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# Quantification of Persistent Organic Pollutants in Various Matrices Using Stir Bar Sorptive Extraction and Isotope Dilution Mass Spectrometry

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# QUANTIFICATION OF PERSISTENT ORGANIC POLLUTANTS IN VARIOUS MATRICES USING STIR BAR SORPTIVE EXTRACTION AND ISOTOPE DILUTION MASS SPECTROMETRY

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By

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# ABSTRACT

## QUANTIFICATION OF PERSISTENT ORGANIC POLLUTANTS IN VARIOUS MATRICES USING STIR BAR SORPTIVE EXTRACTION AND ISOTOPE DILUTION MASS SPECTROMETRY

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Dissertation supervised by Dr. H. M. Skip Kingston

A method was developed to quantify persistent organic pollutants (POPs) in a wide range of matrices including wastewater, dietary supplements, and human whole blood using stir-bar sorptive extraction, GC-MS/MS, and isotope dilution mass spectrometry (IDMS). The method enabled accurate, precise, sensitive, and efficient quantification of POPs in these matrices. Compared with calibration curves, IDMS provided measurements with a higher level of accuracy and precision, especially at lower measured concentrations. The use of GC-MS/MS enabled a lower limit of quantification compared with GC-MS. A reverse-IDMS method was performed to further eliminate biases from the labelled concentrations of the commercially available standards.

12 commercially available plant-extract based dietary supplement samples were analyzed using this method. Polycyclic aromatic hydrocarbons (PAHs) including

naphthalene, acenaphthene, phenanthrene, and benzo[a]pyrene were detected in most of the products with mean concentrations over 1 ng/g. Organochlorine pesticides (OCPs) were detected less frequently than PAHs in these products, and none of the OCPs had mean concentrations over 1 ng/g. These results were compared with existing guidelines and none of the analytes in the samples were found to be above the daily allowable limits. The method was also applied to analysis of 10 human whole blood samples acquired from a blood bank in Northern California. On average, 10 POPs were detected in each sample. The mean xenobiotic body-burden was calculated for each sample and ranged from 0.719 to 1.12 ng/g. This method has demonstrated analytical advantages and will be further applied in the study of environmental and human health.

# DEDICATION

I dedicate my dissertation work to my family. I feel grateful for their lifelong love and support. A special thanks to my lovely wife, Xiaohan, who has been standing by my side through life's ups and downs.

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# Chapter 1: Introduction

## 1.1 Persistent organic pollutants

Persistent organic pollutants (POPs) are highly stable organic chemicals that resist photolytic, biological, and chemical degradation. They persist in the environment, bioaccumulate through the food chain, and may adversely impact human health and the environment.[1] POPs have been released to the environment over the past decades mainly due to human activities. Widely distributed and accumulated over these decades, POPs have become one of the high-priority environmental and human health concerns around the globe. POPs are highly resistant to degradation due to their stable structures. They are semi-volatile, which allows them to enter the atmosphere in the vapor phase or adsorb on atmospheric particles that can be transported over long distances.[2-5] Because of their hydrophobic structures, most POPs readily pass through the phospholipid structure of biological membranes from the surrounding medium and accumulate in the living organism.[2, 6]

Intended to address the widespread environmental and human health issues caused by POPs, the Stockholm Convention, under the United Nations Environmental Program, was adopted in 2001 and entered into force in 2004. The Stockholm Convention requires its parties to take measures to eliminate or reduce the release of POPs into the environment and aims to restrict the production and use of POPs and protect human health and the environment.[1] 12 POPs were initially listed in the convention including aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans

(PCDFs). Since 2009, the treaty has been amended by adding 16 new POPs such as hexachlorocyclohexanes, chlordecone, hexabromobiphenyl, perfluorooctane sulfonic acid, polychlorinated naphthalenes, and short-chain chlorinated paraffins, etc.[1]

Based on the sources, POPs can be placed in two categories, 1) products intentionally produced for one or more purposes, or 2) unintentionally formed as by-products in industrial processes or other human activities. Additionally, minor quantities of POPs can be created from natural processes.[7] Based on the molecular structures, POPs can be represented by two subgroups: polycyclic aromatic hydrocarbons (PAHs) and halogenated hydrocarbons such as organochlorine pesticides (OCPs), PCBs, polybrominated diphenyl ethers (PBDEs), PCDDs, and PCDFs.[5]

### 1.1.1 Polycyclic aromatic hydrocarbons

PAHs are generated from natural sources such as forest fires, volcanoes, and biogenic sources[8] or anthropogenically from sources such as the exhaust of motor vehicles, petroleum refineries, combustion of industrial and domestic wastes, and chemical engineering processes.[9-11] PAHs have been widely found in the environment including atmosphere, water, sediments, and food products.[11-13] These PAHs have been linked to increased risks of DNA damages, chromosomal aberrations, and cancers especially leukemia.[14-18]

Naphthalene, the first member of the PAH group, is a common micropollutant in drinking water. After entering the human body, it covalently binds to molecules in liver, kidney and lung tissues, thereby enhancing its toxicity.[19] Acute exposure to naphthalene is known to cause haemolytic anaemia and nephrotoxicity.[19] Another frequently studied

PAH, benzo[a]pyrene, can be found in smoked meats, tobacco smoke, and exhaust emissions and has been linked to cancers such as lung cancer and colorectal adenoma etiology.[20-22]

### 1.1.2 Organochlorine pesticides

OCPs have been extensively used in agriculture globally. Although the production and application of some OCPs have been banned in developed countries for decades, they are still widely present in water, soils, sediments, the atmosphere, fish, and food products due to their high persistence and semi-volatile properties.[23-29] Many OCPs have been recognized as endocrine disrupters which can interfere with the hormonal system and consequently damage the reproductive and immune systems of exposed individuals and may cause reproductive diseases such as breast cancer and prostate cancer.[1, 30-32]

DDT is one of the earliest and most well-known synthetic OCPs in the world. Although it has been banned in most developed countries since the 1970s, it is still widely distributed in the environment and living organisms. The chemical stability and associated lipophilicity result in DDT being only slowly eliminated by most living creatures.[33] It can be metabolized into dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), which share similar traits and may have even higher toxicity.[34] DDT as well as its metabolites may lead to pancreatic cancer, neurological dysfunction, and reproductive diseases.[33, 35]

1.1.3 Others

Polychlorinated biphenyls (PCBs) are mixtures of chlorinated hydrocarbons that have been used extensively since the 1930s in various industrial uses, such as plasticizers, adhesives, waxes, lubricating oils, heat exchange fluids, dielectrics in transformers and large capacitors, and paint additives in carbonless copy paper due to their physico-chemical properties such as chemical inertness, resistance to heat, non-flammability, low vapor pressure and high dielectric constant.[5, 7] The PCB family consists of 209 possible congeners ranging from three monochlorinated isomers to the fully chlorinated decachlorobiphenyl isomer.[8] Although the production of PCBs has been banned in most countries since the 1970s, leakage from old equipment, building materials, stockpiles and landfill sites remains a continued threat of PCB emission.[8] PCBs are believed to interfere with thyroid hormones, estrogens, and androgens and inhibit various metabolic enzymes.[36] They have also been linked with adverse health effects such as hepatoxicity, neurodevelopmental toxicity, and carcinogenesis.[37-38]

PBDEs share some features and commonalities with PCBs and are extensively used as an additive flame retardant in various plastic materials, polyurethane foam, and heavy textiles, such as carpets and curtains.[7] These PBDEs are believed to be slowly released over the life of the plastics, foams and other products and make their way into the food chain and the human population.[39] Unlike PCBs, PBDEs are still produced and in use worldwide, although penta-, octa- and, deca-BDEs have been banned in Europe and several states in the United States.[40-41] Since the chemical structure of PBDEs and their metabolites closely resembles thyroid hormones and thus bind with high affinity to thyroid hormone transport protein, PBDEs can interfere with thyroid function, disrupt

hormonal balance, and cause neurotoxic effects.[39-40, 42] Children and young adults are believed to be more prone to developmental dysfunctions as a consequence of PBDE exposure.[40]

PCDDs and PCDFs, also referred to as dioxins and furans, respectively, are two chemically similar groups of chlorinated aromatic compounds. Dioxins have 75 and furans have 135 possible congeners.[5] These compounds are generally formed unintentionally as by-products of various industrial and combustion processes such as production of steel and fuel combustion.[8] To assess the toxicity of PCDDs and PCDFs, relative toxicity factors are assigned to individual dioxins and furans by comparing with the toxicity of 2,3,7,8-tetrachlorodibenzodioxin, the most potent congener within these groups of compunds.[43] 17 out of the 210 dioxins and furans were found to have significantly higher toxicity than the other congeners.[5] Some known health effects of PCDDs and PCDFs include peripheral neuropathies, fatigue, depression, immunotoxicity, carcinogenicity, and adverse effects on reproduction, development, and endocrine functions.[5, 43]

Chlorpyrifos is a widely-used organophosphate insecticide that is generally believed to have relatively low toxicity compared with other organophosphate insecticides like parathion.[44] However, evidence has pointed to adverse health effects of chlorpyrifos exposure on the nervous system and it has been associated with developmental issues such as lowered intelligence quotients of school-aged children.[44-47] California, which consumes a large amount of chlorpyrifos each year, began the legislative process to ban this pesticide completely in 2019.[48] Chlorpyrifos is not generally considered as a POP. However, due to its similar analytical property to POPs

and the emerging environmental and human health concerns against it, chlorpyrifos was included in the study.

## 1.2 Exposomics

The exposome, as a new concept, was first brought forward in 2005 and was defined as life-course environmental exposures from the prenatal period onwards.[49] This concept of exposome was used to illustrate the entireness of environmental exposure. As a complement to the genome, the exposome may provide important clues for the understanding of human chronic diseases.[50] During the past decade, the concept of "exposome" has been further developed and refined by the scientific community. The exposome is no longer restricted to exogenous chemicals entering the body from the environment. It also takes into account the endogenous sources from the internal chemical environment.[51-52] Such an emphasis on the internal chemical environment is essential to incorporate chemicals not only from polluted air and water, but also from diet, smoking, drugs, radiation, and endogenous processes like inflammation, stress, lipid peroxidation, and infections.[51]

The exposome is generally considered to consist of three overlapping and intertwining domains: 1) specific external exposures, 2) general external exposures, and 3) internal exposures. Specific external exposures include chemical contaminants and environmental pollutants, radiation, infection, occupation and medical interventions, diet, and lifestyle factors such as tobacco and alcohol. This has been the major focus of exposomics and epidemiological studies seeking to correlate environmental risk factors to diseases. General external exposures include factors such as social, economic, and

psychological influences like social capital, education, financial status, psychological and mental stress, and climate. Internal exposures include metabolism, gut microflora, inflammation, oxidative stress, lipid peroxidation, aging, and other natural biological processes.[53-54] A diagram of the three domains of the exposome and their interactions is presented in Figure 1.1.

Strategies for characterizing exposomes can be either "bottom-up" or "top-down". A diagram of these two strategies is shown in Figure 1.2. In the "bottom-up" strategy, all target chemicals in each external source of an individual's exposure such as air, water, and diet are measured. Although this approach has the advantage of relating exposures directly to their direct sources, it requires a heavy workload of sampling and measurements and neglects the essential features of an individual's internal chemical environment.[51-52] On the contrary, the "top-down" approach employs strategies to measure all target chemicals and their metabolites as well as biomarkers in an individual's blood. This approach investigates both exogenous and endogenous chemicals in the internal chemical environment. Once the exposures of interest are identified in the blood samples, additional testing could be employed to determine their sources.[51-52] However, since it is not currently feasible to measure all chemicals in the blood, exposomics research has been focusing initially on classes of substances that are known to be biologically active and associated with diseases, including reactive electrophiles, endocrine disruptors, immune modulators, receptor-binding agents, metals, and POPs.[51-52] The "top-down" strategy has been applied in exposome studies to analyze the connection between certain chemicals and diseases.[55-57] For chemicals that are difficult to be directly measured, it would be beneficial to investigate the related physiological

processes such as metabolism, as these processes typically generate products that can serve as biomarkers in the blood.[52]

Understanding the potential link between the exposure to environmental pollutants, such as POPs, and human health has been an important emphasis in exposomics research. Recent studies have focused on developing effective methods to quantify POPs in the human body for the purposes of improving human health as well as disease diagnosis and prevention.[56, 58-59] In this work, quantitative methods were developed to analyze POPs in a wide range of matrices including human blood, dietary supplements, drinking water and wastewater, air, and food products. These methods are used in exposomics research to determine the sources and concentrations of human's exposure to POPs.

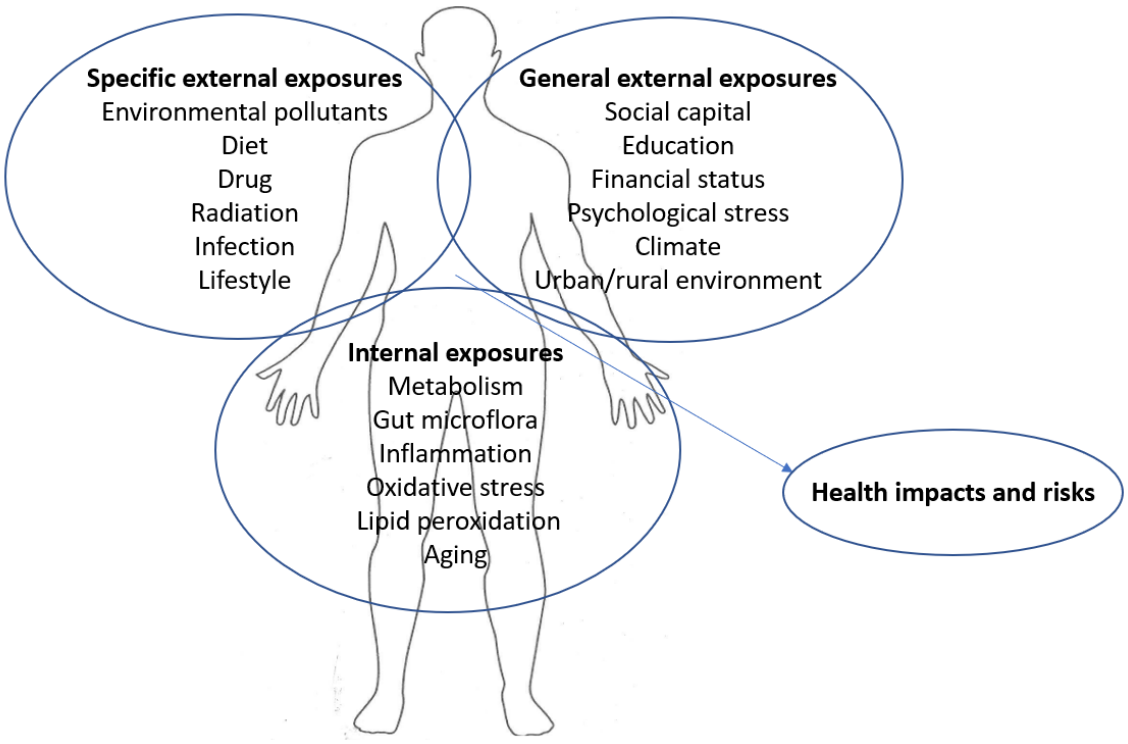


Figure 1.1 Three domains of exposome defined by Wild.[53] Examples of specific external, general external, and internal exposures are given in the diagram.

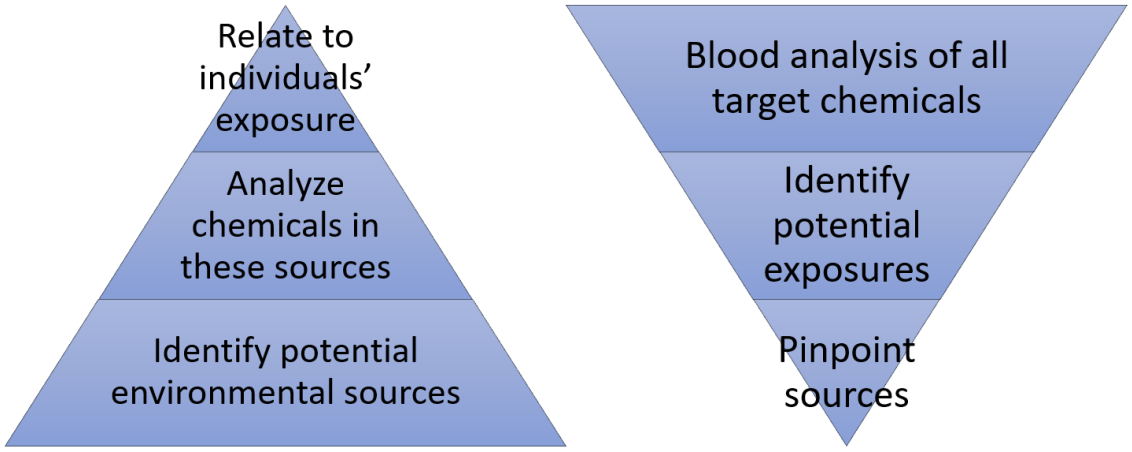


Figure 1.2 "Bottom-up" (left) and "top-down" (right) strategies for characterizing exposomes.

# Chapter 2: Methodology

## 2.1 Extraction

### 2.1.1 Stir-bar sorptive extraction

Stir-bar sorptive extraction (SBSE) was first described as a novel extraction technique in 1999.[60] During the past two decades, SBSE has evolved into an extremely powerful extraction and preconcentration technique for solventless and miniaturized sample preparation in a wide range of applicable areas. Since the introduction of this technique, over 900 papers on SBSE have been published.[61]

With SBSE, the solutes are extracted from the sample matrix into a polymer coating on a glass magnetic stir-bar. The extraction is controlled by the partition coefficient of the analytes between the polymer coating and the sample matrix as well as by the phase ratio between the polymer coating and the sample volume.[62-63] The polymeric coating is typically polydimethylsiloxane (PDMS), which has several specific characteristics that make it the most widely used sorptive extraction phase. Analytes can be absorbed into and retained within the bulk of the PDMS instead of being retained on the surface, and the retaining capacity of PDMS for a certain compound is not influenced by the presence of other analytes since each analyte has its own partition equilibrium into the PDMS phase. In addition, the thermal stability of PDMS facilitates analysis under relatively high temperature conditions.[63] Another analytical attribute of PDMS is that its degradation fragments contain characteristic silicone mass fragments that can easily be discerned by using mass spectrometry.[60]

Stir-bar sorptive extraction coupled with GC-MS has been used to extract and analyze mainly hydrophobic organic compounds in aqueous samples. This combined

technique has demonstrated advantages such as low detection limits, high analyte recoveries, good repeatability, relative simplicity, and automation.[58, 64-66] SBSE consists of two major steps: extraction and desorption. In the extraction step, the stir-bar is added to a sample vial and the liquid sample is stirred. After extraction, the stir-bar is removed and rinsed with deionized water and is then ready for desorption.[63] Thermal desorption is typically followed by the GC to recover and separate the analytes extracted by the stir-bar. The thermal desorption unit (TDU) of the instrument thermally desorbs the analytes from the stir-bar and injects them into the GC. The TDU consists of two programmable temperature vaporizers (PTVs). The first PTV is heated to desorb the analytes from the stir-bar; while the second, the cooled injection system (CIS), cryofocuses the desorbed analytes before they are injected into the GC. Since the thermal desorption can take up to 15 min, the cryogenic process is required to significantly minimize the chromatographic peak width.[63] Compared with conventional extraction methods such as liquid-liquid extraction and solid-liquid extraction, this newer method is more environmental friendly as it minimizes the use of solvents and residual toxic wastes.

### 2.1.2 Other extraction methods

Some other commonly applied extraction methods for analysis of POPs include solid phase microextraction (SPME), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), and ultrasonic assisted extraction (UAE). These extraction methods are used to increase the diffusion and desorption rate of analytes from the sample matrix to the solvent and thus require less solvent.[67]

Similar to SBSE, SPME was first invented in 1990 and quickly gained popularity due to its simplicity and effectiveness in rapid sampling.[68] It is a modified syringe-like apparatus with fused silica fiber coated on the needle. The extraction involves exposing the fiber in the vapor phase above a gaseous, liquid, or solid sample or immersing the fiber in a liquid sample. After equilibration, the SPME fiber is removed from the sample and the analytes are thermally desorbed for subsequent analysis.[69-71]

MAE is a process of using microwave energy to heat solvents in contact with the sample in order to partition analytes from the sample matrix into the solvent. Microwave energy is a non-ionizing form of electromagnetic radiation, with a frequency of 2.45 GHz for most commercially available microwave ovens, that causes molecular motion by ion migration and dipole rotation, and does not normally cause changes in molecular structure. The extraction process is based on the efficient heating of materials by microwave dielectric heating effects and is dependent on the ability of the reagents to absorb microwave energy and convert it to heat.[72]

PLE is a sample preparation technique that derived from supercritical fluid extraction (SFE) in the 1990s.[73] It combines elevated temperature and pressure with liquid solvents to achieve fast and efficient extraction of the analytes from a solid sample matrix. The elevated temperature results in a decrease in solvent viscosity which helps to disrupt the solute-matrix interactions and increases the diffusion coefficients. Meanwhile, the elevated pressure facilitates the penetration of the solvent into the matrix, which also favors the extraction of the analytes.[73-74]

UAE utilizes ultrasonic waves which have frequencies above 20 kHz to assist in the extraction by producing cavitation, vibration, crushing, mixing and other

comprehensive effects in media.[75-76] The main driving force for the extraction effects of sonication is believed to be the cavitation phenomena. When the ultrasound propagates through a medium, it induces a series of compressions and rarefactions in the molecules of the medium. Such alternating pressure changes result in formation and collapse of bubbles in the medium, and consequently lead to significant liquid circulation currents coupled with intense turbulence, which facilitates the extraction.[75-77]

SBSE, SPME, MAE, PLE, and UAE have been extensively applied to analysis of POPs in a wide range of samples. In some studies, more than one extraction techniques are combined for optimal extraction efficiency.[78-81] Recent studies that utilized these extraction techniques for analysis of POPs are summarized in Table 2.1.

As one of the most commonly used sorbent-based extraction techniques for POPs analysis, SBSE offers many advantages for extraction of POPs in various matrices as previously discussed. However, this method has certain limitations. For example, SBSE is generally not effective for extraction of relatively polar compounds due to the non-polar nature of PDMS coating. Effort has been made to extend the applicable polarity range of compounds by modifying the sorbent material of stir-bars.[82-84] Additionally, a typical clean-up procedure for the stir-bars after use involves soaking in multiple solvents and high temperature heating. This procedure is time consuming and can take 5-6 hours to complete.

Vacuum assisted sorbent extraction (VASE), a sorbent based extraction technique developed recently,[85] is an alternative approach to overcome the above limitations.[86] VASE utilizes sorbent traps called sorbent pens (SPs) to perform headspace extraction at vacuum condition. The SPs are packed with a large quantity of sorbent materials which

are approximately 10 times the volume of SBSE and 500 times the volume of SPME.[86] To accelerate the extraction kinetics, reduce the sampling time, and extend the range of analytes, the in-vial extraction is performed in a vacuum environment. After extraction, the SPs are thermally desorbed at a GC injection port, followed by GC-MS analysis. Compared with SBSE and SPME, VASE has advantages such as less carryover, higher durability, improved sensitivity due to larger sorbent surface area, and ability to use a series of sorbents in SPs to recover a wider range of compounds ranging from volatile to semi-volatile.

Table 2.1 Studies in the past decade using SBSE, SPME, MAE, PLE, or UAE for analysis of POPs.

| Analytes | Sample matrices | Extraction methods | References |
|---|---|---|---|
| POPs, e.g. PCBs, PBDEs, etc. | Human serum | SBSE | Boggess, et al.[58] |
| POPs, e.g. PCBs, PBDEs, etc. | Human serum | SBSE | Boggess, et al.[56] |
| POPs, e.g. PAHs, OCPs, etc. | Human whole blood | SBSE | Hao, et al.[59] |
| POPs, e.g. PAHs, OCPs, etc. | Dietary supplements | SBSE | Hao, et al. |
| OCPs | Textiles | SPME | Zhu, et al.[87] |
| PAHs, OCPs, PCBs | Natural and artificial soils | SPME | Bielska, et al.[88] |
| OCPs | Human serum | SPME | Koureas, et al.[89] |
| PAHs, OCPs, and PCBs | River water | SPME | Hu, et al.[90] |
| PCBs and PBDEs | Soil and fish | MAE | Wang, et al.[91] |
| Pesticides | Airborne particulate matter | MAE | Coscolla, et al.[92] |
| PAHs | Fish | MAE | Ramalhosa, et al.[93] |
| Pesticides | Milk formula | MAE | Fang, et al.[94] |
| PAHs | Grilled meat | MAE | Kamankesh, et al.[95] |
| Pesticides | Herbs | PLE | Du, et al.[96] |
| OCPs and PCBs | Marine samples, e.g. fish, squid, shrimp, etc. | PLE | Helaleh, et al.[97] |
| PAHs, PCBs, and PBDEs | Atmospheric particulate matter | PLE | Clark, et al.[98] |
| PCBs | Shellfish | UAE | Zhou, et al.[99] |
| PCBs | small-size biological tissues | UAE | Pena-Abaurrea, et al.[100] |
| OCPs and PCBs | Soil | UAE | Flores-Ramírez, et al.[101] |

## 2.2 Isotope dilution mass spectrometry

Isotope dilution mass spectrometry (IDMS) is a quantification method that involves spiking stable isotope analogs of target analytes into sample matrix. A known amount of isotopically enriched analog of an analyte of interest is spiked into the sample prior to extraction. After equilibration between the sample and the spike, the resulting isotope ratio is measured by mass spectrometry. By using this isotope ratio, the concentration of the analyte in the sample is calculated. The ratio of the signal intensity of a target analyte (A) with a natural isotope distribution to the signal intensity of its stable heavy-labeled isotope analog (B) is equal to the ratio of the concentration of the target analyte to the concentration of its isotopically labelled analog. Specifically:

Eq. 1.1 $$R_m = \frac{A_s C_s W_s + A_{sp} C_{sp} W_{sp}}{B_s C_s W_s + B_{sp} C_{sp} W_{sp}}$$

In this equation, $R_m$ is the measured isotope ratio of A to B. $A_s$ and $B_s$ are fractions of A and B in the sample, respectively. $A_{sp}$ and $B_{sp}$ are fractions of A and B in the spike, respectively. Examples demonstrating calculations of these fractions can be found in previous published literature.[102] $C_s$ is concentration of the target analyte in the sample and $C_{sp}$ is concentration of the spike (in nmol/g). $W_s$ and $W_{sp}$ are weights of the sample and the spike, respectively. In this equation, each term is known or can be determined by mass spectrometry except $C_s$. Therefore, the direct mathematical IDMS equation to calculate the concentration of the target analyte in the sample, $C_s$, is as follow:

Eq. 1.2 $$C_s = \frac{C_{sp} W_{sp} (A_{sp} - R_m B_{sp})}{W_s (R_m B_s - A_s)}$$

Unlike calibration curves or calibration curves with internal standards, IDMS is a direct quantification method that avoids the need for a series of dilutions and external

calibrations. By spiking the sample with isotope analogs, each IDMS measurement is its own "calibration". For IDMS, once the equilibrium between the sample and the spike is achieved, variation of analyte recovery will not affect the quantification results.[103] Factors that typically impact the recoveries such as partial loss of the analytes, interferences during the analysis, and instrument signal drift, will not influence accuracy and precision of the measurement as these factors have the same effects on the analytes and their corresponding isotope analogs so that "$R_m$" is not influenced. Additionally, IDMS methods are less time-consuming than calibration curve methods since the procedure to create calibration curves using standards with different concentrations is not necessary for IDMS. Given that at least five standards with different concentrations are needed to create a calibration curve for each analyte, the time for analysis using IDMS is less than one sixth of the analysis time using calibration curves. "Isotope dilution" has been used in several recent studies for quantification.[104-106] However, these "isotope dilution" methods still employed calibration curves since the isotope spikes were added as internal standards. IDMS, in contrast, is a direct mathematical quantification method that facilitates quantification using a mathematical algorithm. IDMS has been applied to analysis of organic compounds in a wide range of sample matrices and was demonstrated to be able to significantly improve the quantitative results by lowering the measurement errors and uncertainties.[58-59, 107-108] Table 2.2 summarizes recent studies using IDMS for analysis of organic compounds. More applications of IDMS are described in the EPA Method 6800.[109]

Table 2.2 Studies in the past decade using IDMS for analysis of organic compounds.

| Analytes | Sample matrices | Instruments | References |
|---|---|---|---|
| Methylmercury | Fish tissues | GC-MS | Castillo, et al.[110] |
| Glyphosate and methylphosphonic acid | Drinking water | ESI-TOF-MS and APCI-Q-TOF-MS | Wagner, et al.[102] |
| POPs, e.g. PCBs, PBDEs, etc. | Human serum | GC-MS | Boggess, et al.[58] |
| Mercury species, e.g. methyl- and ethylmercury | Human whole blood | GC-ICP-MS | Rahman, et al.[111] |
| Reduced, oxidized and total glutathione | Biological samples, e.g. red blood cell and saliva | LC-MS/MS | Fahrenholz, et al.[107] |
| Drugs, e.g. morphine, heroin, etc. | Synthetic urine | ESI-TOF-MS | Wagner, et al.[108] |
| POPs, e.g. PCBs, PBDEs, etc. | Human serum | GC-MS | Boggess, et al.[56] |
| Glutathione and drug metabolite | Human whole blood | LC-MS/MS | Kingston, et al.[112] |
| POPs, e.g. PAHs, OCPs, etc. | Human whole blood | GC-MS/MS | Hao, et al.[59] |
| POPs, e.g. PAHs, OCPs, etc. | Dietary supplements | GC-MS/MS | Hao, et al. |

# Chapter 3: Quantification of POPs in human whole blood

## 3.1 Introduction

Persistent organic pollutants (POPs) are organic compounds that resist photolytic, biological, and chemical degradation, persist in the environment, bioaccumulate through the food chain, and may lead to adverse impacts on human health and the environment.[113] POPs have been released to the environment over the past decades mainly due to human activities. Widely distributed and accumulated over these decades, POPs have become one of the high-priority environmental and human health concerns around the globe. POPs are highly resistant to degradation due to their stable structures. The carbon-halogen bonds of the halogenated POPs can resist hydrolysis especially when halogens are attached to an aromatic ring.[6] POPs are also semi-volatile, which allows them to enter the atmosphere either in the vapor phase or adsorb on atmospheric particles and thus be transported over long distances.[2-4] POPs are typically lipophilic and this property of POPs leads to their propensity to readily pass through the phospholipid structure of biological membranes from the surrounding medium and accumulate in fatty tissue of the organism.[2, 6]

Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) are two categories of POPs that are present ubiquitously in the environment.[114] PAHs are generated anthropogenically from exhaust of motor vehicles, petroleum refineries, combustion of industrial and domestic wastes, chemical engineering processes, etc.[9-11] PAHs have been associated with DNA damages, chromosomal aberrations, and cancers especially leukemias.[14-18] OCPs have been extensively used in agriculture worldwide. Although the production and application of some OCPs have been banned in developed countries for decades, they are still widely present in water, soils, sediments, the

atmosphere, fish, and food products due to their high persistence and semi-volatile properties.[23-29] Many OCPs have been recognized as endocrine disrupters, which can interfere with the hormonal system and consequently damage the reproductive and immune systems of exposed individuals and may cause reproductive diseases such as breast cancer and prostate cancer.[30-32, 113] Quantification of PAHs and OCPs in human whole blood and demonstration of the analytical figures of merit of the method were the overarching aims of this study.

