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On-line flow injection molecularly imprinted solid phase extraction for the preconcentration and determination of 1-hydroxypyrene in urine samples

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ABSTRACT

New analytical strategies tend to automation of sample pre-treatment and flow analysis techniques provided a number of enhanced analytical methods allowing high throughput. Flow techniques are usually faster, more robust and more flexible than their batch equivalents. In addition, flow methods use less sample and reagent amounts and reduce analytical costs and waste.

A flow injection solid-phase extraction pre-concentration system using a molecularly imprinted polymer (MIP) packed micro-column was developed for the determination of 1-hydroxypyrene in human urine with fluorescence detection. The pre-concentration of 1-hydroxypyrene on the MIP was carried out based on the specific retention of analyte by on-line introducing the sample into the micro-column system. Methanol and dichloromethane mixture was used to elute the retained analyte for fluorometric analysis. Important influencing factors were studied in detail, in batch and in flow (MISPE procedure optimisation, sample and eluent volumes, flow rate, dimensions of MIP micro-column and amounts of packing material, etc).

To the best of our knowledge, this is the first on-line flow injection molecularly imprinted solid phase extraction for the pre-concentration and determination of hydroxylate PAH metabolite in urine samples.

The optimised method was successfully applied to the determination of 1-Hydroxypyrene in spiked urine samples, with recoveries in the range of 74–85% and RSD < 4.6%. Under optimum experimental conditions, the linearity concentration range used was 10–400 μgL^{-1}, $R^2 > 0.996$. We obtained limit of detection and quantification of 3.1 μgL^{-1} and 10.5 μgL^{-1}

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are important environmental and dietary pollutants [1,2] with carcinogenic and mutagenic potential [3]. Usually, biomonitoring of PAHs exposure is based on the measurement of urinary hydroxylated metabolites (OH-PAHs) and several biomarkers have been proposed [4–7]. However,1-hydroxypyrene(1-OHP) is commonly used as a biological indicator of exposure to PAHs in environmental and occupational health studies [8–10]. In the last years, many analytical methods have been published for determination of 1-OHP and other PAH metabolites. Different chromatography methods coupled to mass spectrometry [11,12] or liquid chromatography with fluorescence detection [6,7,9,13] have been mainly used for de- termination of these compounds. However, liquid chromatography with fluorescence detection (LC-FLD) is extensively employed for determination of 1-OHP in most routine laboratories, since it is a cheap and simple technique, in comparison to others and it is as sensitive as other methods.

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 [14,15]. On the other hand, the new analytical strategies tend to automation of sample pre-treatment and flow-analysis techniques provided a number of enhanced analytical methods allowing high throughput [11,16,17].

Consequently, several pre-treatment methodologies have been proposed to improve the sensitivity and specificity in the OH-PAHs determination. Solid-phase extraction (SPE) with different types of sorbents [7,12,18–20] immunoextraction [21], solid phase microextraction (SPME) [22], stir bar sorptive extraction (SBSE) [23] and liquid–liquid extraction (LLE) [16] have been used in the developed methods. Fluorometric analysis on solid sorbent elements has been also proposed [24] and other pre-treatment systems have been studied [25].

In response to the need for automation sample pre-treatment, flow injection analysis (FIA) is unquestionable improvement of efficiency and precision from mechanisation of the whole measuring system, especially if entire procedure involves some two- phase processes of sample treatment conducted on-line [26]. FIA analysis is a simple, rapid and versatile technique that is firmly established and presents several advantages compared to similar determinations performed, with widespread application in chemical analysis. These advantages have led to an extraordinary development of FIA in the last years.

Following these trends, some automated procedures were developed for PAHs analysis [27–30] however few procedures were applied to OH-PAHs determinations. Column-switching systems coupled to HPLC were used for determining monohydroxy[a] benzopyrene isomers [31] or 1-OHP [32]. In other studies, auto- mated off-line solid phase extraction were developed for 3-hydroxybenzo[a]pyrene quantitation [13,33] and a Rapid Trace SPE system was optimised for measuring 23 urinary PAH metabolites [11].

In this context, we have developed a flow injection solid-phase extraction for the pre-concentration and determination of 1-OHP in urine samples, using a molecularly imprinted polymer (MIP) as sorbent material. Compared with traditional sorbents, MIPs are much more effective and have been successfully applied to the isolation and pre-concentration of analytes in complex samples in the last years [34]. On the other hand, flow techniques are usually faster, more robust and more flexible than their batch equivalents [16], using less sample and reagent amounts and reducing analy- tical costs and wastes.

