CHAPTER 1 : INTRODUCTION

1.0 Introduction

1.1 Bisphenol A (BPA)

Bisphenol A (BPA) had been used in the plastics industry since 1940s and 50s. It is a building block of polycarbonate plastics often used in food and beverage containers, including baby bottles, and it is also used as an additive in other plastics. BPA is also a component of epoxy resins used for dental materials, including dental sealants, and lining of food and beverage containers as well as numerous other products (Steinmetz *et al*., 1997).

Besides, BPA is the highest volume chemicals produced worldwide with over six billion pounds produced each year (Burridge, 2003). It is used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and in many plastic consumer products including toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment and tubing, and consumer electronics (Wetherill *et al*., 2007).

Additional uses for BPA include items that we come in contact with daily at home and in the workplace including the coating of CDs, DVDs, electrical and electronic equipment, automobiles, sports safety equipment, thermal paper, recycled paper and carbonless paper often used in register receipts (Steinmetz *et al*., 1998).

Polymerized, BPA molecules are linked by ester bonds that are subject to hydrolysis when exposed to high temperatures or to acidic or basic substances (Steinmetz *et al*., 1998). Studies have shown that BPA can leach from polycarbonate plastics and from epoxy resins and other products in contact with food and drink, and as a result, routine ingestion of BPA is presumed (Markey *et al*., 2001). According to Wetherill *et al*. (2007), the BPA has been shown to leach from food and beverage containers, and some dental sealants and composites under normal conditions of use.

Studies have also determined that BPA can be measured in humans in serum, urine, amniotic fluid, follicular fluid, placental tissue, and umbilical cord blood. The levels of total BPA (free and conjugated) in human blood and other fluids are higher than the concentrations that have been reported to stimulate a number of molecular endpoints in cell culture *in vitro* (Wetherill *et al*., 2007).

In addition, BPA is the subject of hot debates regarding "low dose" toxicological effects (EFSA, 2008), i.e., effects at doses clearly below the present tolerable daily intake (TDI) of 0.05 μg/kg body weight (EFSA, 2006). If such effects were proven to be pertinent for human health, exposure to sources so far considered minor might become relevant. Dermal exposure to BPA from thermal printer paper might be such a source (Biedermann *et al.*, 2010).

1.2 Thermal Paper

The thermal or thermosensitive printers have been widely employed in our daily life. Their ease of use, convenience and fast throughput have encouraged a broad application base to be developed. Nowadays, it can be found in most supermarkets, restaurants, petrol stations, modes of public transport, fax, ATM units, laboratory and medical instruments such as spectrophotometer, electrocardiograph, etc. Thermal printing requires special type of thermal paper that used exclusively for fax machines, ATM receipts, store bills, bus tickets and in laboratory and medical instruments such as spectrophotometer, electrocardiograph, etc. (Jasuja and Singh, 2011).

Thermal papers were introduced by National Cash Register Company in 1968 using the color forming reaction between leuco dyes and co-reactants (NCR, 1969). The paper includes the printing ink covering the whole surface on the side to be printed. The colorant consists of a leuco dye, i.e., a molecule that can adopt two forms, one of which is colorless. On printing, a thermal head causes the components to melt and react with each other, causing the dye to become dark (Biedermann *et al.*, 2010).

When paper subjected to heat, it produce black color which is indicative of the presence of unsubstituted leuco dyes while presence of substituted fluoran compounds form different color(s) such as red, green, yellow, black (Muthyala, 2002). Accordingly, this relatively stable, inexpensive and fast process has been exploited in the thermal paper industry since the late 1960s (NCR, 1969).

1.3 Objective of Study

The aim of this study is to determine the concentration of BPA in thermal papers using the High Performance Liquid Chromatography (HPLC) equipped with a diode array detector.

CHAPTER 2 – MATERIALS AND METHODS

2.0 Materials and Methods

2.1 Chemical and Reagents

Bisphenol A, 99+% purity was obtained from Sigma-Aldrich, Inc. (St. Louis, USA). The stock solution was prepared by dissolving the appropriate amount of standards in Methanol before being stored at 4°C in the refrigerator. The solution was further diluted to yield the suitable working solutions with Methanol. All chemical solvents including Methanol and Acetonitrile used for HPLC operation were purchased from Merck Chemical Company.

2.2 Instrumentation

High performance liquid chromatography was performed using Shimadzu Prominence HPLC system equipped with a diode array detector was used for the analysis and separation. The HPLC system consist of DGU-20A3 Online Degasser, LC-20AD Solvent Deliver Unit, CTO-20AC column oven and SIL-20ACHT HPLC auto-sampler.

The detection wavelength was set starting from 190 to 400 nm where the BPA detected at 230 nm. The analytical column used was Chromolith® RP-8e column (100 mm x 4.6 mm, particle size 5µm). The column temperature at 40°C was controlled by column heater and the guard column used to protect the analytical column (20 mm x 4.6 mm, particle size 5µm).

The condition of HPLC parameter was optimized by low pressure gradient elution with mobile phase consisted of Acetonitrile-Water with 0.1% TFA (50:50, v/v). The total flow rate was maintained at 1.0 mL/min with 20 μL injection volume.

