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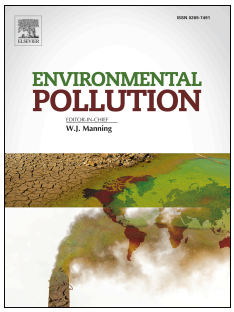

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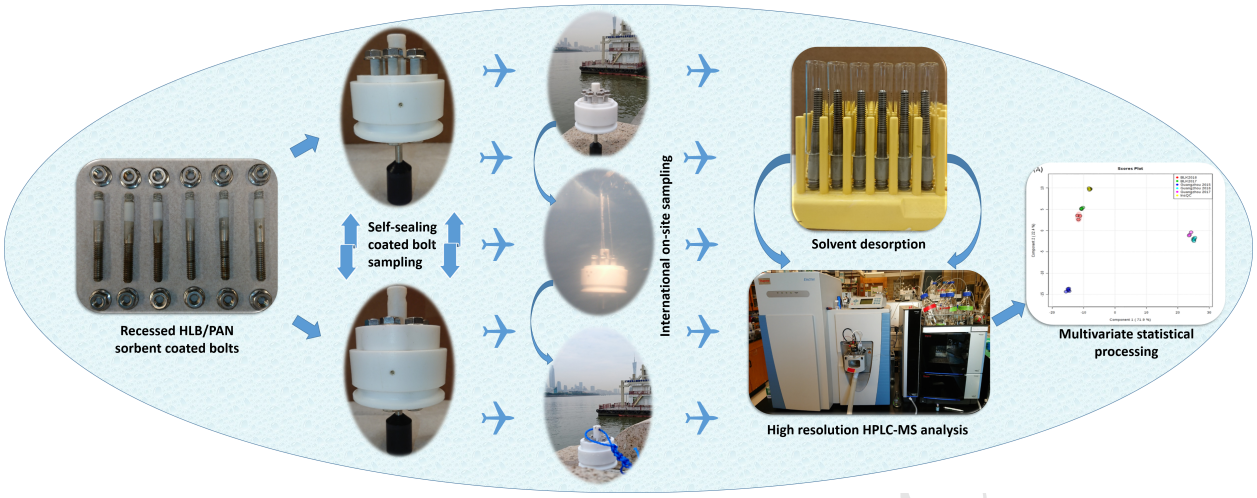

Introducing a mechanically robust SPME sampler for the on-site sampling and extraction of a wide range of untargeted pollutants in environmental waters

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Abstract

The present study introduces a mechanically robust, sealable SPME sampler for the on-site sampling and extraction of a wide range of untargeted pollutants in environmental waters. Spray-coating and dip coating methodologies were used to coat the surfaces of six stainless steel bolts with a layer of HLB/PAN particles, which served as the extractive substrate in the proposed device. In addition, this sampler was designed to withstand rough handling, long storage times, and various environmental conditions. In order to identify whether the sampler was able to stabilize extracted compounds for long periods of time, the effects of storage time and temperature were evaluated. The results of these tests showed no significant differences in the quantity and quality of the extracted chemicals following 12 days storage at room temperature, thus confirming the device's suitability for use at sampling sites that are far away from the laboratory facilities. The proposed device was also used to perform extraction and untargeted analyses of river waters in five different geographical locations. The constituent chemicals in the samplers were analyzed and determined using high-resolution HPLC-Orbitrap MS. Toxin and Toxin-Target Database was used as a reference database for toxins and environmental contaminants. Ultimately, over 80 tentative chemicals with widely varying hydrophobicities ranging within -2.43< logP <11.9—including drugs, metabolites, wide ranges of toxins, pesticide, and insecticides—were identified in the samplers used in the different rivers. The log P values for the tentative analytes confirmed that the introduced device is suitable for the extraction and trace analysis of wide ranges of targeted and untargeted pollutants.

47

48 **Capsule**

49 The present study introduces a mechanically robust SPME sampler device for the on-site
50 sampling and extraction of a wide range of the pollutants in environmental waters.

51 **1. Introduction**

52 The determination and trace analysis of chemicals in environmental water samples is a
53 challenging task that requires sampling, extraction, and enrichment before analysis.

54 Grab sampling may not be without its downsides as it may be difficult to ensure the
55 stability of the analytes in the sample solution during transport back to the laboratory. This
56 instability is especially problematic in samples that have ongoing biological activity. Therefore,
57 it would be ideal if sampling and sample preservation could be performed in-situ using device
58 with a sealed design. Moreover, in real environmental sample analysis, especially when the
59 sampling site is far away from the laboratory, a specific sampler design is required to ensure that
60 the extracted compounds are kept safe and unchanged until they reach the laboratory for the final
61 analysis step. In addition, mechanically robust sampling devices and technologies are required
62 for environmental sampling sites with harsh conditions, such extreme temperature, pressure, and
63 pH, as well as high flow rates (Poole et al., 2017).

64 Liquid chromatography-mass spectrometry (LC-MS) equipped with an electrospray
65 ionization (ESI), is one of the most important and widely used analytical methods for
66 multivariate, untargeted methodologies . However, LC-MS analysis can be limited by matrix
67 effect, which occurs when ionization is enhanced or suppressed due to other components in a
68 complex matrix. As such, researchers have confirmed that sample preparation is the key to

minimizing the presence of interfering compounds in complex matrices before analysis (van Leeuwen and de Boer, 2007).

Moreover, trace chemicals in water samples are comprised of a wide variety of analytes with various chemistries, polarities, solubilities, and chemical stabilities. Hence, at present, no single method is able to detect all of the chemicals in environmental water samples. Thus, there is a pressing need for a non-selective and non-destructive sample preparation method that enables the extraction of a variety of compounds without the loss, degradation, or transformation of analytes. In this direction, the main aim of the present study was to develop a sampling device and an extractive approach that enables analysis of a wide range of chemicals in environmental water samples.

Solid-phase microextraction (SPME) encompasses a wide range of geometries and configurations that have evolved comprehensively into almost every field of analytical chemistry, specifically for the analysis of environmental and water samples (Kenessov et al., 2016; Poole et al., 2017; Xu et al., 2016). SPME techniques have long been known to be reliable methods for combining sampling and sample preparation into a single step (Bojko et al., 2012; Pawliszyn, 2003; Reyes-Garcés et al., 2018). This reputation has largely been due to the development of a wide variety of SPME geometries that have improved speed, sensitivity, and convenience of use. As molecules are extracted into the pore space of sorbent particles, they can be removed from a given aqueous matrix (Pawliszyn, 2003). Not only does this form of extraction eliminate the need to transport large volumes of water, but it also allows an extracted compound to be stabilized more effectively, thus preventing additional reactions—particularly further breakdowns due to biochemical processes (Reyes-Garcés et al., 2018).

In the present study, we introduce a new SPME sampling and extraction device that can be used for environmental water sample analysis. Given the above-noted requirements, we sought to design an ideal sampling and extraction device that is capable of stabilizing extracted compounds on the sorbent coatings for extended periods in ambient conditions and detecting a wide range of targeted and untargeted analytes. In order to further test proof of concept, we used our device to analyze untargeted pollutants in water samples from five different rivers in China (Wuhan, Shanghai, Guangzhou, and Dalian rivers) and Canada (Grand River).

