CAPILLARY ELECTROPHORESIS AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BISPHENOL A (BPA) IN ENVIRONMENTAL WATERS

by

AMNA FAROOQ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

In

THE FACULTY OF SCIENCE
GRADUATE STUDIES

Thesis examining committee:

Kingsley Donkor (PhD), Associate Professor and Thesis Supervisor,
Department of Physical Sciences (Chemistry)
Heidi Huttunen-Hennelly (PhD), Associate Professor and Committee member,
Department of Physical Sciences (Chemistry)
Naowarat (Ann) Cheeptham (PhD), Associate Professor and Committee member,
Department of Biological Sciences
James K. Kariuki (PhD), Associate Professor and External Examiner,
University of Alberta, Augustana Campus

April 2015

THOMPSON RIVERS UNIVERSITY
(Kamloops)

Abstract

Bisphenol A (BPA) and its structural analogues (BPF, BPS, BPP, BPZ, BADGE, BPAF, BPAP) are used in the production of polycarbonate plastics and epoxy resins. Most of them have proven endocrine disruptive effects in humans and in other life forms in very low concentrations. BPA is of particular interest as it is mass produced and released into the environment as a result of human activity and accumulates in aquatic and terrestrial environments. Recent studies have revealed the presence of BPA in fresh water resources which is not only a threat to the fresh water ecosystems but also to humans because the usual source of drinking water is from rivers and streams. Presence of bisphenols in the environment is crucial and use of analytical techniques for their chemical separation and subsequent analysis is necessary for efficient environmental monitoring of these compounds. This study used capillary electrophoresis (CE) and liquid chromatography-mass spectrometry (LC/MS) to develop sensitive analytical protocols for quantification of BPA present in environmental, swimming pool and tap water samples from the Kamloops region. Standard addition and internal standard calibration approaches were used to quantify BPA in the water samples. In addition, a CE method was developed to simultaneously separate eight bisphenol analogues, BPA, BPF, BPS, BPZ, BPP, BPAP, BPAF and BADGE. Optimization of experimental parameters such as pH, buffer concentration, detection wavelength, applied voltage and buffer additives enabled the successful baseline separation of all the analogues.

Acknowledgements

I am thankful to Allah; who has created me and endowed me with everything that I have today in my life. If I would have to count up the favors of Allah on me, never would I be able to count them. Allah is the best companion, glorifying Him is everything that is in heavens and earth. A belief that He is with me, made the toughest tasks of life easy and manageable.

I am eternally indebted to my parents, Mohammed Aslam and Safia Aslam, for their unconditional love and endless support. Their prayers and encouragement have made a big difference in my life. Big time thanks to my husband, Farooq Haider, for his continuous love and support. In his presence, I feel happy, grounded, and enjoy the tiny moments in life. I also want to acknowledge my sister, Ayesha Chaudhry for her emotional and moral support at times when I needed it the most. I am very much thankful to my supervisor, Dr. Kingsley Donkor, for his continuous guidance and support throughout the M.Sc. program. He has made an inevitable contribution in my academic career. He inspired me to work hard and encouraged me to strive higher than I could ever imagine. It is well said that a teacher affects eternity; he can never tell where his influence stops. Thanks for the rigorous training and challenges that you made me face; they have given me the courage to be leading every race. I owe you a debt of gratitude. I am also thankful to my M.Sc. Committee members, Dr. Heidi Huttunen-Hennelly and Dr. Naowarat (Ann) Cheeptham, for their endless guidance, support and encouragement. Thanks to my external examiner, Dr. James Kariuki, for his time and effort in my defense. I am extremely grateful to Dr. Wendy Gardner, the M.Sc. program coordinator at TRU for always being there to help and for providing best guidance and support. Lastly, I thank the TRU Department of Physical Sciences (Chemistry) for providing resources and labs for this work.

To my beloved parents and husband!

Table of Contents

List of Abbreviations

1. Bisphenol A (2,2-bis-(4-hydroxyphenyl) propane; BPA)
2. Bisphenol A diglycidyl ether (2,2-Bis[4-(glycidyloxy) phenyl] propane); BADGE)
3. Bisphenol AF (4,4'-(hexafluoro-isopropylidene) diphenol; BPAF)
4. Bisphenol AP (4,4′-(1-Phenylethylidene) bisphenol; BPAP)
5. Bisphenol F (4,4'-dihydroxydiphenylmethane; BPF)
6. Bisphenol P (4,4'-(1,4-phenylenediisopropylidene) bisphenol; BPP)
7. Bisphenol S (4,4'-sulfonyldiphenol; BPS)
8. Bisphenol Z (4,4'-cyclohexylidenebisphenol; BPZ)
9. Bisguaiacol F (BGF)
10. CE (Capillary Electrophoresis)
11. LC/MS (Liquid Chromatography/Mass Spectrometry)
12. EOF (Electroosmotic Flow)
13. CZE (Capillary Zone Electrophoresis)
14. CGE (Capillary Gel Electrophoresis)
15. CEC (Capillary Electrochromatography)
16. CIEF (Capillary Isoelectric Focusing)
17. CITP (Capillary Isotachophoresis)
18. MEKC (Micellar Electrokinetic Chromatography)
19. UV (Ultraviolet)
20. PDA (Photodiode array)
21. LIF (Laser Induced Fluorescence)
22. FDA (U.S. Food and Drug Administration)
23. EPA (U.S. Environmental Protection Agency)
24. MIP (Molecular Imprinted Particles)
25. ESI (Electrospray Ionization)
26. APCI (Atmospheric Pressure Ionization)
27. APPI (Atmospheric Pressure Photoionization)
28. TOF (Time of Flight)
29. LOD (Limit of Detection)
30. LOQ (Limit of Quantitation)

31. BW (Body Weight)
32. NOAEL (No Observed Adverse Effect Level)
33. LOAEL (Lowest Observed Adverse Effect Level)

List of Tables

List of Figures

Chapter 1
Introduction and Overview

Bisphenols

Bisphenol-A (BPA) and other bisphenols are found in polycarbonate plastics and epoxy resins. Polycarbonate plastics are often used in consumer goods and containers that store food and beverages, such as water bottles. Epoxy resins are used to coat the inside of metal products, such as food cans and bottle tops [1]. Thus, the major human exposure route to bisphenols is via ingestion of contaminated food and water, with over 90% of adults in many countries having detectible amounts in their urine. There are many types of bisphenols including bisphenol (A, AB, AF, AP, B, BP, C, E, F, G, M, S, P, PH, TMC, and Z) [1,2].

Bisphenols and similar other chemical compounds are introduced into the environment by humans and they are capable of disrupting the endocrine systems of higher life forms such as wildlife, fish and humans themselves [3]. The consequences of such disruptions can be profound because of the crucial role hormones play in controlling living organism's growth and development. BPA is one of the highest volume chemicals produced world-wide. Current estimates indicate that more than 6 billion pounds of BPA are released into the atmosphere each year [3,4].

BPA was first synthesized by A.P Dianin in 1891 and was investigated for potential commercial use in 1930s during a search for synthetic estrogen [4]. BPA's estrogenic activity was confirmed by tests but another structurally related synthetic compound called diethylstilbestrol (DES) was prove to be far more potent than BPA in a classical estrogenicity assay of vaginal cornifaction [4]. The use of BPA as a synthetic estrogen was therefore abandoned in favor of DES which was administered to pregnant women from late 1940s to 1971 to prevent multiple pregnancy related problems including miscarriages and premature births [5].

Bisphenol A (BPA) is produced by condensation of phenol and acetone in the presence of HCl or sulphonated styrene DVB catalysts. The reaction is acid catalyzed at 60-80°C with a molar ratio of phenol to acetone ranging from 3:1 to 10:1 [6]. The condensation reaction gives a mixture of BPA and its isomer *o,p*-BPA and small amounts of impurities including trisphenol and polyphenols. The latter impurities are formed by the reaction of phenol with trace amounts of mesityl oxide which itself is a product of self-condensation

of acetone followed by dehydration [6,7]. Figure 1.1 shows chemical reaction for BPA formation

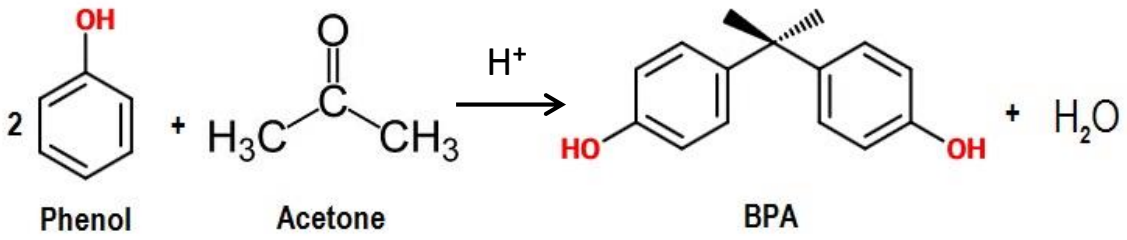


Figure 1.1. Chemical reaction for BPA formation

BPA is a monomer used in the manufacturing of polycarbonate. Polycarbonate, in turn, is used in a wide array of plastic products with new applications continuously being developed [2]. Polycarbonate is routinely subjected to heat treatment and BPA has been detected in the thermal degradation products formed during this thermal treatment [2]. It has also been well documented that polymerization reactions may not be fully complete and that a significant proportion of unreacted products can be recovered from these plastics [8,9].

BPA is mainly used as a material for the production of epoxy resins and polycarbonate plastics. With the increased use of the substances made of polycarbonate plastics and epoxy resins human exposure to BPA has also increased [1,2,10]. Environment (aquatic environment, air and soil) can be one source of BPA contamination for humans but the primary route is through foods contaminated with BPA. On the basis of several studies, the daily human intake of BPA is < 1 µg/kg BW/day [11]. BPA toxic levels for human beings are documented to be 50 mg/kg/day [12]. BPA is considered to be dangerous for aquatic life in even at µg/L (ppb) levels. Table 1.1 summarizes various physical and chemical properties of BPA.

Table 1.1. Key Facts about BPA based on Staples et al. 1998

PROPERTY	VALUE
Molecular weight	228 gm/mol
Empirical formula	$(CH_3)_2C(C_6H_4OH)_2$
Specific gravity	1.09 - 1.19 gm/cm^3
Boiling point	398 °C
Melting point	150 - 155 °C
IUPAC ID	2,2-bis (4 hydroxyphenyl) propane
Solubility	120 - 300 mg/L at pH 7
Density	1.20 g/cm³
Octane Water Partition Coefficient (Log K_{ow})	3.4 (3.3 - 3.8)
$t_{1/2}$ water & wastewater	2.5 - 4 days
Vapor pressure	8 10^{-10} - 4 10^{-7} mm Hg
pKa value	9.59 - 11.3
Henry's constant	10^{-10} Atm-m^3/mol
Publicly Owned Treatment Works (POTW) effluent	8 - 25 µg/L
Bioconcentration factor	5 – 68
Biodegradation	76 - 95% in 28 days
POTW treatment efficiency	92 - 99.8%
Photodegradation in water	Limited
Photo-oxidation in water	$t_{1/2}$ = 6 - 160 days

BPA Contamination

Estimated Amounts of BPA released in the Environment

Environmental Protection Agency (EPA) of the United States (U.S.) estimated amount of BPA released into the environment. In 2002, the estimates were 85,300 kg of stack and fugitive emissions to air, 3,500 kg directly to water, 1,100 kg to water after 90% removal in treatment plants, and an additional 10,000 kg to water from indirect sources such as landfills [14]. In the U.S., BPA had a maximum level of 420 ng/L in drinking water treatment plants, and up to 3642 ng/L at domestic wastewater treatment plants [15]. According to the European Union (E.U) risk assessment report, levels of BPA released into the environment are similar to that in the U.S. Approximately, 2,140 kg of BPA was released to air and 86,500 kg was estimated to be released to water [14].

Sources of BPA Contamination

BPA is a mass produced chemical and because of its wide spread use in plastic industry, it can be released in to the environment during its manufacturing process. It is released as fugitive dust from closed systems during the processing, handling, and transportation of the plastic material [14]. Elevated temperature results in increased vapor pressure of compounds, high temperature used during the manufacturing process results in release of molten BPA into the environment [14]. BPA has also been found to leach into the environment from plastics and thermographic papers found in landfills, and PVC pipelines used for transporting water [15-17].

Aquatic Environment

The solubility of BPA in water ranges from 120 to 300 µg/mL [18]. BPA can be found in wastewater from factories that produce it because it is not completely removed during wastewater treatment. The wastewater containing BPA can be a source of contamination for the aquatic environment [18,19].

Recently, high levels of BPA were identified in leachates from a waste landfill and was reported that the levels of BPA in the leachates of a hazardous waste landfill ranged from 1.3 to 17,200 ng/mL (average 269 ng/mL) [19,20]. Since these leachates are discharged during treatment, the concentration of BPA in effluent is considerably lower. It was found that the levels of BPA in four landfill leachates ranged from 15 to 5400 ng/mL, but ranged from 0.5 to 5.1 ng/mL in effluents after treatment [19]. However, these effluents that contain BPA

after leachate treatment are known to be a source of BPA contamination in the aquatic environment [18-20].

BPA in river waters can be degraded under aerobic conditions but not under anaerobic conditions [21-23]. It was found that most bacteria isolated from river waters can biodegrade BPA, but there were differences in the BPA removal rates and bacteria belonging to *Pseudomonas* sp. strain showed high BPA biodegradability (about 90%). Moreover, a *Streptomyces* sp. strain isolated from river water has high BPA degradability (less than 90% for 10 days) [24]. These results show that BPA degrading bacteria are widely distributed in river water.

In spite of BPA degradation in river water by bacteria, however, half-lives averaging 3-5 days may be long enough to have an effect on aquatic organisms. Several studies have suggested that no, or very low BPA contamination is present in aquatic organisms [25]. It was also found that the bile of fish near sewage treatment plants contained estrogenic substances at levels 10^4 to 10^6 times higher than those in the water [25].

Air

The photo-oxidation half-life for BPA has been calculated to be between 0.74 h and 7.4 h from a study using the atmospheric oxidation program [26]. Moreover, the transport potential of BPA to air is much lower (less than 0.0001%) than that to water (about 30%) or soil (about 68%) [26]. It is recently reported that the concentrations of BPA ranged from 2 to 208 ng/m^3 in three of seven air samples (a plastic workplace, a residence and an office building) [27]. Generally, the possibility of inhaling high BPA levels from air is very low. However, workers in companies that produce BPA-based products are an exception. It is also reported that the concentration of urinary BPA was higher in epoxy resin sprayers (average 1.06 μmol/mol creatinine) than in workers with jobs that did not involve bisphenol A diglycidyl ether (BADGE) use (average 0.52 μmol/mol creatinine) [28]. BADGE is the reaction product of 1 mole of BPA with 2 moles of epichlorohydrin and BPA is a metabolite of BADGE [29].

Soil

The soil adsorption coefficient (K_{OC}) values of BPA ranged from 314 to 1524 when calculated using a water solubility of 120 ng/L and an octanol–water partition coefficient (K_{OW}) of 3.32 [30]. These absorption values mean that BPA released to ground or surface water can be

absorbed to soil or sediments. In fact, the levels of BPA in sediments are higher than those in surface waters [31,32].

Half-life for BPA in soil is less than 3 days from a study using ^{14}C-BPA and soil. The major route of dissipation of ^{14}C-BPA in soil was the formation of bound residues [33]. However, BPA contamination in soil can be positively correlated with human densities because of an increase in BPA pollution by human wastes such as domestic and industrial wastes [34]. It seems that human wastes are the major source of BPA contamination in soil.

Plastic Substances

BPA can migrate from polycarbonate plastics. It is found that BPA concentrations eluted from new and used polycarbonate baby bottles were below 1.0 - 3.5 ppb and below 1.0 - 6.5 ppb, respectively, but were 10 - 28 ppb from used and scratched bottles [35]. Similar results were obtained for new and old polycarbonate cases. Similarly, it was also found that BPA levels in the first (new baby bottles), second (51 days of use) and third tests (169 days of use) were 0.2, 8.4 and 6.7 μg/dm^2, respectively [36].

The high levels of BPA migration from used polycarbonate containers compared to those from new ones have been studied too, which relates to the degradation of the polymer. The carbonate linkages in new containers are rather stable, but can hydrolyze in hot water or at an alkaline pH [35,36]. This means that BPA can migrate from plastics after washing and sterilization in alkaline solutions or in hot water. The more polycarbonate containers are used, the higher the possibility of BPA migration from them [36,37].

Moreover, BPA migration from plastics may be higher in food simulating liquids than in water. In studies of BPA migration from polycarbonate plastics conducted with the use of food-simulating liquids; BPA levels in ethanol and acetic acid differed with storage time and temperature but were higher than that in the water [37-39].

Human Exposure to BPA

BPA can leach into food from the epoxy resin lining of cans and from consumer products such as polycarbonate tableware, food storage containers, water bottles, and baby bottles [40]. Additional traces of BPA can leach out of these products when they are heated at high temperatures. Recent studies also suggest that the public may be exposed to BPA by handling cash register receipts and thermal papers [41]. Research is needed to determine how much BPA from these papers enters the body and how it gets there. The National

Institute of Environmental Health Sciences expects to support more research to determine if BPA in receipts poses a risk to human health [42].

Health Implications of BPA

Endocrine disruption

BPA is an endocrine disruptor, numerous studies have found that laboratory animals exposed to low levels of BPA have elevated rates of diabetes, mammary and prostate cancers, decreased sperm count, reproductive and neurological problems [3,43-45]. Early developmental stages appear to be the period of greatest sensitivity to its effects and some studies have linked prenatal exposure to later physical and neurological difficulties [5, 44-46]. Regulatory bodies have determined safety levels for humans, but those safety levels are currently being questioned or are under review as a result of new scientific studies.

Skin and Eye contact

If BPA comes into contact with the eye as a result of being handled improperly, it may cause moderate irritation of the eye with corneal injury. Dust may irritate eyes [47]. A brief contact is nonirritating to the skin. However, prolonged or repeated contact may cause skin irritation. Prolonged contact may cause an allergic skin reaction, especially when combined with exposure to ultraviolet radiation from the sun or other sources. In Europe and North America, BPA is classified as a skin sensitizer. Neither short duration nor prolonged skin contact is likely to result in absorption of harmful amounts of BPA [47].

Ingestion and Inhalation

Small amounts swallowed accidentally or incidentally by handling BPA are not likely to cause injury. Swallowing larger amounts repeatedly can cause damage to the liver or kidneys [47]. BPA found in the dust may irritate the membranes of the nose and throat [47].

Other Implications

The weight of the evidence from animal studies shows that BPA does not have the potential to be a carcinogen. BPA has not been shown to cause adverse effects on reproduction or the development of offspring in animal studies unless the doses were high enough to be toxic to the mother and the fetus. Animals that were fed high doses of BPA exhibited effects on the liver and kidney [47]. Figure 1.2 shows BPA exposure effects.

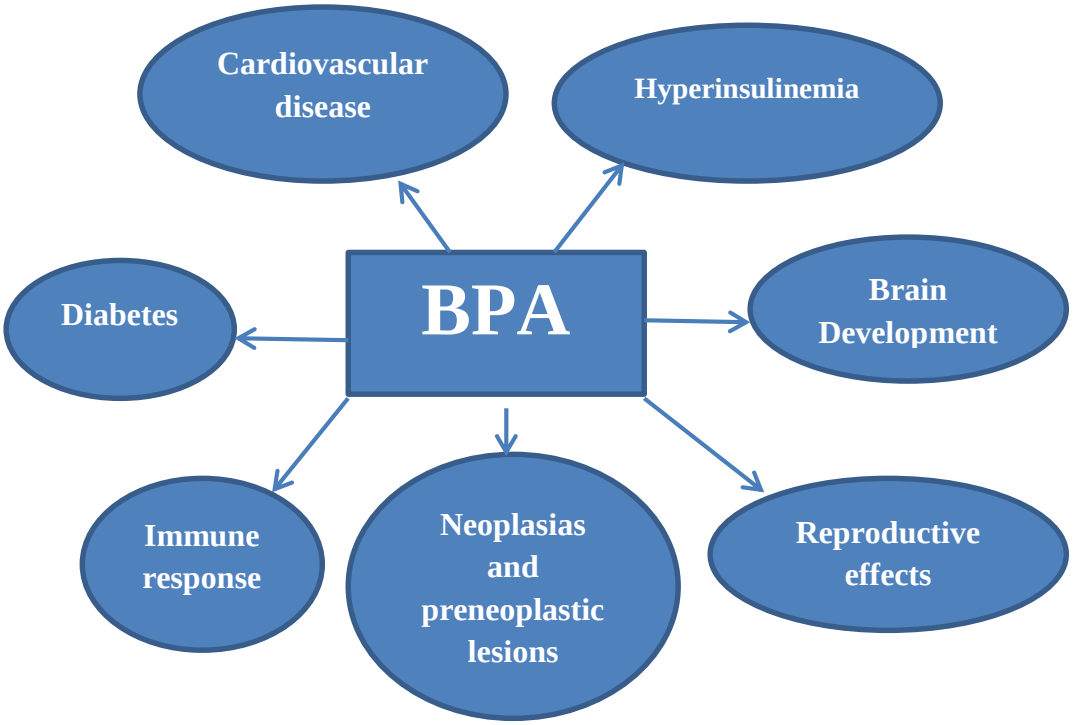


Figure 1.2. BPA exposure effects

Environmental Fate, Transport, and Bio-uptake of BPA

According to McKay level 1 modeling (which estimates the distribution of a contaminant in different environmental compartments) about 25% of an environmental release of BPA would be found in soil, 25% in sediment and 50% in water with less than 1% in biota [48].

Glycosylation

Plants can rapidly absorb BPA through their roots from water and metabolize it to several glycosidic compounds. Glycosylation, the main route of BPA metabolism in plants, leads to loss of estrogenicity of the parent compound. BPA mono- and di-b-D-glucopyranosides show reduced or no estrogenic activity in *in vitro* tests [49]. Two oxidative enzymes, peroxidase and polyphenol oxidase, are associated with BPA metabolism [50,51].

Photolysis and Photo-oxidation

Photolysis and photo-oxidation are the main non-biological pathways of BPA break down in the aquatic environment. Photodegradation of BPA is slow in pure water, but in the presence of the following, it is accelerated.

- Dissolved organic matter including humic and fulvic acid [52-54]
- Reactive oxygen species including hydroxyl and peroxyl radicals and singlet oxygen [54-56]
- Ions including ferric and nitrate ions [53,54]

Indoor Dust

In artificial indoor streams, DT50 values (time when 50% of initial BPA disappeared) were about 1 day [57].

Bio-uptake and Degradation by Fish

BPA has been found in a number of market seafood species. In Singapore, 13.3 – 213.1 µg/kg of BPA was found in prawn, crab, blood cockle, white clam, squid, and fish purchased from local supermarkets, indicating the potential for human exposure by eating contaminated seafood [58]. Zebrafish initially eliminated parent BPA with a half-life of 1.1 h. In a second phase it had a half-life of 39 h. Metabolites included sulfate and glucuronic acid conjugates [59].

Methods for Removal of BPA from the Environment

The concern of BPA as an environmental pollutant has triggered research in finding alternatives for BPA and in the possible removal of BPA from the environment.

Enzymatic Degradation

Several studies have investigated the use of enzymes for the removal of BPA from waters. Polycyclic aromatic hydrocarbons, especially the phenolic compounds have been removed by polymerization catalyzed by peroxidase enzymes [60]. A microbial peroxidase enzyme, coprinus cinereus peroxidase, efficiently removed BPA from an aqueous solution; complete removal was attained in 30 min [60].

Recombinant Plants

Scientists recently have used recombinant DNA technology to produce recombinant tobacco plants containing a gene for lignin peroxidase. The transgenic plant produced lignin peroxidase in the roots of the plants for the removal of BPA. These plants were able to remove aqueous BPA four times greater than control plants [61].

Chitosan-based BPA removal

The use of chitosan gels, powders, and porous beads in a solution of BPA and tyrosinase can result in complete removal of BPA [62]. Polyphenol oxidase (PPO) is used for the quinone oxidation of BPA followed by the use of chitosan beads for removal of the quinone product. The optimum condition for PPO oxidation of BPA is at pH 7.0 and at 40 °C. Complete removal of BPA by the adsorption of the quinone derivative on chitosan beads was achieved in 4-7 h [62].

Electrocatalysis

An electrocatalytic approach has also been used in the degradation and removal of BPA from water [63,64]. Electrochemical oxidation of BPA has been attempted with carbon electrodes; polymerization of BPA in the solution resulted in inactivation of the carbon electrode because of the deposition of polymer film on the electrode [63]. In order to improve the stability and reusability, the "Electrodes Ionic Liquids" (ILs) have been used. PbO_2-ILs/Ti electrodes were able to electrocatalyze the degradation of BPA up to pH 9 [64].

Novel Materials

Novel materials have been designed and developed for the removal of BPA. Titanium oxide (TiO_2) powder with a zeolite adsorbent has been used as a photo-catalyst for the degradation and removal of BPA from water. Under UV-irradiation 100 mg of TiO_2 powder or sheet was able to remove more than 90% BPA from 50 mL of 100 µM

solution after 24 or 72 h [65]. Molecular imprinted particles (MIP) have been developed for BPA selection and removal from water. The binding capacity of the BPA-MIP, for saturation, was noted to be 30.26 µmol BPA/g MIP [66].

Enzymatic degradation and removal of BPA is effective, resulting in up to 100% removal. However, the use of enzymes can turn to be inefficient for industrial use because of the possible inactivation of the enzymes and time they take to carry out a reaction.

Electrocatalysis can help achieve high removal of BPA in less time. The concern with electrocatalysis is the stability and cost of the materials used. Novel materials such as MIP are being developed and investigated for the removal of BPA. The materials mentioned here are able to remove BPA from water, but their efficiency for large scale operations still need to be investigated.

Bisphenol Analogues

Following the widespread use of BPA, concerns have been raised regarding its leaching from packaging and storage containers into food and beverages. Many studies have reported BPA as an environmental contaminant, showing its occurrence in environmental compartments such as air, water, soil, sediment, indoor dust and human tissues [25-27]. Keeping in mind the harmful health effects of BPA, several companies have voluntarily taken BPA out of their plasticware and canned food packaging. However, they have substituted it with other analogues of BPA such as BPS, BPP, BPZ, BPAP, BPAF and BPF [67,68]. These structural analogues are not well researched but recent studies revealed that they are still harmful. Nevertheless, companies are using "BPA-free" labeling to differentiate their products as more environmental friendly [67-69].

Bisphenol analogues are a group of chemicals with two hydroxyphenyl functional groups. These include bisphenol A [2,2-bis (4-hydroxyphenyl) propane; BPA], bisphenol A diglycidyl ether [2,2-bis (4-glycidyloxyphenyl) propane]; BADGE], bisphenol AF [4,4'-(hexafluoro-isopropylidene) diphenol; BPAF], bisphenol AP [4,4′-(1-Phenylethylidene) bisphenol ; BPAP], bisphenol F [4,4'-dihydroxydiphenylmethane; BPF], bisphenol P [4,4'-(1,4-phenylenediisopropylidene) bisphenol; BPP], bisphenol S [4,4'-sulfonyldiphenol; BPS] and bisphenol Z [4,4'-cyclohexylidenebisphenol; BPZ]. These analogues are also threatening to become environmental contaminants in the future [67,68]. A number of

analogues are currently in production and consumption and among those, the most widely used are of BPF, BPAF, BPS and BADGE. Table 1.2 shows structures and chemical names for the eight bisphenol analogues analyzed in this study.

Bisphenol F

Bisphenol F is a bisphenol derivative with antioxidant activities. Bisphenol F has been reported to exhibit estrogen agonistic properties. BPF is used to make epoxy resins and coatings in lacquers, varnishes, liners, adhesives, plastics, water pipes, food packaging and dental sealants. BPF has been reported to induce DNA strands breaks [70]. BPF genotoxicity depends on the metabolic capabilities of cells [71].

Bisphenol AF (BPAF)

BPAF is a fluorinated compound related to bisphenol A in which the two methyl groups are replaced with trifluoromethyl groups. BPA binds with human estrogen-related receptor gamma (ERR-γ); BPAF binds alpha and beta receptors but ignores ERR-γ. Instead, BPAF activates ERR-α and binds to and disables ERR-β [72].

Bisphenol S (BPS)

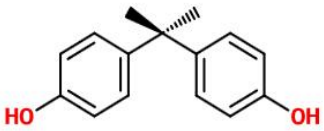
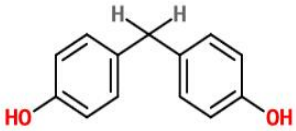
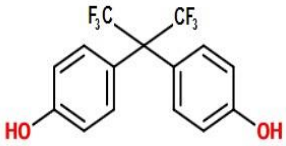
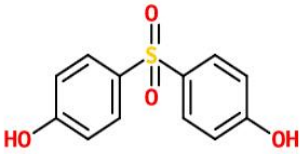
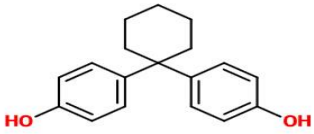
BPS has two phenol functional groups on either side of a sulfonyl group. It is an analogue of bisphenol A (BPA) in which the dimethylmethylene group [$C(CH_3)_2$] is replaced with a sulfone group (SO_2) [73]. BPS has become common as a plasticizing agent following the widespread bans on the use of BPA due to its estrogen-mimicking properties, and bisphenol S can now be found in a variety of common consumer product [74,75]. Bisphenol S also has the advantage of being more stable to heat and light than BPA and besides that it also has the endocrine disruptive effects [76].

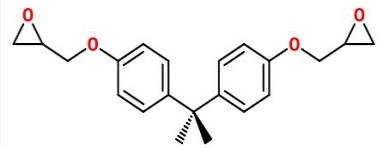
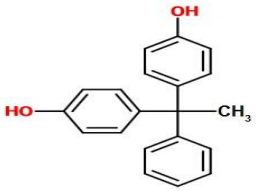
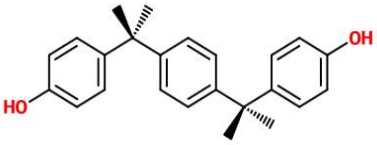
Bisphenol A diglycidyl ether (BADGE)

BADGE is another bisphenol analogue used as a constituent of epoxy resins. It is a derivative of BPA and glycidol which is used in epoxy resins for its cross-linking properties [77-79]. Food cans have an epoxy resin coating to prevent food interaction with the metal. They are resistant to most solvents and can bond to a metal substrate [77,78]. The production of epoxy resins uses BPA diglycidyl ether (BADGE) which is formed by a reaction of BPA with epichlorohydrin [77,79]. Residues of unreacted BPA present in BADGE can migrate into food [78,79]. Additionally, non-crossed linked residues of BADGE in the can coating can migrate into the food which can be accelerated

at elevated temperatures. BADGE has proven endocrine disruptive effects in experimental animals [77-79].

Table 1.2. BPA and its structural analogues (Sigma Aldrich)

Compound	Structure	Chemical Name
BPA		2,2-bis (4 hydroxyphenyl) propane
BPF		4,4'-dihydroxydiphenyl methane
BPAF		4,4'-(hexafluoro-isopropylidene) diphenol
BPS		4,4'-sulfonyldiphenol
BPZ		4,4'-cyclohexylidenebisphenol

Compound	Structure	Chemical Name
BADGE		2,2-Bis[glycidyloxy)phenyl] propane
BPAP		4,4′-(1-Phenylethylidene) bisphenol
BPP		4,4'-(1,4-phenylenediisopropylidene) bisphenol

Safe Alternative for BPA

With the increasing evidence that BPA in the environment may cause adverse health effects there is a desire to develop alternatives for BPA which are safe for all life forms. Some of the newer plastics include carbon dioxide based carbonate polymers, cyclic olefin copolymers and biobased polyhydroxyalkanoates [80]. One of the most successful new polymers, called Tritan, is a polyester copolymer made from dimethyl terephthalate. Tritan is a strong competitor against polycarbonates in consumer products due to its strength, clarity, and temperature resistance [80].

A new compound called bisguaiacol F (BGF) made from lignin as an alternative to BPA is being developed by researchers at the University of Delaware. The compound BGF has a similar molecular shape to BPA and is expected to have properties like BPA that can be useful in polymers such as polycarbonates, epoxy resins, and even in polystyrene, PVC, etc. [80]. BGF is structurally similar to BPA, with two hydroxyphenyl groups. It is synthesized by reacting two lignin breakdown products, vanilyl alcohol and guaiacol [81]. Figure 1.3 shows chemical reaction for BGF formation.

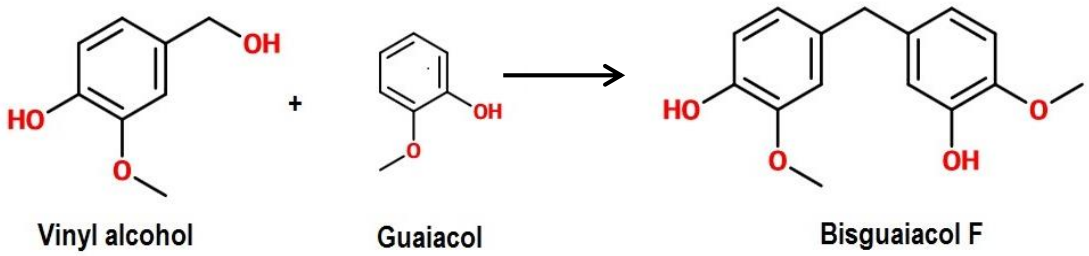


Figure 1.3. Chemical reaction for bisguaiacol F (BGF) formation

FDAs Current Perspective on BPA

According to FDA's current assessment, BPA is safe at very low levels that occur in some food which is based on review by FDA scientists after several studies. With the concerns expressed in the last few years about the safety of BPA, the FDA initiated additional studies to help determine whether or not BPA is safe as it is currently used in food packaging and containers. Some of these studies have been completed and others are ongoing. The FDA's studies are being conducted by the agency's National Center for Toxicological Research (NCTR). The results from studies so far support FDA's assessment that the use of BPA in food packaging and containers is safe [82].

Canadian Government's Stand on BPA

In 2008, Environment Canada released its final "Screening Level Risk Assessment" for BPA. As part of this assessment, Environment Canada proposed adding BPA to Schedule 1 of the Canadian Environmental Protection Act (CEPA 1999) [83]. In their review, Canadian authorities have stated that "the potential impacts of bisphenol A in the Canadian environment are of sufficient magnitude to warrant use of a precautionary approach in response to uncertainties in the evaluation of risk" [84].

Health Canada's Food Directorate has concluded that the current dietary exposure to BPA through food packaging is not expected to pose a health risk to the general population, including newborns and infants [85].

Evaluation of Risk to Public Health through BPA Exposure

There are a few questions needed to be answered in order to determine risk posed on health of human beings through BPA exposure. The questions are as follows:

Does BPA in Low Doses Exhibit Deleterious Effects on Human?

BPA does not bind to α-fetoprotein, thus exposure of fetuses and neonates to even low doses could alter organogenesis and histogenesis [86]. Moreover, recent studies have revealed a variety of pathways through which BPA can "stimulate" cellular responses at very low concentrations, below the levels where BPA is expected to bind to the classical nuclear or genomic estrogen receptors (ERs) [87].Thus, low levels of BPA appear to act via mER, GPR30, ERs positioned in non-classical locations such as the cytosol and mitochondria, as well as other receptors. These receptors are likely to be present in different cell types at various developmental times and response stages, low-dose BPA exposure could have profoundly diverse effects on the same organ at different life stages [4].

Are Humans Exposed to Truly Significant Levels of BPA?

Since 1999, more than a dozen studies using a variety of different analytical techniques have measured free, unconjugated BPA concentrations in human serum at levels ranging from 0.2 - 20 ng/mL (µg/L) serum [88]. The relatively high levels of BPA in the serum of pregnant women, umbilical cord blood, and fetal plasma indicate that BPA crosses the maternal-fetal placental barrier. BPA has also been measured in human urine from several populations around the world. These studies confirm widespread human exposure to BPA, as suspected from the studies of BPA in blood [88]. A 2005 study conducted by the U.S. Centers for Disease Control and Prevention (CDC) detected BPA in 95% of urine samples from a reference population of 394 American adults using isotope dilution gas chromatography/mass spectrometry with average levels of total BPA in male and female urine of 1.63 and 1.12 ng/mL (µg/L), respectively [88]. Importantly, in some cases, the concentrations of total BPA (unconjugated and conjugated) in human blood and other tissues and fluids were higher than those that stimulated a number of molecular endpoints in cells cultured *in vitro* and appeared to be within the range of the levels of BPA in animal studies [89].

Does Human Exposure Occur Exclusively through the Oral Route?

Few studies have estimated total BPA exposure. Using data from environmental (water, air, soil) and food (can inner surfaces, plastic containers) contamination, it is estimated that daily human intake of BPA is less than 1 µg/kg BW/day [11]. Alternatively, the European Commission's Scientific Committee on Food estimated BPA exposure to be 0.48–1.6 µg/kg BW/day from food sources alone [90]. Two additional studies were conducted to estimate BPA exposure levels in young children. The first examined their potential exposures at home and in daycare [91]. BPA was detected in indoor and outdoor air samples, floor dust, and play area soil, and in liquid and solid foods in both locations at similar levels. Based on these environmental levels, the average BPA exposure level for young children was estimated at 42.98 ng/kg BW/day. A second observational study examining BPA exposures in 257 preschool children verified that BPA could be found in more than 50% of indoor air, hand wipe, solid food, and liquid food samples and suggested that 99% of exposures of preschool children originated from the diet; the estimated exposure from dietary sources was 52–74 ng/kg BW/day, and the estimated inhalation exposure was found to be 0.24–0.41 ng/kg BW/day [92].

Additional studies have shown that BPA can be found in dust samples, indoor and outdoor air, sewage leachates and water samples from around the world [87]. Thus, humans are potentially exposed to low doses of BPA through routes other than the verified oral exposures.

Is BPA Inactivated by Conjugation in the Digestive System?

The liver plays an essential role in BPA metabolism in both animals and humans. Through glucuronidation the liver metabolizes and facilitates excretion of both endogenous and exogenous compounds. Liver enzymes responsible for glucuronidation of BPA produce BPA glucuronide, the major BPA metabolite in animals and humans that has little or no estrogenic activity [93]. BPA is also conjugated *in-vivo* to BPA sulfate by phenol sulfotransferases found in the liver; sulfation of BPA abolishes its estrogenic activity [94]. Detailed, systematic studies have not yet determined the proportion of BPA that is metabolized to BPA glucuronide and BPA sulfate [94]. A small study suggests there may be gender differences in the concentrations of BPA metabolites in urine, with

women having higher levels of BPA sulfate and men having higher levels of BPA-glucuronide, but studies with larger sample sizes are needed to verify this finding [95]. It has been assumed that oral intake leads to complete inactivation of BPA. However, pharmacokinetic studies indicate that not all BPA is conjugated by the liver. In rodents, conjugated BPA is deconjugated by enzymes in the lower intestine and colon [96]. Studies also indicate that humans produce glucuronidases in their digestive tracts, with increasing production throughout infancy until adult levels are reached at 4 years of age, so conjugated BPA may be deconjugated and activated by infants during the digestive process [96]. This may be true for human fetuses and neonates as well.

Chapter 2

Instrumental Techniques

Capillary Electrophoresis

CE is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage [97]. Electrophoresis is defined as migration of charged ions under the influence of electric field. Arnes Tiselius was the first scientist who showed the capability of electrophoresis in free solution in an experiment for separation of proteins in 1930 [98]. His work remained unnoticed until another scientist Hjerten introduced the use of capillaries for carrying out electrophoresis in the 1960's [98]. The use of capillary to carry out electrophoresis solved problems associated with traditional electrophoresis methods. Use of capillary enhanced the efficiency and operating capabilities of traditional electrophoresis, thin dimensions of the capillaries increased the surface to volume ratio which eliminated overheating while operating at high voltages [97-100].

Basic Theory

CE is a powerful separation technique in which separation of the charged molecules is accomplished with the help of a fused silica capillary (typically 25-100 μm inner diameter) under the influence of an applied electric field. For the formation of an electric field along the migration path, an electrically conducting medium for the flow of electric current is required. The addition of a background electrolyte (BGE) in the solvent provides this continuum needed which does not change with time. BGE is often a buffer which not only maintains the pH but can also selectively influences the ionic mobilities and enhance resolution [97-103].

Electrophoretic Mobility

Electrophoretic mobility is the solute's ability to move through the buffer solution in response to applied electric field. The positively charged ions (cations) move towards the negatively charged cathode and negatively charged ions (anions) move towards the positively charged anode inside the capillary. Neutral species do not respond to electric field and thus remain stationary [97-99]. The electrophoretic mobility of an ion is proportional to the charge on the ion and inversely proportional to its radius, and is also directly dependent upon the magnitude of the applied electric field. The ion undergoes a force that is equal to the product of the net charge and the electric field strength. It is also

influenced by a drag force which is equal to the product of the translational friction coefficient and the velocity [97-100].

This leads to the expression for *electrophoretic mobility*:

$$\mu_{EP} = q/f = q/6\pi\eta r$$

where,

"f" for a spherical particle is given by the Stokes' law

"η" is the viscosity of the solvent

"r" is the radius of the ion

The rate at which these ions migrate is dictated by the charge-to-mass ratio. The actual velocity of the ions is directly proportional to E, the magnitude of the electrical field and can be determined by the following equation:

$$v = \mu_{EP}E$$

This relationship shows that a greater voltage will quicken the migration of the ionic species.

Electroosmotic Mobility

The electroosmotic mobility is also called electroosmotic flow (EOF). EOF is caused by applying high-voltage to an electrolyte-filled capillary. It refers to the movement of BGE in response to applied electric field. Generally, when an electric field is applied to a capillary filled with an aqueous BGE solution, the BGE moves towards the cathode. This occurs because the walls of the silica capillary are electrically charged. The surface of the silica capillary contains large number of silanol groups (Si-OH). At pH greater than 2 or 3 the SiOH groups lose a proton to become silanoate ions (SiO^-). The capillary wall then has negative charges, which attract and tightly bind positively charged cations from the BGE to form an inner and fixed layer at the capillary. These cations are not sufficient to neutralize all the negative charges, and other cations are more loosely bound, forming an outer mobile layer. These two layers constitute the double layer. So the inner cation layer is stationary, while the outer layer is free to move along the capillary. The applied electric

field causes the free cations to move toward the cathode creating a powerful bulk flow thus producing the electroosmotic flow [97-103].

The rate of the electroosmotic flow is governed by the following equation:

$$\mu_{EOF} = \frac{\varepsilon\zeta}{4\pi\eta E}$$

where,

"ε" is the dielectric constant of the solution

"η" is the viscosity of the solution

"E" is the field strength

"ζ" is the zeta potential.

The EOF works best with a large zeta potential between the cation layers, a large diffuse layer of cations to drag more molecules towards the cathode, low resistance from the surrounding solution so that all the SiOH groups are ionized [100]. Figure 2.1 shows EOF generation due to applied voltage.

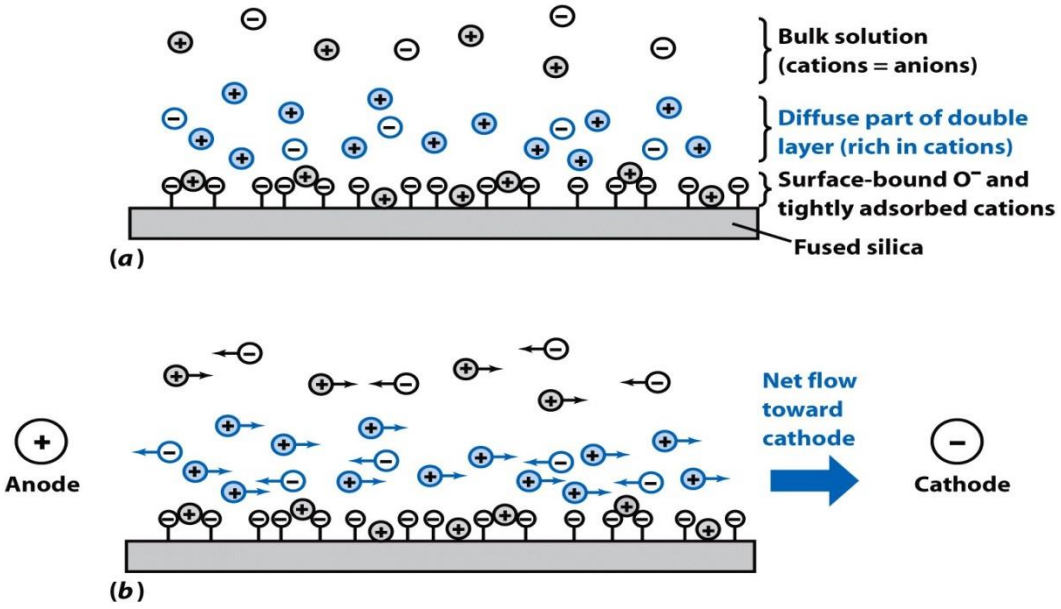


Figure 2.1. Electroosmotic flow due to applied voltage (Ref: Harris, D.C. Qualitative Chemical Analysis, Seventh Edition. W.H. Freeman and Company, 2007).

Capillary Electroseparation Methods [97-100]

There are six widely known capillary electroseparation methods.

1. Capillary zone electrophoresis (CZE).
2. Capillary gel electrophoresis (CGE).
3. Micellar electrokinetic capillary chromatography (MEKC).
4. Capillary electrochromatography (CEC).
5. Capillary isoelectric focusing (CIEF).
6. Capillary isotechophoresis (CITP).

These electroseparation methods can also be categorized into continuous and discontinuous systems as shown in the Figure 2.2.

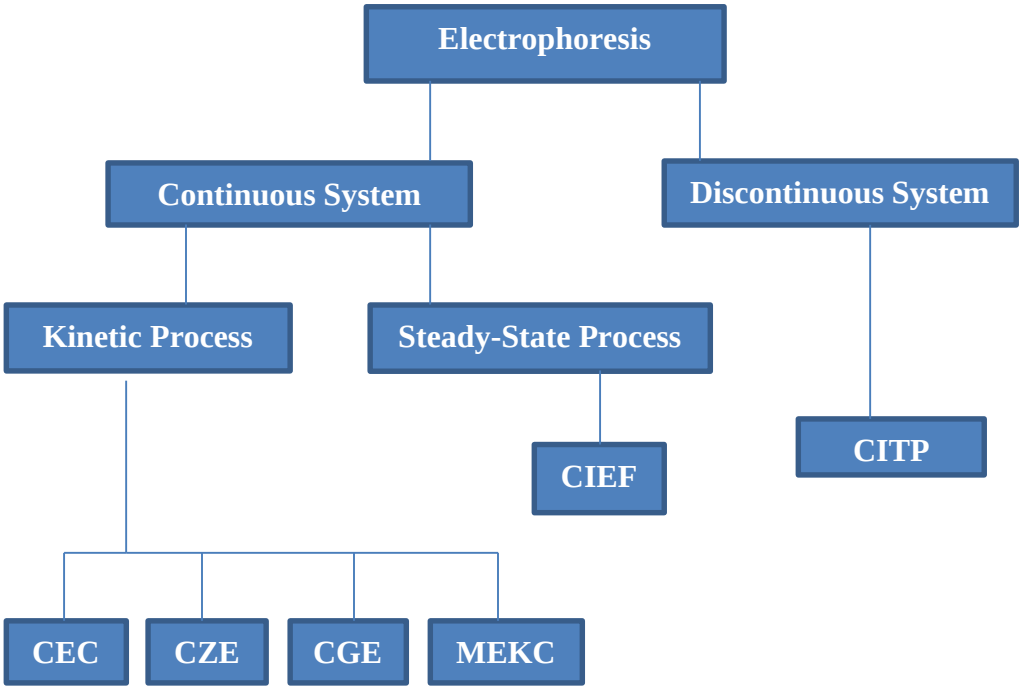


Figure 2.2. Categorization of electrophoresis techniques

Capillary Zone Electrophoresis (CZE)

Capillary zone electrophoresis (CZE) is also known as free solution capillary electrophoresis. It is the most commonly used technique of the six CE methods. A mixture in a solution can be separated into its individual components quickly and easily. The separation is based on the differences in electrophoretic mobility, which is directly proportional to the charge on the molecule, and inversely proportional to the viscosity of the solvent and radius of the ion. The velocity with which the ion moves is directly proportional to the electrophoretic mobility and the magnitude of the electric field [97,98].

Inside the fused silica capillaries the EOF drags bulk solvent along with it towards the cathode. Anions in solution are attracted to the positively charged anode, but get dragged towards the cathode under the influence of EOF. Cations with the largest charge-to-mass ratios separate out first, followed by cations with reduced ratios, neutral species, anions with smaller charge-to-mass ratios, and finally anions with greater ratios. The

electroosmotic velocity can be adjusted by altering pH, the dielectric constant of the BGE, the viscosity of the solvent, ionic strength and voltage [97,98].

Capillary Gel Electrophoresis (CGE)

In CGE, separation is based on the difference in solute size as the particles migrate through the gel. Gels are useful because they minimize solute diffusion which results in zone broadening, prevent the capillary walls from adsorbing the solute, and limit the heat transfer by slowing down the molecules. A commonly used gel apparatus for the separation of proteins is capillary SDS-PAGE. It is a highly sensitive system and only requires a small amount of sample [97,98].

Capillary Electrochromatography (CEC)

Capillary electrochromatography **(**CEC) involves the use of a packed column similar to chromatography. The mobile liquid passes over the silica wall and the particles. An EOF occurs because of the presence of charges on the stationary surface. CEC and CZE are both similar because of the presence of a plug-type flow compared to the pumped parabolic flow that increases band broadening [97,98].

Capillary Isoelectric Focusing (CIEF)

Capillary isoelectric focusing (CIEF) is a technique commonly used to separate peptides and proteins because they mostly are composed of zwitterionic molecules [97,98]. At a certain pH, known as isoelectric pH or pI, the zwitterionic molecules have an equal number of positive and negative charges; although they are charged, they behave as if they are neutral because their positive and negative charges cancel each other [97,98]. As a result, these molecules have no tendency to migrate in an electric field. At a pH below the pI, the molecule is positive, and then negative when the pH is above the pI because the charge changes with pH. A pH gradient can be used to separate molecules in a mixture. Special reagents called ampholytes are used to create a pH gradient. The ampholytes are mixture of buffers with a range of pKa values [97,98]. During a CIEF separation, typically no EOF is used (EOF is removed by using a coated capillary). When the voltage is applied, the ions will migrate to a region where they become neutral (pH = pI). The anodic end of the capillary sits in acidic solution (low pH), while the cathodic end sits in basic solution (high pH). Compounds of equal isoelectric points are “focused”

into sharp segments and remain in their specific zone, which allows for their distinct detection [97,98].

Capillary Isotachorphoresis (CITP)

Capillary isotachorphoresis (CITP) is the only method to be used in a discontinuous system. The analyte migrates in consecutive zones and each zone length can be measured to find the quantity of sample present [97,98].

Micellar Electrokinetic Chromatography (MEKC)

In micellar electrokinetic chromatography (MEKC), separation takes place in an electrolyte solution which contains a surfactant at a concentration above the critical micellar concentration (*cmc*), which is the threshold concentration at which micellization occurs[97-100]. The micelles behave as pseudo-stationary phase in the buffer. Solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed of micelles. MEKC can therefore be considered as a hybrid of electrophoresis and chromatography. It is used for the separation of both neutral and charged analytes, maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis [97-100]. One of the most widely used surfactants in MEKC is the anionic surfactant, sodium dodecyl sulphate (SDS). Cationic surfactant such as cetyltrimethylammonium bromide (CTAB) results in formation of cationic micelles and zwitterionic surfactants such as CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) results in formation of zwitterionic micelles [97-104]. Figure 2.3 shows the MEKC process.

