Dispersive Magnetic Solid Phase Extraction Sorbent of Octadecyl/Silica/Magnetite Nanoparticles for the Determination of Tetracyclines in Water Samples

Watchara Kaewsuwan

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Chemistry
Prince of Songkla University
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Author	Mr. Watchara Kaewsuwan
Major Program	Chemistry

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

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

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ชื่อวิทยานิพนธ์ ตัวดูดซับของแข็งอนุภาคแม่เหล็กเคลือบซิลิกาและออกตะเดซิลสำหรับวิเคราะห์สารเตตราไซคลีนในน้ำ
ผู้เขียน นายวัชระ แก้วสุวรรณ์
สาขา เคมี
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บทคัดย่อ

วิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อพัฒนาตัวดูดซับของแข็งอนุภาคแม่เหล็กเคลือบซิลิกาและออกตะเดซิล ($C_{18}/SiO_2/Fe_3O_4$ NPs) สำหรับสกัดและเพิ่มความเข้มข้นสารเตตราไซคลีนในตัวอย่างน้ำ อนุภาคแม่เหล็กช่วยในการแยกตัวดูดซับออกจากตัวอย่างน้ำได้ง่ายและรวดเร็วโดยใช้แท่งแม่เหล็กภายนอก ชั้นซิลิกาช่วยป้องกันการรวมกลุ่มของอนุภาคแม่เหล็กในขณะทำการสกัดตัวอย่าง และออกตะเดซิลช่วยเพิ่มความสามารถในการดูดซับสารเตตราไซคลีน ได้ศึกษาสภาวะที่เหมาะสมเพื่อเพิ่มประสิทธิภาพในการสกัด ได้แก่ ปริมาณของตัวดูดซับ พีเอชของน้ำตัวอย่าง อัตราเร็วในการคนตัวอย่าง เวลาที่ใช้ในการสกัด และสภาวะในการคายการดูดซับ ภายใต้สภาวะที่เหมาะสม วิธีที่พัฒนาขึ้นให้ช่วงความเป็นเส้นตรงตั้งแต่ 0.0020 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร สำหรับออกซีเตตราไซคลีน และ 0.010 ถึง 1.0 ไมโครกรัมต่อมิลลิลิตร สำหรับเตตราไซคลีนและคลอเตตราไซคลีน มีขีดจำกัดในการตรวจวัด 0.002 ไมโครกรัมต่อมิลลิลิตร สำหรับออกซีเตตราไซคลีน และ 0.01 ไมโครกรัมต่อมิลลิลิตร สำหรับเตตราไซคลีนและคลอเตตราไซคลีน ตัวดูดซับที่พัฒนาขึ้นมีประสิทธิภาพในการสกัดที่ดีโดยมีค่าร้อยละการได้กลับคืนในช่วง 82 ถึง 88 เปอร์เซ็นต์ และมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 10 เปอร์เซ็นต์ วิธีที่พัฒนาขึ้นสามารถประยุกต์ใช้สำหรับการสกัดและวิเคราะห์สารเตตราไซคลีนในตัวอย่างน้ำได้ นอกจากนี้ตัวดูดซับที่พัฒนาขึ้นสามารถใช้งานได้ง่าย สะดวกรวดเร็ว ราคาถูก และมีประสิทธิภาพในการสกัดสูง

Thesis title Dispersive Magnetic Solid Phase Extraction Sorbent
of Octadecyl/Silica/Magnetite Nanoparticles for the
Determination of Tetracyclines in Water Samples
Author Mr. Watchara Kaewsuwan
Major Program Chemistry
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Abstract

This thesis aimed to develop a new magnetic solid phase extraction sorbent of magnetite nanoparticles coated with silica and octadecyl ($C_{18}/SiO_2/Fe_3O_4$) for the extraction and preconcentration of tetracyclines in water samples. The magnetite nanoparticles facilitated a convenient magnetic separation of sorbent from an aqueous sample using an external magnet, the silica layer helped to prevent the aggregation of the magnetite nanoparticles and the octadecyl layer helped to enhance the adsorption ability. To obtain the highest extraction efficiency with the shortest analysis time, the effect of various parameters were optimized including the amount of sorbent, sample pH, stirring rate, extraction time and desorption conditions. Under the optimum conditions, the calibration curves were linear over the concentration range of 0.0020 to 1.0 µg mL^{-1} for oxytetracycline and 0.010 to 1.0 µg mL^{-1} for tetracycline and chlortetracycline. The limit of detections were 0.002 µg mL^{-1} for oxytetracycline and 0.01 µg mL^{-1} for tetracycline and chlortetracycline. The satisfactory recoveries were obtained in the range of 82 to 88% with the relative standard deviations were less than 10%. The developed method was successfully applied for the extraction and determination of tetracyclines from water samples with a several advantages such as simplicity, rapid and convenience, cost-effectiveness and high extraction efficiency.

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The successful completion of this thesis would be quite impossible without the help of many people. I wish to express my gratitude to those who have contributed to the completion of this thesis:

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Watchara Kaewsuwan

The Relevance of the Research Work to Thailand

The purpose of this Master of Science Thesis in Chemistry (Analytical chemistry) is to develop and evaluate the performance of a magnetic sorbent of magnetite nanoparticles coated with silica and octadecyl ($C_{18}/SiO_2/Fe_3O_4$) for the extraction and determination of tetracyclines in water samples followed by high performance liquid chromatography with diode-array detector (HPLC-DAD).

The developed method is simplicity, rapid and convenience, cost-effectiveness and high extraction efficiency which can be applied for the extraction and determination of tetracyclines in environmental water samples by several governments and private sector in Thailand, the Ministry of Public Health, Ministry of Agriculture and Cooperative, Ministry of Environment and Ministry of Education.

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List of abbreviations

AOAC	Association of analytical communities
C_6H_5	Phenyl
C_8	Octyl
C_{18}	Octadecyl
CE	Capillary electrophoresis
CN	Cyanopropyl
CTC	Chlortetracycline
CW/TPR	Carbowax/templated resin
DAD	Diode-array detector
EU	European Union
FID	Flame ionized detector
FLD	Fluorescence detector
FT-IR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HLB	Hydrophilic Lipophilic Balanced
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICH	International Conference on Harmonisation
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MIP	Molecularly imprinted polymer
MNPs	Magnetic nanoparticles
MRLs	Maximum residue limits
MS	Mass spectrometer
MSPE	magnetic solid phase extraction
MWCNTs	Multiwall carbon nanotubes
NH_2	Aminopropyl
NPs	Nanoparticles

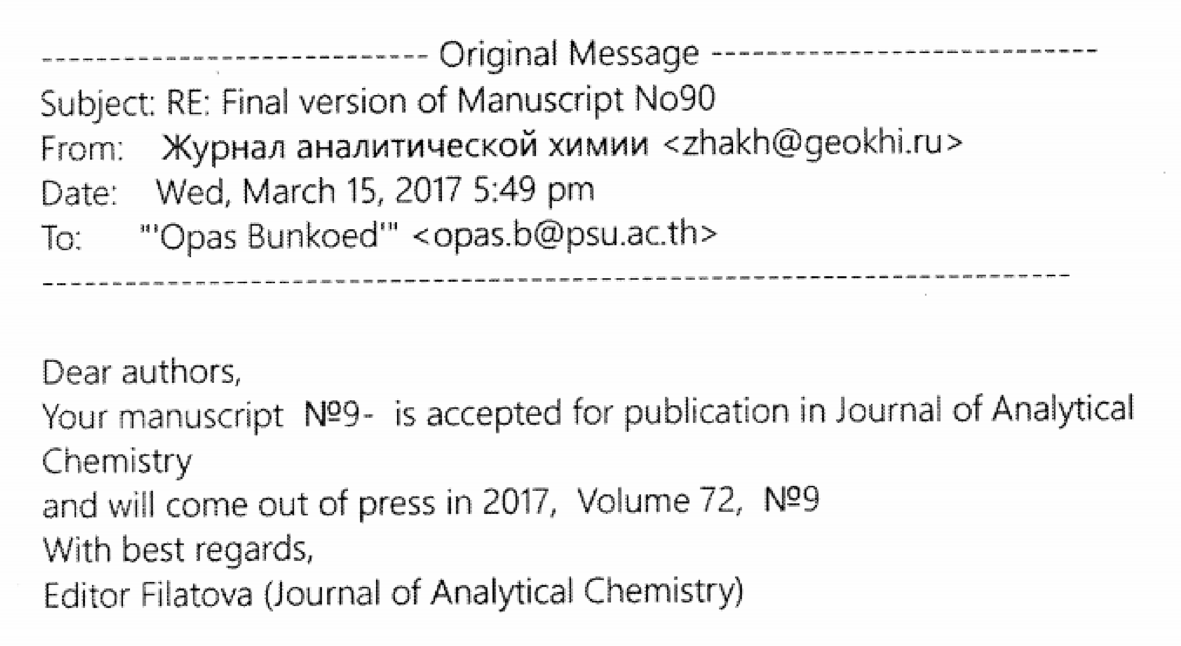
List of abbreviations (Continued)

OTC	Oxytetracycline
PANI	Polyaniline
PPy	Polypyrrole
PS	Polystyrene
PVA	Poly (vinyl alcohol)
R^2	Coefficient of determination
RSDs	Relative standard deviations
SEM	Scanning electron microscopy
SD	Standard deviation
S/N	Signal-to-noise ratio
SPE	Solid phase extraction
SPME	Solid phase microextraction
TC	Tetracycline
TEOS	Tetraethyl orthosilicate
TLC	Thin layer chromatography
UV-Vis	Ultraviolet-visible
U.S. FDA	United State Food and Drug Administration
VSM	Vibrating sample magnetometer
WHO	World Health Organization

List of publication

Paper: **Kaewsuwan, W.,** Kanatharana, P., and Bunkoed, O. Dispersive Magnetic Solid Phase Extraction Using Octadecyl Coated Silica Magnetite Nanoparticles for the Extraction of Tetracyclines in Water Samples.

Manuscript (Accepted), Journal of Analytical Chemistry.



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Dear authors,

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and will come out of press in 2017, Volume 72, №9

With best regards,

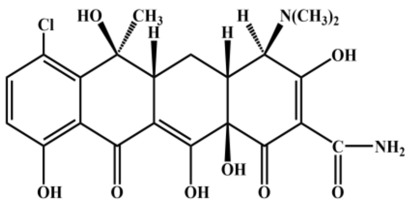
Editor Filatova (Journal of Analytical Chemistry)

1. Introduction

1.1 Background and the rationale

Tetracyclines (TCs) are a group of antibiotics extensively used for the prophylactic control of bacterial infections in human and veterinary medicine. It is also misused for growth promotion in the farming industry (Yang *et al.*, 2013; Jiao *et al.*, 2015). Tetracyclines antibiotics, oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC), **(Figure 1.1)** are the second most widely used in the world (Erşan *et al.*, 2013) due to their easy availability, satisfactory therapeutic properties, low costs and broad spectrum of antimicrobial activity against a wide range of both Gram-positive and Gram-negative bacteria (Solliec *et al.*, 2015). However, their extensively used with animals some serious problems on human health have emerged, some human pathogens that were originally sensitive to tetracyclines are now resistant, and some tetracyclines are toxic (Evaggelopoulou and Samanidou, 2012). For these reasons, regulatory agencies have enacted decisions that keep these substances under control. The World Health Organization (WHO) and European Union (EU) have established a maximum residue limits (MRLs) of 0.10 mg L^{-1} for oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC). The U.S. Food and Drug Administration has set the tolerance of 0.30 mg L^{-1} for the combined residues of OTC, TC and CTC in milk (Fritz and Zuo, 2007). Therefore, it is important to develop a suitable method for the determination of tetracyclines.

Oxytetracycline (OTC) Tetracycline (TC)



Chlortetracycline (CTC)

Figure 1.1 The chemical structures of tetracyclines

Several analytical methods have been reported for the determination of tetracyclines *i.e.*, ion chromatography (IC) (Ding and Mou, 2000; Moats, 2000), thin layer chromatography (TLC) (Oka *et al.*, 1994), capillary electrophoresis (CE) (Chen and Gu, 1995; Ibarra *et al.*, 2011) and High performance liquid chromatography (HPLC) (Cinquina *et al.*, 2003; Yang *et al.*, 2013). Among these methods, HPLC is the most widely used because of its high sensitivity, good selectivity and precision (Yang *et al.*, 2013). However, the direct detection of tetracyclines in real samples are usually difficult because of their low concentrations and effected by many interferences. Therefore, sample preparation method is required prior to instrumental analysis.

Various sample preparation methods have been reported for the extraction and preconcentration of tetracyclines such as liquid liquid extraction (LLE) (Zhao *et al*., 2004), solid phase extraction (SPE) (Ben *et al*., 2008) and solid phase microextraction (SPME) (Lock *et al.*, 1999). However, traditional liquid liquid extraction methods are time-consuming, tedious step and require a large volume of organic solvents (Tsai *et al.*, 2010). A conventional SPE procedures are more efficient according to their ability to achieve high recoveries. According to previous reported, Oasis HLB cartridges provided acceptable performance for the extraction of tetracyclines from various samples (Škrášková *et al.*, 2013). It is designed to retain both hydrophobic and hydrophilic compounds with a high capacity (Li *et al.*, 2008; Xu *et al.*, 2008). However, traditional SPE cartridges and equipment are expensive, the operations are quite tedious and time-consuming (Liu *et al.*, 2014a). In addition, the advantages of SPME method such as the reduction of organic solvent requirements, simplicity and save preparation time (compared to LLE and SPE). However, SPME method has some disadvantages including expensive, the fiber is fragile and has limited lifetime and sample carry-over during extraction (Tsai *et al*., 2009). To overcome these problems, a new sample preparation technique called magnetic solid phase extraction (MSPE) has received increasing attention due to its simplicity, short extraction time, low consumption of organic solvents and easy separation of magnetic sorbents from various samples (Sukchuay *et al.*, 2015). However, bare magnetic nanoparticles (Fe_3O_4) are prone to aggregate, easy to oxidize and low selectivity in complex matrices. Therefore, these magnetic nanoparticles were modified with specific ligands to make them more selective (Bagheri *et al.*, 2014). Silica is one of the most ideal coating material due to it can prevent the aggregation of Fe_3O_4 nanoparticles and improve their chemical stability (Nodeh *et al.*, 2015). However, the hydrophilic silica would not be applicable for the extraction of tetracyclines. In this work, an additional coating of an octadecyl (C_{18}) was used to improve the adsorption capacity of tetracyclines via hydrophobic interactions. The developed $C_{18}/SiO_2/Fe_3O_4$ sorbent was applied to extract tetracyclines from water samples and high performance liquid chromatography (HPLC) was used for the separation and quantification of tetracyclines.

