Journal of Chromatography B, 957 (2014) 53–59

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of sulfadiazine in Jiaozhou Bay using molecularly imprinted solid-phase extraction followed by high-performance liquid chromatography with a diode-array detector

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ARTICLE INFO

Article history:
Received 5 October 2013
Accepted 8 February 2014
Available online 12 March 2014

Keywords:
Sulfadiazine
Molecularly imprinted polymer
Solid-phase extraction
Seawater
Jiaozhou Bay

ABSTRACT

A highly selective sample cleanup procedure featuring molecularly imprinted solid-phase extraction (MISPE) was developed for the isolation and determination of sulfadiazine (SDZ) in seawater samples from Jiaozhou Bay, China. The molecularly imprinted polymer (MIP) was prepared using SDZ as the template molecule and methacrylic acid as the functional monomer. The MIP was used as a selective sorbent for the solid-phase extraction of SDZ. An off-line MISPE method followed by high-performance liquid chromatography (HPLC) with diode-array detection was established for the analysis of SDZ. Good linearity of the MISPE column for SDZ standard solutions was obtained within 0–200 $\mu g\,L^{-1}$ ($R^2 > 0.99$). The recoveries of spiked seawater samples were satisfactory as high as 88%. Finally, seven samples in Jiaozhou Bay were determined and there was no sulfadiazine found except #2 and #5 sample. The concentrations were respectively 0.33 $\mu g\,L^{-1}$ and 0.28 $\mu g\,L^{-1}$, and the relative standard deviations were 1.35% and 4.13% ($n = 3$).

1. Introduction

Aquaculture production has significantly increased because of intensive farming. Different antibiotics are used to treat infections caused by various bacterial pathogens of fish [1–3]. Sulfadiazine (SDZ), a sulfonamide, is a main antibacterial agent used for fish disease treatment [4,5]. Sulfonamides show relatively long half-lives and potential carcinogenic effects; thus, they are a critical concern in aquatic environments [6,7]. As such, developing reliable analytical methods to facilitate the rapid, sensitive, and selective determination of SDZ from seawater is an urgent necessity.

Several techniques, such as fluorescence probes, capillary zone electrophoresis, liquid chromatography with UV, enzyme-linked immunosorbent assay, and tandem mass spectrometry, have been developed for SDZ determination [8–12]. These techniques are accurate, precise, and robust; however, some of them require extraction, clean-up, and enrichment, all of which are time- and solvent-consuming during actual operation. Solid-phase extraction (SPE) uses both a solid phase and a liquid phase to isolate an analyte or a type of analyte from a solution. SPE is usually used to clean up chromatographic or other analytical samples before analyte(s) quantification [13–15]. A disadvantage of classical SPE sorbents (e.g., C8 and C18), however, is their low selectivity. Thus, several new sorbents, such as molecularly imprinted polymers (MIPs) and immunosorbents, have been developed to meet the SPE requirement of selectivity [16,17]. Coupling of MIPs with SPE can combine the advantages of both molecular recognition and traditional separation methods. Thus, molecularly imprinted SPE (MISPE) may achieve higher enrichment and clean-up efficiencies than traditional SPE cartridges because of the coupling of the high specificity, selectivity, and sensitivity of MIP and the high resolving power of SPE [18–20].

Most methods for SDZ determination are used to analyze food or feed stuffs [21,22], biological fluids [23], and environmental samples, including wastewater, ground water, and surface water [24–26]. Few methods have been used for determining SDZ from seawater samples. As the seawater matrix is more complicated than the freshwater matrix, SDZ analysis of seawater samples may also be expected to be more difficult than the analysis of freshwater samples. To the best of our knowledge, no method for SDZ detection in seawater samples based on the MIPSE procedure has yet been developed.

Jiaozhou Bay is a typical semi-enclosed bay situated in the southern part of Shandong Peninsula (35°58′–36°18′N,

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http://dx.doi.org/10.1016/j.jchromb.2014.02.053
1570-0232/

120°04′–120°23′E). Given rapid progresses in industrial and aquaculture development, Jiaozhou Bay is significantly affected by anthropogenic activities and its water quality has deteriorated over the last two decades. Many studies have been conducted on the pollution status of the bay, mainly focusing on nutrients, heavy metals, alkylphenols, and benzene hydrocarbons [27–31]. There has been little report on SDZ in Jiaozhou Bay. Our work thus represents the first attempt to separate and determine SDZ in Jiaozhou Bay using MISPE technology.

In this study, an SDZ-imprinted polymer was prepared through the bulk polymerization method using SDZ as the template molecule, methacrylic acid (MAA) as the functional monomer, and ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent. The obtained MIP showed high affinity toward SDZ and was successfully applied as a special SPE sorbent for selective extraction of SDZ from seawater samples in Jiaozhou Bay. The aim of the present work was to design a simple and effective method, dedicated to selectively recognize and determinate SDZ in seawater samples based on MISPE. SDZ in Jiaozhou Bay was successfully detected using the developed method.

