Talanta 143 (2015) 71–76

Contents lists available at ScienceDirect

Talanta

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

Synthesis of a molecularly imprinted polymer for the isolation of 1-hydroxypyrene in human urine

M. Serrano, M. Bartolomé, A. Gallego-Picó *, R.M. Garcinuño, J.C. Bravo, P. Fernández

Department of Analytical Sciences, Faculty of Sciences, National University of Distance Education (UNED), E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form
23 April 2015
Accepted 30 April 2015
Available online 8 May 2015

Keywords:
1-Hydroxypyrene
MISPE
HPLC-FLD
Urine

ABSTRACT

In the last decades, the assessment of polycyclic aromatic hydrocarbons' exposure has generated great interest and 1-hydroxypyrene (1-OHP) has been commonly used as a biological indicator of exposure to PAHs in many studies in environmental and occupational health. In this research, a molecularly imprinted polymer (MIP) was synthesised by precipitation polymerisation using 1-OHP as template, methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker, and acetonitrile as porogen. MIP was used as solid-phase extraction (SPE) material for sample pretreatment and isolation of 1-OHP and posterior detection in a reversed-phase HPLC-FLD. Various parameters including washing solvent, elution solvent and volume affecting the extraction efficiency of the polymer have been evaluated to achieve the isolation of 1-OHP from urine samples and to reduce non-specific interactions. Cross-selectivity was studied in the presence of other structural analogues of the 1-OHP as hydroxyphenantrene isomers.

The method was validated over a concentration range of 0.15–2.00 μg L^{-1}, $R^2 > 0.998$. Recovery values were in the range of 78–90% and RSD < 6.7%. The limits of detection and quantification were 0.05 μg L^{-1} and 0.17 μg L^{-1}, respectively.

In our knowledge, it is the first time that this methodology is applied for analysing urinary hydroxypyrene and it has been demonstrated that this specific MISPE–HPLC-FLD method offers a fast and simple alternative to determine 1-OHP in human urine samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of compounds extensively distributed in air, soil, water, food and many occupational environments. For the general population, the major routes of exposure to PAHs are from diet, smoking and indoor air [1,2]. PAHs ubiquity and carcinogenic and mutagenic potential [3] have received great concern in the last decades and have generated, from toxicological viewpoint, great interest [4]. The PAHs metabolism is complex and they are quickly biotransformed to hydroxylated metabolites (phase I), and then further are transformed to glucuronide or sulphate conjugates (phase II) [5–7]. Usually, biomonitoring of PAHs exposure is based on the measurement of urinary hydroxylated metabolites (OH-PAHs) and several biomarkers have been proposed [4,8–10]. However, 1-hydroxypyrene (1-OHP) is commonly used as a biological indicator of exposure to PAHs in many studies in environmental and occupational health [11,12]. Although some authors have questioned 1-hydroxypyrene as a reliable bioindicator of measured dietary PAH [13], in a subsequent review, the concentration profile of OH-PAHs excreted in urine, as well as correlations among different OH-PAH analytes, was evaluated and the authors concluded that the highest concentration of urinary PAH exposure was the major factor determining high urinary concentration of 1-OHP [14]. The results of this and other studies have demonstrated that 1-OHP may be now largely considered a solid biomarker of exposure to pyrene and other PAHs, as pyrene is found in most PAH mixtures [7,11,14,15].

In the last years, many analytical methods have been published for the determination of 1-OHP and other hydroxylated metabolites in human and animal samples [7] including chemiluminescent enzyme-immunoassay [16], gas chromatography coupled to mass spectrometry (GC–MS), or coupled to high-resolution mass spectrometry (CG–HRMS) [17–19], two-dimensional gas chromatography with a flame ionisation detector (GCxGC) [20], and liquid chromatography tandem mass spectrometry (LC–MS/MS) [21–23]. Synchronous fluorescence spectrometry (SFS) has also been proposed as screening method [24–27]. However liquid chromatography with fluorescence detection (LC-FLD) has been extensively applied for determination of 1-OHP in very different matrices

* Corresponding author. Tel.: +34 91 398 73 64; fax: +34 91 398 83 79.
E-mail address: agallego@ccia.uned.es (A. Gallego-Picó).

http://dx.doi.org/10.1016/j.talanta.2015.04.092
0039-9140/

[9,10,28,29], since it is a cheap and simple technique, in comparison to others. On the other hand, LC-FLD is as sensitive as other methods and can be easily employed in most routine laboratories. Nevertheless the most important challenge in the analysis of OH-PAHs and other trace contaminants is the extraction and isolation of analytes from complex matrices, thus new selective methods are required in order to achieve low concentration levels [30,31]. Accordingly, several analytical methodologies have been proposed to improve the sensitivity and specificity in the OH-PAHs determination. Solid-phase extraction (SPE) with different types of sorbents [6,10,17,23,32,33], immunoextraction [34], solid phase microextraction (SPME) [35,36], stir bar sorptive extraction (SBSE) [5,37] and liquid–liquid extraction (LLE) [20,32] have been used as sample pretreatments. In addition, fluorometric analysis on solid sorbent elements has been also studied [25] and other pretreatment systems have been proposed [38].

Recent trends in sample preparation and purification include the development of advanced adsorbent materials. Different reviews have presented the significant increase in the use of molecularly imprinted polymers (MIPs) as selective materials for solid-phase extraction and other sample treatments [39,40]. Compared with traditional sorbents, MIPs are the most effective sorbents and have been successfully applied to the isolation of analytes in foods, biological and environmental samples in the last years.

