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Electronic supplementary materials

Conjunction of a new ultrasonic assisted dispersive soild phase extraction method with HPLC-DAD for trace determination of diazinon in biological and water media

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2.1 Reagents and materials

All reagents with analytical grade were prepared from Merck (Darmstadt, Germany, www.merck.de) and used without subsequent purification. A stock solution (1000.0 mg L^{-1}) of diazinon (degrees of purity were >98%) was prepared by dissolving an appropriate amount of diazinon in ethanol. The applied standard solutions for extraction procedures were prepared daily via serial dilutions of the stock solution with deionized water. Ammonia solution (25%), isopropanol, methanol, hydrochloric acid (HCl) (37%), sodium hydroxide, sodium chloride, tetra isopropyl titanate, graphite powder (325 mesh), potassium permanganate ($KMnO_4$), concentrated sulfuric acid (H_2SO_4) and phosphorus pentoxide (P_2O_5) was purchased from Merck Company (Darmstadt, Germany). Acetone, $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ was purchased from Carlo-Erba (Via R Merendi, 22, 20010 Cornaredo MI, Italy). Hydrogen peroxide (H_2O_2) was purchased from Chem-Lab (De Arend 2, 8210 Zedelgem, Belgium).

2.2. Apparatus

Diazinon was analyzed using an Agilent 1260 liquid chromatograph equipped with a diode array detector (DAD). The separation column used was an Agilent Eclipse-XDB-C18 analytical column (250 mm × 4.6 mm, 5 µm). The mobile phase was methanol/water (50/50) and the flow rate was 1 mL min^{-1}. Injection volume of solution was 20 µL. The pH was measured at 25 ± 1 ºC with a digital Metrohm 827 Ion analyzer (Herisau, Switzerland) equipped with a combined glass-calomel electrode.

2.3. Sample pretreatment of studied biological samples

For analysis of plasma and milk samples, 5.0 mL of these samples were spiked with diazinon at the desired concentration levels and then acidified with 200.0 µL hydrochloric acid (37.0%) to disturb the molecules protein bindings. Then, 150.0 µL trichloroacetic acid (TCA) was added to denature the proteins [1]. These processes eventually led to precipitation of

proteins. Subsequently, the samples were centrifuged at 5000 rpm for 10.0 min. An amount of 3.5 mL of the supernatant was transferred to the centrifuge glass vial and diluted to 15.0 mL with ultrapure water by using 10.0 mL pipette. For urine sample, 5.0 mL spiked urine were centrifuged at 5000 rpm for 10.0 min and then diluted to 15.0 mL with ultrapure water.

2.5. Synthesis of the sorbent

2.5.1. Synthesis of graphene-oxide

Graphene-oxide (GO) was prepared using Hummer's method. Graphite powder (1.00 g, 325 mesh) and H_2SO_4 (95%) (23 mL) were added to a 250 mL conical flask and the mixture was stirred with a magnetic bar. Sodium nitrate (0.50 g) was added and the resulting mixture was cooled to 0 °C. Under vigorous agitation, $KMnO_4$ (3.00 g) was added slowly and the mixture was stirred for 1h while the temperature was kept below 35 °C. Then, H_2O (45 mL) was added slowly to the reaction mixture and the solution was stirred for 30 min at 90 °C. Next, H_2O_2 (10 mL of a 30% solution) and deionized water (140 mL) were added to the mixture. Then resulting precipitate was centrifuged and washed repeatedly with HCl (5%, 3 × 15 mL) and ethanol. The mixture was then vacuum dried at 60 ^{o}C. The GO was obtained as a brown powder.

2.5.2. Synthesis of magnetic graphene oxide

To obtain magnetic graphene oxide, at first 1.0 g of GO was added to 200 mL deionized water and then dispersed using stirring for 1 h. Then, under vigorous stirring 2.0 g $FeCl_2.4H_2O$ and 5.2 g $FeCl_3.6H_2O$ with molar ratio of Fe^{3+}:Fe^{2+}= 2:1 was added to the solution under Ar atmosphere. After that, 15 mL of ammonia solution (%25) was added to the suspension at the 80 ºC. After 15 min the solid was separated by a magnet and washed three times with 0.1 mol L^{-1} NaCl solution.

