**GC-MS Sample Preparation Protocol for Organic Acids in Urine**

1. **Scope**

This document applies to the preparation and derivatization of urine samples for organic acid analysis by GC-MS.

1. **Objective**

Illustrate the protocols used for the extraction and derivatization of urinary organic acids for GC-MS analysis.

1. **Materials**
   1. Urine
   2. HPLC water
   3. 75 g/L (75 mg/mL) methoxyamine hydrochloride solution in pyridine
   4. BSTFA reagent
   5. 3.64 mM cholesterol as internal standard solution
   6. QC synthetic mixture (organic acid mixture, 5 mM)
   7. Ethyl acetate
   8. Hexane
2. **Equipment**
   1. 1.5 mL Eppendorf tubes
   2. Centrifuge
   3. Adjustable pipet with tips
   4. GC sample vials with 400 uL inserts
   5. Speed Vac Concentrator
   6. Vial Incubator
3. **Extraction and Derivatization Protocol**
   1. Preparation of blank: 200 μL HPLC water and 40 uL methoxyamine HCl in 2mL glass vials.
   2. Preparation of QC: 100 μL QC mix (200 μM) and 100 μL HPLC water, and 40 μL methoxyamine HCl in 2 mL glass vials.
   3. Pipet samples (200 μL urine) and 40 μL (75 g/L in H2O) methoxyamine HCl into 2 mL glass vials.
   4. Incubate samples at 60 ºC for 30 minutes.
   5. Transfer samples to 1.5 mL Eppendorf tubes.
   6. Add 20 uL of internal standard (cholesterol, 3.64 mM), and 600 uL of ethyl acetate. Vortex thoroughly for 1 minute. Spin samples at 10000 RPM for three minutes.
   7. Take 500 μL of the supernatant, and put into a new 2 mL glass vial.
   8. Add 600 μL of ethyl acetate to the Eppendorf tube. Vortex thoroughly for one minute. Spin samples at 10000 RPM for three minutes.
   9. Take 500 μL of the supernatant, and put into the 2 mL glass vial containing the previous supernatant (combine the supernatant from the two extractions).
   10. Evaporate samples to dryness under nitrogen with heat (35 ºC)
   11. Add 160 μL of Hexane and 40 μL of BSFTA.
   12. Incubate samples at 70 to 90 ºC for 15 minutes.
   13. Transfer samples to 250 μL insert (you can use the same vial).
   14. Refrigerate samples until analysis.
   15. 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.
   16. 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.