2.3 Sampling

10 printed thermal papers were collected from various thermal printers of automated teller machines, retail stores, petrol station, auto-pay parking machines, and credit and debit card machines.

2.4 Sample Extraction

100 mg pieces of samples were weighed and placed into Teflon capped vials. 10 mL of Methanol was further added into the vials before being sonicated at room temperature for 1 minute. The Methanol extracts was transferred into round bottom flasks by Pasteur pipettes and rotary evaporated at 50°C. The extraction step repeated to extract any residual BPA that not extracted in the first extraction. Both extract residues were combined and redissolved in Methanol. 1 mL aliquot of Methanol was further filtered via syringe equipped with 0.20 μm PTFE filter prior HPLC analysis.

In addition, a non-thermal paper that used as reagent blank was extracted and analyzed like thermal paper for control. The non-thermal papers that had being spiked with suitable amount of BPA standard also being prepared for recovery calculation purpose. All papers were further extracted and injected into HPLC system for analysis.

2.5 Method Validation

Method validation is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (ISO 17025, 2005). Validation of analytical methods is recognized as a potentially weak link in the quality chain of laboratories. The validation procedures need to be considered in the context of fitness for purpose and cost–benefit criteria (King, 2003).

In addition, method validation is critical to the application of any method, to ensure the viability of the method before the costly exercise of a formal collaborative trial and to provide evidence of the reliability of analytical methods if collaborative trial data are not available (Thompson *et al*., 2002).

2.5.1 Recovery

According to Chapman (2005), accuracy referred to the closeness of measured values, observations or estimates to the real or true value (or to a value that is accepted as being true. ASTM (2002) stated accuracy as the closeness of agreement between an observed value and accepted reference value.

Besides, the accuracy also can be measured by % Recovery where the formula as per below:-

% Recovery (% R) = Analytical Value x 100/ True Value

The typical and acceptable values of % Recovery is within the range 80-120 %.

CHAPTER 3 – RESULTS AND DISCUSSION

3.0 Results and Discussion

3.1 Sampling

10 printed thermal papers were obtained from various thermal printers of automated teller machines, retail stores, petrol station, auto-pay parking machines, and credit and debit card machines. Most of the samples were collected from the retail stores and automated teller machines. According to Biederman *et al.* (2010), the thermal paper was easily identified by its ability to turn dark upon heating.

3.2 Sample Extraction

100 mg pieces of samples had been selected to be the ideal weight of sample during extraction based on earlier experiments conducted by Mendum *et al.* (2011) and Biederman *et al.* (2010) on BPA content in thermal paper. Mendum *et al.* (2011) had used Ethanol to extract BPA from the paper, while Biederman *et al.* (2010) preferred the mixture of Methanol and water.

Double extraction had been performed on the thermal papers to increase the extraction efficiency where more than 99.0 % extraction efficiency recorded in the study. The extraction efficiency also being increased due to Methanol properties used compared to other solvents. The sonication process also further increased the efficiency since the step ensures all the BPA content in the thermal paper was extracted completely.

3.3 Sample Analysis

All samples were filtered first via syringe equipped with 0.20 μm PTFE filter prior HPLC analysis to remove any impurities and prevent HPLC column being clogged or contaminated.

The HPLC guard column also had the same function like the filter.

HPLC used had been selected to determine the BPA content in thermal paper instead of GC-FID in the work of Mendum *et al.* (2011). The condition of HPLC parameter was optimized by low pressure gradient elution with mobile phase consisted of Acetonitrile-Water with 0.1% TFA (50:50, v/v). The total flow rate was maintained at 1.0 mL/min with 20 μL injection volume. The detection wavelength was set starting from 190-400 nm where the BPA detected at 230 nm.

3.4 BPA Peak Identification and its Retention Time

The identification of BPA peak and its retention time had been performed using the BPA 1000 ppm standard. It is being prepared by dissolving the BPA solid in the Methanol before being stored at 4°C in the refrigerator. The standard solution then being subjected to HPLC and BPA peak was detected at 230 nm with retention time of 3.296 min. The chromatogram of BPA 1000 ppm standard peak was shown in the Figure 3.1 below.

