A new Sorbent of Polypyrrole/Silica/Magnetite Nanoparticles for the Extraction of Sulfonamides from Water Samples

Thanyaporn Sukchuay

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Author Mrs. Thanyaporn Sukchuay

Major Program Chemistry

Major Advisor:

.....

(Dr. Opas Bunkoed)

Co-advisor:

.....

(Assoc. Prof. Dr. Proespichaya Kanatharana)

Examining Committee:

.....Chairperson

(Assoc. Prof. Dr. Lupong Kaewsichan)

.....Committee

(Dr. Pamornrat Kueseng)

.....Committee

(Dr. Opas Bunkoed)

.....Committee

(Assoc. Prof. Dr. Proespichaya Kanatharana)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Master of Science Degree in Chemistry

.....

(Assoc. Prof. Dr. Teerapol Srichana)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

.....Signature
(Dr. Opas Bunkoed)
Major Advisor

.....Signature
(Mrs. Thanyaporn Sukchuay)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

.....Signature

(Mrs. Thanyaporn Sukchuay)

Candidate

ชื่อวิทยานิพนธ์ ตัวดูดซับอนุภาคแม่เหล็กชนิดใหม่เคลือบซิลิกาและพอลิไพโรล สำหรับสกัดซัลโฟนาไมด์ในน้ำ

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สาขา เคมี

ปีการศึกษา 2558

บทคัดย่อ

วิทยานิพนธ์นี้ได้พัฒนาตัวดูดซับชนิดใหม่ คือตัวดูดซับอนุภาคแม่เหล็กเคลือบซิลิกาและพอลิไพโรล (polypyrrole/SiO_2/Fe_3O_4) สำหรับสกัดและเพิ่มความเข้มข้นสารซัลโฟนาไมด์ในตัวอย่างน้ำ โดยนำอนุภาคแม่เหล็กขนาดนาโนเมตร (Fe_3O_4) เคลือบด้วยซิลิกาและพอลิไพโรล ซึ่งอนุภาคแม่เหล็กช่วยในการแยกสารที่สนใจออกจากตัวอย่างน้ำได้ง่ายและรวดเร็ว ซิลิกาช่วยเพิ่มพื้นที่ผิวสัมผัสทำให้ชั้นของพอลิไพโรลเคลือบได้ดีขึ้น พอลิไพโรลช่วยเพิ่มประสิทธิภาพสกัด เนื่องจากสามารถเกิดอันตรกิริยาแบบ π-π ระหว่างพอลิไพโรลและซัลโฟนาไมด์ เพื่อให้ได้ประสิทธิภาพในการสกัดสูงสุด ได้ศึกษาสภาวะที่เหมาะสม ได้แก่ ปริมาณของตัวดูดซับของแข็ง พีเอชของสารตัวอย่าง เวลาที่ใช้ในการสกัด ชนิดและปริมาณตัวทำละลายที่ใช้ชะสารตัวอย่าง เวลาและอุณหภูมิที่ใช้ในการชะสาร และผลของเกลือ ภายใต้สภาวะที่เหมาะสม วิธีที่พัฒนาขึ้นให้ช่วงความเป็นเส้นตรงตั้งแต่ 0.30 ถึง 200 ไมโครกรัมต่อลิตร สำหรับซัลฟาไดอะซีนและซัลฟาเมอราซีน และให้ช่วงความเป็นเส้นตรงตั้งแต่ 1.0 ถึง 200 ไมโครกรัมต่อลิตร สำหรับซัลฟาเมทธาซีนและซัลฟาโมโนเมททอกซีน มีขีดจำกัดการตรวจวัด 0.30 ไมโครกรัมต่อลิตรสำหรับซัลฟาไดอะซีนและซัลฟาเมอราซีน และ 1.0 ไมโครกรัมต่อลิตร สำหรับซัลฟาเมทธาซีนและซัลฟาโมโนเมททอกซีน ตัวดูดซับที่พัฒนาขึ้นมีประสิทธิภาพในการสกัดที่ดี โดยมีค่าร้อยละการได้กลับคืนอยู่ในช่วง 86.7 ถึง 99.7 เปอร์เซ็นต์ และมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 6 เปอร์เซ็นต์ ตัวดูดซับที่พัฒนาขึ้นมีข้อดี คือ มีราคาถูก (2.50 บาทต่อชิ้น) ให้ผลการทำซ้ำที่ดี สามารถนำไปประยุกต์ใช้ในการวิเคราะห์สารซัลโฟนาไมด์ในตัวอย่างน้ำในสิ่งแวดล้อมได้ โดยวิธีที่พัฒนาขึ้นนี้ให้ผลการวิเคราะห์ที่ถูกต้อง แม่นยำ และมีความน่าเชื่อถือ

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Abstract

This thesis aimed to develop a new magnetic solid phase extraction sorbent of polypyrrole/silica/magnetite nanoparticles (polypyrrole/SiO_2/Fe_3O_4) for the extraction and preconcentration of sulfonamides in water samples by coating magnetite nanoparticles with silica and polypyrrole. The Fe_3O_4 nanoparticles provided a simple and fast separation method for the analytes in water samples. The silica coating increased the surface area that helped to increase the polypyrrole layer. The polypyrrole provided a high extraction efficiency due to the π-π interactions between the polypyrrole and sulfonamides. Several parameters that affected on the extraction efficiencies, i.e., the amount of sorbent, pH of the sample, extraction time, desorption solvent, time and volume, desorption temperature, ionic strength were investigated. Under optimum conditions, the method was linear over the range 0.30 – 200 μg L^{-1} for sulfadiazine and sulfamerazine, and 1.0 – 200 μg L^{-1} for sulfamethazine and sulfamonomethoxine. The limit of detections were 0.30 μg L^{-1} for sulfadiazine and sulfamerazine, and 1.0 μg L^{-1} for sulfamethazine and sulfamonomethoxine. The results showed the recoveries were in the range of 86.7-99.7% with relative standard deviations of < 6 %. This developed sorbent is cost-effective (0.07 USD per sample) and provided a good sorbent-to-sorbent reproducibility. This simple and rapid method was successfully applied to efficiently extract sulfonamides from water samples with a high extraction efficiency and it provided a good accuracy, precision and reliability.

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List of Abbreviations

AOAC	Association of analytical communities
CHI	Chitosan
CE	Capillary electrophoresis
CW/DVB	Carboxywax/divinylbenzene
DAD	Diode-array detector
DLLE	Dispersive liquid liquid extraction
EIA	Enzyme immunoassay
FLD	Fluorescence detector
FT-IR	Fourier transform infrared spectroscopy
MIP	Molecularly imprinted polymer
MMIP	Magnetic molecularly imprinted polymer
MNPs	Magnetic nanoparticles
MRL	Maximum residue limit
MS	Mass spectrometer
MSPE	Magnetic solid phase extraction
MWCNTs	Multiwall carbon nanotubes
NP	Normal phase
PANI	Polyaniline
PDMS/DVB	Polydimethylsiloxane/divinylbenzene
PPyNPs	Polypyrrole nanoparticles
RP-HPLC	Reversed phase-High performance liquid chromatography
RSD	Relative standard deviation
SD	Standard deviation
SDZ	Sulfadiazine
SEM	Scanning electron microscope

List of Abbreviations (Continued)

SMT	Sulfamethazine
SMX	Sulfamonomethoxine
SMZ	Sulfamerazine
SPME	Solid phase micro extraction
SPE	Solid phase extraction
UV-vis	Ultraviolet-visible

List of Publication

Paper **Sukchuay, T.,** Kanatharana, P., Wannapob, R., Thavarungkul, P., and Bunkoed, O. A polypyrrole/silica/magnetite nanoparticles as a sorbent for the extraction of sulfonamides in water samples.
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CHAPTER 1

Introduction

1.1 Background and Rationale

Sulfonamides are one types of antibiotic that have been used as effective chemotherapeutics in veterinary medicine practice because of their cost effectiveness and wide spectrum antimicrobial activity. They are used in veterinary medicine in pure formulations or in combination with other antibiotics to combat infectious disease, to promote growth and increase the productivity of livestock, poultry and aquaculture (Dmitrienko *et al.*, 2014). They are active against a broad spectrum of both gram-positive and negative bacteria including species of the genus *Streptococcus*, *Staphylococcus*, *Escherichia*, *Neisseria*, *Shigella*, *Salmonella*, *Nocardia*, *Chlamydia* and *Clostridium*. In addition, they have been used againt protozoa, parasites, and fungi (Baran *et al.*, 2011). A variety of sulfonamides are mostly employed in animal husbandry and intensive aquaculture farms such as sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX) (**Fig. 1.1**) because of their commercial availability and low cost. Therefore, it can be contaminated in environment.

Several research work reported the contamination of sulfonamides in environmental water, sewage, livestock and aquaculture wastewater, river and coastal waters. Sulfamethoxazole were detected in sewage or heavily sewage-impacted waters in five tropical asian countries in the concentration of 1720 ngL^{-1}(Vietnam), 802 ngL^{-1} (Philippines), 538 ngL^{-1} (India), 282 ngL^{-1} (Indonesia) and 76 ngL^{-1} (Malaysia) (Shimizu *et al.*, 2013). Sulfamethazine (0.076 to 0.22 µg L^{-1}) and sulfadimethoxine (0.046 to 0.068 µg L^{-1}) were found in priwells formerly used as sources of drinking water in Washington, USA (Batt *et al.*, 2006). Sulfanilamide, sulfadiazine, sulfamerazine, sulfamethoxypyridazine and sulfamethazine were found in selected Malaysian swine wastewater in the range of 5.15 to 93.75 ng L^{-1} (Malintan and Mohd, 2006). Due to their persistence for a long time, which lead to concern of widespread antibiotic resistant bacteria and resistance genes in the aquatic

environment (Kim *et al.*, 2013). Therefore, it is important to develop a suitable method for the determination of sulfonamides.

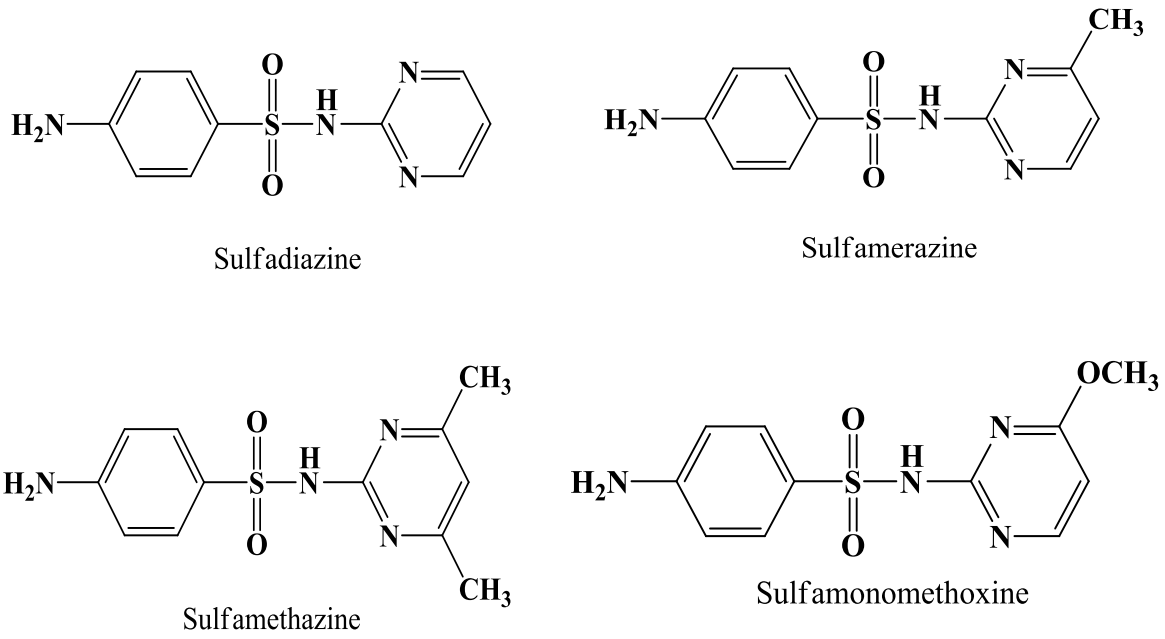


Fig. 1.1 The molecular structure of sulfonamides

Several methods have been reported for the determination of sulfonamides including, capillary electrophoresis (CE) (Tong *et al.*, 2013), enzyme immunoassay (EIA) (Galarini *et al.*, 2014; Zhou *et al.*, 2014) gas chromatography (GC) (Reeves, 1999), high performance liquid chromatography (HPLC) (Malintan and Mohd, 2006; Tao *et al.*, 2009; Zhang *et al.*, 2011b). Among these methods, HPLC has been widely used because of its high sensitivity and good selectivity (Lin and Huang, 2008). Due to the relatively low amount of sulfonamides in environmental water samples together with the complexity of the matrices, a sample preparation method is generally required prior to instrumental analysis.

Various sample preparation methods have been employed for the extraction and preconcentration of sulfonamides such as dispersive liquid-liquid extraction (DLLE) (Herrera-Herrera *et al.*, 2013b), liquid-liquid-liquid microextraction (LLLM) (Lin and Huang, 2008), hollow fiber-based liquid phase microextraction (HF-LPME) (Ramos Payán *et al.*, 2011), cloud point extraction (Zhang *et al.*, 2011b), stir bar

sorptive extraction (Huang *et al.*, 2009; Yu and Hu, 2012), solid phase microextraction (SPME) (McClure and Wong, 2007), solid phase extraction (SPE) (Fang *et al.*, 2006; Raich-Montiu *et al.*, 2007; Shi *et al.*, 2011; Sun *et al.*, 2014) and micro solid phase extraction (μSPE) (Díaz-Álvarez *et al.*, 2014). Among these, SPE is one of the most widely used due to it provides high extraction efficiency, good selectivity, and reproducibility. However, traditional SPE cartridges and equipment are expensive and the operation is quite tedious. To overcome this problem, magnetic solid phase extraction (MSPE) have been developed. It has attracted much attention since the sorbent can easily be separated from the sample solution using an external magnetic field, which makes the separation easier and faster (Hashemi *et al.*, 2014). This technique has more advantages than the conventional SPE sorbent which need to be packed in an SPE column or SPE cartridge and avoiding the time consuming process of loading large-volume of sample. Moreover, magnetic sorbents can be reused after a simple washing operation. However, naked Fe_3O_4 nanoparticles tend to aggregate, are prone to oxidation and are not selective toward complex matrices (Zhang *et al.*, 2011a). Therefore, the surface of these magnetic nanoparticle have been modified with specific ligands to make them selective and appropriate sorbents (Zhang *et al.*, 2011b). There are many works reported the use of magnetic nanoparticles composited or coated with others materials as magnetic nanosorbent such as graphene (Luo *et al.*, 2011; (Wang *et al.*, 2015), graphene oxide (Pan *et al.*, 2014; Yan *et al.*, 2014), multiwalled carbon nanotube (Xu *et al.*, 2013; Bunkoed and Kanatharana, 2015), carbon (Yang *et al.*, 2014), hydrophilic carbon (Geng *et al.*, 2012), C18 (Liu *et al.*, 2014), molecularly imprinted polymer (Zhou *et al.*, 2015) and silica (Yamini *et al.*, 2015). Among these, silica (SiO_2) is one of the most ideal coating layers on the surface magnetic nanoparticles since it can prevent the Fe_3O_4 nanoparticles aggregating and improve their chemical stability (Hashemi *et al.*, 2014).

Silica nanoparticles is becoming a promising and important approach in the development of surface modification of magnetic nanoparticles. This approach prevents the agglomeration of particles as well as providing an environment for the transferring of hydrophobic iron oxide nanoparticles into a hydrophilic system. Surface modification of nano-sized inorganic cores with different inorganic shells to form a core/shell type nanostructure has become an important route to obtain

functional nanomaterials since it can provide interesting physical and chemical properties (Tie *et al.*, 2007). However, the hydrophilic SiO_2 would not be applicable for the extraction of slightly or non polar compounds. Therefore, it was used to composite or modified with other materials for the improvement the hydrophobic interaction between analytes and sorbent. There are several works reported the use of silica (SiO_2) coated magnetic nanoparticles and composited or modified with other materials such as polyaniline (Wang *et al.*, 2014b), phenyl (Ibarra *et al.*, 2014; Tang *et al.*, 2014), chitosan (Ren *et al.*, 2013), ionic liquid (He *et al.*, 2014), mesoporus (de Souza *et al.*, 2014), polyaniline-graphene oxide (Su *et al.*, 2014).

Recently, polymers are widely used as coating layer to adsorb various compounds. Because of its ability to establish π–π interactions, and excellent chemical, mechanical and thermal stability. Polypyrrole is one of conducting polymers which are widely used for the extraction of varoius compounds. In this work, the new magnetic solid phase extraction of the polypyrrole/SiO_2/Fe_3O_4 sorbent was developed. Polypyrrole was used as the coating material because it contains a conjugated π structure that can adsorb sulfonamides via π-π interactions. Moreover, polypyrrole can easily be prepared by chemical polymerization under mild condition (Mehdinia *et al.*, 2012). The sorbent can be simply prepared by coating of silica on magnetic nanoparticles and follow by polypyrrole polymer. The polypyrrole/SiO_2/Fe_3O_4 sorbent was then applied to extract sulfonamides such as sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX) by high performance liquid chromatography (HPLC). To obtain the best extraction efficiency, affecting parameters were optimized, i.e., amount of sorbents, extraction time, sample pH, effect of ionic strength, type and volume of the eluting solvent. The aim is to provide a reliable, simple, highly sensitive and environmental friendly method for the monitoring of sulfonamides in environmental water samples such as tap, canal and lake waters.

1.2 Chemical properties of sulfonamides

Sulfonamides are white or slightly yellowish, odorless powders, and some have a bitter taste. Most of these substances are poorly soluble in water. The solubility of sulfonamides in acids and alkali is conditioned by their amphoteric properties, which are due to the presence of the basic aromatic amino group (pK_{a1} 2-2.5) and the amide group, which contains a labile hydrogen atom with acidic properties (pK_{a2} 5-8). The acidic properties of sulfonamides are more pronounced than their basic properties, and thus sulfonamides are positive charged in acidic medium at pH <2, neutral at pH 3-5, and negatively charged at pH >5 (Kümmerer, 2009).

1.3 Toxicity of sulfonamides

Sulfonamides have the potential to cause a variety of untoward reactions, including commonly in the form of allergic reactions, disbacteriosis, suppression of enzyme activity, alteration of the intestinal microflora, and promotion of sustainable forms of pathogens (Dmitrienko *et al.*, 2014), urinary tract disorders, haemopoietic disorders, porphyria and hypersensitivity reactions. When it was used in large doses, it may cause a strong allergic reaction. Two of the most serious are Stevens-Johnson syndrome and toxic epidermal necrolysis (Lyell syndrome)

1.4 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) has been widely used for the quantification of sulfonamides due to its high sensitivity and good precision. (Sun *et al.*, 2009; Ramos Payán *et al.*, 2011; Shi *et al.*, 2011; Sun *et al.*, 2014; Yan *et al.*, 2014). HPLC is a separation technique that can be used for the analysis of organic molecules and ions which is based on mechanisms of adsorption, partition and ion exchange, depending on the type of stationary phase used which involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Reversed-phase HPLC is generally employed for the separation and determination of sulfonamides. This type of chromatography is performed on a

nonpolar stationary phase with a polar mobile phase. In RP-HPLC the separation is based on the partition of the analyte between the stationary phase and the mobile phase. It is frequently practiced using a bonded phase on silica, with the bonded moiety (ligand) being a hydrocarbon chain (e.g., C8, C18). The mobile phase in RP-HPLC is typically a partially aqueous, partially organic solvent and is more polar than the stationary phase. The solute molecules are in equilibrium between the hydrophobic stationary phase and partially polar mobile phase, the direction of the equilibrium determining a stronger or a weaker retention of the analytes (Moldoveanu and David, 2013c). A typical HPLC system (**Fig.1.2**) consists of the mobile phase container, a pump to carry on the mobile phase and sample through the system, an injector or autosampler to allow sample introduction, a column for separation of analytes, a detector to detect the separated analytes, and a data processor to assist in interpretation results. To obtain the best performance, *i.e.*, high sensitivity, good separation, and short analysis time, some parameters need to be study such as mobile phase composition, stationary phase, types and wavelength of detector.

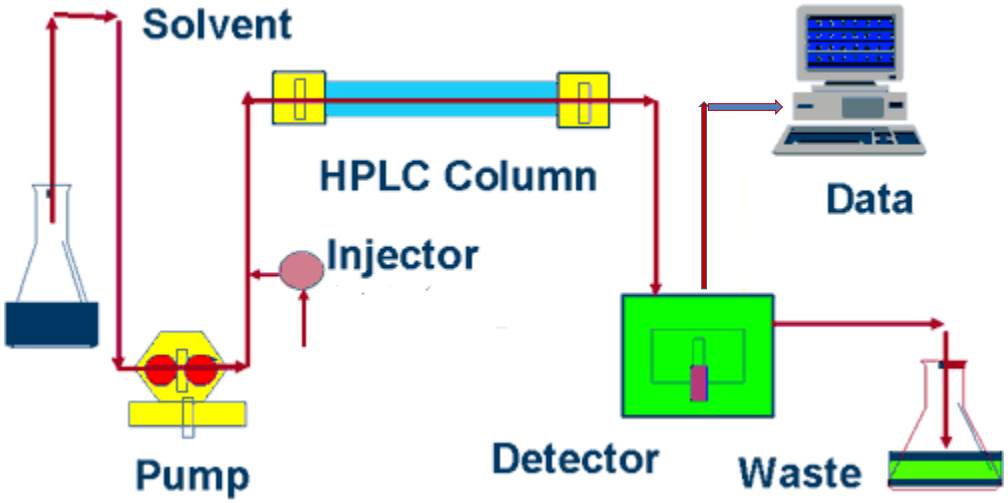


Fig. 1.2 A schematic of an HPLC system

1.4.1 Mobile phase

The mobile phase is one of critical step for effecting a successful separation. The solvents are selected depending on the type of HPLC, the nature of the analytes, the choice of the stationary phase, and also the type of detection used for the analyte measurement. The interaction in RP-HPLC is considered to be the hydrophobic forces. This force is caused by the energies resulting from the disturbance of the dipolar structure of the solvent (Moldoveanu and David, 2013b). Sulfonamide is ampholytic compound which could have a tailing peak in reversed-phase chromatography. The tailing in reversed phase liquid chromatography is caused by the residue silanol groups and metal impurities in traditional phase column materials. This behavior can be reduced by mean of mobile phase with high acidity and ionic strength. The most of solvents of mobile phase which have been used for the determination of sulfonamides, it consisted of 0.3% formic acid and acetronitrile (Herrera-Herrera *et al.*, 2013a), acetic acid (1%) and methanol (Sun *et al.*, 2009), formic acid (0.1%) and formic acid (0.1%) in acetonitrile (Ibarra *et al.*, 2014), acetic acid (0.5%) and acetonitrile (Malintan and Mohd, 2006), acetic acid (1%) and acetonitrile (Shi *et al.*, 2011). In this work, the mobile phase was a mixture of 0.20% aqueous acetic acid and acetonitrile 70:30 (V/V). The flow rate was set 0.70 mL min^{-1} with isocratic condition.

