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Determination of azoxystrobin and chlorothalonil using a methacrylate-based polymer modified with gold nanoparticles as solid-phase extraction sorbent

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Abstract

This paper describes a novel and sensitive method for extraction, preconcentration and determination of two important widely used fungicides, azoxystrobin and chlorothalonil. The developed methodology is based on solid-phase extraction (SPE) using a polymeric material functionalized with gold nanoparticles (AuNPs) as sorbent followed by high-performance liquid chromatography (HPLC) with diode array detector (DAD). Several experimental variables that affect the extraction efficiency such as the eluent volume, sample flow rate and salt addition were optimized. Under the optimal conditions, the sorbent provided satisfactory enrichment efficiency for both fungicides, high selectivity and excellent reusability (>120 re-uses). The proposed method allowed the detection of 0.05 μg L^{-1} of the fungicides and gave satisfactory recoveries (75-95%) when it was applied to drinking and environmental water samples (river, well, tap, irrigation, spring and sea waters).

Keywords: azoxystrobin; chlorothalonil; gold nanoparticles; polymer-based material; solid-phase extraction; HPLC-DAD.

Introduction

Azoxystrobin (AZO, methyl (2E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) and chlorothalonil (CLT, 2,4,5,6-tetrachloroisophthalonitrile) (Fig. 1) are broad spectrum pesticides used as fungicides in a great variety of crops diseases. AZO is a systemic fungicide from the strobilurin chemical family, with eradicant, protectant and traslaminar properties. It acts inhibiting mitochondrial respiration, spore germination and mycelial growth, and shows antisporulant activity [1]. However, AZO has low toxicity for birds, mammals, bees and other non-target terrestrial organisms (arthropods and earthworms) [2]. CLT is a contact fungicide from the chloronitrile group, which has some activity as a bactericide, microbiocide, algaecide, insecticide, and acaricide, and prevents spore germination and zoospore motility [1]. It also presents activity against a number of turf diseases, with potential to deactivate several fungal enzymes at different points. It occupies the third position among the most widely used fungicide in the USA [3]. CLT irritates eyes and skin and it has been classified as probable human carcinogen by the US Environmental Agency (EPA) [4].

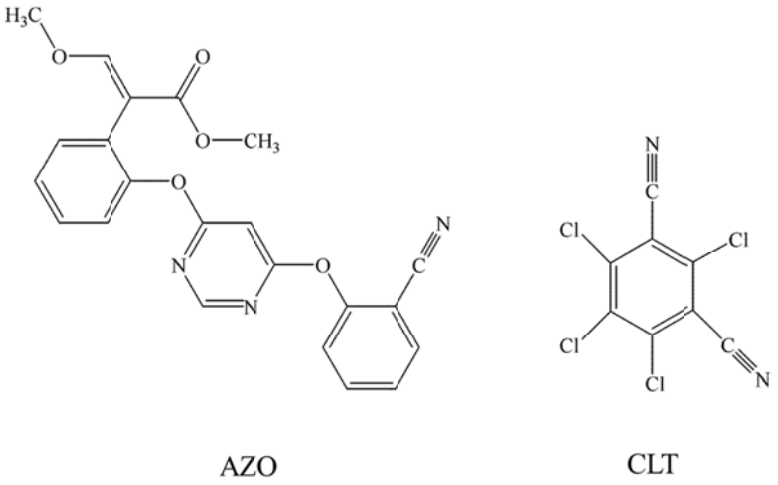

Fig. 1 Molecular structures of AZO and CLT

In order to reduce the risk of resistance in target pathogens, the rotation of strobilurin with protectant fungicides, like CLT, has been proposed [5]. Moreover, there are commercial formulations containing both fungicides, which increase the possibility that both compounds may be found together in food and environmental samples. The typical determination methods of AZO and CLT pesticides are gas chromatography (GC) coupled with mass spectrometry (MS) [1, 6-10] or electron capture detector [11-13], and less often HPLC with UV detection [14].

