Sample preparation of blood plasma or serum samples for GCTOF analysis

Version: 1
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Summary: This SOP describes sample extraction and sample preparation for primary metabolism profiling by gas chromatography / time of flight mass spectrometry (GCTOF)

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Centrifuge	Eppendorf	5415 D
Calibrated pipettes 1-200 µl and 100-1000µl		
Eppendorf tubes 1.5 mL, uncolored		Cat. No. 022363204
ThermoElectron Neslab RTE 740 cooling bath		
MiniVortexer	VWR	58816-121
Orbital Mixing Chilling/Heating Plate	Torrey Pines Scientific Instruments	
Speed vacuum concentration system	Labconco Centrivap cold trap	
Precision balance with accuracy ± 0.1mg		
2mL crimp vials with Target Micro-Serts		
Agilent Electronic crimper and decapper		
Acetonitrile LCMS quality	JT Baker #9829-02	
Isopropanol HPLC solvent	JT Baker #9095-02	
pH paper 5-10	EMD Chem. Inc. Gibbstown NJ 08027	
Nitrogen line with pipette tip		
Methoxyamine hydrochloride [MeOX]	Aldrich: Cat. No. 226904	
Pyridine	Acros Organics	Cat. No. 270970
N-methyl-N-(trimethylsilyl)-trifluoroactamide [MSTFA]	Aldrich	Cat. No. 394866
FAME markers (refer to FAME marker SOP for preparation)		

Protocol:

Starting material:
Plasma/serum: 30 µl sample volume or aliquot

1. **Preparation of extraction mix before experiment:**
   1. Check pH of acetonitrile and isopropanol (pH7) using wetted pH paper
   2. Acetonitrile, isopropanol and water are mixed in volumes in proportion 3 : 3 : 2
   3. Rinse the extraction solution mix for 5 min with nitrogen with small bubbles. Make sure that the nitrogen line was flushed out of air before using it for degassing the extraction solvent solution

2. **Sample Preparation:**
   1. Switch on bath to pre-cool at –20°C (±2°C validity temperature range)
   2. Gently rotate or aspirate the blood samples for about 10s to obtain a homogenised sample.
   3. Aliquot 30µl of plasma sample to a 1.0 mL extraction solution. The extraction solution has to be pre-chilled using the ThermoElectron Neslab RTE 740 cooling bath set to -20°C.
   4. Vortex the sample for about 10s and shake for 5 min at 4°C using the Orbital Mixing Chilling/Heating Plate. If you are using more than one sample, keep the rest of the sample on ice (chilled at <0°C with sodium chloride).
   5. Centrifuge samples for 2min at 14000 rcf using the centrifuge Eppendorf 5415 D.
   6. Aliquot two 450µL portions of the supernatant. One for analysis and one for a backup sample. Store the backup aliquot in -20°C freezer.
   7. Evaporate one 450µL aliquots of the sample in the Labconco Centrivap cold trap concentrator to complete dryness.
   8. The dried aliquot is then re-suspended with 450 µL 50% acetonitrile (degassed as given above).
   9. Centrifuged for 2 min at 14000 rcf using the centrifuge Eppendorf 5415.
   10. Remove supernatant to a new Eppendorf tube.
   11. Evaporate the supernatant to dryness in the Labconco Centrivap cold trap concentrator.
   12. Submit to derivatization.

3. **Derivatization**
   - Prepare 40mg/mL MeOX solution in pyridine. Weigh out methoxyamine hydrochloride in 1.5mL Eppendorf tube on balance and add appropriate amount of pyridine.
   - Vortex MeOX solution and sonicate at 60^{o}C for 15 minutes to dissolve.
   - Add 10 µL of 40mg/mL Methoxyamine hydrochloride [MeOX] solution to each dried sample and standard
   - Shake at maximum speed at 30ºC for 1.5 hours.
   - To 1mL of MSTFA and add 10 µL of FAME marker. Vortex for 10sec.
   - Add 91 µL of MSTFA + FAME mixture to each sample and standard. Cap immediately.
   - Shake at maximum speed at 37 ºC
   - Transfer contents to glass vials with micro-serts inserted and cap immediately.
   - Submit to GCTOF MS analysis

4. **Quality assurance**
   - For each sequence of sample extractions, perform one blank negative control extraction by applying the total procedure (i.e. all materials and plastic ware) without biological sample.
   - Use one commercial plasma/serum pool sample per 10 authentic subject samples as control. If no suitable commercial blood sample is available, prepare a large pool sample during the

thawing/mixing step by aliquoting 100 ul per 1 ml plasma sample, and aliquot such pool sample for 1 pool extract per 10 authentic subject samples.

- Prepare at least one NIST plasma extract in the same manner

DISPOSAL OF WASTE: Collect all chemicals in appropriate bottles and follow the disposal rules. Collect residual plasma / serum samples in specifically designed red 'biohazard' waste bags.

References:

Fiehn O, Kind T (2006) Metabolite profiling in blood plasma. In: Metabolomics: Methods and Protocols. Weckwerth W (ed.), Humana Press, Totowa NJ