May 15, 2019

UC Davis - Metabolomics: Sample preparation for GCTOF analysis

DOI
dx.doi.org/10.17504/protocols.io.ytjfwkn

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DOI: dx.doi.org/10.17504/protocols.io.ytjfwkn

External link: https://mmpc.org/shared/document.aspx?id=121&docType=Protocol

Protocol Citation: Oliver Fiehn 2019. UC Davis - Metabolomics: Sample preparation for GCTOF analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ytjfwkn

Protocol status: Working
We use this protocol and it's working

Created: March 04, 2019

Last Modified: May 15, 2019

Protocol Integer ID: 21067

Keywords: GCTOF analysis

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 Catalog #**5415D
- Calibrated pipettes 1-200 ul and 100-1000 ul
- Eppendorf tubes 1.5 mL uncolored **Eppendorf Catalog #**022363204
- ThermoElectron Neslab RTE 740 cooling bath **Catalog #**RTE 740 cooling bath
- MiniVortexer **VWR International (Avantor) Catalog #**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 **Agilent Technologies**
- Acetonitrile LCMS quality **JT Baker Catalog #**9829-02
- Isopropanol HPLC solvent **JT Baker Catalog #**9095-02
- pH paper 5-10 **Merck Millipore (EMD Millipore) Catalog #**108027
- Nitrogen line with pipette tip
- Methoxyamine hydrochloride [MeOX] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 226904
- Pyridine **Acros Organics Catalog #**270970-4X25ML
- N-methyl-N-(trimethylsilyl)-trifluoroactamide [MSTFA] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**394866
- FAME markers (refer to FAME marker SOP for preparation)

Note:

Eppendorf, RRID:SCR_000786
Sigma-Aldrich, RRID:SCR_008988

Before start

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 60ºC 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.*