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Group-selective molecularly imprinted polymer solid-phase extraction for the simultaneous determination of six sulfonamides in aquaculture products

Xizhi Shi [a,*], Yuan Meng [a], Jinghua Liu [a], Aili Sun [a], Dexiang Li [a], Chunxia Yao [b], Yin Lu [a], Jiong Chen [a]

[a] Key Laboratory of Applied Technology of Marine Biology, Ministry of Education, Faculty of Life Science and Biotechnology, Ningbo University, 818 Fenghua Road, Ningbo 315211, PR China

[b] Institute for Agri-food standards and Testing Technology, Shanghai Academy of Agriculture Science 2901 Beidi Road, Shanghai 201106, PR China

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ABSTRACT

Group-selective molecularly imprinted polymers (MIPs) made from sulfonamides (SAs) using functional monomer methacrylic acid (MAA) were synthesized. The derived molecularly imprinted solid-phase extraction (MISPE) cartridges were developed for the purification and enrichment of aquatic products. The optimum template molecule and the ratio of the functional monomer to the template for obtaining group selectivity to SAs were sulfadimethoxine (SDM) and 4:1, respectively. The MIPs were characterized by Brunauer–Emmett–Teller (BET), scatchard plot, and chromatography analysis, all of which demonstrate better chromatographic behavior and group-selectivity of MIPs for SAs compared with those of corresponding NIPs. The extraction conditions of MISPE for six SAs were optimized; the method precision and accuracy were satisfactory for the fish and shrimp samples at 0.05, 0.1, and 0.2 mg kg^{-1} spiked levels. Recoveries ranging from 85.5% to 106.1% (RSD, 1.2–7.0%, $n = 3$) were achieved. The limits of detection ($S/N = 3$) and quantitation ($S/N = 10$) in the shrimp and fish samples were achieved from 8.4 to 10.9 μg kg^{-1} and from 22.4 to 27.7 μg kg^{-1}, respectively. Therefore, the obtained MIPs and MISPE can be employed for the enrichment and clean-up of SAs. This paper presents a new analytical method which enables the simultaneous determination and quantification of SAs in aquaculture products.

1. Introduction

Sulfonamides (SAs) are investigated worldwide to prevent and control a great variety of bacterial diseases in intensive aquaculture production [1,2]. However, the extensive use of SAs usually results in aquaculture water pollution which has serious side effects such as potential carcinogenic effects on humans [3,4]. Therefore, the usage of SAs is regulated and detected in aquatic environments and aquaculture products. To ensure food safety and human health, China and other countries have set a maximum residue limit (MRL) of 100 μg kg^{-1} for the sum of SAs in edible tissue [5]. Several quantitative analytical methods have recently been reported to detect the presence of SAs in different matrices. These include liquid-chromatography (LC) coupled with mass spectrometry, ultraviolet absorbance detection, fluorescence and electrochemical detection, gas chromatography (GC)–mass spectrometry and GC, capillary electrophoresis (CE), and immunochemical methods [6–10]. Furthermore, sample matrices are usually complex, the concentrations are relatively low, and different SAs drugs are often abused; these conditions make the direct determination of the residues of SAs difficult. Therefore, sample isolation and purification procedures are needed to minimize potential matrix effects and play an important role in the analysis of SA multi-residues.

Solid-phase extraction (SPE) is the most popular method for the clean-up and preconcentration of antibiotic multi-residues in the analysis of complex matrix samples [11]. Conventional SPEs lack selectivity, sensitivity, and capacity, leading to low determination sensitivity and the suppression or enhancement of the analyte signal due to strong matrix effects [12]. Molecularly imprinted polymers (MIPs), a new type of intelligent polymers, are synthetic materials with highly cross-linked three-dimensional network binding sites. The shape, size, and functionalities of MIPs are complementary to the target analyte and exhibit powerful characteristics of high selectivity, sensitivity, and capacity [13,14]. MIPs have been successfully used as selective sorbent materials (molecularly imprinted solid-phase extraction, MISPE) for the isolation and preconcentration of trace analytes from the complex matrix [15].

Most reported MIPs of SAs were prepared using sulfamethazine (SMZ) as the template molecule. However, there are only a few reports on the application of MISPE for sample clean-up and preconcentration of single target analytes. In this work, MIPs with group specificity for SAs in aquaculture samples were prepared using methacrylic acid (MAA) as the functional monomer and SDM as the template. Furthermore, a multi-residue detection method

* Corresponding author. Tel.: +86 574 87600551; fax: +86 574 87608347.
E-mail address: sxzsal78@yahoo.com.cn (X. Shi).