Stir-bar sorptive extraction (SBSE) has become a widely applied method for analysis of POPs since it was first described as a novel solvent free extraction technique in 1999.[60] The solutes are extracted from the sample matrix into a polymer coating on a glass magnetic stir bar. The extraction is controlled by the partitioning coefficient of the analytes between the polymer coating and the sample matrix as well as by the phase ratio between the polymer coating and the sample volume.[62-63] The polymeric coating is polydimethylsiloxane (PDMS), which has several specific characteristics that make it the most widely used sorptive extraction phase. The thermal stability of PDMS facilitates analyses under relatively high temperature conditions.[63] In addition, analytes can be absorbed into and retained within the bulk of the PDMS instead of being retained on the surface, and the retaining capacity of PDMS for a certain compound is not influenced by the presence of other analytes since each analyte has its own partitioning equilibrium into the PDMS phase. An analytical attribute of PDMS is that its degradation fragments contain characteristic silicone mass fragments that can easily be discerned by using mass spectrometry.[60] Stir-bar sorptive extraction coupled with GC/MS has been used to extract and analyze mainly hydrophobic organic compounds in aqueous samples. This combined

technique has demonstrated advantages such as low detection limits, high analyte recoveries, good repeatability, relative simplicity, and automation.[58, 64-66] Compared with traditional extraction methods such as liquid-liquid extraction, this newer method is more environmentally friendly since it minimizes the use of solvents and residual toxic wastes.

The exposome is defined as life-course environmental exposures from the prenatal period onwards.[49] It has been recognized as an environmental factor that impact human health, triggering metabolic changes and diseases.[51, 56] Understanding the potential link between the exposure to environmental pollutants, such as POPs, and human health is an important emphasis within a relatively new scientific field called exposomics. Recent studies have focused on developing effective methods to quantify POPs in the human body for the purposes of improving human health as well as disease diagnosis and prevention.[56, 58] A method that can provide accurate, precise, and sensitive quantitative measurements of POPs in the blood is essential to study the link between an individual's exposure to POPs and their health effects. In this study, a quantitative method using stir bar sorptive extraction-thermal desorption-gas chromatography-tandem mass spectrometry (GC/MS/MS)-isotope dilution mass spectrometry (IDMS) was developed to quantify POPs in human whole blood samples. In a previous study, an SBSE-GC/MS-IDMS method was used to analyze POPs in human serum samples.[58] By utilizing GC/MS/MS, this new method was expected to reach lower limit of quantifications, which is important in analyzing POPs at low concentration levels. Additionally, a reverse-IDMS method was demonstrated for the first time in this work. Reverse-IDMS is a quantitative method that enables verifying and recalibrating the concentration of standards of the analytes. Since the

results of a majority of studies typically rely on the labelled concentration of commercially available standards, a method that can verify and recalibrate these standards is essential.

3.2 Materials and experiments

The natural standards of the analytes (naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, δ-HCH, dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyltrichloroethane (DDT), and chlorpyrifos (CPS)) were obtained from Restek (Bellefonte, PA). The isotopic labelled standards of the analytes (naphthalene-D8, acenaphthene-D10, fluorene-D10, phenanthrene-D10, fluoranthene-D10, pyrene-D10, benzo[a]anthracene-D12, -D12, benzo[b]fluoranthene-D12, benzo[k]fluoranthene-D12, benzo[a]pyrene-D12, indeno[1,2,3-cd]pyrene-D12, benzo[ghi]perylene-D12, α-HCH-$^{13}$C6, β-HCH-$^{13}$C6, γ-HCH-$^{13}$C6, δ-HCH-$^{13}$C6, DDE-$^{13}$C12, DDD-$^{13}$C12, DDT-$^{13}$C12, and CPS-D10) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA). These standards were diluted to concentrations of 100-1000 ng/g and stored in a cold room (-20 °C).

The extraction was performed using 10 mm x 0.5 mm (length × film thickness) PDMS stir bars supplied by Gerstel (Mülheim an der Ruhr, Germany). The stirring process was carried out using a multiple-position magnetic stirring plate (Gerstel) at a stirring rate of 1200 rpm. After stirring for one hour, the stir bar was taken out of the matrix with tweezers, rinsed with deionized water, and carefully dried with clean cloth. Then the stir

bar was placed in a desorption tube and the tube was loaded in a tray and introduced sequentially into the TDU. The sample loading and handling were performed by a dual-head robotic multi-purpose sampler (MPS-2, Gerstel). A cooled injection system (CIS-6, Gerstel) was used as the injector for the GC/MS/MS system (7890B GC, 7010 MS/MS, Agilent Technologies, Santa Clara, CA). Figure 3.1 shows SBSE of human blood samples in sample vials on stirring plate.

The experimental parameters were adapted from previous SBSE methods.[58, 63] Desorption temperature of the TDU was set at 290 ºC. The analytes were desorbed under helium in the TDU and then were sent to CIS and cryofocused at -10 ºC by liquid nitrogen for 15 minutes. The CIS (with Tenax TA packed glass liner) was then heated at 12 ºC per second to 300 ºC to transfer the analytes to the GC column. The column used was HP-5 MS column (Agilent, 30 m × 0.25 mm internal diameter, 0.25 μm film thickness, 5%-phenyl methylpolysiloxane). The carrier gas was helium, at a flow rate of 1.2 mL/minute. The GC oven temperature was ramped at 10 ºC per minute from 40 ºC to 290 ºC, and then held at 290 ºC. After electron ionization, the analytes were analyzed by the triple-quad mass analyzer. Identification and quantification of analytes was conducted using the multiple reaction monitoring (MRM) mode. The MRM transitions of the analytes and the isotopes are shown in Table 3.1. Data analysis and IDMS calculation was performed using Agilent MassHunter Workstation software and Microsoft Excel. The peak areas of the analytes and isotopes were used for IDMS calculations.

*Discussion of Alternative Extraction Methods*

As an alternative extraction method, solid phase microextraction (SPME) was comparable to SBSE based on current literature. SPME has previously been investigated

for whole blood analysis in our research lab. Nonetheless, we experienced several issues with SPME for whole blood analysis. The main issue using SPME was that the fiber coating swelled when heated and then was easily damaged while being removed from the sample vial. As a result, when used in whole blood sample analysis, the SPME fiber usually had a lifespan of only a few uses. On the other hand, the stir bars utilized in SBSE have a lifespan usually between 50-100 uses. Eventually, we employed SBSE as our extraction method as it was considered the most effective method to couple with GC/MS/MS and IDMS.


Figure 3.1. Sample vials containing human whole blood sample stirring on a magnetic stirring plate.

Table 3.1 GC/MS/MS method parameters of the natural analytes and their isotopes.

| | RT (min) | Precursor (Da) | Product (Da) | | RT (min) | Precursor (Da) | Product (Da) | CE (V) |
|---|---|---|---|---|---|---|---|---|
| Naphthalene | 8.964 | 128 | 127 | Naphthalene-D8 | 8.923 | 136 | 134 | 15 |
| Acenaphthene | 13.024 | 152 | 151 | Acenaphthene-D10 | 12.956 | 162 | 160 | 30 |
| Fluorene | 14.224 | 165 | 164 | Fluorene-D10 | 14.156 | 175 | 173 | 30 |
| Phenanthrene | 16.449 | 178 | 177 | Phenanthrene-D10 | 16.394 | 188 | 186 | 15 |
| Fluoranthene | 19.267 | 202 | 201 | Fluoranthene-D10 | 19.227 | 212 | 210 | 5 |
| Pyrene | 19.780 | 202 | 201 | Pyrene-D10 | 19.739 | 212 | 210 | 5 |
| Benzo[a]anthracene | 22.653 | 228 | 227 | Benzo[a]anthracene-D12 | 22.612 | 240 | 238 | 5 |
| Chrysene | 22.747 | 228 | 227 | Chrysene-D12 | 22.693 | 240 | 238 | 5 |
| Benzo[b]fluoranthene | 25.161 | 252 | 251 | Benzo[b]fluoranthene-D12 | 25.106 | 264 | 262 | 5 |
| Benzo[k]fluoranthene | 25.228 | 252 | 251 | Benzo[k]fluoranthene-D12 | 25.173 | 264 | 262 | 5 |
| Benzo[a]pyrene | 25.997 | 252 | 251 | Benzo[a]pyrene-D12 | 25.942 | 264 | 262 | 5 |
| Indeno[1,2,3-cd]pyrene | 29.948 | 276 | 275 | Indeno[1,2,3-cd]pyrene-D12 | 29.852 | 288 | 286 | 25 |
| Benzo[ghi]perylene | 31.041 | 276 | 275 | Benzo[ghi]perylene-D12 | 30.918 | 288 | 286 | 25 |
| α-HCH | 15.531 | 181 | 145 | α-HCH-$^{13}$C6 | 15.531 | 187 | 151 | 10 |
| β-HCH | 16.111 | 181 | 145 | β-HCH-$^{13}$C6 | 16.111 | 187 | 151 | 10 |
| γ-HCH | 16.233 | 181 | 145 | γ-HCH-$^{13}$C6 | 16.233 | 187 | 151 | 10 |
| δ-HCH | 16.732 | 181 | 145 | δ-HCH-$^{13}$C6 | 16.732 | 187 | 151 | 10 |
| DDE | 20.304 | 246 | 176 | DDE-$^{13}$C12 | 20.303 | 258 | 188 | 30 |
| DDD | 21.088 | 235 | 165 | DDD-$^{13}$C12 | 21.087 | 247 | 177 | 20 |
| DDT | 21.776 | 235 | 165 | DDT-$^{13}$C12 | 21.775 | 247 | 177 | 20 |
| CPS | 18.518 | 314 | 258 | CPS-D10 | 18.436 | 324 | 260 | 15 |

## 3.3 Results and discussions

### *Method Validation*

After optimizing the experimental parameters, the method was validated by quantifying POPs with known concentrations in blank-subtracted bovine whole blood samples. Ideally, a human whole blood standard reference material containing the analytes can be used for validation of the method. However, currently such a standard reference material was not found on the market. Instead, we spiked the bovine whole blood samples with certified standards of the analytes followed by recovery tests. Into each sample vial, 8 mL of deionized water, 2 mL of acetonitrile, and 200 µL of bovine whole blood were added by mass. The certified natural standards of the analytes were spiked into the bovine whole blood samples to create reference standards at four different concentrations (0.100, 0.321, 1.23, and 3.41 ng/g for PAHs; 0.0396, 0.127, 0.487, and 1.35 ng/g for OCPs; 0.109, 0.349, 1.34, and 3.70 ng/g for CPS). These concentrations were shown as concentration 1, 2, 3, and 4 in the later discussions. The isotopically enriched standards were spiked into these reference standards to quantify the natural analytes using IDMS. At each concentration level, five replicate samples were analyzed (n=5). The measurements of the spiked analytes in the reference standards were compared with the theoretical values at the four different concentrations. The units of these measurements were converted to ng/g. Except for acenaphthene, no statistical difference between the measured values and the theoretical values was observed, which indicated the accuracy of the method for most of the analytes. For acenaphthene, reverse-IDMS method was employed to recalibrate and verify the labelled concentration of the isotope and natural standard. This will be further discussed in later sections. Percent error

of the measurements compared with the theoretical values and the relative standard deviations (RSDs) were shown in Table 3.2. Except for acenaphthene, the error of the analytes ranged from 2.51% to 10.8% and the RSD was in a range of 6.30-15.3%. The mean error of all the analytes (excluding acenaphthene) was 6.52% with a mean RSD of 9.27%. The limit of quantifications (LOQs) of the analytes using this method were also listed in Table 3.2. The LOQs were calculated using the mean plus ten times standard deviation of a set of blank measurements (n=5). These LOQs were approximately one order of magnitude lower than the LOQs reported in previous SBSE-GC/MS methods [58, 109, 115-117] which was likely due to the application of the MRM feature of the GC/MS/MS.

Table 3.2 Percent errors, RSDs, and LOQs of the measurements of the spiked analytes in the bovine whole blood using SBSE-GC/MS/MS-IDMS (n=5). Error (%) = | measured mean value – theoretical mean value | / theoretical mean value x 100%. Mean percent errors and mean RSDs were the mean values of the percent errors and RSDs determined at the four different spiking concentrations.

| | Mean Error (%) | Mean RSD (%) | LOQ (ng/g) |
|---|---|---|---|
| Naphthalene | 9.18 | 8.11 | 0.0758 |
| Acenaphthene | 35.4 | 8.29 | 0.0102 |
| Fluorene | 2.51 | 7.90 | 0.0107 |
| Phenanthrene | 9.16 | 8.89 | 0.0143 |
| Fluoranthene | 5.17 | 8.45 | 0.0106 |
| Pyrene | 10.8 | 9.31 | 0.0106 |
| Benzo[a]anthracene | 8.85 | 8.83 | 0.0116 |
| Chrysene | 7.06 | 8.98 | 0.0100 |
| Benzo[b]fluoranthene | 6.20 | 11.2 | 0.0670 |
| Benzo[k]fluoranthene | 2.53 | 11.0 | 0.0483 |
| Benzo[a]pyrene | 3.76 | 11.7 | 0.0666 |
| Indeno[1,2,3-cd]pyrene | 4.77 | 13.1 | 0.0167 |
| Benzo[ghi]perylene | 5.29 | 15.3 | 0.0173 |
| α-HCH | 3.36 | 6.65 | 0.0162 |
| β-HCH | 10.3 | 9.73 | 0.0266 |
| γ-HCH | 6.70 | 7.47 | 0.0161 |
| δ-HCH | 7.50 | 8.91 | 0.0221 |
| DDE | 8.17 | 7.17 | 0.0171 |
| DDD | 6.17 | 6.30 | 0.0103 |
| DDT | 4.41 | 9.16 | 0.0185 |
| CPS | 8.63 | 8.19 | 0.0259 |

*Comparison between IDMS and Calibration Curves*

The measurements using the IDMS method were compared with measurements using calibration curves. Standard five-point calibration curves were created for each analyte. The isotope standards were added as internal standards (IS) to create calibration curves with IS for each analyte. The comparison of percent error of the measurements using IDMS, calibration curves, and calibration curves with IS is shown in Table 3.3. Mean percent errors and RSDs of the measurements using IDMS, calibration curves, and calibration curves with IS were calculated excluding acenaphthene. The results are listed in Table 3.4. At concentration 1, the mean error of the measurements using calibration curves was over 80%. For naphthalene, α-HCH, β-HCH, γ-HCH, and δ-HCH the errors were over 100%. By adding IS the mean error of measurements at concentration 1 was improved to 39.0%. However, the mean RSD was at a high level of 31.9%. As a comparison, the mean error and RSD of measurements using IDMS were at relatively lower levels (10.6% and 14.6%, respectively). At concentration 2, the mean error of the measurements significantly decreased for IDMS, calibration curves, and calibration curves with IS. These mean errors were 5.66%, 20.3%, and 5.78%, respectively. At concentration 3 and 4, the mean error of calibration curves continued decreasing to 16.8% and 8.60%, whereas the mean errors of IDMS and calibration curves were similar compared with concentration 2. From concentration 2 to concentration 4, the mean errors of IDMS and calibration curves with IS were within the range of 4.89-6.81%. At concentration 1 and 2, the mean RSDs of the measurements using IDMS were 14.6% and 7.33%, which were lower than using calibration curves (18.3% and 26.0%, respectively) and calibration curves with IS (31.9% and 12.1%, respectively). At concentration 3 and 4,

calibration curves with IS had the lowest mean RSDs of 5.38% and 5.05%. The mean RSDs of IDMS were slightly higher at 8.53% and 6.85%. The mean RSDs of calibration curves (8.11% and 9.71%, respectively) were higher than calibration curves with IS, however, these mean RSDs decreased compared to concentration 1 and 2.

A graphic comparison of the measurements using IDMS, calibration curves, and calibration curves with IS is shown in Figure 3.2. Fluorene was selected as an example to present the results. IDMS had observable advantages in accuracy and precision compared with the other two methods at the lowest measured concentration. At the other three higher measured concentrations, calibration curves and calibration curves with IS had improved accuracy and precision. However, IDMS had consistently high level of accuracy and precision with a mean error of 2.67% and RSD of 7.03%. These results coordinated with the conclusions from previous studies[58, 108] that the accuracy and precision of the measurements using calibration curves worsened when approaching the LOQ of the method. This work demonstrated the capability of IDMS to maintain quantitative accuracy and precision consistently.

Table 3.3 Errors (%) of measurements of the spiked analytes in bovine whole blood at the four different spiking concentrations. Results showing comparison between IDMS and calibration curves (CC) with and without internal standards (IS) added (n=5).

| | Concentration 1 | | | Concentration 2 | | | Concentration 3 | | | Concentration 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS |
| Naphthalene | >100 | 47.3 | 24.7 | 19.3 | 1.24 | 1.42 | 26.6 | 5.15 | 4.51 | 2.82 | 5.14 | 6.08 |
| Acenaphthene | 75.0 | 20.5 | 41.0 | 9.11 | 1.34 | 34.3 | 27.6 | 2.84 | 32.7 | 3.04 | 5.31 | 33.6 |
| Fluorene | 82.9 | 21.0 | 2.02 | 19.2 | 4.08 | 0.780 | 26.2 | 8.39 | 4.07 | 0.222 | 12.9 | 3.16 |
| Phenanthrene | 99.4 | 25.0 | 22.6 | 15.1 | 0.107 | 6.96 | 26.0 | 10.2 | 6.57 | 3.68 | 9.71 | 0.559 |
| Fluoranthene | 65.0 | 7.38 | 2.86 | 12.3 | 1.76 | 4.50 | 23.7 | 3.76 | 5.23 | 4.61 | 4.48 | 8.08 |
| Pyrene | 69.9 | 31.1 | 7.96 | 11.9 | 0.857 | 11.9 | 22.4 | 8.03 | 11.7 | 0.169 | 12.5 | 11.7 |
| Benzo[a]anthracene | 34.3 | 61.3 | 6.55 | 0.527 | 11.0 | 9.60 | 6.30 | 5.81 | 8.55 | 1.29 | 10.3 | 10.7 |
| Chrysene | 55.0 | 14.0 | 14.9 | 0.455 | 2.44 | 5.61 | 21.3 | 1.61 | 7.04 | 9.28 | 11.1 | 0.704 |
| Benzo[b]fluoranthene | 55.0 | 30.7 | 8.25 | 0.0653 | 5.10 | 7.49 | 9.43 | 7.97 | 0.854 | 9.24 | 3.46 | 8.19 |
| Benzo[k]fluoranthene | 56.3 | 79.8 | 1.03 | 13.8 | 11.2 | 3.13 | 28. 4 | 8.73 | 2.70 | 34.3 | 16.5 | 3.24 |
| Benzo[a]pyrene | 19.2 | 4.15 | 4.12 | 42.3 | 5.54 | 6.21 | 32.8 | 1.77 | 2.95 | 30.1 | 0.472 | 1.77 |
| Indeno[1,2,3-cd]pyrene | 3.06 | 80.6 | 9.52 | 23.1 | 16.2 | 2.27 | 23.1 | 1.38 | 4.87 | 10.6 | 4.99 | 2.43 |
| Benzo[ghi]perylene | 32.3 | 37.9 | 15.0 | 16.4 | 3.33 | 0.391 | 1.91 | 2.87 | 2.60 | 6.25 | 6.57 | 3.19 |
| α-HCH | >100 | 34.8 | 11.6 | 36.9 | 3.21 | 0.273 | 23.9 | 4.75 | 0.589 | 0.0791 | 7.00 | 1.01 |
| β-HCH | >100 | 14.4 | 16.1 | 16.7 | 11.2 | 9.12 | 16.6 | 2.04 | 6.09 | 3.87 | 6.48 | 9.71 |
| γ-HCH | >100 | 13.8 | 16.3 | 33.9 | 6.30 | 6.89 | 27.1 | 0.997 | 1.74 | 0.783 | 0.751 | 1.88 |
| δ-HCH | >100 | 36.8 | 13.1 | 40.2 | 6.18 | 6.89 | 7.52 | 1.19 | 6.38 | 5.67 | 2.430 | 3.61 |
| DDE | 48.7 | 5.21 | 10.3 | 48.8 | 0.666 | 5.59 | 0.833 | 0.775 | 9.43 | 18.8 | 3.31 | 7.32 |
| DDD | 76.4 | 85.5 | 2.35 | 14.9 | 10.21 | 9.17 | 5.06 | 4.85 | 7.18 | 7.67 | 6.04 | 6.01 |
| DDT | 40.1 | 90.7 | 8.08 | 29.0 | 2.15 | 6.10 | 1.11 | 16.3 | 0.0921 | 16.5 | 6.69 | 3.37 |
| CPS | 22.0 | 59.6 | 13.9 | 11.4 | 12.8 | 8.91 | 4.91 | 2.98 | 6.76 | 6.17 | 5.20 | 4.99 |

Table 3.4 Mean percent error and relative standard deviations (RSDs) of the measurements of the spiked analytes (excluding acenaphthene) in bovine whole blood at four different spiking concentrations using IDMS, calibration curves, and calibration curves with internal standards (IS) for quantification (n=5).

| | | **Concentration 1** | **Concentration 2** | **Concentration 3** | **Concentration 4** |
|---|---|---|---|---|---|
| CC | Error (%) | 82.5 | 20.3 | 16.8 | 8.60 |
| | RSD (%) | 18.3 | 26.0 | 8.11 | 9.71 |
| CC with IS | Error (%) | 39.0 | 5.78 | 4.98 | 6.81 |
| | RSD (%) | 31.9 | 12.1 | 5.38 | 5.05 |
| IDMS | Error (%) | 10.6 | 5.66 | 4.99 | 4.89 |
| | RSD (%) | 14.6 | 7.33 | 8.53 | 6.58 |

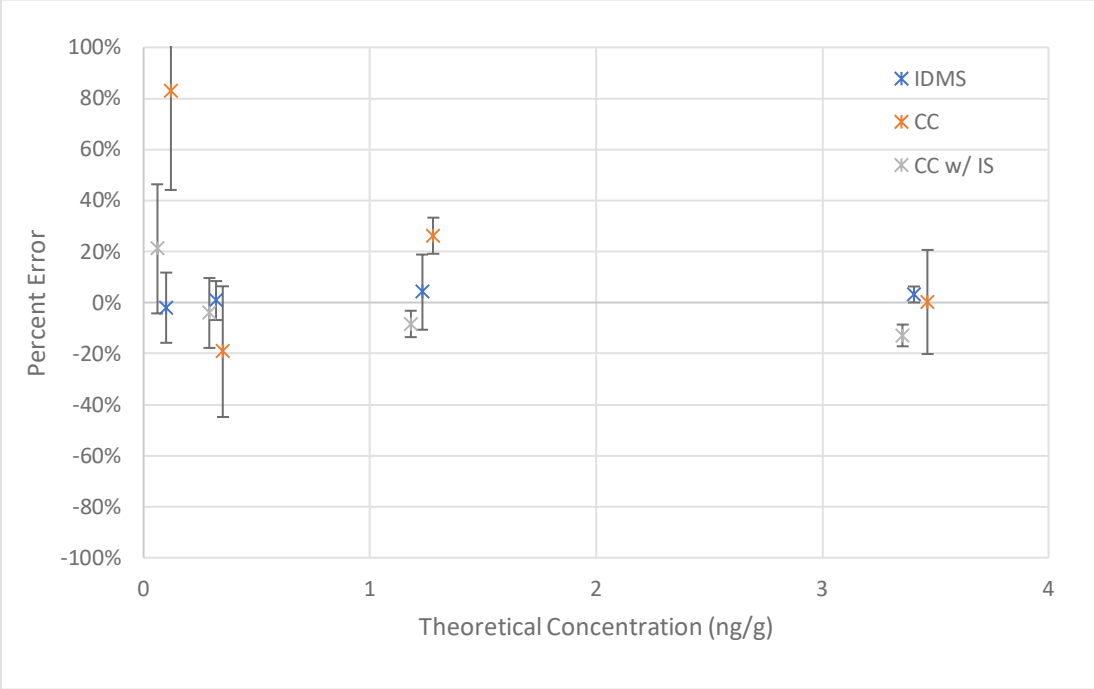


Figure 3.2 Comparing percent error of measurements of spiked fluorene in bovine whole blood at the four different spiking concentrations using IDMS, calibration curves, and calibration curves with IS for quantification (n=5, 95% CI).

