CONTAMINATION OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN FOOD PACKAGING

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ABSTRACT

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been detected in the blood of individuals from a number of regions and countries throughout the world. Consumer products such as textiles, carpets, cookware, and food packaging expose a portion of humans to PFOS and PFOA. This research aims to optimize the method parameters for sample pretreatment and to determine PFOS and PFOA contamination in food packaging made of paper using pressurized liquid extraction (PLE) followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The study found that the optimum conditions of PLE were 30 minutes static extraction time with a flush volume of 100% cell volume and one extraction cycle under 80°C and 1000 psi. The extraction technique validated the absolute recovery from PFOS and PFOA fortified control samples at three different levels (5, 50, and 200 ng g^{-1}). The average recoveries were always higher than 79 % with relative standard deviation (RSD) lower than 11%. PFOS and PFOA were extracted from 34 food packaging samples (instant food cups, microwave-popcorn bags, beverage cups, ice cream cups, fast food containers, dessert containers, and baking paper) collected in Thailand by using the optimum PLE technique. PFOS and PFOA were detected in all kinds of collected samples extracted by methanol with the average concentration of 4.89 and 2.87 ng g^{-1}, respectively. The average amount of PFOS and PFOA migrated from food packaging samples through contact with saliva simulant were 2.47 and 2.84 ng g^{-1}, respectively. PFOS and PFOA were migrated from paper sample by saliva simulant at almost the same concentration levels with the average concentration values obtained by methanol extract sample. The estimation of PFOS and PFOA intake amounts in Asian adults from daily food packaging were about 0.01 ng $(kg\ bw)^{-1}\ day^{-1}$ for both compounds. Comparisons with the total daily water intake standard of PFOS and PFOA are more than twice these estimated values. This suggests that the PFOS and PFOA from food packaging is one of the major concerns for exposure of PFOS and PFOA to the human body.

KEY WORDS: PFOS / PFOA / FOOD PACKAGING/ PRESSURIZED LIQUID EXTRACTION / LC-MS/MS

68 pages

การปนเปื้อนของเปอร์ฟลูออโรออกเทนซัลโฟเนต (PFOS) และเปอร์ฟลูออโรออกทาโนอิคแอซิด (PFOA) ในบรรจุภัณฑ์อาหาร

CONTAMINATION OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN FOOD PACKAGING

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บทคัดย่อ

เปอร์ฟลูออโรออกเทนซัลโฟเนต (PFOS) และเปอร์ฟลูออโรออกทาโนอิคแอซิด (PFOA) มีการตรวจพบในเลือดของมนุษย์จากหลากหลายประเทศทั่วโลก ซึ่งผลิตภัณฑ์สำหรับการอุปโภคและบริโภค เช่น สิ่งทอ พรม เครื่องครัว และบรรจุภัณฑ์อาหารนั้นเป็นสาเหตุหนึ่งที่ก่อให้เกิดความเสี่ยงต่อ PFOS และ PFOA เข้าสู่ร่างกายมนุษย์ การวิจัยนี้จึงมีวัตถุประสงค์เพื่อหาวิธีการที่เหมาะสมในการวิเคราะห์หาปริมาณ PFOS และ PFOA ในบรรจุภัณฑ์อาหารประเภทกระดาษ ตลอดจนเพื่อศึกษาหาปริมาณการปนเปื้อนของ PFOS และ PFOA ในบรรจุภัณฑ์อาหารประเภทกระดาษโดยใช้เทคนิคการสกัดแบบ Pressurized liquid extraction (PLE) และนำไปวิเคราะห์ด้วยเครื่อง LC-MS/MS จากผลการศึกษาพบว่าเทคนิคการสกัดแบบ PLE ที่เหมาะสมนั้น ใช้ระยะเวลาการสกัด 30 นาที และใช้จำนวนรอบในการสกัดเพียง 1 รอบ ด้วยปริมาณตัวทำละลาย 100% ของขนาดเซลล์บรรจุตัวอย่าง ภายใต้อุณหภูมิ 80 องศาเซลเซียส และความดัน 1,000 พีเอสไอ และสภาวะที่เหมาะสมของเทคนิคการสกัดที่ได้นี้มีค่าเปอร์เซนต์การได้กลับคืนของ PFOS และ PFOA ในแต่ละระดับความเข้มข้นของ PFOS และ PFOA (5, 50, and 200 นาโนกรัมต่อกรัม) มากกว่า 79% และค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 11% เมื่อนำเทคนิคการสกัดแบบ PLE ที่เหมาะสมนี้ไปใช้ในการหาปริมาณการปนเปื้อนของ PFOS และ PFOA ในตัวอย่างบรรจุภัณฑ์อาหารประเภทกระดาษจำนวน 34 ตัวอย่าง (ถ้วยบะหมี่กึ่งสำเร็จรูป ถุงไมโครเวฟสำหรับป๊อปคอร์น แก้วเครื่องดื่ม ถ้วยไอศครีม หีบห่ออาหารฟาสฟู้ด หีบห่อขนม และกระดาษซับน้ำมัน) จากประเทศไทย พบว่ามีการปนเปื้อนในตัวอย่างทุกประเภทที่สกัดด้วยตัวทำละลายเมทานอล โดยมีความเข้มข้นเฉลี่ยของ PFOS และ PFOA เท่ากับ 4.89 และ 2.87 นาโนกรัมต่อกรัม ตามลำดับ ในขณะที่ปริมาณของ PFOS และ PFOA ที่ออกมาจากบรรจุภัณฑ์อาหารด้วยการชะจากน้ำลายเทียมนั้นมีปริมาณเท่ากับ 2.47 และ 2.84 นาโนกรัมต่อกรัม ตามลำดับ ซึ่งจะเห็นว่าปริมาณที่ได้นี้มีค่าใกล้เคียงมากกับการสกัดหาปริมาณทั้งหมดของ PFOS และ PFOA ในบรรจุภัณฑ์อาหารด้วยตัวทำละลายเมทานอล สำหรับการประเมินหาปริมาณการได้รับ PFOS และ PFOA ต่อวันผ่านทางบรรจุภัณฑ์อาหารในคนเอเชียนั้น มีค่าประมาณ 0.01 นาโนกรัมต่อกิโลกรัมของร่างกายมนุษย์ต่อวัน ซึ่งเมื่อเปรียบเทียบกับค่ามาตรฐานปริมาณการได้รับ PFOS และ PFOA ต่อวันผ่านทางน้ำดื่มพบว่าค่ามาตรฐานนี้มากกว่าเพียง 2 เท่า จึงแสดงให้เห็นว่า PFOS และ PFOA ในบรรจุภัณฑ์อาหารนั้นเป็นเรื่องสำคัญที่ควรคำนึงถึงสำหรับแนวโน้มการปนเปื้อนของ PFOS และ PFOA เข้าสู่ร่างกายมนุษย์

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LIST OF ABBREVIATIONS

°C	Celsius Degree
%RSD	Relative Standard Deviation (%)
C6	6 Carbon Atom Chain
C8	8 Carbon Atom Chain
EFSA	European Food Safety Authority
EPA	Environment Protection Agency
g	Gram
L	Liter
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
mg	Milligram
mL	Milliliter
mM	Milli-Mole
m/z	Mass To Charge Ratio
ng	Nanogram
pH	Potential Hydrogen
PFCs	Perfluorinated Compounds
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PLE	Pressurized Liquid Extraction
POPs	Persistent Organic Pollutants
SD	Standard Deviation
USEPA	United States Environmental Protection Agency

CHAPTER I
INTRODUCTION

1.1 Background of study

Perfluorinated compounds (PFCs) have increasingly attracted global concerns because they are globally distributed, environmentally persistent, bio-accumulative, and potentially harmful to wildlife and humans. The most commonly used compounds in these groups are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Trudel *et al.*, 2008). In the year 2000, growing concern about this class of chemicals resulted in the announcement of the largest producer, 3M Company, to phase out the production of PFOS. Since then, a number of papers reporting environmental concentrations of PFOS and PFOA have been published. PFOS was recently included as a persistent organic pollutant (POP) in Annex B of the Stockholm Convention (Stockholm Convention Secretariat, 2009).

PFOS and PFOA are synthetic organic chemicals consisting of a fully fluorinated carbon chain and a sulfonate group or carboxylic group, respectively (Lau *et al.*, 2004). The structures of PFOS and PFOA are the combination of hydrophilicity from function groups and hydrophobicity from fluorinated structure. The presence of strong C-F bonds makes them chemically and thermally very stable resistant to hydrolysis, photolysis, and microbial degradation or metabolism. PFOS and PFOA have been observed to persist in the environment, bioaccumulate in human and animal tissue, biomagnify in food chains, and have potential significant impacts on human health and the environment (Lau *et al.*, 2004; EFSA, 2008).

PFOS and PFOA are bio-accumulated and have various toxicities toward living organisms, including humans (EFSA, 2008). The toxicology of PFOS and PFOA has been extensively reviewed. Hepatotoxicity, developmental toxicity, immunotoxicity, hormonal effects, and a carcinogenic potency in animal studies are the effects of main concern (Wilhelm *et al.*, 2009). The concentrations of various PFCs have been detected in the blood of individuals from a number of regions and

countries throughout the world (Olsen *et al.*, 2003; Ehresman *et al.*, 2007; Vassiliadou *et al.*, 2010). The half-lives of human serum elimination of PFOS and PFOA have been estimated at 5.4 and 3.8 years, respectively (Olsen *et al.*, 2005). However, the origin of the human blood contamination by PFCs is not quite well understood yet (Jogsten *et al.*, 2009). Human exposure to PFOS and PFOA is due to a variety of environmental and product-related sources. To mitigate any future risks associated with PFOS and PFOA, there is thus an urgent need for improved understanding of the pathways of human exposure**.**

Due to their properties, PFOS and PFOA have been widely used in industrial such as electronics, cosmetics, firefighting foams, cookware, and water and grease repellent coatings for fabrics and paper packaging for containing food (Ostertag *et al.*, 2009). Consumer products cause a major portion of the human exposure to PFOS and PFOA such as clothes, textiles, carpets, cookware, and paper packaging. Food packaging made of paper may be an important source of perfluorinated chemicals in humans such as in microwave popcorn bag (Begley *et al.*, 2005), which the chemicals can be released gradually during microwave heating and leak into the food or the vapors. Food packaging materials come directly into contact with food, so it is of great significance to accurate determine the PFOA and PFOS at trace levels in food packaging materials.

Paper is the most widely used as packaging material. The surface of paper is treated to improve paper properties, including physical strength, oil/grease resistance, and wettability. Food packaging products made of paper material are usually coatings/additives with PFOS and PFOA for oil and water resistance (Begley *et al.*, 2005; Harada *et al.*, 2009). Thus, Food packaging is important to be estimated for dietary exposure of PFOS and PFOA contamination.

To date, there is little information concerning human exposure to PFCs through the diet (Jogsten *et al.*, 2009). Furthermore, the information is very little on the contamination of PFOS and PFOA in food packaging that has the potential to migrate from perfluorochemical coatings/additives into food. Therefore, the analysis of PFOS and PFOA leached from the package into its contents is important for quality assurance and food safety.

This research aims to monitor the amount of PFOS and PFOA in food packaging product made of paper material and study the migration of PFOS and PFOA from food packaging to human body through saliva simulant. Moreover, this study was conducted to simulate the leaching factors of PFOS and PFOA in food packaging under simulation of temperature with watery, pH and oiliness conditions. In order to achieve the main goal, suitable analytical procedures for reliable quantification of trace amount of the target analytes were developed or optimized. This research included the development and validation of methodology in order to indicate the reliable method to determine the concentration of PFOS and PFOA in food packaging products made of paper material.

The concentration of PFOS and PFOA contaminated in food packaging could be a useful database, which would be beneficial in reminding a people and government to avoid of using PFOS and PFOA contaminated packaging. For Thailand, the information of PFOS and PFOA contamination and consumption guidelines in food packaging has not been available yet. Thus this study would be the first database for Thailand about contamination of PFOS and PFOA in food packaging.

1.2 Objectives of study

The overall objective of this research was to investigate the amount of PFOS and PFOA in food packaging products made of paper material. The specific objectives were as follows:

1. To study the effects of parameters for pressurized liquid extraction (PLE) technique to extract PFOS and PFOA from food packaging including extraction time, extract solvent volume and extraction technique.

2. To study the amount of PFOS and PFOA in food packaging products made of paper material.

3. To study the migration of PFOS and PFOA from food packaging to human body through saliva simulant.

4. To study the leaching factors of PFOS and PFOA in food packaging under simulate conditions of temperature with watery/acidic and oiliness.

1.3 Scope of study

The scopes of study were as follows:

1. Investigation the contamination of PFOS and PFOA in food packaging products made of paper material, which usually coatings/additives with PFOS and PFOA for oil and water resistance. All paper materials were purchased from the domestic and international brands of restaurants/cafes located in Bangkok, Thailand. Food packaging samples were included instant food cup, microwave-popcorn bag, beverage cup, ice cream cup, fast food container, dessert container and baking paper.

2. Pressurized liquid extraction (PLE) technique was developed to quantify the amount of PFOS and PFOA extracted from food packaging samples through contact with methanol extraction. In addition, the extraction solvent in this study was the saliva stimulant, which synthetic in the laboratory for study the migration of PFOS and PFOA from food packaging. Ethanol/water (1:9, v/v) and ethanol/water (19:1, v/v) were also extraction solvent, which used for simulate the watery/acidic and oiliness condition.

3. Influence parameters of pressurized liquid extraction (PLE) technique, including static extraction time, flush volume and number of extraction cycles were carefully evaluated by extracted concentration of PFOS and PFOA from food packaging sample in low level (ng g^{-1}).

4. All samples were analyzed by Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

CHAPTER II
LITERATURE REVIEW

2.1 Perfluorinated compounds (PFCs)

In 1970 was the first recognized of the PFCs occurrence in the environmental and has continuously increased since then. PFCs classes are the perfluorinated sulfonates (PFSAs) and the perfluorinated carboxylates (PFCAs) and the most commonly measured compounds in these classes are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Both groups have polar and nonpolar domains can lessen water surface tension more than hydrocarbon-based surfactants, and are more powerful wetting agent. PFOS and PFOA are the final products of degradation of numerous fluorochemicals, intensively used in industry. Due to their high stability and low level of biodegradability, they remain in the environment (Ropers *et al*., 2009). Since 2000 there has been a great deal of activity by research laboratories, regulatory authorities and industry to classify, monitor and regulate these pollutants.

2.1.1 Production

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are synthesized fully fluorinated organic compounds. Organic fluorochemicals are compounds in which one or more carbon-hydrogen (C - H) bounds are replaced by carbon-fluorine (C - F) bounds. PFOS and PFOA have been synthesized in laboratory and in fill-scale manufacturing operations. They have been synthesized via two main techniques, electrochemical fluorination (ECF) or telomerization (Lau *et al.*, 2004) as shown in Figure 2.1 and 2.2. ECF is based upon the electrolysis of a hydrocarbon analogue of the target PFC in liquid hydrogen fluoride. A feedstock of perfluorooctanesulfonyl fluoride (POSF) is produced from ECF and undergoes reaction with other chemicals to form various PFCs. Telomerization is based upon the

polymerization of an unsaturated perfluoroalkene in the presence of perfluoroalkyl iodide.

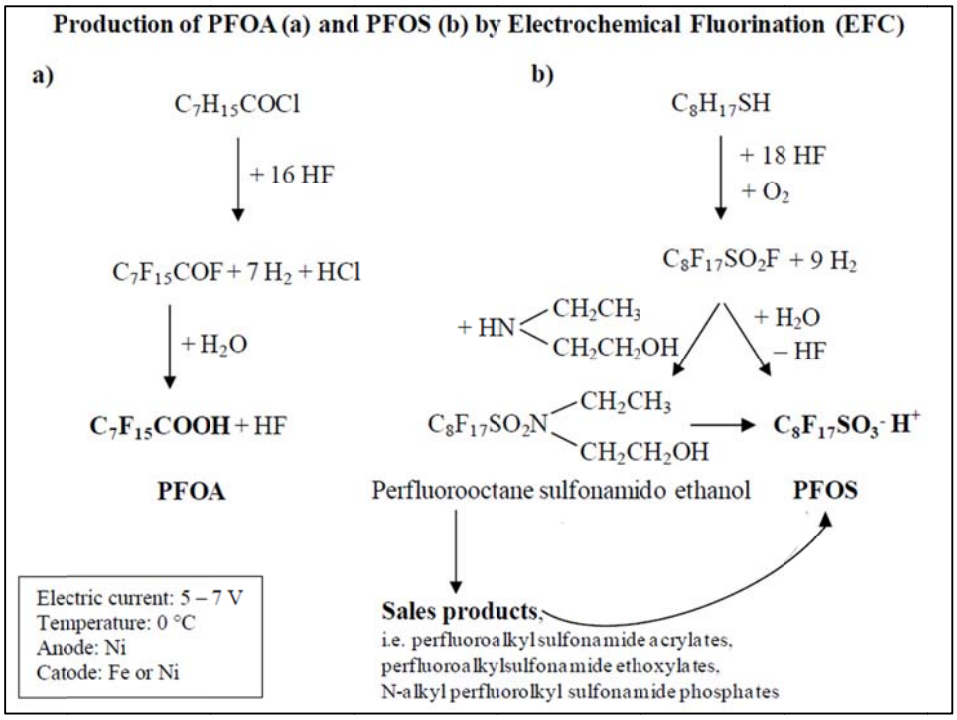


Figure 2.1: Electrochemical Fluorination Process (Schultz ***et al.***, 2003)

