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Monitoring of Five Sulfonamide Antibacterial Residues in Milk by In-Tube Solid-Phase Microextraction Coupled to High-Performance Liquid Chromatography

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A simple, rapid, and sensitive method for the quantitative monitoring of five sulfonamide antibacterial residues in milk was developed by coupling in-tube solid-phase microextraction (SPME) to high-performance liquid chromatography with an ultraviolet detector. A poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary column was selected as the extraction medium for this on-line technique. To obtain optimum extraction efficiency, several parameters relating to in-tube SPME were investigated. By simple extraction with ethanol, dilution with phosphate buffer solution, and centrifugation, the sample solution then could be directly injected into the device for extraction. The calculated detection limits for sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine sodium, and sulfacetamide sodium were 2.0, 2.8, 1.7, 2.5, and 22 ng/mL, respectively. The method was linear over the range of 20−5000 ng/mL (100−5000 ng/mL for sulfacetamide sodium) with a correlation coefficient R^2 value >0.9980. Excellent method reproducibility was found by intra- and interbatch precisions, yielding the relative standard deviations of <10.0 and <9.94%, respectively. The proposed method was proved to be robust in monitoring sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine sodium, and sulfacetamide sodium residues in milk.

KEYWORDS: Sulfonamide antibacterials; milk; in-tube SPME-HPLC; poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary

INTRODUCTION

Sulfonamides (SAs) represent a class of antibacterial compounds. They have been widely used in food-producing animals for therapeutic, prophylactic, and growth-promoting purpose (*1*). Improper use of SAs in the dairy industry, such as excessive administration and inappropriate withdrawal period, may result in SA residues in milk. The presence of sulfonamide residues in milk is of great concern because some of the compounds such as sulfamethazine are carcinogen (*2*), and all of them can promote occurrence of the antibiotic-resistant bacteria, which leads to inefficiency of this medicine for the therapeutic use (*3*).

To ensure milk safety for the consumers, several authorities around the world have established maximum residue limits (MRL) in milk. The European Union (EU) set a MRL of 100 ng/g of SAs as a total in milk (regulation EC/281/96). The Ministry of Agriculture of the People's Republic of China established a MRL of 100 ng/g for the sum of SAs and a MRL of 25 ng/g for sulfamethazine only (*4*).

Therefore, the analytical method for monitoring SA residues in milk is required to be simple, rapid, precise, inexpensive, and capable of detecting the residues below the MRL. High-performance liquid chromatography (HPLC), as an analytical technique, has been given much attention in this field. Coupled to ultraviolet (UV) detection, HPLC has been successfully applied to determine several SA residues in milk (*5*−*17*). When using mass spectrometry (MS) detection, analysis at the parts per billion level was achieved (*18*−*20*). On the other hand, the complexity of milk samples requires a simple and effective sample preparation technique for drug analysis. Several pretreatment methods, such as solid-phase extraction (SPE) (*17*, *19*), liquid−liquid extraction (LLE) (*5*, *6*, *8*−*11*, *16*, *18*), and matrix solid-phase dispersion (MSPD) (*7*), have been applied for the extraction of sulfonamide antibacterials from milk. However, these methods demand complex extraction and cleanup procedures or large volumes of sample and solvent or the use of toxic solvents such as chloroform, dichloromethane, and hexane. To overcome those problems, Furusawa successfully applied a shield column (*15*) or an Ultrafree-MC centrifugal ultrafiltration unit (*13*, *14*) for the determination of SA residues in milk, both of which utilized nontoxic ethanol as the only organic solvent. Bogialli developed a simplified, cheap, and nontoxic method to extract 12 SAs from whole milk, which was based on the MSPD technique by using hot water as extractant (*20*). Yang developed an on-line microdialysis system coupled to HPLC, and a simple, fast, and eco-friendly method for determination of six sulfonamides in milk was accomplished (*12*).

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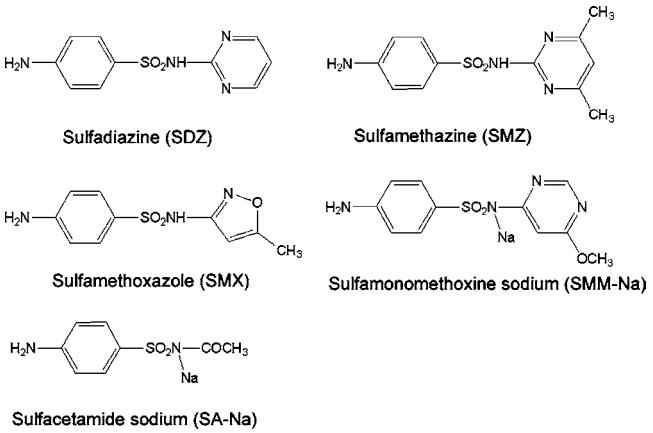

Figure 1. Chemical structures of five sulfonamide antibacterials.

