# MOLECULARLY IMPRINTED POLYMER BASED-MICRO-SOLID PHASE EXTRACTION OF BISPHENOL A AND SULFONAMIDE ANTIOBIOTICS IN AQUEOUS SAMPLES

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# MOLECULARLY IMPRINTED POLYMER BASED-MICRO-SOLID PHASE EXTRACTION OF BISPHENOL A AND SULFONAMIDE ANTIBIOTICS IN AQUEOUS SAMPLES

**by**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AALLME | Alcoholic assisted dispersive liquid-liquid microextraction |
| ACN | Acetonitrile |
| AD | Amperometric detection |
| AED | Atomic emission detection |
| APTES | 3-aminopropyl triethoxysilane |
| BAμE | Bar adsorption microextraction |
| BET | Brunauer-Emmett-Teller |
| Bio-DLLME | Bio-dispersive liquid-liquid microextraction |
| BPA | Bisphenol A |
| CE | Capillary electrophoresis |
| CNT | Carbon nanotubes |
| $C_{18}$ | Octadecyl carbon chain |
| DCM | Dichloromethane |
| DLME | Dispersive liquid microextraction |
| DLLME | Dispersive liquid-liquid microextraction |
| DLLME-IL | Dispersive liquid-liquid microextraction-ionic liquid |
| DMSPE | Dispersive micro-solid phase extraction |
| D-μ-SPE | Dispersive micro-solid phase extraction |
| EDCs | Endocrine disrupting chemicals |
| EF | Enrichment factor |
| EFSA | European Food Safety Authority |
| EOC | Emerging organic contaminant |
| EtOH | Ethanol |
| EU | European Union |
| FESEM | Field emission scanning electron microscope FT-IR |
| FLD | Fluorescence detection |
| GAC | Green Analytical Chemistry |
| GCB | Granulated carbon black |
| GC-FID | Gas chromatography-flame ionization detector |
| GC-MS | Gas chromatography-mass spectrometry |
| HAc | Acetic acid |
| HCl | Hydrochloric acid |
| HF-LPME | Hollow fiber liquid phase microextraction HF-SPME |

| | |
|---|---|
| HPLC-DAD | High performance liquid chromatography diode array detector |
| IL-MB-LPME | Ionic liquid magnetic bar liquid phase microextraction |
| ISP | Isopropanol |
| It-SPME | In tube-solid phase microextraction |
| KBr | Potassium bromide |
| LC-MS | Liquid chromatography- mass spectrometry |
| LLE | Liquid-liquid extraction |
| LPME | Liquid phase microextraction |
| LOAEL | Lowest-observed-adverse-effect level |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MeOH | Methanol |
| MEPS | Microextraction in packed syringe |
| $MgSO_4$ | Magnesium sulphate |
| MIP | Molecularly imprinted polymer |
| MISG | Molecularly imprinted silica gel |
| MMM | Mixed matrix membrane |
| MMMM | Mixed matrix membrane microextraction |
| MMHESPE | Magnetic mixed hemimicelles solid-phase extraction |
| MISPE | Molecularly imprinted solid phase extraction |
| MRL | Maximum residual limit |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| MWCNT | Multiwalled carbon nanotubes |
| NaOH | Sodium hydroxide |
| $NH_4OH$ | Ammonium hydroxide |
| NaCl | Sodium chloride |
| NISG | Non-imprinted silica gel |
| ND | Not detected |
| NOAEL | No-observed-adverse-effect level |
| OASIS HLB | OASIS hydrophilic lipophilic balance |
| PAHs | Polycyclic aromatic hydrocarbons |
| PLE | Pressurized liquid extraction |
| PP | Polypropylene |

| | |
|---|---|
| PPCP | Pharmaceuticals and personal care products |
| RR | Relative recovery |
| RSD | Relative standard deviation |
| SAs | Sulfonamide antibiotics |
| SDME | Single drop microextraction |
| SDZ | Sulfadiazine |
| SEM | Scanning electron microscope |
| SFO | Solidification of floating organic |
| SLM-MIB-SPE | Supported liquid membrane-molecularly imprinted bead-solid phase extraction. |
| SML | Specific migration limit |
| SMM | Sulfamonomethoxine |
| SMX | Sulfamethoxazole |
| SMX-MISG | Sulfamethoxazole-molecularly imprinted silica gel |
| SPE | Solid phase extraction |
| SPE-DLLME-SFO | Solid phase extraction-dispersive liquid-liquid microextraction solidify floating organic droplet |
| SSLLME | Supramolecular solvent liquid-liquid microextraction TDI |
| TDI | Total daily intake |
| TEOS | Tetraethoxysilane |
| UA-DSPME | Ultrasound assisted-dispersive solid phase microextraction |
| UHPLC | Ultra high performance liquid chromatography |
| USAE | Ultrasound assisted emulsification USFDA |
| USFDA | United State Food and Drug Administration |
| UV | Ultraviolet |
| VALLME | Vortex assisted liquid-liquid microextraction |
| WHO | World Health Organization |
| μ-SPE | Micro-solid-phase extraction |

## LIST OF SYMBOLS

| | |
|---|---|
| Cm | Centimetre |
| ˚C | Degree Celsius |
| °C/min | Degree Celsius per minute |
| G | Gram |
| *g* force | Gravity force |
| g $mL^{-1}$ | Gram per millilitre |
| H | Hour |
| log*P* | Partition coefficient |
| M | Molar |
| Mg | Milligram |
| mg $L^{-1}$ | Milligram per litre |
| Min | Minute |
| mL | Millilitre |
| mL $min^{-1}$ | Millilitre per minutes |
| Mm | Millimetre |
| Nm | Nanometre |
| $pK_a$ | Acid dissociation constant |
| $R^2$ | Correlative of determination |
| □f $kg^{-1}$ | Microgram per kilogram |
| µg $L^{-1}$ | Microgram per litre |
| µL | Volume per volume |
| µm | Micrometre |
| µ mol $L^{-1}$ | Micro mole per litre |
| v/v | Volume per volume |
| w/w | Weight per weight |
| % | Percent |