2. Experimental

2.1. Chemical and materials

LC-MS grade methanol, water, and acetonitrile were obtained from Fisher Scientific Canada (Markham, ON, Canada). Formic acid (FA), ammonium acetate, dimethylformamide (DMF), 150 kDa polyacrylonitrile (PAN), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (Oakville, ON, Canada). The 18-8 stainless steel nuts, bolts (with dimensions of 8 × 5 × 7 cm), and springs were purchased from Spaenaur (Kitchener, ON, Canada), while the Teflon coated springs (Swagelok model 177-R3A-K1-B) were purchased from Swagelock Inc. (Sarnia, ON, Canada). Rare-earth magnets were purchased from Lee Valley Tools (Waterloo, ON, Canada), and the Teflon sampler bodies were sourced and constructed by the University of Waterloo Science Machine Shop (Waterloo, ON, Canada). The plastic 300 μL vials and the amber 2 mL glass vials, along with pre-pierced polytetrafluoroethylene (PTFE)/silicone septa that were used in puncture tests, were purchased from Canadian Life Sciences (Peterborough, ON, Canada). Finally, hydrophilic−lipophilic balanced (HLB) particles were obtained from Waters Corporation (Wilmslow, U.K.).

2.2. Instrumental analysis

A Thermo Accela autosampler-HPLC and an Exactive Orbitrap MS (Thermo Fisher Scientific, San Jose, CA, USA) were used to separate and analyze the untargeted analytes. Separation was conducted using a Supelco Discovery Pentafluorophenyl (PFP) HS F5 column (100 mm × 2.1 mm id × 3 μm film thickness) (Supelco, Millipore-Sigma Bellefonte, PA, USA). The HPLC-MS conditions were configured in accordance with previous studies conducted by our group (Vuckovic and Pawliszyn, 2011). The mobile phase was comprised of Phase A (99.9:0.1 % water/FA) and Phase B (0.99.9:0.1 % acetonitrile/FA). The LC was operated in gradient mode with the following sequence: 100 % A from 0 to 3.0 min; linear gradation to 10 % A from 3.0 to 25.0 min; and, finally, an isocratic hold at 10 % A until 34.0 min. This process consisted of a total flow rate of 300 μL min^{-1} and a total run time of 40 min per sample, including a 6 min column re-equilibration period.

The analyses were performed using both positive and negative electrospray ionization (ESI). The injection volume for each analysis was 10 μL; the samples were stored at 4 ^{o}C on the autosampler while waiting for injection. All injections were performed in randomized order, and instrument QCs and pool QCs were periodically run in order to verify instrument performance. MS acquisition was performed using automatic gain control (AGC) = balanced (1,000,000 ions) with a 50,000 resolution at 2 Hz. The injection time onto the C-trap was 100 ms. The sheath gas (arbitrary units), auxiliary gas (arbitrary units), sweep gas (arbitrary units) volumes used were 30, 10, and 5, respectively, the selected ESI voltage was 4.0 kV (-2.9 negative mode), and the capillary and vaporizer temperatures were each 300 ^{o}C. The MS acquisition also used a range of 100-1000 m/z for the positive and negative ESI reversed-phase methods. External instrument mass calibrations were performed every 48 hrs in order to maintain a range of 2 ppm for all ions.

2.3. Data Processing and Statistical Analysis

Data processing was performed using a multi-stage technique. First, the raw data files obtained from the HPLC-MS were converted to a mzXML format using the free Proteowizard software, MSconvert. The parameters used during this process included a mz level=1 filter, 64-bit binary encoding precision, and the write index option. Next, the converted files were imported into the R-based software, MZmine 2, for further peak filtering and detection (Olivon et al., 2017). After being imported, scan-by-scan filtering was performed on the data using a 5-data-point Savitzky-Golay filter. A mass peak list was then generated using exact mass detection with an m/z range of 99-1000 m/z and a mass tolerance of 5.0 ppm. This mass list was then imported into chromatogram builder, which was set to have a minimum peak height of 10,000 (arbitrary) and a minimum width of 0.017 min. Later, these rebuilt chromatograms were deconvoluted using a Savitzky-Golay filter with a minimum peak height of 10,000 (arbitrary) and a peak width setting of 0.017-1.0 minutes. Following this step, the generated peak list was filtered using an m/z-range of 99-1000, an RT range of 0.8-35 minutes, and a peak width of 0.017-1.0 minutes. A compiled aligned peak list table was then generated using a 5 ppm mass tolerance, 5 % retention time tolerance, and weighting values of 10 and 20 (arbitrary) for RT and m/z, respectively. Finally, the peaks associated with the instrument and sampler blanks were manually removed, and the peaks list rows filter was used once again on the compiled aligned peak list, but with the minimum peaks per row set to 3. This secondary filtering removed erroneous single detections, which was statistically prudent as there were always 5-6 replicates per sample. The processed aligned peak list was then exported as a CSV file, which was then imported into SIMCA-14 multivariate data processing software (Umetrics, Malmo, Sweden). Principle component analysis was then performed using Pareto scaling in order to determine the significant features of the various samples, as well as to test their data fit.

For untargeted analysis, data processing was again performed via a multi-step process. First, the raw data files obtained from the HPLC-MS were converted to an mzXML format using the free Proteowizard software, MSconvert (Adusumilli and Mallick, 2017). Once converted, these files were imported into XCMS online for furthering peak filtering and detection (Chambers et al., 2012). The peakpicking algorithm that was selected was centWave, which was set with the following parameters: 3 ppm, a minimum peak width of 10 s, and a maximum peak width 60 s. Alignment was achieved using the following parameters: minfrac 1, mzwid 0.015, and a bw of 5 seconds. The samples from the different rivers were processed separately using the positive/negative ESI modes. Eventually, the xMSannotator package (version 1.3.2) was used to annotate the peak lists for each different sample group that were obtained via XCMS online (Uppal et al., 2017). In order to cross-reference the detected toxins and environmental contaminants, the Toxin and Toxin Target Database (T3DB) database was used (Wishart et al., 2015). The results were refined manually to identify compounds with a confidence level of 3, as well as unique matched candidates in the T3DB. Chem-spider was used to extract values of the log P, using ACD/LogP or ChemAxon calculator (Levin et al., 2016). The T3DB was used to determine the chemotaxonomic class/subclass and the main application/source of each chemical. The results were evaluated via principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), which were both conducted using MetaboAnalyst. As part of this analysis, the obtained features went through several manual filtrations; for example, features where the ratio between the average sample-signal intensity and the average blank-signal intensity was less than 5 were removed. In addition, features where the RSD for pooled QC was more than 30 % were also removed. These criteria have been recommended for untargeted and metabolomic studies by HRMS.

