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Additional Information

Extraction and preconcentration of organophosphorus pesticides in water by using a polymethacrylate-based sorbent modified with magnetic nanoparticles

S. Meseguer Lloret^{a*}, S. Torres-Cartas^a, M. Catalá-Icardo^a, E.F. Simó-Alfonso^b, J.M. Herrero-Martínez^{b*}

^a Instituto de Investigación para la Gestión Integrada de Zonas Costeras, Universitat Politècnica de València, C/Paraninf 1, 46730 Grao de Gandía, Spain

^b Department of Analytical Chemistry, University of Valencia, C/Doctor Moliner 50, 46100 Burjassot, Valencia

* Corresponding authors:

Dra. Susana Meseguer Lloret
Tel: +34962849333
Fax: +34962849309
e-mail: sumello@qim.upv.es

Dr. Jose Manuel Herrero Martínez
Tel: +34963544062
Fax: +34963544436
e-mail: jmherrer@uv.es

Abstract

A polymethacrylate-based sorbent modified with magnetic nanoparticles (MNPs) has been synthesized and used as sorbent for solid-phase extraction (SPE) and magnetic solid-phase extraction (MSPE) of three organophosphorus pesticides (phosmet, pirimiphos-methyl and chlorpyrifos) in water samples followed by high-performance liquid chromatography-diode array detection (HPLC-DAD). The sorbent was prepared from a glycidyl methacrylate-based polymer, modified with a silanizing agent, followed by immobilization of MNPs on the surface of the material. The sorbent was characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). Comparative studies of this support were done both in conventional SPE cartridge and MSPE approach. Several extraction parameters (loading pH, elution solvent, eluting volume and loading flow rate) were investigated in detail. Under optimal conditions, the proposed sorbent gave an excellent enrichment efficiency of analytes and detection limits between 0.01 and 0.25 $\mu\text{g L}^{-1}$. The recoveries of organophosphorus pesticides in spiked water samples were in the range of 71-98%, and the developed sorbent showed a high reusability (up to 50 uses without losses in recovery). The proposed method was satisfactorily applied to the analysis of these pesticides in water samples from different sources.

Keywords: Organophosphorus pesticides, magnetic polymer-based material, solid-phase extraction, water analysis

1. Introduction

Organophosphorus pesticides (OPPs) have been extensively employed to protect agricultural crops and plantations due to their insecticidal properties. Pre- and post-harvest treatments have been applied to protect fruits and cereals from weeds, biting and sucking insects, and pests in storehouses, among others. After the treatment of the crops or plantations, the wastewater that contains the remains of these pesticides can reach drinking or superficial waters, causing adverse effects in the environment and human health. Therefore, the presence of residues of these compounds in waters must be monitored.

According to the EU Directive on water quality (98/83/EC) [1], the maximum allowed concentration for individual or total pesticides in drinking water is 0.10 and 0.5 $\mu\text{g L}^{-1}$, respectively. Then, the analysis of pesticides at these very low concentration values requires the development of sensitive and selective analytical techniques. In this sense, several analytical techniques for the analysis of pesticide residues mainly based on gas (GC) or liquid (LC) chromatography combined with a sensitive detection technique such as mass spectrometry (MS) [2, 3] and chemiluminescence [4, 5], among others, have been reported.

Pre-concentration and clean-up of samples is usually a pretreatment step required to reach the established detection limits of these pollutants. In order to enhance sensitivity, different pretreatment methods have been applied to the determination of OPPs in environmental water samples. Dispersive liquid-liquid microextraction (DLLME) [6-9] has been extensively applied, whereas SPE with C18-based material and solid-phase microextraction (SPME) [6, 10-13] are the most common techniques used for enrichment of these pesticides. However, these SPE methods have some disadvantages, e.g. long extraction time, column blocking, limited selectivity, poor reusability, whereas for SPME the fibers are fragile and relatively expensive. In this sense, the development of new SPE sorbents with enhanced properties is highly desirable.

Recently, magnetic materials have been employed as conventional or batch SPE sorbents (namely, magnetic solid-phase extraction, MSPE) based on the use of functionalized magnetic nanomaterials (FMMs) adsorbents [14]. Applying an external magnetic field without any additional centrifugation or filtration step can easily and quickly isolate these resulting materials. Moreover, these materials are relatively stable and reusable for the preconcentration and enrichment of key analytes in environmental or biological samples. Although there are different FMMs, the most frequently employed nanoparticles (NPs) are magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) due to its easy preparation, functionalization and operation [15]. Among the FMMs developed, magnetite octadecylsilane [16, 17], magnetite carbon coated [18], magnetite/reduced graphene oxide [19], and fatty acid coated magnetic [20] nanoparticles have been recently employed in the determination of OPPs in water samples, all in dispersive mode. Microspheres of maghemite-chitosan [21] have also been employed for the dispersive MSPE of OPPs in fruits. However, some of these FMMs (e.g. C18-modified magnetic particles) are difficult to disperse in water samples due to their very hydrophobic surface; as a result, its extraction efficiency is reduced. Moreover, a magnetite C18 sorbent has been employed in the determination of OPPs in fruits and vegetables [22], but in this case, the extraction was carried out in a cartridge filled with the composite material, however, this interesting alternative has been scarcely employed.

On the other hand, these FMMS are generally coated with silica or polymer-based materials, and there are few reports that have described the use of bare magnetic nanoparticles (MNPs) for selective extraction of certain organic compounds [23]. Consequently, a different strategy would be to immobilize the bare MNPs onto silica or polymeric support and the developed platform could be used for capture phosphorus-based compounds.

Glycidyl methacrylate (GMA) is a common monomer employed in the synthesis of monolithic columns, which due to its porous structure, high permeability and low backpressure, has been mainly used for separation techniques [24]. In recent years, there is an increasing interest in the use of monolithic materials for sample preparation [25]. Thus, GMA polymers contain oxirane rings that react with ligands (such as ammonia or ethylene diamine) to allow the attachment of organic material or metallic NPs such as gold [26] or silver [27], providing good adsorption capacity and high selectivity properties. However, few works have used GMA as a platform for the attachment of MNPs [28]. Thus, Krenkova *et al.* have described a stable coating of iron oxide NPs via electrostatic interactions of citrate ions on the surface of these NPs with a quaternary amine functionalized poly(GMA-co-ethylene dimethacrylate) monolith for selective isolation of low-abundant phosphopeptides from complex protein digests [28].

