



Aptamer-modified magnetic nanoparticles for extraction of atrazine in environmental water samples

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ABSTRACT

In this work, a novel aptamer magnetic sorbent was prepared and used as magnetic solid-phase extraction sorbent for selective isolation of atrazine (ATZ) in water samples. The magnetic oligosorbent was fabricated by immobilizing DNA aptamer onto the surface of vinylized magnetic nanoparticles (VMNPs) via “thiol-ene” click chemistry reaction. After optimization of different aptamer coupling parameters, the following experimental conditions were found to give best results: 100 mg VMNPs incubated with 1 $\mu\text{mol L}^{-1}$ thiol-modified aptamer for 5 h at 55 °C. The resulting oligosorbent was characterized by different instrumental techniques such as transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR) and inductively coupled plasma mass spectrometry (ICP-MS). Then, several parameters of the extraction protocol were investigated to achieve the best ATZ extraction performance. The optimized aptamer-based magnetic solid-phase extraction (Apt-MSPE) protocol followed by HPLC with diode-array detection gave detection and quantification limits for ATZ of 0.005 $\mu\text{g L}^{-1}$ and 0.018 $\mu\text{g L}^{-1}$, respectively. The applicability of this protocol was demonstrated by analyzing several environmental water samples from different sources with recovery values from 85.5 to 94% and relative standard deviations less than 2.5%. Moreover, the developed Apt-MNPs sorbent could be re-used at least 12 times, without losses in its extraction capacity.

1. Introduction

Triazines are still used worldwide for controlling weeds in crop production, which causes the persistent contamination of aquatic environments and a subsequent health risk for ecosystems and humans [1,2]. Among triazines, atrazine (ATZ) is one of most employed herbicides, and regulations regarding its maximum residue limit in drinking water have been established by both US Environmental Protection Agency (3 $\mu\text{g L}^{-1}$ of ATZ) [3] and European Community (0.1 $\mu\text{g L}^{-1}$ individual pesticide) [4], because it can persist in soil and waters from several months to many years, and the exposure to this compound can affect endocrine, cardiovascular, immunological and reproductive human systems. [5,6].

All these considerations make mandatory the determination of triazines, and ATZ in particular, in environmental samples.

Due to this growing concern, many analytical methods have been recently developed for the analysis of triazines in water samples, among which gas chromatography (GC) and high-performance liquid chromatography (HPLC) with mass spectrometry (MS) [7–11] and UV [12,13] detectors have been reported.

Nevertheless, despite the advances in instrumentation during the last years, sample preparation is necessary for the evaluation of trace levels of triazines in the aquatic environment, but also for removing sample matrix interferences. In this context, various sample preparation techniques have been developed for the extraction of triazines from water

Abbreviations: ACN, acetonitrile; AIBN, azobisisobutyronitrile; Apt-MSPE, aptamer-based magnetic solid-phase extraction; ATZ, atrazine; BB, Binding buffer; DACT, 2,3-diamino-6-chloro-S-triazine; DEA, des-ethyl atrazine; DIA, des-isopropyl atrazine; LPME, liquid-phase microextraction; MeOH, methanol; MNP, magnetic nanoparticle; MSPE, magnetic solid-phase extraction; PROM, prometryn; SELEX, Systematic Evolution of Ligands by Exponential enrichment; SIM, simazine; TBAZ, terbutylazine; TBAZ-OH, terbutylazine-2-hydroxy; TCEP, tris[2-carboxyethyl]phosphine; TEM, transmission electron microscopy; Tris, tris(hydroxymethyl)amino-methane; VMNP, vinylized MNP; VTMS, vinyltrimethoxysilane.

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samples [14] with good results such as solid-phase extraction (SPE) [12,15,16], solid-phase microextraction (SPME) [17], QuEChERS [18], and liquid-phase microextraction (LPME) [19,20], among others.

In the last years, magnetic solid-phase extraction (MSPE) has been demonstrated to be a highly efficient, simple, low-cost and environmentally friendly strategy for the extraction of target compounds from complex matrices [21]. In MSPE, magnetic materials, which can be easily separated from the matrix by an external magnet, are used as sorbents for the extraction of target analytes. The performance of the adsorbent is a key factor on the extraction efficiency in MSPE. In this sense, different types of magnetic adsorbents such as magnetic carbon-based materials [22–24], magnetic silica-based materials [25] and magnetic framework composites [26] have been reported for the pre-concentration of these pollutants in different matrices.

Moreover, in order to increase selectivity of MSPE procedures, several affinity ligands such as molecularly imprinted polymers (MIPs) and aptamers have received a growing interest in the last years [27,28]. Indeed, various magnetic MIPs have been developed and used for the extraction of triazines [29,30]. However, the preparation of MIPs requires a large amount of the target analyte (or one of its analogue), which could be a handicap when the study of a scarce or expensive analyte is carried out. Moreover, analyte removal from the MIP is sometimes time-consuming and tedious depending on the physico-chemical properties of both analyte and monomers. Also, these systems show high cross-reactivity, exhibiting high recognition to related and sometimes to apparently unrelated molecules.

Aptamers, frequently referred as “synthetic antibodies”, are relatively short (20–60 nucleotides) single-stranded DNA or RNA molecules that bind with high affinity and specificity to target compounds, such as small molecules, peptides or proteins. They are commonly selected from a random bank by an *in vitro* combinatorial selection method called Systematic Evolution of Ligands by Exponential enrichment (SELEX). These selective ligands are attractive alternatives to antibodies due to their easy, highly reproducible synthesis, large stability and convenient chemical modification, among other features [31]. Aptamers show also certain benefits over MIPs such as their reproducible production, which provides a homogeneity of the binding sites, limited cross-reactivity and their easy implementation in aqueous medium, which renders their applicability more straightforward than that of MIPs [32,33]. Recently, several methodologies [34–36] have been developed for aptamers combined with functional magnetic nanoparticles (MNPs) to achieve selective sample extraction or detection.

Regarding ATZ, different variants of SELEX have been described to identify aptamers with good affinity and specificity toward this target compound [37–39]. Indeed, these aptamers have been used to develop biosensors for the monitoring of this pollutant in soil and water samples [6,40–42]. Until now, the application of aptamer in MSPE as efficient and selective materials for ATZ extraction has not been reported.