2.4. Preparation of the coated bolt SPME device

Initially, the coated bolts were prepared using a spray-coating methodology previously reported by our group (Mirnaghi et al., 2011; Musteata et al., 2007). Briefly, 150 kDa of PAN was dissolved in DMF to make a 10 % PAN solution. 10 mL of this solution was then mixed with 1.0 g of 30 µm HLB particles. Next, 3 mL of DMF was added to the mixture to create a sprayable slurry. However, before applying the slurry, the stainless steel bolts were etched by immersing them in an open beaker of concentrated HCl under sonication for 10 minutes. Following etching, the steel bolts were coated with approximately 10-12 coats of slurry using an Aldrich glass sprayer (Sigma-Aldrich, Oakville, ON, Canada). Each coated bolt was then placed in a modified GC oven at 150 ^{o}C to dry. The coated bolts were then cleaned and conditioned via immersion in a 50:50 methanol/water solution.

For bolts prepared with a recessed extraction phase etching was performed for 1.5 hours, which resulted in a 30 µm indentation on the stainless steel surface. Dip coating was then performed using a programmable actuator in order to allow the bolts to be immersed in the PAN/HLB/DMF slurry up to the edge of the etched surface. Furthermore, due to the availability of the sorbent, a smaller and more strongly adsorbing 5 µm HLB particle was used. Unlike the other set of bolts, this set was only dip coated in 2 coats of the slurry, with each coated bolt being thermally set in a modified GC oven at 150 ^{o}C. The excess coating was then removed from the head of the bolt using a scalpel blade, and the coating was cleaned and conditioned via immersion in 50:50 methanol/water solution.

2.5 Construction of the coated bolt sealable SPME sampler

As can be seen in Figure 1, the coated bolt sealable SPME sampler was constructed out of a chemically and thermally resistant polytetrafluoroethylene (PTFE) body with 6 drilled

positions to hold 6 coated bolts per sampler. Beyond serving as the extraction phase, these 18-8 stainless steel bolts of 0.63 cm thickness (McMaster Carr, Elmhurst, Il, USA) also served to hold the two PTFE portions together. A 316 stainless steel spring with a spring constant of 2.91 kgf/cm and 1.22 cm of compression travel (McMaster Carr, Elmhurst, Il, USA) was machined into the center of the sampler. This spring served to hold the 2 PTFE components apart, sealing the sampler during transportation and storage. To overcome the force provided by the spring during sampling 6 rare earth magnetics (Lee Valley Tools, Waterloo, ON, Canada) having diameter of 0.953 cm with individual magnetic strength of 2.27 kg were placed with an aligned magnetic fields into both PTFE components. Lastly, to assist with operation of the sampler a beveled edge and push rod were incorporated into the sampler body.

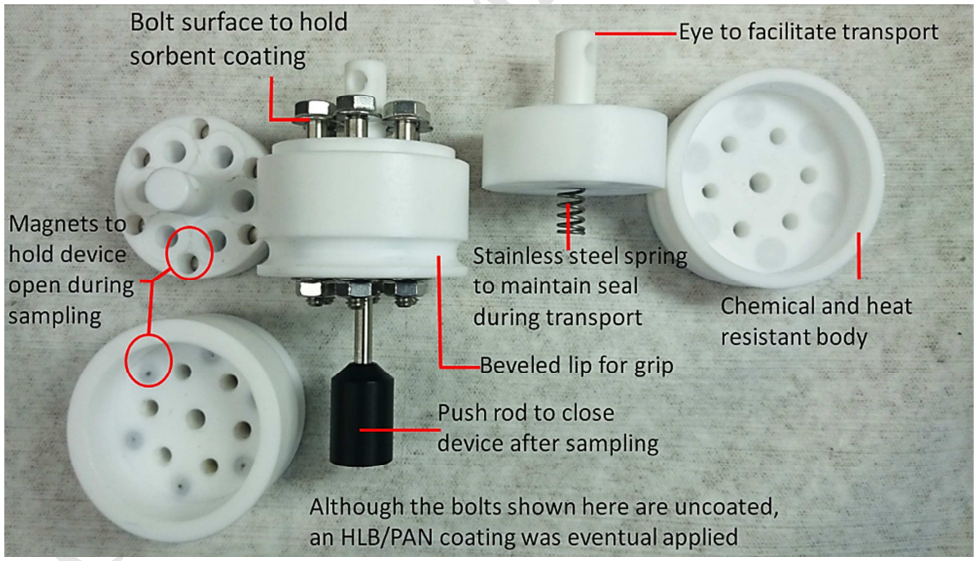


Fig. 1. Breakdown of the magnetic- and spring-locking coated-bolt SPME device.

2.6. Analytical desorption procedure

Desorption of was carried out by placing the coated bolt in a narrow, high-density polyethylene (HDPE) centrifuge tube such that the coated side of the bolt was immersed in 800

µL of desorption solvent, which was comprised of 50:50 ACN/H_2O. Next, the tubes were placed in a Benchmark Scientific Benchmixer XL multi-tube vortexer (Mandel Scientific, Toronto, ON) and agitated at 1200 revolutions per minute for 75 minutes. Following desorption, the solutions were transferred to 2 mL amber glass vials for storage and analysis. Pool QCs were prepared by removing 100 µL of solution from each individual sample and mixing them in a single 2 mL vial. These solutions were stored at -80 ^{o}C while awaiting analysis in order to ensure analyte stability.

2.7. Assessment of the storage temperature and time

In order to confirm that the sealable sampler design was capable of stabilizing the extracted compounds on the sorbent coating, real-world samples were collected immediately downstream from the outflow pipe of an undisclosed waste-water treatment facility along the Grand River in Southern Ontario using 3 different devices giving 18 coated bolts total. These samples were then stored under various conditions for up to 12 days. The samplers were deployed on-site via kayak, and sampling was performed for 1 hour. The measured ambient river temperature was 6.5 ^{o}C, while the temperature at the treatment facility's outflow fluctuated slightly around 20 ^{o}C. Following sampling, the devices were sealed using the springe loaded sealing mechanism and transported back to the laboratory. Solvent desorption was then immediately performed on 4 of the coated bolts, while the remaining devices were stored within the sealable sampler bodies at: A) room temperature for 3 days; B) room temperature for 12 days; and C) -80 ^{o}C in a freezer for 12 days. To best randomize the experiment, 2 bolts were taken from samplers 1 and 2 for immediate desorption, after 3 days of room temperature storage and after 12 days of room temperature storage while the entirety of sampler 3 was placed in the freezer.

2.8. Long-distance sampling of various river sampling sites

After confirming that coating's ability to stabilize the extracted compounds, several samplers were taken to 4 locations in China, including Guangzhou (November 2015, October 2016, and March 2017), Shanghai (November 2015), Dalian (October 2016), and Wuhan (November 2015), and one location in Canada, Ontario (Grand River) (October 2015). One sampler containing 6 replicate TF-SPME coated bolts was deployed at a unique river system in each of these locations. These samplings included: a 7 hour 15 minute overnight sampling of the Pearl River in Guangzhou (23° 6' 23.60"N, 113° 17' 35.12"E); a 7 hour 30 minute overnight sampling of an unnamed tributary of the Huangpu River (31° 9' 0.64"N, 121° 26' 13.02"E), which was downstream from a suspected waste water treatment facility nearby in Shanghai; a 7 hour midday/evening sampling of the Yangzee River in Wuhan (30° 34' 37.32"N, 114° 17' 37.59"E); and an 8 hour night/midnight sampling of the Xinghai River in Dalian (38° 53' 22.93"N, 121° 35' 37.82"E). All samplings were performed by suspending the TF-SPME sampler approximately 1 meter from the riverbed using a rope. In order to evaluate whether the sampler might absorb any chemicals/contamination during transportation, one sampler was considered a blank sampler. This sampler was taken on the trip but was never deployed on-site. Following sampling, all samplers were quickly rinsed using bottled water, dabbed dry with a Kimwipe, clicked into the sealable position and wrapped with aluminum foil, and then placed into a Zip-lock bag and stored at ambient temperatures until returning to the laboratory in Waterloo, Canada, for desorption and analysis.

