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# Simultaneous Determination of BPA and BPS Using UV/Vis Spectrophotometry and HPLC

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# SENIOR THESIS APPROVAL

This Honors thesis entitled

**"Simultaneous Determination of BPA and BPS Using UV/Vis Spectrophotometry and HPLC"**

written by

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and submitted in partial fulfillment of
the requirements for completion of
the Carl Goodson Honors Program
meets the criteria for acceptance
and has been approved by the undersigned readers.

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# 2. ABSTRACT

Bisphenol A (BPA) has been one of the most used plasticizers with more than 4.8 million tons produced in 2012. BPA is also an endocrine disruptor that has been linked to adverse health effects such as cancer, obesity, behavioral and mood changes, lowered fertility, developmental changes and more in humans and other animals. The evidence of the toxicity of BPA, even at very low levels, has caused many countries to limit its use, especially in baby bottles and other baby-related hard plastic items. In these items, BPA has been replaced with other bisphenols, such as Bisphenol S (BPS). BPS is more stable and heat resistant than BPA. However, BPS is also an endocrine disruptor and can behave like BPA in cellular activities. Also, studies have shown more dermal penetration of BPS than BPA, and it has been linked to similar adverse health effects.

In this research, methods were developed to simultaneously determine concentrations of BPA and BPS using UV-VIS Absorption Spectrophotometry and High Pressure Liquid Chromatography (HPLC).

These methods were applied to water:methanol (1:1) samples exposed to different kinds of plastics, food cans and thermal receipt paper to test for leaching of BPA and/or BPS.

The concentrations determined ranged from w-x for BPA and y-z from BPS. The latter concentrations were above the Total Daily Intake approved by the Food and Drug Administration or the European Food Safety Authority. The methods used suggested that other chemicals leached out in addition to BPA and BPS. Time (0–2 weeks) and temperature (22-70°C) were varied to simulate everyday use of these products.

The effectiveness of the UV-VIS Absorption Spectrophotometry in determining concentrations of BPA and BPS in samples was compared to HPLC using statistical analysis. And this method proved to be effective.

# 3. ACKNOWLEDGEMENTS

Putting together this project and publication was a long and strenuous achievement. I take this time to be grateful for the assistance of the following because I could have not gone through it all without them.

- Dr. Sara Hubbard, the research director and first publication reviewer
- Dr. Joe Bradshaw, provided help with HPLC
- Dr. J.D. Patterson Summer Research Program, program that gave me the opportunity to be a research scientist
- Dr. Marty Perry, publication reviewer
- Dr. Byron Eubanks, publication reviewer
- Alpha Tau Honors Program, gave me the opportunity to make this publication
- Ouachita Baptist University, school where I performed my research project

# 4. DEDICATION

This publication is dedicated to

***My Family***

Protogene NSENGUMUREMYI & Marie Josée HAKIZIMANA, my dear parents

Leandre NSENGUMUREMYI, my dear brother

***and***

***All the people that have supported me and helped me develop into the person that I am today.***

# 5. ABOUT THE AUTHOR

Jean Eudes Benecyo was born on November 28, 1993 in Bujumbura, Burundi. He lived there with his parents until they moved to Kigali, Rwanda in 2000. At a young age, he started learning French to supplement to his native language Kinyarwanda. He later learned English while pursuing science classes in high school. He excelled at sciences in the Rwanda National Advanced Level Examination given to all the students in the country at the end of high school and was selected as one of the student recipients of the Rwanda presidential scholarship. This scholarship gave him an opportunity to pursue his undergraduate studies at Ouachita Baptist University where he plans to graduate on May 14th, 2016 with honors before pursuing his post graduate studies in medicine. In his time at Ouachita Baptist University, he has matured as a scientist and learned a lot from his biology major, business administration, and chemistry minors. His favorite motto is "Si isti et istae, cur non ego" (=If they [did it], why not me?) because to him, the sky is only the beginning.

# 6. VIDEO SUMMARY OF THE PROJECT

Please visit my YouTube channel under the name "Jean Eudes Benecyo" and look for the video named *Chemicals are leaching out of plastics into our drinks, our food, and even our skin: A chemical research study on Bisphenol A and Bisphenol S.*

This video provides a brief summary of the research with a focus on the importance of the research and what was achieved through the research in a way that is very understandable to the non-scientific community and any other viewer.

# 7. INTRODUCTION

During the summer of 2016, a research project was started by Jean Eudes Benecyo. This research had focused on developing a quantitative method that could determine simultaneously concentrations of bisphenol A and/or bisphenol S in a solution and applying the method in real world samples.

## 7.1. WHY DOES THIS RESEARCH MATTER?

After exhibiting links to several health problems[1], bisphenol A was reduced on the market and bisphenol S was introduced in the plastic industry. However, bisphenol S has been showing links to similar health problems to bisphenol A. These chemicals are endocrine disruptors[2] which mimic regular hormones and cause unforeseen cellular responses (**Error! Reference source not found.**).

[1] For links to health problems caused by BPA and BPS, see Section 7.2: SCIENTIFIC LITERATURE ON HEALTH EFFECTS CAUSED BY BISPHENOL A AND BISPHENOL S (on page 13)

[2] For more details on what endocrine disruptors are, see Section 7.1.1: HISTORY OF BISPHENOL A AND BIPHENOL S (on page 13)

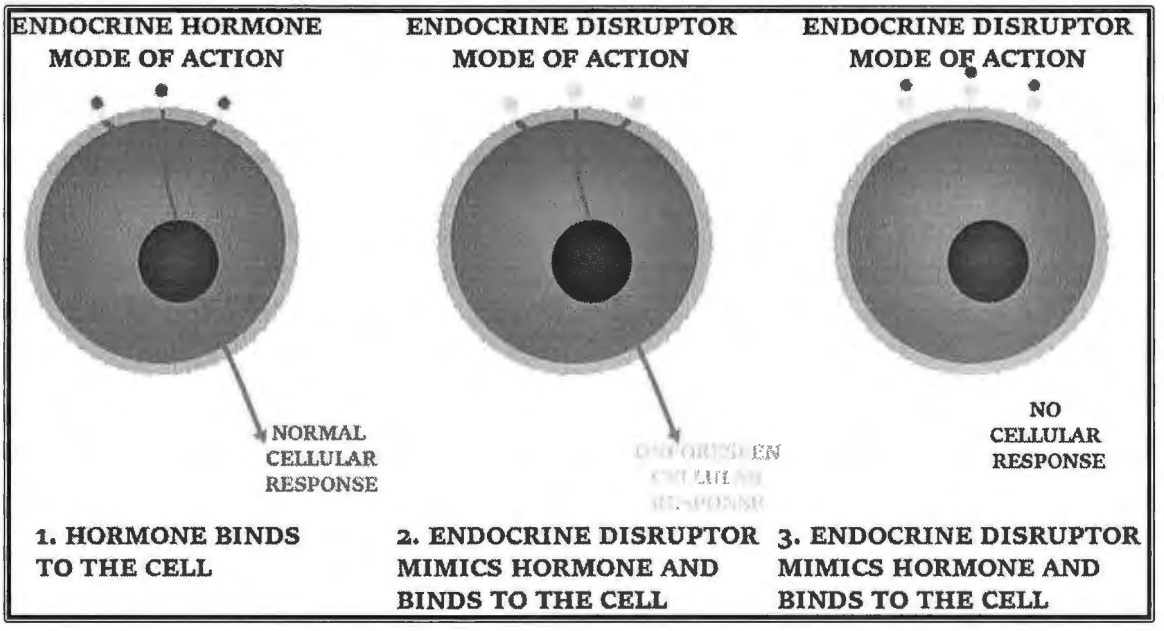


*Figure 1: How endocrine hormones work compared to endocrine disruptors*

The problem with plastics or most items is that manufacturers do not tell or are not required to reveal the chemicals used or their concentrations within their products. This is fair since if they gave it out, other companies would reproduce their products. But it results in the consumer not knowing the chemical composition of a given item, which makes it difficult to tell how much bisphenol A and/or bisphenol S are contained in a given item.

Our goal was to develop a method that can determine how much of these chemicals leach out of plastics or other samples. The chemical concentrations can then tell us the risk of exposure from plastics and other items. And as previously mentioned, the beauty of the UV/VIS Spectrophotometry method is that the majority of institutions can afford it and it takes a very short time to tell how much chemicals are present at an efficiency quite comparable to the HPLC method.

All these aforementioned reasons made this research project important because it developed an efficient method that can determine how much chemicals bisphenol A (BPA) and/or bisphenol S (BPS) are in a given solution. One widely used and well-known

quantitative method in analytical chemistry is High Pressure Liquid Chromatography (HPLC). This method allows for separation of mixtures and quantitative determination of component concentrations. The HPLC method is highly trusted on accuracy and precision in determining the concentration and presence of chemicals. The downside of HPLC is that it can be rather expensive.

Our goal was to create a new efficient and less time consuming method compared to the HPLC method.[3] Our method uses the UV/VIS Spectrophotometry. It is also affordable and has very easy steps to follow compared to the HPLC while conserving similar efficacy as determined later in the study. There is a high availability of UV/VIS spectrophotometers at most institutions, so it is very reasonable for the replication of this study at several different institutions.

This research project used the developed method to determine the occurrence of BPA and BPS in solutions. Bisphenol A and bisphenol S are two chemicals widely used in hard plastics.[4] This makes our exposure to them easy.

### 7.1.1. HISTORY OF BISPHENOL A AND BIPHENOL S

Bisphenols are chemical compounds characterized by two phenol rings (Figure 2). There are several bisphenols[5] but bisphenol A and bisphenol S are the most widely used.

