Articles

The Development of a Semiautomated Procedure for the Synthesis and Screening of a Large Group of Molecularly Imprinted Polymers

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A method for synthesis and evaluation of molecularly imprinted polymers (MIPs) on a semiautomated miniature scale is reported. This technique combines molecular imprinting with the combinatorial chemistry approach, allowing rapid screening and optimizations of libraries of MIPs. The polymers were prepared and evaluated in situ by rebinding utilizing powder dispensing and liquid handling systems. MIPs were prepared by a combinatorial approach using methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide, and styrene as functional monomers, and acetonitrile and toluene as porogenic solvents. A drug substance having aromatic, hydroxyl, –O–CONH₂ functional groups was selected as the template molecule for this study. The MIP library results demonstrated that the polymer prepared with MAA as functional monomer shows the strongest binding affinity, and therefore, is preferred for the preparation of this particular template molecule. Due to the low consumption of reagents, and more importantly, the demonstrated ability of this method to effectively identify optimal imprinting conditions, this small-scale combinatorial protocol is well suited for fast and efficient screening and optimizations of MIPs.

1. Introduction

Molecularly imprinted polymers (MIPs) have been shown to possess unique and predetermined selectivity for target analytes. MIPs can selectively recognize a template molecule used in the imprinting process even in the presence of compounds having similar structure and functionality to the template.[1–10]

The pharmaceutical industries have continuously invested heavily in research and development for the production of novel drug substances. The demand for rapid and effective analytical strategies of this industry that drive improvements in the quality of their products results in a constant search for new analytical methods. The advantages of MIPs, e.g. physical robustness, resistance to high pressure and temperature, and tolerance of different solvents and media, have led to modest increases in their use in the pharmaceuticals sector. MIPs have been implemented in various applications, including sample preparation, as stationary phases for analytical separations, and as analyte recognition materials in affinity assays.[11–16]

Solid phase extraction (SPE) based on molecularly imprinted polymers (MIPs) is a novel approach for sample preparation and preconcentration. The implementation of MIPs in SPE devices for the separation and detection of drugs and drug metabolites has great potential in the pharmaceutical industry. The control of pharmaceutical impurities is a critical issue. HPLC methods applicable to the analysis of drug substances/drug products should be able to separate the active pharmaceutical ingredients (API) from the impurities and degradation products. Analysis of these trace amounts of impurities in the presence of a large quantity of API is problematic, in particular because the impurities are usually structurally related to the API. The use of imprinted polymers as separation media for drug substance and drug product analyses is particularly important for the isolation of degradation products and impurities from the API.

In this work, MIPs that exhibit a high binding specificity for a drug substance were synthesized as sorbents in small scale. These sorbents, when produced in bulk, could be used as solid phase extraction devices for the isolation of impurities and degradation products in drug substances and drug products. This isolation allows for impurity profiling in the absence of the API.[17] As coelution between impurities, degradation products, and the API are frequently a significant concern, this approach is likely to be a useful addition to

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Table 1. Polymerization Mixture Compositions Used To Build an MIP Combinatorial Library

polymer no.	DS (mmol)	MAA (mmol)	acrylamide (mmol)	styrene (mmol)	4-VP (mmol)	acetonitrile	chloroform (ml)	EDMA (mmol)	AIBN (mmol)
1	0.1	0.4				0.3		2	0.05
2	0.1	0.4				0.5		2	0.05
3	0.1	0.4					0.3	2	0.05
4	0.1	0.4					0.5	2	0.05
5	0.1		0.4			0.3		2	0.05
6	0.1		0.4			0.5		2	0.05
7	0.1		0.4				0.3	2	0.05
8	0.1		0.4				0.5	2	0.05
9	0.1				0.4	0.5		2	0.05
10	0.1				0.4		0.5	2	0.05
11	0.1			0.4		0.3		2	0.05
12	0.1			0.4		0.5		2	0.05
13	0.1			0.4			0.3	2	0.05
14	0.1			0.4			0.5	2	0.05

HPLC method development strategies as a technique to demonstrate specificity.

