รายงานวิจัยฉบับสมบูรณ์

พัฒนาตัวดูดซับบนอนุภาคแม่เหล็กสำหรับวิเคราะห์ซัลโฟนาไมด์

Development of a magnetic solid phase extraction sorbent for the determination of sulfonamide

ผศ. ดร. โอภาส บุญเกิด

โครงการวิจัยนี้ได้รับทุนสนับสนุนจากเงินงบประมาณแผ่นดิน
มหาวิทยาลัยสงขลานครินทร์
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Development of a magnetic solid phase extraction sorbent for the determination of sulfonamide

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กิตติกรรมประกาศ

โครงการวิจัยนี้ได้รับสนับสนุนจากเงินงบประมาณแผ่นดิน มหาวิทยาสงขลานครินทร์ สัญญาเลขที่ SCI580881S ผู้วิจัยขอขอบคุณสถานวิจัยการวิเคราะห์สารปริมาณน้อยและไบโอเซนเซอร์ ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์สำหรับสถานที่ทำวิจัยและเครื่องมือวิจัยที่เกี่ยวข้อง

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พฤษภาคม 2559

บทคัดย่อ

ตัวดูดซับของแข็งอนุภาคแม่เหล็กชนิดใหม่ พอลิไพโรลเคลือบซิลิกาและอนุภาคแม่เหล็กนาโนได้ถูกสังเคราะห์ขึ้นสำหรับสกัดและเพิ่มความเข้มข้นสารซัลโฟนาไมด์ในตัวอย่างน้ำ โดยอนุภาคแม่เหล็กขนาดนาโนช่วยให้การแยกตัวดูดซับออกจากน้ำตัวอย่างได้ง่ายและรวดเร็ว ชั้นซิลิกาช่วยเพิ่มพื้นที่ผิวทำให้สามารถเคลือบพอลิไพโรลได้มากขึ้น ชั้นพอลิไพโรลที่เคลือบอยู่บนซิลิกาช่วยเพิ่มประสิทธิภาพการสกัดสารซัลโฟนาไมด์โดยเกิดอันตรกิริยาแบบ π-π และไฮโดรโฟบิก เพื่อให้ได้ประสิทธิภาพของการสกัดที่ดีที่สุด ได้ศึกษาปัจจัยต่างๆ ดังนี้ ปริมาณตัวดูดซับ พีเอชของสารตัวอย่าง เวลาและอุณหภูมิของการสกัด ความแรงของไอออนในตัวอย่าง และสภาวะที่เหมาะสมของการคายการดูดซับ ภายใต้สภาวะที่เหมาะสม วิธีที่พัฒนาขึ้นมีช่วงความเป็นเส้นตรงในช่วง 0.30 ถึง 200 ไมโครกรัมต่อลิตร สำหรับสารซัลฟาไดอะซีนและซัลฟาเมราซีนและมีช่วงความเป็นเส้นตรงตั้งแต่ 1.0 ถึง 200 ไมโครกรัมต่อลิตรสำหรับสารซัลฟาเมธาซีนและซัลฟาโมโนเมท๊อกซีน โดยมีขีดจำกัดการตรวจวัดเท่ากับ 0.30 ไมโครกรัมต่อลิตร สำหรับสารซัลฟาไดอะซีนและซัลฟาเมราซีนและ 1.0 ไมโครกรัมต่อลิตร สำหรับสารซัลฟาเมธาซีนและซัลฟาโมโนเมท๊อกซีน วิธีที่พัฒนาขึ้นสามารถทำได้ง่ายและรวดเร็ว โดยมีประสิทธิภาพสูงในการสกัดสารซัลโฟนาไมด์ทั้ง 4 ชนิดซึ่งมีร้อยละการได้กลับคืนอยู่ในช่วง 86.7 ถึง 99.7 เปอร์เซนต์ โดยมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 6 เปอร์เซนต์

Abstract

A magnetic solid phase extraction sorbent of polypyrrole/silica/magnetite nanoparticles were 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^{-1} for sulfadiazine and sulfamerazine, and 1.0-200 μg L^{-1} for sulfamethazine and sulfamonomethoxine. The limits of detections were 0.30 μg L^{-1} for sulfadiazine and sulfamerazine and 1.0 μg L^{-1} for sulfamethazine and sulfamonomethoxine. This simple and rapid method was successfully applied to efficiently extract sulfonamides from water samples. It showed 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 %.

บทสรุปผู้บริหาร
(Executive Summary)

บทนำ

ปัจจุบันมีการใช้ยาปฏิชีวนะกันอย่างแพร่หลายในวงการแพทย์และเกษตรกรรมซึ่งมีการนำมาใช้ในการรักษาโรคของคนและสัตว์ แต่หากมีการใช้เกินความจำเป็น หรือผิดวิธี อาจทำให้ประสบปัญหาการดื้อยา ทำให้รักษาโรคไม่ได้ผล ดังนั้นจึงจำเป็นต้องมีการตรวจวิเคราะห์การตกค้างของยาปฏิชีวนะเหล่านี้ทั้งในอาหารและสิ่งแวดล้อม โดยเทคนิควิเคราะห์ที่นิยมใช้ได้แก่ เทคนิคแก๊สโครมาโทกราฟีและเทคนิคโครมาโทรกราฟีของเหลวสมรรถนะสูง แต่เนื่องจากการตกค้างของยาปฏิชีวนะในสิ่งแวดล้อมและในอาหารมีปริมาณน้อยและในตัวอย่างมีตัวรบกวนมาก ดังนั้นจึงจำเป็นต้องมีขั้นตอนการเตรียมตัวอย่างที่เหมาะสมก่อนการวิเคราะห์ ซึ่งเทคนิคที่มีการนำมาใช้ในการเตรียมตัวอย่าง ได้แก่ เทคนิคการสกัดด้วยตัวทำละลาย (Liquid Liquid Extraction) เทคนิคการสกัดด้วยตัวดูดซับของแข็ง (Solid Phase Extraction: SPE), การสกัดด้วยตัวดูดซับของแข็งปริมาณน้อย (Solid Phase Microextraction: SPME) แต่เทคนิคดังกล่าวมีข้อด้อย คือมีขั้นตอนการสกัดที่ยุ่งยาก ใช้สารอินทรีย์ปริมาณมากและใช้เวลาในการสกัดนาน