3.5.3. Synthesis of TiO_2/magnetic graphene

To prepare TiO_2/magnetic graphene, magnetic graphene oxide was first synthesized and the resulting nano-particles were used as seed for the TiO_2 coating. For the TiO_2 coating, 1 g of nanosized magnetic graphene oxide were dispersed in 60 mL of isopropanol and stirred for 1h. Then the dispersion was transferred to an erlenmeyer flask where 70 mL of distilled water (Milli-Q, Millipore System) and 200 mL of isopropanol were added and the mixture was stirred for 30 min. Next, 7 mL of tetra isopropyl titanate was added and the final reaction mixture was stirred for 10 h. Temperature was controlled below 35°C during stirring steps. The resulting catalytic particles were separated magnetically, washed three times with acetone and dried overnight in an oven at 60 °C. The prepared magnetic nanosorbent were calcined at 400 °C for 2 h to form TiO_2 in mixed phase (anatase and rutile phase) and reduce GO to graphene.

3.1. Characterization

The XRD patterns of the Fe_3O_4, magnetized graphene oxide and TiO_2/ magnetic graphene are shown in Figure 1S (ESM). For Fe_3O_4, There are six characteristic peaks occur at 2θ of 30.2, 35.6, 43.2, 53.7, 57.2 and 62.8, which correspond to indices (220), (311), (400), (422), (511) and (440), respectively well indexed to the typical cubic inverse spinel structure (JCPDS card No .19-629). This revealed that Fe_3O_4, magnetic graphene oxide, TiO_2 / magnetic graphene were composed of Fe_3O_4, and the Fe_3O_4 nanoparticles remained unchanged after each functionalization step (no decomposition or converting to Fe_2O_3). No graphene peaks are found in the magnetized graphene oxide and the observation is related to the disordered of distance between graphene sheets after the attachment of Fe_3O_4 particles [2].

The XRD patterns of TiO_2/magnetic graphene show peaks on 2θ = 25.4, 37.59, 38.75, 48.21, 55.23, 62.89, 68.28, 70.03, and 76.61. The XRD patterns of TiO_2/magnetic graphene display the presence of both anatase and rutile phase in the structure. On the other hand, no

diffraction peak of GO was distinguished in the XRD patterns of the TiO_2/magnetic graphene, suggesting that the GO was reduced to graphene during the calcination process.

Energy dispersive X-ray (EDX) spectrum was used for elemental analysis of synthesized magnetized nanosorbent. EDX of TiO_2/magnetic graphene (Figure 2S (ESM)) revealed the existence of Fe, C, O, and Ti ,which exhibited the existence of TiO_2 and graphene nanosheets.

Elemental mapping of C, O, Ti and Fe in synthesized materials after each step was given in Figure 3S (ESM). Elemental mapping of TiO_2/magnetic graphene revealed that TiO_2 nanoparticles dispersed uniformly on graphene nanosheets. On the other hand, the presence of C, O, Ti and Fe on the elemental mapping of magnetized nanosorbent can prove the successful synthesis of TiO_2/magnetic graphene.

To further characterize of the prepared sorbent, FT-IR spectrum was measured. In the FT-IR spectrum of magnetic graphene oxide, the peak at 1740 cm^{-1} could be ascribed to the C=O stretching modes of graphene oxide. Furthermore, the IR absorption at 1059 cm^{-1}, 1390 and 1640 cm^{-1} are ascribed to alkoxy C-O, C-OH stretching vibration and C=C skeleton vibration, respectively. The analysis of surface oxygen groups of TiO_2/magnetic graphene showed that the intensities of peaks of surface oxygen functional groups (C-O and C-OH) decrease and C=O band disappears and the observation ascribed to the reduction of graphene oxide during the hydrothermal process. Furthermore, in the IR spectrum of TiO_2/magnetic graphene the broad IR bands around 600-800 cm^{-1} are attributed to the stretching vibration of Ti-O-Ti bonds in TiO_2 [3].

