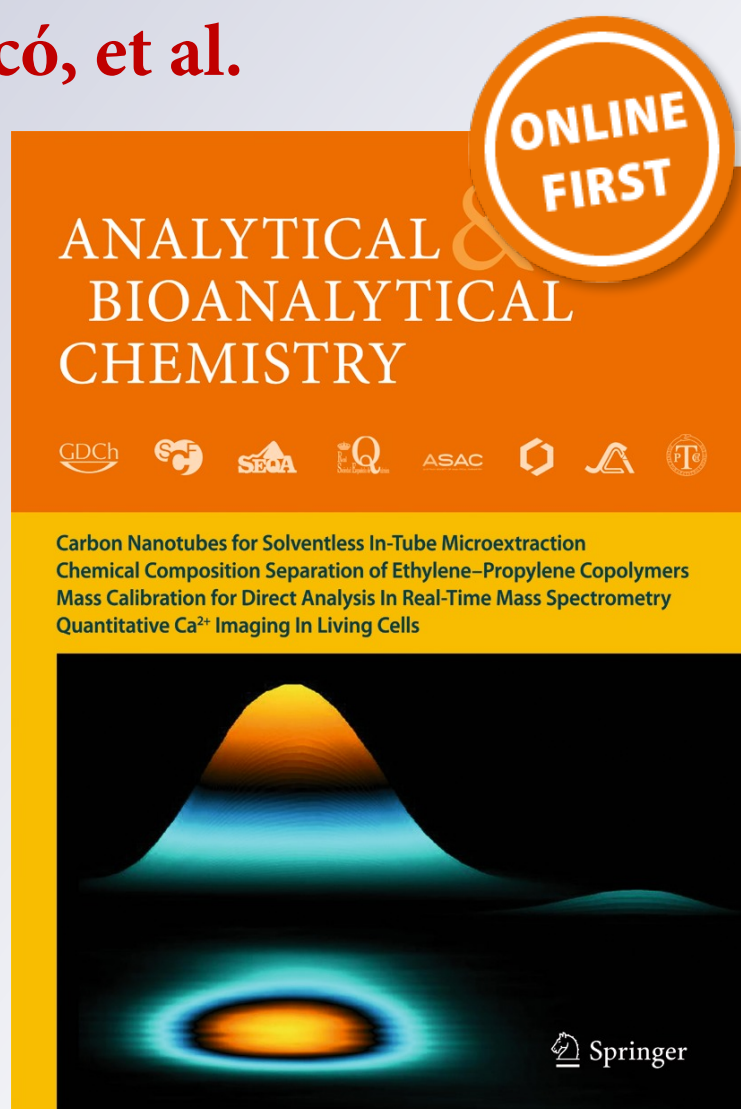*Silica supported Fe₃O₄ magnetic nanoparticles for magnetic solid-phase extraction and magnetic in-tube solid-phase microextraction: application to organophosphorous compounds*

Y. Moliner-Martinez, Yosmery Vitta, Helena Prima-Garcia, R. A. González-Fuenzalida, Antonio Ribera, P. Campíns-Falcó, et al.

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Silica supported Fe$_3$O$_4$ magnetic nanoparticles for magnetic solid-phase extraction and magnetic in-tube solid-phase microextraction: application to organophosphorous compounds

**Y. Moliner-Martinez · Yosmery Vitta ·
Helena Prima-Garcia · R. A. González-Fuenzalida ·
Antonio Ribera · P. Campíns-Falcó · Eugenio Coronado**

Abstract This work demonstrates the application of silica supported Fe$_3$O$_4$ nanoparticles as sorbent phase for magnetic solid-phase extraction (MSPE) and magnetic on-line in-tube solid-phase microextraction (Magnetic-IT-SPME) combined with capillary liquid chromatography–diode array detection (CapLC-DAD) to determine organophosphorous compounds (OPs) at trace level. In MSPE, magnetism is used as separation tool while in Magnetic-IT-SPME, the application of an external magnetic field gave rise to a significant improvement of the adsorption of OPs on the sorbent phase. Extraction efficiency, analysis time, reproducibility and sensitivity have been compared. This work showed that Magnetic-IT-SPME can be extended to OPs with successful results in terms of simplicity, speed, extraction efficiency and limit of detection. Finally,

wastewater samples were analysed to determine OPs at nano-grams per litre.

