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A rapid and versatile microfluidic method for the simultaneous extraction of polar and non-polar basic pharmaceuticals from human urine

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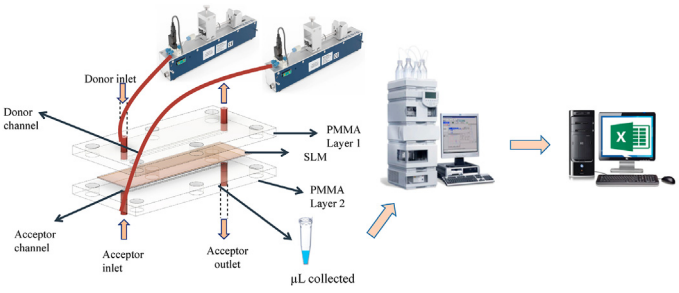
HIGHLIGHTS

- An efficient microfluidic device for the simultaneous extraction of polar and non-polar basic drugs is proposed.
- A new supported liquid membrane based on a mixture of 2:1 (v/v) tributyl phosphate and dihexyl ether is proposed.
- The microfluidic extraction system showed good long-term stability with same membrane.
- The liquid phase microextraction device offered efficiencies over 78% in urine samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In sample preparation, simultaneous extraction of analytes of very different polarity from biological matrixes represents a challenge. In this work, verapamil hydrochloride (VRP), amitriptyline (AMP), tyramine (TYR), atenolol (ATN), metopropol (MTP) and nortriptyline (NRP) were used as basic model analytes and simultaneously extracted from urine samples by liquid-phase microextraction (LPME) in a microfluidic device. The model analytes (target compounds) were pharmaceuticals with $0.4 < \log P < 5$. Different organic solvents and mixtures of them were investigated as supported liquid membrane (SLM), and a mixture of 2:1 (v/v) tributyl phosphate (TBP) and dihexyl ether (DHE) was found to be highly efficient for the simultaneous extraction of the non-polar and polar model analytes. TBP reduced the intrinsic hydrophobicity of the SLM and facilitated extraction of polar analytes, while DHE served to minimize trapping of non-polar analytes. Sample and acceptor phase composition were adjusted to pH 12 and pH 1.5, respectively. Urine samples were pumped into the microfluidic system at 1 µL min-1 and the extraction was completed in 7 min. Recoveries exceeded 78% for the target analytes, and the relative standard deviation ($n = 4$) was below 7% in all cases. Using five microliters of SLM, the microfluidic

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extraction system showed good long-term stability, and the same SLM was used for more than 18 consecutive extractions.

1. Introduction

In the last decade, liquid phase microextraction (LPME) systems have been investigated and developed with the aim of minimizing the use of hazardous organic solvent. Liquid phase microextraction is a widely used technique for the treatment of samples and has been conducted in different formats, such as dispersive liquid-liquid extraction (DLLE) [1–3], hollow fiber liquid phase microextraction (HF-LPME) [4–6], single drop microextraction (SDME) [7–9], and electromembrane extraction (EME) [10–12]. LPME has been successfully applied to both biological, environmental and food samples and there are two different modes of work depending on the nature of one of the phases. The analyte is extracted from the sample to a microliter volume of acceptor, which can be an organic solvent [13,14] or an aqueous solution [15,16]. In the analytical field, sample pretreatment systems are commonly used as a cleaning, preconcentration and extraction procedure. In addition, the development of improvements in these procedures and the advantages they present have attracted the interest of the scientific community, not only for their development but also using these systems as a routine procedure. LPME [17–19] and EME [20–22] are well-known techniques and have been developed extensively, especially for biomedical environmental applications [23–26] or even food applications [17]. Both LPME and EME provide preconcentration and efficient sample clean up.

In both LPME and EME, analytes are extracted across a supported liquid membrane (SLM), which is a microliter volume of organic solvent immobilized by capillary forces in the pores of a polymeric membrane. The chemical composition of the SLM play a central role in both LPME and EME, and different solvents are used for different analytes depending on their acid/base properties and based on their polarity [27–33]. Therefore, simultaneous extraction of bases and acids, or simultaneous extraction of polar and non-polar analytes, represent a challenge. The EME system has been shown to be a good option for the simultaneous extraction of acids and bases [34–36]. These systems have been optimized mainly for compounds of similar polarity, providing enrichment factors of between 300 and 350 [34], 76–130 [35] and good recoveries (60–80%), but the latter requiring extraction times of 45 min [36]. In these cases, different SLMs have been used for acidic or basic compounds sharing the same donor phase. Furthermore, EME has been previously studied for the extraction of compounds of different polarity, either for acidic or basic compounds using a different membrane according to acidity and polarity [37]. The use of eutectic solvents proved to be an option for the extraction of compounds of different polarity using different SLMs and obtaining efficiencies higher than 85%.

