Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 225–233

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A simple and highly selective molecular imprinting polymer-based methodology for propylparaben monitoring in personal care products and industrial waste waters

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ARTICLE INFO

Article history:
Received 8 May 2017
Received in revised form 31 October 2017
Accepted 1 November 2017
Available online 4 November 2017

Keywords:
Molecularly imprinting polymer
Propylparaben
Personal care products
HPLC-UV

ABSTRACT

In this work, a novel molecularly imprinted polymer (MIP) proposed as solid phase extraction sorbent was developed for the determination of propylparaben (PP) in diverse cosmetic samples. The use of parabens (PAs) is authorized by regulatory agencies as microbiological preservative; however, recently several studies claim that large-scale use of these preservatives can be a potential health risk and harmful to the environment.

Diverse factors that influence on polymer synthesis were studied, including template, functional monomer, porogen and crosslinker used. Morphological characterization of the MIP was performed using SEM and BET analysis. Parameters affecting the molecularly imprinted solid phase extraction (MISPE) and elution efficiency of PP were evaluated. After sample clean-up, the analyte was analyzed by high performance liquid chromatography (HPLC). The whole procedure was validated, showing satisfactory analytical parameters. After applying the MISPE methodology, the extraction recoveries were always better than 86.15%; the obtained precision expressed as RSD% was always lower than 2.19 for the corrected peak areas. Good linear relationship was obtained within the range 8–500 ng mL^{-1} of PP, $r^2 = 0.99985$. Lower limits of detection and quantification after MISPE procedure of 2.4 and 8 ng mL^{-1}, respectively were reached, in comparison with previously reported methodologies. The development of MISPE-HPLC methodology provided a simple an economic way for accomplishing a clean-up/preconcentration step and the subsequent determination of PP in a complex matrix. The performance of the proposed method was compared against C-18 and silica solid phase extraction (SPE) cartridges. The recovery factors obtained after applying extraction methods were 96.6, 64.8 and 0.79 for MISPE, C18-SPE and silica-SPE procedures, respectively. The proposed methodology improves the retention capability of SPE material plus robustness and possibility of reutilization, enabling it to be used for PP routine monitoring in diverse personal care products (PCP) and environmental samples.

1. Introduction

The use of parabens (PAs) is authorized by FDA (Food and Drug Administration) and the local sanitary authority ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica) as microbiological preservative to avoid the presence of fungi and microorganisms in food, drink, pharmaceuticals and personal care products (PCPs) [1]. The most used PAs are methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP). These compounds are found in almost all types of cosmetics and their mixtures are widely used because of their synergistic effects [2]. Although these products are included in the list of allowed substances, maximum concentration limits have been imposed. The European Union accepts the use of PAs in a maximum total concentration of 0.8% (w/w) [3], while they must be individually used in a maximum concentration of 0.4% (w/w).

Recent studies claim that long or high exposure to PAs can be a potential health risk, although traditionally it has been con-

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https://doi.org/10.1016/j.jpba.2017.11.002
0731-7085/

sidered that these products have low toxicity. Some toxic effects reported are the potential influence on the incidence of breast cancer [4,5] their role as endocrine disruptors [6], a relationship with male infertility [7] and allergic contact dermatitis contribution [8]. Because of this, some patents of products free of PAs were developed in recent years and an increasing number of companies are still working in the field [9,10]. An additional problem associated with the massive and permanent use of PCPs is the release of PAs through domestic wastewater into the environment, thus there is concern about potential long-term effects on humans and wildlife [11,12].

Taking the parabens toxicity in consideration, the Scientific Committee on Consumer Safety of the European Commission in its publication "Opinion on parabens" of 2013, modified PP and BP values considered safe for PCPís consumer. Since October 2014 the sum of their individual concentrations should not exceed 0.14% (w/w) being currently allowed up to 0.19% (w/w) [13]. In addition, the presence of this combination is prohibited in PCPs for children under the age of 3 [13–15]. Nevertheless, in our country PP in combination with MP is the far used as PCP preservative.

Several techniques are often used to quantify prohibited or restricted ingredients in PCP. However, considering the sensitivity and selectivity requirements, in most cases sample pretreatment steps are necessary [12]. For this purpose, solid phase extraction (SPE) and solid phase microextraction (SPME) [16,17] are the most common alternative because of their simplicity and effectiveness in reducing the amount of organic solvents used. Nevertheless, the low selectivity associated with traditional SPE often leads to the co-extraction of many components of the matrix. Several strategies for improving SPE selectivity were reported, usually involving physical or chemical modifications on solid surface of extractants. Among the novel approaches, molecularly imprinted polymers (MIP) are synthetic materials capable of selectively interact with specific chemical functional group [18–21]. The most significant benefits of the MIPs are the low cost of synthesis, high mechanical strength, chemical stability, and re-use skills [22]. Use of MIPs for increasing selectivity is of particular interest when the sample is complex and the presence of interferences could hamper the final quantification [23].

The feasibility of SPE using MIPs (MISPE) as adsorbent for parabens quantification in complex matrices was demonstrated by Nuñez [24] and Beltran [25]. Furthermore, the use of MIPs in combination with modern separation techniques, allowed the achievement of better results than using traditional SPE cartridges in terms of enrichment of the analyte and clean-up efficiency.

In this work, synthesis of MIPs was developed for PP extraction from real samples of PCP and effluent followed by HPLC analysis. Among the parabens, PP was chosen as target molecule due to its massive use as preservative in PCP in combination with MP. Nevertheless, the concentration allowed for PP is stricter than MP because of its higher toxicity, and therefore a selective and sensitive methodology is necessary for monitoring PP in studied samples. Summarizing, the proposed methodology combines the inherent high extraction specificity and efficacy of MIPs to the high resolving power of instrumental separation methods [19].