*Recalibration and validation of the purchased standards using reverse-IDMS*

Isotopically enriched standards purchased from commercial manufacturers are generally considered to have "accurate" labelled concentrations. However, there is a need for a method that can validate and recalibrate the concentrations of these standards. When considering the concentration of the target analyte in the sample, i.e. $C_s$, as known, the IDMS equation can be modified to a reverse-IDMS equation to calculate the concentration of the isotope in the spike, i.e. $C_{sp}$.

Solving IDMS Eq. 1.1 for $C_{sp}$:

Eq. 3.1 $$C_{sp} = \frac{C_s W_s (A_s - R_m B_s)}{W_{sp}(R_m B_{sp} - A_{sp})}$$

Eq. 3.1 is the reverse-IDMS equation that was used to calculate and verify the concentration of the isotope in the spike. The results were compared to the labelled concentrations for verification and recalibration. In this study, the measured concentrations of acenaphthene using IDMS were on average 35.4% higher than the expected values. This error was suspected to originate from the inaccuracy of the labelled concentration of either the isotopically enriched standard or the natural standard used to create the reference standard. Reverse-IDMS was performed using two additional natural standards at two concentrations to test whether the labelled concentration of acenaphthene-D10 was accurate as labelled. The natural standards were obtained from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Hampton, NH). The blank-subtracted bovine whole blood samples were spiked with acenaphthene-D10 at two different concentrations and then spiked with the natural standard of acenaphthene for quantification of acenaphthene-D10. Analysis was performed using SBSE-GC/MS/MS and Eq. 3.1 was applied for quantification. Expected and measured concentration of

acenaphthene-D10 at two different concentrations were listed in Table 3.5. When the additional natural standards were used the mean error between the measured concentrations of acenaphthene-D10 and the theoretical concentrations decreased to 5.43%. There was no statistical difference between the expected concentrations and the measured concentrations of acenaphthene-D10 using the two additional natural standards, indicating that the labelled concentration of the isotopically enriched standard was accurate. Therefore, it verified that the labelled concentration of acenaphthene in the natural standard that was used to create the reference standard was not accurate as labelled, which resulted in the 35.4% error. This work demonstrated that reverse-IDMS can be a valuable tool to trace the inaccuracies of measurements and has the potential to be employed to troubleshoot for analytical errors and to perform method validation.

Table 3.5 Expected and measured concentration of acenaphthene-D10 using two different natural standards with reverse-IDMS at two different concentrations (ng/g, n=5, 95% CI). Natural standard from Sigma Aldrich is listed as “Natural Standard 1” and natural standard from Fisher Scientific is listed as “Natural Standard 2”.

| | Natural Standard 1 | | Natural Standard 2 | |
|---|---|---|---|---|
| | Concentration 1 | Concentration 2 | Concentration 1 | Concentration 2 |
| Expected Acenaphthene-D10 Concentration | 0.400 ± 0.032 | 1.15 ± 0.02 | 0.411 ± 0.037 | 1.16 ± 0.03 |
| Measured Acenaphthene-D10 Concentration | 0.439 ± 0.031 | 1.17 ± 0.03 | 0.376 ± 0.031 | 1.14 ± 0.03 |

*Quantification of Human Whole Blood Samples*

After development, optimization and validation, the method was used to analyze human whole blood samples obtained from Stanford Blood Center (Palo Alto, CA). Donors of the blood center typically come from within a 30-mile radius of the San Francisco Bay area. Ten whole blood samples were randomly selected and deidentified. These samples were listed as 1-10 in Table 3.6. Approximately 200 mg of the whole blood was added to each sample vial with 8 mL of deionized water and 2 mL of acetonitrile and then analyzed using SBSE-GC/MS/MS-IDMS. Number of detection and concentration range of these analytes were listed in Table 3.7. Naphthalene, α-HCH, DDD, DDE were detected in all ten blood samples; phenanthrene, benzo[a]pyrene, γ-HCH, and DDT were detected in at least eight blood samples; benzo[a]anthracene, chrysene, benzo[k]fluoranthene, and chlorpyrifos were detected in none of the ten samples. On average, 10 analytes were detected in each blood sample; 14 were detected in sample No. 1 which was the highest number detected among the ten samples and 8 were detected in sample No. 5, 8, and 10 which was the lowest number detected. Most analytes detected had average concentration below 1 ng/g. However, naphthalene, DDE, and benzo[a]pyrene had average concentrations above 1 ng/g (1.53, 1.57, and 1.84, respectively). The average concentration of all quantified analytes were grouped into one variable termed mean xenobiotic body-burden (MXB) [56]. Sample No. 9 had an MXB of 1.12 ng/g which was the highest among the ten samples and sample No. 5 had an MXB of 0.719 ng/g which was the lowest. On average the MXB was 0.897 ng/g in each sample. MXB has been reported to be associated with certain human health issues such as autism spectrum disorder [56, 118].

Table 3.6. Measurements of the analytes in ten human whole blood samples from Stanford Blood Center using SBSE-GC/MS/MS-IDMS (n=5, 95% CI). Units of these measurements were converted to ng/g. Results below limit of quantification are shown as N/A.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 5.14 ± 1.55 | 1.19 ± 0.34 | 1.16 ± 0.23 | 0.618 ± 0.133 | 0.892 ± 0.145 | 0.972 ± 0.210 | 1.31 ± 0.28 | 1.93 ± 0.39 | 1.01 ± 0.15 | 1.04 ± 0.10 |
| Acenaphthene | 0.515 ± 0.047 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Fluorene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0.116 ± 0.027 | N/A | N/A |
| Phenanthrene | 0.189 ± 0.027 | 0.144 ± 0.023 | 0.0823 ± 0.0169 | 0.164 ± 0.035 | 0.159 ± 0.021 | N/A | 0.0867 ± 0.0184 | 0.318 ± 0.050 | 0.256 ± 0.055 | 0.230 ± 0.065 |
| Fluoranthene | 0.0643 ± 0.0151 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0.0905 ± 0.0176 |
| Pyrene | 0.108 ± 0.024 | 0.103 ± 0.019 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[a]anthracene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Chrysene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[b]fluoranthene | N/A | N/A | N/A | N/A | N/A | 1.76 ± 0.15 | N/A | N/A | 2.46 ± 0.30 | N/A |
| Benzo[k]fluoranthene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[a]pyrene | N/A | 1.88 ± 0.33 | N/A | 2.74 ± 0.22 | 2.33 ± 0.87 | 1.56 ± 0.16 | 2.29 ± 0.48 | 1.17 ± 0.26 | 1.33 ± 0.15 | 2.38 ± 0.23 |
| Indeno[1,2,3-cd]pyrene | 0.306 ± 0.068 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[ghi]perylene | 0.289 ± 0.047 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| α-HCH | 0.828 ± 0.148 | 0.336 ± 0.068 | 0.828 ± 0.216 | 0.401 ± 0.087 | 0.386 ± 0.078 | 0.514 ± 0.112 | 0.664 ± 0.101 | 0.333 ± 0.091 | 0.291 ± 0.072 | 0.494 ± 0.118 |
| β-HCH | 1.04 ± 0.19 | N/A | 0.584 ± 0.137 | 0.564 ± 0.094 | N/A | 0.870 ± 0.190 | 1.15 ± 0.28 | N/A | 0.544 ± 0.174 | N/A |
| γ-HCH | 0.473 ± 0.094 | 0.541 ± 0.099 | 0.571 ± 0.136 | 0.694 ± 0.180 | 0.250 ± 0.117 | 0.511 ± 0.078 | 0.564 ± 0.121 | N/A | 0.305 ± 0.070 | N/A |
| δ-HCH | 0.877 ± 0.213 | 0.550 ± 0.168 | 0.800 ± 0.167 | 0.690 ± 0.130 | N/A | 0.837 ± 0.147 | 1.26 ± 0.357 | N/A | N/A | N/A |
| DDE | 1.18 ± 0.24 | 1.43 ± 0.19 | 3.51 ± 0.34 | 1.09 ± 0.17 | 0.756 ± 0.164 | 2.73 ± 0.28 | 0.958 ± 0.172 | 0.911 ± 0.143 | 3.89 ± 0.15 | 1.91 ± 0.20 |
| DDD | 0.535 ± 0.036 | 0.513 ± 0.085 | 0.495 ± 0.048 | 0.539 ± 0.07 | 0.482 ± 0.045 | 0.550 ± 0.054 | 0.530 ± 0.073 | 0.488 ± 0.051 | 0.512 ± 0.037 | 0.575 ± 0.090 |
| DDT | 0.729 ± 0.050 | N/A | 0.556 ± 0.130 | 0.963 ± 0.092 | 0.501 ± 0.104 | 0.519 ± 0.098 | 0.567 ± 0.073 | 0.595 ± 0.109 | 0.610 ± 0.120 | 0.954 ± 0.191 |
| CPS | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |

Table 3.7 Number of the whole blood sample each analyte was detected in and their concentration range analyzed using SBSE-GC/MS/MS-IDMS (n=5). Results below limit of quantification are shown as N/A.

| | Number of detections | Concentration range (ng/g) |
|---|---|---|
| Naphthalene | 10 | 0.618 – 5.14 |
| Acenaphthene | 1 | 0 – 0.515 |
| Fluorene | 1 | 0 – 0.116 |
| Phenanthrene | 9 | 0 – 0.318 |
| Fluoranthene | 2 | 0 – 0.0905 |
| Pyrene | 2 | 0 – 0.108 |
| Benzo[a]anthracene | 0 | N/A |
| Chrysene | 0 | N/A |
| Benzo[b]fluoranthene | 2 | 0 – 2.46 |
| Benzo[k]fluoranthene | 0 | N/A |
| Benzo[a]pyrene | 8 | 0 – 2.74 |
| Indeno[1,2,3-cd]pyrene | 1 | 0 – 0.306 |
| Benzo[ghi]perylene | 1 | 0 – 0.289 |
| α-HCH | 10 | 0.291 – 0.828 |
| β-HCH | 6 | 0 – 1.15 |
| γ-HCH | 8 | 0 – 0.694 |
| δ-HCH | 6 | 0 – 1.26 |
| DDE | 10 | 0.756 – 3.89 |
| DDD | 10 | 0.482 – 0.575 |
| DDT | 9 | 0 – 0.954 |
| CPS | 0 | N/A |

## 3.4 Conclusions

An accurate, precise, sensitive, and efficient method was developed for quantification of POPs in human whole blood samples. IDMS was compared with calibration curves and was demonstrated to be able to increase accuracy and precision of the measurements especially at lower measured concentrations. A reverse-IDMS method was developed to verify and recalibrate labelled concentrations of commercially available standards. Finally, this validated SBSE-GC/MS/MS-IDMS method was applied to quantify POPs in human whole blood samples. The mean concentration of the quantified analytes was also calculated for each sample. The method developed in this study enables accurate, precise, and sensitive quantitative measurements of POPs in the blood and is important to study and understand the link between an individual's exposure to POPs and their adverse effects on human health. The small blood sample volume required by this method can facilitate the use of a minimally invasive finger-stick sampling instead of traditional vein blood draw sampling. This method is also capable of further applications in exposomics. Human whole blood samples from various regions can be analyzed to study the regional and demographic distribution of POPs. This can contribute to investigation of presence and concentration of the agricultural and industrial sources of POPs and their impacts in different communities and regions.

# Chapter 4: Quantification of POPs in dietary supplements

## 4.1 Introduction

Persistent organic pollutants (POPs) are highly stable organic chemicals that resist photolytic, biological, and chemical degradation. They persist in the environment, bioaccumulate through the food chain, and may adversely impact human health and the environment.[113] POPs have been released to the environment over the past decades mainly due to human activities. Widely distributed and accumulated over these decades, POPs have become one of the high-priority environmental and human health concerns around the globe. POPs are highly resistant to degradation due to their stable structures. They are also semi-volatile, which allows them to enter the atmosphere either in the vapor phase or adsorb on atmospheric particles that are transported over long distances.[2-4] Because of their hydrophobic structures, most POPs readily pass through the phospholipid structure of biological membranes from the surrounding medium and accumulate in the living organism.[2, 6]

Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) are two categories that are present ubiquitously in the environment.[114] PAHs are generated naturally or anthropogenically from sources such as the exhaust of motor vehicles, petroleum refineries, combustion of industrial and domestic wastes, and chemical engineering processes.[9-11] PAHs have been linked to increased risks of DNA damages, chromosomal aberrations, and cancers such as leukemia.[14-18] OCPs have been extensively used in agriculture globally. Although the production and application of some OCPs have been banned in developed countries for decades, they are still widely present in water, soils, sediments, the atmosphere, fish, and food products due to their high persistence and semi-

volatile properties.[23-29] Many OCPs have been recognized as endocrine disrupters which can interfere with the hormonal system and consequently damage the reproductive and immune systems of exposed individuals and may cause reproductive diseases such as breast cancer and prostate cancer.[30-32, 113] Chlorpyrifos is a widely-used organophosphate insecticide that has been associated with the alteration of brain cell development in rats, leading to changes in cognitive impairment, especially during early neonatal exposure [119]. California, which consumes a large amount of chlorpyrifos each year, began the legislative process to ban this pesticide completely in 2019.[59]

POPs have been found in children's blood[56] and blood samples from a blood bank in Northern California.[59] Human's exposure to these toxins typically occurs through diet, drinking water, and air.[120] Dietary supplements are suspected to be one of the sources of the exposure. Dietary supplements are products intended to supplement the diet and typically contain dietary ingredients such as vitamins, minerals, herbs, fiber, botanical extracts, amino acids, and other substances that increase an individual's dietary intake.[121-122] More than half of adults in the US take dietary supplements daily or occasionally.[20] The majority of botanicals or herbals that are used as raw materials of dietary supplements are farmed using conventional agricultural practices that may involve pesticide application.[123] In addition, POPs consumed in different agricultural or industrial regions can be transported over long distances and contaminate the botanicals that are used in dietary supplement formulations. Thus, POPs in dietary supplements can be a source of xenobiotic toxins in the human body that can adversely impact human health. Dietary supplements from worldwide have been analyzed and found to contain POPs such as PAHs, polychlorinated biphenyls, polybrominated diphenyl ethers, and pesticides.[124-128]

Physicians and nutritionists generally suggest taking dietary supplements to adjust metabolism and improve health without considering the toxins they may contain. Currently, no routine method exists for the extraction and quantitative determination of POPs in dietary supplements. Therefore, the development of an effective and efficient extraction and analysis protocol for POPs in dietary supplements is important to assure product quality, public safety, and regulatory compliance.

Stir-bar sorptive extraction (SBSE) is a solid phase extraction technique which was first described in 1999.[60] A glass magnetic stir-bar with a polymer coating, typically polydimethylsiloxane (PDMS), was employed to extract analytes from the sample matrix. The extraction is controlled by the partition coefficient of the analytes between the PDMS coating and the sample matrix, as well as by the phase ratio between the PDMS coating and the sample volume.[62-63] After the analytes are extracted, the stir-bar is removed from the sample matrix for thermal desorption. The thermal desorption unit (TDU) thermally desorbs the analytes from the stir-bar and the cooled injection system (CIS) cryofocuses the desorbed analytes and then injects them into the GC for separation. With SBSE, the sample pretreatment procedure and the amount of solvents used are minimized compared with multiple-step extractions that are typically employed for analysis of POPs in solid samples. SBSE coupled with GC-MS has been used to extract and analyze mainly hydrophobic organic compounds in aqueous samples. This platform has demonstrated advantages such as low detection limits, high analyte recoveries, good repeatability, and relative simplicity and automation.[58, 64-66] To quantify 21 POPs including PAHs, OCPs, and chlorpyrifos in 12 plant-extract based dietary supplement products that are commercially available off-the-shelf in the US, a quantitative method using SBSE, gas chromatography-

tandem mass spectrometry (GC-MS/MS), and isotope dilution mass spectrometry was developed.

4.2 Materials and experiments

The unlabeled natural standards of the analytes (naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, δ-HCH, p,p'-dichlorodiphenyldichloroethylene (DDE), p,p'-dichlorodiphenyldichloroethane (DDD), p,p'-dichlorodiphenyltrichloroethane (DDT), and chlorpyrifos) were obtained from Restek (Bellefonte, PA). The isotopic labelled standards of the analytes (naphthalene-D8, acenaphthene-D10, fluorene-D10, phenanthrene-D10, fluoranthene-D10, pyrene-D10, benz [a]anthracene-D12, chrysene-D12, benzo[b]fluoranthene-D12, benzo[k]fluoranthene-D12, benzo[a]pyrene-D12, indeno[1,2,3-cd]pyrene-D12, benzo[ghi]perylene-D12, α-HCH-$^{13}$C6, β-HCH-$^{13}$C6, γ-HCH-$^{13}$C6, δ-HCH-$^{13}$C6, DDE-$^{13}$C12, DDD-$^{13}$C12, DDT-$^{13}$C12, and chlorpyrifos-D10) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA). These standards were diluted to concentrations of 100-1000 ng/g in acetone and stored in a cold room (-20 °C).

Dietary supplement samples investigated in this study were plant-extract based products in the form of either tablet or powder. The samples in tablet form were homogenized by trituration using Retsch Grindomix GM200 (Haan, Germany), whereas samples in fine powder form were directly used in the next step. Approximately 1 g of the processed dietary supplement sample, 10 mL of solvents, and a stir-bar, were added to each

sample vial for SBSE. The extraction was performed using 10 mm x 0.5 mm (length × film thickness) PDMS stir-bars supplied by GERSTEL (Mülheim an der Ruhr, Germany). The stirring process was carried out using a multiple-position magnetic stirring plate (GERSTEL) at a stirring rate of 1200 rpm. After stirring for one hour, the stir-bar was taken out of the matrix with tweezers, rinsed with deionized water, and carefully dried with a clean wipe. Then the stir-bar was placed in a desorption tube which was then placed in a tray and introduced sequentially into the TDU. The sample loading and handling were performed by a dual-head robotic multi-purpose sampler (MPS-2, GERSTEL) with full automation. A cooled injection system (CIS-6, GERSTEL) was used as the injector for the GC-MS/MS instrument (7890B GC, 7010 MS/MS, Agilent Technologies, Santa Clara, CA).

The experimental parameters were adapted from previous SBSE methods.[58-59, 63] Desorption temperature of the TDU was set at 290 ºC. The analytes were desorbed under helium in the TDU and then sent to CIS and cryofocused by liquid nitrogen at -10 ºC for 15 min. The CIS with Tenax TA packed glass liner was then heated at 12 ºC per second to 300 ºC to transfer the analytes to the GC column. The column used was HP-5 MS column (Agilent, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness, 5%-phenyl methylpolysiloxane). The carrier gas was helium, at a flow rate of 1.2 mL/min. The GC oven temperature was ramped at 10 ºC/min from 40 ºC to 290 ºC, and then held at 290 ºC. After electron ionization (70 eV; 230 ºC), the analytes were analyzed by the triple-quad mass analyzer. Identification and quantification of analytes were conducted using the multiple reaction monitoring (MRM). The MRM transitions of the analytes and the isotopes were described previously.[59] Data analysis and IDMS calculation was performed

using Agilent MassHunter Workstation software and Microsoft Excel. The peak areas of the analytes and isotopes were used for calculations.

## 4.3 Results and discussions

### *Optimization of Extraction Procedure*

Extraction solvents and time of the SBSE method were optimized using a set of recovery experiments. The unlabeled natural standards of the analytes were spiked into a plant-extract based dietary supplement product in fine powder form to create a reference standard at a concentration of 10 ng/g. Relative recoveries of the analytes using different extraction procedures were compared to determine the optimized extraction solvents and time. The extraction time was set at 1h to compare relative recoveries of different extraction solvents including: 1) 10 mL deionized water, 2) 2 mL methanol + 8 mL deionized water, 3) 5 mL methanol + 5 mL deionized water, 4) 2 mL acetonitrile + 8 mL deionized water, and 5) 5 mL acetonitrile + 5 mL deionized water. Relative recoveries of the spiked analytes in dietary supplement sample using these different extraction solvents were shown in Figure 4.1. The 2 mL acetonitrile + 8 mL deionized water had the highest recoveries for over half of the analytes and showed the overall highest recovery. The 10 mL deionized water as well as 2 mL methanol + 8 mL deionized water presented higher recoveries for analytes with lower molecular weight such as naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, α-HCH, β-HCH, γ-HCH, and δ-HCH. However, these relatively more polar solvents had poor recoveries for analytes like benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, DDE, DDD, and DDT. Therefore, 2 mL acetonitrile + 8

mL deionized water was selected for the extraction protocol of the method. Using 2 mL acetonitrile and 8 mL deionized water as extraction solvents, different extraction time (30, 60, 90, and 120 min) were investigated. Relative recoveries of the spiked analytes in dietary supplement sample with different extraction time were shown in Figure 4.2. The relative recovery of most analytes reached equilibrium at 60 min. These results correlate with previous SBSE methods applied in other matrices.[58-59, 63]

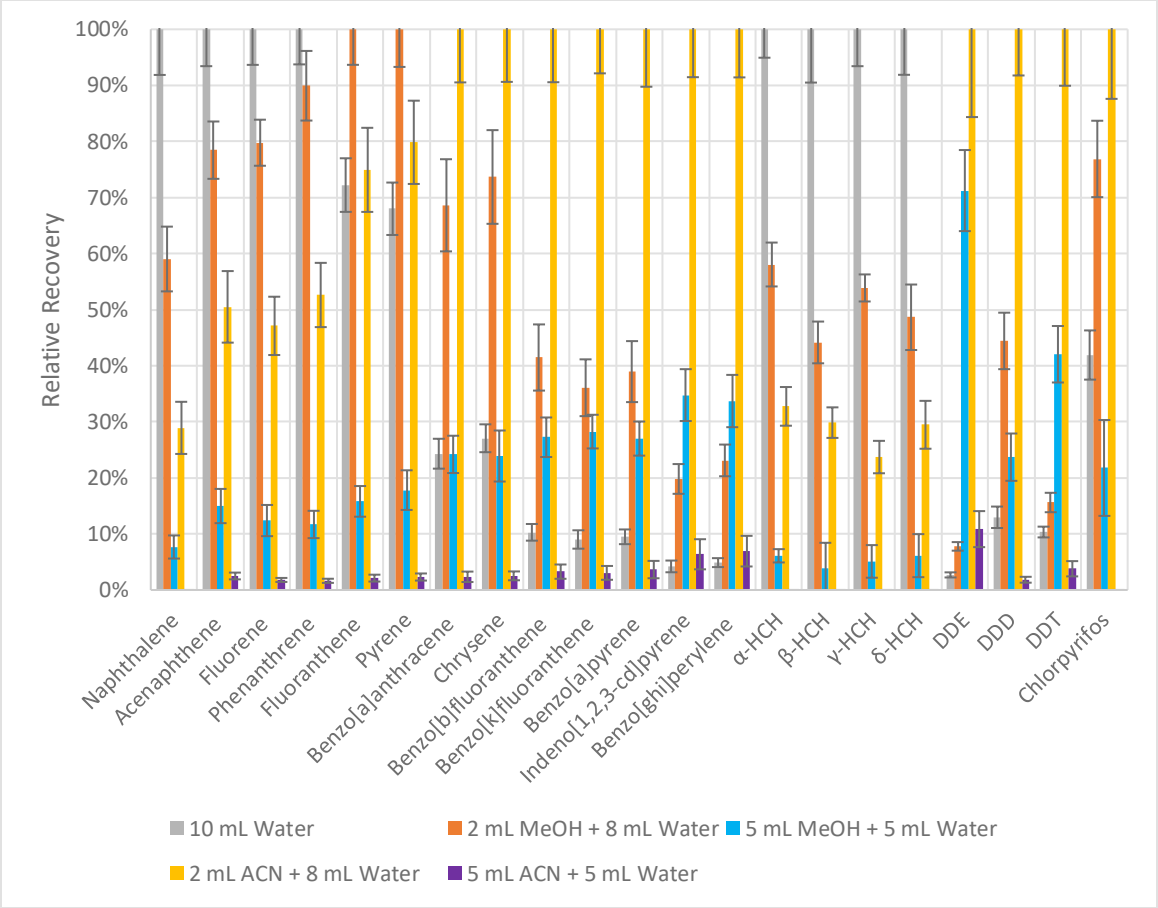


Figure 4.1. Relative recovery of the spiked analytes in dietary supplement sample using different extraction solvents (n=5, 95% CI). The relative recovery of each analyte was normalized to a 0-100% scale.

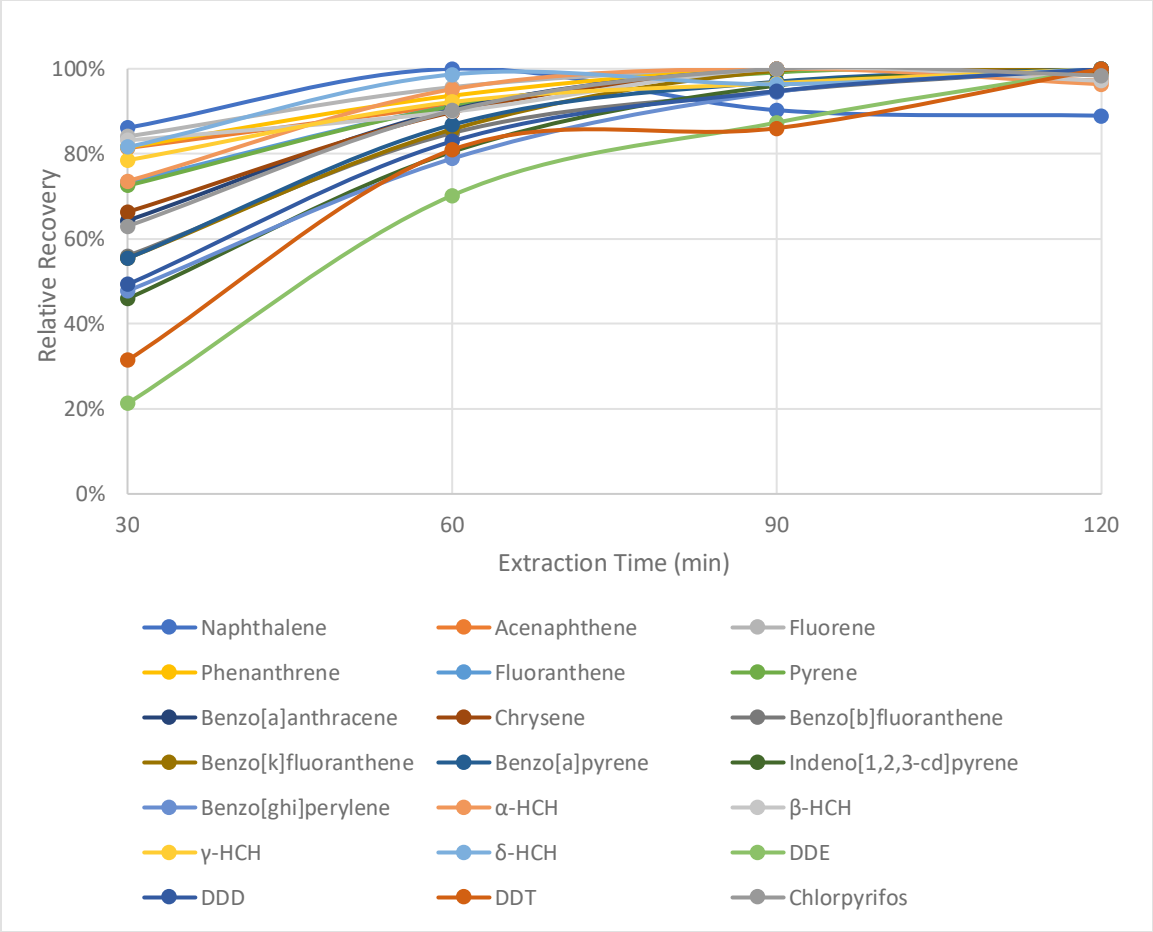


Figure 4.2. Relative recovery of the spiked analytes in dietary supplement sample using different extraction time (n=5). The relative recovery of each analyte was normalized to a 0-100% scale.