3.2.2 Amount of sorbent

In UA-d-SPE, the amount of sorbent used is closely correlated with the degree of adsorption of analytes. To test this, the influence of the sorbent quantity on the extraction recovery of diazinon was evaluated over the span of 30-70 mg. As shown in Figure 4S (ESM), the mean

extraction recovery for all the analytes increased with an increase in the amount of sorbent from 30 up to 50 mg, and thereafter leveled off. This could be explained by the fact that by increasing the amount of sorbent the area required for the adsorption of the analytes is increased up to a certain level (i.e., 50 mg). Nevertheless, a further rise in the sorbent amount led to a relatively high consumption of desorption solvent, which in turn, reduced the analytes enrichment. As can be deducted from Figure 4S (ESM), the application of 50 mg sorbent provided the satisfactory extraction recovery for the target analytes. Thus, 50 mg was considered to be the optimum for the next studies.

3.2.5. Elution studies

Following the adsorption, the implication of a proper organic solvent for the elution of the analytes from the sorbent is an important factor in a UA-d-SPE. As the adsorption of diazinon onto the sorbent is based on π-π and hydrophobic interactions, the various elution solvents of different polarities were considered (methanol, acetone and acetonitrile) to break down the above interactions. Amongst the mentioned three solvents, acetonitrile provided the highest desorption efficiency followed by methanol and acetone as indicated in Figure 5S (ESM). It should be noted that 1 ml of each solvent were tested in the elution studies. Meanwhile, elution solvents was subjected to ultrasonic vibration for 2 min. Therefore, 1 ml of acetonitrile was applied as the optimum elution solvent in the following experiments.

3.3. Reusability of the sorbent

In a further development, to demonstrate the reusability and recyclability of the sorbent, the recovery of diazinon adsorbed onto the sorbent was investigated using the elution solvent (acetonitrile). The sorbent recovery over four continuous cycles of reuse was tested. It was found out that a large fraction of the adsorbed diazinon (>90%, at 50 µg/l) could be recovered under the optimized condition (data not shown). While, a low recovery loss (within 10%) for

the analytes was observed for the reused sorbent after 4 cycles, both of which, indicate good reusability of the applied sorbent.

References:

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[3] Y. Yang, Y. Li, J. Wang, J .Wu, D .He (2017) Graphene-TiO 2 mesoporous spheres assembled by anatase and rutile nanowires for efficient NO photooxidation. Q An J Alloys Compd 699: 47-56.

Table 1S: A comparison between extraction efficiency of the sorbent, pure titanium dioxide and pure hydrophobic sorbent (like polystyrene-divinyl benzene) at optimized conditions. (It should be mentioned that the particle size of TiO_2 nanoparticles is about 150 nm and the particle size of polystyrene-divinyl benzene is about 200 nm. Furthermore the amount of applied sorbents for the experiment was 50 mg)

Sorbent	Extraction recovery (%) after 5 min	Adsorption capacity (mg g^{-1})
TiO_2/magnetic graphene	> 99%	22.4 mg g^{-1}
Pure TiO_2	45%	6.1 mg g^{-1}
Polystyrene-divinyl benzene	65%	12.9 mg g^{-1}

Figure 1S. The XRD patterns of magnetic nanoparticle, magnetic GO and TiO_2/magnetic graphene.