2. Materials and methods

2.1. Reagents and apparatus

Propylparaben (PP), methylparaben (MP), benzoic acid (BA), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) and benzoyl peroxide (BPO) were supplied by Sigma-Aldrich (St. Louis, MO, USA, https://www.sigmaaldrich.com). Methanol was of HPLC grade while toluene, ethanol and acetic acid were of analytical grade, and they all were purchased from Sintorgan (Buenos Aires, Argentine, https://www.sintorgan.com.ar). Ultrapure water was obtained from an ultrafiltration equipment Millipore Barnstead EasyPure II (Buenos Aires, Argentina, https://www.thermofisher.com/ar/es/home.html).

Silica and C-18 SPE cartridge (Enviro Clean®) for analytical use were employed sample pretreatment, in order to establish comparisons to synthetized MIPs columns. Centrifugation was performed by a centrifugal Beckman TJ-6 (Buenos Aires, Argentine, http://www.bioesanco.com.ar).

2.2. Chromatographic conditions

The chromatographic analysis was performed using an HPLC system equipped with a Gilson 322 pump controller, a Rheodyne injector with 20 μL loop, a Gilson 170 UV–vis diode array detector (Buenos Aires, Argentine, http://www.bioesanco.com.ar). The separative column used was a Phenomenex Gemini 5 μm C18 110 Å (150 × 4.6 mm) (Buenos Aires, http://www.omnilab.com.ar).

2.3. Polymer preparation

MIPs were prepared by bulk polymerization according to the non-covalent approach [26] by dissolving appropriate quantities of both, the template molecule and the functional monomer in toluene. For the synthesis, PP was used as a template molecule, MAA as a functional monomer, toluene as a porogen, the crosslinker selected was EGDMA and BPO was chosen as the radical initiator.

The mixture of the mentioned constituents was incubated for one hour, and after that, the crosslinking agent and initiator were added. Then the mixture was purged with nitrogen for 2 min, sealed and placed in a glycerin bath at 60 °C for 24 h to induce the polymerization.

Once the MIP synthesis was completed, the removal of the template was performed using a mixture of methanol/acetic acid (9:1, v/v).The mixture was stirred in a vortex and the remaining solid was separated by centrifugation at 5000 rpm for 15 min. This operation was repeated 8 times until the template was not detected in the supernatant which was monitored by HPLC-UV. As a control, a non-imprinted polymer (NIP) was simultaneously synthesized in the same conditions without the addition of a template molecule.

2.4. Polymer characterization

The polymers were characterized by scanning electron microscopy (SEM, http://labmem.unsl.edu.ar)LEO 1450VP (variable pressure).

Textural characterization of samples under study was carried out by N_2 adsorption – desorption at 77 K using an Autosorb 1-MP manometric adsorption equipment (USA, http://www.quantachrome.com/index.html), where the samples were previously degassed at 50 °C for 12 h, up to a residual pressure smaller than 0.5 Pa. The specific surface area (S_{BET}) was obtained with the Brunauer, Emmet and Teller (*BET*) method [27]. The total pore volume (V_{TP}) was obtained by the Gurvich's rule at a relative pressure of 0.99 [28]. The pore size distribution of the samples under study was obtained by the *VBS* method for cylindrical pore geometry [29,30] using the adsorption branch data.

2.5. Sample preparation

The analytical sample consisted in three brands of wipes used for baby hygiene (2 containing PP and 1 "PAs free"), antibacterial wipes (without PP stated in its composition), industrial wastewater, and the same effluent with the addition of PP standard.

In the case of wipes, sample was placed in a beaker with 20 mL of MilliQ water, after boiling for 1 min, the resultant liquid was filtered and the entire process repeated once. The two sample aliquots were combined and made up to 50 mL with MilliQ water, and then 8 mL of this solution was taken and diluted to a final volume of 10 mL with ethanol.

The wastewater sample was collected from an industrial area and two aliquots of 8 mL were taken and diluted to a final volume of 10 mL with ethanol, in one of the effluent samples 1 mL of a PP standard was added to the previous dilution with ethanol.

2.6. Adsorption study of PP-MIP complex

In order to obtain the maximum adsorption of the target molecule, the amount of polymer as well as the interaction solvent and the binding time were evaluated in a batch study. The effect of the amount of polymer on the PP-MIPs binding was studied within the range 5–25 mg. The influence of the interaction solvents on the MIP recognition properties was evaluated by using the following media: water, acetonitrile, methanol, ethanol, propanol, *n*-hexane, toluene and their mixtures in different proportions. The interaction time was investigated from 1 to 15 min. The influence of the pH of the sample on the binding interaction was evaluated within the range 5.0–9.0.

A standard PP solution was prepared by diluting the analyte in a mixture ethanol/water in a 2:8 relation to obtain a final concentration of 0.020 mg mL^{-1}. For the pH study, the water was replaced by phosphate buffer solutions within a pH range of 5.0–9.0. The binding procedure was as follows: one milliliter of PP standard was added to an appropriate amount of sorbent in a polypropylene eppendorf tube. The mixture was vortexed at 1600 rpm for 2 min and then stirred in shaker at 120 rpm at room temperature for each studied binding time. Thereafter, the supernatant was separated by centrifugation at 5000 rpm for 15 min and analyzed by HPLC-UV. For interference study, mixture of standard solutions of PP and MP (1:1) was performed as described above.

