

# รายงานวิจัยฉบับสมบูรณ์

พัฒนาตัวดูดซับชนิดใหม่สำหรับการสกัดและตรวจวิเคราะห์สารเตตราไซคลีน  
ในน้ำและอาหาร

Development of a new sorbent for the extraction and determination  
of tetracycline in water and food samples

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โครงการวิจัยนี้ได้รับทุนสนับสนุนจากเงินรายได้มหาวิทยาลัยสงขลานครินทร์  
ประจำปีงบประมาณ 2558 รหัสโครงการ SCI581211S

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## กิตติกรรมประกาศ

โครงการวิจัยนี้ได้รับสนับสนุนจากงบประมาณเงินรายได้มหาวิทยาลัยสงขลานครินทร์ ประจำปี 2558 สัญญาเลขที่ SCI581211S ผู้วิจัยขอขอบคุณ สถานวิจัยการวิเคราะห์สารปริมาณน้อยและไบโอเซนเซอร์ ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์สำหรับสถานที่ทำวิจัยและเครื่องมือวิจัยที่เกี่ยวข้อง

โอภาส บุญเกิด  
พฤษภาคม 2560

## บทคัดย่อ

อนุภาคแม่เหล็กขนาดนาโนเคลือบด้วยชั้นซิลิกาและออกตาเดซิลถูกสังเคราะห์ขึ้นเพื่อใช้เป็นตัวดูดซับของแข็งอนุภาคแม่เหล็กสำหรับสกัดสารในกลุ่มเตตราไซคลีนในตัวอย่างน้ำ อนุภาคแม่เหล็กช่วยให้สามารถแยกตัวดูดซับออกจากตัวอย่างน้ำได้ง่าย ชั้นของออกตาเดซิลช่วยเพิ่มความสามารถในการดูดซับ และชั้นซิลิกาช่วยป้องกันการจับกลุ่มกันของอนุภาคแม่เหล็ก ภายใต้สภาวะที่เหมาะสมของการตรวจวัด ได้แก่ ปริมาณตัวดูดซับ พีเอชของตัวอย่าง อัตราเร็วของการคนสารตัวอย่าง เวลาในการสกัด ชนิดและปริมาณของตัวทำละลายที่ใช้ในการคายการดูดซับ พบว่าให้ประสิทธิภาพในการสกัดที่ดีโดยมีร้อยละของการได้กลับคืนอยู่ในช่วง 82 ถึง 88 มีช่วงความเป็นเส้นตรงตั้งแต่ 0.002 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร สำหรับออกซีเตตราไซคลีน และ 0.01 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร สำหรับเตตราไซคลีนและคลอเตตราไซคลีน ตามลำดับ โดยวิธีที่พัฒนาขึ้นนี้มีข้อดีหลายประการ ได้แก่ สามารถเตรียมตัวดูดซับและใช้งานได้ง่าย มีราคาถูก และมีประสิทธิภาพในการสกัดสูง



### Abstract

Magnetite nanoparticles coated with silica and hydrophobic octadecyl layers were successfully synthesized and used in magnetic solid phase extraction of tetracyclines from water samples. The magnetite nanoparticles facilitated a convenient magnetic separation of sorbent from an aqueous sample, the octadecyl layer helped to enhance the adsorption ability and the silica layer helped to prevent the aggregation of the magnetite nanoparticles. The effect of various parameters on the extraction efficiency were optimized including the amount of sorbent, sample pH, stirring rate, extraction time and desorption conditions. Under the optimum conditions, the recoveries were in the range of 82 to 88%, the calibration curves were linear over the concentration range of 0.002 to 1.0  $\mu\text{g/mL}$  for oxytetracycline and 0.01 to 1.0  $\mu\text{g/mL}$  for tetracycline and chlortetracycline, respectively. The developed method had several advantages such as simplicity, convenience, cost-effectiveness and high extraction efficiency.

## บทสรุปผู้บริหาร (Executive Summary)

### บทนำ

ปัจจุบันมีการใช้ยาปฏิชีวนะกันอย่างแพร่หลายทั้งในวงการแพทย์และเกษตรกรรมซึ่งมีการนำมาใช้ในการรักษาโรคของคนและสัตว์ ตัวอย่างของยาปฏิชีวนะที่มีการนำมาใช้ เช่น เตตราไซคลิน ซัลโฟนาไมด์ เป็นต้น แต่หากมีการใช้สารเหล่านี้เกินความจำเป็นหรือผิดวิธี อาจทำให้ประสบปัญหาเกิดการดื้อยาทำให้การรักษาโรคไม่ได้ผลและถ้ามีการตกค้างในอาหารและสิ่งแวดล้อมจะเป็นอันตรายต่อมนุษย์ ดังนั้นจึงจำเป็นต้องมีการตรวจวิเคราะห์การตกค้างของยาปฏิชีวนะเหล่านี้ทั้งในอาหารและสิ่งแวดล้อม โดยเทคนิควิเคราะห์ที่นิยมใช้ได้แก่ เทคนิคแก๊สโครมาโทกราฟี และเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง เนื่องจากเทคนิคเหล่านี้มีความไววิเคราะห์สูง แต่เนื่องจากการตกค้างของยาปฏิชีวนะในสิ่งแวดล้อมและในอาหารมีปริมาณน้อยและในตัวอย่างมีตัวรบกวนมาก ดังนั้นจึงจำเป็นต้องมีขั้นตอนการเตรียมตัวอย่างที่เหมาะสมก่อนการวิเคราะห์ ซึ่งเทคนิคที่มีการนำมาใช้ในการเตรียมตัวอย่าง ได้แก่ เทคนิคการสกัดด้วยตัวทำละลาย (Liquid Liquid Extraction) เทคนิคการสกัดด้วยตัวดูดซับของแข็ง (Solid phase extraction) และการสกัดด้วยตัวดูดซับของแข็งปริมาณน้อย (Solid phase microextraction) แต่เทคนิคดังกล่าวมีข้อด้อย คือมีขั้นตอนการสกัดที่ยุ่งยาก ใช้สารอินทรีย์ปริมาณมาก ใช้เวลาในการสกัดนานและมีราคาแพง

ดังนั้นโครงการวิจัยนี้จึงสนใจพัฒนาตัวดูดซับชนิดใหม่บนอนุภาคแม่เหล็ก (Magnetic solid phase extraction) ในลักษณะคอมโพสิตร่วมกับ Sol-gel และวัสดุพอลิเมอร์ชนิด C18 ( $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C18}$ ) เพื่อเพิ่มขีดความสามารถในการตรวจวิเคราะห์สารเตตราไซคลินที่ปนเปื้อนปริมาณน้อย โดยวิธีการสกัดที่พัฒนาขึ้น สามารถทำได้ง่าย มีขั้นตอนการสกัดที่ไม่ยุ่งยาก โดยให้ประสิทธิภาพของการสกัดและตรวจวิเคราะห์เทียบเท่ากับตัวดูดซับที่มีจำหน่ายทางการค้าซึ่งมีราคาแพงแต่ตัวดูดซับที่พัฒนาขึ้นมีราคาถูกและเป็นมิตรกับสิ่งแวดล้อม นอกจากนี้ตัวดูดซับที่พัฒนาขึ้นสามารถนำกลับมาใช้ซ้ำได้

### วัตถุประสงค์

เพื่อพัฒนาตัวดูดซับบนอนุภาคแม่เหล็กแบบใหม่สำหรับวิเคราะห์ยาปฏิชีวนะกลุ่มเตตราไซคลินที่ตกค้างปริมาณน้อยในตัวอย่างอาหารและสิ่งแวดล้อมด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง

## สรุป

งานวิจัยนี้เป็นการพัฒนาตัวดูดซับชนิดใหม่สำหรับสกัดและเพิ่มความเข้มข้นของสารกลุ่มเตตราไซคลินในตัวอย่างน้ำร่วมกับการตรวจวิเคราะห์ด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง (HPLC) โดยตัวดูดซับที่พัฒนาขึ้นคือ อนุภาคแม่เหล็กขนาดนาโนเคลือบด้วยชั้นซิลิกาและออกตาเดซิล เพื่อให้ได้ประสิทธิภาพของการสกัดสูงสุด ใช้เวลาและตัวทำลายน้อย จึงได้หาสภาวะที่เหมาะสมของการสกัด โดยได้สภาวะที่เหมาะสมดังนี้ ปริมาณตัวดูดซับ 100 มิลลิกรัม พีเอชของตัวอย่างอยู่ในช่วง 5-6 อัตราเร็วของการคนสารตัวอย่าง 1500 รอบต่อนาที เวลาในการสกัด 20 นาที โดยใช้กรดออกซาลิก 60 มิลลิโมลาร์ในเมทานอล ปริมาตร 4 มิลลิลิตรเป็นตัวทำละลายที่ใช้ในการคายการดูดซับ

ภายใต้สภาวะที่เหมาะสมของการสกัดพบว่าช่วงความเป็นเส้นตรงของออกซีเตตราไซคลินอยู่ในช่วง 0.002 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร เตตราไซคลินและคลอเตตราไซคลินอยู่ในช่วง 0.01 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร ขีดจำกัดการตรวจวัด (LOD) ของออกซีเตตราไซคลินเท่ากับ 2 ไมโครกรัมต่อลิตร เตตราไซคลินและคลอเตตราไซคลินเท่ากับ 10 ไมโครกรัมต่อลิตร ตามลำดับ

เมื่อเปรียบเทียบประสิทธิภาพในการสกัดสารกลุ่มเตตราไซคลินของตัวดูดซับที่พัฒนาขึ้นกับตัวดูดซับชนิด C18 ที่มีจำหน่ายทางการค้าและพบว่าตัวดูดซับทั้งสองชนิดให้ประสิทธิภาพในการสกัดสารกลุ่มเตตราไซคลิน ไม่แตกต่างกันอย่างมีนัยสำคัญแต่เมื่อเปรียบเทียบขั้นตอนการสกัดพบว่าวิธีการสกัดที่พัฒนาขึ้นสามารถทำได้ง่ายกว่า และใช้เวลาน้อยกว่า อีกครั้งตัวดูดซับที่พัฒนาขึ้นมีราคาถูก ตัวดูดซับที่พัฒนาขึ้นสามารถนำกลับมาใช้ซ้ำได้อย่างน้อย 5 ครั้ง ซึ่งทำให้สามารถลดค่าใช้จ่ายของการเตรียมตัวอย่างลง โดยได้ประยุกต์ใช้วิธีที่พัฒนาขึ้นตรวจวิเคราะห์การปนเปื้อนของสารกลุ่มเตตราไซคลิน ในแหล่งน้ำต่างๆ ได้แก่ น้ำก๊อก น้ำจากแหล่งธรรมชาติ และน้ำเสีย โดยพบการปนเปื้อนของสารเตตราไซคลินปริมาณน้อยในน้ำเสีย จากผลการศึกษาร้อยละการได้กลับคืนจากการเติมสารมาตรฐานในน้ำตัวอย่างที่ความเข้มข้นต่างๆ พบว่าให้ร้อยละการได้กลับคืนอยู่ในช่วง 82 ถึง 88 เปอร์เซ็นต์

# ภาคผนวก 1

ผลงานตีพิมพ์ชิ้นที่ 1

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## Dispersive Magnetic Solid Phase Extraction Using Octadecyl Coated Silica Magnetite Nanoparticles for the Extraction of Tetracyclines in Water Samples

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**Abstract** - Magnetite nanoparticles coated with silica and hydrophobic octadecyl layers were successfully synthesized and used in magnetic solid phase extraction of tetracyclines from water samples. The magnetite nanoparticles facilitated a convenient magnetic separation of sorbent from an aqueous sample, the octadecyl layer helped to enhance the adsorption ability and the silica layer helped to prevent the aggregation of the magnetite nanoparticles. The effect of various parameters on the extraction efficiency were optimized including the amount of sorbent, sample pH, stirring rate, extraction time and desorption conditions. Under the optimum conditions, the recoveries were in the range of 82 to 88%, the calibration curves were linear over the concentration range of 0.002 to 1.0 µg/mL for oxytetracycline and 0.01 to 1.0 µg/mL for tetracycline and chlortetracycline, respectively. The developed method had several advantages such as simplicity, convenience, cost-effectiveness and high extraction efficiency.

