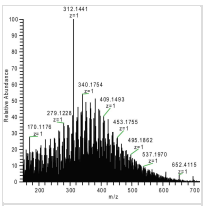

Feb 16, 2022

Protocol for PPL Solid Phase Extraction for Dissolved Organic Matter (DOM) Sample Preparation for LC-MS/MS Analysis

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mthukral

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General Remarks:
Protocol for PPL Solid Phase Extraction (SPE) for sample preparation for LC-MS/MS analysis of DOM. Using a SPE vacuum station, this protocol can be adapted for large (>200 mL) or smaller sample volumes (<200 mL). The general procedure is written for larger volumes with notes embedded with procedural modifications for smaller samples. Examples of recommendations for sample size are 1 L for environmental samples, and 50-100 mL for a laboratory culture. Avoid materials with silicone coating and PEG contaminants (e.g. silicon tubing and sterivex and supor filters).

Citation: mthukral , Irina Koester, Daniel Petras, Ralph Riley Torres, Allegra Aron, Emily Gentry, Xavier Siwe-Noundou, Rosemary Dorrington, Kerry Mcphail, Aaron Hartmann, Lihini Aluwihare Protocol for PPL Solid Phase Extraction for Dissolved Organic Matter (DOM) Sample Preparation for LC-MS/MS Analysis https://dx.doi.org/10.17504/protocols.io.b4x8qxrw

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protocol

Petras D, Koester I, Da Silva R, Stephens BM, Haas AF, Nelson CE, Kelly LW, Aluwihare LI and Dorrestein PC (2017) High-Resolution Liquid Chromatography Tandem Mass Spectrometry Enables Large Scale Molecular Characterization of Dissolved Organic Matter. Front. Mar. Sci. 4:405. doi: 10.3389/fmars.2017.00405

Solid-Phase-Extraction, Dissolved Organic Matter, Mass Spectrometry, Sample Preparation

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Feb 11, 2022

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Materials

General Materials
Agilent SPE Vacuum Manifold (Part Nr.: 12234100)
HDPE vacuum carboy (Thermo scientific, 2226-0050PK) as solvent waste container
Bulkheads for carboy cab (6149-0001PK)
Rubber vacuum tubing
Vacuum pump (e.g. Vacuubrand MD 4C or cheap option)

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Nalgene HDPE 1L sample bottles (Part Nr.: 2002-0032)
Glass inserts for autosampler vials (Thermo scientific 03-375-3A)

Material for Sample Tubing (optional for larger volumes)
Polypropylene or teflon tubing ⅛ inch (teflon preferred)
Luer adapters (Cole Palmer Part Nr.: VV-30800-18)
Luer stopcocks (Cole Palmer Part Nr.: EW-30600-01)
Glass 1 mL serological pipettes
Shrink tape/tubing
Teflon tape
Heat gun

Material for Reservoirs (optional for small volumes)
Agilent 60 mL reservoirs (Part Nr.: 12131012)
Agilent stopcock (12131005) or Luer stopcocks (Cole Palmer Part Nr.: EW-30600-01)

SPE Cartridges
Agilent Bond Elut PPL cartridges, 200 mg , 3 mL, for larger volumes (Part Nr.: 12105005)
Agilent Bond Elut PPL cartridges 50 mg, for smaller samples (Part Nr.:12105002)
Agilent cartridge adapter caps (Part Nr.: 12131001)

Solvents
Trace metal grade HCl, 37% (Sigma aldrich 339253)
LC-MS Grade H2O (VWR BDH83645.400)
LC-MS Grade MeOH (Fisher Scientific MX0486-1)

Material for Sample Elution
PPL Elution Rack (Custom made part, details including vector graphic for laser cutting can be found in the supplemental information)
2 mL LC glass Vials (Thermo scientific 03-377B)
LC Vial Caps (Thermo scientific 03-379-124)
50 mL Plastic syringe with luer lock
1000 uL pipette and pipette tips
Formic acid (part number)
LC vial insert (part number)
Trace metal grade hydrochloric acid

Material for Extraction Efficiency
Scale
Hamilton syringe (100-250uL) or 100 uL autopipette and pipette tips
Bulb pipette 15-20mL
Thermo Scientific VOA vials with septum cap (SB36-0040)

