

ORIGINAL RESEARCH PAPER

Graphene Quantum Dots/Eggshell Membrane Composite as a Nano-sorbent for Preconcentration and Determination of Organophosphorus Pesticides by High-Performance Liquid Chromatography

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ABSTRACT

In this study graphene quantum dots/eggshell membrane nanocomposite (GQDS/ESM) is prepared and used as an efficient solid-phase extraction (SPE) sorbent for preconcentration of organophosphorus pesticides (OPPs) from aqueous solutions. The retained analytes on the sorbent are stripped by acetonitrile and subsequently are determined by high-performance liquid chromatography. Various parameters affecting the extraction efficiency of OPPs on the GQDS/ESM, such as solution pH, amount of nano-sorbent, sample loading flow rate, elution conditions and sample volume are investigated. The results demonstrated that the proposed method has a wide dynamic linear range (0.05–100 ng mL⁻¹), good linearity (R²>0.997) and low detection limits (0.006–0.32 ng mL⁻¹). High enrichment factors are achieved ranging from 110 to 140. In the optimum experimental conditions, the established method is successfully applied for the determination of OPPs in spiked water samples (well, tap, shaft and canal) and apple juice. Satisfactory recovery results show that the sample matrices under consideration do not significantly affect the extraction process.

Keywords: Eggshell; Graphene Quantum Dots; Nano-Sorbent; Pesticides

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INTRODUCTION

Up to date, solid-phase extraction (SPE) has been the most popular sample pretreatment techniques due to its several advantages including high enrichment performance, low solvent-cost, easy automation over some conventional techniques such as liquid-liquid extraction and Soxhlet extraction. The choice of SPE adsorbents plays an important role in the extraction process. In recent years, many efforts have been paid to development of various materials as SPE adsorbents such as alkyl-bonded silica (C8, C18), styrene-divinylbenzene copolymers [1,2], mixed hemimicelle/admicelle derived from surfactant-coated mineral oxides [3–7], nanomaterials [8–12], and molecular imprinted

polymers [13]. Majority of SPE adsorbents mentioned above are based on chemical structure. So, it is crucial to search for economical, easily available and environmental friendly biomaterials as SPE adsorbents.

The interwoven and coalescing fiber network of the ESM and the presence of abundant functional groups such as carboxyl, hydroxyl, amino, phosphoryl, and thiol facilitate the binding of the various metal species.[14] Eggshell membrane (ESM) is a cheap, green and easily achieved a biological material which can be used as an adsorbent in SPE. It is mainly composed of a thin inner and a thick outer membrane including typical collagen protein, amino acids, saccharides and lipids

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[15, 16] with a complex lattice network composed of stable and water-insoluble protein fibers. So, it could be considered as a promising candidate for SPE adsorbent due to attractive features such as the high specific surface area, excellent chemical stability and high density of surface functional groups including amines, amides and carboxylic [17].

Graphene sheets smaller than 100 nm are called GQDS which show various electronic and optoelectronic properties due to quantum confinement and edge effects [18]. Due to the ultrahigh specific surface area [19] GQDS are more sensitive to micro-environmental changes in comparison with another carbon-based materials. So far, much effort has been paid to the biological [20, 21] and catalysis applications of GQDS [23].

Organophosphorus pesticides (OPPs) are a group of chemical compounds used for the control and elimination of insects in agriculture [23-25]. During development, neurologic effects of OPPs exposure, even at low levels, may be detrimental because neurotransmitters, including acetylcholine which play essential roles in the cellular and architectural development of the brain [26, 27].

Contamination of surface water and groundwater with hazardous compounds has attracted increasing attention in recent decades. Therefore, for the sake of human health and environmental pollution control, the determination of trace OPPs in environmental samples is of tremendous importance. According to the European Union (EU) Directive on water quality (98/83/CE) [28], the maximum admissible concentration (MAC) for pesticides is 0.1 mgL⁻¹ for each individual substance and 0.5 mgL⁻¹ is the maximum allowed for the total concentration of all organophosphorus. Development of an efficient analytical method to detect such contaminants is an important topic for environment protection.