In this work, an 1-OHP molecularly imprinted polymer and a flow injection solid-phase extraction pre-concentration system using MIP packed micro-column was developed for the determination of 1-OHP in human urine with fluorescence detection. The pre-concentration of analyte on the MIP was carried out based on the specific retention of 1-OHP by on-line introducing the sample into the micro-column system. Important influencing factors were studied in detail, in batch and in flow system (MISPE procedure optimisation, sample and eluent volumes, flow rate, dimensions of MIP micro-column and amounts of packing material, etc). This method was validated in spiked blank urine collected from healthy newborns.

2. Material and methods

2.1. Reagents and instruments

Standards of 1-hydroxypyrene (1-OHP) in crystalline solid and in acetonitrile solution (at a concentration of 10 ng μL^{-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). Ultra pure 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 solutions in ACN was used for preparation of working solutions. In cross-selectivity experiments, OHPHE isomers were used and working solutions of them were prepared in acetonitrile by appropriate dilution of standard solutions purchased. All working solutions were daily prepared and were stored in amber coloured flasks to prevent photodegradation.

Fluorescence intensity was measured using a model LS-50B luminescence spectrometer (Perkin-Elmer, Beaconsfield, UK). Instrumental parametes and processing data were controlled by FL Winlab software. The excitation and emission wavelengths were set at 242 nm and 388 nm respectively. A Perimax peristaltic pump (Spetec, Erding, Germany), a six ways injection valve (Omnifit, Cambridge, UK) with a 250 µL sampling loop, and a 100 µL quartz flow-through cell (Hellma 176.051-QS) in the sample compartment of the spectrofluorometer, were used to set-up the FIA manifold. A 1.8 cm long, 0.8 mm i.d. PTFE column filled with MIP was connected to the FI system.

An ETHOS SEL microwave oven (Milestone, Sorisole, Italy) was used to remove the template from the imprinted polymer. Instrumental parameters were controlled by MLS-easy WAVE-com- biCHEM 3.5.2.0 software.

In adsorption and batch experiments, an Agilent 1230 Infinity HPLC coupled to a Hewlett Packard 1046A luminescence detector was used.

2.2. Samples

PAH 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 FIA 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: 10–400 μg L^{-1}.

2.3. Synthesis of molecularly imprinted polymer

Synthesis of MIP was carried out following previous research [20] with minor modifications. In order to avoid potential over- pressure on the flow injection system a bulk polymerisation strategy has been performed, which allowed choosing the particle size. In this procedure 10 mg of crystalline 1-OHP were used as template, 23.3 μL of MAA as functional monomer, 350 μL of EGDMA as crosslinker, 79.7 mg of AIBN as polymerisation reaction initiator and ACN (0.622 mL) as porogen. The template, functional monomer and crosslinker ratio chosen was 1:6:40. The total solution was purged with nitrogen and sonicated during 10 min in an ultrasonic bath (Selecta Ultrasons, Spain). The polymerisation was started at room temperature and subsequently, the polymerisation mixture was transferred to a thermostated bath set at 60 °C for a period of 24 h. Afterwards the polymer obtained was maintained at 60 °C in an oven for 24 h to complete the cure of the polymer.

The MIP monolith was crushed, mechanically ground, and wet- sieved using methanol to deliver polymer particulates. In this work, the sieved selected fraction was larger than 50 mm and less than 100 mm.

Non-imprinted polymer (NIP) was prepared and processed in the same manner as the imprinted polymer but in the absence of template (1-OHP).

2.4. Template extraction

A critical phase in the MIP preparation is the extraction of the template (1-OHP) from the MIP. Usually, the template was removed from the MIP by Soxhlet extraction with methanol until 1-OHP was not detected in the washing solution by fluorescence (λex = 242 nm, λem = 388 nm). In the present work, the template underwent microwave-assisted extraction (MAE) with the aim of reducing this time and increasing extraction efficiency. Standard MAE conditions were as follows: MIP and 30 mL of methanol were placed in the extraction vessels and exposed to MAE over a 5 min heating ramp period up to 100 °C. This temperature was then maintained for 20 min. After extraction, the vessel content was transferred to a vacuum filtration system for removal of the sol- vent. This process was performed three times in order to ensure the total extraction of the template and washing solutions were kept for posterior extraction efficiency studies. Finally, the polymer particles were dried at 60 °C in a hot air oven and stored at room temperature prior to use.

Non-imprinted polymer (NIP) was also extracted in the same method.

