Original Research

Preparation and Application of Molecularly Imprinted Polymers for Five Pyrethroid Insecticides

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Abstract

Molecularly imprinted polymers (MIPs) that had high selectivity for five pyrethroid pesticides with high detection and exceedance rates were synthesized through precipitation polymerization using ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture as double template molecules, acrylamide as a functional monomer, ethylene glycol dimethacrylate as a crosslinking agent, and acetonitrile as a porogen. The imprinted polymers prepared were then characterized and evaluated through scanning electron microscopy (SEM) and an equilibrium adsorption experiment. The results showed that the MIPs had specific adsorption capacities for five pyrethroid pesticides. A solid-phase extraction column was prepared using the synthesized polymers as the adsorption material, and a molecularly imprinted solid-phase extraction-gas chromatography equipped with an electron capture detector (MI-SPE-GC-ECD) method was confirmed for the determination of five pyrethroid pesticide residues in agricultural products. A good linear relationship was present between the peak area and the concentration of pyrethroid insecticides within the range of 2-100 μg/kg. The correlation coefficient (R^2) was greater than 0.9930, and the minimum limits of detection and quantification were 15.5-25.8 and 54.3-88.1 ng/L, respectively. Under different levels of additives, the standard recovery of pyrethroid pesticides in the samples varied from 72.8% to 110.2%, and the relative standard deviation (RSD) was less than 5.5%. In conclusion, this method enables the rapid separation and efficient enrichment of multiple pyrethroid pesticides in agricultural products in a single step.

Keywords: pyrethroid pesticides, molecularly imprinted polymers, solid-phase extraction, actual sample

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Introduction

Pyrethroid pesticides are a new class of broad-spectrum insecticides that are synthesized by chemical methods and have structures similar to natural pyrethroids [1, 2]. They are widely used in the production of vegetables, fruits, cotton, and grain crops [3-5] due to their high efficiency, low toxicity, low residue, and environmental friendliness [6]. Currently, the most popular products used for agricultural control in the pyrethroid insecticide market primarily include cypermethrin and beta-cypermethrin, cyhalothrin and beta-cyhalothrin, cyfluthrin, deltamethrin, and fenvalerate [7, 8]. However, studies have shown that pyrethroid insecticides are light, have heat stability, are hydrophobic, degrade slowly in the natural environment [9-11], and remain stable in environmental substrates and agricultural products for a long time [12]. Hence, pyrethroid pesticides are difficult to remove when they are transmitted into the biological organisms through the food chain, and long-term accumulation will cause serious damage to the immune, nervous, and genetic systems of humans and other mammals [13, 14].

The issue of pyrethroid insecticide residues has raised concerns both domestically and internationally. In response, various countries have established maximum residue limits (MRLs) for different pyrethroid insecticides. For instance, the MRL of beta-cyhalothrin in frozen vegetables and fruits and fresh or frozen vegetables regulated by the European Union is 0.01-0.3 mg/kg [15]. The MRLs of cyhalothrin and beta-cyhalothrin in pears and watermelons are 0.1 mg/kg, and the MRL of beta-cypermethrin in root and potato vegetables is 0.01 mg/kg according to GB 2763-2021 in China [16]. With attention to health and food safety, the MRL of pyrethroid pesticides in food is becoming lower, which requires higher requirements for pyrethroid pesticide detection technology. Currently, the detection of pyrethroid pesticides relies on large instruments, and the primary detection methods include high-performance liquid chromatography (HPLC) [17-19], high-performance liquid chromatography-mass spectrometry (HPLC-MS) [20], gas chromatography (GC) [21, 22], and gas chromatography-mass spectrometry (GC-MS) [23, 24]. However, due to the low residues of these types of insecticides in agricultural products and the presence of an extremely complex interference matrix in actual samples, the sensitivities of conventional detection methods are greatly limited. In addition, there are a few disadvantages in the application of mature solid-phase extraction techniques in sample pretreatment. There is no specific selectivity for the target compound; hence, other interfering substances are extracted simultaneously with the target substance during the extraction and purification process. This results in a failure to achieve the enrichment and purification effects of the target compound. Therefore, it is of considerable practical importance to

research and develop efficient, rapid, selective, and high throughput sample pretreatment techniques for realizing the trace multi-residue detection of pyrethroid pesticides in complex matrices.

The appearance of the molecular imprinting technique (MIT) has a very practical application value for solving these problems due to the complex matrix and low content of the target [25]. MIT is a technology for preparing molecularly imprinted polymers with specific pore structures and recognition sites according to the structure of the target compound, and it has strong specificity for the target substance, good stability, and practicality [26]. It can quickly separate and enrich trace amounts of a target substance in a sample matrix [27]. Currently, molecularly imprinted polymers have been used as solid-phase extraction adsorbents for the pretreatment of complex samples that can effectively realize the analysis and detection of trace substances in a complex sample matrix [28-31]. Ming Zhang et al. formulated a molecularly imprinted solid-phase extraction (SPE) in combination with a gas chromatography (GC) method by using a phenyl ether-biphenyl eutectic mixture as a template for the determination of pyrethroid pesticides in honey samples [32]. Xizhi Shi et al. produced molecularly imprinted polymers with deltamethrin and cypermethrin as template molecules, which were successfully employed for the determination of five pyrethroid insecticides in aquaculture seawater by combining gas chromatography-electron capture detection [33]. However, currently, most MIPs use the pesticide compounds themselves or single structural analogs as templates for imprinting preparation methods, and the prepared polymer cannot produce specific effects on a variety of pyrethroid target molecules because the template used is difficult to elute and has a single recognition site. The employment of two or more structural analogs or fragments as dummy templates can accomplish simultaneous recognition, extraction, and separation of more than one analyte.

The structure of pyrethroid compounds shows that ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture are important intermediates and benzene ring structures of pyrethroid compounds, and these two compounds are structural fragments of pyrethroid compounds. To further increase the recognition and adsorption sites and improve the adsorption efficiency of pyrethroid pesticides, this study intends to combine ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture to develop a double dummy template molecularly imprinted polymer. The synthesis and adsorption properties of MIPs were then optimized and characterized. These MIPs were subsequently utilized as packing material to construct a molecularly imprinted solid-phase extraction (MISPE) column. A method combining molecularly imprinted solid-phase extraction with gas chromatography and electron capture detection (MI-SPE-GC-EDC) was then developed to effectively determine five pyrethroid pesticides known for their

high detection rates and associated risks in fruits and vegetables, including pears, apples, and cucumbers.