Due to the low concentrations and the complexity of environmental matrices, the determination of pesticides often requires a pretreatment step, which involves extracting target compounds and cleaning them up prior to chromatographic analysis. SPE is the most popular sample preparation procedure and it is widely employed in a great variety of sample matrices, including environmental water samples. This can be explained taking into account its advantages such as high recoveries and enrichments factors, simplicity or easy of automation [15-17]. The choice of appropriate SPE sorbent is a critical factor to achieve high extraction efficiency and preconcentration level [17]. Within commercial sorbents, silica-based materials modified with long chain alkyl groups (C8 or C18) have been used popularly as SPE adsorbents [15]. However, these reversed-phase materials have several shortcomings such as the presence of residual silanol groups, the narrow pH stability range and the limited reusability [15].

Organic polymer-based monolithic materials are an interesting alternative to develop new stationary phases for its application in both separation techniques and sample pretreatment. These polymers present chemical stability in a wide pH range (from 1 to 14), higher loading capacity and fewer secondary interactions than silica-based stationary phases. Besides, they are ease to prepare and their pore structure and chemical surface can be modified to tailor the extraction efficiency [18-19]. Highly cross-linked polymers and nanomaterials incorporated to polymeric structures, [20] have been described as interesting approaches to increase the surface area, which has been a common limitation of the conventional polymeric sorbents, especially for the retention of small molecules. Glycidyl methacrylate (GMA)-based monolith polymer is an excellent reactive support to produce stationary phases due their reactive epoxide functionalities [19]. In fact, several nanomaterials have been incorporated to these

polymeric structures, being AuNPs one of the most studied [20, 21]. Their large surface-to-volume ratio, stability, biological compatibility and unique optical and molecular-recognition properties have favored its widespread application [22]. Among the implemented strategies, the most commonly approach used is the functionalization of the porous surface of these polymers with thiol or amine groups followed by the covalent attachment of the AuNPs due to the strong affinity of gold for these groups [21, 23-25].

Most of the applications related to pesticides determination involving AuNPs are supported on electrochemical sensors, often based on molecularly imprinted polymers containing this nanomaterial [26-29]. Despite the potential of AuNPs, its combination with organic polymers to perform as preconcentrating supports of pesticides has been scarcely studied [24, 30]. Thus, a GMA-co-ethylene dimethacrylate (EDMA) monolith or its powder has been functionalized with AuNPs for surface-enhanced Raman scattering (SERS) determination of phosmet and disulfoton [24, 30]. Also a methoxy-mercapto-poly(ethylene glycol) polymer [31] has been used to conjugate several Au shapes, including these NPs for SERS enhancement. Recently, Vergara *et al.* [25] have proposed a powdered GMA-co-EDMA based polymer modified with AuNPs for SPE of proteins. This SPE procedure allowed the extraction and purification of these biomacromolecules since its preconcentration was not required.

The aim of the present work is to study the retention behavior of this sorbent against small molecules (pesticides) and their application to environmental matrices, whose low concentrations makes preconcentration mandatory. Thereby, a SPEcombined with HPLC/DAD method is proposed for the determination of AZO and CLT in natural water samples. Both fungicides have been selected because, as commented above, they are formulated together in several preparations and, moreover, both contain a cyano group in their chemical structure, which has good affinity for gold surfaces. The influence of different experimental parameters on the extraction efficiency and preconcentration was optimized. In addition, the applicability of the proposed method was demostrated by the simultaneous determination of both fungicides in some drinking and environmental waters. This work represents the first application of methacrylate materials modified with AuNPs as SPE sorbents for the extraction and preconcentration of fungicides from water samples prior to HPLC-DAD analysis.