Introduction

Hybrid magnetic nanoparticles (NPs) have been widely used to develop new (micro)extraction techniques. In this context, most applications are based on their use for magnetic solid-phase extraction (MSPE) or dispersive (micro)solid-phase extraction (D-(μ)SPE). These adsorbents exhibit superpara-magnetic behaviour that makes them especially attractive for MSPE because they can be concentrated and separated from solutions easily at low magnetic field gradients [1, 2]. These methods, however, require several sample pretreatment steps. In this respect, in-tube solid-phase microextraction (IT-SPME) can overcome this limitation as it facilitates sample processing, especially in hyphenated liquid chromatography (LC) techniques. Nevertheless, the main drawback of IT-SPME is the poor extraction capacity. Recently, we reported the use of silica supported Fe$_3$O$_4$ magnetic nanoparticles (MNPs) as sorbent phase for IT-SPME (Magnetic-IT-SPME) [3]. In this approach, a magnetic hybrid material was immobilized on the surface of a bared fused silica capillary column and utilized as injection loop providing quantitative extraction efficiencies for pharmaceutical emerging pollutants.

Herein, the objective of this work has been to study the adsorption of organophosphorous compounds (OPs) on a silica supported Fe$_3$O$_4$ NPs sorbent material. This knowledge

Y. Moliner-Martinez · R. A. González-Fuenzalida ·
P. Campíns-Falcó (✉)
Departamento de Química Analítica, Facultad de Química, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain
e-mail: pilar.campins@uv.es

Y. Vitta
School of Chemistry, Faculty of Science, Central University of Venezuela Paseo los Ilustres, Urb. Valle Abajo, 20513 Carracas, Venezuela

H. Prima-Garcia · A. Ribera · E. Coronado
Instituto de Ciencia Molecular (ICMol), Universidad de Valencia, Catedrático José Beltrán 2, 46980 Paterna, Valencia, Spain

has been used to develop MSPE and Magnetic IT-SPME extraction procedures. Additionally, the hybrid material has also been proved useful for IT-SPME, increasing the number of existing adsorbent phases for this technique. Chlorfenvinphos and chlorpyrifos (OPs) are widely used pesticides. The European Directive 2008/105/EC includes those compounds as priority pollutants due to their hazardous nature, persistence and water solubility and gives environmental quality standards for both OPs [4]. The results showed the potential application of Magnetic-IT-SPME for these pollutants. The paper is mainly focused for improving on-line IT-SPME in two ways: introducing new phases and searching new interactions in order to achieve better extraction efficiencies.

Experimental section

MSPE procedure

Fifty milligrams of magnetic sorbent material was added to 20 mL of standards or water samples. After stirring during 10 min, the sorbent was isolated from the solution with a Nd disk magnet. The sorbent was air-dried to eliminate the excess water (30 s). Subsequently, the analytes were extracted with 500 μL of methanol and isolated with the magnet. Then, 1 mL of water was added and the extracts were processed (see Electronic supplementary material).

Magnetic-IT-SPME procedure

Magnetic-IT-SPME was carried out by the approach developed in our group [3] (see Electronic supplementary material). Aliquots of 3 mL of the standards or samples were directly loaded into the magnetic capillary column at 10 μL/s using a syringe at different magnetic fields (B). The schematic diagram of the system is shown in Fig. S1 (Electronic supplementary material). The analytes were desorbed from the capillary column combining the mobile phase and the change of magnetic field polarity. Finally, the analytes were separated in the analytical column. After each injection, the capillary column was cleaned with 300 μL of methanol.

Water samples

Wastewater samples (effluents) were collected at different water treatment plants of the Comunidad Valenciana. Samples were directly analysed. After the arrival to the laboratory, water samples were stored in dark in brown glass flasks at 4 °C until analysis. Each sample was analysed in triplicate and at room temperature. External calibration was used for quantitation.

