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Biphasic extraction metabolites in algal matrix or periphyton V.2

Forked from a private protocol

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvw1zxdlmk/v2

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OPEN ACCESS

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External link: https://metabolome.u-bordeaux.fr/en/plateforme-bordeaux-metabolome-english/

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

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Keywords: metabolites, environnemental metabolomics, biphasic extraction, algal matrix, periphyton, lipids on an algal matrix, biphasic extraction, different steps of extraction, h2o mixture for the simultaneous extraction, liquid extraction, lipid metabolite, metabolite, extraction, simultaneous extraction, algal matrix, lipid, periphyton the purpose, periphyton, h2o mixture

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Abstract

The purpose of this instruction is to specify the different steps of extraction of metabolites and lipids on an algal matrix or periphyton previously freeze-dried and stored in a freezer at -80 °C .

Principle:
Use of a MeOH/MTBE/H2O mixture for the simultaneous extraction of primary, secondary and lipid metabolites.
Extraction is done by combining liquid/solid and liquid/liquid extraction that is carried out twice to optimize yields.

Materials

Consumable

Benchtop with extractor hood
2 mL tubes with fl at cap skirt in PP + 0.5mm microbeads
Ice blocks and ice cubes
Micropipettes + tips
Amber Vials 1.8mL with cryopreservation label
150mL glass bottles
UPLC grade solvents
2 beakers

Internal standards and matrix for control

100 mg of spike matrix (control condition or reference matrix)
PE tracer solution (15:0) @ 100 ng.µL-1 (or ppm)
Internal "polar" lipid standards @ 33.3 ng.µL-1 (or ppm)
Mix SST (50 mg/L)
Pesticide mix (3.3 mg/L)
Mix IS pesticides (2.5 mg/L)
Mix IS metabolites (50 mg/L) + (100mg/L)

Solvents

Ultra pure water :H2O
ACETONITRILE :ACN
METHANOL : MEOH
ISOPROPANOL : ISO

Equipment

Ultrasonic cleaner USC100T	NAME
Ultrasonic tank	TYPE
VWR	BRAND
6	SKU

Equipment

Labconco™ FreeZone™ 2.5L -84°C Benchtop Freeze Dryer	NAME
FreeZone	BRAND
10-400-014	SKU
https://www.fishersci.com/shop/products/2-5l-84c-bt-fd-sst-115v-60hz/10400014	LINK

Precision scale

Equipment

scale SARTORIUS	NAME
scale	TYPE
SARTORIUS	BRAND
MSA 36P-000DH	SKU

Equipment

FastPrep-24 5G	NAME
Bead beater	TYPE
MP Biomedicals	BRAND
116005500	SKU
https://uk.mpbio.com/fastprep-24-5g-instrument	LINK

Equipment

5430R	NAME
refrigerate centrifuge	TYPE
Eppendorf	BRAND
5430 R	SKU

Equipment

new equipment

NAME

Genevac EZ-2

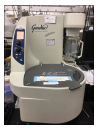
BRAND

EZ-2

SKU

https://www.spscientific.com/Products/Centrifugal_Evaporators___Sample_Concentrators/Genevac/EZ-2_Series/EZ-2_Series/

SPECIFICATIONS



Protocol materials

- MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34875-100ML
- Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641
- MilliQ water
- Acetonitrile ULC-MS **Biosolve Catalog #**0001204101BS
- Isopropanol HyperSolv CHROMANORM **VWR International (Avantor)**

Safety warnings

Use lab coat, gloves and safety glasses and laboratory fume hood. You can refere to the safety rules of the lab.

All waste generated must be treated properly.

Sample traceability is paramount, so it is necessary to optimize sample naming and storage as well as all reagents and stock solutions.

Gravimetric monitoring of stock solutions is carried out.