1.2 Objective

To develop a new magnetic solid phase extraction sorbent for the extraction and preconcentration of tetracyclines in water samples.

1.3 Analytical methods

Several analytical techniques have been reported for the determination of tetracyclines. High performance liquid chromatography (HPLC) is the most commonly used because of its high sensitivity, good selectivity and precision (Yang *et al.*, 2013). HPLC is a separation technique for identify of each individual target analytes based on mechanism of adsorption, partition or ion exchange depend on the type of stationary phase and chemical properties of analytes. A typical setup of an HPLC system as shown in **Figure 1.2**, which consists of the mobile phase container to hold the solvent, a pump to deliver the desired flow rate and mobile phase through the system, an injector or autosampler to introduce the sample into the continuously flowing mobile phase that carrier the sample through the column, the column contains the stationary phase to separate of target analytes, a detector to generates a signal proportional to the amount of target analytes, a data processor to provide a data acquisition. In order to achieve the highest performances *i.e.*, good separation and short analysis time, some parameters need to be optimized such as composition of mobile phase, flow rate of mobile phase and detection wavelength.

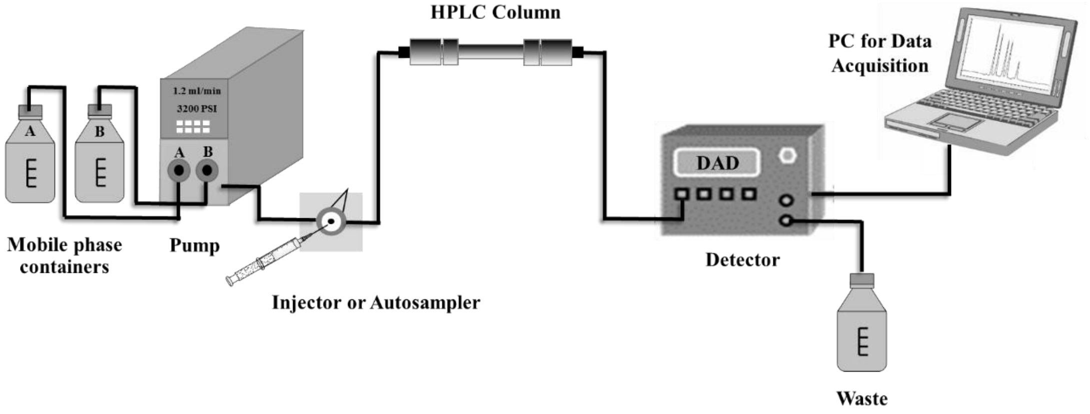


Figure 1.2 A typical setup of an HPLC system

Mobile phase of an HPLC system generally is a mixture of water and organic solvent. The choice of mobile phase depends on the mode of HPLC operation. Since, the chemical structures of tetracyclines are amphoteric compounds which can be interacted with any residual of silanol groups on the silica support surface containing in the column (stationary phase), producing tailing peaks. Therefore, the addition of some acid in mobile phase is normally required. The mobile phase for the separation and determination of tetracyclines such as methanol : acetonitrile : 0.02 M oxalic acid solution (10 : 20 : 70 % v/v) (Yang *et al.*, 2013), 0.01 M oxalic acid : acetonitrile : methanol (60 : 25 : 15 % v/v) (Cinquina *et al.*, 2003) and 0.1% formic acid : acetonitrile : methanol (75 : 18 : 7 % v/v) (Andersen *et al.*, 2005). In this work, the mobile phase was a mixture of methanol : acetonitrile : 0.01 M oxalic acid (5 : 18 : 77 % v/v) with the flow rate of 1.0 mL min^{-1}.

The stationary phase is the most important part for the separation of target analytes. Various types of stationary phases have been used for the separation of organic compounds such as octyl (C_8), octadecyl (C_{18}), phenyl (C_6H_5), cyanopropyl (CN) and aminopropyl (NH_2). In this work, the separation of tetracyclines were carried out using a reverse phase VertiSep^{TM} UPS-C_{18} column (4.6 x 150 mm, 5 µm particle size).

The chromatographic detector is a capable for the identification of eluting components in the mobile phase. The specific property of detectors response to any compounds based on collective changes in composition of sample and mobile phase. A number of analytical methods have been used for the quantification of tetracyclines using high performance liquid chromatography (HPLC) coupled with different detection modes such as ultraviolet-visible (UV-Vis) (Wang *et al.*, 2008; Zhou *et al.*, 2009), diode-array detector (DAD) (Shalaby *et al.*, 2011; Feng *et al.*, 2016), fluorescence (FLD) (Peres *et al.*, 2010; Mesgari Abbasi *et al.*, 2012) and mass spectrometer (MS) (Ben *et al.*, 2008; Salvia *et al.*, 2015). In this work, DAD was chosen for the detection and quantification of tetracyclines.

The advantages of DAD over the UV-Vis detector such as high resolution and low noise. It has multiple diode array to obtain the information over a wide range of wavelengths at one time, which is a merit of the DAD detector. The sensitivity depends on the molar absorption coefficient of the target analytes. It is used for the simultaneous monitoring of several absorbing component at different wavelengths in order to select the best wavelength for analysis.

1.4 Sample preparation techniques

Sample preparation techniques are important for the determination of target analytes in complexes matrix. It is used to preconcentrate and clean-up the samples before an instrumental analysis (Wang *et al.*, 2008). Various sample preparation techniques have been reported for the extraction and preconcentration of tetracyclines such as liquid liquid extraction (LLE) (Zhao *et al.*, 2004), solid phase extraction (SPE) (Ben *et al.*, 2008) and solid phase microextraction (SPME) (Lock *et al.*, 1999). Recently, a new approach called magnetic solid phase extraction (MSPE) has also been developed for the extraction and preconcentration of organic compounds (Rodriguez *et al.*, 2010; Ibarra *et al.*, 2011; Tolmacheva *et al*., 2015). A summarized of sample preparation techniques for the extraction of tetracyclines are showed in **Table 1.1**. In this work, MSPE was applied for the extraction and preconcentration of tetracyclines from water samples.

MSPE has been developed to overcome the problems of a conventional SPE and LLE techniques. MSPE technique is based on the use of a magnetic sorbent added into the solution containing the target analytes. Under the stirring, the analytes adsorbed on the surface of a magnetic sorbent. After extraction, the analytes were desorbed from a magnetic sorbent with the appropriate organic solvents and can be easily separated by an external magnet without centrifugation or filtration of the sample (Ibarra *et al.*, 2015). This technique has been used for the extraction and preconcentration of target analytes from different matrices due to its simplicity, short extraction time, low consumption of organic solvents and high extraction efficiency (Chen *et al.*, 2015; Sukchuay *et al.*, 2015). The application of MSPE for the determination of various target analytes from different samples as shown in **Table 1.2**.

Table 1.1 Sample preparation techniques for the extraction and preconcentration of tetracyclines

Extraction methods	**Sample**	**Extractants**	**Extraction time (min)**	**Sample amount (mL)**	**LOD (µg L^{-1})**	**Recovery (%)**	**References**
LLE	Milk	Hexane	< 15	10	10-25	88-96	(Zhao *et al.*, 2004)
SPE	Swine wastewater	Oasis HLB cartridge	400	200	0.005-0.091	70-137	(Ben *et al.*, 2008)
SPME	Milk	CW/TPR	-	3.5	4-40	-	(Lock *et al.*, 1999)
MSPE	River water	HCPs-Fe_3O_4 adsorbent	20	100	-	95-97	(Tolmacheva *et al.*, 2015)
MSPE	Milk	silica based magnetic support	20	50	0.01	91-97	(Rodriguez *et al.*, 2010)
MSPE	Milk	Fe_3O_4-SiO_2-phenyl modified	15	200	2-9	92-103	(Ibarra *et al.*, 2011)

LLE = Liquid liquid extraction; SPE = Solid phase extraction; SPME = Solid phase microextraction; MSPE = Magnetic solid phase extraction

Table 1.2 The application of a magnetic solid phase extraction (MSPE) technique

Analytes	**Sample**	**Materials coating**	**Analytical method**	**LOD (μg L^{-1}, kg^{-1})**	**Recovery (%)**	**References**
Cephalosporins	Milk	C_{18}-Fe_3O_4@mSiO_2	LC-MS/MS	0.06-0.09	85-93	(Liu *et al*., 2014b)
PAHs	Water	Magnetic C_{18} microspheres	GC/MS	0.8-36	35-99	(Liu *et al*., 2009)
Tetracyclines	Milk	Fe_3O_4-SiO_2-phenyl modified	Capillary electrophoresis	2-9	92-103	(Ibarra *et al*., 2011)
Methylprednisolone	Rat plasma	C_{18}-MNPs	HPLC-DAD	10	92-96	(Yu *et al*., 2010)
PAHs	Water	Fe_3O_4/MWCNTs/ alginate	HPLC-FLD	0.005-0.010	92-98	(Bunkoed and Kanatharana, 2015)
Nitrophenols	Water	Fe_3O_4@PPy NPs	HPLC-UV	0.3-0.4	84-109	(Tahmasebi *et al*., 2013)
Phthalate esters	Packaged food	magnetic-MWCNTs-PVA cryogel	GC-FID	26.3-36.4	70-118	(Makkliang *et al*., 2015)

Table 1.2 The application of a magnetic solid phase extraction (MSPE) technique (Continued)

Analytes	**Sample**	**Materials coating**	**Analytical method**	**LOD ($\mu g\ L^{-1}$, kg^{-1})**	**Recovery (%)**	**References**
Fluoroquinolones	Water	Fe_3O_4@SiO_2@ P(VI-*co*-DB	HPLC-DAD	0.20-1.46	52-105	(Huang *et al.*, 2013)
Benzodiazepines	Biological fluids	Fe_3O_4/PANI	HPLC-UV	0.2-2.0	84-99	(Asgharinezhad *et al.*, 2014)
Sulfonamides	Water	polypyrrole/SiO_2/ Fe_3O_4	HPLC-DAD	0.30-1.0	87-100	(Sukchuay *et al.*, 2015)
Phenolic compounds	Water	Fe_3O_4@C@PANI	GC-MS	2.52-29.7	85-111	(Meng *et al.*, 2011)
Estrogens	Milk	MNPs/PPy	LC-MS/MS	0.0051-0.0667	83-109	(Gao *et al.*, 2011)
Carbamate pesticides	Tomatoes	G-MNPs	HPLC-DAD	0.58-2.06	90-102	(Li *et al.*, 2015)

1.5 Magnetic solid phase extraction sorbent

Magnetic nanoparticles (MNPs) are an alternative and a convenient material for the preparation of sorbent with magnetic properties, it was commonly used as the supporting materials combine with MSPE method because of their small particle size and high surface area (Wierucka and Biziuk, 2014). Several advantages of MNPs are that they can be easily functionalized through the attachment of organic or inorganic substance to the particle surface which can increase the selectivity. In this work, the synthesis of Fe_3O_4 NPs were prepared by a chemical co-precipitation method (Huang *et al.*, 2013; Bunkoed and Kanatharana, 2015). The Fe_3O_4 NPs was formed according to the following reaction (Lee *et al.,* 2012; Asgharinezhad *et al.*, 2014);

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \xrightarrow{\text{Co-precipitation}} Fe_3O_4 + 4H_2O$$

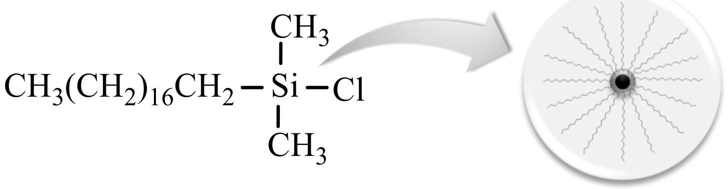
The physical and chemical properties of magnetic nanoparticles such as size, shape, and composition of the magnetic nanoparticles depend on the synthesis method, the ratio of $FeCl_2{\cdot}4H_2O/FeCl_3{\cdot}6H_2O$, the reaction time and reaction temperature. The application of magnetic nanoparticles (MNPs) were successfully applied for the preconcentration of various target analytes in different samples including biological samples (Chiang *et al.*, 2005; Zhou *et al.*, 2013), food samples (Ibarra *et al.*, 2011; Chen and Li, 2013) and environmental samples (Sun *et al.*, 2009; Bunkoed and Kanatharana, 2015). However, magnetic sorbent based on magnetic nanoparticles (Fe_3O_4 NPs) are prone to aggregation, oxidation and not selective in the complex matrices. Therefore, the surface of Fe_3O_4 NPs were functionalized with surfactants to improve the selectivity and chemical stability which can introduce abundant adsorption sites and different functional groups of magnetic nanoparticles.

