Molecularly Imprinted Solid-Phase Extraction in the Analysis of Agrochemicals

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The molecular imprinting technique is a highly predeterminative recognition technology. Molecularly imprinted polymers (MIPs) can be applied to the cleanup and preconcentration of analytes as the selective adsorbent of solid-phase extraction (SPE). In recent years, a new type of SPE has formed, molecularly imprinted polymer solid-phase extraction (MISPE), and has been widely applied to the extraction of agrochemicals. In this review, the mechanism of the molecular imprinting technique and the methodology of MIP preparations are explained. The extraction modes of MISPE, including offline and online, are discussed, and the applications of MISPE in the analysis of agrochemicals such as herbicides, fungicides and insecticides are summarized. It is concluded that MISPE is a powerful tool to selectively isolate agrochemicals from real samples with higher extraction and cleanup efficiency than commercial SPE and that it has great potential for broad applications.

Introduction

Food shortage is currently a serious problem. Large amounts of agrochemicals are employed to increase production yields. In particular, insecticides, fungicides, herbicides and plant growth regulators are sprayed on crops through the entire growing period. Because many agrochemicals are harmful to human health, it is necessary to detect their residuals. A complete analysis method includes sample collection, sample preparation, and isolation, identification and quantification of analytes. However, sample preparation and isolation of analytes are the most important, tedious and time-consuming steps, and a possible source of inaccuracy and imprecision of the overall analysis. Therefore, solid-phase extraction (SPE) is widely used for sample purification and preconcentration of analytes. SPE was introduced in the early 1970s. It avoids and minimizes the disadvantages of liquid–liquid extraction (LLE). Compared to LLE, SPE reduces the required time, especially if automated methods are used; it can handle small samples (50–100 μL) and requires small volumes of solvent (1). The principles of SPE are as follows: when the samples go through the column with extraction sorbents, part or all of them are adsorbed; one or several mixed solvents are used to wash off the impurities; finally, a small amount of solvent is used to elute the analytes. Depending on the adsorbent properties, the SPE sorbents can be divided into normal phase, reversed phase, ionic and other special sorbents (2). Commercially available phases for SPE based on silica and bonded silica have been used for a wide range of analytes. The traditional solid-phase sorbents use the interaction difference between the solid sorbent and analytes to separate the analytes. Because the interactions between them are nonspecific, it is possible for several components to be simultaneously extracted.

The selectivity of the column is poor and the SPE column is expensive. Because the source and composition of the sample has become more complex, the traditional SPE technique cannot meet the requirements.

The molecularly imprinted technique originated from immunology. In recent years, molecularly imprinted polymers (MIPs) have drawn much attention due to their outstanding advantages such as stability, predetermined recognition ability, relative ease and low cost of preparation, and potential application to a wide range of target molecules (3). MIPs are cross-linked polymers with specific binding sites for a particular analyte. As shown in Figure 1, the template and monomer(s) are first mixed to form a stable prepolymerization complex in a selected solvent. Second, the polymerizations are initiated in the presence of a suitable cross-linker. Third, the traditional bulk polymerization is ground and sieved to an appropriate particle size. Finally, the template is removed and some cavities are left. These cavities are able to selectively rebind the analytes from a complex mixture. MIPs are widely applied to chromatographic separation, SPE, bionicsensing, enzyme simulated catalysis and drug development and screening. The use of SPE with MIP is referred to as molecularly imprinted solid-phase extraction (MISPE). Compared with classical SPE, MISPE is very efficient for extraction and cleanup. Anderson et al. (4) compared MISPE with classical SPE in the detection of benzodiazepines in postmortem hair samples. The results showed qualitative agreement and MISPE showed higher extraction efficiency for the samples. Tang et al. (5) prepared a bensulfuron-methyl (BSM) molecularly imprinted polymer and applied it to sample pretreatment. The recoveries of BSM on MIP and C18 cartridges were compared in the purification of soybean extract; the results demonstrated that the recovery of the MIP extraction was 46.03% higher than that of C18. MISPE is usually combined with another analytical method to detect the residuals of agrochemicals. The work load of sample pretreatment is approximately 70% of agrochemical analysis and very time consuming. MISPE is a fast and effective method of sample pretreatment. In recent years, it has rapidly developed in the field of agrochemical analysis. However, only one review has been published about the application of MISPE in agrochemical detection (6). This review focused on the preparation and application of MISPE for the analysis of pesticide residues from food matrix. There are also several reviews focused on the application of MISPE to environmental and biological samples (7–11). The application to pesticide residues in food was only mentioned in individual examples. Therefore, the application of MISPE in the determination of agrochemicals is systematically summarized in this review. The comparison between MISPE and commercial SPE is also discussed.
MIPs, which showed high selectivity (13). Covalent imprinting means that the monomer and template interact with covalent interactions, such as electrostatic forces, hydrogen binding or Van der Waals forces. There are so many advantages of non-covalent imprinting: for instance, it is easy to prepare the template/monomer complex and to remove the template from the polymers. In 1993, Vlatakis et al. synthesized theophylline MIPs, which showed high selectivity (13). Covalent imprinting means that the monomer and template interact with covalent power such as Schiff’s bases, borate, acetal ketone and ester. However, the interactions are so strong that it is difficult to thoroughly remove the template. MIPs are produced by synthesizing specific sugar or amino acid derivatives that contain a polymerizable function such as vinylphenylboronate (14). Preparing semi-covalent imprinting polymers is similar to the method for non-covalent imprinting polymers. It has the advantages of non-covalent and covalent imprinting (3).

