

Oct 30, 2025

Version 2

🌐 Expanding the clinical use of DBS: through a metabolomic and a lipidomic evaluation_Mat&Meth V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbwkyggpk/v2>



Jérémy nteiro^{1,2,3}, cyrielle.clement⁴, Antoine Lefèvre^{1,2}

¹Université de Tours, INSERM, Imaging Brain & Neuropsychiatry iBrain U1253, 37032, Tours, France;

²Plateforme de Métabolomique et d'Analyses Chimiques, US61 ASB, Université de Tours, CHRU Tours, Inserm, Tours, France;

³MetaboHUB-Tours, Tours, France;

⁴MetaboHUB-MetaToul-Lipidomique, MetaboHUB-ANR-11-INBS-0010, Inserm U1297, Université Paul Sabatier Toulouse III, 31432 Toulouse, France



Jérémy Monteiro

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbwkyggpk/v2>

Protocol Citation: J  r  my nteiro, cyrielle.clement , Antoine Lef  vre 2025. Expanding the clinical use of DBS: through a metabolomic and a lipidomic evaluation_Mat&Meth. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.81wgbwkyggpk/v2>Version created by **J  r  my Monteiro**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 28, 2025

Last Modified: October 30, 2025

Protocol Integer ID: 230969

Keywords: metabolomics, lipidomics, targeted, untargeted, quantification, extraction, serum, Dried Blood Spot, biological information, LC-MS, SFC-MS, lipidome of serum, screening of metabolome, clinical use of db, metabolomic, metabolome, metabolite, lipidome, lipid, serum, clinical use, db, extraction to the data analysis

Funders Acknowledgements:

Agence Nationale de la Recherche

Grant ID: ANR-11-INBS-0010 MetaboHUB

Agence Nationale de la recherche au titre de France 2030

Grant ID: ANR-21-ESRE-0035

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

WARNING : this protocols is connect to the corresponding research paper about DBS and serum.

The only authors described here are the one which have developped or carry out the analysis.

In this protocole, multiple analysis are described to quantify metabolites and lipids & screening of metabolome and lipidome of serum and DBS. For every analysis, the whole process is described, from the extraction to the data analysis.

If some information are missing, please report to the corresponding research paper, in the Mat&Meth section or in the supplementary data.

Image Attribution

Laurent Galineau (Plateforme Imagerie Préclinique, US-61 ASB, Université de Tours, CHRU Tours, Inserm, Tours, France ; Université de Tours, INSERM, Imaging Brain & Neuropsychiatry iBrain U1253, 37032, Tours, France)
Camille Dupuy (Plateforme de Métabolomique et d'Analyses Chimiques, US61 ASB, Université de Tours, CHRU Tours, Inserm, Tours, France; MetaboHUB-Tours, Tours, France; Université de Tours, INSERM, Imaging Brain & Neuropsychiatry iBrain U1253, 37032, Tours, France)

Guidelines

Metabolomics is the “omics” science studying metabolites and can meet the need for a deeper insight into biological data. It offers a real-time snapshot of a metabolic state and so reflect immediate physiological changes. By revealing metabolic profiles, metabolomics allows for precise diagnosis, tailored treatments, and proactive health management, ensuring highly personalized and effective interventions.

(Nordström, A., Lewensohn, R. *Metabolomics: Moving to the Clinic. J Neuroimmune Pharmacol* **5**, 4–17 (2010). <https://doi.org/10.1007/s11481-009-9156-4>)



Materials

Mass Spectrometers

- Q-Exactive or equivalent
- Xevo TQ-XS or Xevo TQ-A
- SFC-G2-TOF or equivalent

Others

- agitators
- micro-pipettes (1000 μ L, 200 μ L, 20 μ L)
- planar agitator
- centrifuge
- SpeedVac
- N₂-flow evaporator
- falcon
- hemolyse tube
- eppendorf (1.5 mL)
- glass vial (2 mL)

Protocol materials

- ☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor)
- ☒ Methanol HiPerSolv CHROMANORM VWR International (Avantor)
- ☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich)
- ☒ Isopropanol HyperSolv CHROMANORM VWR International (Avantor)
- ☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich)
- ☒ Formic acid >99% for LC-MS VWR International (Avantor) Catalog #84865.260
- ☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor)
- ☒ Methanol HiPerSolv CHROMANORM VWR International (Avantor)
- ☒ formiate ammonium VWR International (Avantor)
- ☒ Aluminium oxide power Merck MilliporeSigma (Sigma-Aldrich)
- ☒ Calibration Solution Pierce LTQ Velos ESI Positive Ion Thermo Fisher
- ☒ Calibration Solution Pierce ESI Negative Ion Thermo Fisher
- ☒ IROA MSMLS, OAMLS, BACMSLS, FAMLS Merck MilliporeSigma (Sigma-Aldrich)
- ☒ L-tryptophan Merck MilliporeSigma (Sigma-Aldrich) Catalog #T0254
- ☒ Indoxyl-3-sulfate Merck MilliporeSigma (Sigma-Aldrich)
- ☒ Kynurenic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #K3375
- ☒ indole-3-Lactic Acid Merck MilliporeSigma (Sigma-Aldrich)
- ☒ water LC-MS Chromasolv Fisher Scientific Catalog #15665350
- ☒ Ammonium acetate Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1542-500G
- ☒ 3-nitrophenylhydrazine Merck MilliporeSigma (Sigma-Aldrich)
- ☒ acetic-13C2-acid Toronto Research Chemicals Inc
- ☒ butanoic-d7-acid Toronto Research Chemicals Inc
- ☒ valeric-d9-acid Toronto Research Chemicals Inc
- ☒ n-(3-dimethylaminopropyl)-n-ethylcarboiimide Merck MilliporeSigma (Sigma-Aldrich)
- ☒ 3-indolepropionic-d2-acid Toronto Research Chemicals Inc
- ☒ melatonin d4 Toronto Research Chemicals Inc
- ☒ 3-hydroxykynurenine 13C2,15N1 Toronto Research Chemicals Inc
- ☒ L-kynurenine d4 Toronto Research Chemicals Inc
- ☒ 5-hydroxy-L-tryptophan d4 Toronto Research Chemicals Inc
- ☒ 1H-indole-d5-3-acetamide Toronto Research Chemicals Inc



- ⊗ indole-3-aldehyde 13C8 **Toronto Research Chemicals Inc**
- ⊗ 3-hydroxyanthranilic-d3-acid **Toronto Research Chemicals Inc**
- ⊗ tryptamine d4 **Toronto Research Chemicals Inc**
- ⊗ L-tryptophan d5 **Toronto Research Chemicals Inc**
- ⊗ serotonin d4 **Toronto Research Chemicals Inc**
- ⊗ indoxyl-13C6 sulfate potassium salt **Toronto Research Chemicals Inc**
- ⊗ 5-hydroxyindole-3acetic-d5-acid **Toronto Research Chemicals Inc**
- ⊗ indoleacetic-d4-acid **Toronto Research Chemicals Inc**
- ⊗ tryptophane **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ tryptophol d4 **Toronto Research Chemicals Inc**
- ⊗ picolinic-d3-acid **Toronto Research Chemicals Inc**
- ⊗ kynurenic-d5-acid **Toronto Research Chemicals Inc**
- ⊗ Quinolinic-d3-acid **Toronto Research Chemicals Inc**
- ⊗ N-acetylserotonine d3 **Toronto Research Chemicals Inc**
- ⊗ indole-3-aldehyde **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ melatonin **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ 5-hydroxyindole acetic acid **Toronto Research Chemicals Inc**
- ⊗ picolinic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ indoxyl-3-sulfate **Toronto Research Chemicals Inc**
- ⊗ indole-3-lactic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ 5-hydroxytryptophane **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ kynurenine **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ 3-hydroxyanthranilic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ N-acetylserotonine **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ quinolinic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ 3-hydroxykynurenine **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ xanthurenic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ tryptophol **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ indole-3-acetic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ indole-3-propionic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ⊗ serotonin **Merck MilliporeSigma (Sigma-Aldrich)**



- ✕ tryptamine **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ indole-3-acetamide **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ kynurenic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ deoxycholic acid d5 **Toronto Research Chemicals Inc**
- ✕ lithocholic acid d4 **Toronto Research Chemicals Inc**
- ✕ cholic acid d5 **Toronto Research Chemicals Inc**
- ✕ cholic acid sulfate d4 **Toronto Research Chemicals Inc**
- ✕ glycocholic acid d4 **Toronto Research Chemicals Inc**
- ✕ taurocholic acid d4 **Toronto Research Chemicals Inc**
- ✕ ursodeoxycholic acid d4 **Toronto Research Chemicals Inc**
- ✕ chenodeoxycholic acid d4 **Toronto Research Chemicals Inc**
- ✕ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ acetic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ propionic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ butyric acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ isobutyric acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ valeric acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ isovaleric acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ taurochenodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ taurohyocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ glycohyocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ taurodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ glycodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ lithocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ glycochenodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ lithocholic acid sulfate **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ tauroolithocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ cholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ glycolithcholic acid **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ cholic acid sulfate **Merck MilliporeSigma (Sigma-Aldrich)**
- ✕ taurocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**



