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Selective magnetic solid-phase extraction of amide herbicides from fish samples coupled with ultra-high-performance liquid chromatography with tandem mass spectrometry

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Running Title Selective magnetic-SPE extraction of amide herbicides from fish

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A list of the non-standard abbreviations

magnetic solid-phase extraction (MSPE)

liquid chromatography-tandem mass spectrometry (LC-MS/MS)

ultra-high-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS)

molecularly imprinted polymers (MIPs)

magnetic molecularly imprinted polymers (MMIPs)

carbon nanotubes (CNTs)

magnetic CNTs (MCNTs)

magnetic dummy molecularly imprinted polymer (MDMIPs)

magnetic dummy non-molecularly imprinted polymer (MDNIPs)

transmission electron microscopy (TEM)

vibrating sample magnetometry (VSM)

ethylene dimethacrylate (EGDMA)

Keywords fish samples; selective extraction; amide herbicides; magnetic solid-phase extraction

ABSTRACT

An efficient magnetic dummy template molecularly imprinted polymer nanocomposite was prepared using multi-walled carbon nanotubes as a support and metolachlor deschloro as a dummy template. The obtained nanocomposites were characterized using Fourier transform infrared spectroscopy, vibrating sample magnetometry, scanning electron microscopy and transmission electron microscopy. The adsorption performance of the obtained nanocomposites was evaluated through binding experiments, including static adsorption, kinetic adsorption, and selective recognition studies. The obtained nanocomposites were successfully applied as selective sorbents for the magnetic solid-phase extraction of seven amide herbicides (alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl, and dimethachlor) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) from fish samples. Under the optimized conditions, the limit of detection was 0.01–0.1 μg kg^{-1}. The obtained recoveries of the amide herbicides from the fish samples were in the range of 88.0 to 102.1% with a relative standard deviation of less than 7.5%. This method, which eliminated the effect of template leakage on qualitative and quantitative analysis was found to be superior to the methods reported in the literature. The results indicated that it could be successfully applied to analyze amide herbicides in fish samples with satisfactory recoveries.

1 Introduction

Recently, with the quality improvement and efficiency model advocated by the Ministry of Agriculture and Rural Affairs of China in the "Implementation Plan for Rural Revitalization Science and Technology Support Actions" breeding methods such as rice-fish, rice-loach, and rice-crab interactions have been promoted throughout the country. The pesticides used in the cultivation fields are washed by rainwater, surface runoff, and other effects into ponds, which leads to the residual pollution of the pond water by the herbicides. Dan et al. screened herbicides in shellfish breeding bases in 13 counties along the coast of the Shandong province in China, and found that the detection rate was as high as 69.7% [1]. Moreover, the residues of amide herbicides have been found in the environment abroad [2].

The amide herbicides, alachlor and butachlor are classified as highly toxic pesticides in China. Alachlor, acetochlor, and butachlor are classified as B-2 carcinogens, while metolachlor is classified as a C carcinogen by the US Environmental Protection Agency [3]. According to reported research, alachlor and acetochlor can significantly increase the exchange frequency of sister chromosomes in human lymphocytes [4] and reduce the survival rate and motility of human sperm [5]. Butachlor is mutagenic, which increases the probability of chromosomal aberrations [6]. In addition, amide herbicides are 500–10000 more toxic to aquatic organisms than mammals [7]. For example, butachlor at high concentrations can induce aberrations in the number of chromosomes in rice field eels and induce cracks and breaks in chromosomal monomers [8].

The current analytical methods for the determination of amide herbicides mainly include high performance liquid chromatography (HPLC) [9,10], gas chromatography (GC) [11,12], and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [13-15], among which ultra-high-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) has been widely applied for the determination of multi-residue pesticides in different samples owing to its high selectivity and reliability. Generally, due to the high level of impurities in fish, purification is a necessary step to obtain more accurate qualitative and quantitative results.

Among the promising alternatives to solid sorbents, molecularly imprinted polymers (MIPs) that offer high selectivity [16] are widely utilized in solid-phase extraction (SPE) [17,18]. The special selectivity of MIPs is postulated to be due to the surface modification of the polymer matrices in the presence of cross-linking agents, functional monomers, and template molecules. Thus, the structure of specific pores, which are complementary to the template molecules in size, shape, and binding

sites, determines the selective response of the MIPs because it affects the mass transfer and access of the analytes to the specific adsorption sites. However, while one of the analytes was used as the template, leakage of the residual template molecules is one of the most significant challenges associated with the application of magnetic MIPs (MMIPs). The most important problems include the leakage of the template, the cumbersome process of packing the SPE column, and the time-consuming process of loading large-volume samples [19]. A dummy template was employed to address these problems [20].

Among the handling methods for treating fish samples, SPE is typically used for the enrichment of analytes with low organic solvent consumption. However, when the number of samples is large, the simultaneous detection speed of the SPE limits its application in sample pretreatment. Magnetic solid-phase extraction (MSPE), which can efficiently shorten the operation time is a more suitable alternative. In the MSPE process, the magnetic sorbents are dispersed directly in the extract to adsorb the analytes. If the adsorbent is selective, the matrix interference can be reduced by using an MIP. After the extraction process, the magnetic sorbents were quickly collected from the extract using an external magnet [21-23].

In the MSPE process, the surface area of the magnetic sorbents is a very important factor that can affect the properties of the MMIPs. Owing to their high chemical stability and large surface area, carbon nanotubes (CNTs) have been established to be an outstanding carrier for improving the adsorption properties. Therefore, CNTs were used as the supporter and magnetic Fe_3O_4 was used as the core to synthesize magnetic dummy MIPs (MDMIPs), and the complex separation procedure was avoided to achieve rapid separation from the complex matrix by applying an external magnet without centrifugation. A highly selective and easily reusable analysis procedure was thus developed [24].

In the present work, the MDMIPs were synthesized and characterized using vibrating sample magnetometry (VSM), Fourier-transform infrared (FTIR) spectrometry, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In addition, the separation efficiency was investigated through several experiments, including Scatchard analysis and kinetic absorption studies. The selective recognition ability was also estimated. Finally, the developed MDMIPs were successfully applied as sorbents in the MSPE process followed by ultra-high-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) for the selective preconcentration of seven amide herbicides (alachlor, acetochlor, pretilachlor,

butachlor, metolachlor, diethatyl ethyl, and dimethachlor) from fish samples.

2 Materials and Methods

2.1 Materials and reagents

Mass grade acetonitrile (ACN), methanol, and formic acid were purchased from Fisher Scientific (Waltham, MA, USA). CNTs were obtained from Shenzhen Nanotech Port (Shenzhen, China). The standards of the amide herbicides (alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl, and dimethachlor) and an internal standard were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Nitric acid was obtained from Merck (Merck, Darmstadt, Germany). Metolachlor deschloro, sodium acetate, $FeCl_3 \cdot 6H_2O$, ethylene glycol, isopropanol, ammonia, tetraethyl orthosilicate, methacrylic acid, ethylene dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), and acetic acid were obtained from Anpel (Anpel, China). Purified water was obtained using a Milli-Q system (Millipore, Billerica, MA, USA). An ACQUITY BEH C18 column (2.1 × 50 mm, particle size: 1.7 μm) was used to separate the analytes.