Inspired by high affinity and selectivity of aptamers, in this work, a novel aptamer-based magnetic (Apt-MSPE) sorbent was developed, and the conditions of the extraction protocol were optimized for the enrichment of ATZ from environmental water samples. The Apt-MSPE protocol was combined with HPLC-DAD, which allowed the determination of ATZ at low levels necessary to guarantee an environmental control of this pollutant and assure the human health.

2. Experimental

2.1. Chemicals, reagents and solutions

ATZ was purchased from Acros organics (Geel, Belgium). Other triazine herbicides such as simazine (SIM), prometryn (PROM), terbutylazine (TBAZ), terbutylazine-2-hydroxy (TBAZ-OH); ATZ metabolites such as des-ethyl atrazine (DEA) and des-isopropyl atrazine (DIA); and other organic reagents such as vinyltrimethoxysilane (VTMS), azobisisobutyronitrile (AIBN) and tris(hydroxymethyl)aminomethane (Tris)

were acquired from Sigma-Aldrich (Seelze, Germany), whereas 2,3-diamino-6-chloro-S-triazine (DACT) and tris[2-carboxyethyl]phosphine (TCEP) were acquired from TCI (Tokio, Japan).

Reagents such as KCl, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, NH_3 , HCl and H_3PO_4 were from Scharlab (Barcelona, Spain), and NaCl, NaH_2PO_4 and NaOH from Pan-reac (Barcelona, Spain) were employed. Iron salts such as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were acquired from Alfa Aesar (Kandel, Germany). Moreover, organic solvents such as ethanol, HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany), and anhydrous- N_2 was supplied by Abelló Linde (Valencia, Spain). Ultra-pure water was obtained with a Puranility TU6 water purification system from VWR (Barcelona, Spain) provided with a 0.2 μm filter.

The 5'-thiol-modified single-stranded DNA aptamer against ATZ [37] with a C6 spacer arm (5'-SH-C6TAC TGT TTG CAC TGG CGG ATT TAG CCA GTC AGT G -3') was synthesized and purified by HPLC by Integrated DNA Technologies (IDT, Leuven, Belgium). The aptamer was dissolved in nuclease-free sterile water (VWR) to obtain a final concentration of 100 $\mu\text{mol L}^{-1}$.

The binding buffer (BB) composed of 20 mM Tris, 100 mM NaCl, 2 mM MgCl_2 at pH 7.4 was daily prepared from a concentrated BB solution (420 mM Tris, 2100 mM NaCl, 42 mM MgCl_2) stored at 4 °C.

Stock solutions (400 $\mu\text{g mL}^{-1}$) of the triazine herbicides or ATZ metabolites were prepared in MeOH and kept at 4 °C. The standard solutions that were subjected to the Apt-MSPE process (1.5–12.5 $\mu\text{g L}^{-1}$) were daily prepared by appropriate dilution of the stock solution in BB.

For the construction of external calibration curve, standard solutions of ATZ (0.02–0.5 mg L^{-1}) were daily prepared by diluting the stock solution in MeOH.

Eppendorf vials (1.5 mL) (Eppendorf, Hamburg, Germany) and polypropylene tubes (160 mL) (VWR) were also used. Before chromatographic analysis, all solutions were filtered with nylon or PTFE Phenex filter membranes (0.22 $\mu\text{m} \times 13 \text{ mm}$) from Phenomenex (Madrid, Spain).

2.2. Apparatus and instrumentation

Laboratory apparatus such as a Nahita 631 Plus drying oven (Vidrafoc, Valencia, Spain), an EppendorfTM thermomixer model F2.0, an Eppendorf centrifuge MiniSpin c (Eppendorf, Madrid, Spain), and a Rotabit orbital agitator (JP Selecta, Barcelona, Spain) were employed.

The morphology of the magnetic materials was characterized by transmission electron microscopy (TEM) using a Jeol, Tokyo, Japan) model JEM-1010 microscope operated at 100 kV. Fourier-transform infrared (FTIR) spectra of materials were carried out using a Tensor 27 spectrometer from Bruker (Bremen, Germany). It was constituted by a DLaTGS detector and a Dura Sample IR II attenuated total reflection (ATR) accessory from Smiths Detection Inc. (Warrington, UK) equipped with a nine-reflection diamond/ZnSe DuraDisk plate.

The aptamer concentration in initial solution (before coupling reaction), supernatant (after coupling reaction) and washing fractions was measured by UV-vis spectra with a HP 8453 diode array spectrophotometer (Hewlett Packard, Germany) equipped with a 100 μL quartz cell and UV-vis Chemstation software (Rev A.02.04a). The determination of phosphorous content in bare and aptamer-modified MNPs was also done by using an inductive coupling plasma equipment connected to mass spectrometer (ICP-MS) model 7900 (Agilent Technologies, Waldbronn, Germany).

Chromatographic analysis was performed using an Agilent 1260 Infinity HPLC instrument (Agilent Technologies) equipped with a quaternary gradient pump, a solvent degasser system, an autosampler, and a diode-array detector (DAD). The chromatographic system was controlled by an OpenLAB CDS LC ChemStation from Agilent (B.04.03).

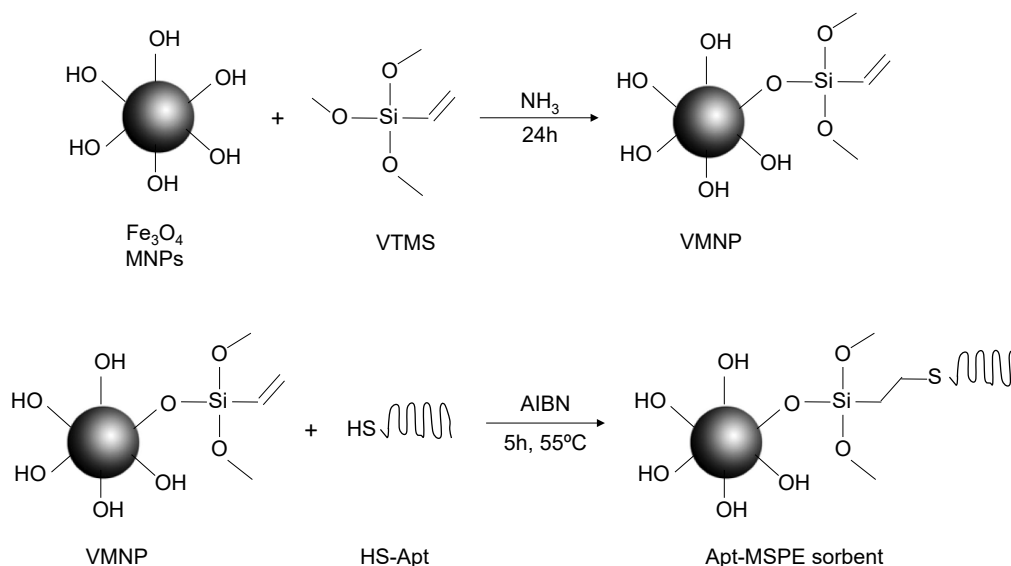


Fig. 1. Preparation scheme of synthesis of VMNPs and aptamer-MSPE sorbent via “thiol-ene” click reaction.