⊗ deoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ ursodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ hyocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ glyoursodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ tauroursodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ chenodeoxycholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ glycocholic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ aconitic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ fumaric acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ malic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ citric acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ isocitric acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ ketoglutaric acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ succinic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ itaconic acid **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ lactic acid **Merck MilliporeSigma (Sigma-Aldrich)**



Troubleshooting

Problem

Retrieving a punch from a dry blank blotter paper

Solution

Soaked them with LC-MS grade water and then punch them

Problem

Normalisation of quantification in DBS

Solution

Not used in this study but if you can, measured the hematocrit level of every patient during sampling session

Problem

DBS storage

Solution

Dedicate a space at -80°C or -20°C for your DBS with a desiccator

Problem

Contamination with 3-nitrophenylhydrazine

Solution

LC-MS system: be careful if you shrink gradient length Pipettes: avoid (if possible) to use only 1 pipette for all
→ prefer one pipette per type of solution

Safety warnings

- ⚠ Be really carefull with DBS handling (no contact with skin, don't breath on it) and storage (humidity, UV exposition, temperature and long-time storage).

Ethics statement

DBS and serum samples were collected at the same time, **from 4 healthy patients**. All samples were obtained in accordance with French legislation (Loi Jardé). In French hospitals with a research mission, the use of sample leftovers for research purposes is based on the principle of no opposition. According to Loi Jardé, no requirement of Ethical Committee or Institutional Review Board was required because no new sampling was required. This law also stipulates that patients must be fully informed of this information/practice and patients must have the possibility, at any time, of objecting to the use of their samples in research. The analyses were always performed in accordance with confidentiality rules. Data were coded without mention of first and last names, and the results were produced in a way that does not allow patients to be identified. Patients did not invoke their right to have a right of access, rectification, portability and limitation of the processing of data and/or biological samples.

Before start

- Dried Blood Spot (DBS) represent the whole blank blotter paper with several blood soaked spots : only these spots are interesting so it is mandatory to retrieve them as 3 mm diameter disc called a punch, with specific equipment : a puncher (available in mostly all hospitals)
- If you are going to do some comparison between DBS and other blood sample, try to have all you sample from same patient(s)

A.1. Large Targeted Metabolomics

8h

1 Extraction of DBS

- 1.1 In the whole DBS card Whatman 903™, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

- 1.2 Put the 2 punches in a 1.5 mL Eppendorf vial

- 1.3 Add 200 µL of

⊗ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

⊗ Methanol HiPerSolv CHROMANORM VWR International (Avantor) /

⊗ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (4:4:2)

- 1.4 Vigourously vortexed during 00:01:00

1m

- 1.5 Planar agitation : 01:00:00 , Room temperature , strength 8/10

1h

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

1.6

 10000 x g, 4°C, 00:10:00

10m

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

1.7

Transfert  150 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

1.8

Dry for  01:00:00 under vacuum at  40 °C

1h

1.9

Dried extract is redissolved with  75 µL of:



- For reversed-phase analysis:

☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (2:8)

- For HILIC analysis:

☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (8:2)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

1.10 Vigorously vortexed during ⌚ 00:01:00 at 🌡 Room temperature

1.11 Planar agitation: ⌚ 00:30:00 , 🌡 Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

1.12 Transfert whole aliquot in a  200 μL 96-well plates (1 for reversed-phase; 1 for HILIC)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

1.13 Injection Volume (μL) : 10



2 Extraction of Serum

2.1 Make aliquots of  50 μL of serum

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

2.2 Add  150 µL of cold

 Methanol HiPerSolv CHROMANORM VWR International (Avantor) ( -20 °C)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

2.3 Vigourously vortexed during  00:01:00

2.4 Let it  00:20:00 at  -20 °C

20m

2.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND



2.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

2.7 Transfert  150 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

2.8 Dry for  01:00:00 under vacuum at  40 °C

2.9 Dried extract is redissolved with  100 µL of:

- For reversed-phase analysis:

☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (2:8)

- For HILIC analysis:

☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (8:2)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

2.10 Vigorously vortexed during ☒ 00:01:00 at ☒ Room temperature

2.11 Planar agitation: ☒ 00:30:00 , ☒ Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

2.12 Transfert whole aliquot in a  200 µL 96-well plates (1 for reversed-phase; 1 for HILIC)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

2.13 Injection Volume (µL) : 5



3 LC parameters #1 - Reversed-Phase

3.1 Phase A:

- 200 mL + 5.5 mL * [Number of injections]

-  Water milliQ Merck MilliporeSigma (Sigma-Aldrich) + 0.1%

-  Formic acid >99% for LC-MS VWR International (Avantor) Catalog #84865.260

- Stir vigorously during  00:01:00 by hand

- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

3.2 Phase B:

- 200 mL + 3.8 mL * [Number of injections]
-  Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** + 0.1%

 Formic acid >99% for LC-MS **VWR International (Avantor)** Catalog #84865.260
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

3.3 Chromatographic System

Equipment

Ultimate 3000	NAME
UHPLC	TYPE
Dionex Softron	BRAND
SRD-3400	SKU
Pump, Column Compartment, AutoSampler	SPECIFICATIONS

3.4 Install the column in the oven

Equipment

SecurityGuard ULTRA Cartridges

NAME

UPLC C18 2.1mm ID Columns

TYPE

Phenomenex

BRAND

AJ0-8782

SKU

Equipment

Kinetex 1.7µm XB-C18 100A

NAME

Column

TYPE

Phenomenex

BRAND

00F-4498-AN

SKU

LC Column 150 × 2.1mm

SPECIFICATIONS

3.5 Adjust the temperature ( 55 °C) and let it heat the core of the column

3.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

3.7 Prepare the piston "lubrication" solution (90%

⊗ Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

⊗ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

⌚ 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

3.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B

- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

3.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

3.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	0.5	2
	0	0.5	2
	2	0.5	2
	6	0.5	30
	9	0.5	75
	9.5	0.5	100
	11.8	0.5	100
	12	0.5	2
	13.7	0.5	2

4 LC parameters #2 - HILIC

4.1 Phase A:

- 200 mL + 2.6 mL * [Number of injections]
-  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** +  10 millimolar (mM)
-  formiate ammonium **VWR International (Avantor)** + 0.1%
-  Formic acid >99% for LC-MS **VWR International (Avantor) Catalog #84865.260**
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

4.2 Phase B:

- 200 mL + 3.8 mL * [Number of injections]
- [95% of
 - ☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) & 5% of
 - ☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich)] + [M] 10 millimolar (mM)
 - ☒ formiate ammonium VWR International (Avantor) + 0.1%
 - ☒ Formic acid >99% for LC-MS VWR International (Avantor) Catalog #84865.260
- Stir vigorously during ⌚ 00:01:00 by hand
- Ultrasound during ⌚ 00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

4.3 Chromatographic System

Equipment

Ultimate 3000	NAME
UHPLC	TYPE
Dionex Softron	BRAND
SRD-3400	SKU
Pump, Column Compartment, AutoSampler	SPECIFICATIONS

4.4 Install the column in the oven



Equipment

CORTECS UPLC HILIC 1.6 μ M

NAME

Column

TYPE

Waters

BRAND

4.5 Adjust the temperature ( 40 °C) and let it heat the core of the column

4.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH

NAME

Ultrasound bath

TYPE

VWR

BRAND

142-0084

SKU

230V 50Hz 370VA

SPECIFICATIONS

4.7 Prepare the piston "lubrication" solution (90%

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH

NAME

Ultrasound bath

TYPE

VWR

BRAND

142-0084

SKU

230V 50Hz 370VA

SPECIFICATIONS

4.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B
- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

4.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 95%B

4.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	0.5	95
	0	0.5	95
	.9	0.5	95
	4.8	0.5	82
	9	0.5	10
	11.2	0.5	10
	11.4	0.5	95



	Retention (min)	Flow (mL/min)	%B
	13.7	0.5	95

5 Mass Spectrometry

5.1 Mass Spectrometer

Equipment

Q-Exactive

NAME

Mass Spectrometer

TYPE

ThermoScientific

BRAND

HESI Source

SPECIFICATIONS

MS Parameters :

