



Faculty of Resource Science and Technology

**METHOD DEVELOPMENT FOR ANALYSIS OF BISPHENOL A USING FOURIER
TRANSFORM INFRARED (FTIR)**

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(56380)**

**Bachelor of Science with Honours
(Resource Chemistry)
2019**

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UNIVERSITY MALAYSIA SARAWAK

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Final Year Project Report

Masters

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**Method Development for Analysis of Bisphenol A Using Fourier Transform Infrared
(FTIR)**

**LEE MEE YI
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An undergraduate thesis dissertation is submitted in partial fulfilment of the requirement for the degree of Bachelor of Science with Honours (Resource Chemistry)

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Method Development for Analysis of Bisphenol A Using Fourier Transform Infrared (FTIR)

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ABSTRACT

The monomer of plastic, bisphenol A (BPA) was found leached from polycarbonate bottle into the drinking water. The method of BPA analysis using Fourier Transform Infrared (FTIR) is not well studied, hence the objectives of the study are to develop the calibration curve for analysis of BPA using FTIR and to determine the method sensitivity. The leaching of BPA was tested for its factors of the duration and temperature by investigating the water sample in contact with the incorporated BPA-PET for 15, 30, 60, 120 mins and temperature at room temperature, 50 °C and 70 °C for 10 mins. The method of extraction used is liquid-liquid extraction (LLE) and the suitable solvent for BPA analysis is dichloromethane (DCM). The calibration curve for analysis of BPA using FTIR was developed and the detection limit of FTIR was 1.0 ppm. The leaching of BPA increased with the duration of time and temperature.

Key words: Bisphenol A (BPA), Fourier Transform Infrared (FTIR), dichloromethane (DCM), liquid-liquid extraction (LLE), leaching factors.

ABSTRAK

Monomer plastik, bisphenol A (BPA) didapati meluntur dari botol polikarbonat ke dalam air minuman. Kaedah analisis BPA dengan menggunakan Fourier Transform Infrared (FTIR) belum dipelajari dengan baik, oleh itu objektif kursus ini adalah untuk membangunkan kurva penentuan bagi analisis BPA dengan menggunakan FTIR dan untuk menentukan kepekaan kaedah. Pelepasan BPA diuji untuk faktor-faktor jangka masa dan suhu dengan menyiasat sampel air yang berinteraksi dengan BPA-PET selama 15, 30, 60, 120 minit dan suhu pada suhu bilik, 50 °C dan 70 °C selama 10 minit. Kaedah pengekstrakan yang digunakan adalah pengekstrakan cecair-cecair dan pelarut yang sesuai untuk analisis BPA adalah diklorometana. Kurva penentuan bagi analisis BPA menggunakan FTIR dikembangkan dan had pengesanan untuk FTIR adalah 1.0 ppm. Pelepasan BPA telah meningkat dengan peningkatan tempoh masa dan suhu.

Kata kunci: Bisphenol A (BPA), Fourier Transform Infrared (FTIR), diklorometana, pengekstrakan cecair-cecair, faktor pelepasan

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List of Abbreviations

ANOVA	analysis of variance
ATR	attenuated total reflection
DCM	dichloromethane
DFT	density functional theory
ELISA	enzyme-linked immunosorbent assay
FTIR	Fourier Transform Infrared
GC-FID	Gas Chromatography Flame Ionization Detection
GC-MS	Gas Chromatography Mass Spectrometry
HDPE	high density polyethylene
HPLC-DAD	High Performance Liquid Chromatography - Diode-Array Detection
HPLC-FD	High Performance Liquid Chromatography - Fluorescence Detection
KBr	potassium bromide
LLE	liquid-liquid extraction
MeOH	methanol
NH ₄ OH	ammonium hydroxide
PC	polycarbonate
PET	polyethylene terephthalate
SCF	scientific committee on food
SPE	solid-phase extraction
SPI	society plastic industry
TDI	tolerable daily intake

Chapter 1.0 Introduction

Bisphenol A (BPA) is an organic synthetic compound with the chemical formula $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OH})_2$ which contain a group of diphenylmethane and two hydroxyphenyl. The chemical structure of BPA is shown in Figure 1. It is a colorless solid that is soluble in organic solvents but poorly soluble in water.