Materials and Methods

Chemicals

Acrylamide (AA), methacrylate (MAA), 4-vinylpyridine (4-VP), trimethylolpropane trimethacrylate (TRIM), ethylene glycol dimethacrylate (EGDMA), and azodiisobutyronitrile (AIBN) were purchased from the Aladdin Reagent Corporation (Shanghai). Ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture were acquired from Beijing Honghu United Chemical Products Co., Ltd. (Beijing, China). The above reagents were all of analytical grade. HPLC-grade acetic acid, methanol, and acetonitrile were obtained from Thermo Fisher Technologies (Shanghai). Pyrethroid pesticide standard solutions ($\geq$ 98% purity), including cypermethrin (CYP), cyfluthrin (CYF), deltamethrin (DEL), cyhalothrin (CYH), and fenvalerate (FEN), were obtained from Anpu Experimental Technology Co., Ltd. The cucumber, pear, and apple samples were purchased from local supermarkets.

Instruments and Conditions

Scanning electron microscope (SEM) images were captured using a SU8010 microscope (Hitachi, Japan). The experiments were carried out on a GC-2010 Plus GC (Shimadzu, Japan) furnished with an electron-capture detector (ECD). A DB-5MS capillary column (30 m×0.32 mm×0.25 μm) was utilized for the separation. High-purity nitrogen was used as a carrier gas, and the flow rate was 1.30 mL/min. The column temperature program was from 60ºC to 180ºC at 20ºC·min^{-1}, maintained for 1 min, rose 10ºC·min^{-1} to 220ºC, and then increased 2ºC·min^{-1} to 250ºC, held for 3 min, and finally rose 30ºC min^{-1} to a final temperature of 280ºC, which was maintained for 30 min. The temperatures of the injector and detector were established at 280ºC and 300ºC, respectively. The carrier gas injection mode was a splitless inlet with an injection volume of 1 μL.

Preparation of the MIPs

The pyrethroid pesticide molecularly imprinted polymer was prepared using precipitation polymerization. A total of 0.5 mmol of ethyl chrysanthemate (Eth) and 0.5 mmol of phenyl ether-biphenyl eutectic mixture (Diphyl) were dissolved in 40 mL of acetonitrile in a glass flask. Subsequently, different amounts of AA and MAA were added to the solution, and the resultant solution was further shaken for 30 min to enable the template molecule and functional monomer to fully act. After adding different amounts of EGDMA and TRIM and 20 mg of AIBN, the mixture was deoxygenated with nitrogen for 10 min and sealed. The glass flask was then placed in a water bath at 60ºC and shaken at 160 rpm for 24 h. At the end of the reaction, the resulting polymer was eluted repeatedly with an acetic acid-methanol (2:8, v/v) solution until the template molecules were no longer detected on an ultraviolet (UV) spectrophotometer. Finally, the acquired MIP powder was washed three times with methanol to eliminate excess acetic acid and then dried under a vacuum oven at 40ºC. The preparation diagram of the MIP is presented in Fig. 1a).
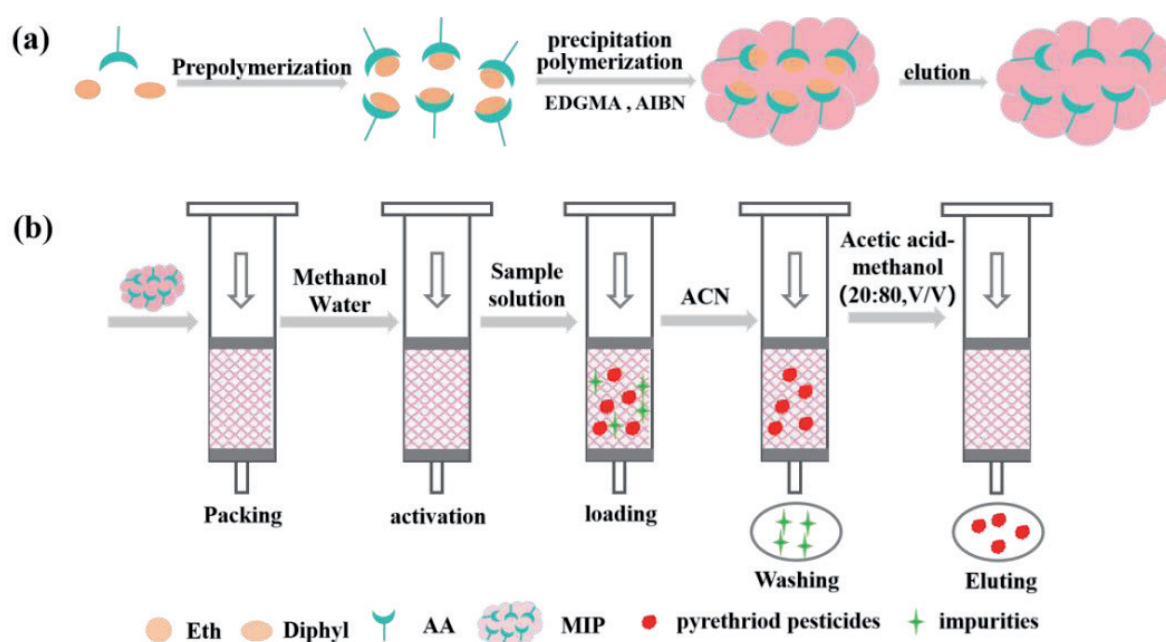

Fig. 1. a) The preparation diagram of the MIP and b) the application of the MIP-SPE.

As a comparison, the non-imprinted polymer (NIP) was synthesized following the same procedure without the template.

Adsorption Properties of the MIPs

Five pyrethroid pesticides that are detected more frequently and pose greater safety risks were selected for the kinetic and isothermal adsorption tests. First, 50 mg of the MIP and 50 mg of the NIP were measured and placed in a 2 mL centrifuge tube. Second, 2 mL of pyrethroid methanol solutions with different mass concentrations were added sequentially, and the supernatant was centrifuged by shaking for a certain time at room temperature. Finally, the contents of the target substances in the supernatant were identified through the use of gas chromatography equipped with an electron capture detector (GC-ECD). Equation (1) was adopted to calculate the adsorption capacity of the polymers, and the adsorption kinetics and isothermal adsorption curves of the MIP and NIP were drawn to ascertain the optimal adsorption saturation time and mass concentration:

$$Q = (C^0 - C^e)V/m \qquad (1)$$

where Q represents the adsorption of the polymer to the target after adsorption equilibrium (mg/g); C_0 indicates the initial content of the target substance methanol solution (mg/g); C_e stands for the content of the target in the supernatant after adsorption equilibrium; V refers to the volume of the target substance methanol solution; and m is the weighing capillary for the MIP or NIP.

