

Pre-Concentration and Determination of Tetracyclines Antibiotics Residues in Water Samples Using RGO/Fe₃O₄ Nanocomposite as Extraction Sorbent

Ungku Amirul Arif Ungku Abdullah¹, Nor Suhaila Mohamad Hanapi^{1*}, Wan Nazihah Wan Ibrahim¹, Nursyamsyila Mat Hadzir¹, Nurzaimah Zaini¹, Ahmad Lutfi Anis², and Noorfatimah Yahaya³

¹School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

²Faculty of Applied Sciences, Universiti Teknologi MARA, 94300 Kota Samarahan, Sarawak, Malaysia

³Integrative Medicine Cluster, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, 13200 Bertam Kepala Batas, Penang, Malaysia

* **Corresponding author:**

tel: +603-55435744

email: norsuhaila979@uitm.edu.my

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Abstract: Existing methods used in tracing Tetracyclines' antibiotics (TCAs) residues which pose serious environmental problems, consume high amounts of organic solvents, are time-consuming, and are relatively expensive. A simple and effective magnetic solid-phase extraction (MSPE) based on reduced graphene oxide/magnetite (RGO/Fe₃O₄) nanocomposite sorbent was successfully developed for preconcentration and extraction of TCAs residues from water samples. The analytes were determined by high-performance liquid chromatography with a diode-array detector (HPLC-DAD). The synthesized nanocomposite was characterized using Fourier-transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), and field emission scanning electron microscopy (FESEM). Sample pH, amount of adsorbent, sample volume, extraction time, desorption time, and desorption solvent were evaluated and optimized. Under optimized conditions, the method demonstrated good linearity over the concentration range of 0.05–1.0 mg L⁻¹ with the coefficient of determination (R^2) \geq 0.9978. Limit of detection (LOD) and limit of quantification (LOQ) were 0.006–0.011 mg L⁻¹ and 0.019–0.036 mg L⁻¹, respectively. The accuracy and precision of the developed method were proven by good analyte recovery (89.77–106.33%) and acceptable precision with relative standard deviation, $RSD \leq$ 5.54%. The results showed that magnetic solid RGO/Fe₃O₄ could be a suitable adsorbent in the preconcentration and extraction of TCAs in water samples.

Keywords: magnetic solid-phase extraction; reduced graphene oxide/magnetite; tetracycline antibiotics; water samples

■ INTRODUCTION

Tetracycline antibiotics (TCAs) are a natural or semisynthetic group of antibiotics frequently used to treat bacterial infections in human and veterinary medicine [1]. Due to their low cost, low toxicity, and broad-spectrum antimicrobial activity, TCAs have been used extensively in the medicinal field for prophylactic, therapeutic, and growth-promoting purposes [2]. However, TCAs usually would not be completely absorbed and metabolized within the body system [3]. For

this reason, residues of these compounds would be excreted and released to the environment water source [4-5]. However, studies have suggested that the frequent abusive consumption of antibiotics may cause toxicity in the body. Hence, many studies have been actively conducted to find efficient analytical techniques for trace determination of antibiotic pollutions in the environment.

Several analytical techniques, such as liquid-liquid extraction [6] and solid-phase extraction [7], are commonly performed for this purpose. However, most

existing methods utilize high consumption of organic solvents, are rather time-consuming, and relatively expensive with low extraction efficiencies [8-9]. For this reason, magnetic solid-phase extraction (MSPE), a miniaturized technique derived from an exhaustive technique called solid-phase extraction (SPE), was introduced. This technique offers excellent features such as simple, low cost, sensitive, high reusability, and minimum organic consumption and is considered an environmentally friendly method [7]. MSPE sorbents are prepared by incorporating two types of materials - organic material with high sorption capacity and an inorganic material with excellent magnetic properties [10]. One exceptional property of MSPE is that prepared magnetic adsorbents can be isolated from sample solutions with ease using an external magnetic field [11].

Recently, magnetite nanoparticles (MNPs) provided the most promising materials with good adsorption efficiency for water remediation applications, with further modification using carbon-based materials such as graphene oxide (GO) and carbon nanotubes [12-13]. MNPs are well accepted due to their super-paramagnetic nature, low toxicity, and biocompatibility. Fe_3O_4 is the most commonly used MNPs to coat adsorbent materials [14].

To date, there are growing demands for magnetic reduced graphene oxide (RGO/ Fe_3O_4) nanocomposite, which has emerged as a viable adsorbent material used in MSPE [15]. Reduced graphene oxide (RGO) is an allotrope of carbon consisting of a single layer of sp^2 -bonded carbon atoms, densely packed in a honeycomb lattice, which is also one of graphene oxide (GO) derivatives [16]. RGO has a lower number of oxygen-containing functional groups in comparison to GO. Therefore, RGO is sometimes more preferred due to its lesser disruption of conjugated π electrons. In addition, it has many potential applications such as drug delivery, sensors, and wastewater treatment, owing to its large specific surface area and remarkable physical and chemical properties [17]. Attributed to oxygen-containing functional groups on its surface, such as hydroxyl, epoxy, and carbonyl groups, RGO has good dispersibility in water and polar organic solvents, which allows it to form a stable aqueous suspension and

rendering the nanosheets nearly impossible to be recovered. As a means to overcome this problem, RGO is often magnetized using MNPs [17].

Various methods for the synthesis of RGO/ Fe_3O_4 nanocomposite have been reported in the literature, such as the chemical co-precipitation method [18], the solvothermal method [19], a hydrothermal method [20], covalent bonding method [21], and electrochemical method [22]. However, some of the major drawbacks of these methods are that they are multistep. In addition, they rely on numerous synthetic conditions such as the ratio of $\text{Fe}^{2+}/\text{Fe}^{3+}$, ionic strength, and temperature, and they employ highly toxic reducing agents such as hydrazine [23]. In order to solve these problems, a simple, green, and efficient in situ redox synthesis of RGO/ Fe_3O_4 nanocomposite has been developed [24]. The synthesis can be performed via a one-step procedure, which eliminates the usage of toxic reducing agents.

This new generation of RGO/ Fe_3O_4 nanocomposite was employed as a magnetic adsorbent for MSPE of TCAs residues from water samples in the present study. The work employs RGO/ Fe_3O_4 nanocomposite, synthesized from a one-step in situ redox synthesis method as a sorbent to extract four selected TCAs from the tap, lake, spring, and river water samples using MSPE prior to HPLC-DAD. The RGO/ Fe_3O_4 -based MSPE method provides a rapid yet simple and excellent extraction performance. In addition, the procedures support the concept of green chemistry as they can be considered significantly economical and environmentally friendly.

