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Can We Re-use "Single-Use" Solid Phase Extraction Cartridges?

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Can We Re-use "Single-Use" Solid Phase Extraction Cartridges?

by

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DEDICATION

I dedicate this thesis to my father for inspiring me as a young girl to understand the "magic" of mathematics and science. Thank you for including me in your late night study sessions filled with blues, dogs, and moments of pure joy when you solved the problem.

I also dedicate this thesis to my mother for teaching me about the natural environment in which I live, and the part I play within it.

Additionally, I dedicate this work to my brother, Jesse, and sister, Emily. I earn this Masters of Science in your name, Jesse, as you have taught me how precious the gift of life is, and how fortunate I am to have been able to achieve this goal because no one is entitled to a tomorrow. Emily, you have shown me what kind patience looks like in the face of tremendous obstacles. I have made it here with you as an inspiration.

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ABSTRACT

Organic and inorganic compounds are present as contaminants in varying concentrations throughout our water cycle. Examples of these contaminants include the endocrine disrupting compounds (EDCs) bisphenol-A (BPA) and 17β-estradiol (E2) from plastics and pharmaceutical use. It can be necessary to obtain the concentration of these compounds within the water cycle for analysis by interested parties such as research groups, regulatory agencies, and private organizations. These concentrations, however, can be too dilute within the initial sample for analysis. Therefore it is necessary to concentrate the compound of interest (analyte) prior to analysis. One such way to do this is by way of Solid Phase Extraction (SPE).

SPE uses a small cartridge which contains chromatographic packing material to chemically extract analytes from a water sample onto a solid phase. To increase concentration, these analytes are then transferred (eluted) to a substantially smaller volume of organic solvent for eventual analyses. These commercially available cartridges are relatively inexpensive, approximately $5 each. However, these cartridges are labeled as single use. In large-scale analyses, this can quickly add up to a sizable percentage of the analysis budget. Additionally, sizable waste volumes can be generated from these analyses in the form of non-degradable polypropylene plastic. If these cartridges can be re-used, material costs as well as waste volumes can be substantially reduced. However, little is known regarding how the quality of analysis degrades with cartridge re-use. The objective of this project is to evaluate the number of times SPE cartridges can be reused without compromising the results of the subsequent analyses.

Based on a review of prior literature, I identified and developed protocols for extracting analytes (BPA and E2) from water via SPE, then analyzing them with gas chromatography and mass spectrometry (GC-MS). These protocols have been developed to mimic those employed by research labs, industry, and other entities for which the results of this study would be most applicable. The only deviation is the re-use of the cartridge rather than disposal and replacement. One type of commercially available SPE cartridge (Oasis HLB, Waters Inc., Milford, MA) was used and two water types were tested. The water was spiked with fixed concentrations of BPA and E2, and then analyzed by way of SPE/GC-MS. For both water types, I performed multiple SPE runs on 10 cartridges each. I tracked the history of GC-MS peak areas, which indicate apparent analyte concentration. Peak area data were analyzed as a function of the number of analyses performed (run number), and evaluated for statistically significant changes as well as overall trends. Statistically significant change and/or trends would indicate that the cartridge had exceeded the maximum allowable number of re-uses and would thereby identify the number of times the "single-use" cartridge can reliably be re-used.

Peak area history for 20 SPE runs per cartridge for pure water samples and 10 SPE runs for wastewater effluent showed no statistically significant changes or trends on peak area. This indicates that cartridges can be re-used at least 10 times without compromising the integrity of water sample analysis for the EDCs considered in this study.

CHAPTER 1: INTRODUCTION

Organic and inorganic compounds are present as contaminants in varying concentrations throughout our water cycle. Some major pollutant types include solvents, heavy metals, salts, minerals, fertilizers and nutrients**,** pesticides, petroleum distillates, pharmaceuticals and personal care compounds (PPCPs), and plasticizers. Heavy metals, salts, and minerals are examples of inorganic pollutants [1]. PPCPs and plasticizers are examples of organic pollutants [2]. Certain pollutants are further classified as Endocrine Disrupting Compounds (EDCs) due to their interference with the body's endocrine system. Two examples of EDCs are the plasticizer bisphenol-A (BPA), used in the manufacturing of plastics, and 17β-estradiol (E2), used in pharmaceutical products [3]. A wide variety of health effects have been linked to these contaminants [4]. For instance, EDCs have been linked to human reproductive abnormalities [5], reduced testosterone production and reduced sperm counts in male rats [6], long-term impacts on intellectual functions in children [7], delayed effects on central nervous system functions in infants [8], as well as skewed gender ratio distribution in crustaceans [9], fish [3], and rats [10].

To protect public health, concentrations of pollutants are evaluated and monitored within the water cycle. This can be done by interested parties such as research groups, regulatory agencies, and private organizations. Typically concentrations of EDCs in water are measured using analytical instruments such as gas chromatography with mass spectrometry (GC-MS) or liquid chromatography with mass spectrometry (LC-MS) [11]. However, concentrations of EDCs can be too dilute within the initial sample for analysis. Therefore, it is necessary to concentrate

the compound of interest (analyte) prior to analysis. One such way to do this is by way of solid-phase extraction (SPE) [11].

SPE uses a chromatographic packing material within the column of a small cartridge which chemically extracts analytes from solution, transferring the analytes from the aqueous phase to the solid phase [12]. These analytes, once concentrated within this cartridge column, can then be eluted by a substantially smaller volume of organic solvent [13]. The net result is a transfer of the analytes from a large volume of aqueous sample at a low concentration to a small volume of organic solvent at a higher concentration. Methods following the SPE process, such as chemical derivatization, can be employed to further prepare the analytes for analysis [13]. The final analysis of the compounds is typically performed using either LC-MS or GC-MS depending on the nature of the chemical [13].

SPE is used to prepare samples for GC-MS or LC-MS analysis in a wide variety of applications. Examples of these applications include: analysis of human plasma [14]; determining E2 concentrations in drinking water [3]; analysis of BPA levels in body fluids and tissues derived from individuals exposed to the wastewater of polycarbonate plastic production [15].

A central component to the SPE method is the cartridge. This commercially available device is relatively inexpensive, approximately $5 to $10 each. However, these cartridges are labeled as single use. For instance, the SPE cartridge manufacturer Waters lists on their website (http:// http://www.waters.com) “these SPE cartridges are intended for single use only”. In large-scale analyses, this can quickly add up to an appreciable cost. Additionally, sizable waste volumes can be generated from these analyses in the form of non-degradable polypropylene plastic.

Large-scale analyses using SPE have associated large costs and large waste volumes. Following are two hypothetical scenarios in which SPE is used. Both of these scenarios were based on real data provided to me by two US water laboratories (asking to remain anonymous) from within the industry. For example, take a large regional laboratory. They have good equipment, well-trained staff, and an efficient SPE system so unnecessary cartridge waste is minimized. They may use approximately 1,500 cartridges per year and pay $5 per cartridge. This translates to approximately $7,500 total cost and 1,500 cartridges disposed of per year. Now take a small mobile laboratory. Their SPE Standard Operating Procedure is a little out of date and inefficient, so for every cartridge used there is one extra cartridge wasted. This laboratory tests between 250-500 samples per year. Because of the extra cartridge waste they, instead, use 500-1,000 cartridges per year. Due to the laboratory's small size, they pay more for their cartridges; approximately $15/ ea. Therefore this small scale laboratory, per year, is spending $7,500-$15,000 on SPE cartridges and throwing away 500-1000 cartridges.

If these cartridges can be re-used without sacrificing or compromising the reliability of the analysis, material costs as well as waste volumes can be substantially reduced. However, little is known regarding how the quality of analysis degrades with cartridge re-use. One study found that SPE disks (similar to SPE cartridges) could be used four times in the preparation of samples for analysis of pyrethroid pesticides by gas chromatography with electron capture detection (GC-ECD) [16]. However, reuse analysis was limited to no more than four times; any reuse above four times was not investigated [16]. Additionally, the study was limited to one type of analyte (pyrethroid), one type of SPE medium (C18 disks), and one type of water [16]. To the best of my knowledge, this is the only study that has examined the potential for re-use of SPE devices.

