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# Preparation and application of sulfaguanidine-imprinted polymer on solid-phase extraction of pharmaceuticals from water

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ABSTRACT

The molecularly imprinted polymers (MIPs) with sulfaguanidine as a template, methacrylic acid, 4-vinylpyridine, and 2-hydroxyethyl methacrylate as functional monomers, ethylene glycol dimethacrylate as a cross-linker and 2,2′-azobis-isobutyronitrile as an initiator have been prepared through the cross-link reaction of polymerization. Solid-phase extraction (SPE) procedure for the extraction of sulfaguanidine from water samples using the prepared MIPs and non-imprinted (NIPs) was evaluated. The best MIP in combination with commercial sorbents was applied for simultaneous extraction of eight pharmaceuticals. New SPE cartridges were prepared by combination of optimal produced MIP and Oasis HLB in 6 mL of polypropilene SPE reservoir.

The developed method which includes new SPE cartridge ($MIP_{MAA}$-Oasis HLB, 400 mg/6 mL) and thin-layer chromatography was validated. The method provides a linear response over the concentration range of 0.5–150 μg/L, depending on the pharmaceutical with the correlation coefficients > 0.9843 in all cases except for norfloxacin (0.9770) and penicillin G procaine (0.9801). Also, the method has revealed low limits of detection (0.25–20 μg/L), good precision (intra and inter-day), a relative standard deviation below 15% and recoveries above 95% for all eight pharmaceuticals. The developed method by using newly prepared SPE cartridge has been successfully applied to the analysis of production wastewater samples from pharmaceutical industry.



## 1. Introduction

In recent years, there has been growing concern about the occurrence of pharmaceuticals in the aquatic system [1,2]. As analytical techniques become more sensitive and more widely deployed, an increasing number of drugs are being detected. Several investigations have gathered evidence that substances of pharmaceutical origin are often not eliminated during the wastewater treatment and also not biodegraded in the environment [3]. Their incomplete removal by the wastewater treatment plants (WWTPs) is referred to as the major source of their release into the environment [4–6].

Consequently, a very sensitive research method is required for the study of the presence of pharmaceutical residues in the environmental samples, particularly wastewater. Simultaneous analysis of several groups of compounds with quite different physico-chemical characteristics generally requires a compromise in the selection of experimental conditions, which in some cases, means failing to obtain the best performance for each compounds. However, developing a multi-group method is rewarding as it can be applied in routine analysis, providing a large amount of data [7]. Several chromatographic methods have been developed for the pharmaceutical analysis in the environmental samples [7–13].

Solid-phase extraction (SPE) is routinely used in many laboratories for preconcentration and/or clean-up steps in the analysis of complex samples due to the advantages of simplicity, rapidness and little consumption of organic solvents. Despite to the popularity of SPE, new solid sorbents have appeared as an alternative to the conventional sorbents with the aim of achieving a more selective preconcentration of target analytes. Thus, immunosorbents and molecularly imprinted polymers (MIPs) appear as excellent candidates to accomplish this requirement [14].

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Molecularly imprinted polymers (MIPs) are an artificially produced macromolecular material, which has prearrangement of structure and specific molecular recognition ability [15] as a result of MIPs synthesis in the presence of target molecules named templates. After the template is removed from the resulting polymer matrix, binding sites having the size and shape complimentary to the template are generated [16].

Except template the components involved in the production of a MIP are functional monomer, cross-linking agent and initiator of radical polymerization. To obtain the highest affinity of the MIP towards the target analyte, careful selection of the monomer composition used in the production of the MIP is crucial. Choosing the right functional monomer is very important because this will determine, on one hand, the stability of the complex formed before and during the polymerization process and, on the other hand, the subsequent ability of the MIP to interact selectively with the target molecule [17]. In the last few years MIPs have been increasingly exploited as selective sorbents in molecularly imprinted solid-phase extraction (MISPE). To date, the main application of MIPs has been the extraction of analytes from biological samples [18–23] and environmental samples [1,2,16,24–31]. Biofluids such as plasma, serum and urine have been the most analysed biomatrices [18–21,23] with only few studies performed to the extraction of compounds from tissue samples [22]. Water is also a "leader" in environmental samples [1,2,16,24,25,27–31] unlike the soil [25,26] and sediments which also present unexplored area of MIPs application [32]. For example, in papers differently prepared MIPs for different purpose and for different type of analytes; sulfonamides (SAs) and methacrylic acid (MAA) for quantification of SAs in aquaculture product [33], organophosphorus pesticides (OPPs) and MAA for use in MIP-SPE sample enrichment of selected OPPs in fruit sample [34], acidic pharmaceutical and EDGMA for the selective extraction of a group of structurally related compounds such as acidic pharmaceuticals [35], methandrostenolone (MA) and chitosan for extraction of MA [36], and emodin with MAA and multiwall carbon nanotubes for purification and enrichment of emodin from kiwi fruit root [37] could be found. The reason for such a big growth of the interest in preparation of different MIPs is in the fact that they can be synthesized easily with high yields and low cost.

