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11

Sample Preparation via Molecularly Imprinted Polymers (MIPs) in LC-MS Bioanalysis

Myriam Díaz-Álvarez and Antonio Martín-Esteban

11.1 Introduction

The main objectives of sample preparation are the removal of unwanted matrix components which might eventually affect extraction, pre-concentration, and detection of target analytes or conversion of (if needed) the analytes to a more suitable form for detection. More recently, new objectives such as improvement of selectivity in extraction, miniaturization, and reduction in use of organic solvents have been set (Smith 2003). In order to fulfill such requirements, sample treatment techniques such as solid-phase extraction (SPE) and solid-phase microextraction (SPME), among others, have been incorporated to bioanalytical laboratories. However, those techniques still suffer from lack of selectivity even with extensive optimization. As an alternative, antibodies immobilized onto an adequate support, called immunosorbents, were proposed some years ago in order to overcome the issue of assay selectivity. The inherent selectivity of antibodies provides an excellent cleanup of the analytes of interest from the sample matrix. Several analytical methods based on immobilized antibody have been developed for the determination of a variety of analytes in food, environmental, and biological samples (Hennion and Pichon 2003). However, generation of suitable antibodies is difficult, time-consuming, and expensive. Furthermore, it is often difficult to guarantee its success. Besides, it is important to point out that once the antibodies have been obtained, they have to be immobilized onto an adequate support, which may result in poor antibody orientation or even complete denaturation. Because of these limitations, an alternative approach to synthesize host molecules, which can recognize targeted guest species, has been developed. This approach is called “molecular imprinting.”

Molecular imprinting, shown schematically in Figure 11.1, is based on the preparation of a highly cross-linked polymer around a template (the analyte) in the presence of a suitable monomer. The template

and monomer(s) are first mixed in order to form a stable pre-polymerization complex in a selected solvent. Subsequently, the polymerization is initiated in the presence of a suitable cross-linker. After polymerization, the polymer is ground and sieved to an appropriate particle size, and the template is removed, leaving cavities complementary in shape, size, and functionality to the template. These cavities are able to selectively rebind, in given conditions, the analyte (the template) from a complex mixture. The preparation of molecularly imprinted polymers (MIPs) is easy and inexpensive and is a powerful tool for providing selectivity to the analytical process (Tamayo et al. 2007; Bui and Haupt 2010; Martín-Esteban and Sellergren 2010; Hu et al. 2013; Martín-Esteban 2013).

11.2 Preparation of MIPs

As mentioned above, the first step in the preparation of MIPs involves the prearrangement of the template and monomer(s). The template molecule associates with the functional monomer(s) to form a covalent or a non-covalent bonded complex. The covalent approach is attributed to Wulff and Sarhan (1972), who described polymer preparation with chiral cavities for the separation of racemic mixtures. This first example of imprinted polymer was based on the reversible formation of ester linkages between a sugar and phenylboronic acid, which was derivatized with a vinyl group. Imprinting with covalent interactions allows the cavity structures to be probed in detail but this system is not very flexible when choosing the functional monomer(s) and the template species, restricting its application to only a few molecules.

A more flexible approach, known as non-covalent imprinting, was introduced by Arshady and Mosbach (1981). In this approach, the template–monomer interactions are based on hydrogen bonding, electrostatic interactions, and/or metal ion coordination. Therefore,

Figure 11.1 Principle of molecular imprinting.

it covers a wide range of monomers and templates, thus increasing its range of application in chemical analysis. This flexibility has led to a spectacular increase in the number of applications published during past years. This chapter will, therefore, focus on the variables that affect the performance of non-covalent imprinted polymers and their applications.

The template, monomer(s), cross-linker, and solvent (porogen) used are obviously the key parameters for the obtainment of a successful selective MIP. All the parameters mentioned have a strong influence on the overall performance of MIPs in terms of affinity, selectivity, loading capacity, etc. The proper selection of these parameters will ensure the appropriate properties of the MIPs for the intended application.

