A simple and fast Double-Flow microfluidic device based liquid-phase microextraction (DF-µLPME) for the determination of parabens in ~~environmental~~ water samples

María Ramos-Payan[a,b,c]*, Santiago Maspoch[c], Andreu Llobera[a]

[a]*Microelectronic National Centre of Barcelona, 08193 Bellaterra, Barcelona, Spain*

[b]*Department of Analytical Chemistry, Faculty of Chemistry, University of Sevilla, c/Prof. García González s/n, 41012, Seville, Spain*

[c]*Department of Analytical Chemistry, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*

Abstract

A fast double-flow microfluidic based liquid phase microextraction (DF-µLPME) combined with a HPLC-UV procedure using diode array detection has been developed for the determination of the four most widely used parabens: Ethyl 4-hydroxybenzoate (EtP), Propyl 4-hydroxybenzoate (PrP), Butyl 4-hydroxybenzoate (BuP) and IsoButyl 4-hydroxybenzoate (iBuP) in ~~environmental~~ water samples. Parabens have successfully been determined in environmental (lake and river water) samples with excellent clean up, high extraction efficiency and good enrichment factor using double-flow conditions. The microfluidic device consists of two micro-channels, which contain the acceptor and sample solution separated by a flat membrane (support liquid membrane). The sample (0.32 mM HCl) and acceptor phase (5.6 mM NaOH) are delivered to the µLPME at 10 µL min^{-1} and 1 µL min^{-1} flow rate, respectively. The extraction efficiencies are over 84 % for all compounds in ~~environmental~~ water samples with enrichment factors within the range of 9-11 and recoveries over 80 %. The procedure provides very low detection limits between 1.6 and 3.5 µg L^{-1}. The extraction time and the volume required for the extraction are 5 minutes and 50 µL, respectively; which are greatly lower compared to any previous extraction procedure for parabens analysis. In addition, this miniaturized

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DF- µLPME procedure significantly reduces costs compared to not only the existing methods for paraben detection, but also to the existing analytical techniques for sample preparation.

Keywords: microextraction, sample preparation, microfluidic, parabens, ~~environmental~~ water samples.

1. Introduction

Sample treatment procedures and methodologies of analysis are in continuous development in order to overcome adverse influences of matrix components on target analyte signals in real sample analysis. In recent years, the miniaturization of analytical chemistry techniques is becoming a dominant trend as it removes limitations presented by current analysis technologies. Microfluidic devices present significant environmental and economic advantages since the consumption of sample, solvent and reagent are lower. In addition, it has been demonstrated their used on sample pretreatment resulting in high selectivity, sensitivity, a good clean-up and short time of analysis.

Parabens are alkyl esters of p-hydroxybenzoic acid which are a group of compounds widely used as antimicrobial agent in food and drinks [1–3]; as well as preservatives and bactericides in cosmetics, personal care and pharmaceuticals [4]. Methylparaben and ethylparaben are the most commonly used due to their shorter ester chain and higher solubility in water [4]. There is a trend to reduce their use since their endocrine disrupting potential, along with the discovery of these chemical compounds in the breast tissue of patients with breast cancer, raised wide discussion about parabens impact and safety [5,6]. Therefore, they have been regulated by the European Union (EU) countries [7-9]. Parabens are continuously released in the aquatic environment and methyl, ethyl, propyl and butylparabens have been detected in water samples at the ng L^{-1} level [10] and more recently in soils and sediments at the ng kg^{-1} range [11], being the domestic and industrial wastewater vias which contribute the most to their direct introduction into aquatic media.

Up to date, paraben have been mostly extracted by using traditional solid phase extraction (SPE) from aqueous samples [13–16] and in the last years, solid phase microextraction (SPME) was also

used for parabens determination [17–19]. Paraben have also been extracted by liquid phase microextraction (LPME) procedures, one of the most known examples of sample preparation, as for example hollow fiber LPME in two [20] and three phases [21], dispersive liquid-liquid microextraction (DLLME) [22], membrane-assisted liquid–liquid extraction (MALLE) [23], dynamic hollow fiber liquid phase microextraction (DHF-LPME) [24], single drop microextraction (SDME) [25]. All those procedures use low solvent volumes, are low-cost and offer high enrichment factor. Recently, we published a brief ~~revision~~ review of the literature concerning the traditional determination of parabens and new developments in cosmetic and environmental samples [26]. Recently, parabens have been extracted using electromembrane extraction (EME) [27] based on a LPME where the analytes are extracted through the membrane due to an electrical field applied to both sides. The authors completed the extraction of parabens in 40 minutes from 10 mL of sample increasing the enrichment factor obtained compared to a previous HF-LPME procedure.

