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Journal of Chemical and Pharmaceutical Research, 2014, 6(5):54-58

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Polymer monolithic rods microextraction coupled with high performance liquid chromatography for the analysis of trimethoprim, sulfadiazine and sulfamethoxazole in honey samples

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ABSTRACT

Novel polymer monolithic rods were prepared by in situ polymerization in a glass capillary and then coupled with high-performance liquid chromatography (HPLC) for the analysis of trimethoprim, sulfadiazine, and sulfamethoxazole. The rods showed homogeneity, uniform surface and micropores, good solvent-resistant ability, and excellent adsorptive ability onto antibacterial synergist compounds and sulfonamides. The extraction conditions were optimized, including extraction solvent, desorption solvent, extraction time, desorption time, and shaking frequency. A method of determining trimethoprim, sulfadiazine, and sulfamethoxazole through polymer monolithic rods microextraction coupled with HPLC was developed. The linear range was 20 μg/L to 200 μg/L, and the detection limits ranged within 10.6 μg/L to 15.5 μg/L. The proposed method was successfully applied to trimethoprim, sulfadiazine, and sulfamethoxazole analysis in spiked honey samples.

Key words: Polymer monolithic rods microextraction; sample preparation; sulfonamides; trimethoprim.

INTRODUCTION

Monolithic materials, which have the advantages of easy preparation, high stability, fast mass transfer, and easy modification, were first proposed by Hjerten et al. [1]. These materials have been used as enzyme reactors [2], high-performance liquid chromatography (HPLC) stationary phases [3], capillary electrochromatography stationary phases [4, 5], ion-chromatography capillary anion exchange columns [6, 7], and sample enrichment adsorbents [8]. Polymer monoliths are usually obtained by in situ polymerization in a particular reaction vessel, such as test tubes, capillaries, and stainless steel columns. High molecular polymers [9] and molecularly imprinted polymers [10] are common forms.

Sulfonamides are attracting increased attention because of their residues in food products and their potential carcinogenicity [11]. Sulfonamides are usually used to treat of bacterial infections in animal husbandry and aquaculture. The residue of sulfonamides is inevitable. Trimethoprim is a kind of antibacterial synergist often used in conjunction with sulfa drugs [12] to substantially increase their antibacterial effect [13]. These two compounds are usually simultaneously determined in milk [14], meat [15-17], and environmental [18, 19] samples.

In this paper, novel polymer monolithic rods were prepared by co-polymerization in a glass capillary and then used as microextraction monolithic material for analyzing sulfadiazine, sulfamethoxazole, and trimethoprim. The polymer monolith was characterized by electron microscopy and solvent-resistance tests. The microextraction conditions and extraction performance were studied. HPLC was also used for the simultaneous analysis of trace trimethoprim, sulfadiazine, and sulfamethoxazole in honey samples.

EXPERIMENTAL SECTION

Sulfadiazine was purchased from Alfa Aesar (Lancaster, UK). Sulfamethoxazole was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Trimethoprim was purchased from Hubei Hengshuo Biochemical Co. Ltd. (Wuhan, China). Acetonitrile and methanol (HPLC grade) were obtained from LAB-SCAN (Bangkok, Thailand). Water used for HPLC was doubly distilled and filtered through a 0.45 μm nylon filter. Other chemicals were analytical pure. Glass capillary (1 mm and 0.3 mm diameter, 100 mm length) was obtained from West China University of Medical Sciences Instrument Plant.

Preparation of polymer monolith

The capillary was cleaned with distilled water and dried at 120 °C. One end of the capillary was flame sintered. Exactly 0.17 mL of methacrylic acid was dissolved in 10 mL of acetonitrile in a conical flask. Then, 5.75 mL of trimethylolpropane trimethacrylate and 120 mg of azoisobutyronitrile were added. The mixture solution was degassed in an ultrasonic bath for 5 min. The solution was then transferred into the sintered capillary using a syringe. The other end of the capillary was capped with polytetrafluoroethylene film. The capillary was then vertically submerged into 60 °C water, and polymerization was performed. The capillary was pulled out 2.5 h later. The polymer monolith was pushed out with a 0.3 mm-diameter capillary. The polymer was transferred into a test tube and deoxygenized in a nitrogen stream for 5 min. Then, the test tube was capped, submerged into 60 °C water for 24 h, and dried at 120 °C for 3 h in a nitrogen atmosphere. Finally, the polymer was intercepted at 0.5 cm length. The 0.5 cm polymer monolith was eluted with methanol-acetic acid (9:1, v/v) to remove the unreacted compounds and impurities until it could not be monitored by HPLC.

Characterization of polymer monolith

Morphological evaluation of the polymer monolith was performed by scanning electron microscopy with a QUANTA scanning electron microscope (FEI, USA). Solvent-resistance ability was also examined by immersing the polymer monolith in different solvents.