**Keywords:** magnetic nanoparticles, silica, octadecyl, tetracyclines

Tetracyclines are widely used in therapy for the prophylactic control of bacterial infections in human and veterinary medicine and as food additives for growth promotion [1]. They are the second most extensively used antibiotics in the world[2] due to their availability, satisfactory therapeutic properties, low costs and very broad spectrum of antimicrobial activity[3]. However, because of their extensive use with animals some serious problems for human health have emerged, as some human pathogens that were originally sensitive to tetracycline are now resistant, and some tetracyclines are toxic[4]. Therefore, regulatory agencies have enacted decisions that keep these substances under control.

Consequently, the determination of tetracyclines in water samples is very important to protect humans from any disturbances caused by tetracyclines. HPLC has been extensively used for the determination of tetracyclines because of its good precision[1,5]. However, direct determination of tetracyclines in real samples is usually difficult because of their very low concentrations; therefore, sample preparation methods are required prior to instrumental analysis. Liquid-liquid extraction (**LLE**)[3] and solid phase extraction (**SPE**) are often used for the extraction of tetracycline from various sample matrixes[6]; however, traditional LLE methods are time-consuming, tedious and require large amounts of organic solvents[7]. SPE procedures are more efficient according to their ability to achieve high recoveries. According to previous reports, Oasis HLB cartridges provided acceptable performance in the SPE of tetracyclines from various samples[4, 8-10]. However, commercial SPE sorbent and equipment are expensive, the operations are quite tedious and time-consuming[11]. To overcome some of these problems, a new SPE technique called magnetic solid-phase extraction (**MSPE**) has received increasing attention due to its simplicity, short extraction time, low solvent consumption, and easy separation of magnetic sorbent from samples solution [12]. However, bare magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$ ) are prone to aggregation, oxidation and are not selective in complex matrices. Therefore, these magnetic nanoparticles

were modified with specific ligands or composited with others materials to improve selectivity[13]. Silica is nearly ideal coating material because it can preclude  $\text{Fe}_3\text{O}_4$  nanoparticles from aggregation and enhance their chemical stability [14-16]. However, the hydrophilic silica would not be suitable for the extraction of tetracyclines. Consequently, in this work, an additional coating of an octadecyl ( $\text{C}_{18}$ ) groups was used because it can adsorb tetracyclines via hydrophobic interactions. The  $\text{C}_{18}/\text{SiO}_2/\text{Fe}_3\text{O}_4$  sorbent was then utilized to extract tetracyclines from water samples, and HPLC was used for the subsequent separation and determination of tetracyclines. The aim was to provide a simple, rapid and environmentally friendly method for the determination of tetracyclines in environmental samples. Oxytetracycline, tetracycline, and chlortetracycline were chosen as test compounds to investigate the performance of the  $\text{C}_{18}/\text{SiO}_2/\text{Fe}_3\text{O}_4$  sorbent.

## EXPERIMENTAL

**Reagents and chemicals.** Acetonitrile, methanol, ethanol, toluene and oxalic acid were from Merck (Darmstadt, Germany). Ammonium hydroxide was from J.T. Baker (Bangkok, Thailand). Iron(III) chloride hexahydrate, iron(II) chloride tetrahydrate, tetraethylorthosilicate, trimethylamine solution, dimethyloctadecylchlorosilane( $\text{C}_{18}$ ), oxytetracycline (**OTC**), tetracycline(**TC**), and chlortetracycline (**CTC**) were from Sigma-Aldrich (Steinheim, Germany). Oasis HLB sorbent (500 mg) were from the Water Corporation (Milford, USA). Stock solutions of tetracyclines (1000 mg/L) were prepared in methanol and stored at 4°C. Deionized water was obtained from a Maxima ultrapure water system (ELGA, England).

**Analytical instrumentation.** Chromatographic analyses were performed using an Agilent 1100 HPLC system (Agilent Technologies, Germany). Data acquisition was performed using Chem Station software. The separation of tetracyclines was performed on a reverse phase

Vertisep<sup>TM</sup> UPS-C<sub>18</sub> column (4.6 × 150 mm, 5 μm particle size, Vertical Chromatography Co., Thailand). Tetracyclines were separated in the reverse phase mode, the mobile phase was a mixture of methanol: acetonitrile: 0.01 M oxalic acid (5: 18: 77, v/v), and the flow rate was 1.0 mL/min with a 20 μL injection volume. Absorbance was monitored at 355 nm.

**Preparation of the C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> sorbent.** The synthesis of C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> sorbent involved three steps as shown in Fig. 1a. First, Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized following a reported method based on chemical co-precipitation [17]. Briefly, FeCl<sub>3</sub>·6H<sub>2</sub>O (3.50 g) and FeCl<sub>2</sub>·4H<sub>2</sub>O (1.50 g) were dissolved in 100 mL of deionized water and stirred at 80°C, then 10 mL of ammonia (30%, v/v) was added dropwise into the solution, and stirring was continued for 30 min in an atmosphere of nitrogen gas. After the reaction, the obtained magnetic particles were separated from the solution by an external magnet, washed with deionized water (100 mL, three times) to remove any unreacted chemicals and dried in an oven at 60°C for 4 h.

Second, the silica-coating on the surface of Fe<sub>3</sub>O<sub>4</sub> nanoparticles was achieved using a sol-gel process [12]. Fe<sub>3</sub>O<sub>4</sub> nanoparticles (2.0 g) were added into the solution containing 50 mL of deionized water, 100 mL of ethanol and 2.0 mL of ammonia (30% v/v) under stirring for 10 min. Then, 2.0 mL of tetraethylorthosilicate was added into the solution under stirring at 500 rpm and the mixture was stirred for 12 h at 40 °C. The SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> nanoparticles were collected by an external magnet, washed with 5 mL of ethanol and deionized water three times, and then dried in an oven at 60°C for 4 h.

Finally, for the C<sub>18</sub>-functionalization, SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> nanoparticles (3.0 g) were added to 90 mL of toluene. The slurry was heated to 80°C and 10 mL of trimethylamine and 1.0 mL of dimethyloctadecylchlorosilane was added. The mixtures were then refluxed for 24 h. The C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> nanoparticles were obtained and collected by magnet, washed with ethanol and deionized water two times, dried in an oven at 60°C for 3 h.



**Extraction of tetracyclines using magnetic solid phase extraction.** The magnetic solid phase extraction procedure is shown in Fig. 1b: 100 mg of  $C_{18}/SiO_2/Fe_3O_4$  sorbent was added into the extraction bottle and conditioned with 2.0 mL of methanol and deionized water, respectively. Then, 5.0 mL of a water sample was added and stirred for 20 min. After that, the sorbent with the adsorbed tetracyclines was separated by magnet and the supernatant solution was discarded. The analytes were then desorbed from the  $C_{18}/SiO_2/Fe_3O_4$  sorbent with 4.0 mL of 60 mM oxalic acid in methanol using sonication for 20 min. The desorption solution was then evaporated to dryness at 60 °C, redissolved with 1.0 mL of the mobile phase, and filtered with a PTFE filter (0.22  $\mu$ m) before analysis by HPLC with diode array detector (DAD).

**Application of the  $C_{18}/SiO_2/Fe_3O_4$  sorbent.** The developed  $C_{18}/SiO_2/Fe_3O_4$  sorbent was applied for the extraction and determination of tetracyclines in tap water, river water and wastewater. Tap water samples were collected from the laboratory, river water was collected from Hat Yai city, Thailand, and wastewater samples were collected from a household effluent from Songkhla province, Thailand. All water samples were filtered across a 0.45  $\mu$ m membrane and stored in amber glass bottles at 4°C until they were analyzed.

## RESULTS AND DISCUSSION

**Characterization of the  $C_{18}/SiO_2/Fe_3O_4$  sorbent.** Characterization of the synthesized magnetic nanoparticles ( $Fe_3O_4$ ,  $SiO_2/Fe_3O_4$ ,  $C_{18}/SiO_2/Fe_3O_4$ ) was achieved using Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM) and Vibrating sample magnetometer (VSM). FT-IR spectroscopy was employed to ensure a proper chemical modification of the magnetic nanoparticles. As shown in Fig. 2a, the absorption peaks at 587  $cm^{-1}$  represented the stretching vibration of the Fe-O-Fe group of magnetite. Silica-coating on the surface of the  $Fe_3O_4$  nanoparticles was observed at 1077  $cm^{-1}$  that was

attributed to the Si-O-Si group of the silica layer, this peak indicated the presence of a silica layer on the surface of Fe<sub>3</sub>O<sub>4</sub>. The absorption peaks at 1605 and 3405 cm<sup>-1</sup> appearing in all the FT-IR spectra were assigned to the absorbed water (-OH group) or the silanol groups (Si-OH) of silica. The absorption peaks that appeared at 2920 and 2850 cm<sup>-1</sup> represented the asymmetric and the symmetric vibrations of the -CH<sub>2</sub> group in the -(CH<sub>2</sub>)<sub>17</sub>CH<sub>3</sub> chain, and indicated that the C<sub>18</sub> group was successfully attached onto the surface of the SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> nanoparticles. The SEM images of the C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> sorbent is illustrated in Fig. 2b, the average diameter of the C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> NPs was approximately 75 ± 10 nm and was nearly spherical in shape. Fig. 2c shows VSM magnetization curves of Fe<sub>3</sub>O<sub>4</sub>, SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> and C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> and the maximum saturation magnetizations were measured at 56.95, 51.05 and 47.97 emu/g, respectively. The decrease in the magnetic strength resulted from the nonmagnetic shell of silica and C<sub>18</sub>, respectively. However, all of the magnetic nanoparticles had a magnetic strength sufficient for the magnetic separation from solution with a magnet, and C<sub>18</sub>/SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub> was still able to produce a rapid separation within 30 s using the magnet. The developed sorbents could be dispersed in the water sample and conveniently isolated from the aqueous media utilizing an external magnet (Fig.2d).

**Optimization of the magnetic solid phase extraction.** In order to achieve a high extraction efficiency, with a short analysis time and low solvent consumption, several experimental parameters including the type of sorbent, the amount of sorbent, sample pH, stirring rate, extraction time and desorption conditions were investigated. The optimization was performed by extracting a spiked water sample containing 0.2 µg/mL of each tetracycline. The obtained recovery was used to evaluate the extraction efficiency.

*Sorbent type.* The extraction capabilities of the uncoated magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>), silica coated magnetic nanoparticles (SiO<sub>2</sub>/Fe<sub>3</sub>O<sub>4</sub>), and octadecyl coated silica-magnetic

nanoparticles ( $C_{18}/SiO_2/Fe_3O_4$ ) were investigated by extracting tetracyclines from spiked water samples. The results (Fig.3) show that bare  $Fe_3O_4$  had low adsorption ability for tetracyclines (3–4%).  $SiO_2/Fe_3O_4$  improved the adsorption ability of tetracycline and this may be due to hydrogen bonding or a hydrophilic interaction; however, the extraction efficiency (15–23%) was not sufficient for application in a real sample. The recoveries for all the tested tetracyclines had a significant increase in the presence of octadecyl ( $C_{18}$ ) groups, which indicated that octadecyl had a significant influence on the extraction process that led to the adsorption of tetracyclines via hydrophobic interaction.

*Effect of the amount of sorbent.* The amount of sorbents directly affected the extraction efficiency of the analytes. The amount of the sorbent was investigated over the range of 50 to 200 mg. As shown in Fig. 4a, the recoveries of tetracyclines increased with the increasing amounts of sorbent from 50 to 100 mg, and then remained almost constant, hence 100 mg of the sorbent was sufficient to extract the tetracyclines from the water samples and was selected for further experiments.