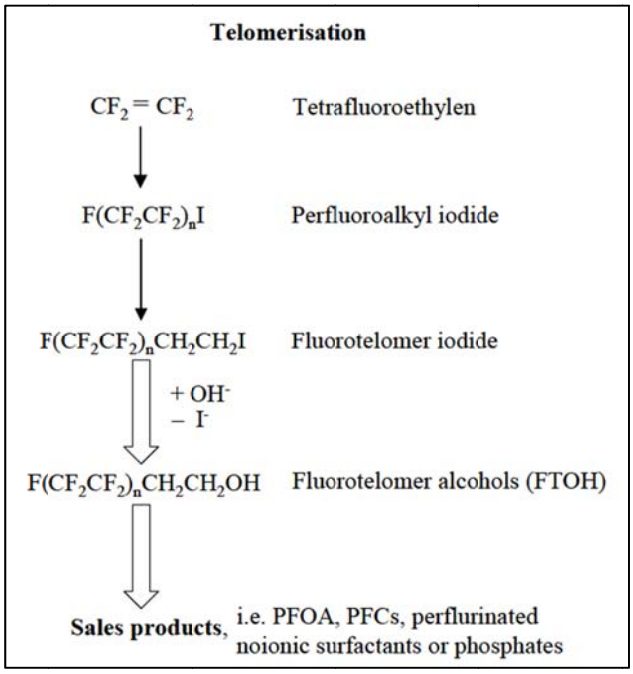
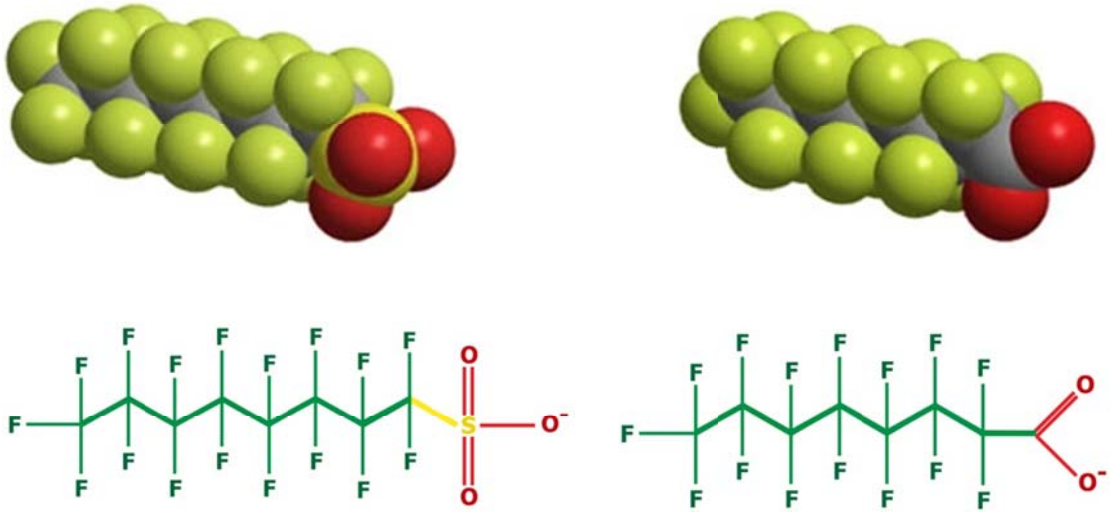


Figure 2.2: Telomerisation Process (Kissa, 2001)

ECF process was ceased after 3M Company phased out its product of PFCs in 2000. However, telomerization process is still applied in industries to produce PFCs (Qiu *et al*., 2007). Present production is primarily in China and Brazil since the increased regulations and restrictions in North America and the European Union, and therefore products are now undergoing enhanced transport to southern Asia, Australia and South America (Carloni, 2009).

2.1.2 General physicochemical properties

PFOS and PFOA have eight carbons (C8), which all hydrogen atoms in the carbon-hydrogen bonds are replaced by the element fluoride. Carbon - fluorine bonds are very strong and extremely stable. Thus, they have properties similar to fluorocarbons as they are fluorocarbon derivatives. PFOS and PFOA are a surface-active agent whose physical properties are governed by a hydrophilic head group and tail is both hydrophobic and oleophobic. Their chemical structures are shown in Figure 2.3. The functional group, as the carboxylic or sulfonic group, can affiliate with water and make PFCs hydrophilic (Qiu *et al*., 2007). Table 2.1 is shown the general properties of PFOS and PFOA.



Perfluorooctane sulfonate (PFOS) **Perfluorooctanoate (PFOA)**

$C_8F_{17}SO_3^-$ **$C_8F_{15}COO^-$**

Figure 2.3: Structural formula of PFOS and PFOA

Table 2.1: General physicochemical properties of PFOS and PFOA

Properties	PFOS	PFOA
Appearance	White powder	White powder
Molecular weight, g mol^{-1}	500.13	414.07
pK_a	-3.27	2.5
Melting point	> 400 ºC	52 – 54 ºC
Boiling point	Not measurable	189 ºC
Speccific Gravity	2.05	1.70
Solubility in pure water,	519 mg L^{-1} at 20 ºC	9,500
Vapour pressure	3.31 x 10^{-4} Pa at 20 ºC	4.2 Pa at 25 ºC
Half – life (in human serum)[a]	5.4 years	3.8 years

Source: EFSA, 2008; Qiu, 2007, a = Olsen *et al.,* 2007

They do not occur naturally in the environment, and due to their low pK_a values, they are present in solutions as anions at pH 7. For PFOA both forms, the free acid and the anion, are present in the environment whereas PFOS only occurs in its anionic form. The free acid is expected to completely dissociate in water, leaving the anionic carboxylate in the water and the perfluoroalkyl chain on the surface (USEPA, 2002). PFOA and PFOS are not expected to be volatilized significantly at environmental conditions; therefore they will be bound to particles in the atmosphere (OECD, 2002). Olsen et al. (2005) gave half-life values of 5.4 and 3.8 years for PFOS and PFOA, respectively, based on an investigation performed on 26 pensioners from two fluorochemical manufacturing plants.

2.1.3 Uses

The application of PFCs are widely used in many industrial processes such as textile, carpet and leather protection, metal plating, paper and packaging protection, firefighting foams, industrial and household cleaning products (surfactants), coatings and coating additives, photographic industry, photolithography, semiconductors, antireflective coatings, hydraulic fluids for the aviation industry, pesticides, medical applications, mining and oil surfactants, flame retardants and adhesives. These applications represent a minor part of known PFCs applications.

Since the addition of PFOS to the Stockholm Convention on persistent organic pollutants (POPs), use and production has become restricted and limited (under annex B) to semiconductor industry production of photo-resists, etching components and anti-reflective coatings, use in aviation oil hydraulics, metal plating, medical devices, reservoirs of aqueous film forming foams (AFFF) containing PFOS and insect baits for ant control. Exemptions are also in place for uses in oil drilling, liquid crystal display (LCD) production, treatments for carpets, leather and upholstery, papers and packaging, rubber and plastics (The POPs, 2010).

Uses of PFOS

- Surface-active agents in aqueous media
- Chemical intermediate; acid catalyst for photoresists
- Surfactant in aqueous firefighting foam
- Surfactant for alkaline cleaners; emulsifier in floor polish
- Mist suppressant for metal plating baths
- Surfactant for etching acids for circuit boards
- Pesticide active ingredient for anti-bait traps

Uses of PFOA

- Dielectric liquid material proposed for use to replace PCBs in transformers.
- Used to produce PFOA salts – processing aids in production of fluoropolymers and fluoroelastomers.
- Additive in aqueous firefighting foam, cosmetics, greases and lubricants, paints, polishes and adhesives, fluorinated surfactants.

2.1.4 Toxicology

As a class, these perfluorochemicals share unique physical and chemical properties that are related to their commercial applications as well as potentials for toxicity. The toxicology of PFOS and PFOA has been extensively reviewed. As far as risks are concerned there are many uncertainties, including the effects, fate and exposure of humans as well as other vertebrates and organisms in the environment. It

is, however, clear that perfluorinated compounds are extremely persistent as a group can be transported over great distances, and that some are bioaccumulative and toxic. There are indications that levels in mammals in the Arctic are increasing. PFOA and PFOS do not accumulate in the fatty tissue as most of the POPs, but in liver, kidney and bladder which consequently leads to the bio-magnification in the food-chain. The summary on relevant properties of toxic PFOS and PFOA is showed in Table 2.2.

Table 2.2: Summary on relevant properties of toxic PFOS and PFOA

	Detail
PFOS	***Toxicity*** - Absorbed orally and distributed to plasma/the liver - Elimination from urine, faeces, childbirth and lactation(Harada *et al.,* 2005) - Toxicity noted in lab. animals (Sprague-Dawley rats and monkeys) ***Tolerable daily intake (TDI)*** - 300 ng (kg body weight) $^{-1}$ d^{-1} (COT, 2006a) - 150 ng (kg body weight) $^{-1}$ d^{-1} (EFSA Journal, 2008) ***Regulatory Highlight*** Additions of PFOS to Stockholm convention annex B in August 2009, restricting use to industrial use, where no alternatives were available.
PFOA	***Toxicity*** - Metabolic effects in newborn mice - Developmental toxicity in mice ***Tolerable daily intake (TDI)*** - 3000 ng (kg body weight) $^{-1}$ d^{-1} (COT, 2006b) - 1500 ng (kg body weight) $^{-1}$ d^{-1} (EFSA Journal, 2008) ***Regulatory Highlight*** Voluntary reduction of PFOA by 95 % in 2010 and complete phase out of stack emissions by the USA‘s top 8 producers by 2015 (USEPA, 2010).

2.1.5 Concentration in human

The presence of organofluorine compounds in human blood was already published at the end of the 1960s. In 1976, the 3M Company started the medical monitoring of employees involved with PFOA production. Mean concentrations of up to 10 μg ml^{-1} were found (3M, 1999). In 1997, the 3M Company reported the presence of PFOS in commercial. In the beginning of 2000 the monitoring of PFOS was extended to blood from the not directly exposed population. PFOS is the most dominant PFC in human blood and PFOA the next most abundant. Karrman *et al*. reviewed PFOS and PFOA concentrations in human blood from different countries ranging from 60 - 10060 ng ml^{-1} (Karrman *et al*. 2009). The pathway leading to human exposure is still not well established. Surprisingly, similar concentrations as for adults were also detected in blood samples from children (OECD, 2002). Food intake may be a route for human exposure.

2.2 Pressurized liquid extraction

Pressurized liquid extraction (PLE) also known as Pressurized fluid extraction (PFE) and by the trade name "Accelerated Solvent Extraction (ASE)" was introduced in 1996, the ASE model is shown in Figure 2.4. In PLE, the solid or semisolid sample is placed in a closed container (cell), and sand, sodium sulfate or hydromatrix is often used as a dispersant in the cell. Solvent is then added to the cell at the start of the heating cycle. During the heating cycle, solvent is pumped in and out of the cell to maintain the pressure and to perform the number of static cycles indicated by the user. As with many of the methods mentioned above, the use of elevated temperatures increases the kinetics of the extraction, while the application of pressure prevents the solvent from boiling. PLE has been used in a variety of applications, including environmental, food and biological samples.

The advantages of PLE are the high sample throughput, low solvent consumption, and high potential of being used routinely. However, one drawback of PLE for all classes of analyses is that wet samples require a drying step prior to analysis when using a non-polar extraction solvent. The extraction schematic of ASE employed DIONEX ASE200 model as shown in Figure 2.5.

Figure 2.4: Accelerated Solvent Extraction, DIONEX ASE200 model

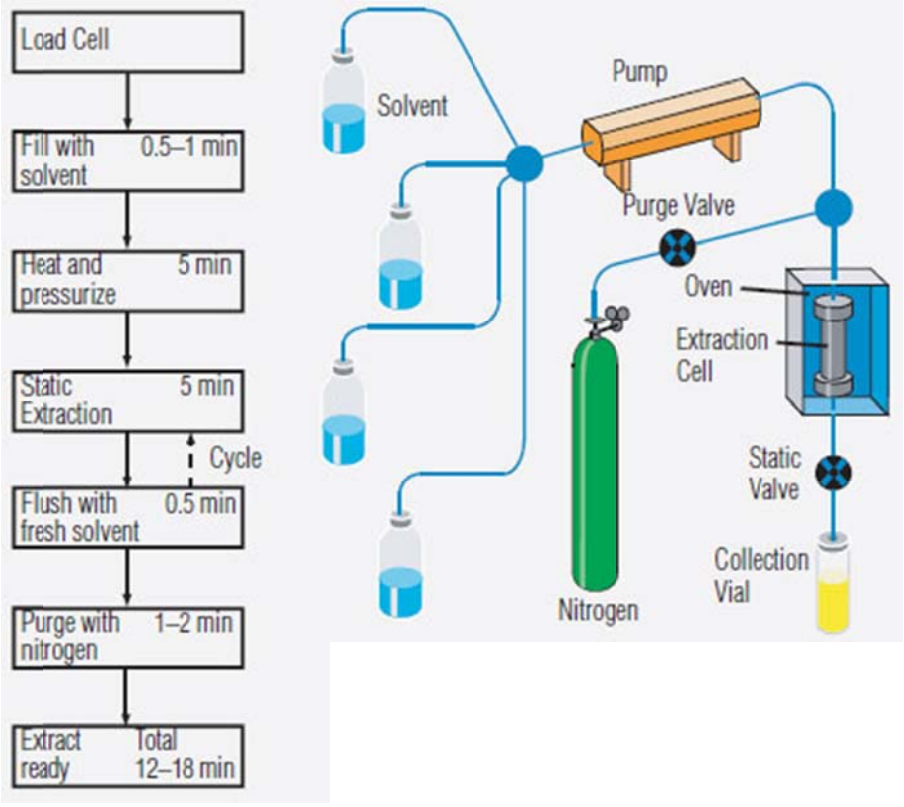


Figure 2.5: ASE schematic

2.3 Liquid chromatography coupled with tandem mass spectrometry

LC-MS is an instrument having separation capability of High Performance Liquid Chromatography (HPLC of simply LC) and detection and quantification power of Mass Spectrometry (MS). If more than one MS is used (i.e., in tandem) then the term is LC tandem MS or LC-MS/MS.

2.3.1 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a liquid chromatography in which high pressure is applied to allow the solution to pass through the column. One essential requirement for HPLC is that sample must be dissolved into a liquid solvent that pass through the column. HPLC is now one of the most powerful tools in analytical chemistry, with the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid.

Essential component of HPLC are:

i) Solvent (or mobile phase)
ii) Pump (to adjust flow rate of solvent at a pressure level)
ii) Auto sampler or sampling port
iv) HPLC column having packed materials (or stationary phase)
v) Detector (absorbance detector, fluorescence detector, etc.)

Retention mechanism of majority of HPLC cases are based on the polarity of molecules:

i) Normal phase: This case the mobile phase is non-polar (organic solvent) and stationary phase is polar. This is similar to the normal chromatography
ii) Reverse phase: This case the mobile phase is polar (e.g., water) and stationary phase is non-polar, i.e., it is reverse to normal chromatography

2.3.2 Tandem Mass Spectrometry (MS/MS)

Mass Spectrometer is an instrument designed to separate gas phase ions according to their mass (m)-to-change (z) ratio (m/z). Although MS is used to measure the exact mass, the importance is not only the mass but also the change. Heart of the MS is an analyzer that separates the gas phase ions. Analyzer uses electric or magnetic or combination of both to move the ions from the place they are produced to the detector. The analyzer is operated under high vacuum so that the ions can travel to the detector with a sufficient yield.

Combining the HPLC system with the MS is not simple because solute of target compound in the sample should be ionized into a gas phase ion. Ions should be generated such that there is no effect of solvent (mobile phase) while adequate vacuum level is still maintained.

MS is composed of three fundamental parts:

i) Ionization source

ii) Analyzer (i.e., mass to charge "m/z" analyzer)

iii) Detector (e.g., electric multiplier)

2.4 PFOS and PFOA contamination in consumer products

Powley *et al.*, (2005) was study to investigate the amount of PFOA extracted from the surface of commercial frying pans. DuPount fluoropolymer was coated on the frying pan. In this study, the simulated cooking conditions were obtained to extract PFOA from pan. The extraction conditions were under 100 and 125°C by using ethanol/water mixture as an extraction solvent. All samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Detection and quantification limits as low as 100 pg cm^{-2} were demonstrated. This result shown that none of fluoropolymer treated frying pan sample had shown the PFOA concentration when extracted under simulated cooking conditions.

Begley *et al.*, (2005) was study the contamination of perfluorinated compound in food-contact substances in low level. The potential source of oral

exposure to perfluorinated compound was showed in food-contact substance. The commercial products are used perfluorinated compound for coating/additive, which perfluorinated compound has the unique physicochemical properties to make the non-stick coating product. PFOS is a residual impurity in some paper coatings used for food contact and PFOA is a processing aid in the manufacture for many purposes including non-stick cookware. The study was presented the migration tests and residual amounts of perfluorinated chemical in the level of mg kg^{-1} and low μg kg^{-1} range, respectively. PFOA is present in microwave popcorn bag paper at amounts as high as 300 μg kg^{-1}.

Vestergren *et. al.*, (2008) was study the exposure of humans to PFOS and PFOA. The uptake into the human body and resulting daily doses were estimated by using a Scenario-Based Risk Assessment (SceBRA) model. The model was varied pathway of these compounds to human body. The physiological and behavioural are differences of age and gender, the exposure and resulting doses for seven consumer groups were calculated. The estimated PFOS and PFOA chronic doses of a general population of an industrialized country were ranged from 3.9 to 520 and 0.3 to 140 ng $(kg\ bw)^{-1}\ day^{-1}$, respectively. The relative importance of precursor-based doses of PFOS and PFOA was estimated to be 2–5% and 2–8% in an intermediate scenario and 60–80% and 28–55% in a high-exposure scenario. The results could be indicates that sub groups of the population may receive a substantial part of the PFOS and PFOA doses from precursor compounds, even though they are of low importance for the general population. Fast food consumption and fraction of food packaging paper treated with PFCs were influential parameters for determining the doses of PFOA.

Ostertag *et al.*, (2009) was study to estimate dietary exposure to PFCs among Inuit in northern Canada. These compounds were measured in traditional foods collected in Nunavut between 1997 and 1999. The average of daily dietary exposure was calculated in 754 individuals' person who has the ranging from 210 to 610 ng $person^{-1}$ or 0.6 – 8.5 ng $(kg\ bw^{-1})$. This results could be found that PFCs was statistically significantly higher in men in the 41– 60 year age group ($p < 0.05$) than younger men (<40 years old) and women from the same age group.