The blank sample was tested using the DB 37/T 3406-2018 method: determination of triazine, amide, and dinitroaniline herbicide residues in fishery products using GC-MS [25]. Real fish samples (including crucian carp, grass carp, bighead carp, and shrimp) were collected from fishponds and rivers in different cities in China.

2.2 Standard solutions

Stock solutions of each amide herbicide were prepared in ACN at a concentration of 1.0 mg mL^{-1} and stored at -18 °C in the dark. A mixed standard solution was prepared in ACN at a concentration of 100.0 μg mL^{-1} by diluting the stock standard solutions and stored at -18 °C. The working solutions were prepared by diluting the mixed standard solution with 50% ACN before use and stored at 4 °C for each analysis.

2.3 Preparation and characterization of adsorbent

The MDMIPs were synthesized using a procedure previously developed by our research group [26]. The preparation involves the synthesis of magnetic CNTs (MCNTs), followed by their surface modification with a layer to provide additional functional groups to facilitate their interaction with the amide herbicides. Activated CNTs were prepared by stirring the CNTs (600 mg) while refluxing in nitric acid (90 °C, 6 h). The CNTS were subsequently washed with deionized water and dried. A mixture of activated CNTs (0.40 g), sodium acetate (3.60 g), $FeCl_3 \cdot 6H_2O$ (1.40 g), and ethylene glycol (80.0 mL) was heated at 200 °C for 12 h in an autoclave, and then cooled to room

temperature. The precipitate (MCNTs) was washed with deionized water and dried. The MCNTs (100 mg) were dispersed in deionized water (4.0 mL) and isopropanol (50.0 mL) via ultrasonication (15 min), and then ammonia (5.0 mL) and tetraethyl orthosilicate (2.0 mL) were added. The mixed solution was stirred at 25 °C for 12 h and washed with deionized water in the presence of a permanent magnet. The obtained modified MCNTs were then dissolved in methanol (30.0 mL) in a flask. The MDMIPs were prepared using a noncovalent imprinting technique. The template molecule, metolachlor deschloro (0.137 g) was mixed with the functional monomer, methacrylic acid (0.1866 mL) in 30 mL methanol which acted as a polymerization solvent, and the mixture was stirred for 12 h. The modified MCNTs (200 mg), EGDMA (2.07 mL), and AIBN (50 mg) were then added to the above solution. To remove dissolved oxygen, the solution was stirred under vacuum for 3 min. The mixture was then heated to reflux under N_2 gas at 60 °C for 24 h. The obtained product was rinsed with ethanol until the supernatant was clear. The template molecule was then extracted from the product with a 90/10 (v/v) MeOH/acetic acid mixture using a Soxhlet extraction system for 72 h. Finally, the obtained product was dried in air. Similarly, the MDNIPs were prepared following the same procedure in the absence of metolachlor deschloro.

The MDMIPs were characterized using FTIR (FT-IR 360, Nicolet, Madison, WI) spectroscopy, VSM (Quantum Design Instrument, SanDiego, CA), SEM (TESCAN Mira4 XMU, USA Inc.) and TEM (Tecnai G2-F30 Field Emission Gun microscope, FEI, Inc., Hillsboro, OR).

2.4 Binding experiment

In the kinetic adsorption experiments, the binding kinetics of the MDMIPs (10 mg) were measured by dispersing them in a solution (2 mL) containing 200 μg mL^{-1} acetochlor. The mixtures were mechanically shaken at room temperature for different times (1, 3, 5, 10, 20, 30, 40, 60, 100, 120, and 150 min). The supernatant was then isolated using a permanent magnet and analyzed using UPLC-MS/MS.

In the static equilibrium adsorption experiments, screw-capped centrifuge tubes were used as batch reactor systems. Each tube contained 10 mg of MDMIPs and MDNIPs dispersed in solutions (2 mL) at different concentrations (1, 5, 10, 25, 50, 150, 200, 400, 500, and 750 mg L^{-1}). The amount of acetochlor bound to the MDMIPs and MDNIPs was determined using UPLC-MS/MS.

For the adsorption selectivity experiments, a standard mixed solution of the amide herbicides (alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl, dimethachlor, and atrazine) was incubated with the MDMIPs or MDNIPs, and the extraction procedure was performed

as described previously for the kinetic adsorption experiments.

2.5 Application to fish samples

The fish samples were homogenized using a blender (Braun Blender FP3010) for 10 min. The samples (5.0 g) and internal standards were placed in centrifuge tubes and extracted with 10.0 mL of ACN for 10 min by vortexing and ultrasonic vibration. The homogenates were centrifuged at 4500 rpm for 3 min, the supernatant in the centrifuge tube was collected, and 50 mg of the MDMIPs was added. After incubation for 15 min at 250 rpm, the MDMIPs were removed using a permanent magnet and washed with 3 mL of water. Subsequently, 3 mL (1 mL added 3 times) of 5% acidic methanol (acetic acid/methanol =5/95. V/V) was used to elute the seven amide herbicides. The eluent solution was then concentrated with N_2 gas and re-dissolved to 0.5 mL with ACN/water/formic acid (5:95:0.1, v/v/v). Finally, the treated samples were subjected to UPLC-MS/MS for quantitative analysis (Fig. 1).

2.6 Instrumental analysis

A C18 column combined with a UPLC system was used for the separation of the amide herbicides in the chromatographic part. The mobile phase A was 0.1% formic acid in Milli-Q water, and the mobile phase B was ACN. The gradient program was set as follows: 0–2.0 min, 5% B; 2.0–4.2 min, gradient increased to 95% B; 4.2–6.4 min, held at 95% B; 6.4–6.5 min, gradient decreased to 5% B; 6.5–8.0 min, held at 5% B. The column heater was maintained at 30 °C. The flow rate was 300 μL min^{-1}.

The column eluent was monitored using an XEVO TQ-S (Waters, Milford, MA) instrument equipped with an electrospray ionization interface in positive mode (ESI+). The optimized parameters were set as follows: capillary, 3.0 kV; source offset, 30 V; cone, 20 V; desolvation temperature, 450 °C; nebulizer, 7.0 Bar; cone gas, 250 L h^{-1}; desolvation gas, 800 L h^{-1}; source temperature, 150 °C. The multiple reaction monitoring (MRM) transitions for the target compounds were as follows: alachlor: 270.0→238.1*, collision energy (CE) =11 eV; 270.0→162.1, CE=19 eV. Acetochlor: 270.0→224.1*, CE=10 eV; 270.0→148.1, CE=18 eV. Pretilachlor: 312.1→252.1*, CE=15 eV; 312.1→176.2, CE=28 eV. Butachlor: 312.1→238.1*, CE=11 eV; 312.1→162.2 , CE=23 eV. Metolachlor: 284.1→252.1*, CE=15 eV; 284.1→176.2, CE=25 eV. Diethatyl ethyl: 312.1→238.2*, CE=26 eV; 312.1→162.2, CE=16 eV. Dimethachlor: 256.1→224.1*, CE=14 eV; 256.1→148.2, CE=24 eV. Atrazine, 216.1→174.1*, CE=17 eV; 216.1→132.1, CE=22 eV, where * represents the quantitative ion.