2.3. Synthesis and vinylization of MNPs

First, Fe_3O_4 MNPs were synthesized via chemical co-precipitation method and subsequently vinylized with VTMS to obtain VMNPs (Fig. 1). Both procedures were described in our previous paper [43] and can be found in the Electronic Supplementary Material (ESM).

2.4. Synthesis of aptamer-functionalized magnetic solid phase extraction sorbent

Before the synthesis, the thiol-modified aptamer solution ($100 \mu\text{mol L}^{-1}$) was subjected to the IDT aptamer reduction protocol with TCEP [44] to reduce the disulfide bond and release the thiol group. After that, aptamer folding treatment was applied by heating the solution at $95 \text{ }^\circ\text{C}$ for 10 min and cooling at $4 \text{ }^\circ\text{C}$ for 10 min in presence of 10 mM Tris buffer pH 7.4.

Then, the folded thiol-modified aptamer against ATZ was immobilized on VMNPs. The synthesis of the Apt-MSPE sorbent was accomplished by means of “thiol-ene” click reaction (see Fig. 1) following a previous work [45], but with modifications. 100 mg of VMNPs (weighed in an vial) were mixed with 0.9 wt% AIBN solution (prepared in $\text{ACN}:\text{H}_2\text{O}$ (50:50 v/v)), and then $10 \mu\text{L}$ of $100 \mu\text{mol L}^{-1}$ solution of the folded thiolated aptamer were added to the vial and the mixture was vortexed for 1 min. Afterwards, the vial was gently shaken (900 rpm) in a thermomixer at $55 \text{ }^\circ\text{C}$ for 5 h to complete the “thiol-ene” click reaction. After the reaction, the synthesized Apt-MSPE sorbent was magnetically separated from the supernatant with the aid of an external neodymium magnet, and the MNPs coated with aptamer were washed three times with 10 mM Tris buffer pH 7.4. The amount of bound aptamer and the coupling efficiency were calculated by subtraction of the aptamer amount in the supernatants and washing fractions from that in the initial solution from UV measurements (absorbance at 260 nm). Afterwards, the Apt-MNPs were resuspended in BB and stored at room temperature until its use.

2.5. Adsorption experiments

Several adsorption experiments were carried out to assess the binding ability of the developed Apt-MNP sorbent. The kinetic adsorption tests were carried out by adding 100 mg of the sorbent to a 1 mL solution of $300 \mu\text{g L}^{-1}$ of ATZ, and stirring it for different times (5–60 min) at $25 \text{ }^\circ\text{C}$. On the other hand, isothermal adsorption experiments were

carried out similarly, by stirring 100 mg of the sorbent in 1 mL of solutions with different concentrations ($0.05\text{--}70 \text{ mg L}^{-1}$) for 5 min at $25 \text{ }^\circ\text{C}$. In both cases, after the adsorption, the remaining concentration of ATZ in the solution was determined by HPLC-UV. The adsorption amounts were calculated as $Q = (C_0 - C) V/m$, where C_0 and C are the initial and final concentrations, V is the solution volume, and m is the mass of sorbent.

2.6. Sample preparation

Six real water samples were collected from different sources: mineral (S1), river (S2), wetland (S3), well (S4), tap (S5) and ditch (S6). The samples were collected in amber glass bottles, filtered in the laboratory with $0.22\text{-}\mu\text{m}$ nylon filters (Phenomenex, Torrance, CA, USA) and stored at $4 \text{ }^\circ\text{C}$ in the refrigerator. Before extraction, an aliquot of water sample was taken, and it was properly diluted with concentrated BB to reach the final BB composition.

2.7. Apt-MSPE protocol

First, 100 mg of Apt-MNPs were mixed with 10 mL of standard solution of ATZ (concentration between 1.5 and $12.5 \mu\text{g L}^{-1}$) (or sample) and 0.5 mL of concentrated BB solution in a polypropylene tube. Then, the mixture was stirred for 15 min at $25 \text{ }^\circ\text{C}$ to completely trap the target analyte. After that, the sorbent was magnetically isolated from the solution and the supernatant was discarded. Next, ATZ was desorbed from the Apt-MNP sorbent using 1.0 mL of MeOH under vortex agitation for 5 min. The magnetic sorbent was separated by an external magnet from the organic solvent. Then, the eluate was transferred to a 2 mL microcentrifuge tube and evaporated under nitrogen flow at room temperature. The residue was dissolved in 0.25 mL MeOH for HPLC analysis. Finally, an aliquot ($20 \mu\text{L}$) of the resulting solution was injected into the HPLC-DAD system.

2.8. Chromatographic analysis

The chromatographic analysis of ATZ, its metabolites (DACT, DEA and DIA) and other triazines (TBAZ-OH, SIM, PROM and TBAZ) was carried by using a Zorbax Eclipse Plus C18 ($10 \text{ cm} \times 4,6 \text{ mm I.D.}$; $3,5 \mu\text{m}$ particle size, Agilent Technologies) column. Isocratic elution was employed using a mobile phase containing 50% of aqueous phase (containing 15 mM phosphoric acid at pH 3.0) and 50% ACN at a flow