- Scan Range: 58-870 m/z
- Resolution MS1: 70,000
- Resolution MS2: 15,000
- Polarity: POS or NEG
- Microscans: 1
- Lock mass: None
- AGC Target: 1E6
- Maximum Inject Time: 250
- Sheath Gas Flow Rate: 50
- Aux Gas Flow rate: 15
- Sweep Gas flow Rate: 1
- Spray Voltage (kV): +/-2.5
- Capillary Temperature (°C): 325
- S-lens RF level: 60
- Aux gas Heater temperature (°C): 400

5.2 Cleaning the source:

- Set source temperature to  50 °C

- Let it "cool" 01:00:00
- Unplug the Ion MAX API source with HESI probe attached
- Unscrewed the Sweep Cone and the capillary
- Immediately plug another cleaned capillary
- Clean the sweep cone with wet
 - ⊗ Aluminium oxide power **Merck MilliporeSigma (Sigma-Aldrich)**
- Rinse thoroughly with
 - ⊗ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**
- In a glass beaker, cover completely the Sweep Cone and the capillary with
 - ⊗ Methanol HiPerSolv CHROMANORM **VWR International (Avantor)**
- Ultrasound during 00:10:00

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

- Let it dry
- Replace the cone and the Ion Max API source with HESI probe
- Set source temperature back to normal

5.3 Reinstall the source & allow it to return to analysis conditions

5.4 Calibration:

- POS: ⊗ Calibration Solution Pierce LTQ Velos ESI Positive Ion **Thermo Fisher**
- NEG: ⊗ Calibration Solution Pierce ESI Negative Ion **Thermo Fisher**
- RMS < 0.4/0.4 in both ionisation mode

Equipment

Fusion 100T

NAME

syringe pump

TYPE

Chemyx Inc.

BRAND

5.5 Prepared a solution for Performance Evaluation (during sequence injection):

- Solvent : ☒ Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) / ☒ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (1:1)
- ☒ L-tryptophan Merck MilliporeSigma (Sigma-Aldrich) Catalog #T0254 at
- ☒ Indoxyl-3-sulfate Merck MilliporeSigma (Sigma-Aldrich) at
- ☒ Kynurenic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #K3375 at
- ☒ indole-3-Lactic Acid Merck MilliporeSigma (Sigma-Aldrich) at

5.6 Once calibrated, sequences can be run

5.7 Typical Sequences:

Sample Type	Injection Volume (µL)
Equilibrium(=sample) *	5



Sample Type	Injection Volume (μL)
10	
Blank Solvent	5
Performance Evaluation * 3	5
Blank Solvent	5
Sample QC	5
Sample *10	5
Sample QC	5
...	5
Last Sample QC	5
Blank Solvent * 3	5

6 Data Analysis

6.1 Metabolite Identification :

- m/z and RT of a chemical library (



IROA MSMLS, OAMLS, BACSMLS, FAMLS Merck MilliporeSigma (Sigma-Aldrich)

) checked with

Software

Thermo Scientific Xcalibur

NAME

- MS² acquired on QC samples
- File exported : list of m/z and RT



6.2 Features detection:

- Peak Picking on all samples is done by using Workflow4Metabolomics
- Automatic attribution of the precedent list

Software

Workflow4Metabolomics

NAME

doi: 10.1093/bioinformatics/btu813

DEVELOPER

[Galaxy](#)

REPOSITORY

<https://workflow4metabolomics.usegalaxy.fr/>

SOURCE LINK

6.3 Fusion of modalites = sorting redundancies:

- Only the best modality is kept for 1 metabolite, based on RT > Dead Volume (0-1 min) and/or lower CV(metabolite) on QCs

A.2. Quantification of Short-Chain Fatty Acids (SCFA)

6h

7 Extraction of DBS

7.1 In the whole DBS card Whatman 903™, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

7.2 Put the 2 punches in a 1.5 mL Eppendorf vial

7.3 Add 200 µL of  Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)**

/  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (4:4:2)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

7.4 Vigourously vortexed during  00:01:00

7.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

7.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

7.7  50 μ L of supernateant is derived in a 1 mL 96-wells plate

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

8 Extraction of Serum

8.1 Make aliquots of  50 μ L of serum



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

8.2 Add  150 μ L of cold Methanol HiPerSolv CHROMANORM VWR International (Avantor) ( -20 °C)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

8.3 Vigourously vortexed during  00:01:008.4 Let it  00:20:00 at  -20 °C8.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

8.6

 10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

8.7

 50 µL of supernateant is derived in a 1 mL 96-wells plate

9

Calibration Curve

9.1

Prepared independant 1mg/mL solution for

 acetic acid Merck MilliporeSigma (Sigma-Aldrich) ,

 propionic acid Merck MilliporeSigma (Sigma-Aldrich) ,



⊗ butyric acid Merck MilliporeSigma (Sigma-Aldrich) ,
⊗ isobutyric acid Merck MilliporeSigma (Sigma-Aldrich) ,
⊗ valeric acid Merck MilliporeSigma (Sigma-Aldrich) and
⊗ isovaleric acid Merck MilliporeSigma (Sigma-Aldrich)

These solutions are called SM.

9.2 Then, prepared "**SG0**" solution :

A	B	C
Metabolite	Volume of SM required (μL)	Concentration SGO (pmol/mL)
Acetic acid	72	300000.00
Butyric acid	3.5	10000.00
Isobutyric acid	3.5	10000.00
Isovaleric acid	4.1	10000.00
Propanoic acid	5.9	20000.00
Valeric acid	4.1	10000.00
Vtot SM (μL)	93	/
V(H ₂ O) (μL)	3 907	/

9.3 Prepared **SG1** solution, which is a dilution by 100 of SG0

9.4 Calibration Curve:



	A	B	C	D	E	F	G	H
	From which ?	SG1	SG1	SG1	SG0	SG0	SG0	SG0
	Calibration Curve Solutions	L1	L2	L3	L4	L5	L6	L7
	V(SG), μL	75	250	500	25	75	250	500
	V(H ₂ O), μL	925	750	500	975	925	750	500

Preparation of calibration curve solutions (a solution si named Lx; x from 1 (less concentrated) to 7 (most concentrated))

	A	B	C	D
	Solution	QC Low	QC Middle	QC High
	V(SG), μL	20	200	400
	V(H ₂ O), μL	980	800	600

Preparation of calibration curve QC, all from SG0

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

10 Derivatization

10.1 All information are available in the following papers:

Citation

Monteiro, J. , Lefèvre, A. , Dufour-Rainfray, D. , Oury, A. , Chicheri, G. , Galineau, L. , Blasco, H. , Nadal-Desbarats, L. and Emond, P (2025). Multi-Compartment SCFA Quantification in Human. American Journal of Analytical Chemistry.

[10.4236/ajac.2024.156012](https://doi.org/10.4236/ajac.2024.156012)

LINK

10.2 Before derivatization reactions, the following solutions must be prepared in a volume > [50 µL * number of sample]:

- (A) An internal standard solution of

⊗ acetic-13C2-acid **Toronto Research Chemicals Inc** ,

⊗ butanoic-d7-acid **Toronto Research Chemicals Inc** and

⊗ valeric-d9-acid **Toronto Research Chemicals Inc** at 1 nM in

⊗ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**

- (B) A 2.5% pyridine solution in

⊗ Methanol HiPerSolv CHROMANORM **VWR International (Avantor)**

- (C) A 105 nM solution of EDC (

⊗ n-(3-dimethylaminopropyl)-n-ethylcarbodiimide **Merck MilliporeSigma (Sigma-Aldrich)**

) in ⊗ Methanol HiPerSolv CHROMANORM **VWR International (Avantor)**

- (D) A 175 nM 3-NPH (

⊗ 3-nitrophenylhydrazine **Merck MilliporeSigma (Sigma-Aldrich)**) solution in

⊗ Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /

⊗ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:1)

10.3 Derivatization reaction:

- Successively add to your sample  50 µL of A, B C and D, in this order

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

10.4 Let react  01:00:00 at  4 °C

10.5 Quench the reaction with  250 µL of acidified water (1 % of

 Formic acid >99% for LC-MS **VWR International (Avantor) Catalog #84865.260**

in  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

10.6 Cover the vials or the 96-wells plate

11 LC parameters

11.1 Phase A:

-  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** + 0.1%
-  Formic acid >99% for LC-MS **VWR International (Avantor) Catalog #84865.260**
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

11.2 Phase B:

-  Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** + 0.1%
-  Formic acid >99% for LC-MS **VWR International (Avantor) Catalog #84865.260**
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