1.4.2 Stationary phase

The stationary phase is a powerful tool for the separation of constituents of complex mixtures, and its selection is critical for the success of the analysis which are being carried through the system by the mobile phase. The retention of the analyte on the stationary phase is dependent on the contact surface area between the nonpolar moiety of the analyte molecule and the stationary phase, both immersed in the aqueous eluent. For this reason an analyte with a larger hydrophobic surface area is more retained on the stationary phase, resulting in longer retention time compared with an analyte with a smaller hydrophobic surface (Moldoveanu and David, 2013a). The stationary phases are widely used such as C18, C8, phenyl, cyano, and perhaps

other functionalities. Different commercial C18 columns also often show different separation selectivities, usually based on differences in silica supports or stationary phase (Kirkland, 2004). In this study, a reversed phase VertiSep^{TM} pH endure C_{18} column (5 µm particles size, 250 mm x 4.6 mm I.D.) was used for the separation of sulfonamides.

1.4.3 Detectors

The detection is based on the fact that the molecules of the sample have physicochemical properties different from those of the mobile phase. The measured properties are determined by the nature of the compound to be analyzed and that of the mobile phase. The choice of a specific property for detection depends on factors such as the extent of difference in a property from that of the mobile phase, sensitivity of the detector to the specific property, and availability of the detector (Moldoveanu and David, 2013b). The analyte detection can be performed using a variety of detectors, some of which provide qualitative information for the compound. There are many types of detector used for the quantification of sulfonamides, *i.e.,* ultraviolet-visible (UV-vis) (Sun *et al.*, 2009; Tao *et al.*, 2009; Huang *et al.*, 2012; Yan *et al.*, 2014), diode-array detector (DAD) (Hela *et al.*, 2003; Yu *et al.*, 2012; Lian *et al.*, 2014), fluorescence (FLD) (Raich-Montiu *et al.*, 2007; Arroyo-Manzanares *et al.*, 2014) and mass spectrometer (MS) (Gao *et al.*, 2010; Xu *et al.*, 2013; Zhao *et al.*, 2014a; Kung *et al.*, 2015).

Ultraviolet-visible (UV-vis) detector is often considered to be one of the most widespread detection techniques combined with liquid chromatography. UV-vis detector is based on compounds selectively absorbing radiation in a concentration-dependent (Beer-lambert law) manner. A basic system consists of a radiation source, wavelength selector, and detector to measure the absorbance of the eluent passing through a flow cell (Shackman, 2013). When working in this mode, the mobile phase has a distinct absorptivity and the peaks are negative (lower absorptivity). The detector and its data processing unit must be able to handle positive and negative

signals. An optical system with high energy and a large dynamic measurement range are necessary.

Diode-array detector (DAD) is commonly used to record the ultraviolet and visible (UV-vis) absorption spectra of samples that are passing through a high-pressure liquid chromatograph. This enables qualitative information to be gathered about the samples. An advantage to diode array detectors is the ability to select the best wavelength for analysis. The most flexible design uses photodiode array detection with the full continuum of radiation passing through the flow cell followed by a fixed grating that spectrally resolves the emission onto an array of multiple detection units (Shackman, 2013). Some features to consider when choosing a diode array detector include resolution, wavelength range, low noise, baseline stability, peak integration and an interchangeable flow cell design.

Fluorescence detector (FLD) is a highly selective and sensitive detection of either natively fluorescent or derivatizable analytes. The selectivity offers the ability to isolate detectable compounds from relatively complicated matrices, reducing the burden on the separation itself. Sensitivity can be one to several orders of magnitude better than absorbance detectors when using lamp-based radiation sources. (Shackman, 2013). The FLD is based on the principle that some compounds fluoresce when bombarded with UV light. The fluorescence process is consist of three stages; (i) Formation of one or more excited state(s) by absorption (excitation), (ii) non-radiative transitions between excited states and (iii) energy loss accompanied by emission of radiation (White and Errington, 2005). If the compound of interest fluoresces this is a very sensitive detector. The analyte is excited by light commonly at 253.7 nm from a low pressure mercury lamp. The light is absorbed, the molecule is excited, and it then gives off light of a different wavelength. This wavelength is monitored by a detector which sits at right angles to the UV light source used to excite the analyte. Analytes that are present at trace levels are commonly derivatized so that they can be detected by fluorescence.

1.5 Sample preparations

Sample preparation is a key step prior to detection of analytes in various matrices. It must be performed in order to extract analytes from different matrices, to eliminate interfering effects of associated components and to reduce the detection limit (Dmitrienko *et al.*, 2014). Sample preparation can be achieved by employing a wide range of techniques, but all methods have the same goal to remove potential interference, increase the concentration of an analyte, convert an anlyte into a more suitable form, and provide a robust, reproducible method that is independent of variations in the sample matrix (Pavlović *et al.*, 2007)**.** An appropriate sample preparation needs to minimize procedure step, reduces analysis time, and enhances extraction efficiency, sensitivity and selectivity. The selective extraction of analytes is based on differences in their chemical and physical properties. These typically include molecular weight, charge, solubility (hydrophobicity), polarity, or differences in volatility. The sample preparation techniques for the extraction and preconcentration of sulfonamides are dispersive-liquid-liquid microextraction (DLLME), solid phase microextraction (SPME), solid phase extraction (SPE) and magnetic solid phase extraction (MSPE). A summary of sample preparation techniques for the determination of sulfonamides are shown in **Table 1.1**.

Table 1.1 Sample preparation techniques for the determination of sulfonamides

Extraction Method	Sample	Extraction time (min)	Sample volume (mL)	Extractants	LOD ($\mu g\ L^{-1}$)	Recovery (%)	References
Dispersive liquid-liquid microextraction	water	3	5	Chloroform	0.41-9.87	78-117	(Herrera-Herrera *et al.*, 2013b)
Liquid-liquid-liquid microextraction	water	45	12	Organic solvent	0.11-0.77	86-109	(Lin and Huang, 2008)
Solid phase microextraction	meat	40	15	PDMS/DVB,	16-39	-	(Lu *et al.*, 2007)
Solid phase microextraction	waste water	20	25	CW/DVB	9.04-55.3	29-112	(Balakrishnan *et al.*, 2006)
Micro-solid phase extraction (Molecularly imprinted polymer)	water	20	4.5	MIP sorbent	0.2-3.0	70-120	(Díaz-Álvarez *et al.*, 2014)
Micro-solid phase extraction	milk	20	10	polypropylene	4.52-10.63	72-84	(Huang *et al.*, 2012)
In-line solid phase extraction	water	15	4.75	HLB particles	0.38-0.56	52-109	(Lara *et al.*, 2009)
Dispersive solid phase extraction	pork	1	20	MWCNTs	103-116	62-83	(Hou *et al.*, 2013)

Table 1.1 Sample preparation techniques for the determination of sulfonamides (Continued)

Extraction Method	Sample	Extraction time (min)	Sample volume (mL)	Extractants	LOD ($\mu g\ L^{-1}$)	Recovery (%)	References
Solid phase extraction	water	25	250	HLB cartridges	0.15-1.0	70-104	(Raich-Montiu *et al.*, 2007)
Solid phase extraction (Molecularly imprinted polymer)	shrimp, fish	5	5	MIP/SPE	8.4-10.9	85.5-106	(Shi *et al.*, 2011)
Stir bar sorptive extraction	milk	10	4	C_{18}	0.9-10.5	68-120	(Yu and Hu, 2012)
Magnetic solid phase extraction	water	20	1	Fe_3O_4/Graphene oxide	50-100	67-120	(Shi and Ye, 2014)
Magnetic solid phase extraction	water	1	5	Fe_3O_4/SiO_2/ graphene	0.09-0.16	74-104	(Luo *et al.*, 2011)
Magnetic solid phase extraction	milk	15	10	Fe_3O_4/SiO_2/ phenyl	7-14	82-115	(Ibarra *et al.*, 2014)
Magnetic solid phase extraction	water	15	500	Magnetic mixed hemicelles/SPE	0.024-0.033	70-102	(Sun *et al.*, 2009)

1.5.1 Liquid liquid extraction (LLE)

LLE is based on a transfer of analyte from the aqueous sample to a water-immiscible solvent (Nováková and Vlčková, 2009). It is involved the relative solubility of analyte in two immiscible phases and governed by the equilibrium distribution or partition coefficient. Extraction of an analyte is achieved by the differences in solubilizing power (polarity) of the two immiscible liquid phases (Ridgway *et al.*, 2007). However, this technique has some disadvantages, i.e., use of large sample volumes and toxic organic solvents, time-consuming, environmentally harmful, unsuitability for hydrophilic compounds (Nováková and Vlčková, 2009), matrix interferences and emulsion formation. For the extraction of sulfonamides, $CHCl_3$ was used as extraction solvent and acetonitrile was used as disperser (Herrera-Herrera *et al.*, 2013b).

1.5.2 Solid phase extraction (SPE)

SPE involves a liquid–solid partition, where the extracting phase is a solid sorbent and it has been used extensively to remove and concentrate trace organic compounds from liquid samples. The key factor in SPE is the choice of sorbent, which controls selectivity, affinity and the capacity of SPE. A choice of sorbents are available using different mechanisms for extraction or retention of analytes (Ridgway *et al.*, 2007). The use of newly synthesized solid materials as sorbents in SPE is a clear trend. All of them promise better selectivity and efficiency towards the target analytes. SPE has become the most popular sample preparation method and was commonly used for a wide range of compounds prior to LC analysis. It has many advantages compared with LLE, such as high preconcentration factor, low consumption of organic solvents and easy to operate (Li *et al.*, 2007). Using SPE, the separation occurs based on the analyte partition coefficient between the mobile phase and the solid sorbent. The solid phase retains the analyte during removal of the aqueous phase and subsequent washing to remove various components. Analyte was then be desorbed by solvent or thermal desorption (Atapattu and Rosenfeld, 2013).
SPE procedures are normally consist of four steps as shown in **Fig. 1.3.**

Conditioning: The sorbent was conditioned with a suitable solvent to improve the reproducibility of the analytes retention and to reduce the impurities on the surface of sorbents (Poole *et al.*, 2000).

Sample loading: The sample is added to the cartridge with strong retention of analyte. The sample should be passed through the cartridge without allowing the cartridge to dry out.

Washing: The sorbent was washed with a solvent of intermediate strength for the removal of interferences that are more weakly retained than the analyte. A small volume of organic solvent may be added to the wash solution to aid in the removal of more hydrophobic interferences, but care must be taken that the analyte of interest is not removed at the same time.

Eluting: The retained of analyte on the solid phase was removed with a suitable solvent that greater affinity for the analytes.

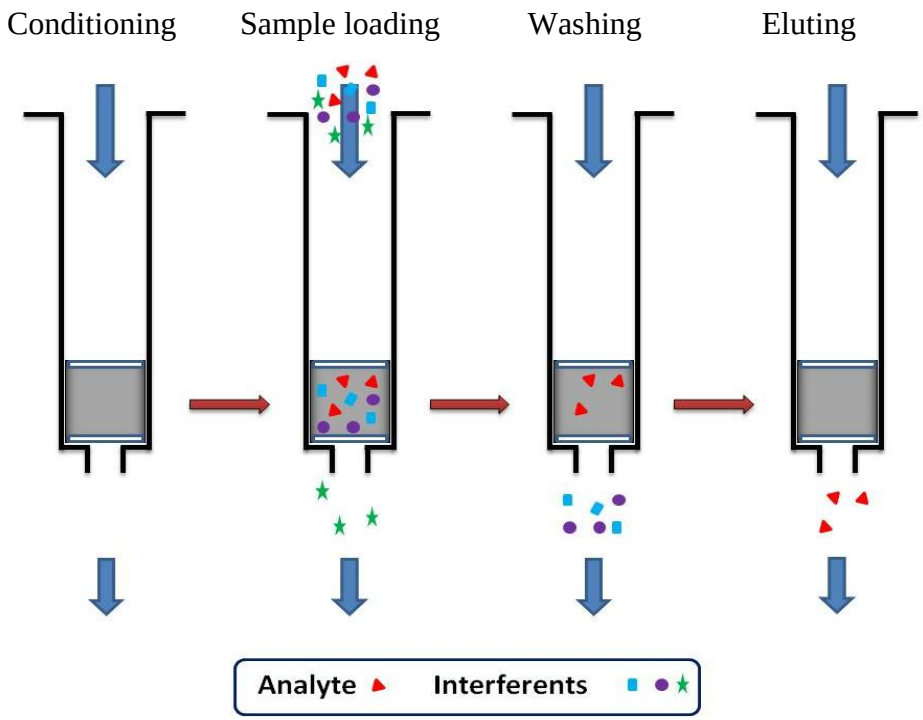


Fig 1.3 Schematic representative the SPE procedure for the extraction of analyte

There are many variations of this technique due to the extraction process, the shape and size of the sorbent bed, and the type of absorbent material. There are different types of solid phase adsorbents have been reported for the extraction of sulfonamides such as OASIS hydrophilic lipophilic balanced (HLB) cartridges (Koesukwiwat *et al.*, 2007; Pailler *et al.*, 2009), C18-coated stir bar (Yu and Hu, 2012) and molecularly imprinted polymer (MIP) (Shi *et al.*, 2011; Díaz-Álvarez *et al.*, 2014).

1.5.3 Solid phase microextraction (SPME)

SPME is based on the partition equilibrium of the analytes between the liquid or gaseous sample and a thin layer of adsorbent material, which is generally coated onto the fused silica fiber. The coating material of the SPME fiber plays a key role for the extraction since it affects the partitioning of the analytes between the coating and sample matrix, and consequently affects the extraction efficiency of the SPME method. An ideal fiber coating should have high extraction efficiency, high thermal and solvent stability, long life time and strong adhesion between the coating and a fused silica fiber (Li *et al*., 2014). In addition, SPME is potentially more cost-effective than SPE since an individual fiber can be used for multiple extractions and very little solvent is required for the overall process (McClure and Wong, 2007). There are different types of polymeric material were used as an adsorbent layer for the extraction of sulfonamides such as polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Lu *et al.*, 2007), carboxywax/divinylbenzene (CW/DVB) (Balakrishnan *et al.*, 2006).

1.5.4 Magnetic solid phase extraction (MSPE)

MSPE is a simple and reproducible method that can be a promising alternative to standard SPE and LLE. Due to the magnetic nature of adsorbents and the selectivity of magnetic separations, the technique can also be used for the preconcentration of analytes from samples containing suspended solids. The magnetic adsorbents in sample preparations provide a faster and easy way for the removal of

the adsorbents from sample matrix, as the phase separation can be conveniently realized by an external magnetic field (Xu *et al.*, 2013). It avoided the time-consuming column-passing process of loading large volume samples in traditional SPE. In MSPE, magnetic sorbent is added to the solution or suspension containing the target analyte. The analyte is adsorbed onto the magnetic sorbent whether under stirring or standing. The sorbent with captured analyte is then recovered from the suspension using an appropriate magnetic separator. The analyte is consequently eluted from the sorbent and analyzed (Chen *et al.*, 2011b). The separation mechanism using magnetic nanoparticles depends on the type of sorbent, and is connected with the interaction of analyte molecules with the surface functional groups of the bed, as in classical extraction in the solid phase. The following types of interactions have been recognized : ionic, dipole-dipole, dipole-induced dipole, hydrogen bonding and dispersion forces. In a reversed phase (RP) system, where the surface of the sorbent possesses a weak polar or non-polar nature, interactions are mostly hydrophobic and van der Waals. In a normal-phase (NP) system, the bed surface possesses a polar nature, which causes polar analytes and analytes having polar fragments to undergo sorption on the surface of the sorbent. In this case, the retention mechanism of analytes is based on hydrogen bonding, dipole-dipole interactions and π-π interactions. More stable interactions, such as chemical bonding, are not used in practice for separation because of their irreversibility (Wierucka and Biziuk, 2014). The application of MSPE for different analytes are as shown in **Table 1.2.**

Table 1.2 Application of magnetic solid phase extraction

Analyte	Sample	Magnetic material coating	Analytical method	LOD ($\mu g\ L^{-1}$)	Recovery (%)	References
B-agonists	Pork, pig liver	MMIP	HPLC-FLD	0.52-1.04	82-90 , 80.4-86.8	(Hu *et al.*, 2011)
macrolide	foodstuff	MMIP	HPLC-UV	0.015-0.2 $\mu g\ g^{-1}$	82.5-111.8	(Zhou *et al.*, 2015)
nitrophenol	water	Fe_3O_4/PPy NPs	HPLC-UV	0.3-0.4	84-109	(Tahmasebi *et al.*, 2013b)
PAHs	water	Fe_3O_4/C	HPLC-FLD	0.15-0.91 ng L^{-1}	79.5-110	(Yang *et al.*, 2014)
PAHs	water	Fe_3O_4/MWCNTs/ alginate	HPLC-FLD	5-10 ng L^{-1}	92.0-97.7	(Bunkoed and Kanatharana, 2015)
pyrethroids	tea drinks	Fe_3O_4/C/PANI	HFLC-UV	0.025-0.032	72.1-118.4	(Wang *et al.*, 2014b)

Table 1.2 Application of magnetic solid phase extraction (Continued)

Analyte	Sample	Magnetic material coating	Analytical method	LOD ($\mu g\ L^{-1}$)	Recovery (%)	References
bisphenol A	water	Fe_3O_4/C/CHI	HPLC-FLD	1.92	99.4-102.6	(Geng *et al.*, 2012)
PAHs	water	Graphene/Fe_3O_4/ polythiophene	GC/MS	0.009-0.020	83-107	(Mehdinia *et al.*, 2015)
sulfonamides	milk	Fe_3O_4/SiO_2/P(MAA-co-EGDMA)	LC/MS/MS	0.5-49.5 $ng\ L^{-1}$	87.6-115.6	(Gao *et al.*, 2010)
sulfonamides	milk	Fe_3O_4/SiO_2/phenyl	HPLC-DAD	7-14	81.88-114.98	(Ibarra *et al.*, 2014)

1.6 Materials for MSPE sorbent

Magnetic nanoparticles is one of the most exciting prospects in current analytical nanotechnology because they can be easily separated from a matrix by using a magnetic field without retaining residual magnetism after removal of the field. The preparation of a magnetic material usually involves three steps, including the synthesis of magnetic particle, the coating of the magnetic core and the modification of the resultant core-shell structure (Giakisikli and Anthemidis, 2013). Functionalized magnetic nanoparticles have distinct advantages over conventional adsorbents because of their selective adsorptivity, favourable water dispersibility, and biocompatibility (Bao *et al.*, 2014). The effectiveness of the materials depends on the characterization of superparamagnetic and have a high magnetic saturation value, which means analyte-loaded sorbent can easily be separated from the sample solutions via an external magnetic field, the small particles and usually on the nanoscale which have large surface area, high adsorption capacity and rapid adsorption rate, low amount of sorbent and short equilibrium time, specific functionalities that can be selective for the analyte, can be recycled (Chen *et al.*, 2011b). MNPs are alternative to conventional SPE due to their small particle size, characterized by a high specific surface area and sorption capacity, and high selectivity regarding analytes with shorten the duration of the extraction process - a small amount of sorbent. The advantages of MNPs such as it can be produced in large quantity using a simple method with a relatively low cost compared with the commercial SPE adsorbents of C18, and C8, their adsorption capacity is expected to be high by considering their large surface area, they have strong magnetic properties and high conducting capacity, and they have convenient and rapid collection of analyte from adsorbent surface using of magnet elution, which avoids time-consuming column passing or filtration (Li *et al.*, 2012). The utilization of iron oxide nanomaterials has received much attention due to their unique properties, such as extremely small size, high surface-area-to-volume ratio, surface modifiability, excellent magnetic properties and great biocompartibility (Xu *et al.*, 2012). Magnetic nanoparticles are prepared by a chemical co - precipitation method (Lee *et al.*, 2012). The method was involved the dissolution of a mixture of a solution of $FeCl_3 \cdot 6H_2O$

and $FeCl_2 \cdot 4H_2O$ in deionized water. The resulting solution was vigorously stirred at 80 °C and ammonium hydroxide was then added dropwise into the solution and it was vigorously stirred for 1 hour under nitrogen atmosphere. Then, the black magnetic particles were separated using a magnet and washed with 100 mL of water three times, and then dried at 60 °C in an oven for 24 h. The chemical reaction of Fe_3O_4 precipitation is expected as follows (Kim *et al.*, 2001; Jiang *et al.*, 2014):

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4\,(\text{black colloidal particles}) + 4H_2O \quad (1.1)$$

The shape, size and quality of the magnetic particles is extremely sensitive to synthesis conditions such as reaction time, temperature, reactant concentration, type and amount of catalyst, quality of solvent, etc. A commercially available magnetic beads usually feature a spherical morphology and a nano size of particles that may be functionalized with different active groups (Aguilar-Arteaga *et al.*, 2010). Magnetic nanoparticle are generally coated with polymers, surfactants, inorganic materials or low molecular weight chelating agents (Borlido *et al.*, 2013). It has been successfully applied in many fields, i.e., biosensor application (Zacco *et al.*, 2007; Li *et al.*, 2015; Zhang *et al.*, 2015), food analysis (Chen *et al.*, 2015; Ebrahimzadeh *et al.*, 2015; Makkliang *et al.*, 2015; Su *et al.*, 2015) medical application (Hałupka-Bryl *et al.*, 2015; Müller *et al.*, 2015), environmental contamination (Bunkoed and Kanatharana, 2015) and antibiotic residues (Gao *et al.*, 2012; Niu *et al.*, 2012; Ma *et al.*, 2014).