■ EXPERIMENTAL SECTION

Materials

The chemicals used in this study, namely graphene oxide (GO), ferric chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), ammonium hydroxide (NH_4OH), ethanol, methanol, acetone, acetonitrile, and oxalic acid, were purchased from Sigma Aldrich (St. Louis, MO, USA). All these chemicals were of analytical reagent grade and were used without any further purification. HPLC grade organic solvents, namely acetonitrile and methanol, were purchased from Merck Chemicals (Darmstadt, Germany), and 18.2 M Ω

ultrapure water was used (Millipore Direct, QTM). Standards of oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), and doxycycline (DC) were procured from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

The quantification of TCAs compounds was performed using HPLC equipped with a diode array detector and a manual injector. The chromatographic separation of TCAs was carried out using the ZORBAX Eclipse Plus C₁₈ column (2.1 × 100 mm, 3.5 mm) from Agilent at 25 °C. The separation was performed using a gradient mobile phase consisting of methanol, acetonitrile, and 0.03 M oxalic acid (A:B:C) with an initial composition of 0:8:92 for 1 min 0:18:82 for 2 min and remained isocratic for 5 min at the ratio 5:20:75 [25]. The composition was then set at 10:25:65 for 3 min, 15:20:65 for 1 min, and 15:25:60 for 3 min. The flow rate of the mobile phase was fixed at 0.2 mL min⁻¹, and then 5 µL of the sample was injected into the system. The TCAs compounds have different absorption maxima, and thus the detection wavelengths analyte was set at 360 nm for TC and OTC, 370 nm for CTC, and 350 nm for DC.

Procedure

Preparation of stock and standard solution

Stock solutions of TC, OTC, CTC, and DC were prepared at a concentration of 1000 mg L⁻¹ by dispersing 10 mg of each standard in 100 mL of methanol. A standard mixture of 100 mg L⁻¹ was prepared as an intermediate solution by diluting 10 mL of each stock solution with methanol to a final volume of 100 mL. Subsequently, a series of standard solutions were prepared at five (5) different concentrations as calibration standards by diluting each standard with appropriate volumes of ultrapure water. For optimization purposes, ultrapure water was spiked with the standard mixture for a final concentration of 1 mg L⁻¹. All solutions were refrigerated at 4 °C before analysis.

Water samples

The applicability of the developed method was demonstrated using RGO/Fe₃O₄ nanocomposite in the MSPE procedure for trace determination of TCAs from actual water samples, namely, tap, lake, spring, and river

water. All samples were collected in Selangor, Malaysia. Tap water was collected from the laboratory in Universiti Teknologi MARA (UiTM), Shah Alam; lake water is from Tasik Seksyen 7, Shah Alam, while both spring and river water were collected from Batu 16, Hulu Langat. The collected samples were filtered using 0.45 µm Nylon filter paper to remove any suspended particles and were stored in polyethylene bottles at 4 °C before analysis.

Preparation of RGO/Fe₃O₄ nanocomposite

RGO/Fe₃O₄ nanocomposite was prepared by a simple, one-step deposition of Fe₃O₄ onto the surface of GO nanosheets at ambient temperature. The method was adopted from previously reported literature with minor modifications [26]. First, 25 mg of GO nanosheets was dissolved in 20 mL of ultrapure water by ultrasonication for 1 h. Next, the pH of the mixture was adjusted to 11 by slowly adding drops of 25% ammonia solution. Under continuous stirring, 125 mg of FeCl₂·4H₂O was added very slowly into the mixture. Black suspensions of RGO/Fe₃O₄ nanocomposite were formed immediately. The mixture was left agitated for 3 h to allow complete bonding between Fe₃O₄ and RGO nanosheets. The RGO/Fe₃O₄ nanocomposite suspensions were separated using a centrifuge at 4000 rpm and washed with ultrapure water 3 times. The nanocomposite residue was dried overnight in a hot air oven at a temperature of 60 °C.

Characterization

The synthesized RGO/Fe₃O₄ nanocomposite along with Fe₃O₄ nanoparticles, GO, and RGO nanosheets were characterized using X-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM), and Fourier-transform infrared spectroscopy (FTIR) [21,27-28]. The XRD analysis of GO nanosheets, Fe₃O₄ nanoparticles, and RGO/Fe₃O₄ nanocomposite was performed using PANalytical Empyrean XRD in the range of 2θ from 10°–80° at a scanning rate of 3° min⁻¹ with Cu-Kα radiation (λ = 1.54056 Å) under the acceleration voltage of 40 kV and a current of 40 mA. The morphology of the studied materials was characterized using Hitachi S-4800 FESEM (Japan) with a generator voltage of 15 kV. The FTIR spectra were recorded using Avatar 360 FTIR (Thermo Fisher Scientific, Waltham, MA).

Magnetic solid-phase extraction (MSPE) procedure

The MSPE procedure was adopted from previous literature with minor alterations [26]. The MSPE procedure was initialized as follows: 10 mg of the synthesized RGO/Fe₃O₄ nanocomposite was dispersed in 8 mL of the water sample. The mixture was sonicated for 5 min to allow adsorption of TCAs onto the adsorbent surface. Next, the adsorbent was separated from the sample matrix with the aid of an external magnetic field, and the supernatant liquid was decanted. The adsorbent was then dispersed into 500 μ L of ethanol and sonicated for 2 min to elute the analytes that were trapped on its surface. Subsequently, the adsorbent was removed, and the eluent was evaporated completely using nitrogen blow. Finally, the dried analyte was reconstituted with 100 μ L of methanol and filtered using a 0.45 μ m PTFE syringe filter. For analysis, 5 μ L of the solution was injected into the HPLC system. A summary of this process is depicted in Fig. 1. Several MSPE parameters were optimized by changing one factor at a time, and the optimal conditions were used in all subsequent experiments. The performance of this procedure was compared with previously reported methods from literature.

Method validation and data analysis

The MSPE performance of the synthesized RGO/Fe₃O₄ nanocomposite for the extraction of the

selected TCAs was quantified and evaluated with respect to the linearity, limit of detection (LOD), the limit of quantification (LOQ), precision (repeatability and reproducibility), and accuracy.

Calibration curves. Calibration standard curves were determined using linear regression: $y = ax + b$, where y is the peak area, a is the slope, x is the respective concentration, and b is the intercept. The linearity responses of TCAs were evaluated using a series of standards at five concentrations ranging from 0.05–1.0 mg L⁻¹, where the coefficient of determination (R^2) should be at least 0.95.