Preparation of MIPs
Many polymerization methods can be applied to prepare MIPs, such as bulk polymerization, in situ polymerization, suspension polymerization, dispersion polymerization, precipitation polymerization, two-step or multistep swelling polymerization and surface polymerization. Traditional bulk polymerization is the most widely used method to prepare MIPs. Monomer, template, cross-linker and initiator are dissolved in solvent in a test tube. The mixture is sparged with nitrogen to remove oxygen and the test tube is sealed under vacuum. When the polymerization is finished under appropriate conditions, the MIP particles are ground and sieved. After that, the particles are washed with appropriate solvents in a Soxhlet extractor until no residue of the template is found in the rinses. This method is very simple and the reaction condition can easily be controlled. MIPs are applied in the research of high-performance liquid chromatography (HPLC) (15–22), gas chromatography (GC) (23–27) and capillary electrophoresis (28, 29). However, due to their irregular sizes and shapes, the application of this method is limited.

To overcome the disadvantages of traditional bulk polymerization, several polymerization methods have been proposed. The microspheres of the regular shape can be produced by dispersion polymerizations (30). In this method, the monomer, template and cross-linker are dissolved in organic solvents. The mixture solution is transferred to the water with stirring. After that, initiator is added to finish the polymerization. These procedures can produce high-quality MIP beads in the 5 to 50 µm size range. Because the procedure is finished in non-covalent solvent, the production is suitable for non-covalent environments. Therefore, this method is limited to some extent. Recently, a new precipitation polymerization method was developed (22, 31–38). In dispersion polymerization, primary particles swell in a polymerization medium and the polymerization takes place in particles, whereas in precipitation polymerization, primary particles do not swell in a polymerization medium and the polymerization proceeds in the medium. However, there is no sharp distinction between them (30, 39). In precipitation polymerization, monomer, template, cross-linker and initiator are dissolved in solvent. After removing oxygen and synthesizing, the polymers are centrifuged or filtered to remove templates. This method can produce a good yield of high-quality products in one step. Unfortunately, because the binding sites are inside the network, the mass transfer of target molecules is very slow. Two-step or multistep swelling polymerization (40–48) is another common method for preparing MIP beads. MIPs of uniform size within the range of 5–100 µm can be produced by these methods. However, it takes time to obtain the polymerization. Suspension polymerization (49–58) is one of the most common and simplest approaches for the production of MIP beads. Conventional suspension polymerization uses water as a continuous phase to suspend a droplet of prepolymerization mixtures (template molecule, functional monomer, initiator, porogen and cross-linker) in the presence of a stabilizer or surfactant. However, hydrogen bonding interactions between a template molecule and a functional monomer is unexpectedly weakened by the water that is used as continuous phase. Recently, two new suspension polymerization techniques have been developed based on droplet prepolymerization mixtures formed in liquid perfluorocarbon or mineral oil. These liquids are used as a continuous phase instead of water. This kind of continuous phase does not interfere with hydrogen bonding or electrostatic interactions between a template molecule and a functional monomer. Both procedures can produce high-quality MIP beads in the 5 to 50 µm size range, whereas in the conventional suspension polymerization procedure, the continuous phase, water, is incompatible with most non-covalent imprinting procedures. The liquids used in the process of new suspension polymerization are rather expensive. Recently, a surface-initiated polymerization (17, 59–69) was applied to prepare an MIP film grafted on the surface of beads. The procedure consists of initially grafting the initiator to the surface of spherical particles and conducting the polymerization reaction of monomers on the surface of the supports. The binding sites are on the surface, so mass transfer is very fast and the template is easily washed. In addition, in situ polymerization (18, 70–72) is a very simple and
direct method for preparing MIPs for SPE separation or HPLC. Template molecules, monomers, cross-linker and porogen are poured into a stainless steel tube. After the removal of template molecules, the MIP column can be directly connected to the HPLC system for online SPE.

