https://dx.doi.org/10.21577/0103-5053.20230093

J. Braz. Chem. Soc., Vol. 34, No. 11, 1641-1651, 2023

Article

Polyvinyl Alcohol/Pectin-Based Hydrogel as Sorptive Phase for the Determination of Freely Dissolved Parabens in Urine Samples by LC-DAD

Andressa Marinho[a] *and Bruno José G. da Silva*[*,a]

[a]*Departamento de Química, Universidade Federal do Paraná (UFPR), 81531-980 Curitiba-PR, Brazil*

An extraction phase based on hydrogel disks of polyvinyl alcohol and pectin was developed, characterized, and evaluated for the extraction of four endocrine disruptors (methylparaben, ethylparaben, propylparaben and butylparaben) in free form in human urine samples with subsequent determination by liquid chromatography coupled with a diode array detector (LC-DAD). The proposed hydrogel showed easy synthesis, was made of low-cost and non-toxic polymers, and showed that it has an amphiphilic character. Gels with lower swelling indexes (175%) and more concentrated dispersions of polymers (P20PC2.0) showed lower relative standard deviation (RSD) values. Under optimized solid phase extraction (SPE) conditions, the LC-DAD method presented limits of quantification of 0.1 μg mL^{-1}, accuracy values ranged from 101.3 to 118.2%, while the inter-assay precision ranged from 1.5 to 17.4%. In addition, the polyvinyl alcohol (PVOH)/pectin-based material presented the possibility of reuse at least 15 times, without presenting carryover effect and physical degradation. In this way, the hydrogel shows promise for the extraction of organic compounds with different polarities in biological matrices.

Keywords: hydrogel, polyvinyl alcohol, pectin, human urine, parabens, solid phase extraction

Introduction

Currently, the sample preparation step has become indispensable for the development of analytical methods. This is justified by the complexity of the matrices (environmental, food and biological) and the low concentration of the compounds of interest in them.[1] Thus, the extraction techniques aim to promote a pre-concentration of the analytes and eliminate interference from the matrix, consequently resulting in less damage to the analytical system.[2] In this sense, the liquid-liquid extraction (LLE) and solid phase extraction (SPE) techniques stand out; however, the SPE has advantages over LLE, such as providing higher pre-concentration and reduction of the matrix effect, using smaller volumes of organic solvent, generating a smaller amount of waste and being a less laborious technique.[3-5] However, commercially available extractor phases for application in SPE have certain disadvantages due to the cost of the commercial phase, especially those that can operate in a smaller polarity range and the fact that they are disposable, the latter of which entails significant expenses with consumables and waste generation.[6-8] In this way, many studies[9-14] have been conducted in the development of new extractor phases, so that they are more selective, with a stronger interaction between analyte and sorbent phase, higher chemical and/or physical stability, and the possibility of reuse and lower cost. These phases consist of molecularly imprinted polymers (MIP), restricted access materials (RAM), nanofibers, metalorganic structure and hydrogels.[9-14]

The hydrogel can be defined as a non-fluid colloidal network or polymeric network that expands throughout its entire volume by a fluid, this being water.[15] They have been developed as sensitive stimulus materials, that is, they present responses to the finest changes in the space that contains them, whether physical (temperature, light, pressure, magnetic field), chemical (ionic strength, pH) or biological changes (enzymes, antibody/antigen). In addition, the presence of hydrophilic groups in its structure allows the passage of a large amount of water without disintegrating the material. Due to these characteristics, the material has great potential for application as sorptive phase.[16-19] In the literature, some works are reported using hydrogel for this purpose, such as, for the extraction of hormones,[20] anti-inflammatories[21] and alkaloids[22] in water samples, as well as for the extraction of antibiotics[23] in food samples and pesticides[24] on vegetables. However,

*e-mail: bruno.quimica@ufpr.br
Editor handled this article: Andréa R. Chaves (Associate)

most applications of hydrogels as a sorptive phase are for application in environmental and food matrices, and for the extraction of compounds in biological matrices. There are only seven works reported in the literature, and these present applications of the hydrogel for the extraction of aromatic polycyclic hydrocarbons,[25] cancer biomarkers,[26] atenolol[27] and anti-inflammatory drugs[28] in urine samples; the extraction of antifungals[29] and antidepressants[30] in human plasma; and parabens[31] in human milk samples. However, this small number of research papers broadens the horizon for exploring the application of the hydrogel as a sorbent phase, especially for biological matrices.

Thus, in the present work, hydrogel disks were developed by combining polymers of synthetic origin (polyvinyl alcohol) and of natural origin (pectin) for application in SPE. And for this purpose, the compounds methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB) were selected, as they are a class of preservatives widely used in hygiene and cosmetic products, and are currently recognized as endocrine disruptors because they alter the functioning of the human body and cause a hormonal imbalance.[32-36] Therefore, the monitoring of these compounds has become increasingly important.

Hence, the objective of the present work is the application of hydrogel disks for the extraction of parabens in human urine samples and subsequent determination by liquid chromatography coupled to a diode array detector (LC-DAD).

Experimental

Reagents and materials

High purity standards ≥ 99.0% of the analytes, MPB, EPB, PPB, BPB and the internal standard (IS), atrazine (ATZ), were provided by Sigma-Aldrich (Saint Louis, USA). High performance liquid chromatography (HPLC) grade solvents employed were acetonitrile (ACN) and methanol (MeOH), supplied by Biograde (Quebec, CA). Ultrapure water with controlled resistivity of 18.2 MΩ cm, purified by the Millipore-Simplicity UV system (Bedford, USA) was used.

The polymers used in the synthesis of the hydrogels were polyvinyl alcohol (PVOH) (> 99% hydrolyzed, molar mass (MM): 89,000-98,000 g mol^{-1}) provided by Sigma-Aldrich (Saint Louis, USA). Citrus pectin (PC) was purchased at Municipal Market of the city of Curitiba, PR, Brazil and was purified through the dialysis process using regenerated cellulose membrane with molecular weight cut off (MWCO) from 12 to 14 kDa (Spectra/Por®) and was packed as a dispersion of 10% PC and immersed in a bath of distilled water for 72 h and then lyophilized for 120 h. The crosslinking agent, anhydrous citric acid, was supplied by Qhemis (Washington, D.C., USA).