Limits of detection and quantification. The limit of detection (LOD) and the limit of quantification (LOQ) values were determined using linear regression where $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$, where σ is the standard deviation of the lowest concentration, and S is the slope of the calibration graph.

Repeatability. The repeatability (intra-day precisions) and reproducibility (inter-day precisions) of the proposed method were expressed in terms of relative standard deviation (RSD). The intra-day precisions were determined using a triplicate measurement of the lowest concentration level and were prepared on the same day. Meanwhile, to determine the inter-day precisions, the same procedure is repeated for three consecutive days.

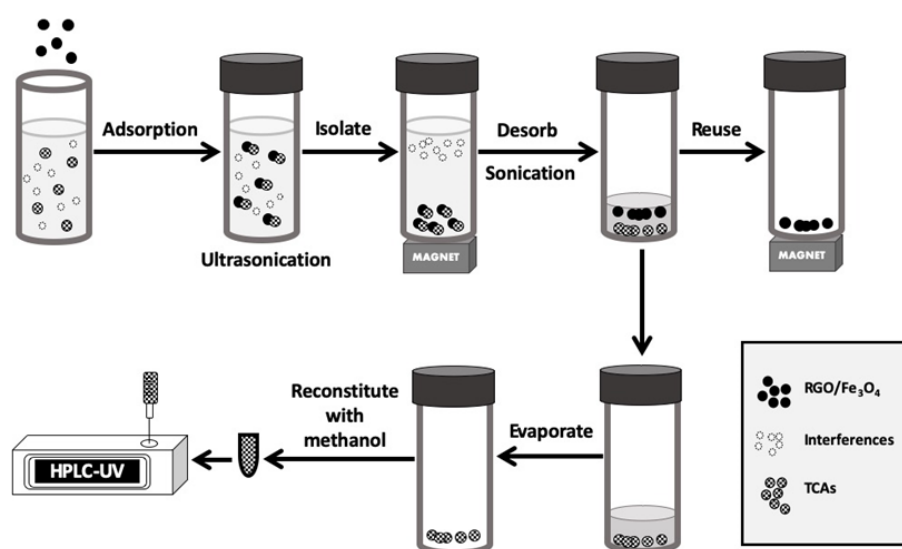


Fig 1. MSPE procedure of TCAs analyte from water using RGO/Fe₃O₄ nanocomposite

Recovery. The method's accuracy, expressed in terms of % recovery (R%), was determined by fortifying real water samples with each targeted analyte at a final concentration of $500 \mu\text{g L}^{-1}$. Although there has been no official guideline established for the recovery of spike pharmaceuticals in water, the acceptable value should normally range from 70–120% with $\text{RSD} \leq 20\%$ [29-30].

RESULTS AND DISCUSSION

System Performance

The performance of HPLC-DAD was calibrated under optimal conditions to ensure the instrument meets the desired and intended performance standards. A series of TCAs standard mixture at five different concentrations were injected into the system. Good separation peaks of TC, OTC, CTC, and DC were obtained within 14 min. Calibration curves of the selected TCAs were plotted, and linear ranges were established between $2\text{--}10 \text{ mg L}^{-1}$ with a coefficient of determination (R^2) greater than 0.999.

Preparation of RGO/Fe₃O₄ Nanocomposite

Fe₃O₄ nanoparticles were incorporated onto the surface of RGO nanosheets by a simple electrostatic self-assembly method [28]. This process involved a redox reaction between the two main precursors to produce RGO/Fe₃O₄ nanocomposite. The chemical scheme of this reaction is shown in Fig. 2. GO became highly negative

when dispersed into the water due to the ionization of the carboxylic acid and phenolic hydroxyl groups present on its surface. Fe²⁺ ions were deposited onto the surface of GO nanosheets. GO acts as an oxidizing agent. GO was reduced to RGO and effectively increased the oxidation state of Fe²⁺ to Fe³⁺. The formation of Fe₃O₄ on the surface of RGO occurred via two distinct pathways. The first pathway manifested in alkaline conditions, where Fe³⁺ reacted with the excess Fe²⁺ to form Fe₃O₄ on the GO surface. The second pathway involves the hydroxyl group that attached itself to the GO surface, which reacted with Fe²⁺ to form Fe₃O₄ on the GO surface. During the redox reaction, the polar oxygenated functional groups on the GO nanosheets serve as the anchoring sites for the Fe₃O₄ nanoparticles, consequently preventing severe agglomeration of the MNPs.

Characterization of Magnetic Nanoparticles RGO/Fe₃O₄ Nanocomposite XRD

The phase and structure for GO nanosheets, Fe₃O₄ nanoparticles, and RGO/Fe₃O₄ nanocomposite were determined as shown in Fig. 3. GO shows a basal reflection (002) sharp peak at $2\theta = 14.1^\circ$ indicating oxygen-rich functional group and water molecules between the layer of GO nanosheets. In Fig. 3(b), the XRD pattern of Fe₃O₄ nanoparticles was reflected by the six distinctive diffraction peaks at $2\theta = 30.2^\circ, 35.6^\circ, 43.3^\circ,$