**MISPE Modes**

The first study of MISPE was made by Sellergren in 1994 (73). Since then, several researchers have developed more MISPEs in different formats. There are two modes of MISPE: online and offline MISPE.

**Offline MISPE mode**

Currently, the offline MISPE mode (Figure 2) is widely used. The primary advantages of this mode are that the entire operation is simple and easy and there are more choices of solvents and additives. Therefore, it has a higher enrichment rate and selectivity. In this mode, a small amount of imprinted polymer (typically 50–500 mg) is packed in a cartridge. The cartridge is conditioned, loaded, washed and eluted. After these steps, the target compounds are isolated from real samples.

In the conditioning step, the cartridge should first be washed by solvent to remove the residues. The cartridge should be conditioned by the loading solvent to maximize the MIP interactions with the target analyte in the sample. Loading the sample is one of the most important procedures in MISPE. The loading solvent is selected by the kind of porogen and template/monomer interactions during polymerization. There are two methods of sample loading. One is aqueous loading and the other is organic loading. Aqueous loading utilizes the hydrophobicity of the sample to combine with the polymers. This loading method can load many samples, but its selectivity is very poor and the interfering substances are also adsorbed. To protect the binding sites, a low polarity solvent should be chosen for the organic loading of samples. The commonly used low polarity solvents are acetonitrile, chloroform, dichloromethane and toluene. Washing is the most important step in MISPE. The purpose of this step is to maximize the specific interaction between MIP and analytes and to simultaneously elute the interfering contaminants. It is difficult to find appropriate solvents for common extraction. Although MIPs have high selectivity, it is easy to separate the contaminants under appropriate conditions. There are many washing solvents. These solvents are usually chosen according to the properties of the templates and the interfering contaminants. Generally, a low polarity organic solvent is used in this step, such as chloroform, dichloromethane, toluene or their mixtures. Eluting is the last procedure in MISPE. To obtain high enrichment factors and quantitative recoveries, it is necessary to use small volumes of solvent. Generally, polar solvents are used, with a small amount of acid or alkali additives such as trifluoroacetic acid and triethylamine. After elution, the eluate is dried and the dry residues are dissolved in an appropriate solvent. The target analytes are analyzed by a specific method.

Offline mode is time-consuming. With the lengthened time, error is increased.

**Online modes**

An online mode means that an MISPE column is linked to another instrument and the processes of concentration, separation and detection are automatic. In this mode, a pre-column packed with the MIP particles is placed before the analytical column or in the loop of the injector. The column is loaded with the sample. After the interfering substances are washed off, the analytes are eluted by the mobile phase, separated in a chromatographic column and determined by a detection system. For example, this method is applied by Zhang et al. (74) for the detection of metribuzin herbicide. With the injection valve in the loading position (Figure 3A), the metribuzin sample is loaded...
into the preconcentration column by Pump 1 and metribuzin is absorbed and concentrated in the column. After loading, the valve is turned to the injection position to connect the preconcentration column to Pump 2 of the analytical system (Figure 3B). The metribuzin is transferred with the mobile phase to the analytical system. This approach is suitable for the direct analysis of metribuzin.