Results and discussion

Adsorption isotherms

The magnetic hybrid material is a highly porous network formed by Fe_3O_4–cetyltrimethylammonium bromide (CTAB) micelles and surfactant micelles embedded along the silica matrix (see Fig. S2a, Electronic supplementary material) providing the hydrophobic sorbent material [5]. Besides, the calculated atomic spin flip time (τ_0) demonstrated that the NPs are non-interacting (data not shown). Additionally, Fe_3O_4 NPs along the silica matrix are well dispersed, isolated and susceptible to small magnetic fields [3].

Langmuir and Freundlich adsorption isotherms were examined to illustrate the nature of the interaction between the hybrid material and the OPs. The correlation coefficients (R^2) (see Table S1, Electronic supplementary material) indicated that the adsorption can be better demonstrated by the Langmuir isotherm compared to the Freundlich model. This result suggested that the analytes were adsorbed on the homogeneous adsorption sites that CTAB micelles created in the hybrid material. This phenomenon is supported by the previously reported results where the adsorption properties were mainly due to hydrophobic interactions between the alkyl chain of CTAB micelles and the analytes.

MSPE procedure

To test the performance of the hybrid material in the determination of OPs, the following extraction conditions: amount of sorbent, volume of sample, contact time and elution solvent were first optimized at 50 mg, 20 mL, 60 min and 500 μL of methanol, respectively (see Fig. S3, Electronic supplementary material). Finally, 1 mL of water was added to the methanolic extracts (500 μL) and processed (see Electronic supplementary material). The absolute extraction efficiencies for chlorfenvinphos and chlorpyrifos were 92±4 and 97±3 %, respectively. Figure 1a illustrates the chromatogram obtained for a mixture of OPs. As can be seen, other compounds present in water were also eluted under the experimental conditions. Nevertheless, they did not interfere in the determination of OPs.

The viability of the use of the hybrid material for OP detoxification of waters was also investigated. Detoxification efficiency was determined as a function of the concentration in the supernatant (see Electronic supplementary material for further details). The results after the removal procedure demonstrated good removal efficiencies: 95±5 and 100±6 % for chlorfenvinphos and chlorpyrifos, respectively. Figure 1b shows the chromatogram obtained for a spiked water sample before and after the detoxification procedure.

In this context, the stability of the sorbent material was studied (see Fig. S4, Electronic supplementary material). The

