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UC Davis - Metabolomics: Sample preparation for GCTOF analysis

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Metabolomics Protocols & Workflows

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We use this protocol and it's working

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ABSTRACT

Summary:

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

References:

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

MATERIALS

MATERIALS

- ☒ Centrifuge **Eppendorf Centrifuge Catalog #5415D**
- ☒ Calibrated pipettes 1-200 µl and 100-1000 µl **Contributed by users**
- ☒ Eppendorf tubes 1.5 mL uncolored **Eppendorf Centrifuge Catalog #022363204**
- ☒ ThermoElectron Neslab RTE 740 cooling bath **Contributed by users Catalog #RT 740 cooling bath**
- ☒ MiniVortexer **VWR Scientific Catalog #58816-121**
- ☒ Orbital Mixing Chilling/Heating Plate **Torrey Pines Scientific Instruments**
- ☒ Speed vacuum concentration system **Labconco Centrивap cold trap**
- ☒ Precision balance with accuracy $\pm 0.1\text{mg}$ **Contributed by users**
- ☒ 2mL crimp vials with Target Micro-Serts **Contributed by users**
- ☒ Agilent Electronic crimper and decapper **Agilent Technologies**
- ☒ Acetonitrile LCMS quality **JT Baker Catalog #9829-02**
- ☒ Isopropanol HPLC solvent **JT Baker Catalog #9095-02**
- ☒ pH paper 5-10 **EMD Chem. Inc. Catalog #108027**
- ☒ Nitrogen line with pipette tip **Contributed by users**
- ☒ Methoxyamine hydrochloride [MeOX] **Sigma Aldrich Catalog # 226904**
- ☒ Pyridine **Acros Organics Catalog #270970-4X25ML**
- ☒ N-methyl-N-(trimethylsilyl)-trifluoroacetamide [MSTFA] **Sigma Aldrich Catalog #394866**
- ☒ FAME markers (refer to FAME marker SOP for preparation) **Contributed by users**

Note:

Eppendorf, [RRID:SCR_000786](#)

Sigma-Aldrich, [RRID:SCR_008988](#)

BEFORE START INSTRUCTIONS

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 ($\pm 2^{\circ}\text{C}$ validity temperature range)
- (2). Gently rotate or aspirate the blood samples for about 10s to obtain a homogenised sample.
- (3). Aliquot 30 μL 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 $<0^{\circ}\text{C}$ with sodium chloride).
- (5). Centrifuge samples for 2min at 14000 rcf using the centrifuge Eppendorf 5415 D.
- (6). Aliquot two 450 μL portions of the supernatant. One for analysis and one for a backup sample. Store the backup aliquot in -20°C freezer.
- (7). Evaporate one 450 μL aliquots of the sample in the Labconco Centrивap 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 Centrивap 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°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 30°C 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 °C
- 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.*