Although polymer preparation by noncovalent imprinting is relatively simple, optimized MIPs are seldom produced.[18–21] Potential issues, such as low binding specificity, template leaching, slow kinetic transfer, and low affinity, can be minimized with optimization.[22,23] Our approach to optimize the main factors affecting the molecular recognition process involves a combinatorial-chemistry-based method. Molecular imprinting using the combinatorial chemistry model allows for rapid screening of combinatorial libraries of MIPs to permit identification of a candidate monomer with the desired levels of capacity and selectivity for a given target molecule.[4,24,25]

In molecular imprinting, intermolecular interactions between the template molecule and the functional monomers play a significant role in molecular recognition. Various functional monomers have been studied against different template molecules; however, this generality makes it difficult to select the appropriate monomers for a given template. Selection of the various functional monomers usually involves time-consuming trial and error, or intuition. Therefore, the development of a semiautomated imprinting process to facilitate production of high performance MIPs is desirable.[26] A library of MIPs was prepared and screened using a semiautomated system consisting of an AutoDose (AutoDose America Inc., Iselin, NJ) powder dispenser and a Gilson (ManSci Inc., Tonawanda, NY) liquid handler. "Mini" MIPs were prepared in situ in individual glass vials.

Solubility assessment of a drug substance is critical in selecting the appropriate conditions for synthesizing polymer with optimized binding capability. Therefore, solvent solubility study was also conducted prior to polymerization. Subsequently, release and rebinding tests of the template molecule in the polymer matrix were performed. Due to the low consumption of reagents, this small-scale protocol is well suited for automation of a combinatorial study.

2. Experimental

2.1. Materials. Methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide, styrene, cross linking monomer ethylene glycol dimethacrylate (EDMA), and free radical initiator, 2,2-azobisisobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Toluene from EMD (Gibbs-town, NJ), HPLC-grade acetonitrile from Fisher Scientific (Fair Lawn, NJ) and Milli-Q water were used. All reagents used in the solvent solubility study were purchased from Aldrich Chemical Co (Milwaukee, WI). Details on the exact structure of the drug substance on which the method is tested and discussed cannot be included here owing to the proprietary nature of the substance; some general structural features of the template are that it is aromatic with hydroxyl and –O–$CONH_2$ functional groups; thus it is a good candidate for imprinting.

2.2. Polymerization. For molecular imprinting, an appropriate amount of functional monomer (approximately 0.40 mmol) was introduced to interact with the drug substance (0.1 mmol) in the selected porogenic solvent. The solution was prepared in a 20 mL scintillation vial. Then, the functional groups on the monomers were positionally fixed with the chemical cross-linker ethylene glycol dimethacrylate (EDMA) (2.00 mmol). The volume of the cross-linker was maintained at about 50% of the total volume of the polymer mixture. Following the dissolution of 0.05 mmol azobis-isobutyronitrile (AIBN) as initiator, the mixture was saturated with dry nitrogen for 10 min, and kept in the oven at 60 °C overnight.

The drug substance, functional monomer (acrylamide), and AIBN were dispensed into the vials using a programmed Autodose powdernium. Other reagents for molecular imprinting were dispensed with a programmed liquid handler (Gilson 215). The drug substance was mixed with different functional monomers (MAA, 4-VP, acrylamide, styrene) and the selected porogenic solvent in a 20 mL vial (Table 1).

2.3. Instrumentation. All the solutions were prepared and dispensed automatically by the programmed Gilson model 215 liquid handler, equipped with 735 software. The drug substance and selected components were dispensed into individual vials using an AutoDose powdernium equipped with Powdernium MTM 2004 software (Figure 1).