In another online MISPE mode, the molecularly imprinted column is directly linked to detector with no chromatographic column. Therefore, preconcentration and separation of analytes can be directly conducted on one MIP column. In this method, the rapid elution of analytes is quite important. If the analytes are strongly retained or interfering substances are retained by the imprinted microcolumn, pulses of polar solvents containing variable amounts of organic solvents are needed. When the analytes are retained in the imprinted sites by interactions based on hydrogen bounds, a single pulse of a polar solvent is sufficient to elute them. Mullett and Lai used this method to detect theophylline in serum (75).

**MISPE application in the analysis of agrochemicals**

Contemporary analytical methods require high sensitivity for detecting and quantifying agrochemicals. Because agrochemicals are present at low concentrations, the common imprinted polymers for MISPE of agrochemicals have been prepared by different polymerization methods, such as bulk polymerization, precipitation polymerization, two-step swelling polymerization, suspension polymerization and dispersion polymerization. A few MIPs have been synthesized by semi-covalent imprinting. Silica grafted with MIP has also been developed as an SPE sorbent.

**MISPE application in herbicides**

Table I lists the applications of MISPE in herbicides. Most MIPs used in MISPE procedures for herbicides are prepared by the non-covalent imprinting technique and bulk polymerization method. Some MIPs are synthesized by precipitation polymerization, suspension polymerization and one-step or multi-step swelling polymerization. Silica grafted with MIPs has also been made. In only one study, the MIPs were synthesized by the semi-covalent procedure. Most MISPE procedures are conducted in offline mode.

Applications of MISPE to the extraction and determination of triazine herbicides from various samples have attracted much attention. Triazine herbicides are used every year in large quantities. Cacho *et al.* (22) prepared MIPs by using propazin as the template to extract triazines from soil and vegetables by precipitation polymerization. Breton *et al.* (76) prepared MIPs by using cyanazine as a template. Their research showed that a combination of photosynthetic-based biosensor and a preconcentration step using MIP allowed the detection of photosynthesis-inhibiting herbicides in water at the level required by the European community. Hu *et al.* (17) prepared a novel prometryne MIP-coated solid-phase microextraction (SPME) fiber and applied it to determine triazines in complicated samples. The results showed that the prometryne MIP-coated fibers possessed special selectivity and affinity to the seven triazines and the sensitivity of the determination of triazines was obviously enhanced by the MIP-coated SPME–HPLC method. Turiel *et al.* (28) synthesized a propazine imprinted polymer using acetonitrile or toluene as porogen. The polymer prepared in toluene showed the best performance. Finally, the MISPE procedure was applied to the cleanup of water, soil and corn sample extracts and the triazines were determined by micellar electrokinetic chromatography (MEKC). Djozan and Ebrahimii (24) produced a monolithic SPME fiber on the basis of MIP to selectively extract triazines from tap water, onion and rice. The triazine herbicides were analyzed by GC and GC–mass spectrometry (MS).

Moreover, MISPE has been applied to the extraction of phenylurea and sulfonylurea herbicides. Tamayo *et al.* (77) prepared two different MIPs by precipitation polymerization using linuron or isoproturon as templates. The MIPs were used as selective sorbents in MISPE procedures for the isolation of several phenylurea herbicides in carrot, potato, corn and pea sample extracts. These herbicides were determined by HPLC–ultraviolet (UV) at 244 nm. Cacho *et al.* (78) synthesized four different MIPs based on methacrylic acid (MAA) by using fenuron or isoproturon as templates and acetonitrile or toluene as porogen. The results showed that fenuron polymers and the polymer prepared by using toluene as porogen were highly selective. The MIPs were applied as a new sorbent to extract phenylureas in ground water and soil samples. Tamayo and Martin-Esteban (18) synthesized MIPs and packed them in stainless steel HPLC columns (125 × 4.6 mm). Finally, the optimum imprinted column was directly used for the LC–UV screening of phenylurea herbicides from vegetable sample extracts without any previous cleanup step at a low concentration level in less than 10 min. Tang *et al.* (5) prepared an MIP by precipitation polymerization by using BSM as the template molecule. The procedure of MISPE was optimized to extract sulfonylureas in soybean samples and a high recovery was obtained. The recoveries of MIP for nicosulfuron (NS), methsulfuron-methyl (MSM), BSM and tribenuron-methyl (TBM) were also compared with those of C18 SPE cartridges; the results demonstrated that the recovery values of MIP were 29.34% for NS, 24.37% for MSM, 46.03% for BSM and 5.01% for TBM. She *et al.* (79) synthesized an MIP for sulfonylurea herbicides by precipitation polymerization by using chlorsulfuron as the template molecule. The MIPs were packed into cartridges. After extraction, the samples were determined by LC–tandem mass spectrometry (MS-MS).