At present, a number of methods have been used for the pre-concentration and determination of OPPs in aquatic samples like ionic liquid-based dispersive liquid-liquid microextraction [29], SPE [30], solid phase microextraction [31], single-drop microextraction [32] and cloud point extraction

[33]. However, most of these methodologies are laborious and time-consuming and low detection limits cannot be achieved. Recently, carbon-based graphene has attracted wide interest due to its high surface area, significant adsorption capacity, a variety of benzene rings and rich π - π electron arrangement [34,35].

Considering the good potential of GQDs for effective interaction with some organic molecules, and high biocompatibility, as well as the surface activity of ESM, resulted developing GQDs/ESM nanocomposite as a novel adsorbent for SPE. The goal of this study is to investigate the performance of GQDs/ESM for preconcentration and determination of OPPs in water samples and fruit juices using an off-line SPE followed by reversed phase HPLC-UV method.

EXPERIMENTAL

Instrumentation

A Jasco HPLC system consisted of a PU-1580 isocratic pump, a Rheodyne 7725i injector with a 10 μ L loop (Rheodyne, Cotati, CA, USA) and a UV-1575 spectrophotometric detector was used in the experiment. The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface. The data were processed using BORWIN software (version 1.50). An analytical 250 mm \times 4.6 mm ID, 5 μ m particle, Perfectsil Target ODS-3 column (MZ-Analysentechnik, Germany) with a ODS-3 pre column (10 \times 4.0 mm I.D., 5 μ m), which was maintained at ambient temperature, was employed for separation. Scanning electron microscopy images were obtained using an S-4800 field emission scanning electron microscope (FESEM) (Hitachi, Tokyo, Japan).

Chemicals and water samples

Pesticide analytical standards include malathion (Mala), diazinon (Diaz), phosalone (Phos) and chlorpyrifos (Chlor) were provided by Fluka (Germany) (Table 1 shows their structures and IUPAC names). All pesticide standards were of

Table 1: Results of regression analysis on calibration curves, linear range, precision, enrichment factors, LODs and LOQs

Analytes	Regression equation	LR ^a (ng mL ⁻¹)	r ²	LOD ^b (pg mL ⁻¹)	LOQ ^c (pg mL ⁻¹)	RSD(%) (n=3, 20 ppb)	EF ^d
Malathion	Y=1363X+1710	0.1-50	0.999	32.0	106.7	4.2	110
Diazinon	Y=16515X+7060	0.05-100	0.999	6.0	20	5.6	125
Phosalone	Y=4545X+12831	0.05-100	0.997	24.0	80.0	6.3	140
Chlorpyrifos	Y=3052X+46681	0.3-50	0.999	8.0	27.0	6.8	135

^a Linear range; ^b Limits of detection (S/N=3); ^c Limits of quantification (S/N= 10); ^d Enrichment factor

97.7 – 99.5% purity. Standard solutions of each compound at a concentration of 100 mg L⁻¹ were prepared in methanol and stored at 4 °C. Methanol LC-grade from Merck (Darmstadt, Germany) was used for standard preparation and chromatographic analysis. Sodium hydroxide and hydrochloric acid used for pH adjustment and sodium chloride used for ionic strength studies which were purchased from Merck (Darmstadt, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). Graphene oxide was purchased from Azar Kimia Nanotechnology Co., Tabriz, Iran, which was synthesized according to modified Hummer's method [36].

The recovery studies were carried out using tap water, shaft water, well water, canal water, and apple juice samples. Tap water sample was collected freshly from our laboratory (Azarbaijan Shahid Madani University, Tabriz, Iran) and well water came from Khoshakan deep groundwater in Khoy (Iran). Canal water (Dizaj, Khoy, Iran) was picked up a few days before analysis. Shaft water sample was collected from Gheshlagh (West Azarbaijan Province, Iran) and apple juice was from SUN ICH (Tehran, Iran). All water samples were collected in brown bottles, stored in the dark place at 4 °C until analysis.

Fresh hen eggs were obtained from a local supermarket. The eggs albumen and yolk were removed and the broken eggshells were immersed in 1% acetic acid solution for 30 min in order to obtain the ESM easily [37]. Then it was washed with adequate ultra-pure water.

