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Molecularly Imprinted Polymer Online Solid-Phase Extraction Coupled with High-Performance Liquid Chromatography−UV for the Determination of Three Sulfonamides in Pork and Chicken

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A selective imprinted amino-functionalized silica gel sorbent was prepared by combining a surface molecular imprinting technique with a sol–gel process for online solid-phase extraction−HPLC determination of three trace sulfonamides in pork and chicken muscle. The imprinted functionalized silica gel sorbent exhibited selectivity and fast kinetics for the adsorption and desorption of sulfonamides. With a sample loading flow rate of 4 mL min^{-1} for 12.5 min, enhancement factors and detection limits for three sulfonamides ($S/N = 3$) were achieved. The precision (RSD) for nine replicate online sorbent extractions of 5 μg L^{-1} sulfonamides was less than 4.5%. The sorbent also offered good linearity ($r^2 > 0.99$) for online solid-phase extraction of trace levels of sulfonamides. The method was applied to the determination of sulfonamides in pork and chicken muscle samples. The prepared polymer sorbent shows promise for online solid-phase extraction for HPLC determination of trace levels of sulfonamides in pork and chicken samples.

KEYWORDS: Sulfonamides; sol–gel; molecularly imprinted polymers; solid-phase extraction; high-performance liquid chromatography

INTRODUCTION

Sulfonamides are a group of synthetic antibiotics which play an important role in veterinary medicine and have been widely used in animal feed. However, the use of these compounds may result in residues in animal-derived food products. This is particularly an issue when the withdrawal period following treatment is too short to allow clearance. As a consequence of the extensive usage of sulfonamides, considerable attention has been paid to the potential human health risk due to their carcinogenic potency and possible role in the development of antibiotic resistance (*1, 2*). To ensure food safety for consumers, the European Union and other countries, including China, have established a maximum residue limit (MRL) of 100 μg kg^{-1} for sulfonamides in foods of animal origin such as meat, milk, and eggs (*3*). Many methods have been described for the determination of resides of sulfonamides in animal tissues. These can be generally divided into two groups. The first group comprises screening methods such as thin-layer chromatography and enzyme immunoassay (*4, 5*), which are fast and inexpensive but may give false positive results. Second, there are quantitative methods including capillary electrophoresis (*6*) gas chromatography, high-performance liquid chromatography (HPLC; see Abbreviations Used) (*7, 8*), and gas chromatography or HPLC coupled with mass spectrometry (*9, 10*). Gas chromatography is relatively difficult because sulfonamides must be volatilized prior to analysis due to the high polarity and low volatility of sulfonamides (*11*). HPLC with mass spectrometer detection is expensive, and many laboratories do not have access to this instrumentation. In addition, HPLC alone is insufficient for direct determination of trace sulfonamides residue in food for its poor detection limits. Instead, trace sulfonamides residue must be preconcentrated in food samples so that they can be detected by HPLC.

Due to the complex nature of meat, samples are often pretreated to remove protein, fat, and reduce potential interference from the sample matrix. The most common treatments are liquid–liquid extraction and solid-phase extraction (SPE) (*12*). Various solid-phase extraction materials were used to pretreat the matrix and concentrate sulfonamides, such as C_{18} (*7, 13, 14*), strong cation-exchange cartridge (*15*), neutral alumina (*16*), XAD-4 (*17*), and multiwalled carbon nanotubes (*18*). In addition, matrix solid-phase dispersion has been used in multiresidue analysis for sulfonamides (*17, 19*). However, these materials are not specific to sulfonamides and usually absorb some coextracts, which interfere with the determination of the target compound. Thus, additional time and pretreatment steps are required to remove the interfering compounds. To improve recovery, large quantities of organic solvents are used, which may be harmful to the environment. For conventional sorbents,

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these difficulties are magnified when the analytes are present at low concentrations.