*Effect of the pH of sample.* The pH of the samples was a significant parameter that affected adsorption of the analytes. Tetracyclines are amphoteric compounds with three protonated functional groups. The  $pK_a$  values of tetracyclines in aqueous solution are around 3.3, 7.5 and 9.0[18]. In aqueous samples, the three groups can undergo a protonation-deprotonation reaction and form cationic form ( $pH > pK_{a1}$ ), zwitterionic form ( $pK_{a1} < pH < pK_{a2}$ ) or anionic form ( $pH > pK_{a3}$ )[19] and the zwitterionic forms are favorable for extraction[20]. Therefore, the effect of the pH of the samples on the extraction efficiency in the range of 4.0–9.0 was investigated. The optimal extraction recoveries were obtained at pH 5.0 to 6.0 (Fig.4b) due to the adsorption of tetracyclines on the sorbent that involved hydrophobic interactions in the presence of the zwitterionic form. The recoveries of

tetracycline decreased at a lower pH than 5.0 due to the protonation of the dimethylamino group that occurred in acidic conditions, while the recoveries also decreased at pH values higher than 6.0 due to the deprotonation of the tetracyclines to give the anionic form that led to a decrease in the hydrophobic interaction between the tetracyclines and the sorbents. However, the pH of the normal water samples was between 5.5 and 6.0, so the samples could be directly analyzed without adjusting the pH values.

*Effect of the stirring rate.* The stirring rate can affect the extraction efficiency because it altered the mass transfer of analytes towards the sorbent. Therefore, the stirring rate was investigated over the range of 500 to 2000 rpm. As shown in Fig. 5a, the recovery increased as the agitation speed increased from 500 to 1500 rpm and then remained almost constant. Therefore, 1500 rpm was chosen for further experiments.

*Effect of the extraction time.* The extraction time was investigated between 10 and 40 min (Fig. 5b). The recovery of tetracyclines increased rapidly when the extraction time was increased from 10 to 20 min and then remained almost constant. Therefore, 20 min was chosen for the next experiments.

*Effect of the desorption conditions.* Since, the adsorption of tetracyclines onto the  $C_{18}/SiO_2/Fe_3O_4$  sorbent was based on hydrophobic interaction, the desorption solvents with different polarities were investigated *i.e.*, methanol, acetonitrile and a mixture of methanol and oxalic acid. The results showed that oxalic acid in methanol provided the highest extraction efficiency (Fig.6a). The concentration of oxalic acid in methanol was further studied by varying between 5.0 and 80 mM (Fig. 6b). The recoveries of analytes increased with the increasing concentration of oxalic acid in methanol and reached a maximum at 60 mM. This was due to the tetracyclines in a cationic form could be desorbed easily from the sorbent. Therefore, 60 mM of oxalic acid in methanol was selected for further studies.

The desorption time was also investigated in the range of 10 to 40 min (Fig.6c). The recoveries increased with increasing desorption time of up to 20 min and then remained almost constant. Therefore, the desorption time of 20 min was selected for further experiments.

The desorption volume was investigated in the range of 2.0 to 8.0 mL (Fig.6d).The recoveries of all the analytes increased with an increasing volume of eluent from 2.0 to 4.0 mL. Therefore, 4.0 mL of desorption solvent was chosen for subsequent experiments.

**Reproducibility and reusability.** Different preparations of the  $C_{18}/SiO_2/Fe_3O_4$  sorbent were investigated for batch-to-batch reproducibility by preparing six sorbents using the same conditions but at different times. These sorbents were used to extract tetracycline (0.2  $\mu\text{g}/\text{mL}$ ) from spiked deionized water. The averages recoveries from the six batches were: oxytetracycline  $84.2\pm 1.4\%$ , tetracycline  $82.4\pm 1.0\%$  and chlortetracycline  $84.7 \pm 1.7\%$ . The relative standard deviations (**RSDs**) were less than 6% and indicated that the preparation procedure of the  $C_{18}/SiO_2/Fe_3O_4$  sorbent had a good reproducibility.

To evaluate the reusability of the developed sorbent, after desorption the used  $C_{18}/SiO_2/Fe_3O_4$  sorbent was washed with 2.0 mL of methanol and 5.0 mL of deionized water with sonication. After washing, the effect of carry-over of the target analytes was investigated, the same sorbent was used to extract the blank sample (no analytes) and no response of tetracycline was detected. This result indicated no carry-over of the analytes. The developed sorbent can be reused five times with recoveries of tetracyclines  $>80\%$ .

**Comparative studies.** The extraction efficiency of the developed  $C_{18}/SiO_2/Fe_3O_4$  sorbent was compared to a commercial Oasis HLB sorbent. The average recoveries of the tetracyclines ranged from 83 to 87% and 83 to 85% for  $C_{18}/SiO_2/Fe_3O_4$  and Oasis HLB, respectively. The recoveries of the two sorbents were no significant difference ( $P > 0.05$ ).

Therefore, the  $C_{18}/SiO_2/Fe_3O_4$  sorbent can be used as an alternative sorbent for the extraction of tetracyclines from water samples. The advantages of  $C_{18}/SiO_2/Fe_3O_4$  sorbent over the commercial SPE sorbent were simpler use and possibility to reuse for up to 5 times, whereas no commercial SPE sorbent could be reused. In addition, the developed method is faster (40 min) than conventional SPE method (60 min).

**Performance of the developed method.** The analytical performances of the developed method, *i.e.* linear range, limit of detection (**LOD**) and limit of quantification (**LOQ**) were investigated under the optimal conditions. Good linearity was obtained in the concentration range of 2.0-1000  $\mu\text{g/L}$  for OTC and 10.0-1000  $\mu\text{g/L}$  for TC and CTC ( $R^2 > 0.999$ ). The RSDs were less than 10%, showing good repeatability of the developed method. The LODs ( $S/N=3$ ) were 2.0  $\mu\text{g/L}$  for OTC and 10.0  $\mu\text{g/L}$  for TC and CTC, and the LOQs ( $S/N=10$ ) were 8.0  $\mu\text{g/L}$  for OTC, and 40.0  $\mu\text{g/L}$  for TC and CTC.

**Analysis of real water samples.** The developed method was used to determine tetracyclines in tap, river and wastewater samples. The typical chromatograms of the spiked water samples (0.2  $\mu\text{g/mL}$ ) with extraction using  $C_{18}/SiO_2/Fe_3O_4$  sorbent and without extraction are shown in Fig. 7. Only low concentrations of TC were detected in the wastewater samples (Table 1). To investigate the accuracy of the developed method, the water samples were spiked with TC at the concentrations of 0.02, 0.10 and 0.20  $\mu\text{g/mL}$ . The recoveries of tetracyclines were in the range of 82.2 to 87.7% with RSDs < 10% (Table 2). The acceptable recoveries indicated that there was no effect from the matrix components in the real water samples. The results implied that the developed method was suitable for the determination of tetracyclines in water samples.

**Comparison of  $C_{18}/SiO_2/Fe_3O_4$  with other sorbents.** A comparison of the developed method with other reported methods for the extraction and determination of tetracyclines is

presented in Table 3. In this work, only 100 mg of sorbents was sufficient to extract tetracyclines from water samples, while conventional SPE cartridge required 500 mg of sorbent. Although some methods used even less sorbent [20], the reusability and LODs were not investigated for method [20]. The recoveries of the developed method were either comparable [21, 22] or better than those with the other methods [23-25]. This is because tetracyclines in the samples solution can be easily adsorbed onto the sorbent, that provided a high adsorption capacity. Compared to the conventional SPE sorbent, the developed method is easier and faster because of the sorbent being easy to separate from the sample solution using an external magnet. In addition, the developed sorbent can be reused 5 times, which helps to reduce analysis time and costs.

## CONCLUSIONS

The developed  $C_{18}/SiO_2/Fe_3O_4$  sorbent was successfully synthesized and applied by a magnetic solid phase extraction process for the determination of tetracyclines in water samples. Coating of the magnetic nanoparticles with silica and  $C_{18}$  groups increased the adsorption ability of tetracyclines because of hydrophobic interactions, improved the dispersibility and stability of the sorbents. The developed sorbent exhibited several advantages including a convenient and rapid collection of analytes from the sample solution using a magnet, avoiding the time-consuming column passing and filtration steps encountered in SPE. The sorbent was produced at a relatively low cost and had high adsorption capacity compared to a commercial SPE cartridge. Moreover, the developed method provided a high extraction efficiency, good reproducibility and reusability of the sorbent. The developed sorbent was successfully applied to extract tetracyclines from real water samples and would be potentially useful for the extraction of other less polar compounds.

## ACKNOWLEDGMENTS

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Figure captions to the manuscript of Kaewsuwan W, et al.

**Fig. 1.** Synthesis of  $C_{18}/SiO_2/Fe_3O_4$  sorbent (a) and extraction of tetracyclines using the  $C_{18}/SiO_2/Fe_3O_4$  sorbent (b).

**Fig. 2.** FT-IR spectra of the magnetic nanoparticles: 1-  $Fe_3O_4$ , 2-  $SiO_2/Fe_3O_4$ , 3-  $C_{18}/SiO_2/Fe_3O_4$  (a), SEM image of  $C_{18}/SiO_2/Fe_3O_4$  sorbent (b), VSM curves (c) and dispersion of  $C_{18}/SiO_2/Fe_3O_4$  sorbents and its separation by a magnet (d).

**Fig. 3.** Extraction efficiency for oxytetracycline, tetracycline, and chlortetracycline.

**Fig. 4.** Effect of the amount of  $C_{18}/SiO_2/Fe_3O_4$  sorbent (a) and sample pH (b) on the recoveries of tetracyclines.

**Fig. 5.** Effect of the stirring rate (a) and extraction time (b) on the recoveries of tetracyclines.

**Fig. 6.** Effect of the type of desorption solvent (a), concentration of oxalic acid in methanol (b), desorption time (c) and desorption volume (d) on the recoveries of tetracyclines.

**Fig. 7.** Chromatograms of spiked wastewater sample (0.2  $\mu\text{g/mL}$ ) without extraction (1) and with extraction using  $C_{18}/SiO_2/Fe_3O_4$  sorbent (2)

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**Table 1.** Concentration of tetracyclines determined in real water samples

Water sample	Concentration, $\mu\text{g/mL}$		
	OTC	TC	CTC
Tap water 1	ND*	ND	ND
Tap water 2	ND	ND	ND
River water 1	ND	ND	ND
River water 2	< LOQ	ND	ND
Wastewater 1	$0.010 \pm 0.002$	ND	ND
Wastewater 2	$0.012 \pm 0.003$	ND	ND

\* Not detected.

**Table 2.** Recoveries of tetracyclines in real water samples

Water samples	Added, $\mu\text{g/mL}$	Recovery $\pm$ SD*, %		
		OTC	TC	CTC
Tap water	0.020	87 $\pm$ 3	87 $\pm$ 3	86 $\pm$ 4
	0.10	83 $\pm$ 1	83 $\pm$ 4	82 $\pm$ 3
	0.20	84 $\pm$ 3	83 $\pm$ 2	85 $\pm$ 5
River water	0.020	88 $\pm$ 4	85 $\pm$ 5	86 $\pm$ 4
	0.10	84 $\pm$ 3	83 $\pm$ 4	84 $\pm$ 3
	0.20	84 $\pm$ 3	84 $\pm$ 4	86 $\pm$ 5
Wastewater	0.020	87 $\pm$ 3	85 $\pm$ 4	84 $\pm$ 3
	0.10	83 $\pm$ 2	85 $\pm$ 3	84 $\pm$ 2
	0.20	84 $\pm$ 3	83 $\pm$ 4	87 $\pm$ 3

\* Standard deviation.