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[3] The comparison between the two methods is discussed in details in Section 9.3.1: DETERMINATION OF KNOWN BPA AND BPS CONCENTRATIONS (on page 26)

[4] For the different places/items that contain BPA and BPS, see Section 7.1.2: SOURCES OF EXPOSURE (on page 13)

[5] There is a long list of bisphenol derivatives which depend on the structures attached to the carbon in the center of the structure or the structures that replace R' and/or R'' in Figure 2. Examples are bisphenol A, bisphenol AP, bisphenol AF, bisphenol B, bisphenol BP, bisphenol C…

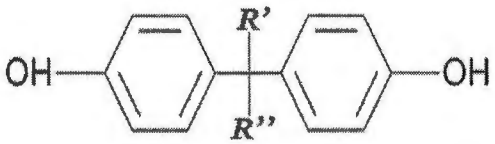


*Figure 2: Bisphenol general structure*

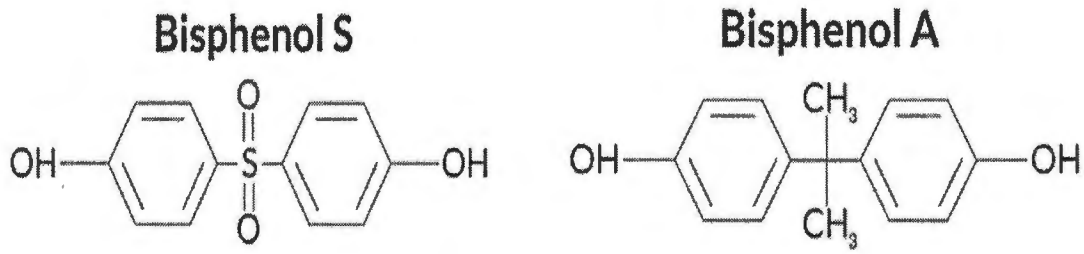


*Figure 3: Bisphenol S and Bisphenol A structures*

These chemicals have not been in use for a sufficient time to allow reliable data on how harmful they are. At this point in time, only links to various health problems have been shown and several countries have banned or limited their use in consumer products (Caliendo).

The following is a timeline of events concerning bisphenol A and all subsequent quotations in the timeline are drawn from an article by Packaging Digest[6]. In 1891, Russian chemist Aleksandr Dianin synthesized bisphenol A for the first time, but it was not until 1905 that it was officially mentioned in a scientific paper by Thomas Zincke. In the 1930s, British chemist Charles Edward Dodds recognized bisphenol A as an artificial estrogen because of its estrogen-like properties. In 1953, polycarbonate resin was invented and bisphenol A began to be used in polycarbonate products all over the world.

In 1960, The FDA approved the use of bisphenol A in consumer products like water bottles, baby bottles, food containers and epoxy linings for metal-based food and beverage

[6] See http://www.packagingdigest.com/shipping-containers/history-bpa or (Caliendo) in the section on page 44

cans. In 1992, Dr. David Feldman of Stanford University discovered that bisphenol A could migrate from his polycarbonate test tubes into specimens and could then mimic estrogen in the specimen and cause unforeseen cellular response at low doses (Caliendo).

In 1996, according to the Environmental Working Group, the FDA estimated that through canned food, adults and infants are exposed to 11μg and 7μg of bisphenol A daily respectively. In 1997, the University of Missouri-Columbia found that low-level exposure to BPA may harm the prostate. Over the next decade, the list of publications on health problems from bisphenol A grew to over 100.

Between 1998 and 2003, due to consumer concerns about the toxic effects of BPA, Japanese industries reduced the use of bisphenol A. Then it replaced its BPA-containing epoxy resin can liners with BPA-free polyethylene terephthalate (PET) in many products. As a result of these changes, Japanese risk assessors have found that no bisphenol A is detectable in canned foods or drinks, and blood levels of bisphenol A in Japanese have declined. In 2008, The European Commission and European Food Safety Authority (EFSA) determined BPA-based products, such as polycarbonate plastic and epoxy resins, are safe for consumers and the environment when used as intended. Health Canada released results of its human health screening assessment on BPA. It declared BPA "toxic" because of reproductive and developmental toxicity and environmental effects. The country bans the import, sale and advertising of polycarbonate baby bottles containing BPA due to these concerns. A report by the U.S. National Toxicology Program (NTP) found "some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to BPA," with that exposure coming from polycarbonate (PC) baby bottles and infant cups. Between the Health Canada announcement and the NTP findings, a majority of retailers and bottle manufacturers switched from PC to alternatives. The Natural Resources Defense

Council (NRDC) takes a stand against BPA as a whole, and asks the FDA to eliminate the chemical from all food packaging (Caliendo).

In 2009, six U.S. companies that produce baby bottles decide to stop using BPA in their products. The Endocrine Society publishes its scientific report on endocrine-disrupting chemicals that says there is strong evidence chemicals that interfere with the hormone system can cause serious health problems. It recommends that people reduce their exposure.

In November 2009, Consumer Reports released a new study about the dangers of BPA from canned foods and cautioned readers to "seek alternatives," including using glass containers when heating food in the microwave.

In 2010, the FDA joined other health agencies to express "some concern" over BPA safety. The FDA supported industry's actions to remove BPA from baby bottles, feeding cups, the lining of formula cans and other food cans, but did not provide any details or a timeframe for these voluntary actions. Health Canada released a new study of BPA exposure levels in canned foods. Its conclusion was "that current dietary exposure to BPA through food packaging is not expected to pose a health risk to the general population, including newborns and infants."

In 2011, the European Union banned BPA in baby bottles. China proposed to ban BPA in anything used to contain food or drink for children. About twenty six U.S. states proposed legislation that would ban certain uses of BPA. Many bills failed, but some are moving forward. The NRDC sues the FDA, and asks the court to compel the agency to respond. The court eventually issues a consent decree requiring FDA to make a final decision on NRDC's petition by Mar. 31, 2012 (Caliendo).

On March 3, 2012, the FDA ultimately decided not to ban BPA from food and beverage packaging. Currently eleven states have banned BPA from baby bottles and

children's sippy cups. California passes the Toxin-Free Infants and Toddlers Act, banning BPA from baby bottles and children's sippy cups. The American Medical Assn. announces its support of tighter restrictions on products containing BPA. France votes to ban BPA in all food containers by Jan. 1, 2014, and by Jan. 1, 2013, for food packages, materials and containers for infants and young children.

In March 2013, Rep. Edward Markey (D-MA) asks the FDA to ban the chemical's use in formula containers, reusable food containers and in canned foods and beverages. The last two petitions are rejected, but the FDA accepts the petition on infant formula, and says it plans to collect comment from the public before making a final decision. The FDA says it will try to complete a scientific review within the next 90 days. (Caliendo)

According to an article by Nature[7], bisphenol S was first made in 1869 as a dye. But it was only introduced recently in the manufacture of plastics and other items as concerns had been raised about BPA. For example it was introduced in cash-register receipts in 2006. Bisphenol S or bisphenol A can act as a dye or paint in thermal receipt paper, where its combination with heat from the printer results in the black ink that we see on receipt papers. This recent introduction in consumer products makes it difficult to research potential health effects because few researchers have studied its toxicity. The few that did have already linked bisphenol S to adverse health effects[8] similar to that of bisphenol A.

## 7.1.2. SOURCES OF EXPOSURE

As mentioned in the previous section, bisphenol S has been in use for a longer time than bisphenol A. Though first intended for other uses, these chemicals were later introduced

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[7] See http://www.nature.com/news/toxicology-the-plastics-puzzle-1.15038 or (Nature) in the section on page 44

[8] For more details about research on bisphenol S, see Section 7.2: SCIENTIFIC LITERATURE ON HEALTH EFFECTS CAUSED BY BISPHENOL A AND BISPHENOL S (on page 17)

as plasticizers. Bisphenol S was first used in 1869 as a dye whereas bisphenol A was first synthesized in 1891. But it was not used in plastics until recently which is why little is known about bisphenol S when compared to bisphenol A.

We get exposed to these chemicals mainly from handling plastics or other products which contain bisphenol A/bisphenol S. Heating such items increase the exposure because more leaching can occur as a consequence. These chemicals can then enter our system through food or drink stored inside the items or even through items like dental sealants.

We can also get exposed to bisphenol A and bisphenol S by dermal absorption. Recent studies showed that bisphenol A and bisphenol S can enter your blood through the tiny pores on the skin. The biggest concern is that because most soaps facilitate their dilution, cleaning your hands with soap after handling items containing these chemicals can result in more dermal absorption[9]. So it is better not to use soap after handling the different items that have bisphenol A or bisphenol S. Images of some items that may contain BPA and/or BPS are shown in Figure 4.

[9] For more details about research on bisphenol S and bisphenol A, see Section 7.2: SCIENTIFIC LITERATURE ON HEALTH EFFECTS CAUSED BY BISPHENOL A AND BISPHENOL S (on page 17)


*Figure 4: Some sources of exposure to bisphenol A and bisphenol S*

We can get exposed from a range of items (Figure 4). Some of them cause a bigger problem than others. For example, a helmet made from BPA/BPS does not cause a problem because of the way it is used. But receipt papers can cause a problem for cashiers who handle a lot of them daily. Similarly, heating milk or formula in the microwave before giving it to children increases exposure from leaching. These chemicals leach more when the temperature is risen. So the use of these plastics can affect the level of exposure. However, the problem with these chemicals is that they accumulate in our system and we get multiple different sources of exposure every day. When combined can cause long-term problems as found in the study done on cashiers and the one done on zebrafish[10].

[10] Extensive details on these studies can be found in Section 7.2: SCIENTIFIC LITERATURE ON HEALTH EFFECTS CAUSED BY BISPHENOL A AND BISPHENOL S (on page 19)

## 7.2. SCIENTIFIC LITERATURE ON HEALTH EFFECTS CAUSED BY BISPHENOL A AND BISPHENOL S

Bisphenol A and Bisphenol S are two aromatic chemicals with quite similar structures[11] (Figure 5). These structural similarities might be the reason behind the similar health effects that they cause on the organism.

