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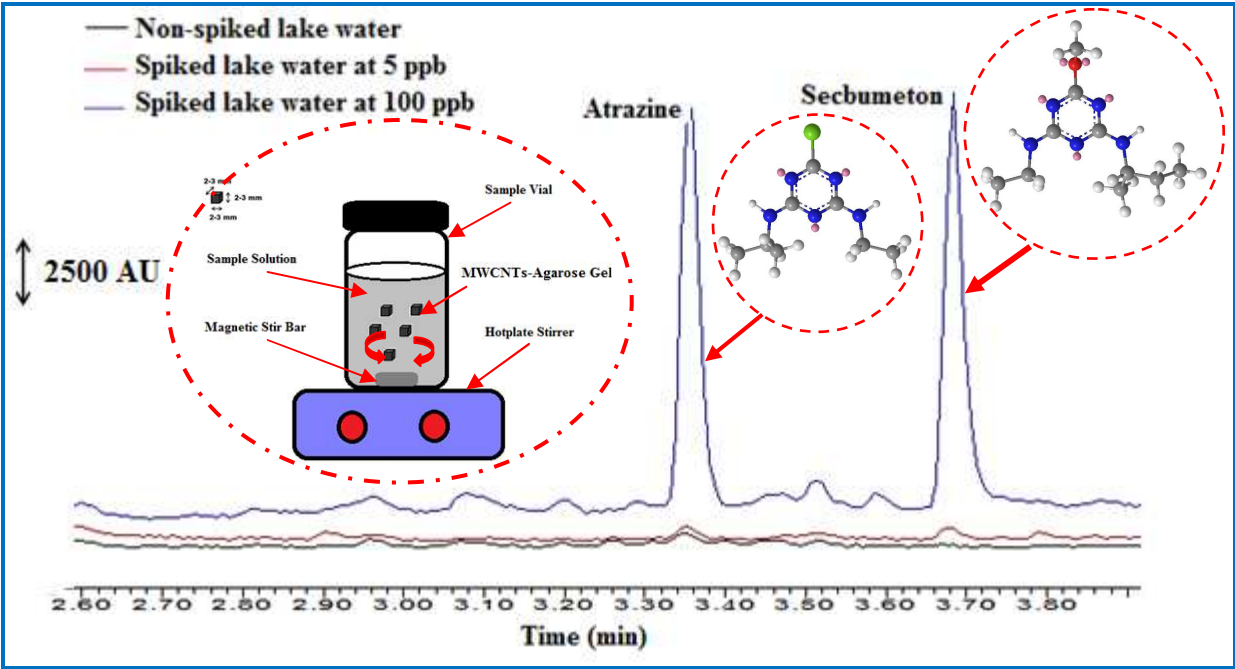
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Graphical Abstract



Multi-walled carbon nanotubes-agarose gel micro-solid phase extraction for the determination of triazine herbicides in water samples

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Abstract: In this work, a new extraction procedure termed multi-walled carbon nanotubes-agarose gel micro-solid phase extraction (MWCNTs-AG- μ SPE) combined with gas chromatography-mass spectrometry (GC-MS) was developed and used for the determination of

triazine herbicides in water samples. High performance synthetic adsorbent, MWCNTs-AG, was prepared by using a simple stirring method. Different stoichiometric ratios of MWCNTs and agarose were mixed in deionized water for at least 24 h at room temperature in order to obtain homogeneous MWCNTs dispersed agarose solution . Two selected triazine herbicides namely atrazine (ATR) and sebumeton (SEC) were employed as model compounds. Several important parameters such as the effects of MWCNTs concentration, type of conditioning solvent, extraction time, type of desorption solvent and desorption time were comprehensively optimized. Under the optimized conditions, the calibration results showed good linearity with the correlations of determination (r^2) in the range of 0.9925-0.9981 for both analytes. Limits of detection (LODs) were found to be $0.319 \mu\text{g L}^{-1}$ and $0.340 \mu\text{g L}^{-1}$ for ATR and SEC, respectively while the LOQs were found to be $1.064 \mu\text{g L}^{-1}$ and $1.136 \mu\text{g L}^{-1}$ for ATR and SEC, respectively. High relative recoveries were obtained in the range of 84.9-108% for the analytes with relative standard deviation of $<15.2\%$ ($n = 3$). The results showed that MWCNTs-AG- μ SPE method is superior as compared to AG- μ SPE for both analytes. This new method was successfully applied for the determination of triazine herbicides in selected water samples. Atrazine was found to be present in the lake water sample by having a signal higher than the LODs and lower than LOQs. The proposed technique proved to be simple and requires relatively short extraction time and low amounts of organic solvents and thus contributes towards green chemistry.

Keywords: Agarose gel, Multi-walled carbon nanotubes, Solid phase microextraction, Triazine herbicides, Water samples.

1. Introduction

Triazine herbicides are extensively used as selective herbicides for the control of broadleaf and grassy weeds in many agricultural crops over the past years. Triazine herbicides are considered one of the most important classes of chemical pollutants due to their toxicity and usually reported present in water, soils and organism due to their high persistency [1]. Due to their high toxicity, triazines have been included in the list of human carcinogens, its metabolites present serious adverse effects such as hormone disrupting, birth defects, reproductive cancers, and weight loss of mother and embryos during gestation [2]. Because of the widespread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets. Thus, a suitable and efficient analytical technique is important in order to eliminate the environmental pollution that becomes a threat to human health. However, due to the small amount of these compounds present in environmental samples, they cannot be directly detected from samples by using any analytical instruments such as high performance liquid chromatography (HPLC), gas chromatography (GC) and etc.. Thus, it is necessary to introduce a new eco-friendly sample preparation/extraction technique for the determination of triazine herbicides in environmental samples.