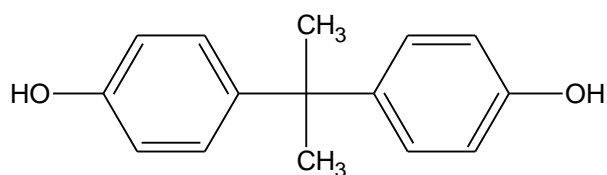


Figure 1: Molecular structure of bisphenol A.

According to Rykowska *et al.* (2005), BPA is widely used in the production of epoxy resin and polycarbonate plastic. It was treated as a non-toxic compound for many years with no negative impacts on humans and animals. BPA-based products were commonly used as vessels for storage of food, drinking water and medicines. BPA is latter found as a xenobiotic endocrine disruptor that hinders the balance of hormonal system in human and animal (Rykowska *et al.*, 2005).

Plastic bottles are labelled according to the resin coding system of 1-7 by the Society Plastic Industry (SPI). The number indicates the type of plastic used and determines whether the plastic is recyclable and reusable. The health issue concerned is the migration of BPA from polycarbonate water bottles to the drinking water (Cooper *et al.*, 2011). Polycarbonate bottles are labelled number 7 according SPI. BPA is the monomer used in manufacturing of polycarbonate bottles. Nowadays, the marketed polycarbonate bottles are replaced with polyethylene terephthalate (PET), labelled as 1. This is mainly aim to mitigate the issue concerning leaching of BPA.

The leaching of BPA can take place in canned food sealed with epoxy resin (LaKind, 2013). The epoxy-based resin is the most common type of resin used in canning. It is produced by condensation of epichlorohydrin and BPA yielding bisphenol A diglycidyl ethers (LaKind, 2013). The consumption of contaminated foods and drinks exposure one to accumulation of BPA in the body. Scientific Committee on Food (SCF) declared that the tolerable daily intake (TDI) of BPA is 0.01 mg/kg body weight (BW)/day (Rykowska *et al.*, 2005).

The instrumental method used in the analysis of BPA are chromatography technique. Gas Chromatography (GC) with Mass Spectrometry (GC-MS), Flame Ionization Detection (GC-FID) and High Performance Liquid Chromatography (HPLC) with Fluorescence Detection (HPLC-FD), Diode-Array Detection (HPLC-DAD) are the analytical instruments that commonly used for the analysis of BPA determination. The detection limit of the GC-MS is 0.2 ng/L (Elobeid *et al.*, 2012) and GC-FID is 0.3 µg/L (Rykowska *et al.*, 2005). For the HPLC- FD and HPLC- DAD, the detection limit are 0.3 ng/L (Omer *et al.*, 2016) and 0.36 mg/L (Rosa *et al.*, 2018) respectively. Besides, the determination of BPA also performed by using the nanostructure ionic liquid modified sensor which are the voltametric sensor with detection limit of 0.04 µM (Nikand & Khalilzadeh, 2016).

The problem statement is the BPA was determined using FTIR by Ullah *et al.* (2016) however, the method sensitivity is not well studied. Therefore, the objectives of the study are to develop the calibration curve for analysis of BPA using FTIR and to determine the method sensitivity.

Chapter 2.0 Literature Review

2.1 Extraction of BPA

2.1.1 Solid-phase extraction

In the study of Rykowska *et al.* (2005), a new sorbent for solid-phase extraction was used in BPA determination of drinking water. The sorbent was chemically bonded ketamine groups and these sorbents have better chromatographic properties compared to the common C18 sorbent. The detection limits of this technique was approximately 0.3 µg/L and the recovery results was in the range of 92.6% to 97.3%. The sorbent was prepared by immersing the dry silica in a mixture of anhydrous xylene and 3-aminopropyltriethoxysilane and boiled the mixture for 12 hours. The xylene and hexane were used to extract the unreacted silane. Next, the extractant was dried under vacuum and exposed to hexamethyldisilazane as the “end capping” reaction. After that, the amino groups was bonded using a derivative of 3-pentano-2,4-dione. The reaction was performed in anhydrous xylene for 12 hour under continuous stirring. The final product was extracted and the silica was dried under vacuum. The solid phase extraction was performed on the columns with C18 sorbent and silica modified with ketoimine groups. Before introducing the samples, the columns were conditioned by 5 mL of a mixture of the methyl chloride and methanol which in 1:1 v/v ratio, 5 mL of methanol, and 15 mL of deionized water. The conditioned columns were loaded with 500 mL of water sample. After sample loading, the sorbent was dried for 10 minutes under vacuum and 3 mL of methanol was used to elute the pre-concentrated compounds. The samples were measured by using Gas chromatography equipped with Flame Ionization Detector (GC-FID) (Rykowska *et al.*, 2005). Mead and Seaton (2011) stated that the SPE offers the advantages of minimizing the organic solvent used, selectively partitioning the analytes from aqueous phase by