In the last years, LPME [38–41] and EME [42–47] have been successfully implemented in microfluidic devices, requiring less sample and organic solvent. Due to very short diffusion distances and operation under dynamic conditions, kinetics are fast and recoveries are high in microfluidic devices. Simultaneous extraction of acids and bases, and polar and non-polar bases, have been reported with electromembrane extraction under microfluidic conditions [48,49]. However, there are no studies on simultaneous microfluidic LPME of basic pharmaceuticals within a large log P window and six different basic drugs have been selected for the study: verapamil hydrochloride (VRP), amitriptyline (AMP), tyramine (TYR), atenolol (ATN), metopropol (MTP) and nortriptyline (NRP). Their therapeutic dose is [50]: 0.1–1, 0.02–0.2, 0.05–0.3, 0.035–0.5, 0.01–0.25 μg mL^{-1} for ATN, NRP, AMP, MTP and VRP, respectively.

For this reason, in this work, an efficient microfluidic method is proposed for simultaneous liquid-phase microextraction of polar and non-polar pharmaceuticals from microliter volumes of human urine samples. The composition and polarity of the supported liquid membrane was developed and tuned carefully to facilitate extraction in a large log P window. Operational parameters were optimized, and the method was evaluated for basic pharmaceuticals in the log P range −0.4 to 4.9.

2. Experimental

2.1. Chemicals and sample solutions

Formic acid, hydrochloric acid, sodium hydroxide, methanol, 2-nitrophenyl octyl ether (NPOE), 1-octanol, 1-decanol, dihexyl ether (DHE), tributyl phosphate (TBP), verapamil hydrochloride (VRP), amitriptyline hydrochloride (AMP), tyramine (TYR), atenolol (ATN), metopropol (MTP), and nortriptyline (NRP) were purchased from Fluka_Sigma_Aldrich (Madrid, Spain). A Celgard 2500 membrane of 25 μm thickness, 55% porosity, and 0.21 × 0.05 μm pores was obtained from Celgard (Charlotte, NC, USA). 100 mg L^{-1} stocks solutions for all analytes were prepared in methanol. All daily working dilutions were prepared using ultrapure water from a Milli-Q Plus (Elga, Purelab option S-R 7–15) (Madrid, Spain).

2.2. Liquid chromatography

An Agilent 1100 series (Barcelona, Spain) liquid chromatography apparatus equipped with a G1312A Bipump system was used for liquid chromatography. This was equipped with a STAR RP-18e column (75 mm × 4.0 mm × 3 μm) from VWR (Barcelona, Spain), and a Kromasil1 100 A C18 (20 mm × 4.6 mm × 5 μm) guard column from Scharlab S.L. (Barcelona, Spain). Column temperature was 30 °C. An autosampler G1313A was used for sample injection (5 μL). 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}. An initial elution gradient was programmed as follows: from 0% to 2% B for 2 min, then from 2% B to 100% B for another 27 min, this condition was kept for 2.5 min, followed by 5 min re-equilibration. The wavelengths used for DAD was 200 nm for VRP, NRP and AMP; and 280 nm for TYR, ATN, MTP. Chromatogram was completed in 29 min and retention times were 3.4, 9.8, 17.9, 25.5, 27.6 and 28.1 min for TYR, ATN, MTP, VRP, NRP and AMP, respectively.

2.3. Fabrication and setup of the microfluidic device

An Epilog Mini 24–30-W laser cutter was used for the device fabrication, under ablation conditions of 40% for writing speed and power, a resolution of 1500, and a frequency of 5000, as described in our previous work Santigosa et al. [40]. The device (Fig. 1) contained two channels each of 25.0 × 3.0 × 0.13 mm (length × width × depth), on two poly(methyl methacrylate)

(PMMA) layers. Two inlets/outlet Teflon tubes (1.5 mm od and 0.12 mm i.d) were located at the ends of the channels, the acceptor and sample solutions were introduced through two holes where the tubes were placed. A 28 mm length × 5 mm width piece of flat polypropylene (PP) membrane (25 μm thickness, 55% porosity) was used to separate the acceptor channel and the sample channel. PP was impregned with optimal organic solvent (5 μL of TBP:DHE (2:1, v/v)) using a micropipette. Inlets Teflon tubes were connected to two separate micro-syringe pumps (Cetoni GmbH, Korbussen, Germany). Acceptor (pH 1.5) and sample (pH 12.0) solutions were pumped into the microfluidic channels at 1 μL min^{-1}. The extraction was completed in 7 min, and acceptor phase was collected by a micropipette and transferred to a micro-insert for its analysis by liquid chromatography.

2.4. Preparation of real samples

Non-diluted and 1:1 diluted urine samples from a 29-year-old female healthy volunteer (staff working on the laboratory) were spiked with model analytes at three different levels (0.75, 1 and 2 mg L^{-1}) prior consent. All samples were adjusted to pH 12.0 (with NaOH) and filtered through Pall Nylaflo™ nylon membrane filter 0.45 μm (Pall Corporation, Ann Arbor, MI, USA) prior to extraction. The samples were delivered into the microfluidic system at 1 μL min^{-1} requiring a final sample volume amount below 100 μL for at least 5 repetitive extractions.