The surface of magnetic nanoparticles (Fe_3O_4 NPs) are generally coated with a layer of silica gel, surfactants and polymers. Silica is becoming a promising and commonly used for the fabrication of silica-coated magnetic nanoparticles (SiO_2/Fe_3O_4 NPs), which favors the dispersion in liquid media and protects them from leaching in an acidic condition (Deng *et al.*, 2005). Moreover, the existence of abundant of silanol groups (Si-OH) on the silica layer, SiO_2/Fe_3O_4 NPs could be easily activated to provide the surface with various functional groups (Deng *et al.*, 2005). The synthesis of SiO_2/Fe_3O_4 NPs usually prepared by the sol-gel polymerization method as follows the Stober process (Stöber *et al.*, 1968; Sukchuay *et al.*, 2015). Which involved the layer of silica was formed around the surface of magnetic nanoparticles by hydrolysis and condensation of tetraethyl orthosilicate (TEOS) as the following reactions (Jankiewicz *et al.*, 2012);

$$Si(OC_2H_5)_4 + 4H_2O \xrightarrow{\text{Hydrolysis}} Si(OH)_4 + 4C_2H_5OH$$

$$Si(OH)_4 \xrightarrow{\text{Condensation}} SiO_2 + 2H_2O$$

To increase the adsorption capability and efficiency of the target analytes, polymer modified or functionalized are attracting interest because of a unique properties of each polymer. Various types of polymer for coating materials such as polyaniline (PANI) (Asgharinezhad *et al.*, 2014), polypyrrole (PPy) (Tahmasebi *et al.*, 2013) and polystyrene (PS) (Tolmacheva *et al.*, 2015) have been regarded as the most important conduction polymers owing to their stability and straight forward synthesis. Among these polymers and materials, octadecyl (C_{18}), **(Figure 1.3)** is widely used for the extraction and determination of various organic compounds such as cephalosporins (Liu *et al.*, 2014b), methylprednisolone (Yu *et al.*, 2010), phthalic acid esters (Guo *et al.*, 2014), organophosphorous pesticides (Shen *et al.*, 2007), endocrine disruptors (Synaridou *et al.*, 2014) and polycyclic aromatic hydrocarbons (Liu *et al.*, 2009; Zhang *et al.*, 2010). It can be interacted with organic compounds through a hydrophobic interaction.



$$CH_3(CH_2)_{16}CH_2-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{Si}}-Cl$$

Figure 1.3 The chemical structure of octadecyl (C_{18})

2. Results and discussion

2.1 Optimization of the HPLC-DAD conditions

The optimum HPLC-DAD conditions for the determination of tetracyclines as follows. The composition of mobile phase was a mixture of methanol : acetonitrile : 0.01 M oxalic acid (5 : 18 : 77 % v/v). The flow rate of mobile phase was 1.0 mL min^{-1} and absorption wavelength was 355 nm. Under the optimum conditions which provided a good separation, a sharp peak and short analysis time for oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC) with the retention times of 4.28, 5.51 and 10.13 min, respectively **(Figure 2.1)**.

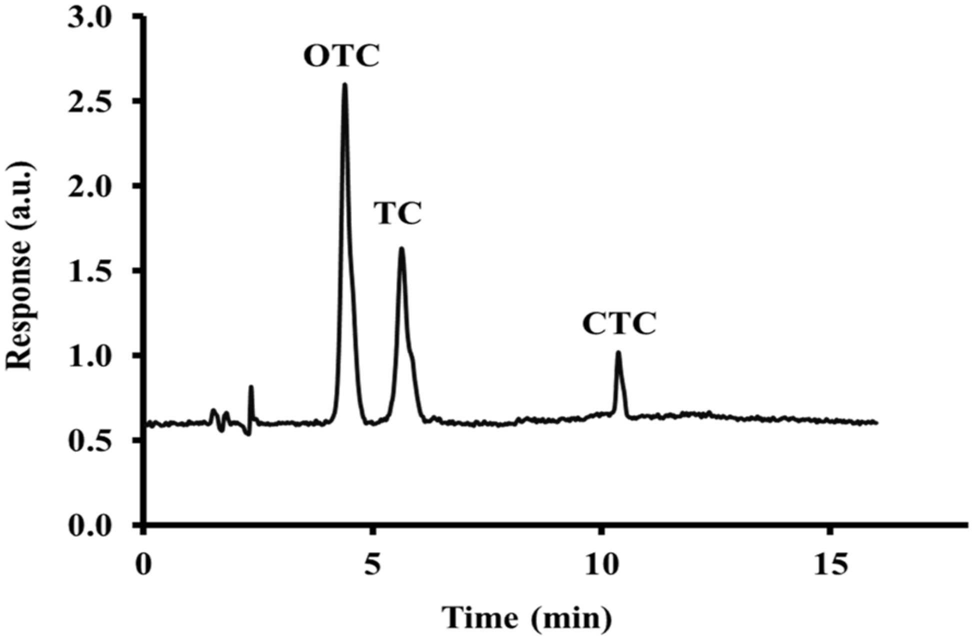


Figure 2.1 The chromatograms of oxytetracyclines (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.2 Preparation and characterization of $C_{18}/SiO_2/Fe_3O_4$ sorbent

The synthesis of $C_{18}/SiO_2/Fe_3O_4$ sorbent involved with three steps. Fe_3O_4 NPs were prepared following a reported based on chemical co-precipitation (Lee *et al.*, 2012; Bunkoed and Kanatharana, 2015). Briefly, The $FeCl_3 \cdot 6H_2O$ (3.50 g) and $FeCl_2 \cdot 4H_2O$ (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 above solution and stirring was continued for 30 min in atmosphere of nitrogen gas. After the reaction, the obtained magnetic nanoparticles 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 onto the surface of Fe_3O_4 nanoparticles was achieved using a sol-gel process (Stöber *et al.*, 1968; Sukchuay *et al.*, 2015). Fe_3O_4 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 dropwise into the solution under stirring 500 rpm and the mixture solution was stirred and heated for 12 h at 40°C. The SiO_2/Fe_3O_4 nanoparticles were collected by an external magnet, washed with 5 mL of ethanol and deionized water for three times, and then dried in an oven at 60°C for 4 h.

Finally, for the C_{18}-functionallization (Liu *et al.*, 2009), SiO_2/Fe_3O_4 nanoparticles (3.0 g) were added in 90 mL of toluene. The slurry was heated to 80°C and 10 mL of trimethylamine and 1.0 mL of dimethyloctadecylchlorosilane were added. The mixtures were then refluxed for 24 h. The $C_{18}/SiO_2/Fe_3O_4$ NPs were obtained and collected by magnet, washed with ethanol and deionized water two times, dried in an oven at 60°C for 3 h.

The characterization of the synthesized magnetic nanoparticles, comprising Fe_3O_4, SiO_2/Fe_3O_4 and $C_{18}/SiO_2/Fe_3O_4$ were investigated using Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM) and Vibrating sample magnetometer (VSM) techniques. The FT-IR spectroscopy was employed to ensure a proper chemical modification on the surface of the magnetic nanoparticles as shown in **Figure 2.2a**, the absorption peaks at 587 cm^{-1} represented the stretching vibration of the Fe-O-Fe group of magnetite nanoparticles (Fe_3O_4 NPs).

Silica-coating on the surface of the Fe_3O_4 NPs 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 the Fe_3O_4 NPs. The absorption peaks at 1605 and 3405 cm^{-1} 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^{-1} represented the asymmetric and the symmetric vibrations of the $–CH_2$ group in the $–(CH_2)_{17}CH_3$ chain and indicated that the C_{18} group was successfully attached onto the surface of the SiO_2/Fe_3O_4 NPs. The SEM image of the $C_{18}/SiO_2/Fe_3O_4$ sorbent is illustrated in **Figure 2.2b**, the average diameter of the $C_{18}/SiO_2/Fe_3O_4$ NPs was approximately 75 ± 10 nm and was nearly spherical in shape. The VSM magnetization curves of Fe_3O_4, SiO_2/Fe_3O_4 and $C_{18}/SiO_2/Fe_3O_4$ sorbent as shown in **Figure 2.2c**, 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_{18}, respectively. However, all of the magnetic nanoparticles had a magnetic strength sufficient for the magnetic separation from the solution with a magnet and $C_{18}/SiO_2/Fe_3O_4$ was still able to produce a rapid separation process within 30 second using the magnet. Moreover, the developed sorbents could be dispersed in the water sample and conveniently isolated from the aqueous media utilizing an external magnet **(Figure 2.2d)**.

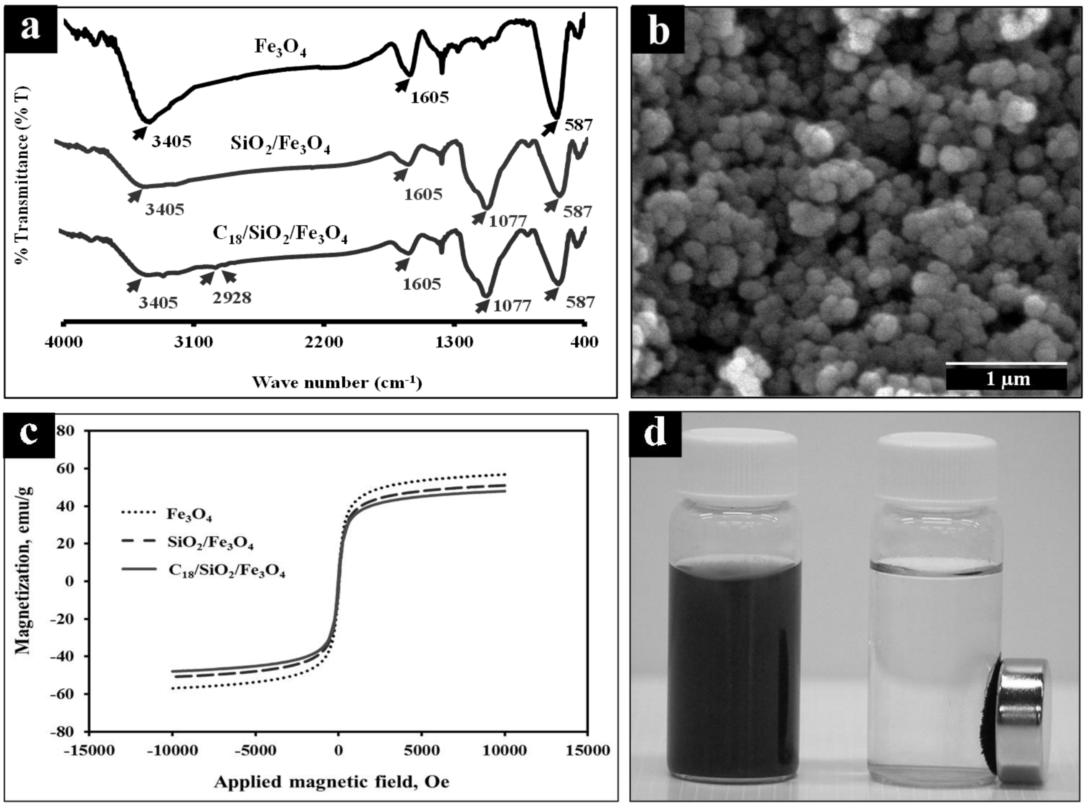


Figure 2.2 (a) FT-IR spectra of Fe_3O_4, SiO_2/Fe_3O_4 and $C_{18}/SiO_2/Fe_3O_4$, (b) SEM image of $C_{18}/SiO_2/Fe_3O_4$ sorbent, (c) VSM curves of Fe_3O_4, SiO_2/Fe_3O_4 and $C_{18}/SiO_2/Fe_3O_4$, (d) The dispersion (left) and separation (right) process of $C_{18}/SiO_2/Fe_3O_4$ sorbent and collected by an external magnet

2.3 Optimization of magnetic solid phase extraction

The MSPE procedure for the extraction of tetracyclines as shown in **Figure 2.3**. 100 mg of $C_{18}/SiO_2/Fe_3O_4$ sorbent was added into the extraction bottle and firstly conditioned with 2.0 mL of methanol and 5.0 mL of deionized water for 5 min, respectively. Then, 5.0 mL of spiked water sample was added into the extraction bottle and stirred for 20 min. After that, the sorbent with the adsorbed tetracyclines was separated rapidly by an external magnet and the supernatant was discarded. The analytes were 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 solution was evaporated to dryness at 50°C. Finally, the residues were redissolved with 1.0 mL of the mobile phase, and then filtered through a PTFE filter (0.22 µm) before analysis by HPLC-DAD system.

In order to achieve the highest extraction efficiency with a short analysis time and low solvent consumption. Several experimental parameters including types of sorbent, amount of sorbents, sample pH, stirring rate, extraction time and desorption conditions were investigated. The optimization was performed by extracting a spiked water sample containing 0.20 µg mL^{-1} of each tetracycline. The optimum condition of one parameter was obtained, and it was used to evaluate the extraction efficiency of the next parameters.

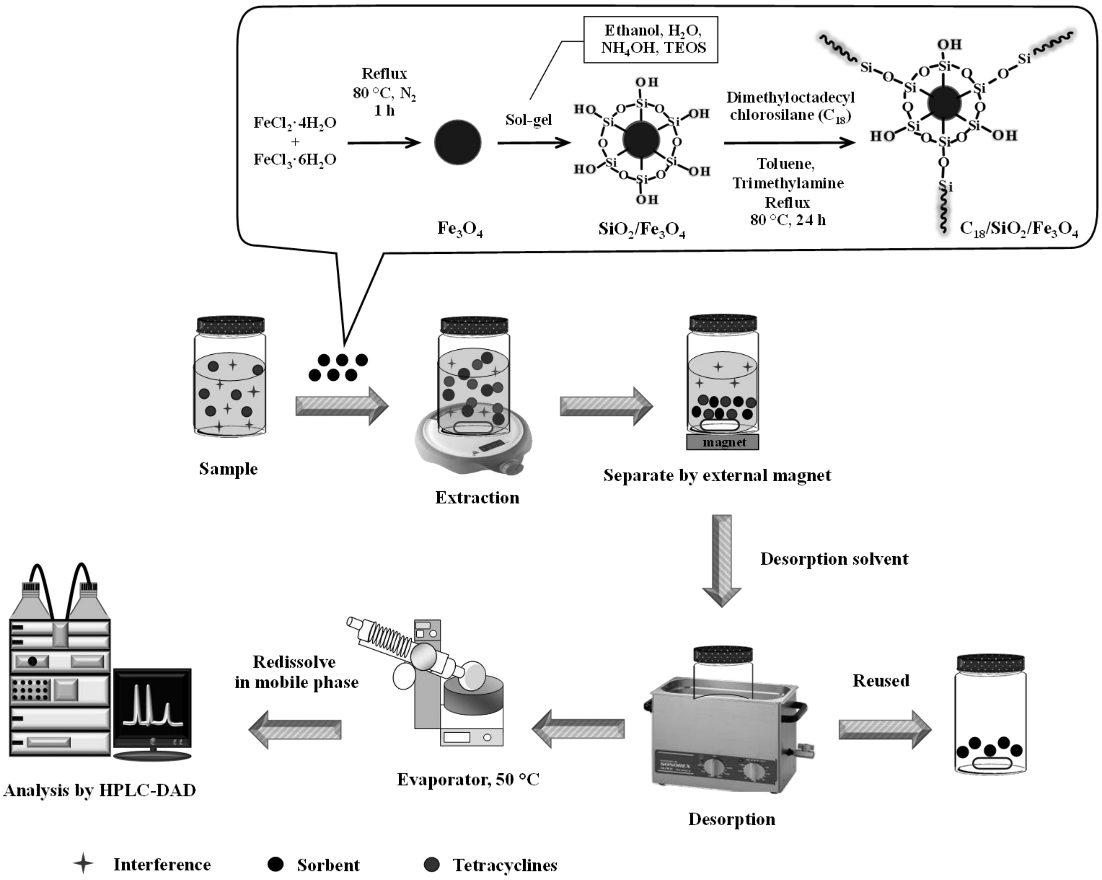


Figure 2.3 The MSPE procedures for the extraction of tetracyclines using $C_{18}/SiO_2/Fe_3O_4$ sorbent

2.3.1 Effect of the types of sorbent

The extraction capabilities of the uncoated magnetic nanoparticles (Fe_3O_4), silica coated magnetic nanoparticles (SiO_2/Fe_3O_4) and octadecyl coated silica-magnetic nanoparticles ($C_{18}/SiO_2/Fe_3O_4$) were investigated by extracting tetracyclines from spiked water samples. The results indicated that **(Table 2.1** and **Figure 2.4)** bare Fe_3O_4 had low adsorption ability for tetracyclines (3-4%). SiO_2/Fe_3O_4 improved the adsorption ability of tetracyclines 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 of all tetracyclines had a significant increase in the presence of octadecyl (C_{18}) groups, which indicated that octadecyl had a significant influence on the extraction efficiency of tetracyclines via hydrophobic interaction.

