Determination of Triclosan in Personal Care Products and Swimming Pool Samples by Liquid Chromatography-Mass Spectrometry

Devin Chattu

Chemistry
Supervisor: Kingsley Donkor

1. Abstract

Antibacterial agents are extremely common in everyday personal care products, such as toothpastes, facial cleansers, hand soaps, body washes, cosmetics, and numerous other products. Due to their widespread use, the active antibacterial agents in these products have been detected in public water samples, such as from pools and rivers. One antibacterial agent under scrutiny at this time is triclosan. Although its effects on human health are controversial and largely unknown, it has been reported to have an effect on the endocrine system. It should also be noted that industries are now avoiding the use of triclosan since very minute quantities can pose a severe risk to marine life in aquatic ecosystems. The purpose of this research was to develop a sensitive, rapid liquid chromatography-mass spectrometry (LC/MS) method to detect triclosan in personal care products and public water samples. The experimental conditions, such as column temperature, solvents, flow rate, analyte extraction methods, and experimental procedure, were all optimized to find the best experimental conditions for detecting triclosan in the samples. The ability to detect triclosan in personal care products, as well as in pool and river water samples, will hopefully encourage consumers to reduce or avoid the use of triclosan containing products. Using the optimized method developed, the average concentration of triclosan in the personal care product samples ranged from 10 ppm to 4741 ppm. The average concentrations in the pool water and river water samples were 49 ppb and 72 ppb, respectively.

2. Acknowledgements

I would like to thank my supervisor, Dr. Kingsley Donkor, for giving me the opportunity to conduct research under his guidance, as well as for proposing research that is in an area of my personal interest. I would also like to thank him for all of his support, patience, and ideas. I

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3. Introduction

3.1 *Triclosan*

Triclosan, also known as 5-chloro-2-(2,4-dichlorophenoxy)phenol, is a synthetic compound that has been used for decades. As an active antibacterial agent and preservative, it has been incorporated into a huge number of widely used household and personal care products.[1] These products include footwear, carpets, plastic toys, kitchen wear, toothpastes, deodorants, creams, lotions, bath gels, shampoos, cosmetics, face cleansers, hand soaps, dish soaps, and even wound sprays. With such widespread use, it's no wonder that there is concern about triclosan's long-term effects on human health and the environment.

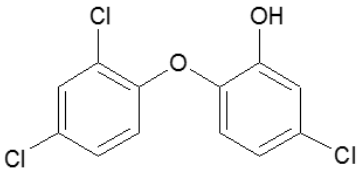

Figure 1. Chemical structure of triclosan

The mechanism of triclosan's antibacterial action involves the selective binding of triclosan to the active site of the enzyme enoyl-acyl carrier protein reductase (ENR).[2] This enzyme is responsible for fatty acid synthesis within bacterial cells. These fatty acids are required for cell membrane synthesis and without the ability to synthesize new cell membranes, cells cannot proliferate. The complex that actually disables the enzymes is a stable ENR-NAD+-Triclosan complex at the active site (NAD+ is nicotinamide adenine dinucleotide). This enzyme is not present in human cells so triclosan is generally regarded as safe. However, recent studies have shown that high concentrations (50 – 100 μM) may impact human hormone regulation by altering steroidogenesis.[3] Steroid based hormones include estradiol and progesterone, which are secreted by the placenta during pregnancy. The effect of triclosan appears to be to increase the secretion of these two hormones. Other adverse health effects are largely unknown at this time. Triclosan does not appear to bioaccumulate in humans, as it is quickly metabolized and excreted. This property allows the assumption that triclosan is safe for large scale use.

Although triclosan is so far known to be benign with respect to its effects on human health, the main concern lies in its huge potential to be an environmental and ecological hazard. Over 95% of triclosan use is in consumer products that are disposed of in residential drains.[4] Most wastewater treatment plants are capable of removing 72 – 94% of triclosan.[5] A considerable amount is still left remaining, and that unremoved triclosan makes its way into lakes, rivers, soils, sediments, and swimming pools. Since only one molecule is needed to permanently inhibit an ENR enzyme, triclosan is effective at extremely low concentrations. The minimum inhibitory concentration as an active bactericide in store products is 500 ppb, but concentrations lower than that can still pose a severe risk to marine life in aquatic environments.[6] Triclosan is also very stable for long periods of time. Its presence and long term exposure to bacteria in the environment can promote environmental antibacterial resistance, which is an issue of great concern.[1] Lastly, triclosan may become a more toxic chlorinated dioxin upon exposure to UV light, which is generously provided by the sun in the environment, further increasing its hazardous potential.[1,5]