*Method Validation*

After optimizing the experimental parameters, the method was validated by quantifying POPs with known concentrations in blank-subtracted dietary supplement samples. Ideally, a dietary supplement standard reference material containing the analytes would be used for validation of the method. However, such a standard reference material is not currently available. Instead, we spiked commercially available dietary supplement samples with certified standards of the analytes followed by recovery experiments. A plant-extract based dietary supplement product in fine powder form was used to create the reference standards. Approximately 1 g of the dietary supplement sample, 8 mL of deionized water, and 2 mL of acetonitrile were added by mass into each sample vial. The certified unlabeled natural standards of the analytes were spiked into the dietary supplement samples to create reference standards at four different concentrations (0.103, 0.333, 1.13, and 3.36 ng/g for PAHs; 0.0409, 0.132, 0.447, and 1.33 ng/g for OCPs; 0.112, 0.362, 1.23, and 3.65 ng/g for chlorpyrifos). These concentrations were referred to as concentration 1, 2, 3, and 4 in later discussions. These reference standards were spiked with isotopically enriched standards to quantify the natural analytes using IDMS. Five replicates were performed at each concentration level. Measurements of the spiked analytes in the reference standards were compared with the theoretical values at the four different concentrations. The units of these measurements were converted to ng/g. For most of the analytes (except acenaphthene), there was no statistical difference between the measured values and the theoretical values, which confirmed the accuracy of the method. For acenaphthene, a reverse-IDMS method was employed to verify and recalibrate the labelled concentration of the isotope and natural standards, which will be

further discussed later. Percent errors of measurements of the spiked analytes in the dietary supplement samples at the four different spiking concentrations were shown in Table 4.1. Percent errors of the measurements compared with the theoretical values and the relative standard deviations (RSDs) were shown in Table 4.2. Except for acenaphthene, the error of the analytes was in a range of 3.08-14.8%, whereas RSD ranged from 4.48% to 12.9%. The mean error of the PAHs (excluding acenaphthene) was 6.81% with a mean RSD of 8.29%. The mean error of the OCPs was 8.16% with a mean RSD of 8.75%. The mean error of all the analytes (excluding acenaphthene) was 7.24% with a mean RSD of 8.26%.

No standard approach has been published before to determine limit of detection (LOD) or limit of quantification (LOQ) for IDMS. Nevertheless, in general either an empirical or a statistical method can be employed to calculate LOD and LOQ.[129] The empirical approach consists of analyzing a series of samples containing increasingly lower concentrations of analyte. The LOD/LOQ is the lowest concentration at which the results still satisfy the predetermined acceptance criteria.[129] A signal to noise (S/N) ratio of 3 is typically set as criteria for LOD.[130] For LOQ, the required S/N ratio can vary from 5 to 20, depending on the guidelines.[131] Multiple statistical approaches have previously been described based on calibration curves.[132-133] In this study, the LOQs were determined using the mean value plus ten times the standard deviation of 20 repetitive measurements of matrix blank samples.

LOQ of each analyte of interest is provided in Table 4.2. These LOQs ranged from 0.0931 ng/g (naphthalene) to 0.00899 ng/g (chrysene). The mean LOQ of the PAHs

was 0.0301 ng/g, whereas the mean LOQ of the OCPs was 0.0227 ng/g. On average, the mean LOQ for all the analytes was 0.0276 ng/g.

Table 4.1. Errors (%) of measurements of the spiked analytes in the dietary supplement samples at the four different spiking concentrations. Results showing comparison between IDMS and calibration curves (CC) with and without internal standards (IS) added (n=5).

| | Concentration 1 | | | Concentration 2 | | | Concentration 3 | | | Concentration 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS | cc | cc w/ IS | IDMS |
| Naphthalene | >100 | 92.8 | 6.83 | 49.6 | 51.0 | 1.68 | 10.3 | 23.5 | 6.67 | 4.52 | 15.8 | 9.87 |
| Acenaphthene | 47.6 | 27.7 | 42.7 | 24.7 | 5.29 | 34.0 | 4.11 | 8.11 | 35.1 | 9.74 | 9.17 | 34.8 |
| Fluorene | 49.0 | 14.5 | 6.22 | 25.6 | 1.75 | 10.1 | 2.84 | 15.4 | 5.74 | 11.6 | 12.2 | 8.30 |
| Phenanthrene | 99.2 | 63.5 | 6.64 | 43.6 | 11.1 | 2.99 | 12.1 | 8.85 | 0.227 | 0.838 | 7.83 | 2.45 |
| Fluoranthene | 65.6 | >100 | 17.8 | 31.8 | 69.0 | 12.3 | 0.239 | 25.4 | 11.1 | 7.39 | 17.4 | 7.77 |
| Pyrene | 49.1 | 28.7 | 7.70 | 14.9 | 0.409 | 2.51 | 7.26 | 10.4 | 9.24 | 9.42 | 10.7 | 8.14 |
| Benz[a]anthracene | 24.6 | 14.6 | 9.99 | 31.3 | 2.73 | 1.12 | 45.1 | 8.18 | 4.96 | 31.5 | 4.76 | 6.51 |
| Chrysene | 8.61 | 14.7 | 19.1 | 2.90 | 3.14 | 5.63 | 21.7 | 14.4 | 4.55 | 14.3 | 12.1 | 4.93 |
| Benzo[b]fluoranthene | 33.3 | 13.5 | 6.01 | 36.5 | 8.25 | 2.11 | 49.3 | 8.72 | 6.18 | 26.4 | 2.04 | 10.6 |
| Benzo[k]fluoranthene | 39.0 | 38.3 | 6.54 | 37.7 | 1.87 | 12.1 | 44.9 | 19.0 | 9.71 | 25.3 | 14.9 | 4.23 |
| Benzo[a]pyrene | 52.0 | 22.7 | 7.22 | 45.5 | 5.91 | 8.75 | 52.5 | 19.5 | 5.03 | 32.8 | 11.4 | 4.38 |
| Indeno[1,2,3-cd]pyrene | 55.4 | 45.8 | 11.7 | 59.1 | 3.23 | 10.4 | 58.6 | 16.7 | 5.61 | 39.5 | 10.8 | 1.17 |
| Benzo[ghi]perylene | 42.9 | 37.0 | 4.61 | 42.1 | 1.57 | 7.53 | 46.5 | 13.8 | 1.67 | 25.0 | 12.4 | 0.183 |
| α-HCH | >100 | 2.04 | 11.2 | 41.1 | 4.60 | 7.95 | 0.232 | 3.95 | 7.83 | 12.6 | 3.29 | 10.0 |
| β-HCH | 93.0 | 22.4 | 4.40 | 30.0 | 0.163 | 9.87 | 2.15 | 7.42 | 7.17 | 10.6 | 2.16 | 3.29 |
| γ-HCH | >100 | 24.7 | 16.1 | 54.2 | 0.320 | 2.54 | 13.6 | 9.39 | 2.28 | 6.34 | 4.93 | 8.29 |
| δ-HCH | 51.0 | 41.7 | 19.4 | 14.1 | 10.2 | 19.6 | 36.5 | 12.4 | 10.6 | 44.4 | 4.17 | 9.72 |
| DDE | 67.6 | 3.24 | 14.9 | 77.2 | 1.83 | 7.75 | 79.2 | 10.7 | 7.63 | 72.7 | 0.754 | 8.63 |
| DDD | 42.9 | 57.6 | 11.6 | 54.4 | 5.92 | 4.37 | 70.0 | 5.06 | 1.17 | 54.3 | 3.78 | 2.69 |
| DDT | 73.9 | >100 | 4.47 | 77.2 | 9.67 | 0.965 | 74.6 | 4.64 | 5.17 | 72.3 | 7.27 | 8.88 |
| CPS | 41.4 | 12.4 | 9.65 | 41.6 | 0.726 | 9.23 | 47.9 | 2.83 | 5.18 | 42.6 | 11.2 | 0.324 |

Table 4.2 Percent errors, RSDs, and LOQs of the measurements of the spiked analytes in the dietary supplement samples using SBSE-GC-MS/MS-IDMS (n=5). Error (%) = | measured mean value – theoretical mean value | / theoretical mean value x 100%. Mean percent errors and mean RSDs were the mean values of the percent errors and RSDs determined at the four different spiking concentrations.

| | Mean Error (%) | Mean RSD (%) | LOQ (ng/g) |
|---|---|---|---|
| Naphthalene | 6.26 | 8.00 | 0.0931 |
| Acenaphthene | 36.6 | 6.46 | 0.0115 |
| Fluorene | 7.60 | 5.95 | 0.0126 |
| Phenanthrene | 3.08 | 9.20 | 0.0147 |
| Fluoranthene | 12.2 | 8.69 | 0.00980 |
| Pyrene | 6.90 | 7.76 | 0.00979 |
| Benz[a]anthracene | 5.65 | 8.18 | 0.0121 |
| Chrysene | 8.55 | 8.68 | 0.00899 |
| Benzo[b]fluoranthene | 6.22 | 8.25 | 0.0720 |
| Benzo[k]fluoranthene | 8.16 | 8.12 | 0.0472 |
| Benzo[a]pyrene | 6.35 | 8.29 | 0.0668 |
| Indeno[1,2,3-cd]pyrene | 7.23 | 10.6 | 0.0178 |
| Benzo[ghi]perylene | 3.50 | 7.78 | 0.0152 |
| α-HCH | 9.25 | 5.98 | 0.0182 |
| β-HCH | 6.18 | 12.9 | 0.0301 |
| γ-HCH | 7.29 | 6.96 | 0.0179 |
| δ-HCH | 14.8 | 12.3 | 0.0355 |
| DDE | 9.73 | 11.4 | 0.0232 |
| DDD | 4.95 | 4.48 | 0.0130 |
| DDT | 4.87 | 7.22 | 0.0210 |
| Chlorpyrifos | 6.09 | 4.48 | 0.0299 |

*Comparison between IDMS and Calibration Curves*

Measurements using the IDMS method were compared with measurements using calibration curves. Standard five-point matrix-matched calibration curves were created for each analyte. Isotope standards were added as internal standards (IS) to create calibration curves with IS for each analyte. Mean percent errors and RSDs of the measurements using IDMS, calibration curves, and calibration curves with IS are shown in Table 4.3. At concentration 1, the mean error of the measurements using calibration curves was over 60%. For naphthalene, α-HCH, and γ-HCH the errors were over 100%. By adding IS the mean error of measurements at concentration 1 decreased to 41.6%. As a comparison, the mean error of measurements using IDMS was 10.1%. At concentration 2, the mean error of the measurements significantly decreased for all the three methods. Nevertheless, IDMS and calibration curves with IS had significantly lower mean error than calibration curves. At concentrations 3 and 4, although the mean errors of calibration curves were reduced, these errors were still over 25%. The mean errors of IDMS and calibration curves with IS at the two higher concentrations were similar compared with concentration 2. From concentrations 2 to 4, the mean errors of IDMS were within a range of 5.89-6.89%, whereas the mean errors of calibration curves with IS ranged from 8.52% to 11.8%. Generally, the RSDs of the measurements decreased from lower to higher measured concentrations for all three methods. At concentration 1, the mean RSDs of the measurements using IDMS were significantly lower than calibration curves and calibration curves with IS. At concentrations 2 to 4, IDMS and calibration curves with IS had comparable results. The mean RSDs of the calibration curves with IS ranged from 5.83% to 7.74%. Similarly, the mean RSDs of IDMS were in the range of 4.56-7.86%.

The mean RSDs of calibration curves were higher than IDMS and calibration curves with IS at all four measured concentrations.

A graphic comparison of the measurements using IDMS, calibration curves, and calibration curves with IS is shown in Figure 4.3. Phenanthrene was selected as an example to present the results. IDMS had observable advantages in accuracy and precision compared with calibration curves especially at the two lower measured concentrations. At the two higher measured concentrations, calibration curves had improved accuracy and precision. Compared with IDMS, calibration curves with IS had similar RSDs at all measured concentrations, however, the error at the lowest concentration was relatively high at 63.5%. Overall, IDMS had a consistently higher level of accuracy and precision over the entire analytical range with a mean error of 3.07% and RSD of 9.20%. These results correlated with the conclusions from previous studies[58-59, 108] that the accuracy and precision of the measurements using calibration curves worsened when approaching the LOQ of the method. This work demonstrated the capability of IDMS to maintain quantitative accuracy and precision consistently.

Table 4.3. Mean percent error and RSD of the measurements of the spiked analytes (excluding acenaphthene) in the dietary supplement samples at four different spiking concentrations using IDMS, calibration curves, and calibration curves with IS for quantification (n=5).

| | | Concentration 1 | Concentration 2 | Concentration 3 | Concentration 4 |
|---|---|---|---|---|---|
| CC | Error (%) | 61.6 | 39.8 | 32.4 | 26.4 |
| | RSD (%) | 30.0 | 16.6 | 10.2 | 12.1 |
| CC with IS | Error (%) | 41.6 | 9.46 | 11.8 | 8.52 |
| | RSD (%) | 20.5 | 7.74 | 6.64 | 5.83 |
| IDMS | Error (%) | 10.1 | 6.98 | 5.89 | 6.02 |
| | RSD (%) | 13.9 | 7.86 | 6.69 | 4.56 |

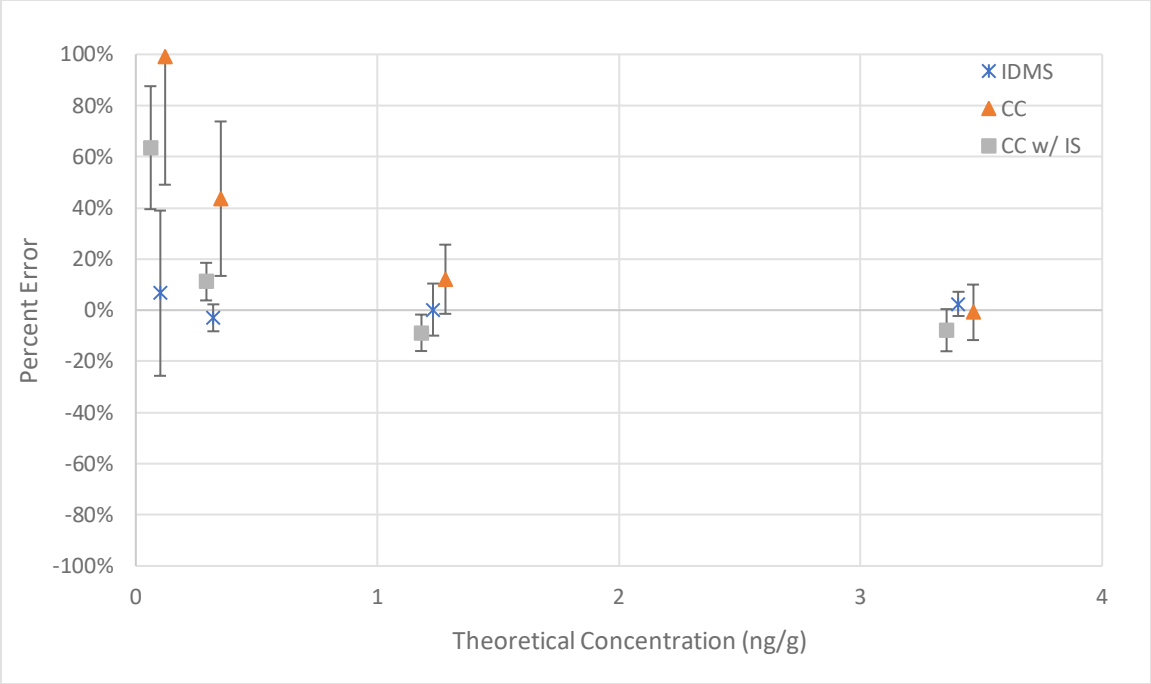


Figure 4.3 Comparing percent error of measurements of spiked phenanthrene in dietary supplement samples at the four different spiking concentrations using IDMS, calibration curves, and calibration curves with IS for quantification (n=5, 95% CI).

*Comparison between GC-MS/MS and GC-MS*

For comparison, the analytes were also quantified using SBSE-GC-MS-IDMS. The GC-MS instrument employed was 7890B GC, 5975C MS by Agilent Technologies (Santa Clara, CA). The stir-bars, the auto-samplers, the TDU and CIS system, and the experimental parameters were the same as employed in the GC-MS/MS method. Selected ion monitoring mode was used for quantification of the analytes. The LOQs of the analytes using the GC-MS method was shown in Table 4.4. These LOQs ranged from 9.26 ng/g (δ-HCH) to 0.457 ng/g (pyrene). The mean LOQ of the PAHs was 1.52 ng/g, whereas the mean LOQ of the OCPs was 4.98 ng/g. On average the mean LOQ for all the analytes was 2.72 ng/g. Compared with the LOQs using GC-MS/MS, the LOQs using GC-MS were approximately two orders of magnitude higher. In analysis of POPs at low concentrations, the improvements in LOQ using GC-MS/MS are crucial. The SBSE-GC-MS-IDMS method was used to analyze the spiked analytes in the dietary supplement reference standards at concentrations 1-4 which were mentioned in the earlier discussions. At concentrations 1-3, the majority of the measurements were not applicable since most of the spiking concentrations were below the LOQs. The percent errors and RSDs of the measurements at concentration 4 were shown in Table 4.4. The errors ranged from 1.44% to 16.2% with a mean value of 8.20%, excluding acenaphthene. The RSDs were in a range of 5.84-14.2% with a mean value of 9.56%. These errors and RSDs were mostly higher than using GC-MS/MS at the same concentration. This work demonstrated the use of GC-MS/MS improved accuracy and precision of the measurements at low concentration levels.

Table 4.4. Percent errors and RSDs of measurements of the spiked analytes in the dietary supplement samples at concentration 4 using SBSE-GC-MS-IDMS. LOQs of the analytes using SBSE-GC-MS-IDMS are also shown. Results below limit of quantification are shown as N/A.

| | Error (%) | RSD (%) | LOQ (ng/g) |
|---|---|---|---|
| Naphthalene | 3.91 | 8.41 | 1.62 |
| Acenaphthene | 30.1 | 6.77 | 1.19 |
| Fluorene | 2.19 | 5.84 | 0.940 |
| Phenanthrene | 5.05 | 8.58 | 0.529 |
| Fluoranthene | 1.44 | 9.22 | 0.513 |
| Pyrene | 9.87 | 12.1 | 0.457 |
| Benz[a]anthracene | 4.77 | 7.16 | 0.654 |
| Chrysene | 8.71 | 9.07 | 0.641 |
| Benzo[b]fluoranthene | 14.3 | 11.1 | 1.66 |
| Benzo[k]fluoranthene | 16.2 | 12.1 | 1.43 |
| Benzo[a]pyrene | 12.6 | 10.2 | 1.57 |
| Indeno[1,2,3-cd]pyrene | N/A | N/A | 3.97 |
| Benzo[ghi]perylene | N/A | N/A | 4.62 |
| α-HCH | N/A | N/A | 5.47 |
| β-HCH | N/A | N/A | 7.41 |
| γ-HCH | N/A | N/A | 6.99 |
| δ-HCH | N/A | N/A | 9.26 |
| DDE | N/A | N/A | 1.66 |
| DDD | N/A | N/A | 1.84 |
| DDT | N/A | N/A | 2.25 |
| Chlorpyrifos | 11.1 | 14.2 | 2.55 |

*Recalibration and validation of the purchased standards using reverse-IDMS*

Isotopically enriched standards purchased from commercial manufacturers are generally considered to have "accurate" labelled concentrations. Laboratories frequently do not validate their purchased standards, which may lead to inaccuracies and biased data. Therefore, there is a need for a method that can be used to confirm the concentrations of these standards shown in the suppliers' certificate of analysis. For this purpose, the concentration of the isotope can be verified by a reverse-IDMS equation, which is modified from the IDMS equation, to calculate "$C_{sp}$".

Solving Eq. 1.1 for $C_{sp}$:

Eq. 4.2 $$C_{sp} = \frac{C_s W_s (A_s - R_m B_s)}{W_{sp}(R_m B_{sp} - A_{sp})}$$

Eq. 4.2 is the reverse-IDMS equation that was used to calculate the concentration of the isotope in the isotopically enriched standard. The results were compared to the labelled concentrations for verification. In this study, the measured concentrations of acenaphthene using IDMS were on average 36.6% higher than the expected values. This was likely originated from inaccurate labelled concentration of either the isotopically enriched standard or the natural standard used to create the reference standard. Reverse-IDMS method was performed using two additional natural standards from independent sources at two concentrations to test whether the labelled concentration of acenaphthene-D10 was accurate as labelled. The two additional natural standards were obtained from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Hampton, NH). Analysis was performed using SBSE-GC-MS/MS and Eq. 4.2 was applied for quantification. When the two additional natural standards were used, there was no statistical difference between the measured concentrations and the theoretical concentrations of acenaphthene-D10

using the two additional standards, indicating that the labelled concentration of the isotopically enriched standard was accurate. Therefore, it demonstrated that the labelled concentration of acenaphthene in the natural standard that was used to create the reference standard was not accurate as stated by the supplier, which resulted in the 36.6% error. The application of reverse-IDMS was also demonstrated in a previous study.[59] These results indicated that reverse-IDMS can be uniquely valuable in tracing biases and inaccuracies of measurements and performing method validation.

*Analysis of Real Samples*

After development, optimization, and validation, the method was used to analyze plant-extract based dietary supplement products that are commercially available in the US. Twelve products from seven different brands were selected for analysis. These products were deidentified and referred to as sample 1-12. Information of each product is listed in Table 4.5. The method discussed in the method validation section was used for quantification. Measurements of the analytes in the 12 commercially available dietary supplement samples are shown in Table 4.6. These quantification results are shown in Figure 4.4. PAHs with lower molecular weight were detected frequently in these samples. Naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, benz[a]anthracene, and chrysene were found in all of the 12 samples. Pyrene was detected in 11 samples. As a comparison, OCPs were not detected as frequently as PAHs. DDT was the most frequently detected OCP. Eight samples were found to contain DDT. The PAHs were found at a higher concentration level. Naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[a]pyrene had average concentrations over 1 ng/g in the tested samples. Acenaphthene and phenanthrene had

mean concentrations over 3 ng/g (3.64 and 5.88 ng/g, respectively). Chlorpyrifos was detected in half of the 12 dietary supplement samples, with a mean concentration of 1.76 ng/g. On average, approximately 12 analytes were detected in each sample. A total of 16 analytes were detected in sample No. 5, which was the highest number detected. Eight were detected in sample No. 4, which was the lowest number detected. Sample No. 1-4 and 6-7 had mean toxin concentrations below 1 ng/g, whereas the rest of the samples had above-1 ng/g mean toxin concentrations. Sample No. 12 had a mean toxin concentration of 3.20 ng/g, which was the highest among all the samples.

Based on the serving instruction of each dietary supplement product, the concentration of each quantified analyte was converted to daily intake amount (ng/day) and daily intake amount per body weight (ng/kg/day) and are shown in Table 4.7. The guidelines for some of the analytes from the Agency for Toxic Substances and Disease Registry (ATSDR) and the California Office of Environmental Health Hazard Assessment (OEHHA) Proposition 65 were listed in Table 4.8. Comparing the quantification results with these guidelines, none of the samples had analytes that exceeded the daily allowable levels. However, for sample No.8, the daily intake amount of benzo[a]pyrene was 30.8 ng/day, which approached approximately half of the no significant risk level set by the OEHHA Proposition 65. For sample No. 5 and 12, the daily intake amount of benz[a]anthracene was 3.52 and 5.02 ng/day, respectively. Both of these results were between 10-20% of the no significant risk level set by the OEHHA under Proposition 65 regulations, which is 33 ng/day. In addition, the daily intake amount of β-HCH in sample No. 5 and γ-HCH in sample No. 10 were 12.5 and 10.9 ng/day, respectively, both of which were within 3% of the OEHHA Proposition 65 no significant

risk level (500 and 600 ng/day, respectively). For the rest of the analytes in the 12 tested dietary supplement samples, the daily intake amounts were generally smaller than 1% of the daily allowable levels.

Evaluating our results under the ATSDR and the OEHHA Proposition 65 guidelines, none of the quantified analytes in the dietary supplement products exceeded the thresholds set for individual toxins. However, at present none of the existing regulations and guidelines concerning the POPs take into account the potential additive and synergistic effects of these toxins. As this study demonstrated, many POPs co-exist as "chemical cocktails" in complex mixtures and the additive effects of these chemicals can be substantial.[120] Another concern to both the public and regulatory authorities has been the possibility of some chemicals enhancing or amplifying the effect of other chemicals, so that they jointly exert a larger adverse effect than predicted.[134] A previous study has demonstrated that the total toxin burden from multiple POPs in children with autism was positively associated with the severity of their diagnosis measured by Autism Diagnostic Observation Scale.[56] More research is in need to further understand the additive and synergistic effects of the co-occurring pollutants in different matrices, and an essential step forward for the regulatory authorities is to address these combined effects of the co-occurring toxins in their guidelines.

