
Bulk pigment extraction from lake sediments

Consumables and equipment

Chemical reagents

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

  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.

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 **5 mL**.

  Tape the labels.
  Two sets are needed, for unfiltered and filtered solvent.

- Polypropylene (PP) small syringes with Luer Lock for filtering (**5 mL**).

  One-time use.

- PTFE hydrophobic filters of **0.2 m**.

  One-time use.

> **!** Important
>
> Always check size and fit compatibility for syringes and filters.

- 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.
  If dishwasher is unavailable do it manual.

- Dry in the drying oven.
- Close with aluminum foil (cleaned with technical acetone).
- Bake in the furnace in **380 °C**.

> **!** Baking
>
> Minimum **3 h**.
> Never bake measurement glass.

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** or CNS to calculate organic matter.
- 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**.
  With minimal **OM** or **TOC** concentrations weigh **1.00 g** and more if needed.
- 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.
- 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**).
- Prepare blank sample from acetone.

Reconstitution and filtering

- Add **5 mL 100%** acetone to the amber vial #1 (unfiltered) 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 **5 mL** syringe, attach the **0.2 µm** PTFE hydrophobic filter on.
- Pipette from the amber vial #1 (unfiltered) directly with a Pasteur pipette into the syringe.
- Pass the solvent through the filter into the amber vial #2 (filtered).
- Loose the filter from the syringe to get all solvent out.
- Close the vial with a cap and store 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.
- 27.03.2023, MZ – NAU final version 1.

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