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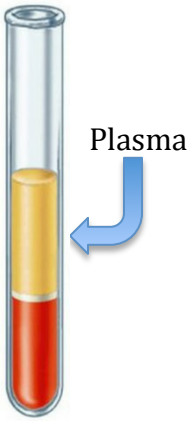
# Sample Collections for LM-SPM Profiling

## I. Human plasma collection and preparation for LC-MS/MS profiling

*Materials and reagents needed:*

A. 21-Gauge butterfly needle *(BD tube 21GX3/4"x12" REF#367326)*
B. Heparin (*Sagent Pharmaceuticals 1000 units/mL REF#25021-400-10*)
C. Collection syringe *(BD tube 10 mL REF#309604)*
D. 15 mL polypropylene tube (*Corning REF#430791)*
E. Deuterium-labeled stock *(Cayman Chemical: #314010 $d_4$-PGE$_2$, #10007737 $d_5$-LXA$_4$, #320110 $d_4$-LTB$_4$, #320110 $d_4$-LTB$_4$, #334230 $d_8$-5S-HETE, #11184 $d_5$-RvD$_2$)*
F. 100% Methanol (not LC/MS grade, *Sigma REF#34860-4X4L-R*)
G. Disposable culture tube *(Pyrex REF#30816444)*

*Plasma collection (3-5 mL needed for LC-MS/MS):*




1. Using a 21-Gauge butterfly needle[A], draw 10 mL of blood into a collection syringe[C] containing 100 µL of heparin[B] (10 units heparin/1 mL of blood)
2. Transfer blood from syringe into a 15 mL polypropylene tube[D]
3. For plasma separation, centrifuge for 20 min at 120 G (no brake to ensure that layers will not be disrupted)
4. Using a plastic Pasteur pipette, transfer plasma (the upper fraction) into a pre-labeled 15 mL polypropylene tube[D]
5. Cap tube and store at -20°C until used for solid phase extraction (*in a research setting,* top with nitrogen gas and cap)

*Protein precipitation (critical step):*

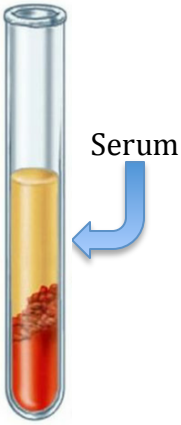
1. *(Performed in profiling lab)* Prepare deuterium-labeled standard by diluting stock[E] of 500 pg/µL to 500 pg/mL with 100% methanol[F] (1:1000 concentration)
2. Mix solution with Vortex Genie
3. Bring sample up to 4X the original volume by adding:
    a. 1 mL prepared deuterium-labeled standard
    b. X mL of 100% methanol[F] (EX: if 3 mL plasma is collected, add 1 mL of standard and 11 mL methanol)
4. Incubate for ~30 min at -20°C
5. Let tubes sit out at room temperature for ~2-3 min, then centrifuge for 10 min at 751 G (no brake)

6. *Optional duplicate analysis*, transfer half of the solution into a disposable culture tube[G] for solid phase extraction and store the remainder of the sample at -20°
7. Prepare sample and perform solid phase extraction

## II. Human serum collection and preparation for LC-MS/MS profiling

*Materials and reagents needed:*

A. A. 21-Gauge butterfly needle *(BD tube REF#367326, 21GX3/4"x12")*
B. Collection syringe *(BD tube 10 mL REF#309604)*
C. 10 mL serum separator tube (*BD tube REF#367820)*
D. 15 mL polypropylene tube (*Corning REF#430791)*
E. Deuterium-labeled stock *(Cayman Chemical: #314010 $d_4$-$PGE_2$, #10007737 $d_5$-$LXA_4$, #320110 $d_4$-$LTB_4$, #320110 $d_4$-$LTB_4$, #334230 $d_8$-5S-HETE, #11184 $d_5$-$RvD_2$)*
F. 100% Methanol (not LC/MS grade, *Sigma REF#34860-4X4L-R)*
G. Disposable culture tube *(Pyrex REF#30816444)*




*Serum collection (3-5 mL needed for LC-MS/MS):*

1. Using a 21-Gauge butterfly needle[A], draw 10 mL of blood into a collection syringe[B] WITHOUT anticoagulant
2. Transfer blood to a 10 mL serum separator tube[C]
3. Incubate at 37°C with 5% $CO_2$ for 24 h
6. Centrifuge for 20 min at 120 G (no brake to ensure that layers will not be disrupted)
4. Using a plastic Pasteur pipette, transfer serum (the upper fraction) into a pre-labeled 15 mL polypropylene tube[D]
5. Cap tube and store at -20°C until used for solid phase extraction (*in a research setting,* top with nitrogen gas and cap)

*Protein precipitation (critical step):*

1. *(Performed in profiling lab)* Prepare deuterium-labeled standard by diluting stock[E] of 500 pg/µL to 500 pg/mL with 100% methanol[F] (1:1000 concentration)
2. Mix solution with Vortex Genie
3. Bring sample up to 4X the original volume by adding:
    a. 1 mL deuterium-labeled standard
    b. X mL of 100% methanol[F] (EX: if 3 mL serum is collected, add 1 mL of standard, 11 mL methanol)
4. Incubate for ~30 min at -20°C