3. Results and discussion

3.1. Design considerations and mechanical robustness of the coated bolt sealable sampler

In order to survive the harsh environmental conditions imposed by real-world sampling settings (such as high flow rates, high/low temperatures, pH, pressure), many design features needed to be considered and optimized during the construction of our sealable HLB/PAN-coated-screw SPME sampler (Fig. 1). Firstly, in-addition to having a solid, compression-resistant body, the solid PTFE shell was designed to survive very high temperatures while also providing maximal chemical resistance in the sampling environment. Another important aspect of the sampler is its spring-assisted sealable design, which incorporates a magnetic locking system that allows the sampler to be held open during sampling (Fig. 2A). Once sampling is complete, the user can simply press on the push rod, which separates the magnets and allows the spring to hold the device in the sealed position (Fig. 2B). This design effectively protects the sorbent coating from convection and open-bed diffusion during storage pre- and post-sampling. The incorporation of the six, large-diameter (0.63 cm thick), coated 18-8 stainless steel bolts directly within the sampler body is also advantageous. In addition to providing the sampler with superb physical strength under load, the large-diameter coated bolts also provide a significant increase in available surface area and, by extension, sorbent coating.

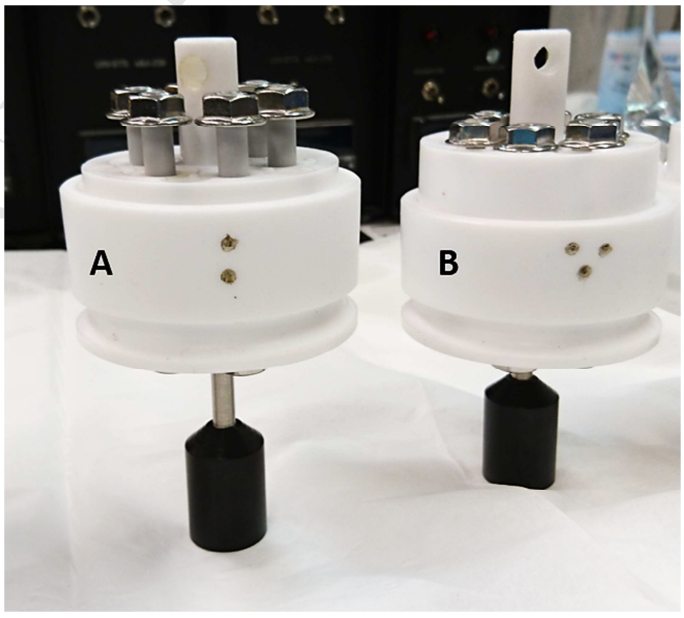


Fig. 2. Magnetic- and spring-locking coated-bolt SPME device shown in: A) sampling position, and B) sealed position.

The coated bolts can be considered a unique SPME morphology that requires proper optimization. The SPME bolts that were used in the first set of samplers were prepared using an older spray-coating method (Mirnaghi et al., 2011; Musteata et al., 2007). However, it was later found that these coatings were prone to stripping when operated within the sampler body. This stripping was caused by the leading edge of the sorbent coating (Fig. 3B) catching on the edge of the cylindrical walls of the PTFE sampler's body during operation. To address this limitation, we decided to employ a recessed coating methodology, which produced a coated surface with a diameter that was equal to or less than the unetched portions of the stainless steel bolts (Poole et al., 2017b). In addition, the recessed coating method was found to produce a much smoother and uniform coating, which may have been partly due to the smaller 5 µm HLB particles that were used.(Gómez-Ríos et al., 2018) Furthermore, since the leading edge of the sorbent coating was protected within the recession, it could no longer catch on the cylindrical edge of the PTFE sealing body (Fig. 3C). Although these coatings were much thinner and contained less volume than the previous design, the available surface area was relatively identical. It is important to note that although efficacy would likely not be affected for shorter extraction times (<30 minutes), a lesser amount of compound may be extracted for longer samplings such as the ~7 hours used throughout the study. This holds especially true for low molecular weight compounds which can diffuse to the sorbent coating more quickly, and have a low coating sampling partitioning coefficient allowing for equilibrium to be reached sooner.

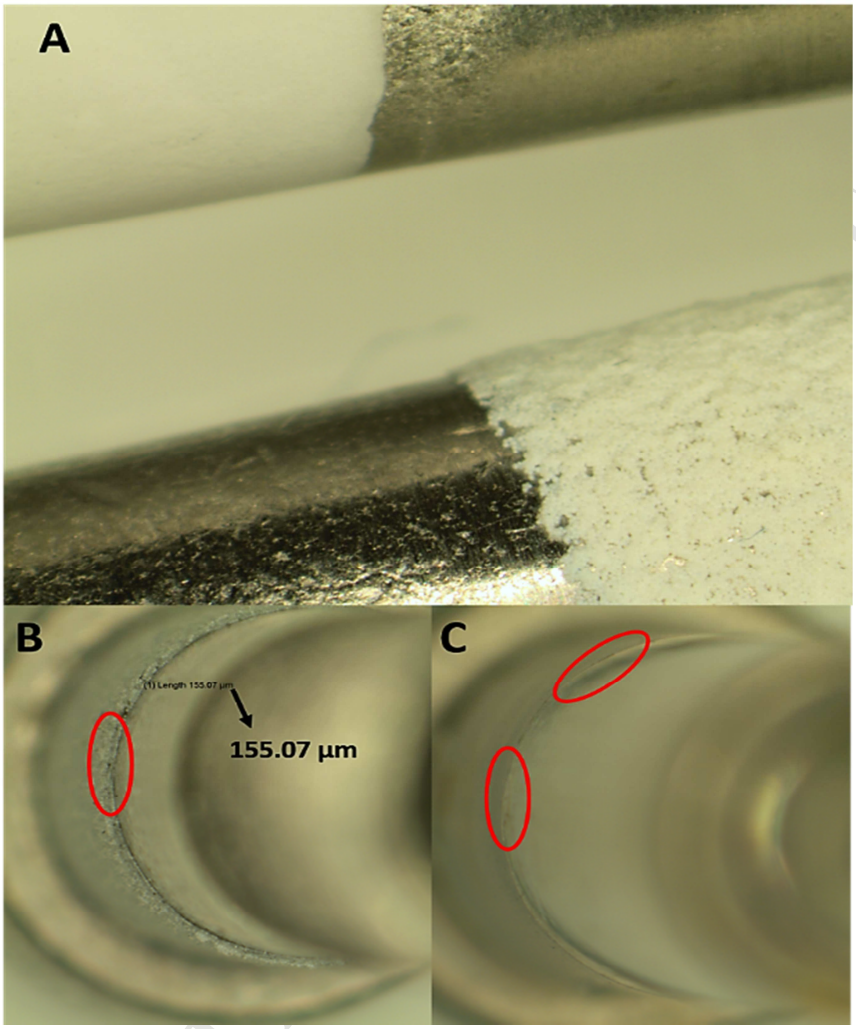


Fig. 3. Comparison of the recessed and non-recessed coated SPME bolt showing: A) side-by-side view of both devices; B) top-down view showing the raised edge (155 µm) of the non-recessed device; (30 µm d. particle) and C) top-down view showing the smooth edge of the recessed device (5 µm d. particle).