After the binding procedure was optimized, 25 mg of the synthetized polymer was placed inside of a polypropylene syringe (3.0 mL) between two glass wool layers of 2 mm width each in a homemade preconcentration column. The extraction process for each 5.0 mL sample aliquot was performed by applying vacuum at the column lower end. The applied flow rate was 1.3 mL min^{-1}. Once the analyte was adsorbed on the MIP material, the sorbent was washed using 2.0 mL ultra-pure water in order to remove non-adsorbed substances. With the aim of comparing results, the study was also performed over 2 commonly used SPE cartridges (silica and C18 sorbents).

2.7. Elution study of PP-MIP complex

After adsorption study previously described, the influence of the nature and the volume of the solvent on the analyte desorption was studied. With this objective, different ethanol/water mixtures and volumes within the range 0.20–5 mL were put in contact with the MIP-PP. Then were stirred in a vortex for 1 min and centrifuged at 5000 rpm for 15 min. The particulate-free supernatant was injected into the HPLC instrument for PP quantification. For control purposes the same procedure was applied to the NIP-PP complex.

Considering the results obtained in the desorption study, the analyte elution was investigated by employing 1.0 mL of an ethanol/water (8:2 v/v) mixture at a flow rate of 1.3 mL min^{-1}. The eluate was then filtered before its injection into HPLC system for analysis.

2.8. HPLC analysis

For PP monitoring in both, supernatants and eluates, 1 mL aliquots were taken out for quantifying the analyte by HPLC-UV. Samples were filtered through 0.45 μm nylon membrane filters (26 mm diameter) (Buenos Aires, http://www.microclar.com/index.php) prior to injection into the chromatographic system. Then, a sample volume of 20 μL was injected into HPLC system and analyzed in the isocratic mode, using as mobile phase a solution composed by 65% methanol and 35% buffer potassium phosphate monobasic (KH_2PO_4, 6.8 gL^{-1}). A constant flow rate was set at 1.3 mL min^{-1}, the target analyte was monitored at λ = 227 nm and its retention time was approximately 4 min. The PP adsorbed amount to MIP/NIP was calculated by subtracting the unbound analyte from the initial concentration of the analyte. All the experiments were done in triplicate unless mentioned otherwise.

3. Results and discussion

3.1. Polymer synthesis

MIPs were prepared by bulk polymerization according to the non-covalent approach because of the simplicity of the procedure without losing the necessary selectivity. In addition, non-covalent approach allowed that interactions between monomers and template were easily obtained when all components were mixed in solution.

The influence of the porogen on the polymer synthesis yielding and the polymer retention capability was evaluated by testing acetonitrile and toluene. The use of toluene showed the best performance and produced MIPs with improved selectivity for PP; thus, it was selected as porogen for the following experiments.

Selection of the functional monomer and the crosslinker was based on the results observed in previous works [24]. Thus, PP was used as a template molecule, MAA as functional monomer. The crosslinker chosen was EGDMA and BPO was chosen as the radical initiator. For the BPO selection, the facility to produce free radicals by thermo-decomposition and its wide availability were taken in account. The procedure was carried out as described in the experimental section.

Six molar ratios between the template and functional monomer (1:1, 1:2, 1:4, 1:6, 1:8 and 1:16) were assayed and the results were expressed in terms of PP retention. The PP retention was slightly enhanced with increasing monomer concentrations, reaching the highest level as starting from 1:6. Afterward, from molar ratios of 1:6 to 1:16 no improvements were observed and therefore, a molar ratio of 1:8 was selected. Finally, mixture template:monomer:crosslinker at molar relation of 1:8:25 was employed for polymer synthesis. Taking into account MAA monomers interacted with the template molecule by hydrogen bonds, it could be observed that this proportion led to a good compromise between adsorption capacity-selectivity. The excess functional monomer resulted in the increased number of binding sites.

After optimization, it was selected a relation of 20 parts for the sum of monomer plus crosslinker and 80 parts corresponding to the porogen. This result was in agreement with those obtained in previous works [26,31]. In addition, the BPO consisted in the 1% of the total monomer weight used.

3.2. Morphological characterization of polymer

The morphology of the optimized polymer was investigated using BET N_2 adsorption–desorption analysis along with SEM.