Therefore, the overall objective of this thesis is to determine how many times selected SPE cartridges can be reused to prepare samples for chemical analysis. Specifically, I used Oasis HLB ® cartridges (Waters Inc., Milford, MA) to prepare two types of water samples (purified water and final treated wastewater from a wastewater treatment plant) spiked with known concentrations of BPA and E2. I evaluated the water samples using GC-MS and looked for statistically significant deviations in the results in order to determine if the cartridges can be re-used and, if so, how many times. For each water type, results were compared based on cartridge run number for the two analytes and control standard. The successful completion of this project will enable laboratories to simultaneously provide high quality data while reducing waste and saving money.

CHAPTER 2: MATERIALS AND METHODS

2.1 Overview

In order to address the objective of this paper, which is to determine the reusability of the SPE cartridge, single-use cartridges were used and reused under application of a standard SPE procedure. I used 10 cartridges for spiked purified water, running each 20 times. I also used 10 cartridges for spiked treated wastewater, running each 10 times. Samples obtained from this SPE procedure were analyzed using GC-MS, which produced chromatograms with peak areas representing analyte concentrations. The peak areas were evaluated for any statistically significant deviation between reuse. The point at which values were found to significantly deviate indicated when a cartridge began showing signs of failure.

2.2 Materials

2.2.1 SPE Cartridges

This analysis uses a 150 mg HLB SPE cartridge with a barrel volume of 6 mL. The manufacturer was Oasis (Milford, MA) with a part number 186003365.

2.2.2 Chemicals

This analysis used the chemicals listed in Table 2.1 for stock preparation, sample preparation, and analysis. Figure 2.1 is the chemical structure of BPA and Figure 2.2 is the chemical structure of E2.

Table 2.1: Chemicals used in this analysis

Chemical	Purity (%)	Manufacture	Location	Part Number
Bisphenol A (BPA)	99	Sigma Aldrich	St. Louis, MO	239658-50G
17β Estradiol (E2)	98	Sigma Aldrich	St. Louis, MO	E8875-250MG
4-Nonylphenol	99	Acros	Geel, Belgium	416240010
BSTFA-TMCS*	n/a	Fluka	Metropolis,IL	15238-5ML
Methanol	99.9	Fisher	Waltham, MA	A412-4
Nitrogen gas	99.5	Airgas	Tampa, FL	NI300

*N,O-bis(Trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane

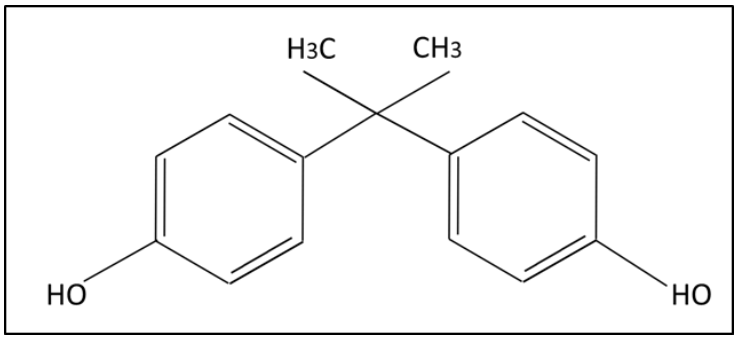

Figure 2.1: Chemical structure of bisphenol A

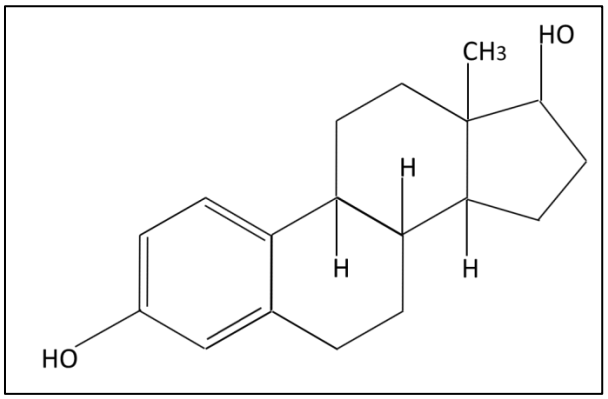

Figure 2.2: Chemical structure of estradiol

2.2.3 Water Tested

This analysis used deionized water and wastewater effluent. The water was prepared as described in the following two sections.

2.2.4 Purified/Deionized (DI) Water

The Environmental Engineering laboratory at the University of South Florida (USF) has access to deionized water, purified on-site as follows. Raw tap water initially hits a 1 μm filter. It

is then run through activated carbon tanks for chlorine and organic removal. It then goes through a mixed bed deionization tank to remove ions, then through a mixed bed deionization polishing tank. Following, it hits a 50.8 cm 0.2 μm filter for bacterial control. Finally, UV light (200 nm wavelength lamp) is used for any remaining bacterial control. The system operates in a loop, travelling from the purification system, out into the laboratory to the DI tap where DI water can be dispensed, and any unused DI water is sent back to the purification system to be treated again. The system is maintained by Purification Technologies.

Table 2.2: Components of the DI purification system

Component	Manufacturer	Location	Part Number
Mixed bed deionization tank	Structural	Milwaukee, WI	1047200811060120
Carbon filter tank	Structural	Milwaukee, WI	CH30546-10010102-10
Micro-filter (rating 0.2, 20" length)	Global	Port Washington, NY	GHPS0.2A20C16S
UV Water Purifier	Mighty Pure	Hauppauge, NY	MP36C

2.2.5 Wastewater Effluent

Wastewater effluent was collected from the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, Florida. The treated wastewater was collected after the denitrification filtration process and before disinfection. Directly prior to sample preparation, the treated wastewater was filtered using 0.22μm membrane filters (Millipore; item # GVWP04700).

2.3 Stock and Sample Preparation Method

An initial stock solution containing the analytes must be created prior to any SPE runs. All samples used in the SPE runs are prepared using this stock solution so as to maintain consistent initial analyte concentrations throughout the data set. Procedures to create this stock solution can be found in Table 2.3a and procedures to prepare the water samples can be found in Table 2.3b. The SPE method in section 2.4 discusses the use of an internal standard. The procedure to create the internal standard stock solution can be found in Table 2.3c.

Table 2.3a: BPA and E2 stock solution preparation

Solution	Soution Type	Materials	Method
BPA and E2 stock	Organic	1. Bisphenol A (BPA) 2. 17β Estradiol (E2) 3. Methanol 4. Scale	1. Mix 0.02 grams each of BPA and E2 into 200ml of methanol 2. Place on shaker table at level 5 for 15 minutes 3. Store in freezer

Table 2.3b: Aqueous sample preparation

Solution	Soution Type	Materials	Method
Aqueous sample preperation	Aqueous	1. BPA and E2 stock solution 2. Water type of choice (i.e. DI water, treated wastewater, etc. 3. Syringe	1. Remove BPA and E2 stock solution from freezer 2. Place on shaker table at level 5 for 15 minutes 3. Mix 10µl BPA and E2 stock solution with 1L water using syringe

Table 2.3c: Internal standard stock solution preparation

Solution	Soution Type	Materials	Method
NP Internal Standard	Aqueous	1. Nonylphenol (NP) 2. Methanol 3. Either a scale for solid NP or syringe for liquid NP	1. Mix 0.02 grams or 22µl nonylphenol into 200ml of methanol 2. Place on shaker table at level 5 for 15 minutes 3. Store in freezer

2.4 SPE Method

The SPE procedure employed in this study was designed to mimic as closely as possible that which may be found in a typical water quality or commercial lab in order to increase the relevance and applicability of this study's results. Method steps were compiled by way of an extensive literature review [11,12,16–25], consultation with field experts, and interviews with commercial lab managers as well as a review of their SPE lab procedure documents. Table 2.4 gives a breakdown of each method step, and its objectives.

