Assessment of styrene-divinylbenzene polymer (PPL) solid-phase extraction and non-targeted tandem mass spectrometry for the analysis of xenobiotics in seawater

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Abstract

Anthropogenic imprints have become a fundamental part of most ecosystems. Our chemical footprint is often detected using targeted approaches, whereas xenobiotics are embedded within the large pool of dissolved metabolites, altered by biotic and abiotic mechanisms. Thus, it is necessary to simultaneously study anthropogenic signals entwined with the variety of organic signatures that exist in aquatic environments. However, methods for non-targeted analysis of natural metabolites are not always well suited for the analysis of pollutants. Here, we report the reassessment of styrene-divinylbenzene polymer-based Priority Pollutant (PPL) solid-phase extraction (PPL-SPE), which is typically used to extract marine dissolved organic matter (DOM) for biogeochemical studies, to analyze a set of xenobiotics commonly observed in coastal North Pacific seawater. After PPL extraction and analysis by nontargeted liquid chromatography tandem mass spectrometry (LC–MS/MS), we successfully detected 23 out of 25 selected pharmaceuticals, personal care products, biocides, perfluorocarbons, and polymer additives in a complex marine DOM sample using positive and negative electrospray ionization. We tested two pH conditions to mimic typical marine DOM extraction studies and found mean recovery rates of xenobiotics were approximately 10% higher in seawater pH (pH ~ 8) than in acidified samples (pH ~ 2) for both negative and positive modes, although overall, mean recovery rates were 10% lower in negative mode. Our results indicate that PPL-SPE in combination with non-targeted LC–MS/MS is capable of capturing the tested set of xenobiotics, thus allowing the repurposing of biogeochemical sampling strategies as well as existing DOM samples and MS data for the subsequent assessment of anthropogenic impacts in marine environments.

On our planet, humans have left their chemical footprint in nearly every environment, and few environments are more impacted than our coastal water bodies. For example, modern industrial activities constantly introduce a multitude of structurally diverse organic contaminants (e.g., pharmaceuticals, fertilizers and other biocides, personal care products [PCPs], industrial chemicals) into the coastal environment. Many of these xenobiotics find their way into marine ecosystems, where they can have negative effects on the environment (Johnston and Roberts 2009; Keil et al. 2011; Halpern et al. 2015; Kivenson et al. 2019). To provide data for risk assessment management and mitigation, not only from a public health and economic perspective but also from a marine ecology standpoint, coastal waters have long been monitored for anthropogenic pollutants (Kimbrough et al. 2008).

Analytical methods designed for the evaluation of organic pollution in marine systems are typically targeted to specific...
compounds or categories of pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organotins, PCPs, hormones and pharmaceuticals, perfluorocarbons (PFCs) and brominated flame retardants (Magi and Di Carro 2018). These targeted methods are restricted to the analysis of a limited number of known chemicals but have the benefit of being quantitative and precise. Alternatively, non-targeted analysis (NTA) aims to examine temporal and spatial variability of all known and unknown compounds in a given system but typically lacks quantitative power. In the past decade, the implementation of high-resolution mass spectrometry (HR-MS) has made NTA more feasible for resolving individual molecules in studies of environmental pollutants (Schymanski et al. 2014b; Shaul et al. 2015; Gosetti et al. 2016; Petrie et al. 2016; Hollender et al. 2017; Rathgeb et al. 2017; Verkh et al. 2018; Lara-Martín et al. 2020; Tian et al. 2020, 2021).

Solid-phase extraction (SPE) is typically first applied in molecular studies of marine dissolved organic matter (DOM) to both enrich the organic yield of the tens of thousands of natural and anthropogenic organic molecules and desalt the seawater samples (Lara-Martín et al. 2020; Petras et al. 2021b). For studies in a biogeochemical context, Priority Pollutant (PPL) resin (Bond Elut, Agilent), a reverse phase, hydrophobic, styrene-divinylbenzene polymer, is typically used as a stationary phase for SPE-DOM. Samples are first acidified to pH 2 to increase the extraction yield of organic acids and allow PPL to achieve around 40–60% extraction efficiency of marine dissolved organic carbon (DOC) and outcompete many alternative resins (Dittmar et al. 2008). While the most common stationary phase for studies of emerging pollutants is Oasis HLB resin (Richardson and Kimura 2016), PPL outperforms Hydrophilic-Lipophilic Balanced (HLB) resins (47% to 42%) in the extraction of coastal DOC (Arellano et al. 2018). After extraction, HR-MS is typically utilized to obtain molecular information of compounds that are accessible for electrospray ionization within the seawater samples (Patriarca et al. 2020a). Recently, high-resolution Orbital ion trap mass spectrometers have been employed for NTA of DOM in part because their fast scan speed (e.g., less than 1 Hz @ R = 100,000) enables direct coupling with ultra-high-performance liquid chromatography (UHPLC) (Hawkes et al. 2018b; Patriarca et al. 2020b) and tandem mass spectrometry (MS/MS) (Petras et al. 2017; Hawkes et al. 2018a; Qiu et al. 2020). Along with improved data acquisition, recent advancements in MS analysis such as feature-based molecular networking in the Global Natural Product Social Molecular Networking (GNPS) environment have greatly improved the capability to annotate numerous chemical compounds from MS/MS spectra, including molecular formula assignment, structure and class annotations with fragmentation trees and other in silico MS/MS search algorithms, and statistical significance estimations (Ruttles et al. 2016; Wang et al. 2016; Schuebert et al. 2017; da Silva et al. 2018; Dührkop et al. 2019, 2021; Ludwig et al. 2020; Nothias et al. 2020; Schmid et al. 2021). These technological advances facilitate both non-targeted screening of anthropogenic compounds as well as the chemical characterization of the DOM chemotype.