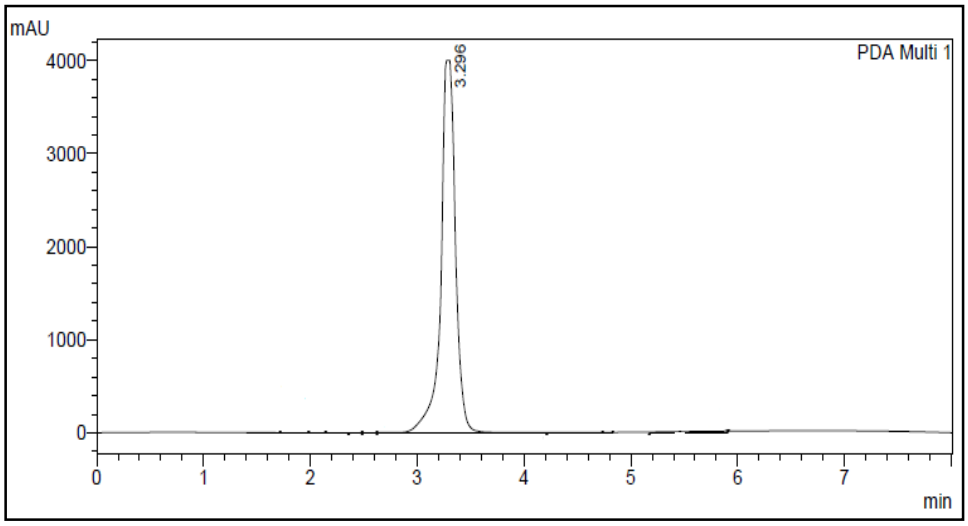

Figure 3.1: The chromatogram of BPA 1000 ppm standard peak and its retention time.

3.5 Sample Peak Identification and its Retention Time

All the thermal papers were subjected to extraction and HPLC analysis for BPA content determination. Most of the samples were found out contained BPA where the highest recorded from the credit card machine with 0.119 g/g BPA corresponded to 11.9 % of relative recovery. Meanwhile, the lowest BPA content obtained from the retail store machine with the value of 0.0842 g/g BPA and 8.4 % relative recovery.

Besides, the Automated Teller Machine 2 reported highest BPA content compared to other automated teller machines with 0.117 g/g BPA corresponded to 11.7% of relative recovery. In the other hand, BPA content was not detected in the thermal paper collected from the debit card machine.

The result of BPA concentrations in the thermal papers and non-thermal paper (control) summarized in the Table 3.1 for reference.

Table 3.1: BPA concentrations in thermal printing papers.

Source	**Retention Time**	**BPA (g/g)**
Auto-Pay Parking Machine	3.272	0.0904 $\pm$ 0.001
Credit Card Machine	3.271	0.119 $\pm$ 0.007
Debit Card Machine	-	n.d
Petrol Station Machine	3.262	0.111 $\pm$ 0.003
Retail Store Machine	3.265	0.0842 $\pm$ 0.002
Automated Teller Machine 1	3.246	0.116 $\pm$ 0.008
Automated Teller Machine 2	3.242	0.117 $\pm$ 0.007
Automated Teller Machine 3	3.240	0.112 $\pm$ 0.005
Automated Teller Machine 4	3.249	0.0951 $\pm$ 0.002
Automated Teller Machine 5	3.246	0.101 $\pm$ 0.009
A4 Paper (Non-thermal)	-	n.d

The chromatograms for each samples and its retention time can be referred below in Figure 3.2 to Figure 3.11.

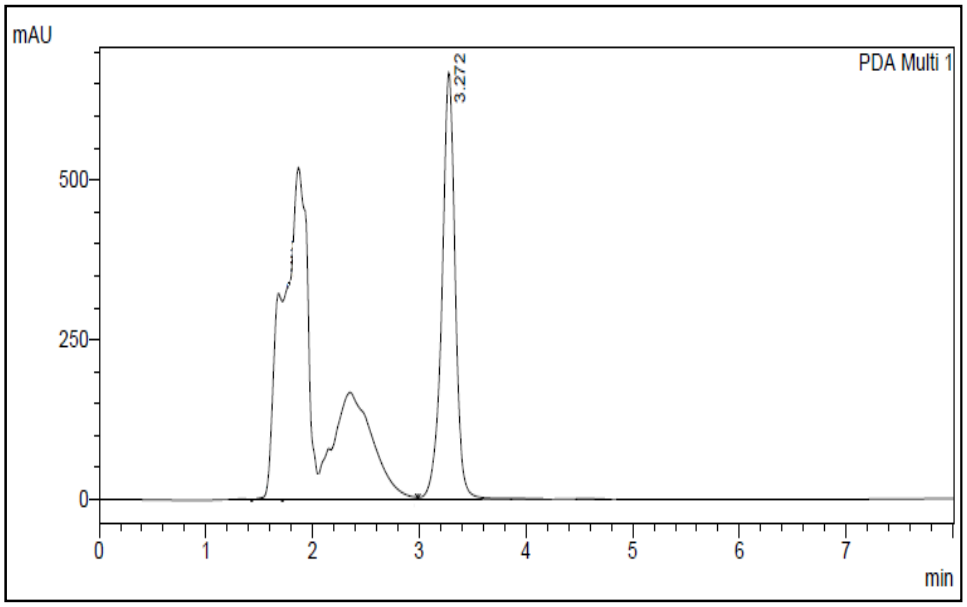

Figure 3.2: The chromatogram of Auto-Pay Parking Machine sample and its retention time.