Another promising sample preparation technique is in-tube solid-phase microextraction (SPME). The technique was developed by Pawliszyn and co-workers in 1997 and utilized an open tubular capillary with an inner surface coating as the extraction medium (*21*). Coupled to HPLC, on-line analysis is achieved. Due to its simple, fast, and solventless features, this method has received great attention. In addition, by integrating sample extraction, preconcentration, and introduction into a single step, this on-line method can provide better accuracy, precision, and sensitivity than those off-line methods.

In the present paper, we applied in-tube SPME coupled to HPLC with UV detection to the simultaneous determination of five sulfonamide antibacterial (**Figure 1**) residues, which are commonly used as veterinary medicines in China and around the world, in milk. A poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary column was selected as the extraction medium, which has successfully been used in the analysis of drugs in biological fluids (*22*−*24*). On the basis of this method, no toxic solvents were used, and simple, rapid, and sensitive analysis was accomplished in the meanwhile.

MATERIALS AND METHODS

Reagents and Chemicals. Ethylene glycol dimethacrylate (EGDMA) was purchased from Acros. Methacrylic acid (MAA), 2,2′-azobis(2-methylpropionitrile) (AIBN), dodecanol, and toluene were obtained from Shanghai Chemical Co. Ltd. and were of analytical reagent grade. Double-distilled water was used for all experiments.

Sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfacetamide sodium (SA-Na), and sulfamonomethoxine sodium (SMM-Na) were obtained from the Northeastern Pharmaceutical Factory (Shenyang, China). All five compounds were prepared as 1 mg/mL mixture solutions in methanol and stored at 4 °C in the dark. With these standard solutions, the sample solution was spiked to the concentration for experiments.

In-Tube SPME-HPLC Device. A poly(MAA-EGDMA) monolithic capillary column (15 cm × 0.25 mm, i.d.) was used as microextraction medium and was prepared according to an in situ polymerization method described previously (*22*). The chemical structure of the polymer monolithic stationary phase is displayed in **Figure 2**. Prior to every extraction, the capillary column was conditioned first by methanol and then by buffer solution.

The schematic diagram of the in-tube SPME-HPLC system used for the study is illustrated in **Figure 3** (*22*). The whole device consisted of a pre-extraction segment and an analytical segment. In the present paper, the pre-extraction segment included a six-port valve (valve 1), a Shimadzu LC-10AT pump (pump A) (Shimadzu, Tokyo, Japan), and a PEEK tube (0.03 in. i.d., 0.7 mL total volume); the analytical segment was composed of a P230 pump (pump B) (Dalian Elite Co.), a six-port valve (valve 2), and an FL2200 variable-wavelength UV detector (Fuli Analytical Instrument Co., Zhejiang, China). Connecting valves

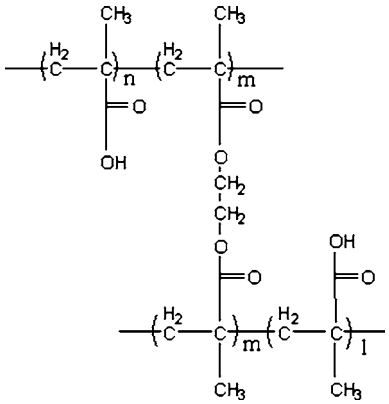

Figure 2. Chemical structure of poly(MAA-EGDMA) monolithic capillary.

1 and 2 with a PEEK tube, on-line extraction, desorption, and separation were achieved.

The procedure used with this device has been described in detail in our previous papers (*22*−*24*) and is listed in **Table 1**. As shown in **Figure 3A**, the extraction process does not interfere with the separation process. Therefore, extraction and separation could be simultaneously performed, and consequently the whole analysis time is reduced. Extraction and desorption flow rates in the present experiments were 0.04 and 0.02 mL/min, respectively.

HPLC-UV Conditions. The instrument used for the study has been described in the above section, which is not discussed here again. The analytical column was a Hypersil ODS column (200 × 4.6 mm i.d.; 5 μm), which was purchased from Agilent Technologies (Palo Alto, CA). The optimized mobile phase for desorption and separation was methanol−0.02 mol/L Na_2HPO_4 buffer solution (3:7, v/v; pH 3.0), and the flow rate was kept at 0.5 mL/min. The detection was performed at 269 nm with the UV detector for all five analytes.

Preparation of Milk Samples. Analyte-free whole milk was purchased from retail markets and stored at −4 °C in the dark before use. The five sulfonamide antibacterials were directly spiked into 1 mL milk samples over a range of 20−5000 ng/mL. Then 0.6 mL of ethanol was added into the spiked milk samples and ultrasonicated for 60 s. After 60 s, the samples were diluted with 0.02 mol/L Na_2HPO_4 buffer solution (pH 2.5) to 10 mL. Finally, the samples were centrifuged at 12000*g* for 6 min, and the supernatant liquids were extracted. Blank milk sample was prepared the same as above but without the compound-spiking step.

