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MOLECULAR IMPRINTING ON SURFACE OF SILICA PARTICLES FOR THE SELECTIVE EXTRACTION OF BENZYLPARABEN IN FLOW SYSTEM APPLIED TO COSMETICS AND WATER SAMPLES

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Abstract

Molecular imprinted polymer coated Silica (Si-MIP) particles were prepared using non-covalent approach. The Si-MIP material was employed as selective solid phase extraction sorbent for the determination of benzylparaben (BP) in samples of environmental and cosmetic origin. The preconcentration step was studied firstly in a batch system and subsequently it was optimized and adapted to an automatized flow system and the eluate was then analyzed by HPLC-UV. The whole procedure was validated, showing satisfactory analytical parameters. The methodology was applied to real samples showing extraction recoveries always better than

98.85%. A linear response within the concentration range of 1.11×10^{-5} - 0.025 mg mL^{-1} was obtained for BP (r^2=0.9998). The limits of detection and quantification after Si-MIP-HPLC procedure were 3.34 and 11.16 ng mL^{-1}, respectively. The developed methodology provided a simple an economic way for accomplishing a clean-up/preconcentration step and the subsequent determination of BP in a complex matrix. The showed selectivity plus robustness and the possibility of reutilization, enable it to be used for BP routine monitoring in personal care products (PCP) and environmental samples.

1. Introduction

The so called emerging contaminants can be broadly defined as any chemical or microorganism that is not commonly monitored in the environment but has the potential to cause known or suspected adverse ecological or human health effects. This group encompasses compounds usually used on pharmaceuticals and personal care products (PCP) and are progressively accumulated on natural aquatic resources. The presence of these contaminants in natural resources could have been for a long time, but may not have been recognized until new detection methods were developed.

The parabens are widely used as preservatives and antimicrobial especially in cosmetics and personal care products. However, their widespread production and use can result in their leaking into environment, becoming potential emerging contaminants [1, 2]. A few years ago, the European Commission [3] banned the use of five parabens in cosmetic products: Isopropylparaben, Isobutylparaben, Phenylparaben, Benzylparaben and Pentylparaben. Nevertheless, these parabens are still employed with frequency in PCPs.

Among several PCPs, sunscreen products are considered one of the major sources for the parabens release to the environment because of the continually increasing frequency of use worldwide. These products contribute to the contamination of oceans, lakes and rivers, increasing the health risk due to exposure, especially important in the case of children. Some toxic effects of parabens 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].

The analysis of emerging contaminants in water resources, demands the development of accurate sample preparation methods. These pollutants are usually found in very low concentrations in matrices that generally are of complex nature. A suitable sample pre-treatment methodology should enhance sensitivity and selectivity of the whole analytical procedure [9]. For this purpose, solid phase extraction (SPE) and solid phase microextraction (SPME) [10,11] have been widely used because of many advantages such as the low requirements of organic solvents and the easy operation. However, in several occasions the selectivity obtained needs to be improved due to the co-extraction of other sample components.

Recently, our group has synthesized molecularly imprinted polymers (MIP) capable of interacting selectively with parabens [12]. In addition to selectivity, the most significant benefits of MIPs are the low cost of synthesis, high mechanical strength, chemical stability, and re-use skills [13-16]. However, retention ratio of target and its analogues was low, and therefore, a further separative technique was necessary for the target determination indicating that specificity could be improved.

Recent studies have described the enhancement of MIP extraction capability by means of the polymer printed on the surface of inorganic particles [17]. The use of this type of structure allows improving the MIP rebinding capacity and the site accessibility to target species. Some

benefits associated with the application of the surface molecular imprinting technique on silica particles are suitable chemical and physical stability, good mechanical strength, and robustness, among others [18-20]. In this paper, the synthesized imprinted polymers technique applied to silica surface particles was used to improve the selective extraction of BP. The dynamic interaction properties that flow system offers were studied in order to enhance the specific target retention and the general properties of the extractive material.