Material and methods

Chemicals and reagents

Chlorothalonil (CLO) (99.3%), azoxystrobin (AZO) (99.4%), glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA) and trisodium citrate were purchased from Sigma-Aldrich (Steinheim, Germany, http://www.sigmaaldrich.com). Azobisisobutyronitrile (AIBN) was from Fluka (Buchs, Switzerland, http://www.sigmaaldrich.com). HPLC gradient grade acetonitrile (ACN) and methanol (MeOH) were from Merck (Darmstadt, Germany, http://www.merck.com). AuNP suspension (particle size, 20 nm, stabilized with sodium citrate, $6.54 \cdot 10^{11}$ particles per mL, $1.09 \cdot 10^{-9}$ M), cyclohexanol and 1-dodecanol were from Alfa Aesar (Landcashire, United Kingdom, http://www.alfa.com). Ethanol and ammonia were from Scharlab (Barcelona, Spain, http://www.scharlab.com). A ultra-pure water system Puranity TU 6 from VWR (Germany, http://www.vwr.com) was used for water purification.

Stock solutions of CLT and AZO (500 mg L^{-1}) were prepared by dissolving appropriate amounts of each pesticide in ACN, and working standard solutions were obtained by dilution of the stock solutions with deionized water.

Instrumentation

Chromatographic analysis was carried out in an HPLC equipment from Jasco Analytica (Madrid, Spain, http://www.jasco-europe.com), composed of a PU-2089 quaternary gradient pump, an AS-2055 autosampler with a 100 μL injection loop and a MD-2018 photodiode array detector. The system was controlled using the LC-NETII/AFC interface also supplied by Jasco. Acquisition and data treatment was performed using the ChromNAV software (version 1.17.01).

Preparation and modification of GMA-based material

The preparation of GMA-co-EDMA polymeric material was based in a previous work [25]. Briefly, a polymerization mixture was prepared in a 10 mL glass vial by weighting GMA (20 wt%), EDMA (5 wt%), cyclohexanol (70 wt%) and 1-dodecanol (5 wt%). AIBN (1 wt% with respect to the monomers) was added as thermal initiator. This mixture was sonicated for 5 min and then purged with nitrogen to remove oxygen for 10 min. The polymerization was carried out in an oven at 60ºC for 24 h. Next, the polymeric material was washed with ethanol to remove the porogenic solvents and possible unreacted monomers. Then, the monolithic bulk material was ground with a mortar and sieved with a steel sieve with sizes between 125 and 200 μm. In order to functionalize the powdered material, it was treated with aqueous 4.5 M ammonia in a round bottomed-flask at 60ºC (water bath) for 2 h under continuous stirring. After completion of the reaction, the material was washed with ultra-pure water to remove the excess of ammonia until the pH of eluate was neutral.

Functionalization of amino-modified GMA-co-EDMA material with AuNPs

The amino-modified powder material (*ca.* 800 mg) was mixed with AuNPs solution (*ca.* 70 mL) and then the mixture was allowed to react under stirring for 20 h. A pink coloured GMA-co-EDMA powder (resulting from the attachment of AuNPs) was obtained. Then, the material was washed with 38.8 mM sodium citrate solution at pH 6.6 in order to remove the non-attached AuNPs onto the amino-modified material. The AuNP-modified material was characterized by SEM (see Electronic Supplementary Material, Fig. S1) and its Au content (0.5 wt%) was also established by colorimetric method [25].

SPE protocol and water samples

The SPE cartridges were prepared as follows. 200 mg of AuNP-modified polymer were packed between two frits (1/16', 20 μm, Análisis Vínicos, Tomelloso, Spain, http://www.analisisvinicos.com)

into a 3 mL empty propylene disposable SPE cartridge (Análisis Vínicos). Activation of the sorbent was done with ACN (1.5 mL) and water (1.5 mL). Then, an appropriate volume (12.5 mL) of fungicide standard solution was loaded on the SPE material at a flow rate of 0.35 mL min^{-1}. The retained analytes were eluted with an optimal volume (3 × 0.3 mL) of ACN and the extract was properly diluted with deionized water and was injected into HPLC system. The same procedure was applied to prepare a blank sorbent constituted by the GMA-co-EDMA polymer (200 mg).

Six water samples from different origins were selected for validating the optimized method. These included river, well, tap, irrigation, spring and sea waters. All the samples were taken from Valencia (Spain). They were collected in amber glass bottles and stored in the dark at 4ºC until analysis, performed before 48 h. In order to remove sand and other suspected solid matters, samples were filtered over a 0.45 μm nylon membrane filters (Phenomenex, Torrance, CA, USA, http://www.phenomenex.com/).