In order to resolve this problem, a simple, efficient and green sample preparation technique is required to replace other conventional extraction techniques. In recent years, an alternative solvent minimized sample preparation approach, solid phase microextraction (SPME), has gained considerable attention. The advantages of the SPME method are that this procedure is simpler and faster than liquid-liquid extraction (LLE) and solid phase extraction

(SPE). Up to now, there are many studies have been reported by utilizing various types of adsorbent in SPME for the determination of triazine herbicides in environmental samples [3, 4]. Currently, Xin Wang and his co-workers have introduced an alternative sorbent which is a carbon nanotube-incorporated with polymer monolith for the determination of six triazine herbices in water samples [5]. From this study, the extractability performances of CNTs sorbent was increased when this material was incorporated with the polymer. Thus, based on these previous mentioned studies, the development or modification of new adsorbent is very much needed in SPME to increase the extraction performances in SPME.

Agarose, a natural polymer consisting of alternating D-galactose and 3,6-anhydro-L-galactose, is extracted from seaweed [6]. Recently, some new innovative techniques termed agarose film liquid phase microextraction (AF-LPME), agarose gel liquid phase microextraction (AG-LPME) for the determination of selected polycyclic aromatic hydrocarbons (PAHs) in water [7, 8], solid-phase membrane tip extraction (SPMTE) for the determination ofazole antifungal drugs in human plasma [9] and non-steroidal anti-inflammatory drugs in human urine [10], two-phase electrodriven membrane extraction for the determination of tricyclic antidepressants in aqueous matrices [11] and portable micro-solid phase extraction for the determination of polycyclic aromatic hydrocarbons in water samples [12] have been demonstrated. These techniques offer inexpensive and selective approaches for the enrichment of analytes and fulfilled green chemistry requirements. The same laboratory explored the extractability of agarose by immobilizing the agarose film with MWCNTs to serve as adsorbent in solid phase microextraction (SPME) for the determination of selected polycyclic aromatic hydrocarbons (PAHs) in green tea beverages [13]. This study claimed that AF-MWCNTs-SPME

method is suitable for trace analysis of environmental pollutants by obtaining high enrichment factor of analytes.

Carbon nanotubes (CNTs), which were discovered by Iijima in 1991 [14] are one of the most widely studied carbon nanomaterials and can serve as excellent adsorbents because of their hollow and layered structure and large specific surface area [15]. CNT adsorbents can be classified into three types: single-walled CNTs (SWCNTs), multi-walled CNTs (MWCNTs), and functionalized CNTs. Such materials have already played an important role as an adsorbent in SPE [16, 17], as a coating material in solid phase microextraction [18, 19] and as packed sorbent in micro-solid phase extraction [20, 21].

In this study, a new approach of micro-solid phase extraction method is introduced by utilizing an agarose gel impregnated with MWCNTs (MWCNTs-AG) as high performance synthetic adsorbent for the determination of triazine herbicides in water samples. Agarose gel is considered as a green polymer due to its biodegradable nature and MWCNTs has very unique thermal and mechanical properties. Thus, we considered that this new approach of MWCNTs-AG- μ SPE can be as an alternative method to minimize the use of chemicals and organic disposal. In addition, the extraction setup design is simplified by directly dipping small piece of cut gel in stirred sample solution. Like another established micro-solid phase extraction, this extraction procedure involves analyte desorption followed by solvent desorption but without involving fibers as an extraction medium.

2. Experimental

2.1 Chemicals and Reagents

Two triazine herbicides, atrazine (ATR) and secbumeton (SEC) were selected as model compounds, were purchased from Riedel-deHaën (Seelze, Germany). HPLC grade tetrahydrofuran (THF), isopropyl alcohol (IPA), methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Multi-walled carbon nanotubes (MWCNTs) (specific surface area $>233 \text{ m}^2 \text{ g}^{-1}$, purity $>95\%$, 8-15 nm outer diameter \times 50 μm in length) were purchased from Sun Nanotech (Jiangxi, China). Agarose (molecular grade) was obtained from Promega (Madison, USA). Deionized water of at least $18 \text{ M}\Omega$ was purified by NanoUltra-pure water system (Barnstead, USA). Hot plate magnetic stirrer (Favorit, Malaysia) and stirring bar ($12 \times 4 \text{ mm}$) were used to agitate the sample solutions during extraction.

2.2 GC-MS Conditions

Analysis of triazines herbicides were performed by injecting 1 μL aliquot of the extract into Agilent Technologies (Milan, Italy), gas chromatograph model 6890N equipped with Agilent Technologies (Milan, Italy), 5973 inert MS detector. Separations were carried out on a HP Ultra-2 capillary column ($25 \text{ m} \times 0.2 \text{ mm}$ i.d. and $0.33 \mu\text{m}$ film thicknesses). Helium gas was used as carrier gas at a flow rate of 1.0 mL min^{-1} . The oven temperature program employed for separation of triazine herbicides is as follows: 190°C for 1 min; increased at 5°C min^{-1} to 210°C held for 0 min. The GC oven temperature and the injection port temperature were maintained at 300°C . All injections were made in the split mode (20:1) ratio. Chromatographic data were processed using MSD Chemstation software.