2. Materials and methods

2.1 Reagents and solutions

Methylparaben (MP), propylparaben (PP), benzylparaben (BP), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) and benzoyl peroxide (BPO) were supplied by Sigma-Aldrich (St. Louis, MO, USA, https://www.sigmaaldrich.com). Silica Gel G (Type 60) was purchased from Merck (Darmstadt, Germany). 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 ultrafiltration equipment Millipore Barnstead EasyPure II (Buenos Aires, Argentina, https://www.thermofisher.com/ar/es/home.html).

2.2 Instrumentals and apparatus

Chromatographic separations were carried out by UHPLC focused Thermo Scientific Dionex Ultimate 3000 Series equipped with Dionex Ultimate 3000 autosampler, Quaternary separation system Dionex Ultimate 3000 Pump and Diode Array detector DAD-3000 (RS). An Zorbax Eclipse XDB-C18 (4.6 x 150mm; 5 μm; Agilent) column was employed for all chromatographic analysis. Sample volume of 20 μL (standard solution/eluate) was injected and

analyzed in the isocratic mode, using as mobile phase a solution composed by 65% methanol and 35% buffer potassium phosphate monobasic (KH2PO4, 6.8 g.L^{-1}). A constant flow rate was set at 1.3 ml min^{-1}, monitored at λ= 227 nm All the experiments were done in triplicate unless mentioned otherwise.

Infrared diffuse reflectance spectroscopy (DRIFTS) study for syntethised sorbents was obtained using Shimadzu Fourier transform infrared spectrophotometer (IRAffinity^{-1}) Spectra (averaging of 50 scans) were collected in the spectral range from 2500 to 500 cm−1, at a spectral resolution of 2 cm−1. Scanning electron microscopy (SEM) images were obtained using an instrument LEO 1450VP (variable pressure) (http://labmem.unsl.edu.ar).

The flow system was performed using Gilson minipuls 3 peristaltic pump and Gilson PhtalateFree PVC pump tubes (2.06 mm ID purple/purple) for all sample preconcentration step. A two positions Rheodyne valve with three channels (six ways) was employed for sample/solution selection. Centrifugation was performed by a centrifugal Beckman TJ-6 (Buenos Aires, Argentine, http://www.bioesanco.com.ar).

2.3 Preparation of the printed silica particles (Si-MIP)

For the synthesis of molecular imprinting polymers coated silica (Si-MIPs), BP was used as a template molecule. Methacrylic acid was used as functional monomer, and toluene as porogen, ethylene glycol dimethacrylate as crosslinking agent and benzoyl peroxide as initiator. The MIP was printed on silica gel particles that were used as inert support. Simultaneously, for comparison purposes Si-NIPs and a bulk MIP were synthesized.

Si-MIPs were prepared by mass polymerization according to the non-covalent approach, by dissolving the template molecule and the functional monomer in toluene. The pre-polymerization mixture was incubated for one hour, and after this, the cross-linking agent, the

initiator and silica gel were added. The mixture was purged with nitrogen for 2 min, and placed into a glycerin bath at 60° C for 2 and 4 h to induce polymerization.

After this, the supernatant was removed, and the polymer was spread on a watch glass and allowed to dry. The removal of the template was carried out with a mixture solution of methanol: acetic acid (9: 1, v / v) by stirring the solution in a vortex and then, the supernatant was separated by centrifugation at 5000 rpm for 15 min. This operation was repeated 8 times until the template was not detected by HPLC-UV in the supernatant from Si-MIPs (both 2 and 4 h), Si-NIPs (2 and 4 h) and MIP.

2.4 Samples description and treatment

Two samples were analyzed for BP monitoring. One consisted in a water sample collected from a local swimming pool; the other was a commercial semisolid sunscreen with not PAs content declared.

For the analysis of the water sample, six aliquots (10 mL) of filtered water (by 0.45 μm nylon membrane filters) were taken from the swimming pool and stored at 5º C until assay.

In the case of the sunscreen, six aliquots of 1.0 g were weighed and 25 mL of water were added. The samples were warmed out for 10 min and then made up to 50 mL, centrifuged and 10 mL the supernatant filtered through 0.45 μm nylon membrane filters (26 mm diameter). The filtered solutions were collected for further preconcentration step.