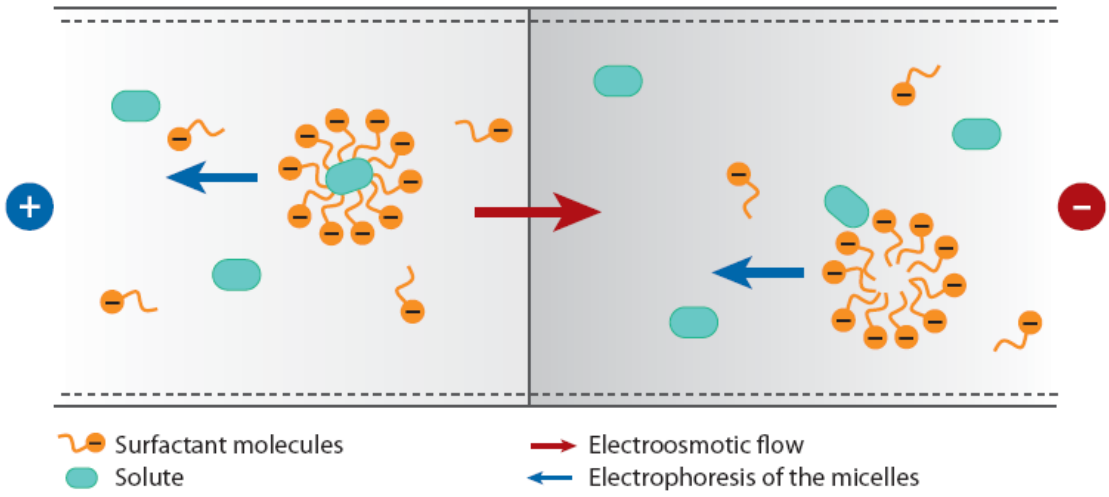


Figure 2.3. Micellar electrokinetic chromatography (MEKC) process

At neutral and alkaline pH, a strong EOF is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulphate (SDS) is used as the surfactant, the electrophoretic migration of the anionic micelles will be towards the anode (opposite direction). As a result of this the overall migration velocity will be slowed down compared to the bulk flow of the BGE solution. In case of neutral solutes, the analyte can partition between the micelle and the aqueous buffer, and has no electrophoretic mobility, so the analyte migration velocity will depend only on the partition coefficient between the micelle and the BGE.

For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelles and BGE and on the electrophoretic mobility of solute in the absences of micelles [100,104].

Since the mechanism in MEKC of neutral and weakly ionized solutes is essentially chromatographic, the migration of solute and resolution can be rationalized in terms of retention factor of the solute *(k)*, also referred to as mass distribution ratio *(Dm)*, which is the ratio of number of moles of solute in the micelles to those in the mobile pahes [100,104].

For a neutral compound, *k* is given by

$$\mathrm{k} = \frac{t_r - t_0}{t_0 \times (1 - \frac{t_R}{t_{mc}})} = \mathrm{K} \times \frac{V_s}{V_m}$$

t_R = migration time of the solute

t_0 = analysis time of an unretained solute

t_{mc} = micelle migration time

K = partition coefficient of the solute

V_S = volume of the micellar phase

V_M = volume of the mobile phase

Likewise, the resolution between two closely-migrating solutes (R_s) is given by:

$$\mathrm{R_S} = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_b}{k_b + 1} \times \frac{1 - \frac{t_0}{t_{mc}}}{1 + k_a \times \frac{t_0}{t_{mc}}}$$

N = number of theoretical plates for one of the solutes

α = Selectivity

k_a and k_b = Retention factors for both solutes respectively

Parameters of MEKC

The main parameters considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

Instrumental parameters

Voltage

Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production (Joule heating) which results in change in temperature and viscosity of the buffer in the cross-section of the capillary. This effect can be significant with high conductivity buffers such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution [105].

Temperature

Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the viscosity of the buffer. These parameters contribute to the shift in migration time of the solutes. A good cooling system can improve the reproducibility of the migration time for the solutes [105].

Capillary

The dimensions of the capillary, such as length and internal diameter, contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electric fields (at constant voltage), increase migration time and improve the separation efficiency. The internal diameter of the capillary helps to control heat dissipation and consequently the sample band broadening [105].

Electrolytic solution parameters

Buffer pH

Change in pH does not modify the partition coefficient of non-ionized solutes but it can modify the EOF in uncoated capillaries. The EOF decreases with the decrease in buffer pH and therefore increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time [105].

Surfactant type and concentration

Surfactant in MEKC is referred to as a pseudo-stationary phase. It acts in the same way as the stationary phase in chromatography. It affects the resolution since it modifies separation and selectivity [105].

Organic solvents

In order to improve MEKC separation of sparingly soluble or hydrophobic compounds, organic modifiers such as methanol, propanol and acetonitrile can be added to the electrolytic solution. The addition of these organic modifiers decreases migration time and the selectivity of the separation [104,105]. Since, the addition of organic modifiers affects the *cmc*, a given surfactant concentration can be used only within a certain percentage of organic modifier before the micellization is inhibited or adversely affected, resulting in the absence of micelles [104,105]. Therefore, the dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible; in some cases the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically [104,105].

Additives for Chiral Separations

For the separation of enantiomers using MEKC, a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions. Micelles that have a moiety with chiral discrimination properties include salts of *N*-dodecanoyl-L-amino acids, bile salts, etc., which contain micellized chiral surfactants [105].

Other Additives

Several strategies can be carried out to modify selectivity by adding chemicals to the buffer. The addition of several types of cyclodextrins to the buffer can also be used to reduce the interaction of hydrophobic solutes with the micelles, thus increasing the selectivity for the compound [105].

Detection Methods

Separation by capillary electrophoresis can be detected by several detection devices. The three most common detectors employed are as follows:

1. UV Detector
2. Photodiode Array Detector
3. Laser Induced Fluorescence Detector

UV Detector

The majority of commercial systems use UV or UV-Visible absorbance as their primary mode of detection. In these systems, a section of the capillary itself is used as the detection cell which enables detection of separated analytes without loss of resolution [106,107].

The UV detector optics include an ultraviolet light source, selectable wavelength filters, aperture, capillary and a single photodiode detector. The light source is the deuterium lamp with a wavelength range of 190 - 600 nm [106,108]. Two lenses focus and direct the output of the lamp through one of the wavelength selecting filters located in the rotating wheel behind the capillary cartridge [106-108]. Figure 2.4 shows UV detector optics layout.

The working mechanism is based on measurement of absorbance of solutions through a capillary having certain path length. The concentration of absorbing analyte is directly proportional to absorbance which corresponds to Beer's law [106,107]. The Beer's law is represented by an equation as follows:

$A = \mathcal{E}bc$

"$\mathcal{E}$" is the extinction coefficient or molar absorptivity.

"c" is the concentration of analyte

"A" is measurement of absorbance of solution

"b" is pathlength of cell

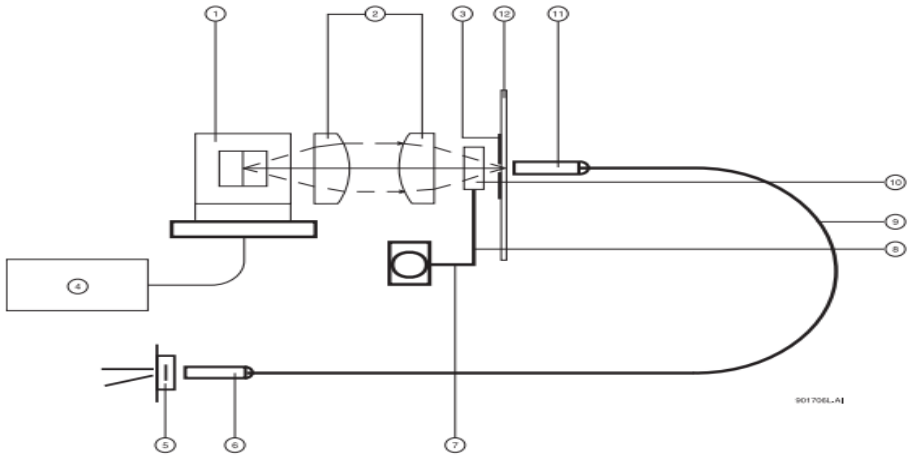


Figure 2.7 UV Optics Layout

1.	Capillary Aperture	2.	Fused Silica Lenses
3.	Deuterium Lamp	4.	Lamp Power Supply
5.	Photodiode	6.	Fiber Optic Connection
7.	Motor	8.	8 Position Filter Wheel
9.	Filter Position (i.e. 214 nm)	10.	Fiber Optic Cable
11.	Fiber Optic Connector	12.	Capillary

Figure 2.4. UV detector optics layout (Ref: Beckman Coulter P/ACE MDQ^{TM} User's Guide)

Photo Diode Array (PDA) Detector

The PDA detector uses the absorbance of light to detect the presence of analytes as they pass through the detection window. Besides that, PDA detector can provide spectral analysis of samples. Spectral signatures obtained in this way can be useful in identifying unknowns [106].

Laser Induced Fluorescence (LIF) Detector

The LIF detector consists of detector module, the LIF interconnect module and a laser module. It uses a laser light source. A 488 nm argon-ion laser and a 635 nm diode laser are mostly used. Other lasers can also be adapted. The LIF detector can use dual lasers and dual photodetectors, making it a true dual wavelength. A fiber cable transmits excitation light from the laser to the capillary in the cartridge. Substances in the capillary which fluoresce at the laser wavelength are detected [106].

Advantages of Using CE

Capillary electrophoresis (CE) is a powerful technique for the separation of charged metabolites, offering high analyte resolution. The advantages of CE involve ease of automation, small sample size, robust separation efficiency and short duration of analysis [97,98]. CE provides unparalleled resolution in comparison to chromatography. An open tubular column eliminates multiple paths and reduces the plate height and improves resolution. There is no stationary phase in capillary electrophoresis, which eliminates the ‘mass transfer” term from the *Van Deemter*’s equation, which comes from the time needed for the analyte to equilibrate between the mobile and stationary phases. Longitudinal diffusion is the only source of peak broadening in capillary electrophoresis [97].

$$H = A + B/u + C u$$

Multiple Paths **Longitudinal diffusion** **Equilibration Time**

where, H is the plate height (which is proportional to the variance of a peak), u is the linear flow rate, and A, B and C are constants for a given column/ capillary and stationary phase.

Capillary electrophoresis generates 50000 - 500000 theoretical plates, which is an order of magnitude better performance than chromatography [97].

Liquid Chromatography-Mass Spectrometer (LC/MS)

Liquid chromatography-mass spectrometry (LC/MS) is a high performance (pressure) liquid chromatography (HPLC) system attached to a mass spectrometer. The liquid chromatography (LC) system separates compounds in the liquid phase based on their relative affinities for the stationary phase of the column. Compounds that have a stronger affinity for the stationary phase take longer time to elute through the column than those with a weaker affinity for the stationary phase [109-111]. Figure 2.5 shows a schematic diagram of liquid chromatograph and Figure 2.6 shows a schematic of mass spectrometer.

Resolution using liquid chromatography is not well pronounced and peak overlap is often observed. In order to overcome the limitations associated with the liquid chromatographic (LC) separation a mass spectrometer is attached to the LC system. The mass spectrometer gives masses of all the components present in peak which can be a very good starting point to identify the peak [109].

Columns for liquid chromatographic systems are packed with different kinds of stationary phase particles. Selection of column is such that it enables adequate separation of analytes, it should be compatible with the liquid chromatographic systems flow requirements and it also needs to be inert to reactions with the eluent, analytes, or matrix of the samples. C18 columns are most commonly used [109-111].

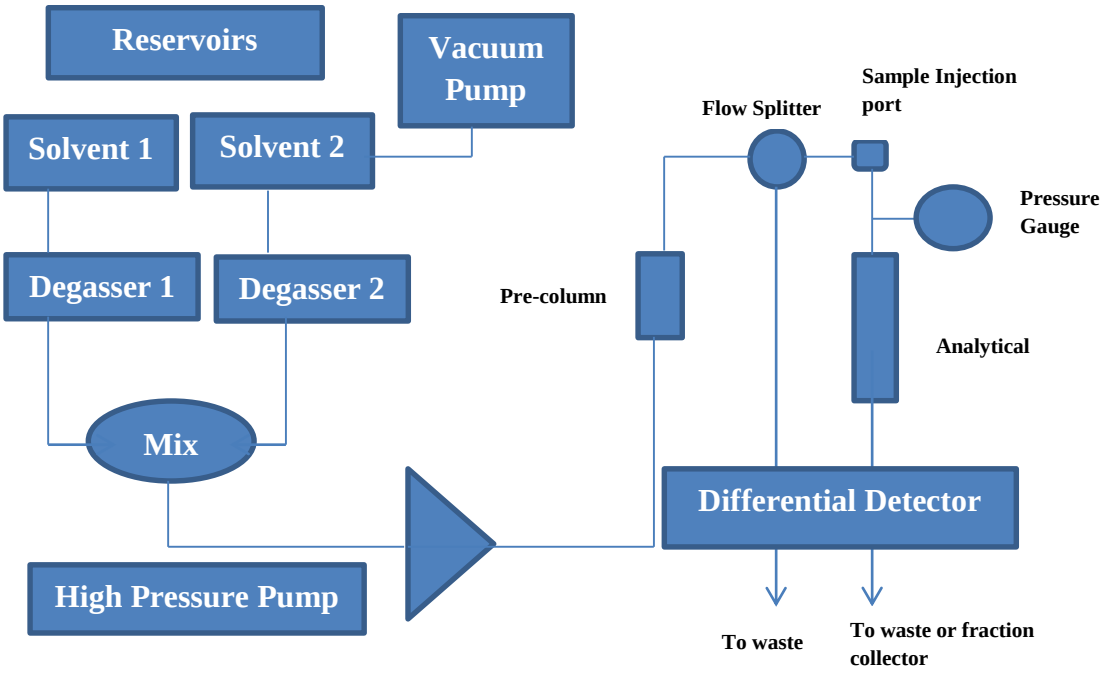


Figure 2.5. Schematic of liquid chromatograph

Mass Spectrometric Detection

Mass spectrometry systems detect analytes by placing a charge on them, followed by separation based on the mass to charge ratio of the analyte. After the ionization of analyte, the charged analyte of specific mass to charge ratio then reaches the detector where it is converted into a signal which is interpreted by a computer. Literature suggests that placing a charge on the analyte (ionization) is usually the limiting factor in LC/MS detection [110].

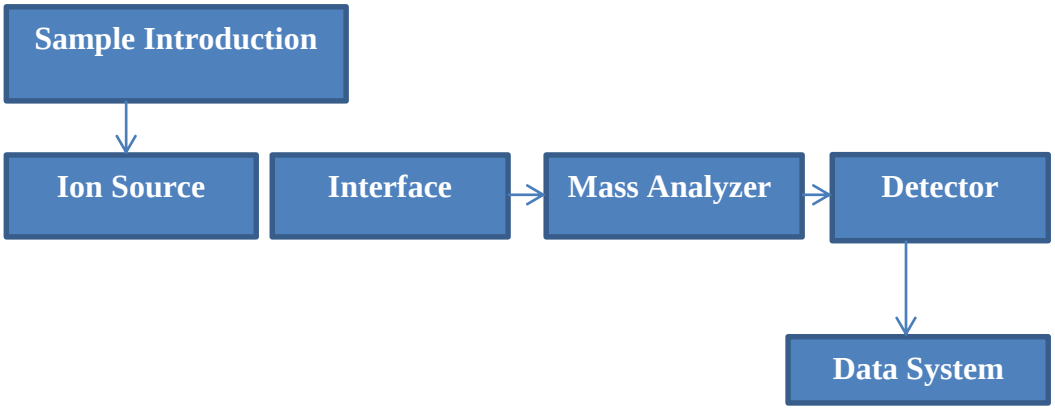


Figure 2.6. Schematic of mass spectrometer

Eluent

An ideal eluent should be capable of dissolving the analytes, and causes them to elute with adequate peak separation. It should be volatile and inert to reacting with the analyte during the liquid chromatography separation. It must also provide adequate ionization of the analyte in the ionization interface. It is hard for one solvent to perform all these tasks, so a gradient of multiple solvents and ionization agents are combined to make an ideal eluent [112].

Types of Adsorption Chromatography

Adsorption chromatography can be done in two ways depending on the polarity of the stationary phase [112].

i. Normal-Phase Chromatography
ii. Reversed-Phase Chromatography

Normal-Phase Chromatography

Normal-phase chromatography separates analytes based on their affinity for a polar stationary surface such as silica; hence it is based on analyte ability to engage in polar interactions such as hydrogen-bonding and dipole-dipole interactions with the sorbent surface. It uses a non-polar, non-aqueous mobile phase such as, chloroform, and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase [112].

Reversed-Phase Chromatography

Reversed-phase chromatography (RPC) has a non-polar stationary phase and an aqueous moderately polar mobile phase. Hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and hydrophilic molecules in the mobile phase will pass through the column and are eluted first. It utilizes stationary phases which are organochlorosilane with an R group of n-octyl (C8) or n-octyldecyl (C18) hydrocarbon chain. It is usually carried out using a buffered aqueous phase as a polar mobile phase; the pH of the buffer should be less than 7.5 since the silica substrate can hydrolyze in basic solution. Reversed phase chromatography is the most common technique because it applies to a very wide range of molecules including charged and polar molecules. It also allows precise control of variables such as organic solvent type

and concentration, pH, and temperature. RPC columns are efficient and stable. It is a very robust technique [112,113].

Solvents in Reversed-Phase Chromatography

The reversed-phase solvents are by convention installed on the HPLC. Solvent A usually is an aqueous solvent and the solvent B is an organic solvent such as acetonitrile, methanol, propanol, etc. So the solvent A usually is HPLC grade water with 0.1% acid and solvent B is generally an HPLC grade organic solvent such as acetonitrile or methanol with 0.1% acid. The acid is used to improve the chromatographic peak shape and to provide a source of protons in reversed phase LC/MS. Most commonly used acids are formic acid, trifluoroacetic acid, and acetic acid [113].

Ionization Agents

Ionization agents are added to the eluent to act as an ionization aid by increasing the efficiency of ionization of the analyte, and act as an ionization buffer by providing a matrix that is consistent between samples causing even ionization of the analyte. The ionization aid can have a large effect upon the efficiency of ionization in the ionization interface [109,110-113]. There are three main types of ionization agents:

- Acidic ionization agent - Formic acid
- Weakly acidic ionization agent - Ammonium formate
- Alkaline ionization agent - Ammonium carbonate.

Ionization techniques in LC/MS

The most widely used ionization techniques in liquid chromatography-mass spectrometry (LC/MS) are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) [109-111].

Electrospray Ionization (ESI)

Electrospray ionization is the most widely used interface in LC/MS applications, and it is a soft ionization technique. ESI operates by pumping the solution of sample through a stainless steel capillary needle at a rate of few microliters per minute. The needle is charged (3 - 6 kV) with respect to a cylindrical electrode that surrounds the needle [110-114]. The resulting charged spray of fine droplets then passes through a desolvating capillary, where evaporation of solvent and attachment of charge to the

analyte molecule takes place. As evaporation of solvent occurs, the droplets become smaller, their charge density increases and desorption of ions in to the ambient gas occurs. The ESI inlet allows the adjustment of capillary temperature, capillary and source voltages, along with physical alignment of the spray nozzle [110-114].
ESI should obtain better ionization efficiencies at lower flow rates through the LC column. The optimal flow rate will be a compromise between retention time, peak broadening and analyte concentration in the eluent. ESI is reported to show a correlation between ion intensity detected at the mass spectrometer and the concentration of the analyte in the eluent. The correlation shows the larger the concentration of analyte in the eluent the larger the detected ion intensity. Reducing the flow of eluent through an ESI interface can enhance the detection of the analyte [109,100,111,114]. Figure 2.7 shows electrospray ion source and Figure 2.8 shows desorption of ions from solution in electrospray system.

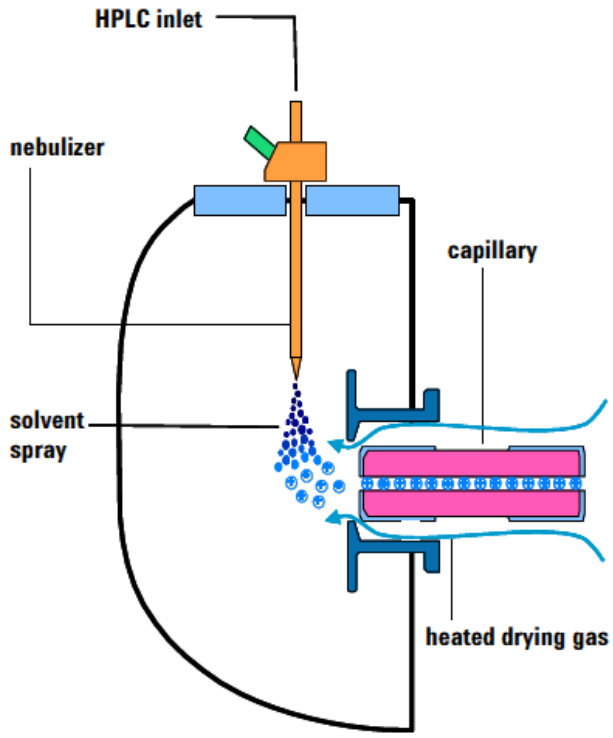


Figure 2.7. Electrospray ion source (Ref: Agilent Technologies, User Manual for LC-MS System, 2011)

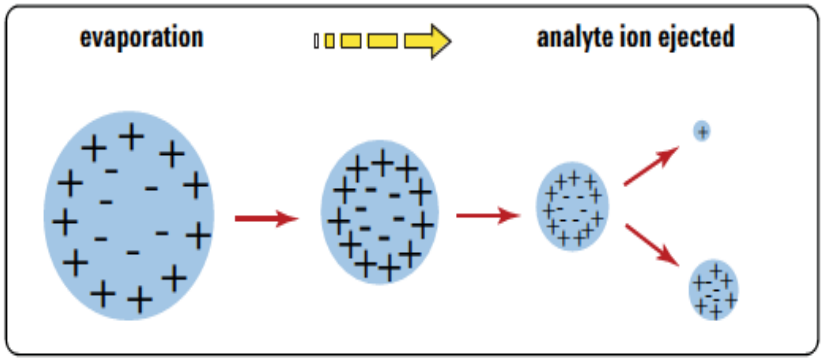


Figure 2.8. Desorption of ions from solution (Ref: Agilent Technologies, User Manual for LC-MS System, 2011)

Atmospheric Pressure Chemical Ionization (APCI)

APCI is a slightly harder ionization technique than ESI, but still considered soft. APCI can ionize compounds that are less polar and normally not ionized by ESI. It operates by exposing analytes in the solvated matrix to an elevated temperature in the source capillary to convert the analytes and solute to the gaseous phase before spraying the gas towards a charged 'probe' which ionizes the analyte via complex mechanisms. The partially solvated analyte ions are then exposed to conditions to aid removal of the solvate and unionized molecules from the charged analytes, before guiding the de-solvated charged analytes into the mass spectrometer almost analogous to ESI. APCI generally induces a larger degree of thermal decomposition of the analytes than the softer ESI. APCI is particularly suitable for analysis of non-volatile and thermally stable analytes and is ineffective for ionizing compounds with low vapor pressures and are thermally labile such as sugars [109,110,114].

Atmospheric pressure photoionization (APPI)

All mass spectrometers require the molecules to be in the gas phase and charged (positively or negatively ionized). In this technique, UV light photons are used to ionize sample molecules. The technique works well with nonpolar or low-polarity compounds not efficiently ionized by other ionization sources [109-111].

First the sample **(**analyte**)** is mixed with a solvent. Depending on the type used, the solvent could increase the number of ions that are formed. The liquid solution is then vaporized with the help of a nebulizing gas such as nitrogen, and then enters an ionization

chamber at atmospheric pressure. There, the mixture of solvent and sample molecules is exposed to ultraviolet light from a krypton lamp. The photons emitted from this lamp have a specific energy level (10 electron volts, or eV) that is high enough to ionize the target molecules, but not high enough to ionize air and other unwanted molecules. So only the analyte molecules proceed to the mass spectrometer to be measured [109-111].

Mass Analyzers

There are several types of mass analyzers that can be used for the separation of ions in a mass spectrometry such as, Quadrupole mass analyzer, Time of Flight mass analyzer, Magnetic Sector mass analyzer (double focusing, single focusing), Electrostatic Sector mass analyzer, Quadrupole Ion Trap mass analyzers and Orbitrap mass analyzers. Each mass analyzer has its own special characteristics and applications. The choice of mass analyzer is based upon the application, cost, and performance desired. The two mostly employed mass analyzers in LC/MS systems are Time of Flight analyzers and Quadrupole analyzers [110,111].

Time of Flight Mass Analyzer

In this mass analyzer system, ions are separated according to their velocities or time of flight. Ions from the ions source are extracted and accelerated to high velocities with the help of an electric field into an analyzer consisting of a long straight "drift tube" [109]. The ions pass along the tube until they reach a detector. The ions exiting from the ion source differ in their masses and that's why they have different velocities [109,110,111]. Heavier ions have a velocity less than that of the lighter ions. The velocity of an ion is inversely proportional to its mass. The distance from the ion's origin to the detector is fixed; the time taken for an ion to traverse the analyzer in a straight line is inversely proportional to its velocity and directly proportional to its mass. Thus, each m/z value has its characteristic time-of-flight from the source to the detector [109,110,111].

The kinetic energy of an ion leaving the ion source is:

$$T = eV = \frac{mv^2}{2}$$

The ion velocity, v, is the length of the flight path, L, divided by the flight time, t:

$$v = \frac{L}{t}$$

Substituting this expression for v into the kinetic energy equation, we can derive the working equation for the time-of-flight mass spectrometer:

$$\frac{m}{e} = \frac{2Vt^2}{L^2}$$

or, rearranging the equation to solve for the time-of-flight:

$$t = L\sqrt{\frac{m}{e}\frac{1}{2V}}$$

Quadrupole Analyzer

The quadrupole is the most widely used analyzer due to its ease of use, mass range covered, good linearity for quantitative work, resolution and quality mass spectra. Quadrupole is an analyzer that separates ions on the basis of their m/z ratio by means of electric field only [109-111].

The quadrupole is composed of two pairs of parallel, cylindrical, metallic rods. One set of rods is at positive electrical potential and the other one is at negative potential. A combination of direct current (DC) potential and radio frequency (AC) potential is applied on each set of rods. The accelerated ionic beam from the ionic source passes through a collimating hole that is aligned with the space between the four rods. Positive ions entering the space between the electrodes are attracted by the rods which are negatively charged. Similarly, ions which are negatively charged are attracted by the rods which are positively charged. The relative charge on the sets of rods is continuously changing and this causes the ions to follow an irregular oscillating path between the rods. Only those ions that can pass through the space between the rods strike the exit hole and are measured by the detector and the rest of ions strike one of the rods and are not detected [109-111].

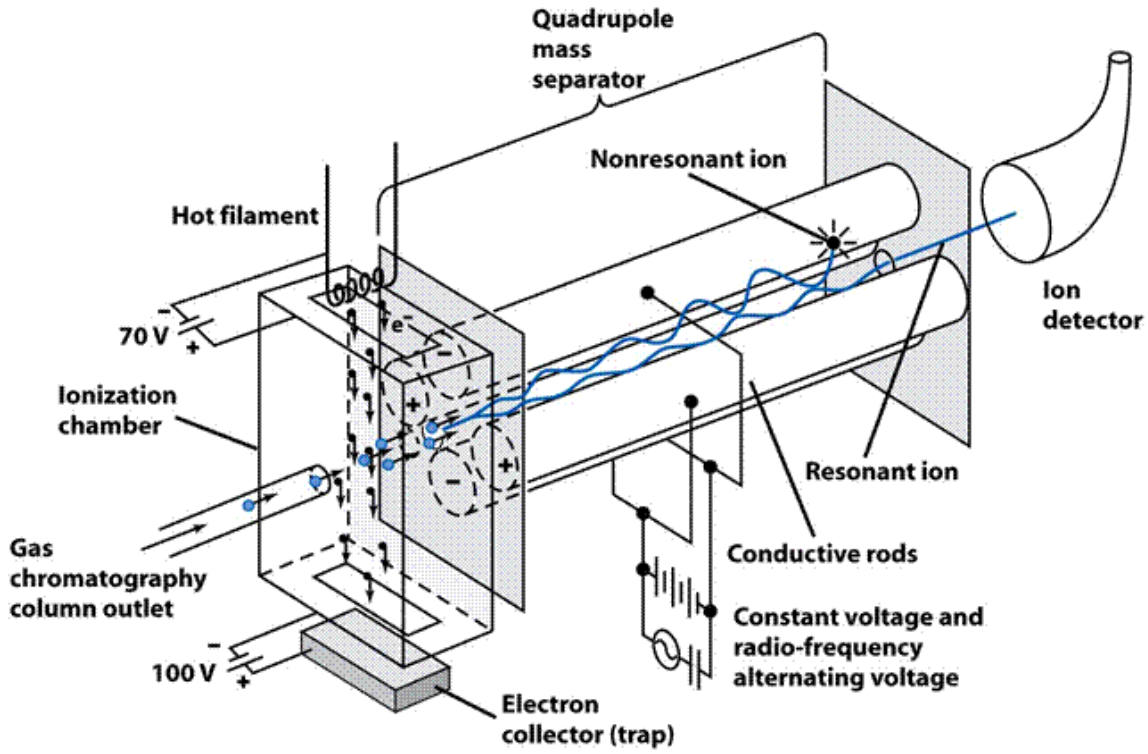


Figure 2.9. Schematic of quadrupole analyzer (Ref: Harris, D.C. Quantitative Chemical Analysis, Seventh Edition. W.H. Freeman and Company, 2007)

Advantages of LC/MS

Higher Selectivity and Sensitivity

Liquid chromatography-mass spectrometry with electrospray ionization operating in the multiple reaction monitoring modes is a standard technique for targeted quantitation because of its well-known selectivity and sensitivity [115].

Multiple Compound Screening

More recently, LC/MS replaces traditional GC methods for multi-compound screening because of its ability to analyze a wider range of food and environmental contaminants in single analysis [115].

QuEChERS (quick, easy, cheap, effective, rugged, and safe) [116]

Simple extraction procedures, such as QuEChERS, allow the efficient and reproducible extraction of hundreds of compounds from simple to complex matrices. The dilution of extracts helps to minimize possible matrix effects. In addition, the direct injection of water samples into LC/MS has gained popularity to avoid time-consuming and labor-intensive sample preparation. QuEChERS is based on acetonitrile extraction with partitioning using

$MgSO_4$ followed by a dispersive solid phase extraction (SPE) cleanup. This procedure has successfully been applied to extract pesticides, antibiotics, mycotoxins, and other compounds effectively and reproducibly from a variety of food commodities [116].

Compound Identification

The capability to perform MS/MS fragmentation is a great tool to identify and detect compounds. Typically, the ratio of two multiple reaction monitoring (MRM) transitions, is used for identification, but the acquisition of enhanced product ion spectra and library searching provides an added degree of confidence and reduces the risk of false positive and false negative results [117,118].

Goals of Research

The overarching goals of the research are as follows:

- Development of rapid and sensitive methods to separate and quantify BPA and its structural analogues on CE and LC/MS.
- Establish an analytical protocol for environmental monitoring of BPA.
- Analysis of environmental water samples for detection and quantification of BPA by using CE and LC/MS.

Research Hypothesis

- Possibility of separation of all bisphenol analogues (BPA, BPF, BPS, BPP, BPZ, BPAF, BADGE, BPAP) using a single protocol by CE.
- As we rely on substances made of plastics in our everyday life; there's a strong interest in the detection of BPA in the environmental and swimming pool waters.
- Presence of BPA in environmental waters would not be negligible.

Significance of the Research Project

BPA and its structural analogues are endocrine disruptors and are widely used as building blocks in plastics materials. BPA is a toxic compound according to Section 64 of the Canadian Environmental Protection Act, 1999 [88]. It is reported to have a hazard quotient of 2.24 at a concentration of 1.6 mg/L in river water [119]. BPA and other similar compounds have been detected in environmental waters which is not only a threat to the aquatic life but also to the human beings and wildlife. These contaminants enter the aquatic environment due to incomplete removal during wastewater reclamation processes and from the leachates from the hazardous landfills [120].

Access to safe drinking water is essential to sustain life and maintain good health. Protecting water at its source (including lakes, rivers) is the first step in ensuring that each individual has access to safe drinking water. Stopping contaminants from getting into drinking water sources provides a line of defense for the protection of ecosystems as well as health of human beings. Aside from BPA entering the natural environment, humans have direct exposure to BPA through the use of polycarbonates and epoxy resins used in food storage containers. For this reason, development of methodologies to detect and quantify BPA and its structural analogues using sensitive analytical instruments is necessary, so that efficient environmental monitoring of these compounds is made possible. This study used CE-UV and LC/MS to develop protocols for BPA detection and quantification present in environmental waters and swimming pool waters.

Studies in the past have revealed presence of BPA in fresh and seawaters. Many investigations have looked at the amount of human exposure to BPA via interaction with the environment and use of BPA based consumer products. A large-scale study conducted in Canada found that BPA concentration is 37 mg/kg in sewage sludges, 149 mg/L in industrial wastewaters and 5 mg/L in freshwater resources [121]. In Europe and North America, BPA is classified as a skin sensitizer; neither short duration nor prolonged skin contact is likely to result in absorption of harmful amounts of BPA [47]. These revelations encouraged us to analyze both environmental and swimming pool waters.

Chapter 3

Materials and Methods

Instrumentation and Capillary Conditioning

A Beckman P/ACE™ System MDQ capillary electrophoresis unit (Fullerton, CA) equipped with ultraviolet detector was employed for all CE analysis. Separations were carried out on a 50 µm (I.D.) × 365 µm (O.D.) × 50 cm (L_T) bare-fused silica capillary (Polymicro Technologies, Phoenix, AZ). The capillary temperature was controlled by a circulating liquid fluorocarbon coolant system. Bisphenols were detected at 214 nm using direct absorbance, 20 kV (normal polarity), and at a constant temperature of 25 °C. A new bare fused-silica capillary was first rinsed with methanol at 30 psi for 30 min to remove any debris or particulates. Then it was rinsed with 1 M NaOH at 20 psi for 20 min to open and remove all the siloxane bridges on the capillary surface and recover a maximum of deprotonated silanol groups. It was then flushed with deionized water at 20 psi for 15 min. The capillary was rinsed with 0.1 M NaOH for 15 min and the run buffer for 15 min at 20 psi every day prior to use. Before each injection, the capillary was rinsed for 5 min with 0.1 M NaOH, 3 min with 18 MΩ water, and finally 5 min with the run buffer. All experiments were performed using the same capillary. Samples were injected at a 5 sec interval and a pressure of 0.5 psi (3.45 kPa).The capillary was filled with deionized water and the ends immersed in vials of water when not in use.

The pH meter used in all experiments was a Symphony SB90M5 pH meter (VWR, Buffalo Grove, IL, USA). All solutions were filtered with a 0.45-µm Nylon syringe filter prior to analysis.

The schematic diagram of CE is shown in Figure 3.1 and the CE system used in the experiments is shown in Figure 3.2.

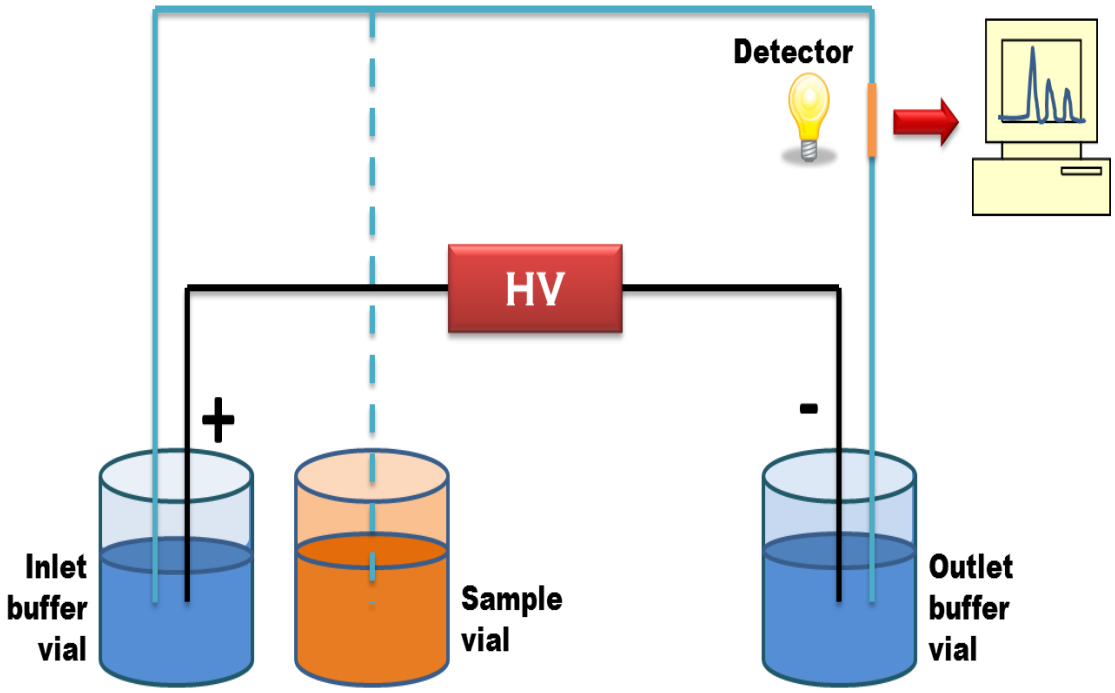


Figure 3.1. Schematic diagram of capillary electrophoresis

Figure 3.2. Capillary electrophoresis (CE) system used in the experiments

Reagents

BPA, BPF, BPS, BPZ, BPP, BPAF, BPAP and BADGE were purchased from Sigma Aldrich Canada Ltd., Oakville, Ontario, Canada. Sodium phosphate monobasic ($NaH_2PO_4.H_2O$) and methanol were purchased from Fisher Scientific Company, Fairlawn, New Jersey, USA. Sodium hydroxide was purchased from EMD Chemicals, Gibbstown, New Jersey, USA. Hydrochlororic acid (HCl) was purchased from Caledon Laboratory Chemicals, Georgetown, Ontario, Canada. Sodium dodecyl sulphate was purchased from VWR, Mississauga, Ontario, Canada.

Preparations of Solutions

Standard Preparation

Standard stock solutions for all the bisphenol analogues were prepared in a volumetric flask using HPLC grade methanol. All stock solutions were filtered by using 0.45 µm Nylon syringe filters. Sodium hydroxide solutions (0.1 M and 1.0 M) were prepared in a volumetric flask using 18 MΩ water. A 1M HCl solution was also prepared using volumetric flask. All solutions were filtered using 0.45 µm filters.

Sample Preparations

All samples were filtered using 0.45 µm Nylon filters. Standard addition method was used for analysis of samples. For each sample, five sample vials were prepared for running on CE. In each sample vial 200 µL of filtered sample was added. The first sample vial contained 200 µL of sample and 300 µL of HPLC grade methanol. For the remaining vials increasing concentrations of 1000 ppm standard BPA stock solution were added, such as 5 µL, 10 µL, 15 µL and 20 µL. Each sample vial was then topped up with quantity sufficient HPLC grade methanol to make the volume up to 500 µL.

Sample Collection

Environmental water samples were collected from eight different locations within and outside the city of Kamloops; these include river water and lake water samples. Samples were collected from South Thompson River (Riverside Park, Pioneer Park), McArthur Island Park, Kamloops Lake, Shuswap Lake, Louis Lake, Paul Lake and Adams River. Swimming pool (both indoor and outdoor) water samples from eight different locations within the city were also collected and analyzed. Tap water samples were also collected from each site to see the difference in the amounts of BPA present in tap waters and environmental and swimming pool waters. Three samples were collected from each site, averaged concentration of BPA among set of three samples was calculated. All samples were stored in a refrigerator at 7 - 8 °C in amber glass sample containers before analysis.

Background Electrolyte (BGE) Preparation

Two methods were developed on CE for the analysis of BPA and its structural analogues. Method (I) used a BGE of 25 mM phosphate monobasic and 20 mM sodium dodecyl sulphate (SDS) solution of pH 2.50 ± 0.05, made using 18 MΩ water. The pH of the solution was obtained by adjusting with 0.1 M HCl and 0.1 M NaOH. The solution was filtered using a 0.45 µm filter.

In Method (II) a BGE of 40 mM sodium tetraborate decahydrate and 15 mM SDS solution of pH 9.50 ± 0.05 was made with 18 MΩ water. The pH of the solution was obtained by adjusting with 0.1 M HCl and 0.1 M NaOH. The solution was filtered using a 0.45 µm filter. Figure 3.3 shows a flow chart on how to make the phosphate and SDS buffer. Similarly Figure 3.4. shows a flow chart on how to make a borate and SDS buffer.

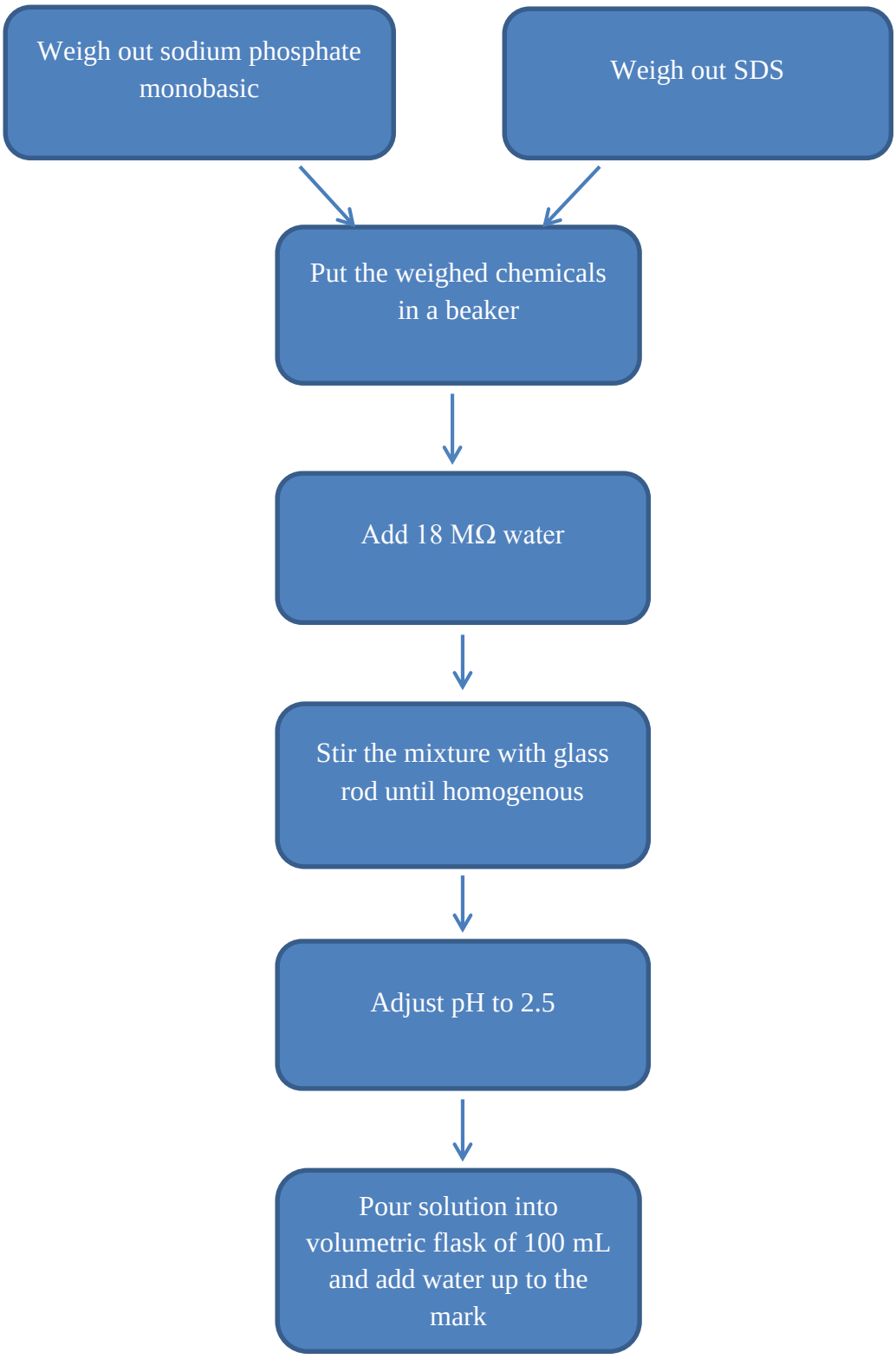


Figure 3.3. A flow chart on how to make the phosphate and SDS buffer

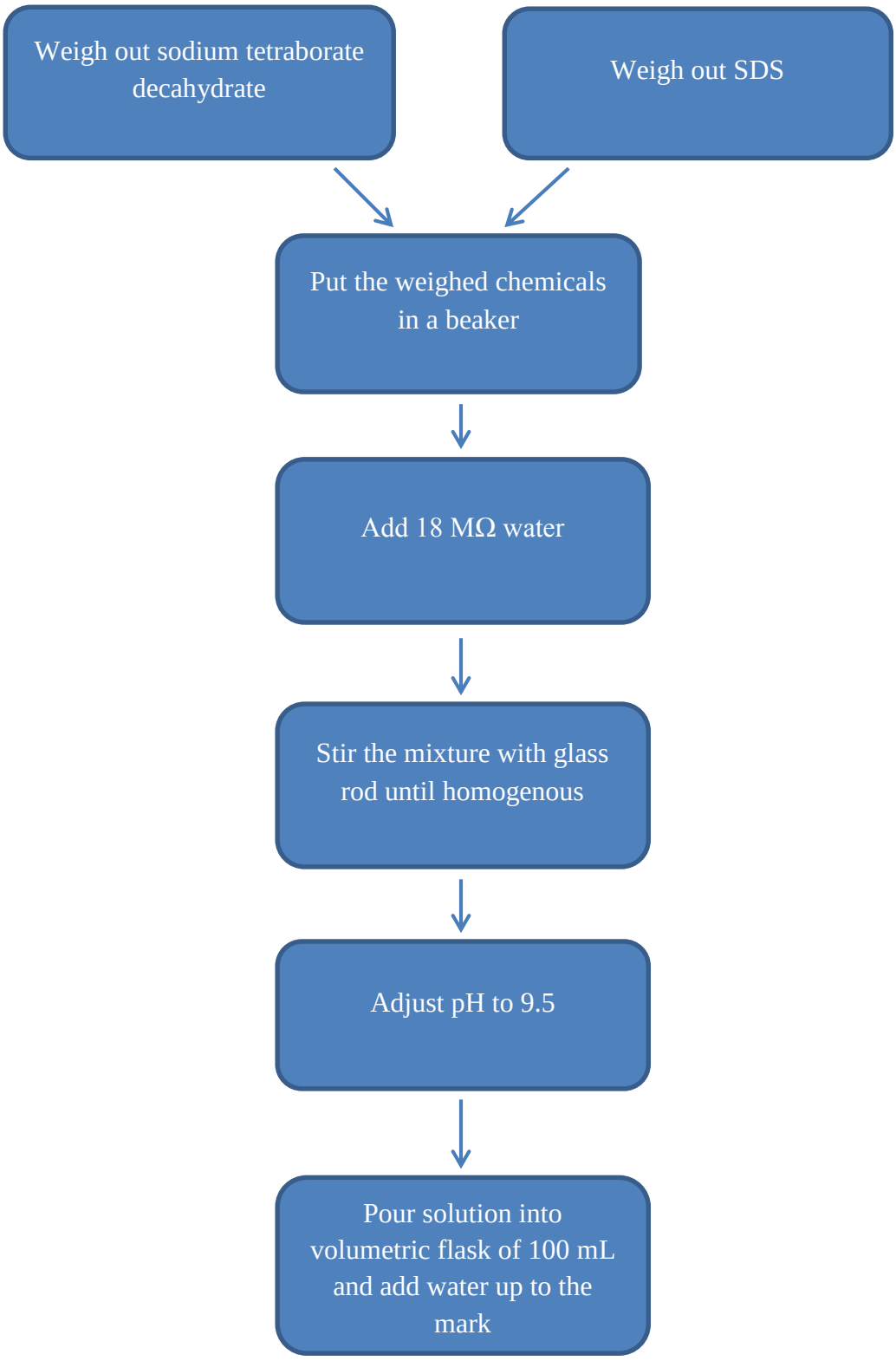


Figure 3.4. A flow chart on how to make a borate and SDS buffer

Capillary Electrophoresis Conditions

At the beginning of each run, the capillary was rinsed with 0.1 M NaOH for 5 min at 20 psi. Following that, the capillary was rinsed with water for 3 min at 20 psi. After that the capillary was rinsed with BGE for 5 min at 20 psi. In case of the separations of mixture of all the bisphenol analogues including BPA, BPF, BPS, BPP, BPZ, BPAF, BPAP and BADGE, the separation was performed for 30 min at 15 kV with a ramp time of 0.17 min using normal polarity. Real samples were only analyzed for the presence of BPA, in that case the separation time was reduced to 8 min at 20 kV with a ramp time of 0.17 min using normal polarity. A list of optimized CE parameters is shown in Table 3.1.

Table 3.1. Optimized CE parameters used for the analysis of bisphenol A and analogues

Parameters	Method (I) & Method (II)
Capillary	Fused silica, 50 μm O.D. x 50 cm total length (40 cm to detector)
Operating Temperature	25 °C
Detection	UV, 214 nm (direct)
Background Electrolyte (BGE)	Method (I) 25 mM phosphate and 20 mM SDS; pH 2.5 Method (II) 40 mM borate and 15 mM SDS; pH 9.5
Rinse Pressure	20 psi: 5.0 min (0.1 M NaOH), 3.0 min (Water), 5.0 min (BGE)
Injection of Sample	Pressure, 0.5 psi for 5.0 s
Separation Voltage	+ 20 kV
Separation Time	Method I (10 min) Method II (30 min), 0.17 min ramp time

LC/MS Instrumentation and Parameters

All analyses were carried out on an Agilent 1200 series HPLC system (Agilent Technologies, Mississauga, ON, Canada) coupled to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) spectrometer equipped with electrospray ionization (ESI) source (gas temperature: 300 °C; drying gas: 8 L/min; nebulizer: 15 psig; sheath gas temperature: 350 °C; sheath gas flow: 8 L/min; Vcap: 3500 V). BPA was analyzed in negative ion mode and mass spectra were collected between 200 and 700 m/z. A sample volume of 2 µL was injected to the LC and the flow rate was set to 0.6 mL/min. Separation was achieved on a Zorbax Extend-C18 column (100 mm × 2.1 mm; 1.8 µm particle size; Agilent, Canada) kept at a constant temperature of 40 ± 0.2 °C. Mobile phase used composed of 40% A and 60% B. A = H_2O + 0.1% acetic acid; B = acetonitrile + 0.1% acetic acid. Diluted samples and standards were analyzed without using gradient elution. LC/MS system used in the experiments is shown in Figure 3.5.

Figure 3.5. Liquid chromatography-mass spectrometer (LC/MS) used in the experiments

Reagents

Bisphenol A (BPA) was purchased from Sigma Aldrich Canada Ltd., Oakville, Ontario, Canada. HPLC grade methanol was purchased from Fisher Scientific Company, Fairlawn, New Jersey, USA. Acetic acid and acetonitrile were purchased from EMD Chemicals, Gibbstown, New Jersey, USA. HPLC grade water was purchased from Caledon Laboratory Ltd, Georgetown, Ontario, Canada.