Table 2.1 The recoveries of tetracyclines with the different types of sorbent (*n*=3)

Types of sorbent	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
Fe_3O_4	4.4 ± 1.0	3.5 ± 1.6	4.7 ± 1.4
SiO_2/Fe_3O_4	21.2 ± 5.9	15.4 ± 5.9	23.4 ± 3.6
$C_{18}/SiO_2/Fe_3O_4$	84.4 ± 2.7	82.6 ± 1.6	84.8 ± 3.8

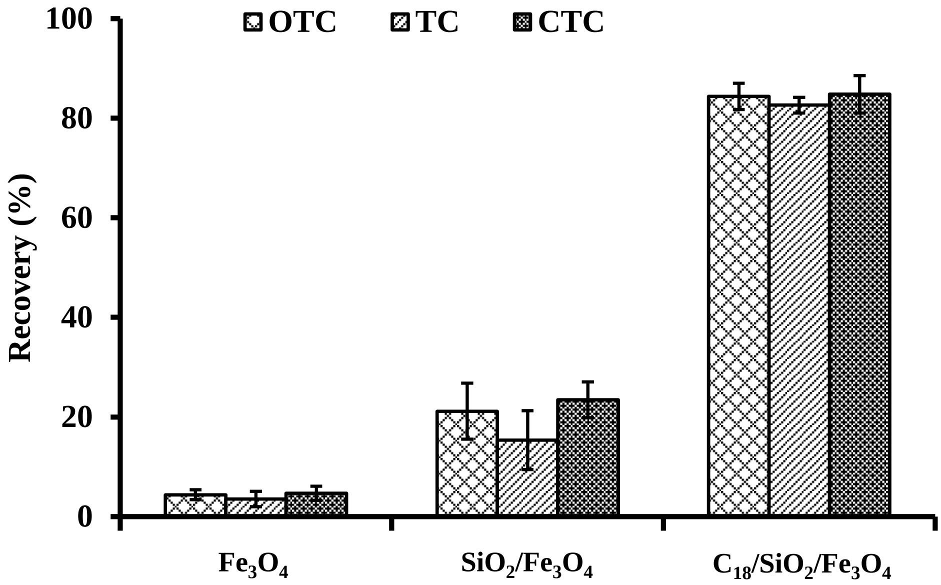


Figure 2.4 The extraction efficiency of different sorbents for the determination of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.3.2 Effect of the amount of $C_{18}/SiO_2/Fe_3O_4$ sorbent

The amount of the sorbent directly affected on the extraction efficiency of the analytes. The amount of the $C_{18}/SiO_2/Fe_3O_4$ sorbent was investigated over the range of 50 to 200 mg as shown in **Table 2.2** and **Figure 2.5**. The recoveries of tetracyclines increased with the increasing of amount of sorbent from 50 to 100 mg, and then remained almost constant. Therefore, 100 mg of the sorbent was enough for the extraction of tetracyclines from the water samples and it was selected for further experiments.

Table 2.2 The recoveries of tetracyclines using the different amount of sorbents (*n*=3)

Amount of sorbent (mg)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
50	64.5 ± 2.4	61.9 ± 2.5	72.2 ± 3.5
100	82.7 ± 2.3	79.9 ± 2.2	82.2 ± 1.4
200	86.1 ± 3.2	83.5 ± 2.9	85.3 ± 3.9

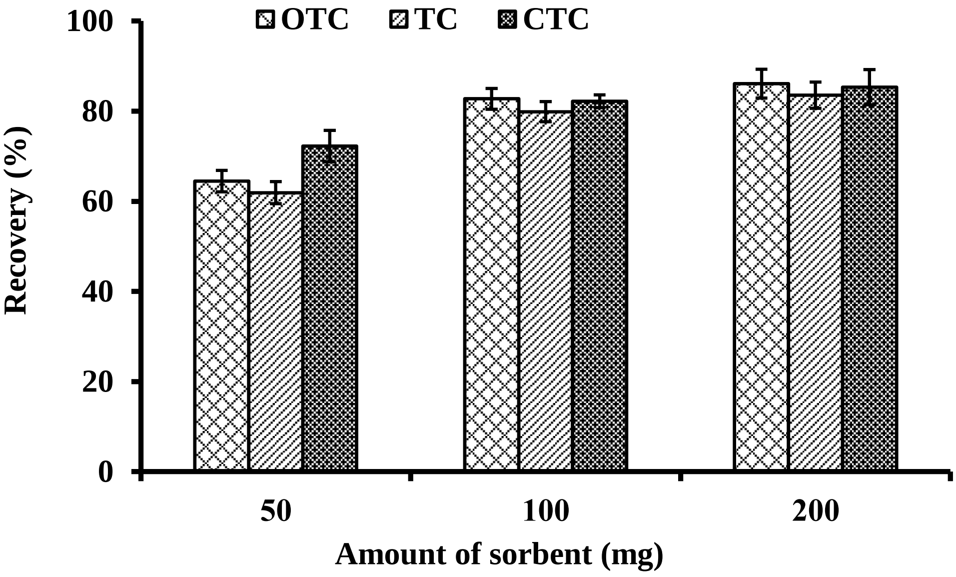


Figure 2.5 Effect of the amount of $C_{18}/SiO_2/Fe_3O_4$ sorbents on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.3.3 Effect of the sample pH

The sample pH was a significant parameter that affected the adsorption ability of the analytes. Tetracyclines are amphoteric compounds with three protonated functional groups. The p*K*a values of tetracyclines in aqueous solution are around 3.3, 7.5 and 9.0 (Ibarra *et al.*, 2011). In aqueous samples, the three groups can undergo a protonation-deprotonation reaction and from cationic species (pH > p*K*a1), zwitterionic species (p*K*a1 < pH < p*K*a2) or anionic species (pH > p*K*a3) (Hung *et al.*) and the zwitterionic forms are favorable for extraction (Tolmacheva *et al.*, 2015). 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 was obtained at pH 5.0 to 6.0 **(Table 2.3** and **Figure 2.6)** due to the adsorption of tetracyclines on the sorbent that involved hydrophobic interactions in the presence of the zwitterionic form. The recoveries of tetracyclines decreased at a pH values lower 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 species that led to a decrease in the hydrophobic interaction between the tetracyclines and the sorbent. However, the pH of the normal water samples was between 5.5 and 6.0. So, the water samples could be directly analyzed without adjusting the pH values.

Table 2.3 The recoveries of tetracyclines at the different sample pH (*n*=3)

Sample pH	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
4.0	33.1 ± 3.4	28.5 ± 2.9	42.1 ± 4.0
5.0	78.7 ± 2.2	76.7 ± 2.9	83.3 ± 2.1
6.0	80.1 ± 3.9	77.9 ± 3.8	80.7 ± 2.8
7.0	59.1 ± 2.3	57.8 ± 3.3	71.7 ± 2.1
9.0	39.91 ± 0.77	39.0 ± 1.7	54.6 ± 2.0

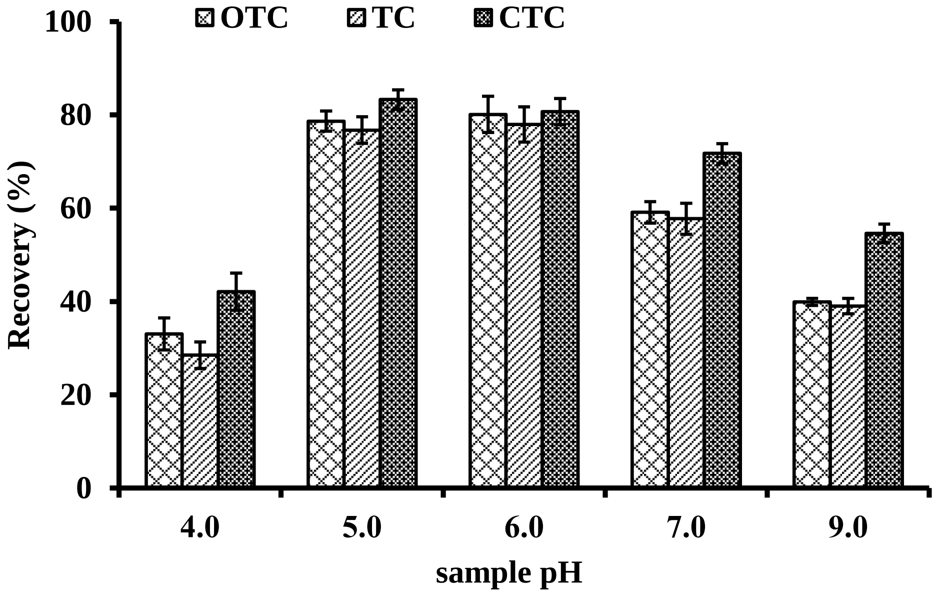


Figure 2.6 Effect of the sample pH on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.3.4 Effect of the stirring rate

The stirring rate can affect the extraction efficiency because of it altered the mass transfer of analytes towards the sorbent. In this work, the stirring rate was investigated in the range of 500 to 2000 rpm as shown in **Table 2.4** and **Figure 2.7**. 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.

Table 2.4 The recoveries of tetracyclines with the different stirring rate (*n*=3)

Stirring rate (rpm)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
500	31.3 ± 2.0	29.6 ± 1.6	34.0 ± 2.5
1000	57.2 ± 1.9	53.8 ± 1.6	62.4 ± 3.0
1500	80.5 ± 2.4	78.2 ± 3.1	80.5 ± 1.4
2000	79.4 ± 2.0	77.6 ± 3.7	78.6 ± 2.5

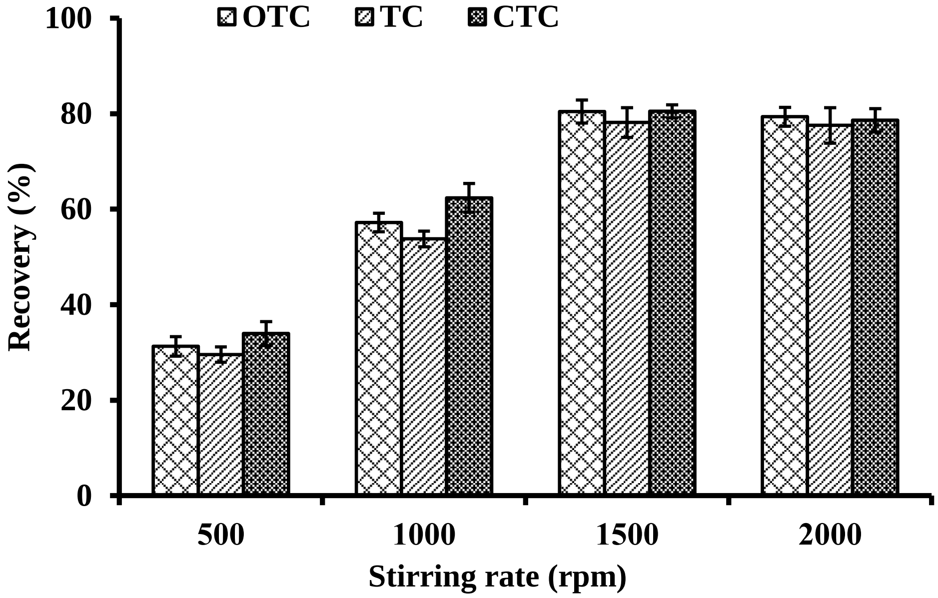


Figure 2.7 Effect of the stirring rate on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.3.5 Effect of the extraction time

The extraction time was investigated in the range of 10 and 40 min. The results as shown in **Table 2.5** and **Figure 2.8**. The extraction efficiency 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.