**Table 3.** Comparison of the developed method with other methods for the determination of tetracyclines

Extraction method	Sample	Sample amount	Extractants	LOD	Recovery, %	Preconcentration factor	References
MSPE	Water	100 mL	Hyper-crosslinked polystyrene -Fe <sub>3</sub> O <sub>4</sub> (20 mg)	-	95-97	25	[20]
SPE	Honey	3 g	DSC-phenyl cartridges (500 mg)	15-30 ng/g	92-96	6	[21]
SPE	Water	100 mL	Hyper-crosslinked polystyrene (30 mg)	0.6-2.0 µg/L	94-98	100	[22]
SPE	Chicken meat and liver	5 g	C <sub>18</sub>	4-10 ng/g	69-93	-	[23]
SPE	Water	100 mL	SPE-C <sub>18</sub> (500 mg)	3.1-3.8 µg/L	72-93	500	[24]
MIMM	Milk	4 g	Molecularly imprinted magnetic microsphere	7.4-19.4 µg/kg	75-94	4	[25]
MSPE	Water	5 mL	C <sub>18</sub> /SiO <sub>2</sub> /Fe <sub>3</sub> O <sub>4</sub> (100 mg)	2-10 µg/L	82-87	5	This work

*Notations:* SPE - Solid phase extraction, MIMM - Molecularly imprinted magnetic microsphere, MSPE - Magnetic solid phase extraction.

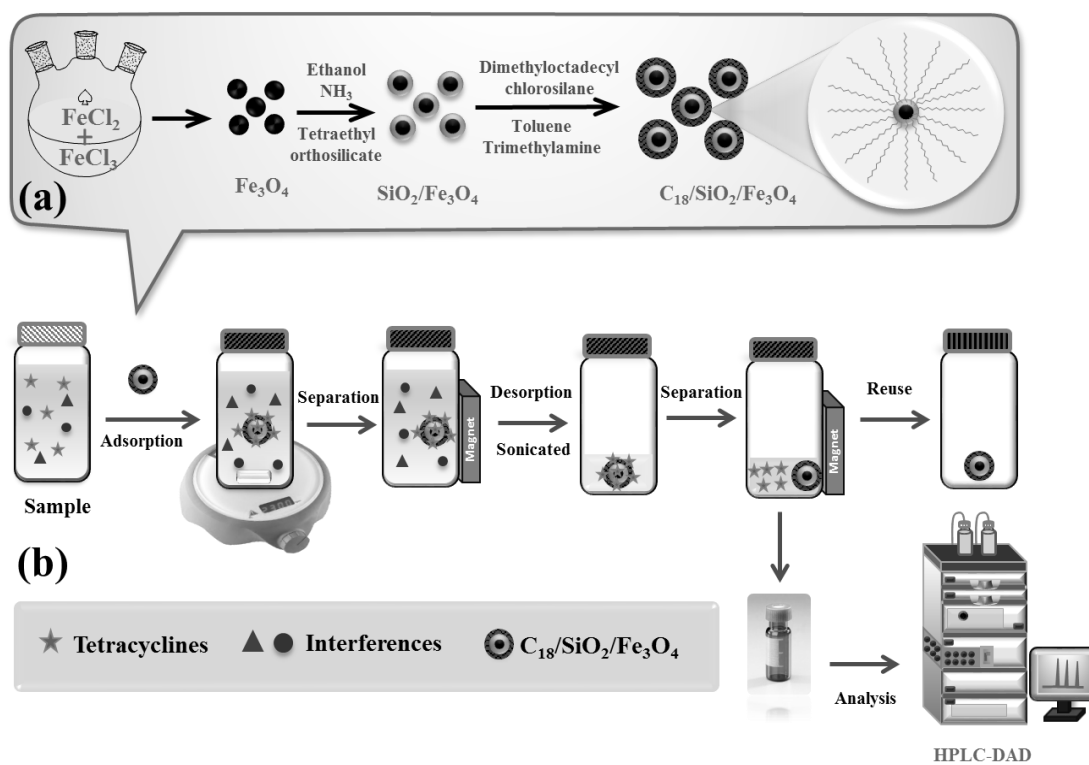


Fig. 1. Kaewsuwan W, et al.

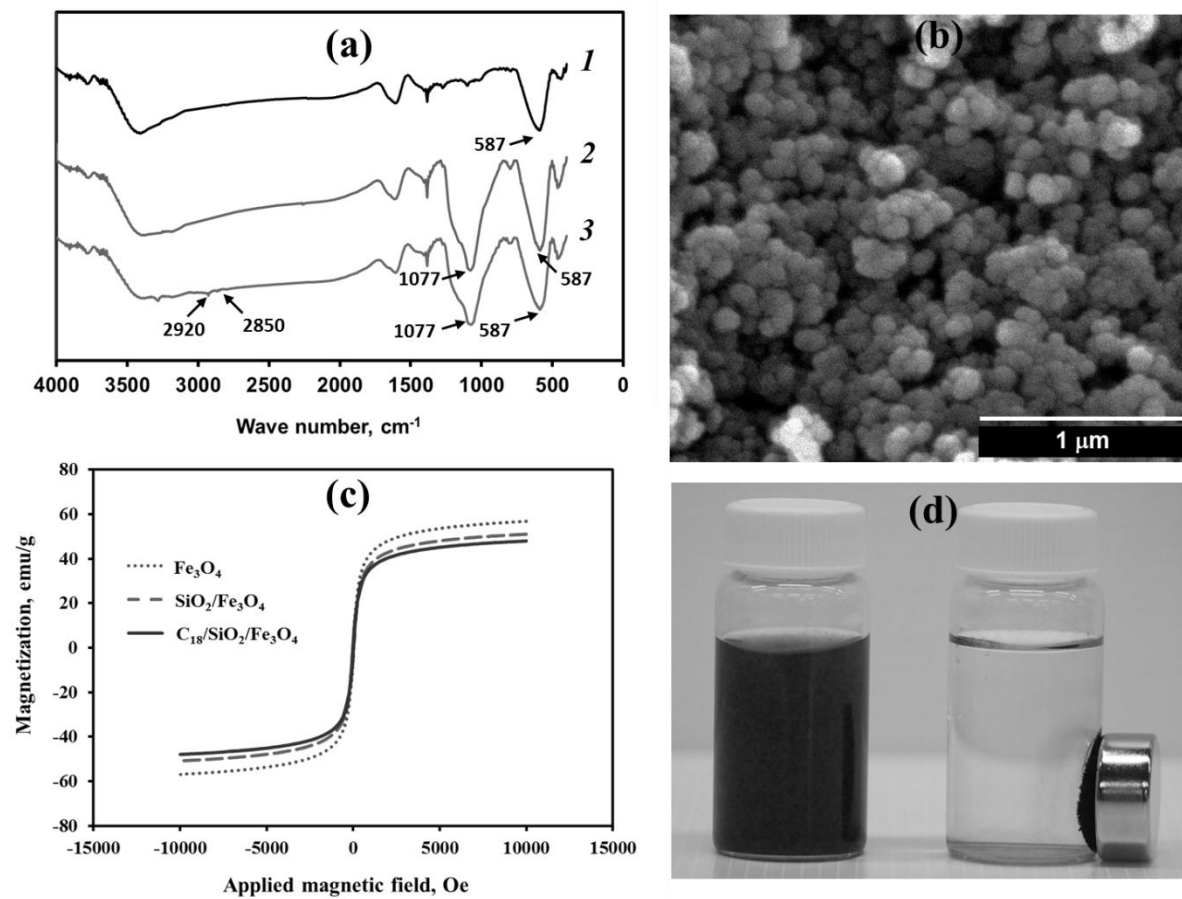


Fig. 2. Kaewsuwan W, et al.



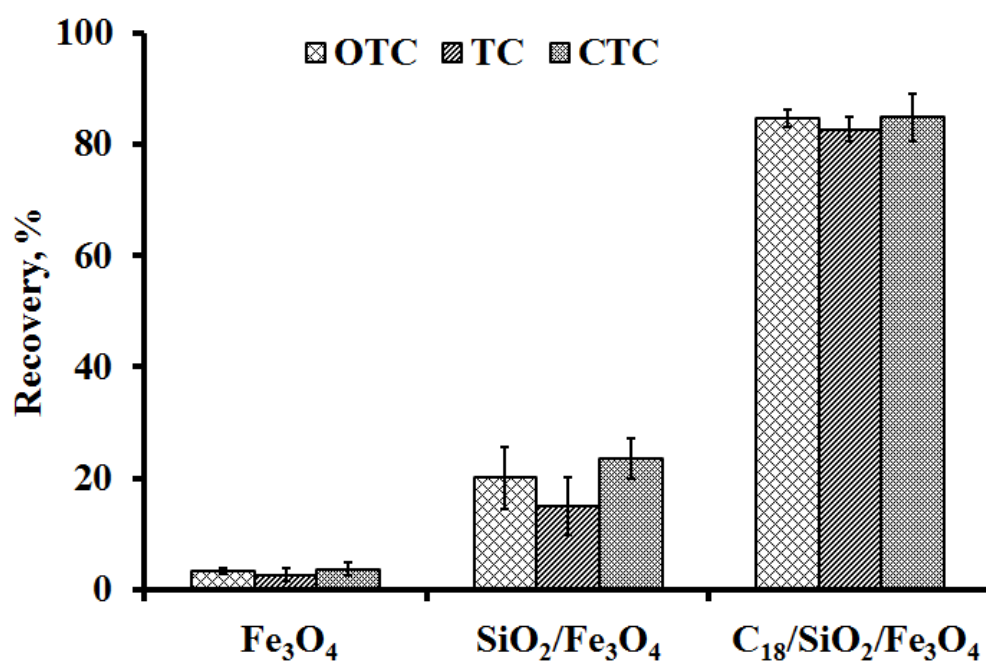


Fig. 3. Kaewsuwan W, et al.

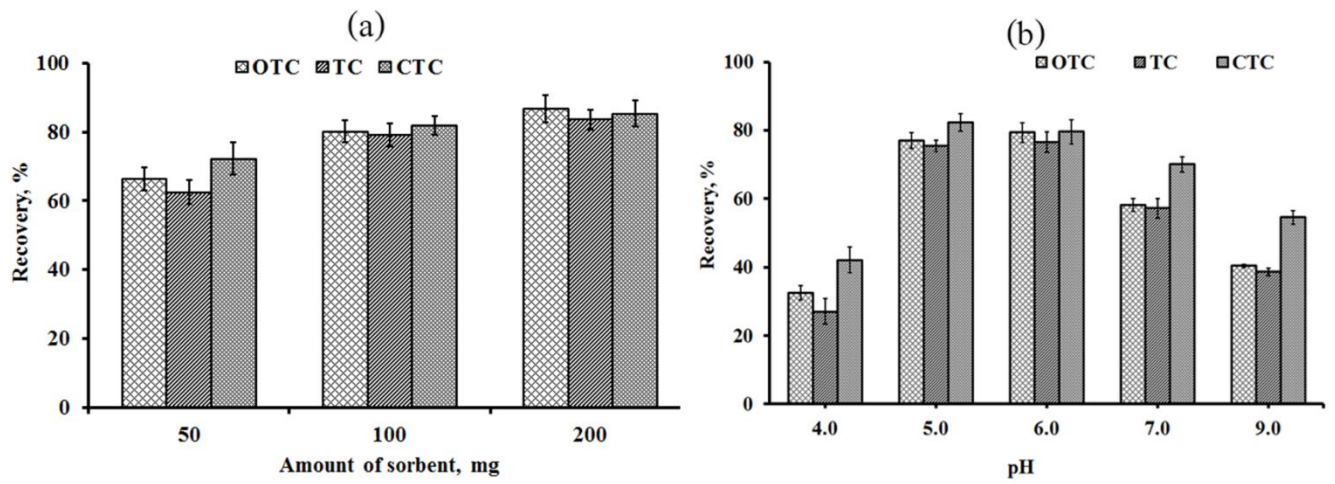


Fig. 4. Kaewsuwan W, et al.