3 Results and Discussion

3.1 Optimization of the preparation method

Density functional theory simulations were usually conducted to optimize the synthesis of molecularly imprinted polymers. The geometries of the monomer, template, and template-monomer complex in each case were optimized using the Hartree-Fock level of theory with the 6–31G(d) basis set. The optimization was followed by a frequency analysis to ensure the absence of imaginary frequencies (as could be seen in Fig. 2). The single point energy and binding energy was calculated by the energy of the template−functional monomer, template and functional monomers. The energy of metolachlor deschloro, alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl and dimethachlor was -2.06×10^{6} KJ mol^{-1}, -3.16×10^{6} KJ mol^{-1}, -3.16×10^{6} KJ mol^{-1}, -3.47×10^{6} KJ mol^{-1}, -3.47×10^{6} KJ mol^{-1}, -3.27×10^{6} KJ mol^{-1}, -3.56×10^{6} KJ mol^{-1} and -3.06×10^{6} KJ mol^{-1}, respectively. Combined with the above energy, the selection of monomers reported in the previous literature [27] and the experimental results, metolachlor deschloro was selected as the template and methacrylic acid was selected as the functional monomer, and the molar ratio of the metolachlor deschloro and methacrylic acid was 1:4 in the reaction condition.

3.2 Characterization of the adsorbent

The FTIR spectrum of the MDMIPs is shown in Fig. 3A. The peaks are observed at 3442 cm^{-1} (stretching mode of the O-H group), 2929 cm^{-1} (telescopic vibration of the C-H group), 1728 cm^{-1} (C-O stretching vibration), 1435 and 1381 cm^{-1} (C-H bending vibration), and 1138 cm^{-1} (C-O stretching vibration). The results confirmed the successful synthesis of the MDMIPs.

The magnetic hysteresis curve revealed that the MDMIPs exhibited typical superparamagnetic behavior with little remanence and coercivity (Fig. 3B). Despite a saturation magnetization of 16.04 emu g^{-1}, the well-dispersed MDMIPs were susceptible to external magnetic fields and could be easily isolated from the solution within 1 min. The excellent magnetic responsiveness of the MDMIPs simplified the separation and purification process and saved the manipulation time. The MDMIPs were thus established as a promising absorbent for practical applications.

The morphology of MDMIPs was evaluated using SEM and TEM (Fig. 3C and Fig. 3D). The straight tubular CNTs and spherical magnetic nanoparticles have been successfully combined. And there is an imprinting layer on the surface of the MDMIPs. These results confirmed the successful synthesis of the MDMIPs.

3.3 Adsorption properties of the adsorbent

3.3.1 Adsorption isotherm

The modes of binding and site distributions involved in the interaction of acetochlor with the adsorbent surfaces of the MDMIPs were initially evaluated by performing binding experiments, in which fixed amounts of the MDMIPs were incubated with different concentrations of acetochlor until equilibrium was reached. The binding capacity of the MIPs was calculated according to the following equation: $Q=(C_i - C_f)*V/m$, where Q (μg mg^{-1}) represents the equilibrium adsorption capacity of the MDMIPs toward acetochlor, C_i (μg mL^{-1}) is the initial solution concentration, C_f (μg mL^{-1}) is the final solution concentration, V (mL) is the volume of the solution tested, and m (mg) is the mass of the MDMIPs. The binding capacities were used to plot the adsorption isotherms (shown in Fig. 4A). According to the adsorption data, the adsorption capacity of the MDMIPs was consistently higher than that of the MDNIPs at each point.

The binding data of the MDMIPs and MDNIPs were further processed via Scatchard analysis [28] using the Scatchard equation ($Q/C=(Q_{max}-Q)/K_d$), where Q (mg g^{-1}) represents the binding capacity, C (mg L^{-1}) is the concentration of free acetochlor at equilibrium, Q_{max} is the maximum binding capacity, and K_d is the equilibrium dissociation constant at the binding site. The Q_{max} and K_d values were calculated from the intercept and slope of the linear plot of Q/C versus Q. The apparent Q values were as follows: $Q_{MDMIPs\_max\_1}$=10.8 mg g^{-1}, $Q_{MDNIPs\_max\_2}$=31.1 mg g^{-1} (shown in Fig. 4B), and Q_{MDNIPs_max} =15.1 mg g^{-1} (shown in Fig. 4C). The value of Q_{MDMIPs_max} (Q_{MDMIPs_max} = $Q_{MDMIPs\_max\_1}$+ $Q_{MDMIPs\_max\_2}$) was calculated to be 41.9 mg g^{-1}. From this data, the occurrence of two adsorption binding modes can be inferred between the MDMIPs and acetochlor molecules. Upon saturation of the specific adsorption point, the substance was bound to the non-specific adsorption site.

3.3.2 Dynamic adsorption properties

The kinetics of adsorption are of special importance in controlling the mechanism of the adsorption process and the process efficiency. Therefore, to investigate the adsorption kinetics [29], the pseudo-first-order "$\log(q_{e1}-q_t)=\log q_{e1}-k_1*t$" and the pseudo-second-order "$t/q_t=1/(k_2*q_{e2}^2) + t/q_{e2}$" models were applied, where q_e and q_t (mg g^{-1}) represent the concentrations of acetochlor bound to the MDMIPs at equilibrium and time t (min), respectively, and k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) represent the rate constants for adsorption. As shown in Fig. 4D, the capacity of the MDMIPs to adsorb acetochlor increased rapidly with time in the range of 0–30 min. Under pseudo-first-order

conditions, the values of k_1 and q_{e1} were calculated to be 0.0098 min^{-1} and 31.3 mg g^{-1}. Under pseudo-second-order conditions, the value of k_2 was 0.00089 g mg^{-1} min^{-1} and that of q_{e2} was 41.5 mg g^{-1} (the correlation coefficient exceeded 0.995). Fitting of the curves to both the models indicated that the pseudo-second-order model was more suitable for explaining the adsorption behavior of the MDMIPs than the pseudo-first-order model.

3.3.3 Selectivity study

To demonstrate the ability of the obtained MDMIPs to selectively adsorb the target molecules in a mixed system containing other compounds, the selectivity of the MDMIPs and the imprinting effect were evaluated using the relative selectivity coefficient. The values of the static distribution coefficient δ, selectivity coefficient α, and relative selectivity coefficient β were obtained using the following equations [30]: $\delta=Q/C$, $\alpha=\delta_{(herbicide)}/\delta_{(atrazine)}$, $\beta=\alpha_{(MDMIPs)}/\alpha_{(MDNIPs)}$, where Q represents the amount of bound MDMIPs, and C represents the initial concentration. The values of δ, α, and β are listed in Table 1. The β values of alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl, and dimethachlor toward atrazine were 2.07, 2.30, 2.25, 2.30, 1.96, 1.93, and 2.26, respectively, which might be a result of the imprinting effect, molecular size recognition, and the interactions between the functional groups of the targets and imprinted cavities.

3.4 Optimization of the sample pretreatment conditions

The extraction experiments were performed using three replicates. The parameters affecting the efficiency of the MSPE process were investigated by analyzing the crucian carp. The amount of MDMIPs, shaking time, and shaking rate were optimized during the adsorption process, and the results showed that the amide herbicides were adsorbed by 50 mg of the MDMIPs via shaking for 15 min at 250 rpm. The elution conditions, including the eluent solvent and elution time, were investigated. When one condition was changed, the others were fixed at their optimum values.