However, there is a major trend in recent years towards the development of new analytical miniaturized technologies able to perform faster, more powerful and versatile analysis. When the LPME is down-scaled in a microfluidic device, the diffusion path is shorter and the transport phenomena is faster. Very few contributions can be found in the literature for miniaturized LPME [28,29] and there is none of them related to the extraction of parabens. LPME into microfluidic devices is still under development. This microfluidic device is a modification of our previous system where the depth~~ep~~ has been decreased 40 µm in order to accelerate the transport phenomena by passive diffusion.

In this work, for the first time a DF-µLPME procedure into a chip combined with HPLC-DAD (diode array) ~~determination~~ detection has been developed for the determination of the four most commonly used parabens in water samples. Compared to the last article published for paraben determination, this new miniaturized microfluidic device significantly reduce the extraction time ~~by 8x and the sample consumption by 100x~~and sample consumption by a factors of eight and 100

respectively. Furthermore, the organic solvent amount is drecreased and DF-µLPME provides very high extraction efficiencies and good enrichment factor. The proposed DF-µLPME-chip procedure is the easiest method up to date, it can be considered as a "green method", is low-cost and simple handling, it can be reusable and allow to change the membrane a number of arbitrary times. In addition, compared to our previous µLPME device, this new geometry accelerated transport phenomena and offered higher enrichment factors together with high extraction efficiencies.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical-reagent grade. Ethyl 4-hydroxybenzoate (EtP), Propyl 4-hydroxybenzoate (PrP), Butyl 4-hydroxybenzoate (BuP), IsoButyl 4-hydroxybenzoate (iBuP), 1-octanol, dihexyl ether, 2-nitrophenyl octhyl ether (NPOE), 1-heptanol, formic acid, sodium hydroxide, chloride acid, methanol were purchased from Fluka–Sigma–Aldrich (Madrid, Spain). All solutions and dilutions were prepared using ultrapure water from a Milli-Q Plus water purification system (Elga, purelab option S-R 7-15 (Madrid, Spain)).

Aqueous working solutions of parabens were daily prepared by adequate dilutions from methanolic 100 mg L^{-1} stock solutions stored at 4ºC. A membrane (Celgard 2500) of 25 µm thickness, 55% porosity, and 0.21 µm x 0.05 µm pores was obtained from Celgard (Charlotte, NC, USA).

2.2 Fabrication of the DF-µLPME

Fig. 1 shows a scheme of the reusable DF-µLPME. As well known, LPME works under passive diffusion of the compounds. This new geometry of the micro-device pretends to improve our previous micro-chip devices in order to decrease the diffusion path and accelerate the transport phenomena. In this case, the channels (composed of two symmetrical patterned plates of poly(methyl methacrylate)) had a length of 15 mm, depth of 40 µm and a width of 2 mm. The front side used a channel as donor (sample) solution, whereas the channel on the back side was used as acceptor

phase. For the ease of fluidic handling, 1.5 mm diameter size in/outlets were drilled in both plates and Teflon sleeves were fixed.

A polypropylene membrane piece of 17 mm length x 3 mm width was placed carefully over one of the channels, and it was impregnated with 1 µL of dihexyl ether using a micropipette. As a result of capillary forces, the solvent was immediately immobilized into the polypropylene membrane turning its appearance from white to transparent. This process was visually inspected. Afterwards, both methacrylate plates of the µLPME were ~~alienated~~ aligned and fixed together using four screws, resulting in a new geometry for the µLPME with the dimensions for a single channel of 29×25×6 mm. An arbitrarily large number of extraction channels can be implemented and independently addressed by increasing the size of both plates.

A laser cutter (Epilog Mini 24-30 W) was used to fabricate this chip. Given the different polymer plates that can be patterned with this laser, poly(methyl methacrylate) (PMMA) was selected for its facet quality (i.e. low roughness) and processing speed. A writing speed of 45%, power of 18%, a resolution of 1500 and a frequency of 5000 provided with the best quality.