11.2.1 Template and Monomer(s)

The first step in the preparation of MIPs is the prearrangement of the template and the monomer(s) in a solvent. As the selection of the monomer depends upon the characteristics of the template, the template should contain in its structure functional chemical groups capable of interacting with the monomer(s) with sufficient strength to form a stable complex (Sellergren 1999). Thus, for templates containing acidic groups, Brönsted basic functional monomers (e.g. 2- or 4-vinylpyridine [VPy]) are preferably choice, whereas for basic templates, acidic functional monomers (e.g. methacrylic acid [MAA]) are used. In the case of weakly polar to apolar templates, with few polar interaction sites, it may be beneficial to use amphiphilic monomers, stabilizing the monomer-template assemblies by hydrophobic and van der Waals forces. Although such rule of thumb is valid, for some systems monomer selection is more complex than expected. The selection is best carried out by using high-throughput polymer synthesis techniques, proper experimental design, and combinatorial techniques. Typically, a combinatorial library of MIPs in small-scale (mini-MIPs) is prepared using different

monomers, cross-linkers, and porogens. The ability of obtained mini-MIPs to recognize target analyte is evaluated by equilibrium rebinding-elution experiments. Subsequently, a scaled-up version of the optimum polymer is prepared (Lanza and Sellergren 2004; Villoslada and Takeuchi 2005; Martin-Esteban and Tadeo 2006). The selection of suitable monomers may also be carried out by computational methods. This approach relies on molecular modeling software to design and screen a virtual library of monomers against the desired template. Through this approach it is possible to calculate binding energies and to predict template-monomer interaction positions, making it easier to select the best functional monomer (Piletsky et al. 2001; Chianella et al. 2006).

Besides, it has to be pointed out that since the template-monomer interactions are governed by an equilibrium process, a large amount of monomer should be used in order to drive the equilibrium toward the formation of the template-monomer complex. In general, a template-monomer molar ratio of 1 : 4 should provide the needed stability for the formed complex. However, the excess of free monomers leads to the formation of nonspecific binding sites, therefore, when using such MIP materials to conduct SPE, the loading, washing, and elution conditions must be correctly selected, as described later.

The solvent used in the pre-polymerization step is also of prime importance since it has a direct influence on the strength of the template-monomer interaction. In general, solvents with a low dielectric constant, such as chloroform and toluene, offer an adequate medium to stabilize hydrogen bonding and/or electrostatic interactions between monomer(s) and templates. Solvents with higher dielectric constants (i.e. acetonitrile) have also been used but the polymers obtained usually show a lower affinity to rebinding the template. Protic solvents, such as water and methanol, are not recommended since they disrupt the template-monomer hydrogen-bonding interactions. Polymerizable host monomers have been designed to form a strong complex with a given template in solution

and, in some cases, this has resulted in a quantitative yield of high-energy binding sites (stoichiometric imprinting), which can recognize the template also under aqueous conditions (Wulff and Knorr 2001; Hall et al. 2005).

Finally, the size and shape of the template has a remarkable influence on the selectivity of the obtained polymers. In general, slight difference in the structure of the template near the functional group responsible for the interaction with the monomer may have an impact on the selectivity of the imprinted polymers. In some cases, this difference prevented the polymers from binding to structurally related compounds (Sellergren et al. 1988; Ramstrom et al. 1996). However, in other cases, the impact seemed negligible (Tamayo et al. 2003).

11.2.2 Cross-linker

In order to preserve the stability of the template–monomer complex during the polymerization process and to increase polymer porosity, a high degree of cross-linking is necessary. The most commonly used cross-linkers are ethylene glycol dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TRIM), and divinylbenzene (DVB). It has been reported that at least 50% of the total monomer in a MAA-system has to be EGDMA, otherwise no recognition can take place (Sellergren 1989). The presence of a cross-linker not only preserves the binding sites but also has a direct influence on the physical and chemical properties of the polymeric matrix. EGDMA is the cross-linker often used in methacrylate-based systems, because it provides mechanical and thermal stability, good wettability, and rapid mass transfer.

11.2.3 Porogen

Apart from its influence on the strength of template–monomer interaction during the prearrangement step, the solvent (porogen) plays an important role in the morphology of the formed polymer in terms of specific surface area and pore diameter. In general, low surface area and low macroporosity are associated with low template recognition in the subsequent rebinding experiments owing to the slow analyte diffusion to the binding sites located in micropores.