Characterization of the MIPs

The surface morphology and structure of the synthesized polymers were characterized by scanning electron microscopy (SEM).

Preparation of the Molecularly Imprinted Solid-Phase Extraction (MI-SPE) and Optimization of the Solid-Phase Extraction Conditions

The synthesized molecularly imprinted polymer powder was filled into the solid-phase extraction (SPE) columns by using the dry packing method. As illustrated in Fig. 1b), first, a sieve plate with a pore size of 20 μm was fitted into the bottom of a cylindrical tube, and then 50 mg of the MIP was filled in the column. Finally, the packing material was compacted from the top with the sieve plate. A total of 2 mL of a 10 mg/L pyrethroid standard solution was added to the prepared MI-SPE, and the loading solution was then optimized according to the recovery rate of the target compound.

Actual Sample Processing

A total of 2.0 g each of pear, apple, and cucumber samples were added to a mixed standard solution containing five types of pyrethroid pesticides. The samples were weighed and subsequently placed in a 50 mL centrifuge tube. Totals of 10 mL of acetonitrile, 4 g of anhydrous magnesium sulfate, and 1 g of sodium chloride were then added to the samples. This was then homogenized at high speed for 5 min, left to stand for 30 min, centrifuged at 4000 r/min for 5 min, and extracted repeatedly three times. The supernatants were then combined, and 1 mL of the supernatants was used for the purification. The purification process followed the optimized extraction conditions of the molecularly imprinted solid-phase extraction (MI-SPE) column, and the target analyte was detected using GC-ECD.

Results and Discussion

Selection of Double Template Molecules

In the selection of template molecules, pyrethroid insecticides are composed of chrysanthemum acid esters and halogenated derivatives of their acids and alcohols, while ethyl chrysanthemate is an important intermediate of pyrethroid insecticides, and phenyl ether-biphenyl eutectic mixture is also a benzene ring structure that most pyrethroid insecticides have. These two template molecules are the primary structural frameworks of pyrethroid insecticides. In theory, it is feasible to prepare polymers using ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture as double template molecules. Fig. 2 indicates that in the actual adsorption experiment, the adsorption capacity of the molecularly imprinted polymers prepared by employing double template molecules (ethyl chrysanthemate: phenyl ether-biphenyl eutectic mixture = 1:1, n/n) was superior to that of molecularly imprinted polymers prepared by using a single template. This may be due to the double template molecules that can make the polymer produce a large
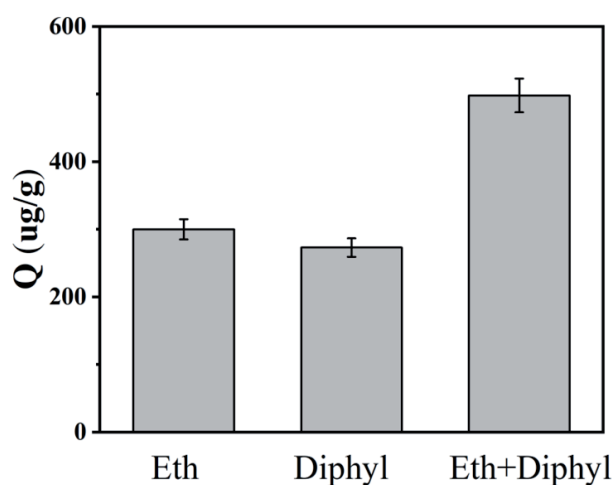

Fig. 2. Adsorption capacities of the different template molecules (Eth: ethyl chrysanthemate, Diphyl: phenyl ether-biphenyl eutectic mixture).

number of imprinted cavities that are more matched with the structure of the target analytes and simultaneously increase the adsorption capacity of the target substances [34, 35]. Therefore, ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture were chosen as double template molecules for the preparation of the pyrethroid pesticide molecularly imprinted polymer.

The Screening and Optimization of the Preparation System for the Molecularly Imprinted Polymers

A precipitation polymerization approach was employed to produce the pyrethroid pesticide molecularly imprinted polymer [36]. To obtain a pyrethroid pesticide molecularly imprinted polymer with a high adsorption capacity and strong specificity, the polymer preparation system was screened and optimized. MAA, AA, and 4-VP were selected as functional monomers in this experiment. It was found that the prepared polymer was a cloudy, milky white liquid when 4-VP was used as the monomer through the preparation experiment of the polymer. Although the white solid was precipitated, it easily agglomerated after template elution and vacuum drying. Therefore, 4-VP was not suitable as the monomer for preparing the polymer, and MAA and AA were selected as monomers for the subsequent experiment. The binary crosslinking agents, EGDMA and TRIM, were selected as crosslinking agents. Table 1 shows the types of monomers and crosslinking agents, and the ratios of monomers, templates, and crosslinking agents in the polymer preparation process were optimized. The results showed that when ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture were mixed in a ratio of 1:1 as template molecules with AA as the functional monomer, EGDMA as the crosslinking agent (the molar ratio of the three substances was

1:4:20), and acetonitrile as the pore-forming agent, the prepared polymer had the highest adsorption capacity. At this point, MIPs with good adsorption capacities for pyrethroid pesticides were obtained. Therefore, this system was chosen as the final preparation system for the polymer.

Morphological and Structural Characterization of the MIPs

The imprinted polymer (MIP) and non-imprinted polymer (NIP) prepared in this experiment were characterized using a scanning electron microscope. As shown in Fig. 3, the left image shows the appearance and morphology of the MIP, which shows that there are abundant reticular pore structures similar to honeycomb shapes, and there are many irregularities on the polymer surface. This was because after the template molecules in the MIP were eluted, imprinting cavities that matched the target molecule were generated on the surface, increasing the binding sites of the polymer to the target molecule [37]. The right image shows the NIP without template molecules, and the particles in the NIP aggregates exhibit irregular states with varying particle sizes, relatively flat surfaces, and no imprinted pores. Compared with the NIP, the MIP had a higher adsorption performance for pyrethroid pesticides.