3.4.1 Amount of the adsorbent

During the adsorption process, the MDMIPs were dispersed in the fish extracts. The aim was to use the minimum amount of MDMPs to obtain satisfactory recoveries of the amide herbicides. Different masses of the sorbent (30–80 mg) were investigated (Fig. 5A). Satisfactory recoveries were achieved using 50 mg of the MDMIPs, and with an increase in the mass, the extent of recovery did not change significantly. Thus, 50 mg of the MDMIPs were used for the subsequent experiments.

3.4.2 Shaking time

The shaking time is also an important factor in the adsorption process. The extraction of the amide herbicides from the fish samples required sufficient time to reach equilibrium to obtain satisfactory recoveries. According to Fig. 5B, satisfactory recoveries were obtained in 15 min, which was deemed the optimum condition.

3.4.3 Shaking rate

The amide herbicides were shaken to increase their extraction efficiency. As shown in Fig. 5C, linear recoveries were observed when the shaking rate was in the range of 100 rpm to 300 rpm. The amide herbicides were extracted via complete exposure to the MDMIPs. Optimized exposure was achieved at a shaking rate of 250 rpm.

3.4.4 Eluent solvent

To optimize the recoveries of the amide herbicides, different elution solvents, including methanol, ACN, a mixture of methanol with acetic acid (v:v=95:5), and ACN mixed with acetic acid (v:v=95:5) were selected. As can be seen in Fig. 5D, compared with the eluent devoid of acetic acid, the solution with acetic acid showed higher recoveries. The highest recoveries were obtained using methanol with acetic acid (v:v=95:5).

3.4.5 Elution time

Under the optimized conditions, the eluent solvent (1 mL) was used for the extraction of the amide herbicides by sonicating for 30 s. The result is shown in Fig. 5E, which indicates that successive extraction for three times using 1 mL of the solvent led to satisfactory extraction and only a slight increase in the extraction efficiency was observed when the extraction was performed more than three times. Therefore, the use of 1 mL solvent for three successive extractions was selected as the optimized condition.

3.4.6 Reuse of the adsorbent

After the extraction process, the MDMIPs were easily collected, washed, and dried. The reuse times of the MDMIPS were evaluated based on the recoveries. As shown in Fig. 5F, the recoveries of the amide herbicides were greater than 75.8%, even in the fourth cycle. It was established that the MDMIPs could be used four times without any noticeable decrease in the adsorption capacity.

3.5 Validation of the methodology

The developed method that combined MSPE with UPLC-MS/MS was validated. Quantitative calibration curves were obtained from the matrix-matched calibration plots of the analytes. The correlation coefficients of the seven amide herbicides were greater than 0.995, indicating a linear

relationship. The limits of detection (LODs) of the seven amide herbicides were in the range of 0.01–0.1 μg kg^{-1}. The recoveries of the amide herbicides were in the range of 88.0–102.1% with the relative standard deviation (RSD) ranging from 0.4 to 7.5% for the first use of the MDMIP. The intraday (three replicates) and interday RSD (six replicates) were less than 6.2 and 7.5%, respectively. Matrix effects were corrected using matrix-matched calibration. The quantitative matrix effects were calculated at three concentration levels (at three spiking level), by comparing the analyte peak areas of the analytes in the mobile phase to the analyte peak area of the samples spiked after extraction. The matrix effects were in the range of -6.1 to 7.9%. The details related to the method are listed in Table 2, and the total ion chromatogram (TIC) of the spiked sample (0.5 μg kg^{-1}) is shown in Fig. 6.

3.6 Comparison of the proposed method with other methods

This method was compared with other methods for the determination of amide herbicides in fish samples. As can be seen in Table 3, compared with other previous reports, a lower LOD or limit of quantification (LOQ) was obtained. Owing to the selectivity of the MDMIPS and the magnetic separation of the adsorbents from the sample matrix, this method allows batch processing of the samples in a shorter time. In contrast to the non-purification method for detecting amide herbicides in fish samples, the detection sensitivities increased with the number of repetitions of the extraction process. In addition, the MDMIPs could be reused three times after the adsorption/desorption processes after due exposure to an external magnetic field.

3.7 Application to real samples

This method was further applied to analyze real fish samples (including crucian carp, grass carp, bighead carp, and shrimp) collected from fishponds or rivers in different cities. Butachlor was detected at a concentration of 1.89 μg kg^{-1}in one crucian carp sample. The concentration of the sample obtained by the national standard method was 1.75 μg kg^{-1}. In general, the results were consistent with those obtained using the national standard methods published by the Chinese government. The concentration was below the maximum residue limit (MRL) of 10.0 μg kg^{-1} stated in the Commission Regulation No 149/2008 [37]. However, according to the DB 37/T 3406-2018 national standard methods, the extract from the fish sample was subjected to the following steps: combination of the extract→ redissolution→ rotary evaporation→ gel permeation chromatography (GPC) purification (23 min)→ rotary evaporation (approximately 10 min)→ redissolution→ SPE purification (14.5 min)→ nitrogen blow drying. The extract from the fish sample was purified using

GPC and SPE before GC-MS analysis. In this method, the extract from the fish sample was subjected to the following steps: shaking for extraction (15 min)→ MDMIP purification→ elution of the analytes→ nitrogen blow drying. The matrix interference was reduced by purifying the MDMIPs. The MDMIPs used in the MSPE have thus been established to be a prudent choice for the simultaneous and selective enrichment of low levels of amide herbicides in fish samples.

4 Concluding remarks

In this study, MDMIPs were prepared using metolachlor deschloro as a dummy template. The obtained MDMIPs exhibited a high adsorption capacity and selectivity toward the detection of amide herbicides. The developed MSPE combined with an UPLC-MS/MS method was successfully applied for the analysis of seven amide herbicides (alachlor, acetochlor, pretilachlor, butachlor, metolachlor, diethatyl ethyl, and dimethachlor). The developed method achieved low LODs, appreciable recoveries, low RSDs, and sufficient linearity and was highly feasible for the detection of herbicides in fish samples. With further research, the MDMIP method can be improved and its application scope expanded to the analysis of various organic pollutants present in fish products.

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References

1. Qiao D., Liu X., Zhang H., Luo J., Han D., Gong X., Zhang X. Pollution characteristics and risk assessment of herbicide residues in shellfish from Shandong coastal area. *China Fish. Qual. Stand.* 2017, 7, 22-29.
2. Bonansea R. I., Amé M. V., Wunderlin D. A. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River basin (Argentina). *Chemosphere* 2013, 90, 1860-1869.