Extraction procedure

Extraction experiment was performed in a 100 mL conical flask. Four polymer monolith rods were used in each extraction. The extraction solution volume was 30 mL, and the shaking frequency was 150 rpm. After extraction, the rods were taken out, inserted into a 2 mL glass bottle, and desorbed with 1.5 mL of desorption solution by ultrasonication. Then, 20 μL of desorbed liquid was injected for HPLC analysis.

Chromatographic conditions

A Dionex-3000 HPLC (Dionex, USA) equipped with a DAD detector and a C18 column (250 mm × 4.6 mm i.d., 5 μm packing, J & K Scientific LTD) was used for separation and detection. All compounds were determined at 270 nm. The mobile phase was acetonitrile/0.01% (v/v) phosphoric acid solution (20:80, v/v) at the flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

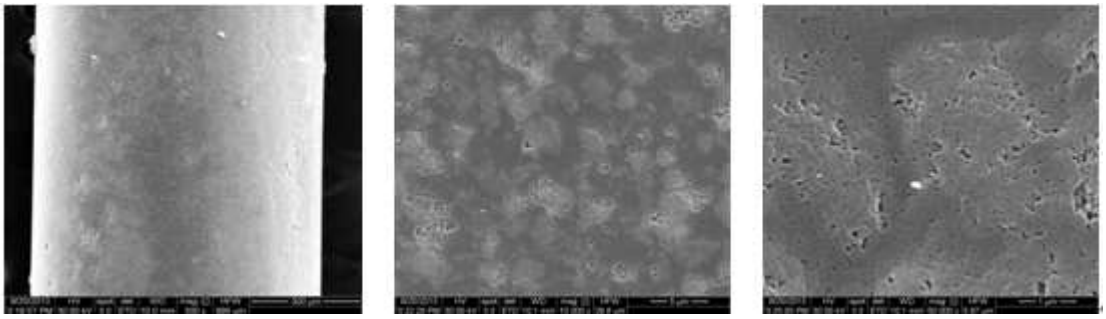
Characterization of polymer monolith

The polymer monolithic rods were investigated with a scanning electron microscope. The surface structures of the rods under 300×, 10 000×, and 50 000× magnification are shown in Fig. 1. The rods with a uniform surface were obtained using a capillary as a mold. Micropores were generated on the surface with a porogen during polymeration. These pores were beneficial to the adsorption of analytes. The solvent-resistance ability of the rods was also investigated. Methanol, acetonitrile, water, acetone, benzene, chloroform, toluene, and methanol-acetic acid (9:1, v/v) were used for the solvent-resistance study. After immersing for 1 h in each solvent, the rods remained intact without any damage. Therefore, the rods showed good solvent-resistance ability and were suitable for adsorption and desorption in the abovementioned solvents.

Optimization of polymer monolith microextraction conditions

The optimum extraction solvent was investigated by selecting a series of test solvents (Fig. 2). The concentration of the three analytes in each solvent was 50 μg/L. Better extraction effects for the polymer monolith were achieved in water and hexane possibly due to the polarity of analytes. The partition coefficient of the analytes was higher in polar organic solvents but lower in weakly polar organic solvents and water. When the extraction solvent was either water or hexane, the analytes were easily adsorbed by the polymer monolith. The highest adsorption amounts were obtained in hexane. Thus, hexane was selected as the extraction solvent. The effect of desorption solvent on the polymer monolith was also investigated. Methanol, acetonitrile, methanol–acetic acid (9:1, v/v), and acetonitrile–acetic acid (9:1, v/v) were selected for the study. Results showed that the best desorption effects were

achieved in methanol for trimethoprim, in acetonitrile-acetic acid (9:1, v/v) for sulfadiazine, and in acetonitrile for sulfamethoxazole. Taking into account the simultaneous analysis of the three analytes, methanol–acetic acid (9:1, v/v) was selected as a desorption solvent, and the vast majority of the adsorbates were desorbed. A good desorption effect was also achieved in the polar solvents methanol and acetonitrile but with poor extraction efficiency. The extraction time and desorption time were also optimized. The extraction time was varied from 30 min to 240 min. Extraction equilibrium was reached at 180 min (Fig. 3). Desorption time was also studied from 2 min to 20 min, and desorption was performed in an ultrasonic bath. Desorption equilibrium reached 10 min (Fig. 4). Therefore, 180 and 10 min were selected as extraction time and desorption time, respectively. The shaking frequency was also studied. Increasing the shaking frequency can enhance the extraction amount. When the shaking frequency was 150 rpm, the extraction amounts were very close to the extraction equilibrium (Fig. 5). Meanwhile, the extraction solution splashed at a much higher shaking frequency. Therefore, 150 rpm was selected in subsequent experiments.