Molecularly imprinted polymers (MIPs) are synthetic materials which can selectively recognize a guest molecule or related analogous compounds and which can be obtained simply and rapidly. They possess molecular recognition sites designed for a specific molecule or a family of compounds. Molecular imprinting is a process by which functional and cross-linking monomers are copolymerized in the presence of the target analyte, which acts as a molecular template (*20*). Subsequent removal of the template leaves behind binding sites that are complemented to the target analyte in the resultant MIPs. MIPs are inexpensive, resistant to elevated temperatures and pressures, and inert toward acids, bases, metal ions, and organic solvents (*21*). MIPs have been used successfully in several fields including artificial receptors (*22*), antibody mimics (*23*), enzyme mimics (*24*), capillary electrophoresis and liquid chromatography for the resolution of racemates (*25*), binding assays (*26, 27*), sensors (*28*), and solid-phase extraction (*20, 29*). For example, in recent years, MIPs have been used for the solid-phase extraction of chloramphenicol, clenbuterol, triazine, herbicides, caffeine, steroids, scopolamin, trimethoprim, local anesthetics, and nerve agent degradation products from biological and food stuff matrices (*30*). Most of the imprinted sorbents used in molecularly imprinted solid-phase extraction were prepared using methacrylic acid and ethylene glycol dimethacrylate as monomers (*31*) and synthesized by bulk polymerization. Most of these materials exhibit high affinity and selectivity, but the fabrication process is complex, removing the template is difficult, and the kinetics of the sorption/desorption process is often unfavorable.

MIPs for sulfamethazine have been used as the stationary phase for HPLC (*32*) and as the solid-phase extractant (*31*). In those studies the MIPs were synthesized by bulk polymerization. In the current study, MIPs for sulfamethazine were synthesized on the surface of silica gel using a molecular imprinting sol–gel technique, which avoids the disadvantages of bulk polymerization. The polymer was packed into a stainless steel preconcentration column which replaced the conventional sample loop on the six-port injector valve of the HPLC to enrich and detect sulfamethazine, sulfathiazole, and sulfamerazine. To our knowledge, there are no studies regarding MIPs which have a high affinity for three sulfonamides and no research regarding an online solid-phase extraction method using MIPs of sulfonamides as the sorbent coupled with high-performance liquid chromatography.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. Silica gel (80–120 mesh, Qingdao Ocean Chemical Co., Qingdao, China) was used as the support to prepare the SMZ-imprinted functionalized sorbent. Sulfamethazine (Sigma) was used as a template molecule. Tetraethoxysilicane (TEOS) and 3-aminopropyltriethoxysilane (APTES; Wuhan University Chemical Factory, Wuhan, China) were used as cross-linking and functional monomers, respectively. Acetonitrile (Sigma, HPLC grade) as solvent, methanol (Tianjin Chemical Co., China, analytical grade), and acetic acid (Tianjin Chemical) were used for elution of the template. Potassium ferrocyanide and zinc sulfate (Tianjin Chemical) were used as precipitators of protein in samples. Ractopamine, sulfathiazole, sulfamerazine, and estriol were purchased from Sigma. The mobile phase used for HPLC experiments consisted of a mixture of methanol (Sigma, HPLC grade) and water (pH = 3.25, the ratio was 22:78), which was filtered through a 0.45 μm filter prior to use. Doubly deionized water (DDW, 18.2 MΩ cm^{-1}) obtained from a WaterPro water system (Labconco Corp., Kansas City, MO) was used throughout the experiments. All reagents used were of at least analytical grade. A stock solution of three sulfonamides, ractopamine, and estriol was prepared by dissolving 10 mg of each in 10 mL of methanol and stored at –20 °C in the dark. Fresh stock solution was prepared weekly and stored at 4 °C in the dark. The working solutions were adjusted daily.

Apparatus and Equipment. The high-performance liquid chromatographic system consisted of two LC-10AT VP pumps and a Shimadzu SPD-10A VP ultraviolet detector and RF-10Avp fluorescence detector (EX, 226 nm; EM, 305 nm; Shimadzu, Kyoto, Japan). All separations were achieved on an analytical reversed-phase column (Alltech-C_{18} 5 μm, 4.6 mm × 25 cm long, Alltech, Deerfield City, IL) at a mobile-phase flow rate of 1.0 mL min^{-1} under isocratic conditions at a column temperature of 35 °C. Class-vp software was used to acquire and process spectral and chromatographic data. A Model FIA-3100 flow injection system (Vital Instruments, Beijing, China) was used for online MIP preconcentration. Tygon pump tubes were used to deliver the sample solution. Small-bore (0.5 mm i.d.) PTFE tubes were adapted for all connections, which were kept the shortest possible length to minimize dead volume. An UV–visible spectrophotometer (Bio-Rad, Hercules City, CA, USA) was used in the static adsorption test. An ultrasonicator was used during preparation of the samples.