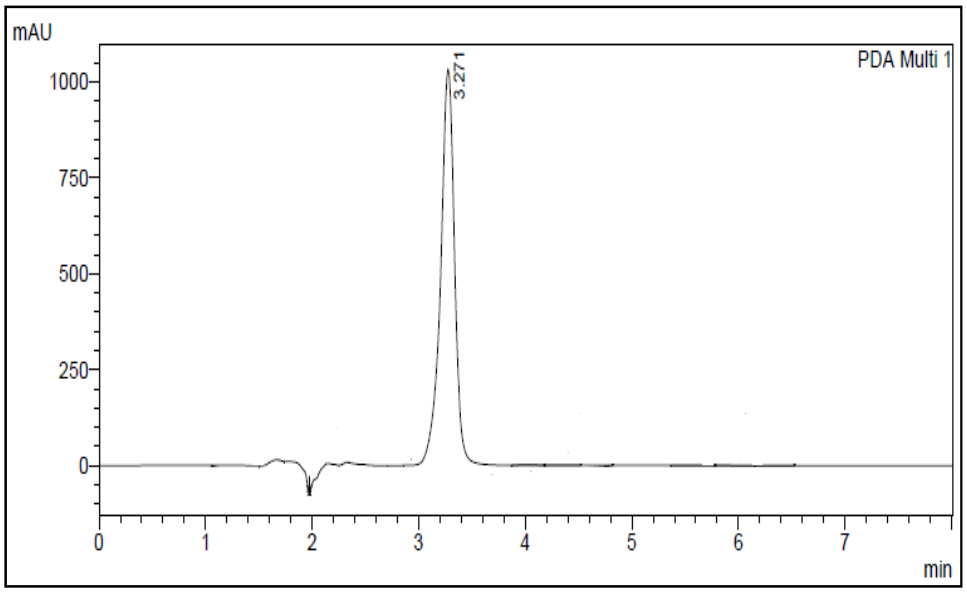

Figure 3.3: The chromatogram of Credit Card Machine sample and its retention time.

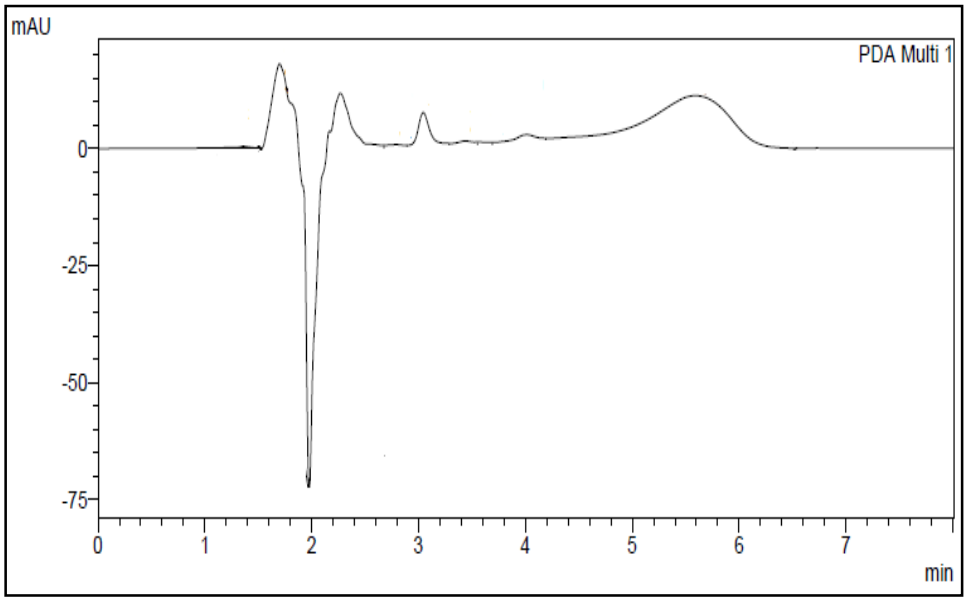

Figure 3.4: The chromatogram of Debit Card Machine sample.

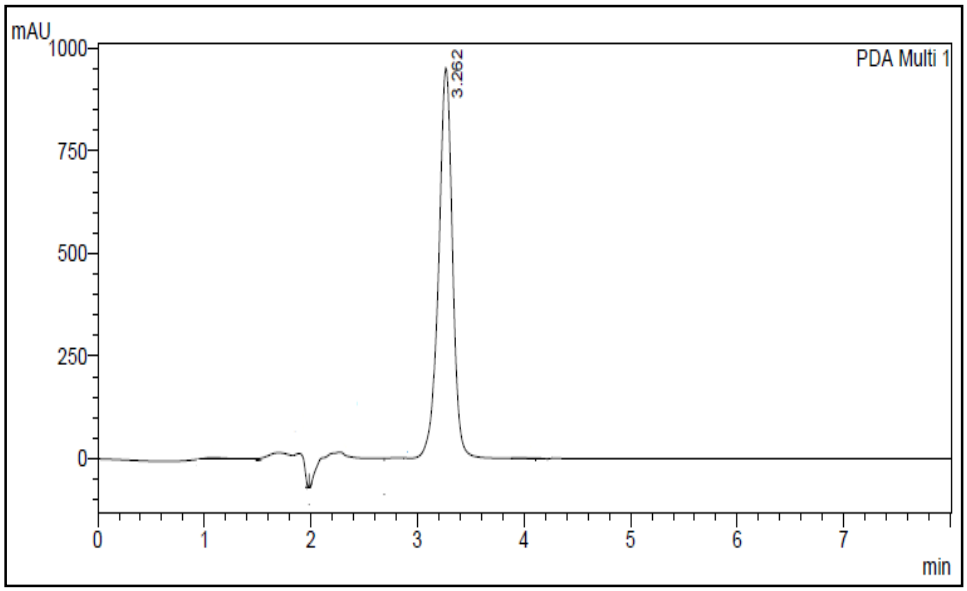

Figure 3.5: The chromatogram of Petrol Station Machine sample and its retention time.