As stated earlier, the polymer obtained by bulk polymerization has to be ground and sieved to the desired particle size (25–50 µm). This process is tedious and time-consuming, and the particles obtained are often irregular in size and shape. In general, only 50% or less of the total amount of polymer is useful for analytical purposes and some binding sites of the polymers are partially destroyed during grinding, which results in a considerable loss of loading capacity of the imprinted polymer versus the theoretical loading capacity considering the amount of template used in preparation.

To overcome the challenges mentioned above, different polymerization strategies have been proposed by several authors for the direct preparation of imprinted polymers, which enables spherical particles to be obtained in the desired particle size. Among those strategies, the most commonly used ones are: (i) imprinting in the pores of preformed beaded silica (Yilmaz et al. 2002; Abou-Hany et al. 2015), (ii) a two-step swelling technique using water as a suspension medium (Haginaka and Sakai 2000; Sambe et al. 2007), and (iii) precipitation polymerization (Wang et al. 2003; Turiel et al. 2005). More recently, the synthesis of core-shell particles has received special attention. In the core-shell particles, since the MIP is located in the outer surface of the particle (“shell”), the mass-transfer of target analytes to the binding sites is improved (Sellergren et al. 2002; Tamayo et al. 2005; Barahona et al. 2010).

Once the polymer has been prepared, it is necessary to remove the template from the polymer in order to free the binding sites. This step is usually carried out by washing the polymer repeatedly with a solvent capable of disrupting the template–monomer interactions. However, it is known that, even after exhaustive washing by different methodologies (Ellwanger et al. 2001), trace templates remain in the polymer network. On the other hand, it is also true that in the vast majority of publications, no template bleeding has been reported at a concentration level that might interfere with the intended applications.

In any case, the best approach to address the bleeding issue is the use of an analogue of the target analyte as a template. By this approach, the bleeding of the template, if any, does not interfere with the quantification of the target analyte. This approach was originally proposed by Andersson et al. (1997) for the selective extraction of sameridine on a MIP synthesized using a template compound closely related to the target analyte. However, the use of template-analogue imprinted polymers often leads to an inferior molecular recognition of the MIPs as compared to those prepared using the analyte itself as the template. A very interesting alternative, proposed by Kawaguchi et al. (2005) is the use of a stable isotope-labeled compound as the template molecule. Isotope imprinting has been further exploited for the determination of bisphenol A (BPA) in water samples. In this case, BPA-d₁₆ was used as a template and BPA-¹³C₁₂ was added to the sample as surrogate standard before SPE. Subsequently, the extracted sample was analyzed by LC-MS. Figure 11.2 shows the LC-MS single ion monitoring chromatograms of BPA, BPA-¹³C₁₂, and BPA-d₁₆ in river water sample using SPE as the sample preparation method. As can be seen, leaking of template BPA-d₁₆ occurs but it does not affect the quantification of analyte BPA.

Figure 11.2 Single ion monitoring chromatograms of BPA, BPA- $^{13}\text{C}_{12}$, and BPA- d_{16} in river water samples subjected to MISPE by LC-MS.
Source: Reprinted from Kawaguchi et al. (2005) with permission from Elsevier.

11.3 MIPs for Sample Preparation in Bioanalysis

11.3.1 Molecularly Imprinted Solid-phase Extraction (MISPE)

The use of MIPs as selective sorbent materials allows for a customized sample preparation. Clean extracts using MIP in SPE, known as molecularly imprinted SPE (MISPE), can be obtained with minimal sample pretreatment and is by far the most advanced MIP technique (Bui and Haupt 2010; Hu et al. 2013; Martin-Esteban 2013) with many successful applications as summarized in Table 11.1.

There are several configurations of MISPE, including (i) batch SPE, where the MIP is incubated with the

sample, (ii) conventional off-line SPE, where the MIP is packed into cartridges, and (iii) in-line or online SPE.