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based on sufficiently purified and enriched MISPE coupled with HPLC–UV analysis was developed and confirmed to be applicable for the simultaneous determination of six SAs residues in aquatic products.

2. Experimental

2.1. Chemicals and materials

Sulfadiazine (SDZ), sulfamerazine (SM), sulfameter (SME), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfadimethoxine (SDM), and methacrylic acid (MAA) were purchased from Sigma–Aldrich (Steinheim, Germany). Cross-linker ethylene glycol dimethacrylate (EGDMA) was obtained from Fluka (Steinheim, USA). The initiator 2, 2′-azobis (2-isobutyronitrile) (AIBN) was purchased from the China National Pharmaceutical Group Corporation (Shanghai, China). Acetonitrile and methanol, both of HPLC grade, were obtained from Fisher Scientific Co. (USA). All other reagents were of analytical grade.

2.2. Synthesis of MIPs for SAs

The preparation of imprinted polymers for SAs was performed as follows. The pre-polymerization solution consisted of 1 mmol template molecule, 5 mL of cross-linker EGDMA, 120 mg of the initiatior AIBN, and the required amounts of functional monomers MAA dissolved in 15 mL of acetonitrile prepared in a round bottom flask. The solutions were mixed by ultrasonic wave under a stream of nitrogen for 10 min to remove the oxygen. Polymerization was initiated by immersing the flask in a water bath at 60 °C and reacted for 24 h. The bulk polymers obtained were crushed and sieved following repeated sedimentation in acetone to remove the fine particles. Polymer particles with sizes less than 60 μm were collected and washed successively with methanol/formic acid (90/10, v/v) until no further SAs could be detected by HPLC–UV analysis. Methanol was then used to remove the template and the remaining unreacted functional monomers. Non-imprinted polymers (NIPs) were prepared under the same procedure and conditions except for the absence of the template.

2.3. Brunauer–Emmett–Teller (BET) analysis

The pore size distribution and surface area of the polymers were measured using a micromeritics ASAP 2020 analyzer (Norcross, GA) and analyzed by the Brunauer–Emmett–Teller (BET) method. A 500 mg quantity of the dried polymer was used for analysis. All samples were degassed at 150 °C for 24 h under nitrogen flow prior to measurement. The nitrogen adsorption/desorption isotherms were recorded at 77 K. The Barret–Joyner–Halenda (BJH) method was applied to acquire the pore size.

2.4. Chromatographic evaluation of MIPs

Polymer particles were slurried in isopropyl alcohol and packed into stainless steel HPLC columns (4.6 mm id × 100 mm) using an air-driven fluid pump (Alltech, USA) with 200 bar packing backpressure. The chromatographic columns were equilibrated and evaluated using acetonitrile as the mobile phase at a rate of 1.0 mL min^{-1} throughout the experiment. The loading volume was 20 μL, the column was at room temperature, and the wavelength for detection was 270 nm. Acetone was used as a void volume marker.

The retention factors were calculated using the equation of $k = (t - t_o)/t_o$, where t and t_o were the retention times of the analytes and void volume marker, respectively. The imprinted factor (*IF*) was calculated as $IF = k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} were the retention factors of each analyte in the MIPs and NIPs column, respectively. The selectivity factor (α) was calculated by the equation, $\alpha = k_1/k_2$, where k_1 is the retention factor of RES and k_2 is the retention factor of RES analogues. The retention index (*RI*) were calculated from the equation $RI = \alpha_{NIP}/\alpha_{MIP}$ [16].

2.5. Binding experiments

Equilibrium binding experiment was performed by adding 40 mg polymers to a 2 mL acetonitrile solution of SDM with sequentically various concentrations from 0.02 to 4.0 mmol L^{-1} and incubated for 24 h at 25 °C. The suspension was centrifuged, and the supernate was evaporated to dryness under nitrogen atmosphere. It was then re-dissolved with the HPLC mobile phase. The amount of SDM bound to polymers (*B*) was obtained by subtracting the free SDM concentration [SDM] from the initial concentration. Scatchard analysis was provided by the Scatchard equation: $B\text{max}/[\text{SDM}] = (B\text{max} - \text{B})/K_D$, where K_D is the association dissociation constant, and *B*max is the apparent maximum binding capacity. Therefore, K_D and *B*max were calculated from the slope and intercept of the *B*/[SDM] versus *B* plot, respectively [16].

2.6. MISPE analysis

MISPE and NIP SPE (NISPE) were prepared by packing the slurry of 25 mg dried polymer into 1 mL SPE cartridges (Supelco, USA) with two frits at each end. The cartridges were sequentially conditioned with 1 mL methanol and 1 mL acetic acid/water (1/99, v/v) before sample loading. The analytes were eluted with 3 mL methanol/acetic acid (9/1, v/v) at each step. The elutes were evaporated to dryness under a stream of nitrogen and re-dissolved with 0.5 mL of the mobile phase for further HPLC analysis.