Adsorption Properties of the MIPs

Five typical pyrethroid pesticides with high detection frequency and great potential safety hazards were selected, and their dynamic and static adsorption experiments were conducted. This allowed for the determination of the adsorption capacity of the molecularly imprinted polymer synthesized under the optimal

Table 1. Screening and optimization of the polymer preparation systems.

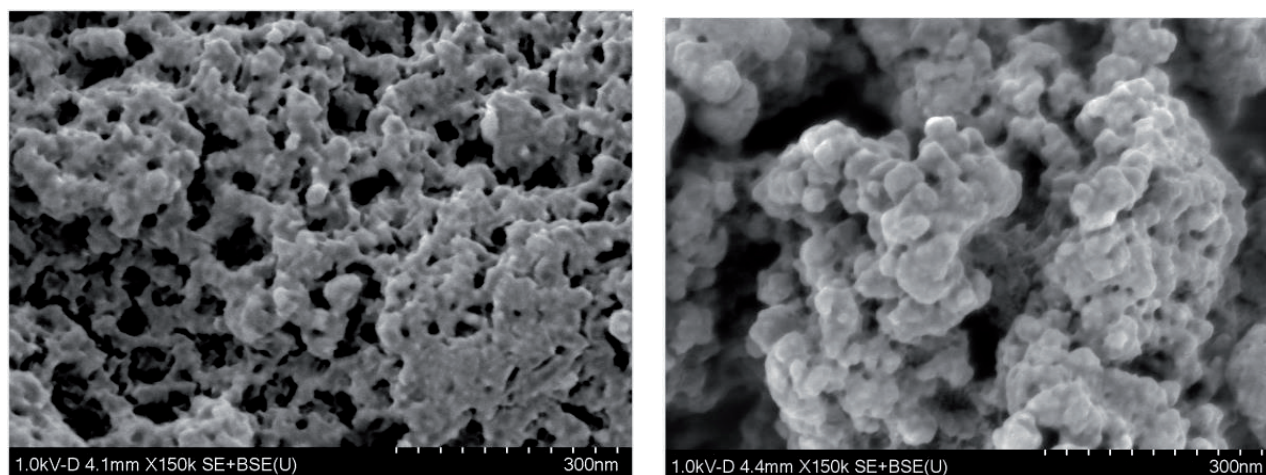| Template molecule	Functional monomer	Crosslinking agent	Porogen	Proportion	Average adsorption capacity (μg/g)
Ethyl chrysanthemate/ phenyl ether-biphenyl eutectic mixture	MAA	EDGMA	Acetonitrile	1-2-20	320
				1-4-20	347
				1-6-20	294
		TRIM		1-2-10	195
				1-4-10	231
				1-6-10	176
	AA	EDGMA		1-2-20	403
				1-4-20	457
				1-6-20	384
		TRIM		1-2-10	217
				1-4-10	184
				1-6-10	143

Fig. 3. SEM images of the MIP (left) and the NIP (right).

polymerization system. The dynamic and static adsorption curves were drawn, and their performance was evaluated. It is evident from the dynamic adsorption curve in Fig. 4 (a-e) that the dynamic adsorption patterns of the MIP and NIP for the five pyrethroid pesticides were basically the same. With an increase in time, the adsorption curve gradually showed an upward trend until it reached equilibrium after 120 min. Thus, 120 min was regarded as the saturation adsorption time.

The static adsorption curve in Fig. 4 (f-j) indicates that the MIP and NIP exhibited good adsorption performances for the five pyrethroid pesticides in target solutions with different concentration gradients. The adsorption capacities of the MIP and NIP for cypermethrin presented unsaturated states; that is, the adsorption capacities displayed upward trends with the growth of the pesticide concentration, while the adsorption capacities for beta-cypermethrin, cypermethrin, fenvalerate, and deltamethrin gradually increased with the increase in the pesticide concentration and then achieved a balance. At a low concentration of pyrethroid pesticides, the adsorption capacity of the MIP was similar to that of the NIP, which may result from the nonspecific property of the polymer. At high concentrations, the adsorption capacity of the MIP for the target substance was greater than that of the NIP. When the concentration exceeded 40 mg/L, the increase in concentration caused the specific binding site of the polymer to play a role. Hence, the MIP has a specific adsorption capacity for the target substance.

Study of the Specific Adsorption of the MIPs

Organophosphorus pesticide (parathion-methyl) was selected to investigate the class-specific adsorption performance of the pyrethroid molecularly imprinted polymer. Fig. 5a) shows that the adsorption results of the MIP and NIP for parathion-methyl, cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, and fenvalerate were 248, 440, 420, 420, 438, 431 µg/g and 159, 205, 194, 183, 187, 158 µg/g, respectively. The adsorption capacity

of the MIP for pyrethroid pesticides was significantly greater than that of organophosphorus pesticides, which indicated that the synthesized MIP exhibited class-specific adsorption for pyrethroid pesticides.

Establishment of the Pyrethroid Pesticide Molecularly Imprinted Solid-Phase Extraction Method

In order to obtain the MIP-SPE with a specific adsorption and enrichment effect, the extraction conditions of the MIP-SPE prepared through the method described in the Materials and Methods Section were optimized. We selected different loading solutions (acetonitrile, 80% acetonitrile-water, methanol, 80% methanol-water, and 50% methanol-water) to analyze the retention effect of the target substances in the MIP-SPE (Fig. 5b)). The results showed that when 80% acetonitrile-water was selected as the loading solution, the retention effect of the target analyte in the MIP-SPE was the best.

To reduce the interference of impurities in the sample matrix, methanol, acetonitrile, and water were selected as the leachates. It can be concluded from Fig. 5c) that when water and methanol were selected as the leachates, the target substance was eluted and lost, which reduced the recovery rate. When acetonitrile was used as the eluent, the recovery rate of the target substance was enhanced, which may have been due to an increase in the specific force between the target substance and the binding site.

