

GC-MS Sample Preparation Protocol for Blood Serum / Plasma

1. Scope

This document applies to the preparation and derivatization of blood serum / plasma samples for analysis by GC-MS.

2. Objective

Illustrate the protocols used for the extraction and derivatization of serum / plasma metabolites for GC-MS analysis.

3. Materials

- 3.1. Serum or Plasma Sample
- 3.2. 8:1 Methanol (HPLC Grade): Water (double distilled) solution
- 3.3. 200 mg/10 mL (20 µg/mL) methoxyamine hydrochloride solution in pyridine
- 3.4. MSTFA derivatization reagent
- 3.5. 0.4 mg/mL Ribitol as internal standard solution
- 3.6. QC synthetic mixture (amino acid mixture: Val, Phe, Ser, Gly, Leu)
- 3.7. Hexane (HPLC grade) and Alkane Standard (C8-C20 and C20-C40)

4. Equipment

- 4.1. 1 mL Eppendorf tubes
- 4.2. Centrifuge
- 4.3. Adjustable pipet with tips
- 4.4. GC sample vials with 250 µL inserts
- 4.5. Vortex (touch mixer)
- 4.6. Speed Vac Concentrator
- 4.7. Vial Incubator

5. Extraction and Derivatization Protocol

- 5.1. Pipet 100 μL of serum / plasma into one mL Eppendorf tube, note the sample number and condition in lab book. Also, Pipet the same volume of HPLC water and QC mixture into two Eppendorf tube as Blank and QC.
- 5.2. Add 10 μL of internal Ribitol solution (0.4 mg/mL) as internal standard solution into each sample, Blank and QC.
- 5.3. Add 800 μL of 8:1 Methanol:Water solution, into each sample, Blank and QC.
- 5.4. Vortex these mixtures for one minute each, let them sit in fridge for 30 minutes with occasional shaking.
- 5.5. Centrifuge samples for 10 minutes at 10000 rpm.
- 5.6. Transfer 200 μL of the supernatant into 400 μL glass insert in 2 mL glass vial to be used for GC analysis.
- 5.7. Speed Vac samples under heat and vacuum for 3 or 4 hours then use the lyophilizer for further drying for another 1.5 ~ 2 hours till they are completely dry.
- 5.8. Add 40 μL of methoxyamine HCL to each vial, and incubate at room temperature for overnight 16 hours.
- 5.9. Add 50 μL MSTFA derivatization agent to each vial.
- 5.10. Incubate vial for 1.5 ~2 hours on hot plate at 80°C, vortex sample several times throughout the incubation process.
- 5.11. Mix 25 μL of Alkane Standard with 75 μL of Hexane (HPLC grade) as Alkane Standard solution in the insert.
- 5.12. Refrigerate sample until analysis.
- 5.13. First analyze Hexane, Alkane Standard solution, QC mixture, and Blank using GC-MS. If they all look good (check peaks and Ribitol signal), then proceed to run samples.
- 5.14. In one sequence, set ~20 samples to run. Must run QC and Hexane in every 10 samples. And also run QC and Hexane at the end of each sequence.