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1

A Suspect Screening Analysis for Contaminants of Emerging Concern in Municipal Wastewater and Surface Water Using Liquid-Liquid Extraction and Stir Bar Sorptive Extraction.

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Abstract

The presence of contaminants of emerging concern (CECs) in wastewater effluent and surface waters is an important field of research for analytical scientists. This study takes a suspect screening approach to wastewater and surface water analysis using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). Two extraction procedures, traditional liquid-liquid extraction (LLE) and stir bar sorptive extraction (SBSE), were utilized and evaluated for their application to wastewater and surface water samples. Both techniques were evaluated regarding their recovery rates, range of compound classes extracted, and on their application to discovery of CECs. For the 14 surrogate compounds analyzed, LLE was able to extract all of them in each matrix with a recovery range of 19% to 159% and a median value of 74%. For SBSE, the recovery rates ranged from 19% to 117% with the median value at 66%, but only 8 of the compounds were able to be extracted because of the polarity bias for this extraction method. A new method of SBSE calibration was also developed using direct liquid injection of the internal standards before desorption of the stir bars. Initial findings indicate increased sensitivity and a greater range of unknown analyte recovery for

SBSE, especially in the more dilute effluent and surface water samples. With the methods used in this study, SBSE has a concentration factor of approximately 416, improving that of LLE, which is 267. Suspect screening analysis was utilized to tentatively identify 32 CECs in the samples, the majority of which were pharmaceuticals and personal care products. More CECs were found using SBSE than LLE, especially in the surface water samples where 13 CECs were tentatively identified in the SBSE samples compared to 6 in the LLE samples.

Introduction

The American Society of Civil Engineers graded the US wastewater infrastructure a D+ in their 2017 Infrastructure Report Card. With repair and expansion costs totaling over $271 billion [1], many of the country's wastewater treatment plants (WWTPs) are not effectively removing all contaminants from current waste streams. These outdated WWTPs were not originally designed to treat modern contaminants of emerging concern (CECs) which range from pharmaceuticals and personal care products to nanomaterials and flame retardants. Primary and secondary treatment technologies, commonly utilized at WWTPs, are not effective for the removal of contaminants resistant to microbial degradation or polar compounds which demonstrate low sorption to sludge and biosolids materials; categories in which many CECs fall[2,3]. WWTP effluent is one of the primary contributors of CECs to environmental waters where they have the potential to impact both human health and the aquatic ecosystem. Many classes of CECs have been found in drinking water [4–6] and have even made their way into the once pristine arctic ecosystem[7,8]. Due to the lack of CEC regulatory monitoring, information regarding environmental persistence, toxological effects, and potential biological impacts are not well known[9].

Typical analytical methods for CEC analysis utilize gas or liquid chromatography (GC or LC) coupled to tandem mass spectrometry (MS/MS) for a predetermined set of compounds[10]. Such targeted approaches allow for low detection limits and reliable quantification, but much of the information about the rest of the sample is left unknown. For a more complete analysis of complex samples, suspect screening methods have been applied, in which databases of chemical suspects are utilized for the tentative chemical or class identification of unknown components, without the initial need for reference standards[11–13]. One technique that is particularly powerful when combined with suspect screening analysis is comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). GC×GC allows for enhanced separation through both increased peak capacity and sensitivity, which is a significant improvement for the separation of the thousands of compounds present in complex environmental samples. The addition of the fast TOFMS detector allows for chemical or class identification of analytes based on their mass spectral comparison to spectral libraries and chemical suspect databases.

Current trends in aqueous sample preparation show a shift in the research and preparation of extraction techniques towards more environmentally friendly techniques [14,15]. While solvent extensive techniques like liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are still commonly used, microextraction techniques such as stir bar sorptive extraction (SBSE), solid-phase microextraction (SPME), and dispersive liquid-liquid microextraction (DLLME) are growing in use [16,17]. There are even multiple regulatory SPME methods, including EPA, ISO, and ASTM, for the extraction of organic contaminants in environmental matrices [18]. Miniaturized sorptive extraction methods have grown in popularity because, when paired with direct thermal desorption, organic solvents and lengthy concentration steps are eliminated[19].

SBSE is similar to classical SPME but the sorptive phase, usually polydimethylsiloxane (PDMS), is coated on a magnetic stir bar and the volume of extraction phase is much greater, allowing for greater extraction efficiency and sorbent capacity[19]. The commercial PDMS Twister stir bar from Gerstel has been shown to have a wide linear range, from low ppt to 100 ppm analyte concentration [20]. SBSE has been used in a variety of sample matrices, ranging from human urine to beer and wine, with limits of detection in the low ng/L range[21]. The greatest limitation to this technique is the chemical selectivity of the commercially available PDMS phase. Due to the non-polar nature of this phase, polar compounds are not efficiently extracted and will exhibit poor recovery.

The goal of this study was to utilize two extraction techniques, LLE and SBSE, for GC×GC-TOFMS analysis of CECs in wastewater samples. The two extraction methods were chosen as they represent the traditional US EPA recommended method (LLE) and a commercially available sorbent based microextraction method (SBSE). The extraction methods were ultimately evaluated for their extraction efficiencies and overall range of extractable analytes. A novel form of internal standard addition was applied for the SBSE, making quantification more directly comparable to the LLE quantification. The US EPA CompTox Chemicals Dashboard was used for the suspect screening database and the majority of CECs tentatively identified in the samples were classified as personal care products and pharmaceuticals.

Materials and Methods

Chemicals and Reagents

Standards were chosen based on the target compounds lists contained in US EPA Method 8270D which represent environmentally-relevant compound classes including a broad range of acidic

and basic compounds. All standards were purchased from Restek (Bellefonte, PA, USA). A full list of the chemicals and reagents is included in the supporting information.