The above sample preparation procedure was simple and rapid, required no complex extraction and cleanup procedures, and demanded no large volumes of sample and solvent. Moreover, no use of toxic solvents was also achieved. As shown in **Figure 2**, poly(MAA-EGDMA) monolithic material is a kind of polymer sorbent that possesses a polar group, a carboxyl acid group, in the hydrophobic bone structure and has been demonstrated to be biocompatible in dealing with biological samples (*22*, *25*). Thus, whereas the target compounds are adsorbed onto the extraction phase through hydrophobic interaction, irreversible adsorption of proteins and fats does not occur due to the presence of a hydrophilic pendant group (carboxyl acid group) in the acidic condition. Therefore, the present sample preparation required no additional steps to eliminate the fats of the milk sample prior to extraction.

RESULTS AND DISCUSSION

In-Tube SPME Desorption and HPLC Separation. After extraction of the analytes, on-line desorption followed. Through switching the six-port valve (valve 2) of the analytical segment to the injection position, desorption was simply accomplished by driving the mobile phase through the capillary column. Therefore, it was important to ensure the selected mobile phase not only provided complete desorption of the extracted analytes from the poly(MAA-EGDMA) monolithic capillary column but also obtained high selectivity and sensitivity. In addition, for

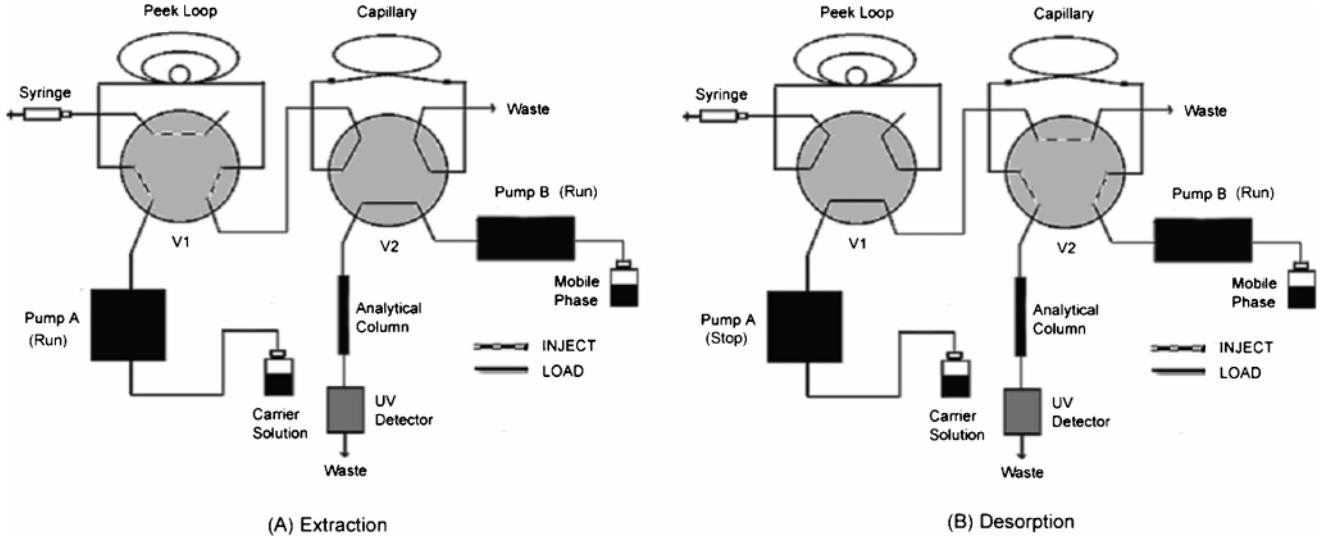

Figure 3. Construction of in-tube SPME-HPLC device.

Table 1. Program for In-Tube SPME Process

sequence	event	pump A	pump B	valve 1	valve 2
1	fill the PEEK tube with methanol	run	run	load	load
2	condition the capillary with methanol	run	run	inject	load
3	condition the capillary with H_2O and fill the PEEK tube with sample	run	run	load	load
4	begin extraction	run	run	inject	load
5	end extraction and flush the capillary with H_2O	run	run	load	load
6	elute the analytes from the capillary	stop	run	load	inject
7	separate the analytes and return to sequence 1	run	run	load	load

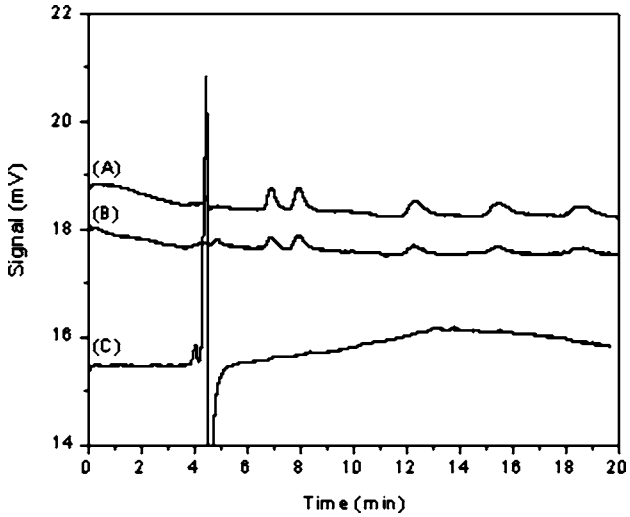

Figure 4. Carry-over study: (**A**) after 7 min of desorption; (**B**) after 10 min of desorption; (**C**) after 12 min of desorption.