Jogsten *et al.,* (2009) was study the source of PFCs from food processing and packaging which might migrate to the diet. The level of PFCs were determined in many food sample such as veal steak, pork loin, chicken breast, black pudding, liver lamb, marinated salmon, foie gras of duck, frankfurt, sausages, and chicken nuggets. PFOS was the most frequently detected, being found in 8 of the 20 food items analyzed, while PFOA was not much detected in sample. According to the results of the present study, it is not sufficiently clear if cooking with non-stick cookware, or packaging some foods, could contribute to a higher human exposure to PFCs.

CHAPTER III
MATERIALS AND METHODS

3.1 Framework of the study

This chapter describes a methodological framework to be implemented in the study. The overall methodologies to be obtained the results are shown in Figure 3.1.

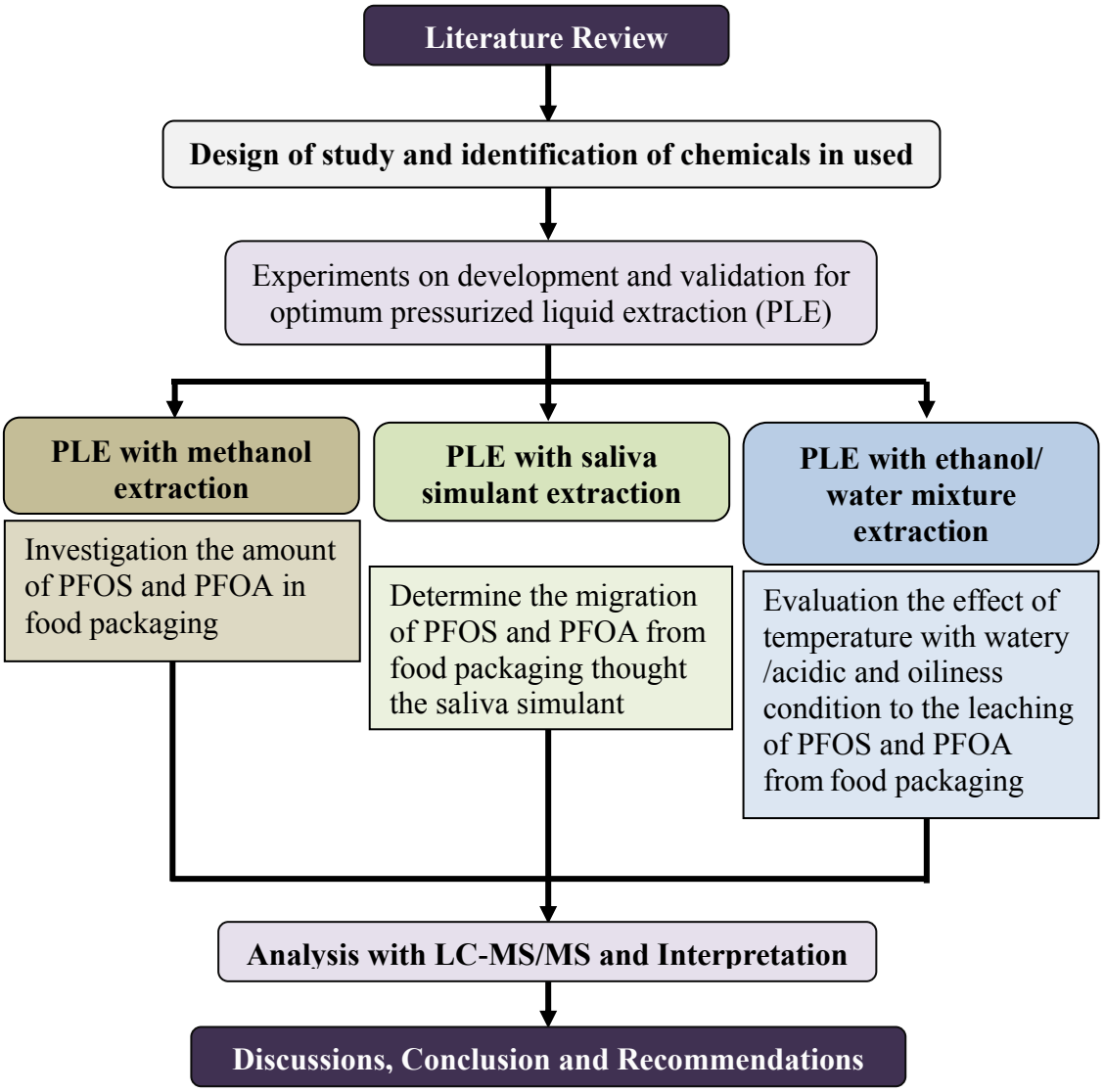


Figure 3.1: Framework of the Study

3.2 Experimental Location

The experiment of this research was conducted in the environmental laboratory of the Civil and Environmental Engineering Department, Faculty of Engineering, Mahidol University, Salaya, Nakornpathom, Thailand**.**

3.3 Chemicals

3.3.1 List of all used chemicals

Chemicals used in the extraction processes and LC-MS/MS analyses were included the following:

- Perfluorooctane sulfonate, PFOS standard 98% purity, 500.13 g mol^{-1}, (#328 - 61592, Wako Company, Japan)
- Perfluorooctanoic acid, PFOA standard > 95% purity, 414.07 g mol^{-1}, (#163 - 09542, Wako Company, Japan)
- Methanol, HPLC grade, > 99.99% purity (MERCK Inc., Germany)
- Acetonitrile, HPLC grade, > 99.8% purity (MERCK Inc., Germany)
- Ammonium acetate, 99.9999% purity (Sigma-Aldrich Inc., USA)
- Glacial acetic acid, ACS grad (MERCK Inc., Germany)
- Denatured Ethanol, ACS grad (MERCK Inc., Germany)
- Potassium chloride, > 99% purity (Sigma-Aldrich Inc., USA)
- Potassium carbonate, > 99% purity (MERCK Inc., Germany)
- Dipotassium hydrogen phosphate, > 99% purity (MERCK Inc., Germany)
- Sodium chloride > 99.5% purity (Sigma-Aldrich Inc., USA)
- Calcium chloride dihydrate, > 99.5% purity (MERCK Inc., Germany)
- Magnesium chloride hexahydrate, > 99% purity (MERCK Inc., Germany)
- 1 N Hydrochloric acid (MERCK Inc., Germany)
- Methanol, ACS grade, > 97% purity (MERCK Inc., Germany)

3.3.2 Standards and fortification solutions

A stock standard solution of PFOS was prepared at a concentration of 200 µg mL^{-1} by dissolving 10 mg of the standard chemical in 50 mL methanol (HPLC grade). A stock standard solution of PFOA was prepared at a concentration of 200 µg mL^{-1} by dissolving 10 mg of the standard chemical in 50 mL methanol (HPLC grade).

Mixed standard solution of PFOS and PFOA was prepared at a concentration of 0.1 µg mL^{-1} by dilution of the stock standard solutions. The stock solution of 25 µL PFOS and 25 µL PFOA were added and adjusted the volume of mixed standard solution to 50 mL by methanol (HPLC grade). The Mixed standard solution of PFOS and PFOA was conducted to prepare the calibration standard.

To prepare 5, 50 and 200 ng g^{-1} fortification for spike 2 g of paper sample with 100 µL. Fortification solutions of 0.1, 1.0 and 4.0 µg mL^{-1} PFOS and PFOA were prepared by diluting the stock standard solutions with methanol (HPLC grade).

All standards and fortification solutions were stored in polypropylene bottle and kept in refrigerator at 4 ºC.

3.3.3 Synthesis of saliva simulant

The saliva solution has the following compositions as shown in Table 3.1. The saliva simulants was prepared with 0.82 mM magnesium chloride, 1.0 mM calcium chloride, 3.3 mM di-potassium hydrogen phosphate, 3.8 mM potassium carbonate, 5.6 mM sodium chloride, and 10 mM potassium chloride.

Table 3.1: The compositions of saliva simulant

Compound	Formula	Concentration, mmol l^{-1}
Potassium chloride	KCl	10.0
Potassium carbonate	K_2CO_3	3.8
Dipotassium hydrogen phosphate	K_2HPO_4	3.3
Sodium chloride	NaCl	5.6
Calcium chloride	$CaCl_2$	1.0
Magnesium chloride	$MgCl_2$	0.82

The potassium and sodium salts were dissolved in distilled water before adding the magnesium and calcium salts and making up to 1 L. The pH of the solution was adjusted to 6.8 by dropwise addition of 1N hydrochloric acid. The synthesis of saliva simulant was shown the step as in Figure 3.2 and 3.3 (Earls *et al.*, 2003).

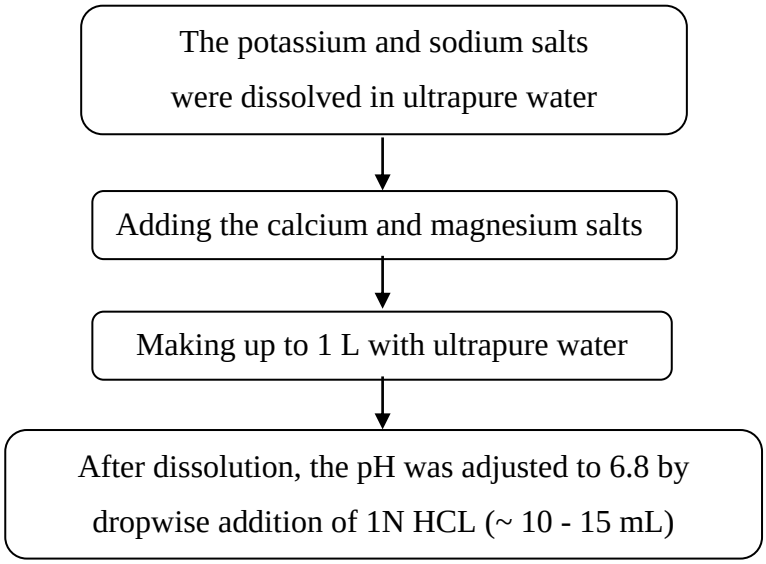


Figure 3.2: Flowchart of saliva simulant synthesis

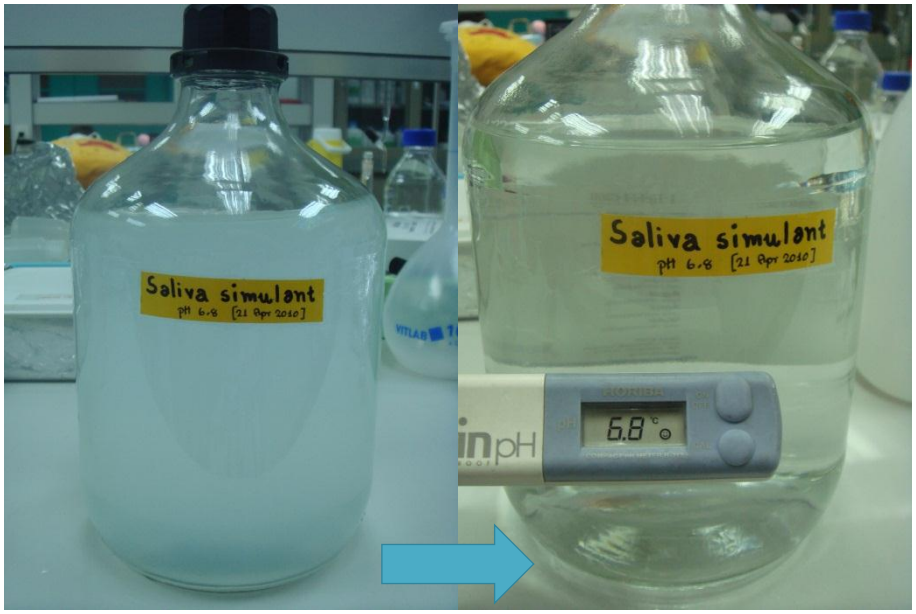


Figure 3.3: pH adjustment of saliva simulant synthesis

3.4 equipment

The details of equipment used in this study are given below:

- Liquid chromatography tandem mass spectrometry (LC-MS/MS), Agilent 1200 SL HPLC and Agilent 6400 MS/MS, Agilent Technologies, Japan
- Accelerated Solvent Extraction, Dionex ASE 200 model
- Centrifuge, Hettich UNIVERSAL 320R model
- Nitrogen purge, Eyela MG 2200 model
- Analytical balance
- Vortex mixer
- pH meter

3.5 Precaution of the experiment

As PFOS and PFOA are ubiquitous environmental contamination and adsorption with glassware, considerable care was taken to avoid sample contamination. All labware and equipment used for PFOS and PFOA preparation or experiment are made from plastic (polypropylene grade) and washed twice with methanol, and then washed twice with ultrapure water prior to use.

3.6 Food packaging sample

3.6.1 Sample collection

All paper materials were purchased from the domestic and international brands of restaurants/cafes located in Bangkok, Thailand. They represented typical paper packaging for containing food. The paper sample were instant food cup, microwave-popcorn bag, beverage cup, ice cream cup, fast food container, dessert container and baking paper. All package samples were fresh packages and have never used to contain diet.

Paper material of noodle cup was used as representative of paper packaging for contained food. The represent sample used to find out the optimum

extraction method because this kind of paper packaging is widely use product. After find out the optimum extraction method, totally 34 samples of food packaging made of paper (10 instant food cups, 3 microwave-popcorn bags, 3 beverage cups, 2 ice cream cups, 8 fast food containers, 7 dessert containers and 1 baking paper) were obtained to determine the concentrations of PFOS and PFOA.

3.6.2 Sample preparation

Before the analysis of paper samples, the printing and outside layer of the containers were deliberately removed with the aid of a cutter. The remaining paper was cut into approximately 5mm x 5mm size pieces by scissors and keep into desiccator. The cut pieces of each paper sample was weighed to 2 g and used in the analysis. The preparation of food packaging sample was showed in Figure 3.4.

Figure 3.4: Food packaging sample preparation

3.7 Experimental procedure

To accomplish the objectives of study, the experimental processes were divided in three phases and described as follows:

3.7.1 Development of extraction technique

This phase was carried out in order to optimize the extraction method for extract PFOS and PFOA in food packaging products made of paper material. Pressurized liquid extraction (PLE) technique was optimized the influence parameter by using methanol as extraction solvent. The procedures are described as follows:

3.7.1.1 The effect of static extraction time on PLE technique

One of the influence parameter on PLE is a static extraction time. In this study, the PLE method was varied this parameter at 5, 10, 20, and 30 min with flush volume of 100% cell volume and one extraction cycle. The sample was heated to 80°C with 1000 psi pressurized. No preheating was done, and the purge time was 60 second. Static extraction time were compared the effectiveness. Three replicates of each batch experiment were extracted for analysis.

3.7.1.2 The effect of flush volume and extraction cycle on PLE technique

After find out the optimum extraction time on PLE technique, the extraction solvent volume was varied by flush volume and number of cycles. The flush volume is amount of solvent to flush through the cell following the static heating step, expressed as a percentage of cell volume. Number of times to perform the static heating and flushing steps was described as number of cycles (Dionex, 1999). The sample prepared for three replicate in each batch experiment. The batches experiment described as in Table 3.2. The optimum static extraction time was selected to operate the extraction step.

Table 3.2: Experimental condition for PFOS and PFOA extraction on PLE technique

Batch No.	**1**	**2**	**3**	**4**	**5**	**6**
Flush volume (%)	20	20	20	60	60	100
Extraction cycle	1	2	3	1	2	1

3.7.1.3 Ensuring of the selective PLE technique

Selectivity extraction technique is needed to assurance the extraction quality. With the percent recovery of analysis method, the recovery experiment was performed by spiking paper sample. This paper sample was obtained from washing paper sample with 20 mL methanol for 30 min on wrist action shaker. The sample was then centrifuged for 5 min at approximately 3000 rpm (Stadalius *et al.*, 2006). After washed, the sample was separate from methanol and keep in desiccator for dried.

The extraction technique was validated the absolute recovery from PFOS and PFOA fortified control samples at three different levels (5, 50, and 200 ng g^{-1}). The 2 g washed paper samples were fortified by spiking 100 µL of PFOS and PFOA fortification solution at concentration levels of 0.1, 1.0 and 4.0 µg mL^{-1}. Seven replicates of sample were performed at each level along with three paper blank sample.

3.7.2 Investigation of PFOS and PFOA contamination in food packaging

This phase was used to provide the data for product-specific exposure assessment for PFOS and PFOA contamination in food packaging products. The precise and accurate PLE technique from the first phase is proposed for the extraction of PFOS and PFOA in all food packaging samples.

3.7.2.1 Concentration of PFOS and PFOA in food packaging

Optimum of PLE technique with methanol extraction was determined the overall concentration of PFOS and PFOA in food packaging from domestic and international brands of restaurants and cafes located in Bangkok, Thailand. All samples were prepared and analyzed in duplicate.

3.7.2.2 Migration of PFOS and PFOA from food packaging through contact with saliva simulant

Saliva simulant was used as an extraction solvent in optimized PLE technique to migrate the PFOS and PFOA from food packaging sample. The part of determination an exposure partway deals with mouthing conducted to understand the potential human health. All samples were prepared and analyzed in duplicate.

3.7.3 Simulated condition of PFOS and PFOA leaching from food packaging

Further study was conducted packed conditions simulation, which is batch experiment under optimized PLE technique. Watery and acidic foods were simulated by using an ethanol/water mixture of 1:9, and an ethanol/water mixture of 19:1 was used to simulate oily or fatty foods. The simulations are consistent with FDA guidelines. The simulation conditions were varied with temperature at 40, 80, 120, 160, 200 °C and room temperature. Each batch was prepared and analyzed sample in triplicate.

3.8 Pressurized liquid extraction (PLE) technique

The PLE was done using an Accelerated Solvent Extraction, Dionex ASE 200 system. Two grams of each paper sample were inserted directly into a 33 mL stainless steel ASE cell. This ASE cell was filled up with stainless steel balls. Cellulose filter (Dionex, P/N 049458) was placed at the bottom of the extraction cell. The sample was then fortified (spike) with 100 µL PFOS and PFOA mixed solution, if needed. The PFOS and PFOA solution delivers directly to the sample. The fortified sample is allowed to air dry for ~ 10 min (The spot was visibly gone within 10 min of 100 µL application) at room temperature to ensure the appropriate distribution in the sample. The ASE cell was placed on Dionex ASE 200 model. The PLE extractions were performed with the extraction solvent and heated to 80°C with 1000 psi pressurized. No preheating was done, and the purge time was 60 second. After extraction, the liquid phase sample was decanted into 50 mL polypropylene bottom for

subsequent solvent extract preparation. The steps in ASE cell sample preparation were illustrated as the following Figure 3.5.