2.7. HPLC analysis

HPLC analysis was performed on a Shimadzu LC-10-Avp HPLC system consisting of a UV detector and Venusil XBP C18 (4.6 mm × 250 mm, 5 μm, Agela Technologies Inc). The column was kept at ambient temperature. The mobile phase consisted of acetic acid/water (1/99, v/v, solvent A) and acetonitrile (solvent B). The gradient elution program was done as follows: isocratic conditions started with 72% A:28% B and the first linear gradient from 28% to 32% B in 8 min, then from 32% to 50% B in 4 min; a third gradient from 50% to 75% B in 8 min was kept for 2 min at the flow rate of 1 mL min^{-1}. The wavelength of determination was performed at 270 nm.

2.8. Extraction of SAs from aquatic products

The shrimp and fish used in this work were purchased from one local market and confirmed to have no detectable SAs by HPLC–UV analysis. The shrimp and fish samples (5.0 g) were precisely weighed and spiked with three levels of SAs at 0.05, 0.1, and 0.2 mg kg^{-1} and then left for at least 15 min. For MISPE extraction, 10.0 mL 1% acetic acid in water was added to the shrimp and fish samples, vortexed, and ultrasonicated for 5 min. Then, the mixture was centrifuged at 5.0×10^3 g for 5 min, and the supernatant was collected and passed through MISPE/NISPE cartridges. After applying the sample, the MISPE/NISPE cartridge was washed with 1.0 mL 5% acetonitrile in water (1% acetic acid). Finally, the elution step was performed using 3.0 mL methanol/acetic acid (9/1, v/v) at 0.5 mL min^{-1}, and the eluting solutions were dried under a stream of nitrogen at 40 °C. The residue was re-dissolved with 0.5 mL 28% acetonitrile in water and filtered through 0.22 μm filter for subsequent HPLC analysis.

Table 1
Compositions and evaluation on the retention of SAs of polymers.

Polymer	Template	Template:monomer:cross-linker	Recovery(%)[a]					
			SDZ	SM	SME	SMZ	SMX	SDM
MIP1	SDM	1:02:40	62.9	67.4	62.4	49.5	63.2	60.7
NIP1	–	0:02:40	30.3	28.6	32.2	26.3	35.4	20.4
MIP2	SDM	1:04:40	85.7	86.9	92.8	90.3	86.2	89.3
NIP2	–	0:04:40	34.8	31.7	34.9	31.2	39.4	29.4
MIP3	SDM	1:06:40	72.6	74.9	75.1	56.6	80.5	76.9
NIP3	–	0:06:40	35.3	32.0	34.8	31.9	33.2	25.0
MIP4	SME	1:01:40	62.1	68.3	72.4	55.3	70.4	49.7
NIP4	–	0:01:40	36.2	33.6	36.8	32.8	45.7	26.3
MIP5	SME	1:02:40	77.0	80.6	83.2	65.5	82.1	55.7
NIP5	–	0:02:40	30.3	28.6	32.2	26.3	35.4	20.4
MIP6	SME	1:04:40	62.8	64.4	61.0	52.5	67.4	44.1
NIP6	–	0:04:40	34.8	31.7	34.9	31.2	39.4	29.4
MIP7	SMZ	1:01:40	53.1	52.8	54.6	54.4	50.6	39.8
NIP7	–	0:01:40	36.2	33.6	36.8	32.8	45.7	26.3
MIP8	SMZ	1:02:40	66.6	68.2	69.9	75.8	67.0	47.3
NIP8	–	0:02:40	30.3	28.6	32.2	26.3	35.4	20.4
MIP9	SMZ	1:04:40	57.5	54.4	57.6	59.2	58.7	43.8
NIP9	–	0:04:40	34.8	31.7	34.9	31.2	39.4	29.4

[a] $n = 3$; The water was used as loading solution. The concentration of loading solutions was 0.1 mg kg^{-1} and the volume of loading solution was 1 mL, washing with 1 mL acetonitrile, elution with 3 × 1 mL methanol.