2.3. Preparation of standard solutions and real samples

Stock solution of $1000 \mu\text{g mL}^{-1}$ of each triazine herbicides (atrazine, and secbumeton) was prepared by weighing 10 mg of each triazine standard into a 10 mL volumetric flask, then dissolved in methanol and kept in the refrigerator. Standard solutions for studying the extraction performance were prepared by spiking the standard solutions with the model analytes at concentration of $0.1 \mu\text{g mL}^{-1}$ in deionized water. Lake water sample was collected from the UTM lake (Johor, Malaysia) and fresh tap water was obtained in the laboratory of the Universiti Teknologi Malaysia, Skudai. These samples were collected in bottles pre-cleaned with acetone. For the lake water samples, the samples were filtered through a Whatman filter paper No. 1 (Maidstone, England). The samples were stored in freezer at -20°C until analysis.

2.4. Preparation of adsorbent (MWCNTs-AG and Agarose Gel)

MWCNTs-AG was prepared in various weight ratio of agarose with MWCNTs by stirring with magnetic bar. Agarose (800 mg) was dissolved in 100 mL hot deionized water and controlled amount of MWCNTs were dispersed into agarose solution. Mechanical stirring for at least 24 h was applied at room temperature in order to obtain homogeneous MWCNTs dispersed agarose solution. The amount of MWCNTs (50, 80, 100, 120, 140 and 160 mg) were optimized to obtain the higher extraction efficiency of analytes. The solution was allowed to cool and gel at room temperature for at least 30 min in the 100 mL beaker. Then, the gel was taken out from the beaker and was carefully cut into small pieces of cube ($3 \times 3 \times 3$ mm in length) using a small knife. To produce a consistent size of the cube, a ruler was used for measuring the size of cube during cutting process. Fig. 1 shows images of the agarose gel (a) before and (b) after cutting

process to form a cube. The preparation of agarose gel cube was prepared with the same procedures as mentioned above without the addition of MWCNTs.

2.5. Multi-walled carbon nanotubes-agarose gel-micro-solid phase extraction (MWCNTs-AG- μ SPE) and agarose gel micro-solid phase extraction (AG- μ SPE)

The experimental set-up is illustrated in Fig. 1(c). Briefly, the prepared MWCNTs-AG and agarose gel cube was conditioned in isopropyl alcohol to wet and activate the sorbent surface for 3 min. The MWCNTs-AG cube was placed in the spiked sample solution that was stirred at optimized agitation rate of 1000 rpm (result not shown). During the extraction process, the gel cube was tumbled freely in the 10 mL sample solution. After 20 min extraction, the MWCNTs-AG cube was removed and rinsed with deionized water to remove any possible surface contaminations. Finally, the MWCNTs-AG cube was placed in a 300 μ L safe-lock tube which was filled with 300 μ L of organic solvent. The analyte was desorbed under ultrasonication for specified time (3-20 min) and 1.0 μ L of the final extract was injected into the GC-MS system for quantification. These procedures were applied for MWCNTs-AG- μ SPE and AG- μ SPE.

2.6. Optimization parameters in MWCNTs-AG- μ SPE

In this study, several extraction parameters were comprehensively studied and optimized to enhance the extraction efficiency of analytes. MWCNTs concentration, type of conditioning solvent, extraction time, desorption solvent and desorption time were comprehensively optimized before the application of the proposed method to the analysis of water samples. Each experiment was performed in triplicate. 10 mL of deionized water sample spiked with 100 μ g L⁻¹ of triazine herbicides was used for optimization of the extraction parameters. The initial

condition of the extraction are; conditioning solvent, isopropyl alcohol; extraction time, 20 min; desorption solvent, acetonitrile (300 μ L); desorption time, 10 min.

2.7. Analytical characterization of the method

The extraction method was assessed for linearity, relative recovery, relative standard deviation (RSDs), limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ can be expressed as a concentration at a specified signal-to-noise ratio obtained from samples spiked with analyte. A signal-to-noise ratio 3:1 is generally considered acceptable for LOD and 10:1 is acceptable for LOQ [22].

3. Results and Discussion

3.1. Effect of MWCNT concentration

Various concentrations of MWCNTs immobilized within agarose in the range of 0.05–0.16% (w/v) were investigated. It was found that the extraction efficiency initially increased with increasing concentration of MWCNTs (Fig. 2). However, when more than 0.14% (w/v) of MWCNTs was used, no additional enhancement of extraction efficiency was observed. It is important to point out that a very high concentration of MWCNTs percentage loading reduces the mechanical strength of the gel in holding the MWCTs particles and therefore resulting in depletion of adsorbent. Thus, incomplete desorption process of analytes resulted in the decreasing of extraction recovery [23]. By considering these results, 0.14% (w/v) of MWCNTs was used in all subsequent experiments.

3.2. Type of conditioning solvent

Conditioning solvent was required in MWCNTs-AG- μ SPE in order to increase the wettability and activate the active sites of the MWCNTs. As MWCNTs is hydrophobic in nature, thus this parameter is unavoidable. In this study, conditioning solvents with water miscibility properties and varies polarities were applied as conditioning solvents (water, acetonitrile (ACN), methanol (MeOH), tetrahydrofuran (THF) and isopropyl alcohol (IPA). From Fig. 3, it was observed that gel conditioned with IPA gave highest extraction efficiencies for all targeted analytes. IPA is a mid-polar solvent and it is the most suitable organic solvent to overcome the wettability issue and enhance the interaction between hydrophobic adsorbent with the mid-polar analyte studied.