3. US Environmental Protection Agency Questions and Answers. Conditional Registration of Acetochlor. US EPA, Washington DC, 1994, 18 1994.
4. Ribas G., Carbonell E., Creus A., Xamena N., Marcos R. Genotoxicity of humic acid in cultured human lymphocytes and its interaction with the herbicides alachlor and maleic hydrazide. *Environ. Mol. Mutagen.* 1997, 29, 272-276.
5. Grizard G., Ouchchane L., Roddier H., Artonne C., Sion B., Vasson M. P., Janny L. In vitro alachlor effects on reactive oxygen species generation, motility patterns and apoptosis markers in human spermatozoa. *Reprod. Toxicol.* 2007, 23, 55-62.
6. Sinha S., Panneerselvam N., Shanmugam G. Genotoxicity of the herbicide butachlor in cultured human lymphocytes. *Mutat. Res.* 1995, 344, 63-67.
7. Kovriznych J., Urbancikova M. Acute toxicity of acetochlor for zebrafish (Danio rerio) and guppy (Poecilia reticulata). *Ekol. Bratisl.* 1998, 17, 449-456.
8. Hu G., Chen J., Wu W., Qu J., Fan L., Wu J. Mutagenesis of Butachlor to Cells of Monopterus albus. *J*, 25. *Guangdong Ocean Univ.*, 43-46 2005.
9. Zhang L., Yu R., Yu Y., Wang C., Zhang D. Determination of four acetanilide herbicides in brown rice juice by ionic liquid/ionic liquid-homogeneous liquid–liquid micro-extraction high performance liquid chromatography. *Microchem. J.* 2019, 146, 115-120.
10. Arias P. G., Martínez-Pérez-Cejuela H., Combès A., Pichon V., Pereira E., Herrero-Martínez J. M., Bravo M. Selective solid-phase extraction of organophosphorus pesticides and their oxon-derivatives from water samples using molecularly imprinted polymer followed by high-performance liquid chromatography with UV detection. *J. Chromatogr. A* 2020, 1626, 461346-461352.
11. Bodur S., Borahan T., Ates N., Bakırdere S. Sensitive determination of acetochlor, alachlor, metolachlor and fenthion utilizing mechanical shaking assisted dispersive liquid–liquid microextraction prior to gas chromatography-mass spectrometry. *Bull. Environ. Contam. Toxicol.* 2020, 105, 460-467.
12. Yang J., Li Q., Wang L., Shao J., Mei W., Wang L. Development and application of a dispersive solid-phase extraction method for the simultaneous determination of chloroacetamide herbicide residues in soil by gas chromatography-tandem mass spectrometry (GC-MS/MS). *Int. J. Environ. Anal. Chem.* 2019, 99, 282-296.
13. Xu Z., Chen M., Hou X., Liu D. Simultaneous determination of seven pesticide residues in

sesame oil using QuERChERS-high-performance liquid chromatography-tandem mass spectrometry. *Chin. J. Anal. Chem.* 2020, 48, 928-936.

14. Liu S., Huang X., Hu K., Jin Q., Zhu G. Development of a multiresidue method for endocrine-disrupting pesticides by solid phase extraction and determination by UHPLC-MS/MS from drinking water samples. *J. Chromatogr. Sci.* 2020, 58, 195-202.
15. González-Gómez L., Morante-Zarcero S., Pérez-Quintanilla D., Sierra I. Simultaneous determination of furanic compounds and acrylamide in insect-based foods by HPLC-QqQ-ms/ms employing a functionalized mesostructured silica as sorbent in solid-phase extraction. *Foods* 2021, 10, 1557-1571.
16. Wang Y., Jin X., Zhao D., Guo X., Li R. Molecularly imprinted solid-phase extraction coupled with gas chromatography for the determination of four chloroacetamide herbicides in soil. *Anal. Methods* 2015, 7, 6411-6418.
17. Mahmoudpour M., Torbati M., Mousavi M., Guardia M., Dolatabadi J. Nanomaterial-based molecularly imprinted polymers for pesticides detection: Recent trends and future prospects. *TrAC Trends Anal. Chem.* 2020, 129, 115943-115956.
18. Hu T., Chen R., Wang Q., He C., Liu S. Recent advances and applications of molecularly imprinted polymers in solid-phase extraction for real sample analysis. *J. Sep. Sci.* 2021, 44, 274-309.
19. Liu L., Yang M., He M., Liu T., Chen F., Li Y., Feng X., Zhang Y., Zhang F. Magnetic solid phase extraction sorbents using methyl-parathion and quinalphos dual-template imprinted polymers coupled with GC-MS for class-selective extraction of twelve organophosphorus pesticides. *Mikrochim. Acta* 2020, 187, 503-514.
20. Hu J., Zhu S., Chen S. E., Liu R., Sun J., Zhao X. E., Liu H. Multiplexed derivatization strategy-based dummy molecularly imprinted polymers as sorbents for magnetic dispersive solid phase extraction of globotriaosylsphingosine prior to UHPLC-MS/MS quantitation. *Mikrochim. Acta* 2020, 187, 373-383.
21. Lin X. P., Wang X. Q., Wang J., Yuan Y. W., Di S. S., Wang Z. W., Xu H., Zhao H. Y., Zhao C. S., Ding W., Qi P. P. Magnetic covalent organic framework as a solid-phase extraction absorbent for sensitive determination of trace organophosphorus pesticides in fatty milk. *J. Chromatogr. A* 2020, 1627, 461387-461396.
22. Yang J., Wang Y., Pan M., Xie X., Liu K., Hong L., Wang S. Synthesis of magnetic metal–

organic frame material and its application in food sample preparation. *Foods* 2020, 9, 1610-1635.

23. Casado-Hidalgo G., Pérez-Quintanilla D., Morante-Zarcero S., Sierra I. Mesostructured silica-coated magnetic nanoparticles to extract six opium alkaloids in poppy seeds prior to ultra-high-performance liquid chromatography-tandem mass spectrometry analysis. *Foods* 2021, 10, 1587-1614.
24. Li F., Gao J., Li X., Li Y., He X., Chen L., Zhang Y. Preparation of magnetic molecularly imprinted polymers functionalized carbon nanotubes for highly selective removal of aristolochic acid. *J. Chromatogr. A* 2019, 1602, 168-177.
25. Determination of triazine, amide, dinitroaniline herbicide residues in fishery products by gas chromatography mass spectrometry Chinese Standard Method (DB 37/T 3406-2018) 2018, 1-12.
26. Gao L., Qin D., Chen Z., Wu S., Tang S., Wang P. Determination of sulfonamide antibiotics in fish and shrimp samples based on magnetic carbon nanotube dummy molecularly imprinted polymer extraction followed by UPLC-MS/MS. *Electrophoresis* 2021, 42, 725-734.
27. Li C., Qi X., Zhang Q., Liu J., Yan S. Computer simulation study on the optimization of functional monomer for tyramine molecularly imprinted polymer. *Comput. Appl. Chem.* 2015, 32, 83-86.
28. Qin D., Wang J., Ge C., Lian Z. Fast extraction of chloramphenicol from marine sediments by using magnetic molecularly imprinted nanoparticles. *Mikrochim. Acta* 2019, 186, 428-437.
29. Li W., Chen N., Zhu Y., Shou D., Zhi M., Zeng X. A nanocomposite consisting of an amorphous seed and a molecularly imprinted covalent organic framework shell for extraction and HPLC determination of nonsteroidal anti-inflammatory drugs. *Mikrochim. Acta* 2019, 186, 76-85.
30. Li H., Xie T., Ye L., Wang Y., Xie C. Core-shell magnetic molecularly imprinted polymer nanoparticles for the extraction of triazophos residues from vegetables. *Microchim. Acta* 2017, 184, 1011-1019.
31. Li Y., Wang M., Yan H., Fu S., Dai H. Simultaneous determination of multiresidual phenyl acetanilide pesticides in different food commodities by solid-phase cleanup and gas chromatography-mass spectrometry. *J. Sep. Sci.* 2013, 36, 1061-1069.
32. Qu Z., Bai X., Zhang T., Yang Z. Ultrasound-assisted extraction and solid-phase extraction for

the simultaneous determination of five amide herbicides in fish samples by gas chromatography with electron capture detection. *J. Sep. Sci.* 2017, 40, 1142-1149.

