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RESEARCH PAPER

Fast Determination of Residual Sulfonamides in Milk by In-Tube Solid-Phase Microextraction Coupled with Capillary Electrophoresis-Laser Induced Fluorescence

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Abstract: A fast, feasible, eco-friendly method for determination of sulfonamides using in-tube solid-phase microextraction (IT-SPME) coupled with capillary electrophoresis-laser induced fluorescence was developed. The graphene-embedded porous polymer monolithic column prepared via in-situ UV polymerization was proposed as the IT-SPME column. The method was applied to determine residual sulfonamides in a real milk sample, and an amount of 3.7 ng mL^{-1} sulfamethazine was detected, which was much lower than the maximum residue limit.

Key Words: In-tube solid-phase microextraction; Capillary electrophoresis-laser induced fluorescence; Sulfonamides

1 Introduction

The issue of antibiotic residues in food is of great public health concern[1–3]. Sulfonamides (SAs), as one of the oldest classes of antibiotics, have been widely used as bacteriostatic agents due to their function in preventing the growth of bacteria, treating the microbial infections and promoting rapid growth for poultry and other animals[4–6]. Previous reports showed that SAs could be detected in animal-original foods such as milk[7,8], honey[9], meat[10], etc. Food containing SAs residues can cause certain harm to human body, and produce a series of side effects. To ensure food safety, maximum residue limits (MRL) have been established for SAs. China has issued a MRL of 100 ng mL^{-1} for total SAs in animal-original foods and a MRL of 25 ng mL^{-1} for sulfamethazine[11]. Therefore, developing simple, rapid, and sensitive methods to analyze and detect SAs is still a crucial issue for evaluating food safety.

Extraction is a widely used sample preparation technique, such as liquid-liquid extraction[12,13], solid phase extraction (SPE)[14,15] and matrix solid-phase dispersion extraction[16,17], etc. SPE is a common mean for extracting SAs. However, the conventional SPE is time-consuming and reagent-wasting. It not only increases the experiment cost and sample consumption, but also causes the pollution to the environment. Relative to the SPE, solid phase microextraction (SPME) is simpler and has lower cost. SPME has been proposed at the end of last century and developed so far[18,19]. SPME can reduce the consumption of sample and solvent, and shorten the time for extraction. Monolithic column in tube solid phase microextraction (IT-SPME) is one class of SPME[20,21], and it has excellent extraction capability due to the features of bicontinuous structure and double pore distribution. Its good permeability can lead to fast elution as well, which is helpful to achieve high-throughput analysis. Furthermore, the large surface area of extraction can not only reduce the dosage of organic solvents, but also improve the miniaturization and automation of device[22]. Moreover, monolithic column IT-SPME can realize on-line analysis combined with other analysis instruments.

In this work, an IT-SPME method coupled with capillary electrophoresis-laser induced fluorescence detection (IT-CE-

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LIF) was developed to enrich, separate, and detect SAs to realize a simple, rapid, sensitive, and environmentally friendly analysis of residual SAs in milk.

2 Experimental

2.1 Reagents and materials

Sodium acetate anhydrous, acetic acid, and macrogol 6000 were purchased from Fuchen Chemical Reagent Factory (Tianjin, China). Sodium phosphate was obtained from Beijing Chemical Works (Beijing, China). Fluorescamine was purchased from Alfa Aesar China (Tianjin) Chemicals Co. Ltd. (Tianjin, China). Ethylene glycol dimethacrylate, *n*-butyl methacrylate, *N,N*-dimethylformamide, 2,2-dimethoxy-2-phenylacetophenone, and 3-(trimethoxysilyl)propyl methacrylate (*γ*-MAPS) were purchased from J&K Scientific Ltd. (Beijing, China). Reduced graphene oxide was purchased from Graphene Supermarket (Reading, MA, USA). The fused silica capillary was provided by Polymicro Technologies (Phoenix, AZ, USA).

Sulfadiazine (SDZ), sulfamerazine (SMR) and sulfamethazine (SMZ) of analytical grade were purchased from Alfa Aesar China (Tianjin) Chemicals Co. Ltd. (Tianjin, China). Individual stock solutions (10.00 mg mL^{-1}) were prepared by dissolving appropriate amounts of the three SAs in 10 mL of anhydrous methanol and stored at 4 °C. A series of diluted solutions were prepared daily by dilution of the standard solutions with anhydrous methanol.