**PENGEKSTRAKAN MIKRO FASA PEPEJAL BERDASARKAN MOLEKUL CETAKAN POLIMER BAGI BISFENOL A DAN ANTIBIOTIK SULFONAMIDA DALAM SAMPEL AKUEUS**

## ABSTRAK

Tesis ini memfokuskan tentang pembangunan, pengesahan dan penggunaan kaedah penyediaan sampel baharu bagi penentuan bisfenol A (BPA) dan antibiotik sulfonamida dalam matrik akueus. Suatu kaedah pengekstrakan mikro yang cepat berasaskan pengemulsian dibantu ultrabunyi pengekstrakan mikro fasa pepejal menggunakan polimer cetakan molekul (USAE-MIP-μ-SPE) telah dibangunkan bagi penentuan BPA di dalam air, minuman dan cecair akueus di dalam makanan bertin digabungkan dengan kromatografi cecair berprestasi tinggi-pengesanan tatasusun diod (HPLC-DAD). Di bawah keadaan optimum, USAE-MIP-μ-SPE menunjukkan kelinearan yang baik dalam julat kepekatan 0.5–700 μg $L^{-1}$ dengan had pengesanan rendah (0.07 μg $L^{-1}$), pengembalian analit yang baik (82.2–118.9 %) dan kebolehulangan yang boleh terima (RSD %=0.7–14.2 %, *n*=3) dengan faktor pengayaan sebanyak 49 bagi BPA. Kaedah yang dibangunkan ini telah menunjukkan kepilihan tertumpu dan kebolehgunaan semula yang baik bagi pengektrakan BPA dan justeru, USAE-MIP-μ-SPE adalah mudah, cepat dan mesra sekitaran. Suatu pengekstrakan mikro membran campuran matriks yang mudah berasaskan gel silika cetakan molekul (MISG-MMMM) telah dibangunkan bagi penentuan tiga antibiotik sulfonamida terpilih di dalam sampel air. Gel silika cetakan molekul (MISG) telah disediakan mengunakan teknik cetakan permukaan dengan proses sol-gel. Kaedah MISG-MMMM telah menunjukkan had pengesanan rendah yang baik dalam julat 0.06–0.17 μg $L^{-1}$ bagi sulfametoksazol, sulfadiazin dan sulfamonometoksin dengan

kelinearan yang baik ($R^2 \geq 0.995$) dalam julat kepekatan 1–500 μg $L^{-1}$, pengembalian yang baik (80–96 %) dan kebolehulangan yang boleh terima (RSD %=1.0–7.2 % $n$=3). MISG-MMMM mengelakkan operasi pengekstrakan yang rumit dan jumlah pelarut (200 μL) dan pengerap (15 mg) yang sangat sedikit. MISG-MMMM bermanfaat dari segi keringkasan, kemudahan dalam operasi dan penjimatan kos ysng ketara. Kaedah USAE-MIP-μ-SPE dan MISG-MMMM yang dibangunkan dengan gabungan HPLC-DAD terbukti pantas, ringkas, kos yang rendah, cekap dan hanya memerlukan jumlah pelarut dan pengerap yang sedikit sekaligus menekankan keupayaannya sebagai kaedah pengektrakan alternatif kearah kimia analisis hijau.

**MOLECULARLY IMPRINTED POLYMER BASED-MICRO-SOLID PHASE EXTRACTION OF BISPHENOL A AND SULFONAMIDE ANTIBIOTICS IN AQUEOUS SAMPLES**

## ABSTRACT

This thesis focuses on the development, validation and application of new sample preparation methods for the determination of bisphenol A (BPA) and sulfonamide antibiotics (SAs) in aqueous matrices. A rapid microextraction method based on ultrasound-assisted emulsification micro-solid phase extraction incorporated with molecularly imprinted polymer (USAE-MIP-μ-SPE) was developed for the determination of BPA in water, beverages and the aqueous liquid in canned foods prior to high performance liquid chromatography diode array detector (HPLC-DAD). Under the optimized conditions, USAE-MIP-μ-SPE showed a good linearity over a concentration range of 0.5–700 μg $L^{-1}$ low limit of detection (0.07 μg $L^{-1}$), good analyte recoveries (82.2–118.9 %) and acceptable repeatability (RSD=0.7–14.2 %, $n$=3) with the enrichment factor of 49. The developed method demonstrated good selectivity and reusability for extraction of BPA and hence, the USAE-MIP-μ-SPE is simple, rapid, inexpensive and environmentally friendly. A facile mixed matrix membrane microextraction based on molecularly imprinted silica gel sorbent (MISG-MMMM) was developed for the determination of three selected sulfonamide antibiotics in water samples. The molecularly imprinted silica gel (MISG) was prepared by a surface imprinting technique with a sol-gel process. MISG-MMMM method demonstrated good limit of detection, in the ranges of 0.06–0.17 μg $L^{-1}$ for sulfamethoxazole, sulfadiazine and sulfamonomethoxine with a good linearity ($R^2$≥0.995) in the concentration of 1–500 μg $L^{-1}$, good recoveries (80-

96%) and repeatability (RSD = 1.0–7.2, *n*=3). MISG-MMMM avoids tedious extraction operations and requires minute amount of solvent (200 μL) and sorbent (15 mg). MISG–MMMM is beneficial in term of simplicity, ease of operation and delivers significant cost advantages. The developed USAE-MIP-μ-SPE and MISG-MMMM methods combined with HPLC-DAD proved to be rapid, simple, low-cost, efficient and require only small amount of solvent and sorbent highlighted their capability as alternative extraction approach towards green analytical chemistry.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research background

In recent years, many research have been developed to investigate on the existence of organic pollutants in the environment. There has been increasing in concern among researchers and regulatory agencies regarding the fate of the unintentional interaction of human and the environments with man-made chemicals. This is due to their potential adverse effects toward human and the environments. There are several groups of man-made chemical compounds that emerged as particularly relevant and are widely used around the globe such as disinfectant by-product, pesticides, herbicides, surfactant, dyes, plasticizers, veterinary and pharmaceutical drugs and their metabolites and endocrine disrupting compounds. This research focuses on bisphenol A (BPA) as one of the endocrine disrupting chemicals (EDCs) and sulfonamide antibiotics (SAs) compounds.