After the µLPME was closed, two separate micro-syringe pumps (Cetoni GmbH, Korbussen, Germany), each operated with a 1000 µL gastight syringe (Hamilton SYR 1 mL 1001 TLL-SAL) were used to pump the sample and acceptor solutions to the µLPME. A 0.32 mM HCl donor solution was continuously distributed to the µLPME at 20 µL min^{-1}. Additionally, a 5.6 mM NaOH aqueous solution as acceptor phase was continuously delivered to the µLPME at 1 µLmin^{-1}. The polypropylene membrane separated both solutions in the µLPME. A micropipette was used to collect the acceptor solution after each extraction and the extract was analyzed by HPLC.

This microchip-device gives the possibility to be used as many times as necessary allowing the membrane exchange when it is needed.

2.4. Chromatographic conditions

The HPLC system consisted of an Agilent 1100 series (Barcelona, Spain) liquid chromatograph equipped with a G1312A Bipump systems. The injector was an autosampler G1313A allowing an injection volume of 5 µL. Separations were carried out at 25 ºC using a LiChroCART1 75-4 Purosphere STAR RP-18e 3mm (75 mm x 4.0 mm i.d.) (VWR, Barcelona, Spain) proceeded by a guard column Kromasil1 100 Å, C18, 5mm (20 mm x 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of 0.1% formic acid (pH 2.6) (component A) and methanol (component B) at a flow rate of 0.5 mL min^{-1}. Separation was performed at 25ºC. An initial 65% component B was used in isocratic mode for 2 min, then a linear elution gradient was programmed from 65% to 80% (B) for 4 min and from 805 % to 85 % B for another minute. Three minutes were waited between injections which allowed re-equilibration of the column to the initial conditions.

The wavelengths used for DAD were 255 nm for all parabens. The chromatogram was completed in 7 minutes and the retention time was 3.3, 4.8, 6.7 and 6.9 for EtP, PrP, BuP and iBu, respectively.

2.5. Preparation of water samples

Superficial water samples were obtained from different lakes and rivers located in Cataluña: river Besos (RBE), river Ripoll (RRI), river Sec (RSE), River Riera de Rubi (RRU) and lake Petit (LPE) from (Barcelona, Spain).

All samples were filtered through Pall NylafloTM nylon membrane filter 0.45 µm (Pall Corporation, Ann Arbor, Michigan, USA) and stored at 4ºC prior to microextraction procedure. Water samples were adjusted to pH 3.5 with HCl before extractions.

2.6. Calculations of extraction efficiency and enrichment factor

The enrichment factor (EF_i) for the analyte *i* was calculated according to the following equation (1):

$$EF_i = \frac{C_{f,a,outlet}}{C_{i,s,inlet}} \quad (1)$$

where $C_{f,a,outlet}$ is the concentration of the analyte i at the outlet of the acceptor channel and $C_{i,s,inlet}$ is the initial concentration of the analyte in the sample. $C_{f,a,outlet}$ was determined by HPLC UV-detection using external calibration. The extraction efficiency (*R, recovery*) was defined as the fraction of analyte transferred to the acceptor phase from the sample. Considering the same extraction time for both (acceptor and sample solution), the acceptor and sample flow rate (v_a and $v_s, respectively$) determinenated the fraction of volume between both phases. Under non stopped-flow conditions, the extraction efficiency (ER %) was calculated according to the following equation (2):

$$EE\ (\%) = \frac{C_{f,a,outlet}}{C_{i,s,inlet}}\ x\ \frac{v_a}{v_s}\ x\ 100 = EF_i\ x\ \frac{v_a}{v_s}\ x\ 100 \qquad (2)$$

3. Results and discussion

3.1. Principle of the extraction

The structure of the device used for DF-µLPME is shown in figure 1. The analytes of interest contained in the sample solution (channel 1) are extracted through a porous membrane into the acceptor solution (channel 2).

Since lL-iquid phase microextraction is based on an extraction principle due to a passive diffusion with a strong pH gradient between two phases, the pH value in the sample solution was under the pKa values of the analytes and the pH value in the acceptor phase was over their pKa. On the basis of the pKa values of the analytes within the range 8.1-8.8, HCl and NaOH were used as sample and acceptor phase, respectively in order to ensure uncharged analytes in the sample solution and negatively charged analytes in the acceptor phase. Once the extraction was completed after 5 minutes, the extract collected from the acceptor phase (outlet) was injected into a HPLC for analysis. The microfluidic device worked in double-flow conditions and each membrane could be reused for consecutive extractions.