3. Results and discussion

3.1. Theoretical considerations

Polar and non-polar model analytes were extracted from aqueous sample pH 12, through organic SLM, and into aqueous acceptor pH 2. For a given analyte, the following partition coefficients controlled the process:

$$K_1 = \frac{C_{eq,SLM}}{C_{eq,s}} \quad (1)$$

$$K_2 = \frac{C_{eq,a}}{C_{eq,SLM}} \quad (2)$$

$C_{eq,SLM}$, $C_{eq,s}$ and $C_{eq,a}$ are the analyte equilibrium concentrations in the SLM, sample and acceptor, respectively. The overall analyte partition coefficient K between the donor and acceptor can be expressed as the product of K_1 and K_2:

$$K = \frac{C_{eq,a}}{C_{eq,s}} = K_1 \times K_2 \quad (3)$$

The analyte extraction recovery at equilibrium is related to the partition coefficients as expressed in the following equation [51]:

$$R(\%) = \frac{K \times V_a}{K \times V_a + K_1 \times V_{SLM} + V_s} \times 100\% \quad (4)$$

V_a, V_s, and V_{SLM} denote the volumes of acceptor, sample, and SLM, respectively. Analyte kinetics are controlled by K_1, and can be modelled using the following equation [51]:

$$C_s(t) = C_s^0 \cdot \exp\left(-\frac{A_{SLM} D_{SLM} K_1}{V_s h} t\right) \quad (5)$$

here, $C_s(t)$ is the analyte concentration in the donor as a function of time, C_s^0 is the initial analyte concentration in the donor ($t = 0$), A_{SLM} is the surface area of the SLM, h is the thickness of the SLM, and D_{SLM} is the diffusion coefficient of the analyte in the SLM. The following discussions of experimental work are anchored in Equations (1)–(5).

3.2. Supported liquid membrane selection

In the first series of experiments, the composition of the supported liquid membrane was studied. Five different organic solvents were selected as SLM for simultaneous extraction of polar and non-polar basic compounds based on previous work [32,33,40]: 1-octanol, 1-decanol, NPOE, DHE and TBP. As well-known for LPME of basic analytes, the compounds must be in their neutral form in the sample and positively charged in the acceptor phase. This way, analytes are extracted from the sample to the acceptor phase through the SLM. Table 1 shows the molecular structure, log P and pKa of model analytes. The preliminary conditions of the rest of the parameter were selected based on the literature for the extraction of these compounds and their pKa value [33]. Thus, 5 mM of phosphate buffer pH 2.0 was used as acceptor, and 5 mM phosphate buffer pH 12.0 spiked with model analytes at 3 mg L^{-1} was used as sample. Both acceptor and sample were pumped at 1 μL min^{-1}. Table 2 summarizes the extraction efficiencies obtained with each of the tested solvents.

1-Octanol (OCT, log P = 3.0) was tested as the first SLM. With this solvent, K_1 and K_2 were calculated for the model analytes based on computer software [51], and from these values theoretical equilibrium recoveries were estimated based on Equation (4)

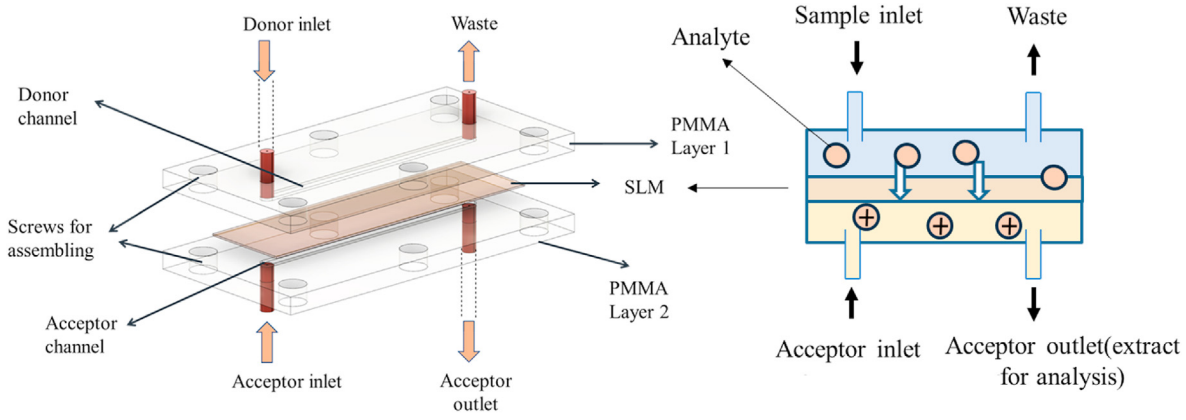


Fig. 1. Microfluidic device scheme.

Table 1
Molecular structure, log P and pKa of model analytes.