EDCs are substances that may disturb the endocrine system in human body. This class of compound may able to mimic the hormones in the body, thus may alter the signals that been carried by the hormone. As the result, it will affect the normal function of tissues and organs (National Institute of Health, 2010). The existence of EDCs in water is a concern of special environmental relevance due to the toxicity of these substances (Cai *et al*., 2003). Among phenolic EDCs, BPA triggers the most

concern from regulatory authorities and scientists due to its high production, extensive usage and prevalent occurrence in the environment (Mei *et al*., 2011).

Antibiotics are extensively used as medicine for the prevention and treatment of bacterial infectious diseases in human and animals. An important but often disregarded aspect of antibiotic use in the fate of antibiotic residue entering the environment (Sun *et al*., 2009; Baran *et al*., 2011; Białk-Bielińska *et al*., 2011). Among the antibiotic groups, SAs are categorized as one of the important antibacterial compounds. SAs have been used widely in both human and animals due to their low cost, broad spectrum activities, and effectiveness as growth promoter (Tong *et al*., 2013). Trace amount of SAs and its metabolites were often reported been detected in environment. This is due to incomplete absorption and metabolism in human and animals, improper way of drugs disposal or unexpected spill during distribution (Díaz-Cruz *et al*., 2003).

The most typical analytical instruments used for determination of EDCs and pharmaceutical compounds are high performance liquid chromatography (HPLC) coupled with fluorescence(FD), ultraviolet (UV) detection (Xu *et al*., 2011), or mass spectrometry (MS), gas chromatography (GC) with mass spectrometry (MS) or atomic emission detection (AED) (Chiavarino *et al*., 1998) and capillary electrophoresis (CE) system with electrochemical detection (ED) (Chu *et al*., 2009). Regardless of the development of highly efficient analytical instrumentations for determination of targeted analytes in samples, most of them cannot handle the matrix directly (Farhadi *et al*., 2012). Thus, sample preparation procedure is crucial to remove major interferences and to improve sensitivity for instruments.

Sample preparation plays important roles in analytical chemistry as it will determine the success of the chemical analysis procedure. In the present study, new molecularly imprinted polymers were used as sorbent in microextraction systems as sample preparation methods for the determination of BPA and SAs in aqueous matrices. Molecularly imprinted polymer has been successfully applied for the extraction of BPA and sulfonamides in different modes of extraction such as SPE (Zhang *et al*., 2006), pipette tips (Brigante *et al*., 2017), supported liquid membrane (Álvarez *et al*., 2014) and coated with magnetic nanoparticles (Xu *et al*., 2011). To the best of our knowledge, this is the first use of molecularly imprinted polymers incorporated with new microextraction systems for BPA and SAs analysis.

## 1.2 Problem statement

Sample preparation is commonly carried out using conventional extraction method such as solid phase extraction (SPE) and liquid-liquid extraction (LLE). However, most of the conventional extraction method suffers from number of drawbacks. LLE, produces clean extracts, but it has a number of drawbacks that limits its use as a sample preparation method including low analyte recoveries, formation of emulsion, limited selectivity and is time consuming when large amount of samples and toxic organic solvents are involved (Tong *et al*., 2013). Although SPE eliminates some drawbacks encountered in LLE however, the manual operation of SPE involves multi-steps and is exhaustive as well as time consuming. Moreover, most of the conventional sorbents are lack in selectivity. Typical SPE sorbents such as $C_{18}$, granulated carbon black (GCB), and carbon nanotubes (CNT), were synthesized and used for the extraction of BPA and SAs antibiotics. However, most

of the conventional sorbent show low retention for polar compounds and are not reusable (Rykowska *et al*., 2005; Sadeghi *et al*., 2016; Wang *et al*., 2016). In order to overcome the drawbacks related to the conventional methods, researchers have committed to the invention of micro-scale sample preparation incorporated with more selective sorbent, molecularly imprinted polymer (MIP). Microextraction offers the major benefits of high enrichment factor of analytes, solvent saving, rapid and efficiency analysis and the process of extraction is based on the equilibrium mechanism. The use of MIP or molecularly imprinted silica gel (MISG) in microextraction systems are expected to increase and enhance the sensitivity of BPA and SAs detection by analytical instrumentations.

## 1.3 Objectives

The aim of the study is to develop new microextraction methods using commercially sourced MIP and synthesized MISG as sorbent for the determination of BPA and SAs, respectively. The specific objectives are to:

a) develop and validate ultrasound-assisted emulsification based on molecularly imprinted polymer micro solid phase extraction (USAE-MIP-μ-SPE) combined with high performance liquid chromatography diode array detector (HPLC-DAD) for the determination of BPA in water, beverages and aqueous canned foods.
b) compare the analytical performance of the developed USAE-MIP-μ-SPE method with commercial SPE-$C_{18}$ method.
c) synthesis and characterize the molecularly imprinted silica gel (MISG).

d) develop and validate molecularly imprinted silica gel (MISG) for mixed matrix membrane microextraction (MISG-MMMM) coupled to HPLC-DAD for the determination of SAs in water samples.