Procedure of Preparation of SMZ-Imprinted Amino-Functionalized Silica Gel Sorbent. *Activation of Silica Gel.* The procedure for activating the silica gel was similar to that reported previously (*33*). The procedure was as follows: silica gel (16 g, 80–120 mesh) was mixed with 120 mL of 33% methanesulfonic acid in a three-necked flask and refluxed while stirring for 8 h. Following this, the activated silica gel was obtained by filtration, washed with DDW to neutral, and dried under a vacuum at 70 °C for 8 h.

Preparation of the SMZ Polymer. An 85 mg amount of SMZ and 6 mL of APTES were dissolved in 10 mL of acetonitrile by stirring and heating at 60 °C for 30 min. Then activated silica gel (0.5 g), TEOS (2 mL), and acetic acid (1 mL) were added and stirred for an additional 20 min. The mixture was incubated for 12 h at 60 °C in a water bath. The product was then recovered by filtration, washed with methanol, and dried under a vacuum at 100 °C for 10 h. Prior to drying, the polymer was extracted using 500 mL of a Soxhlet device (12 h in a 10% acetic acid and methanol solution).

Preparation of the SMZ-Imprinted Sol−Gel Sorbent. The SMZ polymer, 25 mL of methanol, and 25 mL of 1 mol L^{-1} HCl were added to a stoppered 100 mL flask and stirred for 2 h to remove the SMZ. The product was isolated by filtration, washed with methanol, neutralized with 0.1 mol L^{-1} NaOH, and washed to neutral pH with pure water. Finally, the sorbent was dried under vacuum at 80 °C for 12 h.

Nonimprinted polymer (NIP) containing no template was also prepared using the same procedure.

Adsorption Test. *Kinetic Adsorption Test.* a 50 mg amount of sorbent was added to 10 mL of 30 mg L^{-1} SMZ acetonitrile solution. The mixture was mechanically shaken for set times at room temperature and then separated centrifugally. The unbound SMZ in the supernatant was measured by UV spectrometry.

Static Adsorption Test. To measure the adsorption capacity of the polymer, 50 mg of SMZ-imprinted or nonimprinted sorbent was mixed with different concentrations of SMZ dissolved in acetonitrile and shaken for 30 min. The resulting supernatant was measured for unbound SMZ by UV spectrometry at 270 nm. The same procedure was applied to test the static adsorption of the nonimprinted polymer.

Competitive Adsorption Test. Adsorption and competitive recognition studies were performed with sulfamethazine, sulfathiazole, sulfamerazine, ractopamine, and estriol (**Figure 1**). The SMZ-imprinted polymer (50 mg) was added to a flask containing 10 mL of 10 mg L^{-1} sulfamethazine, sulfathiazole, sulfamerazine, ractopamine, and estriol acetonitrile mixed solution, shaken at room temperature for 30 min, and then separated centrifugally. HPLC was used to measure the five unextracted target molecules.

Extraction of Pork and Chicken Meat. A 2 g amount of fresh homogenized pork or chicken sample was added to a 25 mL tube containing 5 mL of acetonitrile and ultrasonicated for 10 min in an ultrasonic bath. The mixture was then filtered, and 5 mL of saturated potassium ferrocyanide and 2 mL of 30% zinc sulfate were added to the filtrate. This mixture was heated for 5 min in a water bath (75 °C) and filtered, and 5 mL of acetonitrile was used to wash the residues.