2.5 Validation Process

The validation of the methodology was performed as is stated in the international guidelines [21] by evaluating accuracy, precision, linearity, limits of detection and quantification and selectivity.

The procedure was carried out by preparing standard solutions of BP and structural analogs or potential interfering substances such as MP and PP each at a concentration of 1.00 mg mL^{-1}. In the case of fortification study, the standards were added to a set of six aliquots of 1.00 g of sunscreen sample and a set of six aliquots of 10 mL of water sample, in both cases without any treatment. The standards were added within the concentration range of 0.05 – 1.25 mg g^{-1} and 1.11x10^{-5} - 0.025 mg mL^{-1} for cosmetic sample and water sample respectively. Afterward, the spiked samples were proceeded as described before (section 2.4) and analyzed by triplicate. The recovery from the spiked samples was calculated.

2.6 Binding and desorption evaluation in batch

In order to obtain the maximum adsorption of the target molecule, the amount of Si-MIP material used for the binding step, as well as the binding time were evaluated in a batch study. The effect of the amount of Si-MIP on the BP binding was evaluated within the range 5.0-25.0 mg. 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. The obtained results from Si-MIP were compared to Si-NIP and MIP.

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 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, a mixture of standard solutions of MP and PP was added to the BP standard.

The influence of the nature and the volume of the elution solvent selected for the analyte desorption was studied. For this purpose, different ethanol/water mixtures and volumes within

the range 0.20–5 mL were put in contact with the Si-MIP/BP complex. Then were stirred in a vortex for 1 min and centrifuged at 5000 rpm for 15 min. The particulate-free supernatants were injected into the HPLC instrument for BP quantification. The same procedure was applied to the Si-MIPs (2 h), Si-NIPs (2 and 4 h) and MIP.

2.7 Automatized solid phase extraction (SPE) studies. Preconcentration manifold

The automatized SPE was carried out using a homemade preconcentration column consisted in 25 mg of synthesized Si-MIP packed inside a polypropylene syringe (1.0 mL). The sorbent material was placed between two glass wool layers of 2 mm width each. For analyte adsorption step, the valve was set in position 1. In this position, 10 mL of filtered sample solutions were propelled by a peristaltic pump and flow through the column to the waste (flow rate 1.0 mL min^{-1}). Following, the removal of sample matrix/potential interferences was performed using 2 mL of ultrapure water and afterward, valve position was set to 2 for BP desorption using 0.5 mL of the eluent. The collected eluate was then analyzed by HPLC system (Figure 1).

3. Results and discussion

3.1 Polymer synthesis and Characterization

Infrared diffuse reflectance spectroscopy (DRIFTS) was employed to study the chemical structures of the different polymers. The band about 1640 cm^{-1} was the bending vibration of the hydroxyl substituent on the surface of silica gel (Figure 2 a). In figure 2 (b and c) is showed the surface silica particle modified with MIP polymer (2h and 4h of synthesis respectively), while in figure 2 d is showed the MIP. Three strong bands can be seen at 1730, 1460 and 1380 cm^{-1} (Fig. 2 b, c and d). These bands were attributed to the stretching and bending vibration of methyl and methylene, the band at 1730 cm^{-1} indicated the existence of the C=O group, indicating that the surface of silica particles was printed.

Figure 3 shows a SEM image from the Si-MIP obtained at synthesis times of 2 and 4 hours. SEM analysis allowed to study the morphology, size and porosity of the particles. It was observed that when the synthesis lasted 4 h (Figure 3 A2 and B2), the surface was covered in greater proportion than in the case where the synthesis lasted 2 hours (Figure 3 A1 and B1).

3.2 Specific recognition of NIP, MIP, Si-NIP and Si-MIP

Among the numerous analogues of parabens, we have selected the largest molecule of the series as template molecule (BP) for performing the synthesis on the surface of silica particles. BP was also chosen as target molecule to prove the existence of an imprinting effect instead of polar interaction forces between analyte and adsorbate. It could be assumed that using BP as the template the generated cavities could host the smaller analogues. For specific recognition study, we have compared the BP retention capacity for all synthesized polymers described previously on batch system in presence of potential interference such as MP and PP, as described in section 2.6. This study included the nature and proportion of solvents used for elution, resulting ethanol/water (8:2, v/v) the best eluting solution. The binding and desorption parameters such as pH, binding time, amount of polymer and volume elution solvent that were selected are shown in table 1.