5. Let tubes sit out at room temperature for ~2-3 min, then centrifuge for 10 min at 751 G
6. *Optional duplicate analysis*, transfer half of the solution into a disposable culture tube[G] for solid phase extraction and store the remainder of the sample at -20°
7. Prepare sample and perform solid phase extraction

## II. Human tissue preparation for LC-MS/MS profiling

*Materials and reagents needed:*

A. 15 mL polypropylene tube (*Corning REF#430791)*
B. Deuterium-labeled stock *(Cayman Chemical: #314010 $d_4$-$PGE_2$, #10007737 $d_5$-$LXA_4$, #320110 $d_4$-$LTB_4$, #320110 $d_4$-$LTB_4$, #334230 $d_8$-5S-HETE, #11184 $d_5$-$RvD_2$)*
C. 100% Methanol (not LC/MS grade, *Sigma REF#34860-4X4L-R)*
D. Disposable culture tube *(Pyrex REF#30816444)*

*Protein precipitation (critical step):*

1. *(Performed in profiling lab)* Zero scale using a 15 mL polypropylene tube[A]
2. Using clean forceps, transfer tissue into tube and weigh
3. Prepare deuterium-labeled standard by diluting stock[B] of 500 pg/μL to 500 pg/mL with 100% methanol[C] (1:1000 concentration)
4. Mix solution with Vortex Genie
5. Bring sample up to 2X the original volume by adding:
    a. 1 mL prepared deuterium-labeled standard
    b. X mL of 100% methanol[C] (EX: if 3 mL tissue is obtained, add 1 mL of standard, 2 mL methanol)
6. Homogenize the tissue in solution using an electric tissue homogenizer
7. Incubate for ~45 min at -20°C
8. Let tubes sit out for ~2-3 min, then centrifuge for 10 min at 751 G
9. *Optional duplicate analysis*, transfer half of the solution into a disposable culture tube[D] for solid phase extraction and store the remainder of the sample at -20°
10. Prepare sample and perform solid phase extraction

## IV. Animal tissue (mouse, monkey, rabbit) preparation for LC-MS/MS profiling

*Materials and reagents needed:*

A. 15 mL polypropylene tube (*Corning REF#430791)*
B. Deuterium-labeled stock *(Cayman Chemical: #314010 $d_4$-PGE$_2$, #10007737 $d_5$-LXA$_4$, #320110 $d_4$-LTB$_4$, #320110 $d_4$-LTB$_4$, #334230 $d_8$-5S-HETE, #11184 $d_5$-RvD$_2$)*
C. 100% Methanol (not LC/MS grade, *Sigma REF#34860-4X4L-R)*
D. Disposable culture tube *(Pyrex REF#30816444)*

*Protein precipitation (critical step):*

1. *(Performed in profiling lab)* Zero scale using a 15 mL polypropylene tube[A]
2. Using clean forceps, transfer tissue into tube and weigh
3. Prepare deuterium-labeled standard by diluting stock[B] of 500 pg/μL to 500 pg/mL with 100% methanol[C] (1:1000 concentration)
4. Mix solution with Vortex Genie
5. Bring sample up to 2X the original volume by adding:
    a. 1 mL prepared deuterium-labeled standard
    b. X mL of 100% methanol[C] (EX: if 3 mL tissue is obtained, add 1 mL of standard, 2 mL methanol)
6. Homogenize the tissue in solution using a glass pestle tissue homogenizer
7. Incubate for ~30 min at -20°C
8. Let tubes sit out for ~2-3 min, then centrifuge for 10 min at 751 G
9. *Optional duplicate analysis*, transfer half of the solution into a disposable culture tube[D] for solid phase extraction and store the remainder of the sample at -20°
10. Prepare sample and perform solid phase extraction

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**Solid phase extraction (SPE) for LM-SPM Profiling**

Lipid Extraction:

After addition of internal standards and protein precipitation with methanol, bring down sample volume to 1 mL using a gentle stream of nitrogen

Add 9 mL of acidified water (pH 3.5)

Using the vacuum manifold:

Condition solid phase extraction cartridge (Biotage Isolute C18 100 mg/3mL; part no. 222-0010-B) by eluting 3 mL of methanol followed by 3 mL of double-distilled water

Pass sample through column slowly (ensure sample elutes drop-wise from cartridge)

Wash cartridge with 3 mL double-distilled water

Make sure column is as dry as possible by applying vacuum and using a kim-wipe to dry inside of cartridge

Wash with 3 mL hexane

Place borosilicate glass tubes under SPE cartridge and elute 3 mL methyl formate (SPMs)

Remove methyl formate fraction tubes; replace with clean borosilicate tubes and elute 3 mL methanol (for collection of cysteinyl leukotrienes and SPM sulfide conjugates).

Evaporate samples with gentle stream of nitrogen

Reconstitute with 50µL of 1:1 methanol:water

Vortex and centrifuge

Transfer to Eppendorf tubes

Centrifuge at max speed for 5 min in Eppendorf tube to remove additional precipitant

Transfer to auto-sample tube to run on LC-MS