The stock solutions of each analyte and the IS were prepared individually by dissolving the high purity standards in MeOH (HPLC grade), resulting in concentrations of 1.0 g L^{-1}. The working solutions were prepared from the stock solutions, containing the four analytes, at a concentration of 100.0 and 10.0 μg mL^{-1}, and a IS working solution at a concentration of 100.0 μg mL^{-1}; such solutions were stored away from light at –20 °C.

LC-DAD analyzes

For the chromatographic determinations, a liquid chromatograph Varian® (Santa Clara, USA), model LC920, equipped with a Rheodyne® injector (with a 20.0 μL loop) and a diode array detector (DAD) was used. For the chromatographic separation, an octadecylsilane column (C18, 5 μm, 150 mm × 4.6 mm) was used, with a similar stationary phase guard column. The mobile phase composition was 50% ultrapure water (solvent A) and 50% ACN (solvent B) in an isocratic manner for a period of 10 min. The analyzes were performed at room temperature (25 °C), the injection volume was 50 μL and the wavelength of 254 and 270 nm were monitored for the four analytes and 222 nm for the IS.

Preparation of the hydrogel disks

Initially, individual aqueous dispersions of PVOH (10, 15, 20% m/v) were prepared using heating (ca. 95 °C) and stirring (120 min) and of dialyzed PC (1.0, 1.3, 1.5, 2.0, 2.7 and 3.0% m/v) using stirring (120 min).

The synthesis of the hydrogel disks was performed following the methodology described by Sampaio *et al.*[20] For this, a dispersion was prepared in the proportion 1:1 (m/m) of the individual dispersion of PVOH with the individual dispersion of dialyzed PC. This new dispersion was under magnetic stirring for 30 min, then an amount of citric acid corresponding to 10% of the total mass of the polymers was added. In order to solubilize the citric acid, agitation was used for 10 min. Then, approximately 1.0 g of the dispersion was transferred to a 10 mL beaker, which served as a mold for the disks. These gels were then transferred to an oven at 60 °C for a period of 15 h, for crosslinking and drying. After this period, the hydrogels were removed from the molds and cut to the appropriate size for coupling to the extraction device. Figure 1 presents a detailed scheme for the synthesis of hydrogel disks.

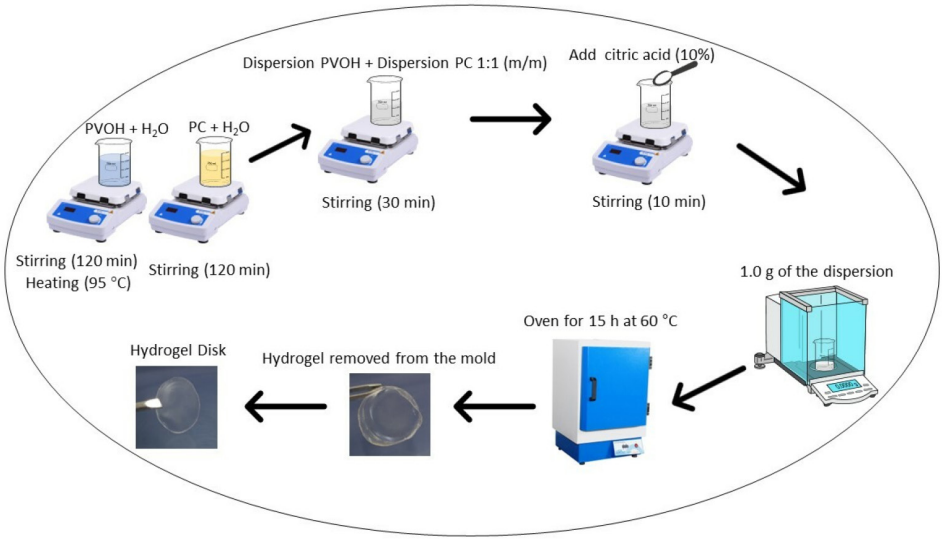

Figure 1. Scheme for the preparation of the hydrogel disks.

The hydrogel disks were synthesized by evaluating: different proportions between the PVOH:PC polymers, that is, 10:1 and 7.5:1, respectively, as well as the concentration of the individual polymer dispersions, keeping the proportions fixed between polymers.

Sample preparation

The extraction devices consisted of a reusable polycarbonate syringe filter holder support for a 25 millimeter diameter membrane filter inside where the extraction disks were placed together with an O-ring. For the extraction process, a manifold system was used, where the extraction devices were positioned and to which a 20 mL syringe without the plunger was attached so that the sample could be poured. Figure 2 shows the device represented.

Initially, the conditioning of the hydrogel disk was performed by percolation of 20.0 mL of a mixture H_2O:MeOH 1:1 (v/v) followed by 20.0 mL of ultrapure water followed by sample percolation through the material. The hydrogel disk was then dried for 30 min. The desorption of the analytes occurred by the percolation of MeOH through the extraction disk with a flow rate of 3 mL min^{-1}, the extract obtained was dried and reconstituted in 1 mL of mobile phase, and then analyzed by LC-DAD. The entire extraction procedure, from the conditioning step to the elution step, took place with the aid of a vacuum, at a pressure of 5 mmHg.

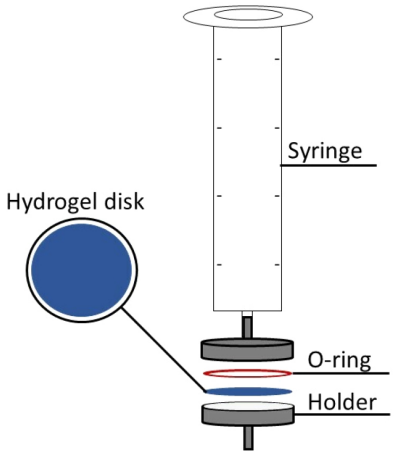

Figure 2. Schematic of the hydrogel-SPE device.

Optimization

The optimal extraction conditions were evaluated using a 2^3 factorial design with triplicate of the central point, containing the following parameters: sample volume (10, 20 and 30 mL), sample percolation flow rate (1.0, 2.0 and 3.0 mL min^{-1}), and pH (3.0, 5.0 and 7.0) (Table S1, Supplementary Information section). The optimal conditions of analyte desorption, either in steps or direct, with volumes of up to 5 mL of methanol, were evaluated in a univariate way, taking into account the number of elutions.

