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Determination of non-steroidal anti-inflammatory drugs in water and urine using selective molecular imprinted polymer extraction and liquid chromatography

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ABSTRACT

A selective solid-phase extraction was employed for the improvement of the determination of non-steroidal anti-inflammatory drugs (NSAIDs) in continental water and urine samples. Ketoprofen, naproxen, diclofenac, and ibuprofen were selected as target analytes due to they are the most frequently administered and consumed NSAIDs. These compounds were extracted using molecular imprinted polymers and determined by liquid chromatography with diode array (DAD), and tandem-mass spectrometry (MS–MS) detectors. Performance of DAD and MS–MS detectors was evaluated throughout this study. The obtained limits of quantification, after a 50-fold preconcentration solid-phase extraction, varied from 20 to 30 $\mu g\,L^{-1}$ for DAD, and from 0.007 to 0.017 $\mu g\,L^{-1}$ for MS–MS for both types of sample matrixes. Quantitative recoveries were found for blank-samples spiked at different NSAIDs concentration levels, ranging from 0.05 to 10 $mg\,L^{-1}$ for urine and from 0.5 to 500 $\mu g\,L^{-1}$ for water. The proposed methodology was applied for the determination of NSAID residues in urine of prescribed individuals, and continental waters.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutic agents [1], being diclofenac, ibuprofen and naproxen the most frequently administered. Taken singly or in combination with other classes of drugs, they relieve symptoms across multiple clinical indications, including short and long term pain states and a range of musculoskeletal disorders [2]. In the last years, the analysis of NSAIDs has gained an increased attention in the scientific literature, probably related to the long-term side effects of those compounds. Acute overdose or chronic abuse of NSAIDs can cause gastrointestinal [3] and cardiovascular complications [4]. So, NSAIDs residues in prescribed individuals, using urine, plasma or blood as biological matrices have been monitored [5,6]. On the other hand, the occurrence of NSAIDs in the environment has drawn significant consideration because they are not efficiently eliminated in sewage treatment plants and then, they are released into the surface water. In this scenario, it is easy to understand the need of sensitive and selective analytical procedures for the determination of NSAID residues, both, in biological and environmental samples.

The determination of NSAIDs is usually carried out by chromatographic techniques. Gas chromatography requires the use of a previous derivatization step [7] and thus, liquid chromatography (LC) is typically employed using ultraviolet (UV) [8], fluorescence (FD) [9], mass spectrometry (MS) [10] or tandem mass spectrometry (MS-MS) [11] detectors. Direct determination of NSAIDs in environmental and biological samples is problematic because of the low analyte concentration and matrix complexity. So, an adequate sample pretreatment becomes a crucial step prior to chromatography analysis. In this sense, several strategies based on the use of liquid–liquid extraction have been developed, such as: hollow-fiber liquid phase microextraction [12], ionic liquid based dynamic liquid phase microextraction [13], ionic liquid-dispersive liquid–liquid microextraction [14] and dispersive liquid–liquid microextraction based on solidification of floating organic droplets [15]. Nevertheless, solid-phase extraction (SPE) is the most used extraction and preconcentration technique for NSAIDs determination, using classical silica and polymeric materials [16], but also based on solid-phase microextraction [17] and stir bar sorptive extraction [18,19]. In the last years, the use of analyte-specific sorbents; such as molecularly imprinted polymers (MIPs) have received increased

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consideration for the selective extraction of NSAIDs from water samples, allowing highly selective retention mechanism based on molecular recognition [20–22]. In this sense, the use of MIPs for the extraction and clean-up of ibuprofen [23] and naproxen [24] from urine samples has also been proposed, being no precedent for the use of MIPs as selective SPE method for the analysis of several NSAIDs simultaneously in urine samples.

In this paper the main objective was the proposal of MIP-SPE for the specific extraction and clean-up in the simultaneous determination of NSAID traces in urine and environmental water samples, using different detectors, including UV and MS-MS. The proposed methods were validated in terms of linearity, sensitivity, selectivity, trueness and precision. Finally, real samples were analyzed in order to check the NSAIDs residue levels in prescribed individual urine and also in continental waters from rivers, lakes, irrigation ditches and sewage plants, using the most appropriate LC detector in each case.

2. Experimental

2.1. Reagents and instrumentation

LC chromatography determinations were performed in an Agilent 1100 Series (Santa Clara, CA, USA) equipped with a G1322A degasser, a G1311A quaternary pump and a G1315 diode array detector (DAD). A Kromasil 100C_{18} column (250 × 2.0 mm, 5 μm), employed for LC separations, was obtained from Scharlau (Barcelona, Spain).

Ultra-high-performance liquid chromatography (UHPLC) was made on a Waters Acquity UPLC system from Waters (Milford, MA, USA), equipped with a binary solvent delivery system, an autosampler, and a BEH C_{18} (2.1 × 50 mm, 1.7 μm) column. MS–MS acquisitions were performed in a Waters Acquity triple quadrupole MS detector, equipped with a Z-spray electrospray ionization source.