For comparative purpose, the shrimp and fish samples were also pre-treated with a 3.0 mL (500 mg) SCX SPE cartridges (Varian, CA, USA). 5.0 g anhydrous sodium sulfate and 20.0 mL methylene chloride was added into the fish and shrimp samples, respectively. The samples were homogenized and then centrifuged at 3500 rpm for 5 min at 4 °C. The supernatant was transferred into a 250-mL round-bottom flask. The residues were extracted two times with 20.0 mL methylene chloride. The supernatants were merged together and concentrated to 2–3 mL at 40 °C by the rotatory evaporator. Further, 5.0 mL of methylene chloride–acetone (60:40, v/v) were added and mixed with the concentrated sample and loaded onto the cartridge. After applying the sample, the cartridge was sequentially washed with 2.0 mL of methylene chloride–acetone (60:40, v/v) and 2.0 mL of acetone. The elution step was performed using 4.0 mL of 0.4 mol L^{-1} ammonium acetate-acetone (50:50, v/v), and the eluate was evaporated to dryness under a stream of nitrogen and re-dissolved with 0.5 mL 28% acetonitrile in water and filtered through 0.22 μm filter for subsequent HPLC analysis.

3. Results and discussion

3.1. Preparation of molecularly imprinted polymers

The different chemical structures and properties of SAs (synthetic antimicrobial agents that possess amphoteric character in the presence of SO_2NH_2 groups) make the simultaneous extraction and purification of SAs difficult. Hence, the efficient extraction and clean-up of group-selective MISPE has attracted increasing attention in recent years. Generally, the molecular recognition of MIPs is acknowledged to arise from three-dimensional cavities that are complementary in both shape and chemical functionality to templates or similar molecules. The shape and functional groups in the template play a significant role in molecular recognition by MIPs [17]. The type of template and the molecular ratio of the functional monomer to the template (Table 1) for this study were investigated to determine differences in the efficiency of MISPE binding (Fig. 1).

As shown in Table 1, the polymers of MIP2, 5 and 8 had the higher recoveries in contrast to other polymers synthesized with the same template and the corresponding NIPs, indicating that proper molar ratios of functional monomer to template are very important to enhance the recognition ability of MIPs. Furthermore, MIP2 shows the highest recovery for six SAs at 85.7–92.8%, while those of the corresponding NIPs was 29.4–39.4%. The specific adsorption recoveries of MIP2 for SAs are 46.8–59.9%, while those of MIP5 and MIP8 were 35.3–52% and 26.9–49.5%, respectively. The adsorption recoveries and specificity of MIP2 were both higher than those of MIP5 and MIP8, indicating that the specific affinity of MIPs depends on the type of template and different ratios of functional monomer to the template. The size and shapes of the different side chains and a common core chemical structure of p-aminobenzene of the SAs results in a greater effect on specific molecular recognition and cross-reactivity in MIPs, respectively [17,18]. MIP2 possessed the optimum imprinting effects and was selected for further experiments.

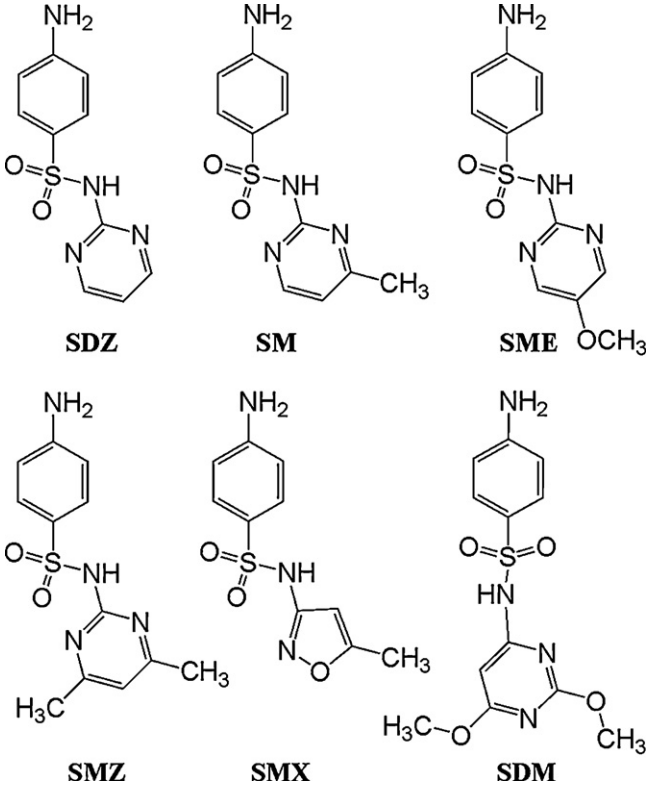

Fig. 1. The chemical structure of the SAs.

Table 2
Comparison of the pore structure characteristics of the MIPs and NIPs.

Type	$a_{s,BET}$ (m^2/g)[a]	Total pore volume (cm^3/g)	Average pore diameter (nm)
MIP2	294.39	0.70	10.89
NIP2	237.34	0.59	9.89
MIP5	273.94	0.66	9.61
NIP5	255.34	0.60	9.41
MIP8	269.98	0.65	9.62
NIP8	255.34	0.60	9.41

[a] $n = 3$.