Physical-chemical characterization of the hydrogel

The extracting phase was characterized by means of physical-chemical tests of swelling degree (SI) and water loss. Both tests were performed in triplicate and at room temperature. To assess the degree of swelling, the dry hydrogel was weighed (M_O) and then submerged in 30 mL of ultrapure water, then every 30 min, for 180 min, the mass of the swollen hydrogels (M_t) was measured, and the degree of swelling evaluated according to equation 1.

$$SI(\%) = \left(\frac{M_t - M_0}{M_0} \right) \times 100 \quad (1)$$

For the water loss (WL) study, the hydrogel was submerged in 30 mL of water overnight, thus ensuring that it reached swelling equilibrium (M_{eq}). Then, the hydrogel mass variation was monitored for 240 min, with intervals of 30 min (M_t), and the percentage of water loss was calculated, based on equation 2.

$$WL(\%) = \left(\frac{M_{eq} - M_t}{M_{eq}} \right) \times 100 \quad (2)$$

Method validation parameters

Stability

The stability parameter of parabens, both in biological fluids and in solvent, is already well established in the literature. In this way, a review was carried out to respect for these values.

Linearity, LLOQ and LOD

Linearity was evaluated for the concentration range, with five levels, from 0.1 to 1.0 μg mL^{-1}, based on studies[37,38] that used the same chromatographic technique for the determination of parabens in urine. For this purpose, human urine samples were used as a blank enriched with the analytes in the aforementioned concentration range, with the IS at the fixed concentration of 0.5 μg mL^{-1}. The assay was performed in triplicate.

The lower limit of quantification (LLOQ) was determined as the lowest concentration on the analytical curve, with relative standard deviation (RSD) values of up to 20% and accuracy between 80-120%. The limit of detection (LOD) was determined using equation 3.

$$LOD = \frac{3.3 \times sd}{a} \quad (3)$$

where sd is the standard deviation of the intercept and a is the slope of the analytical curve.

Precision

The reproducibility of the method was determined through precision tests, which were evaluated according to the RSD values for each of the concentrations on the analytical curve. These values must be less than 20 and 15% for LLOQ and other points on the curve, respectively.

Accuracy

Accuracy was evaluated through the proximity between the actual concentration value and the value obtained through the interpolation of points in the equations of the analytical curve defined for each analyte. This value must comprise the range of 80-120% and 85-115%, for LLOQ and other points, respectively.

Reuse of the sorptive phase

The same hydrogel disk was subjected to fifteen consecutive extractions performed on two consecutive days. Between each extraction, a cleaning step was performed with 20.0 mL of H_2O:MeOH 1:1 (v/v) followed by 20.0 mL of ultrapure water, to avoid the carryover effect. As the extractions were conducted on different days, the extraction device containing the hydrogel disk was stored submerged in ultrapure water at 4 °C to ensure swelling equilibrium.

Real samples

The developed method was applied to 3 different urine samples, donated by male and female volunteers aged between 28 and 65 years. Samples were fortified with analytes and internal standard (IS), both at a concentration of 0.5 μg mL^{-1}. Samples were analyzed in triplicate. The use of human urine for the development of this work was approved by the Research Ethics Committee of the Health Sector of the Federal University of Paraná, CAAE 4626712120000102.

Results and Discussion

LC-DAD analyzes

The chromatogram obtained for the separation of the four parabens is shown in Figure 3. The elution order of the compounds depends on their interaction with the stationary phase. As reverse phase chromatography was used, where the stationary phase is non-polar, the order of elution will be as follows: first the MPB, which is the most polar because it has the smallest alkyl chain of the ester group and therefore less interaction with the stationary phase. Like this, basically, by adding the carbon chain linked to the ester group of parabens, the second, third and fourth chromatographic peak corresponds to, respectively, EPB, PPB and BPB, the latter presenting the longest elution time.

Composition of the sorptive phase

The evaluation of the extracting phase was carried out based on two parameters. First, it was evaluated the proportion between the constituent polymers which are 7.5:1 and 10:1 PVOH:PC, respectively. The second evaluation was the mass variation that keeps the aforementioned

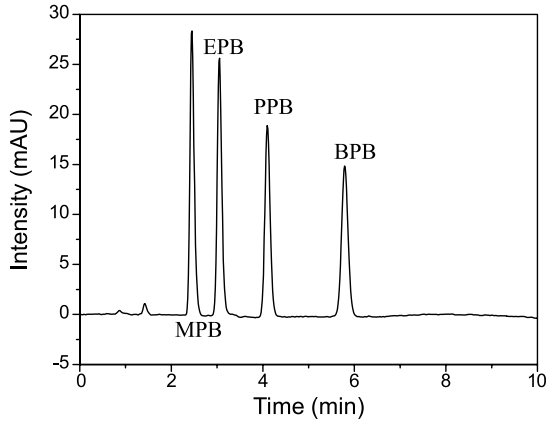

Figure 3. Chromatogram of paraben standards in methanol 1 μg mL^{-1}.

proportions fixed and changing the concentrations of the polymer dispersions used. Since PVOH dispersions above 20% have high viscosity and are difficult to handle, this concentration was selected as the maximum working limit. In addition, PVOH concentrations lower than 10% turn out to be a very malleable gel and deform inside the support used for the extractions leading to high inaccuracies and sample loss during the SPE processes. Therefore, PVOH concentration lower than 10% was selected as the minimum working concentration.[20]

Figure 4 shows the average peak area of each analyte for the hydrogels, P10PC1.0, P15PC1.3, P20PC2.0, P10PC1.3, P15PC2.0 and P20PC2.7. To differentiate each of the evaluated hydrogels, the acronym PxPCy was used, where x presents the concentration of the individual dispersion of PVOH and y the concentration of the individual dispersion of PC, both in percentage (m/v).

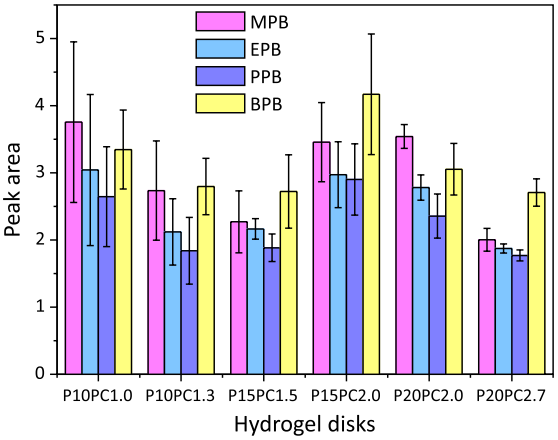

Figure 4. Average areas of chromatographic peaks (n = 4) of parabens according to the composition of the hydrogel used in the extraction of the analytes, at the concentration of 5.0 μg mL^{-1}.