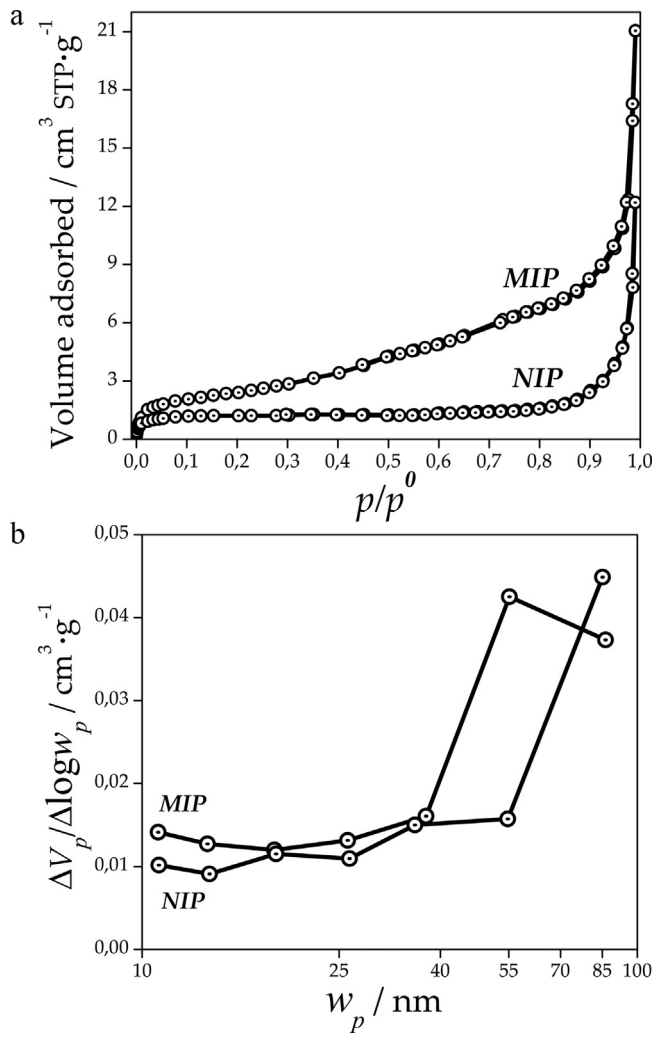

Fig. 1. (a). N_2 adsorption – desorption isotherm at 77 K of NIP and MIP polymers. (b). Pore size distributions for NIP and MIP polymers.

The N_2 adsorption – desorption isotherm at 77 K of the NIP and MIP samples are shown in Fig. 1(a). These materials present a Reversible Type II isotherm (without hysteresis loop), typical for nonporous or macroporous adsorbents [32]. The adsorption isotherms of both polymers quickly increase the N_2 adsorbed volume at relative pressures higher than 0.9, due to the N_2 adsorption on larger mesopores or macropores, as can be seen in Fig. 1(b) where these samples exhibit pores with sizes higher than 40 nm. The V_{TP} of MIP and NIP polymers were 0.03 and 0.02 $cm^3\ g^{-1}$, respectively. The S_{BET} of the MIP and NIP samples were 10 and 6.5 $m^2\ g^{-1}$, respectively. The increase of the specific surface area can be related with the formation of a shape-selective cavity that is complementary to the template and is absent in the non-imprinted polymer.

In accordance with BET study, in SEM analysis it was observed that the MIP particles showed a smaller size with a mean diameter of approximately 2.157 μm while in the case of NIP the mean diameter was 5.776 μm (Fig. 2).

3.3. Selection of solvent for interaction

The high selectivity of MIP polymer is mainly due to the formation of a cavity specific for a particular analyte. There are several variables that affect it, such as the nature of the solvent used to establish the interaction, the time and stirring mode and the quantity of the MIP used, among others.

The binding capacity of MIPs was evaluated putting in contact with PP standards in several dissolution media: water, acetonitrile, methanol, ethanol, propanol, *n*-hexane, toluene and their mixtures in different proportions. Although the solvent used as porogen for the synthesis of MIPs was described in several reports as the most suitable medium for MIP-PP interaction, it was not applicable for the present methodology [26]. Considering that the template molecule was poorly retained when using toluene as the dissolution medium of PP other dissolution solvents were tested. After experimental procedures, mixtures ethanol/water in 2:8 and 1:9 relation, showed almost quantitative retention (91% and 97%, respectively) (Fig. 3).

Nevertheless, the 2:8 (v/v) ethanol/water ratio was considered the most appropriate solvent because it is not only adequate for the MIP-PP interaction but it enhanced the polymer selectivity as well. This last statement is based on the results of HPLC –UV analysis of both MIP and NIP supernatants. In the case of 1:9 ratio, analyte retention on MIPs was high; however, no significant differences to NIP were found. On the contrary, when using a molar ratio 2:8 the selective retention of MIP was improved. This was also observed in the synthesis of a MIP used for PAs recognition under similar experimental conditions to ours [24].

3.4. Evaluation of the polymer cavity specificity

The efficiency of the imprinting effect can be correctly studied by means of IPB (imprinting induced promotion of binding factor) that describes the quality of the interaction better than the amount of analyte that is bound by MIP (A_{MIP}) [33]. IPB is defined as:

$$IPB = (A_{MIP} - A_{NIP})/A_{NIP}, \text{ where} A_{NIP} \text{is the analyte that is bound by NIP.}$$

The selective retention of the MIP-PP and NIP-PP complexes was evaluated by calculating IPB factors in systems with different interaction solvents. The IPB factors of mixtures that showed the high retention percentages were 0.176 and 0.005 for ethanol/water relations of 2:8 and 1:9, respectively. For this reason, the 2:8 relation mixture was selected as the interaction solvent that exhibited the best performance.

The existence of a specific complementary cavity to PP was evaluated by performing a binding assay with a standard mixture of PP and a related compound with similar physicochemical properties and commonly combined in cosmetic formulations such as BA and MP. Using this equation, IPB obtained for PP was 0.176, while for BA and MP were 0.031 and 0.039, respectively. These results demonstrated a selective binding between MIP and PP when it is compared to analogue molecules.

3.5. Amount of polymer and binding time

After the selection of solvent for interaction, the whole procedure was performed to investigate the necessary amount of polymer for obtaining the maximum adsorption of PP. The study was carried out within a dry weight range of 5–25 mg of MIP as described. It was found that 20 mg of MIP were needed to reach the maximum adsorption percentage. When the amount of polymer was increased above 20 mg, no improvement in the quantity of retained PP could be observed.

In addition, the effect of the binding time on the PP adsorption percentage was evaluated within a time interval of 1–45 min. No appreciable differences were observed by modifying the binding time; thus, 20 min was selected because a slight improvement in terms of retention percentage was obtained.