Previous studies have reported compound-specific recoveries of a set of metabolite standards by PPL-SPE and targeted LC–MS/MS (Johnson et al. 2017), yet the capability of PPL-SPE in combination with non-targeted MS/MS to recover common xenobiotics has not been systematically assessed. Simultaneously characterizing natural and anthropogenic organic compounds in marine ecosystems would benefit from a method that can capture both natural and anthropogenic compounds to identify direct markers of pollution, natural toxins, chemical cues, and other compounds that structure microbial communities and their interactions. Furthermore, many biogeochemical and environmental metabolomics studies have utilized PPL-extracted DOM and this number continues to grow. Therefore, the enormous volume of archived samples and existing data from marine ecosystems could be repurposed to identify and track anthropogenic imprints around the world over time. The main goal of this study was to assess the capabilities of PPL-SPE and non-targeted LC–MS/MS to retrieve xenobiotics from the marine DOM pool.

Fig. 1. Classification (a) and ionization mode detection (b) of xenobiotics used in this study.
We examined the recovery rates of a mixture of xenobiotic standards in seawater by PPL-SPE and non-targeted UHPLC–MS/MS. We selected 25 compounds (Fig. 1), observed in our own published data from the San Diego coast (Petras et al. 2017, 2021a) as well as in studies from different coastal environments (Lara-Martin et al. 2020), including public non-targeted MS/MS data (e.g., the data sets, MSV000085786, MSV000083889 MSV000087006 on massive.ucsd.edu) that are of environmental concern and/or used as tracers of anthropogenic pollution. These compounds represent a group of xenobiotics commonly introduced into the environment, including biocides, drugs such as antibiotics and alkaloids, perfluorinated compounds, polymer additives, and PCPs. We assessed overall recovery from a seawater organic matter (OM) matrix, linearity of signal response, and the presence of potential degradation products under both acidified and nonacidified PPL-SPE conditions.

**Materials and procedures**

Authentic standards of xenobiotics included hexa-(methoxymethyl) melamine, 1,10-phenanthroline, sulfamethoxazole, erythromycin, isoxaben, imazamox, caffeine, perfluorooctanoic acid, perfluorohexanoic acid, perfluorobutanesulfonic acid, perfluorooctanesulfonic acid, methamphetamine, cocaine, benzylecgonine, and heroin. Details about source and purity are included in Supporting Information Table S1 and structures in Supporting Information Table S2. Standards were dissolved and mixed in methanol (LC–MS grade, Fisher Scientific) to yield final stock concentrations of 100 μg mL⁻¹. Absolute quantities of 0, 0.1, 1, 10, and 100 ng of xenobiotics were added volumetrically using the stock solution into glass vials with 1000 μL of methanol (LC–MS grade, Fisher Scientific). The standard solutions were dried in a vacuum centrifuge (Centrivap, Labconco) and stored at −80°C for 24 h, which we considered the longest time expected between sampling and extraction during fieldwork, all samples were extracted through PPL cartridges with a bed mass of 200 mg (Bond Elut, Agilent) accordingly to our previous studies (Petras et al. 2017, 2021b). Before use, the cartridges were rinsed and activated with three cartridge volumes of methanol (LC–MS grade, Fisher Scientific) and conditioned with three cartridge volumes of water (LC–MS grade, Fisher Scientific) at pH 2 (acidified with HCl, 37% p.a., trace metal grade, J.T. Baker) or pH 7 (no acid addition), respectively. Samples were pulled through the cartridges at a flow rate of around 10 mL min⁻¹ with the help of a vacuum SPE station. After extraction, the PPL cartridges were rinsed with three cartridge volumes of pH 2 or pH 7 water to remove the remaining salt. Rinsing with pH 7 water may have influenced the retention and recovery of xenobiotics for our untreated samples. No impact of pH on salt solubility was observed. After drying with nitrogen gas, the cartridges were eluted with 2 mL of methanol (LC–MS grade, Fisher Scientific) into glass vials. The extracts were dried in a vacuum centrifuge (Centrivap, Labconco) and stored at −80°C until further analysis.