2.5. Batch mode binding experiment

An adsorption experiment was carried out to study binding properties of MIP and NIP. In this batch experiment 20 mg of MIP or NIP were added to 2.5 mL of solution of 1-OHP with various concentrations ranging from 0.5 to 60 mg L^{-1}. The mixture was incubated for 24 h at room temperature stirring at 450 rpm. The supernatants and polymer were isolated by centrifugation and filtration (0.45 mm). The concentration of unbound 1-OHP in the supernatant was analysed by HPLC-FD [20]. All the model para- meters were evaluated by both non-linear regression and linear least-squares method using OriginPro v. 8.0 software of OiginLab Corp. (Northampton, U.K.).

2.6. Flow injection procedure

The flow injection system developed in this work is shown in Fig.1. Experiments using imprinted and non-imprinted polymer were carried using the following procedure. The procedure was started by flowing the carrier solution using a peristaltic pump, at a flow rate of 1 mL min^{-1} through the microcolumn until a stable baseline fluorescence signal was achieved. At this point, 250 µl of sample was injected into the capillary column using the injection valve, and the 1-OHP remained adsorbed to the imprinted poly- mer. Subsequently, a washing step using a mixture of ACN/water (95:5, v:v) as washing solution was carried out in order to remove potential interferents. After that, the eluent solution was flowed through the column removing the adsorbed 1-OHP. The relative intensity of fluorescence was measured continuously using selected wavelength (λex = 242 nm, λem = 388 nm) and slit width of 2.5 for excitation and emission. The resulting peak due to the pass of the 1-OHP through the flow cell was recorded and the peak height was used for quantitation.

The micro-column of the system was prepared with 11.3 mg of dried MIP packed in a 1.8 cm long, 0.8 mm i.d. PTFE with the aid of a syringe.

3. Results and discussion

3.1. MIP preparation and extraction of the template

Depending on the polymerisation method used, MIPs can have various physical configurations and the volume of porogen plays an important role in the polymer morphology. However, It has been found that the crushed bulk polymer with irregularly shaped particles, and the precipitation polymer agglomerates prepared by polymerisation have similar micro-structure [35] and similar binding site are obtained for selective extraction of analyte. In this work, MIP was synthesised with a molar ratio of 1:6:40 (template/ MAA/EGDMA). Theoretically, the imprinting template-memory mainly depends on the interaction between the template and the functional monomer and consequently, MAA was chosen as functional monomer due to its widely and recognised ability to form hydrogen bonds, and the weakly polar acetonitrile was chosen as porogen because strongly polar solvents could counteract the formation of affinity bonds for template. The selected particle size was between 50 mm and 100 mm. The particle size was chosen for the best use of the FI system. Particles < 50 mm produced an overpressure on the system, while those 100 mm led to broad fluorescence peaks.

For the optimised procedure 1 mL min^{-1} flow through a 1.8 cm long and 0.8 mm internal diameter micro-column was utilised.

Additionally, the step for removing the imprinted 1-OHP was improved using microwave-assisted extraction (MAE). The effectiveness of the extraction was evaluated by fluorometric analysis of the washing solutions containing the extracted template collected after the successive MAE extractions. Extraction results showed that the complete removal of the template from the MIP to occur during the first extraction and successive extractions are consequently unnecessary. This procedure reduces the time required for achieving efficient extraction of the template in the routine post-treatment step with imprinted polymers.

3.2. Binding properties and adsorption isotherms

Equilibrium isotherm plays an important role in the predictive modelling for analysis and design of adsorption systems. Fig. 2 shows the sorption capacity for 1-OHP by MIP and NIP. In the low concentrations of 1-OHP, the amount of analyte was not enough to saturate the specific binding cavities. Nevertheless, the 1-OHP concentration increases, leads to the occupancy of almost all the specific imprinted sites.

The equilibrium relationship is described by adsorption isotherms (Q vs. C_e). This relationships depend on the type of ad- sorption that occurs, multi-layer, chemical, physical adsorption, etc.

The adsorption capacity value (Q, mg g^{-1}) was evaluated from the difference of the initial and the measured free 1-OHP concentrations in the supernatant by Eq. (1):

$$Q = \frac{(C_0 - C_e) \times v}{w} \quad (1)$$

where C_0 and C_e are the initial and equilibrium adsorption concentrations of the analyte, respectively, v is the volume of the solution and w is the mass of polymer. The maximum adsorption capacity of the MIP particles was 2.14 mg g^{-1} that was higher than that of the NIP particles (0.85 mg g^{-1}). This corresponds to specific interactions between the template and the residual of functional monomer in the MIP. Non-specific rebinding in the NIP is due to hydrogen bonds, electrostatic interactions and van der Waals forces between the template and the residual functional groups in polymer.