ดังนั้นโครงการวิจัยนี้จึงสนใจพัฒนาตัวดูดซับชนิดใหม่บนอนุภาคแม่เหล็ก (Magnetic solid phase extraction) ที่เคลือบด้วยซิลิกาและวัสดุพอลิเมอร์ชนิดพอลิไพโรล (Fe_3O_4/SiO_2/polypyrrole) เพื่อเพิ่มขีดความสามารถในการตรวจวิเคราะห์สารซัลโฟนาไมด์ที่ปนเปื้อนปริมาณน้อย

วัตถุประสงค์

แพื่อพัฒนาตัวดูดซับบนอนุภาคแม่เหล็กแบบใหม่สำหรับวิเคราะห์ยาปฏิชีวนะกลุ่มซัลโฟนาไมด์ที่ตกค้างปริมาณน้อยด้วยเทคนิคโครมาโทรกราฟีของเหลวสมรรถนะสูง

สรุป

โครงการวิจัยนี้เป็นการพัฒนาตัวดูดซับของแข็งอนุภาคแม่เหล็กชนิดใหม่ โดยใช้พอลิไพโรลเคลือบซิลิกาและอนุภาคแม่เหล็กนาโนสำหรับสกัดและเพิ่มความเข้มข้นสารซัลโฟนาไมด์ในตัวอย่างน้ำร่วมกับเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง โดยอนุภาคแม่เหล็กขนาดนาโนช่วยให้สามารถแยกตัวดูดซับออกจากน้ำตัวอย่างได้ง่ายและรวดเร็วโดยใช้แท่งแม่เหล็ก ชั้นซิลิกาช่วยเพิ่มพื้นที่ผิวทำให้สามารถเคลือบพอลิไพโรลได้มากขึ้นและชั้นพอลิไพโรลที่เคลือบอยู่บนซิลิกาช่วยเพิ่มประสิทธิภาพการสกัดสารซัลโฟนาไมด์โดยเกิดอันตรกิริยาแบบ π-π และไฮโดรโฟบิก จากการศึกษาสภาวะที่เหมาะของเทคนิคโครมาโทรกราฟีของเหลวสมรรถนะสูงในการตรวจวิเคราะห์สารซัลโฟนาไมด์ทั้ง 4 ชนิด คือ ใช้อะซีโตไนไตรน์และกรดอะซิติก (0.2 %) เป็นเฟสเคลื่อนที่ในอัตราส่วน 70:30 เปอร์เซนต์โดยปริมาตร อัตราการไหล 0.7 มิลลิลิตรต่อนาที วัดค่าการดูดกลืนแสงที่ความยาวคลื่น 270 นาโนเมตร และปริมาตรสารตัวอย่าง 20 ไมโครลิตร เพื่อให้ได้ประสิทธิภาพของการ

จากการศึกษาเปรียบเทียบประสิทธิภาพการสกัดสารซัลโฟนาไมด์ของตัวดูดซับที่พัฒนาขึ้นกับตัวดูดซับทางการค้าพบว่าตัวดูดซับที่พัฒนาขึ้นให้ร้อยละการได้กลับคืน 84-95 เปอร์เซ็นต์ และตัวดูดซับทางการค้าให้ร้อยละการได้กลับคืน 84 ถึง 96 เปอร์เซ็นต์ ซึ่งตัวดูดซับทั้งสองให้ผลที่ไม่แตกต่างกันอย่างมีนัยสำคัญ ดังนั้นสามารถสรุปได้ว่าวิธีที่พัฒนาขึ้นสามารถนำมาใช้ในการสกัดสารซัลโฟนาไมด์ในตัวอย่างน้ำได้ โดยมีข้อดีกว่าตัวดูดซับทางการค้า เช่น มีราคาถูก ใช้งานได้ง่าย นอกจากนี้ตัวดูดซับที่พัฒนาขึ้นสามารถนำกลับมาใช้ซ้ำได้ 16 ครั้ง โดยยังคงให้ประสิทธิภาพในการสกัดสารซัลโฟนาไมด์มากกว่า 80 เปอร์เซ็นต์แสดงว่าตัวดูดซับที่พัฒนาขึ้นมีความเสถียรที่ดี

ภาคผนวก

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Research Article

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 solid-phase extraction (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

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Abbreviations: MSPE, magnetic solid-phase extraction; **SDZ**, sulfadiazine; **SMZ**, sulfamerazine; **SMT**, sulfamethazine; **SMX**, sulfamonomethoxine; **TEOS**, tetraethylorthosilicate

used because it contains a conjugated π structure 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