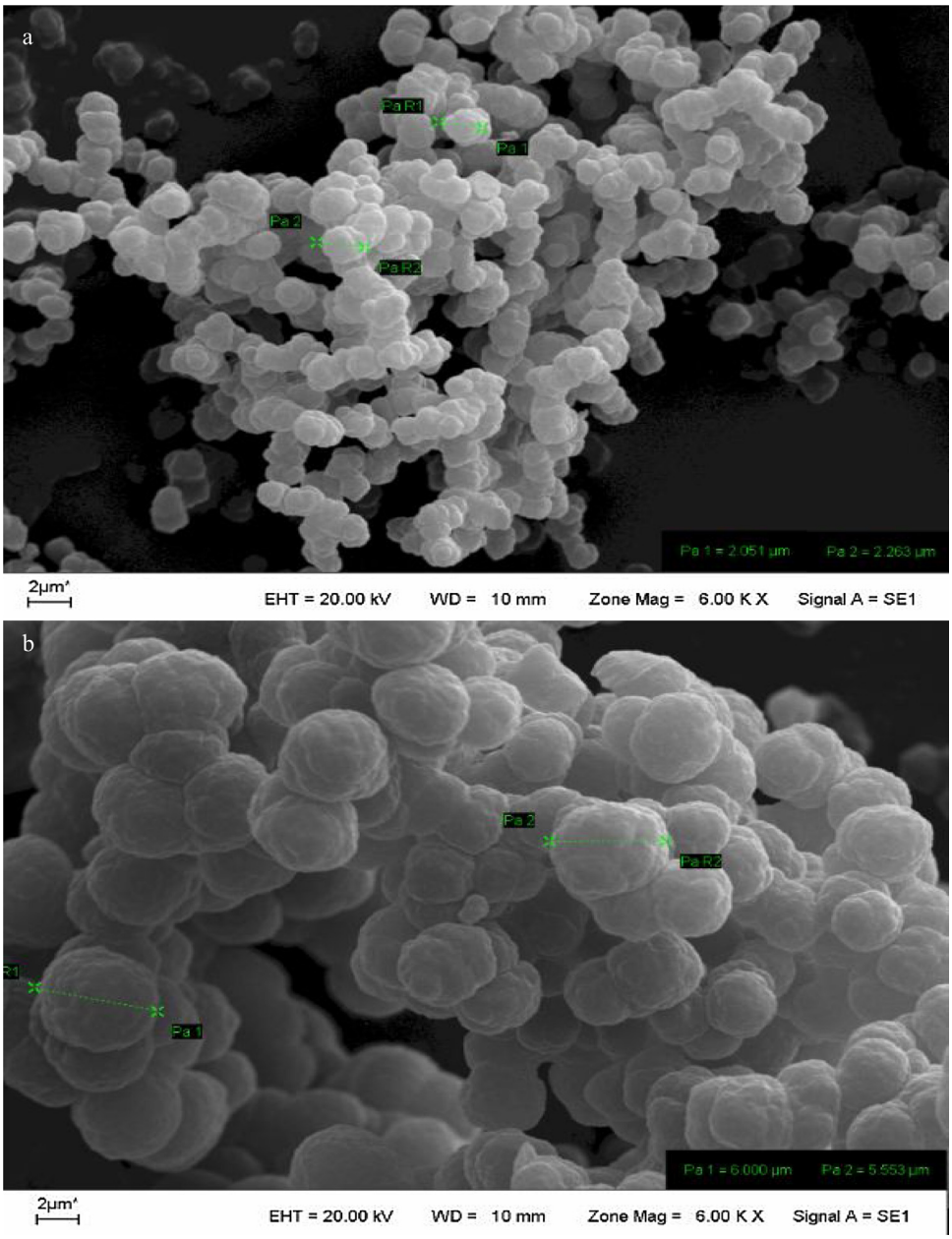

Fig. 2. Scanning electron microscopy (SEM) images of (a) MIP polymer and (b) NIP polymer.

3.6. *Influence of the pH of the sample on the binding interaction*

The pH of the baby wipes is generally within the range 5.5–6.5 and the pH of the environmental samples analyzed varied between 6.0–7.0 ±0.5. Considering that the pH of samples can influences the interaction between the analyte and the polymer, this parameter was studied within the range 5.0–9.0. The procedure was applied as was explained in the Materials and Methods section and the retention capability was evaluated. The retention percentage in all the cases was higher than 93%, it can be concluded that the retention was not affected by the pH of the sample.

3.7. *Preconcentration parameters*

Several factors may affect the preconcentration efficacy of synthetized polymers in flow-mode, extrinsically to its binding capacity. For this purpose, some experimental parameters of flow preconcentration were evaluated such as flow rate, polymers amount, and column size.

The flow rate of elution solvent was studied within the range 0.5-3.0 mL min^{-1}, no significant improvements on extraction efficacy up to 1 mL min^{-1} was observed. Therefore, sample/standard flow rate of 1.0 mL min^{-1} was chosen for its preconcentration procedure.

According to the obtained results from the batch system the amount of synthetized polymer used to fill the preconcentration column was 10–30 mg. A maximum retention capacity was observed when 20 mg of MIP were used for PP retention, while an excess of adsorbent led to a larger flow pathway resulting in a larger elution time. Therefore, 20 mg of MIP material was used for filling the preconcentration column, in addition a 3 mL syringe (0.5 cm inner diameter) demonstrated to be the most suitable recipient.