Preparations of Solutions

Standards Preparation

Standard stock solution of BPA was prepared in a volumetric flask using HPLC grade methanol. Stock solutions were filtered by using 0.45 µm Nylon syringe filters. Mobile phase solvents (Solvent A: + H_2O + 0.1% acetic acid; Solvent B: acetonitrile + 0.1% acetic acid) were filtered using 0.45 µm filters.

Samples Preparation

All samples were filtered using 0.45 µm filters. Internal calibration method was used for analysis of samples. In each sample vial 700 µL of filtered water sample was added along with 25 ppm of triclosan. The sample volume was then topped up with HPLC grade methanol to make volume up to 1500 µL.

Calibration Standards

Calibration standards to be analyzed on LC/MS were prepared with increasing concentrations of BPA and a constant concentration of triclosan (25 ppm) and all the sample vials were topped up to the mark of 1500 µL using HPLC grade methanol. Calibration curves were constructed by plotting the peak areas of BPA (for the analyte) versus analyte concentrations, using at least six calibration points in a curve (2, 5, 10, 25, 50, 75).

LC/MS Conditions

Before analyzing the standards or samples the instrument was tuned and calibrated in 3200 m/z mass range, the polynomial plot for calibration fitted within error ±2 ppm and the instrument was set in extended dynamic range. A list of LC/MS optimized parameters are shown in Table 3.2.

Table 3.2. Optimized LC/MS parameters used in the analysis of BPA

Parameters	Method
Injection Volume	2 µL
Flow Rate	0.6 mL/min
Solvents	A (H_2O + 0.1% AA); B (ACN + 0.1% AA)
Run Time	10 min
Column Temperature	40 ºC
Gas Temperature	350 ºC
Ion Source	ESI
Ion Polarity	Negative
Drying Gas	8 L/min
Capillary Voltage	1500 V

Chapter 4

Method Validation

CE Method Validation

Percent Recovery Results of CE Method

Percent recovery is the calculation of the percentage of how much of the original substance was obtained at the end of the analysis. So it is mass of how much we obtained divided by mass we started with times 100.

Calculated by:

$$\textbf{\% Recovery} = \frac{\boldsymbol{Spiked}_{(exp)}}{\boldsymbol{Spiked}_{(true)}} \times 100$$

where,

Spiked $_{(true)}$ = Spiked Concentration

U = Unspiked Concentration

Spike $_{(exp)}$ = (S) $_{true}$ − (U)

The recovery of BPA was determined at low (10 ppm) and high concentrations (40 ppm) by comparing the peak area of the analyte in the samples with peak areas of unspiked sample analyte.

From each category of the samples such as the river, lake, swimming pool and tap water samples, one representative sample was selected and percent recovery calculations were made for four concentrations i.e., 10 ppm, 20 ppm, 30 ppm and 40 ppm using calibration equation Y = 759.54x – 97.3. Good percent recoveries for the selected samples were obtained ranging from 80% - 115%. The percent recovery data is compiled in Table 4.1.

.

Table 4.1. Percent recovery results for four water samples on CE

Sample Name	Spiked Concentration (ppm)	Recovered Concentration (ppm)	% Recovery
Pool Water (P3-A)	10	11.4	114.8%
	20	21.3	106.6%
	30	32.0	106.9%
	40	40.7	101.9%
Paul Lake	10	9.0	90.0%
	20	21.2	106.4%
	30	32.3	107.6%
	40	43.0	107.6%
Riverside Park	10	9.0	90.4%
	20	18.4	92.4%
	30	30.8	102.6%
	40	39.1	97.8%
Tap Water (P2-B)	10	10.8	108.4%
	20	20.9	104.9%
	30	28.5	95.0%
	40	45.6	114.2%

Interday and Intraday Precision Studies of CE Method

To carry out method validation and to ascertain the reproducibility of the proposed method on capillary electrophoresis instrument, intraday and interday precision studies were carried out. Intraday study was carried out by analyzing BPA standards made with HPLC grade methanol at four different concentrations including 500 ppb, 1 ppm, 3 ppm and 6 ppm. These BPA standards were analyzed at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as percent relative standard deviation (%RSD). The results of intraday and interday studies showing the repeatability of %RSDs are summarized in Table 4.2. Good repeatability was obtained for the peak area ratios (%RSD < 10%) and migration times (%RSD < 10%) during an intraday calibration of triplicate per each standard (n = 3). To

calculate the precision of the calibrations among 3 days, standard concentration levels of 500 ppb, 1 ppm, 3 ppm and 6 ppm were analyzed in triplicate in three consecutive days and quantified using calibration equations.

Table 4.2. Intraday and interday precision (%RSD) of BPA on CE

Concentration	Day 1		Day 2		Day 3	
	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD
500 ppb	6.8	0.8	7.0	3.2	8.3	6.2
1 ppm	2.8	0.4	6.6	3.2	4.8	0.5
3 ppm	2.2	1.9	3.7	2.5	8.1	2.0
6 ppm	1.0	2.0	2.6	8.2	5.5	10.0

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of CE method

The LOD calculated as the concentration that will give a response with a signal-to-noise (S/N) ratio of 3, were all in lower ppm range and the LOQ calculated as the concentration that will give a response with S/N ratio of 10 were also in lower ppm range. The LOD and LOQ for CE method are shown in Table 4.3.

Table 4.3. Limit of detection (LOD) and limit of quantitation (LOQ) for CE method

BPA	LOD (ppm)	LOQ (ppm)	Calibration Equation	R^2
	0.0106	0.0363	y = 759.54x – 97.3	0.9708

LC/MS Method Validation

Percent Recovery Results of LC/MS Method

The recovery of BPA was determined at low (100 ppb) and high concentrations (1 ppm) by comparing the peak area of the BPA in the samples with peak areas of unspiked sample analyte.

From the river, lake, swimming pool and tap water samples, one representative sample was picked and percent recovery calculations were made using external calibration equation Y = 102.65x + 6455.3 (50 ppb - 1 ppm). Good percent recoveries for the selected samples were obtained ranging from 80% - 110%. The percent recovery data is compiled in Table 4.4.

Table 4.4. Percent recovery results for four water samples on LC/MS

Sample Name	Spiked Concentration (ppb)	Recovered Concentration (ppb)	% Recovery
P1-A	100	88.1	88.1
	200	179.1	89.5
	500	496.7	99.3
	1000	803.1	80.3
Pioneer Park River Water	100	91.5	91.5
	200	191.9	95.9
	500	483.9	96.7
	1000	920.6	92.0
Pioneer Park Tap Water	100	110.3	110.3
	200	200.2	100.1
	500	493.2	98.6
	1000	989	98.9
Paul Lake	100	83.1	83.1
	200	183.3	91.6
	500	481.8	96.3
	1000	995.0	99.5

Chromatograms for Percent Recovery

Percent recovery was calculated at four different concentrations of 100 ppb, 200 ppb, 500 ppb and 1000 ppb. The chromatograms and mass spectra for Pioneer Park and Swimming pool (P1-A) water samples are shown in Figures 4.1 - 4.4.

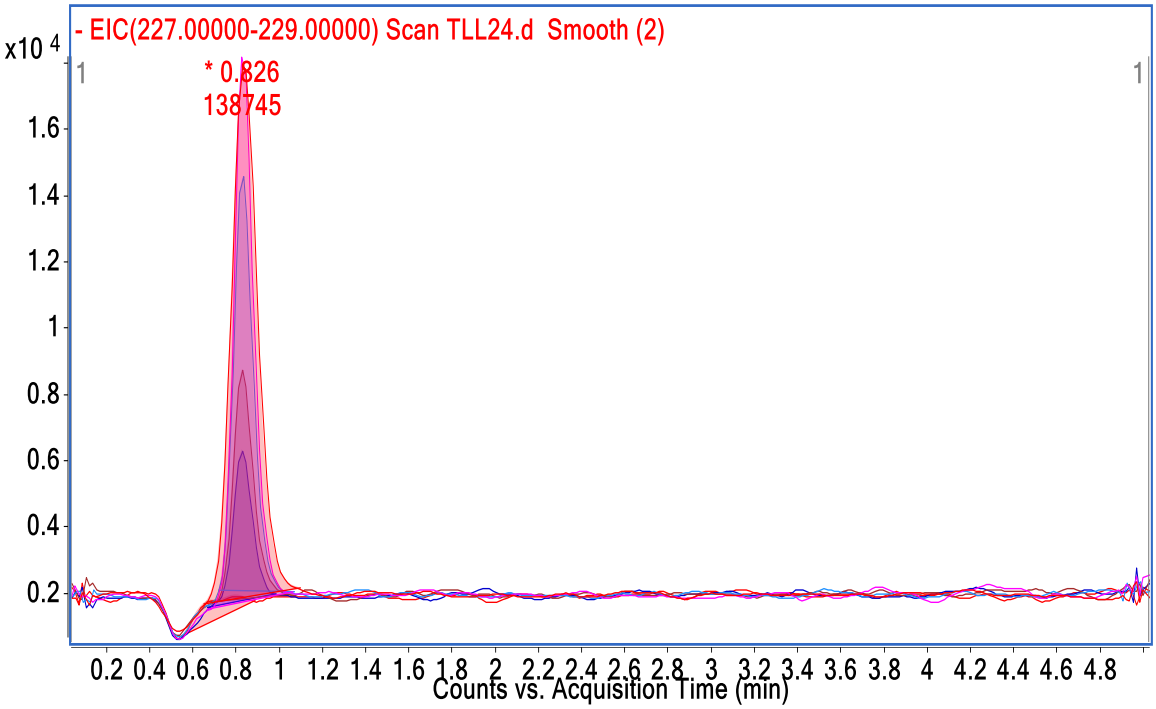


Figure 4.1. Chromatograms for Pioneer Park water sample (y-axis is counts and x-axis is time in min)

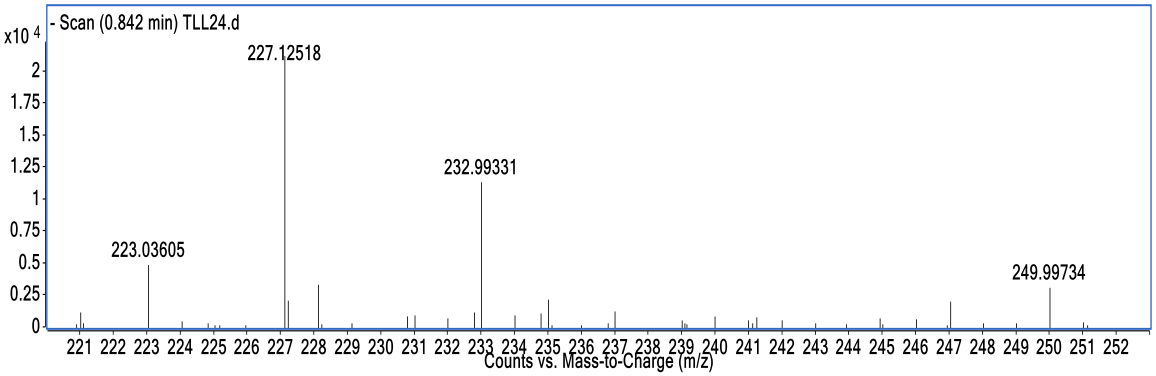


Figure 4.2. Mass spectrum for Pioneer Park water sample (y-axis is signal intensity and x-axis is time in min)

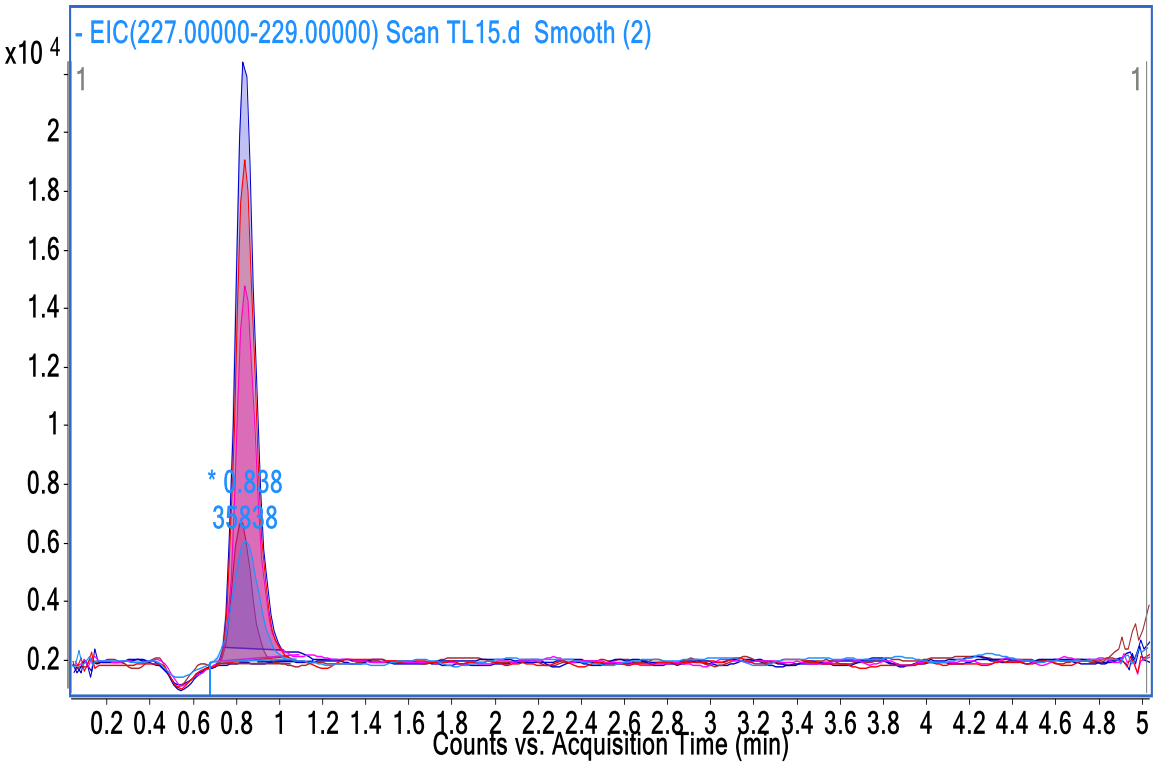


Figure 4.3. Chromatograms for swimming pool water sample P1-A (y-axis is the counts and x-axis is time in min)

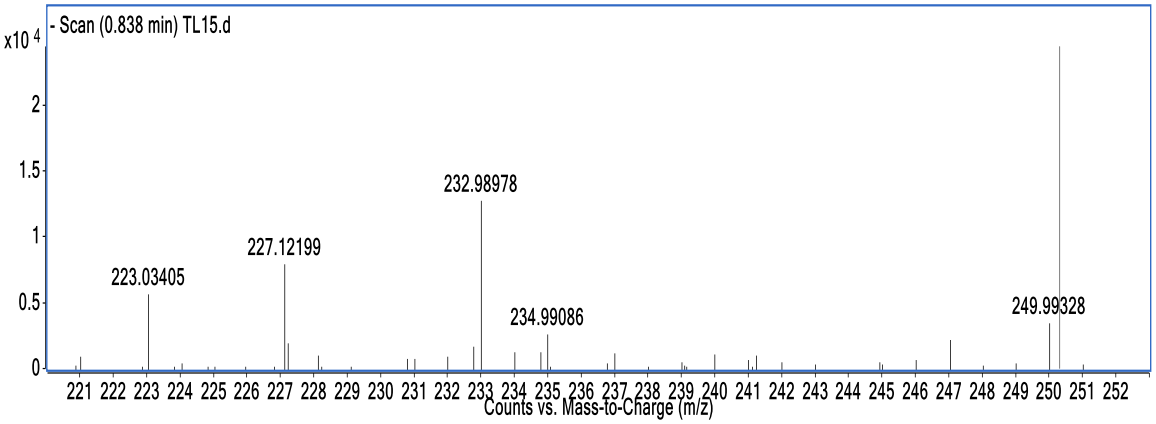


Figure 4.4. Mass spectrum for swimming pool water sample P1-A (y-axis is signal intensity and x-axis is time in min)

Interday and Intraday Precision of LC/MS Method

In order to ascertain the reproducibility of the proposed method on the LC/MS instrument, intraday and interday precision studies were carried out. For intraday study, BPA standards made with HPLC grade methanol were analyzed at five different concentrations including 250 ppb, 500 ppb, 1 ppm, 5 ppm and 25 ppm. Triclosan was also added into the BPA standards to be analyzed as an internal standard in a constant amount. For instance, the 250 ppb, 500 ppb and 1 ppm BPA standard had 250 ppb triclosan added while for 5 ppm and 25 ppm BPA standards added concentration of triclosan was 25 ppm.

BPA standards were analyzed at three different times in a day for the intraday study. The same procedure was followed for three successive days to determine interday precision. The results were reported as percent relative standard deviation (%RSD). The results from migration times and peak areas again showed a good reproducibility with percent RSD of 1 to 7 percent for peak areas and between 0.6 to 6 percent for the migration times.

The results of intraday and interday studies showing the repeatability of %RSDs are summarized in the tables below. Excellent repeatability was obtained for the peak area ratios (RSD < 10%) and migration times (RSD < 10%) during an intraday calibration of triplicate per each standard (n = 3). To calculate the precision of the calibrations between 3 days, standard concentration levels of 250 ppb, 500 ppb, 1 ppm, 5 ppm and 25 ppm were analyzed in triplicate in three consecutive days and quantified using calibration equations. Table 4.5 and Table 4.6 show interday and intraday precision studies for peak areas and migration times of both BPA and triclosan.

Table 4.5. Intraday and interday precision (%RSD) for BPA on LC/MS

Concentration	Day 1		Day 2		Day 3	
	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD
250 ppb	3.6	4.5	3.3	1.2	3.3	1.2
500 ppb	2.0	3.7	1.9	1.2	1.9	1.3
1 ppm	4.2	3.5	2.7	0.6	2.6	1.2
5 ppm	0.3	1.9	2.6	3.6	2.6	1.2
25 ppm	6.9	1.3	4.1	1.2	4.1	2.5

Table 4.6. Intraday and interday precision (%RSD) for triclosan by LC/MS

Concentration	Day 1		Day 2		Day 3	
	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD	Peak Area %RSD	Migration Time %RSD
250 ppb	2.2	0.9	2.9	4.2	4.0	1.1
25 ppm	1.1	1.5	2.6	2.7	2.3	1.1

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of LC/MS Method

The LOD was calculated as the concentration that will give a response with a signal-to-noise (S/N) ratio of 3, and were all in lower ppm range and the LOQ calculated as the concentration that will give a response with a S/N ratio of 10 were also in lower ppb range. The LOD and LOQ values are shown in Table 4.7.

Table 4.7. Limit of detection (LOD) and Limit of quantitation (LOQ) for LC/MS method

	LOD (ppb)	LOQ (ppb)	Calibration Equation	R^2
BPA	6.32	21.09	y = 0.008x + 0.0061	0.9986

Chapter 5

Results and Discussion

Capillary Electrophoresis (CE) Analysis of Samples by Standard Addition

Method of standard addition was used to analyze all the samples. It is a quantitative analysis approach used in situations where sample matrix also contributes to the analytical signal. This is referred to as the matrix effect. When matrix effects appear and matrix-matched calibration samples are not available, the standard addition method is considered as the calibration method of choice. It involves adding known amounts of standard to one or more aliquots of the sample solution, compensating for a sample constituent that enhances or depresses the analyte signal. Although, standard addition is a well-established approach for overcoming matrix effects, it is time consuming and requires a larger number of measurements per sample.

In order to analyze real samples on the CE instrument, five measurements per sample were made. A standard addition plot was obtained by spiking samples with four known concentrations of analyte (BPA) and plotting the concentration of spiked BPA on the x-axis and the corresponding peak areas on the y-axis. So, five sample vials were prepared to analyze a single sample. A constant volume of sample (200 µL) was added to each sample vial. Then a series of increasing volumes of stock solution were added to these sample vials. The standard stock solution was not added to the first sample vial, so it contained 200 µL of filtered sample and 300 µL of HPLC grade methanol to make the volume up to 500 µL. The second vial contained 200 µL of sample and 5 µL of 1000 ppm BPA standard (10 ppm) and 295 µL of HPLC grade methanol. The third vial also had constant volume of sample + 10 µL (20 ppm) of 1000 ppm BPA stock. The fourth sample vial contained constant volume of sample and 30 ppm of BPA standard. The fifth sample vial contained sample + 40 ppm BPA standard. Finally, each vial was made up to the mark of 500 µL with methanol and vortex well. The concentration and volume of the stock solution added was chosen to increase the concentration of the unknown considerably in each succeeding vial.

As area under the peak corresponds to the amount of the analyte present in a sample, so peak areas were obtained from the electropherograms for all the succeeding sample vials. The first sample vial had no manual addition of BPA (corresponds to unknown concentration); upon analysis it gave us a peak. The peak areas were then plotted on the y-axis of a graph, with the concentrations of the unknown and known standards plotted on the x-axis and an equation of the line was obtained.

When the resulting line was extrapolated to the x-axis, the point of intersection of the abscissa corresponds to the concentration of the BPA present in the sample. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. We ignored negative sign associated with the reading of the unknown.

In this work, after measuring the response for a series of standard addition solutions, we plotted the results and used equation of the line, y = mx + b and put y = 0 to get the value of x.

Y = mx + b

where

x = concentration of the unknown

m = slope

b = y intercept

A common standard addition plot for one of the samples is shown in Figure 5.1.

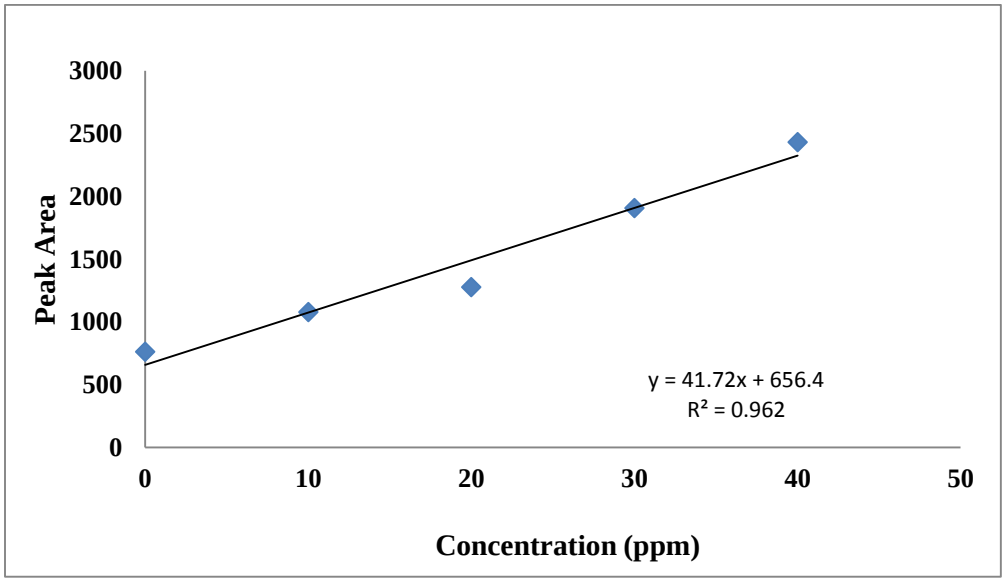


Figure 5.1. BPA standard addition plot by CE

Analysis of Swimming Pool Water Samples by CE

The swimming pool waters were analyzed for the presence of BPA. The analysis time was set at 8 min. Migration time for BPA was 2 min. Distinct sharp peaks have been observed and areas under the peaks were plotted against the added concentrations of the standards to obtain a standard addition plot. Swimming pool water samples analysis resulted in excellent reproducible data compared to the environmental water samples because of having fewer matrixes in them. BPA was detected in considerable amounts in the pools water. Reason behind it could be the presence of swimmers, most pools had plastic dividers and some had plastic liners. These factors can contribute to the presence of BPA in these swimming pool waters.

Levels of BPA were different for different pools and they are represented in the form of bar charts in the Figure 5.2. There were some pool water samples, where BPA was not detected at all. These pools have been disinfected with sodium hypochlorite pucks. The reaction of BPA with sodium hypochlorite results in degradation of BPA along with formation of other derivatives of BPA. So, it was concluded that this disinfection method has resulted in possible degradation of BPA. The degradation and kinetics of sodium hypochlorite oxidation of BPA has been studied earlier. The results from the studies showed that BPA degradation takes place with sodium hypochlorite and it follows pseudo-first-order kinetics [122]. The pH value also influences the degradation of BPA greatly. The pseudo-first-order rate constant of the reaction between BPA and HOCl reached to the maximum during pH 8 - 9 [122]. The BGE used to carry out separation also worked between the pH range of 8.5 - 10.5. The removal efficiency of BPA would increase with increasing temperature [122]. All swimming pool samples are coded from P1 – P8, where “As” represent the actual swimming pool water samples and “Bs” represent the tap water samples from the same pool. Figure 5.2 shows concentrations of BPA present in swimming pool water samples by CE.

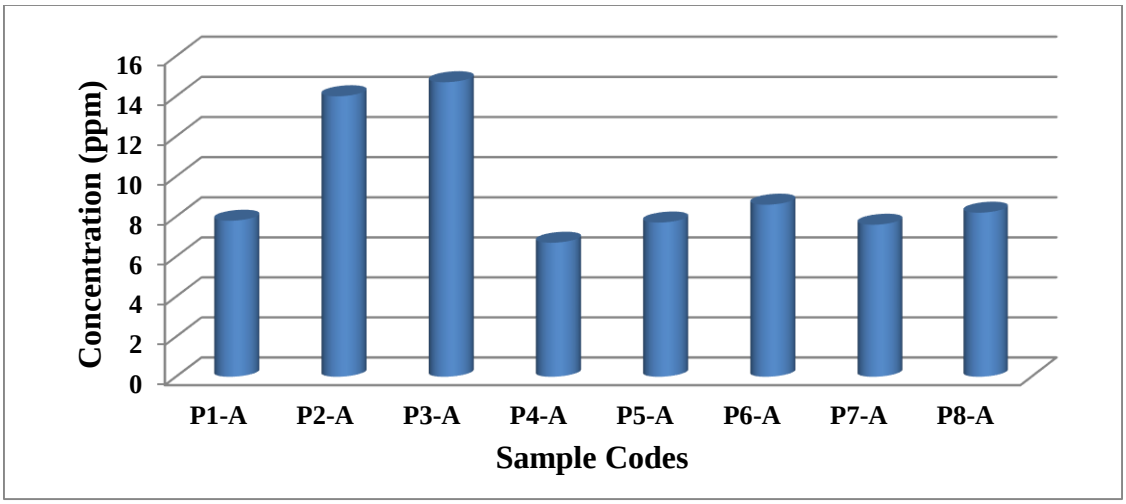


Figure 5.2. Analysis of swimming pool water samples by CE

Analysis of Tap Water Samples by CE

In the beginning, the tap water samples were thought to act as control for this study but the results were not as expected. Considerable levels of BPA were detected in most of the tap waters samples. The reason for the presence of BPA in tap water could possibly be the polyvinyl chloride (PVC) pipelines and the relining of the pipelines. Relining is a process of recoating the inside of the water pipe instead of replacing the old pipes with new ones. The recoating of the drinking water pipes is often done with an epoxy resin containing BPA or BADGE which can result in leaching of these chemical compounds. The concentration of BPA in the tap water samples are shown in Figure 5.3.

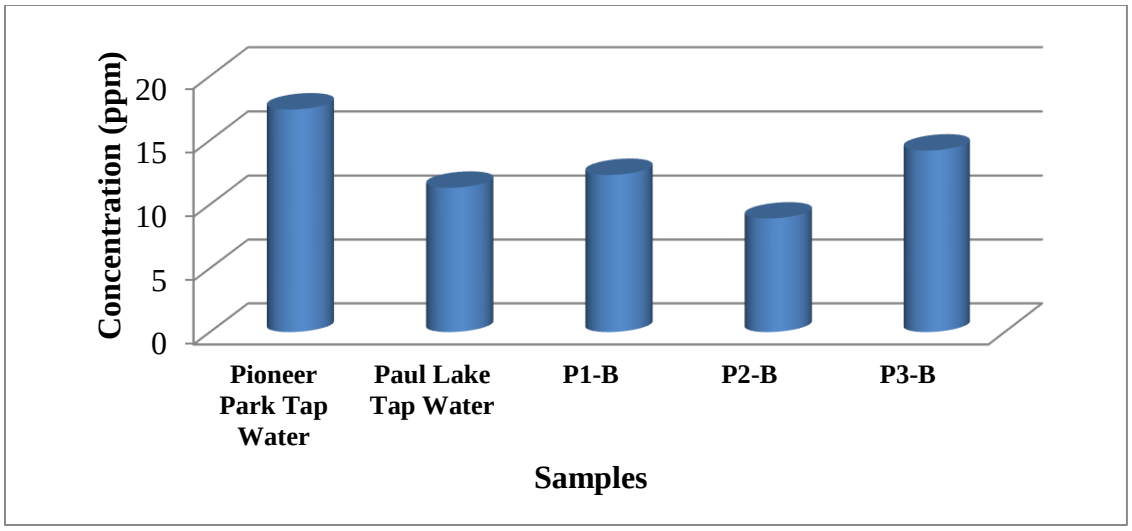


Figure 5.3. Analysis of tap water samples by CE

Analysis of River Water Samples by CE

River water samples were analyzed on CE using the standard addition method of analysis. Most river waters had BPA levels less than 10 ppm. Riverside Park river water sample has the highest level of BPA up to 8 ppm. While majority of the river waters have BPA levels of about 5 ppm. BPA levels were calculated from the three different samples from each location and then an average concentration of BPA was calculated and used to plot the bar graph shown in Figure 5.4 below.

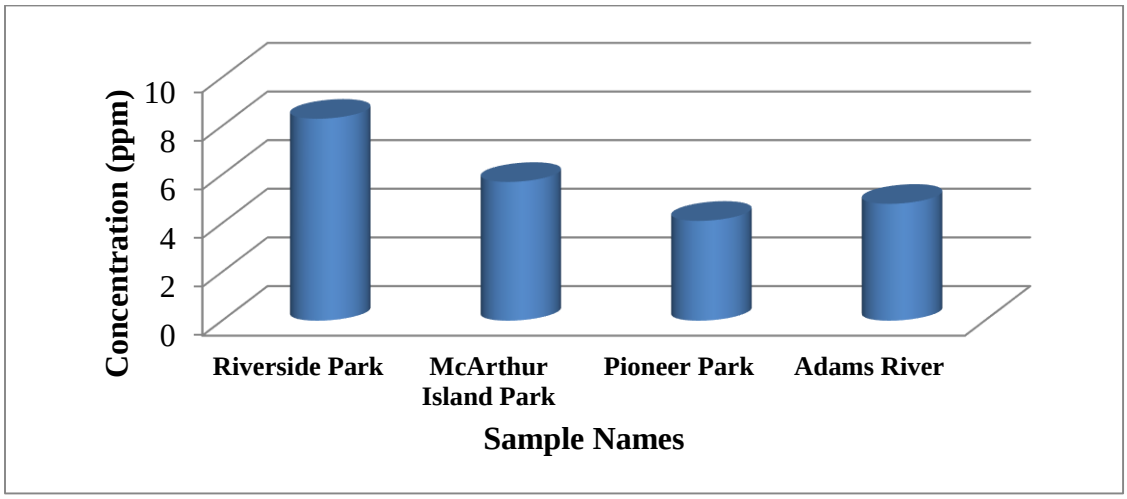


Figure 5.4. Analysis of river water samples by CE

Analysis of Lake Water Samples by CE

Water samples were collected from four different lakes near the Kamloops region and analyzed. The concentrations of BPA present in these samples are shown in Figure 5.5. It can be seen that the lake water samples have BPA levels less than 15 ppm. Only Paul lake water sample was found to have BPA concentration of more than 10 ppm while the other three lake water samples were less than the 10 ppm mark.

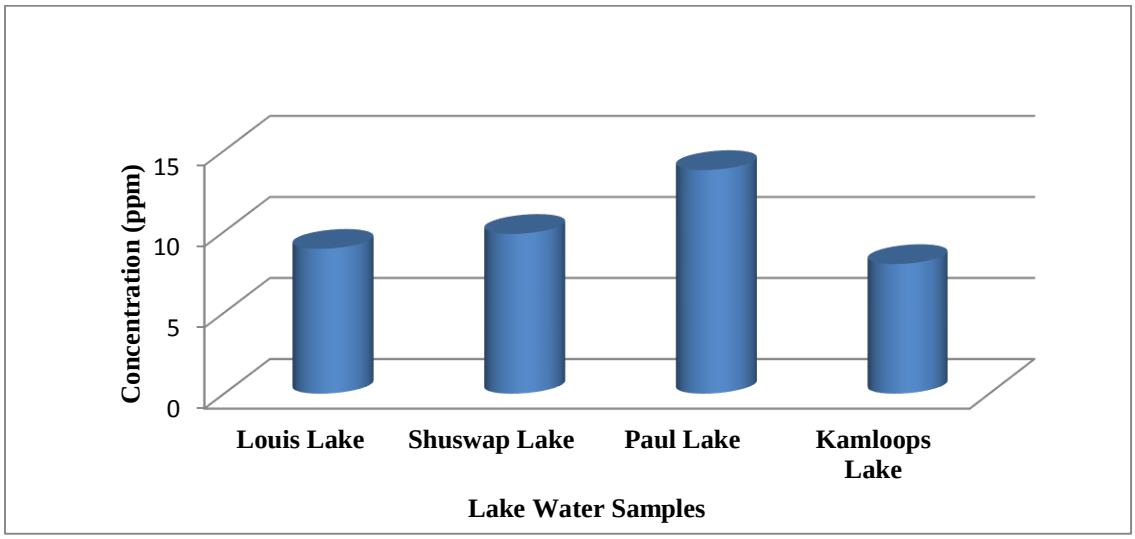


Figure 5.5. Analysis of lake water samples by CE

BPA concentrations obtained from all samples (including set I, II and III) are shown in Tables 5.1, 5.2 and 5.3. All concentrations were found to be in the lower ppm range.

Table 5.1. Standard addition data from analysis of samples set I

No. of Observations.	Water Sample	Equation	R^2	Concentration. Standard Addition (ppm)
1	Riverside Park	Y= 284.92x + 1864	0.8106	6.5
2	Pioneer Park	Y= 153.94x + 277	0.975	1.7
3	Shuswap Lake	Y= 1914.5x + 14945	0.9783	7.8
4	Louis Lake	Y= 1744.7x + 8610.5	0.9162	4.9
5	McArthur Island Park	Y= 1568.3x + 6106.4	0.9571	3.8
6	Kamloops Lake	Y= 55.769x + 601.23	0.9063	10.7
7	Adams River	Y= 973.39x + 4204.8	0.9866	4.3
8	Paul Lake Tap Water	Y = 20.83x + 437.6	0.9549	21.0
9	Pioneer Park Tap Water	Y= 225.37x + 3457.2	0.9451	15.3
10	Paul Lake	Y= 1831.7x + 20106	0.9432	10.9
11	P1-A	Y= 500.89x + 739.8	0.9444	1.4
12	P1-B	Y= 54.59x + 640.6	0.9806	11.7
13	P2-A	Y= 601.14x + 10029	0.8797	16.6

No. of Observations.	Water Sample	Equation	R^2	Concentration. Standard Addition (ppm)
14	P2-B	Y= 23.71x + 188.8	0.947	7.9
15	P3-A	Y= 59.65x +1095	0.9937	18.3
16	P3-B	Y= 41.72x + 656.4	0.962	15.7
17	P4-A	Y= 75.31x + 362.2	0.8756	4.8
18	P5-A	Y= 249.79x + 4120.9	0.8747	16.5
19	P6-A	Y= 40.28x + 271.2	0.9427	6.7
20	P7-A	Y=169.16x + 1684	0.8017	9.9
21	P8-A	Y= 400.22x + 7193.4	0.9758	17.9

Table 5.2. Standard addition data from analysis of samples set II

No. of Observations	Water Sample	Equation	R^2	Concentration Standard Addition (ppm)
1	Riverside Park	Y= 341.9x + 3062.4	0.9383	8.9
2	Pioneer Park	Y= 292.24x + 1916	0.9386	6.5
3	Shuswap Lake	Y= 75.96x + 792.2	0.9713	10.4
4	Louis Lake	Y= 931.67x + 4000.8	0.972	4.2
5	McArthur Island Park	Y= 121.78x + 815.2	0.9788	6.6
6	Kamloops Lake	Y= 214.6x + 953	0.8539	4.4
7	Adams River	Y = 2317.5x + 13966	0.934	6.0
8	Paul Lake Tap Water	Y= 120.86x + 749.6	0.9516	6.2
9	Pioneer Park Tap Water	Y= 292.42x + 1916	0.9386	6.5
10	Paul Lake	Y= 1927.6x + 24052	0.9425	12.4
11	P1-A	Y=197.98x + 2221	0.903	11.2
12	P1-B	Y= 58.32x + 750	0.8241	12.8
13	P2-A	Y= 85.35x + 826	0.9809	9.6

No. of Observations	Water Sample	Equation	R^2	Concentration Standard Addition (ppm)
14	P2-B	Y= 545.43x + 4443.2	0.9469	8.1
15	P3-A	Y= 276.67x + 1740.8	0.9948	6.2
16	P3-B	Y= 85.04x + 867.8	0.985	10.2
17	P4-A	Y= 63.18x + 538.2	0.9770	8.5
18	P5-A	Y= 631.88x + 2151.8	0.945	3.4
19	P6-A	Y= 126.67x + 1136.2	0.9348	8.9
20	P7-A	Y= 223.71x + 1086.8	0.9021	4.8
21	P8-A	Y= 338.16x + 1368	0.987	4.0

Table 5.3. Standard addition data from analysis of samples set III

No. of Observations	Water Sample	Equation	R^2	Concentration Standard Addition (ppm)
1	Riverside Park	Y= 227.71x + 2202.4	0.924	9.6
2	Pioneer Park	Y= 4046.9x + 16757	0.9571	4.1
3	Shuswap Lake	Y= 103.46x + 1175.1	0.9133	11.3
4	Louis Lake	Y= 238.62x + 7720	0.6565	17.6
5	McArthur Island Park	Y= 1316.6x + 9204.2	0.9085	6.9
6	Kamloops Lake	Y= 101.5x + 926.8	0.8543	9.1
7	Adams River	Y = 1197.6x + 5174.8	0.9977	4.3
8	Paul Lake Tap Water	Y= 113.62x + 791.6	0.9079	6.9
9	Pioneer Park Tap Water	Y= 148.78x + 4535.3	0.9653	30.4
10	Paul Lake	Y= 125.62x + 2295.2	0.9775	18.2
11	P1-A	Y= 730.96x + 7319.2	0.8324	10.0
12	P1-B	Y= 66.94x + 848.4	0.9805	12.6
13	P2-A	Y= 137.36x + 2165.4	0.8358	15.7
14	P2-B	Y= 1309.2x + 14057	0.9456	10.7

No. of Observations	Water Sample	Equation	R^2	Concentration Standard Addition (ppm)
15	P3-A	Y= 524.7x + 10233	0.9586	19.5
16	P3-B	Y= 38.109x + 642.02	0.9058	16.8
17	P4-A	Y= 422.26x + 15470	0.8543	6.9
18	P5-A	Y= 272.68x + 881	0.9735	3.2
19	P6-A	Y= 589.03x + 6069	0.9164	10.3
20	P7-A	Y= 188.15x + 1541.2	0.9224	8.1
21	P8-A	Y= 393.26x + 1102.8	0.9507	2.8

Chemical Separation of BPA, BPF and BADGE by CE

CE Method (I) comprising of a background electrolyte (BGE) of 25 mM phosphate monobasic and 20 mM sodium dodecyl sulphate (SDS) solution of pH 2.5 was employed to achieve the chemical separation of three bisphenol analogues, BPA, BPF and BADGE shown in Figure 5.6. Optimization of experimental parameters such as pH, buffer concentration, detection wavelength, applied voltage and buffer additives enabled the successful baseline separation of these three analogues.

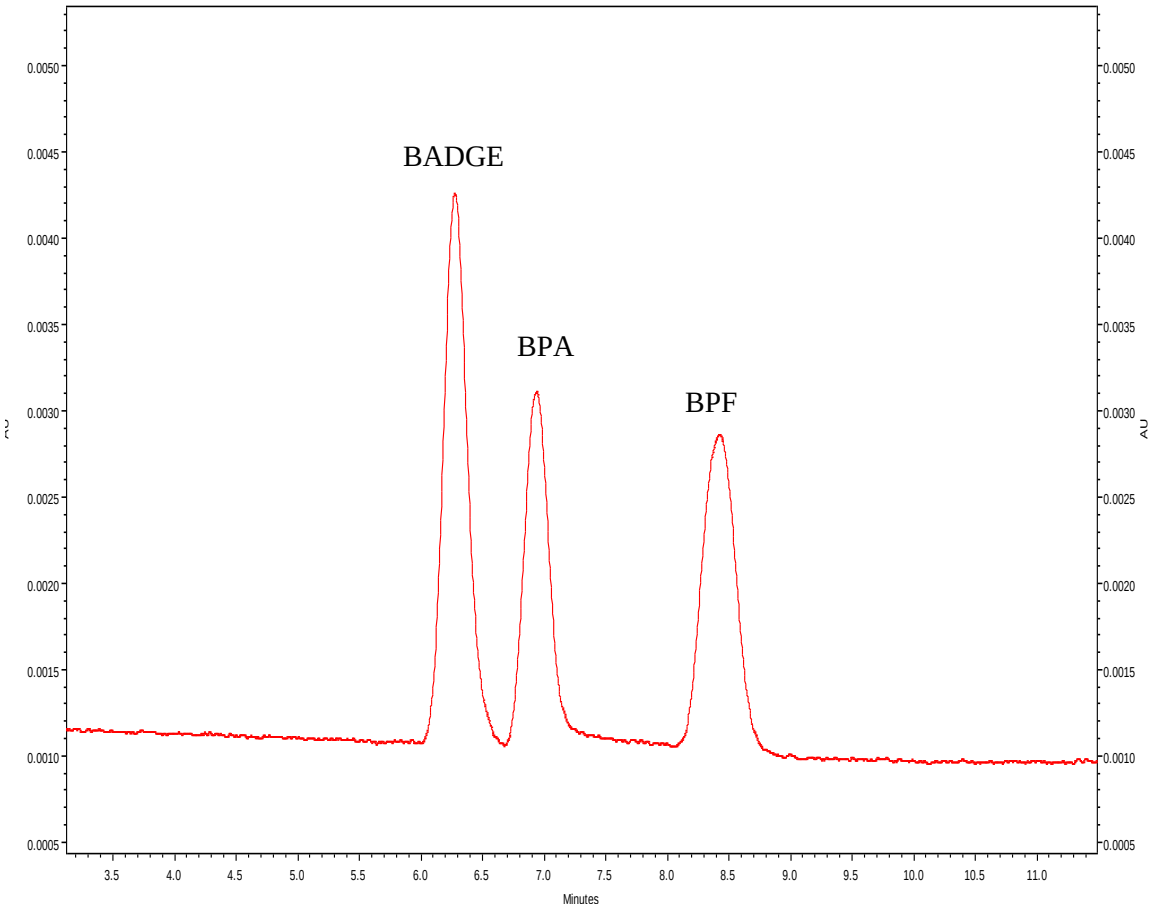


Figure 5.6. Separation of BPA, BPF and BADGE using CE

Development of CE Protocol for Separation of Eight Bisphenol Analogues

CE Method (II) comprising of a BGE of 40 mM sodium tetraborate decahydrate and 15 mM SDS solution of pH 9.5 was used to simultaneously separate BPA, BPF, BPS, BPZ, BPP, BADGE, BPAP, BPAF and BPF. Optimization of experimental parameters such as pH, buffer concentration, detection wavelength, applied voltage and buffer additives enabled the successful baseline separation of all the analogues.

In order to identify the peaks, nine sample vials were prepared. In each vial 50 µL of standard stock solution (1000 ppm) of each bisphenol analogue (BPA, BPF, BPS, BPZ, BPP, BADGE, BPAP, BPAF and BPF) was added. The first sample vial left unspiked. The rest of the vials contained the standard mix plus 100 µL of spiked analogue whose peak had to be determined in the mixture. For example, if BPA peak has to be identified; in a sample vial, 50 µL of each analogue including the BPA was added, after that the vial was additionally spiked with 100 µL of BPA. The BPA peak grew resulting in confirmation and identification of the peak. Migration time was considered as another factor for confirmation and identification of the peaks. Individual peaks had distinct retention times. Spiking and retention times led to the identification of the peaks in the Figure 5.7. The BPAP peak is smaller as compared to the peaks for other bisphenol analogues. It is possible that BPAP is less sensitive to the developed protocol. Further optimization of the developed protocol can result in better sensitivity for the BPAP, whereas, for the rest of the analogues the sensitivity is very good. All of the analogues are nicely separated and baseline resolved.

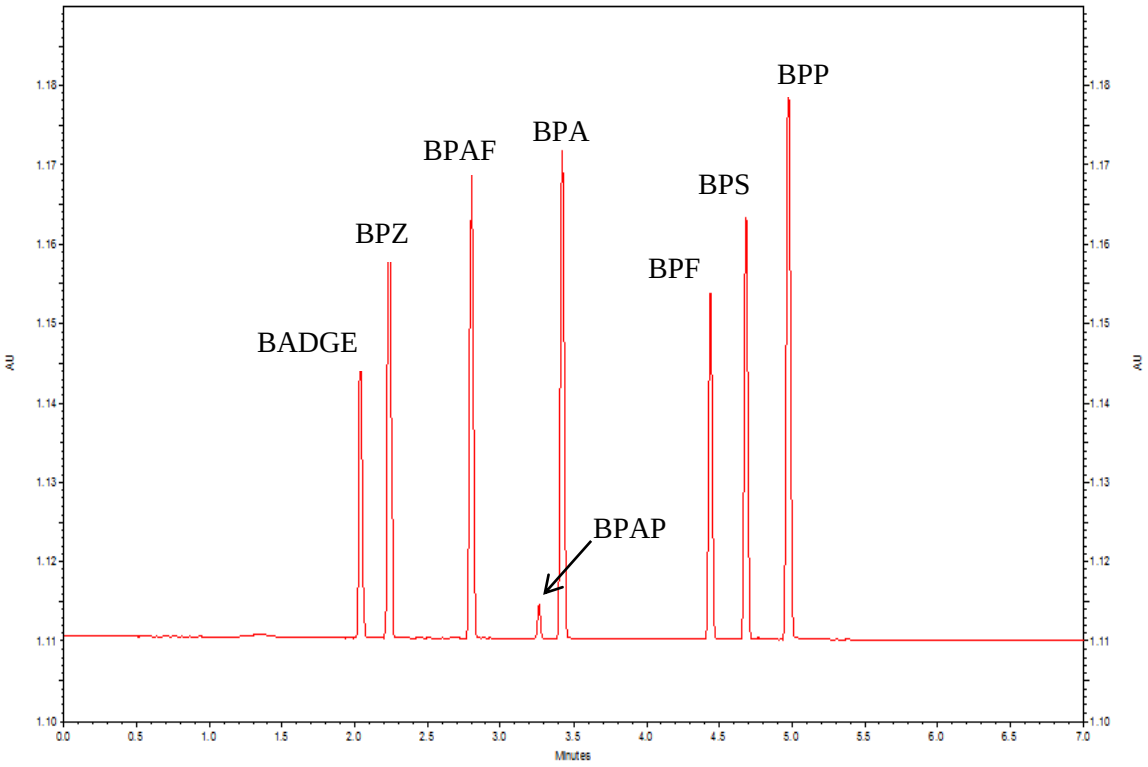


Figure 5.7. Separation of BPA, BPF, BPS, BPP, BPZ, BADGE, BPAF, BPAP on CE

Standard Addition Sample Electropherograms

Two representative samples were chosen from each category of the samples such as river, lake, swimming pool and tap water samples. The electropherograms showing increasing concentrations of BPA for the chosen water samples are shown in Figures 5.8 – 5.15.