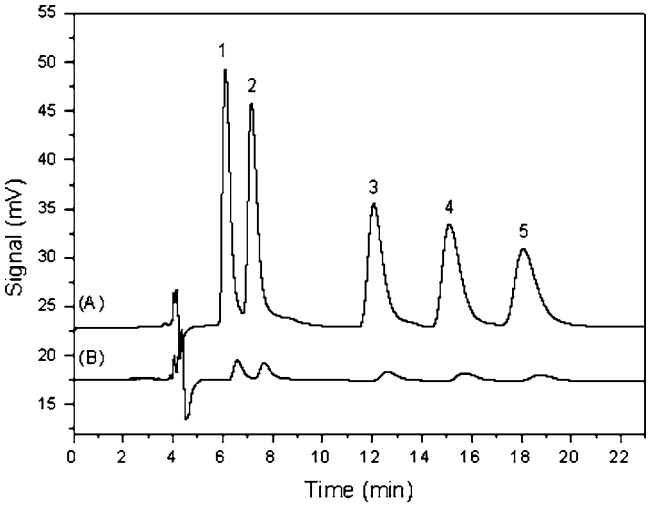

Figure 5. HPLC chromatograms of five sulfonamide antibacterials obtained by in-tube SPME-HPLC (**A**) and direct injection of the standard sample (**B**). Sulfonamide antibacterials were spiked at 500 ng/mL in water. The volume for direct injection was 20 μL. Carrier solution was 0.02 mol/L Na_2HPO_4 buffer solution (pH 3.0). Extraction time was 10 min. Other SPME and HPLC conditions were outlined under Materials and Methods. Peaks: 1, sulfacetamide sodium; 2, sulfadiazine; 3, sulfamethazine; 4, sulfamethoxazole; 5, sulfamonomethoxine sodium.

the milk sample analysis, it also had to make the analytes avoid interference from the inherent components of milk in the chromatographic analysis. After optimization, methanol−0.02 mol/L Na_2HPO_4 buffer solution (3:7, v/v; pH 3.0) was found to be suitable for desorption and separation.

The desorption time was investigated in the range of 7−12 min. After each desorption, the capillary was not immediately washed and conditioned by methanol and buffer solution, but flushing of the capillary by the mobile phase was allowed to continue for another 3 min to investigate the carry-over. HPLC chromatograms are displayed in **Figure 4**. The results showed that carry-over of the five compounds was not found after 12 min. Besides, no peak broadening or tailing was found in the following chromatographic analysis. Therefore, 12 min was selected for the desorption time.

Method Performance of Aqueous Samples. Figure 5 shows the typical chromatograms of five compounds obtained by in-tube SPME-HPLC and direct injection into the LC column. Using this in-tube SPME-HPLC device, on-line extraction, preconcentration, and desorption resulted in the achievement of large volume injection of sample solutions without peak

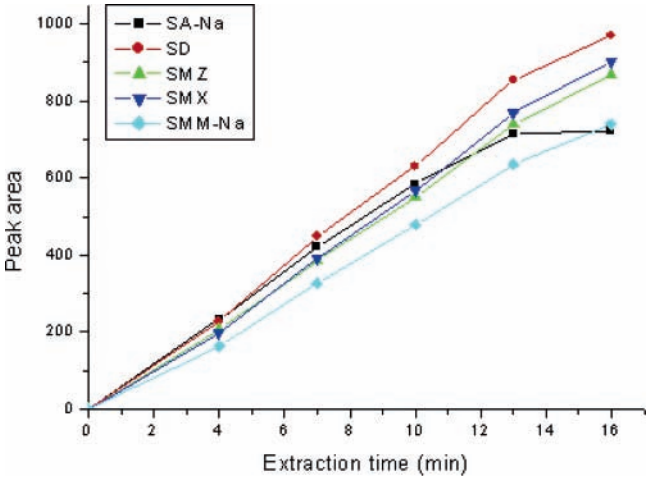

Figure 6. In-tube SPME-HPLC extraction time profile of five sulfonamide antibacterials. Sulfonamide antibacterials were spiked at 500 ng/mL in water. Other SPME and HPLC conditions were the same as **Figure 5**.