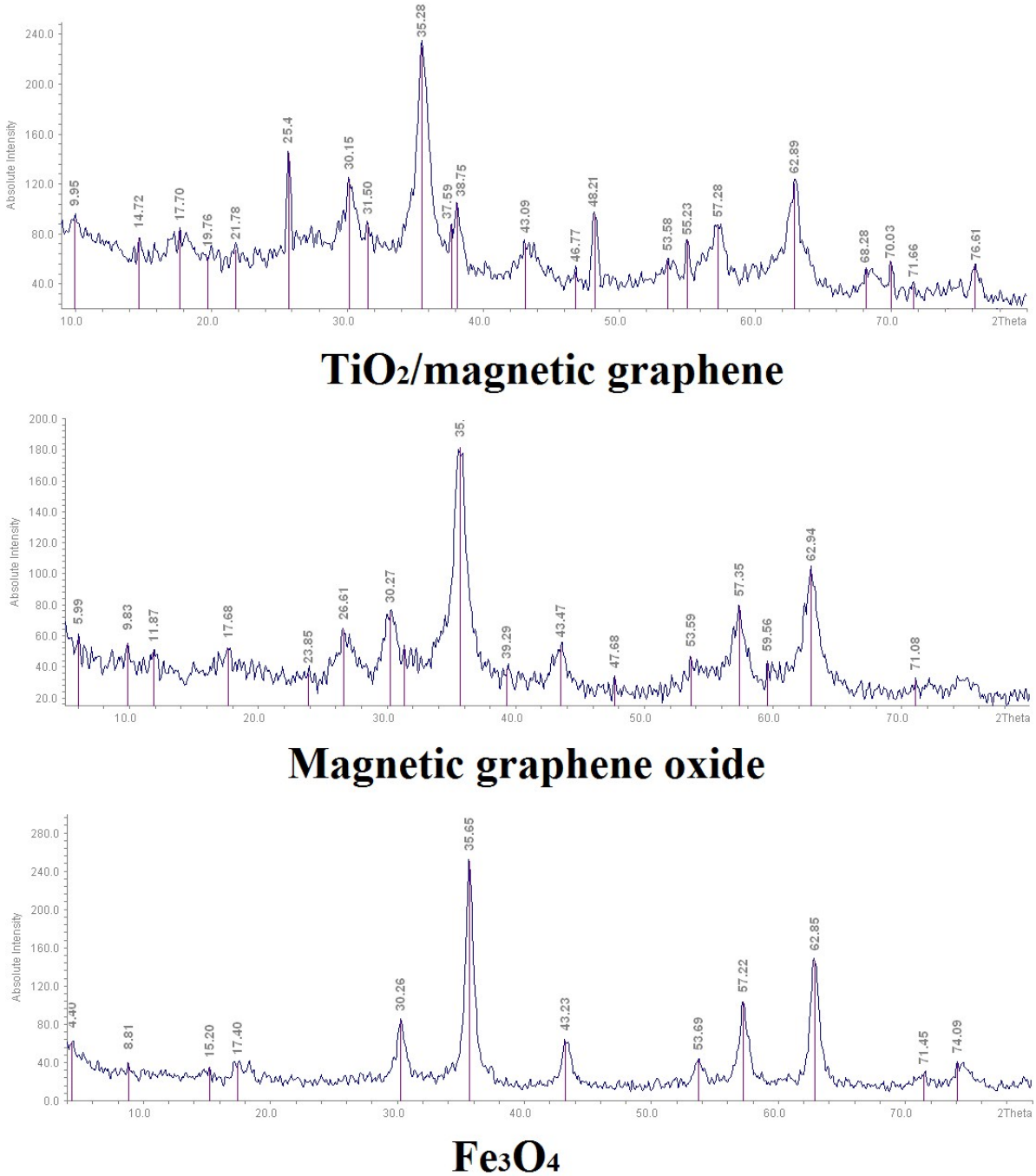

Figure 2S. Energy-dispersive X-ray spectroscopy analysis of magnetic nanoparticle, magnetic graphene oxide and TiO_2/magnetic graphene.