In order to further improve the performance of magnetic materials, in this work, a novel composite material that combines the advantages of Fe₃O₄ and GMA polymer is described for the selective analysis OPPs. The sorbent was prepared from a ground GMA-based polymer modified with a silanizing agent for a subsequent attachment of magnetite nanoparticles. Thus, the immobilization of Fe₃O₄ on the surface of amino silane modified methacrylate polymer provided bare MNPs that are able to interact with functional groups of P-O, present in OPPs [29, 30]. The prepared material was evaluated both in a SPE cartridge as well as in MSPE mode for preconcentration of three OPPs pesticides (chlorpyrifos, pirimiphos-methyl and phosmet). Several extraction parameters and other variables (breakthrough volume and reusability) influencing the analytical performance were established. After the SPE or MSPE, LC-DAD analysis was carried out. The optimized procedure was applied to the determination of the three OPPs in surface water samples from different sources. To our knowledge, up to now, there was no published investigation devoted to this type of platforms and to its potential as adsorbent for OPPs.

2. Material and methods

2.1 Chemicals and reagents

The chemical reagents employed for the polymer synthesis and its subsequent functionalization were: glycidyl methacrylate (GMA) from Sigma-Aldrich (St. Louis, MO, USA); ethylene glycol dimethacrylate (EDMA) purchased from Scharlab (Barcelona, Spain); cyclohexanol, 1-dodecanol, (3-aminopropyl)trimethoxysilane (APTMS) and acetone from Alfa-Aesar (Karlsruhe, Germany), azobisisobutyronitrile (AIBN) from Fluka (Buchs, Switzerland); anhydrous-N₂ from Abelló Linde (Barcelona, Spain). Magnetite nanoparticles (MNPs) were synthesized with: FeCl₂·4H₂O, FeCl₃·6H₂O from Panreac (Barcelona, Spain) and ammonia from Scharlab.

The pesticide standards were: chlorpyrifos (CLO, 99.9%) from Sigma-Aldrich, and pirimiphos-methyl (PIR, 99.5%) and phosmet (PHO, 99.4%) from Fluka.

For SPE and HPLC procedures, the employed reagents were: acetic acid, ethanol, hydrochloride acid, sodium hydroxide, sodium carbonate and tetrahydrofuran (THF) from Scharlab, and acetonitrile (ACN), propanol and methanol (MeOH) from Merck (Darmstadt, Germany).

For the preparation of aqueous solutions, an ultrapure water system Puranitiy TU 6 from VWR (Radnor, PA, USA) was used.

Stock solutions of CLO, PIR and PHO ($2000 \mu\text{g mL}^{-1}$) were prepared by dissolving appropriate amounts of each pesticide in ACN. A mixture of the three pesticides ($50 \mu\text{g mL}^{-1}$ of PHO and $125 \mu\text{g mL}^{-1}$ of PIR and CLO) in ACN was prepared, and working standard solutions were obtained by dilution of this mixture with ultrapure water. All pesticide solutions were stored in darkness at 4°C and remain stable for a month.

2.3 Instrumentation

A drying oven (JP Selecta, Barcelona, Spain) was employed to carry out the polymerization and functionalization procedures.

TEM images of MNPs were obtained using a Jeol (Tokyo, Japan) model JEM-1010 microscope operated at 100 kV. For this purpose, the nanoparticles were dispersed in pure ethanol by sonication, and 0.05 mL of this suspension was placed on a 200-mesh Cu grid coated with a holey amorphous carbon film. The ethanol was evaporated prior to TEM analysis. Images were obtained using a MegaView III camera provided with the AnalySIS image data acquisition system. A Hitachi S-4800 integrated with backscattered electron detector and a QUANTAX 400 energy dispersive spectrometer (Bruker, Germany) was employed to obtain SEM micrographs. For these measurements, the materials were sputter-coated with a thin layer of Au/Pd.

Fourier transform infrared spectroscopy (FT-IR) spectra of powdered materials (GMA-*co*-EDMA polymer and the polymer modified with MNPs) were obtained with a Jasco 4100 type A spectrophotometer (Jasco, Easton, MD) fitted with a single reflection attenuated total reflectance (ATR) accessory. Spectra were collected from 4000 to 500 cm^{-1} in the absorbance mode at a 2 cm^{-1} resolution with 100 scans. Magnetic properties were measured with a Quantum Design MPMS-XL 5 SQUID magnetometer. Vibrating sample magnetometry (VSM) data were taken at 300 K in the range of $-15\,000$ Oe (-1.5 Tesla) to $+15\,000$ Oe ($+1.5$ Tesla). The magnetization saturation value (M_{sat}) was the maximum magnetization observed, and was taken at $15\,000$ Oe. Chromatographic determination of pesticides was made with an Agilent chromatographic system (Sta. Clara, CA, USA) consisted of a quaternary gradient pump (Agilent 1260 Infinity), a photodiode array detector (DAD, Agilent 1200 Series) and 7125 Reodyne manual injection valve (Rohnert Park, CA, USA) furnished with a $200 \mu\text{L}$ loop. The instrument was controlled by the Agilent ChemStation Software (Rev B.04.03). The separation column was a Brisa LC2, C18 $5\mu\text{m}$, 150×4.6 mm from Teknokroma (Barcelona, Spain).

2.4 Synthesis of MNPs

MNPs were synthesized by the well-known co-precipitation method [31] from $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ in the presence of aqueous ammonia with a ratio 2:1. Thus, 180 mL of 0.062 M Fe (III) and 0.031 M Fe (II) aqueous solution were placed into a two-neck round-bottom flask with a glass condenser. The solution was stirred under nitrogen until 50°C. Then, 12.5 mL of concentrated ammonia were added under vigorous stirring, and the reaction was carried out for 30 min at 50°C. Afterwards, the temperature was raised to 90°C and the mixture was kept at this temperature for 30 min.

The MNPs were separated from the reaction mixture with a neodymium magnet, and washed with water and ethanol several times to remove the excess of reagents. Then, the MNPs were dried in an oven at 60°C for 30 min. Then, TEM characterization of MNPs was done, showing diameters of *ca.* 12 nm (see Fig. 1).