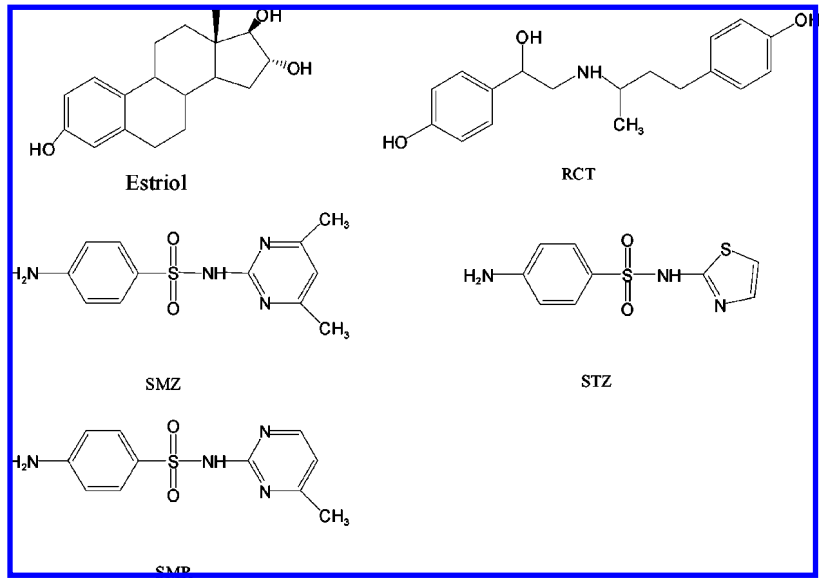

Figure 1. Structures of estriol, ractopamine, and three sulfonamides.

The filtrate was then refiltered with a 0.22 μm cellulose acetate film and transferred into a 100 mL calibrated flask and diluted to the 100 mL mark with DDW for enriching online.

Procedures for Online SPE-HPLC Determination of Sulfonamides Using the Imprinted Sorbent. To evaluate the applicability of the imprinted functionalized silica gel sorbent for online SPE-HPLC determination of trace sulfonamides, a cylindrically shaped microcolumn (1.5 cm × 4 mm i.d.) packed with 50 mg of the imprinted functionalized silica gel sorbent was prepared. The process of the online SPE preconcentration coupled to HPLC for determination of sulfonamides in pork and chicken is as follows: first, the sample solution was introduced onto the SPE microcolumn at a flow rate of 4.5 mL min^{-1} while the HPLC injector valve is in the load position. This results in the sulfonamides being preconcentrated by the sorbent-packed precolumn, and the unwanted water is sent to the waste. Second, the analytes adsorbed on the SPE microcolumn were eluted in the back-flush mode by the HPLC mobile phase at a flow rate of 1.0 mL min^{-1} into the chromatographic separation column for 1.5 min by switching the HPLC valve from "load" to "inject". As such, the sample band in the microcolumn was compressed into a narrow band before entering the analytical column and the band broaden effect was reduced. Third, the HPLC injector valve was turned to the load position for the next sample preconcentration cycle while the analytes were separated in the chromatographic separation column to improve sample throughput. Chromatograms were recorded and stored on the hard disk of the computer. Areas at 270 nm were calculated and used for data evaluation.

RESULTS AND DISCUSSION

Synthesis Method and Reacting Mechanism of the MIPs for Sulfamethazine. There are siloxane groups (Si−O−Si) in the bulk and silanol groups (Si−OH) on the silica gel surface. The silanol groups are responsible for chemical modifications that may occur on the silica surface. Methanesulfonic acid (33%) was chosen as the activator for increasing the amount of silanol groups in order to satisfy the requirements for reactions with other materials in the current study because of its strong catalytic action, high boiling point, and its ability to be used repeatedly.

Taking into consideration the molecular structure, the size of the R-group, the potential for harm, and the extent of use of sulfonamides, sulfamethazine was chosen as a template molecule to synthesize MIPs that selectively adsorb sulfonamides. In the process of polymerization, sulfamethazine combined with 3-aminopropyltriethoxysilane to form the functional silica gel compounds. In the presence of tetraethoxysilicane, the functional silica gel compounds of sulfamethazine integrated with the silica gel into a polymer of sulfamethazine. Then, the sulfamethazine molecule was removed by mixture of HCl and methanol. In this way, the sulfamethazine−MIP was formed, with distinct binding sites for sulfonamides.

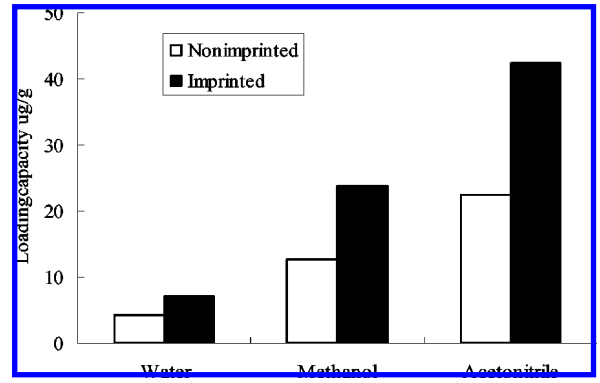

Figure 2. Effect of solvent on the binding of 30 mg L^{-1} SMZ onto 50 mg of sorbent in different solvents.