For LC–MS/MS analysis, all standards and samples were resuspended in 100 μL MeOH/H₂O/formic acid (FA) (80/19/1). A total of 10 μL were injected into a Vanquish reverse-phase UHPLC system (Thermo Fisher Scientific). Retention time drifts were controlled with a quality control mix of six standards evenly distributed in the chromatogram (Sulfamethazine, Sulfamethizole, Sulfachloropyridazine, Sulfadimethoxine, Amityprylamine, and Coumarin-314) at the beginning and the end of the sequence. A C18 porous core shell column (Kinetex C18, 150 × 2 mm, 1.8 μm particle size, 100 Å pore size, Phenomenex) was used as a stationary phase. The mobile phase consisted of solvent A H₂O + 0.1% FA and solvent B acetonitrile +0.1% FA. The flow rate was set to 0.5 mL min⁻¹. Samples were eluted with a linear gradient from 0 to 0.5 min, 5% B, 0.5 to 8 min, 5% to 50% B, 8 to 10 min, 50% to 99% B, followed by a 2-min washout phase at 99% B and a 3-min reequilibration phase at 5% B. Data-dependent acquisition (DDA) of MS/MS spectra was performed in positive and negative modes. ESI parameters were set to 52 AU ion source voltage, 14 AU auxiliary gas temperature, and 400°C auxiliary gas temperature. The spray voltage was set to 3.5 kV and the inlet capillary to 320°C. Then, 50-V lens level was applied. MS scan range was set to 150–1500 m/z with a resolution at m/z 200 (R_m200) of 70,000 with one microscan. The maximum ion injection time was set to 100 ms with automated gain control (AGC) target of 1.0 × 10^6. Up to 5 MS/MS spectra per MS1 survey scan were recorded in DDA mode with R_m200 of 17,500 with one microscan. The maximum ion injection time for MS/MS scans was set to 100 ms with an AGC target of

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3.0 \times 10^5 \text{ ions and a minimum 5\% C-trap filling. The MS/MS precursor isolation window was set to m/z 1. Normalized collision energy was set to a stepwise increase from 20\% to 30\% to 40\% with z = 1 as default charge state. MS/MS scans were triggered at the apex of chromatographic peaks within 2–15 s from their first occurrence. Dynamic precursor exclusion was set to 5 s. Ions with unassigned charge states were excluded from MS/MS acquisition as well as isotope peaks.}

Recoveries of xenobiotics at pH 2 and pH 8 were estimated in comparison to the standard series for 1, 10, and 100 ng in MeOH/H2O/FA (80/19/1). One-liter aliquots of pure water (LC–MS grade, Fisher Scientific) were extracted with PPL cartridges as described before and used as process blanks for PPL extraction. From data-dependent LC–MS/MS analysis, raw spectra were converted to .mzXML files using ProteoWizard (Chambers et al. 2012), and ion features were generated from extracted ion chromatograms (XIC) using MZmine2.53 and linked to their tandem mass spectra, which provides a combination of relative abundance (XIC) and qualitative (MS/MS) information (Pluskal et al. 2010). For peak picking in positive mode, an intensity threshold of $1 \times 10^4$ for MS1 spectra and $5 \times 10^2$ for MS/MS spectra were used; in negative mode, an intensity threshold of $5 \times 10^4$ for MS1 spectra and $2.5 \times 10^2$ for MS/MS spectra were used. For MS1 chromatogram building, a 5 ppm mass accuracy, and a minimum peak intensity of $2 \times 10^5$ for positive mode and $1 \times 10^5$ for negative mode were set, with a minimum time span of 0.02 min. XIC were deconvoluted using the local minimum search algorithm with the chromatographic threshold set to 1%, a minimum relative height of 1%, a minimum absolute height of $2 \times 10^5$ for positive mode and $1 \times 10^5$ for negative mode, a minimum ratio of 1 for peak top/edge, and a minimum peak duration of 0.05 min. After chromatographic deconvolution, MS1 features were linked to MS/MS spectra within 0.01 m/z mass and 0.1 min retention time windows. Isotope peaks were grouped using the isotope grouper module and features from different samples were aligned with 5 ppm mass tolerance and 0.1 min retention time tolerance. MS1 features that were not accompanied by an MS/MS spectrum were not considered further. MS1 features that did not occur in at least two samples and/or did not contain a minimum of two peaks per isotope pattern were filtered out as well. After filtering, gaps in the feature matrix were filled with the peak finder multithreaded algorithm with a retention time tolerance of 0.15 min, a mass tolerance of 5 ppm, and an intensity tolerance of 10\%. Finally, peak areas were exported in a feature table as .csv file and corresponding consensus MS/MS spectra as .mgf file.