Lake Water Samples Analysis

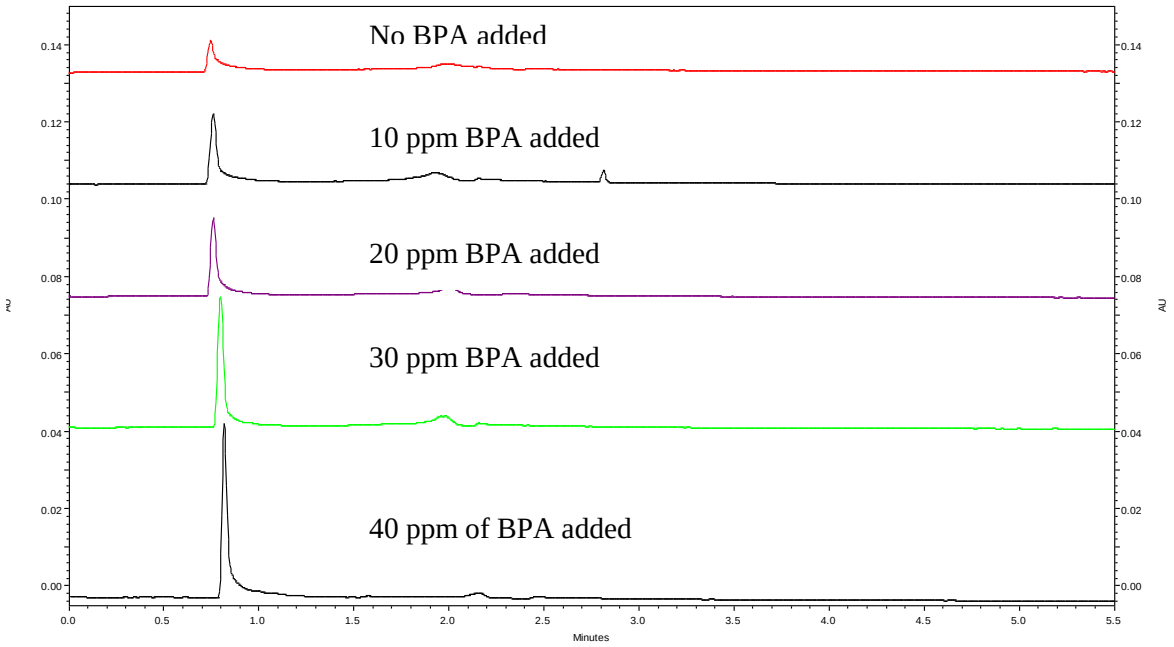


Figure 5.8. Louis Lake water sample electropherograms

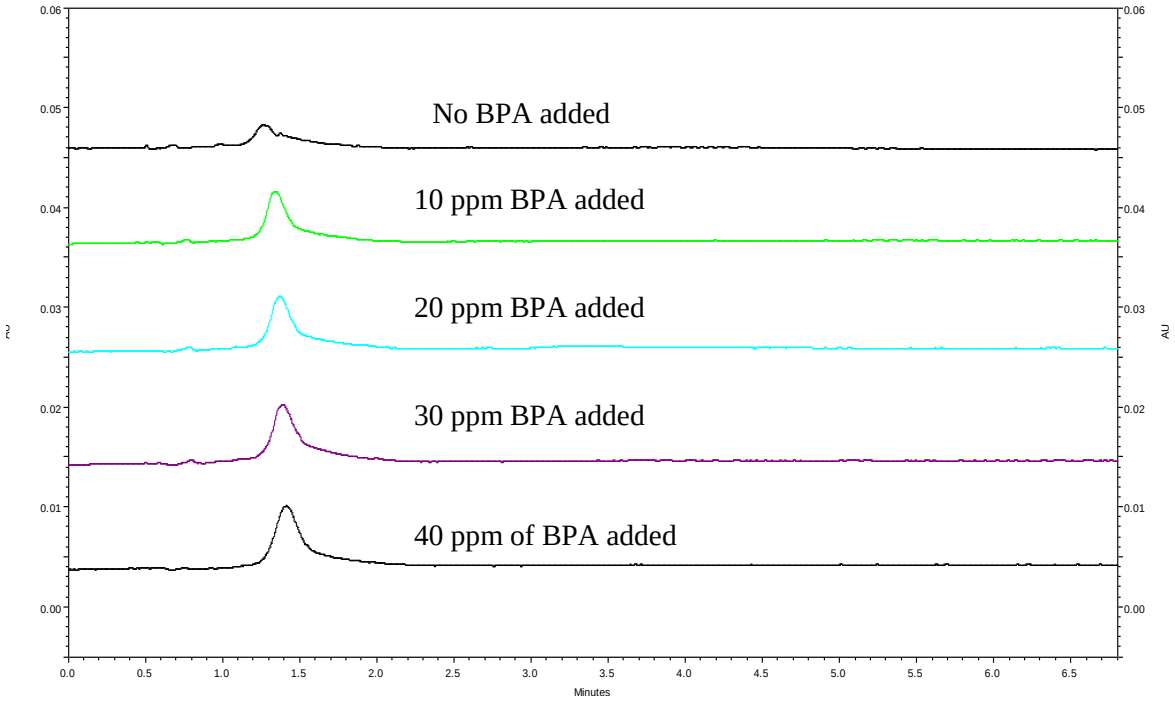


Figure 5.9. Shuswap Lake water sample electropherograms

River Water Samples Analysis

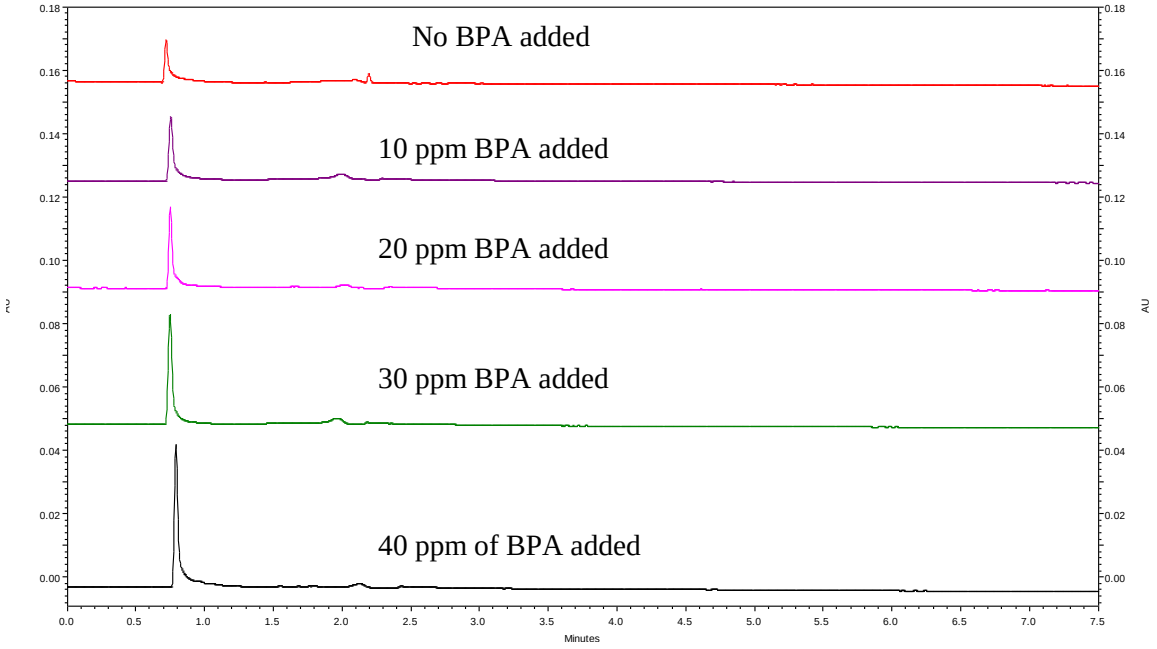


Figure 5.10. Adams river water sample electropherograms

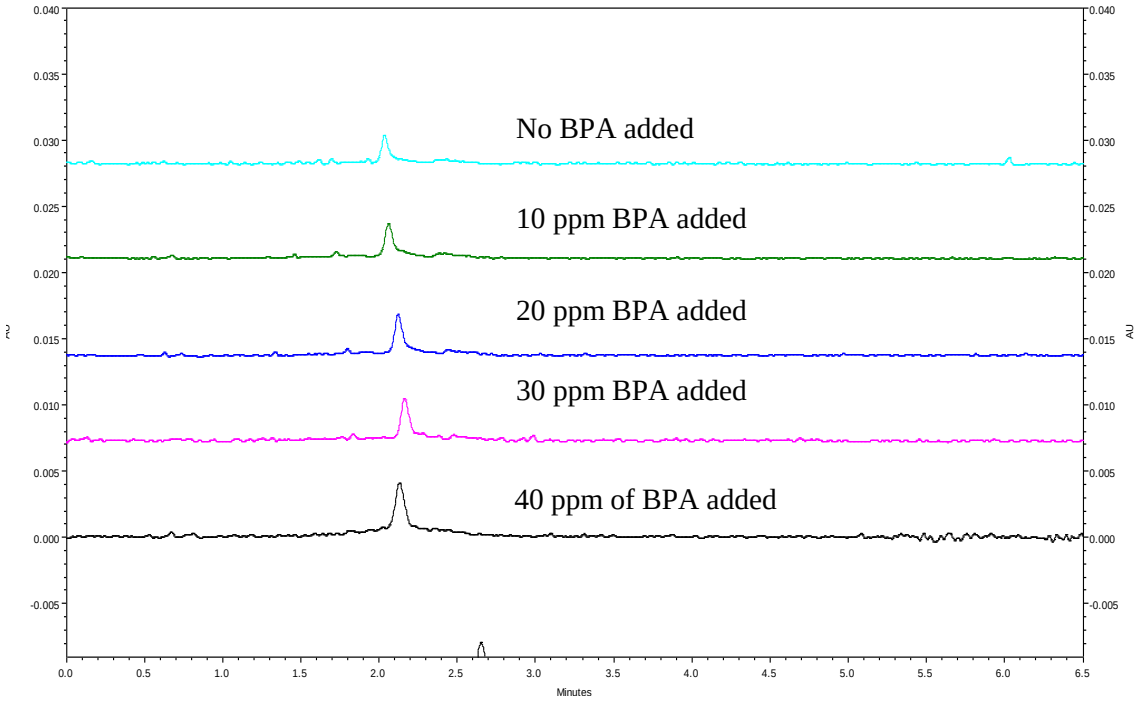


Figure 5.11. Riverside Park water sample electropherograms

Tap Water Sample Analysis

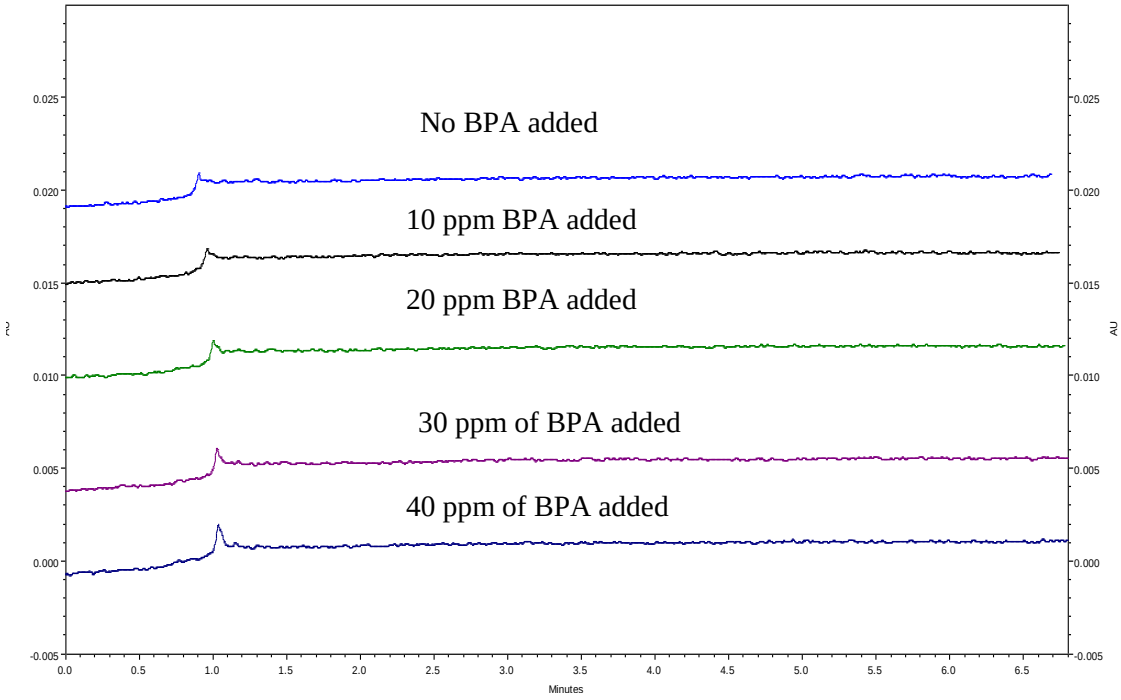


Figure 5.12. Swimming pool tap water (P1-B) sample electropherograms

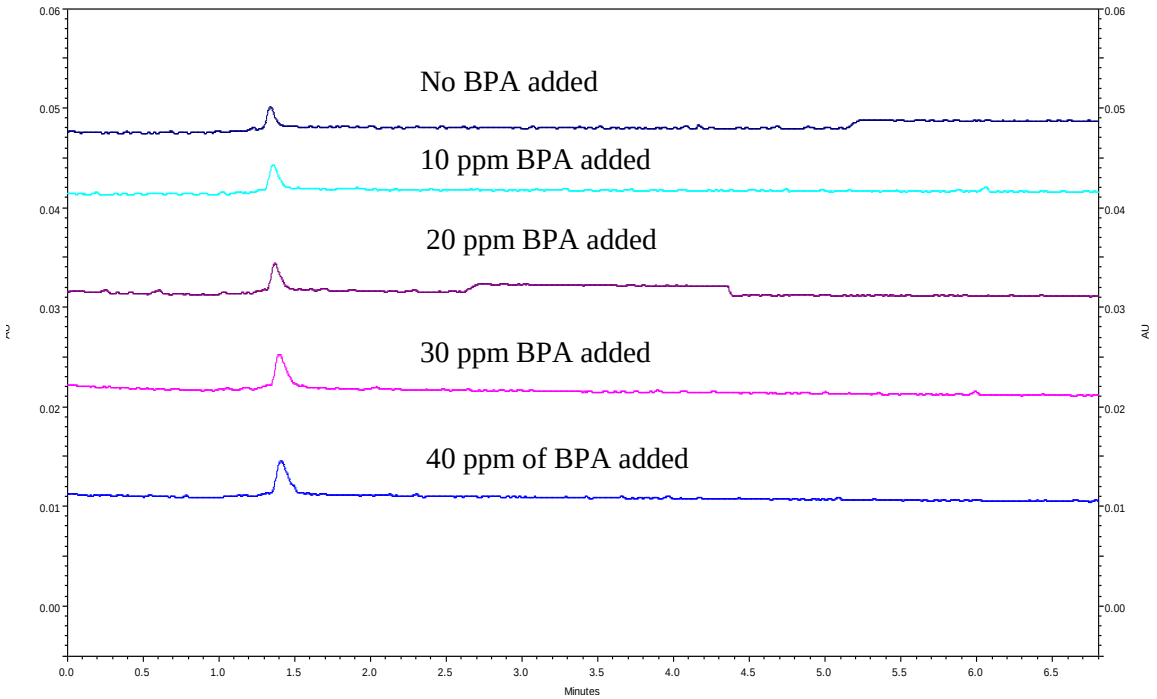


Figure 5.13. Pioneer Park tap water sample electropherograms

Swimming Pools Water Samples

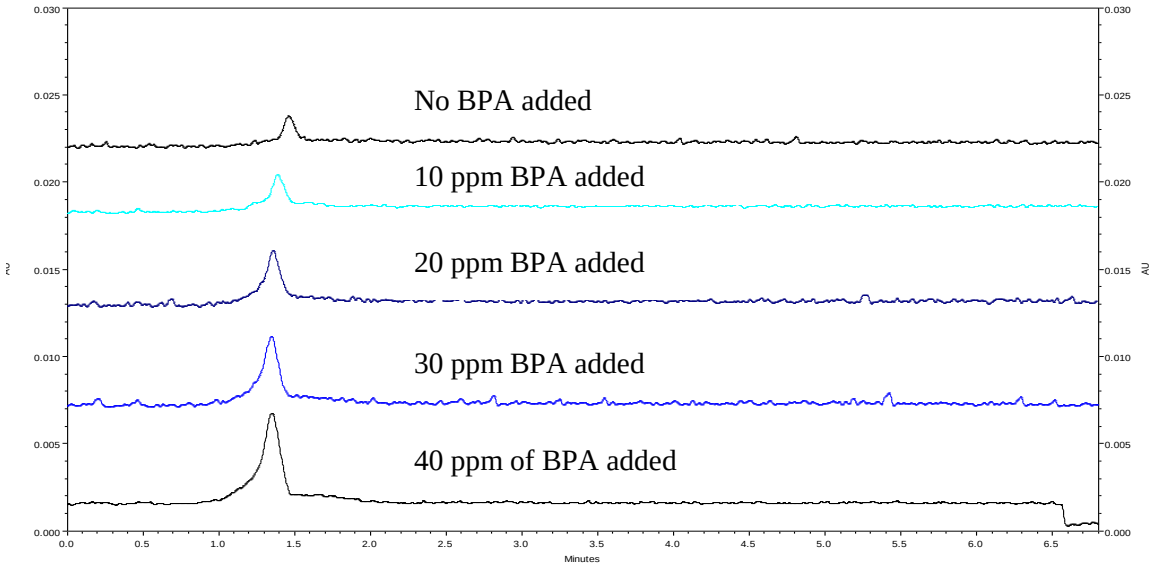


Figure 5.14. Swimming pool (P1-A) water sample electropherograms

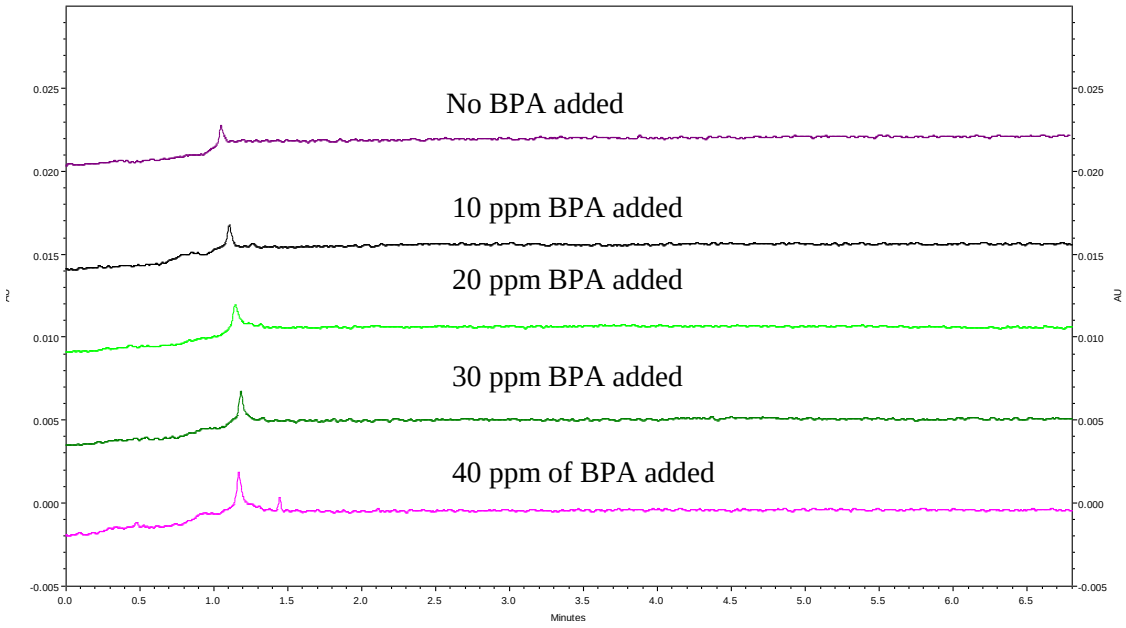


Figure 5.15. Swimming pool (P7-A) water sample electropherograms

Liquid Chromatography-Mass Spectrometry (LC/MS) Analysis by Internal Standard Calibration Approach

Internal standard approach was used to analyze water samples on LC/MS. The use of internal standard in a chemical analysis greatly improves the method accuracy and precision. The criterion for selecting the internal standard is that it should behave similarly to the analyte but provide a signal that can be distinguished from that of the analyte. Ideally, any factor that affects the analyte signal will also affect the signal of the internal standard to the same degree. Thus, the ratio of the two signals will exhibit less variability than the analyte signal alone. Internal standards are often used in chromatography and mass spectrometry. They are used to correct for variability due to analyte loss in sample storage and treatment.

Whenever samples are handled or prepared in any way, additional errors are introduced from the many variables involved with the sample preparation, such as volume or weight measurement errors, losses on surfaces of containers and because of evaporation, contamination, transfer errors, etc. The internal standard aims to compensate for these potential sources of errors. By adding a surrogate (compound of similar chemical attributes to the analyte of interest) of known amount to the sample prior to sample preparation or analysis, the surrogate should experience the same changes as the analyte of interest. By developing a calibration curve based on the relative response of the target analytes to the amount of the surrogate, much of the variation can be removed.

In this study, triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] was used as the internal standard. It is a chlorinated aromatic compound similar in properties to BPA. Triclosan is stable and it does not interfere with the analyte, it is also similar in structure to BPA which ensures it will behave in a similar way like the analyte. Figure 5.16 shows a comparison of triclosan and BPA structure. Triclosan is commercially used in soaps, shampoos, deodorants, toothpastes, mouth washes and cleaning supplies. It is also part of consumer products, including kitchen utensils, toys, bedding, socks and trash bags.

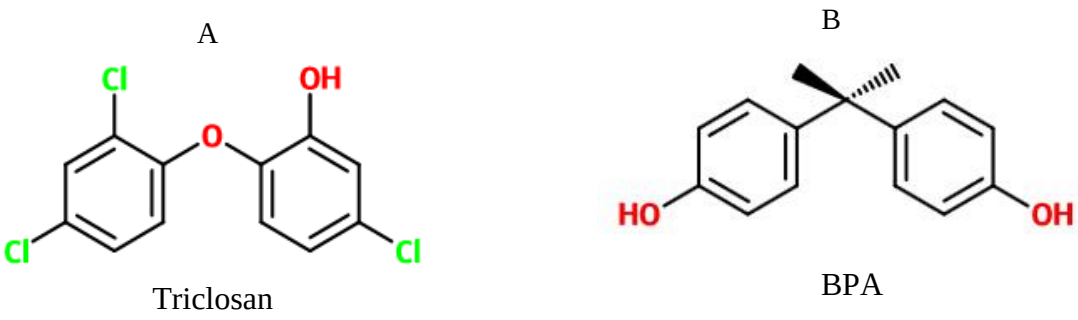


Figure 5.16. Structures, (A) Triclosan; (B) BPA

An internal calibration curve was constructed with BPA standards ranging from 2 ppm to 75 ppm. In each standard a constant amount of internal standard was added (25 ppm) triclosan. Peak area ratios of BPA to triclosan were used to plot the calibration curve. The internal standard calibration curve used to calculate concentrations of BPA in water samples is shown in Figure 5.17.

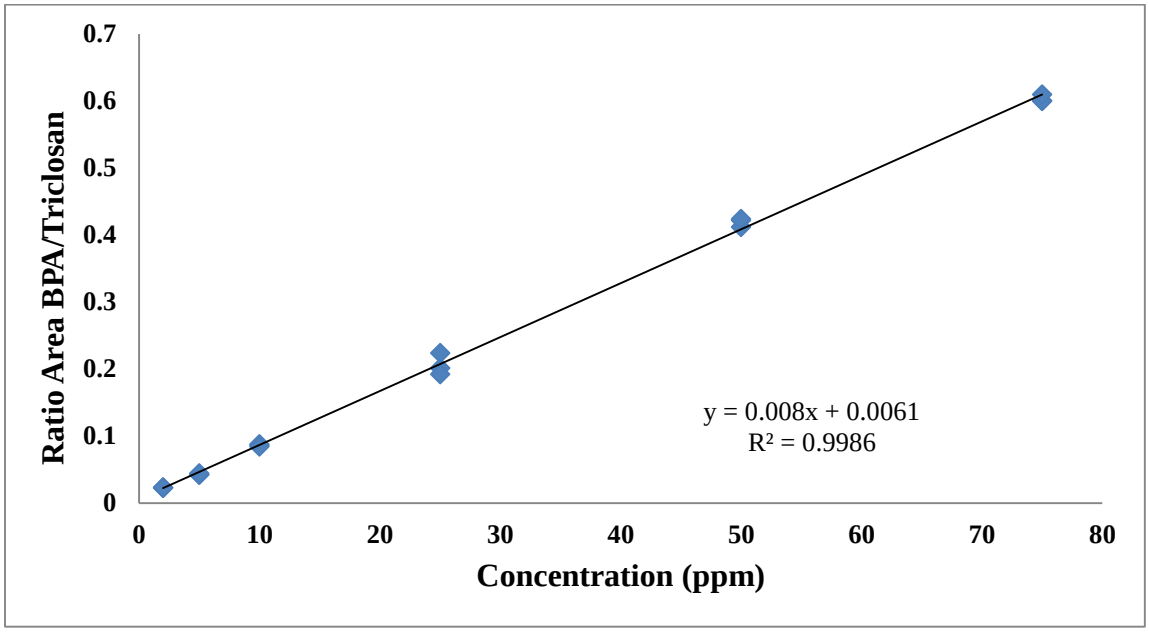


Figure 5.17. BPA internal standard calibration curve by LC/MS

LC/MS Sample Analysis

Extracted ion chromatograms (EIC's) including mass spectra for BPA and triclosan (internal standard) in the water samples are shown in Figures 5.18 – 5.41. In an extracted ion chromatogram one or more mass-to-charge (m/z) values representing one or more analytes of interest are extracted from the entire data set for a chromatographic run. EIC allows the mass spectrometer to be used as a selective detector where we can display data only for peaks of interest in cluttered total ion chromatograms (TIC). So they are created via a data mining process. Chromatograms are created by plotting the intensity of the signal observed at a chosen (m/z) value as a function of retention time where the x-axis represents time and the y-axis represents signal intensity.

Two sample chromatograms were chosen from each category of the water samples, such as, river, lake, swimming pool and tap water samples. BPA has a molar mass of 228.2 and after removal of a proton during ionization process; the mass spectrum shows a peak with (m/z) 227.2. Similarly, the molar mass of triclosan is 289.5 and mass peak for it shows at m/z 288.9 in the mass spectra. They are created via a data mining or data analysis process using the Agilent Mass Hunter software which comes with the LC/MS instrument.

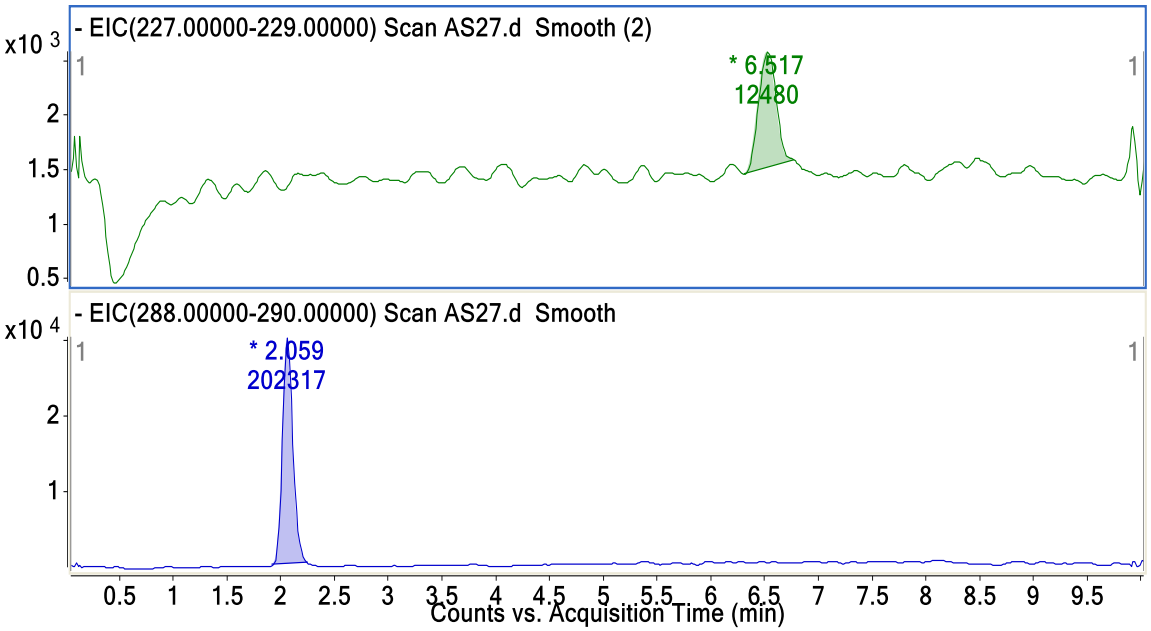


Figure 5.18. Chromatograms showing BPA and triclosan (IS) presence in Pioneer Park water sample (y-axis is counts and x-axis is time in min)

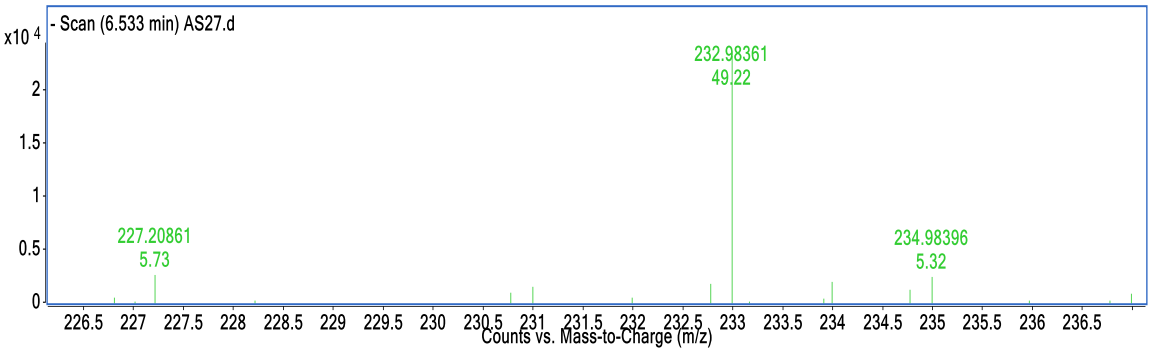


Figure 5.19. Mass spectrum for BPA present in Pioneer Park water sample (y-axis is signal intensity and x-axis is time in min)

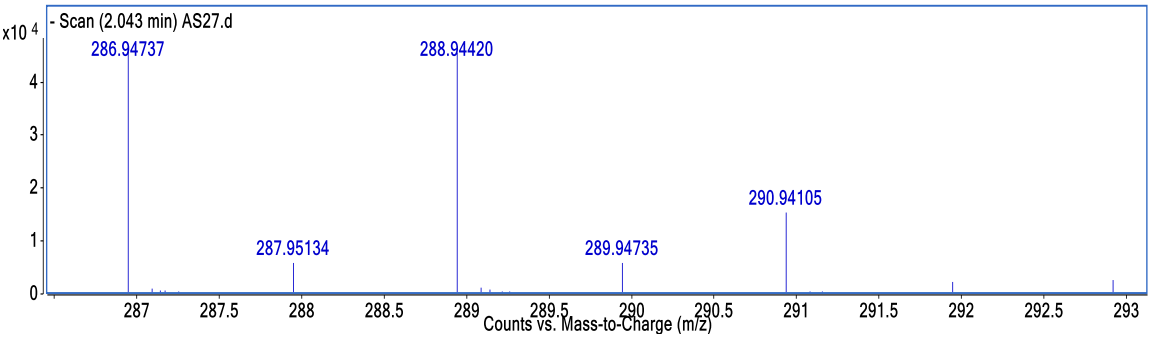


Figure 5.20. Mass spectrum for triclosan added in Pioneer Park water sample (y-axis is signal intensity and x-axis is time in min)

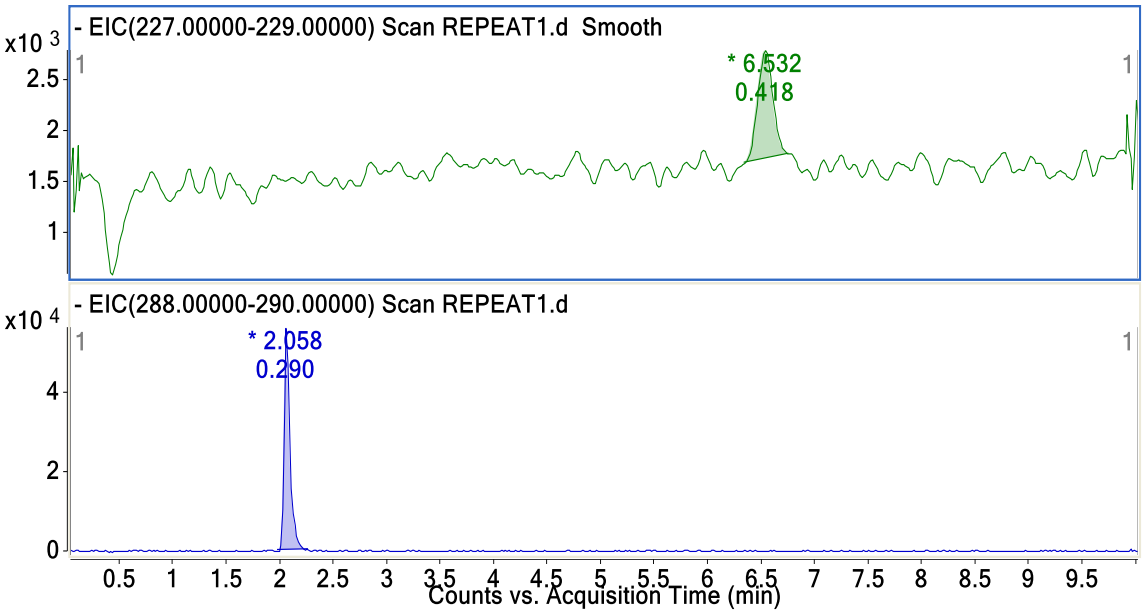


Figure 5.21. Chromatograms showing BPA and triclosan (IS) presence in McArthur Island Park water sample (y-axis is counts and x-axis is time in min)

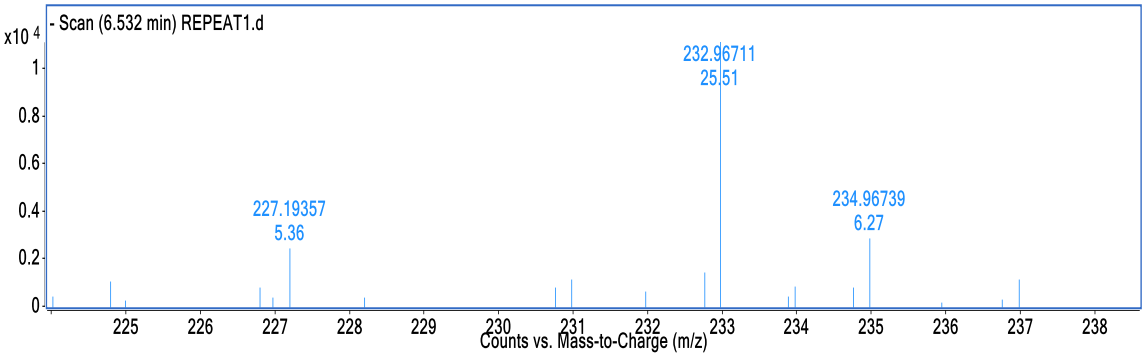


Figure 5.22. Mass spectrum for BPA in McArthur Island Park water sample (y-axis is signal intensity and x-axis is time in min)

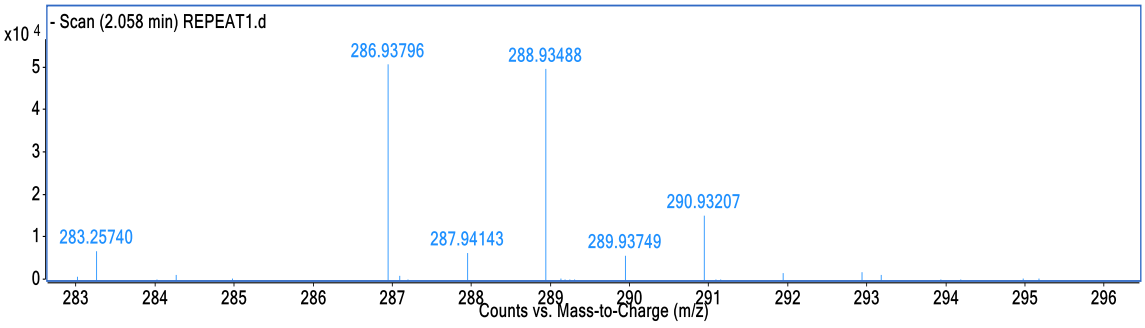


Figure 5.23. Mass spectrum for triclosan added in McArthur Island Park water sample (y-axis is signal intensity and x-axis is time in min)

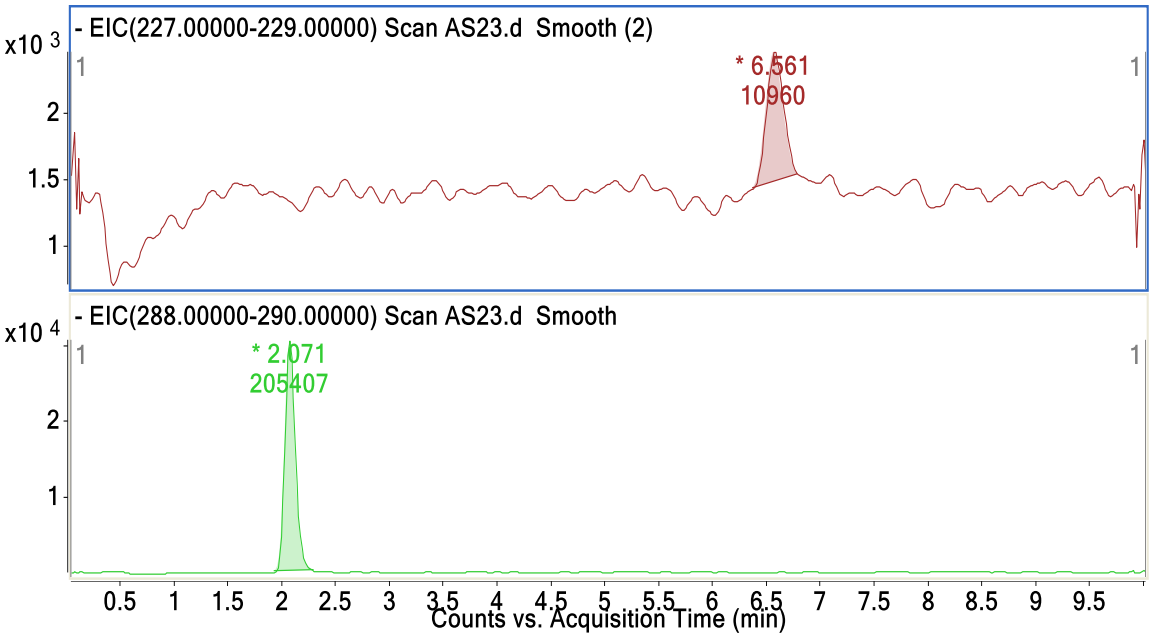


Figure 5.24. Chromatograms showing presence of BPA and triclosan (IS) in swimming pool water sample P2-A (y-axis is counts and x-axis is time in min)

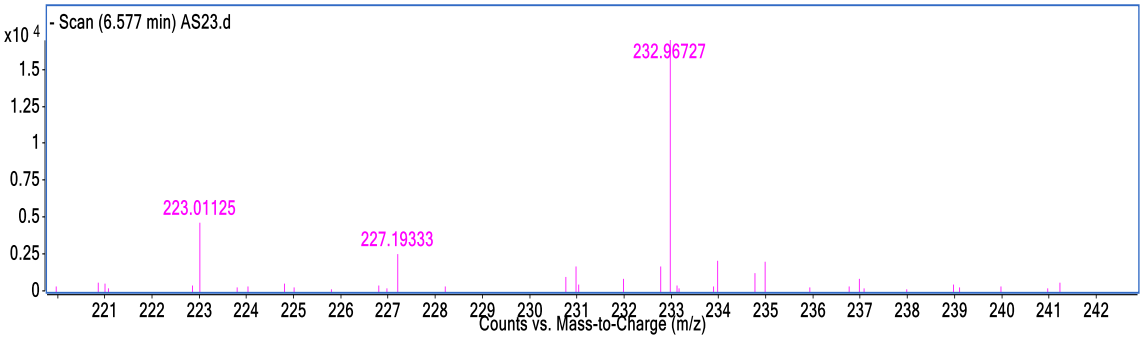


Figure 5.25. Mass spectrum for BPA present in swimming pool water sample P2-A (y-axis is signal intensity and x-axis is time in min)

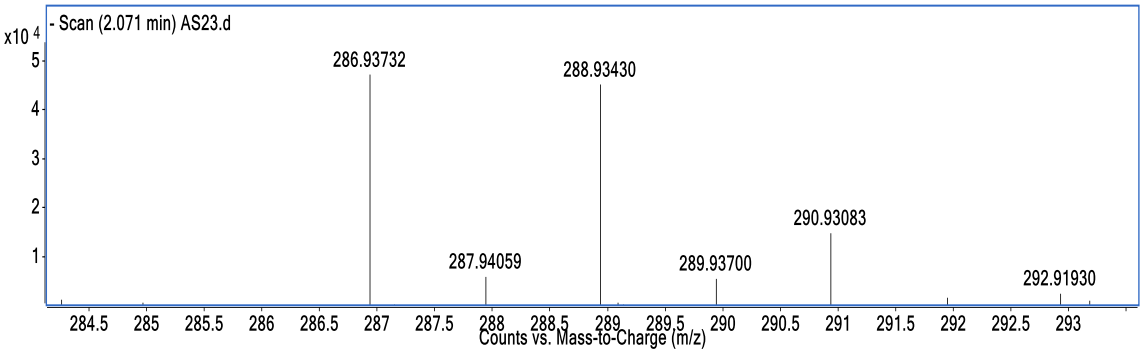


Figure 5.26. Mass spectrum for triclosan (IS) added in swimming pool water sample P2-A (y-axis is signal intensity and x-axis is time in min)

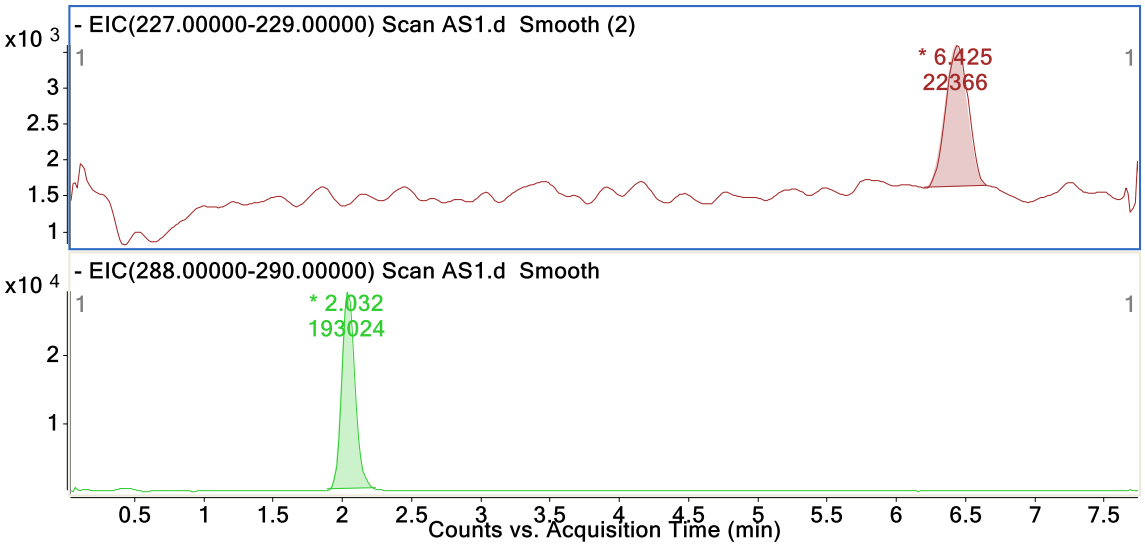


Figure 5.27. Chromatograms showing BPA and triclosan (IS) presence in swimming pool water sample P1-A (y-axis is counts and x-axis is time in min)

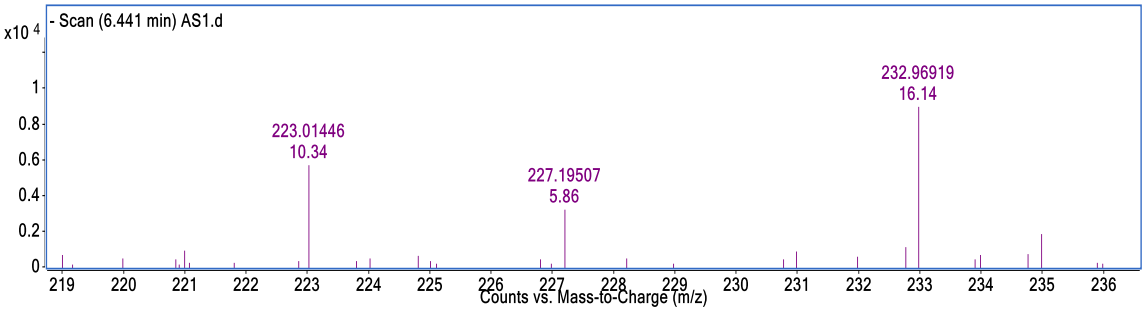


Figure 5.28. Mass spectrum for BPA in swimming pool water sample P1-A (y-axis is signal intensity and x-axis is time in min)

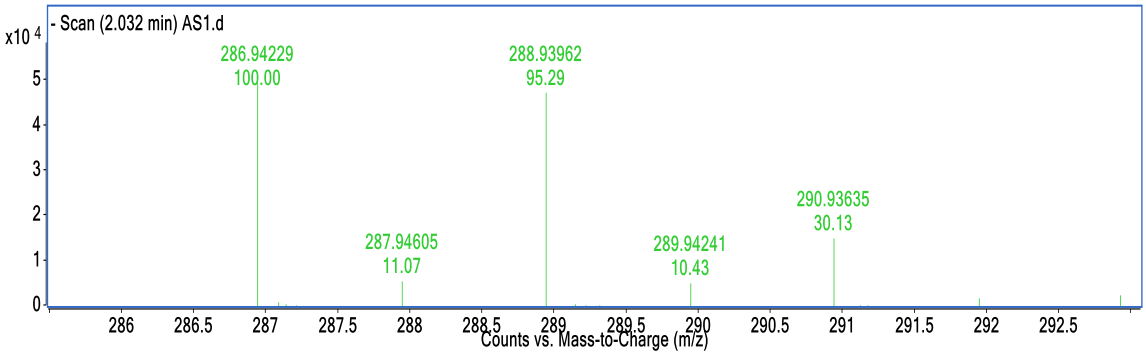


Figure 5.29. Mass spectrum for triclosan (IS) added in swimming pool water sample P1-A (y-axis is signal intensity and x-axis is time in min)

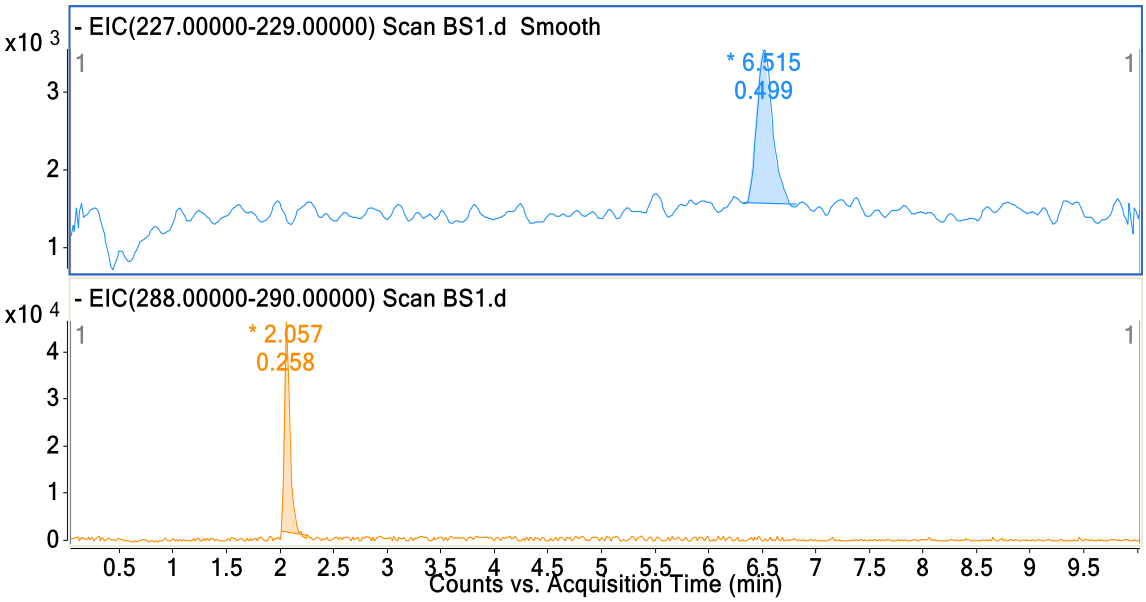


Figure 5.30. Chromatograms showing BPA and triclosan presence in Louis Lake water sample (y-axis is counts and x-axis is time in min)

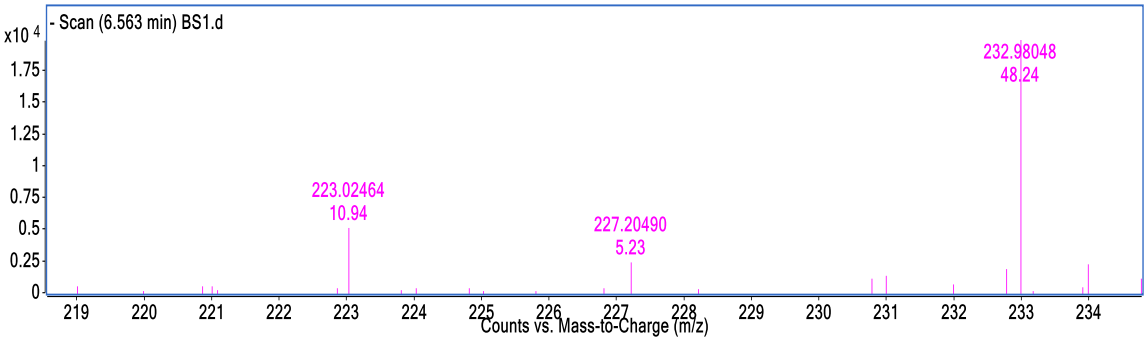


Figure 5.31. Mass spectrum for BPA present in Louis Lake water sample (y-axis is signal intensity and x-axis is time in min)

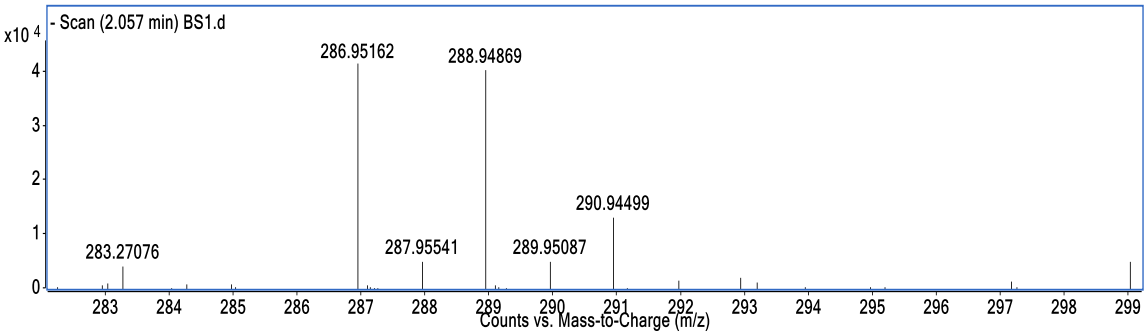


Figure 5.32. Mass spectrum for triclosan (IS) added in Louis Lake water sample (y-axis is signal intensity and x-axis is time in min)

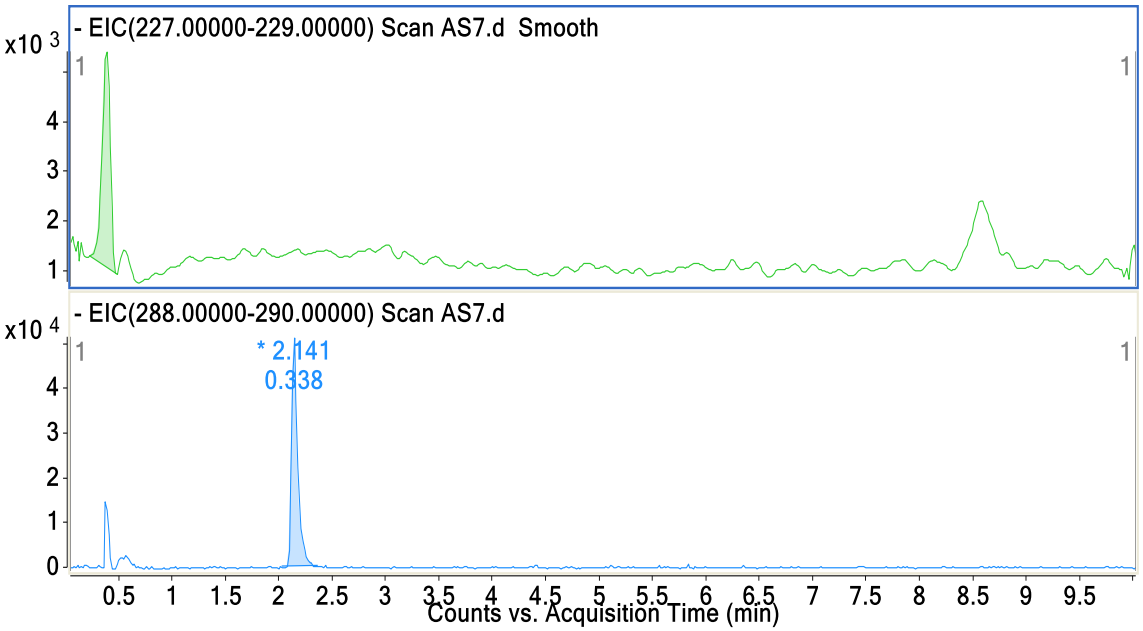


Figure 5.33. Chromatograms for presence of BPA and Triclosan (IS) present in Paul Lake water sample (y-axis is counts and x-axis is time in min)

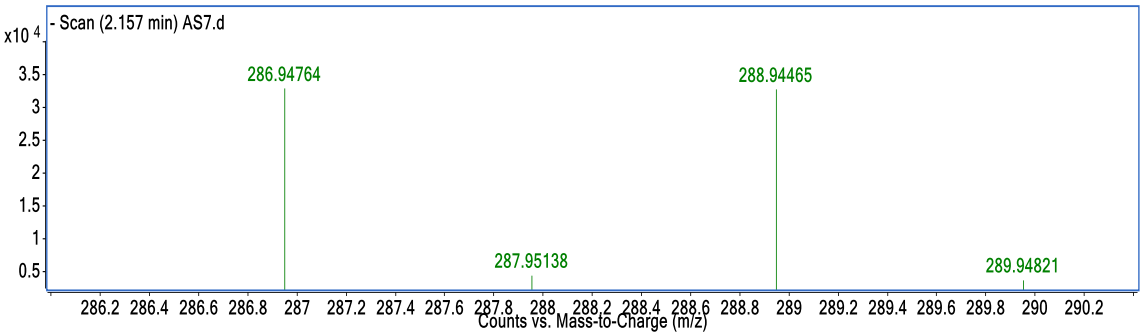


Figure 5.34. Mass spectrum for BPA present in Paul Lake water sample (y-axis is signal intensity and x-axis is time in min)