11.3.1.1 Off-line Protocols

Typically, a small amount (15–500 mg) of imprinted polymer is packed into polyethylene cartridges. Then, as it is shown in Figure 11.3, the sample is commonly loaded onto the MIP cartridge in a low-polarity solvent, in which the specific interactions are maximized. After a washing step to remove unwanted components that are nonspecifically bound to the polymeric matrix, a solvent is added to disrupt the non-covalent interactions between the analyte and the imprinted polymer to elute the analytes, ideally free of interfering matrix components, and the final extract is further analyzed by chromatographic techniques. Samples such as urine, serum, or plasma are usually

Table 11.1 Examples of MISPE in pharmaceutical analysis and bioanalysis.

Analytes	Sample and matrix	Mode	Reference
Pentamidine	Urine	In-line	Sellergren (1994)
Sameridine	Plasma	Batch	Andersson et al. (1997)
Theophylline	Serum	In-line, PE	Mullett and Lai (1998)
Theophylline	Plasma	In-line, DPE	Mullett and Lai (1999)
Theophylline	Serum	Off-line	Li et al. (2009)
β -Agonists	Urine	Off-line	Van Hoof et al. (2005)
Clenbuterol	Urine	Off-line	van Hout et al. (2003)
Cephalexin	Serum	In-line, DPE	Lai and Wu (2003)
Metformin	Plasma, urine	In-line, DPE	Lai and Feng (2003)
Verapamil and its metabolites	Urine, plasma, and cell cultures	Online, RAM	Mullett et al. (2004)
Diphenylphosphate	Flame retardants in urine	Off-line	Moller et al. (2004)
Ropivacaine	Plasma	Off-line	Cobb and Andersson (2005)
Ropivacaine	Plasma	Online	Abdel-Rehim et al. (2006)
Ropivacaine and bupivacaine	Plasma	Online	Cobb et al. (2007)
Trimethoprim	Urine	Off-line	Hu et al. (2005)
MNAL (tobacco-specific nitrosamine)	Urine	Off-line	Xia et al. (2005)
Cocaine and metabolites	Urine	Off-line, Mag	Sánchez-González et al. (2016)
Mycophenolic acid	Plasma of transplant patients	Off-line	Yin et al. (2006)
Alfuzosin	Plasma and soil	Off-line	Chapuis et al. (2006)
Diazepam and metabolites	Hair	Off-line	Ariffin et al. (2007)
Carbamazepine	Urine and waste water	Off-line	Beltran et al. (2007)
Benzodiazepines	Hair	Off-line	Anderson et al. (2008)
Zidovudine and stavudine	Serum	Off-line	Duy et al. (2009)
Methamphetamine, amphetamine, and methylenedioxyphenylalkylamine designer drugs	Blood	Off-line	Kumazawa et al. (2012)
Diethyl thiophosphate and diethyl dithiophosphate	Urine	Off-line	Santos et al. (2012)
Cannabinoids	Urine, oral fluid	Off-line	Cela-Pérez et al. (2016)
Urinary benzene metabolites	Urine	Off-line	Chauhan et al. (2015)
Biochanin A, daidzein, and genistein	Urine	Off-line	Chrzanowska et al. (2015)
Morphine	Urine	Off-line, MWCNTs	Kolaei et al. (2016)
Venlafaxine and metabolites	Plasma	Off-line	Miranda et al. (2016)

PE, pulsed elution; DPE, differential pulsed elution; RAM, restricted access material; Mag, magnetic; MWCNTs, multiwalled carbon nanotubes.

pre-diluted with a low-polarity solvent (i.e. acetonitrile) to maximize the non-covalent interactions between the analyte and the binding sites of MIPs during the loading step. However, aqueous samples can also be directly loaded onto MIP cartridges. In this case, MIPs behave like a reverse-phase sorbent and thus both target analytes and matrix components are retained through nonspecific interactions. Then, the washing solvent has to be suitable

for removing the matrix components and, more important, of redistributing nonspecifically bound analytes to the selective imprints. Unfortunately, the success of such procedure is not always achieved. Lately, efforts have been directed toward the synthesis of water-compatible MIPs by incorporating hydrophilic surface properties to the polymer in order to reduce nonspecific hydrophobic interactions (Wan et al. 2015).

Figure 11.3 Molecularly imprinted solid-phase extraction (MISPE) procedure.