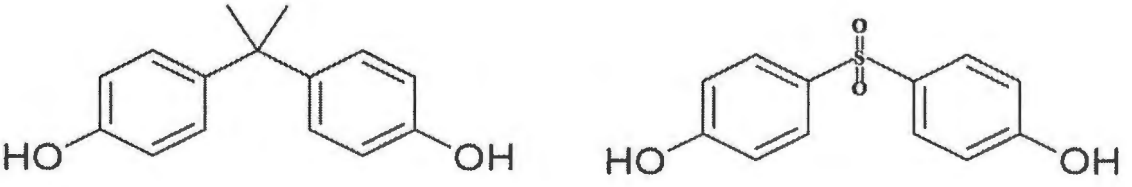


*Figure 5: Structures of bisphenol A and bisphenol S (left: BPA, right: BPS)*

Health problems have been linked to the leaching of these chemicals from their source into food or liquid. For example, bisphenol A is mostly used in polycarbonate where it can leach over time according to the following reaction. This reaction can still happen given a methanol or other alcohols or groups with the hydroxyl group.

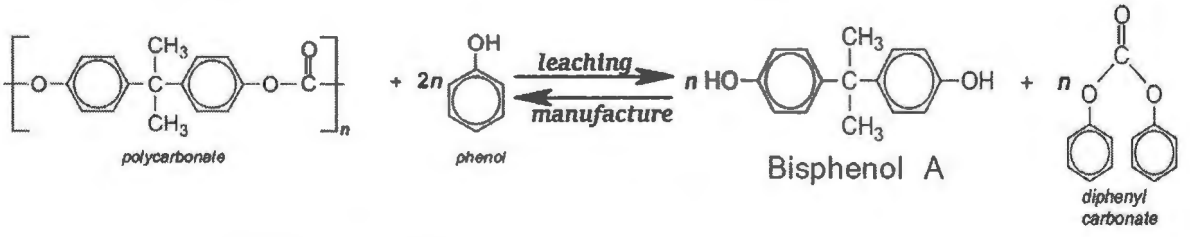


*Figure 6: Reaction between polycarbonates and bisphenol A*

In 2014, the EFSA (European Food and Safety Association) reevaluated the total daily intake[12] for bisphenol A from 50 µg/kg of body weight/day to 4 µg/kg of body weight/day. The EFSA also mentioned that the highest estimates for dietary exposure and for

[11] For details about the chemical structures of BPA and BPS, refer to Section 7.1.1: HISTORY OF BISPHENOL A AND BIPHENOL S and Figure 3 (on page 13)

[12] The total daily intake (TDI) is a concentration above which a chemical is not safe anymore. The TDI for BPA is 4 µg/kg of body weight/day. The TDI for BPS has not been determined yet.

exposure from a combination of sources are three to five times lower than the new total daily intake. (EFSA, No consumer health risk from bisphenol A exposure).

However, a new risk assessment of bisphenol A is going on as new scientific evidence raised concerns about the effects of bisphenol A on the immune system of fetuses and young children (EFSA, No consumer health risk from bisphenol A exposure). This evidence is being reviewed by the EFSA and an updated bisphenol A risk assessment is expected in 2018 (EFSA). The new evidence comes from two research studies by research groups. The results of the first study suggested that a decrease in the defense of the immune system occurred when exposed to a low dose of BPA (dose equivalent to the new total daily intake). The results were consistent with a decreased number of helper T cells, regulatory T cells and dendritic cells in spleen and mesenteric lymph nodes of BPA-exposed rats. (Ménard, Lencina and Leveque). The latter study showed that the body tries to fight off bisphenol A as it is viewed as a foreign chemical. This result was in accordance with the increase of activated T lymphocytes observed in spleen of BPA-exposed rats compared to controls (Ménard).

Previous research has also raised problems about the use of bisphenol A or bisphenol S in the manufacture of consumer products. According to a study done in 2008, bisphenol A exhibits similar to effects seen in response to the model estrogens, diethylstilbestrol and ethinylestradiol. [13] For most effects in this study, the potency of bisphenol A was determined to be approximately 10 to 1,000-fold less than that of diethylstilbestrol or ethinylestradiol. The researchers were confident that adult exposure to bisphenol A affects the male reproductive tract, and that long-lasting, organizational effects in response to developmental exposure to bisphenol A occur in the brain, the male reproductive system, and metabolic

[13] These are derivatives of estrogen and diethylstilbestrol is also an endocrine disruptors.

processes. They suggested that further confirmation about how adult exposure to bisphenol A affects the brain, the female reproductive system, the immune system was needed (Richter).

In a study conducted in 2001, pregnant female rats were exposed to low doses of bisphenol A. The effects of the exposure were studied in the offspring. The offspring exhibited an apparent increase in body weight since birth and it continued through adulthood. The females showed altered patterns in their estrous cycles. Also levels of plasma luteinizing hormone were lower than normal in adulthood. The conclusion of the study was that rats have sensitivity to BPA during the perinatal period (BS, MK and DA). Similar studies have shown the following data. In male rodents, after exposure to bisphenol A, there was decreased sperm counts, delayed puberty, feminization of reproductive organs, and atrophy of reproductive organs. And in female rodents, after exposure to bisphenol A, there was accelerated puberty, decreased fertility, altered estrous cyclicity, and ovarian malfunction (Vrooman, Oatley and Griswold).

According to an article by Environmental Health Perspectives, a study done on a rat pituitary cell line in 2013 revealed that bisphenol S disrupts membrane-initiated E2-induced cell signaling, leading to altered cell proliferation, cell death, and PRL release at 10-15 M levels (Viñas and Watson). These results suggest that bisphenol S can be linked to some similar effects caused by bisphenol A. Another study during the same year revealed that zebrafish exposed to 0.5µg/L of bisphenol S (1/6 of maximum concentration detected in environment) had fewer eggs, malformed offspring and higher estrogen to testosterone levels than untreated zebrafish (Ji). In a recent study from 2015, scientists provided more evidence that both bisphenol A and bisphenol S cause alterations in brain development leading to hyperactivity in zebrafish (Kincha, Ibhazehiebo and Jeong).

We get exposed to these chemicals very often. For example, a study done in 2012 revealed that 81% of 315 urine samples analyzed from USA and Asia (China, India, Japan, Korea, Kuwait, Malaysia, and Vietnam) contained bisphenol S ranging between 0.2 - 21ng/mL with the highest concentrations being from Japan and the USA (Liao, Liu and Alomirah).

The problem with these chemicals is people can also be exposed to them through the skin. In 2014, a study revealed that using a hand sanitizer after exposure to BPA led to increased dermal penetration of bisphenol A (Hormann, Vom Saal and Nagel).

While bisphenol A and bisphenol S exposure has been linked to health problems, the quantity seems to be low in the environment compared to the total daily intake. But with accumulation, these chemicals can build up, potentially weakening our organisms.

## 7.3. INTRODUCTION TO THE RESEARCH AND HYPOTHESIS

Bisphenol A and Bisphenol S, our chemicals of interest are widely used in products we use daily. When they leach out of these products, they can enter our system where they have been proved to be harmful.

This research project developed a method that can simultaneously determine either BPA or BPS, or both chemicals simultaneously. Several plastics are dumped each year in lakes and other bodies of water. In such places, having a method that can determine both chemicals simultaneously could be very useful. Additionally, this method could be used to determine if BPA or BPS is present in samples.

Bisphenol A has been in use for a longer time compared to Bisphenol S. According to this, our expectations were to find more occurrence of bisphenol A than bisphenol S in tested samples from the target products. After bisphenol S was introduced as a bisphenol A

substitute, it was introduced in the manufacturing process of several products. This meant to us that if we find BPA-free or bisphenol A – free products, they likely contain bisphenol S.

Our research conditions tried to replicate how the different product items are used. Different conditions of time and temperature were applied. Our hypothesis was that as time and/or temperature increased, leaching would occur more readily. In other words, we expected that time and temperature would positively correlate with leaching concentrations of bisphenol A and bisphenol S.

# 8. MATERIALS AND METHODS

## 8.1. OVERVIEW

This research first focused on developing a quantitative method that could be used to determine concentrations of either bisphenol A or bisphenol S in a solution with 1:1 methanol:water. After a method was developed, it was used to determine concentrations of mixed quantities of bisphenol A and bisphenol S in a solution with 1:1 methanol:water. The method used was the UV/VIS Spectrophotometry. The effectiveness of this method was compared to the High Pressure Liquid Chromatography method using statistical analysis. It was applied in real world samples to determine concentrations of bisphenol A and bisphenol S in mixtures.

## 8.2. UV/VIS SPECTROPHOTOMETRY

### 8.2.1. REAGENTS

HPLC grade Bisphenol A (99+% BPA), HPLC grade Bisphenol S (98+% BPS), HPLC grade methanol, HPLC grade water were purchased from Sigma-Aldrich. Nitric acid was also purchased from Sigma-Aldrich for glassware cleaning purposes and other cleaning

purposes.

### 8.2.2. GLASSWARE AND OTHER SUPPLIES

A marker, sticky papers, scissors, aluminum foil, parafilm, goggles, face mask, gloves, spatulas, graduated cylinders, beakers, volumetric flasks, volumetric pipettes, and graduated pipettes were provided for the experiments. Glassware was cleaned with 1:1 nitric acid/water[14] and dried before and after an experiment to ensure purity of chemicals and accuracy of measurements.

### 8.2.3. EXPERIMENT CONDITIONS

All the reagents were stored at room temperature. Glassware was heated in the oven at 120 °C for at least 2 hours prior to use to insure they were dry. The different samples used for the research were heated in the oven according to the different set experiment conditions. Conditions of time (0–2 weeks) and temperature (22-70°C) varied from experiment to experiment. All samples were collected in triplicates to ensure precision.