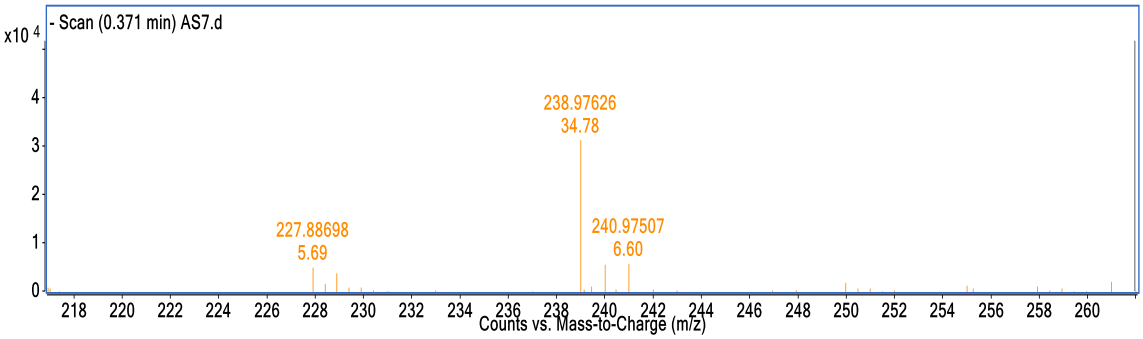


Figure 5.35. Mass spectrum for triclosan (IS) added in Paul Lake water sample (y-axis is signal intensity and x-axis is time in min)

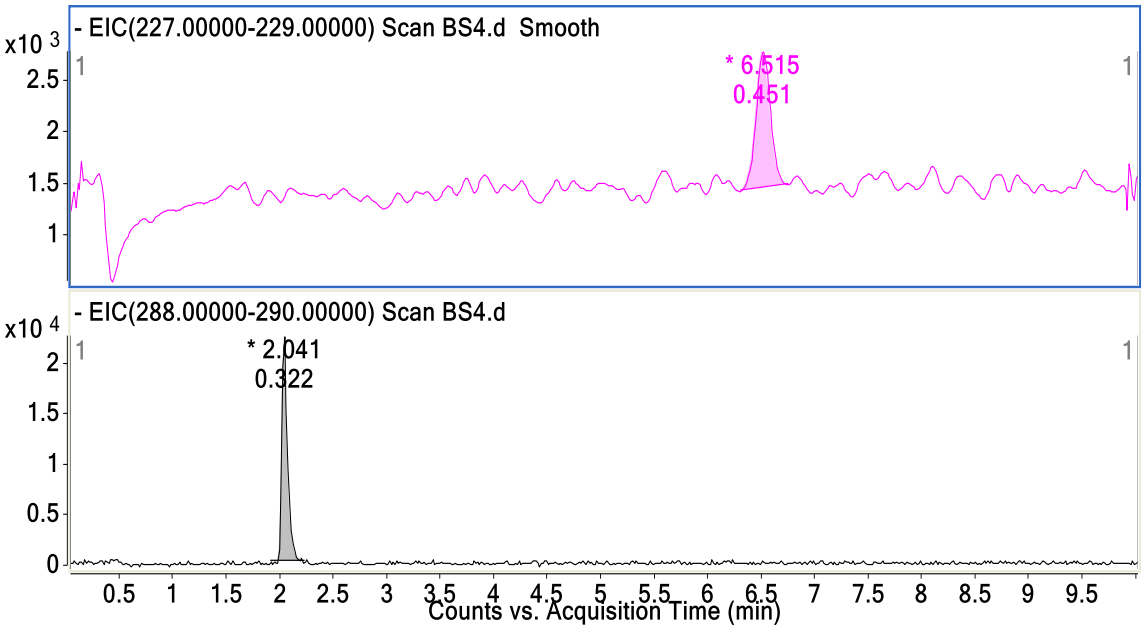


Figure 5.36. Chromatograms for BPA and triclosan presence in Pioneer Park tap water sample (y-axis is counts and x-axis is time in min)

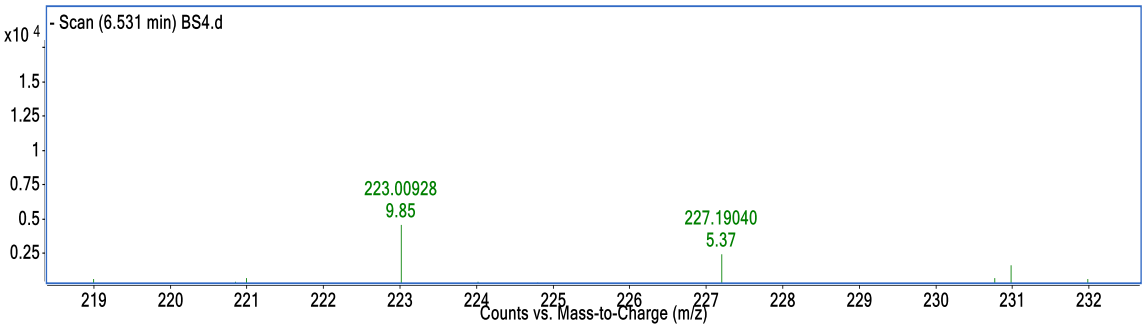


Figure 5.37. Mass spectrum for BPA present in Pioneer Park tap water sample (y-axis is signal intensity and x-axis is time in min)

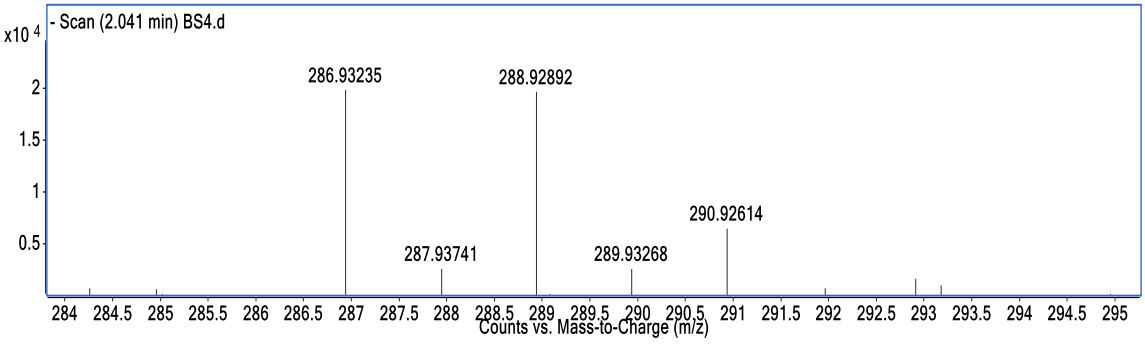


Figure 5.38. Mass spectrum for triclosan added to Pioneer Park tap water sample (y-axis is signal intensity and x-axis is time in min)

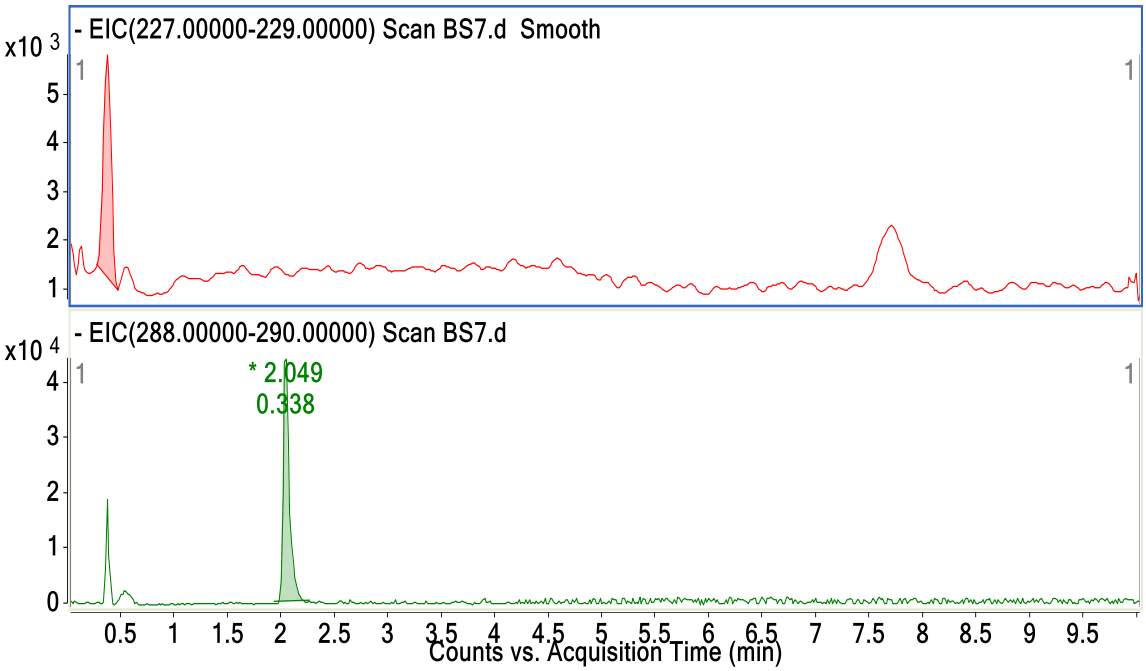


Figure 5.39. Chromatograms showing BPA and triclosan presence in Paul lake tap water sample (y-axis is counts and x-axis is time in min)

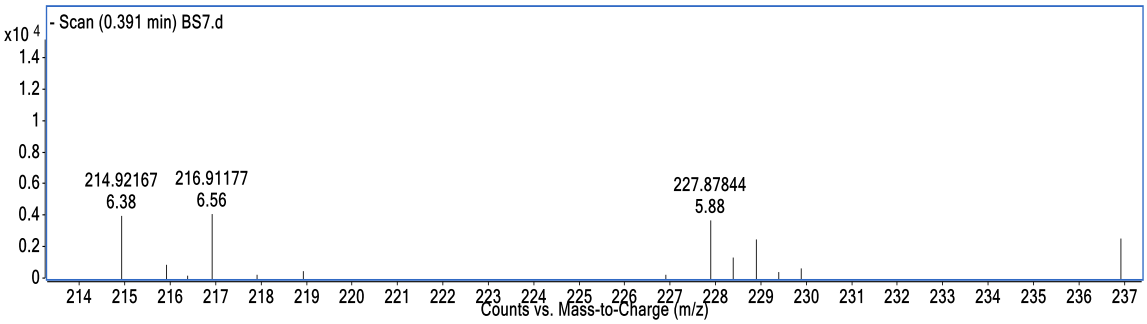


Figure 5.40. Mass spectrum for BPA present in Paul Lake water sample (y-axis is signal intensity and x-axis is time in min)

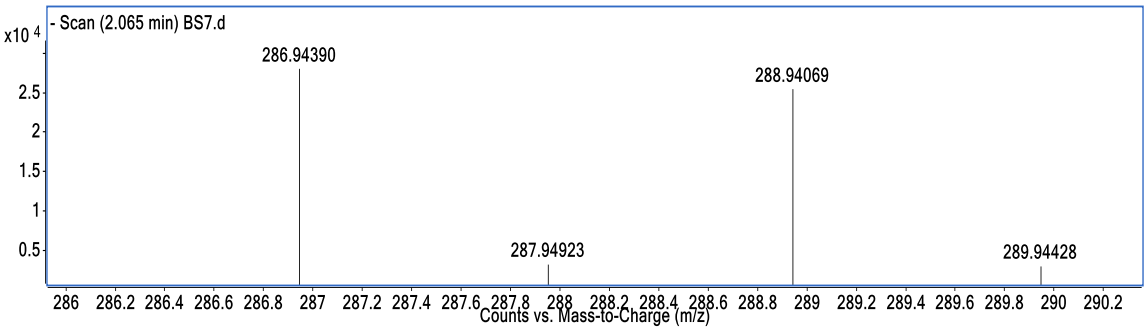


Figure 5.41. Mass spectrum for triclosan added in Paul Lake water sample (y-axis is signal intensity and x-axis is time in min)

Internal Calibration Data from LC/MS Sample Analysis Using Calibration Curve Y = 0.008x + 0.0061 (2 ppm – 75 ppm)

Concentrations of BPA determined in all the environmental, swimming pool and tap water samples (including samples set I, II and III) are given in Table 5.4.

Table 5.4. Internal calibration data from LC/MS analysis

No. of Observations	Sample Name	Set (I) Concentration (ppm)	Set (II) Concentration (ppm)	Set (III) Concentration (ppm)
1	Riverside Park	1.9	5.4	3.2
2	Pioneer Park	3.9	6.9	2.9
3	Shuswap Lake	2.9	4.6	2.6
4	Louis Lake	6.7	4.6	5.9
5	McArthur Island Park	3.9	4.2	2.4
6	Kamloops Lake	7.9	9.5	8.8
7	Adam’s River	2.2	3.6	2.6
8	Paul Lake Tap Water	4.4	5.2	5.3
9	Pioneer Tap Water	8.2	14.7	11.4
10	Paul Lake	7.9	10.7	8.3
11	P1-A	6.8	6.7	11.7

No. of Observations	Sample Name	Set (I) Concentration (ppm)	Set (II) Concentration (ppm)	Set (III) Concentration (ppm)
12	P1-B	6.7	16.1	4.4
13	P2-A	11.7	5.9	11.2
14	P2-B	3.0	2.1	5.4
15	P3-A	7.2	9.1	10.4
16	P3-B	10.1	9.9	7.2
17	P4-A	3.9	5.4	2.3
18	P5-A	3.6	5.9	2.7
19	P6-A	4.3	6.2	5.2
20	P7-A	8.9	15.4	6.2
21	P8-A	11.9	9.9	12.2

Sample Analysis on LC/MS

LC/MS method offers a rapid, practical, accurate and robust alternative for estimating BPA concentrations in variety of environmental waters. LC/MS method developed can detect BPA at 50 ppb level using triclosan as internal standard. Levels of BPA in environmental waters were in the lower ppm range (less than 15 ppm). BPA concentrations quantified in all environmental water samples were below human toxic BPA level. LC/MS results were in close agreement with the CE results. Concentrations of BPA present in samples analyzed by LC/MS are given in Figure 5.42. Concentrations of BPA present in the water samples analyzed by LC/MS with standard deviation error bars are shown in appendix Figure E.2.

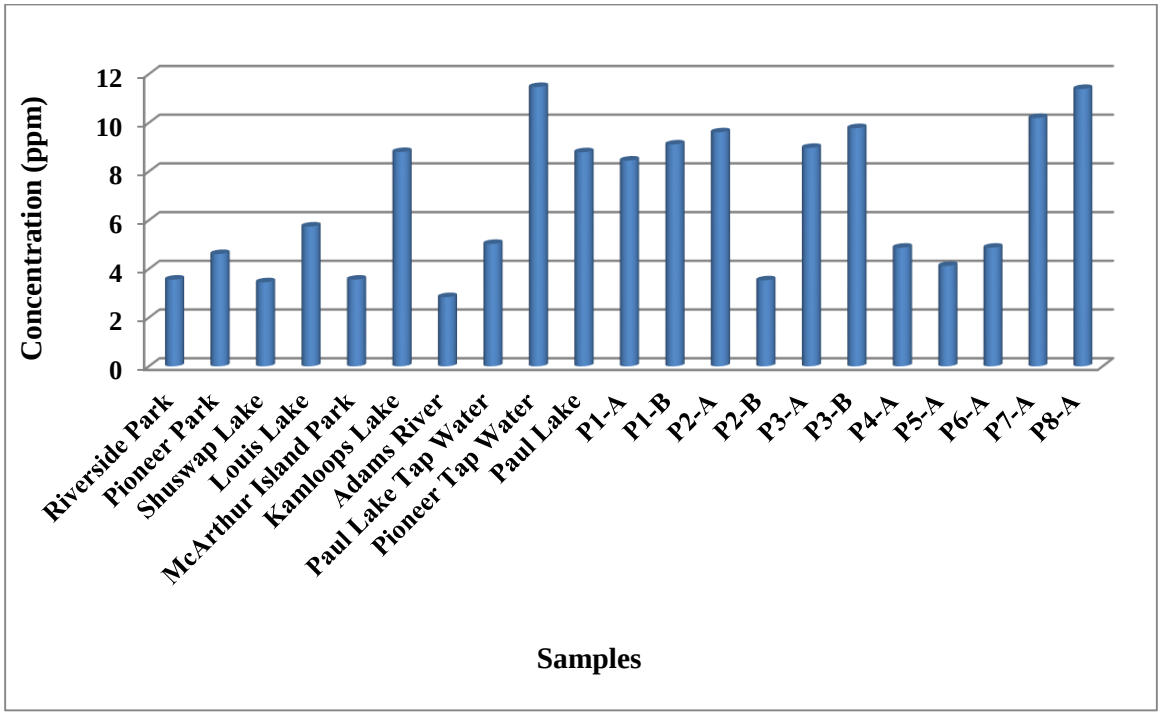


Figure 5.42. Concentrations of BPA present in samples analyzed by liquid chromatography-mass spectrometry (LC/MS)

Comparison of CE Standard Addition Results with LC/MS Internal Standard Results

The samples were analyzed on two different instruments CE and LC/MS. Two different techniques were used to account for the variation in sample analysis due to matrix effect, evaporation and other losses of analyte during sample preparation. It enhances the precision in our results. CE and LC/MS results were in close agreement with each other. CE standard addition concentrations were relatively higher than the LC/MS concentrations. A comparison of results from CE and LC/MS is shown in Figure 5.43. A comparison of results from CE and LC/MS with standard deviation error bars is shown in the appendix Figure E.3.

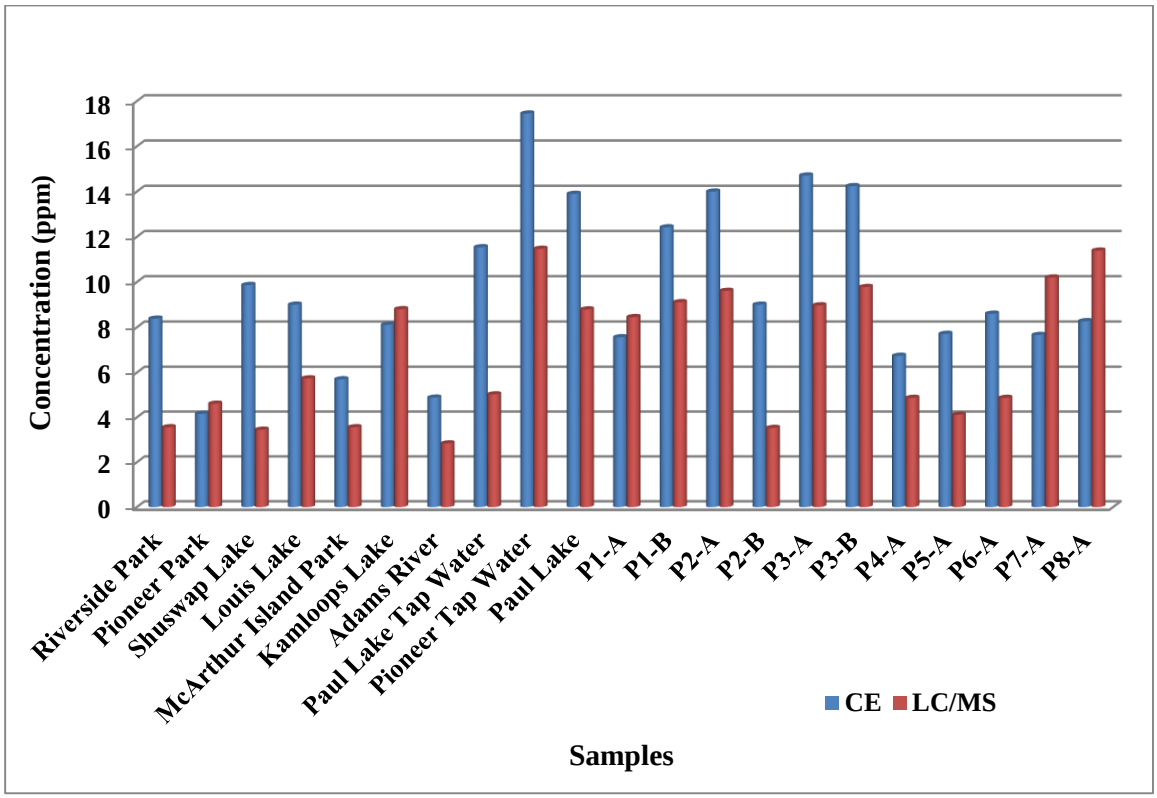


Figure 5.43. Comparison of capillary electrophoresis (CE) and liquid chromatography-mass spectrometry (LC/MS) results

Conclusions

Bisphenols, especially BPA, is produced in large amounts every year in the world. As far as the consumer demands are concerned there is over 6 billion pounds of BPA produced per year. Bisphenol A is critically important to the production of plastics used in thousands of products. It is therefore critically important for the regulatory agencies to take a strong stance in protecting the public's health. Bisphenol A binds to estrogen receptors (alpha and beta nuclear receptors and plasma-membrane bound receptors). Exposure to very low doses early in development alters the breast and prostate tissues in ways that increase the risk of developing cancer later in life and disrupts brain development and behavior. Low dose exposure may also increase the risk of developing insulin resistant diabetes and obesity and disrupt chromosomal alignment, resulting in one of the most common causes of miscarriage in humans.

Its estrogenicity, once considered weak, is now known to be much more potent. Most importantly, these effects are being reported at levels found in the human body. Wildlife is also exposed to similar kinds of threats with exposure to BPA. The data collected so far in the field of environmental toxicology is sufficiently robust to raise concerns about the potentially harmful impact of endocrine disrupting chemicals on humans and on other life forms. Extrapolation of evidence from animal studies to humans must be done cautiously because differences among species and strains have been reported regarding a variety of parameters. All of these pieces of evidence should encourage regulatory agencies to apply the precautionary principle and thus ban or substitute those chemicals that are likely to be harmful to the normal development of humans and wildlife. In the NTP report, the most recent statement by the FDA's commissioner, and a report from Health Canada classifying BPA as a human and environmental toxin all suggest a potential change in the perception of the regulatory community toward recognizing the risk posed by BPA exposure.

Water is the most essential and prime necessity of life. It is an essential requirement for the life supporting activities. Surface water generally available in rivers and lakes is used for drinking purposes. Aquatic organisms also need a healthy environment to live and need to have adequate nutrients for their growth. The productivity of the fresh water ecosystem depends on the physicochemical characteristics of the water bodies. Presence of BPA in environmental waters is not only a concern for the fresh water ecosystem but also to

humans. Waste water containing BPA can be a source of BPA contamination in to the surface water resources, BPA leaches from the hazardous waste landfills and enter in to the lakes and rivers. Tap water samples where water is pumped in to the taps through PVC pipes have high concentrations of BPA. BPA is not detected in those pool waters, which were disinfected by sodium hypochlorite pucks.

The methods developed for BPA detection and quantification using both CE and LC/MS were successful and offer rapid, practical, accurate and robust alternative for estimating BPA concentrations in variety of environmental waters. Levels of BPA determined in environmental waters were in the lower ppm range less than 18 ppm. BPA concentrations quantified in all environmental water samples studied were below human toxic BPA level of 50 ppm. A rapid and sensitive LC/MS method that was developed in this work can detect BPA at 50 ppb level using triclosan as internal standard. Simultaneous baseline separation was achieved for BPA, BPF, BPS, BPZ, BPP, BPAP, BPAF and BADGE using a protocol developed on CE. The LC/MS and CE results were found to be in close agreement. Both the CE and LC/MS methods were highly reproducible with %RSDs less than 10%.

Future Work

BPA can rapidly degrade in environmental waters because of the light and the action of microscopic organisms such as bacteria and algae. Water samples from rivers and lakes are needed to be analyzed on regular intervals. BPA has a half-life of 2.5 - 4 days; analysis of water samples on weekly basis can help establish the average amount of BPA present in a particular environmental water sample, at a given time. This can help determine the accurate concentration of BPA which the aquatic life is exposed to. Water samples from the waste water treatment plants are also needed to be analyzed in order to have an idea of the consumption of BPA in everyday life. Fresh water resources near the wastewater treatment plants and hazardous landfills must be analyzed to comprehend the sources of BPA leaching in to the environmental waters.

Swimming pool and environmental water samples for this study were collected in summer of 2014. Water samples are needed to be collected in the spring and at other times of year in order to determine, if the concentration of BPA fluctuates at different times in the year. Also, the samples were collected when there were lots of swimmers present in the pools and on beaches. Sediments were also present in some of the environmental waters. Sediments could possibly have BPA in them; presence of swimmers can also contribute to the quantity of BPA that is being introduced in the environmental and swimming pool waters. So the major source of BPA is yet to be identified.

Sample pretreatment using solid phase extractions is needed to be carried out in order to compare results to the simplified method developed in this work. Separation of other analogues was successfully achieved using the CE protocol in this thesis but due to time constraints no quantification of these analogues were done. So for the future it would be interesting to quantify the other analogues of bisphenols particularly BPS in the water samples because BPS is being used to replace BPA these days.

Analyzing the effect of bacteria on BPA is also very interesting. Bacteria of *Pseudomonas* sp. strain and *Streptomyces* sp. strain profoundly degrade BPA in the environmental waters under certain conditions. The optimized condition for these bacteria to biodegrade BPA needs to be determined. Other species of bacteria which can degrade or metabolize BPA in environmental waters are yet to be known.

A large number of publications on the toxicity and endocrine activity of BPA in animals have been reported and all of them have declared different levels of BPA that can harm different species of animals. Extrapolation of the findings from animal studies to human beings has led to controversies about the safety of BPA among the scientists and in national and state legislatures and also in the general public. It has now become important that government agencies organize drug style safety trials of BPA in humans as much basic information about how BPA behaves in the human body is still unknown.

References

1. Cyclicor Polymers (http://www.cyclicor.com) Date: 17-10-**2014**.
2. Olmo, M.; Gonzallez, A.; Navas, N.A.; Vilchez, J.L. Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry. *Anal. Chim. Acta.*, **1997,** *346*, 87-92.
3. Rubin, B.S. Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *J. Steroid Chem. Mol. Biol.*, **2011,** *127*, 27-34.
4. Dodds, E.C.; Lawson, W. Synthetic estrogenic agents without the phenantherene nucleus. *Nature*, **1936,** *137*, 996-997.
5. McLachlan, J.A. Prenatal exposure to diethylstilbestrol (DES): A continuing story. *IJE*, **2006**, *35*, 868-870.
6. Sheemol, V.N.; Unni, I.R.; Goppinathan, C. Catalysis by heteropoly acids: Formation of BPA from phenol and acetone. *Indian J. Chem. Technol.*, **2001**, *8*, 298-300.
7. The AMBERLYST (http://www.amberlyst.com/bpa.htm) Date: 17-10-**2014**.
8. Paseiro, P.; Simal, J.L.; Paz, S.; Lopez, P.; Simal, J. Kinetics of the hydrolysis of bisphenol F diglycildyl ether in water-based food simulants. Comparison with bisphenol A diglycidyl ether. *J. Agric. Food Chem.*, **1993,** *40*, 868-872.
9. He, M.; Urban, M.W.; Bauer, R.S. Exudation processes in hydrogenated bisphenol-a-based epoxy coatings: Spectroscopic study. *J. Appl. Polym. Sci.*, **1993,** *49*, 345-359.
10. Kang, J.; Kondo, F.; Katayama, Y. Human exposure to bisphenol A. *Toxicology*, **2006**, *226*, 79-89.
11. Dorn, P.B.; Chou, C.S.; Gentempo, J.J. Degradation of bisphenol A in natural waters. *Chemosphere*, **1987**, *16*, 1501-1507.
12. BPA General Toxicity (http://www.bisphenol-a.org/human/herGeneralToxicity.html) Date: 6-9-**2014**.
13. Toxicological Profile for BPA, September 2009. Office of Environmental Health Hazard Assessment.

14. Cousins, I. T.; Staples, C. A.; Klecka, G. M.; Mackay, D. A multimedia assessment of the environmental fate of Bisphenol A. *Hum. Ecol. Risk Assess.*, **2002**, *8*, 1107-1135.
15. Huang, Y.Q.; Wong, C.K.C.; Zheng, J.S.; Bouwman, H.; Barra, R.; Wahlström, B.; Neretin, L.; Wong, M.H. Bisphenol A (BPA) in China: A review of sources, environmental levels, and human health impacts. *Environ. Int.*, **2011**, *10*, 4-10.
16. Yasuhara, A.; Shiraishi, H.; Nishikawa, M.; Yamamoto, T.; Nakasugi, O.; Okumura, T.; Kenmotsu, K.; Fukui, H.; Nagase, M.; Kawagoshi, Y. Organic components in leachates from hazardous waste disposal sites. *Waste Manag. Res.*, **1999**, *17*, 186-197.
17. Asakura, H.; Matsuto, T.; Tanaka, N. Behavior of endocrine-disrupting chemicals in leachate from MSW landfill sites in Japan. *Waste Manag.*, **2004**, *24*, 613-622.
18. Yamada, K.; Urase, T.; Matsuo, T.; Suzuki, N. Constituents of organic pollutions in leachates from different types of landfill sites and their fate in the treatment processes. *J. Jpn. Soc. Water Environ.*, **1999**, *22*, 40–45.
19. Yamamoto, T.; Yasuhara, A.; Shiraishi, H.; Nakasugi, O. Bisphenol A in hazardous waste landfill leachates. *Chemosphere*, **2001**, *42*, 415–418.
20. Kang, J.H.; Kondo, F. Bisphenol A degradation by bacteria isolated from river water. *Arch. Environ. Contam. Toxicol.*, **2002a**, *43*, 265–269.
21. Kang, J.H.; Kondo, F. Effects of bacterial counts and temperature on the biodegradation of bisphenol A in river water. *Chemosphere.*, **2002b**, *49*, 493–498.
22. Klecka, G.M.; Gonsior, S.J.; West, R.J.; Goodwin, P.A.; Markham, D.A. Biodegradation of bisphenol A in aquatic environments: river die-away. *Environ. Toxicol. Chem.*, **2001**, *20*, 2725– 2735.
23. Kang, J.H.; Ri, N.; Kondo, F. *Streptomyces* sp. strain isolated from river water has high bisphenol A degradability. *Lett. Appl. Microbiol.*, **2004**, *39*, 178–180.
24. Larsson, D.J.; Adolfsson, M.; Parkkonen, J.; Pettersson, M.; Berg, A.H.; Olsson, P.E. Ethinyloestradiol-an undesired fish contraceptive? *Aquatic Toxicol.*, **1999**, *45*, 91–97.
25. Kang, J.H.; Kondo, F. Bisphenol A degradation in river water is different from that in seawater. *Chemosphere*, **2005,** *60*, 1288–1292.

26. Rudel, R.A.; Brody, J.G.; Spengler, J.D.; Vallarino, J.; Geno, P.W.; Sun, G.; Yau, A. Identification of selected hormonally active agents and animal mammary carcinogens in commercial and residential air and dust samples. *J. Air Waste Manage. Assoc.*, **2001**, *51*, 499–513.
27. Hanaoka, T.; Kawamura, N.; Hara, K.; Tsugane, S. Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. *Occup. Environ. Med.*, **2002**, *59*, 625–628.
28. Poole, A.; Herwijnen, P.; Weideli, H.; Thomas, M.C.; Ransbotyn, G.; Vance, C. Review of the toxicology, human exposure and safety assessment for bisphenol A diglycidyl ether (BADGE). *Food Addit. Contam.*, **2004**, *21*, 905–919.
29. Howard, P.H. Handbook of Environmental Fate and Exposure Data, Vol. 1. Lewis Publishers, Chelsea, MI. **1989**.
30. Bolz, U.; Hagenmaier, H.; Korner, W. Phenolic zenoestrogens in surface water, sediments, and sewage sludge from Baden-Wurttemberg, south-west Germany. *Environ. Pollut.*, **2001**, *115*, 291– 301.
31. Heemken, O.P.; Reincke, H.; Stachel, R.; Theobald, N. The occurrence of xenoestrogens in the Elbe River and the North Sea. *Chemosphere*, **2001**, *45*, 245–259.
32. Fent, G.; Hein, W.J.; Moendel, M.J.; Kubiak, R. Fate of ^{14}C-bisphenol A in soils. *Chemosphere*, **2003**, *51*, 735–746.
33. Kawahata, H.; Ohta, H.; Inoue, M.; Suzuki, A. Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki islands in Japan within coral reefs and adjacent river mouths. *Chemosphere*, **2004**, *55*, 1519–1527.
34. Takao, Y.; Lee, H.C.; Ishibashi, Y.; Kohra, S.; Tominaga, N.; Arizono, K. Fast screening method for bisphenol A in environmental water and infood bysolid-phase microextraction (SPEM). *J. Health Sci.*, **1999**, *45*, 39-45.
35. Brede, C.; Fjeldal, P.; Skjevrak, I.; Herikstad, H. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. *Food Addit. Contam.*, **2003**, *20*, 684–689.
36. Biles, J.E.; McNeal, T.P.; Begley, T.H. Determination of bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates. *J. Agric. Food Chem.*, **1997a**, *45*, 4697–4700.

37. Biles, J.E.; McNeal, T.P.; Begley, T.H.; Hollifield, H.C. Determination of bisphenol A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. *J. Agric. Food Chem.,* **1997b**, *45*, 3541–3544.
38. Philo, M.R.; Jickells, S.M.; Damant, A.P.; Castle, L. Stability of plastic monomers in food-simulating liquids under European Union migration test conditions. *J. Agric. Food Chem.,* **1994**, *42*, 1497–1501.
39. Olmo, M.; Gonzalez, A.; Navas, N.A.; Vilchez, J.L. Determination of BPA in water by gas chromatogharphy-mass spectrometry. *Anal. Chim. Acta,* **1997**, *346*, 87-92.
40. Geens, T.; Aerts, D.; Berthot, C.; Covaki, A.; Pussemier, L.; Lacomte, P,; Goeyens, L. A review of dietary and non-dietary exposure to BPA. *Food Chem. Toxicol.,* **2012**, *50*, 3725-3740.
41. National Institute of Environment Health Sciences (www.niehs.nih.gov)
42. Connor, J.C.; Chapin, R.E. Critical evaluation of observed adverse effects of endocrine active substances on reproduction and development, the immune system, and the nervous system. *Pure Appl. Chem.,* **2003**, *75*, 2099–2123.
43. Rubin, B.S. An endocrine disruptor with wide spread exposure and multiple effects. *J. Steroid Biochem. Mol. Biol.,* **2011**, *127*, 27-34.
44. Okada, H.; Tokunaga, T.; Liu, X.; Takayanagi, S.; Matsushima, A.; Shimohigashi, Y. Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. *Environ. Health Perspect*., **2008**, *116*, 32–8.
45. Vom, S.F.S.; Myers J.P. Bisphenol A and risk of metabolic disorders. *JAMA,* **2008**, *300*, 1353–1355.
46. Hengstler, J.G.; Foth, H.; Gebel, T.; Kramer, P.J.; Lilienblum, W.; Schweinfurth, H.; Volkel, W.; Wollin, K.M. A Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. *Crit. Rev. Toxicol.,* **2011**, *41*, 263-291.
47. Product Safety Assessment, Bisphenol A (www.dow.com/productsafety/finder/) Date: 3-1-**2015**.
48. Staples, C. A.; Dorn, P.B.; Klecka, G.M.; Branson, D.R.; O'Block, S.T.; Harris, L.R. A review of the environmental fate, effects and exposures of bisphenol A. *Chemosphere,* **1998**, *36*, 2149-2173.

49. Morohoshi, K.; Shiraishi, F.; Oshima, Y.; Koda, T.; Nakajima, N.; Edmonds, J.S.; Morita, M. Synthesis and estrogenic activity of bisphenol A mono- and di-beta-Dglucopyranosides, plant metabolites of bisphenol A. *Environ. Toxicol. Chem.*, **2007**, *22*, 2275-2279.
50. Kang, J. H.; Aasi, D.; Katayama, Y. Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. *Crit. Rev. Toxicol.*, **2007**a, *37*, 607-625.
51. Kang, J. H.; Kondo, F.; Katayama, Y. Human exposure to bisphenol A. *Toxicology*, **2006**, *226*, 79-89.
52. Chin, Y.P., Penney, L.; Miller, L.Z.; Cawley, K.; Weavers, L.K. Photosensitized degradation of bisphenol A by dissolved organic matter. *Environ. Sci. Technol*., **2004**, *38*, 5888-5894.
53. Peng, Z. E.; Feng, W.; Deng, N. Photodegradation of bisphenol A in simulated lake water containing algae, humic acid and ferric ions. *Environ. Pollut.*, **2006**, *144*, 840-846.
54. Zhan, M.; Yang, X.; Xian, Q.; Kong, L. Photosensitized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances. *Chemosphere*, **2006**, *63*, 378-386.
55. Sajiki, J.; Yonekubo, J. Degradation of bisphenol-A (BPA) in the presence of reactive oxygen species and its acceleration by lipids and sodium chloride. *Chemosphere*, **2002**, *46*, 345-354.
56. Sajiki, J.; Yonekubo, J. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. *Chemosphere*, **2003**, *51*, 55-62.
57. Licht, O.; Jungmann, D.; Ladewig, V.; Ludwichowski, K. U.; Nagel, R. Bisphenol-A in artificial indoor streams: I. Fate and effects on aufwuchs. *Ecotoxicology*, **2004**, *13*, 657-666.
58. Basheer, C.; Lee, H. K; Tan K. S. Endocrine disrupting alkylphenols and bisphenol-A in coastal waters and supermarket seafood from Singapore. *Mar. Pollut. Bull.*, **2004**, *48*, 1161-7.

59. Lindholst, C.; Wynne, P. M.; Marriott, P.; Pedersen, S. N.; Bjerregaard, P. Metabolism of bisphenol A in zebrafish (Danio rerio) and rainbow trout (Oncorhynchus mykiss) in relation to estrogenic response. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, **2003**, *135*, 169-77.
60. Sakurai, A.; Toyoda, S.; Sakakibara, M. Removal of Bisphenol A by polymerization and precipitation method using *Coprinus cinereus* peroxidase. *Biotech. Letters*, **2001**, *23*, 995-998.
61. Sonoki, T.; Kajita, S.; Uesugi, M.; Katayama, Y.; Iimura, Y. Effective removal of Bisphenol A from contaminated areas by recombinant plant producing lignin peroxidase. *J Pet. Environ. Biotech.*, **2011**, *2*, 105-110.
62. Kimura, Y.; Yamamoto, M.; Shimazaki, R.; Kashiwada, A.; Matsuda, K.; Yamada, K. Use of chitosan for removal of Bisphenol A from aqueous solutions through quinone oxidation by polyphenol oxidase. *J App. Polymer Sci.*, **2011**, *124*, 796-804.
63. Kuramitz, H.; Nakata, Y.; Kawasaki, M.; Tanaka, S. Electrochemical oxidation of Bisphenol A. Application to the removal of Bisphenol A using carbon fiber electrode. *Chemosphere*, **2001**, *45*, 37-43.
64. Ju, P.; Fan, H.; Guo, D.; Meng, X.; Xu, M.; Ai, S. Electrocatalytic degradation of Bisphenol A in water on a Ti-based PbO2-ionic liquids (ILs) electrode. *Chem. Eng. J.*, **2012**, *179*, 99-106.
65. Fukahori, S.; Ichiura, H.; Kitaoka, T.; Tanaka, H. Photocatalytic decomposition of Bisphenol A in water using composite TiO2-zeolite sheets prepared by a papermaking technique. *Environ. Sci. Technol.*, **2003**, *37*, 1048-1051.
66. Ren, Y.; Ma, W.; Ma, J.; Wen, Q.; Wang, J.; Zhao, F. Synthesis and properties of Bisphenol A molecular imprinted particle for selective recognition of BPA from water. *J Colloid Interface Sci.*, **2012**, *367*, 355-361.
67. Rosenmai, A.K.; Dybdahl, M.; Pedersen, M.; Alice, B.M.; Wedebye, E.B.; Taxvig, C.; Vinggaard, A.M. Are structural analogues to bisphenol A safe alternatives? *Toxicol Sci.*, **2014**, *16*, 35-47.
68. BPA Free Why It Matters (www.thermos.com/bpa.aspx) Date: 27-11-**2014**.

69. (Bisphenol-A, (http://www.wholefoodsmarket.com/about-our-products/food-safety/bisphenol) Date: 19-11-**2014**.
70. Cabaton, N.; Dumont, C.; Severin, I.; Perdu, E.; Zalko, D.; Cherkaoui-Malki, M.; Chagnon, M.C. Genotoxic and endocrine activities of bis(hydroxyphenyl)methane (bisphenol F) and its derivatives in the HepG2 cell line. *Toxicology*, **2009**, *255*, 15-24.
71. Audebert, M.; Dolo, L.; Perdu, E.; Cravedi, J.P.; Zalko, D. Use of the gamma H2AX assay for assessing the genotoxicity of bisphenol A and bisphenol F in human cell lines. Arch Toxicol **2011**, *85*, 1463-73.
72. Science News (https://www.sciencenews.org/article/another-plastics-ingredient-raises-safety-concerns) Date: 3-12-**2014**.
73. Liao, C.; Liu, F.; Kannan, K. Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol a residues". *Environ. Sci. Technol.*, **2012**, *46*, 6515–22.
74. Liao, C.; Liu, F.; Guo, Y.; Moon, H. B.; Nakata, H.; Wu, Q.; Kannan, K. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: Implications for human exposure. *Environ. Sci. Technol.*, **2012**, *46*, 9138.
75. Kuruto-Niwa, R.; Nozawa, R.; Miyakoshi, T.; Shiozawa, T.; Terao, Y. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. *Environ. Toxicol. Pharmacol.*, **2005**, *19*, 121-130.
76. Jenna Bilbrey "BPA-Free Plastic Containers May Be Just as Hazardous". *Scientific American*. (Aug 11, 2014).
77. Rauter, W.; Dickinger, G.; Zihlarz, R.; Lintschinger, J. Determination of Bisphenol A diglycidyl ether (BADGE) and its hydrolysis products in canned oily foods from the Austrian market, *Z. Lebensm. Unters. Forsch. A*, **1999**, *208*, 208–211.
78. Coulier, L.; Bradley, E.L.; Bas, R.C.; Verhoeck, K.C.M; Driffield, M.; Harmer, N.; Castle, L. Analysis of reaction products of food contaminants and ingredients: Bisphenol A diglycidyl ether (BADGE) in canned foods. *J. Agric. Food. Chem.*, **2010**, *58*, 4873-4882.

79. Hyoung, U.J.; Yang, Y.J.; Kwon, S.K.; Yoo, J.H.; Myoung, S.C.; Kim, S.C.; Hong, Y.P. Developmental toxicity by exposure to bisphenol A diglycidyl ether during gestation and lactation period in Sprague-Dawley male rats. *J. Prev. Med. Public Health.* **2007**, *40*, 155-61.
80. Tullo, A. H. Breaking in the new. *Chem. Engn. News*, **2011**, *89*, 10-14.
81. BPA substitute (http://www.rsc.org/chemistryworld/2014/03/green-safe-bpa-substitute-bgf-lignin-paper) Date: 17-9-**2014**.
82. Public Health focus, Bisphenol A: Use in Food Contact Application. (http://www.fda.gov/newsevents/publichealthfocus/ucm064437.htm) Date: 17-9-**2014**.
83. Environment Canada (http://ec.gc.ca/epe-epa/default.asp?lang=En&n=B1029F82-1) Date: 17-9-**2014**..
84. Environment Canada **(**http://www.hc-sc.gc.ca/fn-an/securit/packag-emball/bpa/index-eng.php**)**. Screening Assessment for the Challenge Phenol, 4,4' -(1-methylethylidene)bis-(Bisphenol A) Chemical Abstracts Service Registry Number 80-05-7. **2008**. Date: 17-9-**2014**.
85. Health Canada (www.hc-sc.gc.ca). Date: 17-9-**2014**.
86. Vandenberg, L.N.; Hauser, R.; Marcus, M.; Olea, N.; Welshons, W.V. Human exposure to bisphenol A (BPA). *Reprod. Toxicol.*, **2007**, *24*, 139–177.
87. Calafat, A.M.; Kuklenyik, Z.; Reidy, J.A.; Caudill, S.P.; Ekong, J.; Needham, J.L. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ. Health Perspect.*, **2005**, *113*, 391–395.
88. Le, H.H.; Carlson, E.M.; Chua, J.P.; Belcher, S.M. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicol. Lett.*, **2008**, *176*, 149–156.
89. Opinion of the scientific committee on bisphenol A. **2002**, (http://europa.eu.int/comm/food/fs/sc/scf/out128_en.pdf) Date: 10-10-**2014**.
90. Wilson, N.K.; Chuang, J.C.; Lyu, C.; Menton, R.; Morgan, M.K. Aggregate exposure of nine preschool children to persistent organic pollutants at day care and at home. *Environ. Epidemiol.*, **2003**, *13*, 187-202.

91. Wilson, N.K.; Chuang, J.C.; Morgan, M.K.; Lordo, R.A.; Sheldon, L.S. An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. *Environ. Res.*, **2007**, *103*, 9–20.
92. Yokota, H.; Iwano, H.; Endo, M.; Kobayashi, T.; Inoue, H.; Ikushiro, S.; Yuasa, A. Glucuronidation of the environmental oestrogen, bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver. *Biochem. J.*, **1999**, *340*, 405–409.
93. Suiko, M.; Sakakibara, Y.; Liu, M.C. Sulfation of environment estrogen-like chemicals by cytosolic sulfotransferases. *Biochem. Biophys. Res. Commun.*, **2007**, *267*, 80-84.
94. Kim, Y.H.; Kim, C.S.; Park, S.; Han, S.Y.; Pyo, M.Y.; Yang, M. Gender differences in the levels of bisphenol A metabolites in urine. *Biochem. Biophys. Res. Commun.*, **2003**, *312*, 441-448.
95. Sakamoto, H.; Yokota, H.; Kibe, R.; Sayama, Y.; Yuasa, A. Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. *Biochim. Biophys. Acta.*, **2002**, *1573*, 171-176.
96. Mykkanen, H.; Tikka, J.; Pitkanen, T.; Hanninen, O. Fecal bacterial enzyme activities in infants increase with age and adoption of adult-type diet. *J. Pediatr. Gastroenterol. Nutr.*, **1997**, *25*, 312-316.
97. Yan Xu. Tutorial of Capillary Electrophoresis. *The Chemical Educator*, **1996**, 2, 1430-4171.
98. CHEMWIKI Capillary Electrophoresis: (http://chemwiki.ucdavis.edu/Analytical_Chemistry/Instrumental_Analysis/Capillary Electrophoresis) Date: 19-9-**2014**.
99. Basic Principles and Modes of Capillary Electrophoresis by Harry Whatley (http://link.springer.com/chapter/10.1007%2F978-1-59259-120-6_2) Date: 19-9-**2014**.
100. Deyl, Z.; Mikshik, I.; Tagliaro, F. Advances in capillary electrophoresis. *Forensic Sci. Int.*, **1998**, *92*, 89-124.

101. Kok, W. Capillary Electrophoresis: Instrumentation and Operation, *Chromatographia*, **2000**, *4*, 44-51.
102. Li, S.F.Y. Capillary Electrophoresis: Principles, *Practice and Applications*, *J. of Chrom. Library*, **1992**, *52*, 155-160.
103. Weinberger, R. Practical Capillary Electrophoresis 2nd ed, **1999**.
104. Hancu, G.; Simon, B.; Rusu, A.; Mircia, E.; Gyeresi, A. Principles of Micellar Electrokinectic Capillary Chromatography applied in Pharmaceutical Analysis. *Adv. Pharm. Bull.*, **2013**, *3*, 1-8.
105. Deeb, S.E.; Iriban, A.M.; Gust, R. Review: MEKC as a powerful growing analytical technique. *Electrophoresis*, **2011**, *32*, 166–183.
106. Beckman Coulter P/ACE MDQ™ User's Guide.
107. Separation Science, premier learning for analytical chemists (http://www.sepscience.com/Information/Archive/CE-Solutions/1438-/CE-Solutions-6-Detection-in-Capillary-Electrophoresis) Date: 22-9-**2014**.
108. Agilent Technologies (http://www.chem.agilent.com/library/support/documents/f01002.pdf) Date: 22-9-**2014**.
109. Pitt, J.J. Principles and applications of liquid chromatography mass spectrometry in clinical biochemistry. *Clin. Biochem. Rev*., **2009**, *30*, 19–34.
110. Waters LC/MS booklet. (http://ccc.chem.pitt.edu/wipf/Waters%20LC-MS%20primer.pdf) Date: 17-9-**2014**.
111. Hewlett Packard, A Primer, Basics of LC/MS. (http://www.chem.agilent.com/library/support/documents/a05296.pdf) Date: 17-9-**2014**.
112. TOSOH: The Chemistry of Innovation (http://www.separations.eu.tosohbioscience.com/servicesupport/techsupport/resourcecenter/principlesofchromatography) Date: 17-9-**2014**.
113. An Ion Source tutorial by Andrew Guzzetta. (http://www.ionsource.com/tutorial/chromatography/rphplc.htm) Date: 17-9-**2014**.
114. Agilent Technologies, User Manual for LC-MS System.

115. Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: Better by GC-MS or LC-MS/MS? *Mass. Spectrom. Rev.*, **2006**, *25*, 838–865.
116. QuEChERS (www.quechers.com) Date: 28-6-**2014**.
117. AB SCIEX (http://www.absciex.jp/Documents/Downloads/Literature/mass-spectrometry-cms_073088.pdf) Date: 18-12-**2014**.
118. Wu, Z.; Huang, Z.; Lehmann, R. The application of chromatography-mass spectrometry: methods to metabolomics. *Chromatographia*, **2009**, *69*, 23–32.
119. Chen, T.C.; Shue, M.F.; Yeh, Y.L.; Hsieh, C.Y.; Kuo, Y.T.; Kuo, C.T. Variation, correlation, and toxicity of phenolic endocrine-disrupting compounds in surface water. *J. Environ. Sci. Health A - Environ. Sci. Eng. Tox. Hazard.*, **2009**, *44*, 1244-1250.
120. McClellan, K.; Halden, R.U. Pharmaceutical and personal care products in archived US biosolids from the 2001 EPA national sewage sludge survey. *Adv. Water. Res.*, **2010**, *44*, 658-668.
121. Lee, H. B.; Peart, T. E. Bisphenol A contamination in Canadian municipal and industrial wastewater and sludge samples. *Water Qual. Res. J. Can.*, **2000**, *35*, 283-289.
122. Wang XJ, Gao NY, Sun XF, Xu B. Influencing factors and kinetics of oxidation of bisphenol A in water with sodium hypochlorite. **2007,** *28*, 2544-9.