## 1.4 Scope of study

This study emphasized on the application of MIP and MISG incorporated to the newly developed microextraction systems for the extraction and preconcentration of BPA and SAs respectively. The terms USAE-MIP-μ-SPE and MISG-MMMM are derived from a combined microextraction methods of ultrasound assisted emulsification (USAE) while MMMM derived from mixed matrix membrane microextraction, respectively. The MIP was commercially sourced from Supelco, Sigma Aldrich company and MISG was prepared through a sol-gel method. In this technique, the MISG was synthesized on the surface of silica gel by using 3-aminopropyl triethoxysilane (APTES) and tetraethoxysilane (TEOS) as the crosslinkers. Characterization of the synthesized MISG was performed by Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), field emission scanning electron microscopy (FESEM) for the morphology and Brunauer-Emmett-Teller (BET) for the surface area analysis. BPA and SAs were used as model analytes in this study. In the first objective, the strategy is based on the emulsification of a microvolume of organic solvent by ultrasound radiation that could facilitate the mass-transfer process between two immiscible phases, which increase the surface of contact between both phases will results in an increment of the extraction efficiency in a short time (Becerril-Bravo *et al*., 2010). The extraction efficiency of USAE-MIP-μ-SPE for BPA was assessed using HPLC-DAD, whilst,

for the second objective, the synthesized MISG was immobilized in the mixed matrix membrane through the dispersion of MISG in agarose base matrix. MISG-MMMM efficiency was investigated for SAs compounds, namely sulfomethoxazole (SMX), sulfamonomethoxine (SMM) and sulfadiazine (SDZ) using HPLC-DAD. Several effective parameters on the microextraction systems such as amount of sorbent, effect of salt addition and pH, sample volume, extraction and desorption time, were optimized thoroughly. Validation studies were carried out for both microextraction methods in terms of their linearity, limits of quantification (LOQs), limits of detection (LODs), repeatability and recovery. USAE-MIP-μ-SPE method was successfully applied for the determination of BPA in beverages, drinking and mineral water, pond and lake water, and canned food samples. On the other hands, MISG-MMMM was employed for the determination of SAs in water samples.

## 1.5 Significance of study

In-line with the environmental concerns and green chemistry concepts, sample preparation methods that are efficient, rapid, inexpensive and environmentally friendly are necessary for any analytical process. The developed USAE-MIP-μ-SPE is beneficial in term of fast extraction time, minimum solvent requirement, inexpensive, ease of operation and provided sensitive determination of BPA compound. Emulsification solvent has been introduced in this study to facilitate the extraction process and thus, shortened the extraction time. Furthermore, different application of membrane was introduced in study. In USAE-MIP-μ-SPE, the porous membrane was used as μ-SPE device that act as filter to protect the MIP from interferences when been applied in complex samples. The benefit of using membrane

protected is its allow the extraction, clean up and preconcentration of analyte to be done simultaneously (Sajid, 2017). Another alternative mode of microextraction system developed in this study is the MMMM incorporated with MISG sorbent for the extraction and preconcentration of SAs compounds. In this study, the developed membrane could be simply modified to different experimental designs and sample size requirements (Mukhtar and See, 2016). USAE-MIP-μ-SPE and MISG-MMMM systems were reported here for the first time for the determination of organic pollutants, and the results indicated they have great potential as alternative 'green' microextraction methods over the most commonly sample preparation methods.

## 1.6 Outline of thesis

This study investigates the synthesis and application of MIP and MISG for the determination of selected organic pollutants, BPA and SAs in aqueous matrices. This thesis consists of five chapters. Chapter 1 describes an overview of the study and Chapter 2 compiles the properties of BPA and SAs with its analytical methods, sample preparation methods including conventional and microextraction methods, microextraction methods for BPA and SAs, and the applications of MIP or MISG in sample preparation methods. Chapter 3 was divided into two parts, in which the first part describes the methodology for a rapid USAE-MIP-μ-SPE for HPLD-DAD determination of BPA and part 2 elaborates methodology on a facile MISG-MMMM for HPLC-DAD determination of SAs in water samples.

Chapter 4 was also divided into two parts. The first part discusses results and discussion on optimization study, method validation and application to real samples

of USAE-MIP-μ-SPE for BPA analysis while the second part explores on the synthesis and characterization of MISG sorbent. The optimization and validation data of MISG-MMMM were also presented in this part. The performance of USAE-MIP-μ-SPE and MISG-MMMM coupled with HPLC-DAD were evaluated for the analysis of BPA and SAs in aqueous matrices. Several important microextraction parameters such as amount of sorbent, sample volume, extraction and desorption time, salt addition, sample pH and desorption solvent were studied.

The final chapter, Chapter 5 covers the overall conclusions and future directions for further studies. This chapter summarizes the main thesis findings of each part and discussed the overall analytical performance of the developed microextraction and analytical methods. Future recommendations are presented for possible further investigations.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Overview

This chapter discussed the fundamental aspects of the organic pollutants and their properties. Besides, the established conventional and alternative extraction methods that were developed for the determination of bisphenol A (BPA) and sulfonamides antibiotics (SAs) were summarized.

## 2.2 Organic pollutants

Contamination happen when a foreign elements or compounds was introduced into the environment at a level that will disturb the system from functioning well. Nowadays, with the emerging of chemical sectors in the country that provide products and solution for the agriculture, medical and household have increases the demand of organics chemical production. As the demand in chemicals increases, more chemicals especially organic chemicals have been produced and release to the environment. Although some of the chemicals may not be a health threat to human and the ecosystem but it may have potential to bring harm to the environment in the future. Besides, some of the chemicals were produced for the purposed to be toxic such as pesticides, herbicides and others. Scientists have been monitoring the releasing of contaminant into the environment such as water, air and

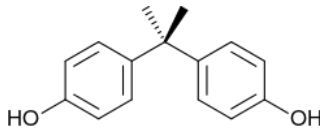
soil for many years. Besides, many articles have shown the present of contaminant in the environment.