### 8.2.4. UV/VIS APPARATUS AND OPERATING CONDITIONS

BPA and BPS were simultaneously determined using the Vernier UV-VIS Spectrophotometer with Logger Pro 3 software.[15]

The UV/VIS Spectrophotometer used in this research project could go up to 2 absorbance units and had wavelengths values that were in the range of 225-800nm. But for

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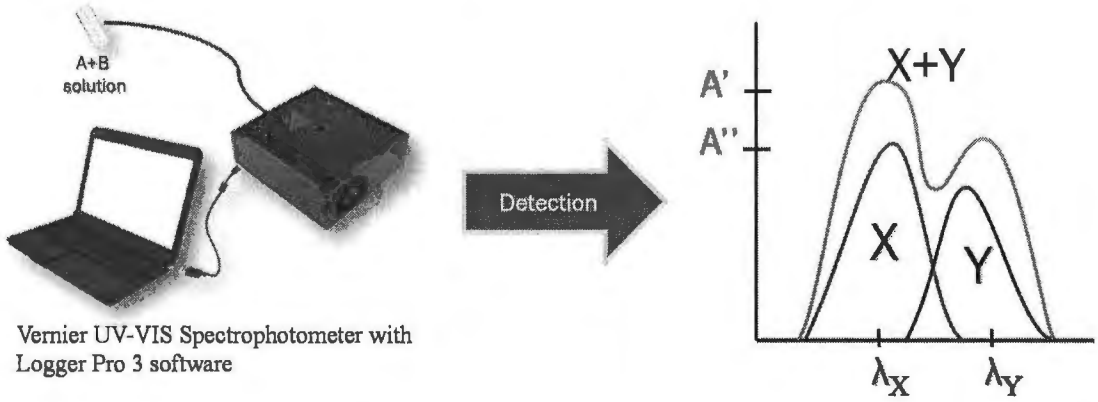
[14] This is preferable because soap can disrupt light and cause background reading in case some soap is not dried in the glassware.

[15] Refer to **Error! Reference source not found.**

our research project, we were only interested in absorbance values between 240-300nm because this was the area where BPA and BPS peaks could be seen since it is their range of wavelength emission.

## 8.2.5. UV/VIS SPECTROPHOTOMETRY QUANTITATION

All samples were collected in triplicate to further ensure precision. Samples used filled three quarters of the 1cm cell used in the Vernier UV-VIS Spectrophotometer. This approximates to a 1.5mL sample. A schematic of the instrument is shown in Figure 7.




$$A' = \varepsilon'_X.b.[X] + \varepsilon'_Y.b.[Y]$$
$$A'' = \varepsilon''_X.b.[X] + \varepsilon''_Y.b.[Y]$$

*Figure 7: UV/VIS Spectrophotometry method*

1. A solution mix of chemical A and B was introduced in the Vernier UV-VIS Spectrophotometer that is connected to a computer with the Logger Pro 3 software.

2. The solution mix was run in the program and absorbance data at each wavelength of light between 225-800nm were obtained.

3. A curve was obtained from Excel by using the absorbance and wavelength data obtained from the Logger Pro 3 software. Absorbance values are represented on the y-axis

and the wavelength data are represented on the x-axis. This curve shown above shows the solution mix curve and the curve for running each chemical independently.

## 8.3. HPLC APPARATUS AND OPERATING CONDITIONS

The HPLC method for BPA/BPS Analysis was developed by injecting 100 µL into Waters Nova-Pak C 18 Column 3.9x150 mm 60 Å 4µm with a mobile phase of water/acetonitrile (60:40) and a flow rate of 1.0 mL/min; Detector: UV (230 nm); Carrier Gas: Helium, 20 mL/min (non-continuous).

This method was used solely to validate the results of the UV/ VIS Spectrophotometry. All samples were also collected in triplicates to further ensure precision.

## 8.4. ITEMS USED FOR THE APPLICATION OF THE UV/VIS SPECTROPHOTOMETRY METHOD

When the UV/VIS Spectrophotometry method was fully developed, it was applied to several items to determine if our method could monitor the leaching of BPA and/or BPS from real-world samples (Figure 8).

Most of the items used were bought at a local Walmart store in Arkadelphia the day before they were used in the lab analytical experiments. But one of the items (P5 in Figure 8) had been in use for five years and another one (Item 13 in Figure 8) had been sitting for three weeks prior. The rest of the items were bought a day prior to the beginning of their use.


*Figure 8: Variety of items used in the application of the UV/VIS Spectrophotometry method*

## 8.5. PREPARATION OF SAMPLES USED IN THE ITEMS

Items used in this project consisted of plastics, food cans and thermal receipt paper. For each of these items, samples were prepared in triplicates according to the following method to ensure good results. The choice of using a 1:1 methanol:water solution was to help increase solubility and to match solvents used in previous experiments (Rachel and Bailey's theses). They were specifically used because of their polarity, affordability, and simplicity. As polar solvents, these chemicals can dissolve BPA and BPS.

In plastics, a 200mL 1:1 methanol:water solution was added in each plastic and monitored according to the different time and temperature conditions assigned. A sample was later taken from the solution and used in the UV/VIS spectrophotometer.

The thermal receipt paper was placed in a 200mL 1:1 methanol:water solution that was monitored according to the different time and temperature conditions assigned. To determine how much BPA and/or BPS leached out, the paper was filtered out and a sample was taken from the solution and used in the UV/VIS spectrophotometer.

For food cans, two different experiments were performed. The first experiment consisted of removing the contents of the food cans and cleaning the inside and then adding a 200mL 1:1 methanol:water solution that was monitored according to the different time and temperature conditions assigned. A sample was later taken from the solution and used in the UV/VIS spectrophotometer. The second experiment consisted of filtering out the solid particles in the food content and taking a sample from the filtered mix of food content and determining how much of BPA and/or BPS was in that solution.

## 8.6. ASSIGNED TIME AND TEMPERATURE CONDITIONS

In order to simulate the use of the items in Figure 8 (on page 26), different conditions of time and temperature were used throughout the experiments. The conditions of time (0–2 weeks) and temperature (22-70°C) varied from experiment to experiment.

All the items were grouped in five different groups (Group I – Group V). Each group had items that are quite similar in their use. Their uses or functions resulted in the same set of conditions of time and temperature being applied to the members of the group during experimentation.

For Group I consisting of plastics P1-P9 (Figure 8 on page 26), the following varying conditions of time and temperature were applied:

- The 1:1 methanol:water solution was prepared then preheated to 70°C in a beaker. Then it was poured in the plastic item after which the plastic was placed and kept in an oven at 70°C for 2hrs. A sample was taken out of the solution from the plastics after the time was done and used in a spectrophotometer.
- The previous plastic and solution were kept in the oven at 70°C for 3 days. Then a second sample was taken out of the solution to see if there was an increase of leaching chemicals over time. The sample was used in a spectrophotometer.

- The previous plastic and solution were then kept at room temperature (24°C) for 4days. Then a third sample was taken and used to determine if more leaching had occurred.
- Plastics were cleaned and a new 1:1 methanol:water solution was introduced in them. Then the plastics and solutions were then kept at room temperature (24°C) for 3days. Then a sample was taken and used to determine how much leaching had occurred.
- For this last experiment, some plastics were chosen that might be exposed to the sunlight for an afternoon while someone is outdoor for example when someone is playing sports and place their water bottle on the field so that (s)he can come back and drink some water when (s)he tired. The chosen plastics were placed on the window of the lab facing the sun for a weekend (It was during the summer and the temperature in that weekend varied between 32-38°C with the lab kept at room temperature or 24°C).

For Group II consisting of the thermal receipt paper or item #10 (Figure 8 on page 26), the following conditions of time and temperature were applied:

- the 1:1 methanol:water solution was prepared at room temperature (24°C) in a beaker where the thermal receipt paper was placed for 5hrs before the mixture was filtered and a sample was taken out of the filtered solution and used in a spectrophotometer.

For Group III consisting of food cans or items #11, #12, and #13 (Figure 8 on page 26), the following varying conditions of time and temperature were applied:

- The food content was preserved at room temperature (24°C) until a sample was taken after filtration of the content in one of the two sets of experiments. This was the first set of experiments that consisted of testing the original food content.
- In the second set of experiments which tested leaching from the food cans' lining to the 1:1 methanol:water solution introduced in the cans after the food content was removed, the solution of interest was monitored according to the following conditions:
    - ✓ The 1:1 methanol:water solution was prepared at room temperature (24°C) and after it was introduced in the food can, it was kept inside for 2hrs at room temperature before a sample was taken
    - ✓ This previous solution was left at room temperature (24°C) in the same can for 1day after the first sample was taken and before a second sample was taken to see if there was an increase of leaching chemicals over time.

For Group IV consisting of plastic P14 (Figure 8 on page 26), the following conditions of time and temperature were applied:

- The 1:1 methanol:water solution was prepared then preheated to 80°C in a beaker. Then it was poured in the plastic P14 after which the plastic was placed and kept in an oven at 80°C for 30mins to simulate how people usually take hot food in their take along and cover them with aluminum paper to keep the food hot until they can get home to eat it or place it in the refrigerator. A sample was taken out of the solution from the plastic after the time was done and used in a spectrophotometer.

For Group V consisting of plastics P15 and P16 (Figure 8 on page 26), the following conditions of time and temperature were applied:

- The 1:1 methanol:water solution was prepared then preheated to 70°C in a beaker. Then it was poured in the plastic P15 after which the plastic was placed and kept in an oven at 70°C for 30mins to simulate the average time people take to eat the food from their bowls. A sample was taken out of the solution from the plastic after the time was done and used in a spectrophotometer. The plastic P16 was placed meanwhile in the remaining preheated solution for 30mins to simulate using a fork while eating or using plastic cooking utensils to mix hot food.

# 9. DATA AND RESULTS

## 9.1. UV/VIS SPECTROPHOTOMETRY

The UV/VIS Spectrophotometry method was the main method used for the research project. This method is easy and affordable. It is very fast too because the detection time is under 1min. So if this method happened to be effective compared to the other more established methods such as HPLC, it would be such a successful project.