The samples were evaluated using an Agilent 1100 series LC system consisting of G1311AA QuatPump, a degasser (G1322A Degasser), a UV detector (G1315B DAD), an automatic sample injector (G1313A ALS), and a reverse-phase column 4.6 × 50mm Agilent Zorbax SB-18 C-18 (1.8 μm) column from Agilent technologies (Palo Alto, CA). Data were collected and analyzed using Agilent ChemStation software.
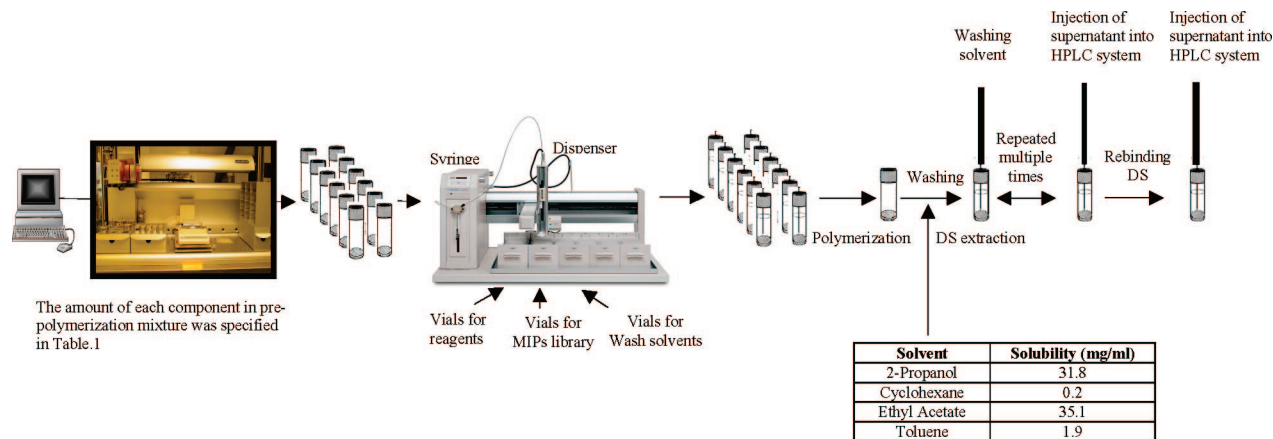
Solvent	Solubility (mg/ml)
2-Propanol	31.8
Cyclohexane	0.2
Ethyl Acetate	35.1
Toluene	1.9

Figure 1. Workflow for a semiautomated system for the synthesis and evaluation of MIP libraries.

2.4. Solubility Study. A solvent solubility assay was prepared using the automated system described above. Powdered drug substance was weighed and dispensed by the Autodose Powdernium directly into HPLC Filter vials with 0.45 μm nylon filter (P/N:35539, Thomson Instruments, Clear Brook, VA). Ten mg of drug substance was added into each HPLC vial with tolerances of $\pm$ 0.5mg.

A total of 36 different solvents in various combinations were added to the HPLC vials using the Gilson liquid handler. Total liquid volume added was 0.3 mL for each filter vial. The samples were then sealed and shaken for one hour, after which the filter vials were compressed to transfer the contents through the filter.

The drug substances were analyzed using a 4.6 × 50mm Agilent Zorbax SB-18 C-18 (1.8 μm) column. The mobile phase used was 0.5% (v/v) trifluoroacetic acid (TFA) in water (eluent A) and 0.5% TFA (v/v) in acetonitrile (eluent B), at 2 mL/min (45 °C). The following gradient was employed: time 0 min 95:5 A:B (v/v), 2.5 min 5:95 A:B (v/v), and 2.55 min until end 95:5 A:B (v/v). Standards used to calibrate the assays were prepared at a concentration of 1.0 mg/mL in 50:50 ACN:H$_2$O (v/v), a typical mobile phase used in methods development studies.

The data was then processed and analyzed using Empower (Waters Corporation, Milford, MA). Table 2 shows the 36 different solvents used to create the solubility profile of the drug substance.

2.5. Initial Screening. After polymerization, the four sets of MIPs were screened using four different solvent systems selected based on their performance in the solubility study. Two mL of each solvent mixture selected was dispensed into the MIP vials. After incubation and shaking for 24 h, the supernatant from each vial was analyzed by HPLC to quantify the amount of free drug substance in solution. In the initial screening, 2-propanol showed the highest drug substance release in the first wash. Therefore, 2-propanol was used in the washing step for extraction of the drug substance from the polymer matrix.

2.6. Routine Screening. Two mL of 2-propanol was dispensed into each of the glass vials and incubated for 2 h. The supernatant was then removed and analyzed by HPLC to quantify the amount of drug substance released into solution. This step was repeated five times. 2 mL of 95:5 2-propanol:acetic acid (v/v) solution was then dispensed into