Synthesis of graphene quantum dots (GQDs)

In this study, the hydrothermal method was used for the synthesis of GQDs from graphene oxide as precursor [38]. The graphene oxide was deoxidized in a tube furnace at 250 °C for 2 h by a heating rate of 5 °C min⁻¹ in a nitrogen atmosphere. The obtained graphene sheets were oxidized in concentrated H₂SO₄ (10 mL) and HNO₃ (30 mL) for 15 h under mild ultrasonication (500 W, 40 kHz). The oxidized graphene sheets were diluted and purified with microporous membrane and re-dispersed in deionized water. Then the suspension was heated at 200 °C for 10h in an autoclave. The resulting black suspension was filtered with microporous membrane and a brown filter solution was obtained. To remove larger graphene nanoparticles, the colloidal solution was dialyzed in a dialysis bag (retained molecular weight: 3500 Da) overnight and GQDs were obtained having stability for more than 3 months.

Preparation of GQDs/ESM nanocomposite

Firstly, the broken fresh eggshell was incubated in diluted 1% acetic acid at 25°C for 30 min. Afterward, the ESM was easily separated and cleaned with a copious amount of twice distilled water. Then, the ESM was immersed in GQDs solution (5.0 mg mL⁻¹) and placed in the ultrasonic bath for 30 min. After changing the color of ESM from purple to brown in the presence of GQDs, the constructed nanocomposite (GQDs/ESM) was immersed in ultra-pure water for 20 min to remove the excess of GQDs from the nanocomposite[37]

A digitally controlled peristaltic pump (Azarkim, peristaltic pump Model 120, supplied by Azar Kimia Nanotechnology Co., Tabriz, Iran) was used to pass the analyte samples and solvents through the column containing GQDs/ESM nano-sorbent at a controlled flow rate.

Column preparation

GQDs/ESM was employed to create the solid-phase extraction column as follows: the column was prepared by introducing 100 mg of GQDs/ESM into an empty polypropylene cartridge using the dry packing method. Two polypropylene frits were situated on the both side of the sorbent in order to prevent it from washing out. Before loading the sample, the column was cleaned with 1 mL of eluent solution and conditioned by passing 2 mL of deionized water through the column prior to each use. Then, a 150.0 mL portion of the sample solution was passed through the column at a flow rate of 2.0 mL min⁻¹ by using the peristaltic pump.

General procedure

Aliquots of 150.0 mL sample or aqueous standard solution containing OPPs with a concentration of 100 ng mL⁻¹ for each analyte (pH = 9) was passed through the GQDs/ESM nano-sorbent in a column at a flow rate of 2.0 mL min⁻¹. After loading, the retained analytes on the column was stripped by acetonitrile, which acts as eluent. The retained OPPs on the sorbent was completely eluted by 1 mL acetonitrile. The concentration of eluted analyte was determined by HPLC-UV method.

Chromatographic conditions

The isocratic mobile phase consisted of methanol-phosphate buffer (25 mM) (pH=4) in the ratio of 80:20 v/v, flowing through the column at a flow rate of 1 mL min⁻¹ [39]. The eluent was monitored using UV detection at a wavelength 225

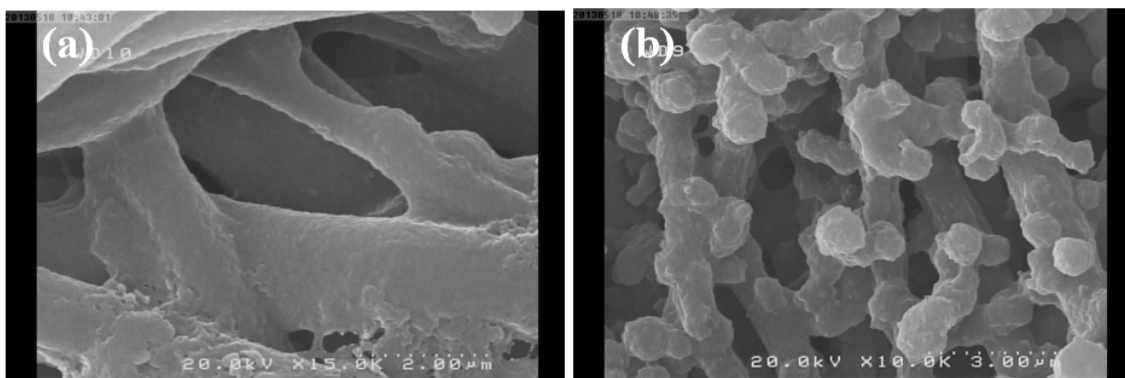


Fig. 1: FESEM images of ESM (A) and GQDs doped ESM (B)