For feature-based molecular networking and spectrum library matching the .mgf file was uploaded to GNPS (gnps.ucsd.edu) (Wang et al. 2016; Nothias et al. 2020). The minimum cosine score to define spectral similarity was set to 0.7. The precursor and fragment ion mass tolerances were set to 0.01 Da and the minimum matched fragment ions to 4. When analog search was performed, the maximum mass difference was set to 100 Da, and node information was matched with the MS1 peak areas from the feature table. Molecular networks were visualized with Cytoscape 3.7 (Shannon et al. 2003). The chemical proportionality of networked compounds between the pH 2 and pH 8 sample groups was calculated using the Chemical Proportionality (ChemProp) workflow in GNPS. The ChemProp score displays the ratio between two connected compounds between two groups (e.g., pH 2 and pH 8) and can be interpreted as directionality within the molecular network that indicates a change of abundance of related compounds such as in a chemical transformation (Petras et al. 2021a).

Assessment
Detection of the standard mix
Combined non-targeted LC–MS/MS and feature-based molecular networking identified the 25 pharmaceuticals, PCBs, biocides, PFCs, and polymer additives used in our study (Fig. 1). This group of commonly found xenobiotics spans a wide range of physicochemical properties, such as polarity and acidity (details included in Supporting Information Table S2). From the total number of xenobiotics, 19 compounds were detected in positive mode, while 9 compounds were detected in negative mode ESI (Fig. 1). PFCs and saccharin were exclusively captured by negative mode. Glyphosate was found in the standard mix in positive mode, but omitted from the further discussion as it was not found in the extractions likely due to insufficient affinity to the PPL resin deduced from its rapid elution (high polarity) off the chromatography column during LC separation. All remaining detected molecules show linear signal responses in the standard mix with correlation coefficients ($R^2$) higher than 0.995, except for heptadecafluorooctanesulfonic acid ($R^2 = 0.984$) in negative mode and octinoxate ($R^2 = 0.974$) in positive mode.

Xenobiotic recoveries at pH 2 and pH 8
Blank-corrected average recoveries of xenobiotics at pH 2 and pH 8 in both positive and negative modes are shown in Table 1. For recoveries of the individual spiked concentrations of 1, 10, and 100 ng L$^{-1}$, see Supporting Information Table S3. These recoveries are in comparison with the standard mix dilutions and therefore include matrix and ion suppression/enhancement effects. In positive mode, caffeine (37\% at pH 2, 92\% at pH 8), heroin (61\% at pH 2, 94\% at pH 8), imazamox (80\% at pH 2, 66\% at pH 8), and lincomycin (71\% at pH 2, 105\% at pH 8) held the highest recoveries, while erythromycin and hexa (methoxymethyl) melamine were recovered but in low amounts of 0.1\% to 0.2\%. In negative mode, perfluorooctanesulfonic acid (75\% at pH 2, 87\% at pH 8) and perfluorobutanesulfonic acid (61\% at pH 2, 68\% at pH 8) held the highest recoveries, while lincomycin held the lowest (0.8\% at pH 2, 21\% at pH 8).
Table 1. Recoveries and standard deviations (in parentheses) of xenobiotics from this study in positive and negative mode at pH 8 and pH 2. Recoveries taken from the literature on previous solid-phase extraction liquid chromatography mass spectrometry studies are also shown in positive and negative mode as well as reported values of the xenobiotics in marine environments.