Fig. 5c) shows that methanol eluted the target substance from the solid-phase extraction column to varying degrees. Therefore, when investigating the influence of the eluent on the target recovery rate, not only were different volumes of methanol selected, but acidified methanol was also chosen as the eluent to compare the target recovery rate. Fig. 5d) shows that the use of 3 mL of 20% acetic acid-methanol as the eluent improved the target recovery rate. Therefore, 3 mL of 20% acetic acid-methanol was selected as the eluent.
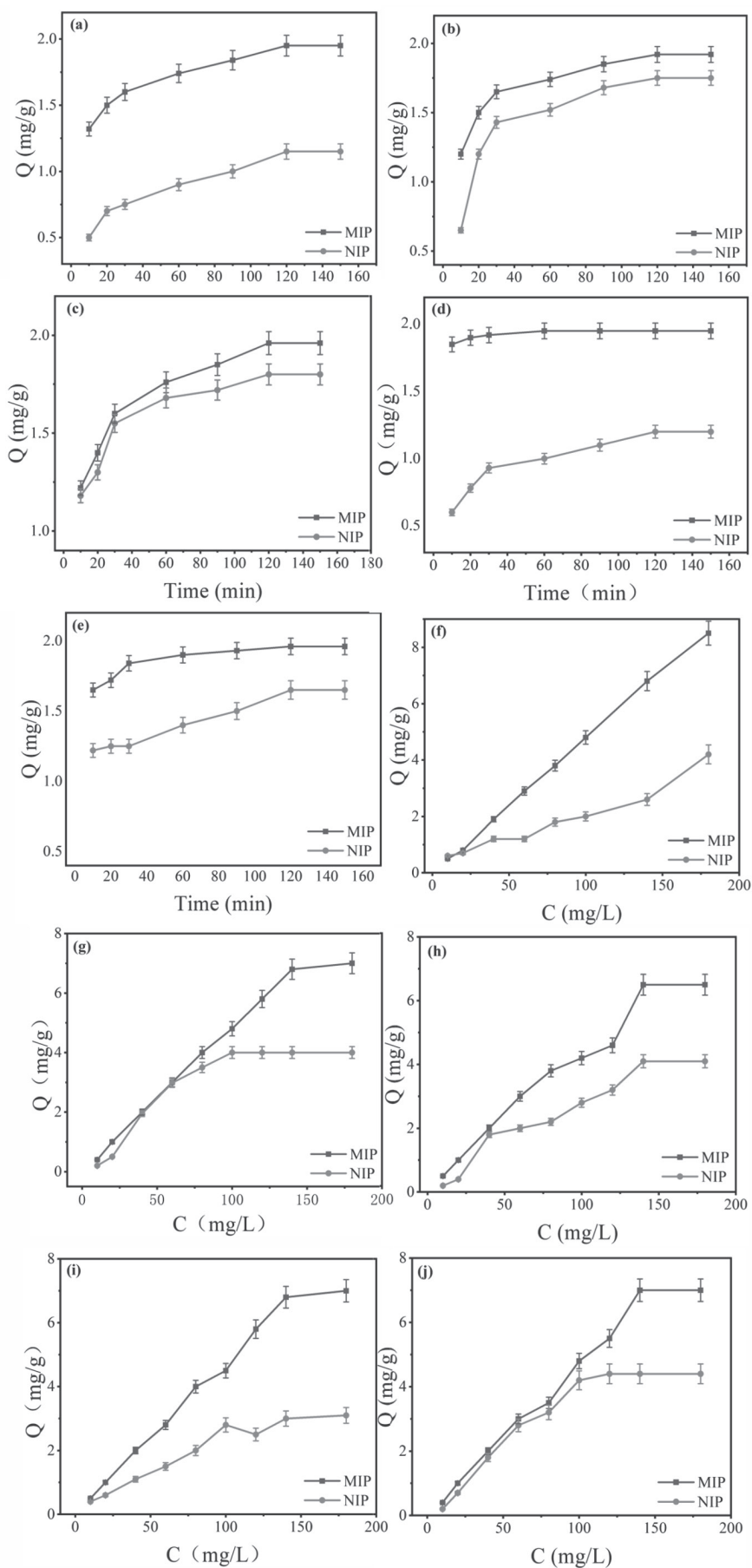
Fig. 4. The dynamic adsorption curves (a-e) and the static adsorption curves (f-j) of the MIP and NIP. (a, f) cyfluthrin; (b, g) cyhalothrin; (c, h) cypermethrin; (d, i) fenvalerate; (e, j) deltamethrin.
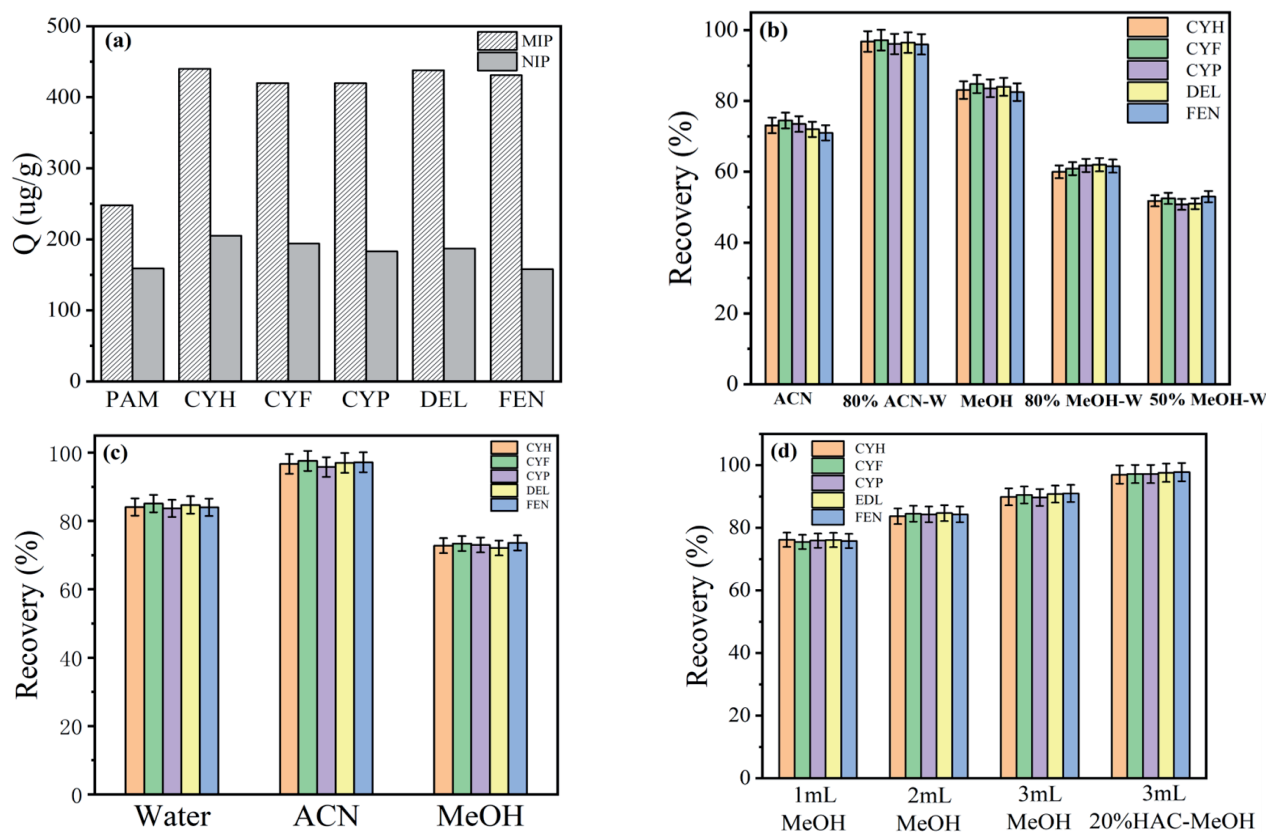
Fig. 5. The adsorption capacity of both the MIP and NIP for the various types of pesticides a) and a comparison of the recovery rates of the five pyrethroid pesticides among the different loading solutions b), Leachate c) and eluent d) (PAM: parathion-methyl; CYH: cyhalothrin; CYF: cyfluthrin; CYP: cypermethrin; DEL: deltamethrin; FEN: fenvalerate).