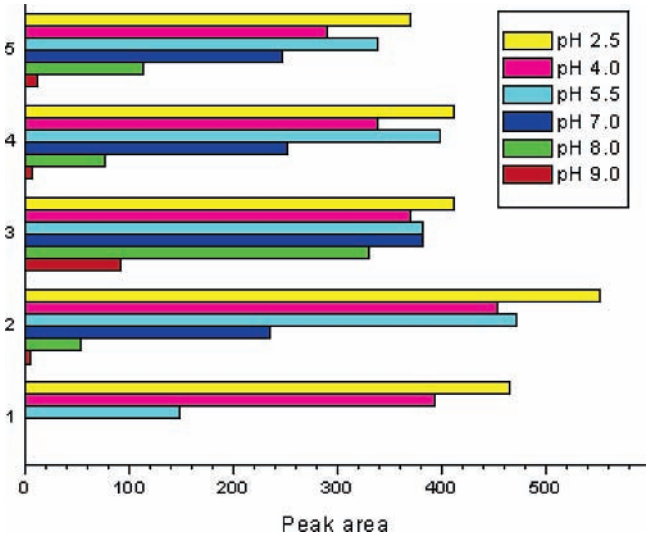

Figure 7. Optimization of the pH of the sample matrix. Sample solutions of five sulfonamide antibacterials spiked at 500 ng/mL were prepared with 0.02 mol/L phosphate buffer at pH 2.5–7.0 and with 0.02 mol/L Tris-HCl buffer at pH 8.0–9.0. Carrier solution was double-distilled water. Extraction time was 7 min. Other SPME and HPLC conditions were the same as **Figure 5**. 1, sulfacetamide sodium; 2, sulfadiazine; 3, sulfamethazine; 4, sulfamethoxazole; 5, sulfamonomethoxine sodium.

broadening. Therefore, a notable decrease in the limit of detection (LOD) was successfully accomplished by in-tube SPME.

For in-tube SPME, extraction efficiency can be estimated using the absolute amount of analytes extracted, which is calculated from experimental measurements with the expression (*26*)

$$n_A = FA = (m/A_d)A$$

where n_A is the amount of analyte extracted by SPME, F is the detector response factor, which can be obtained by comparing the amount of analyte (m) injected to the area counts (A_d) obtained through direct injection, and A is the response obtained by SPME. Using the previously determined separation and elution conditions, a 400 μL standard aqueous solution with five sulfonamides spiked at 500 ng/mL was extracted. The extracted amounts, calculated by comparing the resulting peak area to that obtained by 20 μL of direct injection (10 ng of analytes injected), were 121.6 ng (SA-Na), 126.0 ng (SDZ), 127.2 ng (SMZ), 121.5 ng (SMX), and 120.0 ng (SMM-Na), respectively. Therefore, the corresponding extraction efficiencies were 60.8, 63.0, 63.6, 60.8, and 60.0%, respectively. Compared to the existing open tubular capillary for the in-tube SPME device (*26*−*29*), the extraction efficiencies using this monolithic capillary are higher. This is mainly due to the increased ratio of the surface area of extraction phase in contact with the sample solution to the volume of sample solution.

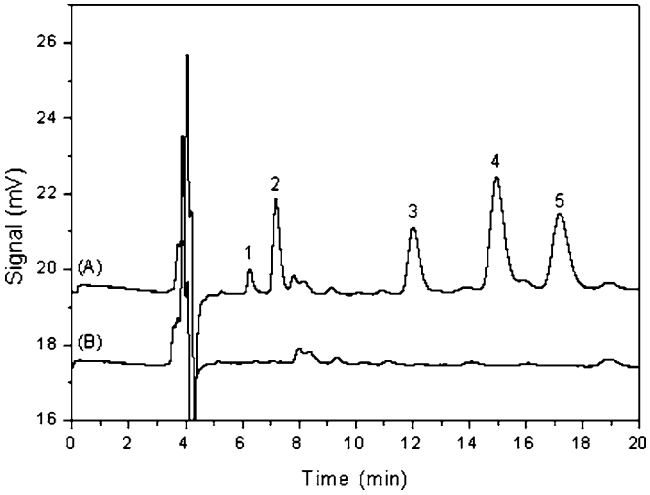

Figure 8. HPLC chromatograms of five sulfonamide antibacterials obtained by in-tube SPME-HPLC from spiked milk sample (**A**) and blank milk sample (**B**). Sample solution consisted of five compounds spiked at 500 ng/mL. Extraction time was 16 min. Carrier solution was 0.02 mol/L Na_2HPO_4 buffer solution (pH 2.5). Other SPME and HPLC conditions were the same as in **Figure 5**. Peaks: 1, sulfacetamide sodium; 2, sulfadiazine; 3, sulfamethazine; 4, sulfamethoxazole; 5, sulfamonomethoxine sodium.