First, all evaluated hydrogel disks were capable of extracting all parabens. Based on the Figure 4, it is possible to verify that the hydrogels with a lower concentration of polymers presented a higher relative standard deviation (RSD) value, for example, the P10PC1.0 gel presented deviations in the range of 17.6% for BPB and up to 37.0% for MPB, while for the P10PC1.3 gel the deviations showed values of up to 27.0% for MPB and PPB. The extraction disks with the highest concentrations of PVOH and PC dispersions showed a lower RSD value, and the gel with the highest concentration of polymers, P20PC2.7, had the lowest RSD value of 3.6% for EPB and up to 8.4% for MPB.

A single-factor analysis of variance (ANOVA), using Origin[39] software, was then applied to verify whether there was a significant difference between the gels, that is, whether any hydrogel had a higher extraction efficiency. With the aid of the statistical test, a significant difference was found in the peak area means for MPB and BPB, for a 95% confidence interval. However, the EPB and PPB showed no significant difference. To find out which hydrogels showed a significant difference between them, the Tukey's test was applied, and for MPB, the disks that showed a significant difference were P10PC1.0 and P20PC2.7, with the former showing a significantly greater response. As for the BPB, the difference occurred in the disks P20PC2.7 and P15PC2.0 and also P15PC2.0 and P15PC1.5. Based on the results obtained through ANOVA, it was decided to exclude the hydrogels that showed a significant difference for at least one of the analytes. Thus, the P15PC1.5 and P20PC2.7 hydrogels were discarded. The remaining hydrogels did not show a significant difference for any of the analytes, so the selection was based on the RSD value, and the hydrogel that presented the lowest RSD value was then selected. Consequently, the P20PC2.0 disk was selected as the sorbent phase and used in subsequent steps.

Characterization of the sorptive phase

Degree of swelling

The graph in Figure 5a shows the swelling behavior of the hydrogels evaluated as an extractor phase. For all evaluated hydrogels, the swelling equilibrium was reached in the first measurement, in 30 min, which presents an advantage for the SPE process, because the faster the swelling, the faster the material will be able to perform the extractions. Furthermore, increasing the polymer concentration caused the hydrogel to exhibit a lower degree of swelling. The more concentrated dispersions of polymers have a higher number of reactive sites for crosslinking, therefore they have a higher crosslinking density, thus increasing the retraction force of the polymeric network, which is the opposite force to swelling, and as a result, they present a lesser degree of swelling.[40]

While the swelling percentage of the P10PC1.3 hydrogel was about 240%, that of the P20PC2.0 hydrogel was

approximately 175%. Thus, it is noted that the hydrogel disks that presented a lower RSD value for the extraction were those that presented a lower degree of swelling, giving evidence that the degree of swelling can interfere in the extraction process. According to Sampaio *et al.*,[20] in hydrogels that absorb greater amounts of water, that is, those that use less concentrated dispersions of polymers, the distance between the polymer chains is greater, which makes it difficult for the analyte to be retained by the sorbent phase. Another consequence of greater water absorption is that, due to the greater distance between the chains, the diffusion of solutes is greater. This greater retention difficulty and increased diffusion may explain the higher RSD value of extractions with hydrogels of less concentrated dispersions, when compared to those of more concentrated dispersions.

Water loss

The water loss of hydrogel disks obtained for this study are shown in Figure 5b. The hydrogels with PVOH concentrations of 10 and 15% showed behavior very similar to the water loss process, which was around 60%. For PVOH concentrations of 20%, the percentage of water loss was lower, around 53% for the P20PC2.0 hydrogel and 49% for the P20PC2.7.

As water loss is a parameter directly proportional to the degree of swelling, lower concentrations end up leading to higher rates of solvent and solute diffusion from the inside to the outside of the polymeric network, thus showing that this can be the cause of higher RSD values of hydrogels that used less concentrated polymer solutions.[20]

Optimization

Extraction

With the sorbent phase selected (P20PC2.0), a 2^3 factorial design was used with triplicate of the central point, and the best extraction conditions for more than 75% of the analytes were obtained with pH 3.0, volume of 30 mL and sample percolation flow rate of 1 mL min^{-1} (Table S2, Supplementary Information section); these conditions were then selected as extraction conditions in subsequent tests.

According to the Pareto chart (Figure S1, Supplementary Information section), only the PPB and BPB analytes showed significant main and interaction effects. PPB had a first-order effect in relation to pH, while BPB had a third-order effect.

This change in the PPB extraction efficiency is related to the composition of the extracting phase. When the pH of the medium is greater than the pK_a of PVOH (4.76), the hydroxyl ions produce hydrolysis of the residual acetate groups and leading to a structure with a high ionic charge density. The high ionic charge density causes repulsion between the polymeric chains which increases the distance between the chains and generates a greater degree of swelling and greater diffusion of analytes.[41] In addition, PC, regardless of the degree of methoxylation, has a pK_a in the range of 3.55-4.10, so at pH < pK_a, the structure is in its neutral form, forming hydrogen bonds between the hydroxyl groups of the pectin and the carboxyl groups of the residual acetates, which in turn decreases the distance between the chains and increases the retraction force, resulting in a lower degree of swelling.[42] Consequently, for both polymers used in the synthesis, a lower pH promotes a lower degree of swelling, which in turn leads to less diffusion of solutes, favoring extraction.

Larger sample volumes lead to a greater amount of analyte matter, while lower sample percolation rates lead to longer contact times between the analytes and the extractor phase, which generates two distinct results. The first result be greater interaction with the extractor phase and better extraction efficiency, and is generally observed