using different solid phases and large volume of sample can be passed through the SPE column.

2.1.2 Liquid-liquid extraction

Elobeid *et al.* (2012) investigated BPA in various brands of drinking bottled water in Riyadh, Saudi Arabia. Seven brands bottled water were extracted using liquid-liquid extraction strategy. Dichloromethane (DCM) was used in the liquid-liquid extraction and the extract was concentrated under a gentle stream of nitrogen. The analysis of the BPA in water sample was conducted using Gas Chromatography/Mass Spectrometer (GC-MS). The recovery was in the range of 79 – 94%. The result of analysis showed high concentration of BPA in all seven brands of bottled water with the mean concentrations of 4.03 ng/L and 7.5 ng/L at 25 °C indoor and 40 °C outdoor, respectively (Elobeid *et al.*, 2012).

2.2 Analysis of BPA

The chromatography technology which has a higher sensitivity is commonly used in the analytical analysis of BPA. In the determination of BPA using GC-MS, Elobeid *et al.* (2012) used the capillary column Elite-5-MS (30 M x 0.25 mm, 0.5 µm film thickness) for separation and the helium as the carrier gas with constant flow rate at 0.1 mL/min. The temperature for both ion source and quadrupole was set at 150 °C. The injection of 1 µL sample was in splitless mode at an inlet temperature of 300 °C. The calibration standard curve with concentration ranging from 0.2 to 1.0 ng/L was used in the concentration determination. Mead and Seaton (2011) used the GC-MS with DB-5 column (J&W Scientific DB-5 with 0.25 µm phase, 0.25 mm i.d., 30m) and the

concentration of the calibration curve was ranging from 10 to 250 ng/ μ L.

In the study of BPA determination using HPLC, Sadeghi *et al.* (2016) used the HPLC Knauer with Chromgate software and a multi-wavelength fluorescence detector. The separations were done using H5-ODS C₁₈ column (15 cm x 4.6 mm, with 5 μ m particle size) from Anachem. The mobile phase used was the mixture of water and acetonitrile in the ratio of 55:45 at a flow rate of 1 mL/min in isocratic elution mode. The detection wavelength were 224 nm for excitation and 308 nm for emission. Omer *et al.* (2016) analyzed the standards and water samples with a Perkin Elmer Flexar FX-10 HPLC system with a fluorescence detector. Separations were carried out on Mini Column (6.0 \times 1.0 cm i.d.). The column used in the HPLC system was Perkin Elmer C8 (150 mm x 4.6 mm, 5 μ m) and the mobile phase used was the mixture of acetonitrile and water with the ratio of 60:40. The detection limit of this method was 0.30 ng/mL.

Besides using GC-MS and HPLC, there are some electrochemical and voltammetric sensor used for BPA determination. In the study of Deng *et al.* (2014), the electrochemical sensor based on an acetylene black paste electrode modified with molecularly imprinted chitosan–graphene composite film was developed for a sensitive and selective detection of BPA. The modification of the imprinted electrochemical sensor was successfully used to detect BPA in plastic bottled drinking water.

According to Ullah *et al.* (2016), the functional groups used for BPA determination in FTIR spectroscopy are C-H and O-H stretching. A complementary FTIR spectroscopic study of BPA was presented to understand the behavior of BPA molecule. The peaks of BPA molecule were calculated based on the Density Functional Theory (DFT) from 400 cm^{-1} to 4000 cm^{-1} with configuration B3LYP 6-311G++ (3df 3pd). The calculated absorption peaks agree reasonably well

with the experimental peaks after scaling with scale factor of 0.9679, except C-H and O-H stretching vibrations (Ullah *et al.*, 2016).