3.2. BET analysis

Generally, the optimal type of template and the proper molar ratios of the functional monomer to the template are very important in the formation of highly group-selective imprinting cavities. As the average pore size diameter and surface area increase, the molecule has higher accessibility, and the functional groups have a better capacity for the templates in the pores. This likely results in a higher rate of rebinding that has good selectivity and affinity for MISPE [16]. The imprinting and recognition mechanism as well as the effects of shape and size on the analytes of MIPs selectivity, the structure characteristics of MIP2, MIP5, MIP8, and corresponding NIPs were all studied (Table 2). The specific surface area of each sample was measured for 3 times by the BET nitrogen adsorption method and calculated the average. MIPs exhibited larger surface area and cavities than those of corresponding NIPs. MIP2 had the largest cavities and surface area. The surface area, total pore value, and average pore diameter of MIP2 showed a large increase compared with those of NIP2. The surface area, total pore value, and average pore diameter of MIP5 and MIP8 had only a slight increase, indicating that the increase in surface area and cavities in MIP2 is likely due to the imprinting effect and the difference in molecular template and the ratio of template to functional monomer [19]. Therefore, MIP2 was selected for further experiments.

3.3. Chromatographic evaluation

The SAs have the same core functional structures and different side chain groups which lend group-selective recognition of the SAs. These properties also enable high recoveries of SAs on MIP2 (Table 1) and provide better demonstration. To gain insight into the group-selective mechanism of MIP2, the cross-reactivity of the polymer was studied via the chromatographic characteristics of SDZ, SM, SME, SMZ, SMX, and SDM. The chromatography columns were successively washed thoroughly with methanol/formic acid (90/10, v/v) and acetonitrile to remove possible interferences until a stable baseline was obtained. After equilibrating the columns with acetonitrile, the elution of SAs was performed, and the retention factors (k) on the MIP2 and NIP2, as well as the corresponding *IF*, α and *RI*, were calculated. As shown in Table 3, the values of k for SAs on MIPs were higher than those on NIPs, meanwhile, the *IF* values obtained for MIP2 were higher than 1.9. From these data, it is obvious that MIP2 exhibit imprinting effects and higher affinity for SAs than the NIP2. Furthermore, the specific shape and binding sites take on a strong recognition of the analytes. However, the values

Table 3
Chromatographic characteristics of MIP2 ($n = 3$).

Analyte	*k*mip	*k*nip	*IF*	α	*RI*
SDZ	0.89	0.29	3.11	0.89	1.36
SM	0.95	0.32	2.94	0.84	1.28
SME	0.56	0.29	1.92	1.44	0.84
SMZ	0.95	0.39	2.41	0.84	1.05
SMX	0.68	0.25	2.78	1.17	1.21
SDM	0.80	0.35	2.29	1.00	1.00

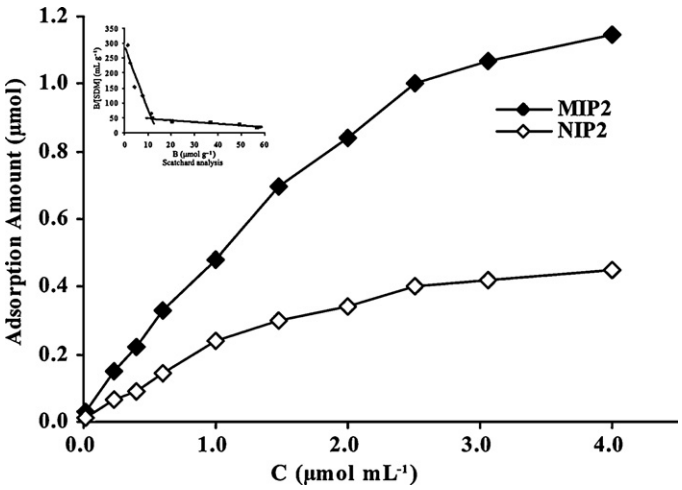

Fig. 2. Adsorption equilibrium isotherm of the MIP2 and NIP2. C, the initial concentration of SDM.

of α and *RI* for SAs were very similar, indicating that MIP2 had a similar re-binding capability and high cross-reactivity to SAs. MIP2 exhibits the highest *IF* for SDZ according to the chemical structures of these compounds; this result can be attributed to the fact that the smallest side chain size of SDZ may result in the high mass transfer of SDZ on MIP2. The shape and size selectivity of the cavities can be a very effective mechanism for the imprinting effect to discriminate different analytes. Overall, these results may be explained by the recognition ability of MIP2 on SAs caused by the complementary binding sites in the polymer. The shape, size, and functional group also contribute to make MIP2 a useful group-recognition material.