1.6.1 Silica coated magnetic nanoparticles

Silica is often used as a core material for the fabrication of core shell nanostructures, mainly due to its unique properties. Spherical silica particles, with diameters ranging from 5 nm to a few microns, suitable for use in the synthesis of core-shell nanostructures, it can be prepared by using various methods. Silica functionalization of magnetic materials is another widespread method of modification because it offers several advantages such as it can be easily coated onto the surface of iron oxide through the sol-gel process, can be easily modified with various silane-

coupling agents or other compounds (e.g., titania and zirconia), are more stable than bare iron oxides and silica can protect iron oxides from leaching under acidic conditions. Finally, silica can also provide a chemically-inert surface for application in biological systems and prevent iron oxides from agglomerating (Li *et al.*, 2013).

Stober method is the most well-known technique which was applied through a sol - gel reaction, which involved the use of alkoxy silane (mainly tetrathoxysilane, TEOS) in basic media to form Fe_3O_4/SiO_2 (Lee *et al.*, 2012). This method is relatively simple, effective and works well for larger silica particles with diameters of hundreds nanometer to a few microns. The Stober method involves mixing of ethanol and ammonia (catalyst) often with a small amount of water followed by quick or slow stepwise addition of TEOS, ($Si(C_2H_5O)_4$), under continuous stirring. During synthesis of silica particles, TEOS undergoes hydrolysis and condensation to form silica framework, which can represented by the following reactions (Jankiewicz *et al.*, 2012)

$$Si(C_2H_5O)_4 + 4\,H_2O \longrightarrow Si(OH)_4 + 4C_2H_5OH \qquad (1.2)$$

$$Si(OH)_4 \longrightarrow SiO_2 + 2H_2O \qquad (1.3)$$

The use of silica as a coating layer to the magnetite nanoparticles not only in enhancing the advantages of their high biocompatibility, hydrophilicity, dielectric property and stability against degradation but also facilitates easy surface modification due to the availability of abundant silanol group (-SiOH) on the surface (Abbas *et al.*, 2014). The Fe_3O_4/SiO_2 has been developed by using modified polyol process in one step of synthesis. The product showed excellent stability against oxidation annealed at 600 ^oC in presence of hydrogen gas (Abbas *et al.*, 2014). Silica coated magnetic nanoparticles have been applied in many applications as shown in **Table 1.3.**

Table 1.3 Application of silica coated magnetic nanoparticles

Analyte	Sample	Sorbent	References
Heavy metal	Waste water	EDTA-chitosan/SiO_2/Fe_3O_4	(Ren *et al.*, 2013)
Cu(II) , Pb(II)	Water samples	Fe_3O_4/SiO_2/Triethylenetetramine	(Mahmoud *et al.*, 2013)
U(VI)	Waste water	Fe_3O_4/SiO_2/Amidoxime	(Zhao *et al.*, 2014b)
Flavonoids	Urine samples	Hemimicells/Fe_3O_4/SiO_2	(He *et al.*, 2014)
sildenafil and its metabolite	biological samples	Methylcellulose coated-Fe_3O_4/SiO_2/phenyl	(Tang *et al.*, 2014)
sulfonamides	environmental water samples	Fe_3O_4/ SiO_2/graphene	(Luo *et al.*, 2011)
sulfonamides	poultry feed	Fe_3O_4/SiO_2/MIP	(Kong *et al.*, 2012)
sulfonamides	milk samples	Fe_3O_4/SiO_2/phenyl	(Ibarra *et al.*, 2014)

1.6.2 Polymer modified magnetic nanoparticles

Magnetic nanoparticles coated with polymer have attracted tremendous interest, especially conducting polymer. There are many typed of polymer have been used as a coating materials on the magnetic nanoparticles, such as polyaniline (Asgharinezhad *et al.*, 2014; Su *et al.*, 2014; Wang *et al.*, 2014b), polystyrene (Jainae *et al.*, 2010), polythiophene (Tahmasebi *et al.*, 2013a; Mehdinia *et al.*, 2015) and polypyrrole (Bhaumik *et al.*, 2013; Bagheri *et al.*, 2014). Among these polymer materials, polypyrrole (**Fig. 1.4**) is one of the most widely used that contributes in many different extraction mechanisms including π-π interactions, polar interactions, anion-exchange and hydrogen bonding.. It becomes one of the most important conducting polymers due to its appreciable environmental stability, easier synthesis, solubility in different solvents (Chakraborty *et al.*, 2012). It was used as an excellent sorbent material for the extraction of a wide variety of organic compounds, i.e., pesticide (Ahmadi *et al.*, 2008; Mollahosseini and Noroozian, 2009; Chen *et al.*, 2011a; Jafari *et al.*, 2014) naproxen (Bagheri *et al.*, 2014), mycotoxin (Yu and Lai, 2007), auxins (Wang *et al.*, 2014a), nitrophenols (Tahmasebi *et al.*, 2013b), phthalate ester (Asadollahzadeh *et al.*, 2010), metal (Bhaumik *et al.*, 2013; Sahmetlioglu *et al.*, 2014) and antibiotics (Szultka *et al.*, 2010; Liu *et al.*, 2012; Mazzotta *et al.*, 2012).

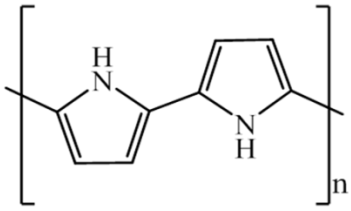


Fig. 1.4 The molecular structure of polypyrrole

1.7 Objective of the research

To develop a new magnetic solid phase extraction sorbent of polypyrrole/SiO_2/Fe_3O_4 for the extraction and preconcentration of sulfonamides from water samples.

1.8 Benefits

It is expected to obtain a new sorbent that has a great potential for the extraction of sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX) in water samples with the advantages of easy to use, inexpensive and reusable.

CHAPTER 2

Results and discussion

2.1 Synthesis of polypyrrole/SiO_2/Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were first prepared by a chemical co-precipitation method (Han *et al.*, 2012). Briefly, 4.70 g of $FeCl_3$•$6H_2O$ and 1.70 g of $FeCl_2$•$4H_2O$ were dissolved in 80 mL of deionized water. Under vigorous stirring at 80^oC, 10 mL of ammonium hydroxide (30% v/v) was added dropwise into the solution over a period of 5 min and the stirring was continued for another 55 min during which time the magnetic particles were formed. The particles were then separated using a magnet, washed three times with 100 mL of water, and then dried at 60^oC in an oven for 24 h.

For SiO_2 coating, 2.0 g of Fe_3O_4 nanoparticles were added into a solution containing 100 mL of ethanol, 50 mL of deionized water and 2.0 mL of ammonium hydroxide (30% v/v) and sonicated for 15 min., 2.0 mL of tetraethyl orthosilicate (TEOS) was added dropwise into the solution while stirring at 300 rpm, 40 ^oC, after which the solution was heated for 12 h. The SiO_2/Fe_3O_4 particles were collected by a magnet, washed with 20 mL of methanol followed by 20 mL of deionized water, respectively, and dried at 60 ^oC in an oven for 6 h.

In the next step, the SiO_2/Fe_3O_4 particles were washed with 5.0 mL of 2-propanol before being incubated in 5.0 mL of pyrrole monomer for 1 h then washed twice with 10 mL of 2-propanol to remove the pyrrole monomer residue. To polymerize the pyrrole, 0.64 g of $FeCl_3$ (oxidant) was dissolved in 20 mL of 2-propanol and added into the pyrrole/SiO_2/Fe_3O_4 in a rotator tube. The polymerization was completed on a rotator mixer for 9 h. The polypyrrole/SiO_2/Fe_3O_4 were then washed twice with 10 mL of 2-propanol, methanol and deionized water, respectively. The particles were dried at 60 ^oC in an oven for 6 h.

2.2 Optimization of HPLC conditions

To obtain the best performance (high sensitivity, good resolution, and short analysis time) a number of the operation conditions of the HPLC were optimized for the analysis of sulfonamides. The optimum absorbing wavelength of sulfonamides was first investigated. Then a mixed standard solution of four sulfonamides was analyzed at the optimum wavelengths (with a mobile phase flow rate of 0.70 mL min^{-1}, a 30 ^{o}C column temperature and a 20 µL sample volume) to obtain the optimum compositions of the mobile phase (% acetic acid and acetronitrile).

The optimum HPLC conditions for the determination of sulfonamides were found to be as follows. The mobile phase was a mixture of 0.20% acetic acid and acetronitrile (70:30 v/v). The optimum ratio of mobile phase was considered from the resolution (Rs = 1.70) that provided the acceptable values (Rs > 1.5) (Snyder and Kirkland, 1979). At the ratio of 0.20% acetic acid (80%) and acetronitrile (20%), although the resolution was acceptable (Rs= 2.0), sulfonamides were retained on the stationary phase longer due to the increasing of hydrophobic interaction. While the ratio of 0.20% acetic acid (60%) and acetronitrile (40%), It showed a poor resolution, Rs < 0.5, due to the mobile phase has more hydrophobic then it increased the interaction between analyte and mobile phase, thus, the retention time was become early. A flow rate of mobile phase was 0.70 mL min^{-1}. The optimum absorption wavelength was at 270 nm. These conditions provided a good peak separations of sulfadiazine, sulfamerazine, sulfamethazine and sulfamonomethoxine with retention times of 8.04, 9.63, 11.03 and 13.48 min, respectively (**Fig 2.1**).

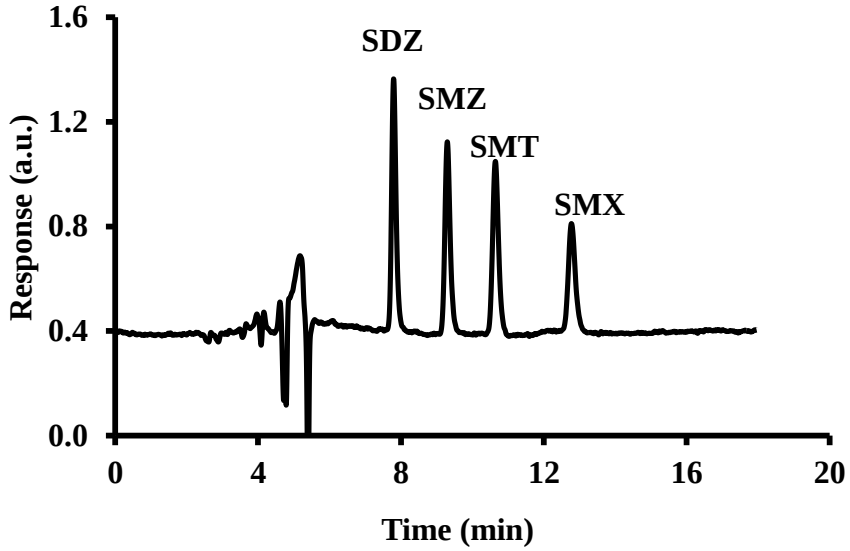
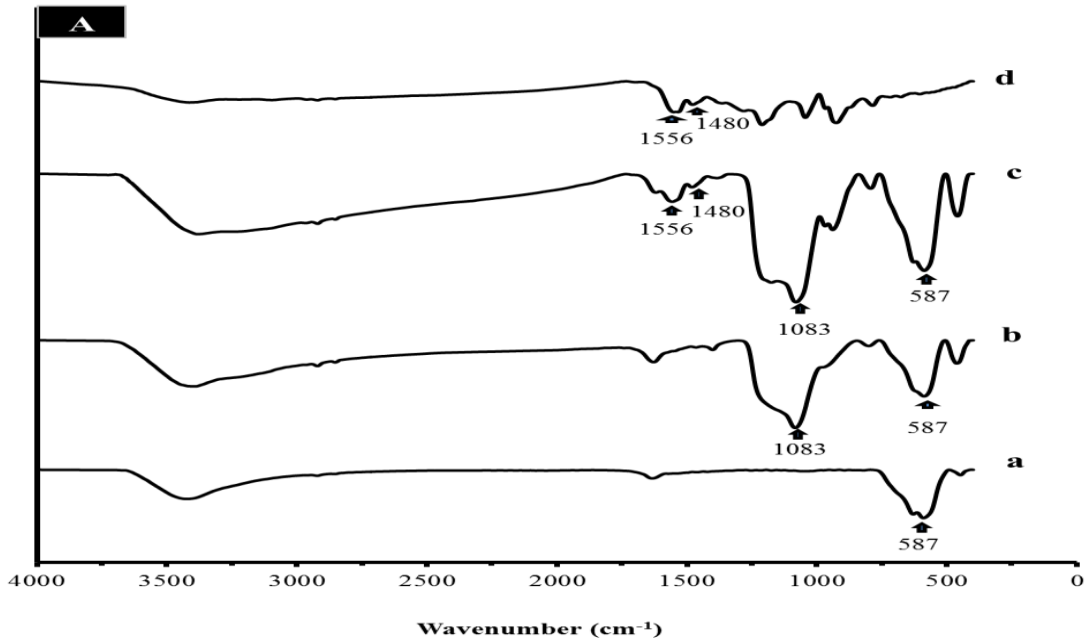
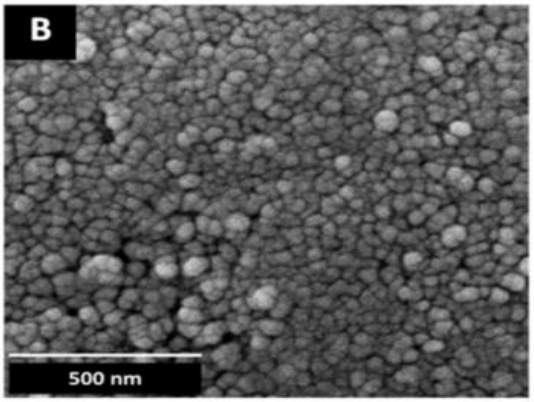


Fig 2.1 Chromatograms of sulfadiazine, sulfamerazine, sulfamethazine and sulfamonomethoxine (10 μg L^{-1})

2.3 Characterization of polypyrrole/SiO_2/Fe_3O_4 sorbent

The FT-IR spectra of the Fe_3O_4, SiO_2/Fe_3O_4 and polypyrrole/SiO_2/Fe_3O_4 nanoparticles are shown in **Fig. 2.2A**. It can be seen that the characteristic peak of Fe_3O_4 appeared at 587 cm^{-1} (Fe–O stretching). The presence of an absorption peak at 1083 cm^{-1} (Si-O-Si) indicated the formation of a silica coating on the Fe_3O_4 surface. The peaks of 1556 cm^{-1} and 1480 cm^{-1} in **Fig. 2.2A** (c and d),were related to the absorption of the pyrrole ring, and indicated the successful coating of the polypyrrole onto the surface of SiO_2/Fe_3O_4 nanoparticles. The SEM morphology of the polypyrrole/SiO_2/Fe_3O_4 (**Fig. 2.2B**) confirmed that the magnetic sorbent were fairly uniform in size and shape, with and average particle size of 70 $\pm$ 10 nm (n=100). **Fig. 2.2C** illustrates the dispersion and agglomeration processes of the polypyrrole/SiO_2/Fe_3O_4 nanoparticles. The homogeneously dispersed magnetic nanoparticle adhered to the side wall of the vials when the external magnetic field was applied, and the solution became transparent within 1 min.





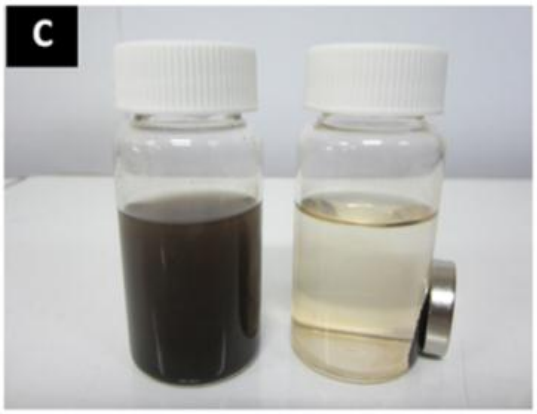


Fig. 2.2 (A) FT-IR spectra of Fe_3O_4 (a), SiO_2/Fe_3O_4 (b) , polypyrrole/SiO_2/Fe_3O_4 (c) and polypyrrole (d), (B) SEM image of polypyrrole/SiO_2/Fe_3O_4, (C) the dispersion (left) and separation (right) process of polypyrrole/SiO_2/Fe_3O_4 sorbent by an external magnet

2.4 Optimization of the magnetic solid phase extraction procedure

Polypyrrole/SiO_2/Fe_3O_4 nanoparticles were used as the MSPE sorbent in an extraction procedure. The initial conditions used in the extraction were as follows. After 100 mg of polypyrrole/SiO_2/Fe_3O_4 the nanoparticles were conditioned in a vial with 2.0 mL of methanol and deionized water, respectively, 5.0 mL of spiked water sample was added and stirred for 30 min. The sorbents, with bound sulfonamides, were then separated using a magnet and the solution was descanted. The analytes were desorbed from the sorbent with 5.0 mL of methanol by sonication for 30 min. The desorption solvent was then evaporated to dryness at 50^{o}C, redissolved in 0.5 mL of methanol and filtered through a PTFE filter (0.22 μm) for further HPLC analysis.

To obtain the maximal extraction efficiency, the main affecting parameters were optimized by varying one parameter at a time, while the others were kept constant. These included the amount of the sorbent, sample pH, extraction time, desorption conditions and effect of the ionic strength. Each experiment was done in triplicate. The extraction efficiency was evaluated in terms of recovery. The optimizations were performed using 5.0 mL of water sample (known to be free of sulfonamides) spiked with a standard solution to obtain a final concentration of 20 μg L^{-1} of each sulfonamide that could be detected by the DAD. When an optimal condition of a parameter was obtained it was used for the optimization of the next parameter.

2.4.1 Type of sorbent

The extraction capabilities of the magnetic (Fe_3O_4), silica coated magnetic (SiO_2/Fe_3O_4) and polypyrrole coated silica-magnetic nanoparticles (polypyrrole/SiO_2/Fe_3O_4) are shown in **Table 2.1** and **Fig. 2.3.** The recoveries of the sulfonamides significantly increased in the presence of polypyrrole, and indicated that the polypyrrole had a significant influence on the extraction process because it can adsorbes sulfonamide via the π-π interaction.

Table 2.1 Recoveries of sulfonamides (20 µg L^{-1}) at various type of sorbent (n=3)

Type of sorbent	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
Fe_3O_4	8.3±2.3	10.51±0.65	6.07±0.61	3.86±0.43
SiO_2/Fe_3O_4	7.78±0.73	14.0±1.4	4.7±1.1	5.6±1.1
Polypyrrole/SiO_2/Fe_3O_4	88.5±4.1	86.1±4.1	85.1±3.1	90.7±4.8

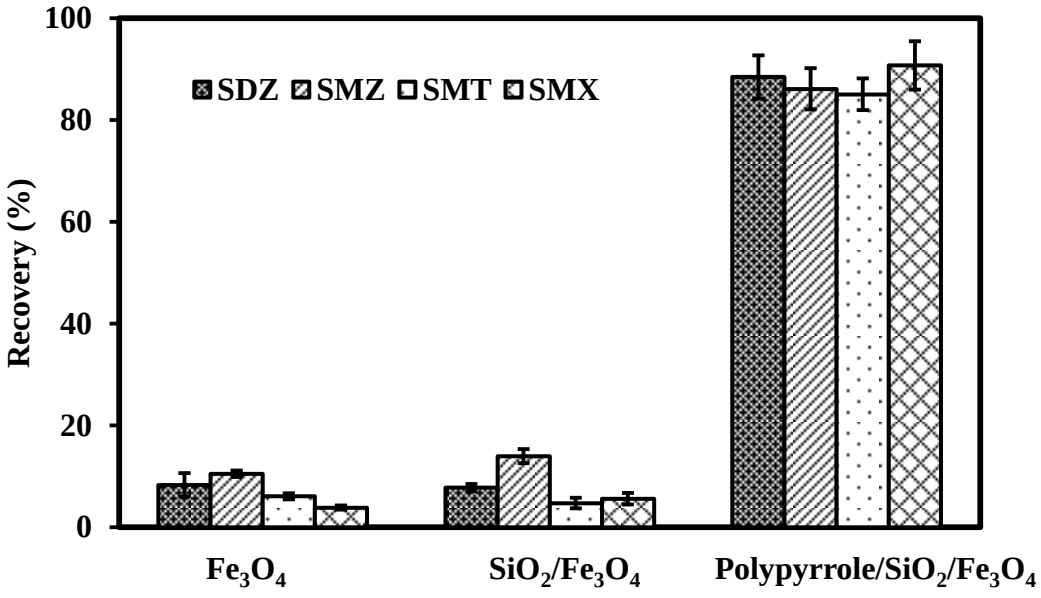


Fig. 2.3 Effect of type of sorbents on the recoveries of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.2 Amount of polypyrrole/SiO_2/Fe_3O_4 sorbent

The effect of the amount of polypyrrole/SiO_2/Fe_3O_4 was investigated over the range of 10, 20, 40, 60, 80 and 100 mg. The amount of sorbent that provided the highest adsorption efficiency was selected. The recoveries of sulfonamides at various amount of polypyrrole/SiO_2/Fe_3O_4 sorbent were shown in **Table 2.2** and **Fig. 2.4.** The recoveries increased with the sorbent amount from 10 to 20 mg, and then remained constant. That is, 20 mg of sorbent was sufficient for the extraction of sulfonamides and was selected for subsequent experiments. This is much less than a traditional C18 SPE cartridge, using ca. 50–100 mg of sorbents, The result indicated that the developed polypyrrole/SiO_2/Fe_3O_4 nanoparticle sorbent had a high adsorption efficiency, and a satisfactory extraction efficiency was achieved by using a much lower amount of sorbent than any previous commercial SPE sorbents.