| Compound name                  | Positive mode pH 8 recoveries | Positive mode pH 2 recoveries | Negative mode pH 8 recoveries | Negative mode pH 2 recoveries | Literature recoveries (positive mode) | Literature recoveries (negative mode) | Observed conc. range. (ng L⁻¹) | Citation                                                                                   |
|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---------------------------------------|---------------------------------------|---------------------------------|------------------------------------------------------------------------------------------|
| Benzoylecgonine                | 59% (23%)                     | 59% (5%)                      | —                             | —                             | 80–110% (HLB, pH ~ 8), 49–109% (HR-X, pH ~ 7) | —                                     | 2.7–54                         | Nödler et al. (2014) and Fontes et al. (2019)                                           |
| Caffeine                       | 92% (32%)                     | 37% (13%)                     | —                             | —                             | 18–68% (HLB, pH ~ 8)                   | —                                     | 4.3–649                        | Pereira et al. 2016; Biel-Maeso et al. 2018; McKenzie et al. 2020                        |
| Carbamazepine                  | 43% (22%)                     | 37% (18%)                     | —                             | —                             | 18–139% (HLB, pH ~ 8), 82–120% (HR-X, pH ~ 8) | —                                     | 0.01–1410                      | Wille et al. 2010; McKenzie et al. 2020; Almeida et al. 2021                            |
| Clarithromycin                 | 26% (10%)                     | 6% (2%)                       | —                             | —                             | 69% (HLB, pH ~ 8)                     | —                                     | 0.2–9.4                         | Biel-Maeso et al. (2018)                                                            |
| Cocaine                        | 43% (19%)                     | 42% (9%)                      | —                             | —                             | 49–109% (HR-X, pH ~ 7)                | —                                     | 12–400                          | Pereira et al. 2016 and Fontes et al. (2019)                                           |
| Dibenzylamine                  | 44% (5%)                      | 46% (6%)                      | —                             | —                             | —                                     | —                                     | —                              | —                                                                                     |
| Erythromycin                   | 1% (1%)                       | 0.1% (0.2%)                   | —                             | —                             | 92% (HLB, pH ~ 8)                     | —                                     | 0.3–2.3                        | Biel-Maeso et al. (2018)                                                            |
| Ethynylestradiol               | 51% (15%)                     | 46% (11%)                     | —                             | —                             | 18% (HLB, pH ~ 8)                     | —                                     | 1.7–8.0                        | Beck et al. (2005)                                                                |
| Heroin                         | 94% (57%)                     | 61% (46%)                     | —                             | —                             | 89% (HLB, pH ~ 6)                     | —                                     | —                              | Jiang et al. (2014)                                                              |
| Hexa (methoxy-methyl)melamine  | 2.2% (0.4%)                   | 0.2% (0.1%)                   | —                             | —                             | —                                     | —                                     | 1.0–28                         | Tian et al. (2020)                                                               |
| Imazamox                       | 66% (18%)                     | 80% (15%)                     | 18% (8%)                      | 9% (3%)                       | 65% (PPL, pH ~ 2)                     | —                                     | —                              | Petras et al. 2021b and Munaron et al. (2012) and Petras et al. 2021b                 |
| Imazapyr                       | 41% (16%)                     | 64% (11%)                     | 21% (8%)                      | 5% (4%)                       | 56% (C18), 40% (PPL, pH ~ 2)           | —                                     | 1.5–15                         | —                                                                                     |
| Igarol                         | 52% (18%)                     | 40% (6%)                      | —                             | —                             | 35% (PPL, pH ~ 2)                     | —                                     | 0.3–3                          | —                                                                                     |
| Isoxaben                       | 43% (17%)                     | 35% (14%)                     | 15% (9%)                      | 14% (13%)                     | 115% (HLB, pH ~ 8)                    | —                                     | 0.04–6.1                       | Biel-Maeso et al. 2018 and Kötke et al. (2019)                                        |
| Lincomycin                     | 105% (41%)                    | 71% (15%)                     | 21% (11%)                     | 1% (1%)                       | 69% (PPL, pH ~ 2)                     | —                                     | 0.3–2.0                        | Tian et al. (2020) and McKenzie et al. (2020)                                         |
| Methamphetamine               | 56% (13%)                     | 41% (10%)                     | —                             | —                             | 3–17% (HLB, pH ~ 8)                   | —                                     | —                              | Thomas and Hilton (2004)                                                            |
| N-acetylsulfamethoxazole        | 40% (16%)                     | 32% (6%)                      | —                             | —                             | 56% (strata-X, pH 3)                  | —                                     | <20                            | —                                                                                     |
| Perfluorobutanesulfonic acid   | —                             | —                             | 68% (26%)                     | 61% (24%)                     | 94% (HLB, pH ~ 8)                     | —                                     | 0.2–4.4                        | Hong et al. (2015) and Tian et al. (2020)                                             |
| Perfluorohexanoic acid         | —                             | —                             | 38% (18%)                     | 29% (5%)                      | 69–82% (HLB, pH ~ 8)                  | —                                     | 3.8–14                         | Fauvelle et al. (2018) and Tian et al. (2020)                                         |

(Continues)
Except for octinoxate, all detected xenobiotics in positive mode had linear signal responses at pH 2 and pH 8 from 0 to 100 ng L$^{-1}$, with correlation coefficients ($R^2$) $>0.986$, and in most cases, $>0.990$ (Supporting Information Fig. S1). In negative mode, signal responses were also linear, with correlation coefficients ($R^2$) higher than 0.990, except for perfluorooctanoic acid ($R^2 = 0.948$) and perfluorohexanoic acid ($R^2 = 0.901$) (Supporting Information Fig. S1). Octinoxate’s recovery could not be calculated due to the high background exhibited by the seawater samples both at pH 2 and pH 8 at that specific m/z value. Consequently, this compound was not included in further analysis and discussion.