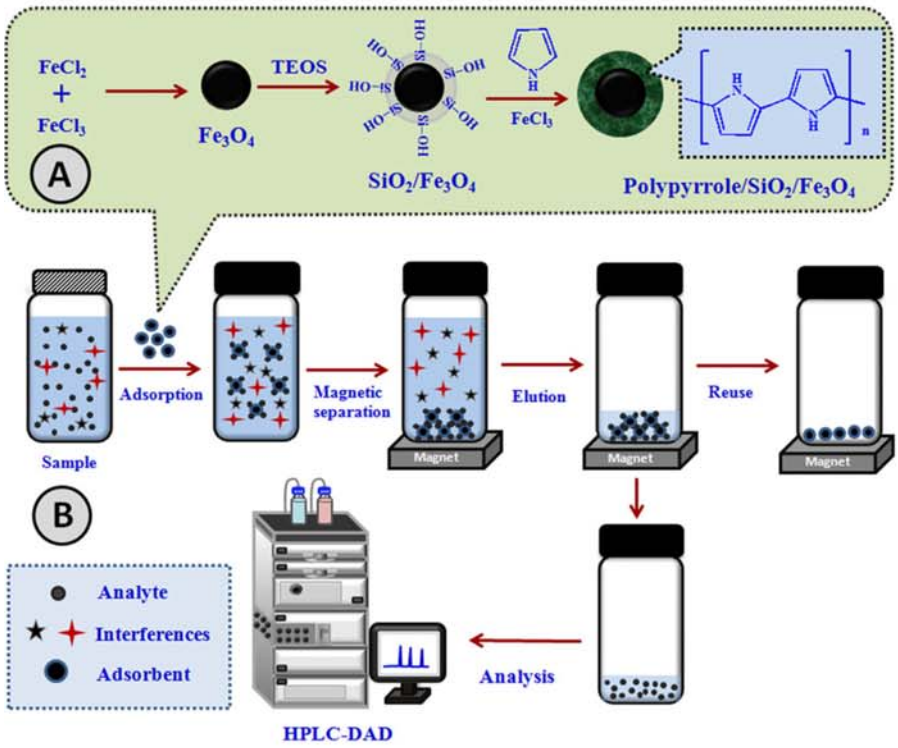

Figure 1. A schematic diagram representative the synthesis of polypyrrole/SiO_2/Fe_3O_4 nanoparticles sorbent (A) and the extraction of sulfonamides using the MSPE sorbent (B).

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.

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. S1. The recoveries of the sulfonamides significantly

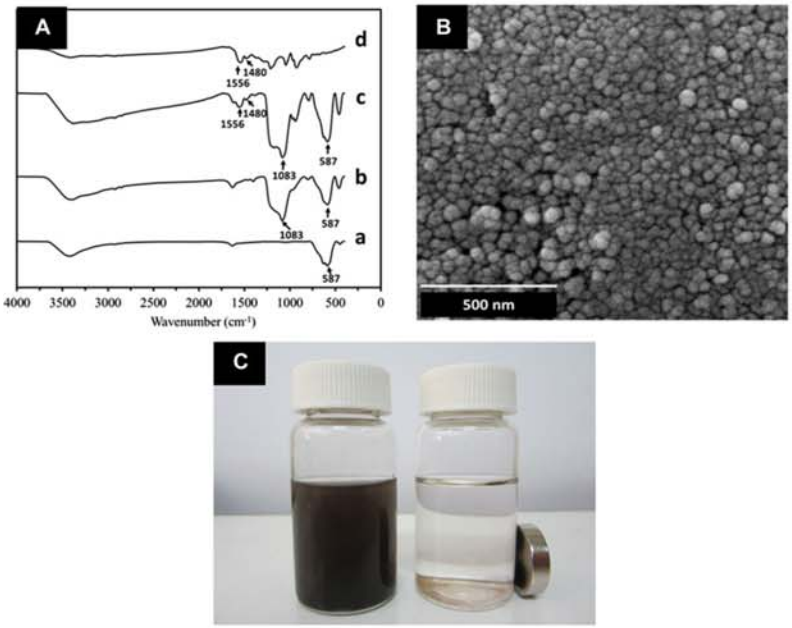

Figure 2. (A) FTIR spectra of Fe_3O_4 (a), SiO_2/Fe_3O_4 (b), polypyrrole/SiO_2/Fe_3O_4 (c), and polypyrrole (d), (B) SEM image of polypyrrole/SiO_2/Fe_3O_4, (C) the dispersion (left) and separation (right) process of polypyrrole/SiO_2/Fe_3O_4 sorbent by an external magnet.

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.

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. S2). 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 (Supporting Information Fig. S3). 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. S4). 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. S5). 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.

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

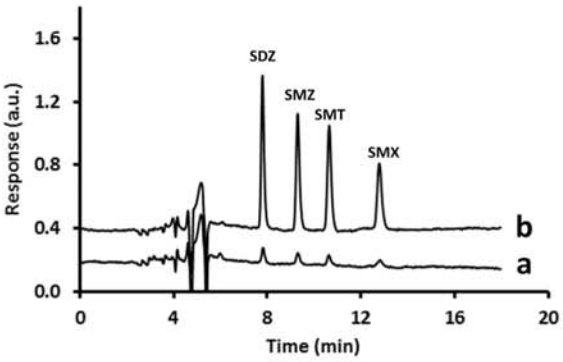

Figure 3. Chromatograms of spiked water sample canal 1 (10.0 μg/L) without extraction (A) and with extraction using polypyrrole/SiO_2/Fe_3O_4 sorbent (B).

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 a slightly nonpolar (propanol, ethyl acetate) and a nonpolar (hexane) solvents were used a low desorption efficiency was obtained (Supporting Information Fig. S6) 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. S7). As for the desorption time 20 min was sufficient to obtain the maximum desorption efficiency for all analytes (Supporting Information Fig. S8).