11.3 Chromatographic System

Equipment

Acquity I-Class UPLC	NAME
Chromatography	TYPE
Waters	BRAND

11.4 Install the column in the oven

Equipment

Kinetex 1.7µm XB-C18 100A	NAME
Column	TYPE
Phenomenex	BRAND
00F-4498-AN	SKU
LC Column 150 × 2.1mm	SPECIFICATIONS

11.5 Adjust the temperature ( 55 °C) and let it heat the core of the column

11.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

11.7 Prepare the piston "lubrication" solution (90%  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

11.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B
- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

11.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

11.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	0.6	15
	0	0.6	15
	2	0.6	15
	4	0.6	25
	6	0.6	40

	Retention (min)	Flow (mL/min)	%B
	8	0.6	50
	9	0.6	85
	9.5	0.6	0
	12.4	0.6	0
	14.5	0.6	15

11.11 Injection volume :  5 µL for both samples

12 Mass Spectrometry

12.1 Mass Spectrometer

Equipment

Xevo TQ-XS, with an ESI probe

NAME

Mass Spectrometer

TYPE

Waters

BRAND

MS Parameters :

- MRM mode
- Polarity : NEG
- Capillary Voltage : 3 kV
- Desolvation Temperature: 450 °C
- Source Temperature: 150 °C
- Nebuliseur Gas Flow: 7 bar
- COne Gas Flow Temperature: 150°C
- Desolvation Gas Flow: 1200 L/hr

MS method

	Meta bolite	RT (min)	Parent ion (m/z)	Daughter ion (m/z)	CE (-eV)	Cone Voltage (V)	Internal Standard	Dynamic Range of Concentration (nM)	LOQ a²(nM)	CO (%)
	Acetic Acid	1.67	194.1	152.01	14	20	Acetic- ¹³ C ₂ -Acid	638 - 425,000	0.42	4.47
	Propionic Acid	2.50	208.1	165.1	16	20	Butyric-d ₇ -Acid	300 - 200,000	0.27	6.53
	Isobutyric Acid	3.60	222.1	179.1	14	20	Butyric-d ₇ -Acid	30 - 20,000	0.04	13.22
	Butyric Acid	3.73	222.1	179.1	14	20	Butyric-d ₇ -Acid	338 - 225,000	0.42	0.62
	Isovaleric Acid	5.25	236.1	193.1	14	20	Valeric-d ₉ -Acid	18 - 12,000	0.12	15.76
	Valeric Acid	5.50	236.1	193.1	14	20	Valeric-d ₉ -Acid	140 - 46,500	0.10	2.67
	Acetic- ¹³ C ₂ -Acid	1.67	196.1	152.01	14	20	-	-	-	-
	Butyric-d ₇ -Acid	3.72	229.1	186.1	14	20	-	-	-	-
	Valeric-d ₉ -Acid	5.44	245.1	202.1	14	20	-	-	-	-

Retention time, fragmentation parameters of SCFA and range of concentrations of the calibration curve

13 Data Analysis

- 13.1 Analysis are validated only if Low, Middle and High QC at the beginning and at the end of the sequences have a CV < 15 %



Software

MassLynx

NAME

Waters

DEVELOPER

A.3. Quantification of Tryptophan (TRP) intermediates

6h

14 Internal Standards Solutions

Citation

Lefèvre A, Mavel S, Nadal-Desbarats L, Galineau L, Attucci S, Dufour D, Sokol H, Emond (2019). Validation of a global quantitative analysis ... Talanta.

[10.1016/j.talanta.2018.11.09404](https://doi.org/10.1016/j.talanta.2018.11.09404)

LINK

The internal standards required are:

- ⊗ Quinolinic-d3-acid **Toronto Research Chemicals Inc** ,
- ⊗ 3-hydroxyanthranilic-d3-acid **Toronto Research Chemicals Inc** ,
- ⊗ 5-hydroxyindole-3acetic-d5-acid **Toronto Research Chemicals Inc** ,
- ⊗ melatonin d4 **Toronto Research Chemicals Inc** ,
- ⊗ picolinic-d3-acid **Toronto Research Chemicals Inc** ,
- ⊗ 3-hydroxykynurenine 13C2,15N1 **Toronto Research Chemicals Inc** ,
- ⊗ L-kynurenine d4 **Toronto Research Chemicals Inc** ,
- ⊗ indoleacetic-d4-acid **Toronto Research Chemicals Inc** ,
- ⊗ kynurenic-d5-acid **Toronto Research Chemicals Inc** ,
- ⊗ tryptamine d4 **Toronto Research Chemicals Inc** ,
- ⊗ tryptophane **Merck MilliporeSigma (Sigma-Aldrich)** ,
- ⊗ L-tryptophan d5 **Toronto Research Chemicals Inc** ,

- ⊗ serotonin d4 **Toronto Research Chemicals Inc** ,
- ⊗ indoxyl-13C6 sulfate potassium salt **Toronto Research Chemicals Inc** ,
- ⊗ 5-hydroxy-L-tryptophan d4 **Toronto Research Chemicals Inc** ,
- ⊗ 1H-indole-d5-3-acetamide **Toronto Research Chemicals Inc** ,
- ⊗ tryptophol d4 **Toronto Research Chemicals Inc** ,
- ⊗ N-acetylserotonine d3 **Toronto Research Chemicals Inc** ,
- ⊗ indole-3-aldehyde 13C8 **Toronto Research Chemicals Inc** &
- ⊗ 3-indolepropionic-d2-acid **Toronto Research Chemicals Inc**

14.1 Mix in one 15 mL falcon tube all the following to create the solution "SEI" :

A	B	C
Internal Standard	Volume SMO (μL)	Volume SM1 (μL)
Quinolinic-d3-acid	17	
3-hydroxyanthranilic-d3-acid	31	
5-hydroxyindole-3acetic-d5-acid		118
melatonin d4		94
picolinic-d3-acid	10	
3-hydroxykynurenine 13C2,15N1	26	
L-kynurenine d4	65	
indoleacetic-d4-acid	14	
kynurenic-d5-acid	2.3	
tryptamine d4	2.4	
xanthurenic-d4-acid	2.5	
L-tryptophan d5	335	
serotonin d4		86
indoxyl-13C6 sulfate potassium salt	51	
5-hydroxy-L-tryptophan d4		134
1H-indole-d5-3-acetamide		54

	A	B	C
	tryptophol d4	2.6	
	indole-3-lactic-d5-acid	13	
	N-acetylserotonine d3		177
	indole-3-aldehyde 13C8	6.1	
	3-indolepropionic-d2-acid	3.8	
	Water to complete to 10 mL (μL)	8753	

Homogeneized and then prepared a dilution by 40 in a 20 mL Flacon, with

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** : this solution is call SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

15 Standards Solutions & Calibration Curve

The standards required are :  picolinic acid **Merck MilliporeSigma (Sigma-Aldrich)**

,  3-hydroxykynurenine **Merck MilliporeSigma (Sigma-Aldrich)** ,

 quinolinic acid **Merck MilliporeSigma (Sigma-Aldrich)** ,

 serotonin **Merck MilliporeSigma (Sigma-Aldrich)** ,

 5-hydroxytryptophane **Merck MilliporeSigma (Sigma-Aldrich)** ,

 kynurenine **Merck MilliporeSigma (Sigma-Aldrich)** ,

 3-hydroxyanthranilic acid **Merck MilliporeSigma (Sigma-Aldrich)** ,



- ⊗ tryptamine Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ tryptophane Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ 5-hydroxyindole acetic acid Toronto Research Chemicals Inc ,
- ⊗ indoxyl-3-sulfate Toronto Research Chemicals Inc ,
- ⊗ N-acetylserotonine Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ xanthurenic acid Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ indole-3-acetamide Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ kynurenic acid Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ indole-3-lactic acid Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ indole-3-aldehyde Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ tryptophol Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ indole-3-acetic acid Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ melatonin Merck MilliporeSigma (Sigma-Aldrich) ,
- ⊗ indole-3-propionic acid Merck MilliporeSigma (Sigma-Aldrich)

15.1 Mix in one 4 mL glass tube all the following :

A	B	C
Metabolite	Volume SM0 (μL)	Volume SM1 (μL)
picolinic acid	17	
3-hydroxykynurenine	31	
quinolinic acid		118
serotonin		94
5-hydroxytryptophane	10	
kynurenine	26	
3-hydroxyanthranilic acid	65	
tryptamine	14	
tryptophane	2.3	
5-hydroxyindole acetic acid	2.4	