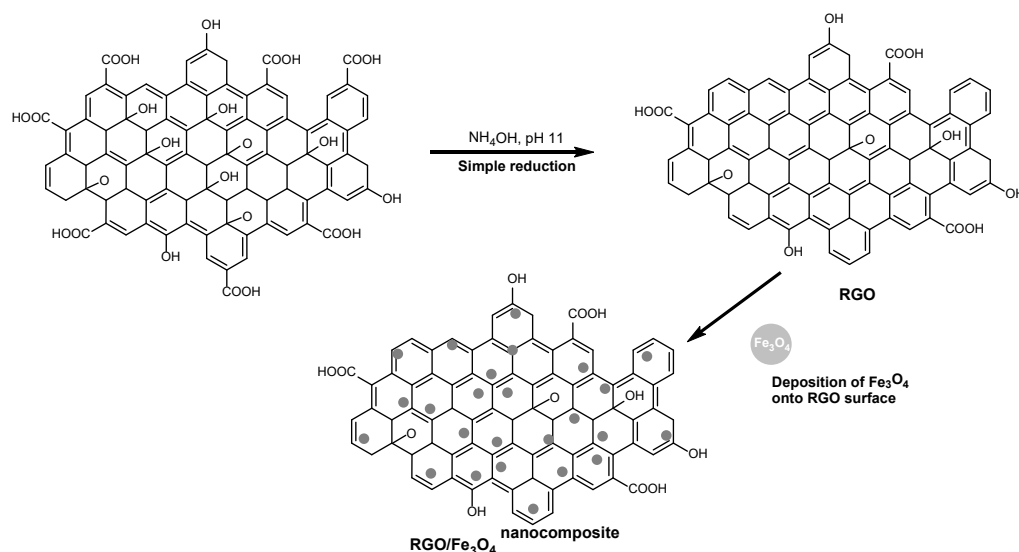


Fig 2. Chemical scheme of the synthesis of RGO/Fe₃O₄ nanocomposite

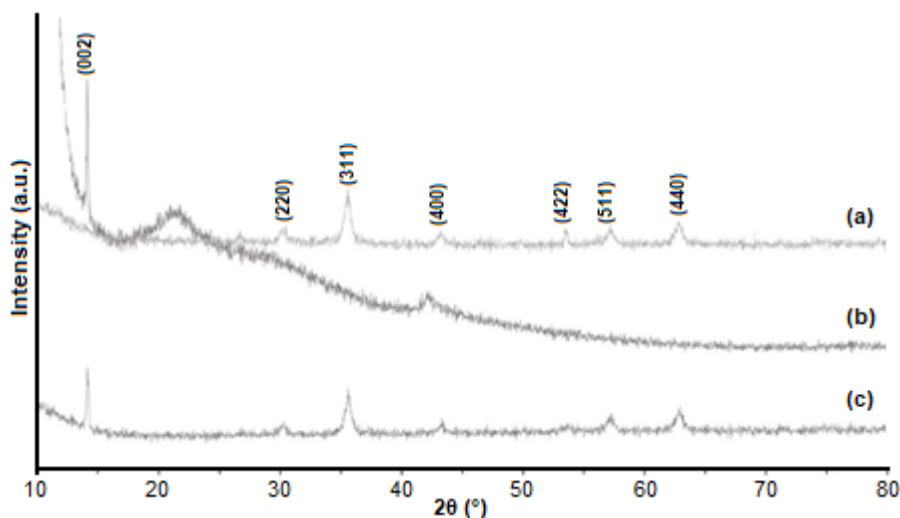


Fig 3. XRD patterns of (a) Fe_3O_4 nanoparticles, (b) GO nanosheets, and (c) RGO/ Fe_3O_4 nanocomposite

53.7°, 57.3°, and 62.8° corresponding to (220), (311), (400), (422), (511), and (440) crystal planes of Fe_3O_4 , respectively. The XRD pattern of Fe_3O_4 nanoparticles suggested that the MNPs possess good crystallinity, indicated by the distinct diffraction peaks and the absence of impurities. As shown in Fig. 3(c), the synthesized RGO/ Fe_3O_4 nanocomposite possesses all the characteristic diffraction peaks that matched the crystal planes of both GO nanosheets (14.1°) and Fe_3O_4 nanoparticles ($2\theta = 30.2^\circ, 35.6^\circ, 43.3^\circ, 53.7^\circ, 57.3^\circ, \text{ and } 62.8^\circ$). The average crystallite size of the nanocomposite calculated using the Scherrer equation is 24.76 nm. Hence, it can be inferred that RGO/ Fe_3O_4 nanocomposites were successfully synthesized, and the crystalline structure of Fe_3O_4 remained unchanged upon synthesis.

FESEM

The FESEM images of all the studied samples are shown in Fig. 4. The micrograph in Fig. 4(a) depicts the smooth and wrinkled surface of GO nanosheets with a distinctive layered appearance. Fig. 4(b) shows the FESEM image of Fe_3O_4 nanoparticles. The MNPs appeared as a spherical-like structure with a wide distribution of particle size and an apparent tendency to agglomerate. After RGO/ Fe_3O_4 nanocomposite formation, the Fe_3O_4 nanoparticles were distributed on the surface of RGO nanosheets, which has become corrugated due to loss of oxygen-containing functional groups. The energy-dispersive X-ray (EDX) analysis was

performed to determine the elemental mapping of the RGO/ Fe_3O_4 nanocomposite. The EDX spectrum in Fig. 4d indicates that the adsorbent contained 25.23 at % of iron (Fe), 43.39 at % of oxygen (O), and 29.97 at % of carbon (C). The results estimated the major compositions of the adsorbent. Furthermore, it also provided evidence that substantiated the embedment of Fe_3O_4 onto the surface of the RGO.

FTIR

FTIR spectra of the studied samples are shown in Fig. 5. The strong and broad peak that appeared in the high-frequency area at 3421 cm^{-1} for synthesized RGO/ Fe_3O_4 nanocomposite (Fig. 5(a)) can be attributed to the O–H stretching and bending of the free or adsorbed water in RGO and Fe_3O_4 . The characteristic peaks that appeared at $1636, 1201, \text{ and } 1051\text{ cm}^{-1}$ denote the C=C stretching of the aromatic ring, C–O–H bending of the phenolic group, and C–O stretching of the epoxy group in RGO nanosheets, respectively. The apparent peak at 587 cm^{-1} is a resonance to the Fe–O bond stretching vibration characteristic to Fe_3O_4 . Compared to the FTIR spectra of individual RGO (Fig. 5(b)) and Fe_3O_4 (Fig. 5(d)), all the characteristic peaks are present in the synthesized RGO/ Fe_3O_4 nanocomposite, supporting the evidence observed in XRD patterns (Fig. 3) and FESEM images (Fig. 4), which indicates that Fe_3O_4 was successfully incorporated onto the surface of RGO.