In SPME, the surface area of the extractive phase plays the most important role in determining both the extraction time required and the amount of the extraction. As can be seen in Table 1, among all of the current SPME-HPLC morphologies, the coated-screw format provides the largest amount of available sorbent and, more importantly, surface area. This large (250 mm^2) surface area is needed to achieve adequate sensitivity during the relatively short sampling times. As such, one could expect a signal improvement of a factor of 22 times over a comparable

320 HLB/PAN SPME fiber in the pre-equilibrium regime of extraction.(Grandy et al., 2018, 2016;
321 Jiang and Pawliszyn, 2012)

Table 1. Comparative physical dimensions of coated HPLC SPME fibers, TFME blades, and the coated-bolt sampler.

	Coated diameter. (mm)	Coating thickness (µm)	Coating length (cm)	Coating vol. (mm^3)	Coating surface area (mm^2).
HPLC SPME fiber*	0.27	45.00	1.50	0.39	11.10
TFME blade (Cudjoe et al., 2009)	2.55 **	120.00	2.00	12.20	102.00
Coated bolt (spray)	6.65	150.00	1.20	37.30	251.00
Coated bolt (recessed)	6.40	25.00	1.20	6.20	241.00

* As per characteristics of commercially available (Millipore-Sigma) SPME-LC fiber probes
** Coated width of blade

322

323

324 **3.2. Effects of storage temperature and time**

325 One of the explicit purposes in designing the coated bolt sampler was to provide a way of
326 stabilizing the extracted compounds on the sorbent coating for extended periods in ambient
327 conditions. To this end, identical real-world extractions were performed at the outflow pipe of an
328 undisclosed waste-water treatment facility along the Grand River (Southern Ontario, Canada)
329 using multiple devices, which were then stored for varying amounts of time under different
330 conditions. The device's storage stability was assessed using a one-way ANOVA with a 95 %
331 level of confidence (Table 2). For this test, 10 low molecular weight features were randomly
332 selected, with none showing significant differences in the amount of analyte remaining on the
333 sorbent coating, even following 12 days of storage at room temperature. This was encouraging as
334 lower molecular weight compounds would be the most likely to be lost via volatilisation if the
335 sealing of the sampler were insufficient. This result is depicted graphically in Fig. 4, which
336 shows the relative signal of the response generated from the pooled QC sample. Since the pooled

337 QC was prepared by mixing a small portion from each extract, it was encouraging to see that it
338 generated a similar signal to that of the samples. However, as the error bars of Fig. 4 indicate, the
339 pooled QC data, which was generated from 7 replicate injections from the same vial, produced
340 noticeably less error than the pooled data from the individual coated bolts, with % RSD's
341 ranging from 5-12 % and 9-20 %, respectively. Although these figures potentially indicate
342 variation in inter-bolt reproducibility, at less than 20 %, this variability is within the acceptable
343 range for on-site sampling methodologies. Furthermore, it is important to note that internal
344 standards have typically been added to the sample solution during in-lab extractions in most
345 other TFME-HPLC studies that have assessed method reproducibility (Mirnaghi et al., 2013,
346 2011; Strittmatter et al., 2012). This is not to say that internal standard correction is poor
347 practice; rather, internal standard correction is generally quite prudent whenever possible, as it
348 can account for any unknown errors that may arise during desorption, liquid extract storage,
349 instrumental analysis, or variability between SPME devices (Mirnaghi et al., 2013, 2011;
350 Strittmatter et al., 2012).
351
352

Table 2. ANOVA testing at 95 % confidence demonstrating consistent signal of select compounds stored on the HLB/PAN-coated-bolt sealable sampler over the entirety of the 12-day room temperature storage stability period (F_{crit} = 3.71) (n=4)

Exact Mass	RT	Empirical formula	Tentative compound class	F Value	%RSD
107.0858	19.93	C8H10	Xylenes	2.08	20
120.0559	10.21	C6H5N3	Benzotriazoles	0.92	16
135.0749	11.71	C12H20N4OS	Isomethiozin	0.77	16
143.1069	13.49	C8H14O2	Carboxylic acids	1.20	16
182.0095	16.92	C8H7NS2	Methylthiobenzothiazoles	1.47	13
189.1639	19.93	C5H6ClN3O	Chloro-methoxypyrazin-amines	3.71	17
199.0968	11.59	C8H18O5	Tetra ethylene glycols	1.87	19
213.0429	18.84	C10H14O4	Carboxylic acids	2.03	16
309.2039	21.24	N/D	Multiple possibilities	1.67	13

315.0098	21.63	N/D	Multiple possibilities	0.40	9

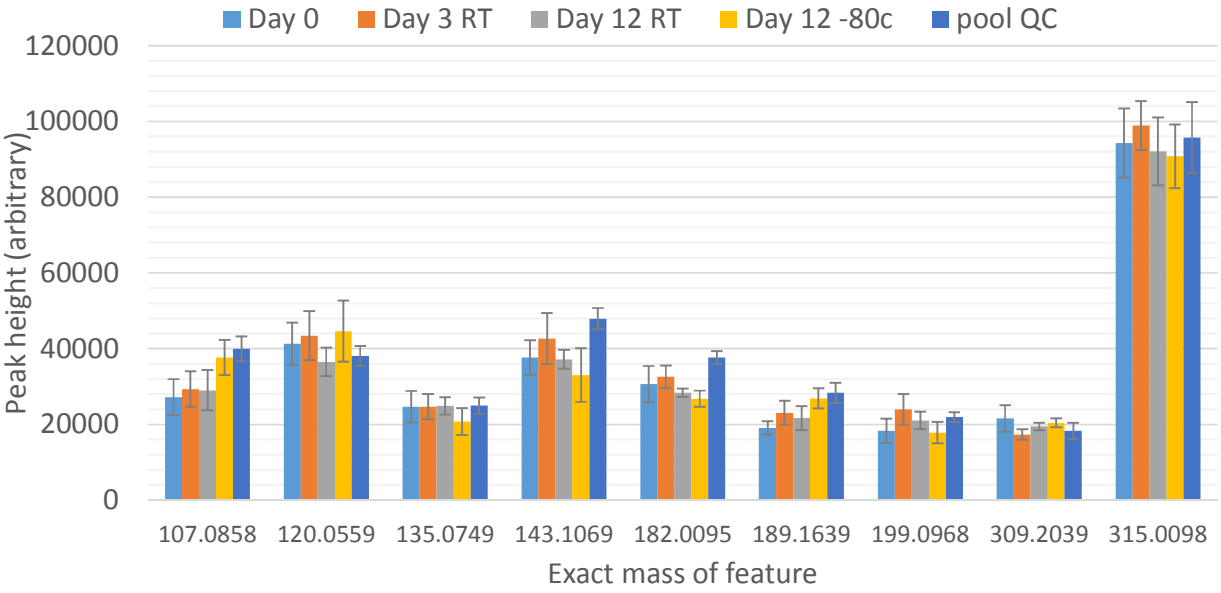


Fig. 4. Stability of randomly selected volatile features on the HLB/PAN-coated-bolt SPME samplers: 2 hours following extraction; after 3 days of storage at room temperature; after 12 days of storage at room temperature; after 12 days of storage at -80 °C; and replicate extractions from the pooled QC. (n=4)
* M/Z 213.0429 not shown due to scaling issues.