SupelMIP SPE-NSAIDs molecular imprinted polymer (25 mg, 3 mL), Supelclean ENVI-18 octadecyl silica C_{18} SPE tubes (500 mg, 3 mL), and Visiprep^{TM} SPE vacuum manifold were obtained from Supelco (Bellefonte, PA, USA). All solvents used were LC grade or higher. Methanol and acetonitrile were purchased from Scharlau. Ultrapure water used in all experiments (with a resistivity higher than 18.2 MΩ) was obtained from a Milli-Q unit (Millipore, Bedford, MA). Acetic acid and ammonium formate (96%) were purchased from Panreac (Barcelona, Spain).

Ibuprofen, ketoprofen, diclofenac sodium salt, naproxen and other pharmaceutical compounds were purchased from Sigma-Aldrich (Steinheim, Germany) and concentrated stock solutions were prepared in methanol and stored at 4 °C in amber glass vials. Formulations containing 600 mg for ibuprofen, 500 mg for naproxen and 25 mg for ketoprofen were purchased from local pharmacies.

2.2. Extraction of NSAIDs

A commercially available MIP specific for NSAIDs was used and the procedure recommended by the supplier was adapted as indicated. MIP cartridge was equilibrated with 1 mL acetonitrile, 1 mL methanol and 1 mL 10 mM ammonium formate (pH 3) in water under gravity flow. A sample volume, from 1 to 50 mL, was adjusted to pH 3 by the addition of 100 mM ammonium formate in water (ratio sample-buffer 10:1 v/v) and loaded through the MIP cartridge at an approximate speed of 2 mL min^{-1} using a SPE vacuum manifold. Cartridge was washed with 1 mL deionized water and 1 mL 40% (v/v) acetonitrile in water. Vacuum was applied through the cartridge after the washing step to remove residual moisture and dry the cartridge. Finally, NSAIDs were eluted with 1 mL methanol, filtered using 0.22 μm PTFE syringe filters, and directly analyzed by the corresponding LC-DAD and UHPLC–MS–MS systems.

A conventional octadecyl silica C_{18} solid phase was also used in a SPE procedure in order to compare the selectivity obtained by the proposed MIPs. The employed extraction protocol for C_{18} supports was based on that found in the literature [25].

2.3. HPLC-DAD determination

A 20 μL volume of sample extract/standard solution was directly injected into the system using 0.8 mL min^{-1} mobile phase flow and 20 °C column temperature. The employed mobile phase was (A) 0.1% (v/v) acetic acid in water and (B) acetonitrile. The gradient was adjusted to obtain an adequate resolution of the studied NSAID compounds, being 0 min 70% B, 12 min 95% B, and 14 min 95% B. Table 1 shows the selected absorbance conditions employed for the determination of each NSAID. The analyte peaks were identified based on their retention times at the corresponding wavelength (see Fig. 1).

2.4. UHPLC–MS–MS determination

Samples were analyzed by UHPLC–MS–MS when a high sensitive analysis was required. A 5 μL injection volume and a mobile phase flow rate of 0.4 mL min^{-1} were employed. The mobile phase consisted of (A) 0.1% (v/v) formic acid in water and (B) methanol. The gradient started at 10% B, was linearly increased to 95% in 4 min, and then maintained for 1 min. Acquisitions were performed using

Table 1
Detector parameters and analytical figures of merit for the determination NSAIDs by liquid chromatography with diode array ultraviolet (DAD) and tandem-mass spectrometry (MS-MS) detectors.

Equipment	Parameter	Ketoprofen	Naproxen	Diclofenac	Ibuprofen
LC-DAD	Retention time (min)	5.98	6.15	8.68	8.99
	λ (nm)	255	230	274	230
	Linear range (mg L^{-1})	1–50	1–50	1–50	1–50
	R^2	0.998	0.997	0.998	0.998
	iLOD (mg L^{-1}) [a]	0.4	0.3	0.4	0.4
	LOQ (μg L^{-1}) [b]	30	20	30	30
UHPLC–MS-MS	Retention time (min)	3.18	3.23	3.61	3.57
	MRM [c]	255 (ES+) → **105**, 77	231 (ES+) → **185**,170	294 (ES+) → **250**, 214	205 (ES−) → **161**, 159
	Linear range (mg L^{-1})	0.005–0.100	0.001–0.100	0.005–0.100	0.005–0.100
	R^2	0.997	0.999	0.999	0.997
	iLOD (mg L^{-1}) [a]	0.0007	0.0003	0.0007	0.0008
	LOQ (μg L^{-1}) [b]	0.015	0.007	0.017	0.016

[a] Instrumental limit of detection established for standard solutions.
[b] Limit of quantification using 50 mL for sample.
[c] Multiple-reaction-monitoring settings (quantitation ion in bold).