Table 2. Linear Regression Data for SPME of Five Sulfonamide Antimicrobials from Milk Samples[a]

compound	linear dynamic range (ng/mL)	regression line			LOD (ng/mL)	LOQ (ng/mL)
		slope	intercept	R^2 value		
SA-Na	100–5000	0.0168	0.8393	0.9980	22	75
SDZ	20–5000	0.0716	0.0433	0.9999	2.0	6.7
SMZ	20–5000	0.0791	1.5286	0.9996	2.8	9.4
SMX	20–5000	0.1752	1.9214	0.9996	1.7	5.5
SMM-Na	20–5000	0.1275	−0.7041	0.9997	2.5	8.3

[a] Number of data points: 8. Extraction flow rate and desorption flow rate were 0.04 and 0.02 mL/min, respectively. Extraction time was 16 min. Carrier solution was 0.02 mol/L Na_2HPO_4 buffer solution (pH 2.5). Desorption solution was methanol–0.02 mol/L Na_2HPO_4 buffer solution (3:7, v/v; pH 3.0). Flow rate for chromatographic separation was 0.5 mL/min. UV detection wavelength was set at 269 nm.

Optimization of In-Tube SPME Conditions. Several parameters such as extraction time and pH value of the sample matrix were investigated, which had influence on the extraction efficiency for in-tube SPME (*30*). The extraction time profile of five sulfonamide antibacterials was monitored by progressing longer periods of sampling time for a 500 ng/mL standard solution. The flow rate of the carrier solution was kept at 0.04 mL/min, and the extraction time was increased from 4 to 16 min, corresponding to 160−640μL of sample volume. As shown in **Figure 6**, the amount of sulfadiazine, sulfamethazine, sulfamethoxazole, sulfacetamide sodium, and sulfamonomethoxine sodium extracted, presented as the peak area, increased greatly and rapidly when the extraction time rose from 4 to 13 min. Increasing the extraction time beyond 13 min did not result

Table 3. Precisions

compound	intrabatch precision (RSD %; $n = 5$) low (20 ng/mL)	medium (300 ng/mL)	high (5000 ng/mL)	interbatch precision (RSD %; $n = 3$) low (20 ng/mL)	medium (300 ng/mL)	high (5000 ng/mL)
SA-Na[a]	3.97	0.89	2.84	4.42	1.00	3.74
SDZ	7.70	5.80	1.51	6.42	4.13	2.71
SMZ	5.18	1.26	1.01	2.79	4.18	0.78
SMX	10.0	2.96	2.78	8.58	4.92	0.17
SMM-Na	7.92	3.18	0.78	9.94	4.62	1.06

[a] The low, medium, and high concentrations for SA-Na were 100, 700, and 5000 ng/mL, respectively.

Table 4. Recoveries of Five Sulfonamide Antimicrobials Spiked into Milk Samples

compound	recovery (%; $n = 3$) low (20 ng/mL)	medium (300 ng/mL)	high (5000 ng/mL)
SA-Na[a]	11.5	14.7	13.9
SDZ	44.8	46.6	43.4
SMZ	52.1	51.3	57.6
SMX	83.5	95.7	96.5
SMM-Na	93.2	86.5	84.6

[a] The low, medium, and high concentrations for SA-Na were 100, 700, and 5000 ng/mL, respectively.

in a proportional increase in the extracted amount for sulfacetamide sodium, whereas the equilibrium value for the other four compounds was not obtained even after 16 min of sampling time. To achieve sufficient analysis sensitivity within a short period time, 16 min of extraction time was selected for the analysis of milk samples.

The effects of the pH value of the sample matrix on the extraction efficiency of sulfonamides were evaluated using several buffer solutions with pH 2.5−9.0. As shown in **Figure 7**, a higher extraction efficiency for all five sulfonamides was obtained at lower pH value, and an obvious decrease was found when the pH was increased. Even no sulfacetamide sodium molecule was extracted onto the capillary column at a pH value of >7.0. The pK_a values of SA, SDZ, SMZ, SMX, and SMM are 5.4, 6.4, 7.5, 5.4, and 6.5, respectively (*31*, *32*), so the molecules could be deprotonated and possess a negative charge as the solution pH value is higher than the pK_a of the compounds. On the other hand, the molecule of the polymer monolithic material could also be deprotonated and possess a negative charge at a higher pH value. Therefore, the presence of repulsive interaction of homogeneous charges between the target compounds and extraction phase results in the extraction efficiency decreasing at higher pH. In addition, the ion-exchange interaction between the protonated drugs and the acidic pendant groups of the polymer is enhanced at lower pH of the sample matrix, which leads to an increase in the extraction efficiency. Therefore, the sample matrix was adjusted to pH 2.5 using 0.02 mol/L Na_2HPO_4 buffer solution for the following experiments.

Analysis of Five Sulfonamide Antibacterials in Milk. *Addition of a Rinsing Step before Elution.* For analysis of milk sample, it is worth noting that proteins and fats of the milk appeared in the capillary during the extraction process, and thus the presence of residual proteins and fats will result in pollution and clogging of the analytical column in the following analysis. Therefore, a rinsing step before elution to remove the residual proteins and fats in the capillary was required. Because the extraction capillary shows biocompatibility, it was easily realized by using 0.02 mol/L Na_2HPO_4 buffer solution (pH 2.5) as the carrier solution to flush the monolithic column immediately after the extraction. In the present work, 7 min was enough for the rinsing step.