Synthesis and Optimization of the MIPs for Sulfamethazine. A number of factors effected the synthesis of MIPs for sulfamethazine. The solvent was a important factor which effected the formation of hydrogen. Although both methanol and acetonitrile can dissolve 3-aminopropyltriethoxysilane and tetraethoxysilicane, the weakly polar acetonitrile was chosen as solvent because strongly polar solvents counteract the formation of hydrogen bonds. Activator has important function in synthesis of molecularly imprinted polymers. Hydrochloric acid is often used as an activator during the synthesis of molecularly imprinted sol–gel. However, HCl is a strong acid, and thus interferes with the sulfamethazine/3-aminopropyltriethoxysilane bond. Therefore, acetic acid was chosen as the activator and HCl was used as an eluent to remove sulfamethazine in the polymer. The functional sites were protonated after removal of the template molecule by methanol and hydrochloric acid. Sodium hydroxide (0.1 mol L^{-1}) was used to destroy the protonation. The proportions of reactants were optimized for synthesis as follows: the highest adsorbent capacity for sulfamethazine was measured using 10 mL of acetonitrile, 85 mg of sulfamethazine, 6 mL of 3-aminopropyltriethoxysilane, 2 mL of tetraethoxysilicane, 0.5 g of activated silica gel, and 1 mL of 0.1 mol L^{-1} acetic acid. We also tested the effect of different

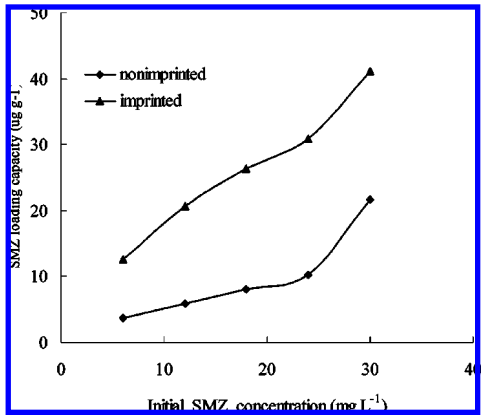

Figure 3. Loading isotherm of SMZ onto the imprinted and nonimprinted sorbents.

temperatures (room temperature and 50, 60, 70, and 80 °C) and found that the polymers have a higher adsorbent capacity at 60 °C.

Choice of Adsorbent Solvent. Sulfamethazine can be dissolved in water, methanol, and acetonitrile. A 50 mg amount of sulfamethazine imprinted or nonimprinted solvent was equilibrated with 10 mL of 30 mg L^{-1} SMZ dissolved in water, methanol, or acetonitrile, respectively, to investigate the effect of solvent on the uptake capacity. The results are shown in **Figure 2**. We found that the polymer had maximal uptake capacity for SMZ dissolved in acetonitrile. Hydrogen bonding is the primary force joining sulfamethazine and 3-aminopropyltriethoxysilane. The strong polarity of solvents such as water and methanol can interfere with the formation of hydrogen bonds. Because of its weak polarity, acetonitrile was chosen as the solvent in the current study.

Uptake Kinetics of Sulfamethazine by the Imprinted Functionalized Silica Gel Sorbent. The uptake kinetics of sulfamethazine by the imprinted functionalized silica gel sorbent were also examined. A 50 mg amount of SMZ-imprinted sorbent was equilibrated for different times with 10 mL of 30 mg L^{-1} sulfamethazine dissolved in acetonitrile. The results indicate that the imprinted sorbent has fast uptake kinetics, and binding equilibrium was obtained within 20 min. If the concentration of sulfamethazine was lower, the time to saturation would be correspondingly shorter. The rapid adsorption kinetics of the imprinted sorbent is an obvious advantage for its application in the online solid-phase extraction. This suggests that the surface imprinting greatly facilitates diffusion of the analyte to the binding sites.