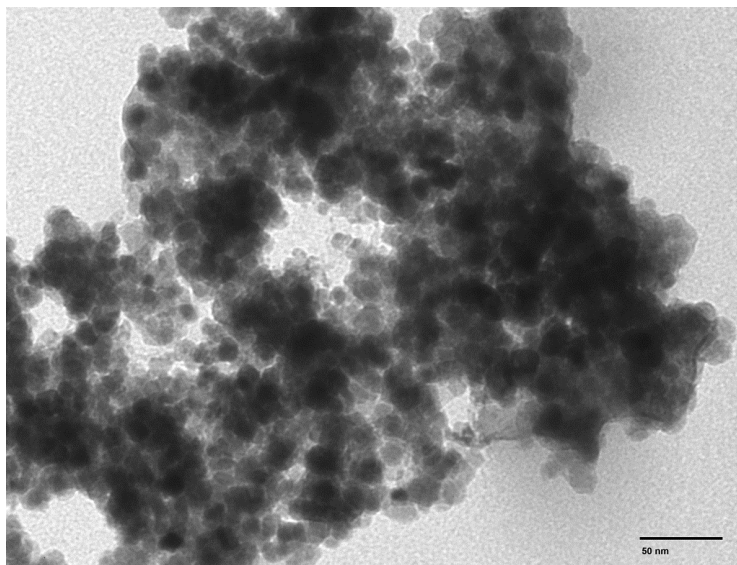


Fig. 1 TEM image of synthesized MNPs

2.5 Preparation and silanization of GMA-*co*-EDMA material

The synthesis of GMA-*co*-EDMA polymeric material was based on a previous work [32]. Briefly, a polymerization mixture composed of monomers (20 wt% GMA, 5 wt% EDMA) and porogenic solvents (70 wt% cyclohexanol and 5 wt% 1-dodecanol) was prepared in a 10 mL glass vial. Furthermore, AIBN (1 wt% with respect to the monomers) was added as initiator. This mixture was firstly sonicated for 10 min and next purged with nitrogen for 10 min in order to remove oxygen. The polymerization was carried out in a drying oven at 60°C for 20 hours. After this, the polymeric material was washed with MeOH in order to remove the porogenic solvents and possible unreacted monomers, and was next dried at 60°C. Then, the bulk material was ground with a mortar, sieved with a steel sieve (particle sizes between 125 and 200 μm) and stored in a desiccator.

Three grams of the synthesized powder material were treated with 75 mL of 0.65 M APTMS in acetone in a glass flask at 60°C (water bath) for 3 hours under stirring. Afterwards, the material was filtered and washed with 20 mL ethanol and dried at 60°C. The powdered poly-(GMA-*co*-EDMA) functionalized with APTMS was stored in a desiccator.

2.6 Functionalization of silanized GMA-based material with MNPs

A dispersion of MNPs in ethanol-water (1:1, v/v) at $1000 \mu\text{g mL}^{-1}$ was prepared, sonicated for 15 min and shaken for 1 min with a vortex. The silanized modified GMA powder material (2.50 g) was added into a 60 mL propylene syringe (with two PTFE frits at the bottom). Several fractions (15×5 mL) of the MNP dispersion were passed through the modified GMA-based material under continuous stirring, and the remaining liquid was removed by gravity at a flow rate of 0.5 mL min^{-1} . After that, a brown coloured MNPs-modified material was obtained. Then, the material was washed with 100 mL of ethanol-water (1:1, v/v) solution and 15 mL of pure ethanol to remove the excess of MNPs. Afterwards, the material was dried in an oven at 60°C and stored in a desiccator.

2.7 SPE protocol and sample preparation

SPE cartridges were prepared by placing MNPs-modified material (150 mg) between two frits (20 μm , Análisis Vínicos, Tomelloso, Spain) into 3 mL empty propylene SPE cartridges (Análisis Vínicos). The sorbent was washed with 15 mL of ethanol-water (1:1, v/v) solution and subjected to vacuum to compact the material. The sorbent was subsequently conditioned with MeOH (5 mL), THF (1.5 mL), water (5 mL) and 0.05% acetic/acetate buffer at pH 5 (5 mL). Next, a known volume of deionized water or sample water spiked with pesticides of interest and adjusted at pH 5 (with 5% acetic/acetate buffer solution) was passed through the cartridge at a flow rate of 2.3 mL min^{-1} . After that, the sorbent was rinsed with 0.05% acetic/acetate buffer solution at pH 5 (1 mL) and dried under vacuum for 10 min. Retained pesticides were eluted with THF (2.5 mL). The same procedure was also applied to prepare a blank sorbent constituted by the GMA-co-EDMA polymer (150 mg).

To perform MSPE experiments, a neodymium magnet was employed in all the steps to separate the MNPs from the supernatant solution. First, 350 mg of MNPs placed in a vial were conditioned with 5 mL of MeOH, 2.5 mL of THF, 5 mL of deionized water and 5 mL of 0.05% acetic/acetate buffer (pH 5.0). After that, a 100 mL water sample adjusted at pH 5.0 (with 5% acetic/acetate) was added. The mixture was sonicated in the ultrasonic bath for 5 min, and shaken for 5 min. The vial was placed on the neodymium magnet for 15 min to let MNPs settle down. Then, the supernatant solution was discarded, and the sorbent was washed with 2 mL of 0.05% acetic/acetate buffer solution (pH 5) and vortexed for 0.5 min. The target analytes were desorbed with 8.0 mL of THF, sonicated for 1.0 min, and shaken for 1 min more.

Three water samples from different sources (tap, river and irrigation) were collected. They were filtered under vacuum through 0.45 μm nylon filters (Phenomenex, Torrance, CA, USA). Before SPE procedure, the pH of samples was properly adjusted at pH 5.0 by adding adequate volumes of 5% acetic/acetate buffer solution.

These real water samples were analyzed (in triplicate) according to the optimum SPE procedure. Since these samples were free of the pesticides of interest, they were spiked with the three pesticides (simultaneously) at two levels of concentration (26 and 50 ng mL^{-1} for PHO and 65 and 125 ng mL^{-1} for PIR and CLO). SPE experiments were also performed in triplicate for each concentration level.