Sample Collection

During mid-March, water samples were collected from the Bellefonte WWTP in Bellefonte, PA. 500 mL samples were taken in triplicate from the pre-treatment influent tank, post treatment effluent tank and from the Spring Creek, about 15 meters downstream of the WWTP outfall site. These samples were collected in clean, 500 mL amber glass jars with PTFE closures. Three method blanks, which also serve as trip blanks, of 500 mL Milli-Q water were prepared and taken to the sampling sites in the collection cooler. The samples were stored at 4˚C until extraction within 7 days of collection. No pre-treatment steps were done prior to the extractions.

Liquid-Liquid Extraction

Samples were extracted using a modified USEPA Method 3510C Separatory Funnel liquid-liquid Extraction, a brief summary of the method is included here, the complete procedure is reported elsewhere.[22] For each sample, a 400 mL aliquot of water was measured for extraction into a 2 liter separatory funnel. The three surrogate standard mixes were spiked into the samples to yield a final extract concentration of 200 ng/mL, except for the influent samples which were spiked 4 times higher for planned extract dilution. Each sample was serially extracted 3 times under both basic (pH of 11) and acidic (pH of 2) conditions using 30 mL of dichloromethane for each step. Samples were concentrated using Kuderna-Danish evaporative concentration to ~10 mL followed by nitrogen blowdown to ~1 mL and reconstituted to 1.5 mL with dichloromethane. Extracts were stored at 4°C until analyzed.

Stir Bar Sorptive Extraction

The stir bar extraction and desorption procedure were optimized before extraction of WWTP and Spring Creek samples, using Milli-Q water spiked with the surrogate mixes and 8270 Megamix, a mixture of 76 environmentally relevant organic contaminants. The SBSE method, adapted from León et al [23], was optimized with respect to surrogate concentration, stir bar spin time, and salting out effects. The extractions were carried out using commercial Twister stir bars, 10 mm length x 0.5 mm film thickness, 24 μL polydimethylsiloxane phase (PDMS) (Gerstel, Inc., Linthicum, MD, USA). Prior to extraction, the stir bars were solvent conditioned in an 80:20 mix of methanol and acetonitrile overnight then conditioned further in the Thermal Desorption Unit (TDU) (Gerstel, Inc.) at 300 ºC for 30 minutes with 80 mL/min desorption flow. The optimized extraction procedure is as follows: 10.0 mL of sample water and 3 g of NaCl were added to a 20 mL headspace vial (except for the influent which was diluted 1:5 to avoid overloading the stir bars). The surrogate mixes were spiked at a concentration of 20 pg/mL in solution (200 pg/stir bar in the 10 mL sample) then the stir bars were placed in the water samples and set to stir at ~1200 rpm for 4 hours on a multi-position stir plate (Cole-Parmer, Vernon Hills, IL, USA). Method blank stir bars were identically prepared using the Milli-Q water taken in the sampling cooler as the water sample. Preconditioned stir bars were also spun in Milli-Q water without the addition of surrogates to serve as PDMS stir bar blanks to ensure chromatographic peaks were not due to stir bar contamination. After extraction, stir bars were kept in a freezer until instrument analysis.

Instrumentation and GC×GC

GC×GC measurements were carried out with a Pegasus 4D GC×GC-TOFMS instrument (LECO Corp., St. Joseph, MI, USA). The gas chromatograph was a 7890A GC system (Agilent Technologies, DE, USA) equipped with a Gerstel Multipurpose Sampler (MPS-2, Gerstel, Inc.).

The column ensemble consisted of a 60 m x 0.25 mm ID x 0.25 µm film thickness Rxi-5 Sil MS (Restek Corp.) coupled to a 1.1 m x 0.25 mm ID x 0.25 µm film thickness Rtx-200 (Restek Corp.). Helium carrier gas was at a constant flow rate of 2.00 mL/min. The primary oven program was as follows: initial temperature of 40 ˚C held for 1.50 min with a single temperature ramp of 3.50 ˚C/min to 315 ˚C with a final hold time of 10.00 min. The secondary oven temperature program was offset by 5 ˚C positive to the primary oven program, the modulator temperature offset was 20 ˚C, and transfer line temperature was set to 300 ˚C. The modulation period was 2.00 seconds with a 0.60 second hot pulse. The MS was operated in electron ionization mode at 70 eV. The collected mass range was 50 – 550 amu with an acquisition rate of 200 spectra/second and the mass defect was set at -20 mu/100u.

For the liquid-liquid extraction samples, 1 µL of the sample was injected into a standard split/splitless injector using a Sky 4.0 mm ID single taper inlet liner with glass wool (Restek Corp.). The inlet was run in splitless mode at 250 ˚C with a 90 second inlet purge time. For all liquid samples, the internal standard (IS) mix was added into calibration standards and samples at 200 ng/mL, immediately before injection.

For the stir bar extracted samples, the thermal desorption analysis was optimized for desorption temperature, flow, and time, along with the cryogenic trapping temperature. The programmed temperature vaporizer (PTV) inlet contained a TDU/CIS liner with glass wool (Gerstel, Inc.) and was maintained in solvent vent mode with splitless inlet transfer. The stir bars were placed in the TDU tubes with small amounts of glass wool added in the bottom to increase surface area for the IS. Liquid IS was fortified into the TDU tube at 200 ng/mL using the MPS system TDU liquid option. Adding the liquid IS into the TDU tube allows for normalization between samples and can account for injection and ionization variance. The TDU desorption and the cooled injection

system (CIS) (Gerstel, Inc.) temperature parameters can be found in table 1. The TDU was run in splitless desorption mode and the CIS was used in standard heater mode with cryo-cooling using liquid nitrogen.

