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Untargeted Metabolomics: Extraction of Plasma Metabolites V.2

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We use this protocol and it's working

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MetEx+

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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

- ☒ water LC-MS Chromasolv **Fisher Scientific Catalog #15665350**
- ☒ Alanine-C13 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #489867**
- ☒ ampicillin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #59349**
- ☒ Acetonitrile ULC-MS **Biosolve Catalog #0001204101BS**
- ☒ Formic acid 99.0+% qualite LC/MS optima **Fisher Scientific Catalog #10780320**
- ☒ D-Glucose-1-¹³C **Merck MilliporeSigma (Sigma-Aldrich) Catalog #297046**
- ☒ Amiloride hydrochloride hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7410**
- ☒ Ammonia solution 25% for HPL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5438300100**
- ☒ Ethylmalonic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #102687**
- ☒ Metformine (1,1-Dimethylbiguanide hydrochloride) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D150959**
- ☒ Atropine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0132**
- ☒ Imipramine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I7379**
- ☒ AMMONIUM CARBONATE, 99.999% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #379999**
- ☒ Colchicine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C9754**
- ☒ 2-aminoanthracene **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A38800**
- ☒ Dihydrostreptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D7253**
- ☒ Roxithromycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #R4393**
- ☒ L-Aspartic acid-¹⁵N **Merck MilliporeSigma (Sigma-Aldrich) Catalog #332135**
- ☒ prednisone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6254**
- ☒ 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**



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 MeOH containing internal standards (IS) at 37.5 ng/mL

extraction solvent :

 Methanol LC-MS CHROMASOLV Fisher Scientific Catalog #15614740

Internal standards :

 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 10mM pH10.5 (with Ammonia solution 25%)+ ES* / Acetonitrile (40:60, v/v) for ZIC-pHILIC analysis or ultrapure water / acetonitrile (95:5, v/v), containing 0.1% formic acid and ES* for C18 analysis.

reagents for mobile phases:

 water LC-MS Chromasolv **Fisher Scientific Catalog #15665350** AMMONIUM CARBONATE, 99.999% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #379999** Ammonia solution 25% for HPL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5438300100** Acetonitrile ULC-MS **Biosolve Catalog #0001204101BS** Formic acid 99.0+% qualite LC/MS optima **Fisher Scientific Catalog #10780320**

*ES (external standards : mixture of 14 authentic chemical standards covering the mass range of interest :

 D-Glucose-1-¹³C **Merck MilliporeSigma (Sigma-Aldrich) Catalog #297046** , L-Aspartic acid-¹⁵N **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** , Alanine-C13 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #489867**

⊗ 2-aminoanthracene Merck MilliporeSigma (Sigma-Aldrich) Catalog #A38800

⊗ ampicillin Merck MilliporeSigma (Sigma-Aldrich) Catalog #59349

⊗ Dihydrostreptomycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #D7253

⊗ Roxithromycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #R4393

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 extracts in 3 volumes of Ammonium carbonate 10mM pH10.5 (with Ammonia solution 25%)+ ES* / Acetonitrile (40:60, v/v) for ZIC-pHILIC analysis or ultrapure water / acetonitrile (95:5, v/v), containing 0.1% formic acid and ES* for C18 analysis.

reagents for mobile phases:

⊗ water LC-MS Chromasolv Fisher Scientific Catalog #15665350

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ampicillin Merck MilliporeSigma (Sigma-Aldrich) Catalog #59349



Dihydrostreptomycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #D7253



Roxithromycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #R4393

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)