In order to improve mass transfer of target analytes, some in situ polymerization strategies have also been proposed. In this regard, Du et al. (2012) described the synthesis of kinetin molecularly imprinted monolith in a syringe by an in-situ polymerization technique. The synthesis procedure is relatively simple and can be easily performed in any laboratory. Similarly, in-situ monolithic molecularly imprinted polymer sol-gel packed tips (MMSTs) have been prepared and evaluated for the extraction of lung cancer biomarker l-tyrosine (Tyr) from human plasma and urine samples by LC-MS/MS (Moein et al. 2014).

The in-situ synthesis of MIPs on the surface of micro-filtration glass-fiber membranes in multi-well filter plates has been also proposed (Ceolin et al. 2009; Renkecz et al. 2011, 2012). Briefly, the synthesis procedure is as follows: firstly, membranes of a 24-well filter plate are washed with methanol and dried before use. Then, 30–50 µl aliquots of polymerization mixture are transferred onto the filter plate membranes under oxygen-free argon atmosphere. The plate is covered with UV transparent cling film and placed under a UV lamp for plate irradiation for three hours. After polymerization, the template is removed by successive washing and ready for SPE experiments. The observed MIP on the surface of the modified membranes forms a veil-like web between adjacent fibers, whereas inside the membrane the polymer is partly deposited in clusters on the glass fibers. This approach is a cost-effective, one-step procedure for preparing MIP-composite membranes and offers a viable alternative to existing MISPE cartridges.

11.3.1.2 Online Protocols

In this format, a small precolumn packed with the imprinted polymer (typically about 50 mg) is placed in the loop of a six-port injection valve. After sample loading and washing out interfering components, the analytes are eluted by the mobile phase and then separated on the analytical column. However, the lack of compatibility of the elution solvent necessary to desorb analytes from the MIP precolumn and the mobile phase for chromatography on the analytical column make it difficult to develop such online MISPE methods. This problem can be circumvented by online directing the eluent from the MIP precolumn to the injection loop and subsequently injected onto the chromatographic system (Theodoridis et al. 2004), or by mixing the organic elution solvent with an aqueous-rich solvent before reaching the analytical column (Ou et al. 2006). However, such approaches increase the complexity of the online system and, therefore, require the incorporation of extra-instrumentation (i.e. pumps).

On the other hand, in order to prevent a large amount of matrix components reaching the MIP precolumn, Mullett et al. (2004) reported a two-step online SPE-LC-MS procedure for the determination of verapamil and its metabolites in urine, plasma, and cell cultures. The procedure involved a restricted access material (RAM) cartridge and a verapamil-imprinted polymer (Figure 11.4). In brief, the raw sample was loaded onto the RAM cartridge where the analytes were retained by the alkyl hydrophobic-bonded phase, while hydrophilic electro-neutral diol groups bound to the external surface of the spherical particles protected the sorbent from binding to

proteins in the sample. After this first cleanup, the analytes were desorbed from the RAM precolumn using acetonitrile, and transferred to the MIP precolumn. Finally, the analytes retained by the MIP were desorbed by the mobile phase and transferred to the analytical column for chromatographic separation. Using this approach, verapamil and its metabolites were easily quantified by LC-MS in <35 minutes, including sample preparation, selective fractionation, chromatographic separation, and detection.

Combining the RAM and the MIP properties into one single material has been proposed to eliminate the fractionation step using the RAM precolumn. Haginaka et al. (1999) reported that hydrophilized MIPs, prepared by postgrafting a hydrophilic polymer, providing RAM properties, on the surface of a preformed MIP, allowed for the repeated direct injection of plasma samples for the determination of (*S*)-naproxen with minor fouling by sticky proteins. Alternatively, the direct inclusion of a hydrophilic comonomer in the imprinting polymerization was proposed (Dirion et al. 2003). It was anticipated that this could contribute to defining the imprinted site in addition to creating a more hydrophilic polymer matrix. Such approach was successfully applied to the determination of ropivacaine and bupivacaine in plasma samples (Cobb et al. 2007).