Fig. 1 **a** Chromatogram obtained for a mixture of chlorfenvinphos and chlorpyrifos (10 $\mu g\ L^{-1}$) using MSPE procedure. **b** Chromatogram obtained for a spiked wastewater sample (3 and 4 $\mu g\ L^{-1}$ for (*1*) chlorfenvinphos and (*2*) chlorpyrifos, respectively) before (*dashed line*) and after (*solid line*) the removal procedure
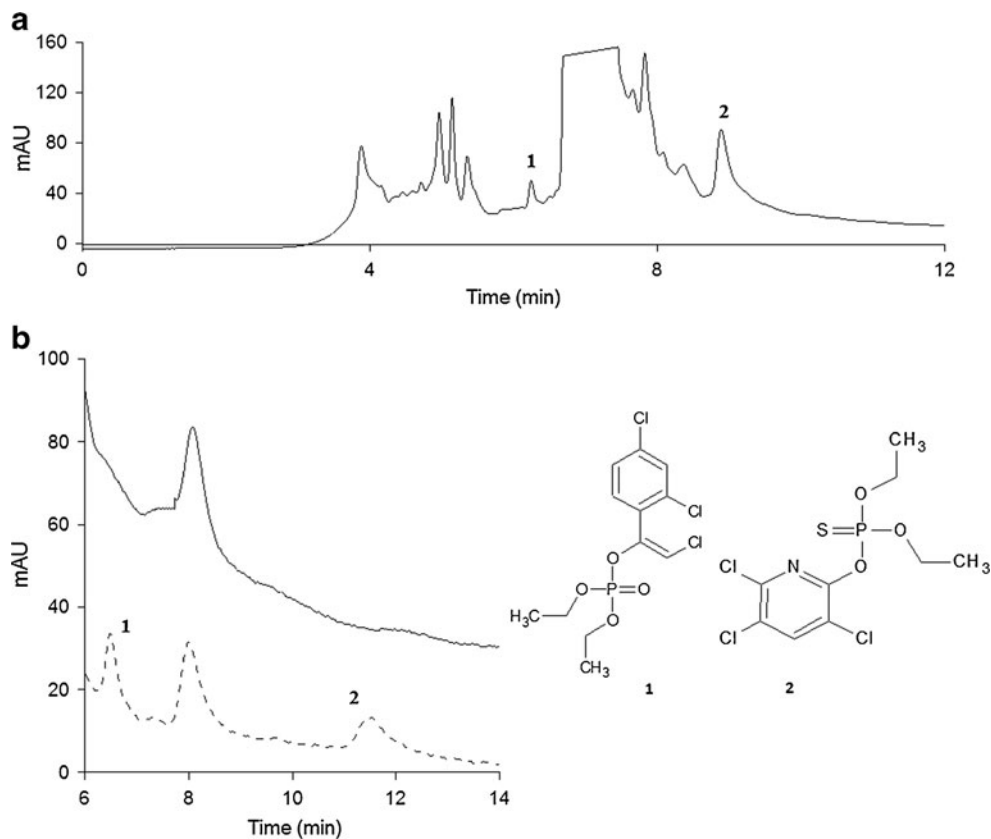
Fe/Si ratio remained constant; thus, Fe leaching was negligible in 40 uses. Nevertheless, the Br/Si ratio decreased in 20 uses. Besides, the percent adsorption of the analytes remained constant after 20 uses. These results indicated that Fe_3O_4-CTAB and CTAB micelles are structural units of the sorbent material and leaching was negligible providing clean extracts when the number of uses was below 20. Therefore, the hybrid material is a suitable, magnetically separable adsorbent to remove OP pollutants at trace level.

Magnetic-IT-SPME procedure

An improved approach of the silica supported Fe_3O_4 NPs is the immobilization as adsorbent phase on the bared surface of a capillary column (see Fig. S2b, Electronic supplementary material). In this approach, a mixture of the target analytes was processed and the extraction efficiency was evaluated as a function of B. The results for both analytes are presented in Fig. 2. Both analytes showed a similar tendency with a maximum in $B=200$ G, giving rise to absolute extraction efficiencies of 60 ± 3 and 84 ± 5 % for chlorfenvinphos and chlorpyrifos, respectively.

The study of the extraction efficiency if $B=0$ G provided values of 33 ± 2 and 64 ± 3 % for chlorfenvinphos and chlorpyrifos, respectively. Besides, the absolute extraction efficiencies obtained with existing sorbent phases such as polymer polydimethylsiloxane (PDMS) (TRB5) were estimated in the range of 25–29 % for both analytes. Therefore, the hybrid material improved the extraction efficiency of OPs when this capillary column was used as injection loop in a conventional IT-SPME (without a B). Alternatively, a significant improvement of the extraction efficiencies was achieved if an external B is applied. In the first case, hydrophobic interactions governed the adsorption capacity of the target analytes. Meanwhile, in the Magnetic IT-SPME approach, the extraction efficiency is improved by combining the hydrophobic interactions and the magnetic forces generated by the nanomagnets.

The processed sample volume was also optimized (see Fig. S5, Electronic supplementary material). The high processed volumes suggested a strong interaction between the analytes and the adsorbent phase, which agreed with the results obtained when $B=0$ G. The chromatogram obtained for a mixture of chlorfenvinphos and chlorpyrifos is depicted in Fig. 2c. Notice that the pretreatment step is fully automated with this approach.

Analytical performance

Table 1 shows the analytical parameters obtained for both procedures. Good linearity was obtained and precision (percent RSD) was also suitable.