3.4. Adsorption capacity of MIPs

The heterogeneous population of binding sites and the binding capacity of MIPs to the template were evaluated by the adsorption isotherm experiment and subsequent Scatchard analysis. The SDM adsorption results of the MIPs and NIPs are shown in Fig. 2. The affinity of SDM on MIP2 is much higher than those on NIPs; a non-linear profile was obtained, suggesting that the binding sites in MIP2 are heterogeneous with respect to the affinity for SDM. Scatchard analysis shows that the curve characterized by two cutting lines reveals that the binding sites in MIP2 can be classified as high-affinity binding sites and low-affinity binding sites. The dissociation constant for high-affinity binding sites is 0.048 $\mu mol\,mL^{-1}$, and the corresponding value of *B*max is 13.74 $\mu mol\,g^{-1}$. The dissociation constant for low-affinity binding sites is 1.74 $\mu mol\,mL^{-1}$, and the corresponding value of *B*max is 91.55 $\mu mol\,g^{-1}$ which may demonstrate that the specific binding affinity of MIP2 is caused by the imprinting effect.

3.5. MISPE analysis

Successful selective recognition due to hydrophobic interactions and chemically and sterically complementary to the analytes is possible in aqueous solutions by optimizing loading, washing, and eluting conditions [20]. The washing step done with the appropriate solution is especially crucial to improve specific interactions further and to remove non-specific affinity between the analytes and the MIP binding sites [21]. In this study, acetonitrile containing 0–10% acetic acid was tested to find the appropriate loading solutions. A 1 mL solution spiked with 0.1 $mg\,kg^{-1}$ of the six SAs was percolated through MIP2 cartridges followed by washing with 1.0 mL acetonitrile and eluting with a 3 mL methanol:HAc (9/1, v/v) mixture solution. As the percentage of acetic acid increased

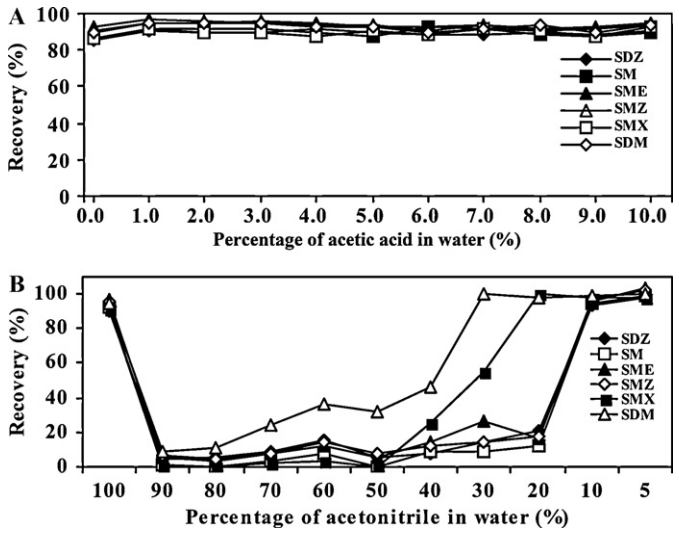

Fig. 3. Recoveries of SAs on MIP2 cartridges under different loading and washing solutions. The concentration of SAs was 0.1 mg kg^{-1}, the volume of loading and washing solution were both 1 mL.

from 0% to 10%, there was no obvious increase in the recovery and decrease in non-specific affinity. However, SAs are typical amphoteric compounds; the low pH value can decrease interaction with the sample matrix and greatly improve extraction efficiency [22]. Therefore, before loading, the MISPE cartridges were sequentially conditioned with 1 mL methanol and 1 mL 1% acetic acid in water. Furthermore, acetonitrile in 1% acetic acid water solutions at different ratios was investigated to optimize the washing condition. Fig. 3B shows that when 1% acetic acid water in acetonitrile is used as the washing solvent, high recoveries on MIPs are obtained for six SAs. However, increasing water from 80% to 90% in acetonitrile results in the low recoveries of the six SAs. This indicates that water may interfere with hydrogen bonding. In addition to shape recognition, ion-exchange and hydrophobic interaction were also dominant effects. When the water (1% acetic acid) content is lower than 10%, shape selectivity and ion-exchange effect played a significant role in the recognition of MIP systems. Furthermore, when acetonitrile 5% in water (1% acetic acid) is used as the washing solvent, almost no SAs are found in the washing fractions from MIP2. This indicates that this solvent may be used as a washing solution to improve MISPE recovery in aquatic products. Finally, the 3 mL methanol:HAC (9/1, v/v) solution was used as the eluting solution after the column was dried for further experiments. A flow rate of 0.5 mL min^{-1} was explored for obtaining high extraction efficiency.