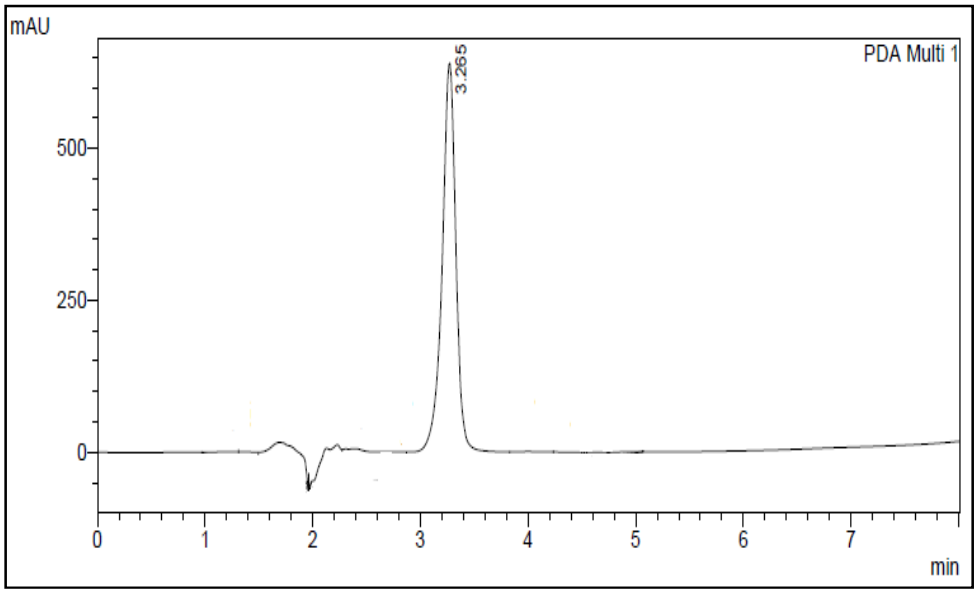

Figure 3.6: The chromatogram of Retail Store Machine sample and its retention time.

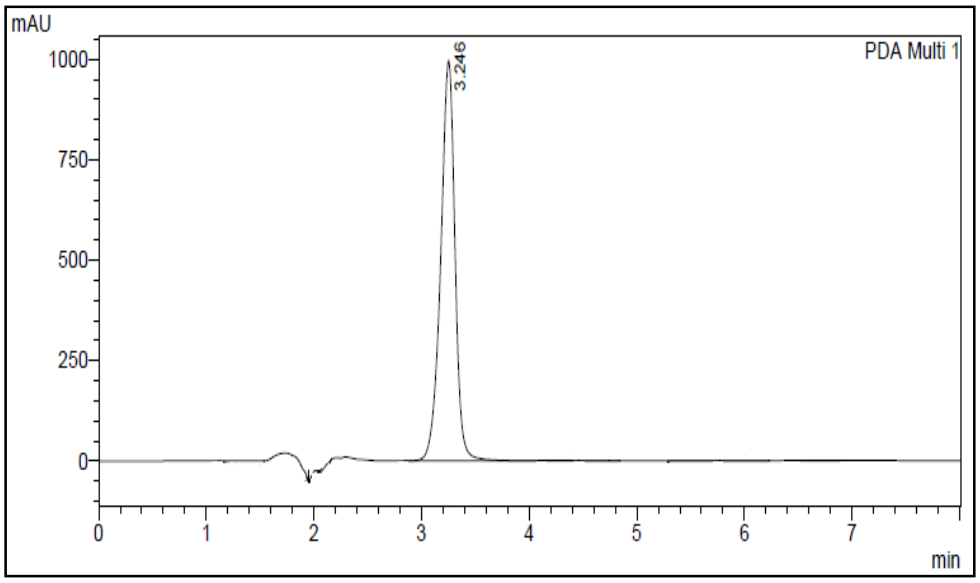

Figure 3.7: The chromatogram of Automated Teller Machine 1 sample and its retention time.