Data Analysis

All data were processed using the ChromaTOF software (LECO Corp.) version 4.50.8. Baseline computing above/through the noise was performed and peak finding procedures with a signal to noise ratio of greater than 100 were applied. Initial data screening of unknowns was performed by spectral comparison of the compounds with the NIST 2011 library. Substances exhibiting a similarity of higher than 70% were considered for closer inspection. Peaks detected in the method blanks and measured samples were compared. Sample peaks in excess of 10 times that of the method blank peaks were retained for further analysis. Solvent and column bleed peaks were also excluded. Suspect screening was carried out using the US EPA CompTox Chemicals Dashboard, which contains 875,000 environmentally relevant chemicals, including those specific to wastewater and surface water contamination.

Calibration and Quantification

Internal standard calibration and quantification was performed for the surrogates in the liquid and stir bar samples to calculate the extraction recoveries. The calibration curves were analyzed using ChromaTOF software and quantification was completed using the average response factor for each surrogate and the relevant internal standard. Liquid injection calibration standards were analyzed over a concentration range of 10-2,000 ng/mL for each compound. The stir bars were spun in calibration solutions containing the surrogates in concentrations ranging from 1.0– 200 pg/mL, corresponding to 10-2,000 pg/stir bar assuming 100% recovery.

Results and Discussion

Method Development of SBSE

SBSE is often highlighted for its simplicity and sensitivity but this method requires additional optimization of the extraction and GC thermal desorption procedures for best results.

Extraction Optimization

The conditions optimized for extraction included surrogate spiking concentration, stir bar spin time, and the addition of salt. Other parameters, such as sample volume, spin speed, and the addition of organic modifiers, may also be optimized but the authors chose to use the common literature values for these.[20,21]

The Twister PDMS stir bars used in this study have a 0.5 mm thick sorbent phase. This translates to a phase volume of about 24 µL compared to the 0.5 µL for a typical 100 µm SPME fiber.[20] Because of this greater phase volume there is increased sorption capacity, but special attention must be paid to the concentration of surrogates and analytes in the sample to ensure they are in the linear range for the stir bar and the GC instrument. Overloading the stir bar or the GC column and detector can be a problem, especially when analyzing complex and concentrated samples, such as wastewater influent. For this study, a 10 mL sample volume was chosen and extraction recovery compounds were added at 20 pg/mL, corresponding to 200 pg on GC column. In SBSE, matrix competition effects can occur, leading to lower extraction efficiency especially for certain polar compounds. This competition effect is increased when the matrix components are strongly retained by the phase and the analytes of interest are less strongly extracted. Because of this, the influent samples were diluted 1:5 to prevent overloading the extraction phase and to achieve better surrogate recovery.

Multiple extraction times were tested with the goal of increasing the recovery of the polar analytes ($\log K_{ow}$<2.5) without jeopardizing the recovery of the more non-polar analytes ($\log K_{ow}$ >2.5) while using the shortest extraction time possible. The testing times were 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours and 16 hours, with triplicate stir bars spun at each time. Upon GC×GC analysis, the IS mix was added onto the stir bars for relative area comparison. For the low $\log K_{ow}$ compounds, the highest sorption to the stir bar occurred at 30 minutes, but 4, 8, and 16 hours also produced acceptable recovery. The higher $\log K_{ow}$ compounds demonstrated poor sorption at 30 minutes and reached a maximum at 4 hours. Although the low $\log K_{ow}$ compounds exhibited the highest recovery at 30 minutes, the 4-hour spin time was chosen in order to not negatively impact any high $\log K_{ow}$ compounds while maintaining acceptable recovery of the traditionally less sorptive compounds.

For polar compounds, absorption into the PDMS phase is minimal but can be increased by the addition of a salt modifier. Increasing the ionic strength of the sample solution can shift the equilibria towards the extracting phase and allow for better extraction recovery of polar compounds. This "salting out" effect has been reported in several studies to slightly increase the K_{ow} of polar compounds, allowing for better extraction recovery in PDMS phase stir bars. [24,25] The addition of salt may also negatively affect the sorption of non-polar analytes[26], but this may be prevented using sequential SBSE. In sequential SBSE, the stir bar is first spun without the addition of a modifier. It is then removed and re-spun in the same solution with the modifier added.[27] This method allows for the non-polar analytes to first be extracted without the addition of the salt. Here, sequential and regular SBSE were tested using the addition of 20% NaCl (w/v), 30% NaCl and no salt added. Figure 1 shows the effects of salt on the extraction of 9 compounds characterized as low $\log K_{ow}$ (<2.5), mid $\log K_{ow}$ (2.5-5.0), and high $\log K_{ow}$ (>5.0). All responses were normalized

to the IS and plotted as a logarithmic function of the relative response of the no salt sample. The addition of 30% salt is observed to greatly increase the recovery of the polar compounds while not having negative effects on the mid and non-polar compounds. The relative responses of the lower molecular weight Polycyclic Aromatic Hydrocarbons (PAHs) ($logK_{ow}$ 3.2 to 3.9) were increased with the addition of salt, and the larger PAHs ($logK_{ow}$ 5.5 to 6.9) did not show significant differences between the no salt and the salt added extraction, this is displayed in Figure S1. The sequential extraction was slightly more effective for 3 compounds, but in general, it did not improve the response for the rest of the compounds and therefore was not used to minimize overall extraction time. The concentration of 30% NaCl was selected as it increased the response of the more polar compounds without negatively impacting the nonpolar compounds.

Thermal Desorption Optimization

Apart from the extraction parameters, the thermal desorption must also be optimized for best results. These parameters include the desorption temperature and flow as well as the inlet trapping temperature. In this study, the desorption temperatures tested were from 250 °C to 300°C at intervals of 10°C. Analyte response increased with increasing desorption temperature, but so did PDMS background from the stir bar. The high levels of background from the stir bar were observed in the chromatograms and potentially interfere with analytes of interest. As a result, 280°C was chosen as the desorption temperature to reduce the PDMS background and still maintain effective transfer of a broad range of analytes, including those of higher molecular weight. Another TDU parameter is the desorption flow, which should be optimized to effectively transfer desorbed compounds from the TDU into the CIS. Known as the "Back Inlet Purge Flow" in the ChromaTOF software, this is the flow through the TDU tube during desorption. Flow rates of 75 and 50 mL/min were tested. Analytes were more effectively transferred at the 50 mL/min flow. A flow rate of 75

mL/min was determined to be too great and caused analyte loss likely due to inefficient transfer into the CIS.