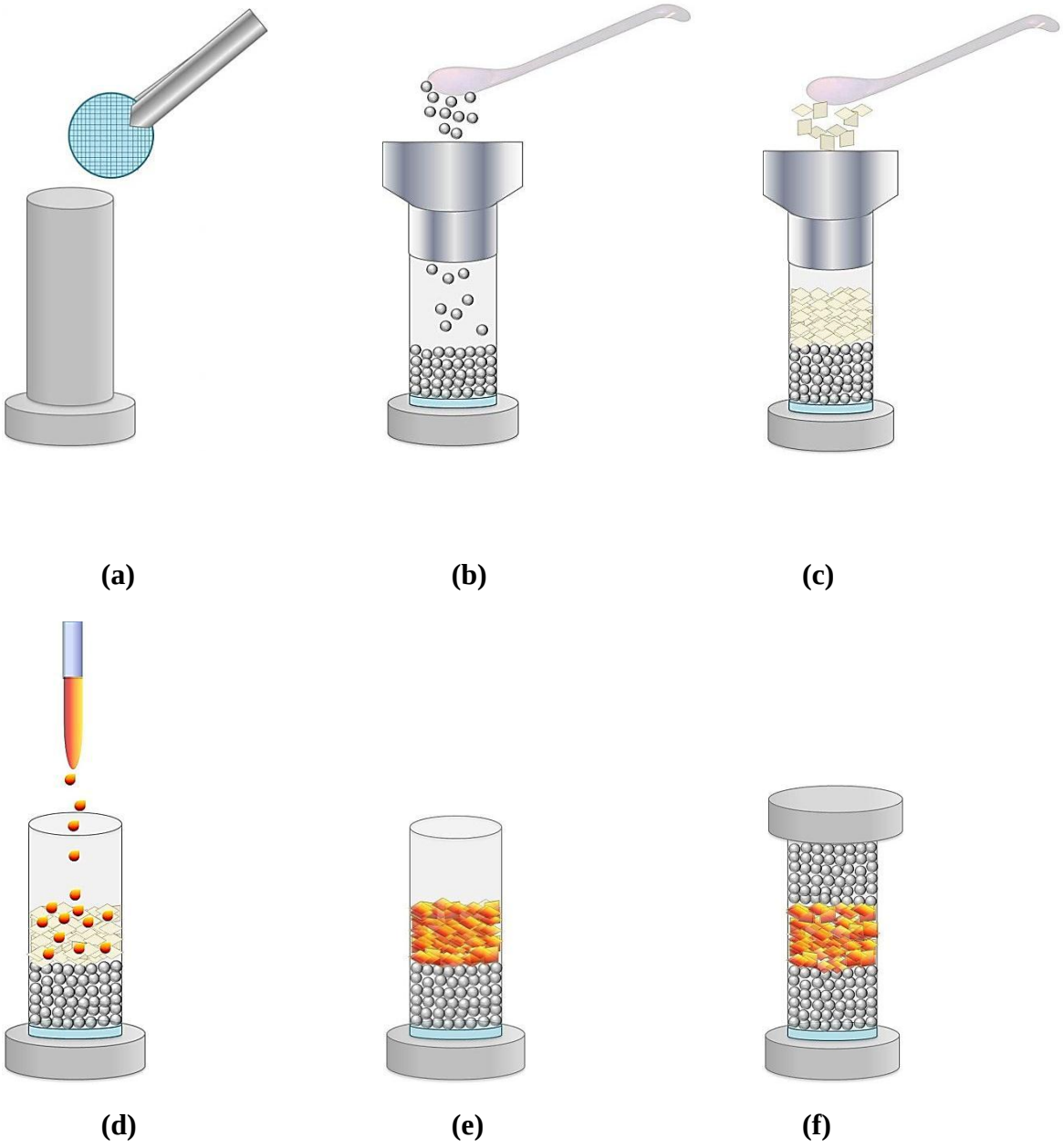


Figure 3.5: Steps in ASE cell sample preparation for PLE process
(a) Cellulose filter was placed at the bottom of the ASE cell (b) ASE cell was filled up with stainless steel balls (c) Two grams of paper sample was inserted directly into the ASE cell (d) 100 µL of PFOS and PFOA solution spikes directly to the sample, if needed (e) The fortified sample is allowed to air dry for ~ 10 min (f) ASE cell was filled up with stainless steel balls and capped the cell

3.9 Preparation of extracted sample for analysis

3.9.1 Extracted sample in methanol

After the PLE technique, each of the extracted samples in methanol was prepared into 2 mL centrifuge tube by adding 1 mL of extracted sample and 1 mL of ultrapure water into the tube, and capped. The addition of ultrapure water helps to precipitate and remove soluble sample components (Mawn *et al.*, 2005). The tubes were shaken to mix homogenously and then centrifuged for 20 minute at 12000 RPM and 25°C to remove any suspended particles in extracted sample. After centrifugation, the liquid phase sample was transferred into vial for analysis by LC-MS/MS.

3.9.2 Extracted sample in saliva simulant

After the PLE technique, the extracted sample in saliva simulant was prepared into 2 mL centrifuge tube, by adding 2 mL of extracted sample into the tube, and capped. The sample tubes were centrifuged for 20 min at 12000 RPM and 25°C to precipitate any suspended particles. Then, 1 mL of the extracted sample was transferred into 8 mL polypropylene tube. The sample tube placed on a Nitrogen purge (Eyela, MG 2200 model) to evaporate under high purified nitrogen gas at 50ºC for 30 minutes or until the sample in tube is completely dried. The sample was reconstituted into 1 mL with acetonitrile, then shook and transferred into vial for analysis by LC-MS/MS.

3.9.3 Ethanol/water extract sample

Further studies were conducted to simulate food contained condition. Ethanol/water mixtures were used to extract paper sample at difference temperature. In order to carry out solvent extraction at each elevated temperature, a PLE apparatus was used Accelerated Solvent Extractor (ASE). The 1:9 ethanol/water and 19:1 ethanol/water extraction were prepared and extract the paper sample by the same process with the saliva stimulant extract sample. Then, the sample was transferred into vial for analysis by LC-MS/MS.

3.10 The calibration standard preparation

The calibrated concentrations of PFOS and PFOA mixed solution ranging from 0.5 to 10 µg L^{-1} were prepared in 50:50 (v/v) methanol/ultrapure water and 40:60 (v/v) acetonitrile/ultrapure, which solutions matched to the final preparation of extracted sample.

3.10.1 The calibration standard for the methanol extract sample

The calibration standards of PFOS and PFOA were prepared in 7 concentration levels at 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg L^{-1} in a composition of 50:50 v/v methanol and ultrapure water.

3.10.2 The calibration standard for saliva simulant and ethanol/water extract sample

The calibration standards were prepared in 40:60 v/v acetonitrile and ultrapure water by added PFOS and PFOA mixed standard into the composition at 7 concentration levels of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg L^{-1}.

3.11 Analysis

The analysis was performed using an Agilent 1200 SL high performance liquid chromatography (HPLC, Agilent Technologies, United States) interfaced to an Agilent 6400 triple quadrupole mass spectrometer (MS/MS, Agilent Technologies, United States). The HPLC column was an Agilent Eclipse XDB-C_{18} 4.6 x 50 mm, 1.8 µm particle sizes and Agilent Eclipse Plus C_{18}, 2.1 x 100 mm, 1.8 µm particle sizes and maintained at a temperature of 40°C. The sample injection volume was 10 µL. The mobile phase used was comprised of (A) 10 mM ammonium acetate (CH_3COONH_4) in ultrapure water and (B) HPLC grade acetonitrile (CH_3CN). The column was flushed using a gradient that increased the acetonitrile to 90% by the process started with initial condition of 45% (B), increased to 50% (B) at 5.0 min, then to 60% (B) at 5.5 min, held at 60% (B) for 4.5 min, went up to 90% (B) at 15 min, along with a flow rate of 0.25 mL min^{-1}. The mobile phase program is found in Table 3.3.

Table 3.3: HPLC column flushing gradient

Time (min)	% A	% B	Flow (mL min^{-1})
0.0	55	45	0.25
5.0	50	50	0.25
5.5	40	60	0.25
10.0	40	60	0.25
15.0	10	90	0.25

The mass spectrometer parameters were optimized to transmit the parent ions, fragment them and monitor the daughter ions. The MS/MS was operated to detect liquid sample in electrospray ionization (ESI) negative mode with capillary voltage 3500 V. Analyte ions were monitored by using multiple reaction monitoring (MRM) mode. Ions selected were 499 (parent) for PFOS and 413 (parent) for PFOA. Ions monitored were 80 (daughter) for PFOS and 369 (daughter) for PFOA. Nitrogen was the collision gas, the collision energy were 55 V for PFOS and 5 V for PFOA. The PFOS and PFOA retention time (RT) under these conditions were 10.9 and 4.8 min respectively. The MS/MS characteristics for the target compounds were shown in Table 3.4.

Table 3.4: Mass spectrometer characteristics for PFOS and PFOA

Target Compound	**Retention time (min)**	**Parent ion (m/z)**	**Productivity ion (m/z)**	**Dwell time* (ms)**	**Collision energy (V)**
PFOS	10.9	499	80	50	55
PFOA	4.8	413	369	50	5

**Dwell time: pause between mass ranges*

The instrument was calibrated for PFOS and PFOA by seven concentration levels of PFOS and PFOA mixed solution in the range of 0.05 – 10.00 µg L^{-1}. Most samples were analyzed shortly after preparation. Otherwise, they were stored in the refrigerator in polypropylene vials at 4°C and analyzed within 1 week. Following Table 3.5 shows the summary of LC-MS/MS instrumentations and their optimized conditions for quantification of PFOS and PFOA.

Table 3.5: Summary of analytical operation setting condition for liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

	Parameters	**Operating Conditions**				
LC	Instrument	Agilent 1200 SL HPLC				
	Column	Agilent Eclipse XDB - C_{18}, 4.6 x 50 mm, 1.8 µm and Plus C_{18}, 2.1 x 100 mm, 1.8 µm				
	Mobile phase	A: 10mM CH_3COONH_4/H_2O B: CH_3CN				
	Gradient	Time (min)	0.0	5.0	5.5	10.0	15.0
		% CH_3CN	45	50	60	60	90
	Flow	0.25 (mL min^{-1})				
	Injection volume	10 µL				
	Column temp.	40°C				
MS/MS	Instrument	Agilent 6400 triple quadrupole mass spectrometer				
	Type	Triple state quadrupole				
	MS/MS operation	MRM (multiple reaction mode)				
	Source	ESI (electrospray ionization)				
	Polarity	Negative				
	Gas temp.	300°C				
	Gas flow	10 L min^{-1}				
	Nebulizer	50 psi				
	Capillary voltage	3500 V				

3.12 Calculation of uptake doses

The result to be obtained from this research study was estimated the uptake doses of PFOS and PFOA from food packaging to dietary pathway. The exposure assessment (expressed in ng (kg bw)$^{-1}$ day^{-1}) was calculated according to equation 3.1, which is based on the algorithm from Trudel *et.al.*, (2008), for the estimation of adult exposure.

Equation 3.1: Equation for uptake doses for food contact paper material pathway modeled of this study (Trudel *et.al.*, 2008)

$$D_{fcm} = \frac{C_{pc} \cdot r_{migr} \cdot MF_{pc} \cdot f_{food_{pc}} \cdot A_{contact} \cdot t_{contact}}{m_{bw}} \cdot F_{uptake}$$

This equation shows the uptake doses is a function of;

C_{pc} : concentration of PFOA in food contact material (ng cm^{-2})

r_{migr} : migration rate of PFOA from food contact material into food (hour^{-1})

MF_{pc} : market fraction of food contact material treated with PFCs

f_{food_pc}: contact frequency of food with treated materials (day^{-1})

$A_{contact}$: contact area of food with contact material (cm^2),

$t_{contact}$: contact time of food with contact material (hour)

m_{bw} : body weight (kg)

F_{uptake} : uptake fraction of PFOS and PFOA via the gastrointestinal tract

Trudel *et.al.*, (2008) modeled this pathway for PFOA only but in this study this pathway was also used for PFOS. Begley *et.al.*, (2005) have demonstrated the fluorotelomers may migrate from food contact materials into food with a rate of approximately $5 \cdot 10^{-4}$ min. This rate is adopted here for PFOS and PFOA. The fraction of PFC containing food contact materials on market is assumed to be 10%, 50%, and 100% in the low-exposure, intermediate, and high-exposure scenarios. The contact frequency of food with treated materials is assumed to be once per month in the low-exposure scenarios, in the intermediate and high-exposure scenarios, the frequency of contact is set equal to the frequency of consuming fast food in adult

Europe population. The contact time of food with treated materials is set to 0.25 hour, 0.5 hour, and 1 hour. The uptake fraction is applied to all consumer groups and equals 66%, 80%, and 91% in the low-exposure, intermediate-exposure, and high-exposure scenarios. The summarized of assumed parameter values shown in Table3.6.

Table 3.6: The assumed parameter value for three scenarios exposure

Parameter	Unit	Low	Int	High
MF_{pc}	%	10	50	100
f_{food_pc}	day^{-1}	0.03	0.1	0.4
$t_{contact}$	hour	0.25	0.5	1
F_{uptake}	%	66	80	91

CHAPTER IV
RESULTS AND DISCUSSION

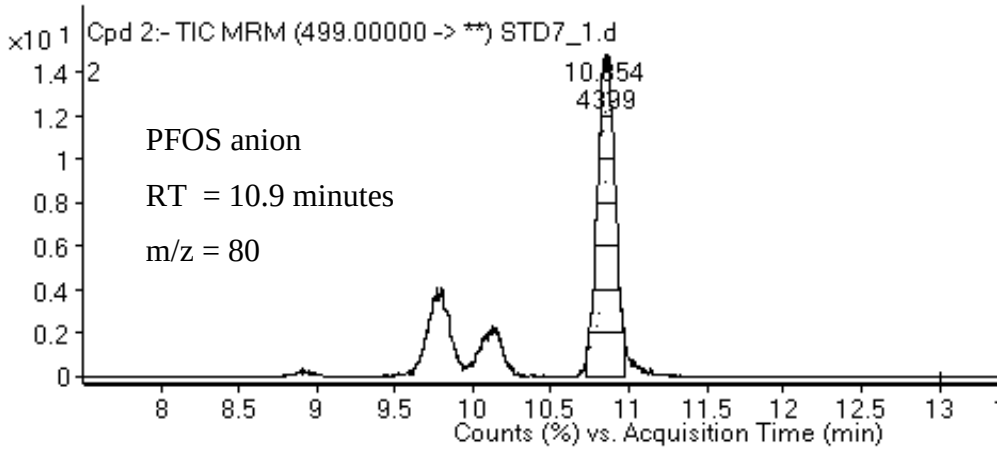
4.1 Analytical method performance

The analysis was performed using an Agilent 1200 SL high performance liquid chromatography (HPLC, Agilent Technologies, Japan) interfaced to an Agilent 6400 triple quadrupole mass spectrometer (MS/MS, Agilent Technologies, Japan). Calibration curves were obtained by dilution of PFOS and PFOA mixed standard solutions. The calibrated concentrations of PFOS and PFOA ranging from 0.05 to 10 µg L^{-1} were prepared in 50:50 (v/v) methanol/ultrapure water and 40:60 (v/v) acetonitrile/ultrapure, which solutions matched to the final preparation of extracted sample. The different component in PFOS and PFOA mixed standard solution between the 50:50 (v/v) methanol/ultrapure water solution and 40:60 (v/v) acetonitrile/ultrapure water solution could confirm the different component of standard solution by observing and determining the slope of standard curve. If the solution effect is not presented, both slopes of the standard curve and the spiked sample calibration should be the same. Table 4.1 shows the standard and the spiked sample calibration equations. Their slopes were not much different, indicating the interference was not occurred from the solution compositions. The calibration curves of PFOS and PFOA in 50:50 (v/v) methanol and ultrapure water solution were linear response. Correlation of determination (R^2) was 0.9999 for both PFOS and PFOA. In case of the calibration curves of PFOS and PFOA in 40:60 (v/v) acetonitrile and ultrapure water solution, R^2 of PFOS and PFOA were 0.9998 and 0.9999, respectively. R^2 achieved from this study were greater than 0.9995, which is the required level for the accepted accuracy to verify of linearity. The example chromatogram of PFOS and PFOA at 10 ppb is shown in Figure 4.1, with the retention times (RT) of PFOS and PFOA at 10.9 and 4.8 minutes, respectively. Selected ion of PFOS and PFOA were 80 and 369, respectively.

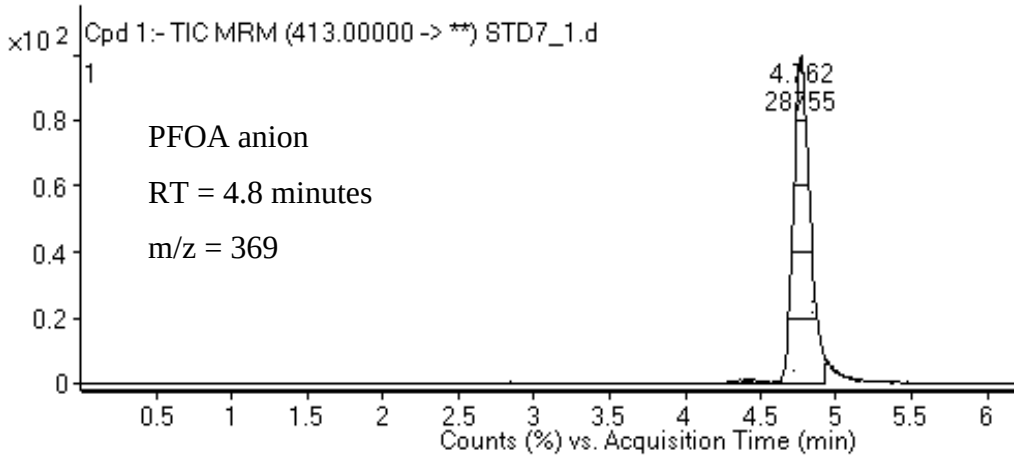
Table 4.1: Linear regression equations of standard PFOS and PFOA

Calibration curves of PFOS and PFOA	PFOS (C8-S)		PFOA (C8-A)	
	Linear regression equation	R^2	Linear regression equation	R^2
in methanol:water, 50:50 (v/v)	y = 427.8319 x	0.9999	y = 1379.0101x	0.9999
in acetonitrile:water, 40: 60 (v/v)	y = 436.7081x – 42.80	0.9998	y = 928.7694x + 44.35	0.9999

y is the response and x is the concentration ($\mu g\ L^{-1}$)



(a)



(b)

Figure 4.1: Example chromatograms of (a) PFOS and (b) PFOA at 10 ug L^{-1}

4.2 Development of the pressurized liquid extraction (PLE) technique

This study was determined the extraction efficiency of pressurized liquid extraction (PLE) technique. PLE technique was extracted PFOS and PFOA from food packaging products made of paper material. Influence parameters of PLE technique were carefully evaluated by extracted concentration of sample in low level (ng g^{-1}). The parameters for evaluation of PLE technique were static extraction time, flush volume and number of flush cycles. In the part of extraction technique development, paper cup of instant noodle was used as the representative of paper sample to extract with PLE technique.

