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Article

Matrix Solid-Phase Dispersion Extraction of Sulfonamides from Blood

Yupu Zhang, Xu Xu, He Liu, Yujuan Zhai, Ye Sun, Shuo Sun, Hanqi Zhang, Aimin Yu and Yinghua Wang*

College of Chemistry, Jilin University, Changchun 130012, P.R. China

*Author to whom correspondence should be addressed: email: analchem@jlu.edu.cn

Matrix solid-phase dispersion extraction was applied to the extraction of sulfadiazine, sulfamerazine, and sulfamethazine from human and animal bloods. The separation and determination of the analytes were carried out by high-performance liquid chromatography. The effects of the types of the dispersion adsorbents and elution solvents were investigated, and the highest recovery was obtained when diatomaceous earth was used as the dispersion adsorbent, while acetone was used as the elution solvent. Under the optimal conditions, the linear range for determining the sulfonamides in blood samples was 0.020–10.0 μg/mL, and the average recoveries of the three sulfonamides were higher than 87.5%.

Introduction

Sulfonamides are commonly used to prevent chronic bacterial infections and promote the growth of animals (1). However, if inappropriately used, the sulfonamides have some side effects. For example, sulfamerazine may be a kind of carcinogen resulting in hypothyroid and have been known to produce thyroid tumors in rodents (2, 3), causing allergic hypersensitivity reactions, and reducing the therapeutic effectiveness of these drugs on humans (4). The other kinds of sulfonamides are widely known to cause allergic reactions, such as emiction and hemopoiesis turbulence (5–8). It has become quite necessary to detect sulfonamides in foods, tissues, and blood. Therefore, the development of methods for monitoring sulfonamides is of great significance.

A large amount of works have been done to monitor sulfonamides by high-performance liquid chromatography (HPLC) (5, 9–12) based on some extraction methods, such as solvent extraction (11–13), magnetic mixed hemi micelles solid-phase extraction (MMHSPE) (14), liquid–liquid–liquid microextraction (LLLME) (15), solid-phase extraction (SPE) (16–20), solid phase microextraction (18), polymer monolith microextraction (PMME) (19), and matrix solid-phase dispersion (MSPD) (13, 20). These extraction methods are often applied to the extraction of target analytes from biological samples including muscle (9, 21, 22), fat (21), liver (21, 23), kidney (21, 23), hair (24), milk (13, 17, 25–29), honey (10, 30), eggs (13, 19, 31), blood (32), and urine (33). However, compared with the classical extraction methods, MSPD has more advantages in the consumptions of solvent and sample (34, 35). Since MSPD was introduced in 1989 by Barker (36), it has been applied by many researchers. The MSPD has been applied to the extraction of sulfonamides from chicken (20), eggs, and milk (13). However, there is no research to extract sulfonamides from the blood by MSPD. Liquid–liquid extraction (LLE) was applied to the extraction of the sulfadiazine and trimethoprim from the plasma (32). In LLE, the samples were deproteinized with acetonitrile and extracted with ethyl acetate. The extraction was carried out in several steps. When the proposed MSPD was applied, deproteinization and extraction can be finished in one step, the operation was simpler and the time for the sample preparation was shorter compared with LLE. This work was focused on the investigation of MSPD extraction of the sulfonamides from human and animal bloods.

Experimental

Chemicals and reagents

Sulfadiazine (SDZ), sulfamerazine (SMR), and sulfamethazine (SMZ) were purchased from Chinese Medical and Biological Products Institute (Beijing, China) and structures of the compounds are shown in Figure 1. The mixed stock solution containing 100.0 μg/mL SDZ, 100.0 μg/mL SMR, and 100.0 μg/mL SMZ was prepared by dissolving the compounds in methanol. The working solutions were prepared by diluting the stock solution with mobile phase and stored at 4°C.

Diatomaceous earth (approximately 400 meshes) was purchased from Wako Pure Chemical Industries (Osaka, Japan). The silica gel (60–325 meshes), neutral alumina (200 meshes), and C_{18} were obtained from Chinese Medical and Biological Products Institute. C_{18} was sequentially washed with *n*-hexane, dichloromethane, and methanol and then dried naturally. Diatomaceous earth, silica gel, and neutral alumina were baked at 650°C for 4 h, dried at 100°C for 2 h, cooled, and stored in the desiccator. The neutral alumina was deactivated with deionized water (m/m, 4:100) before use. HPLC-grade methanol was obtained from Fisher Scientific (Pittsburgh, PA). Water was purified through a distilling apparatus (Ronghua Company, Jiangsu, China) and filtered through the Millipore filter (Billerica, MA). Analytical reagent grade methanol, *n*-hexane, cyclohexane, petroleum ether, dichloromethane, acetonitrile, and acetone were purchased from Beijing Chemical Factory (Beijing, China). The mobile phase was filtered through a 0.45-μm nylon membrane.