nm. The mobile phase was filtered through a 0.22 mm membrane-type GV filter (Millipore). A 40 kHz and 138 W ultrasonic bath water at controlled temperature (bath model LBS2-FALC instruments, Italy) was applied to degassing the mobile phase.

RESULTS AND DISCUSSION

Characterization of nano-sorbent (GQDs/ESM)

Fig. 1 shows FESEM images of typical surface regions of ESM (A) and GQDs/ESM (B), obtained at 15000 and 10000 magnifications respectively. ESM has an intricate lattice network composed of highly cross-linked protein fibers. This membrane is composed of interlinked and coalescing fibers ranging from 1 to 3 μm in diameter, and micropores about 5-10 μm in size. The structure of ESM allows facile incorporation of GQDs into the semipermeable ESM which results in a homogeneous bionanocomposite throughout the whole membrane. Fig. 1B shows the FESEM image of GQDs/ESM, representing that GQDs appropriately doped in ESM protein fibers. It can be seen that piecemeal GQDs sheets on the ESM fibers were constructed, and a uniform and three-dimensional GQDs film was formed. The porous and layered structure of GQDs/ESM and uniform distribution of GQDsparticles on ESM fibers is beneficial to the adsorption of the analyte on sorbent as well as easy desorption of retained analyte by an eluent solvent due to effective contacts.

Optimization of SPE conditions

Comparative study of ESM and GQDs/ESM as SPE adsorbent

Fig. 2 shows the extraction efficiencies of analytes with ESM and GQDs/ESM adsorbents prepared at same conditions. As can be seen, ESM shows low efficiency in the extraction of OPPs. The presence

of graphene sheets in GQDs/ESM composite increased the extraction efficiency. This promising result can be related to inherent characteristics of GQDs such as high surface to the volume ratio, high capacity of sorbent, the presence of small sheets that have π - π interactions and the presence of many hydroxyl functional groups resulting the polarity of GQDs.

Effect of pH

The effect of pH on the retention of OPPs was investigated by applying the proposed extraction and elution procedure to the sample solutions. The pH of each solution was adjusted to values ranging from 4 to 12 with a minimum volume of 0.01 mol L⁻¹ HNO₃ and/or NaOH. It was found that the recovery

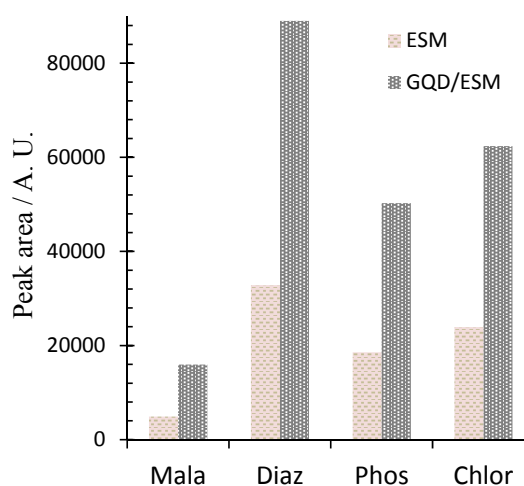


Fig. 2: Comparative chromatographic responses observed for OPPs after preconcentration by ESM and GQDs/ESM as sorbents. Conditions: adsorbent amount: 100 mg; sample volume: 150 mL; concentration of each analyte: 100 ng mL⁻¹; flow rate: 2 mL min⁻¹; desorption solvent: acetonitrile; NaCl concentration: 200 mM; pH = 9