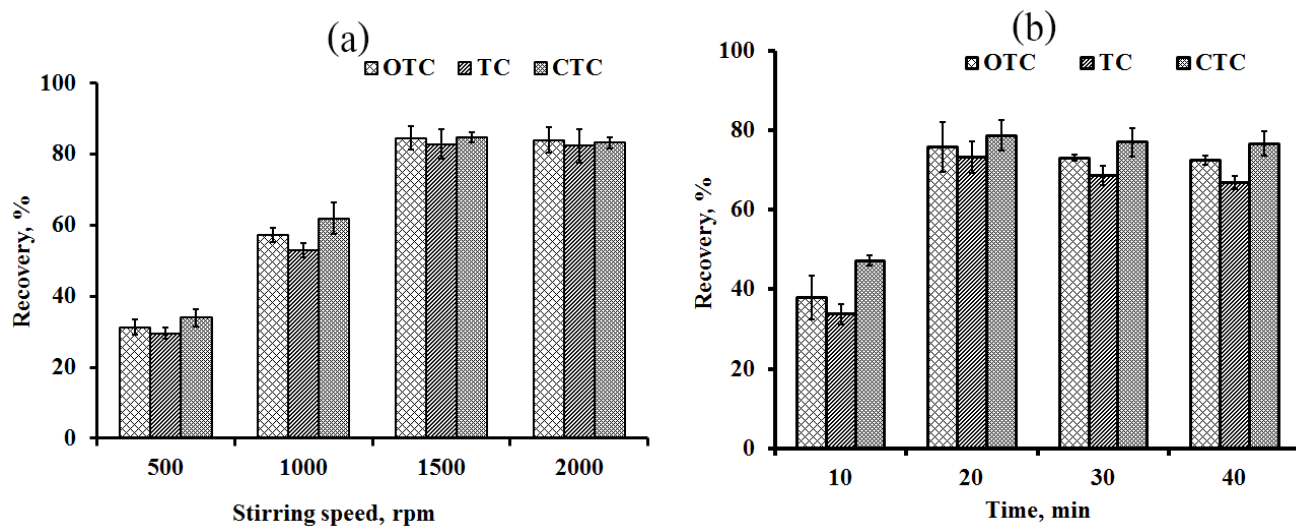


Fig. 5. Kaewsuwan W, et al.

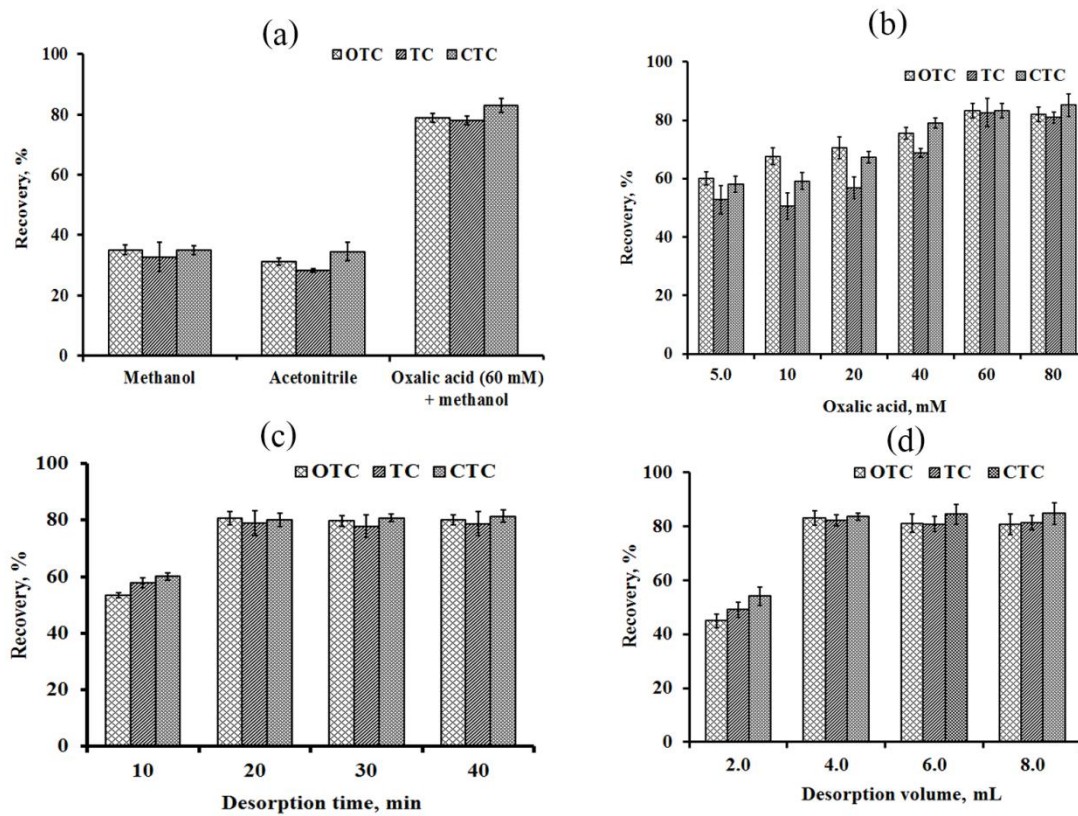


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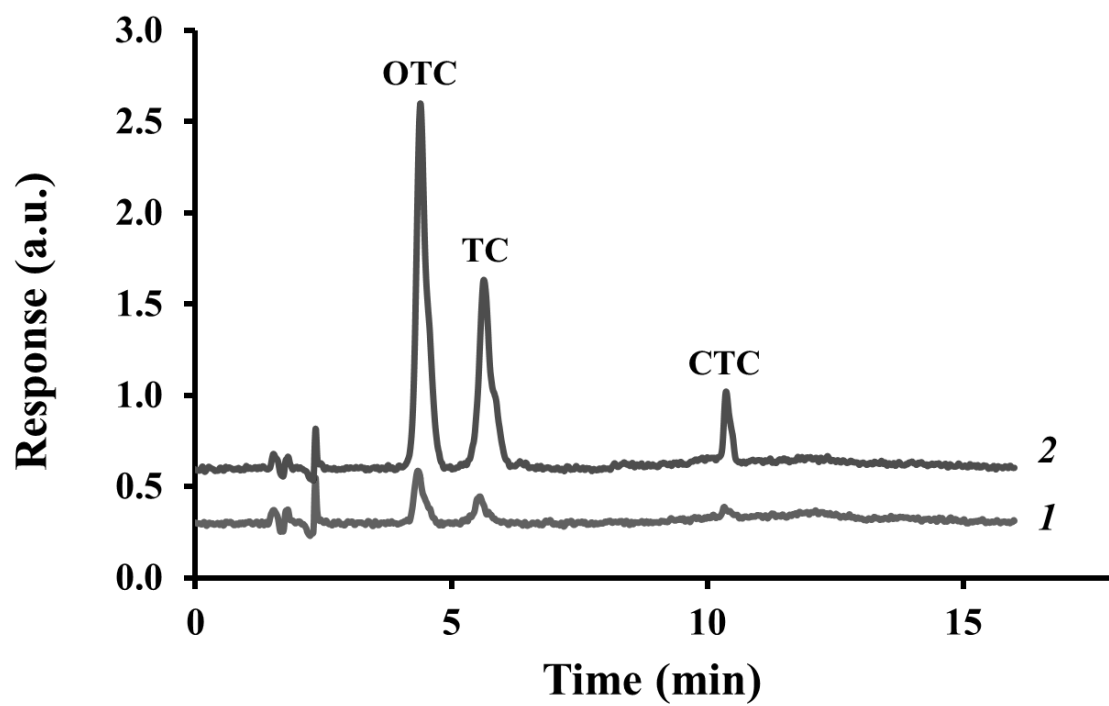


Fig. 7. Kaewsuwan W, et al.

## ภาคผนวก 2

ผลงานตีพิมพ์ชั้นที่ 2

ผลงานตีพิมพ์เพิ่มเติมนอกเหนือจากข้อเสนอโครงการวิจัย

## Polyaniline-coated magnetite nanoparticles incorporated in alginate beads for the extraction and enrichment of polycyclic aromatic hydrocarbons in water samples

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### ABSTRACT

A magnetic solid-phase extraction sorbent consisting of polyaniline-coated magnetite nanoparticles entrapped in alginate beads (PANI/alginate/Fe<sub>3</sub>O<sub>4</sub>) was successfully synthesised and applied for the extraction and preconcentration of polycyclic aromatic hydrocarbons (PAHs) in water samples. The magnetite nanoparticles helped to provide an easy and rapid isolation of the sorbent from the sample solution using an external magnet. The alginate beads helped to increase the surface area for polyaniline coating. The polyaniline-coated alginate/magnetite composite helped to increase the extraction efficiency due to the  $\pi$ - $\pi$  interactions between the polyaniline and the PAHs. Various parameters that affected the extraction efficiencies were optimised including the polymerisation time, the amount of sorbent, sample pH, extraction time, ionic strength, and desorption conditions. Under the optimal conditions, a linear response was achieved in the concentration range of 0.040–50.0  $\mu\text{g L}^{-1}$ , and the limit of detection was 0.010  $\mu\text{g L}^{-1}$ . This simple, convenient, cost-effective, and environmentally friendly method was successfully applied for the extraction and enrichment of PAHs in water samples. The recoveries ranged from 86.0% to 97.8% with a relative standard deviation <10 %.

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Magnetic solid-phase extraction; polyaniline; alginate; polycyclic aromatic hydrocarbons

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent pollutants in the environment that are composed of two or more fused aromatic rings [1]. They are produced from the incomplete combustion of organic materials and released into the environment via atmospheric fallout, urban run-off, municipal effluents, oil spillage, and industrial and vehicle emissions [2]. Due to their carcinogenic and mutagenic toxicity, the European Union (EU) and the United States Environmental Protection Agency (US EPA) have listed them as priority pollutants [3,4]. Moreover, some PAHs also cause endocrine disruption of important endocrine functions [5]. Therefore, the determination of PAHs in the environment is important, however, they are usually present at trace levels in environmental samples at

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concentrations that are lower than the detection limit of analytical instruments. Therefore, a suitable sample preparation method is generally required prior to instrumental analysis to preconcentrate the target PAHs.

Various sample preparation methods have been reported for the extraction and preconcentration of PAHs such as solid-phase extraction (SPE) [6–8], solid-phase microextraction (SPME) [9,10], stir bar sorptive extraction (SBSE) [11], and dispersive liquid–liquid microextraction (DLLME) [2,12]. Among these methods, SPE is one of the most extensively used for the extraction and enrichment of PAHs due to its high extraction efficiency [7]. However, it still has some drawbacks, such as time-consuming and tedious operations. To overcome these problems, a new SPE mode called magnetic solid-phase extraction (MSPE) has attracted much attention [13–15]. In the MSPE procedure, the magnetic adsorbents used to adsorb the analytes can be easily and rapidly isolated from sample solutions using an external magnet [16,17]. Several types of magnetic materials were used as MSPE sorbents such as zero-valent iron (Fe) [18], maghemite ( $\gamma$  Fe<sub>2</sub>O<sub>3</sub>) [19], and magnetite (Fe<sub>3</sub>O<sub>4</sub>) [20]. However, bare magnetic nanoparticles have a low selectivity towards the target analytes and a low stability in acid aqueous media [21]. Therefore, modifications to the magnetic sorbents with high-affinity coating materials are usually required to improve their extraction efficiency and selectivity [16,22–24]. Polyaniline (PANI) is a good choice of such a high-affinity material for the extraction of PAHs due to its large benzene ring system that contributes to strong interactions with aromatic compounds via  $\pi$ – $\pi$  interactions. Moreover, it also has a good chemical stability, large surface areas, a permeable porous structure, relatively low cost, and is easy to synthesis [25,26]. Polyaniline could be also polymerised in the presence of support media to produce an adsorbent with a defined shape [27,28].