Sulfonamides are among the most widely used antimicrobials in human and veterinary medicine, and several SPE methods have been developed for their extraction from water and clean-up from biological matter [38–42]. Although sulfonamides are often analysed a group of antibiotics in the environment, most reported MIPs of SAs were prepared using sulfamethazine [14,43] or sulfamethoxazole [1,43,44] as the template molecule. Sulfaguanidine (SGUA) belongs to a sulfonamide group of antibiotic which are widely used in medicine, animal husbandry and veterinary practice. But many authors [39,40] when developing methods for sulfonamides do not include sulfaguanidine in it. Other authors [13,42], during the optimization procedure dropped sulfaguanidine from further study due to recoveries less than 10%. Reason for that probably lies in its physico-chemical properties. Namely, the structures in sulfaguanidine contain a strong basic group ($pK_a$ 12.1) and are positively charged at most conditions, which make SPE extraction difficult by HLB cartridges [13].

In this paper, sulfaguanidine was used as template molecule based on previously published literatures [13,41,42,45] where it is evident that sulfaguanidine makes a problem during extraction procedure from water. According to our knowledge, preparation of MIP with SGUA template has not been published yet. Sulfaguanidine-imprinted polymers were synthesized using three different kinds of functional monomers (methacrylic acid (MAA), 4-vinylpyridine (4-VPy), 2-hydroxyethyl methacrylate (HEMA)) in a polar organic solvent acetonitrile. The recognition abilities of the synthesized polymers were studied. The goal of this work was not only to develop and apply the prepared MIPs to selective analysis of SGUA and its analogues from water sample but to apply the best MIP in the combination with the commercial sorbents for simultaneous analysis of different pharmaceutical classes from the water samples. According to that, new SPE cartridges were prepared in order to achieve the best extraction recovery for eight investigated pharmaceuticals, not only for SGUA. Investigated pharmaceuticals belong to the antibiotic group.

Extraction efficiency experiments were checked by thin-layer chromatography (TLC).

After the optimal combination of sorbents was selected, the performance of the MISPE–TLC method was validated and the method was successfully applied to the determination of pharmaceutical presence in the production wastewater samples from pharmaceutical industry.

## 2. Experimental

### 2.1. Pharmaceuticals, standards and reagents

The studied pharmaceuticals are as follows: trimethoprim (TMP), oxytetracycline (OTC), enrofloxacin (ENRO), norfloxacin (NOR), sulfaguanidine (SGUA), sulfamethazine (SMETH), sulfadiazine (SDIAZ) and penicillin G/procaine (PGP). All pharmaceutical standards were of analytical grade ( $>99\%$) and obtained from Veterina Animal Health Ltd. (Kalinovica, Croatia). The chemical structures of the pharmaceuticals included in this study are shown in Fig. 1.

A stock standard solution of the eight pharmaceutical compounds was prepared by dissolving accurately weighed amounts of powdered standards in methanol. The mass concentrations of pharmaceutical compounds in the mixture were 5 mg/L for NOR, 10 mg/L for ENRO, and 100 mg/L for SMETH, SDIAZ, TMP, OTC, PGP, and SGUA. The standard working solution (2.5 mg/L for NOR, 5 mg/L for ENRO, and 50 mg/L for the others) was obtained by dilution of the stock standard solution with methanol.

Standard solutions of three (SGUA, SDIAZ, SMETH) and four pharmaceuticals (SGUA, SDIAZ, SMETH, TMP) were prepared in the same way but concentrations of all pharmaceuticals in mentioned solutions were 50 mg/L. Concentration of SGUA in standard solution of sulfaguanidine was 50 mg/L also.

All mentioned standard solutions were stored protected from light at 4 °C and all used solvents were HPLC-grade supplied by Kemika (Zagreb, Croatia).

Chemicals for the polymer syntheses were methacrylic acid (MAA), 4-vinylpyridine (4-VPy), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EDGMA) all of them from Sigma-Aldrich. The initiator 2,2′-azobis-isobutyronitrile (AIBN) was supplied by AKZO Chemie (Delfzijl, The Netherlands), and before use was recrystallized repeatedly from cold methanol and dried in a vacuum oven.

In this work chromatographic plates HPTLC silica gel 60 $F_{254}$ $10\times20\ cm^2$ and HPTLC CN $F_{254}$ $10\times10\ cm^2$ or $10\times20\ cm^2$, purchased from Merck (Darmstadt, Germany) were used. For solid-phase extraction 200 mg Oasis HLB (Waters, Milford, Massachusetts) and Strata X (Phenomenex, Torrance, USA) cartridges were used. The polypropilene SPE empty reservoir (3 mL) and adequate 20 μm polyethylene frits (PTFE) were purchased from Agilent (Santa Clara, CA, USA).

Water sample free of antibiotics was taken from the wellspring near Borčec, north-west Croatia.