicroLiter	07-0000-101	270µL cap. /100pk	
GC glass insert cap	MicroLiter	08-0040A	Crimp caps	
LC glass insert cap	MicroLiter	07-0045R	Snap caps	

Scientist: _____ Date: _____