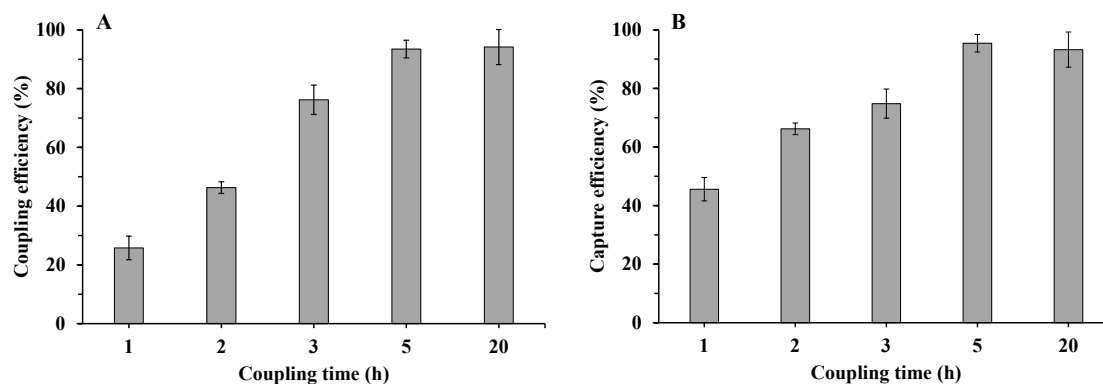


Fig. 2. Influence of coupling time on aptamer immobilization onto MNPs (A) and capture efficiency of ATZ of Apt-MNPs prepared at different coupling times (B). Coupling conditions (trace A): 100 mg vinylized MNPs, thiol aptamer solution (final concentration, $1 \mu\text{mol L}^{-1}$), initiator (0.9 wt% AIBN in 1 mL ACN:H₂O (50:50 v/v)), “thiol-ene” click reaction at 55 °C. Retention conditions (trace B): 5 mL aqueous standard solution of ATZ ($60 \mu\text{g L}^{-1}$), 100 mg Apt-MNPs, adsorption time 30 min at 25 °C. Error bars show the standard deviation of the results ($n = 3$).

rate of 0.8 mL min^{-1} for ATZ and the other triazines. The chromatographic analysis of ATZ metabolites required less content of ACN in the initial mobile phase of (such as 10% or 30%). In all cases, the injection volume was 20 μL , and detection wavelength was set at 225 nm.

2.9. Method validation

The quantitation of ATZ was validated in terms of linearity range, sensitivity, precision and accuracy. The linear range was established by analyzing standard solutions of ATZ at concentrations ranging from 0.5 to $12.5 \mu\text{g L}^{-1}$. An external calibration approach was used. The instrumental limits of detection (LOD) and quantification (LOQ) were experimentally obtained as the concentration of ATZ that provided a signal-to-noise ratio (S/N) of 3 and 10, respectively.

On the other hand, the reproducibility in the preparation of Apt-MNP sorbent was investigated with aqueous solution samples containing $7.5 \mu\text{g L}^{-1}$ of ATZ in BB solution. The relative standard deviation (RSD) of ATZ recovery was calculated.

The accuracy of the method was evaluated through a recovery study, where different blank water samples were fortified with the target analyte at three concentration levels (1, 2.5 and $7.5 \mu\text{g L}^{-1}$).

3. Results and discussion

3.1. Preliminary studies of aptamer coupling onto MNPs and characterization

Thiol aptamer specific to ATZ was covalently coupled onto the surface of vinylized MNPs (VMNPs) by “thiol-ene” click chemistry to prepare aptamer-MNPs (Apt-MNPs). Several parameters such as coupling time and aptamer content were investigated.

In order to investigate coupling time, coupling efficiency and capture efficiency of target analyte were examined. These experiments were performed in triplicate. Coupling efficiency was firstly evaluated by quantifying the aptamer at 260 nm before and after coupling reaction, as indicated in Section 2.4. The Apt-MNPs were washed several times to evaluate whether the binding was covalent or by physical adsorption onto surface. The results (depicted in Fig. 2A) demonstrated that aptamer was covalently coupled to the surface of MNPs. As shown in this figure, a gradual increase in the coupling efficiency with increasing coupling time from 1 to 5 h is found, reaching a plateau after this time, with an average coupling efficiency of approximately 94%. The maximum coverage density (in pmol/mg) of the Apt-MNP sorbent was 92.5 after 5 h of coupling reaction.

Then, the capture efficiency of the Apt-MNPs prepared at different coupling times for ATZ extraction was evaluated. To perform these

retention studies, ultrapure water solutions (5 mL) containing $60 \mu\text{g L}^{-1}$ of ATZ were used. Other experimental conditions selected were: 100 mg Apt-MNPs and adsorption time for 30 min at 25 °C. In order to evaluate the capture (retention) efficiency, the percentage of captured herbicide over the total initial amount before and after retention step was found out. As shown in Fig. 2B, the retention efficiency of resulting Apt-MNPs increased with their preparation (coupling) time, which is consistent with the previous trend. Hence, 5 h was selected as coupling time for further experiments.

Next, aptamer content was studied. Different concentrations of aptamer solution from 0.5 to $5 \mu\text{mol L}^{-1}$ were used to prepare Apt-MNPs. The results showed that concentrations above $0.5 \mu\text{mol L}^{-1}$ gave satisfactory capture efficiencies (>90%), achieving the maximum response of ATZ captured with a level of aptamer at $1 \mu\text{mol L}^{-1}$, and kept almost constant when a higher concentration of aptamer was used.

Therefore, the reaction conditions employed to achieve a maximum aptamer coupling onto MNPs were: 100 mg VMNPs were mixed with a thiol aptamer solution (final concentration, $1 \mu\text{mol L}^{-1}$) and with thermal initiator (0.9 wt% AIBN in 1 mL ACN:H₂O (50:50 v/v)). The reaction mixture was incubated for 5 h at 55 °C.

Also, the morphological structure of the prepared magnetic nanoparticles, the vinylized MNPs and Apt-MNPs, were characterized by transmission electron microscope (TEM). As shown in Fig. S1, both magnetic nanomaterials showed similar sizes with average diameters of 15 nm and 17 nm, respectively.

