Journal of Chromatography A, xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Supported liquid membrane-protected molecularly imprinted beads for micro-solid phase extraction of sulfonamides in environmental waters

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ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 9 April 2014
Accepted 11 April 2014
Available online xxx

Keywords:
Sulfonamides
Aqueous samples
Micro-solid phase extraction
Supported liquid membrane
Hollow fiber
Imprinted polymers

ABSTRACT

In this work, molecularly imprinted polymer (MIP) beads have been prepared and evaluated for the development of a supported liquid membrane-protected micro-solid phase extraction method for the analysis of sulfonamides (SAs) in aqueous samples. The performance of MIP beads was firstly evaluated in cartridges by conventional solid-phase extraction for the simultaneous analysis of SAs. Afterward, beads were packed into a polypropylene hollow fiber protected by an organic solvent immobilized in the pores of the capillary wall. During the process, the analytes were extracted from the aqueous sample to the immobilized organic solvent and then selectively retained by the MIP beads located inside the capillary. The effect of various experimental parameters as sample pH, time and stirring-rate among others, were studied for the establishment of optimum rebinding conditions. Relative recoveries for all sulfonamides tested in river and reservoir water samples by the proposed method using 100 mL water sample spiked with 50 μg L-1 of each sulfonamide were within 70–120%, with a relative standard deviation (RSD) <10% ($n = 3$). The detection limits (LODs) were within 0.2–3 μg L^{-1}, depending upon the sulfonamide and the type of water used.

1. Introduction

Nowadays, a large variety of synthetic organic compounds are used in enormous quantities for a range of purposes and thus, there is a growing concern of their occurrence in the environment. Particularly important is the presence and eventual toxicity of the so-called "emerging organic contaminants" (EOCs) both in the terrestrial and aquatic environment. Besides, despite of their wide occurrence in the environment they are not commonly monitored due to the lack of regulation [1]. EOCs include a wide array of different compounds including pharmaceuticals and personal care products (PPCPs), pesticides, veterinary products, industrial compounds/by-products, food additives as well as engineered nano-materials [2].

In particularsulfonamides (SAs), antibacterial agents derived from sulfanilamide (*p*-aminobenzenesulfonamide), are used in human medicine but mainly in animal husbandry and intensive aquaculture production [3]. Several studies have already established that these compounds enter the environment by means of wastewater effluents from municipal treatments plants, hospital effluents and livestock activities among others [2], making necessary the development of sensitive and selective analytical methods for SAs determination in environmental samples.

Surface water samples are complex matrices rich in organic matter and thus sample preparation becomes a key step that includes the isolation of the analytes from interfering compounds and the preconcentration up to adequate levels for measurement.

Traditionally, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been two of the most widespread extraction techniques in laboratories. However, last decade has led to the development of new micro-extraction techniques such as solid-phase microextraction (SPME) [4], where the analytes are retained in a micro-extraction phase and then desorbed as concentrated extracts for their analytical determination, and liquid-phase microextraction (LPME), where a water immiscible organic sorbent is immobilized as a thin supported liquid membrane (SLM) in the pores of a porous membrane. The subsequent use of a porous polypropylene hollow fiber (HF) as supporting membrane lead to a new process called HF-LPME [5–7].

In parallel, molecularly imprinted polymers (MIPs) have been proposed as selective sorbents for the extraction and clean-up of target analytes from bio-, environmental and food samples.

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http://dx.doi.org/10.1016/j.chroma.2014.04.038
0021-9673/

MIPs are synthetic polymers typically obtained by copolymerizing a monomer with a cross-linker in the presence of a template molecule. The monomers are chosen considering their ability to interact with the functional groups of the template molecule. After polymerization, the template is removed leaving complementary cavities and thus MIPs are able to selectively rebind the template (and related compounds) being separated from matrix-interfering compounds [8–10].

The combination of MIPs and HF-LPME was recently proposed for the selective extraction of triazines in sludge water, watermelon, milk and urine samples [11], and TBZ in citrus-juice samples [12], reaching LODs low enough to permit the satisfactory analysis of target analytes in real samples. In both publications, a molecularly imprinted fiber was protected by a porous polypropylene capillary impregnated with a water-immiscible organic phase. Thus, the complete extraction procedure involves two simultaneous processes: HF-LPME of the analyte from the aqueous sample to an organic acceptor solution through a SLM; and SPME of the analyte from the organic acceptor solution to a MIP-fiber inside the polypropylene hollow capillary. Finally, after extraction is complete, MIP-fiber is taken off the polypropylene hollow capillary for washing and elution of target analytes. It is important to stress that such approach overcomes in a simple manner the problems of selective recognition of MIPs in aqueous samples. However, it is important to point out that this procedure is not only tedious and time consuming but also a bit risky since the fibers are rather fragile and it is necessary to handle them cautiously in order to prevent their breakage.

In the present work, a new approach for the combination of MIPs and HF-LPME is proposed. The device consists of few milligrams of imprinted beads packed into the lumen of a porous polypropylene capillary in order to protect and separate them from aqueous media. Then, a water immiscible organic solvent is immobilized within the pores of the membrane and the target analytes are extracted from aqueous samples to an organic solvent where MIPs perform selective recognition. Finally, the analytes can be easily eluted from polypropylene capillary and further analyzed by chromatographic techniques. Molecularly imprinted beads have been prepared for the determination of SAs in surface water samples using sulfadimethoxine as template molecule.