3.6. Analysis of SAs in aquatic products

SPE provides a simple and effective extraction and purification method for complex sample matrixes. However, conventional SPE usually lack selectivity and are easily subject to the co-extraction of sample matrix constituents like proteins and carbohydrates, which may reduce the lifetime of LC-column and recovery [16]. Hence, rapid, accurate, and lower consumption of organic solvent extraction and purification methods are necessary for the determination of SAs. For MISPE and NISPE, to decrease the binding of SAs to the sample matrix, 1% acetic acid in the sample was selected for adjusting pH during the experiment protocol and for precipitating protein. After centrifugation, the supernatants were directly applied to the MISPE or NISPE cartridges. In this study, the validation of the developed analytical method was carried out by applying the procedure as described in the section "Extraction of SAs from aquatic products." The results of mean quantitative recoveries and repeatability (RSD) of SA-spiked fish and shrimp samples are summarized in Table 4. Compared with the recoveries after NISPE and commercial SCX SPE cartridges, the mean quantitative recoveries after MISPE are improved and in the range of 85.5–106.1% at three different spiked levels with RSD values in the range of 1.2–7.0% in all cases. These reports showed the good applicability of the method for the quantitative detection of SAs in aquatic samples. As shown in Table 5, under the optimum conditions of MISPE, the

Table 4
Analysis of SAs in the spiked shrimp and fish samples on MISPE, NISPE and SCX SPE (n = 3).

	Sulfonamide	Spiked concentration					
		0.05 mg kg^{-1}		0.1 mg kg^{-1}		0.2 mg kg^{-1}	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Shrimp[a]	SDZ	90.5	6.2	90.3	4.5	90.8	1.9
	SM	106.1	1.9	93.1	7.0	96.2	1.3
	SME	98.5	4.4	96.4	4.0	97.9	3.0
	SMZ	93.4	3.5	92.8	4.1	96.1	3.1
	SMX	102.2	1.2	97.4	6.3	100	2.0
	SDM	97.7	2.1	90.7	1.5	91.1	2.6
Fish[a]	SDZ	103.8	5.4	93.5	2.6	91.2	3.8
	SM	97.6	4.1	103.7	4.9	92.5	3.5
	SME	97.7	3.9	88.8	5.5	87.3	4.3
	SMZ	94.7	6.7	87.8	6.5	86.6	5.2
	SMX	94.4	5.7	90.5	3.5	88.9	4.0
	SDM	93.8	4.6	93.6	5.6	85.5	3.1
Shrimp[b]	SDZ	70.7	8.7	73.5	5.9	65.4	4.0
	SM	68.3	4.6	67.7	7.4	60.7	3.4
	SME	72.5	6.2	77.3	4.4	69.3	4.8
	SMZ	68.4	4.9	73.6	4.7	61.5	3.7
	SMX	75.4	4.1	67.2	7.3	73.0	4.1
	SDM	63.2	5.0	59.8	3.6	55.1	4.9
Fish[b]	SDZ	75.9	7.0	68.3	4.4	73.0	7.5
	SM	64.1	5.7	67.9	6.7	65.6	5.5
	SME	71.3	5.1	64.3	6.1	69.0	7.5
	SMZ	64.8	7.2	61.9	7.0	70.7	5.3
	SMX	77.3	6.8	70.5	3.6	73.7	4.2
	SDM	60.5	5.5	57.5	6.1	54.3	6.2
Shrimp[c]	SDZ	81.2	3.4	84.0	5.1	83.2	6.2
	SM	83.5	6.3	85.7	8.6	84.3	4.7
	SME	83.0	5.9	84.1	5.4	86.7	5.1
	SMZ	84.0	7.1	86.8	4.6	78.3	6.6
	SMX	85.7	3.8	83.3	7.1	75.3	5.7
	SDM	86.2	5.8	84.9	4.1	82.4	6.8
Fish[c]	SDZ	80.0	7.3	82.2	3.1	85.0	4.7
	SM	84.2	6.9	83.4	5.2	84.6	5.8
	SME	82.9	5.8	85.0	6.5	76.3	4.8
	SMZ	84.9	6.7	84.2	8.5	83.2	4.2
	SMX	82.2	5.1	81.0	5.0	81.7	7.6
	SDM	84.8	5.5	80.5	6.1	77.3	5.8

[a] Analysis of SAs in the spiked shrimp and fish samples on MISPE.
[b] Analysis of SAs in the spiked shrimp and fish samples on NISPE.
[c] Analysis of SAs in the spiked shrimp and fish samples on SCX SPE.