Evaluation of Static Adsorption. To evaluate the combined capability of the MIPs to bind SMZ, the uptake capacity of imprinted or nonimprinted polymers was studied at room temperature. The absorption isotherm curve is shown in **Figure 3**. The results indicate that the uptake capacity of the imprinted materials (up to 42.1 $\mu g\ g^{-1}$) was higher than that of nonimprinted materials (up to 21.7 $\mu g\ g^{-1}$). During the process of producing the imprinted polymer, the presence of SMZ molecules causes the ligand to arrange regularly and form a tridimensional chemical structure (*33*). The SMZ specific cavity is formed after the imprint molecule is eluted. Whereas the direction of the matchbody was changed easily because of the agility of -CH_2−CH_2−CH_2- conjoined to a functional -NH_2 group, the geometrical structure changed in a large range, and the combined sites of matchbody to imprint molecule were uncertain and resulted in the low adsorption capacity of the material.

Selectivity of MIPs for Sulfonamides. Sulfamethazine, sulfathiazole, sulfamerazine, ractopamine, and estriol were selected to validate the selectivity of the MIPs to sulfonamides. As shown in **Table 1**, the uptake capacity of the SMZ-imprint sorbent for sulfamethazine, sulfathiazole, and sulfamerazine is higher than that of nonimprinted sorbent. In contrast, estriol did not absorb to either and ractopamine was absorbed more by the nonimprinted sorbent. The distribution coefficients for the three sulfonamides on the imprinted sorbent were 41.25, 69.54, and 388.24, respectively. The distribution coefficients for ractopamine and estriol on the imprinted sorbent were 7.36 and 0, respectively. The selectivity coefficients for the three sulfonamides were 5.6, 9.6, and 52.8 for the imprinted sorbent and 0.9, 5.2, and 9.2 for nonimprinted sorbent. The MIPs showed selectivity due to the tailor-made cavity which is selective for sulfonamides. **Table 1** also illustrates that the adsorption to the imprinted sorbent varied with each of the sulfonamides. Adsorption was highest for sulfathiazole and least for sulfamethazine. As shown in **Figure 1**, the structures of the three sulfonamides were analogous with the only difference occurring in the size of the R-group. Sulfathiazole suffers less steric hindrance due to its simple structure.

Table 1. Competitive Loading of Sulfonamides, Estriol, and Ractopamine by SMZ-Imprinted and Nonimprinted Sorbents[a]

parameter	sorbents	imprinted	nonimprinted
loading capacity, $\mu g\ g^{-1}$	sulfamethazine	17.1	8.9
	sulfamerazine	25.2	6.7
	sulfathiazole	66	43.8
	ractopamine	3.5	7.8
	estriol	0	6.25
K_d	sulfamethazine	41.25	14.36
	sulfamerazine	69.54	30.15
	sulfathiazole	388.24	155.87
	ractopamine	7.36	16.99
	estriol	0	8.29
K	sulfamethazine	5.6	0.9
	sulfamerazine	9.5	1.5
	sulfathiazole	52.8	9.2
K'	sulfamethazine	5.75	
	sulfamerazine	6.25	
	sulfathiazole	6.59	

[a] $K_d = \{(C_i - C_f)/C_f\}\{\text{volume of solution (mL)}\}/\{\text{mass of gel (g)}\}$, where C_i and C_f represent the initial and final concentrations, respectively; k is the selectivity coefficient; $K = K_d(\text{sulfonamides})/K_d(\text{RAC})K'$ is the relative selectivity coefficient; and $K' = k_{\text{imprinted}}/k_{\text{nonimprinted}}$.

Table 2. Figures of Merit for the Online Solid-Phase Extraction Coupled with HPLC for Determination of Trace Sulfonamides

	enrichment factors[a]	detection limit ($S/N = 3$) (ng L^{-1})	peak area precision ($n = 9$) (%, RSD)
sulfathiazole	435	4.6	2.53
sulfamerazine	352	5.1	3.01
sulfamethazine	314	7.3	4.21

[a] Compared with direct injection of a 20 μL sample solution.

Application of the Imprinted Sorbent to Selective Online SPE-HPLC Determination of Three Sulfonamides. The applicability of the imprinted amino-functionalized silica gel to online SPE-HPLC determination of trace sulfonamides was evaluated. The chemical and flow variables such as sample acidity, sample loading flow rate, and eluent (type, concentration, and flow rate) were optimized to achieve sensitivity and precision for the extraction and elution of sulfonamides.