Water samples were subjected to the process described above directly and after spiking with both pesticides at two fortification levels: 0.8 and 3.2 μg L^{-1} for CLO and 1.7 and 6.6 μg L^{-1} for AZO (values about 5 and 20 times higher than the limit of quantification (LOQ) of each pesticide when 12.5 mL of sample were processed). Three replicates of each concentration level were prepared.

HPLC procedure

HPLC separation was performed with a Kinetex C18 100 x 4.6 mm (2.6 μm particle size) core-shell column from Phenomenex, in conjunction with a security guard UHPLC C18 column from Jasco Analytica. A mobile phase containing a mixture of ACN and water (55:45, v/v) was used, and the flow rate was set at 1 mL min^{-1}. An aliquot (100 μL) of the standard or sample solutions was injected into the HPLC system. The UV spectra were recorded between 200 and 400 nm, and quantification was performed at 232 and 203 nm for CLO and AZO, respectively.

Results and discussion

Optimization of SPE procedure

In order to achieve an appropriate extraction performance of the AuNP-modified material as SPE sorbent for preconcentrating CLT and AZO, several experimental parameters including type and volume of extraction solvent, extraction flow rate, sample volume and ionic strength were studied. The optimization of the SPE was performed using standard solutions of both fungicides. All presented results were obtained from the mean value of three replicates.

In order to establish the effectivity of each step in the SPE procedure, a preliminary study was done by collecting the fractions from the loading step (5 mL of a standard solution containing 100 μg L^{-1} of each fungicide), the washing step (1 mL of water) and the elution step (2.8 mL of ACN). These

experiments showed higher recoveries (>90%) in the loading step, whereas minor losses (<7%) were observed in the loading step.

In SPE procedure, the selection of an appropriate solvent is important to elute all the retained analytes on the sorbent surface to achieve thus the highest recovery. The solvents used in this study were ACN, MeOH and a mixture of both (1:1, v/v). The extraction efficiency of fungicides obtained with these solvents is depicted in Fig. 2a. As it can be seen, the performance of ACN was significantly better than its mixture with MeOH or pure MeOH in eluting the CLT off the SPE cartridge. The volume of eluting solvent depends on kinetic properties of the SPE bed and it is also an important factor that could affect the extraction efficiency [17]. Thus, the elution volume must be large enough for achieving the complete extraction of the fungicides, but as small as possible in order to avoid unnecessary dilutions and to get the highest enrichment factor and to avoid breakthrough of undesirable matrix components. Fig. 2b shows the results of studying the effect of volume of eluting solvent. From this figure, recoveries of 84% and 98% could be achieved for CLT and AZO, respectively, when the volume of ACN was higher than 0.9 mL. In order to obtain a high enrichment factor, 0.9 mL of ACN was adopted for elution for further studies.