2. Experimental

2.1. Reagents

Sulfadimethoxine (SDM), sulfadiazine (SDZ), sulfapyridine (SP), sulfamerazine (SM), sulfamethazine (SMZ), sulfamonomethoxine (SMM), sulfachloropyridazine (SCP) and sulfamethoxazole (SMX) were purchased from Sigma-Aldrich and the chemical structures are shown in Fig. 1. Stock standard solutions (1 g L^{-1}) were prepared in acetonitrile and stored at −22 °C. Methacrylic acid (MAA), divinylbenzene-80 (DVB-80) and 2, 2′-azo-bis-isobutyronitrile (AIBN) were purchased from Sigma-Aldrich. HPLC-grade toluene, acetonitrile (ACN), methanol (MeOH) and sodium chloride were purchased from Scharlab (Barcelona, Spain). MAA and DVB-80 were freed from stabilizers by distillation under reduced pressure and by passing through a short column packed with neutral alumina (Aldrich), respectively. AIBN was recrystallized from MeOH prior to use. All other chemicals were used as received. Water was obtained from a Milli-Q purification system (Molsheim, France). Formic acid, acetic acid and sodium hydroxide were purchased from Panreac (Barcelona, Spain). The Q3/2 polypropylene hollow fiber used to support the organic phase was purchased from Membrana (Wuppertal, Germany) with an internal diameter of 600 μm, 200 μm of wall thickness and 0.2 μm pores.

2.2. Polymer preparation

The polymers were prepared by precipitation polymerization in a fashion similar to the general procedure described by Wang et al. [13]. SDM (0.09 mmol), MAA (0.36 mmol), DVB-80 (3.60 mmol), and AIBN (0.33 mmol) were dissolved in 12.5 mL of a 25:75 (v/v) toluene:acetonitrile mixture in a 20 mL glass tube. The tube was sealed by means of a screw-cap and introduced into a temperature controllable incubator equipped with a low-profile roller (Barloworld Scientific, Staffordshire, UK); the latter allowed for the slow rotation (24 rpm) of the tube about its long axis over the course of the polymerization. The temperature was ramped from room temperature to 60 °C over 2 h and then maintained at 60 °C for a further 24 h. At the end of this period, the reaction mixture had the appearance of a milky suspension. The polymer particles were separated from the polymerization mixture by vacuum filtration trough a nylon membrane filter. The template was removed with 150 mL of 1:1 v/v methanol: acetic acid mixture and finally washed with methanol (100 mL). A non-imprinted polymer (NIP) was prepared in the same manner than MIP but without the addition of template. Finally, obtained polymers were dried at room temperature and stored in amber glass vials at ambient temperature.

Scanning electron micrographs of both MIP and NIP were imaged at Centro de Microscopía Electrónica “Luis Bru” (Universidad Complutense de Madrid) using a JEOL JM-6400 (Peabody, MA). Particle size distributions were measured using ImageJ software [14].

2.3. Rebinding study

Prior to packaging the synthesized MIP beads inside the capillaries, their ability to recognize SAs was evaluated by molecularly imprinted solid-phase extraction (MISPE). With this purpose, about 100 mg of polymer were placed in an empty 6 mL glass extraction cartridge. Before the first use, polymers were conditioned with 50 mL of ACN and 50 mL of toluene. Once the polymer was conditioned, 1 mL of a mixture of SAs in toluene was loaded onto the polymer and washed with 1 mL of a 90/10 (v/v) toluene:ACN mixture. Then, the polymer was dried under vacuum and analytes eluted with 1 mL of ACN. An aliquot of 200 μL of the obtained extracts was evaporated to dryness under a gentle nitrogen stream and reconstituted in 200 μL of 25/75 (v/v) ACN:water adjusted to pH 2.5 with formic acid. Finally, the extracts were analyzed by HPLC as indicated below (Section 2.7). Between extractions, 5 mL of a 90/10 (v/v) methanol:acetic acid mixture was percolated through the cartridge, followed by 5 mL of methanol, 5 mL of ACN and 5 mL of toluene.

2.4. Preparation of polypropylene capillaries packed with MIP beads

MIP beads were introduced into the lumen of the porous polypropylene capillary in the form of slurry. About 100 mg of MIP beads were homogenized in 1 mL of acetonitrile for 5 min by an ultrasonic water bath (290 W, 50/60 Hz, S 40 H Elmasonic) supplied by Elma (Singem, Germany). Then, with the help of a 1 mL medical syringe, the slurry was introduced into the lumen of a 5 cm polypropylene capillary with the lower end sealed by mechanical pressure with the help of a pliers. The excess of solvent leaked trough the pores of the membrane leaving the MIP beads inside the lumen. After filling, the other capillary end was also closed by mechanical pressure.

Please cite this article in press as: M. Díaz-Álvarez, et al., J. Chromatogr. A (2014), http://dx.doi.org/10.1016/j.chroma.2014.04.038