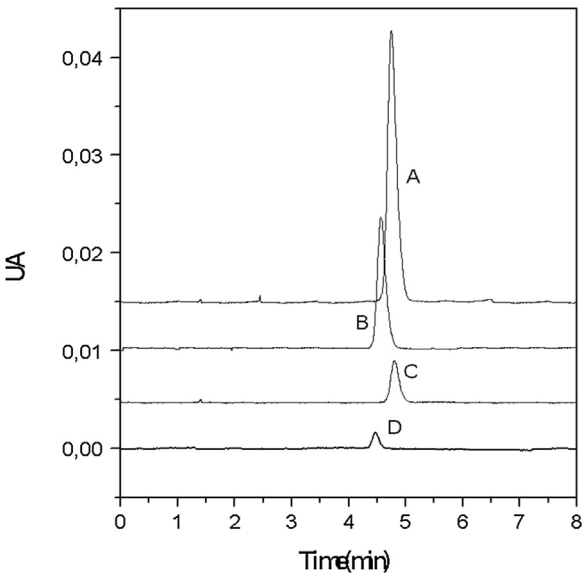

Fig. 3. Chromatograms from the supernatant obtained after MIP-PP interaction. Dissolution medium of PP standards composed by ethanol/water: a) 4:6 relation; b) 3:7 relation; c) 2:8 relation; d) 1:9 relation. Chromatographic conditions: see Section Materials and Methods.

3.8. Selection of solvent and volume for elution

After the interaction of the different elution solvents with the MIP-PP complex, the supernatant was injected into the HPLC instrument. An almost quantitative PP elution was observed when using a molar ratio 8:2 (v/v) of an ethanol/water solution. The elution procedure was repeated with additional 200 μL solvent and subsequently analyzed by HPLC. It was seen that the 98% of added PP was eluted with the first aliquot, while in the second 200 μL aliquot, the remaining PP was completely eluted. To study the possibility of carry over effect, after an extraction and elution procedure another run was performed without loading the sample. This was investigated at the maximum concentration level of PP studied (500 ng mL^{-1}). Obtained chromatograms showed that there was no carry over effect between runs.

In addition, no appreciable improvements were observed by increasing the 200 μL elution volume. As a result, the lowest volume that allowed the elution of 98% of the PP was 200 μL; thus, it was chosen for the following studies.

3.9. Validation

Analytical validation of this methodology was performed according to international guidelines of ICH (International Conference on Harmonization) [34]. Commercial wipes samples used for baby hygiene, PAs free, served as reference during validation of the proposed analytical method.

The evaluation of linearity of the MISPE-HPLC-UV methodology was carried out with a PCP "PAs free" samples spiked with standard solutions of PP with concentrations ranging from 24 to 500 ng mL^{-1}. The developed procedure was applied to the spiked samples in triplicate at six levels concentration and the obtained corrected peak areas were used to plot calibration curves. The calibration equations were obtained by the least-squares linear regression method and used for unknown concentrations calculation. The analytical values obtained are shown in Table 1.

The obtained F-test value (13036.81 at a 95 confidence level) was lower than the tabulated, showing that the method has a linear behavior. The analysis of variance (ANOVA) was calculated using MINITAB15. A probability (p) value lower than 0.001 in the ANOVA indicated the statistical significance at 95% confidence level. The amount of standard, which could be detected with a signal-to-noise ratio ≥ 3 was considered to be the limit of detection (LOD). The limit of quantitation (LOQ) was calculated as the analyte concentration that can be accurately and reliably determined with a signal-to-noise ratio ≥10. LOD and LOQ were evaluated based on the signal background obtained with the analysis of a diluted standard solution (n = 6) (Table 1). For ensure that LOQ was quantified with an acceptable accuracy and precision, it was evaluated considering the standard deviation and the coefficient of variation. The accuracy inter assay for the LOQ concentration of 8 ng mL^{-1}, was 2.12 (expressed as SD, n = 6) and the precision inter assay, was 13.34 (expressed as CV%, n = 6).

Table 1
Method validation regarding linearity, LOD, LOQ, repeatability, and intermediate precision (retention time and peak area) for PP.

Analytical Parameters		
Linear range (ng mL^{-1})		8–500
Regression Equation	Slope	1.28×10^9
	Intercept	44593.81
	r^2	0.9998
LOD (ng mL^{-1})	HPLC	17.2
	HPLC-MISPE	2.4
LOQ (ng mL^{-1})	HPLC	57.3
	HPLC-MISPE	8.0
Precision		
Retention time CV% (n = 6)	Repeatability	1.10
	Intermediate Precision	1.70
Peak area CV% (n = 6)	Repeatability	0.88
	Intermediate Precision	2.19

The precision was evaluated at two levels, in terms of method repeatability and intermediate precision. Repeatability refers to the variability when the method is performed by the same analyst over a short timescale while intermediate precision relates to precision when one or several factors are changed in the method. In both cases, precision was expressed by relative standard deviations (RSDs) of the retention times and the corrected peak areas.

The repeatability of the whole analytical procedure was evaluated by applying the methodology to 2 aliquots of PCP "PAs free" spiked with PP and analyzing by HPLC in triplicate within the same day. The repeatability for PP determination was less than 1.1% for the retention time and 0.88% for the corrected peak area. In the case of intermediate precision determination, the method factors that were changed included the day of operation and the sources of reagents or electrolytes. Thus, the entire analytical procedure was performed during three consecutive days to the spiked "PAs free" sample and the obtained RSDs values were always better than 1.7% for the retention time and 2.1% for the corrected peak area. To investigate the reuse ability of the synthesized polymers, 5 cycles of adsorption-desorption per day were performed. The procedure was carried out at the same conditions as previously described and was repeated during 5 consecutive days, no appreciable differences in the results were observed.