Table 4.5 Deidentified sample number, brand, form, and simplified description of the 12 dietary supplement samples analyzed.

| Sample number | Brand | Form | Simplified product description |
|---|---|---|---|
| 1 | A | Tablet | Multivitamin for energy and metabolism with vegetable ingredients |
| 2 | A | Tablet | Multivitamin for pregnant women with vegetable ingredients |
| 3 | A | Tablet | Multivitamin for energy and metabolism with vegetable ingredients |
| 4 | A | Tablet | Multivitamin for pregnant women with vegetable ingredients |
| 5 | B | Tablet | Multivitamin for pregnant women with vegetable and fruit ingredients |
| 6 | B | Tablet | Multivitamin for pregnant women with vegetable and fruit ingredients |
| 7 | B | Tablet | Multivitamin for pregnant women with vegetable and fruit ingredients |
| 8 | C | Tablet | Herbal based multivitamin for women |
| 9 | D | Powder | Herbal supplement for prostate health |
| 10 | E | Powder | Herbal supplement for urinary tract health |
| 11 | F | Powder | Herbal supplement for urinary tract health |
| 12 | G | Powder | Herbal supplement for female health |

Table 4.6 Measurements of the analytes in the 12 commercially available dietary supplement samples using SBSE-GC-MS/MS-IDMS (n=5, 95% CI). Units are in ng/g. Results below limit of quantification are shown as N/A.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 1.90 ± 0.414 | 2.03 ± 0.395 | 2.28 ± 0.253 | 1.90 ± 0.428 | 3.53 ± 0.607 | 2.42 ± 0.410 | 2.61± 0.179 | 3.89 ± 0.713 | 6.45 ± 1.71 | 0.917 ± 0.194 | 3.53 ± 0.335 | 4.25 ± 0.646 |
| Acenaphthene | 0.852 ± 0.0464 | 0.879 ± 0.164 | 0.519 ± 0.0807 | 1.01 ± 0.130 | 23.3 ± 4.18 | 0.265 ± 0.0421 | 5.65 ± 0.590 | 6.46 ± 0.470 | 0.743 ± 0.0739 | 0.175 ± 0.0298 | 0.819 ± 0.0925 | 2.97 ± 0.542 |
| Fluorene | 0.459 ± 0.158 | 0.549 ± 0.111 | 0.759 ± 0.128 | 1.74 ± 0.352 | 0.772 ± 0.136 | 0.388 ± 0.0564 | 0.803 ± 0.0403 | 1.94 ± 0.298 | 1.71 ± 0.354 | 0.609 ± 0.107 | 1.91 ± 0.191 | 7.14 ± 1.26 |
| Phenanthrene | 2.31 ± 0.321 | 1.24 ± 0.154 | 1.65 ± 0.193 | 1.60 ± 0.184 | 2.66 ± 0.352 | 1.15 ± 0.166 | 1.83 ± 0.204 | 3.25 ± 0.204 | 5.55 ± 0.733 | 11.1 ± 0.253 | 12.1 ± 1.48 | 26.0 ± 1.72 |
| Fluoranthene | 1.87 ± 0.132 | 0.725 ± 0.0719 | 0.928 ± 0.0621 | 0.589 ± 0.101 | 2.10 ± 0.254 | 0.626 ± 0.108 | 1.92 ± 0.181 | 1.22 ± 0.238 | 2.12 ± 0.340 | 4.35 ± 0.152 | 5.71 ± 0.820 | 10.5 ± 0.706 |
| Pyrene | 1.91 ± 0.258 | 0.494 ± 0.0902 | 0.616 ± 0.0971 | 0.265 ± 0.0243 | 2.79 ± 0.470 | 0.496 ± 0.0690 | 2.23 ± 0.313 | 2.42 ± 0.348 | N/A | 5.42 ± 0.350 | 6.72 ± 1.36 | 6.72 ± 1.06 |
| Benz[a]anthracene | 0.539 ± 0.0922 | 0.212 ± 0.0340 | 0.124 ± 0.0186 | 0.584 ± 0.111 | 0.453 ± 0.0635 | 0.126 ± 0.0219 | 0.383 ± 0.0692 | 1.05 ± 0.183 | 1.37 ± 0.240 | 0.507 ± 0.109 | 0.838 ± 0.0976 | 1.82 ± 0.259 |
| Chrysene | 0.779 ± 0.151 | 0.101 ± 0.0169 | 0.182 ± 0.0354 | 1.67 ± 0.332 | 0.726 ± 0.141 | 0.255 ± 0.0410 | 0.725 ± 0.0461 | 2.10 ± 0.282 | 1.64 ± 0.338 | 1.39 ± 0.202 | 0.787 ± 0.140 | 2.00 ± 0.217 |
| Benzo[b]fluoranthene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[k]fluoranthene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[a]pyrene | N/A | N/A | N/A | N/A | 1.49 ± 0.242 | 0.691 ± 0.120 | N/A | 17.3 ± 2.79 | 2.43 ± 0.560 | N/A | N/A | N/A |
| Indeno[1,2,3-cd]pyrene | 0.887 ± 0.192 | 0.111 ± 0.0273 | N/A | N/A | 0.415 ± 0.0944 | N/A | 0.207 ± 0.0337 | N/A | 1.43 ± 0.236 | N/A | 0.674 ± 0.0546 | N/A |
| Benzo[ghi]perylene | 1.03 ± 0.164 | 0.167 ± 0.0329 | 0.625 ± 0.0112 | N/A | 0.501 ± 0.0718 | N/A | 0.272 ± 0.0511 | N/A | 1.79 ± 0.329 | N/A | 1.49 ± 0.268 | 2.68 ± 0.355 |
| α-HCH | N/A | N/A | N/A | N/A | N/A | 0.105 ± 0.0197 | 0.252 ± 0.0413 | N/A | N/A | 1.01 ± 0.105 | N/A | N/A |
| β-HCH | N/A | N/A | N/A | N/A | 1.61 ± 0.169 | N/A | N/A | 1.96 ± 0.232 | N/A | N/A | N/A | N/A |
| γ-HCH | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 6.80 ± 0.992 | N/A | N/A |
| δ-HCH | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| DDE | N/A | N/A | N/A | N/A | 0.382 ± 0.0782 | N/A | 0.239 ± 0.0433 | N/A | N/A | 0.618 ± 0.133 | 0.768 ± 0.140 | N/A |
| DDD | N/A | N/A | N/A | N/A | 0.132 ± 0.246 | 0.187 ± 0.0392 | 0.228 ± 0.0657 | N/A | N/A | 0.622 ± 0.0950 | 1.16 ± 0.105 | N/A |
| DDT | N/A | 0.747 ± 0.143 | 0.446 ± 0.0956 | N/A | 0.298 ± 0.0265 | 0.157 ± 0.0145 | N/A | 0.539 ± 0.106 | N/A | 1.62 ± 0.314 | 0.687 ± 0.142 | 1.59 ± 0.222 |
| Chlorpyrifos | 0.436 ± 0.0740 | N/A | N/A | N/A | 1.24 ± 0.102 | N/A | 0.371 ± 0.0767 | N/A | N/A | 1.34 ± 0.105 | 16.2 ± 1.88 | 1.51 ± 0.229 |

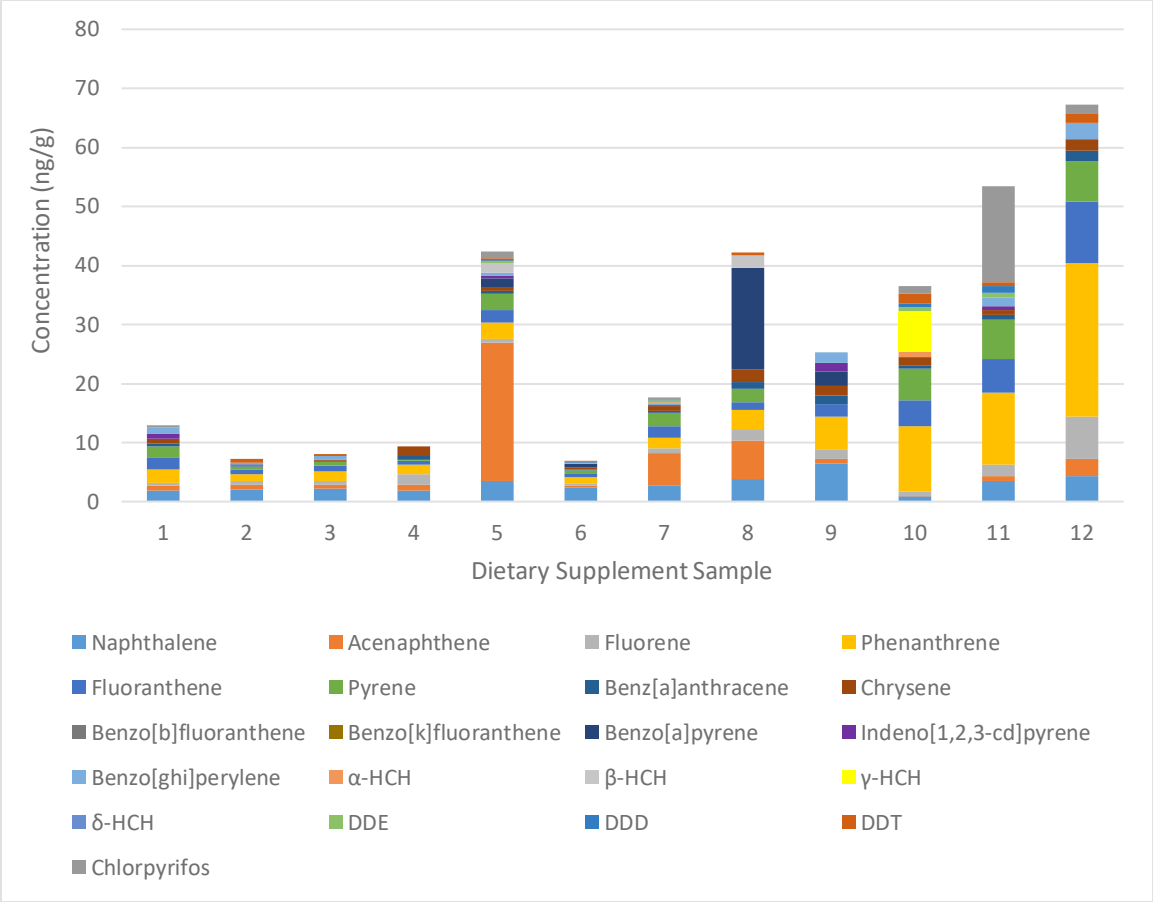


Figure 4.4 Measurements of the analytes in the 12 commercially available plant-extract based dietary supplement samples using SBSE-GC-MS/MS-IDMS (n=5).

Table 4.7 Daily intake of the analytes in the 12 commercially available dietary supplement samples based on the serving instruction of the individual product (n=5). For each analyte the results in the upper row are shown in the unit of ng/kg/day and the results in the lower row are shown in the unit of ng/day. Results below limit of quantification are shown as N/A.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 0.0751 | 0.0811 | 0.0902 | 0.0760 | 0.354 | 0.0651 | 0.0610 | 0.0893 | 0.104 | 0.0190 | 0.0945 | 0.151 |
| | 5.82 | 6.28 | 6.98 | 5.88 | 27.4 | 5.04 | 4.72 | 6.91 | 9.30 | 1.47 | 7.31 | 11.7 |
| Acenaphthene | 0.0337 | 0.0351 | 0.0206 | 0.0405 | 2.34 | 0.00713 | 0.132 | 0.148 | 0.0119 | 0.00362 | 0.0220 | 0.106 |
| | 2.60 | 2.72 | 1.60 | 3.14 | 181 | 0.551 | 10.2 | 11.5 | 1.07 | 0.281 | 1.70 | 8.18 |
| Fluorene | 0.0181 | 0.0219 | 0.0302 | 0.0695 | 0.0775 | 0.0105 | 0.0188 | 0.0445 | 0.0275 | 0.0126 | 0.0512 | 0.255 |
| | 1.40 | 1.70 | 2.34 | 5.38 | 6.00 | 0.810 | 1.46 | 3.44 | 2.47 | 0.979 | 3.96 | 19.7 |
| Phenanthrene | 0.0912 | 0.0493 | 0.0658 | 0.0641 | 0.267 | 0.0311 | 0.0429 | 0.0746 | 0.0892 | 0.231 | 0.325 | 0.927 |
| | 7.06 | 3.82 | 5.09 | 4.96 | 20.7 | 2.41 | 3.32 | 5.78 | 8.01 | 17.9 | 25.2 | 71.8 |
| Fluoranthene | 0.0740 | 0.0290 | 0.0369 | 0.0235 | 0.210 | 0.0169 | 0.0450 | 0.0281 | 0.0340 | 0.0903 | 0.153 | 0.375 |
| | 5.73 | 2.24 | 2.86 | 1.82 | 16.3 | 1.31 | 3.48 | 2.17 | 3.05 | 6.99 | 11.8 | 29.0 |
| Pyrene | 0.0755 | 0.0197 | 0.0245 | 0.0106 | 0.280 | 0.0134 | 0.0523 | 0.0556 | N/A | 0.112 | 0.180 | 0.240 |
| | 5.85 | 1.53 | 1.90 | 0.818 | 21.7 | 1.03 | 4.05 | 4.30 | | 8.71 | 13.9 | 18.6 |
| Benz[a]anthracene | 0.0213 | 0.00848 | 0.00495 | 0.0233 | 0.0455 | 0.00341 | 0.00898 | 0.0241 | 0.0220 | 0.0105 | 0.0224 | 0.0649 |
| | 1.65 | 0.656 | 0.383 | 1.81 | 3.52 | 0.264 | 0.695 | 1.87 | 1.98 | 0.816 | 1.74 | 5.02 |
| Chrysene | 0.0308 | 0.00404 | 0.00725 | 0.0668 | 0.0729 | 0.00687 | 0.0170 | 0.0482 | 0.0263 | 0.0289 | 0.0211 | 0.0712 |
| | 2.38 | 0.312 | 0.561 | 5.17 | 5.64 | 0.532 | 1.31 | 3.73 | 2.36 | 2.23 | 1.63 | 5.51 |
| Benzo[b]fluoranthene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[k]fluoranthene | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Benzo[a]pyrene | N/A | N/A | N/A | N/A | 0.149 | 0.0186 | N/A | 0.398 | 0.0390 | N/A | N/A | N/A |
| | | | | | 11.5 | 1.44 | | 30.8 | 3.50 | | | |
| Indeno[1,2,3-cd]pyrene | 0.0350 | 0.00443 | N/A | N/A | 0.0417 | N/A | 0.00484 | N/A | 0.0229 | N/A | 0.0181 | N/A |
| | 2.71 | 0.343 | | | 3.23 | | 0.375 | | 2.06 | | 1.40 | |
| Benzo[ghi]perylene | 0.0406 | 0.00667 | 0.0249 | N/A | 0.0503 | N/A | 0.00637 | N/A | 0.0288 | N/A | 0.0401 | 0.0955 |
| | 3.15 | 0.516 | 1.92 | | 3.90 | | 0.493 | | 2.58 | | 3.10 | 7.39 |
| α-HCH | N/A | N/A | N/A | N/A | N/A | 0.00284 | 0.00590 | N/A | N/A | 0.0209 | N/A | N/A |
| | | | | | | 0.219 | 0.457 | | | 1.62 | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| β-HCH | N/A | N/A | N/A | N/A | 0.162<br>12.5 | N/A | N/A | 0.0450<br>3.48 | N/A | N/A | N/A | N/A |
| γ-HCH | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0.141<br>10.9 | N/A | N/A |
| δ-HCH | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| DDE | N/A | N/A | N/A | N/A | 0.0384<br>2.97 | N/A | 0.00560<br>0.433 | N/A | N/A | 0.0128<br>0.994 | 0.0206<br>1.59 | N/A |
| DDD | N/A | N/A | N/A | N/A | 0.0132<br>1.02 | 0.00505<br>0.391 | 0.00534<br>0.413 | N/A | N/A | 0.0129<br>1.00 | 0.0310<br>2.4 | N/A |
| DDT | N/A | 0.0298<br>2.31 | 0.0177<br>1.37 | N/A | 0.0299<br>2.31 | 0.00424<br>0.328 | N/A | 0.0124<br>0.957 | N/A | 0.0337<br>2.61 | 0.0184<br>1.43 | 0.0567<br>4.39 |
| Chlorpyrifos | 0.0172<br>1.33 | N/A | N/A | N/A | 0.124<br>9.63 | N/A | 0.00869<br>0.673 | N/A | N/A | 0.0278<br>2.15 | 0.435<br>33.7 | 0.0538<br>4.16 |

To calculate the results in the unit of ng/kg/day, mean female body weight of 77.4 kg[135] was used for sample 1-8 and 10-12, and mean male body weight of 89.8 kg[135] was used for sample 9.

Table 4.8 Minimal risk levels set by ATSDR and no significant risk levels in the Proposition 65 of OEHHA for the analytes. Analytes without specific guidelines are shown as N/A.

| | **ATSDR-Minimal Risk Level (mg/kg/day)** | **OEHHA Proposition 65- No Significant Risk Level (µg/day)** |
|---|---|---|
| Naphthalene | 0.6 | 5.8 |
| Acenaphthene | 0.6 | N/A |
| Fluorene | 0.4 | N/A |
| Phenanthrene | N/A | N/A |
| Fluoranthene | 0.4 | N/A |
| Pyrene | N/A | N/A |
| Benz[a]anthracene | N/A | 0.033 |
| Chrysene | N/A | N/A |
| Benzo[b]fluoranthene | N/A | 0.096 |
| Benzo[k]fluoranthene | N/A | N/A |
| Benzo[a]pyrene | N/A | 0.06 |
| Indeno[1,2,3-cd]pyrene | N/A | N/A |
| Benzo[ghi]perylene | N/A | N/A |
| α-HCH | N/A | 0.3 |
| β-HCH | N/A | 0.5 |
| γ-HCH | N/A | 0.6 |
| δ-HCH | N/A | N/A |
| DDE | N/A | 2 (DDE, DDD, and DDT combined) |
| DDD | N/A | 2 (DDE, DDD, and DDT combined) |
| DDT | 0.00005 | 2 (DDE, DDD, and DDT combined) |
| Chlorpyrifos | 0.001 | 0.0001 (mg/kg/day; child-specific refence dose) |

## 4.4 Conclusions

This study elucidated the development of an accurate, precise, sensitive, and efficient quantification method for POPs in plant-extract based dietary supplements. IDMS was compared with calibration curves and was demonstrated to have advantages in improving accuracy, precision, and efficiency of the analysis. GC-MS/MS was compared with GC-MS and was able to lower the LOQs by approximately two orders of magnitude. The optimized and validated method was used to quantify POPs in 12 commercially available plant-extract based dietary supplements in the US. PAHs such as naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[a]pyrene were detected in most of the products. DDT was the most frequently detected OCP and was found in 8 products. On average, 12 analytes were detected in each sample with a mean concentration of 1.31 ng/g. These measurements were converted to daily intake amount and compared with the existing guidelines. None of the quantified analytes in the investigated dietary supplement products exceeded the thresholds set for individual toxins.

# Chapter 5: Analysis of POPs in wastewater and drinking water

## 5.1 POPs in wastewater – Updating the EPA Method 625

### 5.1.1 Introduction

The US Environmental Protection Agency (EPA) Method 625 requires a liquid-liquid extraction (LLE) with methylene chloride as solvent for analysis of POPs in wastewater and other environmental samples.[136] Methylene chloride is an organic solvent with high volatility and acute inhalation and dermal exposure to it may cause irritation, fatigue, nausea, coma, and even death. In addition, methylene chloride has also been linked to reproductive and developmental effects and cancer.[137] A large amount of hazardous waste containing methylene chloride is generated every year for analysis of wastewater and other environmental samples using the current method. Additionally, as a conventional extraction technique, LLE is time consuming and typically involves excessive operational procedures in analytical laboratories. Therefore, a green and efficient sample preparation technique for an updated EPA Method 625 is in need. Compared with LLE, SBSE is more efficient and environmentally friendly. If LLE is replaced by SBSE for extraction, not only would the efficiency of the method be improved, but also significantly less hazardous waste would be generated in the sample preparation process. In this study, an SBSE method was used to analyze wastewater and toxicity characteristic leaching procedure (TCLP) samples with fortified POPs. The results were compared with other participant laboratories and provided to the EPA to update the EPA Method 625.

5.1.2 Materials and experiments

Wastewater and TCLP samples with fortified POPs were provided by the Independent Laboratories Institute (Washington DC). Concentration of the fortified analytes in these samples were unknown to all participated laboratories. Isotopic labelled standards of the analytes (1,2,4-trichlorobenzene-D3, naphthalene-D8, 2-chloronaphthalene-D7, acenaphthene-D10, 4-chlorophenyl phenyl ether-D5, 4-bromophenyl phenyl ether-D5, di-n-octyl phthalate-D4, chrysene-D12, benzo(k)fluoranthene-D12, hexachlorobezene-$^{13}$C6, fluorene-D10, anthracene-D10, pyrene-D10) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA). These standards were diluted to concentrations of 100-1000 ng/g in acetone and stored in a cold room (-20 °C).

10 mL of the sample and a stir-bar were added to each sample vial for SBSE. The extraction was performed using 10 mm x 0.5 mm (length × film thickness) PDMS stir-bars supplied by GERSTEL (Mülheim an der Ruhr, Germany). The stirring process was carried out using a multiple-position magnetic stirring plate (GERSTEL) at a stirring rate of 1200 rpm. After stirring for one hour, the stir-bar was taken out of the matrix with tweezers, rinsed with deionized water, and carefully dried with a clean wipe. Then the stir-bar was placed in a desorption tube which was then placed in a tray and introduced sequentially into the TDU. The sample loading and handling were performed by a dual-head robotic multi-purpose sampler (MPS-2, GERSTEL) with full automation. A cooled injection system (CIS-6, GERSTEL) was used as the injector for the GC-MS instrument (7890A GC, 5975C MS, Agilent Technologies, Santa Clara, CA).

The experimental parameters were adapted from previous SBSE methods.[58-59, 63] Desorption temperature of the TDU was set at 290 ºC. The analytes were desorbed under helium in the TDU and then sent to CIS and cryofocused by liquid nitrogen at -10 ºC for 15 min. The CIS with Tenax TA packed glass liner was then heated at 12 ºC per second to 300 ºC to transfer the analytes to the GC column. The column used was HP-5MS column (Agilent, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness, 5%-phenyl methylpolysiloxane). The carrier gas was helium, at a flow rate of 1.2 mL/min. The GC oven temperature was ramped at 10 ºC/min from 40 ºC to 290 ºC, and then held at 290 ºC. After electron ionization (70 eV; 230 ºC), the analytes were analyzed by the quadrupole mass analyzer. Data analysis and IDMS calculation was performed using Agilent MassHunter Workstation software and Microsoft Excel. The peak areas of the analytes and isotopes were used for calculations.

### 5.1.3 Results and discussions

Figure 5.1 shows measurements of 13 analytes in five different matrices: wastewater, wastewater with 20% acetonitrile, deionized water, TCLP, and TCLP with 20% acetonitrile using SBSE-GC-MS-IDMS. Analytes in all five matrices showed similar results for most of the analytes. For fluorene and anthracene, the measured concentrations in wastewater with 20% acetonitrile were slightly higher than other matrices. RSDs of measurements of the analytes in these matrices were 6.57%, 1.19%, 3.91%, 2.63%, and 2.68%, respectively. These results showed that measurements using this method were not significantly affected by different matrices. However, the matrices with 20% of acetonitrile added helped improve recovery and precision of measurements.

As discussed in Chapter 3 and 4, adding a small amount organic solvent such as acetonitrile or methanol can help reduce the amount of analytes that can potentially stick to the wall of the glass sample vials, and thus result in higher recovery of analytes.[80]

The fortified concentrations of the analytes in the wastewater and TCLP samples were provided by the Independent Laboratories Institute. These concentrations were used to calculate percent errors of the measurements. Table 5.1 shows the fortified concentrations of each analyte and the accuracy and precision of the results from our lab at Duquesne University (DU) and other participant laboratories. GERSTEL, one of the 23 participant laboratories in updating the EPA Method 625, used the same extraction method as DU. However, instead of IDMS, it employed calibration curves as quantification approach. Other participated laboratories used conventional extraction methods such as LLE or solid phase extraction (SPE) and calibration curves for quantification.

DU results showed that acenaphthene, di-n-octyl phthalate, anthracene, and pyrene had relatively higher levels of accuracy with errors below 5.10%, whereas 1,2,4-trichlorobenzene, 4-chlorophenyl phenyl ether, and fluorene had relatively lower levels of accuracy with errors above 29.5%. Errors of other analytes ranged from 13.9% to 18.0%. GERSTEL had a similar level of accuracy (with mean error of 16.7%) to DU (17.4 %). All the 23 participant laboratories had a mean error of 31.7%. Compared with the average level of accuracy of all participant laboratories, results of DU and GERSTEL laboratories showed not only overall lower error but also higher level of accuracy for almost all the individual analytes. DU also showed a higher level of precision compared with the average level of all participant laboratories. All analytes in DU had RSDs between 4.42%

and 9.06% and the mean RSD (6.57%) is significantly smaller than the average RSD (23.8%) of all participant laboratories.

The fortified wastewater samples were also analyzed at Applied Isotope Technologies (AIT) to demonstrate transferability of the method. AIT lab had the same instruments as DU and these samples were analyzed using the SBSE-GC-MS-IDMS method at AIT. Experimental conditions and parameters were kept identical at DU and AIT. Figure 5.2 shows measurements of the 13 fortified analytes in the wastewater samples at DU and AIT laboratories. For all the analytes, there was no statistically significant difference between the measurements at DU and AIT, which demonstrated good transferability of the method.

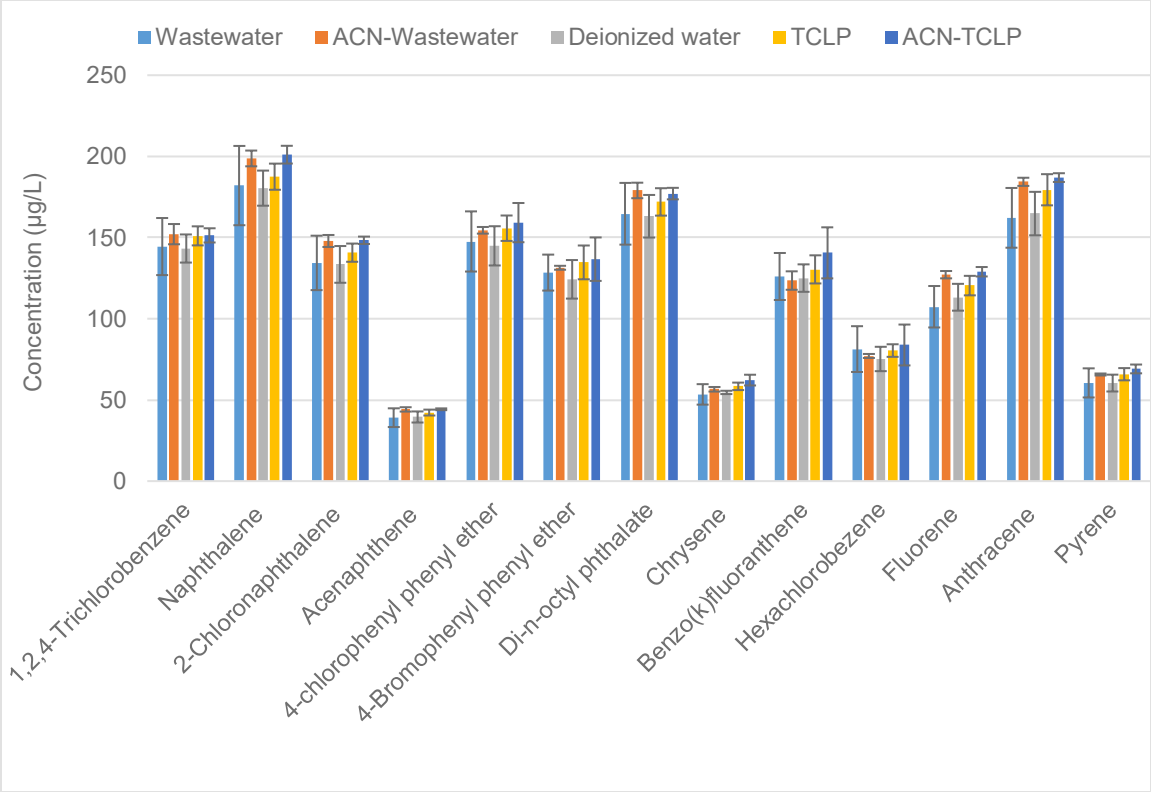


Figure 5.1 Measured concentration of the fortified analytes in five different matrices: wastewater, wastewater with 20% acetonitrile, deionized water, TCLP, and TCLP with 20% acetonitrile using SBSE-GC-MS-IDMS (n=3, 95% CI).