For evaluation of the imprinting ability, the imprinting factor (IF) was calculated and the value was 2.5. IF is defined as the Eq. (2):

$$IF = \frac{Q_{MIP}}{Q_{NIP}} \quad (2)$$

where Q_{MIP} and Q_{NIP} are the adsorption capacity of MIP and NIP towards 1-OHP, respectively. Imprinting factor is a measure of the strength of interaction of the imprinted polymer towards the template molecule and it has been shown that there was a positive correlation of the interaction strength with the imprinting factor, meaning that imprinted polymers exhibiting good performance, in terms of high imprinting factor value, should

interact strongly with the template molecule and therefore afford greater retention in relation to the non-imprinted polymer [36].

Many isotherms model have been reported in literature to successfully calculate the binding properties of MIPs [37] but several two-parameter isotherm models are commonly used in modelling the adsorption data, such as Langmuir or Freundlich. Modelling the adsorption isotherm data is an essential way for predicting and comparing the adsorption performance. In this work, Langmuir and Freundlinch isotherm models were used to adsorption data of MIP.

The linear form of the Langmuir isotherm expression is re- presented by the Eq. (3):

$$\frac{1}{Q} = [\frac{1}{Q_{max}K_L}] \times \frac{1}{C_e} + \frac{1}{Q_{max}} \quad (3)$$

where C_e is the concentration of solution at equilibrium (mg L^{-1}), Q is the corresponding adsorption capacity (mg g^{-1}), Q_{max} (mg g^{-1}) and K_L (L mg^{-1}) are constants which are related to adsorption capacity and energy or net enthalpy of adsorption, respectively. Langmuir isotherm model is a model that describes monolayer adsorption based on the assumption that all the ad- sorption sites have equal template affinity and that adsorption at one site does not affect adsorption at an adjacent site.

The standard errors (S.E.) for each parameter were used to measure the goodness-of-fit. Apart from S.E., the correlation coefficient (R^2) was also used to determine the best-fitting isotherm to the experimental data (Table 1).

The Q_{max} and K_L values for MIP were 1.32 mg g^{-1} and 0.09 L mg^{-1}, respectively with $R^2 = 0.948$. NIP values were 0.30 mg g^{-1} and 0.48 L mg^{-1} for Q_{max} and K_L, and $R^2 = 0.799$. These clearly show that the MIP values of Q_{max} was higher that NIP, and Langmuir model fit satisfactory MIP adsorption data.

On the other hand, the Freundlich isotherm is introduced as an empirical model, where Q represents the amount adsorbed per amount of adsorbent at the equilibrium, C_e represents the equilibrium concentration, and K_F and n are parameters that depend on the adsorbate and adsorbent.

The linear form of the Freundlich isotherm expression is represented by the Eq. (4):

$$\log Q = \log K_F + \frac{1}{n} \log C_e \quad (4)$$

where K_F and n are Freundlich constants which correspond to adsorption capacity and adsorption intensity, respectively. For MIP, the obtained values were 0.100 for K_F and 1.26 for n with

$R^2 = 0.985$. The K_F and n values for NIP were 0.072 and 1.85, respectively with $R^2 = 0.876$. Freundlich isotherm model considers heterogeneous surfaces and is based on the idea that the adsorption depends on the energy of the adsorption sites. The n constant value of 1 indicates to the heterogeneous nature of the surface of the adsorbent and unfavourable adsorption. The situation $n > 1$ is most common and may be due to a distribution of surface sites or any factor that causes a decrease in adsorbent-adsorbate interaction with increasing surface density [37].

Results suggest that both Langmuir isotherm model and the Freundlich can generate satisfactory fit to the experimental MIP data. The results revealed that Freundlich model

was more applicable to estimate the affinity distributions than the Langmuir model according to the correlation coefficients (R^2). The Freundlich isotherm model is the earliest known relationship describing the non-ideal and reversible adsorption, which can be applied to multilayer adsorption, on the basis of an assumption concerning the energetic surface heterogeneity. Additionally, a Scatchard analysis of binding of 1-OHP to the MIP was carried out by Eq. (5).

$$Q/C = \frac{(Q_{max} - Q)}{K_d} = (1/K_d) \times Q + Q_{max}/K_d \quad (5)$$

where K_d and Q_{max} are the equilibrium dissociation constant and the apparent maximum number of binding sites, respectively, and C is the adsorbed concentration of 1-OHP.

The Scatchard graph (Fig. 3) obtained was not linear and suggested that the binding sites in the MIP were not uniform and two classes of binding sites with differing K_d values existed in the MIP.