Despite this, new polymerisation strategies need to be explored to improve MIPs efficiency. Bulk polymerisation has traditionally been used for preparing MIPs. Moreover, it is necessary to crush and sieve the polymer to generate particles with an appropriate size for use. This process affects the imprinted sites and MIPs present a heterogeneous binding site distribution limiting their applicability range [39,41]. To overcome these limitations, new polymerisation strategies have been studied to obtain MIPs with proper physical characteristics (size, porosity, pore volume, and area) [41]. On the other hand, only some of these alternatives have been used for the preparation of MISPE protocols [39], such as multi-step swelling and polymerisation and precipitation polymerisation [41]. In this context, precipitation polymerisation seems to be an attractive and easy method.

To our knowledge, MIP applications in the analytical determination of PAHs are limited [42,43] and in the literature, only two works reported the application of MIP for the determination of hydroxylated metabolites in soils [44] and microalgae cultures [45].

In this study, 1-OHP molecularly imprinted polymer was synthetised by precipitation polymerisation to be used as sorbent in a MISPE procedure for urinary PAH metabolites. Once the MISPE parameters were optimised, the procedure was validated using spiked human urine samples according to real values analysed in biomonitoring assays. Recent human biomonitoring studies have provided around 0.2 $\mu g\,L^{-1}$ of 1-OHP in urine of Spanish adult population, lower than other values obtained from other European Countries [46].

2. Experimental

2.1. Chemicals and solutions

Standards of 1-hydroxypyrene (1-OHP) in crystalline solid and in acetonitrile solution (at a concentration of 10 $\mu g\,L^{-1}$), and hydroxyphenantrene (OHPHE) isomer standards (1-OHPHE, 2-OHPHE, 3-OHPHE and 4-OHPHE) at a concentration of 10 $\mu g\,L^{-1}$ were purchased from Dr. Ehrenstofer (Germany). Ethylene glycol dimethacrylate (EGDMA) and methacrylic acid (MAA) were obtained from Sigma-Aldrich (Spain). 2,2-azo(bis)-isobutyronitrile (AIBN) and dichloromethane (DCM) were obtained from Fluka (Switzerland). HPLC-grade solvents used were acetonitrile (ACN) and methanol (MeOH), these were purchased from Scharlau (Spain). Ultrapure water (18.2 $M\Omega\,cm^{-1}$ quality) was obtained using a Milli-Q water system (Millipore Ibérica, Spain) and used for all dilutions. All other reagents used were of analytical grade or better.

Crystalline solid of 1-OHP was used for the preparation of MIP and the standard solution in ACN was used for preparation of working solutions. The working solutions of 1-OHP were prepared daily in acetonitrile. In cross-selectivity experiments, OHPHE isomers were used and working solutions of them were prepared daily in acetonitrile by appropriate dilution of standard solutions purchased.

All solutions were stored in amber-coloured flasks to prevent photodegradation.

2.2. Samples

PAHs free urine samples were collected from healthy newborns and stored without preservatives at −20 °C until use. Before processing, samples were allowed to thaw at room temperature and then homogenised by gently shaking.The biological fluid was directly injected into the chromatographic system after only filtration using Whatman PPW/GMF polypropylene filters (0.45 μm).

The working standard solutions were used to spike the PAHs free urine to cover calibration range 0.15–2.00 $\mu g\,L^{-1}$.

2.3. Instrumentation and chromatographic conditions

Chromatographic analysis was performed on an Agilent 1230 Infinity HPLC coupled to a Hewlett Packard 1046A luminescence detector, operated with Chemstation for LC Agilent system. Samples were manually injected using a 20 μL injector loop.

The stationary phase was a Zorbax Eclipse PAH (150 × 4.6 mm^2, 3.5 μm) linked to a security guard cartridge Salva Eclipse PAH (12.5 × 4.6 mm^2, 5 μm) from Agilent Technologies (Spain). The chromatographic separation was carried out at constant temperature (25 °C). The mobile phase used was a mixture of methanol and water (88:12) pumped at a flow-rate of 1 $mL\,min^{-1}$ in isocratic mode. Excitation and emission wavelengths of the fluorescence detector were fixed at 242 and 388 nm, respectively. The chromatographic procedure was optimised to analyse 1-OHP and OHPHE isomers.

2.4. Synthesis of molecularly imprinted polymers

MIP was synthesised using 10 mg 1-OHP as a template, MAA (23.3 μL) as functional monomer, EGDMA (350 μL) as crosslinker, AIBN (79.7 mg) as polymerisation reaction initiator and ACN (8.95 mL) as porogen.

The polymerisation was carried out in a 35 mL glass vial, and a molar ratio of 1:6:40 (template/MAA/EGDMA) was maintained in excess of AIBN. The molar relation 1:24 between template-porogen allowed the preparation of imprinted beads. Polymerisation was carried out under high dilution conditions, in order to synthesise uniform crosslinked polymer microspheres [47]. The total solution was purged with nitrogen and sonicated during 10 min in an ultrasonic bath (Selecta Ultrasons, Spain).

The polymerisation process was thermally initiated and maintained at 60 °C for a period of 24 h into a thermostated bath with magnetic stirring at 100 rpm. Afterwards, the polymer particles were collected by centrifugation and the template and unreacted chemicals were removed with methanol in a Soxhlet extractor. A nylon mesh of 25 μm (Safer NYTAL, Switzerland) was used to hold