The binding time was evaluated within the interval of 1-15 min as described in experimental section. Non changes on binding percentage were observed along the study; thus, 15 min was selected for batch study in order to assure the maximum binding. In order to investigate the necessary amount of polymer, a quantity of 5 mg of polymer was considered the minimum needed to assure the total adsorption of BP. After 5 mg, quantities of polymer were gradually increased until non improvement on adsorption was observed. The surrounding electrolytic properties such as pH and ionic strength have few or no effects on the interaction between BP and polymers. This responds to the fact, among other causes, that parabens are non-ionic compounds at the studied pH range.

With the optimized experimental conditions, the retention capacity, expressed as % recovered (Table 2) that showed the best performance was Si-MIP (4h) being 73.5, 40.3, and 85.5 for MP, PP and BP, respectively. Therefore, Si-MIP (4h) was chosen for further studies as the sorbent material for flow preconcentration study.

Based on the results obtained in the batch study, we worked on the adaptation of the procedure to flow automatized system as was described in experimental section. The optimization of the flow system preconcentration system was carried out by testing the following parameters: sampling time, elution stream and elution profile. The eluates were collected in fractions of 250 µL each, and then analyzed by HPLC-UV. From the obtained chromatogram of the first fraction, we could observe that more than 90% of adsorbed BP was eluted together with almost insignificant quantities of MP and PP. From the second fraction of collected eluate, the complete desorption of retained BP was achieved without the loss of selectivity, since detected quantities of MP and PP were still low. Comparing the performance of Si-MIP respect of the selectivity of BP retention for batch and flow system, the last showed a great improvement due to the insignificant retention for the case of the analogues MP and PP (Table 2).

After flow system parameters study, the final eluate volume chosen was 500 µL of an ethanol/water (8:2 v/v) mixture at a flow rate of 1.00 mL min^{-1}. Working under these experimental conditions, the obtained chromatogram for the analysis of BP in presence of similar concentration levels of MP and PP is shown in Fig 4.

Therefore, we can conclude that the synthesized Si-MIP polymer presents specific cavities for the target molecule (BP). In addition, becoming in a flow system process, the dynamic interaction between host-target molecules avoid the no target analogues retention. In

addition, several advantages were accomplished, allowing the synthesis of a polymer product with chemical and physical stability, good mechanical strength and robustness.

3.3 Preconcentration parameters

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

The flow rate of elution solvent was studied within the range 0.25-3.00 mL min^{-1}, no significant improvement on extraction efficacy up to 1.00 mL min^{-1} was observed. Therefore, sample/standard flow rate of 1.00 mL min^{-1} was chosen for the preconcentration procedure.

According to the obtained results from the batch system the studied range of the amount of Si-MIP filled the preconcentration column was 10-40 mg. From 20 mg, BP retention capacity was reached within the studied BP concentration. Therefore, 20 mg of Si-MIP was used as packing material for preconcentration column.

The recovery factor (R%) was calculated as follows: R% = (PFr / PFt). 100

Where PFr is the real preconcentration factor and PFt is theoretical preconcentration factor, and they were 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 method was 85.5

3.4 Validation

Analytical validation of this methodology was performed according to international guidelines of ICH (International Conference on Harmonization) [20].

For the evaluation of the linearity of the MISPE-HPLC-UV methodology, the standard of BP was studied within the range of 1.11×10^{-5} - 0.025 mg mL^{-1}. The obtained corrected peak areas were used to plot calibration curve and the corresponding equation was obtained by the least-squares linear regression method. Calibration curve was linear over the studied concentration range, with the regression coefficient (r^2) of 0.9998 for determination of BP (Table 3). The calculated F value was higher than tabulated critical value for a significant level of 0.05 showing that the regression adjust found has a slope significantly different from zero.