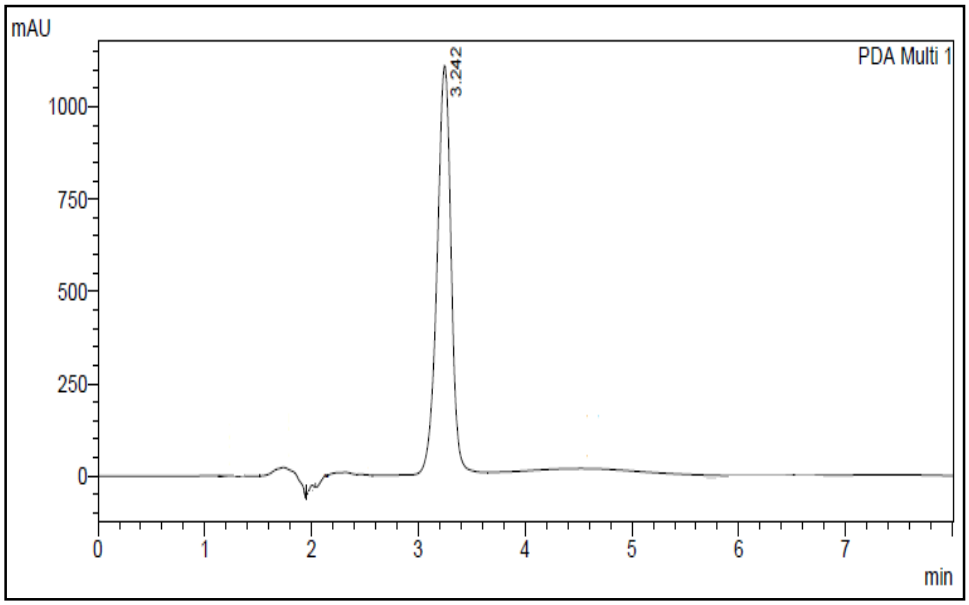

Figure 3.8: The chromatogram of Automated Teller Machine 2 sample and its retention time.

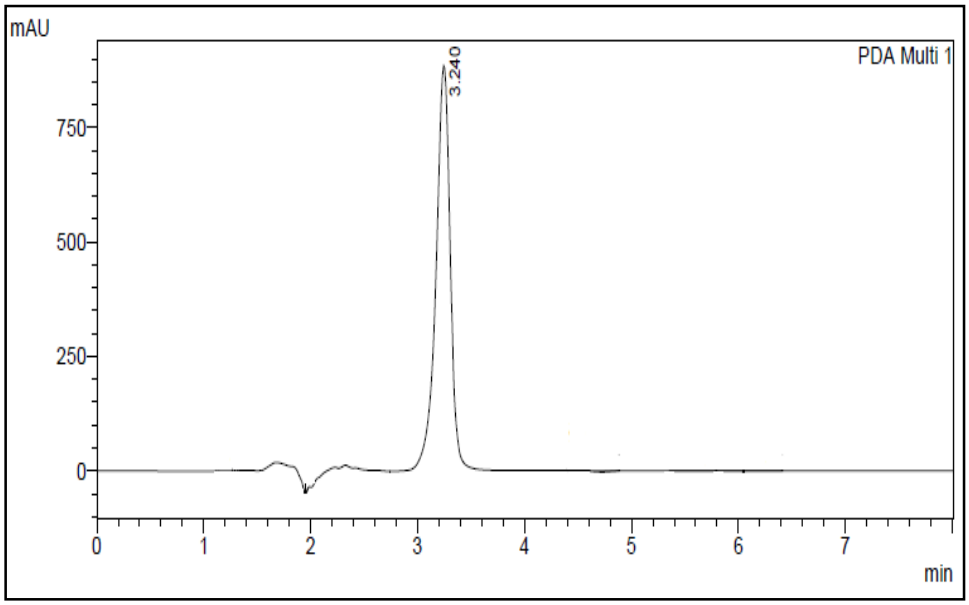

Figure 3.9: The chromatogram of Automated Teller Machine 3 sample and its retention time.

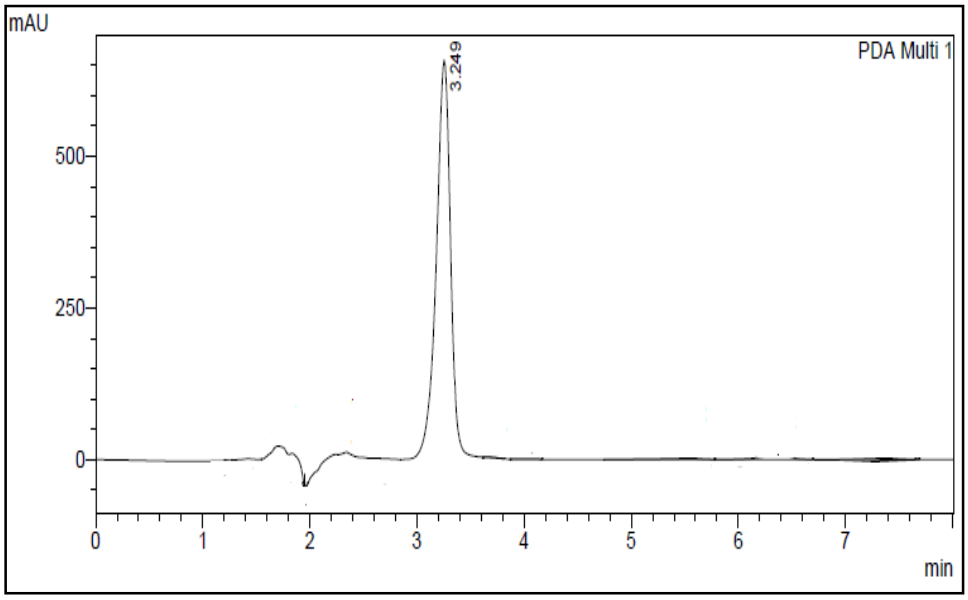

Figure 3.10: The chromatogram of Automated Teller Machine 4 sample and its retention time.