11.3.1.3 In-line Protocols

Due to the high selectivity of MIPs, the direct coupling of a MIP column in-line with the detection system might allow the extraction, enrichment, separation, and determination of target analytes in single step. MISPE with direct in-line UV-detection was first described by Sellergren (1994) for the determination of pentamidine in urine. In that work, after loading 100 ml of diluted urine sample, the column was washed with 100 ml of a buffer solution (pH 9) and pentamidine was eluted with ~1.5 ml of buffer at pH 3. The selective elution allowed for the successful detection of pentamidine free of co-extractives by UV at 270 nm. However, since a large volume of sample was loaded, a high amount of matrix components were retained on MIP columns by hydrophobic interactions, which made it necessary to use a large volume of washing solutions.

MISPE with pulsed elution (MISPE-PE) was proposed as an alternative, which was exemplified in the determination of theophylline in chloroform-diluted serum samples (Mullett and Lai 1998). After sample loading and washing off the interfering components, a 20 µl of methanol was injected to elute theophylline allowing its detection by UV at 270 nm (Mullett and Lai 1998). MISPE-PE has been subsequently improved by applying successive pulses of 20 µl of different solvents, known as MISPE

Figure 11.4 Experimental configuration of the online two-step SPE-LC procedure for the determination of verapamil and its metabolites using a restricted access material (RAM) cartridge and a verapamil-imprinted polymer. Source: Reprinted from Mullett et al. (2004) with permission from Elsevier.

Figure 11.5 Schematic of preparation of molecularly imprinted microSPE device. Source: Reprinted from Sánchez-González et al. (2015) with permission from Elsevier.

with differential pulsed elution (MISPE-DPE), in order to wash out remaining interfering components and the nonspecifically retained analyte fraction (Mullett and Lai 1999). Both approaches allow for analyte enrichment through injection of large volumes of sample, leading to a high analytical sensitivity.

11.3.1.4 Other Protocols

The use of magnetic nanoparticles as support for different stationary phases in batch SPE is one of the most active research area in sample preparation. Upon sample extraction, such modified magnetic particles can be separated from the matrix by a simple magnet, thereby avoiding tedious filtration and/or centrifugation steps. The surface of magnetic particles can be modified by the incorporation of MIPs, which provides the needed selectivity for the extraction (Chen and Li 2012; Hu et al. 2013). One of the simplest ways to synthesize magnetic MIPs is the encapsulation of magnetite nanoparticles in silica through a typical sol-gel reaction (Hu et al. 2013). Silica shells are easily modifiable and, thus, desirable functional groups can be grafted to the surface of the nanoparticles. For example, vinyl groups, which are necessary for the subsequent surface imprinting, can be introduced onto particle surface by reaction with 3-methacryloyloxypropyltrimethoxysilane (MPS). Finally, the MIPs are formed on the surface of modified magnetic particles by the copolymerization of vinyl groups with typical imprinting monomers and cross-linkers in the presence of the template molecule. This procedure has been successfully used for the extraction of sterols in serum (Zhang et al. 2011) and cocaine and its metabolites in urine (Sánchez-González et al. 2016), among others.

A rather simple approach, so-called molecularly imprinted micro-solid-phase extraction (MI-MSPE) has

been proposed. The MI-MSPE (Figure 11.5) device comprises MIP particles enclosed within a porous polypropylene (PP) flat-sheet membrane envelope. Once the MI-MSPE device is prepared, it is conditioned and placed in the sample. The device tumbles freely within the sample solution during extraction for a certain period of time under stirring. After extraction, the device is manually removed with the help of tweezers and placed in a vial for elution of analytes. This approach has been successfully applied to the selective extraction of cocaine and its metabolites from human urine (Sánchez-González et al. 2015). Alternatively, MIP pills have been used for the extraction of cannabinoids from urine and oral fluids. In this approach, the MIP pill is added to the sample and sonicated for a certain period of time. After that, the MIP pill is transferred to a clean tube for elution of target analytes (Lendoiro et al. 2014).

11.3.2 MIPs in Other Sample Preparation Techniques

Other sample preparation techniques, such as SPME or stir-bar sorptive extraction (SBSE), have been incorporated to analytical laboratories as an alternative to SPE. However, the variety of commercially available fibers (or stir bars) is limited and extraction is based on nonselective absorption (i.e. polar analytes are attracted to polar phases). In this regard, the incorporation of MIPs to these techniques would ideally provide a powerful analytical tool with the characteristics of both technologies (simplicity, flexibility, and selectivity).