Overall, most compounds exhibited good linearity over the three orders of magnitude tested, thus highlighting the semi-quantitative power of the method. Except for caffeine, the compounds were not detected in the nonspiked (“0 ng”) sample. This result is not surprising, as our sampling location (Scripps Pier) did not have any wastewater or river input, boatyards in its proximity, and no significant rainfall during or immediately before sample collection. For reference, XIC showing the baseline signal corresponding to each compound are included in Supporting Information Table S4. Supporting Information Table S5 also provides values for the integrated signal in the nonspiked sample.

For the remaining 23 compounds, response factors were calculated as the ratio between the peak area and the initially spiked xenobiotic mass in nanograms (Supporting Information Table S6). Figure 2a shows violin plots of response factors for xenobiotics at pH 2 and pH 8 for all concentrations (positive and negative modes). A paired t-test (two tails) for response factors gave a $p$-value of 0.109 for positive mode and 0.097 for negative mode. Differences were not statistically significant for the 95% confidence level. Figure 2b shows violin plots of recoveries for xenobiotics at pH 2 and pH 8 for all concentrations (positive and negative modes). A Grubbs test for outliers was performed and results above the 95% confidence threshold level were not used in this statistical analysis. A paired $t$-test (two tails) for recoveries gave a $p$-value of 0.008 for positive mode and 0.002 for negative mode and thus differences were statistically significant for 95% confidence level. These results suggest that the variation between pH 2 and pH 8 does not have a significant influence on the instrument response for the selected xenobiotics, while it seems to have an effect on the recoveries (i.e., the ratio between the peak areas of xenobiotics in these samples and the peak areas in the standard series). However, mean recovery at pH 2 (37%) is still 82% of the mean recovery at pH 8 (45%) for compounds detected in positive mode. For compounds detected in negative mode, the mean recovery at pH 2 (24%) is 73% of the mean recovery at pH 8 (33%). The relationship between pKa and xenobiotic recovery was tested; however, no correlation was observed for our sample group.

### Table 1. Continued

| Compound name | Previous SPE LC-MS studies | Observed conc. range (ng L$^{-1}$) | Literature recoveries (negative mode) | Literature recoveries (positive mode) |
|---------------|---------------------------|-----------------------------------|---------------------------------------|---------------------------------------|
| Perfluorooctanesulfonic acid | 70–95% (HLB, pH ~ 8) | 8% (56%) | 75% (38%) |
| Perfluorooctanoic acid | 42% (53%) | 5% (28%) | 42% (53%) |
| Saccharin | 8% (6%) | 8% (6%) | 11% (73%) |
| Sulfamethoxazole | 16% (7%) | 10% (9%) | 16% (7%) |
| Fauvelle et al. (2018) and Tian et al. (2020) | 0.05–1.40 | 113–124% (HLB, pH ~ 8) | 93% (HLB, pH ~ 8), 5–69% (HLB, pH ~ 2), 81–119% (HR-X, pH ~ 8) |
| Fauvelle et al. (2018) and Tian et al. (2020) | 2.0–7.1 | 5.2% (HLB, pH ~ 8) | 93% (HLB, pH ~ 8), 5–69% (HLB, pH ~ 2), 81–119% (HR-X, pH ~ 8) |
| Brunovský et al. (2017) | 0.49–5.23 | 0.49–5.23 | 0.49–5.23 |
| Wille et al. 2010; Bayen et al. 2016; Béziat et al. (2019) | 0.06–99 | 0.06–99 | 0.06–99 |
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Analysis of xenobiotics in seawater
Molecular networking view on the role of pH

Besides the aforementioned 25 xenobiotics, a total number of 4182 ion features (defined as single chromatographic peaks of a given m/z value with an assigned MS/MS spectra) were identified in positive mode, and 2157 in negative mode. To explore this chemical space, molecular networks based on MS/MS spectral similarity were generated using the GNPS feature-based molecular networking workflow (Nothias et al. 2020). Figure 3 shows the global network Cytoscape visualization for positive and negative modes, where clusters related to the xenobiotics selected in this study are highlighted. Zoomed-in sub-networks for cocaine, benzoylecgonine, heroin, imazapyr, and imazamox are shown. Based on their MS/MS similarity, related compounds are located in the same sub-network. For example, morphine, 6-acetylmorphine, and 6-acetylcodeine (see Fig. 3 for molecular structures) are linked to heroin with mass differences of m/z 42.011 (acetyl) and 14.011 (methylene), showcasing the ability of this method to chart the chemical space around xenobiotics including side products and impurities from the standards as well as potential metabolic and abiotic degradation products, including modifications resulting from pH adjustment.