Table 5
LOD and LOQ of the MISPE method for SAs in shrimp and fish samples.

Samples	Analyte	LOD (μg/kg)[a]	LOQ (μg/kg)[b]
Shrimp	SDZ	10.7	24.4
	SM	9.8	27.7
	SME	10.5	26.7
	SMZ	9.6	25.3
	SMX	8.8	26.6
	SDM	8.4	25.3
Fish	SDZ	9.7	24.3
	SM	10	24.9
	SME	10.9	26.7
	SMZ	10.2	25.4
	SMX	9.1	23.9
	SDM	8.7	22.4

[a] Signal-to-noise ratio is 3.
[b] Signal-to-noise ratio is 10.

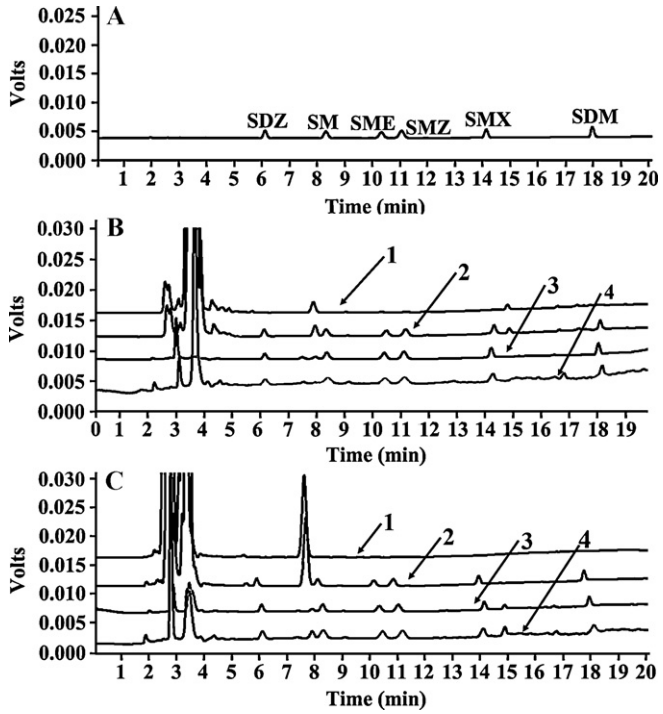

Fig. 4. The chromatographic profiles at 270 nm from the analysis of the spiked sample at 0.1 mg kg^{-1} with MISPE, NISPE or SCX SPE cartridges. A: the standard substance; B: shrimp, 1: blank sample, 2: spiked sample with NISPE cartridges, 3: spiked sample with MISPE, 4: spiked sample with SCX SPE; C: fish, 1: blank sample, 2: spiked sample with NISPE cartridges, 3: spiked sample with MISPE, 4: spiked sample with SCX SPE.

limits of detection (LOD, $S/N = 3$) in the shrimp and fish samples were in a range of 8.4–10.9 μg kg^{-1} for investigated SAs. The limits of quantification (LOQ, $S/N = 10$) in the shrimp and fish samples were in a range of 22.4–27.7 μg kg^{-1} for investigated SAs. Furthermore, reduction of impurities may be very significant in increasing column lifetime and facilitating the quantification of the analytes. Thus, the clean-up chromatographic diagrams of SAs for the 5 g fish and shrimp samples spiked at 100 μg kg^{-1} concentration were achieved before and after MISPE interaction, NISPE or commercial SCX SPE cartridges. As shown in Fig. 4, compared to the NISPE cartridges and SCX SPE cartridges, after the spiked sample was treated with the MISPE procedure, no interfering peaks were observed and the peaks at the solvent front are considerably reduced, indicating that impurities were mostly removed and that the method has good selectivity. In addition, compared to the commercial SCX SPE cartridge, the developed MISPE exhibited lower consumption of organic solvent and better separation efficiency. The results confirmed the reliability and efficiency of the proposed method for the analysis of SA residues in aquaculture samples.

4. Conclusions

In this paper, group-selective MIPs for six SAs were synthesized by thermal polymerization. The products were evaluated by a series of adsorption experiments, BET and MISPE analysis, all of which demonstrate that MIPs are useful for the selective recognition of SAs. Furthermore, a MISPE method coupled with HPLC–UV was successfully developed to detect six SAs at low concentration levels in aquatic samples, which is superior to conventional SPE method coupled with HPLC–UV in terms of selectivity, time-consuming and reduced matrix effects and exhibits the similar performance of LODs and LOQs with those reported in the previous studies. The developed MISPE is established as a valuable tool for the clean-up and enrichment of six SAs in aquatic products.

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