	A	B	C
	indoxyl-3-sulfate	2.5	
	N-acetylserotonine	335	
	xanthurenic acid		86
	indole-3-acetamide	51	
	kynurenic acid		134
	indole-3-lactic acid		54
	indole-3-aldehyde	2.6	
	tryptophol	13	
	indole-3-acetic acid		177
	melatonine	6.1	
	indole-3-propionic acid	3.8	
	Water to complete to 2 mL (µL)	877	

This solution is called **SG0**

15.2 Dilute SG0 by 100 to created **SG1**

15.3 Prepare a solution of BSA :

- 400 mg BSA
- 10 mL PBS
- Planar agitation for  00:20:00 , strength 8/10 at  Room temperature
- Transfert in another falcon to remove any foalm

20m



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

16 Calibration Curve

16.1 Calibration Curve:

	A	B	C	D	E	F	G	H
	From which ?	SG1	SG1	SG1	SG0	SG0	SG0	SG0
	Calibration Curve Solutions	L1	L2	L3	L4	L5	L6	L7
	V(SG), μL	75	250	500	25	75	250	500
	V(H ₂ O), μL	425	250	0	475	425	250	0
	V(BSA) (μL)	500	500	500	500	500	500	500

Preparation of calibration curve solutions (a solution is named L_x; x from 1 (less concentrated) to 7 (most concentrated))

	A	B	C	D
	Solution	QC Low	QC Middle	QC High
	V(SG), μL	20	200	400

	A	B	C	D
	V(H ₂ O), µL	480	300	100
	V(BSA) (µL)	500	500	500

Preparation of calibration curve QC, all from SG0

17 Extraction of DBS

17.1 In the whole DBS card Whatman 903™, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

17.2 Put the 2 punches in a 1.5 mL Eppendorf vial

17.3 Add  200 µL of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

17.4 Vigourously vortexed during  00:01:00

17.5  00:30:00 at  -20 °C

17.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R	NAME
Centrifuge	TYPE
ThermoScientific	BRAND

17.7 Transfert  175 µL of supernateant in a 2 mL glass vial

Equipment

FinnPipette	NAME
F2	TYPE
ThermoScientific	BRAND

17.8 Evaporate:  01:00:00 ,  40 °C , vacuum

17.9 Add 100 μ L of  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:9)

Equipment	
FinnPipette	NAME
F2	TYPE
ThermoScientific	BRAND

17.10 Vigourously vortexed during  00:01:00

17.11  10000 x g, 4°C, 00:10:00

Equipment	
Sorvall Legend X1R	NAME
Centrifuge	TYPE
ThermoScientific	BRAND

17.12 Transfert  85 μ L in a glass vial



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

18 Extraction of Serum

18.1 Make aliquots of  50 μL of serum

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

18.2 Add  200 μL of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

18.3 Vigourously vortexed during  00:01:00

18.4  00:30:00 at  -20 °C

18.5  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

18.6 Transfert  175 µL of supernateant in a 2 mL glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

18.7 Evaporate:  01:00:00 ,  40 °C , vacuum

18.8 Add 100 µL of  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:9)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

18.9 Vigourously vortexed during  00:01:00

18.10  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R	NAME
Centrifuge	TYPE
ThermoScientific	BRAND

18.11 Transfert  85 µL in a glass vial

Equipment

FinnPipette	NAME
F2	TYPE
ThermoScientific	BRAND

19 LC parameters

19.1 Phase A:

-  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** + 0.5%
-  Formic acid >99% for LC-MS **VWR International (Avantor)** Catalog #84865.260
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

19.2 Phase B:

- ☒ Methanol HiPerSolv CHROMANORM VWR International (Avantor) + 0.5%

☒ Formic acid >99% for LC-MS VWR International (Avantor) Catalog #84865.260
- Stir vigorously during by hand
- Ultrasound during to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

19.3 Chromatographic System

Equipment

Acquity I-Class UPLC	NAME
Chromatography	TYPE
Waters	BRAND

19.4 Install the column in the oven



Equipment

Kinetex 1.7µm XB-C18 100A	NAME
Column	TYPE
Phenomenex	BRAND
00F-4498-AN	SKU
LC Column 150 × 2.1mm	SPECIFICATIONS

19.5 Adjust the temperature ( 55 °C) and let it heat the core of the column

19.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

19.7 Prepare the piston "lubrication" solution (90%  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

19.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B
- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

19.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

19.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	0.4	5
	0	0.4	5
	2	0.4	10
	4	0.4	15
	6	0.4	30

	Retention (min)	Flow (mL/min)	%B
	8	0.4	40
	9	0.4	45
	9.5	0.4	100
	11	0.4	00
	11.5	0.4	5
	13.5	0.4	5

19.11 Injection volume :  5 µL for both samples

20 Mass Spectrometry

20.1 Mass Spectrometer

Equipment

Xevo TQ-XS, with an ESI probe

NAME

Mass Spectrometer

TYPE

Waters

BRAND

21 Data Analysis

21.1 Analysis are validated only if Low, Middle and High QC at the beginning and at the end of the sequences have a CV < 15 %

Software

MassLynx

NAME

Waters

DEVELOPER

A.4. Quantification of Bile Acids

6h

22 Internal Standards (IS) Solutions

- The internal standards required are:

- ☒ cholic acid d5 Toronto Research Chemicals Inc ,
- ☒ cholic acid sulfate d4 Toronto Research Chemicals Inc ,
- ☒ glycocholic acid d4 Toronto Research Chemicals Inc ,
- ☒ taurocholic acid d4 Toronto Research Chemicals Inc ,
- ☒ ursodeoxycholic acid d4 Toronto Research Chemicals Inc ,
- ☒ chenodeoxycholic acid d4 Toronto Research Chemicals Inc ,
- ☒ deoxycholic acid d5 Toronto Research Chemicals Inc ,
- ☒ lithocholic acid d4 Toronto Research Chemicals Inc

- Prepared solution of 1 mg/mL of each IS, called SM

22.1 Mix in one 10 mL Falcon tube all the following to create the solution "SEI" :

A	B
Internal Standard	Volume SM (μL)
Cholic acid d5	4.1
cholic acid sulfate d4	5.4
glycocholic acid d4	9.4
taurocholic acid d4	10.8
ursodeoxycholic acid d4	2.0

A	B
chenodeoxycholic acid d4	2.0
deoxycholic acid d5	1.6
lithocholic acid d4	19.0
Methanol/water (1:1) to complete to 10 mL (μL)	4946

Homogeneized and then prepared 32 mL of a dilution by 40 in a 20 mL Flacon, with  Isopropanol HyperSolv CHROMANORM VWR International (Avantor) : this solution is call SEI_40

23 Standards Solutions & Calibration Curve

- The standards required are :

-  taurohyocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glycohyocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glycodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  lithocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  chenodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glycolithcholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glycocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  cholic acid sulfate Merck MilliporeSigma (Sigma-Aldrich) ,
-  taurochenodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  ursodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glycochenodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  taurodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  lithocholic acid sulfate Merck MilliporeSigma (Sigma-Aldrich) ,
-  taurocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  tauroolithocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  hyocholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  glyoursodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,
-  tauroursodeoxycholic acid Merck MilliporeSigma (Sigma-Aldrich) ,

 cholic acid Merck MilliporeSigma (Sigma-Aldrich) ,

 deoxycholic acid Merck MilliporeSigma (Sigma-Aldrich)

- **Prepare standard solution of 1 mg/mL** for each standard and a diluted one, by 100, for glycolithocholic acid, urosdeoxycholic acid, tauroolithocholic acid and hyocholic acid

23.1 Mix in one 4 mL glass tube all the following :

A	B	C
Metabolite	Volume SMO (μL)	Volume SM1 (μL)
taurohyocholic acid	2.2	
glycohyocholic acid	1.9	
glycodeoxycholic acid	4.5	
lithocholic acid	38	
chenodeoxycholic acid	3.9	
glycolithcholic acid		173
glycocholic acid	9.0	
cholic acid sulfate	2.1	
taurochenodeoxycholic acid	5.2	
ursodeoxycholic acid		157
glycochenodeoxycholic acid	13.5	
lithocholic acid sulfate	2.0	
taurodeoxycholic acid	5.2	
taurocholic acid	5.4	
tauroolithocholic acid		193
hyocholic acid		163
glycoursodeoxycholic acid	4.5	
tauroursodeoxycholic acid	5.0	
cholic acid	4.1	



	A	B	C
	deoxycholic acid	3.9	
	Water to complete to 2 mL (μL)	1202	

This solution is called **SGO**

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

23.2 Dilute SGO by 100 to created **SG1**

23.3 Prepare a solution of BSA :

- 400 mg BSA
- 10 mL PBS
- Planar agitation for  00:20:00 , strength 8/10 at  Room temperature
- Transfert in another falcon to remove any foalm