Table 5.1 Analytes in wastewater: DU results compared with other laboratories (n=3).

| | **Fortified concentrations (µg/L)** | **DU measurements (µg/L)** | **Error (%)** | **GERSTEL measurements (µg/L)** | **Error (%)** | **Average error of 23 participant labs (%)** | **DU RSD (%)** | **Average RSD of 23 participant labs (%)** |
|---|---|---|---|---|---|---|---|---|
| 1,2,4-Trichlorobenzene | 97.5 | 144 | 48.2 | 128 | 31.3 | 39.8 | 6.27 | 26.0 |
| Naphthalene | N/A | 182 | N/A | N/A | N/A | N/A | 7.18 | N/A |
| 2-Chloronaphthalene | N/A | 134 | N/A | N/A | N/A | N/A | 6.40 | N/A |
| Acenaphthene | 39.1 | 37.8 | 3.28 | 32.4 | 1.28 | 28.4 | 7.70 | 23.4 |
| 4-Chlorophenyl phenyl ether | 108 | 148 | 36.5 | 133 | 23.0 | 36.6 | 6.49 | 26.6 |
| 4-Bromophenyl phenyl ether | 113 | 128 | 13.9 | 131 | 16.2 | 33.9 | 4.42 | 25.5 |
| Di-n-octyl phthalate | 172 | 165 | 4.54 | 158 | 8.40 | 14.3 | 6.13 | 12.7 |
| Chrysene | 65.1 | 53.4 | 18.0 | 57.5 | 11.7 | 31.2 | 6.01 | 25.2 |
| Benzo(k)fluoranthene | 148 | 126 | 14.9 | 109 | 26.4 | 29.0 | 6.01 | 22.6 |
| Hexachlorobenzene | 69.9 | 81.3 | 16.4 | 75.9 | 8.59 | 36.0 | 9.06 | 28.3 |
| Fluorene | 82.9 | 107 | 29.5 | 86.7 | 4.55 | 31.3 | 6.19 | 23.0 |
| Anthracene | 161 | 162 | 0.760 | 93.1 | 42.2 | 41.7 | 5.96 | 22.0 |
| Pyrene | 57.6 | 60.5 | 5.10 | 51.7 | 10.2 | 26.5 | 7.59 | 26.1 |

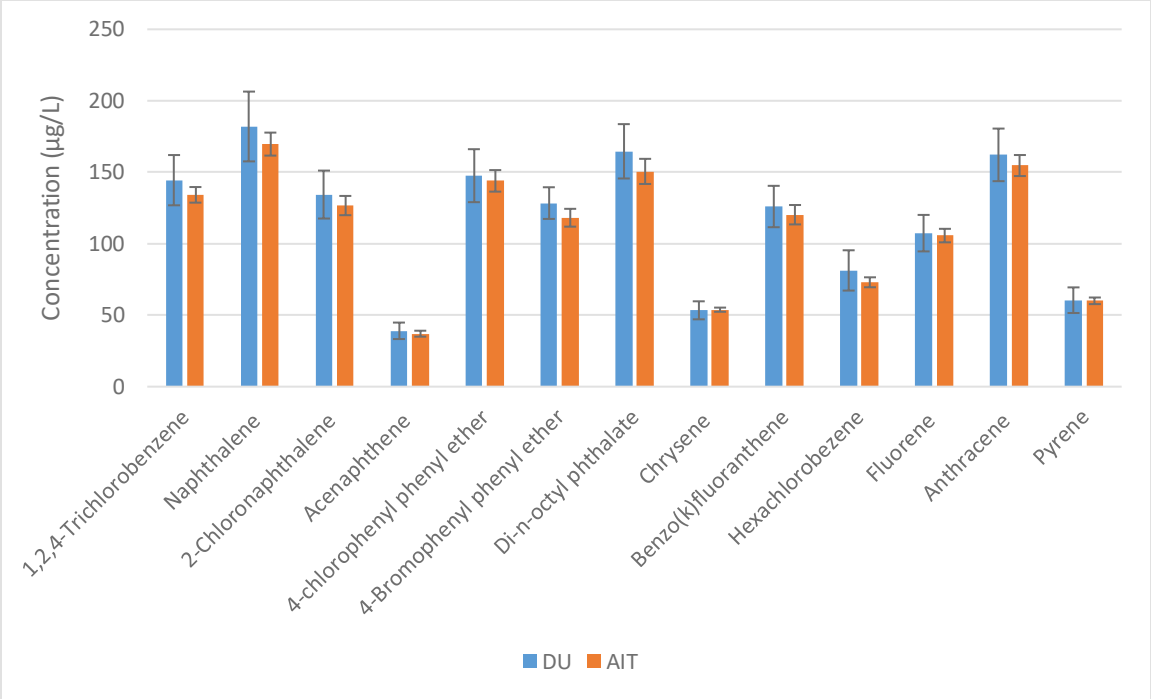


Figure 5.2 Measurements of analytes in fortified wastewater samples in two separate laboratories - DU and AIT using SBSE-GC-MS-IDMS (n=3, 95% CI).

### 5.1.4 Conclusions

This study showed application of SBSE-GC-MS-IDMS on quantification of POPs in wastewater. Compared with conventional extraction techniques such as LLE and SPE, SBSE is greener and more efficient. Additionally, compared with results from other participant laboratories using LLE and SPE for extractions, this method provided a higher level of accuracy and precision. GERSTEL laboratory, which employed SBSE in this comparative study, showed similar results to DU laboratory. SBSE-GC-MS-IDMS was also utilized to analyze fortified POPs in different matrices including wastewater, deionized water, and TCLP samples. For most of the analytes, there was no statistically significant difference in measurements in these different matrices. The same method was employed at AIT laboratory to analyze the same samples to demonstrate transferability of the method. No significant difference was found between measurements at DU and AIT laboratories for all the analytes. Based on these results, we conclude that SBSE-GC-MS-IDMS is an accurate, precise, transferable, efficient, and green method for analysis of POPs in wastewater samples. Considering the analytical advantages and green features of SBSE, replacing the conventional LLE and SPE methods in the EPA Method 625 with SBSE would be an important step forward in future.

## 5.2 POPs in drinking water – Analysis of the EPA Method 525 compounds

### 5.2.1 Introduction

For decades extractions of semivolatile organic compounds (SVOCs) in water samples have been performed by liquid-liquid extraction (LLE) and solid phase extraction (SPE). These conventional extraction techniques have been applied in hundreds of thousands of analytical laboratories and proven effective for routine water analysis. Nonetheless, as the development of new extraction techniques such as solid phase micro-extraction (SPME) and stir-bar sorptive extraction (SBSE) in the 1990s, these conventional extraction methods have been critically challenged in sensitivity, efficiency, and environmental friendliness.[60, 68]

SPME was first introduced in 1990 to address the growing need for rapid and solvent-free sample preparation.[68] This technique provides simultaneous separation and preconcentration for volatile and semivolatile analytes in complex sample matrices. It has been considered an advanced technique over SPE due to shorter analysis time, simpler operation, compatibility with automation, and reduced generation of chemical wastes. However, this technique has limitations such as limited choice of commercially available fibers, fragility of needle and fiber, low extraction capacity, and low chemical and temperature resistance.[138-142]

SBSE has rapidly become a broadly applied sample preparation technique for analysis of SVOCs since it was first introduced in 1999.[60] It offers advantages such as low detection limits, high analyte recoveries, and high temperature resistance.[59-63, 143] Nevertheless, this technique has certain limitations. For example, it is generally not effective for extraction of relatively polar compounds due to the non-polar nature of

PDMS coating. Alterations to the coating or samples matrix can be performed to increase recovery of polar compounds. However, these modifications in turn reduce recovery of non-polar compounds. Matrix effect is another major drawback of SBSE, especially for samples with high organic matter or suspended solid component, such as environmental samples, biological fluids or foods, where adsorption of the analytes onto the organic matter surface competes with the stir bar during the extraction.[116] Furthermore, operations like removing the stir-bars from the sample vial, rinsing, and drying are usually performed manually, which is laborious and can introduce errors.[63] Additionally, a typical clean-up procedure for the stir-bars after use involves multiple-step solvent soaking and high temperature heating. This procedure is time consuming and requires additional apparatus to complete.

Vacuum assisted sorbent extraction (VASE), a sorbent based extraction technique recently developed, has become an alternative approach to overcome the above limitations.[85-86] VASE utilizes sorbent traps called sorbent pens (SPs) to perform headspace extraction at vacuum condition. The SPs are packed with a large quantity of sorbent materials which are approximately 10 times the volume of SBSE and 500 times the volume of SPME.[86] To accelerate the extraction kinetics, reduce the sampling time, and extend the range of analytes, the in-vial extraction is performed in a vacuum environment. After extraction, the SPs are thermally desorbed at a GC injection port, followed by GC-MS analysis. Compared with SBSE and SPME, VASE has advantages such as less carryover, higher durability, improved sensitivity due to larger sorbent surface area, and ability to use a series of sorbents in the SPs to recover a wider range of compounds ranging from volatile to semivolatile.

Based on the concept of VASE, full evaporative vacuum extraction (FEVE) was developed in this work for analysis of SVOCs in drinking water samples. With FEVE, the sample is fully evaporated through a multi-bed SP under vacuum. During water evaporation, relatively volatile analytes are trapped by stronger sorbent beds. Once the water is fully evaporated, heat is applied to the sample vial to promote less-volatile analytes into the vapor phase for capture by weaker sorbent beds of the SP. This combination of vacuum evaporation, secondary heating, and multi-bed sorbent design enables extraction and preconcentration of a wide assortment of SVOCs in a single experiment. Unlike VASE and other extraction technique, during FEVE water in the sample is completely evaporated under vacuum before the transfer of analytes takes place. Therefore, the sorbent does not need to compete with the sample matrix for the analytes and thus enables high recovery of a broad range of SVOCs. After extraction, the SPs are sequenced for automated sample introduction and thermal desorption (TD) followed by GC-MS.

The US EPA Method 525 involves analysis of SVOCs in drinking water, including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), organophosphate pesticides (OPPs), polychlorinated biphenyls (PCBs), and phthalates.[144] These chemicals have been extensively applied, recognized as high priority organic pollutants, and have raised serious environmental and human health concerns worldwide.[11-13, 29, 35, 37, 145-146] An efficient and green method that can provide accurate, precise, and sensitive quantitative measurements of these pollutants is in need. In this study, an FEVE-TD-GC-MS method was developed to analyze 122 SVOCs listed by the EPA Method 525 in drinking water. This list of analytes covered a wide range of SVOCs

from light organophosphate chemicals such as diisopropyl methylphosphonate to heavy six-ring PAHs such as benzo[ghi]perylene.

5.2.2 Materials and instruments

*Materials and chemicals*

2-mL crimp-top glass sample vials were purchased from VWR (Radnor, PA). MS-grade acetone was obtained from Sigma Aldrich (St. Louis, MO). Standards of the SVOC analytes and internal standards were obtained from AccuStandard (New Haven, CT). These standards were diluted to a concentration of 20 µg/mL with acetone and stored in a freezer (-20 °C). Before analysis, these standards were further diluted with acetone into a 4 µg/mL mix as working standard.

*Thermal desorption and GC-MS*

After completion of FEVE the SPs were loaded in a sample tray. The sample handling was performed by a Sample Preparation Rail (SPR; Entech Instruments; Simi Valley, CA) with full automation. The Sorbent Pen Desorption Unit (5800-SPDU; Entech Instruments) was used as the thermal desorption system of the GC-MS (7890B GC, 5977C MS; Agilent Technologies, Santa Clara, CA). After the SPDU was preheated to 200 ºC for 1 min, the SP was desorbed at 260 ºC for 5 min. A UAC-1MS precolumn (10 m × 0. 53 mm × 0.15 µm, methylpolysiloxane; Quadrex Corp, Bethany, CT) was used to provide a delayed split point, enabling analyte preconcentration and backflush. An HP-5MS (Agilent, 30 m × 0.25 mm × 0.25 µm, 5%-phenyl-methylpolysiloxane) was used as analytical column. The carrier gas was helium, at a flow rate of 1.2 mL/min.

Figure 5.3 shows the configuration of SPDU, two column design, and split control of the GC-MS. This design of the instrument enables desorption, delivery, split, backflush,

and analysis of the samples. After the SP is inserted into the SPDU, valves 2 and 4 are on during preheat. Once the desorption starts, valves 1 and 4 are on, enabling preconcentration of SVOC analytes on column 1 and meanwhile splitting out compounds that are more volatile than the lightest analyte of interest. After desorption, valves 1 and 3 are on, allowing the analytes to proceed on column 2. Due to a thicker film on column 2 than column 1, the analytes dynamically refocus on column 2, which results in narrower chromatographic peaks. Furthermore, during this period the SPDU is baked out at 260 ºC to eliminate potential carryover in the SP. After the heaviest analyte of interest elutes out from column 1 and starts separating on column 2, valves 2 and 3 are on to backflush unwanted heavy compounds out of the system. Finally, the SPDU cools down and returns to the idle status where valves 2 and 4 are on and ready for the next sample.

The GC oven temperature was held at 40 ºC for 5 min during sample desorption, ramped at 30 ºC/min to 175 ºC, held for 3 min, then ramped at 4 ºC/min to 200 ºC, and finally ramped at 7 ºC/min to 300 ºC and held for 3 min until the end of the run. The total run time was approximately 36 min. Data acquisition and analysis was performed using Agilent MassHunter Workstation, Entech SPRINT software, and Microsoft Excel. Other GC-MS method parameters including retention time (RT) and quantitative ion (QI) of all target analytes are summarized in Table 5.2.

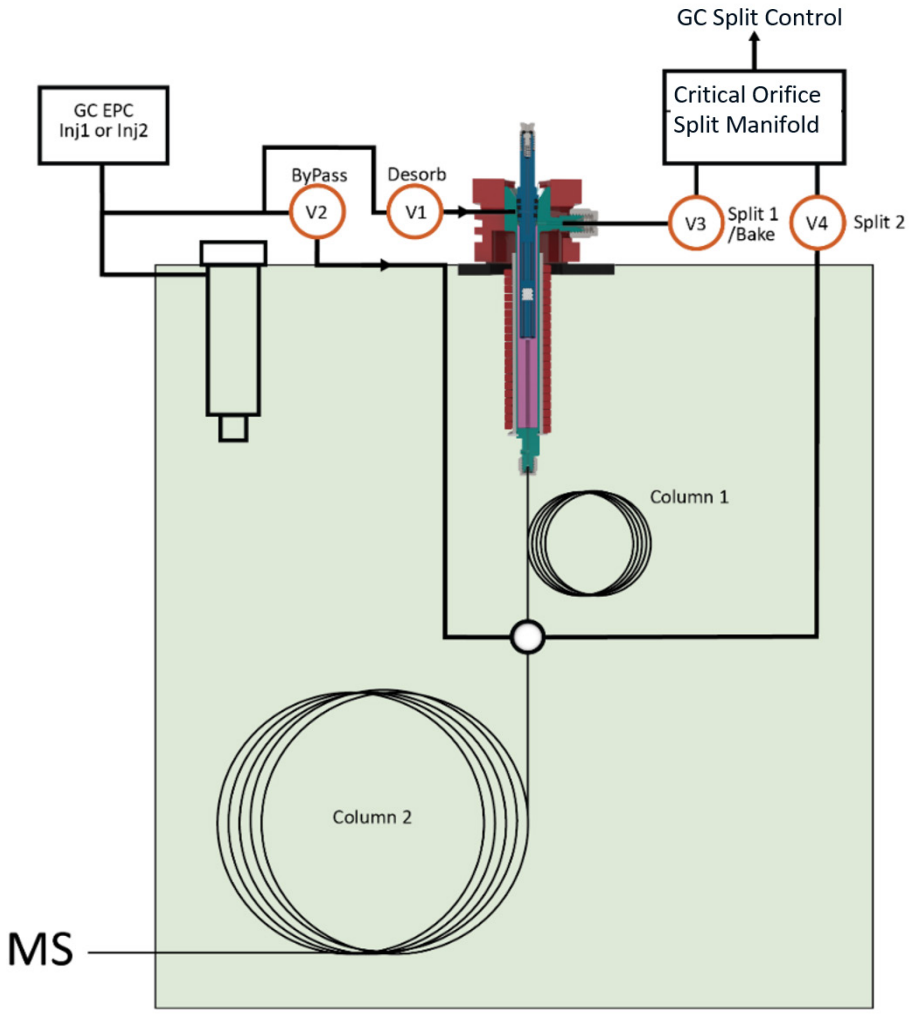


Figure 5.3 Configuration of SPDU, two column design, and split control of the GC-MS.

Table 5.2 GC-MS retention time and QI of 122 target SVOC analytes.

| Compound Name | RT (min) | QI (m/z) |
|---|---|---|
| DIMP | 8.553 | 97 |
| isophorone | 8.911 | 82 |
| dichlorvos | 9.881 | 109 |
| EPTC | 10.823 | 128 |
| mevinphos | 11.425 | 127 |
| butylate | 11.51 | 57 |
| vernolate | 11.651 | 128 |
| dimethylphthalate | 11.661 | 163 |
| 2,6-dinitrotoluene | 11.675 | 165 |
| etridizole | 11.741 | 211 |
| acenaphthylene | 11.774 | 152 |
| pebulate | 11.793 | 128 |
| chlorneb | 12.292 | 193 |
| 2-chlorobiphenyl | 12.292 | 188 |
| BHT | 12.4 | 205 |
| tebuthiuron | 12.405 | 156 |
| 2,4-dinitrotoluene | 12.546 | 165 |
| molinate | 12.664 | 126 |
| 4-chlorobiphenyl | 13.281 | 188 |
| diethylphthalate | 13.318 | 149 |
| fluorene | 13.408 | 165 |
| propachlor | 13.643 | 120 |
| ethoprop | 13.944 | 97 |
| cycloate | 13.996 | 83 |
| chlorpropham | 14.166 | 213 |
| trifluralin | 14.561 | 264 |
| phorate | 14.858 | 75 |
| α-HCH | 14.999 | 181 |
| 2,4'dichlorobiphenyl | 15.032 | 222 |
| hexachlorobenzene | 15.329 | 284 |
| atraton | 15.348 | 196 |
| dimethipin | 15.494 | 54 |
| simazine | 15.498 | 201 |
| prometon | 15.55 | 225 |
| atrazine | 15.677 | 200 |
| β-HCH | 15.743 | 181 |
| propazine | 15.814 | 214 |
| γ-HCH | 16.002 | 181 |
| pronamide | 16.327 | 173 |
| 2,2',5-trichlorobiphenyl | 16.374 | 186 |
| phenanthrene | 16.412 | 178 |

| | | |
|---|---|---|
| anthracene | 16.581 | 178 |
| δ-HCH | 16.661 | 181 |
| terbacil | 16.774 | 161 |
| disulfoton | 16.746 | 88 |
| pentachlorophenol | 16.901 | 266 |
| chlorothalonil | 16.901 | 266 |
| phosphamidon | 17.89 | 127 |
| 2,4,4'-trichlorobiphenyl | 17.98 | 256 |
| acetochlor | 18.182 | 146 |
| vinclozolin | 18.234 | 212 |
| methyl_parathion | 18.196 | 109 |
| metribuzin | 18.243 | 198 |
| simetryn | 18.333 | 213 |
| ametryn | 18.531 | 227 |
| alachlor | 18.545 | 188 |
| heptachlor | 18.573 | 100 |
| prometryn | 18.677 | 241 |
| terbutryn | 19.152 | 226 |
| 2,2',5,5'-tetrachlorobiphenyl | 19.232 | 220 |
| bromacil | 19.293 | 205 |
| dibutyl_phthalate | 19.43 | 149 |
| aldrin | 19.826 | 66 |
| metolachlor | 19.891 | 162 |
| cyanazine | 19.915 | 225 |
| 2,2',3,5'-tetrachlorobiphenyl | 19.962 | 220 |
| chlorpyrifos | 20.028 | 97 |
| ethyl_parathion | 20.033 | 109 |
| triadimefon | 20.15 | 208 |
| dacthal | 20.226 | 301 |
| MGK264(a) | 20.593 | 164 |
| diphenamid | 20.602 | 72 |
| MGK264(b) | 20.956 | 164 |
| heptachlor_epoxide | 21.158 | 353 |
| 2,3',4',5-tetrachlorobiphenyl | 21.309 | 220 |
| chlorfenvinphos | 21.436 | 267 |
| trans-chlordane | 21.93 | 375 |
| pyrene | 22.119 | 202 |
| tetrachlorvinphos | 22.312 | 109 |
| endosulfan-I | 22.335 | 241 |
| cis-chlordane | 22.434 | 375 |
| butachlor | 22.509 | 176 |
| trans-nonachlor | 22.627 | 409 |
| napropamide | 22.712 | 72 |
| profenofos | 23.004 | 339 |

| | | |
|---|---|---|
| 4,4'-DDE | 23.136 | 246 |
| tribufos | 23.145 | 57 |
| dieldrin | 23.155 | 79 |
| 2,3,3',4',6-pentachlorobiphenyl | 23.352 | 326 |
| oxyfluorfen | 23.451 | 252 |
| nitrofen | 23.72 | 283 |
| endrin | 23.795 | 263 |
| endosulfan-II | 24.026 | 195 |
| 2,2',3,4',5'6-hexachlorobiphenyl | 24.106 | 360 |
| chlorobenzilate | 24.134 | 251 |
| 2,3',4,4',5-pentachlorobiphenyl | 24.153 | 326 |
| 4,4'-DDD | 24.36 | 235 |
| ethion | 24.558 | 231 |
| 2,2',4,4',5,5'-hexachlorobiphenyl | 24.836 | 360 |
| endosulfan_sulfate | 25.283 | 272 |
| norflurazon | 25.311 | 145 |
| butylbenzylphthalate | 25.386 | 149 |
| 4,4'-DDT | 25.485 | 235 |
| 2,2',3,4,4',5'-hexachlorobiphenyl | 25.612 | 360 |
| hexazinone | 25.669 | 171 |
| tebiconazole | 25.848 | 125 |
| di(2-ethylhexyl)adipate | 26.088 | 129 |
| chrysene | 26.766 | 228 |
| benzo[a]anthracene | 26.888 | 228 |
| methoxychlor | 27.096 | 227 |
| 2,2',3,4,4',5,5'-heptachlorobiphenyl | 27.505 | 394 |
| di(2-ethylhexyl)phthalate | 27.877 | 149 |
| fenarimol | 28.621 | 107 |
| cis-permethrin | 29.577 | 183 |
| trans-permethrin | 29.761 | 183 |
| benzo[b]fluorancene | 30.392 | 252 |
| benzo[k]fluorancene | 30.467 | 252 |
| benzo[a]pyrene | 31.315 | 252 |
| fluridone | 31.663 | 328 |
| indeno[1,2,3-c,d]pyrene | 34.502 | 276 |
| dibenzo[a,h]anthracene | 34.606 | 278 |
| benzo[g,h,i]perylene | 35.242 | 276 |

5.2.3 Results and discussions

*Design of FEVE*

FEVE sorbent pen (FSP) is a specially designed SP that fits in the 2mL FEVE sample vials. To capture a broad range of SVOCs, the sorbent bed consists of two sorbents, PDMS coated glass beads (lower sorbent) and Tenax TA (upper sorbent), in series. This design of FSP is shown in Figure 5.4. For analysis of more volatile compounds, a stronger third sorbent like Carboxen or Carbosieve can be added in the FSP to create a more retaining sorbent bed. However, for the suite of SVOCs in the study, a third sorbent was not necessary. As shown in Figure 5.5, a 2-mL sample vial with 1 mL of water sample was attached to an FEVE vacuum sleeve and then an FSP was inserted. A silicon O-ring was placed between the top of the sample vial and the bottom of the vacuum sleeve to create a leak-tight seal. After assembled the FEVE assemblies were placed into the FEVE instrument. Figure 5.6 shows the front and top views of the instrument. A top plate was used to squeeze down the two upper vacuum sleeve O-rings against grooves in the vacuum manifold, creating a leak-tight seal. The multi-position design of the manifold allows for up to 30 samples to be extracted simultaneously.

The FEVE process consists of four major steps: vacuum verification, matrix evaporation, water elimination verification, and high temperature diffusive desorption. During vacuum verification, the evacuation valve of the FEVE instrument is turned on for 5 sec then turned off for 1 min. A pressure increase during this period is used to determine whether the system is leak tight. After the vacuum verification standard is met, the matrix evaporation starts. The evacuation valve is kept on at the stage to provide a vacuum environment. $N_2$ flush is turned on for 30 sec in every 5 min to mitigate

condensation of water in the evacuation channels. When the pressure of the instrument drops below 0.1 psia the process advances to water elimination verification, where the sample vials are heated from 30 to 50 ºC. If the pressure increase is less than 0.1 psia per min, the final step, high temperature diffusive desorption begins. The sample vials are heated at 230ºC for 5 min. A pen cooling fan is turned on at the stage to keep the sorbent area of the FSP cool to maximize its adsorption capacity. After the vial heater is cooled down the FSPs are ready for TD-GC-MS analysis. A photo of the FEVE instrument mounted on a vacuum extraction bar (VXB) with water samples loaded is shown in Figure 5.7. The use of VXB enables off-line sample extraction without occupying the GC-MS. The FEVE instrument can also be mounted on the SPR GC-MS autosampler rail for automated sample handling and analysis.


Figure 5.4 Photograph and render of an FSP showing the internal sorbent beds.

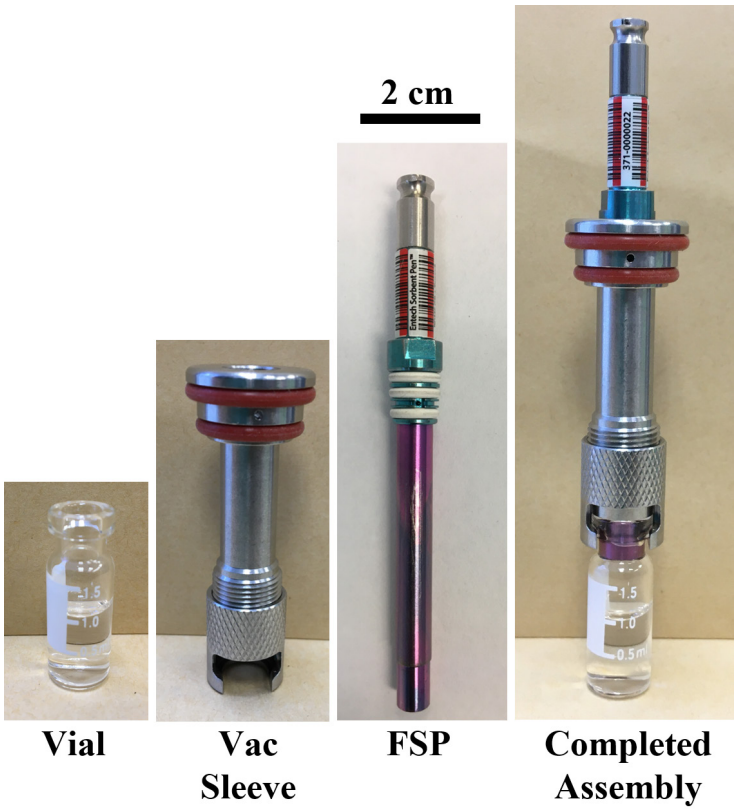


Figure 5.5 Components comprising the FEVE sample assembly, including the 2-mL sample vial (with 1 mL of water sample), FEVE vacuum sleeve (with vial nut and silicon O-rings), and FSP.

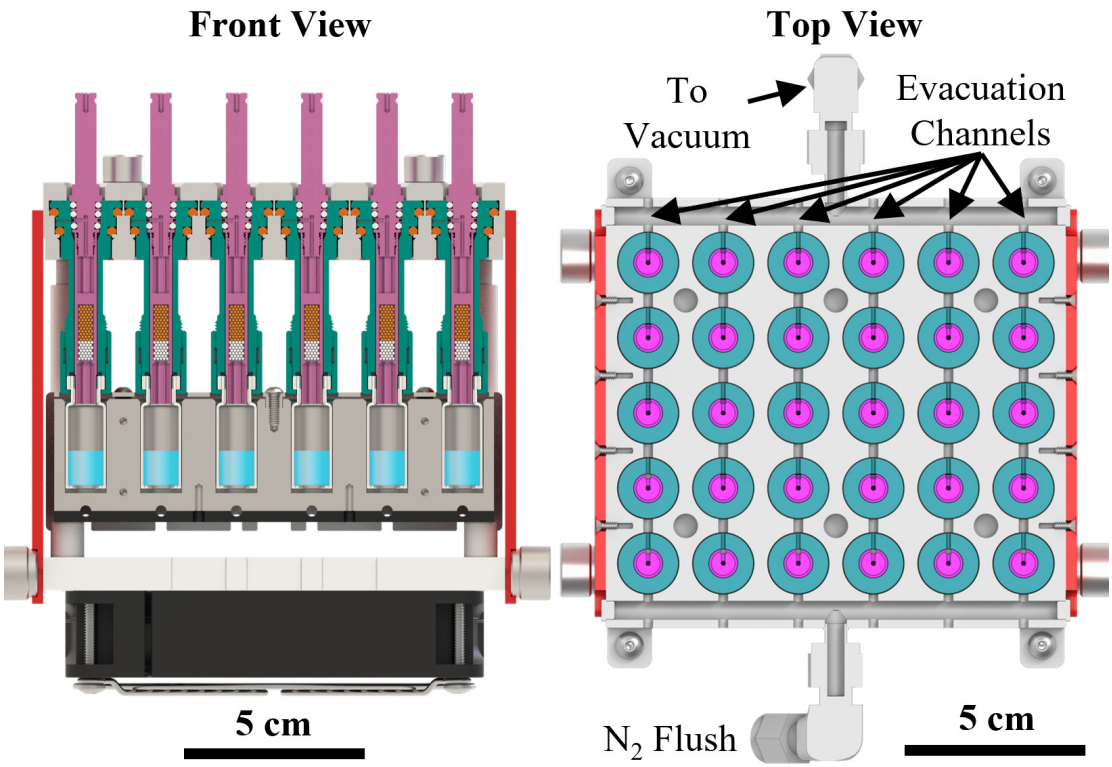


Figure 5.6 Front view of the FEVE instrument with FEVE sample assemblies in place (left). Top-down view of the FEVE vacuum plate showing the nitrogen input, the vacuum output, and the 6 parallel 5-position evacuation channels (right).