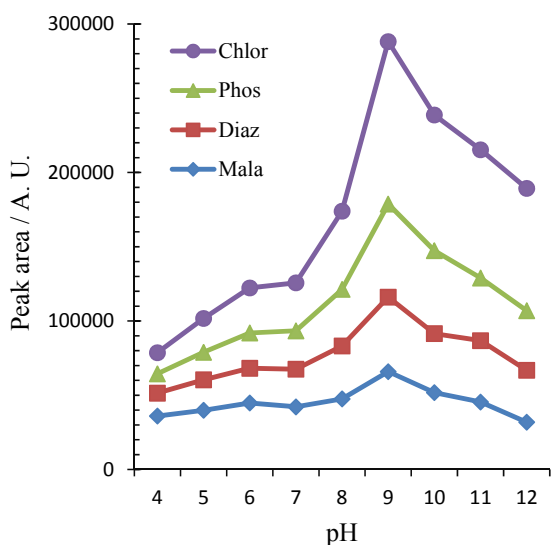


Fig. 3: Effect of solution pH on the extraction efficiency of OPPs by GQDs/ESM nano-sorbent. Conditions: adsorbent amount: 150 mg; sample volume: 150 mL; concentrations of each analyte: 100 ng mL⁻¹; flow rate: 2 mL min⁻¹; desorption solvent: acetonitrile; no salt addition

values increased as the pH increased to 9, and turned off when the solution pH exceeded 9 (Fig. 3). At low pH, a decrease in extraction efficiency may be due to the repulsion between the positive surface of adsorbent and positive ions on OPPs. On the other hand at higher pH, the deprotonation of the phenolic hydroxyl groups of graphene moiety in the graphene-based sorbent converts them into negatively charged phenoxide ions. Following the deprotonation as well as due to the abundance of

OH⁻ ions, extraction efficiency of OPPs decreases because selected OPPs already contains anionic sites such as on Cl, O and S. The dramatically decreased in peak area ratio of OPPs at higher pH (pH 9-12) is due to the fact that pesticides undergo hydrolysis rather than sorption. Therefore, pH 9 was selected as the working pH. We did not use any buffer solution for pH adjusting, because this may affect the retention of OPPs due to the competition of the anionic species for bonding to active sites on the nano-sorbent.

Effect of GQDs/ESM amount

To test the effect of the amount of nano-sorbent on the quantitative retention of pesticides, the extraction was conducted by varying the amounts of the GQDs/ESM from 50 to 250 mg. The results indicated that the quantitative recovery values of OPPs were obtained by using 100 mg of nano-sorbent. Therefore, 100 mg of nano-sorbent was employed in this work.

Effect of sample loading flow rate

The influence of the sample loading flow rate on the recovery was investigated between 1.0 and 9.0 mL min⁻¹. As can be seen in Fig. 4, maximum recovery values for all the analytes were observed at flow rate 2.0 mL min⁻¹. However, higher flow rates led to a continuous decrease in the recovery values as the interaction time between the analytes and the sorbent was decreased. Thus, the loading flow rate of 2.0 mL min⁻¹ was selected for further experiments.

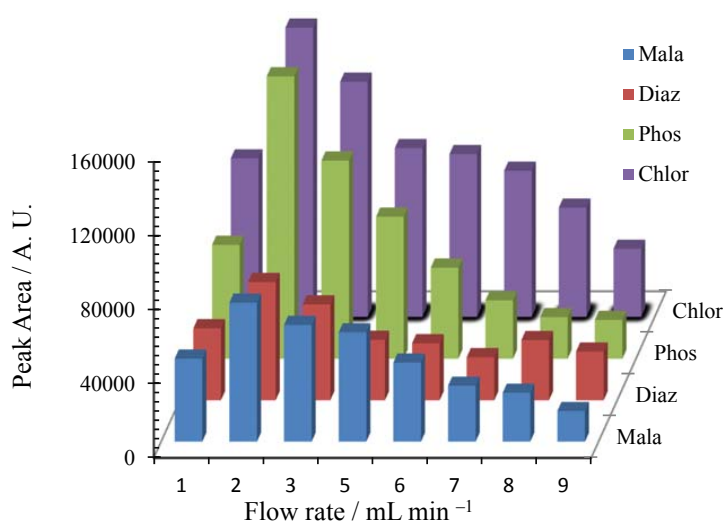


Fig. 4: Effect of sample loading flow rate on the extraction efficiency. Other conditions were as in Fig. 3