Table 2.4: SPE method steps

Step	Method	Step Objective
1. Conditioning/ Equilibrate (not flow rate sensitive)	Pull 5 mL of methanol three times and then 5 mL of DI through cartridge using Büchner flask under mild vacuum	***Conditioning Objective***: To moisten pores in silica packing material in filter column with organic solvent so as to change silica pores from hydrophobic to hydrophilic and allow analyte capture. 99% of chromatographic surface is inside pores. When dry, the silica particle has a hydrophobic ligand which will not allow analyte into pore for capture.
		Equilibrate Objective: To fill pores with water, allowing sample in following steps to penetrate pores
2. Loading (flow rate sensitive)	Pull 0.5 L of 1 µg/L aqueous sample through cartridge at low flow volume using Büchner flask. 1 mL per minute is typical flow rate.	To transfer compounds from the sample to the cartridge. Note: compounds are not solids; this is not a TSS filtration process. Compounds are retained in cartridge by chemical interactions between the sorbent and the compounds.
3. Washing (flow rate sensitive)	Rinse cartridge 1 time with 5 mL of DI water using Büchner flask. 1 mL per minute is typical flow rate.	To wash off potential interferences and to remain consistent with well-established SPE method

Table 2.4 (Continued)

Step	Method	Step Objective
4. Elution (flow rate sensitive)	Pull 5 mL methanol through cartridge using vacuum manifold to a separate container. Container size should be compatible with evaporation apparatus. 1 mL per minute is typical flow rate.	***Objective 1:*** To move analytes from solid phase to organic phase, facilitating subsequent steps ***Objective 2:*** To transfer compounds to a smaller volume of fluid, thus increasing concentration by a factor of 100.
5. Internal Standard	Spike eluent with 5 µL of nonylphenol (NP) stock solution (22 µL NP: 200 mL methanol)	This controlled amount of NP, added after the SPE process phase, is used in the analysis phase to check for potential SPE system errors and normalize peak area obtained from GC-MS
6. Evaporation	Place eluent and its container in evaporation apparatus and evaporate off organic solvent until just a residue remains (evaporate to dryness)	To isolate just the compounds by removing them from the eluent
7. Derivatization	Add 100 µL of derivatization agent BSTFA-TMCS to dry sample and then placing in oven at 65°C for 25 min	The derivatization step, by way of silylation, makes analytes more volatile and easier to detect using the GC-MS

Table 2.4 (Continued)

Step	Method	Step Objective
8. Analysis	Inject 1 µL of sample into GC-MS within 30 minutes of derivatization	To analyze derivatized concentrations of analytes in sample

2.5 GC-MS Method

A PerkinElmer (Boston, MA) GC (Clarus 580) and MS (Clarus 560D) was used in this analysis. The software used was Turbo Mass.

Prior to data acquisition, the scan mode was set using the Turbo Mass software program to the following parameters:

- The inlet line temperature of the GC/MS was set at 170 °C
- The manifold source temperature was set at 160 °C
- The oven temperature was set at maximum temperature of 280 °C
- The electron emission current of GC/MS was 10 µA (70 eV)
- Multiplier voltage was 366 V

Data acquisition was performed in split scan mode measuring the following three compounds and their associated GC-MS specific fragment value (m/z) and typical elution times:

Table 2.5: Analytes with fragment value and typical elution times

Analyte	(m/z)	Typical Elution Times (min)
NP	313	12.0
BPA	357	13.5
E2	416	18.0

The GC oven temperature step-up program was:

- Hold the oven temperature for 1 min at 80°C
- Increase the temperature by 15°C/min to 240°C

- Hold for 1 min
- Increase the temperature by 10°C/min to 280°C
- Hold for 10 min.

The total run time for one sample to complete analysis in the GC-MS was programmed at 26.67 minutes. See Figure 2.3 for an example chromatogram for BPA.

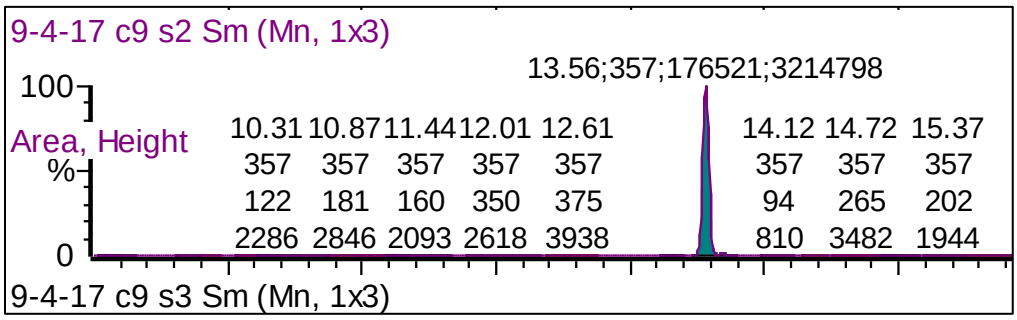

Figure 2.3: Example chromatogram for BPA. 13.56 is the elution time, 357 is the fragment, and 176,521 is the chromatogram peak area.

2.6 Testing and Data Analysis Procedure for SPE Cartridges

In order to obtain high quality data, efforts to reduce interferences from glassware were found to be a critical component to this study. When not in use during analysis, all glassware was kept in a 10% HCl acid bath. Syringes were washed throughout, and after, every sample preparation using methanol.

Samples were prepared (see Section 2.3) using the SPE method (see Section 2.4) and analyzed using a GC-MS (see Section 2.5). Chromatograms with peak areas were obtained for each analyte per sample run using the GC-MS (see figure 2.3 for example).

Chromatogram peak areas were collected for each sample. See Appendix A, Table A.1a, Table A.1b, and Figure A.1 for examples. 10 cartridges were used per water type. Peak area values for BPA and E2 were normalized by dividing the values by the areas of the internal standard peak area. DI water cartridges were run 20 times each. Treated wastewater cartridges were run 10 times each. Peak areas were evaluated and averaged across sample run number. For

example, BPA peak areas for sample 3 across all 10 treated wastewater cartridges were evaluated together for an average, sample standard deviation, and outliers. The average area for each run number was then plotted and evaluated for any observable trends up or trends down to indicate the cartridge had possibly exceeded the maximum allowable number of re-uses.

Prior to evaluating the full data set, outliers were identified and removed using the Interquartile Range[26]. In order to do this, all peak areas (entire data set for NP, and per run for BPA and E2) for a specific water type were collected together and ordered from smallest to largest, then separated in half. The median of the smallest half is called the lower fourth. The median of the upper half is the upper fourth. A measure of spread that is resistant to outliers, the Fourth Spread (f_s), is calculated as such:

f_s= upper fourth – lower fourth

An observation farther than 1.5 times the Fourth Spread ($1.5*f_s$) from the closest fourth (lower or upper) is considered an outlier. Peak area values that were identified as outliers using this method were not included in the analysis.

The two-sided t-test with a 95% confidence was used to evaluate statistical significant variation between Run 1 and Run j (j=2…10 for DI water and j=2…20 for treated wastewater) [26]. The null hypothesis for this test stated: "Any difference in the average peak area between single use and "n" use is due purely to "noise" and does not indicate a change in the cartridge." A t-statistic was calculated for each run number and compared to its critical value [26]. Critical values can be looked up in any standard critical value t distribution table. t-Static values that exceeded the associated critical value indicated the failure of the null hypothesis. This failure was used to represent a "breaking point" which indicated the possibility that the cartridge was no longer re-usable.