3.3. Extraction time

Extraction times in the range of 10 to 25 min were examined in order to determine the equilibrium time required for analytes to partition into acceptor phase. The sample solution was continuously stirred at room temperature at 1000 rpm. From the results obtained (Fig. 4a) it can be seen that the equilibrium was reached at 20 min as it gave the highest extraction efficiency for both analytes. Slight decrease of peak area was observed when longer extraction time (25 min) was applied. This might be due to the increase of the temperature due to the stirring of the solution during a long period of time (> 20 min). Hence, 20 min was chosen as the optimum extraction time.

3.4. Desorption solvent and desorption time

Analytes were desorbed using an organic solvent from the MWCNTs-AG after extraction by using ultrasonication method as the technique was suitable to be applied for reversible adsorption. Several selected organic solvents which are compatible with GC-MS such as MeOH,

ACN, IPA and THF were evaluated (Fig. 4b). Based on the results, THF was found to be the most suitable desorption solvent as it gave the highest peak areas. Therefore, further experiments were carried out using 300 μL of THF as desorption solvent.

The effect of desorption time in the range of 3–20 min (Fig. 4c) of ultrasonication was investigated. It was found that maximum desorption of the analytes was achieved within 5 min of ultrasonication. There was a slight decrease in the peak area observed beyond 5 min of ultrasonication. Therefore, desorption time of 5 min with ultrasonication was chosen for subsequent experiments.

3.5. Comparison study between MWCNTs-AG- μSPE and AG- μSPE

Experiments were carried out to compare the performance of MWCNTs-AG- μSPE and AG- μSPE under their respective optimized conditions using deionized water samples and spiked with the analytes at 100 $\mu\text{g L}^{-1}$. The stirring speed and extraction time employed for both MWCNTs-AG- μSPE and AG- μSPE were 1000 rpm and 20 min, respectively. Result obtained shows that the extraction efficiency of MWCNTs-AG- μSPE for both analytes were significantly higher as compared to those for AG- μSPE .

As shown in Fig. 5, MWCNTs-AG- μSPE method demonstrated better performance in terms of extraction recovery for ATR and SEC when compared to the AG- μSPE . This might be attributed to the increased number of interaction sites introduced in the gel. Higher surface area of MWCNTs also might be provided the sufficient capacity for the adsorption of analytes. In term of mechanism, the interaction between sorbent and analytes only occurred at the surface of the sorbent (do not involve any covalent bonding in the pore/inner space of the sorbent). Positive effects of MWCNTs on extraction efficiency might be due to the hydrophobic and pi-pi

interactions between CNTs and triazine [24]. By considering the results obtained, MWCNTs-AG- μ SPE method was selected for the method validation.

3.6. Linearity, limit of detection (LOD), limit of quantification (LOQ) and relative recoveries

The MWCNTs-AG- μ SPE method was validated for relative recoveries, sample calibration, LOD and LOQ. As can be seen from the Table 1, good linearity from 1 – 1000 $\mu\text{g/L}$ were obtained for both ATR and SEC where the coefficients of determination (r^2) were 0.9925 and 0.9981, respectively. The LODs and LOQs were found to be 0.319 $\mu\text{g L}^{-1}$ and 0.340 $\mu\text{g L}^{-1}$ for ATR and SEC, respectively while the LOQs were found to be 1.064 $\mu\text{g L}^{-1}$ and 1.136 $\mu\text{g L}^{-1}$ for ATR and SEC, respectively.

3.7. Application of MWCNTs-AG- μ SPE for real sample analysis

In order to investigate the practicality of the proposed method for the analysis of triazine herbicides in real samples, the proposed method was applied to the analysis of the atrazine (ATR) and sebumeton (SEC) in the tap and lake water samples. For lake water sample, no peak was detected for SEC and ATR was detected by having a signal higher than the LODs and lower than LOQs. (Fig. 6). For tap water sample, no peak was detected for both analytes (figure not shown). Relative recovery studies were conducted by spiking tap water and lake water samples with ATR and SEC to give a final concentration of 5 and 100 $\mu\text{g L}^{-1}$. Relative recovery were calculated as the percentage of mean values of extracted target analytes peak area (three replicates) against the peak of target area of target analyte derived from the plotted calibration curve. Good average relative recoveries in the range of 84.9% to 108% with relative standard

deviations (RSDs) of less than 15.2% ($n = 3$) (Table 2.) indicated that the precision and accuracy of the present method is acceptable

4. Conclusion

New application of agarose gel-impregnated with multi-walled carbon nanotubes in micro-solid phase extraction was proposed in this work. The proposed method was proven to be a superior as an alternative micro-solid phase extraction method in extracting triazine herbicides as model analytes in water samples. Since there is only a small size of the gel (3 mm \times 3 mm \times 3 mm) is required for each extraction, thus it could be used as disposable single-use adsorbent to avoid any possible carry-over effect. This newly developed technique provides some beneficial advantages such as shorter extraction time, simple extraction set-up design and low consumption of organic solvents. Besides that, the application of MWCNTs-AG as adsorbent in micro-solid phase extraction would give some advantages towards green chemistry as agarose is a biodegradable product.

Acknowledgement

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Figure Captions:

Fig. 1 Images of the agarose gel multi-walled carbon nanotubes (a) before, (b) after cutting process to form a cube and (c) schematic of MWCNTs-AG- μ SPE setup.