### 9.1.1. BPA AND BPS ABSORPTION SPECTRA

Absorption spectra tell us about the amount of light at a particular wavelength absorbed by a specific chemical. They are used in studies to determine and differentiate chemicals. For this method to be used, the chemicals have to be emitting light at different wavelengths, otherwise it is not such a great method to use.

BPA and BPS emit light at two different wavelengths. BPA's highest peak is seen at 272.3nm BPS's highest peak is seen at 255.1nm. This can be observed in the following figure representative of wavelengths at which BPA and BPS emit light.

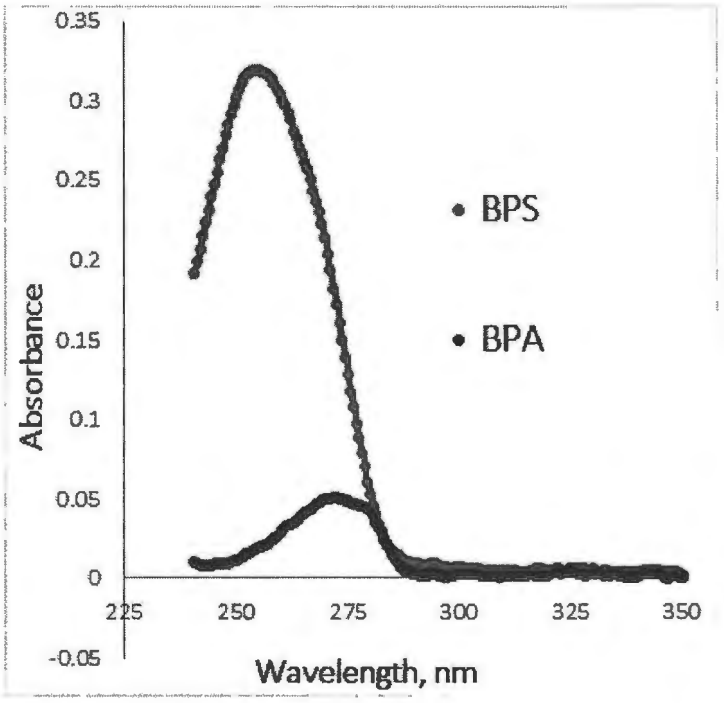



*Figure 9: 3.2 μg/mL BPA/BPS UV-VIS Absorbance Spectra in 1:1 methanol/water*

After these curves are obtained, they can then be used to calculate how concentrated our mixtures, and individual chemicals are.

## 9.1.2. BPA AND BPS CALIBRATION CURVES

Data were accumulated for BPA and BPS independently at different known concentrations of to obtain each absorption calibration curves. Even though the instrument could measure up to 2 absorbance units on the UV/VIS Spectrophotometer, high linear ranges for both chemicals were obtained.

Four calibration curves were derived from absorbance data at 272.3nm and 255.1nm for both BPA and BPS. The ranges at which BPA and BPS could be determined with accuracy were 0.48-120 µg/mL for BPA[16] and 0.06-20 µg/mL for BPS[17].

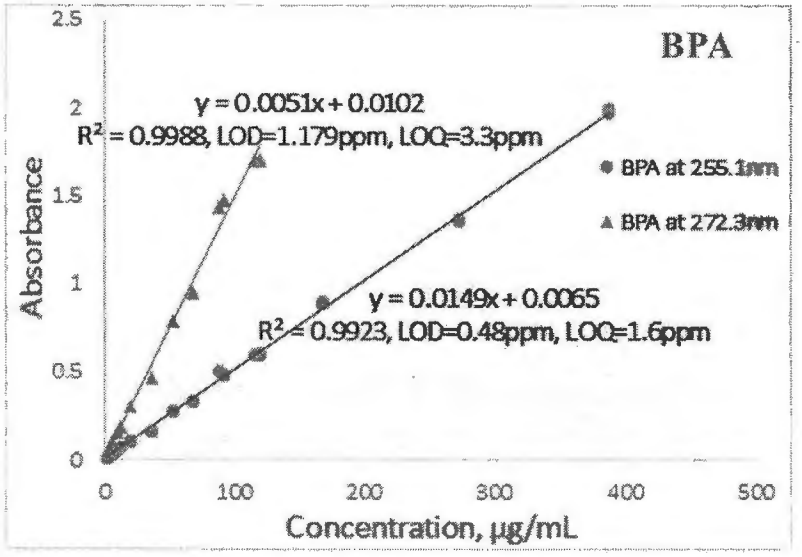



*Figure 10: UV/VIS Absorbance calibration curves of BPA*

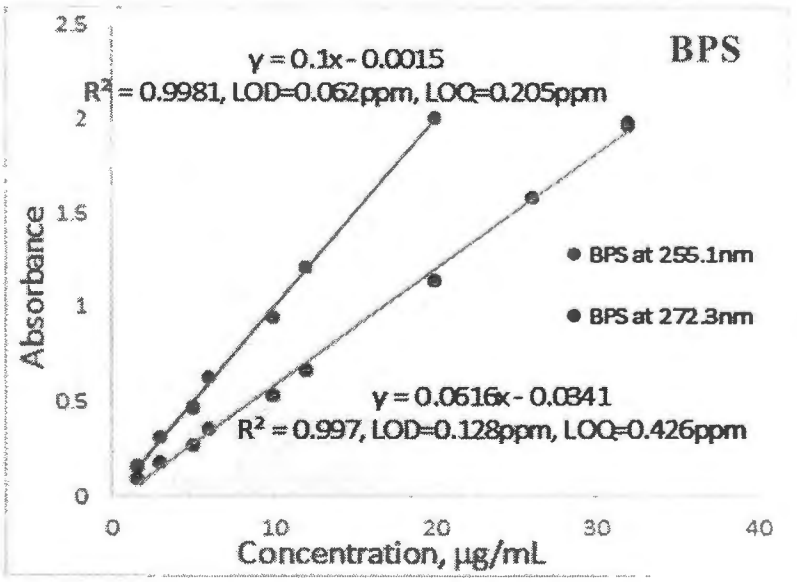



*Figure 11: UV/VIS Absorbance calibration curves of BPS*

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[16] *Figure 10: UV/VIS Absorbance calibration curves of BPA*

[17] *Figure 11: UV/VIS Absorbance calibration curves of BPS*

All the data in the calibration curves were obtained in triplicate, and the $R^2$ values, the limits of detection ($LOD_{BPA}$ at 272.3nm= 0.48μg/mL and $LOD_{BPS}$ at 255.1nm= 0.06μg/mL), the limits of quantitation ($LOQ_{BPA}$ at 272.3nm= 1.60μg/mL and $LOQ_{BPS}$ at 255.1nm= 0.21μg/mL), and the linear ranges (up to 120μg/mL at 273nm for BPA and up to 20 μg/mL at 255.1nm for BPS) showed that this method was precise, accurate and could be effective.

The slopes of the calibration curves were used to determine the molar absorptivities of BPA and BPS at the two wavelengths. The molar absorptivities[18] were the last missing pieces of the absorbance equations.

### 9.1.3. SYSTEM OF EQUATIONS

Concentrations of BPA and BPS in the mixture were calculated using this system of absorbance equations:

$$A' = \varepsilon'_{BPA} * b * [BPA] + \varepsilon'_{BPS} * b * [BPS]$$

$$A'' = \varepsilon''_{BPA} * b * [BPA] + \varepsilon''_{BPS} * b * [BPS]$$

Where A: Absorbance; ε: Molar Absorptivity; b: Cell size (b = 1cm);
[ ]: Concentration in μg/mL;
' : at 255.1nm; '' : at 272.3nm.

By inputting all the known data, the unknown concentrations of our chemicals could be found and compared to the calculated values to determine the effectiveness of the method.

## 9.2. HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

The HPLC method was used to validate the UV/VIS Spectrophotometry method. The HPLC is the most widely used and most effective analytical method. So this part of the

[18] Molar absorptivity is a measure of how well a chemical species absorbs a given wavelength of light.

research project was focused on analyzing how the UV/VIS Spectrophotometry method would compare to it.

## 9.2.1. BPA AND BPS HPLC CALIBRATION CURVES

Chromatography is a method that can be used used to separate a mixture into its components. HPLC is one of the major separation methods used in chemistry that can also allow for quantitation of samples. After multiple trials to adjust at the right retention time and wavelength at which we could see a visible separation of both BPA and BPS, we determined the right settings for our HPLC instrument for the preferred results. These settings are mentioned in the materials and methods section. (on page 25)

Multiple chromatograms were obtained to get HPLC calibration curves. HPLC calibration curves were obtained by preparing a range of known concentrations of either BPA or BPS, one at a time, and then getting all the resulting data plotted. The resulting data made a calibration line from plotting the peak areas against the known concentrations. (Figure 12). The line equation is used to calculate any unknown concentrations when given the peak area from the chromatogram.