(a) **(b)** **(c)**

Fig. 1 Scanning electron micrographs of polymer monolithic rod. (a) 300×, (b) 10000×, (c) 50000×

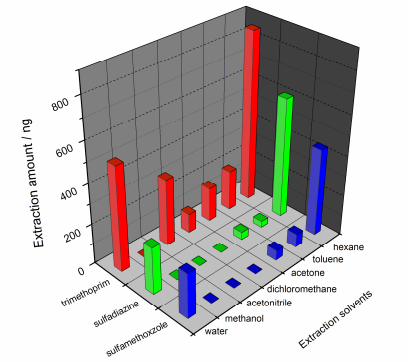


Fig. 2 Extraction amounts of trimethoprim, sulfadiazine and sulfamethoxazole by polymer monolith in different extraction solvents

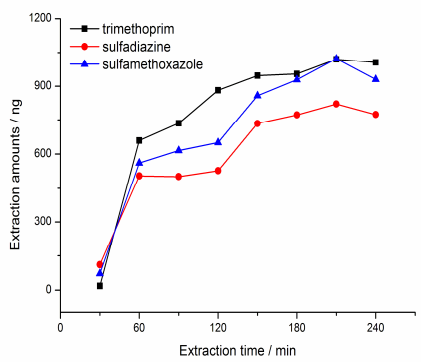


Fig. 3 Extraction amounts of trimethoprim, sulfadiazine and sulfamethoxazole by polymer monolith in different extraction time

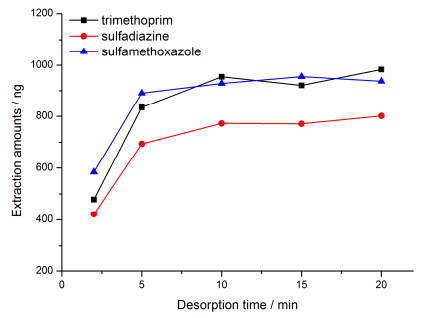


Fig. 4 Extraction amounts of trimethoprim, sulfadiazine and sulfamethoxazole by polymer monolith in different desorption time

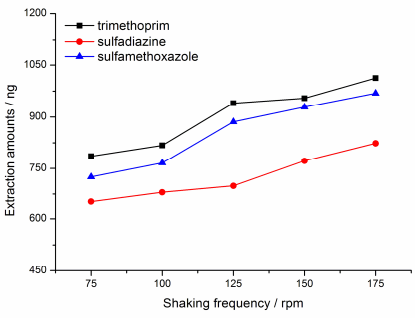


Fig. 5 Extraction amounts of trimethoprim, sulfadiazine and sulfamethoxazole by polymer monolith under different shaking frequency

Extraction performance of polymer monolith

The extraction capabilities of the polymer monolithic rods were investigated with trimethoprim, sulfadiazine, and sulfamethoxazole mixed standard solutions in hexane at 1–1000 μg/L. As shown in Fig. 6, the extraction yield increased with increased concentration from 1 μg/L to 600 μg/L. Extraction capability tended to reach equilibrium beyond 600 μg/L. Fig. 6 shows a good linear relationship between extraction amount and analyte concentration from

1 µg/L to 200 µg/L. The rods can be used for the simultaneous analysis of trimethoprim, sulfadiazine, and sulfamethoxazole. The rods were also used for the extraction of diaveridine, sulfathiazole, and sulfachloropyridazine (Fig. 7). The concentration of extraction solution was 50 µg/L. Results showed that the rods had good adsorption ability onto antibacterial synergist compounds and sulfonamides. The extraction performance of different groups of polymer monolithic rods was also evaluated. Five groups of monolithic rods were selected to extract 50 µg/L mixed standard solutions of trimethoprim, sulfadiazine, and sulfamethoxazole. The relative standard deviations (RSDs) of the extraction amounts of the three analytes were 4.5%, 7.5%, and 4.7%, respectively. Results showed that the rods synthesized by in situ polymerization had good precision.

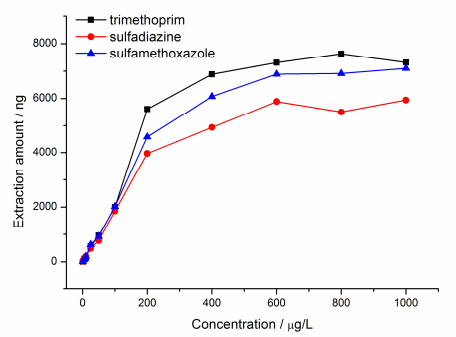


Fig. 6 Extraction amounts of polymer monolith for trimethoprim, sulfadiazine and sulfamethoxazole in different concentration