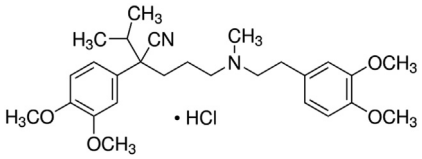
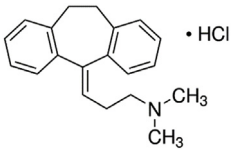
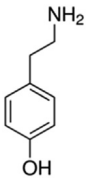
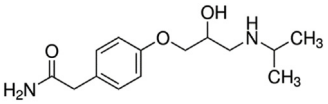
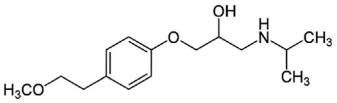
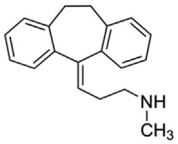
Drug	Structure	log P	pKa
Verapamil		5.04	9.68
Amitriptyline		4.81	9.76
Tyramine		0.68	9.66[base]/10.41[acid]
Atenolol		0.42	9.67
Metoprolol		1.75	9.67
Nortriptyline		4.43	10.47

Table 2
Recoveries (RSD %) of the polar and non-polar basic using different organic solvents as the SLM for μLPME-chip device.

SLM	Extraction efficiency ± SD (%)					
	TYR	ATN	MTP	VRP	NRP	AMP
Octanol	70.0 ± 6.0	78.6 ± 4.3	75.4 ± 1.8	50.4 ± 4.6	41.3 ± 3.3	43.8 ± 3.6
Decanol	25.3 ± 0.9	36.7 ± 3.0	45.6 ± 2.1	80.4 ± 4.5	79.1 ± 4.2	82.7 ± 6.9
NPOE	0.0 ± 0.0	31.9 ± 2.4	62.5 ± 6.1	87.1 ± 4.8	75.6 ± 4.4	95.2 ± 5.5
DHE	5.7 ± 1.4	24.4 ± 5.0	60.4 ± 2.7	72.7 ± 3.2	55.3 ± 3.4	39.5 ± 4.9
TBP	67.6 ± 7.8	80.8 ± 6.4	53.7 ± 4.3	31.3 ± 4.6	71.5 ± 3.2	7.7 ± 2.5
1:1 TBP:NPOE	16.1 ± 1.5	27.5 ± 1.7	48.6 ± 2.8	77.8 ± 5.9	68.7 ± 4.5	43.3 ± 1.2
2:1 TBP:NPOE	30.2 ± 1.7	39.4 ± 2.5	49.3 ± 4.4	94.9 ± 1.9	72.4 ± 2.9	55.3 ± 6.8
1:2 TBP:NPOE	0.0 ± 0.0	27.5 ± 1.7	52.0 ± 1.7	55.3 ± 1.2	43.0 ± 1.6	45.2 ± 2.8
3:1 TBP:NPOE	30.3 ± 2.8	64.8 ± 7.0	74.3 ± 1.9	89.8 ± 5.4	83.9 ± 4.6	53.8 ± 4.9
1:3 TBP:NPOE	0.0 ± 0.0	1.8 ± 3.9	94.8 ± 4.6	99.3 ± 4.5	83.7 ± 2.2	88.9 ± 3.5
1:1 TBP:DHE	56.5 ± 5.6	52.2 ± 5.9	75.1 ± 5.5	79.6 ± 6.2	65.6 ± 3.6	25.0 ± 6.1
2:1 TBP:DHE	78.6 ± 3.5	92.8 ± 4.6	87.2 ± 6.8	97.2 ± 3.3	91.4 ± 3.4	64.5 ± 5.2
1:2 TBP:DHE	15.9 ± 1.3	21.3 ± 1.4	80.4 ± 3.5	78.2 ± 3.7	51.2 ± 2.0	49.5 ± 1.9
3:1 TBP:DHE	55.1 ± 5.9	83.7 ± 6.3	102.4 ± 5.4	84.1 ± 3.2	42.0 ± 1.7	51.3 ± 5.7
1:3 TBP:DHE	0.0 ± 0.0	21.9 ± 4.4	81.9 ± 6.9	74.9 ± 1.1	61.9 ± 2.3	74.8 ± 2.5

(Table 3). For the polar analytes (tyramine, atenolol, and metoprolol), K_1-values were low. The theoretical equilibrium recoveries are very close to 100%, while the experimental values ranged between 70 and 79%. This indicated that the polar analytes were not extracted completely to equilibrium for kinetic reasons. For the non-polar analytes, K_2-values were low, and theoretical recoveries were calculated to 30−43%. The experimental values ranged between 41 and 50%. Kinetics were faster for these analytes, but recoveries were limited by trapping in the SLM (K_2-limitation).

When OCT (log P = 2.58) was replaced by 1-decanol (DEC, log P = 3.47), the intrinsic hydrophobicity of the SLM increased, while the number of hydrogen bond sites remained the same (HBA = HBD = 1). Therefore, K_1-values decreased in general. The polar analytes now suffered from poor transfer into the SLM (K1-

Table 3
Calculated values for K_1, K_2, K, and recovery with 1-octanol as SLM.