MISPE has also been applied to other kinds of herbicides. A method based on chemometrics and quantum chemistry was proposed by Zhang *et al.* to design and synthesize dummy chloroacetamide imprinted polymers (58). Metolachlor deschloro was selected as the template. Compared with commercial SPE columns, MISPE exhibited selective binding properties for chloroacetamide herbicides and the matrix effect was significantly decreased. Baggiani *et al.* (80) prepared bentazene polymers by using non-covalent molecular imprinting polymerization. The MIPs were packed in HPLC columns. The columns showed good recoveries (91–96%) and concentration factors of 3.2–15.2.

**MISPE application in fungicides**

Table II lists the applications of MISPE to the extraction of fungicides in various samples. All MIPs were prepared by the non-covalent imprinting technique and most of the MISPE studies were conducted in the offline mode. Cacho *et al.* (29) prepared molecularly imprinted capillary columns by using thiabendazole...
<table>
<thead>
<tr>
<th>Template</th>
<th>MIP synthesis</th>
<th>Type of analytes</th>
<th>Number of analytes</th>
<th>MISPE mode</th>
<th>Sample</th>
<th>Analytical system</th>
<th>Recovery (%)</th>
<th>Limit of detection</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Terbuthylazine</td>
<td>Non-covalent, bulk</td>
<td>Chlorotriazines</td>
<td>5</td>
<td>Offline</td>
<td>Ground water</td>
<td>LC–diode array detection (DAD)</td>
<td>81 – 96</td>
<td>0.05 – 0.2 μg/L</td>
<td>15</td>
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<td>Feruron or isoproturon</td>
<td>Non-covalent, bulk</td>
<td>Phenylureas</td>
<td>7</td>
<td>Offline</td>
<td>Ground water soil</td>
<td>LC–UV</td>
<td>74.9 – 116.4</td>
<td>—</td>
<td>16</td>
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<td>Prometryn</td>
<td>Non-covalent, surface</td>
<td>Triazines</td>
<td>5</td>
<td>Offline</td>
<td>Soybean</td>
<td>HPLC–UV</td>
<td>81.7 – 119.7</td>
<td>78.0 – 103.5</td>
<td>17</td>
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<td>Non-covalent, in situ</td>
<td>Phenylureas</td>
<td>2</td>
<td>Online</td>
<td>Potato</td>
<td>HPLC–UV</td>
<td>81.0 – 106.1</td>
<td>0.012 – 0.090 μg/L</td>
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<td>Non-covalent, bulk</td>
<td>Sulfonureas</td>
<td>5</td>
<td>Offline</td>
<td>Water</td>
<td>HPLC–UV</td>
<td>85 – 116.4</td>
<td>2 – 9 ng/L</td>
<td>20</td>
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<td>Sulfonureas</td>
<td>5</td>
<td>Offline</td>
<td>Tap water</td>
<td>HPLC–DAD</td>
<td>96 – 102</td>
<td>6 – 13 ng/L</td>
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<td>Triazines</td>
<td>5</td>
<td>Offline</td>
<td>Drinking water</td>
<td>HPLC–UV</td>
<td>88 – 93</td>
<td>0.01 μg/mL</td>
<td>21</td>
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<td>Semi-covalent, precipitation</td>
<td>Triazines</td>
<td>5</td>
<td>Offline</td>
<td>Soil</td>
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<td>89 – 95</td>
<td>0.4 – 2.4 ng/g</td>
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<td>Sulfonureas</td>
<td>4</td>
<td>Offline</td>
<td>Water</td>
<td>HPLC–UV</td>
<td>85.1 – 92.5</td>
<td>15 – 25 ng/L</td>
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<td>Triazines</td>
<td>7</td>
<td>Offline</td>
<td>Water</td>
<td>GC–MS</td>
<td>87.8 – 92.8</td>
<td>20 – 88 ng/mL</td>
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<td>Triazines</td>
<td>5</td>
<td>Offline</td>
<td>Tap water</td>
<td>MEKC–UV</td>
<td>85.1 – 92.5</td>
<td>0.1 μg/kg</td>
<td>58</td>
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<td>Methylthiotriazines</td>
<td>3</td>
<td>Online</td>
<td>River water</td>
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<td>85.1 – 101</td>
<td>25 pg/m</td>
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<td>Chloroacetamides</td>
<td>6</td>
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<td>Cabbage</td>
<td>LC–MS-MS</td>
<td>87.1 – 92.5</td>
<td>0.1 μg/kg</td>
<td>58</td>
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<td>Cyanazine</td>