33. Liu S., Huang X., Jin Q., Zhu G. Determination of a broad spectrum of endocrine-disrupting pesticides in fish samples by UHPLC–MS/MS using the pass−through cleanup approach. *J. Sep. Sci.* 2017, 40, 1266-1272.
34. Cho H. R., Park J. S., Kim J., Han S. B., Choi Y. S. Multiresidue method for the quantitation of 20 pesticides in aquatic products. *Anal. Bioanal. Chem.* 2015, 407, 9043-9052.
35. Shin U., Shin H. Determination of 25 ED s in frog and fish tissue by GC-MS (SIM). *Bull. Korean Chem. Soc.* 2003, 24, 413-420.
36. Fu L., Lu X., Tan J., Wang L., Chen J. Multiresidue determination and potential risks of emerging pesticides in aquatic products from Northeast China by LC–MS/MS. *J. Environ. Sci. (China)* 2018, 63, 116-125.
37. Official Journal of the European Union Temporary MRLs referred to in Articles 2008, Temporary MRLs for substances without MRLs under Directives 86/362/EEC, 86/363/EEC and 90/642/EEC, 16 and 22(1).

Figure captions

Fig. 1 Diagram of the adsorption and resolution process

Fig. 2 Optimized geometries and bond breaking positions of the amide herbicides.

Fig. 3 FTIR spectrum (A), VSM plot (B), SEM image (C) and TEM image (D) of MDMIPs.

Fig. 4 Adsorption isotherms corresponding to MDMIPs and MDNIPs (A); Scatchard analysis of MDMIPs (B) and MDNIPs (C); and kinetics of adsorption by MDMIPs (D).

Fig. 5 Optimization of conditions for the recoveries of seven amide herbicides: amount of MDMIPs (A); shaking time (B); shaking rate (C); eluent solvent (D); elution time (E); and reuse of the MDMIPs (F).

Fig. 6 TIC of the spiked sample (0.5 μg kg^{-1}).

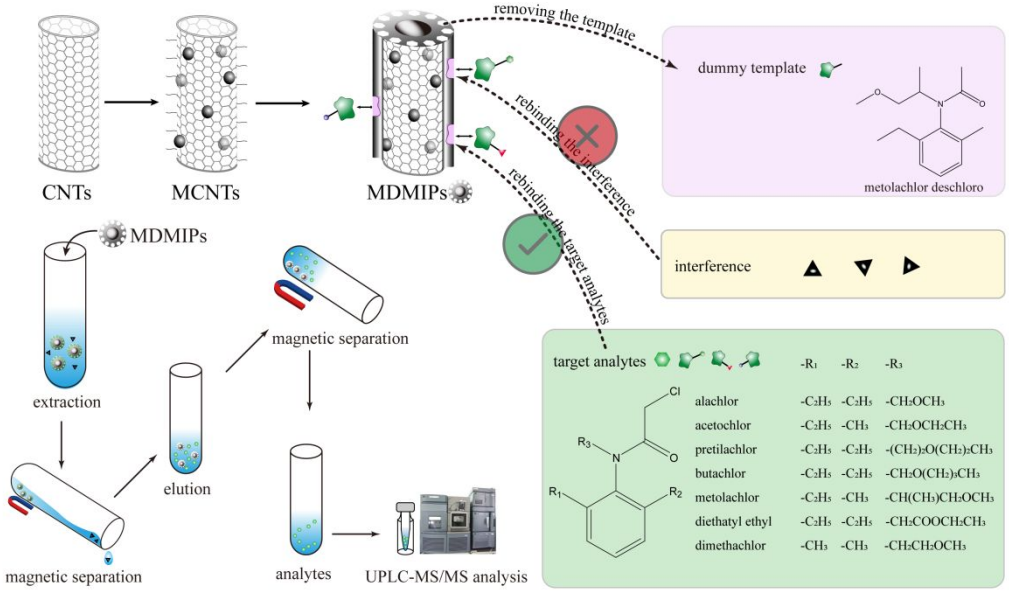

Fig. 1 Diagram of the adsorption and resolution process

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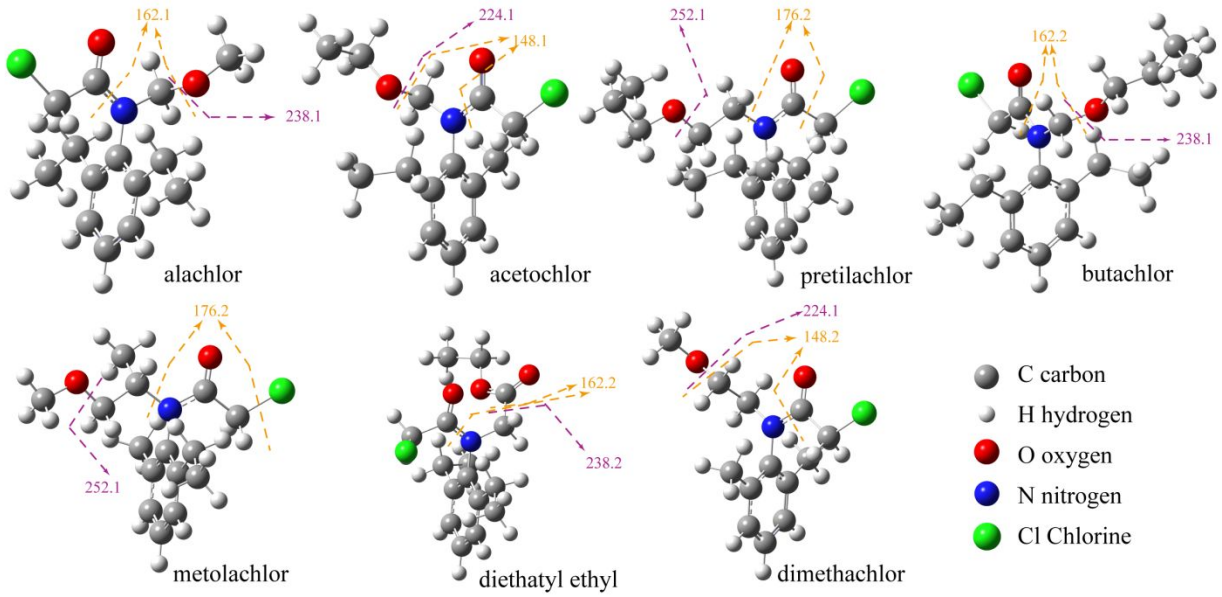

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523 **Fig. 2** Optimized geometries and bond breaking positions of the amide herbicides.