The accuracy, in terms of recovery, was verified by applying the proposed method to the blank PCP sample spiked with known quantities of PP solutions at six concentration levels (n = 6). The study was performed as described previously and the elution liquids were analyzed by HPLC in triplicate. The average concentrations determined for PP were taken as recovery value. In Table 2 it can be seen, the results ranged from 86.15% to 110.50% recovery, which are comparable with the reported by other authors. These results demonstrated that the PCP matrices assayed in this experi-

Table 2
Method validation regarding accuracy.

Aliquots[a]	PP added (ng mL^{-1})	PP recovered (ng mL^{-1})	Recovery ±SD (%)[b]
I	24.8	27.0	109.0 ± 2.2
II	120.0	132.6	110.5 ± 2.2
III	180.0	155.7	86.5 ± 1.6
IV	240.0	206.6	86.1 ± 1.7
V	320.0	304.9	95.3 ± 1.5
VI	500.0	445.5	89.1 ± 1.8

[a] 1 mL PAs free PCP sample spiked with PP.
[b] Recovery = (found value/added value) × 100.

ment had little effect on the extraction efficiency and therefore, the absence of matrix effect.

The specificity of the method was investigated by both peak purity and spiking experiments with pure standard compounds. Peak purity was evaluated by means of the UniPoint^{TM} System Software, using a diode array detector. Comparison of the chromatograms of the spiked PCP sample at the LOQ level with the chromatograms of the non-spiked blank PCP sample aided assessment of the selectivity of the developed method. There appeared to be no interference from sample constituents; also, this was in agreement with the recovery test results.

3.10. MISPE procedure in real samples

The efficiency of this polymer for the clean-up of wipes liquid extracts containing fragrances, humectants, preservatives and other cosmetic ingredients was evaluated. In addition, with the aim of determining the usefulness for environmental analysis, the methodology was applied to industrial wastewater samples.

The study was made on spiked samples at the studied concentration range on 3 different brands of baby wipes. The samples with and without performing the MISPE methodology as described in experimental section were analyzed by HPLC–UV.

With the aim of comparing the extraction efficiency results, the study was also performed by replacing the polymer with a suitable amount of commercially available C18 sorbent (Enviro Clean®, 40–63 μm particle size) and silica sorbent, and placing into a 2.0 mL polypropylene tube. The obtained results showed values within the range 0.021–1.480 mg mL^{-1} and are illustrated in Fig. 4.

Then, the recovery factor (R%) obtained after using MISPE, C18-SPE and silica-SPE cartridges were calculated as follows: R% = (PFr/PFt). 100

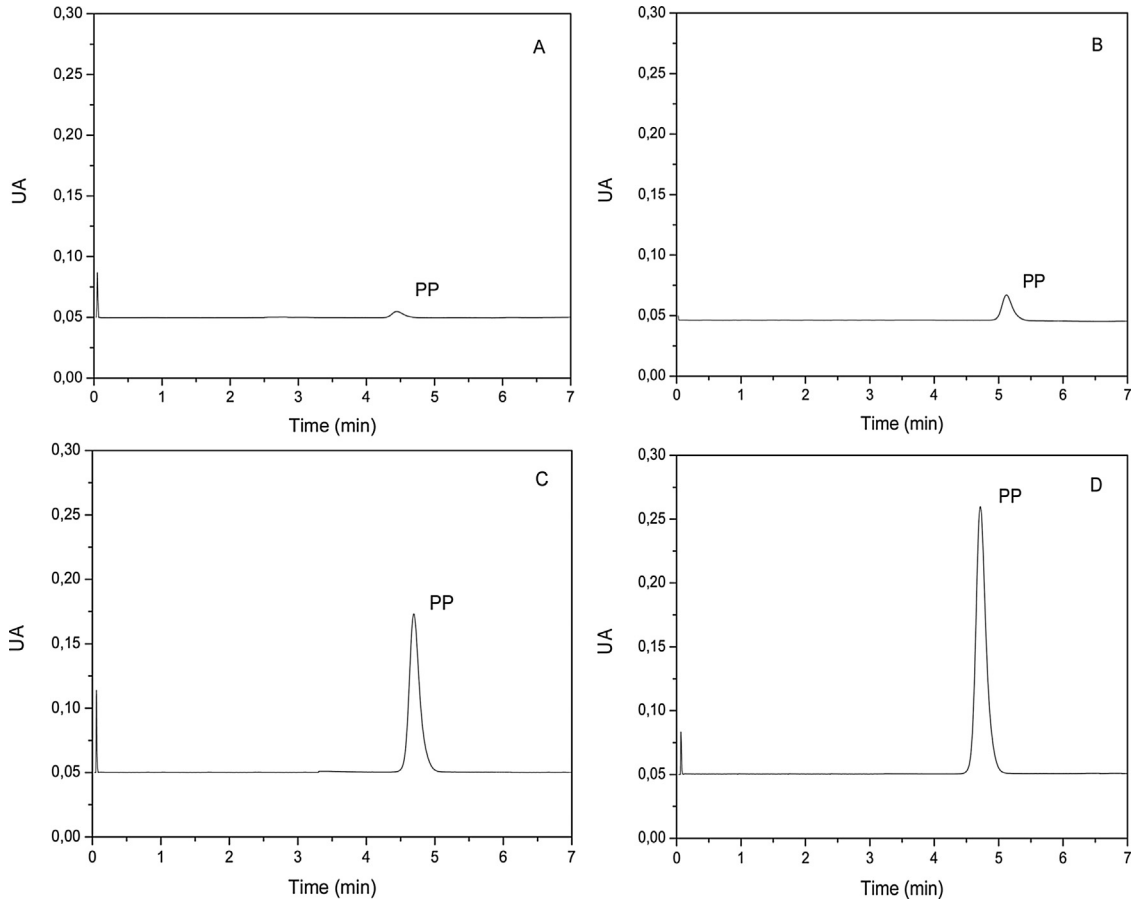

Fig. 4. Chromatograms from the elution liquids obtained before and after sample pretreatment. Sample before pretreatment (A), sample after pretreatment with silica sorbent, C18 sorbent and MISPE sorbent (B, C and D respectively).