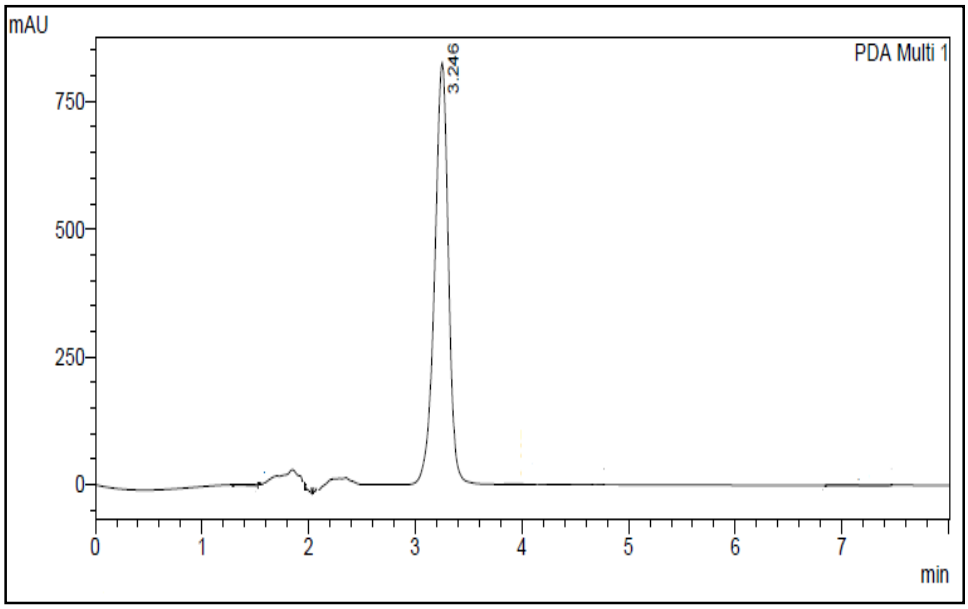

Figure 3.11: The chromatogram of Automated Teller Machine 5 sample and its retention time.

3.6 Method Validation

For validation purposes, a non-thermal paper that used as reagent blank was extracted and analyzed like thermal papers for control. The BPA content was not detected in that non-thermal paper where its chromatograms in the Figure 3.12 below.

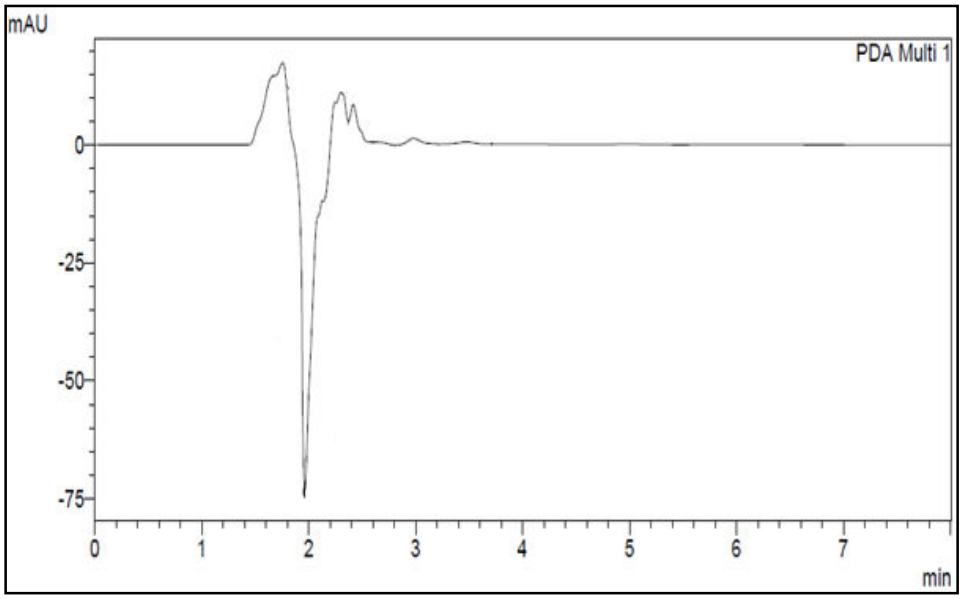

Figure 3.12: The chromatogram of A4 (Non-thermal paper) sample.

In addition, the non-thermal papers that had being spiked with suitable amount of BPA standard also being analyzed for recovery calculation purpose. The % Recovery recorded for spiking with 100 g/g and 500 g/g BPA standard solution were 107.0 % and 111.0% respectively. The chromatograms of the spiking results are shown in the Figure 3.13 and 3.14.