Appendix

Appendix A: Information on BPA

Table A.1. BPA exposure effects on living organisms (U.S EPA Report 2014)

Property	Data	Reference
	Toxicokinetics	
Dermal Absorption	➢ Human skin: 10% of applied mM dose was absorbed. ➢ Pig skin: 10 μg/mL radiolabeled BPA. After 2, 5, and 10 hours of exposure, the total BPA skin content was 3%, 6.9%, and 11.4% of the applied dose, respectively. BPA remained in the skin surface and accumulated primarily in the dermis.	EINECS, 2010; NIOH, 2010
Absorption, Distribution, Metabolism & Excretion	➢ Data located for rats, mice, monkeys, and humans indicate that ingested BPA is rapidly and extensively absorbed from the gastrointestinal tract (up to 85 - 86% in rats and monkeys and essentially 100% of a relatively small dose in humans). ➢ Orally-absorbed BPA undergoes extensive first-pass metabolism. ➢ In all species studied, the major metabolic pathway involved was the conjugation of BPA to BPA-glucuronide. ➢ Approximately 13 - 42% of an administered BPA dose was recovered in the urine of rats as the glucuronide	EINECS, 2010

Property	Data	Reference
	metabolite; 50 - 83% was eliminated in the feces, mostly as free BPA. ➢ Limited excretion in the milk was observed.	
	Acute Mammalian Toxicity	
Acute Lethality		
Oral Lethality	Mouse LD_{50} = 1,600 mg/kg BW; Rabbit LD_{50} = 2,230 mg/kg BW Rabbit LD_{50} = 3,000 – 6,400 mg/kg BW	EINECS, 2010; European Commission, 2000
Dermal and Inhalation Lethality	No deaths among male and female rats (10/sex) exposed to BPA dust at 0.17 mg/L (highest attainable concentration) for 6 hours; transient slight nasal tract epithelial damage was evident.	
	Carcinogenicity	
Carcinogenicity	Based on existing carcinogenicity study data, There is confidence that exposure to BPA: ➢ Exhibits endocrine activity and has estrogenic properties ➢ Estradiol-17β is classified as carcinogenic (IARC); It is likely that exposure to BPA: ➢ May be associated with increased cancers of hematopoietic system and increased interstitial cell tumors ➢ Alters function of microbules ➢ Induces aneuploidy in cells and tissues use a predisposition for preneoplastic	Keri, Ho et al., 2007

Property	Data	Reference
	lesions in adult mammary gland and prostate gland tissues It is possible that exposure to BPA: ➢ Induces *in vitro* cellular transformation ➢ Promotes tumor progression and reduces time to recurrence in advanced prostate cancers with androgen receptor mutations.	
Genotoxicity	➢ Largely negative results in a variety of *in vitro* test systems, including studies with *Salmonella typhimurium*, Chinese hamster V79 cells, Syrian hamster embryo cells, and mouse lymphoma cells. ➢ DNA damage was induced in MCF-7 and MDA-MB-231 cells, DNA adduct formation in Syrian hamster ovary cells, and a number of positive findings have been reported for the potential for BPA to inhibit purified microtubule polymerization, affect the spindle apparatus, and produce aneuploidy in *in vitro* studies with Chinese hamster V79 cells or oocytes from Balb/c or MF1 mice. ➢ FAO/WHO Expert Panel concludes: BPA is not a mutagen in *in vitro* test systems, nor does it induce cell transformation. BPA has been shown to	FAO/WHO, 2011

Property	Data	Reference
	affect chromosomal structure in dividing cells in *in vitro* studies, but evidence for this effect in *in vivo* studies is inconsistent and inconclusive. BPA is not likely to pose a genotoxic hazard to humans.	
	Reproductive Effects	
Reproductive and Fertility Effects	Female effects: There is sufficient evidence in rats and mice that BPA causes female reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 50 mg/kg BW/day and a LOAEL of 500 mg/kg BW/day. Male effects: There is sufficient evidence in rats and mice that BPA causes male reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 50 mg/kg BW/day and a LOAEL of 500 mg/kg BW/day. The joint FAO/WHO Expert Panel reviewed located reproductive and developmental toxicity data for BPA as of November 2010 and noted that most regulatory bodies reviewing the numerous studies on BPA have indicated an oral reproductive and developmental NOAEL of 50 mg/kg BW/day.	Chapin et al. 2008; NTP-CERHR, 2008; FAO/WHO, 2011

Property	Data	Reference
	➢ The joint FAO/WHO Expert Panel reviewed reproductive and developmental toxicity data for BPA located as of November 2010 and noted that most regulatory bodies reviewing the numerous studies on BPA have indicated an oral reproductive and developmental NOAEL of 50 mg/kg BW/day. ➢ Furthermore, changes in brain biochemical signaling, morphometric and cellular endpoints within sexually dimorphic anatomical structures and neuroendocrine end-points were reported at dietary exposures below 5 mg/kg BW/day. Methodological limitations introduce uncertainty in interpretation of the findings.	FAO/WHO, 2011
	Neurotoxicity	
Neurotoxicity Screening Battery (Adult)	There is potential for neurotoxicity effects based on the presence of the phenol structural alert (Estimated)	U.S. EPA, 2010; Professional judgment
	Repeated Dose Effects	
Repeated Dose	The FAO/WHO Expert Panel reviewed the located information regarding repeated-dose oral toxicity of BPA and concluded that results demonstrated effects on the liver, kidney, and body weight at doses of 50 mg/kg BW/day and higher and that the lowest NOAEL was 5 mg/kg BW/day.	FAO/WHO, 2011

Property	Data	Reference
	Skin Sensitization	
Mice	➢ Negative in a modified local lymph node assay of mice administered BPA epicutaneously on the ears at concentrations up to 30% on 3 consecutive days.	EINECS, 2010
	Negative in a local lymph node assay modified to test for photoreactivity in mice administered BPA epicutaneously on the ears at concentrations up to 30% on 3 consecutive days and irradiated with UV light immediately following application.	EINECS, 2010
	Negative in several sensitization tests using guinea pigs.	
Guinea Pigs	➢ Positive in 2/16 guinea pigs receiving BPA (50% in dimethyl phthalate) for 4 hours (occluded) once per week for 3 weeks and single challenge (4 hours occluded) 2 weeks later.	European Commission, 2000; EINECS
Mouse	➢ Negative, mouse; BPA applied as 1% solution in acetone and corn oil for 2 days; induced UV-photosensitization on flank and ears.	European Commission, 2000
	➢ Positive, mouse ear swelling photoallergy test.	
Rabbits	➢ Positive, rabbits; repeated dermal application (30 times over 37 days) of BPA (pure powder) produced moderate swelling and redness; skin turned yellow	NIOSH, 2010

Property	Data	Reference
	followed by dark pigmentation after day 15.	
Humans	➢ Negative in comprehensive medical surveillance data obtained from three BPA manufacturing plants for 875 employees examined for several years where workers were potentially exposed to other chemicals (phenol, acetone) that are not considered to be skin sensitizers.	EINECS, 2010
	➢ Limited human data provide suggestive evidence that BPA may potentially act as a dermal sensitizer, although concomitant exposure to other potential dermal sensitizers may reflect a cross-sensitization response.	EINECS, 2010
	➢ The Joint FAO/WHO Expert Meeting review of the toxicological aspects of BPA concludes that BPA is capable of producing a skin sensitization response in humans.	FAO/WHO, 2011
	Eye Irritation	
Rabbit	Slight to high Irritation	EINECS, 2010; European Comission, 2000
	Immunotoxicity	
Immune System Effects (Included under Repeated Dose)	Rodent studies (direct or *in utero* exposure) suggest that BPA may modulate immune homeostasis, but due to study variations and deficiencies, there is no clear evidence that	Willhite, Ball et al. 2008; FAO/WHO, 2011

Property	Data	Reference
	BPA interferes with immune function.	
	Endocrine Activity	
	➢ The estrogenicity of BPA has since been evaluated using several different kinds of in vitro assays, including binding assays, recombinant reporter systems, MCF-7 cells, rat pituitary cells, rat uterine adenocarcinoma cells, human adenocarcinoma cells, fish hepatocytes (vitellogenin production), and frog hepatocytes (vitellogenin production). According to the NTP-CERHR Expert Panel, there is considerable variability in the results of these studies with the estrogenic potency of BPA ranging over about 8 orders of magnitude.	NTP-CERHR, 2008
	➢ A number of in vivo tests have been conducted with most of the focus on effects on uterine weight in immature or ovariectomized animals. These studies indicate that the potency of BPA in increasing uterine weight varies over ~4 orders of magnitude. According to the NTP-CERHR Expert Panel, oral BPA does not consistently produce robust estrogenic responses and, when seen, estrogenic effects after oral treatment occur at high-dose levels.	NTP-CERHR, 2008
	➢ A limited number of studies have	NTP-CERHR,

Property	Data	Reference
	evaluated androgen activity of BPA. These studies provide little evidence of androgenic effects, but there is limited evidence of antiandrogenicity.	2008
	➢ Positive estrous response; subcutaneous injections of BPA to ovariectomized rats (positive response measured by cornification in vaginal smears).	European Commission, 2000
	➢ Numerous studies were located regarding the behavior of BPA as an estrogen or xenoestrogen in ecological organisms. Important results include findings that BPA increases plasma vitellogenin concentration in freshwater and saltwater fish at a potency in the range of 10^{-4} that of 17β-estradiol and that BPA can bind to the estrogen receptor of fish, albeit at a lower affinity than that of 17β-estradiol.	EINECS, 2010
	➢ BPA can interact with non-classic estrogen receptor systems at similar or lower concentrations than interactions with ERα and ERβ. BPA has a high binding affinity to estrogen-related receptor-γ (ERRγ), an orphan receptor that shares a sequence homology with ERα and ERβ but is not activated by estradiol.	NTP, 2010
	➢ BPA also impacts cellular physiology through rapid signaling mechanisms,	NTP, 2010

Property	Data	Reference
	independent of nuclear hormone receptor activity, to modify the activities of various intracellular signaling networks. Maximal rapid signaling effects for BPA and 17β-estradiol are often observed at similar concentrations.	
	Representative *in vitro* studies	
	Receptor Binding Assays	
	➢ In a human ER binding assay, the relative binding affinity (RBA) of BPA was 0.195% compared to 126% for 17β-estradiol. RBAs for other bisphenol compounds included 0.129% for BPC, 0.0803% for BPAP, 0.0719% for BPF, and 0.0055% for BPS. An RBA of 0.00473% was reported for PHBB.	METI, 2002
	➢ In a competitive ER binding assay using human ERα, the RBA for BPA was 0.32% that of 17β-estradiol. RBAs for other bisphenol compounds included 1.68% for BPC, 1.66% for BPAP, and 0.09% for BPF.	Coleman, Toscano et al. 2003
	➢ In a rat uterine cytosol assay that evaluated ER binding affinity, ER binding affinities for BPA and BPF were approximately 3 orders of magnitude less than that for 17β-estradiol.	Perez, Pulgar et al. 1998
	➢ In a rat uterine cytosolic ER-competitive binding assay, results for BPA, BPS, and PHBB indicated a weak affinity for ER.	Laws, Yavanhxay et al. 2006

Property	Data	Reference
	➢ BPA exhibited weak ER binding activity in preparations from uteri of ovariectomized Sprague-Dawley rats as evidenced by a relative binding affinity (RBA) that was 0.008% of the binding affinity of 17β-estradiol. RBAs for other tested chemicals included 0.003% for PHBB, 0.0009% for BPF, and 0.0007% for the proprietary substituted phenolic compound.	Blair, Branham et al. 2000
	Representative *in vitro* studies	
	Gene Transcription Assays	
	➢ BPA exhibited evidence of estrogenic activity in a yeast (*Saccharomyces cerevisiae*) two-hybrid assay using ERα and the coactivator TIF2. Based on estrogenic activity that was 5 orders of magnitude lower than that of 17β-estradiol, BPA was considered weakly estrogenic. Assessment of other bisphenols resulted in a ranking of relative potency as follows: BPC ≥ BPA > BPF > BPS.	Chen, Michihiko et al. 2002
	➢ BPA exhibited estrogenic activity approximately 10,000-fold less than that of 17β-estradiol in an in vitro recombinant yeast estrogen assay; the estrogenic activities of BPF and PHBB were 9,000-fold and 4,000-fold less than that of 17β-estradiol.	Miller, Wheals et al. 2001

Property	Data	Reference
	➢ BPA exhibited evidence of estrogenic activity in a yeast (*Saccharomyces cerevisiae*) two-hybrid assay using ERα and the coactivator TIF2.	Nishihara, Nishikawa et al. 2000
	➢ In a yeast two-hybrid system (reporter gene assay) using β-galactosidase activity as a measure of estrogenic activity, an estrogenic response was elicited by BPA and BPF but not by BPS.	Hashimoto and Nakamura, 2000
	➢ In a yeast two-hybrid assay (reporter gene assay) using β-galactosidase activity as a measure of estrogenic activity, an estrogenic response was elicited by BPA and BPF.	Ogawa, Kawamura et al. 2006
	➢ In a reporter gene assay of estrogen-induced transcriptional activity, relative activity (RA) for BPA was 0.00278% compared to 81.7% for 17β-estradiol. RAs for other bisphenol compounds included 0.00189% for BPC, 0.000639% for BPF, 0.000254% for BPS, and 0.000184% for BPAP. An RA of 0.000592% was reported for PHBB.	METI, 2002
	➢ In an ER-mediated reporter gene expression assay, BPA induced reporter gene expression at a relative activity (RA) of 2.75×10^{-3} that of 17β-estradiol. RAs for other bisphenol compounds included 5.3×10^{-4} for bisphenol F,	Coleman, Toscano et al. 2003

Property	Data	Reference
	$4.9x10^{-4}$ for bisphenol C, and $9.0x10^{-5}$ for bisphenol AP.	
	➢ In an ERE-luciferase reporter assay using MCF-7 cells, an EC_{50} was 0.63 µM for BPA compared to an EC_{50} of $8.6x10^{-6}$ for 17β-estradiol (i.e., BPA was approximately 5 orders of magnitude less potent than 17β-estradiol at inducing estrogenic activity). EC_{50} values for other bisphenol compounds included 0.42 µM for bisphenol C, 1.0 µM for bisphenol F, and 1.1 µM for bisphenol S.	Kitamura, Suzuki et al. 2005
	➢ In an ERE-luciferase reporter assay using MCF-7 cells in the presence of 17β-estradiol, neither BPA, bisphenol C, bisphenol F, bisphenol S, nor bisphenol M appeared to exert an anti-estrogenic effect.	Kitamura, Suzuki et al. 2005
	Representative *in vitro* studies Progesterone Receptor Induction	
	➢ BPA induced progesterone receptors in cultured human mammary cancer cells (MCF-7), but the magnitude of the induction was not specified.	EINECS, 2010; European Commission, 2000
	➢ In an assay designed to evaluate estrogenic effects on the number of progesterone receptors (PgR) in MCF7 cells, 17β-estradiol, BPA, and	Perez, Pulgar et al. 1998

Property	Data	Reference
	bisphenol F each increased the concentration of PgR by approximately 10 to 15 fold.	
	Representative *in vitro* studies	
	Cell Proliferation Assays	
	➢ In an E-SCREEN test of MCF7 cell proliferation (an indicator of estrogenic activity), the proliferative potency of BPA was approximately 10^{-5} that of 17β-estradiol, suggestive of a weakly estrogenic effect for BPA. The potency of bisphenol F was somewhat less than that of BPA.	Perez, Pulgar et al. 1998
	➢ In a proliferation assay of MCF-7 human breast cancer cells that contain ERα and ERβ and are known to proliferate in response to estrogens, BPA induced a proliferative response that was $2.0x10^{-3}$ that of 17β-estradiol. Proliferative values for other bisphenol compounds included $1.6x10^{-3}$ for bisphenol C, $1.0x10^{-3}$ for bisphenol F, and $6.0x10^{-4}$ for bisphenol AP.	Coleman, Toscano et al. 2003
	➢ In an E-screen test for estrogenicity, BPA and bisphenol F increased proliferation of MCF-7 cells with EC_{50} values of 410 nM and 84.8 nM, respectively, compared to an EC_{50} of 0.0045 nM for 17β-estradiol. The results indicate a weak estrogenic effect	Stroheker, Picard et al. 2004

Property	Data	Reference
	with BPF exerting a more potent effect than BPA.	
	➢ In an E-screen test for estrogenicity, BPA, bisphenol F, and bisphenol S increased proliferation of MCF-7 cells at concentrations in the range of 10^{-4} to 10^{-7} M. BPA appeared to be more effective than bisphenol S or bisphenol F.	Hashimoto, Moriguchi et al. 2001
	➢ BPA increased the rate of proliferation of MCF-7 cells at 3 - 5 orders of magnitude less than that of 17β-estradiol.	EINECS, 2010; European Commission, 2000
	➢ In an assay that measured induction and secretion of pS2 in cultured MCF7 cells (ELSA-pS2 immunoradiometric assay), induction of pS2 by BPA and bisphenol F was approximately 1,000-fold less than that of 17β-estradiol.	Perez, Pulgar et al. 1998
	Representative *in vivo* studies	
	➢ Exposure of immature female rats to BPA (gavage dosing once daily for 4 days) resulted in no apparent effects on uterine weight. BPF-treated rats exhibited significantly increased uterine weight. There were no effects on uterine weight of BPF or BPA treated ovariectromized rats.	Stroheker, Picard et al. 2004
	➢ In uterotrophic assays using ovariectomized mice, BPA treatment at	Kitamura, Suzuki

Property	Data	Reference
	doses in the range of 20 to 500 mg/kg/day for 3 days resulted in dose-related increased relative uterus weights of 147 - 185% that of controls compared to nearly 500% increased uterus weight in mice administered 17β-estradiol at 50 µg/kg/day. This result is indicative of an estrogenic effect *in vivo*.	et al. 2005
	➢ In an uterotrophic assay in which immature female rats were injected with BPF, BPS, or BPM subcutaneously for three consecutive days, observed changes in uterine weight indicated that BPF, BPS, and BPM exerted both estrogenic and anti-estrogenic responses.	Akahori, Makai et al. 2008
	Representative Androgen Assays	
	➢ In an ARE-luciferase reporter assay using a mouse fibroblast cell line (NIH3T3 cells), neither BPA, BPC, BPF nor BPS exerted an androgenic effect.	Kitamura, Suzuki et al. 2005
	➢ In an ARE-luciferase reporter assay using a mouse fibroblast cell line (NIH3T3 cells), BPA inhibited the androgenic activity of dihydrotestosterone. Anti-androgenic responses were elicited by BPC, BPF, and BPS as well.	Kitamura, Suzuki et al. 2005

Property	Data	Reference
	➢ BPA and BPF induced androgenic effects in MDA-MB453 cells transfected with an AR responsive luciferase reporter gene; anti-androgenic effects were elicited in the presence of dihydrotestosterone. Relative potency of the androgenic and anti-androgenic effects elicited by BPA was similar to that of BPF.	Stroheker, Picard et al. 2004
	Representative Thyroid Assays	
	➢ In an assay of thyroid hormonal activity whereby induction of growth hormone production is assessed in GH3 cells, neither BPA nor BPC inhibited growth hormone production.	Kitamura, Suzuki et al. 2005 Kitagawa,Takatori et al. 2003
	➢ BPA did not exhibit thyroid hormone receptor binding in a yeast two-hybrid assay system.	

Table A.2. Enzymes capable of biodegrading and metabolizing BPA

Enzymes	Sources	References
Manganese peroxidase (MnP)	Fungi (Pleurotus ostreatus O-48, *Phanerochaete chrysosporium* ME-446, *Trametes versicolor* IFO-7043, *Phanerochaete chrysosporum* ME-446 and *Trametes versicolor* IFO-6482)	Hirano et al. (2000), Tsutsumi et al. (2001), Suzuki et al. (2003)
Laccase	Fungi (*Phanerochaete chrysosporium* ME-446, *Trametes versicolor* IFO-7043, *Trametes villosa*, *Phanerochaete chrysosporum* ME-446 and *Trametes versicolor* IFO-6482)	Tsutsumi et al. (2001), Fukuda et al. (2001), Uchida et al. (2001), Suzuki et al. (2003)
Peroxidase	Bacteria (*Coprinus cinereus*), plant [soybean and horseradish (*Armoracia rusticana*)]	Sakurai et al. (2001), Caza et al. (1999), Sakuyama et al. (2003)
Polyphenol oxidase	Plant (mushroom)	Yoshida et al. (2002)
Cytochrome P450	Bacteria (*Sphingomonas* sp. strain AO1), mammals (mouse and rat)	Sakurai et al. (2001), Yoshihara et al. (2001)

Enzymes	Sources	References
UDP-glucuronosyltransferase (UGT)	Fish [carp (*Cyprinus carpino*)], mammals (mouse, rat and human)	Yokota et al. (2002), Cappiello et al. (2000), Matsumoto et al. (2002)
Sulfotransferase	Mammal (human)	Suiko et al. (2000), Nishiyama et al. (2002)

Table A.3. Global BPA production capacity (Jaio et al. 2008)

Country/Region	Production Capacity (10^3 tonnes/year)	Percentage
USA	1075	22.9
Taiwan	615	13.1
Japan	611	13.0
Germany	456	9.7
Netherlands	410	8.7
Spain	280	6.0
Korea	260	5.5
Singapore	230	4.9
Belgium	220	4.7
China Mainland	167	3.6
Russia	165	3.5
Thailand	160	3.4
Brazil	27	0.6
Belgium	220	4.7
Poland	12	0.3
Czechoslovakia	8.5	0.2
Sum	4696.5	100

Table A.4. Microorganisms capable of biodegrading and metabolizing BPA

Microorganisms	Strains	References
Planktons	➢ *Chlorella fusca* var. *vacuolata*	Hirooka et al. (2003)
	➢ *Nannochloropsis* sp.	Ishihara and Nakajima (2003)
	➢ *Chlorella gracilis*	Ishihara and Nakajima (2003)
Fungi	➢ *Pleurotus ostreatus* O-48	Hirano et al. (2000)
	➢ *Phanerochaete chrysosporium* ME-446	Tsutsumi et al. (2001)
	➢ *Trametes versicolor* IFO-7043	Tsutsumi et al. (2001)
	➢ *Trametes villosa*	Fukuda et al. (2001)
	➢ *Phanerochaete chrysosporum* ME-446	Uchida et al. (2001)
	➢ *Trametes versicolor* IFO-6482	Suzuki et al. (2003)
	➢ *Aspergillus fumigatus*	Suzuki et al. (2003)
	➢ *Fusarium sporotrichioides* NFRI-1012	Yim et al. (2003)
	➢ *Fusarium moniliforme* 2-2	Chai et al. (2005)
	➢ *Aspergillus terreus* MT-13	Chai et al. (2005)
	➢ *Emericella nidulans* MT-98	Chai et al. (2005)
	➢ *Stereum hirsutum*	Lee et al. (2005)
	➢ *Heterobasidium insulare*	Lee et al. (2005)

Microorganisms	Strains	References
Bacteria	➢ *Psudomonas paucimobilis* FJ-4	Ike et al. (2000)
	➢ *Pseudomonas* sp.	Kang and Kondo (2002a)
	➢ *Pseudomonas putida*	Kang and Kondo (2002a)
	➢ *Streptomyces* sp.	Kang et al. (2004)
	➢ *Sphingomonas* sp. strain AO1	Sasaki et al. (2005)

Table A.5. BPA concentrations in natural surface waters (Flint et al. 2012)

Location	Concentration (µg/L)	Sample	References
USA	12	River water	Kolpin et al. 2002
The Netherlands	21	River water	Belfroid et al. 2002
Japan	19	River water	Crain et al. 2007
Portugal	4	River water	Azevedo et al. 2001
China	0.262	Marine water	Fu et al. 2007
Italy	0.297	River water	Urbatzka et al. 2007
Japan	0.058	Estuarine water	Kawahata et al. 2004
China	0.0925	Estuarine water	Fu et al. 2007

Table A.6. Levels of BPA in canned foods reported by several studies (Kang et al. 2006)

Canned Food	No. of Samples	BPA Concentration (ng/g)	References
Meats	8	130 (17 - 602)	Imanaka et al. (2001)
	5	110 (17 - 380)	Goodson et al. (2002)
	6	21 (<20 - 98)	Thomson and Grounds (2005)
Infant Formula	14	5 (0.1 - 13)	Biles et al. (1997a)
Dairy Products	3	31 (21 - 43)	Kang and Kondo (2003)
Fish	10	22 (ND to 43)	Goodson et al. (2002)
	8	23 (<20 - 109)	Thomson and Grounds (2005)
	9	30 (<5 - 102)	Munguia-Lopez et al. (2005)
Beverages	11	<1 (ND to < 7[a])	Goodson et al. (2002)
	80	18 (ND to 212)	Horie et al. (1999)
	4	<10	Thompson and Grounds (2005)
Vegetables and	10	25 (9 - 48)	Goodson et al. (2002)
Fruits	10	20 (ND to 76)	Brotons et al. (1999)
	33	6 (<10 - 24)	Thomson and Grounds (2005)

ND, not detected.

[a] <7, detected but not quantifiable

Table A.7. BPA concentration in sediments and suspended solids (Flint et al. 2012)

Location	Concentration (µg/kg d.w.)	Sample	Reference
The Netherlands	43	Suspended solid sediments (Marine water)	Vethaak et al. 2005
China	27.3	Suspended solid sediments (River water)	Fu et al. 2007
Okinawa	11	Sediment (Estuarine and marine sites)	Kawahata et al. 2004
Japan	2.7	Sediment (Marine sites)	Kawahata et al. 2004
Italy	<2.0	Sediment (Estuarine sites)	Pojana et al. 2007
Germany	10 - 190	Solid sediments	Fromme et al. 2002
	66 - 343	Sediments	Heemken et al. 2001
	10 - 380	Sediments	Stachel et al. 2003

"d.w.": dry weight

Table A.8. Table BPA concentration in pretreated and treated waters

Location	Concentration (µg/L)	Sample	References
Japan	17,200	Landfill leachate	Yamamoto et al. 2001
	5400	Landfill leachate	Yamada et al. 1999
	370	Paper-mill effluent	Fukazawa et al. 2002
Canada	1.054	Waste water influent	Fernandez et al. 2007
	0.590	Waste water influent	Fernandez et al. 2007
	0.040	Kraft mill effluent	Fernandez et al. 2007
USA	0.049	Waste water influent	Yu and Chu. 2009
	1.7	Untreated septage	Ruedel et al. 1998
Australia	23.03	Waste water influent (combined municipal and industrial)	Al-Rafai et al. 2007
	5.48	Waste water influent (Combined municipal and storm water)	Al-Rafai et al. 2007
	0.14	Waste water influent (Municipal)	Al-Rafai et al. 2007
Belgium	0.006	Textile mill effluent	Loos et al. 2007
Italy	0.005	Waste water effluent	Crain et al. 2007

Table A.9. BPA concentration in ground water, soils, sewage sludge

Location	Concentration	Sample	References
Groundwater (Concentration µg/L)			
USA	1.9	Groundwater (National reconnaissance)	Focazio et al. 2008
	1.41	Groundwater (Impacted by landfill)	Rudel et al. 1998
	0.029	Groundwater (Impacted by waste water recharge)	Rudel et al. 1998
Spain	1.5	Groundwater (Agricultural region)	Latorre et al. 2003
Soils, Sewage, Sludges (Concentration µg/kg d.w.)			
USA	81	Soil amended with biosolid	Kinney et al. 2008
	147	Soil	Kinney et al. 2008
Canada	360	Sewage sludge	Mohapatra et al. 2011
Germany	1363	Sewage sludge	Fromme et al. 2002

“d.w.”: dry weight

Table A.10. Harmful effects of BPA on different organisms (Kang et al. 2007; Flint et al. 2012)

Species	BPA exposure	Effect	References
		Reptiles	
Broad-snouted Caiman (*Caiman latirostris*)	1.4 ppm (90 mg/egg)	Abnormal seminiferous tubules in males	(Stoker et al. 2003)
		Birds	
Japanese Quail (*Coturnix japonica*)	200 mg/g per egg	Oviduct abnormalities in females	(Berg et al. 2001)
White Leghorn Chicken	2 mg/kg of BPA every two days for maximum of 23 weeks	Delayed growth of the male chicken phenotype including the comb, wattle and testis	(Furuya et al. 2006)
		Amphibians	
Wrinkled Frog (*Rana rugosa*)	10^{-7} M for nine days	Tail regression suppressed	(Goto et al. 2006)
Western Clawed Frog (*Silurana tropicalis*)	2.28 mg/L for nine days	Spontaneous metamorphosis inhibited	(Kashiwagi et al. 2008)
Dark Spotted Frog (*Rana nigromaculata*)	200 mg/L for 45 days	Tail flex malformations	(Yang et al. 2005)

Species	BPA exposure	Effect	References
African Clawed Frog (*Xenopus laevis*)	5700 mg/L	Head malformations, scoliosis and organogenesis suppression occur	(Iwamuro et al. 2003)
		Fish	
Atlantic Cod (*Gadus morhua*)	50 µg/L for 3 weeks	Vtg induction	Larsen et al. 2006
Atlantic Salmon (*Salmo salar m. Sebago*)	1000 µg/L 6 days	Yolk sac edema and hemorrhage	Honkanen et al. 2004
Zebra fish (*Danio rerio*)	534 µg/L for 1 week	Vtg induction	Lindholst et al. 2003
Rainbow Trout (*Onchorhynchus mykiss*)	500 µg/L for 1 week	Vtg induction	Lindholst et al. 2003
Turbot (*Psetta maxima*)	59 µg/L for 2 weeks	Altered sex steroids levels	Labadie and Budzinski 2006
Medaka (*Oryzias latipes*)	200 µg/L for 9 days	Embryonic deformities	Pastav et al. 2001
Longchin Goby (*Chasmichthys dolichognathus*)	0.1 time not specified	Inhibit estrogen synthesis	Baek et al. 2003
Guppy (*Poecilia reticulate*)	274 µg/L for 3 weeks	Reduced sperm counts	Haubruge et al. 2001
Goldfish (*Carassius auratus*)	40 µg/L for 4 weeks	Vtg induction	Ishibashi et al. 2001

Species	BPA exposure	Effect	References
Fathead Minnow (*Pimephales promelas*)	160 µg/L for 2 weeks	Vtg induction	Sohoni et al. 2001
European Seabass (*Dicentrarchus labrax*)	10 µg/L for 2 weeks	Vtg induction	Correia et al. 2007
Carp (Cyprinus carpio)	1000 µg/L for 2 weeks	Intersex condition	Mandich et al. 2007
Brown Trout (*Salmo trutta f. fario*)	5 µg/L for 75 days	Complete inhibition of ovulation	Lahnsteiner et al. 2005

Appendix B: Method Validation Results for CE and LC/MS

Table B.1. Percent recovery calculations for CE method

Sample Name	Spiked Concentration (Spiked) $_{true}$	Area	Unspiked Concentration (U)	Spike$_{(exp)}$ = (Spiked)$_{true}$ - (U)	% Rec. = Spiked$_{(exp)}$/ Spiked$_{(true)}$
P3-A	0	1562	2.1		
	10	10289	13.6	11.4	114.8%
	20	17770	23.5	21.3	106.6%
	30	25927	34.2	32.1	106.9%
	40	32533	42.9	40.7	101.9%
Paul Lake	0	46823	61.7		
	10	53662	70.7	9.0	90.0%
	20	62995	83.0	21.2	106.4%
	30	71360	94.1	32.3	107.6%
	40	79539	104.8	43.1	107.6%
Riverside Park	0	7469	9.9		
	10	14340	19.0	9.0	90.4%
	20	21510	28.4	18.4	92.4%
	30	30864	40.7	30.8	102.6%
	40	37186	49.1	39.1	97.8%
P2-B	0	6104	8.1		
	10	14340	19.0	10.8	108.4%
	20	22041	29.1	20.9	104.9%
	30	27752	36.6	28.5	95.0%
	40	40812	53.8	45.6	114.2%

Table B.2. Intraday precision studies for peak areas on CE

Concentration	Peak Area	Standard Deviation	Mean	%RSD
500 ppb	17772, 19289, 19923	1105.2	18994.6	5.81
1 ppm	48306, 41192, 38521	5057.8	42673.0	11.8
3 ppm	60988, 66107, 63470	2559.8	63521.6	4.0
6 ppm	96975, 94975, 95590	1024.4	95846.6	1.0

Table B.3. Intraday day precision studies for migration times on CE

Concentration	Migration Time	Standard Deviation	Mean	%RSD
500 ppb	2.01, 2.09, 2.09	0.04	2.06	2.1
1 ppm	2.16, 2.13, 2.05	0.05	2.12	2.6
3 ppm	2.13, 2.12, 2.17	0.02	2.15	1.1
6 ppm	2.10, 2.12, 2.10	0.01	2.10	0.6

Table B.4. First day interday precision studies for peak areas on CE

Concentration	Peak Area	Standard Deviation	Mean	%RSD
500 ppb	10004, 11216, 11357	743.8	10859	6.80
1 ppm	47988, 45366, 46860	1315.3	46738	2.82
3 ppm	51489, 50547, 52767	1114.2	51601	2.21
6 ppm	94976, 96921, 95927	972.5	95941	1.01

Table B.5. First day interday precision studies for migration times on CE

Concentration	Migration Time	Standard Deviation	Mean	%RSD
500 ppb	1.68, 1.68, 1.71	0.01	1.69	0.81
1 ppm	1.70, 1.71, 1.71	0.01	1.71	0.37
3 ppm	1.76, 1.77, 1.71	0.03	1.75	1.98
6 ppm	2.13, 2.10, 2.02	0.05	2.08	2.02

Table B.6. Second day interday precision studies for peak areas on CE

Concentration	Peak Area	Standard Deviation	Mean	%RSD
500 ppb	13244, 12054, 11572	860.6	12290	7.03
1 ppm	20214, 19469, 22136	1376.1	20606	6.60
3 ppm	28523, 28675, 26793	1045.4	27997	3.71
6 ppm	65754, 69303, 68154	1810.8	67737	2.60

Table B.7. Second day interday precision studies for migration times on CE

Concentration	Migration Time	Standard Deviation	Mean	%RSD
500 ppb	2.10, 2.12, 2.23	0.06	2.15	3.21
1 ppm	1.95, 2.06, 2.05	0.06	2.02	3.21
3 ppm	2.01, 1.91, 1.98	0.05	1.96	2.56
6 ppm	2.00, 1.93, 1.70	0.15	1.87	8.23

Table B.8. Third day interday precision studies for peak areas on CE

Concentration	Peak Area	SD	Mean	%RSD
500 ppb	17460, 15162, 15172	1323.8	15931.3	8.30
1ppm	22532, 24493, 24612	1168.0	23879.0	4.80
3 ppm	30234, 35568, 33746	2711.2	33182.6	8.10
6 ppm	42489, 39975, 38062	2220.2	40175.3	5.51

Table B.9. Third day interday analysis precision studies for migration times on CE

Concentration	Migration Time	SD	Mean	%RSD
500 ppb	2.43, 2.52, 2.23	0.14	2.39	6.20
1 ppm	2.49, 2.51, 2.52	0.01	2.51	0.50
3 ppm	2.51, 2.41, 2.46	0.05	2.46	2.00
6 ppm	2.37, 1.99, 2.01	0.21	2.12	10.00

Table B.10. First interday precision studies for peak areas on LC/MS

Conc.	Peak Area	Standard Deviation	Mean	%RSD
50 ppb	11774, 12107, 10027	1117.2	11302.6	9.8
100 ppb	21596, 19472, 22908	1733.9	21325.3	8.1
250 ppb	24060, 25102, 25855	901.3	25005.6	3.6
500 ppb	59675, 57323, 58783	1187.4	58593.6	2.0
1 ppm	104764, 113777, 111367	4666.2	109969.3	4.2
2 ppm	236947, 228853, 232475	4054.4	232758.3	1.7
5 ppm	454172, 456193, 457048	1476.8	455804.3	0.3
10 ppm	946637, 938220, 876373	38368.6	920410.0	4.1
25 ppm	2197683, 2386478, 2080780	154251.4	2221647.0	6.9
50 ppm	4522522, 4231049, 4292354	153672.8	4348641.6	3.5
75 ppm	6323948, 6318821, 6305650	9439.0	6316139.6	0.2

Table B.11. First day interday precision studies for migration times on LC/MS

Concentration	Migration Time	Standard Deviation	Mean	%RSD
50 ppb	0.76, 0.74, 0.80	0.03	0.76	3.90
100 ppb	0.77, 0.78, 0.85	0.04	0.80	5.45
250 ppb	0.76, 0.78, 0.83	0.03	0.79	4.55
500 ppb	0.77, 0.80, 0.83	0.03	0.80	3.75
1 ppm	0.77, 0.77, 0.82	0.02	0.78	3.55
2 ppm	0.77, 0.77, 0.78	0.01	0.77	0.74
5 ppm	0.75, 0.78, 0.77	0.02	0.76	1.98
10 ppm	0.76, 0.78, 0.78	0.01	0.77	1.48
25 ppm	0.75, 0.76, 0.77	0.01	0.76	1.31
50 ppm	0.75, 0.76, 0.78	0.02	0.76	1.96
75 ppm	0.76, 0.78, 0.77	0.01	0.77	1.29

Table B.12. Second day interday precision studies for peak areas on LC/MS

Conc.	Peak Area	Standard Deviation	Mean	%RSD
50 ppb	10093, 9499, 10024	324.8	9872.0	3.2
100 ppb	17803, 18092, 19672	1006.1	18522.3	5.4
250 ppb	25879, 27003, 25310	861.5	26064.0	3.3
500 ppb	57826, 56602, 58814	1108.1	57747.3	1.9
1 ppm	108165, 113193, 113525	3003.3	111627.6	2.7
2 ppm	238109, 266919, 235141	17553.1	246723.0	7.1
5 ppm	494584, 506013, 479570	13261.9	493389.0	2.6
10 ppm	1018478,1008892, 949060	37617.8	992143.3	3.8
25 ppm	2523137, 2639773, 2431879	104204.8	2531596.3	4.1
50 ppm	4734827, 4643574, 4626752	58152.5	4668384.3	1.2
75 ppm	6560623, 6529370, 6311574	135669.4	6467189.0	2.0

Table B.13. Second day interday precision studies for migration times on LC/MS

Concentration	Migration Time	Standard Deviation	Mean	%RSD
50 ppb	0.77, 0.78, 0.78	0.01	0.77	1.29
100 ppb	0.82, 0.84, 0.83	0.01	0.83	1.20
250 ppb	0.82, 0.84, 0.82	0.01	0.83	1.20
500 ppb	0.82, 0.83, 0.81	0.01	0.82	1.21
1 ppm	0.82, 0.82, 0.81	0.06	0.82	0.69
2 ppm	0.76, 0.83, 0.81	0.03	0.80	3.75
5 ppm	0.80, 0.85, 0.82	0.03	0.82	3.65
10 ppm	0.82, 0.84, 0.81	0.02	0.82	2.43
25 ppm	0.82, 0.82, 0.83	0.01	0.82	1.21
50 ppm	0.81, 0.83, 0.82	0.01	0.82	1.21
75 ppm	0.81, 0.83, 0.82	0.01	0.82	1.22

Table B.14. Third day interday precision studies for peak areas on LC/MS

Concentration	Peak Areas	Standard Deviation	Mean	%RSD
50 ppb	11601, 10093, 11380	814.3	11024.6	7.38
100 ppb	14535, 12057, 13642	1255.0	13411.3	9.35
250 ppb	28403, 26665, 24803	1800.3	26623.6	6.76
500 ppb	59839, 62872, 61703	1529.7	61471.3	2.48
1 ppm	113094, 117807, 114987	2371.6	115296.0	2.05
2 ppm	309057, 293617, 277317	15871.9	293330.3	5.41
5 ppm	540406, 504174, 490817	25658.7	511799.0	5.01
10 ppm	1056558, 1038286, 1029956	13607.1	1041600.0	1.30
25 ppm	2539920, 2481474, 2414643	62685.2	2478679.0	2.52
50 ppm	4782296, 4450351, 4408530	204791.5	4547059.0	4.50
75 ppm	6394943, 6442278, 6463534	35112.07	6433585.0	0.54

Table B.15. Third day interday precision studies for migration times on LC/MS

Concentration	Migration Time	Standard Deviation	Mean	%RSD
50 ppb	0.77, 0.77, 0.791	0.01	0.77	1.29
100 ppb	0.79, 0.80, 0.79	0.01	0.79	1.26
250 ppb	0.79, 0.80, 0.79	0.01	0.79	1.26
500 ppb	0.78, 0.78, 0.77	0.01	0.77	1.29
1 ppm	0.77, 0.79, 0.78	0.01	0.78	1.28
2 ppm	0.77, 0.76, 0.78	0.01	0.77	1.29
5 ppm	0.77, 0.78, 0.79	0.01	0.78	1.28
10 ppm	0.77, 0.77, 0.79	0.01	0.77	1.29
25 ppm	0.77, 0.77, 0.78	0.01	0.77	1.29
50 ppm	0.78, 0.79, 0.78	0.01	0.78	1.28
75 ppm	0.71, 0.79, 0.78	0.04	0.76	5.26

Table B.16. Intraday precision studies for peak areas on LC/MS

Concentration	Peak Area	Standard Deviation	Mean	%RSD
50 ppb	11191, 12423, 13192	1009.3	12268.6	8.23
100 ppb	16120, 17355, 17434	736.8	16969.6	4.34
250 ppb	33432, 28830, 32229	2386.7	31497.0	7.57
500 ppb	67014, 67616, 66826	412.6	67152.0	0.61
1 ppm	118543, 125281, 126925	4441.4	123583.0	3.59
2 ppm	279481, 277763, 275836	1823.4	277693.3	0.65
5 ppm	547964, 516628, 525232	16190.1	529941.3	3.05
10 ppm	1048562,1055162,1022097	17498.8	1041940.3	1.67
25 ppm	2483548, 2477394, 2521536	23907.7	2494159.3	0.95
50 ppm	4552014, 4584501, 4564734	16370.3	4567083.0	0.35
75 ppm	6549246, 6892431, 6621331	180954.5	6687669.3	2.70

Table B.17. Intraday precision studies for migration times on LC/MS

Concentration	Migration Time	Standard Deviation	Mean	%RSD
50 ppb	0.79, 0.77, 0.77	0.01	0.77	1.29
100 ppb	0.79, 0.77, 0.78	0.01	0.78	1.28
250 ppb	0.78, 0.77, 0.78	0.01	0.77	1.29
500 ppb	0.79, 0.71, 0.78	0.04	0.76	5.26
1 ppm	0.77, 0.75, 0.76	0.01	0.76	1.32
2 ppm	0.78, 0.76, 0.77	0.01	0.77	1.29
5 ppm	0.77, 0.78, 0.76	0.01	0.77	1.29
10 ppm	0.78, 0.76, 0.75	0.02	0.76	2.63
25 ppm	0.79, 0.76, 0.76	0.01	0.77	1.29
50 ppm	0.77, 0.77, 0.76	0.01	0.76	1.31
75 ppm	0.78, 0.77, 0.77	0.01	0.77	1.29

Table B.18. Percent recovery for LC/MS method

Sample Name	Spiked Concentration (Spiked) $_{true}$	Area	Unspiked Concentration (U)	Spike$_{(exp)}$ = (Spiked)$_{true}$ - (U)	% Rec. = Spiked$_{(exp)}$/ Spiked$_{(true)}$
	0	7393	9.1		
Pool	100	16445	97.3	88.1	88.1
Water	200	25781	188.2	179.1	89.5
Sample	500	58386	505.9	496.7	99.3
(P1-A)	1000	89840	812.3	803.1	80.3
	0	9291	27.6		
Pioneer	100	18693	119.2	91.5	91.5
Park	200	28992	219.5	191.9	95.9
Water	500	58964	511.5	483.9	96.7
	1000	103794	948.2	920.6	92.0
	0	6700	2.3		
Pioneer	100	18042	112.7	110.3	110.3
Park Tap	200	27259	202.6	200.2	100.1
Water	500	57327	495.5	493.2	98.6
	1000	108221	991.38	989.0	98.9
	0	7774	12.8		
	100	16307	95.9	83.1	83.1
Paul Lake	200	26595	196.1	183.3	91.6
	500	57235	494.6	481.8	96.3
	1000	109911	1007.8	995	99.5

Appendix C: CE Electropherograms of twenty-one water samples

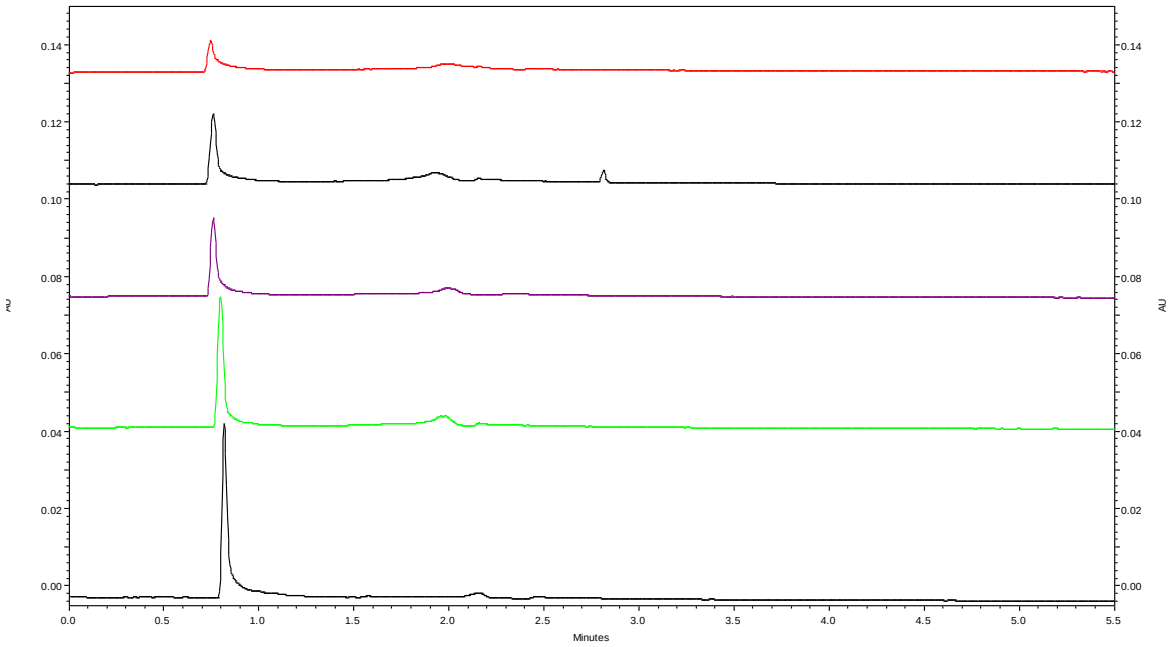


Figure C.1. Louis Lake water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

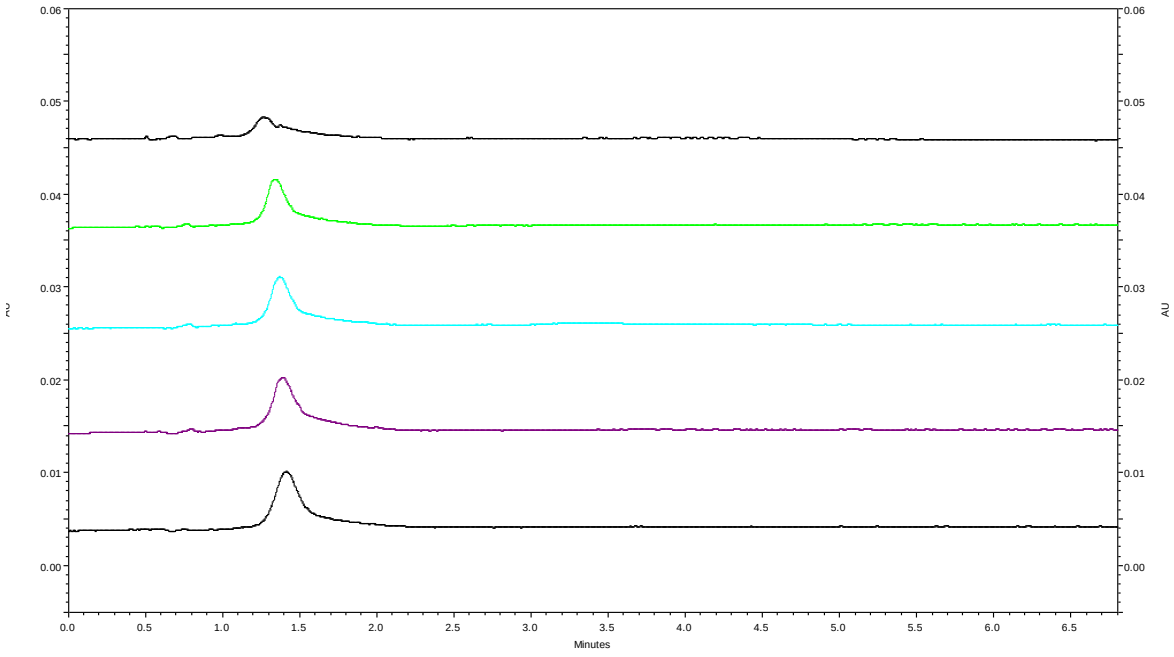


Figure C.2. Shuswap Lake water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

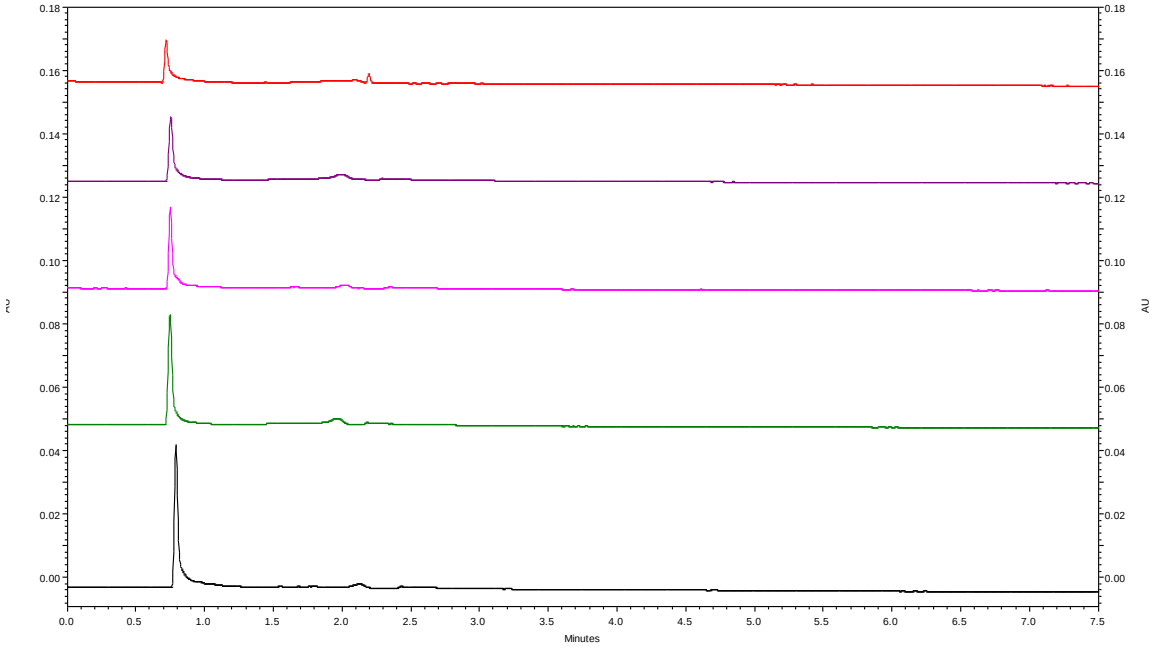


Figure C.3. Adams River water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