In this work, a new MSPE sorbent of polyaniline-coated magnetite nanoparticles that were incorporated into alginate beads (PANI/alginate/Fe<sub>3</sub>O<sub>4</sub>) was developed. The alginate hydrogel was used to increase of the surface area that polyaniline can be coated [29]. PANI was therefore coated onto the surface of calcium alginate bead caged magnetite nanoparticles to improve the extraction efficiency of the sorbent. The developed sorbent was applied for the extraction and enrichment of PAHs in water. Benzo[a]anthracene, Benzo[a]pyrene, and Benzo[b]fluoranthene were selected as test compounds because of their toxicity, mutagenicity and they are grouped as probable human carcinogen. They are also included in the 2007 Priority List of Hazardous Substances.

## 2. Experimental

### 2.1. Chemicals and reagents

Aniline was purchased from Loba (Mumbai, India). Ammonium persulfate ((NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), Iron (II) chloride tetrahydrate (FeCl<sub>2</sub>·4H<sub>2</sub>O), Iron (III) chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was of HPLC grade and was purchased from Merck (Darmstadt, Germany). Calcium chloride dehydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O) was purchased from Ajax Finechem (NSW, Australia). Benzo[a]anthracene (BaA), Benzo[a]pyrene (BaP), Benzo[b]fluoranthene (BbF) standard solutions were purchased from Restek (Bellefonte, USA). Ultrapure water was prepared using a Maxima



ultrapure water system (ELGA, Buckinghamshire, England). Working standard solutions were prepared by diluting the stock standard solution with acetonitrile.

## 2.2. Instrumentation

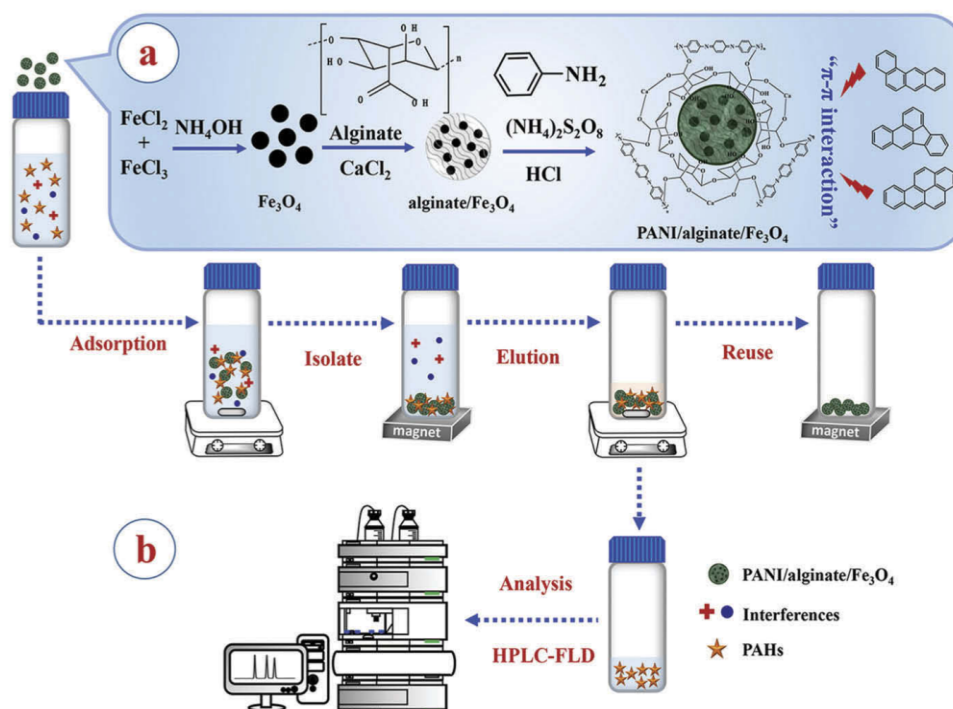
Chromatographic analysis was performed using a Hewlett-Packard 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany). ChemStation software was used for data acquisition and evaluations. The separation was performed on a reverse-phase VertiSep<sup>TM</sup> C<sub>18</sub> column (5 µm particles size, 250 mm× 4.6 mm I.D.) (Restek Bellefonte, USA). The mobile phase was acetonitrile: water (95:5, %v/v) at a flow rate of 1.0 mL min<sup>-1</sup>. The PAHs were detected with a fluorescence detector (FLD) with excitation (Ex) and emission (Em) wavelengths; BaA: Ex = 290, Em = 410; BbF: Ex = 266, Em = 450 and BaP: Ex = 270, Em = 410 nm. The injection volume and the column temperature were 20 µL and 30°C, respectively. The surface morphology of the developed sorbent was observed by scanning electron microscopy (SEM) JSM-5200 (JEOL, Tokyo, Japan). The Fourier transform infrared spectrometer (FT-IR) spectra were determined by FT-IR (Bruker, Germany). The N<sub>2</sub> adsorption and desorption isotherms were acquired using Quantachrome Autosorb 1 (Quantachrome Instruments, USA).

## 2.3. Preparation of polyaniline/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent

Figure 1(a) shows a schematic preparation of polyaniline/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent. Fe<sub>3</sub>O<sub>4</sub> nanoparticles were prepared by a chemical co-precipitation method [30]. Briefly, 5.0 g of FeCl<sub>3</sub> · 6H<sub>2</sub>O and 2.0 g of FeCl<sub>2</sub> · 4H<sub>2</sub>O were dissolved in 100 mL of deionised water and vigorously stirred in a nitrogen atmosphere. The mixed solution was heated and maintained at 80°C, then 10 mL of ammonium hydroxide was added dropwise, and the reaction was continuously refluxed for 1 h. The magnetite nanoparticles were separated from solution using a magnet, then thoroughly washed with 100 mL of distilled water three times, and finally the magnetite nanoparticles were dried at 60°C in an oven for 4 h.

For preparation of the alginate/Fe<sub>3</sub>O<sub>4</sub> composite beads [31], sodium alginate (2.0% w/v) was dissolved in deionised water, 0.20 g of synthesised Fe<sub>3</sub>O<sub>4</sub> was dispersed into 100 mL of the alginate solution by ultrasonication for 1 h to obtain homogenous colloidal suspension solution and this was added dropwise into a calcium chloride solution (5.0% w/v) while continuously stirring. The alginate/Fe<sub>3</sub>O<sub>4</sub> composite beads that formed were kept in the calcium solution for 2 h for the complete bead formation and then separated from the solution by a magnet.

For polyaniline coating, 3.0 mL of the aniline monomer was added into 100 mL of 1.0 M HCl while stirring to obtain the aniline solution and the alginate/Fe<sub>3</sub>O<sub>4</sub> composite beads were added into the aniline solution. Simultaneously, 1.80 g of (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> was added into 100 mL of 1.0 M HCl to obtain a (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution. The (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution was rapidly poured into the aniline solution. Polymerisation was allowed to occur at 30°C for 2 h. The product of the PANI-coated alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent was washed with ethanol and distilled water three times.



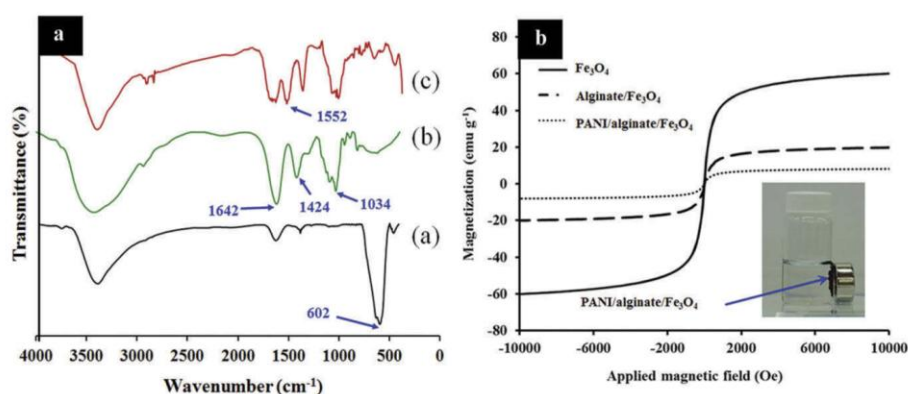
**Figure 1.** Schematic for the preparation of the (a) PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent and (b) the magnetic solid-phase extraction procedure using the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent.

## 2.4. Extraction procedure

The extraction procedure was carried out as shown in Figure 1(b). Firstly, 0.40 g of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbents was added into 10.0 mL of water sample and stirred at 1000 rpm for 20 min for the complete adsorption of the PAHs. Subsequently, the sorbents with the adsorbed PAHs was isolated from the sample solution using a magnet and the supernatant was decanted. Then, the extracted PAHs were desorbed from the sorbents with a desorption solvent while stirring at 1000 rpm for 30 min. The desorption solvent was then evaporated to dryness at 60°C, redissolved in 1.0 mL of acetonitrile and filtered through a PTFE filter (0.22 μm) before being injected into the HPLC system. The PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbents were reused after washing with 2.0 mL acetonitrile and 5.0 mL of deionised water.

## 2.5. Water samples

To indicate the applicability of the developed method, the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbents were applied as the MSPE sorbent for the extraction and enrichment of PAHs in real water samples that is. reservoir water, river water, and wastewater samples. Reservoir water was collected from reservoir of Prince of Songkla University, river water samples were collected from three rivers in Hat Yai city, Songkhla, Thailand and two wastewater samples were collected from a household effluent from Hat Yai City, Songkhla, Thailand. All samples were filtered through the 0.45-μm filter to remove particulate matters and kept in brown bottles at 4°C.



**Figure 2.** FT-IR spectra (A) of (a)  $\text{Fe}_3\text{O}_4$ , (b) Alginate and (c) PANI/alginate/ $\text{Fe}_3\text{O}_4$  and (B) The hysteresis loops of  $\text{Fe}_3\text{O}_4$ , Alginate/ $\text{Fe}_3\text{O}_4$  and PANI/alginate/ $\text{Fe}_3\text{O}_4$ . The inset represents the PANI/alginate/ $\text{Fe}_3\text{O}_4$  beads separated by a magnet.

### 3. Result and discussion

#### 3.1. Characterisation of PANI/alginate/ $\text{Fe}_3\text{O}_4$ sorbent

The preparation of the PANI/Alginate/ $\text{Fe}_3\text{O}_4$  sorbent was confirmed by FT-IR spectroscopy and SEM. Figure 2(a) exhibits the FT-IR spectra of the  $\text{Fe}_3\text{O}_4$ , alginate, PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent. The absorption peak at  $602\text{ cm}^{-1}$  was attributed to the stretching of the  $\text{Fe}_3\text{O}_4$  [32–34]. The absorption peaks at  $1642$ ,  $1424$ ,  $1034\text{ cm}^{-1}$  were related to the stretching of the O=C–O (asymmetric), O=C–O (symmetric), and the C–O of alginate bead [28,35]. The characteristic peaks appeared at  $1552\text{ cm}^{-1}$  (C=C stretching of the benzenoid ring) corresponded with the spectrum of PANI [36,37] and indicated that the polyaniline had been successfully synthesised and coated onto the alginate/ $\text{Fe}_3\text{O}_4$  bead.