Detection Limits, Quantification Limits, and Calibration Curves. **Figure 8** displays the chromatograms obtained for in-tube SPME of milk samples. All five sulfonamide peaks can be seen very clearly on the chromatogram, with no significant interference from any milk components. Thus, quantification of the five compounds could be successfully achieved. Milk samples were spiked over a range of 20−5000 ng/mL for sulfadiazine, sulfamethazine, sulfamethoxazole, and sulfamonomethoxine sodium and at 100−5000 ng/mL for sulfacetamide sodium. As shown in **Table 2**, satisfactory regression coefficients for the calibration curves were obtained. The LOD and limit of quantification (LOQ) for these five compounds in milk samples were determined at a concentration at which signal-to-noise ratios were equal to 3 and 10 under our HPLC-UV detector conditions, respectively. LOD and LOQ data are also displayed in **Table 2**.

Precision, Recovery, and Stability. The reproducibility of the developed method was determined by the intra- and interday precision. The intra- and interday relative standard deviations (RSDs) were investigated with the low, medium, and high concentrations, respectively, according to the calibration curve ranges. Five extractions of a mixture sample solution over a day gave the intraday RSDs. The interday precision was determined by extracting a mixture sample solution that had been independently prepared for 3 continuous days. Data are reported in **Table 3**.

The recovery of the analytes from spiked milk samples was calculated by comparison of the in-tube SPME-HPLC peak areas from the spiked milk samples to those obtained from the standard solutions, and data are shown in **Table 4**. From the data, recoveries of SA-Na, SDZ, and SMZ were low. It is expected that the presence of ethanol and fat in the sample solutions affects the interaction (mainly hydrophobic interaction) between analytes and the extraction phase during the extraction process, which results in low extraction efficiency. In addition, extraction from milk samples can also lead to the loss of analytes.

The monolithic capillary shows high stability because no significant changes, such as capillary column backpressure and extraction efficiency, were found in its extractions over 2 months during this study.

Conclusion. In-tube SPME coupled to HPLC with a poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary as the extraction medium was successfully applied to the simple and rapid determination of five sulfonamide antibacterial residues in milk. In comparison to the pretreatment methods as reported previously, the proposed in-tube SPME-HPLC method is environmentally friendly and inexpensive and easily realizes on-line analysis. In addition, using the in-tube

SPME coupled to HPLC with UV detection, simultaneous analysis was accomplished with high sensitivity. Therefore, the proposed method will be useful and practical in future residue monitoring and in studying the pharmacokinetics of sulfadiazine, sulfamethazine, sulfamethoxazole, sulfacetamide sodium, and sulfamonomethoxine sodium in milk.

LITERATURE CITED

(1) Dixon Holland, D. E.; Katz, S. E. Competitive direct enzyme-linked immunosorbent screening assay for the detection of sulfamethazine contamination of animal feeds. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 784−789.

(2) Littlefield, N. A.; Sheldon, W. G.; Allen, R.; Gaylor, D. W. Chronic toxicity/carcinogenicity studies of sulphamethazine in Fischer 344/N rats: two-generation exposure. *Food Chem. Toxicol.* **1990**, *28*, 157−167.

(3) Haagsma, N.; Pluijmakers, H. G.; Aerts, M. M.; Beek, W. M. Rapid sample preparation methods for analysis of residues of sulfamethazine and its N4-acetyl and desamino metabolites in swine tissue by HPLC. *Biomed. Chromatogr.* **1987**, *2*, 41−45.

(4) The maximum residue limit of veterinary drugs in edible animal food established by Ministry of Agriculture of People's Republic of China. *Chinese J. Vet. Drug* **2003**, *37*, 15−20.

(5) Weber, J. D.; Smedley, M. D. Liquid chromatographic method for determination of sulfamethazine residues in milk: collaborative study. *J. AOAC Int.* **1993**, *76*, 725−729.

(6) Weber, J. D.; Smedley, M. D. Liquid chromatographic determination of sulfamethazine in milk. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 445−447.

(7) Long, A. R.; Short, C. R.; Barker, S. A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk. *J. Chromatogr.* **1990**, *502*, 87−94.

(8) Smedley, M. D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk: collaborative study. *J. AOAC. Int.* **1994**, *77*, 1112−1122.

(9) Furusawa, N. Clean and rapid liquid chromatographic technique for monitoring of oxytetracycline and sulphadimicline in milk without use of organic solvents. *Chromatographia* **2003**, *57*, 317−320.

(10) Smedley, M. D.; Weber, J. D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 875−879.

(11) Perez, N.; Gutierrez, R.; Noa, M.; Diaz, G.; Luna, H.; Escobar, I.; Munive, Z. Liquid chromatographic determination of multiple sulfonamides, nitrofurans, and chloramphenicol residues in pasteurized milk. *J. AOAC Int.* **2002**, *85*, 20−24.