3.3. Off-line solvent studies

In these preliminary studies, loading, washing and elution solvents were selected and optimised. Cartridges packed with the MIP or NIP particles were prepared following the procedure used in our previous work [20]. According to the literature, the solvent used as porogen during polymerisation was chosen as loading solvent. In this case, acetonitrile was chosen as loading solvent in order to allow rebinding of the analyte to specific sites. Ensuring complete remove of the interfering compounds non-specifically retained, different washing solvents were investigated: water, acetonitrile, and mixture methanol-acetonitrile and acetonitrile- water. The analysis of fractions collected from the washing step showed than the best results were obtained using ACN:water (95:5, v:v). In the last, the elution solvent has to be optimised according to its ability to disrupt the analyte polymer interaction. Methanol, methanol:ACN (80:20, v:v), ACN:water (80:20, v:v) and methanol:DCM (85:15, v:v) were studied as elution solvents. In the optimum conditions, the best recovery (78%) was obtained using the mixture of MeOH:DCM (85:15, v:v). The concentration of 1-OHP in this off-line experiment was analysed by HPLC-FD [20].

3.4. Optimisation of FI system variables

Subsequently, other variables influencing the FI system were optimised. Optimum solvents and solutions selected in off-line experiments were studied using the flow injection system shows in Fig. 1. We used acetonitrile as the carrier solvent and sample loading, a mixture of ACN:water (95:5, v:v) as washing solvent and a mixture of polar protic solvent (MeOH) and polar aprotic solvent (DCM) in 85:15 proportions as elution solution. Then, different concentrations of 1-OHP (10–400 mg L-1) in acetonitrile were prepared to assess loading concentration. Afterward, sample volumes of 100, 250, 400 mL and flow rates between 0.1 and 1.5 mL min-1 were tested. Values of 250 mL and 1 mL min-1 were chosen, respectively, as a compromise between sensitivity and good sample throughput. The response signal was obtained after only a short run time (around 1.5 min).

Fig. 4 shows the elution response profile for 1-OHP using MIP and NIP micro-column in the FI system, where continuous line represents the MIP system signal and the NIP system signal is represented by the dotted line. In the imprinted polymer system it can be appreciate how a first fluorescence signal was obtained corresponding to the carrier solution (baseline) (1). After the sample injection, the 1-OHP was totally retained by the MIP and an increase of the fluorescence intensity was not observed (2). Afterwards, the eluent solution was passed through the system and a rapid increase of the fluorescence signal was produced, which once achieved the maximum (3) begun to decrease. Finally, the carrier solution was one more flowed recovering the baseline and becoming the system regenerated and ready for a new injection (4). In the non-imprinted polymer system it can be observed how the 1-OHP was not retained by the NIP flowing with the carrier and producing a small increment in the fluorescence intensity returning immediately to the baseline.

The optimised procedure was applied to urine samples free of 1-OHP spiked with the working solution at different level of concentrations (10 to 400 mg L^{-1}), according to real values ana- lysed in occupationally exposed subjects (coke-oven workers) [16]. Fig. 5 shows response signals corresponding to a sample injection in the optimised FIA system without micro-column (dotted line) and sample injection in the optimised FIA system with MIP micro- column (solid line). In this last response curve it can be observed how washing step (2) removed a small amount of 1-OHP previously the elution step (3).

3.5. Cross-selectivity experiments

In order to investigate the cross-selectivity, FI system was used for determination of 1-OHP in presence of other structural analogues of 1-OHP as hydroxyphenantrene isomers. We have chosen phenanthrene metabolites because they have also received great interest in biological monitoring of PAH cause the PAH rich diet origins elevated excretion of phenanthrene metabolites in urine. Consequently, in a number of studies, phenanthrene metabolites have been utilised for biological monitoring of PAH diet [10]. In aromatic hydrocarbons generally fluorescence and the quantum efficiency usually increases as the number of rings and their degree of condensation increases. Consequently, 1-OHP presents more fluorescence intensity than OHPHE isomers in the same concentration and conditions.

Cross-selectivity experiments were assessed mixing OHPHE isomers and 1-OHP obtaining an unique signal. The fluorescence signal of hydroxyphenanthrene isomer and 1-OHP mixture was measured continuously using selected wavelength (λex = 242 nm, λem = 388 nm) for excitation and emission. The detected peaks due to the passage of the mixture through the flow cell was recorded. The analytes were retained completely into micro-column and the comparative results obtained in load, washing and elution steps, in presence of hydroxyphenantrene isomers were evaluated (Fig. 6). For the interference study, a mixture of 100 μg L^{-1} of all hydroxyphenantrene isomers and 100 μg L^{-1} of 1-OHP were prepared with purchased standards solutions. The optimised procedure described was used to analyse the mixture.