In this study, BPA and SAs were selected as target analytes for the development of new sample preparation methods. The details information on the selected compounds are discussed further in the following section.

### 2.2.1 BPA

Endocrine disrupting compounds (EDCs) are a class of chemical compound that may interfere the function of endocrine system if been exposed at certain doses. Among the EDCs, BPA has causes major concern among researches and public authorities since it is extensively used around the world. Besides, it also has been identified as one of the highest produce chemicals worldwide with more than eight billion pound produced every years and the amount released into the atmosphere is approximately more than one hundred tons yearly (Vandenberg *et al.,* 2010). The physical and chemical properties of BPA are shown in Table 2.1.

Table 2.1. Physical and chemical properties of bisphenol A. Source: National Center for Biotechnology Information.Pubchem Compound Database; CID=6623, https://pubchem.ncbi.nlm.nih.gov/compound/6623 (accessed Dec 12, 2017)

| **Properties** | **Compound** |
|---|---|
| | Bisphenol A |
| IUPAC name | 2,2-bis(4-hydroxyphenyl)propane |
| Chemical Structure |  |
| Empirical formula | $C_{15}H_{16}O_2$ |
| $pK_a$ | 9.65 |
| Boiling point | 220 °C (428°F) |

BPA is commonly used in the production of polycarbonate plastic and epoxy resin (Rubin, 2011). It can be found in line metal cans, toys, water pipes, drinking containers, eyeglass lenses, medical and safety equipment and electronics (Vandenberg *et al.,* 2007). Previous studies revealed that, the BPA may leached out from the food container into the food at the temperature of 40°C when incubate for 8 hours and the concentration of BPA leached out increased with increasing temperature and incubation time (Munguía-López *et al.,* 2005; Cao & Corriveau, 2008; Ehlert *et al.,* 2008; Kubwabo *et al.,* 2009;).

A study by Calafat and his group in 2005 was carried out on the effects of BPA exposure towards animals and humans (Calafat *et al.,* 2005). Based on their findings, it shows that exposure of several dosage of BPA may cause severe effect on human. Various concentration of BPA were also detected in 95 % of urine sample collected from 394 adult in United State (Calafat *et al.,* 2005) and was found in breast milk samples (Sun *et al.,* 2004). BPA is excreted from human through blood

and sweat and can be found in sweat at higher concentration as compared to blood and urine (Genuis *et al.,* 2012). BPA interferes the human endocrine system and may result in epigenetic modification that alters the synthesis of testosterone and estradiol (Galloway *et al.,* 2010). This process will cause anomalous activities in human reproductive organ such as ovarian cysts, irregular cycle (Kato *et al.,* 2003), placental dysfunction and neonatal mortality (Tachibana *et al., 2007)*. As for male, exposure of BPA will caused erectile dysfunction and ejaculation difficulties ( Li *et al.,* 2010).

Due to the adverse effects of BPA towards human, most of the legislative authorities have enacted tolerable daily intake (TDI) and maximum residual limit (MRL) for BPA. According to World Health Organisation (WHO), the TDI for Europe stated by European Food Safety Authority (EFSA) in 2006 was 5 $mg/kg^{-1}$ for no-observed-adverse-effect level (NOAEL) and 120 $mg/kg^{-1}$ for lowest-observed-adverse-effect level (LOAEL), United State, TDI were 2.42 $\mu g/kg^{-1}$ and 0.185 $\mu g/kg^{-1}$ as stated by United State Food and Drug Administration (USFDA) (Food & Authorities, 2009). Recently, in 2015, EFSA has set new TDI amount of BPA to 4 $\mu g/kg^{-1}$ (Materials, Aids, Panel, & Tdi, 2015).

### 2.2.1(a) Extraction methods for BPA

Typical sample preparation methods for BPA include solid phase extraction (SPE) and liquid-liquid extraction (LLE), which are presently the most widely used methods. SPE remove some drawbacks face in LLE and a number of SPE columns have been employed for BPA determination, including $C_{18}$ (Maragou *et al.,* 2006),

OASIS HLB (Coughlin *et al.,* 2011), ion exchange (Regueiro, & Wenzl, 2015) and molecularly imprinted polymer (MIP) (Espenschied & Claus, 2015), which are all commercially available. However, the manual operation of SPE involves multi-steps and is exhaustive as well as time consuming. Additionally, the conventional cartridge-based SPE usually requires a high amount (150-5000 mg) of sorbent for column packing and a huge amount of organic solvent is used in the conditioning and elution steps in SPE (Mijangos, *et al.,* 2015). The columns are relatively expensive and cannot be reused.

The development of micro-scale extraction technique able to overcome the disadvantages of LLE and SPE. Example of microextraction techniques that have been developed for determination of BPA are dispersive liquid-liquid microextraction (DLLME) (Cunha *et al.,* 2015), pressurised liquid extraction (PLE) (Ferrer *et al.,* 2011), dispersive micro-solid phase extraction (DMSPE) (Reyes-Gallardo *et al.,* 2016), coacervative microextraction (Bendito et al., 2009), hollow fiber liquid phase microextraction (HF-LPME) (Tan *et al.,* 2012), vortex assisted liquid-liquid microextraction (VALLME) (Yiantzi *et al.,* 2010), solidification of floating organic drop (SFO) (Sadeghi *et al.,* 2016), and alcoholic assisted dispersive liquid-liquid microextraction (AALLME) (Fatemi *et al.,* 2014).