Table 2. Solubility Profiles of the Drug Substance in 36 Different Solvents

	sample name	solubility (mg/mL)
1	tert-butyl methyl ether	0
2	chloroform	0
3	cyclohexene	0
4	heptane	0.1
5	methylcyclohexane	0.1
6	cyclohexane	0.2
7	isopropyl Acetate	0.2
8	cyclopentane	0.3
9	1-chlorobutane	0.9
10	xylene	1.6
11	toluene	1.9
12	cumene	2.1
13	water	2.3
14	anisole	3.2
15	isobutyl acetate	28.3
16	nitromethane	28.9
17	1-butanol	29.6
18	dimethylformamide	30
19	1-methoxy-2-propanol	30.3
20	1-pentanol	30.4
21	2-methoxyethanol	30.4
22	butyl acetate	30.5
23	trifluoroethanol	30.5
24	2-methyl-1-propanol	30.7
25	1,4-dioxane	30.8
26	trifluoroethanol	31
27	tetrahyrofuran	31.3
28	2-pentanol	31.4
29	3-pentanol	31.6
30	2-propanol	31.8
31	acetonitrile	32.8
32	2-butanone	33.1
33	4-methyl-2-pentanone	33.1
34	methanol	33.2
35	ethanol	34.1
36	ethyl acetate	35.1

each vial using the procedure specified above. This step was repeated twice, followed by rinsing with 2 mL of acetonitrile before the rebinding step.

Rebinding of the drug substance was performed by adding 2 mL of 50 mM of the drug substance standard solution into each vial. The supernatant from each vial was collected and injected into HPLC system for quantification.

3. Results and Discussion

An in situ molecular imprinting protocol was carried out using a programmed, semiautomated procedure. A library of "mini" MIPs were prepared in individual vials and
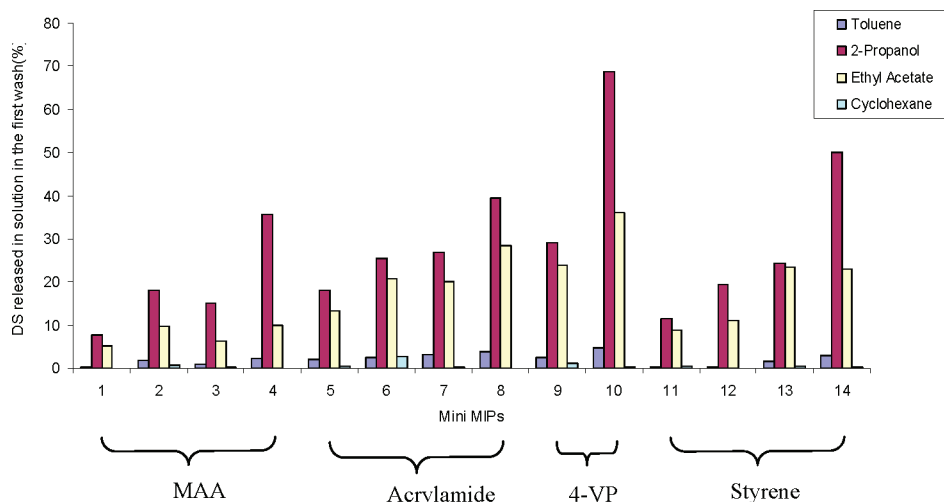
Figure 2. Percent recovery of drug substance (DS) in each of the MIPs for each of four solvents (toluene, 2-propanol, ethyl acetate, cyclohexane). Solubility profiles of the solvents were determined using the amount of drug substance released during extraction relative to the total applied. Initial screening shows 2-propanol has the highest solubility profile, whereas cyclohexane has the lowest.

screened using a semiautomated system consisting an Auto Dose powder dispenser and a Gilson liquid handler. The drug substance, functional monomer, and AIBN were dispensed into the vials using a programmed Autodose powdernium. Other reagents for molecular imprinting were dispensed with a programmed Gilson liquid handler. The polymerization mixture compositions used to synthesize the MIPs is shown in Table 1.

A solvent solubility study was conducted on the drug substance prior to polymerization. Solubility assessment for of a drug substance is critical in selecting appropriate conditions for polymerization. To facilitate hydrogen bonding interactions between the template and monomers, and to determine which solvents best extract the drug substance from the polymer matrix, an array of solvents must be studied.[27] Table 2 shows the 36 different solvents used to create the solubility profile of the drug substance. Four solvents with low polarity were selected based on the solubility of the drug substance in various media. 2-propanol and ethyl acetate provided the higher solubility profiles, 31.8 and 35.1 mg/mL respectively, whereas toluene and cyclohexane gave lower solubility profiles, 1.9 and 0.2 mg/mL, respectively, of the drug substance.