CHAPTER 3: RESULTS

20 samples per cartridge for deionized water, and 10 samples per cartridge for treated wastewater, were collected and analyzed. The following are the final plots per analyte for each water type:

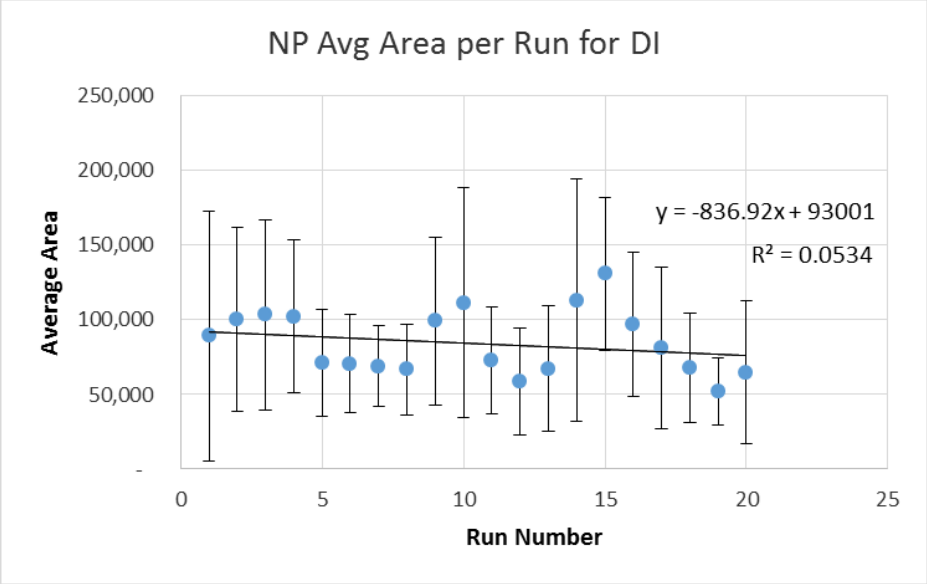

Figure 3.1: NP average peak areas per sample number for DI water

Table 3.1: All nonylphenol peak areas collected from DI water sample run 1 through 20 for cartridge 1 through 10 with outliers removed. Removed outliers are represented by an X. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	NP Outliers Excluded											
Run	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	Average	StDev
1	177,792	234,778	20,709	52,447	53,427	66,962	X	X	17,950	X	8.92E+04	8.36E+04
2	133,056	191,180	47,208	51,486	20,953	113,746	X	X	141,385	X	9.99E+04	6.15E+04
3	X	111,330	49,555	44,821	23,603	127,284	X	215,546	151,388	101,820	1.03E+05	6.35E+04
4	49,251	41,905	156,918	106,843	72,506	132,377	X	97,283	66,175	193,868	1.02E+05	5.12E+04
5	78,164	-	61,895	83,667	33,612	55,100	99,992	X	114,072	41,212	7.10E+04	3.54E+04
6	68,736	38,731	61,895	95,435	67,213	59,287	145,695	X	52,992	43,841	7.04E+04	3.26E+04
7	61,529	65,447	X	120,525	45,340	28,004	96,335	66,167	58,952	75,273	6.86E+04	2.71E+04
8	64,710	57,301	136,982	74,563	30,292	50,323	93,114	62,803	38,056	57,991	6.66E+04	3.04E+04
9	112,588	99,665	86,541	84,972	37,016	63,288	219,396	166,127	40,984	79,301	9.90E+04	5.62E+04
10	107,521	85,637	66,671	-	54,697	74,816	234,041	234,041	42,800	99,674	1.11E+05	7.69E+04
11	49,337	58,809	109,611	-	80,825	67,270	110,940	101,042	39,842	38,400	7.29E+04	3.57E+04
12	62,821	88,995	99,276	36,334	-	85,487	75,970	28,416	2,574	46,723	5.85E+04	3.56E+04
13	55,634	74,105	46,480	78,523	34,162	100,956	147,458	21,136	45,155	-	6.71E+04	4.23E+04
14	54,901	104,047	49,048	242,118	74,900	250,787	180,784	48,840	43,219	79,125	1.13E+05	8.12E+04
15	136,510	157,723	120,631	111,621	17,789	159,488	X	X	185,547	155,025	1.31E+05	5.13E+04
16	56,542	141,619	164,471	90,948	43,179	141,250	X	X	40,612	96,642	9.69E+04	4.82E+04
17	56,499	157,938	93,120	-	30,409	67,358	X	32,719	54,861	152,285	8.06E+04	5.40E+04
18	89,583	115,124	36,010	90,656	32,067	76,968	X	19,408	111,998	36,341	6.76E+04	3.69E+04
19	71,185	82,878	32,458	32,575	31,446	39,234	60,829	43,498	35,081	90,540	5.20E+04	2.26E+04
20	-	100,202	52,971	50,624	92	76,229	25,006	68,343	47,948	159,765	6.46E+04	4.78E+04