Fig. 2 Effect of MWCNTs concentration on MWCNTs-AG- μ SPE of triazine from water sample. Extraction conditions: 0.1 $\mu\text{g mL}^{-1}$ of spiked solution; conditioning solvent, isopropyl

alcohol; extraction time, 20 min; desorption solvent, acetonitrile (300 μL); desorption time, 10 min.

Fig. 3 Effect of conditioning solvent on MWCNTs-AG- μSPE of triazines from water sample. Extraction conditions: 0.1 $\mu\text{g mL}^{-1}$ of spiked solution; MWCNTs concentration, 0.14% (w/v); extraction time, 20 min; desorption solvent, acetonitrile (300 μL); desorption time, 10 min.

Fig. 4 Effect of extraction time (a), effect of desorption solvent (b) and effect of desorption time (c) on MWCNTs-AG- μSPE of triazines from water sample. Extraction conditions: 0.1 $\mu\text{g mL}^{-1}$ of spiked solution; MWCNTs concentration, 0.14% (w/v); conditioning solvent, isopropyl alcohol; desorption solvent, acetonitrile (300 μL); desorption time, 10 min.

Fig. 5 Comparison between MWCNTs-AG- μSPE and AG- μSPE . Extraction conditions: 100 $\mu\text{g/L}$ of spiked solution; MWCNTs concentration, 0.14% (w/v); conditioning solvent, isopropyl alcohol; extraction time, 20 min; desorption solvent, tetrahydrofuran (300 μL); desorption time, 5 min.

Fig. 6 Chromatogram of non-spiked lake water (a), spiked lake water at 5 $\mu\text{g L}^{-1}$ (b), and spiked lake water at 100 $\mu\text{g L}^{-1}$ (c) after MWCNTs-AG- μSPE under the optimum conditions. GC conditions: HP Ultra-2 capillary column (25 m \times 0.2 mm i.d. and 0.33 μm film thickness); temperature program, 190 $^{\circ}\text{C}$ for 1 min, raised to 210 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C min}^{-1}$ and held at 210 $^{\circ}\text{C}$ for 0 min; injection volume, 1 μL .