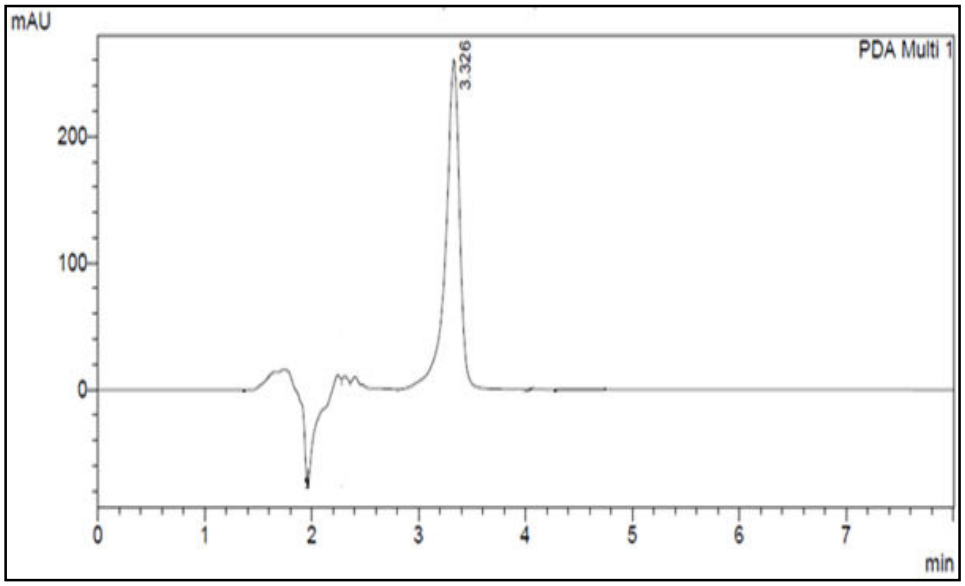

Figure 3.13: The chromatogram of spiking sample with 100 g/g BPA standard solution and its retention time.

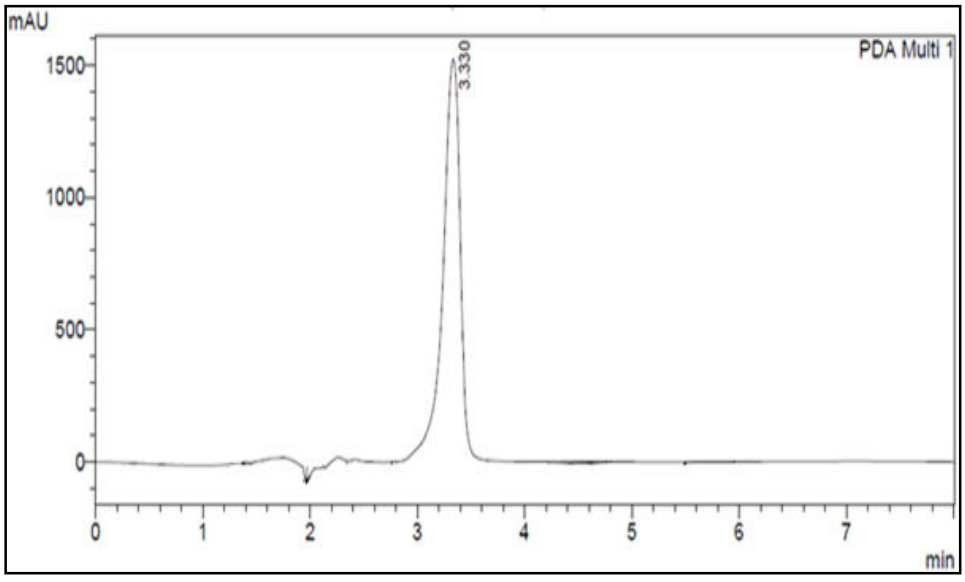

Figure 3.14: The chromatogram of spiking sample with 500 g/g BPA standard solution and its retention time.

CHAPTER 4 – CONCLUSION

4.0 Conclusion

In conclusion, most of the thermal papers that were obtained from various thermal printers contained BPA range from 0.0842 g/g to 0.119 g/g. The BPA content in thermal papers were detected by High Performance Liquid Chromatography (HPLC) equipped with a diode array detector. The highest BPA content recorded from the credit card machine with 0.119 g/g BPA corresponded to 11.9 % of relative recovery.

Meanwhile, the lowest BPA content obtained from the retail store machine with the value of 0.0842 g/g BPA and 8.4 % relative recovery. Besides, the Automated Teller Machine 2 reported highest BPA content compared to other automated teller machines with 0.117 g/g BPA corresponded to 11.7% of relative recovery. In the other hand, BPA content was not detected in the thermal paper collected from the debit card machine.

The extraction method used in this study including double extraction, solvent and sonication used increased the extraction efficiency more than 99.0 %. The non-thermal paper used as control and that paper had also being spiked with BPA standard for recovery calculation purpose. The % Recovery recorded for spiking with 100 g/g and 500 g/g BPA standard solution were 107.0 % and 111.0% respectively.