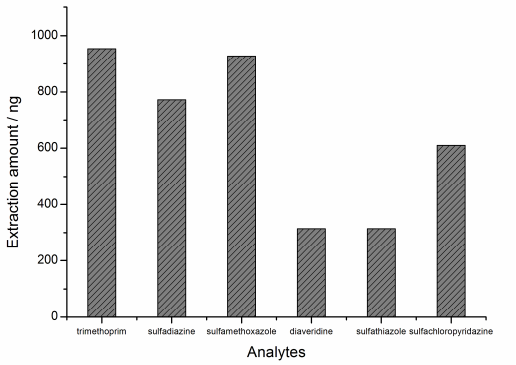


Fig. 7 Extraction amounts of polymer monolith for antibacterial synergist compounds and sulfonamides

Application of polymer monolith microextraction coupled with HPLC

A method of analyzing trimethoprim, sulfadiazine, and sulfamethoxazole by polymer monolithic rod microextraction coupled with HPLC was developed. Table 1 shows that good linearities were achieved within the range of 20–200 µg/L. The detection limits for the three compounds varied from 10.6 µg/L to 15.5 µg/L.

Table 1 The linear range and detection limit (DL) of polymer monolith coupled with HPLC for the detection of trimethoprim, sulfadiazine and sulfamethoxazole

Compound	Linearity			DL[a] (µg/L)
	Range (µg/L)	Equation	r	
Trimethoprim	20-200	$Y = -1.15\times10^{-2} + 7.67\times10^{-4} X$	0.9990	15.5
Sulfadiazine	20-200	$Y = -7.14\times10^{-2} + 7.06\times10^{-3} X$	0.9946	10.6
Sulfamethoxazole	20-200	$Y = -6.41\times10^{-2} + 5.65\times10^{-3} X$	0.9916	12.3

[a] *Detection limits were estimated on the basis of 3:1 signal to noise ratios.*

The spiked honey samples set at two levels (50 and 100 µg/L) were analyzed by the developed method, and the adsorption solution was analyzed by HPLC. Fig. 8(a) to 8(c) shows the chromatograms of the 100 µg/L mixed standard solution, the honey solution sample extracted with the rods coupled with HPLC, and the 100 µg/L spiked honey solution sample extracted with the rods coupled with HPLC, respectively. Results showed that the rods enriched the three analytes from the extraction solution of honey samples. The recoveries of the three compounds were 55.5% to 121.0% (Table 2). These results indicated that this method can be used to extract trace antibacterial synergist compounds and sulfonamides from complex samples.

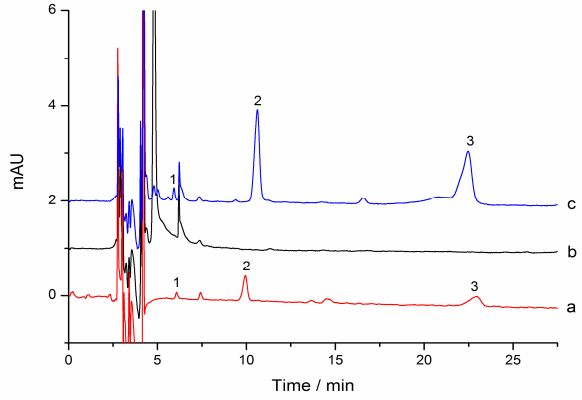


Fig. 8 Chromatograms of honey samples. (a) 100 µg/L mixed standard solution, (b) polymer monolithic rod extraction of honey sample, (c) polymer monolithic rod extraction of 100 µg/L spiked sample. 1: trimethoprim, 2: sulfadiazine, 3: sulfamethoxazole

Table 2 Recoveries of trimethoprim, sulfadiazine and sulfamethoxazole for spiked honey samples (n=4)

Compounds	Honey samples			
	50 (μg/L)		100 (μg/L)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Trimethoprim	55.5	3.5	59.9	4.4
Sulfadiazine	98.3	6.0	100.8	4.0
Sulfamethoxazole	114.1	6.9	121.0	2.3

CONCLUSION

Novel polymer monolithic rods were prepared by situ polymerization in a glass capillary and coupled with HPLC for the analysis of trimethoprim, sulfadiazine, and sulfamethoxazole. The rods showed a uniform surface with micropores, good solvent-resistance ability, and excellent adsorptive ability onto antibacterial synergist compounds and sulfonamides. The rods were successfully used for the simultaneous analysis of trimethoprim, sulfadiazine, and sulfamethoxazole in spiked honey samples, with satisfactory recoveries. These results indicated that the rods can be used for the selective enrichment of trace antibacterial synergist compounds and sulfonamides in complex samples.

Acknowledgements
Authors would like to thank the National Natural Science Foundation of China for financially supporting this research under grant number 21205054 and thank Yunnan Provincial Natural Science Foundation of China under grant number 2011FZ043.

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