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. S9) 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

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

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. S10) 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 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. S11), 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 S1). 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 S1).

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 S2).

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.

5 References

[1] Dost, K., Jones, D. C., Davidson, G., *Analyst* 2000, *125*, 1243–1247.

[2] Lin, A. Y. C., Yu, T. H., Lin, C. F., *Chemosphere* 2008, *74*, 131–141.

[3] Li, J. D., Cai, Y. Q., Shi, Y. L., Mou, S. F., Jiang, G. B., *J. Chromatogr. A* 2007, *1139*, 178–184.

[4] Cribb, A. E., Miller, M., Tesoro, A., Spielberg, S. P., *Mol. Pharmacol.* 1990, *38*, 744–751.

[5] Lin, C. Y., Huang, S. D., *Anal. Chim. Acta* 2008, *612*, 37–43.

[6] Raich-Montiu, J., Folch, J., Compano, R., Granados, M., Prat, M. D., *J. Chromatogr. A* 2007, *1172*, 186–193.

[7] Shi, X., Meng, Y., Liu, J., Sun, A., Li, D., Yao, C., Lu, Y., Chen, J., *J. Chromatogr. A* 2011, *879*, 1071–1076.

[8] Herrera-Herrera, A. V., Hernández-Borges, J., Borges-Miquel, T. M., Rodríguez-Delgado, M. Á., *J. Pharm. Biomed. Anal.* 2013, *75*, 130–137.

[9] Payán, M. R., López, M. A. B., Fernández-Torres, R., Navarro, M. V., Mochón, M. C., *J. Chromatogr. B* 2011, *879*, 197–204.

[10] Wang, X., Chen, N., Han, Q., Yang, Z., Wu, J., Xue, C., Hong, J., Zhou, X., Jiang, H., *J. Sep. Sci.* 2015, *38*, 2167–2173.

[11] Zhang, N., Peng, H., Wang, S., Hu, B., *Microchim. Acta* 2011, *175*, 121–128.

[12] Hashemi, M., Taherimaslak, Z., Rashidi, S., *J. Chromatogr. A* 2014, *960*, 200–208.

[13] Abolghasemi, M. M., Parastari, S., Yousefi, V., *J. Sep. Sci.* 2014, *37*, 3526–3532.

[14] Herrera-Herrera, A. V., Hernandez-Borges, J., Afonso, M. M., Palenzuela, J. A., Rodriguez-Delgado, M. A., *Talanta* 2013, *116*, 695–703.

[15] Shao, M., Zhang, X., Li, N., Shi, J., Zhang, H., Wang, Z., Zhang, H., Yu, A., Yu, Y., *J. Chromatogr. B* 2014, *961*, 5–12.

[16] Rahimi, M., Noroozian, E., *Anal. Chim. Acta* 2014, *836*, 45–52.

[17] Bagheri, H., Roostaie, A., Baktash, M. Y., *Anal. Chim. Acta* 2014, *816*, 1–7.

[18] Tahmasebi, E., Yamini, Y., Seidi, S., Rezazadeh, M., *J. Chromatogr. A* 2013, *1314*, 15–23,

[19] AOAC. Guidelines for single-laboratory validation of chemical methods for dietary supplements and botanicals appendix K, 2012, 1–32.

[20] Diaz-Alvarez, M., Barahona, F., Turiel, E., Martin-Esteban, A., *J. Chromatogr. A* 2014, *1357*, 158–164.

[21] Lara, F. J., Garcia-Campana, A. M., Neususs, C., Ales-Barrero, F., *J. Chromatogr. A* 2009, *1216*, 3372–3379.

[22] Yu, C., Hu, B., *Talanta* 2012, *90*, 77–84.

[23] Huang, X., Qiu, N., Yuan, D., *J. Chromatogr. A* 2009, *1216*, 8240–8245.

[24] Shi, P., Ye, N., *Anal. Methods* 2014, *6*, 9725–9730.

Supporting Information

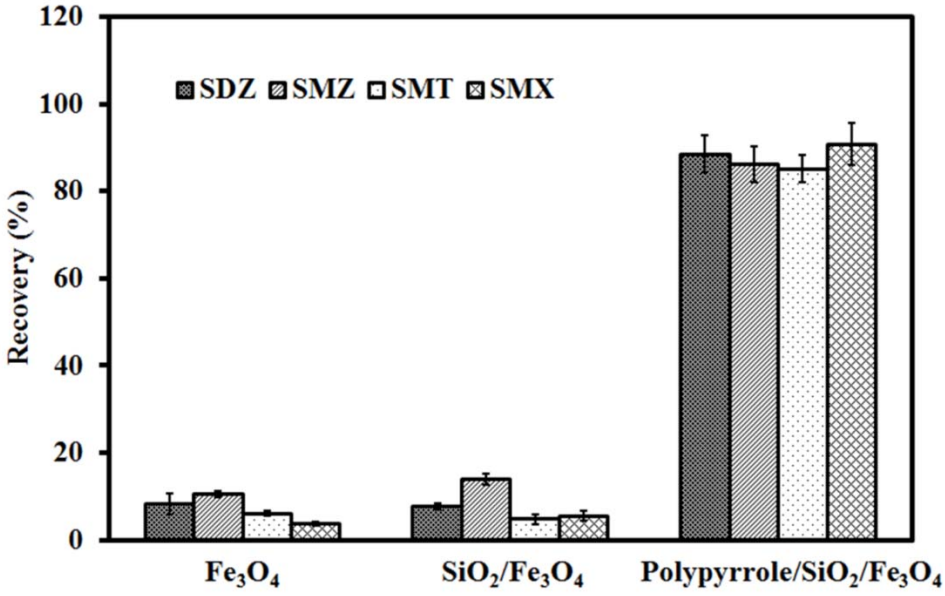

Fig. S1 Effect of different sorbents on the recoveries of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