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Preparation of Materials I

1 Assembly of tubing

Materials

1.1 Cut tubing to desired length (recommended 30 inches/ 75cm) with a precise, flat cut.

1.2 Push tubing firmly into a glass pipette until it reaches the bottom of the pipette tip.

1.3 Seal the top of the pipette with teflon tape and wrap a 5 cm piece of heat-shrink tubing over to cover the junction.

1.4 Heat the shrink tubing carefully with the heat gun until it shrinks and fixes the tube-pipette connection.

1.5 On the other end of the tubing, attach a luer adapter and stopcock. The tubing may need to be heated with the heat gun to allow the luer to fit.

1.6 If DOC analysis from the same bottles is desired, MeOH needs to be avoided. Alternatively, soak adapters, reservoirs and bottles in 3.7% HCl and rinse thoroughly with MilliQ water.

1.7 Image 1 and Image 2: Assembly of tubing.

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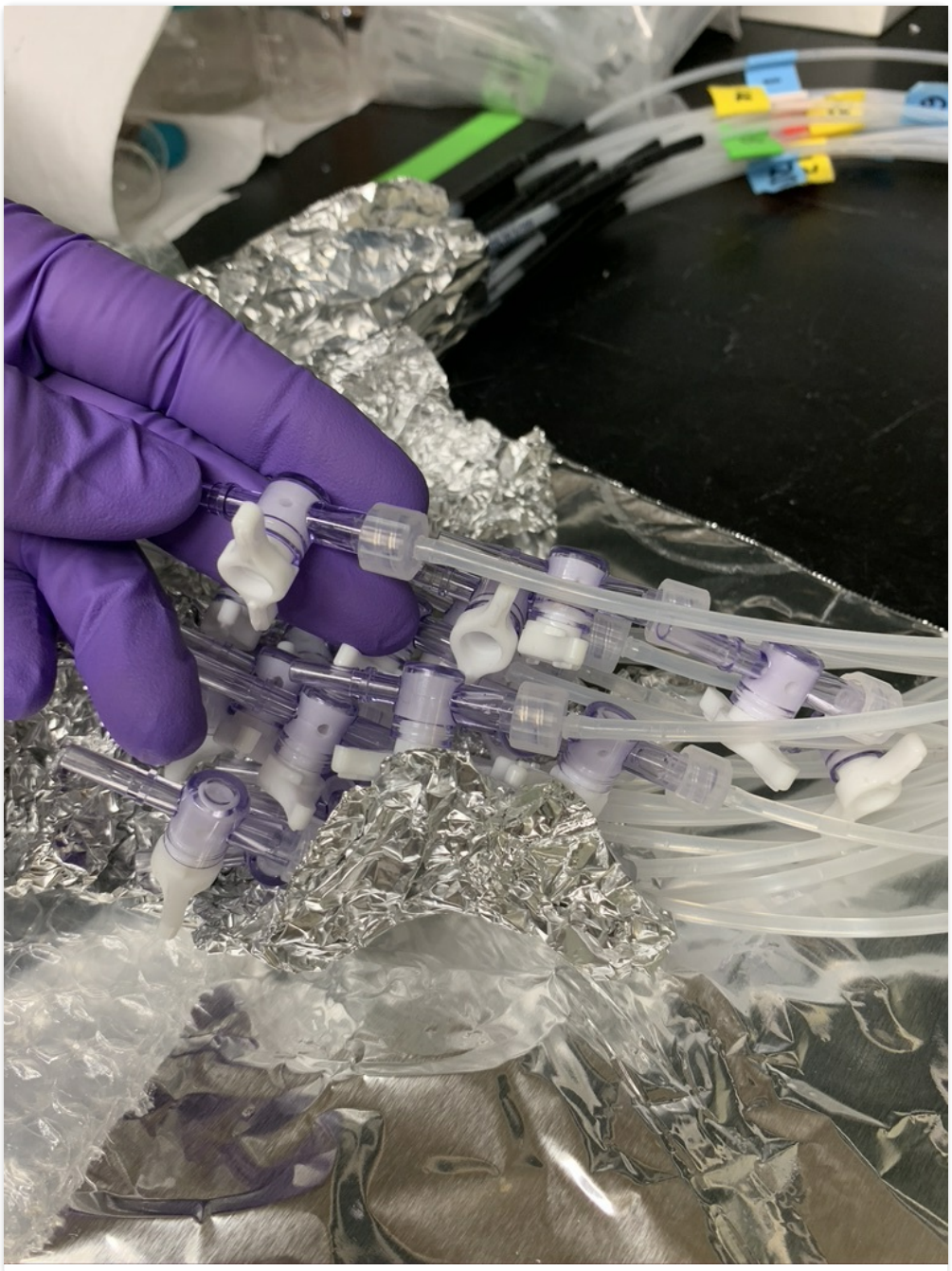