Once thermally desorbed from the stir bar, the analytes are transferred to the CIS inlet which is held at low temperature to trap and retain analytes. For injection, the CIS is heated rapidly to transfer analytes to the GC column. The CIS trapping temperature was evaluated at -150, -120, -100, -80, and -50 °C. There was no observable difference in analyte response for the trapping temperatures at and below -100ºC. Temperatures above -100ºC were not as effective at trapping the more volatile compounds as shown in Figure S2, therefore -100°C was chosen to achieve the best analyte trapping for the broad volatility range and use the least amount of cryogenic coolant.

To the best of the author's knowledge, this is the first study to use TDU liquid injection for the automated addition of IS into the sample tubes prior to instrument analysis. In other studies, the IS was added into the sample matrix before spinning the stir bar. In this case, the IS is accounting for variation from the sample extraction process, such as poor extraction efficiency and surface adsorption. With GC-MS quantification (and in this case GC×GC-TOFMS quantification), it is best practice to also use a separate IS to account for instrument variability that should be added at the same concentration to all samples immediately before analysis.[28] In this study, surrogate compounds were added to the sample matrix prior to extraction in order to calculate the SBSE efficiency and the IS was spiked into the TDU tubes prior to instrument analysis to account for chromatographic and mass spectrometer variance. To test the precision of the IS addition method, the MPS was used to add the IS into a TDU tube with glass wool 4 times. The relative standard deviation (RSD) of the IS areas ranged from 6.1-9.9% which is acceptable for a TOF-MS. The RSD of the IS was also low in the calibration samples, ranging from 4.9 – 11.4%. The inter-sample variability is rather high (up to 59%) for some analytes extracted with SBSE, as can

be seen in Table 2. Adding the IS with the liquid injection prevents this extraction variability from having a detrimental effect on the reliability of the quantified data. This method should also allow for more accurate quantification of analytes extracted by SBSE as the instrument variability will be accounted for with the IS.

Expected Limitations and Advantages of Extraction Methods

Both of the extraction methods studied have advantages and limitations when applied to the analysis of CECs in complex environmental samples. The major shortcoming for the PDMS stir bar technique is the limited chemical selectivity range. Even with the addition of salt, SBSE using PDMS may be incapable of extracting very polar compounds, thus leaving out a wide range of analytes potentially present in the sample. LLE with dichloromethane is commonly used because it extracts an acceptable range of both polar and nonpolar analytes, although, more volatile analytes can be lost in subsequent steps to reduce solvent volume. Analyte loss can be prevented with careful lab procedure such as heating at low temperature during Kuderna-Danish concentration and keeping a low flow rate during nitrogen blow down.

Compared to LLE, SBSE may not be selective for as many analytes, but it is more sensitive, with literature values often seen in the low parts per trillion detection range.[29,30] In LLE, the 400 mL starting volume is extracted and concentrated to 1.5 mL, of which 1 μL is analyzed, therefore the concentration factor is approximately 267. With SBSE, the entire contents of the 24 μL PDMS extraction phase are analyzed from the 10 mL starting volume, resulting in a concentration factor of approximately 416, leading most often to the use of SBSE for trace analysis of dilute samples.

Surrogate Recovery

To compare the efficiency of the extraction methods the surrogate recovery rates were calculated for each sample (n = 3 for each sample type). The surrogate mixtures were added to the LLE water samples prior to extraction to be at a final extract concentration of 200 ng/mL, final volume of 1.5 mL. The surrogate mixture was added to the SBSE samples at 20 pg/mL in the 10 mL water sample. The calibration curves for all 14 surrogate compounds (except for 2-Chlorophenol-d4 in the Spring Creek data set) were linear over the range studied, with correlation coefficients greater than 0.98 and relative standard deviation (RSD) values below 25% for the liquid injection samples. In the SBSE calibrations, 8 of the surrogate compounds were calibrated for the influent/effluent samples and 7 were calibrated for the Spring Creek samples. The 4 acid surrogate compounds (2-fluorophenol, phenol-d6, 2-chlorophenol-d4, and 2,4,6-tribromophenol) were not detected in the calibration stir bars due to either their polarity or insufficient transfer during desorption. The loss of recovery for this class of surrogates demonstrates the major problem with PDMS sorbent based SBSE; its selectivity for non-polar compounds and poor extraction of polar compounds. The other non-calibrated surrogates, triphenyl phosphate and tris-(1,3-dichloro isopropyl) phosphate, showed non-linear responses as their RSDs were above 25%. These compounds are relatively non-polar ($\log K_{ow}$ of 4.49 and 3.27 respectively) and they elute at the end of the analysis. Their poor calibration linearity is suspected to be from poor thermal desorption transfer from the stir bar to the TDU and the TDU to the CIS.

The recovery results for the surrogates in samples are listed in Table 2 and the method blank recovery values are in the supplemental Table S1. The recovery values for LLE and SBSE were similar for the surrogate compounds that could be effectively extracted with each method. For the compounds that were amenable to SBSE, the recovery rates ranged from 19% to 117%, with

the median value at 66%. In SBSE method blanks, the detected surrogate compounds with $\log K_{ow}$ >3 all showed recovery greater than 85%, demonstrating the methods efficient extraction for nonpolar compounds. The sample matrix impacted the SBSE recoveries. Every compound, except 2-fluorobiphenyl and nitrobenzene-d5, were recovered at higher levels in both the effluent and Spring Creek samples than the influent samples despite the 1:5 dilution of these samples. The reduction in influent samples analyte recovery is most likely due to competition from the fatty acids and steroid compounds that dominate the influent samples[29]. The recovery for the surrogate nitrobenzene-d5 indicates increased extraction recovery due to the matrix interactions in both of the wastewater samples compared to the spring creek sample where it was not detected.