3.2 *Liquid Chromatography – Mass Spectrometry*

Liquid chromatography-mass spectrometry (LC/MS) is the coupling of two separate instruments for the separation, quantification, and detection of analytes. Liquid chromatography acts to separate all of the molecular components of a sample based on their polarity. This technique involves the use of a liquid mobile phase and a stationary phase. The mobile phase and stationary phase are either polar or non-polar, with each of the phases being of opposite polarity to each other. The length and width of the column, as well as the packing, influence the properties of the column. In reversed phase-liquid chromatography, the liquid mobile phase consists of a polar solvent, and the column consists of a non-polar packing. Non-polar species that are carried through the column will interact with the non-polar packing of the column to a greater extent than polar species, which will pass through the column uninterrupted. The greater the interaction with the column, the longer the species will be retained, resulting in a longer elution time. The component separation characteristic of liquid chromatography is the result of this varying elution time for each species. The separated components are then transferred to a detector, such as a mass spectrometer.[7,8]

A mass spectrometer operates by performing a number of functions. Molecules that enter the mass spectrometer are first vaporized, then ionized, and then sorted into their mass-to-charge ratio (m/z) before being detected. There are numerous ionization techniques, such as electrospray ionization (ESI). The liquid eluent from an LC/MS is ionized and discharged in a fine spray. The droplets evaporate off solvent, allowing the ionized analyte ions to condense, thereby concentrating the charge within an aerosol droplet. Eventually, a critical point is reached, resulting in a “coulombic explosion” of ions. These ions are accelerated towards a mass analyzer.

The mass analyzer used in this work is a quadrupole time-of-flight (Q-TOF) mass analyzer. The Q-TOF couples the use of an oscillating electric field, and varying flight times of projected ions, to separate the ions (and fragments) based on their m/z ratios. An electron multiplier detector then detects these separated ions and plots them on a mass spectrum, showing relative abundance against m/z ratio.[7,8]

The purpose of this research was to develop a sensitive and rapid method to detect and quantify triclosan in personal care products, as well as in river and swimming pool waters, using reversed phase-liquid chromatography-mass spectrometry. The fact that triclosan is present and can be detected, in these products and water samples will, it is hoped, encourage consumers to consider reducing, or avoiding the use of triclosan-containing products.[7,8]

4. Experimental

4.1 *Instrument and Optimized Instrumental Parameters*

The instrument used in this research was an Agilent 1200 Series High Performance Liquid Chromatography Instrument coupled to an Agilent 6530 Accurate-Mass Quadrupole-Time of Flight (Q-TOF) mass spectrometer with electrospray ionization (Figure 2). The instrumental parameters are outlined in the table below (Table 1).

Table 1: Optimized LC/MS parameters

Injection volume	5 µL
Flow rate	0.4 mL/min
Solvent composition	A: 40 % Water (0.1 % acetic acid) B: 60 % Acetonitrile (0.1 % acetic acid)
Run time	30 min
Column type	C-18
Column temperature	40 °C
Gas temperature	300 °C
Drying gas flow	8.0 L/min
Nebulizer	8 psig
Sheath gas temp	350 °C
Sheath gas flow	10 L/min
Vcap	3000 V
Ion polarity	Negative

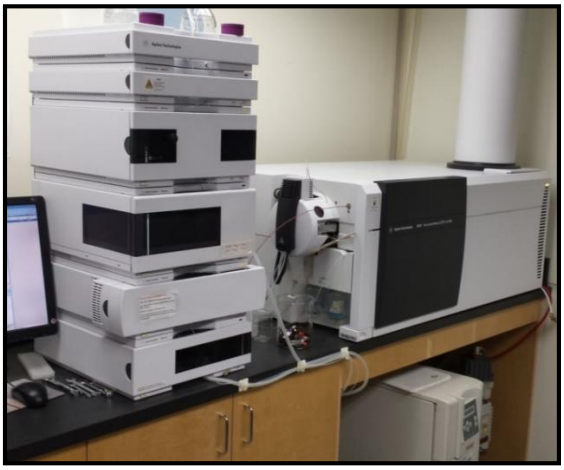

Figure 2. Agilent LC/MS Instrument

4.2 *Materials and Reagents*

Triclosan, bisphenol A (BPA), and sodium hydroxide, were purchased from Sigma-Aldrich (Oakville, ON, Canada). Methanol, acetonitrile, and acetic acid, were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Any water used in standard or sample preparation was deionized 18 mΩ water. All reagents were of analytical grade.