<td>Non-covalent, bulk</td>
<td>Triazines</td>
<td>3</td>
<td>Offline</td>
<td>Water</td>
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<td>85.1 – 92.5</td>
<td>0.1 μg/kg</td>
<td>58</td>
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<td>Phenylureas</td>
<td>4</td>
<td>Offline</td>
<td>Potato</td>
<td>HPLC–UV</td>
<td>80 – 98</td>
<td>—</td>
<td>76</td>
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<td>Offline</td>
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<td>LC–UV</td>
<td>84 – 110</td>
<td>0.3 – 0.9 ng/g</td>
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<td>Sulfonureas</td>
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<td>Offline</td>
<td>Soybean</td>
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<td>76 – 97</td>
<td>0.15 – 1.45 μg/kg</td>
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<td>Maize</td>
<td>LC–MS-MS</td>
<td>75 – 110</td>
<td>0.02 – 0.15 μg/kg</td>
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<td>HPLC–UV</td>
<td>85.1 – 92.5</td>
<td>5 – 15 ng/L</td>
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<td>Environment water</td>
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<td>94 – 115</td>
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<td>98 – 115</td>
<td>—</td>
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<td>Drinking water</td>
<td>HPLC–UV</td>
<td>98 – 97</td>
<td>0.3 – 0.9 ng/g</td>
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<td>Atrazine</td>
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<td>Offline</td>
<td>Water, soil</td>
<td>HPLC–UV</td>
<td>96.8 – 119.5</td>
<td>6.0 ng/L</td>
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<td>Triazines</td>
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<td>Offline</td>
<td>Water</td>
<td>HPLC–UV</td>
<td>96.8 – 119.5</td>
<td>6.0 ng/L</td>
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<td>Triazines</td>
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<td>Water</td>
<td>HPLC–UV</td>
<td>—</td>
<td>—</td>
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<td>Triazines</td>
<td>5</td>
<td>Offline</td>
<td>Water</td>
<td>HPLC–UV</td>
<td>—</td>
<td>—</td>
<td>88</td>
</tr>
</tbody>
</table>
as template. The high selectivity obtained by the MIP–capillary electrochromatography (CEC) procedure allowed the unambiguous detection and quantification of TBZ in citrus samples by direct injection, without any previous cleanup, in less than 6 min. Cacho et al. (98) synthesized benzimidazole imprinted polymers by using thiabendazole as the template and divinylbenzene (DVB) as the cross-linker. They developed two modes of MISPE, online and offline. The online enrichment procedure, based on the use of an MIP-DVB column, was proposed as a fast and automatic method for the screening of benzimidazole compounds in tap, river and well water samples. The offline enrichment of the water samples, followed by HPLC, has also been developed. They also compared the cleanup efficiency of MISPE and C18 SPE. Compared with the MISPE, a much noisier baseline was shown on the chromatogram and it was difficult to determine fenbendazole in all evaluated water samples when the samples were preconcentrated through a C18 disc. Baggiani et al. (99) prepared MIPs by iniferter-mediated grafting on porous chloromethylated polystyrene beads, using pyrimethanil as template. The MIPs were evaluated for use as an SPE sorbent. The pyrimidinic fungicides were extracted from red wine samples and analyzed by HPLC. Preconcentration and quantitative extraction of pyrimethanil from wine samples was shown to be feasible down to 0.1 mg/mL. Hu et al. (100) synthesized MIPs by precipitation polymerization by using tebuconazole as the template. The MIPs were used as the SPE sorbent for the direct extraction of tebuconazole from different samples (cabbage, orange juice, tap water, shrimp, and pannage). The recovery of tebuconazole from C18 SPE was only 59.8%, which is significantly less than that from MISPE (75.8%). The results indicated that MISPE provided better recoveries than C18 SPE and SCX SPE.