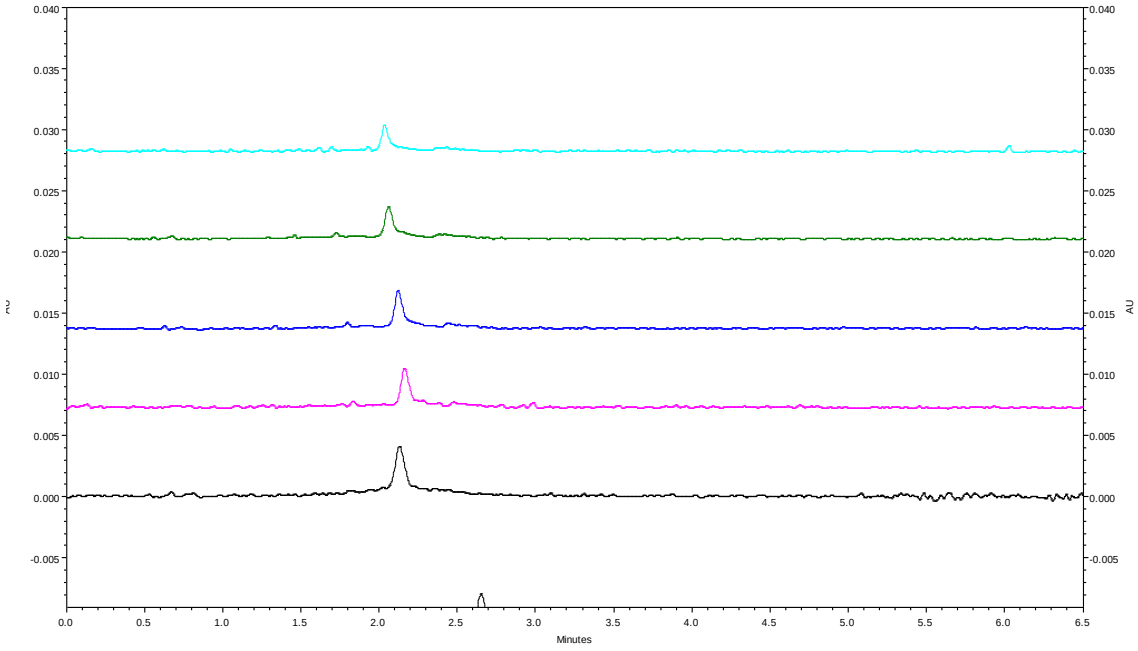


Figure C.4. Riverside Park water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

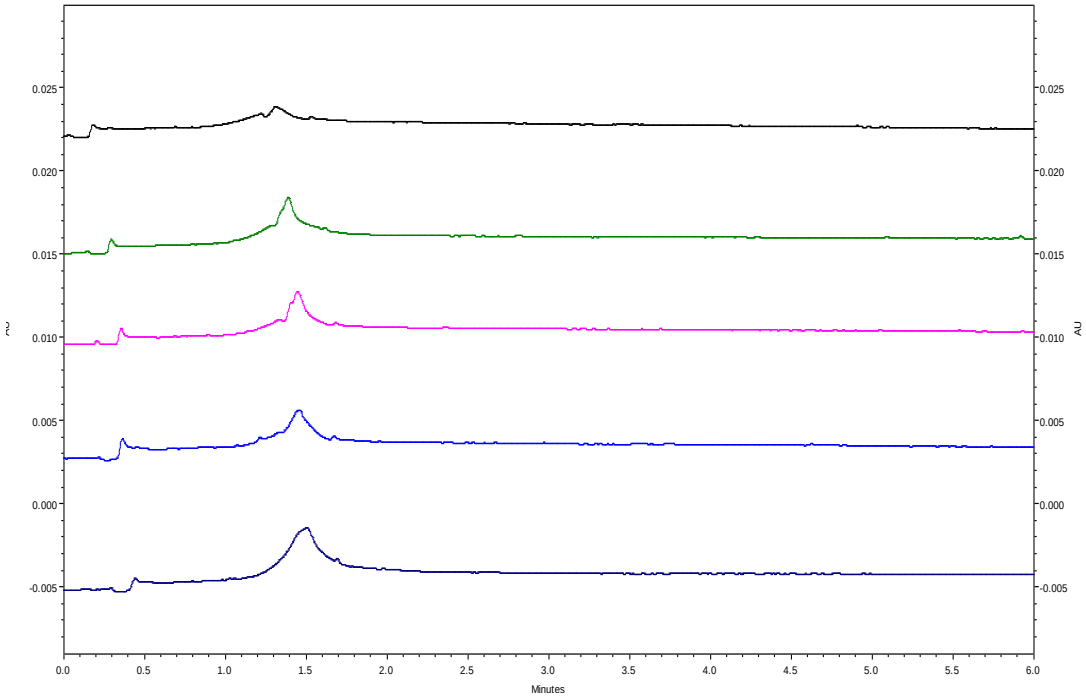


Figure C.5. Swimming Pool tap water (P3-B) sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

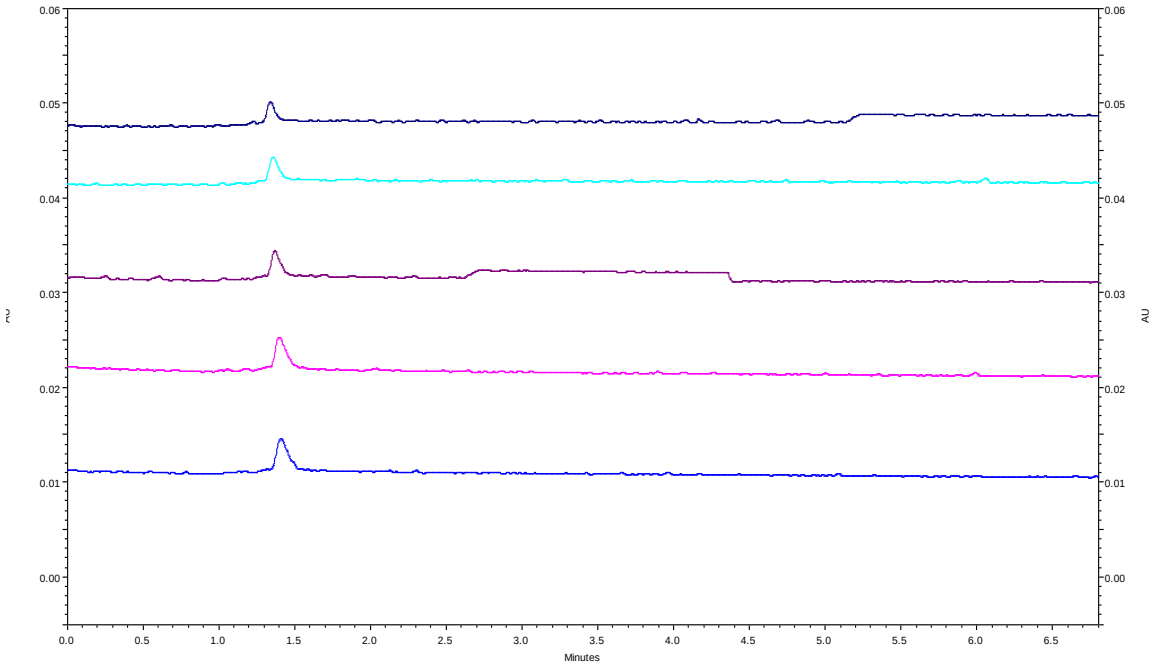


Figure C.6. Pioneer Park tap water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

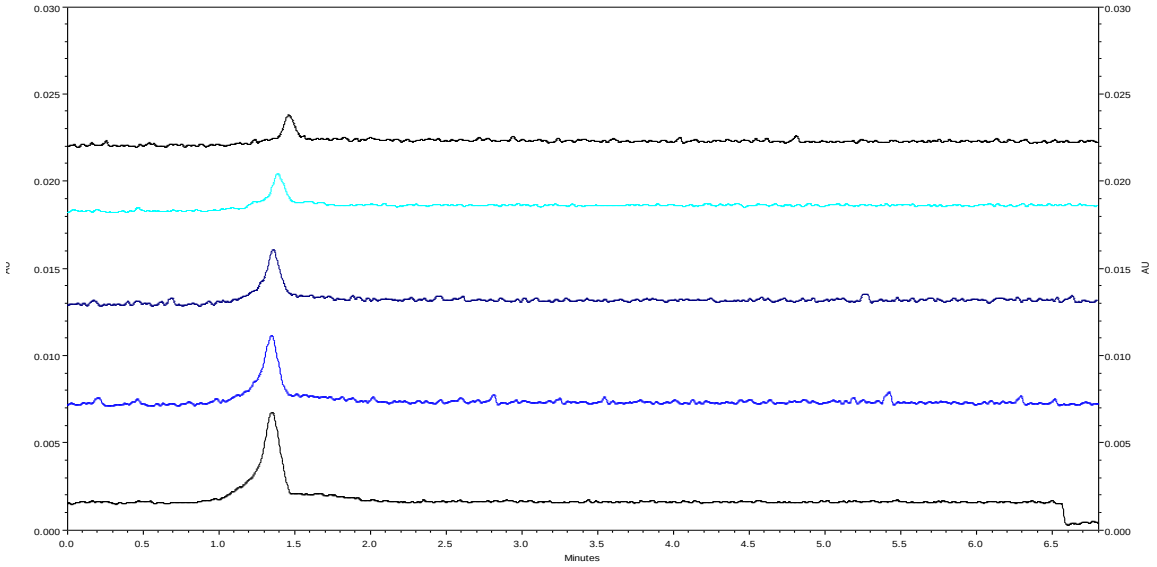


Figure C.7. Swimming Pool (P1-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

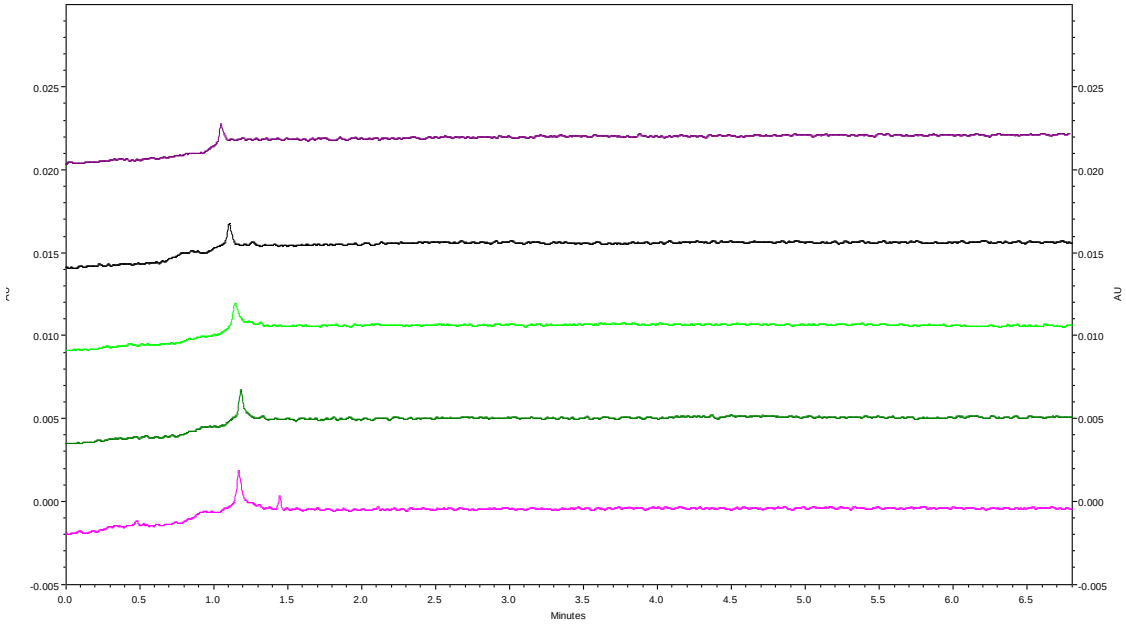


Figure C.8. Swimming Pool (P7-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

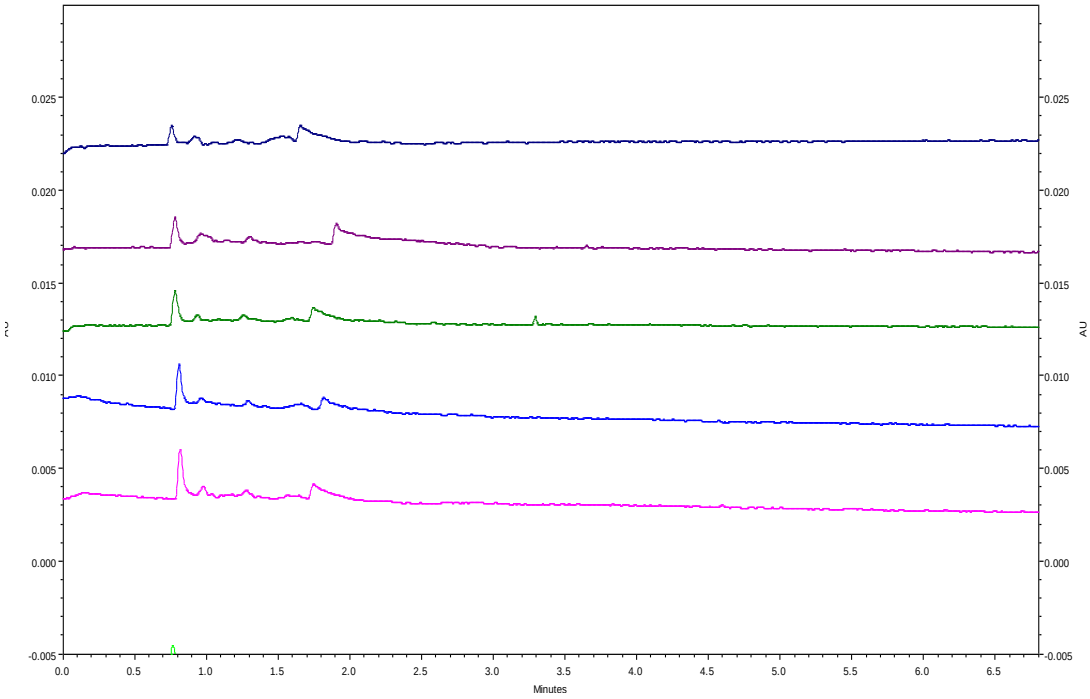


Figure C.9. Pioneer Park water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

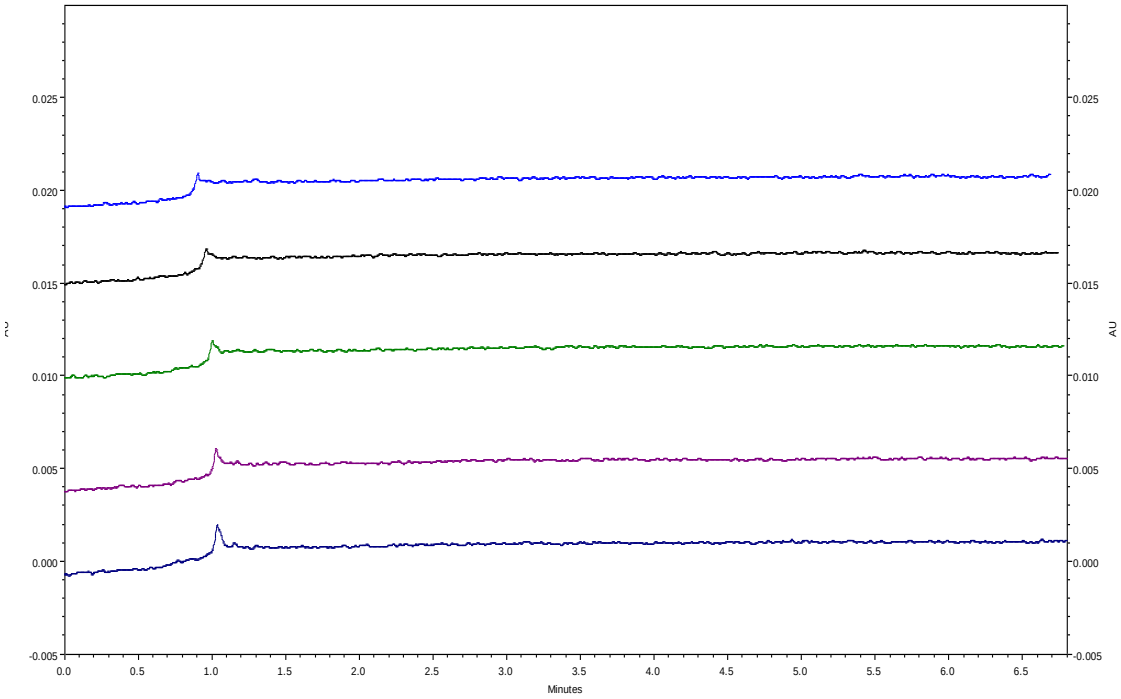


Figure C.10. Swimming Pool (P1-B) tap water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

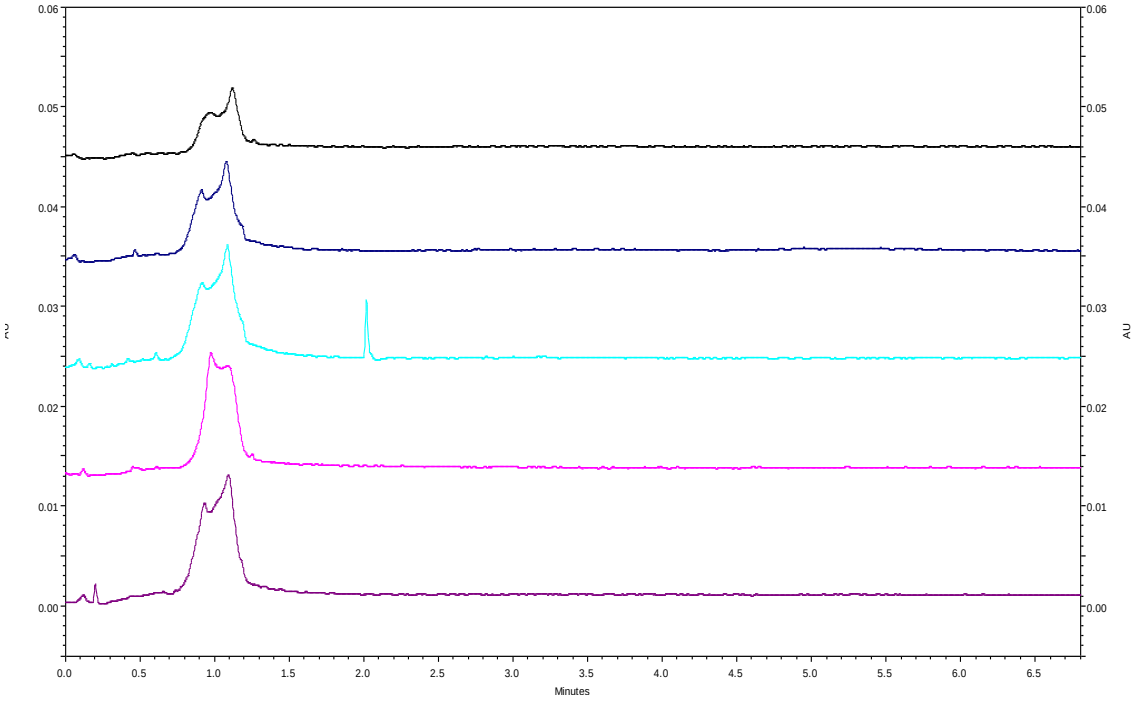


Figure C.11. McArthur Island Park water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

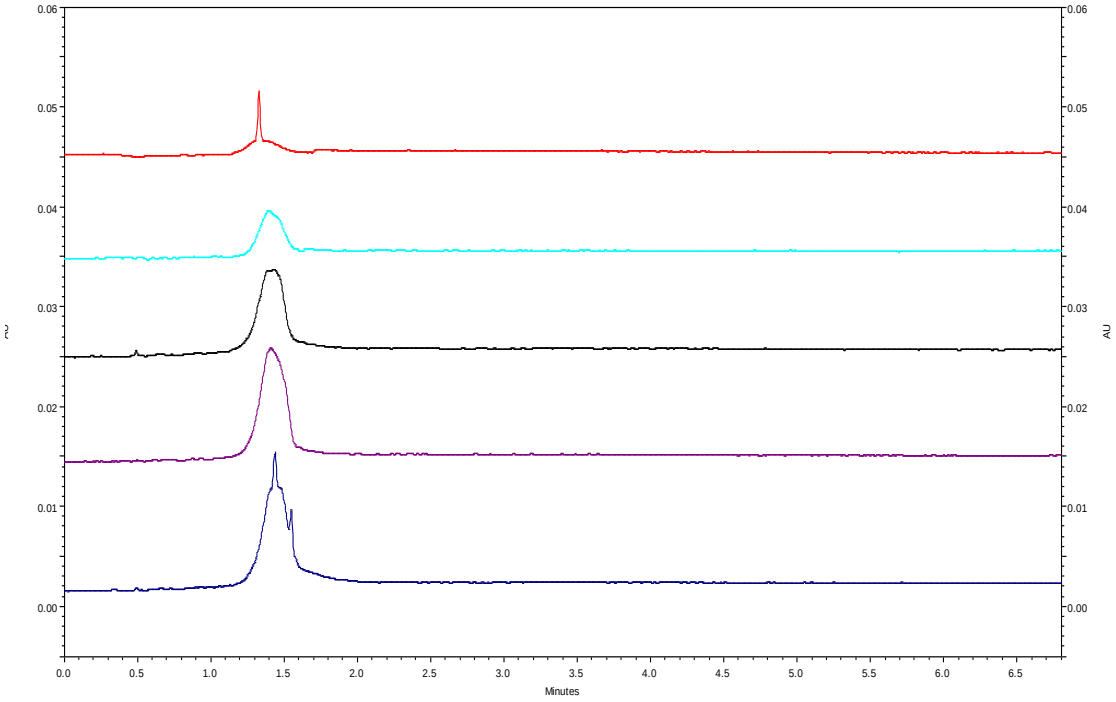


Figure C.12. Swimming Pool (P2-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

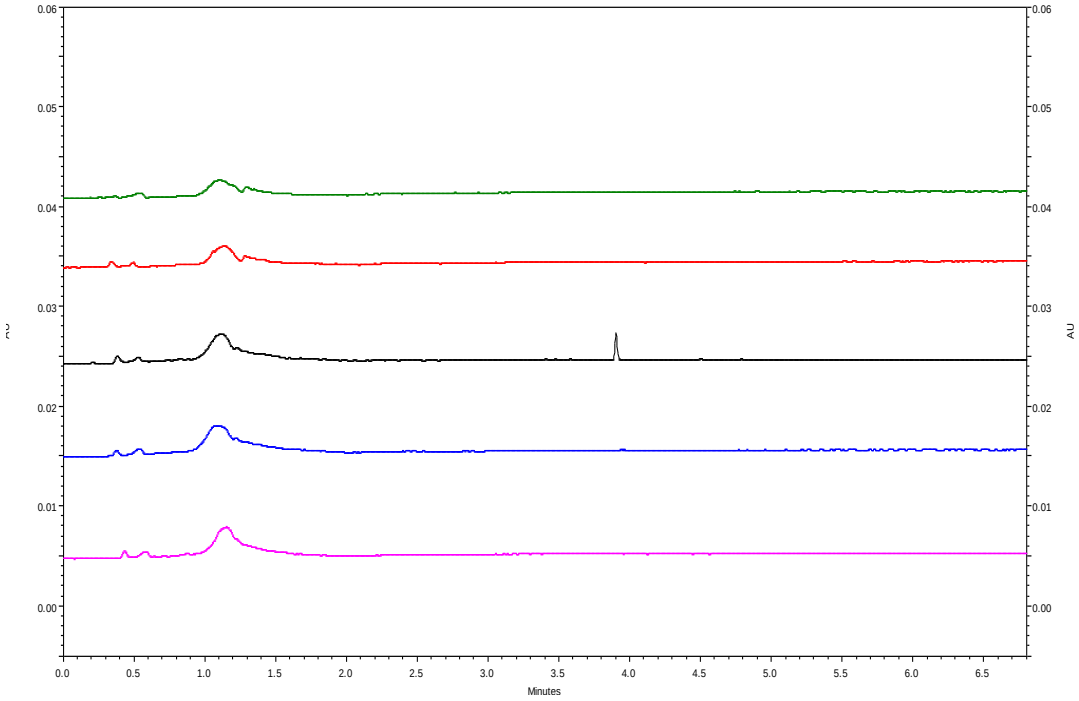


Figure C.13. Swimming Pool tap (P2-B) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

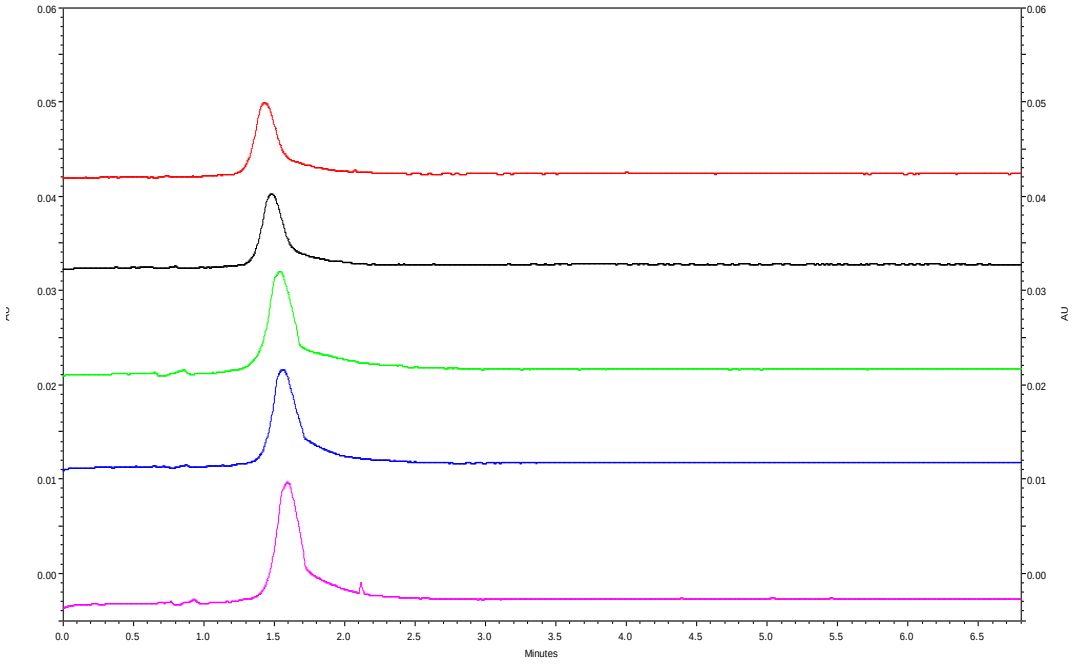


Figure C.14. Swimming Pool (P5-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

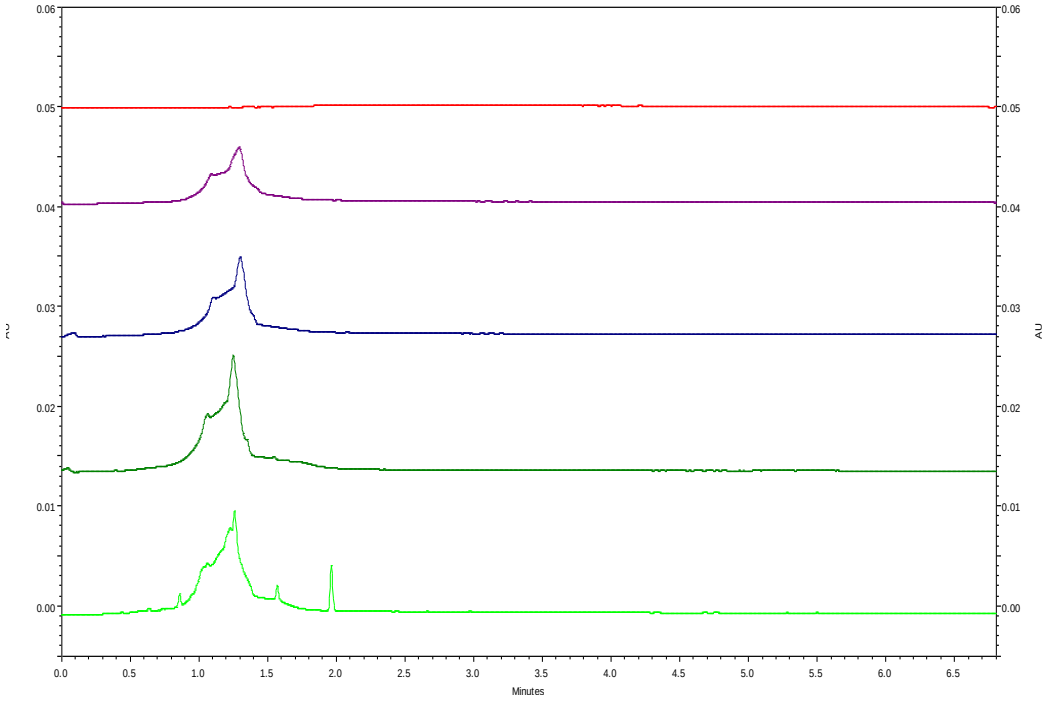


Figure C.15. Paul Lake water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

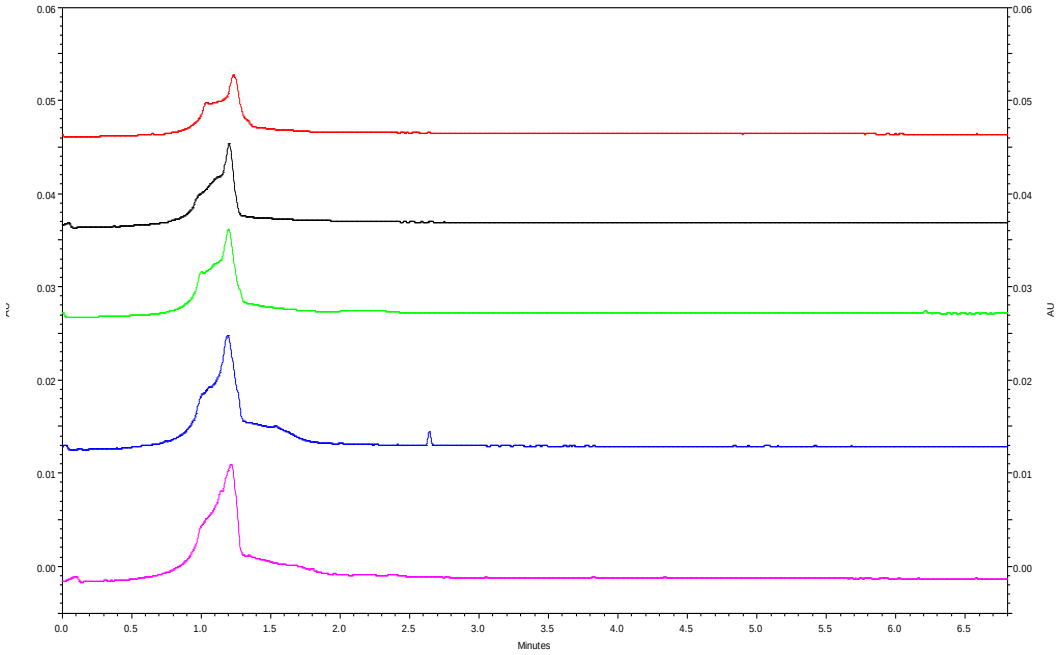


Figure C.16. Swimming Pool (P3-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

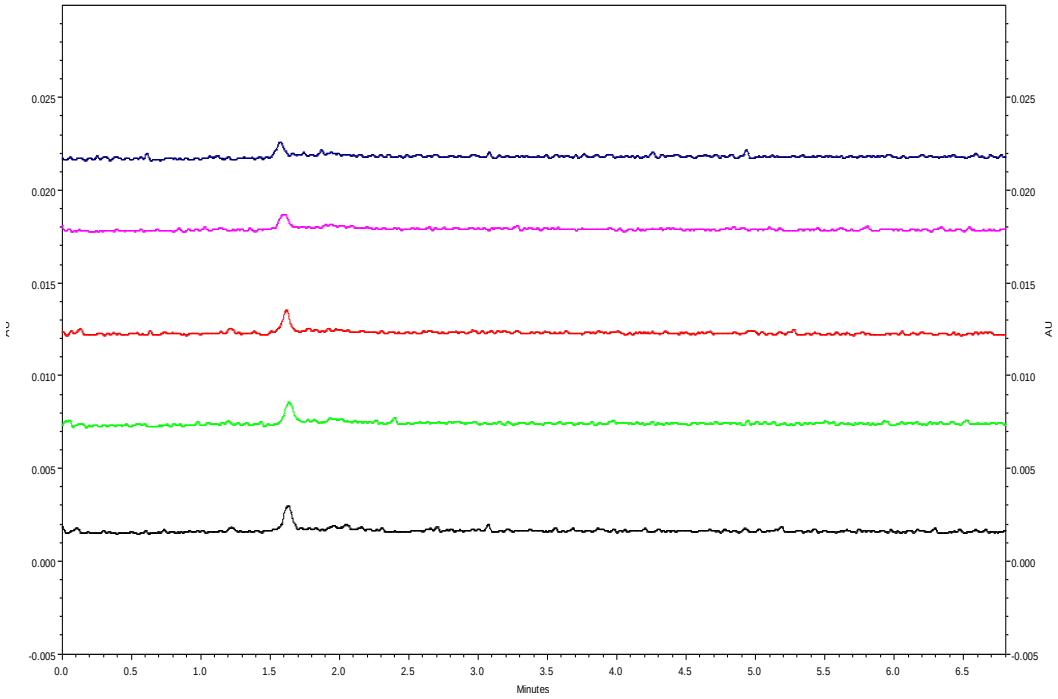


Figure C.17. Paul Lake tap water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

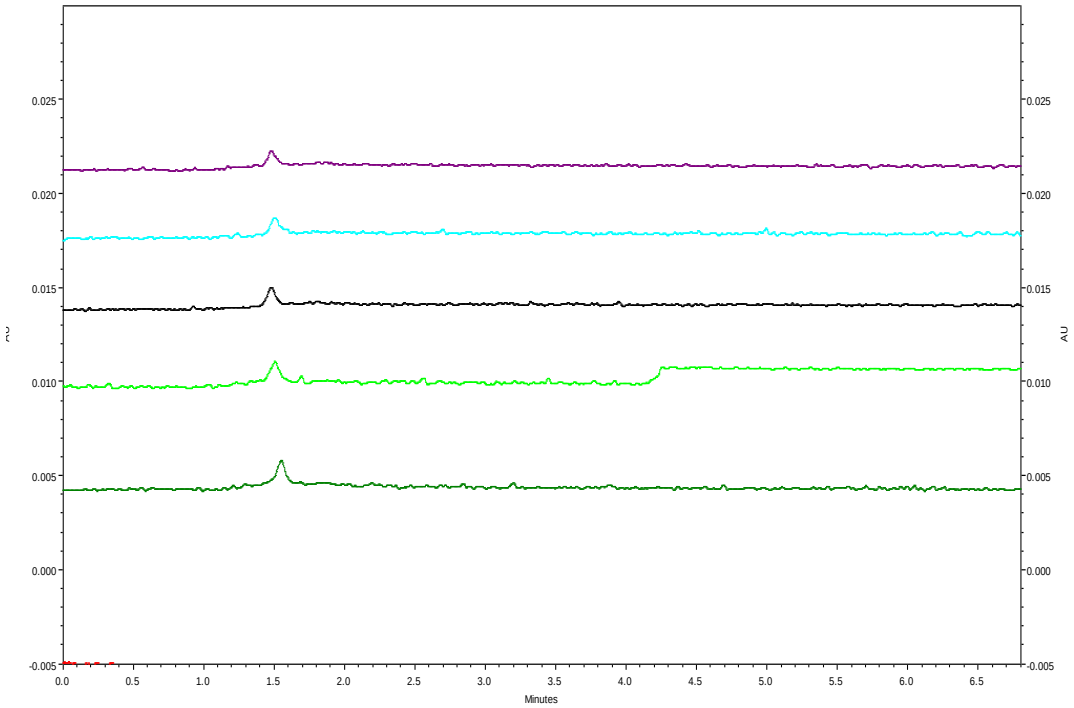


Figure C.18. Kamloops Lake water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

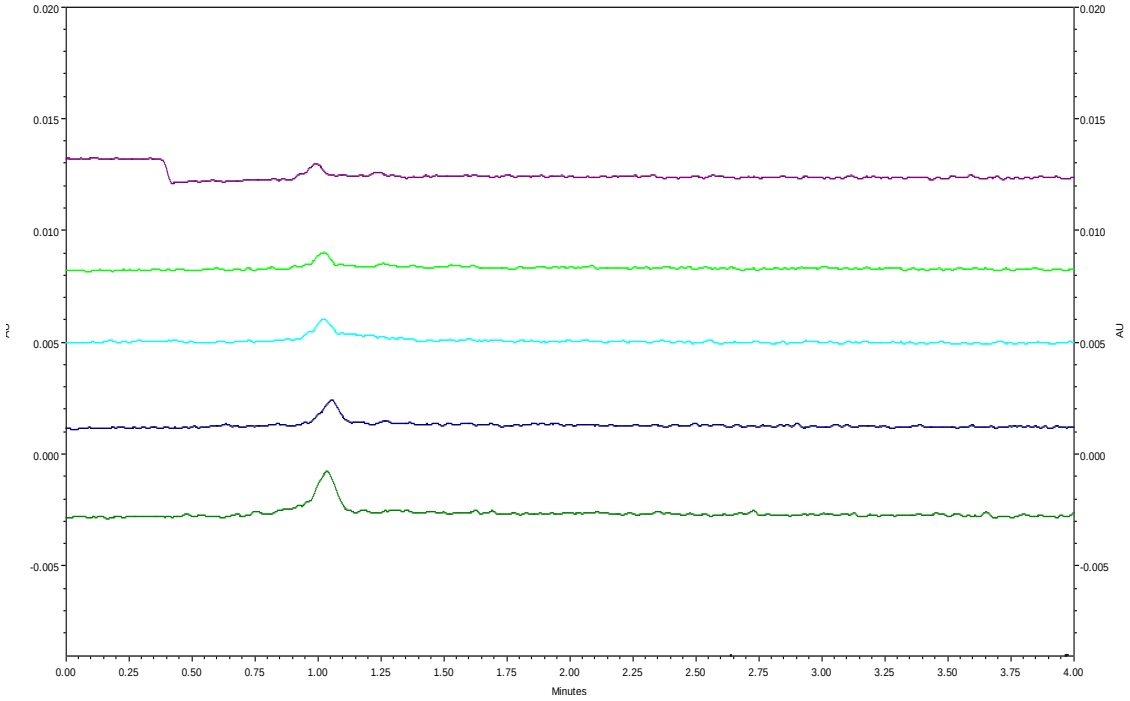


Figure C.19. Swimming Pool (P4-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

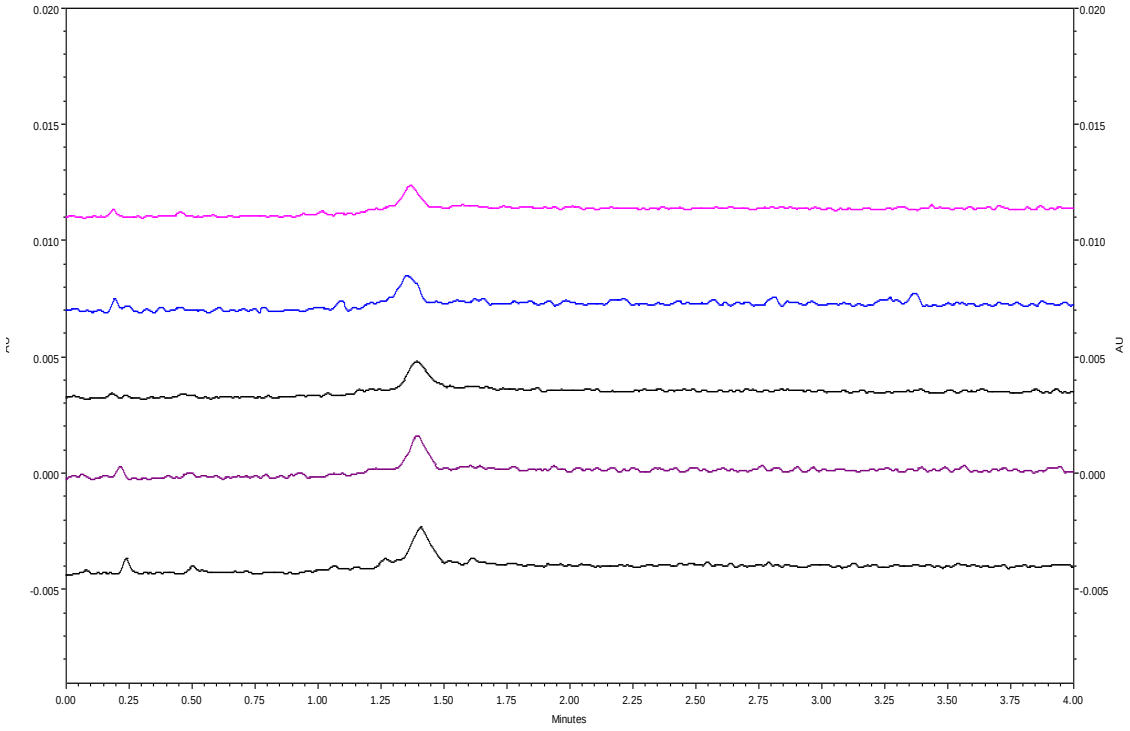


Figure C.20. Swimming Pool (P6-A) water sample electropherograms by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

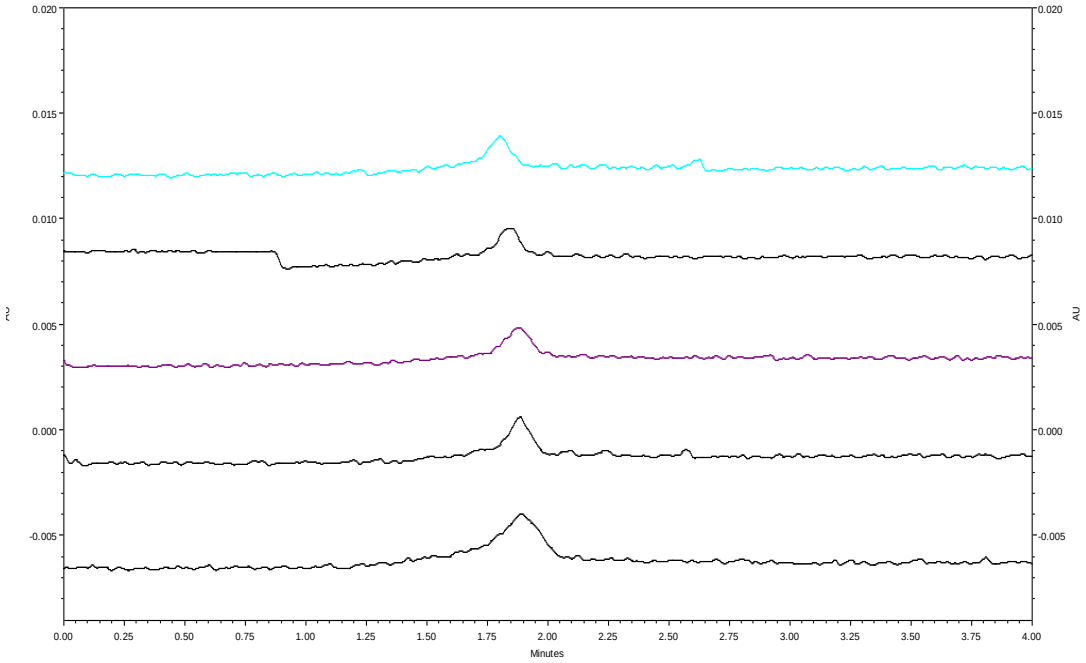


Figure C.21. Swimming Pool (P8-A) water sample by standard addition; BPA spikes added (top to bottom: 0 ppm, 10 ppm, 20 ppm, 40 ppm)

Appendix D: LC/MS Chromatograms of twenty-one water samples

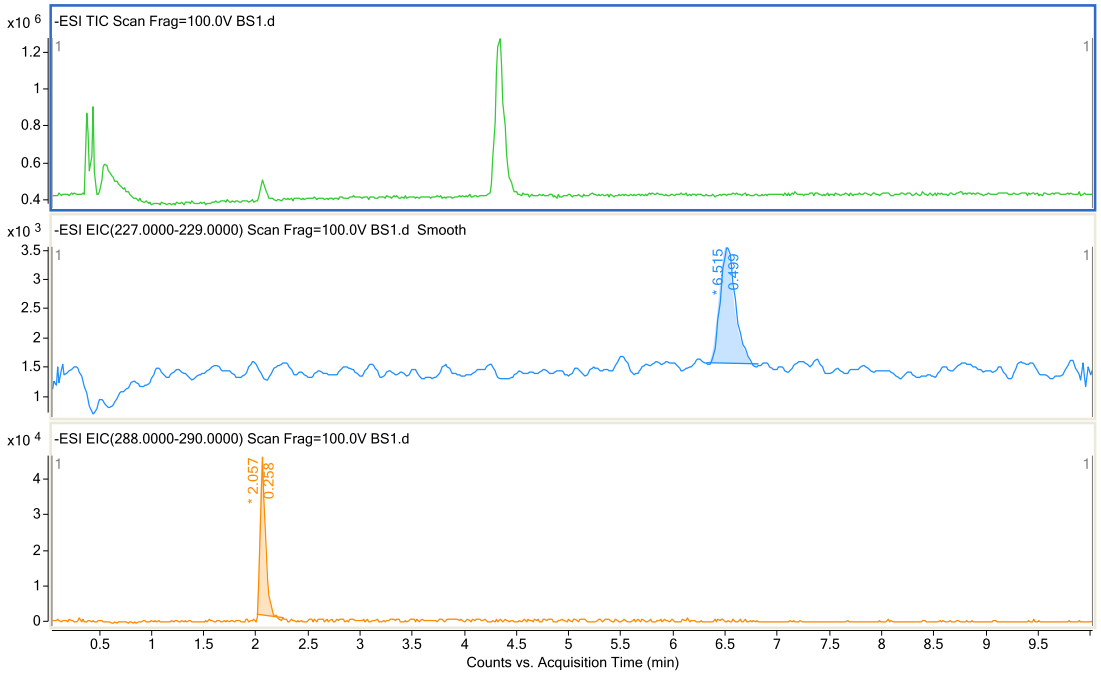


Figure D.1. Louis Lake water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

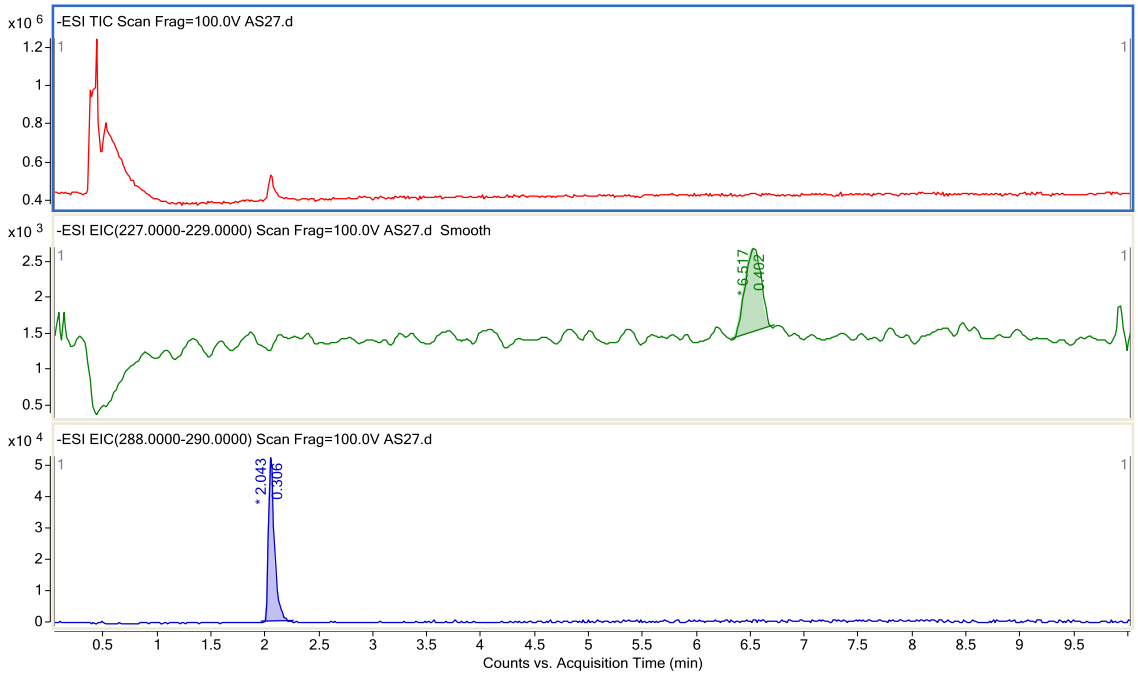


Figure D.2. Pioneer Park water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

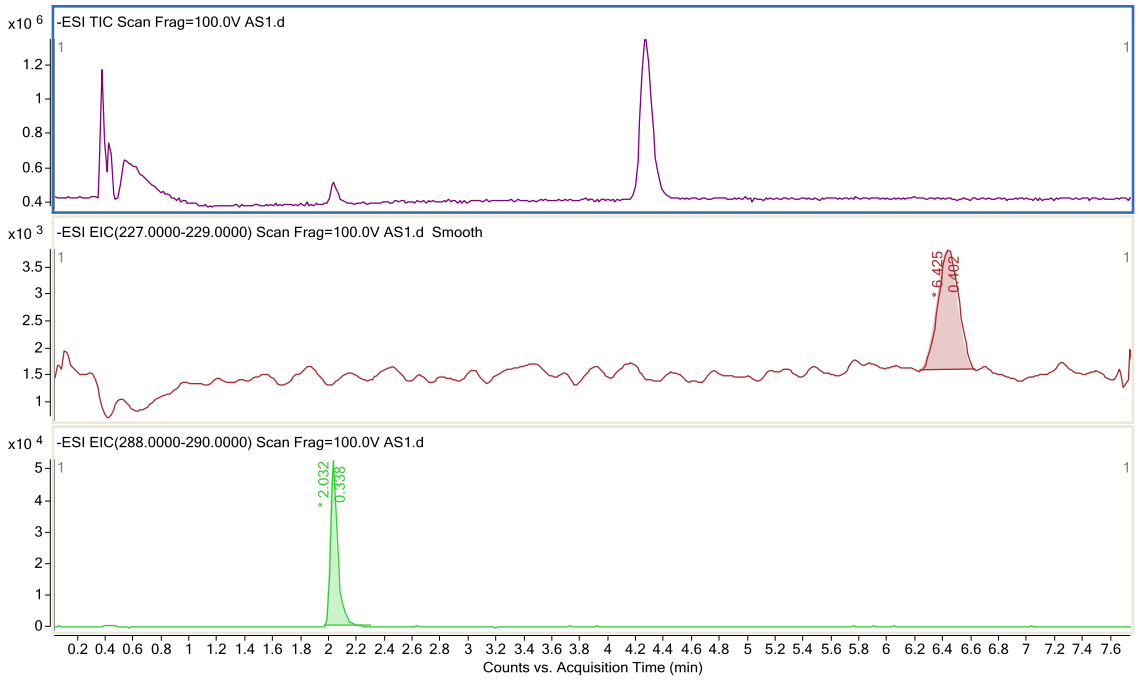


Figure D.3. Swimming Pool (P1-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