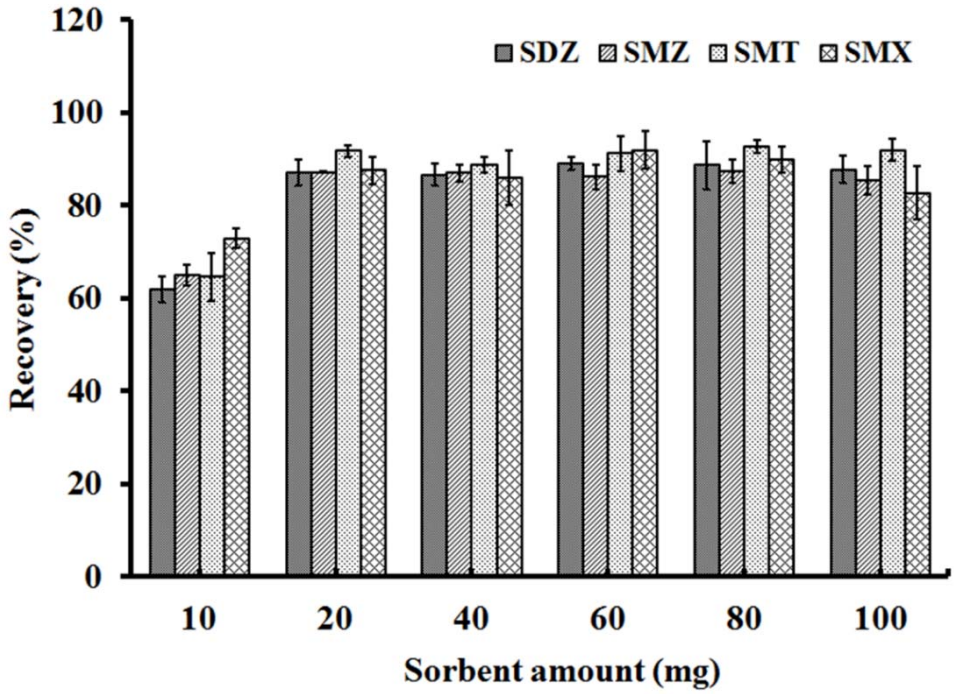

Fig. S2 The effect of the amount of polypyrrole/SiO_2/Fe_3O_4 sorbent on the extraction efficiency of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

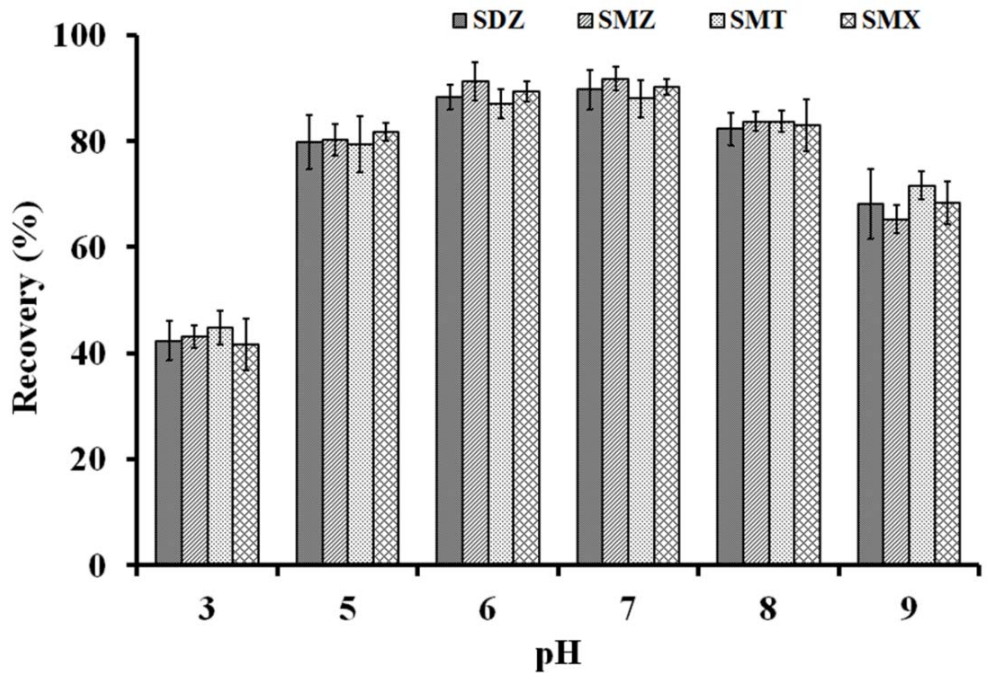

Fig. S3 Effect of the water sample pH on the extraction of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