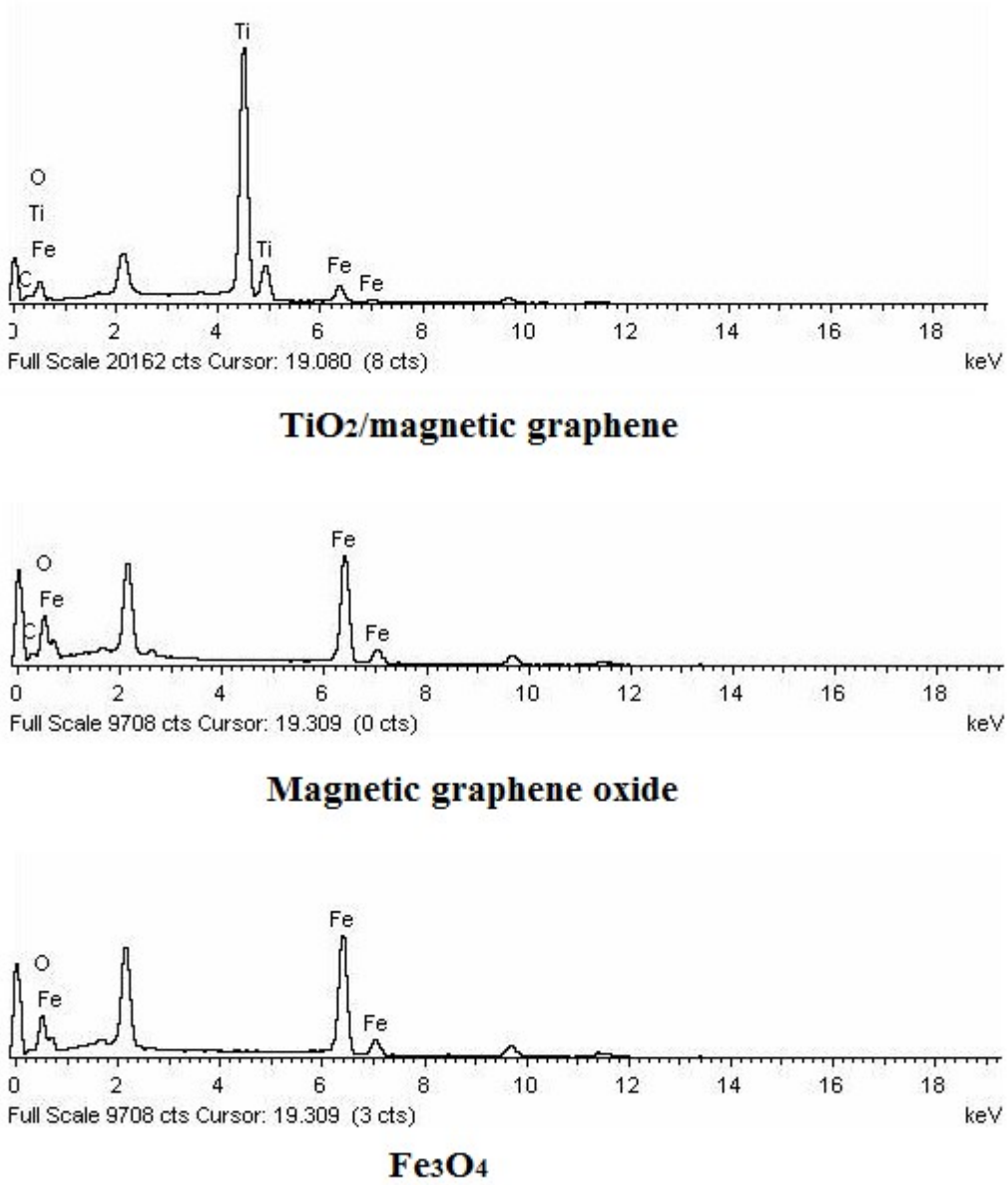

Figure 3S. Elemental mapping studies of magnetic nanoparticle, magnetic GO and TiO_2/magnetic graphene.