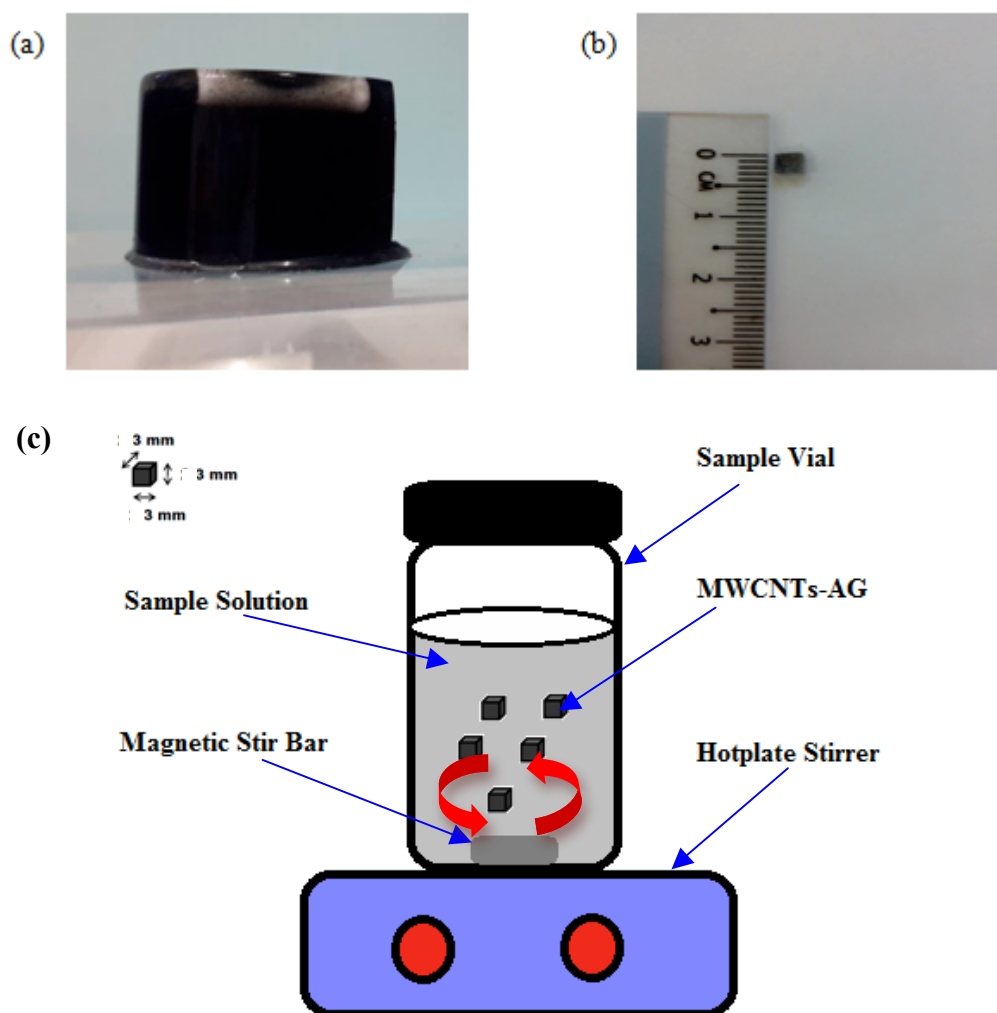


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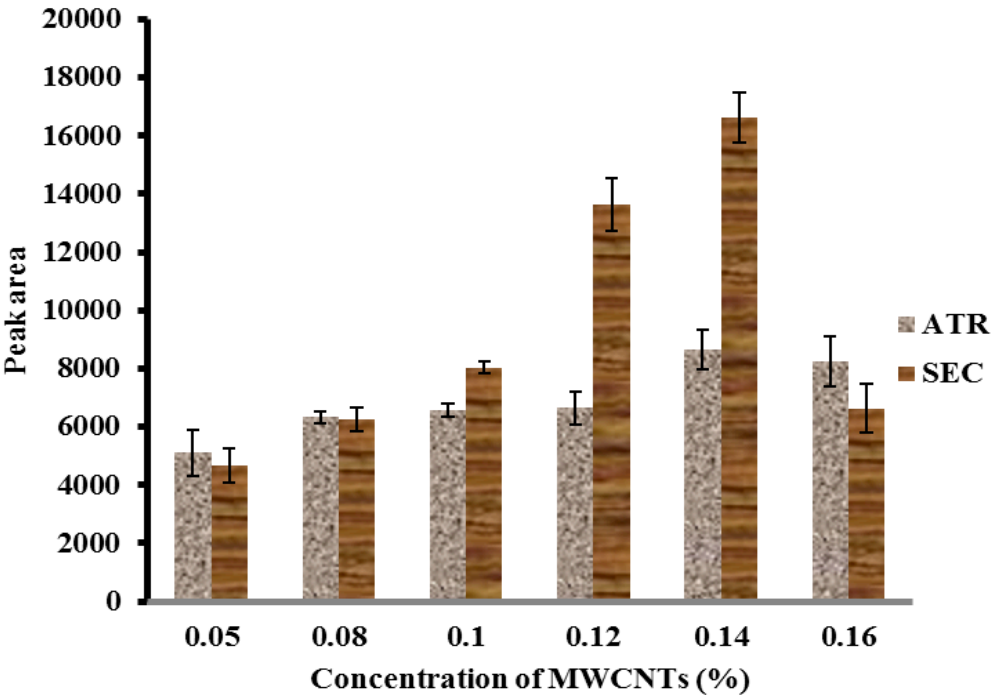


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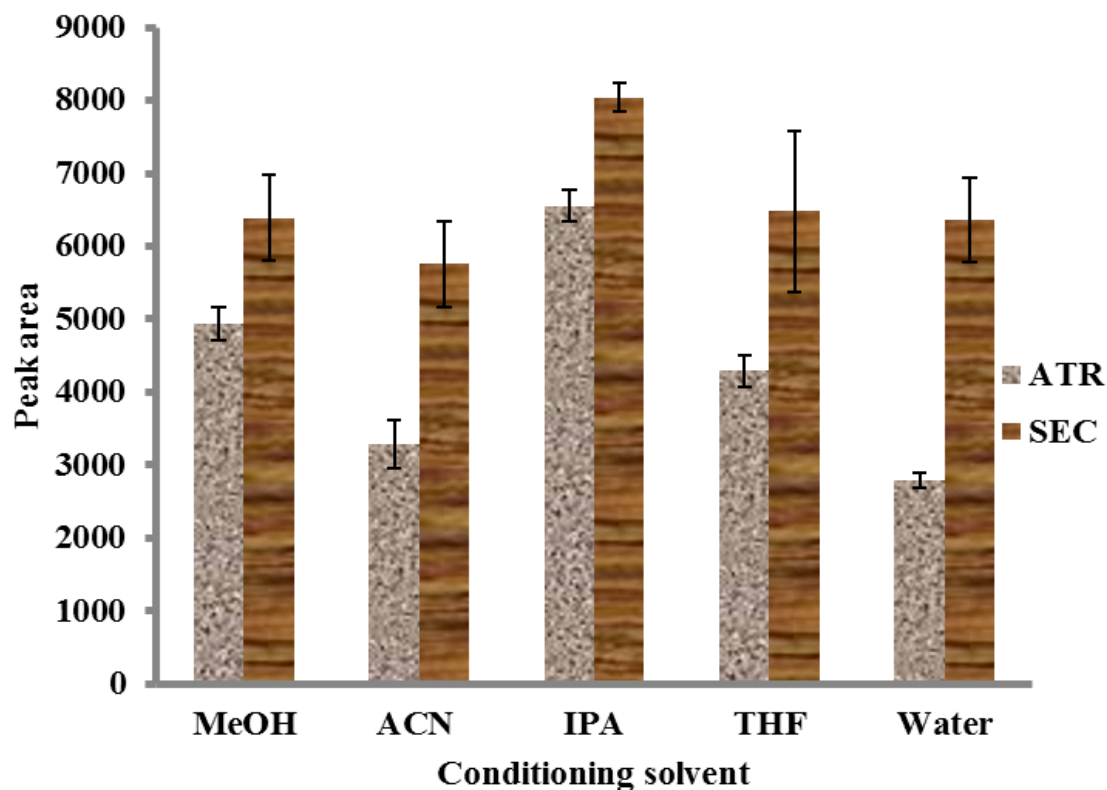


Fig. 3 Effect of conditioning solvent on MWCNTs-AG- μ SPE of triazines from water sample. Extraction conditions: $0.1 \mu\text{g mL}^{-1}$ of spiked solution; MWCNTs concentration, 0.14% (w/v); extraction time, 20 min; desorption solvent, acetonitrile ($300 \mu\text{L}$); desorption time, 10 min.