FT-IR spectroscopy was also used to identify the modification of MNPs with aptamer (see Fig. S2). The presence of an absorption peak at 550 cm^{-1} , which corresponds to the Fe–O of magnetite phase, was present in the synthesized magnetic materials. The presence of an absorption band around 1050 cm^{-1} attributed to the stretching bonds of Si–O–Si, and the peak at 1620 cm^{-1} due to C = C stretching vibrations, confirmed the vinylization process (Fig. S2, trace a). After assembly of thiol aptamer onto MNPs (Fig. S2, trace b), a slight weakening of absorption peak corresponding to the C = C was observed after “thiol-ene” click chemistry, jointly with the emergence of a band at 1650 cm^{-1} , which corresponds to heterocyclic structure (thymine) of aptamer. Additionally, ICP-MS analysis was carried out to corroborate the attachment of aptamer onto MNPs. Thus, Apt-MNPs showed contents of phosphorus ($0.043 \pm 0.001\%$) and sulfur ($0.008 \pm 0.001\%$), whereas a control of MNPs only provided small amounts of phosphorus ($0.0027 \pm 0.0001\%$), probably due to a cross-contamination associated with the reagents’ impurities.

3.2. Optimization of Apt-MSPE protocol

In order to achieve a high extraction performance of Apt-MNPs

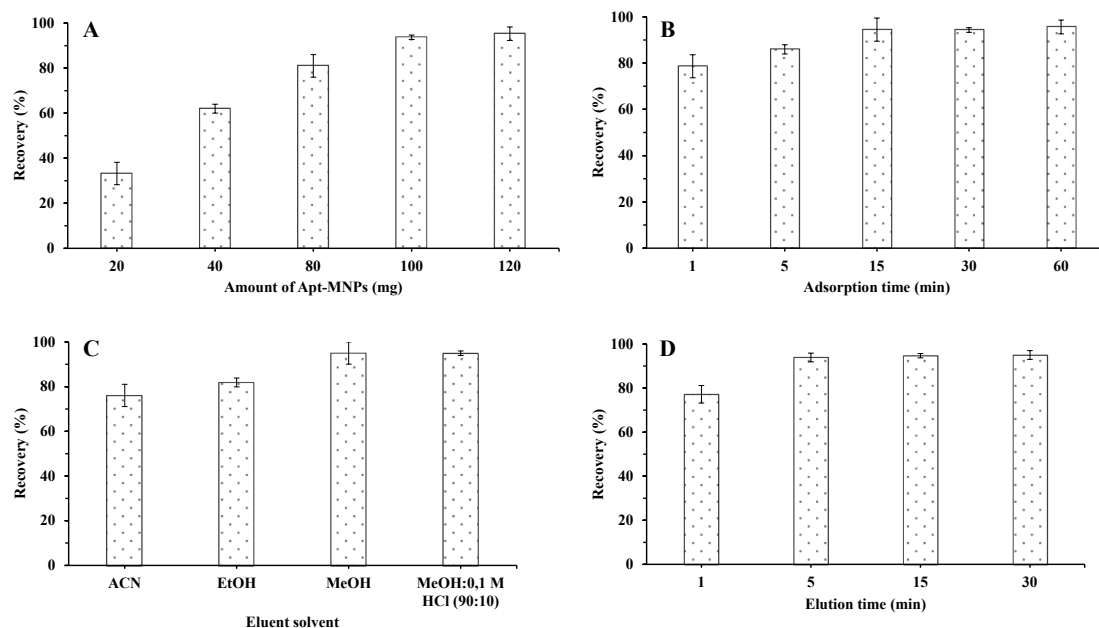


Fig. 3. Effect of several parameters on the extraction recovery of ATZ using Apt-MNPs as sorbent: (A) Amount of Apt-MNPs, (B) adsorption time, (C) elution solvent and (D) elution time. Experimental conditions (except the parameter under investigation): sample volume, 5 mL aqueous standard solution of ATZ ($60 \mu\text{g L}^{-1}$); amount of Apt-MNPs, 100 mg; adsorption time, 30 min; elution time, 15 min; eluent, 1 mL MeOH. Error bars show the standard deviation of the results ($n = 3$).

toward ATZ, several parameters in the capturing and elution steps were investigated. Thus, the effect of the mass of Apt-MNPs, adsorption time, pH and temperature were investigated. These optimization studies were carried out using 5 mL of ultrapure water solution containing $60 \mu\text{g L}^{-1}$ of ATZ. Also, an adsorption time of 30 min was firstly selected in order to ensure high material-analyte contact, and an initial elution with 1 mL of MeOH for 15 min was chosen. The recovery of ATZ was employed to evaluate the extraction efficiency under different conditions. All experiments were performed in triplicate.

Fig. 3A shows the effect of the dosage of Apt-MNPs (from 20 to 120 mg) on the extraction recovery of ATZ. As it can be seen, when the amount of the sorbent increased from 20 to 100 mg, the extraction recoveries of ATZ were significantly improved. At higher amounts, the recovery values kept almost constant, suggesting that 100 mg Apt-MNPs was appropriate for capturing of the target analyte, and it was selected for further studies.

The effect of adsorption time was investigated to provide enough contact and reach and adsorption equilibrium between Apt-MNPs and ATZ. As shown in Fig. 3B, quantitative recoveries of ATZ ($>85\%$) were obtained from 5 min onwards. A slight increase was observed in the extraction yield from 1 to 15 min, and then it remained constant. Therefore, the capturing time was fixed in 15 min from now on.

The influence of pH of the BB on the ATZ recovery was also considered by changing the solution pH between 6.6 and 8. The results showed that no significant variations in ATZ recoveries were observed compared to those obtained at pH 7.4, which is consistent with the optimal BB condition obtained in the SELEX process [37], and this pH was selected for further studies.

The effect of adsorption temperature was also investigated on the extraction efficiency. As shown in Fig. S3, the maximum extraction efficiency was obtained, when the Apt-MNPs and ATZ were incubated at 20°C . Further increasing the temperature resulted in a slight reduction of the recovery. Therefore, an extraction temperature of 20°C was employed for subsequent studies.

Also, eluting conditions have a significant contribution on the extraction efficiency. Thus, eluent and elution time were optimized to efficiently desorb ATZ from Apt-MNPs. The elution of ATZ was studied using different several commonly used organic solvents, i.e., ACN, EtOH

and MeOH. Also, acidic methanol mixture (MeOH:HCl 90:10 (v/v), containing 0.12 M HCl) was considered taking into account that ATZ can be protonated under these conditions, which may be more efficient for the elution. Vortexing was used to facilitate the elution. The results in Fig. 3C showed that MeOH and acidic MeOH provided satisfactory elution ability higher than the other solvents since similar quantitative recovery results were obtained in both cases; being MeOH chosen for simplicity and convenience for further studies.