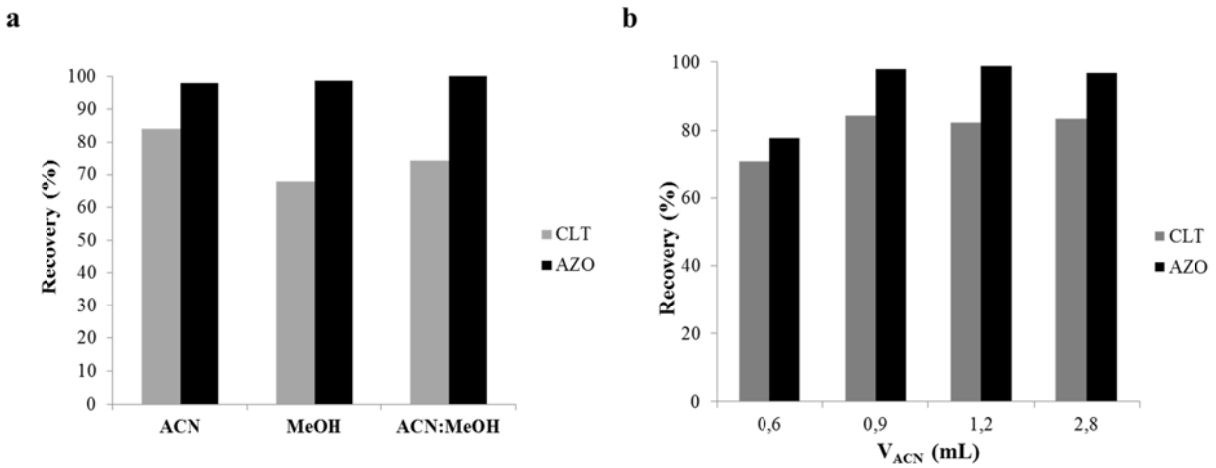

Fig. 2 Effect of (a) type of eluting solvent, (b) volume of eluting solvent (ACN) on recovery of the fungicides. Conditions: volume of standard solution: 5 mL; concentration of two analytes: 12 μg L^{-1}; flow rate: 0.30 mL min^{-1}

The flow rate of sample solution is an important parameter, which has a strong influence on the recoveries, but also in the time of the extraction process. Thus, the sample loading flow rate was studied in the range 0.30 – 0.55 mL min^{-1}. It was found that flow rates over 0.4 mL min^{-1} resulted in a decrease in retention efficiencies of pesticides, which was particularly important in the case of CLT. Consequently, the flow rate was kept at 0.35 mL min^{-1} onwards.

The ionic strength effect in a SPE protocol can be employed to reduce the solubility of analytes in the aqueous phase while enhancing their partitioning into the stationary phase [14]. In order to improve retention of the pesticides, the effect of different percentages of sodium chloride, from 5 to 20% (w/v), in the aqueous medium was studied. However, as shown in Fig. 3, when salt concentration increased, the recoveries for both pesticides decreased, and consequently, the use of NaCl was discarded.

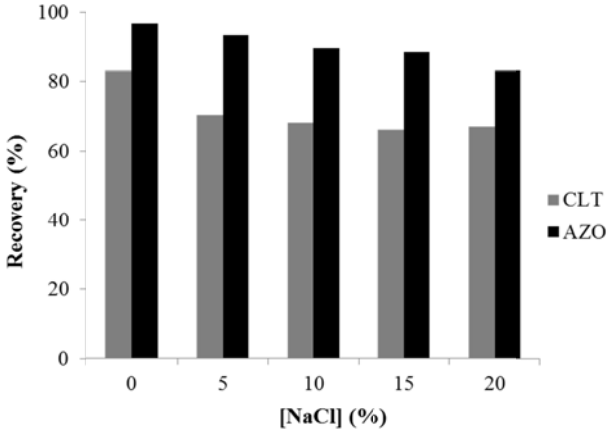

Fig. 3 Effect of the ionic strength (concentration of NaCl) on the recovery of both fungicides. Conditions: volume of standard solution: 5 mL; concentration of two analytes: 40 µg L^{-1}; flow rate: 0.35 mL min^{-1}

An important parameter in SPE is the breakthrough volume, especially when this technique is going to be applied to determine trace elements and preconcentration is mandatory. The breakthrough volume depends on the kinetic properties of the extraction process, especially when short sorbent beds are used since the plate number would be small [32]. For this purpose, different sample volumes (5 - 40 mL) of the standard pesticide solutions were passed through to the SPE material by keeping constant the amount of pesticide (50 ng). The effect of sample volume on the recoveries of both fungicides is given in Fig. 4. Experimental results showed recoveries above 90% for AZO up to 25 mL, whereas a considerable decrease in the recoveries of CLT was observed at volumes from 12.5 mL onwards. For this reason, a volume of 12.5 mL was selected.