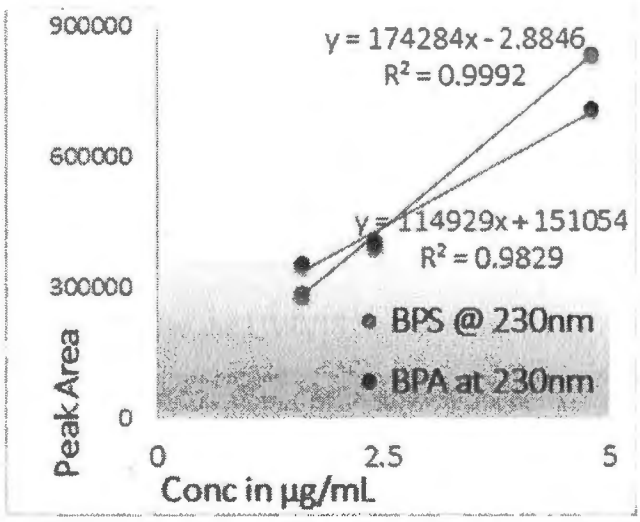



*Figure 12: HPLC calibration curves for BPA and BPS in 1:1 methanol/water*

### 9.2.2. BPA AND BPS SOLUTION MIXTURE HPLC CHROMATOGRAM

A representative chromatogram is shown in Figure 13. This figure shows clear separation between BPA and BPS curves. Using our HPLC settings mentioned in the materials and methods section (on page 25), we determined retention times at which BPA and BPS were detected to be 1.898min and 3.334min respectively. BPA and BPS were quantified after graphing the peak areas under each curve for each against the known concentrations that resulted in those peak areas. This resulted in calibration curves for each of the chemicals. The line equation for each chemical gave us a formula that relates peak areas and concentration. (Figure 12)

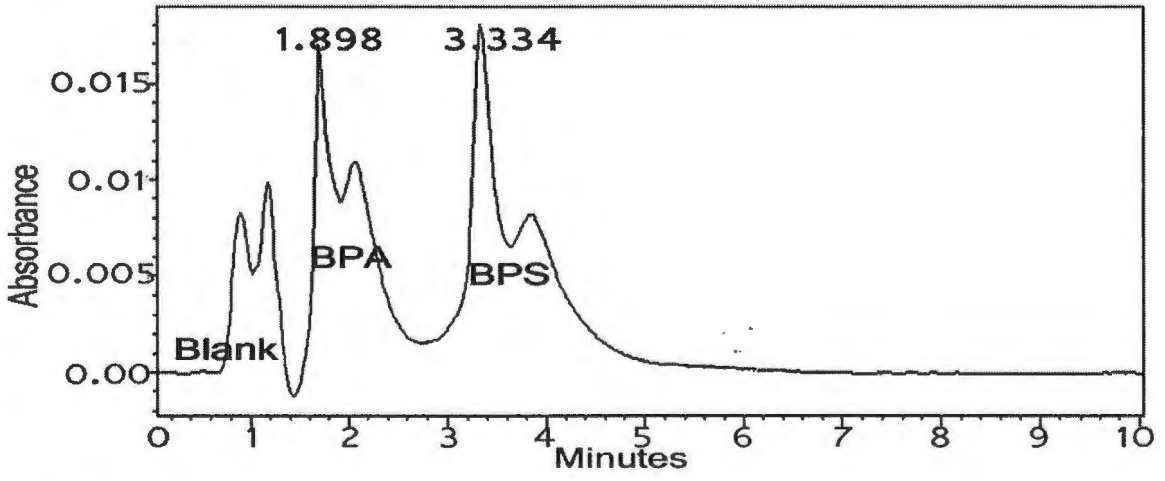



*Figure 13: HPLC Separation of a 3.2 µg/mL BPA and 3.2 µg/mL BPS solution mix in 1:1 methanol/water*

## 9.3. APPLICATION OF UV/VIS SPECTROPHOTOMETRY

We applied the UV/VIS Spectrophotometry method to validate effectiveness before using the method to calculate unknown concentrations of BPA and BPS that leach out from different items routinely encountered.

### 9.3.1. DETERMINATION OF KNOWN BPA AND BPS CONCENTRATIONS

A lot of data were obtained from the UV/VIS Spectrophotometry method before it was accepted as an effective analytical method (Table 1).

*Table 1: Comparison of actual and calculated [BPA] and [BPS] values and determination of the effectiveness of the UV-VIS Absorption Spectrophotometry method by statistical analysis in comparison to the HPLC method*

| | | CALCULATED | | PREPARED | % RSD | % | T test | CALCULATED | % |
|---|---|---|---|---|---|---|---|---|---|
| SAMPLE | | CONC , µg/mL | SD | CONC , µg/mL | CONC | ERROR | result | CONC , µg/mL | ERROR |
| #1 | BPA | 1.49 | 0.134 | 1.60 | 8.40 | 6.60 | NOT SSD | - | - |
| | BPS | 1.47 | 0.033 | 1.60 | 2.07 | 7.97 | SSD | - | - |
| #2 | BPA | 2.54 | 0.195 | 2.40 | 8.14 | -6.01 | NOT SSD | - | - |
| | BPS | 2.13 | 0.049 | 2.40 | 2.03 | 11.20 | SSD | - | - |
| #3 | BPA | 4.60 | 0.375 | 4.80 | 7.82 | 4.25 | NOT SSD | - | - |
| | BPS | 4.15 | 0.094 | 4.80 | 1.97 | 13.51 | SSD | - | - |
| #4 | BPA | 6.41 | 0.739 | 9.60 | 7.70 | 33.24 | SSD | - | - |
| | BPS | 8.80 | 0.197 | 9.60 | 2.05 | 8.38 | SSD | - | - |
| #5 | BPA | 2.65 | 0.281 | 3.20 | 8.79 | 17.26 | NOT SSD | 2.94 | 8.13 |
| | BPS | 2.93 | 0.067 | 3.20 | 2.11 | 8.45 | SSD | 3.13 | 2.19 |
| #6 | BPA | 85.07 | 2.412 | 97.90 | 2.46 | 13.11 | SSD | - | - |
| | BPS | 10.04 | 0.299 | 10.10 | 2.96 | 0.59 | NOT SSD | - | - |
| #7 | BPA | 58.02 | 1.864 | 65.30 | 2.85 | 11.16 | SSD | - | - |
| | BPS | 11.78 | 0.304 | 12.60 | 2.41 | 6.47 | NOT SSD | - | - |
| #8 | BPA | 9.82 | 0.757 | 11.20 | 6.76 | 12.30 | NOT SSD | - | - |
| | BPS | 8.48 | 0.192 | 8.81 | 2.18 | 3.70 | NOT SSD | - | - |
| #9 | BPA | 95.22 | 2.481 | 114.00 | 2.18 | 16.48 | SSD | - | - |
| | BPS | 8.75 | 0.286 | 8.40 | 3.41 | -4.13 | NOT SSD | - | - |

This table presents some sample data obtained from the UV/VIS Spectrophotometry method. Calculated or determined concentrations seem to be close to the prepared or theoretical concentrations.

Taking a closer look at the table shows us a more detailed analysis of how effective the method is. This method is precise. All data had a lower than 9% percent relative standard deviation (% RSD), of which more than half % RSDs were lower than 3% (The % RSD data >5% are highlighted in *green*). By comparing calculated to prepared concentrations, this method showed the extent of accuracy. The percent error was variable in the obtained data but still the concentrations were relatively close as half of the data were not statistically significantly different (all not statistically significantly different data are highlighted in *red*) and about half of the statistically significantly different (all statistically significantly different data are highlighted in *blue*) had percent errors lower than 10%.

So we used all this obtained data to show that our method's accuracy and precision supported the use for real world samples. In addition, this method is affordable and has a very low detection time (<1/2 min).

Sample 5 in Table 1 also shows how the UV/VIS Spectrophotometry data compared to the HPLC data that are shown in the right side of the table. When compared the data obtained from both methods are relatively close and they are both close to the prepared concentration value. The BPA and BPS prepared concentrations in the solution were 3.2μg/mL each. The calculated UV/VIS Spectrophotometry data for BPA and BPS in the solution were 2.65μg/mL and 2.93μg/mL respectively while the HPLC data for BPA and BPS in the solution were 2.94μg/mL and 3.13μg/mL respectively. BPA data in both methods were not statistically significantly different while BPS data were statistically significantly different even though they were much closer to 3.2μg/mL than the BPA data were.

### 9.3.2. APPLICATION TO REAL-WORLD SAMPLES

Scientific literature tells us that BPA and BPS chemicals have been linked to adverse health effects. These chemicals are used in the manufacture of several items we encounter daily and they sometimes leach out of those items and enter our digestive system. (on page 18). This next part of the research was aimed at using our developed method to prove whether these chemicals could leach out of items presented in Figure 8 (on page 26) or Figure 14 (on page 38).


*Figure 14: Variety of items used in the application of the UV/VIS Spectrophotometry method*

## 9.3.3. BPA AND BPS DATA OF THE DIFFERENT ITEMS

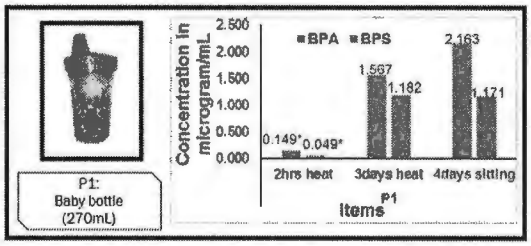
The UV/VIS Spectrophotometry method proved to be effective and so it was used to determine how much of the chemicals was leaching out of the items. Different ranges of data were determined. Most of the data gave us an expected trend. As time and/or temperature were increased, higher concentrations leached out of the items.

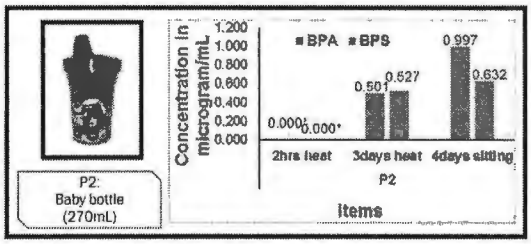
The five different groups[19] that were studied in this research project provided the following data.

