Uniwersytet Gdański

Department of Geomorphology and Quaternary Geology – Procedure

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Bulk pigment extraction from lake sediments

Consumables and equipment

Chemical reagents

- Acetone **100%** (grade **HPLC**): solvent.
- Acetone **technical**: cleaning.
- **Redistilled water (*MilliQ*)**.
- Technical nitrogen.
- Neodisher Laboclean.

  Neodisher LaboClean FLA: liquid, highly-alkaline cleaning, dispergator agent.

Equipment

- Laboratory fume hood.

> Acetone
>
> Acetone (C_3H_6O) is an organic solvent requiring work under the **running fume hood**.
> Acetone is highly **flammable** and is an **irritant.**

- Ultrasound bath.
- Centrifuge.
- Vortex shaker.
- Laboratory furnace for glassware baking.
- Laboratory oven.
- Freeze drier.
- Glass beakers for acetone pippeting.
- Acetone pipette **1000 µl** and tips (for acetone).
- Heating plate.
- Nitrogen evaporator.

  - Needles for N_2 evaporator.
  - Metal rack for amber vials on the heating plate.

  For example *Turbovap* instrument.
- Racks for centrifuge tubes and amber vials.
- Analytical balance, antistatic device and stainless steel spatula.
- Ice bath.

  Plastic tray with cooling packs.
- Crimper (for closing HPLC vials).

Other

- Polypropylene (PP) centrifuge tubes (falcons) **25 ml** or **50 ml** (Corning™).

  Other plastics will melt in contact with acetone.
  One-time use.
- Amber vials with caps **25 ml** or **50 ml**.

  Tape the labels.
- PTFE hydrophobic filters of **0.2 m** (**13 mm**).

  One-time use.
- Polypropylene (PP) small syringes with Luer Lock for filtering (**2 ml**).

  One-time use.

> **!** Important
>
> Always check size and fit compatibility.

- Aluminum foil.
- Glass Pasteur pipettes.

  One-time use.
- Silicone nipples for pipettes.

Cleaning

Amber vials

- Put into soap water with Neodisher Laboclean for few hours.
- Wash in the washing machine.

  Glassware program and Neodisher Laboclean.
- Dry in the drying oven.
- Close with aluminum foil (cleaned with technical acetone).
- Bake in the furnace in **500 °C**.

  Minimum **3 h**.

Amber vials caps

- Rinse with tap water.
- Put into beaker filled with **technical acetone** and **redistilled water (MilliQ)** mixture (**1:1**).
  - Sonicate for **10 minutes**.
- Put into beaker filled with **technical acetone**.
  - Sonicate for **10 minutes**.
- Dry in the drying oven.

  Washing solvents can be used again. Store in the glass bottles.

Evaporator needles

- Put into beaker filled with **technical acetone**.
- Sonicate **3 × 10 minutes**.

Disposal

- Acetone accordingly.
- PP falcons and glass Pasteur pipettes need to be washed and recycled.

Sediment preparation

- Freeze dry.

  High temperature speeds up pigment degradation.

- Loss on ignition **550 °C**.

  Paleoenvironmental Research Laboratory: calculate organic matter from CNS.

- Weigh **0.5 g** homogenized sediments into polypropylene falcons (PP) **25 ml**.

  Clean tools with technical acetone, use antistatic device.
  If there is not enough material, use no less than **0.25 g**.

- Label falcon and cap with sample name.

Pigment extraction

- Prepare workplace, cover surfaces under the fume hood with aluminum foil cleaned with technical acetone.
- Switch off the lights whenever possible.
- Fill glass beaker with **100%** acetone.

  Don't pipette from the bottle.

- Rinse pipette with acetone.

  Pipette into waste beaker.

- Add **5 ml** of acetone to the falcon.

  pipette right, there should be no air bubbles in the tip.

- Shake with vortex for **1 minute**.
- Sonicate for **1 minute**.
- Centrifuge:
  - **10 minutes**.
  - **5000 rpm**.

  This step needs to be adjusted to the centrifuge. Lower rpm requires longer centrifugation.

- Check if supernatant is clear after centrifugation.

  If it is not clear centrifugate again.

- Remove (transfer) supernatant using glass Pasteur pipette into amber vial.

  Label amber vial accordingly.
  Be sure all supernatant is transferred.

- Repeat two times using **5 ml** acetone each time.
- Hold labelled Pasteur pipettes in a glass beaker between the extraction steps.

  Every amber vial and falcon has its own Pasteur pipette.

- Amber vials between extraction steps should be covered from light and stored in the ice bath.

  After **three extraction cycles**, if supernatant still has a strong color continue with **additional extraction steps**.
  It is important that **all samples** are prepared **the same way**.
  Extracts can be stored **for a few days** in the **-20 °C** temperature.

Concentration and reconstitution

Evaporation

- Set heating plate and nitrogen evaporator.
- Evaporate samples to dry residue.

Before use be sure that needles are clean (Section **??**).

- Put amber vials in the rack on the heating plate set to **35 °C**.
- Open nitrogen flow.

Set flow so small flexion of the liquid surface is visible.

- Evaporate to dry residue (around **4** to **6 h**).

Reconstitution and filtering

- Add **2 ml 100%** acetone to the amber vial using **1000 µl** pipette.

Be very precise, check for air bubbles in the pipette tip.

- Homogenize solution with the glass Pasteur pipette.
- Rinse the vial walls and *vortex*.

All pigments need to redissolve in the acetone.

- Take a **2 mL** syringe, attach the **0.2 µm** PTFE hydrophobic filter on.
- Pipette directly with a pateur pipette into the syringe.
- Pass the solvent through the filter into the small HPLC glass vials with the piston.
- Loose the filter from the syringe to get all solvent out.
- Close the HPLC vials with a crimper and store your samples in a labelled sample box at -20 °C.

Credits

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Changelog

- 09.12.2022, MZ – initial version.
- 09.02.2023, MZ – full version.
- 17.03.2023, MZ – update with filtering and callouts.

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