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

24 Calibration Curve

24.1 Calibration Curve:

	A	B	C	D	E	F	G	H
	From which ?	SG1	SG1	SG1	SG0	SG0	SG0	SG0
	Calibration Curve Solutions	L1	L2	L3	L4	L5	L6	L7
	V(SG), μ L	75	250	500	25	75	250	500
	V(H ₂ O), μ L	425	250	0	475	425	250	0



	A	B	C	D	E	F	G	H
	V(BSA) (μL)	500	500	500	500	500	500	500

Preparation of calibration curve solutions (a solution si named Lx; x from 1 (less concentrated) to 7 (most concentrated))

	A	B	C	D
	Solution	QC Low	QC Middle	QC High
	V(SG), μL	20	200	400
	V(H ₂ O), μL	480	300	100
	V(BSA) (μL)	500	500	500

Preparation of calibration curve QC, all from SG0

25 Extraction of DBS

25.1 In the whole DBS card Whatman 903™, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

25.2 Put the 2 punches in a 1.5 mL Eppendorf vial

25.3 Add  400 μL of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

25.4 Vigourously vortexed during  00:01:00

25.5  00:30:00 at  -20 °C

25.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

25.7 Transfert  350 µL of supernateant in a 2 mL glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

25.8 Evaporate:  01:00:00 ,  40 °C , vacuum

25.9 Add 100 µL of  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:1)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

25.10 Vigourously vortexed during  00:01:00

25.11  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

25.12 Transfert  85 μL in a glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

26 Extraction of Serum

26.1 Make aliquots of  50 μL of serum



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

26.2 Add  400 μ L of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

26.3 Vigourously vortexed during  00:01:00

26.4  00:30:00 at  -20 °C

26.5 Transfert  375 μ L of supernateant in a 2 mL glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

26.6 Evaporate:  01:00:00 ,  40 °C , vacuum

26.7 Add 100 µL of  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:1)

26.8 Vigourously vortexed during  00:01:00

26.9  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

26.10 Transfert  85 µL in a glass vial



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

27 LC parameters

27.1 Phase A:

- ☒ Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** /
☒ Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (9:1) + 1 mM
☒ formiate ammonium **VWR International (Avantor)** + 0.1%
☒ Formic acid >99% for LC-MS **VWR International (Avantor)** Catalog #84865.260
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

27.2 Phase B:

- ☒ Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** / ☒ Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** (1:1)
- Stir vigorously during by hand
- Ultrasound during to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS



27.3 Chromatographic System

Equipment

Acquity I-Class UPLC

NAME

Chromatography

TYPE

Waters

BRAND

27.4 Install the column in the oven

27.5 Adjust the temperature ( 60 °C) and let it heat the core of the column

27.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

27.7 Prepare the piston "lubrication" solution (90%

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

27.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B



- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

27.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

27.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0.0	0.6	10
	0.0	0.6	10
	0.1	0.6	10
	9.25	0.6	35
	11.5	0.65	85
	11.8	0.8	100
	12.0	1	100
	12.1	1	100
	12.4	0.85	100
	12.45	0.85	55
	12.5	0.8	10
	12.6	0.7	10
	12.7	0.6	10
	15.0	0.6	10



Citation

Sarafian MH, Lewis MR, Pechlivanis A, Ralphs S, McPhail MJ, Patel VC, Dumas ME, Holmes E, Nicholson JK.
(2015). Bile acids profiling and quantification in ... Analytical Chemistry.

[10.1021/acs.analchem.5b0155609/30/2015](https://doi.org/10.1021/acs.analchem.5b0155609/30/2015)

LINK

27.11 Injection volume :  5 μ L for both samples

28 Mass Spectrometry

28.1 Mass Spectrometer

Equipment

Xevo TQ-XS, with an ESI probe

NAME

Mass Spectrometer

TYPE

Waters

BRAND

29 Data Analysis

29.1 Analysis are validated only if Low, Middle and High QC at the beginning and at the end of the sequences have a CV < 15 %

Software

MassLynx

NAME

Waters

DEVELOPER

A.5. Quantification of TriCarboxylic Acids intermediates

6h

30 Internal Standards (IS) Solutions

- The internal standards required are:
- Prepare a solution of 1 mg/mL of each IS, called **SM**

30.1 Mix in one 10 mL Falcon tube all the following to create the solution "SEI" :

A	B
Internal Standard	Volume SM (μL)
Succinic acid 13C4	4
Fumaric acid 13C4	8
Malic acid 13C4	4
Itaconic acid 13C4	2
Citric acid 13C4	10
Lactic acid 13C2	4
V(water) to complete to 2 mL (μL)	1978

Homogeneized and then prepared 40 mL of a dilution by 40 in a 50 mL Flacon, with

⊗ Water milliQ Merck MilliporeSigma (Sigma-Aldrich) : this solution is call SEI_40

31 Standards Solutions & Calibration Curve



- The standards required are: ☒ citric acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ isocitric acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ aconitic acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ ketoglutaric acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ succinic acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ fumaric acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ malic acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ itaconic acid Merck MilliporeSigma (Sigma-Aldrich) ,
☒ lactic acid Merck MilliporeSigma (Sigma-Aldrich)
- **Diluted one, by 100, for glycolithocholic acid, urosdeoxycholic acid, tauroolithocholic acid and hyocholic acid**

31.1 Mix in one 4 mL glass tube all the following :

A	B
Metabolite	Volume SMO (μL)
Citric acid	115
Isocitric acid	115
Aconitic acid	35
Ketoglutaric acid	29
Succinic acid	24
Fumaric acid	2
Malic acid	27
Itaconic acid	3
Lactic acid	144
Water to complete to 2 mL (μL)	1505

This solution is called **SGO**

31.2 Dilute SGO by 100 to created **SG1**

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

32 Calibration Curve

32.1 Calibration Curve:

	A	B	C	D	E	F	G	H
	From which ?	SG1	SG1	SG1	SG0	SG0	SG0	SG0
	Calibration Curve Solutions	L1	L2	L3	L4	L5	L6	L7
	V(SG), μL	75	250	500	25	75	250	500
	V(H ₂ O), μL	925	750	500	975	925	750	500

Preparation of calibration curve solutions (a solution si named L_x; x from 1 (less concentrated) to 7 (most concentrated))

	A	B	C	D
	Solution	QC Low	QC Middle	QC High
	V(SG), μL	20	200	400
	V(H ₂ O), μL	980	800	600



Preparation of calibration curve QC, all from SG0

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

33 Extraction of DBS

33.1 In the whole DBS card Whatman 903™, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

33.2 Put the 2 punches in a 1.5 mL Eppendorf vial

33.3 Add  200 µL of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

33.4 Vigourously vortexed during  00:01:00

33.5  00:30:00 at  -20 °C

33.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

33.7 Transfert  175 µL of supernateant in a 2 mL glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

33.8 Evaporate:  01:00:00 ,  40 °C , vacuum

33.9 Add 100 μ L of  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** /  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (1:9)

33.10 Vigourously vortexed during  00:01:00

33.11  10000 x g, 4°C, 00:10:00

33.12 Transfert  85 μ L in a glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

34 Extraction of Serum

34.1 Make aliquots of  50 μL of serum

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

34.2 Add  200 μL of SEI_40

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

34.3 Vigourously vortexed during 00:01:00

34.4 00:30:00 at -20 °C

34.5 Transfert 175 µL of supernateant in a 2 mL glass vial

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

34.6 Evaporate: 01:00:00 , 40 °C , vacuum

34.7 Add 100 µL of Methanol HiPerSolv CHROMANORM VWR International (Avantor) /
Water milliQ Merck MilliporeSigma (Sigma-Aldrich) (1:9)

Equipment		
FinnPipette		NAME
F2		TYPE
ThermoScientific		BRAND

34.8 Vigourously vortexed during  00:01:00

34.9  10000 x g, 4°C, 00:10:00

Equipment		
Sorvall Legend X1R		NAME
Centrifuge		TYPE
ThermoScientific		BRAND

34.10 Transfert  85 µL in a glass vial

Equipment	
FinnPipette	NAME
F2	TYPE
ThermoScientific	BRAND

35 LC parameters

35.1 Phase A:

- ☒ Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** + 0.1%
☒ Formic acid >99% for LC-MS **VWR International (Avantor)** Catalog #84865.260
- Stir vigorously during by hand
- Ultrasound during to remove air bubble

Equipment	
Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

35.2 Phase B:

-  Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) + 0.1%
-  Formic acid >99% for LC-MS VWR International (Avantor) Catalog #84865.260
- Stir vigorously during  00:01:00 by hand
- Ultrasound during  00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