	K_1	K_2	K	Recovery (%)
Tyramine	0.29	89	26	99
Atenolol	2.6	$6.6 \bullet 10^2$	$1.7 \bullet 10^3$	99
Metoprolol	57	30	$1.7 \bullet 10^3$	99
Nortriptyline	$2.7 \bullet 10^4$	0.065	$1.7 \bullet 10^3$	30
Amitriptyline	$6.3 \bullet 10^4$	0.49	$3.2 \bullet 10^3$	43
Verapamil	$1.1 \bullet 10^5$	0.029	$3.2 \bullet 10^3$	30

V_s and V_a was set to 15 μL, and V_{SLM} was set to 1 μL.

limitation), while the non-polar analytes improved due to increased K_2-values. In subsequent experiments, we also tested dihexyl ether (DHE, log P = 4.55), 2-nitrophenyl octyl ether (NPOE, log P = 4.86), and tributyl phosphate (TBP, log P = 4.09) as SLMs. With DHE, the intrinsic hydrophobicity was even higher than with DEC, and the solvent has a single HBA site. For this reason, K_1-values were low, and all the analytes suffered from slow kinetics (K_1-limitation). When DHE was replaced by NPOE, the intrinsic hydrophobicity increased slightly, but the number of HBA sites increased from one to three, and the aromatic ring count changed from zero to one. The polar analytes still suffered from K_1-limitation with NPOE, owing to hydrophobicity, but due to hydrogen bond and π-type interactions, the non-polar analytes were extracted with high recoveries. When NPOE was replaced by TBP, the intrinsic hydrophobicity decreased and the number of HBAs increased to four. K_1-values increased significantly, the polar analytes were extracted with high recovery, while the non-polar analytes now suffered from low K_2-values (K_2-limitation).

As illustrated in the discussion above with pure solvents, the extraction efficiency for a given analyte depends on the hydrophobicity of the solvent and the number of functional groups. Increasing the intrinsic hydrophobicity decreases K_1-values (and increases K_2), while increasing the number of functional groups increases K_1 (and decreases K_2). For high recovery, K_1 and K_2 have to be balanced. Due to this, and because the analytes were within a very broad log P-range, we tested binary mixtures of TBP and NPOE, and TBP and DHE (Table 2). The mixtures of TBP and NPOE all provided low recoveries for the polar analytes, due to high intrinsic hydrophobicity caused by NPOE. Very interestingly, the 2:1 ratio of TBP and DHE provided high recoveries (>60%) for all the analytes, regardless of polarity. This SLM clearly balanced hydrophobicity and the activity of functional groups, in such way that both K_1- and K_2-limitations were avoided for the analytes with $0.4 < \log P < 5.0$. Clearly, this is a fine balance, as evidenced by the observation that the performance of the mixtures of TBP and DHE were highly dependent of the compositional ratio of the binary mixture. The TBP and DHE mixture in ratio 2:1 was used in subsequent experiments.

3.3. Acceptor and sample composition

In the following experiment, the composition of the acceptor and sample was optimized. Due to the great difference that exists between the pH of both phases, the stability of the extraction was tested with and without buffer. The effect of phosphate buffer was studied by carrying out different extractions at different buffer concentrations (0 mM, 5 mM, 10 mM and 20 mM). It was observed that the pH changed during the extraction when no buffer was used, however, no change was observed in the pH when using phosphate buffer during the extraction. Maintaining a stable pH during extraction improved extraction efficiencies and reproducibility of the system. On the other hand, the highest recoveries were obtained with 5 mM phosphate buffer (79–85%), while efficiencies decreased as the concentration of buffer was increased. Therefore, a concentration of 5 mM of phosphate buffer was set to study the effect of pH.

In following experiments, sample pH was tested between 6.0 and 12.0, while the acceptor was fixed at pH 2 to keep the analytes positively charged. Fig. 2 shows recoveries as function of sample pH. Due to low log P and high pKa, recovery of ATN increased with increasing pH in the sample up to 12.0. At this pH, the other model analytes still were extracted with high recoveries, and pH 12.0 was selected as the final sample pH. The acceptor pH was studied between 0.5 and 4.0, while sample pH was constant at 12.0 (Fig. 3). Lower efficiencies were observed at pH 0.5 due to ion-pairing. The basic analytes were fully protonated at low pH and ion-pair formation between protonated drug molecules and phosphate ions was increased are partly back-extracted in to the SLM. The highest recoveries were obtained between pH 1.0 and 1.5, and pH 1.5 was selected for subsequent experiments. All experiments with different sample and acceptor pH were studied in triplicate (n = 3) with a relative standard deviation below 7% for all model analytes.