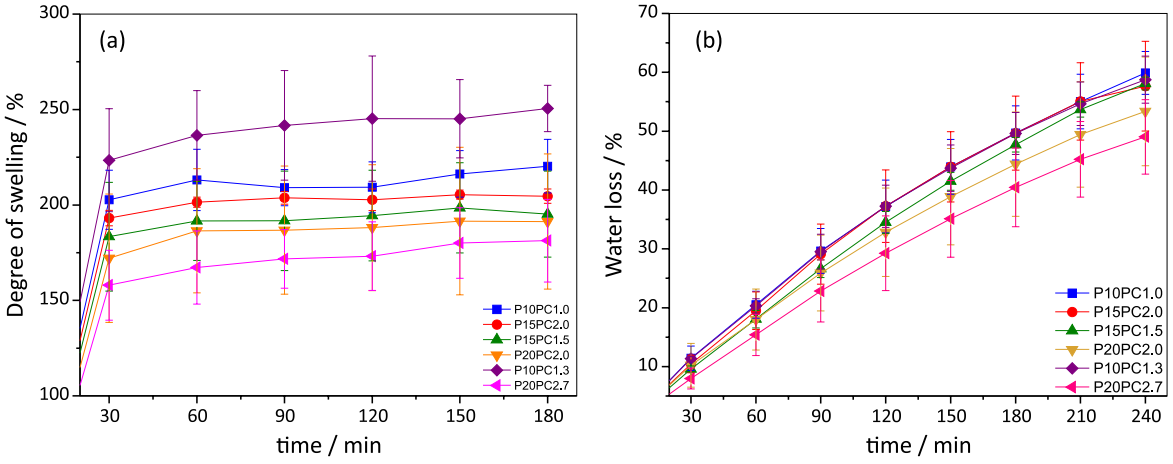

Figure 5. (a) Degree of swelling and (b) water loss of hydrogel disks.

for compounds of lower polarity. The second result, due to longer contact time, can cause the sample itself to promote the desorption of the analytes. This result is more common in compounds of higher polarity and greater affinity with the sample.

The RSD values were calculated using triplicate of the center point, being 26.6, 19.3, 5.4 and 1.8% for MPB, EPB, PPB and BPB, respectively. Higher RSD values for MPB and EPB, analytes of greater polarity, may be correlated with the percolation flow rate of the sample. As previously mentioned, the sample itself may lead to desorption of the analytes due to the contact time. In addition, the greater deviation may be associated with the composition of the extracting phase since PVOH has a more hydrophobic character, hindering water percolation, when compared to more polar hydrogels.[20] In addition through the purification process, PC shows an increase in the degree of methoxylation, that is, the pectin used is classified as high methoxylation (HM),[43] therefore, the increased esterification makes pectin more non-polar. This lower affinity of the polymers with the more polar compounds can explain the higher RSD values presented by them.

Therefore, the third-order interaction effect in relation to BPB, as previously mentioned, is correlated with a lower pH that favors extraction and added to this, a larger sample volume and lower flow rate, increase the amount of matter in the analyte and the contact time with the extractor phase. As BPB is the most non-polar compound among those evaluated, the lowest flow rate does not have negative effects on its interaction with the extractor phase, unlike the more polar ones, thus causing an effect between these factors and increasing the extraction efficiency.

Desorption

First, the desorption of the analytes in stages was evaluated, using the methanol solvent. Thus, for analyte desorption, 6 elution steps with 1.0 mL each were evaluated. Each extract obtained was analyzed separately. The results obtained show that the volumes of 4.0 and 5.0 mL were responsible for more than 88 and 96% of the accumulated area, respectively (Table S3, Supplementary Information section), thus, were evaluated using the direct elution mode. The extracts obtained from each of the volumes mentioned above were dried and reconstituted with 1.0 mL of methanol, with the purpose of matching the final volumes of the extracts of each of the elution modes and making it possible to compare the tests. Using volumes of 4.0 and 5.0 mL, in the direct elution mode and in stages, the latter promoted a better desorption of the analytes, showing a chromatographic peak area gain of approximately 4 times. Analogously to what happens in the LLE, in the SPE, the addition of aliquots of smaller volume of solvent promotes a longer contact time with the analytes, which favors their desorption and thus increases the extraction efficiency, when compared to the addition of a larger volume of solvent in a single step. Hence, for the elution of the analytes in the last stage of the extraction procedure, the elution mode that promoted an accumulation of chromatographic peak area greater than 95% was selected, that is, the mode in stages with a volume of 5.0 mL in 5 additions of 1.0 mL each.

Method validation parameters

Stability

The stability of the four analytes in methanol is two months with storage at 4 °C,[38] and six months when stored at –20 °C.[44,45] Therefore, the maximum period established for the storage of solutions in methanol was for six months at –20 °C.

The stability of parabens in the working matrix, that is, in urine, is 40 days in three temperature conditions, which are, room temperature, at 4 °C and freezing at –20 °C.[37] It also features stability of six months, even going through several freeze-thaw cycles.[46] There are also reports in the literature that the analytes present stability in urine for up to 30 months when stored at –70 °C.[47]

These studies demonstrate that the evaluated analytes are stable in the matrix of interest for a long period of time, at different temperatures and at different cycles of freezing and thawing.

Linearity, LLOQ and LOD

Table 1 presents the linear regression data, the LLOQ and LOD values for each analyte.

Linear regression was satisfactory for the four analytes, with a coefficient of determination (R^2) above 0.99. The LLOQ showed RSD values below 20% and accuracy between 80-120%. It is noteworthy that with the decrease in polarity, there is a decrease in the RSD value for the LLOQ and lower LOD values. This result is consistent with the fact that analytes of lower polarity suffer less the negative effect of the desorption of analytes from the sorbent phase by the matrix itself, in addition to greater interaction with the extractor phase.

Precision and accuracy

Precision and accuracy data are shown in Table 2.

The analytical methodology showed acceptable precision, with RSD values lower than 20% for the LLOQ and 15% for the other points, for the four analytes. As well as an accuracy between 80-120% and 85-115%, for the lowest concentration of the analytical curve and other

Table 1. Linearity parameters

Analyte	Equation of straight line ($y = ax + b$)	Standard errors	R^2	LLOQ			LOD / (μg mL^{-1})
		a/b		Concentration / (μg mL^{-1})	RSD / %	Accuracy / %	
MPB	$y = 0.49627x - 0.02629$	0.021/0.009	0.9926	0.1	17.4	118.2	0.034
EPB	$y = 0.63600x - 0.02141$	0.022/0.006	0.9951	0.1	12.1	109.8	0.020
PPB	$y = 0.96275x + 0.01072$	0.009/0.004	0.9996	0.1	6.4	101.7	0.007
BPB	$y = 1.24171x + 0.02220$	0.017/0.003	0.9992	0.1	3.8	101.3	0.005

MPB: methylparaben; EPB: ethylparaben; PPB: propylparaben; BPB: butylparaben; R^2: coefficient of determination; LLOQ: lower limit of detection; RSD: relative standard deviation; LOD: limit of detection.