In presence of hydroxyphenantrene isomers, signal corresponding to the analytes was not detected in the load step (1). In previous studies the MIP exhibited higher specific binding for 1-OHP than for isomers tested [20]. The presence of the hydroxyl group and the smaller size of OHPHE isomers than 1-OHP, makes that they may easily permeate into the binding sites of MIP and OHPHE isomers exhibited considerable binding by the

MIP com- parable with 1-OHP binding. Consequently, all compounds were retained in the load step. In hydrodynamic conditions, OHPHE competitiveness of 1-OHP and OHPHE isomers also was clearly demonstrate in washing and elution step, and signal recorded in the washing step (2) increased to the detriment of the signal elution (3) when isomers were presents. This result confirmed the 1-OHP and OHPHE affinity by the MIP demonstrated in stationary conditions [20].

3.6. Validation of the procedure

The analytical characteristics of the proposed procedure were evaluated. The calibration curve was constructed by preparing blank urine samples spiked with known amounts of working standard solutions of 1-OHP in a range of 10–400 mg L^{-1}. The linearity of three calibration curves was studied with a model linear regression. The mean calibration equation was $Y = 149.32X + 0.72$. The results showed a good linearity with a mean squared correlation coefficient (R^2) of 0.996. The LOD and LOQ values were calculated at signal-to-noise ratios of 3 and 10 respectively, following IUPAC recommendations. The LOD obtained was 3.1 mg L^{-1} and the LOQ was 10.5 mg L^{-1}, and the precisions (RSD) were < 4.6%. The optimised method was successfully applied to the determination of 1-OHP with recoveries in the range of 74–85% for spiked urine samples providing greater specificity of MIP compared with traditional sorbents. The precision of the method was evaluated with RSD in terms of reproducibility (inter-day precision) and repeatability (intra-day precision). Percentage of recovery and precision are summarised in Table 2.

To our knowledge, flow injection solid-phase extraction pre- concentration or on-line systems applied to determination of PAHs are limited. Coordination polymers were explored as sorbent for flow injection SPE on line coupled with HPLC-UV of PAHs in water [28]. In other studies, three kinds of materials (C30 particles, monolithic silica C18 stationary phase and quartz wool coated with C30) were used as sorbing media of PAHs for the on-line enrichment of aqueous PAHs in micro-column LC [29]. On the other hand, only three works had been reported the application of MIP for hydroxylated metabolites [20,33,38] and the present work is the first flow injection molecularly imprinted solid phase ex- traction for the pre-concentration and determination of 1-OHP in urine samples. The developed procedure is simple, sensitive, selective, fast and cost-effective with short response time (1.5 min) and allows 1-OHP to be detected over a wide range (10– 400 mg L^{-1}). On the other hand, luminescence spectrometer is an equipment usually find in routine laboratories and does not require highly trained personnel. The method provides good re-coveries and offers the possibility of other coupling strategies. Although chromatographic techniques provide reliable results in the analysis of OH-PAH, their experimental procedures are time consuming and expensive. It is within this context that new ana-lytical approaches based on easy-to-use and cost-effective methodology (FIA system) that provide large sample throughput be- come extremely relevant, even if carrying out analytical procedure in FIA system is associated with existing dispersion. This system could be easily used as screening method to evaluated exposure in environmental and occupational health studies.

4. Conclusions

In this work, a molecularly imprinted polymer for 1-OHP was synthesised and a MAE protocol was optimised with the aim of reducing this time and increasing extraction efficiency. A novel flow injection solid-phase extraction pre-concentration system using this molecularly imprinted polymer packed micro-column was developed for the determination of 1-hydroxypyrene in hu- man urine with fluorescence detection. FIA system, offers several advantages in term of considerable decrease in sample volume, reagent consumption and high sample throughput, in comparison to any other techniques applied to PAH analysis and biomonitoring. The proposed procedure shows good selectivity, sensitivity and reproducibility. In addition, this procedure is cheaper than other techniques and offers the possibility of other coupling strategies. The method developed has a good recoveries and short response time (1.5 min) and allows 1-OHP to be detected over a wide range (10–400 mg L^{-1}) with no changes to the analytical procedure.

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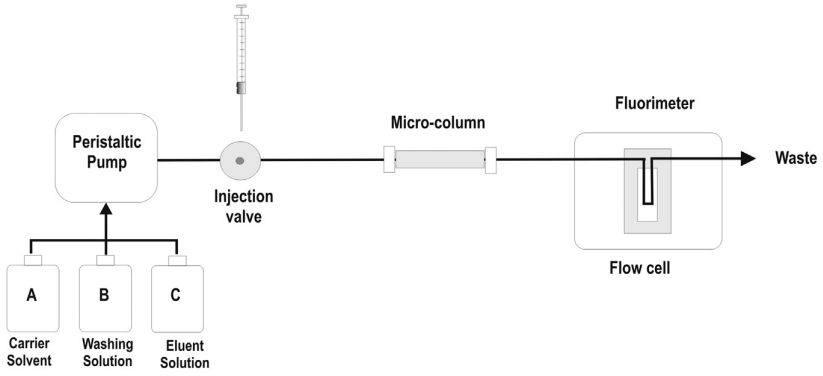

Fig. 1. Flow injection system for determining 1-OHP.