Table 2.5 The recoveries of tetracyclines with the different extraction time (*n*=3)

Extraction time (min)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
10	37.1 ± 4.0	33.1 ± 3.6	47.6 ± 3.2
20	81.1 ± 1.5	76.7 ± 2.0	82.0 ± 2.8
30	78.27 ± 0.92	74.8 ± 3.8	80.5 ± 2.6
40	78.2 ± 2.0	73.3 ± 1.8	80.2 ± 1.7

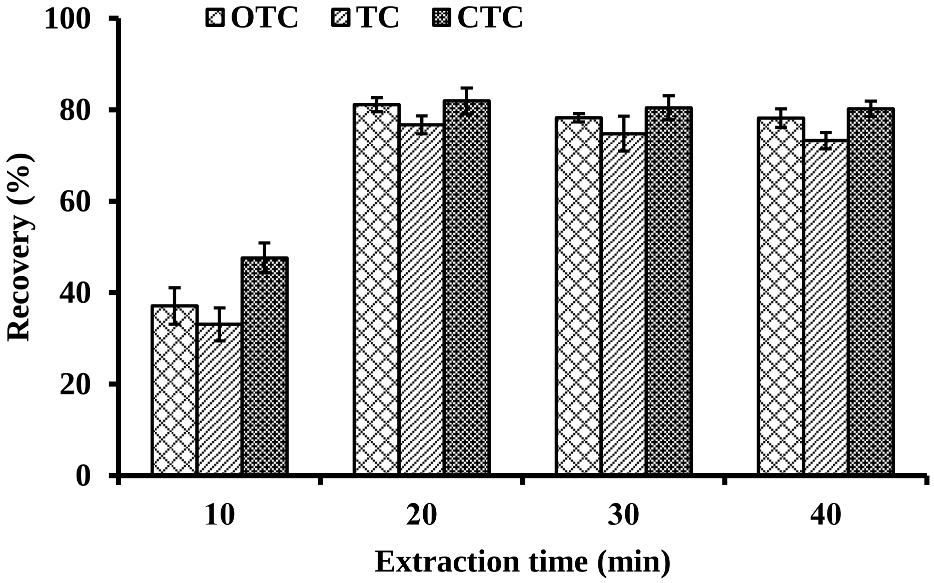


Figure 2.8 Effect of the extraction time on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.3.6 Effect of the desorption conditions

The desorption conditions including desorption solvent, desorption time and desorption volume are important in a MSPE procedure. Due to the adsorption of tetracyclines onto the surface of $C_{18}/SiO_2/Fe_3O_4$ sorbent was based on hydrophobic interaction. Therefore, the effect of 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 **(Table 2.6** and **Figure 2.9)**. The concentration of oxalic acid in methanol was further studied by varying between 5.0 and 80 mM and the results are showed in **Table 2.7** and **Figure 2.10**. 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 effect of desorption time was also investigated in the range of 10 to 40 min. The results as shown in **Table 2.8** and **Figure 2.11**. The recoveries increased with increasing desorption time up to 20 min and then remained almost constant. Therefore, the desorption time of 20 min was selected for further experiments

The effect of desorption volume was also investigated in the range of 2.0 to 8.0 mL and the results are showed in **Table 2.9** and **Figure 2.12**. The recoveries of tetracyclines increased with an increasing volume of eluent from 2.0 to 4.0 mL and then remained almost constant. Therefore, 4.0 mL of desorption solvent was chosen for subsequent experiments.

Table 2.6 The recoveries of tetracyclines with the different type of desorption solvents (*n*=3)

Types of desorption solvent	Recovery (%) ± SD		
	OTC	TC	CTC
Methanol	34.4 ± 2.9	31.8 ± 4.7	35.2 ± 2.0
Acetonitrile	31.8 ± 2.1	27.6 ± 1.6	36.2 ± 2.6
Oxalic acid in methanol (60 mM)	81.3 ± 2.1	80.3 ± 2.0	84.6 ± 2.8

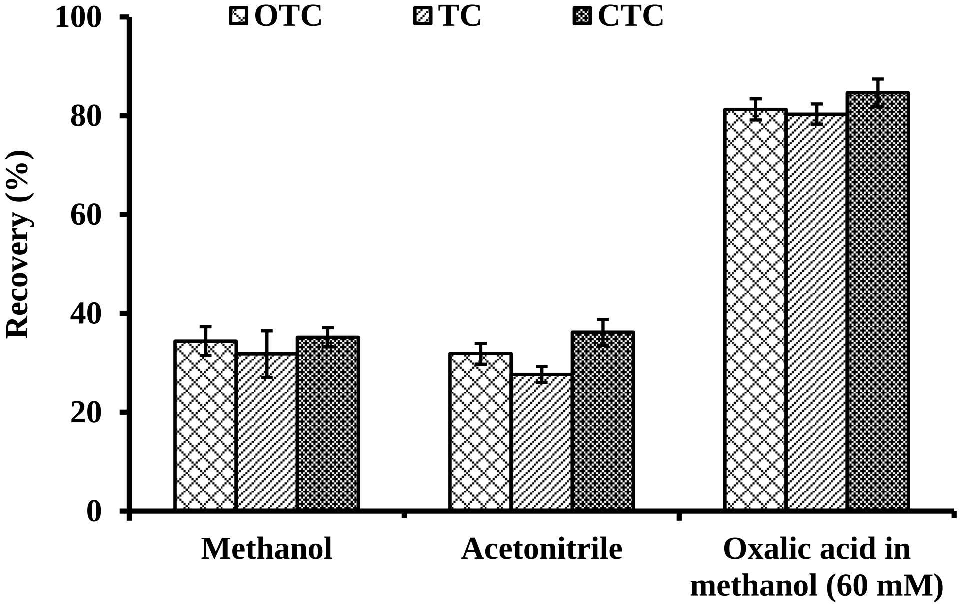


Figure 2.9 Effect of the types of desorption solvent on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

Table 2.7 The recoveries of tetracyclines with the different concentration of oxalic acid in methanol (*n*=3)

Concentration of oxalic acid in methanol (mM)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
5	60.0 ± 2.9	52.8 ± 4.4	58.0 ± 2.9
10	67.7 ± 3.8	50.6 ± 3.8	59.2 ± 2.0
20	70.5 ± 2.0	56.7 ± 1.6	67.4 ± 1.7
40	76.0 ± 2.4	70.9 ± 2.9	78.2 ± 2.3
60	83.9 ± 1.8	83.2 ± 3.2	84.4 ± 1.5
80	82.6 ± 1.9	81.8 ± 1.2	85.9 ± 3.2

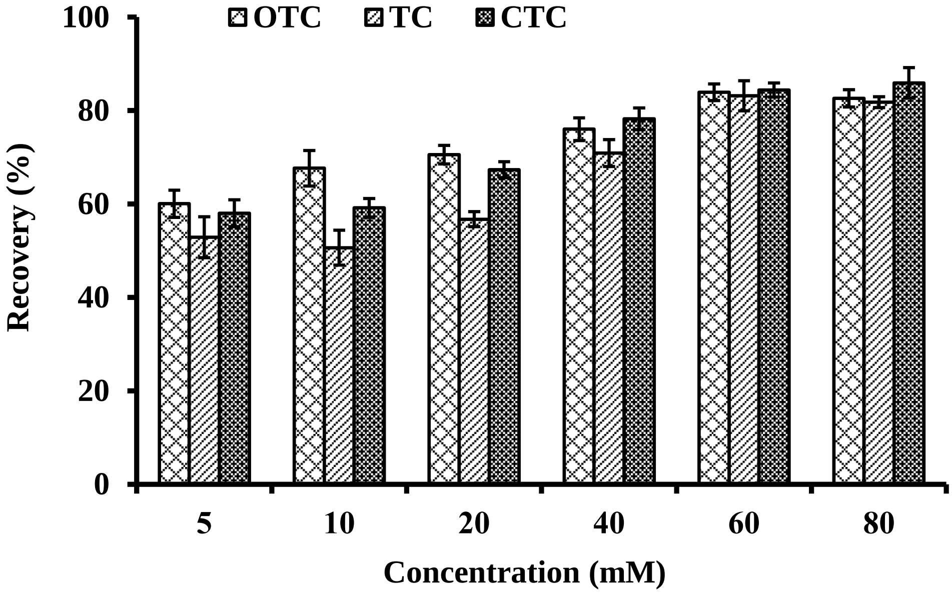


Figure 2.10 Effect of the concentration of oxalic acid in methanol on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

Table 2.8 The recoveries of tetracyclines with the different desorption time (*n*=3)

Desorption time (min)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
10	53.46 ± 0.91	57.8 ± 1.8	60.1 ± 1.3
20	80.6 ± 1.6	79.0 ± 2.2	81.5 ± 2.1
30	80.1 ± 2.4	79.4 ± 2.2	80.8 ± 3.3
40	80.1 ± 1.8	80.7 ± 2.0	80.7 ± 3.2

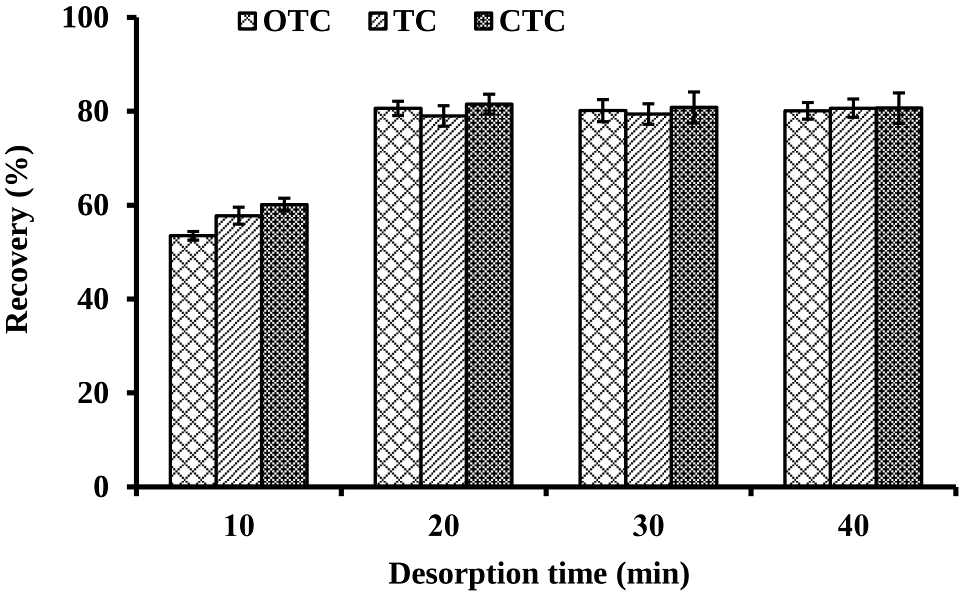


Figure 2.11 Effect of the desorption time on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

Table 2.9 The recoveries of tetracyclines with the different desorption volume (n=3)

Desorption volume (mL)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
2.0	45.0 ± 2.5	49.2 ± 2.8	54.1 ± 3.5
4.0	83.2 ± 1.6	81.7 ± 2.2	84.4 ± 2.0
6.0	82.2 ± 1.4	81.4 ± 2.4	85.2 ± 3.2
8.0	81.5 ± 1.5	82.9 ± 2.5	84.9 ± 2.4

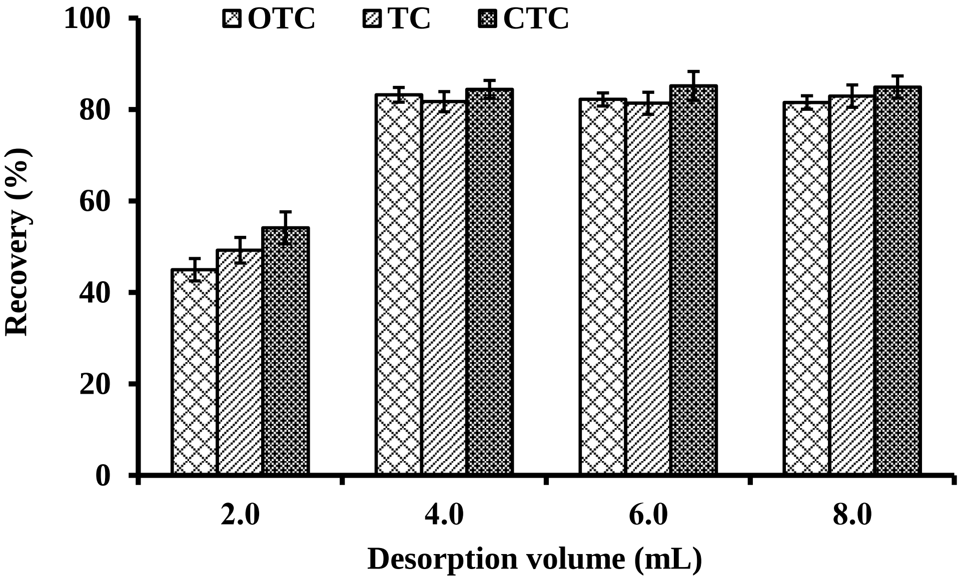


Figure 2.12 Effect of the desorption volume on the recoveries of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

The optimum extraction conditions of the magnetic solid phase extraction (MSPE) using $C_{18}/SiO_2/Fe_3O_4$ sorbent for the determination of tetracyclines are summarized in **Table 2.10**.

Table 2.10 The optimum extraction conditions of MSPE for the determination of tetracyclines (*n*=3)

Parameters	Investigated values	Optimum conditions
Amount of sorbent (mg)	50, 100, 200	100
pH of water samples	4.0, 5.0, 6.0, 7.0, 9.0	5.0-6.0
Stirring rate (rpm)	500, 1000, 1500, 2000	1500
Extraction time (min)	10, 20, 30, 40	20
Desorption solvent	Methanol, Acetonitrile, Oxalic acid in methanol	Oxalic acid in methanol
Concentration of oxalic acid in methanol (mM)	5, 10, 20, 40, 60, 80	60
Desorption time (min)	10, 20, 30, 40	20
Desorption volume (mL)	2.0, 4.0, 6.0, 8.0	4.0

2.4 Analytical performances

Under the optimum conditions of HPLC-DAD and MSPE condition using $C_{18}/SiO_2/Fe_3O_4$ sorbent, the analytical performances *i.e.*, linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and precision were investigated to evaluate the extraction efficiencies of the developed method.

2.4.1 Linearity, limit of detections and limit of quantifications

The linearity of an analytical method is important in a quantitative analysis. It is defined as the ability to obtain the results which are directly proportional to the concentrations of the analytes in the sample. The linear dynamic range was investigated by preparing a mixtures of oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC) in deionized water in the concentration range of 0.0010 µg mL^{-1} to 1.0 µg mL^{-1}. The analytes were extracted by MSPE method (n = 3) and analyzed by HPLC-DAD. The calibration curves were plotted between the peaks area and the concentration of analytes, the linearity was determined by considering the coefficient of determination (R^2) which must be greater than 0.99. The linearity of the developed method as shown in **Table 2.11** and **Figure 2.13**. The results indicated that this method provided a wide linear range and good linearity with coefficient of determination (R^2) greater than 0.9999.

Limit of detection (LOD) is generally determined by the analysis at the lowest concentration of analyte in the samples that can be distinguished between signal response and baseline noise. To establish the minimum concentration of analyte that can be reliably detected. The signal-to-noise ratio greater than or equal 3 ($S/N \geq 3$) was used to consider the LOD (ICH, 1999).

Limit of quantification (LOQ) is generally determined by comparing the measured signals at the lowest concentrations of analyte in the samples with those of blank samples. To establish the minimum concentration of analytes that can be reliably quantified. The signal-to-noise ratio greater than or equal 10 ($S/N \geq 10$) was used to consider the LOQ (ICH, 1999).