The preparation of the first MIP-coated silica fiber was proposed by Koster et al. (2001) for the SPME of brombuterol from human urine. Silica fibers were activated by silylation and subsequently immersed in a polymerization solution composed of clenbuterol, MAA, and

EGDMA in acetonitrile to carry out polymerization during 12 hours at 4°C under irradiation at 350 nm. According to authors, fibers with a polymeric film thickness of ~75 µm were obtained in a reproducible manner. Specific extraction of brombuterol from urine has been obtained on the MIP-coated silica fiber. Recently, key factors influencing both the morphology and the thickness of the obtained MIP coating has been systematically studied in a series of papers dealing with the preparation of different MIP-coated silica fibers. The results obtained are rather promising and further development is expected in the near future (Hu et al. 2008, 2009).

A completely different approach for the direct preparation of MIP fibers (monoliths), using fused silica capillaries as molds, has been proposed (Djozan and Baheri 2007; Turiel et al. 2007). Following a simple experimental procedure, MIP monoliths of 1 cm length are obtained with thickness dependent on the inner diameter of the silica capillary used. Moreover, the obtained fibers were flexible and can be bended to a certain extent.

The use of MIP-coated stir bars prepared by chemical bonding of the MIP to silylated stir bars has been proposed. This procedure is similar to that described above for the preparation of MIP-coated fibers for SPME and has been applied for the determination of β_2 -agonists (Xu et al. 2010) and sulfa drugs (Xu et al. 2011) in different samples.

Finally, the use of water-compatible MIPs in matrix solid-phase dispersion (MSPD) has been demonstrated for the selective extraction of fluoroquinolones from eggs and tissue (Yan et al. 2007), with average recoveries 72.2–114.1%, depending upon the analyte and the sample, and relative standard deviations of <7.0%. The superior performance of MIPs compared to conventional sorbents (C18 and Florisil, recoveries were 24.9–84.6%) was clearly demonstrated. More recently, similar methodologies have been applied to the MSPD of steroids in goat milk (Ganan et al. 2012, 2014).

11.4 Fragment Imprinting

Traditional imprinting is well-suited for small molecules but it fails when the targets are complex, poorly soluble in organic solvents, or simply unavailable in sufficient

quantities for imprinting. In this regard, the imprinting of most biomolecules (i.e. proteins, nucleic acids, and polysaccharides) is a challenging task. In these cases, polymers may need to be synthesized in aqueous media, allowing for solubilization of biomolecules without affecting their native conformation. However, the interactions between the target and the functional monomers commonly used in molecular imprinting in aqueous solution are not optimal.

In order to overcome this problem, fragments of the target protein may be used as templates (Quaglia et al. 2001; Rachkov and Minoura 2001). This approach uses available templates that are compatible with the imprinting, resulting in binding sites, complementary to the protein substructure but able to retain the larger biomolecule. Following this so-called epitope imprinting approach, short peptides (epitope) have been used as templates to generate MIP material for binding larger target containing the epitope sequence. Besides, the combination of epitope imprinting with surface imprinting techniques has led to the obtainment of MIPs that are able to recognize peptides (Titirici and Sellergren 2004) or even proteins (Nishino et al. 2005, 2006) based on just a short terminal peptide sequences.

11.5 Summary

MIPs are well-suited to be incorporated to sample preparation techniques to obtain cleaner sample extracts as compared to some conventional methods. Such cleaner extracts are often accompanied with reduced ion suppression in MS detection. As shown by the numerous recent examples, the quantitative analysis of pharmaceutical drugs, drugs of abuse, or endogenous biomarkers in various biomatrices (urine, serum, plasma, hair, and tissue samples) has been improved by the application of MIPs.

During the last few years, refinement of the use of MIPs in sample preparation has been observed. Further development in the areas of molecular recognition in aqueous media and protein imprinting is expected. Commercialization of certain MIPs will more or less represent a breakthrough of sample preparation in the bioanalysis field.

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