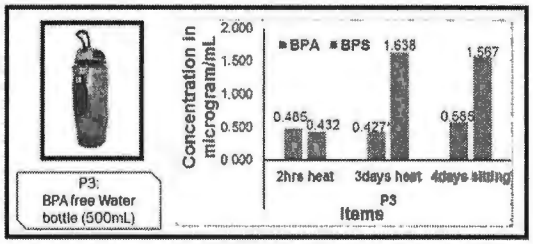
For Group I, two major sets of experiments were conducted. The first set of experimental data is shown below. In this set of data, a 1:1 methanol:water solution was

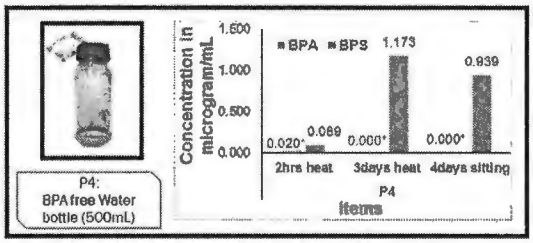
[19] Refer to the groups presented in Section 8.6: ASSIGNED TIME AND TEMPERATURE CONDITIONS (on page 14)

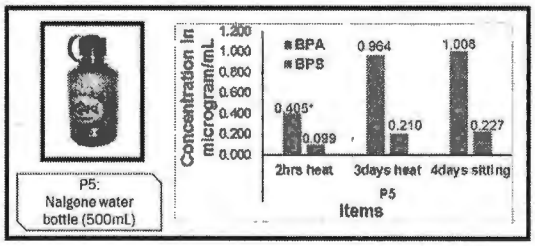
added to the different items and then samples were taken after being heated for 2 hrs, 3 days, and after sitting at room temperature for 4 days.[20]

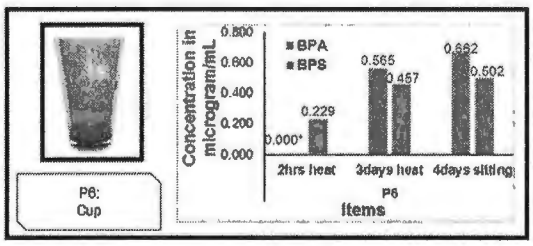


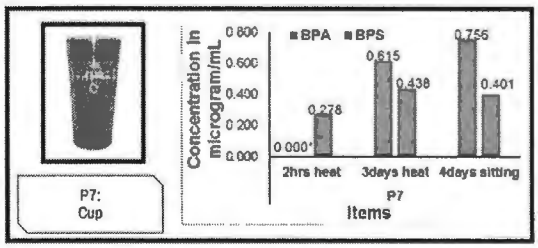



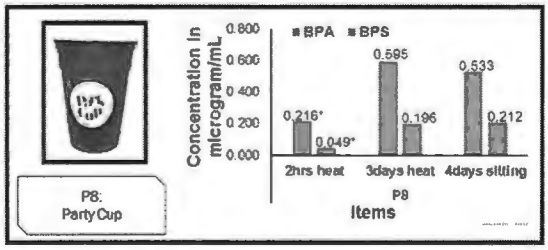



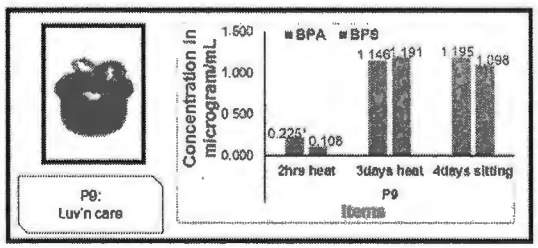




[20] More details of Group I experiments are found in Section 8.6: ASSIGNED TIME AND TEMPERATURE CONDITIONS (on page 14)

All data concentrations that were below the respective BPA and BPS LODs[21] are represented by a number followed by * in the figures.

These experiments were done in order to see if BPA or BPS would leach out and how much would leach out. These different data indicated that leaching was happening mostly at a detectable level. Leaching also occurred in a specific pattern. As time and temperature increased so did the leached concentration as expected. The two BPA free items showed the leaching of BPS that still followed the trend that as time and temperature increase, much concentration was leaching out. The rest showed increasing amounts of BPA leaching out. Most of the data in this set of experiments were above the LOD.

The second set of experiments with the Group I items consisted of taking a new 1:1 methanol:water solution that was added to the items and then placing both the solution and the plastic at room temperature for 3 days. Also a few selected items were exposed to the sun for 3 successive summer afternoons with a 1:1 methanol:water solution that was added to the items to see how much leaching would result.[22]

Here are the data for the second set of experiments with the group I items.

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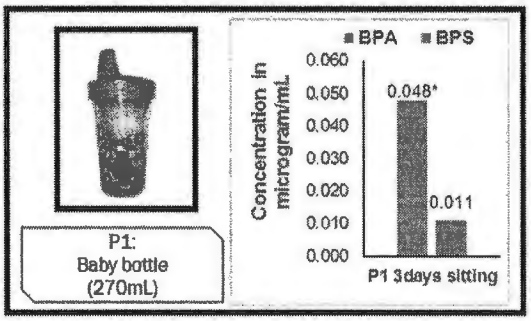
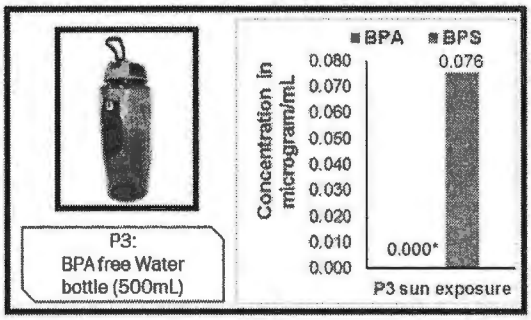
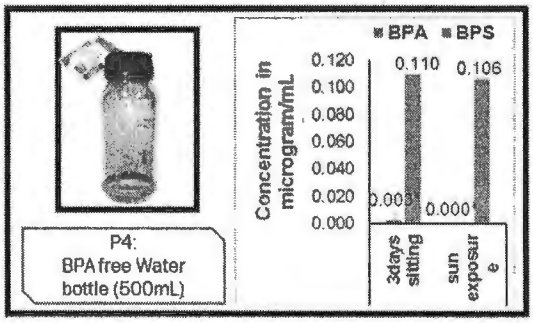
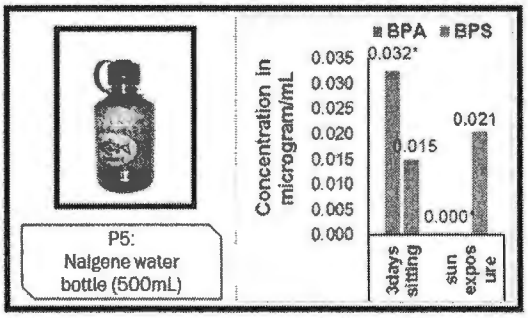
[21] LOD is Limit of detection or the concentration below which our instrument is not as effective compared to calculations with concentrations above the LOD. $LOD_{BPA}$ at 272.3nm= 0.48μg/mL and $LOD_{BPS}$ at 255.1nm= 0.06μg/mL

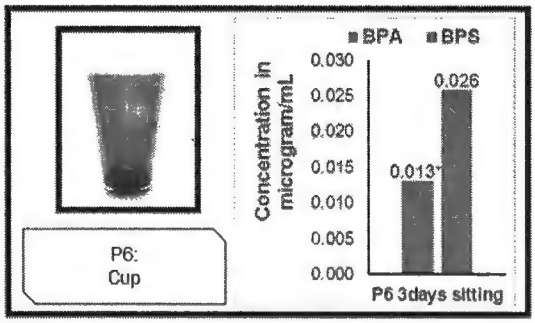
[22] More details of Group I experiments are found in Section 8.6: ASSIGNED TIME AND TEMPERATURE CONDITIONS (on page 14)

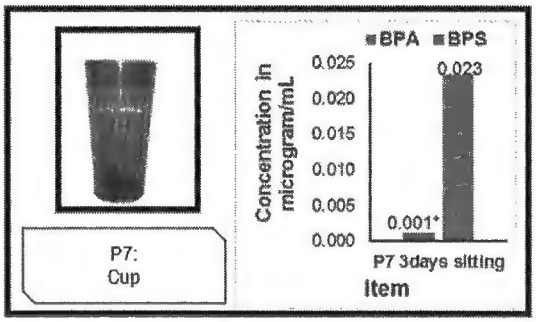


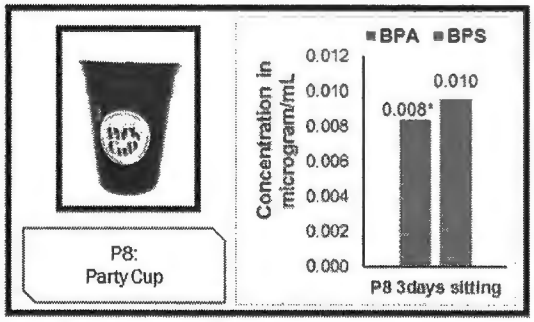



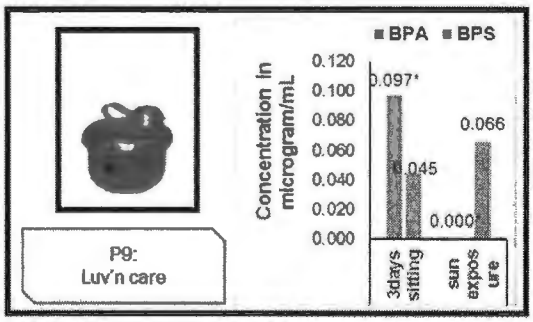



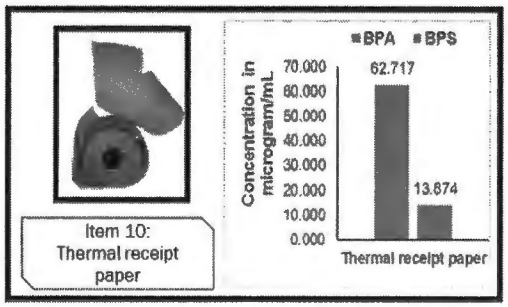




All data concentrations that were below the respective BPA and BPS LODs are represented by a number followed by * in the figures.

All the BPA obtained from this set of experiments were below the LOD.[23] This showed us that undetectable amounts of BPA had leached but because of a high $LOD_{BPA}$, we cannot tell efficiently if the amounts that the instrument measured were true values. Because the amounts were below the LOD, we can say that the leaching was very low. Also if one of the items or its contents are never exposed to heat, that would be the best way to reduce the leaching but unfortunately many people still heat the contents or directly apply heat on the items themselves.