Table 2.2 Recoveries of sulfonamides (20 μg L^{-1}) at various amount of sorbent (n=3)

Amount of sorbent (mg)	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
10	61.8±2.8	64.9±2.2	64.6±5.2	72.9±2.0
20	87.1±2.8	87.2±0.13	91.7±1.3	87.5±2.9
40	86.6±2.4	87.0±1.8	88.7±1.7	85.9±5.9
60	88.9±1.4	86.1±2.7	91.1±3.7	91.9±4.1
80	88.6±5.2	87.3±2.6	92.7±1.5	89.8±2.9
100	87.7±2.9	85.4±3.1	91.9±2.4	82.7±5.7

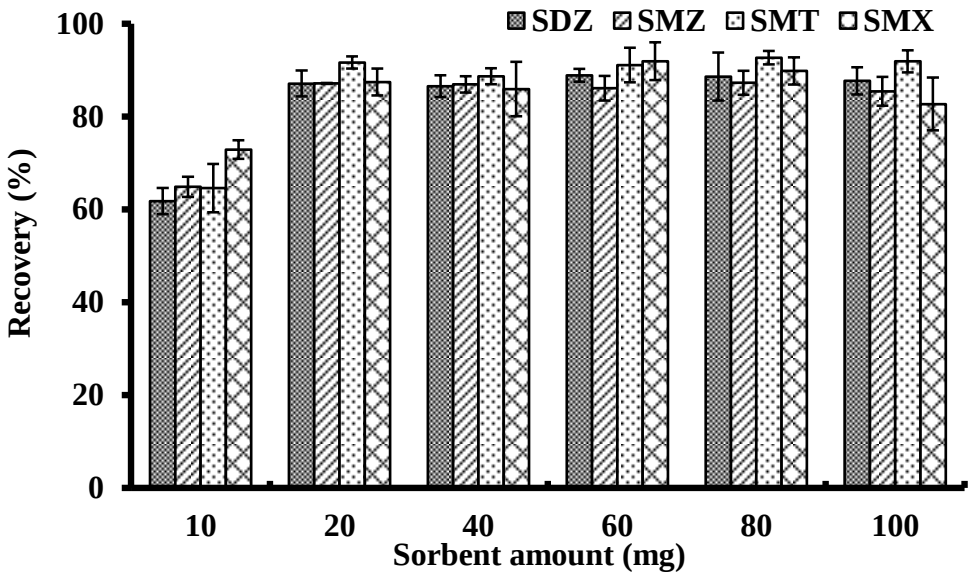


Fig. 2.4 The effect of the amount of polypyrrole/SiO_2/Fe_3O_4 sorbent on the extraction efficiency of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.3 pH of the sample

Sample pH plays an important role in the adsorption of target analytes on the sorbent. Its influence was investigated, i.e., 3.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by adjusting with HCl or NaOH. The results showed that at pH 6.0 and 7.0, in which sulfonamides are in a neutral form (Herrera-Herrera et al., 2013), A high recovery of >80 % was obtained for all tested sulfonamides (**Table 2.3 and Fig. 2.5**). At a sample pH lower than 6.0, the extraction efficiency was reduced, probably because of the protonation of the amine group of the analyte making it more difficult to form hydrogen bond with the sorbent. The extraction efficiency also decreased at a pH value above 7.0 but as a result of the anionic nature of sulfonamides since they would become more negatively charged hence, the π-π interaction between sulfonamides and the sorbent would be reduced. Since, the water samples pHs are normally in the range of 6–7, there will normally be no need to adjust their pHs.

Table 2.3 Recoveries of sulfonamides (20 µg L^{-1}) at various pH of the sample (n=3)

pH	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
3	42.4±3.7	43.1±2.0	44.8±3.3	41.7±4.9
5	79.8±5.2	80.2±2.9	79.5±5.3	81.8±1.7
6	88.3±2.3	91.3±3.6	87.0±2.8	89.4±1.8
7	89.8±3.7	91.8±2.3	88.0±3.5	90.2±1.5
8	82.3±3.2	83.7±1.9	83.7±2.0	83.0±4.8
9	68.2±6.5	65.3±2.6	71.6±2.7	68.3±4.0

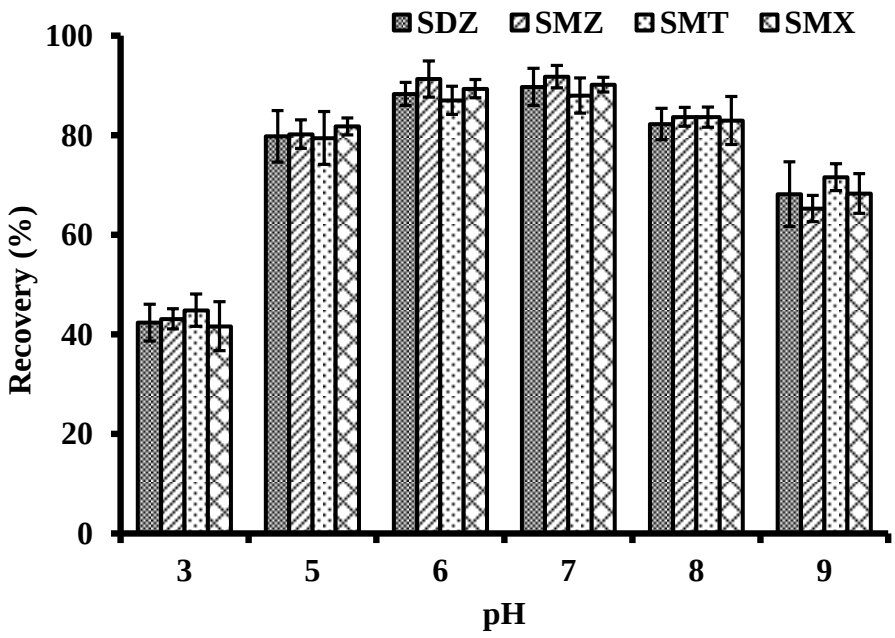


Fig. 2.5 Effect of the water samples pH on the extraction of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.4 Extraction time

Extraction time, the time required for the adsorption of the analyte from the sample solution into the sorbent, was also an important parameter which can affect the extraction efficiency. The extraction time was investigated in the range of 2, 5, 10, 20, 30 and 40 min to achieve the best extraction efficiency with the shortest analysis time. The extraction efficiency increased with extraction time from 2 to 20 min, after which it remained almost constant **(Table 2.4 and Fig. 2.6)**. Therefore, the extraction time of 20 min was selected for further studies.

Table 2.4 Recoveries of sulfonamides (20 µg L^{-1}) at various extraction time (n=3)

Extraction time (min)	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
2	41.98±0.89	43.3±2.9	39.1±2.8	42.5±4.0
5	46.7±5.2	47.2±3.2	46.1±6.9	50.6±4.5
10	67.6±4.0	66.5±4.6	66.1±2.8	66.9±3.0
20	87.7±3.1	83.3±3.6	82.8±3.0	88.3±4.2
30	85.9±2.5	82.67±0.89	86.3±3.7	87.6±3.1
40	80.2±4.0	75.36±0.60	76.7±1.0	81.0±3.2

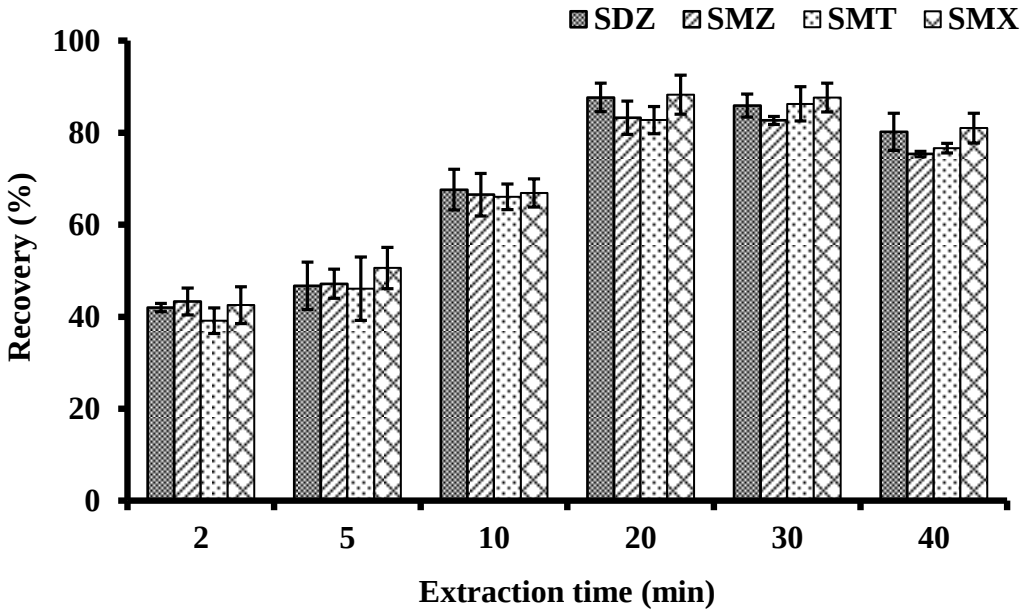


Fig. 2.6 The effect of the extraction time on the recovery of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.5 Desorption solvent, volume and time

The selection of an appropriate solvent to desorb the analytes from the sorbent prior to instrumental analysis was important in a MSPE procedure. Since, the adsorption of sulfonamides onto the polypyrrole/SiO_2/Fe_3O_4 nanoparticles was based on π-π interaction, the desorption solvents of different polarities needed to be considered. Methanol, acetonitrile, acetone, propanol, ethyl acetate and hexane were investigated. The results indicated that when the slightly non-polar (propanol, ethyl acetate) and non-polar (hexane) solvent were used a low desorption efficiency was obtained (**Table 2.5 and Fig. 2.7**) probably because the polypyrrole/SiO_2/Fe_3O_4 sorbent could not be dispersed well in these solvent. For the other three solvents methanol provided the highest desorption efficiency, with acetonitrile and acetone providing a slightly lower value. Therefore, methanol was further employed. Another advantage of using methanol was that, after desorption the solvent must be

removed and methanol evaporated faster than acetonitrile due to its lower boiling point.

To obtain the highest desorption efficiency by using the smallest volume of desorption solvents, its volume was varied from 1.0, 2.0, 3.0, 4.0 and 5.0 mL. The desorption solvent with smallest volume that provided the highest recovery was chosen. The results showed that all analytes could be completely desorbed from the sorbent by sonication with 3.0 mL of methanol (**Table 2.6 and Fig. 2.8**).

The effect of the desorption time on the desorption amount of sulfonamides was also investigated from 5, 10, 20 and 30 min. The shortest desorption time that provided the highest recovery was selected. As for the desorption time 20 min was sufficient to obtain the maximum desorption efficiency for all analytes (**Table 2.7 and Fig. 2.9**).

Table 2.5 Recoveries of sulfonamides (20 μg L^{-1}) at various desorption solvent (n=3)

Type of desorption solvent	**Recovery (%)**			
	SDZ	**SMZ**	**SMT**	**SMX**
Methanol	88.7±2.9	86.1±3.4	86.5±3.5	87.0±3.2
Acetronitrile	85.8±3.5	82.8±2.1	81.5±4.6	82.8±6.1
Acetone	84.5±6.0	80.2±3.4	77.1±4.6	79.4±4.6
Propanol	41.3±3.3	43.3±4.3	40.9±5.0	43.7±2.8
Ethyl acetate	32.8±4.3	31.3±6.4	34.5±3.2	28.6±3.2
Hexane	7.94±0.67	6.73±0.29	6.59±0.58	7.0±1.3

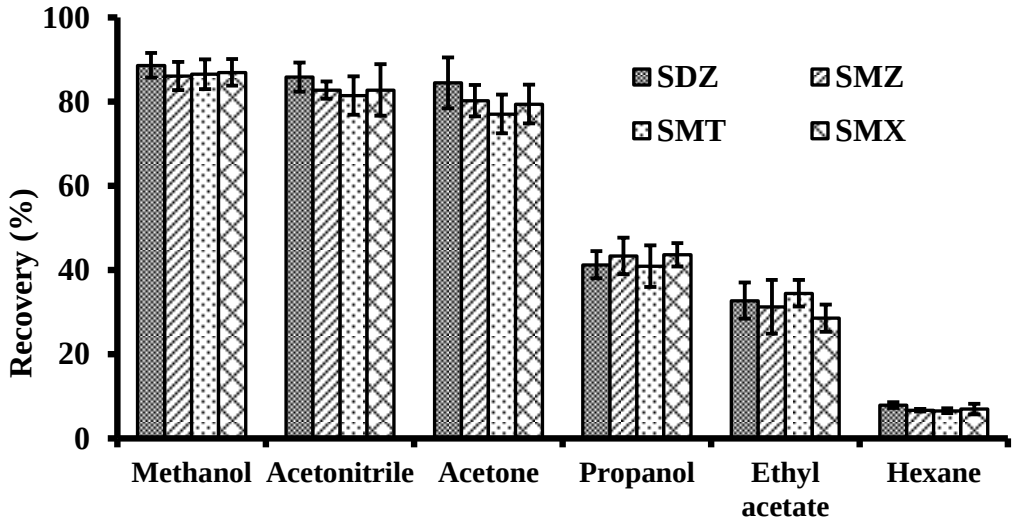


Fig. 2.7 Effect of desorption solvent on the recovery of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

Table 2.6 Recoveries of sulfonamides (20 µg L^{-1}) at various desorption volume (n=3)

Desorption volume (mL)	Recovery (%)			
	SDZ	SMZ	SMT	SMX
1.0	42.0±3.4	42.5±4.5	42.9±5.5	45.0±1.9
2.0	58.7±3.7	59.8±3.9	63.5±1.8	65.1±1.6
3.0	86.2±2.8	88.34±0.68	86.8±3.1	89.9±1.4
4.0	84.8±2.8	86.5±1.9	86.4±1.8	90.6±1.3
5.0	86.4±1.5	91.0±2.3	87.1±1.5	92.2±1.2

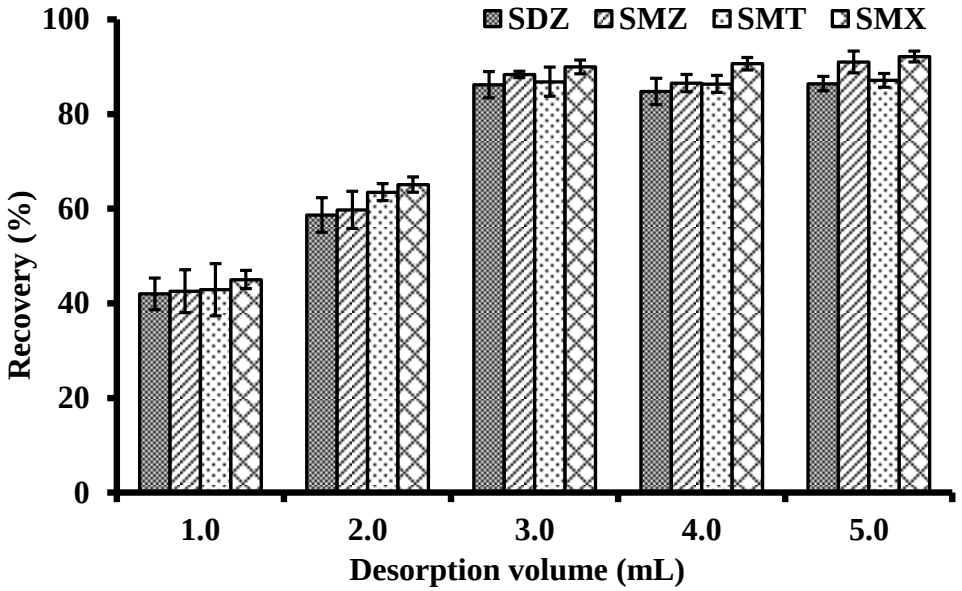


Fig. 2.8 Effect of desorption volume on the recovery of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

Table 2.7 Recoveries of sulfonamides (20 µg L^{-1}) at various desorption time (n=3)

Desorption time (min)	**Recovery (%)**			
	SDZ	**SMZ**	**SMT**	**SMX**
5	70.0±2.3	69.32±0.85	67.9±1.2	63.5±2.7
10	79.4±5.1	77.2±2.2	72.6±4.9	68.8±2.3
20	90.1±2.5	83.8±4.0	82.9±3.0	83.9±5.7
30	84.8±2.2	85.5±2.8	87.4±2.4	83.2±3.4

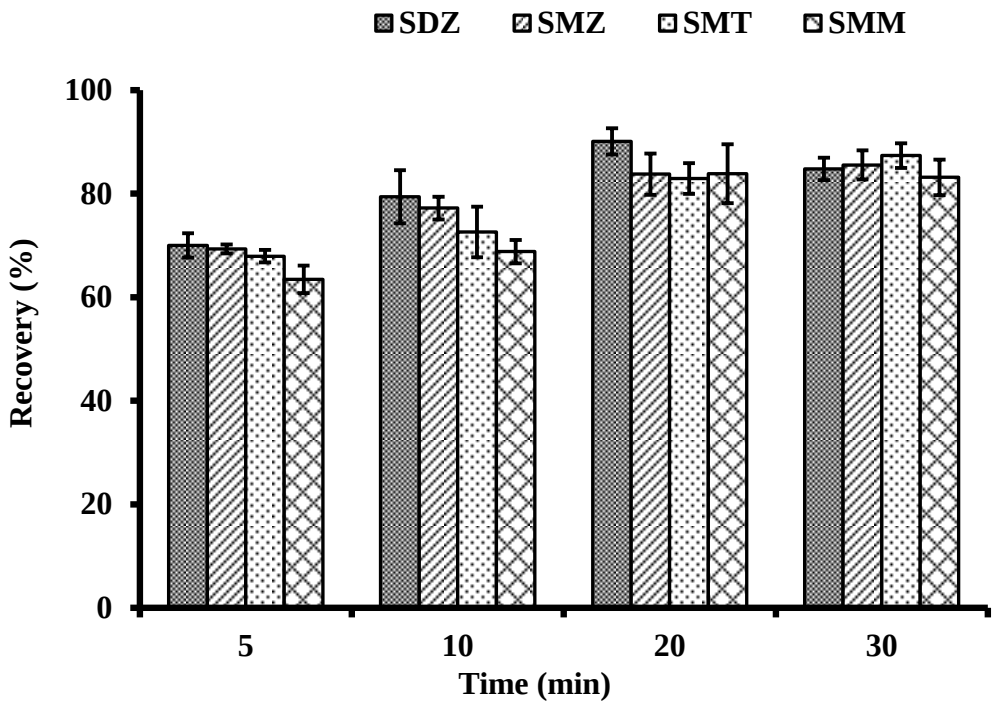


Fig. 2.9 Effect of desorption time on the recovery of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.6 Desorption temperature

The effect of the desorption temperature was studied from 30 to 50 ^{o}C in which the extraction efficiency increased with the desorption temperature and reached the highest level at 45 ^{o}C (**Table 2.8 and Fig. 2.10**). A lower efficiency at a lower desorption temperature may be caused by a slower diffusion rate of the analyte from the sorbent to the desorption solvent while the higher temperature may result in the volatilization of the desorption solvent, thus, the solvent volume was reduced. Therefore, 45 ^{o}C was used for further experiments.

Table 2.8 Recoveries of sulfonamides (20 μg L^{-1}) at various desorption temperature (n=3)

Desorption temperature (^oC)	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
30	85.4±6.2	56.6±4.9	56.8±2.7	60.8±2.0
40	68.8±5.1	65.4±3.5	68.5±3.7	71.4±5.1
45	87.6±4.1	86.9±1.7	86.5±2.1	88.2±4.0
50	84.4±6.2	84.9±3.8	82.6±4.4	86.2±5.6

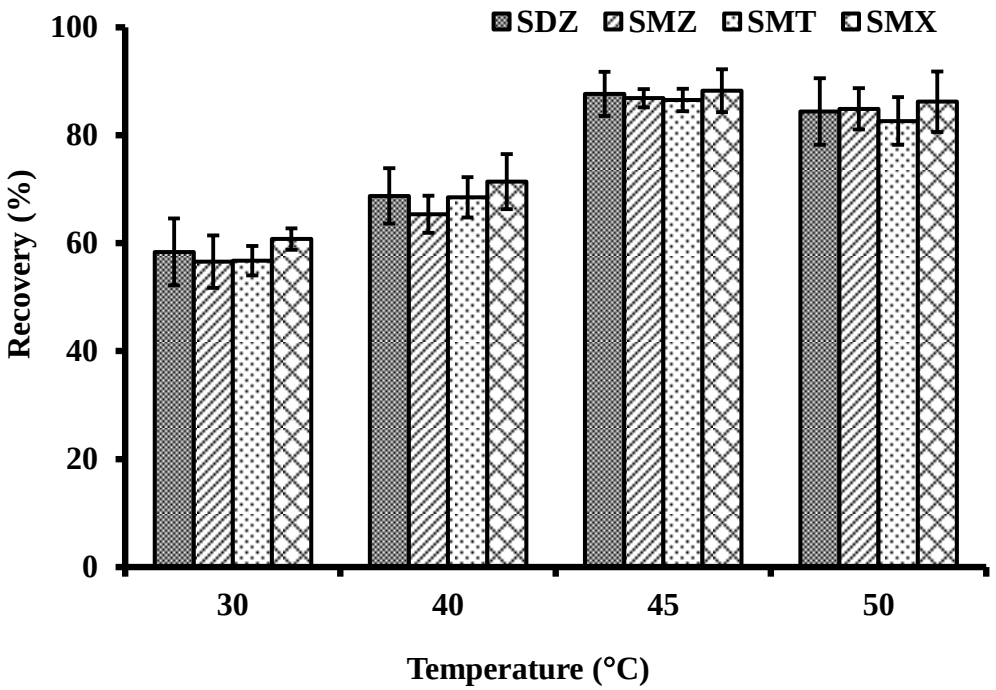


Fig. 2.10 The effect of desorption temperature on the recovery of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

2.4.7 Effect of ionic strength

The mechanism of mass transfer of the analytes in the MSPE can be influenced by the ionic strength (Rahimi and Noroozian, 2014) because the solubility of analytes in the aqueous phase was reduced when the ionic strength was increased, and the analytes that partitioned into the adsorbent was enhanced (Bagheri *et al.*, 2014). The effect of ionic strength on the extraction efficiency of sulfonamides using polypyrrole/SiO_2/Fe_3O_4 sorbent was investigated by varying the concentration of NaCl in the water sample over the range of 0, 2, 5 and 10 % (w/v). The results showed that the ionic strength had a negative effect on the extraction efficiency (**Table 2.9 and Fig. 2.11**) probably because the increase of the salt concentration increased the solution viscosity, hence, this reduced the diffusion rates of analyte from the water to the sorbent, hence, there was decreased extraction efficiency (Tahmasebi *et al.*, 2013). Therefore, no salt was added to the sample solution.