As can be seen from Figure 2, solvents with high solubility profiles have significantly higher drug substance release (the highest is 69% for 2-propanol and 36% for ethyl acetate) compared to those with low solubility profiles (the highest is 4% for toluene and 3% for cyclohexane) during drug substance extraction. In the initial screening, 2-propanol shows the highest drug substance release in the first wash. Therefore, 2-propanol was selected as the washing solvent for the washing step in which the drug substance is extracted from the polymer matrix. These results suggest that the data obtained from solvent solubility study plays a significant role in selecting the appropriate solvent for drug substance extraction prior to the rebinding step. Solvent choice is also important in that it is the solvent that provides the environment in which the template and the functional monomers interact. Figure 3 shows that acetonitrile consistently creates polymers with higher binding capacity than those created
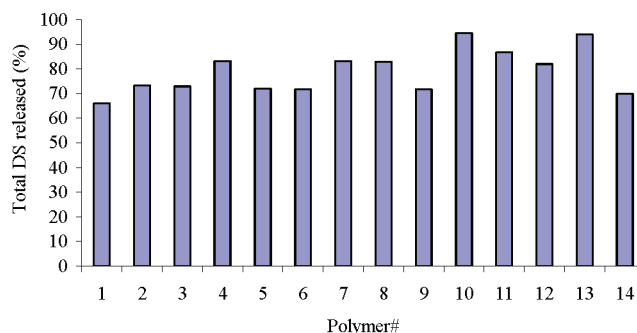

Figure 3. Total drug substance released from the polymer matrix in the extraction process. Based on the hydrogen bonding interaction between the template and monomers, polymers created with MAA have the lowest drug substance release, whereas polymers created with 4-VP have the highest release.

using toluene since the polymers show lower drug substance release during washing. The amount of solvent used to create the polymers also plays an important role in controlling the porosity of the final polymers. Polymers created with higher amounts of solvents are more porous, and therefore, release more drug substance during washing.

The MIP produced using 2-propanol as the washing solvent was selected for further study in the rebinding step. Repetitive rinsing was performed to extract most of the drug substance from the polymer matrix. This was done by rinsing all 14 MIPs with 5 × 2 mL aliquots of 2-propanol, then 2 mL of 95:5 2-propanol:acetic acid (v/v) solution twice, and finally rinsed with 2 mL of acetonitrile. Figure 3 shows the total amount of drug substance released from the 14 MIPs prior to the rebinding step, with polymer no. 1 with the lowest drug substance release (68%) and polymer no. 10 as the highest (95%).

Routine screening in the rebinding step was performed by adding 2 mL of 50 mM of the drug substance solution into each vial (4.3 mL drug substance/vial). After incubating overnight, the supernatant were collected and analyzed by reverse phase HPLC to quantify the concentration of the free drug substance in solution. The amounts of drug substance bound were obtained by subtracting the free drug substance in
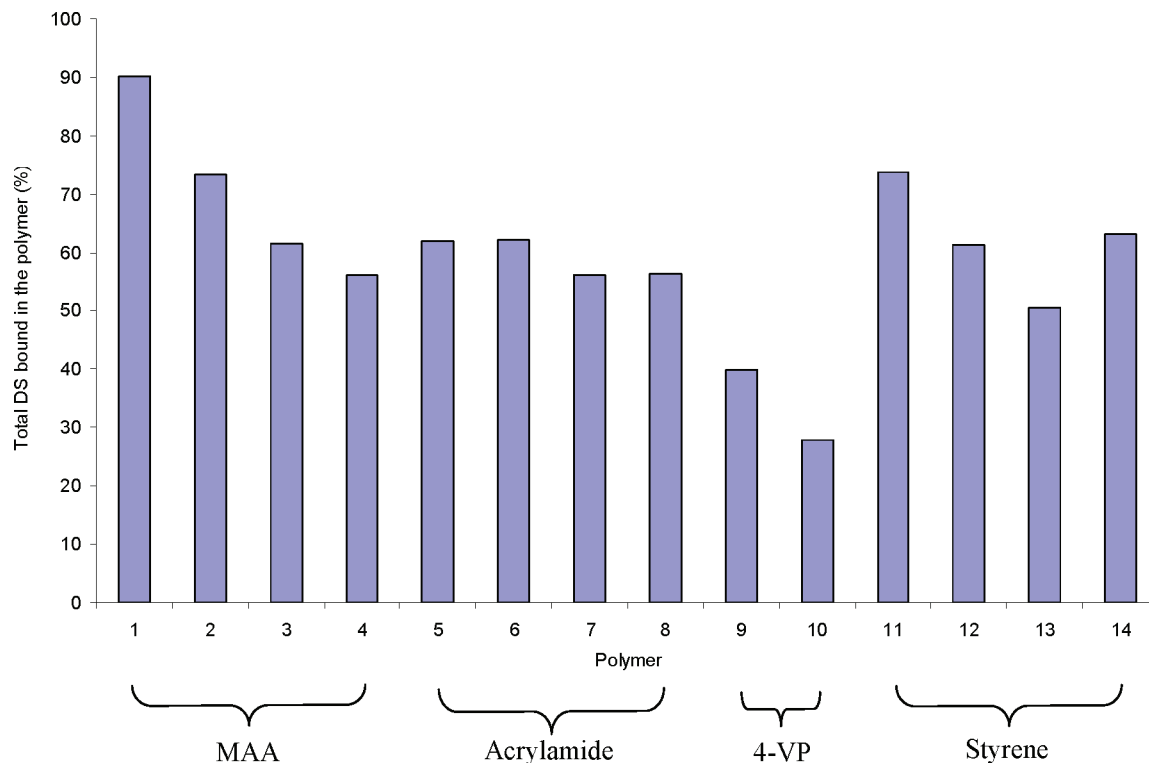
Figure 4. Total fraction of drug substance rebound into each MIP in the rebinding step. From the four monomers initially selected, polymers created with MAA show the highest rebinding of drug substance. Polymer no.1 proved to be the most optimal conditions for the imprinting of this particular drug substance.