For Group II, thermal receipt paper was soaked in a 1:1 methanol:water solution at room temperature in a beaker for 5 hours before filtration and the preparation of a sample were made. The following data were obtained.




These data proved that more BPA leach out of the thermal receipt paper as expected.[24] Even though no one usually soaks receipt papers directly into water, this shows that in case of bad recycling, these papers might end up in lakes and oceans where they can pollute water sources and come back in our systems. Also since BPS especially can enter our system by dermal penetration,[25] receipt papers can cause more problems to people who work in stores,

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[23] LOD is Limit of detection or the concentration below which our instrument is not as effective compared to calculations with concentrations above the LOD. $LOD_{BPA}$ at 272.3nm= 0.48μg/mL and $LOD_{BPS}$ at 255.1nm= 0.06μg/mL

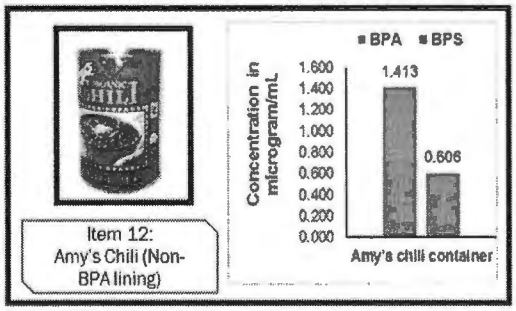
[24] Thermal receipt paper usually contains BPA which, when in contact with heat from the receipt printer, results into a black ink that is seen on receipt papers.

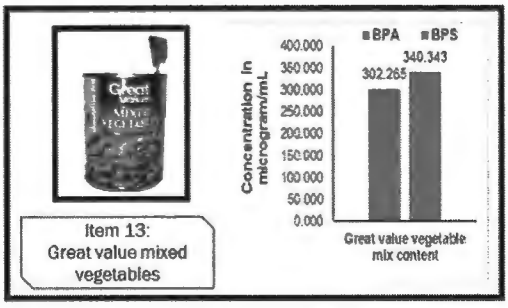
[25] For dermal penetration, refer to Section 7.1.2: SOURCES OF EXPOSURE (on page 9)

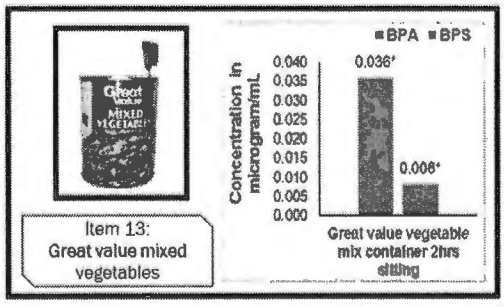
for example. and have to handle receipts all day. The paper used was the size of an average receipt paper (10cm x 6cm) that one might receive at any store to show how much could leach out. This method was not the most efficient way of determining leaching concentrations because it involved degradation of the paper that could cause more of the materials that make the paper mix with our chemicals of interest. Even though such mixed results were a risk, our curves were actual BPA and BPS curves as expected with no apparent noise.

For Group III consisting of food cans, two sets of experiments were conducted. The first consisted of using samples taken from the contents of the food can. The second consisted of introducing a 1:1 methanol:water solution in the food can after removing and cleaning the inside then waiting to determine how time affects the rate of leaching.

The data from the two sets of experiments are shown below. Data labeled as coming from containers mean samples were taken from the 1:1 methanol:water solution while the data labeled as content mean they came from the actual original food content from the food can.

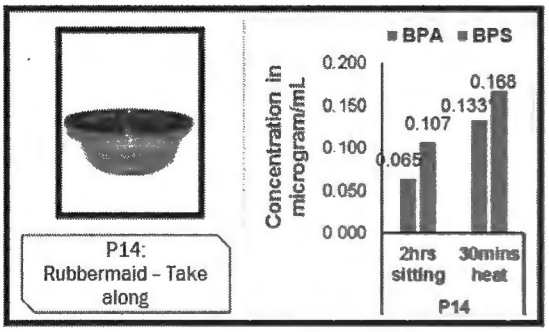


The 1:1 methanol:water added to the containers did not seem to capture detectable amounts of BPA or BPS in the Great Value mixed vegetables. However, detectable amounts were determined from both the Amy's chili container solution and the Great Value mixed vegetables original content. The original content was filtered but since it was made of food, a mix of different chemicals could have resulted in the calculations of the high concentration of BPA and BPS even though our curves were clearly BPA and BPS mixture curves.

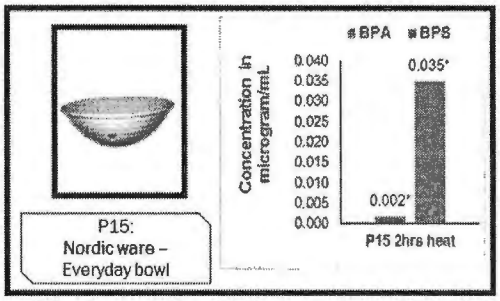
For Group IV consisting of P14, an experiment was set up to mimic typical use. Usually P14 is used to carry leftover food that is often still a little hot at the end of the meal. Some people will use that take along plastic and place it into the microwave to heat the food which increases the leaching as it is shown below.

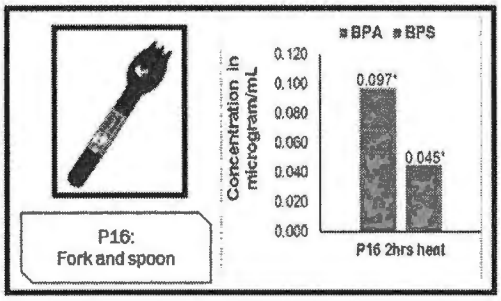



These experimental results show that even heating food for 30mins in the take along plastic will leach considerable amounts of BPA and/or BPS.

It is therefore advised to remove food or any take along contents from the container before one heats the food contents on a clean non-plastic plate.

For Group V consisting of plastics P15 and P16, these plastics were subjected to heat by either placing them in a hot 1:1 methanol:water solution in the case of P16 for 2 hours or by placing a hot 1:1 methanol:water solution in the item in the case of P15.




All the data from the above experiments were lower than the LODs.[26] This might be a result of having low concentrations that leach out or no leaching at all.

Overall, the experiments demonstrated the efficiency of the method even in case of real world samples. The UV/VIS Spectrophotometry is an affordable method and it saves time. The best part of all is that when compared to the HPLC method, the most widely used and trusted analytical chemistry method, it showed quite similar efficacy.

# 10. CONCLUSION

This research project consisted of developing a method that could simultaneously or independently determine BPA and BPS in a solution. The developed method was the UV/VIS Spectrophotometry method that uses light absorption and emission properties of chemicals of interest to determine how much of the chemicals are present.

In the specific case of this research project, the UV/VIS Spectrophotometry was shown to be effective at determining BPA and/or BPS at great ranges that extended from $LOD_{BPA}$ at 272.3nm= 0.48μg/mL up to 120μg/mL at 273nm for BPA and $LOD_{BPS}$ at 255.1nm= 0.06μg/mL up to 20 μg/mL at 255.1nm for BPS for BPS and the limits of

[26] LOD is Limit of detection or the concentration below which our instrument is not as effective compared to calculations with concentrations above the LOD. $LOD_{BPA}$ at 272.3nm= 0.48μg/mL and $LOD_{BPS}$ at 255.1nm= 0.06μg/mL

quantitation are the following $LOQ_{BPA}$ at 272.3nm= 1.60μg/mL and $LOQ_{BPS}$ at 255.1nm= 0.21μg/mL.

High Pressure Liquid Chromatography method (HPLC) was used to validate the method. Calibration curves were obtained, and identical samples were run using both methods. It was found that very similar results were obtained with both HPLC and the UV/Vis method, which indicated that the new method was a reasonable option for the detection of a mixture of BPA and BPS.

There are benefits and limitations at using either of the methods. Benefits of using the UV/VIS Spectrophotometry include the fact that this method is very fast, affordable, user-friendly, and it is comparable to the HPLC method. The limitation is that it does not provide separation of chemicals. And in some mixtures with complex matrices, it needs to be coupled to other methods such the standard addition method[27] to provide efficiency in reading results. Benefits of using the HPLC method are better separation of chemicals and settings can be varied to produce the best separation on the chromatogram and that it is the most used in analytical chemistry. Cons are that HPLC instruments are expensive and data take a very long time to acquire. The HPLC instrument is also less user-friendly compared to the UV/VIS Spectrophotometer.

The UV/VIS Spectrophotometry method was used for testing real world samples. Results showed that there is a positive correlation between the concentration leached and the time and/or temperature change. This conclusion was expected mainly because BPA and BPS can diffuse better in methanol:water solution since they are polar molecules and like dissolves like.

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[27] The standard addition method is an analytical method in which different concentrations of a standard are added to an unknown solution to determine the unknown chemicals that make up the mixture.

After seeing how this method compare to the HPLC, plans are being made so that it can be used in further research to try to test more known and unknown samples and bring the limits of detection even lower while increasing accuracy and precision. This method can be applied to determine two closely related chemicals or any two different chemicals that can absorb and emit light at different wavelengths.

# 11. AUTHOR'S FINAL NOTE

BPA and BPS are chemicals that we are exposed to from a lot of different sources. The best way to limit and reduce the exposure to those chemicals is by carefully using hard plastic items, avoiding heating plastics with food or drink inside, and by not requesting receipts unless they are necessary.

These two chemicals accumulate in our system over time and have been linked to several health problems. And because they can act as hormones, they can induce cellular response change at low concentrations or even cause no cellular response at all.

The developed method during the research project presented in this publication can help determine the level of exposure. But prevention is always better than exposure. The world would be a better place with less unwanted chemicals getting into our organ systems. So limit your exposure to these chemicals.

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