The samples analyzed were Dial® coconut water hand soap, Dial® spring water hand soap, Dial® fresh pear hand soap, Dial® body wash, Solarcaine® first aid spray, Colgate Total® gel toothpaste, Colgate Total® paste toothpaste, Trisan® facial skin cleanser, Life® orange citrus dish soap, Kamloops Tournament Capital Center swimming pool water, and Kamloops Riverside river water. All the manufactured products were purchased from Shoppers Drug Mart® (Kamloops, BC, Canada).

4.3 *Preparation of Standard Solutions and Solvents*

A 1000 ppm triclosan stock solution was prepared by weighing out 10 mg and dissolving it in methanol to a volume of 10 mL. This 1000 ppm solution was diluted to 10 ppm in methanol. The triclosan standards were prepared in 1.5 mL LC/MS vials to concentrations of 50 ppb, 100 ppb, 200 ppb, 400 ppb, 600 ppb, 800 ppb, and 1000 ppb. BPA was used as an internal standard, and was added to these standards to a concentration of 600 ppb. The standards were run in duplicate on the LC/MS to produce a standard curve.

Solvents were prepared by adding 0.250 mL acetic acid to two 250-mL volumetric flasks and diluting to the mark with 18 mΩ water in one flask, and diluting to the mark with acetonitrile in the other flask. Both solvents contained 0.1 % acetic acid by volume.

4.4 *Preparation of Samples*

Approximately 0.15 g of each toothpaste, body wash, facial skin cleanser, dish soap, and coconut water hand soap, were weighed out in Erlenmeyer flasks. A 0.01 M NaOH solution was prepared by dissolving 0.1 g NaOH in 18 mΩ water to a volume of 250 mL. A 10 mL aliquot was pipetted into each of the sample contained in Erlenmeyer flasks. The samples were then sonicated for 10 min in an ultrasonic water bath. The samples were transferred to centrifuge tubes where they were subsequently centrifuged at 3000 rpm for 15 min.[9] The supernatants were collected and filtered using 3 mL 0.45 µm Luer-lock syringes. The samples were pipetted into 1.5 mL LC/MS vials. Then, BPA internal standard was added to each sample vial to a concentration of 600 ppb.

Less viscous consumer products, such as the first aid spray and three hand soaps, were simply diluted with methanol. The first aid spray was diluted 1:5, whereas the hand soaps were diluted 1:10. After dilution, the samples were filtered into 1.5 mL LC/MS vials using 3 mL 0.45 µm Luer-lock syringes. BPA internal standard was added to each sample vial to a concentration of 600 ppb.

The water samples were filtered with 3 mL 0.45 µm Luer-lock syringes, and had BPA added to a concentration of 600 ppb within 1.5 mL LC/MS vials.

All samples were then run on the LC/MS with the instrument parameters set to the parameters listed in Table 1.

5. Results and Discussion

5.1 *Analysis of Standards*

Upon running the triclosan standards through the LC/MS, the produced chromatograms were evaluated. An extracted ion chromatogram (EIC) was used to identify the peaks belonging to triclosan and BPA. For triclosan, an EIC mass range (m/z) of 288.00000 to 290.00000 was used. For BPA, an EIC mass range (m/z) of 227.00000 to 229.00000 was used. All of the EICs showed large definitive peaks within the specified mass ranges. Upon analyzing these peaks for mass spectrum data, all of the standards' mass spectra contained a very prominent peak at the m/z of 287.95, which is only 0.60 less than triclosan's molecular weight of 288.54 g/mol. In other studies, the observed mass peak for triclosan was an identical 287.95.[1] This value is

expected as the MS was operating in negative mode, so a value of (M-1) is expected. All of the standards' mass spectra also contained a very prominent peak at the m/z of 227.11 as well, which is about 1 mass unit less than BPA's molecular weight of 228.29 g/mol.[10] Again, since the MS was operating in negative mode, a value of (M-1) is expected. The EICs and mass spectra for both triclosan (Figure 3 and Figure 4) and BPA (Figure 5 and Figure 6) are shown below.