The accuracy, in terms of recovery, was verified by performing the standard addition method and applying the proposed methodology to real samples. These samples consisted in water collected from a swimming pool and a PCP sample both spiked with known quantities of BP at six concentration levels (n=6). In Table 4 it can be seen, the obtained recovery values ranging from 99.61% to 101.8%.

The repeatability of the whole analytical procedure was evaluated by applying the methodology to 3 aliquots of sunscreen spiked with 1 mg g^{-1} of BP and analyzing by HPLC in triplicate within the same day. The repeatability for BP determination expressed as SD%, was always lower than 0.86. Intermediate precision study was evaluated by changing the day of operation, solutions, reagents and operator. The methodology was applied to the analysis of the sunscreen sample spiked at the same concentration level during three consecutive days, giving a SD% value always lower than 4.6.

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). 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 obtained LOD and LOQ were 0.169 µg g^{-1} and 0.564 µg g^{-1} respectively for sunscreen samples, while for the analysis of swimming pool water were 3.34 ng mL^{-1} and 11.16 ng mL^{-1} respectively.

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.

4. Conclusion

This methodology presented advantages in terms of selectivity, sensitivity, robustness and low cost compared to pre-existing methods for bencylparabene determination in both, potentially contaminated water samples and PCP products. In addition, a relatively simple instrumental of common use like HPLC-UV is necessary. The automatized flow system preconcentration step allowed a selective extraction of the target molecule, providing its sensitive determination at trace level of analyte with reliable accuracy and precision. In addition, it does not involve the use of organic solvents during extraction of parabens which is usually demanding, so it can be considered an environmentally friendly method. The high mechanical strength that the sorbent presents after repeated cycles of use allows its application as a routine quality control of preservatives and environmental emerging contaminant control.

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Figure Captions

Figure 1. Automatized preconcentration manifold

Figure 2. DRIFTS spectra of Silica particule (A), Si-MIP 2h (B), Si-MIP 4h (C) and MIP (D).

Figure 3. Scanning electron microscopy (SEM) images of (A1-B1) Si-MIP 2h polymer and (A2-B2) Si-MIP 4h polymer.

Figure 4. Chromatograms obtained from spiked sunscreen with MP, PP and BP at 0.25 mg g^{-1} after SPE procedure, where: a) MP; b) PP; and c) BP. Chromatographic conditions: see Section Materials and Methods.

Table 1. Parameters evaluated in batch study.

	Studied Range	Selected Value
Binding Time (min)	1 - 15	15
Amount of Polymer (mg)	5.0-25.0	20.0
pH	5.0 - 9.0	Non Applicable
Volume of Elution Solvent (mL)	0.2-1.0	0.5

Parameters evaluated with standard solution of MP, PP, and BP at 0.02 mg mL^{-1} in batch study as described in experimental section.

Table 2. Retention capacity study, expressed as % recovered for the different polymers.

Recovery (%) in Batch System			
	MP	**PP**	**BP**
NIP	42.0	44.5	48.3
MIP	60.5	62.5	85.5
Si-NIP 2h	80.9	57.0	74.3
Si-NIP 4h	78.43	61.3	68.9
Si-MIP 2h	77.1	48.5	82.3
Si-MIP 4h	73.5	40.3	85.5
Recovery (%) in Flow System			
Si-MIP 4h	0.59	11.0	85.5

Retention capacity study, expressed as % recovered for the different polymers, where: MIP= Molecular Imprinting Polymer; NIP=Non-Imprinting polymer; Si-NIP (2h and 4 h)= Non-Imprinting polymer coated silica of 2 h and 4 h of synthesis respectively; and Si-MIP (2h and 4 h)= Molecular Imprinting polymer coated silica of 2 h and 4 h of synthesis respectively.

Table 3 Method validation regarding linearity, LOD, LOQ, repeatability, and intermediate precision (retention time and peak area) for BP in both samples.