### MISPE application in pesticides

The application of MISPE to the extraction and determination of pesticides has attracted much attention, due to the wide distribution of pesticides in plants (Table III). Organophosphorus pesticides are widely used in agriculture because they can effectively control insects and weeds to improve the productivity and quality of the crop. However, their residuals can remain after harvesting or storage. The development of a detection method with high sensitivity is quite necessary to prevent these uncontrolled effects on human health and environmental pollution. Pereira and Rath (101) synthesized an MIP that was able to selectively extract fenitrothion (FNT) from tomatoes. The cleanup and concentrating of FNT before HPLC analysis was performed with three different cartridges placed with the MIP for three days and a fortification level of 5 μg/g. The MIP presented a medium extraction efficiency of 59%. This MIP cartridge could be reused several times after regeneration. Xin et al. (102) prepared an MIP by using a mixture of trichlorfon and monocrotoxophos as a mixed template. Using this imprinted polymer as the sorbent, a new method of MISPE coupled with GC was developed for the determination of organophosphates in vegetables. Xu et al. (103) synthesized the imprinted polymer by a room temperature ionic liquid-mediated bulk polymerization technique, using dichlorvos as the template. The cartridge was packed with MIPS to pretreat the vegetables. This cartridge was compared with the C18 extraction column. The results from the chromatograms demonstrated that the imprinted cartridge had a
higher concentration effect and better selectivity for dichlorvos than the C18 extraction column. Kang et al. (104) prepared an MIP by using diethyl(3-methylureido)(phenyl)methylphosphonate (DEP) as a dummy template by precipitation polymerization. They discovered that the specific recognition primarily depended on the functional group of the analyte with the DEP-MIPs. Xie et al. (105) synthesized an MIP by the precipitation polymerization method by using triazophos (TAP) as the template. In their work, an MIP-chemiluminescence (CL) sensor method was developed for the sensitive, selective determination of primicarb in tomato and pear by combining MIP-polymer monolith microextraction with HPLC–photodiode array (PDA). They also compared the performance of MISPE and C18. The higher cleanup efficiency using MISPE was shown in the chromatogram and the MIP monolith could be reused many times without any deterioration.

**Conclusions**

MIPS are generally used for sample preparation and preconcentration of analytes in the analysis of agrochemicals. They are...
often used as sorbents in the procedure of MISPE, which can selectivity isolate analytes from the sample matrix. Compared with classical SPE, MISPE is highly efficient at extraction and cleanup due to its high selectivity (5, 20, 25, 26, 58, 75, 76, 94, 97, 98, 100, 103). Furthermore, it is possible to reuse MIP-based SPE columns many times without any deterioration (20, 101, 109) and the organic solvent consumption is lower in MISPE (25). Therefore, the cost of MISPE is much lower than commercial SPE. The application of MISPE is classified into two modes, offline and online. Most MISPEs are used in offline mode, including applications in herbicides, fungicides, and pesticides. In online mode, the procedures of purification, preconcentration and separation are accomplished in only one MIP column. This mode has higher accuracy and is easy to automate. The published results showed that MISPE is an efficient method to purify, preconcentrate and extract analytes from various samples. Therefore, online MISPE coupled with other instruments should be used for more applications. However, the high selectivity of MISPE provides efficient extraction for the template molecule and its analogues. When online MISPE is coupled with a separation method such as GC or HPLC, this limits its application in the multiresidue analysis of agrochemicals. Therefore, a future trend should develop multitemplate MIPs that meet the requirements of multiresidue analysis of agrochemicals.

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