Furthermore, the variability of synthesized sorbent or commercially available sorbent has become the contributing factor in alternative sorbent-based extraction methods for determination of BPA. Several previously reported sorbents Some of the adsorbent used for determination of BPA include $C_{18}$ (Maragou et al., 2006), multiwalled carbon nanotubes (Cai *et al*., 2003; Elbashir *et al*., 2016), different types

of magnetic nanoparticles such as magnetic nylon 6 composite (Reyes-Gallardo *et al*., 2016), Fe@MgAl-LDH magnetic nanoparticle (Zhou *et al*., 2017), magnetic activated carbon (Filippou et al., 2016), electrospun nylon 6 nanofibrous membrane (Yan *et al*., 2010), and chemically bonded ketoimine groups (Rykowska *et al*., 2005)

The development of sorbent-based microextraction methods have greatly minimized the chemical consumption and generated less waste. Several analytical methods have been established for the determination of BPA in various types of matrices including high liquid chromatography (HPLC) with different detection systems such as fluorescence detection (FLD) (Sun *et al.,* 2004; Brenn-Struckhofova & Cichna-Markl, 2006; Bendito *et al.,* 2009) mass spectrometry (MS) and tandem mass spectrometry (MS/MS) (Maragou *et al.,* 2006; Coughlin *et al.,* 2011; Khedr, 2013; Vitku *et al.,* 2015), ultraviolet detection (UV) (Yoshida *et al.,* 2001; Filippou *et al.,* 2016; Haeri, 2016), gas chromatography with mass spectrometry (GC-MS) (Jin *et al.,* 2004; Wang *et al.,* 2009; Cunha & Fernandes, 2010; Elobeid *et al.,* 2012; Azzouz *et al.,* 2016; Brigante *et al.,* 2017), gel immunoaffinity chromatography (Brenn-Struckhofova *et al.*, 2006), and capillary electrophoresis with ultraviolet detection (CE) (Zhong *et al.,* 2011; Zhang *et al.,* 2015). Summary of some existing chromatographic methodologies for the detection of BPA is shown in Table 2.2.

Based on the summary, multiwalled carbon nanotubes (MWCNT) has been used as sorbent in SPE for determination of BPA in bottled water (Elbashir *et al*., 2016). MWCNT has advantages in term of high inner volume and surface area, high mechanical strength and stability (Latorre *et al*., 2015). However, MWCNT has low water solubility and have low recoveries in dispersing media (Latorre *et al*., 2015).

Next, magnetic nanoparticle has been used for the extraction of BPA (Xu *et al*., 2011b; Reyes-Gallardo *et al*., 2016). It have several advantages such as reduce the tedious on column SPE procedures, rapid analytes separation and the used of centrifugation and filtration can be discarded (Latorre *et al*., 2015). However it requires a complex synthesis in order to obtained good magnetic sorption material (Latorre *et al*., 2015). Other than SPE, DLLME also can give a promising result. This is due to the facts that DLLME have the unique features in term of simplicity of operation, rapidness and high enrichment factor (Cunha *et al*., 2011). However, the used of GC-MS require the sample to be derivatized. Recently, a combination of SPE and DLLME has been applied for the extraction of BPA (Sadeghi *et al*., 2016). This technique give has the lowest LOD among the others. Besides, the technique shows a great potential in the combination of SPE and DLLME as it has been successfully applied to a wide range of complex samples. Therefore, this study will be focus on the application of more selective sorbent, MIP for μ-SPE with the use of emulsifier to improve the selectivity and recovery for the determination of BPA.

Table 2.2. Summary of some existing chromatographic methodologies of BPA.

| **Matrix** | **Sample preparation** | **Instrumentation** | **LOD ($\mu g/kg^{-1}$)** | **Linear range ($\mu g/kg^{-1}$)** | **Recovery (%)** | **References** |
|---|---|---|---|---|---|---|
| Bottled water | SPE | HPLC-FLD | 0.03 | - | 92.5 | (Elbashir *et al*., 2016) |
| Canned food<br>Powdered milk<br>Honey<br>Fish sample<br>Soft drink | SPE-DLLME-SFO | HPLC-UV | 0.002 | 0.005 -10 | 93.9 -102.1 | (Sadeghi *et al*., 2016) |
| Urine | DLLME | GC-MS | 2.0 | 500-500,000 | 88-93<br>71-75 | (Cunha & Fernandes, 2010) |
| Plastic bottled water | SPME | HPLC-UV | 0.15 | 0.2-20 | 95 | (Yan *et al*., 2010) |
| Milk | DMSPE | HPLC-UV | 3.05 | 10.2-4000 | 86 – 99 | (Reyes-Gallardo *et al*., 2016) |
| Packed food | MIP-MSPE | HPLC-FLD | 0.1 | 0.5-100 | 72 – 113 | (Xu *et al*., 2011b) |

*HPLC-UV: high performance liquid chromatography ultraviolet, HPLC-FLD: high performance liquid chromatography flurocense detector, GC-MS: gas chromatography mass spectrometry, SPE: solid phase extraction, SPME: solid phase membrane extraction, MSPE: magnetic solid phase extraction, MIP-MSPE: molecularly imprinted polymer- magnetic solid phase microextraction, DLLME: dispersive liquid-liquid microextraction, SPE-DLLME-SFO: solid phase extraction-dispersive liquid-liquid microextraction, LOD: limit of detection.

### 2.2.2 Sulfonamide antibiotics

Recently, various types of synthetic organic compounds are used in large quantities with different purposes lead to growing concern of their occurrence in the environment. The presence of these emerging organic contaminants (EOC) will eventually become toxic to the terrestrial and aquatic life due to lack of regulation and in monitoring the substances (Tadeo *et al*., 2012). Examples of EOCs include pesticides, industrial by products, food additives, veterinary products and pharmaceuticals and personal care products (PPCPs) (Lapworth *et al*., 2012).