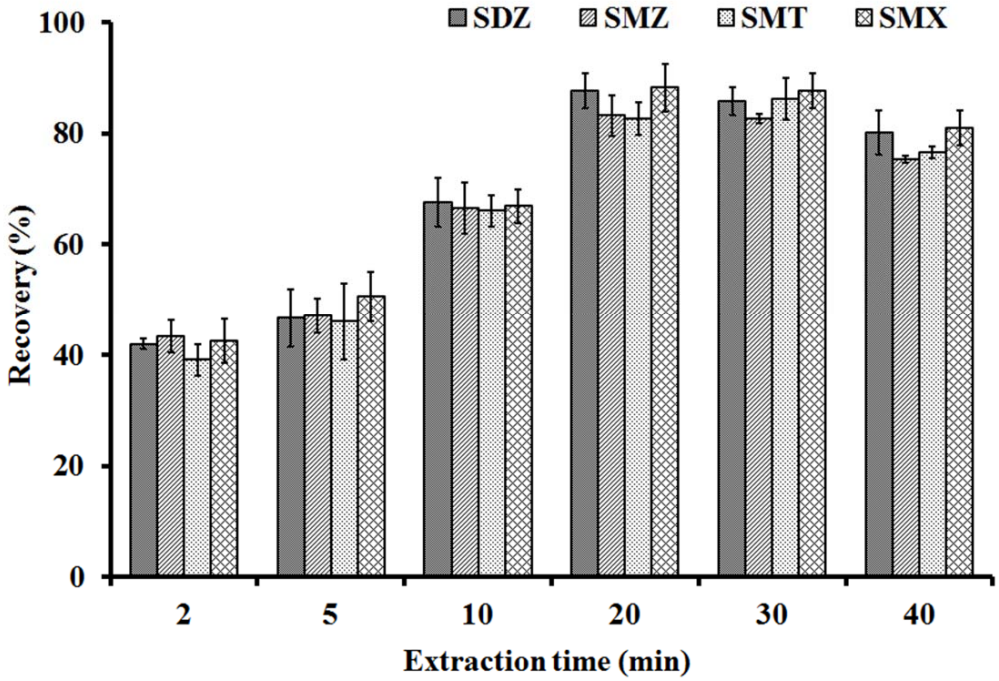

Fig. S4 The effect of the extraction time on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

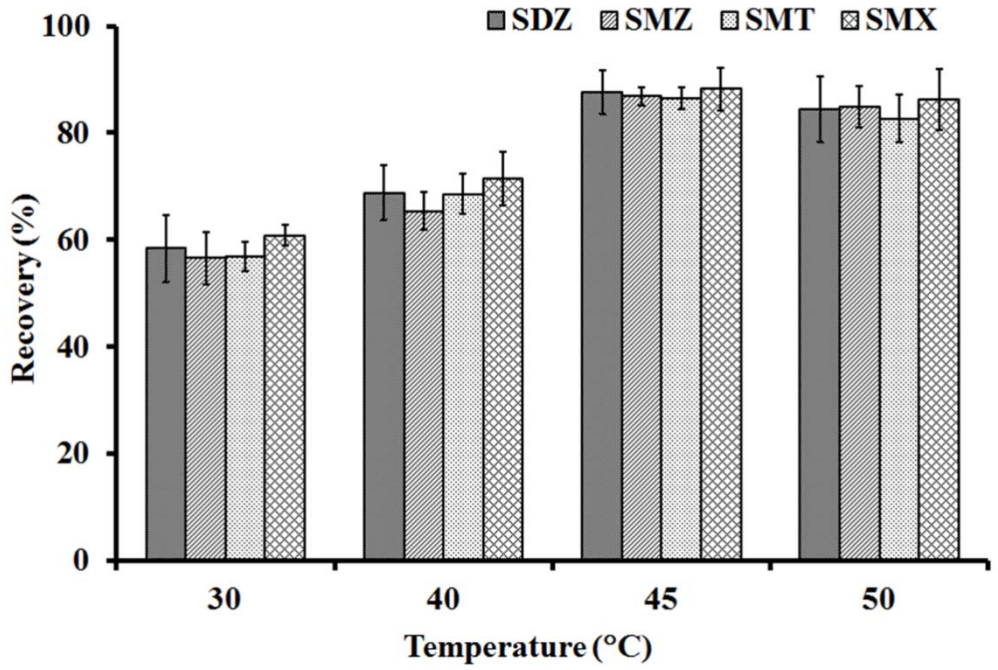

Fig. S5 Effect of the desorption temperature on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

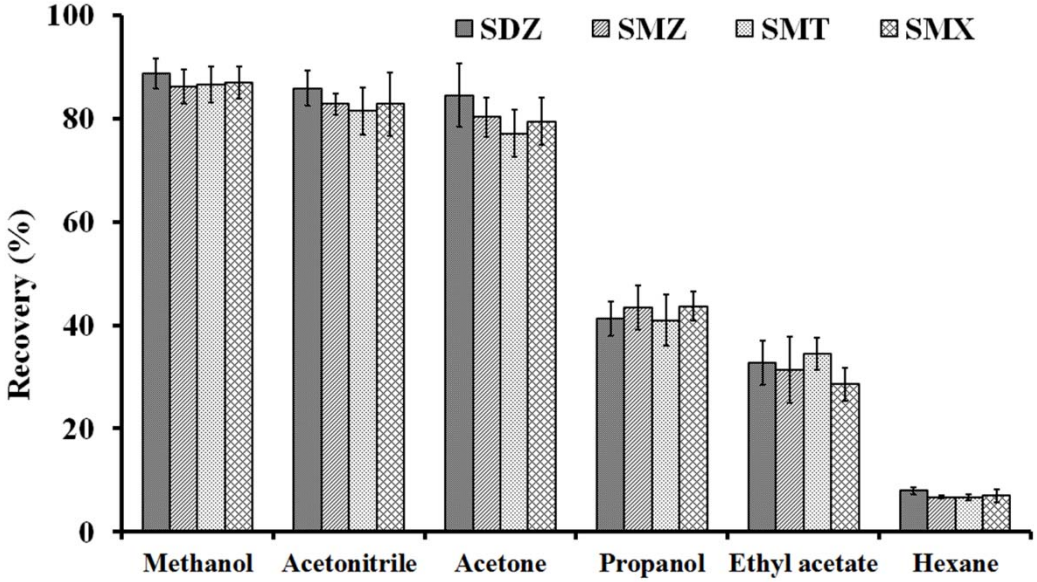

Fig. S6 Effect of the desorption solvent on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