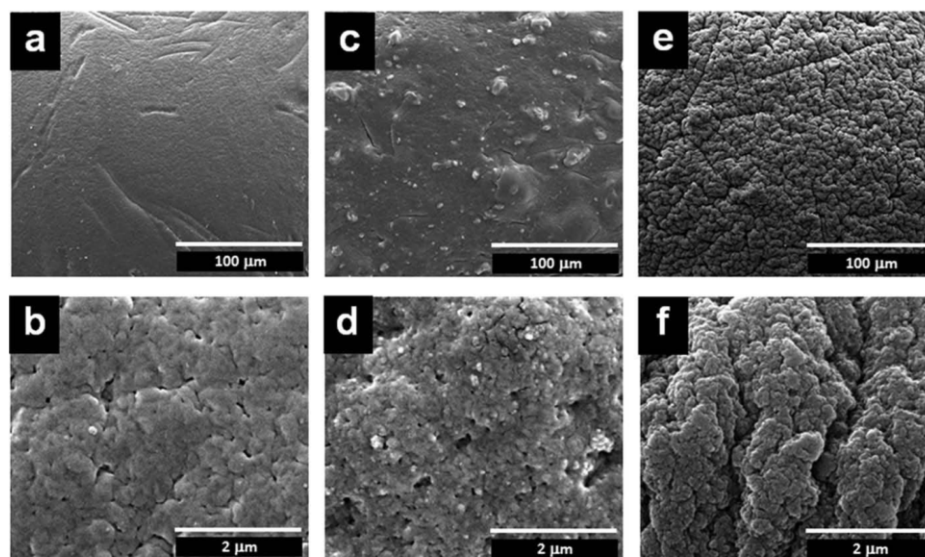
Vibrating sample magnetometry was also used to study the magnetic properties of the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent. Figure 2 (b) shows the magnetic hysteresis loops of  $\text{Fe}_3\text{O}_4$ , alginate/ $\text{Fe}_3\text{O}_4$ , and PANI/alginate/ $\text{Fe}_3\text{O}_4$ . The saturation magnetisation value were  $60.13$ ,  $19.90$ , and  $8.03\text{ emu g}^{-1}$  for  $\text{Fe}_3\text{O}_4$ , alginate/ $\text{Fe}_3\text{O}_4$ , and PANI/alginate/ $\text{Fe}_3\text{O}_4$ , respectively. The magnetic strength of the developed sorbents was sufficient for magnetic separation, and it was rapidly isolated from the solution using an external magnet.

The surface morphology of dried alginate bead,  $\text{Fe}_3\text{O}_4$ /alginate, and PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent were investigated by SEM. The morphology of the alginate bead is shown in Figure 3(a, b). The surface of the alginate bead was rough and showed a high porosity. Figure 3(c, d) demonstrates the distribution of the  $\text{Fe}_3\text{O}_4$  nanoparticles in the alginate bead. Figure 3(e, f) illustrates that the PANI was successfully coated onto the surface of the  $\text{Fe}_3\text{O}_4$ /alginate bead hence this attributed to the large surface area which could offer a high extraction efficiency for the PAHs, the extraction recovery  $>84\%$ . The BET-specific surface area of PANI/alginate/ $\text{Fe}_3\text{O}_4$  was determined by nitrogen adsorption and desorption isotherms as  $27.87\text{ m}^2\text{ g}^{-1}$ .

#### 3.2. Optimisation of the magnetic solid-phase extraction

In order to achieve the highest extraction efficiency with the shortest analysis time and a minimum use of solvent, several parameters such as the type of sorbent, polymerisation





**Figure 3.** SEM image of alginate (a = 500x, b = 20,000x), Fe<sub>3</sub>O<sub>4</sub>/alginate (c = 500x, d = 20,000x) and PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> (e = 500x, f = 20,000x).

time, amount of sorbent, extraction time, desorption conditions, sample pH, and ionic strength were investigated and optimised. The optimisation of extraction conditions were conducted using a spiked deionised water containing 1.0 μg L<sup>-1</sup> of each of the PAHs. The extraction efficiency was evaluated in terms of recovery.

### 3.2.1. Type of sorbent

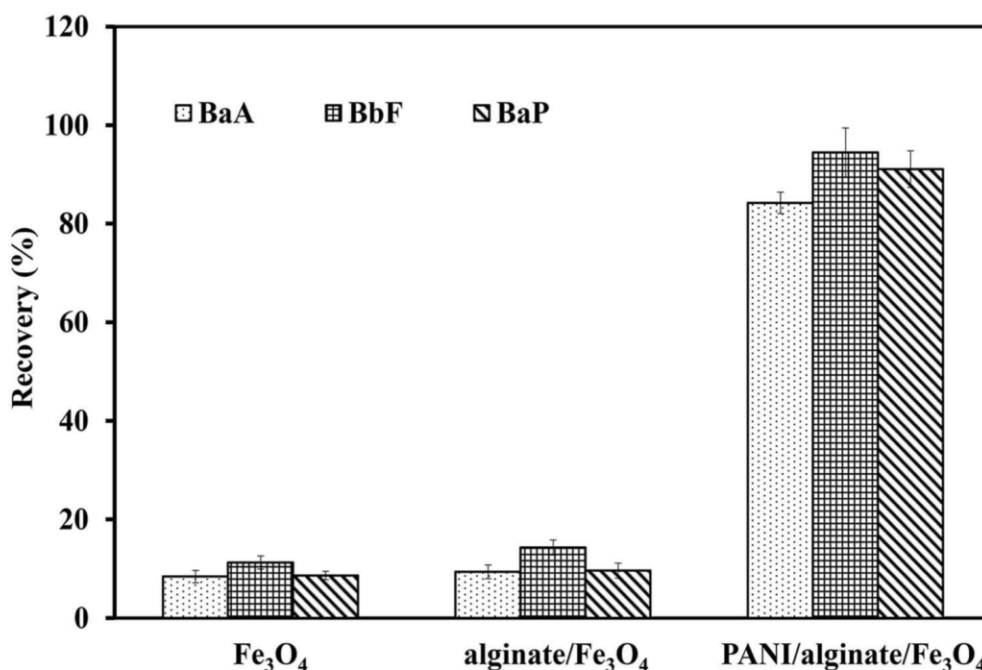
The extraction abilities of Fe<sub>3</sub>O<sub>4</sub>, Fe<sub>3</sub>O<sub>4</sub>/alginate and PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent were first investigated. As shown in Figure 4, Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>/alginate sorbents provided extraction efficiencies that were lower than 20%. While the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent provided a high recovery of >84 %. These results confirmed that PANI helped to increase the adsorption capability of the target PAHs via π–π interactions. Hence, the PANI-coated alginate/Fe<sub>3</sub>O<sub>4</sub> was applied as a MSPE sorbent for the extraction and enrichment of PAHs in water samples.

### 3.2.2. Polymerisation time

To obtain the highest extraction efficiency of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent, the effect of polymerisation time was studied over the range of 1.0–24.0 h. As shown in Figure S1, the extraction efficiency increased with the polymerisation time from 1.0 to 2.0 h and the extraction efficiency was decreased at any polymerisation time above 6 h. This was because polyaniline agglomerated and developed as a layer that was too thick and reduced the surface areas (Figure S2). Therefore, a polymerisation time of 2 h was selected.

### 3.2.3. Amount of sorbent

For the MSPE method, the amount of sorbent was one of the important parameters that influenced the extraction efficiency of the target analytes. To select the appropriate amount of PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent for the extraction of PAHs, different amounts of sorbent were investigated in the range of 0.20–0.80 g. The recoveries of PAHs increased



**Figure 4.** Effect of different sorbents on the recoveries of PAHs ( $1.0 \mu\text{g L}^{-1}$ ) ( $n = 3$ ); 20-min extraction time, 1000 rpm stirring of adsorption and desorption and 30-min desorption time.

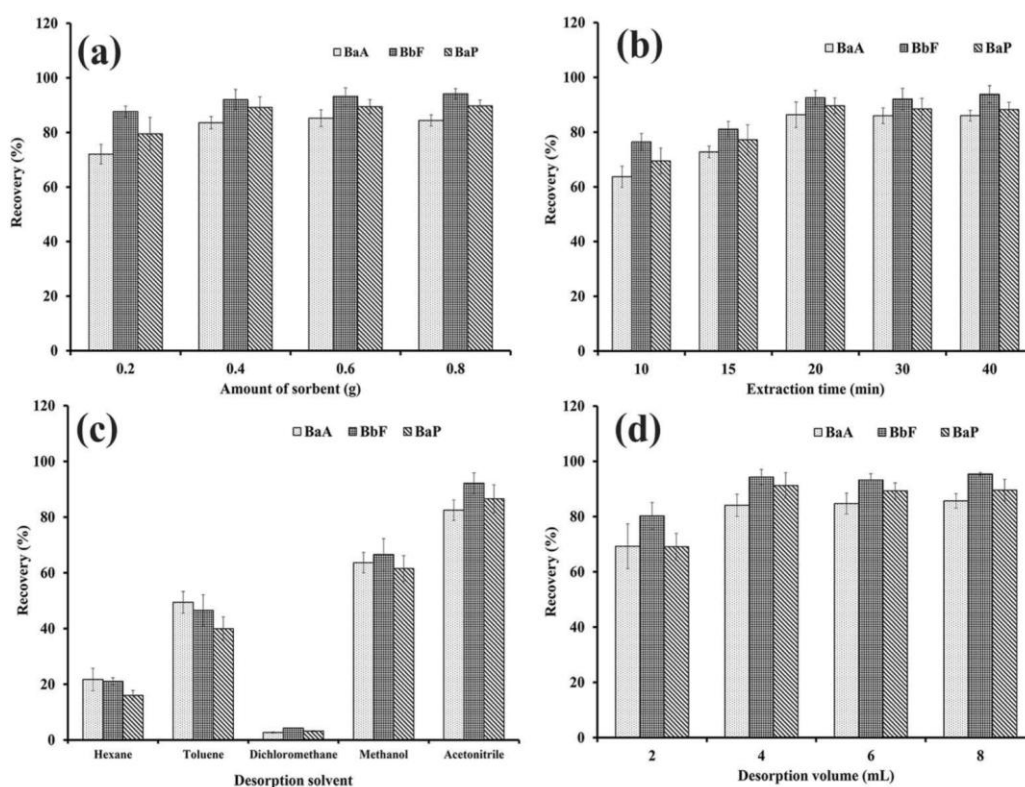
with an increase of the amount of sorbent from 0.20 to 0.40 g (Figure 5(a)), and it remained almost constant with any further increase of sorbent. This indicated that 0.40 g of sorbent was sufficient for the extraction of PAHs in 10.0 mL of a spiked water sample. Therefore, 0.40 g was selected as the optimal sorbent amount and used for further experiments.

### 3.2.4. Extraction time

Extraction time is an important factor that influences the extraction efficiency. To obtain the highest extraction efficiency with the shortest analysis time. The extraction time was investigated in the range of 10–40 min. It can be seen from Figure 5(b), that the extraction efficiency of all target PAHs was increased with extraction times from 10 to 20 min, and then remained almost constant with any further increase of the extraction time up to 40 min. Therefore, to ensure complete adsorption, an extraction time of 20 min was selected as being the optimum.

### 3.2.5. Desorption conditions

After extraction was completed, the analytes were desorbed from the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent. To ensure complete desorption of all target analytes, five organic solvents with different polarities were investigated including hexane, toluene, dichloromethane, methanol, and acetonitrile. Among these solvents, acetonitrile provided the highest recovery (Figure 5(c)), while a less polar solvent (hexane, toluene, and dichloromethane) provided a low desorption efficiency because the developed sorbent could not be dispersed well in these solvents leading to low desorption ability. Therefore,



**Figure 5.** (a) Effect of the amount of sorbent, (b) extraction time, (c) desorption solvent and (d) desorption solvent volume on the recoveries of PAHs ( $1.0 \mu\text{g L}^{-1}$ ) using the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent ( $n = 3$ ).

acetonitrile was further employed for desorption of PAHs from the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent.

In order to obtain complete desorption of PAHs from the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent with a minimal solvent volume and shortest desorption time, the effects of the volume of desorption solvent and the desorption time were investigated. All analytes were completely desorbed from the sorbent with 4.0 mL of acetonitrile (Figure 5(d)) and the desorption time of 30 min was sufficient to obtain the maximum desorption efficiency for all target analytes (Figure S3).