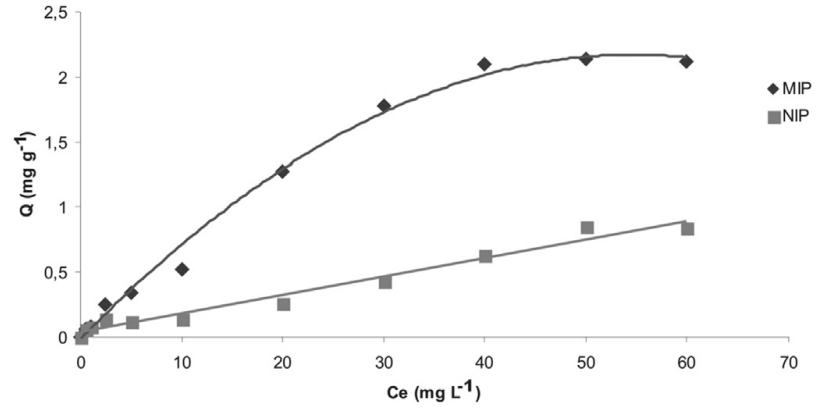

Fig. 2. Experimental adsorption data of the MIP and NIP for 1-OHP.

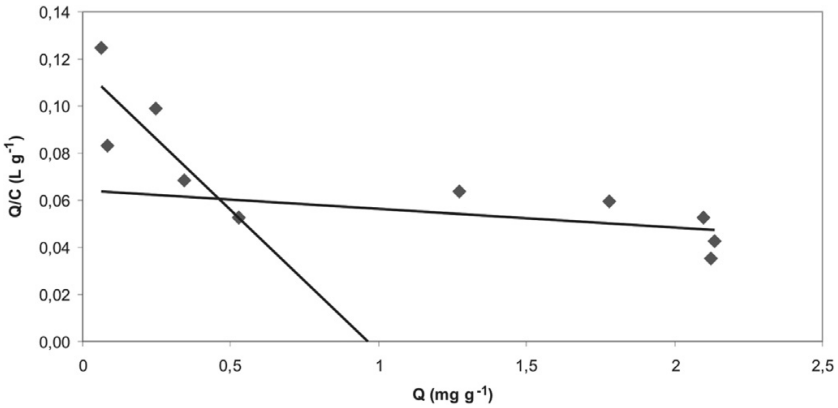

Fig. 3. Scatchard graph of binding of 1-OHP to the MIP.

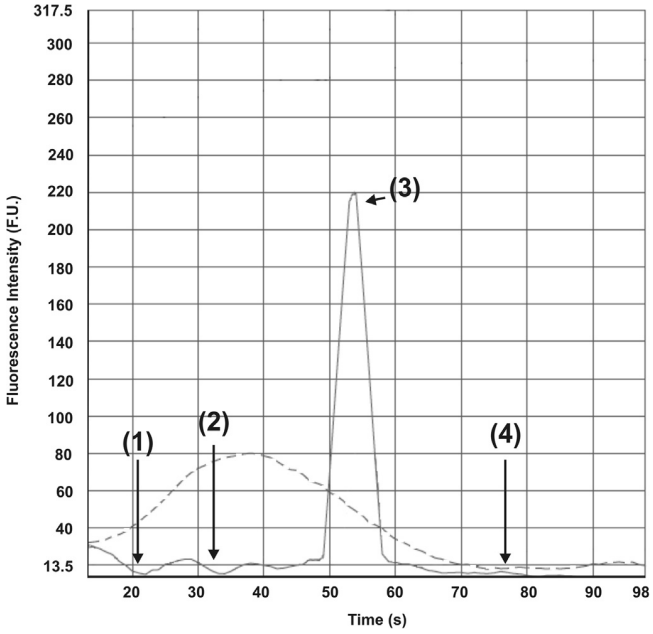

Fig. 4. Response profiles in the FI system for standard solution OHP (100 mg L^{-1}) using NIP system (dotted line) or MIP system (solid line).