3.2. Optimization and evaluation of experimental conditions

Firstly, preliminary experiments were performed to determinate the most suitable organic solvent to be used as SLM. 1-nitrophenyil octyl ether (NPOE), 1-octanol, 1-heptanol and dihexylether were tested. For those experiments, 0.32 mM HCl as sample solution, 5.6 mM NaOH as acceptor solution, and 3 µL min^{-1} and 1 µL min^{-1} as sample and acceptor flow rate, respectively, were used. The µLPME was opened for replacing the membrane after each organic solvent test. The organic solvent optimization was done at 1 µL min^{-1} as donor and acceptor flow rate. As seen in Table 1, the extraction efficiency was similar for all organic solvents, although the best results were obtained when dihexylether was used as support liquid membrane, which was consequently used as SLM for the rest of the study.

In a next experiment, the donor and acceptor phase composition were optimized. For this optimization, the donor and acceptor flow rate were 3 µL min^{-1} and 1 µL min^{-1}, respectively for all experiments. First, the donor phase (containing 0.5 mg100 µg mL^{-1} of each paraben) was tested within a pH range of 1-4, while the acceptor phase kept fixed at pH 11.5. The extraction was completed in 5 minutes. As it can be seen in Figure 2, parabens could be extracted within a wide range of pH, although it was observed a slight increase of the peak area at pH 3.5 for Et-P and Bu-P. There was no influence in the acceptor donor phase pH value on the extraction procedure, especially for iBu-P and Pr-P where the peak areas remains practically constant. The same experiments were carried out for the optimization of the acceptor phase concentration. In this case, the donor phase concentration was fixed at pH 3.5 and aqueous NaOH solutions with pH values within a range of 10-12 were tested. A study of degradation for each compound at different times was realized over pH 12 to check their stability at high pH during their extraction, observing a slight degradation over pH 12. The figure 3 shows that the highest peak areas were obtained at pH 11.75 for all compounds. The peak areas dramatically decreased at pH 10 and parabens were not extracted at pH values lower than 10. Consequently, a pH of 3.5 and 11.75 were fixed as sample and acceptor composition, respectively, for the study of the flow rate. Regarding figure 2 and 3, each pointFigure captions of

figure2 and 3 ~~was tested~~were based on 3 replicate experiments resulting in a relative standard deviation (RSDs %) below 4 % for all analytes.

The study of the donor and acceptor flow rate was investigated. The donor flow rate was tested between 1-30 µL min^{-1} while the acceptor flow rate was kept constant at 1 µL min^{-1}. The stability of the SLM was checked when high donor flow rates were tested. Over flow rates of 60 µL min^{-1}, there was a high pressure difference across the system resulting in low reproducibility. Figure 4 shows the extraction efficiencies obtained at each flow rate. As it can be seen, the extraction efficiencies decreased as the donor flow rate increased significantly due to the decrease residence time of the sample. The highest extraction efficiencies were obtained at 1 µL min^{-1} flow rate for all compounds. However, the enrichment factor increased when the donor flow rate increased (data not shown). The extraction efficiency decreased significantly over 10 µL min^{-1} for all compounds (EE ≤ 50 %). The election of the donor flow rate will be based in order to develop a method which offer simultaneously good enrichment factor and extraction efficiency. Since the extraction efficiency were over 73-85 % for all compounds at 10 µL min^{-1} resulting in good enrichment factor within the ranges of 8-10, the donor flow rate was fix at 10 µL min^{-1}. The influence of the acceptor flow rate was tested within the range of 1-4 µL min^{-1} while the donor flow rate was kept constant at 10 µL min^{-1}. Figure 5 shows the effect of the acceptor flow rate versus the enrichment factor. The enrichment factor decreased significantly when the acceptor flow rate increased and the best results were obtained using an acceptor flow rate of 1 µL min^{-1}. Each point from figure 4 and 5 was based on 3 replicate experiments resulting in a relative standard deviation (RSDs %) below 3 % for all analytes.