Table 2.9 Recoveries of sulfonamides (20 µg L^{-1}) at various concentration of NaCl (n=3)

Concentration of NaCl (%w/v)	**Recovery (%)**			
	SDZ	**SMZ**	**SMT**	**SMX**
0	89.4±3.8	87.7±1.5	85.0±2.7	86.7±3.5
10	88.2±2.1	85.6±5.6	83.8±4.9	85.1±2.6
20	77.9±5.5	76.8±3.5	77.6±3.3	79.9±1.4
30	74.3±4.3	74.3±4.9	74.9±2.4	76.6±4.0

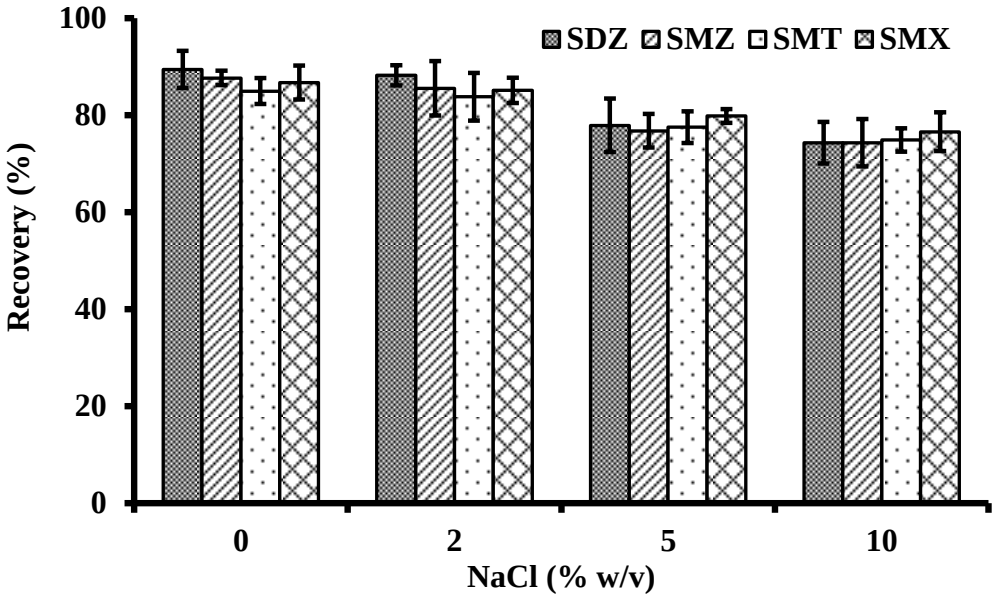


Fig. 2.11 The Effect of ionic strength on the recoveries of sulfonamides; sulfadiazine (SDZ),sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

Summary of optimum conditions for the MSPE using polypyrrole/SiO_2/Fe_3O_4 sorbent are shown in **Table 2.10.**

Table 2.10 Optimization of magnetic solid phase extraction for the determination of sulfonamides using polypyrrole/SiO_2/Fe_3O_4 sorbent

Parameter	**Investigated values**	**Optimum conditions**
Amount of polypyrrole/SiO_2/Fe_3O_4 sorbent (mg)	10, 20, 40, 60, 80, 100	20
pH of the sample	3, 5, 6, 7, 8, 9	6,7
Extraction time (min)	2, 5, 10, 20, 30, 40	20
Desorption solvent	Methanol, Acetonitrile, Acetone, Propanol, Ethyl acetate, Hexane	Methanol
Desorption volume (mL)	1.0, 2.0, 3.0, 4.0, 5.0	3.0
Desorption time (min)	5, 10, 20, 30	20
Desorption temperature (^oC)	30, 40, 45, 50	45
Concentration of NaCl % (w/v)	0, 2, 5, 10	0

2.5 Analytical performances and method validation

Under the optimum conditions of the polypyrrole/SiO_2/Fe_3O_4 sorbent and HPLC system, the analytical performances were evaluated, i.e., the linear range, the limit of detection (LOD), the limit of quantification (LOQ), the precision and accuracy, the reproducibility and the reusability.

2.5.1 Linear range

The linearity of an analytical method is important in a quantitative analysis. It is defined as the ability to obtain the results which are directly proportional to the concentrations (amount) of the analyte in a sample (within a given concentration range). The linear range of the developed method was investigated using standard solutions of SDZ, SMZ, SMT and SMX in the concentrations ranged from 0.10 to 200 μgL^{-1} (triplication for each concentration). The linear ranges were plotted between the peak area versus the concentration of analytes ($\mu g\ L^{-1}$), the linear range is achieved when the coefficient of determination (R^2) is equal or greater than 0.99. The calibration plot of the peak areas versus sulfonamides concentrations in spiked deionized water provided a wide linear range and good linearity ($R^2 > 0.997$) as shown in (**Table 2.11, Fig 2.12 – 2.15**)

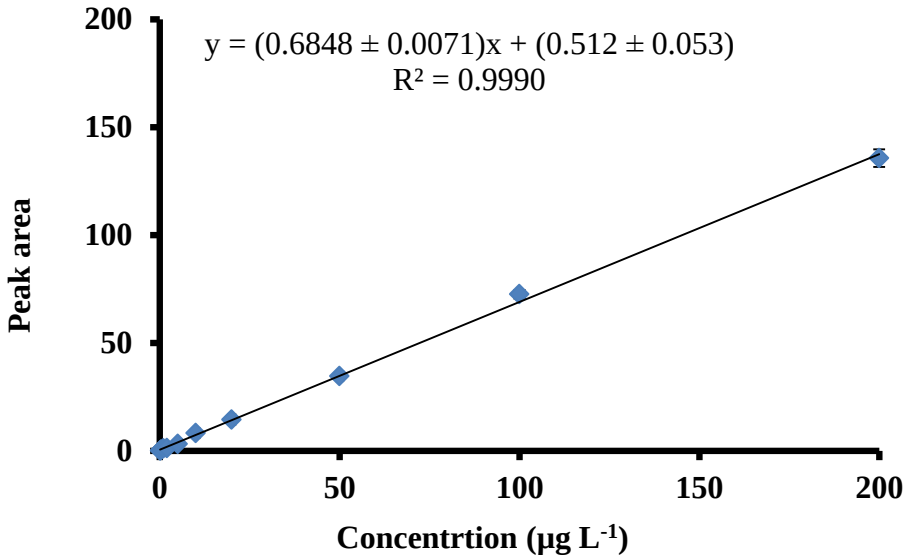


Fig. 2.12 Linearity of sulfadiazine

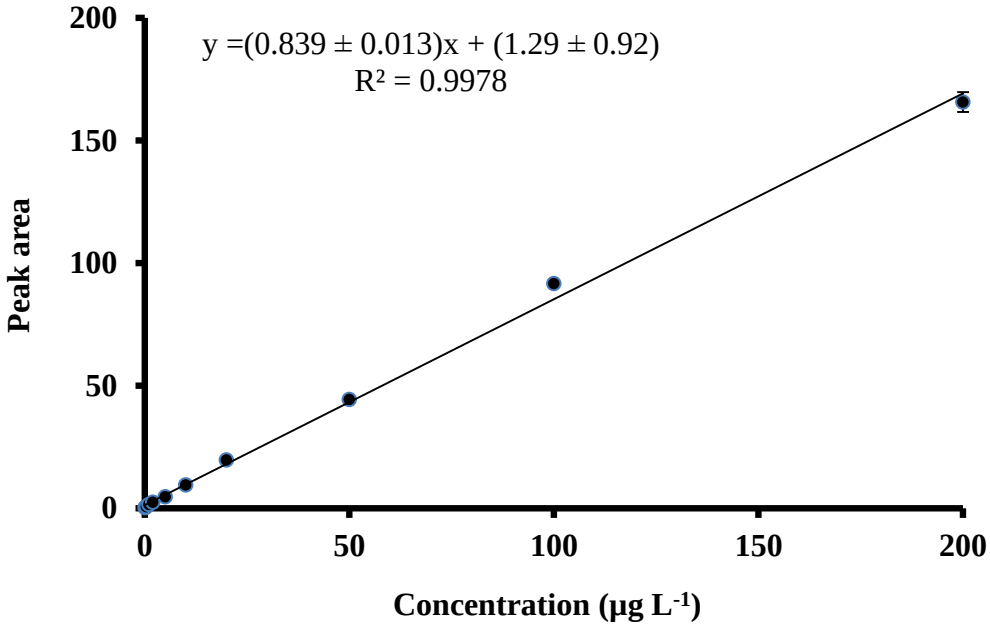


Fig. 2.13 Linearity of sulfamerazine

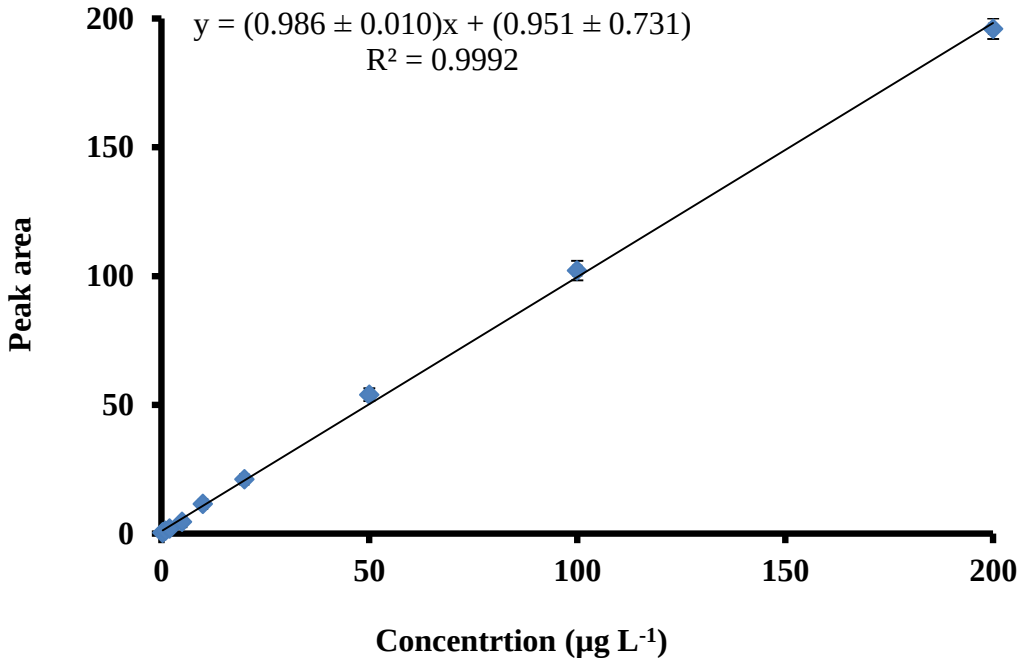


Fig. 2.14 Linearity of sulfamethazine

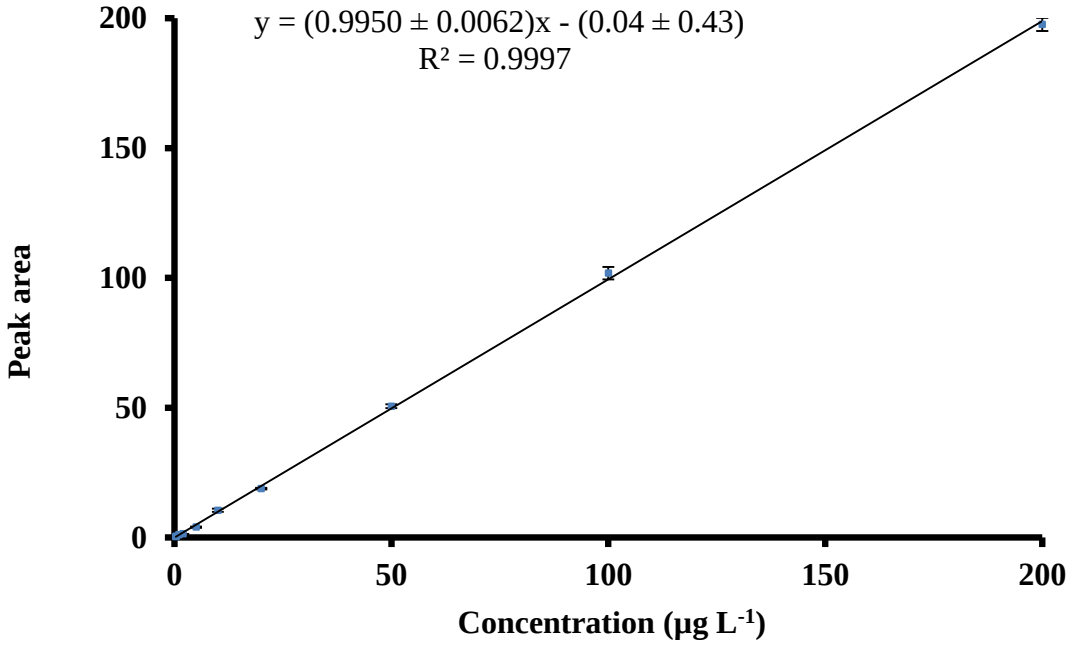


Fig. 2.15 Linearity of sulfamonomethoxine

2.5.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is the smallest amount or concentration of an analyte in the test sample that can be reliably distinguished from zero (IUPAC, 2002). The LOD is determine by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected according to base on the signal-to-noise ratio of 3 (S/N=3) (ICH, 1999) .

The limit of quantification (LOQ) is the lowest concentration of analyte in the sample can be determined with acceptable performance (EURACHEM, 1998). The LOQ is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably quantified according to based on signal-to-noise ratio of 10 (S/N=10) (ICH, 1999).

The LOD and LOQ were very low in the μg L^{-1} as shown in **Table 2.11**.

Table 2.11 Analytical performances of polypyrrole/SiO_2/Fe_3O_4 sorbent for the extraction of sulfonamides

Compounds	**Linear range (μg L^{-1})**	**Regression linear equation**	**R^2**	**LOD (μg L^{-1})**	**LOQ (μg L^{-1})**
SDZ	0.30 – 200	y=(0.685±0.007)x+(0.512±0.053)	0.9990	0.30	1.0
SMZ	0.30 – 200	y=(0.839±0.013)x+(1.29±0.92)	0.9978	0.30	1.0
SMT	1.0 – 200	y=(0.986±0.010)x+(0.95±0.73)	0.9992	1.0	4.0
SMX	1.0 – 200	y=(0.995±0.006)x-(0.04±0.43)	0.9997	1.0	4.0

2.5.3 Precision

Precision is the closeness of agreement between independent test results obtained under optimum conditions. It is usually determined in terms of the relative standard deviations (RSDs). Five water samples spiked with the standard SDZ, SMZ, SMT and SMX solution at the concentrations of 5.0, 20.0 and 100 µg L^{-1} were analyzed (five replications for each concentration). The RSDs from each concentration were used to indicate the precision of the developed method. The RSDs were less than 6%. These values were better than that of the accepted RSDs as recommended by the AOAC method (RSD=15%) indicating that the developed method based on the use of the polypyrrole/SiO_2/Fe_3O_4 sorbent and analysis by HPLC-DAD are suitable and reliable to be used as the sample preparation technique for the determination of sulfonamides at low concentration.

2.5.4 Accuracy

The accuracy of an analytical procedure can be described as the closeness of agreement between the true value or an accepted reference value and the found value. The recovery was performed by spiking a mixture of sulfonamides standard into water samples (known to be free of sulfonamides) to obtain the concentration of 5.0, 20.0 and 100 µg L^{-1}. The spiked sample were extracted and analyzed under the optimum conditions. The recovery (%) is calculated as (AOAC, 2012):

$$\text{Recovery (\%)} = (Cf - Cu) \times 100/Ca$$

where

Ca is the calculated (not analyzed) concentration of analyte added to the test sample

Cf is the concentration of the fortified (spiked)

Cu is the concentration of the unfortified (control sample)

The recoveries of all tested sulfonamides in water samples were in the range of 86.7 to 99.7 % (**Table 2.12**). The recoveries were in the acceptable range of 70-110% (AOAC, 2012) indicated that there was no effect from the matrix composition of the water samples. It can be concluded that the developed sorbent is suitable for extraction and determination of trace sulfonamides in real water samples.

Table 2.12 Recoveries of sulfonamides in real water samples (n=5)

Water samples	Added ($\mu g\ L^{-1}$)	Recovery (%)			
		SDZ	SMZ	SMT	SMX
Tap water	5	93.8 ± 4.2	94.8 ± 2.7	97.1 ± 1.4	95.6 ± 3.7
	20	89.8 ± 5.3	93.7 ± 4.3	96.6 ± 2.4	94.2 ± 3.6
	100	91.8 ± 4.5	86.7 ± 3.1	90.8 ± 5.3	90.2 ± 4.1
River water	5	94.8 ± 4.0	95.5 ± 2.0	93.6 ± 3.7	94.2 ± 4.2
	20	89.2 ± 2.0	92.0 ± 1.0	93.4± 3.8	91.8 ± 3.0
	100	86.7 ± 3.0	90.0 ± 3.0	93.0 ± 1.5	91.2 ± 4.8
Lake water	5	95.0 ± 2.9	99.7 ± 4.6	93.7 ± 4.2	97.0 ± 1.6
	20	97.7 ± 2.5	91.9 ± 1.0	96.2 ± 4.2	95.4 ± 3.3
	100	93.0 ± 2.1	90.1 ± 4.9	87.6 ± 3.0	92.1 ± 3.1

2.5.5 Reusability

Reusability of the developed sorbent was investigated to reduce preparation time of the sorbent and reduce the analysis cost. Five pieces of the polypyrrole/SiO_2/Fe_3O_4 sorbent were used in this study. Each piece was used to extract SDZ, SMZ, SMT and SMX from the standard solution (20 µg L^{-1}) and after desorption step, the target analytes were quantified. After desorption the used polypyrrole/SiO_2/Fe_3O_4 sorbent was washed by stirred for 1 min in 2.0 mL methanol and 2.0 mL deionized water, respectively, prior to next used. The extraction procedures were then repeated until the recoveries decreased to below 80 % of the initial value. After washing the sorbent was tested with a blank sample where no HPLC response was observed which indicated that there was no carry-over of the analytes. The developed sorbent could be reused for up to 16 times **(Fig 2.16)** while maintaining recoveries of sulfonamides > 80 % which were in acceptable value 80-110% (AOAC, 2012).

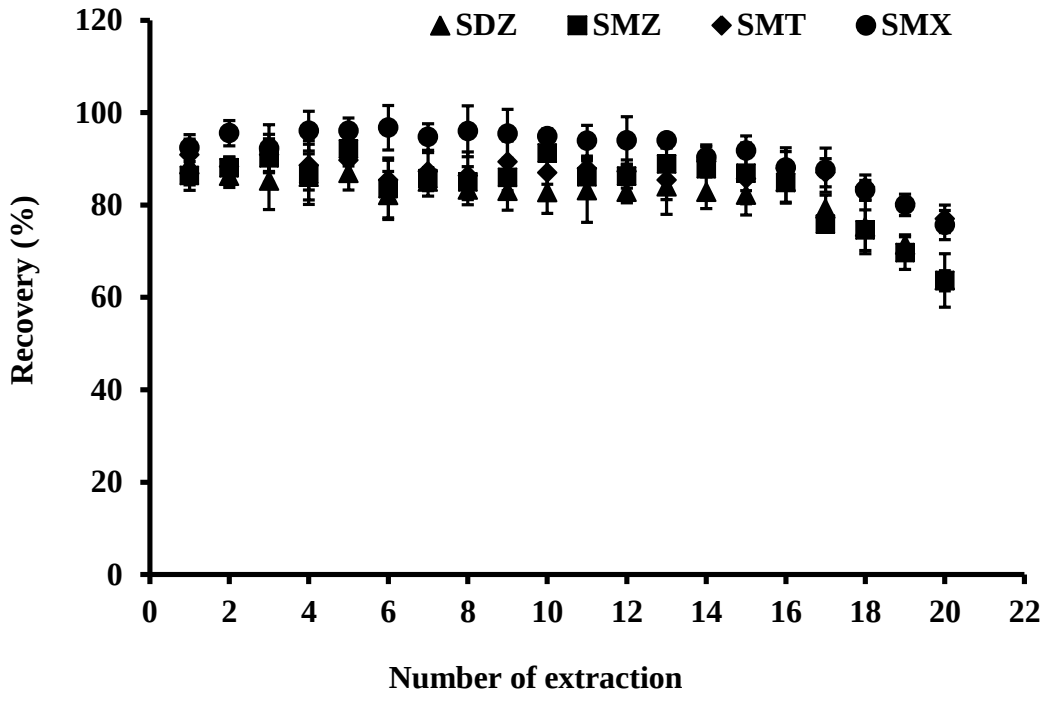


Fig. 2.16 Reusability of the polypyrrole/SiO_2/Fe_3O_4 sorbent for extraction of sulfonamides (20.0 µg L^{-1}) from spiked deionized water (n=5)

2.5.6 Reproducibility

The preparation of the polypyrrole/SiO_2/Fe_3O_4 sorbent was investigated in term of batch-to-batch reproducibility. Six different batches were used to extract sulfonamides (20.0 μg L^{-1}) in spiked deionized water under the same conditions. The averages of the recoveries and relative standard deviations (%RSD) were used to indicate the batch-to-batch reproducibility for each analyte. The results are as shown in **Table 2.13**. These were better than the acceptable values recommended by the AOAC (recovery = 80-110%, RSD = 32%), and indicated that the preparation procedure of the polypyrrole/SiO_2/Fe_3O_4 sorbent has a good reproducibility.