Preparation of Materials II

2 Washing of Material

2.1 For LC-MS/MS sample collection, bottles, connectors and tubing should be rinsed with MeOH followed by MilliQ water before use.

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2.2 If DOC analysis from the same bottles is desired, MeOH needs to be avoided. Alternatively, soak adapters, reservoirs and bottles in 3.7% HCl and rinse thoroughly with MilliQ water.

Preparation of Materials III

3 Setup of SPE station

3.1 Connect SPE station to a HDPE vacuum carboy as a solvent waste container, which is also connected to a vacuum pump as shown in figures 2 and 3.

3.2 Image 3 and Image 4: Connection of SPE station to solvent waste container and to vacuum pump.

Citation: mthukral , Irina Koester, Daniel Petras, Ralph Riley Torres, Allegra Aron, Emily Gentry, Xavier Siwe-Noundou, Rosemary Dorrington, Kerry Mcphail, Aaron Hartmann, Lihini Aluwihare Protocol for PPL Solid Phase Extraction for Dissolved Organic Matter (DOM) Sample Preparation for LC-MS/MS Analysis https://dx.doi.org/10.17504/protocols.io.b4x8qxrw

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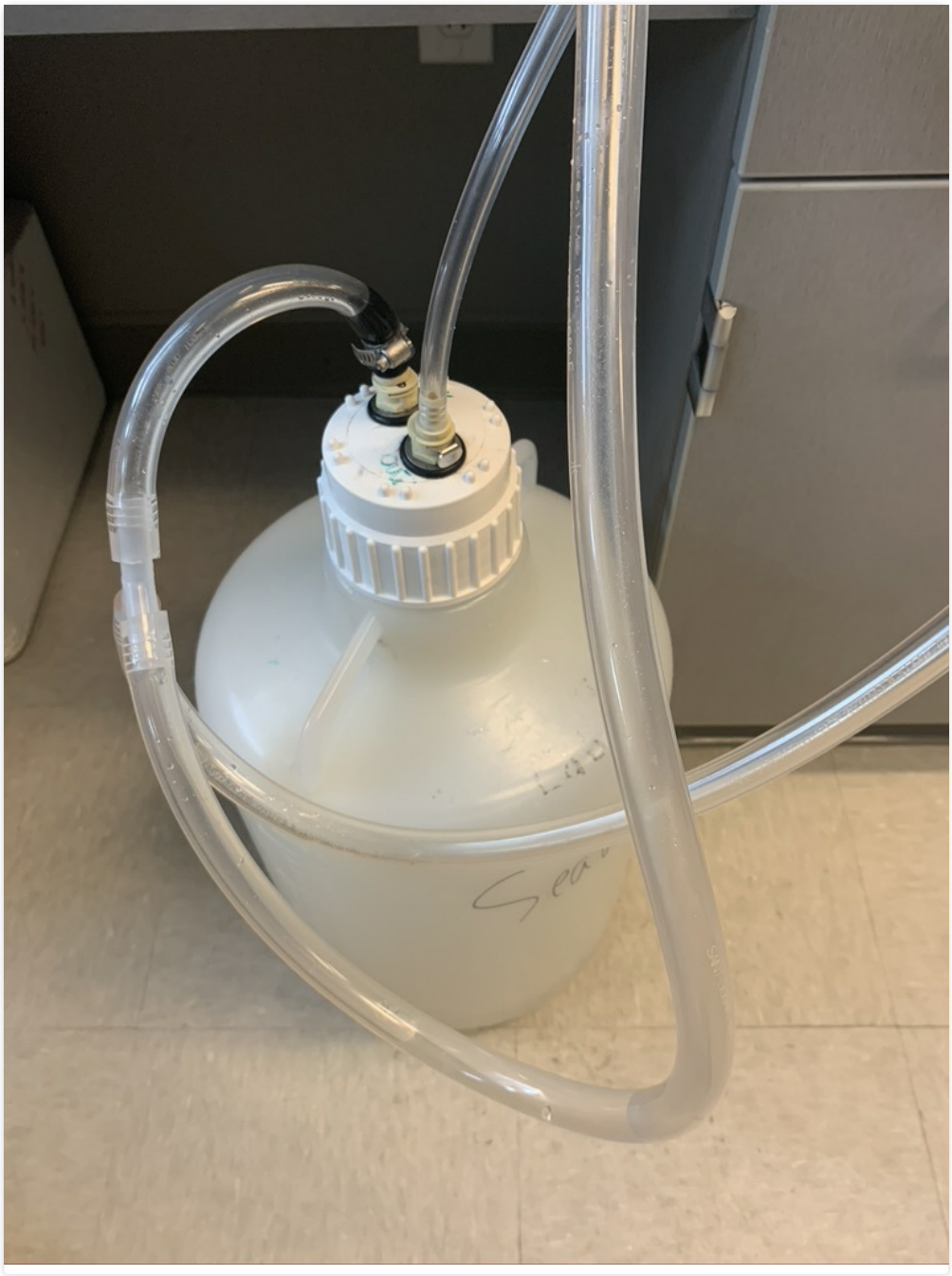
3.3 Image 5: Set up of the Waste Container Cap