2.8 HPLC procedure for pesticide analysis

The chromatographic separation of the three OPPs (PHO, PIR and CLO) was carried out using a gradient elution with ACN and water as follows: 65% ACN for the first 3 min; then a linear gradient to 85% ACN in 0.5 min, and this composition was held for 5 min and then back to the initial conditions (0.5 min). The injection volume was 200 μL , the flow rate was kept at 1 mL min^{-1} and the column temperature was ambient. In this study, complete separation of the three analytes could be accomplished within 12 min. The selected wavelengths for the three pesticides were: 222, 248 and 200 nm for PHO, PIR and CLO, respectively.

3. Results and discussion

3.1 Preparation and characterization of the MNPs-modified material

Due to the easy modification of epoxy groups, a poly(GMA-co-EDMA) monolith was first prepared as a generic support for MNPs immobilization. For this purpose, this polymer was ground into fine powder and then functionalized with MNPs in a two step-procedure. Thus, epoxide groups of polymerized GMA were allowed to react with APTMS to afford a polymer with amino silane functionalities. Then, a MNPs colloidal dispersion was used to functionalize the resulting amino silanized material until the polymer surface was fully covered with MNPs. It was visually confirmed by a change of color in the powdered monolith from white to brown. The route for the synthesis of MNPs-modified material is shown in Fig. 2.

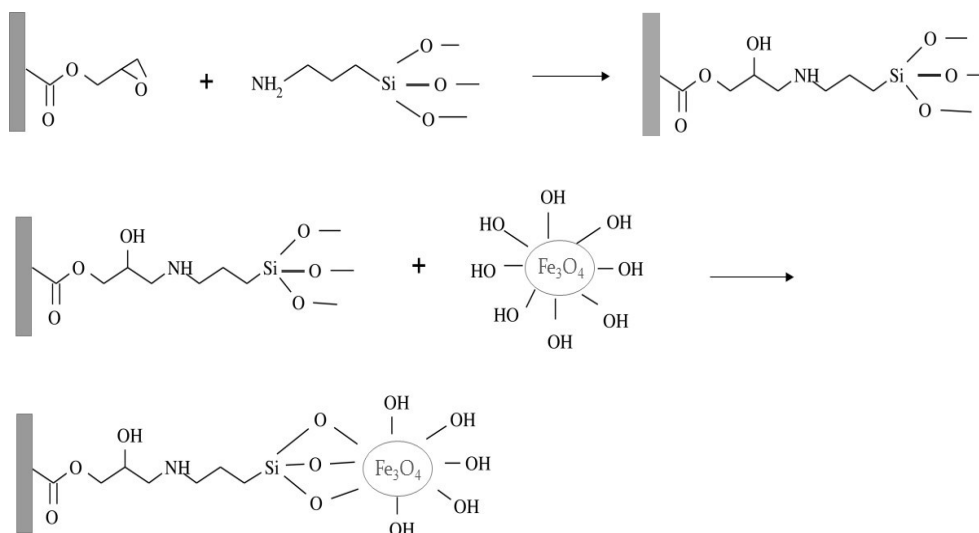


Fig. 2 Scheme of preparation of poly(GMA-co-EDMA) material and its modification with APTMS and immobilization of MNPs.

In addition, SEM micrographs of this material were taken, where the MNPs immobilized onto the polymer surface could be easily distinguished (Fig. 3).

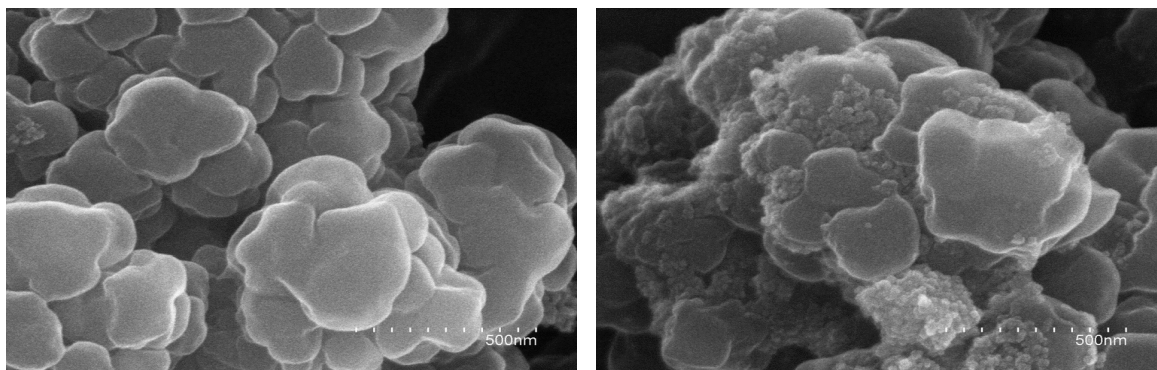


Fig. 3 SEM micrographs of GMA-co-EDMA powder material without MNPs (a) and functionalized with MNPs (b).

FT-IR spectroscopy was also employed to verify the immobilization of MNPs onto the surface of the amino silanized methacrylate polymer. As shown in Fig. 4, the successful coating of Fe_3O_4 onto the surface of amino-modified polymer was proven with the presence of a strong peak at 508 cm^{-1} corresponding to the Fe-O bond. This band appeared shifted to low wavenumbers compared with that of bare Fe_3O_4 and aminosilane-coated Fe_3O_4 [33, 34]. This phenomenon can be explained by changes in the local chemical bonding environment of Fe-O (covalent attachment of Fe-O-H groups to the silanized GMA-based material).

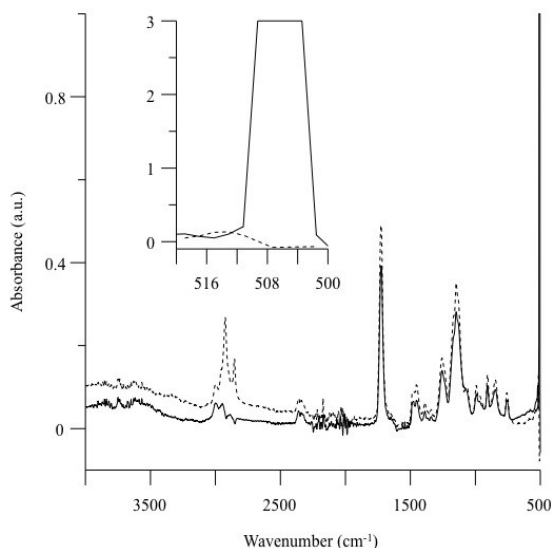


Fig. 4 FT-IR spectra of GMA-co-EDMA polymer (dashed line) and Fe_3O_4 immobilized onto amino silanized GMA-based material (continuous line).