Finally, the influence of salt addition on extraction efficiency of target analytes was investigated in the concentration range of 0–20% (w/v) of NaCl. NaCl is commonly added to sample solution in extraction procedures as a salting-out agent to improve recovery of the analytes. Since ~~enhancement~~ higher recoveries were achieved with 10 % NaCl, salt was added to the sample for DF-µLPME

procedure. The salt addition effect was tested at optimal conditions and it was observed an increase of the extraction efficiency of 19 %, 6 %, 28 % and 23 % for EtP, PrP, iBuP and BuP, respectively, when 5 % NaCl (w/v) was used. However, the extraction efficiencies decreased at higher % NaCl. Table 2 shows the extraction efficiency and enrichment factor for DF-µLPME procedure with salt addition.

3.3. Method evaluation

A 10-point (in triplicate) calibration curve was constructed using a least-square linear regression analysis of standard mixtures (5 % NaCl added) at different concentrations. Using the selected DF-µLPME conditions, several aqueous pH 3.5 solutions containing each compound, were injected into the microfluidic liquid phase microextraction procedure and analyzed according to the described HPLC procedure. Detection and quantification limits were calculated as three and ten times the standard deviation of the background signal, respectively. Subsequently, peak areas were plotted as function of concentration. A linear relationship was obtained for all the model analytes, and r^2 values exceeded in all cases 0.9996 (see table 2). Repeatability was tested based on 4 replicate experiments from 20 and 50 µg L^{-1} samples in 0.32 mM HCl, obtaining RSD values all below 4 % . The interday repeatability (n = 3, 5 days) were below 4% for all analytes. As seen in table 2, very low detection and quantitation limits between 1.6-3.5 and 5-11.6 µg L^{-1} were obtained, respectively. The reproducibility of the device using the same membrane and different ones was tested based on 10 replicates experiments, resulting in RSD values all below 3%. Under optimized conditions, the DF-µLPME provided extraction efficiencies in the range from 84 to 100 % depending of their partition coefficients and good enrichment factor between 8.5 and 10.2 were obtained. The performance of the DF-µLPME was compared with a traditional electromembrane extraction recently published [27] for parabens determination, where similar quantitation limits were obtained due to its higher enrichment factor, however extraction efficiencies were below 9 % , the extraction time was completed after 40 minutes and the sample volume required was 200 times higher (10000 µL).

3.4. ~~Environmental w~~Water analysis using microfluidic µLPME

River and lake water samples were collected from different points: river Besos (RBE), river Sec (RSE), river Riera de Rubi (RRU) and lake Petit (LPE), and river Ripoll (RRI). Water samples spiked at three different levels (12, 25 and 50 µg L^{-1}) were analyzed according to the proposed procedure. All water samples were analyses with no spiking ~~drug~~ compound so as to evaluate the original presence of parabens. Table 4 shows the recoveries for the parabens in each type of water sample. In all cases, the recoveries were over 80% with standard deviations lower than 2 %. In addition, an excellent clean-up was obtained with no sample dilution which implies a great advantage compared to traditional LMPE extraction which required much higher sample volume consumption. Figure 6 shows a representative chromatogram of a spiked lake water sample at 14 µg L^{-1}. As it can be seen, our DF-µLPME offers excellent baselines demonstrating the clean-up efficiency of this method. On the other hand, no paraben was detected in the analyzed water samples. Table 4 shows the comparison of the proposed DF-µLPME method with other extraction methods for parabens. This DF-µLPME procedure provides the highest efficiency of extraction with the smallest sample volume analysis and in the shortest time (over 84 % extraction efficiency, 50 µL of sample volume and 5 minutes) compared to previous methods for extraction of parabens. Some procedures using SPE or hollow fiber provided a ~~bit higher~~somewhat better LOQ due to its higher enrichment, however they require 200 times higher sample volume and 12 times longer extraction times. Additionally, most methods require sample conditioning prior to extraction while DF-µLPME is performed in one single step. This DF-µLPME device is also reusable and allows more than ten consecutive extractions. To conclude, DF-µLPME is a very fast potential method for high efficient extraction even with low microliters from ~~environmental~~ water samples.

4. Conclusions

This work presents for the first time a double-flow microfluidic µLPME reusable for the determination of parabens in ~~environmental~~ water samples. The new geometry of this miniaturized

device DF-µLPME gives many advantages over traditional LPME and also EME used for the determination of parabens recently published [27], such as: (1) decrease the organic solvent used as SLM, (2) ~~reduce the extraction time by 8x~~an eightfold reduction of the extraction time, (3) ~~reduce~~ a 100fold reduction of the sample consumption volume ~~by 100x,~~ (4) more simple handling (5) reduce cost instrumentation since the device is reusable, (6) allow multi-extraction using the same membrane, and (7) provide simultaneously very high extraction efficiencies (> 84 %) and good enrichment factor (9-10). In addition, this device offers low LOD and LOQ and recoveries over 84 % for all parabens in ~~environmental~~ water samples resulting in excellent base lines. Furthermore, compared to our previous µLPME device [30], the new geometry also accelerated transport phenomena by decreasing the length path and the depth~~ep~~, and offered higher enrichment factors together with high extraction efficiencies.