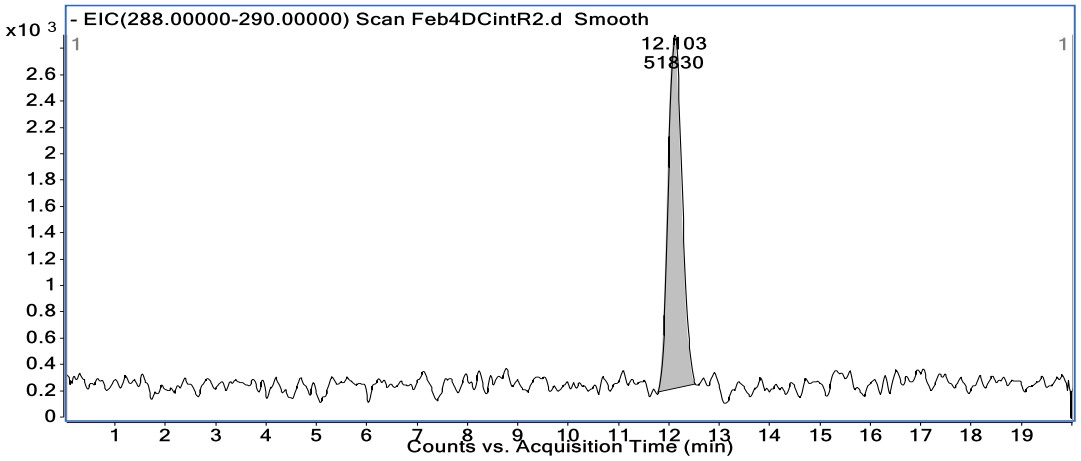

Figure 3. Triclosan EIC of the 1000 ppb standard.