The LLE recovery values ranged from 19% to 159%, with the median value of 74%. As a group, the acid surrogate mix compounds demonstrated poor recovery, although LLE was able to extract them, unlike SBSE. The poor recovery of these analytes is likely attributed to losses during the concentration steps due to their volatility. In addition, the acid surrogates are also more soluble in water than in dichloromethane. Complex environmental samples often suffer from matrix interaction enhancement effects.[31] This is best demonstrated by the Spring Creek samples, where 7 of the surrogates had recoveries over 100%.

Suspect Screening Analysis for CECs

Complex samples produce large, complex datasets that require a data processing workflow to identify significant features within the samples. This is often accomplished through the following basic steps: initial discovery of peaks (usually thousands per sample), reduction in the number of peaks by removing irrelevant background, solvent, and column bleed, tentative identification of remaining compounds, and confirmation of these identifications.[32] For the discovery of peaks,

the samples were processed and compared to a reference method blank for each sample set. In order for an analyte to be added to the peak list it must be exclusive to the samples or, if it is present in the blank, it's peak area must be over 20% more abundant in the samples than the reference method blank. The number of peaks was further reduced through removing those that were not identified by spectral matches with the NIST library, when match criteria was set to greater than 800 (80% match) on average in both similarity and reverse matching. In suspect screening analysis, the compounds of interest are compared to a list of relevant suspect compounds. In this study, the US EPA Comptox Chemistry Dashboard was utilized and the specific lists that were searched can be found in the supplemental information.

Between the WWTP and Spring Creek samples, a total of 32 suspect analytes were tentatively identified, ranging from pharmaceuticals and personal care products to industrial products and waste. The Venn diagrams in figure 2 compare the identified analytes based on their extraction technique and sample location. For a complete list of analytes, similarity and reverse library match values, and reported functional use see table S.2. The $\log K_{ow}$ values for the suspect analytes range from -0.07 (caffeine) to 5.95 (homosalate). Both LLE and SBSE methods were effective at extracting the majority of analytes in the influent and effluent waters, but SBSE was more effective for the Spring Creek samples. LLE of Spring Creek samples found only 1 compound (cedrol) not detected in SBSE, while SBSE extracted an additional 7 compounds not found in LLE. This is most likely due to the increased sensitivity of SBSE compared to LLE, which is observed in the range of calibration standards. For the liquid samples the low calibration standard was 10 ng/mL. For the stir bar samples, the low calibration standard was 1.0 pg/mL, 10,000 times more sensitive. The WW effluent goes into Spring Creek after tertiary disinfection therefore the contaminants from the spring samples are more dilute. SBSE was able to extract

more of the trace analytes due to the greater concentration factor and sensitivity. The stir bars also performed unexpectedly well over a wide range of polarities, even extracting some of the compounds with low logK_{ow} values such as maltol (log K_{ow} = 0.07), benzothiazole (log K_{ow} = 1.90), and caffeine (log K_{ow} = -0.07). Caffeine was found in both influent and effluent samples using LLE, but only in the influent for SBSE. It is suspected that caffeine in the influent was extractable by SBSE because of its high concentration but it could not compete with more concentrated analytes at the lower concentration in the effluent, therefore it was not detected.

This data also demonstrates the inefficiencies of the WWTP for removing CECs. Only 2 compounds, Thymol and Ibuprofen, were found to be removed to below detection limits from the influent samples after analysis with each method. Out of the 27 suspect analytes identified in the effluent, 13 were also identified in Spring Creek. This is most likely due to the sensitivity of SBSE analysis on the diluted Spring Creek analytes. It was also interesting, though not unexpected, to find the pesticide Atrazine and the herbicide precursor and degradation product 3,4-Dichloro-benzenamine in the Spring Creek water. Central Pennsylvania is an agricultural area and agricultural runoff is common in local streams and rivers. Even more pesticides and herbicides would be expected to be found in the Spring Creek samples with a pesticide specific targeted search.

Because this study utilizes GC×GC-TOFMS with suspect screening analysis, analytes from a wide range of chemical classes and functional use were tentatively identified. Combining comprehensive extraction methods with multidimensional chromatography allows for a more complete analysis of samples compared to targeted methods. All of these analytes have been identified individually in targeted analysis [33–35] but it is uncommon for multiple compound classes to be identified in one study, highlighting the importance of suspect screening analysis.

Conclusion

In this study, two extraction methods were evaluated for the suspect screening analysis of CECs in wastewater influent, effluent, and discharge impacted surface water through GC×GC-TOFMS. Both LLE with dichloromethane and SBSE with PDMS yielded similar recovery results and linearity for the selected surrogates and calibration mix. A new method of SBSE internal standard calibration was developed utilizing the TDU liquid option to add IS directly before chromatographic analysis. This method modification should provide for an analytical benefit as compared to adding the IS to the sample before extraction. SBSE requires some method optimization before use to expand its selectivity range, but it is a more sensitive and greener technique that can also be automated. LLE utilizes a large amount of organic solvent and is time consuming but has a larger range of compound classes that can be extracted. CECs were extracted effectively using both SBSE and LLE of the WW samples, but SBSE extracted a larger number of analytes in both cases. As a result of the higher concentration factor, SBSE was especially advantageous for extracting the trace components in the Spring Creek samples. Because this study utilizes GC×GC-TOFMS with suspect screening analysis, analytes from a wide range of chemical classes and functional use were tentatively identified. Combining comprehensive extraction methods with multidimensional chromatography allows for a more complete analysis of samples compared to targeted methods. All of the 32 suspect analytes have been identified individually in targeted analysis [33–35] but this study highlights the importance of suspect screening analysis to identify more compounds in complex samples.