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Figure 1. Schematic of a double-flow liquid phase microextraction on a microfluidic device (DF-µLPME)

Figure 2. Optimization of the donor phase composition. SLM: dihexylether, flow rate (donor phase): 3 µL min^{-1}, flow rate (acceptor phase): 1 µL min^{-1}, acceptor phase composition: pH 11.75

Figure 3. Optimization of the acceptor phase composition. SLM: dihexylether, flow rate (donor phase): 3 µL min^{-1}, flow rate (acceptor phase): 1 µL min^{-1}, donor phase composition: pH 3.5

Figure 4. Extraction efficiency versus sample flow rate. SLM: dihexylether, flow rate (acceptor phase): 1 µL min^{-1}, donor phase composition: pH 11.75 and acceptor phase composition: pH 3.5

Figure 5. Extraction enrichment versus acceptor phase flow rate. SLM: dihexylether, flow rate (donor phase): 10 µL min^{-1}, donor phase composition: pH 11.75 and acceptor phase composition: pH 3.5

Figure 6. Chromatogram of a spiked ~~environmental~~ river water sample containing the four parabens at 14 µg L^{-1}. Extraction time: 5 minutes. SLM: diexylether; donor phase composition: pH 11.75 and acceptor phase composition: pH 3.5. Sample volume required: 50 µL. No sample dilution.

Table 1. Recoveries (RSD %) of the parabens using different organic solvents as the SLM for DF-µLPME.

Table 2. Calibration parameters, method detection limit (MLOD), method quantitation limit (MLOQ), extraction efficiencies and enrichment factor for all analytes for the proposed DF-µLPME procedure

Table 3. DF-µLPME/HPLC recoveries (average of three determinations ± standard deviation) from spiked ~~environmental~~ water samples

Table 4. Comparison of figures of merit of DF-µLPME with other analytical techniques for determination of parabens.

Fig 1

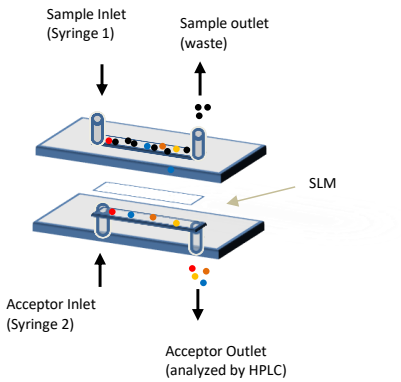
Sample Inlet
(Syringe 1)
Sample outlet
(waste)
SLM
Acceptor Inlet
(Syringe 2)
Acceptor Outlet
(analyzed by HPLC)

Fig 2

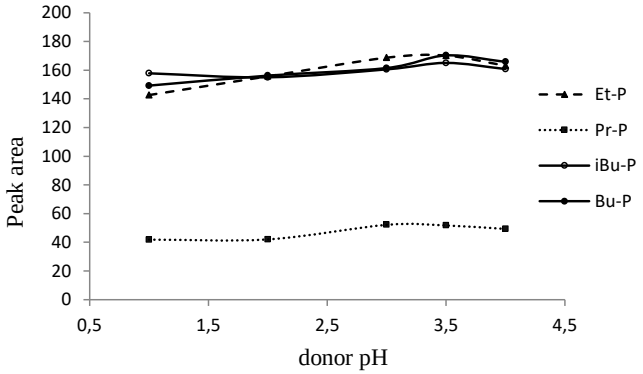
200
180
160
140
120
100
80
60
40
20
0
Peak area
0,5
1,5
2,5
3,5
4,5
donor pH
Et-P
Pr-P
iBu-P
Bu-P