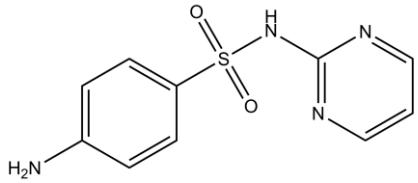
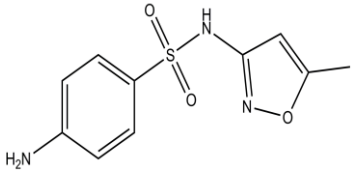
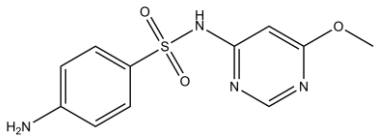
SAs are one of the oldest antibiotics that have been used as antimicrobial drugs in humans, animals and intensive aquaculture production (Tolika *et al*., 2011). SAs are derivatives of sulfanilic acid and were widely administered as potent chemotherapeutics in veterinary medicine practice due to their wide-spectrum antimicrobial activity and cost effectiveness (Yu *et al*., 2017). Previous study showed that, there are more than 10,000 sulfanilamide derivatives were synthesized and about 40 types of these antibiotics were used in medical and veterinary practice (Yu *et al*., 2017). In fact, SAs have been extensively used in food producing animals and it was estimated that 60 % of animals were exposed to sulfonamides at certain ages of their live (Bogialli & Corcia, 2009).

Although the biological half-life of SAs is 10 hours (Boothe, 2012), recent studies show that, there is possibility that these compounds enter the environment through wastewater effluents from hospital effluent, treatment plants, and industrial livestock production among others. Moreover, SAs residues were also detected in the

environmental water, soils and wastewater samples (Babić *et al.,* 2006; Tong *et al.,* 2009; Wei *et al.,* 2011). In order to prevent the worst scenario, regulatory authority of European Union (EU), United State Food Department Agency (USFDA) has established a strict regulation and the maximum residual limit (MRL) of SAs at 100 μg/kg.

Unintentional intake of sulfonamides will contribute to development of bacteria that resist to the antibiotic or commonly known as antibiotic resistant bacteria (Baran *et al.,* 2011). Although, there are no significant effects have been reported, the accumulation of sulfonamides drugs in various organisms in food chain may lead to increment of toxic effect induced by it (Migliore *et al.,* 1996; Sukul *et al.,* 2006). Moreover, at the environmental exposure level, this drug may inhibit the growth of human embryonic cell with 30 % decrease in cell proliferation compared to controls (Pomati *et al.,* 2006). Therefore, as a prevention step, other authority agency has developed strict regulatory limits also at 100 μg/kg to prevent contamination of sulfonamides in food matrices. Chemical structure and characteristics of selected sulfonamides are described in Table 2.3.

Table 2.3. Chemical structures of selected SAs. Source: National Center for Biotechnology Information.Pubchem Compound Database; CID=46782960,https://pubchem.ncbi.nlm.nih.gov/compound/46782960 (accessed Dec.12,2017).

| **Properties** | **Compounds** | | |
|---|---|---|---|
| Common name | Sulfadiazine | Sulfamethoxazole | Sulfamonomethoxine |
| IUPAC name | 4-amino-N-pyrimidin-2-ylbenzenesulfonamide | 4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide | 4-amino-N-(6-methoxypyrimidin-4-yl)benzenesulfonamide |
| Chemical Structure |  |  |  |
| Empirical formula | $C_{10}H_{10}N_4O_2S$ | $C_{10}H_{11}N_3O_3S$ | $C_{11}H_{12}N_4O_3S$ |
| $pK_a$ | 6.36 | 6.16 | 6.67 |
| Melting point | 255.5 | 167 | 204 |
| log*P* | 0.39 | 0.89 | 1.03 |

### 2.2.2(a) Extraction methods for SAs

Numerous analytical methods have been developed for the determination, extraction and identification of sulfonamides (SAs) such as high performance liquid chromatography with fluorescence detection (FLD) (Costi *et al*., 2010; Tsai *et al*., 2010), ultraviolet detection (UV) (Gao *et al*., 2010; Salami & Queiroz, 2011; Díaz-Álvarez *et al*., 2014; Wu *et al*., 2015), ultra high performance liquid chromatography with mass spectrometry (UHPLC-MS) and tandem mass spectrometry (UHPLC-MS/MS) (Thompson & Noot, 2005; Lu *et al*., 2007; Xu *et al*., 2013; Spielmeyer *et al*., 2014; Jia *et al*., 2016;), and capillary electrophoresis with ultraviolet detector (CE-UV) (Fuh & Chu, 2003).

Due to the fact that the present of most SAs are at relatively low concentration in the environmental samples, clean-up and preconcentration techniques are necessary for determination of SAs prior to instrument analysis. Most of the developed preconcentration techniques were more focus on SPE with the used of different type of sorbents such as $C_{18}$ (Salami & Queiroz, 2011), OASIS hydrophilic lipophilic balance (HLB) (Malintan & Mohd, 2006), florisil column (Granja *et al*., 2008), and cation exchange sorbent (Hela *et al*., 2003). Although the developed methods able to determine SAs at trace level, but it is labour intensive, lack of sensitivity and time consuming (Sun *et al*., 2009). In order to improve the sensitivity and capability of the extraction method, several microextraction method incorporated with novel sorbent have been developed for the microextraction and preconcentration of sulfonamides to overcome drawbacks of conventional SPE and LLE. Examples of previously developed microextraction method include headspace

microextraction hollow fiber based liquid phase microextraction (HS-LPME) (Payán *et al*., 2011), ionic liquid based microwave-assisted (Xu *et al*., 2011), ionic liquid magnetic bar (IL-MB) (Wu *et al*., 2015) and using supramolecular solvent extraction (Costi *et al*., 2010). As for sorbent based microextraction methods have been introduced for determination of sulfonamides such as different type of magnetic nanoparticles such as magnetic multiwalled carbon nanotubes (Xu *et al*., 2013), magnetic hypercrosslinked polystyrene (Tolmacheva *et al*., 2016), metal-organic framework/graphite oxide (MIL-101(Cr)@GO) (Jia *et al*., 2016), molecularly imprinted polymer (Díaz-Álvarez *et al*., 2014; Gao *et al*., 2010). A summary of some previously reported methods for the determination of SAs is shown in Table 2.4.