The VSM data for the MNP-composite material showed approximately 1/3 the saturation magnetization of the pure MNPs, with $M_{\text{sat}} = 24.52 \text{ emu g}^{-1}$. Some loss of magnetization is expected when the MNPs were attached to methacrylate polymer because the polymer does not contribute to the overall magnetization (Fig. 5). This decrease in magnetization is comparable to other magnetic composite materials that have been made with polymers such as polystyrene [35] and poly(methylmethacrylate) [36].

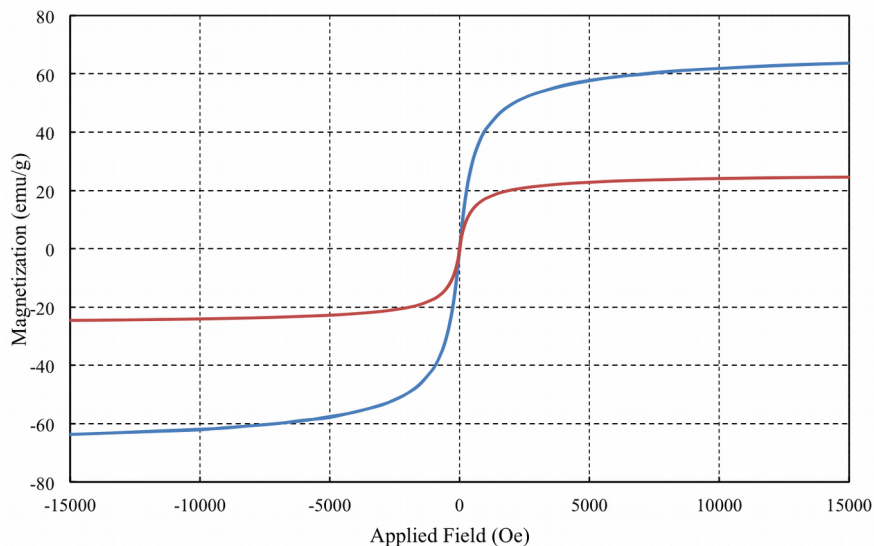


Fig 5 Magnetization data at 300 K for MNPs (blue) and MNP-composite material (red) from -15000 Oe to 15000 Oe

3.2 Optimization of SPE procedure with MNPs-modified material

The potential of methacrylate polymer modified with MNPs was evaluated firstly as traditional SPE sorbent for the enrichment of three OPPs (PHO, PIR and CLO) in aqueous samples. For this purpose, several parameters of the SPE procedure, such as loading pH, elution solvent and elution volume, flow rate, breakthrough volume and reusability were carefully investigated. During optimization of SPE conditions, an aqueous solution containing 100 ng mL^{-1} of PHO and 250 ng mL^{-1} of PIR and CLO was employed as test mixture.

The adsorption of analytes onto mineral oxide surfaces is strongly influenced by its charge density, which can be modified by the acidity of the sample solution [23, 37]. Thus, the influence of pH on the recoveries by changing the pH value of sample solution between 3.5 and 9 was studied. For this study, the loading and washing step was adequately adjusted to pH conditions to achieve the highest extraction efficiency. Prior to the loading of the sample, a conditioning step of cartridge was done (see experimental details in SPE protocol and sample preparation section), and ACN was firstly used as eluting solvent. As shown in Fig. 6, the recoveries of the three analytes at pH 5.0 reached high values; thus, the recoveries of

PHO and PIR were close to 90%, whereas for CLO it was near 70%. However, the presence of CLO was not observed in the collected fractions from loading and washing steps; therefore, it can be concluded that the key for increasing CLO recovery probably resided in the improvement of the elution step.

The selection of an appropriate solvent is of major concern for the optimization of SPE process. For this purpose, different elution solvents were assayed: MeOH, propanol, ACN and THF.

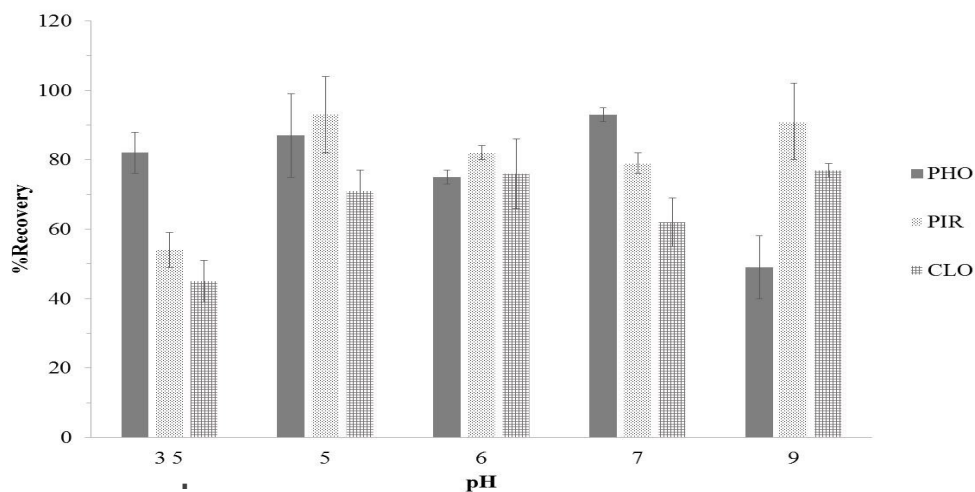


Fig. 6 Effect of the pH value of the sample solution on the recoveries of OPPs using MNPs-modified material as SPE sorbent. Conditions: concentrations (100 ng mL^{-1} PHO, 250 ng mL^{-1} for PIR and CLO); sample volume, 5 mL; eluent volume, 4 mL; flow rate, 1 mL min^{-1} .