3.4. Sample flow rate

Sample flow rate was investigated under optimal experimental conditions as described above. The trend of the donor and acceptor flow rate has been previously studied with other compounds [38,39], observing that the extraction efficiency decreases when the donor and acceptor flow rate increases, being a more accentuated decrease in the acceptor. This is because the residence time of both the analytes and the acceptor phase within the extraction channel is low. Therefore, under this criterion, a low acceptor flow rate was

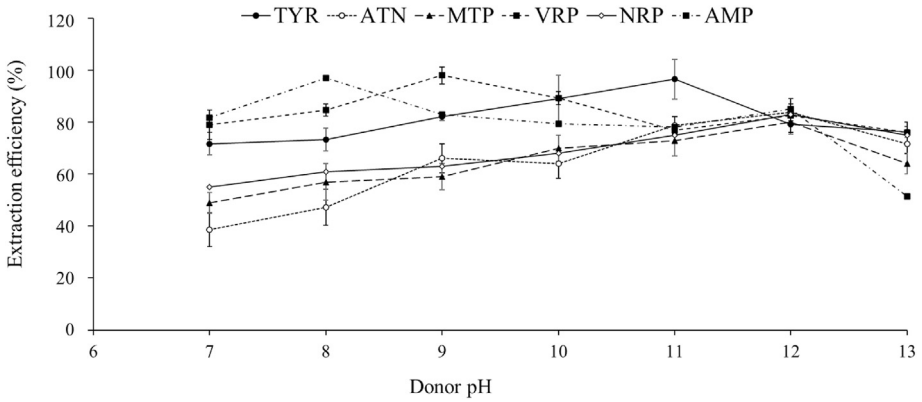


Fig. 2. Optimization of sample composition. SLM: 2:1 TBP:DHE, 5 mM phosphate buffer (pH 2) as acceptor, flow rate: 1 μL min^{-1} (acceptor and sample).

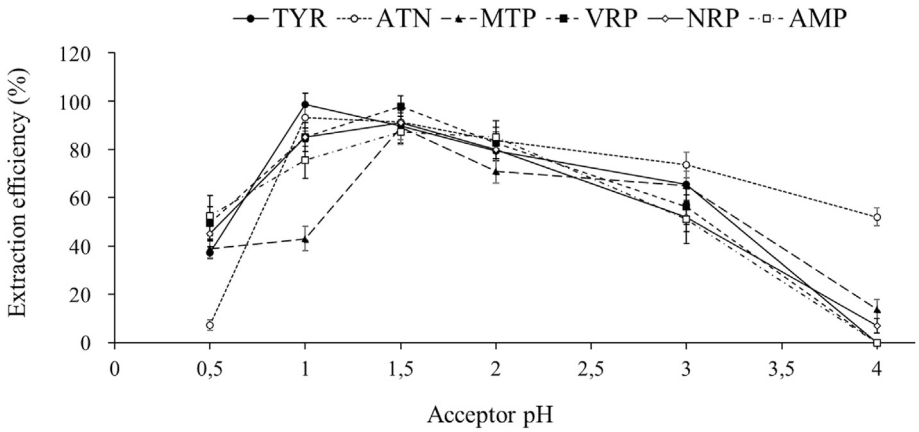


Fig. 3. Optimization of acceptor composition. SLM: 2:1 TBP:DHE, 5 mM phosphate buffer (pH 12) as sample, flow rate: 1 μL min^{-1} (acceptor and sample).

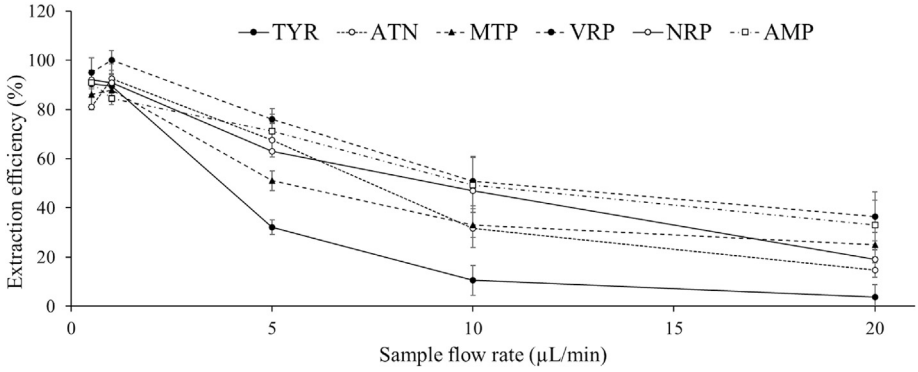


Fig. 4. Recovery versus sample flow rate. SLM: 2:1 TBP:DHE, 5 mM phosphate buffer (pH 1.5) for acceptor phase and 5 mM phosphate buffer (pH 12)for sample solution, flow rate: 1 μL min^{-1} (acceptor and sample).

set to study the influence of the donor flow rate. The sample flow rate was tested between 0.5 and 20 μL min^{-1} while the acceptor flow rate was kept constant at 1 μL min^{-1}. As seen in Fig. 4, recoveries decreased significantly when increasing the sample flow, and with the highest efficiency at 0.5 and 1 μL min^{-1}. When there is a process of passive diffusion and slow mass transfer, the analyte needs a longer residence time in the extraction channel to achieve better efficiencies. Otherwise, the analyte cannot be successfully extracted when the donor flow is increased since the contact of the analyte with the SLM decreases. A flow rate of 1 μL min^{-1} was selected to decrease the extraction time prior analysis by HPLC. Under optimal conditions, recoveries between 84 and 100% were obtained. Each experimental point was tested in triplicate with relative standard deviation below 6% at low flow rate below 5 μL min^{-1}. However, the relative standard deviation increased when at flow rate above 5 μL min^{-1}, due to decreased SLM stability.