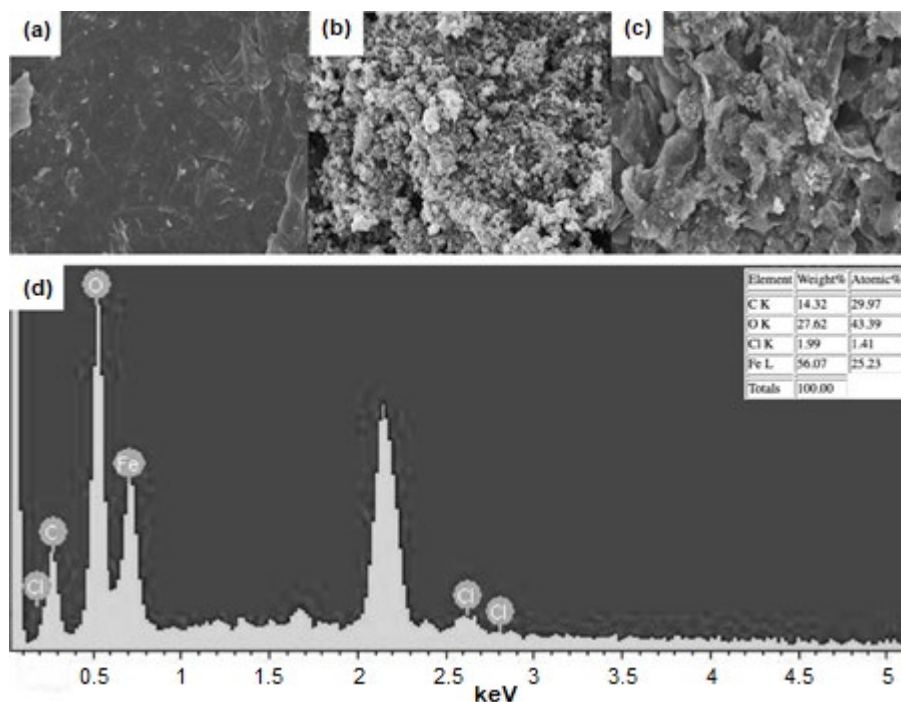


Fig 4. FESEM images of (a) GO nanosheets, (b) Fe_3O_4 nanoparticles, (c) RGO/ Fe_3O_4 nanocomposite, and (d) EDX spectra of RGO/ Fe_3O_4 nanocomposite

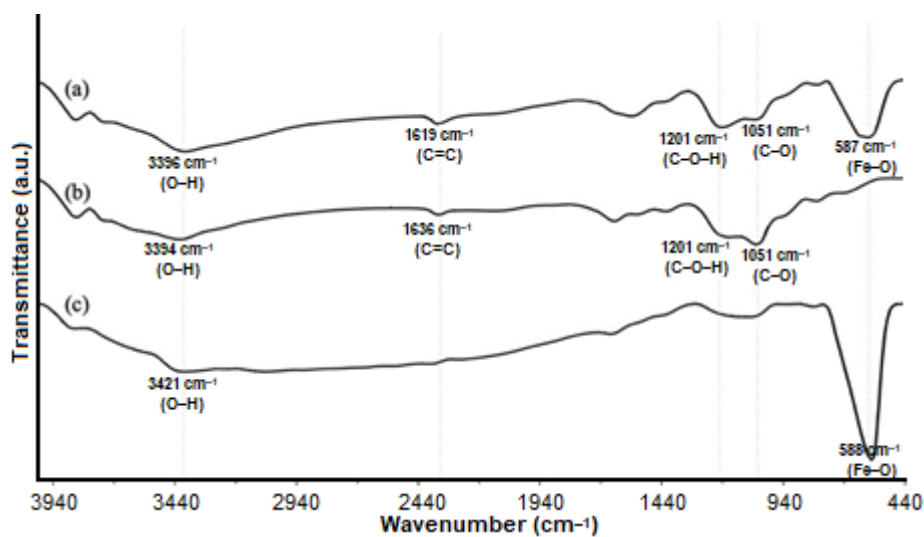


Fig 5. FTIR spectra of (a) RGO/ Fe_3O_4 nanocomposite, (b) RGO nanosheets, and (c) Fe_3O_4 nanoparticles

Optimization of Magnetic Reduced Graphene Oxide Extraction Conditions

The MSPE procedure was optimized in order to achieve the highest recovery, lower the consumption of solvents and materials and shorten the analysis time duration. The parameters involved in this study were the sample pH, amount of adsorbent, sample volume,

extraction time, desorption time, and desorption solvent. In addition, the ionic strength of the sample solution was excluded due to its negligible influence on the TCAs recoveries [31-33]. The optimization experiments were conducted using ultrapure water spiked with TCAs analyte at a final concentration of 1 mg L^{-1} to simulate a real sample.

Effect of sample pH

The pH of the sample plays a significant role, which influences the sorption behavior of TCAs on the surface of RGO/Fe₃O₄ nanocomposite. The point of zero charges (pH_{pzc}) of the nanocomposite surface is estimated to be approximately at pH 3.6. Therefore, the modification of the sample pH influences the protonation–deprotonation transition of the oxygen moieties that are attached on its surface; where the surface becomes acidic with a positive net charge when the sample pH is lower than that of the pH_{pzc} (pH < pH_{pzc}), and vice versa [34]. Additionally, TCAs are ionizable organic compounds, which chemical speciation is strongly dependent on the pH of the media [35]. Thus, TCAs may exist as (1) cations (TCH⁺) at pH < 4 through the protonation of dimethylammonium group; (2) zwitterions (TCO²⁻-N⁺) at pH 3.5–7.5 through deprotonation of the phenolic diketone moiety, or (3) anions (TCO²⁻) by deprotonation of the tricarbonyl system and phenolic diketone moiety.

The effect of the sample pH was investigated in the range of 2–12 to control the electrostatic interactions of TCAs and RGO/Fe₃O₄ nanocomposite. As can be seen, the recovery of TCAs abruptly increased and reached a point of maxima when the sample pH was adjusted from around 4 to 6 (pH > pH_{pzc}) due to stronger electrostatic interactions between the TCAs zwitterions and the net negative charge of the moderately basic surface of the nanocomposite. However, with a further increase of the pH (pH > 7), the adsorption of TCAs can be seen progressively decreased due to stronger electrostatic repulsive force between the TCAs anions and the net negative charge of the strongly basic surface of the

nanocomposite. Therefore, pH 4 was selected and used in all subsequent experiments.

Effect of the amount of adsorbent

The amount of adsorbent is another important parameter to be considered in the MSPE procedure. Generally, an increase in the amount of adsorbent will increase the number of adsorption sites that may occupy the adsorbate particles during the extraction procedure [36–37]. In order to determine the minimum amount of adsorbent sufficient to extract the total amount of TCAs per sample, the influence of the mass of RGO/Fe₃O₄ nanocomposite on the recovery of the targeted TCAs was investigated in the range between 5 and 25 mg. The recoveries of TCAs increased when the weight of RGO/Fe₃O₄ nanocomposite was increased from 5 to 10 mg (Fig. 6). However, a further increase in the adsorbent weight from 10 to 50 mg did not improve the recovery of TCAs. From the data, it can be concluded that 10 mg of RGO/Fe₃O₄ nanocomposite would provide a sufficient number of active sites to occupy the total amount of TCAs in the 8 mL of sample solution. For this reason, the weight of RGO/Fe₃O₄ nanocomposite was kept constant at 10 mg in all subsequent experiments.