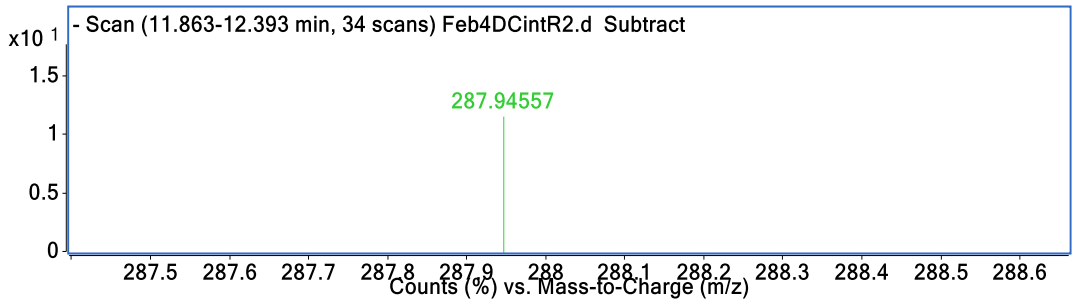

Figure 4. Triclosan mass spectrum of the 1000 ppb standard

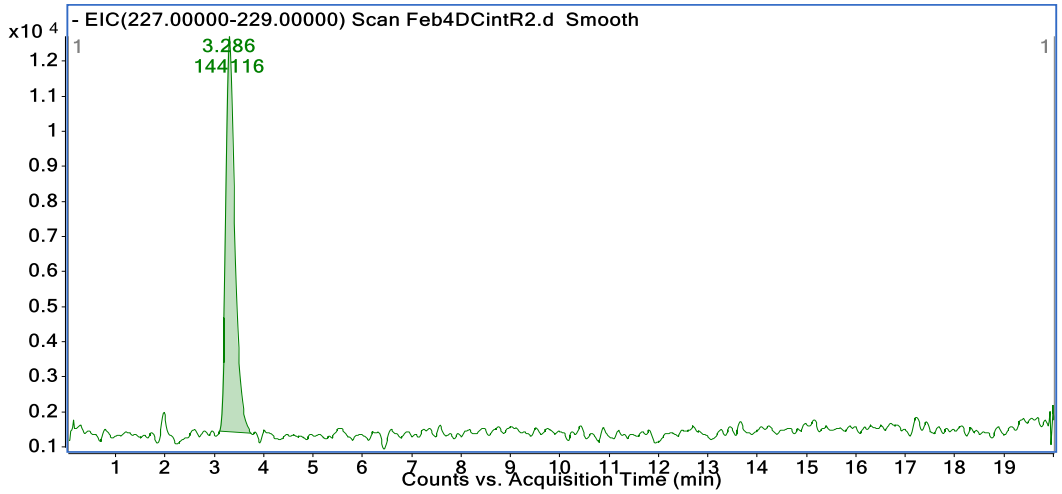

Figure 5. EIC of 600 ppb BPA in the 1000 ppb standard

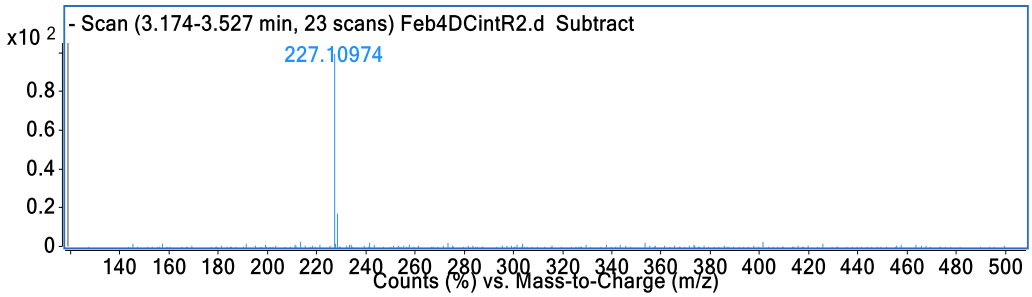

Figure 6. Mass spectrum of 600 ppb BPA in 1000 ppb triclosan standard

In order to determine the concentration of triclosan in the consumer products and water samples, a standard curve had to be generated from the standards. Using the instrument's Mass Hunter software, the peak areas of the triclosan and BPA peaks within their respective EICs were determined. The internal standard approach was used so that a triclosan/BPA peak area ratio was plotted against triclosan standard concentration to generate the standard curve (Figure 7). A coefficient of linearity (R^2) value of 0.96 was obtained. Although this value was fairly decent, a better value could have been achieved if lower concentrations of the internal standard had not been used. This is because baseline signal noise was beginning to become an issue at the 50 ppb level, which may have contributed to a less than desirable R^2 value.