The influence of sample pH on the online extraction of 5 $\mu g\ L^{-1}$ of sulfonamides was studied in the pH range of 4–8 at a sample flow rate of 4 mL min^{-1}. Our results showed that the chromatography peak areas of sulfonamides were maximal at

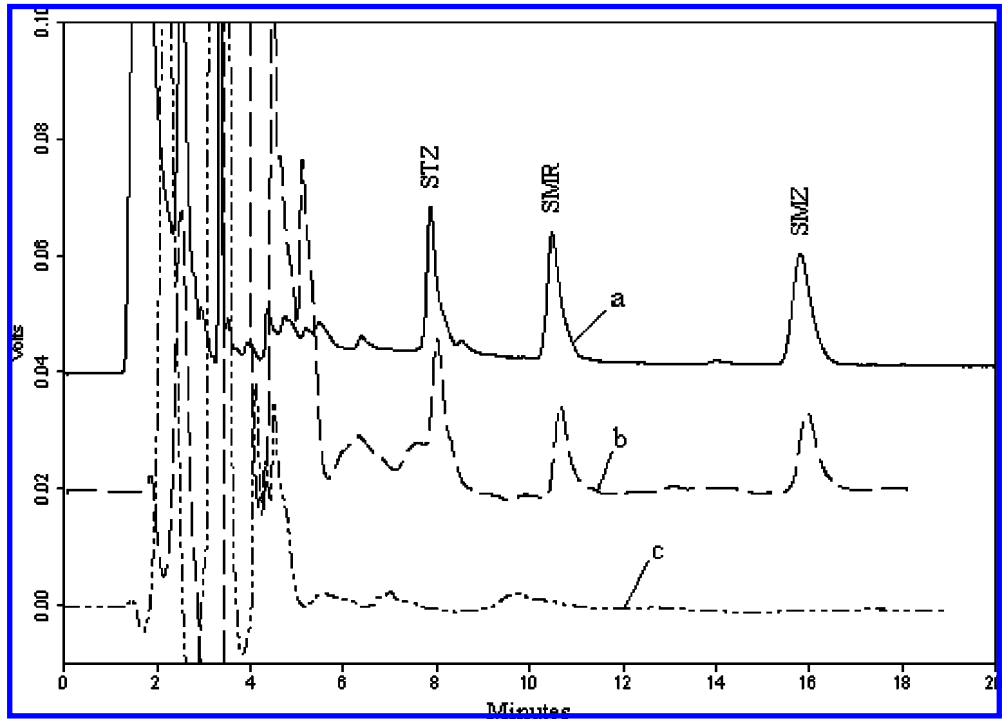

Figure 4. Chromatograms used a UV detector of 50 mL standard mixture solution of 5 μg L^{-1} of three sulfonamides, a pork sample spiked with 3 μg L^{-1} of three sulfonamides, and a blank pork sample with online solid-phase extraction preconcentration ((**a**) standard mixture solution; (**b**) pork sample spiked three sulfonamides; (**c**) blank pork sample).

Table 3. Recoveries of Samples Spiked Sulfonamides (Mean (RSD%), $n = 3$)

	pork muscle			chicken muscle		
	0.5 ng L^{-1}	1 ng L^{-1}	3 ng L^{-1}	0.5 ng L^{-1}	1 ng L^{-1}	3 ng L^{-1}
sulfathiazole	78.3 (3.6)	79.8 (2.1)	83.6 (1.9)	71.9 (4.5)	76.2 (3.5)	77.1 (2.7)
sulfamerazine	75.4 (2.9)	75.3 (2.5)	76.3 (5.3)	68.1 (1.6)	73.1 (5.6)	75.2 (4.8)
sulfamethazine	69.3 (3.9)	72.4 (3.1)	73.5 (2.6)	67.3 (4.3)	70.6 (2.1)	72.1 (1.4)

sample pH $\sim$ 7. The effect of sample loading time on the online solid-phase extraction of 5 μg L^{-1} sulfonamides was tested at a sample loading flow rate of 4 mL min^{-1}. The chromatographic peak area increased almost linearly as sample loading time increased up to at least 20 min. Studies on the effect of sample loading flow rate on the online solid-phase extraction of 5 μg L^{-1} indicated that the chromatographic peak area increased linearly with an increase in the sample loading flow rate up to 6.0 mL min^{-1}. These results also suggest that the kinetics for adsorption of sulfonamides by the imprinted sorbent were rapid. The linearity for the chromatographic peak area against sample loading time and sample loading flow rate in the present online solid-phase extraction system offers great potential for enhancement by increasing sample loading rates and sample loading time without losing extraction efficiency.