Also, different elution times (in the range 1–15) were investigated in this study. As shown in Fig. 3D, when the elution time increased from 1 to 5 min, the extraction recovery of ATZ ascended significantly, and then it remained stable. Therefore, 5 min was selected for subsequent experiments.

Next, the possibility of concentrating the final extract by evaporation and re-dissolution was assessed. For this purpose, methanolic solutions of ATZ were evaporated at room temperature with either air flow or under an N_2 stream, and then reconstituted in smaller MeOH volumes. No significant loss of the analyte (recoveries around 90%) was observed using $250 \mu\text{L}$ as final reconstitution volume, with either of the procedures. However, better precision results (variations below 10%) were obtained with nitrogen evaporation, and this protocol was selected for analyte preconcentration.

In order to achieve the maximum enrichment of ATZ different sample volumes (between 1 and 20 mL) of standard ATZ solution were investigated, being subjected to the optimized MSPE protocol. As shown in Fig. S4, large recoveries (around 90%) up to 10 mL were obtained for the tested analyte. Higher volumes than 10 mL led to a decrease in the recovery values. Consequently, this volume was adopted for the analysis of real water samples and, taking into account the final established reconstitution volume of 0.25 mL, the optimized Apt-MSPE protocol provided a theoretical enrichment factor of 40.

The reusability of the magnetic affinity sorbent was also evaluated by applying repeatedly the Apt-MSPE protocol (see Section 2.7) to extract an aqueous standard solution of ATZ at $15 \mu\text{g L}^{-1}$. As shown in Fig. S5, the Apt-MNPs can be reused at least 12 times, giving extraction yields higher than 80%.

Also, the storage stability of the Apt-MNPs was investigated over a 30-day period. When these were stored in BB solution at room

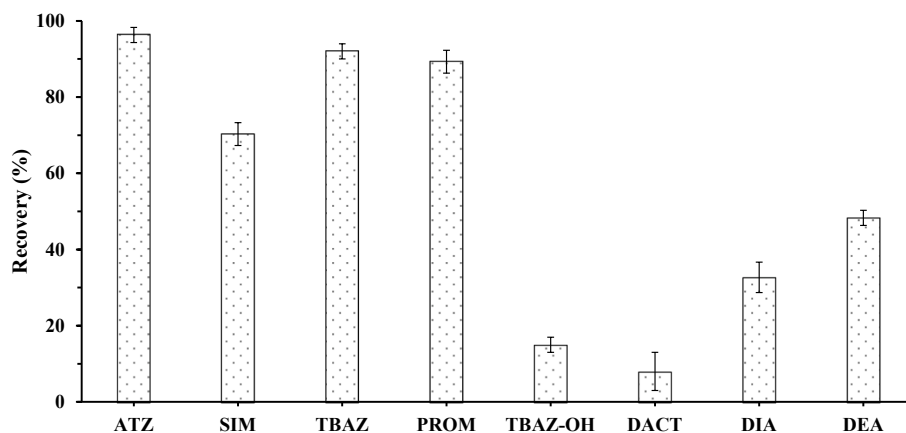


Fig. 4. Comparison of extraction efficiency of several triazine analogues and ATZ metabolites using the Apt-MNPs sorbent. Experimental conditions: sample volume, 10 mL standard solutions of triazine analogues, ATZ and its metabolites ($7.5 \mu\text{g L}^{-1}$); amount of Apt-MNPs, 100 mg; adsorption time, 15 min; elution time, 5 min; eluent, 1 mL MeOH. Error bars show the standard deviation of the results ($n = 3$).

temperature and measured periodically (every week), they showed that no significant change of extraction efficiency ($>85\%$) was observed over this period.

3.3. Adsorption ability of Apt-MNP sorbent

In order to examine the adsorption process of ATZ onto the developed sorbent, the adsorption kinetics and binding isotherms of ATZ onto the Apt-MNP sorbent were considered [47].

Regarding the kinetic study, the adsorption of ATZ onto the sorbent was quite fast, and only five minutes were needed to ensure its quantitative adsorption (Fig. S6A). Then, two adsorption kinetic models (pseudo-first and pseudo-second-order models [47]) were applied to the experimental data to understand the probable mechanism involved in ATZ adsorption on Apt-MNP. These models can be described as follows:

$$\ln(Q_e - Q_t) = \ln Q_e - k_1 \cdot t \quad (1)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 \cdot Q_e^2} + \frac{t}{Q_e} \quad (2)$$

where k_1 is the equilibrium rate constant of the pseudo-first order model, k_2 is the equilibrium rate constant of the pseudo-second order model, and Q_e and Q_t are the amount of ATZ adsorbed at equilibrium and any time, respectively.

As can be seen in Fig. S6B, the adsorption of ATZ fitted perfectly well with the pseudo-second-order model (Eq. (2), $R^2 = 0.9999$), in contrast to the pseudo-first-order model (Eq. (1), $R^2 = 0.5338$). The fitting curve parameters for both models are listed in Table S1.

On the other hand, the adsorption capacity of Apt-MNP sorbent was considered on the basis of adsorption isotherms using Langmuir (Eq. (3)) and Freundlich (Eq. (4)) models [47]:

$$\frac{C_e}{Q_e} = \frac{1}{Q_{\max} \cdot k_L} + \frac{C_e}{Q_{\max}} \quad (3)$$

$$\ln Q_e = \ln k_F + \frac{1}{n} \ln C_e \quad (4)$$

where k_L is the Langmuir isotherm constant (L mg^{-1}), Q_{\max} is the maximum adsorption capacity (mg g^{-1}), k_F is the Freundlich constant related to the adsorption capacity of the sorbent (mg g^{-1}), and n is the Freundlich constant related to adsorption intensity of the sorbent.

Thus, the experimental data were adjusted to these adsorption isotherm models, being these curves depicted in Fig. S7 and the calculated parameters of these models listed in Table S2. The resulting isothermal adsorption curves fitted better to the Langmuir model ($R^2 =$

0.9999) than to the Freundlich one ($R^2 = 0.9491$). In this sense, the maximum adsorption capacity (Q_{\max}) obtained from the Langmuir model was 0.32 mg g^{-1} , which was very similar to the experimental value (0.31 mg g^{-1}).