4.2.1 Effect of static extraction time on PLE

Static extraction times on PLE technique were investigated for different time period at 5, 10, 20 and 30 minutes. These results are shown in Figure 4.2. The X-axis shows the extraction time on PLE technique in the unit of minute and the Y-axis shows the extracted concentration of PFOS and PFOA from paper samples in the unit of ng g^{-1}. In the line graph, concentration of PFOS was shown in the black line with circle (–●–) and the grey line with triangle (–▲–) was the concentration of PFOA. The extracted efficiency of different extraction time period for PFOS and PFOA were increased with increasing the static extraction time. Also, the trend of extracted concentrations of both target compounds was related when increasing the static extraction time. The extracted concentration of PFOS and PFOA were almost constant after 20 minutes. Thus, for the different static extraction times, static extraction time of 30 minutes was the most efficient in extracting PFOS and PFOA from food packaging sample.

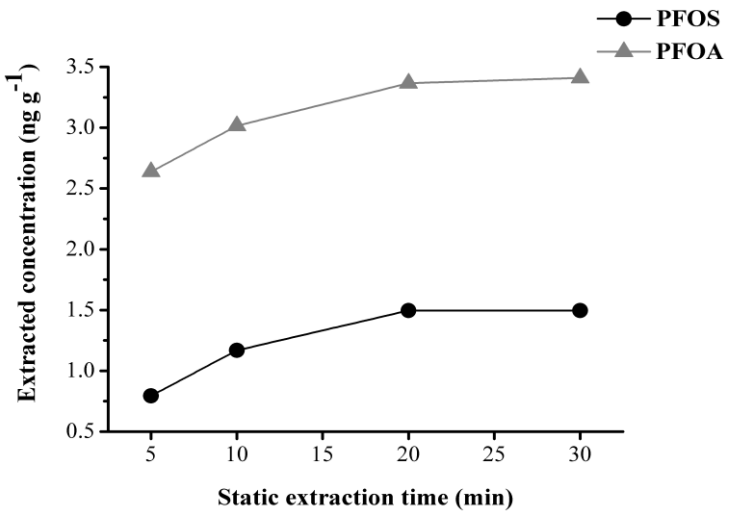
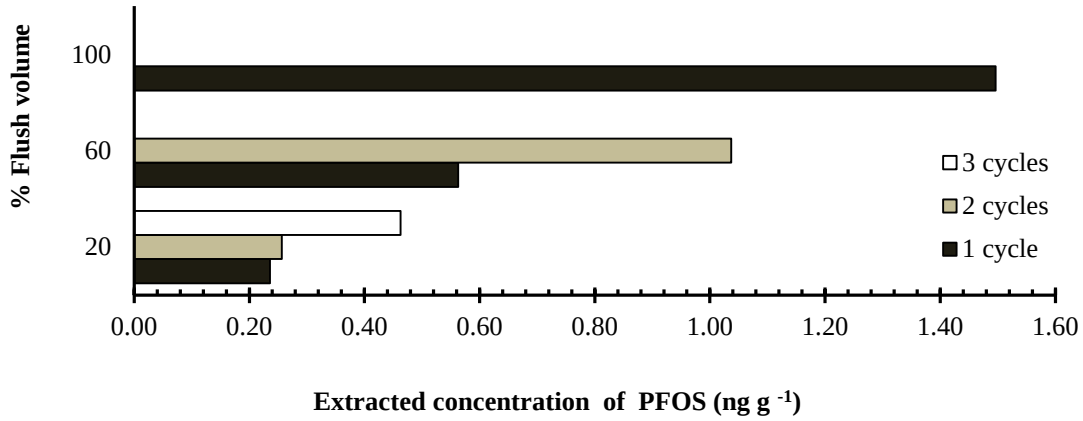


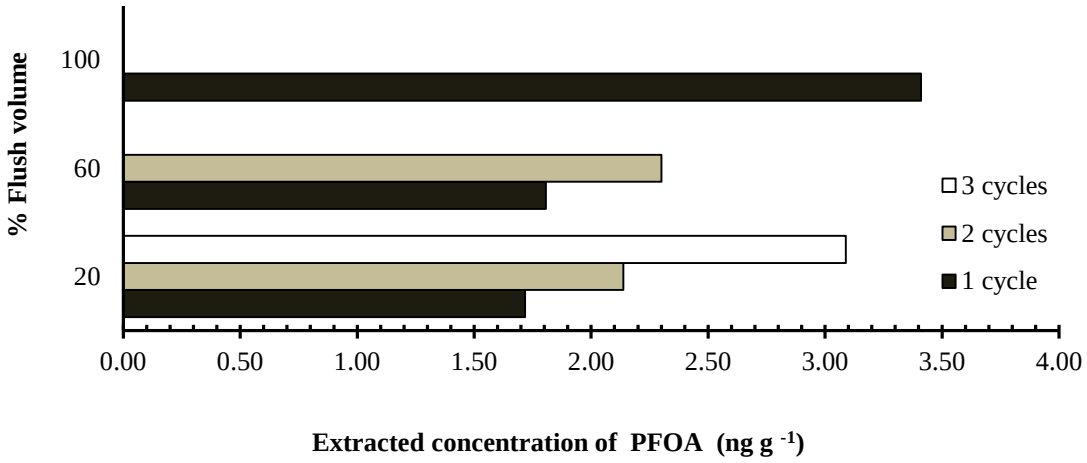
Figure 4.2: Effect of static extraction time on PLE technique to extract PFOS and PFOA in paper sample (noodle cup)

4.2.2 Effect of flush volume and extraction cycle on PLE

Figure 4.3 shows that flush volume and extraction cycle of PLE effect to extracted PFOS and PFOA in paper samples. The X-axis shows the extracted concentration of PFOS (Figure 4.3 (a)) and PFOA (Figure 4.3 (b)) in the unit of ng g^{-1}. The Y-axis shows the percentage of flush volume at 20%, 60% and 100%. The number of extraction cycle were shown in different color of columns, one extraction cycle was showed in black column, for two and three extraction cycles were shown in grey and white columns, respectively. The results show that when increase number of extraction cycles, it appeared that PLE was more efficient in extraction of PFOS and PFOA. Also in flush volume, the extraction of PFOS and PFOA was more efficient when increasing flush volume. However, the extracted sample was deeply colored and contained some co-extracted materials and the totally time for extraction process with PLE were increased when increasing the number of extraction cycle. Thus, the method of the 100% flush volume couple with one cycle was the most efficient to extract PFOS and PFOA in paper sample.



(a)



(b)

Figure 4.3: Effect of flush volume and extraction cycle on the pressurized liquid extraction to extract (a) PFOS and (b) PFOA in paper samples

4.2.3 Ensuring of the selective PLE technique

As can be seen from the results 4.2.1 and 4.2.2, the study found that the optimum condition of PLE technique was 30 minutes static extraction time with flush volume of 100% cell volume and one extraction cycle under temperature of 80°C and 1000 psi. The selected method was then validated in washed paper sample at three different levels of PFOS and PFOA fortified control samples (low, medium, and high). Average percent recoveries and standard deviations (SD) at each fortification level for PFOS and PFOA in paper samples are summarized in Table 4.2. The average

recoveries were always higher than 79 % with relative standard deviation (RSD) lower than 11% in seven control samples for each level. Optimization of the PLE procedure was established based on the recovery data, accuracy, precision, and repeatability of the method. The results showed that the developed extraction method could be satisfactorily used to determine PFOS and PFOA concentrations in paper packaging materials.

Table 4.2: Recoveries of PFOS and PFOA on the optimum PLE technique for extracted PFOS and PFOA in food packaging

	Amount Fortified (ng/g)*	**Recovery**		
		Min - Max (%)	**Avg. ± SD (%)**	**RSD (%)**
PFOS	**5**	70.20 - 86.40	79.46 ± 6.14	7.73
	50	74.51 - 96.24	86.53 ± 9.26	10.70
	200	79.94 - 98.42	89.72 ± 7.44	8.29
PFOA	**5**	84.48 – 94.56	90.14 ± 3.36	3.73
	50	84.92 – 99.48	92.39 ± 6.10	6.60
	200	89.81 – 99.77	92.86 ± 3.62	3.90

* *Number of replicates = 7 for each fortification level*

4.3 PFOS and PFOA in food packaging

The optimized PLE technique was applied to determine PFOS and PFOA in food packaging samples. Totally 34 samples were purchased from the domestic and international brands of restaurants/cafes located in Bangkok, Thailand. The details of paper packaging samples were showed in Table 4.3.

Table 4.3: Details of food packaging samples

Categories	Brand	Detail	Area size, dm^2/g
Instant food cup	Brand #1	Noodle cup	0.35
		Instant rice porridge cup	0.35
	Brand #2	Noodle cup	0.40
		Instant rice porridge cup	0.40
	Brand #3	Instant rice porridge cup	0.40
	Brand #4	Instant rice porridge cup	0.30
Microwave-popcorn bag	Brand #5	Microwave-popcorn bag	1.20
	Brand #6	Microwave-popcorn bag	1.30
Beverage cup	Brand #7	Hot cup	0.40
	Brand #8	Hot cup	0.30
	Brand #9	Cool cup	0.35
Ice cream cup	Brand #10	Ice-cream cup	0.40
	Brand #11	Ice-cream cup	0.45
Fast food container	Brand #12	Fried-chicken box	0.40
		french-fried bag	2.00
		french-fried wrapper	2.40
		Fried-chicken Wrapper	2.50
	Brand #13	Fried-chicken box	0.40
		french-fried bag	2.20
		french-fried box	0.50
		Hamburger wrapper	3.50
Dessert container	Brand #14	Pretzels box	0.35
		Pretzels wrapper	2.10
	Brand #15	Donut box	0.35
		Donut wrapper	4.40
	Brand #16	Donut box	0.30
		Donut wrapper	4.65
		Donut bag	2.25
Baking paper	Brand #17	Wrapper	2.45

4.3.1 Concentration of PFOS and PFOA

Optimum of PLE technique with methanol extraction was determined the overall concentration of PFOS and PFOA in food packaging from domestic and international brands of restaurants and cafes located in Bangkok, Thailand. All samples were prepared and analyzed in duplicate. Figure 4.4 shows those contamination levels of PFOS and PFOA in the collected paper samples. The X-axis were categories of food packaging samples including instant food cups (IFC), microwave-popcorn bags (MPB), beverage cups (BRC), ice cream cups (ICC), fast food containers (FFC), dessert containers (DSC), and baking papers (BKP). The average concentration of the target PFCs were 4.89 and 2.87 ng g^{-1} in PFOS and PFOA, respectively. Among the PFOS, the highest average concentration was found in fast food containers, followed by beverage cups, baking papers, ice cream cups, instant food cups, dessert containers and microwave-popcorn bags. The highest average concentration of PFOA was found in fast food containers, followed by ice cream cups, baking papers, instant food cups, beverage cups, microwave-popcorn bags and dessert containers**.**

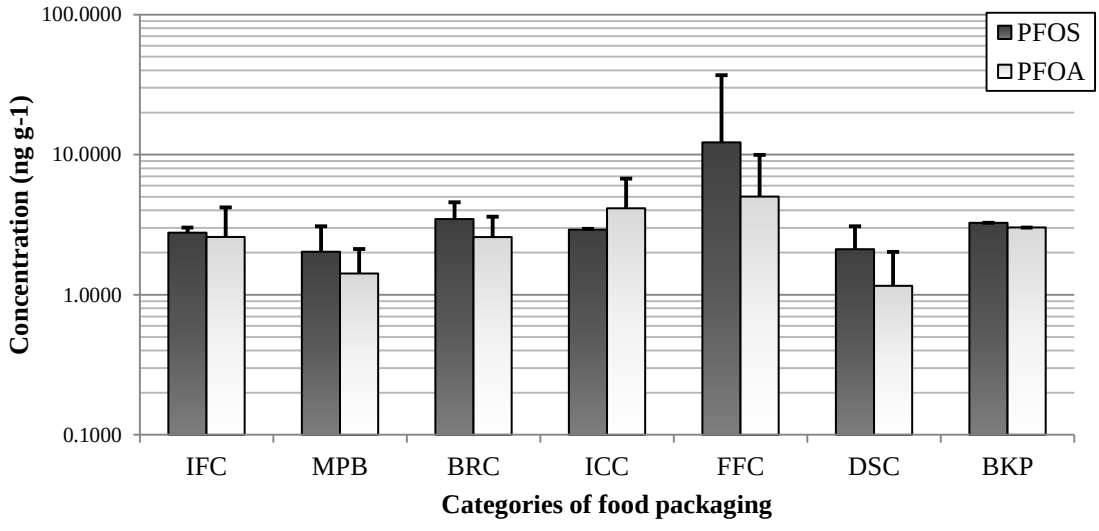


Figure 4.4: Concentration of PFOS and PFOA in food packaging

Both of PFOS and PFOA was found the highest concentrations in fast food containers at the concentration level of 36.99 and 9.99 ng g^{-1}, respectively. All

samples in this category of paper packaging were found the contamination of PFOS and PFOA as shown in Table 4.4. The concentration of PFOS ranged from 3.01 to 36.99 ng g^{-1} and that of PFOA from 1.43 to 9.99 ng g^{-1}.

Table 4.4: Concentration of PFOS and PFOA in fast food container

Sample	Brand	PFOS (ng g^{-1})	PFOA (ng g^{-1})
Fried-chicken box	#1	5.02	6.43
French-fried bag	#1	17.21	8.73
French-fried wrapper	#1	21.98	9.99
Fried-chicken Wrapper	#1	7.34	5.90
Fried-chicken box	#2	36.99	1.54
French-fried bag	#2	3.01	1.43
French-fried box	#2	3.45	4.70
Hamburger wrapper	#2	3.01	1.46
		$\bar{X}$ = 12.25	$\bar{X}$ = 5.02

**samples were prepared in duplication for each sample*

4.3.2 Migration of PFOS and PFOA from food packaging through contact with saliva simulant

Migration methods are intended to determine the extent to which the PFOS and PFOA can be extracted from food packaging under conditions relevant to real – life activities. Determination of PFOS and PFOA migrated from food packaging by saliva simulant using PLE technique is shown in Figure 4.5. The X-axis were categories of food packaging samples including instant food cups (IFC), microwave-popcorn bags (MPB), beverage cups (BRC), ice cream cups (ICC), fast food containers (FFC), dessert containers (DSC), and baking papers (BKP). The average concentration of the target PFCs were 2.47 and 2.84 ng g^{-1} in PFOS and PFOA, respectively. The highest concentration of PFOS was found in fast food containers, followed by microwave-popcorn bags, ice cream cups, instant food cups, beverage cups, and dessert containers. The highest concentration of PFOA was also found in fast food containers, followed by microwave-popcorn bags, dessert containers, instant

food cups, ice cream cups, and beverage cups. Both of PFCs compounds were not found in a baking paper.

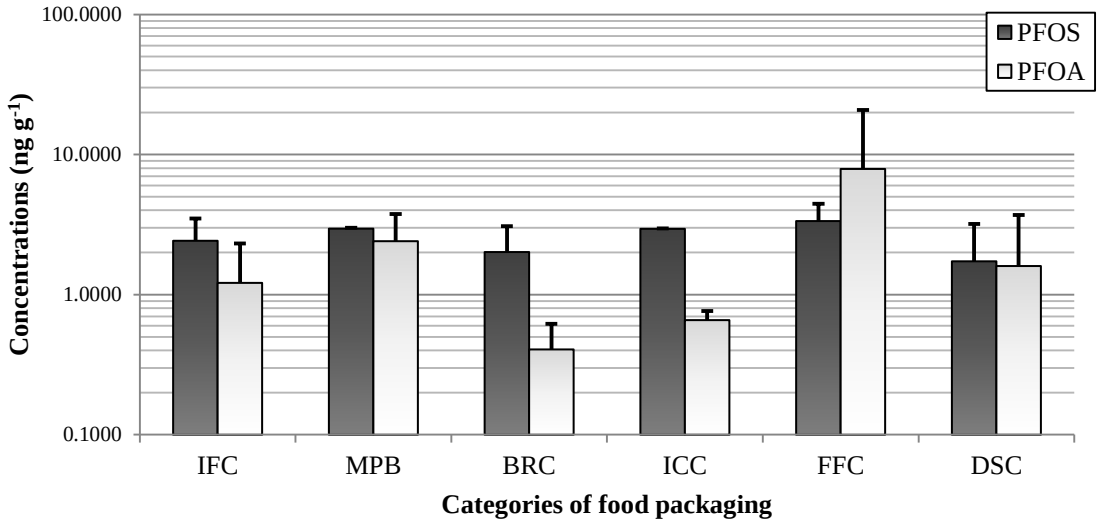


Figure 4.5: Concentration of PFOS and PFOA migrate from food packaging by saliva simulant

Table 4.5: Migration of PFOS and PFOA by saliva simulant extraction in fast food container

Sample	Brand	PFOS (ng g^{-1})	PFOA (ng g^{-1})
Fried-chicken box	#1	3.66	6.06
French-fried bag	#1	4.45	6.56
French-fried wrapper	#1	2.97	8.94
Fried-chicken Wrapper	#1	3.52	4.41
Fried-chicken box	#2	3.01	7.09
French-fried bag	#2	3.20	5.85
French-fried box	#2	3.01	20.86
Hamburger wrapper	#2	3.03	3.48
		$\bar{X}$ = 3.36	**$\bar{X}$ = 7.91**

**samples were prepared in duplication for each sample*

Both of PFOS and PFOA was found the highest concentration in fast food container sample at the concentration of 4.45 and 20.86 ng g^{-1}, respectively. All samples in this category of paper packaging were found the contamination of PFOS and PFOA as show in Table 4.6. The concentration of PFOS ranged from 2.97 to 4.45 ng g^{-1} and that of PFOA from 3.48 to 20.86 ng g^{-1}.