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Figures and Tables

	TDU	CIS
Initial Temperature (°C)	30	-100
Delay/Equilibrium Time (min)	0.50	0.20
Ramp Rate (°C/min)	720	720
End Temperature (°C)	280	280
Final Hold Time (min)	6.00	5.00

Table 1: TDU desorption and CIS temperature information for the SBSE samples analysis.

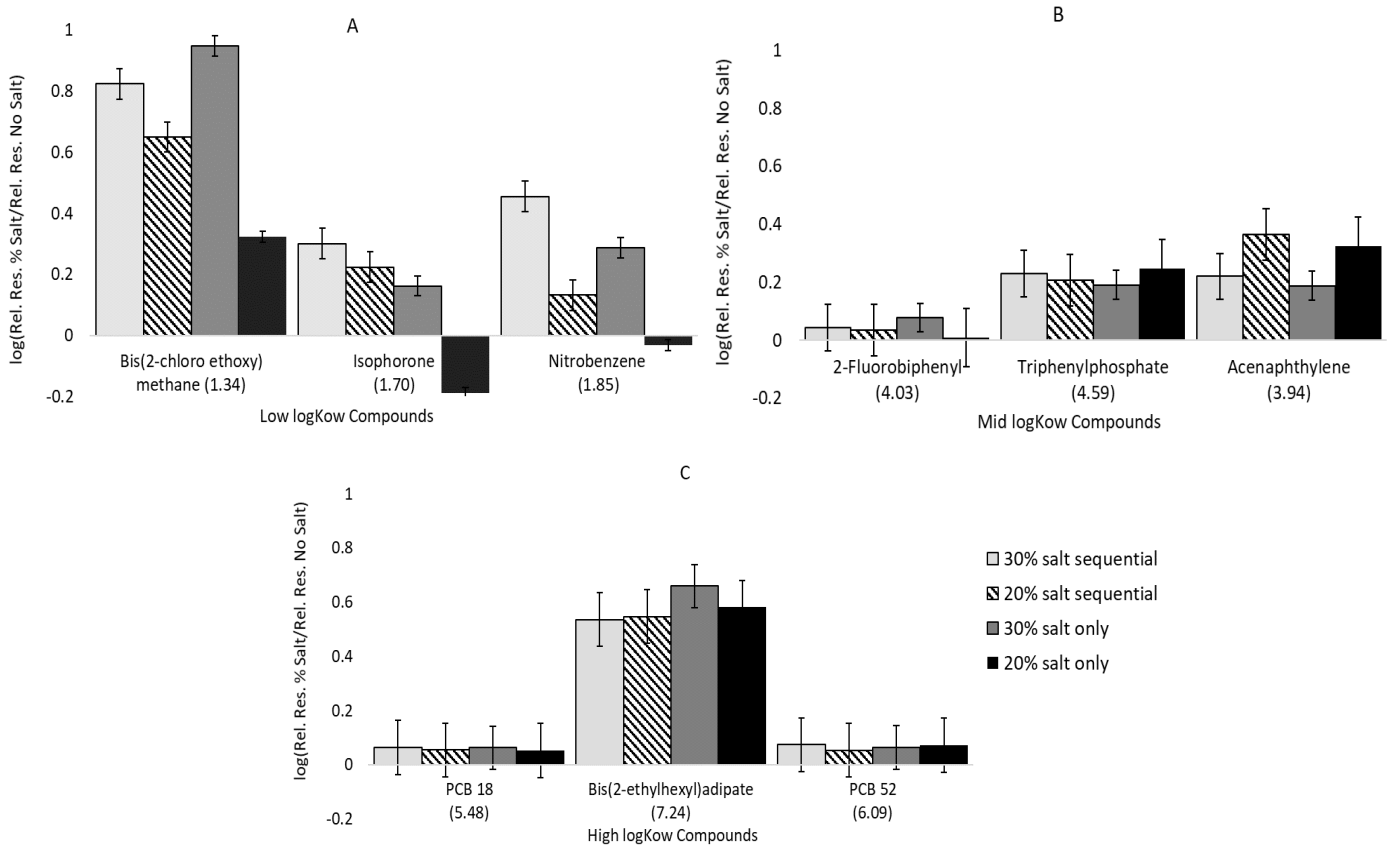

Fig. 1: Relative response of compounds when spun in sample solutions of different salinity logarithmically normalized against the sample with no salt added. A: low $logK_{ow}$ compounds, B: mid $logK_{ow}$ compounds, C: High $logK_{ow}$ compounds. Error bars are standard deviation. $LogK_{ow}$ values are experimental or predicted values from the US EPA Chemistry Dashboard https://comptox.epa.gov/dashboard