For simplicity, the optimum HPLC mobile phase was used to elute the adsorbed sulfonamides from the imprinted amino-functionalized silica gel-packed column. The time required for quantitative desorption of the adsorbed sulfonamides when the HPLC injector valve was in the "inject" position was evaluated in order to determine when the HPLC injector valve should turn to the load position for the next online solid-phase extraction during the HPLC separation of the analytes. By studying the effect of different desorption times when the mobile phase acted as an eluent, we found that the chromatographic peak area of the sulfonamides increased rapidly from 0 to 1.0 min, increased slightly between 1.0 and 1.3 min, and then leveled off in the range of 1.3–2.0 min. Accordingly, a desorption time of 1.5 min was selected to ensure the complete stripping of the adsorbed sulfonamides from the MIPs. Once the adsorbed sulfonamides were quantitatively stripped from the imprinted amino-functionalized silica gel-packed column, the HPLC injector valve was turned to the load position for the next preconcentration cycle, so that the current HPLC separation and the subsequent preconcentration proceeded in parallel. Our results showed that setting the HPLC mobile phase at a flow rate of 1.0 mL min^{-1} for 1.5 min was efficient for quantitative elution of the adsorbed sulfonamides from the microcolumn packed with SMZ-imprinted functionalized silica gel sorbent. Furthermore, the three sulfonamides were separated completely.

Performance for the Present Online SPE Coupled with HPLC Using the Developed Imprinted Functionalized Silica Gel Sorbent. The analytical characteristics of the online solid-phase extraction coupled with HPLC for the determination of sulfonamides developed in this study are given in **Table 2**. The peak areas were linear with $r^2 > 0.99$ over a concentration range of 0.1−12 μg L^{-1} for the three sulfonamides. The precision (RSD) for the nine replicate online SPE of sulfonamides (5 μg L^{-1}) were 2.53, 3.01, and 4.21 for sulfathiazole, sulfamerazine, and sulfamethazine, respectively. The detection limits (3 N) of sulfathiazole, sulfamerazine, and sulfamethazine were 4.6, 5.1, and 7.3 ng L^{-1}, respectively. With a sample loading flow rate of 4 mL min^{-1} for 12.5 min extraction, the enrichment factors obtained by comparing the slopes of the linear portion of the calibration curves directing injection and online extraction were

435, 352, and 314 respectively for sulfathiazole, sulfamerazine, and sulfamethazine compared to direct injection of 20 μL of sample solution.

To evaluate the usefulness of the developed method, pork and chicken samples from a local market were spiked with three levels of sulfonamides (0.5, 1, and 3 ng L^{-1}) and analyzed. Typical chromatograms are shown in **Figure 4**. At each concentration, three measurements were performed (**Table 3**). We achieved good recoveries of pork and chicken muscle ranging from 69.3 to 83.6% and from 67.3 to 77.1%, respectively.

Conclusion. A simple procedure was developed to synthesize a highly selective imprinted amino-functionalized silica gel sorbent by combining a surface molecular imprinting technique with a sol–gel process. The prepared material shows high affinity, selectivity, capacity, and good site accessibility for sulfonamides. This is a promising method for selective adsorption and determination of sulfonamides from pork and chicken muscle matrices by online SPE-HPLC.

ABBREVIATIONS USED

HPLC, high-performance chromatography; MRL, maximum residue limit; SPE, solid-phase extraction; TEOS, tetraethoxysilicane; APTES, 3-aminopropyltriethoxysilane; MIPs, molecularly imprinted polymers; SMZ, sulfamethazine; DDW, doubly deionized water; STZ, sulfathiazole; SMR, sulfamerazine; RSD, relative standard deviation; RCT, ractopamine.

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