Talanta 153 (2016) 177–185

Contents lists available at ScienceDirect

Talanta

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

Comprehensive evaluation of imidazole-based polymers for the enrichment of selected non-steroidal anti-inflammatory drugs

Dieter Schemeth [a], Christoph Kappacher [a], Matthias Rainer [a,*], Ramona Thalinger [b], Günther K. Bonn [a,c]

[a] Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 80/82, A-6020 Innsbruck, Austria
[b] Institute of Physical Chemistry, University of Innsbruck, Innrain 80/82, A-6020 Innsbruck, Austria
[c] Austrian Drug Screening Institute, Innrain 66a, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
2 March 2016
Accepted 3 March 2016
Available online 4 March 2016

Keywords:
Non-steroidal anti-inflammatory drugs
Optimization
Solid phase extraction
Humane urine
UHPLC-qTOF-MS

ABSTRACT

This study reports the comparison of four manufactured imidazole-based copolymers and two commercially available hydrophilic sorbents for the solid phase extraction (SPE) of selected non-steroidal anti-inflammatory drugs (NSAID). Different hydrophilic copolymers were obtained by a suspension polymerization using a styrene-based and a methacrylate-based cross-linker and by single step modifications for enhancing the ion-exchange character. SPE protocols were optimized for both non-modified and modified sorbents and applied for the enrichment of selected NSAID using all six copolymers.

Comparison and evaluation were carried out by determining recovery rates of standard mixtures at different concentration levels ranging from 0.5 mg L^{-1} to 10 mg L^{-1} and by the enrichment of spiked human urine at two concentration levels. In order to gain insight into the complexity of the biological sample and its reduction after solid phase extraction, UHPLC–MS analysis and following database comparison was performed for the three mixed-mode strong anion-exchange sorbents. In order to prove the applicability of the modified imidazole-based polymers for the enrichment of NSAID in surface water, river water or groundwater, solid phase extraction was performed with 10 ppb of NSAID which resulted into enhanced enrichment by a hundredfold.

1. Introduction

In many European countries non-steroidal anti-inflammatory drugs (NSAID) are used in quantities of more than hundreds of tons a year with increasing tendency [1,2]. Apart from their anti-inflammatory effects they show analgesic, antipyretic and blood-thinning activities and are used in human medicine and veterinary medicine [3,4]. In general NSAID exert their effects by inhibiting the binding pocket of cyclooxygenase enzymes (COX1 and COX2) which are responsible for the formation of prostaglandins [5–7]. The bioavailability of the most common NSAID is almost 100% but small amounts of non-absorbed drugs and their metabolites are released by urinary system [4,8]. The determination of the concentration levels of pharmaceuticals and their metabolites in urine is of both clinical and toxicological interest including pharmacokinetic studies, forensic controls or assisting monitoring for therapeutic intervention [9–12].

However, due to the use of NSAID in high quantities in medical treatment, the detection of pharmaceuticals in terrestrial and aquatic systems has increased in the last few years and thus has become a significant regulatory and scientific concern worldwide [13,14]. Apart from contaminated municipal wastewater, groundwater and even coastal marine waters can be affected by active pharmaceutical compounds due to leaching from soil [15,16]. Several studies indicate that NSAID disrupt the endocrine system, induce pathogen resistance and disturb natural microbial communities when humans and microbial fauna are long-time exposed to low concentrated pharmaceuticals or to their active metabolites [17–19]. Some of the drugs can be metabolized within a few days but their degradation products can be even more toxic [20]. Furthermore, commonly drugs are continuously added to the ecosystem and lead to pseudo-persistence, especially in river water and groundwater [15,21].