The extraction efficiency of the three OPPs obtained from the elution with these four solvents is shown in Fig. 7. As it can be seen, THF gave the best recoveries for the OPPs tested. After that, the THF volume was varied between 1 and 4 mL at elution flow rate of 1 mL min^{-1} and the recoveries of pesticides increased with increasing THF volume up to 2.5 mL, being this value selected for further studies.

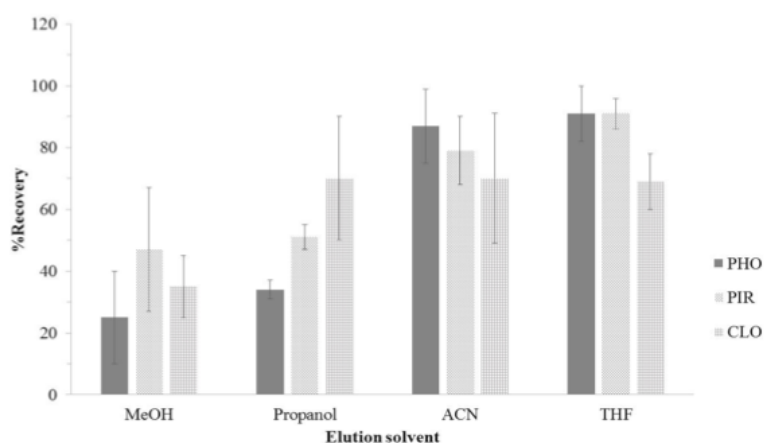


Fig. 7 Effect of the type of the eluting solvent on the recovery of the OPPs. Conditions: volume of standard solution: 5 mL; concentration of analytes: 100 ng mL^{-1} PHO, 250 ng mL^{-1} for PIR and CLO; eluent volume, 4 mL; flow rate: 1 mL min^{-1} .

The flow-rates of the sample loading and elution steps in a SPE protocol are important parameters, which not only affect the recoveries of analytes, but also controls the analysis time. Firstly, the flow rate of the sample loading was varied between 1-3.6 mL min⁻¹. It was found that the recoveries remained above 82% up to 2.3 mL min⁻¹. However, higher flow rates gave a decrease in the recoveries values between 5-20%. Then, a sample loading flow rate of 2.3 mL min⁻¹ was adopted. Secondly, the flow rate of the elution step was varied between 0.5-2.3 mL min⁻¹ but no differences in recoveries were obtained.

Under the selected conditions, the SPE protocol was also applied with the GMA-co-EDMA sorbent (without MNPs), and recoveries near 55% were obtained for the three OPPs, being quite lower than those found for the material modified with MNPs. These results demonstrated the key role of the attached MNPs onto polymer in the adsorption of these pesticides.

To achieve reliable analytical results and high enrichment factor, it is important to obtain satisfactory recoveries for all analytes in as large volume of sample solutions as possible. In order to evaluate the breakthrough volume, variable volumes (between 10 mL and 500 mL) of the OPPs mixture solution were passed through the SPE material by keeping constant the total amount of loaded pesticides (0.5 µg PHO, 1.25 µg PIR and 1.25 µg CLO). Fig. 8 shows the recoveries of the three pesticides obtained for each tested volume. As it can be seen, high recoveries (83-98%) up to 100 mL were achieved for the three analytes. However, higher volumes (>100 mL) led to a decrease in the recovery values. Consequently, 100 mL was adopted as volume for the analysis of real water samples.

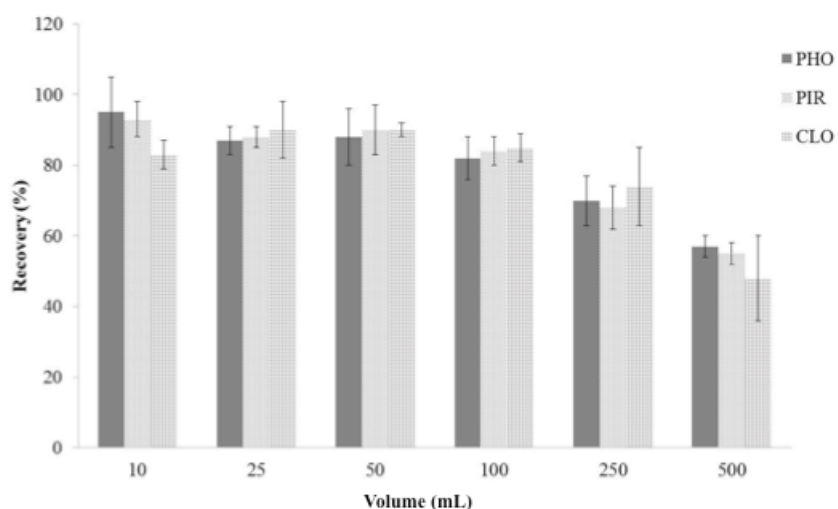


Fig. 8 Effect of sample volume on the recoveries of three OPPs by using the MNPs-modified material as SPE sorbent. Conditions: sample solution 0.5 µg PHO, 1.25 µg PIR and 1.25 µg CLO dissolved in different sample volumes; volume of eluent 2.5 mL; flow rate 2.3 mL min⁻¹.

The reusability of the commercial SPE cartridge has been a drawback for a long time because of the risk of pore closure and sample contamination. A commercial cartridge generally can be used no more than five times for water sample analysis and once for more complex samples. The stability and reusability of our sorbent were investigated by reusing the material to extract analytes from water samples and their spiked solutions continuously for 2 months. Thus, washing with 10 mL water and 5 mL MeOH reactivated

the cartridge after each use, and no carryover and memory effects were observed. The results showed that cartridges can be re-used at least for 50 times with satisfactory recoveries comprised between 80-97%, with variation coefficients below 10%.

Next, a MSPE protocol using the magnetic composite materials under application of magnetic field was developed. For this purpose, several parameters optimized in the conventional SPE procedure were adapted to MSPE. The amount of adsorbent is an important parameter to obtain the maximum recovery in this technique. For this purpose, different amounts of magnetic sorbent (75-350 mg) were applied to extract the OPPs from 100 mL deionized water spiked with 100 ng mL⁻¹ of PHO, and 250 ng mL⁻¹ of PIR and CLO. The extraction recovery improved when the magnetic composite material was increased due to the fact that a higher amount of adsorbent in the solution leads to a higher surface area and greater availability of more adsorption sites of sorbent. Recoveries between 42-68% were achieved with a MNPs amount of 350 mg and a single elution step with 4 mL of THF. A further increase in the sorbent dosage did not improve the extraction recoveries. A second elution with 4 mL of THF enhanced the recoveries to 58-81%. In addition, ultrasonication improved the extraction of the analytes. In liquids, the bubbles formed by the sonication of solutions burst and result in the production of high local temperatures and pressure gradients. These localized high-energy environments may increase the efficiency of desorption of the analytes from a solid sorbent [38, 39]. In this study, it was shown that the elution step assisted by sonication significantly improved the recoveries to the desired results (86-88%).