Table 2.13 Recoveries of sulfonamides after extraction with polypyrrole/SiO_2/Fe_3O_4 sorbent

Number of sorbent	Recoveries (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
1	89.8	91.8	88.0	90.2
2	90.1	83.8	82.9	83.9
3	87.1	87.2	91.7	87.5
4	87.7	83.3	82.8	88.3
5	88.7	86.1	86.5	86.9
6	86.2	88.3	86.8	90.0
Average	88.2	86.7	86.5	87.8
RSD (%)	1.7	3.6	3.9	2.7

2.5.7 Comparison of the sorbents

The efficiency of the developed sorbent was compared to a commercial SPE sorbent, HLB. HLB was conditioned with 2.0 mL of methanol followed by 2.0 mL of deionized water and then loaded with 5.0 mL of spiked water sample (20 µg L^{-1} of sulfonamides), desorbed with 3.0 mL of methanol and detected by HPLC-DAD. The average recoveries of the two sorbents were statistical compared by the paired *t*–test. The results showed that similar recoveries were obtained (**Table 2.14 and Fig. 2.17**) and there were no significant difference between the two sorbents ($P > 0.05$). The advantages of the developed method over the traditional SPE sorbent that they were simpler to use, required a shorter extraction time and used less solvents consumption. In addition, the polypyrrole/SiO_2/Fe_3O_4 sorbent could be reused for a maximum of 16 times, whereas any commercial HLB sorbent cannot be reused.

Table 2.14 Recoveries of sulfonamides (20 µg L^{-1}) after extraction with polypyrrole/SiO_2/Fe_3O_4 sorbent and the commercial HLB cartridges (n=3)

Type of sorbent	Recovery (%)			
	SDZ	**SMZ**	**SMT**	**SMX**
Polypyrrole/SiO_2/Fe_3O_4	89.2±2.1	89.0±2.5	88.6±1.6	92.0±3.9
HLB	87.7±3.4	90.7±4.4	89.0±3.6	91.4±4.8

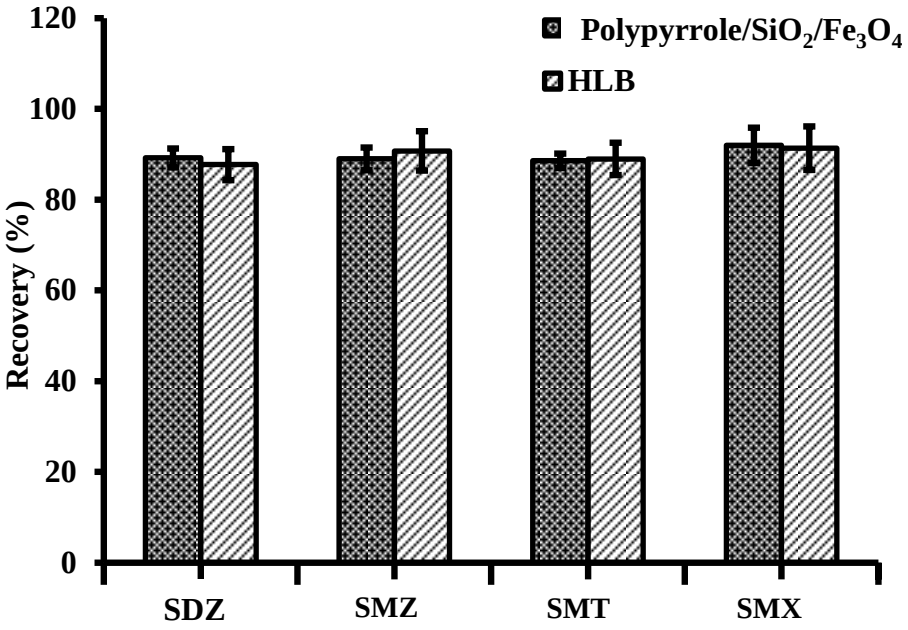


Fig. 2.17 The extraction efficiency of sulfonamides in spiked deionized water, a comparision between the polypyrrole/SiO_2/Fe_3O_4 sorbent and the conventional HLB cartridge

2.6 Analysis of sulfonamides in water samples

Qualitative analysis was carried out by comparing the retention time of the SDZ, SMZ, SMT and SMX from the chromatogram of an unknown sample to the one of spiked standard solution in the sample under the same conditions.

The developed polypyrrole/SiO_2/Fe_3O_4 sorbents were applied to extract sulfonamides from tap water from a laboratory, other samples were collected from Wong and U-Tapao canals in Hat Yai city, Songkhla, Thailand, and Songkhla Lake, Thailand. Each water sample was filtered through a 0.45 μm membrane to remove suspended particles and stored in a brown glass bottle at 4 ^{o}C. These samples were extracted and analyzed under the optimum conditions.

The optimum condition of the polypyrrole/SiO_2/Fe_3O_4 sorbent and HPLC-DAD system were used to determine SDZ, SMZ, SMT and SMX in tap

water, canal and lake water samples. The average retention time of SDZ, SMZ, SMT and SMX in the water samples and spiked samples were similar (**Fig. 2.18**).

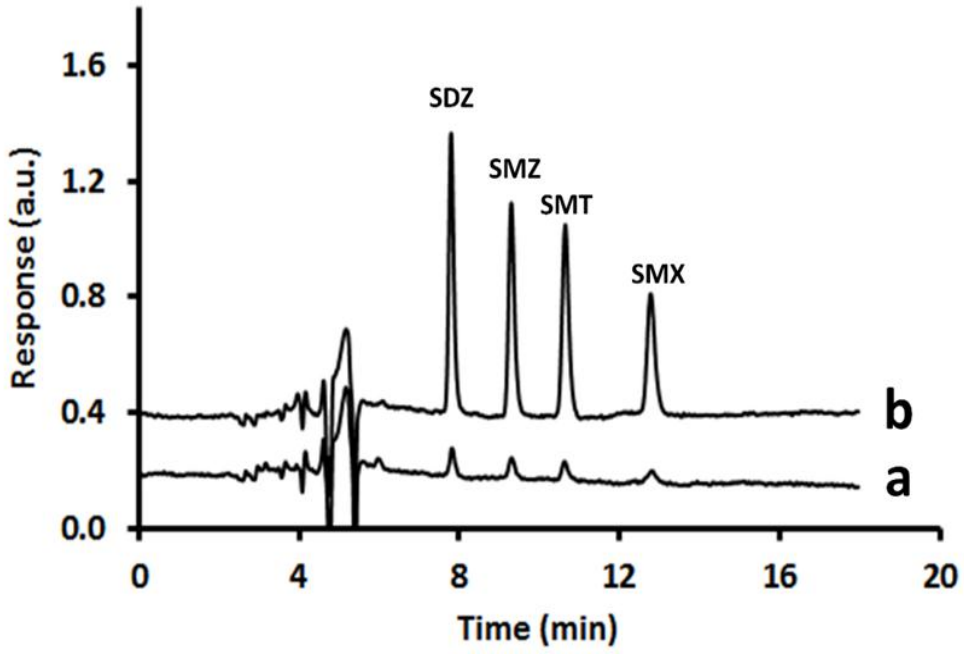


Fig. 2.18 Chromatograms of spiked water sample canal (10.0 µg L^{-1}) without extraction (a) and with extraction using polypyrrole/SiO_2/Fe_3O_4 sorbent (b)

The matrix interferences have a significant effect on the sample preparation procedure. The interfering compounds may strengthen or reduce the signal, and the magnitude of the effect may also depend on the concentration (Bruce *et al.*, 1998). Prior to the quatitative analysis of SDZ, SMZ, SMT and SMX in the real samples, the effect of the sample matrix on the analytical performances of the developed method was evaluated. Each type of the samples was spiked with known concentrations of mixture of sulfonamides standard solution to obtain the spiked samples over the concentration of 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg L^{-1}. A standard solution of SDZ, SMZ, SMT and SMX was also prepared in 5.00 mL of deionized water in the same concentration range. Both the spiked samples and the

standard solutions were extracted and analyzed under optimum conditions. The effect of the matrix interferences were evaluated by comparing the slopes of the standard calibration curve and the spiked curve using two-way ANOVA (analysis of variance). Their slopes were compared, when the slope of the matrix matched and the standard calibration curves were significantly different, the matrix matched calibration curve must be used to analyze the sulfonamides in real samples. In contrast, the standard calibration curve can be used for the analysis if their slopes showed no significant differences.

In this work, the matrixes were not significant different for all type of water samples (P>0.05). Examples of standard and matrix matched calibration curve of sulfonamides are shown in **Fig 2.19 to 2.22**. The standard and matrix matched calibration curve parameters of sulfonamides are summarized in **Table 2.15**. Then standard curve was used for the quantitative analysis of sulfonamides in water samples.

Table 2.15 The comparison between standard and matrix matched calibration curve of sulfonamides obtained from MSPE followed by HPLC-DAD

Sulfonamides	Regression equation		R^2		*P*-value
	Standard curve	Spiked curve	Standard curve	Spiked curve	
sulfadiazine	y=(0.7199±0.0090)x+(0.01±0.41)	y=(0.724±0.018)x+(0.41±0.85)	0.9991	0.9975	0.43
sulfamerazine	y=(0.9064±0.0090)x+(0.47±0.41)	y=(0.945±0.028)x-(0.6±1.3)	0.9964	0.9995	0.76
sulfamethazine	y=(1.023±0.020)x+(0.8±1.0)	y=(0.963±0.032)x+(3.2±1.6)	0.9989	0.9967	0.88
sulfamonomethoxine	y=(1.026±0.010)x-(0.85±0.53)	y=(0.992±0.029)x+(0.9±1.5)	0.9997	0.9974	0.34

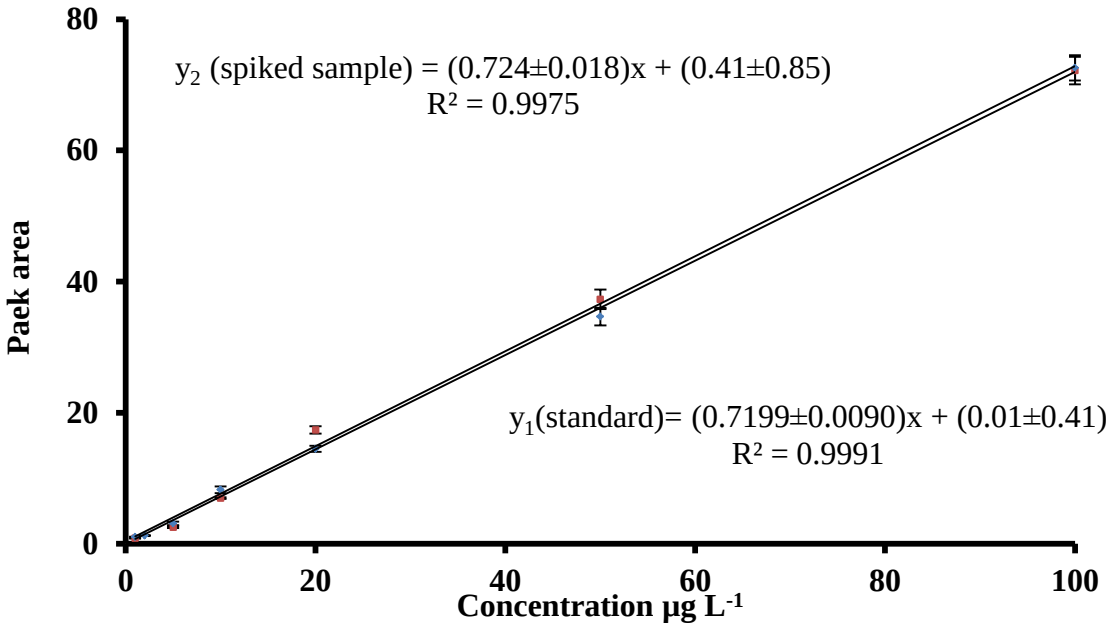


Fig. 2.19 Standard curve and spiked curve of sulfadiazine in water sample

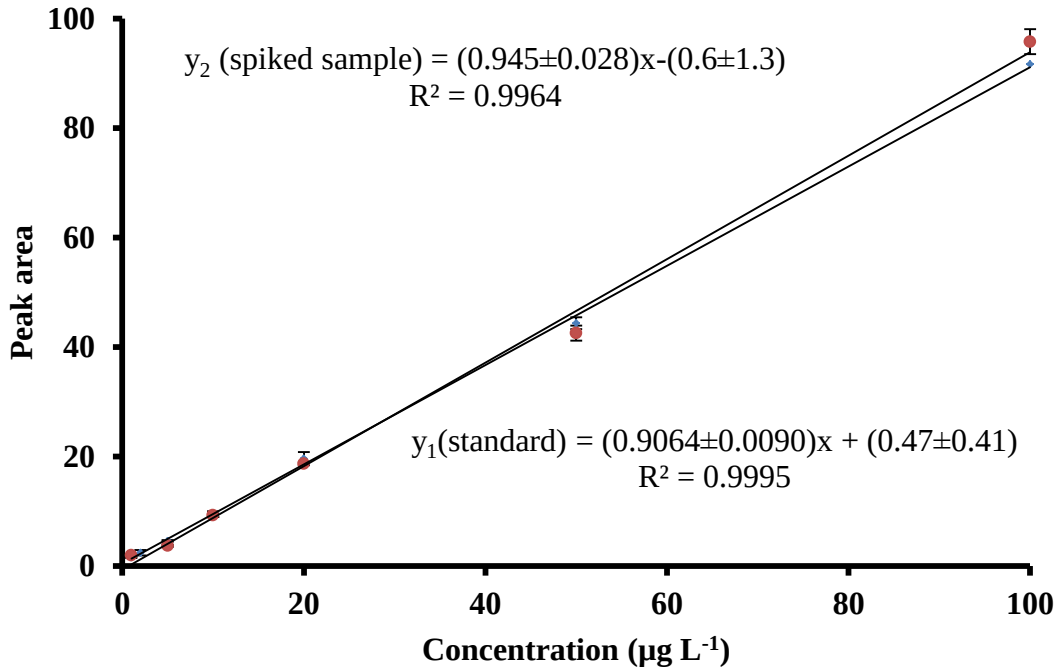


Fig 2.20 Standard curve and spiked curve of sulfamerazine in water sample

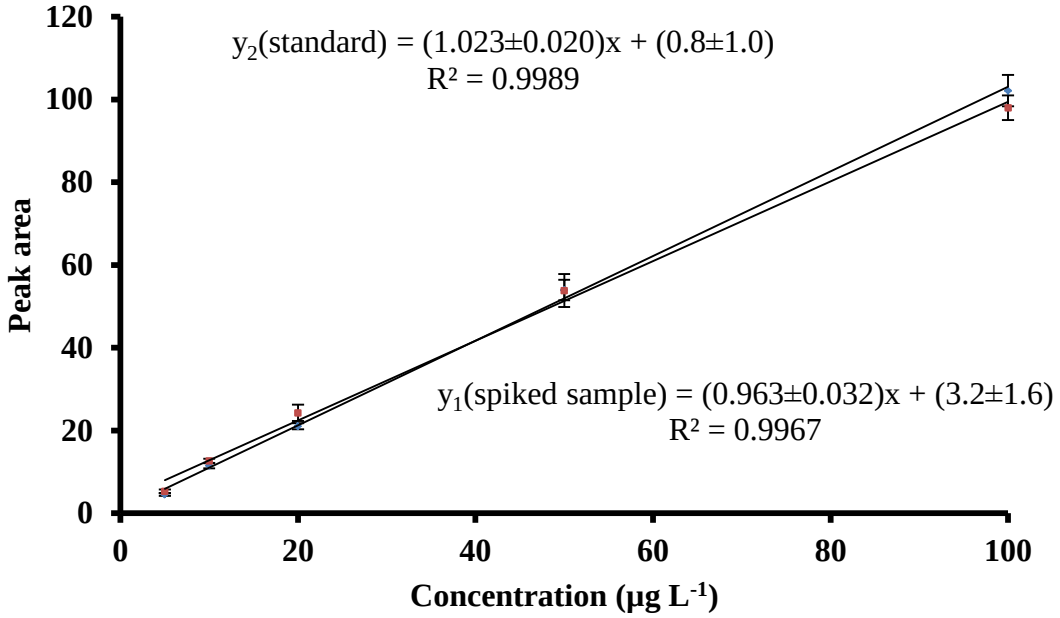


Fig. 2.21 Standard curve and spiked curve of sulfamethazine in water sample

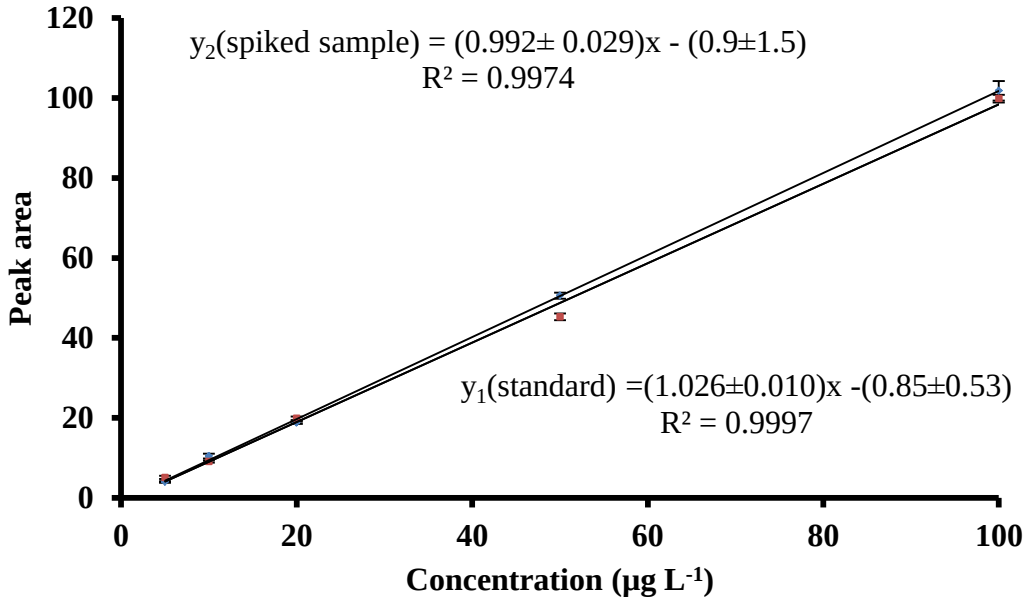


Fig. 2.22 Standard curve and spiked curve of sulfamonomethoxine in water sample

CHAPTER 3

Concluding remarks

A novel, simple and cost effective magnetic solid phase extraction of polypyrrole/SiO_2/Fe_3O_4 sorbent has been developed and successfully applied for the extraction of sulfonamides in water samples, followed by HPLC-DAD analysis. The developed method provides a good linear range 0.30 – 200 µg L^{-1} for sulfadiazine and sulfamerazine, 1.0 – 200 µg L^{-1} for sulfamethazine and sulfamonomethoxine. The limit of detections (LODs) were 0.30 µg L^{-1} for sulfadiazine and sulfamerazine and 1.0 µg L^{-1} for sulfamethazine and sulfamonomethoxine. This method has a good reproducibility with relative standard deviations (RSDs) in the range 1.7 – 3.9%, which were in the acceptable value by AOAC (RSD=32%). The recoveries were in the range of 86.7 to 99.7% and RSDs were less than 6%. The results were in the acceptable range by the AOAC method (Recovery = 80-110%, RSD=32%). It can be indicated that the developed method based on the use of the polypyrrole/SiO_2/Fe_3O_4 sorbent is suitable for extraction and determination of trace sulfonamides in real water samples.

A comparison between the performance of the developed methods and the previously reported method are summarized in **Table 3.1**. In the case of the LODs, the values from this work were within the same range (Raich-Montiu *et al.*, 2007; Lin and Huang, 2008; Lara *et al.*, 2009; Díaz-Álvarez *et al.*, 2014) or better than some methods (Huang *et al.*, 2009; Yu and Hu, 2012; Herrera-Herrera *et al.*, 2013). However, for two of these that have the similar LODs (Raich-Montiu *et al.*, 2007; Lin and Huang, 2008) they required longer extraction time and larger sample volumes.

For the other two (Lara *et al.*, 2009; Díaz-Álvarez *et al.*, 2014) although the extraction conditions were similar to the develop method, their recoveries were not as good. When considered the recoveries, the performance of the developed method was either comparable (Raich-Montiu *et al.*, 2007; Lin and Huang, 2008) or better than (Huang *et al.*, 2009; Lara *et al.*, 2009; Yu and Hu, 2012; Díaz-Álvarez *et al.*, 2014) other methods. That is, the main advantage of this work is the short extraction time (20 min) combines with the small sample volume that can provide very good performances. This is because the target analytes in the solution can easily be adsorbed onto the sorbent that provided a large adsorption capacitiy. The other advantage is the sorbent can be separated rapidly from the sample solutions using an external magnetic field. Moreover, it can be reused at least 16 times, with a relatively low cost (0.07 USD per sample) which helps to reduce analysis cost and time. This indicates that the proposed new method has better accuracy and is also probably much cheaper to use. This can certainly be applies for the determination of other aromatic compounds such as polycyclic aromatic hydrocarbons.