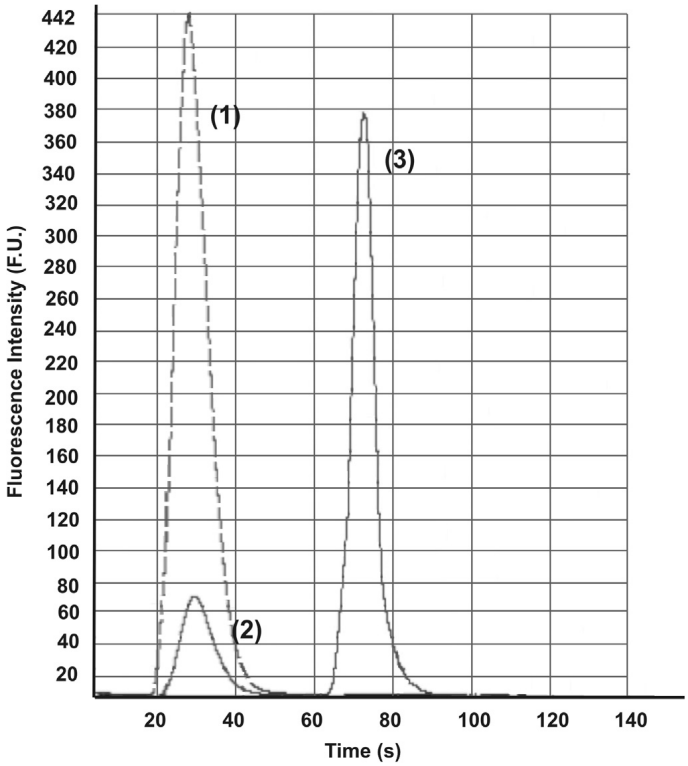

Fig. 5. Response profiles in the FI system for 1-OHP (400 mg L^{-1}). Signal for urine sample without micro-column (1) and with MIP micro-column: (2) washing step and (3) elution step.

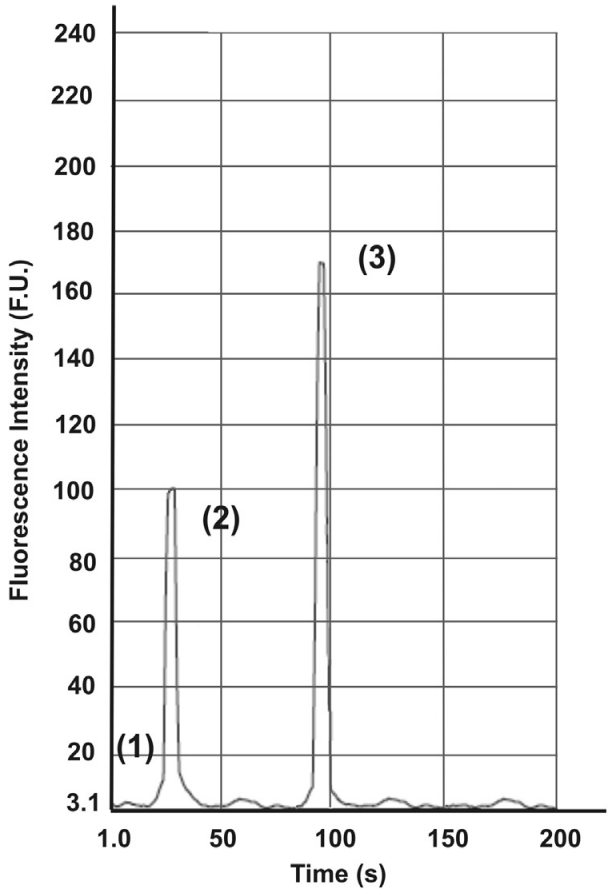

Fig. 6. Response profile in the FI system for 1-OHP (100 mg L^{-1}) and hydroxyphenantrene isomers mixture (100 mg L^{-1}). (1) loading step, (2) washing step elution step.

Table 1. Langmuir and Freundlich isotherm parameters obtained by non-linear fitting for MIP and NIP polymers.

Model	Parameters	MIP		NIP	
		Value	S.E.	Value	S.E.
Langmuir	$a = 1/Q_{max}$	0.760	0.467	3.383	0.885
	$b = 1/(K_L Q_{max})$	8.313	0.647	7.021	1.225
	R^2	0.948	–	0.779	–
Freundlich	$a = \log K_F$	- 0.997	0.038	- 1.141	0.080
	$b = 1/n$	0.794	0.023	0.539	0.066
	R^2	0.985	–	0.876	–

Table 2. Precision and recovery percentages of 1-OHP in the proposed method.

	Concentration level (mg L^{-1})	Precision (%)	Recovery (%) Mean±SD
Intra-day precision ($n = 3$)	10	3.8	85±5
	100	1.0	74.07±0.8
	400	4.4	81±4
Inter-day precision (n¼=3)	10	0.5	85±3
	100	4.5	83±4
	400	4.5	81±4