Porcine blood was purchased from local food market (Changchun, China). Human and white rat blood samples were obtained from the Medicine Institute of Jilin University (Changchun, China). The spiked samples containing SDZ, SMR, and SMZ at concentration levels of 0.2 and 2.0 μg/mL were prepared by spiking the working solutions into the blood samples. At each concentration level, three replicate samples were prepared to evaluate the recoveries and precision.

Apparatus

UV-1700 Ultraviolet Spectrophotometer was purchased from Shimadzu Technologies (Kyoto, Japan). Shimadzu HPLC–20AB Class VP HPLC system (Shimadzu Technologies, Kyoto, Japan) equipped with a UV detector was used. The chromatographic

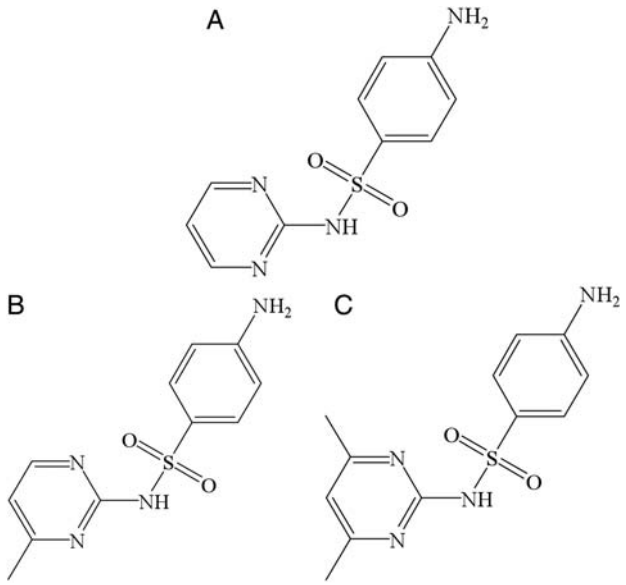

Figure 1. Structures of the sulfonamides: (A) sulfadiazine (SDZ), (B) sulfamerazine (SMR), (C) sulfamethazine (SMZ).

column was a C_{18} column (250 × 4.6 mm i.d., 5 μm, Agela Technologies, Tianjin, China). The sample injection volume was 20 μL and the temperature of the column was controlled at 25°C. The mobile phase consists of water (A) and methanol (B). The gradient elution condition is as follows: 0–3 min (75% A), 3–6 min (65% A), 6–9 min (55% A), 9–12 min (45% A), and 12–16 min (35% A). The flow rate of the mobile phase was kept at 1.0 mL/min, and the absorbance was measured at the wavelength of 269 nm.

Preparation of sample

One gram of diatomaceous earth (dispersion adsorbent) and 0.25 mL of blood were placed in the agate mortar. The sample and the dispersion adsorbent were blended using a pestle. After the sample dispersed completely, the homogeneous mixture was transferred into a glass column (300 × 15 mm i.d.) with a layer of adsorbent cotton on the bottom of the column. A thin layer of adsorbent cotton was added at the top of the sample mixture. The column was eluted with a suitable volume of elution solvent by gravitational flow. The eluate was evaporated to dryness, and 1 mL of the HPLC mobile phase was added to dissolve the residue. The resulting sample solution was filtered through a 0.45-μm membrane. Twenty microliters of the sample solution was injected into the HPLC system for analysis.

Results and Discussion

Optimization of MSPD conditions

Dispersion adsorbents and elution solvents

In the MSPD, the dispersion adsorbent was used to abrade the sample. When the sample is disrupted with the mortar and pestle, the dispersion adsorbent plays a role for breaking the sample into smaller pieces. At the same time, the disrupted sample is adsorbed onto the surface of the dispersion adsorbent based on the polarities of the dispersion adsorbent and the sample. The polar sample is easily adsorbed on the surface of the polar dispersion adsorbent, and the non-polar sample is adapted to the adsorption on the surface of the non-polar dispersion adsorbent. Therefore, the type of the dispersion adsorbent is important and should be selected.