The LOD and LOQ of the developed method for the determination of tetracyclines as shown in **Table 2.11**.

Table 2.11 Analytical performances of the developed $C_{18}/SiO_2/Fe_3O_4$ sorbent for the extraction and determination of tetracyclines

Analytes	Linear range (µg mL^{-1})	Regression line equation	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
OTC	0.0020 – 1.0	y = (153.76 ± 0.22)x - (0.024 ± 0.085)	0.9999	0.0020	0.0080
TC	0.010 – 1.0	y = (95.37 ± 0.38)x - (0.07 ± 0.18)	0.9999	0.010	0.040
CTC	0.010 – 1.0	y = (19.11 ± 0.16)x + (0.069 ± 0.074)	0.9999	0.010	0.040

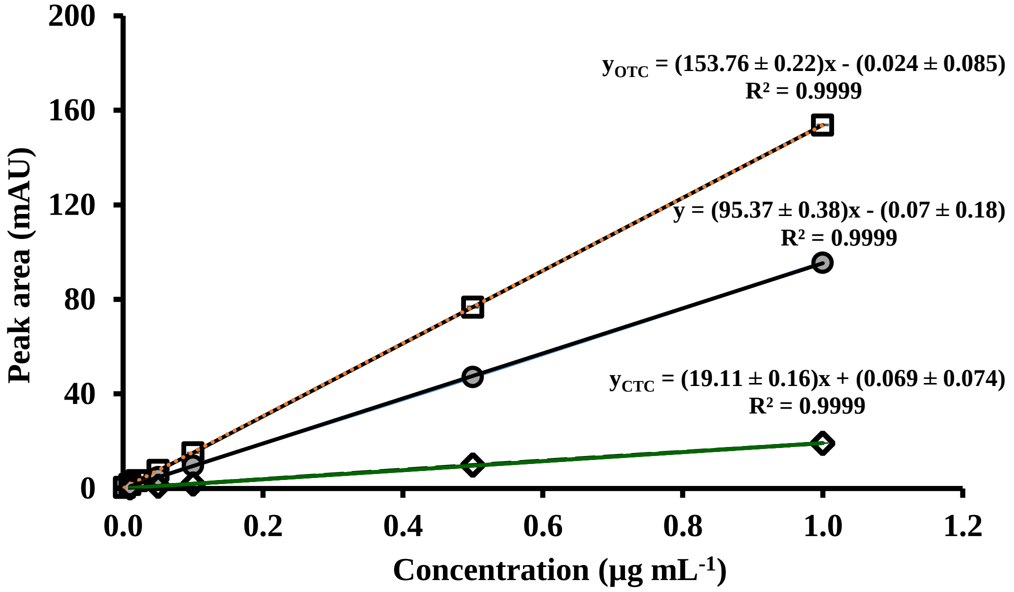


Figure 2.13 The linearity of oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.4.3 Reproducibility

The reproducibility of the $C_{18}/SiO_2/Fe_3O_4$ sorbent was investigated in term of batch-to-batch reproducibility, by preparing six different batches under the same conditions but at different times. These sorbents were used to extract tetracyclines (0.2 μg mL^{-1}) from spiked deionized water. The averages recoveries from the six different batches were 84.2 ± 1.4% for OTC, 82.4 ± 1.0% for TC and 84.7 ± 1.7% for CTC. The relative standard deviations were lower than 6% **(Table 2.12** and **Figure 2.14)**, which better than the acceptable values as recommended by the AOAC (AOAC, 2013) (RSDs = 16%). This result indicated that the preparation procedures of the $C_{18}/SiO_2/Fe_3O_4$ sorbent had a good reproducibility.

Table 2.12 The recoveries of tetracyclines with the different lots of the $C_{18}/SiO_2/Fe_3O_4$ sorbent (*n*=3)

Lots of sorbent	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
1	85.7 ± 1.9	83.8 ± 3.7	84.2 ± 3.7
2	84.2 ± 2.1	81.2 ± 2.8	85.2 ± 3.9
3	85.2 ± 1.2	81.9 ± 2.7	84.5 ± 1.4
4	84.0 ± 2.2	81.3 ± 1.5	84.2 ± 3.0
5	85.9 ± 2.1	84.8 ± 2.3	85.6 ± 2.4
6	83.6 ± 3.6	81.8 ± 1.6	84.8 ± 2.4
Average	84.75 ± 0.78	82.47 ± 0.83	84.73 ± 0.92
RSD (%)	0.92	1.0	1.1

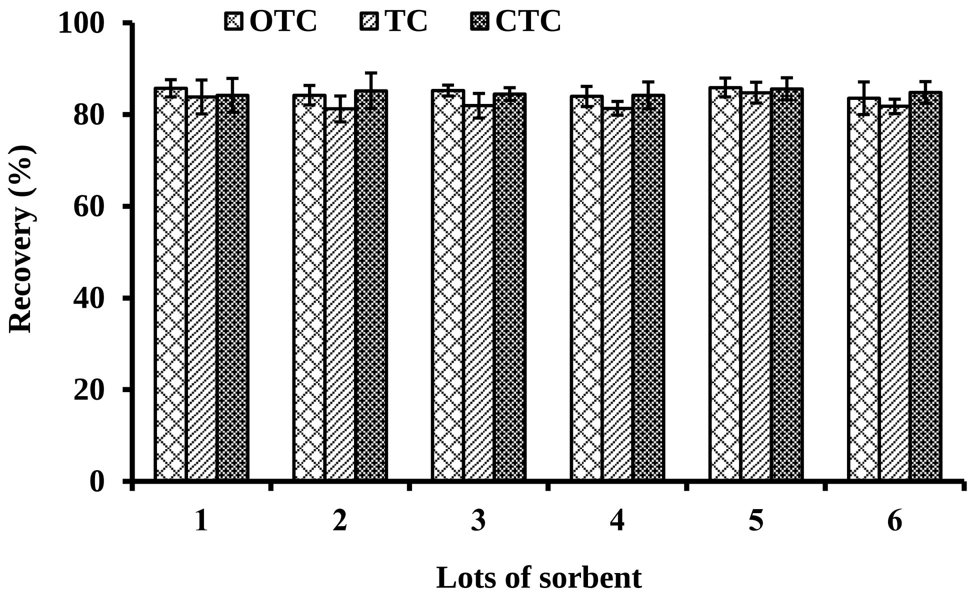


Figure 2.14 The reproducibility of the $C_{18}/SiO_2/Fe_3O_4$ sorbent for the extraction of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.4.4 Reusability

To evaluate the reusability of the developed $C_{18}/SiO_2/Fe_3O_4$ sorbent which helps to reduce the preparation time of the sorbent and also reduce analysis cost. After desorption of the analytes from the $C_{18}/SiO_2/Fe_3O_4$ sorbent it was washed with 2.0 mL of methanol and 5.0 mL of deionized water by sonication before the next used. After washing, the washing solvent was tested again and compared with the response of blank sample (no analytes) and no response of tetracyclines were detected. It indicated that no carry-over of the analytes. The developed $C_{18}/SiO_2/Fe_3O_4$ sorbent could be reused up to five times which the recoveries of tetracyclines > 80% **(Table 2.13** and **Figure 2.15)**; acceptable values in the range of 80-110% recommended by AOAC (AOAC, 2013).

Table 2.13 The recoveries of tetracyclines using $C_{18}/SiO_2/Fe_3O_4$ sorbent (*n*=3)

Reusability (Time)	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
1	83.2 ± 1.6	82.2 ± 3.7	84.2 ± 1.6
2	83.9 ± 2.3	81.5 ± 3.8	82.7 ± 2.9
3	83.0 ± 2.2	81.9 ± 3.7	82.3 ± 4.3
4	81.8 ± 3.2	81.3 ± 1.5	81.4 ± 1.8
5	81.4 ± 3.3	80.1 ± 3.6	80.6 ± 4.0
6	77.2 ± 1.2	72.5 ± 3.6	74.1 ± 4.2

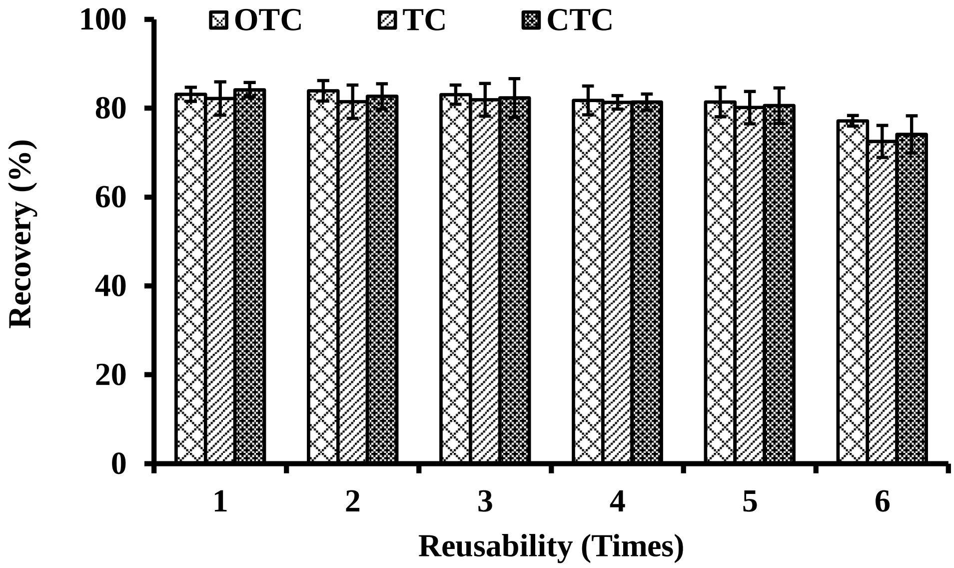


Figure 2.15 The reusability of the $C_{18}/SiO_2/Fe_3O_4$ sorbent for the extraction of tetracyclines; oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)

2.4.5 Comparative studies with the commercial sorbent

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% for $C_{18}/SiO_2/Fe_3O_4$ and 83 to 85% for Oasis HLB, respectively **(Table 2.14** and **Figure 2.16)**. The recoveries of both sorbents were no significant difference ($P > 0.05$). It can be concluded that, 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 are simpler to use and can be reused up to 5 times, whereas commercial SPE sorbent cannot be reused.

Table 2.14 Comparison between $C_{18}/SiO_2/Fe_3O_4$ sorbent and Oasis HLB cartridge (*n*=3)

Types of sorbent	**Recovery (%) ± SD**		
	OTC	**TC**	**CTC**
$C_{18}/SiO_2/Fe_3O_4$	85.3 ± 2.7	82.9 ± 2.6	86.6 ± 2.8
Oasis HLB	83.0 ± 1.7	81.9 ± 2.7	85.2 ± 2.0

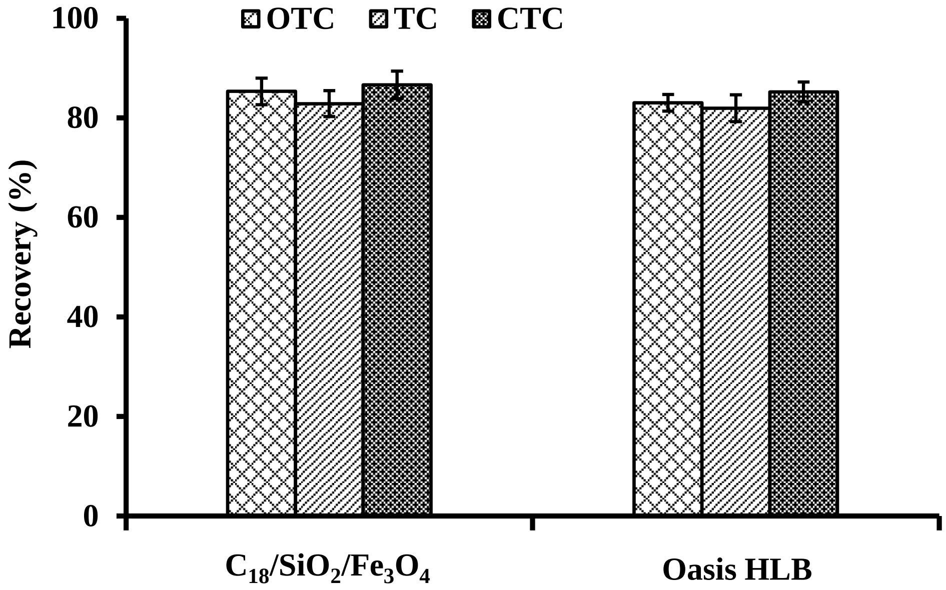


Figure 2.16 The extraction efficiency of tetracyclines in spiked deionized water, a comparison between the $C_{18}/SiO_2/Fe_3O_4$ sorbent and Oasis HLB sorbent

2.4.6 Analysis of tetracyclines in water samples

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 samples were collected from two rivers in Hat Yai city, Thailand and wastewater samples were collected from a household effluent from Songkhla province, Thailand. All water samples were filtered through 0.45 μm membrane and stored in amber glass bottles at 4°C until they were analyzed. A typical chromatograms of tetracyclines of spiked water samples (0.2 μg mL^{-1}) with and without extraction using $C_{18}/SiO_2/Fe_3O_4$ sorbent as shown in **Figure 2.17**. Only low concentrations of oxytetracycline were detected in the wastewater samples **(Table 2.15)**.