The recoveries of all the analytes studied obtained via conventional SPE (83-98%) and MSPE approach (86-88%) were comparable, although slightly better results were found in the first approach. On the other hand, the SPE cartridges require less amounts of sorbent and smaller elution volumes, which makes this protocol cost-effective. Moreover, the SPE protocol simplifies the handling of more samples simultaneously, which speeds the preconcentration process of OPPs. In particular, MSPE allowed processing 1 sample per hour (*ca.* 8 samples in an 8 h working day), whereas a rate of 10 samples per hour using vacuum manifold (*ca.* 80 samples per day) could be achieved with SPE methodology.

3.3 Figures of merit

The analytical performance of method was established using the optimum conditions found in using cartridge mode in combination with HPLC with DAD detection. The calibration curves for the three OPPs were constructed by preconcentrating several working standards of the mixture prepared in ultrapure water (100 mL) at different concentration levels. A linear correlation ($r > 0.998$) between peak area and concentration was achieved for all analytes. Regression equations and linear ranges are summarized in Table 1. The limits of detection (LOD) and quantification (LOQ) were experimentally obtained as the concentration of the analyte that provided a signal-to-noise ratio (S/N) of three and ten, respectively [40]. As shown in Table 1, the LODs varied between 0.01 and 0.25 ng mL⁻¹.

The precision of the SPE-HPLC-DAD method (intra- and inter-day conditions) was also evaluated by calculating the relative standard deviation (%RSD) of spiked ultrapure water samples analyzed in triplicate per day during three days. The results given in Table 1 indicate that precision was satisfactory with RSD values below 5.4%.

Table 1 Analytical figures of merit for the developed SPE-LC-DAD method (processed volume 100 mL).

Pesticide	Calibration			Precision, n=3			
	Regression equation ^a $A=(a\pm s_a) + (b\pm s_b)\cdot C$ (n, r ² , S _{y/x})	Linear interval (ng mL ⁻¹)	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)	Spiked level (ng mL ⁻¹)	Intra-day, %RSD	Inter-day, %RSD
PHO	$A=(-0.5 \pm 1.5) + (17.3 \pm 0.5)\cdot C$ (15, 0.99, 2)	0.05 - 16	0.01	0.03	5	4	1.9
PIR	$A=(0.9 \pm 1.3) + (5.45 \pm 0.16)\cdot C$ (15, 0.99, 1.7)	0.25 - 40	0.05	0.16	12.5	4.1	5.4
CLO	$A=(5.7 \pm 1.2) + (4.77 \pm 0.17)\cdot C$ (15, 0.99, 1.6)	1.5 - 40	0.25	0.83	12.5	4	3.4

^aA is peak area, C is analyte concentration (in ng mL⁻¹).

3.4 Analysis of water samples

Three different water samples were used to validate the preconcentration method based on the use of MNPs-modified material as SPE sorbent. None of the three target pesticides was found in any of the real water samples analyzed. Thus, the real samples were spiked with the three OPPs (simultaneously) at two concentration levels.

Table 2 %Recoveries of target pesticides in spiked water samples (n=3).

Water Sample	PHO Spiked level		PIR Spiked level		CLO Spiked level	
	26 ng mL ⁻¹	50 ng mL ⁻¹	65 ng mL ⁻¹	125 ng mL ⁻¹	65 ng mL ⁻¹	125 ng mL ⁻¹
Tap	83 ± 4	91.9 ± 1.3	79 ± 4	92.5 ± 1.9	71 ± 4	85.0 ± 1.9
Irrigation	85 ± 3	88.6 ± 1.0	83 ± 2	91.2 ± 0.8	85 ± 4	95 ± 5
River	94 ± 8	87 ± 2	98 ± 11	86 ± 3	81 ± 4	80 ± 4

The measured spiked recoveries are depicted in Table 2, and it was found that the recoveries of three pesticides were good in the range of 71-98%. Fig. 9 shows the chromatograms from blank and spiked irrigation water sample.

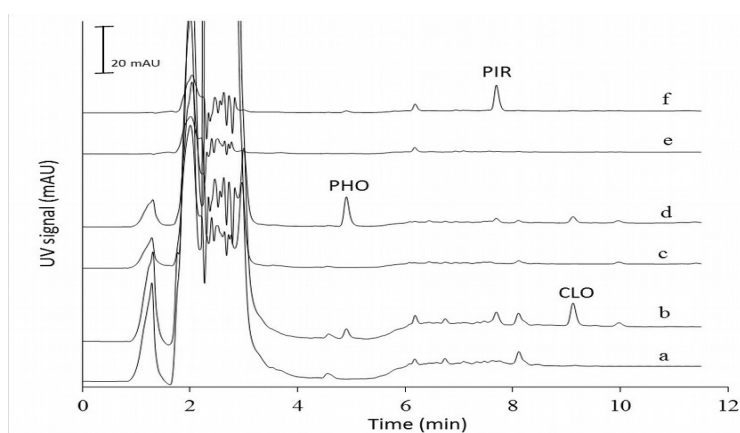


Fig. 9 Chromatograms of irrigation water sample unspiked (traces (a), (c) and (e) at 200, 222 and 248 nm, respectively) and spiked with 50 ng mL⁻¹ for PHO, and 125 ng mL⁻¹ for PIR and CLO (traces (b), (d) and (f) at 200, 222 and 248 nm, respectively).

3.5 Comparison with other extraction procedures

Table 3 compares the characteristic features for the proposed method with other extraction methods reported in the literature for the determination of OPPs in water samples. Regarding recovery values obtained in this study, these were similar to those found in most reported studies with the exception of those given in references [7] and [29], where the recoveries of CLO were quite low.