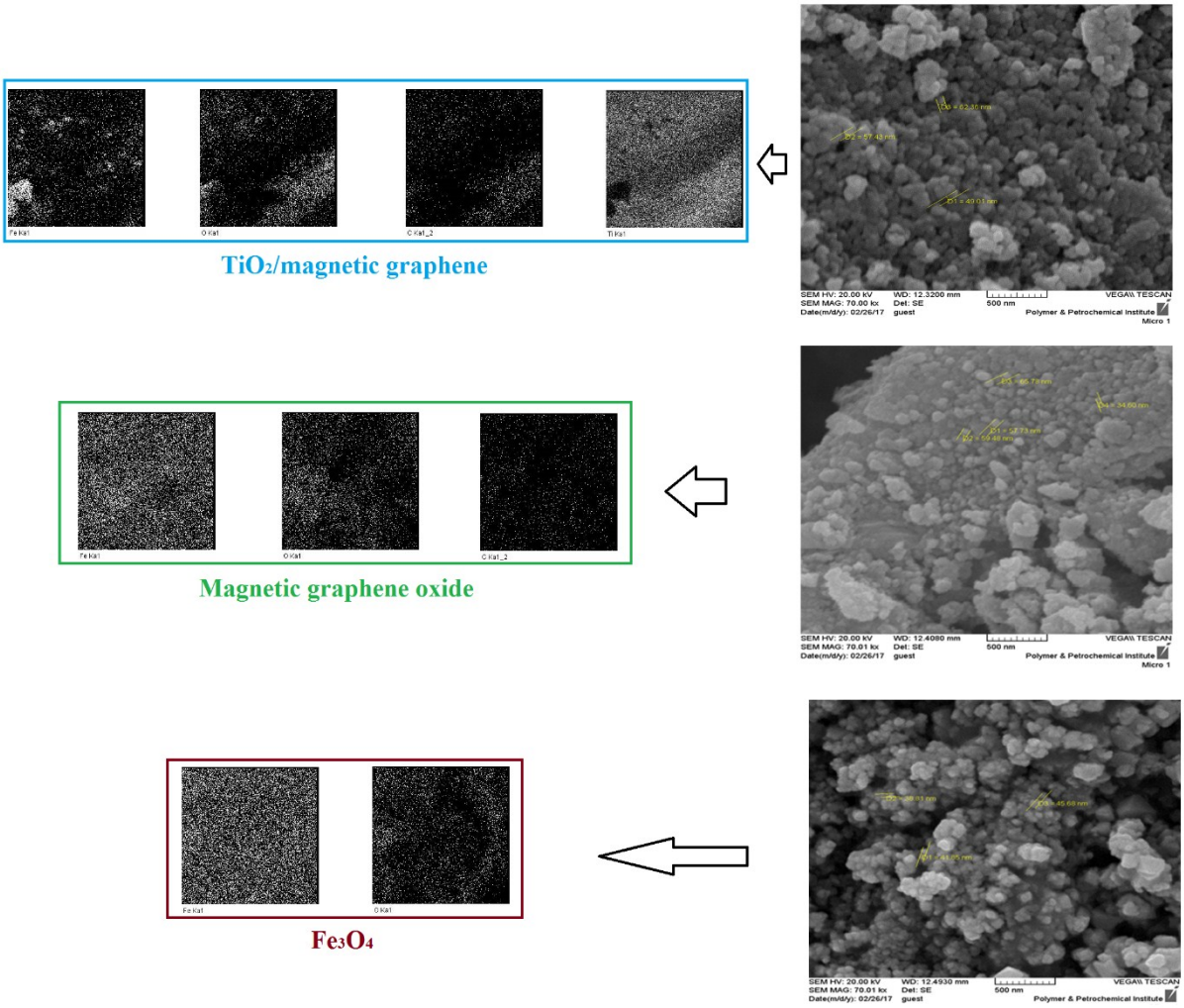

Figure 4S. Effect of adsorbent amount on the extraction recovery of diazinon (solution's pH: 7.0; sonication time: 5 min, salt amount: no salt addition, desorption condition: 1 mL acetonitrile under 2 min sonication).