At the same time, the efficiency of the extraction is relevant to the kind of elution solvent. The blood consists of water, proteins, carbohydrates, fats, and various inorganic species. Therefore, the selected elution solvent should satisfy such requests: (*i*) the polarities of the target analyte and the elution solvent are similar, so the target analyte can be eluted by the elution solvent; (*ii*) these biomolecules, such as proteins, carbohydrates, and fats, which may impair the column of HPLC, cannot be eluted. To select the optimum dispersion adsorbent and the elution solvent, several dispersion adsorbents and elution solvents were studied. The silicagel, diatomaceous earth, alumina, and C_{18} were used as dispersion adsorbents, and *n*-hexane, cyclohexane, petroleum ether, dichloromethane, acetone, acetonitrile, methanol, and deionized water were used as elution solvents in this study. The spiked porcine blood samples at the analyte concentration level of 0.5 μg/mL are prepared for this purpose. The experimental results showed that the *n*-hexane, cyclohexane, and petroleum ether cannot elute the target analytes, and the methanol and deionized water were not suitable to separate the target analytes from the blood because the blood can be dissolved in the solvents. The experimental results are shown in Table I, which indicates that the recoveries for the analytes are highest when acetone was used as an elution solvent. The recoveries are similar when C_{18} and diatomaceous earth were used as dispersion adsorbents. However, the expense of C_{18} was greater than that of diatomaceous earth. Considering this, the diatomaceous earth was used as the dispersion adsorbent in further experiments.

Effect of the amount of dispersion adsorbent

The amount of dispersion adsorbent has an effect on the extraction yields. Because the sample is blood, which is a viscous liquid, the smaller the amount of the dispersion adsorbent in the mixture of the dispersion adsorbent and the blood, the more viscous the mixture becomes, and the more difficult the

Table I
Effects of the Adsorbents and the Elution Solvents on the Recoveries

Adsorbent	Elution solvent	Recovery (%)		
		SDZ	SMR	SMZ
Silicagel	Acetonitrile	46.5	43.8	38.6
	Dichloromethane	10.7	10.6	8.1
	Acetone	69.1	56.3	57.3
Alumina	Acetonitrile	25.0	30.0	36.7
	Dichloromethane	8.3	7.8	7.1
	Acetone	47.9	51.5	56.7
C_{18}	Acetonitrile	44.7	48.3	61.1
	Dichloromethane	48.3	58.8	61.3
	Acetone	96.8	74.1	94.8
Diatomaceous earth	Acetonitrile	39.9	41.6	41.0
	Dichloromethane	24.0	20.0	19.3
	Acetone	94.8	90.0	96.8

dispersion of the target analytes. The effect of the amount of the dispersion adsorbent on recoveries was studied, and the experimental results are shown in Figure 2. It is obvious that the extraction yields are highest when the amount of dispersion adsorbent was 1.0 g.

Washing solvent

The washing solvents are often required before elution to remove the interferents. *n*-Hexane, cyclohexane, and petroleum ether were used because their polarities are low and dissimilar from the target analytes. Figure 3 indicates that when the washing solvents were used, very few peaks in the chromatogram of the washing eluate were observed. The experimental results indicate that very few interfering compounds can be removed by using the washing solvent. Considering this, the samples were prepared without washing.

Volume of elution solvent

Acetone was used as elution solvents, the volume of elution solvent directly determines whether the target analytes is eluted completely. In most applications, 8 mL of elution solvent was used. Some reports indicated that most target analytes were eluted in the first 4 mL of elution solvent (4, 36, 39–43). The effect of the volume of the elution solvent was studied when the diatomaceous earth was used as a dispersion adsorbent and acetone was used as an elution solvent. The experimental results shown in Figure 4 indicate that the recoveries of SDZ, SMR, and SMZ increase when the solvent volume increases from 6 mL to 10 mL. The recoveries of SDZ, SMR, and SMZ are 94.8%, 90.0%, and 96.8%, respectively, when the volume of the elution solvent is 10 mL. When the volume of the elution solvent increased from 10 mL to 14 mL, no significant increase of recoveries was observed. So in the experiment, the selected volume of elution solvent was 10 mL.

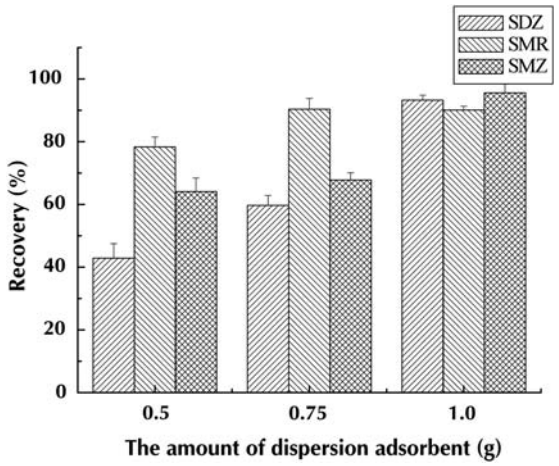

Figure 2. The effect of the amount of the dispersion adsorbent on the recoveries.