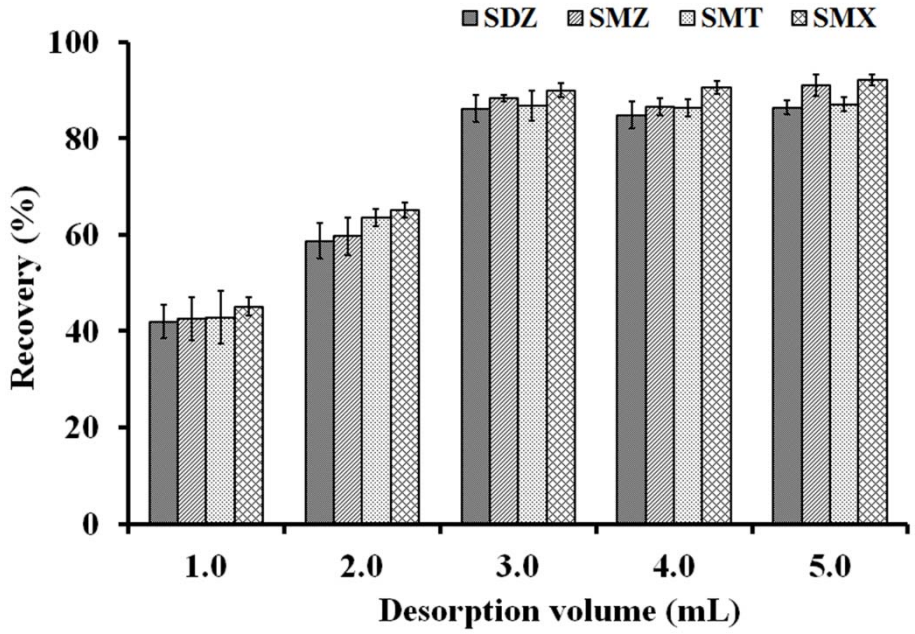

Fig. S7 Effect of the desorption volume on the recovery of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

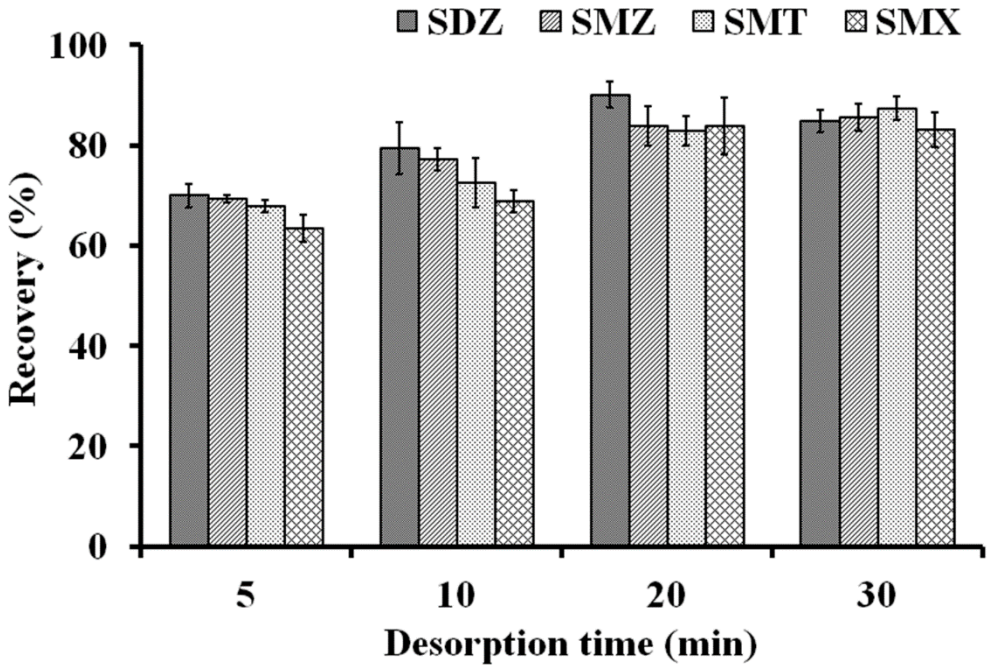

Fig. S8 Effect of desorption time on the recovery of sulfonamides**;** sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