Effect of ionic strength

Generally, addition of salt decreases the solubility of analytes in aqueous samples and enhances their partitioning into the adsorbent or organic phases. For investigating the influence of the ionic strength on the extraction performance, several experiments were performed by adding varying NaCl amounts from 0 to 300 mM. The results demonstrated an improvement in the extraction efficiency for all the analytes when NaCl amount increased up to 200 mM. Increasing NaCl amount more than 200 mM causes a small decrease in the extraction efficiency. Therefore, 200 mM NaCl was used in further experiments (Fig. 5).

Type and volume of eluent solvent

The eluent reagent and its volume should be carefully taken into account as major parameters. For this reason, various stripping reagents such as methanol, ethanol, acetonitrile, acetone, and n-hexane were tested to find the best stripping solution for the retained OPPs. Among these reagents, acetonitrile provided higher recovery values. It was found that 1.0 mL was sufficient for eluting all the analytes from the column completely.

Sample volume and pre-concentration factor

The effect of sample solution volume on the OPPs loading on the sorbent was investigated by passing 50 - 300 mL sample solutions spiked with the fixed amount of each analyte (2.5 mg) at a flow rate 2.0 mL min^{-1} according to the recommended procedure. Recovery of OPPs was found to be quantitative when sample volume was chosen in the range 50.0 - 150.0 mL. For sample solution larger than 150.0 mL, the recovery decreased for

all analytes. Considering 150.0 mL as aqueous sample volume which was preconcentrated in 1.0 mL acetonitrile and analyzed by using HPLC-UV at 225 nm, the values of enrichment factors for malathion, diazinon, phosalone, and chlorpyrifos were calculated as 110, 125, 140 and 135, respectively.

Validation of the method

Under optimized conditions, analytical features of the proposed method such as linear range (LR), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ), enrichment factor (EF) and precision were examined (Table 1). To obtain the precision of the method, replicated analysis of spiked water samples were carried out for three times, and relative standard deviation (RSD) values were calculated by the obtained peak area of each analyte. All the analytes exhibited good linearity with the coefficient of determinations ranging from 0.997 - 0.999. The LODs values based on signal-to-noise ratio (S/N) of 3, were $6.0 - 32.0 \text{ pg mL}^{-1}$, and the LOQs, based on signal-to-noise ratio (S/N) of 10, were $20 - 160.7 \text{ pg mL}^{-1}$. The LOD values easily and conveniently meet the MAC of 0.1 ng mL^{-1} for one organophosphorus pesticide and 0.5 ng mL^{-1} for the total concentration of these pesticides in drinking water set by EU.

Application to real samples

To test the reliability of the proposed procedure, the method was employed for the analysis of four environmental water samples (tap, well, shaft and canal) and apple juice and the results were shown in Table 2. Results of initial analysis confirmed that they were free of target analytes. The accuracy of the presented method was evaluated by the

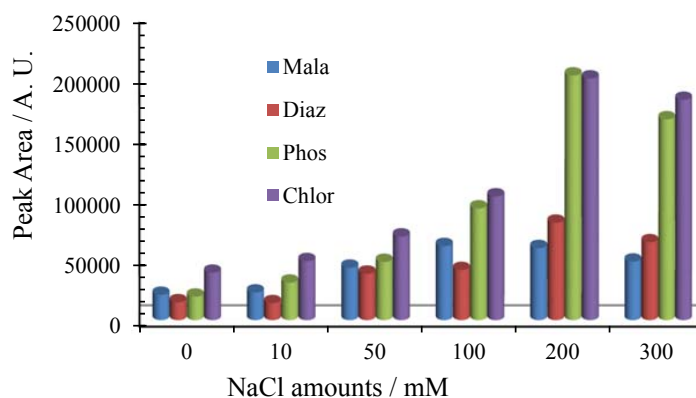


Fig. 5: Effect of ionic strength (NaCl/mM) on the extraction efficiency. Other extraction conditions were as in Fig. 3

Table 2: Results of determination and recoveries of various real samples spiked with OPPs.