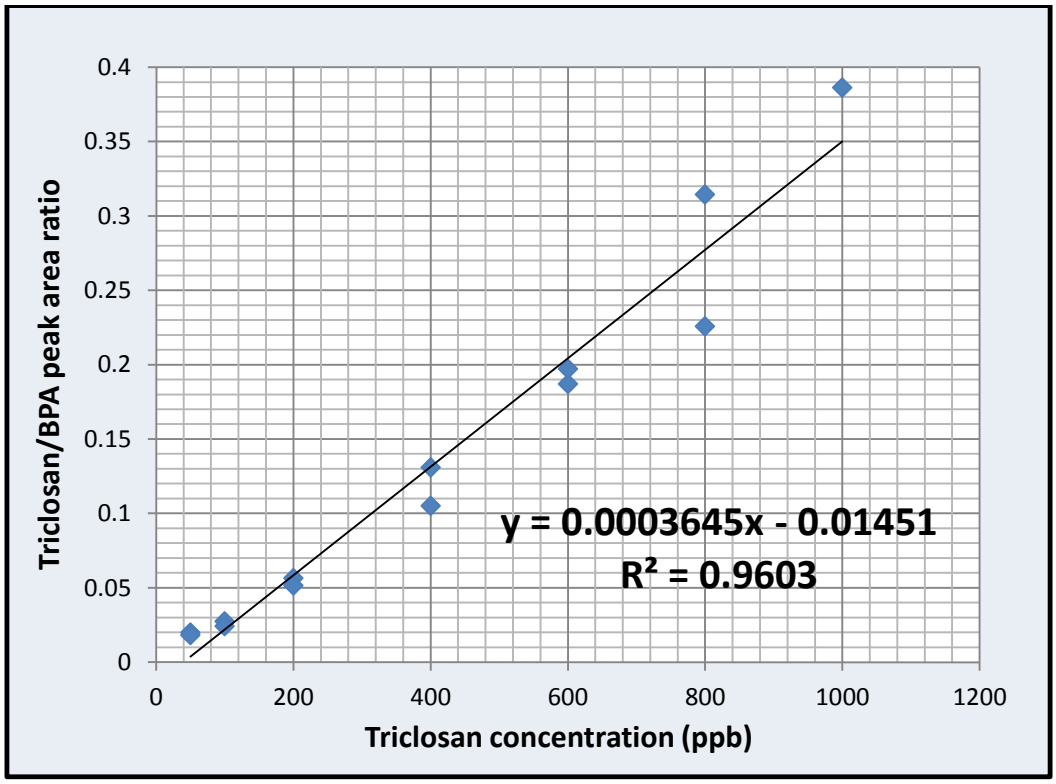

Figure 7. Triclosan with a BPA internal standard calibration curve

5.2 *Analysis of Samples*

The run time, along with the rest of the experimental parameters, was kept the same throughout the entire experiment due to the varying elution time of triclosan in the sample runs. Every sample was run in duplicate, just like the standards. EICs for each sample were analyzed in the same mass ranges as the standards to detect triclosan (m/z range of 288.00000 – 290.00000) and BPA (227.00000 – 229.00000). Prominent peaks for both triclosan and BPA were found in almost every sample run. The Solarcaine® First Aid spray EICs and mass spectra are shown below (Figures 8, 9, 10, and 11). One of the two toothpaste runs did not show a triclosan peak. A possible reason for this could be due to temporary column clogging. A triclosan peak was also not detected in one of two Riverside river water samples. This could likely be due to extremely low triclosan levels, rendering the triclosan peak indistinguishable from the baseline signal noise peaks.