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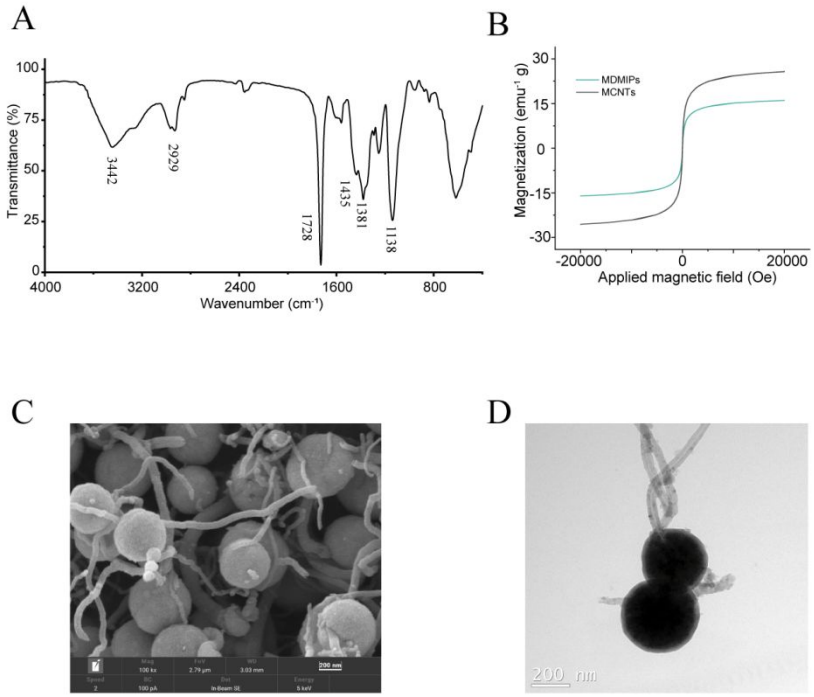

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530 **Fig. 3** FTIR spectrum (A), VSM plot (B), SEM image (C) and TEM image (D) of MDMIPs.

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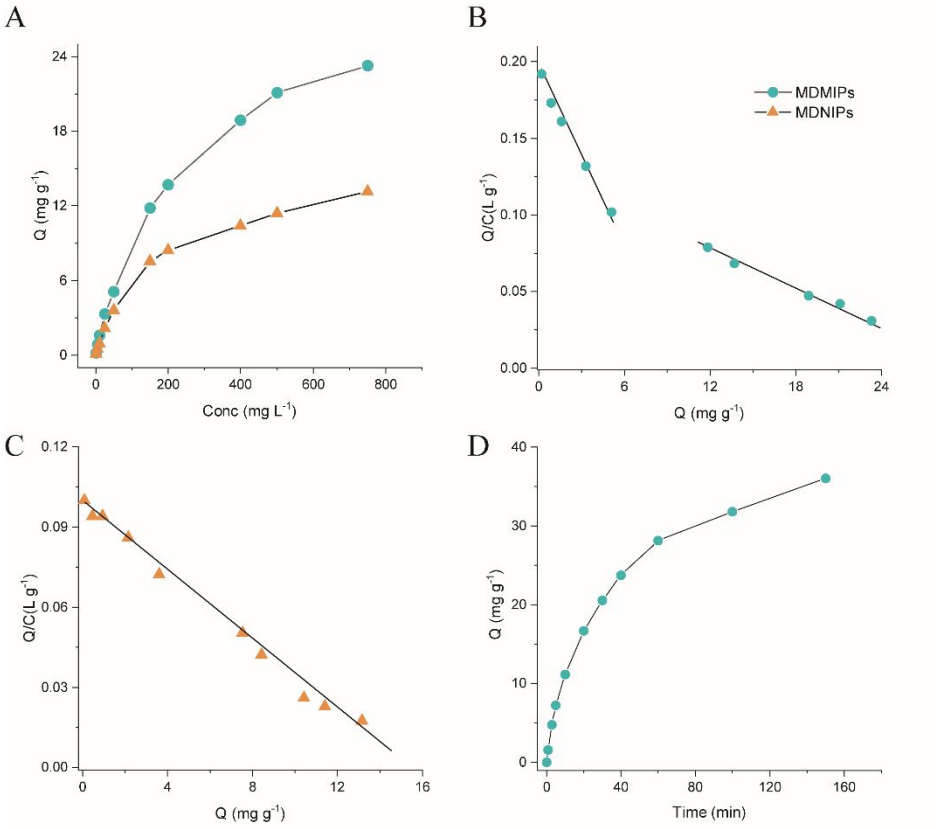

535

536 **Fig. 4** Adsorption isotherms corresponding to MDMIPs and MDNIPs (A); Scatchard analysis of

537 MDMIPs (B) and MDNIPs (C); and kinetics of adsorption by MDMIPs (D).

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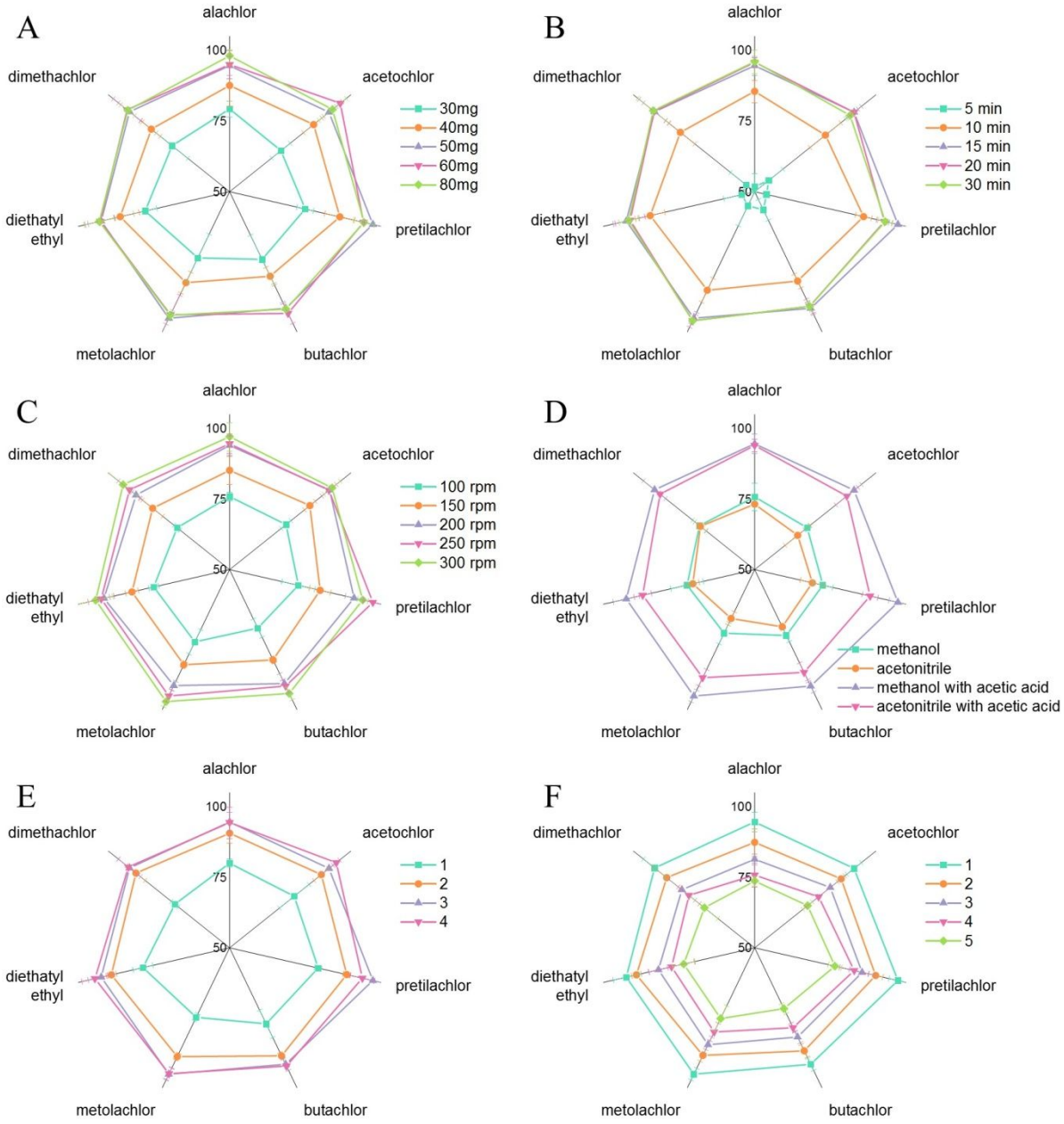