35.3 Chromatographic System

Equipment

Acquity I-Class UPLC	NAME
Chromatography	TYPE
Waters	BRAND

35.4 Install the column in the oven

Equipment

Kinetex 1.7µm XB-C18 100A	NAME
Column	TYPE
Phenomenex	BRAND
00F-4498-AN	SKU
LC Column 150 × 2.1mm	SPECIFICATIONS

35.5 Adjust the temperature ( 55 °C) and let it heat the core of the column

35.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

35.7 Prepare the piston "lubrication" solution (90%

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

35.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B
- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

35.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

35.10 Gradient:

	Retention (min)	Flow (mL/min)	%B
	0.0	0.6	10
	0.0	0.6	10
	0.1	0.6	10



	Retention (min)	Flow (mL/min)	%B
	9.25	0.6	35
	11.5	0.65	85
	11.8	0.8	100
	12.0	1	100
	12.1	1	100
	12.4	0.85	100
	12.45	0.85	55
	12.5	0.8	10
	12.6	0.7	10
	12.7	0.6	10
	15.0	0.6	10

Citation

Sarafian MH, Lewis MR, Pechlivanis A, Ralphs S, McPhail MJ, Patel VC, Dumas ME, Holmes E, Nicholson JK.
(2015). Bile acids profiling and quantification in ... Analytical Chemistry.

[10.1021/acs.analchem.5b01556](https://doi.org/10.1021/acs.analchem.5b01556)09/30/2015

LINK

35.11 Injection volume :  5 μ L for both samples

36 Mass Spectrometry

36.1 Mass Spectrometer



Equipment

Xevo TQ-XS, with an ESI probe	NAME
Mass Spectrometer	TYPE
Waters	BRAND

37 Data Analysis

- 37.1 Analysis are validated only if Low, Middle and High QC at the beginning and at the end of the sequences have a CV < 15 %

Software

MassLynx	NAME
Waters	DEVELOPER

B.1. Untargeted Lipidomics

8h

38 Extraction of DBS

- 38.1 In the whole DBS card Whatman 903TM, retrieved 2 punches (1 punch = 3 mm disc) with a puncher
WARNINGS:
- a punch must be entirely soaked in blood
 - if possible, do not punch near the exterior of the blood droplets of the DBS
- 38.2 Put the 2 punches in a 1.5 mL Eppendorf vial

38.3 Add  1000 µL of

 Isopropanol HyperSolv CHROMANORM VWR International (Avantor)

38.4 Vigourously vortexed during  00:01:00

38.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

38.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

38.7 Transfert  900 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

38.8 Dry for  02:00:00 under vacuum at  40 °C

2h

38.9 Dried extract is redissolved with  100 µL of:

 Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** /

 Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** /

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (6:3:1)

38.10 Vigorously vortexed during  00:01:00 at  Room temperature

38.11 Transfert whole aliquot in a  200 µL 96-well plates



Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

39 Extraction of Serum

39.1 Make aliquots of  50 μL of serum

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

39.2 Add  950 μL of cold

 Isopropanol HyperSolv CHROMANORM VWR International (Avantor) ( -20 °C)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

39.3 Vigourously vortexed during  00:01:00

39.4 Let it  00:20:00 at  -20 °C

39.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

39.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

39.7 Transfert  900 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

39.8 Dry for  02:00:00 under vacuum at  40 °C

2h

39.9 Dried extract is redissolved with  100 µL of:

 Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** /

 Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** /

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (6:3:1)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

39.10 Vigorously vortexed during  00:01:00 at  Room temperature

39.11 Transfert whole aliquot in a  100 µL 96-well plates

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

40 LC parameters

40.1 Phase A:

- 200 mL + 4.0 mL * [Number of injections]
-  Water milliQ Merck MilliporeSigma (Sigma-Aldrich) /
-  Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /

Isopropanol HyperSolv CHROMANORM VWR International (Avantor) (5:3:2)

- 1 mM of formiate ammonium VWR International (Avantor)
- Stir vigorously during 00:01:00 by hand
- Ultrasound during 00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

40.2 Phase B:

- 200 mL + 4.0 mL * [Number of injections]
- Water milliQ Merck MilliporeSigma (Sigma-Aldrich) /
Acetonitrile HiPerSolv CHROMANORM VWR International (Avantor) /
Isopropanol HyperSolv CHROMANORM VWR International (Avantor) (1:9:90)
- 10 mM of formiate ammonium VWR International (Avantor)
- Stir vigorously during 00:01:00 by hand
- Ultrasound during 00:10:00 to remove air bubble

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

40.3 Chromatographic System

Equipment

Ultimate 3000	NAME
UHPLC	TYPE
Dionex Softron	BRAND
SRD-3400	SKU
Pump, Column Compartment, AutoSampler	SPECIFICATIONS

40.4 Install the column in the oven



Equipment

ACQUITY UPLC BEH C18 (2.1×100×1.7) NAME

Column TYPE

Acquity BRAND

40.5 Adjust the temperature ( 60 °C) and let it heat the core of the column

40.6 Prepare the cleaning solvents (same solvent as the recovery solvent) & ultrasound it during  00:05:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH NAME

Ultrasound bath TYPE

VWR BRAND

142-0084 SKU

230V 50Hz 370VA SPECIFICATIONS

40.7 Prepare the piston "lubrication" solution (90%

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** and 10%

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**) & ultrasound it during

 00:10:00 to remove air bubbles

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

40.8 Purge the system:

- 1 time at 100%A and 1 time at 100%B with open valve
- 2 times with open valve, 2 times with close valve at 50%A and 50%B
- Set the flow at 2 mL/min at 50%A and 50%B during  00:10:00
- Check that the pression is stable; otherwise : purge again

40.9 Balancing the system: Gradually reach the flow rate of 0.5 mL/min at 2%B

40.10

Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	0.4	10
	0	0.4	10
	2.7	0.4	45
	2.8	0.4	53
	8	0.4	60
	8.1	0.4	80

	Retention (min)	Flow (mL/min)	%B
	11.5	0.4	80
	12	0.4	100
	13	0.4	100
	13.2	0.4	10

40.11 Volume of injection (μL):  5 μL for both samples

41 Mass Spectrometry

41.1 Mass Spectrometer

Equipment

Q-Exactive

NAME

Mass Spectrometer

TYPE

ThermoScientific

BRAND

HESI Source

SPECIFICATIONS

MS Parameters :

- Scan Range: 250-1500 m/z
- Resolution MS1: 70,000
- Resolution MS2: 15,000
- Polarity: POS or NEG
- Microscans: 1
- Lock mass: None
- AGC Target: 1E6
- Maximum Inject Time: 200
- Sheath Gas Flow Rate: 45
- Aux Gas Flow rate: 10
- Sweep Gas flow Rate: 1

- Spray Voltage (kV): +/-3.5
- Capillary Temperature (°C): 250
- S-lens RF level: 25
- Aux gas Heater temperature (°C): 350

41.2 Cleaning the source:

- Set source temperature to  50 °C
- Let it "cool"  01:00:00
- Unplug the Ion MAX API source with HESI probe attached
- Unscrewed the Sweep Cone and the capillary
- Immediately plug another cleaned capillary
- Clean the sweep cone with wet

 Aluminium oxide power **Merck MilliporeSigma (Sigma-Aldrich)**
- Rinse thoroughly with  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)**
- In a glass beaker, cover completely the Sweep Cone and the capillary with

 Methanol HiPerSolv CHROMANORM **VWR International (Avantor)**
- Ultrasound during  00:10:00

Equipment

Ultrasonic Cleaner USC - TH	NAME
Ultrasound bath	TYPE
VWR	BRAND
142-0084	SKU
230V 50Hz 370VA	SPECIFICATIONS

- Let it dry
- Replace the cone and the Ion Max API source with HESI probe
- Set source temperature back to normal

41.3 Reinstall the source & allow it to return to analysis conditions

41.4 Calibration:

- POS: Calibration Solution Pierce LTQ Velos ESI Positive Ion **Thermo Fisher**
- NEG: Calibration Solution Pierce ESI Negative Ion **Thermo Fisher**
- RMS < 0.4/0.4 in both ionisation mode

Equipment

Fusion 100T

NAME

syringe pump

TYPE

Chemyx Inc.