2.3 Factors affecting the leaching of BPA

Cooper *et al.* (2011) studied about the BPA contamination of water occurred in the different types of reusable drinking bottles, marketed as alternatives to BPA-containing polycarbonate plastics. The reusable plastic, aluminum and stainless steel water bottles were evaluated whether BPA migrated into water using a sensitive and quantitative BPA-specific competitive enzyme-linked immunosorbent assay (ELISA). At room temperature the concentration of BPA migrating from polycarbonate bottles ranged from 0.2 to 0.3 mg/L. Under identical conditions BPA migration from aluminum bottles lined with epoxy-based resins vary depending on the manufacturer where the concentration ranges from 0.08 to 1.9 mg/L. Leena *et al.* (2016) stated that the migration of BPA from polycarbonate to the water is encouraged by increased acidity and temperature, as well as the mechanical cleaning and cleaning using detergents.

2.3.1 Effect of temperature

The experiment carried out by Leena *et al.* (2016) showed that the concentration of BPA present in water exposed to direct sunlight was in range from 7.90 to 16.85 ng/mL which is higher than the water samples stored at room temperature in range from 3.10 to 6.24 ng/mL. Elobeid *et al.* (2012) also proved that the increased temperature promote the leaching of BPA, the concentration of BPA in water increased from 4.03 ng/L (indoor storage) to 7.5 ng/L (outdoor storage). Based

on Dornath (2010), the BPA in water is difficult to be detected if the water is stored at room temperature (24 °C) or the temperature of dishwasher (55-65 °C). However, when the polycarbonate bottles is exposed to an autoclave at 120 °C for 2 hours, BPA was found to leach in detectable amounts (> 10 ppb).

2.3.2 Effect of storage duration

Fan *et al.* (2014) studied the effect of storage temperature and duration on leaching of BPA from polyethylene terephthalate drinking water bottles. The effect of storage temperature was investigated at temperature 4 °C, 25 °C and 70 °C for 7 days and the effect of storage duration was conducted at 70 °C for 1, 2 and 4 weeks. The median value of the BPA concentrations in 16 brands of polyethylene terephthalate (PET) bottles was 7.27 ng/L, 10.44 ng/L and 23.4 ng/L for storage at 4°C, 25°C and 70 °C, respectively. The results showed the BPA concentration increased with storage duration (Fan *et al.*, 2014).

2.4 BPA analysis in Malaysia and other countries

According to Ghazali and Johari (2015), the investigation of endocrine disrupting compounds (EDCs) such as plasticizers, pesticides and surfactants in Malaysia started in 1999 and became critical due to the difference in temperature, weather and economic status which are different from other countries. BPA is a type of EDCs that is widely used in manufacturing processes of polycarbonate plastic and epoxy resin. The banned of BPA bottles in Malaysia had been reported by The Star (Ghazali & Johari, 2015).

Ghazali and Johari (2015) reported the level of BPA monitored worldwide in water sample. In Malaysia, the analysis of BPA showed the BPA concentration of 0.0035 – 0.0598 $\mu\text{g/L}$ when tap water was used as sample and 0.0007 – 0.0059 $\mu\text{g/L}$ when bottled mineral water was used as sample. The BPA level of the bottled water in France was detected in the range of 0.07 – 4.21 $\mu\text{g/L}$ while the BPA concentration was 0.002 – 0.0297 $\mu\text{g/L}$ when drinking water treatment plant was used as sample. For the sample of river water, the BPA concentration detected in Germany, United States, China and Taiwan were 0.009 – 0.776, 0.0009 – 0.044, 0.0191 – 0.106 and 0.01 – 44.65 $\mu\text{g/L}$ respectively. In Japan, the BPA concentration detected was 0.20 – 370 $\mu\text{g/L}$ when final effluent in the waste paper recycling plants leachate was used as the sample. For the human exposure to BPA, the reference dose of BPA taken per day is 0.05 mg/kg. Hence, the BPA analysis was performed to control the human exposure to BPA (Ghazali & Johari, 2015).