To sum up, 3 mL of methanol and 3 mL of water were employed as the activation solution, 1 mL of 80% acetonitrile-water target solution was used as the loading solution, 1 mL of acetonitrile was taken as the leachate, and 3 mL of 20% acetic acid-methanol was regarded as the eluent. The flow rate was controlled at 1 mL/min. Under these conditions, the recovery rate of the pyrethroid target substances was the maximum.

Methodology Evaluation

Linear Range and Detection Limit

In this experiment, a pear was chosen as the sample matrix, and the blank sample extracting solution

purified using the MIP-SPE was used to prepare the five pyrethroid pesticide mixed standard solutions with concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg/L. The standard curve was plotted with the concentrations of the five pyrethroid pesticides as the x-axis and the peak area as the y-axis. The signal-to-noise ratios (S/N) that equaled 3 and 10 were the limits of detection and quantification for each component, respectively. Table 2 and Fig. 6 reveal that the five pyrethroid pesticides had excellent linear relationships within the range of 0.01-0.5 mg/L, the correlation coefficients were all above 0.9930, and there were favorable chromatographic separation effects. When the S/N equaled three, the minimum limit of detection (LOD) of the pyrethroid pesticides in the pear samples ranged

Table 2. The linear equation, correlation coefficient (R^2), linear range, LOD, and LOQ of the five pyrethroid pesticides.

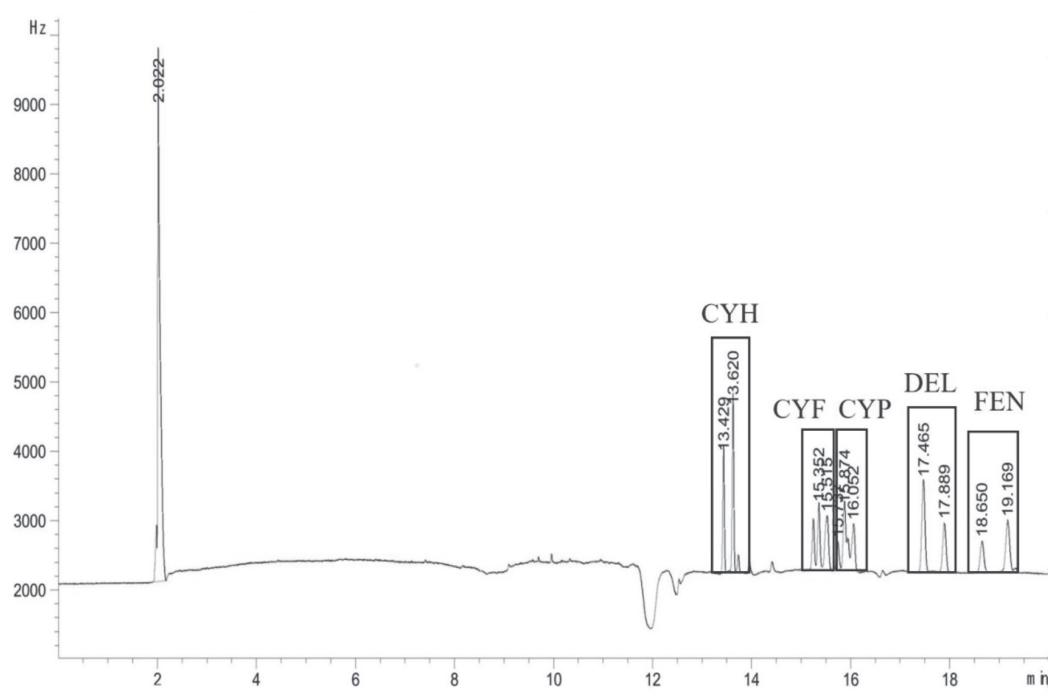| Pyrethroid pesticides	Linear equation	Correlation coefficient (R^2)	Linear range mg/L	LOD ng/L	LOQ ng/L
CYP	Y = 372460X + 7135.9	0.9949	0.01-0.5	25.6	87.8
CYF	Y = 381850X + 1893.6	0.9932	0.01-0.5	23.4	79.8
DEL	Y = 383015X + 4716.7	0.9940	0.01-0.5	25.8	88.1
CYH	Y = 371140X + 8560.1	0.9935	0.01-0.5	15.7	54.3
FEN	Y = 260529X + 3951.4	0.9932	0.01-0.5	18.9	65.1

Fig. 6. Standard chromatogram for the five pyrethroid pesticides according to the GC-ECD (0.02 mg/L).