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540 **Fig. 5** Optimization of conditions for the recoveries of seven amide herbicides: amount of MDMIPs

541 (A); shaking time (B); shaking rate (C); eluent solvent (D); elution time (E); and reuse of the

542 MDMIPs (F).

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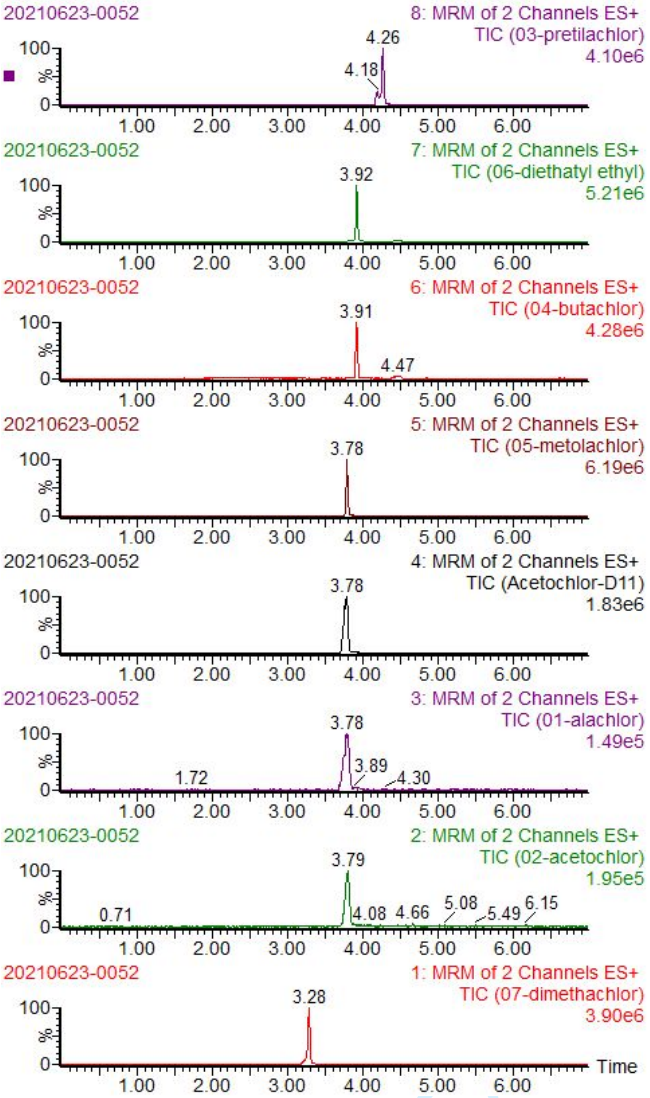

Fig. 6 TIC of the spiked sample (0.5 μg kg^{-1}).

547 **Table 1** Selectivity parameters of MDMIPs and MDNIPs

compounds	MDMIPs		MDNIPs		Relative selectivity coefficient (β)
	Static distribution coefficient (δ)	Selectivity coefficient (α)	Static distribution coefficient (δ)	Selectivity coefficient (α)	
alachlor	75.88	4.92	35.36	2.38	2.07
acetochlor	75.37	4.89	31.53	2.12	2.30
pretilachlor	78.00	5.06	33.40	2.25	2.25
butachlor	72.20	4.68	30.22	2.03	2.30
metolachlor	73.46	4.76	36.14	2.43	1.96
diethatyl ethyl	73.30	4.75	36.53	2.46	1.93
dimethachlor	72.72	4.72	30.95	2.08	2.26
atrazine	15.42	-	14.86	-	-

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549

550 **Table 2** Recovery, precision, LOD, LOQ, and linearity toward the detection of amide herbicides in spiked fish

551 samples.

Analytes	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Range (μg kg^{-1})	Matrix effect (%)	Spiking level (1*LOQ)		Spiking level (5*LOQ)		Spiking level (10*LOQ)	
					Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
alachlor	0.1	0.2	0.2-10	-4.1~-1.4	88.0	3.0	94.6	3.4	94.6	1.5
acetochlor	0.1	0.2	0.2-10	-2.5~2.3	89.0	1.9	95.1	3.3	92.6	1.0
pretilachlor	0.02	0.05	0.05-5	0.7~5.3	96.8	5.8	102.1	3.2	101.1	6.2
butachlor	0.05	0.1	0.1-10	-0.6~4.7	91.3	4.0	95.8	2.4	95.6	0.4
metolachlor	0.01	0.02	0.02-2	-6.1~-0.7	95.4	2.8	99.7	2.3	98.2	1.5
diethatyl ethyl	0.1	0.2	0.2-10	2.5~7.9	94.3	3.3	96.7	4.7	98.3	0.8
dimethachlor	0.01	0.02	0.02-2	1.6~6.4	88.0	4.7	95.4	5.0	93.7	1.6

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554 **Table 3** Comparison of the developed method with other methods for the determination of amide herbicides in
555 aquatic products

Samples (numbers of amide herbicides)	Sample purification	Determination	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	RSD (%)	References
fishery products (5)	GPC → SPE	GC-MS	0.3-0.5	1.0-1.5	70.0-120.0	<15	[25]
fish (5)	SPE	GC-MS	3-5	10	66.9-110.6	2.0-19.0	[31]
Fish (5)	SPE	GC-ECD	0.19~0.42	0.63~1.39	71.2-92.6	<4.7	[32]
fish (2)	Pass-through cleanup	UPLC-MS/MS	0.10-0.16	0.33-0.53	71.8-116.5	<15.3	[33]
Eel and shrimp (1)	QuEChERS	LC-MS/MS	-	≤5.0	68.4-125.2	≤14.1	[34]
Frog and Fish Tissue (1)	SPE	GC-MS	0.3	-	77.2-106.0	6.4-6.8	[35]
aquatic products (2)	GPC	LC-MS/MS	0.011-0.027	0.036-0.091	50.0-106.0	9-20	[36]
aquatic product (7)	MDMIPs	UPLC-MS/MS	0.01-0.1	0.02-0.2	88.0-102.1	<7.5	This method

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