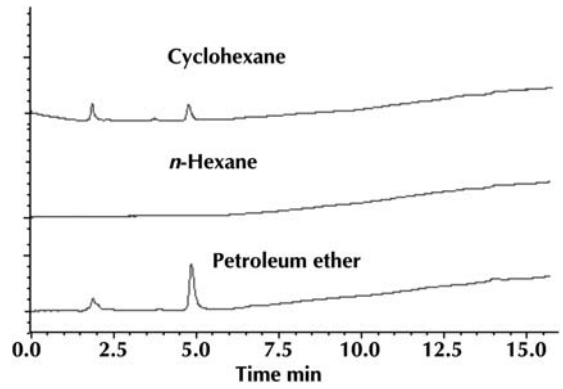

Figure 3. The effect of the washing solvents.

HPLC performances

Sulfonamides have absorption in the UV spectrum because of the benzene ring in the structure of sulfonamides. Many research studies indicated that the maximum absorption wavelengths of the sulfonamides were between 250–280 nm (11,

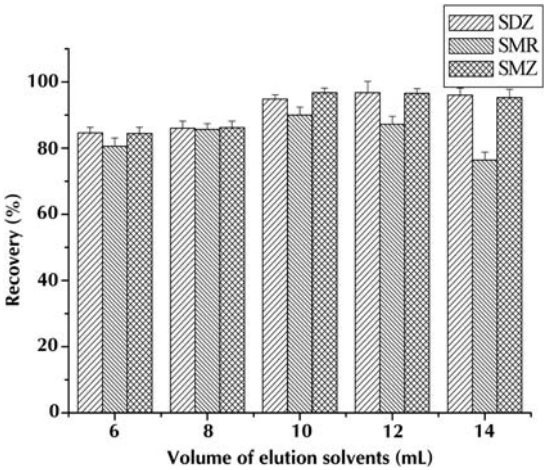

Figure 4. The effect of the volume of the elution solvent on the recoveries.

Table II
The Limit of Detection and the Linear Range

Analyte	Regression equation	Correlation Coefficient	Concentration range (μg/mL)	LOD (μg/mL)
SDZ	A = −844.8 + 94795.2C	0.99992	0.0050–2.50	0.0014
SMR	A = −529.4 + 92494.1C	0.99996	0.0050–2.50	0.0015
SMZ	A = −712.4 + 88418.2C	0.99996	0.0050–2.50	0.0021

Table III
The Repeatability of the Assays

Analyte	Concentration (μg/mL)	Repeatability RSD (%) ($n = 3$)
Sulfadiazine (SDZ)	0.01	0.76
	0.25	2.66
	1.0	0.50
Sulfamerazine (SMR)	0.01	2.17
	0.25	1.04
	1.0	0.26
Sulfamethazine (SMZ)	0.01	2.12
	0.25	1.45
	1.0	0.87