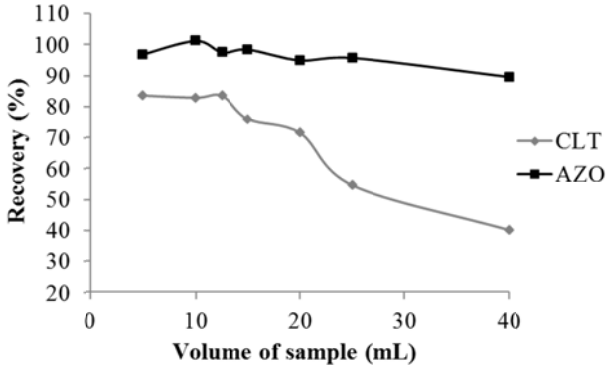

Fig. 4 Effect of sample volume on the recoveries of AZO and CLT using the AuNP-modified polymer material as SPE sorbent. Conditions: sample solution, 50 ng of each fungicide dissolved in different sample volumes; volume of eluent: 0.9 mL; flow rate: 0.35 mL min^{-1}

Also, a comparison in terms of retention was performed with a generic GMA-based polymer used as sorbent under the optimal conditions found for the material modified with AuNPs. Thus, this generic polymer produced up to a decrease of 35% in recovery values, which confirmed the large selectivity of the AuNPs modified material.

Regarding to the mechanism of interaction between the selected fungicides and the sorbent material, it could be explained by taking into account the affinity of cyano group by noble metal nanoparticle surfaces. Thus, the adsorption of aromatic nitriles on a metal surface can be made through three coordinating sites, namely the nitrogen lone pairs, the CN π system and the π system of the benzene ring. In organometallic chemistry, nitriles are generally known to have a σ-type coordination to metal atoms via the nitrogen lone-pair electrons. In fact, SERS studies on several aromatic nitriles having both conjugated and unconjugated CN π systems have shown that adsorption via this CN π system has a significant influence on the binding mechanism for most aromatic nitriles on Au and Ag [33].

Analytical performance of the method

The optimized SPE procedure was validated with respect to linearity, inter- and intra-day precision, limits of detection (LOD) and quantification (LOQ). Calibration curves were prepared at six levels and each calibration level was injected twice. Next, linear calibration plots were obtained by representing analyte peak area versus standard concentration (μg L^{-1}). External calibration curves were employed since the slopes of calibration curves based on standard solutions and those obtained with spiked sample solutions tested did not differ significantly. The linear dynamic ranges and correlation coefficients are given in Table 1.

The precision of the SPEcombined with HPLC method was evaluated by studying the intra- and inter-day reproducibilities of extractions of 12.5 mL of spiked water samples at two concentration levels. The intra-day precision was determined by analyzing six replicates within a given day, whereas the inter-day precision was estimated by analyzing four series of three independent experiments carried out on four different days (see Table 1). The method showed a good precision with relative standard deviation (RSD) values below 7%. The limit of detection (LOD) of each pesticide was calculated as 3 times the standard deviation of the peak area, s, divided by the slope of the calibration curve [34]. The values of s were obtained by processing, with the SPE developed method, six aqueous solutions containing known low concentrations of the pesticides that fulfill the signal-to-noise ratio of 3. Limit of Quantification (LOQ) was obtained as 3.3 times the LOD values. The LOD obtained for CLT (0.05 μg L^{-1}) was below the maximum residue limit imposed by current regulations (0.1 μg L^{-1} for individual pesticides in drinking water) [35]. In the case of AZO, a volume of 25 mL was processed to obtain the same LOD, and it could be performed without losses due to its higher breakthrough volume. The resulting LODs were lower or similar than the ones reported in literature for the simultaneous determination of both pesticides (see Section *Comparison with other methods*).

The stability and potential regeneration of the SPE sorbent were also investigated. The column can be easily reused after regeneration with 1.0 mL of deionized water and 1.0 mL of ACN, respectively, and is stable for up to 120 adsorption-elution cycles without significant decrease in the recoveries for both fungicides.