	Compound	Cal. Mass (m/z)	log Kow	LLE WW Influent %R±RSD	LLE WW Effluent %R±RSD	LLE Spring Creek %R±RSD	SBSE WW Influent %R±RSD	SBSE WW Effluent %R±RSD	SBSE Spring Creek %R±RSD
Acid Surrogate Standard Mix (3/90 SOW)	2-Fluorophenol	112	1.82	79 ± 11	60 ± 1	35 ± 22	ND	ND	ND
	Phenol-d6	99	1.54	26 ± 5	25 ± 3	106 ± 8	ND	ND	ND
	2-Chlorophenol-d-4	132	2.22	94 ± 22	68 ± 8	64 ± 9	ND	ND	ND
	2,4,6-Tribromophenol	62	4.40	26 ± 32	19 ±12	93 ± 40	ND	ND	ND
Base Neutral Surrogate Standard Mix (3/90 SOW)	1,2- Dichlorobenzene-d4	150	3.44	65 ± 17	58 ± 6	159 ± 14	68 ± 5	72 ± 8	117 ± 1
	2-Fluorobiphenyl	172	4.03	61 ± 21	55 ± 4	65 ± 19	93 ± 6	81 ± 12	90 ± 3
	Nitrobenzene-d5	82	1.82	66 ± 13	66 ± 10	86 ± 22	43 ± 1	39 ± 1	ND
	p-Terphenyl-d14	244	5.51	74 ± 22	79 ± 11	118 ± 10	19 ± 59	34 ± 3	54 ± 9
QuEChERS Internal Standard Mix for GC-MS Analysis	PCB 18	186	5.24	66 ± 19	81 ± 5	101 ± 16	66 ± 37	98 ± 4	102 ± 9
	PCB 28	186	5.72	65 ± 19	75 ± 9	106 ± 13	39 ± 41	61 ± 20	73 ± 7
	PCB 52	220	8.83	67 ± 20	82 ± 14	89 ± 4	44 ± 49	63 ± 15	66 ± 14
	Triphenylmethane	165	5.11	68 ± 19	86 ± 8	95 ± 6	38 ± 43	58 ± 23	76 ± 11
	Triphenyl phosphate	77, 326	4.59	71 ± 9	86 ± 12	103 ± 14	NLR	NLR	NLR
	Tris-(1,3-dichloro isopropyl)phosphate	75, 77	3.27	97 ± 20	112 ± 3	81 ± 7	NLR	NLR	NLR

Table 2: Calibration Mass, LogK_{ow}, % Recovery and % Relative Standard Deviation for the LLE and SBSE samples. %RSD (n=3). ND, non-detected analyte. NLR, non-linear response in calibration (only high concentration response observed).

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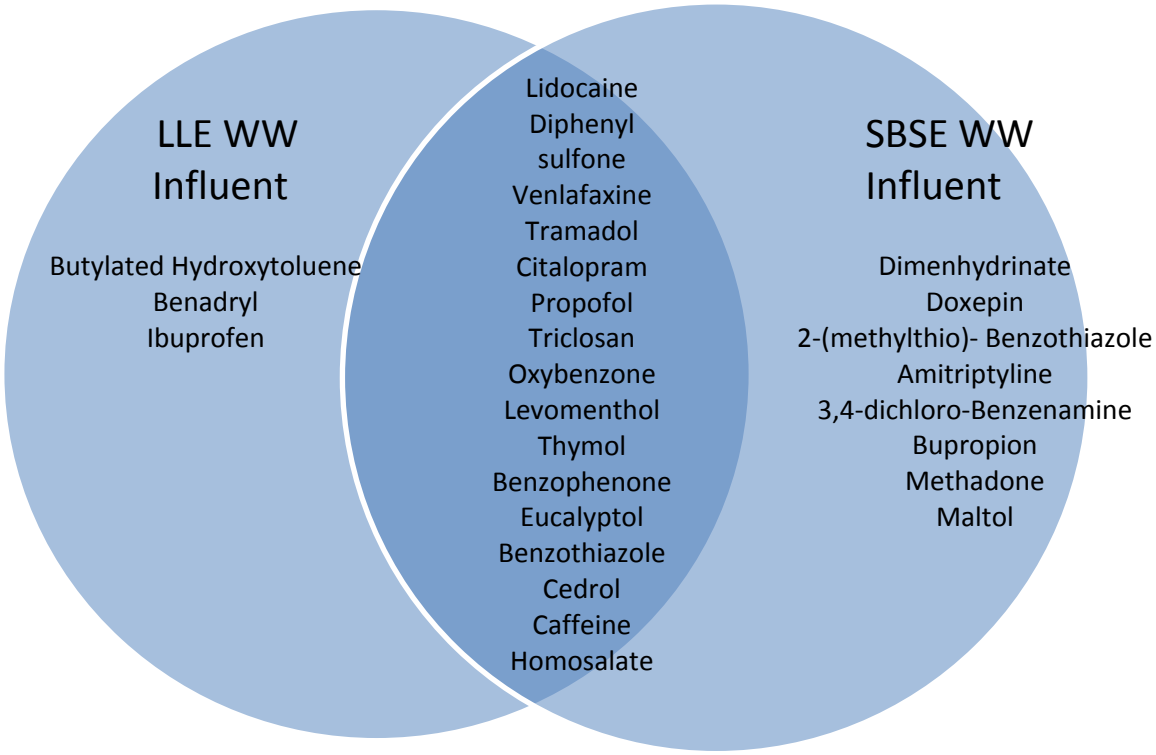
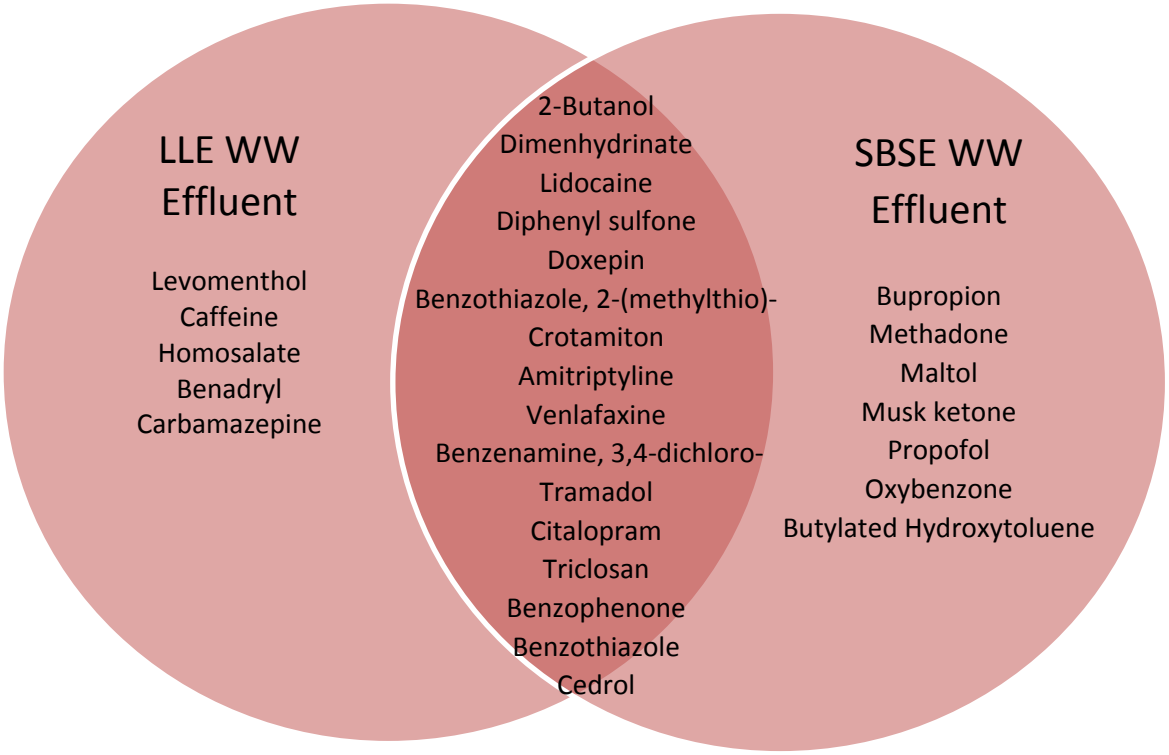
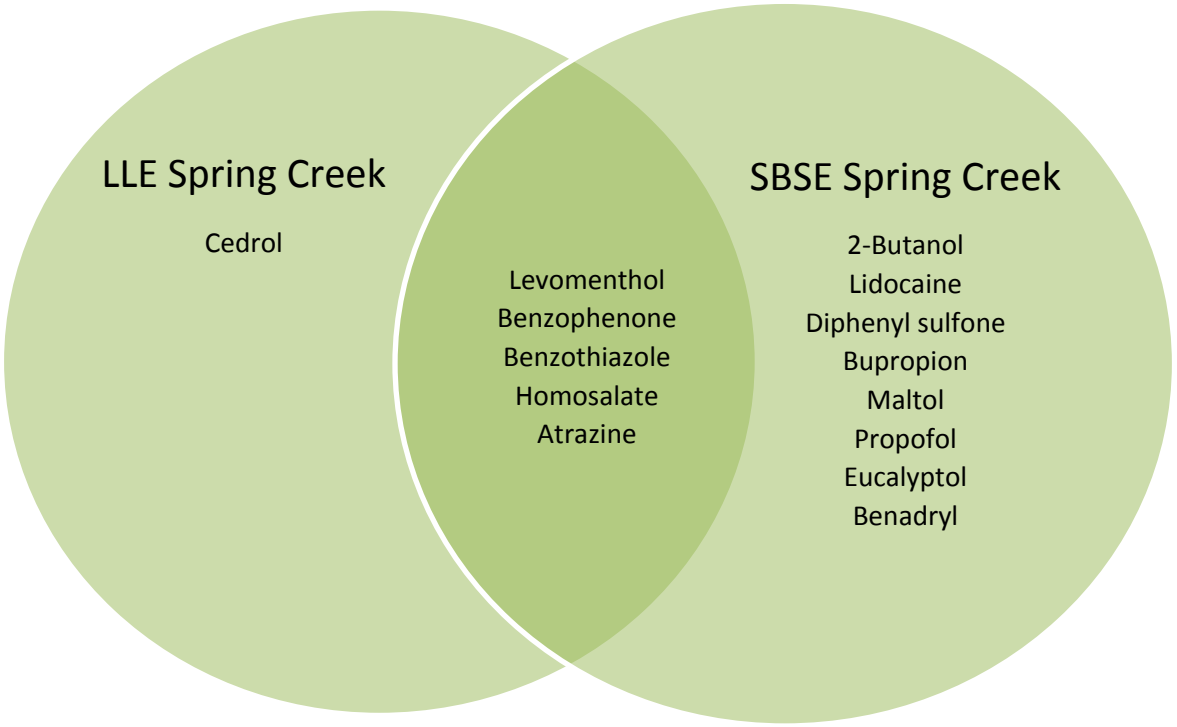
431