Effect of sample volume

In order to determine the maximum suitable volume of sample per unit weight of adsorbent, the influence of sample volume on the sorption behavior of TCAs on RGO/Fe₃O₄ nanocomposite was studied in the range between 2 and 10 mL. From Fig. 7(a), it can be observed that the recovery of TCAs gradually increased with the increase of sample volume from 2 to 8 mL. With

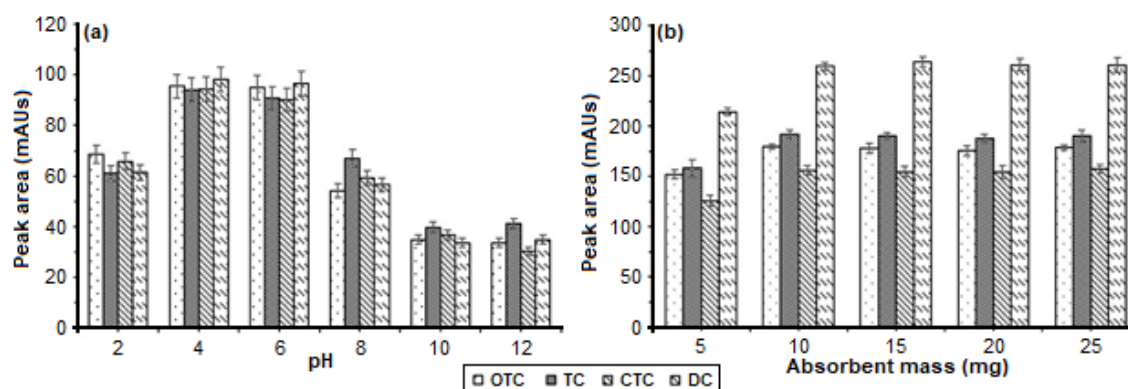


Fig 6. Effect of (a) sample pH (b) amount of adsorbent on the extraction of TCAs using RGO/Fe₃O₄ nanocomposite

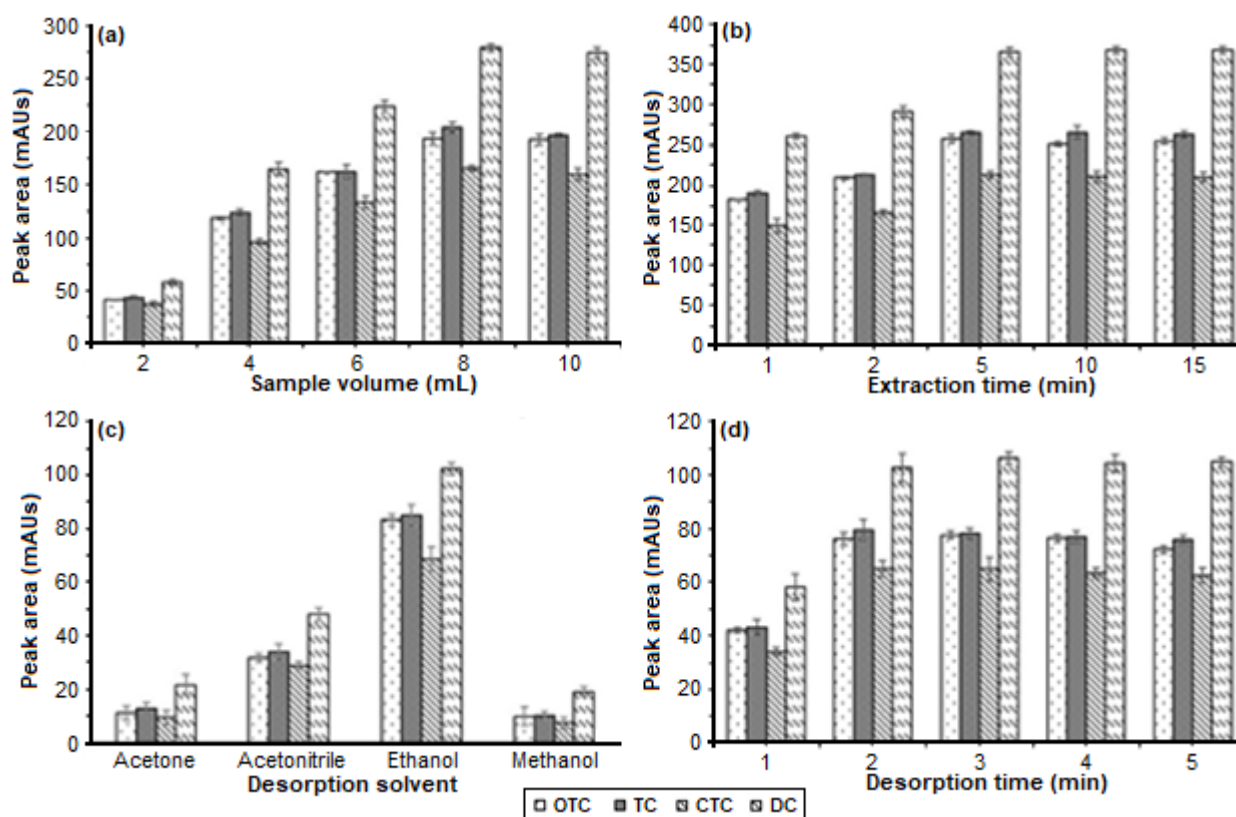


Fig 7. Effect of (a) sample volume, (b) extraction time, (c) desorption solvent, and (d) desorption time on the extraction of TCAs using RGO/Fe₃O₄ nanocomposite

a further increase in sample volume above 8 mL, the recovery remained constant. Therefore, increasing the sample volume should also increase TCAs' recovery, as a larger volume of sample would result in a larger total amount of TCAs molecules available to occupy the active sites on the surface of RGO/Fe₃O₄ nanocomposite [38]. However, it is also important to note that the maximum amount of analyte adsorbed onto the adsorbent surface is limited by the weight of adsorbent used during the extraction procedure and the overall adsorption kinetics of TCAs when the volume of the sample is increased [39]. From the results obtained, it was deduced that the maximum applicable volume of sample per 10 mg of RGO/Fe₃O₄ nanocomposite is 8 mL. For this reason, the sample volume was set at 8 mL in all subsequent experiments.

Effect of extraction time

The effect of extraction time was studied to determine the time required for the complete adsorption

of TCAs onto the surface of RGO/Fe₃O₄ nanocomposite. A sufficient amount of time must be given for a close MSPE system to achieve adsorption equilibrium, at which the rates of adsorption and desorption become constant [40]. In this experiment, the adsorption of TCAs was facilitated by the use of ultrasonication. The influence of extraction time between the analyte and adsorbent was investigated by varying the sonication time from 1 to 15 min. The experimental results in Fig. 7(b) demonstrate that the recovery of TCAs increased gradually as the sonication time progressed from 1 to 5 min. This suggests that the adsorption of TCAs increases over time as it allows a higher frequency of effective contacts between the adsorbent particles and adsorbate molecules in a given sample. After 5 min, the recovery of TCAs remained constant, indicating that the adsorption equilibrium has been achieved and that the adsorption process has completed. Therefore, the MSPE procedure was carried out employing 5 min of extraction time in all further stages of this work.