In summary, microextraction in packed syringe has improved the extraction recovery and successfully applied in complex sample such as eggs (Salami & Queiroz, 2011). However, it required multistep procedures since the sample need to be introduced to packed syringe repeatedly in order to ensure the target analyte in sample has been completely extracted. Next is bar adsorption microextraction (Ide *et al*., 2016) where the principle of this technique is to immobilize the sorbent. The sorbent is packed in a 'container' or stick at a 'base' such as polypropylene or bar. The main advantages of these techniques are multistep of clean up process and used of solvents can be avoided (Moreda-Piñeiro & Moreda-Piñeiro, 2015). However, several report stated a low recoveries when using this techniques (Moreda-Piñeiro & Moreda-Piñeiro, 2015).

Besides, the liquid phase extraction also has been modified to improve the performance of the extraction. The used of green organic such as ionic liquid

(IL) show a promising result. Ionic liquid magnetic bar liquid phase microextraction (IL-MB-LPME) has been successfully developed and applied for the extraction of sulfonamides in butter samples (Wu et al., 2015). High extraction recovery was obtained is in the range from 72.9-103.5 %. IL is one of the green solvents with low toxicity compared to typical organic solvents, with characteristics of high surface tension, biocompatible and high solvation that make IL able to dissolve in wide range of compounds (Abdelhamid., 2016). However, the drawback of using IL is it have high viscosity that will lower the mass transport rate, rate of diffusion thus causes longer extraction time (Abdelhamid., 2016). Next, Supramolecular solvent liquid-liquid microextraction (SSLLME) that used of reverse of decanoic acid that been dispersed in solution such as tetrahydrofuran before added to the sample. Then, the solution been separated from sample by centrifugation (Spietelun *et al*., 2014). The advantages of this technique are it consumes less organic solvent and have high pre-concentration factors (Spietelun *et al*., 2014). However, this technique requires specially-designed centrifuge cone for the production of the supramolecular solvent (Spietelun *et al*., 2014)

In order to improve the extraction efficiency, sorbent that is more selective toward the analyte could be the solution. It could improve the extraction efficiency in complex matrices and reduce the time needed to complete the extraction process. This study will be focus on developing a more selective sorbent and simple extraction method that could improve the efficiency of the extraction.

Table 2.4. Summary of some previously reported methods for the determination of SAs.

| Matrix | Extraction mode | Instrumentation | LOD (μg/kg$^{-1}$) | Linear range (μg/kg$^{-1}$) | Recovery (%) | References |
|---|---|---|---|---|---|---|
| Blood | SPE | HPLC-UV | 0.0014 | 0.02-10 | 81.3-106.8 | (Zhang *et al.,* 2012) |
| Eggs | MSPE | LC-MS/MS | 1.4 | 10-1000 | 74-96 | (Xu *et al.,* 2013) |
| Water | (BAμE) | HPLC-DAD | 0.08 | 0.16-8.0 | 63.8-81.2 | (Ide *et al.,* 2016) |
| Eggs | MEPS | LC-DAD | 30 | 30-300 | 94-111 | (Salami & Queiroz, (2011) |
| Meat | SSLLME | LC-FD | 104 | 0.08-15 | 44-105 | (Costi *et al.,* 2010) |
| Butter | IL-MB-LPME | HPLC-UV | 1.36 | 6.0-300 | 72.9-103.5 | (Wu *et al.,* 2015) |

*HPLC-DAD: high performance liquid chromatography diode array detector, HPLC-UV: high performance liquid chromatography ultraviolet, LC-DAD: liquid chromatography diode array detector, LC-MS: liquid chromatography mass spectrometry, LC-MS/MS: liquid chromatography tandem mass spectrometry, LC-FD: liquid chromatography flurocense detector MEPS: microextraction in packed syringe, BAμE: bar adsorption microextraction, MSPE: Magnetic solid phase extraction, IL-MB-LPME: ionic liquid magnetic bar liquid phase microextraction, SSLLME: supramolecular solvent liquid-liquid microextraction, SPE: solid phase extraction.

## 2.3 Extraction and preconcentration methods for chemical analysis

Analytical protocol covers various important steps including sampling, sample pre-treatment, extraction of the target analyte, detection of the analyte, quantification and data handling. In separation chemistry, sample preparation step plays crucial roles since it will determine the quality of the analytical result and the time taken to complete the analysis.

There are several conventional extraction methods that are frequently used for the extraction of organic compounds from different matrices such as pressurized liquid extraction (Dorival-García, Zafra-Gómez, Navalón, & Vílchez, 2012), microwave-assisted extraction (Sanchez-Prado *et al.,* 2015), and SPE (Babić *et al.,* 2006; Javanbakht *et al.,* 2010).

### 2.3.1 Liquid-liquid extraction

One of the oldest extraction methods used for the extraction of organic compound is Liquid-liquid extraction (LLE). LLE is also known as solvent extraction. LLE extraction mechanism is based on the different distribution of the components that being separated between two liquid phases (Figure 2.1.). The separation is depended on the mass transfer of the component to be extracted from the first liquid phase to second liquid phase (Berger *et al.,* 2005). The organic phase phases is dispersed into droplet and in continuous phase in order to obtain a large and significant mass-transfer interface. Therefore, LLE is usually performed in mixer-settler equipment or using an extraction column, that usually equipped with rotating