Protocol MSU_MSMC_006

Methoximation and *tert*-butyldimethylsilylation derivatization of amino acids and organic acids for GC/MS analysis

Version 1.2

Last updated on Nov 17, 2020

Reagents

Methoxyamine hydrochloride (Sigma-Aldrich #89803)

Pyridine (dry), (Sigma-Aldrich #270970-4X25ML)

N-Methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) containing 1% *tert*-butyldimethylsilyl chloride (TBDMSCl), in sealed glass ampules (Sigma-Aldrich #M-108)

Supplies

1.7-mL polypropylene microcentrifuge tubes with locking caps (VWR #490016-245)

Calibrated 1000-µL pipetter and pipet tips

Calibrated 10-µL pipetter and pipet tips

Vortexer

Ultrasound water bath

Labeled amber autosampler vials (BMB Stores #21140 with low volume (250 µL) glass inserts (BMB Stores #51832085) and PTFE-lined screw caps (BMB Stores #06718904)

Oven or heated block

Analytical balance (to 0.1 mg)

Spatula, precleaned

Samples

Use extracts of <u>one</u> of the following (after evaporation of solvents to dryness, typically in a screw cap vial; can use autosampler vials with inserts; extraction details are in separate SOPs):

- blood serum or plasma (30 µL)
- urine (50-100 µL)
- Cell cultures (10^7 cells)
- Cell culture media (50 µL)
- Homogenized tissue: 2-25 mg of tissue

Procedure

1. Set oven (or heated block) temperature to 60˚C.
2. Prepare labels for microcentrifuge tubes and GC vials
3. Use a spatula to weigh 0.040 g of methoxyamine hydrochloride into a 1.7 mL microcentrifuge tube labeled as “40 mg/mL methoxyamine-HCl in pyridine”
4. Transfer 1000 µL of dry pyridine into the above microcentrifuge tube; seal the tube.
5. Vortex briefly, then ultrasonicate for 15 minutes; ensure that all of the solid has dissolved before proceeding.
6. Add 100 µL of the methoxyamine-HCl/pyridine solution to each dried sample, blank, QC sample, and calibration standard.
7. Heat tubes at 60˚C for 12-24 hours; allow tubes to cool to room temperature.
8. Add 100 µL of MTBSTFA + 1% TBDMSCl and seal the tube.
9. Heat tubes at 60˚C for 12-24 hours; allow tubes to cool to room temperature.
10. Transfer 50 µL of each reaction mixture to an autosampler vial equipped with low-volume insert; cap the vial and transfer it to the GC/MS autosampler.
11. Transfer the remaining reaction materials to a separate labeled autosampler vial (no insert necessary) for storage.