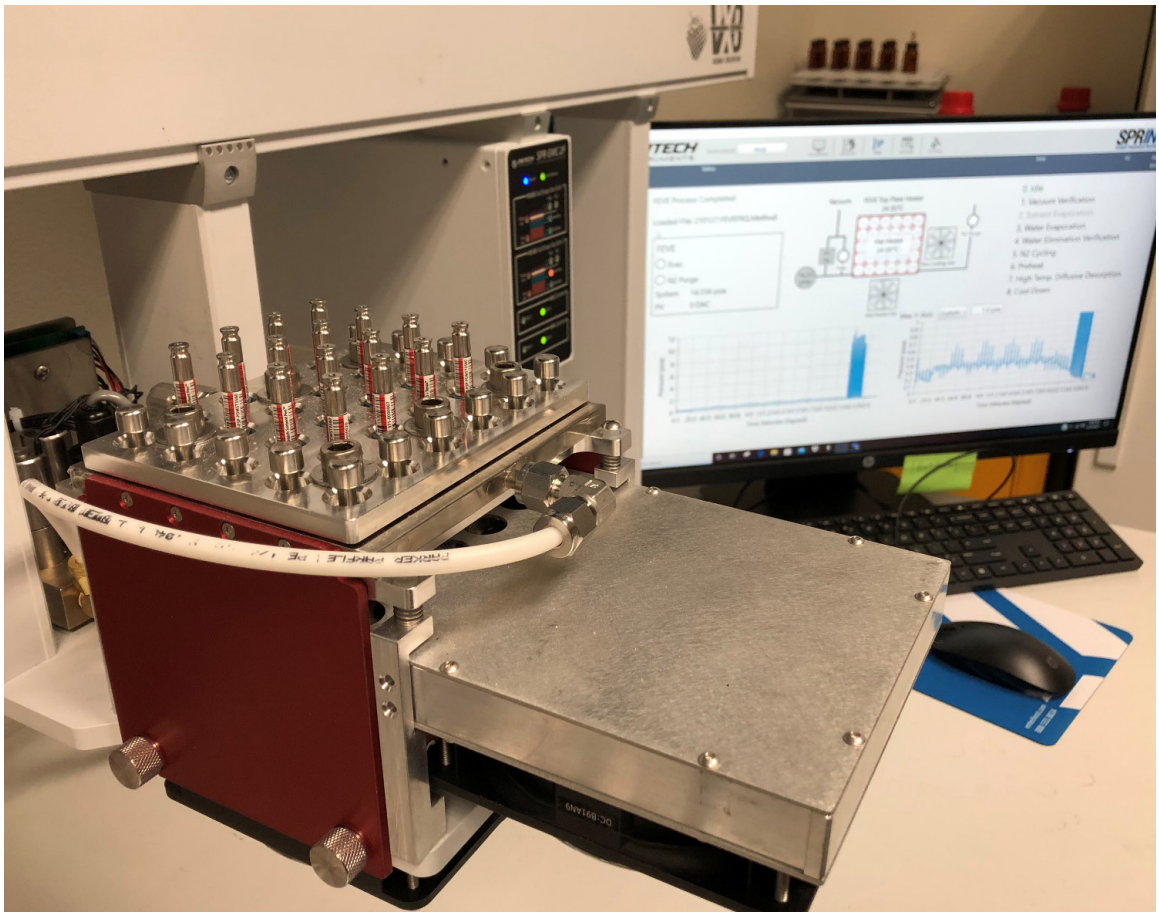

Figure 5.7 FEVE instrument mounted on a vacuum extraction bar (VXB) with water samples loaded.

*Desorption temperature optimization*

1 µL of the working standard mix was spiked on the bottom screen of the SP. The SPs were placed in FEVE assemblies and went through the FEVE extraction process. No water sample was used for desorption method optimization. After FEVE these SPs were analyzed with different desorption temperature to optimize the method. Desorption time was set at 5 min for optimization of desorption temperature. Recovery of each analyte using different desorption temperatures was normalized to 100%.

Desorption temperatures of 170, 200, 230, and 260 ºC were compared for each analyte. Based on the trend of recovery vs desorption temperature, the behavior of the 122 SVOC analytes can be placed in three categories. Figure 5.8 and 5.9 present relative recoveries of PAHs and PCBs, respectively. For most of these PAHs and PCBs, their recoveries reached maximum at 230 ºC. Most of the analytes from the other SVOC groups also fell in this category. Figure 5.10 shows relative recoveries of several OPPs. For these compounds, their recoveries significantly increased when the desorption temperature raised to 260 ºC. Figure 5.11 indicates relative recoveries of several OPPs and OCPs. These analytes showed increased recovery with higher desorption temperature from 170 to 260 ºC. Furthermore, the breakdown products of Tenax significantly increased when desorption temperature was higher than 260 ºC. With all these factors considered, 260 ºC was selected as the optimal desorption temperature for all target analytes.

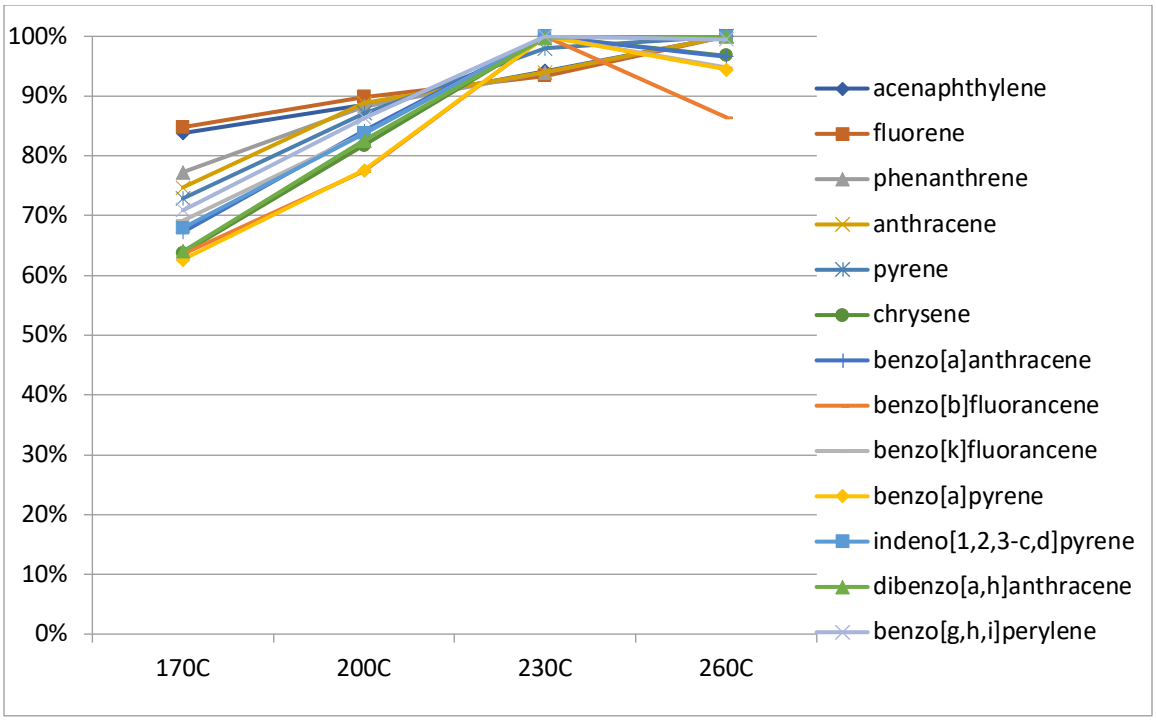


Figure 5.8 Relative recovery of PAHs using different desorption temperatures (n=2).

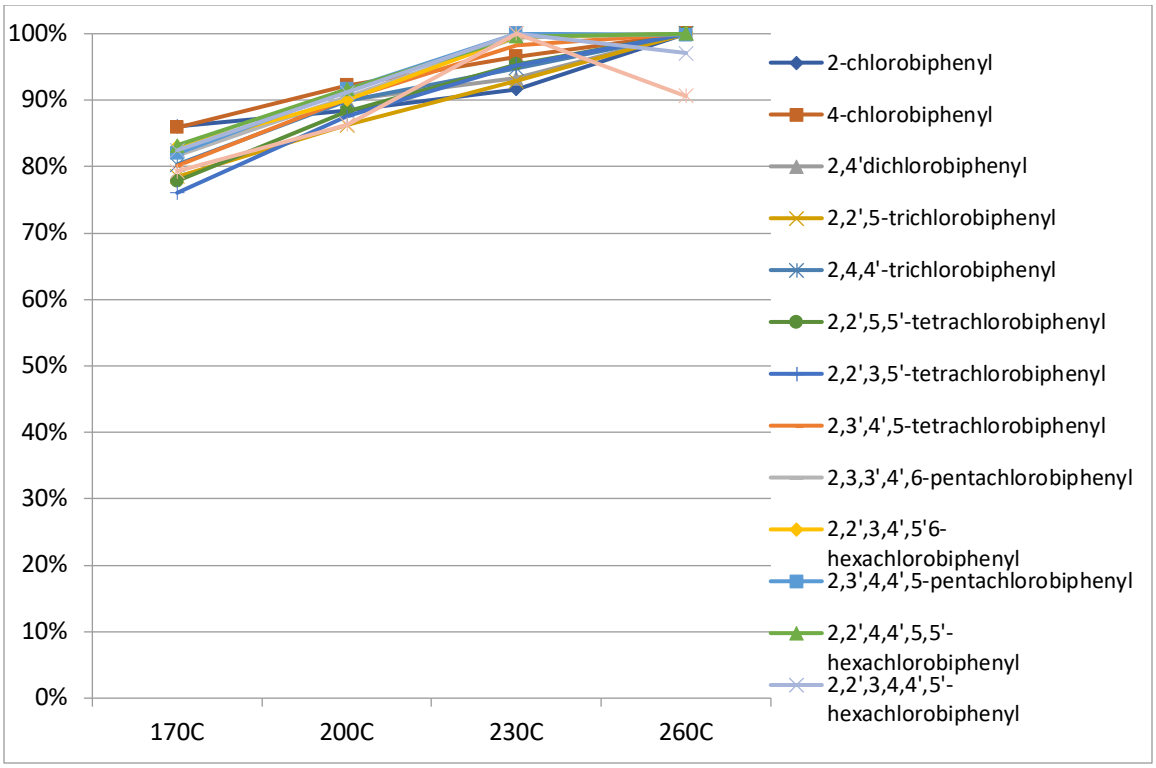


Figure 5.9 Relative recovery of PCBs using different desorption temperatures (n=2).

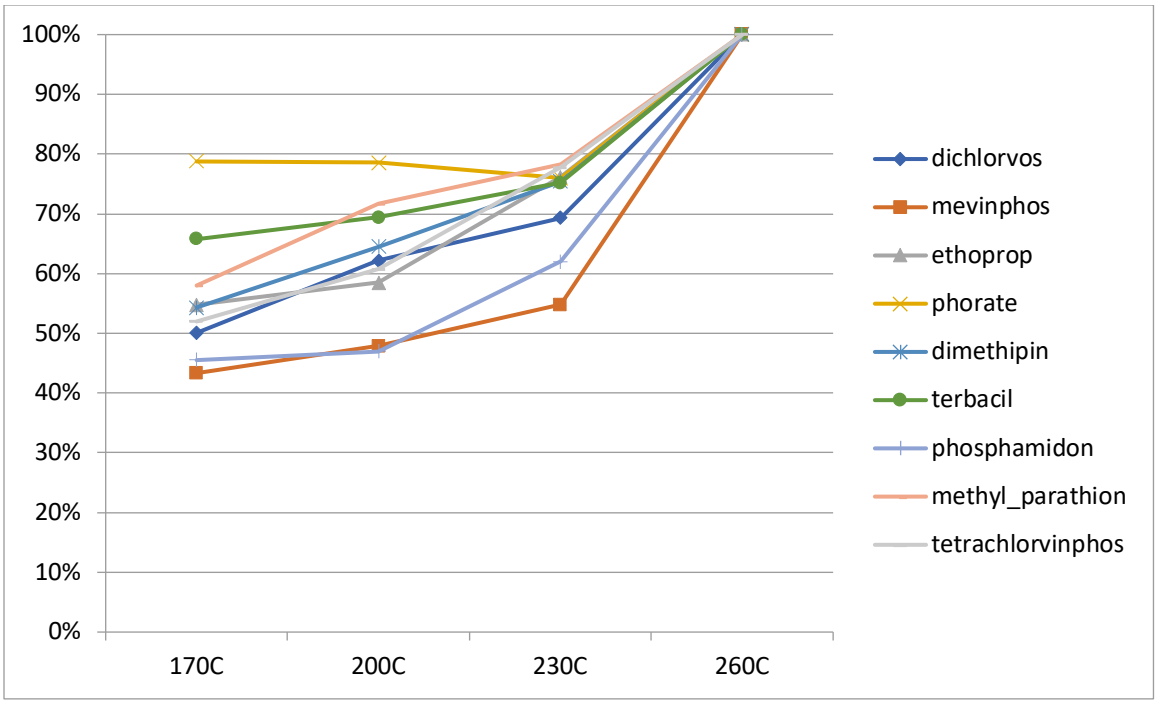


Figure 5.10 Relative recovery of selected OPPs using different desorption temperatures (n=2).

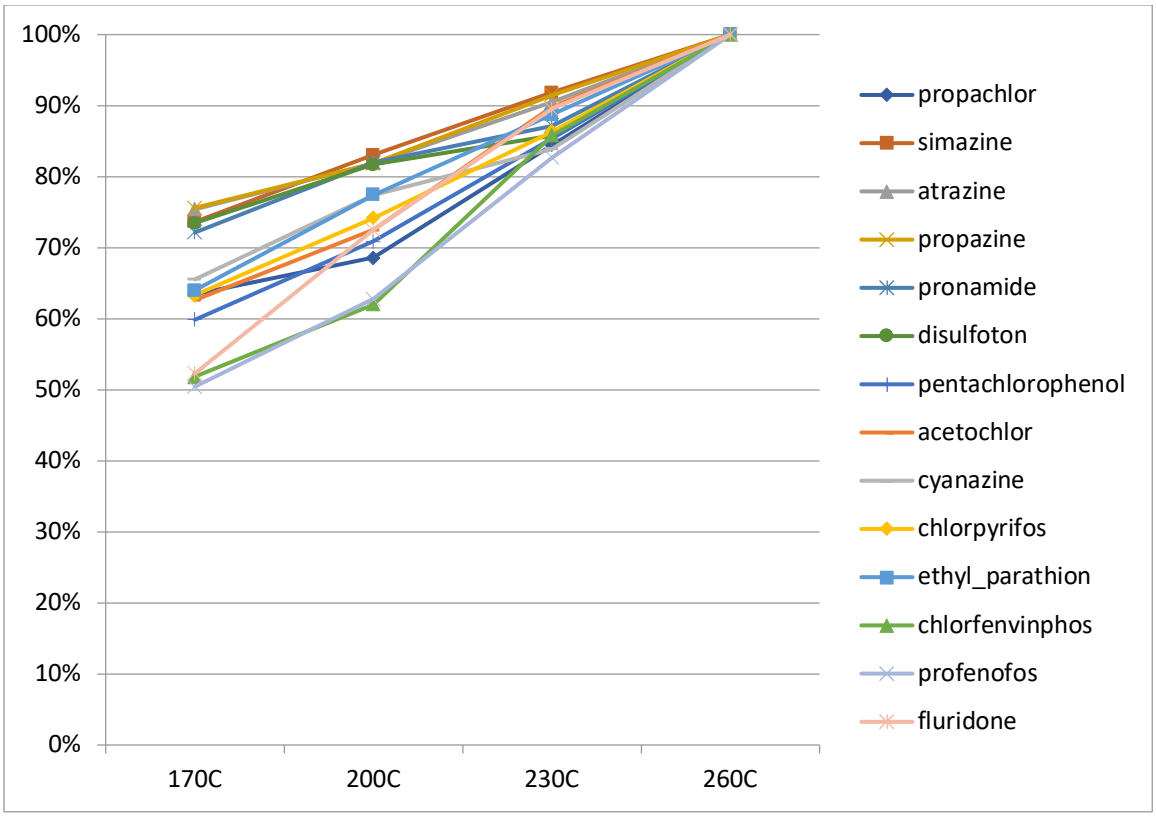


Figure 5.11 Relative recovery of selected OCPs and OPPs using different desorption temperatures (n=2).

### 5.2.4 Conclusions

This study demonstrates the design and development of a novel sample preparation technique, FEVE. Compared with conventional extraction techniques such as LLE and SPE, it is more efficient and environmentally friendly. Compared with other solvent-free sample preparation techniques developed in the 1990s such as SPME and SBSE, it offers advantages such as less carryover, higher durability, improved sensitivity due to larger sorbent surface area, and ability to use a series of sorbents in the SPs to recover a broad range of compounds ranging from volatile to semivolatile. The design of the FEVE instrument has been completed. Preliminary data has shown its feasibility in replacing the older sample preparation techniques for analysis of SVOCs in drinking water. The next step forward will be developing a quantification method using FEVE-TD-GC-MS to demonstrate its analytical figures of merit such as accuracy, precision, sensitivity, etc.

# Chapter 6: Conclusions

## 6.1 Persistent organic pollutants and the exposome

POPs are highly stable organic chemicals that resist photolytic, biological, and chemical degradation. They are persistent yet mobile in the environment, bioaccumulate through the food chain, and can harm the environment and human health. These features have made them become a fast-growing concern over the globe in the past decades. PAHs, OCPs, PCBs, PBDEs, PCDDs, and PCDFs are categories of POPs that have been generated, applied, and distributed worldwide.

The exposome is defined as life-course environmental exposures from the prenatal period onwards. Different from previous concepts in environmental and human health, the exposome focuses on the entireness of environmental exposure, as a complement to the genome. Both exogenous and endogenous sources of exposure are incorporated in the concept of the exposome. Either “bottom-up” or “top-down” can be used to characterize the exposome. The “top-down” approach has been widely applied to analyze the connection between chemicals and human diseases. An important emphasis in exposomics research has been understanding the potential link between the exposure to environmental pollutants, such as POPs, and human health.

## 6.2 Stir-bar sorptive extraction and Isotope Dilution Mass Spectrometry

SBSE is a sorptive extraction technique based on PDMS polymer coating of a magnetic stir-bar. SBSE is typically coupled with GC-MS for analysis of POPs. After extraction, analytes extracted by the stir-bar is thermally desorbed in the TDU and

injected into the GC by the CIS. Compared with conventional extraction methods such as LLE, SBSE is a greener and more efficient sample preparation technique.

IDMS is quantification method involves spiking isotope analogs of target analytes into the sample matrix. After equilibration between the sample and the spike, the resulting isotope ratio is measured by mass spectrometry. Concentration of the analyte in the sample is calculated using the isotope ratio. Compared with conventional quantification methods such calibration curves, IDMS avoids the need for a series of dilutions and external calibrations, and thus is less time consuming and more accurate and precise. IDMS has been applied in a wide range of sample matrices for various groups of analytes and was demonstrated to be able to significantly improve the quantitative results by lowering the measurement errors and uncertainties.

### 6.3 Quantification of POPs in human whole blood

SBSE-GC-MS/MS-IDMS was used to analyze POPs in human whole blood samples. Bovine whole blood samples were used for method development, optimization, and validation. LOQs of the analytes were between 0.01-0.08 ng/g, which demonstrated high level of sensitivity of the method. IDMS was compared with calibration curves and showed a higher level of accuracy and precision. A reverse-IDMS method was developed to verify and recalibrate labelled concentrations of commercially available standard. This method is essential for quality assurance and control of IDMS measurements. Ten human whole blood samples from Stanford Blood Center were analyzed using SBSE-GC-MS-IDMS. On average, 10 POPs were detected in each sample. The mean xenobiotic body-burden values were in a range of 0.719 to 1.12 ng/g. This method has demonstrated

analytical advantages and will be further applied in the study of environmental and human health.

## 6.4 Quantification of POPs in dietary supplements

SBSE-GC-MS/MS-IDMS was utilized to analyze POPs in dietary supplement samples. GC-MS/MS was compared with GC-MS and was able to lower the LOQs by approximately two orders of magnitude. Twelve plant-extract based dietary supplement products were obtained off-shelf from supermarket stores in the US. On average, 12 analytes were detected in each sample with a mean concentration of 1.31 ng/g. These measurements were converted to daily intake amount and compared with the existing guidelines. None of the quantified analytes in the investigated dietary supplement products exceeded the thresholds set for individual toxins. However, additive and synergistic effects of co-occurring pollutants need to be considered in the guidelines in future as numerous POPs can co-exist in the products.

## 6.5 Analysis of POPs in wastewater and drinking water

SBSE-GC-MS-IDMS was applied to quantification of fortified POPs in wastewater samples to help update the EPA Method 625. The results were compared with other participant laboratories. Both mean percent error and RSD of measurements at DU were significantly smaller than the average level of other participant laboratories. The wastewater samples were also analyzed at AIT laboratory using the same method. There was no statistically significant difference between the measurements at DU and AIT for

all the analytes. These results demonstrated a high level of accuracy, precision, and transferability of the method.

A novel sample preparation technique, FEVE, is designed and developed to analyze SVOCs in drinking water. These analytes are listed in the EPA Method 525 which cover a broad range of SVOCs. The design of the FEVE instrument has been completed and optimized. Preliminary data has shown its feasibility in replacing the older sample preparation techniques such as LLE and SPE for analysis of SVOCs in drinking water. A quantitative FEVE-TD-GC-MS method will be developed.

## 6.6 Outlook

During the past decades, the rapid development of analytical techniques has made it possible to accurately and precisely quantify a broad range of organic pollutants at low concentrations in complex matrices with efficient and green approaches. The advancement of analytical techniques has also spurred a growing awareness on the exposome from both the public and scientific community. Nonetheless, to better understand the connections between environmental pollutants and human health, more analytical methods and data are still in need. More human health related guidelines from the authorities are essential for future environmental pollutant regulation. As for the future development of sample preparation technique, greener, more convenient, and less costly will be the direction.

REFERENCES


1. Stockholm Convention. http://www.pops.int/Home/tabid/2121/Default.aspx.
2. Jones, K. C.; Voogt, P. d., Persistent organic pollutants (POPs): state of the science. *Environ Pollut* **1999,** *100* (1-3), 209-221.
3. Beyer, A.; Mackay, D.; Matthies, M.; Wania, F.; Webster, E., Assessing long-range transport potential of persistent organic pollutants. *Environmental Science & Technology* **2000,** *34* (4), 699-703.
4. Ali, U.; Syed, J. H.; Malik, R. N.; Katsoyiannis, A.; Li, J.; Zhang, G.; Jones, K. C., Organochlorine pesticides (OCPs) in South Asian region: a review. *Science of the Total Environment* **2014,** *476*, 705-717.
5. Ritter, L.; Solomon, K.; Forget, J.; Stemeroff, M.; O'Leary, C., Persistent organic pollutants: an assessment report on: DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, dioxins and furans. *International Programme on Chemical Safety (IPCS)* **1995**.
6. El-Shahawi, M.; Hamza, A.; Bashammakh, A.; Al-Saggaf, W., An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta* **2010,** *80* (5), 1587-1597.
7. Breivik, K.; Alcock, R.; Li, Y. F.; Bailey, R. E.; Fiedler, H.; Pacyna, J. M., Primary sources of selected POPs: regional and global scale emission inventories. *Environmental Pollution* **2004,** *128* (1-2), 3-16.
8. Vallack, H. W.; Bakker, D. J.; Brandt, I.; Broström-Lundén, E.; Brouwer, A.; Bull, K. R.; Gough, C.; Guardans, R.; Holoubek, I.; Jansson, B., Controlling persistent organic pollutants–what next? *Environmental Toxicology and Pharmacology* **1998,** *6* (3), 143-175.
9. Baek, S.; Field, R.; Goldstone, M.; Kirk, P.; Lester, J.; Perry, R., A review of atmospheric polycyclic aromatic hydrocarbons: sources, fate and behavior. *Water, Air, and Soil Pollution* **1991,** *60* (3-4), 279-300.
10. Christensen, E. R.; Bzdusek, P. A., PAHs in sediments of the Black River and the Ashtabula River, Ohio: source apportionment by factor analysis. *Water research* **2005,** *39* (4), 511-524.
11. Bansal, V.; Kim, K.-H., Review of PAH contamination in food products and their health hazards. *Environment international* **2015,** *84*, 26-38.
12. Chen, B.; Xuan, X.; Zhu, L.; Wang, J.; Gao, Y.; Yang, K.; Shen, X.; Lou, B., Distributions of polycyclic aromatic hydrocarbons in surface waters, sediments and soils of Hangzhou City, China. *Water research* **2004,** *38* (16), 3558-3568.
13. Ravindra, K.; Sokhi, R.; Van Grieken, R., Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. *Atmospheric Environment* **2008,** *42* (13), 2895-2921.
14. Mastrangelo, G.; Fadda, E.; Marzia, V., Polycyclic aromatic hydrocarbons and cancer in man. *Environmental Health Perspectives* **1996,** *104* (11), 1166-1170.
15. Baird, W. M.; Hooven, L. A.; Mahadevan, B., Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. *Environmental and Molecular Mutagenesis* **2005,** *45* (2-3), 106-114.
16. Bocskay, K. A.; Tang, D.; Orjuela, M. A.; Liu, X.; Warburton, D. P.; Perera, F. P., Chromosomal aberrations in cord blood are associated with prenatal exposure to

carcinogenic polycyclic aromatic hydrocarbons. *Cancer Epidemiology, Biomarkers & Prevention* **2005,** *14* (2), 506-511.
17. Xue, W.; Warshawsky, D., Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicology and Applied Pharmacology* **2005,** *206* (1), 73-93.
18. Deziel, N.; Rull, R.; Colt, J.; Reynolds, P.; Whitehead, T.; Gunier, R.; Month, S.; Taggart, D.; Buffler, P.; Ward, M., Polycyclic aromatic hydrocarbons in residential dust and risk of childhood acute lymphoblastic leukemia. *Environmental Research* **2014,** *133*, 388-395.
19. Samanta, S. K.; Singh, O. V.; Jain, R. K., Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *TRENDS in Biotechnology* **2002,** *20* (6), 243-248.
20. Tancell, P.; Rhead, M.; Trier, C.; Bell, M.; Fussey, D., The sources of benzo [a] pyrene in diesel exhaust emissions. *Science of the Total Environment* **1995,** *162* (2-3), 179-186.
21. Denissenko, M. F.; Pao, A.; Tang, M.-s.; Pfeifer, G. P., Preferential formation of benzo [a] pyrene adducts at lung cancer mutational hotspots in P53. *Science* **1996,** *274* (5286), 430-432.
22. Sinha, R.; Kulldorff, M.; Gunter, M. J.; Strickland, P.; Rothman, N., Dietary benzo [a] pyrene intake and risk of colorectal adenoma. *Cancer Epidemiology and Prevention Biomarkers* **2005,** *14* (8), 2030-2034.
23. Wang, Y.; Zhang, S.; Cui, W.; Meng, X.; Tang, X., Polycyclic aromatic hydrocarbons and organochlorine pesticides in surface water from the Yongding River basin, China: Seasonal distribution, source apportionment, and potential risk assessment. *Science of the Total Environment* **2018,** *618*, 419-429.
24. Ding, Y.; Harwood, A. D.; Foslund, H. M.; Lydy, M. J., Distribution and toxicity of sediment-associated pesticides in urban and agricultural waterways from Illinois, USA. *Environmental Toxicology and Chemistry* **2010,** *29* (1), 149-157.
25. Verweij, F.; Booij, K.; Satumalay, K.; van der Molen, N.; van der Oost, R., Assessment of bioavailable PAH, PCB and OCP concentrations in water, using semipermeable membrane devices (SPMDs), sediments and caged carp. *Chemosphere* **2004,** *54* (11), 1675-1689.
26. Bidleman, T. F.; Leone, A. D., Soil–air exchange of organochlorine pesticides in the Southern United States. *Environmental Pollution* **2004,** *128* (1-2), 49-57.
27. Sapozhnikova, Y.; Bawardi, O.; Schlenk, D., Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. *Chemosphere* **2004,** *55* (6), 797-809.
28. Jaward, F. M.; Farrar, N. J.; Harner, T.; Sweetman, A. J.; Jones, K. C., Passive air sampling of PCBs, PBDEs, and organochlorine pesticides across Europe. *Environmental Science & Technology* **2004,** *38* (1), 34-41.
29. Schecter, A.; Colacino, J.; Haffner, D.; Patel, K.; Opel, M.; Päpke, O.; Birnbaum, L., Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. *Environmental Health Perspectives* **2010,** *118* (6), 796-802.
30. Snedeker, S. M., Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. *Environmental Health Perspectives* **2001,** *109* (suppl 1), 35-47.