Effect of desorption solvent

The selection of a suitable organic solvent is important to ensure the complete elution of trapped analytes from the surface of RGO/Fe₃O₄ nanocomposite [41-42]. In this study, the influence of different types of organic solvents was investigated to enhance the recovery of analytes. Arranged according to the increasing order of polarity, the solvents used were acetone, acetonitrile, ethanol, and methanol. The volume of the desorption solvent was fixed at 500 μ L. The results shown in Fig. 7(c) show that the use of acetone as a desorption solvent yields the lowest recovery and is therefore impotent for the desorption of the targeted TCAs. Although methanol is the most polar solvent used in this experiment, it has failed to yield the best recovery percentage and could not effectively break the adsorbent-adsorbate bond between the two phases since both TCAs, and RGO/Fe₃O₄ nanocomposite were also hydrophilic in nature [43-44]. The highest recoveries for all the targeted TCAs were obtained using ethanol, presumably due to its higher compatibility and stronger hydrogen bond and dipole-dipole intermolecular interactions with the targeted TCAs, thus enabling effective desorption of TCAs from the nanocomposite surface. Therefore, ethanol was selected as the desorption solvent in all subsequent experiments.

Effect of desorption time

Desorption time is the time required for the analyte to be eluted from the adsorbent surface [45]. In this study, the elution of TCAs from the prepared RGO/Fe₃O₄ nanocomposite was performed using an ultrasonic bath. The effect of desorption time was studied for 0–5 min to determine the minimum amount of time required for complete elution. Fig. 7(d) shows that the recovery of TCAs improved significantly within 2 min. However, further increase in sonication time did not result in any improvement of the recovery of analytes. This fact demonstrates that the recovery of TCAs increases as time progresses before finally reaching a saturation state, indicating that the state of sorption equilibrium between the bulk phase (sample solution) and the adsorbent surface has been achieved. Based on this, it is acceptable to assume that the completion of the desorption process

can be achieved within 2 min. For this reason, the desorption time of 2 min was selected in all further experiments.

Reusability

Reusability of the synthesized RGO/Fe₃O₄ nanocomposite is a crucial property that has a notable impact on the proposed method's economic and environmental aspects [46]. In this work, the reusability study was conducted to determine the number of times the adsorbent can be regenerated and reused before any significant degradation in the extraction performance can be noticed. The nanocomposites' reusability was evaluated by collecting and washing the used nanocomposite with a sufficient amount of methanol, 0.03 M oxalic acid, and ultrapure water to remove any remains of TCAs molecules from the previous extraction. The adsorbent was then dried overnight in an oven at 60 °C. The same MSPE procedure was further repeated for 10 cycles. The concentration of TCAs solution was fixed at 1 mg L⁻¹. The data of TCAs recoveries were recorded at every two intervals of the extraction cycle. The results obtained suggest that the recoveries of the targeted TCAs remain almost consistent without significant loss of performance up to 10 cycles (Fig. 8). Thus, the results also confirmed that RGO/Fe₃O₄ nanocomposite can be regenerated and reused and has a great potential in real sample preparation.

Method Validation

Under optimal MSPE conditions, several key parameters such as linearity, the limit of detection (LOD) and limit of quantification (LOQ), precision, and accuracy were validated to evaluate the analytical performance of the developed RGO/Fe₃O₄-MSPE-HPLC-DAD method. The linearities of the method can be determined by plotting the calibration curves for all TCAs analytes using a series of standards at five (5) different concentrations within the selected range of 0.05–1 mg L⁻¹. Good linearities were achieved for the targeted TCAs with coefficients of determination (R^2) of 0.9989, 0.9991, 0.9978, and 0.9992 for TC, OTC, CTC, and DC. The LODs at which the signal-to-noise ratios are

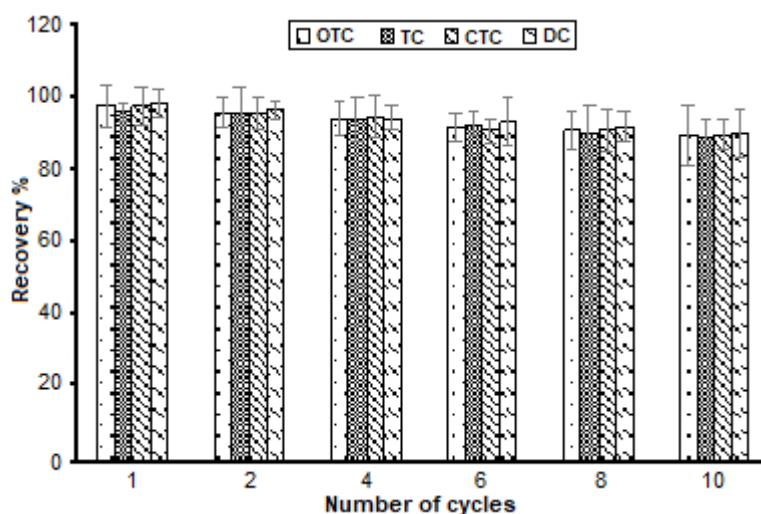


Fig 8. Reusability of RGO/Fe₃O₄ nanocomposite for the extraction of TCAs

equal to 3, were established ranging from 0.006–0.011 mg L⁻¹. Meanwhile, the LOQs at which the signal-to-noise ratios are equal to 10, were established ranging from 0.019–0.036 mg L⁻¹. Both the LODs and LOQs lie well below the maximum residual limit (MRL) of TCAs set by the European Union, which is 100 µg kg⁻¹ (equivalent to 0.1 mg L⁻¹) in food products [47]. Furthermore, the precisions of the method were evaluated in terms of repeatability and reproducibility using triplicate extraction data (n = 3) from the intra-day test (three tests on the same day) and inter-day test (repetition of triplicate MSPE procedure for three consecutive days) using 8 mL of ultrapure water spiked with TCAs analytes

at a final concentration of 1 mg L⁻¹. Precisions of the intra-day and inter-day obtained, expressed in terms of RSD, were 1.39%–3.38% and 2.75%–5.59%, respectively. The analytical evaluation of the RGO/Fe₃O₄-MSPE-HPLC-DAD method is summarized in Table 1.