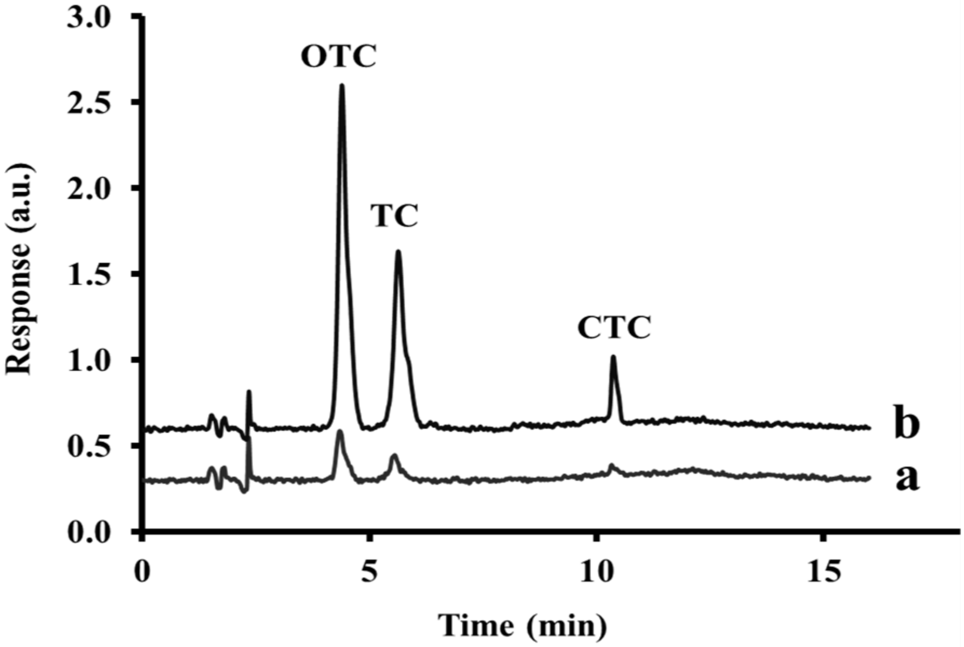


Figure 2.17 A typical chromatograms of tetracyclines of spiked water samples without extraction (a) and with extraction using $C_{18}/SiO_2/Fe_3O_4$ sorbent (b)

Table 2.15 Concentration of tetracyclines in real water samples

Water samples	**Concentration (μg mL^{-1}) ± SD**		
	OTC	**TC**	**CTC**
Tap water 1	N.D.	N.D.	N.D.
Tap water 2	N.D.	N.D.	N.D.
River water 1	N.D.	N.D.	N.D.
River water 2	< LOQ	N.D.	N.D.
Wastewater 1	0.0100 ± 0.0020	N.D.	N.D.
Wastewater 2	0.0120 ± 0.0030	N.D.	N.D.

N.D. = Not detected

2.4.7 Accuracy and precision

The accuracy of the developed method was evaluated by considering of the recovery (%). The water samples were spiked with standard tetracyclines to obtain the final concentrations of 0.020, 0.10 and 0.20 µg mL^{-1}. The recovery (%) was calculated as following equation (AOAC, 2013).

$$\text{Recovery (\%)} = (C_f - C_u) \times 100/C_a$$

Where; C_f is the concentration of analytes in fortified (spiked sample)

C_u is the concentration of analytes in the unfortified (real or blank sample)

C_a is the calculated concentration of analyte added in the sample

The average recoveries of tetracyclines in water samples were in the range of 82 to 88% with RSDs of < 10% **(Table 3)**, the acceptable recovery are in the range of 80 to 110% (AOAC, 2013). These results indicated that there were no effect from the matrix components in the real water samples. The results were implied that the developed method was suitable for the extraction and determination of tetracyclines from water samples.

Table 2.16 The recoveries of tetracyclines in real water samples

Samples	**Added (μg mL^{-1})**	**Recovery (%) ± SD**		
		OTC	**TC**	**CTC**
Tap water	0.020	86.6 ± 3.3	86.5 ± 2.4	86.0 ± 4.1
	0.10	83.2 ± 1.3	82.6 ± 4.4	82.2 ± 2.9
	0.20	84.1 ± 3.3	82.5 ± 2.1	85.2 ± 4.7
River water	0.020	87.7 ± 3.8	84.6 ± 4.7	86.0 ± 4.1
	0.10	83.9 ± 2.6	82.6 ± 3.6	84.1 ± 2.5
	0.20	84.1 ± 3.3	83.8 ± 4.1	85.9 ± 5.3
Wastewater	0.020	87.2 ± 2.8	84.5 ± 3.6	84.2 ± 3.1
	0.10	82.9 ± 2.1	84.5 ± 3.4	84.2 ± 2.2
	0.20	84.1 ± 3.3	83.1 ± 4.2	87.0 ± 3.4

The precision of the developed method was investigated and refers to the percentage relative standard deviation (%RSD). The standard tetracyclines were spiked in water samples to obtain the final concentration of 0.020, 0.10 and 0.20 µg mL^{-1}; with five replications for each concentration. The spiked samples were extracted and analyzed under the optimum condition. The RSDs for each concentration were lower than 6%, the acceptable value recommended by AOAC equal to 8%. The result indicated that the developed method has a good precision.

2.5 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 are summarized in **Table 3.1**. In this work, only 100 mg of the 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 (Tolmacheva *et al.*, 2015). However, the reusability and LODs were not investigated. (Tolmacheva *et al.*, 2015). The recoveries of the developed method were either comparable (Vinas *et al.*, 2004; Udalova *et al.*, 2015) or better than those other methods (Zhu *et al.*, 2001; Shalaby *et al.*, 2011; Lv *et al.*, 2013). This is because tetracyclines in the samples solution can be easily adsorbed onto the sorbent, which 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 cost.

Table 2.17 Comparison of the developed method with other methods for the determination of tetracyclines

Extraction method	**Sample**	**Extractants**	**Sample amount**	**LOD**	**Time (min)**	**Recovery (%)**	**Preconcentration factor**	**References**
SPE	Honey	DSC-phenyl cartridge (500 mg)	3 g	15-30 ng/g	45	92-96	6	(Vinas *et al.*, 2004)
SPE	Water	Hyper-crosslinked polystyrene (30 mg)	100 mL	0.6-2.0 µg/L	3	94-98	100	(Udalova *et al.*, 2015)
SPE	Chicken meat and liver	C$_{18}$	5 g	4-10 ng/g	25	69-93	-	(Shalaby *et al.*, 2011)
SPE	Water	SPE-C$_{18}$ (500 mg)	100 mL	3.1-3.8 µg/L	15	72-93	500	(Zhu *et al.*, 2001)
MSPE-MIP	Milk	Molecularly imprinted magnetic microsphere	4 g	7.4-19.4 µg/kg	25	75-94	4	(Lv *et al.*, 2013)
MSPE	Water	Hyper-crosslinked polystyrene-Fe$_3$O$_4$ (20 mg)	100 mL	-	20	95-97	25	(Tolmacheva *et al.*, 2015)
MSPE	Water	C$_{18}$/SiO$_2$/Fe$_3$O$_4$ (100 mg)	5 mL	2-10 µg/L	20	82-87	5	This work

SPE = Solid phase extraction; MSPE = Magnetic solid phase extraction; MIP = Molecularly imprinted polymer

3. Concluding remarks

The developed $C_{18}/SiO_2/Fe_3O_4$ sorbent was successfully synthesized and applied as a magnetic solid phase extraction for the extraction and determination of tetracyclines in water samples. Coating of the magnetics nanoparticles with silica and C_{18} groups helped to increase the adsorption ability of tetracyclines via hydrophobic interactions, improve the dispersibility and stability of the sorbents. The developed sorbent exhibited several advantages including a convenient and rapid collection of the 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 the sorbent can be reused. The developed sorbent was successfully applied to determine tetracyclines from real water samples and would be potentially useful for the extraction of other less polar compounds.

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Publication

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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 (Fe_3O_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 Fe_3O_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 (C_{18})

groups was used because it can adsorb tetracyclines via hydrophobic interactions. The $C_{18}/SiO_2/Fe_3O_4$ 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 $C_{18}/SiO_2/Fe_3O_4$ 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, dimethyloctadedylchlorosilane (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 ChemStation software. The separation of tetracyclines was performed on a reverse phase Vertisep^{TM} UPS-C_{18} 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 $C_{18}/SiO_2/Fe_3O_4$ sorbent. The synthesis of $C_{18}/SiO_2/Fe_3O_4$ sorbent involved three steps as shown in Fig. 1a. First, Fe_3O_4 nanoparticles were synthesized following a reported method based on chemical co-precipitation [17]. Briefly, $FeCl_3\cdot 6H_2O$ (3.50 g) and $FeCl_2\cdot 4H_2O$ (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_3O_4 nanoparticles was achieved using a sol-gel process [12]. Fe_3O_4 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_2/Fe_3O_4 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_{18}-functionallization, SiO_2/Fe_3O_4 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 were added. The mixtures were then refluxed for 24 h. The $C_{18}/SiO_2/Fe_3O_4$ 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 ºC, redissolved with 1.0 mL of the mobile phase, and filtered with a PTFE filter (0.22 µm) 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 μm 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_3O_4. The absorption peaks at 1605 and 3405 cm^{-1} 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^{-1} represented the asymmetric and the symmetric vibrations of the $–CH_2$ group in the– $(CH_2)_{17}CH_3$ chain, and indicated that the C_{18} group was successfully attached onto the surface of the SiO_2/Fe_3O_4 nanoparticles. The SEM images of the $C_{18}/SiO_2/Fe_3O_4$ sorbent is illustrated in Fig. 2b, the average diameter of the $C_{18}/SiO_2/Fe_3O_4$ NPs was approximately 75 ± 10 nm and was nearly spherical in shape. Fig. 2c shows VSM magnetization curves of Fe_3O_4, SiO_2/Fe_3O_4 and $C_{18}/SiO_2/Fe_3O_4$ 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_{18}, respectively. However, all of the magnetic nanoparticles had a magnetic strength sufficient for the magnetic separation from solution with a magnet, and $C_{18}/SiO_2/Fe_3O_4$ 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_3O_4), silica coated magnetic nanoparticles (SiO_2/Fe_3O_4), 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 p*K*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 from cationic form ($pH > pKa1$), zwitterionic form ($pKa1 < pH < pKa2$) or anionic form ($pH > pKa3$) [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 was 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 μg/mL) from spiked deionized water. The averages recoveries from the six batches were: oxytetracycline 84.2 ± 1.4%, tetracycline 82.4 ± 1.0% and chlortetracycline 84.7 ± 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 µg/L for OTC and 10.0-1000 µg/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 µg/L for OTC and 10.0 µg/L for TC and CTC, and the LOQs ($S/N = 10$) were 8.0 µg/L for OTC, and 40.0 µg/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 µg/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 µg/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 magnetics 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.

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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 µg/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, µg/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 ± 0.002	ND	ND
Wastewater 2	0.012 ± 0.003	ND	ND

* Not detected

Table 2. Recoveries of tetracyclines in real water samples

Water samples	Added, µg/mL	Recovery ± SD*, %		
		OTC	TC	CTC
Tap water	0.020	87 ± 3	87 ± 3	86 ± 4
	0.10	83 ± 1	83 ± 4	82 ± 3
	0.20	84 ± 3	83 ± 2	85 ± 5
River water	0.020	88 ± 4	85 ± 5	86 ± 4
	0.10	84 ± 3	83 ± 4	84 ± 3
	0.20	84 ± 3	84 ± 4	86 ± 5
Wastewater	0.020	87 ± 3	85 ± 4	84 ± 3
	0.10	83 ± 2	85 ± 3	84 ± 2
	0.20	84 ± 3	83 ± 4	87 ± 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_3O_4 (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_{18}	4-10 ng/g	69-93	-	[23]
SPE	Water	100 mL	SPE-C_{18} (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	$C_{18}/SiO_2/Fe_3O_4$ (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.

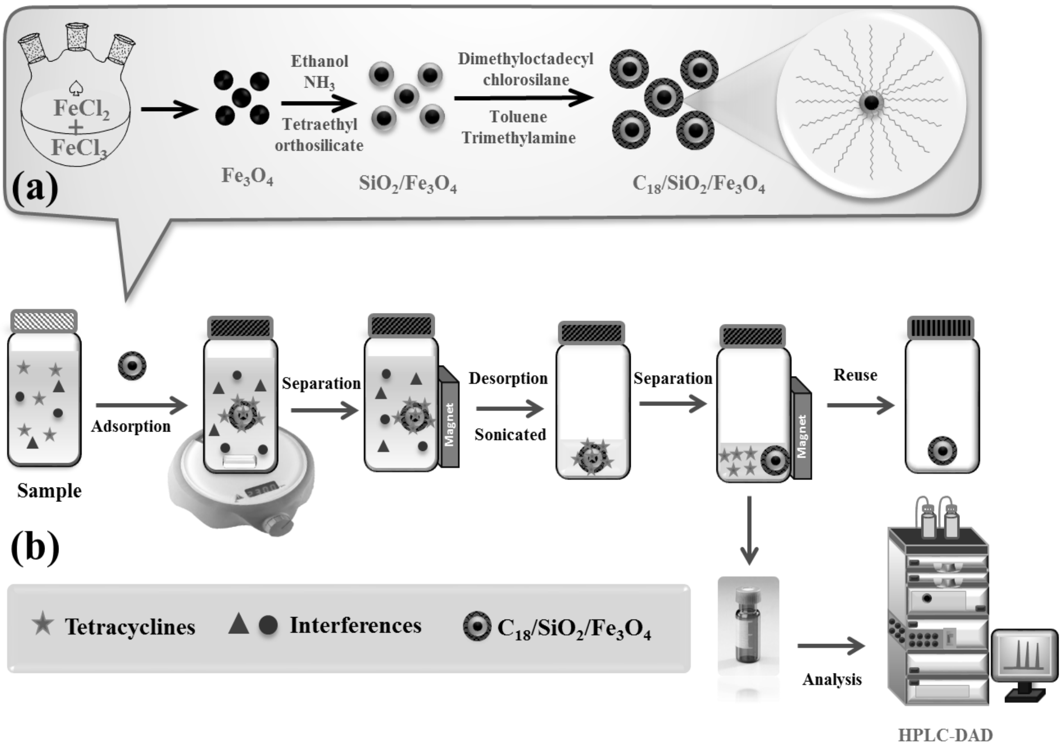


Fig. 1. Kaewsuwan W, et al.