Furthermore, the semi-quantitative information obtained from this study allows the prioritization of potential (bio)chemical and pH related transformations. Chemical Proportionality (ChemProp) is a data analysis workflow for molecular networks that makes use of mass shifts between connected nodes and displays the variation in the peak area ratio between two related features, for example, over temporal or spatial coordinates (Petras et al. 2021a). In this case, ChemProp was used to prioritize the formation of compounds during pH shifts. An absolute ChemProp value higher than 2, which is the cutoff that we employ in this analysis, implies a 100-fold variation or higher in the ratio between two related features (e.g., 10-fold decrease of the reactant and a 10-fold increase of the product). Supporting Information Fig. S2 shows ChemProp values vs. absolute m/z change for positive and negative modes data along with the frequency of each m/z change. The highest frequency changes are those corresponding to m/z ± 14.016, ± 28.031, and ± 44.026, which correspond to methyl (CH₃), ethyl (C₂H₄), and carboxylic...
(COO) functional group masses. From the total number of feature pairs in our network, only 1.5% (71 of 4645 network edges) showed an absolute ChemProp value higher than 2 in positive mode and 3.1% (75 of 2425 network edges), in negative mode, which indicated that only a small number of detected compounds exhibited pH-related transformations that could be detected by isolation and analysis methods employed here. Moreover, the number of features detected at pH 2 and 8 was

![Molecular network of features detected in positive (a) and negative (b) mode. Nodes represent MS/MS spectra, which are connected based on their spectral similarity. Selected sub-networks are highlighted and circled (left). Nodes are labeled with the parent ion m/z values. Edges (dashed lines) are labeled with the mass difference between two nodes. The pie chart indicates the relative distribution of precursor intensity between standard, pH 2, and pH 8 samples. Stereochemistry is not shown. Roman numerals indicate community confidence level in compound annotation where matches are either standard (I), library match (II), or proposed structure (III) (Sumner et al. 2007; Schymanski et al. 2014).](image-url)
comparable. In the seawater samples, 4025 features were detected at pH 2 and 4082 at pH 8 in positive mode. In negative mode, 2020 features were detected at pH 2 and 2066 at pH 8. While the focus of our study was mainly on xenobiotics, we did not observe that the extraction pH significantly alters the chemotype of the PPL-extracted OM in general. This analysis provides a window into how ChemProp can be used to assess putative transformations within the chemotype from a global perspective. However, additional experiments would be needed to confirm the structures of putative transformation products and identify potential sources.

**Discussion**

This article assesses the semi-quantitative recovery of 25 xenobiotics in seawater by PPL-SPE and non-targeted LC-MS/MS, which is traditionally employed in qualitative biogeochemical studies of OM. While this method is used to study naturally occurring molecules in the marine environment, we have shown that it is also capable of semi-quantitatively assessing at least part of the anthropogenic footprint of coastal waters. While this is by no means a replacement for quantitative studies, NTA can be used to help design future targeted experiments. The ability to examine the footprint of human interference along with a fraction of the natural OM pool may enable a more comprehensive examination of how anthropogenic activities influence microbial metabolism and ecosystem health.

From a methodological perspective, changes in OM extraction conditions such as pH did not dramatically impact the chemical signal of the standard mix, thus enabling the identification of xenobiotics at low pH conditions that increase the extraction yield of marine OM. The slightly higher average recovery rate at pH 8 could be explained by the higher proton affinity (e.g., amino groups) of most of the tested compounds. The use of positive and negative ionization modes further allowed the detection of dissimilar compounds, covering a broader part of the chemical space. While ESI negative mode is well suited to detect carboxylate-containing compounds such as PFAs, data generated in ESI positive mode is more appropriate to analyze nitrogen-containing compounds, such as several of the xenobiotics selected in this study. Although a combination of positive and negative modes data provides insight into a larger array of compounds, its application in future studies will depend on the availability of time and funds for sample analysis. The linear MS response with increasing concentrations observed for most of the selected xenobiotics also demonstrates the semi-quantitative value of this approach.

While we did not intend to experimentally benchmark compound specific extraction efficiencies of PPL vs. other commonly applied SPE resins, a comparison of the typical extraction efficiency reported in the literature (and by no means comprehensive) showed that PPL-SPE falls into a similar range (Table 1). As stated above, the average recovery in positive mode at pH 8 is 45% (range 0–114%) and at pH 2 is 37% (range 0–96%), which is lower than the average recovery of 69% (ranging between 3% and 139%) obtained in targeted LC-MS studies that use positive mode and HLB resin. In negative mode, average recoveries of 33% (range 8–68%) at pH 8 in our study were also lower than the average recovery rate of 78% published in other studies. We also found several papers that reported recovery rates with HR-X resin, another polystyrene-divinylbenzene-based SPE resin similar to PPL (see Table 1). Also here, the average recovery was higher with an average of 90% (ranging between 49% and 120%). Besides general higher extraction efficiencies of the SPE material, a possible explanation for the higher recoveries (often >100%), could be the targeted nature of these studies, which allows for optimal selection of both extraction parameters (e.g., resins and elution solvents to minimize interference by natural OM) and analysis parameters (e.g., optimization of ESI settings to mitigate or even reverse possible matrix ion suppression effects).

