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Version 1

## Untargeted Metabolomics: Extraction of Plasma Metabolites V.1

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**We use this protocol and it's working**

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## Disclaimer

N/A

## Abstract

The extraction of plasma metabolites for untargeted metabolomics involves methanolic protein precipitation from a fixed volume of fasting plasma, aiming to maximize coverage of the plasma metabolome. The proposed protocol includes both external and internal metabolite standards in the extraction process to answer to our quality control (QC) procedures. The expected outcome is a normalized and controlled extraction process, allowing for the accurate comparison of relative metabolite abundances across all samples within the large cohorts.

## Image Attribution

N/A

## Guidelines

N/A

## Protocol materials

-  Methanol LC-MS CHROMASOLV Fisher Scientific Catalog #15614740
-  Dimetridazole Merck MilliporeSigma (Sigma-Aldrich) Catalog #31707
-  2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0326
-  2-methyl-4-chlorophenoxyacetic acid (MCPA) Merck MilliporeSigma (Sigma-Aldrich) Catalog #45555
-  Dinoseb Merck MilliporeSigma (Sigma-Aldrich) Catalog #45453
-  L-Aspartic acid-<sup>15</sup>N Merck MilliporeSigma (Sigma-Aldrich) Catalog #332135
-  Atropine Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0132
-  Colchicine Merck MilliporeSigma (Sigma-Aldrich) Catalog #C9754
-  Imipramine hydrochloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #I7379
-  Acetonitrile ULC-MS Biosolve Catalog #0001204101BS
-  AMMONIUM CARBONATE, 99.999% Merck MilliporeSigma (Sigma-Aldrich) Catalog #379999
-  Ethylmalonic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #102687
-  Formic acid 99.0+% qualite LC/MS optima Fisher Scientific Catalog #10780320
-  Amiloride hydrochloride hydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7410
-  Ammonia solution 25% for HPL Merck MilliporeSigma (Sigma-Aldrich) Catalog #5438300100
-  prednisone Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6254
-  Metformine (1,1-Dimethylbiguanide hydrochloride) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D150959
-  water LC-MS Chromasolv Fisher Scientific Catalog #15665350
-  D-Glucose-1-<sup>13</sup>C Merck MilliporeSigma (Sigma-Aldrich) Catalog #297046

## Before start

To analyze plasma samples, it is essential to collect all samples using the same anticoagulant (preferably EDTA for LC-HRMS analyses)

For human samples analyses in France, a CODECOH ("**Déclaration ou autorisation des activités de conservation et de préparation à des fins scientifiques d'éléments issus du corps humain**") must be submitted at least 2-3 months in advance to ensure approval before sample reception.



## Randomisation

- 1 Reorganize plasma samples in random order on dry ice
- 2 Store at  -80 °C until aliquoting

## Sample aliquoting and quality control sample constitution

- 3 Thaw plasma samples (keep them fresh on ice)
- 4 Aliquoting : 50µL (or 25µL) depending on available volume
- 5 Create a quality control (QC) : pool of a same volume (~20µL) of all samples
- 6 Aliquoting QC : 50µL (or more) depending on available volume
- 7 Store at  -80 °C until sample extraction

## Sample extraction

- 8 Thaw plasma samples (keep them fresh on ice)
- 9 Performe metabolite extraction twice (HILIC and C18) from 25µL or 50µL of plasma
- 10 Add 4 volumes (100µL or 200 µL) of cold  
 Methanol LC-MS CHROMASOLV Fisher Scientific Catalog #15614740 containing  
internal standards (IS) et 37.5 ng/mL ((  
 Dimetridazole Merck MilliporeSigma (Sigma-Aldrich) Catalog #31707 ,

2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0326**

2-methyl-4-chlorophenoxyacetic acid (MCPA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #45555**

Dinoseb **Merck MilliporeSigma (Sigma-Aldrich) Catalog #45453**

11 Vortex  00:00:05 5s

12 Incubate on ice  01:30:00 until complete protein precipitation 1h 30m

13  20000 x g, 4°C, 00:15:00 15m

14 Collect supernatants

15 Dry supernatants under a nitrogen stream using a

#### Equipment

|              |       |
|--------------|-------|
| TurboVap LV  | NAME  |
| Evaporator   | TYPE  |
| BIOTAGE      | BRAND |
| TurboVap® LV | SKU   |

 30 °C

16 Store at  -80 °C until analysis



## sample reconstitution

15m 5s

17 Resuspend dried extracts in 3 volumes of

 AMMONIUM CARBONATE, 99.999% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #379999**

10mM pH10.5 (with

 Ammonia solution 25% for HPL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5438300100**)+ ES\* /  Acetonitrile ULC-MS **Biosolve Catalog #0001204101BS** (40:60, v/v) for ZIC-pHILIC analysis or water LC-MS Chromasolv **Fisher Scientific Catalog #15665350** / Acetonitrile ULC-MS **Biosolve Catalog #0001204101BS** (95:5, v/v), containing 0.1% Formic acid 99.0+% qualite LC/MS optima **Fisher Scientific Catalog #10780320**

and ES\* for C18 analysis. \*ES (external standards : mixture of 9 authentic chemical standards covering the mass range of interest :

 D-Glucose-1-<sup>13</sup>C **Merck MilliporeSigma (Sigma-Aldrich) Catalog #297046** , L-Aspartic acid-<sup>15</sup>N **Merck MilliporeSigma (Sigma-Aldrich) Catalog #332135** , Ethylmalonic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #102687** , Amiloride hydrochloride hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7410**,  prednisone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6254** , Metformine (1,1-Dimethylbiguanide hydrochloride) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D150959**,  Atropine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0132** , Colchicine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C9754** , Imipramine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I7379** )18 Vortex  00:00:05

5s

19  20000 x g, 4°C, 00:15:00

15m

20 Transfer supernatant into 0.2 mL vials



## QC reconstitution

15m 5s

21 Resuspend dried QCextracts in 3 volumes of

AMMONIUM CARBONATE, 99.999% Merck MilliporeSigma (Sigma-Aldrich) Catalog #379999

10mM pH10.5 (with

Ammonia solution 25% for HPL Merck MilliporeSigma (Sigma-Aldrich) Catalog #5438300100

) + ES\* / Acetonitrile ULC-MS Biosolve Catalog #0001204101BS (40:60, v/v) for ZIC-pHILIC analysis or

water LC-MS Chromasolv Fisher Scientific Catalog #15665350 /

Acetonitrile ULC-MS Biosolve Catalog #0001204101BS (95:5, v/v), containing 0.1%

Formic acid 99.0+% qualite LC/MS optima Fisher Scientific Catalog #10780320

and ES\* for C18 analysis. \*ES (external standards : mixture of 9 authentic chemical standards covering the mass range of interest :

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, Atropine Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0132 ,

Colchicine Merck MilliporeSigma (Sigma-Aldrich) Catalog #C9754 ,

Imipramine hydrochloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #I7379 )

22 Vortex 00:00:05

5s

23 20000 x g, 4°C, 00:15:00

15m

24 Transfer supernatant into 0.2 mL vials



25 Prepare 2-fold, 4-fold and 8-fold diluted QC samples

## Protocol references

Boudah *et al.* **Annotation of the human serum metabolome by coupling three liquid chromatography methods to high-resolution mass spectrometry.** J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Sep 1:966:34-47. DOI: [10.1016/j.jchromb.2014.04.025](https://doi.org/10.1016/j.jchromb.2014.04.025)