Real sample analysis

The developed method was applied to analyze three (3) different water samples, namely tap, river, and spring water, to verify the developed RGO/Fe₃O₄-MSPE-HPLC-DAD method's performance in the real application. The results shown in Table 2 indicate that no trace of TCAs can be found in each of the samples. The accuracy of the proposed method was evaluated by spiking the samples

Table 1. Analytical data of RGO/Fe₃O₄-MSPE- HPLC-DAD method for the determination of TCAs in water

Analyte	Linearity, mg L ⁻¹	R ²	LOD, mg L ⁻¹	LOQ, mg L ⁻¹	RSD (n = 3)	
					Intra-day	Inter-day
OTC	0.05–1.0	0.9991	0.008	0.026	2.39	2.75
TC	0.05–1.0	0.9989	0.009	0.029	1.39	4.15
CTC	0.05–1.0	0.9978	0.011	0.036	2.54	5.59
DC	0.05–1.0	0.9992	0.006	0.019	3.38	3.14

Table 2. Analysis of TCAs in four different water samples

Water Sample	OTC		TC		CTC		DC	
	R%	RSD%	R%	RSD%	R%	RSD%	R%	RSD%
Tap	92.67	2.18	92.38	1.32	98.41	1.94	96.69	2.01
Lake	89.82	5.69	91.85	3.31	93.35	6.59	90.84	4.90
Spring	95.74	3.22	94.87	4.03	89.77	4.22	103.41	3.27
River	99.15	2.76	106.33	2.93	96.58	5.48	93.16	5.54

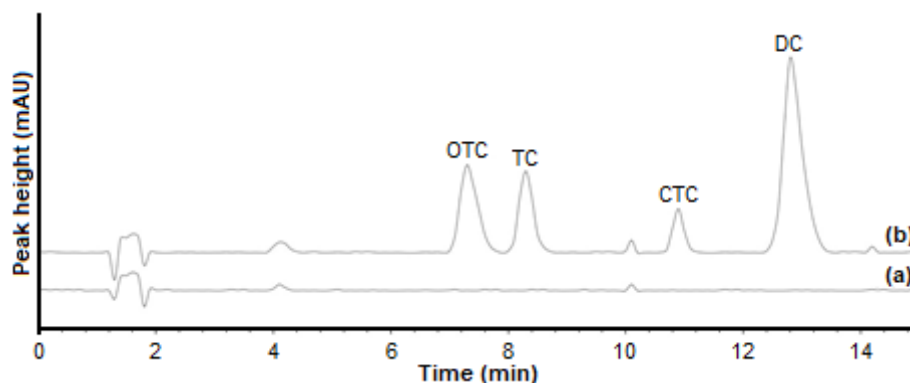


Fig 9. HPLC-DAD chromatogram of (a) blank sample and (b) spiked sample (0.5 mg L^{-1} for each analyte)

Table 3. Comparison of the analytical performance of the developed method with other methods for the extraction and determination of TCAs in a water sample

Extraction technique	Sorbent	Sample	LOD, $\mu\text{g L}^{-1}$	RSD, %	Recovery, %	Ref
MSPE-HPLC-DAD	RGO/ Fe_3O_4	Water	6–11	1.39–5.59	89.8–106.3	[This work]
SPE-HPLC-MS	C18 cartridge	Water	3.1–3.8	4–11	72–93	[44]
SPE-LVSS-CE	C18 cartridge	Milk	19.93	1.7–9.7	-	[48]
MSPE-DLLME	$\text{Fe}_3\text{O}_4@/\text{SiO}_2@/\text{GO}-\beta\text{-CD}$	Water	1.8–2.9	78.5–109.3	1.6–6.3	[37]
MSPE-UPLC-TUV	$\text{Fe}_3\text{O}_4@/\text{SiO}_2@/\text{FeO}$	Water	0.027–0.107	< 4	91.0–104.6	[49]
μ -SPE-HPLC-UV	PET/GO nanofiber	Honey	15.3	4.9	89–94	[36]
SALLME- HPLC-UV	-	Urine	0.17	8	91–105	[50]
SPE-UPLC-Q/TOF MS	Oasis HLB	Urine	0.138	4.1–7.8	90	[51]
SPE-HPLC-DAD	Oasis HLB	Water	3.5–6.5	<14.5	82.1–114.7	[52]

with a standard mixture of TCAs at a final concentration of 0.5 mg L^{-1} . The chromatogram of blank and spiked water samples is presented in Fig. 9. Satisfactory recoveries were obtained for all TCAs analytes ranging from 89.77%–106.33%. Meanwhile, analyses on the triplicates gave acceptable RSDs ranging from 1.32% and 6.59%.

Comparative Study

The MSPE performance of RGO/ Fe_3O_4 nanocomposite for the extraction of the selected TCAs from the water was compared with other reported methods in terms of LOD, RSD percentage, and recoveries percentage (Table 3). The developed method exhibited LOD comparable to conventional SPE technique based on C₁₈ cartridge and μ -SPE based on PET/GO nanofiber. However, relatively lower LOD values may be caused by the quantification instruments used, not due to the developed adsorbent's capability for extraction of analytes from samples. Nevertheless, it is

worth noting that the developed adsorbent offers advantages compared to previously reported methods, including high percentage MSPE recoveries ($\geq 89.8\%$) for real samples with satisfactory RSD percentage ($\leq 5.59\%$). In addition to this, the desorption rate was fast (3 min ultrasonication assisted) with good reusability (10 adsorption-desorption cycles).

CONCLUSION

A magnetic solid-phase extraction (MSPE) technique based on a new generation RGO/ Fe_3O_4 nanocomposite has been established and demonstrated as an efficient alternative technique for rapid separation, preconcentration, and enrichment of OTC, TC, CTC, and DC from water samples. Remarkable extraction of TCAs can be achieved due to the ability of the nanocomposite to form π - π interactions and electrostatic force with the analytes. The nanocomposite was synthesized using a quick, easy, and green technique, which eliminates multistep procedures and

the consumption of toxic reducing agents. The MSPE-RGO/Fe₃O₄ technique was optimized, evaluated, and compared with other existing methods. It offers several notable advantages regarding good linearity, low LOD and LOQ, acceptable precision, and satisfactory accuracy. In addition, the RGO/Fe₃O₄ nanocomposite sorbent is reusable up to 10 times. Thus, this newly developed technique provides a rapid, more efficient, and comparable performance for rapid extraction, preconcentration, and trace determination of TCAs from water samples.

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