To ensure that the noted reproducibility was not just associated with the 10 randomly selected features, principal component analysis was applied to the dataset to see if any grouping could be observed between the coated bolts in the different storage conditions. Appropriately, no clustering was observed between samples in the related PCA-plot (Fig. S1), indicating that any separation among the samples was likely due to random background noise. This is to be expected as multivariate approaches base separation on the most significant features in a given dataset. When no actual statistical differences exist between samples, the PCA algorithm will begin to identify random noise and artifacts as the most significant factor driving sample separation, thus resulting in a randomly distributed PCA plot like the one seen in Fig. S1. Furthermore, because

the samples were so similar, even the pooled QC data was found to exhibit poor grouping on the PCA plot, despite the good performance of instrumental QC data.

It is also important to note that although quantitative analysis was not the primary goal of this study it would still be quite feasible to develop a quantitative methodology for these devices. To perform such a calibration the best approaches would likely be Kinetic calibration either with or without internal standard best described in Ouyang *et al*. research cited here.(Ouyang, 2008; Ouyang et al., 2009)

3.3. Application of the designed device for the untargeted analysis of river samples

After testing the device's design and functionality, it was tested in real-world field conditions, namely, in the untargeted analysis of samples from rivers in five different geographical sampling sites (see Section 2.6).

In order to evaluate the device's extraction and analytical performance, MetaboAnalyst was used to process the resultant data, with PLS-DA being conducted in both positive and negative ionization mode for all devices. The PLS-DA results for the blank sampler (this device was taken on the trip, but was never deployed on site) and the sampler used at the Guangzhou sampling site (2017) are shown in Fig. 5. As can be seen from the plots, the instrumental QCs are clustered tightly and show very clear separation, which demonstrates the repeatable performance of the analytical instruments. In addition, the blank sampler and the sampler used at the Guangzhou site showed two distinct clusters. This gives some indication that the samplers did not absorb a significant amount of contamination during the trip, and, more importantly, that the obtained signals from the deployed sampler were exclusively due to compounds collected at the sampling site. Moreover, the proximity of the pooled QC data in the region of the deployed

sampler indicates that the most significant features were indeed related to the actual sampling site and not contaminants resulting from international travel.

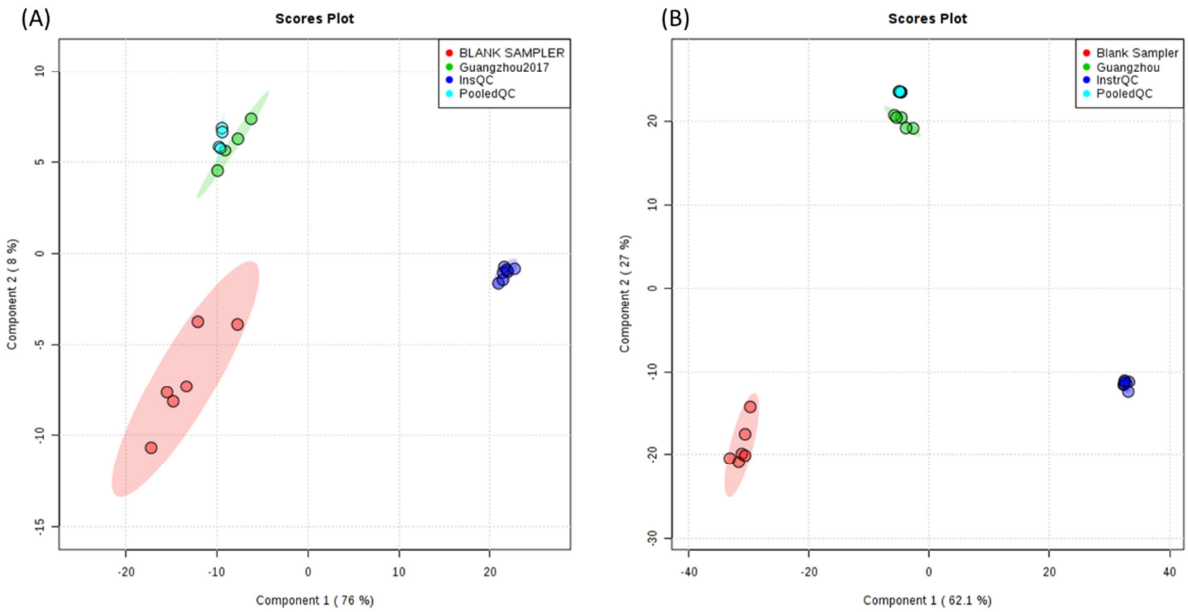


Fig. 5. PLS-DA score plots for the sampler deployed at Guangzhou sampling site and the blank sampler, which was taken on the same trip, but was never deployed on site. A) Positive ESI mode, B) Negative ESI mode.

Fig. 6. shows the PLS-DA score plot for the same sampling site (Guangzhou) over 3 consecutive years. As can be seen, there is a high level of clustering for the instrumental QC and the blank sensor indicating stable response of the analytical instrument. Greater levels of similarity in the features were observed in the samples taken in 2016 and 2017 than in those taken in 2015. This was confirmed by analyzing the data using xMSannotator and the annotated list (Table S1). The Dalian sampling site produced the same results as the Guangzhou over the same sampling period (Fig. 7).