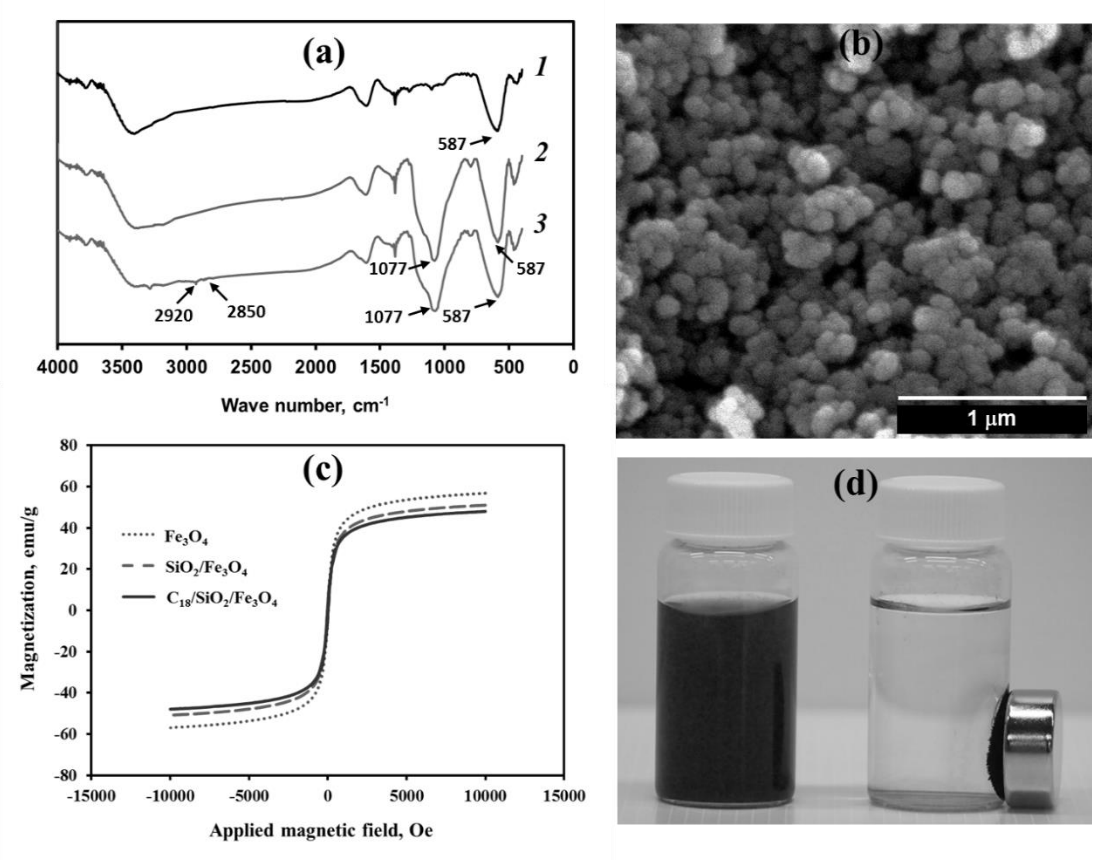


Fig. 2. Kaewsuwan W, et al.

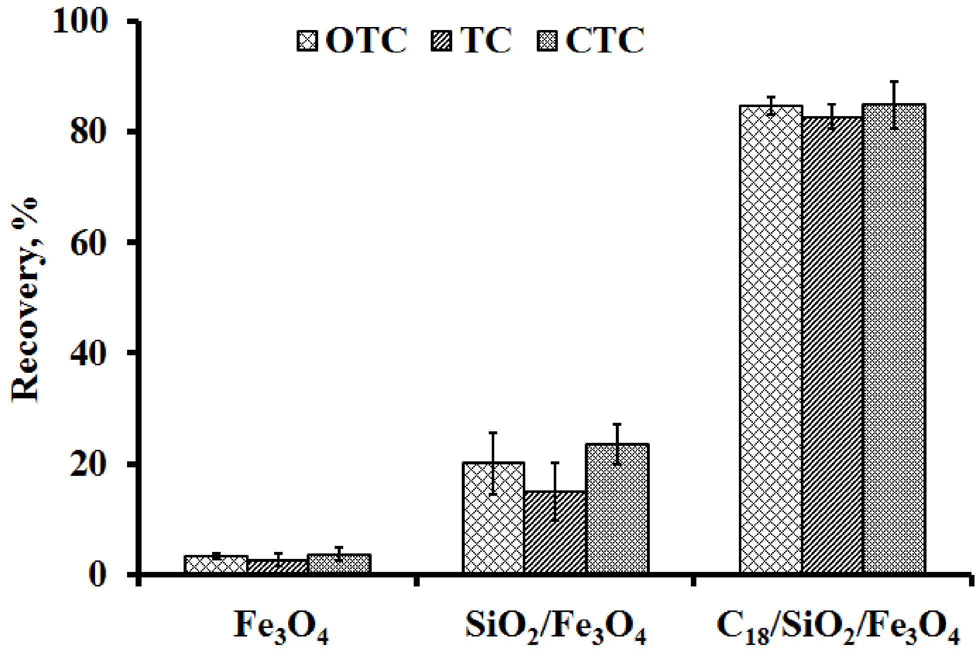
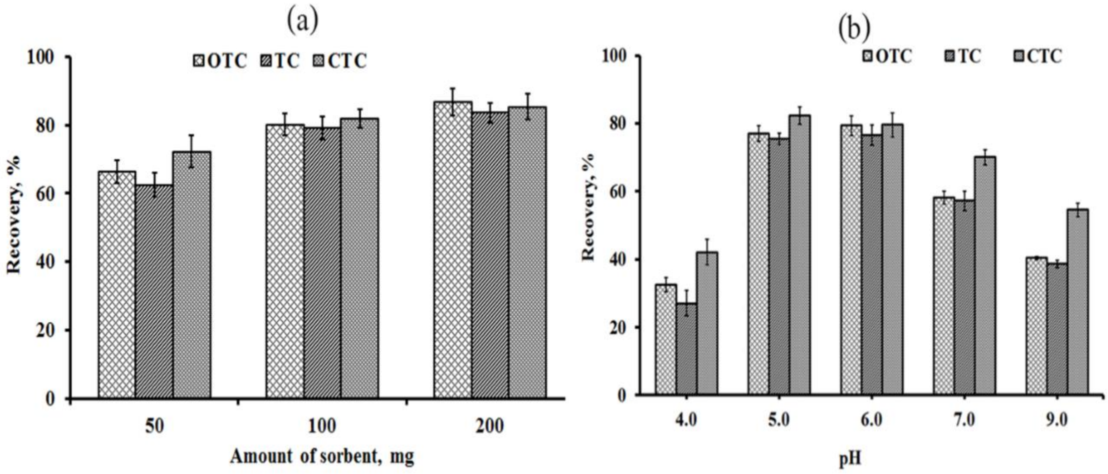


Fig. 3. Kaewsuwan W, et al.



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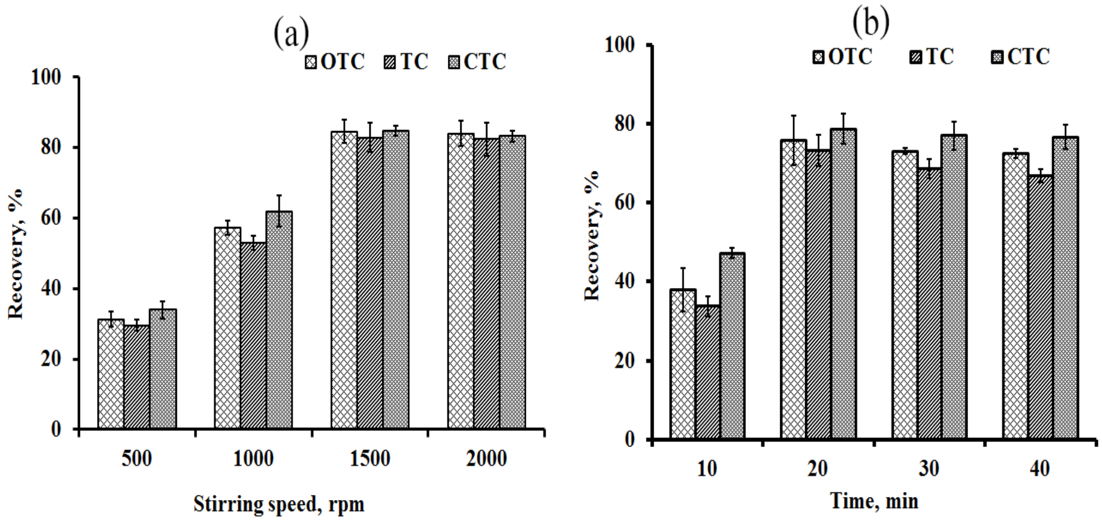

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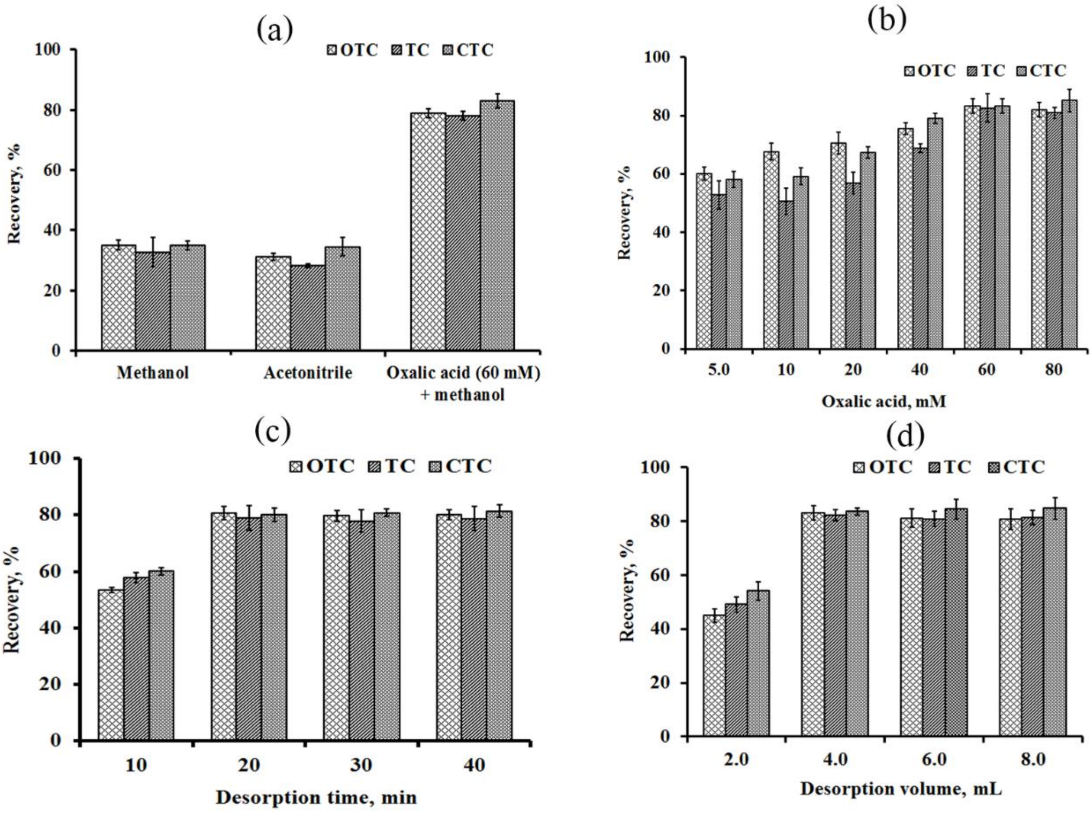

Fig. 6. Kaewsuwan W, et al.

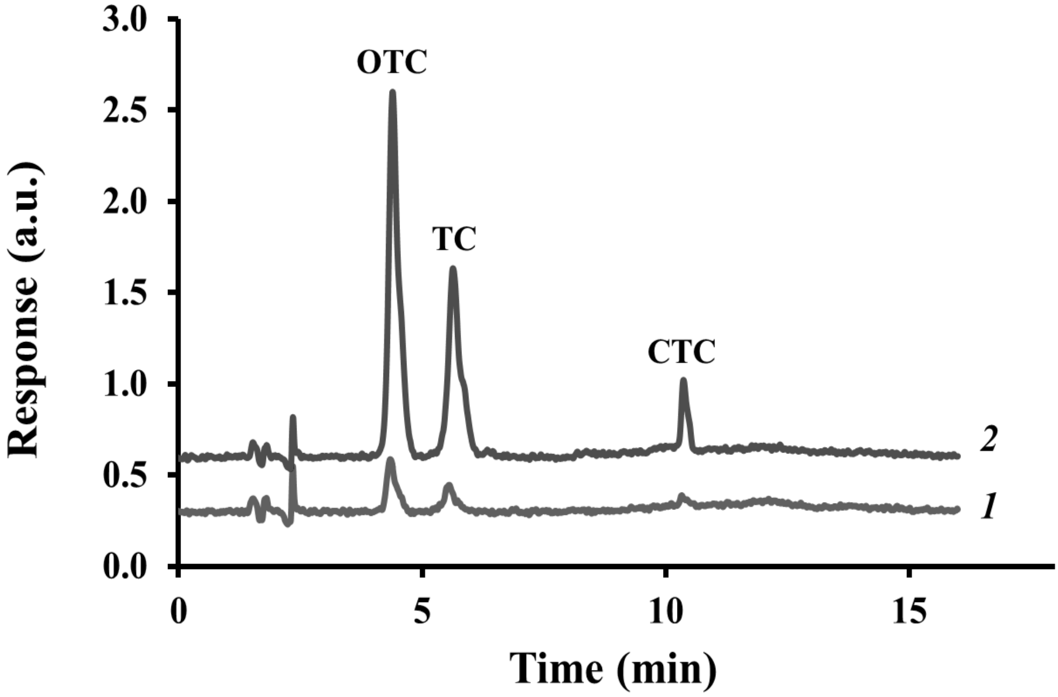

Fig. 7. Kaewsuwan W, et al.

VITAE

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List of Poster presentations and Publication

Poster presentations

Kaewsuwan, W., Kanatharana, P., and Bunkoed, O. "Development of a magnetic solid phase extraction for the determination of tetracyclines in water samples" The International Congress for Innovation in Chemistry (PERCH-CIC Congress IX 2016), 26th - 29th June, 2016, Pattaya, Chonburi, Thailand.

Kaewsuwan, W., Kanatharana, P., and Bunkoed, O. "A magnetic adsorbent of octadecyl/silica/magnetite nanoparticles for the extraction of tetracycline from water samples" Pure and Applied Chemistry International Conference (PACCON 2017), 2nd - 3rd February, 2017, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand.

Publication

Kaewsuwan, W., Kanatharana, P., and Bunkoed, O. "Dispersive Magnetic Solid Phase Extraction Using Octadecyl Coated Silica Magnetite Nanoparticles for the Extraction of Tetracyclines in Water Samples"
Manuscript (Accepted), Journal of Analytical Chemistry.