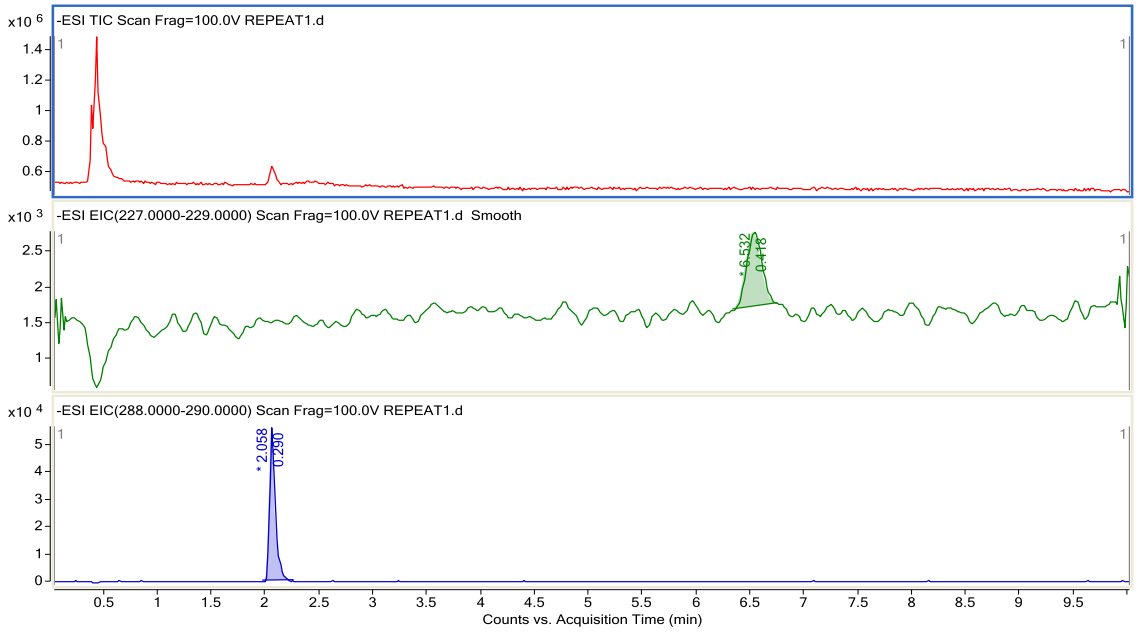


Figure D.4. McArthur Island Park water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

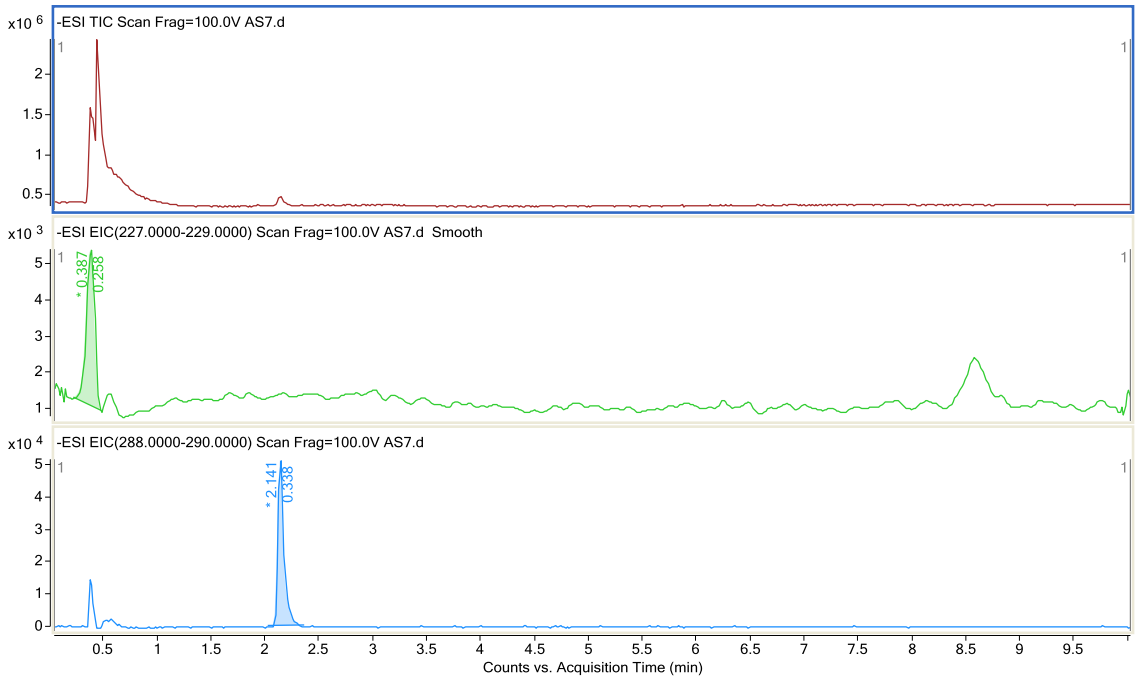


Figure D.5. Paul Lake water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

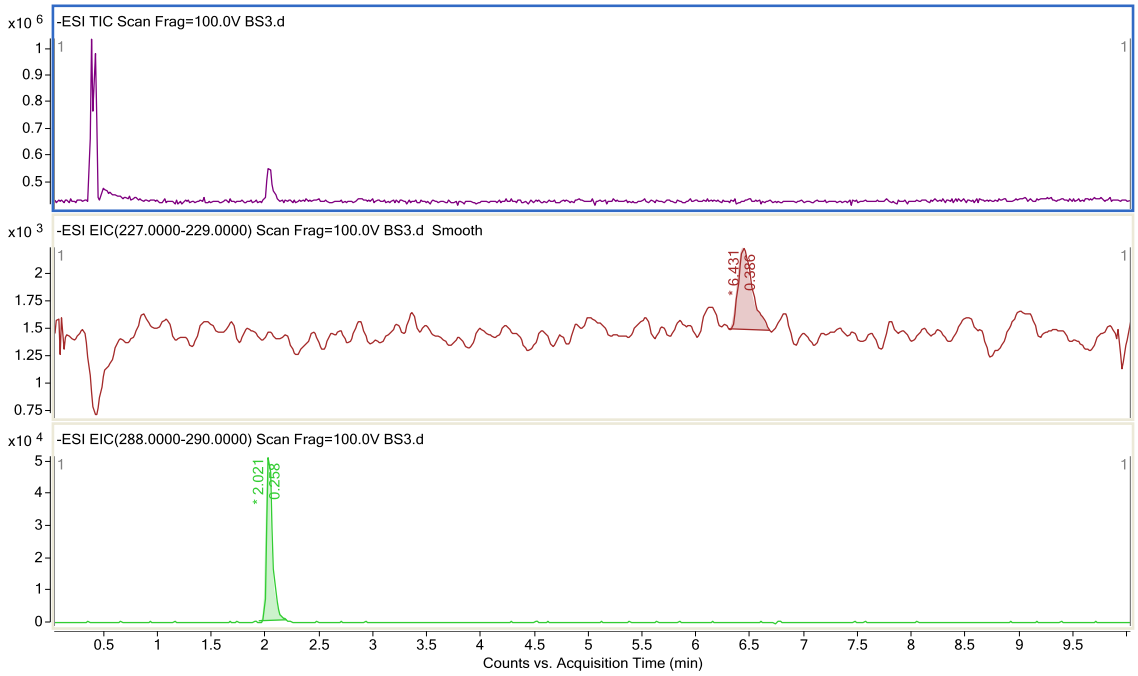


Figure D.6. Swimming Pool (P5-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

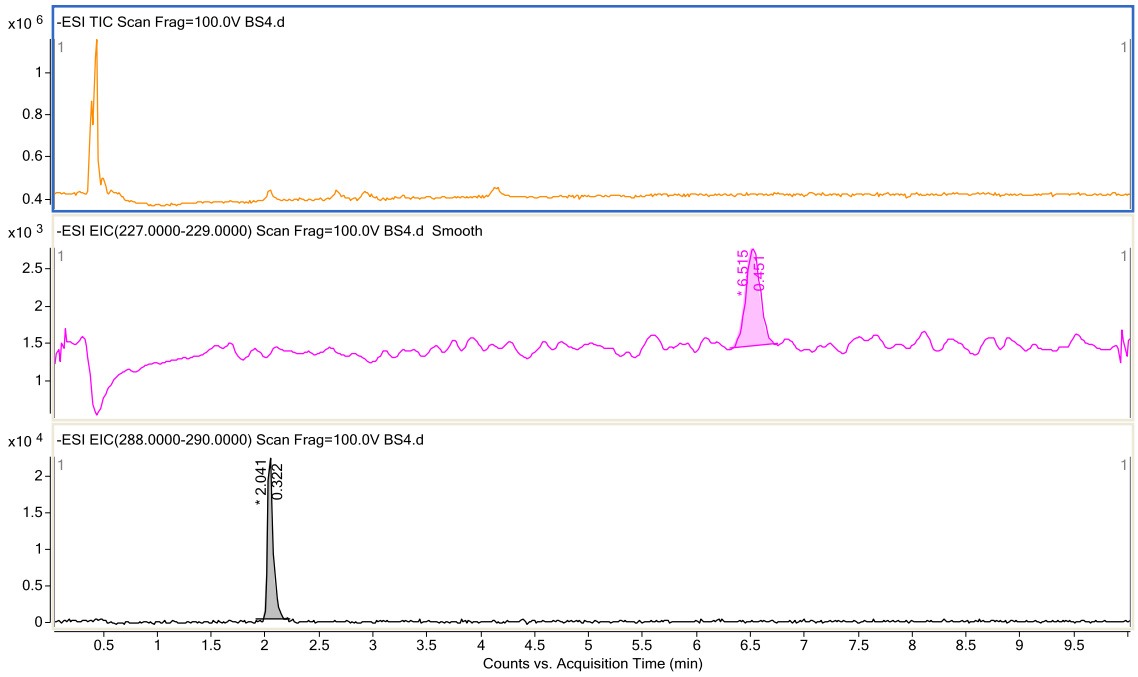


Figure D.7. Pioneer Park tap water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

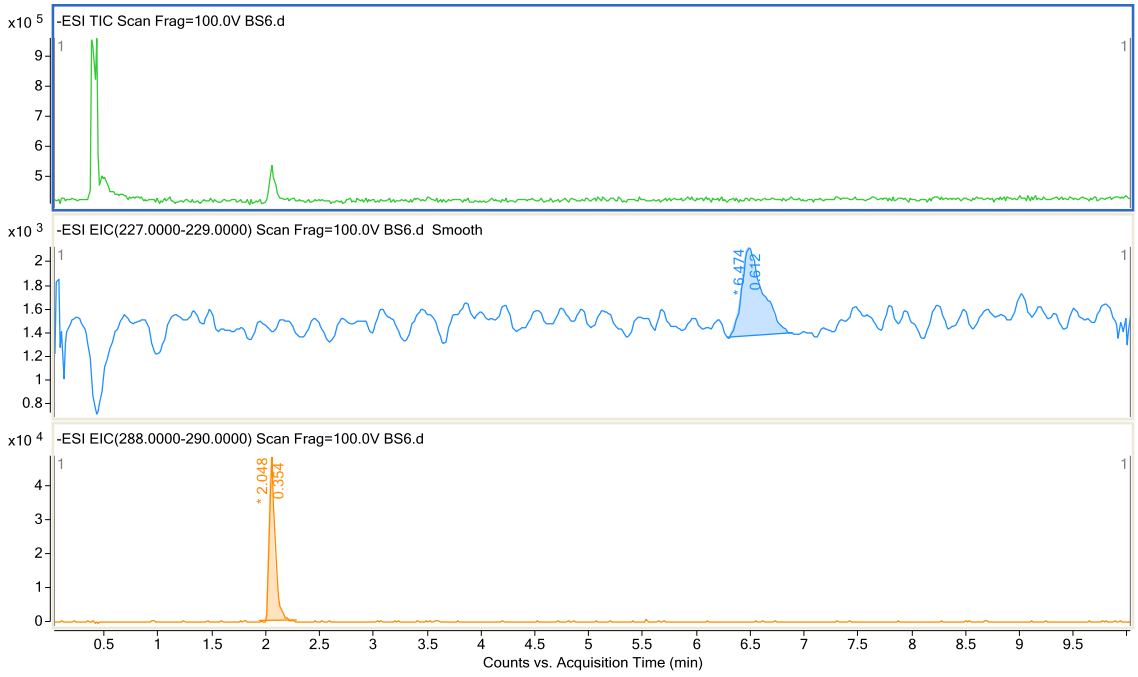


Figure D.8. Swimming Pool (P8-A) sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

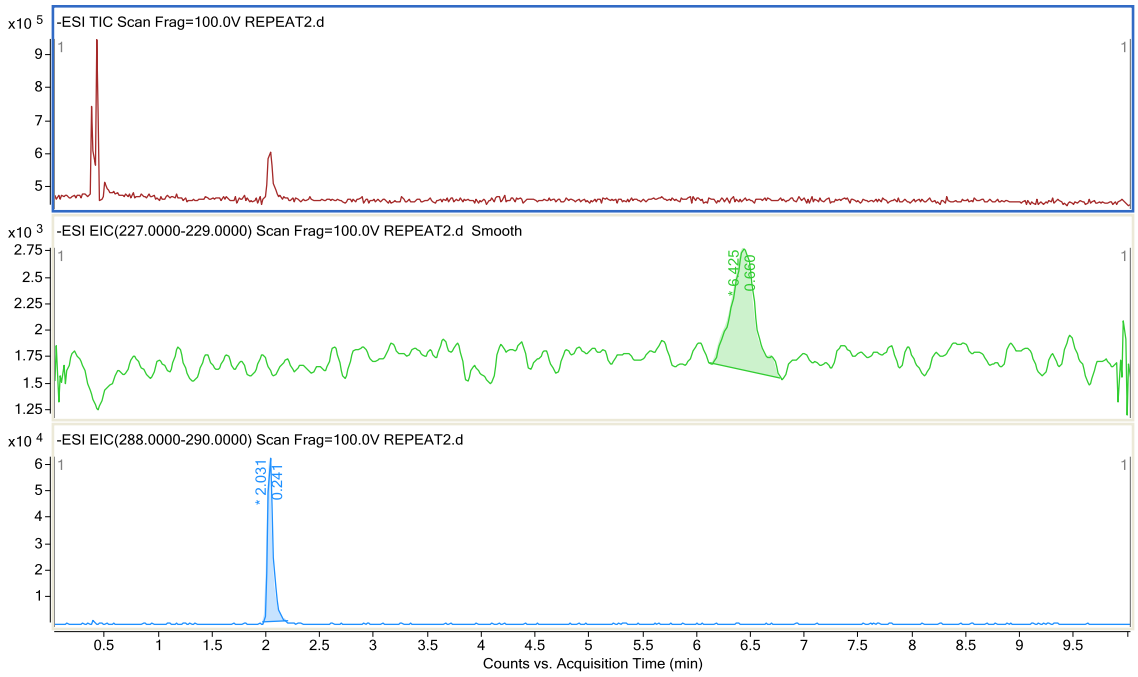


Figure D.9. Kamloops Lake water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

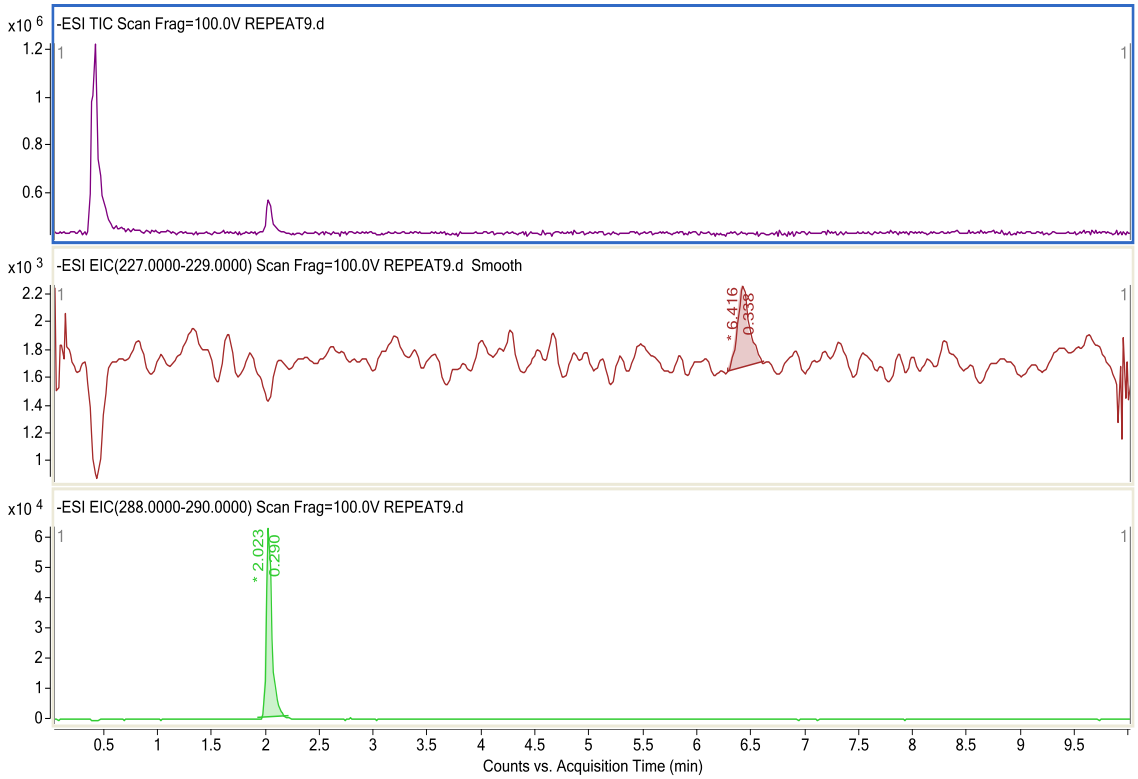


Figure D.10. Swimming Pool (P3-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

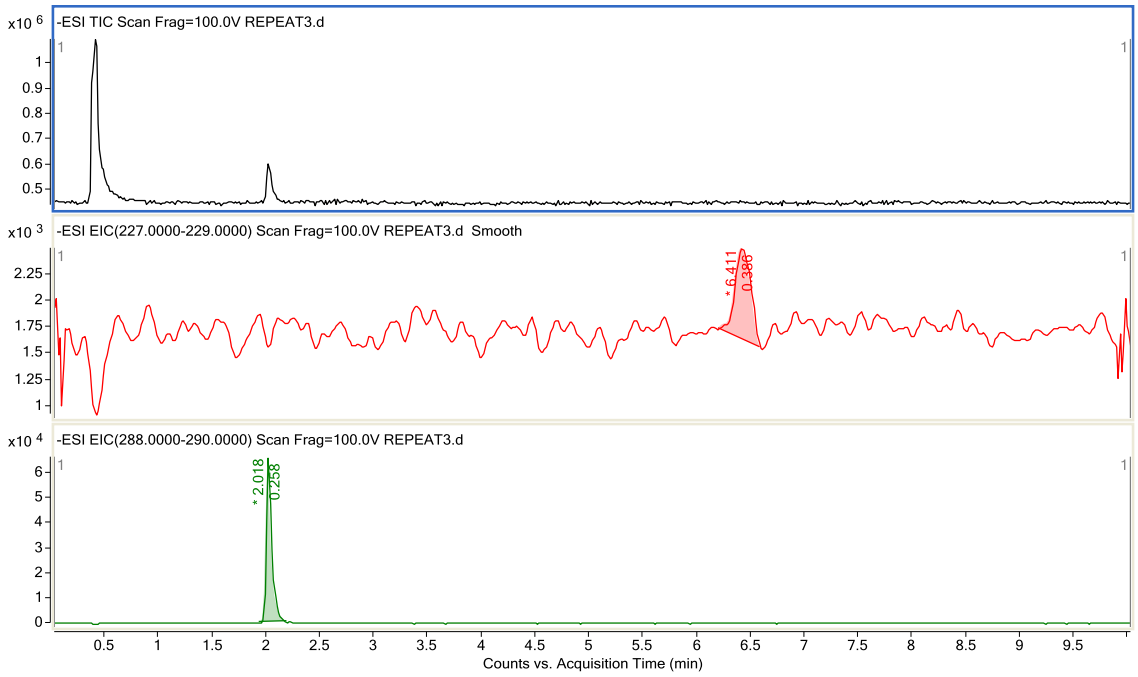


Figure D.11. Swimming Pool (P4-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

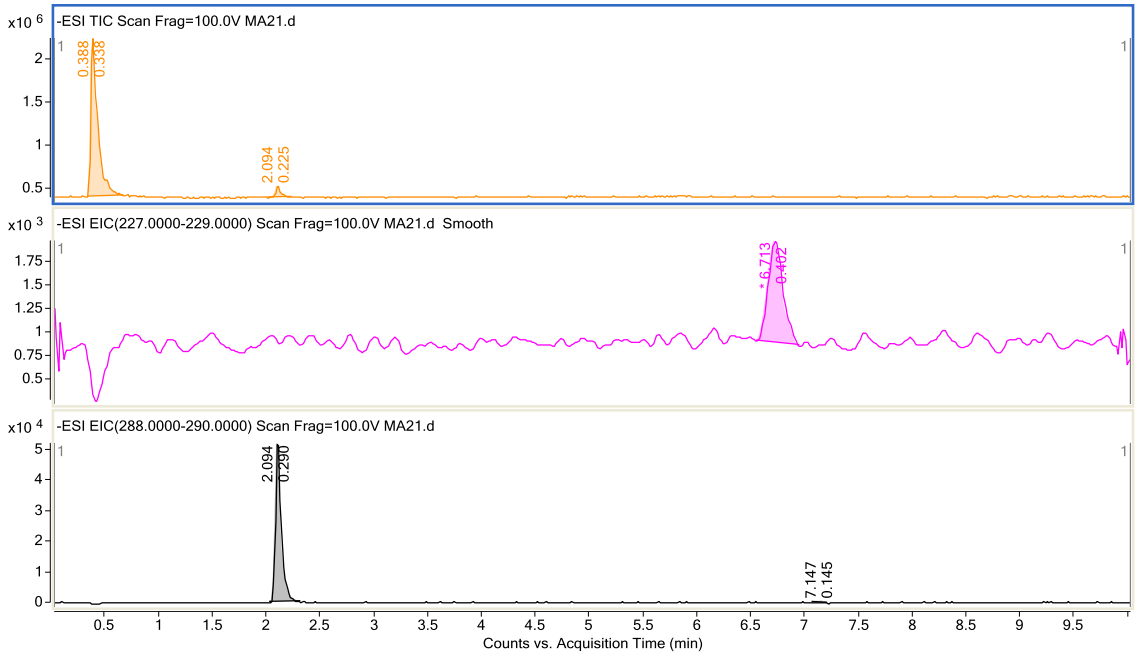


Figure D.12. Swimming Pool (P3-B) tap water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

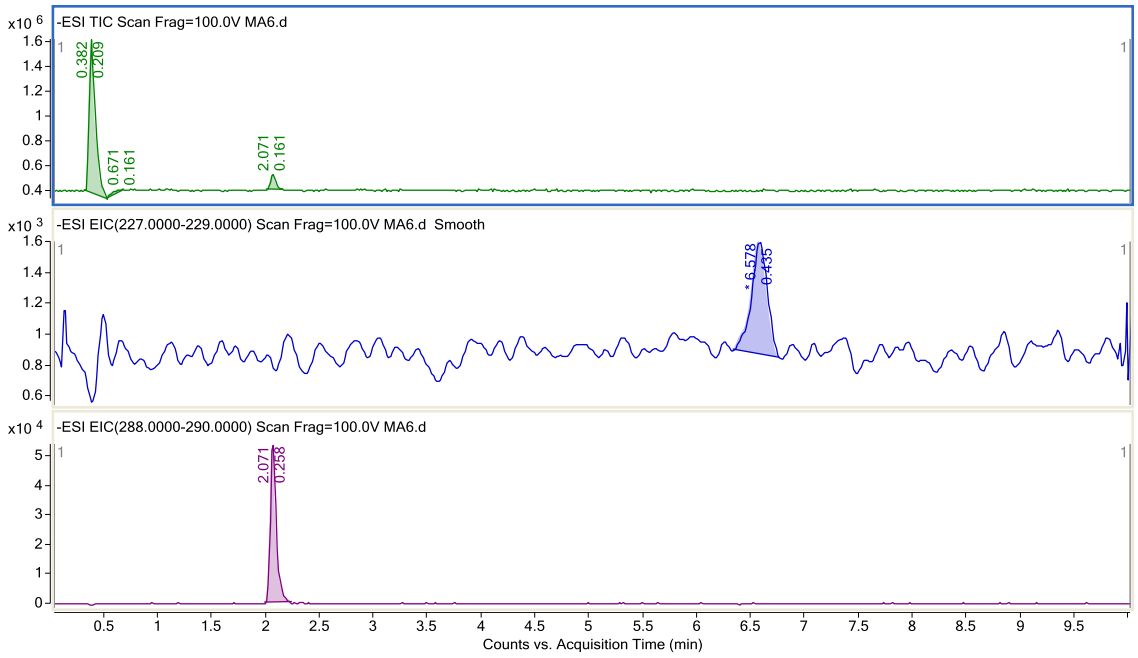


Figure D.13. Swimming Pool (P2-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

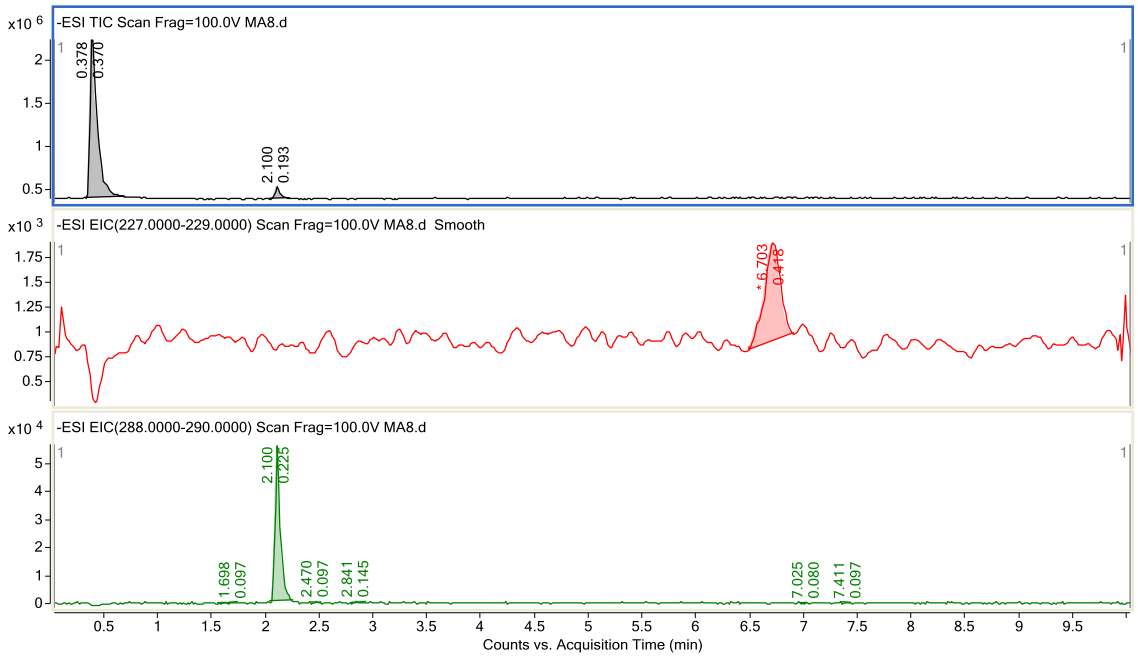


Figure D.14. Swimming Pool (P7-A) water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

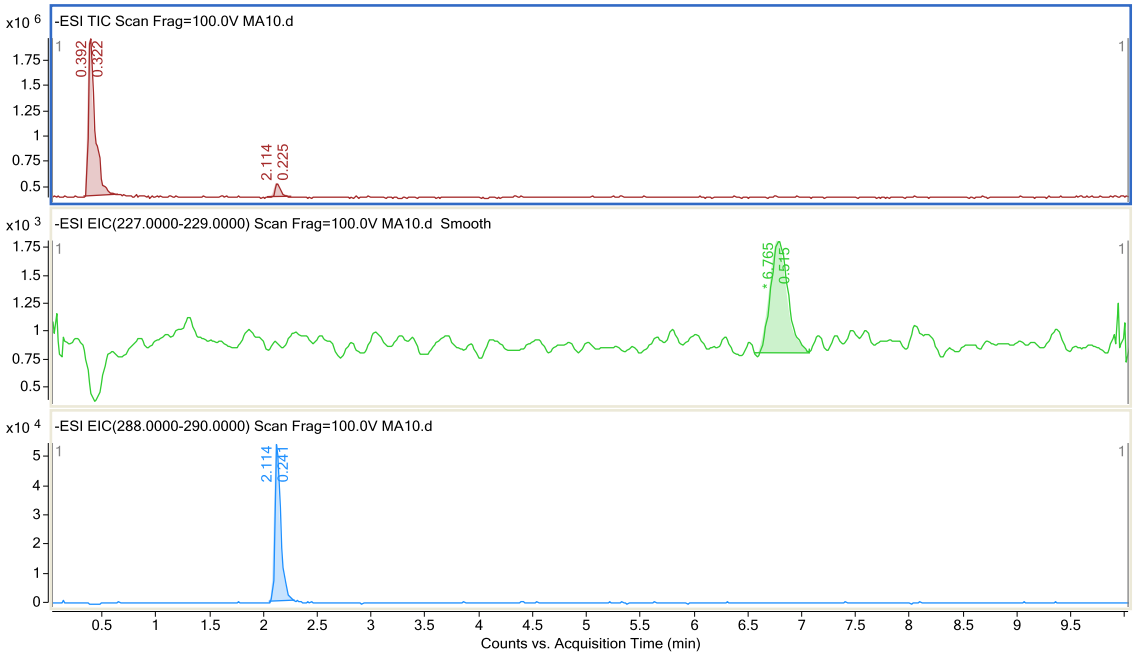


Figure.D.15. Swimming Pool (P6-A) water sample chromatogram; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

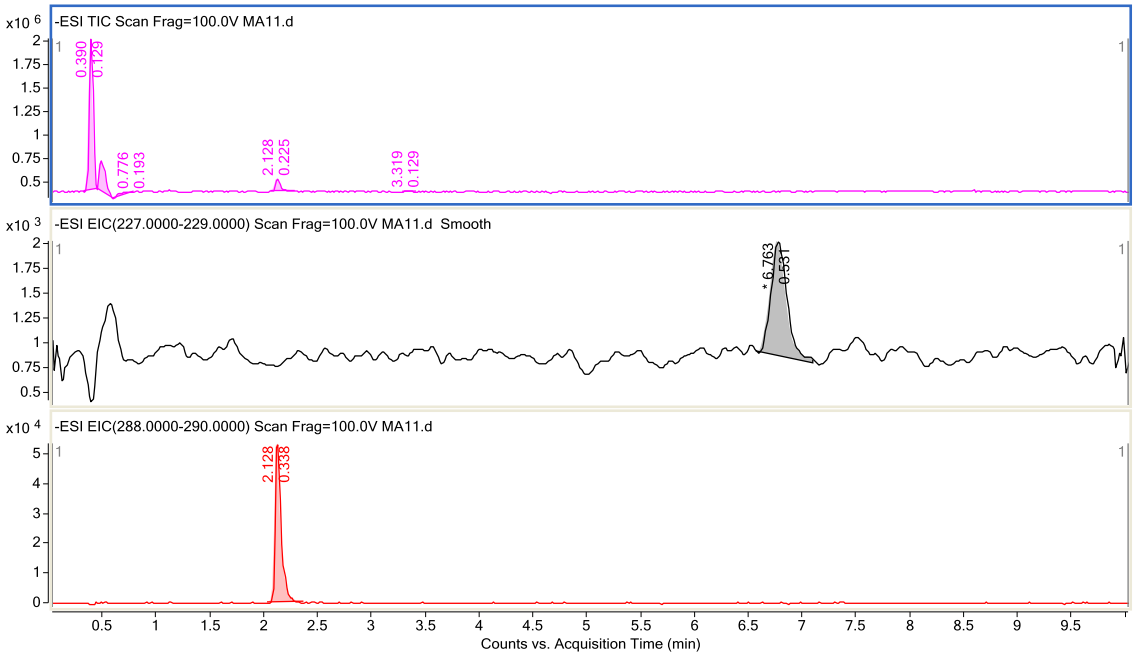


Figure. D.16. Paul Lake tap water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

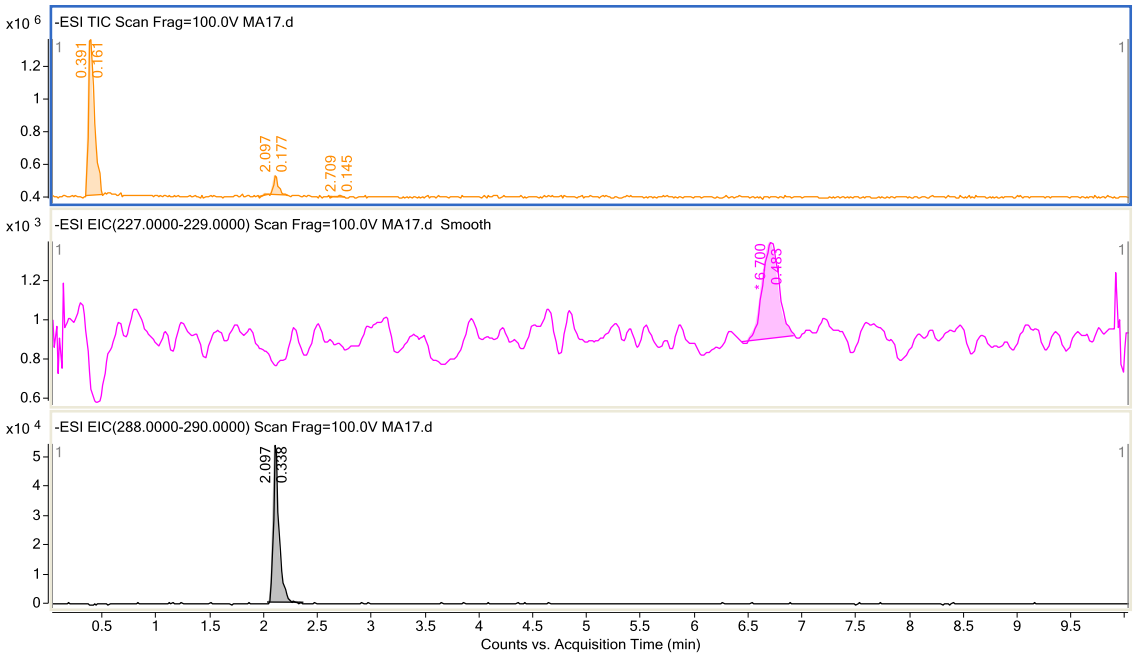


Figure. D.17. Riverside Park water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

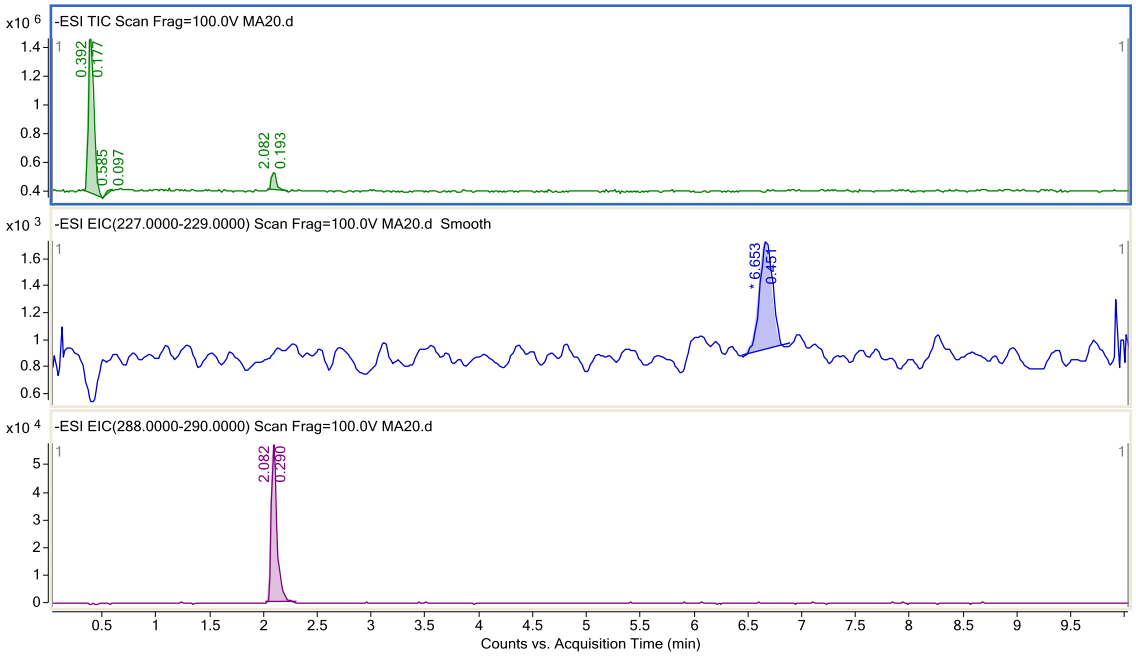


Figure. D.18. Adams River water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

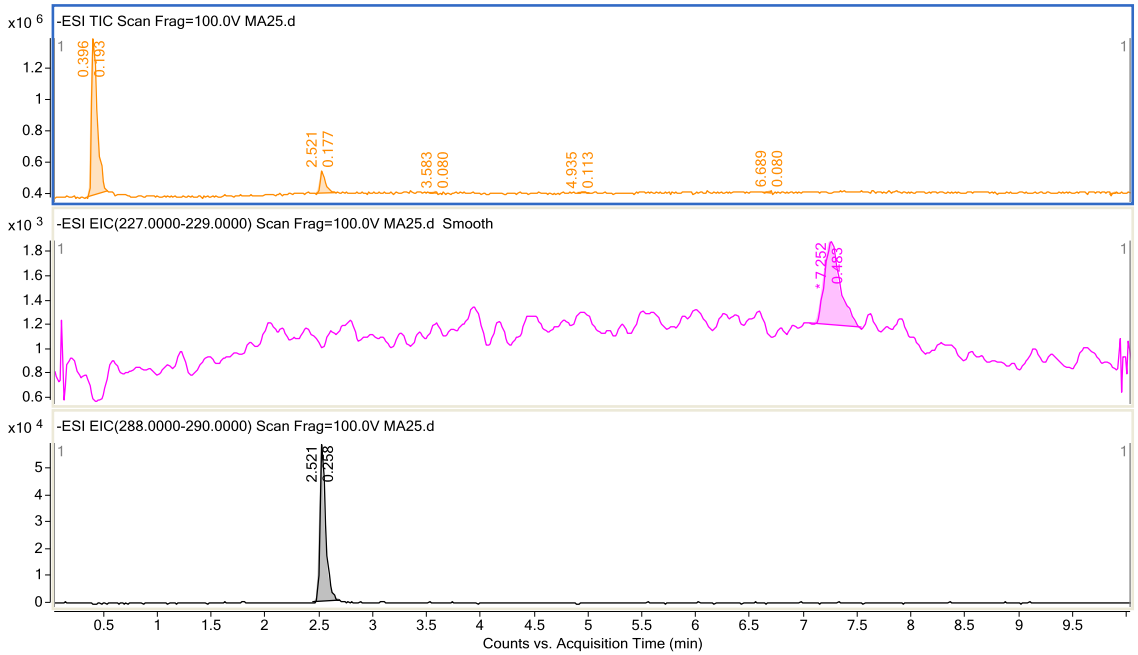


Figure. D.19. Shuswap Lake water sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

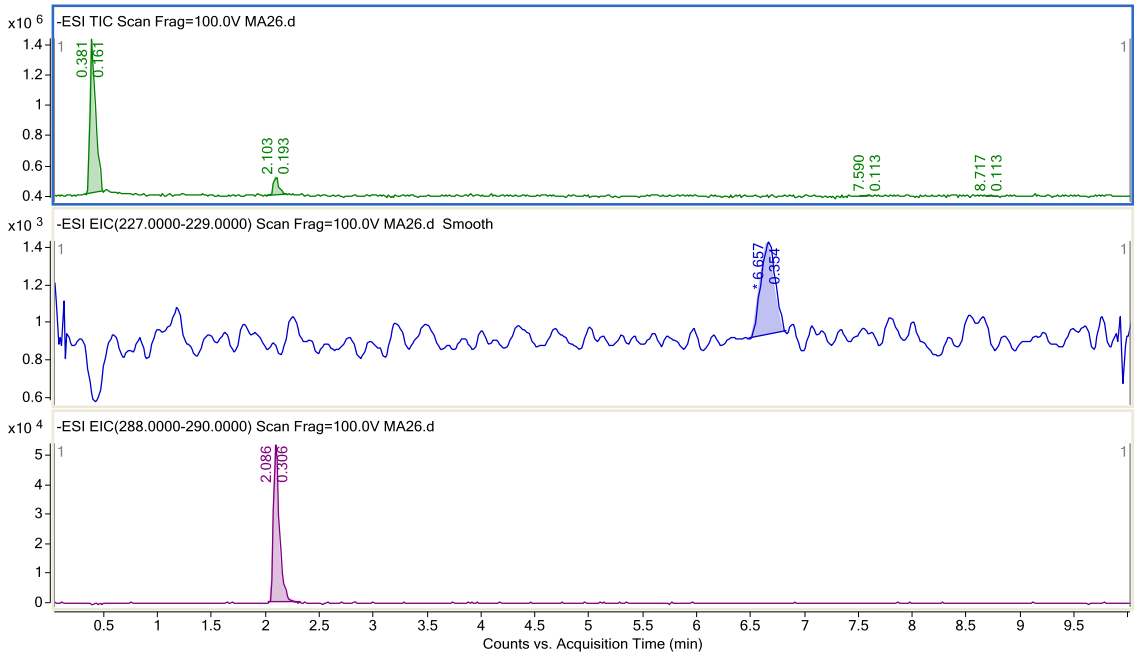


Figure. D.20. Swimming Pool tap water (P1-B) sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

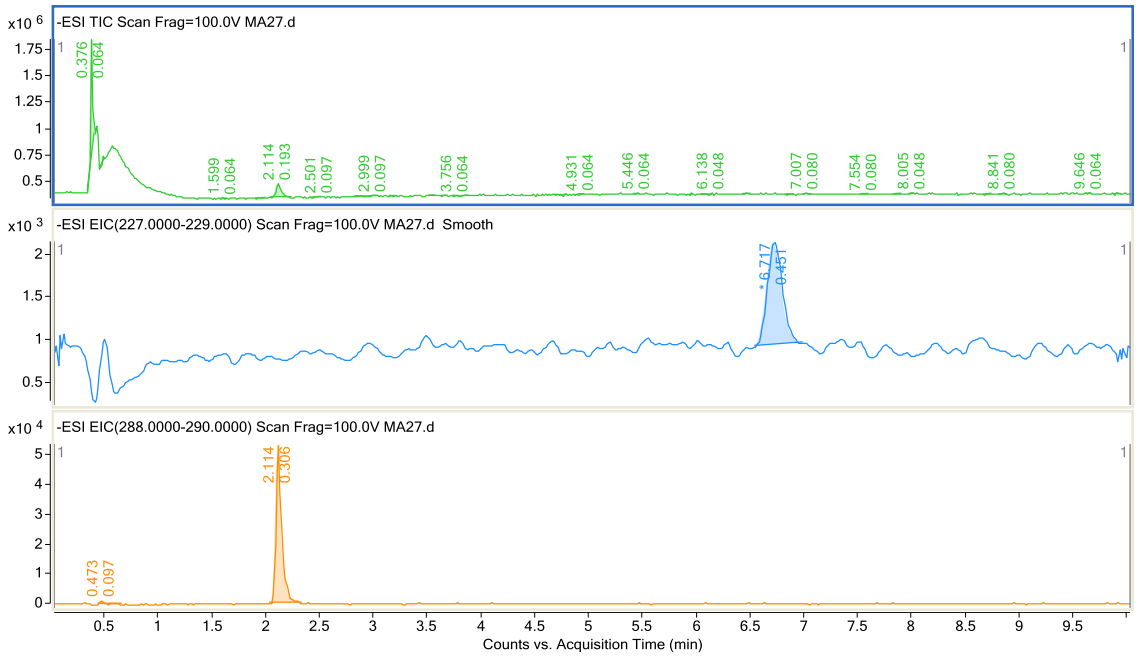


Figure. D.21. Swimming Pool tap water (P2-B) sample chromatograms; (top to bottom: TIC, EIC of BPA and EIC of triclosan)

Appendix E: Concentrations of BPA present in Environmental Water Samples with Standard Deviation Error Bars

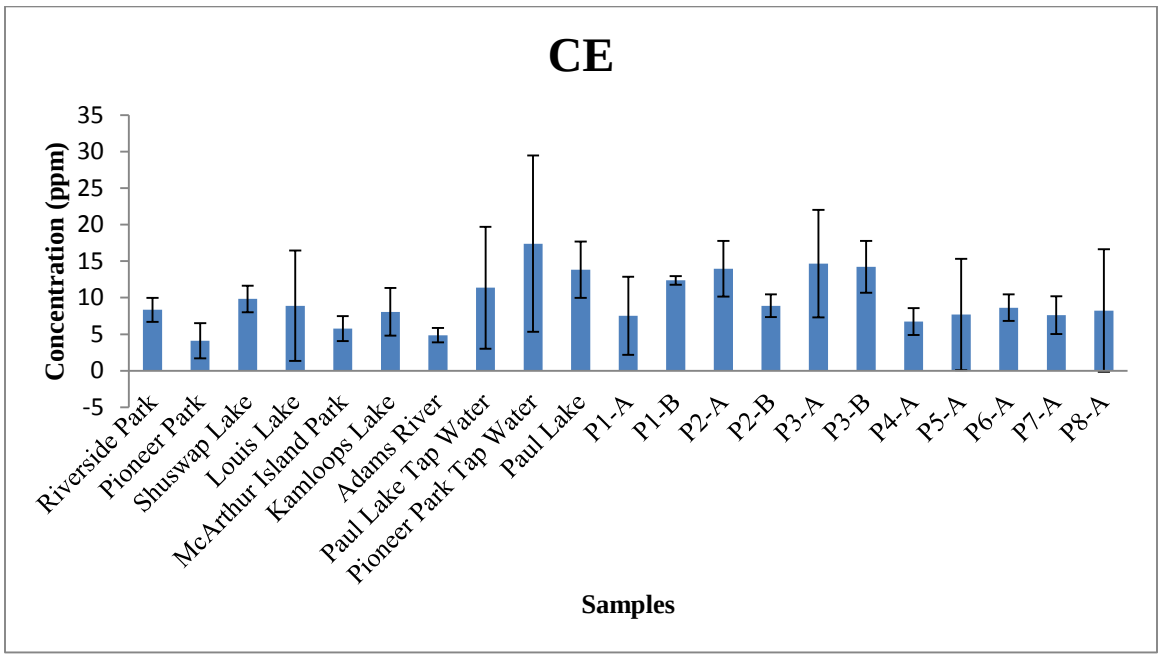


Figure E.1. Concentration of BPA in samples analyzed by CE

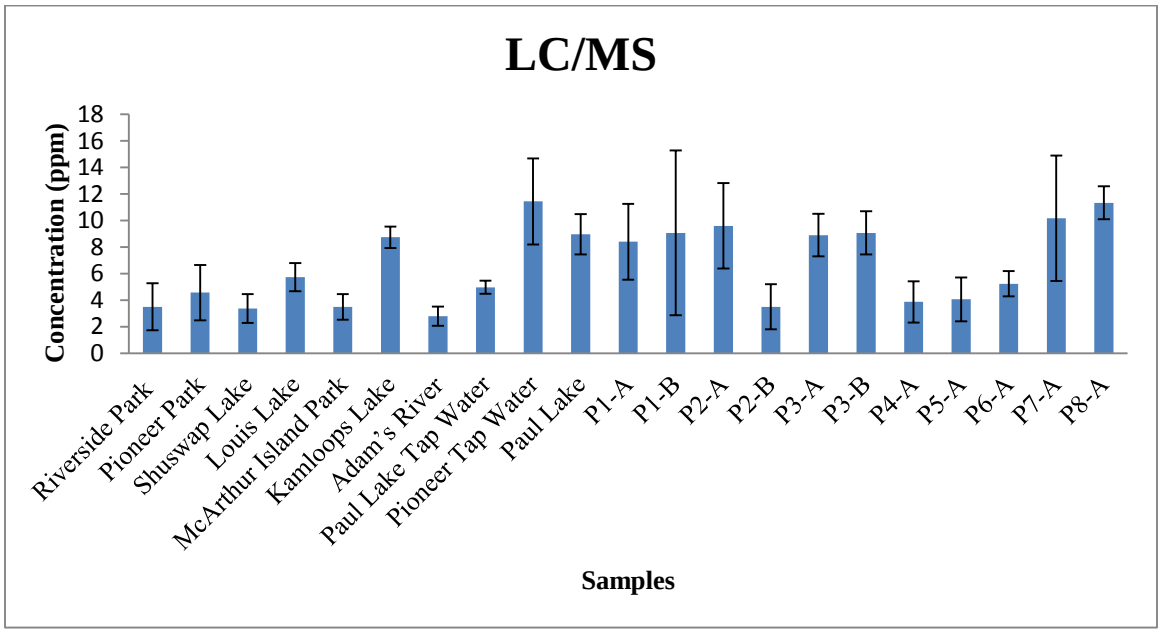


Figure E.2. Concentrations of BPA in samples analyzed by CE

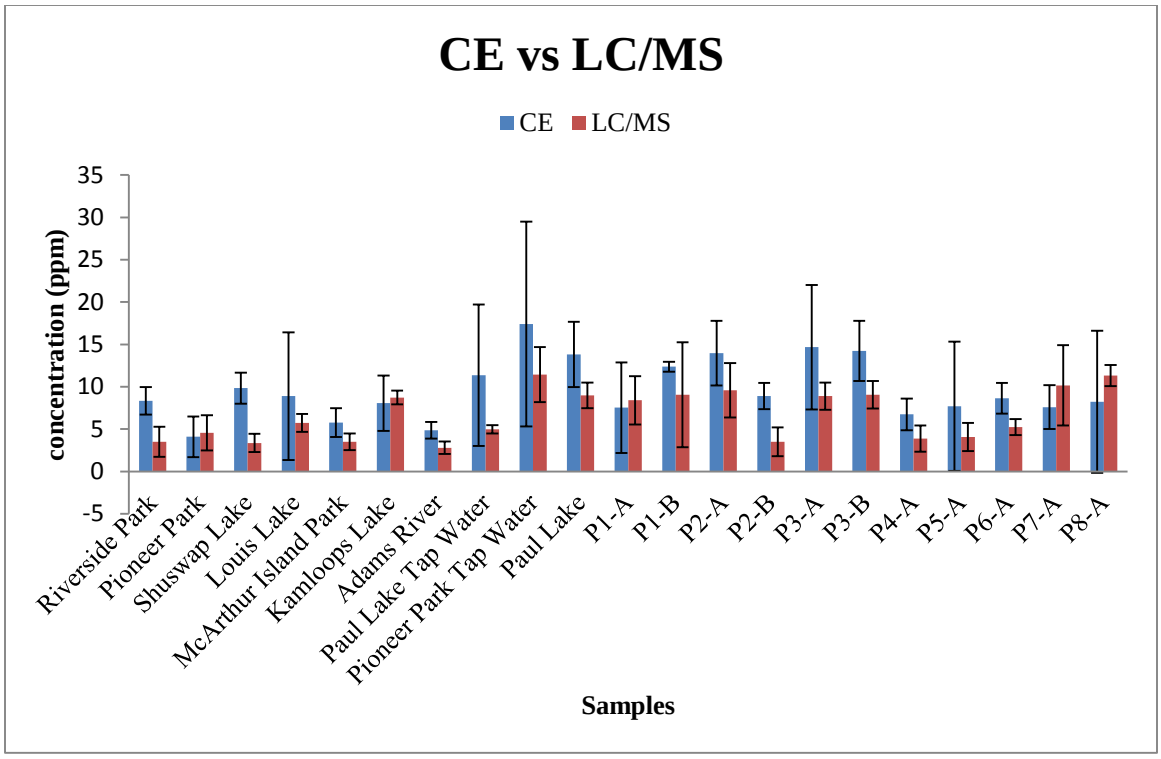


Figure E.3. Comparison of results from CE and LC/MS