Table 3. Recovery rates and RSDs (n = 3) of the five pyrethroid pesticides in the different agricultural products.

| Substrate | Addition levels | | | | | |
| | 0.01 mg/kg | | 0.02 mg/kg | | 0.05 mg/kg | |
	Average recovery rates (%)	Relative standard deviations (%)	Average recovery rates (%)	Relative standard deviations (%)	Average recovery rates (%)	Relative standard deviations (%)
Pear	81.6-94.8	0.6-4.8	72.8-98.5	1.3-4.2	87.6-10.2.8	1.2-3.6
Cucumber	74.8-94.6	0.3-5.5	81.8-104.6	1.7-3.1	77.8-110.2	1.7-2.3
Apple	77.4-89.4	0.6-2.3	75.4-102.4	2.2-5.1	79.4-106.4	0.6-2.6

between 15.5 and 25.8 ng/L, and when the S/N equaled 10, the minimum limit of quantification (LOQ) ranged from 54.3 to 88.1 ng/L. Therefore, this experiment met the quantitative determination requirements of pyrethroid pesticide residues in agricultural products. The detection limit, linear range, and recovery rate of the method were 0.011-0.018 ng/g, 0.1-200 ng/g, and 94.3-102.7%, respectively.

Addition and Recovery Experiment

Three levels of addition and recovery experiments were set up for the blank samples of pears, apples, and cucumbers, with each concentration repeated three times, and the recovery rate was ascertained using GC-ECD. The experimental outcomes are displayed in Table 3, and the recovery rates of the five pyrethroid pesticides varied from 72.8 to 110.2%, and the relative standard deviations (RSDs, n = 3) ranged between 0.3 and 5.5%.

Conclusions

In this study, a pyrethroid pesticide molecularly imprinted polymer was synthesized for the first time using ethyl chrysanthemate and phenyl ether-biphenyl eutectic mixture as double template molecules. They were prepared using precipitation polymerization with acrylamide as a functional monomer, ethylene glycol dimethacrylate as a crosslinking agent, and acetonitrile as a pore-forming agent. The selective adsorption effect of the polymer was assessed in an experiment that demonstrated that the polymer had good selectivity and adsorption toward pyrethroid pesticides. The MIP-SPE prepared with this polymer was filler-enriched, and five pyrethroid pesticides were separated into different agricultural product samples, which greatly reduced the matrix interference in the GC-ECD detection. The analytical method of the pyrethroid pesticides established in this study extracted, purified, and enriched the samples in a single step, which saved

the time of sample pretreatment and reduced the application of organic reagents. The accuracy, precision, and sensitivity of the method fulfilled the demands of residue detection and could be applied for detection in actual samples. Future research can focus on further optimizing the synthesis conditions of molecularly imprinted polymers to enhance their adsorption capacity and selectivity. Additionally, integrating MIPs with advanced detection technologies, such as surface-enhanced Raman spectroscopy, could lead to more efficient and accurate methods for monitoring the safety of agricultural products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

1. CHEN Y., LAI J.H., ZHANG M.M., ZHAO T.Y., WANG S., LI J.L., LIU S.L. Status of pyrethroid pesticide pollution in agricultural products and technologies for its removal: A Review. Food Science. **43** (9), 285, **2022**.

2. TANG W.X., WANG D., WANG J.Q., WU Z.W., LI L.Y., HUANG M.L., XU S.H., YAN D.Y. Pyrethroid pesticide residues in the global environment: An overview. Chemosphere. **191**, 990, **2018**.

3. HUANG X.D., LIU H.F., XU X.M., CHEN G. Magnetic composite based on carbon nanotubes and deep eutectic solvents: preparation and its application for the determination of pyrethroids in tea drinks. Foods. **12** (1), **2023**.

4. ZHU X.D., JIA C.H., ZHENG Z.T., FENG X.Y., HE Y., ZHAO E.C. Solid-phase extraction combined with dispersive liquid-liquid microextraction for the determination of pyrethroid pesticides in wheat and maize samples. Journal of Separation Science. **39** (23), 4621, **2016**.

5. ZHU Q.Y., YANG Y., ZHONG Y.Y., LAO Z.T., O'NEILL P., HONG D., ZHANG K, ZHAO S.Q. Synthesis, insecticidal activity, resistance, photodegradation and toxicity of pyrethroids (A review). Chemosphere. **254**, 126779, **2020**.

6. AHAMAD A., KUMAR J. Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring. Journal of Hazardous Materials Advances. **10**, 100284, **2023**.

7. YUAN H.Y., LI B., WEI J., LIU X.W., HE Z.Y. Ultra-high performance liquid chromatography and gas chromatography coupled to tandem mass spectrometry for the analysis of 32 pyrethroid pesticides in fruits

and vegetables: A comparative study. Food Chemistry. **412** (30), 135578, **2023**.

8. LIANG Z., ABDELSHAFY A.M., LUO Z.S., BELWAL T., LIN X.Y., XU Y.Q., WANG L., YANG M.Y., QI M., DONG Y.Y., LI L. Occurrence, detection, and dissipation of pesticide residue in plant-derived foodstuff: A state-of-the-art review. Food Chemistry. **384** (1), 132494, **2022**.

9. ZHAN H., HUANG Y.H., LIN Z.Q., BHATT P., CHEN S.H. New insights into the microbial degradation and catalytic mechanism of synthetic pyrethroids. Environmental Research. **182**, 109138, **2020**.

10. PITZER E.M., WILLIAMS M.T., VORHEES C.V. Effects of pyrethroids on brain development and behavior: Neurotoxicology and Teratology. **87**, 106984, **2021**.

11. BHATT P.K., RENE E.R., HUANG Y.H., LIN Z.Q., PANG S.M., ZHANG W.P, CHEN S.H. Systems biology analysis of pyrethroid biodegradation in bacteria and its effect on the cellular environment of pests and humans. Toxicology. **9** (6), 106582, **2021**.

12. ZHAO M.R., CHEN F., WANG C., ZHANG Q., GAN J.Y., LIU W.P. Integrative assessment of enantioselectivity in endocrine disruption and immunotoxicity of synthetic pyrethroids. Environmental Pollution. **158**, 1968, **2010**.

13. HAN J.J., ZHOU L.Q, LUO M., LIANG, Y.R., ZHAO, W.T., WANG P., ZHOU Z.Q., LIU D.H. Nonoccupational exposure to pyrethroids and risk of coronary heart disease in the Chinese population. Environmental Science & Technology. **51**, 664, **2017**.

14. ANILAVA K., ABHIK G. Biomarkers of type II synthetic pyrethroid pesticides in freshwater fish. Biomed Research International. **9**, 928063, **2014**.

15. KUCHHEUSER P., BIRRINGER M. Pesticide residues in food in the European Union: Analysis of notifications in the European Rapid Alert System for Food and Feed from 2002 to 2020. Food Control. **133**, 108575, **2022**.