Limits of detection (LODs) for chlorfenvinphos and chlorpyrifos with the MSPE–capillary LC–diode array detection (MSPE-CapLC-DAD) were 100 and 50 ng L^{-1}, respectively. Note that these values allow the use of this procedure to control the contamination of water with OPs (maximum

Fig. 2 Extraction efficiency (percent) as a function of the B obtained for **a** 10 μg L^{-1} chlorfenvinphos and **b** 3 μg L^{-1} chlorpyrifos. Injection 200 μL, $B_{retention}=B$ (gauss) and $B_{elution}=B$ (gauss) (reversed polarity). **c** Chromatograms of a mixture of 10 μg L^{-1} chlorfenvinphos (*1*) and 2 μg L^{-1} chlorpyrifos (*2*), $B_{retention}=200$ G and $B_{elution}=200$ G (reversed polarity). Injection 3 mL, mobile phase: methanol/water 95:5 (*v/v*) 6 μL min^{-1}
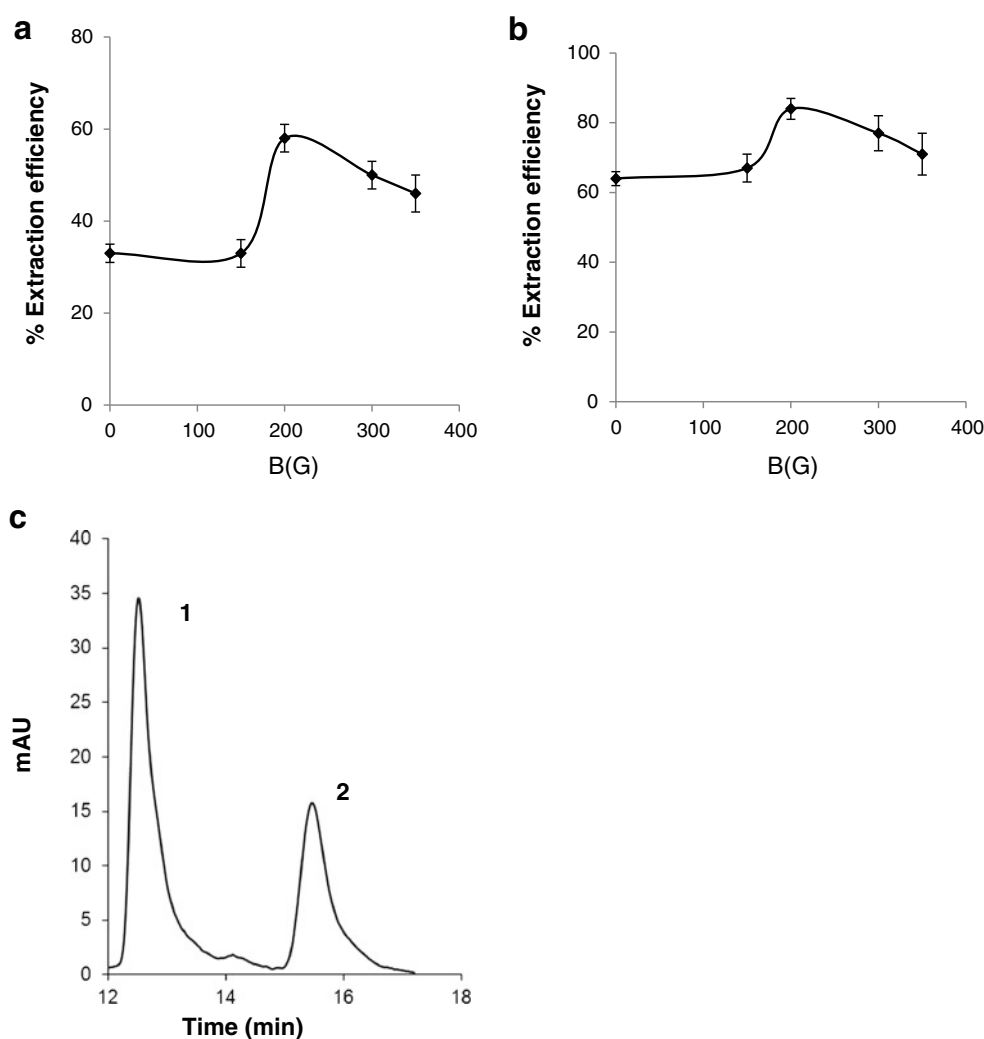
acceptable concentration (MAC) values are 300 and 100 ng L^{-1} for chlorfenvinphos and chlorpyrifos, respectively). The Magnetic-IT-SPME (see Table 1) approach improved the LODs compared to those achieved with existing sorbent phases such as PDMS for OPs in accordance with the best efficiencies achieved. It should be noted that these experiments were carried out by using a short particulate column as analytical column (see Electronic supplementary material). The use of a longer particulate column or a monolithic capillary column would even improve the sensitivity of this approach. No further research has been made because the achieved LODs are suitable for testing the European Directive [4].