4.4 Comparison of PFOS and PFOA concentration with areas of paper packaging

4.4.1 PFOS and PFOA concentration in food packaging

Weight based concentration are converted into per-area concentration by calculating the area in one gram of paper samples in Table 4.3. The extracted samples by methanol extraction had an average concentration of PFOS and PFOA at 8.57 and 5.03 ng dm^{-2}, respectively, while the average concentration of PFOS and PFOA were 4.80 and 4.55 ng dm^{-2}, respectively for the extracted sample by saliva simulant extraction. The PFOS and PFOA were detected in almost all food packaging samples made of paper. A detailed overview of the results is given in Table 4.6.

The extracted samples by methanol have highest concentration of PFOS in fast food container samples at level of 92.48 ng dm^{-2}, and for PFOA the highest concentration of extracted sample by methanol was ice cream cup samples at the level of 16.91 ng dm^{-2}. PFOS and PFOA migration of food packaging samples by saliva simulant have the highest concentration in beverage cup samples at the level of 10.26 ng dm^{-2} for PFOS and 41.71 ng dm^{-2} in fast food container samples for PFOA. The data from this study shows low levels of PFOS and PFOA (< 100 ng dm^{-2}) presented in the food packaging samples, indicating that not all brands of food packaging samples included PFOS and PFOA as coating materials on the food-contact side or as additive into the paper material. The previous study showed the level of PFOA analysis in paper products from USA with the highest concentration level at 290 µg kg^{-1} in microwave-popcorn bag but in some paper products were not detected PFOA such as sandwich wrapper, hamburger wrapper and french-fried box (Begley *et al.*, 2005). In this study the highest concentration of PFOA was in ice cream cup at 16.91 ng dm^{-2} (~7.27 µg kg^{-1}) as shown in Table 4.6, which the concentration of the sample

was less than the microwave-popcorn bag in the previous study around 40 times. Some food packaging samples were bought from small restaurants/cafes and may not necessarily coated/added with fluorochemicals for saving cost when the owners bought the food packaging from industry or wholesale store, which may explain the absence of detectable PFOS and PFOA level in some samples.

Table 4.6: The PFOS and PFOA concentration with area of food packaging

Sample (n)*	Avg. Area, $dm^2\ g^{-1}$	PFOS, ng dm^{-2}				PFOA, ng dm^{-2}			
		Methanol		Saliva simulant		Methanol		Saliva simulant	
		$\bar{x}$	(max-min)	$\bar{x}$	(max-min)	$\bar{x}$	(max-min)	$\bar{x}$	(max-min)
Instant food cup (10)	0.36	7.76	(9.83-4.21)	6.60	(8.72-0.00)	7.31	(12.02-2.36)	3.35	(6.61-0.00)
Microwave-popcorn bag (3)	1.23	1.69	(2.56-0.00)	2.41	(2.51-2.26)	1.18	(1.77-0.12)	1.93	(2.90-1.35)
Beverage cup (3)	0.35	9.83	(11.45-8.43)	5.90	(10.26-0.00)	7.14	(10.29-3.01)	1.19	(2.06-0.00)
Ice cream cup (2)	0.43	6.89	(7.37-6.41)	6.97	(7.35-6.59)	10.15	(16.91-3.39)	1.57	(1.91-1.23)
Fast food container (8)	1.74	16.86	(92.48-0.86)	3.74	(9.15-0.86)	5.16	(16.07-0.42)	10.88	(41.71 -0.99)
Dessert container (7)	2.06	4.16	(10.04-0.00)	4.05	(9.92-0.00)	1.43	(5.79-0.09)	3.11	(12.34-0.00)
Baking paper (1)	2.45	1.33	-	0.00	-	1.23	-	0.00	-
Average	**1.18**	**8.57**		**4.80**		**5.03**		**4.55**	

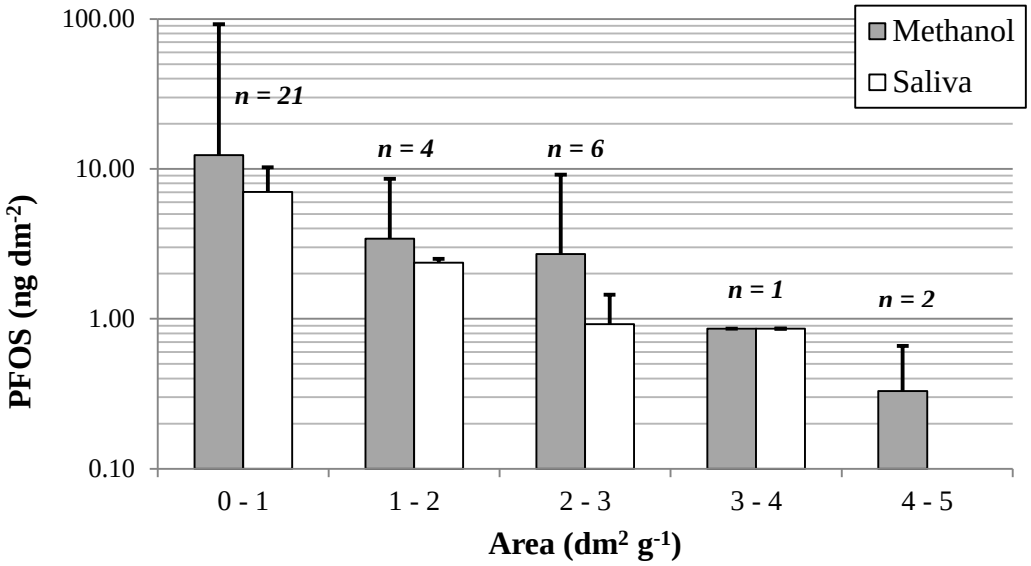
**Sample (n): Number of sample, samples were prepared in duplication for each sample*

Jogsten et al. (2009) investigated the concentrations of PFCs in food and packaged food in Spain. Their study showed that PFOS was the compound most frequently detected, which range from 0.01 – 0.33 ng g^{-1} and the concentration of PFOA was only found in one sample at 0.675 ng g^{-1}. Meanwhile the average concentrations of PFOS and PFOA in food packaging of this study were ~10.11 and ~5.94 ng g^{-1} (Table 4.6), respectively, which was higher than in packaged food of Jogsten et al. (2009)'s study. Concentration of PFOS and PFOA found in both

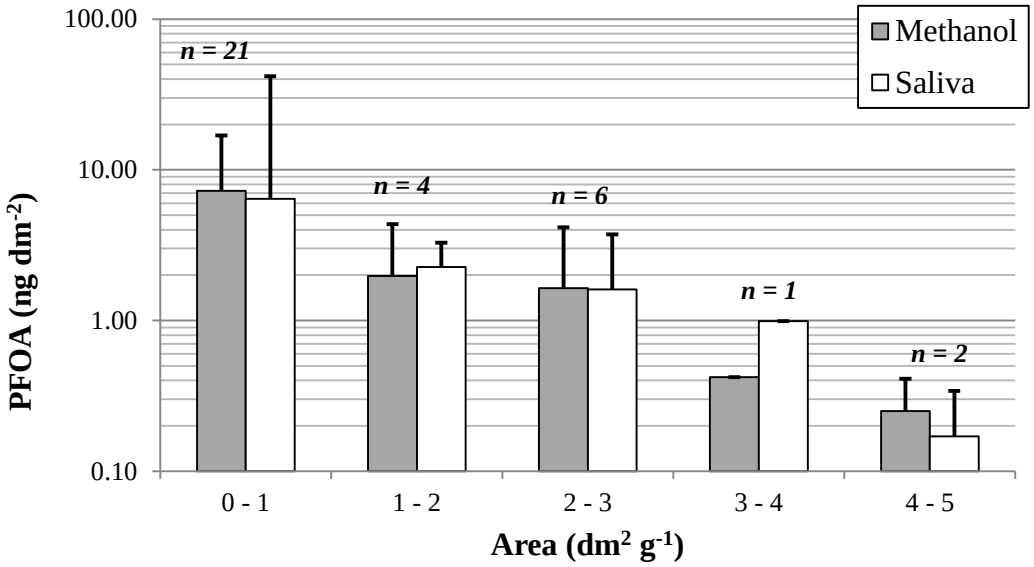
packaged food and food packaging items suggesting that food packaging might play as a source of human exposure to PFOS and PFOA through the diet.

4.4.2 Comparison between the areas of paper packaging effected to the migration of PFOS and PFOA

Comparison of the PFOS and PFOA concentration in food packaging samples by the area size in one gram of paper sample at the value range between ≤ 1 to 5 dm^2 g^{-1}. In one gram of paper sample, the area size was the inversion of the thickness of paper sample. If they have more area size in one gram, it means that they have less thickness. In Figure 4.6, the results showed that the concentration of PFOS and PFOA extracted from paper samples by methanol and saliva simulant were comparable. The results presented a significant relation of PFOS and PFOA concentration to the area size. The concentrations increased when the area size of paper samples were decreased. The highest concentration was in the area of paper sample ≤ 1 dm^2 g^{-1}. The food packaging samples in the area at ≤ 1 dm^2 g^{-1} were instant food cup, ice cream cup and some kinds of fast food container such as fried-chicken box and french-fried box. This group of paper samples has the most thickness, the average concentration level of PFOS and PFOA in 10 cm x 10 cm of paper sample area were 12.38 and 7.25 ng, respectively. For the saliva simulant, PFOS and PFOA can be leached from the group of the most thickness paper sample at the level of 7.01 and 6.41 ng, respectively. The results indicated that less area size (more thickness) in one gram of paper samples might be coated or added with larger quantities of the PFOS and PFOA in paper materials. Moreover, this group of paper sample has widely use in food and beverage packaging. They can easily leach into food and beverages from the food packaging made of paper. Saliva simulant can migrate the PFOS and PFOA from paper sample at high level of concentration almost the same level with the paper sample extracted by methanol extraction (Figure 4.6). Therefore, the potential significant impact on human health is high when consuming the food and beverage which contained in paper packaging.



(a)



(b)

Figure 4.6: (a) PFOS and (b) PFOA leached from the difference area size in one gram of food packaging samples

4.5 Simulated condition of PFOS and PFOA leaching from food packaging

4.5.1 Simulation of watery and acidic foods condition

A mixture of 1:9 ethanol/water was used to simulate watery and acidic foods. The results as shown in Figure 4.7, the simulation conditions were varied with temperature at 40, 80, 120, 160, 200 °C and room temperature as shown in the X-axis of Figure 4.7. The Y-axis was showed the extracted concentration of PFOS and PFOA in unit of ng g^{-1}. The extracted concentration levels of PFOS was showed in the black circle marker (●) and grey triangle marker (△) was the extracted concentration levels of PFOA. Extracted concentrations of PFOS were constant from the room temperature to 160°C. Although, the extracted concentrations of PFOS at different temperature were not much different, but the temperature at 80 °C have the highest extracted concentration of PFOS. The temperature of 80ºC is the food temperature, which expected to reach during containing food after cooking. In case of PFOA the extracted concentration was increased when increasing the temperature until 160°C. The PFOA extractable under simulated condition of watery and acidic food was highest concentration at temperature of 160ºC. Powley (2005) simulated watery and acidic foods to extract PFOA from cookware by used PLE with 1:9 ethanol/water at the temperature of 125ºC. The previous study found that PFOA was reached from cookware at the concentration level of < 10 ng dm^{-2}. Comparing to this study, the paper sample have the approximately area at 0.35 dm^2 g and the PFOA concentration at 120ºC was about 6 ng g^{-1}, which can be calculated the PFOA concentration to the value of 17 ng dm^{-2}. An ethanol/water mixture of 1:9 could not extract PFOS and PFOA from paper sample at temperature of 200ºC because the paper sample was burned under this condition. The temperature at 200ºC could not extract PFOS and PFOA, the cause might be related to the boiling point of these chemical. In case of PFOA had the boiling point at 189ºC, might be after this temperature point PFOA is possible to degrade. Further study, should set the temperature at this point to study the effect of boiling point to degrade these chemical with high pressure.

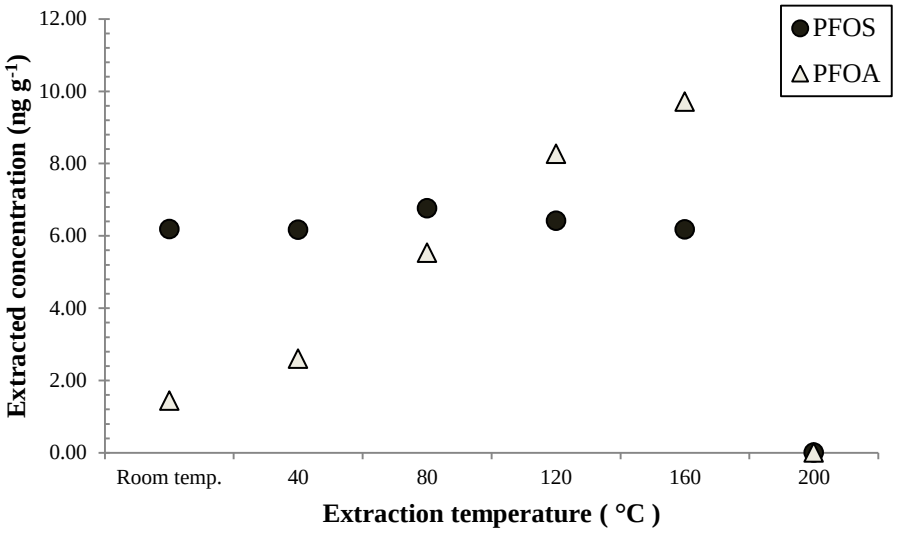


Figure 4.7: PFOS and PFOA leaching concentration under the simulation of watery and acidic foods

4.5.2 Simulation of fatty or oily foods condition

A mixture of 19:1 ethanol/water was used to simulate fatty or oily foods. The simulation conditions were varied with temperature at 40, 80, 120, 160, 200 °C and room temperature as shown in the X-axis of Figure 4.8. The Y-axis was showed the extracted concentration of PFOS and PFOA in unit of ng g^{-1}. The extracted concentration levels of PFOS was showed in the black circle marker ( ) and grey triangle marker ( ) was the extracted concentration levels of PFOA. Extracted concentration of PFOS was not extracted when the temperature were room temperature and 40ºC. Extracted concentration of PFOS was nearly constant from the temperature of 80 - 160°C. In case of PFOA the extracted concentration was increased when increasing the temperature from 40ºC to 160°C. The PFOA extractable under simulated condition of oily food was highest concentration at temperature of 160ºC. Also the same with 1:9 ethanol/water, an ethanol/water mixture of 19:1 could not extract PFOS and PFOA from paper sample at temperature of 200ºC because the paper sample was burned under this condition. The previous study, Powley (2005) found that the extraction of 19:1 ethanol/water mixture at the temperature of 125ºC could be extracted PFOA from cookware less than < 10 ng dm^{-2}. Comparing to this study, the

paper samples had the approximately are at 0.35 dm^2 g and the PFOA concentration at 120 ºC was about 2.5 ng g^{-1}, which can be calculated the PFOA concentration to the value of 7 ng dm^{-2}.