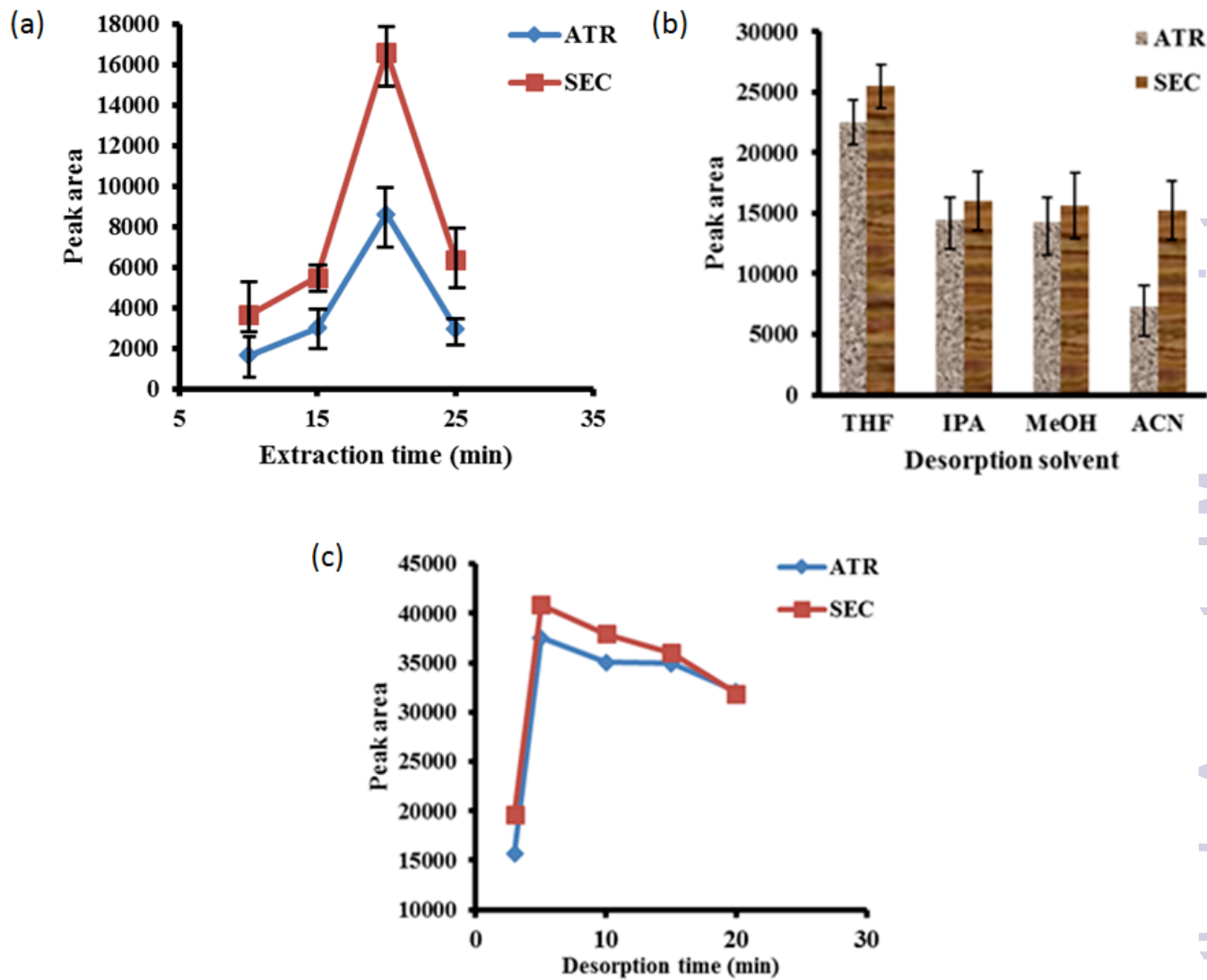


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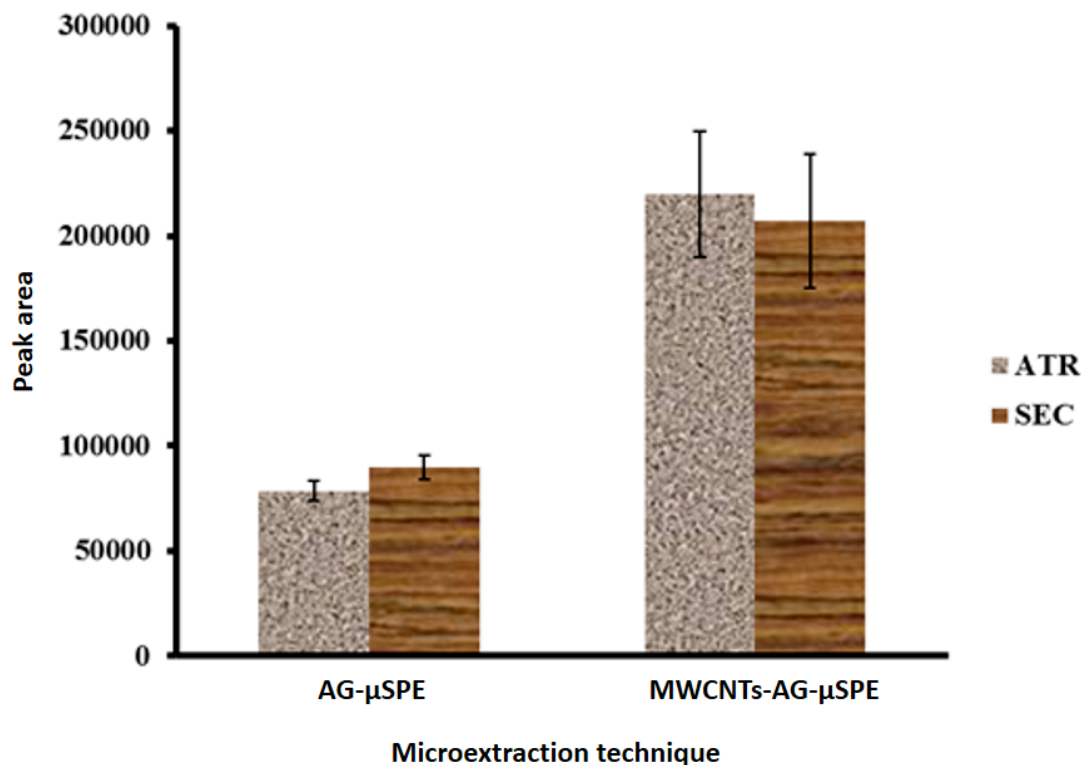