31. Xu, X.; Dailey, A. B.; Talbott, E. O.; Ilacqua, V. A.; Kearney, G.; Asal, N. R., Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in US adults. *Environmental Health Perspectives* **2009,** *118* (1), 60-66.
32. Vos, J. G.; Dybing, E.; Greim, H. A.; Ladefoged, O.; Lambré, C.; Tarazona, J. V.; Brandt, I.; Vethaak, A. D., Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology* **2000,** *30* (1), 71-133.
33. Beard, J.; Collaboration, A. R. H. R., DDT and human health. *Science of the Total Environment* **2006,** *355* (1-3), 78-89.
34. Yu, H.-Y.; Bao, L.-J.; Liang, Y.; Zeng, E. Y., Field validation of anaerobic degradation pathways for dichlorodiphenyltrichloroethane (DDT) and 13 metabolites in marine sediment cores from China. *Environmental Science & Technology* **2011,** *45* (12), 5245-5252.
35. Colosio, C.; Tiramani, M.; Maroni, M., Neurobehavioral effects of pesticides: state of the art. *Neurotoxicology* **2003,** *24* (4-5), 577-591.
36. Ulbrich, B.; Stahlmann, R., Developmental toxicity of polychlorinated biphenyls (PCBs): a systematic review of experimental data. *Archives of toxicology* **2004,** *78* (5), 252-268.
37. Safe, S. H., Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Critical reviews in toxicology* **1994,** *24* (2), 87-149.
38. Park, H.-Y.; Hertz-Picciotto, I.; Sovcikova, E.; Kocan, A.; Drobna, B.; Trnovec, T., Neurodevelopmental toxicity of prenatal polychlorinated biphenyls (PCBs) by chemical structure and activity: a birth cohort study. *Environmental Health* **2010,** *9* (1), 51.
39. McDonald, T. A., A perspective on the potential health risks of PBDEs. *Chemosphere* **2002,** *46* (5), 745-755.
40. Siddiqi, M. A.; Laessig, R. H.; Reed, K. D., Polybrominated diphenyl ethers (PBDEs): new pollutants–old diseases. *Clinical Medicine & Research* **2003,** *1* (4), 281-290.
41. Dodson, R. E.; Perovich, L. J.; Covaci, A.; Van den Eede, N.; Ionas, A. C.; Dirtu, A. C.; Brody, J. G.; Rudel, R. A., After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. *Environmental science & technology* **2012,** *46* (24), 13056-13066.
42. Meerts, I. A.; Van Zanden, J. J.; Luijks, E. A.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, Å.; Brouwer, A., Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicological sciences* **2000,** *56* (1), 95-104.
43. Van den Berg, M.; Birnbaum, L.; Bosveld, A.; Brunström, B.; Cook, P.; Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W., Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental health perspectives* **1998,** *106* (12), 775-792.
44. Whitney, K.; Seidler, F.; Slotkin, T., Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. *Toxicology and Applied Pharmacology* **1995,** *134* (1), 53-62.

45. Mie, A.; Rudén, C.; Grandjean, P., Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. *Environmental Health* **2018,** *17* (1), 1-5.
46. Rauh, V.; Arunajadai, S.; Horton, M.; Perera, F.; Hoepner, L.; Barr, D. B.; Whyatt, R., Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environmental health perspectives* **2011,** *119* (8), 1196-1201.
47. Grandjean, P.; Landrigan, P. J., Neurobehavioural effects of developmental toxicity. *The lancet neurology* **2014,** *13* (3), 330-338.
48. California Environmental Protection Agency - California Acts to Prohibit Chlorpyrifos Pesticide. https://calepa.ca.gov/2019/05/08/california-acts-to-prohibit-chlorpyrifos-pesticide/.
49. Wild, C. P., Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology Biomarkers & Prevention* **2005,** *14* (8), 1847-1850.
50. Escher, B. I.; Hackermüller, J.; Polte, T.; Scholz, S.; Aigner, A.; Altenburger, R.; Böhme, A.; Bopp, S. K.; Brack, W.; Busch, W., From the exposome to mechanistic understanding of chemical-induced adverse effects. *Environment international* **2017,** *99*, 97-106.
51. Rappaport, S. M., Implications of the exposome for exposure science. *Journal of Exposure Science and Environmental Epidemiology* **2011,** *21* (1), 5-9.
52. Rappaport, S. M.; Smith, M. T., Environment and disease risks. *science* **2010,** *330* (6003), 460-461.
53. Wild, C. P., The exposome: from concept to utility. *International journal of epidemiology* **2012,** *41* (1), 24-32.
54. Vrijheid, M., The exposome: a new paradigm to study the impact of environment on health. *Thorax* **2014,** *69* (9), 876-878.
55. Rappaport, S. M.; Barupal, D. K.; Wishart, D.; Vineis, P.; Scalbert, A., The blood exposome and its role in discovering causes of disease. *Environmental health perspectives* **2014,** *122* (8), 769-774.
56. Boggess, A.; Faber, S.; Kern, J.; Kingston, H. S., Mean serum-level of common organic pollutants is predictive of behavioral severity in children with autism spectrum disorders. *Scientific Reports* **2016,** *6*, 26185.
57. Lyall, K.; Croen, L. A.; Sjödin, A.; Yoshida, C. K.; Zerbo, O.; Kharrazi, M.; Windham, G. C., Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. *Environmental Health Perspectives* **2017,** *125* (3), 474-480.
58. Boggess, A. J.; Rahman, G. M.; Pamukcu, M.; Faber, S.; Kingston, H. S., An accurate and transferable protocol for reproducible quantification of organic pollutants in human serum using direct isotope dilution mass spectrometry. *Analyst* **2014,** *139* (23), 6223-6231.
59. Hao, W.; Kingston, H. M. S.; Dillard, A.; Macherone, A.; Stuff, J.; Pamuku, M., Quantification of Persistent Organic Pollutants in Human Whole Blood Samples Using Stir Bar Sorptive Extraction Coupled with GC/MS/MS and Isotope Dilution Mass Spectrometry. *Microchemical Journal* **2020,** *153*, 104279.

60. Baltussen, E.; Sandra, P.; David, F.; Cramers, C., Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *Journal of Microcolumn Separations* **1999,** *11* (10), 737-747.
61. David, F.; Ochiai, N.; Sandra, P., Two decades of stir bar sorptive extraction: A retrospective and future outlook. *TrAC Trends in Analytical Chemistry* **2019,** *112*, 102-111.
62. David, F.; Sandra, P., Stir bar sorptive extraction for trace analysis. *Journal of Chromatography A* **2007,** *1152* (1-2), 54-69.
63. Prieto, A.; Basauri, O.; Rodil, R.; Usobiaga, A.; Fernández, L.; Etxebarria, N.; Zuloaga, O., Stir-bar sorptive extraction: a view on method optimisation, novel applications, limitations and potential solutions. *Journal of Chromatography A* **2010,** *1217* (16), 2642-2666.
64. León, V.; Alvarez, B.; Cobollo, M.; Munoz, S.; Valor, I., Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry: I. Method optimisation. *Journal of Chromatography A* **2003,** *999* (1-2), 91-101.
65. Popp, P.; Keil, P.; Montero, L.; Rückert, M., Optimized method for the determination of 25 polychlorinated biphenyls in water samples using stir bar sorptive extraction followed by thermodesorption-gas chromatography/mass spectrometry. *Journal of Chromatography A* **2005,** *1071* (1-2), 155-162.
66. Prieto, A.; Zuloaga, O.; Usobiaga, A.; Etxebarria, N.; Fernández, L., Development of a stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry method for the simultaneous determination of several persistent organic pollutants in water samples. *Journal of Chromatography A* **2007,** *1174* (1-2), 40-49.
67. Xu, W.; Wang, X.; Cai, Z., Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: A review. *Analytica Chimica Acta* **2013,** *790*, 1-13.
68. Arthur, C. L.; Pawliszyn, J., Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical chemistry* **1990,** *62* (19), 2145-2148.
69. Holt, R., Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. *Journal of Chromatography A* **2001,** *937* (1-2), 107-114.
70. Balasubramanian, S.; Panigrahi, S., Solid-phase microextraction (SPME) techniques for quality characterization of food products: a review. *Food and Bioprocess Technology* **2011,** *4* (1), 1-26.
71. Aulakh, J.; Malik, A. K.; Kaur, V.; Schmitt-Kopplin, P., A Review on solid phase micro extraction—high performance liquid chromatography (SPME-HPLC) analysis of pesticides. *Critical reviews in analytical chemistry* **2005,** *35* (1), 71-85.
72. Reyes, L. H.; Mar, J. L. G.; Hernández-Ramírez, A.; Peralta-Hernández, J. M.; Barbosa, J. M. A.; Kingston, H. S., Microwave assisted extraction for mercury speciation analysis. *Microchimica Acta* **2011,** *172* (1-2), 3-14.
73. Camel, V., Recent extraction techniques for solid matrices—supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst* **2001,** *126* (7), 1182-1193.

74. Mustafa, A.; Turner, C., Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica chimica acta* **2011,** *703* (1), 8-18.
75. Tiwari, B. K., Ultrasound: A clean, green extraction technology. *TrAC Trends in Analytical Chemistry* **2015,** *71*, 100-109.
76. Wen, C.; Zhang, J.; Zhang, H.; Dzah, C. S.; Zandile, M.; Duan, Y.; Ma, H.; Luo, X., Advances in ultrasound assisted extraction of bioactive compounds from cash crops–A review. *Ultrasonics sonochemistry* **2018,** *48*, 538-549.
77. Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M., Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry* **2017,** *34*, 540-560.
78. Camino-Sanchez, F. J.; Zafra-Gomez, A.; Perez-Trujillo, J. P.; Conde-Gonzalez, J. E.; Marques, J. C.; Vilchez, J. L., Validation of a GC-MS/MS method for simultaneous determination of 86 persistent organic pollutants in marine sediments by pressurized liquid extraction followed by stir bar sorptive extraction. *Chemosphere* **2011,** *84* (7), 869-81.
79. Martinez-Parreno, M.; Llorca-Porcel, J.; Valor, I., Analysis of 51 persistent organic pollutants in soil by means of ultrasonic solvent extraction and stir bar sorptive extraction GC-MS. *Journal of separation science* **2008,** *31* (20), 3620-9.
80. Ochiai, N.; Sasamoto, K.; Kanda, H.; Yamagami, T.; David, F.; Tienpont, B.; Sandra, P., Optimization of a multi-residue screening method for the determination of 85 pesticides in selected food matrices by stir bar sorptive extraction and thermal desorption GC-MS. *Journal of separation science* **2005,** *28* (9-10), 1083-1092.
81. Sandra, P.; Tienpont, B.; David, F., Multi-residue screening of pesticides in vegetables, fruits and baby food by stir bar sorptive extraction–thermal desorption–capillary gas chromatography–mass spectrometry. *Journal of Chromatography A* **2003,** *1000* (1-2), 299-309.
82. Cacho, J. I.; Campillo, N.; Viñas, P.; Hernández-Córdoba, M., Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC–MS. *Journal of pharmaceutical and biomedical analysis* **2013,** *78*, 255-260.
83. Cacho, J. I.; Campillo, N.; Viñas, P.; Hernández-Córdoba, M., Stir bar sorptive extraction polar coatings for the determination of chlorophenols and chloroanisoles in wines using gas chromatography and mass spectrometry. *Talanta* **2014,** *118*, 30-36.
84. Zhou, Q.; Qian, Y.; Qian, M. C., Analysis of volatile phenols in alcoholic beverage by ethylene glycol-polydimethylsiloxane based stir bar sorptive extraction and gas chromatography–mass spectrometry. *Journal of Chromatography A* **2015,** *1390*, 22-27.
85. Jeleń, H. H.; Gaca, A.; Marcinkowska, M., Use of sorbent-based vacuum extraction for determination of volatile phenols in beer. *Food Analytical Methods* **2018,** *11* (11), 3089-3094.
86. Trujillo-Rodríguez, M. J.; Anderson, J. L.; Dunham, S. J.; Noad, V. L.; Cardin, D. B., Vacuum-assisted sorbent extraction: An analytical methodology for the determination of ultraviolet filters in environmental samples. *Talanta* **2020,** *208*, 120390.

87. Zhu, F.; Ruan, W.; Liu, L.; Lai, R.; Zeng, F.; Ouyang, G., Determination of organochlorine pesticides in textiles using solid-phase microextraction with gas chromatography–mass spectrometry. *Microchemical Journal* **2013,** *110*, 280-284.
88. Bielská, L.; Šmídová, K.; Hofman, J., Solid phase microextraction of organic pollutants from natural and artificial soils and comparison with bioaccumulation in earthworms. *Ecotoxicology and environmental safety* **2014,** *100*, 44-52.
89. Koureas, M.; Karagkouni, F.; Rakitskii, V.; Hadjichristodoulou, C.; Tsatsakis, A.; Tsakalof, A., Serum levels of organochlorine pesticides in the general population of Thessaly, Greece, determined by HS-SPME GC–MS method. *Environmental research* **2016,** *148*, 318-321.
90. Hu, Q.; Liu, S.; Liu, Y.; Fang, X. a.; Xu, J.; Chen, X.; Zhu, F.; Ouyang, G., Development of an on–site detection approach for rapid and highly sensitive determination of persistent organic pollutants in real aquatic environment. *Analytica chimica acta* **2019,** *1050*, 88-94.
91. Wang, P.; Zhang, Q.; Wang, Y.; Wang, T.; Li, X.; Ding, L.; Jiang, G., Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. *Analytica Chimica Acta* **2010,** *663* (1), 43-48.
92. Coscollà, C.; Castillo, M.; Pastor, A.; Yusà, V., Determination of 40 currently used pesticides in airborne particulate matter (PM 10) by microwave-assisted extraction and gas chromatography coupled to triple quadrupole mass spectrometry. *Analytica chimica acta* **2011,** *693* (1-2), 72-81.
93. Ramalhosa, M. J.; Paíga, P.; Morais, S.; Sousa, A. M.; Gonçalves, M. P.; Delerue-Matos, C.; Oliveira, M. B. P. P., Analysis of polycyclic aromatic hydrocarbons in fish: optimisation and validation of microwave-assisted extraction. *Food chemistry* **2012,** *135* (1), 234-242.
94. Fang, G.; Lau, H. F.; Law, W. S.; Li, S. F. Y., Systematic optimisation of coupled microwave-assisted extraction-solid phase extraction for the determination of pesticides in infant milk formula via LC–MS/MS. *Food chemistry* **2012,** *134* (4), 2473-2480.
95. Kamankesh, M.; Mohammadi, A.; Hosseini, H.; Tehrani, Z. M., Rapid determination of polycyclic aromatic hydrocarbons in grilled meat using microwave-assisted extraction and dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry. *Meat science* **2015,** *103*, 61-67.
96. Du, G.; Xiao, Y.; Yang, H. R.; Wang, L.; Song, Y. l.; Wang, Y. T., Rapid determination of pesticide residues in herbs using selective pressurized liquid extraction and fast gas chromatography coupled with mass spectrometry. *Journal of separation science* **2012,** *35* (15), 1922-1932.
97. Helaleh, M. I.; Al-Rashdan, A.; Ibtisam, A., Simultaneous analysis of organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) from marine samples using automated pressurized liquid extraction (PLE) and Power Prep™ clean-up. *Talanta* **2012,** *94*, 44-49.
98. Pena, M. T.; Casais, M. C.; Mejuto, M. C.; Cela, R., Development of a sample preparation procedure of sewage sludge samples for the determination of polycyclic aromatic hydrocarbons based on selective pressurized liquid extraction. *Journal of Chromatography A* **2010,** *1217* (4), 425-435.

99. Zhou, Q.; Sang, Y.; Wang, L.; Ji, S.; Ye, J.; Wang, X., Determination of polychlorinated biphenyls by GC/MS with ultrasound-assisted extraction from shellfish. *Frontiers of Agriculture in China* **2010,** *4* (4), 489-493.
100. Pena-Abaurrea, M.; de la Torre, V. G.; Ramos, L., Ultrasound-assisted extraction followed by disposable pipette purification for the determination of polychlorinated biphenyls in small-size biological tissue samples. *Journal of Chromatography A* **2013,** *1317*, 223-229.
101. Flores-Ramirez, R.; Medellin-Garibay, S.; Castillo, C.; Ilizaliturri-Hernandez, C.; Zuki-Orozco, B.; Batres-Esquivel, L.; Diaz-Barriga, F., Application of focused ultrasound-assisted extraction to the determination of persistent organic pollutants (POPs) in soil samples. *Bulletin of environmental contamination and toxicology* **2015,** *95* (2), 207-214.
102. Wagner, R.; Wetzel, S. J.; Kern, J.; Kingston, H., Improved sample preparation of glyphosate and methylphosphonic acid by EPA method 6800A and time-of-flight mass spectrometry using novel solid-phase extraction. *Journal of Mass Spectrometry* **2012,** *47* (2), 147-154.
103. Sargent, M.; Harrington, C.; Harte, R., *Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry (IDMS)*. Royal Society of Chemistry: Cambridge, UK, 2002.
104. Liao, W.; Ghabour, M.; Draper, W. M.; Chandrasena, E., Lowering detection limits for 1, 2, 3-trichloropropane in water using solid phase extraction coupled to purge and trap sample introduction in an isotope dilution GC-MS method. *Chemosphere* **2016,** *158*, 171-176.
105. Duhamel, N.; Slaghenaufi, D.; Pilkington, L. I.; Herbst-Johnstone, M.; Larcher, R.; Barker, D.; Fedrizzi, B., Facile gas chromatography–tandem mass spectrometry stable isotope dilution method for the quantification of sesquiterpenes in grape. *Journal of Chromatography A* **2018,** *1537*, 91-98.
106. Frank, S.; Hofmann, T.; Schieberle, P., Quantitation of benzene in flavourings and liquid foods containing added cherry-type flavour by a careful work-up procedure followed by a stable isotope dilution assay. *European Food Research and Technology* **2019**, 1-6.
107. Fahrenholz, T.; Wolle, M. M.; Kingston, H. S.; Faber, S.; Kern, J. C.; Pamuku, M.; Miller, L.; Chatragadda, H.; Kogelnik, A., Molecular speciated isotope dilution mass spectrometric methods for accurate, reproducible and direct quantification of reduced, oxidized and total glutathione in biological samples. *Analytical Chemistry* **2015,** *87* (2), 1232-1240.
108. Wagner, R. L.; Boggess, A. J.; Wetzel, S. J.; Kingston, H. S., Sensitive and stable pre-calibrated solid-phase extraction columns for environmental and forensic quantification using isotope dilution mass spectrometry. *Analytical Methods* **2015,** *7* (10), 4285-4294.
109. Zhao, B.; Zhang, S.; Zhou, Y.; He, D.; Li, Y.; Ren, M.; Xu, Z.; Fang, J., Characterization and quantification of PAH atmospheric pollution from a large petrochemical complex in Guangzhou: GC–MS/MS analysis. *Microchemical Journal* **2015,** *119*, 140-144.
110. Castillo, A. n.; Rodríguez-González, P.; Centineo, G.; Roig-Navarro, A. F.; Alonso, J. I. G. a., Multiple spiking species-specific isotope dilution analysis by

molecular mass spectrometry: simultaneous determination of inorganic mercury and methylmercury in fish tissues. *Analytical chemistry* **2010,** *82* (7), 2773-2783.
111. Rahman, G. M.; Wolle, M. M.; Fahrenholz, T.; Kingston, H. S.; Pamuku, M., Measurement of mercury species in whole blood using speciated isotope dilution methodology integrated with microwave-enhanced solubilization and spike equilibration, headspace–solid-phase microextraction, and GC-ICP-MS analysis. *Analytical chemistry* **2014,** *86* (12), 6130-6137.
112. Kingston, H. M.; Pamukcu, M., Accurate measurement of glutathione for disease diagnosis and drug metabolite screening. Google Patents: 2018.
113. Stockholm Convention - What are POPs. http://chm.pops.int/TheConvention/ThePOPs/tabid/673/Default.aspx.
114. He, W.; Chen, Y.; Yang, C.; Liu, W.; Kong, X.; Qin, N.; He, Q.; Xu, F., Optimized Multiresidue Analysis of Organic Contaminants of Priority Concern in a Daily Consumed Fish (Grass Carp). *Journal of Analytical Methods in Chemistry* **2017,** *2017*.
115. Pérez-Carrera, E.; León, V. M. L.; Parra, A. G.; González-Mazo, E., Simultaneous determination of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in seawater and interstitial marine water samples, using stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry. *Journal of Chromatography A* **2007,** *1170* (1-2), 82-90.
116. Camino-Sánchez, F.; Rodríguez-Gómez, R.; Zafra-Gómez, A.; Santos-Fandila, A.; Vílchez, J., Stir bar sorptive extraction: recent applications, limitations and future trends. *Talanta* **2014,** *130*, 388-399.
117. Dominguez, I.; González, R. R.; Liébanas, F. J. A.; Vidal, J. L. M.; Frenich, A. G., Automated and semi-automated extraction methods for GC–MS determination of pesticides in environmental samples. *Trends in Environmental Analytical Chemistry* **2016,** *12*, 1-12.
118. Lyall, K.; Croen, L. A.; Sjödin, A.; Yoshida, C. K.; Zerbo, O.; Kharrazi, M.; Windham, G. C., Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. *Environmental health perspectives* **2016,** *125* (3), 474-480.
119. Levin, E. D.; Addy, N.; Nakajima, A.; Christopher, N. C.; Seidler, F. J.; Slotkin, T. A., Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. *Developmental Brain Research* **2001,** *130* (1), 83-89.
120. Könemann, W.; Pieters, M., Confusion of concepts in mixture toxicology. *Food and Chemical Toxicology* **1996,** *34* (11-12), 1025-1031.
121. U.S. Food and Drug Administration- What is a dietary supplement? http://www.fda.gov/AboutFDA/Transparency/Basics/ucm195635.htm.
122. National Institute of Health- Office of Dietary Supplement. http://ods.od.nih.gov/factsheets/DietarySupplements-HealthProfessional/.
123. Chen, Y.; Al-Taher, F.; Juskelis, R.; Wong, J. W.; Zhang, K.; Hayward, D. G.; Zweigenbaum, J.; Stevens, J.; Cappozzo, J., Multiresidue Pesticide Analysis of Dried Botanical Dietary Supplements Using an Automated Dispersive SPE Cleanup for QuEChERS and High-Performance Liquid Chromatography–Tandem Mass Spectrometry. *Journal of agricultural and food chemistry* **2012,** *60* (40), 9991-9999.
124. Rawn, D. F.; Breakell, K.; Verigin, V.; Nicolidakis, H.; Sit, D.; Feeley, M., Persistent organic pollutants in fish oil supplements on the Canadian market:

polychlorinated biphenyls and organochlorine insecticides. *Journal of food science* **2009,** *74* (1), T14-9.
125. Marti, M.; Ortiz, X.; Gasser, M.; Marti, R.; Montana, M. J.; Diaz-Ferrero, J., Persistent organic pollutants (PCDD/Fs, dioxin-like PCBs, marker PCBs, and PBDEs) in health supplements on the Spanish market. *Chemosphere* **2010,** *78* (10), 1256-62.
126. Ruiz-Delgado, A.; Martínez-Domínguez, G.; Romero-González, R.; López-Ruiz, R.; Frenich, A. G., Determination of polycyclic aromatic hydrocarbons in soy isoflavone nutraceutical products by gas chromatography coupled to triple quadrupole tandem mass spectrometry. *Journal of separation science* **2016,** *39* (3), 528-536.
127. Chen, Y.; Lopez, S.; Hayward, D. G.; Park, H. Y.; Wong, J. W.; Kim, S. S.; Wan, J.; Reddy, R. M.; Quinn, D. J.; Steiniger, D., Determination of multiresidue pesticides in botanical dietary supplements using gas chromatography–triple-quadrupole mass spectrometry (GC-MS/MS). *J Agr Food Chem* **2016,** *64* (31), 6125-6132.
128. Szarka, A.; Turková, D.; Hrouzková, S., Dispersive liquid-liquid microextraction followed by gas chromatography–mass spectrometry for the determination of pesticide residues in nutraceutical drops. *Journal of Chromatography A* **2018,** *1570*, 126-134.
129. Armbruster, D. A.; Tillman, M. D.; Hubbs, L. M., Limit of detection (LQD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clinical chemistry* **1994,** *40* (7), 1233-1238.
130. Desimoni, E.; Brunetti, B., About estimating the limit of detection by the signal to noise approach. **2015**.
131. González, O.; Blanco, M. E.; Iriarte, G.; Bartolomé, L.; Maguregui, M. I.; Alonso, R. M., Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect. *Journal of Chromatography A* **2014,** *1353*, 10-27.
132. Long, G. L.; Winefordner, J. D., Limit of detection. A closer look at the IUPAC definition. *Analytical chemistry* **1983,** *55* (7), 712A-724A.
133. Foley, J.; Dorsey, J., Clarification of the limit of detection in chromatography. *Chromatographia* **1984,** *18* (9), 503-511.
134. Cedergreen, N., Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. *PloS one* **2014,** *9* (5), e96580.
135. Frank, S.; Hofmann, T.; Schieberle, P., Quantitation of benzene in flavourings and liquid foods containing added cherry-type flavour by a careful work-up procedure followed by a stable isotope dilution assay. *European Food Research and Technology* **2019,** *245* (8), 1605-1610.
136. EPA Method 625.1- Base/Neutrals and Acids by GC/MS. **2014**.
137. *Toxicological File for Methylene Chloride*; US Department of Health and Human Services: 2000.
138. Dietz, C.; Sanz, J.; Cámara, C., Recent developments in solid-phase microextraction coatings and related techniques. *Journal of Chromatography A* **2006,** *1103* (2), 183-192.
139. Spietelun, A.; Pilarczyk, M.; Kloskowski, A.; Namieśnik, J., Current trends in solid-phase microextraction (SPME) fibre coatings. *Chemical Society Reviews* **2010,** *39* (11), 4524-4537.

140. Merkle, S.; Kleeberg, K. K.; Fritsche, J., Recent developments and applications of solid phase microextraction (SPME) in food and environmental analysis—a review. *Chromatography* **2015,** *2* (3), 293-381.
141. Piri-Moghadam, H.; Alam, M. N.; Pawliszyn, J., Review of geometries and coating materials in solid phase microextraction: opportunities, limitations, and future perspectives. *Analytica chimica acta* **2017,** *984*, 42-65.
142. Jalili, V.; Barkhordari, A.; Ghiasvand, A., A comprehensive look at solid-phase microextraction technique: A review of reviews. *Microchemical Journal* **2020,** *152*, 104319.
143. Hao, W.; Kingston, H. S.; Dillard, A.; Stuff, J.; Pamuku, M., Quantification of persistent organic pollutants in dietary supplements using stir bar sorptive extraction coupled with GC-MS/MS and isotope dilution mass spectrometry. *Food Additives & Contaminants: Part A* **2020**, 1-14.
144. EPA Method 525.3 - Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS). **2014**.
145. Kwong, T. C., Organophosphate pesticides: biochemistry and clinical toxicology. *Therapeutic drug monitoring* **2002,** *24* (1), 144-149.
146. Kamrin, M. A., Phthalate risks, phthalate regulation, and public health: a review. *Journal of Toxicology and Environmental Health, Part B* **2009,** *12* (2), 157-174.