Concerning the LODs, most of these studies have been focused on the determination of CLO, in this sense, our LOD value was better than those reported using either SPE with conventional C18 cartridges [11, 12] or DLLME [13]. However, the LOD achieved for this pesticide was higher than other sample pretreatments (such as LDS-VSLLME [9], HS-SPME [13] or SPE combined with DLLME [6]). However, it should be mentioned that few studies have been reported for the simultaneous determination of the two [17, 20] or three pesticides [41] investigated. Thus, the LODs assayed in this study were similar [20] or even better [17] than those described using magnetic functionalized materials in combination with highly sensitive and sophisticated techniques (GC-MS). However, the method developed in this study provided satisfactory LODs using common equipment accessible in most analytical laboratories. Also, our LOD values for PIR and CLO were similar to the ones obtained by Catalá-Icardo *et al.* [41], where a more sensitive detection (chemiluminiscence) was used.

The proposed SPE material developed here could be favourably compared with recent reports related to the use of FMM-based adsorbents for pesticide extraction and preconcentration [17, 18, 20]. Regarding to the preparation of sorbent few simple steps are required, and moreover our protocol simplifies the handling of more samples simultaneously and speeds the preconcentration process of pesticides. In particular, this method allowed a sample throughput of 10 samples h⁻¹ (see data above), whereas a rate of 1-2 samples h⁻¹ could be achieved with other FMM-based protocols [17, 18, 20].

A significant strength of the proposed sorbent is its large reusability (see data above). In this regard, in the related studies of FMM-based materials or even in other extraction devices [17, 18, 20, 6, 11, 12, 41], this important parameter was not evaluated. This excellent reusability combined with the possibility of manufacturing several SPE cartridges (*ca.* 15) from the bulk MNPs-modified material (see Functionalization of silanized GMA-based material with MNPs section) undoubtedly makes this protocol economically attractive.

Additionally, due to the small mass sorbent employed (150 mg), which is lower than habitual packed SPE cartridges [11,12, 41] or even some FMM-based material [20], lower volumes of harmful organic solvents (2.5 mL THF) were required for sample elution, which was in agreement with the requirements of Green Analytical Chemistry.

In any case, the SPE procedure developed here constitutes a good alternative for the effective and selective extraction of these pesticides since it can be performed in short time, with low cost, large reusability and environmentally friendly, which certainly makes it suitable for the analysis of real water samples.

Table 3 Comparison of the proposed method with other recently developed extraction methods for the analysis of CLO, PIR and PHO in water samples.

Analytical Method	Extraction procedure	Pesticide	LOD (ng mL ⁻¹)	Added Conc. (ng mL ⁻¹)	%Recovery	Reference
LC-MS/MS	SPE, C18 (500 mg)	CLO	8.6	20-100	37-51	[11]
GC-FPD	SPE, C18 (100 mg) + DLLME, chlorobenzene (12 µL)	CLO	0.0003	0.05	88.9%	[6]
LC-UV	SPE, C18 (500 mg)	CLO	3	1000	85	[12]
	SPE, MWCNT (100 mg)	CLO	0.004	1000	94	
GC-ECD	HS-SPME, polythiophene fiber	CLO	0.02	0.03-1.5	96.2-100.7	[13]
LC-UV	DLLME, [C ₈ MIM][PF ₆] (43.6 mg)	CLO	5	75-1000	101.8-113.4	[7]
LC-UV	LDS-VSLLME, 1-undecanol (80 µL)	CLO	0.08	2-30	95.5-98.7	[9]
LC-UV	MSPE, Fe ₃ O ₄ /C MNPs (97.4 mg)	CLO	0.0043	2	90.6-101.4	[18]
LC-DAD	UASEME, C ₆ H ₅ Cl (150 µL)	PHO	0.1	10-100	87.2-102	[8]
GC-MS	MSPE, C18FMM (10 mg)	CLO	3	No data	No data	[17]
		PIR	4.6			
GC-MS	SPE, PA-Fe ₃ O ₄ -MNPs (400 mg)	CLO	0.03	0.2	68.6-71.7	[20]
		PIR	0.03	0.2	71.6-78.9	
LC-CLD	SPE, Strata X (200 mg)	CLO	0.01	0.7-1.4	40.4-81.7	[41]
		PIR	0.02	1.4-2.8	71.2-103.3	
		PHO	0.005	0.6-1.2	68.8-109	
LC-DAD	SPE, GMA-co-EDMA-MNPs (150 mg)	CLO	0.25	65-125	71-95	This study
		PIR	0.05	65-125	79-98	
		PHO	0.01	26-50	83-94	

Abbreviations: FPD: Flame photometric detection; HP-SPME, Headspace SPME; UASEME: Ultrasound assisted surfactant-enhanced emulsification microextraction; [C₈MIM][PF₆] : 1-octyl-3-methylimidazolium hexafluorophosphate; LDS-VSLLME: Low-density solvent-based vortex-assisted surfactant-enhanced-emulsification liquid-liquid microextraction; C18FMM: C18 Functionalized magnetic microspheres; Fe₃O₄/C MNPs: Carbon coated Fe₃O₄ Nanoparticles; PA-Fe₃O₄-MNPs: Palmitic acid coated magnetic nanoparticles; LC-CLD: LC post-column with chemiluminescence detection.

4. Conclusions

In this work, a novel SPE sorbent based on a silanized GMA-co-EDMA polymer modified with MNPs has been synthesized and applied to the extraction of three OPPs (PHO, PIR and CLO) in water samples, followed by their analysis using HPLC-DAD. To our knowledge, it was the first time using a polymeric support of bare MNPs to extract pesticides in these matrices. Thus, the main attributes of our proposed sorbent are: i) the bare Fe surface of the polymeric support constitutes a good and selective platform for adsorption of OPPs, and ii) the magnetic functionalized material could be used both in SPE cartridges as in dispersive mode (MSPE), since it showed good dispersibility in water. The MSPE method was shorter in terms of processing time than conventional approach, but it needed more sorbent and had slightly lower recoveries and sample throughput than the SPE cartridge protocol. In summary, the present SPE method is simple, cost-effective, selective and sensitive, and showed a large reusability of material (up to 50 reuses without significant variations in recoveries).

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Conflict of interest

The authors declare that they have no conflict of interest.

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