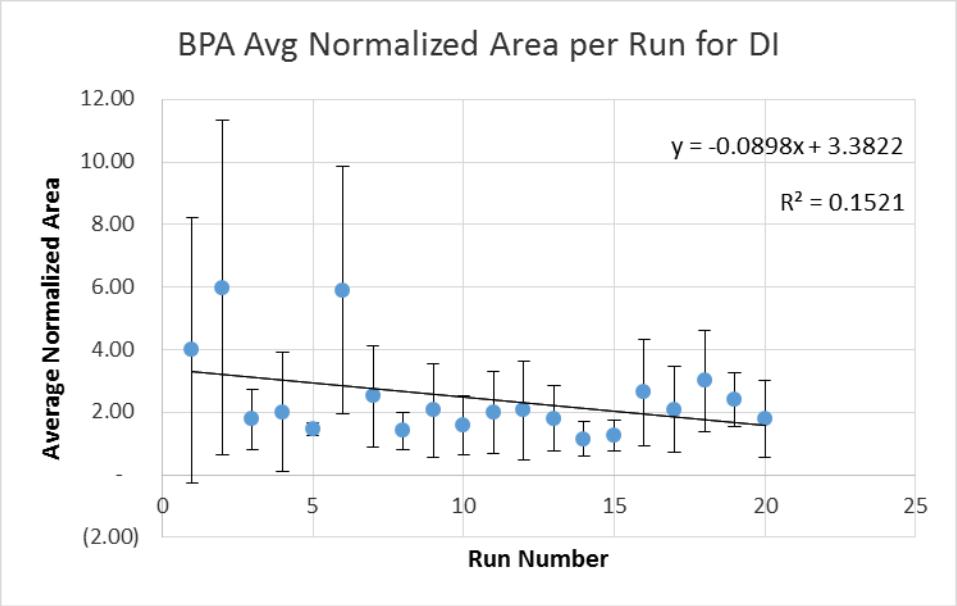

Figure 3.2: BPA average normalized peak areas per sample number for DI water

Table 3.2a: All bisphenol peak areas collected from DI water, sample run 1 through 20 for cartridge 1 through 10 with outliers removed. Removed outliers are represented by an X. Because nonylphenol is the internal standard and is used to normalize the EDC peak areas, all non-outlier BPA peak areas were also removed if the nonylphenol peak area from the same cartridge number and run number was an outlier. BPA runs with an associated NP outlier are highlighted in yellow. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	BPA Ratios Outliers Excluded											
Run	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	Average	StDev
1	1.31	0.82	2.02	1.67	7.98	2.22		X	11.85		3.98	4.23
2	1.13	0.83	3.79	12.06	10.70	12.03			1.33		5.98	5.36
3		1.77	3.24	2.54	X	1.15		0.22	1.51	1.94	1.77	0.97
4	X	5.12	0.54	0.72	4.71	0.62		0.66	2.40	1.17	1.99	1.90
5	1.73	X	1.49	1.70	1.24	1.60	1.26	X	1.24	1.53	1.47	0.20
6	13.86	8.50	2.80	X	6.73	5.69	1.10		3.64	4.90	5.90	3.96
7	2.82	2.00		4.71	1.72	X	1.46	1.60	5.16	0.65	2.51	1.61
8	2.13	1.20	0.76	X	1.47	2.65	1.09	1.11	1.17	1.07	1.40	0.60
9	1.60	1.30	1.15	0.80	4.97	3.37	1.02	0.66	3.90	1.82	2.06	1.49
10	1.50	1.69	2.12	-	2.29	3.21	1.29	0.72	0.38	1.01	1.58	0.96
11	4.24	2.83	1.17	-	1.16	2.70	1.83	1.14	0.20	2.70	2.00	1.32
12	2.59	1.18	1.14	5.03	-	1.57	-	0.57	-	2.31	2.06	1.57
13	1.72	0.58	2.15	1.36	X	0.91	1.52	2.90	3.24	-	1.80	1.05
14	0.45	1.07	1.55	0.66	2.23	0.59	1.02	X	1.45	1.25	1.14	0.56
15	1.08	2.11	1.31	1.71	X	0.68			1.03	0.82	1.25	0.51
16	2.82	4.73	0.57	3.27	4.67	0.61			3.33	1.05	2.63	1.70
17	3.04	1.00	1.01	-	4.27	X		2.13	2.16	1.00	2.09	1.36
18	3.25	1.80	4.45	0.72	5.23	5.06		2.36	1.33	2.82	3.00	1.63
19	X	1.51	3.27	2.04	2.81	3.41	1.84	1.68	3.58	1.47	2.40	0.86
20	-	0.25	3.41	1.06	X	3.00	1.41	2.30	2.32	0.49	1.78	1.23

Table 3.2b: Average bisphenol peak areas per run, collected from DI water, and associated t-statistic.

Run	Average	Critical value ($t_{0.025,v}$)	2 sided t-statistic
1	3.98		
2	5.98	2.201	0.774
3	1.77	2.447	1.351
4	1.99	2.306	1.147
5	1.47	2.447	1.569
6	5.90	2.179	0.903
7	2.51	2.365	0.866
8	1.40	2.447	1.601
9	2.06	2.365	1.154
10	1.58	2.447	1.478
11	2.00	2.447	1.202
12	2.06	2.365	1.152
13	1.80	2.447	1.337
14	1.14	2.447	1.767
15	1.25	2.447	1.701
16	2.63	2.365	0.791
17	2.09	2.365	1.136
18	3.00	2.365	0.581
19	2.40	2.447	0.974
20	1.78	2.447	1.335

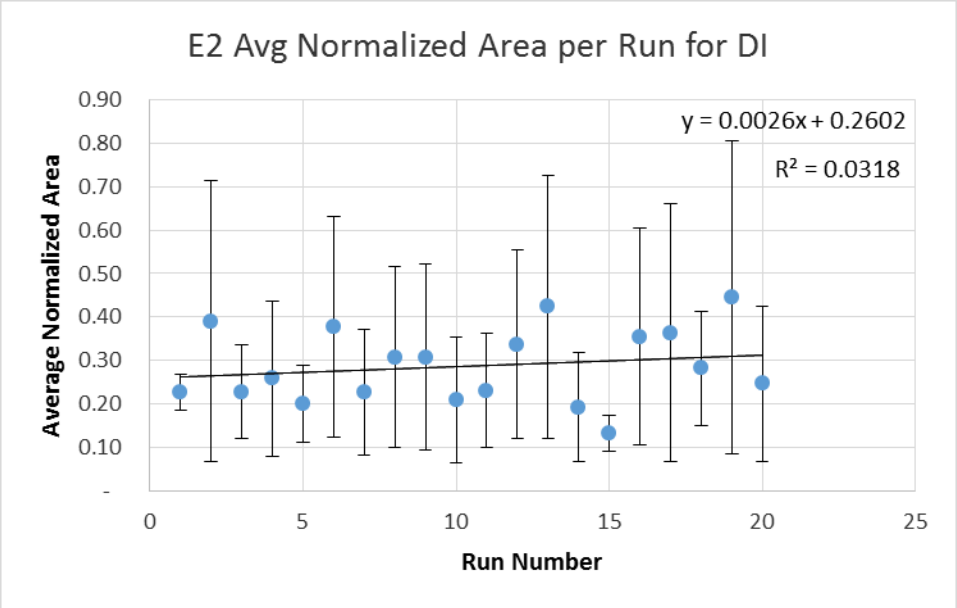

Figure 3.3: E2 average normalized peak areas per sample number for DI water

Table 3.3a: All estradiol peak areas collected from DI water, sample run 1 through 20 for cartridge 1 through 10 with outliers removed. Removed outliers are represented by an X. Because nonylphenol is the internal standard and is used to normalize the EDC peak areas, all non-outlier E2 peak areas were also removed if the nonylphenol peak area from the same cartridge number and run number was an outlier. E2 runs with an associated NP outlier are highlighted in yellow. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	E2 Ratios Outliers Excluded											
Run	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	Average	StDev
1	0.22	0.21	0.24	0.29	X	0.17			X		0.23	0.04
2	0.19	0.12	0.27	0.48	1.01	0.55			0.11		0.39	0.32
3		0.31	0.35	0.25	X	X		0.06	0.14	0.25	0.23	0.11
4	X	0.60	0.10	0.13	0.27	0.46		0.16	0.22	0.14	0.26	0.18
5	0.26	-	0.28	0.15	0.14	0.27	0.14		0.19	0.17	0.20	0.09
6	X	0.60	0.24	0.87	0.40	0.40	0.09		0.21	0.21	0.38	0.25
7	0.39	0.29		0.31	X	X	0.17	0.00	0.35	0.09	0.23	0.14
8	0.30	0.17	0.12	0.67	0.58	0.31	0.14	0.52	0.14	0.15	0.31	0.21
9	0.22	0.64	0.19	0.14	0.65	0.51	0.10	0.11	0.31	0.21	0.31	0.21
10	0.23	0.38	0.11	-	0.31	0.43	0.16	0.12	0.03	0.12	0.21	0.14
11	X	0.33	0.19	-	0.32	0.35	0.26	0.11	0.03	0.26	0.23	0.13
12	0.42	0.13	0.19	0.65	-	0.24	0.32	0.14	0.66	0.29	0.34	0.22
13	0.21	0.68	0.37	0.20	1.01	0.13	0.20	0.55	0.44	-	0.42	0.30
14	0.08	X	0.21	0.09	0.28	0.07	0.14	0.47	0.20	0.19	0.19	0.13
15	0.17	0.14	0.16	0.16	X	0.07			0.16	0.07	0.13	0.04
16	0.40	0.76	0.08	0.26	0.61	0.12			0.48	0.13	0.35	0.25
17	0.46	0.14	0.17	-	0.56	0.96		0.24	0.28	0.10	0.36	0.30
18	0.18	0.25	0.43	0.09	0.49	0.33		0.33	0.14	0.29	0.28	0.13
19	0.85	0.06	0.35	1.20	0.37	X	0.23	0.27	0.48	0.20	0.45	0.36
20	-	0.02	0.28	0.51	X	X	0.20	0.36	0.29	0.06	0.25	0.18

Table 3.3b: Average estradiol peak areas per run, collected from DI water, and associated t-statistic. t-Statistic values which exceed the associated critical value have been highlighted in yellow.