3.4. Selectivity of the Apt-MSPE sorbent

To evaluate the selectivity of the prepared magnetic oligosorbent, several triazine analogues (such as SIM, TBAZ, PROM and TBAZ-OH) as well as ATZ metabolites (DACT, DEA and DIA) were evaluated. Chemical structures of these compounds are shown in Fig. S8. To perform these selectivity experiments, the same LC conditions (see Section 2.8) that those used for ATZ were adopted for triazine analogues. Indeed, the triazine analogues (TBAZ-OH, SIM, TBAZ and PROM) eluted at different retention times than ATZ (a chromatogram of this mixture is given in Fig. S9). Concerning to the ATZ metabolites (DACT, DEA and SIA), these compounds eluted at the front of the chromatogram due to its higher polarity than ATZ and other triazines, being necessary to modify the mobile phase conditions (see Section 2.8). In any case, the extraction efficiency of each compound (at level of $7.5 \mu\text{g L}^{-1}$) can be investigated under the optimized Apt-MSPE conditions. As shown in Fig. 4, moderate or low cross-binding activities were evidenced on structurally related herbicides such as SIM and TBAZ-OH; however, TBAZ and PROM showed a larger retention, since these compounds have only small structural differences with ATZ, the aptamer-based sorbent showed a high recognition ability. Usually, this is not a problem since it is generally agreed in the aptamer research field that it is particularly more challenging to find specific aptamers for small molecules than for proteins [46], largely because of the less binding motifs on small molecules. On the other hand, metabolites of ATZ showed low recovery values, which can likely be due to their higher polarity (presence of one or more amino functional group in their structure) compared to ATZ (see Fig. S8). These results confirm the satisfactory selectivity of Apt-MNP sorbent.

3.5. Analytical figures of merit

The figures of merit of the optimized Apt-MSPE coupled with HPLC-DAD method were evaluated, and several analytical parameters were established according to that described in Section 2.9. A good linearity ($r > 0.999$) was observed in the range $0.5\text{--}12.5 \mu\text{g L}^{-1}$. The enrichment factor for ATZ was calculated by comparison of the slopes of the calibration equations before ($Y = (274 \pm 2) \cdot C_{\text{ATZ}} + (-1.3 \pm 0.4)$, $r = 0.9998$, where $Y = \text{peak area}$ and $C_{\text{ATZ}} = \text{ATZ concentration expressed as } \mu\text{g L}^{-1}$), and after the extraction process ($Y = (10560 \pm 130) \cdot C_{\text{ATZ}} +$

Table 1

Recovery study of ATZ in spiked water samples analyzed following the Apt-MSPE protocol.

Sample	Type of water	ATZ concentration ($\mu\text{g L}^{-1}$)	Recovery (%) \pm s (n = 3)
S1	Mineral	1	106 \pm 5
		2.5	92 \pm 4
		7.5	94 \pm 8
S2	River	1	102 \pm 6
		2.5	93.7 \pm 1.8
		7.5	92.9 \pm 1.5
S3	Wetland	1	94 \pm 4
		2.5	89 \pm 2
		7.5	87.8 \pm 1.4
S4	Well	1	98 \pm 5
		2.5	91 \pm 3
		7.5	88 \pm 8
S5	Tap	1	93 \pm 2
		2.5	87.6 \pm 1.9
		7.5	85.5 \pm 0.1
S6	Ditch	1	95 \pm 3
		2.5	90 \pm 2
		7.5	87 \pm 7

(-2.7 ± 0.6 , $r > 0.9994$). The resulting enrichment factor was close to 40, the theoretical value. The instrumental LOD and LOQ were 0.2 and $0.72 \mu\text{g L}^{-1}$ ATZ, respectively. The LOD and LOQ values for the proposed method were calculated considering the obtained instrumental values and enrichment factor for ATZ giving 0.005 and $0.018 \mu\text{g L}^{-1}$, respectively.

The precision of preparation reproducibility of Apt-MNPs was also evaluated as indicated in Section 2.9, giving RSDs values of ATZ recovery below 3.0% (n = 3) in one batch and lower than 4.9% (n = 3) among different batches.

3.6. Analysis of real water samples

To demonstrate the applicability of the Apt-MNPs sorbent, the

present method was applied to the determination of ATZ in six environmental water samples from different sources by following the same protocol as for standard solutions. ATZ was not detected in none of the samples analyzed. Next, validation samples were prepared using these samples fortified with ATZ at different concentration levels: 1, 2.5 and $7.5 \mu\text{g L}^{-1}$. As shown in Table 1, satisfactory recovery values (between 85.5 and 106%) were obtained in all cases, which indicate the good accuracy of the method. Fig. 5 shows the chromatograms of blank and spiked water sample using the developed Apt-MSPE-HPLC-DAD method.

3.7. Comparison with other extraction protocols

The analytical features of the Apt-MSPE-HPLC-DAD method presented in this work were compared with other recently published MSPE methods coupled with HPLC systems employed to analyze atrazine or a group of triazines in water samples (Table 2). As can be seen, the recoveries, precision, adsorption time of our method were similar to other works reported in the literature. Concerning enrichment factor and adsorption capacity, although our sorbent gave lower values than previous studies, the values obtained were good enough to extract and preconcentrate the usual concentrations of ATZ in real water samples. Besides, the present method showed lower LOD value than other methods, and it provided similar values to when high sensitive and sophisticated technique (like UPLC-MS/MS) was used [30,48,49]. Other remarkable feature of our method was the reusability of the Apt-VMNP sorbent. This type of data was not always reported and, among those studies that includes this information, only the sorbent developed by Tong et al. [50] could be used >12 times. This parameter makes our method a suitable alternative to the analysis of ATZ at trace levels. All these good features combined with the use of common equipment accessible (such as DAD detector) in most analytical laboratories make this procedure an attractive alternative for the analysis of trace ATZ in environmental matrices.