Figure 2: Venn diagrams of all sample types showing the differences and commonalities of the analytes extracted with LLE and SBSE.






LLE Spring Creek
Cedrol
Levomenthol
Benzophenone
Benzothiazole
Homosalate
Atrazine
SBSE Spring Creek
2-Butanol
Lidocaine
Diphenyl sulfone
Bupropion
Maltol
Propofol
Eucalyptol
Benadryl

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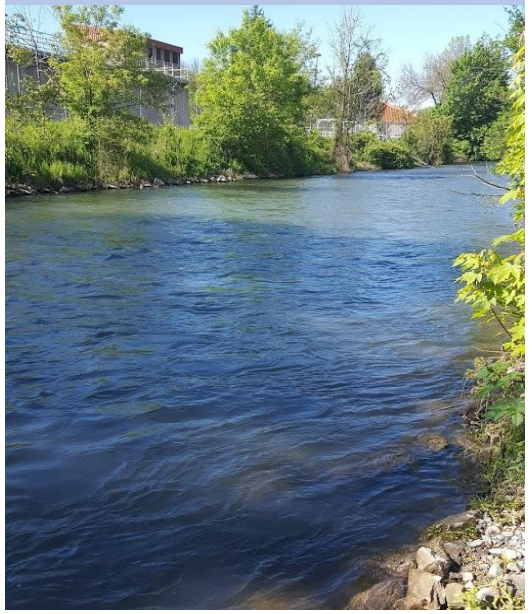
520

Contaminants of Emerging Concern?

Municipal Wastewater Treatment Plant

Receiving Surface Water



Liquid-liquid Extraction

Stir Bar Sorptive Extraction

Comprehensive Two-Dimensional Gas Chromatography