Where PFr is the real preconcentration factor and PFt is theoretical preconcentration factor, and they are calculated by:

PFr = Cf/Ci, being Cf and Ci the final concentration and the initial concentration respectively.

PFt = Vi/Vf, being Vi and Vf the initial volume and final volume respectively.

The R% obtained after applying extraction methods were 96.6, 64.8 and 0.79 for MISPE, C18-SPE and silica-SPE procedures, respectively. It can be seen that $R\%_{MISPE}$ is significantly greater than the obtained with the traditional SPE methods.

The methodology selectivity was evaluated in real samples that contain MP in their composition. It was observed that the retention capacity was 25% higher for PP when compared against MP. In the case of wipes "PAs free", the analyte could be detected in one sample and only after MISPE process was applied.

Samples of environmental concern were also investigated, giving as a result that PP was not detected in the industrial wastewater analyzed samples. The procedure was applied on PP spiked samples, after quantification the obtained recoveries were analyzed and no matrix effects were observed. The LOD and LOQ achieved for this methodology were adequate to determine the PAs at trace levels, required for environmental studies. In comparison to some recently reported works, the sensitivity of this methodology was approximately one order of magnitude higher [25].

The methodology developed in this work is more convenient in terms of simplicity considering that previously reported methods require additional steps of sample preparation such as agitation, centrifugation [35] heater-stirrer and control of solvent temperature to avoid memory effects [36]. Even more, in the case of all the informed liquid-phase microextraction methods for separating parabens, they require pHs lower than 6 because at these conditions its undissociated form can be extracted efficiently [37]. Furthermore, longer sample treatment time [38] is needed and therefore the required total time for analysis is higher.

Additionally, the reported SPE methods involve additional steps for sample evaporation and the subsequent reconstitution with high volumes of organic solvents [39], and an agitation stage for both the retention and elution processes [40]. Specifically, methodologies that involve the use of MISPE for the extraction of parabens use higher amount of polymer for the column assembly, which means greater cost with the same extraction efficiency. It also noteworthy that the column re-conditioning step is simpler than similar reported methods, leading to a higher versatility for the present methodology [24].

In conclusion, the proposed methodology implies shorter sample treatment times in comparison with other already informed methods, and eliminates additional steps of agitation, pH adjustments and centrifugation. In addition, the present procedure avoid the use of large volumes of pollutant solvents being also convenient in terms of selectivity and sensitivity.

4. Conclusion

In this work, the synthesis of MIPs for the solid phase extraction of PP in real PCP and environmental samples was developed. The procedure was optimized in order to obtain a target-selective adsorbent. The effectiveness, selectivity, robustness and reutilization capability of synthetized MIPs were evaluate.

The main factor that affected the performance of MIPs, was solvent composition, for both interaction and elution steps. Among studied solvents, the ethanol/water mixtures at 2:8 (v/v) allowed specific MIP-PP interaction while in inverse proportion (8:2 v/v) resulted in an effective eluent (higher than 99% of PP elution). This polarity inversion of solvents led to the removal of PP from MIP binding sites. Specific retention of PP-MIP was evaluated by means of IPB factors, which were compared with a frequently associated analogue molecules such as BA and MP, being higher for PP. Additionally, SEM and BET analysis were performed, showing a greater surface area for MIP when it is compared to NIP at the same synthesis conditions.

The methodology was validated and applied to real samples consisting of personal care products and industrial wastewater samples. Lower limits of detection and quantification, (2.4 ng mL^{-1} and 8 ng mL^{-1}, respectively) were reached, in comparison with previously reported methodologies. It was demonstrated that the synthetized MIP was able to preconcentrate PP even at trace level concentrations, exhibiting its potential for samples of environmental concern. As adsorbent material, preconcentration capacity of synthesized MIPs was comparatively higher than traditional SPE cartridges. However, the main advantage of the application of MIP, rather than increasing sensitivity is the potential to target specific analytes. The use of MIPs, allowed a simple, rapid and effective sample pretreatment previous direct analysis by high resolution separation techniques without additional labor-intensive and time demanding stages. Its robustness and the possibility of reutilization, enable that this methodology can be used as a tool for PP routine monitoring in diverse PCP and environmental samples. Furthermore, the proposed sample treatment employing MIPs replaced the use of long-chain organic solvents by environmentally friendly reagents, leading to a green analysis method for PP.

Funding

This work was supported by the National University of San Luis [Project No. 2/1316], CONICET [PIP-1122050100395CO], and Laboratorio de Control de Calidad de Medicamentos UNSL, Argentina. The authors wish to thank INQUISAL-CONICET (Instituto de Química de San Luis-Consejo Nacional de Investigaciones Científicas y Tecnológicas). The authors have declared no conflict of interest.

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