### 3.2.6. Effect of sample pH and ionic strength and sample volume

Since, the sample pH may affect the extraction efficiency and the stability of the sorbent. Therefore, the effect of sample pH on the extraction efficiency of the target PAHs was investigated by adjusting it with hydrochloric acid or sodium hydroxide solutions in the range of 2.0–9.0. As shown in Figure S4, the extraction efficiency showed no significant changes when the pH of the sample solution was changed, this is because the PAHs exist as neutral molecules. Therefore, for convenient analysis, there is no need to adjust the pH of the samples. The effect of ionic strength was also studied due to its ability to decrease the solubility of analytes in the sample solution and cause an increase in the extraction efficiency. In this work, the effect of ionic strength on the extraction efficiency of PAHs was investigated by adding NaCl to the sample solution over the concentration



range of 0–15% w/v. As shown in Figure S5, the extraction efficiency was independent of the ionic strength. Hence, there is no need to add NaCl to the sample solution. These results indicated a good stability of the developed sorbent in various conditions.

The influence of sample volume on the extraction efficiency of PAHs was investigated in the range of 5.0–50.0 mL and the results as shown in Figure S6. The recovery was reduced when the sample volume was greater than 10.0 mL. Therefore, 10.0 mL was selected as an optimum sample volume for the further experiment.

### 3.2.7. Effect of adsorption and desorption stirring rate

The extraction efficiency of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent depended on the partition rate of analytes between sample solution and sorbent. The stirring rate was an effective way to increase the mass transfer of analytes from sample solution to the sorbent. Therefore, the effect of stirring rate were investigated from 500 to 1500 rpm. The results showed that the recovery increased with stirring rate and reached a maximum at 1000 rpm (Figure S7). Therefore, 1000 rpm was selected as an optimum stirring rate.

The effect of desorption stirring rate was also investigated in the range of 500–1500 rpm. It was observed that the recovery increased when the desorption stirring rate increased up to 1000 rpm and became constant at higher rate (Figure S8). Therefore, the desorption stirring rate of 1000 rpm was chosen for further experiment.

### 3.3. Reproducibility, reusability, and adsorption capacity

The preparation reproducibility of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent was evaluated in terms of lot-to-lot reproducibility. Six different lots of sorbent were prepared under the same conditions but with different times that were used to extract PAHs in spiked deionised water at 1.0 µg L<sup>-1</sup>. The relative standard deviations (RSDs) were lower than 6% (Figure S9), and these were better than the acceptable values recommended by the AOAC (<16%). This indicated that the process for preparation of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent had a good reproducibility.

In order to investigate the reusability of the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent, after the desorption of the analytes from the sorbent, to eliminate the memory effect, it was washed with 2.0 mL of acetonitrile and 5.0 of deionised water and no response of target analytes were observed which indicated that no carry-over was obtained. As shown in Figure S10, the recoveries were almost unchanged after six cycles. These results indicate that the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent could be reused six times.

The adsorption capacity of PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent for BaA, BbF, and BaP was found to be 1.25, 1.19, and 1.15 µg g<sup>-1</sup>, respectively.

### 3.4. Comparative studies

The extraction efficiency of PAHs using the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent were compared with a commercial SPE sorbent (Sep-Pak C18 plus). As shown in Figure S11, the average recoveries were from 86.1% to 95.0% and 85.7% to 92.7% for the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent and Sep-Pak C18 plus cartridge, respectively. The average recoveries of the developed sorbent and Sep-Pak C18 plus were compared by the statistical *t*-test [38] and there was no significant difference between the two sorbents (*P* > 0.05). However, the

developed sorbent showed several advantages over the commercial SPE sorbent such as simpler to use and lower cost. Moreover, the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> composite sorbent could be reused up to six times, whereas the commercial SPE sorbent cannot be reused. Therefore, the PANI/alginate/Fe<sub>3</sub>O<sub>4</sub> sorbent can be used as an alternative sorbent for the extraction and enrichment of PAHs.

### 3.5. Analytical performance

Under the optimum extraction conditions, the analytical performance of the developed method was evaluated including its linear range, limit of detection (LOD) and limit of quantification (LOQ) and the result are summarised in Table S1. The developed method has a wide linearity over the range of 0.040–50.0 µg L<sup>-1</sup> with a coefficient of determination (R<sup>2</sup>) of greater than 0.999. The repeatability of the developed sorbent was investigated to evaluate the precision of the method and the RSDs was less than 10%, indicated that it had a good repeatability. The LOD and LOQ based on the signal-to-noise ratios of 3 and 10 were 0.01 and 0.04 µg L<sup>-1</sup>, respectively. The LOD and LOQ values were much lower than the maximum contaminant level (MCL) in the surface water, that is. 0.2 µg L<sup>-1</sup> for the BbF and BaP and 0.1 µg L<sup>-1</sup> for BaA, respectively. Therefore, the developed method can effectively be used to determine BaA, BbF, and BaP in water samples.

### 3.6 Application of the developed method for the determination of PAHs in water samples

To demonstrate the applicability and reliability of the developed method, it was applied for the determination of PAHs in reservoir water, river water, and wastewater samples. The quantitative results are listed in Table 1. None of target PAHs was detected in the reservoir water, while the average concentration of PAHs in the river waters and waste waters in the range of not-detectable to 0.06 µg L<sup>-1</sup>. The presence of PAHs may be due to incomplete combustion from the vehicle emissions deposited into the water samples. For waste water, PAHs are produced by cooking that are released into the effluents [2].

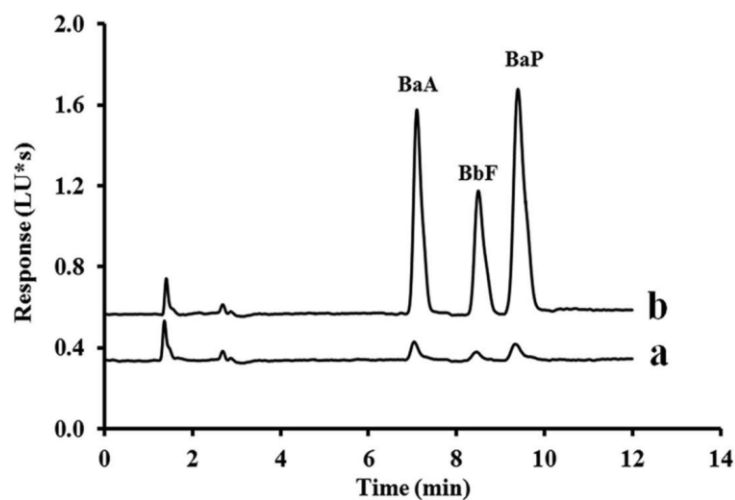
The accuracy of the developed method was validated by spiking standard PAHs into real water samples at three concentrations (i.e 0.10, 0.50, and 2.0 µg L<sup>-1</sup>) and was then extracted and detected under optimum conditions. As listed in Table S2, the recoveries of BaA was lower than BbF and BaP due to its polar is more than BbF and BaP. However, the recoveries of the three PAHs were in the range of 86.0 ± 5.3% to 97.8 ± 8.3% which

**Table 1.** Concentration of PAHs in real water samples.

Water sample	Concentration (µg L <sup>-1</sup> )		
	BaA	BbF	BaP
Reservoir water	ND	ND	ND
River water 1	ND	<LOQ	<LOQ
River water 2	0.048 ± 0.007	0.046 ± 0.006	0.052 ± 0.009
River water 3	<LOQ	<LOQ	<LOQ
Waste water 1	<LOQ	<LOQ	<LOQ
Waste water 2	0.059 ± 0.008	0.040 ± 0.004	<LOQ

ND: not detectable.





**Figure 6.** HPLC chromatograms of target PAHs in spiked wastewater samples ( $1.0 \mu\text{g L}^{-1}$ ) (a) before and (b) after the extraction using the PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent.

are within the acceptable values (70–125%) of the AOAC guidelines. Satisfactory recoveries demonstrated no effect from the matrix compositions of the water samples. **Figure 6** illustrates a typical chromatogram of PAHs in spiked wastewater samples at  $1.0 \mu\text{g L}^{-1}$  before and after extraction using PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent. This result clearly demonstrated that the developed PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent could be applied for the extraction and determination of trace PAHs in water samples that contain different matrix interferences.

### 3.7. Comparison of the developed method with other methods

The analytical performance of this method for the extraction and determination of PAHs in water samples was compared with other existing methods. As shown in **Table 2**, the LOD of this method is lower than several methods [6,24,39–44]. Although, some methods provided a lower LOD, however, these methods required a larger sample volume [45] and longer extraction time [8]. The recoveries for this work were comparable [41,43] or better than others methods [6,8,24,39–41,44,45]. Hence, the analytical performance of the developed method was acceptable for trace analysis of PAHs.

## 4. Conclusion

In this work, polyaniline-coated magnetite nanoparticles incorporated into alginate beads was successfully synthesised and applied as a MSPE sorbent for the extraction and enrichment of PAHs in water samples. The coating of the composited alginate/ $\text{Fe}_3\text{O}_4$  beads with polyaniline not only improved the adsorption ability of the PAHs but also provided a high extraction efficiency ( $86.0 \pm 5.3$  to  $97.8 \pm 8.3\%$ ) and a good stability and reproducibility. The low detection limit was  $0.01 \mu\text{g L}^{-1}$  and that was below the MCL values required by the US EPA. The PANI/alginate/ $\text{Fe}_3\text{O}_4$  sorbent exhibited a convenient and rapid collection of the sorbent from water samples without a column passing or

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

Methods	Sorbents	PAHs	LOD ( $\mu\text{g L}^{-1}$ )	Recovery (%)	Extraction time (min)	Sample volume (mL)	References
DLLME-HPLC-FLD	Organic solvent	BaA, BbF, BaP	0.05–0.08	86–95	-	4	[41]
SPE-HPLC-UV	Multiwalled carbon nanotubes	BaA, BbF	0.009–0.015	79–118	125	500	[8]
SPME-HPLC-DAD	Gold-functionalised stainless steel wire	BaP	0.10	85–120	15	15	[42]
SRSE-GC-MS	Poly(ethyleneglycol dimethacrylate)/graphene	BaA, BbF, BaP	0.019–0.056	63–99	120	50	[43]
MES-HPLC-FID	PANI/Zn-film/stainless steel	BaP	0.05	82–110	30	1	[44]
MSPE-GC-MS	Magnetic N-doped carbon nanotubes	BaA	0.36	81–105	1	4	[39]
MSPE-HPLC-FLD	Ionic liquid-coated $\text{Fe}_3\text{O}_4$	BaA, BaP	0.0003–0.0017	76–105	10	300	[45]
MSPE-HPLC-UV	Graphitic carbon nitride/ $\text{Fe}_3\text{O}_4$ composite	BaA	0.05	80–99	10	100	[6]
MSPE-HPLC-UV	$\text{Fe}_3\text{O}_4$ /graphene oxide	BaA, BbF, BaP	0.09–0.13	77–103	10	50	[24]
MSPE-UPLC-DAD	Magnetic nanoparticles–nylon 6 composite	BbF	0.05	80–111	30	25	[40]
MSPE-HPLC-FLD	PANI/alginate/ $\text{Fe}_3\text{O}_4$ sorbent	BaA, BbF, BaP	0.01	86–98	20	10	This work

DLLME: dispersive liquid–liquid microextraction; SPE: solid-phase extraction; SPME: solid-phase microextraction; SBSE: stir bar sorptive extraction; MES: microextractor stick; MSPE: magnetic solid-phase extraction.

filtration step that was time consuming. In addition, the developed novel sorbent was easily prepared, cost-effective and environmentally friendly. The developed method was successfully applied to determine BaA, BbF, and BaP in real water samples. It could be applied for the determination of PAHs in other samples.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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