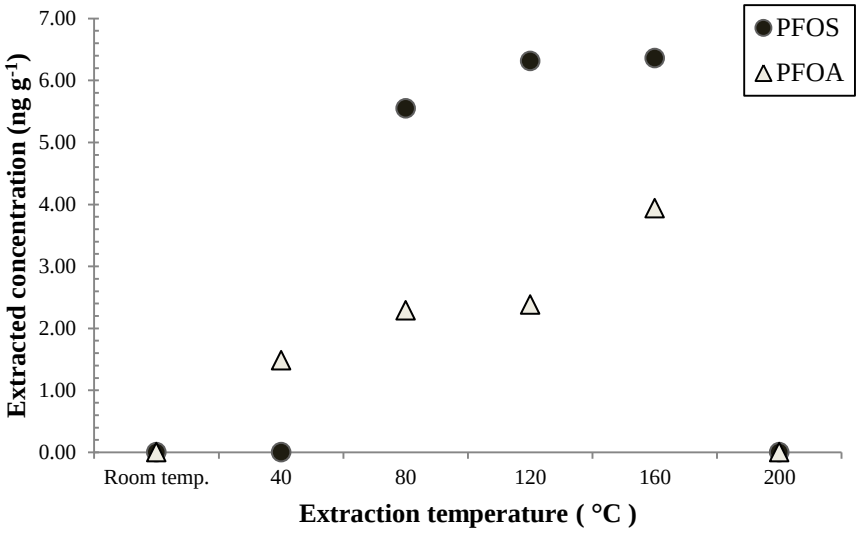


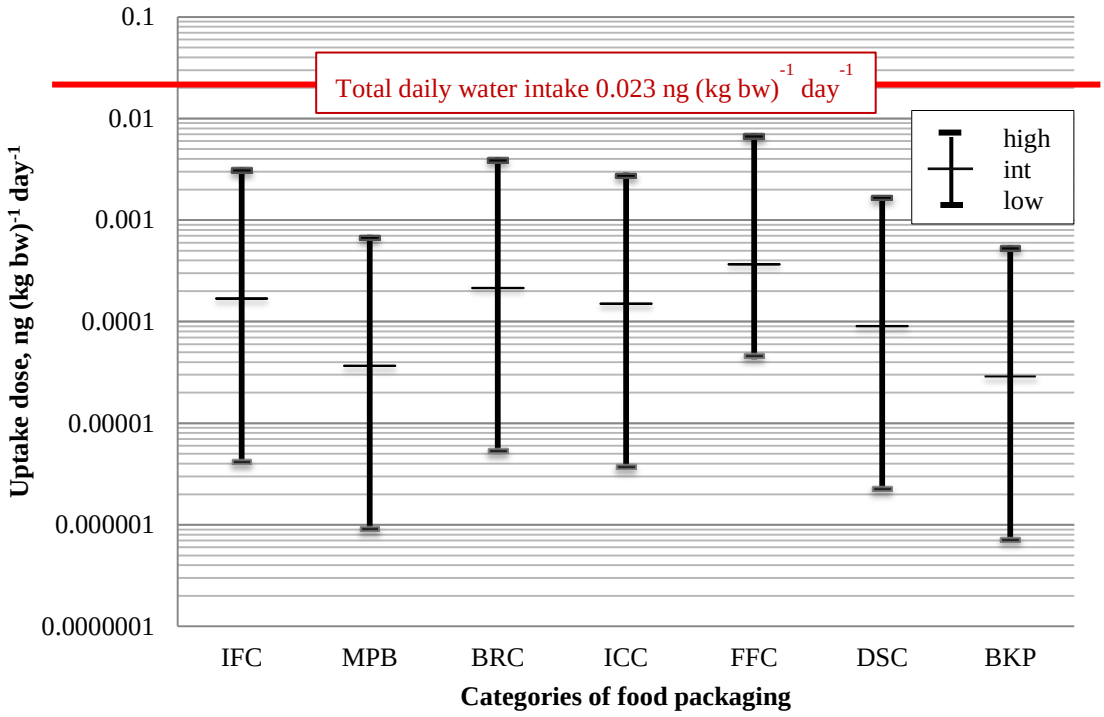
Figure 4.8: PFOS and PFOA leaching concentration under the simulation of oily food

4.6 Dietary exposure to PFOS and PFOA

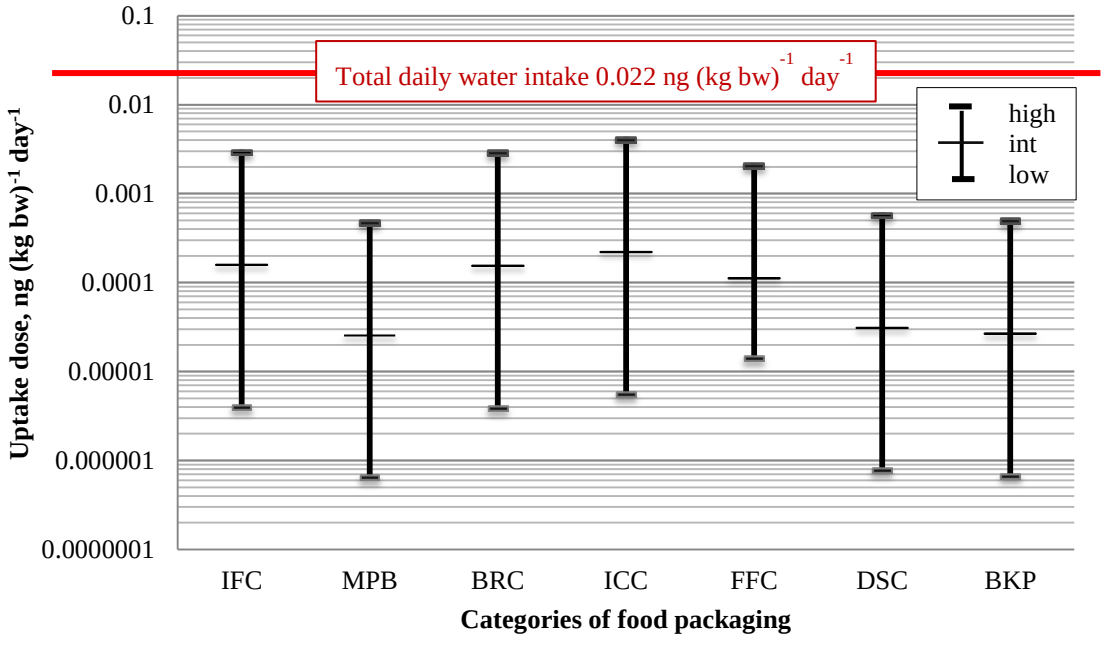
Contaminated food packaging products have been reported previously as important pathways of human exposure. Further, this study uses concentrations of PFOS and PFOA in food packaging samples to derive estimates of human exposure that are compared to the current EFSA tolerable daily intake values (TDIs) for these compounds (PFOS = 150 ng (kg bw) $^{-1}$ day^{-1}, PFOA = 1500 ng (kg bw) $^{-1}$ day^{-1}). These are a threshold value, which if surpassed are expected to produce adverse health effects. Due to the uncertainty involved within the assessment parameters, three scenarios of exposure have been used to deem whether at any of these levels the body burden of people could cross the TDI threshold. Scenarios represent realistic situations where exposure occurs in the everyday life of consumers. The exposure assessment was conducted for three scenarios to create a low, intermediate and high exposure value. For the purposes of dietary exposure estimates, an average body weight of 55 kg is assumed for the adult Asian population (FHO/WHO, 2005). The contact area of

food with contact material is assumed to be 200 cm^2, reflecting the use of such materials in various applications.

The results of estimating food packaging exposure to PFOS and PFOA are displayed in Figure 4.9. The X-axis is shows the categories of food packaging samples including instant food cups (IFC), microwave-popcorn bags (MPB), beverage cups (BRC), ice cream cups (ICC), fast food containers (FFC), dessert containers (DSC), and baking papers (BKP). The Y-axis is shows the exposure assessment (expressed in ng $(kg\ bw)^{-1}\ day^{-1}$) was calculated according to equation 3.1, which is based on the algorithm from Trudel *et.al.*, (2008), for the estimation of adult exposure. The results indicate that contaminated food packaging samples under the high-exposure of PFOS for adults uptake doses was highest in fast food container samples at 0.0067 ng $(kg\ bw)^{-1}\ day^{-1}$. UK FSA (2009) was given the value of total daily dietary intake for PFOS at 1 – 10 ng $(kg\ bw)^{-1}\ day^{-1}$. The drinking water concentrations were derived from Fromme *et al.*, (2009), Germany, the total daily water intake of PFOS was 0.023 ng $(kg\ bw)^{-1}\ day^{-1}$. The high- exposure of PFOA for adults uptake doses was highest in ice cream cups at 0.0040 ng $(kg\ bw)^{-1}\ day^{-1}$. The total daily dietary intake for PFOA was given at >0.55 – 10 ng $(kg\ bw)^{-1}\ day^{-1}$ by UK FSA and the total daily water intake of PFOA was 0.22 ng $(kg\ bw)^{-1}\ day^{-1}$ (Fromme *et al.*, 2009). Exposure pathway of PFOS and PFOA in food packaging was nearly the value of daily water intake. For both PFOS and PFOA the total exposure expected to be received by the general population is not believed to cause ill-health, because it remains well below the TDI thresholds.



(a)



(b)

Figure 4.9: Uptake dose of (a) PFOS and (b) PFOA from food packaging products in ng per kg body weight per day for low-exposure (low), intermediate-exposure (int), and high-exposure (high) scenario

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Application of pressurized liquid extraction (PLE) technique was investigated for the extraction of PFOS and PFOA from food packaging product made of paper material. Influence parameters of PLE were carefully evaluated by extracted concentration of sample in low level (ng g^{-1}). The study found that the optimum condition of PLE was 30 minutes static extraction time with flush volume of 100% cell volume and one extraction cycle under 80°C and 1000 psi. When developing a method for the PLE technique, there are many parameters to consider. In this study optimized PLE procedure was validated the absolute recovery from PFOS and PFOA fortified control samples at three different levels (5, 50, and 200 ng g^{-1}). The average recoveries were always higher than 79 % with relative standard deviation (RSD) lower than 11% in seven control samples for each level.

These results data from the analysis of 34 food packaging product made of paper material samples were contaminated with PFOS and PFOA and could be migrated from the packaging by saliva simulant. The average concentration of PFOS was 4.89 ng g^{-1} and 2.87 ng g^{-1} for PFOA. The concentrations of PFOS and PFOA were highest in fast food container samples at 36.99 ng g^{-1} and 9.99 ng g^{-1}, respectively. Almost all target analytes were detected in food packaging samples. The amount of PFOS and PFOA migrated from food packaging samples through contact with saliva simulant were 4.80 and 4.55 ng dm^{-2}, respectively. Saliva simulant can leach PFOS and PFOA from the group of the most thickness paper sample ($\leq$ 1 dm^2 g^{-1}) at the level of 7.01 and 6.41 ng dm^{-2} for PFOS and PFOA, respectively. The data presented in this study showed that the food packaging appeared to be a significant source of PFOS and PFOA to human exposure. These contaminants may enter to human by consuming food which contained in paper packaging.

5.2 Recommendations

The results can be used to provide the data for product-specific exposure assessment of PFOS and PFOA contamination in paper food packaging. The toxicity and the migration behavior of PFOS and PFOA are less known and require more attention. To ensure the safety of food packaging made of paper, these compounds should also be regulated. Despite considerable research in recent years, there remain numerous research gaps relating to the environmental impacts, movement, and toxicity of PFCs such as analysis of the actual migration of PFCs from their sources and the mechanisms involved, and more detailed study of the presence of PFOS and PFOA in human exposure.

REFERENCES

3M Company, (1999). Perfluorooctane sulfonate: Current summary of human sera, health and toxicity data. 3M Company, 21 January 1999.

Begley, T. H., White, K., Honigfort, P., Twaroski, M. L., Neches, R., & Walker, R. A. (2005). Perfluorochemicals: Potential sources of and migration from food packaging. [Article]. *Food Additives & Contaminants, 22*(10), 1023-1031. DOI: 10.1080/02652030500183474

Carloni, D. (2009). Perfluorooctane Sulfonate (PFOS) Production and Use: Past and Current Evidence (pp. 56): UNIDO.

COT (Committee on toxicity of chemicals in food, consumer products and the environment).(2006). COT statement on the tolerable daily intake for perfluorooctane sulfonate. http://www.food.gov.uk/multimedia/ pdfs/cotstatementpfos200609.pdf 2006a.

COT (Committee on toxicity of chemicals in food, consumer products and the environment). (2006). COT statement on the tolerable daily intake for perfluorooctanoic acid. http://www.food.gov.uk/multimedia/pdfs/ cotstatementpfoa200610.pdf 2006b.

Dionex. (1999). *ASE® 200 Accelerated Solvent Extractor Operator's manual*: Dionex Corporation.

Earls, A. O., Axford, I. P., & Braybrook, J. H. (2003). Gas chromatography-mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles. *Journal of Chromatography A, 983*(1-2), 237-246. DOI: 10.1016/s0021-9673(02)01736-3

EFSA. (2008). Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA) and their salts. *EFSA Journal, 653*, 1-131. DOI: 10.2903/j.efsa.2008.653

Ehresman, D. J., Froehlich, J. W., Olsen, G. W., Chang, S.-C., & Butenhoff, J. L. (2007). Comparison of human whole blood, plasma, and serum matrices

for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. *Environmental Research, 103*(2), 176-184. DOI: 10.1016/j.envres.2006.06.008

Fromme, H.; Tittlemier, S.A.; Volkel, W.; Willhelm, M.; & Twardella, D.(2009) Perfluorinated compounds – exposure assessment for the general population in western countries. *Int. J. Hygiene and Environ. Health¸ 212,* 239-270.

Harada, K., & Koizumi, A. (2009). Environmental and biological monitoring of persistent fluorinated compounds in Japan and their toxicities. *Environmental Health and Preventive Medicine, 14*(1), 7-19. DOI: 10.1007/s12199-008-0058-5

Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., & Koizumi, A. (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion, *Environ. Res.*, 99 (2), 253-261.

Jogsten, I. E., Perelló, G., Llebaria, X., Bigas, E., Martí-Cid, R., Kärrman, A., & Domingo, J. L. (2009). Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. *Food and Chemical Toxicology, 47*(7), 1577-1583. DOI: 10.1016/j.fct.2009.04.004

Kärrman, A., Harada, K. H., Inoue, K., Takasuga, T., Ohi, E., & Koizumi, A. (2009). Relationship between dietary exposure and serum perfluorochemical (PFC) levels--A case study. *Environment International, 35*(4), 712-717. DOI: 10.1016/j.envint.2009.01.010

Kissa, E., (2001). Fluorinated Surfactants and Repellents, Second Ed. Marcel Dekker, New York.

Lau, C., Butenhoff, J. L., & Rogers, J. M. (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicology and Applied Pharmacology, 198*(2), 231-241. DOI: 10.1016/j.taap.2003.11.031

Mawn, M. P., McKay, R. G., Ryan, T. W., Szostek, B., Powley, C. R., & Buck, R. C. (2005). Determination of extractable perfluorooctanoic acid (PFOA) in

water, sweat simulant, saliva simulant, and methanol from textile and carpet samples by LC/MS/MS. *The Analyst, 130*(5), 670-678.

OECD (Organisation for Economic Co-operation and Development), (2002). Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. ENV/JM/RD(2002)17/FINAL. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology, Environment Directorate, Organisation for Economic Co-operation and Development (Paris). Available at URL http://www.oecd.org/dataoecd /23/18/2382880.pdf

Olsen, G. W., Mair, D. C., Reagen, W. K., Ellefson, M. E., Ehresman, D. J., Butenhoff, J. L., & Zobel, L. R. (2007). Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations in American Red Cross blood donors. *Chemosphere, 68*(1), 105-111. DOI: 10.1016/j.chemosphere.2006.12.031

Olsen, G., Ehresman, D., Froehlich, J., Burris, J., Butenhoff, J.,. (2005). Evaluation of the half-life ($t_{1/2}$) of elimination of perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHS) and perfluorooctanoate (PFOA) from human serum. *In: An International Symposium on Fluorinated Alkyl Organics in the Environment. Univ.Toronto (abstract TOX 017).*

Olsen, G. W., Church, T. R., Miller, J. P., Burris, J. M., Hansen, K. J., Lundberg, J. K., & Zobel, L. R. (2003). Perfluorooctanesulfonate and Other Fluorochemicals in the Serum of American Red Cross Adult Blood Donors. [Article]. *Environmental Health Perspectives, 111*(16), 1892-1901.

Ostertag, S. K., Tague, B. A., Humphries, M. M., Tittlemier, S. A., & Chan, H. M. (2009). Estimated dietary exposure to fluorinated compounds from traditional foods among Inuit in Nunavut, Canada. *Chemosphere, 75*(9), 1165-1172. DOI: 10.1016/j.chemosphere.2009.02.053

Powley, C. R., Michalczyk, M. J., Kaiser, M. A., & Buxton, L. W. (2005). Determination of perfluorooctanoic acid (PFOA) extractable from the surface of commercial cookware under simulated cooking conditions by LC/MS/MS. *The Analyst, 130*(9), 1299-1302.

Qiu, Y. (2007). *Study on treatment technologies for perfluorochemicals in wastewater.* Doctoral Dissertation, Kyoto University, Japan. Available at URL http://hdl.handle.net/2433/44143

Ropers, M. H., Durand, S., Veyrand, B., Beaumal, V., Marchand, P., Anton, M., & Le Bizec, B. (2009). Contamination of food by fluorinated surfactants - Distribution in emulsions and impact on the interfacial protein behaviour. *Food Hydrocolloids, 23*(4), 1149-1155. DOI: 10.1016/j.foodhyd. 2008.10.001

Schultz, M.M., Barofsky, D.F., & Field, J.A. (2003). Fluorinated Alkyl Surfactants. *Environ. Eng. Sci,* 20, 487-501.

Stadalius, M., Connolly, P., L'Empereur, K., Flaherty, J. M., Isemura, T., Kaiser, M. A., & Noguchi, M. (2006). A method for the low-level (ng g-1) determination of perfluorooctanoate in paper and textile by liquid chromatography with tandem mass spectrometry. *Journal of Chromatography A, 1123*(1), 10-14. DOI: 10.1016/j.chroma.2006.03.037

Stockholm Convention Secretariat. (2009). Governments unite to step-up reduction on global DDT reliance and add nine new chemicals under international treaty. Retrieved from Stockholm Convention on persistent organic pollutants (POPs) website: http://chm.pops.int/Convention/Pressrelease/ COP4Geneva8May2009/tabid/542/language/en-US/Default.aspx

The POPs. (2010). *Stockholm Convention on persistent organic pollutants,* UNEP owned website:http://chm.pops.int/Home/tabid/36/mctl/ViewDetails/Event ModID /1007/EventID /85/xmid/1854/language/en-US/Default.aspx, 2010

Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I. T., & Hungerbühler, K. (2008). Estimating Consumer Exposure to PFOS and PFOA. *Risk Analysis, 28*(2), 251-269. DOI: 10.1111/j.1539-6924.2008. 01017.x

USEPA, Stewardship Program. (2010). *2010/2015 PFOA Stewardship program, U.S. Environment Protection Agency (EPA).* Available at URL http://www. epa.gov/oppt/pfoa/ pubs/stewardship/index.html

USEPA. (2002). Revised draft hazard assessment of perfluorooctanoic acid and its salts (pp. 107): Office of Pollution Prevention and Toxics Risk Assessment Division.