It is important to point out that feature-based molecular networking provides level 2 IDs (spectrum-library matches) but propagates those annotations between files via exact mass and retention time (Sumner et al. 2007; Schymanski et al. 2014b; Nothias et al. 2020). In very complex samples, such as DOM, there is a chance that other co-eluting compounds have the same molecular formula and thus exact mass which could lead to false-positive detection. The best way to avoid false positive propagation is to manually examine MS/MS spectra of individual files in which a compound was annotated. In some samples, a compound may have only been detected by MS1 (as this is more sensitive), but the absence of an MS2 spectrum in that sample does not contradict the annotation. The confirmation of a matching MS2, however, will provide further confidence or a nonmatching MS2 spectrum can exclude a false-positive annotation. In this study, we manually inspected all samples for matching MS2 spectra of the spiked standards. We recognize, however, that manual inspection is only practical for a limited number of compounds and files and should rather be part of the final data interpretation after computational annotation and statistical prioritization.

The present study was restricted to a selected, though chemically diverse, group of xenobiotics commonly found in Southern California coastal waters (Petras et al. 2021b). The semi-quantitative power of this method has the ability to guide targeted studies of numerous pollutants found in marine environments. The most interesting benefit of this method, however, is the ability to uncover abiotic and metabolic transformations of pollutants in seawater. Moving forward, when analyzing the distribution of xenobiotics in environmental samples, chemically diverse compounds should be included to determine, for example, whether particular structural and physicochemical properties result in discernible temporal and
spatial trends in the environment. Moreover, with the aid of growing MS/MS data repositories, newly emerging pollutants or classes of pollutants could be potentially identified and compared to existing data sets from all over the world, thus allowing for the retrospective analysis of environmental data using this workflow (Alygizakis et al. 2018; Wang et al. 2020; Petras et al. 2021b). Besides the tremendous scientific benefit from repurposing archived DOM samples and LC–MS/MS data, the molecular networking approach applied here to examine potential chemical transformations offers a framework to prioritize and identify new xenobiotic derivatives, such as microbial and abiotic degradation products, and also flag emerging contaminants.

Data availability statement

Raw and processed mass spectrometry data are available through the MassIVE repository (massive.ucsd.edu) through the following identifier: MSV000085025. MS/MS spectra for positive and negative mode for 25 pharmaceuticals, personal care products, biocides, PFCs, and polymer additives used in our study were added to the GNPS reference library. The Feature-Based Molecular Networking results can be accessed under the following links: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=835f74d7d73b42a28ca392f6e86b663 (positive ESi mode) and https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=bfe50f5fcd44ca391e366f925e497 (negative ESi mode). All XICs and MS/MS spectra of the study can be viewed online through the GNPS Dashboard (Petras et al. 2021c) in the following links: Negative mode: https://gnps-lcms.ucsd.edu/?xic_mz=&xic_formula=&xic_peptide=&xic_tolerance=0.5&xic_ppm_tolerance=10&xic_tolerance_unit=Da&xic_rt_window=&xic_norm=False&xic_file_grouping=MZ&xic_integration_type=AUC&show_ms2_markers=True&ms2marker_color=blue&ms2marker_size=5&ms2_identifier=None&show_lcms_2nd_map=True&map_plot_zoom=%7B%7D&map_plot_quantization_level=Medium#%7B%7D&map_plot_color_scale=c2acd54953a81&map_plot_zoom_level=1&map_plot_color=blue&map_plot_quantization_level=Medium#%7B%7D&map_plot_color_scale=c2acd54953a81&map_plot_quantization_level=Medium#%7B%7D&map_plot_color_scale=c2acd54953a81&map_plot_quantization_level=Medium#%7B%7D&map_plot_color_scale=c2acd54953a81

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Acknowledgments
L.C., K.P., and D.P. were supported by the Understanding and Protecting the Planet initiative of the University of California San Diego.

R.R.T. was supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-2038238. Any opinions, findings, and conclusions, or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. D.P. was supported by the Deutsche Forschungsgemeinschaft through the CMFI Cluster of Excellence (EXC 2124). Open access funding enabled and organized by Projekt DEAL.

Conflict of interest
Pieter C. Dorrestein is a scientific advisor for Sirenas LLC and Cybele.