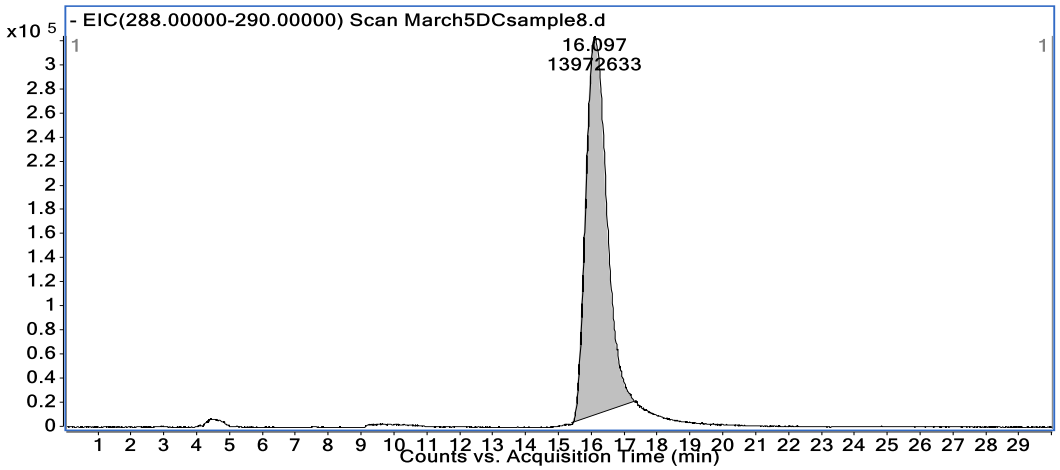

Figure 8. EIC of triclosan in a Solarcaine® sample

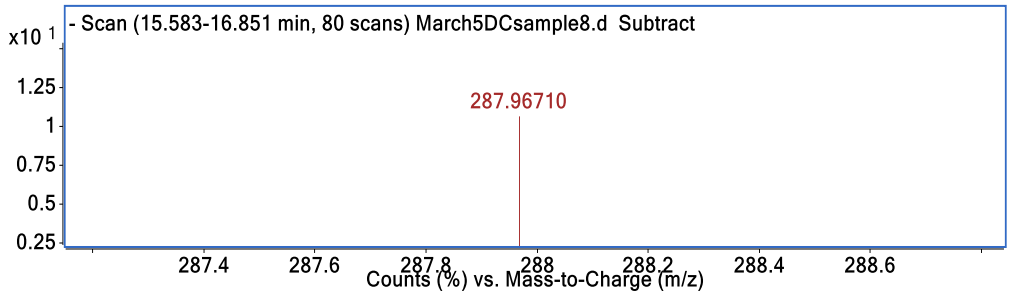

Figure 9. Mass spectrum of triclosan in a Solarcaine® sample

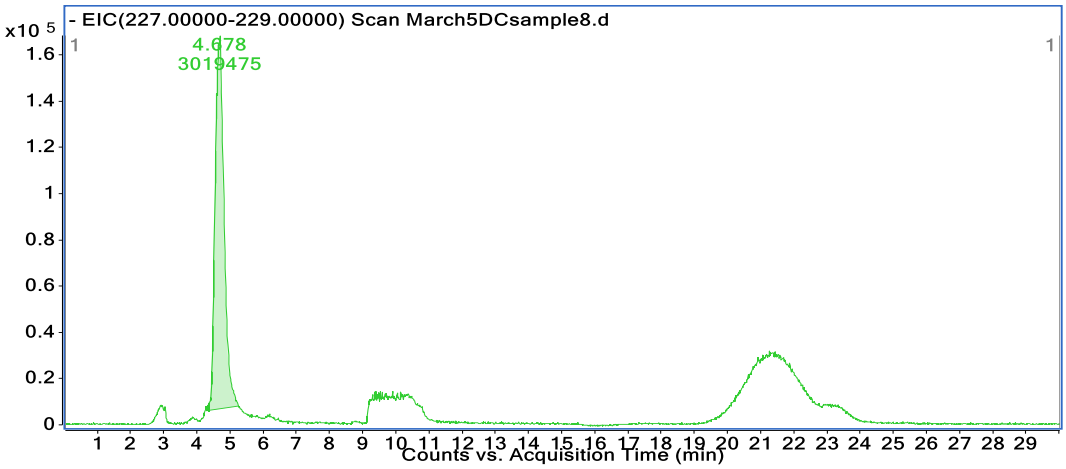

Figure 10. EIC of 600 ppb BPA in a Solarcaine® sample

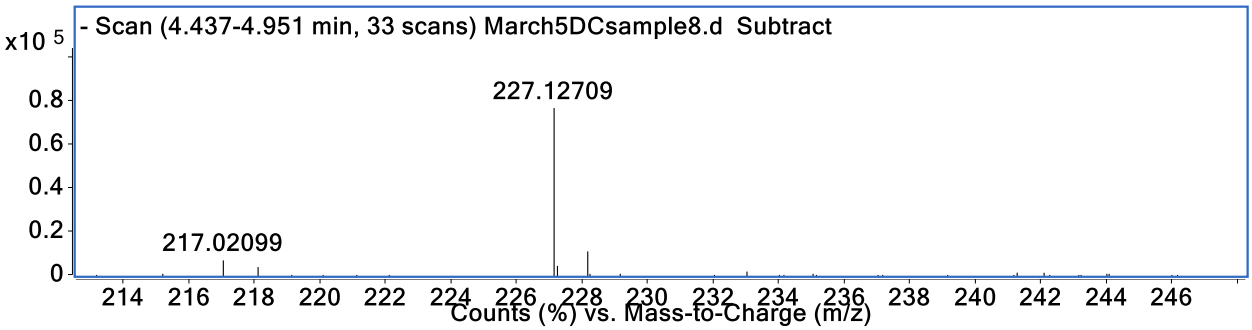

Figure 11. Mass spectrum of 600 ppb BPA in a Solarcaine® sample

Mass Hunter software was again used to determine the EIC peak areas of triclosan and BPA in each sample. The triclosan/BPA peak area ratio and the equation of the standard curve were used to determine the triclosan concentration. Working backwards through the dilution calculations, the original sample triclosan concentrations were determined. The table below shows the average of obtained original sample concentrations.