4. Conclusions

In this work, a novel efficient and selective sorbent was synthesized by immobilization of aptamer against ATZ onto the surface of MNPs by “thiol-ene” click reaction. Conjugation conditions of aptamer to these particles were optimized in order to achieve the highest efficiency coupling as well as to obtain the greatest capture efficiency of ATZ. The resulting magnetic oligosorbent was characterized and several

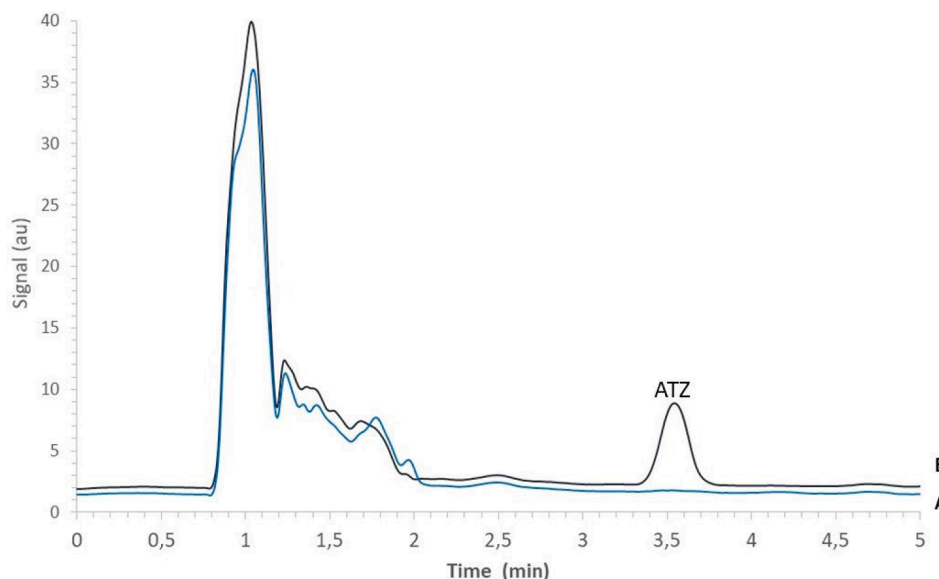


Fig. 5. HPLC chromatograms of a spiked water sample ($7.5 \mu\text{g L}^{-1}$ ATZ) (A) before (B) after applying the proposed Apt-MSPE protocol.

Table 2

Comparison of the proposed method with other recent magnetic extraction methods for the analysis of triazines in water samples.

Analytes	Water samples	Extraction procedure// Analytical method	Recovery (%)	LODs ($\mu\text{g}/\text{L}$)	Adsorption capacity (mg/g)	Enrichment factor	Sorbent reusability	Adsorption time	Ref
AMET, ATZ, CYAN	River, pond and farmland surface waters	IL-MG MSPE // HPLC-UV	97–101	0.09–15	8.27	83	–	15 min	[53]
AMET, dimethametryn, PROM, terbutryn, terbometon, trietazine	Commercial, tap, river and ground waters	MHFB-ILME // HPLC-DAD	73–109	0.15–0.48	–	66.7	–	16 min	[54]
AMET, ATZ, PROM, SIM	Irrigation and river waters	ZIF-8/SiO ₂ @Fe ₃ O ₄ MSPE// HFCLC-UV	89–100	0.18–0.72	–	125	–	3 min	[55]
ATZ, CYAN, PROP, SIM	River and swamp waters	Propazine-MMIP d-SPE // HPLC-DAD	75–94	0.16–0.51	–	200	–	30 min	[29]
ATZ	Farmland and lake and river and tap waters	Fe ₃ C/MnO/GC microspheres MSPE // HPLC-UV	87–107	0.01	–	50	25 times	10 min	[52]
ATZ	River and waste waters	US-d-LPME and MSPE // HPLC-UV	98–110	0.3	–	200	–	4 min	[56]
AMET, ATZ, PROM, SIM, terbutryn,	Farmland, reservoir, river, tap well and waters	MPC-CNC MSPE //UHPLC-MS/MS	74–117	0.003–0.007	–	75	12 times	16.5 min	[49]
AMET, ATZ, desmetryn, PROM, PROP, SIM	Pond, river and tap waters	MWNTs@Fe ₃ O ₄ @MIR MSPE // UHPLC-MS/MS	89–98	0.007–0.068	7.07	6.6	5 times	10 min	[50]
AMET, ATZ, desmetryn, PROM, PROP, SIM	Lake water	MIR MSPE // UHPLC-MS/MS	89–98	0.02–0.38	2.06	3.3	6 times	10 min	[51]
ATZ	Dich, mineral, river, tap, well and wetland	Apt-MSPE // HPLC-UV	86–106	0.005	0.32	40	12 times	15 min	This work

Abbreviations: AMET: ametryn; CYAN: cyanazine, dCG: graphitic carbon, d-SPE: dispersive solid-phase extraction; GC: graphitic carbon; IL-MG: Ionic liquid-magnetic graphene composite; MHFB-ILME: Magnetic hollow-fiber bar-ionic liquid microextraction; MMIP: magnetic molecularly imprinted polymer; MPC-CNC: Magnetic partially carbonized cellulose nanocrystals; MWNTs@Fe₃O₄@MIR: magnetic multi-walled carbon nanotubes imprinting resin; PROM: prometryn; PROP: propazine; UHPLC: Ultra-high performance liquid chromatography; US-d-LPME: ultrasonic assisted dispersive liquid phase microextraction; ZIF: zeolitic imidazolate frameworks.

parameters of the Apt-MSPE protocol were also investigated in detail. Under the optimal conditions, the combination of this protocol with HPLC-DAD analysis led to high extraction recovery values, low LOD, satisfactory enrichment factor (40) and suitable sorbent reusability (at least 12 times without losses in its extraction capacity). The whole method was successfully applied to the analysis of ATZ in real water samples from different sources with high selectivity and preconcentration ability, which demonstrated the promising characteristics of the Apt-MNP sorbent for trace level ATZ determination. To our knowledge, this work reports for the first time the application of “thiol-ene” click chemistry to the preparation of aptamer-modified magnetic nanomaterials for the extraction of this pollutant, being this strategy and the resulting magnetic oligosorbent attractive alternatives for convenient, efficient and selective extraction and preconcentration of harmful environmental pollutants and biological molecules in environmental, food and clinical fields.

CRediT authorship contribution statement

Sagrario Torres-Cartas: Methodology, Formal analysis, Investigation, Writing – original draft. **Susana Meseguer-Lloret:** Methodology, Formal analysis, Investigation, Writing – original draft. **Mónica Catalá-Icardo:** Writing – review & editing, Supervision. **Ernesto Francisco Simó-Alfonso:** Writing – review & editing, Supervision, Resources. **José Manuel Herrero-Martínez:** Writing – review & editing, Supervision, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2023.108902>.

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