Run	Average	Critical value ($t_{0.025,v}$)	2 sided t-statistic
1	0.23		
2	0.39	2.447	1.335
3	0.23	2.447	0.054
4	0.26	2.306	0.502
5	0.20	2.201	0.718
6	0.38	2.365	1.656
7	0.23	2.365	0.028
8	0.31	2.23	1.205
9	0.31	2.23	1.169
10	0.21	2.20	0.326
11	0.23	2.23	0.115
12	0.34	2.23	1.570
13	0.42	2.26	2.032
14	0.19	2.23	0.723
15	0.13	2.306	3.857
16	0.35	2.365	1.422
17	0.36	2.306	1.370
18	0.28	2.23	1.202
19	0.45	2.306	1.813
20	0.25	2.306	0.311

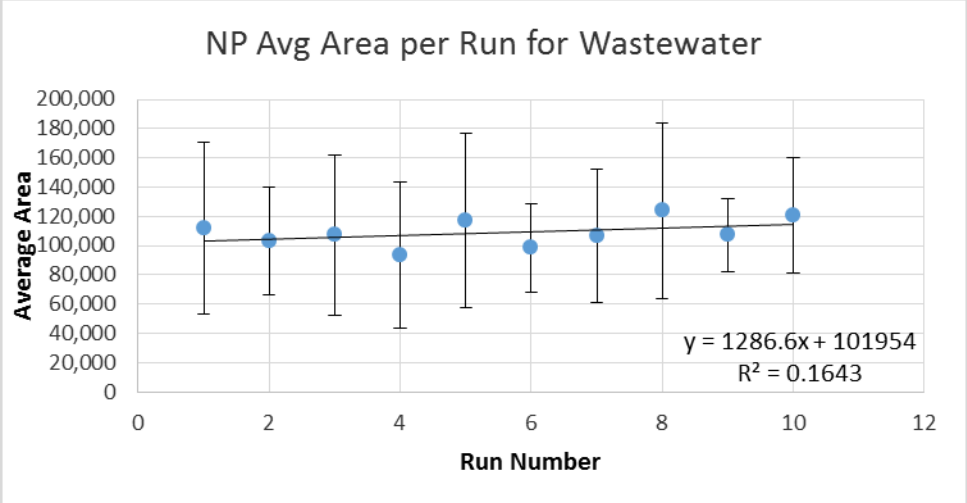

Figure 3.4: NP average peak areas per sample number for treated wastewater

Table 3.4: All nonylphenol peak areas collected from treated wastewater sample run 1 through 10 for cartridge 11 through 20 with outliers removed. Removed outliers are represented by an X. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	NP Outliers Excluded											
Run	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	Average	StDev
1	211,659	200,002	105,911	135,982	79,627	131,424	50,040	88,791	32,803	85,118	1.12E+05	5.88E+04
2	138,152	X	145,051	112,295	133,460	127,939	64,428	41,775	85,780	79,664	1.03E+05	3.66E+04
3	137,158	223,102	57,057	91,871	163,217	115,253	37,759	68,084	91,001	87,011	1.07E+05	5.51E+04
4	176,018	186,148	62,378	113,197	78,501	58,284	89,019	55,582	66,203	49,713	9.35E+04	4.98E+04
5	128,050	96,571	97,874	133,672	185,213	144,571	77,389	71,846	-	-	1.17E+05	5.97E+04
6	72,724	118,398	98,911	128,930	138,537	134,489	86,396	83,166	47,651	77,945	9.87E+04	3.03E+04
7	93,276	189,108	104,333	61,774	182,187	108,924	94,962	88,535	49,054	94,161	1.07E+05	4.55E+04
8	154,348	173,394	125,392	132,305	66,383	92,835	120,391	127,287	-	-	1.24E+05	6.00E+04
9	116,522	151,515	137,849	89,399	73,209	91,956	92,022	X	102,887	109,490	1.07E+05	2.49E+04
10	171,916	87,328	82,825	155,109	75,637	91,999	167,231	X	148,102	107,472	1.21E+05	3.92E+04

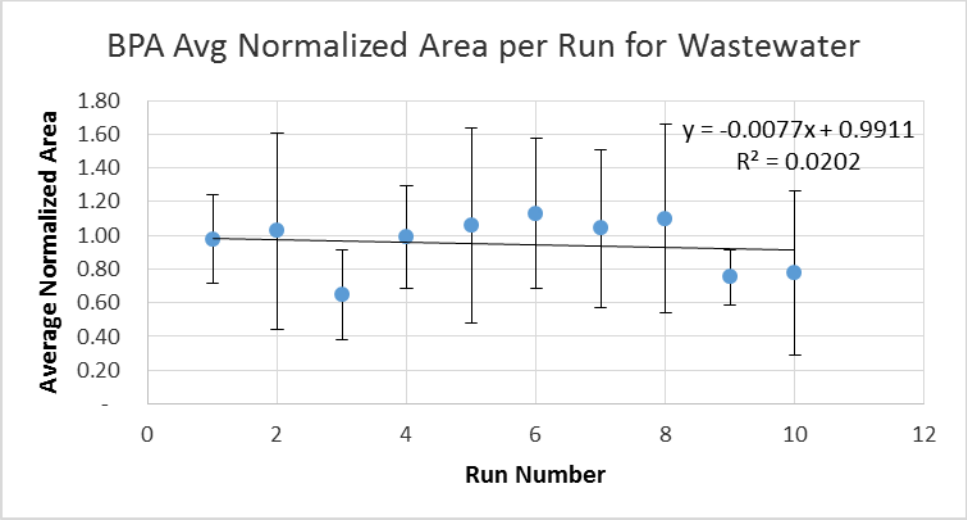

Figure 3.5: BPA average normalized peak areas per sample number for treated wastewater

Table 3.5a: All bisphenol peak areas collected from treated wastewater sample run 1 through 10 for cartridge 11 through 20 with outliers removed. Removed outliers are represented by an X. Because nonylphenol is the internal standard and is used to normalize the EDC peak areas, all non-outlier BPA peak areas were also removed if the nonylphenol peak area from the same cartridge number and run number was an outlier. BPA runs with an associated NP outlier are highlighted in yellow. Removed outliers are represented by an X. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	BPA Ratios Outliers Excluded											
Run	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	Average	StDev
1	0.70	0.76	X	0.90	1.03	0.61	1.39	1.32	1.08	0.99	0.98	0.26
2	0.45		0.48	0.61	0.82	0.82	1.56	1.39	2.06	X	1.03	0.58
3	0.60	0.40	0.32	0.52	0.41	0.95	1.07	0.85	X	0.72	0.65	0.26
4	X	0.57	X	1.04	0.46	1.15	1.21	1.30	1.03	1.13	0.99	0.30
5	0.48	0.73	1.39	X	0.80	1.15	1.50	1.37	-	-	1.06	0.58
6	0.66	1.42	0.70	1.21	0.77	1.16	1.95	1.52	0.58	1.33	1.13	0.45
7	0.59	0.58	0.72	1.15	0.95	1.43	0.59	1.83	1.53	X	1.04	0.47
8	1.02	0.67	0.61	0.89	1.23	1.33	1.46	1.58	-	-	1.10	0.56
9	0.77	0.43	0.96	0.74	0.78	0.96	0.64		0.80	0.69	0.75	0.16
10	0.14	0.84	0.63	0.42	1.86	0.86	1.04		0.49	0.68	0.77	0.49

Table 3.5b: Average BPA peak areas per run, collected from treated wastewater, and associated t-statistic. t-Statistic values which exceed the associated critical value have been highlighted in yellow.