SAMPLE	Sunscreen		Swimming pool water	
Linear range	0.564 - 1250 µg g^{-1}		1.11x10^{-5} - 0.025 mg mL^{-1}	
Regression Equation	Slope	113 (3)	Slope	5713 (4)
	Intercept	98.3 (3)	Intercept	0.6 (3)
	r^2	0.99 (3)	r^2	0.999 (1)
LOD	169 (5) ng g^{-1} (n=3)		3 (4) ng mL^{-1} (n=3)	
LOQ	564 (6) ng g^{-1} (n=3)		11 (5) ng mL^{-1} (n=3)	
Precision				
Retention time SD% (n=6)			Repeatability	0.25
			Intermediate Precision	0.86
Peak area SD% (n=6)			Repeatability	0.43
			Intermediate Precision	4.60

Table 4. Method validation regarding accuracy

Aliquots[a]	Sunscreen Sample				Swimming Pool Sample		
	Base value[b] (mg g^{-1})	BP added (mg g^{-1})	Recovered[c] (mg g^{-1})	Recovery[d] ±SD (%)[e]	BP added (mg mL^{-1})	Recovered[c] (mg mL^{-1})	Recovery[d] ±SD (%)[e]
I	0.86	0.00	0.00	-	1.11x10-5	1.13x10-5	101.80 (2)
II	0.86	0.05	0.05	99.6 (5)	0.005	0.005	99.530 (5)
III	0.86	0.25	0.25	101.1 (5)	0.010	0.009	98.850 (5)
IV	0.86	0.50	0.49	98.6 (3)	0.015	0.014	99.930 (7)
V	0.86	1.00	1.00	100.39 (6)	0.020	0.020	100.200 (1)
VI	0.86	1.25	1.24	99.9 (3)	0.025	0.025	99.99 (6)

[a]Aliquots of sunscreen samples consisted in 1 g of the semisolid product and 10mL for swimming pool samples.

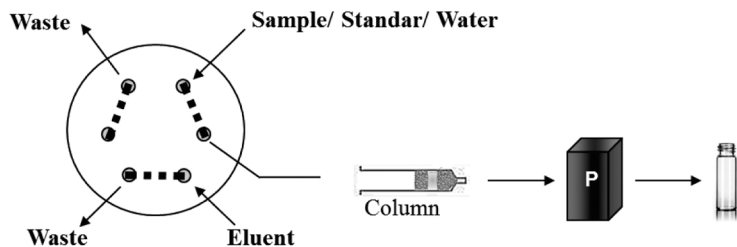[b]Sample without addition BP.

[c]Recovered: found- base value.

[d]Recovery (%): (Recovered/added value) x 100.

[e]n=3

Highlights

- A Si-MIP/HPLC-UV analytical system was developed and validated for benzylparaben analysis.

- The Si-MIP/HPLC-UV procedure was highly selective for its application in complex matrixes.

- The method is simpler and faster than previous and shows high stability.

- Lower LOQ and good recovery factor were obtained with routinely used equipment.

- The whole procedure avoid the use of large volumes of pollutant solvents.
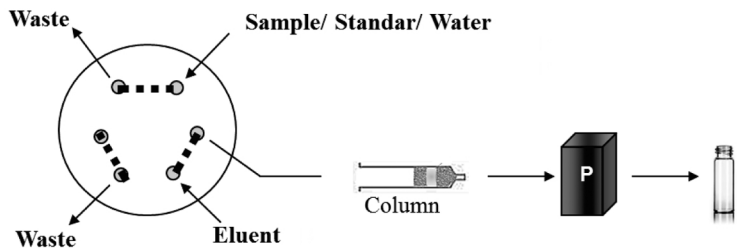
a- Sample/Standar preconcentration step