Fig 3

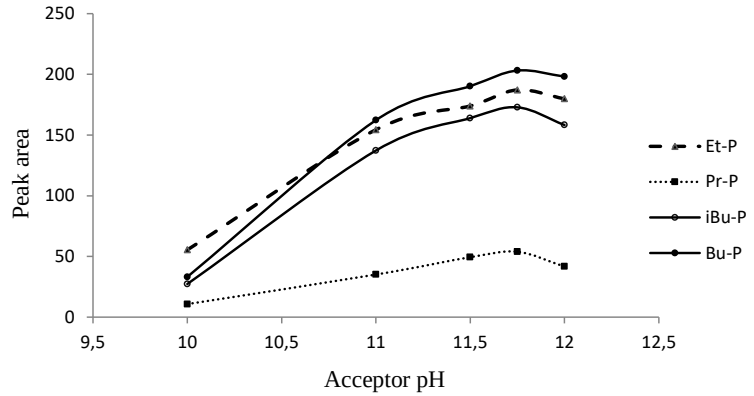
250
200
150
100
50
0
Peak area
9,5
10
10,5
11
11,5
12
12,5
Acceptor pH
Et-P
Pr-P
iBu-P
Bu-P

Fig 4

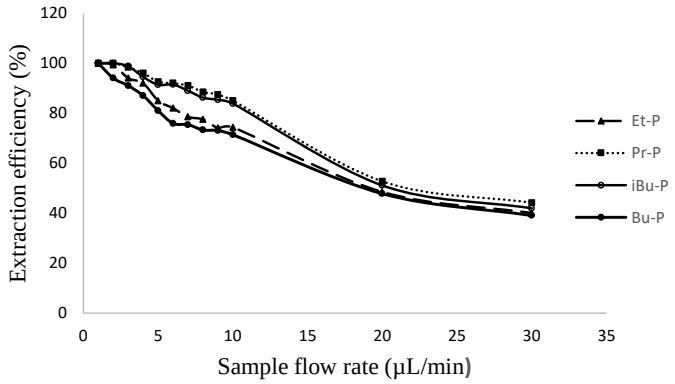
120
100
80
60
40
20
0
Extraction efficiency (%)
0
5
10
15
20
25
30
35
Sample flow rate (µL/min)
Et-P
Pr-P
iBu-P
Bu-P

Fig 5

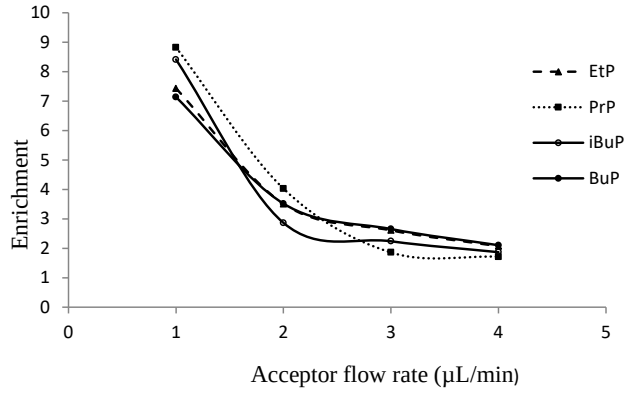
10
9
8
7
6
5
4
3
2
1
0
Enrichment
0
1
2
3
4
5
Acceptor flow rate (µL/min)
EtP
PrP
iBuP
BuP

Fig 6

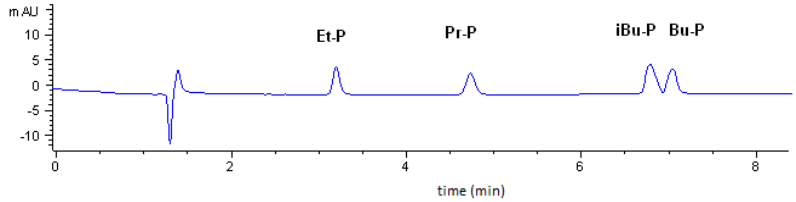

Table 1

	Ethyl 4-hydroxybenzoate	Propyl 4-hydroxybenzoate	IsoButyl 4-hydroxybenzoate	Butyl 4-hydroxybenzoate
NPOE	88 (1)	100 (1)	100 (1)	75 (1)
Dihexylether	100 (1)	100 (1)	100 (2)	89 (1)
1-heptanol	75 (1)	90 (2)	80 (1)	60 (2)
1-octanol	87 (1)	100 (1)	98 (1)	72 (1)