Table 3.1 Comparison of the developed method with other methods for the analysis of sulfonamides

Extraction Method	Sample	Extraction time (min)	Sample volume (mL)	Extractants	LOD (μg L^{-1})	Recovery (%)	References
Magnetic solid phase extraction	water	20	5	polypyrrole/SiO_2/ Fe_3O_4	0.3-1.0	87-100	This work
Dispersive liquid-liquid microextraction	water	3	5	Chloroform	0.41-9.87	78-117	(Herrera-Herrera *et al.*, 2013)
Liquid-liquid-liquid microextraction	water	45	12	Organic solvent	0.11-0.77	86-109	(Lin and Huang, 2008)
Micro-solid phase extraction (Molecularly imprinted polymer)	water	20	4.5	MIP sorbent	0.2-3.0	70-120	(Díaz-Álvarez *et al.*, 2014)
In-line solid phase extraction	water	15	4.75	HLB particles	0.38-0.56	52-109	(Lara *et al.*, 2009)
Solid phase extraction	water	25	250	HLB cartridges	0.15-1.0	70-104	(Raich-Montiu *et al.*, 2007)
Stir bar sorptive extraction	milk	10	4	C_{18}	0.9-10.5	68-120	(Yu and Hu, 2012)
Stir bar sorptive extraction	milk	60	50	Monolithic material	1.3-7.9	55-126	(Huang *et al.*, 2009)
Magnetic solid phase extraction	water	20	1	Fe_3O_4/Graphene oxide	50-100	67-120	(Shi and Ye, 2014)

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Publication

A polypyrrole/silica/magnetite nanoparticles as a sorbent for the extraction of sulfonamides from water samples

Thanyaporn Sukchuay, Proespichaya Kanatharana, Rodtichoti Wannapob, Panote Thavarungkul, and Opas Bunkoed

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Thanyaporn Sukchauy[1,2,3]
Proespichaya Kanatharana[1,2,3]
Rodtichoti Wannapob[1,2,3]
Panote Thavarungkul[1,2,4]
Opas Bunkoed[1,2,3]

[1]Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[2]Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[3]Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[4]Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

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Original Paper

Polypyrrole/silica/magnetite nanoparticles as a sorbent for the extraction of sulfonamides from water samples

A magnetic solid-phase extraction sorbent of polypyrrole/silica/magnetite nanoparticles was successfully synthesized and applied for the extraction and preconcentration of sulfonamides in water samples. The magnetite nanoparticles provided a simple and fast separation method for the analytes in water samples. The silica coating increased the surface area that helped to increase the polypyrrole layer. The polypyrrole-coated silica provided a high extraction efficiency due to the π–π and hydrophobic interactions between the polypyrrole and sulfonamides. Several parameters that affected the extraction efficiencies, i.e. the amount of sorbent, pH of the sample, extraction time, extraction temperature, ionic strength, and desorption conditions were investigated. Under the optimal conditions, the method was linear over the range of 0.30–200 μg/L for sulfadiazine and sulfamerazine, and 1.0–200 μg/L for sulfamethazine and sulfamonomethoxine. The limit of detection was 0.30 μg/L for sulfadiazine and sulfamerazine and 1.0 μg/L for sulfamethazine and sulfamonomethoxine. This simple and rapid method was successfully applied to efficiently extract sulfonamides from water samples. It showed a high extraction efficiency for all tested sulfonamides, and the recoveries were in the range of 86.7–99.7% with relative standard deviations of $< 6\%$.

Keywords: Magnetic nanoparticles / Polypyrrole / Silica / Sulfonamides
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1 Introduction

Sulfonamides are a group of synthetic antibiotics often used to prevent infections, treat diseases, and to promote growth because of their broad-spectrum activities, effectiveness, and low cost [1]. These compounds enter the environment by means of wastewater effluents from municipal treatments plants, hospitals, livestock activities, and the improper disposal of drugs or unexpected spills during manufacturing or distribution [2]. Consequently, traces of sulfonamides have often been detected in environmental waters [3]. Even at trace levels, they can promote the development of antibiotic resistant bacteria, cause allergic reactions in humans, or even possess carcinogenic potency [4].

HPLC has been widely used for the determination of sulfonamides because of its high sensitivity and good precision [5]. Due to the relatively low concentration of sulfonamides in water samples together with the complexity of the matrices, a sample preparation method is generally required before instrumental analysis. Among the various sample preparation techniques that have been reported for extraction and preconcentration of sulfonamides [6]–[9], SPE is one of the most widely used techniques due to its high extraction efficiency. However, traditional SPE cartridges and equipment are expensive and the operation is quite tedious. Recently, magnetic SPE (MSPE) has attracted much attention since the sorbent can be easily separated from the sample solution using an external magnetic field [10] and can be reused after a simple washing operation. However, naked magnetic nanoparticles (Fe_3O_4) tend to aggregate, are prone to oxidation and are not selective toward complex matrices. Therefore, the surfaces of these magnetic nanoparticles have been modified with specific ligands to make them more selective and become more suitable [11]. Silica (SiO_2) is one of the most ideal coating layers since it can prevent Fe_3O_4 nanoparticles aggregating over a wide range of pH values and improve their chemical stability [12]. However, the hydrophilic SiO_2 would not be applicable for the extraction of sulfonamides. Therefore, in this work, an additional coating of a polypyrrole, which can easily be prepared by chemical polymerization under mild conditions [13], was used because it contains a conjugated π struc-

Correspondence: Opas Bunkoed, Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
E-mail: opas.b@psu.ac.th
Fax: +66-7455-8841

Abbreviations: MSPE, magnetic SPE; **SDZ**, sulfadiazine; **SMZ**, sulfamerazine; **SMT**, sulfamethazine; **SMX**, sulfamonomethoxine; **TEOS**, tetraethylorthosilicate

ture that can adsorb sulfonamides by π–π and hydrophobic interactions. The polypyrrole/SiO_2/Fe_3O_4 sorbent was then applied to extract sulfonamides from water samples and then be detected by HPLC. The aim was to provide a reliable, simple, highly sensitive, and environmentally friendly method for the monitoring of sulfonamides in environmental samples. Sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT), and sulfamonomethoxine (SMX), because of their toxicity, were selected as test compounds to investigate the performance of the developed sorbent.

2 Materials and methods

2.1 Reagents and chemicals

Methanol, acetonitrile, acetic acid, and 2-propanol were from Merck (Darmstadt, Germany). Sodium chloride was from Labscan (Bangkok, Thailand). Ethanol and ammonium hydroxide were from JT Baker (Bangkok, Thailand). Iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$), Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), Iron(III) chloride anhydrous ($FeCl_3$), pyrrole, tetraethylorthosilicate (TEOS), SDZ, SMZ, SMT, and SMX were from Sigma–Aldrich (Steinheim, Germany). Oasis HLB (30 mg) was from Waters (Milford, USA). Individual stock solutions of each standards (100 μg/mL) were prepared in methanol and stored at 4°C. Ultrapure water was obtained using a maxima ultrapure water system (ELGA, UK).

2.2 Instrumentation

Chromatographic analysis was performed on a Hewlett-Packard 1100 series HPLC system (Agilent Technologies, Germany). The separations were performed on a reversed-phase VertiSepTM C_{18} column (5 μm particles size, 250 × 4.6 mm id; Restek Bellefonte, USA). The surface morphology of the prepared sorbent was observed by SEM (JSM-5200, JEOL, Tokyo, Japan). The FTIR spectra were determined by FTIR spectroscopy (PerkinElmer, Waltham, MA, USA).

2.3 HPLC conditions

To obtain the best performance (high sensitivity, good resolution, and short-analysis time) a number of the operational conditions of the HPLC were optimized for the analysis of sulfonamides. The optimum absorbing wavelength of the sulfonamides was first investigated. Then a mixed standard solution of four sulfonamides was analyzed at the optimum wavelengths (with a mobile phase flow rate of 0.70 mL/min, a 40°C column temperature and a 20 μL sample volume) to obtain the optimum compositions of the mobile phase (% acetic acid and acetonitrile).

2.4 Synthesis of polypyrrole/SiO_2/Fe_3O_4 nanoparticles

Figure 1A shows a schematic presentation for the synthesis of polypyrrole/SiO_2/Fe_3O_4 nanoparticles. Fe_3O_4 nanoparticles were first prepared by a chemical coprecipitation method [12]. Briefly, 4.70 g of $FeCl_3{\cdot}6H_2O$ and 1.70 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 80 mL of deionized water. Under vigorous stirring at 80°C, 10 mL of ammonium hydroxide (30% v/v) was added dropwise into the solution over a period of 5 min and the stirring was continued for another 55 min during which time the magnetic particles were formed. The particles were then separated using a magnet, washed three times with 100 mL of water, and then dried at 60°C in an oven for 24 h.

For SiO_2 coating, 2.0 g of Fe_3O_4 nanoparticles were added into a solution containing 100 mL of ethanol, 50 mL of deionized water and 2.0 mL of ammonium hydroxide (30% v/v) and sonicated for 15 min. A 2.0 mL of TEOS was added dropwise into the solution while stirring at 300 rpm, and 40°C, after which the solution was heated for 12 h. The SiO_2/Fe_3O_4 particles were collected by a magnet, washed with 20 mL of methanol, 20 mL of deionized water, and dried at 60°C in an oven for 6 h.

The SiO_2/Fe_3O_4 particles were washed with 5.0 mL of 2-propanol before being incubated in 5.0 mL of pyrrole monomer for 1 h then residue monomer was removed from the sorbent. To polymerize the pyrrole, 0.64 g of $FeCl_3$ (oxidant) was dissolved in 20 mL of 2-propanol and added into the pyrrole/SiO_2/Fe_3O_4 in a rotator tube. The polymerization was completed on a rotator mixer for 9 h. The polypyrrole/SiO_2/Fe_3O_4 was then washed twice with 10 mL of 2-propanol, methanol, and deionized water, respectively. The particles were dried at 60°C in an oven for 6 h.

2.5 MSPE procedure

Polypyrrole/SiO_2/Fe_3O_4 nanoparticles were used as the MSPE sorbent in an extraction procedure as shown in Fig. 1B. The initial conditions used in the extraction were as follows. After 100 mg of polypyrrole/SiO_2/Fe_3O_4 the nanoparticles were conditioned in a vial with 2.0 mL of methanol and deionized water, respectively, 5.0 mL of spiked water sample was added and stirred for 30 min. The sorbents, with bound sulfonamides, were then separated using a magnet and the solution was descanted. The analytes were desorbed from the sorbent with 5.0 mL of methanol by sonication for 30 min. The desorption solvent was then evaporated to dryness at 50°C, redissolved in 0.5 mL of methanol and filtered through a PTFE filter (0.22 μm) for HPLC analysis.

To obtain the maximal extraction efficiency, the main affecting parameters were optimized by varying one parameter at a time, while the others were kept constant. These included the amount of the sorbent, sample pH, extraction time, desorption conditions, and effect of the ionic strength. Each experiment was done in triplicate. The extraction efficiency was evaluated in terms of recovery. The optimizations were performed using 5.0 mL of water sample spiked with a standard solution to obtain a final concentration of 20 μg/L for each sulfonamide.

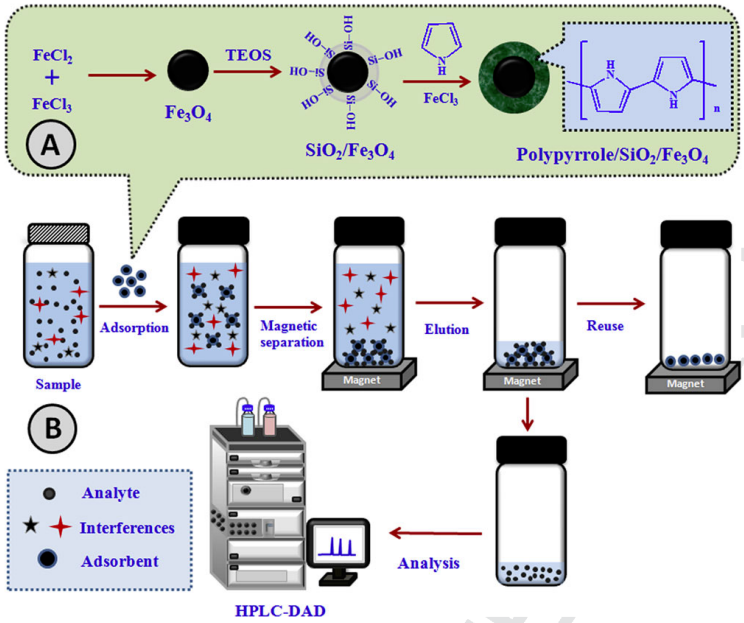

Figure 1. A schematic diagram representative the synthesis of polypyrrole/SiO_2/Fe_3O_4 nanoparticles sorbent (A) and the extraction of sulfonamides using the MSPE sorbent (B).

2.6 Water samples

The developed sorbent was applied to the extraction and preconcentration of sulfonamides in tap, canal, and lake water samples. Tap water was collected from a laboratory; other samples were collected from the Wong and U-Tapao canals in Hat Yai city, Songkhla, Thailand, and Songkhla Lake, Thailand. Each water sample was filtered through a 0.45 μm membrane to remove suspended particles and stored in a brown glass bottle at 4°C.

3 Results and discussion

3.1 Optimum HPLC conditions

The optimum HPLC conditions for the determination of sulfonamides were found to be, mobile phase: a mixture of 0.20% acetic acid and acetonitrile (70:30 v/v), flow rate: 0.70 mL/min, absorption wavelength: 270 nm. These conditions provided a good peak separation for sulfadiazine, sulfamerazine, sulfamethazine, and sulfamonomethoxine with retention times of 8.04, 9.63, 11.03, and 13.48 min, respectively.

3.2 Characterization of polypyrrole/SiO_2/Fe_3O_4

The FTIR spectra of the Fe_3O_4, SiO_2/Fe_3O_4, and polypyrrole/SiO_2/Fe_3O_4 nanoparticles are shown in Fig. 2A. Fe_3O_4 showed a characteristic peak at 587 cm^{-1} (Fe–O stretching). An absorption peak at 1083 cm^{-1} (Si–O–Si) indicated the formation of a silica coating on the Fe_3O_4 surface. The peaks at 1556 and 1480 cm^{-1} in Fig. 2A (c and d) were related to the absorption of the pyrrole ring, and indicated the successful coating of the polypyrrole onto the surface of SiO_2/Fe_3O_4 nanoparticles. The SEM micrograph of the polypyrrole/SiO_2/Fe_3O_4 sorbent (Fig. 2B) confirmed that the magnetic sorbents were fairly uniform in size and shape, with an average particle size of 70 ± 10 nm (n = 100). Figure 2C illustrates the dispersion and agglomeration processes of the polypyrrole/SiO_2/Fe_3O_4 nanoparticles. The homogeneously dispersed magnetic nanoparticles adhered to the side wall of the vials when the external magnetic field was applied and the solution became transparent within 1 min.

3.3 Optimization of the MSPE procedure

3.3.1 Type of sorbent

The extraction capabilities of the Fe_3O_4, SiO_2/Fe_3O_4, and polypyrrole/SiO_2/Fe_3O_4 are shown in Supporting Information Fig. 1. The recoveries of the sulfonamides significantly increased in the presence of polypyrrole, and indicated that the polypyrrole had a strong influence on the extraction process because it adsorbs sulfonamide by π–π and hydrophobic interactions.

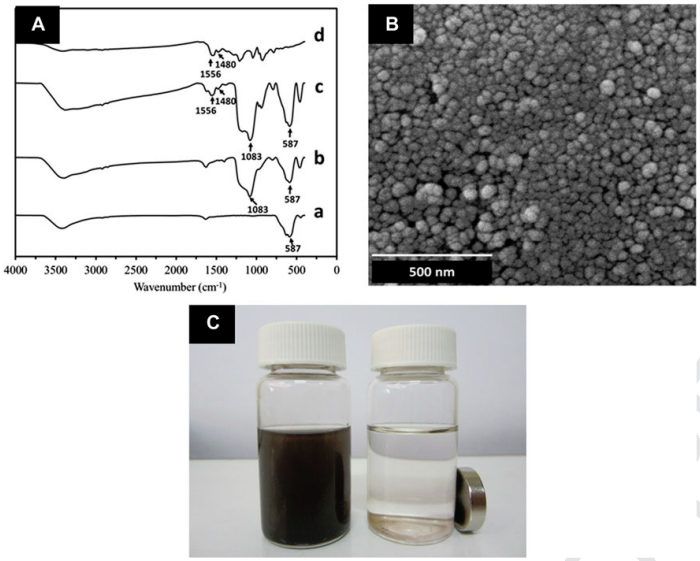

Figure 2. (A) FTIR spectra of Fe_3O_4 (a), SiO_2/Fe_3O_4 (b), polypyrrole/SiO_2/Fe_3O_4 (c), and polypyrrole (d), (B) SEM image of polypyrrole/SiO_2/Fe_3O_4, (C) the dispersion (left) and separation (right) process of polypyrrole/SiO_2/Fe_3O_4 sorbent by an external magnet.

3.3.2 Amount of sorbent

The effect of the amount of polypyrrole/SiO_2/Fe_3O_4 was investigated over the range of 10–100 mg and the recoveries increased with the sorbent amount from 10 to 20 mg, and then remained constant (Supporting Information Fig. 2). That is, 20 mg of sorbent was sufficient for the extraction of sulfonamides and 20 mg was selected for subsequent experiments. This is much less than a traditional C_{18} SPE cartridge, using ca. 50–100 mg of sorbents. The result indicated that the developed sorbent had a high adsorption efficiency, and a satisfactory extraction efficiency was achieved by using a much lower amount than any previous commercial SPE sorbent.

3.3.3 pH of the sample

Sample pH plays an important role in the adsorption of target analytes on the sorbent. Its influence was investigated in the range of pH 3.0–9.0 by adjusting with HCl or NaOH. At a pH 6.0 and 7.0, in which sulfonamides are in a neutral form [14], a high recovery of > 80% was obtained for all tested sulfonamides (Fig. Supporting Information 3). At a sample pH lower than 6.0, the extraction efficiency was reduced, probably because of the protonation of the amine group of the analyte [15] making it more difficult to form a hydrogen bond with the sorbent. The extraction efficiency also decreased at a pH value above 7.0 but as a result of the anionic nature of sulfonamides since they would become more negatively charged [14] hence, the hydrophobic interactions between sulfonamides and the sorbent would be reduced. Since, the water samples pH values are normally in the range of 6–7, there is normally no need to adjust their pH values.

3.3.4 Extraction time

Extraction time, the time required for the adsorption of the analyte from the sample solution into the sorbent, was also important. The extraction time was investigated in the range of 2–40 min to achieve the best extraction efficiency with the shortest analysis time. The extraction efficiency increased with extraction time from 2 to 20 min, after which it remained almost constant (Supporting Information Fig. 4). Therefore, the extraction time of 20 min was selected for further studies.

3.3.5 Desorption temperature

The effect of the desorption temperature was studied from 25 to 50°C in which the extraction efficiency increased with the desorption temperature and reached the highest level at 45°C (Supporting Information Fig. 5). A lower efficiency at a lower desorption temperature may be caused by a slower diffusion rate of the analyte from the sorbent to the desorption solvent while the higher temperature may result in the volatilization of the desorption solvent, thus, the solvent volume was reduced. Therefore, 45°C was used for further experiments.

3.3.6 Desorption solvent, volume, and time

The selection of an appropriate solvent to desorb the analytes from the sorbent before instrumental analysis was important in a MSPE procedure. Since, the adsorption of sulfonamides onto the polypyrrole/SiO_2/Fe_3O_4 nanoparticles was based on π–π and hydrophobic interactions, the desorption solvents of different polarities needed to be considered. Methanol, acetonitrile, acetone, propanol, ethyl acetate, and hexane were investigated. The results indicated that when

Table 1. Recoveries of sulfonamides in real water samples ($n = 5$)

Water samples	Added (μg/L)	Recovery (%)			
		SDZ	SMZ	SMT	SMX
Tap water	5	93.8 ± 4.2	94.8 ± 2.7	97.1 ± 1.4	95.6 ± 3.7
	20	89.8 ± 5.3	93.7 ± 4.3	96.6 ± 2.4	94.2 ± 3.6
	100	91.8 ± 4.5	86.7 ± 3.1	90.8 ± 5.3	90.2 ± 4.1
Canal water	5	94.8 ± 4.0	95.5 ± 2.0	93.6 ± 3.7	94.2 ± 4.2
	20	89.2 ± 2.0	92.0 ± 1.0	93.4 ± 3.8	91.8 ± 3.0
	100	86.7 ± 3.0	90.0 ± 3.0	93.0 ± 1.5	91.2 ± 4.8
Lake water	5	95.0 ± 2.9	99.7 ± 4.6	93.7 ± 4.2	97.0 ± 1.6
	20	97.7 ± 2.5	91.9 ± 1.0	96.2 ± 4.2	95.4 ± 3.3
	100	93.0 ± 2.1	90.1 ± 4.9	87.6 ± 3.0	92.1 ± 3.1

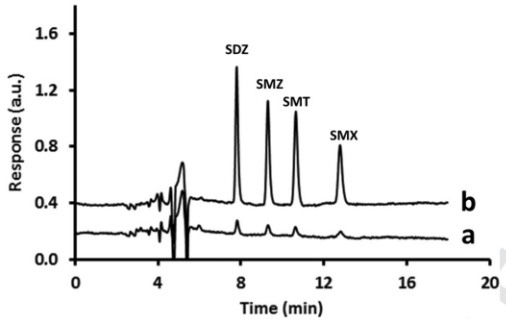

Figure 3. Chromatograms of spiked water sample canal 1 (10.0 μg/L) without extraction (A) and with extraction using polypyrrole/SiO_2/Fe_3O_4 sorbent (B).

a slightly nonpolar (propanol, ethyl acetate) and a nonpolar (hexane) solvents were used a low desorption efficiency was obtained (Supporting Information Fig. 6) probably because the polypyrrole/SiO_2/Fe_3O_4 sorbent could not be dispersed well in these solvents. For the other three solvents, methanol provided the highest desorption efficiency with acetonitrile and acetone providing a slightly lower value. Therefore, methanol was employed. Another advantage of using methanol was that, after desorption the solvent must be removed and methanol evaporated faster than acetonitrile due to its lower boiling point.