Table 2 compares the studied extraction procedures. In MSPE, magnetism is used as a separation tool; this possibility

Table 1 Analytical parameters obtained for chlorfenvinphos and chlorpyrifos with MSPE-CapLC-DAD and Magnetic-IT-SPME-CapLC-DAD

Procedure		Analytical parameters				RSD (%)	
		$a \pm s_a$	$b \pm s_b$ (μg L^{-1})	R^2	LOD (ng L^{-1})	Intraday ($n=3$)	Interday ($n=6$)
MSPE-CapLC-DAD[a]	Chlorfenvinphos	7±2	4.9±0.2	0.9932	100	5	10
	Chlorpyrifos	−2±10	25±1	0.9910	50	7	13
Magnetic-IT-SPME-CapLC-DAD[b]	Chlorfenvinphos	3±20	90±4	0.9947	50	9	14
	Chlorpyrifos	82±53	500±7	0.9997	10	12	15

a monolithic analytical column, *b* particulate analytical column (for more information, see "Experimental section" and Electronic supplementary material)

Table 2 Comparison of some characteristics of the extraction techniques: MSPE, IT-SPME and Magnetic-IT-SPME using the silica supported Fe$_3$O$_4$ NPs as sorbent phase

Magnetism implementation		Sample treatment	Automatization	Analysis time (min)	LOD (ng L^{-1})	
					Chlorfenvinphos	Chlorpyrifos
Separation tool		MSPE (this work)	*	20	100	50
		MSPE (reference [4] SI)	*	20	–	290 (LOQ)[a]
		MSPE (reference [5] SI)	*	>30	–	4.3[b]
Variable in the extraction process	$H=0$	IT-SPME	****	18	85	15
	$H=B$	Magnetic-IT-SPME	****	18	50	10

SI supplementary information

[a] Ten millilitres of sample and GC-MS

[b] Eight hundred millilitres of sample, evaporation step (8 mL of acetonitrile) and HPLC-UV

serves to estimate the concentration of target analytes bearing in mind the legislated values. LODs achieved are compared with those provided by other materials proposed in the literature. However, it is limited due to the lack of automatization and sensitivity, whereas the use of the silica supported Fe$_3$O$_4$ NP capillary column in IT-SPME is notably more efficient, whether in absence of a magnetic field or when a magnetic field is applied. Particularly attractive is the use of Magnetic-IT-SPME. In this case, the magnetic field is a variable in the adsorption process, showing a significant improvement on the extraction efficiency and sensitivity and with a procedure completely automated.

Application to water samples

The methods were applied to the determination of the target analytes in wastewater samples. Chlorfenvinphos and chlorpyrifos were below LOD in the samples with both procedures. Satisfactory recoveries were obtained for spiked samples with mean values of 94±5 and 97±6 %, for chlorfenvinphos and chlorpyrifos, respectively.

Conclusions

This work demonstrated the potential of silica supported Fe$_3$O$_4$ NPs as adsorbent phase for MSPE and Magnetic-IT-SPME to determine OPs in water samples. The results obtained with MSPE-CapLC-DAD were satisfactory, taking into account the MAC values of these pollutants. Additionally, the adsorbent phase has proved to be useful to remove OP

pollutants in water samples. However, more favourable results were achieved with Magnetic-IT-SPME as a pretreatment step yielding extraction efficiencies of up to 60 and 84 % for chlorfenvinphos and chlorpyrifos, respectively. This approach provides a potential extraction procedure that avoids the pretreatment step and improves sensitivity in water analysis.

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