Table 4
Method detection limit (LOD), method quantitation limit (LOQ) and extraction efficiencies at optimal conditions.

	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	R^2	EE[a]
TYR	0.18	0.60	0.9991	90 (4)
ATN	0.14	0.45	0.9996	93 (3)
MTP	0.14	0.45	0.9993	100 (3)
VRP	0.14	0.45	0.9992	100 (5)
NRP	0.18	0.60	0.9991	99 (2)
AMP	0.18	0.60	0.9994	85 (3)

[a] % Extraction efficiency (%RSD, n = 4).

The effect of carry-over was studied at 1 and 3 mg L^{-1} using different membranes and the same membrane in consecutive extractions without observing significant changes in the extraction efficiency of the collected acceptor phase. Under optimal conditions with 1 μL min^{-1} sample flow, the SLM was used for 18 consecutive extractions, and with relative standard deviations between 6 and 7%. After 18 extractions, the membrane must be filled with more organic solvent since the extraction efficiency decreases and an increase of RSD was also observed.

4. Evaluation of analytical performance

The microfluidic LPME method was combined with liquid chromatography and evaluated for the determination of non-polar and polar basic model analytes (pharmaceuticals) at optimal experimental conditions as described above. A calibration curve was constructed using a least-square linear regression analysis at six different concentrations from 0.45 to 10 μg mL^{-1} for ATN, MTP, and VRP and from 0.60 to 10 μg mL^{-1} for TYR, NRP, and AMP, observing a linear relationship with r^2 values over 0.9995 in all cases. Table 4 summarizes the calibration parameters of the method: detection limits, quantitation limits and recoveries in standard solutions. Detection and quantitation limits were based on S/N ratio of 3 and 10, respectively. Method detection limits and quantitation limits were within 0.14–0.18 and 0.45–0.60 μg mL^{-1}, respectively, for all compounds. Extraction efficiencies were 90, 93, 100, 100, 99 and 84% for TYR, ATN, MTP, VRP, NRP and AMP, respectively. Each experimental point was studied in triplicate,

Table 5
Recoveries (average of three determinations ± standard deviation) from spiked non-diluted and 1:1 diluted urine samples.

Samples	Extraction efficiency ± SD (%) (n = 3)					
	TYR	ATN	MTP	VRP	NRP	AMP
Urine 0.75 μg mL^{-1} (non-diluted)	110 ± 5	92 ± 7	105 ± 6	89 ± 3	99 ± 7	84 ± 6
Urine 0.75 μg mL^{-1} (1:1 dilution)	100 ± 7	92 ± 5	110 ± 3	90 ± 2	106 ± 3	89 ± 5
Urine 1 μg mL^{-1} (non-diluted)	100 ± 4	94 ± 3	97 ± 7	95 ± 4	99 ± 6	92 ± 3
Urine 1 μg mL^{-1} (1:1 dilution)	102 ± 5	95 ± 6	101 ± 3	97 ± 7	102 ± 5	94 ± 4
Urine 2 μg mL^{-1} (1:1 dilution)	80 ± 5	78 ± 2	89 ± 4	90 ± 7	79 ± 1	86 ± 6
Urine 2 μg mL^{-1} (non-diluted)	85 ± 7	80 ± 6	97 ± 5	96 ± 2	81 ± 2	92 ± 5

with relative standard deviation below 6% for all analytes. The device and membrane reproducibility was tested using two different devices and different membranes, replacing the membrane three times in ach device, obtaining a relative standard deviation between 4 and 8% for all compounds. Repeatability and intraday repeatability were studied in triplicate at low, medium and high concentration levels of the calibration curve. A relative standard deviation between 4-6% and 5–6% was obtained for repeatability and intraday repeatability, respectively.

