DMPI GC-MS Sample Preparation Protocol for Blood Plasma/Serum

Objective

Illustrate the protocols used for the preparation of plasma/serum for GC-MS analysis.

Materials

1. Plasma or serum sample
2. Protein precipitant: methanol spiked with a retention-time-lock internal standard of 6.25 mg/L perdeuterated myristic acid (C14:0-D27-TMS)
3. Ethyl acetate
4. 18 mg/mL methoxyamine hydrochloride solution in pyridine
5. MSTFA derivatization reagent
6. QC plasma (e.g. NIST SRM 1950 plasma standard)

Equipment

1. 1 mL Eppendorf microcentrifuge tubes, pre-rinsed twice with methanol and dried
2. Centrifuge
3. Adjustable pipet with tips
4. GC vials
5. Vortex
6. SpeedVac sample concentrator
7. Incubator

Sample Extraction and Derivatization

1. Pipet serum/plasma samples and three QC plasma (100 µL each) into 1 mL Eppendorf microcentrifuge tubes.
2. Prepare three blanks using 1 mL Eppendorf mcirocentrifuge tubes.
7. Add 750 µL of protein precipitant (methanol spiked with a retention-time-lock internal standard of 6.25 mg/L perdeuterated myristic acid) to each sample, blank and QC.
3. Vortex these mixtures for one minute each.
4. Centrifuge samples for 5 min at 14,000 rpm (2081 x *g*).
5. Transfer 150 µL of the supernatant into new Eppendorf microcentrifuge tubes.
6. Speed Vac samples for 5 hours, followed by the addition of 100 µL ethyl acetate as an azeotropic drying agent, and another 45 minutes of SpeedVac drying.
7. Add 25 µL of 18 mg/mL methoxyamine hydrochloride in pyridine to each tube, vortex and pulse spin with centrifuge; incubate at 50 ^{o}C for 30 minutes.
8. Add 75 µL of MSTFA to each tube, vortex and pulse spin with centrifuge; incubate at 50^{o}C for 30 minutes.
9. Transfer samples to GC vials for analysis.
10. Analyze one blank and one QC sample first. If the retention times and peak shapes are consistent with established values, proceed to analyze samples.
11. Samples are run in a randomized order, with blank and QC samples at the beginning, middle, and end of every sequence.