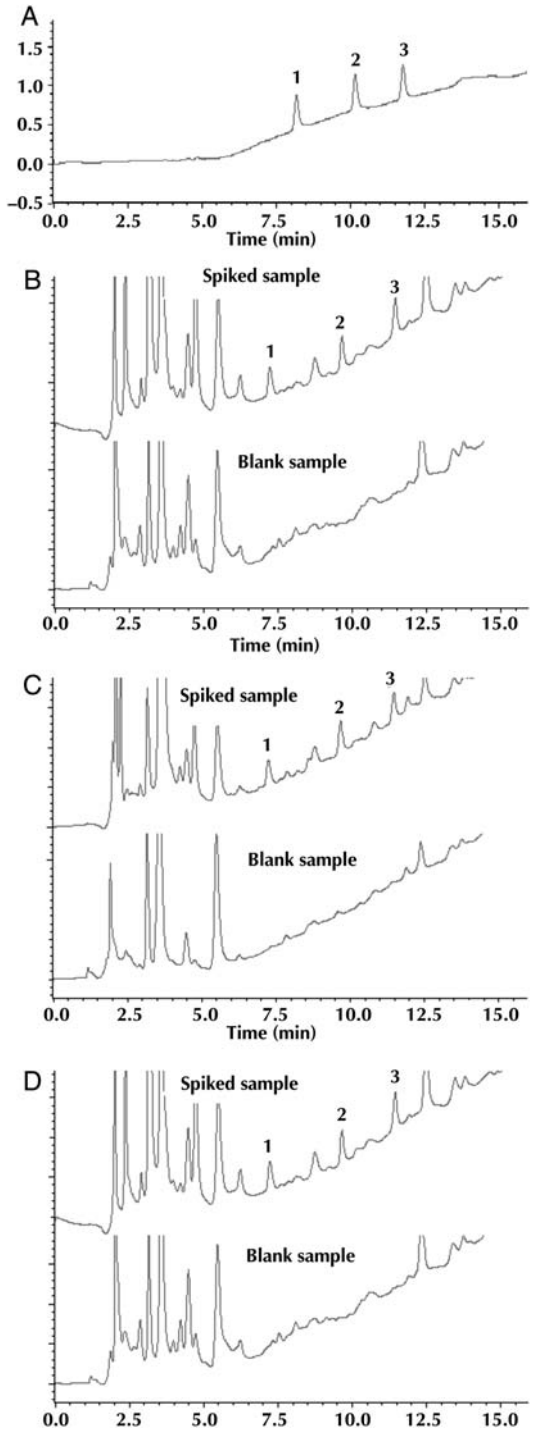

Figure 5. The chromatograms of the standard solution (A), porcine blood (B), human blood (C), and white mouse blood (D). The concentration of the analytes in the standard solution and the spiked samples was 0.05 μg/mL: (1) SDZ, (2) SMR, and (3) SMZ.

38, 39). In the work, 269 nm was selected as the detection wavelength for the analytes.

To construct the calibration curves, standard solutions at concentration levels ranging from 0.0050 to 2.5 μg/mL were analyzed. The relationships between the analyte concentration (C) and the peak area (A) are described in the regression equations, which are listed in Table II. The concentration ranges for determining the analytes are also presented in Table II. Based on preparation of the samples mentioned earlier, 1.0 mL of the sample solution was obtained with 0.25 mL of blood and used for HPLC analysis. Therefore, the concentration range for determining the sulfonamides in practical blood samples should be from 0.020 to 10.0 μg/mL. The limit of quantification (LOQ) is lower than that (0.1 μg/mL) obtained by liquid–liquid extraction (32).

Table IV
Analytical Results of SDZ, SMR, and SMZ in Spiked Samples

Sample	Spiked level (μg/mL)	Sulfadiazine (SDZ)		Sulfamerazine (SMR)		Sulfamethazine (SMZ)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Porcine blood	0.5	94.8	1.56	90.0	1.17	96.8	2.70
	0.05	87.3	2.37	88.5	2.06	105.4	1.63
	0.02	83.2	3.82	102.1	2.26	106.8	10.8
Human blood	0.5	82.3	1.11	92.6	2.61	85.4	0.64
	0.05	81.3	2.72	93.4	1.53	83.4	4.82
	0.02	82.3	1.45	93.7	1.61	84.9	1.56
White mouse blood	0.5	95.3	1.10	92.6	3.50	95.1	1.24
	0.05	91.4	1.99	74.0	2.00	98.3	1.09
	0.02	90.0	1.33	87.7	2.91	93.4	2.98

The limits of detection (LODs) were obtained by following equation: LOD = 3SB/m, where SB represents the standard deviation of the blank signal; m represents the slope of the calibration curve. The obtained LOD values for the three sulfonamides are listed in Table II. The relative standard deviations (RSD) are obtained by analyzing the working solutions. The experimental results are listed in Table III. The RSD values for the three sulfonamides were between 0.26% and 2.66%.

Sample analysis

The accuracy and precision of the proposed method were evaluated by analyzing the samples. The chromatograms for the blank and spiked samples are shown in Figure 5. There is much baseline drift observed in the chromatograms. The drift is due to the quality of methanol, because the baseline drift of the chromatogram of the standard solution is so obvious. The experimental results indicate that the baseline drift in the chromatogram is not be related to the sample preparation. The analytical results of spiked samples are listed in Table IV. From Table IV, it is seen that the proposed method provides good recoveries and reasonable precision for sulfonamides at three concentration levels. The recoveries of the three sulfonamides were between 81.3% and 105.4% with the RSDs between 0.64% and 4.82%. These results indicate that the proposed method is suitable for the extraction of sulfonamides from blood samples.

Conclusion

The obtained results indicate that the proposed MSPD–HPLC method can be applied for the simultaneous determination of SDZ, SMR, and SMZ in the blood samples. The porcine, human, and white rat blood samples were analyzed to evaluate the method. The recoveries of three sulfonamides were

satisfactory. Because blood is liquid, it was convenient to mix the dispersion adsorbent and the sample. Both the diatomaceous earth and acetone are inexpensive and commercially available. These results show that the proposed method is reliable, simple, and inexpensive for extraction of sulfonamides.

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