solution from the initial amount. Figure 4 shows the amount of drug substance bound by each MIP.

This small-scale protocol for synthesis of MIPs was useful in identifying the functional monomers that are most suitable for a given template. For this study, four commonly used functional monomers, methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide, and styrene, were selected to create the MIPs. MAA is an acidic monomer, 4-VP is basic, and acrylamide and styrene are neutral functional monomers.

Polymers 1–4 were synthesized using MAA, polymers 5–8 were synthesized using acrylamide, polymers 9–10 were synthesized with the basic monomer 4-VP, and polymers 11–14 were synthesized using styrene. The drug substance used as the template for all MIPs was a basic molecule with a pK_a of ~12. The exact structural details for the template cannot be included here owing to the proprietary nature of the substance; some general structural features of the template are that it is aromatic with hydroxyl and $-O-CONH_2$ functional groups; thus it is a good candidate for imprinting. Since hydrogen bonding is the major interaction force between the template and the monomers, MAA will be the best choice of monomer since it can form a strong interaction with the basic functional groups. Data in Figure 3 shows, as expected based on the structural features of the template, that MIPs created with MAA have the lowest drug substance release during the washing step, followed by MIPs created with neutral monomers, styrene, and acrylamide, whereas polymers synthesized with 4-VP showed the highest drug substance release. Moreover, the initial screening results also suggest that MIPs synthesized with MAA provide the highest binding affinity of the drug substance.

As illustrated in Figure 4, the highest amount of drug substance was bound by polymer no. 1 (see Table 1 for polymer composition). Therefore, from the four monomers initially selected, MAA proved to be the most suitable functional monomer for the imprinting of the basic drug substance. From the various amounts and combination of components and reagents used to create the 14 MIP libraries, polymer no. 1 was shown to be the most optimal for the synthesis of this MIP.

Conclusion

The screening protocol described in this work consists of two screening steps. The first capitalizes on the amount of drug substance released in various solvents with differing solvation characteristics, and the second is dependent on the amount of drug substance rebound into the imprinted polymer after it is repetitively rinsed to extract the drug substance from the polymer matrix prior to rebinding. This protocol, in concert with a semiautomated combinatorial approach to MIP synthesis, holds great promise in optimization of MIP performance.

Due to the low consumption of reagents, the small-scale protocol is well suited for automation. The results from this study suggest that the semiautomated combinatorial imprinting technique is a promising method for identifying the optimal conditions for MIP preparation for a given molecule. The method has the advantage that the materials used in preparing the MIPs are inexpensive and readily available. The MIP is relatively simple to produce, which is especially important when alternative target receptors (antibodies, aptamers, etc.) are difficult or expensive to obtain. Additional

advantages of MIPs include their ability to withstand high pressures, temperatures, extremes in pH, and a variety of organic solvents.

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