2. Materials and methods

2.1. Materials

SDZ was purchased from Aladdin Reagent Company (Shanghai, China). MAA and 2,2-azoisobutyronitrile (AIBN) were obtained from Kermel Chemical Company (Tianjin, China). EGDMA was obtained from Alfa. Prior to their use, MAA and EGDMA were purified to remove the polymerization inhibitor and AIBN was recrystallized. HPLC-grade acetonitrile and methanol from Merck were used. Unless otherwise specified, all of the reagents used were of analytical grade. All of the water used in the experiments was obtained from a Millipore Milli-Q purification system (Millipore, Bedford, MA, USA). A 1 g L^{-1} SDZ (Aladdin Reagent Company, Shanghai) stock solution was prepared in methanol and stored in the dark at 4 °C.

2.2. HPLC conditions

HPLC analysis was performed on a Hitachi L-2000 series HPLC system containing an L-2130 binary pump, an L-2200 autosampler, an L-2300 column compartment, and an L-2455 diode-array detector to monitor the effluent at 260 nm for SDZ. The analytical column was a 250 mm × 4.6 mm, 5 μm, LaChrom C18 column (Hitachi, Japan), and the column thermostat was set to 30 °C. The mobile phase was acetonitrile–water (25:75, v/v), and the flow rate was set to 1.0 mL/min.

2.3. Preparation of MIP and non-imprinted polymer (NIP)

MIP was prepared using bulk polymerization by dissolving 1 mmol of SDZ, 6 mmol of the functional monomer MAA, and 20 mmol of the cross-linker EGDMA in 15 mL of chloroform in a 50 mL borosilicate glass bottle with a rubber cap. This mixture was rotated at 150 rpm for 6 h at 25 °C to form an imprinting molecule and monomer complex. After addition of 40 mg of AIBN, the solution was saturated with dry nitrogen for 15 min, and the bottle was placed in a water bath (thermostat) at 60 °C for 24 h. After polymerization, the polymer was ground with a mortar and pestle, sieved through 140-mesh and 200-mesh screens to obtain particles with sizes between 75 and 106 μm, and then repeatedly suspended in acetone to remove small particles. The product was extracted with methanol using a Soxhlet apparatus for 48 h. During template removal, the SDZ in the extraction solvents was determined using a UV–vis spectrophotometer at 260 nm until no template molecule was further detected. The product was then dried under vacuum at room temperature.

The corresponding NIP was prepared and washed using the same steps but without the SDZ template.

2.4. Morphological observations

The surface morphology of the particles was determined using a Hitachi S-4800 cold field-emission scanning electron microscope (SEM, Tokyo, Japan). The polymers were prepared by wetting a slide glass with a small drop of the diluted particle dispersion. Before SEM observation, the dried specimen was coated with a thin layer of gold under vacuum conditions. We also evaluated the physical properties of the prepared polymers using BET N_2 adsorption–desorption analysis (Best Instrument, Beijing, China).

2.5. Steady-state binding studies

Bulk polymer particles (20 mg) were weighed and placed in a 4 mL glass bottle with a cap. Approximately 2 mL of SDZ (known concentration) in methanol–water (10:90, v/v) solution was mixed with this polymer. The mixture was slightly shaken using a horizontal shaker for 24 h at 25 °C and then centrifuged for 5 min at 4000 rpm. Final SDZ concentrations were determined using HPLC with a diode-array detector at 260 nm. The amount of SDZ bound to the polymer was calculated by subtracting the amount of free SDZ from the initial amount added to the mixture. SDZ standard solutions in methanol–water (10:90, v/v) were prepared, and a standard calibration curve for SDZ analysis was constructed. The curve was linear (R^2 = 0.998) within the range of 0–100 mg L^{-1}.

2.6. Preparation of MISPE column

MISPE column was prepared by packing the dry MIP (50 mg) in a 1 mL glass syringe (2 cm × 0.9 cm i.d.). The syringe tube was thoroughly cleaned, dried, and attached to two sieve plates at the bottom and top ends. To validate the performance of the MISPE, the corresponding NIP was also packed individually into cartridges to compare their SDZ extraction efficiencies.

2.7. SPE for SDZ standard solution

Before analyte loading, the MISPE column was pre-conditioned sequentially with 2 mL of deionized water and methanol. After passing the SDZ standard solutions in methanol–water (10:90, v/v) at different concentrations through the columns at a flow rate of 0.25 mL/min, the columns were washed with 1 mL of methanol–water (30:70, v/v) at the same flow rate. The analyte retained on the sorbent was eluted with 1 mL of methanol for further HPLC analysis.

2.8. SPE for spiked seawater samples from Jiaozhou Bay

Seven seawater samples were collected from Jiaozhou Bay in Qingdao, China in June 2012 (Fig. 1). Approximately 150 mL of raw seawater was collected at 2 m seawater layer from the water surface in a small boat. The seawater samples obtained were immediately filtered though precombusted (450 °C, 3 h) Whatman GF/F glass-fiber membranes. 30 mL of filtrated seawater samples was decanted into precombusted glass bottles and stored at −20 °C in the dark until further analysis. Seawater samples (4 mL) were spiked with SDZ at concentrations of 0, 5, 10, 15, and 20 μg L^{-1}. Three replicate spiked seawater samples were passed through the MISPE (or NISPE) column, and the column was washed and eluted as described above. The elution fractions were then collected for