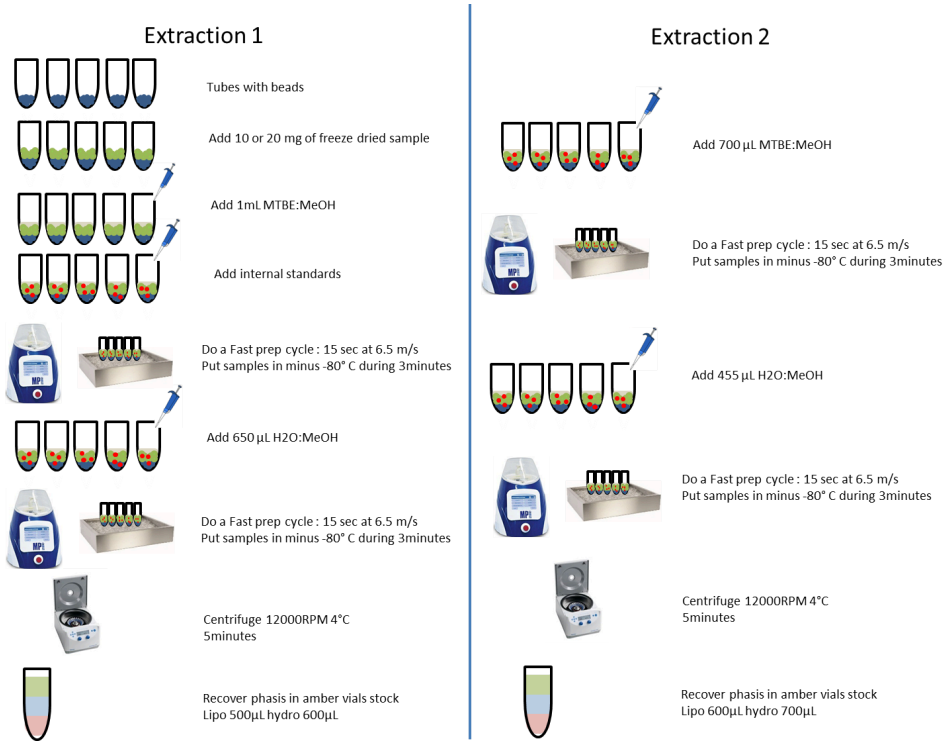
Write down all the weight as well as the volumes and nature of the internal standards added in a laboratory notebook.

Before start

Samples must be freeze dried and store at -80°C
Cleaning of the glassware should be done according to the laboratory laundry protocol using UPLC grade solvents or 8h oven calcination at 450°C.
All reagents used, including water, must be UPLC grade.
As the preservation of samples is done by cold, it is essential that as many steps as possible are done on ice.

EXTRACTION OVERVIEW

1



Extraction overview

PREPARATION

2h

2 EXPERIMENTAL CONDITIONS

Allow 4-6 replicates per experimental sample

o For environmental samples: provide doping with pesticide internal standards (IS) + metabo/lipido IS

o For laboratory samples: provide doping with IS of the investigated compound + metabo/lipido IS

• Provide a Procedural blank = solvent condition (including or not white field) ⇒ 3 replicates

• Provide a condition Blind Blank = solvent (=procedural blank with all IS) ⇒ 2 replicates

• Provide Spikes for validation of the manipulation ⇒ 2 replicates

o Spike (1) reference sample + (SST + Pest + PE:15) pre Extraction. + IS post Extraction

o Spike (2) reference sample + IS post Extraction.

3 PREPARATION OF EXTRACTION MIXTURES – UPLC GRADE (HERE FOR 60 REPLICATES!)

• Prepare an MTBE:MeOH mixture (3:1, v:v) ⇒ 150 mL stored at 4 °C for maximum one week

• Prepare a mixture H2O:MeOH (3:1, v:v) ⇒ 100 mL stored at 4 °C for maximum one week

MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34875-100ML

Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3641

MilliQ water

4 PREPARATION OF BALL TUBES AND VIALS

All these steps can be done the day before.

• Annotate all tubes

• Add 150 mg (± 10mg) of 0.5mm beads to each 2mL tube

• Annotate all 1.8mL amber stock vials (duplicate for lipophilic and hydrophilic fractions)

5 PREPARATION OF CRUSHED ICE OR COLD BLOCKS

• Prepare ice with the ice machine continuously throughout the extraction

• Place cold blocks at -80 °C as well as microtube racks

6 PREPARATION OF MIXTURES FOR DOPING: 5m

SST mix ([M] 50 Parts per Million (PPM)) + pesticide mix ([M] 3.3 Parts per Million (PPM))mix IS pesticides ([M] 2.5 Parts per Million (PPM)) + mix IS metabo ([M] 50 Parts per Million (PPM)) + PE (15:0) ([M] 100 Parts per Million (PPM))

• Ultrasound 00:05:00 at Room temperature

EXTRACTION STEPS 2h

7 PRELIMINARY STEPS 15m

On ice, in the soundcheck room:

• Add 10 mg (+- 1mg) of freeze dried matrix per tube containing the microbeads

• Record the exact mass of sample added

Doping with tracers/surrogates and IS:

o Sample approx: 10 µL IS pesticides + 10 µL IS metabo + 50 µL PE (15:0)

o Lab sample: 10 µL IS of the test compound + 10 µL IS metabo + 10 µL PE (15:0)
o Spike on matrix (or solvent if matrix not available): Extraction yield
Spike 1:
• Pre-extraction: 10 µL mix SST + 10 µL mix Pest + PE (15:0)

o Procedural blank: solvent only
o Blind blank: solvent + 10 µL All IS

In order to guarantee the cold chain:
• For 00:15:00 : Place the tubes and MTBE:MeOH solution at -80 °C ;
H20:MeOH solutions at -20 °C
• Start the Fast Temp on the centrifuge 4 °C

