DOM Inter-lab MS/MS study sample prep
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DOM Extract preparation

200 L of surface seawater were collected at the top of the Ellen Browning Scripps Memorial Pier on the 26th of February 2021 between 11:00 and 19:00 PDT. The seawater was filtered through an AcroPak 0.8/0.45 um Supor membrane filter (Pall Corporation) and subsequently acidified with a total of 260 mL HCl (37%, trace metal grade).
For solid phase extraction (SPE), Agilent Bond Elut PPL styrene-divinylbenzene polymer cartridges with 5 g bed mass and 60 mL column volume were used. The cartridges were activated with 30 mL of MeOH and then rinsed with 30 mL H_2O (pH 2, LCMS grade) and 30 mL MeOH (LCMS grade) followed by an equilibration with 30 mL H_2O (pH 2, LCMS grade).
For sample loading, acidified seawater was pulled with PP tubing connected to a serological glass pipette through 8 SPE PPL cartridges in parallel. We used a vacuum SPE station (Agilent 20 port SPE station) to maintain a flow rate of approximately 20 mL/min/cartridge and a total loading time of 20 h. A process blank was collected using 4 L of acidified H_2O (pH2, LCMS grade) onto a 5 g cartridge using the same SPE protocol as above.
After sample loading, the cartridges were desalted with 60 mL H_20 (pH 2, LCMS grade) and dried under N_2 gas.
After drying, the cartridges were eluted with 20 mL MeOH per cartridge resulting in a total of 200 mL eluted sample to which an internal standard mix was added that contained 5 ug each of domoic acid, kanic acid, isoxaben, irgarol, imazapyr, heroin, methamphetamine and cocaine. The pooled sample was then aliquoted to 100 individual 2mL HPLC vials. The blank sample was eluted with 10 mL MeOH, an aliquoted into 6 2 mL vials. All vials were dried down in a vacuum centrifuge overnight at room temperature. 6 aliquots were weighed after the drying process resulting in 1.8 mg of total organic matter per vial.
Two representative aliquots were redissolved in H_20 (pH 2) for TOC measurements resulting in 30.0 uM (for 2 L) and 0.72 mg C/vial. The overall extraction efficiency was 43.3 % considering the DOC concentration of the initial seawater sample of 69.3 uM carbon.
Samples were stored at -80 degrees, till shipment via 2 day express with initial cooling with -80 C cold packs) to the Uppsala University, Sweden.

Algae Extract Sample preparation

Algae lyophilized cells from *Synechococcus* sp. were purchased from Merck (Product number 491764). 0.83 g cells were suspended in 50 ml methanol (LCMS grade) in a methanol rinsed Falcon tube, and this was sonicated for 5 minutes and centrifuged at 5000 RCF for 10 minutes. The supernatant was decanted to a new Falcon tube, dried down under nitrogen and redissolved in water (MilliQ). This was sonicated and centrifuged, and the supernatant transferred to a 250 ml bottle, diluted to 150 ml and acidified with 150 µL HCl. On addition of the acid, the bright green solution turned a yellow-brown colour. The solution was extracted on a pre-conditioned PPL cartridge (1g; Agilent), and the yellow-brown colour was not retained.

Retained metabolites were eluted with methanol (8ml loaded, 5.32 g retrieved). 300µL was removed and the rest was dried under nitrogen. The extract was redissolved in 2ml methanol, and 0.1 ml was taken for DOC analysis. This 100µL fraction was dried under nitrogen until the weight no longer changed, and redissolved in 36.5 ml MilliQ water. The DOC concentration was determined to be 5.79 µg/ml after subtraction of the blank, indicating 2.1 mg/ml C in the 2ml stock solution. From the stock solution, three new stock solutions with 1800, 600 and 200 µg/ml were prepared in methanol.

Ring-Study Standard Samples

The final sample scheme for the ring study is shown in Figure 1. Final DOM samples were prepared from the dried DOM extracts and the algae extracts. Therefore the 60 dried DOM extracts from San Diego were redissolved in 100 uL MeOH (LCMS garde) from which 50 uL were transferred into new 2 mL glass vials resulting in a total of 120 DOM samples. To 28 vials 25 uL 1800ug/mL algae extract was added (A45M sample). To the next 28 vials 25 ul 600 ug/mL algae extract was added (A15M sample). To the last 28 vials 25 ul 200 ug/mL algae extract was added (A5M sample). Finally 25 uL of 1800ug/mL algae extract was added to 28 new vials to which also 10 uL of internal standards mix was added (2.5 ug/mL of each domoic acid, kanic acid, isoxaben, irgarol, imazapyr, heroin, methamphetamine and cocaine) resulting in a final amount of 25 ng standard per vial (A sample).
For the blank sample, 4 blank aliquots were re-dissolved in each 100 uL MeOH (LCMS grade), pooled and 150 µL of the standard mix was added. 18 uL of the resulting solution was aliquoted into 28 new 2 mL vials.

As QC standard mix we used mixture of Vanillin, Methyl-D-Mandelate, Hippuric acid, Caffeine, Sulfathiazole, Capsaicin, Dihydrocapsicin, Guanosine-5'-monophosphate, Raffinose and glycyrrhizic acid, with a concentration of 100 ug/mL in 50% MeOH (LCMS grade), of which we aliquoted 20 uL to 28 new 2 mL glass vials.
Finally, all vials were dried down in vacuum centrifuge at 35 degrees centigrade, capped and shipped to the participating labs. For shipping non-priority tracked shipment was used without cooling.