Table 2. Precision and accuracy of the hydrogel-solid phase extraction (SPE) procedure

Analyte	Concentration / (μg mL^{-1})	RSD / %	Accuracy / %
MPB	0.1	17.4	118.2
	0.5	10.1	108.0
	1.0	6.4	102.1
EPB	0.1	12.1	109.8
	0.5	14.2	103.3
	1.0	3.4	102.8
PPB	0.1	6.4	101.7
	0.5	1.5	99.8
	1.0	9.0	96.6
BPB	0.1	3.8	101.3
	0.5	5.0	99.5
	1.0	6.8	98.8

MPB: methylparaben; EPB: ethylparaben; PPB: propylparaben; BPB: butylparaben; RSD: relative standard deviation.

points, respectively. In addition, as different hydrogel discs were used for each analytical curve, using PVOH and pectin dispersions prepared on different days, it is possible to state based on the RSD values that the developed material presents satisfactory reproducibility.

Reuse of sorptive phase

The extractor phase showed physical and mechanical stability, since it remained intact and did not show damage, even when exposed to vacuum, water, organic solvent (MeOH) and biological sample (pH 3.0) during extractions, in addition to storage under refrigeration. The results obtained from this test are shown in Figure 6.

The random dispersion of the data indicated that there was no carryover effect, that is, the cleaning and reconditioning step of the sorbent phase proved to be efficient, allowing reuse. The RSD values obtained for the extractions were 29.0, 18.4, 10.4 and 7.0% for MPB, EPB, PPB and BPB, respectively. The greater interaction of the sorptive phase

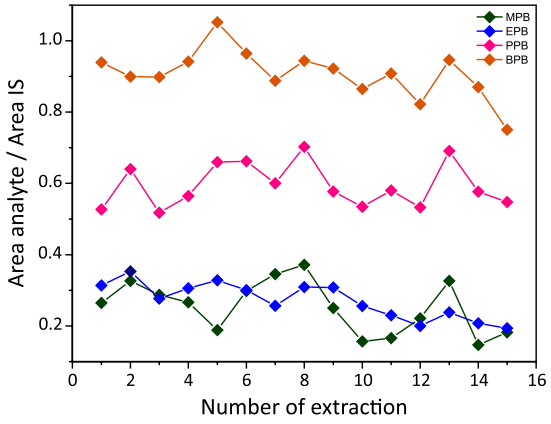

Figure 6. Area analyte/area IS ratio of 15 subsequent extractions for parabens using P20PC2.0 hydrogel.

and the lower negative effect of desorption explain the lower RSD values for PPB and BPB compounds when compared to MPB and EPB. However, it was possible to perform 15 extractions with RSD values < 20% for more than 75% of the studied compounds. It is concluded that despite the need for more careful studies regarding the synthesis and interaction processes, the absence of carryover effect and stability of the hydrogel present a positive result regarding its reuse and may be evaluated in the future for an even greater number of extractions, mainly because there was no tendency to decrease the performance of extraction up to fifteen times of use. In this way, the possibility of reuse leads to a lower cost of the material, in addition to contributing to the principles of green chemistry for less waste generation.

The determined LLOQ and LOD values for parabens are in accordance with that found in the literature using similar analytical techniques (Table 3). It is also observed that works that present lower values of these parameters used detectors more sensitive, such as the mass spectrometer, however, entail a greater cost. Therefore, the developed method has the advantage of not requiring pre-treatment of the sample, which reduces the number of steps in the procedure, in addition to using lower cost equipment, thus making the method cheaper and less time consuming.

Table 3. Comparison of methods for determining MPB, EPB, PPB and BPB in urine samples

Sample preparation	Analytical technique	Pre-treatment urine	LLOQ / (μg mL^{-1})	LOD / (μg mL^{-1})	Reference
DLLME	cLC-UV	enzymatic hydrolysis	0.023-0.036	0.007-0.011	48
FPSE	LC-DAD	–	0.01	0.003	38
FPSE	LC-DAD	–	0.1	0.03	37
MEPS	LC-MS/MS	enzymatic hydrolysis	0.00005-0.00033	0.0001	49
SPE	UPLC-MS/MS	enzymatic hydrolysis	0.0003-0.001	0.00009-0.00037	50
SPE	LC-DAD	–	0.1	0.005-0.034	this work

DLLME: dispersive liquid-liquid microextraction; FPSE: fabric phase sorptive extraction; MEPS: microextraction by packed sorbent; SPE: solid phase extraction; LLOQ: lower limit of detection; LOD: limit of detection; cLC: capillary liquid chromatography; UV: ultraviolet detector; LC: liquid chromatography; DAD: diode array detector; MS/MS: tandem mass spectrometry; UPLC: ultra-high performance liquid chromatography.

Real samples

The spiked samples were evaluated according to precision (RSD) and recovery, expressed in terms of accuracy. The results are shown in Table 4 and the chromatogram is shown in Figure 7.

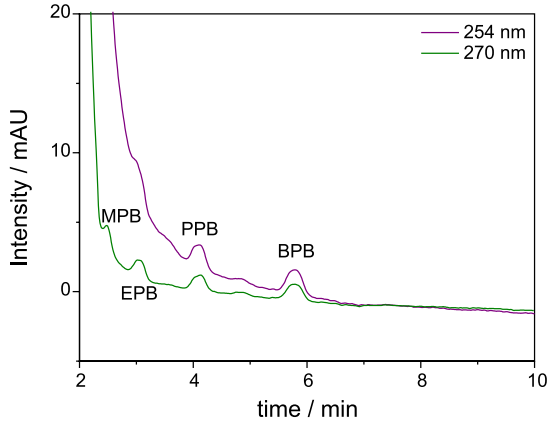

Figure 7. Chromatogram of urine spiked with parabens 0.5 μg.

For the EPB and PPB, the precision values were satisfactory for the three samples, that is, below 15%, as well as the accuracy expressed in terms of recovery, with values between 85 and 115%. BPB, on the other hand, presented acceptable recovery results for the three samples and precision for samples 1 and 2. MPB, on the other hand, was the analyte that presented the most inconsistent discrepant results, with RSD values above 15% and recovery below 85%. As previously explained, the MPB has a log K_{ow} of 1.96, showing a much more polar character than the other analytes evaluated. Thus, there may be competition between the analyte-extractor phase and analyte-matrix, justifying the low precision, as well as possibly causing a greater matrix effect for this compound in the evaluated samples. As human urine compositions vary greatly between individuals, the method did not present a satisfactory result for the determination of MPB in these samples, which does not mean that the method cannot be applied for the determination of this analyte in other samples, since it showed acceptable linearity, precision and accuracy.