Nowadays the analysis of NSAID is performed by gas chromatography [22] or liquid chromatography followed by fluorescence detection [23], UV detection [24], mass spectrometry or tandem mass spectrometry whereby the mass spectrometric detection is

Abbreviations: (NSAID), non-steroidal anti-inflammatory drugs; (P1), poly(N-vinyl imidazole/ethylene glycol dimethacrylate); (P2), poly(N-vinyl imidazole/divinylbenzene); (P3), quaternized poly(N-vinyl imidazole/ethylene glycol dimethacrylate; (P4), quaternized poly(N-vinyl imidazole/divinylbenzene); (QuEChERS), quick, easy, cheap, effective, rugged, and safe

* Corresponding author.
E-mail address: m.rainer@uibk.ac.at (M. Rainer).

http://dx.doi.org/10.1016/j.talanta.2016.03.015
0039-9140/

mostly used due to their low limits of detection [25]. However, sample preparation is still required before chromatographic analysis due to low concentrated compounds of interest and other interfering substances in clinical or environmental samples [26]. Solid phase extraction is one of the most common used tools for sample preparation either in the offline or online mode. Magiera et al. compared seventeen different commercially available sorbents (silica based and polymeric based) for selected drugs in urine including naproxen, ibuprofen and diclofenac but performed only one general protocol for all sorbents [27]. In many other studies, solid phase extraction of acidic NSAID was performed on polymeric sorbents such as Oasis HLB [24], Oasis MAX [28] or Strata X [29] which show a more polar surface compared to common styrene based resins.

Apart from the manufacturer's offered sorbents, research groups published different manufactured sorbents for the enrichment and purification of acidic pharmaceuticals in wastewater and biological samples involving molecularly imprinted polymers [30,31], polar polymeric resins [32] or anion exchange based sorbents [28]. Fontanals et al. prepared an anion exchange resin based on crosslinked polymer-supported imidazolium trifluoroacetate and described the new material by the concept of ionic liquid analogues [33]. The group showed good results for acidic pharmaceuticals comparable to well-established anionic exchange SPE sorbents such as the Oasis MAX sorbent. In a previous study, the same group prepared an imidazole based resin by suspension polymerization and demonstrated the successful enrichment of highly polar standards spiked in Milli-Q water and Ebre river water [34,35].

In this study six different polymers were evaluated for the enrichment of NSAID at different concentration levels, spiked in biological samples and in environmental-simulated samples. Therefore, vinyl imidazole was polymerized with two different cross-linkers, divinylbenzene and ethylene glycol dimethacrylate, by applying suspension polymerization. The polymerization reaction was optimized by changing the protective drop stabilizer and by the choice of an appropriate water to organic phase ratio. The obtained polymers were further derivatized by a substitution with methyl iodide in order to receive strong anion-exchange resins. The two non-modified resins were compared with the similar Oasis HLB and the modified sorbents were tested against Oasis MAX by performing an off-line solid phase extraction approach. Apart from quantitative recovery studies, UHPLC–MS analysis of a complex urine sample before and after solid phase extraction by the strong anion-exchange resins was performed to get more detailed information about the sample complexity and selectivity of the different sorbents.

2. Experimental

2.1. Reagents and materials

Azobisisobutyronitrile, divinylbenzene (80%), ethylene glycol dimethacrylate (99%), N-vinyl imidazole (99%), toluene, poly-vinylalcohol (99% hydrolyzed), (hydroxypropyl)methyl cellulose, dibutylphthalate (99%), sodium acetate (< 99%), salicylic acid (< 99%), acetylsalicylic acid (< 99%), acetone (< 99.9%), naproxen sodium salt (98.0–102.0%), trifluoroacetic acid (< 99%) and formic acid (< 99%) were purchased from Sigma Aldrich (Buchs, Switzerland). Methanol (< 99.9%) and acetonitrile (< 99.9%) were from Carl Roth (Karlsruhe, Germany) and diclofenac and ibuprofen was purchased from Merck (Darmstadt, Germany). Water was collected from a Milli-Q system. The solid phase extraction sorbents Oasis HLB and Oasis MAX (both 30 mg) were from Waters Corp. (Milford, Massachusetts). Particle sieves (25 μm and 50 μm) were from Linker Industrie-Technik GmbH (Kassel, Germany) and empty polypropylene SPE tubes (1 cm^3 and 3 cm^3) with 20 μm polyethylene frits were acquired from Sigma Aldrich (Buchs, Switzerland) and Carl Roth (Karlsruhe, Germany). Human urine was collected from a volunteer. All experiments were performed in compliance with the relevant laws and institutional guidelines.