Waste

Sample/ Standar/ Water

Waste

Eluent

Column
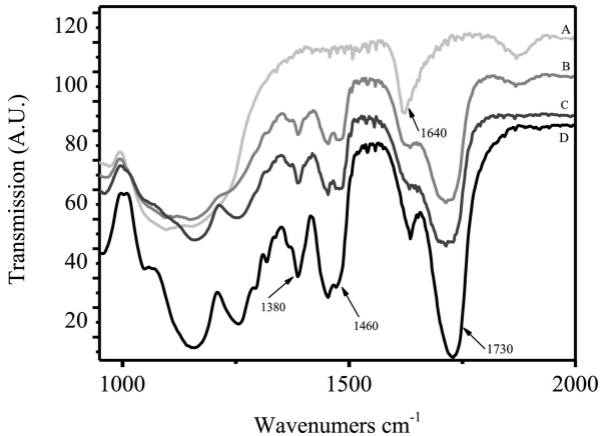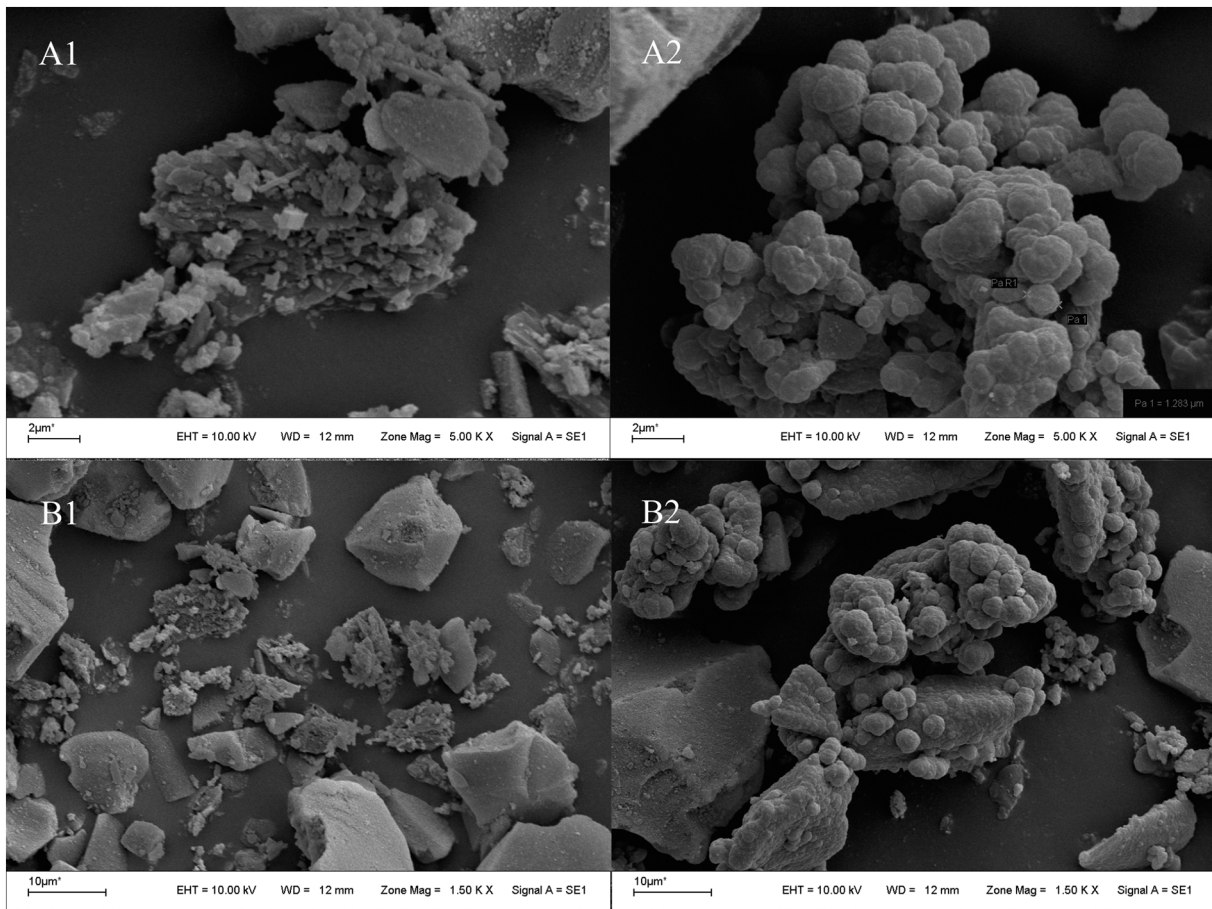
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b – Elution Step

Waste

Sample/ Standar/ Water

Waste

Eluent

Column

P

Figure 1

Figure 2

Figure 3

Figure 4