Run	Average	Critical value ($t_{0.025,v}$)	2 sided t-test
1	0.98		
2	1.03	2.262	0.221
3	0.65	2.120	2.632
4	0.99	2.145	0.080
5	1.06	2.201	0.390
6	1.13	2.145	0.915
7	1.04	2.179	0.355
8	1.10	2.160	0.616
9	0.75	2.160	2.187
10	0.77	2.179	1.097

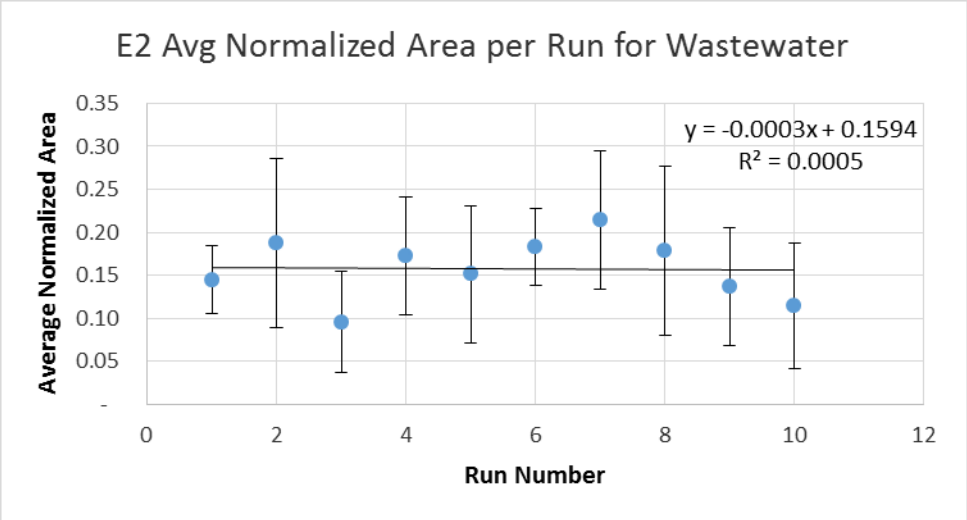

Figure 3.6: E2 average normalized peak areas per sample number for treated wastewater

Table 3.6a: All estradiol peak areas collected from treated wastewater sample run 1 through 10 for cartridge 11 through 20 with outliers removed. Removed outliers are represented by an X. Because nonylphenol is the internal standard and is used to normalize the EDC peak areas, all non-outlier E2 peak areas were also removed if the nonylphenol peak area from the same cartridge number and run number was an outlier. E2 runs with an associated NP outlier are highlighted in yellow. Removed outliers are represented by an X. A dash (-) represents when no data was available (for example, if a test tube fractured while in the oven before an analysis could be performed).

	E2 Ratios Outliers Excluded											
Run	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	Average	StDev
1	0.11	0.14	X	X	0.17	0.10	0.21	0.19	0.11	0.13	0.14	0.04
2	0.08		0.07	0.10	0.15	0.15	0.26	0.24	0.33	0.30	0.19	0.10
3	X	0.06	0.03	0.02	0.07	0.17	0.17	0.13	X	0.12	0.10	0.06
4	X	0.14	0.15	0.08	0.10	0.17	0.20	0.23	0.31	0.16	0.17	0.07
5	0.07	0.14	0.14	0.10	0.13	0.21	0.22	0.21	-	-	0.15	0.08
6	X	0.16	0.23	0.18	0.16	0.20	0.21	0.21	0.08	0.20	0.18	0.04
7	0.14	0.09	X	X	0.16	0.26	0.23	0.28	0.23	0.33	0.21	0.08
8	0.16	0.14	0.09	0.09	0.24	0.20	0.25	0.27	-	-	0.18	0.10
9	0.07	0.08	0.11	0.18	0.11	0.15	0.30		0.13	0.11	0.14	0.07
10	0.01	0.12	0.08	0.04	0.26	0.13	0.16		0.07	0.14	0.11	0.07

Table 3.6b: Average E2 peak areas per run, collected from treated wastewater, and associated t-statistic.

Run	Average	Critical value ($t_{0.025,\nu}$)	2 sided t-test
1	0.14		
2	0.19	2.228	1.20
3	0.10	2.179	1.95
4	0.17	2.160	1.05
5	0.15	2.160	0.23
6	0.18	2.131	1.87
7	0.21	2.228	2.19
8	0.18	2.179	1.00
9	0.14	2.160	0.27
10	0.11	2.179	1.05

CHAPTER 4: DISCUSSION

Though the water samples were prepared with a consistent known concentration of analytes, the results showed variability in the peak area of the chromatograms (representing concentration). Due to the sensitivity of SPE and GC-MS analysis, this is not unusual. BPA contamination from plasticware, contamination from small amounts of residue build-up on equipment, and uneven dried sample residue are all examples of potential sources which can slightly alter the final results.

Peak area results did vary, sometime substantially. Data points deemed to be outliers were removed from the overall dataset (see Chapter 3 for method). Overall, however, no consistent trend up or down indicating steady cartridge degradation was observed. The statistical analysis using the t-test produced some t-statistics which were higher than the associated critical value, resulting in the failure of the null hypothesis and indicating a possible breaking point in the re-use of the cartridge. Because I was using a 95% confidence interval, there was a 5% chance that the null hypothesis would fail. For the most part, the number of times the null hypothesis failed per sample set fell within 5%. BPA in treated wastewater exceeded 5%, but the number of times the null hypothesis failed was not substantially greater than other sample sets so the percentage could be attributed to the small sample size. Additionally, no consistency or logical pattern in these breaking point was observed. For example, a breaking point was observed on run 15 for E2 in DI water. If a failure had occurred, it would be expected that at least a certain percentage of run 15-20 would also fail. This did not occur. Additionally, there was not a

particular run number that consistently failed either per water type, EDC type, or overall. Therefore no cartridge failure was identified.

Based on the results of this study, the single-use Oasis HLB cartridge appears to be reusable for at least 20 purified water samples with no organic matrix, and at least 10 times for filtered wastewater samples containing an organic matrix. As no failure of the cartridge was observed, and no quantifiable maximum number of re-uses was identified, re-use could exceed these values.

CHAPTER 5: SUMMARY, CONCLUSION, AND RECOMMENDATIONS

The results of this study indicate that the SPE single-use Oasis HLB cartridge is, in fact, re-usable to at least 20 re-uses for purified water with no organic matrix, and at least 10 re-uses for filtered water with an organic matrix.

To the best of my knowledge, there is only one published study evaluating the re-use of single-use SPE discs. Therefore there are a number of knowledge gaps which still need to be filled. For example, does the manufacturer and type of SPE cartridge make a difference? Does the water type make a difference? I would recommend more studies be performed using multiple types/ manufacturers of SPE cartridges and discs using water types with a variety of organic matrices. If evaluation is to include a statistical analysis, does using a larger sample size better the approximation (based on the Central Limit Theorem [26]? Does using a larger sample size result in the observation of an eventual cartridge failure?) I would also recommend a studies be carried out until either a trend up/ down, or a breaking point, is identified to indicate quantifiable cartridge failure. Is there a physical/ chemical explanation for an observed failure in a cartridge? It may also be helpful to perform a study which focuses specifically on the materials of the cartridge. For instance, how does the silica of the solid phase behave over multiple cartridges re-uses. Micro-imaging and chemical analysis could offer helpful knowledge as to why a cartridge would or would not fail.

In performing this study, I found contamination prevention was a critical component to SPE, regardless whether or not the cartridge was being re-used or not. Unused glassware was

stored in an acid bath. Equipment such as the injection needle and evaporation apparatus were routinely cleansed with methanol. As I was analyzing for BPA, it was necessary for me to reduce as much plasticware in the study as possible. Otherwise, due to the sensitivity of SPE, plastic exposure led to spiked BPA results.

LIST OF REFERENCES

[1] G. Tchobanoglous, E.D. Schoeder, Water quality : characteristics, modeling, modification, Prentice Hal, 1985.

[2] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, et al., Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance, Environ. Sci. Technol. 36 (2002) 1202–1211. doi:10.1021/es011055j.

[3] H. Kuch, K. Ballschmiter, Determination of Endocrine-Disrupting Phenolic Compounds and Estrogens in Surface and Drinking Water by HRGC-(NCI)-MS in the Picogram per Liter Range, Environ. Sci. Technol. 35 (2001) 3201–3206. doi:10.1021/es010034m.

[4] T. Colborn, F.S. vom Saal, A.M. Soto, Developmental effects of endocrine-disrupting chemicals in wildfire and humans, Environ. Health Perspect. 101 (1993) 378–384. doi:10.1289/ehp.93101378.