Divinylbenzene and N-vinyl imidazole were extracted three times with 10% (v/v) NaOH solution and distilled under vacuum. Ethylene glycol dimethacrylate was purified through a basic alumina column before usage.

2.2. Instrumentation

Analysis of different solid phase extraction fractions were carried out on an Ultimate 3000 RSLC micro system (Dionex-Thermo Scientific, Dreieich, Germany) with a VWD-3400RS UV detector, using a Supelco Discovery C18 column (3 μm; 0.32 × 100 mm; Bellefonte, PA-USA). For all runs, a 45-nl flow cell was applied. The column oven temperature was set at 40 °C and signals were detected at 220 nm and 232 nm. The mobile phases were water with 0.1% TFA (phase A) and acetonitrile (phase B). Washing steps and eluted fractions of the standard mixtures were separated by a delayed gradient elution. For 1 min the system was set at 10% B and afterwards increased to 80% B within 5 min. The flow rate was set to 11 μl min^{-1}. For the determination of urine samples, the system was set at 1% B for 2 min and increased to 80% B within 12 min at a flow rate of 11 μl min^{-1}.

UHPLC–MS analysis of the spiked urine sample was performed on an Ultimate 3000 (Dionex – Thermo Scientific; Dreieich, Germany), using an Agilent Zorbax Eclipse Plus C18 column (1.8 μm; 2.1 × 100 mm; Santa Clara, CA-USA). The column oven temperature was set at 40 °C. The mobile phase A was water with 0.1% formic acid and phase B was acetonitrile. After an isocratic flow of 2% B at 0.5 ml min^{-1} for 4 min, a gradient was run from 2% B to 80% B within 16 min. Mass spectrometric detection was performed by a Maxis Impact (qTOF-MS, Bruker; Bremen, Germany) in both modes, positive and negative.

Elementary analysis was performed on a 2400 CHN Elemental Analyzer (Perkin Elmer; Waltham, USA) and BET measurements were carried out by a Nova Station A (Quantachrome Instruments; Boynton Beach, FL-USA).

2.3. Polymer synthesis

For the synthesis of poly(N-vinyl imidazole/divinylbenzene) AIBN (1% (m/m) of total monomer content) was dissolved in a mixture of 17.7 ml toluene and 2.1 ml dibutylphthalate. One half part of the organic phase was added to 6 ml of divinylbenzene and the other half was added to 4 ml N-vinyl imidazole. Both fractions were ultra-sonicated (Bandelin Sonorex; Berlin-Germany) for 5 min before purging with argon for further 5 min. In a 250 ml three-necked round bottom flask equipped with a mechanical stirrer and a reflux condenser 50 ml of a 0.5% (m/m) (hydroxypropyl) methyl cellulose and the vinyl imidazole fraction were added and flushed with argon. The mixture was stirred under moderate rates for 30 min at 80 °C. Subsequently, the divinylbenzene fraction was added and reacted for approximately 40 h. For the suspension polymerization of poly(N-vinyl imidazole/ethylene glycol dimethacrylate), divinylbenzene was exchanged by an equimolar amount of ethylene glycol dimethacrylate. After the reaction was stopped, the obtained polymers were washed excessively with methanol and acetone. For the derivatization of one gram dried polymer, 4.5 ml acetonitrile and 2 ml methyl iodide were added and the reaction was carried out under inert atmosphere for 24 h at room temperature and 2 h at 60 °C. The modified polymers were washed excessively with methanol, water and acetone and stored under inert gas.