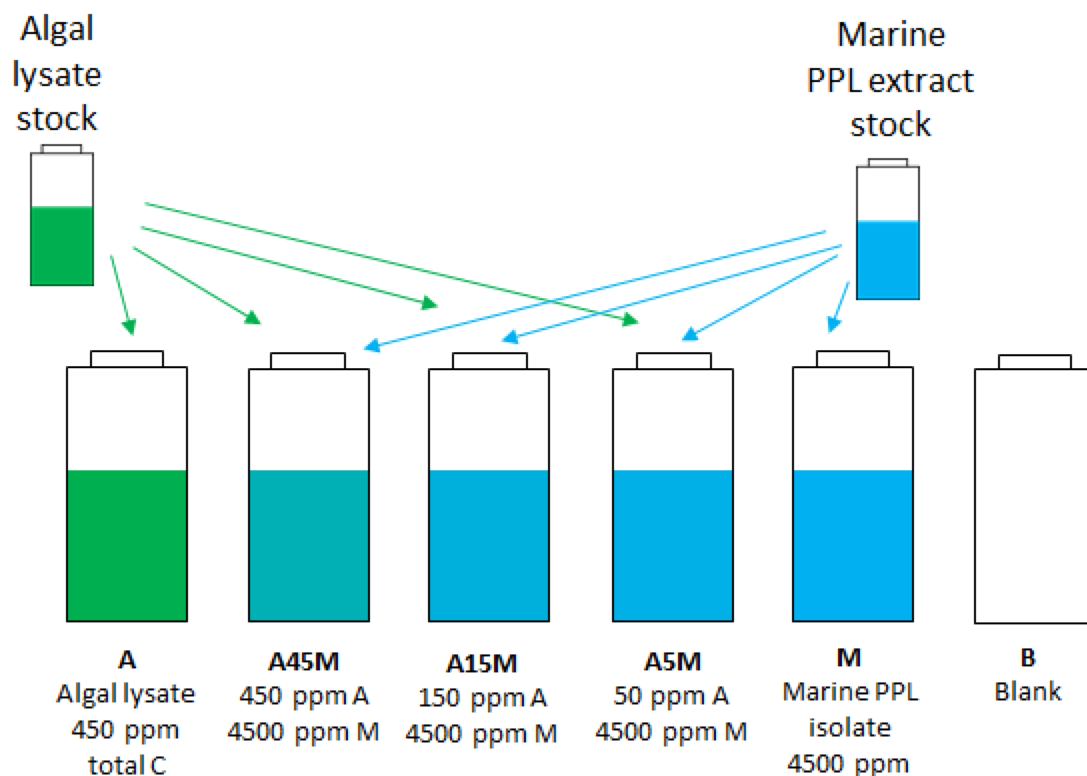

Figure 1: Sample Mixing Scheme

LC-MS/MS analysis

For LC-MS/MS analysis DOM, algae and QC samples were re-dissolved in 200 uL 50% MeOH (LCMS grade) and PPL-Planks were redissolved in 100 uL 50% MeOH (LCMS grade) and transferred into a micro insert. Final concentrations are displayed in table 1 of which 5-10 uL were injected per LC-MS run.

NOTE: The majority of labs to date (Jun 26th 2021) have injected 5µL, meaning a total of 60µL injected and 140µL remaining, which can be stored at -20degC for future analysis.

Table 1: Final concentrations of standard samples

Sample	A	A45M	A15M	A5M	M	PPL-BL	QC-Mix
Marine DOM [mg/mL]	0	4.5	4.5	4.5	4.5		
Algae Extract [ug/mL]	450	450	150	50	0.0		
Internal Standards [ug/mL]	0.12	0.12	0.12	0.12	0.12	0.12	
QC-Standards [ug/mL]							10

For LC-MS/MS data acquisition the following standardized parameters were used between all participating labs. For LC separation a C18 column with 2 mm diameter and between 10 and 15 cm length was used. As mobile phase H2O (A) and Acetonitrile (B) with each 0.1% FA were used. Flow rate was set to 0.4 mL/ min and a linear two step gradient started at 5 % B then started to increase at 30 sec from 5-50% B at 7 min followed by an increase to 99% B at 10 min, a 3 min washout phase at 99% B, and a 4 min equilibration phase at 5% B (method length = 17 min). MS data was continuously acquired for the full 17 min in MS1 pos and neg as well as DDA MS/MS pos and neg mode. Each sample was analysed in triplicate in random order in each positive and negative ESI mode, except PPL-BL and QC-Mix, which were measured once in each mode.

Table 2: Exact masses of internal standards

Compound	Molecular Formula	Mass [M-H+]+	Mass [M+Na+]+	Mass [M-H+]-
Domoic acid	$C_{15}H_{21}NO_6$	312.1441639	334.1261085	310.1296109
Kanic acid	$C_{10}H_{15}NO_4$	214.1073845	236.089329	212.0928315
Isoxaben	$C_{18}H_{24}N_2O_4$	333.1808837	355.162828	331.1663308
Irgarol	$C_{11}H_{19}N_5S$	254.1433929	276.125338	252.1288399
Imazapyr	$C_{13}H_{15}N_3O_3$	262.1186179	284.100563	260.1040649
Heroin	$C_{21}H_{23}NO_5$	370.1648993	392.146844	368.1503464
Methamphetamine	$C_{10}H_{15}N$	150.1277261	172.109671	148.113173
Cocaine	$C_{17}H_{21}NO_4$	304.1543347	326.13628	302.1397817