16. National food safety standard-Maximum residue limits for pesticides in food, GB 2763-2021, Beijing (China). **2021**.

17. NIKDEL M., NEZAMI A.E., YAMINI Y., NEZHADB E.G., NAZARIPOUR A., BAGHANI M., RAHIMZADEGAN M. Optimized three-phase hollow fiber liquid-phase automated microextraction and its application in ultra-trace analysis of three pyrethroids in water and fruit samples using high-performance liquid chromatography-diode array detection. Separation Science Plus. 7, 2300143, **2024.**

18. MA K.X., ZHANG M.X., MIAO S.C., GU X.Y., LI N., CUI S.H., YANG J. Magnetic solid-phase extraction of pyrethroid pesticides in environmental water samples with $CoFe_2O_4$-embedded porous graphitic carbon nanocomposites. Journal of Separation Science. **41**, 3441, **2018**.

19. ZHANG J, WANG Y., YU J., WANG Q.Y., KHATTAK K.N., YANG X.P. Determination of pyrethroids in water samples by dispersive solid-phase extraction coupled with high-performance liquid chromatography. Water Environment Research. **94**, e10813, **2022**.

20. HOU M.Y., ZANG X.H., WANG C., WANG Z. The use of silica-coated magnetic graphene microspheres as the adsorbent for the extraction of pyrethroid pesticides from orange and lettuce samples followed by GC-MS analysis. Journal of Separation Science. **36**, 3242, **2013**.

21. XUE J.J., XU Y.J., LIU F.M., XUE J., LI H.C., PENG W. Comparison of different sample pre-treatments for multi-residue analysis of organochlorine and pyrethroid pesticides in chrysanthemum by gas chromatography

with electron capture detection. The Journal of Separation Science. **47**, 1311, **2024**.

22. ZHANG Y.P., HU D.Y., ZENG S., LU P., ZHANG K., CHEN L., SONG B. Multiresidue determination of pyrethroid pesticide residues in pepper through a modified QuEChERS method and gas chromatography with electron capture detection. Biomedical Chromatography. **30**, 142, **2016**

23. GAO X., GUO H., WANG J.W., ZHAO Q.B. Sensitive and rapid determination of pyrethroids in human blood by gas chromatography-tandem mass spectrometry with ultrasound-assisted dispersive liquid-liquid microextraction. Drug Testing and Analysis. **10**, 1131, **2018**.

24. POSTA S.D., GALLO V., GENTILI A., GHERARDI M., GARA L.D., FANALI C. Low transition temperature mixture-based extraction of 14 pesticides from tomato samples and their high-performance liquid chromatography-tandem mass spectrometry analysis. Journal of Chromatography A. **1717** (22), 404690, **2024**.

25. ANTONIO M.E. Green molecularly imprinted polymers for sustainable sample preparation. Journal of Separation Science. **45**, 233, **2022**.

26. CHEN L.X., XU S.F., LI J.H. Recent advances in molecular imprinting technology: current status, challenges and highlighted applications. Chemical Society Reviews. **40**, 2922, **2011**.

27. CHEN L.X., WANG X.Y., LU W.H., WU X.Q., LI J.H. Molecular imprinting: perspectives and applications. Chemical Society Reviews. **45**, 2137, **2016**.

28. BAGHERI A.R., ARABI M., GHAEDI M., OSTOVAN A., WANG X., LI J., CHEN L., WANG X.Y., LI J.H., CHEN L.X. Dummy molecularly imprinted polymers based on a green synthesis strategy for magnetic solid-phase extraction of acrylamide in food samples. Talanta. **195**, 390, **2019**.

29. GUO X.C., XIA Z.Y., WANG H.H., KANG W.Y., LIN L.M., CAO W.Q., ZHANG H.W., ZHOU W.H. Molecularly imprinted solid phase extraction method for simultaneous determination of seven nitroimidazoles from honey by HPLC-MS/MS. Talanta. **166**, 101, **2017**.

30. ARABI M., OSTOVAN A., BAGHERI A.R., GUO X.T., WANG L.Y., LI J.H., WANG X.Y., LI B.W., CHEN L.X. Strategies of molecular imprinting-based solid-phase extraction prior to chromatographic analysis. Trac-Trends in Analytical Chemistry. **128**, 115923, **2020**.

31. OLCER Y.A., DEMIRKURT M., DEMIR M.M., EROGLU A.E. Development of molecularly imprinted polymers (MIPs) as a solid phase extraction (SPE) sorbent for the determination of ibuprofen in water. Rsc Advances. **50**, 31441, **2017**.

32. ZHANG M., HE J., SHEN Y.Z., HE W.Y., LI Y.Y., ZHAO D.X., ZHANG S.S. Application of pseudo-template molecularly imprinted polymers by atom transfer radical polymerization to the solid-phase extraction of pyrethroids. Talanta. **178**, 1011, **2018**.

33. SHI X.Z., LIU J.H., SUN A.L, LI D.X., CHEN J. Group-selective enrichment and determination of pyrethroid insecticides in aquaculture seawater via molecularly imprinted solid phase extraction coupled with gas chromatography-electron capture detection. Journal of Chromatography A. **1227**, 60, **2012**.

34. MENG A.C., LEJEUNE J., SPIVAK D.A. Multi-analyte imprinting capability of OMNiMIPs versus traditional molecularly imprinted polymers. Journal of Molecular Recognition. **22**, 121, **2009**.

35. XIE X.W., MA X.G., GUO L.H., FAN Y.M., ZENG G.L., ZHANG, M.Y., LI J. Novel magnetic multi-templates molecularly imprinted polymer for selective and rapid removal and detection of alkylphenols in water. Chemical Engineering Journal. **357**, 56, **2019**.

36. LIU J., SONG H., LIU J., LIU Y., LI L., TANG H., LI Y.C. Preparation of molecularly imprinted polymer with double templates for rapid simultaneous determination of melamine and dicyandiamide in dairy products. Talanta. **134**, 761, **2015**.

37. GAO R.X., HAO Y., ZHAO S.Q., ZHANG L.L., CUI X.H. LIU D.H., TANG Y.H., ZHENG Y.S. Novel magnetic multi-template molecularly imprinted polymers for specific separation and determination of three endocrine disrupting compounds simultaneously in environmental water samples. RSC Advances. **4**, 56798, **2014**.