Samples		Mala	Diaz	Phosa	Chlor
Well water	Initial	ND ^a	ND ^a	ND ^a	ND ^a
	Found ^b	4.83	3.02	5.08	3.08
	RR ^{c,d} % (RSD%) ^e	96.6(4.1)	100.7(5.1)	101.6(2.1)	102.7(4.8)
Tap water	Initial	ND	ND	ND	ND
	Found ^b	4.86	2.95	4.89	2.95
	RR ^{c,d} % (RSD%) ^e	97.2(4.4)	98.3(3.7)	97.8(3.4)	98.3(3.3)
Shaft water	Initial	ND	ND	ND	ND
	Found ^b	5.032	2.89	5.21	3.09
	RR ^{c,d} % (RSD%) ^e	100.6(3.9)	96.3(5.4)	104.2(5.1)	103.0(3.4)
Canal water	Initial	ND	ND	ND	ND
	Found ^b	5.01	2.92	5.13	3.05
	RR ^{c,d} % (RSD%) ^e	100.2(4.2)	97.3(4.1)	102.6(3.9)	101.7(3.9)
Apple juice	Initial	ND	ND	ND	ND
	Found ^b	4.82	2.96	4.91	2.87
	RR ^{c,d} % (RSD%) ^e	96.4(5.3)	98.7(2.9)	98.2(5.1)	95.6(4.9)

^aNot detected.; ^bSpiked concentrations of Mala, Diaz, Phos and Chlor are 5, 3, 5, 3 ng mL⁻¹, respectively.;

^cRelativercovery.; ^dPercentage ratio of the found and spiked concentrations; ^eObtained for three determinations.

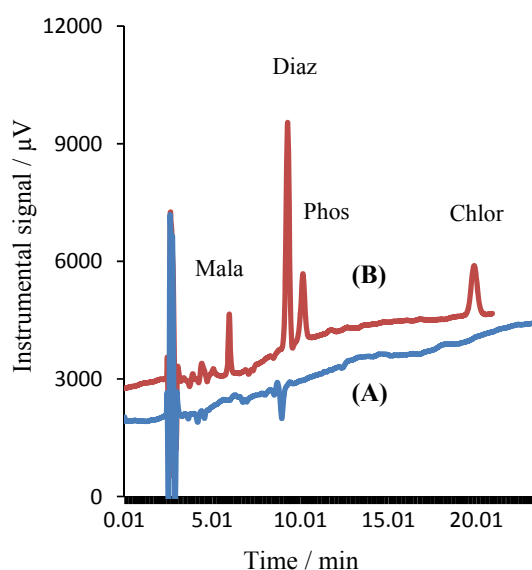


Fig. 6: SPE-HPLC-UV chromatograms: (A) Well water sample; (B) Well water sample spiked with 5 ng mL⁻¹ Phos, 5 ng mL⁻¹ Mala, 2 ng mL⁻¹ Chlo and 2 ng mL⁻¹ Diaz obtained for the target analytes using the proposed method

recovery test carried out with spiked samples. Relative recoveries (RR%) for the analysis of OPPs in spiked samples using the proposed method based on three replicate extractions and determinations are shown in Table 2, which indicated that the recoveries for the four OPPs were in the range 82.0% – 113.3% with R.S.D.s between 2.1% and 6.3%. Typical chromatograms of spiked water samples were shown in Fig. 6.

CONCLUSIONS

In this study, the prepared GQDs/ESM nanocomposite has shown excellent potential for use as a new SPE sorbent for OPPs extraction from aqueous solution. GQDs/ESM is a good choice to use for separation and preconcentration of pollutants in aqueous samples due to its low cost compared with commercially available sorbents. It can be concluded that the coupling of novel GQDs/ESM as SPE nano-sorbent with HPLC method can exhibit excellent selectivity, repeatability, and ease of operation in optimum conditions. The method can be successfully applied to determine OPPs in water samples and fruit juices.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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