The influence of the volume of methanol was also investigated. The results showed that all analytes could be completely desorbed from the sorbent by sonication with 3.0 mL of methanol (Supporting Information Fig. 7). As for the desorption time 20 min was sufficient to obtain the maximum desorption efficiency for all analytes (Supporting Information Fig. 8).

3.3.7 Effect of ionic strength

The mechanism of mass transfer of the analytes in the MSPE can be influenced by the ionic strength [16] because the solubility of analytes in the aqueous phase was reduced when the ionic strength was increased, and the analytes that partitioned into the adsorbent was enhanced [17]. The effect of ionic strength was investigated by varying the concentration of NaCl in the water sample over the range of 0–10% w/v. In this system, the ionic strength had a negative effect on the extraction efficiency (Supporting Information Fig. 9) probably because the increase of the salt concentration increased the solution viscosity, hence, this reduced the diffusion rates of analyte from the water to the sorbent, hence, there was decreased extraction efficiency [18]. Therefore, no salt was added to the sample solution.

3.4 Reproducibility and reusability

The preparation of the polypyrrole/SiO_2/Fe_3O_4 sorbent was investigated for batch-to-batch reproducibility. Six different batches were used to extract sulfonamides (20.0 μg/L) in spiked deionized water under the same conditions. The averages of the recoveries of the six batches were: sulfadiazine 88.2 ± 1.5%, sulfamerazine 86.7 ± 3.1%, sulfamethazine 86.5 ± 3.3%, and sulfamonomethoxine 87.8 ± 2.7% with RSDs in the range 1.7–3.9%. These were better than the acceptable values recommended by the AOAC (recovery = 80–110%, RSD = 32%), and indicated that the preparation procedure of the polypyrrole/SiO_2/Fe_3O_4 sorbent has a good reproducibility.

The reusability of the sorbent was also investigated. After desorption the used polypyrrole/SiO_2/Fe_3O_4 sorbent was washed by stirring for 1 min in 2.0 mL methanol and 2.0 mL deionized water, respectively. After washing the sorbent was tested with a blank sample where no HPLC response was observed indicated that there was no carryover of the analytes. The sorbent could be reused for up to 16 times (Supporting Information Fig. 10) while maintaining recoveries of sulfonamides >80% (acceptable value 80–110%) [19].

3.5 Comparative studies

The efficiency of the developed sorbent was compared to a commercial SPE sorbent, HLB. HLB was conditioned with

Table 2. Comparison of the developed method with other methods for the determination of sulfonamides

Extraction method	Sample	Extraction time (min)	Sample volume (mL)	Extractants	LOD (μg/L)	Recovery (%)	References
Dispersive liquid–liquid microextraction	Water	3	5	Chloroform	0.41–9.87	78–117	[8]
Liquid–liquid–liquid microextraction	Water	45	12	Organic solvent	0.11–0.77	86–109	[5]
Micro-solid phase extraction (Molecularly imprinted polymer)	Water	20	4.5	MIP sorbent	0.2–3.0	70–120	[20]
In-line solid phase extraction	Water	15	4.75	HLB particles	0.38–0.56	52–109	[21]
Solid phase extraction	Water	25	250	HLB cartridges	0.15–1.0	70–104	[6]
Stir bar sorptive extraction	Milk	10	4	C_{18}	0.9–10.5	68–120	[22]
Stir bar sorptive extraction	Milk	60	50	Monolithic material	1.3–7.9	55–126	[23]
Magnetic solid phase extraction	Water	20	1	Fe_3O_4/Graphene oxide	50–100	67–120	[24]
Magnetic solid phase extraction	Water	20	5	polypyrrole/SiO_2/Fe_3O_4	0.3–1.0	87–100	This work

2.0 mL of methanol followed by 2.0 mL of deionized water and then loaded with 5.0 mL of spiked water sample (20 μg/L of sulfonamides), desorbed with 3.0 mL of methanol and detected by HPLC. Similar recoveries were obtained (Supporting Information Fig. 11), and the average recoveries of the two sorbents were compared statistically by the paired *t*–test and there were no significant difference between the two sorbents ($p > 0.05$). The advantages of the developed method over the traditional SPE sorbent are that they were simpler to use and required a shorter extraction time. In addition, the polypyrrole/SiO_2/Fe_3O_4 sorbent could be reused for a maximum of 16 times, whereas the commercial HLB sorbent cannot be reused.

3.6 Analytical performance

The performances of the developed method, i.e. linearity, LOD, and LOQ, were investigated under the optimal conditions. The calibration plot of the peak areas versus sulfonamides concentrations in spiked deionized water provided a wide and good linearity ($r^2 > 0.997$) (Supporting Information Table 1). The RSDs for each concentration were less than 10%. While the LOD (S/N = 3) and LOQ (S/N = 10) were also very low in the μg/L range (Supporting Information Table 1).

3.7 Real sample analysis

To assess the practical applicability of the developed method, it was applied to determine sulfonamides in tap, canal, and lake water samples. The chromatograms of the spiked water samples (10.0 μg/L) without extraction and with extraction using polypyrrole/SiO_2/Fe_3O_4 sorbent are shown in Fig. 3. In the real water samples, only a low concentration of sulfadiazine was detected in the canal and lake water samples (Supporting Information Table 2).

To evaluate the accuracy of the developed method, the water samples were spiked with the sulfonamides standard solutions to obtain the concentrations of 5.0, 20.0, and 100 μg/L. The recoveries of all tested sulfonamides in the water samples were in the range of 86.7–99.7% (Table 1) with the RSDs of less than 6%. The recoveries were in the acceptable range of 80–110% [19] and indicated that there was no effect from the matrix composition of the water samples. It can be concluded that the developed sorbent was suitable for the extraction and determination of trace sulfonamides in real water samples.

3.8 Comparison of the developed method with other methods

Several sample preparation methods have been reported for the analysis of sulfonamides in various sample matrices and the performances of these methods are summarized in Table 2. In the case of the LODs, the values from this work were within the same range [5, 6, 20, 21] or better than some methods [8, 22–24]. However, for two of these that have similar LODs [5, 6] they required a longer extraction time and larger sample volumes. For the other two [20, 21] although the extraction conditions were similar to the developed method, their recoveries were not so good. When the recoveries were considered, the performance of the developed method was

either comparable [5,6] or better than [20–23] the other methods. This clearly indicates that the developed method has high extraction efficiency and sensitivity. This is because the target analytes in the solution can be easily adsorbed onto the sorbent that provided a large adsorption capacity. The other advantage is that the sorbent can be separated rapidly from the sample solutions using an external magnetic field. Moreover, it can be reused for at least 16 times, which helps to reduce analysis costs and time. This indicates that the proposed new method has a better accuracy and is also probably much cheaper to use.

4 Conclusions

In this work, a MSPE polypyrrole/SiO_2/Fe_3O_4 sorbent was developed and successfully used for the extraction of sulfonamides from in water samples, followed by HPLC analysis. The magnetic property provided a convenient and fast separation of the sorbent from the water sample by applying an external magnetic field. Other advantages included it was simple to prepare with a relatively low cost (0.4 USD per sample) and could be reused or at least 16 times without loss of extraction efficiency, so the total cost of time and analysis was reduced. In addition, the developed method provided a low detection limit, good accuracy, precision, and reproducibility that were suitable for the determination of trace sulfonamide contamination in environmental water samples. This could certainly be applied for the determination of other aromatic compounds such as polycyclic aromatic hydrocarbons.

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The authors declare that there are no conflicts of interest.

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Supporting Information

A polypyrrole/silica/magnetite nanoparticles as a sorbent for the extraction of sulfonamides from water samples

Thanyaporn Sukchauy[1,2,3], Proespichaya Kanatharana[1,2,3], Rodtichoti Wannapob[1,2,3], Panote Thavarungkul[1,2,4] and Opas Bunkoed[1,2,3*]

[1]Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[2]Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[3]Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[4]Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

***Corresponding author:** Opas Bunkoed

Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

Email address: opas.b@psu.ac.th; Fax: +66 7455 8841

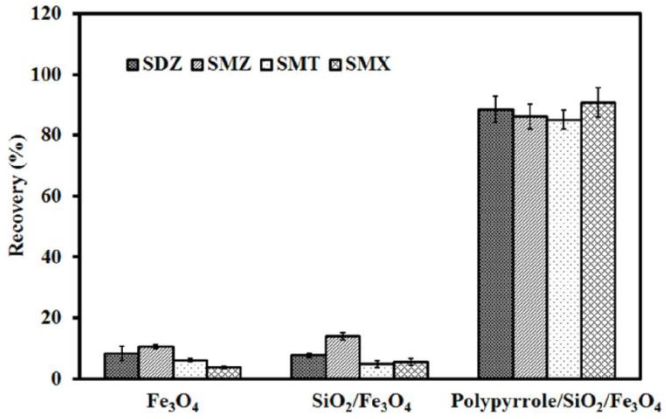

Fig. S1 Effect of different sorbents on the recoveries of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

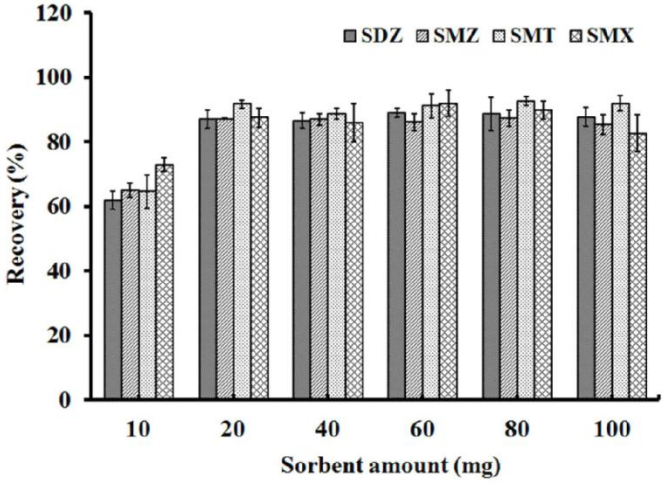

Fig. S2 The effect of the amount of polypyrrole/SiO_2/Fe_3O_4 sorbent on the extraction efficiency of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

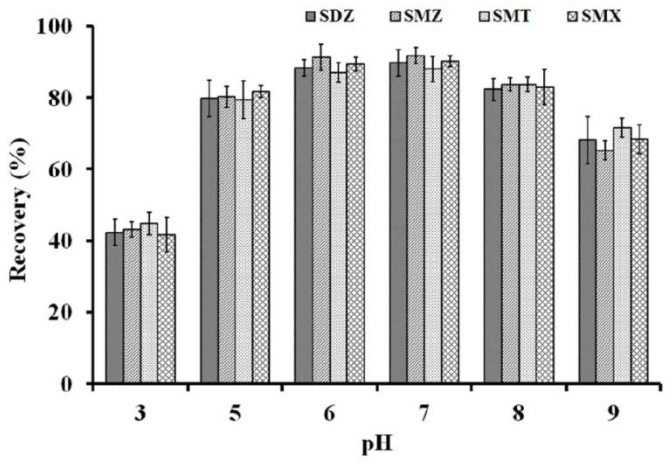

Fig. S3 Effect of the water sample pH on the extraction of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

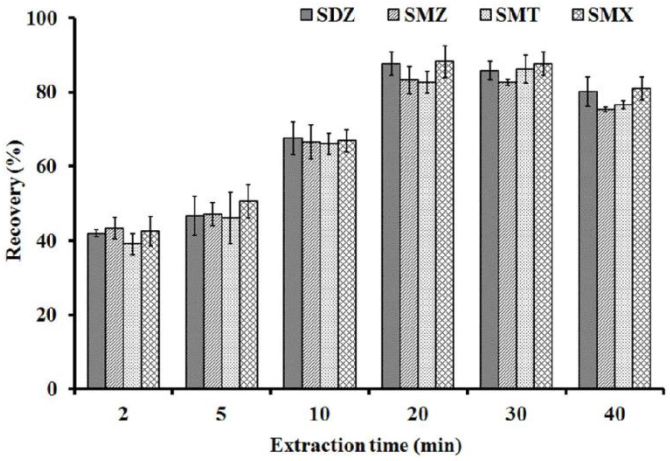

Fig. S4 The effect of the extraction time on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

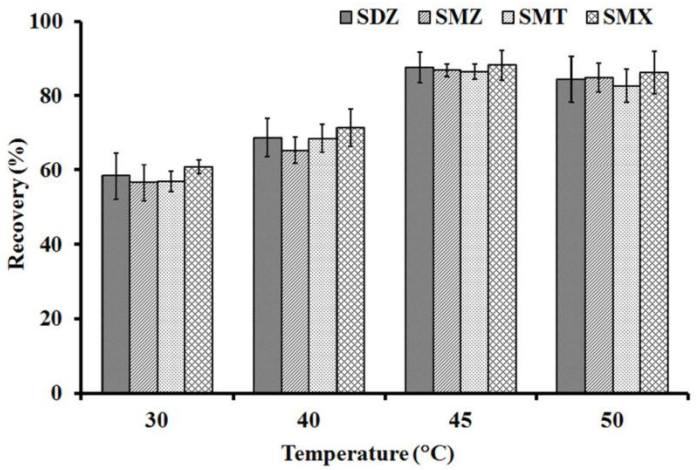

Fig. S5 Effect of the desorption temperature on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

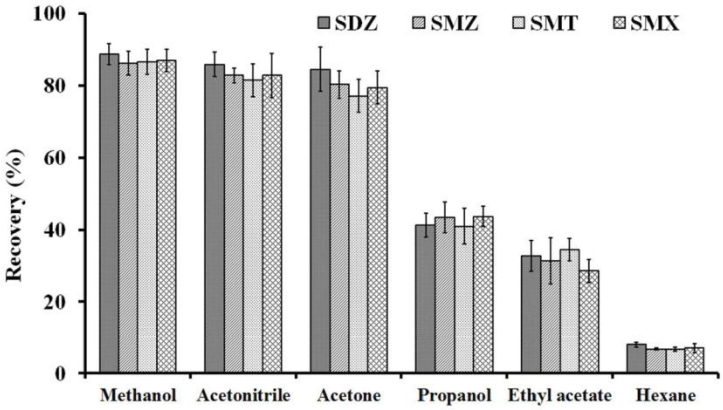

Fig. S6 Effect of the desorption solvent on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

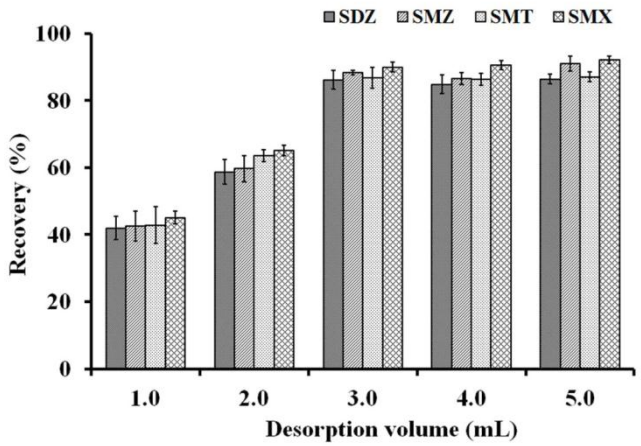

Fig. S7 Effect of the desorption volume on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

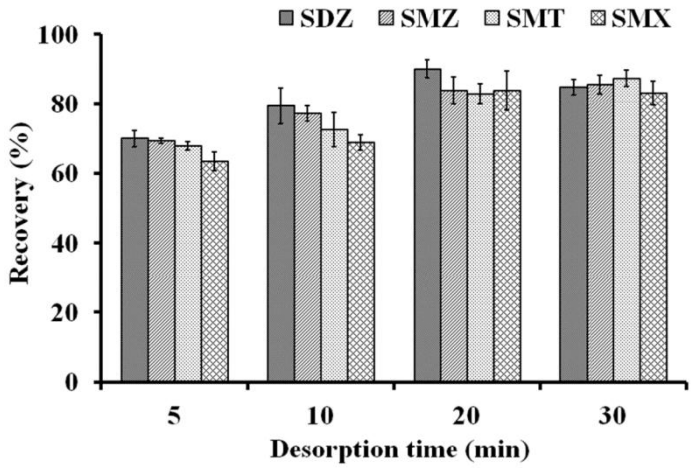

Fig. S8 Effect of desorption time on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

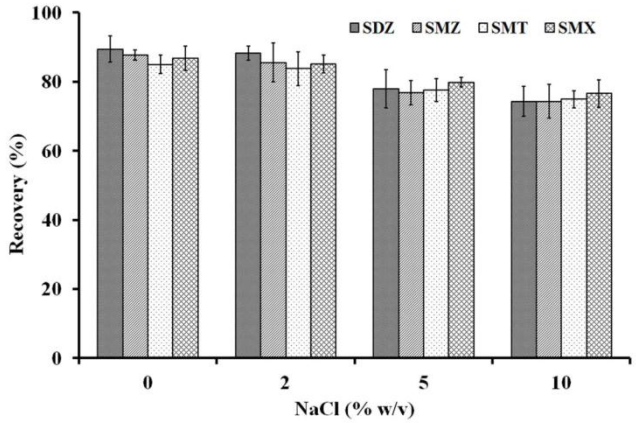

Fig. S9 The Effect of ionic strength on the recoveries of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

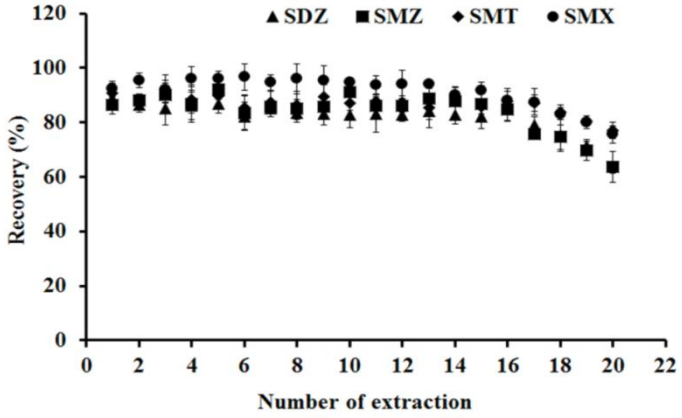

Fig. S10 Reusability of the polypyrrole/SiO_2/Fe_3O_4 sorbent for extraction of 20.0 μg L^{-1} of sulfonamides from spiked deionized water (n=5).

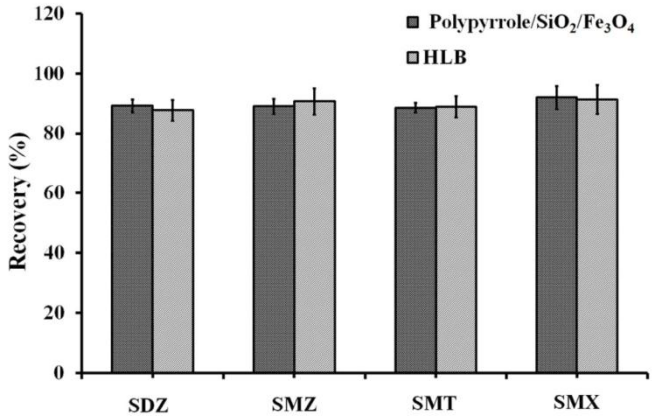

Fig. S11 The extraction efficiency of sulfonamides in spiked deionized water, a comparision between the polypyrrole/SiO_2/Fe_3O_4 sorbent and the conventional HLB SPE cartridge

Table S1. Analytical performance of the polypyrrole/SiO_2/Fe_3O_4 sorbent

Compounds	Linear range ($\mu g\ L^{-1}$)	Regression line equation	R^2	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)
SDZ	0.30 – 200	y=(0.6850±0.0071)x+(0.512±0.053)	0.9990	0.30	1.0
SMZ	0.30 – 200	y=(0.839±0.013)x+(1.29±0.92)	0.9978	0.30	1.0
SMT	1.0 – 200	y=(0.986±0.010)x+(0.95±0.73)	0.9992	1.0	4.0
SMX	1.0 – 200	y=(0.9950±0.0062)x-(0.04±0.43)	0.9997	1.0	4.0

Table S2. Concentration of sulfonamides determined in real water samples

Water sample	Concentration ($\mu g\ L^{-1}$)			
	SDZ	SMZ	SMT	SMX
Tap water 1	ND	ND	ND	ND
Tap water 2	ND	ND	ND	ND
Canal water 1	6.46 ± 0.51	ND	ND	ND
Canal water 2	ND	ND	ND	ND
Lake water 1	7.50 ± 0.33	ND	ND	ND
Lake water 2	3.45 ± 0.81	ND	ND	ND

ND–Not detected

VITAE

Name Mrs. Thanyaporn Sukchuay

Student ID 5610220037

Education Attainment

Degree	**Name of Institution**	**Year of Graduation**
Bachelor of Science (Agro-Industry)	Prince of Songkla University	1995

Scholarship Awards during Enrolment

Faculty of Science, Prince of Songkla University (grant no. SCI580881S), The Center of Excellence for Innovation in Chemistry (PERCH-CIC), the National Research University Project of Thailand, Trace Analysis and Biosensor Research Center, the Commission on Higher Education, Ministry of Education and Graduate School.

Work-Position and Address

Food Technologist at Songkhla Fish Inspection and Research Center, Fish Inspection and Quality Control Division, Department of Fisheries, Ministry of Agriculture and Cooperatives

79/2 Wichieanchom Road, Tambon Boyang, Muang Songkhla, Thailand

List of Publication

Sukchuay, T., Kanatharana, P., Wannapob, R., Thavarungkul, P. and Bunkoed, O. "A polypyrrole/silica/magnetite nanoparticles as a sorbent for the extraction of sulfonamides from water samples." Accepted manuscript online : 8 Sep 2015/ DOI : 10.1002/jssc.201500766.