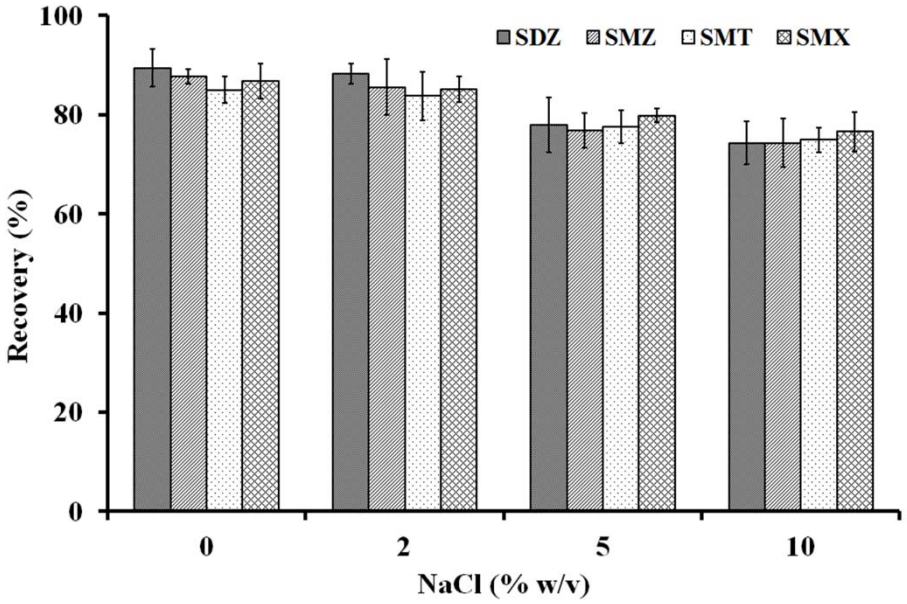

Fig. S9 The Effect of ionic strength on the recoveries of sulfonamides; sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethazine (SMT) and sulfamonomethoxine (SMX)

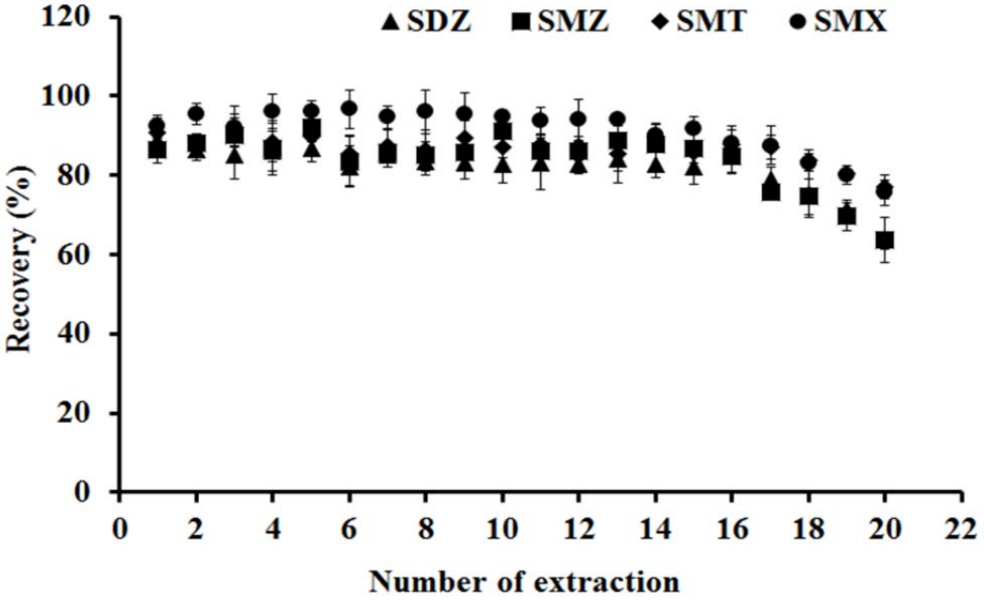

Fig. S10 Reusability of the polypyrrole/SiO_2/Fe_3O_4 sorbent for extraction of 20.0 μg L^{-1} of sulfonamides from spiked deionized water (n=5).

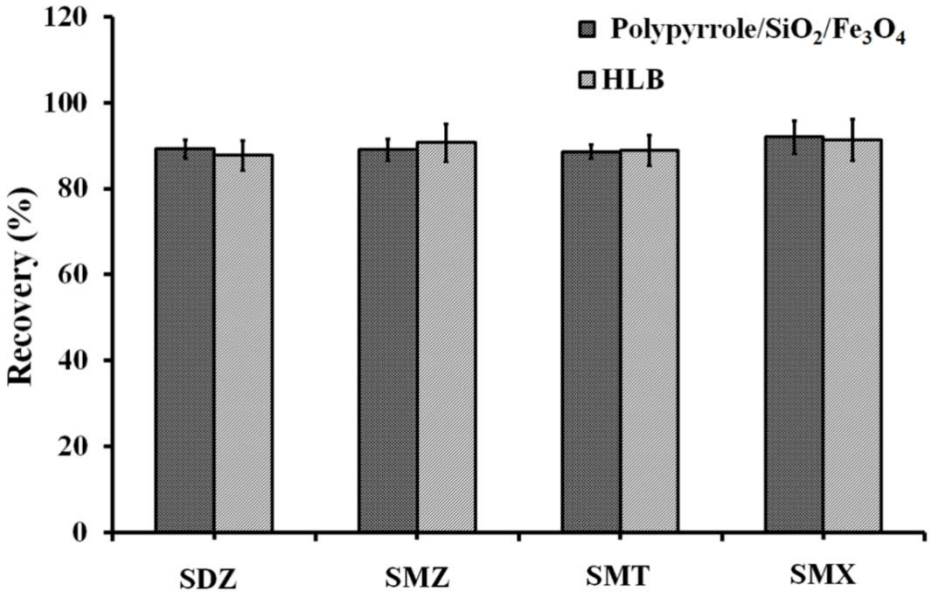

Fig. S11 The extraction efficiency of sulfonamides in spiked deionized water, a comparision between the polypyrrole/SiO_2/Fe_3O_4 sorbent and the conventional HLB SPE cartridge

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

Compounds	Linear range (μg L^{-1})	Regression line equation	R^2	LOD (μg L^{-1})	LOQ (μg 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

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