[a] Sample: 1 µL min^{-1} of HCl at pH 3.5 containing the four drugs each at 100 µg L^{-1}; acceptor: 1 µL min^{-1} of NaOH at pH 11.75; extraction time: 5 min. NPOE: 2-Nitrophenyl octyl ether

Table 2

	MLOD (µg L^{-1})	MLOQ (µg L^{-1})	R^2	EE *	Ef
Ethyl 4-hydroxybenzoate	3.5	11.6	0.9994	84 (3)	8.4
Propyl 4-hydroxybenzoate	1.8	6	0.9997	95 (2)	9.5
IsoButyl 4-hydroxybenzoate	1.6	5.2	0.9995	102 (3)	10.2
Butyl 4-hydroxybenzoate	2.6	8.8	0.9994	88 (2)	8.8

*% Extraction efficiency (%RSD, n=3)

Table 3

	Spiked level (µg L^{-1})	RRU	RBE	RRI	LPE	RSE
Ethyl 4-hydroxybenzoate	12	93.1 ± 0.9	80.7 ± 0.6	80.5 ± 0.8	85.0 ± 1.6	91.4 ± 0.2
	25	92.4 ± 1.5	81.2 ± 0.9	81.5 ± 1.6	85.1 ± 0.6	92.6 ± 0.7
	50	92.7 ± 1.6	80.9 ± 1.4	82.0 ± 1.1	85.9 ± 0.8	92.1 ± 0.9
Propyl 4-hydroxybenzoate	12	85.3 ± 1.2	83.9 ± 0.2	95.0 ± 0.6	84.8 ± 0.9	80.7 ± 0.7
	25	86.4 ± 1.1	84.5 ± 0.9	96.7 ± 1.5	84.5 ± 0.6	82.0 ± 1.4
	50	85.0 ± 0.8	84.9 ± 0.8	97.0 ± 1.9	85.3 ± 1.1	82.8 ± 1.1
IsoButyl 4-hydroxybenzoate	12	92.6 ± 0.7	81.6 ± 0.4	82.1 ± 1.3	83.1 ± 1.3	85.7 ± 0.4
	25	95.4 ± 0.4	82.7 ± 1.4	82.5 ± 1.7	83.7 ± 0.8	85.9 ± 1.8
	50	96.0 ± 0.9	83.4 ± 1.1	85.6 ± 1.0	84.4 ± 1.2	87.3 ± 0.9
Butyl 4-hydroxybenzoate	12	88.1 ± 1.6	77.9 ± 0.6	85.4 ± 0.9	81.8 ± 0.9	85.0 ± 1.5
	25	87.4 ± 1.4	75.1 ± 1.2	87.3 ± 0.6	82.9 ± 0.8	87.6 ± 0.6
	50	88.6 ± 0.8	78.4 ± 1.5	88.0 ± 0.8	83.4 ± 1.6	87.4 ± 1.2

Tabla con formato

Table 4

Analytical method	Analyte	Matrix	Sample Volume (mL)	LOQ (μg L^{-1})	EF	EE %	Extraction time (min)	Reference
SPE	MeP, EtP	Water	500	0.001-0.2	-	-	> 60	13
SPE	MeP, EtP, PrP	Water	70	0.5	170-253	-	> 60	16
SPME	MeP, EtP, PrP	Aqueous		> 123	-	-	> 30	17
SPME	MeP, EtP, PrP BzP	Water	10	0.004-0.02	-	-	> 60	19
DLLME	MeP, EtP, PrP, BuP,	Water	10	6-37	70-210	-	> 20	22
HF-LPME[a]	MeP, EtP, PrP	Water and urine	8	100-300	21-154	-	40	20
HF-LPME[b]	MeP, EtP, PrP, BuP, iPrP iBuP. BzP	Water	3.5	0.5	3-16	24-60	30	21
DHF-LPME	MeP, EtP, PrP	Cosmetics	20	20-50	30-300	3-30	40	24
MALLE	MeP, EtP, PrP, BuP, iPrP iBuP. BzP	Water	18	0.0003-0.005	-	40-90	> 60	23
EME-HPLC/UV	EtP, PrP, BuP, iBuP. BzP	Water	10	2.4-5	32-49	< 8	40	27
DF-μLPME-HPLC/UV double-flow	EtP, PrP, BuP, iBuP	Water	0.05	5-12	9-10	84-100	5	This work

[a] Hollow fiber liquid phase microextraction- 2 phases
[b] Hollow fiber liquid phase microextraction- 3 phases