Table 4. Results of the application of the methodology developed (n = 3)

Analyte	Sample		
	1	2	3
MPB			
Added concentration / (μg mL^{-1})	0.50	0.50	0.50
Measured concentration / (μg mL^{-1})	0.40	0.44	0.53
RSD / %	20.4	36.2	30.1
Recovery / %	80.7	88.0	105.6
EPB			
Added concentration / (μg mL^{-1})	0.50	0.50	0.50
Measured concentration / (μg mL^{-1})	0.48	0.50	0.54
RSD / %	14.2	15.0	6.6
Recovery / %	97.1	100.2	107.2
PPB			
Added concentration / (μg mL^{-1})	0.50	0.50	0.50
Measured concentration / (μg mL^{-1})	0.51	0.56	0.51
RSD / %	10.8	13.6	10.0
Recovery / %	102.0	112.9	102.0
BPB			
Added concentration / (μg mL^{-1})	0.50	0.50	0.50
Measured concentration / (μg mL^{-1})	0.52	0.59	0.55
RSD / %	10.8	14.6	18.2
Recovery / %	105.2	117.9	110.5

MPB: methylparaben; EPB: ethylparaben; PPB: propylparaben; BPB: butylparaben; RSD: relative standard deviation.

Therefore, the method developed for the determination of parabens, in its free form, in other words, unconjugated, in urine samples can be satisfactorily applied, and for parabens with a higher log K_{ow} value there is an increase in precision and accuracy.

Conclusions

In this work, it was possible to develop a non-toxic and low-cost sorbent phase, composed of natural polymers, which have the advantage of being biodegradable, and synthetic polymers, which are capable of conferring physical resistance to the material. In addition, the hydrogels used in SPE were capable of promoting the extraction of the four paraben endocrine disruptors. The parabens were evaluated and shown to have differences in polarity with log K_{ow} in the range of 1.96-3.57, showing amphiphilic character phases, and have a lower cost when compared to commercial phases, such as hydrophilic-lipophilic balance (HLB). However, one of the main objectives in the development of new sorbent materials is that they can be reused. The evaluated hydrogel showed chemical and physical resistance and could be reused for at least 15 times, which leads to a reduction in the cost of the material and contributes to a lower generation of waste. Furthermore, results have shown that in addition to repeatability, the hydrogel also exhibits appropriate reproducibility. Thus, it was possible to use this material, as a sorbent phase, in the development of an analytical methodology that allowed the determination of parabens unconjugated in a biological matrix, such as urine, expanding the applications of the material. However, it is worth mentioning the need for further studies regarding the interaction processes between the hydrogel and the analytes, in addition to the use of this same material for other classes of compounds and different matrices.

Supplementary Information

Supplementary data (referring to the optimization stage of the extraction) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the financial support and scholarships.

References

1. Zhang, C.; Xing, H.; Yang, L.; Fei, P.; Liu, H.; *Chin. J. Chem. Eng.* **2022**, *42*, 245. [Crossref]
2. Xu, L.; Qi, X.; Li, X.; Bai, Y.; Liu, H.; *Talanta* **2016**, *146*, 714. [Crossref]
3. Buszewski, B.; Szultka, M.; *Crit. Rev. Anal. Chem.* **2012**, *42*, 198. [Crossref]
4. Filippou, O.; Bitas, D.; Samanidou, V.; *J. Chromatogr. B* **2017**, *1043*, 44. [Crossref]
5. Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J.; *TrAC, Trends Anal. Chem.* **2015**, *73*, 19. [Crossref]
6. Barbosa, M. O.; Ribeiro, R. S.; Ribeiro, A. R. L.; Pereira, M. F. R.; Silva, A. M. T.; *Sci. Rep.* **2020**, *10*, 22304. [Crossref]
7. de Noronha, B. V.; Fernando Bergamini, M.; Marcolino Jr., L. H.; da Silva, B. J. G.; *J. Sep. Sci.* **2018**, *41*, 2790. [Crossref]
8. Smith, R. M.; *J. Chromatogr. A* **2003**, *1000*, 3. [Crossref]
9. Andrade-Eiroa, A.; Canle, M.; Leroy-Cancellieri, V.; Cerdà, V.; *TrAC, Trends Anal. Chem.* **2016**, *80*, 641. [Crossref]
10. An, J.; Dong, Z.; Zhang, W.; Yan, Y.; Kang, W.; Lian, K.; *Microchem. J.* **2021**, *168*, 106475. [Crossref]
11. Zhang, S.; Yao, W.; Fu, D.; Zhang, C.; Zhao, H.; *J. Sep. Sci.* **2018**, *41*, 1864. [Crossref]
12. Kaur, R.; Kaur, R.; Rani, S.; Malik, A. K.; Kabir, A.; Furton, K. G.; *J. Sep. Sci.* **2019**, *42*, 862. [Crossref]
13. Orachorn, N.; Klongklaew, P.; Bunkoed, O.; *Microchem. J.* **2021**, *171*, 106827. [Crossref]
14. Huang, Y.; Wang, D.; Liu, W.; Zheng, L.; Wang, Y.; Liu, X.; Fan, M.; Gong, Z.; *Food Chem.* **2020**, *316*, 126378. [Crossref]
15. Alemán, J. V.; Chadwick, A. V.; He, J.; Hess, M.; Horie, K.; Jones, R. G.; Kratochvíl, P.; Meisel, I.; Mita, I.; Moad, G.; Penczek, S.; Stepto, R. F. T.; *Pure Appl. Chem.* **2007**, *79*, 1801. [Crossref]
16. Brudno, Y.; Mooney, D. J.; *J. Control. Release* **2015**, *219*, 8. [Crossref]
17. El-Husseiny, H. M.; Mady, E. A.; Hamabe, L.; Abugomaa, A.; Shimada, K.; Yoshida, T.; Tanaka, T.; Yokoi, A.; Elbadawy, M.; Tanaka, R.; *Mater. Today Bio* **2022**, *13*, 100186. [Crossref]
18. Castilhos, N. D. B.; Sampaio, N. M. F. M.; da Silva, B. C.; Riegel-Vidotti, I. C.; Grassi, M. T.; Silva, B. J. G.; *Carbohydr. Polym.* **2017**, *174*, 507. [Crossref]
19. Peppas, N. A.; Khare, A. R.; *Adv. Drug Delivery Rev.* **1993**, *11*, 1. [Crossref]
20. Sampaio, N. M. F. M.; Castilhos, N. D. B.; da Silva, B. C.; Riegel-Vidotti, I. C.; Silva, B. J. G.; *Molecules* **2019**, *24*, 40. [Crossref]
21. Ling, H.; Wu, G.; Li, S.; Zhou, Q.; Li, C.; Ma, J.; *Chin. J. Chromatogr.* **2022**, *40*, 323. [Crossref]
22. Jacumasso, S. C.; de Alvarenga, G.; de Lazzari, A. C.; Sampaio, N. M. F. M.; Silva, B. J. G.; Marchesi, L. F.; Vidotti, M.; Riegel-Vidotti, I. C.; *Appl. Sci.* **2022**, *12*, 10609. [Crossref]
23. Klongklaew, P.; Kanatharana, P.; Bunkoed, O.; *Food Chem.* **2020**, *309*, 125685. [Crossref]
24. Gao, Y.; Gao, M.; Chen, G.; Tian, M.; Zhai, R.; Huang, X.; Xu, X.; Liu, G.; Xu, D.; *Food Chem.* **2021**, *352*, 129187. [Crossref]
25. González-Martín, R.; Pacheco-Fernández, I.; Maiti, B.; Ayala, J. H.; Afonso, A. M.; Díaz, D. D.; Pino, V.; *J. Chromatogr. A* **2020**, *1619*, 460910. [Crossref]

