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## Preparing water samples for analysis using ultrahigh resolution mass spectrometry V.1

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### ABSTRACT

Solid phase extraction using PPL modified from Dittmar et al. (2008): Dittmar, T.; Koch, B.; Hertkorn, N.; Kattner, G., A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography Methods* 2008, 6, 230-235

### MATERIALS TEXT

Have on hand before you start:

- Apparatus to filter water samples
- Teflon bottles, Teflon tubing, Teflon filter holders, peristaltic pump with Pharmed BPT tubing for the pump head
- Omnipore filters from Millipore (47 mm filters, part number JGWP04700)
- Bond Elut PPL cartridges from Agilent (1 gm / 6 mL cartridges, part number 12255002)
- Extraction manifold, vacuum pump, carboy to collect waste, Teflon tubing
- Combusted glassware:
  - test tubes
  - glass sample vials with Teflon-lined caps
  - glass pipets
- 100% methanol, Optima grade
- 12 M hydrochloric acid, trace metal grade

Two comments:

- (1) The ferrules in the Teflon filter holders are really easy to lose...best to keep the nuts tight most of the time.
- (2) You will want a clamp or two cable ties on the outflow of the peristaltic pump.

### Extraction of organic compounds using the Bond Elut PPL solid phase extraction resin

- 1 Remove biotic and abiotic particulate matter with filtration method of choice. We generally use 0.2 um Omnipore (PTFE, hydrophilic) filters from Millipore in Teflon filter holders with a peristaltic pump. At the pump head, we use PharMed BPT tubing. Depending on the volume of your sample you could alternatively use the glass filtration setup hooked up to a vacuum pump.
- 2 After filtering the water, fold the filter cell side in, and put the filter into a cryovial and freeze at -80°C.
- 3 Save the filtrate.  
Acidify filtrate with concentrated HCl to pH 2-3. Do not let filtrate pH reach values lower than 2. Approximate volume of acid needed: 1 mL / 1 L seawater.  
You can store the acidified filtrate at 4 °C until you are ready to extract it. We usually do not store it for more than ~24 hours in this state.

- 4
  - Pass one cartridge volume of 100% methanol through the PPL cartridge.
  - Use an old test tube inside the filtration manifold to collect the waste methanol.
- 4.1 If you will be filtering a small volume you should rinse the PPL cartridge with one cartridge volume of 0.01M HCl before loading sample, otherwise you are using the first few milliliters of your sample as your rinse to remove the methanol.
- 5
  - Use Teflon tubing to connect the filtered seawater sample to the PPL cartridge.
  - Turn on the vacuum pump to pull the acidified sample through the PPL cartridge.
  - Target flow rate should be less than  $40 \text{ ml min}^{-1}$ .
  - Rinse the PPL cartridge with four cartridge volumes of 0.01 M hydrochloric acid.
  - Dry for 5 minutes by leaving the vacuum pump on (you may need to turn up the pressure on the pump to be sure you pull out the dregs of liquid).
  - Put a fresh glass test tube under the outlet of each sample.
  - Add one cartridge volume of 100% methanol and elute the sample off the PPL. The flow rate should be less than  $2 \text{ ml min}^{-1}$ , and gravity is usually sufficient.
  - Transfer your sample to a glass vial with a Teflon-lined cap.
  - Store samples at  $-20^{\circ}\text{C}$  or lower to minimize sample alteration.
- 6
  - Dry sample in a vacufuge until almost dry, 'almost dry' should be between 5 - 20  $\mu\text{L}$  of fluid.
  - Do not allow samples to be completely dried since you will lose organic compounds.
- 7
  - Neutralize the filtrate with baking soda before discarding it with seawater waste.

#### Calculating carbon concentration of extracellular extracts

- 8
  - The volume you prepare for analysis with the FT-LC or TSQ will depend on the concentration of carbon in your samples. For example, in seawater samples, we filter 2 liters of water which has a concentration of 45 $\mu\text{M}$  NPOC. If the extraction is 100% efficient, we would have an extract of 1 ml with a concentration of 90,000  $\mu\text{M}$
  - $(2000 \text{ ml}) * (45 \text{ } \mu\text{M}) = (1 \text{ ml}) * (x)$
  - $X = 90,000 \text{ } \mu\text{M}$
  - This calculation assumes that you have a final extract of 1ml since you dried down whatever volume ended up eluting off the PPL cartridges. You can then bring up the dried solution in a known volume of whatever solvent you want.
  - For seawater, using  $\sim 1/3$  of the 1 ml extract seems to work for the FT-LC and TSQ.  $2/3$  of the 1 ml extract is too high. Your results may vary depending on the sample since the extraction efficiency varies for different samples.
  - For the TSQ in particular, you need to keep track of the total extract volume in order to figure out the concentration of each metabolite in the original extract. Since the amount eluted off the PPL cartridges is not constant, you can either (1) dry the original extract down and add a known concentration of solvent or (2) measure the final volume of the PPL extract using a clean syringe. Whether you decide to dry everything down all at once, or only dry a portion of the sample is up to you.

#### Prepare samples for analysis using targeted or untargeted mass spectrometry-based analysis

- 9
  - Before starting, solvent rinse a glass syringe with the following solvents: MQ water, methanol, acetone, dichloromethane. Rinse the syringe with each solvent three times, one solvent at a time in the above order.
- 10
  - Use this clean syringe to transfer a known volume of your PPL extract to an 8 ml glass vial. After each sample, rinse the syringe first with methanol and then acetone.
- 11
  - Dry down the methanol that is in the 8 ml amber vial using the Vacufuge until it is 'almost dry' (see above comment about 'almost dry').

- 12 After nearly dry, bring up the samples in 500  $\mu$ L 0.05 - 0.15  $\mu$ g/mL deuterated injection mix (D2 biotin, D6 succinic acid, D4 cholic acid, and D7 indole 3 acetic acid). **Note**: sometimes these should be brought up in a different volume depending on the particular samples. Also depending which method these are being used for they may need to be in different solvents (currently 1% ACN for reversed phase and 95% ACN for HILIC).
- 13 For each sample setup two, 2ml vials with caps and septa appropriate for the autosamplers. Into each 2 ml vial, place a glass insert with the plastic foot.
- 14 For the FT samples, place 200  $\mu$ L in vial, place a glass insert with the plastic foot.  
For the TSQ samples, place 100  $\mu$ L of sample into the insert.
- 15 To create a pooled sample: add 45  $\mu$ L of each sample into an LC vial (without an insert) and vortex it. This should be run as a partial loop between blanks and samples and as a full loop approximately every 6 samples throughout the run. If the volume you have is adequate for that many replicates you can leave it in the 2 ml vial, otherwise you can put 200  $\mu$ L of the pooled sample into multiple vials with inserts to make the volume go further.



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