5. Real samples analysis

Urine samples were collected from one 29-year old healthy adult female volunteer. Each urine sample was spiked at one concentration level (0.75, 1 and 2 μg mL^{-1}) within the linear range, containing all analytes. All samples were spiked and pH adjusted (using NaOH) prior extraction. For 1:1 dilution factor, sample was diluted using MiliQ water. Non-diluted and 1:1 diluted samples (with mili-Q water) were analysed keeping experimental parameters fixed at optimal conditions. All samples were submitted to the microfluidic device for extraction. Recoveries are shown in Table 5. As seen, high recoveries between 84 and 100% were obtained at 0.75 μg mL^{-1} with no sample dilution. A 1:1 dilution did not show an increase in recovery, and therefore both samples can be considered for real sample analysis, in which the sample does not need to be diluted to obtain good recoveries. Slightly lower recoveries were observed in higher concentration spiked samples at 2 μg mL^{-1} (78–89%). Recoveries over 78% were observed in all cases for all compounds with RSD below 7%. SLM stability was studied with the same and different membranes, observing good SLM stability for more than 10 consecutive extractions in both cases. Repeatability and intraday repeatability in human samples were studied in triplicate at 0.75 and 1 mg L^{-1}, observing a relative standard deviation below 6% in both cases. In parallel, the same experiments were carried out in another similar device to check its reproducibility, observing the same efficiencies with an RSD below 7% compared to the other device. The microfluidic method was successfully applied in urine samples as shown in Fig. 5A. Urine samples have been analysed without the proposed microfluidic procedure as shown in Fig. 5B. When both chromatograms are compared, the benefits of sample treatment procedures on complex urine samples can be clearly seen. The membrane acts selectively decreasing the interferences present in the real sample, resulting in an excellent clean-up.

6. Conclusions

In this work, a microfluidic device for simultaneous liquid-phase microextraction of polar and non-polar basic pharmaceuticals is proposed for the first time. The parameters that govern the extraction are slightly different depending on the device used, and it is important to continue researching new innovations that offer versatility to extractions, such as the study of SLM. This device enabled exhaustive (or near-exhaustive) extraction of pharmaceuticals in the log P range 0.4–5 from 10 μL humane urine samples. With only 5 μL organic solvent as supported liquid membrane (SLM), at least 18 samples were extracted with the same SLM. A comprehensive study of the supported liquid membraned (SLM)

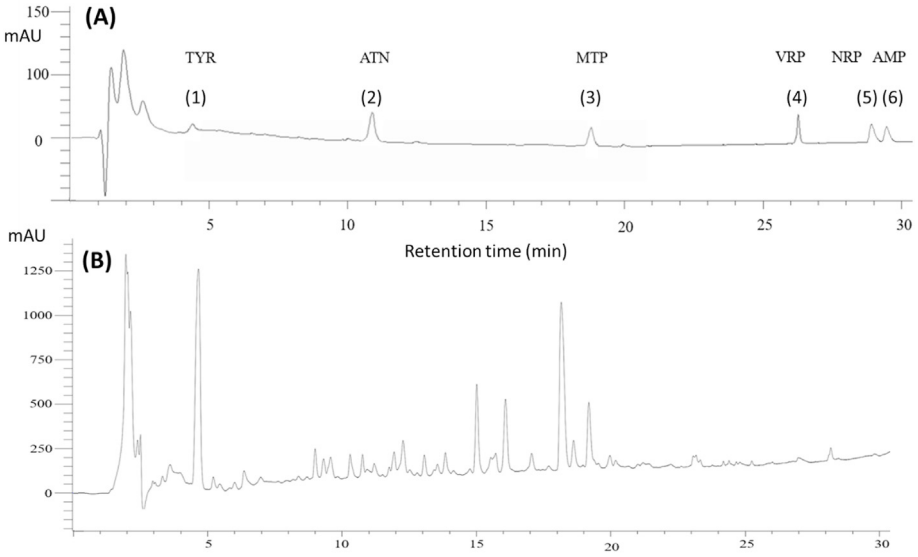


Fig. 5. Chromatogram of spiked human urine at 2 μg mL^{-1} after submitted to the microfluidic device (A) and without (B). (1) TYR, (2) ATN, (3) MTP, (4) VRP, (5) NRP, (6) AMP.

was carried out, and a SLM based on a 2:1 (v/v) mixture of tributyl phosphate (TBP) and dihexyl ether (DHE) was found to be an appropriate compromise SLM. Thus, excess of TBP kept the intrinsic hydrophobicity of the SLM relatively low, and this was mandatory for efficient extraction of the polar model analytes. DHE on the other hand reduced trapping of non-polar model analytes within the SLM, and enabled high recoveries for the model analytes with high log P. This study is based on fundamental aspects with the aim of demonstrating that the versatility of extracting this type of pharmaceutical products in a large log P window can be added to the well-known advantages of microfluidic systems, provided that supported liquid membranes (SLM) are prepared as a mixture of organic solvents with different and very specific characteristics. The compromise SLM thus provided efficient extraction of basic analytes over the entire log P range from 0.4 to 5. The TBP:DHE SLM was mechanically and chemically stable under dynamic conditions, it was compatible with human urine samples, and provided highly efficient clean up.

Ethics declarations

The sample was provided voluntarily and with informed consent.

CRediT authorship contribution statement

Elia Santigosa: Formal analysis, Investigation, Data curation. **Stig Pedersen-Bjergaard:** Formal analysis, and, Writing – original draft. **Pablo Giménez-Gómez:** Resources. **María Muñoz:** Validation, and, Resources. **María Ramos-Payán:** Methodology, Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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