Table 1 Analytical figures of merit of the proposed SPE combined with HPLC/UV method

Pest.	Calibration		Intra-day precision[a], %		Inter-day precision[a], %		LOD	LOQ
	Range μg L^{-1}	Correlation coefficient	Low level[b]	High level[c]	Low level[b]	High level[c]	μg L^{-1}	μg L^{-1}
AZO	2.8-15000	0.9996	2.2	3.5	4.8	3.0	0.05[d]	0.17[d]
CLT	1.7-10000	0.9993	7.3	5.0	6.8	6.4	0.05	0.17

a. Relative standard deviation (RSD, %)
b. Ultra-pure water spiked with 1 and 2 μg L^{-1} of CLT and AZO, respectively
c. Ultra-pure water spiked with 7 and 14 μg L^{-1} of CLT and AZO, respectively
d. Processing volume, 25 mL

Application to real water samples

The applicability of the proposed SPE method was tested by the determination of both fungicides in six complex water samples, namely river, well, tap, irrigation, spring and sea waters. None of the studied fungicides was detected in the original samples. Then, the standard solutions of both fungicides were added to all of the original samples in order to evaluate the validity of the presented method. Fig. 5 shows a representative example of river water sample unspiked (traces a and c) and spiked at a concentration level of 1.7 and 0.8 μg L^{-1} of AZO and CLT, respectively (traces b and d). As shown in Table 2, recoveries between 75 and 95% were found with RSDs smaller than 14%. These results are good taking in account that the acceptable range for recoveries in water samples is usually set between 7 0% and 110%, with a maximum permitted RSD of 20% [36].

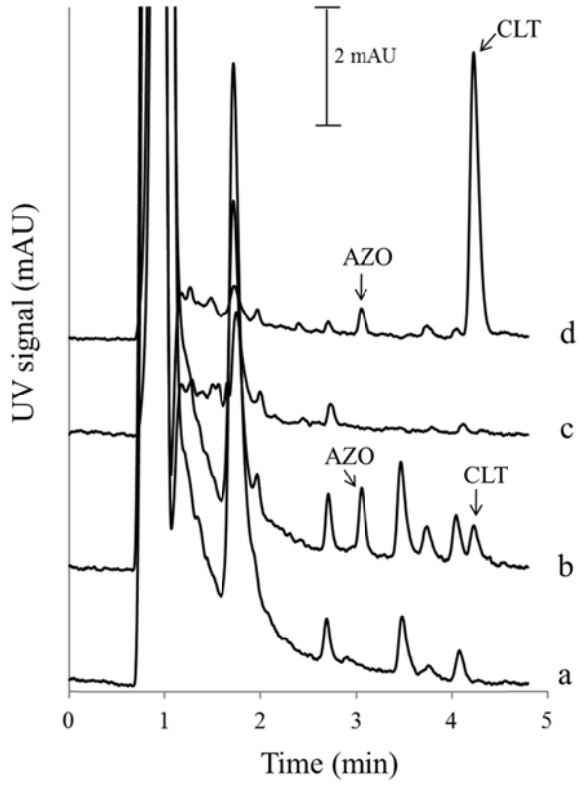

Fig. 5 Chromatograms of river water: blank (traces a and c at 203 and 232 nm, respectively) and spiked with 1.7 and 0.8 μg L^{-1} of AZO and CLT, respectively (traces b and d at 203 and 232 nm, respectively). See text for experimental conditions

Table 2 Analytical performance of the SPE combined with HPLC/UV method for real water samples (n=3)

Sample (pH) (L[a], μS/cm)	Analytes	Spiked level μg L^{-1}	Recovery, % (RSD,%)
Tap water	AZO	1.7	84 (6)
(7.7)		6.6	89 (5)
(397)	CLT	0.8	88 (5)
		3.2	80 (1)
Well water	AZO	1.7	95 (4)
(7.6)		6.6	86 (4)
(1754)	CLT	0.8	91 (6)
		3.2	78 (3)
Spring water	AZO	1.7	82 (3)
(7.8)		6.6	86 (5)
(482)	CLT	0.8	77 (2)
		3.2	76 (3)
River water	AZO	1.7	75 (5)
(8.3)		6.6	87 (3)
(981)	CLT	0.8	81 (4)
		3.2	78 (3)
Irrigation water	AZO	1.7	78 (4)
(8.2)		6.6	80 (3)
(813)	CLT	0.8	77 (7)
		3.2	82 (3)
Sea water	AZO	1.7	87 (11)
(8.1)		6.6	85 (3)
(54100)	CLT	0.8	85 (14)
		3.2	78 (3)