Chapter 3.0 Materials and Methods

3.1 Calibration samples

The stock solution of 1000 ppm BPA was prepared in acetonitrile and dichloromethane (DCM). Two milligrams of BPA standard (Sigma-Aldrich) was weighed and dissolved in 2.0 mL of solvent (Aristiawan *et al.*, 2015). The BPA standard solutions were then diluted from the stock solution to attain concentrations of 0, 1, 10, 50, 100, 300 and 500 ppm. The samples were subjected to analysis with FTIR. For acetonitrile, the solvent was dried in 3Å molecular sieves for 24 hours prior to use. Acetonitrile is very sensitive to moisture and the moisture content in the background acetonitrile could interfere the FTIR analysis when the characteristic OH-group of BPA is examined. According to Williams and Lawton (2010), the treatment of acetonitrile with 3Å molecular sieves readily gave solvent with very low residual moisture content. The molecular sieve was activated before used. Five grams of 3Å molecular sieve was weighed and heated at 80 °C for 5 mins. After heating, the molecular sieve was poured into a bottle with 50.0 mL of acetonitrile and left overnight.

The standard BPA was scanned for the solid sample using the Potassium Bromide (KBr). The KBr was dried at 80 °C overnight to remove moisture. The 5 % of BPA standard samples were prepared by weigh 5 milligrams of BPA standard and make up with KBr to 100 milligrams. The mixture was ground to very fine powder using the mortar and then a disc was formed under high pressure. The disc was then put on the optical path of infrared radiation to take the measurements. All the measurements were taken at room temperature and atmospheric pressure.

3.2 Sample Preparation for leaching study

3.2.1 Leaching factor of interact duration

A 0.005 g of BPA was incorporated with plastic PET of dimensions 2×2 cm. The BPA standard coated on the PET plastic was dissolved by adding a few drops of acetonitrile and left to dry. BPA is highly soluble in the acetonitrile. The incorporated BPA-PET was placed in a petri dish containing 50 mL of distilled water. The expected concentration of BPA in water is 100 mg/L if the BPA leached completely into the water. The petri dish was left for 15, 30, 60 and 120 minutes in duplicates. A blank sample was prepared with uncoated PET plastic. At the end of the time set, 5 mL of water sample was extracted for analysis of BPA via liquid-liquid extraction using DCM. 5 mL of water sample and 5 mL of DCM were added to a separatory funnel and shaken to mix well. After the separation of DCM and water sample, the bottom layer of solution was collected for analysis. This procedure of leaching is according to Rosa *et al.* (2018), Fan *et al.* (2014) and Elobeid *et al.* (2012) with modifications.

3.2.2 Leaching factor of solutions temperature

To examine the leaching of BPA under the influence of temperature, the PET plastic was similarly coated but was left in water bath at room temperature, 50 °C and 70 °C for 10 mins. A set of blank was simultaneously prepared. The petri dish with 50 mL distilled water and incorporated BPA-PET was put on the water bath as shown in Figure 2. At the end of the samples, 10 mL of water samples were extracted according to the aforementioned procedure.



Figure 2: Petri dishes with blank PET sample and incorporated BPA-PET sample put on the water bath.

3.3 FTIR Analysis

The spectra of the standard solutions and sample solutions were obtained using an ATR-FTIR of Thermo Scientific, Nicolet iS10 spectrometer equipped with a diamond crystal accessory. All the spectra were acquired between 4000 cm^{-1} and 525 cm^{-1} and a spectral resolution of 4 cm^{-1} with 32 number of scan for acetonitrile samples and 16 number of scan for DCM samples. The number of scan of DCM samples was reduced to 16 number of scan because DCM is a volatile liquid and its volatility increased the volume of the samples needed for the FTIR analysis. The crystal was cleaned with the absolute ethanol before scanning and different pipettes were used for each samples for the contamination precautions. The background scanning was done as no sample was putting on the crystal and it must be scanned before the sample scanning with same setup with the sample. The samples in the BPA analysis were scanned using FTIR after the samples were freshly prepared to reduce the duration effect of the BPA stability.