Extraction Procedure I

4 Prefiltration (optional)

4.1 If DOM extraction is desired, samples should be filtered with Glass Fiber Filters, (GF/F, 0.7 µm, combusted). Depending on total sample volume, 47 mm filters are appropriate for 0.5-2 L of sample. For larger volumes, larger filter or

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cartridge systems should be used, with special attention paid to potential for carbon contamination (in DOC labs a polysulfone cartridge filter that can be soaked overnight with 0.01N HCl is used, followed by extensive rinsing with MilliQ water until pH paper shows neutral pH of effluent).
Note: Supor or sterivex filter can be used as well if sampling is combined with microbiome sampling. Due to high background contamination from the filter we do not recommend this, however if those filters are used, the first >100 mL of filtrate should be discarded to flush the filters.

Extraction Procedure II

5 Cartridge activation and pre-conditioning

5.1 Remove lids on vacuum station only for ports in use.

5.2 Connect stopcock ends of tubing to an adapter inserted into a PPL cartridge connected to each port (figure 4).

5.3 For smaller samples install 4 parts vertically in this order from base up: stopcock, PPL cartridge, adapter cap, reservoir (Image 6).
Image 6: Set up for larger sample volumes (>200 mL).

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5.4 Image 7: Set up for smaller samples (<200 mL).

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5.5 Add an extra port for LC-MS H2O procedural blank each use.

5.6 Wash cartridges as follows to activate PPL resin, without letting them run dry between washes:

1. 3x column volume 100 % LC-MS grade MeOH.
2. 3x column volume acidified H2O (Prepare as 1 mL 37% HCl into 1 L of LC-MS grade H2O; pH 2.0).
3. 3x column volume 100% LC-MS grade MeOH.
4. 3x column volume acidified H2O.

Extraction Procedure III

6 Sample preparation and loading/extraction

6.1 Acidify samples with concentrated HCl until the pH of the sample reaches 2.0 (typically 0.12% acid for seawater).

6.2 For small volumes (<200 mL): Pour samples into the reservoir and let it drip through slowly: about 1 drop every second (approx. 8 mL/min).

6.3 For larger volumes (>200 mL): Place pipette tip of tubing assembly in sample bottle and turn on and adjust vacuum to a final loading flow rate between 8 and 16 mL/min (slower for smaller volumes)

6.4 Flush PPL with 2 column volumes pH 2.0 H2O to remove salt from the cartridge.

6.5 Dry cartridge under nitrogen gas until the color of the cartridge changes to a light yellow. Shake PPL cartridges to ensure all the resin changes color.