2.2 Apparatus

Capillary electrophoresis experiments were carried out with a laboratory-built system including a high voltage power supply (DW-P303-1ACFO, Dongwen High Voltage, China) and a home-made laser-induced fluorescence (LIF) detector. Separations were performed in bare fused silica capillaries with 75 μm I.D. and the total length of 28.0 cm (23.0 cm effective length).

LIF detector was built in a confocal optical configuration. A 405-nm beam from a 405-nm laser diode (Sanyo) was reflected by a dichroic mirror (455 nm, Omega Optical) and focused onto the capillary through a microscope objective lens (40 ×) bought from Mike photoelectric instrument Co. (Chongqing, China). The emitted fluorescence from the capillary was collimated by the same objective lens, passed through the dichroic mirror, a long-pass filter (515 nm, Omega Optical), and reflected by a mirror to a 200-μm pinhole. Then the fluorescence was collected by a photomultiplier tube (H10722-01 Hamamatsu, Japan). The detector output was captured by a DAQCard-6062E data acquisition card (National Instruments, USB-6361, USA) and analyzed with an in-laboratory program written in Lab view.

The morphology of IT-SPME monolithic capillary was examined by scanning electron microscopy (SEM) (S-4300, HITACHI, Japan).

2.3 Preparation of poly-BERIT-SPME monolithic columns

The IT-SPME monolithic columns were prepared using deep-UV transparent capillaries with 30.0 cm length and 100 μm I.D. via a UV-initiated polymerization method. The capillaries were pre-treated before polymerization according to a previous report[23], which allowed the inner wall of capillaries to vinylize with 3-(trimethoxysilyl) propyl methacrylate (*γ*-MAPS). The preparation of IT-SPME monolithic columns were followed the procedure reported in the literatures[24,25] with minor modifications. Briefly, apre-polymerization solution containing 19.88% (*w/w*) ethylene glycol dimethacrylate (EDMA), 29.82% (*w/w*) *n*-butyl methacrylate (BMA) (EDMA and BMA were filtered through an Al_2O_3 column), 9.94% (*w/w*) PEG-6000, 39.76% (*w/w*) *N,N*-dimethylformamide (DMF), 0.50% (*w/w*) 2,2-dimethoxy-2-phenylacetophenone (DMPA), and 0.10% (*w/w*) reduced graphene oxide (RGO) was mixed ultrasonically into a homogeneous solution and purged with nitrogen for 5 min. Then the solution was loaded into the pre-treated capillary. After both ends of the capillary were sealed with two silicon rubber septa, the capillary was placed in a UV chamber and exposed to 365 nm ultraviolet light for 25 min. Following polymerization, the ends of the capillary were cut off to obtain the desired length and the capillary was flushed with methanol at 2000 psi for 20 min using an HPLC pump (Model 510, Waters, USA) to remove unreacted monomers and porogens. Then poly (BMA-EDMA-RGO) (poly-BER) capillaries were obtained, and ready for use as IT-SPME column.

2.4 Sample preparation

The milk samples were purchased from local supermarket and stored at 4 °C. Three milliliters of milk was added into 27 mL of phosphate buffer solution (0.02 M, pH 5.0) to precipitate protein and extract the analytes. After being vortexed for 5 min, the mixture was centrifuged at 8000 rpm for 20 min at room temperature. Then the supernatant was collected and passed through a 0.22-μm nylon filter membrane. Finally, 2.0 mL of supernatant was taken into a brown bottle for derivatization.

2.5 Derivatization procedure

Fluorescamine solution (3 mg mL^{-1} in acetone) was mixed with sulfonamide working solutions or sample extracts in acetate buffer according to the molar ratio of 1:10. The mixture was allowed to derivatize completely for 30 min at 30 °C.

In this work, 5.0 μL of sodium acetate solution (pH 3.5) and 1.0 μL of fluorescamine solution (3.0 mg L^{-1}, acetone) were