Fig. 5 Comparison between MWCNTs-AG-μSPE and AG-μSPE. Extraction conditions: 100 μg/L of spiked solution; MWCNTs concentration, 0.14% (w/v); conditioning solvent, isopropyl alcohol; extraction time, 20 min; desorption solvent, tetrahydrofuran (300 μL); desorption time, 5 min.

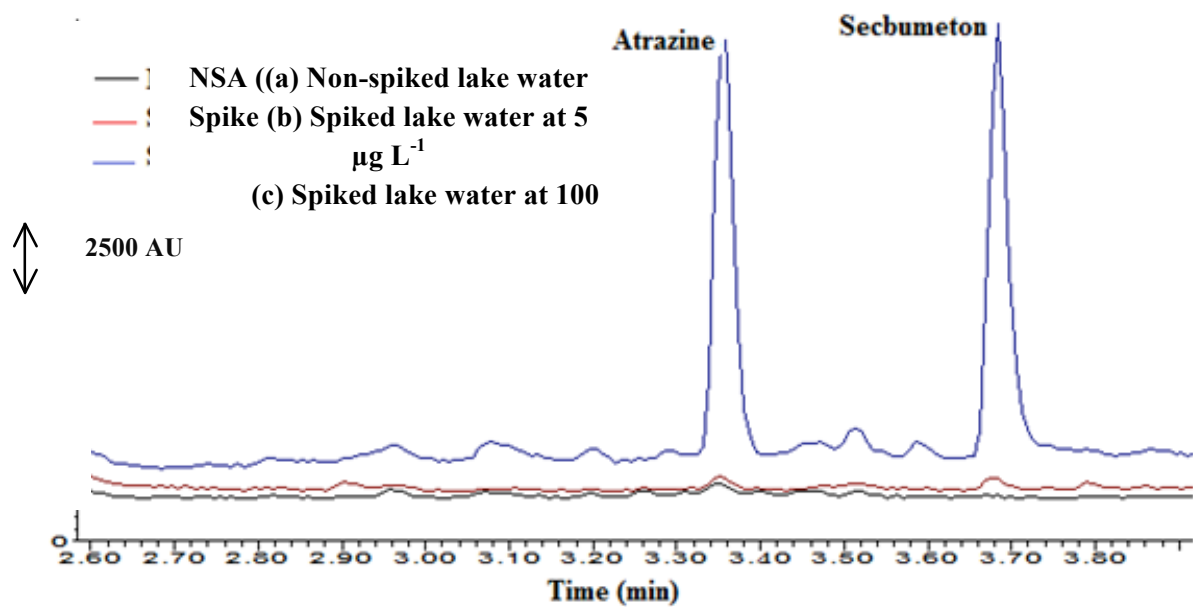


Fig. 6 Chromatogram of non-spiked lake water (a), spiked lake water at 5 $\mu\text{g L}^{-1}$ (b), and spiked lake water at 100 $\mu\text{g L}^{-1}$ (c) after MWCNTs-AG- μSPE under the optimum conditions. GC conditions: HP Ultra-2 capillary column (25 m \times 0.2 mm i.d. and 0.33 μm film thickness); temperature program, 190 $^{\circ}\text{C}$ for 1 min, raised to 210 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C min}^{-1}$ and held at 210 $^{\circ}\text{C}$ for 0 min; injection volume, 1 μL .

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Table 1 Method validation of MWCNTs-AG- μ SPE for the determination of spiked triazine herbicides in tap water samples ($n = 3$)

Triazine	Calibration range, $\mu\text{g L}^{-1}$	Coefficient of determination, r^2	LOD, $\mu\text{g L}^{-1}$	LOQ, $\mu\text{g L}^{-1}$
ATR	1-1000	0.9925	0.319	1.064
SEC	1-1000	0.9981	0.340	1.136

Table 2 Precision and accuracy study of MWCNTs-AG- μ SPE using spiked triazine herbicides in tap and lake water samples ($n = 3$).

Sample	Triazine	Average relative recoveries, % (RSD,%) $5 \mu\text{g L}^{-1}$	$100 \mu\text{g L}^{-1}$
Tap water	ATR	108 (8.42)	104 (3.52)
	SEC	99.3 (15.2)	101 (1.24)
Lake water	ATR	84.9 (15.0)	89.4 (5.20)
	SEC	99.8 (7.03)	90.1 (4.81)

Graphical Abstract