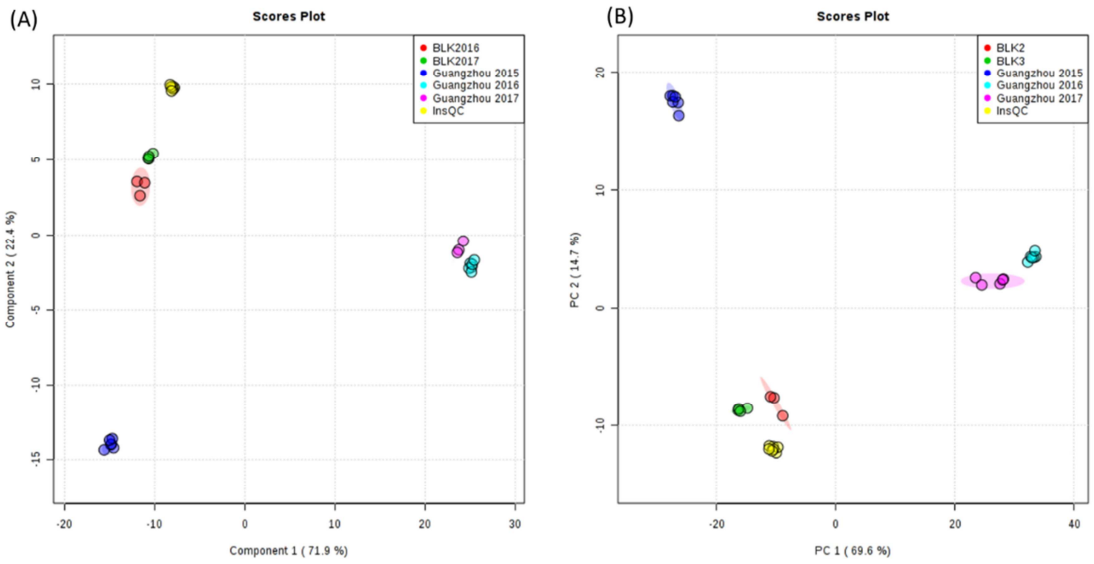


Fig. 6. PLS-DA score plots for the Guangzhou sampling site over 3 consecutive years. A) Positive ESI mode, B) Negative ESI mode.

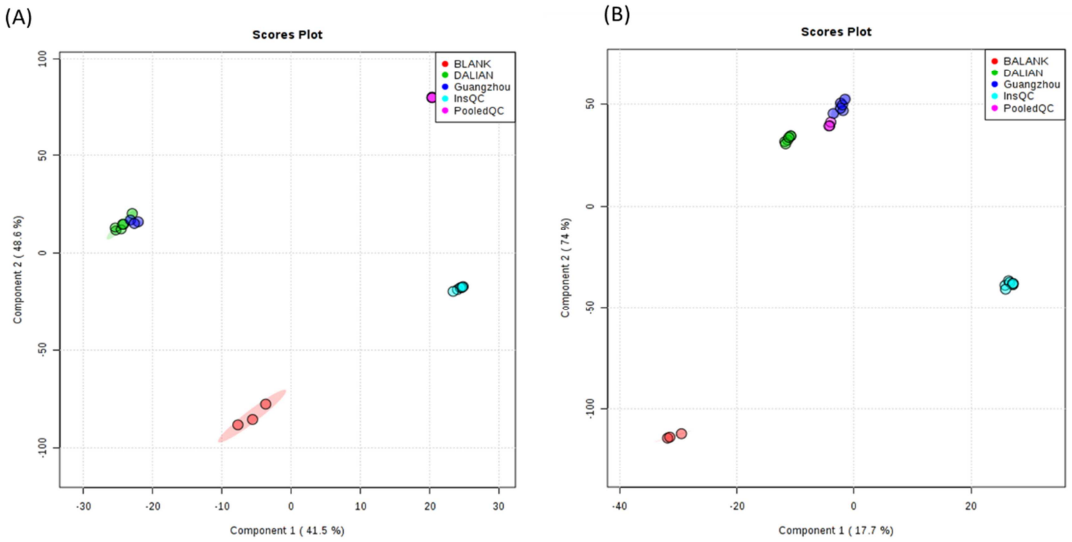
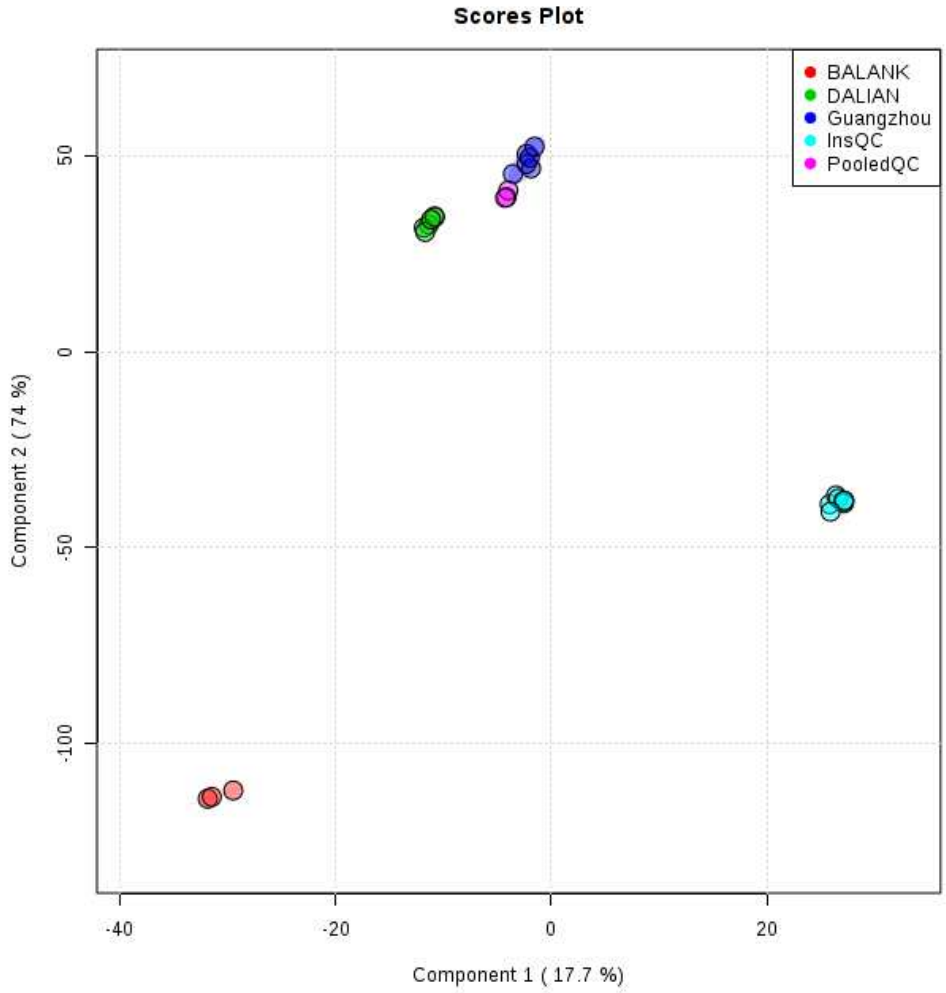


Fig. 7. PLS-DA score plots for the Guangzhou and Dalian sampling sites. A) Positive ESI mode, B) Negative ESI mode.

The validity of PLS-DA plots (Figs. 5-7) was confirmed via cross-validation processing, the results of which are illustrated in Figs. S2-4. These plots were used to find the predictive values of the used model, as they effectively show how strong/valid the statistical analytical

model can be. Q2 is an estimate of the model's predictive ability and is calculated via cross-validation (CV).



In each CV, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples (Predicted Residual Sum of Squares, or PRESS). For convenience, the PRESS is divided by the initial sum of the squares and subtracted from 1 to resemble the scale of the R2. Good predictions will have a low PRESS or a high Q2. Increasing the values of the Q2 by increasing the number of components is confirmation of the statistical model's validity.