6.6 If you are working in the field, this step could be skipped and proceed to the next step of sample storage. The cartridges should then be dried before elution. (Drying is important because addition of methanol for elution can result in methylation of carboxylic acids if residual pH2 water is present in the cartridge).

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Extraction Procedure IV

7 Sample storage of cartridges

7.1 Wrap cartridges individually in aluminum foil and freeze at -80°C, or proceed to elution. Storage at -20°C is also acceptable if -80°C is not available (e.g. during fieldwork). For field work, place samples on dry ice and transport to the lab to freeze.

Extraction Procedure V

8 Sample elution and preparation for LC-MS/MS analysis

8.1 Set up PPL cartridges in PPL Elution Rack with an LC vial beneath each cartridge.

8.2 Elute each cartridge with 2 mL of LC-MS grade MeOH into the LC vial using 1 mL pipette.

8.3 If the measurement of extraction efficiencies is desired, follow steps described in below.

8.4 Cap LC vial for short term storage or proceed directly to dry down the extract in a Centrivap (typically overnight) at room temperature.

8.5 Once dry, store at -80°C or -20 °C or resuspend the dried extract for LC-MS analysis.

8.6 For LC-MS analysis, samples need to be resuspended in 100 uL 80% LC-MS grade MeOH + 0.1% FA by pipetting the solvent up and down over the DOM pellet. The respondent sample should then be transferred to a micro glass insert inside of a 1.5 mL HPLC vial. If the sample does not dissolve completely, it may need to be centrifuged to pellet debris.

8.7 The sample is now ready for LC-MS/MS analysis. Storage after analysis

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should be at -80°C.

9 Carbon Extraction Efficiency:Determination using dried aliquot of SPE-DOM extract

9.1 The sample volume extracted needs to be known

9.2 Weigh combusted GC vials with teflon lined caps and freezer-resistant labels

9.3 Elute SPE-DOM into GC vial and immediately cap to avoid evaporation of the methanol

9.4 Weigh vial with cap and methanol extract

9.5 Calculate the weight difference (full - empty vial). Transfer mass into volume by considering the density of methanol at the temperature of the lab

9.6 Take DOC samples from methanol extract (100uL or more depending on concentration of extract) and transfer into combusted DOC vial (Thermo Scientific VOA vials)

Note: You can use an auto pipette but to be more accurate use Hamilton-syringe (clean 10times with methanol and wipe needle with Kimwipe 10 times before usage and in between each sample)

9.7 Evaporate extracts in DOC vials in a speedvac (RT) or oven (40°C) overnight. Use a piece of combusted aluminum foil to cover the DOC vial loosely so the methanol can evaporate

9.8 After drying: add an exact amount of Milli-Q using a clean bulb pipette to the DOC vial (depending on concentration and volume of the extract aliquot, 10mL is minimum to run the sample on the TOC analyzer).

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9.9 Use the aluminum foil as liner and screw on the DOC cap if measured immediately. For longer storage cap the vial with acid washed and teflon coated caps

9.10 Put the sample for 10min into an ultrasonic bath. If the extract did not dissolve, further agitate and/or pipette back and forth in order to dissolve the dried extract.

9.11 Acidify DOC sample to pH 2 (~ 1mL conc. HCl per liter)

9.12 Measure DOC concentration using a TOC analyzer

9.13 Calculate the SPE-DOC concentration considering all volumes (samples, extract, etc)

9.14 The extraction efficiency is the ratio between SPE-DOC and bulk DOC of the same sample

10 **Carbon Extraction Efficiency: Determination by sampling flow-through**

Note: This method is easier to perform but overestimates the extraction efficiency in comparison to the method described in 8. See Table S1 in the Supplemental Materials of Petras et al. (2017) for comparison. The DOC concentration of flow-through depends on the time of sampling: in the beginning DOC of flow-through will be less than later when the SPE column might get saturated.

10.1 Collect flow-through sample dripping out of the SPE column into combusted DOC vial

10.2 Cap with acid washed teflon coated cap

10.3 Measure DOC concentration (must have at least 10mL of volume for analysis)

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10.4 Subtracting this permeate DOC concentration from the pre-filtered seawater DOC concentration entering the SPE column will result in the “extracted” DOC concentration

10.5 The ratio between “extracted” DOC concentration and the pre-filtered DOC concentration is the extraction efficiency

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