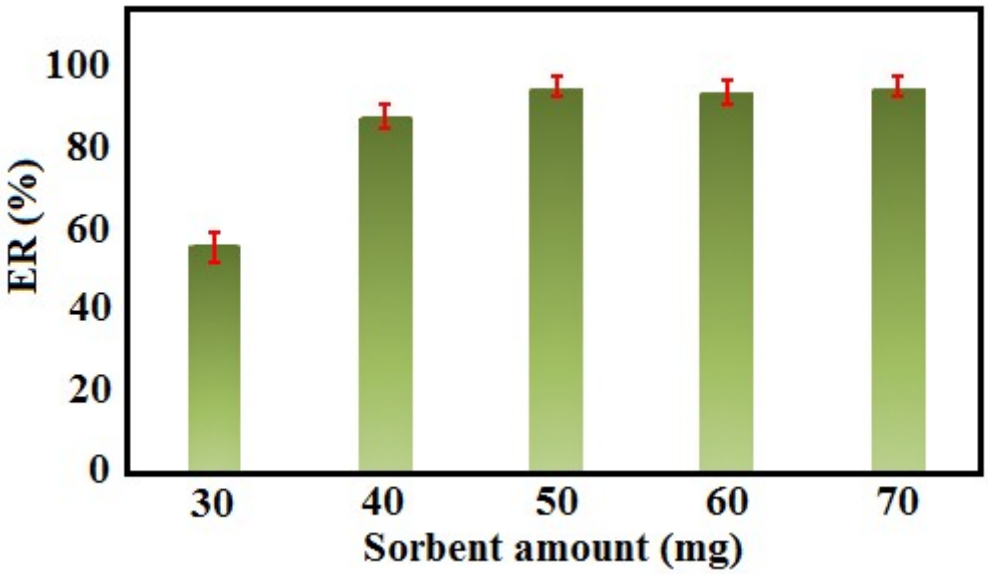
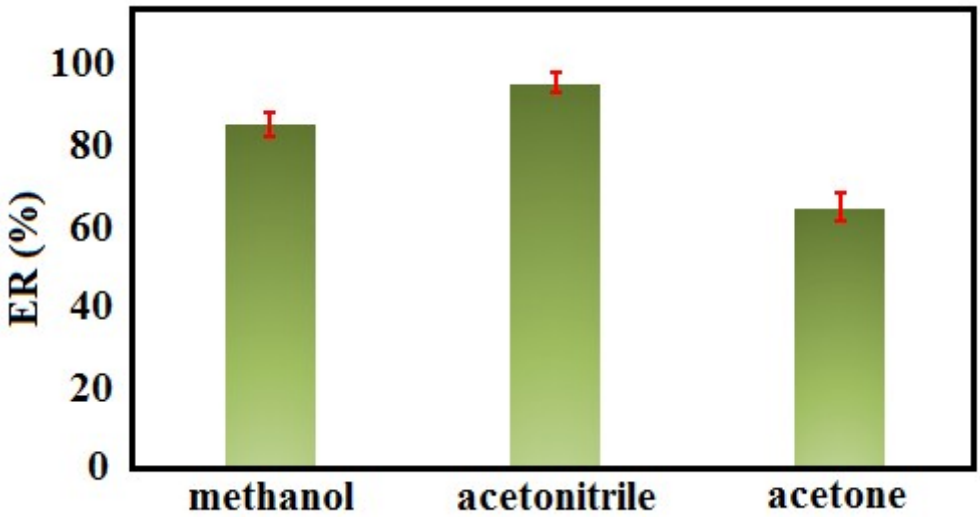
Figure 5S. Effect of desorption condition on the extraction recovery of diazinon (solution's pH: 7.0; sorbent amount: 50 mg, sonication time: 5 min, salt amount: no salt addition).