26. Salami, M.; Talebpour, Z.; Alizadeh, R.; *J. Pharm. Biomed. Anal.* **2022**, *219*, 114902. [Crossref]
27. Basan, H.; Yarımkaya, S.; *Luminescence* **2014**, *29*, 225. [Crossref]
28. Fresco-Cala, B.; Gálvez-Vergara, A.; Cárdenas, S.; *Talanta* **2020**, *218*, 121124. [Crossref]
29. Manouchehri, M.; Seidi, S.; Naseri, M. T.; Rouhollahi, A.; *J. Sep. Sci.* **2022**, *45*, 594. [Crossref]
30. Guzella, C. S.; Souto, D. E. P.; Silva, B. J. G.; *Carbohydr. Polym.* **2022**, *294*, 119810. [Crossref]
31. Sampaio, N. M. F. M.; de Oliveira, B. H.; Riegel-Vidotti, I. C.; da Silva, B. J. G.; *Anal. Bioanal. Chem.* **2022**, 1. [Crossref]
32. Aker, A. M.; Watkins, D. J.; Johns, L. E.; Ferguson, K. K.; Soldin, O. P.; Anzalota Del Toro, L. V.; Alshawabkeh, A. N.; Cordero, J. F.; Meeker, J. D.; *Environ. Res.* **2016**, *151*, 30. [Crossref]
33. Lokhnauth, J. K.; Snow, N. H.; *Anal. Chem.* **2005**, *77*, 5938. [Crossref]
34. Nishihama, Y.; Yoshinaga, J.; Iida, A.; Konishi, S.; Imai, H.; Yoneyama, M.; Nakajima, D.; Shiraishi, H.; *Reprod. Toxicol.* **2016**, *63*, 107. [Crossref]
35. Nowak, K.; Ratajczak-Wrona, W.; Górska, M.; Jabłońska, E.; *Mol. Cell. Endocrinol.* **2018**, *474*, 238. [Crossref]
36. Smarr, M. M.; Sundaram, R.; Honda, M.; Kannan, K.; Louis, G. M. B.; *Environ. Health Perspect.* **2017**, *125*, 730. [Crossref]
37. Tartaglia, A.; Kabir, A.; Ulusoy, S.; Sperandio, E.; Piccolantonio, S.; Ulusoy, H. I.; Furton, K. G.; Locatelli, M.; *J. Chromatogr. B* **2019**, *1125*, 121707. [Crossref]
38. Rigkos, G.; Alampanos, V.; Kabir, A.; Furton, K. G.; Roje, Ž.; Vrček, I. V.; Panderi, I.; Samanidou, V.; *Biomed. Chromatogr.* **2021**, *35*, 4974 [Crossref]
39. *OriginPro* 8.5; OriginLab Corporation, USA, 2010.
40. Peng, Z.; Li, Z.; Zhang, F.; Peng, X.; *J. Macromol. Sci. Part B* **2012**, *51*, 1934. [Crossref]
41. Mansur, H. S.; Sadahira, C. M.; Souza, A. N.; Mansur, A. A. P.; *Mater. Sci. Eng.: C* **2008**, *28*, 539. [Crossref]
42. Mishra, R. K.; Datt, M.; Banthia, A. K.; *AAPS PharmSciTech* **2008**, *9*, 395. [Crossref]
43. Lopes, L. C.; Simas-Tosin, F. F.; Cipriani, T. R.; Marchesi, L. F.; Vidotti, M.; Riegel-Vidotti, I. C.; *Carbohydr. Polym.* **2017**, *155*, 11. [Crossref]
44. Martín-Pozo, L.; Gómez-Regalado, M. C.; Moscoso-Ruiz, I.; Zafra-Gómez, A.; *Talanta* **2021**, *234*, 122642. [Crossref]
45. Van Overmeire, I.; Vrijens, K.; Nawrot, T.; Van Nieuwenhuyse, A.; Van Loco, J.; Reyns, T.; *J. Chromatogr. B* **2019**, *1121*, 96. [Crossref]
46. Zhou, X.; Kramer, J. P.; Calafat, A. M.; Ye, X.; *J. Chromatogr. B* **2014**, *944*, 152. [Crossref]
47. Calafat, A. M.; Weuve, J.; Ye, X.; Jia, L. T.; Hu, H.; Ringer, S.; Huttner, K.; Hauser, R.; *Environ. Health Perspect.* **2009**, *117*, 639. [Crossref]
48. Carrasco-Correa, E. J.; Vela-Soria, F.; Ballesteros, O.; Ramis-Ramos, G.; Herrero-Martínez, J. M.; *J. Chromatogr. A* **2015**, *1379*, 65. [Crossref]
49. Silveira, R. S.; Rocha, B. A.; Rodrigues, J. L.; Barbosa, F.; *Chemosphere* **2020**, *240*, 124951. [Crossref]
50. Dewalque, L.; Pirard, C.; Dubois, N.; Charlier, C.; *J. Chromatogr. B* **2014**, *949-950*, 37. [Crossref]

Submitted: February 28, 2023
Published online: June 12, 2023