As was noted in the introduction, trace chemicals in water samples are comprised of a wide variety of analytes that have various chemistries, polarities, solubilities, and chemical stabilities. One important objective of the current study was to show how the proposed device can be used to extract and analyze a wide range of untargeted compounds. In order to assess whether the proposed device can be used for such applications, the results obtained from the different sampling sites were processed comprehensively. Untargeted environmental analysis can be used to provide insight into the distribution of pollutants in different environmental sites. LC–MS Orbitrap, which is a high-resolution mass instrument (HRMS), has become a widely used platform for such analyses due to its sensitivity, speed, versatility, and reproducibility. ESI is commonly used as an interface to connect LC and MS, as it provides good sensitivity and a high proportion of molecular ions for detection. However, many additional ion signals, such as in-source fragment, adducts, and multi-charge species, are also acquired in untargeted analysis, which leads to complementation in the interoperation of the LC-MS data. An alternative (and sometime complimentary) approach to untargeted analysis involves the statistical and computational analysis of all signals produced by an analytical platform. This approach brings 'annotation' term vs 'identification' term in untargeted metabolomics and analysis. Whereas identification is the process of confirming identity using at least two independent reference standards (e.g., accurate mass and retention time), annotation entails using databases to make tentative matches based on spectral similarity and/or physicochemical properties.

To this end, we elected to use the freely available R package, xMSannotator (Uppal et al., 2017). This package incorporates several utilities and an integrative multi-criteria scoring algorithm designed to improve the annotation of high-resolution metabolomics data. The main purpose of the software is to facilitate metabolite identification in untargeted LC−MS data. In the

present study, this software was used to simplify the data obtained from the high-resolution LC-MS and to create a list of the tentative extracted chemicals using the T3DB (this procedure was described in Section 2.2). Fig. S5 shows the mass profile plots of the unfiltrated features (left) and the tentative annotated features (right) that were obtained after applying XMSannotator. As can be seen from the plots, over a thousand features were obtained at each sampling site (left side); however, by applying the xMSannotator integrative scoring algorithm, it was possible to simplify these data to a tentative list of chemicals, which are shown in the mass profile plots in Fig. S5 (right side). Although it is not possible to identify compounds with 100 % certainty using XCMSannotatoor alone, the results presented in this study are interesting nonetheless. Any of the tentative IDs made in this study should be considered speculative until the appropriate MSn validation or standard confirmation can be conducted. Such validation was beyond the scope of this study though.

Table S-1 summarizes the list of chemicals obtained using the xMSannotator algorithm. As can be seen from the table, over 80 chemicals with different chemotaxonomy classes/subclasses and a wide range of log P values ($-2.43 < \log P < 11.9$) were obtained. As mentioned in the experimental section, PFP-HPLC column was used for the analysis. A previous study conducted by our group proved that the PFP-HPLC column was able to separate the highest number of features, with identified metabolites ranging between $-7 < \log P < 15$ (Mousavi et al., 2015). In that study, it was shown that pentafluorophenyl bonded in PFP column incorporates fluorine atoms on the periphery of the phenyl ring, applying multiple retention mechanisms for separation of both polar and aromatic compounds. These compounds can be retained with a PFP column due to the presence of the electronegative fluorine atoms, which produce an electron deficient phenyl ring. According, in order to enable a wider range of extracts

for analysis, the same HPLC column was used in the current study. Moreover, the present study required an extraction phase that was suitable for a wide range of chemicals; consequently, an HLB coating that was specifically designed for the extraction of low molecular weight, polar, and non-polar compounds was selected. HLB particles are second-generation mesoporous polymers that feature a large surface area. These particles are synthesised using a poly(divinylbenzene-co-N-vinylpyrrolidone) skeleton structure, which helps to balance hydrophobic and hydrophilic interactions, largely due to the presence of aromatic rings in the divinylbenzene and the polar groups present in the lactam ring of the N-vinylpyrrolidone (Poole, 2012). The sorption mechanism of these particles has also been previously studied (Dias and Poole, 2002). That research observed that HLB particles have strong ion-pair electron interactions and a strong affinity towards compounds containing hydrogen bonds with acidic properties. Due to these intermolecular interactions, HLB-coated surfaces are exceptional at retaining compounds with electron-rich structures (aromatic rings) and hydrogen-bonding capabilities (hydrogen-bond donors), it means HLB particles are suitable for extracting a wide range of chemicals.

Here, in the present study, as can be seen in Table S-1, a wide range of chemicals from different groups was obtained using the proposed device, including: amino acids, peptides, acrylic acids (and derivatives), benzofurans, benzoic acid (and derivatives), biphenyl (and substitute derivatives), carboxylic acids (and derivatives), coumarins (and derivatives), cytochalasans, dihydrofurans, indoles (and derivatives), isoindole (and derivatives), morpholines, methoheterocylcliclic compounds, organo-post-transition metal compounds, organofluorines, organonitrogen compounds, organooxygen compounds, organic phosphoric acids (and derivatives), piperidines, prenol lipids, purines (and derivatives), phenanthrenes (and

510 derivatives), perylenequinones, quinone and hydroquinone lipids, and retinoid.(Pon et al., 2014,
511 2009; Wishart et al., 2015)

512 Most of these chemicals are primarily used in the agricultural industry as herbicides,
513 pesticides, and/or fungicides. Moreover, many of them are used in personal care products, which
514 are considered an important and inevitable group of emerging contaminants. (Pon et al., 2014,
515 2009; Wishart et al., 2015) This could be expected from the ever-increasing level of chemical
516 contaminations of environmental sources, which are consequence of different anthropogenic
517 activities.

518 **4. Conclusion**

519 A new, mechanically robust sampling and extractive TF-SPME device that incorporates
520 recessed HLB/PAN-coated bolts with a large surface area was designed and deployed on-site. As
521 the results of this research show, enclosing the bolts in a sealable Teflon body made it possible to
522 stabilize analytes extracted from a real sample on the coating for a period of at least 12 days at
523 ambient temperatures. Furthermore, the enclosable design allowed the samplers to be transported
524 using unconventional means and over great distances. In addition to providing the sampler with
525 physical rigidity, the large-diameter cylindrical bolts also offered a large surface area on which
526 the coating could be applied. Indeed, this surface area was calculated as being more than double
527 that of a comparable thin-film blade device and 22 times larger than commercially available
528 SPME-HPLC fibers. Since short sampling times are frequently used in on-site sampling, the
529 increased surface area is critical for ensuring reasonable sensitivity. Finally, a proof-of-concept
530 test of the samplers in real-world field conditions proved that these devices could be used to
531 collect and analyze water samples collected in various environments halfway across the world. In
532 the future, similar yet targeted applications could be explored as internal standards could easily

be pre-loaded and stabilized onto the coated bolts for quantification purposes. Regardless, the results of these field tests indicated that the sealable TF-SPME bolt sampler is suitable for the on-side collection of a wide range of chemical species, which will be a tremendous help in characterizing water contaminants and discovering new chemical pollutants.

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Declaration of Interests

The authors of this manuscript declare no conflict of interests pertaining to this manuscript, financial or otherwise.

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Highlights

- A robust SPME sampler for on-site sampling and extraction is introduced.
- The sampler was applied to detect a wide range of untargeted pollutants in environmental waters.
- At ambient temperatures, the sampler proved capable of stabilizing the extracted chemicals for a period of 12 days.
- The introduced sampler has the potential for use in many applications, particularly in cases when the analysis facilities are far away from the sampling sites.