[5] C. Casals-Casas, B. Desvergne, Endocrine disruptors: from endocrine to metabolic disruption., Annu. Rev. Physiol. 73 (2011) 135–162. doi:10.1146/annurev-physiol-012110-142200.

[6] L.G. Parks, J.S. Ostby, C.R. Lambright, B.D. Abbott, G.R. Klinefelter, N.J. Barlow, et al., The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat, Toxicol. Sci. 58 (2000) 339–349. doi:10.1093/toxsci/58.2.339.

[7] J.L. Jacobson, S.W. Jacobson, Intellectual impairment in children exposed to polychlorinated biphenyls in utero., N. Engl. J. Med. 335 (1996) 783–789. doi:10.1056/NEJM199609123351104.

[8] N. Ribas-Fitó, E. Cardo, M. Sala, M. Eulàlia de Muga, C. Mazón, A. Verdú, et al., Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants., Pediatrics. 111 (2003) e580–e585. doi:10.1542/peds.111.5.e580.

[9] P. Palma, V.L. Palma, C. Matos, R.M. Fernandes, A. Bohn, A.M.V.M. Soares, et al., Assessment of the pesticides atrazine, endosulfan sulphate and chlorpyrifos for juvenoid-related endocrine activity using Daphnia magna, Chemosphere. 76 (2009) 335–340. doi:10.1016/j.chemosphere.2009.03.059.

[10] A.K. Hotchkiss, L.G. Parks-Saldutti, J.S. Ostby, C. Lambright, J. Furr, J.G. Vandenbergh, et al., A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion., Biol. Reprod. 71 (2004) 1852–1861. doi:10.1095/biolreprod.104.031674.

[11] Y. Picó, M. Fernández, M.J. Ruiz, G. Font, Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment, J. Biochem. Biophys. Methods. 70 (2007) 117–131. doi:10.1016/j.jbbm.2006.10.010.

[12] V. Camel, Solid phase extraction of trace elements, Spectrochim. Acta. 58 (2003) 1177–1233. doi:10.1016/S0584-8547Ž03.00072-7.

[13] J.C. Arsenault, Waters Corporation, Beginner's Guide to SPE, Waters Corportation, Milford, 2012.

[14] G.C. Burdge, P. Wright, A.E. Jones, S.A. Wootton, A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction., Br. J. Nutr. 84 (2000) 781–787.

[15] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W. V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2007) 139–177. doi:10.1016/j.reprotox.2007.07.010.

[16] V. Domingues, M. Cabral, A. Alves, C. Delerue-Matos, Use and Reuse of SPE Disks for the Determination of Pyrethroids in Water by GC-ECD, Anal. Lett. 42 (2009) 706–726. doi:10.1080/00032710902721949.

[17] F. Degel, Comparison of new solid-phase extraction methods for chromatographic identification of drugs in clinical toxicological analysis, Clin. Biochem. 29 (1996) 529–540. doi:10.1016/S0009-9120(96)00096-3.

[18] C.F. Poole, A.D. Gunatilleka, R. Sethuraman, Contributions of theory to method development in solid-phase extraction, J. Chromatogr. A. 885 (2000) 17–39. doi:10.1016/S0021-9673(00)00224-7.

[19] S. Rodriguez-Mozaz, M.J. López De Alda, D. Barceló, Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry, J. Chromatogr. A. 1045 (2004) 85–92. doi:10.1016/j.chroma.2004.06.040.

[20] C.F. Poole, New trends in solid-phase extraction, TrAC - Trends Anal. Chem. 22 (2003) 362–373. doi:10.1016/S0165-9936(03)00605-8.

[21] X. Hu, X. Wu, F. Yang, Q. Wang, C. He, S. Liu, Novel surface dummy molecularly imprinted silica as sorbent for solid-phase extraction of bisphenol A from water samples, Talanta. 148 (2016) 29–36. doi:10.1016/j.talanta.2015.10.057.

[22] H.S. Chang, K.H. Choo, B. Lee, S.J. Choi, The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water, J. Hazard. Mater. 172 (2009) 1–12. doi:10.1016/j.jhazmat.2009.06.135.

[23] M.C. Hennion, Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography., J. Chromatogr. A. 856 (1999) 3–54. doi:10.1016/S0021-9673(99)00832-8.

[24] D. Štajnbaher, L. Zupančič-Kralj, Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry, J. Chromatogr. A. 1015 (2003) 185–198. doi:10.1016/S0021-9673(03)01211-1.

[25] W.S. Kim, A. Do, D. Yeh, J. Cunningham, Extraction of bisphenol-A and 17β-estradiol from water samples via solid-phase extraction (SPE), Rev. Anal. Chem. 33 (2014). doi:10.1515/revac-2013-0016.

[26] J.L. Devore, Probability and Statistics for Engineering and the Sciences, 1982. doi:10.2307/1270176.

APPENDIX A: EXAMPLE DATA COLLECTION

Table A.1a: Example collected peak area, and normalization (analyte/NP ratio column). Analyte here is BPA for cartridge 17, Sample 1-10.

Cartridge run	Time	BPA area	Denominator	BPA/NP ratio
0	0	-		
1	13.48	69,388	50,040	1.39
2	13.38	100,529	64,428	1.56
3	13.41	40,470	37,759	1.07
4	13.35	108,121	89,019	1.21
5	13.33	116,455	77,389	1.50
6	13.33	168,222	86,396	1.95
7	13.38	55,936	94,962	0.59
8	13.36	175,223	120,391	1.46
9	13.37	58,635	92,022	0.64
10	13.38	174,340	167,231	1.04

Table A.1b: Statistical evaluation of BPA for cartridge 17, Sample 1-10.

	Time	Area
StDv	0.04	0.40
Max	13.48	1.95
Min	13.33	0.59
Range	0.15	1.36
Average	13	1.24

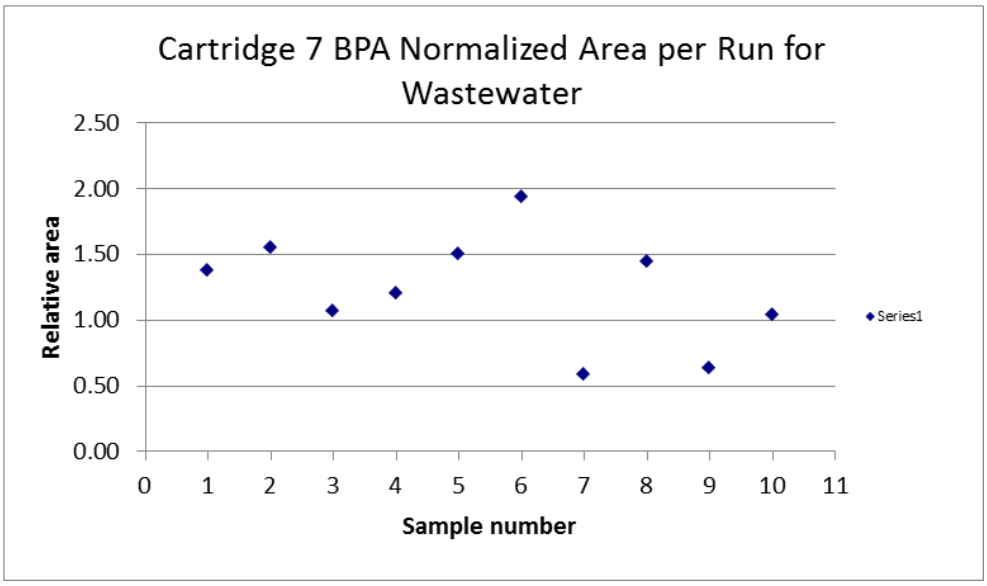

Figure A.1: Plot of peak area for one cartridge over all 10 re-uses. Analyte here is BPA for cartridge 17, Sample 1-10.