BRAND

41.5 Once calibrated, sequences can be run

41.6 Typical Sequences:

Sample Type	Injection Volume (µL)
Equilibrium(=sample) * 10	5
Blank Solvent	5
Performance Evaluation * 3	5
Blank Solvent	5
Sample QC	5
Sample *10	5
Sample QC	5



	Sample Type	Injection Volume (μL)
	...	5
	Last Sample QC	5
	Blank Solvent * 3	5

42 Data Analysis

42.1 Lipids Identification :

Software

LipidSearch

NAME

ThermoScientific

DEVELOPER

42.2 Features detection:

- Peak Picking on all samples is done by using Workflow4Metabolomics
- Automatic attribution of the precedent list

Software

Workflow4Metabolomics

NAME

doi: 10.1093/bioinformatics/btu813

DEVELOPER

[Galaxy](#)

REPOSITORY

<https://workflow4metabolomics.usegalaxy.fr/>

SOURCE LINK



42.3 Fusion of modalities = sorting redundancies:

- Only the best modality is kept for 1 metabolite, based on RT > Dead Volume (0-1 min) and/or lower CV(metabolite) on QCs

B.2. Quantitative Lipidomics

6h

43 Method from the paper :

Citation

Le Faouder, P.; Soullier, J.; Tremblay-Franco, M.; Tournadre, A.; Martin, J.-F.; Guitton, Y.; Carlé, C.; Caspar-Bauguil, S.; Denechaud, P.-D.; Bertrand-Michel, J (2021). Untargeted Lipidomic Profiling of Dry Blood ... Metabolites.

<https://doi.org/10.3390/metabo1105030501>

LINK

- More details on internal standards used and quantification process on :

<https://doi.org/10.5281/zenodo.17184000>

44 Extraction of DBS

44.1 In the whole DBS card Whatman 903TM, retrieved 2 punches (1 punch = 3 mm disc) with a puncher

WARNINGS:

- a punch must be entirely soaked in blood
- if possible, do not punch near the exterior of the blood droplets of the DBS

44.2 Put the 2 punches in a 1.5 mL Eppendorf vial

44.3 Add 1000 µL of

 Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** internal solutions

44.4 Vigourously vortexed during 00:01:00

44.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment		
Titramax 1000		NAME
Shaker		TYPE
Heidolph		BRAND

44.6  10000 x g, 4°C, 00:10:00

Equipment		
Sorvall Legend X1R		NAME
Centrifuge		TYPE
ThermoScientific		BRAND

44.7 Transfert  900 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

44.8 Dry for  02:00:00 under vacuum at  40 °C

44.9 Dried extract is redissolved with  100 µL of:

 Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** /

 Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** /

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (6:3:1)

44.10 Vigorously vortexed during  00:01:00 at  Room temperature

44.11 Transfert whole aliquot in a  200 µL 96-well plates

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

45 Extraction of Serum

45.1 Make aliquots of  50 μ L of serum

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

45.2 Add  950 μ L of cold



Isopropanol HyperSolv CHROMANORM VWR International (Avantor)

solutions

of internal

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

45.3 Vigourously vortexed during  00:01:00

45.4 Let it  00:20:00 at  -20 °C

45.5 Planar agitation :  01:00:00 ,  Room temperature , strength 8/10

Equipment

Titramax 1000

NAME

Shaker

TYPE

Heidolph

BRAND

45.6  10000 x g, 4°C, 00:10:00

Equipment

Sorvall Legend X1R

NAME

Centrifuge

TYPE

ThermoScientific

BRAND

45.7 Transfert  900 µL of supernateant in a glass vial of 1.5 mL

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

45.8 Dry for  02:00:00 under vacuum at  40 °C

45.9 Dried extract is redissolved with  100 µL of:

 Acetonitrile HiPerSolv CHROMANORM **VWR International (Avantor)** /

 Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)** /

 Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** (6:3:1)

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

45.10 Vigorously vortexed during  00:01:00 at  Room temperature

45.11 Transfert whole aliquot in a  100 µL 96-well plates

Equipment

FinnPipette

NAME

F2

TYPE

ThermoScientific

BRAND

46 SFC parameters

46.1 Phase A:

- Supercritical CO2

46.2 Phase B:

-  Water milliQ **Merck MilliporeSigma (Sigma-Aldrich)** /
-  Methanol HiPerSolv CHROMANORM **VWR International (Avantor)** (2:98)
- 20 mM of  formiate ammonium **VWR International (Avantor)**

46.3 Chromatographic System

Equipment

Ultra-Performance Convergence Chromatography (UPC²)^{NAME}

Chromatography^{TYPE}

Waters^{BRAND}

46.4 Install the column in the oven

Equipment

ACQUITY UPC² Torus DEA (100×3×1.7)^{NAME}

Column^{TYPE}

Acquity^{BRAND}

46.5 Adjust the temperature (40 °C) and let it heat the core of the column

46.6

Gradient:

	Retention (min)	Flow (mL/min)	%B
	0	1.2	1
	0	1.2	1
	0.5	1.2	1
	6	1.2	40
	6.1	1.2	65
	9.1	1.1	65
	12.1	1.2	1

47 Mass Spectrometry

47.1 Mass Spectrometer

Equipment

Xevo G2 XS QTof

NAME

Mass Spectrometer

TYPE

Waters

BRAND

MS Parameters :

- Resolution MS1: 16,000 at 400 m/z
- Mode: Full MS
- Scan Range: 50-1500 Da (centroid)
- Polarity: POS or NEG
- Source Temperature (°C): 150



- Spray Voltage (kV): -2.6 & 3
- Desolvation Gas Flow Rate (L/hr): 1000
- Cone Gas Flow Rate (L/hr): 50
- Desolvation temperature (°C): 550

48 **Data Analysis**

48.1 <https://doi.org/10.5281/zenodo.17184000>



Citations

Step 10.1

Monteiro, J. , Lefèvre, A. , Dufour-Rainfray, D. , Oury, A. , Chicheri, G. , Galineau, L. , Blasco, H. , Nadal-Desbarats, L. and Emond, P. Multi-Compartment SCFA Quantification in Human
[10.4236/ajac.2024.156012](https://doi.org/10.4236/ajac.2024.156012)

Step 14

Lefèvre A, Mavel S, Nadal-Desbarats L, Galineau L, Attucci S, Dufour D, Sokol H, Emond. Validation of a global quantitative analysis ...
[10.1016/j.talanta.2018.11.09404](https://doi.org/10.1016/j.talanta.2018.11.09404)

Step 27.10

Sarafian MH, Lewis MR, Pechlivanis A, Ralphs S, McPhail MJ, Patel VC, Dumas ME, Holmes E, Nicholson JK.. Bile acids profiling and quantification in ...
[10.1021/acs.analchem.5b0155609/30/2015](https://doi.org/10.1021/acs.analchem.5b0155609/30/2015)

Step 35.10

Sarafian MH, Lewis MR, Pechlivanis A, Ralphs S, McPhail MJ, Patel VC, Dumas ME, Holmes E, Nicholson JK.. Bile acids profiling and quantification in ...
[10.1021/acs.analchem.5b0155609/30/2015](https://doi.org/10.1021/acs.analchem.5b0155609/30/2015)

Step 39.2

Folch J, Lees M, Stanley. A simple method for the isolation ...
[https://doi.org/10.1016/S0021-9258\(18\)64849-51957](https://doi.org/10.1016/S0021-9258(18)64849-51957)

Step 43

Le Faouder, P.; Soullier, J.; Tremblay-Franco, M.; Tournadre, A.; Martin, J.-F.; Guitton, Y.; Carlé, C.; Caspar-Bauguil, S.; Denechaud, P.-D.; Bertrand-Michel, J. Untargeted Lipidomic Profiling of Dry Blood ...
<https://doi.org/10.3390/metabo1105030501>

Step 45.2

Folch J, Lees M, Stanley. A simple method for the isolation ...
[https://doi.org/10.1016/S0021-9258\(18\)64849-51957](https://doi.org/10.1016/S0021-9258(18)64849-51957)

Step 46

Le Faouder, P.; Soullier, J.; Tremblay-Franco, M.; Tournadre, A.; Martin, J.-F.; Guitton, Y.; Carlé, C.; Caspar-Bauguil, S.; Denechaud, P.-D.; Bertrand-Michel, J. Untargeted Lipidomic Profiling of Dry Blood ...
<https://doi.org/10.3390/metabo1105030501>



Acknowledgements

This work was supported by:

- the MetaboHUB infrastructure funded by the Agence Nationale de la Recherche under the France 2030 program (MetaboHUB ANR-11-INBS-0010 ; MetEx+ ANR-21-ESRE-0035; MetaboHUB (JVCE) ANR-24-INBS-0012)
- the Plateforme de Métabolomique et d'Analyses Chimiques, US-61, MetaboHUB-Tours

We would like to thank:

- the MetaboHUB-MetaToul-Lipidomique platform for the SFC analysis and for welcoming and training Jérémy Monteiro
- the CHRU of Tours for the help for sampling and the handling of samples