[a] L: conductivity

Comparison with other methods

The developed method was compared with a variety of previous reported methods for the simultaneous determination of both fungicides. The distinct features of these multirresidual analytical methods are summarized in Table 3. As can be seen, some works for certain food matrices showed low recoveries for CLT (< 70%) [6, 8-10, 12]. However, the mean recovery values assayed in this work are in the same order of magnitude than those found in previous works for most water and food samples considered [7, 11, 13, 14]. Concerning to the LODs, our values were better or of the same order that those reported using either SPE with conventional cartridges [1, 6] or liquid-liquid extraction [13]. However, the LODs achieved in this work were comparable to the ones obtained by Yang *et al.* [14]. Moreover, the proposed SPE protocol can be accomplished in short time and presents a very large reusability (see data above), which undoubtedly improves their availability for its application to extract these fungicides in different sample matrices.

Table 3 Comparison of the proposed SPE combined with HPLC/UV with other reported methods for the simultaneous determination of AZO and CLT

Method	Matrix	LOD		Recovery, %		Ref.
		AZO	CLT	AZO	CLT	
SPE-GC/MS	Water	0.1 mg L^{-1}	0.3 mg L^{-1}	-	-	[1]
SPE-GC/MS	Malt beverages	5 μg L^{-1}	10 μg L^{-1}	92-94	34-42	[6]
SPE-GC/MS or LP-GC/MS/MS	Vegetables	0.2-18.8 μg kg^{-1}	0.1-1.6 μg kg^{-1}	95-108	88-97	[7]
dSPE-GC/MS	Food	-	-	84-107	21-51	[8]
SFE-GC/MS	Spinach	-	-	92	20	[9]
dSPE-GC-MS/MS	Cereals and dry animal Feed	0.007 mg kg^{-1}	0.01 mg kg^{-1}	101-130	68-94	[10]
SLE/LTP-GC/ECD	Strawberry	0.013 mg kg^{-1}	0.008 mg kg^{-1}	91-102	88-111	[11]
QuEChERS-GC/ECD/NPD	Peppermint	[LOQ: 0.01 mg kg^{-1}]	[LOQ: 0.021 mg kg^{-1}]	84-99	2-106	[12]
LLE-GC/ECD	Peppers	4 μg L^{-1}	1 μg L^{-1}	93-97	91-104	[13]
VAM-IL-DLLME-HPLC/UV	Water	0.04 μg L^{-1}	0.04 μg L^{-1}	89-96	79-85	[14]
SPE-HPLC/UV	Water	0.05 μg L^{-1}	0.05 μg L^{-1}	75-95	76-91	This work

LLE: Liquid-liquid extraction; SFE: Supercritical fluid extraction; SLE/LTP: Solid-liquid extraction with low-temperature partitioning; LP: Low pressure; ECD: electron capture detector; NPD: nitrogen–phosphorus detector. VAM-IL-DLLME: Vortex-assisted magnetic β-cyclodextrin/attapulgite-linked ionic liquid dispersive liquid–liquid microextraction

Conclusions

This paper describes a simple, sensitive, selective and reliable SPE procedure for the simultaneous determination of the fungicides AZO and CLT in water samples using a methacrylate polymer modified with AuNPs as sorbent prior to HPLC analysis. The proposed method has demonstrated to provide high recovery values (average recovery values > 75%), wide linear ranges and low detection limits (0.05 μg L^{-1}) for these analytes, being these values below the limit of detection imposed by current regulations for these compounds in water samples. The advantages of the present SPE method are its simplicity, environmentally sustainability (low consumption of solvents), high selectivity and reusability (more than 120 uses without losses in recoveries). Additionally, the results demonstrated that the tested sorbent exhibits notable merits for trapping fungicides like CLT and AZO or even to concentrate other environmental pollutants (containing amino, cyano or thiol groups in their structure) in water samples or other matrices, which constitutes an attractive perspective for ultra-trace analysis.

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