Vassiliadou, I., Costopoulou, D., Ferderigou, A., & Leondiadis, L. (2010). Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in Greece. *Chemosphere, 80*(10), 1199-1206. DOI: 10.1016/j.chemosphere.2010.06.014

Wilhelm, M., Angerer, J., Fromme, H., & Hölzer, J. (2009). Contribution to the evaluation of reference values for PFOA and PFOS in plasma of children and adults from Germany. *International Journal of Hygiene and Environmental Health, 212*(1), 56-60. DOI: 10.1016/j.ijheh.2007.11.002

APPENDICES

APPENDIX A
FORMULAS AND SPECIMEN CALCULATION

A.1 Stock standard solution

Chemicals standard were prepared at a concentration of 200 µg mL^{-1} by dissolving 10 mg of the chemicals standard in 50 mL methanol. The calculation as in the following;

PFOS (C_8-S)

(10 mg of PFOS / 50 mL) * 1000 mL = 200 mg L^{-1}

PFOA (C_8-A)

(10 mg of PFOA / 50 mL) * 1000 mL = 200 mg L^{-1}

A.2 Mixed standard solution of PFOS and PFOA

The mixed standard solution was prepared at a concentration of 0.1 µg mL^{-1} by dilution of the stock standard solutions. The final volume of mixed standard solution is 50 mL in methanol. The formula in this calculation was;

$$\mathbf{C_1V_1 = C_2V_2}$$

Where; C_1 is an initial concentration

C_2 is a final concentration

V_1 is an initial volume

V_2 is a final volume

$$C_1V_1 = C_2V_2$$

$$(200\ \mu g\ mL^{-1}) * V_1 = (0.1\ \mu g\ mL^{-1})(50\ mL)$$

$$V_1 = 0.025\ mL = 25\ \mu L$$

Thus, added 25 µL PFOS and 25 µL PFOA, and then adjust the volume to 50 mL by methanol (HPLC grade).

A.3 Calibration Standard (7 concentration levels)

The Mixed standard solution of PFOS and PFOA was conducted to prepare the calibration standard. The calibration standard was prepared at a concentration of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg L^{-1} by dilution of the mixed standard solutions of PFOS and PFOA. The final volume of calibration standards were 50 mL in 50% methanol and 40%acetonitrile. The calculation as in the following;

$$\mathbf{C_1V_1 = C_2V_2}$$

Concentration	**Mixed standard Volume**		
0.05 µg L^{-1}	V_1	= (0.05 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 0.025 mL
0.1 µg L^{-1}	V_1	= (0.1 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 0.050 mL
0.5 µg L^{-1}	V_1	= (0.5 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 0.25 mL
1.0 µg L^{-1}	V_1	= (1.0 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 0.50 mL
2.0 µg L^{-1}	V_1	= (2.0 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 1.00 mL
5.0 µg L^{-1}	V_1	= (5.0 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 2.50 mL
10.0 µg L^{-1}	V_1	= (10.0 µg L^{-1}) (50 mL) / (100 µg L^{-1})	= 5.00 mL

A.4 Fortification sample

To prepare 5, 50 and 200 ng g^{-1} fortification for spike 2 g of paper sample with 100 µL. Fortification solutions of PFOS and PFOA were prepared by diluting the stock standard solutions with methanol (HPLC grade).

Low level (5 ng g^{-1})

5 ng g^{-1} = (Conc. * 100 µL) / 2 g

Conc. = 0.1 ng μL^{-1} = 0.1 µg mL^{-1}

Thus, prepared the fortification solution at low level by using the stock solution of PFOS and PFOA as the following calculated volume;

$$C_1V_1 = C_2V_2$$
$$(200\ \mu g\ mL^{-1}) * V_1 = (0.1\ \mu g\ mL^{-1})(50\ mL)$$
$$V_1 = 0.025\ mL = 25\ \mu L$$

Then, added 25 µL PFOS and 25 µL PFOA, and then adjust the volume to 50 mL by methanol (HPLC grade) and spiked this solution to the paper sample at the volume of 100 µL.

Middle level (50 ng g^{-1})

$$50\ ng\ g^{-1} = (Conc. * 100\ \mu L) / 2\ g$$
$$Conc. = 1\ ng\ \mu L^{-1} = 1\ \mu g\ mL^{-1}$$

Thus, prepared the fortification solution at middle level by using the stock solution of PFOS and PFOA as the following calculated volume;

$$C_1V_1 = C_2V_2$$
$$(200\ \mu g\ mL^{-1}) * V_1 = (1\ \mu g\ mL^{-1})(50\ mL)$$
$$V_1 = 0.25\ mL = 250\ \mu L$$

Then, added 250 µL PFOS and 250 µL PFOA, and then adjust the volume to 50 mL by methanol (HPLC grade) and spiked this solution to the paper sample at the volume of 100 µL.

High level (200 ng g^{-1})

$$200\ ng\ g^{-1} = (Conc. * 100\ \mu L) / 2\ g$$
$$Conc. = 4\ ng\ \mu L^{-1} = 4\ \mu g\ mL^{-1}$$

Thus, prepared the fortification solution at high level by using the stock solution of PFOS and PFOA as the following calculated volume;

$$C_1V_1 = C_2V_2$$
$$(200\ \mu g\ mL^{-1}) * V_1 = (4\ \mu g\ mL^{-1})(50\ mL)$$
$$V_1 = 1\ mL$$

Then, added 1 mL PFOS and 1 mL PFOA, and then adjust the volume to 50 mL by methanol (HPLC grade) and spiked this solution to the paper sample at the volume of 100 µL.

A.5 Saliva simulant

The following formula is used for calculate the chemical in synthesis of saliva simulant.

	M	=	mol / L
	mol / L	=	(g / molecular weight) / L
Thus,	M	=	(g / molecular weight) / L
Then,	**g**	**=**	**M * molecular weight * L**

The chemicals,

KCl;	g	=	$10.0 \times 10^{-3} \times 1 \times 74.551$	= 0.7455 g/L
K_2CO_3;	g	=	$3.8 \times 10^{-3} \times 1 \times 138.204$	= 0.5252 g/L
K_2HPO_4;	g	=	$3.3 \times 10^{-3} \times 1 \times 174.174$	= 0.5748 g/L
NaCl;	g	=	$5.6 \times 10^{-3} \times 1 \times 58.443$	= 0.3273 g/L
$CaCl_2$;	g	=	$1.0 \times 10^{-3} \times 1 \times 110.984$	= 0.1110 g/L
$MgCl_2$;	g	=	$0.82 \times 10^{-3} \times 1 \times 95.211$	= 0.0781 g/L

A.6 Concentration of PFOS and PFOA in unit of mg kg^{-1}

To calculate the amount of PFOS and PFOA in unit of mg kg^{-1} is used the following formula.

Concentration of the chemical (mg kg^{-1}) = (A * B) / W

Where,	A	is the concentration from LC-MS/MS (mg L^{-1})
	B	is the liquid volume after extraction (mL)
	W	is the sample weight (g)

APPENDIX B
GRAPH OF CALIBRATION CURVE STANDARDS

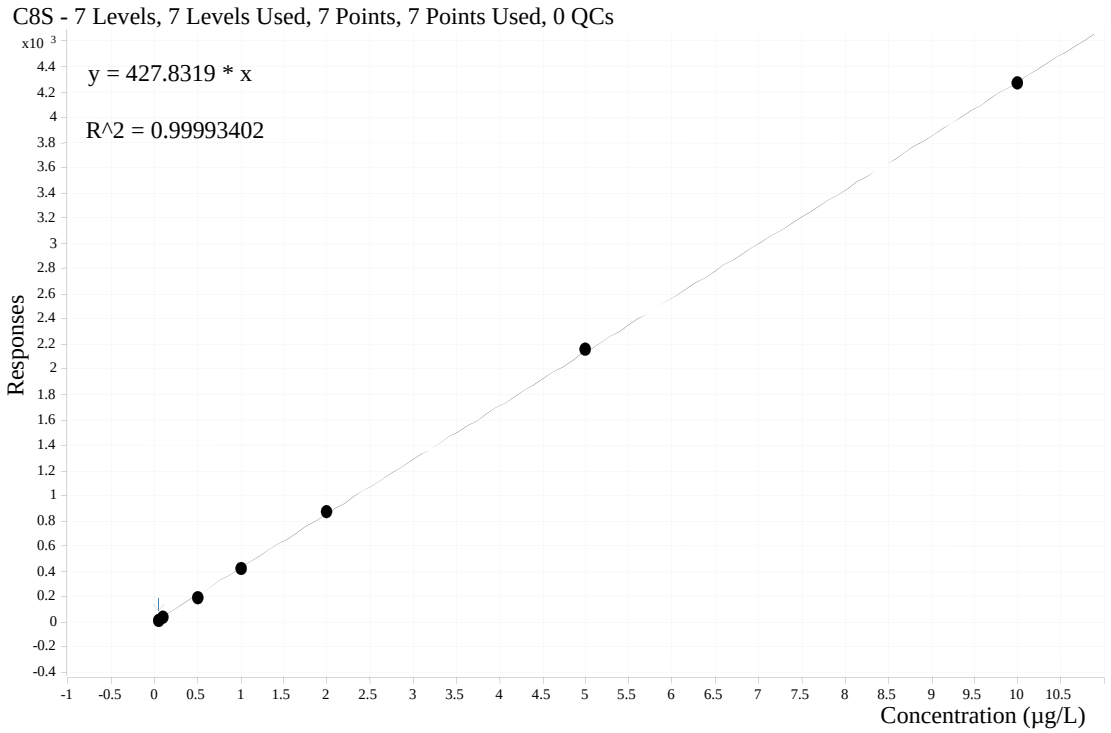
Calibration curves of PFOS and PFOA standard

Calibration curves of PFOS and PFOA standard in 50:50 (v/v) methanol and ultrapure water solution

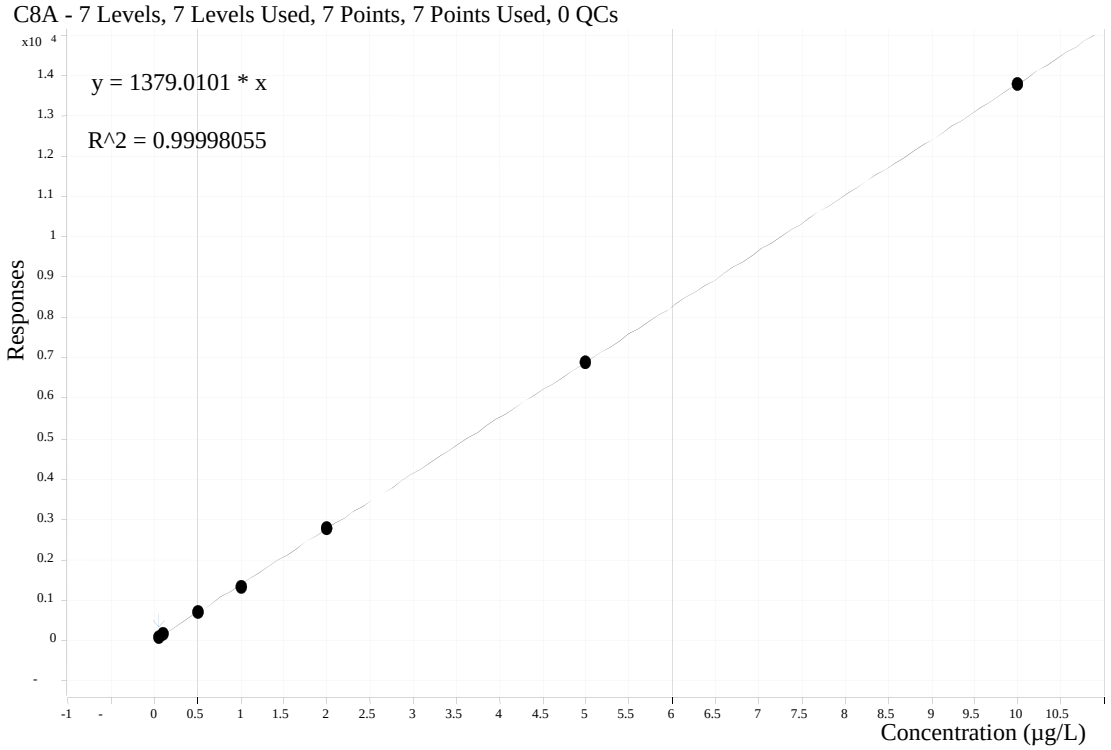
The calibration curves of PFOS and PFOA in 50:50 (v/v) methanol and ultrapure water solution were linear response. Regression correlations (Correlation of determination, R^2) of PFOS and PFOA were 0.9999. The calibration curves of PFOS and PFOA for quantification, consisting of seven level covering 0.05 - 10 µg L^{-1} is shown in Figure A-1.

Calibration curves of PFOS and PFOA standard in 40: 60 (v/v) acetonitrile and ultrapure water solution

Calibration curves of PFOS and PFOA in 40: 60 (v/v) acetonitrile and ultrapure water solution were linear response. R^2 of PFOS and PFOA were 0.9998 and 0.9999, respectively. The calibration curves of PFOS and PFOA range from 0.05 - 10 µg L^{-1} is shown in Figure A-2.

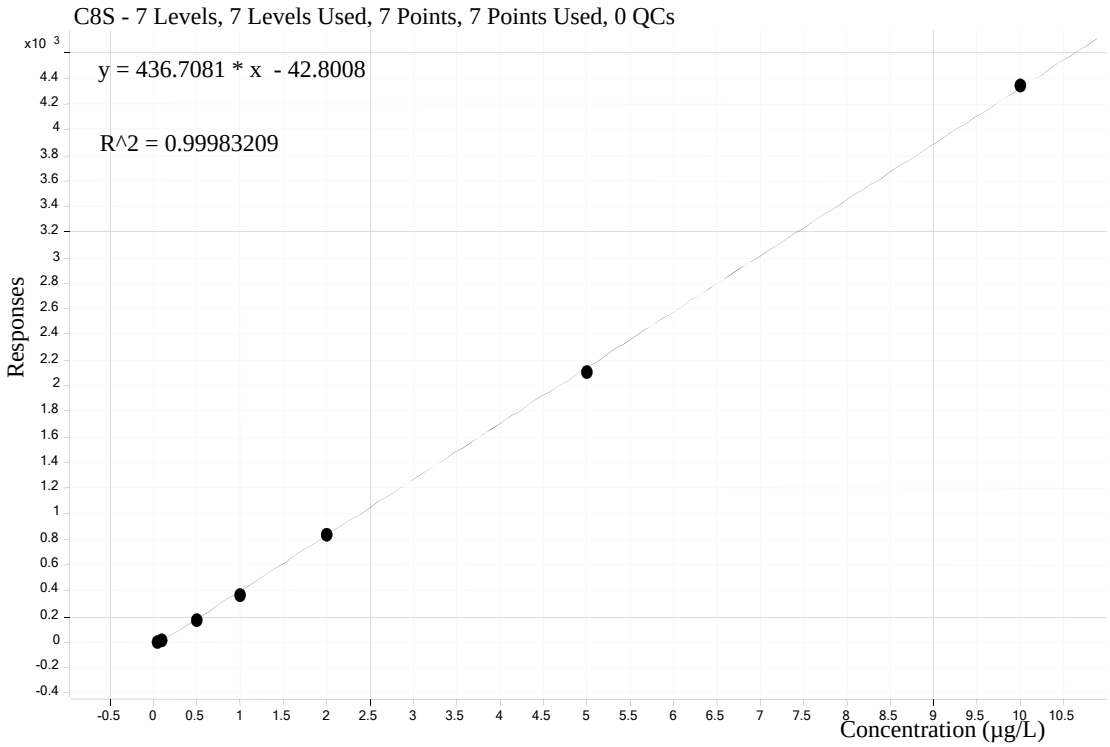


(a)



(b)

Figure A-1: Calibration curves of (a) PFOS and (b) PFOA in 50% methanol



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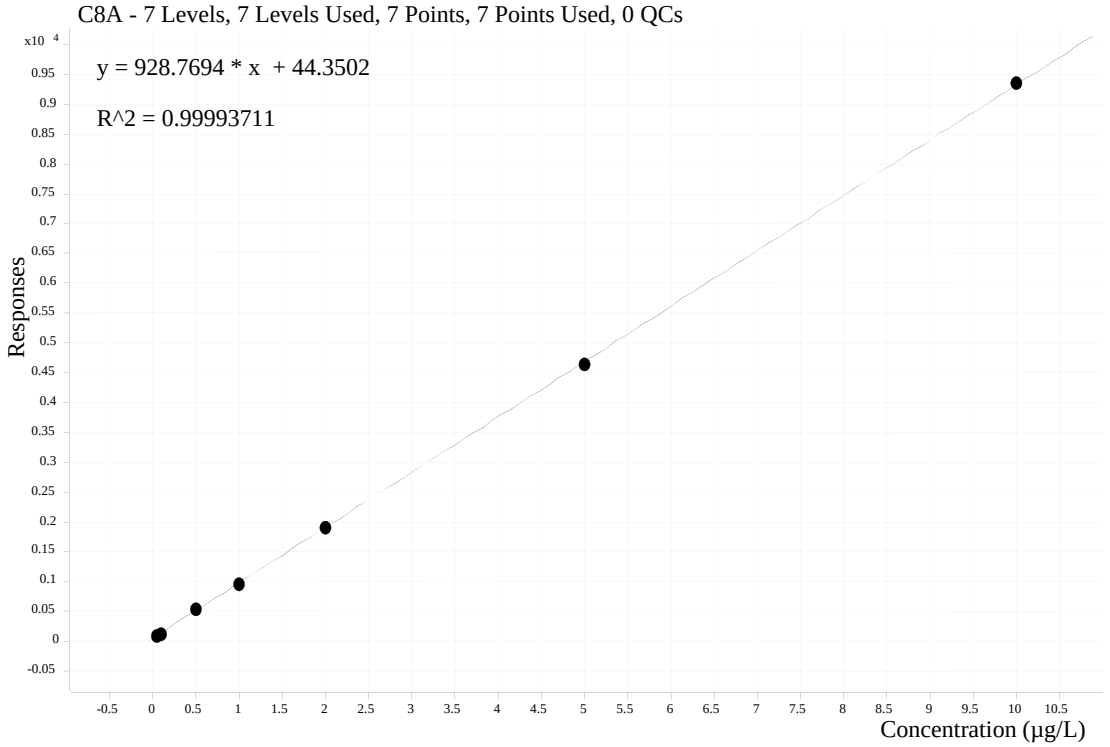

(b)

Figure A-2: Calibration curves of (a) PFOS and (b) PFOA in 40% acetonitrile

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Bangkok, Thailand, 16 pages, March 2011

Somrutai Poothong, Suwanna Kitpati Boontanon, and Narin Boontanon. (2010), Analysis of Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) in paper food packages by pressurized fluid extraction and liquid chromatography coupled with tandem mass spectrometry, *Proceeding of 8th Science and Technology Conference on Science and Technology for Community Development,* Thammasat University, Thailand, 8 pages, March 2010.