8 EXTRACTION 1 14m 45s

Under hood and on ice: Add 1 mL of MTBE:MeOH (3:1) per tube
• Cycle to Fast prep: 00:00:15 at 6.5 m/s
• Place the samples in ice or at -80 °C for 00:03:00
• Do a second cycle in Fast prep: 00:00:15 at 6.5 m/s
• Place the samples in ice or at -80 °C for 00:03:00

Under hood and on ice: Add 650 µL of H2O:MeOH
• Do a third cycle in Fast prep: 00:00:15 at 6.5 m/s
• Place the samples in ice or at -80 °C for 00:03:00
• Centrifugation: 12000 rpm, 4°C, 00:05:00

Under the hood and on ice, recover the phases in vials stocks (amber 1.8mL)
⇒ 500 µL lipophilic above; 600 µL hydrophilic below using a micropipette
Save the base for extraction 2

Equipment	
FastPrep-24 5G	NAME
Bead beater	TYPE
MP Biomedicals	BRAND
116005500	SKU
https://uk.mpbio.com/fastprep-24-5g-instrument	LINK

9 EXTRACTION 2 14m 45s

Under hood and on ice: Add 700 µL of MTBE:MeOH (3:1) per tube

- Cycle to Fast prep: 00:00:15 at 6.5 m/s
- Place the samples in ice or at -80 °C for 00:03:00
- Do a second cycle in Fast prep: 00:00:15 at 6.5 m/s
- Place the samples in ice or at -80 °C for 00:03:00

Under hood and on ice: Add 455 µL of H2O:MeOH

Do a third cycle in Fast prep: 00:00:15 at 6.5 m/s

- Place the samples in ice or at -80 °C for 00:03:00

Centrifugation: 12000 rpm, 4°C, 00:05:00

Under the hood and on ice, recover the phases under the hood in the same vials

⇒ 600 µL lipophilic above; 700 µL hydrophilic below using a micropipette

End of the process

- Weigh the amber stocks after transfer of the extracts

⇒Theoretically: 1.1 mL lipo vs 1.3 mL hydro

- Doping
  - Spike 1 & 2
    - Post-extraction: 10 µL IS metabo + 10 µL IS pesticides

- Store all vials at -80 °C

PREPARATION OF VIALS FOR INJECTION

2h

10 HYDROPHILIC FRACTION UPLC-TOF

35m

- 00:05:00 ultrasound
- 500 µL transfer into clear vials
- Evaporate solvant in Genevac : 30 °C , 00:30:00 hydro mode
- Add 100 µL H2O:ACN 50:50 and transfer into insert

Acetonitrile ULC-MS **Biosolve Catalog #**0001204101BS

- QC pool preparation and dilutions

⇒Pool 10 µL of each sample in amber vial (without insert) and prepare QC/2 QC/4

Boosting the QC pool with leucine 1ppm at 1/100

- Calibration range preparation: SST mix + Pest mix + PE (15:0) with fixed concentration IS metabo and Pest

Equipment

new equipment

Genevac EZ-2

EZ-2

https://www.spscientific.com/Products/Centrifugal_Evaporators___Sample_Concentrato
-2_Series/EZ-2_Series/

11 LIPOPHILIC FRACTION UPLC-TOF

25m

- 00:05:00 ultrasound
- 500 µL transfer into clear vials
- Evaporate solvant in Genevac : 30 °C , 00:20:00 lipo mode

• Add 100 µL ISO:ACN 50:50 and transfer into insert

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

QC pool preparation and dilutions

⇒Pool 10 µL of each sample in amber vial (without insert) and prepare QC/2 QC/4

Boosting the QC pool with leucine 1ppm at 1/100

• Calibration range preparation: SST mix + Pest mix + PE (15:0) with fixed concentration IS metabo and Pest

12 LIPOPHILIC FRACTION HPLC-MS/MS 5m

• 00:05:00 ultrasound

• 125 µL transfer in insert in clear vials

• Dry evaporation under N2 flow at Room temperature

• Addition of the injection solvent for final dilution at 1/2: 250 µL of isopropanol + 50 µL IS polar lipids for subsequent analysis of glycolipids and phospholipids for example

Protocol references

Giavalisco et al.2011 ; Sostare et al. 2018 ; Salem et al. 2017 ; Cajka and Fiehn 2016 ; Pezzati et al.2019 ; Tufi et al.2015