Table 2: Original triclosan sample concentrations

Sample	Concentration
Dial® coconut water hand soap	4741 ppm
Dial® spring water hand soap	41 ppm
Dial® fresh pear hand soap	92 ppm
Solarcaine®	68 ppm
Colgate Total® gel toothpaste	609 ppm
Colgate Total® paste toothpaste	728 ppm
Dial® coconut water hand soap extract	3303 ppm
Trisan® extract	2833 ppm
Dial® body wash extract	3037 ppm
Life® orange citrus dish soap extract	10 ppm
TCC swimming pool water	49 ppb
Riverside river water	72 ppb

One of the original sample concentrations obtained for the coconut water hand soap was discarded as the value was vastly below that obtained for a second sample. The value that was discarded was 12 ppm. This is significantly lower than the 4741 ppm value obtained by a duplicate sample run. The same coconut water hand soap was used in the extraction method as well, and the average original concentration obtained using that method was 3303 ppm. This is much closer to the 4741 ppm value than it is to the 12 ppm value. To further verify that the 12 ppm value was incorrect, it would be necessary to analyze the coconut water hand soap using another analytical technique, such as capillary electrophoresis, and comparing the results.

A comparison to the manufacturers' claim was not possible for the majority of the samples as the triclosan content was not listed. In fact, the only consumer products that did list the triclosan content were the toothpastes. Both Colgate Total® gel and paste toothpastes had a listed triclosan content of 0.3 % w/w (3000 ppm). With obtained values of 609 ppm for the gel, and 728 ppm for the paste, this would equate to a percent recovery of 20.3 % and 24.3 %, respectively. These low recovery yields indicate that a better extraction method needs to be implemented. Perhaps the use of a solid-phase extraction cartridge could help increase the extraction efficiency and triclosan yield.

Analyzing the pool and river water samples, ppb level values (49 – 72 ppb) were found. Although these levels don't appear to be high enough to raise immediate concern, it is important to remember that very minute quantities are needed to be an active bactericide. The fact that triclosan is detectable in these public water samples is enough to raise concern that widespread triclosan use needs to be restricted.

6. Conclusion

A sensitive and rapid method was successfully developed to separate, detect, and quantify triclosan in personal consumer care products and public swimming pool water and river water samples, although the extraction method could likely be improved. Triclosan was successfully detected in all samples. The content was determined to range from 10 ppm to 4740 ppm for consumer products and 49 ppb to 72 ppb for the water samples. The detection of triclosan in public water samples indicates that its industrial scale use in everyday products needs to be reduced, restricted, or avoided. This also signals a need for better wastewater treatment to successfully remove or destroy triclosan before it is released back into the environment.

7. Future Work

There are several things that could be done in future work. Implementing solid-phase extraction could very likely increase the triclosan sample extraction yields dramatically. Running more replicates for each sample would also increase the precision of the obtained data. Improving solvent composition may also help by allowing for better detection of triclosan at lower ppb concentrations. Also, analyzing these same samples using a different analytical technique, such as capillary electrophoresis, and comparing the results, could be a possible future project.

8. Literature Cited

1) Gonzales-Marino, I., Quintana, J. B., Rodriguez, I., Cela, R. *Rapid Commun. Mass Spectrom.*, **2009**, 23, 1756 – 1766.

2) Hua, W., Bennett, E. R., Letcher, R. J. *Environment International*, **2005**, 31, 621 – 630.

3) Honkisz, E., Zieba-Przybylska, D., Wojtowicz, A. K. *Reproductive Toxicology*, **2012**, 34, 385-392.

4) Glaser, A. Beyond Pesticides. The Ubiquitous Triclosan. *A common antibacterial agent exposed*, **2004**, 24, 12 – 17.

5) Lee, D. G., Cho, K., Chu, K. *Chemosphere*, **2015**, 125, 9 – 15.

6) Assadian, O., Wehse, K., Hubner, N., Koburger, T., Bagel, S., Jethon, F., Kramer, A. *GMS Krankenhhyg Interdiszip*, **2011**, 6, 1-7.

7) Donkor, K. CHEM 3100, *Instrumental Analysis Notes*, **2014**. Thompson Rivers University, Kamloops, B.C., Canada.

8) Cinel, B. CHEM 3230, *Organic Spectroscopy Notes*, **2015**. Thompson Rivers University, Kamloops, B.C., Canada.

9) Lu, H., Ma, H., Tao, G. *Spectrochimica Acta Part A*, **2009**, 73, 854 – 857.

10) Bisphenol A MSDS, Sigma-Aldrich. (*Accessed April 20, 2015*). http://www.sigmaaldrich.com/catalog/search?term=bisphenol+A+MSDS&interface=All&N=0&mode=partialmax&lang=en&region=CA&focus=product