(12) Yang, T. C. C.; Yang, I. L.; Liao, L. J. Determination of sulfonamide residues in milk by on-line microdialysis and HPLC. *J. Liq. Chromatogr. Relat. Technol.* **2004**, *27*, 501−510.

(13) Furusawa, N. Simplified determining procedure for routine residue monitoring of sulphamethazine and sulphadimethoxine in milk. *J. Chromatogr. A* **2000**, *898*, 185−191.

(14) Furusawa, N.; Kishida, K. High-performance liquid chromatographic procedure for routine residue monitoring of seven sulfonamides in milk. *Fresenius' J. Anal. Chem.* **2001**, *371*, 1031−1033.

(15) Kishida, K.; Furusawa, N. Application of shield column liquid chromatography for determination of sulfamonomethoxine, sulfadimethoxine, and their N_4-acetyl metabolites in milk. *J. Chromatogr. A* **2004**, *1028*, 175−177.

(16) Furusawa, N. Liquid-chromatographic determination of sulfadimidine in milk and eggs. *Fresenius' J. Anal. Chem.* **1999**, *364*, 270−272.

(17) de Zayas-Blanco, F.; García-Falcón, M. S.; Simal-Gándara. Determination of sulfamethazine in milk by solid phase extraction and liquid chromatographic separation with ultraviolet detection. *Food Control* **2004**, *15*, 375−378.

(18) Abian, J.; Churchwell, M. I.; Korfmacher, W. A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level. *J. Chromatogr.* **1993**, *629*, 267−276.

(19) Cavaliere, C.; Curini, R.; Corcia, A. D.; Nazzari, M.; Samperi, R. A simple and sensitive liquid chromatography−mass spectrometry confirmatory method for analyzing sulfonamide antibacterials in milk and egg. *J. Agric. Food Chem.* **2003**, *51*, 558−566.

(20) Bogialli, S.; Curini, R.; Corcia, A. D.; Nazzari, M.; Polci, M. L. Rapid confirmatory assay for determining 12 sulfonamide antimicrobials in milk and eggs by matrix solid-phase dispersion and liquid chromatography−mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 4225−4232.

(21) Eisert, R.; Pawliszyn, J. Automated in-tube solid-phase microextraction coupled to high-performance liquid chromatography. *Anal. Chem.* **1997**, *69*, 3140−3147.

(22) Fan, Y.; Feng, Y. Q.; Da, S. L.; Shi, Z. G. Poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary for in-tube solid-phase microextraction coupled to high performance liquid chromatography and its application to determination of basic drugs in human serum. *Anal. Chim. Acta* **2004**, *523*, 251−258.

(23) Fan, Y.; Feng, Y. Q.; Da, S. L.; Gao, X. P. In-tube solid-phase microextraction with poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary for direct high-performance liquid chromatographic determination of ketamine in urine samples. *Analyst* **2004**, *129*, 1065−1069.

(24) Wen, Y.; Fan, Y.; Zhang, M.; Feng, Y. Q. Determination of camptothecin and 10-hydroxycamptothecin in human plasma using polymer monolith in-tube solid phase microextraction combined with high-performance liquid chromatography. *Anal. Bioanal. Chem.* **2005**, *382*, 204−210.

(25) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *J. Pharm. Biopharm.* **2000**, *50*, 27−46.

(26) Wu, J. C.; Pawliszyn, J. Polypyrrole coated capillary coupled to HPLC for in tube solid phase microextraction and analysis of aromatic compounds in aqueous samples. *Anal. Chem.* **2001**, *73*, 55−63.

(27) Wu, J. C.; Tragas, C.; Lord, H.; Pawliszyn, J. Analysis of polar pesticides in water and wine samples by automated in-tube solid-phase microextraction coupled with high-performance liquid chromatography−mass spectrometry. *J. Chromatogr. A* **2002**, *976*, 357−367.

(28) Mullett, W. M.; Levsen, K.; Borlak, J.; Wu, J. C.; Pawliszyn, J. Automated in-tube solid-phase microextraction coupled with HPLC for the determination of N-nitrosamines in cell cultures. *Anal. Chem.* **2002**, *74*, 1695−1701.

(29) Wu, J. C.; Pawliszyn, J. Preparation and applications of polypyrrole films in solid-phase microextraction. *J. Chromatogr. A* **2001**, *909*, 37−52.

(30) Kataoka, H. Recent advances in solid-phase microextraction and related techniques for pharmaceutical and biomedical analysis. *Curr. Pharm. Anal.* **2005**, *1*, 65−84.

(31) Agarwal, V. K. High-performance liquid chromatographic methods for the determination of sulfonamides in tissue, milk and eggs. *J. Chromatogr.* **1992**, *624*, 411−423.

(32) Xiao, Y. X.; Xiao, X. Z.; Feng, Y. Q.; Wang, Z. H.; Da, S. L. High-performance liquid chromatography of sulfonamides and quinolones on *p-tert*-butyl-calix[6] arene-bonded silica gel stationary phase. *Talanta* **2002**, *56*, 1141−1151.

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